Development of microbe-assisted phytoremediation of industrially polluted soils in Punjab

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By

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DECLARATION

I hereby declare that the thesis entitled, **"Development of microbe-assisted phytoremediation of industrially polluted soils in Punjab"** submitted for Ph.D. Biotechnology Degree to Department of Biotechnology, Lovely Professional University is entirely original work and all ideas and references have been duly acknowledged. The research work has not been formed the basis for the award of any other degree.

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ABSTRACT

Polluted soil and water pose a worse impact on the food and nutrients quality consumed by human and animal biota. Sources of soil pollution are mainly due to the industrial effluent discharge, which can be classified mainly into metallic and non-metallic pollutant-bearing effluents. In order to tackle with this trouble, a plant-based technology known as 'phytoremediation' is employed to clean up the contaminated lands. Apart from other processes involved in phytoremediation, phytoextraction is considered in the current work. The present study was conducted to 'develop microbial consortium for phytoremediation of industrially polluted regions of Jalandhar (Punjab). To accomplish various objectives, initially, four different sites were selected in the industrial area of Jalandhar that were found to be contaminated with heavy metals in exceedingly large amounts as compared to normal field soil. Nine different bacteria of genus *Pseudomonas, Citrobacter, Ralstonia, Enterobacter* and *Cellulosomicrobium* were isolated, identified on the basis of 16S rRNA sequencing and submitted to NCBI for accession numbers. Along with rhizobacteria, 3 different arbuscular mycorrhizal fungal species were also isolated belonging to genera *Glomus* and *Acaulospora*.

Furthermore, with the combination of all these 9 bacterial and 3 AM fungal species, a microbial consortium was developed which was assessed to check its role in increasing the efficacy of native plant species (*Ricinus communis* and *Canna indica*) to phytoextract heavy metals (Arsenic and Cadmium) from the soil. Different parameters in order to check the potential of microbial consortium, where protein content, enzymatic activities (Catalase, Glutathione reductase, Ascorbate peroxidase and Guaiacol peroxidase), plants physical parameters (height, wet weight and dry weight), percentage DPPH scavenging activity, phytochemical screening (Total Phenolic and Flavonoid content), photosynthetic pigments (Chl a, Chl b, Chl_{a+b}, Chl_{x+c}), heavy metal uptake by plant parts and other phytoremediating factors (BCF, BAF, TF, TI and PC) were determined during 3 months of experimentation period. In all these activities, microbial consortium depicted tremendous outcome with the order of efficacy: microbial consortium>mycorrhizal>rhizobacteria at significant level $p \leq 0.05$. Nevertheless, with increasing concentration of As and Cd, some activities revealed lower values ($p \leq 0.05$) and some expressed higher values. Even photosynthetic pigments showed elevated content in the plants inoculated with the microbial consortium in comparision to other treatments in both the plants. The values of BAF, BCF, TF and TI were found to be >1 after 3rd month that is evident of commendable hyperaccumulating/phytoextraction potential of plants inoculated with the microbial consortium. Hence, native microbes are best opted to fight against the evil of heavy metal pollution.

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PREFACE

This work was conducted in order to evaluate the outcome of phytoremediation to eradicate contaminants (heavy metals) from industrially polluted land. The soil was collected from different sites in the industrial area of Jalandhar, Punjab. Native plants and microbes were used alone and in combination (microbial consortium) for reducing the toxicity of heavy metals in polluted areas.

This study can be proved exceptionally beneficial for people of Jalandhar region to eradicate this potent trouble due to heavy metal toxicity.

In the current research, the investigation has been carried out on:

1. Isolation, identification and multiplication of various native microbes and arbuscular mycorrhizal fungi that are associated with native plant species found in industrially effluent polluted sites.
2. Enzymatic studies were conducted on various parts of the plants inoculated with rhizobacteria, mycorrhiza and microbial consortium along with two concentrations of heavy metals (As and Cd)
3. Evaluation of other phytochemicals, physiological and photosynthetic parameters in the plants (*R. communis* and *C. indica*) inoculated with heavy metals and microbes
4. The efficiency of the developed microbial consortium was evaluated by calculating different phytoremediating parameters for all the inoculated plants.

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INTRODUCTION

INTRODUCTION

Advances in science and technology have enabled humans to misuse natural resources to a great extent mainly soil and water sources with increasing industrial (Miri *et al*., 2016) and anthropogenic activities (Zhang *et al*., 2017a, 2017b). Soil and water contamination leads to decisive environmental and human health concern (Mojiri, 2012). Water, air and soil are said to be contaminated when the pollutants concentration increases above the defined legal standard values (Horta *et al*., 2015). A diverse range of inorganic and organic compounds are responsible for contamination problems, mainly includes heavy metals, dangerous wastes, flammable petroleum products, explosives etc. The growth of the plant is reduced due to heavy metals which further leads in the reduction of chlorophyll content and photosynthetic rates (Imran*et al*, 2016). Some of the agricultural practices such as agrochemicals usage, protract application of urban sewage sludge in agricultural soils and instantaneous industrial activities (Czarnecki and During, 2015) like waste disposal and burning of waste, as well as from anthropogenic activities are liable for adding metals such as iron, lead, arsenic, copper, zinc, nickel, cadmium, cobalt and mercury endlessly to soil (Tchounwou *et al*., 2014). Organic components can be degraded by the microorganism but heavy metal pollutants are needed to be immobilized and physically removed by separate processes for its treatment as compared to organic ones (Jadhav *et al*., 2010).

1.1 Worldwide heavy metals pollution

The group of elements with an atomic density greater than 6g/cm^3 is known as heavy metals. These heavy metals are ubiquitous in the earth's crust (therefore their availability and concentration in water and soil fluctuate from less than 1000 parts per million (ppm=mg kg^{-1}=mgL^{-1}) to few parts per billion (ppb=µgkg^{-1}=µgL^{-1}) (Alloway, 2013). A balanced concentration of heavy metals in soil is harmless to living organisms except some of the metalliferous soils. However, some operational activities such as mining, energy production and agricultural activities have increased the concentration of these heavy metals beyond the required critical-concentration in those areas which were once clean (Blaylock *et al*, 2000). Bioaccumulation and biomagnifications of these elements lead to a variety of lethal effects on the food chain of living organisms when released into the environment (Dembitsky, 2003; Manohar *et al*., 2006). In the areas where the anthropogenic activities are veryhighsome of the major environmental pollutants found there are copper, cadmium, chromium, lead, nickel, arsenic and zinc (the United States Environmental Protection Agency, 1997).

These heavy metals have caused severe toxicity around the world and there are many documented cases of different elements that cause these issues. A report documented the example of severe pollution suffered by many cities of China which is alone polluted with heavy metals in area > 1.0 million Km ^{2} i.e. 100 million hectare (He. *et al*., 2015), one of them is Linfen

which is situated in the coal region of the country; Another report is of the Dominican Republic, Haina which is the site of a former automobile battery recycling smelter and the residents of this area suffer from extensive lead poisoning ; Ranipet, an Indian city which is contaminated by azodyes and chromium by the tannery industries and this affects nearly 3.5 million people (Chottu *et al*., 2009); groundwater of Malwa region of Punjab contaminated with As, Cd, Cr, Hg, Zn, Pb (Sharma *et al*., 2017); sewage water used for irrigation purpose inLudhiana (Punjab) contaminated with Pb, Cr, Cd and Ni (Dheri *et al*., 2007); sewage water containing cadmium used for irrigation purpose in Jalandhar (Sikka *et al*., 2009). Whereas a Russian city Norilsk which accounts for the world's largest heavy metals smelting complex releases annually more than 4 million tons of copper, cadmium, nickel, arsenic, lead, zinc and selenium (WHO, 2008). According to a report by the U.S environment action group (ENS, 2006), all these documented cases are related to the toxicity level caused by different heavy metals due to which the world's most polluted areas endanger the health of more than 10 million people in different countries. Heterogeneous groups are formed by heavy metals due to their varied chemical and biological properties. Most of the heavy metals *viz.* Zn, Cr, Ni, Co, Hg, Pb, Cd, Cu, As are extremely toxic in their soluble (Yu *et al.,* 2016) and elemental forms (Pickering, 1997).

The anthropogenic contamination (Sarwar *et al*., 2016) caused by metals have different sources which includes fuel emission, mining, industrial effluents, military operations, smelting activity, agricultural chemicals, brick kilns, coal combustion and some of small-scale industries viz. metal production units , metal smelting , cable coating and battery manufacturing (Guo *et al*., 2002). Lead (Pb), arsenic (As), mercury (Hg), chromium (Cr), nickel (Ni), cadmium (Cd), cobalt (Co), zinc (Zn), manganese (Mn), aluminium (Al) and copper (Cu) are some of heavy metals commonly found in soils (Tchounwou *et al*.,2012). According to Agency for Toxic Substances and Disease Registry (ATSDR, 2012) and the United States Environmental Protection Agency (USEPA) As, Pb, Cd and Hg are included in the top 20 Hazardous Substances.

Reactive oxygen species (ROS) is produced beyond the toxic limits by all heavy metals. Acute toxicity may be caused in plants by heavy metals by destroying the unity of vital biomolecules or by interrupting the important groups of enzymes and transforming the antioxidant defence mechanism which is the outcome of ROS productivity (Sarwar *et al*., 2010). Certain heavy metals are essential for plants growth, yield and development at lower concentration (Imran *et al*., 2016) but at a higher concentration can pose deleterious effects to organisms including human beings (Roy and McDonald, 2013). Biochemical and physiological processes of plants are affected by these heavy metals which could hinder the growth of the plant and leads to critical death of plant (Xu *et al*., 2009). The toxic levels of heavy metals might damage the cell

membrane and destroy the biomolecules and cellular organelles of plants under ROS stress conditions (Ekmekci *et al*., 2008)

1.2 Remediation of heavy metal contaminated soil: Phytoremediation

Heavy metals contaminate the habitat by their insertion in soil and their persistence for longer period depends on a variety of soil and metal. The processes used for remediation of heavy metal contaminated sites are possibly in-situ (on-site) or ex-situ (off-site), chemical, physical and biological (Zhang *et al.,* 2018). For a productive and cost-effective remediation of the contaminated site often these techniques are used in fusion with one another and aim to lower the total or bioavailable fragment of heavy metal in soil along with the successive assembly in the food chain (Bhargava *et al*., 2012).

Phytoremediation *viz*. vegetative remediation, agro-remediation, green remediation, and botanoremediation are a technology that utilizes plants for remediation and revegetation of contaminated land (Sharma *et al*., 2014). In 1983, the conception of metal-accumulating plants for the cleanup of heavy metal contaminated soil was initially presented but from last 300 years, the execution of the same has been carried out. Attention is still required for the development of cost-effective, environmental friendly, logical and simple methods (Mangkoedihardjo *et al*., 2008). Therefore, Phytoremediation has been proved as an eco-friendly, non-invasive, cost-effective, attractive, energy efficient method for cleaning up the sites with different levels of heavy metal (loid)s (Sabir *et al*., 2014).

Along with other conventional remediation approaches, phytoremediation can be effectively used as a finalizing step for the remedial process and its efficiency is based on innumerable soil and plant factors like bioavailability of metals in soil, physico-chemical properties of the soil, microbial and plant exudates (Conte *et al*., 2016).Similarly, the capabilities of living organisms to uptake sequester, translocate, detoxify, and accumulate the heavy metals from soil are the significant parameters for the efficacy of this process (Markowicz *et al.,* 2016). Phytoremediation comprises diverse applications and procedures which are different in mechanisms/processes, by which plants immobilize, degrade or remove the metals from soil and it uses the plants in a different aspect to eradicate the problem of organic/inorganic contaminants (Ali *et al*., 2013). The process of phytoremediation is basically classified into phytoextraction, phytostabilization and phytoevaporation, (Figure 1) which follow different mechanisms for uptake of metals from soil (Yadav *et al*., 2018). Those plants which assemble a very high concentration of metals are known as hyperaccumulators (Mojiri, 2011).

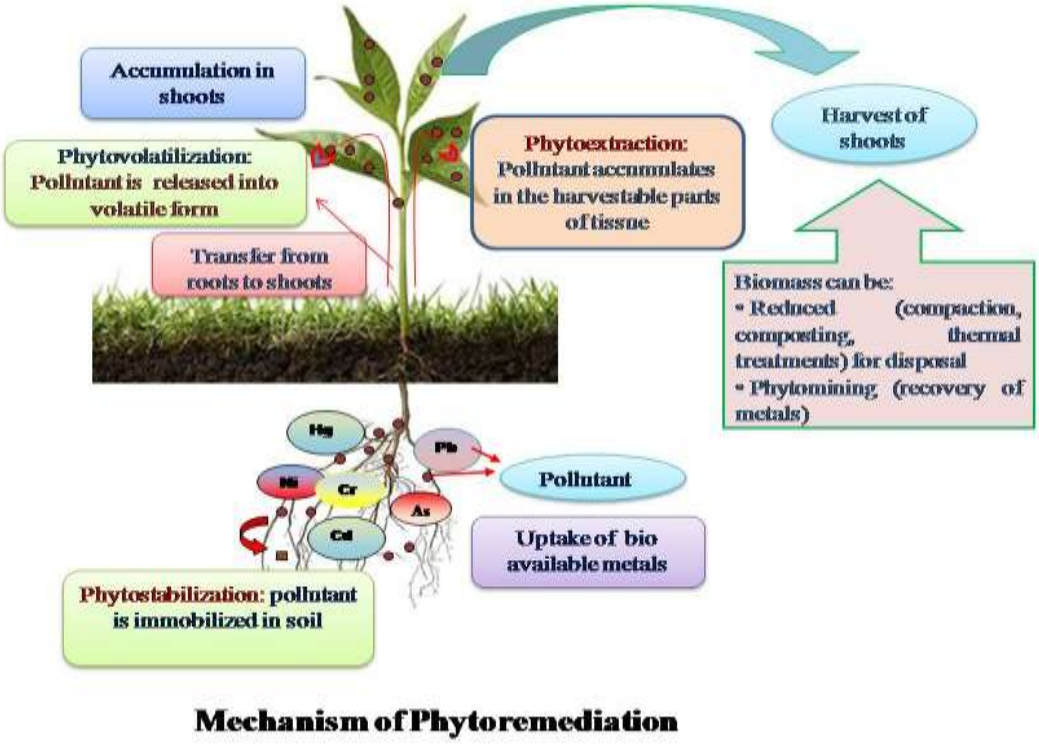


Figure 1: Types and mechanism of phytoremediation

1.3 Mechanism of Phytoremediation

1.3.1Transport and accumulation

According to Crowley *et al* (1991), the protons are released by the roots of the hyperaccumulator plants in the rhizosphere of roots to acidify the soil where metal ions are mobilized and metal bioavailability is increased. The lipophilic cellular membrane acts as the first barrier to entry of ions into the cell due to the metal ions charge. Therefore, following are the secretion types which can facilitate transportation process.

1. Transporter proteins: These proteins are inhabited by specific binding domain which can bind and transport the metal ions into cells from the extracellular space.
2. Nature chelators: EDTA is a chelator which can bind to heavy metal ions and leaves them uncharged, therefore, an uncharged ion possess high mobility and can easily get through the cell membrane. In contrast to EDTA, plants can excrete less toxic and more biodegradable nature chelators. Phytochelatin (PC) and metallothionein (MT) are well-studied nature chelators.
3. Organic acids: In the root-shoot transportation, some organic acids viz. citric and malic acid act as positive bio-reagents to enhance the absorption of heavy metals by roots.

1.3.2 Detoxification

A deleterious effect on cells is posed by hazardous heavy metals by binding to an essential protein, inhibiting the cellular activities and restricting the regulation of the cell. Fortunately, the hyperaccumulator plants protect themselves from the dismissive heavy metal stress by their own mechanism (Gang Wu *et al.*2010). Some of the detoxification mechanisms are:

1. Chelation: The heavy metals are transported, accumulated and detoxified by chelation. Metals ions can be bonded by the ligands of chelators (most often histidine and citrate) (Kramer, U., 2005).
2. Vacuole compartmentalization: The distribution and concentration of metals ions are effectively controlled by vacuolar compartmentalization since vacuole is considered as the main storage place of heavy metals in plant cells. Compartmentalization of vacuole is basically the mechanism of "arresting or imprisoning" metal ions in a limited site so that other parts of cells have no access to these metal ions (Eapen *et al*., 2005). According to Salt *et al* (1999), this mechanism is proved to be true in detoxification and tolerance in Cd.
3. Volatilization: Some plant species convert metal ions into volatile state and avoid the deleterious effects caused by a prolonged stay of heavy metals and their accumulation (Pilon, 2005).

1.4 Hyperaccumulation of heavy metals: Hyperaccumulator plants

According toAli *et al* (2018), term "hyperaccumulators" indicates those plants that accumulate a large amount of one or more metals from the soil. Further, these heavy metals are not retained in the roots but translocated to shoots and accumulated in the aerial parts of the plants mainly in leaves at 100-1000 fold higher concentration as compared to the non-hyperaccumulator plants without any visible phytotoxicity symptoms (Reeves, 2006).

Major steps involved in the hyperaccumulation of heavy metals by plants are:

1. Plasma membrane present in the root cells allows the transportation of heavy metals.
2. Translocation and loading in Xylem
3. Sequestration and detoxification of metals by the cells of the plant.

Two or more than two heavy metals are absorbed and accumulated by most of the hyperaccumulating plants (Jayakumar *et al*., 2014; Yang *et al*., 2002, 2004). Some plants show a remarkable accumulation of metals at very high levels in their aerial parts. Plants showing accumulation of heavy metals in the leaves ($\mu g g^{-1}$) above 10,000 for Mn and Zn, 1000 for Ni, Cu, Pb, As and 100 for Cd, are considered as hyperaccumulator plants for the particular element (Kramer, 2010). About 500 hyperaccumulator plant species that possess heavy metal accumulating traitshave been reported currently which mostly belong to families of

Brassicaceae, Violaceae, Caryophyllaceae, Asteraceae, Poaceae and Fabaceae(Cappa and Pilon-Smits, 2014; Gallego *et al*., 2012; Milner and Kochian, 2008). Major hyperaccumulators of heavy metals belong to the members of Fabaceae and Brassicaceae (Sun *et al*., 2011). Usually, some herbaceous plant with limited biomass is often referred to as hyperaccumulators which have the efficiency to accumulate heavy metals in their aerial parts (Vander *et al*., 2013). Thus it has been suggested that for phytoremediation, plants are desired to have high above ground biomass, high branches root, high growth rate and efficient uptake of heavy metals through translocation to aerial parts (Ali *et al*., 2013).

1.5 Arbuscular mycorrhiza in phytoremediation

Two Greek words 'mycos' and 'rhiza'meaning 'fungus' and 'roots' respectively makes "mycorrhiza" which is defined as a symbiotic relationship between a fungus and a root. This symbiotic association between a fungus and a root of a living plant is responsible for the transfer of nutrients (Brundrett *et al*., 2004). As the mycorrhizal fungi multiply in both soil and roots of the plant, the extra radial hyphae in the soil take nutrition from there and transport it to the root system of the plant. Therefore the plant's root system absorption surface area is increased. According to Sylvia *et al* (1992), as compared to non-mycorrhizal plants, the plants possessing mycorrhizal association possess more potential to withstand the environmental stress. In the Rhizosphere, heavy metals are phytostabilized by the AM fungi by the production of various compounds which results in their precipitation in the soil and they can also chelate them in their cellular structures or absorb them in their cell walls (Gaur and Adholeya, 2004; Gohre and Paszkowski, 2006). Binding of heavy metals by an insoluble glycoprotein viz glomalin, produced by fungal hyphae can affect the process of phytostabilization (Chavez *et al*., 2004).Mycorrhizal plants can accumulate heavy metals in plant shoots or increase their absorption by increasing the heavy metal uptake by the process of phytoextraction (Citterio *et al*., 2005).

In the heavy metal contaminated sites or disturbed areas, plants can easily access to immobile minerals and contribute to plant growth with the help of arbuscular mycorrhizal (AM) fungi (Vivas, *et al.,* 2003). AM fungi can elevate plant health and growth by upgrading mineral nutrition or increasing resistance to abiotic and biotic stress. Moreover, the extrametrical hyphae of AM fungi can bind the loose soil and sand grains into firm aggregates as well as plays a crucial role in plant growth and productivity. In the polluted areas, nutrients like phosphorous, nitrogen and potassium are in limited quantity which is easily accessible by AM fungi (Bheemareddy *et al*., 2011). When the level of heavy metals is elevated in the plant, they are translocated and accumulated in parenchyma cells of the root, where different fungal structures

are present viz vesicles, arbuscule and hyphae (Kaldorf *et al*., 1999). The mechanism which explains the movement of heavy metal to plant roots by AM fungi is as under:

1. Deposition of the heavy metals in the cellular walls or in fungal vacuoles
2. Heavy metal sequestration by Siderophore deposit heavy metal in soil or root apoplasm
3. Heavy metal deposition in fungal or plant cell by metallothioneins or phytochelatins
4. Heavy metal allocation from cytoplasm is carried by metal transporters located at plasmalemma of both symbionts (Galli *et al*., 1994; Leyval *et al*., 1997).

Arbuscular mycorrhizal fungi can increase plant nutrient uptake in the low or medium fertile soil including micronutrients like Fe, Mn, Cu and Zn whereas mycorrhizal plants can reduce the heavy metal concentration in their shoots with an increase in the concentration of these micronutrients in polluted soil (Schutzendubel and Polle, 2002).

1.6 Plant growth promoting rhizobacteria (PGPR) in phytoremediation

Phytoremediation potential depends upon the soil interactions with heavy metals, plants, and bacteria (Ojuederie *et al*., 2017). Figure 2 shows these interactions are affected by various factors like activity and characteristics of plant and rhizobacteria, soil properties, climatic conditions etc.

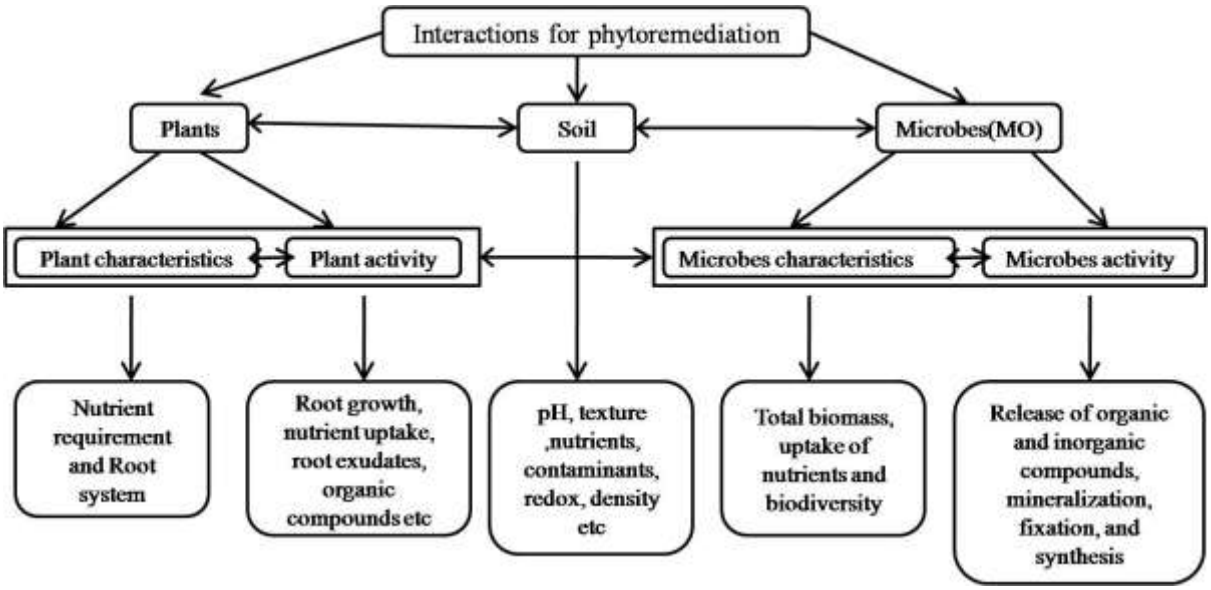


Figure 2: Rhizospheric interactions between plants, soil and microbes

According to Glick (2010), the major determinant for the phytoremediation is the interactions between a large number of different microbes and the plant roots. A major role is played by soil microbes in pest control and growth of the plant, recycling of plant nutrients, soil structure maintenance, detoxification of harmful chemicals (Elsgaard *et al*., 2001).

Therefore, the reduction of phytotoxicity of contaminated soil and remediation capability of plants is enhanced by soil bacteria (Forni*et al*., 2017). A specific association is formed between plant and bacteria in which plant provides a specific carbon source to bacteria that tend the

bacteria to decrease the phototoxic level of contaminated soil (Wenzel, 2009). In contrast, plant roots also provide root exudates and increase ion solubility. Hence, the remediation activity of bacteria related to plant roots is enhanced by these biochemical mechanisms (Rashid, 2018). In addition to that, metal chelating agents known as siderophores are produced by rhizobacteria that play a major role in the procurement of heavy metals (Dimpka,2016).Often, in the soil contaminated with heavy metal plants are iron deficient, therefore these metal chelating-Siderophore produced by rhizobacteria help plants to acquire adequate iron (Burd *et al*.,2000). Plant growth is enhanced by both free-living as well as symbiotic plant growth promoting rhizobacteria (PGPR) (Vessey, 2003). Various microorganisms in the soil enhance the plant enrichment by altering physico-chemical properties along with a change in form of metals in the soil (Zhang *et al*., 2018).

Some of the parameters that are responsible for enhancement of plant growth by both free-living as well as symbiotic bacteria are: availability of phosphorus for uptake by plant, production of plant hormones viz auxins, gibberellins and cytokinins, reduction in the level of plant ethylene, sequestration of trace elements like iron by siderophores for plants, nitrogen fixation for plant, increasing the availability of phosphorus for uptake by plant (Glick *et al*., 2010). PGPR are considered as a good fertilizer for increasing plant growth on heavy metal contaminated site, hence PGPR is used in phytoremediation technology for better plant growth (Burd *et al*.,2000) and elevate detoxification of soil(Mayak *et al*.,2004). In phytoremediation certain properties of plants which enhance phytoremediation rate, such as, high level of biomass production, less contaminant uptake, health and nutrition of plant, are upgraded by PGPR. But the selection of PGPR is very important as its survival and success rate is to be determined when used for phytoremediation practice. Arbuscular mycorrhizal fungi (AMF) paired with PGPR can prove to be an excellent way in increasing the phytoremediation efficiency (Leyval *et al*., 2002). Even though PGPR role is important in phytoremediation but restricted research in this area has been done and field studies are required to support the practice of phytoremediation.

Therefore, the present study focuses on the development of microbial consortium consisting rhizobacteria and arbuscular mycorrhiza fungi (AMF) which are capable of remediating high concentration of heavy metals from the industrially polluted soil with the help of native plants species. Along with monitoring the effect of heavy metals on the native plant species, various antioxidant enzyme activities of plants as well as their photosynthetic capability were studied. Total phenolic and flavonoid content (phytochemicals) in plants was determined along with different phytoremediation parameters including Tolerance index, Biological concentration factor, Biological accumulation factor, Translocation factor and Phytoextraction capacity.

CHAPTER 2

REVIEW OF LITERATURE

REVIEW OF LITERATURE

2.1 Soil pollution

The soil is considered as the biogeochemical agent which is a natural support system of life on earth (Robinson, 2012). The natural flow of contaminants is altered by the current scenario of industrial activity as some of the novel metals are introduced into the environment. The discharge rate of these effluents in soil and water has been boosted up by the increase in the industrialization and urbanization including many other activities like mining farming, military activities, waste practices etc (Zhang *et al.*, 2017a). These practices have contaminated a huge area with elevated levels of organic and inorganic contaminants. In the ecosystem, negative effects are posed by these contaminants and is a great danger to public health when enters into drinking water or food products through leaching (Qing-Hong *et al*., 2012). Therefore "a site where a firm amount of hazardous substances are present due to inhuman activities to a level which can cause a consequential threat to the recipient is required to be managed by a strict action against it" is defined as a contaminated site (Van-Camp L., 2004).

Industries discharge the effluents mostly consisting of toxic substances comprising heavy metals such as copper, lead, cadmium, zinc, nickel and chromium which are considered major pollutants of the environment in high anthropogenic pressure areas (United States Environment Protection Agency, 2001). Therefore, in the environment, their presence is of major concern because they pose a big threat to the environment as well as human life due to their toxic levels and higher bio-accumulating tendency (Jaya Singh *et al*., 2010). The heavy metals are added permanently to the soil as they are not subjected to microbial attack or degradation; therefore they are considered most conventional pollutants in the environment. Hence, heavy metal concentration often overshoots the permissible level found in water, sediments and soil which in turn damage the biological processes (Massa *et al*., 2010). So, being a conventional disposal site of most heavy metals, soil needs to be treated for an eco-friendly environment.

2.1.1 Organic contaminants in soil

Industrialization accounts for the synthesis of substantial diversity of anthropogenic chemical compounds in which some of the chemicals such as nitroaromatic and organ chlorines are synthesized knowingly whereas some other chemicals like PVC (polyvinyl chloride plastic, are produced and burnt to generate noxious undesired by-products. Some other varied blend of hydrocarbons such as crude oil is formed sunken under elevated pressure from the leftover of organic material and can be polished into abundant products (OPEC. 2011). It is used as raw material for several products such as solvents, paints and plastics. Conceivably crude oil is the most utilized non-renewable natural resource, as well as the substances derived from the crude

oil, are the most frequent contaminants of the environment. Mostly plastic products can be burned as they are made up of hydrocarbons, whereas some chlorine-rich products of plastic like PVC make dioxins when burnt (O'Hara K., 1988). Various municipal landfill and dump are often filled with plastic waste; therefore, constructive methods are required for the cleanup of oil-polluted sites as due to the high concentration of carbon in the oil products they are readily utilized by the microorganisms in the environment. Hence, bioremediation is considered as an effective remedy for the treatment of such sites because it is a biological, cost-effective and feasible method. Example of such treatment is the Deepwater Horizon oil spill the USA where direct application of dispersant was done to disperse the oil that elevates the natural level of oil biodegradation (Atlas, 2011).

Polyaromatic hydrocarbons are another organic contaminants found in the environment which are not produced for commercial use as they are formed as a result of forest fires and volcanic eruptions. Whereas, mostly PAHs are originated by the anthropogenic sources like partial combustion of wood, waste and fossil fuels; exhaust of automobiles; and petroleum spills (Dabestani R., 1999). PAHs are transported by wind to remote locations where they are directly deposited on the soil or indirectly added and deposited through agricultural practices. Approximately, 0.7-1 mg/m^2 of PAHs is deposited in soil by atmospheric discharge (Wilcke W., 2007; Johnsen AR., 2007). One more popular explosive which comes in the family of organic contaminants is trinitrotoluene, or TNT, mostly used in buildings and mining. TNT is an interminable contaminant but can be degraded aerobically as well as anaerobically by the microbes (Caballero, 2001).

2.1.2 Inorganic contaminants in soil

Inorganic contaminants mainly include radionuclides (P, U and Cs), plant fertilizers (viz. Phosphate, nitrate etc) and most importantly metals/metalloids (Eg Hg, Cd, As, Cu, Mn, Zn, Se etc). They occur naturally throughout the earth's crust and mainly in the form of negatively or positively ions and their uptake and translocation depend on plant transporters. These metalloids are widely utilized by various industries and in agriculture purpose and released into the environment (Tchounwou *et al*., 2014). Also, by some of the tannery industries, these toxic elements are disposed off in the form of ammonia, hydrogen sulphide and chromium (Chauhan *et al*., 2015). These inorganic contaminants cannot be degraded but can be altered by reduction/oxidation and moving into the plants or by volatilization (Se, Hg) (Dhankher*et al*., 2012).The heavy metal definition is almost debatable but due to some efforts, they can be more precisely called as potential toxic elements (PTE) (Duffus *et al*., 2002). Chromium, cobalt, arsenic, silver, molybdenum, lead, nickel, zinc, iron, copper, silver, cadmium, mercury and

manganese are some of the potentially toxic elements (PTE's) (Alloway, 2013). Unlike other organic molecules, their presence in the environment as a contaminant is persistent.

Elements with an atomic weight between 6.5 and 200.59 and a specific gravity greater than 4, are known as heavy metals (Kennish, 1992). Some heavy metals are required in a trace quantity by living organisms whereas these metals can be injurious to organisms if taken in an excess amount (Berti *et al*., 1996). Heavy metals are divided into two categories; essential and non-essential, where arsenic, cadmium, chromium, mercury, lead, antimony etc belong to non-essential heavy metals where these metals are responsible for surface water and soil contamination (Kennish , 1992). Heavy metals occur in various phases like colloidal, particulate, dissolved and ionic phase as well as possess a prominent affinity for organoclays, organic matter coated oxides and humic acids. Cation exchange capacity, pH, amount of metal, organic carbon content, the redox potential of the system and mineral components oxidation state controls the solubility of heavy metals in groundwater and soil where the soluble forms are mostly ions or unionised organometallic complexes.

According to many reports, heavy metals cause diverse diseases in humans (Sarwar *et al*., 2016) namely chronic anaemia, kidney damage, cognitive impairment, cancer, nervous system, brain, skin, bones (Jarup, 2003) cardiovascular disease etc. As compared to other organic contaminants heavy metals are tremendously resistant to chemically or biologically induced degradation due to which the content of heavy metal (loid) persists for long period in the soil after being introduced into it. Therefore, it is a global concern to observe the content of heavy metals in the agricultural soil and crops that are cultivated on this soil which should not overreach the permissible limits because of the toxic effects associated with the exposure to these heavy metals (Sana Khalid., 2016).Nandkumar *et al*. (1995) reported, the persistence of lead in soil is for a period of 150-5000 years and can exist in soil for more than 150 years after the application of sludge. Likewise, half-life of cadmium is also reported biologically to be more than 18 years. As, pollution posed by heavy metals is not a big issue in worlds most parts, but due to its effect on agricultural productivity it is a major concern. High reactivity rate of heavy metals accounts directly for the senescence, low energy synthesis process and lower growth rate. Xu *et al*(2008) report for the adverse effect of heavy metals on the absorption and transportation of essential elements, which influence the metabolism resulting in lowering growth and reproduction of plants. According to Ruley *et al* (2006), reduction in leaf chlorophyll synthesis and its growth is a result of the elevated level of lead which is more than 30 mg. Lead can cause many issues like the decline in photosynthetic rate, yellowing of young leaves, reduced crop production, lower growth rate, lowering the level of absorption of some vital elements like iron (Fe) from the soil. Some of the anthropogenic sources and harmful effects of toxic heavy metals are discussed in table 1.

Table 1: Anthropogenic sources and harmful effects of heavy metals

Heavy metal	Anthropogenic sources	Harmful effects	References
Arsenic (As)	Wood preservatives and pesticides	As is a phosphate analogue, therefore, hinders the cellular processes like ATP synthesis and oxidative phosphorylation	Thangavel and Subbhuraam (2004), Tripathi *et al*. (2007)
Cadmium (Cd)	Paints, electroplating, phosphate fertilizers, plastic stabilizers	An endocrine disruptor, hinders the calcium regulation in biological systems, mutagenic, carcinogenic renal failure, chronic anaemia	Pulford and Watson (2003), Salem *et al*. (2000) and Awofolu (2005)
Chromium (Cr)	Fly ash, steel industries, tanneries	Side effects causing hair loss	Khan *et al*. (2007), Salem *et al*. (2000)
Copper (Cu)	Fertilizers and pesticides	Kidney and brain damage due to a raised level of Cu, chronic anaemia, liver cirrhosis, intestine and stomach irritation	Khan *et al*. (2007), and Wuana and Okieimen (2011)
Mercury (Hg)	Coal combustion and medical waste	Depression, drowsiness, hair loss, memory loss, damage to the brain, kidney and lungs, ulcers, vision disturbance, memory loss, restlessness.	Wuana and Okieimen (2011), Ainza *et al*. (2010) and Gulati *et al*. (2010)
Nickel (Ni)	Surgical instruments, alloys of steel, automobile batteries, kitchen appliances, industrial effluents	Hair loss causes cancer of the lungs, nose and sinuses, pulmonary toxic, cancer of stomach and throat, immunotoxic, nephrotoxic, genotoxic, reproductive toxic.	Tariq *et al*. (2006), Salem *et al*. (2000), khan *et al*. (2007), Das *et al*. (2008) and , Mishra *et al*. (2010)
Lead (Pb)	Manufacturing of battery, insecticides and herbicides	Reduced intelligence, impaired development in children, learning disabilities, coordination problem, renal failure, cardiovascular disease, short-term memory loss.	Wuana and Okieimen (2011), Padmavathiamma and Li(2007), Wuana and Okieimen (2011) and iqbal (2012)
Aluminium (Al)	Aluminium cookware, deodorants, drinking water, food, beverages and aluminium containing drugs.	Nausea, mouth ulcers, skin rashes, skin ulcers, vomiting, diarrhoea, Alzheimer disease, loss of coordination, memory loss, balance problem.	WHO (1997), Krewski *et al*. (2009)
Antimony (Sb)	Used in fireproofing and plastics, found in battery electrodes,ceramics,pigments frequent use in firearms	Respiratory problems, tissue irritation, RBC haemolysis, skin rashes.	Carson. (1987)
Zinc (Zn)	Plating iron and steel, galvanizing	Nausea, vomiting, diarrhoea, kidney and stomach damage, itching, tingling, stinging on skin	Hess and Schmid (2002)
Manganese (Mn)	Industrial dust and fumes	Affects nervous system, central and peripheral neuropathies.	Neustadt and Pieczenik (2007)

2.1.3 Worldwide pollution of heavy metal

According to Singh *et al* (2003), annual release of heavy metals worldwide have reached 7,83,000 metric ton forthe lead, 22,000 ton for cadmium, 1,35,000 ton for zinc and copper 9,39,000 ton. European Environment Agency (EEA) has reported about the content of toxicity that has been added to the environment because of the anthropogenic activities whichhave increased up to three million sites in its member countries, whereas the need for the cleanup actions is required in 2,50,000 sites. EEA, while taking into consideration the actual trends, founds that this figure will be increased to about 50% by 2025 (EEA 2007; Wber *et al*. 2006). Most abundant soil contaminants are heavy metals, which accounts for polluting various countries frequently including Europe, the US, England, China and India. Out of all these countries, China produces the maximum amount of chromate and releases into the environment (Mahar *et al*., 2016) and the lands contaminated by heavy metals were almost 20 Milli hectare (MHa) including $1/5^{th}$ of the total arable land (Bah *et al*., 2010). A potential risk is posed to the ecological and human health by extensive mining and smelting of lead and zinc ores which results in contaminating soil.

Different sites account for the presence of different metals and the source of every pollutant also varies accordingly. The main constraint for the quality of agricultural land and food safety is caused by a major soil contaminant that is cadmium (Atafar *et al*., 2010). According to a report given by Kurek and Bollag (2004), the change in the composition of the microbial community of soil is affected by the heavy metal contamination in the soil which further affects the characteristics of the soil. Some of the heavy metals (magnesium, calcium, potassium, zinc, chromium, sodium, iron and copper) are required in a certain specific amount and if that amount exceeds the given amount, than these heavy metals can cause toxicity and their deficiency can decelerate the biological functions and processes (Fahad and Bano., 2012; Fahad *et al*., 2014; Fahad *et al*., 2016; Khan *et al*., 2013).

Whereas, there are some toxic metals (Cadmium, mercury, nickel and lead) which when ingested can cause severe health risk in humans (Muhammad *et al*., 2010, 2011). It has been reported in various studies that some of the trace elements like iron, lead, cadmium, chromium, nickel, cobalt, copper, zinc and manganese can be threatening for consumption purpose (Agusa *et al*., 2006; Buschmann *et al*., 2008; Frisbie *et al*., 2009; Luu *et al*., 2009). Among all the heavy metals, arsenic has been proved to be a challenge as a major contaminant in groundwater for the world.

According to Ravenscroft *et al* (2009), nearly 150 million people have been overblown by the ingestion of arsenic from water. Arsenicosis and several other arsenic analogous diseases related to skin and internal cancers in the organs like kidney, lungs and bladder have been affected more

than 7 lakh people in the regions of South and East Asia (World Bank Policy Report, 2005; Schmoll *et al*., 2006; Rahman *et al*., 2009). Smedley and Kinniburgh (2002) reported some other nations of the world that have been overblown by heavy metal contamination are China, India, Bangladesh, Pakistan, Argentina, Mexico, Hungary, Chile, Vietnam, Mexico and several parts of United States of America. Moreover, this heavy metal pollution issue is faced by more than 25 other nations of the world which include India, Pakistan, China, Ghana, Senegal, Kenya, Ethiopia, Mexico, Uganda, Sri Lanka, Argentina, Algeria and Ivory Coast (Rafique *et al*., 2009; Rahman *et al*.,2009).

Apart from all these heavy metal polluted countries, Pakistan is one of them having different areas which are mostly affected by arsenic like Lahore and Kasur, Punjab (Farooqi *et al*., 2007), Manchar lake, Sindh (Arain *et al*., 2008, 2009), Jamshoro, Sindh (Baig *et al*., 2009), Muzaffargarh, Punjab (Nickson *et al*., 2005), Tharparkar, Sindh (Brahman *et al*., 2013) and DG Khan, Punjab (Malana and Khosa., 2013). According to a report given by Arain *et al* (2008), in Hyderabad city, more than 40 people died because of elevated levels of arsenic and other toxic metals in drinking water which was consumed unknowingly by people. Furthermore, increased toxic levels of heavy metals have affected many areas in Punjab, where Pakistan Council of Research in Water Resources (PCRWR) declared six cities including Kasur, Gujranwala, Multan, Vehari, Bahawalpur, Lahore and Sheikhopura (PCRWR, 2005). After the analysis of various reports from literature and physico-chemical analysis of selected sites, two most abundant and toxic heavy metal has been selected for this research work *viz* Arsenic and Cadmium.

2.1.4 Arsenic: Sources and Harmful effects

In 1250 A.D, Albert Magnus discovered a toxic metalloid known as 'Arenic' (As) which belongs to group V and atomic number 33 in the periodic table and is found in both organic and inorganic forms in the environment basically in plants (Artus, 2006). In various countries, arsenic is found in the groundwater beyond the drinking water regulation levels in different areas. Major health issues related to the higher toxic levels of arsenic are diabetes, cardiovascular diseases and cancer (Abernathy *et al*., 2003; Guo, 2004). Apart from this several anthropogenic sources like usage of arsenic-based pesticides and herbicides, mining practices and usage of arsenic contaminated water for irrigation are the major causal agents of soil contamination, predominantly in farmLand ecosystem (Jackson *et al*., 2006; Chen *et al*., 2008; Williams *et al*.,2009). From January 2006, United States Environmental protection agency (USEPA) in context of arsenic contamination has reduced the extreme contamination limit of drinking water from 50 to 10 μgL^{-1} to adequately safeguard human health from deleterious effects of arsenic (USEPA, 2001) because it is considered the topmost hazardous substance in the list of toxic

metals by US Agency for Toxic Substances and Disease Registry.Arsenic holds a position of the 20th number in the terms of its abundance in the earth's crust, 12^{th} number in human body and 14th in seawater (Mandal and Suzuki, 2002). Arsenic contamination is not a major issue in the case of groundwater but also causes toxicity in soil.

According to Rana *et al* (2007), the As content found in the Indian soil is 10 $mgkg^{-1}$ to 20 $mgkg^{-1}$, in Bangladesh, it is found to be lower than 10 $mgkg^{-1}$. The contamination level of arsenic may reach up to 80 mg L^{1} in the areas where irrigation is done with the help of contaminated water and according to an estimation the arsenic builds up in soil is up to 5 kg ha^{-1} $year^{-1}$ (Huq *et al*., 2003). In South Asia recently 10 million people were overblown due to the drinking water contamination by As and around 200,000 to 270,000 people have died worldwide due to cancer caused by the same. Nine out of total 18 districts of West Bengal have been spotted for having the arsenic levels in groundwater above 0.05 mgL^{-1} whereas arsenic affected regions in West Bengal held about 63% of total population and 44% of total area. Rosen and Liu (2009) reports that the safe limit of drinking water 10 μgL^{-1} is exceeded by arsenic contaminated water in China, India and coastal belts of Bangladesh where India along with Bangladesh holds the limit of 50 μgL^{-1} as prescribed by WHO (WHO,2001). Millions of people suffer from the adverse effects of arsenic-polluted drinking water in the areas of Australia, South America, Japan, India and Bengal (Alaerts *et al*., 2001; Mandal and Suzuki, 2002; Ohno *et al*., 2007).

Various anthropogenic sources that release arsenic into the environment greatly differ in their bioavailability and chemical speciation. Commercial waste accounts for 40% As discharge onto land, accordingly mining industry accounts for 16%, coal ash 22% and steel industry discharge 13% (Eisler, 2004). Various cosmetics, fireworks, antifouling agents, pigments, ceramic and glass, electronics and Cu based alloys are manufactured with the help of (As_2O_3) arsenic trioxide (Leonard, 1991). Another use of arsenic is in wood preservation with the help of copper and chromium i.e copper-chromium-arsenate (CCA).As forcontaining, pesticides and herbicides are disposed off by the industries that are the major source of soil and water contamination. In Calcutta, India random discharge of copper acetoarsenite, an arsenical pesticide (Paris Green) from the industrial effluents during its manufacturing lead to soil and groundwater contamination (Chatterjee *et al*., 1999). On the other hand, effluent released during timber treatment is considered as the major source of contamination by arsenic both in terrestrial as well as the aquatic environment in New Zealand (Bolan and Thiyagarajan., 2001). Similarly, As is released into the atmosphere by the combustion of coal where the fly and bottom ash that is generated contains ample amount of arsenic which when disposed off often leads to the contamination of both water and soil (Beretka and Nelson, 1994). Some of the horticulture pesticides which are used in agricultural practices (orchards) often lead to As contamination in different parts of the

world, an example of some pesticides are Paris Green, magnesium arsenate, zinc arsenate, calcium arsenate, lead arsenate and zinc arsenite (Peryea and Creger, 1994). Smith *et al* (1998) reported some of the organoarsenical herbicides viz disodium methanearsonate (DSMA) and monosodium methanearsonate (MSMA) also cause As contamination in soil.

Another report states that arsenical pesticides are used in sheep and cattle dips to control the lice, ticks and fleas that also cause As contamination in soil (Mc Bride *et al*., 1998). In New South Whales a study was conducted which reported around 11 dip sites with a considerable amount of surface soil (0-10 cm) As contamination (37-352 $mgkg^{-1}$) where movement of arsenic was found to be 57-2282 $mgkg^{-1}$ 20-40 cm down in depth of soil profile (Mc Laren *et al*.,1998). Similarly, soil is contaminated by the regular application of As-containing fertilizers which are taken up by plants and finally reaches the food chain (Mc Laughlin *et al*, 1996).

Normally, concentration of As in does not exceed 10 $mgkg^{-1}$ in uncontaminated soil whereas the elevated levels of arsenic in soil is due to the anthropogenic activities (Adriano, 2001) For example, level of As in soil extend up to 9300 $mgkg^{-1}$ near the As mineral deposit areas therefore, As concentrations in soil can extend from more than 1 to 250,000 $mgkg^{-1}$ relying on the attributes of anthropogenic and geogenic sources.

2.1.5 Cadmium: Sources and Harmful effects

Cadmium (Cd) is believed to be 2-20 times more toxic than many other heavy metals (Kabata-Pendias, 2001). Cd pollution in the environment is caused due to both anthropogenic and natural sources where the anthropogenic sources include metal and ore processing, mining, agricultural activities and Cd contaminated sludge and water disposal, added 3-10 times more Cd into the environment as compared to natural sources including forest fire, transport of soil particles by wind and volcanic eruptions (Irwin, 1997). Anthropogenic activities have elevated the level up to 2.2-107 kg release of Cd worldwide in the past five decades (Costa *et al*., 2012). Places which are intensely cultivated and industrially established are considered as most polluted areas by cadmium contamination (Lux *et al*., 2011).

Cd possess a very little biological demand (Prapagdee *et al*.,2013) and hence it is considered relatively more mobile as compared to other heavy metals, in the soil-plant system and it inhabits the ability to move deeper into soil layer or ground /surface water (Selvam and Wong.,2009). In the environment, cadmium is believed to be most hazardous heavy metal as it can easily assemble in organism and at less toxic levels can cause cancer (Grat-ao *et al*., 2012) therefore US Department of Health and Human Services' Agency for Toxic Substances and Disease Registry (2007) ranked this metal with 7^{th} position in the list of top 10 hazardous substances. Cd-contaminated food is the major source of entry of Cd into the human body (Vido *et al*., 2001). Half-life of Cd is 25-30 years, therefore if it is absorbed or ingested may cause

persistentpoisoning (Uraguchi and Fujiwara, 2013) and poses a major risk to society by causing hypercalciuria, interrupting Ca metabolism and formation of kidney stones. Itai-itai is a disease that is generated due to prolonged exposure to Cd, indicated by kidney failure and softening of bones (Nogawa, 1981).

Annually 3×10^3 tonnes of Cd enter the environment (Sanit-a di toppi and Galbbrielli, 1999). A significant role is played by the composition of soil and parent material for Cd distribution in the profile. According to a report, Concentration of Cd may reach upto 10 $mgkg^{-1}$ (Dubois *et al*., 1998; Benitez., 1999) and even upto 22 mg kg^{-1}in another case (Prudente, 1999). A notable assembly of lead is figured as $5.6\text{-}38\times10^6$ kg Cd yr^{-1} in soil worldwide due to human activities (Jasia and Fulekar, 2009). The deleterious effects of Cd are faced by various countries like Belgium, Sri Lanka, Spain, China etc. A major by-product of different anthropogenic activities like mining, metal ore refining and smelting, is cadmium (Stanbrough *et al*., 2013). Metal mines account for the accumulation of Cd in the soil to the range of 980 $mgkg^{-1}$ (Fuge *et al*, 1993) whereas 900-1500 $mgkg^{-1}$ Cd was found in the soil nearsmelting industries (Peters and Shem, 1992). As compared to copper mine tailings, manganese mine tailings usually possess elevated levels of Cd (Li, 2006) and tin mine tailing possess as high as 100 $mgkg^{-1}$(Song *et al*, 2004).

Major sources of cadmium to enter the environment is through industrial waste which include Cd containing batteries, preparation of alloys, paints, plastic manufacturing, and electroplating (Adriano,2001;Cordero *et al*,2004), automobiles, household appliances, parts of airplane, industrial tools, fasteners such as screw, nut bolt etc, rubber curing, curing of rubber, luminescent dials, photography, farming machines and fungicides (Adriano, 2001). Likewise, 332 $mgkg^{-1}$ Cd was found in the reclamation site for battery (Elliott and Brown, 1989), 8.5 $mgkg^{-1}$ in the lead battery recycling site (Superfund Site) in Midwest, USA (Pichtel *et al*,2000), maximum levels of Cd found to be 8.8 $mgkg^{-1}$ at the lead acid battery dump (Pichtel *et al*,2000). High levels of Cd has been found to be present in composted sludge, therefore, it is used as soil amendments and can be easily accumulated by the plants that are grown on Cd contaminated sites (Hassan and Aarts, 2011).

Another source of Cd found in elevated levels in soil is due to the inflated amount of contaminated phosphate fertilizers application to the soil (Booth, 2005; Stephens and Calder, 2005). Apart from these sources, Cd contamination is also found in the agricultural soils which are irrigated with the wastewater. As reported by Ji *et al*. (2011) concentration of Cd was found to be 10 $mgkg^{-1}$ in the surface soil of Shenyang Zhangshi Irrigation Area (SZIA) in China, which was irrigated for more than a decade with wastewater. In the 1960s, due to rapid industrialization paddy fields of some regions in Japan were heavily polluted by Cd (Makino *et al*., 2010). 0.097$mgkg^{-1}$ was found to be an average content of Cd in the normal soil as compared to the Cd-

contaminated soil which is reported to be 3.16 $mgkg^{-1}$in the soil irrigated with wastewater (Cheng. 2003). Rice fields Cd contamination in China have reached up to 50 million kg^{-1} in 1999 as compared to the total cultivable land contaminated with Cd was found to be 13,000 hectares (Wang *et al*., 2010b; Li *et al*., 2009c) and recently 100 million per annum (Ministry of Environmental Protection of China. 2013).

Similarly, black soil of Northeast China also holds 3 $mgkg^{-1}$ of Cd in the paddy fields which overreach the limit recommended by the World Health Organization of less than 0.2 $mgkg^{-1}$ (Schnoor, 2004). 2.6 $mgkg^{-1}$ of Cd has been reported in the rice grains and blood and urine of localities of that area (Xiong *et al*., 2004). Urban-based processes of industries add high amount of heavy metals into the atmosphere in various industrialized countries (Gray *et al*., 2003). In the Netherlands, approximate 420 km^2 of the area are contaminated with different metals like Zn, Pb and Cd along with some areas in Flanders which are estimated to be around 280 km^2 due to the enduring deposition in the atmosphere (OVAM *et al*., 2004). According to Meers *et al* (2010) atmospheric discharge of certain metals like Pb, Zn and Cd account for polluting some regions in Belgium and Netherlands with around 700 km^2 area.

Amongst the common sources of Cd emission in the soil along highways is the aerial emission through car exhaust. For example, the concentration of Cd in soil was found to be very high in the location nearby the highway (7 m) as compared to the one which is far away (200 m) from highway. Apart from the toxic effects on human, elevated levels of Cd poses deleterious effects on plants also. The toxic effects of Cd in the plants are: reduction in nutrient and water uptake, inhibition of photosynthesis and respiration rate of plants, lipid peroxidation enhancement, alterations in gene and protein expression, disturbance in metabolism and elevating accumulation rate of reactive oxygen species (ROS) (Sandalio *et al*., 2001; Sharma and Dubey., 2005; Tanhan *et al*., 2007; Lopez-Millan *et al*., 2009; Semane *et al*.,2010). Therefore, it is a need of the present time to effectively control the pollution that is caused by these metals in the environment (water and soil) by eco-friendly techniques.

2.2 Phytoremediation: strategy for environmental cleanup

In the context of this environmental pollution caused by the different organic and inorganic contaminants, different remediating techniques have been developed out of which some are very expensive and some are cheap and eco-friendly. As, soils contaminated with heavy metals have been a major issue, therefore it has encountered a significant attention at present.

Decontamination of the polluted/contaminated sites by the implementation of various biological processes is a demanding chore because heavy metals endure in the soil and cannot be degraded (Kidd *et al*., 2009; Rajkumar *et al*., 2010, Ma *et al*., 2011a). Extraction and concentration of heavy metals should be carried out in order to remediate the contaminated site by a suitable

technique for appropriate disposal in the landfill sites which are secure for their ejection. Some of the expensive well established and traditional techniques for the remediation of these heavy metals include: physical separation, solidification, burial, thermal processes and electrochemical methods and are frequently detrimental to microbial diversity of soil (Pulford and Watson, 2003; Dermont *et al*., 2008).

Phytoremediation is often referred to as a process in which plants are involved in detoxification/decontamination of polluted sites. Phytoremediation is recommended as a substitute method for the removal of pollutants from soil, water and air with the help of plants and microbes (Sobariu *et al*., 2017) without affecting the biological activity, fertility and structure of soil (Cristaldi *et al*., 2017). The efficiency of phytoremediation depends on diverse factors that include bioavailability of metals, plant species, the property of soil and essence of contaminant (Sreelal and Jayanthi, 2017)

2.2.1 Phytoremediation: Broad categories and their implementation

Phytoremediation is mainly categorized into four different types on the basis of heavy metal remediation methods:

Phytoextraction: removal of heavy metals from the soil by concentrating heavy metals in the harvestable parts of the accumulator plants.

Rhizoremediation: elimination of contaminants from the soil by using microorganisms that are related to the plants.

Phytostabilization: pollutants are stabilized by the use of plants to minimize the environmental stress.

Phytovolatilization: Microbial genes are used by genetic engineering methods to convert toxic contaminants intonon-toxic pollutants.

2.2.1.1 Phytoextraction

The plants uptake heavy metals from the soil and clean-up the environment with this solar driven technique which is based on the plant roots potential to uptake, concentrate and translocate the heavy metals to the harvestable aboveground parts of the plants (Mahar *et al*., 2016; Sreelal and Jayanthi, 2017). Therefore, contamination level of soil is reduced by phytoextraction. As during this process, the heavy metals accumulate in the plant biomass from the soil, therefore, it is easy to treat, dispose of, oxidize and recycle the plant biomass as compared to soil. A load of heavy metals is permanently removed from the soil by phytoextraction as it is suitable to sites where the level of heavy metals is low to moderate and in heavily polluted soil, sustainability of most plant species does not prevail. Phytoremediation is recommended as a substitute method for the removal of pollutants from soil, water and air with the help of plants and microbes (Sobariu *et al*., 2017) without affecting the biological activity, fertility and structure of soil (Sabir *et al*.,

2014). Microorganism in the soil also assists in improving the plant enrichment by altering the forms of metals and physico-chemical properties of soil (Zhang et al., 2018).

Species of the plants which can accumulate an adequate amount of heavy metals in their shoot tissues at a much higher level as compared to the soil or plant species which are nonaccumulating are known as hyperaccumulators (Arshad *et al*., 2008; Shahid *et al*., 2012; Ali *et al*., 2013). Ideally hyperaccumulator plants must possess certain specific properties like capability to accumulate metals at higher levels in the aboveground parts, fast growth with high biomass, toleration of elevated level of heavy metals, abundant root system, adaptation to the local environmental conditions, resistance to pests and diseases, agrochemical treatment, harvestable, usage as forage for animals and most importantly metal translocation from roots to shoots (Alkorta *et al*., 2004: Lestan *et al*., 2008)

As compared to nonaccumulator plants capacity of hyperaccumulator species is 100-500 fold higher to accumulate the heavy metals in the shoots with no effect on the yield (Bhargava *et al*., 2012; Sheoran *et al*., 2016; Mahar *et al*.,2016).The hyperaccumulator plant species possess different requirement of storage and accumulation for different metals therefore the species of plants accumulating more than 100mgkg^{-1} of Cd and Se ,1000 mg/kg As, Ni, Cu and Pb or more than 10,000 mgkg^{-1} of Zn and Mn are known as hyperaccumulator plants (Mahar *et al*.,2016). In leaf dry matter the percentage of some heavy metals has been recorded as Pb ≥ 0.1, Co ≥0.1, Mn ≥1.0, Cd ≥0.01, Sb ≥0.1, Ni ≥0.1, Zn ≥1.0 and Cu ≥0.1 (Verbruggen *et al*., 2009). Presence of high amount of pollutants in the environment, accounts for the highest ranking of the phytoremediation method of metals and metalloids i.e phytoextraction, amongst all the other methods utilized to attain phytoremediation. The other well-known method is Rhizoremediation, which is assisted by microbes for phytoremediation of polluted sites.

Elekes *et al* (2010) assessed seven unlike species of perennial grasses found in the industrial area of Targoviste city for their functionality in phytoremediation potential of heavy metal polluted soil. The results of experiments conclude that accumulation of Sr, Cr, Sn and Zn by *Lolium perenne* can exceed up to 100% and also 410.90% of Sn was accumulated by *Lolium perenne* as well as 704% by *Festuca pratensis*, hence both plants are good hyperaccumulators of Sn. According to an experiment conducted on *Lactuca sativa L*. plants grown in industrial wastewater possess the possibility of phytoremediating some heavy metals (Ni, Cu, Cr and Zn) from wastewater (Naaz and Pandey, 2010).

In 2007, Zhuang *et al*, performed an experiment in the field to assess phytoremediation potential of different plants having higher biomass *viz. Dianthus chinensis, Vetiveria zizanoides, Rumex crispus, two populations of Rumex acetosa and Rumex K-1*(*Rumex upatientia*x, *R. Timschmicus*) as compared to the other metal hyperaccumulators *viz. Viola baoshanensis, Sedum alfredii.* Pb,

Cd and Zn were added to the paddy fields that were used in the experimentation. The rate of phytoextraction by *V. baoshanensis*for Cd was 0.88% and *S. alfredii*for Zn was 1.15% whereas in case of *R. crispus* rate of phytoextraction of Cd and Zn was 0.16 and 26.8 kg ha^{-1}, respectively hence proving it to be good phytoaccumulator of Zn and Cd in the soil.

Anderson *et al* (2010) evaluated the phytoremediation potential of two plants a mash fern (*Thelypteris palustris*) and Asparagus fern (*Asparagus sprengeri*) for arsenic contaminated land from which they concluded that both of these plants can accumulate higher levels of arsenic. Apart from this, an experiment conducted on some aquatic plants has shown the good hyperaccumulating capacity of arsenic from contaminated water. Phytoremediation potential of different aquatic plants *viz.* Water ferns (*Azolla caroliniana, Azolla pinnata* and *Azolla filiculoides*), hydrilla (*Hydrilla verticillata*), water hyacinth (*Eichhornia crassipes*), water spinach (*Ipomoea aquatica*), duckweeds (*Lemna minor, Spirodela polyrhiza, Lemna gibba*), watercress (*Lepidium sativum*) and water cabbage (*Pistia stratiotes*) were studied for arsenic uptake potential and its mechanism (Rai *et al*., 2009).

In 2010, Duo *et al* explored the efficiency of *Lolium perenne L*. to uptake multiple heavy metals from MSW (municipal solid waste) compost and the results suggested an accumulation of heavy metals in different parts of the plant where higher heavy metals content was found in the lower part of shoots than in the upper parts of shoots. *Mirabilis jalapaL.*was found to be an efficient Zn hyperaccumulator in the aboveground tissues in a multi-metal contaminated soil (Sun *et al*., 2011). A study was conducted to evaluate the Pb phytoextraction capability of *Sesbania drummondii*and results show the accumulation of Pb above a threshold level (Israr *et al*., 2011). Zeng *et al* (2011) conducted an experiment to investigate the higher Zn tolerance capability of *Arabis paniculata,* which shows the higher ability of *A. paniculata* to accumulate Zn as compared to *T. caerulescens* and *Sedum afredii*.

Brunetti *et al*., (2011), reported another hyperaccumulator (*B. napus*) which can accumulate the higher amount of Cr, Zn, Cu and Pb in the roots and shoots, where the accumulation of heavy metals was found to be higher in shoots than roots. Monferran *et al* (2012) investigated the phytoextraction potential of *Potamogeton pusillus* for Cr (VI) in the presence of Cu. According to Lariose*t al*., (2012) two clones of poplar were analysed for their phytoextraction and toleration potential of Zn as well as their effect on plant growth. Clone of poplar (Eridano) showed its enhanced capability to accumulate and translocate heavy metal to aerial parts hence proving it an effective Zn- phytoremediator.

Likewise, there are many more hyperaccumulator plants which possess a strong capability to eradicate various heavy metals from the contaminated soil. Some of them have been illustrated in table 2.

Table 2- Hyperaccumulator plant species in accordance with the content of metal accumulation

Plant species	Metal	Metal accumulation ($mgkg^{-1}$)	References
Alyssum markgrafii	Ni	19,100	Bani *et al*., 2010
Aeolanthus biformifolius	Cu	13,700	Chaney *et al*.,2010
Achillea millefolium	Hg	18,275	Jianxu *et al*., 2012
Alyssum heldreichii	Ni	11,800	Bani *et al*.,2010
Azolla pinnata	Cd	740	Rai., 2008
Berkheya coddi	Ni	18,000	Mesjasz-Przyby *et al*.,2004
Corrigiola telephiifolia	As	2010	Garcia-Salgado *et al*.,2012
Deschampsia cespitosa	Cd	226.2	Kucharski *et al*., 2005
Eleocharis acicularis	Cd	2239	Sakakibara *et al*., 2011
	As	1470	Sakakibara *et al*., 2011
Helianthus annuus	Pb	5600	Koptsik 2014
Helianthus tuberosus	Hg	1.89	Aleksandra *et al*.,2008
Haumaniastrum robertii	Co	10,200	Chaney *et al*., 2010
Hordeum spp	Hg	2.35	Rodriguez*et al*., 2007
Medicago sativa	Pb	43,300	Koptsik., 2014
Pteris biaurita	As	2000	Srivastava *et al*., 2006
Pteris cretica	As	1800	Srivastava *et al*.,2006
Pteris vittata	As	8331	Oliveira*et al*., 2014
Rorippa globosa	Cd	>100	Wei *et al*., 2008
Sebertia acuminate	Ni	250,000	Jaffre *et al*., 1976
Silene vulgaris	Hg	4.25	Araceli *et al*., 2012
Solanum sp.	Cd	158	Chen *et al*., 2014
Thlaspi caerulescens	Cd	263	Lombi *et al*., 2002
Thlaspi rotundifolium	Pb	8200	Kopstick., 2014
Sesbania drummondi	Cd	1687	Israr *et al*., 2006
Pteris vitata	As	23,000	Dong ,2005
Thlaspi caerulescens	Cd	80	Banasova and Horak, 2008
Myriophyllum heterophyllum	Cd	21.46	Sivaci *et al*., 2008
Arabis paniculata	Cd	1127(shoots)	Zeng *et al*.,2009
Potamogeton crispus	Cd	49.09	Sivaci *et al*.,2008
Atriplex halimus	Cd	606.51	Nedjimi and Daoud, 2009
Sedum alfredii	Cd	2,183	Jin *et al*, 2009
Sedum alfredii	Zn	13.799	Jin *et al*, 2009
Phytolacca Americana	Mn	32,000	Pollard *et al*., 2009

Brassica juncea	Ni	3916	Fuloria., 2009
Potentilla griffithii	Zn	19,600(leaves)	Hu *et al*., 2009
Rorripa globosa	Cd	218.9	Sun *et al*.,2010
Thlaspi praecox Wulfen	Cd	>1,000(seeds)	Vogel-Mikus *et al*., 2010
Vetiveria zizanioides	Pb	20	Gupta *et al*., 2008
Elsholtzia splendens	Cu	1200	Jiang *et al*.,2004
Brassica juncea	Pb	2400	Bennett *et al*., 2003
Dianthus chinensis	Cd	20	Lai and Chen. 2004
Vetiveria zizanioides	Zn	500	Lai and Chen. 2004
Trigonella foenumgraecum.L	Cr	10	Dheri *et al*.,2007
Averrhoa carambola	Cd	1.6(av)	Li *et al*.,2009
Pennisetum americanum	Zn	600	Zhang *et al*., 2010
Pennisetum atratum	Cd	8	Zhang *et al*.,2010

Apart from all these plants, there are various ornamental plants that can accumulate a substantial amount of heavy metals in different parts. According to a report given by Trampczynska *et al* (2001), *Canna genneralis* is best suited for Pb phytoextraction, which is considered as a significant ornamental that is mostly found in the urban landscape. Another ornamental that is a very efficient hyperaccumulator is, *Pelargonium* (scented geranium) (Saxena *et al*., 1999). It can accumulate metals like Ni, Cd and Pb, from different concentrations of metal solutions.

Prasad and Freitas (2003) reported the presence of scented geranium on multimetal contaminated soils as well as on soils contaminated with a blend of hydrocarbons and metals. Sinha (1999) assessed metal accumulation potential of *Bacopa monnirii L*. under contamination with various metals like Cr, Mn, Cu, Pb and Cd. Order of metal accumulation in roots was found to be Mn>Cr>Cu>Cd>Pb, where accumulation in shoots was found to be less as compared to roots. An experiment conducted by Giordani *et al* (2005) conducted an experiment to determine the ability of different herbaceous crops (Ricinus, tomato and barley) to accumulate Ni under three different treatments levels (150,300 and 600 ppm).

Out of these three crops, spinach was found to be a good accumulator of Ni as compared to other two crops. *Cucurbita pepo, B. juncea, Raphanus sativus var.oleiformis, Zea mays* and *Amaranthus sp.*was found to be efficient hyperaccumulators for various heavy metals like Cd, Ni, Pb, Zn, Cr, Se and Cu (Aggarwal *et al*., 2006). Some of the wild plants such as *Hirsch feldaincana, Conyza discoridies, Cynodon dactylon* and *Sylibum marianum,* have been reported as significant species to accumulate Pb and Zn in a contaminated land (Celestino *et al*., 2006).

2.2.1.1.1 Mechanism for a hyperaccumulation of heavy metals by plants

The process of hyperaccumulation is based mainly on three distinctive features which basically involve: the immense potential of accumulating heavy metals from soil, rapid and efficacious translocation of metals from root-shoot; and the elevated levels of detoxification and seizing the metals in the leaves.

2.2.1.1.2 Heavy metal uptake

Taking into consideration, the model plants for hyperaccumulating capability, i.e *Thlaspi caerulescens* (most ecotopes) and *A. Halleri*, the Cd accumulation in plants is described as under:

In case of these plants, it is clearly demonstrated that by increasing the concentration of zinc, the Cd uptake by the roots of the plant is affected, viz Cd uptake is decreased by the increasing concentration of Zn in soil (Jhao *et al*., 2002). Whereas, the plant *T. caerulescens* (Ganges ecotype) posseses exceptionally great potential to hyper-accumulate Cd in the aerial parts where the uptake of Cd is not hindered by the presence of high concentration of Zn. Hence it is suggested that there is the presence of a specific and coherent independent Cd transport system in the root cells (Lombi .E. *et al*., 2001).

Similarly, the mechanism for As uptake as described by Meharg *et al* (2002), in which the transporters of the chemical analogue phosphate are responsible for As uptake in plant roots in the form of arsenate. Moreover, the ability of As uptake by hyperaccumulating fern is based on the phosphate/arsenate transport systems's (Poynton *et al*.,2004) higher affinity for arsenate in addition on the ability of plant to inflate As bioavailability in the Rhizosphere by lowering the pH via enormous load of dissolved organic carbon (Gonzaga *et al*.,2009) of root exudates. Hence, the decrease in the pH accounts for the capability of water-soluble arsenic, to be taken up by the plant roots (Fitz *et al*., 2002).

2.2.1.1.3 Translocation from root to shoot

In the non-hyperaccumulator plants, mostly the heavy metals are taken up from soil and hold in the root cells followed by their detoxification in the cytoplasm by chelation or stored into the vacuoles.Whereas in the case of hyperaccumulators these heavy metals are translocated to the shoot via xylem more speedily and orderly (Lasat *et al*., 2000). Similarly, in the roots of hyperaccumulator plants, a large amount of small organic molecules are present that can control the metal binding ligands. Free amino acids like nicotinamine and histidine play a vital role in the heavy metal accumulation, which builds sturdy complexes with bivalent cations (Callahan *et al*., 2006). For example, in Ni hyperaccumulation, histidine is considered as the most important ligand.In the case of hyperaccumulator *P.vittata*, the translocation of arsenic to shoots is greater as compared to the non-hyperaccumulator fern.

Arsenic is translocated to shoots predominantly in form of arsenite, which further accounts for more than 90% of As in the xylem sap (Su *et al*.,2008). Glutathione-dependent arsenate reductase briskly reduces arsenate (As V) to arsenite (As III) in the roots of hyperaccumulating ferns (Duan *et al*., 2005).

2.2.1.1.4 Detoxification

A salient feature of hyperaccumulator plants is to detoxify and sequester the contaminants with greater efficiency which in turn enables them to condense large quantity of heavy metals in the aerial organs without being affected by any phytotoxic effect. The higher level of heavy metal accumulation basically affects the leaves where photosynthesis occurs, which is a prime requirement for the survival of plants wherein the major target of these contaminants is the photosynthetic apparatus. Some parts of the leaves like cuticle (Robinson *et al*.,2003), trichome (Kupper *et al*., 2000) and epidermis (Freeman *et al*., 2006) are the sites where heavy metals cause less damage to the photosynthetic apparatus by detoxifying and sequestering them. Subsidiary and guard cells of stomata also eliminate various heavy metals from the leaves (Cosio *et al*., 2005) which results in the preservation of functional stomatal cells from phytotoxic effects of metals.

Heavy metals forms complexes with ligands in the aerial parts of the hyperaccumulator plants where they are detoxified or sequestered likewise they are eradicated from the metabolically active cytoplasm by shifting them into inactive compartments, which mainly include cell walls and vacuoles. In the fronds of hyperaccumulator ferns, As is stored as an inorganic arsenite in the vacuoles (Zhao *et al*., 2009). Apart from this, some organic acids (small ligands) plays a vital role in the detoxification.for instance, the main ligand of nickel in leaves of *T. goesingense* is citrate whereas, acetate and citrate help in binding Cd in the leaves of *S. nigrum*. Moreover, malate is complexed with Cd in *T. caerulescens* and Zn in *A. halleri* (Ueno *et al*., 2008).

2.2.1.2 Rhizoremediation

This is the other method for effectively remediating the contaminated soil by degrading contaminants with the assistance of microorganisms occupying the rhizosphere (Kuiper *et al*., 2004).

2.2.1.2.1 Rhizofiltration

The application of rhizofiltration is to tackle with the discharge of effluents from different industries and acid mine drainage along with agricultural run-off where the contaminants are concentrated in the roots of terrestrial as well as aquatic plants by absorbing, condensing and precipitating them. Some of the metals that are retained within the roots are cadmium, nickel, chromium, lead, copper and zinc (Chaudhry *et al*., 1998). As rhizofiltration has been used to treat the surface and groundwater consisting different concentration of heavy metals like Zn, Pb

and Cr, hence it is a very cost-effective technique. Some of the in situ, as well as ex-situ studies, have been performed on plants such as sunflower, tobacco, spinach, corn and Indian mustard which indicated the great efficiency of this technique for removal of lead from the effluent where the highest capability was shown by *Helianthus annuus* (sunflower).

According to Prasad and Freitas (2003), *Brassica juncea* has been found to accumulate Cr, Cd, Pb, Zn, and Cu along with *H. annus* which accumulates Sr and Pb in hydroponic solutions. Various aquatic plants have been found to be effective in rhizofiltration of heavy metals from the polluted water that includes *Polygonum amphibium* L. (sharp dock), *Lemna minor* L. (duckweed), *Eichhornia crassipes* (water hyacinth), *P. Stratiotes* (water lettuce) etc. *E. crassipes*, an aquatic macrophyte ubiquitously found in lakes, rivers and stream all over the world, is known to be an efficient trace metal accumulator macrophyte (Zhang et al. 2010).

Also, in another experiment conducted by Santos and Lenzi (2000), *Eichhornia crassipes* eliminated lead from the industrially contaminated effluent in a study conducted under controlled conditions in a greenhouse. Due to large biomass and extensive and well-developed root system, *E. crassipes* has been widely utilized for wastewater treatment plants for effectively removing the organic and inorganic contaminants and improving the quality of water.

Apart from lead, *E. crassipes* also accumulates various other heavy metals like Ag, Cd, Pb, As, Cr, Se, Cu etc from wastewater (Zhu et al., 1999). Wang et al (2002) reported the Cd accumulation capability of water hyacinth (462 $mgkg^{-1}$) to be exceptionally greater as compared to other wetland plants. In a similar study conducted by Ingole and Bhole (2003), *E. crassipes* depicted acute accumulation of As, Cr, Hg, Pb, Zn and Ni in hydroponic conditions. Some studies were conducted to determine the phytotoxic effect and uptake capacity of trace metals by water hyacinth (Sindhu et al. 2017; Saha et. al. 2017; Mishra and Maiti 2017).

Phytoremediation of As from contaminated soils and aquatic environments has been studied previously. Upadhyay et al. (2017) studied As accumulation in *Hydrilla verticillata*, *Ceratophyllum demersum,* and *Lemna minor* whereas Niazi et al. (2017) reported *B. napus and B. juncea*for As removal. Singh et al. (2016) found naturally growing *E. crassipes*as a dominant As accumulator. Ismail and Beddri (2009) used it for trace metals removal from petroleum refinery effluents. *Zea mays* have also been identified to possess greater potential for rhizofiltration (bioaccumulation and absorption) of various heavy metals (Cr, Hg and Pb) in a greenhouse experiment conducted by Benavides *et al* (2018).

2.2.1.2.2 Rhizodegradation

Rhizodegradation elevates the degradation of various recalcitrant compounds in the soil by the assistance of plants which activate the microbes near the interface of root and soil. Low molecular weight organic acids, nitrogen and carbon compounds are released to increase the

bioavailability of the contaminants in the soil to the plants, which in return nourishes the microbes in the rhizosphere (White *et al*., 2003). According to Kuiper (2004), there is a two-step enrichment technique in which the potential of biodegradation by bacteria is enhanced after they are harvested from the plant roots which are found in contaminated areas and then these bacteria are made to re-colonize the roots of the plant.

2.2.1.3 Phytostabilization

Other name for phytostabilization is phytorestoration which is a plant-based technique in which the pollution caused by metals is reduced by stabilizing them in the plants rhizosphere by the process of sorption and binding (sequestration) where the availability of metals to the livestock, human and wildlife is lowered by immobilizing them in plant roots (Wong, 2003).The main aim of this technique is to stabilize the metals rather than to remove them from the site,unlike other phytoremediation techniques so that the risk to human health and environment is reduced. Plants that help in phytostabilization acquires many features such as: easy and quick to grow, easy to establish and care for, form thick canopies along with dense root system, magnanimous to the high concentration of metal and the site conditions. This technique is considered more advantageous as compared to other techniques as it is less extortionate, less environmentally indistinct and easily implemented (Berti and Cunningham, 2000).

2.2.1.4 Phytovolatilization

'The transformation of organic to inorganic contaminants which are taken along with water to volatile gaseous forms inside the plant resulting in their release at low concentration into the atmosphere' is defined as phytovolatilization (Mueller *et al*., 2000).Therefore, this technique pollutant are transferred from one compartment to the other but not totally removed (Ali *et al*., 2013; Sharma and Pandey, 2014).

The main aim of this technique is to remove contaminants present in the soil, sediment or water, mainly organic contaminants such as tetrachloroethane, trichloromethane and tetrachloromethane (Yu *et al*, 2006; Miguel *et al*., 2013) and particularly for selected metals such as selenium and mercury from the soil where it is converted from more toxic form (Hg^{2+}) to less toxic form (Hg^{0}) (Wang *et al*., 2012; Oosten and Maggio, 2015). Various efforts have been made in past to remove or reduce the toxic content and achieve phytovolatilization of mercury to a much greater extent by insertion of Hg ion reductase genes in plants like *Nicotiana tobaccum* L. and *Arabidopsis thaliana* L. (Rugh *et al*., 1996).

2.3 Role of different Rhizobacteria in phytoremediation

Plant growth promoting (PGPR) is a term defining rhizospheric bacteria which are dynamically skilled for colonizing roots of plants and stimulating the plant growth. Taking into consideration the plant and host relationship, PGPR can be predominantly divided into two groups: free-living

and symbiotic rhizobacteria (Khan, 2009), which can either conquer cell's interior and survive inside the cells (intracellular PGPR) for e.g. nodule bacteria, or extracellular PGPR, which stay outside the plant cells (e.g. *Azotobacter*, *Bacillus*, *Pseudomonas* etc.). There are three distinct ways in which PGPR affects the growth of a plant:

(1) Various nutrients are taken up from the environment with the assistance of these organisms (Akmakc *et al*., 2006)

(2) Plants are protected from different diseases (Khan *et al*., 2009) and

(3) Particular compounds are synthesized and supplied to plants by them (Glick, 1995).

In general, plant growth is improved by the synthesis of different enzymes, siderophores, vitamins, antibiotics and phytohormone precursors (Ahmad *et al*., 2008) and inhibition of ethylene synthesis. Along with this, synthesis of specific enzymes by PGPR enhances the growth of plants, which results in the physiological amendments in plants. PGPR consists of broad range in which the heavy metal stress is reduced in soil by escalating uptake of metal by the plant as well as by increasing the growth of the plant. Some of PGPR are *Agrobacterium, Azospirillum, Pseudomonas, Arthrobacter, Alcaligens (Ralstonia), Serratia, Bacillus, Burkholderia, Rhizobium and Azotobacter* (Carlot *et al*., 2002; Penrose *et al*., 2003). According to Braud *et al* (2009), PGPR produces siderophores like *Pseudomonas* which can further produce highly soluble metal complexes which are taken up by plants in bulk amount (Wu *et al*., 2009).

Toxic heavy metals are converted to other non-toxic forms by different bacteria which can be easily taken up by plant roots. In the case of Selenium (Se), its accumulation is enhanced in plants by reducing selenate to organic Selenium (Zayed *et al*., 1998). Metal bioavailability is also influenced by the rhizobacteria by amending chemical composition, *viz* organic matter content and pH etc. (Huang *et al*., 2005). Moreover, PGPR (*Pseudomonas menocina*) and arbuscular mycorrhizal fungus (*Glomus intraradices* or *G. Mosseae*), if co-inoculated together can be the best way to increase the activity of antioxidant catalase which can decrease the oxidative damage induced by heavy metal stress. Both microbes and associative hosts must have tolerance in order to outlive and escalate in sites contaminated with metals.

PGPR have developed various mechanisms by which heavy metals can be rendered harmless by immobilization, transformation to another state, and hence making them inactive to permit heavy metal uptake, for surviving under environment stressed by heavy metals (Nies, 1999). The mechanisms are as follows: (1) extrusion- chromosomal mediated events push out the metals from the cell (2) bio-transformation- transformation of higher toxic level to lower toxic level (3) exclusion- target site is kept away from heavy metals (4) accommodation- complexes are formed between metals and metal binding proteins (Kao *et al*., 2006; Umrania 2006) or other cell components (5) methylation and demethylation. Therefore these rhizobacteria function

metabolically in the metal polluted environment with the assistance of these defence mechanisms.

Along with PGPR, plant growth-promoting bacteria (PGPB) are the soil bacteria which are favourable for plants and affect the growth of plant positively. Host plants are well adapted to some suboptimal soil conditions by these soil bacteria and also enhance the phytoremediation efficiency by stimulating the growth of the plant, diminishing the phytotoxic effect of metals, increasing translocation of metals within the plant and amending bioavailability of metals in soil. According to Ma *et al* (2016) in plants,metal accumulation capacity and its translocation may be altered by PGPB by their multiple plant growth promoting traits which include detoxification, accumulation, transformation, sequestration and metal resistance. The success of phytoremediation is inhibited by the critical factor i.e metal phytotoxicity (Shin *et al*., 2012). Rhizobacteria possess great potential to increase mineral and metal mobilization by producing siderophores and organic acids, hence increasing the level of nutrient and heavy metal uptake as a result in improving host plant's phytoextraction potential of metal (Chen *et al*., 2014).

2.3.1 Phytoremediation assisted by Plant growth promoting endophytes (PGPE)

Many researchers have examined a close interaction between plant-microbes heavy metals, for enhancing the phytoremediation potential, in the rhizosphere soils (Dharni *et al*., 2014; Ma *et al*., 2016). For the phytoremediation of metal polluted sites, plants are inoculated with selected and native microbes, the process known as bioaugmentation, which has gained a lot of prominence in recent times (Glick *et al*., 2010). Some research established that plant growth promoting rhizobacteria (PGPR) are capable to reduce phytotoxicity of metal and increase biomass production of plants when they are grown in soil contaminated with metals.

As, the study has been extensively conducted on the effect of PGPR on growth of plant and heavy metal phytoremediation potential in polluted soil (Dhami *et al*., 2013) nevertheless insignificant research has been done on interactions between plant-endophytic bacteria and their ability in phytoremediating soil contaminated with metals (Chen *et al*., 2014; Babu *et al*., 2015). Endophytic bacteria falls in the category of those bacteria which lies below the epidermal layer of cells in the plant tissues and can establish a colony in the internal tissues developing various lifestyles which include mutualism, trophobiotic, symbiotic and mutualism (Schulz and Boyle, 2006).Most of the endophytes emerged from the epiphytic bacterial communities generally, present in the rhizosphere or phyllosphere/other parts of plants; whereas, some endophytes may be mediated by foliar tissues or seeds (Bacon and Hinton, 2007).

2.3.2 Mechanisms of heavy metal accumulation assisted by rhizobacteria

Various mechanisms have been reported by which rhizobacteria can help in the accumulation of heavy metals from soil. Some of them are as under:

2.3.2.1 Secretion by rhizobacteria

Siderophores are metal chelating agents produced by different rhizobacteria, plays a major role in the procurement of various heavy metals and affect the increased bioavailability of soil-bound iron (Kanazawa *et al*., 1994). Basically, the plants growing in the metal contaminated soils are deficient in iron therefore sufficient iron to these plants is made available by PGPB by the production of Siderophores (Burd *et al*., 2000). The growth of roots in various plant species like Indian mustard (Belimov *et al*., 2005) is stimulated by a number of PGPR, which consists of ACC deaminase enzyme that hydrolyses and decreases the ACC content in plants, therefore, decreasing the biosynthesis of ethylene by plants (Hall *et al*., 1996).

Roots and seeds of some plants exude ACC which is further taken up by bacterium and ACC deaminase cleaves it into α-ketobutyrate and ammonia (Glick *et al*., 1998) and results in the root elongation when the released ammonia is utilized by bacteria as a nitrogen source hence plant ethylene is reduced with the reduction in ACC within plant (Penrose and Glick, 2001). Indole Acetic acid (IAA) is also produced by rhizobacteria which are bound to roots or seeds (Patten and Glick, 2002), enhances the growth of plant roots. Different effects are observed in context to the production of IAA like; primary root elongation is promoted by production of low level of IAA, whereas adventitious and lateral root formation (Glick, 1998) is stimulated by production of higher levels of IAA which also results in primary root growth inhibition (Xie *et al*., 1996).

2.3.2.2 Higher surface area-volume ratio

Rhizobacteria probably act as a microbial chelate which is associated with phytoremediation because of their minute size and large contact area due to which the surface area-to-volume and bacterial activities are increased (Karenlampi *et al*., 2000). For example, germination of Indian mustard shows more root hairs when germinated on Se- containing media from bacterial coated axenic seeds and more accumulation of Se is observed (de Souza *et al*., 1999a) as compared to plants that grow from only axenic seeds without bacterial coating.

2.3.2.3 Transformation of toxicity of metals

In soil, the bioavailability of metals to plants is basically a major aspect in the phytoremediation efficiency. Transformation of toxic to non-toxic forms of heavy metals are converted by the native rhizobacteria like in case of Se, it is converted into organic Se and organoselenium forms like SeMet from selenate by bacteria, hence Se accumulation is enhanced in plants at a faster rate into roots (Zayed *et al*., 1998). Major factors that control the bioavailability of heavy metals in soil are pH, organic matter and solubility (Gray *et al*., 2006). Like in case of *Pseudomonas maltophilia* strain, toxicity and mobility of Cr^{6+} were reduced to Cr^{3+} which is immobile and even other toxic ions such as $Hg^{2+,}$ Cd^{2+} and Pb^{2+} are also made less mobile (Park *et al*.,1999).

2.3.2.4 Inhibition of pathogens of plants

Plant pathogens are squashed by divergent mechanisms by PGPR which involve conflict for space and nutrients (Elad and Chet, 1987), production of various antibiotics like pyocyanin and pyrrolnitrin etc (Pierson and Thomashow, 1992) and Siderophore production viz, pseudobactin which restrict the iron availability required for the pathogen's growth (Lemanceau *et al*., 1992). Production of chitinases (lytic enzymes) and β-1,3-glucanases which can degrade glucan and chitin present in the cell wall of fungi (Fridlender *et al*., 1993), toxin degradation produced by pathogen (Duffy and Defago *et al*., 1999) and production of HCN, are some of other important mechanisms taken into consideration for pathogen removal.

2.3.2.5 Transport protein stimulation

Survival and multiplication of bacteria within the host as well as in the environment are dependent on the sequestration and uptake of metals like Zinc (Zn), iron (Fe) and manganese (Mn). Sulphate transport protein (located in root plasma) is stimulated by bacteria, which transports selenate (Leggett and Epstein, 1956).

2.4 Role of Arbuscular Mycorrhizal (AM) fungi in phytoremediation

According to the fossil record, around 450 million years ago, the symbiosis of arbuscular mycorrhizal (AM) symbiosis is very ancient and universal where the interaction occurs between greater than 90% of herbaceous species and innumerable fungal species of phylum Glomeromycota (Redecker *et al*., 2000). Therefore, mycorrhizosphere is developed by various types of mycorrhizal symbioses in which there is the presence of blended root zone (Rhizosphere) and extraradical hyphae (hydrosphere). There is the existence of multiple families of plants in different ecosystems which are associated with arbuscular mycorrhizal fungi worldwide like in arid region (O'Connor *et al*., 2002), tropical areas (Zhao *et al*., 2001) or arctic-alpine region (Muthukumar and Udaiyan, 2000).

Fourteen AM species of *Glomus* spp., *Acaulospora* spp. and *Sclerocystis* spp. were dominantly found in five different medicinal plants, *Centella Asiatic, Ocimum sanctum, Acorus calamus, Oxalis corniculata* and*Mentha arvensis* during a study carried out in Sagar (M.P) by Soni (2007) and Vyas*et al* (2007). Due to the ubiquitous nature, AMF can colonize various plant roots found in terrestrial regions and an immense network of hyphae is formed that can reach up to the nutrients present in soil which are unavailable for the plants to be taken up directly (Smith and Smith, 2012). Apart from this, AMF also enhance the potential of the host to uptake water and nutrient supply by transporting different immobile molecules, phosphorus, micronutrients and nitrogen directly to the roots and damage caused by root pathogens is also diminished by them (St-Arnaud and Vujanovic, 2007).

Therefore, from all these properties, Rilling and Steinberg (2002) depicted that AMF helps to enhance the texture of soil and by aggregating soil particles reduces the risk of erosion as they can withstand exceedingly hard conditions and inhabit most harsh climates, which include trace metal contaminated soils (Zarei *et al*.,2008;Wu*et al*.,2010). The beneficial role is played by AMF in the mobilization and immobilization of metals in soil along with increased bioavailability of metals to plants (Smith and Read, 2008). Different factors are responsible for the uptake and immobilization of trace metals by plants which comprises: species of plant, species of AMF, soils concentration and essence of metals (Audet and Charest, 2009).

According to Wang *et al* (2007) compared to single species of AMF, mixed inoculants appears to be more effectual for example, in an experiment growth and accumulation of heavy metals in *Z. mays* was compared when inoculated with *Glomus caledonium* 90036 or mixture of different species including (*Scutellospora gilmori* ZJ39, *Glomus spp., Gigaspora margarita* ZJ37, *Acaulospora spp. and Gigaspora decipens* ZJ38), where greater extent of colonization was observed in the case of single inoculants i.e *Glomus caledonium* 90036 as compared to the plants that were inoculated with the mixture. Results of the experiment also depicted that concentration of Zn, Pb, Cu and Cd in the shoot wasless with *Glomus caledonium* than with the different species, hence proving the mixed inoculants as more productive than a single one to enhance the efficacy of phytoextraction of heavy metals.

Another experiment conducted by Prasad *et al* (2006) in Jabalpur (M.P) region resulted in the isolation of twenty-two different fungal spores of different genera from the Rhizosphere of *Glycine max* (soybean) after the study on AMF distribution in that area. Similarly, Gai *et al*, (2006) performed an experiment on the Tibetan plateau to study the AMF associated with sedges. Another experiment carried out in lower hills of Himachal Pradesh shows a variety of AMF in the wild plants (Aggarwal *et al*., 2004). Sharma (2004) reported no significant correlation amongst AM fungal spore amount and percentage mycorrhizal infection in some economically significant plants of Uttrakhand. Survey of AM fungi occurrence in different tree species from Western Ghats of Goa (India) was carried out by Khade and Rodrigues (2003). Limited species of AM fungi was reported by Chaurasia *et al* (2005). Another finding reported that major amount of fungal species was found in association with wild plants than crops grown in fields after the survey of fungal diversity in wild plants and field crops in northern China (Gai *et al*., 2004).

Most commonly found AM species in India after the study of arbuscular mycorrhizal diversity was found to be *Glomus spp*. (Mehrotra. 2007).According to a report given by Gupta and Mukherji (2001), 111 AM fungal species were found from India, which is found to be more prevalent in cultivable land than non-cultivable land. The result shows the presence of 2 species

of *Sclerocystis*, 12 species of *Gigaspora*, 15 species of *Scutellospora*, 14 species of *Acaluospora*, 1 species of *Endogone*, 60 species of *Glomus* and 3 species of *Sclerocystis.* There are different categories of AM fungi out of which VAM (vesicular-arbuscular mycorrhizal) is most valuable as in an experiment conducted by Aguilera-Gomez *et al* (1999), VAM plants were found to effectively increase leaf number,root, shoot, leaf area, fruit mass and reproduction rate as compared to non VAM plants. Even at a lower concentration of Phosphorus VAM plants shows higher root colonization whereas no effect was seen in sporulation. According to Chaudhury and Panja (2007), availability of VAM fungi is huge than in virgin soil.

VAM inoculation is also helpful in reducing the diseases caused by plant pathogens like in case of *Coleus forskohlii*, root rot caused by *Fusarium spp*. has been administered by three species of AM fungi and rhizobacteria i.e *Pseudomonas fluorescens, Glomus mosseae* and *Trichoderma viride.* Similarly, in *Piper nigrum* (pepper) infection is caused by *Phytophthora capsici* (soil bacteria) (Sarma *et al*., 2000) and *P. capsici* infection has been managed by *Trichoderma harzianum* (Sarma *et al*., 1997). In *Anethum graveolens*, growth of plant and essential oils concentration was increased up to 90% when inoculated with two species of AM fungi i.e *Glomus fasciculatum* and *Glomus macrocarpum* similarly oil content was increased up to 72% in *Trachyspermum ammi*by same AM fungus but *Glomus fasciculatum* was less effective than *Glomus macrocarpum*(Kapoor *et al*., 2002). After conduction of an experiment on *Coriandrum sativum* by Kapoor *et al* (2004), it was reported that after inoculation of these two AM fungi, the concentration of essential oils was increased up to 40%.

Toussaint *et al* (2007), reported an increase in the content of rosmaric and caffeic production in shoots of *Ocimum basilicum* by inoculation with *Glomus caledonium* and also caffeic acid concentration was increased in the same plant by *G. mosseae*. Two species of AM fungi viz, *Glomus versiforme* and *G. intraradices* were inoculated in onion plant, where the better yield of onion bulbs was shown by *G. versiforme* than bulbs inoculated with *G. intraradices* (Charron *et al*., 2001). In 2001, Gupta*et al* reported that when compared to normal plants, *Mentha piperita* (peppermint) shows an elevated level of shoot biomass as a result of inoculation with 131.1 % of *G. aggregatum*, 877.85 of *G. mosseae* and 145.3% of *G. fasciculatum.*

Douds *et al* (2007), conducted an experiment to test the response of potato when inoculated with AM fungi in a field where high concentration of P was available in two growing seasons and results depicted that in first year yield of tubers (fresh weight) were increased as compared to the second year's response but when compared to control the overall yield of the plant increased by 10-20%. Tawaraya *et al* (2007) reported that *Aloe vera* plants when inoculated with *Gigaspora decipiens* and *Glomus clarum* resulted in increased fresh weight of shoot along with N and P concentration in shoots than in uninoculated plants. Querejeta *et al* in 2007 observed fast growth

in *Pistacia lentiscus* and *Retama sphaerocarpa* shrubs with AM fungi pre-inoculated seedlings than in control plants which are colonized by local AM fungi. Similarly, significant increase in plant height and dry weight along with gain in content of fruit protein was observed in *Capsicum annum* by Sudhanshu and Verma (2006).

Currently, production and usage of AM fungi inoculums are being done by various techniques where, remarkable results are seen in the large scale production of AM fungi inoculums for field inoculation by using appropriate host in pot culture which is easy to perform as compared to other techniques (Harikumar and Potty, 2002). In agriculture system and forest usage, the inoculums should be efficient to show its good effects and under proper production conditions must show effective response of growth in the target plants (Mehrotra, 2005).

Even though, a large quantity of scientific reports related to the efficiency of phytoremediation and growth of plant by the assistance of AM fungi and bacteria have been published but still usage of two microorganisms in sites polluted with heavy metals have limited literature. Yet, in an experiment conducted in Spain, on white clover (*Trifolium repens* L.) two indigeneous species of AM fungi and rhizobacteria viz, *Glomus mosseae* and Cd-adapted bacterium, *Brevibacillus sp*. were inoculated in plant and resulted in huge amount of root biomass and AM and nodule colonization (symbiotic structures). The combination of this inoculum consistently increased N and P content in plant but in AM fungus- bacterium treatments, *Trifolium* plant shows decreased uptake of Cd (Vivas *et al*., 2003). Identical results were observed in the case of *T. repens* plants treated with Zn and Ni along with AM and *Brevibacillus* strains (Vivas *et al*., 2006). Therefore, these experiments reveals the efficiency of indigeneous bacteria or AM fungi in assisting growth of plant throughout phytoremediation process in soils polluted with heavy metals.

In an experiment conducted by Pandey and Pandey in 2005, effect of five pathogenic AM fungi (*Macrophonia phaseolina, Fusarium solani, Pythium aphanidermantum, Rhizotonia solani* and *Sclerotium rolsfii*) with biocontrol agents was seen on seed germination percentage of tomato, brinjal and chilli, where the results depicted 100% emergence of seedling by *T. koningii* and *B. subtilis* in tomato and 76.7% by *T. harzianum* in chilli whereas 16.7% by *B. Subtilis* and *T. harzianum* in brinjal. In another experiment it was proved that *Glomus fasciculatum* increases the growth of tomato plant and fusarium wilt is reduced (Manian *et al*., 2006). Similarly, in 2009, Sharma *et al* observed that biocontrol agent of wilt and root rot in case of *Acacia nilotica* was *Trichoderma viride* and AM fungi.

2.5 Antioxidant enzymes involved in phytoremediation

Plants growing in heavy metal polluted soil encounter stress because of toxicity caused by chemicals and deficiency due to nutrients. Immoderate levels of Reactive oxygen species (ROS)

is produced due to the biotic and abiotic stress which results in detrimental effect at cellular level that can be repaired or diminished by a complex antioxidant system. ROS consists of some free radicals like hydroxyl ion (OH), superoxide anion (O^{2-}), hydrogen peroxide (H_2O_2), singlet oxygen ($1O_2$) and so on. Plants shield themselves against these toxic intermediates of oxygen, plant cells and its organelles (Mitochondria, peroxisomes and chloroplast) by the antioxidant defence system (Khan *et al*., 2008). ROS is always formed in plants in two different ways, firstly, by the leakage ofO_2 electrons from the activities of electron transport in mitochondria, plasma membrane and chloroplasts, Secondly by different metabolic pathways confined in various compartments of cells (Heyno *et al*., 2011).

According to Sharma *et al* (2012), excessive production of ROS in plants is due to interference in cellular homeostatsis which may be caused by some environmental factors such as metal toxicity, salinity, drought, UV-B radiation, chilling and attack by a pathogen. All reactive oxygen species pose extreme harm to organisms at a higher concentration. A cell reaches an oxidative stage when the level of ROS overreaches defence mechanism. If the ROS exceeds the normal level it can be a threat to cells where different processes take place that finally leads to the death of cells, like enzyme inhibition, damage to nucleic acids, peroxidation of lipids, activation of programmed cell death (PCD) pathway and oxidation of proteins (Meriga *et al*., 2004). Hence, excess of ROS is detoxified by an effective antioxidant system, which comprises enzymic as well as nonenzymic antioxidants (Noctor *et al*., 1998).

Non enzymic antioxidants within the cell consists of tocophenols, glutathione, carotenoids, phenols and ascorbate (Gill *et al*.,2010) whereas enzymic antioxidants are guaicol peroxidase (GPX), ascorbate peroxidise (APX), catalase (CAT), superoxide dismutase(SOD), glutathione reductase(GR), dehydroascorbate reductase (DHAR) etc (Noctor *et al*., 1998). Role of these antioxidants is to delete or remove excess of ROS from accumulating to toxic levels and helps the plant to adapt and survive during stress conditions (Devaraj*et al*., 2008). The activities of antioxidant enzymes in the plants stressed with metals are exceedingly uneven depending on the species of plant, metal ions, duration of exposure and concentration (Sharma and Dietz ., 2009).

Therefore in this research work, a part of work has been concentrated to check the activities of these antioxidant enzymes in different plantspartsunder cadmium and arsenic stress. The selected enzymes have been elaborated as under:

2.5.1 Ascorbate peroxidase (APX)

APX is a non-glycosated and heme containing enzyme that makes use of ascorbic acid to remove H_2O_2 that is potentially harmful, via ascorbate-glutathione pathway (Van-Breusegem *et al*., 1995). Main parts of plants are chloroplast and cytosol where APX is active (Asada, 1992) whereas it is also found in mitochondria (Anderson *et al*., 1995). APX plays a major role in

shielding cells of higher plants, euglena, algae and other organisms as well as scavenging ROS. It is involved in scavenging of H_2O_2 in ASH-ASH and in water-water cycles along with employing ASH as donor of electron.

Elevated levels of APX have been observed in many plants during higher stress level. In an experiment, under Cd stress, level of APX activity in leaf was reported to be increased in *V. mungo* (Singh *et al*., 2008), *B. juncea* (Mobin and Khan, 2007), *Ceratophyllum demersum* (Arvind and Prasad, 2005) and *T. aestivum* (Khan *et al*., 2007). *H. Vulgare* also shown increase in APX activity (Simonovicova *et al*., 2004). Shanker *et al* (2005) conducted an experiment on *Vigna radiata* to check the APX activity when 50µM of Cr was added to the nutrient media. Results shows increase in APX activity after 12, 24 and 48 hr after treatment with Cr (VI).

Sharma *et al* (2007) reported maximum activity in roots and shoots of Zn treated *Cicer arietinum*. In another experiment, conducted on Indian mustard (*Brassica juncea* L.) treated with Cr, APX activity was found to increase in leaves with exposure to time and treatment of Cr given to plant (Diwan *et al*., 2010). In 2011, Bahl *et al* performed an experiment to investigate the effect of APX on treatment with 1mM Cr, Pb and Cd in *Typha angustifolia* after 30 days of treatment. Results predicted higher activity of APX in the shoots treated with Pb and Cd whereas decreased APX in Cr treated plants as compared to control. In sunflower (*Helianthus annus*) treated with heavy metals, the APX activity was found to be increased in young seedlings (Nehnevajova *et al*., 2012). *Eruca sativa*, when treated with different concentrations of Zn, resulted in elevated levels of enzyme activity (Ozdener *et al*., 2010).

When compared with a control plant, *Groenlandia densa*, showed a decrease in APX activity with increasing concentration of Cd (Yilmaz and Parlak, 2011). Thounaojan *et al* (2012) studied the effect of Cu in different concentrations and time interval on *Oryza sativa* and results showan increase in APX enzyme progressively. The variant response was observed in different tolerant and sensitive cultivars of wheat for the differential response of APX enzyme under stress (Sgherri *et al*., 2000).

Under drought stress, a significant increase in APX activity was seen in *T. aestivum* cv-C306 (Khanna-Chopra and Selote, 2007) and *Vigna unguiculata* (Manivannan *et al*., 2007). Similarly, in response to salt stress increased APX activity was observed in *Beta vulgaris* (Bor *et al*., 2003), *Lactuca sativa* (Eraslan *et al*., 2007), *Azolla pinnata* (Masood *et al*., 2006) and *Oryza sativa* (Lee *et al*., 2001). Decreased APX activity was observed in different (10) genotypes of *Pisum sativum* affected with Cd toxicity (Metwally *et al*., 2004). Whereas, APX activity was found to be less in *B. napus* under Cd stress (Nouairi *et al*., 2009). Similarly, APX activity was observed to be less in *Ocimum tenuiflorum* under Cr stress (Rai *et al*., 2004) as well as in *B.juncea* (Pandey *et al*., 2005), Ni stress in *Hordeum vulgare* (Tamas *et al*., 2008),increased APX activity under Cr stress

in *Vigna radiate* (Shanker *et al*., 2004), Cd stress in *Helianthus annus* (Gallego *et al*., 2002), Cd in *Pisum sativum*(Romero Puertas *et al*., 2002), Cd in *B. juncea* (Mobin and Khan, 2007), Mn in *Cucumis sativus* (Shi *et al*., 2006), Hg in *Sesbania drummondii* (Israr and Sahi, 2006), Cu in *Ceratophyllum demersum* (Devi and Prasad, 1998).

2.5.2 Catalase (CAT)

Catalases being tetrameric in nature possess the ability to catalyse the oxidation of H_2O_2 into H_2O and O_2 and play a crucial role in detoxification of ROS under conditions of stress (Garg and Manchanda, 2009). The ability tocatalysing to convert hydrogen peroxide into water and oxygen is very high, as it can convert 6 million molecules of H_2O_2 to H_2O and O_2 in a minute. Presence of catalases is most profound in peroxisomes/ glyoxysomes as well as in mitochondria and is very important for removal of H_2O_2 which is released during β-oxidation of fatty acids and photorespiration (Arora *et al*., 2002). It is absent in chloroplasts but most commonly found in other plant tissues. Various studies have been carried out to determine CAT activity in different plants under heavy metal stress.

Decline in CAT activity was observed in *Glycine max* (Balestrasse *et al*., 2001), *Solanum lycopersicum* (Chamseddine *et al*., 2009), *Oryza sativa* (Thounaojam *et al*., 2012), *A. thaliana* (Cho and Seo, 2005), *Phragmites australis* (Lannelli *et al*., 2002), *Helianthus annus* (Nehnevajova *et al*., 2012) and *Capsicum annuum* (Leon *et al*., 2002) but , increase in CAT activity was observed in roots of *V. mungo* (Singh *et al*., 2008), *O. Sativa* (Hsu and Kao, 2004), *C. Arietinum* (Hasan *et al*., 2010), *B juncea* (Mobin and Khan, 2007) and *T. aestivum* (Khan *et al*., 2007) under Cd stress. Yadav *et al* (2010) reported high CAT activity against Cr stress and does not affect any metabolic activity in plants. The investigation was carried out on heavy metals stress and the efficiency of the antioxidative defence system in cells of plants. In *A. thaliana* roots and leaves exposed to Uranium and Cadmium, no alterations were remarked in enzyme capacity. Hence Vanhoudt *et al*(2010) concluded that concentration of metal applied and time of exposure does not affect the level of protein in the enzymes antioxidative defence mechanism as well as under heavy metal stress no function of plants are affected.

Various other studies revealed the increase in CAT activity under varied environmental conditions, like UV-B exposure to *Helianthus annus* (Costa *et al*., 2002), *Phaseolus vulgaris*(Shi *et al*., 2005), *Solanum tuberosum* (Santos *et al*., 2004) and *Arabidopsis thaliana* (Gao and Zhang, 2008), low temperature in *Spirodella polyrrhiza* (Song *et al*., 2006), drought stress in *Vigna unguiculata* (Manivannan *et al*., 2007);salinity stress in *Triticum aestivum* (Raza *et al*., 2007), *Beta maritima* (Bor *et al*.,2003) and *Catharanthusroseus* (Misra and Gupta, 2006); Hyperhydric stress in *Euphorbia millii* (Dewir *et al*.,2006).

CAT activity was increased in context to plants exposed to different metals *viz.* Cr stress in *B. Juncea* (Pandey *et al*.,2005), *Vigna radiata* (Shanker *et al*., 2004) and *Ocimum tenuiflorum* (Rai *et al*., 2004); Cd stress in *Hordeum vulgare* (Patra and Panda, 1998), *Phragmites australis* (Lannelli *et al*., 2002) and *Lemna minor* (Hou *et al*., 2007); Al in *Vigna radiata* (Ali *et al*., 2008a); Hg in *Eichhornia crassipes* (Mart.) (Narang *et al*., 2008); As in *B. juncea* (Gupta *et al*., 2009) and *Pteris vitatta* (Cao *et al*., 2004); Cu in *Ceratophyllum demersum* (Devi and Prasad, 1998), *Prunus cerasifera* (Lombardi and Sebastiani, 2005), *Hordeum Vulgare* (Demirevska-Kepova *et al*., 2006); Pb stress in *Sesbania drummondii* (Ruley *et al*., 2004) and *Pisum sativum* (Malecka *et al*.,2001); Zn stress in *B. juncea* (Prasad *et al*., 1999).

2.5.3 Guaiacol peroxidase (GPX)

Guaiacol Peroxidase is a protein that contains heme and oxidizes various organic or inorganic substances (Asada, 1994), as well as hydrogen peroxide (H2O2), oxidizes Pyrogallol and Guaiacol (aromatic electron donor). GPX consists of two structural Ca^{2+} and four disulfide bridges (Schuller *et al*., 1996) and is mostly found in plants, microbes and animals. Isoenzymes of GPX basically exists in the cell wall, cytosol and tissues of plants localized in vacuoles (Asada *et al*., 1992). Many significant processes like wound healing, defence against abiotic and biotic stress, lignifications of cell walls, biosynthesis of ethylene and degradation of IAA, are associated with GPX (Kobayashi *et al*., 1996). Broadly, GPX is considered as "stress enzyme". GPX activity is promoted by the varied stressful environmental conditions. In *S. polyrrhiza*, increased guaiacol activity was observed in response to low temperature (Song *et al*., 2006) whereas in another report given by Ali *et al* (2005), at a higher temperature, GPX activity was increased in *Phalaenopsis*. Similarly, in other environmental stress conditions, different activities of GPX were observed *viz.* UV-B radiation in *Helianthus annuus* (Costa *et al*., 2002) and *Solanum tuberosum* (Santos *et al*., 2004); exposure of ozone in *Arabidopsis thaliana* (Rao *et al*., 1996). GPX activity was also enhanced in case of drought stress in *Catharanthus roseus* (Jaleel *et al*., 2007), *Triticum aestivum* (Khanna-Chopra and Selote, 2007) and *Glycine max* (Zhang *et al*., 2007).

Salt stress also enhanced the GPX activity in *Beta maritime* and *B. vulgaris* (Bor at al., 2003), *Catharanthus roseus* (Misra and Gupta, 2006), *Oryza sativa* (Lee *et al*., 2001), *Gossypium cv. Pora* (Meloni *et al*., 2003) and *Triticum estivum* (Raza *et al*., 2007). Another experiment conducted by Yoshimura *et al* (2004), resulted in increased GPX activity in transgenic tobacco plants, under high intensity chilling stress (250mM NaCl) and 50µM methyl viologen application under medium light intensity. Stress caused due to heavy metal is also accountable to enhance GPZ activity in *Brassica juncea* (Gupta *et al*., 2009) and *Pteris vitatta* (Cao *et al*., 2004) under As stress; Cr stress in *Vigna radiata* (Ali *et al*., 2008), *Oryza sativa*(Guo *et al*., 2007),

Amaranthus viridis (Liu *et al*., 2008) and *Pistia stratiotes* (Sinha *et al*., 2005); Cd stress in *Lemna minor* (Hou *et al*., 2007), *Lycopersicon esculentum* (Dong *et al*., 2006), *Bacopa monnieri* (Mishra *et al*., 2006), *Capsicum annum* (Leon *et al*., 2002);Hg stress *in Eichhornia crassipes* (Narang *et al*., 2008), *L. esculentum* (Cho and Park, 2000); Cu stress in *B. juncea* (Wang *et al*., 2004) and Mn stress in *Hordeum vulgare* (Demirevska *et al*., 2004) , *Cucumis sativa* (Shi *et al*., 2006).

But in some cases, GPX activity was decreased under heavy metal stress in certain plants like Cd stress in *Typha latifolia* (Fediuc and Erdei, 2002*), Hybrid willow* (*Salix matsudana*, Saliaceae) (Yu *et al*., 2007) and *Thlaspi caerulescens* (Wójcik*et al*., 2002). Enzyme activity is decreased in Wheat leaves caused by Zn and Cr stress (Panda *et al*., 2003). Under Cr and Pb toxicity, enzyme activity was decreased in *Taxithelium napalense* (Choudhury and Panda, 2005).

2.5.4 Glutathione reductase (GR)

Glutathione reductase is a member of the flavoenzyme family, found in both eukaryotes and prokaryotes and play a major role in maintaining GSH level by defending against oxidative stress (May *et al*., 1998). It reduces GSSG to GSH at the same time NADPH is oxidized (Rendon *et al*., 1995). In ASH-GSH cycle, GR is considered as the most potent enzyme that can defend against ROS by assisting GSH in its reduced status (Chamseddine *et al*., 2009). It is found to be located predominantly in Chloroplasts and in small fractions in cytosol and mitochondria (Creissen *et al*., 1994). The tolerance power of plants to different stress conditions is determined by the GSH and GR (Rao *et al*., 2008).

Various experiments were carried out to observe the activity of GR in plant parts under heavy metal stress conditions. In an experiment conducted by Diwan *et al* (2010), GR activity was enhanced in Indian mustard under different time intervals and treatment concentration with Cr. Similarly under Cr stress GR activity was increased in roots of *Vigna radiata.* In sunflower, the GR activity was enhanced in young seedlings when exposed to heavy metals (Nehnevajova *et al*., 2012). Thounaojan *et al* (2012) reported an increase in GR activity in all heavy metal treated plants of *Oryza sativa* as compared to control plant, as well as GR activity, was found to be more in roots than shoots. In presence of an elevated level of Cr, GR activity was declined in *Vigna radiata* leaves (Karuppanapandian *et al*., 2009).

Under drought stress conditions, Chopra and Selote (2007) investigated the increase in Gr activity in *T. aestivum cv.*C306, whereas the decline in activity was observed in *cv. Moti* strain. Wheat leaves show reduced GR activity under heavy metal stress (Panda and Patra, 2000). Ali *et al* (2005) conducted an experiment which resulted in increased GR activity in leaves of *Phalaenopsis* at a higher temperature (40°C), whereas decreased activity in roots was observed. GR activity was increased under salt stress in various plants *viz, Gossypium cv.Pora* (Meloni *et*

al., 2003), *Portulaca alevacea* (Yazici *et al*., 2007), *Beta maritima* (Bor *et al*., 2003) and *Catharanthus roseus* (Misra and Gupta, 2006). According to Yadav *et al* (2010), *Jatropha curcus* plant, involved in detoxification of Cr, showed increased activity of the enzyme under Cr stress. In the alkaline environment (150 mM NaCl) during tissue culture experiment, GR activity was observed to be increased in shoots of *Morus sp.* (Harinasut *et al*., 2003).

In *Sesbania drummondii*, GR activity increased under Cd stress of different concentrations of 50 µM and 100µM, by 62.5 and 56.2% respectively and a significant increase in activity was observed at each treatment than control (Israr *et al*., 2011). Under Cd stress, no variation was found in GR activity in *Groenlandia densa* (Yilmaz and Parlak, 2011). Under hyperhydric stress, GR activity was enhanced in leaves of *Euphorbia millii* (Dewir *et al*., 2006); whereas enzyme activity decreased under cold stress in *Nicotiana tobacum* leaves (Gechev *et al*., 2003). In *Cucumis sativus* GR activity was observed to increase when treated with EBr (Yun-yan *et al*., 2007). In other experiments carried out on different plants like *B. juncea* under Cr stress shown decline in enzyme activity , likewise decrease in GR activity was observed in *Helianthus annus* under CD, Cu and Fe stress (Gallego *et al*., 1996), *Taxithelium napalense* under Cr and Pb stress (Choudhury and Panda, 2005). Therefore, best-adapted plants for phytoremediation are those which can adjust to the toxic levels of heavy metals and can survive under stress conditions.

2.6 Stimulation of microbe bioactivity in the root zone

Bioactivity of microbes (bacteria and fungi) in the rhizosphere can be stimulated by plants with the excretion of bio-enhancing compounds. As compared to normal soil, the concentration of microbes in rhizosphere soil is 10 to 100 times higher. Source of nitrogen and carbon for bacteria in soil is provided by root exudates secreted by plants. The compounds that are secreted by plants mainly consist of carbohydrates, amino acids, phenols and flavonoids. Some of these compounds are elaborated below.

2.6.1 Phenols

According to Randhir *et al* (2004), phenolic compounds are secondary metabolites which are derivatives of phenylpropanoid, pentose phosphate and shikimate pathways in plants. Plants consist of various phenolic compounds. Basically, fruits and vegetables contain phenolic compounds that include stress-linked phytochemicals and pose a major impact on the human health. Therefore, these compounds occur more often as groups of phytochemicals which are physiologically and morphologically important in plants (Iqbal *et al*, 2012). Phenolic compounds can scavenge activity of free radicals and are found both inedible as well as non-edible plants. The antioxidant activity of phenols is mainly due to their redox property (Hsu. 2006). Phenolic compounds have been used in agri-industrial by-products and plants due to its antioxidant activity, its occurrence and its uses (Balasundram *et al*., 2006). A major source of phenolic

compounds in the human diet are beverages, vegetable and fruits. Being a dietary antioxidant in human health and disease, plant polyphenols might shield against the damage caused due to oxidation.

Furthermore, phenols are considered to act as the biomarkers of metal exposure (D.Bialonska *et al*., 2007). When compared to the polluted and non-polluted soils (Márquez-García *et al*., 2009), *E. andevalensis* showed different phenolic concentration and when the same plant was grown in the lab under controlled conditions with cadmium stress, drastic changes were observed in its antioxidant activity (Márquez-García *et al*.,2011). Even the change in glutathione and ascorbate levels was observed along with changes in other antioxidant enzymes activity (Márquez-García *et al*., 2012). Similarly, the experiment was conducted by Krejcarová *et al* (2015) in which phenolic content of seabuckthorn leaves (*Hippophae rhamnoides L Elaeagnaceae*) was determined where the results show the phenolic content increased in the course of the season but decreased content was observed when the plant was collected from another region. Another experiment conducted on *Gynurs procumbens* (*Lour.*) *Merr*, total phenolic content was increased with increased concentration of Cu and Cd therefore the plants (*G. procumbens*) cultivated in heavy metal polluted soil is not safe to consume because of the higher bioaccumulation of metals in plant parts that is above the safety levels than given by WHO (Ibrahim *et al*., 2017).

2.6.2 Flavonoid

Flavonoid compounds possess a wide field of biological effects which include anti-oxidant activity, anti-viral, anti-inflammatory, anti-thrombotic, anti-bacterial and anti-allergic, apart from these effects, risk due to disease of the coronary heart is also reduced by this compound. The source of flavonoid as a natural antioxidant is vegetables, herbs, fruits, wine, tea and spices that are already utilized as nutritional supplements (Patel *et al*., 2010). Basically, flavonoids are found in all plant parts. As per an estimation 2.1% of carbon that is produced by plants is converted into flavonoids or alike analogous compounds (Markham, 1982). Biflavones, methylated derivatives, aglycones and glycosides are some of the major benzo- γ-pyrene derivatives of flavinoids (Swain, 1976). General sugar found in flavinoids is glucose whereas manose, fructose, xylose, rhamnose, galactose, apiose, arabinose are also present in mono or di- or tri- flavinoids glycosides (Markham, 1982). One of the prime groups of naturally occurring phenols is also constituted by flavonoids.

According to a study conducted by Ibrahim (2017) on *Gynura procumbens* (*lour.*) *Marr* the content of flavonoid increased with increase in Cd and Cu concentration in soil but reduced content was observed when metal is applied in a combination of two (Cd+Cu). Whereas, in other cases the same plant shows reduced production of secondary metabolites when grown in heavily polluted soil. In another experiment effect of cadmium on flavonoid content of *Hordeum sativum*

L. was observed was analysed. Results depicted the highest content of flavonoid in leaves (58.3gkg^{-1} dry matter), lowest content in roots (20.0g/kg dry matter). Therefore the descending order for the flavonoid content was in order leaves > shoots > roots. Overall, when the plant was treated with cadmium, a decrease in flavonoid content was observed in all the parts of the plant. We can conclude from this experiment that as many of the flavonoid structures are bound with transistion metals in chelate complexes (rutin-Cu-Zn) (Bai *et al*., 2004) hence the there is a decrease in free favonoid content in Cd treated plants.

In another experiment, Ahmad *et al* (2015) reported a decrease in total phenolic (1.728 mgg^{-1}) and flavonoid (1.087 µgg^{-1}) content in *Piper nigrum. Linn* fruits where the content of heavy metals was found to be within standard limits. Ahmad *et al* (2016) conducted an experiment to check the phenol and flavonoid content in *Brassica juncea* (mustard) plant where a decrease in flavonoid content was observed with increase in Cd concentration whereas an increase in phenol content was observed with same Cd concentration. Similarly, application of Se resulted in similar content of flavonoids and increased the content of phenols in mustard.

2.6.3 Amino acids

Plants consist of amino acids that form protein. Synthesis of amino acids in plants is from the oxygen and carbon that is acquired from hydrogen and air from water in the soil. Overall yield and quality of crops are increased with the help of amino acids as they are absorbed in plants through stomata. Physiological activities of plants are also influenced by amino acids. According to Jezek *et al* (2011), amino acids are the constituents and precursors to proteins and plays a vital role in the development and metabolism of plants. Accumulation of specific amino acids in the plants that are subjected to toxic heavy metals has been observed which can be valuable for plants. Xu *et al* (2012) reported the significant role played by amino acids that are assembled under heavy metal stress in plants, which include signalling molecules and osmolyte as well as detoxification facility and ion transport regulation.

Zemanova *et al* (2013) conducted an experiment to check the content of selected amino acids associated with cadmium stress in *Noccaea caerulescens* and *Arabidopsis halleri.* The result shows that *Arabidopsis halleri* is best adapted to stress conditions as compared to *N. caerulescens* where a change in different amino acids was observed in both plants under different Cd stress conditions. When exposed to heavy metals, various plants have shown to accumulate proline (Talanova *et al*., 2000). In *Deschampsia* and *Silene*, which are metal tolerant plants possess higher content of proline when compared with the non-tolerant plants (Schat *et al*., 1997). In 1987, Smirnoff and Stewart reported the presence large amount of proline in *Deschampsia* which is a non-tolerant ecotype whereas in tolerant ecotype this response was missing. Further, in presence of Cu, Ni and Zn, non-tolerant species of *Silene vulgaris* showed

massive proline accumulation in leaves. In tolerantecotype, the proline content was observed to be 5-6 times higher (Schat *et al*., 1997).

Pant *et al* (2011) conducted an experiment to check the effect of arsenic, cadmium and lead on an amino acid, chlorophyll, ascorbic acid and proline content of *Shorea robusta* seedlings with the application of different concentrations of metals. With the help of UV spectrophotometer, the content of amino acids, proline, ascorbic acid and chlorophyll was measured which showed total amino acid and chlorophyll content was decreased with elevated levels of heavy metals. Strongest effect on amino acid content was due to Cd toxicity followed by lead and arsenic.

2.6.4 Carbohydrate

Carbohydrates are mainly stored in plants which are a product of photosynthesis. Generally, pollution caused by heavy metals unfavourably affects carbohydrate synthesis as well as storage reserve mobility from cotyledon to seedlings. Various experiments were carried out to check the detrimental effects of heavy metals on the carbohydrate content in plants parts under various concentrations.

An experiment was conducted by Mesa *et al* (2016) to check the change in carbohydrate content, mainly starch in leaves and fruits of Abbe Fetal pear trees in different growing seasons. As the degradation of starch started, soluble carbohydrates content was increased. In another experiment conducted by Naquib and Barakat (1989) on *Vicia faba* to check the carbohydrate content in presence of tin and strontium, results show carbohydrate content in leaf was decreased when seeds were soaked in stannous chloride. Sugar beet cv. Monohill was grown in a solution of calcium and observed for sucrose, fructose, starch and glucose content in 5 weeks old plant (Gerger and Betrell, 1992). As per the results obtained, carbohydrate content was affected by Ca or Cd where a reduction in carbohydrate content was due to cadmium and calcium affected the carbohydrate content between storage and growth processes.

Hordeum vulgare L.var 2052 (Barley) was evaluated for some biochemical constituents proline, protein, chlorophyll a, b and carbohydrate content under different concentrations (10, 20 and 30 mM) of cadmium. The effect was seen on all these parameters in comparison to control under these concentrations. Low concentration (10 mM) of Cd resulted in less effect on protein and carbohydrate whereas; a higher concentration of Cd (20 and 30 mM) shows a reduction in these contents (Gubrelay *et al*., 2013). Similarly, in a study conducted by R. Frossard *et al* in 1988, the effect of different heavy metals was seen on the fructose, starch and sugar content in *Lolium multiflorum var. italicum* and *Lolium perenne*. Results suggested that with an increase in the concentration of heavy metals in soil, carbohydrate content was also altered before reaching to toxic levels of heavy metals (Cd, Cu and Ni) in fodder plants. An experiment was conducted by Moya *et al* (1995) to evaluate the effect of external application of abscisic acid (ABA) and

gibberellins (GA_3) on photosynthetic rate and carbohydrate content under stress of heavy metals on *Oryza sativa* (Rice). The result shows the growth inhibition of rice when treated with cadmium and nickel whereas accumulation of carbohydrate was stimulated in seeds, stems and leaves. But when GA was added along with cadmium the result was reversed, where growth was stimulated in seedlings but carbohydrate content was inhibited.

2.7 Hyperaccumulator plants selected for study: *Ricinus communis*

Linnaeus (1753) was the person to first identify genus *Ricinus.* L. and further its description were given by J. Mueller Argavoskii along with allocation to a family called Surge family in Euphorbiaceae (Moshkin V.A., 1986). Being a native species of Ethiopia, tropical Africa, *Ricinus communis* L. possess a historical background in literature since ancient times in world's different regions. Widely it is known as 'Castor oil plant','Wonder tree' or 'Castor bean' In India, it is being cultivated in many dry regions and found as a wild plant in mostly degraded, waste and contaminated soils. Following China and Brazil for the second and third number in cultivation and production of Castor seeds and oil, India is the top producer among 20 nations. It is considered a multipurpose crop due to its peculiar biochemistry, commercial importance and production of useful biomaterial like ricinoleic acid, castor oil, ricinoleyl-sulfate, lithium grease etc (McKeon *et al*., 2016).

Throughout the world, *R. communis* is cultivated majorly in subtropical and tropical regions as a warm season crop and perennial shrub whereas in a temperate climate as an annual plant (Rojer and Rix, 1999). Rainfall and temperature requirement that are considered suitable for its growth are 500 - 600mm and 20 -26°C respectively. Most of the tropical and sub-tropical countries *viz* Brazil, Philippines, India, China, USSR, Argentina and Thailand grow Castor plant but it found worldwide apart from these countries (Perdomo *et al*., 2013). In the warmer parts of India, most of the Castor plants are found, also along the roadside, near habitation as a wild plant and on wastelands, their presence is found in abundance (Faostat, 2011). Conditions required for the growth are slightly acidic (pH 5-6.5), fertile soil, sandy loams and well-drained soils are favourable for growth whereas marshy soil and clay soil with poor drainage are unsuitable for its growth. They are propagated by seeds.

It can be grown in different soil conditions like saline soil and heavy metals contaminated the soil with different elements like cadmium, caesium, copper, manganese (Gupta *et al*., 2007), chromium, arsenic (Melo *et al*., 2009) and nickel. It is known as a good hyperaccumulator, therefore, it can remediate hazardous material and toxic contaminants from the ecosystem (Olivares *et al*., 2013). It is a phytoextractor of Boron and can tolerate levels of Zn, Mn, Cu and Fe. According to Costa *et al* (2012), *Ricinus communis* is an ideal plant for remediating lead and cadmium contaminated soils. When compared to mustard (*Brassica juncea*) plant, *R. communis*

due to the presence of underground and aboveground biomass is considered as an efficient hyperaccumulator of Cd in high quantity (Bauddh *et al*., 2012).

Apart from this, some manipulation in the rhizospheric interactions of the plants grown on metal contaminated soil speeds up the rate of phytoremediation (Ananthi *et al*., 2013). Microbial consortia and some rhizobacteria are the soil microorganisms that inhabit the rhizosphere as an integral part of it. This microbial consortium affects the bioavailability and mobility of heavy metals in soil.

Different studies were conducted to check the efficiency of this hyperaccumulator for remediating contaminated soil with various heavy metals. In an experiment conducted by Bauddh and Singh (2012b, 2014), *B. juncea* was compared with *R. communis* for effective phytoremediation capability of Cd in drought and salinity stress. As a result, when compared to Indian mustard, castor showed more bioaccumulation of proline as self-protection ability. In salinity and drought stress, Cd content was found to be higher in roots of *R. communis* than *B. juncea.* Castor accumulated 17 foldes higher Cd content in roots and 1.5 foldes higher content in shoots as compared to Indian mustard.

Another experiment was conducted by Niu *et al* in 2009 that reported the capability of *R. communis* to accumulate higher content of cadmium and lead in roots and shoots. *R. communis* accumulated 10.54 – 24.61 g lead kg^{-1} dry weight, as a result of an experiment conducted by Romeiro *et al* (2006).

But according to Raskin *et al* (1994), the lead extraction capability of hyperaccumulator plants should be 1.0 g kg^{-1}D.W in tissues. Malarkodi *et al* (2008) found higher accumulation capacity of nickel by *R. communis* (747.3-874.6 mg kg^{-1} dry weight) in normal growth conditions but with the addition of organic manure (poultry manure and farmyardmanure), there was further increase in its accumulation rate as compared to normal field conditions. According to Vwiokoet at el (2006), in the soil contaminated with oil *R.communis* can effectively extract Mn, Pb, V and Ni at a concentration of 1-6% (W/W oil/soil) along with increasing growth of the plant. Similarly, Castor plant can accumulate Barium (Ba) from the soil contaminated with scrap metal residue (Abreu *et al*., 2012).

As reported by Coscione and Berton (2009), *Ricinus communis* possess the excellent potential to sequester barium from soil contaminated with it. When cultivated in soil contaminated with fly ash *R. communis* can extract various heavy metals like zinc, cadmium, chromium, copper, lead, manganese and iron (Pandey, 2013; Coscione and Berton, 2009). Shoot and root biomass of *R. communis* are enhanced by inoculation of the plant with different microbes e.g *Pseudomonas sp.* PsM6 or *P.jessenii* PjM15 (Rajkumar and Freitas, 2008), PGPRs (Romeiro *et al*., 2006), PGPB SRS8 (Ma *et al*., 2011). These PGPRs also help to reduce the toxic level of metals for plant and

when provided as an amendment in the soil, smoothly increase the mobility of heavy metals by *R. communis*. Report given by Adhikari and Kumar (2012) shows the lesser number of cells in the cortex area of roots of *R. communis* after treatment with nickel.

Mahmud *et al* (2008) collected soil and plant samples of groundwater from four different sites in Bangladesh contaminated with arsenic. Plants were selected for phytoremediation of arsenic on the basis of translocation factors (TFs) and bioconcentration factors (BCFs). Based on this selection criteria 49 species of plants were selected that were found to be best suited for their phytoremediation capacity and belongs to 29 families out of which there were three herb species (*Blumea lacera, Ageratum conyzoides* and *Mikania cordata*), two shrubs (*Ricinus communis* and *Clerodendrum trichotomum*) and one fern species (*Dryopterisfilix-mas*).

A study was conducted by Shaheenj *et al* (2006) to compare and assess the accumulation of arsenic, its resistance level, avoidance and tolerance by two species of plants: *Fagopyrum esculentum* L (Common buckwheat) and *Ricinus communis* (Castor bean plant). As a result, *R. communis* shows lower avoidance and higher tolerance to As but the opposite result was observed in buckwheat which shows lower tolerance, higher avoidance and resistance to As. Therefore, the experiment concluded that *R. communis* plants can be the best option for phytoremediating As contaminated soil.

Further, an experiment was conducted by Andreazza *et al* (2013) on *R. communis* plants to check the toxicity of copper after 57 days of cultivation which exhibited higher biomass production. High tolerance index of the plant was reported for the fresh mass of roots and dry mass of shoots, roots show higher bioaccumulation of Cu and were strongly capable for phytostabilization of Cu. According to the authors, *R. communis* during cultivation remarkably did not evacuate P, N and Mg from the soil. It was further noticed that Castor plants manifest a strong potential towards phytoaccumulation of copper, when plants were cultivated in 3 variant types of Cu contaminated soil e.g Cu mining waste consisting 40% native soil and 60% Cu mining waste and two sites having vine-yard soil contaminated with Cu (Inceptisol and Mollisol). The values of phytoaccumulation were 5900, 3052 and 2805 g ha^{-1}.

A comparison was made in 23 genotypes of *R. communis* to evaluate the potential of the plant for uptake and mobility of Cd and DDTs in contaminated soil. For experimentation, the soil was collected which was naturally contaminated with DDT (0.35 mg kg^{-1}) and cadmium (0.42 mg kg^{-1}). Soil samples were further contaminated with DDTs (1.7 mg kg^{-1}) and Cd (2.8 mg kg^{-1}). Variations were seen in plants for uptake and accumulation of Cd in leaf, stem and roots (1.22, 2.27 and 37.63 mg kg^{-1}) and DDTs (0.37, 0.43 and 70.51 mg kg^{-1}) respectively. This study revealed the higher capability of *R. Communis* for removing Cd and DDTs from contaminated soil (Huang *et al*., 2011).

A study was conducted to check the consequence of planting *S. alfredii* with *L. perenne* (ryegrass) and *R. communis* (castor plant) on heavy metals and PAH (polycyclic aromatic hydrocarbon) contaminated soils. The result of co-planting of *S. alfredii* with castor and ryegrass shows enhanced dissipation of anthracene and pyrene than in bare soil or monoculture of *S. alfredii*. This can be due to the increase in the microbial population of soil and their related activities in both the treatments. Therefore, to reduce the PAH and heavy metal contamination from soil, co-panting of *S. alfreddi* with castor and ryegrass can be an effective strategy (Wang *et al*., 2013).Shi and Cai (2009) performed an experiment to check the effect of Cd on eight different energy crops in contaminated soil. The selected crops were tolerant to Cd and when compared to all, four species were found to be more tolerant *viz. Cannabis sativa* (hemp), *Linum usitatissimum* (flax), *R. communis* (castor) and *Arachis hypogaea* (peanut). Hence, the result of the experiment concludes that energy crops can be grown on cadmium contaminated sites in order to be effective against their removal (Phytoremediation).

A similar study was conducted by Bauddh *et al* (2015) on 7 different herbaceous crops to evaluate the phytoremediation capacity of these plants against nickel on contaminated soil. The selected crops are *R. communis* (castor), *Hordeum vulgaris* (barely), *Sorghum vulgare* (sorghum), *B. juncea* (cabbage), *Phaseolus vulgaris* (bean), *S. oleracea* (spinach) and *S. lycopersicum* (tomato); where *R. communis* was found to be a good hyperaccumulator of nickel in its roots and shoots than other crops. Cd and Pb accumulation capability of four plants were studied by Zhi-Xin *et al* (2007) *viz. Medicago sativa* L. (alfalfa), *H. annuus* L.(sunflower), *B. juncea* L.(mustard) and *R. communis* L.(castor) in hydroponic cultures. During 5 weeks of cultivation period, *R. communis* accumulated a considerable amount of both metals.

2.7.1 *Canna indica* L.

Canna indica L (Cannaceae)is a sturdy and monocot plant with broad leaves and fleshy leaves, usually found growing up to a height of 0.5 to 2.5 m according to the variety. It is widely grown in India as an ornamental plant. The plant is green in colour with a single stem. Basically, it is a wetland plant that possesses leaves with an oblong shape and long spikes as an inflorescence. The occurrence of fruit is in form of hard, small and black seeds. Flowers of this plant are bisexual and red in colour. This bright colour of flowers gives it a designation of the valuable ornamental plant where it becomes a good source for extracting natural colourants for purpose of dying in various textile industries (Milow *et al*., 2010).

"Indian shots" is the name given to the plant due to its resemblance with gun pellets. The diameter of the rhizomes of the plant is 3 cm in thickness and abundant tillering (Madhumati *et al*., 2016). Rhizome is rich in high-quality starch and chiefly used in traditional folk medicines (Swarnkar *et al*., 2008; Padal *et al*., 2010). It is most often used as a plant species in urban streets

and parks because of its extended flowering duration, effortless growth and attraction (Cheng*et al*., 2007). This plant is highly tolerable to diseases and has the ability to grow in unfavourable conditions with the production of extensive biomass. It does not enter the food chain as it is non-edible therefore blocks the passage of contaminants. It is a perennial plant that can multiply easily and even if leaves are trimmed it can simply spread or regrow with the help of rhizomes (Karunakaran *et al*., 2017).

It is capable of growing on wastewater and is quite effective to treat wastewater (Priyanka *et al*., 2017). As stated in the literature, it acts as a good hyperaccumulator therefore used by many researchers for their study. Moreover, it can improve water quality by cultivating in constructed wetlands (Grosse *et al*., 2011) and can be proved to be a good hyperaccumulator of cadmium with some constraint at elevated concentrations (Cheng *et al*., 2002a). *Canna indica* has been chiefly known for its phytoremediation capability in order to eradicate toxic heavy metals like copper and zinc along with some fertilizers and pesticides (Mahamadi *et al*., 2011). Its extract is widely used as a good source of natural antioxidant agent, which comprises of excess volume of phenols and flavonoids (Vankar *et al*., 2010).

Long back, according to an experiment conducted by Debnath and Mukherjee (1982), detectable effect of barium chloride on anthocyanin content of *C.indica* was observed Whereas Cheng *et al* (2002) noticed the effect of cadmium on photosynthetic rate of *C.indica*. Cheng *et al* (2007) studied the phytoremediation capability of triazophos by *C.indica* in the hydrophobic system. Phytoremediation potential of *C.indica* for TAP was observed to be 74% as well as it can be an eminent tool for constructed wetlands. Another finding reported the good accumulation capacity of *C.indica* plant for different heavy metals, therefore, it is an efficient hyperaccumulator of heavy metals (Subhashini *et al*., 2014). In a study conducted by Subhashini *et al* (2014), the heavy metal accumulation potential of *C.indica* was observed for various heavy metals viz. Lead, nickel, zinc, cadmium and chromium, where Pb, Zn and Cr were used in phytoextraction process and Ni and Cd in phytostabilization. The final result declared this plant as a very good accumulator of all these metals from soil.

Another experiment was conducted by Gunarathna *et al* (2016) in Kebithigollowa Central College in Anuradhapura district of Sri Lanka, to determine the effect of RO concentrate on the growth of *C.indica* and properties of soil as well as the effectiveness of *C. indica* plant as an agent for phytoremediation was also studied. Results showed a significant reduction in the growth of the plant in presence of RO concentrate when compared to groundwater. After analysis of soil, it was observed that *C. indica* plants can effectively remediate calcium, potassium and nitrogen along with a reduction in electrical conductivity whereas ineffective in removing magnesium, phosphorous and sodium form RO concentrate in the soil. Hence the

experiment concluded that *C.indica* can act as a successful phytoremediation agent to eradicate higher levels of RO concentrate from the soil as well as it can generate dual benefits as a phytoremediator of pollutants while generating earnings.

Cule*et al* (2016) conducted an experiment to explore the phytoremediation potential of *C.indica* in lead-contaminated water. On the 21st day of sampling maximum content of lead (41 mg Pb/L) was found in roots of the plant hence results demonstrated that terrestrial plants can be best suited for rhizofiltration as compared to the aquatic plants where *C. indica* is the best tool in rhizofiltration system for treatment of lead-polluted water. Li*et al* (2013) used two plant species viz. *Eleusine indica* (Goosegrass herb) and *C.indica* (Canna) to remove some heavy metals (Cd, Cr, Cu, Zn, Ni and Pb) from the sewage sludge by potted planting. As a result, Goosegrass showed effective removal of Cu, Ni, Cd and Zn whereas, by *C.indica* Zn, Ni and Cd were removed in soil amended with sludge.

Priyanka *et al* (2017) studied the effect of *Canna indica* on the removal of textile contaminants along with lowering BOD, COD, TDS and TS of water. The result of the study describes the beneficial role of Canna plant in effectively reducing all physical, biological and chemical parameters from textile wastewater. 72 % of total contaminants were reduced from the wastewater received from textile industries where percentage removal of BOD was found to be 93% and COD was 63% and values of TDS, pH and TS were found to be lying within CPCB standards after treatment. Another study was conducted by Shankar *et al* (2016) to investigate the phytoremediation potential of three plants *Canna indica*, Taro plant and *Aloe vera* to reduce the content of D.O, BOD, COD and pH in domestic wastewater. *C indica* was found to be most effective (40%) in reducing all the contaminants and the above-given parameters from wastewater. Madhumathi *et al* (2016) conducted an experiment on two natural wetland plants *Canna indica* and *Cyperus alopecuroides*to check the chromium accumulation capability by these plants along with the estimation of their BCF and TF. As both the plants are good hyperaccumulators, but when compared to *C. alopecuroides, C. indica* accumulated more chromium in its roots and 147 was its BCF. Inspite of this, the translocation of Cr to aerial parts of the plant (leaves and stem) was insignificant because of its lower TF value. Whereas BCF value of *Cyperus* plant was found to be 36 that clearly indicates less accumulation of Cr in roots of this plant but TF was found to be more which indicates the higher translocation of Cr to aerial parts of plants. Hence experiment concludes that *C. alopecuroides* as compared to *C. indica*, is a good translocator are well as an accumulator of Cr.

A similar study was conducted by Karunakaran*et al* (2017) to study the effect of two biochar *viz.* Coconut shell biochar (BCcs) and rice husk biochar (BCrh) on effective removal of chromium by *Cana indica* L. after harvesting the plant at a time interval of 5, 10 and 20 days, chromium

uptake by the plant was observed. Mechanism of Cr uptake by biochar was studied based on the accumulation of metal by plant parts and the results states depending on the variety of biochar used; the soil is remediated from Cr (VI) with the help of *Cana indica* L. by Phytoextraction or phytostabilization. Therefore, biochars can be combined with hyperaccumulating plants by altering their temperature to effectively eradicate Cr from the soil.

Cheng*et al* (2007) concluded from his experimentation that *C.indica* L. possesses the potential of phytoremediating TAP (triazophos) from constructed wetlands. The result shows that 74% of TAP was remediated by *C. indica* in the hydroponic system. Dibyendu (2013) studied the response of antioxidant enzymes on Cu accumulation by *C.indica* L. Cu accumulation was observed to be higher in roots (108-191 $\mu g g^{-1}$ DW) as compared to the accumulation rate in leaves (23.36-40.43 $\mu g g^{-1}$ DW). Antioxidant enzyme ascorbate content does not change in copper-treated roots of *Canna* plant whereas the content of glutathione reductase and dehydroascorbate reductase was increased. Hence by concluding from the results of an experiment, it was inferred that *C. indica* L. possess the calibre to accumulate the higher amount of copper in the roots by preventing its accumulation in aboveground parts.

Many other scientists have carried out different experiments to prove that *R. communis* and *C. indica* are good hyperaccumulators of different heavy metals from contaminated soil as well as water. Therefore, keeping in mind their phytoremediation potential, this study has been designed to study the effect of microbial consortium (Rhizobacteria and Mycorrhiza) on Cd and As contaminated soil along with observing the effect of these heavy metals on the antioxidant enzymes and other photosynthetic parameters of both these test plants under natural conditions.

CHAPTER 3

HYPOTHESIS

HYPOTHESIS

In the present era, heavy metals contamination of soil due to brisk industrialization and urbanization is a crucial problem that is prevailing over other issues. Due to their non-biodegradability, these heavy metals pose various health issues in humans as well as causing other environmental troubles for plants and animals. Hence, there is an acute need to work on this aspect of reducing heavy metals toxicity in soil. To tackle with such a major issue, very eco-friendly, cost-effective and efficient method is used by various researchers i.e phytoremediation, which involves the plants that can readily accumulate contaminants (heavy metals) from the soil into their below and above ground parts thus reducing the toxicity and an excess amount of these heavy metals from soil. The plant species that can accumulate or extract a large amount of heavy metals from soil into their parts effectively are known as hyperaccumulators. Therefore, the present study is designed to check the effect of microbial consortium developed from the native rhizobacterial and arbuscular mycorrhizal fungus species on the native wild species of plants in order to diminish the toxic effects of heavy metals found in contaminated areas. Native microbes possess a great potential to reduce the toxic effects of heavy metals along with various hyperaccumulator plant species.

The aim of this project is to provide an understanding needed to harness rhizobacteria and mycorrhizal associated with the polluted soil and to develop methods to accelerate these processes for the phytoremediation of contaminated environments. This technology has the ability to rejuvenate the contaminated environments effectively. With the exciting new development in this field and focus on interdisciplinary research and using it on gaining the fundamental knowledge necessary to overcome the obstacles facing current technologies and also with respect to ethical, legal, and social issues involved this technology will go a long way in cleaning the environment in near future. We are aware of the consequences of pollutants effect in our day-to-day life. People are working a lot for developing strategies to eradicate this notorious problem. Microorganisms and plants native to these sites can be the best option to fight against this evil. This work can be a milestone for people of Jalandhar, Punjab region, to eradicate certain pollutants.

CHAPTER 4

OBJECTIVES

4. Aim and Objectives

4.1 Background

Due to extensive toxicity caused by the heavy metals in soil, this acute problem needs an efficient method for its treatment. Hence, an eco-friendly, cost-effective and sustainable method known as phytoremediation, is used nowadays for eradicating this problem. This study is also designed in the same context to develop microbial consortium for phytoremediation of industrially polluted soil by native microbes,as native microbial and plant species are helpful in effectively reducing the toxic levels of heavy metals in the soil, thus increasing the fertility of the land and enhancing the crop productivity.

4.2 Objectives

Looking at the aim of phytoremediation by native microbes (rhizobacteria and arbuscular mycorrhizal fungi) and plant species, the following objectives were undertaken in order to accomplish the proposed work:

1. To assess, identify and multiply Rhizobacteria and AM fungi associated with plants around industrial effluent polluted sites of Jalandhar.
2. To determine enzymatic activities of plants associated with precipitation and binding of pollutants.
3. To evaluate the potential of plant–symbiotic rhizobacteria and arbuscular mycorrhiza in grass-system to remove the pollutants.
4. To check the efficacy of microbial consortium incorporated with selected plants for effective phytoremediation of the selected site.

CHAPTER 5

MATERIAL & METHODS

MATERIAL AND METHODS

The research work was conducted to evaluate the potential of plants to accumulate heavy metals in their parts in coordination with the microbial consortium.

5.1 Study area

Prior to finalizing a particular study site, various polluted sites were explored in Jalandhar region which includes local drain at Hardaspur village (Coordinates: 31°14'42“N 75°42'33”E) situated near Lovely Professional University campus, Waryana industrial complex (Coordinates: 31.3350°N 75.5154°E) Kapurthala road, Jalandhar, Focal point (industrial area, Jalandhar) and Leather complex (Coordinates: 31.3312°N 75.5251°E), Kapurthala Road, Jalandhar. Directly or indirectly, various small and large scale industries in these areas are disposing off their partially treated effluents nearby the drains, can become the part of groundwater or enters the nearby agricultural fields.

Farmers unknowingly use this wastewater from drain for irrigation practices and for the present research, we have collected soil samples from these areas (figure 3). But final selection of study area was based on the high pollutant load in Jalandhar (Punjab), located in focal point industrial area (coordinates: 31.3491°N 75.5757°E). This focal point is located at a distance of 12.4 km away from Jalandhar city on the National highway towards Amritsar. Different large and small-scale industries are located in this area like steel, metal, automobiles, textile, batteries etc. Jalandhar has an elevation of 241 meters height that is equivalent to 791 feet. The climate condition of Jalandhar is warm and temperate. Rainfall is much less in winters as compared to summers. Annual temperature of this city is 23.9° C along with 769 mm of precipitation.

5.2 Site description and sampling

Ten different sites were visited and analysed for the exposing of contaminants into the nearby land in Focal point, Jalandhar. Out of these ten, only four sites were selected for this research namely Site 1, Site 2, Site 3 and Site 4 (figure 3). These small-scale industries are surrounded by agricultural and residential land and directly contaminating soil as well as groundwater of that area. From the above-mentioned sites, soil and root samples were collected from a depth of approximately 10-20 cm.

For sampling, the plants were uprooted and soil was collected from the rhizosphere of the plants. From each site, 3-4 subsamples were collected randomly weighing around 1 kg. These soil and root samples were properly sealed in fresh and sterile Ziploc bags and brought back to the laboratory in ice and kept at 4°C for further analysis and experimentation. Before any analysis, soil samples were air-dried, powdered and sieved through a 2mm sieve to manually remove stones and other plant material.

5.2.1 Processing of soil samples

Collected soil samples were divided into two parts for experimentation

1. One part was used for physico-chemical analysis
2. The second part was used for microbiological analysis (isolation of rhizobacteria and mycorrhizal species)

5.3 Physico-chemical analysis of soil samples

ICP-AES (Inductively Coupled Plasma- Atomic Emission Spectrometry) for pre-analysis of heavy metal in soil samples was carried out from Punjab Agriculture University, Ludhiana (Soil testing laboratory) and post-analysis of heavy metal in plant and soil samples by ICP-OES (Inductively coupled plasma-optical emission spectrometry) from Lovely Professional University. Other parameters of soil for pH, P, N, K and soil type were also analysed to be familiar with the nature of soil (sandy, clay, silt) found in this area (figure 3).

Figure 3: Different sites in industrial area Jalandhar selected for sample collection.

5.3.1 pH measurement of soil

Samples of soil collected from various sites were filtered through a sieve to remove the debris and particles greater than 6.3 mm. 30 g of soil was taken in a glass beaker and an equal amount of distilled water was added to it. The slurry was obtained after regular stirring and was properly

covered afterwards. 10-15 minutes time interval was required for the proper stirring of slurry and kept undisturbed for 1 hour to stabilize the pH and temperature. The electrode of pH meter was standardized using standard phosphate buffer with pH 7. Stirring of the sample with glass rod prior to the checking of pH was important. pH value was recorded and the electrode was cleaned with distilled water followed by dabbing with tissue in order to clear the film formed on it (Geotechnical Engineering Bureau, 2007).

5.3.2 Extraction of nutrients available in the soil

Estimation of potassium and sodium was carried out by extracting 5.0 g of soil samples with 100 mL ammonium acetate buffer (~pH 7.0) for half an hour and filtered (APHA, 1998). Following the extraction, an aliquot was collected and nutrients were estimated from the samples.

5.3.2.1 Potassium and Sodium

In a 25 mL beaker, an aliquot was added that was drawn from extracted material and with help of capillary fed into a flame photometer. After the stabilization of flame, reading was recorded on indicator scale. By taking a range of standard solutions of potassium chloride and sodium chloride, a calibration curve was prepared and the total concentration of potassium and sodium was calculated.

$$\text{Potassium/sodium} = \frac{\text{K or Na from standard curve}\left(\frac{\text{mg}}{\text{l}}\right) \times \text{volume of solution}}{10 \times \text{weight of the sample(g)}}$$

5.3.2.2 Phosphorous

Phosphorous and heavy metalswere estimated through the method given by John (1970).

5.3.2.2.1 Reagents

a) Stock solution: In 300 mL distilled water 20 g of ammonium molybdate was dissolved. To this solution, 10N H_2SO_4 (450 mL) was added followed by continuous stirring. Then 0.5 % antimony potassium tartrate (100 mL) was added and final volume make up was done up to 1 L with the addition of distilled water and the solution was stored in a dark coloured glass bottle away from direct light.

b) Working reagent: To 100 mL of stock solution 1.5 g of ascorbic acid was added. This working reagent should always be prepared fresh.

c) Standard solution: 1000 mg/L (1000 ppm) standard stock solution was prepared by dissolving 0.439 g of KH_2PO_4 in distilled water (100 mL). Standard curve was prepared with the range 0.2, 0.4, 0.6, 0.8 and 1.0 µg/mL

d) Diacid mixture: Conc HNO_3 (Nitric acid) and $HClO_4$ (Perchloric acid) were mixed in ratio 4:1 to prepare this diacid mixture.

5.3.2.2.2 Procedure

In a 250 mL digestion flask, 0.5 g of soil was taken and to it, diacid mixture (15 mL) was added. This mixture was made colourless by digesting it in a digestion chamber. The leftover mixture was diluted with distilled water up to 30 mL and filtered with the help of Whatman filter paper no. 1. Further, it was transferred into a volumetric flask of 50 mL and distilled water was added to make final volume 50 mL. 1 mL aliquot was taken from each flask in a volumetric flask of 50 mL and freshly prepared diacid mixture (5 mL) was added to it. With the addition of distilled water final volume was made to 50 mL and after 30 min using UV-Visible spectrophotometer-117 absorbance of the solution was measured at 880 nm.

5.3.3 Heavy metal analysis

A diacid mixture was prepared by mixing concentrated HNO_3 (Nitric acid) and $HClO_4$ (Perchloric acid) in the ratio of 4:1 (V/V). Afterwards, in a 250 mL digestion flask 0.5 gm of soil was taken and to it, diacid mixture was added (15 mL). In a digestion chamber, this mixture was digested till its colour disappears. Further, the leftover mixture was diluted with distilled water to make volume 30 mL and filtered through Whatman filter paper no. 1. This solution is cooled and transferred to a volumetric flask (50 mL) and distilled water was added to make a final volume of the solution up to 50 mL. This solution is then analysed for heavy metals in soil and other plant parts by ICP-AES.

5.4 Community analysis and selection of Plants

Plants found nearby the industrially polluted area belong to different families that include *Saccharum spontaneum, Brassica juncea, Canna indica, Ricinus communis, Solanum nigrum, Parthenium hysterophorus, Arabidopsis thaliana* and *Eichhornia crassipes* (aquatic plant). All these plants are known as good hyperaccumulators, but for this present study, only two were selected for experimentation on the basis of their higher phytoextraction potential and production of larger biomass. As a result, two plants were selected: *Ricinus communis* and *Canna indica.*

5.5 Isolation of Arbuscular mycorrhiza fungal spores

Two different methods were used for the isolation of mycorrhizal fungal spores.

5.5.1 Wet-sieving and decanting method

This method was given by Gerdemann and Nicolson (1963).

Procedure:

1. Around 100 g of rhizosphere soil was added to water (~1000 mL).
2. The mixture requires continuous stirring for 10-15 seconds and let the mixture stand for 1-2 hours so that unwanted coarse particles in the soil settle down properly and the resulted

supernatant was decanted through a series of sieves that are arranged in a descending order of mesh size (710 µm, 500 µm, 250 µm and 53 µm respectively).

3. Sieveate were collected from each sieve separately in different beakers and filtered through Whatman filter paper No. 1 separately.
4. These spores were then picked by a hypodermic needle and analysed under a dissecting microscope after mounting on polyvinyl lactic acid glycerol and stored in glycerine for further experimentation.

5.5.2 Sucrose density gradient centrifugation

This method was stated by Ohms (1957).

Procedure:

1. In a 15 mL centrifuge tube, 5mL of 50 % and 25 % sucrose solution was added to which with help of hypodermic syringe water was added in order to form different layers of the solutions.

2. Suspension of sieving was added to this centrifuge tube and centrifuged for 5 minutes at 3100 rpm.

3. Debris was collected that was accumulated in the middle layer of the centrifuge tube and washed with water.

4. Spores were collected on the filter paper as mentioned in the first method and analysed under a dissecting microscope.

5. Collected spores were stored in glycerine for multiplication and inoculation with plants.

Spores were identified following the manual given by Sheneck and Perez (1990), according to their morphological characteristics that include shape, size, colour, hyphal attachment, bulbous suspension and wall structure.

5.6 Mycorrhizal quantification

By following the method given by Adholeya and Gaur (1994), the estimation of AM fungal spores was done using a modified intersect method. This method includes the compartmentalization and numbering of filter paper to count the number of spores under a dissecting microscope.

5.7 Mycorrhizal root colonization

Mycorrhizal association with roots was analysed to study the interaction between them by "Rapid clearing and staining technique" given by Phillip and Hayman (1970).

Procedure:

1. Roots were washed under tap water to remove the unwanted adherent particles of soilsand then cut into 1 cm long bits.

2. These root pieces were then boiled in 10 % KOH solution in hot water bath for 1 hour at 90° C to remove tannin and colour from them. This solution readily allows the stain penetration into roots by removing host cytoplasm.
3. KOH solution was then poured off and root pieces were washed with distilled water 4-5 times.
4. After washing, roots were treated with 10 % or 5N of dilute HCl for 3-5 minutes to clear the stain in the fungal tissues.
5. Washing was again repeated and root segments were attained with trypan blue (0.5 %) for 24 hours.
6. To destain root tissues, trypan blue was decanted and kept overnight in lactic acid: glycerol (1:1) solution.
7. Finally, roots were mounted on glycerol or lactic acid: glycerol solution in 1:1 ratio and viewed under a microscope for mycorrhiza hyphal association within roots.
8. After analysing the association total root colonization percentage was calculated by the root slide technique given by Giovannetti and Mosse (1980).

The formula used for calculation of percent root colonization was:

$$\text{Percentage mycorrhizal root colonization} = \frac{\text{No. of root segments infected}}{\text{Total no. of root segments studied}} \times 100$$

5.8 Multiplication of Mycorrhiza

Mycorrhizal multiplication was carried out with a host plant (*Sorghum bicolor*) in pots. The quantity of fungal spores was increased in 5-6 months of planting period. Layering was done for this purpose by soil collected from polluted area as well as from the normal field. The spores were preserved as such in the soil for further inoculation with test plant.

5.9 Isolation and screening of Rhizobacteria

Rhizobacteria are considered as the part of rhizomicroflora that provides nutrients to plants and are found in the rhizosphere of polluted and non-polluted soil. In order to prepare a good microbial consortium, we need to isolate these rhizobacteria from different soil samples collected from different polluted sites. The method used for their isolation was a serial dilution of soil. Basic media used for isolation was nutrient agar at a temperature range of 37°C. After the appearance of visual colonies, isolation of pure colonies was carried out using the streakingtechnique. Isolated colonies were selected on the basis of colour, shape and other morphological features of the colonies (Arneja, 2003) and were further purified by sub-culturing technique (Nutrient agar) to obtain pure cultures. The new individual culture indicates growth of single species which are known as stock or pure culture (Arneja, 2003). Glycerol stock was

prepared to preserve the pure cultures for long-term usage. The composition of nutrient agar used is given in table 3:

Table 3: **Composition of nutrient agar**

Constituents	**Quantity g/L**
Peptic digest of animal tissue	5
Sodium chloride	5
Beef extract	1.5
Yeast extract	1.5
Agar	15
Distilled water	1 litre
pH	7.4

Another method used for isolation of rhizobacteria was media enrichment technique for isolating rhizobacteria which are particularly associated with accumulation of heavy metals (Arsenic and cadmium).1 g of soil sample collected from each site was added in 250 mL conical flasks each containing different concentrations of heavy metals (50, 100, 200, 500 and 1000 $mgkg^{-1}$). The mixture was incubated in an orbital shaker incubator at 30°C and 110 rotations per minutes (rpm) for 10 days. After incubation, 1 mL of supernatant was transferred to fresh media containing heavy metals and incubated for 4 days under the same conditions for 3-4 times repeatedly (Ramu *et al*., 2014).

5.9.1 Minimum inhibitory concentration (MIC)

Selection of bacterial cultures for microbial consortium was based on minimum inhibitory concentration test which was performed atvarying concentrations of heavy metals (250, 500, 1000& 2000$mgkg^{-1}$). Heavy metals were selected on the basis of ICP-AES analysis. Bacterial cultures were grown by spreading method on sterile Petri plates followed by addition of selected concentrations of heavy metals in the wells made in the same plates (well diffusion method).

After providing incubation of 24-48 hours at 37°C, plates were analysed for the inhibition zones that appear around the wells. Strains of bacteria were selected on the basis of MIC for identification.

5.9.2 Identification of Rhizobacteria

Nine strains were selected for further experimentation on the basis of MIC that shows the inhibition capability of rhizobacteria toward the variant heavy metal concentrations. These cultures were identified by 16 S rRNA sequencing from Yaazh Xenomics, Tamil Nadu (India). The protocol for bacterial identification is as follows:

1. Lysis/Homogenization: cells were lysed by suspending 1-3 colonies aseptically and mixed with “B cube” lysis buffer (450 µl) in a microcentrifuge tube (2 mL) and by pipetting repeatedly cells were lysed.

2. 4 µl of RNase A and 250 µl of “B cube” neutralization buffer was added

3. The content was vortexed and tubes were incubated at 65°C for 30 minutes in the water bath.

4. Centrifugation of tubes was done for 15 minutes at 10°C at 14,000 rpm.

5. Without disturbing the pellet, resulting viscous supernatant was transferred into a fresh 2 mL microcentrifuge tube.

6. “B cube” binding buffer (600 µl) was added to the content and thoroughly mixed by pipetting followed by incubation at room temperature for 5 minutes.

7. 600 µl of contents were then transferred to a 2 mL spin column placed in a collection tube.

8. Centrifugation was carried out at 14000 rpm for 2 minutes after which flow through was discarded.

9. Spin column and collection tube were reassembled and then remaining 600 µl of lysate were transferred.

10. Centrifugation at 14,000 rpm for 2 minutes was done with discarding flow-through.

11. “B Cube” washing buffer I (500 µl) was added to the spin column and again centrifuged for 2 minutes at 14,000 rpm followed by flow through discarding.

12. Spin column again reassembled and 500 µl “B Cube” washing buffer II was added along with centrifugation (2 minutes at 14,000 rpm) and flow through was discarded.

13. Spin column was transferred to a 1.5mL sterile microcentrifuge tube.

14. 100 µl of “B Cube” elution buffer was added at the middle of the spin column.

15. 5 minutes incubation was given to tubes at room temperature along with centrifugation at 600 rpm.

16. 14 and 15 steps were repeated for complete elution. The DNA is present in the buffer in microcentrifuge tubes.

17. Aliquotes were run on 1% agarose gel to measure DNA concentration.

18. DNA samples were stored for further use at -20°C.

The composition of Taq Mater Mix

- Taq DNA polymerase is supplied in 2X Taq buffer
- 0.4 mM dNTPs
- 3.2 mM $MgCl_2$
- 0.02 % bromophenol blue

Primer details

Primer name	Sequence detail	No of bases
27F	AGAGTTTGATCMTGGCTCAG	20
1492	RTACGGYTACCTTGTTACGACTT	22

5 µl of isolated DNA was added in 20 µl of PCR reaction solution (1.5 µl of forward and reverse primer, 5 µl of deionized water and 12 µl of taq master mix).

PCR was performed using following thermal cycling conditions:

1. **Denaturation**: DNA template was heated for 3 minutes to 94 °C.
2. **Annealing**: Mixture was cooled from 94 °C for 30 sec, 50 °C for 60 sec and 72 °C for 60 sec.
3. **Extension**: Reaction is then heated to 72 °C for 10 minutes.

Purification of PCR Production

Unincorporated PCR primers and dNTPs from PCR products were removed by using Montage PCR Clean-up kit (Millipore).The PCR product was sequenced using the 27F/1492R primers. ABI PRISM® BigDyeTM Terminator Cycle Sequencing Kits with AmpliTaq® DNA polymerase (FS enzyme) were used for sequencing reactions (Applied Biosystems).

Sequencing protocol

16s rRNA universal primers were used for single-pass sequencing on each template. Ethanol precipitation protocol was used for purification of fluorescent-labelled fragments from the unincorporated terminators. The samples were resuspended in distilled water and subjected to electrophoresis in an ABI 3730xl sequencer (Applied Biosystems).

5.9.3 Multiplication of Rhizobacteria

The selected nine strains were further multiplied in nutrient broth for inoculation with the host plants. The nutrient broth was prepared in which loopful of pure bacterial cultures were added and incubated at 30°C for 5-8 days at continuous shaking conditions in an orbital incubator shaker at 120 rpm.

Table 4:Composition of nutrient broth used is as under

Constituents	Quantity in g/L
Distilled water	1 l
Peptone	10
Beef extract/yeast extract	10
Sodium chloride (NaCl)	5
pH	7.3

These cultures were further used as individual and asa microbial consortium (Rhizobacteria + mycorrhiza) for inoculation with the host plant after addition of different concentrations of selected heavy metals.

5.9.4 Synergistic activity (Biocompatibility test) of selected rhizobacteria

Further, synergistic activity (biocompatibility test) of these isolates was conducted following the method described by Nikam *et al* (2007) with slight modifications for in vitro testing.

1. All the selected bacterial cultures were spread on the growth media (nutrient agar) and allowed to grow for 24 hr at 37°C.
2. Five mm size sterilized paper (Whatmann paper no 1) discs impregnated with a bacterial suspension of individual isolates were placed at the distance of 5 mm from the periphery of Petri plates already having growth of cultures inoculated in a different pattern to check the antagonistic effect of bacterial cultures on each other.
3. After placing these discs, cultures were again allowed to grow for 12 hr at 37°C.
4. Finally, the growth was observed which shows that there was no zone of inhibition in any plate; hence all the bacterial cultures are not a competitor but are compatible with each other and can make a good microbial consortium.

5.10 Plant cultivation, treatments and sampling

Selected plants (*Ricinus communis* and *Canna indica*) were treated with different concentrations (50 and 100 $mgkg^{-1}$) of selected heavy metals (As and Cd) to analyse various physiological and chemical parameters.

5.10.1 Pot culture experiment

The effect of heavy metals on *R. communis* and *C. indica* plants under two different concentrations (50 and 100 $mgkg^{-1}$) of two selected heavy metals (cadmium and arsenic) was studied using pot culture. The pot culture experiment was conducted using polypropylene pots in natural environmental conditions. The soil was collected from non-contaminated fields. The soil was alluvium and sandy loam in texture. The soil was air-dried and sieved through a 2 mm mesh before filling in pots. Approximately, 5 kg of air-dried soil was added to each pot. All the experimental set up were carried out in triplicates. The seeds of *R. communis* and rhizomes of *C. indica* were pre treated before sowing in pots.

5.10.1.1 Pre treatment of plant seeds before sowing

In the case of *C. indica* plant, rhizomes were collected after uprooting a good number of whole plants from the fields. Rhizomes were separated from the roots, washed properly with water several times and then kept for some time to dry. For better growth, *C.indica* plants require warm conditions, so the experimentation was started in the month of March when the sun is in its full strength. Most of the rhizomes were planted horizontally with the eye facing upwards around 3-6 inches deep in the soil. Pots were watered thoroughly and allowed to drain as this is also one of the growth requirements of the plant.

R. communis plant was cultivated by seeds that were collected from plants grown in the polluted area. The seeds of this plant are very hard and difficult to grow. Therefore, pre-treatment is required for seeds. For that, seeds are soaked for overnight in distilled water and then treated with 10% (v/v) sodium hypochlorite solution that helps to break the hard coat of the seeds. The plant needs heat and warm temperature for growth (28-38°C) with a good amount of light. 5-6 seeds were sown 1-2 inches deep in each pot and soil was kept moist for efficient growth. After 1 month of planting, thinning was done to keep only 2-3 plants per pot.

5.10.2 Experimental setup

The treatments were designed after intense scanning of literature. Two levels of cadmium and arsenic were added i.e 50 and 100 ppm ($mgkg^{-1}$) to the soil. These metal concentrations were supplied in the form of Cadmium chloride ($CdCl_2$) and Sodium Arsenate (Na_3AsO_4) on the basis of their solubility in soil. These salts are easily taken up by plants (bioavailable). These salts of heavy metals were dissolved in distilled water and added to soil after sowing the seeds and addition of rhizobacterial and mycorrhizal cultures.

Plants without metal treatment served as negative control and those treated with only rhizobacteria and mycorrhizal as positive control along with one normal control without any treatment. Pots were placed in the field conditions to grow a plant in the natural environment. Seeds germinated to reach a plant density of 4-5 after 10-20 days of sowing. Tap water was used as water sources for plants. Other physiological and chemical parameters were estimated in plants and soil in 30, 60 and 90 days old plants. All the plants were harvested after 120 days of treatment. The pot experiment design is described (table 5) as under for both the plants (*R. communis* and *C. indica*)

Table 5: Experimental set up for *R. communis* and *C. indica (*in triplicates) along with rhizobacteria, mycorrhiza and microbial consortium.

Control+PS1	As 50+PS1	As 100+PS1	Cd 50+PS1	Cd100+PS2
Control+PS2	As 50+PS2	As 100+PS2	Cd 50+PS2	Cd 100+PS2
Control+PS3	As 50+PS3	As 100+PS3	Cd 50+PS3	Cd 100+PS3
Control+PS4	As 50+PS4	As 100+PS4	Cd 50+PS4	Cd 100+PS4
Control+HX1	As 50+HX1	As 100+HX1	Cd 50+HX1	Cd 100+HX1
Control+NAP1	As 50+NAP1	As 100+NAP1	Cd 50+NAP1	Cd 100+NAP1
Control+TL1	As 50+TL1	As 100+TL1	Cd 50+TL1	Cd 100+TL1
Control+XL1	As 50+XL1	As 100+XL1	Cd 50+XL1	Cd 100+XL1
Control+BZ1	As 50+BZ1	As 100+BZ1	Cd 50+BZ1	Cd 100+Bz1
Control+G.c	As 50+G.c	As 100+G.c	Cd 50+G.c	Cd 100+G.c
Control+G.k	As 50+G.h	As 100+G.h	Cd 50+G.h	Cd 100+G.k
Control+A.k	As 50+A.k	As 100+A.k	Cd 50+A.k	Cd 100+A.k
Control+All B	As 50+ All B	As 100+All B	Cd 50+All B	Cd 100+All B
Control+All M	As 50+ All M	As 100+All M	Cd 50+All M	Cd 100+All M
Control+ microbial consortim	As50+microbial Consortium	As100+ microbial Consortium	Cd50+ microbial consortium	Cd100+ microbial consortium

(**PS1-PS9** indicates rhizobacterial cultures (1.*Pseudomonas putida*, 2: *Citrobacter freundii*, 3: *Pseudomonas plecoglossicida*, 4: *Pseudomonas fulva*, 5: *Pseudomonas sp*, 6: *Ralstonia insidiosa*, 7: *Enterobacter ludwigii*, 8: *Pseudomonas aeruginosa*, 9: *Cellulosomicrobium funkei.*, **As 50=** arsenic 50 $mgkg^{-1}$, **As 100**= Arsenic 100 $mgkg^{-1}$, **Cd 50**= Cadmium 50 $mgkg^{-1}$, **Cd 100**=cadmium 100 $mgkg^{-1}$, **G.c**= *Glomus claroideum*, **G.h**= *Glomus hoi*, **A.k**= *Acaulospora kintensis*, **All B**= All bacterial cultures, **All M**= All mycorrhizal cultures)

5.11 Determination of Enzymatic activities of plants

Protein estimation and enzyme activities of both plant leaves and roots were analysed and calculated according to the procedure given as under:

5.11.1 Preparation of plant extracts (Roots and leaves of *R. communis* and *C. indica*)

In 3 mL of 100 mM potassium phosphate buffer (pH 7.0), 1 g of plants tissue (leaves and roots) were homogenized using chilled pestle and mortar. Centrifugation of homogenate was done at 15000 rpm at 4 °C for 20 minutes and the resultant (supernatant) was further used as crude enzyme preparation in the estimation of protein content and different enzyme activities, using UV/Vis spectrophotometer.

5.11.2 Estimation of Protein content

The method given by Lowry (1951) was used to measure total soluble protein content in leaves and roots of both plants.

5.11.2.1 Principle

The amino acids, tyrosine and tryptophan present in the protein reduces the components of Folin-Ciocalteu reagent (Phosphomolybdic phosphotungstic) due to which blue colour was developed and also the colour developed by biuret reaction of alkaline cupric tartrate with protein was estimated by Lowry's method.

5.11.2.3 Reagents used

Reagent A-2% Sodium carbonate (Na_2CO_3) in 0.1 N Sodium hydroxide (NaOH)

Reagent B-0.5 % Copper sulphate ($CuSO_4$) in 1% Potassium sodium tartrate ($KNaC_4H_4O_6.4H_2O$)

Reagent C-Reagent A (50 mL) and Reagent B (1 mL) (prepared fresh before using)

Reagent D-FolinCiocalteu reagent

Stock solution- 50 mg of Bovine serum albumin (BSA) dissolved in distilled water (50 mL). Stock standards were diluted to prepare protein working standards.

5.11.2.4 Procedure

In a series of test tubes, an equal amount of standard and sample (0.1 mL) were taken to make final volume 1 mL with distilled water. Distilled water was taken as blank. To each test tube, 5 mL of reagent C was added and allowed to stand for 10 minutes after mixing the solutions properly. Further 0.5 mL of reagent D was added and incubated at room temperature for 30 minutes in dark after proper mixing. After the appearance of blue colour, reading was observed on a spectrophotometer at 660 nm.

5.11.2.5 Calculations

Absorbance *vs* concentration graph was plotted for a standard solution of protein and protein content was estimated from the graph itself. Protein content is expressed in mg g^{-1}fw.

5.12 Enzyme activity

Four oxidoreductase enzymes have been selected for this study *viz.* Catalase, Glutathione reductase, Ascorbate peroxidase and Guaicol peroxidase and their estimation will be done from leaves, stem and roots of the plant according to the standard protocols as under:

5.12.1 Cell-free extracts preparation

Plant material (Roots and leaves) were taken separately, washed with cold distilled water and dabbed dry with filter paper. To achieve maximum extraction of enzymes in leaves and roots, conditions of extraction were standardized with respect to molarity and pH of the buffer. All the steps involved in extraction were carried out at 0-4°C. Maceration of tissue was done in chilled pestle and mortar in 5 mL of 0.1 M phosphate buffer at pH 7.5. Centrifugation of the homogenate was done at 4°C for 20 minutes at 10,000 rpm. After decanting the supernatant it was used as a crude enzyme extract for determining enzyme activity.

5.12.1.1 Catalase (CAT)

The method to determine catalase activity was given by Sinha (1972). 1.0 mL of reaction mixture consists of 0.5 mL of 0.2 M phosphate buffer with pH 7.0, 0.4 mL of 0.2 M hydrogen peroxide and 0.1 mL properly diluted enzyme extract. The reaction mixture was incubated for 5

minutes at 37°C and then followed by addition of 3 mL mixture containing 5 % (w/v) potassium dichromate and glacial acetic acid in ratio 1:3 (v/v). Tubes were heated for 10 minutes in boiling water bath. In one of the test tubes, enzyme extract was added after stopping the reaction and this test tube served as control. The absorbance of the test and control was measured at 570 nm after cooling the tubes. Absorbance of test samples was subtracted from the control sample in order to determine residual H_2O_2 amount in the reaction mixture. The amount of enzyme which catalysis the oxidation of 1 mM of H_2O_2 per minute was defined as one unit of enzyme activity under assay conditions.

5.12.1.1.1 Calculations

Amount of enzyme required to liberate half of the peroxide oxygen from H_2O_2 was described as one unit of enzyme activity.

$$\text{Unitactivity(unit min}^{-1}\text{g}^{-1}\text{ tissue)} = \frac{\text{changein absorbancemin}^{-1} \times \text{totalvolume (mL)}}{\text{Ext. coeff} \times \text{vol. ofsampletaken(mL)} \times \text{wt. ofplanttissue (g)}}$$

Where, Extinction coefficient = 6.93×10^{-3} $mM^{-1}cm^{-1}$

$$\text{Specific activity} = \frac{\text{Unit activity (unit min}^{-1}\text{g}^{-1}\text{ tissue)}}{\text{Protein content (mg g}^{-1}\text{FW)}}$$

5.12.1.2 Ascorbate peroxidase (APX)

Ascorbate peroxidase activity was estimated by the method given by Nakano and Asada (1981). 2.7 mL of reaction mixture contains 2.25 mL of 100 mM phosphate buffer at pH 7.0, 0.2 mL of 0.5 mM ascorbate (ascorbic acid), 0.2 mL of 0.1 mM hydrogen peroxide and 0.05 mL enzyme extract. Initiation of the reaction was done by addition of hydrogen peroxide. Absorbance was measured at 290 nm in a spectrophotometer. Oxidation of ascorbic acid was noted by determining the decrease in absorbance. One unit of enzyme is defined as 1 mole of ascorbic acid oxidized per minute at 290 nm.

5.12.1.2.1 Calculations

Amount of enzyme required to oxidize 1 µM of ascorbatemin^{-1} g^{-1}fw is defined as one unit of enzyme activity.

$$\text{Unit activity (unit min}^{-1}\text{g}^{-1}\text{ tissue)} = \frac{\text{change in absorbance min}^{-1} \times \text{total volume (mL)}}{\text{Ext. coeff} \times \text{vol. ofsample taken (mL)} \times \text{wt. of plant tissue (g)}}$$

Where, extinction coefficient = 2.8 $mM^{-1}cm^{-1}$

$$\text{Specific activity} = \frac{\text{Unit activity (unit min}^{-1}\text{g}^{-1}\text{ tissue)}}{\text{Protein content (mg g}^{-1}\text{FW)}}$$

5.12.1.3 Glutathione reductase (GR)

For measuring enzyme activity method of Halliwell and Foyer (1978) was used. Reaction mixture (2.3 mL) consists of 2 mL of 0.1 M phosphate buffer with pH 7.5, 0.1 mL of 5 mm oxidized glutathione (GSSG), 0.1 mL of 0.2 mM NADPH and 0.1 mL enzyme extract. Due to the oxidation of NADPH decrease in absorbance was measured at 340 nm. Non-enzyme oxidation of NADPH was recorded and subtracted from the value of oxidized NADPH. 1.0 µM of NADPH oxidized per minute is defined as one unit enzyme activity.

5.12.1.3.1 Calculations

Determination of one unit of enzyme activity is the amount of enzyme required to oxidize 1 µm of NADPH $min^{-1}g^{-1}$ FW.

$$\text{Unit activity (unit min}^{-1}\text{g}^{-1}\text{ tissue)} = \frac{\text{change in absorbance min}^{-1} \times \text{total volume (mL)}}{\text{Ext. coeff} \times \text{vol. of sample taken(mL)} \times \text{wt. of plant tissue (g)}}$$

Where, Extinction coefficient = 6.22mM $^{-1}cm^{-1}$

$$\text{Specific activity} = \frac{\text{Unit activity (unit min}^{-1}\text{g}^{-1}\text{ tissue)}}{\text{Protein content (mg g}^{-1}\text{FW)}}$$

5.12.1.4 Guaiacol peroxidise (GPX)

Enzyme activity was measured by minor modifications in the method given by Putter (1971). Reaction mixture consists of 0.05 mL guaiacol solution, 0.03 mL of hydrogen peroxide solution, 3 mL phosphate buffer with pH 7.0 and 1 mL of enzyme extract. The solution was mixed properly and absorbance was noted at 336 nm. Time was noted to increase the absorbance by 0.1. Specific enzyme activity is expressed as µM of hydrogen peroxide reduced min^{-1} (mg protein)$^{-1}$.

5.12.1.4.1 Calculations

Enzyme activity was calculated by the formula givenas:

$$Unit\ activity\ (unit\ min^{-1}g^{-1}\ tissue) = \frac{change\ in\ absorbance\ min^{-1} \times total\ volume\ (ml)}{Ext.coeff \times vol.ofsample\ taken\ (ml) \times wt.of\ plant\ tissue\ (g)}$$

Where, Extinction coefficient = 25.5 $mM^{-1}cm^{-1}$

$$\text{Specific activity} = \frac{\text{Unit activity (unit min}^{-1}\text{g}^{-1}\text{ tissue)}}{\text{Protein content (mg g}^{-1}\text{FW)}}$$

5.13 Stimulation of microbe bioactivity in the root zone (Phytochemical screening)

Plants can stimulate microbe (bacteria and fungi) bioactivity in the root zone (rhizosphere) by excretion of bio-enhancing compounds. The plant-excreted root exudates provide a carbon and

nitrogen source for soil -bacteria. The secondary metabolites secreted by plants mainly includeflavonoids and phenols**,** which are estimated with High-performance thin layer chromatography (HPTLC) techniques. Phytochemical estimation (content of total phenols and flavonoids) is done by the procedure described as under. DPPH scavenging activity was also determined in leaves, stem and roots of *R. communis* and *C. indica* plants.

5.13.1 Preparation of extract

The dried leaves and roots were powdered and extracted with 70% methanol by reflux method. Methanolic extract of all samples was filtered with Whatman filter paper and then evaporated to dryness under vacuum. Final volume makes up was done by addition of 10 mL of methanol and stored at 4°C for further analysis.

5.13.2Determination of Antioxidant and Free Radical Scavenging Properties (DPPH assay)

The radical scavenging activity of selected plants extract against stable DPPH (2, 2-diphenyl-2-picrylhydrazyl hydrate) was determined spectrophotometrically (Farooq and Sehgal. 2017). 1 g of the powdered plant samples of leaves, roots and stem of both the test plants were taken for the antioxidant activity and extracted with methanol (10 mL). The extracts acquired from each of the plant materials were filtered separately and concentrated by vacuum evaporation. When DPPH reacts with an antioxidant compound, it is reduced due to the donation of hydrogen. The solution of DPPH (0.3 mM) was prepared by dissolving 11 mg of DPPH in 50 mL of methanol. The optical density (OD) of DPPH solution was set between 0.8-1 by diluting it with 50 % methanol. Different concentrations of both the plant's parts (leaves, roots and stem) were added separately to 2 mL of DPPH (Mensor *et al*., 2001). After 30 minutes of incubation, the discolouration of the purple to yellow colour was measured at 520 nm. Methanol was taken as blank and 2 mL of DPPH solution was taken as control. The whole experiment was conducted in triplicates (n=3).

5.13.3Total Phenolic content estimation (Folin-Ciocalteu reagent method)

Total phenolic content (TPC) of crude extract of plant parts was determined by Folin-Ciocalteu reagent method (Alhakmani, 2013). A calibration curve was plotted using Gallic acid as the reference standard (20-100µgmL^{-1}). 0.5 mL of leaf extract (100µgmL^{-1}) was mixed with Folin-Ciocalteu reagent (1.5 mL) diluted 1:10 with de-ionized water). To this solution, 3 mL of 7.5 % (w/v) sodium carbonate solution was added for neutralization. The reaction mixture was kept for 30 minutes in dark at irregular shaking condition for the development of colour.

Absorbance was measured at a wavelength of 760 nm using double beam UV-Vis spectrophotometer. The linear regression equation was obtained from the gallic acid standard plot, from which total phenolic content was determined. TPC was expressed as mg/g gallic acid equivalent (GAE) of dry extract and calculated as mean ± SD (n=3).

5.13.4 Total flavonoid content estimation (aluminium chloride calorimetric method)

The method given by Madaan (2011) was used for the determination of total flavonoid content in plant parts using quercetin as the standard for plotting calibration curve. In 80 % ethanol, 10 mg of quercetin was dissolved and diluted to a range of 20, 40, 60, 80 and 100 μgmL^{-1}. In a test tube, 0.5 mL of diluted quercetin standard solution or plant extracts of different concentrations were mixed separately with 1.5 mL of ethanol (95%), 0.1 mL of aluminium chloride (10 %), 0.1 mL of potassium acetate (1 mol/L) and 2.8 mL distilled water. 30 minutes of incubation was given to the test tubes at room temperature to complete the reaction.

With the help of double beam UV-Vis spectrophotometer, absorbance was noted against blank at a wavelength of 415 nm. Total flavonoid content was calculated from the linear regression equation obtained from the calibration curve of quercetin. Flavonoid content was expressed as mg/g of quercetin equivalent (QE) of dry plant extract and calculated as mean ±Sd (n=3).

5.13.5 Qualitative and quantitative estimation of phenols (Gallic acid) and flavonoids (Quercetin) by HPTLC method

For quantitative and qualitative estimation of phenols and flavonoids in plant parts, samples were sent to Punjabi University, Patiala. Dried plant parts (leaves, roots and stem) were extracted with 70% ethanol by using Microwave-assisted extraction (MAE) method (Thakker et. al., 2011).

The F254 HPTLC plates were developed simultaneously in a mobile phase consists of toluene: ethyl acetate: formic acid (4.5: 3: 0.2 v/v/v) at 25 ± 20°C temperature and 30% relative humidity and allowed to travel up to a distance of 8 cm. After development, the plates were air dried and scanned densitometrically at 366 nm for gallic acid and quercetin. The peak areas were recorded. Calibration curves of gallic acid (200-600 nm) and quercetin (200-800 nm) were prepared by plotting peak areas versus concentration.

5.14 Evaluation of the potential of plant–symbiotic rhizobacteria and arbuscular mycorrhiza in association with the microbial consortium in the plants

The third objective includes evaluation of potential plant-symbiotic rhizobacteria and arbuscular mycorrhizal in plants. Different physico-chemical parameters were analysed in order to evaluate the potential of consortium incorporated with plants under heavy metal stress. Plant height, wet weight, dry weight of plant parts along with some photosynthetic parameters (Chlorophyll a, chlorophyll b, Total chlorophyll and carotenoids) content was calculated according to the standard methods cited in the literature.

5.14.1 Determination of physical parameters of plants

From the pot experimentation, the effect of metal treatment (arsenic and cadmium) on the overall growth rate of selected plants was studied. Plants grown in each pot with heavy metal treatment (50 and 100 ppm) were carefully uprooted every 30 days of growth from the time of transplantation. Shoots and roots of plants from each treatment were cleaned thoroughly,

measured in length and weighed separately. Parts of the plant were then oven dried at 60±2°C for 72 hours and weighed again.

5.14.2 Determination of Chlorophyll content

The method given by Makeen (2007) was used to measure total chlorophyll and carotenoid content in plants. Chlorophyll content was measured from tender shoots of plants collected from each treated pot. Chlorophyll a (Chl a) and chlorophyll b (Chl b) were measured at 663 and 645 nm using UV-Vis spectrophotometer. 5 gram of fresh clean leaves were taken and homogenized in 10 mL of extraction solution (10 mL of 0.1 N HH_4OH + 90 mL of acetone). The plant material was carefully ground using pestle and mortar under dark and cold conditions to prepare fin slurry of the tissue sample. The tubes are then kept in the refrigerator for 2 hours and ground again for centrifugation at 5000 rpm for 20 minutes after addition of 80% aqueous acetone. After collecting supernatant dilute it with aqueous acetone to make final volume 20 mL.

Calculations:

Chlorophyll a and b were calculated according to lichtenthaler (2001)

Chlorophyll a (mg/mL) (Chl_a) = 12.25 A663.2 – 2.79 A646.8

Chlorophyll b (mg/mL) (Chl_b) = 21.50 A646.8 – 5.10 A663.2

Where: A646.8 = absorbance at wavelength 646.8

A 663.2 = absorbance at wavelength 663.2

5.14.3 Determination of Total chlorophyll and Carotenoid content

Total chlorophyll content was calculated according to the formula given by Makeen (2007)

$$Chl_{a+b} = (19.54 \times A645) + (8.29 \times A663)$$

From total chlorophyll content, total carotenoid content of plant was calculated using the given formula

$$\text{Carotenoids (mg/mL) } (Chl_{x+c}) = \frac{1000 - A470 - 1.82\ Chla - 85.02\ Chlb}{198}$$

Where A470 = absorbance at wavelength 470 nm

5.15 Evaluating the efficacy of microbial consortium incorporated with selected plants

Phytoremediation capability of all plants in pot experiment was evaluated in vivo. Different parameters were calculated in order to evaluate the efficacy of microbial consortium incorporated with selected plants under different heavy metal treatments.

5.15.1 Heavy metal analysis of soil and plant parts

From each pot, around 5 grams of soil was collected from the rhizosphere region and air dried. Similarly, one plant from each pot was uprooted for executive periods of 30, 60 and 90 days of transplanting. The plants were washed properly with water and air dried for 24 hours and then

oven dried at 60°C. The dried samples of soil and plant were crushed, packed and labelled separately till further analysis. The samples were processed for acid digestion and total metal content with the help of ICP-OES as discussed earlier under metal estimation in soil samples. The same procedure was followed for heavy metals analysis of soil and plant parts (leaves, roots and stem). All sampling and analysis were done in triplicates.

5.15.2 Biological concentration factor (BCF)

Bio-concentration factor was calculated according to Malik *et al* (2010) andYoon *et al* (2006) as metal concentration ratio of plant to soil.

$$\text{BCF} = \frac{\text{metal concentration of plant root (mg/kg)}}{\text{metal concentration of soil (mg/kg)}}$$

5.15.3 Biological accumulation factor (BAF)

Biological accumulation factor was calculated according to the formula given by Malik *et al* (2010) as metal concentration ratio of plant shoot to soil:

$$\text{BAF} = \frac{\text{metal concentration of plant shoot (mg/kg)}}{\text{metal concentration of soil (mg/kg)}}$$

5.15.4 Translocation factor

Translocation factor was calculated according to Cui *et al* (2007) and Bu-Olayan and Thomas (2009) as the ratio of heavy metals in plant shoot to that of the plant root.

$$\text{TF} = \frac{\text{heavy metal in plant shoot (mg/kg)}}{\text{heavy metal in plant root (mg/kg)}}$$

5.15.5 Tolerance index

Tolerance index was calculated according to the formula given by Diwan *et al* (2010)asthe ratio of dry weight of plant with metal treatment to the control plant without any treatment:

$$\text{TI} = \frac{\text{DW of the plant in contaminated soil (mg/kg)}}{\text{DW of the plant in uncontaminated soil (mg/kg)}}$$

5.15.6 Phytoextraction capacity

Finally, the phytoextraction capacity of the plant was calculated by the formula given under

Phytoextraction capacity (PC) = Plant biomass (DW) × Concentration of metal

5.15.7 Statistical analysis

The experiment was conducted in randomised block design taking three triplications of each treatment and the results are expressed as mean±SD. Variability of data and validity of results was confirmed by one-wayanalysis of variance (ANOVA) by SPSS software (version 18). In order to determine the differences among the treatments within respective months between two plants applied with different concentrations of heavy metals, Turkeys test was applied. If the p-value ≤ 0.05, results will be considered statistically significant.

CHAPTER 6

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

The first objective of the study was: To assess, identify and multiply Rhizobacteria and AM fungi associated with plants around industrial effluent polluted sites of Jalandhar (Punjab), which was completed with the following results.

6.1 Study area

Punjab is the sixteenth largest state of India in terms of population and comprises 22 districts and a total of 237 cities. Amongst which Mohali, Ludhiana, Amritsar, Patiala and Jalandhar are the major cities where Ludhiana being the largest and Jalandhar is the oldest city in Punjab. In recent years, Jalandhar has experienced a rapid urbanization and developed high industrialization areas or centres. While considering the aspect of industrial pollution in Punjab, Ludhiana lies on top of the list followed by several others along with Jalandhar and other cities. Major industries that are present in Jalandhar are leather, hand tools, sports, pipe fitting etc. The untreated effluent and waste produced from these industries are disposed off into the drains that carry domestic waste and then treated by the Effluent treatment plants (ETPs) or either released directly into the nearby land. In Jalandhar, the major focus was on the Industrial area, a focal point to study the effect of contaminants on plantation around that area.

6.2 Site description and sampling

Four sites namely, Site 1, Site 2, Site 3 and Site 4 of the focal point, Jalandhar were selected (figure 4) after proper assessment of the area on the basis of addition of major contaminants to nearby land.

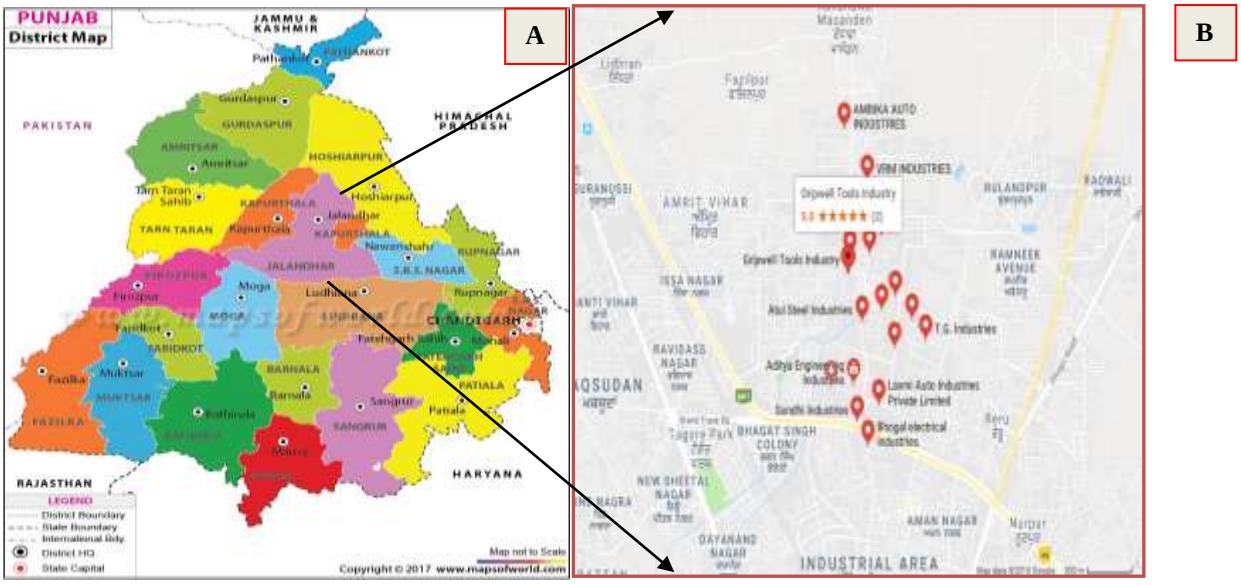


Figure 4: Map showing (A) Geographical region of Punjab. (B) The industrial area of Jalandhar with some major large and small scale industries**.**

Soil samples were collected in triplicates by using quadrate method (Cox, 1990) from each site (figure 4) along with the roots of the plants found in that area. Samples were collected in airtight Ziplock bags and stored at 4°C for other analysis and experimentations.

6.3 Physico-chemical analysis of soil samples from different sites

Due to continuous disposal of untreated effluent into nearby land by some of the small-scale industries and usage of the same land for agriculture purpose, has been considered as a major threat. In order to detect the presence of contaminants in soil nearby these industries, physico-chemical characterization is required prior to any experimentation and conclusion. Hence, physico-chemical characterization of soil samples of all the sites was carried out and shown in table 6. It was observed from the physico-chemical analysis that soil samples from different sites were almost acidic while some samples were near to neutral values of pH.

Samples were collected in triplicates from all sites and marked as site 1, 1a and 1b, 2, 2a, 2b, 3, 3a, 3b and 4, 4a, 4b. The analysis shows that the sites are almost acidic when the mean was calculated. Site 3 was found to be basic in nature, followed by site 4, 1 and 2 with mean pH values of 5.23, 4.67, 5.31 and 4.36 respectively. Total potassium and sodium content were found to be less in contaminated sites as compared to normal field soil but in contrast total, available phosphorus content was significantly less than the content of potassium and sodium in contaminated sites than normal soil. Available phosphorus content was different among all the four sites and was reported to be least in site 4. Also, available potassium content in soil samples from the 1^{st} and 2^{nd} site was almost similar but slightly different from 3^{rd} and 4^{th} site but sodium content was found to be significantly same in almost all the four sites.

One of the most concerned factors that command the conversion of metals from immobile-solid phase to more easily bioavailable forms is pH of the soil (Zhao *et al*., 2012). It is presumed that at higher pH values, less trace metal availability is detected. Apart from metal bioavailability, soil pH also affects the process of metal uptake into roots though this is metal specific (Brown *et al*., 1995). Some features like the formation of rocks, the composition of soil, organic matter and other physical and chemical properties of soil affect the bioavailability of metals (Lokeshwari *et al*., 2006). Generally, the metal interaction chemistry with a matrix of soil is intermediate to the concept of phytoremediation.

Table 6: Physico-chemical analysis of soil samples

Sampling sites	pH	Mean pH value	Available K ($mgkg^{-1}$)	Mean K values	Available P ($mgkg^{-1}$)	Mean P values	Available Na ($mgkg^{-1}$)	Mean Na values
Control	6.91	6.99± 0.075^{c}	28.9	33.73± 5.488^{h}	9.69	9.63± 0.273^{e}	82.7	85.7± 2.690^{k}
	7.03		32.6		9.88		87.9	
	7.05		39.7		9.34		86.5	
Site 1	5.64	5.23± 0.944^{b}	16.5	17.8± 1.153^{f}	6.85	6.59± 0.325^{c}	73.9	70.8± 3.655^{j}
1 a	4.15		18.7		6.71		66.78	
1 b	5.9		18.2		6.23		71.78	
Site 2	3.45	4.67± 1.161^{a}	17.8	18.26± 0.450^{g}	8.52	8.29± 0.252^{e}	69.31	69.6± 3.117^{i}
2 a	4.82		18.3		8.02		66.78	
2 b	5.76		18.7		8.33		72.98	
Site 3	4.92	5.31± 1.431^{b}	16.4	17.1± 0.711^{f}	7.11	7.26± 0.171^{d}	71.57	69.9± 1.824^{i}
3 a	4.12		17.8		7.45		67.98	
3 b	6.9		17.1		7.24		70.34	
Site 4	4.54	4.36± 1.051^{a}	18.1	18.46± 0.907^{g}	5.34	5.37± 0.176^{b}	76.42	72.9± 3.056^{j}
4 a	5.31		19.5		5.56		71.98	
4 b	3.23		17.8		5.21		70.56	

Mean ±SD (n=3) Different alphabets indicates statistically significant difference at P≤0.05 by Turkey's test

6.4 Heavy metal analysis of soil samples

The soil sample was collected in triplicates from the polluted sites and analysed for the presence of heavy metals by ICP-OES. The soil was primarily contaminated with arsenic and cadmium with elevated levels of other metals like cobalt, nickel and lead in contrast to the standard given by Bureau of Indian Standards (BIS). It is well established that contamination caused by heavy metals causes dynamic stress in the environment as well as plants, due to which human beings and animals are at prime risk.

Therefore the present work revealed that heavy metals that were found in the soils contaminated with industrial effluents and were selected for the study, were above the permissible limits prescribed by Bureau of Indian Standards (1983) as shown in table 7. Arsenic was found to be more in all the four selected sites with a concentration of 21.4, 28.11, 21.12 and 20.34 $mgkg^{-1}$ in site 1, site2, site 3 and site 4 respectively. Whereas, cadmium was found to be less compared to arsenic but more than BIS standards, values of 7.25, 7.13, 6.21 and 6.15 $mgkg^{-1}$ of cadmium were found in site 1, site 2, site 3, and site 4 respectively. Heavy metal concentration in soil was found in order: Cr < Cu < Pb < Ni < Co < Cd < As. Therefore, two metals were selected for the present study to check the effectiveness of native wild plant species for phytoremediation potential i.e arsenic and cadmium.

Table 7: Heavy metal analysis of soil sample (ICP-OES) from four different sites compared to normal soil and BIS standards.

Sr. No	Heavy metals	Concentration in polluted soil ($mgkg^{-1}$)				Normal soil ($mgkg^{-1}$)	BIS standards ($mgkg^{-1}$)
		Site 1	Site 2	Site 3	Site 4		
1.	**Arsenic**	**21.14± 1.123 ^{d}**	**28.11± 0.876^{d}**	**21.12± 1.372^{d}**	**20.34± 0.987^{d}**	4.616±0.6783	**20**
2.	Cobalt	47.11± 0.678 ^{f}	52.93± 0.897^{g}	36.34± 1.971^{e}	45.12± 1.782^{f}	0.008±0.367	25-50
3.	Calcium	470.3± 0.987 ^{n}	311.1± 0.456^{m}	639.7± 1.253^{p}	580.2± 2.678^{o}	377.7±1.673	200-400
4.	**Cadmium**	**7.25 ± 0.771^{b}**	**7.13± 0.788^{b}**	**6.21± 1.253^{a}**	**6.15± 1.444^{a}**	0.311±1.378	**3-6**
5.	Chromium	9.299± 1.342^{c}	24.34± 2.313^{d}	53.79± 1.362^{g}	41.41± 1.367^{f}	0.051±1.234	75-100
6.	Copper	91.12± 1.234^{j}	70.79± 1.562^{h}	81.73± 0.562^{i}	58.87± 0.578^{g}	2.004±0.456	135-270
7.	Iron	39.28± 0.678^{e}	79.77± 1.789^{h}	85.95± 0.678^{i}	88.75± 0.567^{i}	16.52±0.466	175-150
8.	Lead	50.11± 1.278^{g}	104.1± 1.452^{k}	145.3± 1.457^{k}	85.67± 0.688^{i}	2.243±0.567	250-500
9.	Magnesium	55.71± 0.678^{g}	22.54± 0.234^{d}	106.6± 0.566^{k}	75.34± 1.546^{h}	126.8±1.567	NA
10.	Manganese	111.4± 1.789^{k}	107.6± 1.368^{k}	101.3± 1.478^{k}	150.6± 0.688^{k}	1.518±1.467	1500-3000
11.	Sodium	220.1± 1.577^{l}	78.21± 1.366^{h}	74.72± 1.378^{h}	350.5± 0.577^{m}	80.79±1.672	NA
12.	Nickel	33.54± 0.578^{e}	100.4± 0.346^{k}	90.13± 0.234^{j}	145.7± 1.245^{k}	0.253±1.463	75-150

Mean ± SD (n=3) Different alphabets indicates statistically significant difference at $P \leq 0.05$ by Turkey's test

6.5 Community analysis and selection of plants (natural population grew in contaminated soil)

Several environmental features like climate, radiations, water content, acidity, aeration and availability of nutrients are responsible for the community of plants found in the contaminated area. This results in weakening of ecosystem which ultimately results in extinction of some plant species from that particular area. Elevated levels of heavy metals in soil had exhibited an adverse effect on the native communities of plants present in contaminated areas. Therefore, in this study, all the sites were analysed for the type of vegetation found in that area. *Saccharum spontaneum, Brassica juncea, Canna indica, Ricinus communis, Solanum nigrum, Parthenium hysterophorus, Arabidopsis thaliana* and *Eichhornia crassipes* (aquatic plant) are some of the plants which were common on all four sites.

Based on the mycorrhizal association and large biomass production, two species were selected for further experimentation viz, *Canna indica* and *Ricinus communis.* According to a case study on native plant species occurring in the industrially polluted area in Bhopal, including *Datura inoxia, Calotropis procera, R. communis* and *Lanta camara* (Waoo*et al*., 2014). Another study revealed the adaptation of 18 different plant varieties in the heavy metal polluted soil. Some of

them were selected for the effective heavy metal removal: *Bidens bipinnata, Artemisia dubia, Polygonum lapatuifolium, Chenopodium ambrosiodes* and *Solanum nigrum* (Anjum*et al*., 2011).

6.6 Mycorrhizal fungus isolation by wet sieving and decanting method (Gerdemann and Nicolson, 1963) and sucrose density gradient centrifugation method (Ohms, 1957).

After an extensive survey of polluted sites and native wild species of plants found in the focal point, industrial area of Jalandhar, four different sites were selected. Soil and root samples were collected from all these sites and were used for mycorrhizal spore and rhizobacterial isolation. Spores of the mycorrhizal fungus were isolated by two methods: Wet-sieving and Decanting method and Sucrose density gradient centrifugation. Most of the spores were found to be *Glomus* and *Acaulospora* according to their morphological characteristics given in Manual of Schenk and Perez (1990). Some of the spores isolated by both the methods are shown in figure 4 and 5 after mounting in polyvinyl lactic acid glycerol (PVLG).

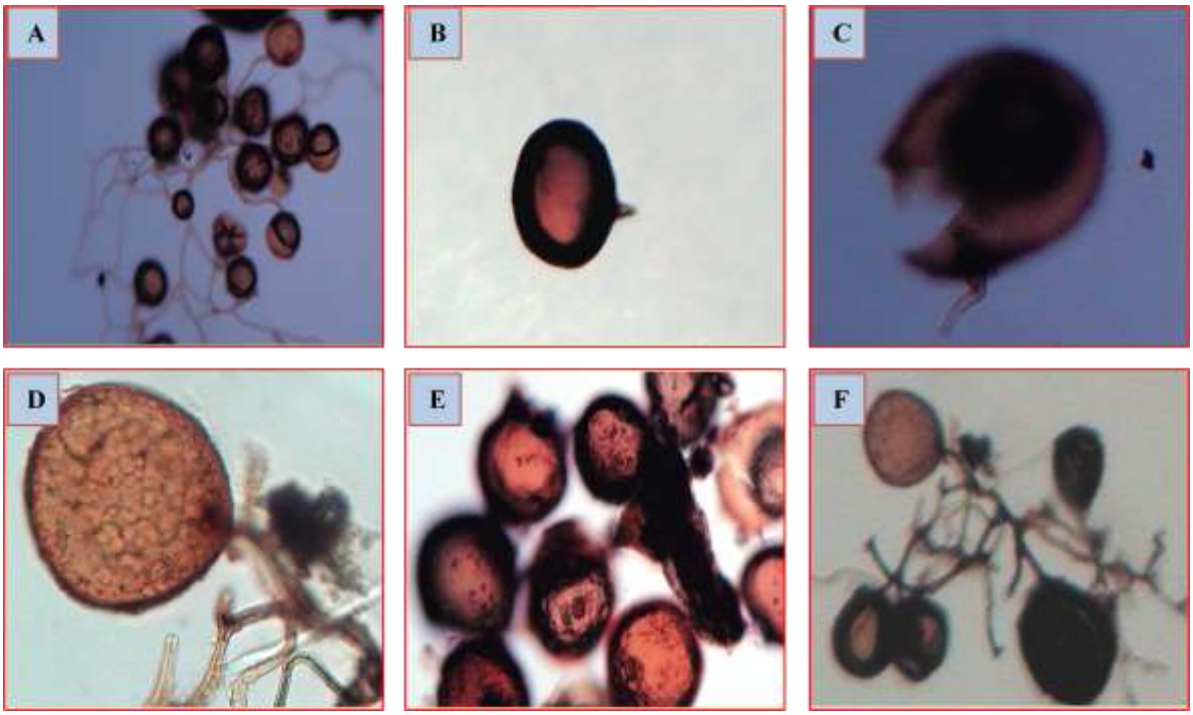


Figure 5: Mycorrhiza spores isolated by wet sieving and decanting and sucrose density gradient centrifugation methods: showing different colonies and group of spores.

(**A**: group of spores, **B**: *Glomus* sp.viewed at 10 X**, C:***Glomus* sp. at 40X, **D:** Group of *Glomus* sp.with hyphal attachment, **E**: Group of different spores, **F:** Group of *Glomus* at 10 X)

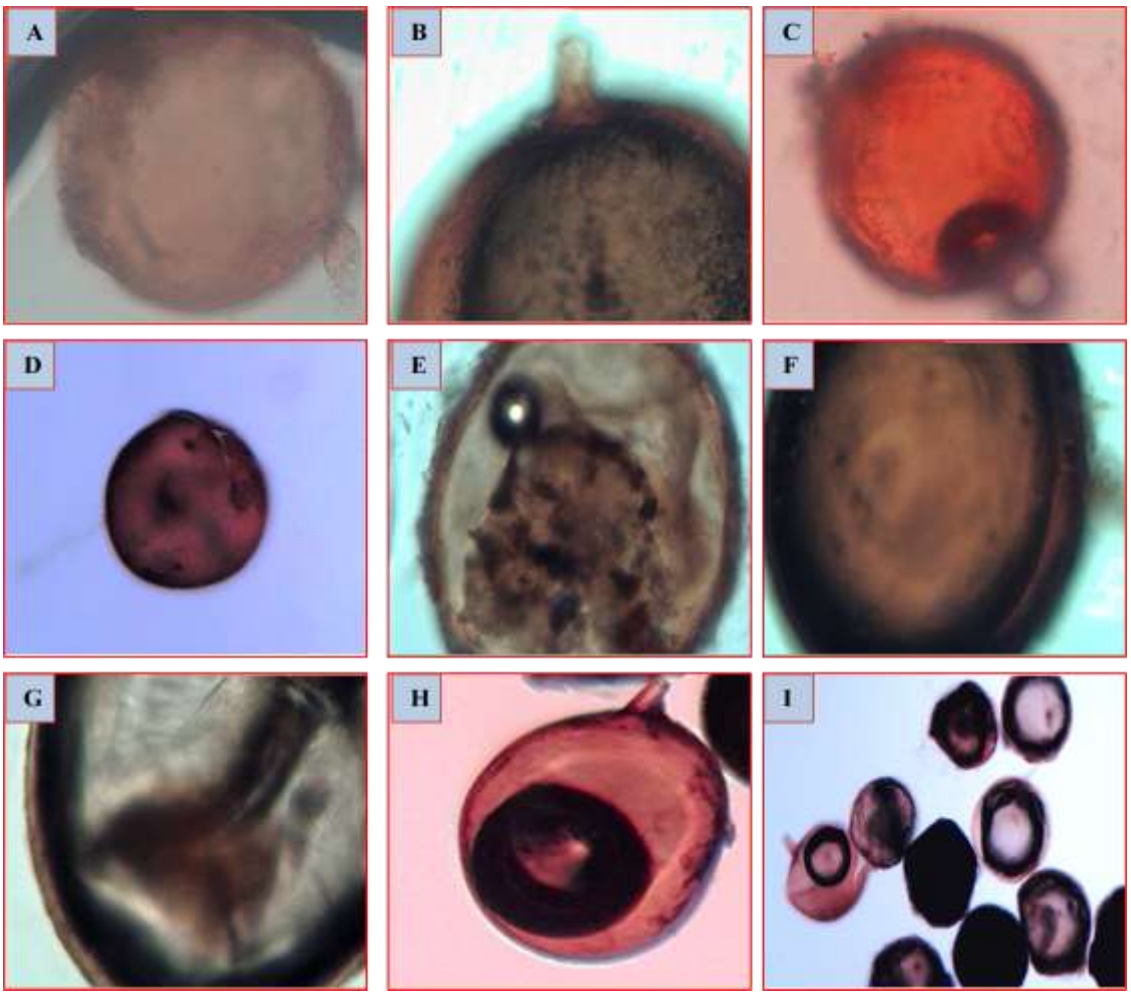


Figure 6: *Glomus* and *Acaulospora* spores viewed under 10X and 40X after staining with PVLG.

(**A-C**: *Glomus* sp with hyphae at 40 X, **D**: *Acaulospora sp* at 10 X.**E-F**: *Acaulospora sp* at 40 X, **G**: *Acaulospora* sp. showing inner morphology, **H**:*Glomus* with hyphae at 10X, **I:** Group of different spores viewed at 10X).

6.7 Mycorrhizal quantification and percentage root colonization

After isolation of AM spores from the selected sites, soil samples were analysed to check mycorrhizal status and biodiversity of spores. Root colonization in mycorrhizal was noticed in respect of vesicles, arbuscules and mycelium. Throughout the study on root samples, divergent mycelium with different shapes and vesicles with almost round, oval and elongated shapes were observed. The wild species of plants found in the selected sites showed overall 52% of root colonization. Where maximum percent colonization was found in site 3 (64.66 %) followed by site 2 (54.33 %) site 1 (44.33%) and site 4 (43.66%). Apart from the calculating percent root colonization, AM spore population for each site was studied. From each site, 2-4 plants were selected for this quantification where 100 g of rhizospheric soil from each plant was processed and a number of AM spores in each soil sample were observed (table 5). The overall number of

spores ranged from 44-95 spores per 100 g of soil. Highest spore count was found in plant species found in site 3 (88.33) followed by site 4, site 4 (57), site 1 (52.33) and site 2 (45).

In present work mainly 2 genera of AM fungi were dominantly found viz: *Glomus sp.* and *Acaulospora sp*. The association between percentage colonization and sporulation alters with different species, nutrient contents of soil and host plant. In this study, a low level of AMF colonization and an average number of sporulation was noted. This may be due to deficiency of fungal biomass and poor root development. Therefore, for further experimentation, three species of AMF were selected *viz: Glomus hoi, Glomus claroideum* and *Acaulospora kentinensis.* These AM fungal strains were procured in large quantities from Centre of Mycorrhizal Research (CMR), The Energy and Resource Institute (TERI), New Delhi.

Our results were found to be in accordance with the investigation carried out by Hassan *et al* (2011) to assess the AMF number in soils and harvested plant roots. Their findings reported the presence of various *Glomus, Scutellospora* species in unpolluted soils. Whereas, *G. intraradices* and *G. viscosum* were found in both polluted and unpolluted soils, but *G. mosseae* and *Glomus* sp.were dominant in trace metal polluted soils. The predominance of *Glomus* sp.in metal polluted soil proposes the heavy metal stress tolerance ability of this species. Similarly, *G. claroideum* isolated from the soil receiving the sludge contaminated with heavy metals showed greater ability to be adapted to the soil contaminated with heavy metals (Del *et al*., 1999). Also, Channasava *et al* (2013) isolated sixteen different AMF species from the mine areas of Yallapur, including *Glomus*, *Acaulospora*, *Gigaspora* and *Scutellospora* species. Lakshman *et al* (2013) reported the presence of *Glomus sp*. in abundance in tropical and mined spoils as compared to other AM fungal species.Therefore in our study, *Glomus* and *Acaulospra* species were predominantly found in association with all the wild species found in all four sites. Vesicles and arbuscules were found in almost all the root samples that were analysed (figure 6).

Table 8: Arbuscular mycorrhizal spore quantification and percent root colonization in selected sites of Jalandhar

Site description	AM spore population per 100 g of soil	Percentage root colonization	Mean values of spore population	Mean value of % root colonization
Site 1	52	48	52.33 ± 8.50^{b}	44.33 ± 12.8^{a}
1 a	61	55		
1 b	44	30		
Site 2	44	45	45 ± 6.55^{a}	54.33 ± 8.14^{b}
2 a	39	58		
2 b	52	60		
Site 3	80	55	88.33 ± 7.63^{c}	64.66 ± 8.38^{c}
3 a	90	69		
3 b	95	70		
Site 4	54	41	57 ± 9.84^{b}	43.66 ± 10.2^{a}
4 a	49	35		
4 b	68	55		

Mean ± SD (n=3) Different alphabets indicates statistically significant difference at $P \leq 0.05$ by Turkey's test

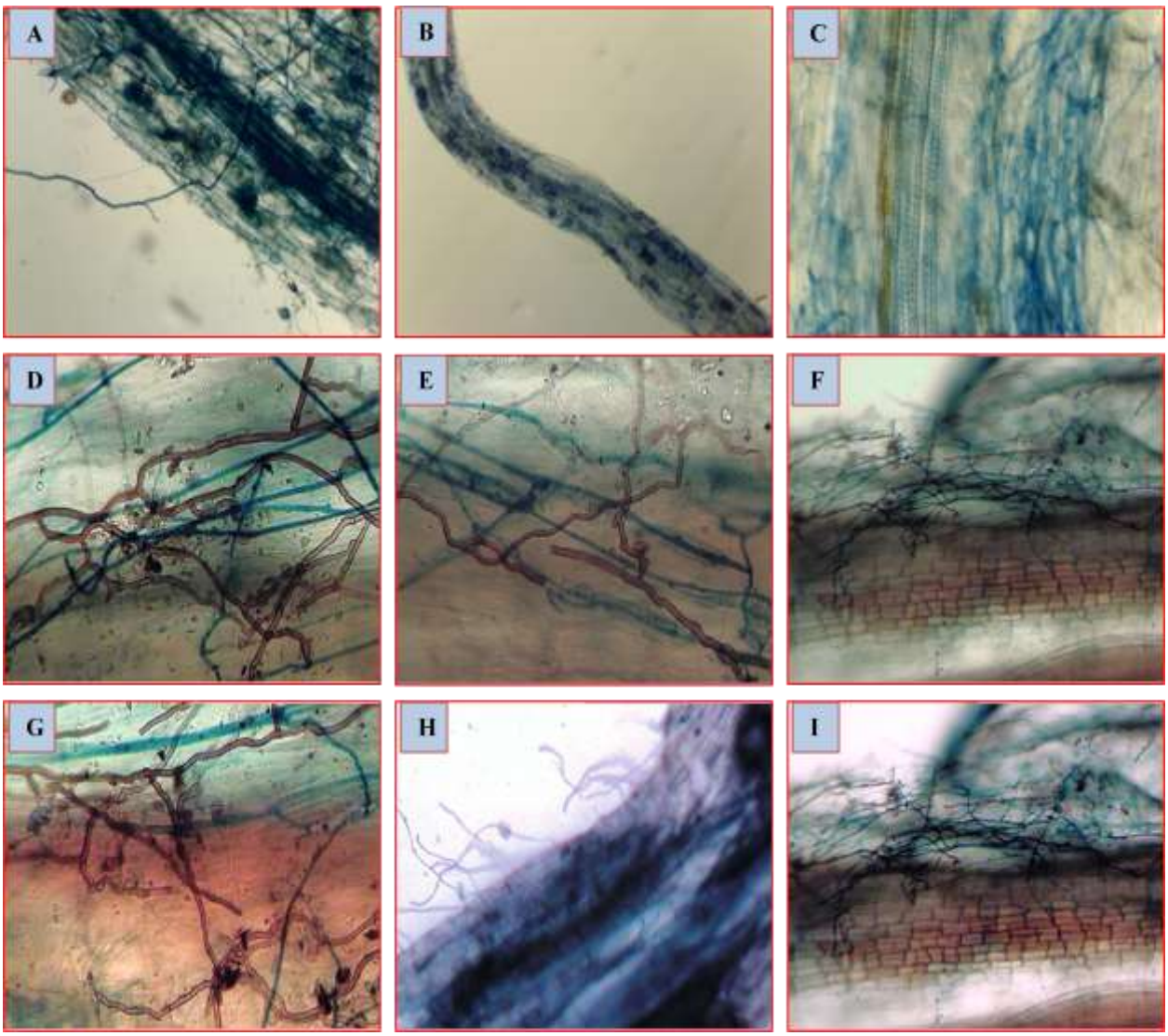


Figure 7: Association of *mycorrhiza* with roots of plants found in polluted sites (A: Root colonization by *Mycorrhiza*, B: Detached vesicles, C: Arbuscule, D: Fungal hypha in the cortex of root, E-I: Root colonization by fungal hypha after staining with trypan blue).

6.8 Multiplication of mycorrhiza with *Sorghum bicolor* in pots

The cultures of mycorrhiza were further inoculated with a host plant *viz*, *Sorghum bicolor* for mass multiplication, as a large quantity of AM culture was required for inoculation with two test plants. For multiplication purpose, each pot was sown with 8-9 seeds of *S. bicolor* along with all the three AM cultures (8 pots for each AM species) separately (figure 8). This experiment was conducted for approximately 3 months. In 1st and 3rd month AM quantification was done which shows that as compared to 1st month, 3rd-month pots have a higher amount of AM spores in each pot. The mean spore population in pots with *G. hoi* was 82±4.312, *G. claroideum* was 89±6.241 and A. *kentinensis* was 92±5.261. Further, these pots with cultures were stored for final experimentation with heavy metals and consortium inoculated with test plants (*R. communis* and *C. indica*).

Figure 8: Pot experimentation for the multiplication of mycorrhizal spores isolated from the soil collected from polluted sites along with cultures procured from CMR, with *Sorghum bicolor* plants.

6.9 Isolation of rhizobacteria (Serial dilution and Media enrichment method)

A total of 32 bacteria were isolated from all four selected sites, out of which only 9 were preferred for microbial consortium on the basis of minimum inhibitory concentration and synergistic activity. Two methods were used for isolation of bacteria i.e Serial dilution followed by media enrichment. In the later method, heavy metals with different concentrations were added to the liquid media (broth) and cultures were further purified on solid media (Nutrient agar). Some of the purified cultures from site 1, site 2, site 3 and site 4 are shown in figure 9, figure 10, figure 11 and figure 12 respectively.

Site 1

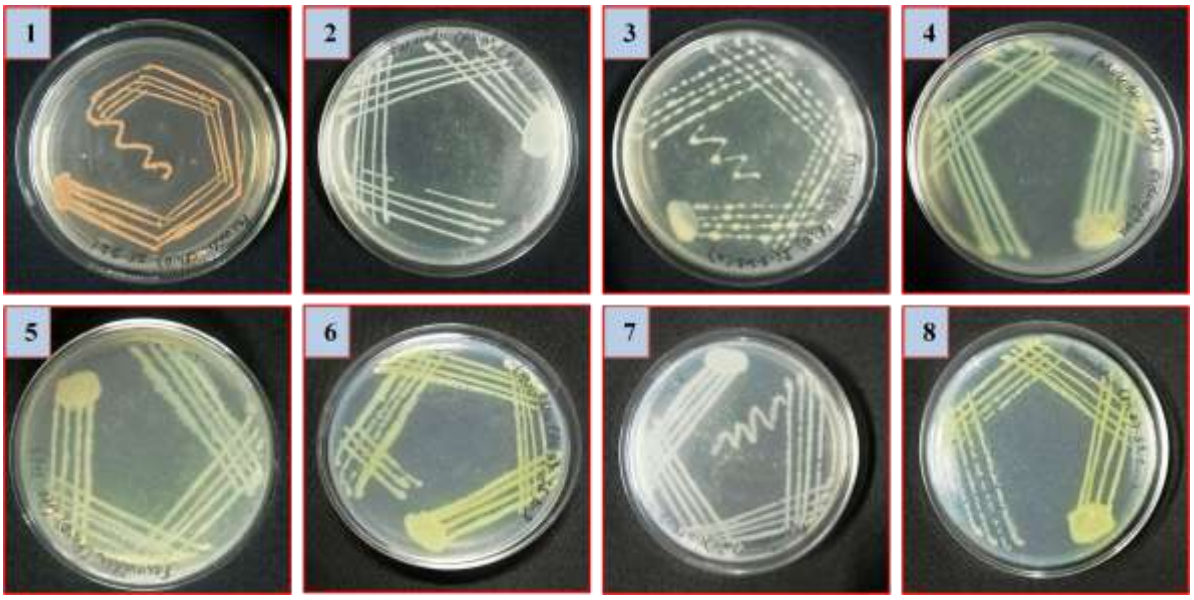


Figure 9: Pure cultures of the rhizobacterial isolates isolated from industrially polluted soil samples around the vicinity of the focal point, Industrial Area, Jalandhar, Punjab. (**Site 1**)

Site 2

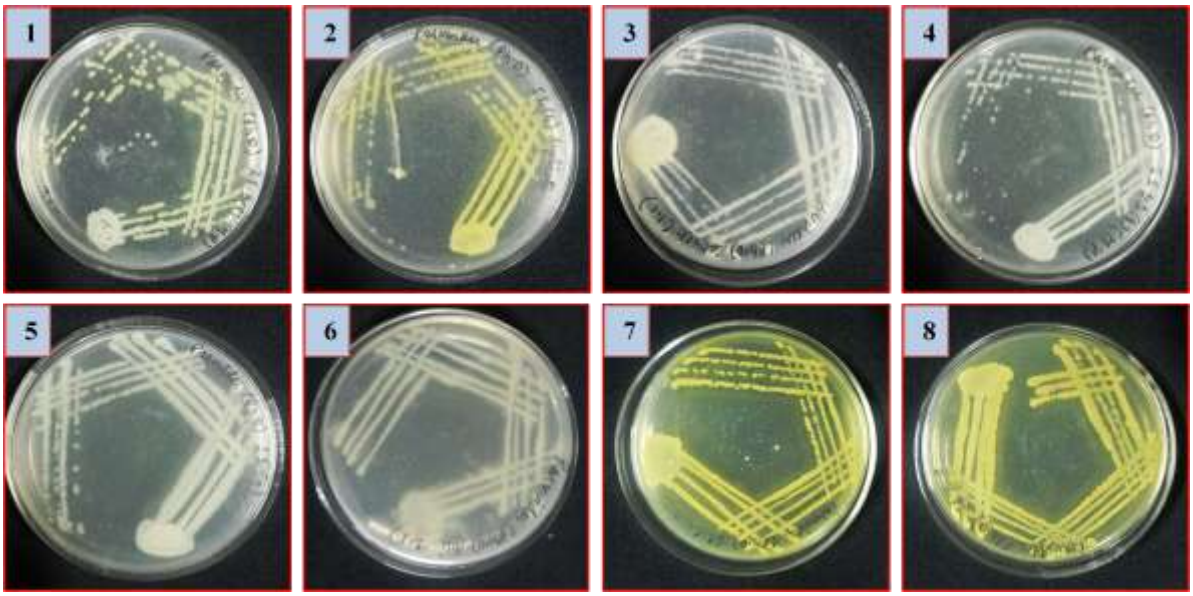


Figure 10: Pure cultures of the rhizobacterial isolates isolated from industrially polluted soil samples around the vicinity of the focal point, Industrial Area, Jalandhar, Punjab. (**Site 2**)

Site 3

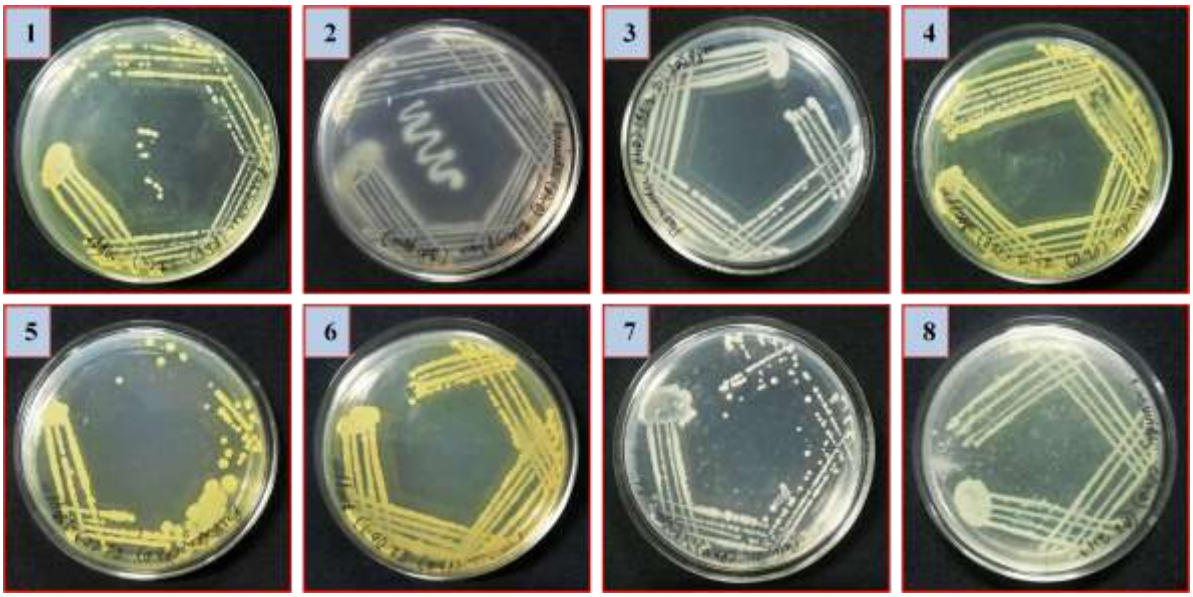


Figure 11: Pure cultures of the rhizobacterial isolates isolated from industrially polluted soil samples around the vicinity of the focal point, Industrial Area, Jalandhar, Punjab. (**Site 3**)

Site 4

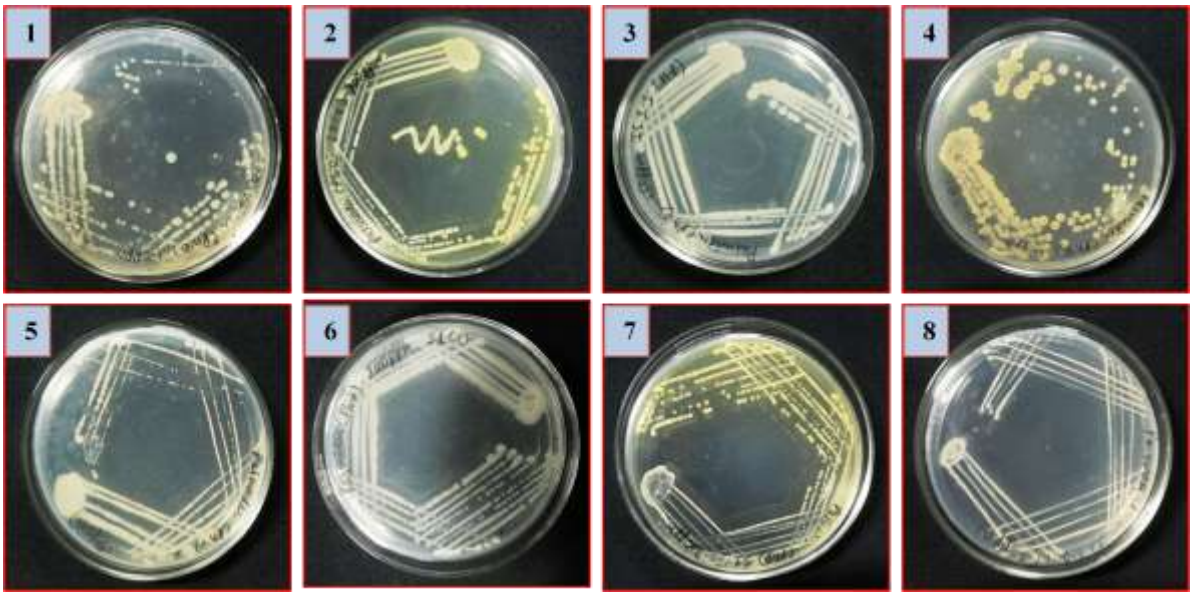


Figure 12: Pure cultures of the rhizobacterial isolates isolated from industrially polluted soil samples around the vicinity of the focal point, Industrial Area, Jalandhar, Punjab. (**Site 4**)

6.10 Minimum inhibitory concentration test (MIC)

Minimum inhibitory concentration testwas performed for all rhizobacterial species against two heavy metals (arsenic and cadmium) at different concentrations (250, 500, 1000 & 2000 mgL^{-1}) which resulted inthe production of the inhibitory zone. Out of all 32bacterial isolates, only nine bacteria have not shown any inhibition against heavy metals and those bacteria were selected for consortium i.e selection was based on the degree of resistance to heavy metals (As and Cd). The selected bacterial cultures are shown in figure 13.

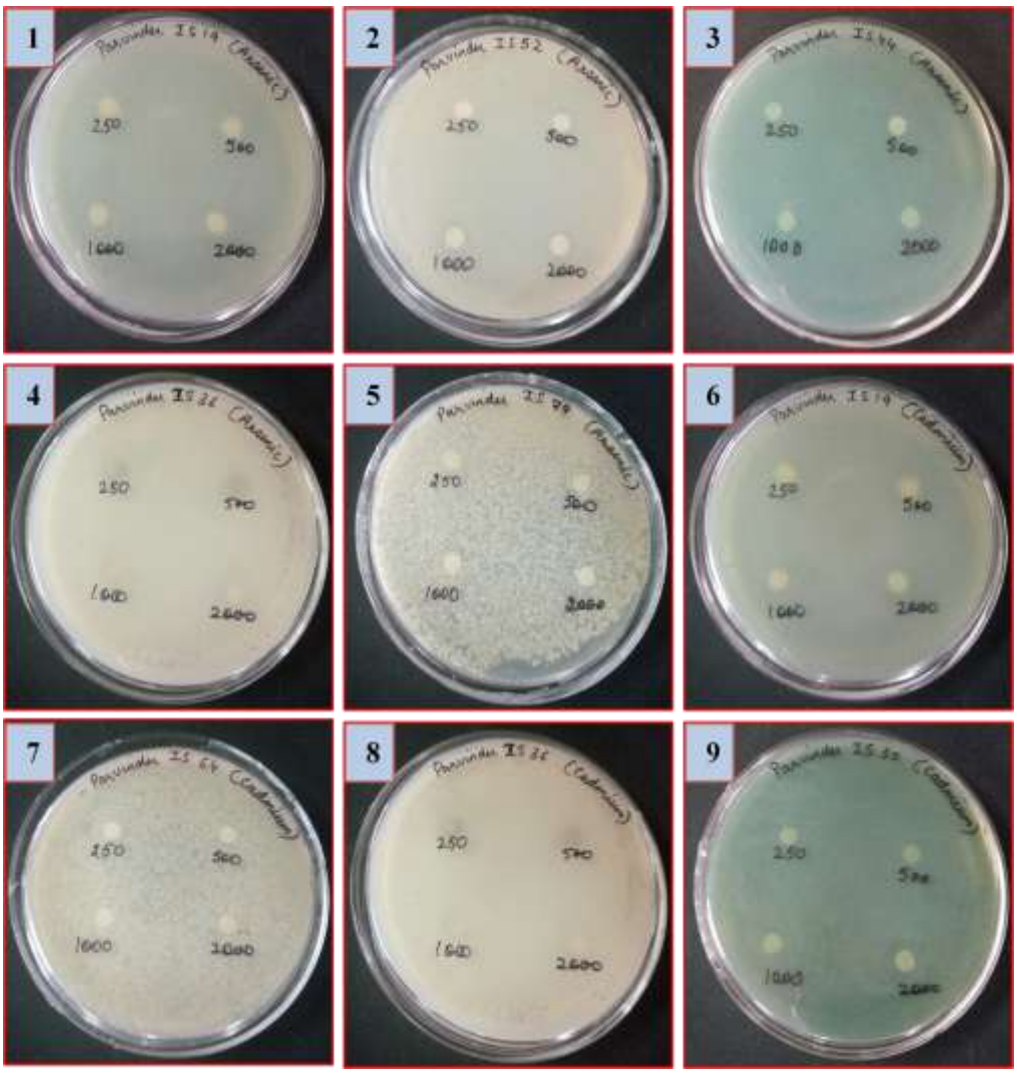


Figure 13: Selected rhizobacteria for microbial consortium that resisted highest concentration of As and Cd (2000 $mgkg^{-1}$) (1: *Pseudomonas putida*, 2: *Citrobacter freundii*, 3: *Pseudomonas plecoglossicida*, 4: *Pseudomonas fulva*, 5: *Pseudomonas sp*, 6: *Ralstonia insidiosa*, 7: *Enterobacter ludwigii*, 8: *Pseudomonas aeruginosa*, 9: *Cellulosomicrobium funkei).*

6.11 Identification of selected rhizobacterial strains

The major step after selection of bacteria was to identify and characterize them, for this 16SrRNA sequencing is required. Identification based on 16S rRNA gene sequencing reveals that the all bacterial isolates belong to genus *Pseudomonas, Citrobacter, Ralstonia, Enterobacter and cellulosomicrobium* and the sequences are deposited in GeneBank under specific accession numbers shown in table 9.

Table 9: Identification of isolated rhizobacteria based on 16s rRNA sequencing with accession numbers deposited in NCBI database (GenBank).

Isolates	Isolates code	Molecular resemblance	Accession no.
1	HX 1	*Pseudomonas putida* strainHX1	MF782681
2	NAP 1	*Citrobacter freundii* strain NAP 1	MF782682
3	TL 1	*Pseudomonas plecoglossicida* strain TL 1	MF782683
4	XL 1	*Pseudomonas fulva* strain XL 1	MF782684
5	BZ 1	*Pseudomonas sp.*strain BZ 1	MF782685
6	PS 1	*Ralstonia insidiosa* strain PS 1	MF828438
7	PS 2	*Enterobacter ludwigii* strain PS 2	MF828439
8	PS 3	*Pseudomonas aeruginosa* strain PS 3	MF828440
9	PS 4	*Cellulosomicrobium funkei* strain PS 4	MF828441

6.12 Synergistic activity (compatibility test) of selected rhizobacterial strains

Synergistic activity of selected rhizobacterial strains was performed according to the method described by Nikam *et al* (2007) with slight modifications as discussed in the material and method section. After observing the growth of all cultures which have not shown any inhibition zone it was clear that all the selected rhizobacteria are not competitors but are compatible with each other and hence can be used as a good microbial consortium against heavy metals Figure 14-22.

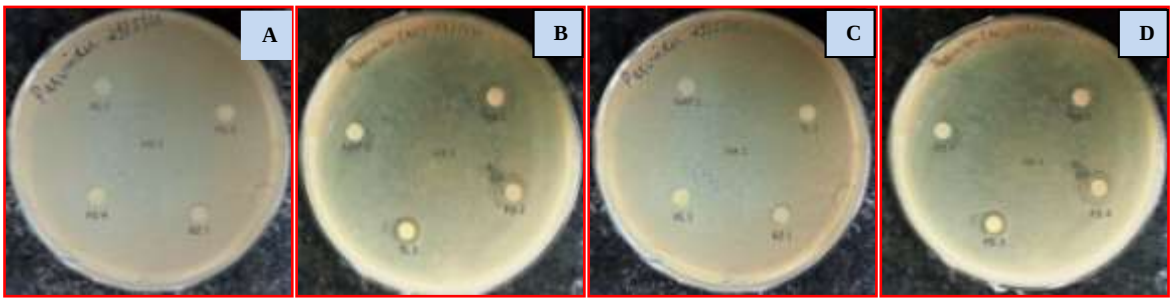


Figure 14: Synergistic activity between all rhizobacterial strains with *Pseudomonas putida strain* HX1by disc diffusion method (where, (**A**) XL1: *Pseudomonas fulva* PS3: *Pseudomonas aeruginosa,* PS4:*Cellulosomicrobium funkei,* BZ1: *Pseudomonas* sp., HX1: *Pseudomonas putida* (**B**) NAP1: *Citrobacter freundii,* PS1: *Ralstonia insidiosa*,PS2:*Enterobacter ludwigii*,TL1: *Pseudomonas plecoglossicida,* HX1: *Pseudomonas putida* **(C)**: NAP1: *Citrobacter freundii*, TL1: *Pseudomonas plecoglossicida,* BZ1: *Pseudomonas* sp., XL1: *Pseudomonas fulva*, HX1: *Pseudomonas putida* **(D)**: PS1: *Ralstonia insidiosa*,PS2:*Enterobacter ludwigii,* PS3: *Pseudomonas aeruginosa,* PS4:*Cellulosomicrobium funkei,* HX1: *Pseudomonas putida*).

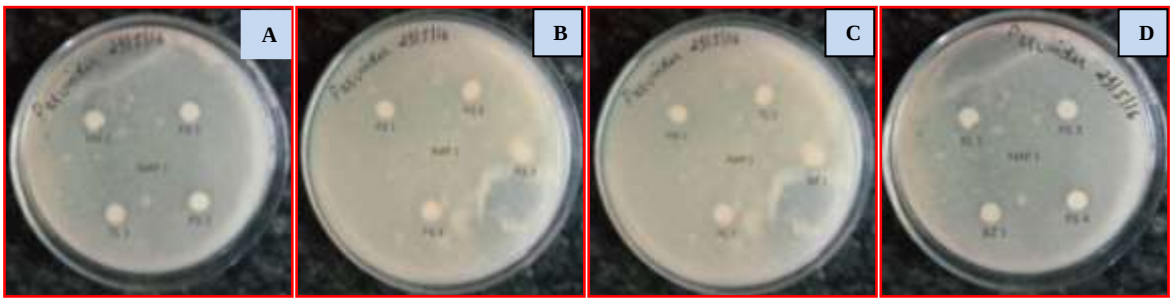


Figure 15: Synergistic activity between all rhizobacterial strains with *Citrobacter freundii*strain NAP1 by disc diffusion method (where, **(A)** HX1:*Pseudomonas putida*, PS1: *Ralstonia insidiosa*, PS2:*Enterobacter ludwigii,* TL1: *Pseudomonas plecoglossicida*, NAP1:*Citrobacter freundii* **(B):** NAP1: *Citrobacter freundii,* PS1:*Ralstonia insidiosa*, PS2:*Enterobacter ludwigii,* PS3: *Pseudomonas aeruginosa*,PS4:*Cellulosomicrobium funkei*, **(C)**:HX1: *Pseudomonas putida*, TL1: *Pseudomonas plecoglossicida*,BZ1: *Pseudomonas* sp**.,** XL1: *Pseudomonas fulva,* NAP1:

Citrobacter freundii (**D):** XL1: *Pseudomonas fulva*, PS3: *Pseudomonas aeruginosa*,PS4:*Cellulosomicrobium funkei*, BZ1: *Pseudomonas* sp**.,** NAP1: *Citrobacter freundii*).

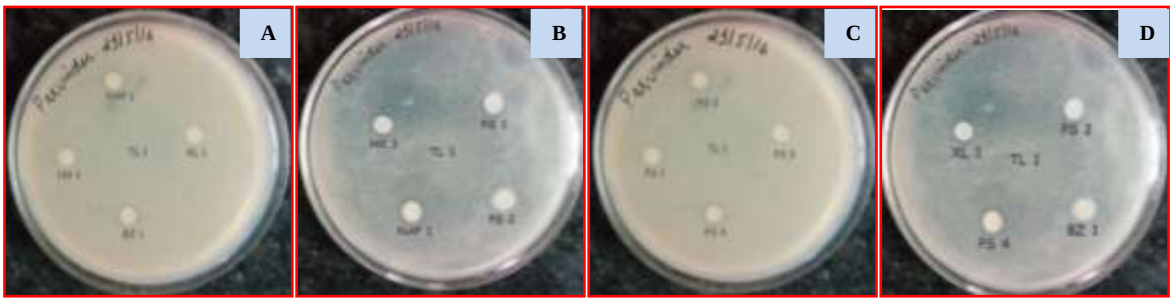


Figure 16: Synergistic activity between all rhizobacterial strains with *Pseudomonas plecoglossicida*strain TL1 with disc diffusion method (where,(**A)** HX1:*Pseudomonas putida*, NAP1: *Citrobacter freundii*,TL1: *Pseudomonas plecoglossicida*, XL1: *Pseudomonas fulva*, BZ1: *Pseudomonas* sp. **(B)** PS1: *Ralstonia insidiosa*, PS2:*Enterobacter ludwigii*, NAP1: *Citrobacter freundii*, HX1:*Pseudomonas putida*, TL1: *Pseudomonas plecoglossicida* **(C**) PS1: *Ralstonia insidiosa*, PS2:*Enterobacter ludwigii*, PS3: *Pseudomonas aeruginosa*, PS4:*Cellulosomicrobium funkei*,TL1:*Pseudomonas plecoglossicida* **(D**) TL1: *Pseudomonas plecoglossicida*, BZ1: *Pseudomonas* sp**.,** XL1: *Pseudomonas fulva*,PS2:*Enterobacter ludwigii*,PS4:*Cellulosomicrobium funkei*,).

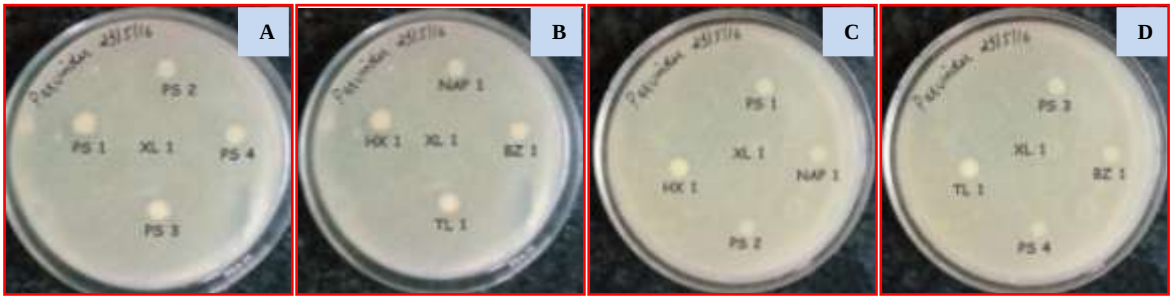


Figure 17: Synergistic activity between all rhizobacterial strains with *Pseudomonas fulva*strain XL1 with disc diffusion method (where,(**A)** PS1: *Ralstonia insidiosa*, PS2:*Enterobacter ludwigii* PS3: *Pseudomonas aeruginosa*, PS4:*Cellulosomicrobium funkei*, XL1: *Pseudomonas fulva* (**B)** HX1:*Pseudomonas putida*, NAP1: *Citrobacter freundii*, TL1: *Pseudomonas plecoglossicida*, XL1: *Pseudomonas fulva*, BZ1: *Pseudomonas* sp. **(C**) PS1: *Ralstonia insidiosa*, PS2:*Enterobacter ludwigii*, NAP1: *Citrobacter freundii*, HX1:*Pseudomonas putida*,XL1: *Pseudomonas fulva* **(D**) TL1: *Pseudomonas plecoglossicida*, PS3: *Pseudomonas aeruginosa*, PS4:*Cellulosomicrobium funkei*, BZ1: *Pseudomonas* sp**.,** XL1: *Pseudomonas fulva*).

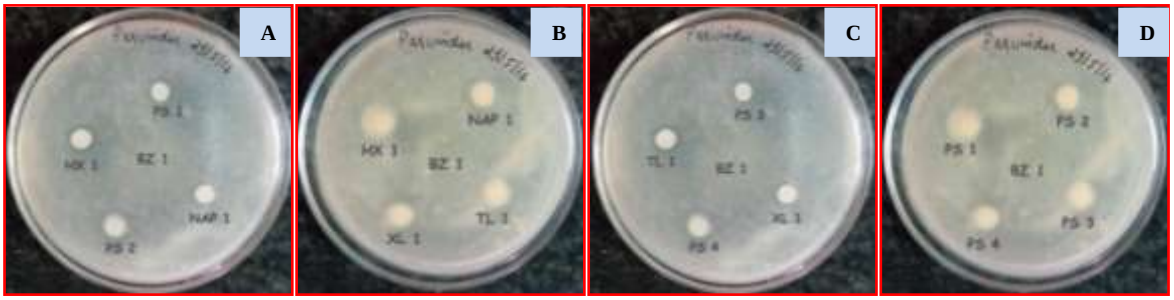


Figure 18: Synergistic activity between all rhizobacterial strains with *Pseudomonas sp*. strain BZ1 with disc diffusion method (where (**A)** PS1: *Ralstonia insidiosa*, PS2:*Enterobacter ludwigii*, NAP1: *Citrobacter freundii*, HX1:*Pseudomonas putida*, BZ1: *Pseudomonas* sp (**B)** XL1: *Pseudomonas fulva*, HX1:*Pseudomonas putida*, NAP1: *Citrobacter freundii*, TL1: *Pseudomonas plecoglossicida*, BZ1: *Pseudomonas* sp. **(C**) PS3: *Pseudomonas aeruginosa*, PS4:*Cellulosomicrobium funkei*, XL1: *Pseudomonas fulva*, TL1: *Pseudomonas plecoglossicida*, BZ1: *Pseudomonas* sp. **(D)** PS1: *Ralstonia insidiosa*, PS2:*Enterobacter ludwigii*, PS3: *Pseudomonas aeruginosa*,PS4:*Cellulosomicrobium funkei*, BZ1: *Pseudomonas* sp**.).**

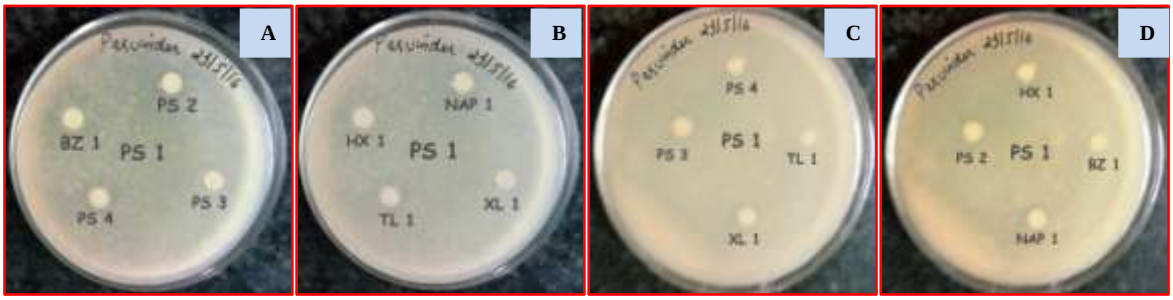


Figure 19: Synergistic activity between all rhizobacterial strains with *Ralstonia insidiosa*strain PS1 with disc diffusion method (where (**A)** PS1: *Ralstonia insidiosa*, PS2:*Enterobacter ludwigii*,PS3: *Pseudomonas aeruginosa*, PS4:*Cellulosomicrobium funkei*, BZ1: *Pseudomonas* sp. (**B)** NAP1: *Citrobacter freundii*, HX1:*Pseudomonas putida*,XL1: *Pseudomonas fulva*, TL1: *Pseudomonas plecoglossicida*, PS1: *Ralstonia insidiosa* **(C**) TL1: *Pseudomonas plecoglossicida*,PS3: *Pseudomonas aeruginosa*, PS4:*Cellulosomicrobium funkei*, XL1: *Pseudomonas fulva*, TL1: *Pseudomonas plecoglossicida*, PS1: *Ralstonia insidiosa* **(D)** BZ1: *Pseudomonas* sp. PS1: *Ralstonia insidiosa*, PS2:*Enterobacter ludwigii*, HX1:*Pseudomonas putida*, NAP1: *Citrobacter freundii*).

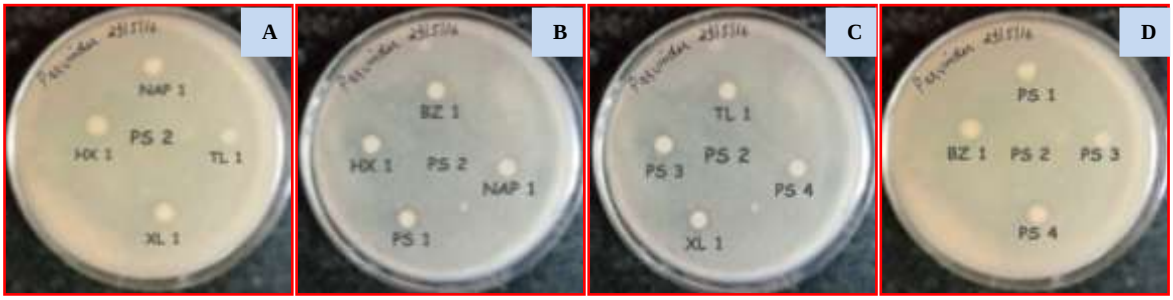


Figure 20: Synergistic activity between all rhizobacterial strains with *Enterobacter ludwiggi*strain PS2 with disc diffusion method (where (**A)** NAP1: *Citrobacter freundii*, HX1:*Pseudomonas putida*,XL1: *Pseudomonas fulva*, TL1: *Pseudomonas plecoglossicida*, PS2:*Enterobacter ludwigii* (**B)** PS1: *Ralstonia insidiosa*, PS2:*Enterobacter ludwigii*, HX1:*Pseudomonas putida* BZ1: *Pseudomonas* sp. NAP1: *Citrobacter freundii* **(C**) PS3: *Pseudomonas aeruginosa*,PS4:*Cellulosomicrobium funkei*, PS2:*Enterobacter ludwigii*, TL1: *Pseudomonas plecoglossicida* XL1: *Pseudomonas fulva1***(D)** BZ1: *Pseudomonas* sp., PS1: *Ralstonia insidiosa*, PS3: *Pseudomonas aeruginosa*,PS4:*Cellulosomicrobium funkei*, PS2:*Enterobacter ludwigii*).

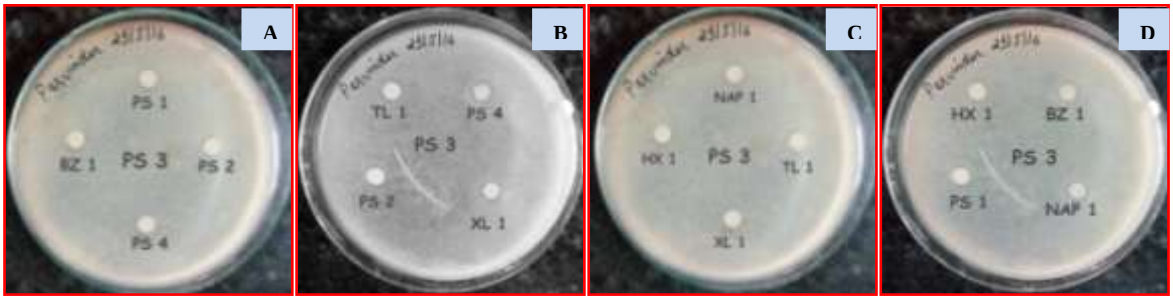


Figure 21: Synergistic activity between all rhizobacterial strains with *Pseudomonas aeruginosa*strain PS3 with disc diffusion method (where (**A)** BZ1: *Pseudomonas* sp., PS1: *Ralstonia insidiosa*, PS3: *Pseudomonas aeruginosa*,PS4:*Cellulosomicrobium funkei*, PS2:*Enterobacter ludwigii* (**B)** PS3: *Pseudomonas aeruginosa*,PS4:*Cellulosomicrobium funkei*, PS2:*Enterobacter ludwigii*, TL1: *Pseudomonas plecoglossicida*XL1: *Pseudomonas fulva* **(C**) NAP1: *Citrobacter freundii*, HX1:*Pseudomonas putida*,XL1: *Pseudomonas fulva*, TL1: *Pseudomonas plecoglossicida*, PS3: *Pseudomonas aeruginosa* **(D)** PS1: *Ralstonia insidiosa*, HX1:*Pseudomonas putida*BZ1: *Pseudomonas* sp. NAP1: *Citrobacter freundii* PS3: *Pseudomonas aeruginosa*).

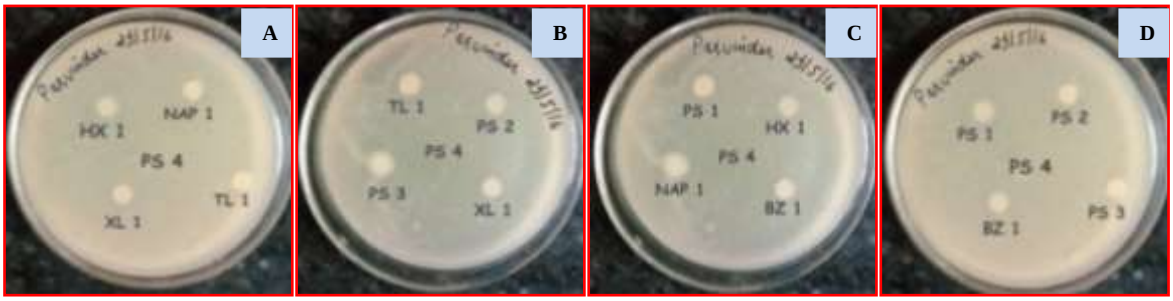


Figure 22: Synergistic activity between all rhizobacterial strains with *Cellulosomicrobium frunkei*strain PS4 with disc diffusion method (where (**A)** NAP1: *Citrobacter freundii*, HX1:*Pseudomonas putida*,XL1: *Pseudomonas fulva*, TL1: *Pseudomonas plecoglossicida*, PS4:*Cellulosomicrobium funkei* (**B)** PS3: *Pseudomonas aeruginosa*,PS4:*Cellulosomicrobium funkei*, PS2:*Enterobacter ludwigii*, TL1: *Pseudomonas plecoglossicida*XL1: *Pseudomonas fulva* **(C**) BZ1: *Pseudomonas* sp., PS1: *Ralstonia insidiosa*, PS4:*Cellulosomicrobium funkei*,NAP1: *Citrobacter freundii*, HX1:*Pseudomonas putida* **(D)** PS1: *Ralstonia insidiosa*,PS2:*Enterobacter ludwigii*,PS3: *Pseudomonas aeruginosa*,PS4:*Cellulosomicrobium funkei*,BZ1: *Pseudomonas* sp.).

6.13 Pot culture experimentation (Experimental Setup)

Seedsof *R. communis* and rhizomes of *C. indica* plants were grown in triplicates with two concentrations of Cadmium and Arsenic (50 and 100 mg kg^{-1}) (figure 23-25). All the plants were watered weekly and monitored to check the effect of heavy metals on plants. Tap water was used asa source of irrigation. Various parameters were studied which includes: protein content, enzymatic study (catalase, ascorbate peroxidase, glutathione reductase and guaiacol peroxidase), phytochemical screening (total phenolic and flavonoid content), HPTLC for phenolic and flavonoid content estimation, photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll and carotenoid content) along with physical parameters of the plants (height, wet weight, dry weight). Further, phytoextraction potential of plants was analysed by various components including heavy metal content in all parts of plants (ICP-OES), bioconcentration factor (BCF), bioaccumulation factor (BAF), translocation factor (TF), tolerance index (TI) and phytoextraction capability (PC).

All these parameters were calculated for the plants inoculated with all rhizobacteria, all mycorrhiza and microbial consortium for consecutive 3 months.

Figure23: Experimental setup for phytoremediation of heavy metals by *Ricinus communis* and *Canna indica*.


A


B


C


D


E


F


G


H


I


J
K
L
M
N
O
P
Q
R
S

Figure 24: Pot experimentationshowing *Ricinus communis* inoculated with arsenic and cadmium (50 and 100 $mgkg^{-1}$) along with rhizobacteria, mycorrhiza and microbial consortium (control and in combination).

(A) C+As 50: control + arsenic 50 $mgkg^{-1}$, **C+As 100**: Control + As 100 $mgkg^{-1}$, **C+Cd 50:** control + cadmium 50$mgkg^{-1}$, **C+Cd 100**: control + cadmium 100 $mgkg^{-1}$,
(B) Control: without any treatment, **C + PS 1**: control + *Ralstonia insidiosa*, **PS1+As 50**: *Ralstonia insidiosa* + arsenic 50 $mgkg^{-1}$, **Const. +As 50**: microbial consortium + arsenic 50 $mgkg^{-1}$, **PS 1+ As 100**: *Ralstonia insidiosa* + arsenic 100 $mgkg^{-1}$, **Const. +As 100**: microbial consortium + arsenic 100 $mgkg^{-1}$
(C)Control: without treatment, **C+PS 2**:Control + *Enterobacter ludwigii*, **PS2+ As 50**: *Enterobacter ludwigii* + arsenic 50 $mgkg^{-1}$, **Const. +As 50**: microbial consortium + arsenic 50 $mgkg^{-1}$,**PS 2+As 100**: *Enterobacter ludwigii* + arsenic 100 $mgkg^{-1}$, **Const. +As 100**: microbial consortium + arsenic 100 $mgkg^{-1}$
(D) Control: without treatment, **C+PS3**:Control+ *Pseudomonas aeruginosa*, **PS 3+As 50:***Pseudomonas aeruginosa* + arsenic 50 $mgkg^{-1}$,**Const. +As 50**: microbial consortium + arsenic 50 $mgkg^{-1}$ **PS 3+As 100**: *Pseudomonas aeruginosa* and arsenic 100 $mgkg^{-1}$, **Const. +As 100**: microbial consortium + arsenic 100 $mgkg^{-1}$

(E)Control: without treatment, **C+PS4**:Control + *Cellulosomicrobium funkei*, **PS 4+ As 50**: *Cellulosomicrobium funkei* + arsenic 50 $mgkg^{-1}$, **Const. +As 50**: microbial consortium + arsenic 50 $mgkg^{-1}$,**PS 4+As 100**: *Cellulosomicrobium funkei* and arsenic 100 $mgkg^{-1}$, **Const. +As 100**: microbial consortium + arsenic 100 $mgkg^{-1}$

(F)Control: without treatment, **C+TL 1**:Control + *Pseudomonas plecoglossicida*, **TL 1+ As 50**: *Pseudomonas plecoglossicida* + arsenic 50 $mgkg^{-1}$, **Const. +As 50**: microbial consortium + arsenic 50 $mgkg^{-1}$,**TL1+As100**: *Pseudomonas plecoglossicida* and arsenic 100 $mgkg^{-1}$, **Const. +As 100**: microbial consortium + arsenic 100 $mgkg^{-1}$

(G)Control: without treatment, **C+NAP 1**:Control + *Citrobacter frundii*, **NAP 1+ As 50**: *Citrobacter frundii* + arsenic 50 $mgkg^{-1}$, **Const. +As 50**: microbial consortium + arsenic 50 $mgkg^{-1}$,**NAP 1+As 100**: *Citrobacter frundii* and arsenic 100 $mgkg^{-1}$, **Const. +As 100**: microbial consortium + arsenic 100 $mgkg^{-1}$

(H)Control: without treatment, **C+XL 1**:Control + *Pseudomonas fulva*, **XL 1+As 50**: *Pseudomonas fulva* + arsenic 50 $mgkg^{-1}$, **Const. +As 50**: microbial consortium + arsenic 50 $mgkg^{-1}$, **XL 1+As 100**: *Pseudomonas fulva* and arsenic 100 $mgkg^{-1}$, **Const. +As 100**: microbial consortium + arsenic 100 $mgkg^{-1}$

(I)Control: without treatment, **C+HX 1**:Control + *Pseudomonas putida*, **HX 1+As 50**: *Pseudomonas putida* + arsenic 50 $mgkg^{-1}$, **Const. +As 50**: microbial consortium + arsenic 50 $mgkg^{-1}$,**HX 1+As 100**: *Pseudomonas putida* and arsenic 100 $mgkg^{-1}$, **Const. +As 100**: microbial consortium + arsenic 100 $mgkg^{-1}$

(J)Control: without treatment,**C+BZ1**:Control + *Pseudomonas sp*., **BZ 1+As 50:** *Pseudomonas sp*. + arsenic 50 $mgkg^{-1}$, **Const. +As 50**: microbial consortium + arsenic 50 $mgkg^{-1}$, **BZ 1+ As100**: *Pseudomonas sp.* + arsenic 100 $mgkg^{-1}$, **Const. +As 100**: microbial consortium + arsenic 100 $mgkg^{-1}$

(K) C**ontrol:** without treatment, **C+ G.h**: control + *Glomus hoi*,**G.h +As 50**: *Glomus hoi* + arsenic 50 $mgkg^{-1}$, **Const. +As 50**: microbial consortium + arsenic 50 $mgkg^{-1}$, **G.h +As 100**: *Glomus hoi* + arsenic 100 $mgkg^{-1}$, **Const. +As 100**: microbial consortium + arsenic 100 $mgkg^{-1}$

(L)Control: without treatment, **C+ G.c:** control + *Glomus claroideum*, **G.c +As 50**: *Glomus claroideum* + arsenic 50 $mgkg^{-1}$, **Const. +As 50**: microbial consortium + arsenic 50 $mgkg^{-1}$**G.c +As 100**: *Glomus claroideum* + arsenic 100 $mgkg^{-1}$, **Const. +As 100**: microbial consortium + arsenic 100 $mgkg^{-1}$

(M)Control: without treatment, **C+ A.k**: control + *Acaulospora kentinensis*, **A.k +As 50**: *Acaulospora kentinensis* + arsenic 50 $mgkg^{-1}$, **Const. +As 50**: microbial consortium + arsenic 50 $mgkg^{-1}$**A.k +As 100**: *Acaulospora kentinensis*+ arsenic 100 $mgkg^{-1}$, **Const. +As 100**: microbial consortium + arsenic 100 $mgkg^{-1}$

(N)Control: without treatment, **C+ PS 1**: control + *Ralstonia insidiosa*, **PS1+ Cd 50**: *Ralstonia insidiosa*+ cadmium 50 $mgkg^{-1}$, **Const.+Cd 50**: microbial consortium + cadmium 50 $mgkg^{-1}$, **PS 1+ Cd 100**: *Ralstonia insidiosa* , **Const. +Cd 100**: microbial consortium + cadmium 100 $mgkg^{-1}$

(O)Control: without treatment, **C+PS 2**:Control + *Enterobacter ludwigii*, **PS 2+ Cd 50:** *Enterobacter ludwigii* + cadmium 50 $mgkg^{-1}$, **Const.+Cd 50**: microbial consortium + cadmium 50 $mgkg^{-1}$, **PS2+Cd 100**: *Enterobacter ludwigii* + cadmium 100 $mgkg^{-1}$, **Const. +Cd 100**: microbial consortium + cadmium 100 $mgkg^{-1}$

(P)Control: without treatment**, C+PS3**:Control+ *Pseudomonas aeruginosa*, **PS 3+ Cd 50**: *Pseudomonas aeruginosa* + cadmium 50 $mgkg^{-1}$, **Const.+Cd 50**: microbial consortium + cadmium 50 $mgkg^{-1}$, **PS 3+Cd 100**: *Pseudomonas aeruginosa* + cadmium 100 $mgkg^{-1}$, **Const. +Cd 100**: microbial consortium + cadmium 100 $mgkg^{-1}$

(Q)Control: without treatment**, C+PS4**:Control+ *Cellulosomicrobium funkei*, **PS 4+ Cd 50**: *Cellulosomicrobium funkei* + arsenic 50 $mgkg^{-1}$,**Const.+Cd 50**: microbial consortium + cadmium 50 $mgkg^{-1}$, **PS 4+As 100**: *Cellulosomicrobium funkei* +arsenic 100 $mgkg^{-1}$, **Const. +Cd 100**: microbial consortium + cadmium 100 $mgkg^{-1}$

(R)Control: without treatment**, C+ TL 1**:Control+*Pseudomonas plecoglossicida*, **TL 1+ Cd 50**: *Pseudomonas plecoglossicida* +cadmium 50 $mgkg^{-1}$,**Const.+Cd 50**: microbial consortium + cadmium 50 $mgkg^{-1}$**TL 1+Cd 100**: *Pseudomonas plecoglossicida* + cadmium 100 $mgkg^{-1}$, **Const. +Cd 100**: microbial consortium + cadmium 100 $mgkg^{-1}$

(S) C**ontrol:** without treatment**, C+XL1**:Control+ *Pseudomonas fulva*, **XL1+ Cd 50**: *Pseudomonas fulva* +cadmium 50 $mgkg^{-1}$, **Const.+Cd 50**: microbial consortium + cadmium 50 $mgkg^{-1}$**XL1+Cd 100**: *Pseudomonas fulva* + cadmium 100 $mgkg^{-1}$, **Const. +Cd 100**: microbial consortium + cadmium 100 $mgkg^{-1}$

(T)Control: without treatment**, C+NAP1**:Control+ *Citrobacter frundii*, **NAP 1+ Cd 50**: *Citrobacter frundii* +cadmium 50 $mgkg^{-1}$, **Const.+Cd 50**: microbial consortium + cadmium 50 $mgkg^{-1}$**NAP 1+Cd 100**: *Citrobacter frundii*+ cadmium 100 $mgkg^{-1}$, **Const. +Cd 100**: microbial consortium + cadmium 100 $mgkg^{-1}$

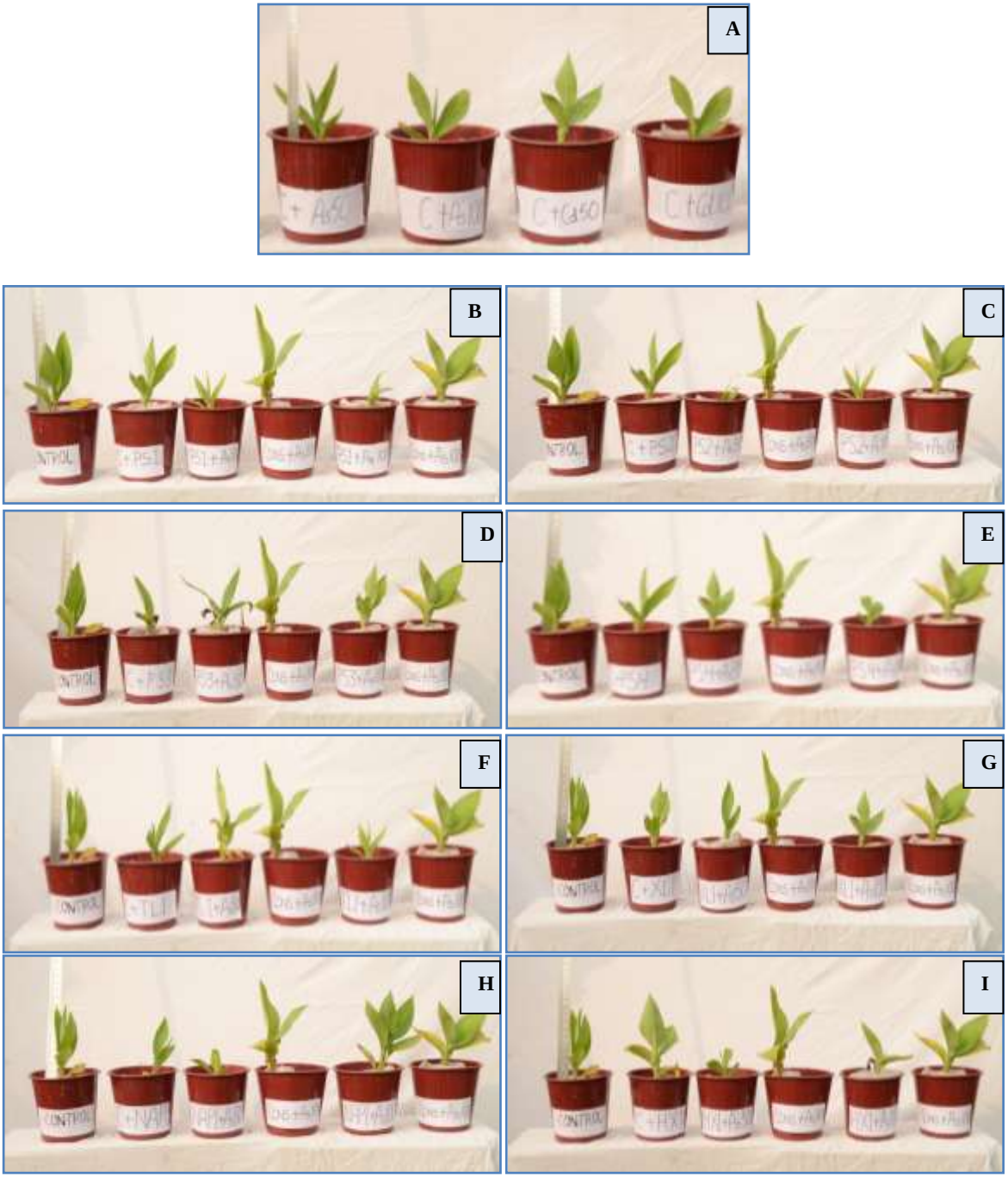
(U)Control: without treatment**, C+HX1**:Control+ *Pseudomonas putida*, **HX 1+ Cd 50**: *Pseudomonas putida* +cadmium 50 $mgkg^{-1}$, **Const.+Cd 50**: microbial consortium + cadmium 50 $mgkg^{-1}$**HX 1+Cd 100**: *Pseudomonas putida* + cadmium 100 $mgkg^{-1}$, **Const. +Cd 100**: microbial consortium + cadmium 100 $mgkg^{-1}$

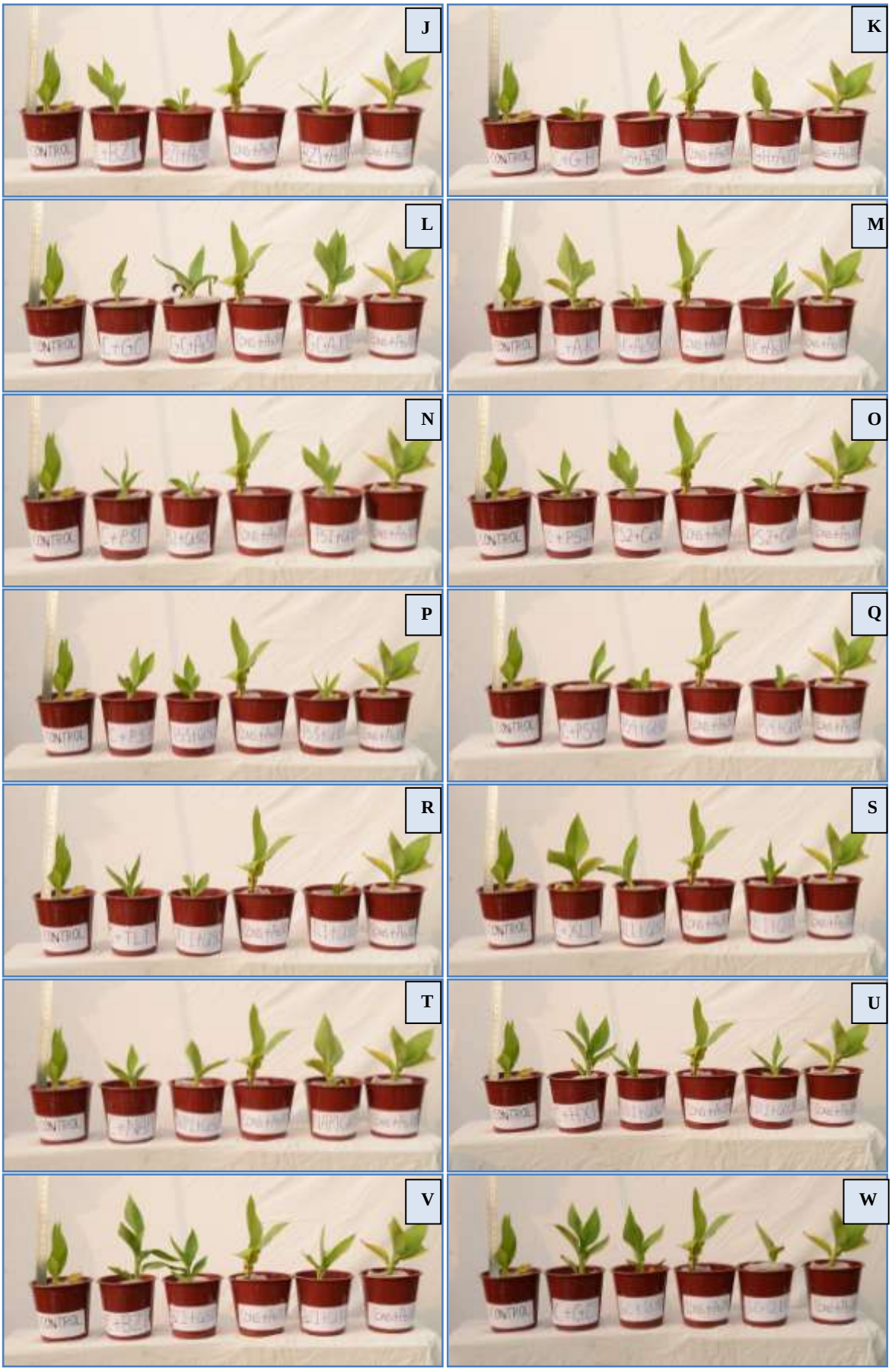
(V)Control: without treatment**, C+BZ1**:Control+ *Pseudomonas sp.*, **BZ1+ Cd 50**: *Pseudomonas sp*. + cadmium 50 $mgkg^{-1}$, **Const.+Cd 50**: microbial consortium + cadmium 50 $mgkg^{-1}$,**BZ1+Cd 100**: *Pseudomonas sp*. +cadmium 100 $mgkg^{-1}$, **Const. +Cd 100**: microbial consortium + cadmium 100 $mgkg^{-1}$

(W)Control: without treatment**, C+G.c:** control + *Glomus claroideum,***G.c +Cd 50**: *Glomus claroideum* + cadmium 50 $mgkg^{-1}$, **Const.+Cd 50**: microbial consortium + cadmium 50 $mgkg^{-1}$,**G.c +Cd 100:***Glomus claroideum* + cadmium 100 $mgkg^{-1}$, **Const. +Cd 100**: microbial consortium + cadmium 100 $mgkg^{-1}$

(X)Control: without treatment**, C+G.h:** control + *Glomus hoi,* **G.h +Cd 50**: *Glomus hoi* + cadmium 50 $mgkg^{-1}$, **Const.+Cd 50**: microbial consortium + cadmium 50 $mgkg^{-1}$, **G.h +Cd 100**: *Glomus hoi* + cadmium 100 $mgkg^{-1}$, **Const. +Cd 100**: microbial consortium + cadmium 100 $mgkg^{-1}$

(Y)Control: without treatment**, C+A.k:** control+*Acaulospora kentinensis*,**A.k +Cd 50**: *Acaulospora kentinensis* and cadmium 50 mgkg^{-1}, **Const.+Cd 50**: microbial consortium+cadmium 50 mgkg^{-1}**A.k +Cd 100**: *Acaulospora kentinensis* + cadmium 100 mgkg^{-1}, **Const. +Cd 100**: microbial consortium + cadmium 100 mgkg^{-1}
(Z)Control: without treatment**, C+All B:** control + all rhizobacteria, **All B+As 50**:All rhizobacteria +arsenic 50 mgkg^{-1}, **All B+As 100**: All rhizobacteria + arsenic 100 mgkg^{-1},**All B+Cd 50**:All rhizobacteria +cadmium 50 mgkg^{-1}, **All B+Cd 100**: All rhizobacteria +cadmium 100 mgkg^{-1},
(AA)Control: without treatment**, C+All M**: control +all mycorrhiza, **All M+As 50**:All mycorrhiza +arsenic 50 mgkg^{-1}, **All M+As 100**: All mycorrhiza +arsenic 100 mgkg^{-1},**All M+Cd 50:** All mycorrhiza + cadmium 50 mgkg^{-1}, **All M+Cd 100**: All mycorrhiza + cadmium 100 mgkg^{-1}).

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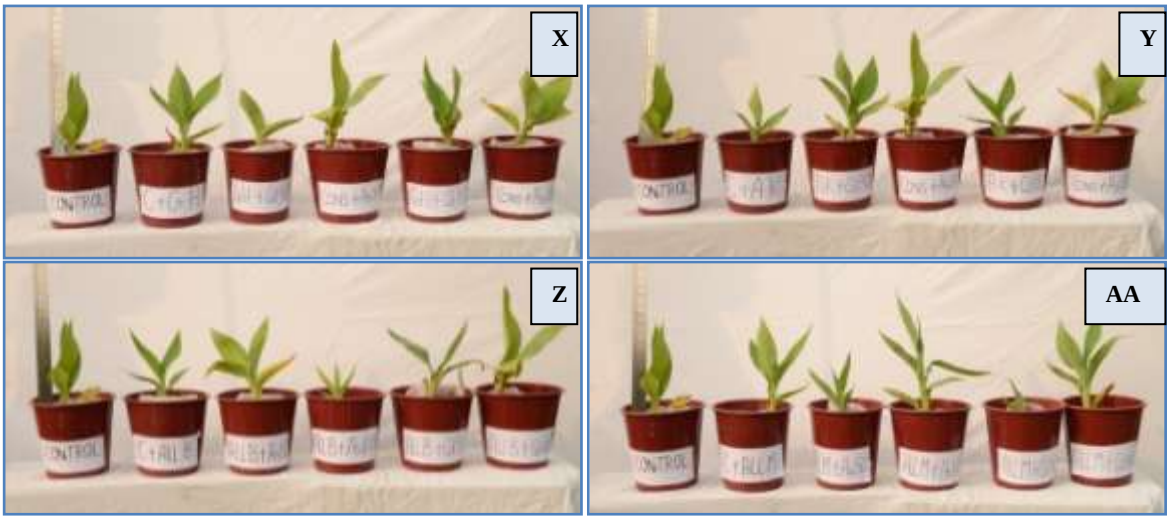

Figure 25: Pot experimentationshowing *Canna indica* inoculated with arsenic and cadmium (50 and 100 $mgkg^{-1}$) along with rhizobacteria, mycorrhiza and microbial consortium (alone and in combination).

(A) C+As 50: control + arsenic 50 $mgkg^{-1}$, **C+As 100**: Control + As 100 $mgkg^{-1}$,**C+Cd 50:** control + cadmium 50$mgkg^{-1}$, **C+Cd 100**: control + cadmium 100 $mgkg^{-1}$
(B) Control: without any treatment, **C + PS 1**: control + *Ralstonia insidiosa*, **PS1+As 50**: *Ralstonia insidiosa* + arsenic 50 $mgkg^{-1}$, **Const. +As 50**: microbial consortium + arsenic 50 $mgkg^{-1}$, **PS 1+ As 100**: *Ralstonia insidiosa* + arsenic 100 $mgkg^{-1}$, **Const. +As 100**: microbial consortium + arsenic 100 $mgkg^{-1}$
(C)Control: without treatment, **C+PS 2**: Control + *Enterobacter ludwigii*, **PS2+ As 50**: *Enterobacter ludwigii* + arsenic 50 $mgkg^{-1}$, **Const. +As 50**: microbial consortium + arsenic 50 $mgkg^{-1}$, **PS 2+As 100**: *Enterobacter ludwigii* + arsenic 100 $mgkg^{-1}$, **Const. +As 100**: microbial consortium + arsenic 100 $mgkg^{-1}$
(D) Control: without treatment, **C+PS3**: Control+ *Pseudomonas aeruginosa*, **PS 3+ As 50:** *Pseudomonas aeruginosa* + arsenic 50 $mgkg^{-1}$,**Const. +As 50**: microbial consortium + arsenic 50 $mgkg^{-1}$ **PS 3+As 100**: *Pseudomonas aeruginosa* and arsenic 100 $mgkg^{-1}$, **Const. +As 100**: microbial consortium + arsenic 100 $mgkg^{-1}$
(E)Control: without treatment, **C+PS4**: Control + *Cellulosomicrobium funkei*, **PS 4+ As 50**: *Cellulosomicrobium funkei* + arsenic 50 $mgkg^{-1}$, **Const. +As 50**: microbial consortium + arsenic 50 $mgkg^{-1}$,**PS 4+As 100**: *Cellulosomicrobium funkei* and arsenic 100 $mgkg^{-1}$, **Const. +As 100**: microbial consortium + arsenic 100 $mgkg^{-1}$
(F)Control: without treatment, **C+TL 1**: Control + *Pseudomonas plecoglossicida*, **TL 1+ As 50**: *Pseudomonas plecoglossicida* + arsenic 50 $mgkg^{-1}$, **Const. +As 50**: microbial consortium + arsenic 50 $mgkg^{-1}$,**TL1+As100**: *Pseudomonas plecoglossicida* and arsenic 100 $mgkg^{-1}$, **Const. +As 100**: microbial consortium + arsenic 100 $mgkg^{-1}$
(G) Control: without treatment, **C+XL 1**: Control + *Pseudomonas fulva*, **XL 1+ As 50**: *Pseudomonas fulva* + arsenic 50 $mgkg^{-1}$, **Const. +As 50**: microbial consortium + arsenic 50 $mgkg^{-1}$, **XL 1+As 100**: *Pseudomonas fulva* and arsenic 100 $mgkg^{-1}$, **Const. +As 100**: microbial consortium + arsenic 100 $mgkg^{-1}$
(H)Control: without treatment, **C+NAP 1**: Control + *Citrobacter frundii*, **NAP 1+ As 50**: *Citrobacter frundii* + arsenic 50 $mgkg^{-1}$, **Const. +As 50**: microbial consortium + arsenic 50 $mgkg^{-1}$, **NAP 1+As 100**: *Citrobacter frundii* and arsenic 100 $mgkg^{-1}$, **Const. +As 100**: microbial consortium + arsenic 100 $mgkg^{-1}$
(I)Control: without treatment, **C+HX 1**: Control + *Pseudomonas putida*, **HX 1+As 50**: *Pseudomonas putida* + arsenic 50 $mgkg^{-1}$, **Const. +As 50**: microbial consortium + arsenic 50 $mgkg^{-1}$, **HX 1+As 100**: *Pseudomonas putida* and arsenic 100 $mgkg^{-1}$, **Const. +As 100**: microbial consortium + arsenic 100 $mgkg^{-1}$
(J)Control: without treatment, **C+BZ1**: Control + *Pseudomonas sp*., **BZ 1+As 50:** *Pseudomonas sp*. + arsenic 50 $mgkg^{-1}$, **Const. +As 50**: microbial consortium + arsenic 50 $mgkg^{-1}$, **BZ 1+ As100**: *Pseudomonas sp.* + arsenic 100 $mgkg^{-1}$, **Const. +As 100**: microbial consortium + arsenic 100 $mgkg^{-1}$
(K) Control: without treatment, **C+ G.h**: control + *Glomus hoi*, **G.h +As 50**: *Glomus hoi* + arsenic 50 $mgkg^{-1}$, **Const. +As 50**: microbial consortium + arsenic 50 $mgkg^{-1}$, **G.h +As 100**: *Glomus hoi* + arsenic 100 $mgkg^{-1}$, **Const. +As 100**: microbial consortium + arsenic 100 $mgkg^{-1}$
(L)Control: without treatment, **C+ G.c:** control + *Glomus claroideum*, **G.c +As 50**: *Glomus claroideum* + arsenic 50 $mgkg^{-1}$, **Const. +As 50**: microbial consortium + arsenic 50 $mgkg^{-1}$**G.c +As 100**: *Glomus claroideum* + arsenic 100 $mgkg^{-1}$, **Const. +As 100**: microbial consortium + arsenic 100 $mgkg^{-1}$
(M)Control: without treatment, **C+ A.k**: control + *Acaulospora kentinensis*, **A.k + As 50**: *Acaulospora kentinensis* + arsenic 50 $mgkg^{-1}$, **Const. +As 50**: microbial consortium + arsenic 50 $mgkg^{-1}$**A.k +As 100**: *Acaulospora kentinensis*+ arsenic 100 $mgkg^{-1}$, **Const. +As 100**: microbial consortium + arsenic 100 $mgkg^{-1}$
(N)Control: without treatment, **C+ PS 1**: control + *Ralstonia insidiosa*, **PS1+ Cd 50**: *Ralstonia insidiosa*+ cadmium 50 $mgkg^{-1}$, **Const.+Cd 50**: microbial consortium + cadmium 50 $mgkg^{-1}$,**PS 1+ Cd 100**: *Ralstonia insidiosa* , **Const. +Cd 100**: microbial consortium + cadmium 100 $mgkg^{-1}$

(O)Control: without treatment, **C+PS 2**: Control + *Enterobacter ludwigii*, **PS 2+ Cd 50:** *Enterobacter ludwigii* + cadmium 50 mgkg^{-1}, **Const.+Cd 50**: microbial consortium + cadmium 50 mgkg^{-1}, **PS2+Cd 100**: *Enterobacter ludwigii* + cadmium 100 mgkg^{-1}, **Const. +Cd 100**: microbial consortium + cadmium 100 mgkg^{-1}
(P)Control: without treatment**, C+PS3**: Control+ *Pseudomonas aeruginosa*, **PS 3+ Cd 50**: *Pseudomonas aeruginosa* + cadmium 50 mgkg^{-1}, **Const.+Cd 50**: microbial consortium + cadmium 50 mgkg^{-1}, **PS 3+Cd 100**: *Pseudomonas aeruginosa* + cadmium 100 mgkg^{-1}, **Const. +Cd 100**: microbial consortium + cadmium 100 mgkg^{-1}
(Q)Control: without treatment**, C+PS4**: Control+ *Cellulosomicrobium funkei*, **PS 4+ Cd 50**: *Cellulosomicrobium funkei* + arsenic 50 mgkg^{-1}, **Const.+Cd 50**: microbial consortium + cadmium 50 mgkg^{-1}**PS 4+As 100**: *Cellulosomicrobium funkei* +arsenic 100 mgkg^{-1}, **Const. +Cd 100**: microbial consortium + cadmium 100 mgkg^{-1}
(R)Control: without treatment**, C+ TL 1**: Control+ *Pseudomonas plecoglossicida*, **TL 1+ Cd 50**: *Pseudomonas plecoglossicida* +cadmium 50 mgkg^{-1}, **Const.+Cd 50**: microbial consortium + cadmium 50 mgkg^{-1}**TL 1+Cd 100**: *Pseudomonas plecoglossicida* + cadmium 100 mgkg^{-1}, **Const. +Cd 100**: microbial consortium + cadmium 100 mgkg^{-1}
(S) C**ontrol:** without treatment**, C+XL1**: Control+*Pseudomonas fulva*, **XL1+ Cd 50**: *Pseudomonas fulva* +cadmium 50 mgkg^{-1}, **Const.+Cd 50**: microbial consortium + cadmium 50 mgkg^{-1}**XL1+Cd 100**: *Pseudomonas fulva* + cadmium 100 mgkg^{-1}, **Const. +Cd 100**: microbial consortium + cadmium 100 mgkg^{-1}
(T)Control: without treatment**, C+NAP1**: Control+ *Citrobacter frundii*, **NAP 1+ Cd 50**: *Citrobacter frundii* +cadmium 50 mgkg^{-1}, **Const.+Cd 50**: microbial consortium + cadmium 50 mgkg^{-1}**NAP 1+Cd 100**: *Citrobacter frundii*+ cadmium 100 mgkg^{-1}, **Const. +Cd 100**: microbial consortium + cadmium 100 mgkg^{-1}
(U)Control: without treatment**, C+HX1**: Control+ *Pseudomonas putida*, **HX 1+ Cd 50**: *Pseudomonas putida* +cadmium 50 mgkg^{-1}, **Const.+Cd 50**: microbial consortium + cadmium 50 mgkg^{-1}**HX 1+Cd 100**: *Pseudomonas putida* + cadmium 100 mgkg^{-1}, **Const. +Cd 100**: microbial consortium + cadmium 100 mgkg^{-1}
(V)Control: without treatment**, C+BZ1**:Control+ *Pseudomonas sp.*, **BZ1+ Cd 50**: *Pseudomonas sp*. + cadmium 50 mgkg^{-1}, **Const.+Cd 50**: microbial consortium + cadmium 50 mgkg^{-1},**BZ1+Cd 100**: *Pseudomonas sp*. +cadmium 100 mgkg^{-1}, **Const. +Cd 100**: microbial consortium + cadmium 100 mgkg^{-1}
(W)Control: without treatment**, C+G.c:** control + *Glomus claroideum,***G.c +Cd 50**: *Glomus claroideum* + cadmium 50 mgkg^{-1}, **Const.+Cd 50**: microbial consortium + cadmium 50 mgkg^{-1},**G.c +Cd 100:***Glomus claroideum* + cadmium 100 mgkg^{-1}, **Const. +Cd 100**: microbial consortium + cadmium 100 mgkg^{-1}
(X)Control: without treatment**, C+G.h:** control + *Glomus hoi*, **G.h +Cd 50**: *Glomus hoi* + cadmium 50 mgkg^{-1}, **Const.+Cd 50**: microbial consortium + cadmium 50 mgkg^{-1}, **G.h +Cd 100**: *Glomus hoi* + cadmium 100 mgkg^{-1}, **Const. +Cd 100**: microbial consortium + cadmium 100 mgkg^{-1}
(Y)Control: without treatment**, C+A.k:** control+*Acaulospora kentinensis*, **A.k +Cd 50**: *Acaulospora kentinensis* and cadmium 50 mgkg^{-1}, **Const.+Cd 50**: microbial consortium+cadmium 50 mgkg^{-1}**A.k +Cd 100**: *Acaulospora kentinensis* + cadmium 100 mgkg^{-1}, **Const. +Cd 100**: microbial consortium + cadmium 100 mgkg^{-1}
(Z)Control: without treatment**, C+All B:** control + all rhizobacteria, **All B+As 50**: All rhizobacteria +arsenic 50 mgkg^{-1}, **All B+As 100**: All rhizobacteria + arsenic 100 mgkg^{-1}, **All B+Cd 50**: All rhizobacteria +cadmium 50 mgkg^{-1}, **All B+Cd 100**: All rhizobacteria +cadmium 100 mgkg^{-1},
(AA)Control: without treatment**, C+All M**: control +all mycorrhiza, **All M+As 50**: All mycorrhiza +arsenic 50 mgkg^{-1}, **All M+As 100**: All mycorrhiza +arsenic 100 mgkg^{-1}, **All M+Cd 50:** All mycorrhiza + cadmium 50 mgkg^{-1}, **All M+Cd 100**: All mycorrhiza + cadmium 100 mgkg^{-1}).

The **second objective** was "to determine enzymatic activities of plants associated with precipitation and binding of pollutants" which was accomplished by obtaining the analytical results of different enzymes analyzed in various parts of the plants.

6.14Estimation of protein content

Heavy metals adversely affect the protein content in roots and leaves of the plant. In the present study, protein content was found to be decreased in leaves and roots of *R. communis* as well as *C. indica* under arsenic stress as compared to the control plants which were not inoculated with heavy metals. Plants that were not inoculated with either heavy metals or microbes, showed comparably higher protein content in all the 3 months of experimentation with a maximum in leaves followed by roots of both the plants. In *R. communis* plants (1^{st} month) inoculated with all rhizobacteria, leaves showed less decline in protein content as compared to control (with only rhizobacterial inoculation) i.e 3.44, 3.43 and 3.38 mg g^{-1} FW (control, As 50 and As 100 mgkg^{-1}) respectively as compared to theroots with same treatment showingcomparatively more decline in protein content in comparison to control *viz,* 1.33, 1.21 and 1.15mg g^{-1} FW (control, As 50 and As 100 mgkg^{-1}) respectively.

When comparing the plants that were inoculated with all mycorrhizal cultures along with two different concentrations of arsenic with all rhizobacteria, the pots with mycorrhizal inoculation shows less protein content in control and inoculated pots *viz,* 3.43, 3.42 and 3.41 in leaves and 1.22, 1.18 and 1.11mg g^{-1} FW in (control, As 50 and 100 mgkg^{-1}) roots respectively. In case of the plants inoculated with microbial consortium and arsenic, protein content was higher in control and inoculated plants as compared to both rhizobacteria and mycorrhiza in both the leaves (3.53, 3.51 and 3.49) and roots (1.47, 1.33 and 1.30) in control, As 509 and As 100 mgkg^{-1}respectively. Similarly, in the 2^{nd} month, the protein content in all the control and inoculated plants with rhizobacteria, mycorrhiza and microbial consortium increased as compared to 1^{st} month but the consistent decline was observed after a 3^{rd} month in all the treated pots. Maximum protein content was observed in microbial consortium treated plants in both leaves (3.96, 3.91 and 3.92) and roots (2.21, 2.11 and 1.94) followed by mycorrhiza (3.96, 3.92 and 3.91 in leaves and 2.10, 1.58 and 1.46 in roots) and rhizobacteria (3.96, 3.92 and 3.77 in leaves and 2.14, 1.62 and 1.501 mg g^{-1} FW in roots (control and As 50 and 100 mgkg^{-1}) respectively (table 10)**.**A similar trend was observed in case of *C. indica* plants, where the protein content in all the control and inoculated plants with rhizobacteria, mycorrhiza and the microbialconsortium was found to be less as compared to plants with *R. communis* plants. As well as the protein content increased in the 2^{nd} month and decreased in the3^{rd} month in both the leaves and roots of all pots. Maximum content in all three months

was observed in plants inoculated with the microbial consortium (leaves and roots) followed by rhizobacteria and mycorrhiza (Figure 28-29).

The plants inoculated with cadmium also reported the adverse effect of this metal on the protein content of the leaves and roots of both plants. In 1st month, protein content was found to be maximum in the plants both *R. communis* and *C. indica* inoculated with microbial consortium, where protein content in leaves was (3.53, 3.43 and 3.42) and (3.54, 3.07 and 3.02mg g^{-1} FW) in control, Cd 50 and 100 mg kg^{-1} respectively and in roots of *R. communis* (1.47, 1.28 and 1.22) *C. indica* (3.31, 3.22 and 3.16mg g^{-1} FW) in control,Cd 50 and 100 mgkg^{-1} respectively. In the 2nd-month protein content was increased in all pots whereas a decline was observed in a 3rd month in leaves and roots of both the plants (control and treated). The protein content was found to be more in all the 3 months in the plants inoculated with microbial consortium followed by rhizobacteria and mycorrhiza. In *R. communis* plant, leaves showed more protein content than roots but in *C. indica* plant roots exhibit more protein content than shoots in all 3 months (table 11).

According to Baudh *et al* (2015), a considerable decline in protein content (33.79 % at 150 mg kg^{-1} of Ni in the soil as compared to control) of *R. communis* plant inoculated with Nickel was observed in 2 months. The study demonstrated that with an increase in the concentration of heavy metals and time period, protein content also decreases which is in accordance with our results. Protein content was found to be less in leaves of *R. communis* plants under heavy metal stress (Cd, Pb, Mn, Zn and Fe) where the concentration of protein was found to be 0.5 fold less in contaminated soil as compared to uncontaminated soil (Ravi *et al*., 2017). Additionally, growth and biomass along with protein content were also affected by heavy metals toxicity due to multiple abiotic factors that lead to the formation of reactive oxygen species (ROS), which causes oxidative damage to proteins and membrane lipids etc. (Singh *et al*., 2010). Results of the present study for protein content indicated that under increasing concentration of heavy metal stress (As and Cd 50 -100 mg kg^{-1}) and time duration protein content decreases in plants, but the plants inoculated with rhizobacteria, mycorrhiza and consortium, possessed significantly higher protein content in leaves and roots as compared to control (without heavy metal treatment). This shows that native rhizobacteria and mycorrhiza species reduces the toxic effects caused by heavy metal on the plant. Here, microbial consortium showed maximum protein content during the course of the study than other inoculants (rhizobacteria, mycorrhiza), hence proved to be more efficient then alone rhizobacteria and mycorrhiza, in reducing the toxic effects of heavy metals on plants protein content.

Table 10: Protein content (mgg^{-1}FW) in leaves and roots of *Ricinus communis* and *Canna indica* inoculated with arsenic (50 and 100 mgkg^{-1}) along with rhizobacteria, mycorrhiza and microbial consortium.

Time duration →	1st month				2nd Month				3rd Month			
Plants →	*R. communis*		*C. indica*		*R. communis*		*C. indica*		*R. communis*		*C. indica*	
Treatment ↓	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots
Control	4.00± 0.004^{a}	2.01± 0.002^{m}	3.99± 0.031^{a}	3.14± 0.032^{h}	4.00± 0.005^{a}	3.01± 0.008^{i}	3.91± 0.021^{b}	3.92± 0.056^{b}	4.00± 0.036^{a}	2.98± 0.053^{a}	3.99± 0.022^{a}	3.91± 0.012^{b}
C + As 50	2.51± 0.011^{k}	1.09± 0.034^{s}	2.01± 0.026^{m}	3.01± 0.014^{i}	2.55± 0.006^{k}	1.32± 0.007^{p}	2.01± 0.008^{m}	2.93± 0.044^{j}	2.41± 0.035^{l}	1.03± 0.072^{s}	1.98± 0.016^{n}	2.14± 0.022^{m}
C + As 100	2.11± 0.021^{m}	1.01± 0.074^{s}	1.09± 0.064^{s}	3.01± 0.071^{i}	2.41± 0.004^{l}	1.23± 0.027^{q}	1.09± 0.091^{s}	2.81± 0.056^{j}	2.32± 0.009^{l}	0.98± 0.077^{t}	1.82± 0.087^{n}	2.11± 0.064^{m}
C +All B	3.44± 0.004^{f}	1.33± 0.058^{p}	3.36± 0.055^{g}	3.18± 0.036^{h}	3.96± 0.050^{a}	2.14± 0.053^{m}	3.41± 0.060^{f}	3.68± 0.009^{d}	3.66± 0.077^{d}	2.02± 0.014^{m}	3.40± 0.027^{f}	3.42± 0.010^{f}
As 50 All B	3.43± 0.004^{f}	1.21± 0.028^{q}	3.18± 0.008^{h}	3.16± 0.047^{h}	3.92± 0.007^{a}	1.62± 0.011^{o}	3.50± 0.005^{e}	3.56± 0.028^{e}	3.60± 0.005^{d}	1.41± 0.008^{o}	3.24± 0.012^{h}	3.22± 0.012^{h}
As 100+All B	3.38± 0.012^{g}	1.15± 0.043^{r}	3.05± 0.004^{i}	3.14± 0.036^{h}	3.77± 0.021^{c}	1.50± 0.024^{o}	3.43± 0.010^{f}	3.40± 0.009^{f}	3.54± 0.063^{c}	1.39± 0.010^{o}	3.16± 0.070^{h}	3.18± 0.029^{h}
C+ All M	3.43± 0.006^{f}	1.22±^{q} 0.009	3.18± 0.028^{h}	2.67± 0.202^{k}	3.96± 0.050^{a}	2.10± 0.007^{m}	3.36± 0.057^{g}	3.81± 0.012^{c}	3.65± 0.065^{d}	1.89± 0.033^{n}	3.26± 0.053^{h}	3.52± 0.009^{e}
As 50 +All M	3.42± 0.007^{f}	1.18± 0.007^{r}	3.15± 0.014^{h}	2.51± 0.137^{k}	3.92± 0.007^{b}	1.58± 0.034^{o}	3.23± 0.008^{h}	3.78± 0.009^{c}	3.09± 0.009^{i}	1.50± 0.008^{o}	3.12± 0.009^{h}	3.51± 0.014^{e}
As 100+All M	3.41± 0.008^{f}	1.11± 0.001^{r}	3.12± 0.003^{h}	2.30± 0.137^{g}	3.91± 0.009^{b}	1.46± 0.084^{o}	3.22± 0.009^{h}	3.61± 0.012^{c}	3.58± 0.004^{c}	1.21± 0.017^{q}	3.11± 0.012^{h}	3.50± 0.007^{e}
C + Const	3.53± 0.006^{e}	1.47± 0.075^{o}	3.54± 0.028^{c}	3.31± 0.008^{g}	3.96± 0.051^{a}	2.21± 0.004^{m}	3.61± 0.092^{d}	3.94± 0.049^{b}	3.74± 0.006^{c}	2.08± 0.004^{m}	3.50± 0.015^{e}	3.69± 0.008^{d}
As 50+Const.	3.51± 0.001^{e}	1.33± 0.012^{p}	3.33± 0.010^{g}	3.21± 0.045^{h}	3.91± 0.010^{b}	2.11± 0.005^{m}	3.61± 0.018^{d}	3.80± 0.007^{c}	3.79± 0.059^{c}	2.05± 0.070^{m}	3.60± 0.016^{d}	3.65± 0.016^{d}
As100+Const	3.49± 0.004^{f}	1.30± 0.007^{p}	3.30± 0.010^{g}	3.24± 0.027^{h}	3.92± 0.009^{b}	1.94± 0.092^{n}	3.41± 0.009^{f}	3.72± 0.012^{c}	3.62± 0.007^{d}	1.72± 0.058^{n}	3.34± 0.080^{g}	3.51± 0.043^{e}

Mean ± SD (n=3) Different small alphabets (a-s) indicates statistically significant difference at P ≤ 0.05 by Turkey's test (**C**: control, **As 50**: arsenic 50 mgkg^{-1}, **As 100**: arsenic 100 mgkg^{-1}, **All B**: all bacterial isolates (1-9), **All M**: All mycorrhiza cultures (1-3), **Const:** microbial consortium (All bacteria + all mycorrhiza)

Table 11: Protein content (mgg^{-1}FW) in leaves and roots of *Ricinus communis* and *Canna indica* treated with Cadmium (50 and 100 mgkg^{-1}) along with rhizobacteria, mycorrhiza and microbial consortium.

Time duration →	1st month				2nd Month				3rd Month			
Plants	***R. communis***		***C. indica***		***R. communis***		***C. indica***		***R. communis***		***C. indica***	
Treatment ↓	**Leaves**	**Roots**	**Leaves**	**Roots**	**Leaves**	**Roots**	**Leaves**	**Roots**	**Leaves**	**Roots**	**Leaves**	**Roots**
Control	4.00± 0.004^{a}	2.01± 0.002^{m}	3.99± 0.031^{a}	3.14± 0.032^{h}	4.00± 0.005^{a}	3.01± 0.008^{i}	3.91± 0.021^{b}	3.92± 0.056^{b}	4.00± 0.036^{a}	2.98± 0.053^{a}	3.99± 0.022^{a}	3.91± 0.012^{b}
C + Cd 50	2.46± 0.002^{l}	1.11± 0.212^{q}	2.10± 0.207^{m}	3.12± 0.094^{i}	3.02± 0.102^{j}	1.24± 0.511^{o}	2.21± 0.222^{l}	3.12± 0.008^{i}	2.08± 0.072^{m}	1.12± 0.002^{q}	2.11± 0.081^{m}	3.01± 0.006^{i}
C + Cd 100	2.11± 0.019^{m}	1.10± 0.009^{q}	2.08± 0.065^{m}	3.10± 0.007^{i}	3.01± 0.012^{j}	1.21± 0.079^{o}	2.21± 0.088^{l}	3.21± 0.067^{h}	1.91± 0.061^{p}	1.12± 0.026^{q}	2.08± 0.054^{m}	3.09± 0.004^{i}
C +All B	3.44± 0.004^{f}	1.33± 0.058^{p}	3.36± 0.055^{h}	3.18± 0.036^{i}	3.96± 0.050^{a}	2.14± 0.053^{m}	3.47± 0.060^{f}	3.68± 0.009^{d}	3.66± 0.077^{d}	2.02± 0.014^{n}	3.40± 0.027^{f}	3.42± 0.010^{f}
Cd 50 + All B	2.45± 0.069^{l}	1.10± 0.003^{q}	2.14± 0.056^{m}	3.15± 0.054^{i}	3.08± 0.009^{j}	1.39± 0.028^{p}	3.21± 0.012^{h}	3.50± 0.007^{e}	2.88± 0.149^{k}	1.21± 0.013^{o}	2.18± 0.016^{m}	3.16± 0.009^{i}
Cd 100 +All B	2.37± 0.006^{l}	1.09± 0.007^{r}	2.03± 0.032^{m}	3.11± 0.015^{i}	3.08± 0.007^{j}	1.31± 0.015^{p}	3.08± 0.017^{j}	3.31± 0.003^{g}	3.06± 0.007^{i}	1.13± 0.033^{q}	2.15± 0.023^{m}	3.11± 0.007^{i}
C+ All M	3.43± 0.006^{f}	1.29± 0.009^{o}	3.18± 0.028^{h}	2.67± 0.202^{e}	3.96± 0.050^{a}	2.10± 0.007^{m}	3.36± 0.057^{g}	3.81± 0.012^{c}	3.65± 0.065^{d}	1.89± 0.033^{n}	3.26± 0.053^{h}	3.52± 0.008^{e}
Cd 50 +All M	2.37± 0.008^{l}	1.00± 0.007^{r}	2.10± 0.005^{m}	2.50± 0.014^{e}	3.64± 0.067^{d}	1.35± 0.055^{p}	3.11± 0.002^{i}	3.67± 0.021^{d}	2.91± 0.003^{k}	1.20± 0.009^{o}	3.11± 0.002^{i}	3.51± 0.005^{e}
Cd 100+AllM	2.37± 0.008^{l}	1.00± 0.005^{r}	2.09± 0.008^{m}	2.25± 0.071^{l}	3.83± 0.065^{c}	1.33± 0.075^{p}	3.10± 0.004^{i}	3.51± 0.011^{e}	2.07± 0.017^{m}	1.11± 0.010^{q}	3.06± 0.028^{j}	3.49± 0.009^{f}
C + Const	3.53± 0.007^{e}	1.47± 0.075^{p}	3.54± 0.028^{e}	3.31± 0.008^{g}	3.96± 0.050^{a}	2.21± 0.004^{l}	3.61± 0.092^{d}	3.94± 0.049^{a}	3.74± 0.006^{c}	2.08± 0.007^{m}	3.50± 0.015^{f}	3.69± 0.005^{d}
Cd 50+Const.	3.43± 0.006^{f}	1.28± 0.013^{o}	3.07± 0.038^{j}	3.22± 0.002^{h}	3.84± 0.062^{c}	1.56± 0.038^{p}	3.46± 0.062^{f}	3.60± 0.016^{d}	3.61± 0.006^{d}	1.37± 0.014^{p}	3.45± 0.067^{f}	3.56± 0.053^{e}
Cd100+Const	3.42± 0.009^{f}	1.22± 0.008^{o}	3.02± 0.007^{j}	3.16± 0.057i	3.90± 0.117^{b}	1.65± 0.017^{p}	3.33± 0.021^{g}	3.56± 0.028^{e}	3.10± 0.006^{h}	1.52± 0.012^{p}	3.27± 0.085^{h}	3.51± 0.012^{e}

Mean ± SD (n=3). Different small alphabets (a-q) indicate statistically significant difference at $P \leq 0.05$ by Turkey's test (**C:** control, **Cd 50:** cadmium 50 mgkg^{-1}, **Cd 100**: cadmium 100 mgkg^{-1}, **All B**: all bacterial isolates (1-9), **All M:** All mycorrhiza cultures (1-3), **Const:** microbial consortium (All bacteria + all mycorrhiza).

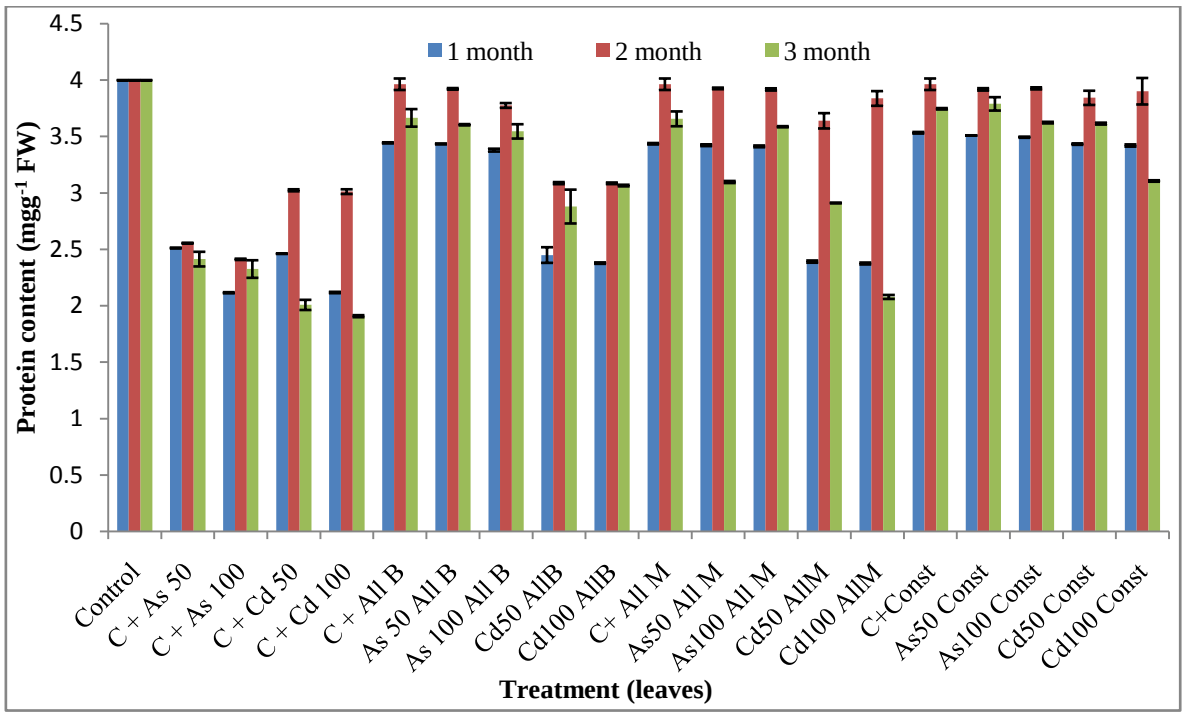

Figure 26: Protein content (mgg^{-1}FW) in leaves of *Ricinus communis* inoculated with arsenic and cadmium (50 and 100 mgkg^{-1}) along with rhizobacteria, mycorrhiza and microbial consortium.

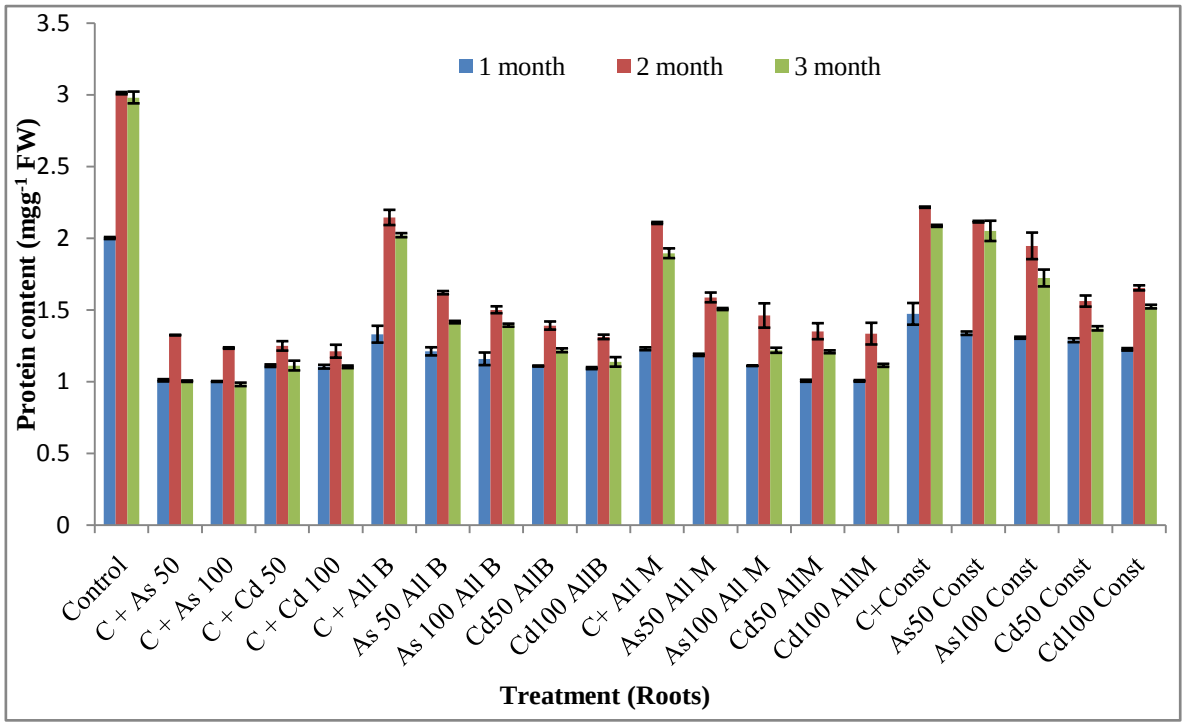

Figure 27: Protein content (mgg^{-1}FW) in roots of *Ricinus communis* inoculated with arsenic and cadmium (50 and 100 mgkg^{-1}) along with rhizobacteria, mycorrhiza and microbial consortium.

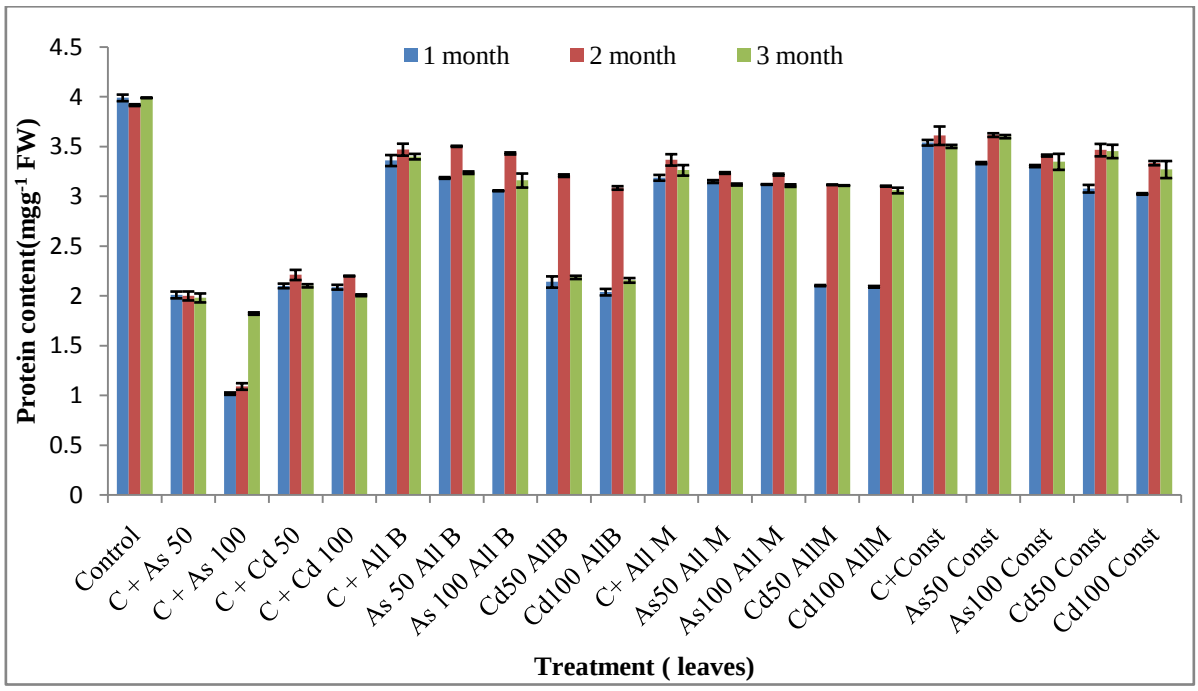

Figure 28: Protein content (mg g^{-1}FW) in leaves of *Canna indica* inoculated with arsenic and cadmium (50 and 100 $mgkg^{-1}$) along with rhizobacteria, mycorrhiza and microbial consortium.

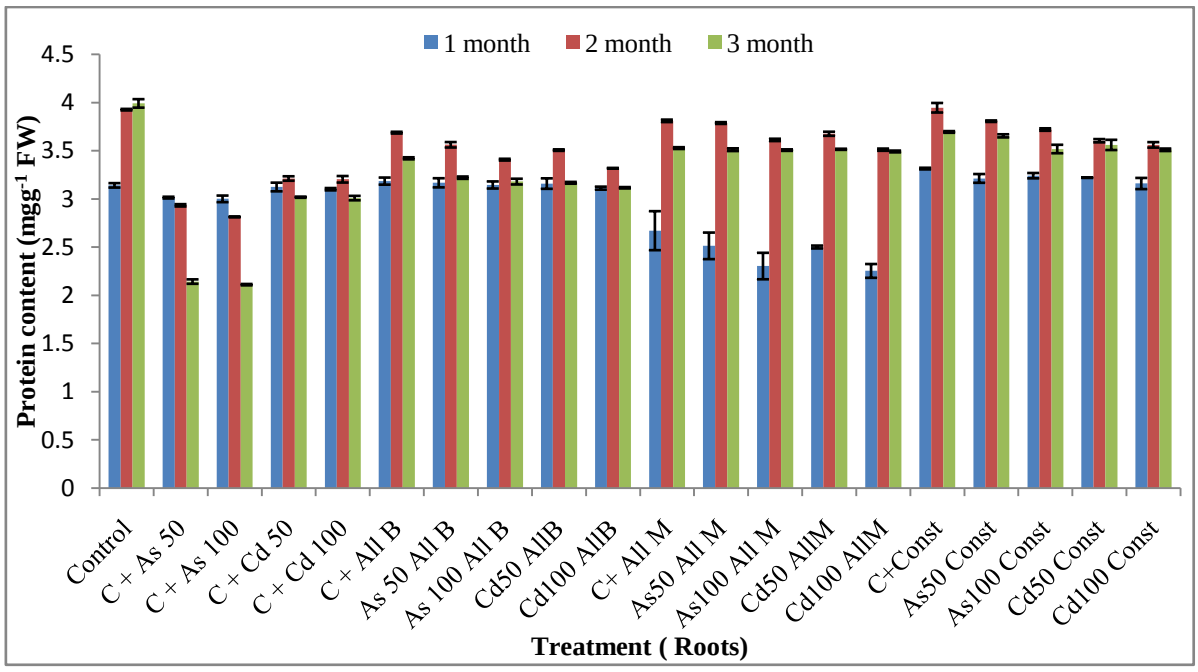

Figure 29: Protein content (mgg^{-1}FW) in roots of *Canna indica* inoculated with arsenic and cadmium (50 and 100 $mgkg^{-1}$) along with rhizobacteria, mycorrhiza and microbial consortium.

6.15 Effect of Arsenic and Cadmium on antioxidant enzymes activity in *Ricinus communis* and *Canna indica*

Various antioxidant enzymes relieve the reactive oxygen species induced by heavy metal stress. Some of such enzymes are Catalase (CAT), Ascorbate peroxidase (APX), Glutathione reductase (GR) and Guaiacol peroxidise (GPX). In the present study, the effect of rhizobacteria, mycorrhiza and microbial consortium inoculated plants (*R. communis* and *C. indica*) under arsenic and cadmium stress (50 and 100 $mgkg^{-1}$) was studied on the enzymatic activity of both the plants.

6.15.1Catalase (CAT) activity in *Ricinus communis* and *Canna indica* inoculated with two concentrations of arsenic and cadmium (50 and 100 $mgkg^{-1}$) along with rhizobacteria, mycorrhiza and a microbial consortium

Both the plants inoculated with arsenic showed a different pattern of catalase activity in 3 months in their leaves and root. In the case of *R. communis* plant catalase activity increased in all the plants inoculated with rhizobacteria, mycorrhiza and microbial consortium in all 3 months, as compared to control plants. After 1stmonth maximum activity was observed in the leaves of *R. communis* i.e 0.016, 0.218 and 0.115 Unit mg^{-1} protein FW in control and plants treated with mycorrhiza (As 50 and 100 $mgkg^{-1}$) respectively, followed by rhizobacteria (0.089, 0.149 and 0.150) and microbial consortium (0.013, 0.131 and 0.079) in control and plants inoculated with mycorrhiza (As 50 and 100 $mgkg^{-1}$) respectively.

After 2nd month activity in leaves of all plants increased in increasing order of microbial consortium (0.129, 0.311 and 0.348),< rhizobacteria (0.125, 0.391 and 0.375) < mycorrhiza (0.228, 0.445 and 0.388) in control and microbial consortium (As 50 and 100 $mgkg^{-1}$) respectively, whereas 3rd month showed decline in catalase activity in both leaves and roots of *R. communis* plant in all treatments where maximum was found in leaves of mycorrhiza pots viz 0.139, 0.370 and 0.294 in control and As (50 and 100 $mgkg^{-1}$) inoculated plants respectivelyfollowed by rhizobacteria (0.139, 0.310 and 0.283) and microbial consortium inoculated plants (0.139, 0.238 and 0.364) in control and As (50 and 100 $mgkg^{-1}$) treated plants respectively.

In roots, CAT activity was found to be less as compared to leaves of *R. communis*in all the 3 months, where maximum activity in roots was observed in control and mycorrhiza (As 50 and 100 $mgkg^{-1}$) treated pots *viz* 0.014, 0.145 and 0.037 respectively, followed by rhizobacteria (0.036, 0.056 and 0.041) and microbial consortium (0.025, 0.043 and 0.031) in control and As 50 and 100 $mgkg^{-1}$inoculated plants after 1st month (figure 30-31).

Whereas after 2nd month CAT activity was found to be maximum in roots of inoculated with rhizobacteria (0.114, 0.137 and 0.129) followed bythe microbial consortium (0.145, 0.121 and 0.132) and finally in mycorrhiza (0.074, 0.115 and 0.117) in control and As 50 and 100 $mgkg^{-1}$ treated plants respectively. After 3rd month, the trend changed from overall decline in CAT activity of roots in all plants as compared to 2nd month but maximum was observed in control and microbial consortium treated plants *viz* 0.068, 0.063 and 0.070 whereas (0.046, 0.056 and 0.061), (0.046, 0.147 and 0.060) in control (with treatment) and rhizobacteria and mycorrhiza (As 50 and 100 $mgkg^{-1}$) treated plants respectively.

Hence it was observed that with an increase in the concentration of arsenic, catalase activity was enhanced in plants with rhizobacterial cultures and decreased in both mycorrhizal and microbial consortium pots after the 1st month. Whereas after 2nd and 3rd-month activity was increased in both mycorrhiza and microbial consortium inoculated plants but decreased in rhizobacteria inoculated plants. In roots, CAT activity decreased with increase in concentration after the 1st month, increases after 2nd month and finally declined after a 3rd month (table 12).

In the case of pots containing *C. indica,* almost different trend was observed where CAT activity increased in all the arsenic (50 and 100 $mgkg^{-1}$) treated plants compared to control (treated and untreated) plants. Leaves and roots of *C. indica* plant showed less CAT activity as compared to *R. communis* plant but maximum CAT activity in root was observed in plants treated with the microbialconsortium in all 3 months. After 1st month, maximum CAT activity was found in leaves of control (microbial consortium treated) and microbial consortium (As 50 and 100 $mgkg^{-1}$) treated *C. indica* plants *viz* 0.060, 0.701 and 0.815 followed by mycorrhiza (0.112, 0.436 and 0.119) and rhizobacteria (0.011, 0.021 and 0.025Unit mg^{-1} protein FW) in control (microbial treated) and As 50 and 100 $mgkg^{-1}$inoculated plants respectively. Here, CAT activity increases with increase in the concentration of arsenic in plants inoculated with rhizobacteria and microbial consortium but the decline in CAT activity was observed in pots consisting of mycorrhiza. In case of *C. indica*roots, after 1st month enhanced CAT activity was observed in microbial consortium inoculated plants (0.039, 0.124 and 0.127) than in mycorrhiza (0.043, 0.039 and 0.055) and rhizobacteria (0.029, 0.030 and 0.047Unit mg^{-1} protein FW) in control (microbial treated) and As 50 and 100 $mgkg^{-1}$treated plants respectively.

After 2nd month, leaves of *C. indica* plant showed enhanced activity in microbial consortium inoculated plants (0.101, 0.797 and 0.880) followed by Mycorrhiza (0.221, 0.591 and 0.090) and rhizobacteria (0.023, 0.032 and 0.038) in control (microbial treatment) and As 50 and 100 $mgkg^{-1}$ inoculated plants respectively. Similar trend was observed in the rootsof *C. indica*, where maximum activity was noticed in microbial consortium (0.109, 0.129 and 0.156), mycorrhiza (0.096, 0.131 and 0.135) and rhizobacteria (0.056, 0.059 and 0.066 Unit mg^{-1} protein FW) in

control (microbial treated) and As 50 and 100 $mgkg^{-1}$inoculated plants respectively. CAT activity increased in both leaves and roots with an increase in the concentration of arsenic in all the plants. *C. indica* plant showed more CAT activity in roots as compared to leaves in all 3 months. Whereas after 2nd month maximum CAT activity in roots of *C. indica* was observed in plants inoculated with microbial consortium followed by mycorrhiza and rhizobacteria. After 3rd month, a significant decrease in CAT activity was observed in leaves and roots, where more decline was seen in case of leaves than in roots, in *C. indica* plants inoculated with rhizobacteria. As after 2ndand 3rd month, maximum CAT activity was found in leaves and roots of *C. indica* plant inoculated with the microbial consortium and least CAT activity was observed in rhizobacteria inoculated plants (table 12).

A study was conducted by Zhang *et al* (2014) on two cultivars of *R.communis* (Zibo 5 and Zibo 8) to check the effect of Cd on antioxidant enzymes. Results demonstrated higher catalase (CAT) activity after Cd treatment in Zibo 8 cultivar as compared to the other one. These findings are in agreement with the present study where CAT activity is increased in leaves and roots of plants at lower concentrations of heavy metals (As and Cd 50 $mgkg^{-1}$) but decreased at higher concentration (As and Cd 100 $mgkg^{-1}$).

As in arsenic-treated plants, similar results were detected in both the plants treated with cadmium (50 and 100 $mgkg^{-1}$). Overall CAT activity in both the plants (leaves and roots) was found to be more than control (microbial treated) plants. Enzyme activity increased after the1st month but a sudden decline was observed after a 3rd month in both the leaves and roots of *R. communis* and *C. indica* plants. After 1st month CAT activity was maximum in leaves and roots of *R. communis* plant inoculated with rhizobacteria, followed by plants inoculated with mycorrhiza and consortium. But after 2nd-month extreme activity was observed in leaves of *R. communis* inoculated with mycorrhiza followed by rhizobacteria and microbial consortium inoculated pots. Whereas in roots, CAT activity was highest in plants inoculated with microbial consortium following rhizobacteria and mycorrhiza treated plants.

After 3rd month CAT activity in leaves of *R. communis* was observed in increasing order of rhizobacteria < microbial consortium < mycorrhiza treated plants but in contrast CAT activity detected in roots was in order rhizobacteria < mycorrhiza < microbial consortium as given in table 13. An almost similar trend was observed in leaves and roots of *C. indica* plant where maximum CAT activity in leaves was detected in rhizobacteria inoculated plants after 1st and 2nd month but plants with microbial consortium possessed maximum activity in the 3rd month. On other hand, roots showed maximum activity in plants with the microbialconsortium in all the 3 months and least in rhizobacteria inoculated plants. Hence, we can conclude that roots of *C.*

indica plant inoculated with microbial consortium remarked excellent CAT activity as compared to leaves (Figure 32-33).

Another study was found to be in accordance with the present work, where the effect of Cu was seen on antioxidant enzymes in leaves and roots of *C. indica*. According to this study, CAT activity was enhanced at lower concentrations of copper and decreased with respect to time and concentration. Also, CAT activity was observed to be 5 times more in roots as compared to leaves of *C. indica* (Dibyendu.2012). During photo-respiration in peroxisomes, CAT removes excess H_2O_2 therefore, CAT activity is considered as one of the most important antioxidant enzyme that eradicates H_2O_2 by converting it into O_2 and H_2O (Noctor *et al*., 2000). Hence, this finding is in accordance with the results of Hegedus *et al* (2001) where a remarkable increase in CAT activity was seen under heavy metal stress.

Similarly, Shah *et al* (2001) reported the enhanced CAT activity in rice seedlings during the early period, under Cd stress in both the roots and leaves. For the removal of toxic peroxide, catalase (CAT) plays a key role, which is an oxidoreductase enzyme that scavenges H_2O_2 by producing oxygen and water (Lin *et al*., 2000) as discussed earlier. In addition to all these experiments, increase in CAT activity was also observed in various plant species treated with different concentrations of heavy metals like copper, lead, zinc etc. by Dinakar *et al*., 2008.

Table- 12: Catalase activity (Unit mg^{-1} protein FW) in leaves and roots of *Ricinus communis and Canna indica* inoculated with Arsenic (50 and 100 mgkg^{-1}) along with rhizobacteria, mycorrhiza and microbial consortium.

Time duration →	1st month				2nd Month				3rd Month			
Plants →	*R. communis*		*C. indica*		*R. communis*		*C. indica*		*R. communis*		*C. indica*	
Treatment ↓	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots
Control	0.016± 0.011^{a}	0.014± 0.001^{a}	0.073± 0.003^{g}	0.022± 0.023^{b}	0.129± 0.076^{k}	0.114± 0.019^{j}	0.044± 0.017^{d}	0.051± 0.009^{e}	0.139± 0.006^{l}	0.121± 0.003^{k}	0.056± 0.006^{e}	0.053± 0.005^{e}
C + As 50	0.064± 0.010^{f}	0.025± 0.006^{b}	0.011± 0.009^{a}	0.019± 0.032^{a}	0.111± 0.067^{j}	0.109± 0.034^{i}	0.028± 0.067^{b}	0.044± 0.055^{d}	0.124± 0.087^{k}	0.118± 0.032^{j}	0.131± 0.018^{k}	0.032± 0.098^{c}
C + As 100	0.111± 0.066^{j}	0.023± 0.023^{b}	0.019± 0.011a	0.011± 0.043^{a}	0.103± 0.016^{i}	0.121± 0.003^{k}	0.101± 0.103^{i}	0.058± 0.211^{e}	0.102± 0.810^{i}	0.024± 0.008^{b}	0.126± 0.007^{k}	0.024± 0.111^{b}
C +All B	0.089± 0.010^{h}	0.036± 0.006^{c}	0.011± 0.005^{a}	0.029± 0.007^{b}	0.125± 0.024^{k}	0.113± 0.007^{j}	0.023± 0.007^{b}	0.056± 0.004^{e}	0.118± 0.008^{j}	0.046± 0.010^{d}	0.063± 0.006^{f}	0.039± 0.003^{c}
As 50 + All B	0.149± 0.004^{l}	0.056± 0.006^{e}	0.021± 0.004^{b}	0.030± 0.004^{b}	0.391± 0.073^{n}	0.137± 0.004^{k}	0.032± 0.012^{c}	0.059± 0.010^{e}	0.310± 0.021^{n}	0.056± 0.004^{e}	0.037± 0.011^{c}	0.050± 0.012^{e}
As 100 + All B	0.150± 0.056^{l}	0.041± 0.013^{d}	0.025± 0.005^{b}	0.047± 0.005^{d}	0.375± 0.076^{n}	0.129± 0.003^{k}	0.038± 0.009^{c}	0.066± 0.014^{f}	0.283± 0.069^{m}	0.061± 0.001^{f}	0.026± 0.008^{b}	0.064± 0.009^{f}
C+ All M	0.116± 0.004^{j}	0.004± 0.007^{s}	0.112± 0.001^{j}	0.043± 0.011^{d}	0.228± 0.021^{m}	0.074± 0.009^{g}	0.221± 0.013^{m}	0.096± 0.007^{e}	0.121± 0.002^{k}	0.046± 0.006^{d}	0.173± 0.076^{r}	0.044± 0.009^{d}
As 50 + All M	0.218± 0.007^{m}	0.145± 0.014^{l}	0.436± 0.007^{n}	0.039± 0.009^{c}	0.445± 0.016^{n}	0.115± 0.004^{j}	0.591± 0.063^{o}	0.131± 0.010^{k}	0.370± 0.035^{n}	0.147± 0.008^{l}	0.279± 0.059^{m}	0.057± 0.002^{e}
As 100 + All M	0.115± 0.003^{j}	0.037± 0.007^{c}	0.019± 0.002^{a}	0.055± 0.016^{e}	0.388± 0.048^{n}	0.117± 0.009^{j}	0.090± 0.012^{h}	0.135± 0.010^{k}	0.294± 0.057^{m}	0.060± 0.004^{f}	0.190± 0.004^{r}	0.069± 0.011^{f}
C + Const	0.113± 0.008^{j}	0.025± 0.001^{b}	0.060± 0.003^{f}	0.009± 0.007^{s}	0.140± 0.012^{l}	0.145± 0.022^{l}	0.101± 0.007^{i}	0.109± 0.007^{i}	0.120± 0.010^{k}	0.068± 0.004^{f}	0.188± 0.003^{r}	0.084± 0.007^{h}
As 50 + Const.	0.131± 0.005^{k}	0.043± 0.016^{d}	0.701± 0.041^{p}	0.020± 0.003^{b}	0.311± 0.014^{n}	0.121± 0.014^{k}	0.797± 0.005^{p}	0.129± 0.008^{k}	0.238± 0.021^{m}	0.063± 0.003^{f}	0.738± 0.072^{p}	0.117± 0.009^{j}
As 100 +Const.	0.079± 0.006^{g}	0.031± 0.002^{c}	0.815± 0.018^{q}	0.127± 0.008^{k}	0.348± 0.009^{n}	0.132± 0.005^{k}	0.880± 0.012^{q}	0.156± 0.007^{l}	0.364± 0.057^{n}	0.070± 0.002^{f}	0.841± 0.057^{q}	0.106± 0.007^{i}

Mean ± SD (n=3).Different small alphabets (a-s) indicate statistically significant difference at $P \leq 0.05$ by Turkey's test (**C:** control**, As 50**: arsenic 50 mgkg^{-1}, **As 100**: arsenic 100 mgkg^{-1}, **All B:** all bacterial isolates (1-9), **All M**: All mycorrhiza cultures (1-3), **Const**: microbial consortium (All bacteria + all mycorrhiza).

Table 13: Catalase activity (Unit mg^{-1}Protein FW) in leaves and roots of *Ricinus communis* and *Canna indica* treated with Cadmium (50 and 100 mgkg^{-1}) along with rhizobacteria, mycorrhiza and microbial consortium.

Time duration →	1st month				2nd Month				3rd Month			
Plants →	*R. communis*		*C. indica*		*R. communis*		*C. indica*		*R. communis*		*C. indica*	
Treatment ↓	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots
Control	0.016± 0.011^{a}	0.014± 0.001^{a}	0.073± 0.003^{f}	0.022± 0.023^{b}	0.129± 0.076^{j}	0.114± 0.019^{i}	0.044± 0.017^{d}	0.051± 0.009^{e}	0.139± 0.006^{k}	0.121± 0.003^{j}	0.056± 0.006^{e}	0.053± 0.005^{e}
C + Cd 50	0.074± 0.001^{f}	0.031± 0.033^{c}	0.013± 0.023^{a}	0.019± 0.062^{a}	0.128± 0.054^{j}	0.115± 0.009^{i}	0.121± 0.006^{j}	0.064± 0.001^{e}	0.141± 0.032^{k}	0.038± 0.012^{c}	0.049± 0.054^{d}	0.039± 0.012^{c}
C + Cd 100	0.063± 0.014^{e}	0.023± 0.023^{b}	0.019± 0.034^{a}	0.011± 0.042^{a}	0.116± 0.066^{i}	0.141± 0.007^{k}	0.118± 0.011^{i}	0.046± 0.013^{d}	0.152± 0.004^{k}	0.031± 0.002^{c}	0.041± 0.004^{d}	0.028± 0.006^{b}
C + All B	0.089± 0.010^{f}	0.036± 0.006^{c}	0.011± 0.005^{a}	0.029± 0.007^{b}	0.125± 0.024^{j}	0.113± 0.007^{i}	0.023± 0.007^{b}	0.056± 0.004^{e}	0.118± 0.008^{i}	0.046± 0.010^{d}	0.063± 0.007^{e}	0.039± 0.005^{c}
Cd 50 + All B	0.467± 0.006^{n}	0.003± 0.004^{q}	0.457± 0.002^{n}	0.033± 0.008^{c}	0.638± 0.009^{o}	0.117± 0.002^{i}	0.571± 0.005^{o}	0.040± 0.012^{d}	0.558± 0.019^{n}	0.043± 0.002^{d}	0.466± 0.014^{n}	0.043± 0.006^{d}
Cd 100 + All B	0.109± 0.002^{h}	0.121± 0.007^{j}	0.111± 0.006^{i}	0.126± 0.007^{j}	0.620± 0.033^{o}	0.139± 0.006^{k}	0.236± 0.007^{m}	0.135± 0.009^{k}	0.409± 0.019^{n}	0.060± 0.003^{e}	0.152± 0.009^{k}	0.037± 0.006^{c}
C + All M	0.116± 0.004^{i}	0.040± 0.007^{d}	0.112± 0.001^{i}	0.043± 0.011^{d}	0.228± 0.021^{m}	0.074± 0.009^{f}	0.221± 0.013^{m}	0.096± 0.007^{g}	0.121± 0.002^{j}	0.046± 0.006^{d}	0.173± 0.076^{l}	0.044± 0.009^{d}
Cd 50 + All M	0.114± 0.004^{i}	0.055± 0.004^{e}	0.153± 0.014^{k}	0.023± 0.008^{b}	0.775± 0.026^{p}	0.098± 0.024^{g}	0.239± 0.006^{m}	0.067± 0.011^{e}	0.791± 0.014^{p}	0.069± 0.002^{e}	0.181± 0.020^{l}	0.055± 0.004^{e}
Cd 100 +All M	0.063± 0.007^{e}	0.044± 0.002^{d}	0.107± 0.019^{h}	0.021± 0.009^{b}	0.648± 0.036^{o}	0.126± 0.007^{j}	0.124± 0.017^{j}	0.077± 0.012^{f}	0.618± 0.008^{o}	0.060± 0.008^{e}	0.183± 0.007^{l}	0.061± 0.006^{e}
C + Const	0.013± 0.008^{a}	0.025± 0.001^{b}	0.060± 0.003^{e}	0.009± 0.007^{q}	0.140± 0.012^{k}	0.145± 0.022^{k}	0.101± 0.005^{h}	0.109± 0.007^{h}	0.120± 0.010^{j}	0.068± 0.004^{e}	0.188± 0.004^{l}	0.084± 0.007^{g}
Cd 50 +Const.	0.124± 0.004^{j}	0.027± 0.002^{b}	0.069± 0.008^{e}	0.107± 0.003^{h}	0.562± 0.022^{n}	0.179± 0.004^{m}	0.101± 0.014^{h}	0.132± 0.009^{k}	0.489± 0.047^{n}	0.056± 0.003^{e}	0.248± 0.015^{m}	0.119± 0.009^{i}
Cd 100+Const.	0.116± 0.003^{i}	0.030± 0.005^{c}	0.055± 0.004^{e}	0.128± 0.008^{j}	0.769± 0.011^{p}	0.167± 0.008^{l}	0.103± 0.013^{h}	0.134± 0.004^{k}	0.685± 0.019^{o}	0.065± 0.009^{e}	0.233± 0.031^{m}	0.111± 0.001^{i}

Mean ± SD (n=3). Different small alphabets (a-q) indicate statistically significant difference at $P \leq 0.05$ by Turkey's test (**C:** control, **Cd 50:** cadmium 50 mg kg^{-1}, **Cd 100:** cadmium 100 mg kg^{-1}, **All B:** all bacterial isolates (1-9), **All M:** All mycorrhiza cultures (1-3), **Const:** microbial consortium (All bacteria + all mycorrhiza).

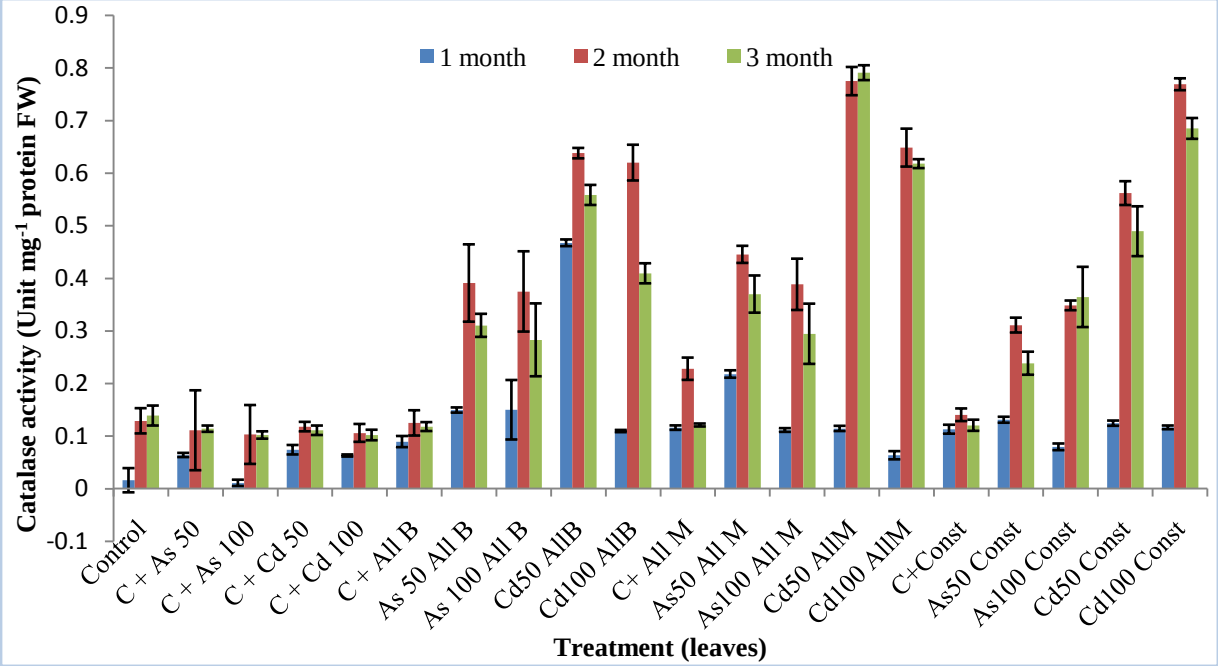

Figure 30: Catalase activity (Unit mg^{-1}Protein FW) in leaves of *Ricinus communis* inoculated with arsenic and cadmium (50 and 100 $mgkg^{-1}$) along with rhizobacteria, mycorrhiza and microbial consortium.

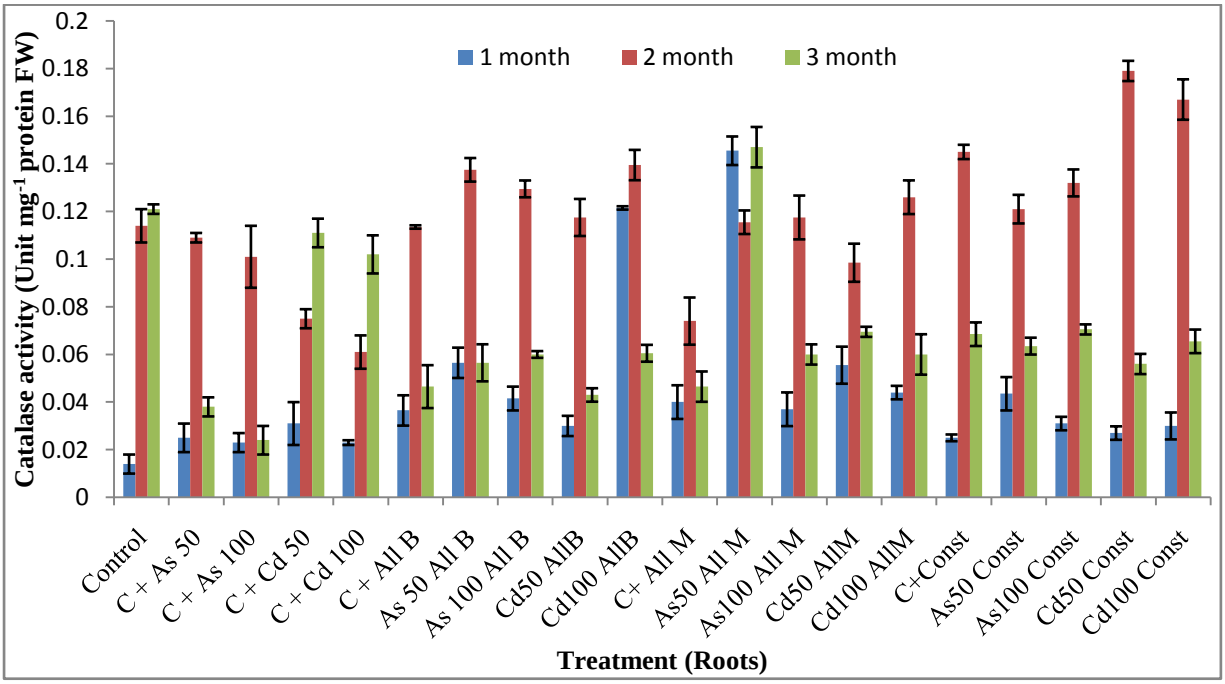

Figure 31: Catalase activity (Unit mg^{-1}Protein FW) in roots of *Ricinus communis* inoculated with arsenic and cadmium (50 and 100 $mgkg^{-1}$) along with rhizobacteria, mycorrhiza and microbial consortium.

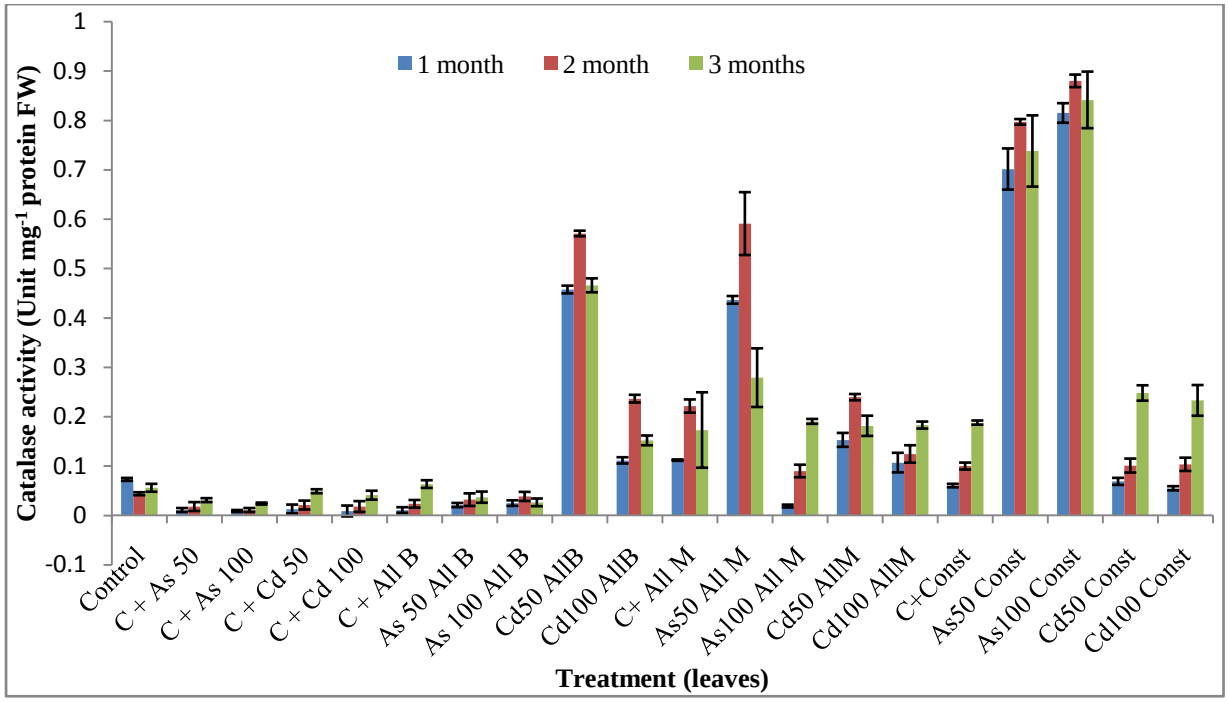

Figure 32: Catalase activity (Unit mg^{-1}Protein FW) in leaves of *Canna indica* inoculated with arsenic and cadmium (50 and 100 $mgkg^{-1}$) along with rhizobacteria, mycorrhiza and microbial consortium.

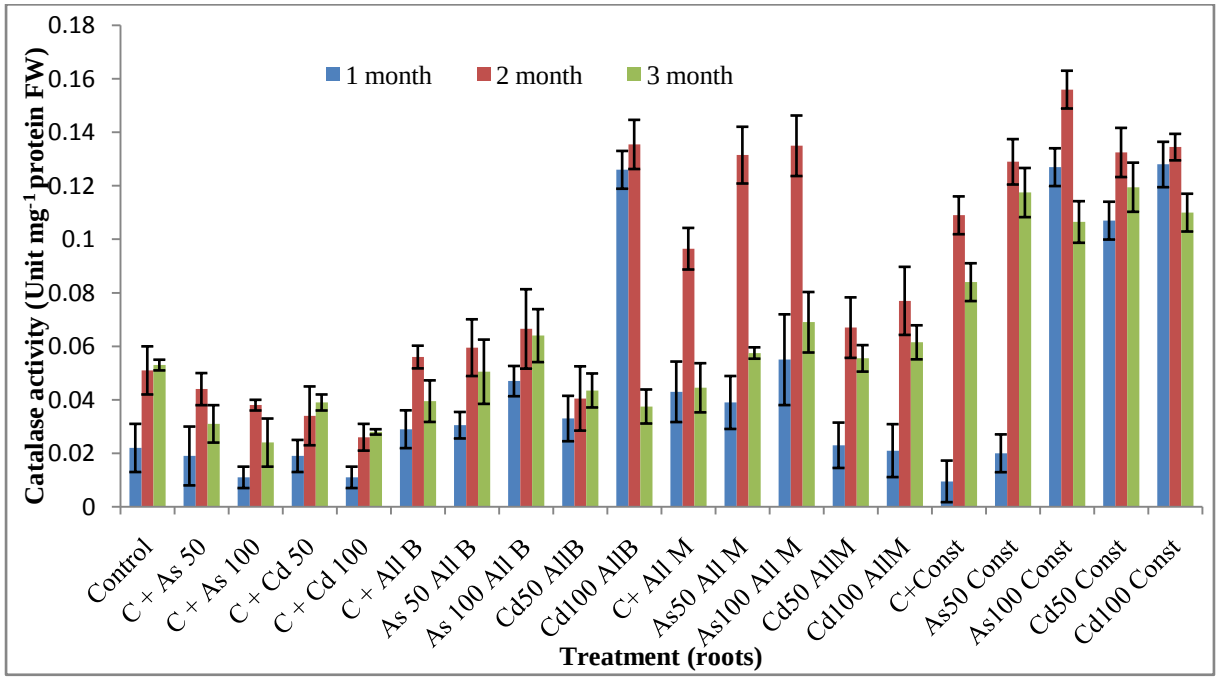

Figure 33: Catalase activity (Unit mg^{-1}Protein FW) in leaves of *Canna indica* inoculated with arsenic and cadmium (50 and 100 $mgkg^{-1}$) along with rhizobacteria, mycorrhiza and microbial consortium.

6.15.2 Ascorbate peroxidase (APX) activity in *Ricinus communis* and *Canna indica* inoculated with two concentrations of arsenic and cadmium (50 and 100 $mgkg^{-1}$) along with rhizobacteria, mycorrhiza and a microbial consortium

R. communis and *C. indica* grew under metal contaminated soil generally showed a significant increase in ascorbate peroxidase activity (APX) in plant parts compared to non stressed plants. In this study, APX activity was observed to be higher in almost all the microbial inoculated plants as compared to all the control plants (without microbial treatment). This can account for the presence of rhizobacterial, mycorrhizal cultures and microbial consortium in the plants that have enhanced the activity of APX. In contrast, in plants treated with only heavy metals and normal control plants (without any treatment) led to a decrease in the activity of APX than microbial treated plants. In addition, APX activity in most of the *R. communis* plants was found to be more pronounced in roots with time.

After 1^{st} and 2^{nd} month of sampling, APX activity was found to be maximum in control and mycorrhiza inoculated*R. communis* plant leaves *viz*, 0.475, 0.369 and 0.322 and 0.696, 0.622 and 0.647 Unit mg^{-1} Protein FWwith two different concentrations of arsenic (control, As 50 and 100 $mgkg^{-1}$) respectively. But after 3^{rd} month, APX activity was enhanced in leaves of *R. communis* plant inoculated with microbial consortium i.e 0.682, 0.677 and 0.671 in control and treated pots (As 50 and 100 $mgkg^{-1}$) respectively followed by mycorrhiza (0.676, 0.670 and 0.675) and rhizobacteria (0.620, 0.560 and 0.556) inoculated plants (in control, As 50 and 100 $mgkg^{-1}$) respectively (figure 34).

In roots of *R. communis* plant inoculated with arsenic and microbial consortium, APX activity was observed to be maximum in all the 3 months compared to the leaves. Also, APX activity increased in both the leaves and roots with time (from 1^{st} to 3^{rd} month) but decrease with increase in the concentration of arsenic in 1^{st} and 3^{rd} month compared to 2^{nd} month. APX activity of *R. communis* plant roots in control and microbial consortium treated plants (As 50 and 100 $mgkg^{-1}$) was found to be 0.405, 0.461 and 0.425 after 1^{st} month, 0.721, 0.90 and 0.956 2^{nd} month and 0.941, 1.206 and 1.122 3^{rd} month followed by mycorrhiza and rhizobacteria inoculated plants (control, As 50 and 100 $mgkg^{-1}$) respectively (figure 35).

Unlike results were found in the leaves and roots of *C. indica* plant inoculated with two arsenic concentrations (50 and 100 $mgkg^{-1}$). APX activity in leaves and roots of *C. indica* was found to be maximum in microbial consortium inoculated plants followed by mycorrhiza and rhizobacteria inoculated plants in all the 3 months. APX activity was also found to enhance with time in all the control and treated pots as given in table 14. APX activity was 0.528, 0.439 and 0.430 in leaves and 0.633, 0.625 and 0.651 in roots of control and microbial consortium inoculated plants (50 and 100 $mgkg^{-1}$) respectively after 1^{st} month. Similarly, in 2^{nd} month leaves

(0.718, 0.711 and 0.708) roots (0.736, 0.739 and 0.752) and 3rd month leaves (0.725, 0.704 and 0.719) and roots (0.737, 0.756 and 0.764 Unit mg^{-1} protein FW) of *C. indica* plant showed maximal APX activity in control and microbial consortium inoculated (As 50 and 100 mg kg^{-1}) plants respectively. Therefore from these results, we can conclude that leaves and roots of *C. indica* plant showed higher APX activity in the plants treated with the microbial consortium in all the 3 months as compared to *R. communis* plant (Figure 34-37).

Our study was found to be in coherence with findings of Zhang *et al* (2015) where 2-fold fold increase in APX activity was observed in the roots of *R. communis* treated with cadmium. Feasible clarification of decline in APX activity in *R. communis* leaves may be due to lack of iron (Fe) in APX- metalloprotein complex (Pandey *et al*., 2002). Different reports on APX activity also suggests the same where APX activity was increased in plants under Cd stress (Mishra*et al*., 2006, 2008; Ahammed *et al*., 2013).

Similarly, the results obtained for *C. indica* were also in accordance with the study of Dibyendu (2013) which showed the increase of APX activity in the leaves as compared to control at increasing concentrations of copper but decreased APX activity in leaves of *C. indica* at higher concentration, which was found in our study as well. Also, roots of *C. indica* possessed higher APX activity than leaves and decrease in APX activity was noticed with increasing concentration in comparison to control (Figure 36-37).

According to Hossain *et al* (1984), reduction in APX activity in lower levels of ascorbate concentration was due to prompt inactivation of isoforms of the chloroplast, especially in H_2O_2 presence. Even, Carvalho (2008) explained that the inhibition of APX was due to the higher potential of excess H_2O_2 to attack it. Since APX is a universal enzyme and strong H_2O_2 scavenger should retain its amount as it may have an unfavourable effect if APX is exported from organelles to cytosol due to the formation of OH radicals via metal catalysed Haber-Weiss reaction (Asada, 1992).

In *R. communis* and *C. indica* plants APX activity, a major component is an Ascorbate-Glutathione pathway that plays a vital role in H_2O_2 scavenging increases with increase in concentration and time period, demonstrating the effectiveness of H_2O_2 scavenging system in both the plants. Enhanced levels of APX under As and Cd generated oxidative stress justify its role in H_2O_2 detoxification. APX showed better efficiency than catalase in destroying H_2O_2 under As and Cd stress which is in agreement with the results of the present study. The reason for this could be the presence of APX throughout the cell and possessing higher substrate similarity in the presence of ascorbic acid as a reductant (Sasaki-Sekimoto*et al*., 2005).

APX activity was also triggered in the *R. communis* and *C. indica* plants under cadmium stress. In *R. communis* plants treated with mycorrhiza and different concentrations of cadmium (50 and

100 mg kg $^{-1}$), leaves showed more APX activity after 1st month followed by microbial consortium and rhizobacteria inoculated plants. After 2nd and 3rd month, the APX activity in leaves of *R. communis* plant increased in the following order of treatment Rhizobacteria < Mycorrhiza < microbial consortium.

The remarkable increase in the APX activity was observed with increase in concentration (50 and 100 mgkg^{-1}) of cadmium in all the 3 months in *R. communis*. Further, roots of *R. communis* plant witnessed increased APX activity in microbial inoculated plants than all control plants in 3 months. Maximum APX activity was seen in roots of the plant inoculated with the microbialconsortium in 3 months. The increasing pattern of APX activity was found to be the same in 3 months in roots of *R. communis* i.e Rhizobacteria < Mycorrhiza < microbial consortium in the roots of *R. communis* plants. Whereas the APX activity in a 1st month was decreased in roots with an increase in the concentration of cadmium but in 2nd and 3rd-month APX activity was increased with an increase in metal concentration. The overall increase in APX was observed in roots of *R. communis* with increasing concentration of heavy metals (arsenic and cadmium) as shown in figure 35.

Apart from activity in leaves and roots of *R. communis* plant, *C. indica* plant also possesses the good capability to overcome the ROS generated during heavy metals stress. In both the leaves and roots of *C. indica*, APX activity was found to be maximal in microbial consortium than in mycorrhiza and rhizobacteria inoculated plants. As already discussed in *R. communis* plant, a significant increase in APX activity was determined from 1st to 3rd month in roots of all the microbial inoculated plants where tremendous results were observed in microbial consortium followed by mycorrhiza and rhizobacteria inoculated plants. Variable results were found in both leaves and roots but maximum APX activity was seen in roots compared to leaves in *C. indica* plant.With increasing concentration of metals, APX activity reduced in plants inoculated with mycorrhiza and rhizobacteria after the 1st month. In contrast, activity in leaves and roots of microbial consortium inoculated plants increased significantly with increase in the concentrationof heavy metals in all the 3 months. After, 2nd and 3rd month overall significant increase in APX activity in roots and leaves were observed in *C. indica* with increasing concentration of Cd (table 15).

Table-14: Ascorbate peroxidase activity (Unit mg^{-1}protein FW) in leaves and roots of *Ricinus communis* and *Canna indica* inoculated with Arsenic (50 and 100 $mgkg^{-1}$) along with rhizobacteria, mycorrhiza and microbial consortium.

Time duration →	1st month				2nd Month				3rd Month			
Plants →	*R. communis*		*C. indica*		*R. communis*		*C. indica*		*R. communis*		*C. indica*	
Treatment ↓	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots
Control	0.128± 0.014^{a}	0.114± 0.012^{a}	0.121± 0.011^{a}	0.191± 0.002^{b}	0.224± 0.017^{e}	0.124± 0.009^{a}	0.134± 0.008^{a}	0.234± 0.006^{e}	0.443± 0.002^{c}	0.236± 0.022^{e}	0.256± 0.005^{d}	0.342± 0.006^{f}
C + As 50	0.198± 0.033^{b}	0.192± 0.002b	0.191± 0.019^{b}	0.201± 0.081^{e}	0.448± 0.005^{c}	0.311± 0.002^{f}	0.413± 0.006^{c}	0.424± 0.004^{c}	0.528± 0.001^{h}	0.343± 0.006^{f}	0.374± 0.009^{f}	0.481± 0.002^{g}
C + As 100	0.184± 0.017^{b}	0.186± 0.003^{b}	0.183± 0.012^{b}	0.194± 0.011^{b}	0.428± 0.006^{c}	0.303± 0.013f	0.405± 0.019^{c}	0.418± 0.006^{c}	0.519± 0.008^{h}	0.329± 0.017^{f}	0.368± 0.013^{f}	0.473± 0.012^{g}
C + All B	0.414± 0.012^{c}	0.201± 0.005^{e}	0.221± 0.002^{e}	0.229± 0.004^{e}	0.617± 0.007^{i}	0.322± 0.014^{f}	0.436± 0.003^{c}	0.446± 0.007^{c}	0.620± 0.002^{i}	0.35± 0.001^{f}	0.545± 0.005^{h}	0.566± 0.003^{h}
As 50 + All B	0.282± 0.001^{d}	0.308± 0.012^{f}	0.198± 0.003^{b}	0.224± 0.004^{e}	0.531± 0.002^{h}	0.564± 0.007^{h}	0.321± 0.009^{f}	0.468± 0.004^{g}	0.560± 0.012^{h}	0.607± 0.006^{i}	0.417± 0.007^{c}	0.561± 0.010^{h}
As 100 + All B	0.206± 0.007^{e}	0.218± 0.007^{e}	0.195± 0.005^{b}	0.237± 0.004^{e}	0.530± 0.004^{h}	0.676± 0.007^{i}	0.441± 0.002^{c}	0.469± 0.004^{g}	0.556± 0.007^{i}	0.717± 0.003^{j}	0.515± 0.008^{h}	0.573± 0.006^{h}
C + All M	0.475± 0.036^{g}	0.320± 0.012^{f}	0.420± 0.012^{c}	0.557± 0.009^{h}	0.696± 0.007^{i}	0.517± 0.008^{h}	0.617± 0.008^{i}	0.607± 0.006^{i}	0.676± 0.021^{i}	0.721± 0.013^{j}	0.667± 0.006^{i}	0.680± 0.012^{i}
As 50 + All M	0.369± 0.010^{f}	0.341± 0.028^{f}	0.434± 0.015^{c}	0.567± 0.006^{h}	0.622± 0.014^{i}	0.678± 0.013^{i}	0.413± 0.010^{c}	0.617± 0.007^{i}	0.670± 0.011^{i}	0.827± 0.019^{k}	0.445± 0.004^{c}	0.695± 0.002^{i}
As 100 + All M	0.322± 0.014^{f}	0.345± 0.062^{f}	0.228± 0.008^{e}	0.575± 0.004^{h}	0.647± 0.002^{i}	0.785± 0.022^{j}	0.608± 0.007^{i}	0.628± 0.003i	0.675± 0.008^{i}	0.88± 0.001^{k}	0.640± 0.012^{i}	0.717± 0.007^{j}
C + Const	0.422± 0.012^{c}	0.405± 0.023^{c}	0.528± 0.007^{h}	0.633± 0.006^{i}	0.672± 0.002^{i}	0.721± 0.013^{j}	0.718± 0.008^{j}	0.736± 0.001^{j}	0.682± 0.012^{i}	0.941± 0.014^{l}	0.725± 0.003^{j}	0.737± 0.004^{j}
As 50 + Const.	0.363± 0.005^{f}	0.461± 0.010^{g}	0.439± 0.014^{c}	0.625± 0.005^{i}	0.606± 0.007^{i}	0.90± 0.015^{l}	0.711± 0.010^{j}	0.739± 0.009^{j}	0.677± 0.008^{i}	1.206± 0.004^{m}	0.704± 0.004^{j}	0.756± 0.001^{j}
As 100 +Const.	0.212± 0.015^{e}	0.425± 0.003^{c}	0.430± 0.010^{c}	0.651± 0.004^{i}	0.635± 0.004^{i}	0.956± 0.077^{l}	0.708± 0.007^{j}	0.752± 0.004^{j}	0.671± 0.011^{i}	1.122± 0.013^{m}	0.719± 0.002^{j}	0.764± 0.003^{j}

Mean ± SD (n=3). Different small alphabets (a-m) indicate statistically significant difference at $P \leq 0.05$ by Turkey's test (**C:** control, **As 50:** arsenic 50 $mgkg^{-1}$, **As 100**: arsenic 100 $mgkg^{-1}$, **All B**: all bacterial isolates (1-9), **All M**: All mycorrhiza cultures (1-3), **Const:** microbial consortium (All bacteria + all mycorrhiza)

Table-15: Ascorbate peroxidase activity (Unit mg^{-1} Protein FW) in leaves and roots of *Ricinus communis* and *Canna indica* inoculated with Cadmium (50 and 100 $mgkg^{-1}$) along with rhizobacteria, mycorrhiza and microbial consortium.

Time duration →	1st month				2nd Month				3rd Month			
Plants →	*R. communis*		*C. indica*		*R. communis*		*C. indica*		*R. communis*		*C. indica*	
Treatment ↓	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots
Control	0.128± 0.014^{a}	0.114± 0.012^{a}	0.121± 0.011^{a}	0.191± 0.002^{b}	0.224± 0.017^{e}	0.124± 0.009^{a}	0.134± 0.008^{a}	0.234± 0.006^{e}	0.443± 0.002^{c}	0.236± 0.022^{e}	0.256± 0.005^{d}	0.342± 0.006^{f}
C + Cd 50	0.389± 0.002^{c}	0.198± 0.004^{a}	0.184± 0.006^{a}	0.211± 0.032^{b}	0.494± 0.007^{d}	0.310± 0.032^{c}	0.381± 0.009^{c}	0.412± 0.003^{d}	0.481± 0.007^{d}	0.313± 0.004^{c}	0.483± 0.009^{d}	0.492± 0.012^{d}
C + Cd 100	0.371± 0.003^{c}	0.184± 0.012^{a}	0.178± 0.023^{a}	0.202± 0.043^{b}	0.481± 0.044^{d}	0.308± 0.022^{c}	0.374± 0.015^{c}	0.408± 0.051^{d}	0.473± 0.045^{d}	0.301± 0.076^{c}	0.472± 0.011^{d}	0.483± 0.009^{d}
C + All B	0.414± 0.012^{d}	0.201± 0.005^{b}	0.221± 0.002^{b}	0.229± 0.004^{b}	0.617± 0.007^{f}	0.322± 0.014^{c}	0.436± 0.003^{d}	0.446± 0.007^{d}	0.620± 0.002^{f}	0.350± 0.001^{c}	0.545± 0.005^{e}	0.566± 0.003^{e}
Cd 50 + All B	0.508± 0.010^{e}	0.619± 0.011^{f}	0.214± 0.007^{b}	0.234± 0.007^{b}	0.573± 0.011^{e}	0.806± 0.007^{h}	0.453± 0.040^{d}	0.454± 0.004^{d}	0.570± 0.002^{e}	0.909± 0.002^{h}	0.539± 0.003^{e}	0.583± 0.006^{e}
Cd 100 + All B	0.520± 0.049^{e}	0.492± 0.008^{d}	0.197± 0.008^{a}	0.245± 0.004^{b}	0.583± 0.007^{e}	0.780± 0.012^{g}	0.463± 0.005^{d}	0.485± 0.005^{d}	0.573± 0.012^{e}	0.990± 0.001^{h}	0.545± 0.003^{e}	0.591± 0.004^{e}
C + All M	0.475± 0.036^{d}	0.320± 0.012^{c}	0.420± 0.012^{d}	0.557± 0.009^{e}	0.696± 0.007^{f}	0.517± 0.005^{e}	0.617± 0.007^{f}	0.607± 0.006^{f}	0.676± 0.021^{f}	0.721± 0.013^{g}	0.667± 0.006^{f}	0.680± 0.012^{f}
Cd 50 + All M	0.519± 0.019^{e}	0.969± 0.006^{h}	0.322± 0.004^{c}	0.563± 0.006^{e}	0.620± 0.012^{f}	1.066± 0.063^{j}	0.621± 0.009^{f}	0.647± 0.005^{f}	0.676± 0.007^{f}	1.109± 0.002^{j}	0.635± 0.008^{f}	0.704± 0.007^{f}
Cd 100 +All M	0.529± 0.010^{e}	0.793± 0.065^{g}	0.206± 0.007^{b}	0.577± 0.006^{e}	0.671± 0.009^{f}	1.116± 0.007^{j}	0.612± 0.006^{f}	0.647± 0.005^{f}	0.696± 0.003^{f}	0.986± 0.006^{i}	0.625± 0.009^{f}	0.712± 0.009^{g}
C + Const	0.422± 0.010^{d}	0.405± 0.023^{d}	0.528± 0.007^{e}	0.633± 0.007^{f}	0.672± 0.002^{f}	0.721± 0.013^{g}	0.718± 0.008^{g}	0.736± 0.007^{g}	0.682± 0.012^{f}	0.941± 0.014^{i}	0.725± 0.007^{g}	0.737± 0.004^{g}
Cd 50 + Const.	0.511± 0.014^{e}	0.797± 0.091^{g}	0.493± 0.011^{d}	0.619± 0.009^{f}	0.669± 0.002^{f}	1.086± 0.062^{j}	0.705± 0.004^{g}	0.771± 0.008^{g}	0.685± 0.004^{f}	1.221± 0.014^{j}	0.723± 0.007^{g}	0.772± 0.009^{g}
Cd 100+Const.	0.545± 0.016^{e}	0.875± 0.074^{h}	0.495± 0.009^{d}	0.629± 0.007^{f}	0.683± 0.010^{f}	1.237± 0.132^{j}	0.713± 0.002^{g}	0.781± 0.004^{g}	0.693± 0.006^{f}	1.322± 0.014^{j}	0.740± 0.007^{g}	0.790± 0.005^{g}

Mean ± SD (n=3). Different small alphabets (a-j) indicate statistically significant difference at $P \leq 0.05$ by Turkey's test (**C**: control, **Cd 50**: cadmium 50 $mgkg^{-1}$, **Cd 100**: cadmium 100 $mgkg^{-1}$, **All B:** all bacterial isolates (1-9), **All M:** All mycorrhiza cultures (1-3), **Const:** microbial consortium (All bacteria + all mycorrhiza)

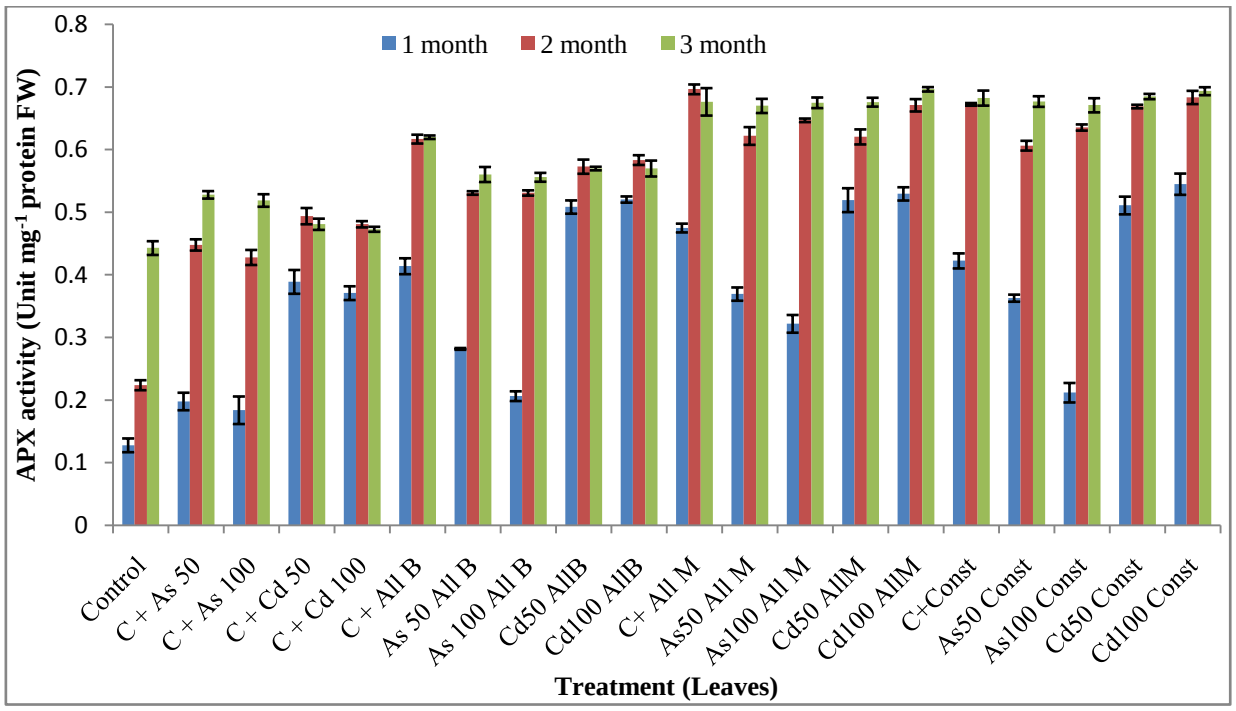

Figure 34: Ascorbate peroxidase activity (Unit mg^{-1} Protein FW) in leaves of *Ricinus communis* inoculated with arsenic and cadmium (50 and 100 $mgkg^{-1}$) along with rhizobacteria, mycorrhiza and microbial consortium.

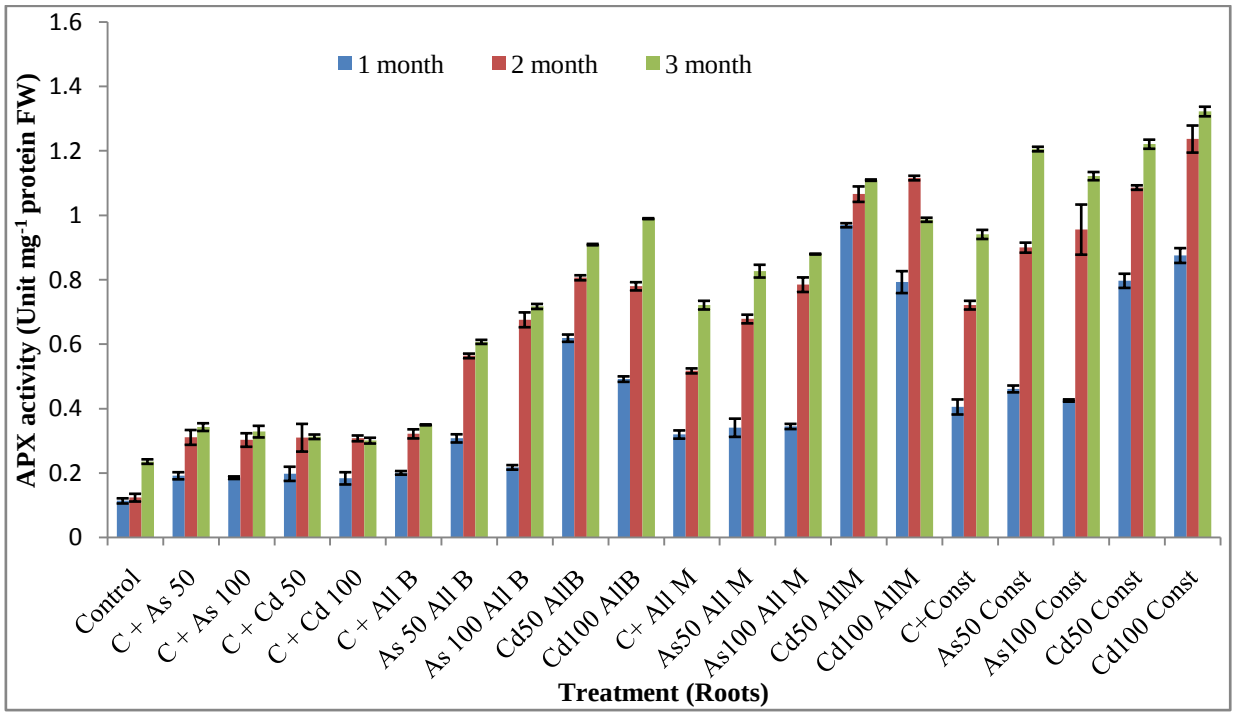

Figure 35: Ascorbate peroxidase activity (Unit mg^{-1} Protein FW) in roots of *Ricinus communis* inoculated with arsenic and cadmium (50 and 100 $mgkg^{-1}$) along with rhizobacteria, mycorrhiza and microbial consortium.

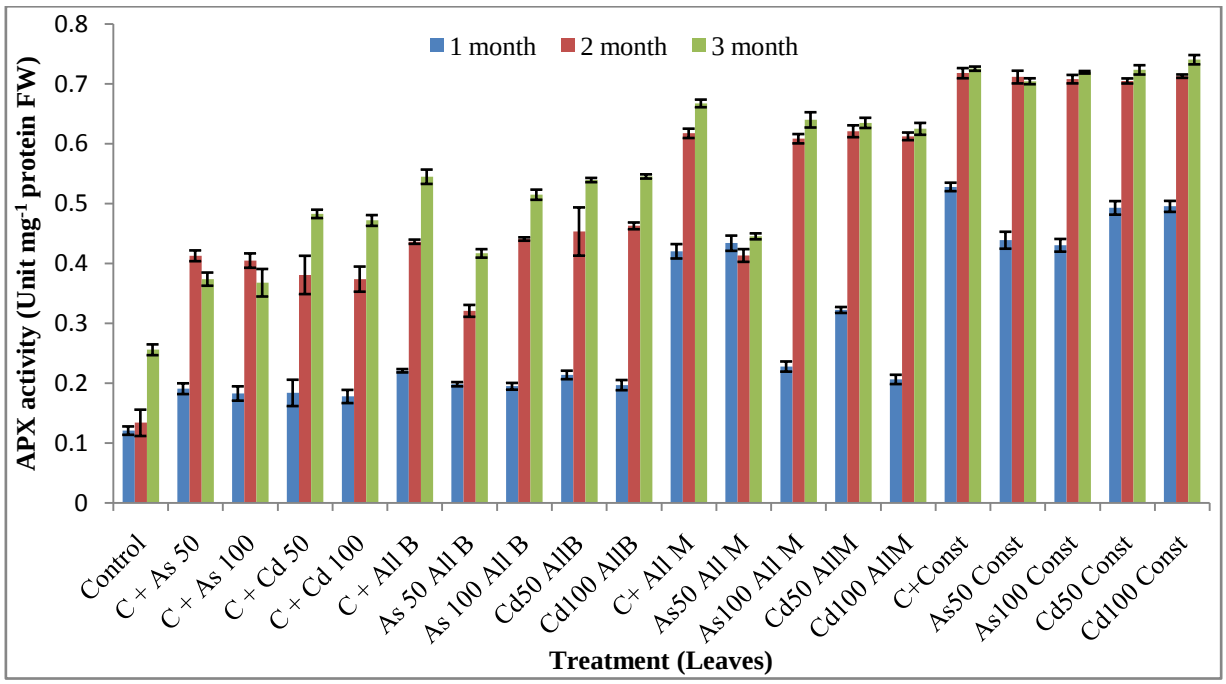

Figure 36: Ascorbate peroxidase activity (Unit mg^{-1} Protein FW) in leaves of *Canna indica* inoculated with arsenic and cadmium (50 and 100 $mgkg^{-1}$) along with rhizobacteria, mycorrhiza and microbial consortium.

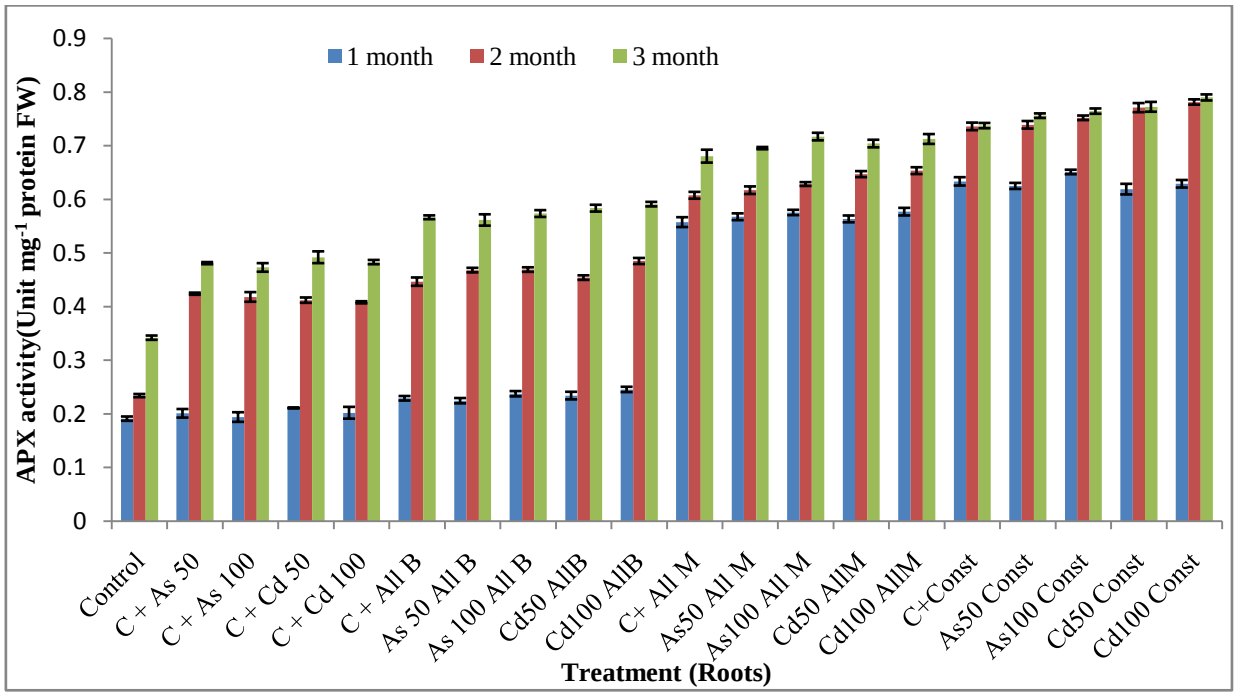

Figure 37: Ascorbate peroxidase activity (Unit mg^{-1} Protein FW) in roots of *Canna indica* inoculated with arsenic and cadmium (50 and 100 $mgkg^{-1}$) along with rhizobacteria, mycorrhiza and microbial consortium.

6.15.3 Glutathione reductase (GR) activity in *Ricinus communis* and *Canna indica* inoculated with two concentrations of arsenic and cadmium (50 and 100 mgkg^{-1}) along with rhizobacteria, mycorrhiza and a microbial consortium

Glutathione reductase (GR) activity was found to be time and concentration-dependent under two different concentrations (50 and 100 mgkg^{-1}) of heavy metals (As and Cd). GR activity in *R. communis* and *C. indica* plant (leaves and roots) under As stress inoculated with Rhizobacteria, mycorrhiza and the microbialconsortium was found to be more than all control plants 3 months of experimentation.

Maximum GR activity was found in both the leaves and roots of *R. communis* plant inoculated with the microbialconsortium in 3 months. 0.015, 0.038 and 0.048 were recorded values of GR activity in leaves of control and microbial consortium (As 50 and 100 mgkg^{-1}) inoculated *R. communis* plant after 1st month and 0.063, 0.071, 0.075 in roots of control and microbial consortium (As 50 and 100 mgkg^{-1}) respectively. After 2nd month GR activity in leaves and roots of microbial consortium inculcated plants was found to be 0.044, 0.060, 0.063 and 0.086, 0.094, 0.104 (control, As 50 and 100 mgkg^{-1}) respectively. Similarly,the 3rd month also showed elevated levels of GR activity in leaves and roots of microbial consortium inoculated pots *viz*, 0.042, 0.058, 0.059 and 0.075, 0.085 and 0.096 (control, As 50 and 100 mgkg^{-1}) respectively. The GR activity was observed in both the leaves and roots of inoculated *R. communis* plants in increasing order of rhizobacteria < mycorrhizal < microbial consortium in the overall experimentation period (1st-3rd month). GR activity significantly increased with increasing concentration of arsenic in leaves and roots in all the 3 months as shown in table 16.

All treated *R. communis* plant roots depicted the highest activity in comparison to leaves of the plant. Identical results were noticed in case of *C. indica* where maximal GR activity was seen in plants impregnated with microbial consortium than rhizobacteria and mycorrhiza inoculated plants. Both the leaves and roots of all the treated plants exhibit the best results during the experimentation period of 3 months where roots had shown more GR activity than leaves (table 16). All 3 months inoculated plants (leaves and roots) showed the GR activity in an increasing order of Rhizobacteria<Mycorrhiza<microbial consortium in *R. communis* plant. However, the overall increase in GR activity in treated *C. indica* plant (leaves and roots) was found with increasing concentration of arsenic with respect to the time period as given in table 16. As both the plants showed maximum GR activity in roots of the microbial consortium implanted pots, hence we can further relate that roots of both the plants accumulated more arsenic as compared to shoots. Indistinguishable results of GR activity were found in cadmium-treated plants where maximum up-regulation of GR activity in leaves and roots of plants inoculated with microbial consortium was observed at 100 mgkg^{-1} i.e higher concentration in both the plants (*R. communis*

and *C. indica*). Here, roots depicted higher GR activity than leaves of both the plants which were again related to higher Cd metal accumulation in roots than shoots (table 17). In all the 3 months of treatment, GR activity was found to be in increasing order as follows: rhizobacteria < mycorrhiza < microbial consortium. Even GR activity in both the plants increased with increasing metal (Cd) concentration (Figure 38-41).

GR activity of the present study was found in accordance with Baudh *et al* (2015). Their study showed a significant change in GR activity under Cd stress in both the leaves and roots of *R. communis*. GR activity was found to be more in roots than leaves of *R. communis* plant as compared to control. GR is present in mitochondria, cytoplasm, chloroplasts and acts as a catalyst for the asc-GSH pathway. SR is used to conserve the GSSH in its reduced form at elevated levels in the cell since GSH in its reduced form is a free radical scavenger. In our study, increased GR activity in both the roots and leaves of *R. communis* and *C. indica* was observed than control. For the root tissues, higher GR activity was seen when compared to leaves. Higher GR activity preserves the NADP+/NADPH ratio and verify the proper functioning of the photosynthetic electron transport chain and leads to a reduction of O_2 and superoxide radicals formation (Sudhakar *et al*., 2001). Similar results were observed for *C. indica* in the study of Dibyendu (2012). Under Cu stress,a significant increase in GR activity was observed in roots and leaves of *C. indica*. Glutathione reductase (GR) is associated with recycling of reduced forms of AsA and GSH along with 3 fold rise in GR activity in *C. indica* leaves that further assists the plant to prolong ascorbic acid and glutathione in a suitable concentration. The stressed plants require the activation of Asc-Glu cycle to tackle with oxidative stress caused by heavy metals. According to a report given by Foyer *et al* (2010), GR is a major enzyme that assists in the GSSG to GSH reduction by oxidation of NADPH to NADP and its key role in fighting with oxidative stress was proposed.

In the present study, it was seen that GR activity was increased in leaves and roots of *R. communis* and *C. indica* plants exposed to As and Cd at two different concentrations. The elevation of GR activity under heavy metal stress can be due to excessive cellular consumption of reduced GSH. The increase in GR activity in leaves and roots of plants in present work was supported by some earlier reports of GR activity up-regulation under heavy metal stress (Diwan *et al*., 2010; Cherif *et al*., 2011). Another report was given by Laspina *et al* (2005) in which *Helianthus annus* showed increased GR activity under Cd and Zn stress (Nehnevajova *et al*., 2012). Therefore, the tolerance developed by the plant against heavy metals is suggested by the increase in CAT, APX and GR activities.

Table-16: Glutathione reductase activity (Unit mg^{-1} Protein FW) in leaves and roots of *Ricinus communis and Canna indica* inoculated with Arsenic (50 and 100 mgkg^{-1}) along with rhizobacteria, mycorrhiza and microbial consortium.

Time duration →	1st month				2nd Month				3rd Month			
Plants →	*R. communis*		*C. indica*		*R. communis*		*C. indica*		*R. communis*		*C. indica*	
Treatment ↓	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots
Control	0.022± 0.003^{c}	0.026± 0.004^{c}	0.009± 0.006^{a}	0.008± 0.022^{a}	0.018± 0.005^{b}	0.052± 0.011^{f}	0.015± 0.051^{b}	0.025± 0.004^{c}	0.022± 0.008^{c}	0.065± 0.009^{g}	0.027± 0.001^{c}	0.026± 0.054^{c}
C + As 50	0.012± 0.019^{b}	0.034± 0.016^{e}	0.011± 0.011^{b}	0.013± 0.004^{b}	0.021± 0.002^{c}	0.048± 0.006^{e}	0.021± 0.009^{c}	0.029± 0.023^{c}	0.031± 0.004^{d}	0.052± 0.009^{f}	0.018± 0.011^{b}	0.016± 0.002^{b}
C + As 100	0.08± 0.005^{a}	0.029± 0.003^{f}	0.008± 0.008^{a}	0.007± 0.001^{a}	0.017± 0.011^{b}	0.034± 0.017^{d}	0.014± 0.004^{b}	0.017± 0.006^{b}	0.021± 0.013^{c}	0.048± 0.041^{e}	0.011± 0.022^{b}	0.008± 0.025^{a}
C + All B	0.015± 0.002^{b}	0.047± 0.002^{e}	0.006± 0.001^{a}	0.012± 0.001^{b}	0.029± 0.006^{c}	0.064± 0.004^{g}	0.019± 0.002^{b}	0.030± 0.002^{d}	0.034± 0.004^{d}	0.063± 0.003^{g}	0.024± 0.003^{c}	0.026± 0.002^{c}
As 50 + All B	0.020± 0.003^{c}	0.055± 0.004^{f}	0.017± 0.002^{b}	0.024± 0.004^{c}	0.051± 0.007^{f}	0.076± 0.002^{h}	0.028± 0.007^{c}	0.038± 0.003^{d}	0.057± 0.006^{f}	0.069± 0.002^{g}	0.034± 0.004^{d}	0.035± 0.004^{d}
As 100 + All B	0.025± 0.002^{c}	0.058± 0.006^{f}	0.019± 0.002^{b}	0.031± 0.003^{d}	0.059± 0.010^{f}	0.083± 0.001^{i}	0.034± 0.004^{d}	0.044± 0.003^{e}	0.058± 0.006^{f}	0.074± 0.004^{h}	0.038± 0.004^{d}	0.040± 0.002^{e}
C + All M	0.017± 0.002^{b}	0.055± 0.003^{f}	0.013± 0.003^{b}	0.022± 0.001^{b}	0.034± 0.003^{d}	0.074± 0.002^{h}	0.024± 0.004^{c}	0.037± 0.004^{d}	0.039± 0.002^{d}	0.063± 0.006^{f}	0.035± 0.005^{d}	0.035± 0.003^{d}
As 50 + All M	0.032± 0.001^{d}	0.055± 0.005^{f}	0.015± 0.003^{b}	0.036± 0.002^{c}	0.058± 0.004^{f}	0.078± 0.006^{h}	0.036± 0.002^{d}	0.044± 0.003^{e}	0.060± 0.002^{g}	0.069± 0.006^{f}	0.046± 0.004^{e}	0.035± 0.004^{d}
As 100 + All M	0.039± 0.004^{d}	0.059± 0.004^{f}	0.024± 0.004^{c}	0.040± 0.002^{e}	0.057± 0.008^{f}	0.085± 0.005^{i}	0.044± 0.004^{e}	0.055± 0.004^{f}	0.062± 0.004^{g}	0.075± 0.006^{h}	0.054± 0.004^{f}	0.045± 0.003^{e}
C + Const	0.015± 0.003^{b}	0.063± 0.002^{g}	0.015± 0.004^{b}	0.026± 0.002^{c}	0.044± 0.009^{e}	0.086± 0.001^{i}	0.038± 0.004^{d}	0.055± 0.003^{f}	0.042± 0.003^{e}	0.075± 0.005^{h}	0.036± 0.004^{d}	0.045± 0.004^{e}
As 50 + const.	0.038± 0.005^{d}	0.071± 0.002^{h}	0.024± 0.001^{c}	0.034± 0.004^{d}	0.060± 0.003^{g}	0.094± 0.004^{j}	0.053± 0.003^{f}	0.056± 0.004^{f}	0.058± 0.008^{f}	0.085± 0.003^{i}	0.055± 0.004^{f}	0.053± 0.003^{f}
As 100 + const.	0.048± 0.003^{e}	0.075± 0.004^{h}	0.039± 0.002^{d}	0.046± 0.002^{e}	0.063± 0.003^{g}	0.104± 0.007^{k}	0.065± 0.004^{g}	0.073± 0.003^{h}	0.059± 0.007^{f}	0.096± 0.004^{j}	0.067± 0.002^{g}	0.061± 0.004^{g}

Mean ± SD (n=3). Different small alphabets (a-k) indicate statistically significant difference at $P \leq 0.05$ by Turkey's test (**C**: control, **As 50**: arsenic 50 mgkg^{-1}, **As 100**: arsenic 100 mgkg^{-1}**, All B**: all bacterial isolates (1-9), **All M**: All mycorrhiza cultures (1-3), **Const:** microbial consortium (All bacteria + all mycorrhiza)

Table-17: Glutathione reductase activity (Unit mg^{-1} Protein FW) in leaves and roots of *Ricinuscommunis and Canna indica* inoculated with Cadmium (50 and 100 $mgkg^{-1}$) along with rhizobacteria, mycorrhiza and microbial consortium.

Time duration →	1st month				2nd Month				3rd Month			
Plants →	*R. communis*		*C. indica*		*R. communis*		*C. indica*		*R. communis*		*C. indica*	
Treatment ↓	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots
Control	0.022± 0.003^{c}	0.026± 0.004^{b}	0.009± 0.006^{a}	0.008± 0.022^{a}	0.018± 0.005^{b}	0.052± 0.011^{f}	0.015± 0.051^{b}	0.025± 0.004^{c}	0.022± 0.008^{c}	0.065± 0.009^{f}	0.027± 0.001^{c}	0.026± 0.054^{c}
C + Cd 50	0.013± 0.002^{b}	0.034± 0.004^{c}	0.014± 0.005^{b}	0.009± 0.001^{a}	0.018± 0.006^{b}	0.038± 0.009^{d}	0.011± 0.003^{b}	0.019± 0.006^{b}	0.021± 0.002^{c}	0.041± 0.001^{e}	0.012± 0.009^{b}	0.013± 0.005^{b}
C + Cd 100	0.008± 0.006^{a}	0.028± 0.008^{b}	0.005± 0.009^{a}	0.003± 0.002^{a}	0.011± 0.006^{b}	0.021± 0.011^{c}	0.006± 0.005^{a}	0.007± 0.002^{a}	0.011± 0.013^{b}	0.034± 0.051^{d}	0.003± 0.003^{a}	0.006± 0.009^{a}
C + All B	0.015± 0.002^{b}	0.047± 0.002^{d}	0.006± 0.001^{a}	0.012± 0.001^{b}	0.029± 0.006^{c}	0.064± 0.004^{g}	0.019± 0.002^{b}	0.030± 0.002^{d}	0.034± 0.004^{d}	0.063± 0.003^{g}	0.024± 0.003^{c}	0.026± 0.002^{c}
Cd 50 + All B	0.025± 0.008^{c}	0.074± 0.004^{g}	0.015± 0.004^{b}	0.028± 0.004^{c}	0.039± 0.012^{d}	0.075± 0.003^{h}	0.024± 0.006^{c}	0.046± 0.004^{e}	0.058± 0.005^{e}	0.076± 0.002^{h}	0.036± 0.002^{d}	0.034± 0.003^{d}
Cd 100 + All B	0.032± 0.004^{d}	0.085± 0.005^{h}	0.024± 0.005^{c}	0.034± 0.004^{d}	0.065± 0.004^{g}	0.089± 0.007^{i}	0.035± 0.004^{d}	0.045± 0.007^{e}	0.061± 0.005^{f}	0.075± 0.004^{h}	0.043± 0.006^{e}	0.042± 0.002e
C + All M	0.017± 0.002b	0.055± 0.003^{e}	0.013± 0.003^{b}	0.022± 0.001^{b}	0.034± 0.003^{d}	0.074± 0.002^{h}	0.024± 0.004^{b}	0.037± 0.004^{d}	0.039± 0.002^{d}	0.063± 0.006^{g}	0.035± 0.005^{d}	0.035± 0.003^{d}
Cd 50 + All M	0.020± 0.004^{c}	0.065± 0.005^{f}	0.036± 0.004^{d}	0.035± 0.004^{d}	0.072± 0.005^{h}	0.076± 0.006^{h}	0.056± 0.003^{f}	0.063± 0.003^{g}	0.068± 0.003^{f}	0.077± 0.006^{h}	0.055± 0.005^{f}	0.055± 0.002^{f}
Cd 100 + All M	0.025± 0.005^{c}	0.078± 0.003^{g}	0.045± 0.004^{e}	0.045± 0.002^{e}	0.069± 0.004^{g}	0.093± 0.003^{j}	0.067± 0.005^{g}	0.066± 0.004^{g}	0.073± 0.006^{g}	0.084± 0.003^{i}	0.067± 0.004^{g}	0.056± 0.003^{f}
C + Const	0.015± 0.003^{b}	0.063± 0.002^{f}	0.015± 0.004^{b}	0.026± 0.002^{c}	0.044± 0.004^{e}	0.086± 0.004^{i}	0.038± 0.004^{d}	0.055± 0.003^{e}	0.042± 0.004^{e}	0.075± 0.005^{h}	0.036± 0.003^{d}	0.045± 0.004^{e}
Cd 50 + Const.	0.034± 0.003^{d}	0.078± 0.007^{g}	0.045± 0.003^{e}	0.045± 0.004^{e}	0.066± 0.007^{g}	0.104± 0.004^{k}	0.076± 0.004^{h}	0.080± 0.002^{i}	0.067± 0.008^{f}	0.095± 0.003^{j}	0.075± 0.005^{h}	0.072± 0.004^{h}
Cd 100+ Const.	0.033± 0.007^{d}	0.083± 0.002^{h}	0.047± 0.002^{e}	0.049± 0.002^{e}	0.071± 0.003^{h}	0.109± 0.002^{k}	0.074± 0.004^{h}	0.074± 0.008^{h}	0.069± 0.001^{f}	0.104± 0.003^{k}	0.077± 0.005^{h}	0.075± 0.005^{h}

Mean ± SD (n=3). Different small alphabets (a-k) indicate statistically significant difference at $P \leq 0.05$ by Turkey's test (**C:** control, **Cd 50:** cadmium 50 $mgkg^{-1}$, **Cd 100:** cadmium 100 $mgkg^{-1}$, **All B:** all bacterial isolates (1-9), **All M:** All mycorrhiza cultures (1-3), **Const:** microbial consortium (All bacteria + all mycorrhiza)

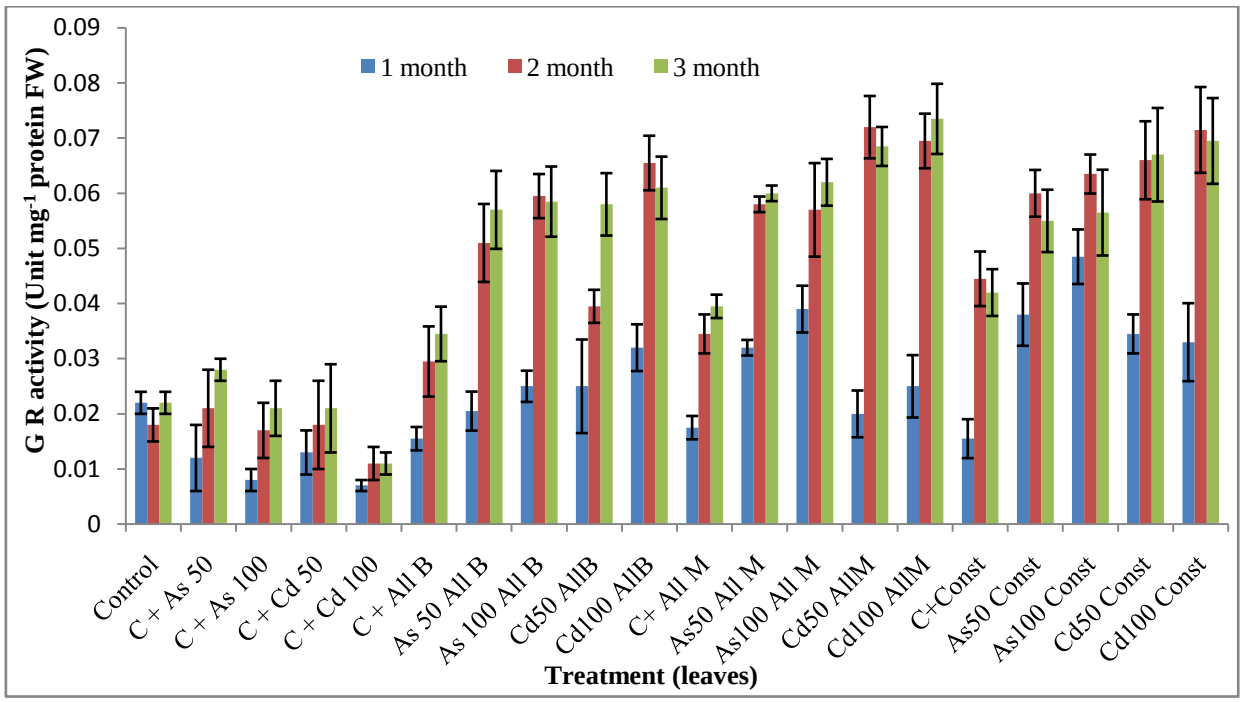

Figure 38: Glutathione reductase activity (Unit mg^{-1} Protein FW) in leaves of *Ricinus communis* inoculated with arsenic and cadmium (50 and 100 $mgkg^{-1}$) along with rhizobacteria, mycorrhiza and microbial consortium.

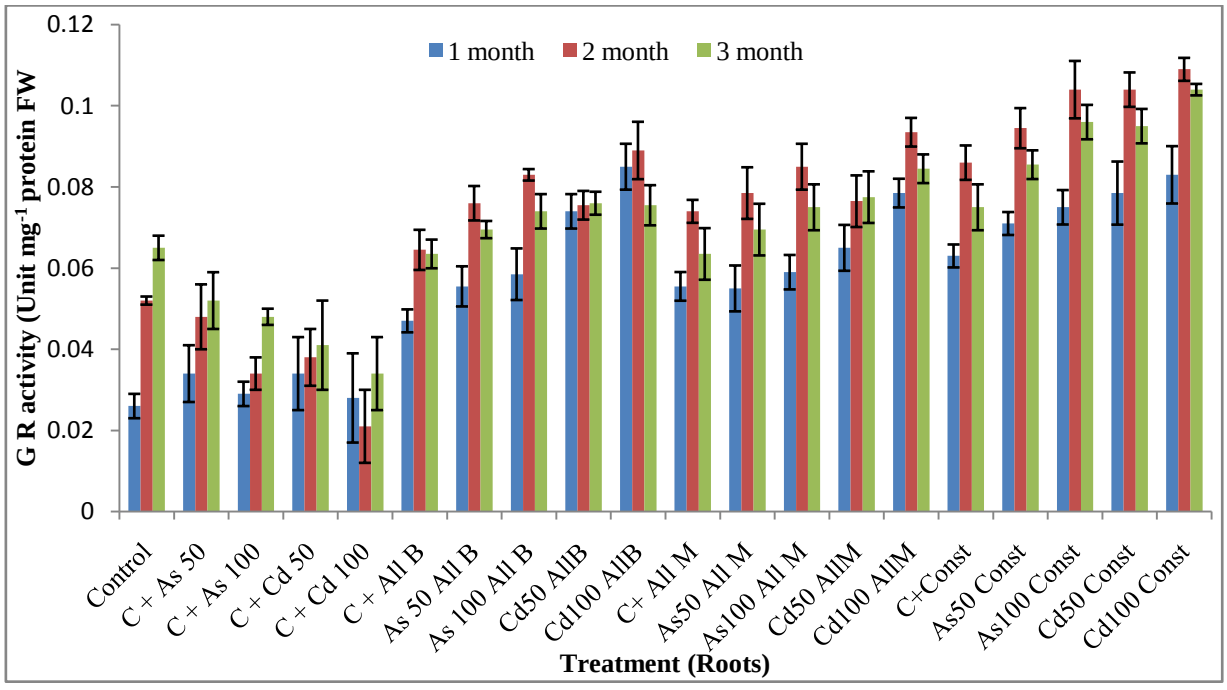

Figure 39: Glutathione reductase activity (Unit mg^{-1} Protein FW) in roots of *Ricinus communis* inoculated with arsenic and cadmium (50 and 100 $mgkg^{-1}$) along with rhizobacteria, mycorrhiza and microbial consortium.

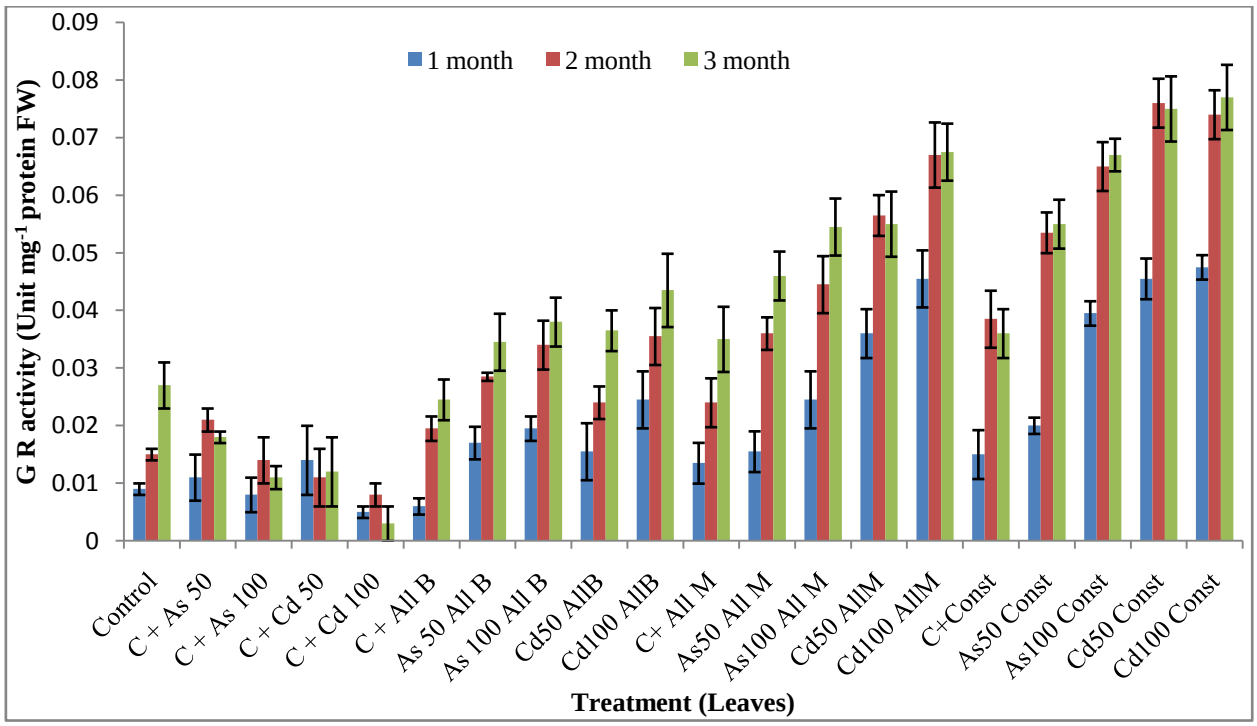

Figure 40: Glutathione reductase activity (Unit mg^{-1} Protein FW) in leaves of *Canna indica* inoculated with arsenic and cadmium (50 and 100 $mgkg^{-1}$) along with rhizobacteria, mycorrhiza and microbial consortium.

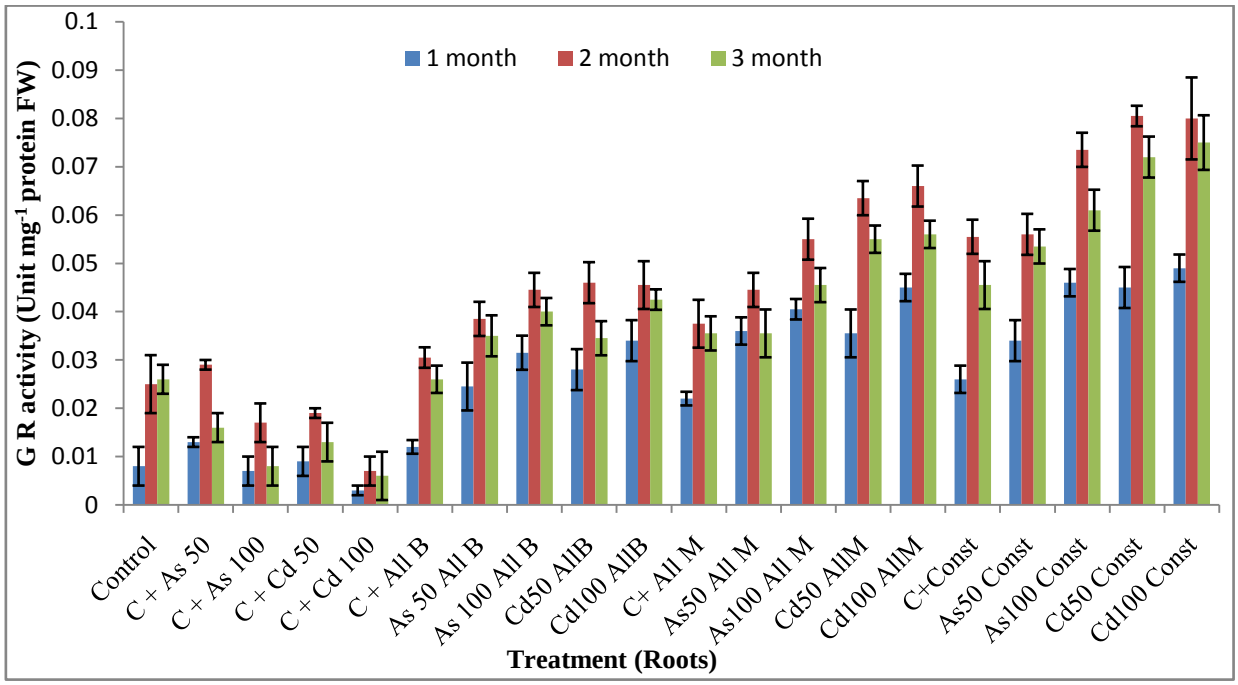

Figure 41: Glutathione reductase activity (Unit mg^{-1} Protein FW) in roots of *Canna indica* inoculated with arsenic and cadmium (50 and 100 $mgkg^{-1}$) along with rhizobacteria, mycorrhiza and microbial consortium.

6.15.4 Guaiacol peroxidase (GPX) activity in *Ricinus communis* and *Canna indica* inoculatedwith two concentrations of arsenic and cadmium (50 and 100 $mgkg^{-1}$) along with rhizobacteria, mycorrhiza and a microbial consortium

Under arsenic stress, leaves and roots of *R. communis* plant showed an increase in GPX activity in all the inoculated plants as compared to control plants. The results found after the1st month were different compared to APX and GR activities in *R. communis* plant (leaves and roots), where GPX activity was not concentration and time-dependent. The results were almost in the same trend as found in CAT activity.

GPX activity increased in leaves and roots of *R. communis* plants impregnated with rhizobacteria, mycorrhiza and microbial consortium in first 2 months, but decreased significantly in the third month. Leaves and roots of rhizobacteria inoculated plants showed a decline in GPX activity in the first 2 months with an increase in the concentration of metal (As 100 $mgkg^{-1}$). In other plants, the same trend was observed wherean increase in GPX activity was seen with increasing concentration of arsenic. Maximum activity was found in leaves and roots of pots inoculated with microbial consortium followed by mycorrhiza and rhizobacteria which was same as in APX and GR activity. After 1st-month GPX activity in leaves of *R. communis* inoculated with microbial consortium was found to 0.216, 0.225,0.228 and in roots was 0.379, 0.440, 0.445 Unit mg^{-1} Protein FWin control and inoculated (As 50 and 100 $mgkg^{-1}$) plants respectively. After a 2ndmonth, the results obtained for GPX activity in leaves (0.273, 0.275, 0.282) and roots (0.430, 0.473, 0.482Unit mg^{-1} Protein FW) was found to be increased in control and microbial consortium (As 50 and 100 $mgkg^{-1}$) inoculated plants respectively.

A significant decrease was seen after 3rd month, where GPX activity in leaves and roots of *R. communis* implanted results were found to be 0.256, 0.251, 0.265 and 0.439, 0.483, 0.485 in control and microbial consortium (As 50 and 100 $mgkg^{-1}$) inoculated plants respectively. Overall increasing order of all the inoculated plants with respect to GPX activity was found to be in the order of rhizobacteria< mycorrhiza <microbial consortium in 3 months of experimentation time. Roots of all inoculated *R. communis* plants displayed more GPX activity than leaves which shows more accumulation of As in roots of the plants (table 18).*C. indica* also demonstrated less GPX activity in both the leaves and roots of all inoculated plants in comparison to *R. communis* plant which can be related to more accumulation of As in leaves and roots of *R. communis* plant than *C. indica* plant. GPX activity was found to be more in all the inoculated plants than the control plants. Maximum GPX activity was observed inmicrobial consortium inoculated pots in both the leaves and roots. The same trend was seen as in *R. communis* with rhizobacteria<mycorrhiza<microbial consortium in increasing order of GPX activity. As the concentration of arsenic increases along with time, GPX activity also increases in all the

inoculated plants but significant reduction was also noticed after 3rd-month sampling in *C. indica* plant (leaves and roots) as shown in table 18.

Under cadmium stress, almost the sametrend in results was observed in leaves and roots of treated *R. communis* and *C. indica* plant with respect to time and concentration. A significant increase in GPX activity after 1st and 2nd month in both the plants with a reduction in GPX activity after the 3rd month was noticed (figure 42-45). Microbial consortium inoculated plants depicted maximal activity in leaves and roots during experimentation period. Therefore, the greatest amount of cadmium was accumulated in roots than leaves in *C. indica* plant in the first two months and accumulation rate significantly decreases with time (table 19).

The increase in GPX activity in roots and leaves of *R. communis* under As and Cd treatments (50 and 100 $mgkg^{-1}$) substantiates the report given by Baudh *et al* (2015) which revealed 16 % increase and 18 % decrease in GPX activity in roots and leaves respectively. GPX plays an important role in the plant to scavenge phospholipid hydroperoxides and hence protects the cell membranes from damage caused by peroxidative (Gueta-Dahan *et al*., 1997). Under biotic and abiotic stresses and heavy metal toxicity, the increase in expressions of many GPXs is observed (Avsian-Kretchmer *et al*., 2004). According to Gupta *et al* (2009), GPX production in plants is induced under metal stress. Another study (Ling *et al*., 2007) proposed the increase in GPX activity in leaves and roots of *C. indica* treated with Cu and Cd. The results demonstrate that under Cu and Cd stress, a significant increase in GPX activity was observed in the roots followed by leaves of *C. indica* as compared to control. This explains that oxidative stress caused by Cu and Cd was overcome by the influence of phytochelatins and an increase in antioxidant enzymes activity to increase the tolerance of *C. indica* against heavy metals.Plants under heavy metal stress showa significant increase in GPOD activity compared to control. Being an induced protein, higher GPX activity discloses that plants can withstand, counteract and devoid the oxidative stress caused by Cd and As.

As indicated by the results, increased GPX activity recommends that this enzyme is an intrinsic defence tool to combat Cd and As-induced oxidative damagein *R. communis* and *C. indica* plants. Being considered as stress enzymes enhanced GPX activity has been reported under toxic levels of various heavy metals like aluminium, copper, cadmium, zinc (Choand Seo.2004). GPX is mainly found in the cytosol, vacuole, cell wall and extracellular spaces. Hence an increase in GPX activity in Cd and As stressed plants might be due to the increase in the liberation of peroxidases that are located in the cell wall.

Table -18: Guaiacol peroxidase activity (Unit mg^{-1} Protein FW) in leaves and roots of *Ricinus communis* and *Canna indica* inoculated with Arsenic (50 and 100 $mgkg^{-1}$) along with rhizobacteria, mycorrhiza and microbial consortium.

Time duration →	1st month				2nd Month				3rd Month			
Plants →	*R. communis*		*C. indica*		*R. communis*		*C. indica*		*R. communis*		*C. indica*	
Treatment ↓	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots
Control	0.111± 0.011^{a}	0.142± 0.009^{c}	0.132± 0.004^{b}	0.165± 0.004^{c}	0.144± 0.006^{c}	0.211± 0.033^{e}	0.133± 0.031^{c}	0.167± 0.005^{c}	0.161± 0.004^{c}	0.219± 0.014^{e}	0.139± 0.071^{b}	0.174± 0.077^{d}
C + As 50	0.121± 0.004^{b}	0.181± 0.009^{d}	0.128± 0.008^{b}	0.174± 0.017^{d}	0.163± 0.001^{c}	0.234± 0.018^{e}	0.143± 0.013^{c}	0.201± 0.022^{e}	0.123± 0.009^{b}	0.234± 0.043^{e}	0.131± 0.007^{b}	0.184± 0.022^{d}
C + As 100	0.131± 0.001^{b}	0.173± 0.007^{d}	0.115± 0.009^{a}	0.163± 0.021^{c}	0.152± 0.011^{c}	0.221± 0.032^{e}	0.133± 0.033^{c}	0.184± 0.009^{d}	0.115± 0.008^{a}	0.218± 0.005^{e}	0.126± 0.007^{b}	0.163± 0.009^{c}
C + All B	0.154± 0.003^{c}	0.235± 0.002^{f}	0.134± 0.005^{b}	0.215± 0.002^{e}	0.164± 0.004^{c}	0.269± 0.002^{f}	0.157± 0.002^{c}	0.226± 0.008^{e}	0.154± 0.002^{c}	0.267± 0.005^{f}	0.152± 0.001^{c}	0.235± 0.006^{e}
As 50 + All B	0.161± 0.002^{c}	0.263± 0.002^{f}	0.183± 0.002^{d}	0.350± 0.006^{h}	0.175± 0.002^{d}	0.275± 0.003^{f}	0.188± 0.005^{d}	0.358± 0.009^{h}	0.166± 0.004^{c}	0.286± 0.002^{f}	0.185± 0.003^{d}	0.375± 0.002^{h}
As 100 + All B	0.140± 0.002^{c}	0.265± 0.004^{f}	0.186± 0.004^{d}	0.307± 0.007^{g}	0.184± 0.003^{d}	0.282± 0.002^{f}	0.194± 0.003^{e}	0.323± 0.001^{g}	0.169± 0.003^{c}	0.286± 0.007^{f}	0.184± 0.002d	0.329± 0.003^{g}
C + All M	0.205± 0.005^{e}	0.321± 0.011^{g}	0.215± 0.002^{e}	0.304± 0.004^{g}	0.242± 0.009^{f}	0.255± 0.002^{f}	0.221± 0.008^{e}	0.324± 0.005^{g}	0.231± 0.003^{f}	0.266± 0.002^{f}	0.208± 0.005^{e}	0.334± 0.003^{g}
As 50 + All M	0.206± 0.003^{e}	0.347± 0.005^{h}	0.274± 0.002^{f}	0.349± 0.003^{g}	0.273± 0.003^{f}	0.385± 0.004^{h}	0.282± 0.001^{f}	0.355± 0.005^{h}	0.260± 0.004^{f}	0.395± 0.003^{h}	0.277± 0.003^{f}	0.367± 0.002^{h}
As 100 + All M	0.222± 0.008^{f}	0.324± 0.009^{g}	0.265± 0.009^{f}	0.330± 0.004^{g}	0.284± 0.004^{f}	0.394± 0.004^{h}	0.274± 0.003^{f}	0.341± 0.004^{h}	0.262± 0.007^{f}	0.369± 0.045^{h}	0.266± 0.002^{f}	0.355± 0.009^{h}
C + Const	0.216± 0.006^{e}	0.379± 0.032^{h}	0.255± 0.006^{f}	0.315± 0.017^{g}	0.273± 0.003^{f}	0.430± 0.005^{i}	0.275± 0.005^{f}	0.336± 0.003^{h}	0.256± 0.004^{f}	0.439± 0.004^{i}	0.267± 0.001^{f}	0.361± 0.003^{h}
As 50 + Const.	0.225± 0.005^{f}	0.440± 0.005^{i}	0.265± 0.002^{f}	0.481± 0.004^{j}	0.275± 0.004^{f}	0.473± 0.003^{j}	0.283± 0.008^{f}	0.497± 0.001^{j}	0.251± 0.004^{f}	0.483± 0.002^{j}	0.275± 0.005^{f}	0.495± 0.005^{j}
As 100+ Const.	0.228± 0.006^{f}	0.445± 0.005^{i}	0.235± 0.003^{e}	0.350± 0.006^{h}	0.282± 0.002^{g}	0.482± 0.007^{j}	0.276± 0.004^{f}	0.380± 0.005^{h}	0.265± 0.003^{f}	0.485± 0.003^{j}	0.273± 0.003^{f}	0.390± 0.005^{h}

Mean ± SD (n=3). Different small alphabets (a-j) indicate statistically significant difference at $P \leq 0.05$ by Turkey's test (**C:** control, **As 50:** arsenic 50 $mgkg^{-1}$, **As 100**: arsenic 100 $mgkg^{-1}$,**All B**: all bacterial isolates (1-9), **All M:** All mycorrhiza cultures (1-3), **Const:** microbial consortium (All bacteria + all mycorrhiza)

Table-19: Guaiacol peroxidase activity (Unit mg^{-1} Protein FW) in leaves and roots of *Ricinus communis* and *Canna indica* inoculated with Cadmium (50 and 100 $mgkg^{-1}$) along with rhizobacteria, mycorrhiza and microbial consortium.

Time duration →	1st month				2nd Month				3rd Month			
Plants →	*R. communis*		*C. indica*		*R. communis*		*C. indica*		*R. communis*		*C. indica*	
Treatment ↓	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots
Control	0.111± 0.011^{a}	0.142± 0.009^{c}	0.132± 0.004^{c}	0.165± 0.004^{c}	0.144± 0.006^{c}	0.211± 0.033^{e}	0.133± 0.031^{d}	0.167± 0.005^{c}	0.111± 0.004^{a}	0.209± 0.014^{e}	0.122± 0.071^{b}	0.156± 0.077^{c}
C + Cd 50	0.231± 0.006^{e}	0.213± 0.033^{e}	0.124± 0.032^{b}	0.198± 0.009^{d}	0.148± 0.007^{c}	0.148± 0.008^{c}	0.214± 0.001^{e}	0.124± 0.007^{c}	0.203± 0.013^{e}	0.241± 0.008^{e}	0.131± 0.065^{b}	0.210± 0.007^{e}
C + Cd 100	0.123± 0.005^{b}	0.194± 0.044^{d}	0.113± 0.005^{a}	0.174± 0.008^{d}	0.133± 0.023^{c}	0.203± 0.009^{e}	0.111± 0.002^{a}	0.183± 0.078^{d}	0.113± 0.006^{a}	0.233± 0.005^{e}	0.124± 0.008	0.201± 0.005^{e}
C + All B	0.154± 0.003^{c}	0.235± 0.002^{e}	0.134± 0.005^{c}	0.215± 0.004^{e}	0.164± 0.004^{d}	0.269± 0.003^{e}	0.157± 0.002^{c}	0.226± 0.002^{e}	0.154± 0.002^{c}	0.267± 0.007^{f}	0.152± 0.001^{c}	0.235± 0.005^{f}
Cd 50 + All B	0.201± 0.002^{e}	0.307± 0.007^{g}	0.155± 0.002^{c}	0.249± 0.003^{e}	0.217± 0.005^{e}	0.334± 0.003^{g}	0.175± 0.003^{d}	0.257± 0.001^{e}	0.209± 0.003^{e}	0.335± 0.004^{g}	0.174± 0.005^{d}	0.265± 0.005^{f}
Cd 100 + All B	0.193± 0.002^{d}	0.347± 0.002^{g}	0.164± 0.003^{c}	0.202± 0.003^{e}	0.206± 0.004^{e}	0.368± 0.004^{g}	0.183± 0.002^{d}	0.236± 0.003^{e}	0.206± 0.003^{e}	0.375± 0.004^{g}	0.170± 0.003^{d}	0.235± 0.005^{f}
C + All M	0.205± 0.005^{e}	0.321± 0.011^{g}	0.215± 0.004^{e}	0.304± 0.005^{g}	0.242± 0.009^{e}	0.255± 0.002^{e}	0.221± 0.004^{e}	0.324± 0.006^{g}	0.231± 0.005^{e}	0.266± 0.002^{f}	0.208± 0.005^{e}	0.334± 0.003^{g}
Cd 50 + All M	0.202± 0.005^{e}	0.349± 0.002^{g}	0.175± 0.005^{c}	0.235± 0.002^{e}	0.266± 0.004^{e}	0.383± 0.003^{g}	0.250± 0.003^{f}	0.242± 0.008^{e}	0.255± 0.005^{f}	0.404± 0.004^{h}	0.244± 0.004^{f}	0.250± 0.001^{f}
Cd 100 +All M	0.228± 0.007^{e}	0.364± 0.005^{g}	0.162± 0.007^{c}	0.375± 0.004^{g}	0.266± 0.004^{e}	0.366± 0.048^{g}	0.245± 0.005^{f}	0.384± 0.003^{g}	0.274± 0.004^{f}	0.428± 0.003^{h}	0.236± 0.003^{f}	0.392± 0.001^{h}
C + Const	0.216± 0.006^{e}	0.379± 0.032^{g}	0.255± 0.005^{e}	0.315± 0.017^{g}	0.273± 0.003^{g}	0.430± 0.005^{h}	0.275± 0.003^{g}	0.336± 0.003^{g}	0.256± 0.002^{f}	0.439± 0.002^{h}	0.267± 0.001^{f}	0.361± 0.005^{h}
Cd 50+ Const.	0.235± 0.007^{e}	0.456± 0.003^{h}	0.282± 0.001^{g}	0.355± 0.005^{g}	0.283± 0.006^{g}	0.475± 0.005^{h}	0.294± 0.005^{g}	0.372± 0.002^{g}	0.266± 0.002^{f}	0.486± 0.003^{i}	0.292± 0.007^{g}	0.380± 0.011^{i}
Cd 100+Const.	0.245± 0.003^{e}	0.466± 0.004^{h}	0.284± 0.005^{g}	0.354± 0.006g	0.291± 0.009^{g}	0.487± 0.003^{h}	0.285± 0.003^{g}	0.368± 0.006^{f}	0.275± 0.004^{g}	0.486± 0.006^{i}	0.278± 0.005^{g}	0.383± 0.002^{i}

Mean ± SD (n=3). Different small alphabets (a-i) indicate statistically significant difference at $P \leq 0.05$ by Turkey's test (**C:** control, **Cd 50:** cadmium 50 $mgkg^{-1}$, **Cd 100:** cadmium 100 $mgkg^{-1}$, **All B**: all bacterial isolates (1-9), **All M:** All mycorrhiza cultures (1-3), **Const:** microbial consortium (All bacteria + all mycorrhiza)

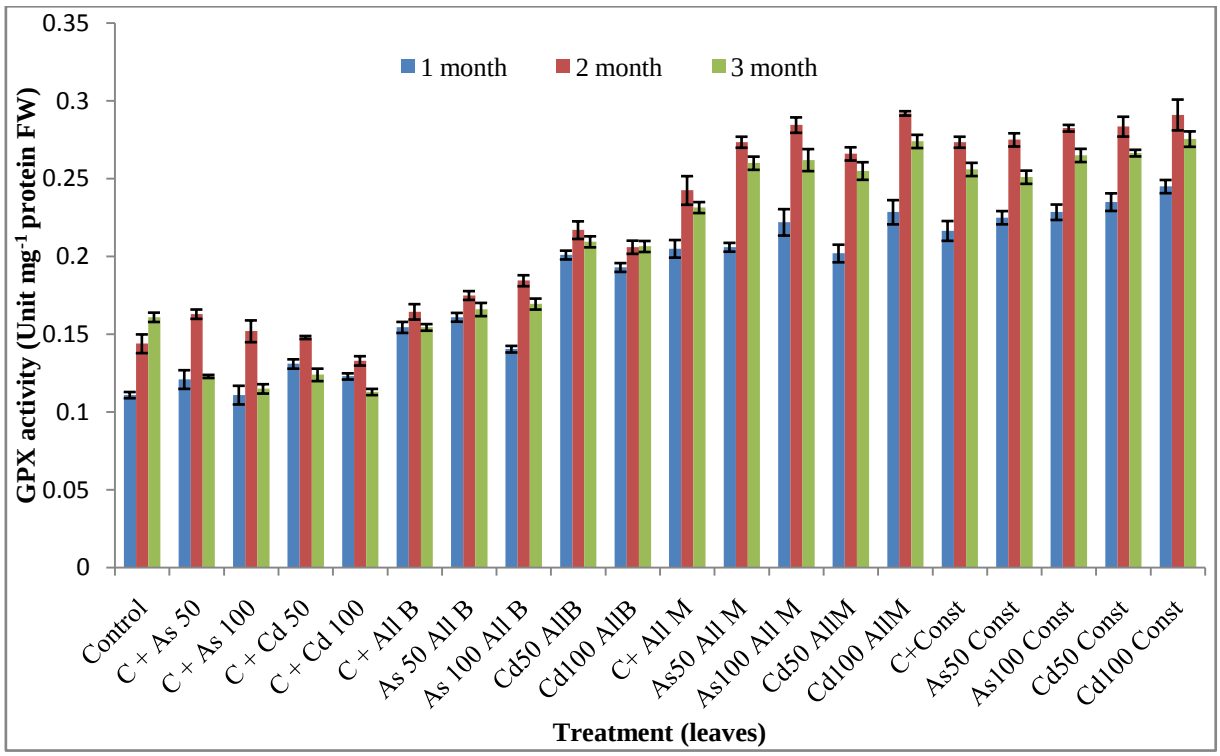

Figure 42: Guaiacol peroxidase activity (Unit mg^{-1} Protein FW) in leaves of *Ricinus communis* inoculated with arsenic and cadmium (50 and 100 $mgkg^{-1}$) along with rhizobacteria, mycorrhiza and microbial consortium.

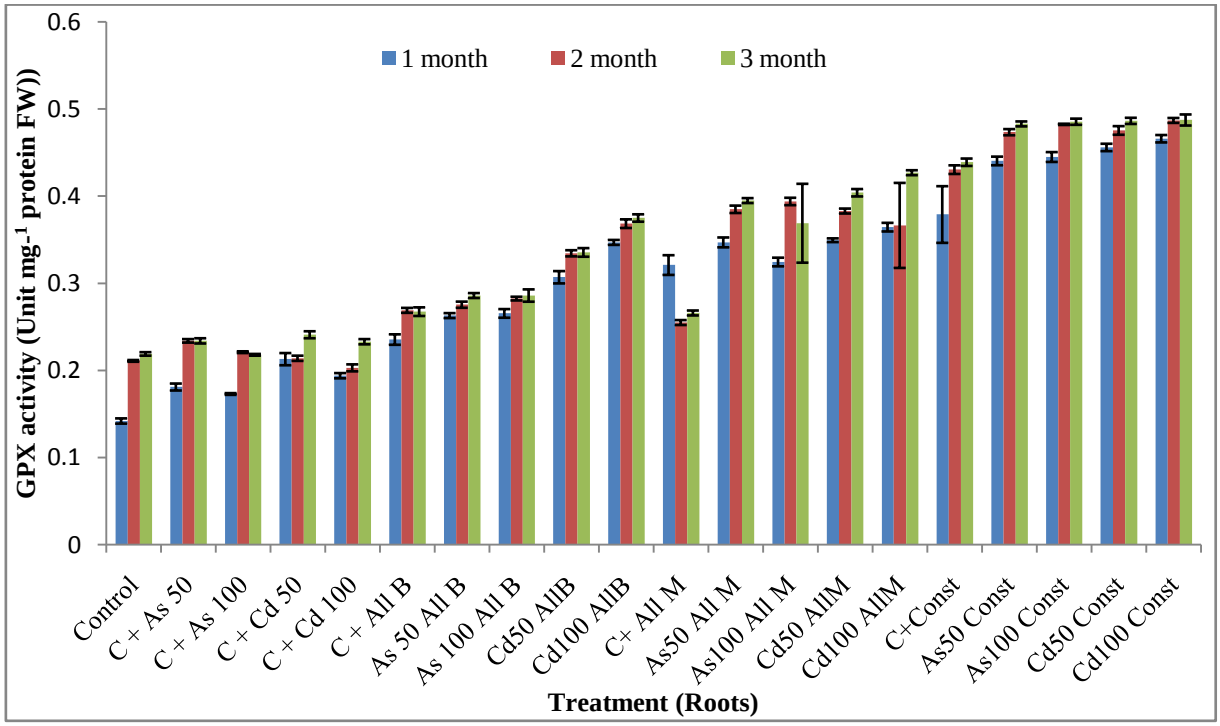

Figure 43: Guaiacol peroxidase activity (Unit mg^{-1} Protein FW) in roots of *Ricinus communis* inoculated with arsenic and cadmium (50 and 100 $mgkg^{-1}$) along with rhizobacteria, mycorrhiza and microbial consortium.

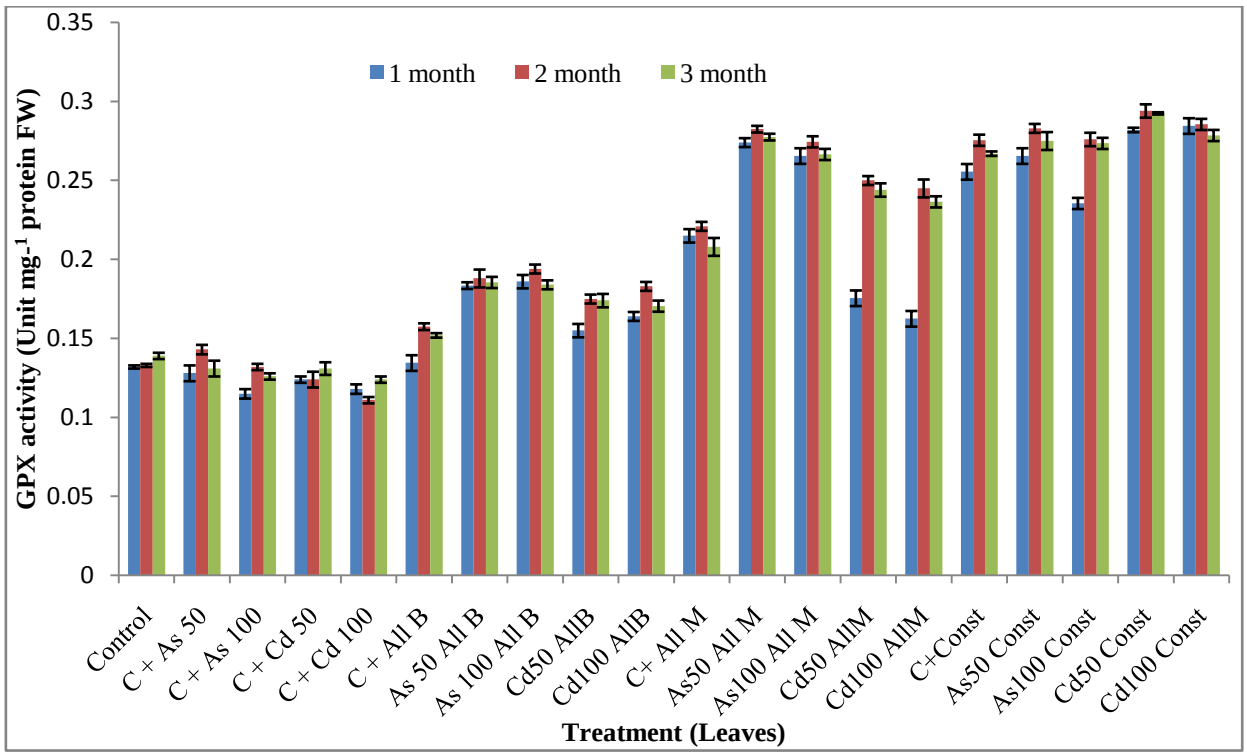

Figure 44: Guaiacol peroxidase activity (Unit mg^{-1} Protein FW) in leaves of *Canna indica* inoculated with arsenic and cadmium (50 and 100 $mgkg^{-1}$) along with rhizobacteria, mycorrhiza and microbial consortium.

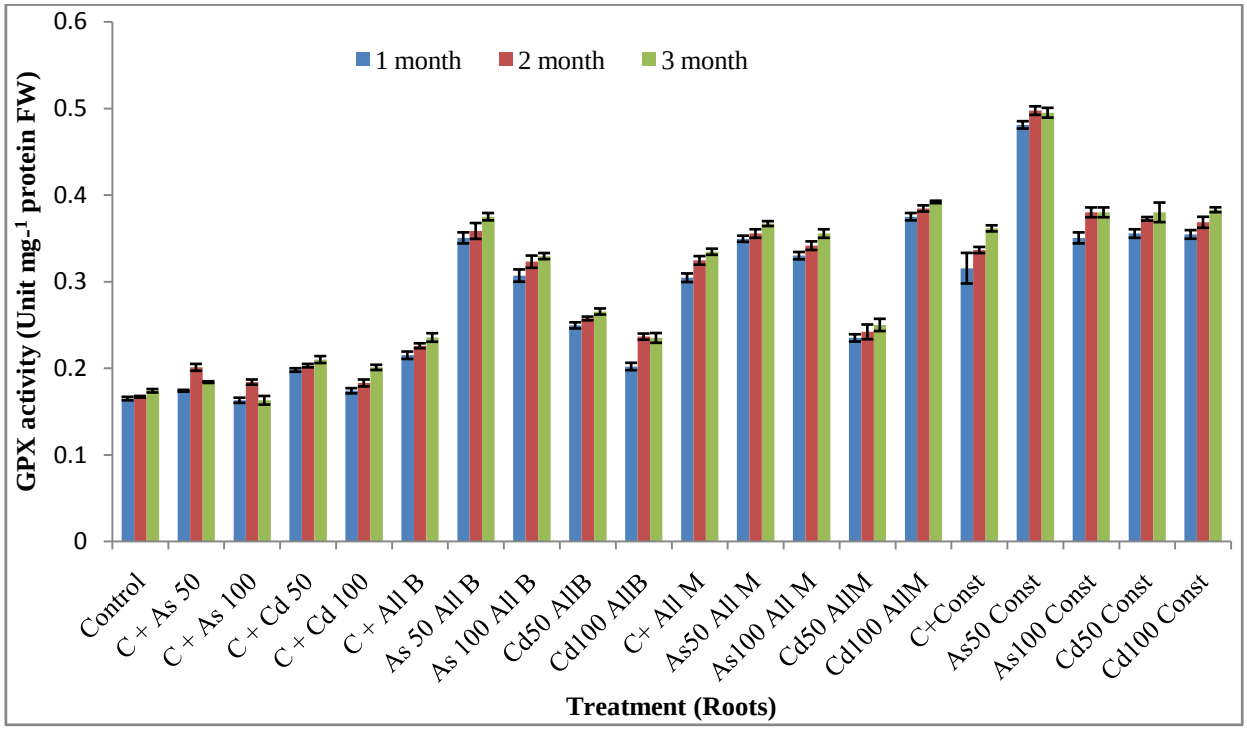

Figure 45: Guaiacol peroxidase activity (Unit mg^{-1} Protein FW) in roots of *Canna indica* inoculated with arsenic and cadmium (50 and 100 $mgkg^{-1}$) along with rhizobacteria, mycorrhiza and bacterial consortium.

6.16 Phytochemical screening of secondary metabolites released by plants under heavy metal stress

Plantsrelease various secondary metabolites (phytochemicals) to combat the stress caused by heavy metals. Total phenolic and flavonoid content along with free radical scavenging (DPPH) potential have been estimated for 3 months in parts of *R. communis* and *C. indica* treated with rhizobacteria, mycorrhiza and microbial consortium.Free radical scavenging potential of plants extracts was determined spectrophotometrically against stable DPPH (2, 2- diphenyl- 2-picrylhydrazyl hydrate)

6.16.1 Determination of DPPH Free Radical scavenging activity (DPPH assay) of *Ricinus communis* and *Canna indica* inoculated with rhizobacteria, mycorrhiza, microbial consortium and heavy metals (As, Cd 50 and 100 $mgkg^{-1}$).

When reaction between DPPH and antioxidant compound takes place, DPPH (stable free radical) gets reduced due to the production of hydrogen. As the concentration of heavy metals increases, percentage scavenging effect on DPPH also increases with respect to increasing concentration (5-15 $\mu g\ mL^{-1}$) of plants extract (*R. communis* and *C. indica*).

Table 20 shows the percentage scavenging activity in parts of *R. communis* treated with arsenic (50 and 100 $mgkg^{-1}$). Results clearly demonstrate the increase in the percentage of DPPH with increasing concentration of plants extract. Even with increasing the heavy metal concentration from 50 to 100 $mgkg^{-1}$, percentage DPPH activity increased significantly in 1st and 2nd month but decreased after the3rd month in all the inoculated plants of *R. communis* as compared to plants treated with arsenic (50 to 100 $mgkg^{-1}$) only. Maximum percentage scavenging activity was observed in the stem of *R. communis* treated with the microbial consortium in all the 3 months with respect to the increasing concentration of plants extract. The values observed for free radical scavenging activity were found to be in the order of leaves < roots < stem of *R. communis* plant inoculated with rhizobacteria < mycorrhiza < microbial consortium. The percent scavenging activity decreases with time (1-3 month). Percentage DPPH activity in 1st month was observed to be 56.7 %, 67.6 % in leaves, 69.3 %, 75.5% in roots and 91.9 %, 87.8% in stem of (15 μgmL^{-1}) microbial consortium and As (50 and 100 $mgkg^{-1}$) treated *R. communis* plants respectively which decreased to 28.6 %, 32.3 % in leaves, 25.8 %, 34.1 % in roots and 44.3 %, 38.9 % in stem of same treatment after 3rd month. Whereas, in mycorrhiza and rhizobacteria inoculated plants significant decrease in DPPH activity was observed from 1st to 3rd month (table 20).A similar trend in results of percentage scavenging activity in *R. communis* plants inoculated with rhizobacteria, mycorrhiza and microbial consortium under cadmium (50 and 100 $mgkg^{-1}$) stress were seen. As compared to control plants (without heavy metals), rhizobacteria inoculated plants showed less DPPH activity compared toinoculated plants (mycorrhiza and microbial

consortium). Even, the percentage scavenging activity in cadmium-treated *R. communis* plants was found to be less in comparisonto those treated with arsenic. Here also, after 1st month percentage scavenging activity (15 µgmL^{-1}) was found to be maximum in microbial consortium inoculate plants of *R. communis* (37.5 %, 42.7 % in leaves, 52.4 %, 58. 9 % in roots and 51.4 %, 71.8% in stem (Cd 50 and 100 mgkg^{-1}) which decreased to 15. 6%, 22.5 % in leaves and 25.6 %, 31.8 % in roots and 35.8 %, 39.8 % in stem after 3rd month respectively in the same treatment. The order for DPPH scavenging activity was found to be rhizobacteria < mycorrhiza <microbial consortium in all the plant's parts during 3 months of experimentation time (table 21). In *C. indica*, DPPH scavenging activity was observed to be significantly less in all the parts, than *R. communis* under arsenic and cadmium stress (50 and 100 mgkg^{-1}). Here also, the scavenging activity decreases with respect to time and increases with respect to the concentration of plants extract in all the treatments.

Maximum DPPH activity was observed in roots of microbial consortium treated *C. indica* plants under As and Cd stress. Percentage scavenging activity was found to be decreased in both the shoots and roots at higher concentration (100 mg kg^{-1}) of arsenic and cadmium with mycorrhiza treated pots in all the 3 months. But in other treatments (microbial consortium and rhizobacteria) scavenging activity increases with increasing metal (As and Cd) concentrations. The percent activity was observed to be 16.9 %, 19.5 % ;11.9 %, 14.1 % ; 8.24 %, 10.8 % in shoots and 22.9 %, 28.9 %; 15.5 %, 16.7 %;10.5 %, 13. 5 % in roots during 1st, 2nd and 3rd month treated with arsenic 50 and 100 mgkg^{-1} respectively (table 22). In cadmium treated pots of *C. indica*, significant less DPPH percent activity was observed in all the treatments, with the order of microbial consortium > mycorrhiza > rhizobacteria (table 23).

The results of the present study were in coherence with M.M Siddiqui *et al* (2014). The results demonstrated that under Cd, Cr and Pb toxicity, DPPH scavenging activity was found to be significantly higher in control plants (*Brassica rapa* var. *turnip*) without heavy metal treatment (87.058 %) compared to the one treated with heavy metals. According to Siddique*et al* (2010) and Fazal *et al*(2011), complex secondary metabolites are produced during the metabolism of plants with some possessing the potential to sequester free radical. But inversely, the scavenging capability of these metabolites decreases under heavy metal stress (Abbasi *et al*., 2012; Ahmad *et al*., 2011). According to our study, DPPH activity was increased in both the plants (*R. communis* and *C. indica*) inoculated with heavy metals along with microbial consortium. Hence this describes the potential of a microbialconsortium to enhance the activity of DPPH under metal stress.

Table-20: Percent (%) DPPH scavenging activity in leaves, roots and stem of *Ricinus communis* inoculated with arsenic (50 and 100 mgkg^{-1}) along with rhizobacteria, mycorrhiza and microbial consortium.

Time duration →		1st month			2nd Month			3rd Month		
Treatment ↓	DPPH (µg/mL)	Leaves	Roots	Stem	Leaves	Roots	Stem	Leaves	Roots	Stem
Control	5	58.1±0.232^{j}	62.2±0.551^{k}	85.6±0.241^{m}	63.4±0.451^{k}	69.5±0.643^{k}	87.7±0.445^{m}	67.4±0.462^{k}	75.7±0.447^{l}	89.2±0.563^{m}
	10	61.8±0.543^{k}	69.6±0.231^{k}	89.9±0.334^{m}	67.5±0.661^{k}	73.8±0.441^{l}	90.7±0.342^{n}	70.6±0.476^{l}	78.8±0.885^{l}	91.7±0.674^{n}
	15	68.7±0.521^{k}	77.1±0.334^{l}	93.6±0.366^{n}	69.5±0.522^{k}	79.4±0.482^{l}	95.6±0.499^{n}	74.7±0.872^{l}	82.9±0.267^{m}	95.9±0.567^{n}
C + As 50	5	6.41±0.213^{d}	6.64±0.344^{d}	7.43±0.223^{d}	3.64±0.336^{b}	3.81±0.667^{b}	5.34±0.912^{c}	1.74±0.671^{a}	2.13±0.743^{b}	3.41±0.842^{b}
	10	11.5±0.223^{f}	20.5±0.334^{g}	24.5±0.367^{g}	4.14±0.628^{c}	4.78±0.885^{c}	10.3±0.553^{e}	1.84±0.648^{a}	3.64±0.663^{b}	3.63±0.673^{b}
	15	14.3±0.563^{f}	28.8±0.774^{g}	29.6±0.563^{g}	8.91±0.674^{d}	9.25±0.679^{e}	22.6±0.680^{g}	3.62±0.783^{b}	8.41±0.334^{d}	8.99±0.237^{d}
C + As 100	5	8.64±0.711^{e}	21.6±0.667^{g}	28.4±0.567^{g}	4.63±0.778^{c}	8.91±0.556^{d}	25.4±0.226^{g}	3.46±0.581^{b}	7.34±0.671^{d}	22.6±0.671^{g}
	10	14.8±0.889^{f}	25.8±0.451^{g}	34.6±0.516^{h}	11.9±0.561^{f}	11.6±0.537^{f}	30.9±0.451^{h}	4.87±0.581^{c}	9.18±0.112^{d}	25.5±0.278^{g}
	15	16.6±0.889^{f}	31.6±0.556^{h}	51.9±0.671^{j}	13.8±0.998^{f}	12.9±0.561^{f}	46.3±0.981^{i}	5.32±0.561^{c}	11.7±0.419^{e}	26.3±0.516^{g}
As50 +All B	5	8.29±0.574^{d}	8.75±0.410^{d}	8.79±0.716^{d}	4.16±0.612^{c}	4.76±0.536^{c}	6.47±0.238^{d}	2.69±0.646^{b}	4.09±0.494^{c}	6.12±0.577^{d}
	10	13.2±0.712^{f}	22.2±0.636^{g}	32.1±2.164^{h}	9.09±0.399^{e}	9.86±0.663^{e}	26.2±0.622^{g}	2.88±0.470^{b}	5.87±0.882^{c}	6.13±0.170^{d}
	15	24.6±0.878^{g}	26.8±1.294^{g}	39.4±0.840^{h}	20.9±0.588^{g}	21.8±6.066^{g}	31.5±0.837^{h}	7.20±0.671^{d}	15.3±1.301^{f}	18.5±0.681^{f}
As100+All B	5	12.9±0.738^{f}	33.8±0.664^{g}	45.8±2.780i	7.90±0.766^{d}	25.9±0.523^{g}	42.6±0.557^{i}	5.32±0.502^{c}	16.3±0.926^{f}	17.7±0.577^{f}
	10	28.3±0.712^{g}	41.2±0.749^{h}	54.1±2.799^{j}	21.7±0.559^{g}	26.7±0.572^{g}	44.2±0.711^{i}	7.61±0.627^{d}	21.9±0.615^{g}	24.6±0.765^{g}
	15	29.3±0.878^{g}	45.5±1.294^{i}	70.7±0.836^{l}	24.5±0.686^{g}	29.7±1.117^{g}	64.9±0.957^{k}	9.60±0.587^{e}	24.3±1.159^{g}	31.3±0.681^{h}
As50 +All M	5	27.9±0.492^{g}	34.8±0.593^{i}	81.5±1.248^{m}	18.1±0.536^{f}	28.3±0.675^{g}	42.4±0.637^{i}	8.31±0.933^{d}	16.8±0.663^{f}	18.4±0.557^{f}
	10	39.4±0.979^{h}	50.9±0.530^{j}	82.7±0.924^{m}	19.2±1.839^{f}	38.9±0.112^{h}	57.6±0.444^{j}	10.9±0.470^{e}	24.7±1.301^{g}	26.6±0.595^{g}
	15	47.4±0.768^{i}	52.4±1.506^{j}	85.2±0.962^{m}	29.5±0.686^{g}	42.6±0.456^{i}	63.9±0.957^{k}	19.6±0.251^{f}	29.4±0.707^{g}	37.8±0.794^{h}
As100+All M	5	21.4±0.574^{g}	44.3±0.445^{i}	55.4±1.170^{j}	16.8±0.612^{f}	28.7±0.557^{g}	33.3±0.477^{h}	7.70±0.502^{d}	18.7±0.332^{f}	24.3±0.582^{g}
	10	39.9±3.829^{h}	52.6±1.223^{j}	62.6±0.714^{k}	29.9±0.479^{g}	38.9±0.162^{h}	43.7±0.764^{i}	8.90±1.097^{d}	25.8±0.523^{g}	29.3±0.595^{g}
	15	42.7±0.658^{i}	58.8±0.645^{j}	68.3±0.962^{k}	31.9±0.784^{h}	39.4±0.342^{h}	45.8±0.957^{i}	15.8±0.671^{f}	26.7±1.117^{g}	36.9±0.908^{h}
As50+Const.	5	39.9±0.476^{h}	61.7±0.156^{k}	83.3±0.876^{m}	15.3±0.154^{f}	35.8±0.919^{i}	61.8±0.223^{k}	18.6±0.653^{f}	18.3±0.675^{f}	34.8±0.345^{h}
	10	48.7±1.213^{i}	66.8±0.543^{k}	86.6±0.562^{m}	19.7±0.342^{f}	43.8±0.615^{i}	66.9±0.124^{k}	22.1±0.352^{g}	22.9±0.453^{g}	39.6±0.412^{h}
	15	56.7±0.356^{j}	69.3±0.245^{k}	91.9±0.231^{n}	22.6±0.543^{g}	45.5±0.763^{i}	73.3±0.342^{l}	28.6±0.245^{g}	25.8±0.444^{g}	44.3±0.223^{i}
As100+Const.	5	52.3±0.113^{j}	59.3±0.494^{j}	78.7±0.432^{l}	29.9±0.654^{g}	43.5±0.763^{i}	71.3±0.116^{l}	19.3±0.351^{f}	26.7±1.011^{g}	32.5±0.921^{h}
	10	59.5±0.245^{j}	66.1±0.643^{k}	83.2±0.443^{m}	32.3±0.435^{h}	44.3±1.279^{i}	77.7±0.134^{l}	29.8±0.674^{g}	32.9±1.449^{h}	35.4±0.501^{h}
	15	67.6±0.562^{k}	75.5±1.142^{l}	87.8±0.143^{n}	36.9±0.652^{h}	57.5±1.442^{j}	79.9±0.543^{l}	32.3±0.453^{h}	34.1±0.572^{h}	38.9±0.456^{h}

Mean ± SD (n=3). Different small alphabets (a-n) indicate statistically significant difference at $P \leq 0.05$ by Turkey's test (**C:** control, **As 50**: arsenic 50 mgkg^{-1}, **As 100**: arsenic 100 mgkg^{-1}, **All B**: all bacterial isolates (1-9), **All M**: All mycorrhiza cultures (1-3), **Const:** microbial consortium (All bacteria + all mycorrhiza)

Table 21: Percent (%) DPPH scavenging activity in leaves, roots and stem of *Ricinus communis* inoculated with cadmium (50 and 100 mgkg^{-1}) along with rhizobacteria, mycorrhiza and microbial consortium.

Time → duration		1st month			2nd Month			3rd Month		
Treatment ↓	DPPH (µg/mL)	Leaves	Roots	Stem	Leaves	Roots	Stem	Leaves	Roots	Stem
Control	5	58.1±0.232^{l}	62.2±0.551^{m}	85.6±0.241^{o}	63.4±0.451^{m}	69.5±0.643^{m}	87.7±0.445^{o}	67.4±0.462^{m}	75.7±0.447^{n}	89.2±0.563^{o}
	10	61.8±0.543^{m}	69.6±0.231^{m}	89.9±0.334^{o}	67.5±0.661^{m}	73.8±0.441^{n}	90.7±0.342^{p}	70.6±0.476^{n}	78.8±0.885^{n}	91.7±0.674^{p}
	15	68.7±0.521^{m}	77.1±0.334^{n}	93.6±0.366^{p}	69.5±0.522^{m}	79.4±0.482^{n}	95.6±0.499^{p}	74.7±0.872^{n}	82.9±0.267^{o}	95.9±0.567^{p}
C + Cd 50	5	4.23±0.545^{d}	4.91±0.998^{d}	5.31±0.870^{e}	2.84±0.561^{b}	2.94±0.567^{b}	3.29±0.667^{c}	1.12±0.561^{a}	1.84±0.671^{a}	2.14±0.519^{b}
	10	9.21±0.561^{g}	16.6±0.571^{h}	18.1±0.561^{h}	3.81±0.671^{c}	4.01±0.671^{d}	7.63±0.819^{f}	1.24±0.167^{a}	2.08±0.156^{b}	2.54±0.456^{b}
	15	11.6±0.356^{h}	21.3±0.451^{i}	22.9±0.567^{i}	6.44±0.556^{f}	7.11±0.636^{f}	13.1±0.699^{h}	2.08±0.511^{b}	4.63±0.881^{d}	6.51±0.331^{f}
C + Cd 100	5	4.61±0.156^{d}	14.9±0.819^{h}	21.8±0.672^{i}	2.45±0.134^{b}	6.66±0.461^{f}	12.6±0.417^{h}	1.29±0.361^{a}	3.41±0.371^{c}	8.44±0.213^{g}
	10	6.24±0.882^{f}	15.6±0.356^{h}	28.5±0.671i	7.84±0.718^{f}	7.49±0.174^{f}	20.8±0.481^{i}	2.43±0.891^{b}	4.84±0.173^{d}	9.89±0.371^{g}
	15	9.13±0.913^{g}	18.4±0.671^{h}	36.5±0.471^{j}	8.91±0.934^{g}	9.13±0.471^{g}	30.9±0.388^{j}	3.64±0.487^{c}	5.66±0.345^{e}	11.6±0.471^{h}
Cd 50+AllB	5	6.34±0.234^{f}	7.81±0.655^{f}	7.91±0.798^{f}	4.12±0.443^{d}	4.38±0.821^{d}	5.32±0.220e	2.15±0.998^{b}	3.25±0.045^{c}	3.82±0.212^{c}
	10	9.13±0.453^{g}	12.5±0.551^{h}	18.7±0.903^{h}	6.61±0.887^{f}	8.55±0.992^{g}	14.5±0.762^{f}	4.16±0.441^{d}	5.35±0.993^{e}	7.81±0.256^{f}
	15	12.5±0.174^{h}	16.8±0.766^{h}	19.5±0.880^{h}	8.18±0.912^{g}	11.2±0.103^{h}	18.8±0.561^{h}	5.18±0.732^{e}	8.25±0.231^{g}	11.5±0.542^{h}
Cd100+AllB	5	8.81±0.442^{g}	23.8±0.771^{i}	32.6±0.111^{j}	5.21±0.905^{e}	13.5±0.832^{h}	22.5±0.599^{i}	3.91±0.912^{c}	9.23±0.332^{g}	13.6±0.321^{h}
	10	14.5±0.923^{h}	28.3±0.421^{i}	39.7±0.099^{j}	8.34±0.431^{g}	15.6±0.223^{h}	25.6±0.431^{i}	5.24±0.872^{e}	9.81±0.419^{g}	14.7±0.451^{h}
	15	18.9±0.651^{h}	32.5±0.761^{j}	48.5±0.118^{k}	12.2±0.461^{h}	19.9±0.209^{h}	38.5±0.491^{j}	7.15±0.112^{f}	11.5±0.321^{h}	22.5±0.445^{i}
Cd50 + AllM	5	23.4±0.998^{i}	24.3±0.881^{i}	55.6±0.189^{l}	16.6±0.441^{h}	18.5±0.084^{h}	26.1±0.487^{i}	9.25±0.431^{g}	11.2±0.874^{h}	15.7±0.556^{h}
	10	29.3±0.112^{i}	38.4±0.871^{j}	56.8±0.029^{l}	18.3±0.449^{h}	23.6±0.992^{i}	29.3±0.412^{i}	11.8±0.523^{h}	15.6±0.912^{h}	16.9±0.512^{h}
	15	32.5±0.214^{j}	40.5±0.666^{k}	59.7±0.910^{l}	21.6±0.781^{i}	29.5±0.712^{i}	32.5±0.223^{j}	13.5±0.571^{h}	17.8±0.521^{h}	18.8±0.561^{h}
Cd100+AllM	5	14.6±0.652^{h}	28.8±0.181^{i}	37.6±0.702^{j}	7.91±0.390^{f}	15.5±0.612^{h}	18.6±0.443^{h}	4.81±0.821^{d}	9.21±0.453^{g}	12.1±0.667^{h}
	10	24.5±0.023^{i}	35.9±0.811^{j}	39.9±0.652^{j}	17.6±0.229^{h}	25.5±0.412^{i}	19.2±0.412^{h}	9.91±0.554^{g}	16.7±0.321^{h}	13.5±0.589^{h}
	15	33.3±0.176^{j}	39.5±0.816^{j}	52.8±0.882^{l}	23.5±0.831^{i}	27.6±0.332^{i}	29.5±0.633^{i}	15.5±0.519^{h}	17.8±0.334^{h}	15.8±0.775^{h}
Cd50+Const.	5	29.5±0.144^{i}	42.5±0.221^{k}	57.9±0.887^{l}	21.5±0.783^{i}	31.5±0.892^{j}	33.5±0.442^{j}	12.6±0.231^{h}	20.9±0.732^{i}	21.7±0.998^{i}
	10	33.6±0.322^{j}	48.1±0.532^{k}	62.5±0.562^{m}	24.5±0.671^{i}	35.5±0.762^{j}	41.8±0.419^{k}	13.9±0.412^{h}	21.8±0.443^{i}	29.8±0.651^{i}
	15	37.5±0.211^{j}	52.4±0.631^{l}	73.8±0.661^{n}	28.7±0.885^{i}	41.5±0.872^{k}	53.5±0.486^{l}	15.6±0.743^{h}	25.6±0.412^{i}	35.8±0.674^{j}
Cd100+Const.	5	31.8±0.221^{j}	43.5±0.871^{k}	51.4±0.119^{l}	23.8±0.991^{i}	29.9±0.341^{i}	27.5±0.238^{i}	13.8±0.571^{h}	18.1±0.449^{h}	16.8±0.661^{h}
	10	38.4±0.231^{j}	52.3±0.901^{l}	66.6±0.291^{m}	26.9±0.322^{i}	40.1±0.251^{k}	43.6±0.367^{k}	18.9±0.883^{h}	29.5±0.244^{i}	32.7±0.841^{j}
	15	42.7±0.224^{k}	58.9±0.710^{l}	71.8±0.118^{n}	30.1±0.412^{j}	42.8±0.258^{k}	53.8±0.413^{l}	22.5±0.124^{i}	31.8±0.412^{j}	39.8±0.655^{j}

Mean ± SD (n=3). Different small alphabets (a-p) indicate statistically significant difference at $P \leq 0.05$ by Turkey's test.(**C**: control, **Cd 50**: cadmium 50 mgkg^{-1}, **Cd 100**: cadmium 100 mgkg^{-1}, **All B:** all bacterial isolates (1-9), **All M:** All mycorrhiza cultures (1-3), **Const:** microbial consortium (All bacteria + all mycorrhiza)

Table 22: Percent (%) DPPH scavenging activity in shoots and roots of *Canna indica* inoculated with arsenic (50 and 100 mgkg^{-1}) along with rhizobacteria, mycorrhiza and microbial consortium.

Time → duration		1st month		2nd Month		3rd Month	
Treatment ↓	DPPH (µg/mL) ↓	Shoots	Roots	Shoots	Roots	Shoots	Roots
Control	5	22.4±1.019^{n}	33.6±0.362^{o}	28.6±0.345^{n}	38.9±0.518^{o}	34.6±0.876^{o}	44.9±0.567^{p}
	10	28.6±0.345^{n}	38.9±0.474^{o}	32.5±0.567^{o}	42.6±0.369^{p}	40.6±0.556^{p}	48.6±0.688^{p}
	15	32.8±0.897^{0}	41.6±0.889^{p}	38.6±0.667^{o}	48.8±0.778^{p}	49.3±0.576^{p}	51.4±0.998^{p}
C + As50	5	4.61±0.134^{d}	5.61±0.889^{e}	3.29±0.892^{c}	2.14±0.879^{b}	1.66±0.471^{a}	1.79±0.461^{a}
	10	5.43±0.998^{e}	5.91±0.882^{e}	4.81±0.721^{d}	3.91±0.472^{c}	2.19±0.371^{b}	2.45±0.374^{b}
	15	8.19±0.327^{f}	9.44±0.371^{g}	6.44±0.381^{e}	6.34±0.376^{e}	3.08±0.398^{c}	3.18±0.371^{c}
C + As 50	5	4.84±0.371^{d}	6.41±0.389^{e}	3.03±0.371^{c}	2.04±0.387^{b}	1.14±0.382^{a}	1.11±0.332^{a}
	10	5.66±0.371^{e}	6.84±0.399^{e}	4.91±0.487^{d}	3.08±0.371^{c}	2.03±0.444^{b}	1.98±0.321^{a}
	15	8.29±0.281^{f}	9.84±0.378^{g}	7.34±0.433^{f}	5.91±0.487^{e}	2.91±0.445^{b}	2.13±0.456^{a}
As50 +All B	5	6.62±1.221^{e}	7.36±0.391^{f}	4.55±0.387^{d}	5.21±0.381^{e}	3.58±0.227^{c}	3.76±0.356^{c}
	10	11.6±0.809^{h}	11.8±0.611^{h}	8.1±0.599^{f}	9.1±0.298^{g}	5.38±0.584^{e}	5.94±0.832^{e}
	15	12.4±0.401^{i}	18.9±0.606^{m}	11.3±0.370^{h}	12.2±0.445^{i}	7.43±0.933^{f}	8.92±0.703^{f}
As100+All B	5	8.12±0.318^{f}	9.11±0.626^{g}	3.72±0.465^{e}	7.13±0.381^{f}	3.10±0.605^{c}	5.78±0.356^{e}
	10	11.4±0.572^{h}	17.9±0.382^{l}	8.4±0.524^{f}	9.93±1.046^{g}	4.66±0.658^{d}	5.03±0.454^{e}
	15	13.5±0.617^{i}	16.8±0.531^{l}	12.4±0.370^{i}	12.6±0.741^{i}	7.51±0.646^{f}	8.60±0.234^{f}
As50+All M	5	10.5±0.557^{h}	18.3±0.704^{m}	10.7±1.550^{h}	12.7±0.915^{i}	6.81±0.757^{e}	8.86±0.356^{f}
	10	14.3±1.473^{k}	21.6±0.611^{n}	11.7±0.674^{h}	13.6±0.523^{j}	7.86±0.950^{f}	9.56±0.302^{g}
	15	14.9±1.765^{k}	26.4±0.682^{n}	12.1±0.296^{i}	15.1±0.667^{k}	9.33±0.358^{g}	12.9±0.703^{i}
As100+All M	5	7.26±0.875^{f}	9.13±0.548^{g}	5.81±0.465^{e}	7.22±0.610^{f}	2.13±0.454^{b}	4.33±0.713^{d}
	10	8.96±0.409^{f}	10.1±0.485^{g}	6.37±0.374^{e}	9.14±0.747^{g}	5.35±0.511^{e}	7.87±0.681^{f}
	15	10.2±0.706^{h}	16.7±0.455^{l}	8.42±0.592^{f}	11.8±0.370^{h}	6.23±0.430^{e}	8.49±0.391^{h}
As50+Const.	5	13.5±0.771^{j}	17.9±0.911^{l}	8.81±0.811^{f}	10.9±0.053^{h}	6.44±0.772^{e}	7.55±0.482^{f}
	10	15.8±0.882^{k}	19.8±0.822^{m}	10.1±0.342^{h}	13.5±0.321^{j}	7.91±0.661^{f}	9.34±0.442^{g}
	15	16.9±0.762^{l}	22.9±0.562^{n}	11.9±0.321^{h}	15.5±0.112^{k}	8.24±0.521^{f}	10.5±0.552^{h}
As100+Const.	5	16.1±0.823^{l}	21.8±0.653^{n}	12.8±0.451^{i}	14.6±0.321^{k}	9.12±0.552^{g}	10.2±0.231^{h}
	10	17.5±0.992^{l}	25.5±0.772^{n}	13.5±0.551^{i}	15.8±0.411^{k}	9.56±0.112^{g}	12.9±0.331^{i}
	15	19.5±0.912^{m}	28.9±0.611^{n}	14.1±0.562^{j}	16.7±0.232^{l}	10.8±0.132^{h}	13.5±0.531^{j}

Mean ± SD (n=3). Different small alphabets (a-p) indicate statistically significant difference at $P \leq 0.05$ by Turkey's test (**C**: control, **As 50**: arsenic 50 mgkg^{-1}, **As 100:** arsenic 100 mgkg^{-1}, **All B:** all bacterial isolates (1-9), **All M:** All mycorrhiza cultures (1-3), **Const:** microbial consortium (All bacteria + all mycorrhiza)

Table 23: Percent (%) DPPH scavenging activity in shoots and roots of *Canna indica* inoculated with cadmium (50 and 100 mgkg^{-1}) along with rhizobacteria, mycorrhiza and a microbial consortium

Time duration →		1st month		2nd Month		3rd Month	
Treatment ↓	DPPH (µg/mL) ↓	Shoots	Roots	Shoots	Roots	Shoots	Roots
Control	5	22.4±0.019^{o}	33.6±0.362^{p}	28.6±0.345^{o}	38.9±0.518^{o}	34.6±0.876^{o}	44.9±0.567^{p}
	10	28.6±0.345^{o}	38.9± 0.474^{p}	32.5±0.567^{p}	42.6±0.369^{p}	40.6±0.556^{p}	48.6±0.688^{q}
	15	32.8±0.897^{p}	41.6± 0.889 ^{p}	38.6±0.667^{p}	48.8±0.778^{q}	49.3±0.576^{q}	51.4±0.998^{q}
C + Cd 50	5	3.69±0.671^{d}	3.28±0.467^{d}	1.84±0.371^{b}	2.04±0.045^{c}	1.04±0.434^{a}	1.02±0.371^{a}
	10	4.23±0.375^{e}	3.94±0.372^{d}	3.19±0.371^{d}	3.29±0.321^{d}	1.81±0.361^{b}	1.34±0.872^{a}
	15	6.14±0.877^{g}	5.64±0.334^{f}	4.84±0.338^{e}	5.66±0.233^{f}	2.93±0.331^{c}	2.11±0.444c
C + Cd 100	5	3.24±0.334^{d}	3.11±0.334^{d}	1.24±0.445^{b}	2.43±0.445^{c}	1.13±0.878^{b}	1.15±0.388^{b}
	10	3.64±0.336^{d}	3.66±0.463^{d}	2.34±0.331^{c}	3.41±0.356^{d}	2.01±0.324^{c}	1.81±0.123^{b}
	15	5.28±0.456^{f}	4.93±0.512^{e}	3.86±0.167^{d}	5.83±0.174^{f}	2.99±0.456^{c}	1.93±0.543^{b}
Cd50+ AllB	5	5.32±0.224^{f}	6.01±0.301^{g}	3.84±0.32^{d}	5.19±0.772^{f}	2.15±0.552^{c}	3.13±0.521^{d}
	10	8.39±0.441^{i}	9.59±0.341^{i}	6.35±0.432^{g}	8.14±0.782^{i}	3.18±0.441^{d}	4.15±0.667^{e}
	15	10.5±0.413^{j}	13.5±0.442^{k}	7.38±0.821^{h}	11.8±0.672^{j}	5.25±0.662^{f}	7.21±0.632^{h}
Cd100+AllB	5	7.32±0.312^{h}	8.35±0.112^{i}	5.32±0.334^{f}	7.09±0.562^{h}	3.11±0.442^{d}	5.25±0.793^{f}
	10	8.91±0.334^{i}	13.2±0.612^{k}	6.18±0.442^{g}	11.2±0.332^{j}	3.25±0.419^{d}	6.29±0.683^{g}
	15	9.35±0.112^{i}	12.8±0.414^{j}	6.93±0.451^{g}	10.3±0.331^{j}	4.01±0.541^{e}	6.58±0.869^{g}
Cd50 +AllM	5	8.25±0.334^{i}	14.5±0.552^{l}	6.21±0.433^{g}	11.8±0.342^{j}	3.35±0.099^{d}	6.15±0.579^{g}
	10	11.9±0.342^{j}	14.9±0.442^{l}	7.63±0.432^{h}	12.1±0.412^{j}	4.15±0.981^{e}	7.35±0.356^{h}
	15	12.8±0.098^{j}	16.3±0.538^{m}	9.31±0.562^{i}	13.3±0.431^{k}	5.35±0.384^{f}	8.18±0.679^{i}
Cd100+AllM	5	6.31±0.341^{g}	7.35±0.456^{h}	4.25±0.401^{e}	5.03±0.445^{f}	2.81±0.643^{c}	3.15±0.362^{d}
	10	6.59±0.312^{g}	8.32±0.517^{i}	4.39±0.456^{e}	6.12±0.453^{g}	3.02±0.862^{d}	3.81±0.767^{d}
	15	9.35±0.412^{i}	13.5±0.448^{k}	6.13±0.342^{g}	10.5±0.449^{j}	3.18±0.561^{d}	4.55±0.681^{e}
Cd50+Const.	5	11.5±0.443^{j}	15.6±0.447^{l}	7.01±0.573^{h}	13.3±0.762^{k}	5.19±0.569^{f}	8.35±0.478^{i}
	10	12.8±0.112^{j}	16.8±0.362^{m}	9.35±0.762^{i}	13.9±0.651^{k}	6.25±0.667^{g}	8.81±0.578^{i}
	15	14.8±0.412^{l}	17.9±0.223^{n}	11.8±0.874^{j}	14.1±0.452^{l}	7.13±0.579^{h}	10.5±0.332^{j}
Cd100+Const.	5	13.9±0.665^{k}	15.3±0.143^{i}	11.3±0.782^{j}	13.5±0.612^{l}	7.15±0.571^{h}	8.25±0.891^{i}
	10	14.9±0.448^{l}	17.5±0.332^{n}	12.6±0.572^{j}	14.8±0.512^{l}	8.13±0.368^{i}	10.3±0.576^{j}
	15	16.7±0.449^{m}	19.3±0.233^{o}	13.9±0.883^{k}	15.9±0.711^{l}	9.19±0.467^{i}	11.8±0.561^{j}

Mean ± SD (n=3). Different small alphabets (a-q) indicate statistically significant difference at $P \leq 0.05$ by Turkey's test. (**C:** control, **Cd 50:** cadmium 50 mgkg^{-1}, **Cd 100:** cadmium 100 mgkg^{-1}, **All B:** all bacterial isolates (1-9), **All M:** All mycorrhiza cultures (1-3), **Const:** microbial consortium (All bacteria + all mycorrhiza)

5.16.2 Total Phenolic Content (TPC) in leaves, roots and stem of *Ricinus communis* and *Canna indica* inoculated with two concentrations of arsenic and cadmium (50 and 100 $mgkg^{-1}$) along with rhizobacteria, mycorrhiza and microbial consortium.

Total Phenolic content in different parts of *R. communis* (leaves, roots and stem) and *C. indica* (Shoot and roots) extracted in methanol under arsenic and cadmium treatment was evaluated in 3 months of experimentation period. Maximum phenolic content was observed in leaves of *R. communis* and shoots of *C. indica* plant in 3 months followed by roots and stem inoculated with the microbial consortium. TPC was found to be more in all inoculated plants (*R. communis* and *C. indica*) compared to all control plants except the control without any treatment (figure 46-50). TPC significantly increased in the first 2 months but decreased in the third month in all parts of *R. communis* and *C. indica* plants. Moreover, a decrease in TPC was noticed with the increase in metal concentration in both the plants. Maximal TPC was detected in the leaves of the plants treated with microbial consortium followed by mycorrhiza and rhizobacteria inoculated plants. 44.3, 52.6, 46.7mg GAE/g DW of TPC was recorded after the1st month in leaves of control and microbial consortium inoculated plants (As 50 and 100 $mgkg^{-1}$) respectively. 40.3, 44, 42.1 and 32.3, 46.2, 44.4 of TPC was observed in roots and stem of control and microbial consortium inoculated plants (As 50 and 100 $mgkg^{-1}$) respectively after 1st-month sampling in *R. communis* plant. Similarly, 38.45, 45.23, 40.2 and 32.5, 37.75, 34.85 mg GAE/g DW was TPC in the shoots and roots of control and microbial consortium impregnated *C. indica* plant (As 50 and 100 $mgkg^{-1}$) respectively after the 1st month.

Likewise after 2nd and 3rd-month sampling maximum activity was found in plants inoculated with the microbialconsortium in both the plants as shown in table 24.Overall range for TPC in all treatments during 3 months was observed in the range 24-65 mg GAE/g DW. *C. indica* plant exhibited less TPC in shoots and roots as compared to *R. communis*. Hence this can be related with higher accumulation of As in the parts of *R. communis* (shoot and roots) which manifests that under heavy metal stress, these secondary metabolites are produced in elevated levels to combat the oxidative stress in plants. But with an increase in metal concentration, the production of these metabolites significantly decreases ($P \leq 0.05$).

After 3rd month of sampling, TPC in both the plants decreased in comparison to 2nd month. This can be due to higher accumulation of metal after a 3rd month in the parts of both the plants which resulted in the decline of TPC**.**An almost similar trend was seen in leaves of both the plants inoculated with two concentrations (50 and 100 $mgkg^{-1}$) of cadmium. Maximum TPC was observed in leaves of both the plants followed by roots and stem in 3 months experimentation period. A remarkable increase in TPC was seen in the case of plants treated with cadmium as compared to plants inoculated with arsenic which infers that both the plants have accumulated

more cadmium than arsenic in their parts. In *R. communis* 44.3, 82.1, 78.4 mg GAE/g DW of TPC was found in leaves of control and microbial consortium inoculated plants whereas, 40.3, 72.4, 68.4 and 32.3, 62.4, 58.4 mg GAE/g DW of TPC was the total phenolic content in roots and stem of microbial consortium inoculated plants (Cd 50 and 100 mgkg^{-}1) respectively after 1st month as given in table 25. In both the plants TPC increased in all parts with respect to time but not concentration, as with increasing concentration TPC decreases. The overall range of TPC in Cd inoculated plants was found to be 28 – 82 mg GAE/g DW in both the plants. In cadmium treated plants, maximum TPC was observed in leaves of microbial consortium inoculated both the plants but in case of *R. communis*, TPC significantly decreased in leaves of microbial consortium inoculated plants from 2nd to 3rd month but not in roots and stem of all treated pots (table 25). Therefore, from the results obtained in TPC of both the plants we can conclude that the microbial consortium developed in this research work, was proven to be effective in reducing the heavy metal toxicity in plant parts than other treatments (rhizobacteria and mycorrhiza).

Our findings are supported by the work done on different plant species including *R. communis* under heavy metal stress to detect the effect on phytochemicals (total phenols and flavonoid content). Results depicted the higher TPC (80.34 mg GAE/g DW) and TFC (13.6 mg GAE/g DW) in the inoculated plants as compared to control pots (72.5 and 12.8 mg GAE/g DW) (Jonnada*et al*., 2015). Also in a study conducted by Marquez *et al* (2011), similar results were found where the effect of Cd was seen on the total phenolic content (TPC) of *Erica andevalensis* plant and the results showed enhanced phenolic content in the plants inoculated with Cd. Maximum TPC was found at 5 µg Cd g^{-1} soil as compared to control plants. But at highest concentration i.e 50 µg Cd g^{-1} soil, TPC decreases as in our present study. This could be due to the phenoxyl radical that acts as prooxidants that finally resulted from the antioxidant reaction (Y. Sakihama *et al*., 2002).

Hence, the plants exposed to thehighest concentration of Cd might have led to a reduction in the synthesis or release of phenolics by an unspecified mechanism to avoid a detrimental effect caused by the production of phenoxy radicals. Another study conducted on *Hippophae rhamnoides*, collected from heavy metal contaminated sites showed increased TPC in the leaves of plants as compared to the control plants, with respect to spring and autumn seasons (Ewa *et al*., 2014). Fazal *et al* (2015) also reported an increase in total phenolic content (TPC) in the leaves and roots of *R. communis* under Cadmium stress where maximum (55 and 59 mg GAE/g DW) TPC was found in 20 mgkg^{-1} of Cd-treated roots and leaves of *R. communis* respectively. Higher TPC content was found in leaves of *R. communis* than roots as compared to control.

Table-24: Total Phenolic Content (TPC) (mg GAE/g DW) in leaves, roots and stem of *Ricinus communis* and shoots and roots of *Canna indica* inoculated with Arsenic (50 and 100 mgkg^{-1}) along with rhizobacteria, mycorrhiza and a microbial consortium

Time duration →	1 Month					2 Month					3 Month				
Plants →	*R. communis*			*C. indica*		*R. communis*			*C. indica*		*R. communis*			*C. indica*	
Treatment ↓	Leaves	Roots	Stem	Shoot	Roots	Leaves	Roots	Stem	Shoot	Roots	Leaves	Roots	Stem	Shoot	Roots
Control	69.3± 0.891^{f}	61.9± 0.115^{f}	58.5± 0.778^{e}	51.5± 0.819^{e}	46.3± 0.667^{d}	71.5± 1.115^{g}	64.7± 1.562^{f}	62.5± 1.432^{f}	59.8± 1.872^{e}	53.4± 0.778^{e}	79.5± 0.768^{g}	68.9± 0.143^{f}	64.5± 0.598^{f}	64.8± 0.781^{f}	59.5± 0.681^{e}
C + As 50	17.4± 0.351^{a}	15.9± 0.665^{a}	12.3± 0.561^{a}	25.5± 0.981^{b}	15.8± 0.418^{a}	29.8± 0.622^{b}	21.7± 0.556^{b}	20.8± 0.223^{b}	29.4± 0.559^{b}	21.3± 0.699^{b}	13.3± 0.167^{a}	12.6± 0.599^{a}	17.8± 0.445^{a}	18.8± 0.777^{a}	15.3± 1.811^{a}
C +As 100	12.3± 0.998^{a}	11.5± 0.151^{a}	9.8± 0.243^{a}	21.8± 1.221^{b}	11.8± 0.887^{a}	23.7± 1.211^{b}	17.8± 0.611^{a}	16.3± 1.182^{a}	25.6± 0.998^{b}	15.6± 0.671^{a}	9.5± 0.116^{a}	8.3± 0.812^{a}	13.9± 0.718^{a}	12.4± 0.162^{a}	9.1± 0.699^{a}
C+All B	28± 0.282^{b}	26.2± 0.282^{b}	24.4± 0.282^{b}	35± 1.412^{c}	23.4± 0.848^{b}	35.9± 1.484^{c}	32.7± 0.989^{c}	34± 1.979^{c}	43± 2.828^{d}	30.9± 0.141^{c}	29.9± 0.989^{b}	25± 1.414^{b}	31.65± 1.767^{c}	39± 1.414^{c}	25.4± 0.867^{b}
As50+AllB	39.6± 0.226^{c}	39.2± 0.228^{c}	32.3± 0.141^{c}	43± 1.413^{d}	25.15± 1.202^{b}	49.75± 1.697^{d}	48.85± 0.353^{d}	43.1± 1.131^{d}	51.7± 0.989^{e}	32.65± 1.909^{c}	43.75± 1.202^{d}	42.35± 1.776^{d}	37.1± 2.687^{c}	47.7± 2.404^{d}	27.15± 1.203^{b}
As100+All B	38± 0.283^{c}	37.4± 0.128^{c}	27.2± 0.288^{b}	36.5± 2.192^{c}	23.25± 1.343^{b}	45.15± 0.212^{d}	45.1± 0.848^{d}	39.3± 1.991^{c}	44.55± 3.606^{d}	30.75± 2.050^{c}	39.15± 2.899^{c}	37.75± 1.767^{c}	33.3± 2.545^{c}	40.55± 2.192^{d}	25.25± 1.343^{b}
C+All M	34.6± 0.284^{c}	32.4± 0.282^{c}	25.4± 0.282^{b}	33.3± 1.834^{c}	28.75± 0.494^{b}	41.65± 1.697^{d}	43.2± 0.565^{d}	35.4± 1.555^{c}	41.3± 0.424^{d}	36.25± 0.212^{c}	35.65± 0.777^{c}	36.7± 2.687^{c}	29.4± 2.545^{b}	37.3± 1.838^{c}	30.75± 0.494^{c}
As 50+All M	48.1± 0.141^{d}	38.4± 0.284^{c}	30.7± 0.282^{c}	40.5± 0.919^{d}	33.15± 0.636^{c}	65.3± 2.757^{f}	48.5± 1.272^{d}	43.35± 2.757^{d}	48.55± 2.333^{d}	40.65± 0.070^{d}	59.35± 0.717^{e}	42.1± 0.887^{d}	37.35± 2.899^{c}	44.55± 0.919^{d}	35.15± 0.636^{c}
As100+All M	42.55± 0.155^{d}	36.2± 0.234^{c}	28.8± 0.141^{b}	38.4± 1.202^{c}	28.3± 1.838^{b}	55.5± 0.636^{e}	46.25± 0.919^{d}	40.35± 1.131^{d}	46.45± 0.212^{d}	35.8± 2.545^{c}	49.5± 0.283^{d}	38.9± 1.838^{c}	38.15± 1.202^{c}	42.45± 1.202^{d}	30.3± 1.838^{c}
C+Const	44.3± 0.144^{d}	40.3± 0.114^{d}	32.3± 0.141^{c}	38.4± 0.636^{c}	32.5± 2.121^{c}	53.65± 2.687^{e}	52.1± 1.555^{e}	36.8± 1.697^{c}	46.45± 0.776^{d}	40± 2.828^{d}	47.65± 0.212^{d}	45.6± 3.676^{d}	30.8± 2.262^{c}	42.45± 0.636^{d}	34.5± 2.121^{c}
As50+Const	52.6± 0.282^{e}	44± 0.287^{d}	46.2± 0.288^{d}	45.2± 1.484^{d}	37.75± 0.353^{c}	64± 0.919^{f}	53.9± 1.979e	71.25± 0.848^{g}	56.7± 0.070^{e}	48.45± 0.633^{d}	58± 0.565^{e}	45.25± 1.060^{d}	65.25± 3.040^{f}	49.15± 0.353^{d}	42.95± 1.344^{d}
As100+Const	46.7± 0.424^{d}	42.1± 0.113^{d}	44.4± 0.285^{d}	40.2± 1.418^{d}	34.85± 1.909^{c}	61.3± 1.979^{f}	52.45± 2.616^{e}	58.05± 1.626^{e}	47.6± 0.878^{d}	45.25± 1.060^{d}	55.3± 1.272^{e}	43.8± 1.697^{d}	56± 1.414^{e}	47.75± 3.606^{d}	39.75± 0.335^{c}

Mean ± SD (n=3). Different small alphabets (a-g) indicate statistically significant difference at $P \leq 0.05$ by Turkey's test.(**C:** control, **As 50:** arsenic 50 mgkg^{-1}, **As 100**: arsenic 100 mgkg^{-1}, **All B**: all bacterial isolates (1-9), **All M:** All mycorrhiza cultures (1-3), **Const**: microbial consortium (All bacteria + all mycorrhiza)

Table-25: Total Phenolic Content (TPC) (mg GAE/g DW) in leaves, roots and stem of *Ricinus communis* and shoots and roots of *Canna indica* inoculated with Cadmium (50 and 100 mgkg^{-1}) along with rhizobacteria, mycorrhiza and a microbial consortium

Time duration →	1 Month					2 Month					3 Month				
Plants →	*R. communis*			*C. indica*		*R. communis*			*C. indica*		*R. communis*			*C. indica*	
Treatment ↓	Leaves	Roots	Stem	Shoot	Roots	Leaves	Roots	Stem	Shoot	Roots	Leaves	Roots	Stem	Shoot	Roots
Control	69.3± 0.891^{f}	61.9± 0.115^{f}	58.5± 0.778^{e}	51.5± 0.819^{e}	46.3± 0.667^{d}	71.5± 1.115^{g}	64.7± 1.562^{f}	62.5± 1.432^{f}	59.8± 1.872^{e}	53.4± 0.778^{e}	79.5± 0.768^{g}	68.9± 0.143^{f}	64.5± 0.598^{f}	64.8± 0.781^{f}	59.5± 0.681^{e}
C + Cd 50	21.3± 0.278^{b}	19.5± 0.672^{a}	18.8± 0.781^{a}	29.5± 0.781^{b}	21.3± 0.748^{b}	34.5± 0.361^{c}	28.9± 0.251^{b}	23.8± 0.371^{b}	35.3± 0.135^{c}	23.3± 1.098^{b}	19.9± 0.667^{a}	22.6± 0.177^{b}	21.9± 0.991^{b}	22.8± 0.119^{b}	17.9± 0.361^{a}
C +Cd100	18.9± 0.361^{a}	15.3± 0.177^{a}	13.9± 0.271^{a}	23.9± 0.661^{b}	19.5± 0.617^{a}	30.9± 1.982^{c}	25.4± 0.271^{b}	19.6± 1.223^{a}	29.8± 1.371^{b}	19.8± 1.291^{a}	12.5± 0.271^{a}	18.9± 0.871^{a}	16.5± 0.361^{a}	18.9± 0.367^{a}	13.7± 0.712^{a}
C+All B	28± 0.282^{b}	26.2± 0.282^{b}	24.4± 0.282^{b}	35± 1.414^{c}	23.4± 0.878^{b}	35.9± 1.484^{c}	32.7± 0.999^{c}	34± 1.979^{c}	43± 2.828^{d}	30.9± 0.141^{c}	29.9± 0.998^{b}	25± 1.411^{b}	31.65± 1.767^{c}	39± 1.414^{c}	25.4± 0.848^{b}
Cd 50+AllB	52.1± 0.134^{e}	43.2± 0.282^{d}	40.6± 0.282^{d}	39.5± 0.636^{c}	24.2± 1.525^{b}	60.5± 0.633^{f}	53.5± 1.131^{e}	55.1± 0.777^{e}	47.05± 0.777^{d}	31.7± 0.834^{c}	54.5± 2.262^{e}	47± 3.252^{d}	49.1± 2.969^{d}	43.05± 0.636^{d}	26.2± 1.345^{b}
Cd 100+All B	44.4± 0.228^{d}	38.8± 0.848^{c}	38.1± 0.141^{c}	37.6± 0.565^{c}	21.3± 1.555^{b}	55.4± 0.848^{e}	46.3± 1,555^{d}	48.65± 1.272^{d}	45.6± 0.847^{d}	28.8± 0.848^{b}	52.3± 0.989^{e}	39.8± 0.565^{c}	42.65± 2.192^{d}	41.6± 0.565^{d}	23.3± 1.535^{b}
C+All M	34.6± 0.328^{c}	32.4± 0.228^{c}	25.4± 0.228^{b}	33.3± 1.838^{c}	28.7± 0.494^{b}	41.65± 1.697^{d}	43.2± 0.565^{d}	35.4± 1.555^{c}	41.3± 0.424^{d}	36.25± 0.212^{c}	35.65± 0.777^{c}	36.7± 2.687^{c}	29.4± 2.545^{b}	37.3± 1.838^{c}	30.75± 0.499^{c}
Cd 50+AllM	62.1± 0.143^{f}	44.8± 1.141^{d}	40.6± 0.282^{d}	43.4± 1.060^{d}	31.5± 1.909^{c}	71.3± 1.626^{g}	58.8± 0.424^{e}	50.9± 1.979^{e}	51.45± 2.474^{e}	39.05± 1.202c	65.3± 0.282^{f}	52.3± 2.545^{e}	44.9± 3.111^{d}	47.45± 1.066^{d}	33.5± 1.909^{c}
Cd 100+AllM	47.2± 0.280^{d}	41.8± 1.414^{d}	32.5± 0.424^{c}	42.3± 1.484^{d}	27.8± 0.989^{b}	64.35± 1.697^{f}	54.9± 0.353^{e}	45.8± 1.484^{d}	50.35± 2.899^{e}	35.3± 0.282^{c}	58.35± 1.343^{e}	48.45± 2.474^{d}	39.8± 3.394^{c}	52± 1.414^{e}	29.8± 0.989^{b}
C+ Const	44.3± 0.114^{d}	40.3± 0.114^{d}	32.3± 0.141^{c}	38.4± 0.636^{c}	32.5± 2.121^{c}	53.65± 2.687^{e}	52.1± 1.555^{e}	36.8± 1.979^{c}	46.45± 0.777^{d}	40± 2.827^{d}	47.65± 0.212^{d}	45.6± 3.676^{d}	30.8± 2.263^{c}	42.45± 0.636^{d}	34.5± 2.122^{c}
Cd 50+Const	82.1± 0.414^{h}	72.4± 0.226^{g}	62.4± 0.281^{f}	47.4± 1.626^{d}	42.5± 1.767^{d}	77± 1.897^{g}	76.9± 0.777^{g}	65.45± 1.626^{f}	65± 0.212^{f}	51.4± 3.676^{e}	71± 3.394^{g}	70.45± 2.899^{g}	56.7± 1.414^{e}	51.8± 1.131^{e}	44.2± 2.969^{d}
Cd100+Const	78.4± 0.222^{g}	68.4± 0.282^{f}	58.4± 0.565^{e}	45.5± 2.757^{d}	42.2± 2.969d	71.45± 1.979^{g}	70.4± 1.343^{g}	52.7± 0.424^{e}	60± 1.414^{f}	49.7± 2.263^{d}	65.45± 2.333^{f}	62.25± 1.060^{f}	56± 1.838e	56± 2.828^{c}	45.9± 4.384^{d}

Mean ± SD (n=3). Different small alphabets (a-g) indicate statistically significant difference at $P \leq 0.05$ by Turkey's test (**C:** control, **Cd 50:** cadmium 50 mgkg^{-1}, **Cd 100:** cadmium 100 mgkg^{-1}, **All B:** all bacterial isolates (1-9), **All M:** All mycorrhiza cultures (1-3), **Const:** microbial consortium (All bacteria + all mycorrhiza)

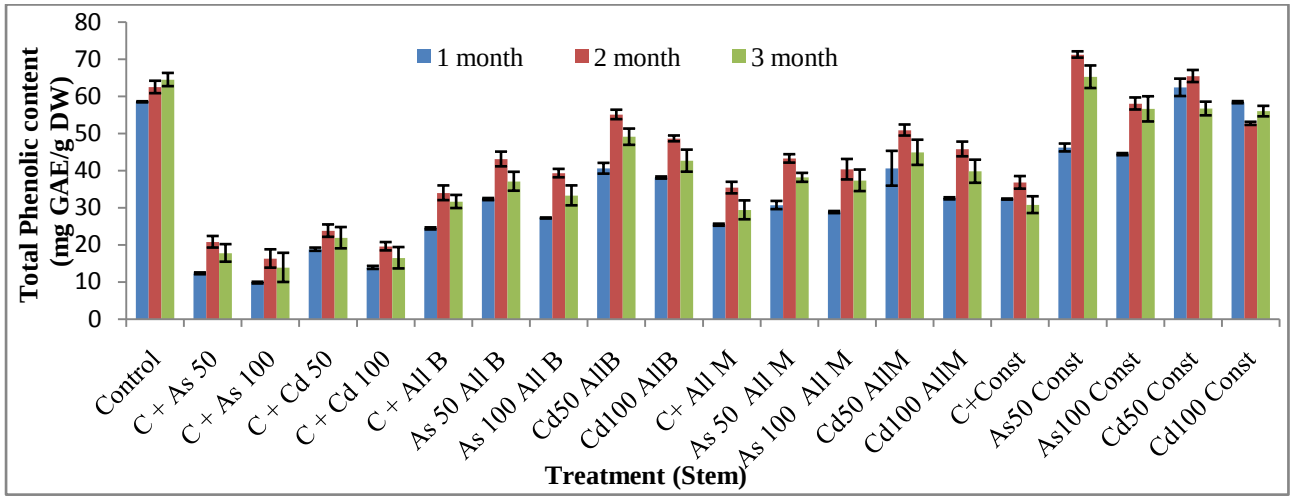

Figure 46: Total Phenolic Content (TPC) (mg GAE/g DW) in the stem of *Ricinus communis* inoculated with arsenic and cadmium (50 and 100 $mgkg^{-1}$) along with rhizobacteria, mycorrhiza and a microbial consortium

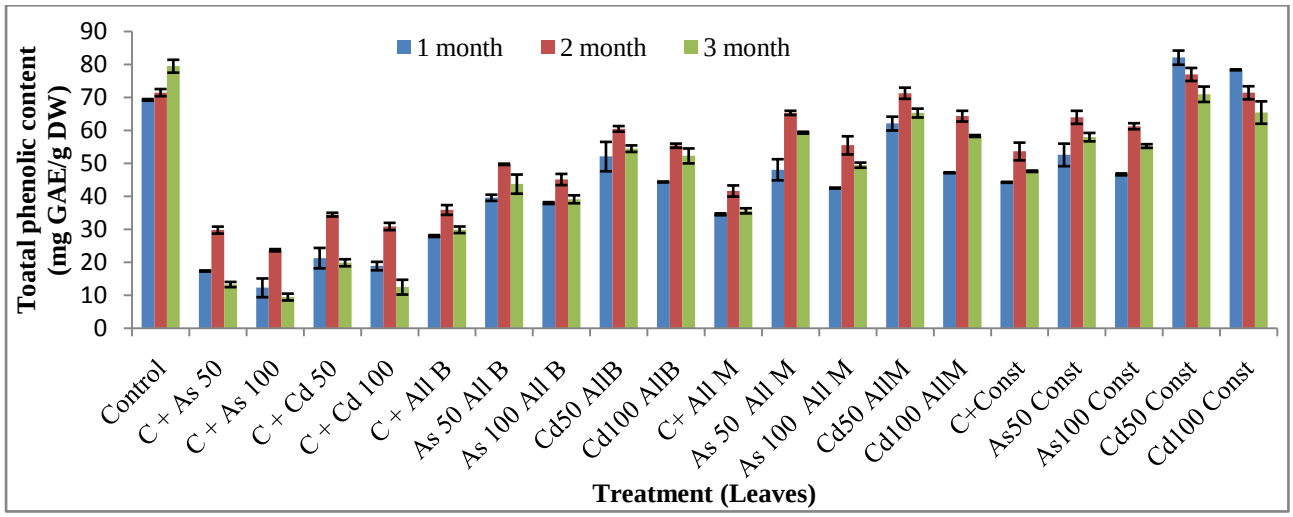

Figure 47: Total Phenolic Content (TPC) (mg GAE/g DW) in leaves of *Ricinus communis* inoculated with arsenic and cadmium (50 and 100 $mgkg^{-1}$) along with rhizobacteria, mycorrhiza and microbial consortium.

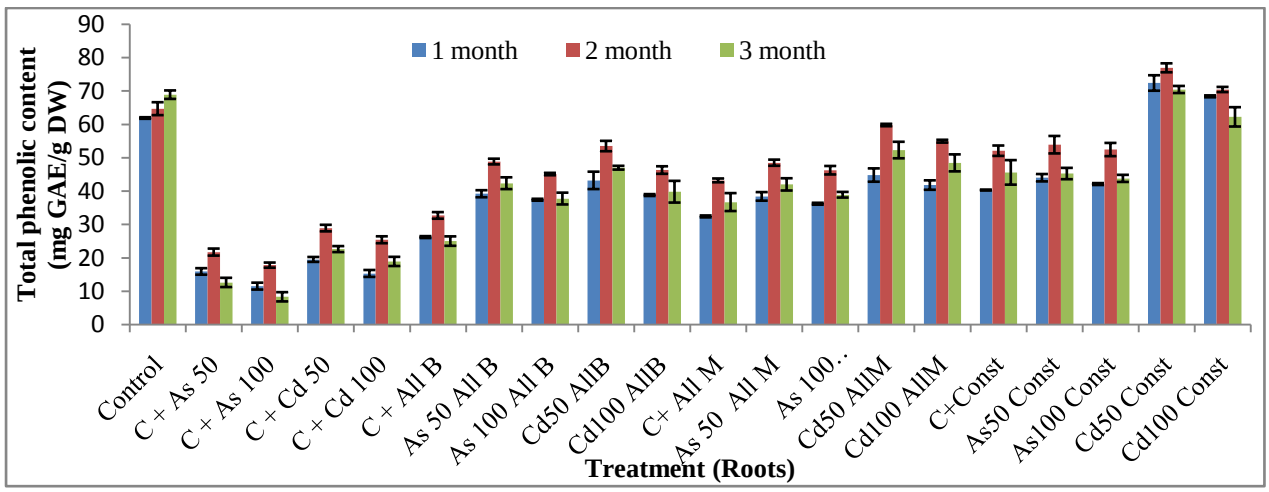

Figure 48: Total Phenolic Content (TPC) (mg GAE/g DW) in leaves of *Ricinus communis* inoculated with arsenic and cadmium (50 and 100 $mgkg^{-1}$) along with rhizobacteria, mycorrhiza and microbial consortium.

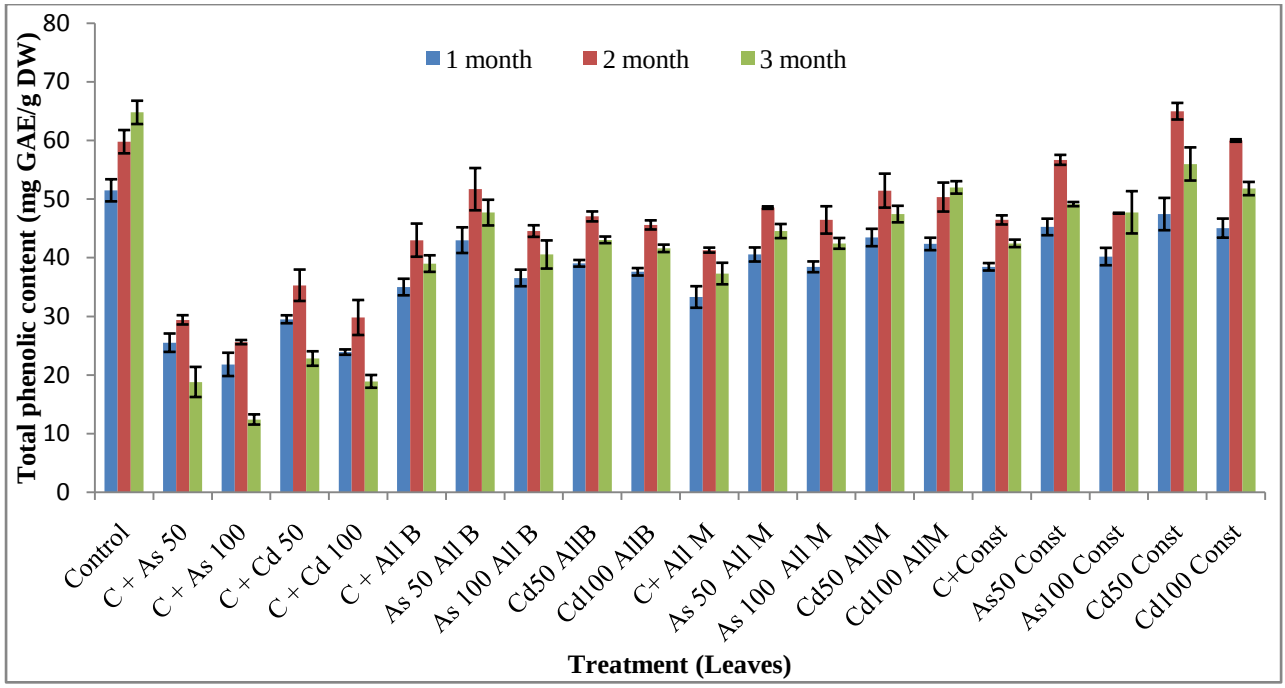

Figure 49: Total Phenolic Content (TPC) (mg GAE/g DW) in leaves of *Canna indica* inoculated with arsenic and cadmium (50 and 100 $mgkg^{-1}$) along with rhizobacteria, mycorrhiza and microbial consortium.

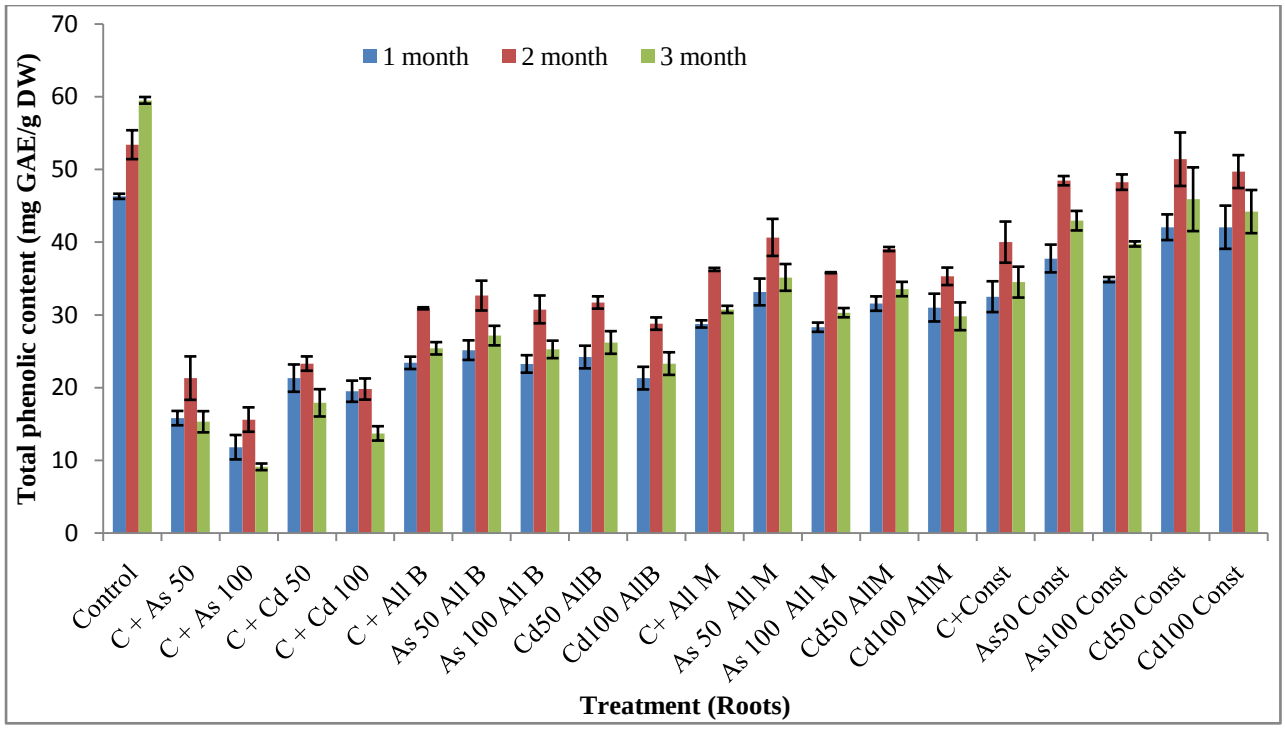

Figure 50: Total Phenolic Content (TPC) (mg GAE/g DW) in roots of *Canna indica* inoculated with arsenic and cadmium (50 and 100 $mgkg^{-1}$) along with rhizobacteria, mycorrhiza and microbial consortium.

6.16.3 Total Flavonoid content (TFC) (mg QE/g DW) in leaves, roots and stem of *Ricinus communis* and shoots and roots of *Canna indica* inoculated with two concentrations of arsenic and cadmium (50 and 100 $mgkg^{-1}$) along with rhizobacteria, mycorrhiza and microbial consortium.

An elevated level of flavonoid content was observed in all the inoculated plants (*R. communis* and *C. indica*) compared to all the treated (microbial and heavy metal) control plants during 3 months experimentation period (figure 51-55). Maximum TFC was noticed in microbial consortium and minimum in rhizobacteria inoculated plants under arsenic stress. Whereas, the TFC was found in an increasing order of stem< roots <leaves in *R. communis* plant and roots< leaves in *C. indica* plant in all the 3 months. *R. communis* plant leaves show 21, 32.6, 31.7 mg QE/g DW TFC in control and microbial consortium inoculated plants (As 50 and 100 $mgkg^{-1}$) respectively after 1st-month sampling. Whereas 28.45, 39, 39.8 and 27.45, 35.6, 33.9 mg QE/g DW of TFC was found in leaves of control and microbial consortium inoculated plants (As 50 and 100 $mgkg^{-1}$) after 2nd and 3rd month respectively (table 26).

As in other activities, TFC was noticed to increase in all the plants from 1st to 2nd month but significantly decreased ($P \leq 0.05$) after the 3rd month. With the increase in As concentration, TFC decreased in all the treated and control pots of both the plants as due to higher As concentration, production of secondary metabolites decreases but increases at a lower concentration. 18.45, 23.6, 21.7 and 11.3, 15.1, 10.6 mg QE/g DW was the TFC in leaves and roots of controland microbial consortium (arsenic 50 and 100 $mgkg^{-1}$) inoculated plants of *C. indica* after 1st month respectively. Similarly,28.5, 31.9, 27.75 and 18.65, 21.65, 20.7 mg QE/g DW of TFC was found in leaves and roots of control and microbial consortium treated pots after 2nd and 3rd months respectively as shown in table 27. Overall TFC was observed to be in the rangeof 3-39 mg QE/g DW in arsenic and microbial treated *R. communis* and *C. indica* plants (table 26).

Almost identical results were remarked in the pots of both the plants under cadmium stress where maximum TFC was observed in microbial consortium inoculated plants and minimum in rhizobacteria inoculated plants. A similar trend of increasing order in both the plants was found to be stem< roots < leaves in *R. communis* and roots <leaves in *C. indica* in all the 3 months. Moreover, cadmium treated plants displayed more content of flavonoid than arsenic-treatedplants. Hence it clearly indicates that Cd was accumulated in higher amount as compared to arsenic in all the treated pots of both the plants. Overall range in all the control and microbial inoculated pots of *R. communis* and *C. indica* plants was found to be from 3-69 mg QE/g DW. Interestingly, TFC also decreases with increasing concentration of heavy metal (Cd) but with time it decreases in both the plants as shown in table 27.

The results of the present work were found to be in accordance with Jonnada *et al* (2015). In their study, five medicinal plants were analysed for their phytochemical properties (TPC and TFC) under heavy metal stress. Results showed that flavonoid content along with phenolic content was enhanced in the plants treated with heavy metals than control plants (untreated). The results of this phytochemical screening were found to be at par with the results of Daniel and Daniang (2011), where these phytochemical (TPC and TFC) found in plants are either synthesized for defence purpose or are the products of plant metabolism. In the study, higher content of flavonoid was found in *R. communis* under stress conditions (13.6 mg/g DW) as compared to control (12.8 mg/g DW). Induction in the biosynthesis of phenolics and flavonoid compounds were observed in wheat (*Triticum aestivum*) under nickel toxicity (Diaz.J *et al*., 2001) and even in maize (*Zea mays*) (Winker. 2002). Also when sprayed with copper sulphate solution (Cu) leaves of *Phyllanthus tenillus* plant showed increased TFC content (Wrinker. 2002) as compared to non treated plants as well as Cd-treated *Phaseolus vulgaris* also depicted the same results (Diaz, J *et al*., 2001).

According to Parry *et al* (1994), increase in flavonoid content under heavy metal stress is due to conjugate hydrolysis but not due to new biosynthesis. Michalak (2006) reported that various phenolic compounds including flavonoids (isoflavones) are induced as a response to multiple stresses (including heavy metal stress). Unidentical results were also noticed in case of *Hordeum sativum L.* (barely) plants. The total flavonoid content was decreased in the roots, shoots and leaves due to toxic effects of Cd in solution. The relatively higher decrease in TFC was found in roots (20-3.05 g/kg DW) followed by shoots (24.2-9.33 g/kg DW) and leaves (58.3 -27.3 g/kg DW). Flavonoid content was found to be decreasing with increasing Cd accumulation in the parts of plants (J. Lachman *et al*. 2005).

This can be explained as, with an increase in Cd concentration, flavonoid content decreases because flavonoids act as efficient chelators of metals (Bai *et al*., 2004). As many flavonoid structures are bound with transition metals in chelate complexes, whose structures were explained by Bai *et al* (2004), thus the decrease in flavonoid content is evidenced under Cd stress. Therefore, Plants use evidently the chelate complex formation with flavonoids for the reduction of heavy metals stress (J. Lachman, 2005).

Table-26: Total Flavonoid content (TFC) (mg QE/g DW) in leaves roots and stem of *Ricinus communis* and shoots and roots of *Canna indica* inoculated with arsenic (50 and 100 mgkg^{-1}) along with rhizobacteria, mycorrhiza and a microbial consortium

Time duration →	1st month					2nd month					3rd month				
Plants →	*R. communis*			*C. indica*		*R. communis*			*C. indica*		*R. communis*			*C. indica*	
Treatment ↓	Leaves	Roots	Stem	Shoots	Roots	Leaves	Roots	Stem	Shoots	Roots	Leaves	Roots	Stem	Shoots	Roots
Control	43.9± 1.221^{e}	24.9± 1.261^{c}	18.8± 0.174^{b}	33.9± 0.238^{d}	19.8± 0.472^{c}	55.8± 0.371^{f}	29.8± 0.832^{c}	22.5± 0.471^{c}	38.3± 0.371^{d}	26.9± 0.361^{c}	59.1± 0.322^{f}	37.6± 0.388^{d}	29.3± 0.361^{c}	44.6± 0.333^{e}	32.8± 0.379^{d}
C + As 50	15.8± 1.332^{b}	9.81± 0.921^{a}	3.41± 0.381^{a}	9.87± 0.712^{a}	6.34± 0.271^{a}	19.8± 0.556^{b}	12.9± 0.661^{b}	6.74± 0.371^{a}	14.9± 0.518^{b}	11.7± 0.537^{b}	14.6± 0.472^{b}	9.81± 0.332^{a}	8.92± 0.361^{a}	11.8± 0.123^{b}	7.74± 0.661^{a}
C + As 100	12.4± 1.922^{b}	6.43± 0.361^{a}	2.34± 0.667^{a}	5.41± 0.446^{a}	4.41± 0.778^{a}	14.6± 0.667^{b}	8.71± 0.771^{a}	4.19± 0.669^{a}	12.3± 0.781^{b}	8.41± 0.589^{a}	11.9± 0.181^{b}	7.43± 0.561a	4.34± 0.671^{a}	8.14± 0.671^{a}	5.63± 0.367^{a}
C + All B	15± 0.282^{b}	11.7± 0.212^{b}	3.7± 0.141^{a}	13.8± 0.282^{b}	7.35± 0.777^{a}	24.2± 0.565^{c}	19.4± 1.131^{b}	10.9± 1.272^{a}	22.55± 0.636^{c}	14.85± 0.212^{b}	21.15± 1.484^{c}	12.65± 1.343^{b}	7.5± 0.424^{a}	16.65± 1.626^{b}	10.1± 0.424^{a}
As 50 +All B	23.15± 0.777^{c}	14.2± 0.565^{b}	5.6± 0.282^{a}	16.5± 0.707^{b}	6.9± 1.272^{a}	32.4± 1.131^{d}	21.8± 1.414^{c}	13.6± 0.494^{b}	24.7± 0.282^{c}	14.95± 1.060^{b}	21± 1.838^{c}	17.05± 1.767^{b}	11.6± 2.262^{b}	18.8± 1.272^{b}	12.55± 0.771^{b}
As 100 + All B	21.2± 1.131^{c}	13.3± 1.272^{b}	5.45± 0.353^{a}	14.6± 0.565^{b}	8.95± 1.343^{a}	30.3± 1.272^{d}	22.5± 1.838^{c}	12.3± 1.484^{b}	25.6± 0.989^{c}	16.45± 0.777^{b}	25.3± 1.131^{c}	17± 1.414^{b}	8.4± 1.414^{a}	19.7± 1.979^{b}	12.8± 1.697^{b}
C + All M	16.65± 0.919^{b}	9.8± 0.565^{a}	7.15± 1.060^{a}	15.1± 0.989^{b}	11.4± 0.848^{b}	25.65± 0.919^{c}	17.8± 0.848^{b}	14.4± 0.212^{b}	26.45± 0.636^{c}	19.85± 1.626^{b}	22.6± 2.545^{c}	13.6± 1.979^{b}	9.95± 0.919^{a}	20.55± 1.626^{c}	15.75± 1.909^{b}
As 50 + All M	23.75± 1.202^{c}	13.1± 2.12^{b}	10.2± 1.131^{b}	15.9± 0.141^{b}	12.4± 0.777^{b}	34.55± 1.343^{d}	19.85± 1.767^{b}	18.8± 1.697^{b}	25.35± 1.484^{c}	21.15± 1.909^{c}	21.15± 1.626^{c}	12.3± 1.838^{b}	12.85± 1.060^{b}	19.45± 2.474^{b}	15.25± 0.353^{b}
As 100 + All M	17.9± 0.424^{b}	11.9± 0.282^{b}	8.55± 0.212^{a}	17.6± 0.565^{b}	12.1± 1.202^{b}	28.55± 1.909^{c}	20.55± 2.050^{c}	16.4± 0.282^{b}	25.85± 1.202^{c}	20.3± 1.697^{c}	27.1± 2.687^{c}	15.05± 1.343^{b}	11.75± 1.202^{b}	19.95± 0.212^{b}	15.15± 1.202^{b}
C+ Const	21± 1.414^{c}	16.6± 1.343^{b}	8.35± 1.060^{a}	18.4± 0.636^{b}	11.3± 1.060^{b}	28.45± 0.777^{c}	22.5± 0.494^{c}	16.4± 0.848^{b}	28.5± 0.070^{c}	18.65± 1.767^{b}	25.45± 2.050^{c}	18.65± 2.050^{b}	10.8± 1.414^{a}	22.05± 0.919^{c}	14.75± 1.200^{b}
As 50 + Const.	32.05± 1.343^{d}	18.5± 0.848^{b}	16.1± 1.272^{b}	23.6± 1.697^{c}	15.1± 2.192^{b}	39.8± 0.424^{d}	26.7± 0.884^{c}	22.2± 1.697^{c}	31.9± 1.621^{d}	21.65± 0.494^{c}	35.6± 1.838^{d}	22.45± 2.050^{c}	19.75± 0.494^{b}	26± 2.687^{c}	16.15± 1.202^{b}
As 100 +Const.	31.7± 0.707^{d}	17.5± 0.494^{b}	12.5± 0.141^{b}	21.7± 2.262^{c}	10.6± 1.414^{b}	39± 1.414^{d}	24.85± 0.919^{c}	20.5± 0.141^{c}	27.75± 1.202^{d}	20.7± 1.697^{c}	33.9± 1.697^{d}	20.85± 2.333^{c}	16.2± 0.565^{b}	21.85± 0.212^{c}	14.85± 0.494^{b}

Mean ± SD (n=3). Different small alphabets (a-f) indicate statistically significant difference at $P \leq 0.05$ by Turkey's test. (**C:** control, **As 50:** arsenic 50 mgkg^{-1}, **As 100:** arsenic 100 mgkg^{-1},**All B:** all bacterial isolates (1-9), **All M:** All mycorrhiza cultures (1-3), **Const:** microbial consortium (All bacteria + all mycorrhiza)

Table-27: Total Flavonoid content (TFC) (mg QE/g DW)in leaves roots and stem of *Ricinus communis* and shoots and roots of *Canna indica* inoculated with Cadmium (50 and 100 mgkg^{-1}) along with rhizobacteria, mycorrhiza and a microbial consortium

Time duration →	1st month					2nd month					3rd month				
Plants →	*R. communis*			*C. indica*		*R. communis*			*C. indica*		*R. communis*			*C. indica*	
Treatment ↓	Leaves	Roots	Stem	Shoots	Roots	Leaves	Roots	Stem	Shoots	Roots	Leaves	Roots	Stem	Shoots	Roots
Control	43.9± 1.221^{e}	24.9± 1.261^{c}	18.8± 0.174^{b}	43.9± 0.238^{e}	19.8± 0.472^{b}	55.8± 0.371^{f}	29.8± 0.832^{c}	22.5± 0.471^{c}	38.3± 0.371^{d}	26.9± 0.361^{c}	59.1± 0.322^{f}	37.6± 0.388^{d}	29.3± 0.361^{c}	44.6± 0.333^{e}	32.8± 0.379^{d}
C + Cd 50	18.8± 0.726^{b}	12.6± 0.112^{b}	5.64± 0.892^{a}	12.3± 0.673^{b}	9.91± 0.782^{a}	22.6± 0.619^{c}	21.8± 0.361^{c}	9.94± 1.281^{a}	16.5± 1.278^{b}	14.9± 0.831^{b}	17.4± 0.371^{b}	13.3± 0.361^{b}	9.93± 0.655^{a}	13.5± 1.283^{b}	7.99± 1.281^{a}
C + Cd 100	14.6± 0.371^{b}	9.91± 0.361^{a}	4.83± 1.032^{a}	8.43± 0.789^{a}	7.48± 1.056^{a}	18.9± 0.998^{b}	19.8± 0.673^{b}	8.14± 0.456^{a}	14.3± 0.785b	12.3± 0.781^{b}	13.8± 1.573^{b}	9.81± 0.998^{a}	7.81± 0.671^{a}	9.87± 0.556^{a}	6.81± 1.573^{a}
C+ All B	15± 0.282^{b}	11.8± 0.212^{b}	3.7± 0.141^{a}	13.8± 0.282^{b}	7.35± 0.777^{a}	24.2± 0.565^{c}	19.4± 1.131^{b}	10.9± 1.272^{a}	22.55± 0.636^{c}	14.85± 0.212^{b}	21.15± 1.484^{c}	12.65± 1.343^{b}	7.5± 0.424^{a}	16.65± 1.626^{b}	10.1± 0.424^{a}
Cd 50 + All B	27.05± 1.202^{c}	17.3± 1.202^{b}	8.15± 0.212^{a}	18± 1.414^{b}	9.2± 0.424^{a}	31.15± 1.626^{d}	24.6± 1.979^{c}	16.25± 0.070^{b}	26.55± 1.484^{c}	17.35± 0.777^{b}	25.2± 1.131^{c}	18.95± 1.343^{b}	12.05± 0.212^{b}	20.65± 2.474^{c}	11.15± 1.202^{b}
Cd 100 + All B	27.3± 1.414^{c}	17.2± 1.484^{b}	7.45± 0.777^{a}	16.1± 1.272^{b}	7± 0.282^{a}	34.6± 0.848^{d}	26.65± 2.050^{c}	14.95± 0.212^{b}	22.9± 1.131^{c}	14.7± 0.565^{b}	29.9± 2.545^{c}	20.6± 0.565^{c}	11.8± 0.989^{b}	17± 0.141^{b}	11.4± 0.848^{b}
C+ All M	16.65± 0.919^{b}	9.8± 0.565^{a}	7.15± 1.060^{a}	15.1± 0.989^{b}	11.4± 0.848^{b}	25.65± 0.919^{b}	17.8± 0.848^{b}	14.45± 0.212^{b}	26.45± 0.636^{c}	19.85± 1.626^{b}	22.6± 2.545^{c}	13.6± 1.979^{b}	9.95± 0.919^{a}	20.55± 1.626^{c}	15.75± 1.909^{b}
Cd 50 + All M	29.65± 1.060^{c}	21.4± 1.767^{c}	9.4± 0.707^{a}	19.4± 0.848^{b}	15.5± 1.343^{b}	37.45± 1.202^{d}	29.6± 0.424^{c}	16.95± 0.212^{b}	27.65± 0.494^{c}	21± 1.414^{c}	26.73± 0.890^{c}	24.6± 0.494^{c}	12.15± 1.343^{b}	23.25± 3.606^{c}	17.4± 0.424^{b}
Cd 100 + All M	29.55± 0.353^{c}	23.6± 1.909^{c}	7.9± 0.989^{a}	18.55± 0.919^{b}	14± 1.414^{b}	36.4± 0.989^{d}	28.6± 2.050^{c}	15.2± 0.282^{b}	27.55± 0.636^{c}	20.15± 1.060^{c}	25.3± 1.414^{c}	21.5± 0.707^{c}	11.65± 0.353^{b}	21.65± 1.626^{c}	16.55± 0.494^{b}
C+ Const	21± 1.414^{c}	16.2± 1.343^{b}	8.35± 1.060^{a}	18.45± 0.636^{b}	11.3± 1.060^{b}	28.45± 0.777^{c}	22.5± 0.494^{c}	16.4± 0.848^{b}	28.5± 0.070^{c}	18.65± 1.767^{b}	25.45± 2.050^{c}	18.65± 2.050^{b}	10.8± 1.414^{b}	22.05± 0.919^{c}	14.75± 1.202^{b}
Cd 50 + Const.	61.5± 1.272^{g}	24.4± 0.636^{c}	16.5± 1.909^{b}	28.05± 1.060^{c}	16.2± 1.343^{b}	69.85± 2.19^{g}	31.45± 0.636^{d}	23.7± 0.424^{c}	38.35± 1.484^{d}	23.7± 1.414^{c}	56.45± 1.060^{f}	25.25± 0.353^{c}	19.2± 0.282^{b}	32.45± 2.474d	19.5± 0.989^{b}
Cd 100 +Const.	44.95± 1.626^{e}	22.5± 0.707^{c}	13.9± 0.070^{b}	23.75± 1.202^{c}	15.6± 1.202^{b}	65.85± 0.919^{g}	31± 2.828^{d}	22± 0.141^{c}	32.3± 1.838^{d}	23.5± 0.565^{c}	53.8± 1.272^{f}	24.45± 0.636^{c}	18.65± 0.777^{b}	26.4± 0.848^{c}	16.7± 2.404^{b}

Mean ± SD (n=3). Different small alphabets (a-f) indicate statistically significant difference at $P \leq 0.05$ by Turkey's test. (**C:** control, **Cd 50:** cadmium 50 mgkg^{-1}, **Cd 100:** cadmium 100 mgkg^{-1}, **All B:** all bacterial isolates (1-9), **All M:** All mycorrhiza cultures (1-3), **Const:** microbial consortium (All bacteria + all mycorrhiza)

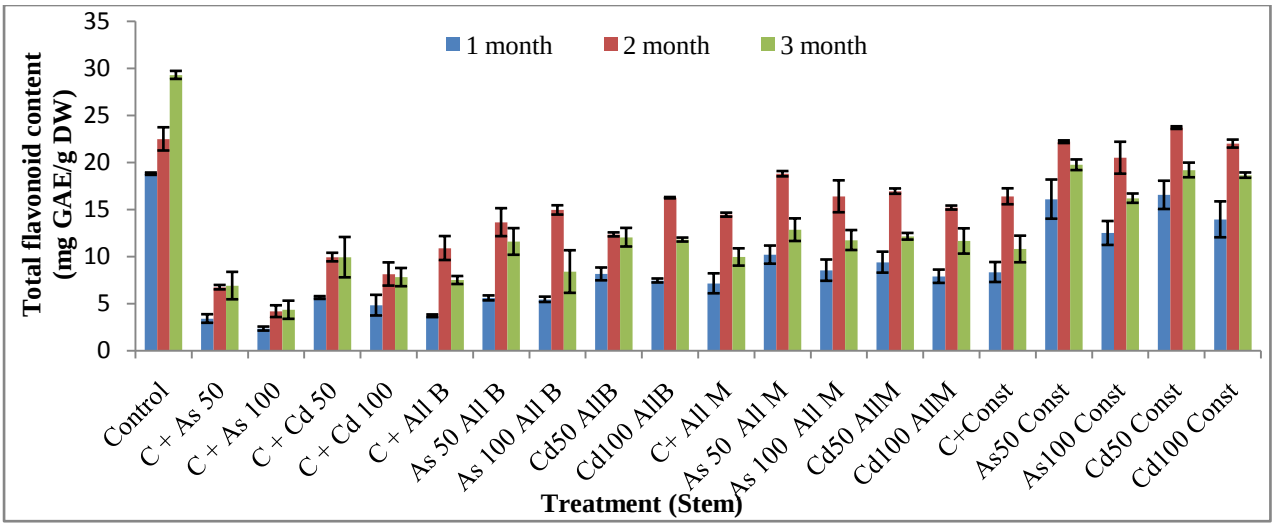

Figure 51: Total Flavonoid content (TFC) (mg QE/g DW) in the stem of *Ricinus communis* inoculated with arsenic and cadmium (50 and 100 $mgkg^{-1}$) along with rhizobacteria, mycorrhiza and microbial consortium.

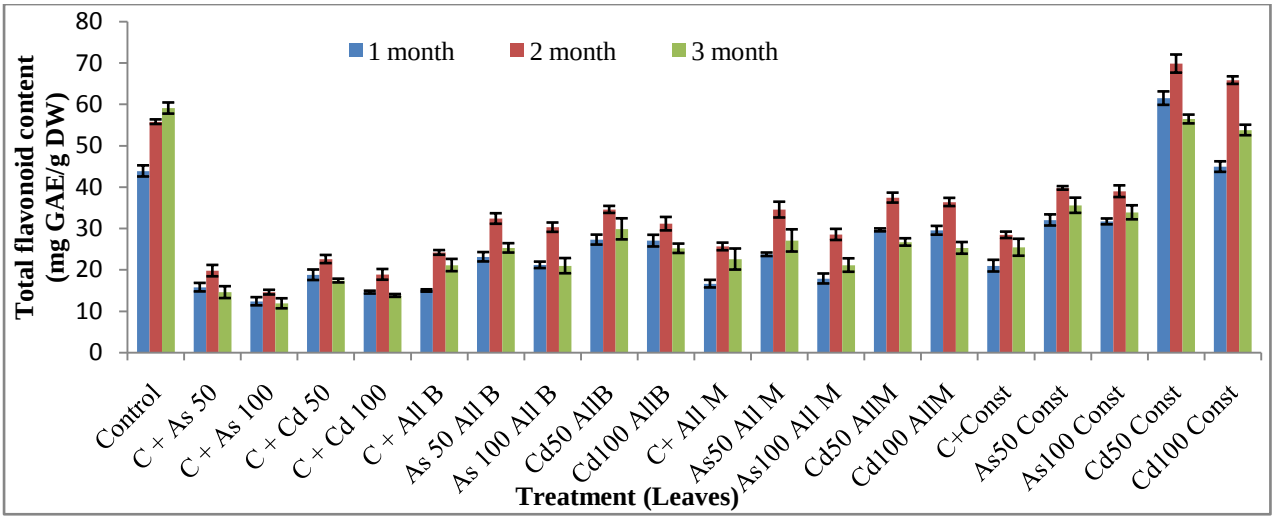

Figure 52: Total Flavonoid content (TFC) (mg QE/g DW) in leaves of *Ricinus communis* inoculated with arsenic and cadmium (50 and 100 $mgkg^{-1}$) along with rhizobacteria, mycorrhiza and microbial consortium.

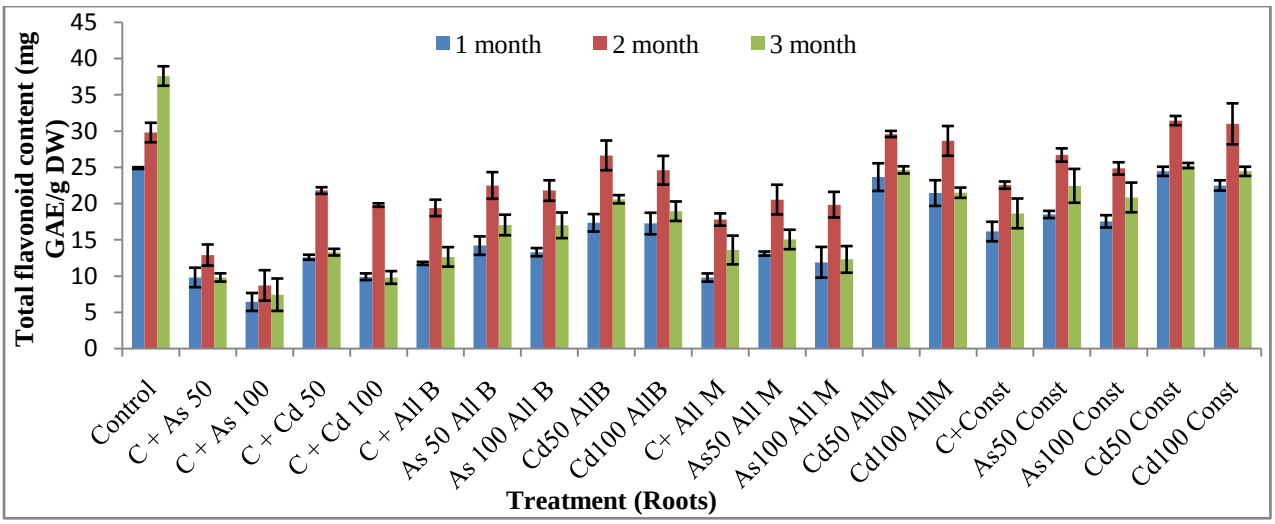

Figure 53: Total Flavonoid content (TFC) (mg QE/g DW) in roots of *Ricinus communis* inoculated with arsenic and cadmium (50 and 100 $mgkg^{-1}$) along with rhizobacteria, mycorrhiza and microbial consortium.

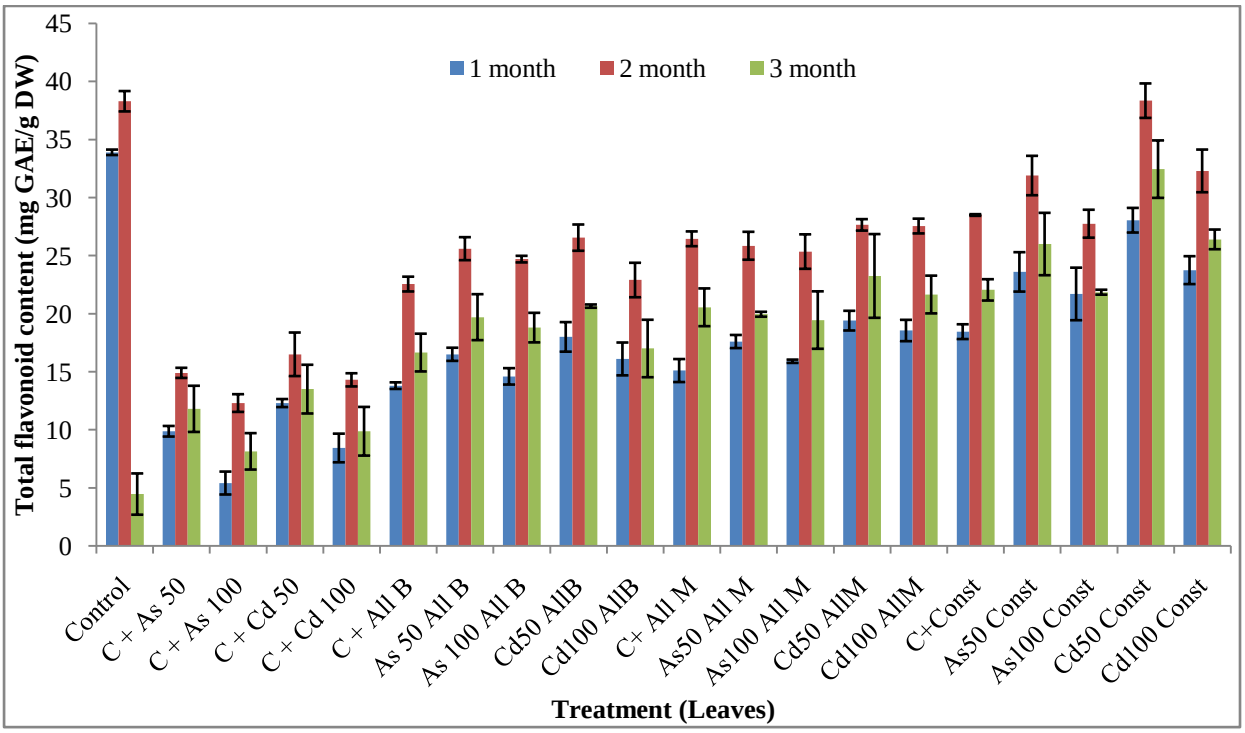

Figure 54: Total Flavonoid content (TFC) (mg QE/g DW) in leaves of *Canna indica* inoculated with arsenic and cadmium (50 and 100 $mgkg^{-1}$) along with rhizobacteria, mycorrhiza and microbial consortium.

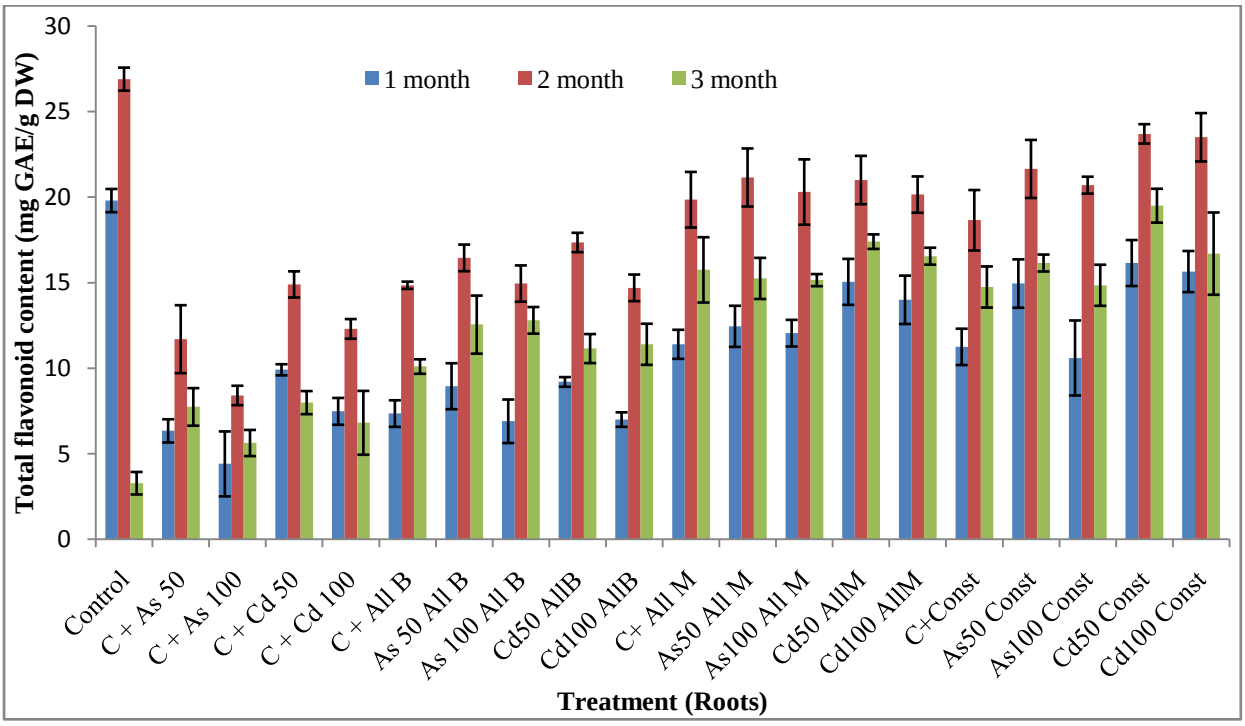

Figure 55: Total Flavonoid content (TFC) (mg QE/g DW) in roots of *Canna indica* inoculated with arsenic and cadmium (50 and 100 $mgkg^{-1}$) along with rhizobacteria, mycorrhiza and microbial consortium.

6.16.4 Qualitative and quantitative estimation of phenols (Gallic acid) and flavonoids (Quercetin) by HPTLC method

The results for the qualitative and quantitative estimation of phenols and flavonoids in different parts of *R. communis* (leaves, roots and stem) and *C. indica* (shoots and roots) plants inoculated with rhizobacteria, mycorrhiza and microbial consortium were estimated by High-performance thin layer chromatography (HPTLC). Dried plant parts (leaves, roots and stem) were extracted with 70% ethanol by using Microwave-assisted extraction (MAE) method and plant samples (leaves roots and stem of *R. communis* and leaves and roots of *C. indica*) were sent to Punjabi University (Patiala) for the analysis and phytochemicals content estimation (figure 56). Gallic acid and Quercetin were validated by the parameters given in table 28.

Figure 56: Dried samples of *Ricinus communis* (leaves, roots and stem) and *Canna indica* (shoots and roots) for HPTLC analysis.

Table 28: HPTLC method validation parameters of Gallic acid and Quercetin compounds

Sr. No.	Parameters	Gallic acid	Quercetin
1	Linearity range (ng/spot)	200-600	200-800
2	r^2 (Correlation coefficient)	0.99	0.98
3	Regression equation	y = 29.276x+10893	y = 29.251x + 27733.1
4	Calculated SD value (CATS software)	2.14	2.45
5	Limit of detection (LOD) (ng) [3 × SD/S]	23	28
6	Limit of quantitation (LOQ) (ng) [10 × SD/S]	87	90
7	Recovery (%)	98.99/100.1/99.98	99.12/99.87/100.02
8	R_f and λ_{max}	0.27; 366	0.52; 366

After running the standards (Gallic acid and Quercetin) along with the leaf, roots and stem of *R. communis* and *C. indica* (shoots and roots) on HPTLC plates, these results were obtained as shown in figure 57-59.

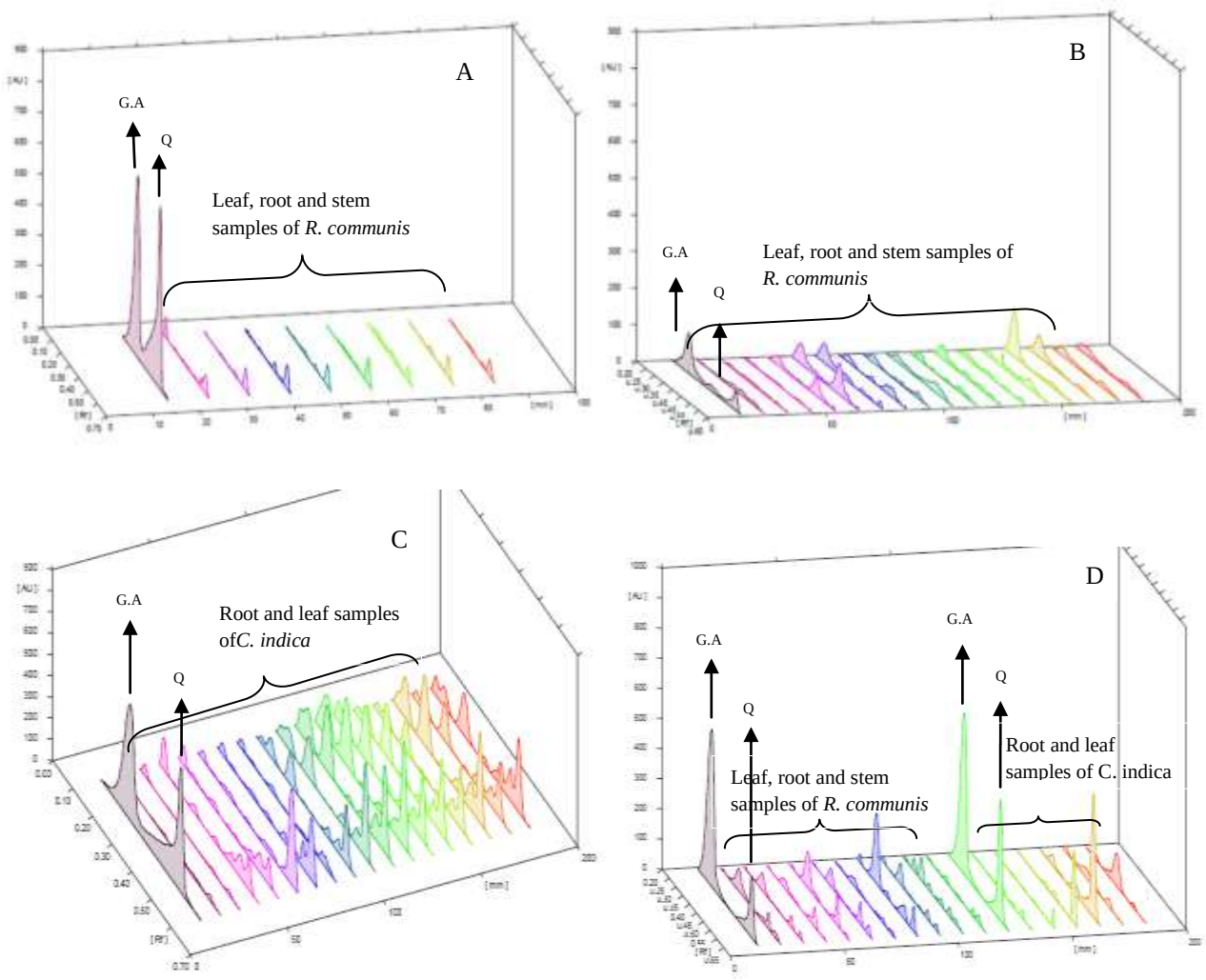

Figure 57: 3-D Densitograms obtained from *Ricinus communis* (leaves, roots and stem) and *Canna indica* (leaves and roots) inoculated with rhizobacteria, mycorrhizal and microbial consortium under As and Cd (50 and 100 $mgkg^{-1}$) stress with standards (Gallic acid and Quercetin) where (**G.A:** Gallic acid and **Q:** quercetin)

(**A**: Leaf, root and stem samples of *R. communis* inoculated with rhizobacteria, mycorrhiza and microbial consortium under As and Cd stress (50 and 100 $mgkg^{-1}$) after 3rd month of sampling with Gallic acid and quercetin as standards, **B:** Leaf, root and stem samples of *R. communis* inoculated with rhizobacteria, mycorrhiza and microbial consortium under As and Cd stress (50 and 100 $mgkg^{-1}$) after 3rd month of samplingwith Gallic acid and quercetin as standards, **C**: Root and leaf samples of *C. indica* inoculated with rhizobacteria, mycorrhiza and microbial consortium under As and Cd stress (50 and 100 $mgkg^{-1}$) after 3rd month of sampling, **D***:* Leaf, root and stem samples of *R. communis* inoculated with rhizobacteria, mycorrhiza and microbial consortium under As and Cd stress (50 and 100 $mgkg^{-1}$) after 3rd month of sampling with Gallic acid and quercetin as standards along with Root and shoot samples of *C. indica* with Gallic acid and quercetin as standards.

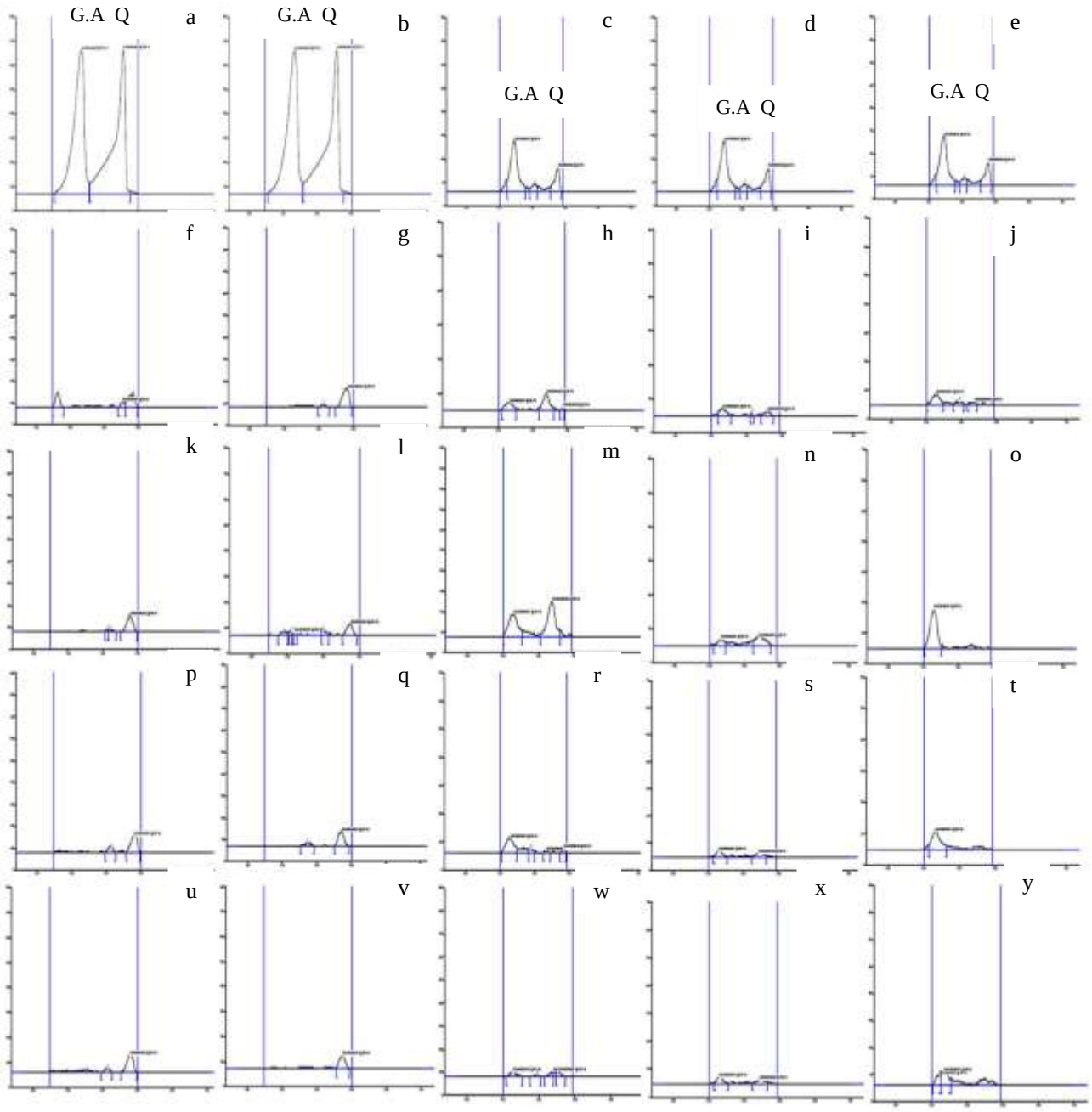

Figure 58: Chromatograms obtained from standard (Gallic acid and Quercetin) and test samples of *Ricinus communis* (leaf, root and stem) and *Canna indica* (shoot and root) inoculated with rhizobacteria, mycorrhiza and microbial consortium along with two concentrations of arsenic and cadmium (50 and 100 $mgkg^{-1}$) where phenolic and flavonoid content was detected.

(where, **G.A**:Gallic acid, **Q**:Quercetin**, a-b** : Standards (G.A and Q) , **c-h**: stem, roots and leaves of *R. communis* (As 50 + microbial consortium)**, i-j**: shoots and roots of *C. indica* (As 50 + microbial consortium), **k-m**: stem, roots and leaves of *R. communis* (As 100 + microbial consortium), **n-o**: shoots and roots of *C. indica* (As 100 + microbial consortium), **p-r**: stem, roots and leaves of *R. communis* (Cd 50 + microbial consortium), **s-t:** shoots and roots of *C. indica* (Cd 50 + microbial consortium), **u-w**: stem, roots and leaves of *R. communis* (Cd 100 + microbial consortium), **x-y** : shoots and roots of *C. indica* (Cd 100 + microbial consortium).

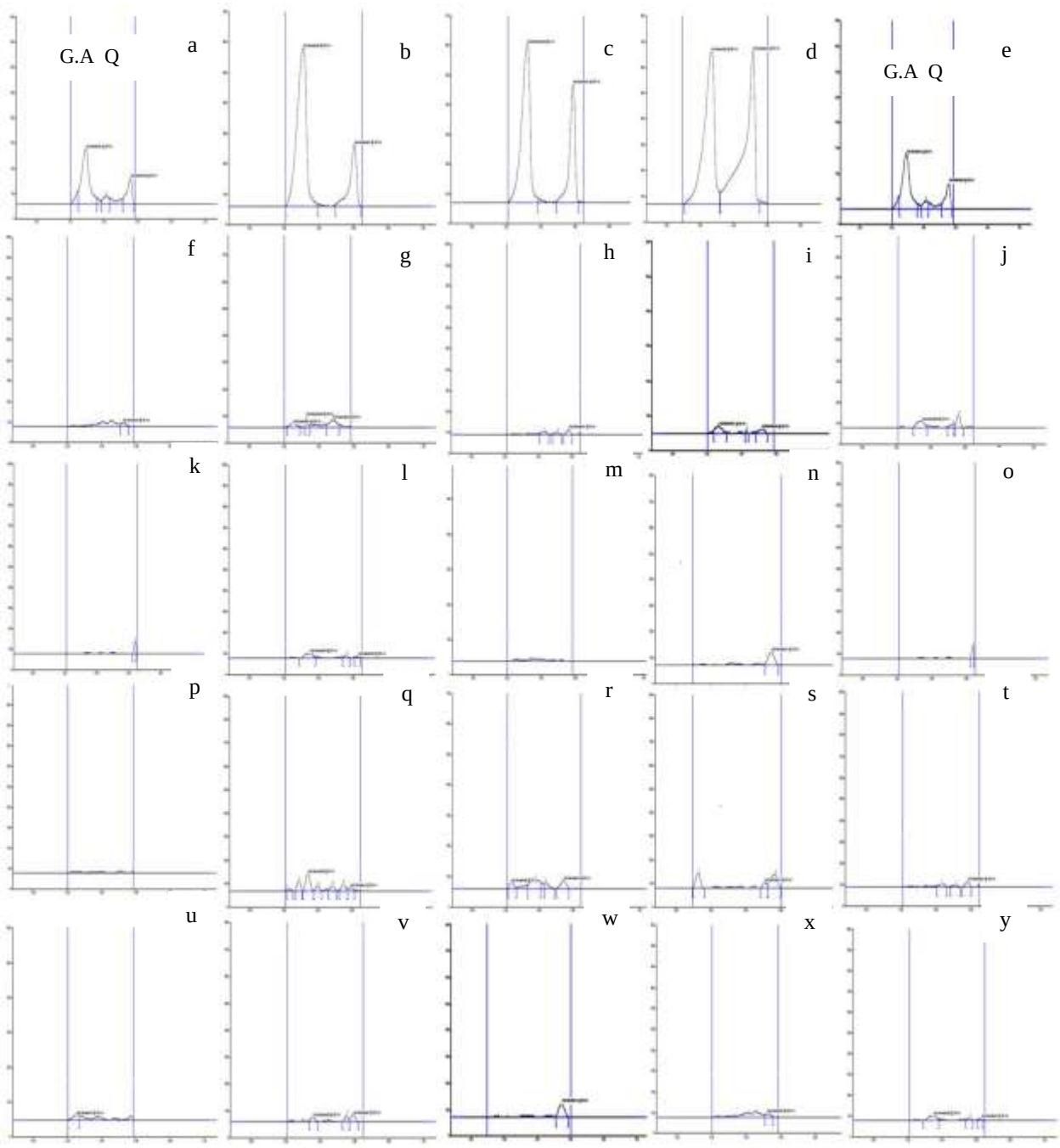

Figure 59: Chromatograms obtained from standard (Gallic acid and Quercetin) and test samples of *Ricinus communis* (leaf, root and stem) and *Canna indica* (shoot and root) inoculated with rhizobacteria, mycorrhiza and microbial consortium along with two concentrations of arsenic and cadmium (50 and 100 $mgkg^{-1}$) where total phenolic and flavonoid content was not detected.

(where, **G.A**:Gallic acid, **Q**:Quercetin**, a-e** : Standards (G.A and Q) , **f-h**: stem, roots and leaves of *R. communis* (As 50 + microbial consortium)**, i-j**: shoots and roots of *C. indica* (As 50 + microbial consortium), **k-m**: stem, roots and leaves of *R. communis* (As 100 + microbial consortium), **n-o**: shoots and roots of *C. indica* (As 100 + microbial consortium), **p-r**: stem, roots and leaves of *R. communis* (Cd 50 + microbial consortium), **s-t:** shoots and roots of *C. indica* (Cd 50 + microbial consortium), **u-w**: stem, roots and leaves of *R. communis* (Cd 100 + microbial consortium), **x-y** : shoots and roots of *C. indica* (Cd 100 + microbial consortium).

After quantification (%) of Gallic acid (phenols) and Quercetin (flavonoids) in *R. communis* (leaves, roots and stem) and *C. indica* (shoots and roots) inoculated with two concentrations of arsenic and cadmium (50 and 100 mg kg^{-1}) along with rhizobacteria, mycorrhiza and microbial consortium, we observed that in some treatments phenol and flavonoid content was not detected (N.D). Whereas plants inoculated with microbial consortium showed maximum phenolic and flavonoid content in leaves, roots and stem (*R. communis*) and shoots and roots (*C. indica*), with maximum phenolic content in roots (0.40 % and 0.38 %) followed by leaves (0.34 % and 0.32 %) and stem (0.09 %, 0.05 %) in pots treated with As 50 and 100 $mgkg^{-1}$ (microbial consortium) respectively in *R. communis*.

Similarly *C. indica* leaves (0.18 %, 0.13 %) possessed more phenolic content than roots (0.11 %, 0.08 %) in microbial consortium inoculated plants. In the control plants (without any treatment), phenolic and flavonoid content were found to be maximum than other control treatments (As and Cd, 50 and 100 $mgkg^{-1}$) in all the parts of both the plants (table 29-32).

Similarly, in *R. communis* plants treated with Cd (50 and 100 $mgkg^{-1}$), maximum phenolic content was obtained in control (without treatment) followed by plants inoculated with the microbialconsortium in order: roots > leaves > stem. Also, in some treatments (*R. communis* and *C. indica*) phenolic content was not detected (N.D) by HPTLC. In *C. indica* (Cd 50 and 100 $mgkg^{-1}$) maximum phenolic content was observed in the leaves of the plants inoculated with the microbialconsortium (0.21 % and 0.20 %) as compared to control (only microbial consortium). On the other hand, both the plants treated with only heavy metals (As and Cd, 50 and 100 $mgkg^{-1}$) showed a significant reduction in phenolic content in all the parts as compared to normal control (without treatment). Almost comparable results were found for Quercetin (flavonoid content) where maximum flavonoid content was acquired in leaves of microbial consortium inoculated plants (*R. communis*) 0.17 % and 0.16 % in As 50 and 100 $mgkg^{-1}$ treatments respectively. Also, maximum flavonoid content was found in all the parts of both the plants in control (without treatment) as compared to other treatments (As 50 and 100 mg kg^{-1}). In *C. indica* (microbial consortium) enhanced flavonoid content was found in roots 0.14 %. 0.12 % of plants treated with As 50 and 100 $mgkg^{-1}$ compared to leaves (0.05 % and 0.03 %) respectively. A similar trend was observed for Cd treatments in both the plants where leaves of *R. communis* showed maximum flavonoid content (0.17 % and 0.16%) in Cd 50 and 100 $mgkg^{-1}$microbial consortium inoculated plants respectively. Similarly, *C. indica* plant showed maximum flavonoid content in leaves than roots inoculated with microbial consortium under Cd stress.

Hence, maximum phenolic and flavonoid content were observed in parts of microbial consortium inoculated plants under heavy metals stress (As and Cd 50 and 100 $mgkg^{-1}$) than any other treatments (table 29-32).

Table 29: Quantification (%) of Gallic acid (Phenols) in leaves, roots and stem of *Ricinus communis* and Shoots and roots of *Canna indica* inoculated with arsenic (50 and 100 mgkg^{-1}) and rhizobacteria, mycorrhiza and microbial consortium.

Plants →	*R. communis*			*C. indica*	
Treatment ↓	Leaves	Roots	Stem	Shoots	Roots
Control	0.23±1.122^{c}	0.25±1.123^{c}	0.15±1.213^{b}	0.25±2.101^{c}	0.22±3.002^{c}
C + As 50	0.15±1.111^{b}	N.D	N.D	N.D	0.12±2.205^{b}
C + As 100	N.D	0.13±2.015^{b}	N.D	N.D	N.D
C + All B	0.06±1.202^{a}	0.07±1.021^{a}	N.D	0.05±1.041^{a}	0.03±1.234^{a}
As 50 + All B	N.D	0.09±1.131^{a}	N.D	0.03±1.022^{a}	N.D
As 100 + All B	0.02±2.001^{a}	N.D	N.D	N.D	N.D
C + All M	0.09±1.003^{a}	0.11±2.012^{b}	N.D	N.D	0.04±1.341^{a}
As 50 + All M	0.18±2.004^{b}	N.D	N.D	N.D	0.06±2.222^{a}
As 100 + All M	N.D	N.D	N.D	N.D	N.D
C + Const.	0.22±2.103^{c}	0.29±1.111^{c}	0.08±1.112^{a}	0.06±2.302^{a}	0.05±2.451^{a}
As 50 + Const.	0.34±2.001^{d}	0.40±1.193^{e}	0.09±1.021^{a}	0.18±2.112^{b}	0.11±2.502^{b}
As 100 + Const.	0.32±1.115^{d}	N.D	N.D	0.13±2.005^{b}	0.08±3.001^{a}

Mean ± SD (n=3).Different small alphabets (a-e) indicate statistically significant difference at $P \leq 0.05$ by Turkey's test (**C**: control, **As 50**: arsenic 50 mgkg^{-1}, **As 100**: arsenic 100 mgkg^{-1}, **All B**: all bacterial isolates (1-9), **All M**: All mycorrhiza cultures (1-3), **Const:** microbial consortium (All bacteria + all mycorrhiza)

Table 30: Quantification (%) of Gallic acid (Phenols) in leaves, roots and stem of *Ricinus communis* and Shoots and roots of *Canna indica* inoculated with cadmium (50 and 100 mgkg^{-1}) along with rhizobacteria, mycorrhiza and microbial consortium.

Plants →	*R. communis*			*C. indica*	
Treatment ↓	Leaves	Roots	Stem	Shoots	Roots
Control	0.23±2.121^{c}	0.25±1.233^{c}	N.D	0.25±2.802^{c}	N.D
C + Cd 50	0.16±1.234^{b}	0.21±2.402^{c}	0.07±2.221^{a}	N.D	0.14±1.501^{b}
C + Cd 100	N.D	0.18±2.705^{b}	0.05±2.104^{a}	0.09±2.871^{a}	0.06±2.004^{a}
C + All B	N.D	N.D	N.D	N.D	N.D
Cd 50 + All B	N.D	N.D	N.D	N.D	N.D
Cd 100 + All B	N.D	N.D	N.D	N.D	N.D
C + All M	0.06±1.602^{a}	N.D	N.D	N.D	0.04±2.672^{a}
Cd 50 + All M	0.09±2.233^{a}	N.D	N.D	N.D	0.06±2.205^{a}
Cd 100 + All M	N.D	N.D	N.D	N.D	N.D
C + Const.	0.21±1.031^{c}	0.33±2.401^{d}	0.05±1.303^{a}	0.09±2.201^{a}	N.D
Cd 50 + Const.	0.19±1.205^{b}	0.42±1.603^{e}	N.D	0.21±1.503^{c}	0.19±1.001^{b}
Cd 100 + Const.	N.D	0.34±1.602^{d}	0.06±1.504^{a}	N.D	0.14±2.104^{b}

Mean ± SD (n=3). Different small alphabets (a-c) indicate statistically significant difference at $P \leq 0.05$ by Turkey's test (**C**: control, **Cd 50:** cadmium 50 mgkg^{-1}, **Cd 100**: cadmium 100 mgkg^{-1}, **All B**: all bacterial isolates (1-9), **All M**: All mycorrhiza cultures (1-3), **Const**: microbial consortium (All bacteria + all mycorrhiza)

Table 31: Quantification (%) of Quercetin (Flavonoid) in leaves, roots and stem of *Ricinus communis* and Shoots and roots of *Canna indica* inoculated with arsenic (50 and 100 mgkg^{-1}) and rhizobacteria, mycorrhiza and microbial consortium.

Plants →	*R. communis*			*C. indica*	
Treatment ↓	Leaves	Roots	Stem	Shoots	Roots
Control	0.19±1.201^{b}	0.16±2.114^{b}	0.15±2.001^{b}	0.16±2.112^{b}	0.19±2.121^{b}
C + As 50	N.D	N.D	0.03±3.563^{a}	N.D	N.D
C + As 100	N.D	0.03±2.102^{a}	N.D	0.03±1.871^{a}	0.02±2.114^{a}
C + All B	0.17±1.033^{b}	0.08±2.211^{a}	0.07±4.004^{a}	N.D	N.D
As 50 + All B	N.D	N.D	N.D	N.D	N.D
As 100 + All B	0.04±2.106^{a}	N.D	N.D	N.D	0.03±1.502^{a}
C + All M	0.14±2.032^{b}	0.03±1.204^{a}	N.D	N.D	0.04±2.231^{a}
As 50 + All M	N.D	N.D	N.D	N.D	N.D
As 100 + All M	0.09±2.305^{a}	N.D	N.D	0.08±2.221^{a}	N.D
C + Const.	N.D	0.08±2.202^{a}	N.D	0.08±1.223^{a}	N.D
As 50 + Const.	0.17±1.203^{b}	0.09±2.215^{a}	0.04±1.202^{a}	N.D	0.14±2.333^{b}
As 100 + Const.	0.16±1.231^{b}	0.07±1.232^{a}	0.02±2.203^{a}	0.03±2.224^{a}	0.12±4.001^{b}

Mean ± SD (n=3). Different small alphabets (a-b) indicate statistically significant difference at $P \leq 0.05$ by Turkey's test. (**C:** control, **As 50**: arsenic 50 mgkg^{-1}, **As 100**: arsenic 100 mgkg^{-1}, **All B**: all bacterial isolates (1-9), **All M**: All mycorrhiza cultures (1-3), **Const:**microbial consortium (All bacteria + all mycorrhiza)

Table 32: Quantification (%) of Quercetin (Flavonoid) in leaves, roots and stem of *Ricinus communis* and Shoots and roots of *Canna indica* inoculated with cadmium (50 and 100 mgkg^{-1}) and rhizobacteria, mycorrhiza and microbial consortium.

Plants →	*R. communis*			*C. indica*	
Treatment ↓	Leaves	Roots	Stem	Shoots	Roots
Control	0.19±2.111^{b}	016±1.343^{b}	N.D	0.16±3.002^{b}	0.19±2.305^{b}
C + Cd 50	N.D	0.09±2.342^{a}	0.03±2.231^{a}	0.13±2.343^{b}	N.D
C + Cd 100	0.19± 2.122^{b}	N.D	N.D	N.D	0.12±2.344^{b}
C + All B	N.D	N.D	N.D	N.D	N.D
Cd 50 + All B	N.D	N.D	N.D	N.D	N.D
Cd 100 + All B	N.D	N.D	N.D	N.D	N.D
C + All M	0.03±3.012^{a}	N.D	N.D	N.D	N.D
Cd 50 + All M	0.05±1.973^{a}	N.D	N.D	0.08±1.981^{a}	0.03±1.991^{a}
Cd 100 + All M	N.D	N.D	N.D	N.D	N.D
C + Const.	N.D	N.D	0.01±2.123^{a}	0.23±1.231^{c}	N.D
Cd 50 + Const.	0.09±2.125^{a}	0.08±2.343^{a}	0.03±2.032^{a}	0.29±2.043^{c}	0.19±2.451^{b}
Cd 100 + Const.	0.07±1.672^{a}	0.05±2.122^{a}	0.02±2.704^{a}	0.24±2.302^{c}	0.17±2.454^{b}

Mean ± SD (n=3). Different small alphabets (a-c) indicate statistically significant difference at $P \leq 0.05$ by Turkey's test. (**C**: control, **Cd 50:**cadmium 50 mgkg^{-1}, **Cd 100**: cadmium 100 mgkg^{-1}, **All B**: all bacterial isolates (1-9), **All M**: All mycorrhiza cultures (1-3), **Const**: microbial consortium (All bacteria + all mycorrhiza)

Objective 3: To evaluate the potential of plant–symbiotic rhizobacteria and arbuscular mycorrhizae in grass-system to remove the pollutants.

5.17.1 Effect of arsenic and cadmium (50 and 100 $mgkg^{-1}$) on plant growth parameters (Plant height, wet weight, dry weight) of *Ricinus communis* and *Canna indica* inoculated with rhizobacteria, mycorrhiza and microbial consortium.

Heavy metals also affect physical parameters of plants, including plants height, wet weight, dry weight. Arsenic is very toxic metal which mostly tends to affectthe height of the plant with increasing concentration. In 3 months of experimentation, plants height is noticed to be affected in plants treated with only As and Cd (50 and 100 $mgkg^{-1}$) as compared to plants inoculated with rhizobacteria, mycorrhiza and microbial consortium and control plants (without As and Cd). Reduced growth in the plants treated with arsenic and cadmium (50 and 100 $mgkg^{-1}$) was observed in both the plants whereas in control plants inoculated with rhizobacteria, mycorrhiza and microbial consortium along with As and Cd, plants length was found to be normal. While with increasing concentration (100 $mgkg^{-1}$) of both the metals, plants height was found to be retarded than lower concentration (50 $mgkg^{-1}$). The results clearly indicate that all the growth parameters were suppressed gradually with the increase in the concentration of As and Cd. A significant decrease ($p \leq 0.05$) was seen in the height of plants with increasing concentration of heavy metals and the growth ceases at a particular time and plants started showing diseased symptoms. As seen in other activities, inspite of heavy metal toxicity (As and Cd), even growth of microbial consortium inoculated plants showed maximum growth in comparison to other treatments (rhizobacteria and mycorrhiza) (table 33-36).According to Hadi *et al* (2015), physiological parameters of *R. communis* under Cd stress were determined after 28 days in hydroponic conditions. In comparison to control, plant height and root length were found to be significantly less in presence of Cd. Plant height reduced significantly at the highest concentration (25 mg/l of Cd) following 20, 15 and 10 mg/L Cd. These results were found to be in accordance with Hadi. F *et al* (2010), Hadi. F *et al* (2009) and Dudka, S *et al* (1996) that suggests root length and plant height is generally reduced in the presence of heavy metals. Many studies were conducted to demonstrate the effect of Cd on the growth of different plants like *Miscanthus* species (H. Guo *et al*., 2016) and *C. indica* (Cheng *et al*., 2007). The results of all these experiments were found to be common where growth was retarded at the highest concentration of Cd in both the plants. Therefore, all these studies were found to be in accordance with the present study where a decrease in growth of plants was observed during 3 months of study, in plants treated with heavy metals (As and Cd), but no significant difference decrease in growth of plants was seen in the plants inoculated with heavy metals along with rhizobacteria, mycorrhiza and microbial consortium. In contrast, the presence of these microbiological agents enhanced the growth of plants under As and Cd toxicity.

Table 33: Effect of arsenic (50 and 100mgkg^{-1}) on physical parameters (height) of *Ricinus communis* (cm) inoculated with rhizobacteria, mycorrhiza and microbial consortium.

Treatment	1 month	2 Month	3 month	Treatment	1 month	2 month	3 month	Treatment	1 month	2month	3month
Control	30.4±1.67^{a}	43.7±6.56^{d}	54.7±4.66^{e}	**C + As 50**	11.3±3.78^{c}	21.8±3.55^{b}	34.6±2.84^{a}	**C + As 100**	11±2.12^{c}	19.5±3.76^{c}	30.4±1.45^{a}
C+PS1	36±5.65^{a}	45±4.24^{d}	60±2.82^{f}	**As50+PS 1**	30.5±2.12^{a}	43.5±3.53^{d}	51±2.82^{e}	**As100+PS 1**	15±3.24^{c}	28±2.44^{b}	38.5±2.53^{a}
C+PS2	25.5±3.53^{b}	34.5±3.23^{a}	49.5±2.12^{d}	**As50+PS 2**	23±2.22^{b}	31.5±3.12^{a}	39±2.42^{a}	**As100+PS 2**	10±2.82^{c}	20±9.89^{b}	29.5±7.77^{b}
C+PS3	21±1.41^{b}	34.5±4.94^{a}	46±4.24^{d}	**As50+PS 3**	21±1.41^{b}	30.5±0.70^{a}	40±1.64^{d}	**As100+PS 3**	12.5±3.53^{c}	23.5±4.94^{b}	34.5±6.36^{a}
C+PS4	16.5±0.70^{c}	27±1.11^{b}	38±2.82^{a}	**As50+PS 4**	15±1.34^{c}	27.5±4.94^{b}	38±4.28^{a}	**As100+PS 4**	12±4.24^{c}	22.5±4.94^{b}	31.5±7.71^{a}
C+HX1	17±4.24^{c}	27.5±3.53^{b}	43±4.04^{d}	**As50+HX1**	15.2±1.06^{c}	25.5±3.53^{b}	36.5±2.12^{a}	**As100+HX1**	10.5±1.22^{c}	19.5±0.70^{c}	28±1.41^{b}
C+NAP1	17.5±2.12^{c}	35.5±2.12^{a}	48±2.82^{d}	**As50+NAP1**	14.5±2.52^{c}	22±3.24^{b}	31±4.24^{a}	**As100+NAP1**	11±2.82^{c}	20±2.62^{b}	29.5±3.53^{b}
C+TL1	16±5.65^{c}	33.5±1.12^{a}	48±2.02^{d}	**As50+TL1**	14.5±3.53^{c}	23±4.33^{b}	33.5±4.94^{a}	**As100+TL1**	13±2.86^{c}	23.5±3.51^{b}	32.5±4.91^{a}
C+XL1	14.5±6.36^{c}	28±5.65^{b}	41.5±3.53^{d}	**As50+XL1**	12.5±3.24^{c}	21.5±4.44^{b}	33.5±3.13^{a}	**As100+XL1**	15±2.78^{c}	26.5±3.52^{b}	34.5±3.56^{a}
C+BZ1	12±1.41^{c}	27±1.82^{b}	41±2.82^{d}	**As50+BZ1**	10±1.01^{c}	17±2.82^{c}	28±4.25^{b}	**As100+BZ1**	16±5.65^{c}	27.5±4.94^{b}	33.5±4.92^{a}
C+ G.c	16±1.41^{c}	30.5±2.12^{a}	45±3.82^{d}	**As50+G.c**	13±2.82^{c}	22.5±6.36^{b}	31.5±6.31^{a}	**As100+G.c**	12±2.72^{c}	21.5±3.12^{b}	30±1.24^{a}
C + G.h	15.5±3.53^{c}	31.5±3.63^{a}	43.5±4.94^{d}	**As50+G.k**	12±2.79^{c}	24.5±3.50^{b}	31.5±3.48^{a}	**As100+G.k**	12.5±2.12^{c}	21.9±2.16^{b}	29.8±1.45^{b}
C+ A.k	17.5±2.12^{c}	33±5.65^{a}	46.5±2.19^{d}	**As50+A.k**	14.5±3.58^{c}	25±5.65^{b}	35.5±3.51^{a}	**As100+A.k**	12±2.88^{c}	22.5±2.11^{b}	31±2.80^{a}
C+ All B	14±5.65^{c}	28±4.15^{b}	41.5±4.94^{d}	**As50+All B**	15.5±3.49^{c}	26.5±6.31^{b}	38±5.65^{a}	**As100+All B**	15.5±2.16^{c}	27.5±2.12^{b}	33±1.41^{a}
C +All M	14±4.55^{c}	28±2.82^{b}	42.5±3.53^{d}	**As50+All M**	18±2.82^{c}	29.5±3.53^{b}	43.5±3.45^{d}	**As100+All M**	12.5±3.51^{c}	25.5±4.91^{b}	31.5±3.51^{a}
C+ Const	15.5±3.12^{c}	31±2.61^{a}	47.5±1.23^{d}	**As50+Const**	21±4.24^{b}	32±4.12^{a}	44.5±4.94^{d}	**As100+Const**	20±2.80^{b}	34.5±3.62^{a}	41.5±3.49^{d}

Mean ± SD (n=3). Different small alphabets **(a-f)** indicate statistically significant difference at P ≤ 0.05 by Turkey's test. (**C**: control, **As 50**: arsenic 50 mgkg^{-1}, **As 100**: arsenic 100 mgkg^{-1}, **All B**: all bacterial isolates (1-9), **All M**: All mycorrhiza cultures (1-3), **Cons**t: microbial consortium (All bacteria + all mycorrhiza), **PS1**:*Ralstonia insidiosa***, PS 2:** *Enterobacter ludwigii***, PS 3:** *Pseudomonas aeruginosa*, **PS 4:** *Cellulosomicrobium funkei*, **HX 1:** *Pseudomonas putida*, **NAP 1:** *Citrobacter freundii*, **TL 1:** *Pseudomonas plecoglossicida* **,XL 1:** *Pseudomonas fulva* ,**BZ 1:** *Pseudomonas sp,* **G.c:** *Glomus claroideum***, G.h :** *Glomus* hoi**, A.k :** *Acaulospora kentinensis*)

Table 34: Effect of cadmium (50 and 100mgkg^{-1}) on physical parameters (height) of *Ricinus communis* (cm) inoculated with rhizobacteria, mycorrhiza and microbial consortium.

Treatment	1 month	2 Month	3 month	Treatment	1 month	2 month	3 month	Treatment	1 month	2month	3month
Control	30.4±1.67^{a}	43.7±6.56^{d}	54.7±4.66^{e}	**C + Cd 50**	12.7±2.56^{c}	21.6±2.78^{b}	32.7±1.56^{a}	**C + Cd 100**	11.6±2.55^{c}	17±2.66^{c}	23.9±2.56^{b}
C+PS1	36±5.65^{a}	45±4.24^{d}	60±2.82^{f}	**Cd 50+PS 1**	12.5±3.53^{c}	22±1.41^{b}	31.5±1.53^{a}	**Cd100+PS 1**	12±4.24^{c}	17.5±3.58^{c}	24±4.34^{b}
C+PS2	25.5±3.53^{b}	34.5±1.33^{a}	49.5±2.12^{d}	**Cd 50+PS 2**	15.5±3.52^{c}	24.5±3.58^{b}	34.5±2.32^{a}	**Cd100+PS 2**	13±2.82^{c}	22±1.82^{b}	30±2.81^{a}
C+PS3	21±1.41^{b}	34.5±4.94^{a}	46±4.24^{d}	**Cd 50+PS 3**	14.5±3.53^{c}	23±1.51^{b}	31±2.52^{a}	**Cd100+PS 3**	10.5±3.52^{c}	20.5±4.94^{b}	28.5±4.95^{b}
C+PS4	16.5±0.70^{c}	27±1.45^{b}	38±2.82^{a}	**Cd 50+PS 4**	16±1.14^{c}	29±4.24^{b}	37±1.24^{a}	**Cd100+PS 4**	15±4.24^{c}	23±2.82^{b}	32.5±2.12^{a}
C+HX1	17±4.24^{c}	27.5±3.53^{b}	43±5.21^{d}	**Cd 50+HX1**	18±2.34^{c}	30±4.54^{a}	38.5±1.53^{a}	**Cd100+HX1**	16.5±2.42^{c}	25±2.81^{b}	32.5±3.53^{a}
C+NAP1	17.5±2.12^{c}	35.5±2.52^{a}	48±3.82^{d}	**Cd50+NAP1**	15±5.04^{c}	25.5±4.91^{b}	32±4.22^{a}	**Cd100+NAP1**	16±4.24^{c}	26±4.54^{b}	33±2.42^{a}
C+TL1	16±5.65^{c}	33.5±2.12^{a}	48±2.82^{d}	**Cd 50+TL1**	16.5±4.06^{c}	25.5±4.93^{b}	33±2.82^{a}	**Cd100+TL1**	15±4.21^{c}	22±2.24^{b}	30.5±2.12^{a}
C+XL1	14.5±6.36^{c}	28±5.65^{b}	41.5±3.53^{d}	**Cd 50+XL1**	14.5±3.52^{c}	24±4.27^{b}	32.5±3.53^{a}	**Cd100+XL1**	12±2.82^{c}	20±2.82^{b}	29.5±2.02^{b}
C+BZ1	12±1.41^{c}	27±2.82^{b}	41±2.42^{d}	**Cd 50+BZ1**	17±3.28^{c}	28±4.29^{b}	35.5±6.36^{a}	**Cd100+BZ1**	13±3.32^{c}	24±3.12^{b}	33±2.82^{a}
C+ Gc	16±1.01^{c}	30.5±2.12^{a}	45±2.82^{d}	**Cd 50+G.c**	13.5±4.94^{c}	22.5±3.53^{b}	29±2.82^{b}	**Cd100+G.c**	13.5±3.53^{c}	23.5±4.24^{b}	31.5±4.94^{a}
C+ Gk	15.5±3.53^{c}	31.5±2.13^{a}	43.5±4.94^{d}	**Cd 50+G.k**	13.5±2.12^{c}	23.5±3.53^{b}	31±2.82^{a}	**Cd100+G.k**	13±1.41^{c}	21.5±0.71^{b}	30.5±0.77^{a}
C+ Ak	17.5±2.12^{c}	33±5.65^{a}	46.5±1.12^{d}	**Cd 50+A.k**	15.5±4.12^{c}	24.5±0.70^{b}	32±2.22^{a}	**Cd100+A.k**	13±2.82^{c}	22.5±4.94^{b}	31±5.65^{a}
C+ All B	14±5.65^{c}	28±5.65^{b}	41.5±4.94^{d}	**Cd 50+AllB**	17±2.89^{c}	29.5±2.12^{b}	38.5±0.70^{a}	**Cd100+All B**	16±1.41^{c}	27±1.14^{b}	34±1.17^{a}
C +All M	14±5.65^{c}	28±2.02^{b}	42.5±3.53^{d}	**Cd 50+All M**	14±5.65^{c}	23±5.65^{b}	31.5±6.36^{a}	**Cd100+All M**	12±2.82^{c}	21.5±3.53^{b}	30±1.43^{a}
C+Const	15.5±2.12^{c}	31±2.82^{a}	47.5±3.33^{d}	**Cd50+Const**	20±5.15^{b}	30.5±4.94^{a}	39±2.80^{a}	**Cd100+Const**	19.5±2.12^{c}	28±2.82^{b}	38±2.88^{a}

Mean ± SD (n=3). Different small alphabets (a-f) indicate statistically significant difference at $P \leq 0.05$ by Turkey's test. (**C**: control, **Cd 50**: cadmium 50 mgkg^{-1}, **Cd 100**: cadmium 100 mgkg^{-1}, **All B**: all bacterial isolates (1-9), **All M**: All mycorrhiza cultures (1-3), **Cons**t: microbial consortium (All bacteria + all mycorrhiza), **PS1**:*Ralstonia insidiosa***, PS 2:** *Enterobacter ludwigii***, PS 3:** *Pseudomonas aeruginosa*, **PS 4:** *Cellulosomicrobium funkei*, **HX 1:** *Pseudomonas putida*, **NAP 1:** *Citrobacter freundii*, **TL 1:** *Pseudomonas plecoglossicida* **,XL 1:** *Pseudomonas fulva* ,**BZ 1:** *Pseudomonas sp*, **G.c:** *Glomus claroideum***, G.h :** *Glomus* hoi**, A.k :** *Acaulospora kentinensis*)

Table 35: Effect of arsenic (50 and 100mgkg^{-1}) on physical parameters (height) of *Canna indica* (cm) inoculated with rhizobacteria, mycorrhiza and microbial consortium.

Treatment	1 month	2 Month	3 month	Treatment	1 month	2 month	3 month	Treatment	1 month	2month	3month
Control	12.6±2.56^{b}	23.7±2.34^{d}	30±3.43^{g}	**C + As 50**	12±2.22^{a}	21±2.45^{d}	27±2.34^{f}	**C + As 100**	10.3±2.11^{c}	18±2.22^{e}	25±2.98^{d}
C+PS1	13.5±2.12^{b}	22±1.41^{d}	28±1.14^{f}	**As50+PS 1**	10±2.78^{c}	18.5±3.23^{e}	23.5±2.23^{d}	**As100+PS 1**	10±4.24^{c}	19±4.33^{e}	24.5±3.55^{d}
C+PS2	14.5±3.55^{a}	23.5±1.12^{d}	25±7.07^{d}	**As50+PS 2**	7±4.43^{c}	17±2.88^{e}	23.5±2.02^{d}	**As100+PS 2**	7.5±3.21^{c}	18±1.21^{e}	23.5±0.79^{d}
C+PS3	12.5±2.11^{a}	24±1.45^{d}	28.5±0.71^{f}	**As50+PS 3**	14±2.13^{b}	22.5±2.12^{d}	27±1.44^{f}	**As100+PS 3**	14.5±2.12^{b}	26±1.13^{e}	31±1.41^{f}
C+PS4	11±1.41^{a}	19±115^{e}	26±2.88^{f}	**As50+PS 4**	10±1.41^{c}	18.5±2.11^{e}	26.5±0.71^{f}	**As100+PS 4**	10±3.82^{c}	18.5±2.12^{e}	24.5±2.06^{d}
C+HX1	9±1.16^{c}	19.5±0.82^{e}	25.5±1.91^{f}	**As50+HX1**	15±1.11^{b}	23±1.23^{d}	28±1.41^{f}	**As100+HX1**	10.1±3.43^{c}	21.5±2.29^{d}	27.5±2.11^{f}
C+NAP1	13±2.62^{b}	22±2.12^{d}	28±1.18^{f}	**As50+NAP1**	15±4.24^{b}	24±2.82^{d}	29±1.13^{f}	**As100+NAP1**	13±2.93^{b}	20.5±2.23^{d}	26±1.82^{f}
C+TL1	12.5±3.51^{a}	20±2.84^{d}	25±1.15^{f}	**As50+TL1**	10.5±4.14^{c}	20.5±6.36^{d}	25.5±6.01^{d}	**As100+TL1**	11±2.12^{a}	19±2.32^{e}	24±2.73^{d}
C+XL1	12±2.65^{a}	21.5±2.11^{d}	26.5±2.02^{f}	**As50+XL1**	10±4.15^{c}	19.5±2.12^{e}	24.5±0.72^{d}	**As100+XL1**	10±2.43^{c}	20±2.11^{d}	29.5±2.31^{f}
C+BZ1	10±1.44^{b}	21.5±2.22^{d}	26.5±2.18^{f}	**As50+BZ1**	10±5.65^{c}	18.5±6.02^{e}	24±5.66^{d}	**As100+BZ1**	13±4.23^{b}	22.5±4.94^{d}	26.5±4.94^{f}
C+ Gc	12.5±3.05^{a}	22±4.66^{d}	27±1.23^{f}	**As50+G.c**	13.5±3.53^{b}	22±2.82^{d}	27±1.41^{f}	**As100+Gc**	15.5±2.12^{b}	25±4.24^{d}	30±4.12^{g}
C+ Gk	8.5±2.22^{c}	19.5±4.23^{e}	25±5.63^{f}	**As50+G.k**	14±4.24^{b}	24±2.24^{d}	29.5±3.51^{f}	**As100+Gk**	13±3.43^{b}	24±1.21^{d}	29±1.41^{f}
C+ Ak	15.5±2.98^{b}	23.5±2.14^{d}	27.5±2.63^{f}	**As50+A.k**	9±2.88^{c}	19.5±0.78^{e}	25±41^{d}	**As100+Ak**	14.5±3.24^{b}	23±2.98^{d}	27.5±2.12^{f}
C+ All B	13.5±3.12^{b}	24.5±3.53^{d}	29.5±3.02^{f}	**As50+All B**	14±2.88^{b}	25.5±2.13^{d}	30±1.09^{g}	**As100+All B**	11±2.72^{c}	21.5±2.03^{d}	27.5±2.92^{f}
C +All M	15±2.18^{b}	24.5±4.92^{d}	29.5±4.22^{f}	**As50+All M**	12.5±3.22^{a}	23±2.76^{d}	28.5±2.13^{f}	**As100+All M**	13±2.84^{b}	21.5±2.98^{d}	27±2.82^{f}
C+Const	14.5±2.23^{b}	24.6±0.77^{d}	29.5±0.83^{f}	**As50+Const**	13±2.67^{b}	24.5±2.13^{d}	37±1.41^{h}	**As100+Const**	11.5±2.08^{a}	22±5.56^{d}	26.5±6.39^{f}

Mean ± SD (n=3). Different small alphabets (a-h) indicate statistically significant difference at $P \leq 0.05$ by Turkey's test. (**C**: control, **As 50**: arsenic 50 mgkg^{-1}, **As 100**: arsenic 100 mgkg^{-1}, **All B**: all bacterial isolates (1-9), **All M**: All mycorrhiza cultures (1-3), **Cons**t: microbial consortium (All bacteria + all mycorrhiza), **PS1**:*Ralstonia insidiosa***, PS 2:** *Enterobacter ludwigii***, PS 3:** *Pseudomonas aeruginosa*, **PS 4:** *Cellulosomicrobium funkei*, **HX 1:** *Pseudomonas putida*, **NAP 1:** *Citrobacter freundii*, **TL 1:** *Pseudomonas plecoglossicida* **,XL 1:** *Pseudomonas fulva* ,**BZ 1:** *Pseudomonas sp,* **G.c:** *Glomus claroideum***, G.h :** *Glomus* hoi**, A.k :** *Acaulospora kentinensis*)

Table 36: Effect of cadmium (50 and 100mgkg^{-1}) on physical parameters (height) of *Canna indica* (cm) inoculated with rhizobacteria, mycorrhiza and microbial consortium.

Treatment	1 month	2 Month	3 month	Treatment	1 month	2 Month	3 month	Treatment	1 month	2 Month	3 month
Control	12.6±2.56^{a}	23.7±2.34^{d}	30±3.43^{f}	**C + Cd 50**	9.3±2.11^{c}	20.2±2.18^{e}	26±2.23^{f}	**C + Cd 100**	10.3±2.21^{c}	19.2±2.10^{e}	25±0.981^{f}
C+PS1	13.5±2.12^{b}	22±1.41^{d}	28±1.14^{d}	**Cd 50+PS 1**	10±2.86^{c}	21.5±2.11^{d}	26.5±2.17^{f}	**Cd100+PS 1**	13.5±3.45^{b}	24±4.32^{d}	29±2.83^{f}
C+PS2	14.5±3.55^{b}	23.5±1.12^{d}	25±7.07^{f}	**Cd 50+PS 2**	14±2.98^{a}	24±4.21^{d}	30±4.23^{f}	**Cd100+PS 2**	10±2.72^{c}	20±4.24^{e}	25.5±3.54^{f}
C+PS3	12.5±2.11^{a}	24±1.45^{d}	28.5±0.71^{f}	**Cd 50+PS 3**	12.5±3.24^{a}	22±5.65^{e}	27±4.23^{f}	**Cd100+PS 3**	10.5±2.65^{c}	19.5±1.70^{e}	23.5±0.73^{d}
C+PS4	11±1.41^{a}	19±115^{e}	26±2.88^{f}	**Cd 50+PS 4**	8±2.17^{c}	18.5±0.56^{e}	24±1.18^{d}	**Cd100+PS 4**	8±1.32^{c}	18.5±2.12^{e}	24±2.61^{d}
C+HX1	9±1.16^{c}	19.5±0.82^{e}	25.5±1.91^{d}	**Cd 50+HX1**	9±2.83^{c}	21.5±0.72^{d}	27±1.41^{f}	**Cd100+HX1**	7±2.13^{c}	17.5±0.82^{e}	23±1.43^{f}
C+NAP1	13±2.62^{b}	22±2.12^{d}	28±1.18^{f}	**Cd50+NAP1**	13±2.78^{b}	22±2.98^{d}	27.5±2.13^{f}	**Cd100+NAP1**	12±2.73^{a}	22±4.13^{d}	27±2.84^{f}
C+TL1	12.5±3.51^{a}	20±2.84^{d}	25±1.15^{d}	**Cd 50+TL1**	12±2.19^{a}	20.5±3.53^{e}	25±4.23^{d}	**Cd100+TL1**	15.5±2.12^{b}	24±2.34^{d}	28.5±2.15^{f}
C+XL1	12±2.65^{a}	21.5±2.11^{d}	26.5±2.02^{f}	**Cd 50+XL1**	11±2.11^{b}	20.5±4.95^{e}	26±4.32^{f}	**Cd100+XL1**	12.5±3.43^{a}	24±2.76^{d}	29±2.11^{f}
C+BZ1	10±1.44^{c}	21.5±2.22^{d}	26.5±2.18^{f}	**Cd 50+BZ1**	10.3±1.41^{c}	20.6±0.70^{e}	25.5±0.81^{f}	**Cd100+BZ1**	12.6±2.16^{a}	23.5±0.18^{d}	29.5±0.23^{f}
C+ Gc	12.5±3.05^{a}	22±4.66^{d}	27±1.23^{f}	**Cd 50+G.c**	10.5±3.23^{c}	20.5±2.14^{e}	26±1.43^{f}	**Cd100+G.c**	9±1.42^{c}	19±2.87^{e}	23.5±3.54^{d}
C+ Gk	8.5±2.22^{c}	19.5±4.23^{e}	25±5.63^{d}	**Cd 50+G.k**	13±4.32^{b}	21.5±2.19^{d}	28±1.01^{f}	**Cd100+G.k**	11.5±2.19^{b}	22.5±3.44^{d}	27.5±3.22^{f}
C+ Ak	15.5±2.98^{b}	23.5±2.14^{d}	27.5±2.63^{f}	**Cd 50+A.k**	12±2.83^{a}	24±2.91^{d}	28±1.14^{f}	**Cd100+A.k**	9±2.13^{c}	21±4.32^{d}	26±4.24^{f}
C+ All B	13.5±3.12^{b}	24.5±3.53^{d}	29.5±3.02^{f}	**Cd 50+AllB**	10.5±2.15^{c}	21±2.42^{d}	26±3.26^{f}	**Cd100+All B**	12.5±3.43^{a}	21.5±2.56^{d}	27±4.24^{f}
C+All M	15±2.18^{b}	24.5±4.92^{d}	29.5±4.22^{f}	**Cd 50+All M**	7.5±3.52^{c}	19.5±0.17^{e}	24±2.87^{d}	**Cd100+All M**	14±2.32^{b}	24±4.32^{d}	29±2.18^{f}
C+Const	14.5±2.23^{b}	24.6±0.77^{d}	29.5±0.83^{f}	**Cd50+Const**	12.5±2.13^{a}	21.5±2.12^{d}	26.5±0.71^{f}	**Cd100+Const**	11±2.32^{b}	21.5±3.45^{d}	31.5±3.563^{f}

Mean ± SD (n=3). Different small alphabets (a-f) indicate statistically significant difference at P ≤ 0.05 by Turkey's test. (**C**: control, **Cd 50**: cadmium 50 mgkg^{-1}, **Cd 100**: cadmium 100 mgkg^{-1}, **All B**: all bacterial isolates (1-9), **All M**: All mycorrhiza cultures (1-3), **Cons**t: microbial consortium (All bacteria + all mycorrhiza), **PS1**:*Ralstonia insidiosa***, PS 2:** *Enterobacter ludwigii***, PS 3:** *Pseudomonas aeruginosa*, **PS 4:** *Cellulosomicrobium funkei*, **HX 1:** *Pseudomonas putida*, **NAP 1:** *Citrobacter freundii*, **TL 1:** *Pseudomonas plecoglossicida* **,XL 1:** *Pseudomonas fulva* ,**BZ 1:** *Pseudomonas sp*, **G.c:** *Glomus claroideum***, G.h :** *Glomus* hoi**, A.k :** *Acaulospora kentinensis*).

From the present study, the results revealed that treatment of arsenic and cadmium at 50 and 100 $mgkg^{-1}$ concentration, affected the fresh weight and dry weight of plants parts (root, stem and leaves) as compared to control plants (without treatment). The change in fresh weight and dry weight in leaf, stem and roots of *R. communis* and *C. indica* were observed during the course of present study where the experimental data reported that fresh weight and dry weight were adversely affected with metal stress as compared to plants without heavy metal treatment. With respect to plants height, fresh weight and dry weight are given for *R. communis* and *C. indica* plants inoculated with rhizobacteria, mycorrhiza and microbial consortium in table 37-40.

Various studies have been performed to study the effect of heavy metals on growth and dry biomass of plants. According to Hadi, F *et al* (2015), total plant (*R. communis*) dry biomass was reduced significantly by Cd treatment in comparison to control (without heavy metal treatment). Also, the dry weight of plants reduced with increasing concentration of Cd (5-25 mg/L). Several other researchers (Hadi, F., 2010; John, R., 2012) also reported the reduced dry weight of plants under metal stress. As dry biomass reduction is considered as major symptoms of toxicity caused by heavy metals in plants. A similar study was performed by Wu *et al* (2016) to check the tolerance of *R. communis* to cadmium. Average shoot biomass decreased with increasing concentration of Cd i.e 10-50 g/L. Even average root biomass was found to be decreased with increasing concentration of cadmium. Baudh *et al* (2016) also explained the effect of Cd on the antioxidant defence system and other growth parameters in two species of plants i.e *R. communis* and *B. juncea*. The results clearly indicated a significant reduction in dry biomass of *R. communis* and *B. juncea* by 26.58 % and 53.84 % respectively. Whereas, 33.84 % and 45.33% reduction in root biomass in *R. communis* and *Brassica* were observed respectively.

Another study was performed by T, Dibyendu (2012) where *C. indica* showed the normal Dry weight of leaves and roots hence indicated the tolerance capabilities of *C. indica* due to Cu-induced oxidative stress. According to a recent study (Javed Ali *et al*., 2018) on phytoextraction of chromium by *Zea Mays* L. (maize), the effect of PGPB along with citric acid was estimated. Combined treatment of *Burkholderia vietnamiensis* and citric acid showed a significant increase in the plant's wet weight and dry weight by 56 % and 50 % respectively. This work is found to be in agreement with the present study where rhizobacteria, mycorrhiza and microbial consortium inoculated plants showed increased plant biomass (root and shoots) under heavy metal stress as compared to control plants (with As and Cd). Therefore, as suggested by Afzal *et al* (2014), phytoremediation potential of polluted soil can be enhanced by Plant-microbe interaction.

Table 37: Effect of arsenic (50 and 100 mgkg^{-1}) on wet weight and dry weight (g) of *Ricinus communis* (root, stem and leaves) inoculated with rhizobacteria, mycorrhiza and microbial consortium.

Time →	1 Month						2 Month						3 Month					
Plant parts →	Root		Stem		Leaves		Root		Stem		Leaves		Root		Stem		Leaves	
Treatment ↓	WW	DW	WW	DW	WW	DW	WW	DW	WW	DW	WW	DW	WW	DW	WW	DW	WW	DW
Control	3.51± 0.091^{d}	1.81± 0.034^{b}	8.01± 0.083i	3.81± 0.221^{d}	3.43± 0.073^{d}	1.61± 0.009^{b}	6.29± 0.051^{g}	2.01± 0.098^{c}	1.06± 0.123^{b}	4.19± 1.211^{e}	6.79± 2.131^{a}	2.31± 0.221^{a}	9.14± 0.324^{j}	3.14± 0.452^{d}	21.9± 0.431^{u}	8.66± 0.572^{i}	18.8± 0.632^{s}	5.01± 0.571^{f}
C+PS1	1.63± 0.084^{b}	0.305± 0.009^{a}	3.53± 0.120^{d}	1.15± 0.077^{b}	1.26± 0.077^{b}	0.28± 0.070^{a}	3.26± 0.169^{d}	0.61± 0.019^{a}	7.07± 0.240^{h}	3.63± 1.711^{d}	2.48± 0.084^{c}	0.46± 0.002^{a}	4.89± 0.254^{e}	0.91± 0.029^{a}	10.6± 0.360^{k}	5.44± 2.566^{f}	4.35± 1.025^{e}	0.81± 0.159^{a}
C+PS2	1.81± 0.014^{b}	0.505± 0.007^{a}	2.93± 0.070^{c}	0.94± 0.049^{a}	2.12± 0.148^{c}	0.48± 0.091^{a}	3.62± 0.028^{d}	1.01± 0.014^{b}	5.86± 0.141^{f}	2.94± 1.385^{c}	4.15± 0.155^{e}	0.97± 0.182^{a}	5.43± 0.042^{f}	1.51± 0.021^{b}	8.79± 0.212^{i}	4.41± 2.078^{e}	7.23± 1.195^{h}	1.73± 0.663^{b}
C+PS3	1.08± 0.035^{b}	0.19± 0.028^{a}	2.15± 0.095^{c}	0.79± 0.042^{a}	2.15± 0.148^{c}	0.41± 0.003^{a}	2.17± 0.070^{c}	0.38± 0.056^{a}	4.31± 0.098^{e}	2.28± 1.074^{c}	4.31± 0.042^{e}	0.83± 0.007^{a}	3.25± 0.106^{d}	0.65± 0.035^{a}	6.46± 0.148^{g}	3.42± 1.612^{d}	8.62± 0.084^{i}	1.45± 0.281^{b}
C+PS4	2.27± 0.049^{c}	0.72± 0.056^{a}	5.59± 0.028^{f}	2.20± 0.035^{c}	2.53± 0.084^{c}	0.38± 0.014^{a}	4.55± 0.098^{e}	1.44± 0.113^{b}	11.1± 0.056^{l}	6.69± 3.153^{g}	5.01± 0.098^{f}	0.77± 0.029^{a}	6.82± 0.148^{g}	2.16± 0.169^{c}	16.7± 0.084^{q}	10.3± 4.73^{k}	8.75± 1.598^{i}	1.36± 0.326^{b}
C+HX1	1.65± 0.070^{b}	0.51± 0.035^{a}	2.81± 0.071^{c}	1.05± 0.007^{b}	1.59± 0.042^{b}	0.20± 0.009^{a}	3.3± 0.141^{d}	1.03± 0.070^{b}	5.62± 0.141^{f}	3.03± 1.428^{d}	3.18± 0.084^{d}	0.40± 0.018^{a}	4.95± 0.212^{e}	1.81± 0.487^{b}	8.43± 0.212^{i}	4.54± 2.142^{e}	6.36± 0.169^{g}	0.71± 0.11^{a}
C+NAP1	1.63± 0.049^{b}	0.47± 0.049^{a}	1.90± 0.106^{b}	0.44± 0.002^{a}	1.12± 0.155^{b}	0.21± 0.020^{a}	3.27± 0.098^{d}	0.95± 0.095^{a}	3.81± 0.212^{d}	1.33± 0.630^{b}	2.14± 0.169^{c}	0.43± 0.041^{a}	4.90± 0.148^{e}	1.64± 0.162^{b}	5.71± 0.318^{f}	2.07± 0.946^{c}	4.28± 0.339^{e}	0.76± 0.225^{a}
C+TL1	1.74± 0.043^{b}	0.65± 0.056^{a}	3.62± 0.120^{d}	1.2± 0.127^{b}	1.88± 0.148^{b}	0.27± 0.016^{a}	3.49± 0.055^{d}	1.3± 0.111^{b}	7.25± 0.240^{h}	3.87± 1.824^{d}	3.62± 0.084^{d}	0.55± 0.032^{a}	5.23± 0.148f	2.25± 0.261^{c}	10.8± 0.360^{k}	5.80± 2.736^{f}	6.35± 1.428^{g}	0.98± 0.254^{a}
C+XL1	2.64± 0.063^{c}	0.76± 0.063^{a}	4.68± 0.098^{e}	1.62± 0.063^{b}	3.14± 0.009^{d}	0.82± 0.007^{a}	5.29± 0.127^{f}	1.53± 0.127^{b}	9.36± 0.197^{j}	4.74± 2.234^{e}	6.18± 0.028^{g}	1.65± 0.011^{b}	7.93± 0.190^{h}	2.65± 0.318^{c}	14.4± 0.296^{o}	7.11± 3.351^{h}	9.27± 0.042^{j}	2.89± 0.552^{c}
C+BZ1	2.14± 0.094c	0.74± 0.063^{a}	5.19± 0.296^{f}	1.65± 0.056^{b}	1.7± 0.028^{b}	0.61± 0.077^{a}	4.29± 0.095^{e}	1.49± 0.127^{b}	10.3± 0.593^{k}	4.83± 2.276^{e}	3.4± 0.056^{d}	1.23± 0.155^{b}	6.43± 0.148^{g}	2.58± 0.304^{c}	15.5± 0.890^{p}	7.24± 3.415^{h}	6.8± 0.113^{g}	2.18± 0.707^{c}
C+Gc	1.74± 0.070^{b}	0.61± 0.042^{a}	3.55± 0.148^{d}	1.18± 0.021^{b}	1.75± 0.063^{b}	0.52± 0.049^{a}	3.48± 0.141^{d}	1.22± 0.084^{b}	7.11± 0.296^{h}	3.6± 1.697^{d}	3.51± 0.127^{d}	1.05± 0.098^{b}	5.22± 0.212^{f}	2.12± 0.282^{c}	10.6± 0.445^{k}	5.4± 2.545^{f}	7.02± 0.254^{h}	1.82± 0.197^{b}
C+G.k	1.69± 0.035^{b}	0.62± 0.049^{a}	3.12± 0.162^{d}	1.54± 0.141^{b}	2.2± 0.098^{c}	0.50± 0.008^{a}	3.39± 0.071^{d}	1.25± 0.044^{b}	6.25± 0.325^{g}	4.92± 2.319^{e}	4.4± 0.197^{e}	1.01± 0.169^{b}	5.08± 0.106^{f}	2.20± 0.615^{c}	9.37± 0.487^{j}	7.38± 3.478^{h}	8.8± 0.395^{i}	1.77± 0.387^{b}
C+A.k	2.45± 0.353^{c}	0.75± 0.042^{a}	4.41± 0.106e	1.76± 0.063^{b}	2.16± 0.091^{c}	0.40± 0.009^{a}	4.9± 0.007^{e}	1.5± 0.084^{b}	8.83± 0.212^{i}	5.43± 2.559^{f}	4.37± 0.127^{e}	0.81± 0.019^{a}	7.35± 1.060^{h}	2.64± 0.678^{c}	13.2± 0.318^{n}	8.14± 3.839^{i}	8.74± 0.254^{i}	1.42± 0.253^{b}
C+All B	1.4± 0.084^{b}	0.38± 0.085^{a}	3.2± 0.11^{d}	1.6± 0.056^{b}	1.18± 0.070^{b}	0.51± 0.078^{a}	2.8± 0.169^{c}	0.76± 0.166^{a}	6.4± 0.226^{g}	4.92± 2.319^{e}	2.36± 0.141^{c}	1.03± 0.156^{b}	4.2± 0.254^{e}	1.3± 0.028^{b}	9.6± 0.339^{j}	7.38± 3.478^{h}	4.72± 0.282^{e}	1.78± 0.090b

C+All M	2.27± 0.063^{c}	0.80± 0.111^{a}	1.56± 0.155^{b}	0.67± 0.703^{a}	2.32± 0.004^{c}	0.18± 0.007^{a}	4.55± 0.127^{e}	1.60± 0.223^{b}	3.12± 0.311^{d}	0.52± 0.247^{a}	4.64± 0.008^{e}	0.37± 0.015^{a}	6.82± 0.190^{g}	2.84± 0.957^{c}	4.68± 0.466^{e}	0.78± 0.371^{a}	9.28± 0.016^{j}	0.65± 0.105^{a}
C+Const	1.12± 0.035^{b}	0.33± 0.014^{a}	2.14± 0.127^{c}	1.02± 0.254^{b}	3.05± 0.098^{d}	0.44± 0.366^{a}	2.25± 0.070^{c}	0.66± 0.028^{a}	4.28± 0.254^{e}	2.52± 1.187^{c}	6.10± 0.196^{g}	0.88± 0.733^{a}	3.37± 0.106^{d}	1.16± 0.282^{b}	6.42± 0.381^{g}	3.78± 1.781^{d}	9.16± 0.294^{j}	1.41± 0.972^{b}
C + As 50	2.64± 0.821^{c}	0.82± 0.561^{a}	4.19± 0.231^{e}	1.06± 0.331^{b}	1.81± 0.082^{b}	0.73± 0.056^{a}	4.66± 0.071^{e}	1.64± 0.045^{b}	8.03± 0.667^{i}	4.09± 0.732^{e}	5.24± 0.321^{f}	1.71± 0.034^{b}	6.66± 0.034^{g}	2.94± 0.561^{c}	12.6± 0.056^{m}	6.03± 1.023^{g}	9.09± 0.892^{j}	2.64± 0.056^{c}
As50+PS1	2.66± 0.042^{c}	0.42± 0.016^{a}	4.61± 0.015^{e}	1.78± 0.016^{b}	1.12± 0.070^{b}	4.73± 0.049^{e}	5.32± 0.084^{f}	0.84± 0.032^{a}	9.22± 0.031^{j}	0.53± 2.511^{a}	2.24± 0.141^{c}	1.59± 0.098^{b}	7.98± 0.127^{h}	1.46± 0.241^{b}	13.8± 0.046^{n}	7.99± 3.767^{h}	4.48± 0.282^{e}	2.77± 0.390^{c}
As50+PS2	1.09± 0.015^{b}	0.34± 0.035^{a}	2.70± 0.012^{c}	0.58± 0.091^{a}	2.65± 0.077^{c}	0.26± 0.008^{a}	2.19± 0.031^{c}	0.69± 0.070^{a}	5.40± 0.025^{f}	1.56± 0.736^{b}	5.31± 0.155^{f}	0.53± 0.016^{a}	3.29± 0.046^{d}	1.22± 0.367^{b}	8.10± 0.038^{i}	2.34± 1.105^{c}	10.6± 0.311^{k}	2.93± 0.159^{c}
As50+PS3	3.51± 0.016^{d}	0.80± 0.005^{a}	7.10± 0.024^{h}	2.31± 0.11^{c}	2.44± 0.036^{c}	0.68± 0.080^{a}	7.02± 0.032^{h}	1.61± 0.011^{b}	14.2± 0.049^{o}	6.71± 3.163^{g}	2.89± 2.901^{c}	1.34± 0.142^{b}	10.5± 0.048^{k}	2.81± 0.589^{c}	21.3± 0.074^{u}	10.6± 4.745^{k}	8.55± 1.594^{i}	2.38± 0.725^{c}
As50+PS4	1.14± 0.006^{b}	0.24± 0.056^{a}	2.42± 0.388^{c}	0.42± 0.001^{a}	2.15± 0.070^{c}	0.47± 0.055^{a}	2.29± 0.012^{c}	0.48± 0.113^{a}	4.85± 0.777^{e}	1.26± 0.595^{b}	4.30± 0.141^{e}	0.94± 0.110^{a}	3.44± 0.019^{d}	0.82± 0.028^{a}	7.27± 1.166^{h}	1.89± 0.893^{b}	8.60± 0.282^{i}	1.66± 0.525^{b}
As50+HX1	1.21± 0.028^{b}	0.41± 0.014^{a}	5.71± 0.078^{f}	1.27± 0.004^{b}	1.67± 0.079^{b}	0.46± 0.075^{a}	2.42± 0.056^{c}	0.82± 0.029^{a}	11.4± 0.156^{l}	3.82± 1.803^{d}	3.35± 0.158^{d}	0.93± 0.151^{a}	3.63± 0.084^{d}	1.43± 0.238^{b}	17.1± 0.235r	5.73± 2.704^{f}	6.70± 0.316^{g}	1.61± 0.067^{b}
As50+NAP1	2.63± 0.078^{c}	0.70± 0.077^{a}	6.53± 0.120^{g}	3.19± 0.098^{d}	2.32± 0.021^{c}	0.74± 0.101^{a}	5.27± 0.156^{f}	1.41± 0.155^{b}	13.7± 0.240^{n}	9.36± 4.412^{j}	4.65± 0.043^{e}	1.49± 0.202^{b}	7.90± 0.235^{h}	2.44± 0.226^{c}	19.6± 0.360^{t}	14.4± 6.618^{o}	9.31± 0.087^{j}	2.58± 0.176^{c}
As50+TL1	2.53± 0.042^{c}	0.41± 0.001^{a}	2.4± 0.113^{c}	0.86± 0.043^{a}	1.6± 0.098^{b}	0.36± 0.009^{a}	5.06± 0.084^{f}	0.83± 0.002^{a}	4.8± 0.226^{e}	2.51± 1.186^{c}	3.2± 0.197^{d}	0.73± 0.018^{a}	7.59± 0.127^{h}	1.46± 0.301^{b}	7.2± 0.339^{h}	3.77± 1.779^{d}	6.44± 0.395g	1.29± 0.292^{b}
As50+XL1	1.68± 0.007^{b}	0.60± 0.008^{a}	2.68± 0.056^{c}	0.68± 0.049^{a}	1.71± 0.035^{b}	0.68± 0.021^{a}	3.37± 0.015^{d}	1.21± 0.016^{b}	5.36± 0.113^{f}	1.95± 0.919^{b}	3.43± 0.070^{d}	1.37± 0.042^{b}	5.05± 0.023^{f}	2.11± 0.398^{c}	8.04± 0.169^{i}	2.92± 1.378^{c}	6.86± 0.141^{g}	2.39± 0.411^{c}
As50+BZ1	2.12± 0.004^{c}	0.80± 0.028^{a}	4.47± 0.070^{e}	1.54± 0.077^{b}	2.70± 0.077^{c}	0.91± 0.091^{a}	4.24± 0.009^{e}	1.60± 0.056^{b}	8.94± 0.141^{i}	4.47± 2.107^{e}	5.41± 0.115^{f}	1.83± 0.183^{b}	6.36± 0.014^{g}	2.79± 0.467^{c}	13.4± 0.212^{n}	6.70± 3.160^{g}	10.8± 0.311^{k}	3.23± 0.968^{d}
As50+G.c	3.28± 0.042^{d}	1.05± 0.001^{b}	6.34± 0.042^{g}	3.53± 0.042^{d}	3.09± 0.035^{d}	1.05± 0.063^{b}	6.56± 0.084^{g}	2.01± 0.014^{c}	12.6± 0.084^{m}	10.5± 4.949^{k}	6.19± 0.070^{g}	2.16± 0.056^{c}	9.84± 0.127^{j}	3.51± 0.685^{d}	19.2± 0.127^{t}	15.7± 7.424^{p}	9.28± 0.106^{j}	3.79± 0.862^{d}
As50+G.k	2.70± 0.134^{c}	0.8± 0.113^{a}	4.62± 0.099^{e}	2.26± 0.205^{c}	2.9± 0.113^{c}	0.62± 0.063^{a}	5.41± 0.268^{f}	1.6± 0.226^{b}	9.24± 0.197^{j}	7.23± 3.408^{h}	5.7± 0.084^{f}	1.25± 0.127^{b}	8.11± 0.403^{i}	2.84± 0.961^{c}	13.8± 0.296^{n}	10.8± 5.112^{k}	11.4± 0.169^{l}	2.21± 0.664^{c}
As50+A.k	1.83± 0.057^{b}	0.19± 0.004^{a}	2.6± 0.094^{c}	0.57± 0.127^{a}	1.54± 0.056^{b}	0.26± 0.001^{a}	3.67± 0.115^{d}	0.39± 0.009^{a}	5.2± 0.197^{f}	1.46± 0.691^{b}	3.18± 0.035^{d}	0.53± 0.002^{a}	5.51± 0.173^{f}	0.68± 0.156^{a}	7.8± 0.229^{h}	2.20± 1.037^{c}	6.37± 0.071^{g}	2.94± 0.185^{c}
As50+All B	1.94± 0.070^{b}	0.71± 0.098^{a}	2.58± 0.056^{c}	0.70± 0.091^{a}	1.71± 0.014^{b}	0.78± 0.035^{a}	3.88± 0.141^{d}	1.42± 0.197^{b}	5.16± 0.113^{f}	1.92± 0.905^{b}	3.42± 0.028^{d}	1.57± 0.070^{b}	5.82± 0.212^{f}	2.52± 0.848^{c}	7.74± 0.169^{h}	2.88± 1.357^{c}	6.84± 0.065^{g}	2.76± 0.678^{c}
As50+All M	2.59± 0.021^{c}	0.78± 0.014^{a}	5.23± 0.155^{f}	2.92± 0.113^{c}	1.66± 0.042^{b}	0.58± 0.028^{a}	5.19± 0.042^{f}	1.56± 0.028^{b}	10.4± 0.311^{k}	8.52± 4.016^{i}	3.32± 0.084^{d}	1.16± 0.056^{b}	7.78± 0.063^{h}	2.72± 0.502^{c}	15.6± 0.466^{p}	12.7± 6.024^{m}	6.64± 0.169^{g}	2.02± 0.311^{c}
As50+Const.	1.44± 0.005^{b}	0.24± 0.003^{a}	3.08± 0.129^{d}	1.50± 0.558^{b}	2.81± 0.704^{c}	0.72± 0.070^{a}	2.88± 0.011^{c}	0.48± 0.007^{a}	6.01± 0.258^{g}	3.33± 1.569^{d}	6.62± 0.009^{g}	1.45± 0.141^{b}	4.33± 0.016^{e}	0.84± 0.159^{a}	9.02± 0.388^{j}	4.99± 2.354^{e}	13.2± 0.019^{n}	2.52± 0.267^{c}
C + As 100	2.41± 0.789^{c}	0.66± 0.009^{a}	4.09± 0.127^{e}	1.01± 0.445^{b}	1.74± 0.099^{b}	0.66± 0.891^{a}	4.13± 0.611^{e}	1.55± 0.781^{b}	8.11± 0.071^{i}	4.01± 0.072^{e}	5.11± 0.119^{f}	1.62± 0.771^{b}	6.31± 0.005^{g}	2.78± 0.065^{c}	1.21± 0.009^{b}	5.91± 0.187^{b}	8.94± 0.212^{i}	2.08± 0.556^{c}

As100+PS1	1.81± 0.036^{b}	0.40± 0.023^{a}	5.53± 0.061^{f}	0.88± 0.144^{a}	2.61± 0.077^{c}	0.73± 0.006^{a}	3.63± 0.073^{d}	0.81± 0.046^{a}	11.6± 0.123^{l}	2.95± 1.391^{c}	5.23± 0.155^{f}	1.47± 0.015^{b}	5.44± 0.110^{f}	1.41± 0.205^{b}	16.5± 0.184^{q}	4.42± 2.087^{e}	10.4± 0.331^{k}	2.58± 0.548^{c}
As100+PS2	1.64± 0.002^{b}	0.10± 0.021^{a}	2.61± 0.007^{c}	0.59± 0.099^{a}	1.06± 0.084^{b}	0.49± 0.021^{a}	3.28± 0.005^{d}	0.21± 0.042^{a}	5.22± 0.015^{f}	1.58± 0.748^{b}	2.12± 0.169^{c}	0.99± 0.042^{a}	4.92± 0.008^{e}	0.36± 0.001^{a}	7.84± 0.023^{h}	2.38± 1.122^{c}	4.24± 0.339^{e}	1.73± 0.277^{b}
As100+PS3	1.21± 0.120^{b}	0.27± 0.004^{a}	1.11± 0.024^{b}	0.15± 0.008^{a}	1.21± 0.014^{b}	0.28± 0.005^{a}	2.43± 0.240^{c}	0.55± 0.008^{a}	2.22± 0.048^{c}	0.44± 0.209^{a}	2.42± 0.028^{c}	0.57± 0.011^{a}	3.64± 0.360^{d}	0.97± 0.181^{a}	3.33± 0.072^{d}	0.66± 0.313^{a}	4.84± 0.056^{e}	1.02± 0.183^{b}
As100+PS4	1.14± 0.021^{b}	0.15± 0.004^{a}	3.25± 0.007^{d}	0.54± 0.071^{a}	1.55± 0.056^{b}	0.61± 0.015^{a}	2.29± 0.042^{c}	0.31± 0.008^{a}	6.50± 0.015^{g}	1.49± 0.705^{b}	3.1± 0.113^{d}	1.22± 0.031^{b}	3.43± 0.063^{d}	0.54± 0.094^{a}	9.75± 0.023^{j}	2.24± 1.058^{c}	6.21± 0.226^{g}	2.12± 0.376^{c}
As5100+HX1	2.14± 0.014c	0.73± 0.035^{a}	2.18± 0.063c	2.2± 0.141^{c}	1.64± 0.049^{b}	0.64± 0.042^{a}	4.28± 0.028^{e}	1.47± 0.071^{b}	4.37± 0.127^{e}	6.3± 2.969^{g}	3.29± 0.098^{d}	1.28± 0.084^{b}	6.42± 0.042^{g}	2.58± 0.643^{c}	6.55± 0.190^{g}	9.45± 4.45^{j}	6.58± 0.197^{g}	2.22± 0.304^{c}
As100+NAP1	1.50± 0.002^{b}	0.72± 0.077^{a}	4.16± 0.009^{e}	0.72± 0.004^{a}	3.36± 0.009^{d}	0.67± 0.042^{a}	3.01± 0.005^{d}	1.45± 0.155^{b}	8.32± 0.019^{i}	2.17± 1.023^{c}	6.72± 0.014^{g}	1.34± 0.004^{b}	4.52± 0.008^{e}	2.51± 0.240^{c}	12.4± 0.029^{m}	3.25± 1.535^{d}	10.8± 0.021^{k}	2.35± 0.482^{c}
As100+TL1	1.65± 0.049^{b}	0.54± 0.049^{a}	3.43± 0.008^{d}	0.61± 0.005^{a}	1.78± 0.003^{b}	0.69± 0.011^{a}	3.31± 0.088^{d}	1.09± 0.055^{b}	6.86± 0.016^{g}	1.83± 0.864^{b}	3.55± 0.007^{d}	1.38± 0.022^{b}	4.96± 0.148^{e}	1.92± 0.558^{b}	10.2± 0.025^{k}	2.74± 1.296^{c}	7.11± 0.014^{h}	2.41± 0.527^{c}
As100+XL1	2.81± 0.045c	0.23± 0.006^{a}	3.81± 0.015d	0.67± 0.001^{a}	1.45± 0.004^{b}	0.51± 0.001^{a}	5.62± 0.091^{f}	0.47± 0.012^{a}	7.62± 0.031^{h}	2.03± 0.958^{c}	2.91± 0.009^{c}	1.02± 0.002^{b}	8.44± 0.137^{i}	0.82± 0.144^{a}	11.4± 0.046^{l}	3.05± 1.438^{d}	5.83± 0.019^{f}	1.79± 0.357^{b}
As100+BZ1	2.11± 0.042c	0.42± 0.005^{a}	5.27± 0.091^{f}	2.66± 0.070^{c}	1.83± 0.062^{b}	0.67± 0.056^{a}	4.22± 0.084^{e}	0.84± 0.011^{a}	10.5± 0.183^{k}	7.83± 3.691^{h}	3.67± 0.125^{d}	1.34± 0.113^{b}	6.33± 0.127^{g}	1.48± 0.280^{b}	15.8± 0.275^{p}	11.7± 5.536^{l}	7.34± 0.251^{h}	2.36± 0.671^{c}
As100+G.c	1.70± 0.007^{b}	0.25± 0.056^{a}	5.45± 0.056f	1.04± 0.064^{b}	1.72± 0.005^{b}	0.48± 0.005^{a}	3.40± 0.001^{d}	0.5± 0.113^{a}	10.9± 0.113^{k}	2.87± 1.356^{c}	3.44± 0.011^{d}	0.96± 0.001^{a}	5.10± 0.002^{f}	0.85± 0.021^{a}	16.3± 0.169^{q}	4.31± 2.034^{e}	6.89± 0.022^{g}	1.69± 0.362^{b}
As100+G.k	2.65± 0.063c	0.51± 0.004^{a}	5.73± 0.007f	2.26± 0.017^{c}	1.63± 0.056^{b}	0.59± 0.021^{a}	5.31± 0.127^{f}	1.03± 0.004^{b}	11.4± 0.155^{l}	6.63± 3.125^{g}	3.26± 0.113^{d}	1.19± 0.042^{b}	7.96± 0.190^{h}	1.80± 0.381^{b}	17.2± 0.233^{s}	9.94± 4.688^{j}	6.52± 0.226^{g}	2.09± 0.494^{c}
As100+A.k	2.75± 0.042^{c}	0.64± 0.063^{a}	4.55± 0.055^{e}	1.89± 0.162^{b}	2.2± 0.028^{c}	0.73± 0.022^{a}	5.5± 0.084^{f}	1.29± 0.127^{b}	9.1± 0.197^{j}	6.03± 2.842^{g}	4.4± 0.056^{e}	1.46± 0.056^{b}	8.25± 0.127^{i}	2.28± 0.678^{c}	13.6± 0.296^{o}	9.04± 4.263^{j}	8.81± 0.113^{i}	2.54± 0.417^{c}
As100+All B	2.94± 0.003c	0.16± 0.006^{a}	3.22± 0.036^{d}	0.42± 0.001^{a}	1.08± 0.007^{b}	0.40± 0.006^{a}	5.88± 0.007^{f}	0.32± 0.012^{a}	6.44± 0.072^{g}	1.28± 0.603^{b}	2.16± 0.014^{c}	0.80± 0.012^{a}	8.82± 0.010^{i}	0.57± 0.137^{a}	9.67± 0.108^{j}	1.92± 0.905^{b}	4.32± 0.028^{e}	1.41± 0.263^{b}
As100+All M	2.78± 0.035^{c}	0.67± 0.055^{a}	4.39± 0.155^{e}	2.65± 0.063^{c}	2.14± 0.063^{c}	0.84± 0.066^{a}	5.57± 0.070^{f}	1.34± 0.111^{b}	8.78± 0.311^{i}	7.83± 3.691^{h}	4.29± 0.127^{e}	1.69± 0.127^{b}	8.35± 0.106^{i}	2.32± 0.275^{c}	13.1± 0.466^{n}	11.7± 5.536^{l}	8.58± 0.254^{i}	2.98± 0.821^{c}
As100+Const.	3.59± 0.021^{d}	0.85± 0.056^{a}	6.7± 0.296^{g}	3.78± 0.282^{d}	3.22± 0.044^{d}	1.22± 0.035^{b}	7.19± 0.042^{h}	1.7± 0.011^{b}	13.4± 0.593^{n}	11.9± 5.628^{l}	6.45± 0.098^{g}	2.45± 0.070^{c}	10.7± 0.063^{k}	2.95± 0.403^{c}	20.1± 0.890^{u}	7.9± 8.442^{g}	12.8± 0.197^{m}	4.31± 0.989^{e}

Mean ± SD (n=3). Different small alphabets (a-u) indicate statistically significant difference at $P \leq 0.05$ by Turkey's test. (,**C**: control, **As 50**: arsenic 50 mgkg^{-1}, **As 100**: arsenic 100 mgkg^{-1}, **All B**: all bacterial isolates (1-9), **All M**: All mycorrhiza cultures (1-3), **Cons**t: microbial consortium (All bacteria + all mycorrhiza), **PS1**:*Ralstonia insidiosa***, PS 2:** *Enterobacter ludwigii***, PS 3:** *Pseudomonas aeruginosa*, **PS 4:** *Cellulosomicrobium funkei*, **HX 1:** *Pseudomonas putida*, **NAP 1:** *Citrobacter freundii*, **TL 1:** *Pseudomonas plecoglossicida* **,XL 1:** *Pseudomonas fulva*,**BZ 1:** *Pseudomonas sp*, **G.c:** *Glomus claroideum***, G.h :** *Glomus* hoi**, A.k :** *Acaulospora kentinensis*, **WW**: wet weight, **DW**: dry weight).

Table 38: Effect of cadmium on wet weight and dry weight (g) of *Ricinus communis* (root, stem and leaves) inoculated with rhizobacteria, mycorrhiza and microbial consortium.

Time →	1 Month						2 Month						3 Month					
Plant parts →	Root		Stem		Leaves		Root		Stem		Leaves		Root		Stem		Leaves	
Treatment ↓	WW	DW	WW	DW	WW	DW	WW	DW	WW	DW	WW	DW	WW	DW	WW	DW	WW	DW
Control	3.51± 0.091^{d}	1.81± 0.034^{b}	8.01± 0.083^{i}	3.81± 0.221^{d}	3.43± 0.073^{d}	1.61± 0.009^{b}	6.29± 0.051^{g}	2.01± 0.098^{c}	1.06± 0.123^{b}	4.19± 1.211^{e}	6.79± 2.131^{g}	2.31± 0.221^{c}	9.14± 0.324^{j}	3.14± 0.452^{d}	21.9± 0.431^{u}	8.66± 0.572^{i}	18.8± 0.632^{s}	5.01± 0.571^{f}
C+PS1	1.63± 0.084^{b}	0.305± 0.009^{a}	3.53± 0.120^{d}	1.15± 0.077^{b}	1.26± 0.077^{b}	0.28± 0.070^{a}	3.26± 0.169^{d}	0.61± 0.019^{a}	7.07± 0.240^{h}	3.63± 1.711^{d}	2.48± 0.084^{c}	0.46± 0.002^{a}	4.89± 0.254^{e}	0.91± 0.029^{a}	10.6± 0.360^{k}	5.44± 2.566^{f}	4.35± 1.025^{e}	0.81± 0.159^{a}
C+PS2	1.81± 0.014^{b}	0.505± 0.007^{a}	2.93± 0.070^{c}	0.94± 0.049^{a}	2.12± 0.148^{c}	0.48± 0.091^{a}	3.62± 0.028^{d}	1.01± 0.014^{b}	5.86± 0.141^{f}	2.94± 1.385^{c}	4.15± 0.155^{e}	0.97± 0.182^{a}	5.43± 0.042^{f}	1.51± 0.021^{b}	8.79± 0.212^{i}	4.41± 2.078^{e}	7.23± 1.195^{h}	1.73± 0.663^{b}
C+PS3	1.08± 0.035^{b}	0.19± 0.028^{a}	2.15± 0.095^{c}	0.79± 0.042^{a}	2.15± 0.148^{c}	0.41± 0.003^{a}	2.17± 0.070^{c}	0.38± 0.056^{a}	4.31± 0.098^{e}	2.28± 1.074^{c}	4.31± 0.042^{e}	0.83± 0.007^{a}	3.25± 0.106^{d}	0.65± 0.035^{a}	6.46± 0.148^{g}	3.42± 1.612^{d}	8.62± 0.084^{i}	1.45± 0.281^{b}
C+PS4	2.27± 0.049^{c}	0.72± 0.056^{a}	5.59± 0.028^{f}	2.20± 0.035^{c}	2.53± 0.084^{c}	0.38± 0.014^{a}	4.55± 0.098^{e}	1.44± 0.113^{b}	11.1± 0.056^{l}	6.69± 3.153^{g}	5.01± 0.098^{f}	0.77± 0.029^{a}	6.82± 0.148^{g}	2.16± 0.169^{c}	16.7± 0.084^{q}	10.3± 4.73^{k}	8.75± 1.598^{i}	1.36± 0.326^{b}
C+HX1	1.65± 0.070^{b}	0.51± 0.035^{a}	2.81± 0.071^{c}	1.05± 0.007^{b}	1.59± 0.042^{b}	0.20± 0.009^{a}	3.3± 0.141^{d}	1.03± 0.070^{b}	5.62± 0.141^{f}	3.03± 1.428^{d}	3.18± 0.084^{d}	0.40± 0.018^{a}	4.95± 0.212^{e}	1.81± 0.487^{b}	8.43± 0.212^{i}	4.54± 2.142^{e}	6.36± 0.169^{g}	0.71± 0.11^{a}
C+NAP1	1.63± 0.049^{b}	0.47± 0.049^{a}	1.90± 0.106^{b}	0.44± 0.002^{a}	1.12± 0.155^{b}	0.21± 0.020^{a}	3.27± 0.098^{d}	0.95± 0.095^{a}	3.81± 0.212^{d}	1.33± 0.630^{b}	2.14± 0.169^{c}	0.43± 0.041^{a}	4.90± 0.148^{e}	1.64± 0.162^{b}	5.71± 0.318^{f}	2.07± 0.946^{c}	4.28± 0.339^{e}	0.76± 0.225^{a}
C+TL1	1.74± 0.043^{b}	0.65± 0.056^{a}	3.62± 0.120^{d}	1.2± 0.127^{b}	1.88± 0.148^{b}	0.27± 0.016^{a}	3.49± 0.055^{d}	1.3± 0.111^{b}	7.25± 0.240^{h}	3.87± 1.824^{d}	3.62± 0.084^{d}	0.55± 0.032^{a}	5.23± 0.148^{f}	2.25± 0.261^{c}	10.8± 0.360^{k}	5.80± 2.736^{f}	6.35± 1.428^{g}	0.98± 0.254^{a}
C+XL1	2.64± 0.063^{c}	0.76± 0.063^{a}	4.68± 0.098^{e}	1.62± 0.063^{b}	3.14± 0.009^{d}	0.82± 0.007^{a}	5.29± 0.127^{f}	1.53± 0.127^{b}	9.36± 0.197^{j}	4.74± 2.234^{e}	6.18± 0.028^{g}	1.65± 0.011^{b}	7.93± 0.190^{h}	2.65± 0.318^{c}	14.4± 0.296^{o}	7.11± 3.351^{h}	9.27± 0.042^{j}	2.89± 0.552^{c}
C+BZ1	2.14± 0.094^{c}	0.74± 0.063^{a}	5.19± 0.296^{f}	1.65± 0.056^{b}	1.7± 0.028^{b}	0.61± 0.077^{a}	4.29± 0.095^{e}	1.49± 0.127^{b}	10.3± 0.593^{k}	4.83± 2.276^{e}	3.4± 0.056^{d}	1.23± 0.155^{b}	6.43± 0.148^{g}	2.58± 0.304^{c}	15.5± 0.890^{p}	7.24± 3.415^{h}	6.8± 0.113^{g}	2.18± 0.707^{c}
C+Gc	1.74± 0.070^{b}	0.61± 0.042^{a}	3.55± 0.148^{d}	1.18± 0.021^{b}	1.75± 0.063^{b}	0.52± 0.049^{a}	3.48± 0.141^{d}	1.22± 0.084^{b}	7.11± 0.296^{h}	3.6± 1.697^{d}	3.51± 0.127^{d}	1.05± 0.098^{b}	5.22± 0.212^{f}	2.12± 0.282^{c}	10.6± 0.445^{k}	5.4± 2.545^{f}	7.02± 0.254^{h}	1.82± 0.197^{b}
C+G.k	1.69± 0.035^{b}	0.62± 0.049^{a}	3.12± 0.162^{d}	1.54± 0.141^{b}	2.2± 0.098c	0.50± 0.008^{a}	3.39± 0.071^{d}	1.25± 0.044^{b}	6.25± 0.325^{g}	4.92± 2.319^{e}	4.4± 0.197^{e}	1.01± 0.169^{b}	5.08± 0.106^{f}	2.20± 0.615^{c}	9.37± 0.487^{j}	7.38± 3.478^{h}	8.8± 0.395^{i}	1.77± 0.387^{b}
C+A.k	2.45± 0.353^{c}	0.75± 0.042^{a}	4.41± 0.106^{e}	1.76± 0.063^{b}	2.16± 0.091^{c}	0.40± 0.009^{a}	4.9± 0.007^{e}	1.5± 0.084^{b}	8.83± 0.212^{i}	5.43± 2.559^{f}	4.37± 0.127^{e}	0.81± 0.019^{a}	7.35± 1.060^{h}	2.64± 0.678^{c}	13.2± 0.318^{n}	8.14± 3.839^{i}	8.74± 0.254^{i}	1.42± 0.253^{b}
C+All B	1.4± 0.084^{b}	0.38± 0.085^{a}	3.2± 0.11^{d}	1.6± 0.056^{b}	1.18± 0.070^{b}	0.51± 0.078^{a}	2.8± 0.169^{c}	0.76± 0.166^{a}	6.4± 0.226^{g}	4.92± 2.319^{e}	2.36± 0.141^{c}	1.03± 0.156^{b}	4.2± 0.254^{e}	1.3± 0.028^{b}	9.6± 0.339^{j}	7.38± 3.478^{h}	4.72± 0.282^{e}	1.78± 0.090^{b}
C+All M	2.27±	0.80±	1.56±	0.67±	2.32±	0.18±	4.55±	1.60±	3.12±	0.52±	4.64±	0.37±	6.82±	2.84±	4.68±	0.78±	9.28±	0.65±

	0.063^{c}	0.111^{a}	0.155^{b}	0.703^{a}	0.004^{c}	0.007^{a}	0.127^{e}	0.223^{b}	0.311^{d}	0.247^{a}	0.008^{e}	0.015^{a}	0.190^{a}	0.957^{c}	0.466^{e}	0.371^{a}	0.016^{j}	0.105^{a}
C+Const	1.12± 0.035^{b}	0.33± 0.014^{a}	2.14± 0.127^{c}	1.02± 0.254^{b}	3.05± 0.098^{d}	0.44± 0.366^{a}	2.25± 0.070^{c}	0.66± 0.028^{a}	4.28± 0.254^{e}	2.52± 1.187^{c}	6.10± 0.196^{g}	0.88± 0.733^{a}	3.37± 0.106^{d}	1.16± 0.282^{b}	6.42± 0.381^{g}	3.78± 1.781^{d}	9.16± 0.294^{j}	1.41± 0.972^{b}
C + Cd 50	2.11± 0.021^{c}	0.88± 0.015^{a}	4.04± 0.551^{e}	1.11± 0.712^{b}	1.88± 0.067^{b}	0.71± 0.052^{a}	4.24± 0.021^{e}	1.71± 0.123^{b}	8.91± 0.189^{i}	4.23± 4.721^{e}	5.63± 0.511^{f}	1.93± 0.331^{b}	6.91± 0.067^{g}	2.99± 0.612^{c}	18.4± 0.345^{s}	7.01± 3.112^{h}	9.03± 0.134^{j}	2.09± 0.502^{c}
Cd50+PS1	3.11± 0.014^{d}	0.92± 0.077^{a}	6.95± 0.084^{g}	4.01± 0.014^{e}	2.78± 0.042^{c}	0.92± 0.077a	6.22± 0.028^{g}	1.85± 0.155^{b}	13.9± 0.169^{n}	12.3± 5.670^{m}	5.56± 0.084^{f}	1.85± 0.155^{b}	9.33± 0.042^{j}	3.21± 0.381^{d}	20.8± 0.254^{u}	8.04± 8.506^{i}	11.1± 0.169^{l}	3.26± 0.926^{d}
Cd50+PS2	2.61± 0.001^{c}	0.66± 0.063^{a}	4.69± 0.155^{e}	2.49± 0.254^{c}	1.65± 0.056^{b}	0.68± 0.021^{a}	5.22± 0.002^{f}	1.33± 0.127^{b}	9.38± 0.311^{j}	6.93± 3.266^{g}	3.3± 0.113^{d}	1.37± 0.042^{b}	7.83± 0.004^{h}	2.30± 0.247^{c}	14.7± 0.466^{o}	5.39± 4.901^{f}	6.6± 0.226^{g}	2.39± 0.411^{c}
Cd50+PS3	2.76± 0.056^{c}	0.71± 0.042^{a}	4.02± 0.042^{e}	1.70± 0.134^{b}	4.56± 0.582^{e}	1.41± 0.526^{b}	5.52± 0.113^{f}	1.42± 0.084^{b}	8.04± 0.084^{i}	4.83± 2.276^{e}	7.62± 0.524^{h}	2.82± 1.165^{c}	8.28± 0.169^{i}	2.5± 0.650^{c}	12.6± 0.127^{m}	7.24± 3.415^{h}	15.2± 1.049^{p}	4.73± 1.040^{e}
Cd50+PS4	3.06± 0.084^{d}	0.30± 0.006^{a}	8.22± 0.388^{i}	1.33± 0.127^{b}	3.4± 0.084^{d}	1.20± 0.002^{b}	6.12± 0.169^{g}	0.60± 0.012^{a}	16.4± 0.777^{q}	3.72± 1.753^{d}	7.04± 0.056^{h}	2.42± 0.028^{c}	9.18± 0.254^{j}	1.06± 0.193^{b}	24.6± 1.166^{u}	5.58± 2.630^{f}	14.8± 0.111^{o}	4.24± 0.905^{e}
Cd50+HX1	1.73± 0.028^{b}	0.66± 0.005^{a}	7.4± 0.579^{h}	1.65± 0.014^{b}	1.74± 0.035^{b}	0.77± 0.014^{a}	3.46± 0.056^{d}	1.33± 0.011^{b}	14.8± 1.159^{o}	4.94± 2.332^{e}	3.49± 0.070^{d}	1.54± 0.028^{b}	5.19± 0.084^{f}	2.32± 0.450^{c}	22.2± 1.739^{u}	7.42± 3.498^{h}	6.98± 0.141^{g}	2.69± 0.494^{c}
Cd50+NAP1	1.58± 0.049^{b}	0.5± 0.042^{a}	2.4± 0.664^{c}	1.06± 0.070^{b}	1.49± 0.028^{b}	0.60± 0.003^{a}	3.17± 0.009^{d}	1.01± 0.084^{b}	4.8± 1.329^{e}	3.03± 1.428^{d}	2.98± 0.056^{c}	1.21± 0.007^{b}	4.75± 0.148^{e}	1.73± 0.205^{b}	7.2± 1.994^{h}	4.54± 2.142^{e}	5.96± 0.113^{f}	2.12± 0.417^{c}
Cd50+TL1	2.08± 0.004^{c}	0.18± 0.004^{a}	6.14± 0.006^{g}	1.15± 0.008^{b}	1.62± 0.077^{b}	0.58± 0.028^{a}	4.17± 0.006^{e}	0.37± 0.009^{a}	12.2± 0.012^{m}	3.43± 1.620^{d}	3.25± 0.155^{d}	1.16± 0.056^{b}	6.25± 0.014^{g}	0.65± 0.147^{a}	18.4± 0.019^{s}	5.15± 2.431^{f}	6.5± 0.311^{g}	2.04± 0.509^{c}
Cd50+XL1	1.96± 0.070^{b}	0.55± 0.084^{a}	2.70± 0.035^{c}	1.25± 0.056^{b}	2.93± 0.091^{c}	1.01± 0.042^{b}	3.92± 0.141^{d}	1.1± 0.169^{b}	5.41± 0.070^{f}	3.63± 1.711^{d}	5.87± 0.183^{f}	2± 0.084^{c}	5.88± 0.212^{f}	1.95± 0.685^{b}	8.11± 0.106^{i}	5.44± 2.566^{f}	11.7± 0.367^{l}	3.51± 0.855^{d}
Cd50+BZ1	1.59± 0.056^{b}	0.31± 0.019^{a}	3.29± 0.008^{d}	1.03± 0.089^{b}	3.03± 0.090^{d}	1.01± 0.002^{b}	3.18± 0.114^{d}	0.62± 0.038^{a}	6.58± 0.016^{g}	2.91± 1.376^{c}	6.07± 0.181^{g}	2.02± 0.005^{c}	4.77± 0.169^{e}	1.09± 0.154^{b}	9.87± 0.025^{j}	4.37± 2.064^{e}	12.1± 0.362^{m}	3.54± 0.725^{d}
Cd50+G.c	2.18± 0.004^{c}	0.67± 0.035^{a}	8.08± 0.053^{i}	3.11± 0.077^{d}	2.78± 0.042^{c}	1.06± 0.056^{b}	4.37± 0.002^{e}	1.35± 0.070^{b}	16.1± 0.106^{q}	9.18± 4.327^{j}	5.56± 0.084^{f}	2.12± 0.169^{c}	6.55± 0.014^{g}	2.37± 0.601^{c}	24.2± 0.159^{u}	13.7± 6.491^{n}	11.2± 0.169^{l}	3.74± 1.046^{d}
Cd50+G.k	2.12± 0.049^{c}	0.22± 0.056^{a}	5.27± 0.091^{f}	2.37± 0.084^{c}	2.63± 0.063^{c}	0.99± 0.007^{a}	4.25± 0.001^{e}	0.44± 0.313^{a}	10.5± 0.183^{k}	6.93± 3.266^{g}	5.27± 0.127^{f}	1.66± 0.448^{b}	6.37± 0.148^{g}	0.75± 0.042^{a}	15.8± 0.275^{p}	6.3± 4.900^{g}	10.5± 0.254^{k}	2.98± 1.372^{c}
Cd50+A.k	2.76± 0.077^{c}	0.55± 0.056^{a}	5.55± 0.098^{f}	2.23± 0.148^{c}	1.54± 0.002^{b}	0.67± 0.022^{a}	5.53± 0.155^{f}	1.11± 0.121^{b}	11.1± 0.197^{l}	7.02± 3.309^{h}	3.08± 0.005^{d}	2.17± 1.173^{c}	8.29± 0.233^{i}	1.94± 0.586^{b}	16.6± 0.296^{q}	6.5± 4.963^{g}	6.16± 0.011^{g}	3.59± 1.286^{d}
Cd50+All B	1.91± 0.039^{b}	0.96± 0.029^{a}	6.46± 0.009^{g}	2.41± 0.004^{c}	3.24± 0.063^{d}	1.49± 0.014^{b}	3.82± 0.079^{d}	1.93± 0.059^{b}	12.9± 0.019^{m}	7.23± 3.412^{h}	6.49± 0.127^{g}	2.98± 0.028^{c}	5.73± 0.118^{f}	3.40± 0.789^{d}	19.3± 0.029^{t}	8.8± 5.118^{i}	12.9± 0.254^{m}	5.22± 1.103^{f}
Cd50+All M	2.9± 0.014^{c}	0.72± 0.056^{a}	5.17± 0.077^{f}	2.53± 0.028^{c}	2.74± 0.070^{c}	1.07± 0.056^{b}	5.8± 0.028^{f}	1.44± 0.113^{b}	10.3± 0.155^{k}	7.53± 3.412^{h}	5.48± 0.141^{f}	2.14± 0.113^{c}	8.7± 0.042^{i}	2.54± 0.707^{c}	15.5± 0.233^{p}	5.2± 5.324^{f}	10.9± 0.282^{k}	3.76± 0.954^{d}
Cd50+Const.	2.15± 0.042^{c}	0.46± 0.063^{a}	4.69± 0.049^{e}	2.84± 0.042^{c}	2.93± 0.006^{c}	0.68± 0.006^{a}	4.3± 0.008^{e}	0.93± 0.127^{a}	9.39± 0.098^{j}	8.43± 3.973^{i}	5.86± 0.012^{f}	1.36± 0.012^{b}	6.45± 0.127^{g}	1.65± 0.551^{b}	14.8± 0.148^{o}	6.01± 5.961^{g}	11.7± 0.025^{l}	2.39± 0.506^{c}
C + Cd 100	2.51± 0.006^{c}	0.71± 0.078^{a}	4.09± 0.512^{e}	1.03± 0.088^{b}	1.74± 0.321^{b}	0.64± 0.072^{a}	4.12± 0.012^{e}	1.13± 0.032^{b}	7.93± 0.121^{h}	3.81± 2.211^{d}	4.81± 0.041^{e}	1.63± 0.029^{b}	6.12± 0.55^{g}	2.81± 0.112^{c}	12.1± 0.043^{m}	5.64± 3.041^{f}	8.45± 0.054^{i}	2.93± 0.112^{c}

Cd100+PS1	1.25± 0.009^{b}	0.22± 0.005^{a}	3.36± 0.008^{d}	1.60± 0.004^{b}	1.30± 0.007^{b}	0.45± 0.002^{a}	2.50± 0.019^{c}	0.44± 0.011^{a}	6.72± 0.016^{g}	4.81± 2.263^{e}	2.60± 0.016^{c}	0.91± 0.004^{a}	3.75± 0.029^{d}	0.77± 0.137^{a}	10.8± 0.025^{k}	4.21± 3.402^{e}	5.21± 0.033^{f}	1.61± 0.332^{a}
Cd100+PS2	1.65± 0.012^{b}	0.41± 0.007^{a}	4.47± 0.006^{e}	2.84± 0.042^{c}	3.23± 0.049^{d}	0.41± 0.023^{a}	3.30± 0.024^{d}	0.82± 0.055	8.94± 0.132^{i}	8.69± 4.096^{i}	6.47± 0.098^{g}	0.83± 0.046^{a}	4.95± 0.036^{e}	1.45± 0.319^{b}	13.4± 0.199^{n}	6.03± 6.145^{g}	12.9± 0.197^{m}	1.45± 0.213^{b}
Cd100+PS3	2.27± 0.009^{c}	0.13± 0.005^{a}	4.45± 0.025^{e}	1.79± 0.008^{b}	2.63± 0.022^{c}	0.69± 0.021^{a}	4.55± 0.019^{e}	0.26± 0.014	8.9± 0.050^{i}	5.35± 2.525^{f}	5.26± 0.084^{f}	1.39± 0.042^{b}	6.83± 0.029^{g}	0.46± 0.073^{a}	13.3± 0.076^{n}	8.03± 3.788^{i}	10.5± 0.169^{k}	2.44± 0.565^{c}
Cd100+PS4	2.16± 0.077^{c}	0.23± 0.005^{a}	4.77± 0.141e	2.66± 0.087^{c}	1.50± 0.018^{b}	0.52± 0.003^{a}	4.33± 0.155^{e}	0.47± 0.012	9.54± 0.282^{j}	7.83± 3.691^{h}	3.01± 0.036^{d}	1.05± 0.007^{b}	6.49± 0.233^{g}	0.83± 0.188^{a}	14.3± 0.424^{o}	7.74± 5.536^{h}	6.03± 0.073^{g}	1.84± 0.361^{b}
Cd100+HX1	1.47± 0.002^{b}	0.14± 0.006^{a}	3.64± 0.110^{d}	1.58± 0.028^{b}	2.75± 0.063^{c}	1.02± 0.056^{b}	2.95± 0.004^{c}	0.28± 0.012	7.29± 0.220^{h}	4.80± 2.264^{e}	5.51± 1.272^{f}	2.04± 0.113^{c}	4.43± 0.006^{e}	0.50± 0.124^{a}	10.9± 0.331^{k}	4.21± 3.396^{e}	11.2± 0.254^{l}	3.59± 0.919^{d}
Cd100+NAP1	2.65± 0.056^{c}	0.83± 0.056^{a}	6.3± 0.070^{g}	3.35± 0.098^{d}	3.28± 0.121^{d}	1.27± 0.369^{b}	5.3± 0.131^{f}	1.66± 0.127	12.6± 0.141^{m}	9.84± 4.638^{j}	6.57± 0.243^{g}	2.54± 0.738^{c}	7.95± 0.169^{h}	2.92± 0.784^{c}	18.9± 0.212^{s}	9.76± 6.957^{j}	13.1± 0.486^{n}	4.57± 2.191^{e}
Cd100+TL1	5.42± 0.702^{f}	0.29± 0.005^{a}	5.64± 0.042^{f}	2.64± 0.002^{c}	1.57± 0.049^{b}	0.67± 0.056^{a}	10.8± 1.404^{k}	0.58± 0.011	11.2± 0.084^{l}	7.93± 3.737^{h}	3.15± 0.989^{d}	1.34± 0.113^{b}	16.2± 2.106^{q}	1.02± 0.225^{b}	16.9± 0.127^{q}	5.23± 1.022^{f}	6.31± 0.197^{g}	2.36± 0.671^{c}
Cd100+XL1	1.47± 0.254^{b}	0.5± 0.127^{a}	3.32± 0.134^{d}	1.38± 0.070^{b}	1.75± 0.056^{b}	0.67± 0.021^{a}	2.94± 0.509^{c}	1.01± 0.254	6.65± 0.268^{g}	4.29± 2.022^{e}	3.5± 0.113^{d}	1.35± 0.042^{b}	4.41± 0.763^{e}	1.79± 0.799^{b}	9.97± 0.403^{j}	3.37± 3.033^{d}	7.7± 0.226^{h}	2.37± 0.551^{c}
Cd100+BZ1	1.73± 0.063^{b}	0.61± 0.077^{a}	2.69± 0.035^{c}	0.99± 0.021^{a}	1.69± 0.042^{b}	0.64± 0.042^{a}	3.47± 0.127^{d}	1.23± 0.555	5.39± 0.070^{f}	3.03± 1.428^{d}	3.38± 0.084^{d}	1.28± 0.053^{b}	5.20± 0.190^{f}	2.18± 0.707^{c}	8.05± 0.106^{i}	3.54± 2.142^{d}	6.76± 0.169^{g}	2.22± 0.304^{c}
Cd100+G.c	1.49± 0.091^{b}	0.45± 0.056^{a}	2.85± 0.063^{c}	0.63± 0.056^{a}	1.45± 0.494^{b}	0.68± 0.106^{a}	2.99± 0.183^{c}	0.9± 0.111	5.71± 0.127^{f}	1.77± 0.834^{b}	2.90± 0.988^{c}	1.37± 0.212^{b}	4.48± 0.275^{e}	1.55± 0.120^{b}	8.56± 0.191^{i}	2.65± 1.251^{c}	5.82± 1.977^{f}	2.43± 0.855^{c}
Cd100+G.k	1.59± 0.091^{b}	0.5± 0.056^{a}	2.82± 0.033^{c}	0.82± 0.066^{a}	1.15± 0.077^{b}	0.62± 0.120^{a}	3.19± 0.183^{d}	1.11± 0.101	5.65± 0.155^{f}	2.61± 1.230^{c}	2.31± 0.155^{c}	1.25± 0.240^{b}	4.78± 0.275^{e}	1.73± 0.155^{b}	8.47± 0.233^{i}	3.91± 1.845^{d}	4.62± 0.311^{e}	2.14± 0.021^{c}
Cd100+A.k	1.42± 0.031^{b}	0.50± 0.084^{a}	2.54± 0.063^{c}	0.96± 0.077^{a}	2.78± 0.229^{c}	0.88± 0.166^{a}	2.85± 0.063^{c}	1.04± 0.169	5.09± 0.127^{f}	2.73± 1.286^{c}	5.56± 0.458^{f}	1.76± 0.333^{b}	4.28± 0.095^{e}	1.78± 0.651^{b}	7.63± 0.190^{h}	3.09± 1.931^{d}	9.65± 1.166^{j}	3.14± 1.207^{d}
Cd100+All B	1.08± 0.021^{b}	0.31± 0.002^{a}	5.60± 0.017^{f}	0.82± 0.017^{a}	2.67± 0.077^{c}	0.77± 0.005^{a}	2.17± 0.042^{c}	0.62± 0.005	11.2± 0.035^{l}	2.51± 1.183^{c}	5.35± 0.155^{f}	1.55± 0.011^{b}	3.25± 0.063^{d}	1.09± 0.210^{b}	16.8± 0.053^{q}	3.76± 1.775^{d}	10.7± 0.311^{k}	2.71± 0.567^{c}
Cd100+All M	1.17± 0.004^{b}	0.211± 0.001^{a}	4.82± 0.077^{e}	1.94± 0.006^{b}	1.75± 0.070^{b}	0.55± 0.014^{a}	2.34± 0.009^{c}	0.42± 0.002	9.46± 0.155^{j}	5.85± 2.759^{f}	3.5± 0.141^{d}	1.1± 0.282^{b}	3.51± 0.014^{d}	0.73± 0.144^{a}	14.4± 0.233^{o}	7.46± 4.138^{h}	7.7± 0.282^{h}	1.93± 0.438^{b}
Cd100+Const.	1.44± 0.452^{b}	0.53± 0.197^{a}	2.73± 0.056^{c}	0.67± 0.028^{a}	1.85± 0.005^{b}	0.67± 0.001^{a}	2.88± 0.905^{c}	1.06± 0.395	5.46± 0.113^{f}	1.95± 0.919^{b}	3.70± 0.011^{d}	1.34± 0.002^{b}	4.32± 1.357^{e}	1.92± 1.067^{b}	8.19± 0.169^{i}	2.92± 1.378^{c}	7.41± 0.022^{h}	2.34± 0.479^{c}

Mean ± SD (n=3). Different small alphabets (a-u) indicate statistically significant difference at $P \leq 0.05$ by Turkey's test. (**C**: control, **Cd 50**: cadmium 50 mgkg^{-1}, **Cd 100**: cadmium 100 mgkg^{-1}, **All B**: all bacterial isolates (1-9), **All M**: All mycorrhiza cultures (1-3), **Cons**t: microbial consortium (All bacteria + all mycorrhiza), **PS1**:*Ralstonia insidiosa***, PS 2:** *Enterobacter ludwigii***, PS 3:** *Pseudomonas aeruginosa*, **PS 4:** *Cellulosomicrobium funkei*, **HX 1:** *Pseudomonas putida*, **NAP 1:** *Citrobacter freundii*, **TL 1:** *Pseudomonas plecoglossicida* **,XL 1:** *Pseudomonas fulva*,**BZ 1:** *Pseudomonas sp*, **G.c:** *Glomus claroideum***, G.h :** *Glomus* hoi**, A.k :** *Acaulospora kentinensis*, **WW**: wet weight, **DW**: dry weight).

Table 39: Effect of arsenic (50 and 100 mgkg^{-1}) on fresh weight and dry weight (g) of *Canna indica* (Root and shoot) inoculated with rhizobacteria, mycorrhiza and microbial consortium.

Time duration →	1St Month				2nd Month				3rd Month			
Plant parts →	Root		Shoot		Root		Shoot		Root		Shoot	
Treatment ↓	WW	DW	WW	DW	WW	DW	WW	DW	WW	DW	WW	DW
Control	2.12± 0.032^{c}	0.17± 0.044^{a}	3.69± 0.116^{d}	0.44± 0.071^{a}	9.84± 0.033^{j}	1.98± 0.078^{b}	14.3± 0.051^{o}	5.11± 0.088^{f}	16.3± 0.134^{q}	5.21± 0.098^{f}	21.5± 0.234^{t}	7.03± 0.041^{h}
C+PS1	2.35± 0.049^{c}	0.24± 0.042^{a}	4.37± 0.113e	0.48± 0.091^{a}	10.26± 0.035^{k}	2.46± 0.035^{c}	15.3± 0.071^{p}	5.44± 0.049^{f}	16.75± 0.212^{q}	5.16± 0.070^{f}	22.65± 0.353^{t}	8.35± 0.056^{i}
C+PS2	3.39± 0.028^{d}	0.41± 0.044^{a}	5.66± 0.077^{f}	1.82± 0.084^{b}	11.07± 1.477^{l}	3.15± 0.056^{d}	17.2± 0.056^{q}	6.19± 0.063^{g}	17.6± 0.424^{r}	6.16± 0.042^{g}	23.7± 0.282^{t}	9.18± 0.091^{j}
C+PS3	3.15± 0.056^{d}	0.25± 0.056^{a}	5.17± 0.077^{f}	1.61± 0.007^{b}	12.07± 0.091^{m}	3.06± 0.042^{d}	17.17± 0.084^{r}	6.12± 0.063^{g}	17.55± 0.494^{r}	6.07± 0.056^{g}	23.55± 0.494^{t}	9.16± 0.070^{j}
C+PS4	3.56± 0.033^{d}	0.24± 0.043^{a}	5.76± 0.008^{f}	1.85± 0.084^{b}	13.01± 0.042^{n}	4.20± 0.047^{e}	17.76± 0.042^{r}	6.04± 0.756^{g}	18.35± 0.353^{s}	7.1± 0.082^{h}	24.06± 0.073^{t}	9.08± 0.777^{j}
C+HX1	2.10± 0.036^{c}	0.15± 0.057^{a}	4.08± 0.091^{e}	0.47± 0.063^{a}	10.08± 0.070^{k}	2.07± 0.063^{c}	15.17± 0.070^{p}	5.28± 0.056^{f}	16.7± 0.141^{q}	5.14± 0.035^{f}	22.5± 0.042^{t}	8.66± 0.749^{i}
C+NAP1	2.48± 0.056^{c}	0.29± 0.077^{a}	4.81± 0.091^{e}	0.49± 0.106^{a}	10.52± 0.042^{k}	2.24± 0.046^{c}	15.8± 0.053^{p}	5.48± 0.077^{f}	16.45± 0.494^{q}	5.14± 0.014^{f}	22.78± 0.042^{t}	8.88± 0.077^{i}
C+TL1	2.11± 0.091^{c}	0.18± 0.084^{a}	3.78± 0.077^{d}	0.46± 0.088^{a}	10.12± 0.063^{k}	2.06± 0.073^{c}	14.2± 0.059^{o}	5.17± 0.071^{f}	16.35± 0.212^{q}	5.23± 0.028^{f}	21.3± 0.282^{t}	7.12± 0.120^{h}
C+XL1	3.13± 0.063^{d}	0.21± 0.056^{a}	5.38± 0.084^{f}	1.03± 0.784^{b}	10.16± 0.042^{k}	2.16± 0.070^{c}	16.37± 0.084^{q}	5.47± 0.028^{f}	16.6± 0.424^{q}	5.52± 0.049^{f}	21.8± 0.084^{t}	7.23± 0.148^{h}
C+BZ1	3.07± 0.084^{d}	0.19± 0.021^{a}	5.28± 0.056^{f}	1.75± 0.056^{b}	10.24± 0.056^{k}	2.25± 0.056^{c}	16.17± 0.091^{q}	5.40± 0.035^{f}	16.55± 0.353^{q}	5.53± 0.028^{f}	22.35± 0.056^{t}	8.07± 0.057^{i}
C + G.c	2.69± 0.120^{c}	0.28± 0.056^{a}	4.92± 0.098^{e}	0.51± 0.035^{a}	10.06± 0.077^{k}	2.15± 0.059^{c}	15.95± 0.056^{p}	5.77± 0.054^{f}	16.25± 0.212^{q}	5.16± 0.063^{f}	22.52± 0.098^{t}	8.37± 0.070^{i}
C + G.k	3.25± 0.056^{d}	0.25± 0.055^{a}	5.21± 0.106^{f}	1.65± 0.049^{b}	12.45± 0.046^{m}	3.19± 0.035^{d}	16.86± 0.073^{q}	5.88± 0.063^{f}	17.45± 0.353^{r}	6.18± 0.091^{g}	22.38± 0.049^{t}	8.17± 0.014^{i}
C + A.k	3.15± 0.057^{d}	0.32± 0.058^{a}	5.38± 0.098^{f}	1.59± 0.056^{b}	12.14± 0.045^{m}	3.16± 0.033^{d}	16.36± 0.070^{q}	5.85± 0.049^{f}	17.3± 0.383^{r}	6.26± 0.063^{g}	22.22± 0.063^{t}	8.05± 0.056^{i}
C + All B	3.11± 0.062^{d}	0.25± 0.051^{a}	5.33± 0.063^{f}	1.58± 0.084^{b}	12.07± 0.070^{m}	3.15± 0.055^{d}	16.99± 0.021^{q}	6.07± 0.049^{g}	17.45± 0.353^{r}	7.12± 0.021^{h}	23.08± 0.049^{t}	9.17± 0.084^{j}
C + All M	3.24±	0.35±	5.49±	1.69±	12.19±	3.25±	17.12±	6.14±	17.65±	7.08±	23.25±	9.22±

	0.049^{d}	0.054^{a}	0.070^{f}	0.049^{b}	0.063^{m}	0.056^{d}	0.127^{r}	0.049^{g}	0.353^{r}	0.042^{h}	0.048^{t}	0.056^{j}
C + Const	3.63± 0.028^{d}	0.45± 0.055^{a}	6.07± 0.071^{g}	1.07± 0.082^{b}	13.18± 0.042^{n}	4.25± 0.041^{e}	17.82± 0.084^{r}	6.48± 0.044^{g}	18.6± 0.424^{s}	5.25± 0.035^{f}	23.8± 0.055^{t}	9.51± 0.046^{j}
C + As 50	2.01± 0.071^{c}	0.15± 0.078^{a}	3.58± 0.087^{d}	0.34± 0.031^{a}	9.12± 0.062^{j}	18.8± 0.022^{s}	12.4± 0.021^{m}	4.93± 0.051^{e}	15.4± 0.126^{p}	5.03± 0.422^{f}	19.3± 0.098^{t}	6.84± 0.054^{g}
As50+PS1	3.25± 0.065^{d}	0.37± 0.063^{a}	5.07± 0.071^{f}	0.52± 0.078^{a}	13.11± 0.049^{n}	2.11± 0.056^{c}	17.15± 0.042^{r}	6.08± 0.049^{g}	18.55± 0.494^{s}	7.08± 0.042^{h}	23.17± 0.077^{t}	9.13± 0.077^{j}
As50+PS2	2.07± 0.070^{c}	0.23± 0.066^{a}	3.20± 0.120^{d}	0.34± 0.042^{a}	10.09± 0.035^{k}	1.90± 0.120^{b}	15.12± 0.127^{p}	5.06± 0.070^{f}	16.4± 0.442^{q}	5.25± 0.035^{f}	22.06± 0.076^{t}	8.16± 0.042^{i}
As50+PS3	3.35± 0.049^{d}	0.24± 0.049^{a}	5.41± 0.035^{f}	1.35± 0.049^{b}	13.09± 0.070^{n}	4.23± 0.077^{e}	16.70± 0.049^{q}	6.51± 0.042^{g}	18.35± 0.212^{s}	7.31± 0.007^{h}	22.24± 0.113^{t}	8.38± 0.063^{i}
As50+PS4	3.51± 0.033^{d}	0.32± 0.056^{a}	6.2± 0.113^{g}	1.85± 0.056^{b}	13.06± 0.071^{n}	4.11± 0.035^{e}	17.1± 0.056^{r}	6.12± 0.059^{g}	18.7± 0.282^{s}	7.19± 0.098^{h}	23.14± 0.049^{t}	9.13± 0.063^{j}
As50+HX1	3.45± 0.052^{d}	0.21± 0.064^{a}	6.04± 0.070^{g}	1.70± 0.097^{b}	13.12± 0.056^{n}	4.10± 0.106e	17.06± 0.073^{r}	6.11± 0.084^{g}	18.4± 0.282^{s}	7.15± 0.021^{h}	23.13± 0.061^{t}	9.1± 0.084^{j}
As50+NAP1	3.12± 0.059^{d}	0.26± 0.035^{a}	5.16± 0.079^{f}	1.36± 0.035^{b}	13.08± 0.056^{n}	4.08± 0.077^{e}	16.18± 0.077^{q}	5.71± 0.049^{f}	18.55± 0.353^{s}	7.14± 0.014^{h}	22.13± 0.062^{t}	8.18± 0.062^{i}
As50+TL1	3.13± 0.071^{d}	0.27± 0.061^{a}	5.27± 0.084^{f}	1.06± 0.073^{b}	12.61± 0.042^{m}	3.26± 0.032^{d}	16.2± 0.075^{q}	5.74± 0.042^{f}	17.7± 0.282^{r}	6.2± 0.070^{g}	22.19± 0.091^{t}	8.35± 0.053^{i}
As50+XL1	3.06± 0.070^{d}	0.19± 0.042^{a}	5.23± 0.074^{f}	1.52± 0.091^{b}	12.65± 0.054^{m}	3.24± 0.049^{d}	17.25± 0.047^{r}	6.08± 0.063^{g}	17.3± 0.282^{r}	6.13± 0.071^{g}	23.25± 0.057^{t}	9.15± 0.035^{j}
As50+BZ1	3.11± 0.073^{d}	0.21± 0.077^{a}	5.21± 0.091^{f}	1.59± 0.070^{b}	12.09± 0.071^{m}	3.25± 0.056^{d}	16.42± 0.042^{q}	6.17± 0.056^{g}	17.5± 0.282^{r}	6.20± 0.063^{g}	22.40± 0.035^{t}	8.22± 0.049^{i}
As50+G.c	3.50± 0.067^{d}	0.24± 0.049^{a}	5.75± 0.091^{f}	1.67± 0.056^{b}	13.01± 0.282^{n}	3.12± 0.021^{d}	17.11± 0.028^{r}	6.29± 0.070^{g}	17.7± 0.228^{r}	6.78± 0.044^{g}	23.23± 0.063^{t}	9.16± 0.063^{j}
As50+G.k	3.65± 0.054^{d}	0.28± 0.035^{a}	5.86± 0.079^{f}	1.78± 0.063^{b}	13.31± 0.035^{n}	4.23± 0.056^{e}	17.29± 0.120^{r}	6.45± 0.059^{g}	18.35± 0.353^{s}	7.64± 0.049^{h}	23.37± 0.070^{t}	9.23± 0.064^{j}
As50+A.k	1.80± 0.049^{b}	0.17± 0.084^{a}	3.29± 0.113^{d}	0.43± 0.056^{a}	9.15± 0.106^{j}	1.7± 0.127^{b}	12.57± 0.091^{m}	4.56± 0.042^{e}	15.2± 0.141^{p}	5.14± 0.049^{f}	24.9± 6.936^{t}	7.18± 0.070^{h}
As50+All B	3.63± 0.035^{d}	0.37± 0.056^{a}	5.85± 0.084^{f}	1.82± 0.084^{b}	13.29± 0.070^{n}	4.08± 0.070^{e}	17.8± 0.056^{r}	6.30± 0.049^{g}	18.65± 0.212^{s}	7.25± 0.056^{h}	23.9± 0.014^{t}	9.57± 0.028^{j}
As50+All M	3.54± 0.049^{d}	0.36± 0.035^{a}	5.76± 0.063^{f}	1.50± 0.066^{b}	13.23± 0.074^{n}	4.06± 0.063^{e}	17.6± 0.035^{r}	6.19± 0.077^{g}	18.6± 0.424^{s}	7.15± 0.056^{h}	23.78± 0.084^{t}	9.55± 0.056^{j}
As50+Const.	3.75± 0.056^{d}	0.37± 0.048^{a}	5.99± 0.049^{f}	1.92± 0.056^{b}	13.45± 0.049^{n}	4.28± 0.035^{e}	17.8± 0.056^{r}	6.47± 0.059^{g}	18.35± 0.353^{s}	7.09± 0.077^{h}	23.87± 0.077^{t}	9.61± 0.042^{j}
C + As 100	2.16± 0.034^{c}	0.24± 0.033^{a}	5.49± 0.018^{f}	1.08± 0.056^{b}	9.98± 0.034^{j}	1.52± 0.044^{b}	15.9± 0.012^{p}	5.31± 0.087^{f}	15.1± 0.018^{p}	4.99± 0.062^{e}	20.9± 0.034^{t}	7.84± 0.019^{h}

As100+PS1	3.8± 0.052^{d}	0.45± 0.056^{a}	6.07± 0.091^{g}	1.93± 0.063^{b}	13.73± 0.028^{n}	4.48± 0.042^{e}	17.9± 0.084^{r}	6.87± 0.047^{g}	18.45± 0.353^{s}	7.07± 0.056^{h}	24.6± 0.424^{t}	9.86± 0.035^{j}
As100+PS2	2.35± 0.054^{c}	0.25± 0.065^{a}	4.79± 0.028^{e}	0.58± 0.098^{a}	10.18± 0.084^{k}	1.65± 0.049^{b}	13.48± 0.056^{n}	4.94± 0.044^{e}	16.6± 0.424^{q}	5.11± 0.035^{f}	21.45± 0.494^{t}	7.15± 0.056^{h}
As100+PS3	3.26± 0.042^{d}	0.24± 0.049^{a}	5.83± 0.071^{f}	1.61± 0.091^{b}	12.85± 0.056^{m}	2.80± 0.657^{c}	17.08± 0.083^{r}	6.08± 0.056^{g}	17.7± 0.282^{r}	6.15± 0.035^{g}	23.75± 0.212^{t}	8.19± 0.028^{i}
As100+PS4	2.56± 0.035^{c}	0.38± 0.063^{a}	5.03± 0.071^{f}	0.63± 0.070a	10.07± 0.077^{k}	1.51± 0.035^{b}	16.35± 0.057^{q}	5.31± 0.098^{f}	16.35± 0.353^{q}	5.35± 0.056^{f}	22.55± 0.494^{t}	8.08± 0.077^{i}
As5100+HX1	2.39± 0.120^{c}	0.35± 0.056^{a}	4.93± 0.169^{e}	0.54± 0.049^{a}	10.09± 0.084^{k}	1.55± 0.056^{b}	15.7± 0.035^{p}	5.16± 0.071^{f}	16.45± 0.494^{q}	5.25± 0.042^{f}	21.5± 0.282^{t}	7.18± 0.072^{h}
As100+NAP1	2.16± 0.042^{c}	0.21± 0.045^{a}	5.59± 0.042^{f}	1.17± 0.075^{b}	9.91± 0.106^{j}	1.48± 0.055^{b}	16.33± 0.088^{q}	5.5± 0.007^{f}	15.6± 0.424^{p}	5.13± 0.056^{f}	22.6± 0.283^{t}	8.14± 0.070^{i}
As100+TL1	3.1± 0.028^{d}	0.20± 0.063^{a}	5.43± 0.084^{f}	1.71± 0.042^{b}	10.25± 0.052^{k}	1.84± 0.049^{b}	16.48± 0.042^{q}	5.31± 0.035^{f}	16.35± 0.353^{q}	5.31± 0.077^{f}	22.7± 0.282^{t}	8.18± 0.074^{i}
As100+XL1	3.27± 0.183^{d}	0.31± 0.047^{a}	6.12± 0.042^{g}	1.86± 0.083^{b}	10.08± 0.055^{k}	1.71± 0.059^{b}	17.15± 0.056^{r}	6.17± 0.077^{g}	16.5± 0.424^{q}	5.28± 0.073^{f}	23.55± 0.353^{t}	9.23± 0.063^{j}
As100+BZ1	3.19± 0.028^{d}	0.25± 0.048^{a}	5.62± 0.098^{f}	1.53± 0.056^{b}	10.54± 0.502^{k}	1.85± 0.049^{b}	17.46± 0.063^{r}	6.36± 0.033^{g}	16.45± 0.333^{q}	5.34± 0.044^{f}	23.35± 0.335^{t}	9.25± 0.049^{j}
As100+G.c	3.06± 0.070^{d}	0.23± 0.056^{a}	5.31± 0.035^{f}	1.21± 0.076^{b}	10.07± 0.071^{k}	1.65± 0.044^{b}	17.12± 0.134^{r}	6.06± 0.063^{g}	16.55± 0.355^{q}	5.29± 0.044^{f}	23.4± 0.424^{t}	9.14± 0.046^{j}
As100+G.k	2.35± 0.048^{c}	0.22± 0.072^{a}	4.62± 0.098^{e}	0.81± 0.042^{a}	9.89± 0.061^{j}	1.50± 0.042^{b}	15.2± 0.056^{p}	5.12± 0.063^{f}	15.55± 0.494^{p}	5.17± 0.077^{f}	21.4± 0.283^{t}	7.16± 0.073^{h}
As100+A.k	2.91± 0.042^{c}	0.18± 0.073^{a}	4.95± 0.091^{e}	1.21± 0.028^{b}	9.99± 0.070^{j}	1.71± 0.058^{b}	16.8± 0.063^{q}	6.08± 0.059^{g}	15.7± 0.282^{p}	5.23± 0.055^{f}	22.6± 0.424^{t}	8.17± 0.084^{i}
As100+All B	3.4± 0.056^{d}	0.27± 0.021^{a}	5.72± 0.046^{f}	1.38± 0.063^{b}	13.16± 0.063^{n}	4.15± 0.056^{e}	17.22± 0.070^{r}	6.95± 0.055^{g}	18.5± 0.424^{s}	7.22± 0.042^{h}	23.7± 0.282^{t}	9.25± 0.056^{j}
As100+All M	3.34± 0.049^{d}	0.26± 0.035^{a}	5.68± 0.035^{f}	1.58± 0.055^{b}	13.27± 0.007^{n}	4.19± 0.049^{e}	17.25± 0.053^{r}	6.97± 0.049^{g}	18.44± 0.352^{s}	7.18± 0.084^{h}	23.4± 0.424^{t}	9.28± 0.049^{j}
As100+Const.	3.25± 0.056^{d}	0.23± 0.035^{a}	5.20± 0.120^{f}	1.22± 0.007^{b}	13.54± 0.502^{n}	4.19± 0.063^{e}	16.8± 0.072^{q}	6.85± 0.056^{g}	18.4± 0.494^{s}	7.07± 0.077^{h}	22.6± 0.424^{t}	8.21± 0.113^{i}

Mean ± SD (n=3). Different small alphabets (a-s) indicate statistically significant difference at $P \leq 0.05$ by Turkey's test. (**C**: control, **As 50**: arsenic 50 mgkg^{-1}, **As 100**: arsenic 100 mgkg^{-1}, **All B**: all bacterial isolates (1-9), **All M**: All mycorrhiza cultures (1-3), **Cons**t: microbial consortium (All bacteria + all mycorrhiza), **PS1**:*Ralstonia insidiosa***, PS 2:** *Enterobacter ludwigii***, PS 3:** *Pseudomonas aeruginosa*, **PS 4:** *Cellulosomicrobium funkei*, **HX 1:** *Pseudomonas putida*, **NAP 1:** *Citrobacter freundii*, **TL 1:** *Pseudomonas plecoglossicida* **,XL 1:** *Pseudomonas fulva*,**BZ 1:** *Pseudomonas sp,* **G.c:** *Glomus claroideum***, G.h :** *Glomus* hoi**, A.k :** *Acaulospora kentinensis,* **WW**: wet weight, **DW**: dry weight).

Table 40: Effect of cadmium on fresh weight and dry weight (g) of *Canna indica* (roots and shoots) inoculated with rhizobacteria, mycorrhiza and microbial consortium.

Time duration →	1 Month				2 Month				3 Month			
Plant parts →	Root		Shoot		Root		Shoot		Root		Shoot	
Treatment ↓	WW	DW	WW	DW	WW	DW	WW	DW	WW	DW	WW	DW
Control	2.12± 0.032^{c}	0.17± 0.044^{a}	3.69± 0.116^{d}	0.44± 0.071^{a}	9.84± 0.033^{j}	1.98± 0.078^{b}	14.3± 0.051^{o}	5.11± 0.088^{f}	16.3± 0.134^{q}	5.21± 0.098^{f}	21.5± 0.234^{t}	7.03± 0.041^{h}
C+PS1	2.35± 0.049^{c}	0.24± 0.042^{a}	4.37± 0.113^{e}	0.48± 0.091^{a}	10.26± 0.035^{k}	2.46± 0.035^{c}	15.3± 0.071^{p}	5.44± 0.049^{f}	16.75± 0.212^{q}	5.16± 0.070^{f}	22.65± 0.353^{t}	8.35± 0.056^{i}
C+PS2	3.39± 0.028^{d}	0.41± 0.044^{a}	5.66± 0.077^{f}	1.82± 0.084^{b}	11.07± 1.477^{l}	3.15± 0.056^{d}	17.2± 0.056^{r}	6.19± 0.063^{g}	17.6± 0.424^{r}	6.16± 0.042^{g}	23.7± 0.282^{t}	9.18± 0.091^{j}
C+PS3	3.15± 0.056^{d}	0.25± 0.056^{a}	5.17± 0.077^{f}	1.61± 0.007^{b}	12.07± 0.091^{m}	3.06± 0.042^{d}	17.17± 0.084^{r}	6.12± 0.063^{g}	17.55± 0.494^{r}	6.07± 0.056^{g}	23.55± 0.494^{t}	9.16± 0.070^{j}
C+PS4	3.56± 0.033^{d}	0.24± 0.043^{a}	5.76± 0.008^{f}	1.85± 0.084^{b}	13.01± 0.042^{n}	4.20± 0.047^{e}	17.76± 0.042^{r}	6.04± 0.756^{g}	18.35± 0.353^{s}	7.1± 0.082^{h}	24.06± 0.073^{t}	9.08± 0.777^{j}
C+HX1	2.10± 0.036^{c}	0.15± 0.057^{a}	4.08± 0.091^{e}	0.47± 0.063^{a}	10.08± 0.070^{k}	2.07± 0.063^{c}	15.17± 0.070^{p}	5.28± 0.056^{f}	16.7± 0.141^{q}	5.14± 0.035^{f}	22.5± 0.042^{t}	8.66± 0.749^{i}
C+NAP1	2.48± 0.056^{c}	0.29± 0.077^{a}	4.81± 0.091^{e}	0.49± 0.106^{a}	10.52± 0.042^{k}	2.24± 0.046^{c}	15.8± 0.053^{p}	5.48± 0.077^{f}	16.45± 0.494^{q}	5.14± 0.014^{f}	22.78± 0.042^{t}	8.88± 0.077^{i}
C+TL1	2.11± 0.091^{c}	0.18± 0.084^{a}	3.78± 0.077^{d}	0.46± 0.088^{a}	10.12± 0.063^{k}	2.06± 0.073^{c}	14.2± 0.059^{o}	5.17± 0.071^{f}	16.35± 0.212^{q}	5.23± 0.028^{f}	21.3± 0.282^{t}	7.12± 0.120^{h}
C+XL1	3.13± 0.063^{d}	0.21± 0.056^{a}	5.38± 0.084^{f}	1.03± 0.784^{b}	10.16± 0.042^{k}	2.16± 0.070^{c}	16.37± 0.084^{q}	5.47± 0.028^{f}	16.6± 0.424^{q}	5.52± 0.049^{f}	21.8± 0.084^{t}	7.23± 0.148^{h}
C+BZ1	3.07± 0.084^{d}	0.19± 0.021^{a}	5.28± 0.056^{f}	1.75± 0.056^{b}	10.24± 0.056^{k}	2.25± 0.056^{c}	16.17± 0.091^{q}	5.40± 0.035^{f}	16.55± 0.353^{q}	5.53± 0.028^{f}	22.35± 0.056^{t}	8.07± 0.057^{i}
C+Gc	2.69± 0.120^{c}	0.28± 0.056^{a}	4.92± 0.098^{e}	0.51± 0.035^{a}	10.06± 0.077^{k}	2.15± 0.059^{c}	15.95± 0.056^{p}	5.77± 0.054^{f}	16.25± 0.212^{q}	5.16± 0.063^{f}	22.52± 0.098^{t}	8.37± 0.070^{i}
C+G.k	3.25± 0.056^{d}	0.25± 0.055^{a}	5.21± 0.106^{f}	1.65± 0.049^{b}	12.45± 0.046^{m}	3.19± 0.035^{d}	16.86± 0.073^{q}	5.88± 0.063^{f}	17.45± 0.353^{r}	6.18± 0.091^{g}	22.38± 0.049^{t}	8.17± 0.014^{i}
C+A.k	3.15± 0.057^{d}	0.32± 0.058^{a}	5.38± 0.098^{f}	1.59± 0.056^{b}	12.14± 0.045^{m}	3.16± 0.033^{d}	16.36± 0.070^{q}	5.85± 0.049^{f}	17.3± 0.383^{r}	6.26± 0.063^{g}	22.22± 0.063t	8.05± 0.056^{i}
C+All B	3.11± 0.062^{d}	0.25± 0.051^{a}	5.33± 0.063^{f}	1.58± 0.084^{b}	12.07± 0.070^{m}	3.15± 0.055^{d}	16.99± 0.021^{q}	6.07± 0.049^{g}	17.45± 0.353^{r}	7.12± 0.021^{h}	23.08± 0.049^{t}	9.17± 0.084^{j}
C+All M	3.24± 0.049^{d}	0.35± 0.054^{a}	5.49± 0.070^{f}	1.69± 0.049^{b}	12.19± 0.063^{m}	3.25± 0.056^{d}	17.12± 0.127^{r}	6.14± 0.049^{g}	17.65± 0.353^{r}	7.08± 0.042^{h}	23.25± 0.048^{t}	9.22± 0.056^{j}

C+Const	3.63± 0.028^{d}	0.45± 0.055^{a}	6.07± 0.071^{g}	1.07± 0.082^{b}	13.18± 0.042^{n}	4.25± 0.041^{e}	17.82± 0.084^{r}	6.48± 0.044^{g}	18.6± 0.424^{s}	5.25± 0.035^{f}	23.8± 0.055^{t}	9.51± 0.046^{j}
C + Cd 50	2.06± 0.045^{c}	0.27± 0.076^{a}	3.35± 0.114^{d}	0.58± 0.067^{a}	9.11± 0.076^{j}	1.48± 0.067^{b}	14.3± 0.044^{o}	4.12± 0.031^{e}	14.9± 0.231^{o}	4.81± 0.067^{e}	21.9± 0.234t	7.04± 0.118^{h}
Cd50+PS1	2.74± 0.035^{c}	0.21± 0.056^{a}	4.90± 0.120^{e}	0.61± 0.091^{a}	11.3± 0.056^{l}	2.55± 0.049^{c}	15.7± 0.056^{p}	5.4± 0.042^{f}	16.45± 0.353^{q}	5.74± 0.049^{f}	21.4± 0.494t	7.35± 0.056^{h}
Cd50+PS2	3.14± 0.049^{d}	0.25± 0.054^{a}	5.32± 0.042^{f}	1.21± 0.098^{b}	12.65± 0.055^{m}	3.14± 0.051^{d}	16.8± 0.049^{q}	5.88± 0.063^{f}	17.5± 0.282^{r}	6.25± 0.065^{g}	22.3± 0.282^{t}	8.20± 0.077^{i}
Cd50+PS3	3.31± 0.043^{d}	0.32± 0.028^{a}	5.19± 0.077^{f}	1.18± 0.106^{b}	12.63± 0.028^{m}	3.08± 0.049^{d}	16.27± 0.028^{q}	5.17± 0.070^{f}	17.7± 0.228^{r}	6.18± 0.007^{g}	22.5± 0.494t	8.18± 0.106^{i}
Cd50+PS4	2.07± 0.077^{c}	0.19± 0.077^{a}	3.28± 0.098^{d}	0.47± 0.084^{a}	9.97± 0.113^{j}	1.83± 0.077^{b}	14.23± 0.063^{o}	4.13± 0.007^{e}	15.4± 0.282^{p}	5.07± 0.071^{f}	21.6± 0.424^{t}	7.29± 0.044^{h}
Cd50+HX1	2.07± 0.056^{c}	0.28± 0.049^{a}	3.37± 0.077^{d}	0.62± 0.049^{a}	9.9± 0.155^{j}	1.55± 0.056^{b}	14.29± 0.049^{o}	4.25± 0.056^{e}	15.7± 0.243^{p}	5.1± 0.054^{f}	22.15± 1.060^{t}	7.31± 0.063^{h}
Cd50+NAP1	3.25± 0.057^{d}	0.26± 0.041^{a}	5.32± 0.063^{f}	1.35± 0.056^{b}	13.1± 0.120^{n}	4.28± 0.045^{e}	16.83± 0.063^{q}	5.87± 0.063^{f}	18.6± 0.345^{s}	7.17± 0.028^{h}	22.5± 0.141^{t}	8.09± 0.070^{i}
Cd50+TL1	3.15± 0.059^{d}	0.25± 0.052^{a}	5.23± 0.077^{f}	1.32± 0.063^{b}	12.8± 0.042^{m}	2.37± 0.028^{c}	16.86± 0.070^{q}	5.89± 0.070^{f}	17.35± 0.353^{r}	6.17± 0.073^{g}	22.35± 0.353^{t}	8.12± 0.077^{i}
Cd50+XL1	3.06± 0.070^{d}	0.15± 0.055^{a}	5.1± 0.056^{f}	1.31± 0.077^{b}	12.6± 0.042^{m}	2.15± 0.055^{c}	16.06± 0.077^{q}	5.21± 0.065^{f}	17.5± 0.424^{r}	6.17± 0.777^{g}	22.25± 0.212^{t}	8.2± 0.089^{i}
Cd50+BZ1	2.22± 0.063^{c}	0.19± 0.070^{a}	4.89± 0.120^{e}	0.48± 0.091^{a}	11.09± 0.048^{l}	2.12± 0.155^{c}	14.7± 0.070^{o}	4.58± 0.056^{e}	16.55± 0.332^{q}	5.75± 0.064^{f}	21.50± 0.572^{t}	7.2± 0.995^{h}
Cd50+G.c	2.33± 0.056^{c}	0.14± 0.028^{a}	4.92± 0.063^{e}	0.52± 0.063^{a}	11.39± 0.021^{l}	2.12± 0.120^{c}	14.85± 0.091^{o}	4.67± 0.014^{e}	16.7± 0.288^{q}	5.65± 0.043^{f}	21.74± 0.346^{t}	7.23± 0.077^{h}
Cd50+G.k	3.21± 0.049^{d}	0.25± 0.056^{a}	5.32± 0.042^{e}	1.17± 0.063^{b}	12.73± 0.007^{m}	2.27± 0.084^{c}	17.13± 0.077^{r}	6.85± 0.056^{g}	17.35± 0.353^{r}	6.12± 0.042^{g}	23.55± 0.494^{t}	9.07± 0.063^{j}
Cd50+A.k	2.70± 0.091^{c}	0.21± 0.084^{a}	5.01± 0.127^{e}	0.58± 0.056^{a}	11.26± 0.035^{l}	1.91± 0.106^{b}	16.55± 0.047^{q}	6.14± 0.049^{g}	16.7± 0.284^{r}	5.73± 0.035^{f}	22.25± 0.353^{t}	8.09± 0.084^{i}
Cd50+All B	2.09± 0.070^{c}	0.17± 0.084^{a}	3.73± 0.077^{d}	0.6± 0.084^{a}	11.05± 0.056^{l}	1.75± 0.049^{b}	14.70± 0.091^{o}	4.21± 0.028^{e}	16.25± 0.211^{r}	5.76± 0.004^{f}	21.15± 0.212^{t}	7.16± 0.070^{h}
Cd50+All M	2.11± 0.079^{c}	0.19± 0.063^{a}	4.21± 0.098^{e}	0.65± 0.065^{a}	10.8± 0.059^{k}	1.55± 0.034^{b}	15.22± 0.098^{p}	5.28± 0.049^{f}	15.5± 0.426^{p}	5.25± 0.566^{f}	21.75± 0.221^{t}	7.22± 0.077^{h}
Cd50+Const.	3.15± 0.053^{d}	0.27± 0.065^{a}	5.32± 0.091^{f}	1.53± 0.070^{b}	13.20± 0.091^{n}	3.06± 0.072^{d}	16.3± 0.028^{q}	5.89± 0.070^{f}	18.4± 0.447^{s}	7.18± 0.004^{h}	22.6± 0.424^{t}	8.08± 0.070^{i}
C + Cd 100	2.15± 0.056^{c}	0.19± 0.012^{a}	4.66± 0.056^{e}	0.71± 0.061^{a}	10.8± 0.033^{k}	1.24± 0.077^{b}	15.7± 0.017^{p}	5.19± 0.078^{f}	14.9± 0.098^{o}	5.04± 0.071^{f}	10.3± 0.019^{k}	7.94± 0.004^{h}
Cd100+PS1	3.34± 0.049^{d}	0.23± 0.035^{a}	5.67± 0.084^{f}	1.7± 0.055^{b}	13.27± 0.063^{n}	3.55± 0.046^{d}	17.17± 0.556^{r}	6.89± 0.071g	18.55± 0.494^{s}	7.29± 0.035^{h}	23.55± 0.494^{t}	9.08± 0.006^{j}

Cd100+PS2	2.16± 0.042^{c}	0.19± 0.079^{a}	4.34± 0.007^{e}	0.60± 0.035^{a}	10.35± 0.056^{k}	1.58± 0.065^{b}	15.31± 0.223^{p}	5.10± 0.106^{f}	15.55± 0.032^{p}	5.18± 0.049^{f}	21.4± 0.426^{t}	7.25± 0.069^{h}
Cd100+PS3	2.25± 0.056^{c}	0.2± 0.007^{a}	4.39± 0.070^{e}	0.57± 0.049^{a}	10.36± 0.042^{k}	1.62± 0.065^{b}	15.35± 0.049^{p}	5.28± 0.063^{f}	15.5± 0.043^{p}	5.28± 0.063^{f}	21.55± 0.335^{t}	7.35± 0.049^{h}
Cd100+PS4	2.21± 0.043^{c}	0.13± 0.035^{a}	3.32± 0.084^{d}	0.46± 0.044^{a}	10.17± 0.066^{k}	1.55± 0.067^{b}	14.33± 0.707^{o}	4.87± 0.091^{e}	15.6± 0.044^{p}	5.34± 0.049^{f}	21.45± 0.006^{t}	7.15± 0.659^{h}
Cd100+HX1	2.14± 0.075^{c}	0.20± 0.120^{a}	3.17± 0.098^{d}	0.5± 0.022^{a}	10.13± 0.063^{k}	1.11± 0.042^{b}	14.08± 0.099^{o}	4.72± 0.848^{e}	15.55± 0.353^{p}	5.46± 0.043^{f}	21.5± 0.555^{t}	7.27± 0.028^{h}
Cd100+NAP1	2.16± 0.042^{c}	0.18± 0.056^{a}	4.62± 0.084^{e}	0.65± 0.065^{a}	10.17± 0.092^{k}	1.50± 0.077^{b}	15.88± 0.097^{p}	5.25± 0.056^{f}	15.3± 0.288^{p}	5.31± 0.065^{f}	22.65± 0.212^{t}	8.2± 0.056^{i}
Cd100+TL1	3.71± 0.044^{d}	0.30± 0.092^{a}	6.16± 0.063^{g}	1.88± 0.047^{b}	13.55± 0.049^{n}	3.61± 0.063^{d}	18.16± 0.079^{s}	7.21± 0.049^{h}	18.55± 0.335^{s}	7.85± 0.065^{h}	24.3± 0.228^{t}	9.15± 0.047^{j}
Cd100+XL1	3.16± 0.073^{d}	0.26± 0.043^{a}	5.32± 0.056^{f}	1.16± 0.070^{b}	13.17± 0.070^{n}	4.35± 0.066^{e}	17.2± 0.008^{r}	6.82± 0.021^{g}	18.5± 0.283^{s}	7.58± 0.065^{h}	23.6± 0.422^{t}	9.2± 0.009^{j}
Cd100+BZ1	2.8± 0.636^{c}	0.25± 0.048^{a}	5.39± 0.077^{f}	1.22± 0.148^{b}	13.09± 0.098^{n}	4.19± 0.005^{e}	17.14± 0.091^{r}	6.13± 0.008^{g}	18.4± 0.284^{s}	7.48± 0.007^{h}	23.35± 0.353^{t}	9.2± 0.113^{j}
Cd100+G.c	2.22± 0.077^{c}	0.2± 0.072^{a}	4.58± 0.078^{e}	0.61± 0.106^{a}	10.21± 0.088^{k}	1.97± 0.092^{b}	15.2± 0.049^{p}	5.22± 0.033^{f}	15.65± 0.353^{p}	5.18± 0.056^{f}	21.83± 0.073^{t}	7.22± 0.063^{h}
Cd100+G.k	2.13± 0.056^{c}	0.18± 0.012^{a}	3.78± 0.084^{d}	0.51± 0.063^{a}	10.09± 0.045^{k}	1.81± 0.035^{b}	14.14± 0.048^{o}	4.18± 0.056^{e}	15.45± 0.555^{p}	5.09± 0.084^{f}	21.45± 0.494^{t}	7.10± 0.064^{h}
Cd100+A.k	3.26± 0.042^{d}	0.21± 0.007^{a}	5.20± 0.120^{f}	1.3± 0.028^{b}	12.54± 0.042^{m}	2.45± 0.005^{c}	17.17± 0.449^{r}	6.15± 0.048^{g}	17.3± 0.283^{r}	6.87± 0.064^{g}	23.6± 0.264^{t}	8.90± 0.021^{i}
Cd100+All B	3.61± 0.079^{d}	0.36± 0.041^{a}	5.76± 0.106^{f}	1.59± 0.072^{b}	13.7± 0.063^{n}	4.60± 0.066^{e}	17.77± 0.054^{r}	6.58± 0.567^{g}	18.75± 0.212^{s}	7.91± 0.034^{h}	23.75± 0.212^{t}	9.14± 0.049^{j}
Cd100+All M	3.48± 0.024^{d}	0.34± 0.049^{a}	5.57± 0.006^{f}	1.63± 0.084^{b}	13.38± 0.076^{n}	4.35± 0.059^{e}	17.29± 0.707^{r}	6.20± 0.043^{g}	18.5± 0.335^{s}	7.24± 0.070^{h}	23.55± 0.212^{t}	9.14± 0.711^{j}
Cd100+Const.	3.08± 0.070^{d}	0.25± 0.035^{a}	5.15± 0.087^{f}	1.3± 0.056^{b}	13.05± 0.053^{n}	4.25± 0.046^{e}	16.8± 0.032^{q}	6.85± 0.055^{g}	18.3± 0.284^{s}	7.25± 0.049^{h}	22.45± 0.553^{t}	8.16± 0.063^{i}

Mean ± SD (n=3). Different small alphabets (a-t) indicate statistically significant difference at $P \leq 0.05$ by Turkey's test. (**C**: control, **Cd 50**: cadmium 50 $mgkg^{-1}$, **Cd 100**: cadmium 100 $mgkg^{-1}$, **All B**: all bacterial isolates (1-9), **All M**: All mycorrhiza cultures (1-3), **Cons**t: consortium (All bacteria + all mycorrhiza), **PS1**:*Ralstonia insidiosa***, PS 2:** *Enterobacter ludwigii***, PS 3:** *Pseudomonas aeruginosa*, **PS 4:** *Cellulosomicrobium funkei*, **HX 1:** *Pseudomonas putida*, **NAP 1:** *Citrobacter freundii*, **TL 1:** *Pseudomonas plecoglossicida* **,XL 1:** *Pseudomonas fulva*,**BZ 1:** *Pseudomonas sp*, **G.c:** *Glomus claroideum***, G.h :** *Glomus* hoi**, A.k :** *Acaulospora kentinensis*, **WW**: wet weight, **DW**: dry weight).

6.17.2: Effect of arsenic and cadmiumon photosynthetic pigments (Chlorophyll a, Chlorophyll b, total chlorophyll and carotenoid contents) of *Ricinus communis* and *Canna indica* plants inoculated with rhizobacteria, mycorrhiza and microbial consortium.

Impacts of two dose levels (50 and 100 $mgkg^{-1}$) of arsenic and cadmium on the photosynthetic system of both the plants were studied. Chlorophyll a, Chlorophyll b, total chlorophyll and carotenoids were calculated in all the 3 months of experimentation with respect to control plants. Chlorophyll a and b content was found to be maximum in the leaves of the microbial consortium inoculated *R. communis* and *C. indica* plants whereas minimum Chl a and Chl b content was seen in mycorrhiza inoculated plants. A significant difference ($p \leq 0.05$) was observed in all the treatments compared to control plants. But the overall result showed enhanced content of chlorophyll a and b in leaves of *R. communis* than *C. indica* inoculated with microbial consortium, mycorrhiza and rhizobacteria.

Both the Chl aand b content decreased with respect to time and metal concentration (As and Cd) of control as well as treated plants. Chlorophyll b content was found to be less in comparison to Chl an in both the plants (control and treated) as given in Table 41. 18.13, 16.83, 15.70 and 5.507, 5.754, 5.819 was Chl a and Chl b content after 1st month of sampling in leaves of control and microbial consortium inoculated (arsenic 50 and 100 $mgkg^{-1}$) *R. communis* plantrespectively. Similarly, 13.85, 13.58, 13.46 and 3.314, 3.17, 2.711 was Chl a and Chl b content in leaves of control and microbial consortium inoculated *C. indica* plant under arsenic stress (50 and 100 $mgkg^{-1}$) respectively after 1st month of sampling. All the values of microbial inoculated plants in *R. communis* and *C. indica* were found to be less than control plants (with microbial treatment and without any treatment) but higher than the control inoculated with only heavy metals.In 3rd month rhizobacteria and microbial consortium inoculated *R. communis* plants displayed less Chl a and b content than *C. indica* plant. Overall Chl a and b content was found to be more in *R. communis* plants than *C. indica*plants inoculated with As and Cd along with rhizobacteria, mycorrhiza and microbial consortium. Unlike arsenic treatment, cadmium inoculated *R. communis* and *C. indica* plants showed variant content of Chl a and b, where control plants presented more content than treated plants.

As in arsenic treatment, microbial consortium inoculated both the plants displayed maximum chlorophyll content followed by rhizobacteria and mycorrhiza inoculated plants during 3 months of experimentation period. In both the metal treatments, chl a content in *C. indica* plant inoculated with rhizobacteria and mycorrhiza, was found to be more than the *R. communis* plant, except microbial consortium inoculated plants in all the 3 months of treatments. After 3rd month of sampling, microbial consortium inoculated *C. indica* plants exhibited more content of Chl a and b than *R. communis* plant (table 42). Therefore, we can conclude that some dissimilar trend

in photosynthetic pigments content (Chl and b) was observed in both the *R. communis* and *C. indica* plant with respect to time which shows the unusual effect of heavy metals on their content as compared to control (figure 60-65)

The results of the present work were in coherence with Javed Ali *et al* (2018). The inoculation of maize plants along with PGPB (*Burkholderia vietnamiensis*) and Citric acid under Cr stress resulted in significant increase in chlorophyll contents to 60 % at 500 ppm of Cr as compared to the industrial contaminated and Cr treated soil only. Even with the increase in Cr concentration, reduction in photosynthetic pigments was noticed in maize which is in accordance with previous reports (Gill *et al*, 2015) that demonstrated decrease photosynthetic pigments in various plant species with increasing Cr concentration. Hegedus *et al* (2001) explained the reason for this decrease as, both the direct and indirect factors are responsible for this like chloroplast inhibition, ultrastructure modifications and with increasing chlorophyllase activity photosynthetic pigments are decomposed (Gill *et al*., 2015). R John *et al* (2008) reported a significant decrease in Chl $_{a}$, Chl $_{b}$ and Chl $_{a+b}$ content with increasing concentration of Cd and Pb. When individual treatment was given (Cd and Pb) marginal increase in Chl a, Chl b and Chl $_{a+b}$ was observed but due to prolonged exposure to 40 mLL^{-1} of Cd (higher concentration) Chl a, Chl b and Chl $_{a+b}$ was declined after 30 days of treatment. Chlorophyll content in plants is reduced under various abiotic stresses (Ahmad *et al*. 2012).

Prasad *et al* (2001) reported the inhibition of chlorophyll biosynthesis under heavy metals stress in various plants. Our results of a decrease in chlorophyll content corroborated with the results of Siedlecka and Krupa (1996) who also found reduced chlorophyll content under heavy metals stress in *Zea mays* and *Acer rubrum*. Hence, the photosynthetic machinery is disrupted due to a loss in chlorophyll content. Another finding by Fikriye *et al* (2005), the effect of Pb, Cu, Cd and Hg on chlorophyll content of *Phaseolus vulgaris* L. (Bean) was estimated and a significant decrease in Chlorophyll content observed with increasing concentration of heavy metals. Along with Cd, many researchers have evaluated the toxic effects of As on photosynthetic pigments of various plants. Fernanda *et al* (2014) performed a similar study where the effect of As with different concentrations was seen on the growth and photosynthetic pigments of *Pistia stratiotes*. A significant decrease in growth of *P. stratiotes* was observed which can be related with the decrease in the photosynthetic pigments followed by a reduction in the photosynthetic process (Mascher *et al*., 2002). Significant reduction in photosynthetic pigments (Chl a, Chl b, chl_{a+b} and chl_{x+c}) were observed in *Pfaffia glomerata* with increasing As stress, in another study performed byGupta*et al*, 2013.

Table 41: Effect of arsenic (50 and 100 mgkg^{-1}) on Chlorophyll a and chlorophyll b content (µgmL^{-1}) in leaves of *Ricinus communis* and *Canna indica* plants inoculated with rhizobacteria, mycorrhiza and microbial consortium.

Time duration →	1st month				2nd month				3rd month			
Plants →	*R. communis*		*C. indica*		*R. communis*		*C. indica*		*R. communis*		*C. indica*	
Treatment ↓	Chl a	Chl b	Chl a	Chl b	Chl a	Chl b	Chl a	Chl b	Chl a	Chl b	Chl a	Chl b
Control	38.8± 0.034^{r}	11.9± 0.034^{l}	32.6± 0.077^{r}	17.8± 0.009^{p}	46.7± 0.045^{s}	19.9± 0.041^{q}	41.6± 0.052^{s}	17.3± 0.267^{p}	52.5± 0.062^{s}	20.3± 0.119^{q}	48.4± 0.045^{s}	19.9± 0.067^{q}
C + As 50	11.21± 0.033^{l}	3.041± 0.056^{d}	11.8± 0.155^{l}	1.814± 0.077^{b}	8.94± 0.051^{i}	1.208± 0.063^{b}	8.61± 0.072^{i}	1.214± 0.251^{b}	5.629± 0.023^{f}	0.543± 0.067^{a}	8.121± 0.034^{i}	0.341± 0.055^{a}
C + As 100	9.41± 0.056^{j}	2.008± 0.077^{c}	8.28± 0.112^{i}	1.623± 0.009^{b}	6.81± 0.056^{g}	1.187± 0.362^{b}	7.34± 0.078^{h}	1.203± 0.124^{b}	4.132± 0.056^{e}	0.419± 0.193^{a}	6.213± 0.045^{g}	0.214± 0.078^{a}
C + All B	16.93± 0.021^{p}	5.072± 0.037^{f}	13.62± 0.122^{n}	2.931± 0.006^{c}	11.73± 0.034^{l}	2.090± 0.647^{c}	11.45± 0.066^{l}	1.679± 0.257^{b}	8.278± 0.046^{i}	0.946± 0.105^{a}	9.37± 0.020^{j}	1.069± 0.091^{b}
As 50 + All B	15.97± 0.040^{o}	3.162± 0.060^{d}	13.40± 0.022^{n}	2.637± 0.076^{c}	12.51± 0.035^{m}	1.217± 0.042^{b}	12.03± 0.892^{m}	1.294± 0.314^{b}	8.184± 0.055^{i}	0.782± 0.052^{a}	9.22± 0.077^{j}	0.958± 0.089^{a}
As 100 + All B	13.87± 0.023^{n}	2.044± 0.052^{c}	13.25± 0.029^{n}	2.415± 0.041^{c}	11.57± 0.070^{l}	1.415± 0.101^{b}	11.47± 0.053^{l}	1.079± 0.223^{b}	7.035± 0.027^{h}	0.833± 0.103^{a}	9.168± 0.055^{j}	0.342± 0.077^{a}
C + All M	16.97± 0.042^{h}	4.442± 0.075^{e}	13± 0.059^{n}	2.82± 0.139^{c}	13.87± 0.024^{n}	0.842± 0.061^{a}	11.30± 0.001^{l}	1.180± 0.244^{b}	7.825± 0.030^{h}	0.687± 0.015^{a}	9.119± 0.042^{j}	0.810± 0.054^{a}
As 50 + All M	12.06± 0.030^{m}	4.697± 0.015^{e}	12.97± 0.122^{m}	2.32± 0.309^{c}	12.51± 0.035^{m}	1.484± 0.075^{b}	9.911± 0.116^{j}	1.538± 0.206^{b}	7.029± 0.039^{h}	0.612± 0.012^{a}	8.271± 0.079^{i}	1.132± 0.081^{b}
As 100 +All M	11.27± 0.014^{l}	2.912± 0.080^{c}	13.02± 0.071^{n}	1.736± 0.142^{b}	11.57± 0.070^{l}	1.145± 0.190^{b}	9.13± 0.078^{j}	1.411± 0.162^{b}	5.952± 0.055^{f}	0.877± 0.109^{a}	8.178± 0.081^{i}	0.957± 0.066^{a}
C + Const	18.13± 0.049^{q}	5.507± 0.193^{f}	13.85± 0.113^{n}	3.314± 0.122^{d}	13.68± 0.033^{n}	3.994± 0.057^{d}	12.27± 0.105^{m}	1.704± 0.071^{b}	8.621± 0.030^{i}	1.698± 0.015^{b}	10.70± 0.078^{k}	1.136± 0.081^{b}
As 50 + Const.	16.83± 0.023^{p}	5.754± 0.052^{f}	13.58± 0.113^{n}	3.17± 0.010^{d}	13.88± 0.048^{n}	1.651± 0.120^{b}	12.13± 0.018^{m}	1.556± 0.107^{b}	8.272± 0.037^{i}	1.752± 0.027^{b}	10.60± 0.072^{k}	0.964± 0.070^{a}
As 100 +Const.	15.70± 0.011^{o}	5.819± 0.038^{f}	13.46± 0.057^{n}	2.711± 0.254^{c}	12.66± 0.056^{m}	2.050± 0.005^{c}	11.56± 0.070^{l}	1.458± 0.101^{b}	8.088± 0.031^{i}	1.473± 0.073^{b}	10.39± 0.035^{k}	0.748± 0.229^{a}

Mean ± SD (n=3). Different small alphabets (a-s) indicate statistically significant difference at $P \leq 0.05$ by Turkey's test. (**C:** control, **As 50**: arsenic 50 mgkg^{-1}, **As 100**: arsenic 100 mgkg^{-1}, **All B**: all bacterial isolates (1-9), **All M**: All mycorrhiza cultures (1-3), **Const:** microbial consortium (All bacteria + all mycorrhiza), **Chl a**: Chlorophyll a, **Chl b**: Chlorophyll b).

Table 42: Effect of cadmium (50 and 100 mgkg^{-1}) on Chlorophyll a and chlorophyll b content (µgmL^{-1}) in leaves of *Ricinus communis* and *Canna indica* plants inoculated with rhizobacteria, mycorrhiza and microbial consortium.

Time duration →	1st month				2nd month				3rd month			
Plants →	*R. communis*		*C. indica*		*R. communis*		*C. indica*		*R. communis*		*C. indica*	
Treatment ↓	Chl a	Chl b	Chl a	Chl b	Chl a	Chl b	Chl a	Chl b	Chl a	Chl b	Chl a	Chl b
Control	38.8± 0.034^{o}	11.9± 0.034^{j}	32.6± 0.077^{0}	17.8± 0.009^{m}	46.7± 0.045^{p}	19.9± 0.041^{n}	41.6± 0.052^{p}	17.3± 0.267^{m}	52.5± 0.062^{q}	20.3± 0.119^{n}	48.4± 0.045^{p}	19.9± 0.067^{n}
C + Cd 50	10.9± 0.045^{i}	2.149± 0.078^{c}	9.91± 0.088^{i}	1.213± 0.004^{b}	7.43± 0.023^{g}	0.874± 0.061^{a}	6.43± 0.035^{f}	1.113± 0.143^{b}	4.419± 0.035^{d}	0.203± 0.115^{a}	7.132± 0.047^{g}	0.321± 0.047^{a}
C + Cd 100	8.74± 0.034^{h}	2.001± 0.025^{c}	7.61± 0.111^{g}	1.014± 0.004^{b}	5.32± 0.023^{e}	0.763± 0.235^{a}	5.18± 0.055^{e}	1.102± 0.234^{b}	4.021± 0.452^{d}	0.189± 0.334^{a}	5.113± 0.234^{e}	0.208± 0.062^{a}
C + All B	16.93± 0.021^{m}	5.072± 0.037^{e}	13.62± 0.122^{l}	2.931± 0.006^{c}	11.73± 0.034^{j}	2.090± 0.647^{c}	11.45± 0.066^{j}	1.679± 0.257^{b}	8.278± 0.046^{h}	0.946± 0.105^{d}	9.375± 0.020^{i}	1.069± 0.091^{b}
Cd50 + All B	12.27± 0.013^{k}	5.608± 0.023^{e}	13.26± 0.091^{l}	2.410± 0.009^{c}	11.43± 0.031^{j}	1.736± 0.056^{b}	11.60± 0.087^{j}	1.341± 0.108^{b}	6.912± 0.065^{f}	0.671± 0.128^{a}	9.126± 0.129^{i}	0.664± 0.240^{a}
Cd 100 + All B	11.44± 0.038^{j}	3.414± 0.044^{d}	13.31± 0.074^{l}	1.554± 0.261^{b}	11.47± 0.038^{j}	1.529± 0.044^{b}	11.21± 0.100^{j}	1.207± 0.329^{b}	6.870± 0.065^{f}	0.251± 0.084^{a}	8.555± 0.086^{h}	0.780± 0.093^{a}
C + All M	16.97± 0.042^{m}	4.442± 0.075^{d}	13± 0.059^{l}	2.82± 0.139^{c}	13.87± 0.024^{l}	0.842± 0.061^{a}	11.30± 0.001^{j}	1.180± 0.244^{b}	7.825± 0.030^{g}	0.687± 0.015^{a}	9.119± 0.042^{i}	0.810± 0.054^{a}
Cd 50 + All M	10.97± 0.057^{i}	6.871± 0.067^{f}	12.95± 0.097^{k}	2.25± 0.184^{c}	10.33± 0.021^{i}	1.594± 0.037^{b}	9.137± 0.041^{i}	1.341± 0.132^{b}	7.177± 0.022^{e}	0.143± 0.076^{a}	8.333± 0.098^{h}	0.791± 0.002^{a}
Cd 100 +All M	11.02± 0.038^{j}	4.726± 0.044^{d}	13.04± 0.164^{l}	1.391± 0.571^{b}	10.27± 0.037^{i}	0.910± 0.102^{a}	8.892± 0.164^{h}	1.352± 0.061^{b}	5.814± 0.049^{h}	0.151± 0.063^{a}	8.139± 0.038^{h}	0.770± 0.084^{a}
C + Const	18.13± 0.049^{n}	5.507± 0.193^{e}	13.85± 0.122^{l}	3.314± 0.122^{d}	13.68± 0.033^{l}	3.994± 0.057^{d}	12.27± 0.105^{k}	1.704± 0.071^{b}	8.621± 0.030^{h}	1.698± 0.015^{b}	10.70± 0.079^{i}	1.136± 0.081^{b}
Cd 50 + Const.	16.80± 0.027^{m}	6.762± 0.083^{f}	13.04± 0.643^{l}	2.941± 3.009^{c}	13.57± 0.020^{l}	3.816± 0.091^{d}	11.51± 0.044^{j}	1.541± 0.090^{b}	8.537± 0.085^{h}	1.632± 0.150^{b}	10.26± 0.024^{i}	0.945± 0.061^{a}
Cd 100+Const.	16.70± 0.020^{m}	6.924± 0.034^{f}	13.31± 0.022^{l}	2.427± 0.019^{c}	13.42± 0.023^{l}	3.899± 0.052^{d}	11.45± 0.071^{j}	1.415± 0.173^{b}	8.366± 0.039^{h}	0.848± 0.012^{a}	10.21± 0.004^{i}	0.698± 0.156^{a}

Mean ± SD (n=3) Different small alphabets (a-q) indicate statistically significant difference at $P \leq 0.05$ by Turkey's test. (**C**: control, **Cd 50**: cadmium 50 mgkg^{-1}, **Cd 100**: cadmium 100 mgkg^{-1}, **All B:** all bacterial isolates (1-9), **All M:** All mycorrhiza cultures (1-3), **Const:** microbial consortium (All bacteria + all mycorrhiza), **Chl a**: Chlorophyll a, **Chl b**: Chlorophyll b).

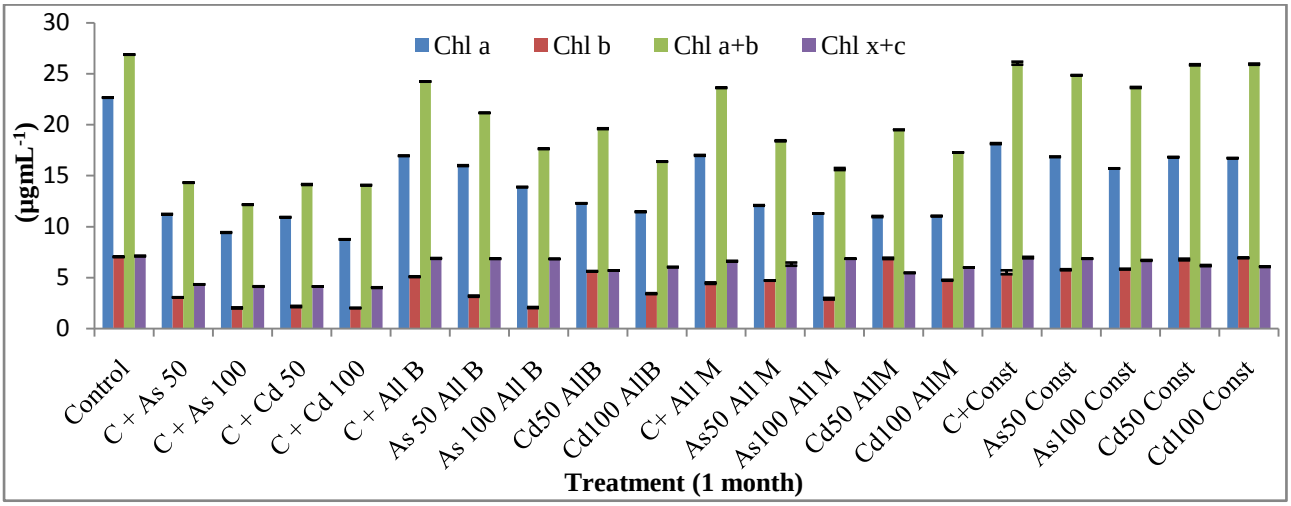

Figure 60: Effect of arsenic and cadmium (50 and 100 mgkg^{-1}) on Chlorophyll a, chlorophyll b,total chlorophyll and carotenoid content (µgmL^{-1}) in leaves of *Ricinus communis* inoculated with rhizobacteria, mycorrhiza and microbial consortium after 1 month.

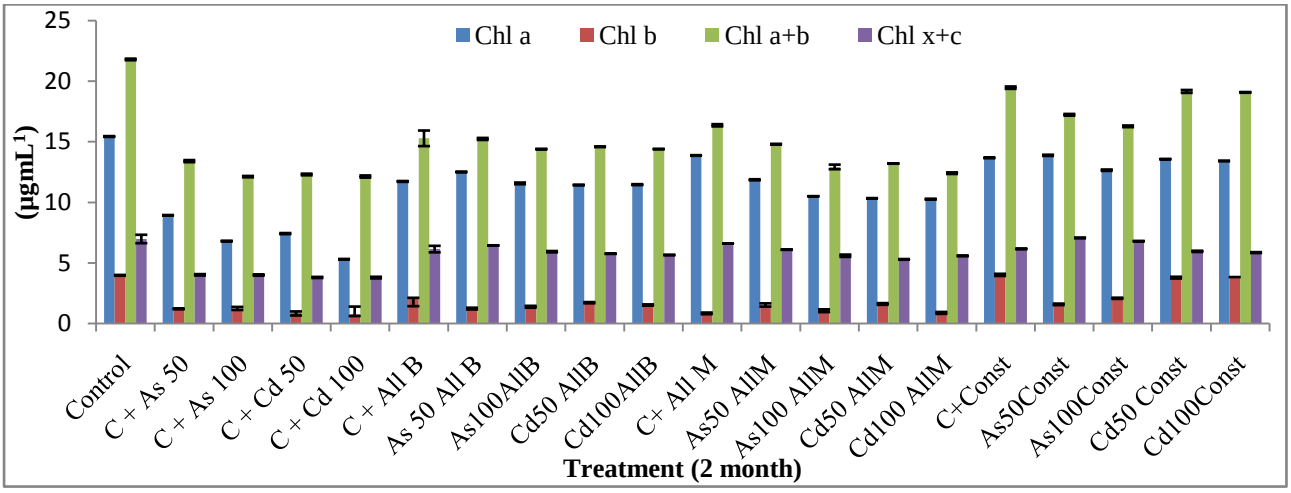

Figure 61: Effect of arsenic and cadmium (50 and 100 mgkg^{-1}) on Chlorophyll a, chlorophyll b, total chlorophyll and carotenoid content (µgmL^{-1}) in leaves of *Ricinus communis* inoculated with rhizobacteria, mycorrhiza and microbial consortium after 2 months.

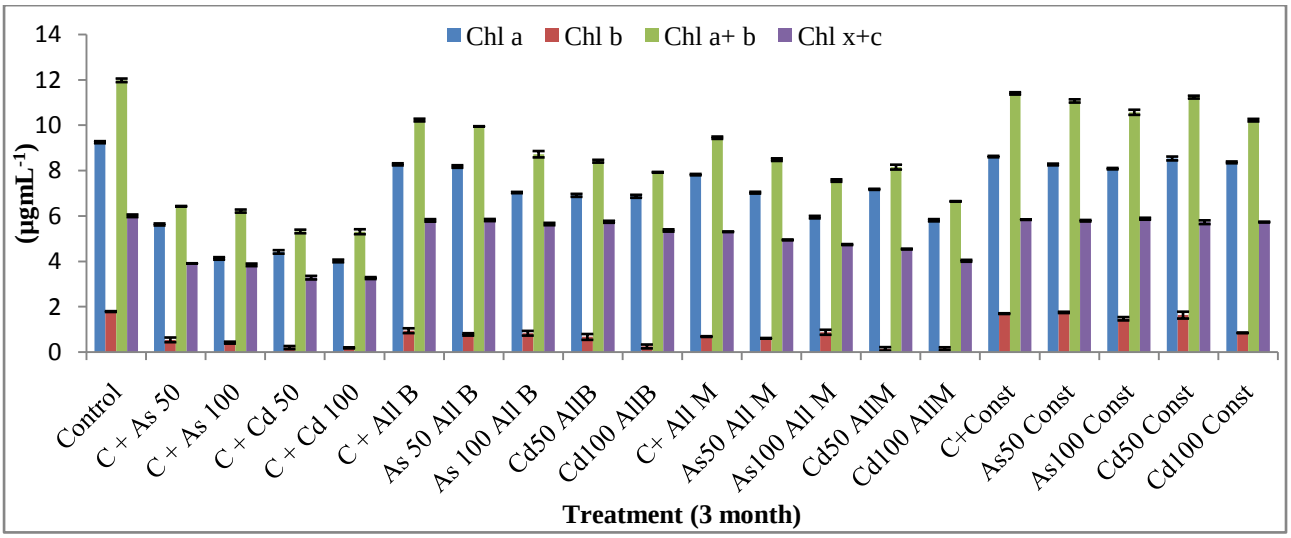

Figure 62: Effect of arsenic and cadmium (50 and 100 mg kg^{-1}) on Chlorophyll a, chlorophyll b, total chlorophyll and carotenoid content (µgmL^{-1}) in leaves of *Ricinus communis* inoculated with rhizobacteria, mycorrhiza and microbial consortium after 3months.

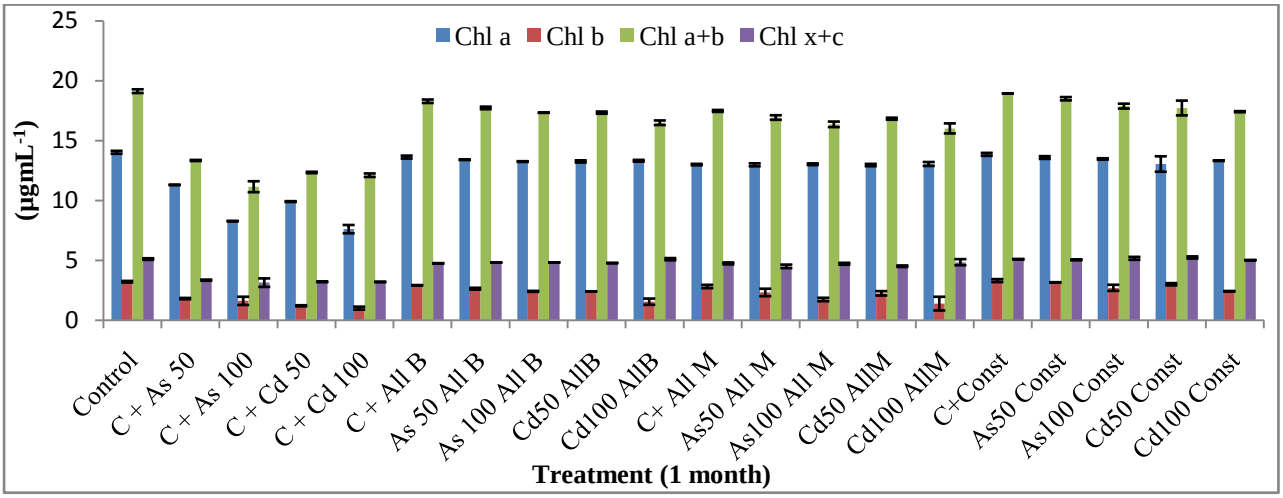

Figure 63: Effect of arsenic and cadmium (50 and 100 mgkg^{-1}) on Chlorophyll a, chlorophyll b, total chlorophyll and carotenoid content (µgmL^{-1}) in leaves of *Canna indica* inoculated with rhizobacteria, mycorrhiza and microbial consortium after 1 month.

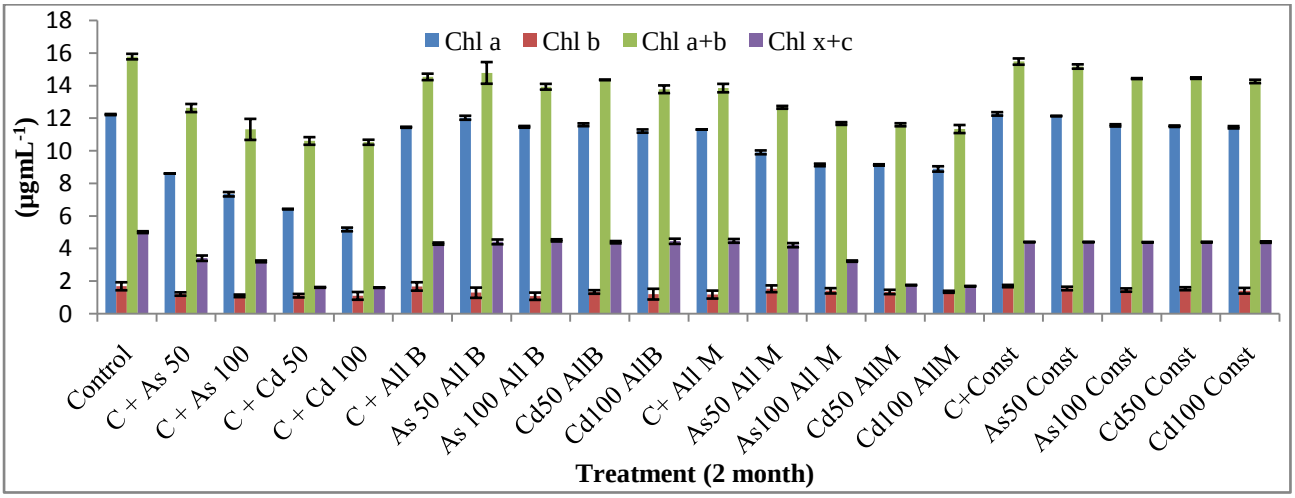

Figure 64: Effect of arsenic and cadmium (50 and 100 mg kg^{-1}) on Chlorophyll a, chlorophyll b, total chlorophyll and carotenoid content (µgmL^{-1}) in leaves of *Canna indica* inoculated with rhizobacteria, mycorrhiza and consortium after 2months.

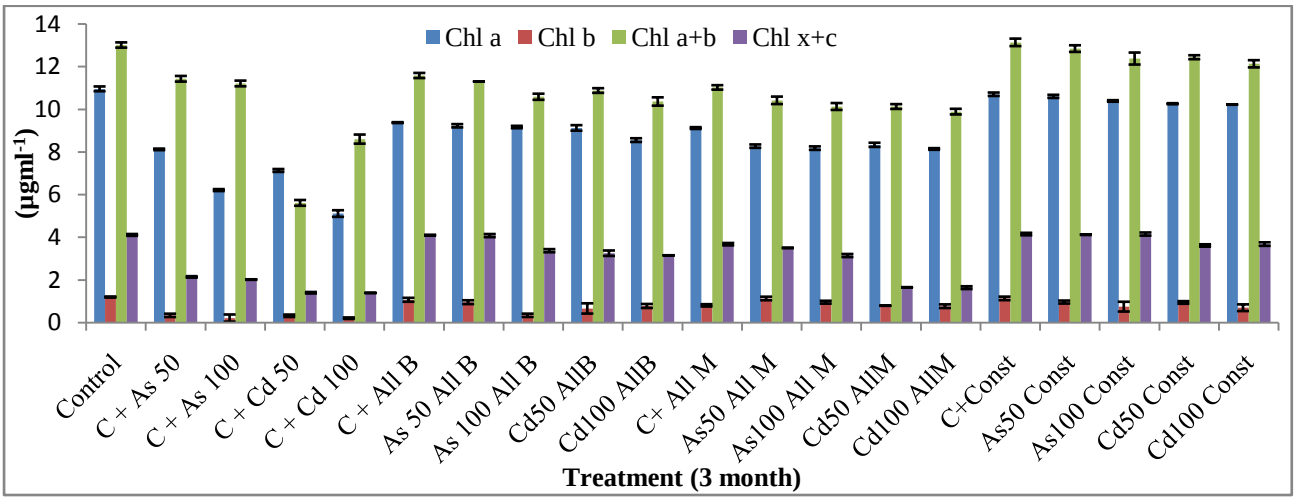

Figure 65: Effect of arsenic and cadmium (50 and 100 mg kg^{-1}) on Chlorophyll a, chlorophyll b, total chlorophyll and carotenoid content (µgmL^{-1}) in leaves of *Canna indica* inoculated with rhizobacteria, mycorrhiza and microbial consortium after 3months.

Total chlorophyll and Carotenoid content of *R. communis* and *C. indica* were also adversely affected by the heavy metals (arsenic and cadmium). The photosynthetic pigments were determined quantitatively from the control and treated plants using formulas (absorption coefficient) at a specific wavelength. The obtained results are represented in the Tables 41-44.

Total chlorophyll and Carotenoids were observed in an almost similar trend in both the plants as Chl and b, where inoculated plants possessed less content as compared to control plants. Total chlorophyll content was maximum in the microbial consortium inoculated plants of *R. communis* and *C. indica* followed by rhizobacteria and mycorrhiza as given in Table 44-45**.** *R. communis* showed more total chlorophyll content in 1^{st} and 2^{nd} month as compared to *C. indica* but after 3^{rd} month *C. indica* depicted higher total chlorophyll content than *R. communis* in all the inoculated plants. Carotenoids were found to be less as compared to Chl $_{a+b}$ in both the plants. With increasing concentration of metals, both the content (Chl $_{a+b}$ and Chl_{x+c}) decreased with respect to time (figure 60-65).

A similar pattern of results was seen in plants under cadmium stress (50 and 100 $mgkg^{-1}$), where maximum Chl $_{a+b}$ and C_{x+c}content in both the plants were found in microbial consortium inoculated plants and minimum in mycorrhiza inoculated plants. In plants treated with cadmium and microbes, more Chl $_{a+b}$ and $C_{x+c}$was found in *R. communis* in comparison to *C. indica*. Therefore, the results of photosynthetic pigments can be related to, a decrease in their contents under heavy metal stress (As and Cd). With increasing concentration of heavy metals, the photosynthetic pigments showeda decline in their content.

According to Farnese*et al* (2014), carotenoids are considered as a part of photosynthetic pigments that plays a major role in the protection of chlorophyll pigment under stress conditions. Hence these carotenoids are even involved in the quenching of photodynamic reactions that leads to loss of chlorophylls, replacement of peroxidation and avoiding the collapse of membranes in chloroplasts. According to a report given by Vajpayee *et al* (2001), Carotenoid content was increased in response to the pollutant in *Pistia stratiotes*. But in the present study, an increase in Carotenoid content was not noticed which accounts for severe damage caused by As concentrations with a prolonged exposure to heavy metals or metalloids.

Therefore this study was found to be in agreement with the present work, where a decrease in Carotenoid content with increasing concentrations of As and Cd was seen in both the plants (*R. communis* and *C. indica*) as compared to the control plants.

Table 43: Effect of arsenic (50 and 100 mgkg^{-1}) on total chlorophyll and carotenoids content (µgmL^{-1}) in *Ricinus communis* and *Canna indica* plants inoculated with rhizobacteria, mycorrhiza and microbial consortium.

Time duration →	1st month				2nd month				3rd month			
Plants →	*R. communis*		*C. indica*		*R. communis*		*C. indica*		*R. communis*		*C. indica*	
Treatment ↓	Chl a+b	C_{x+c}	Chl a+b	C_{x+c}	Chl a+b	C_{x+c}	Chl a+b	C_{x+c}	Chl a+b	C_{x+c}	Chl a+b	C_{x+c}
Control	34.81± 0.023^{o}	12.34± 0.034^{k}	29.44± 0.044^{n}	11.53± 0.008^{j}	48.91± 0.012^{p}	14.68± 0.003^{l}	44.42± 0.223^{p}	12.08± 0.067^{k}	54.82± 0.089^{q}	16.93± 0.059^{l}	51.31± 0.045^{q}	14.38± 0.065^{l}
C + As 50	14.31± 0.013^{l}	4.321± 0.065^{c}	13.34± 0.112^{l}	3.349± 0.045^{b}	13.41± 0.345^{l}	4.031± 0.453^{c}	12.63± 0.234^{j}	3.413± 0.061^{b}	6.431± 0.056^{e}	3.913± 0.027^{b}	11.43± 0.124^{j}	2.143± 0.045^{a}
C + As 100	12.14± 0.034^{j}	4.113± 0.056^{c}	11.15± 0.112^{j}	3.143± 0.056^{b}	13.13± 0.234^{k}	4.013± 0.345^{c}	11.32± 0.351^{j}	3.219± 0.351^{b}	6.219± 0.045^{e}	3.849± 0.059^{b}	11.21± 0.551^{j}	2.019± 0.057^{a}
C + All B	24.23± 0.015^{n}	6.865± 0.037^{e}	18.28± 0.143^{m}	4.749± 0.020^{c}	15.29± 0.643^{l}	6.153± 0.266^{e}	14.54± 0.197^{l}	4.309± 0.067^{c}	10.23± 0.059^{i}	5.807± 0.055^{d}	11.58± 0.120^{i}	4.098± 0.021^{c}
As 50 + All B	21.15± 0.018^{n}	6.836± 0.021^{e}	17.72± 0.106^{m}	4.825± 0.011^{c}	15.23± 0.084^{l}	6.453± 0.011^{e}	14.78± 0.664^{l}	4.416± 0.144^{c}	9.952± 0.007^{h}	5.823± 0.046^{d}	11.30± 0.008^{j}	4.079± 0.073^{c}
As 100+All B	17.62± 0.029^{m}	6.817± 0.001^{e}	17.32± 0.010^{m}	4.830± 0.003^{c}	14.39± 0.028^{l}	5.939± 0.053^{d}	13.93± 0.175^{l}	4.506± 0.066^{c}	8.725± 0.139^{g}	5.648± 0.051^{d}	10.58± 0.143^{i}	3.372± 0.073^{b}
C + All M	23.61± 0.031^{n}	6.590± 0.042^{e}	17.47± 0.081^{m}	4.748± 0.080^{c}	16.36± 0.092^{l}	6.606± 0.001^{e}	13.85± 0.258^{l}	4.477± 0.115^{c}	9.452± 0.051^{h}	5.301± 0.003^{d}	11.02± 0.104^{j}	3.676± 0.055^{b}
As 50 +All M	18.40± 0.051^{m}	6.306± 0.157^{e}	16.92± 0.190^{l}	4.503± 0.146^{c}	14.80± 0.031^{l}	6.101± 0.010^{e}	12.67± 0.087^{k}	4.215± 0.127^{c}	8.485± 0.056^{g}	4.947± 0.019^{c}	10.42± 0.175^{i}	3.500± 0.010^{b}
As100 +All M	15.64± 0.100^{l}	6.840± 0.009^{e}	16.35± 0.230^{l}	4.718± 0.079^{c}	12.93± 0.191^{k}	5.603± 0.095^{d}	11.68± 0.083^{j}	3.23± 0.040^{b}	7.563± 0.053^{f}	4.742± 0.021^{c}	10.13± 0.161^{i}	3.144± 0.072^{b}
C + Const	26.02± 0.148^{n}	6.950± 0.071^{e}	18.94± 0.007^{m}	5.093± 0.026^{d}	19.47± 0.098^{m}	6.171± 0.035^{e}	15.48± 0.192^{l}	4.405± 0.007^{c}	11.40± 0.051^{j}	5.837± 0.007^{d}	13.13± 0.175^{l}	4.151± 0.057^{c}
As 50+ Const.	24.83± 0.029^{n}	6.836± 0.004^{e}	18.49± 0.137^{m}	5.049± 0.041^{d}	17.23± 0.073^{m}	7.074± 0.033^{f}	15.17± 0.133^{l}	4.404± 0.014^{c}	11.07± 0.070j	5.792± 0.033^{d}	12.84± 0.155^{k}	4.11± 0.011^{c}
As 100+Const.	23.64± 0.053^{n}	6.672± 0.041^{e}	17.87± 0.203^{m}	5.171± 0.122^{d}	16.28± 0.068^{l}	6.808± 0.020e	14.43± 0.028^{l}	4.391± 0.017^{c}	10.57± 0.112^{i}	5.881± 0.042^{d}	12.37± 0.281^{k}	4.151± 0.073^{c}

Mean ± SD (n=3) Different small alphabets (a-q) indicate statistically significant difference at $P \leq 0.05$ by Turkey's test. (**C**: control, **As 50**: arsenic 50 mgkg^{-1}, **As 100**: arsenic 100 mgkg^{-1}, **All B:** all bacterial isolates (1-9), **All M**: All mycorrhiza cultures (1-3), **Const:** microbial consortium (All bacteria + all mycorrhiza), **Chl a+b**: total chlorophyll, **C_{x+c}:** carotenoids).

Table 44: Effect of cadmium (50 and 100 mgkg^{-1}) on Total chlorophyll and carotenoids content (µgmL^{-1}) in *Ricinus communis* and *Canna indica* plants inoculated with rhizobacteria, mycorrhiza and microbial consortium.

Time duration →	1st month				2nd month				3rd month			
Plants →	*R. communis*		*C. indica*		*R. communis*		*C. indica*		*R. communis*		*C. indica*	
Treatment ↓	Chl a+b	C_{x+c}	Chl a+b	C_{x+c}	Chl a+b	C_{x+c}	Chl a+b	C_{x+c}	Chl a+b	C_{x+c}	Chla+b	C_{x+c}
Control	34.81± 0.023^{m}	12.34± 0.034^{h}	29.44± 0.044^{l}	11.53± 0.008^{g}	48.91± 0.012^{n}	14.68± 0.003^{i}	44.42± 0.223^{n}	12.08± 0.067^{h}	54.82± 0.089^{o}	16.93± 0.059^{j}	51.31± 0.045^{o}	14.38± 0.065^{i}
C + Cd 50	14.12± 0.056^{i}	4.113± 0.034^{c}	12.34± 0.113^{h}	3.214± 0.034^{b}	12.31± 0.034^{h}	3.813± 0.123^{b}	10.16± 0.023^{g}	1.621± 0.045^{a}	5.321± 0.045^{d}	3.284± 0.037^{b}	8.613± 0.058^{f}	1.401± 0.049^{a}
C + Cd 100	14.06± 0.009^{i}	4.004± 0.045^{c}	12.11± 0.012^{h}	3.201± 0.058^{b}	12.14± 0.234^{h}	3.802± 0.011^{b}	10.53± 0.145^{g}	1.604± 0.056^{a}	5.309± 0.012^{d}	3.266± 0.034^{b}	8.602± 0.187^{f}	1.392± 0.045^{a}
C + All B	24.23± 0.015^{l}	6.865± 0.037^{e}	18.28± 0.143^{j}	4.749± 0.020^{c}	15.29± 0.643^{i}	6.153± 0.266^{e}	14.54± 0.197^{i}	4.309± 0.067^{c}	10.23± 0.059^{g}	5.807± 0.055^{d}	11.58± 0.120^{g}	4.098± 0.021^{c}
Cd 50 + All B	19.59± 0.039^{k}	5.678± 0.007^{d}	17.33± 0.092^{j}	4.779± 0.007^{c}	14.58± 0.023^{i}	5.768± 0.013^{d}	14.35± 0.016^{i}	4.395± 0.070^{c}	8.416± 0.061^{f}	5.746± 0.043^{g}	10.87± 0.109^{g}	3.254± 0.127^{b}
Cd100 +All B	16.36± 0.004^{j}	6.018± 0.029^{e}	16.48± 0.191^{j}	5.101± 0.093^{d}	14.40± 0.004^{i}	5.664± 0.008^{e}	13.78± 0.235^{h}	4.451± 0.158^{c}	7.927± 0.016^{f}	5.364± 0.050^{g}	10.36± 0.194^{g}	3.151± 0.005^{b}
C + All M	23.61± 0.031^{l}	6.590± 0.042^{e}	17.47± 0.081^{j}	4.748± 0.080^{c}	16.36± 0.092^{j}	6.606± 0.001^{e}	13.85± 0.258^{h}	4.477± 0.115^{c}	9.452± 0.051^{f}	5.301± 0.003^{g}	11.02± 0.104^{g}	3.676± 0.055^{b}
Cd 50 +All M	19.48± 0.006^{j}	5.446± 0.003^{d}	16.82± 0.084^{j}	4.514± 0.064^{c}	13.21± 0.015^{h}	5.303± 0.026^{d}	11.60± 0.092^{g}	1.764± 0.006^{a}	8.157± 0.106^{f}	4.543± 0.018^{g}	10.12± 0.111^{g}	1.654± 0.009^{a}
Cd100 +All M	17.27± 0.004^{j}	5.980± 0.002^{d}	16.01± 0.419^{j}	4.850± 0.254^{c}	12.41± 0.065^{h}	5.592± 0.032^{d}	11.34± 0.247^{g}	1.696± 0.015^{a}	6.645± 0.012^{e}	4.027± 0.041^{g}	9.89± 0.131^{f}	1.639± 0.065^{a}
C + Const	26.02± 0.148^{l}	6.950± 0.071^{e}	18.94± 0.007^{j}	5.093± 0.026^{d}	19.47± 0.098^{k}	6.171± 0.036^{e}	15.48± 0.192^{i}	4.405± 0.007^{c}	11.40± 0.051^{g}	5.837± 0.007^{g}	13.13± 0.175^{h}	4.151± 0.057^{c}
Cd 50+Const.	25.86± 0.057^{l}	6.164± 0.056^{e}	17.72± 0.617^{j}	5.247± 0.088^{d}	19.15± 0.120^{k}	5.966± 0.050^{d}	14.46± 0.045^{i}	4.399± 0.024^{c}	11.24± 0.063^{g}	5.728± 0.081^{g}	12.43± 0.092^{h}	3.616± 0.055^{b}
Cd 100+Const.	25.93± 0.059^{l}	6.042± 0.004^{e}	17.41± 0.045^{j}	5.014± 0.026^{d}	19.07± 0.029^{k}	5.863± 0.036^{d}	14.26± 0.102^{i}	4.403± 0.045^{c}	10.22± 0.056^{g}	5.732± 0.019^{f}	12.13± 0.169^{h}	3.681± 0.081^{b}

Mean ± SD (n=3) Different small alphabets (a-o) indicate statistically significant difference at $P \leq 0.05$ by Turkey's test. (**C**: control, **Cd 50**: cadmium 50 mgkg^{-1}, **Cd 100:** cadmium 100 mgkg^{-1}, **All B:** all bacterial isolates (1-9), **All M:** All mycorrhiza cultures (1-3), **Const:** microbial consortium (All bacteria + all mycorrhiza), **Chl a+b**: total chlorophyll, **C_{x+c}:** carotenoids).

6.18 Evaluating the efficacy of microbial consortium incorporated with selected plants.

Final objective includes the evaluation of microbial consortium, rhizobacteria and mycorrhiza inoculated with two different concentrations of heavy metals (As and Cd 50 and 100 $mgkg^{-1}$).Their efficiency was observed after ICP-OES analysis of soil as well as plant parts after every month sampling. Along with heavy metal analysis, various other phytoremediation parameters were calculated by which phytoextraction capability of *R. communis* and *C. indica* was evaluated.

6.18.1 Arsenic and cadmium accumulation in *Ricinus communis* and *Canna indica* (roots and shoots) inoculated with Rhizobacteria, Mycorrhiza and microbial consortium.

In general, there was an increase in metal accumulation in roots, leaves and stem of *R. communis* when metal concentration and exposure time were increased. Accumulation of arsenic was found to be more in roots of both the plants than leaves and stem, with increasing concentration of metal. Roots of *R. communis* accumulated 33.3, 37.85 and 41.65 $mgkg^{-1}$ DW of As (50 $mgkg^{-1}$) in rhizobacteria, mycorrhiza and microbial consortium inoculated plants respectively. Whereas, control plants showed less accumulation of arsenic and cadmium as shown in figure 66-72. With 100 $mgkg^{-1}$ of arsenic treatment at different time intervals (1-3 months), accumulation of As in *R. communis* increased significantly ($p \leq 0.05$) in order stem< leaves<roots. After 3rd month, in roots of *R. communis* maximum accumulation (195.1 $mgkg^{-1}$) was observed at 100 $mgkg^{-1}$ of arsenic and microbial consortium inoculated plants. At 50 $mgkg^{-1}$, arsenic accumulation was 19.2, 23.55, 27.15 and 12.15, 15.35, 19.3 in leaves and stem of *R. communis* plant treated with rhizobacteria, mycorrhiza and microbial consortium respectively after the1st month (table 45). At a concentration of 100 $mgkg^{-1}$ arsenic accumulation in leaves (46.15, 51.8, 64.95), stem (20.8, 27.1, 30.35) and root (63.35, 66.3, 74.35) of rhizobacteria, mycorrhiza and microbial consortium inoculated *R. communis* plants were observed to be more as compared to lower concentration (50 $mgkg^{-1}$).

Like arsenic, Cd accumulation in roots, leaves and stem also progressed with increasing level of Cd treatment and plant growth. Though Cd accumulation and its distribution in parts of *R communis* plant were quite same as As but Cd accumulation was observed to be more than As accumulation in different plant parts. The scheme of Cd distribution in *R. communis* plant was as follows: stem < leaves < roots. The highest Cd accumulation was observed after the3rd month of sampling in roots at 100 $mgkg^{-1}$ Cd and microbial consortium inoculated plants (table 46). After 3rd month of sampling maximum accumulation was noticed in microbial consortium inoculated *R. communis* plants with 100 $mgkg^{-1}$ Cd (196 $mgkg^{-1}$) followed by leaves (164.95 $mgkg^{-1}$) and stem (90.05 $mgkg^{-1}$). The overall range of Cd accumulation in all the pots after the 3rd month was

observed to be in order stem <leaves < roots with a metal concentration of 64-165 $mgkg^{-1}$ in leaves, 132-196 $mgkg^{-1}$ in roots and 35-90 $mgkg^{-1}$ in the stem as given in table 46.

Thus the analysis of this study revealed a very good Cd translocation from roots to shoots, in leaves and stem of *R. communis* after the 3rd month of cultivation under Cd and As stress. Similarly, the concentration of As and Cd metal in roots and shoots of rhizobacteria, mycorrhiza and microbial consortium inoculated *C. indica* plants are given in table 45 and 46. The results showed that *C. indica* accumulated more As and Cd in roots than *R. communis.* The overall range of As and Cd accumulation in roots of microbial consortium inoculated *C. indica* plants were observed to be 126-205 $mgkg^{-1}$ and 130-211 $mgkg^{-1}$ respectively after the 3rd month of sampling. With increasing concentration of both the metals, the *C. indica* plant accumulated more metals from the soil in its parts in an order of roots> shoots. But at 100 $mgkg^{-1}$ concentration of As and Cd, maximum accumulation was observed in shoots after all the 3 months of sampling i.e 92.85, 106.8, 198 and 95, 116.9, 198 mg kg^{-1} in the microbial consortium (As and Cd) inoculated plants of *C. indica* respectively (table 45 and 46).

Therefore, the influence of this study is that at higher concentration of As and Cd, *C indica* accumulated maximum heavy metals in its roots than shoots in association with the microbial consortium, followed by mycorrhiza and rhizobacteria. Hence, the microbial consortium was proved to be very effective in enhancing the hyperaccumulation capability of both the plants, to accumulate metals from the soil in different parts with respect to time and concentration. Also from the Tables 45-48, we have observed that as the concentration of metals (As and Cd) in the parts of the plants increases with time, it is significantly ($p \leq 0.05$) decreased in the soil simultaneously. Hence, we can say that the effective accumulation of heavy metals in plant parts was seen in all the 3 months of experimentation period in *R. communis* and *C indica* (figure 66-72).

Innumerable research has been accomplished by various researchers which contributed to the fact that heavy metals are accumulated by the hyperaccumulator plants either in the roots or in the aerial parts. But if these plants are further inoculated with rhizospheric microbes, the efficacy of these plants to accumulate or uptake heavy metals or contaminants from the soil is enhanced. Therefore, many studies support the present work in this aspect of metal accumulation by plant parts. According to Mukherjee*et al* (2018), endophytic bacterial consortium helps to enhance the As phytoremediation potential of *Solanum nigrum* in association with *Lantana camara* as a host plant. Seven endophytic bacteria were isolated from the rhizosphere of plants grown in contaminated areas. *L. camara* was used as the host plant along with *S. nigrum* which resulted in increased growth as well as phytoremediation potential of *S. nigrum* to accumulate As. Another study was conducted by Ali *et al* (2018) to demonstrate the effect of plant growth promoting

endophyte (*Burkholderia vietnamiensi*s) along with citric acid (CA) in Cr phytoremediation by *Zea mays* L. (maize). The results showed an enhanced phytoextraction potential of the plant in the presence of combined treatment of endophyte and Cd where 50 % increase in Cr bioaccumulation by maize was seen. Therefore, the capability of plant growth promoting bacteria to accelerate the heavy metal uptake by plants either directly or indirectly through various mechanisms was well explained by Glick (2010).

In a study conducted by M. Rajkumar *et al* (2008) almost identical results were noticed. The effect of plant growth promoting bacteria on growth and heavy metal accumulation by *R. communis* was demonstrated. As per the readings, two PGPB (*Pseudomonas sp.* and *Pseudomonas jessenii*) strains showed good hyperaccumulation capabilities of different heavy metals (Ni, Cu and Zn) in combined as well as single treatments. Apart from rhizobacteria, arbuscular mycorrhizal fungi are also well studied for the enhancement in phytoremediation capabilities. Hence, Schneider *et al* (2016) conducted an experiment to check the diversity of A.M fungi in heavy metal contaminated soil and 39 AMF species were isolated from the Rhizosphere of different plants grown in that area (*Vitiveria zizanoides, Pteris vitata, Pteridium aquilinum* and *R. communis).* Hence the result suggests the dominance of AMF diversity in the areas with elevated levels of Pb in the soil. Taking into account the hyperaccumulating potential of plants, *R. communis* and *C. indica* has shown tremendous results in the present study which can be further related to many other reports.

The phytoextraction potential of both these plants can be similar studies conducted by various researchers. A study was conducted by R. Andreazza *et al* (2013) to check the potential of *R. communis* for phytoremediating copper contaminated soil. Findings of the study indicated that *R. communis* is well-suited candidate for efficiently hyperaccumulating Cu contaminated soil with phytoaccumulation value of 2805 g ha^{-1}. Effect of some inorganic and organic amendments was also noticed on the Cd bio-accumulation potential of *Brassica juncea* and *R. communis.*With the help of organic biofertilizers (*Bacillus subtilis* and *Azotobacter chroococum*) and inorganic fertilizers (urea and DAP), increased Cd accumulation was observed in the roots and shoots of both the plants.

Almost similar findings were reviewed on *C. indica*phytoremediating potential by Cule *et al* (2016), Subhashini *et al* (2014), Bose *et al* (2008) and Cheng *et al* (2002).

Table 45: Effect of rhizobacteria, mycorrhiza and microbial consortium on heavy metal accumulation (As 50 and 100 mgkg^{-1}) by leaves, roots and stem of *Ricinus communis* (mgkg^{-1}).

Time duration →	1st month				2nd month				3rd month			
Treatment ↓	**Leaves**	**Roots**	**Stem**	**Soil**	**Leaves**	**Roots**	**Stem**	**Soil**	**Leaves**	**Roots**	**Stem**	**Soil**
Control	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C + As 50	10.4± 1.221^{a}	24.5± 1.271^{b}	9.18± 1.023^{a}	188.1± 0.987^{i}	18.9± 1.634^{a}	32.9± 2.341^{c}	12.5± 1.934^{a}	173.8± 1.845^{i}	34.8± 2.461^{c}	84.3± 1.461^{h}	21.7± 1.944^{b}	98.9± 2.493^{i}
C + As 100	30.9± 2.034^{c}	41.4± 2.104^{d}	11.6± 1.491^{a}	398.4± 1.821^{k}	51.4± 2.042^{e}	73.8± 3.103^{h}	19.9± 2.004^{b}	342.9± 1.034^{k}	112.9± 2.045^{a}	152.4± 1.462^{i}	58.1± 1.945^{e}	159.9± 2.334^{i}
As 50+All B	19.2± 1.272^{b}	33.3± 1.272^{c}	12.15± 0.353^{a}	175.3± 1.343^{i}	27.55± 2.474^{b}	54.5± 3.111^{e}	20.85± 2.050^{b}	143.75± 1.767^{i}	59.2± 2.687^{e}	127.85± 1.484^{i}	30.65± 1.909^{c}	23.2± 7.071^{b}
As 100+AllB	46.15± 3.181^{d}	63.35± 0.636^{f}	20.8± 2.121^{b}	352.8± 0.777^{k}	73.15± 7.141^{g}	90.35± 2.757^{h}	32.95± 2.192^{c}	301.1± 10.11^{k}	130.9± 1.484^{i}	177.1± 8.202^{i}	70.85± 2.757^{g}	59.7± 68.44^{e}
As 50 + All M	23.55± 1.060^{b}	37.85± 2.757^{c}	15.35± 1.484^{b}	172.3± 1.555^{i}	34.8± 0.989^{c}	59.4± 2.969^{e}	24.15± 1.484^{b}	127.45± 6.434i	67.6± 2.121^{f}	132.8± 2.121^{i}	28.35± 2.050^{b}	20.5± 0.848^{b}
As 100+All M	51.8± 0.848^{e}	66.3± 1.555^{f}	27.1± 1.838^{b}	348± 5.303^{k}	80.9± 1.979^{h}	120.8± 2.192^{i}	45.9± 3.394^{d}	248± 4.949^{j}	139.75± 2.192^{i}	186.8± 2.192^{i}	77.45± 0.919^{g}	88.5± 1.979^{h}
As 50 +Const.	27.15± 0.494^{b}	41.65± 1.202^{d}	19.3± 1.131^{b}	155.2± 0.141^{i}	42.75± 2.050^{d}	67.1± 2.545^{f}	33.7± 2.262^{c}	103.5± 3.181^{i}	71.05± 0.353^{g}	135.5± 3.181^{i}	30.1± 1.414^{c}	10.65± 5.020^{a}
As100+Const.	64.95± 3.040f	74.35± 0.777^{g}	30.35± 2.192^{c}	331± 2.474^{k}	86.2± 3.252^{h}	137.4± 5.515^{i}	54.05± 1.626^{e}	220.05± 1.767^{j}	151.4± 2.121^{i}	195.1± 4.525^{i}	79.1± 1.272^{g}	72.65± 1.202^{g}

Mean ± SD (n=3).Different small alphabets (a-k) indicate statistically significant difference at $P \leq 0.05$ by Turkey's test. (**C**: control, **As 50**: arsenic 50 mgkg^{-1}, **As 100**: arsenic 100 mgkg^{-1}, **All B:** all bacterial isolates (1-9), **All M**: All mycorrhiza cultures (1-3), **Const:** microbial consortium (All bacteria + all mycorrhiza), **ND**: not detected).

Table 46: Effect of rhizobacteria, mycorrhiza and microbial consortium on heavy metal accumulation (Cd 50 and 100 mgkg^{-1}) by leaves, roots and stem of *Ricinus communis* (mgkg^{-1}).

Time duration →	1st month				2nd month				3rd month			
Treatment ↓	**Leaves**	**Roots**	**Stem**	**Soil**	**Leaves**	**Roots**	**Stem**	**Soil**	**Leaves**	**Roots**	**Stem**	**Soil**
Control	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C + Cd 50	17.5± 1.345^{b}	22.9± 2.193^{c}	8.51± 0.456^{a}	192.4± 1.053^{i}	24.6± 1.045^{c}	38.9± 2.492^{d}	12.4± 2.034^{a}	167.6± 1.392^{i}	52.8± 2.492^{f}	123.9± 3.472^{i}	24.5± 0.467^{c}	47.5± 0.479^{e}
C + Cd 100	39.9± 1.094^{d}	48.2± 1.943^{e}	17.1± 0.893^{b}	384.1± 1.945^{k}	57.8± 1.046^{f}	74.9± 2.945^{h}	22.8± 3.012^{c}	329.9± 1.935^{k}	122.4± 3.917^{i}	168.9± 2.381^{j}	52.3± 0.889^{f}	152.3± 2.056^{i}
Cd 50+AllB	23.4± 1.555^{c}	34.6± 3.111^{d}	14.1± 0.282^{b}	168.1± 1.697^{i}	40.55± 1.060^{e}	60.05± 2.616^{g}	26.1± 3.111^{c}	121.1± 1.697^{j}	64.95± 4.454^{g}	132.7± 2.262^{j}	35.5± 0.989^{d}	11.65± 1.060^{b}
Cd 100+AllB	53.35± 1.343^{f}	65.15± 1.767^{g}	24.8± 4.949^{c}	343± 1.767^{k}	77.1± 2.545^{h}	102.9± 2.262^{i}	37.65± 2.616^{d}	276± 7.141^{k}	139.6± 1.979^{i}	181.4± 4.879^{j}	76.6± 7.495^{h}	98.2± 8.626^{i}
Cd 50 + All M	27.35± 2.899^{c}	43.85± 1.343^{e}	16.3± 1.414^{b}	158± 3.818^{i}	48.1± 1.697^{e}	74.9± 3.676^{h}	36.5± 2.626^{d}	87.8± 2.828^{i}	69.55± 1.909g	134.4± 3.323^{j}	30.55± 1.767^{d}	13.55± 2.474^{b}
Cd 100+All M	63.95± 3.040^{g}	74.2± 2.687^{h}	31.8± 0.848^{d}	327± 2.121^{k}	93.85± 2.192^{i}	115.3± 4.242^{j}	45.3± 4.242^{e}	240± 2.474^{k}	157.6± 2.404^{i}	187± 2.757^{j}	88.85± 1.484^{i}	62.75± 1.484^{g}
Cd 50 +Const.	37± 1.697^{d}	46.9± 3.394^{e}	23.6± 3.252^{c}	142.2± 1.484^{i}	56.3± 2.969^{f}	67.5± 3.252^{g}	33.15± 2.050^{d}	87.35± 6.434^{i}	71.45± 0.919^{h}	138.2± 2.969^{j}	31.7± 0.565^{d}	5.85± 1.060^{a}
Cd100+Const.	65.15± 1.767^{g}	77.15± 3.747^{h}	34.6± 0.989^{d}	320.3± 2.121^{k}	110.6± 0.989^{j}	133.4± 2.828^{j}	54.1± 1.697^{f}	197.9± 5.656j	164.95± 5.444^{j}	196± 5.515^{j}	90.05± 1.767^{i}	52.2± 1.272^{f}

Mean ± SD (n=3).Different small alphabets (a-j) indicate statistically significant difference at $P \leq 0.05$ by Turkey's test. (**C**: control, **Cd 50**: cadmium 50 mgkg^{-1}, **Cd 100**: cadmium 100 mgkg^{-1}, **All B:** all bacterial isolates (1-9), **All M**: All mycorrhiza cultures (1-3), **Const:** microbial consortium (All bacteria + all mycorrhiza), **ND**: not detected).

Table 47: Effect of rhizobacteria, mycorrhiza and microbial consortium on heavy metal accumulation (As 50 and 100 mgkg^{-1}) by shoots and roots of *Canna indica* (mgkg^{-1}).

Time duration →	1st month			2nd month			3rd month		
Treatment ↓	Leaves	Roots	Soil	Leaves	Roots	Soil	Leaves	Roots	Soil
Control	ND	ND	ND	ND	ND	ND	ND	ND	ND
C+As 50	21.3±1.873^{a}	22.9±2.045^{a}	192.3±2.243^{i}	29.9±1.934^{a}	43.4±1.236^{c}	164±3.841^{i}	68.9±2.945^{e}	113±1.29^{i}	59.9±2.94^{d}
C + As 100	48.9±0.998^{c}	56.6±0.245^{d}	387.8±2.384^{k}	74.8±1.384^{f}	88.9±1.995^{g}	324±4.987^{l}	133.6±2.487^{i}	163±2.341^{i}	192.3±2.193^{i}
As 50 +All B	30.55±1.767^{b}	36.65±2.050^{b}	178.7±2.262^{i}	43.1±1.131^{c}	57.2±1.272^{d}	146±4.313^{g}	83.1±2.545^{g}	126±2.616^{i}	36.55±2.898^{b}
As 100+AllB	67.55±0.919^{l}	74.95±4.030^{f}	350.5±3.889^{k}	96.1±3.959^{h}	109±1.979^{i}	295±6.434^{k}	156.5±2.262^{i}	185±5.939^{k}	153±3.111^{h}
As 50+All M	34.7±1.272^{b}	44.2±2.687^{c}	164.5±2.474^{i}	44.4±1.555^{c}	58.7±0.565^{d}	142±1.060^{i}	91.85±1.909^{h}	133±1.697^{i}	21.85±0.777^{a}
As100+AllM	86.95±1.909^{g}	75.65±3.040^{f}	327.8±2.757^{k}	97.3±2.616^{h}	114±3.394^{i}	279±2.969^{j}	174±2.899^{i}	194±3.181^{i}	125±4.808^{i}
As 50+Const.	41.75±3.040^{c}	52.7±1.272^{d}	151.9±4.242^{i}	51.5±3.181^{d}	64.5±1.343^{e}	131±3.818^{i}	96.75±3.040^{h}	139±2.333^{i}	12.9±0.707^{a}
As100+Const	92.85±1.343^{h}	82.85±1.484^{g}	313±1.767^{k}	106±6.434^{i}	126±3.111^{i}	264±4.454^{j}	198±5.020^{i}	205±1.272^{i}	93.8±3.252^{h}

Mean ± SD (n=3) Different small alphabets (a-k) indicate statistically significant difference at $P \leq 0.05$ by Turkey's test. (**C**: control, **As 50**: arsenic 50 mgkg^{-1}, **As 100**: arsenic 100 mgkg^{-1}, **All B:** all bacterial isolates (1-9), **All M**: All mycorrhiza cultures (1-3), **Const:** microbial consortium (All bacteria + all mycorrhiza)

Table 48: Effect of rhizobacteria, mycorrhiza and microbial consortium on heavy metal accumulation (Cd 50 and 100 mgkg^{-1}) by shoot and roots of *Canna indica* (mgkg^{-1}).

Time duration →	1st month			2nd month			3rd month		
Treatment ↓	Leaves	Roots	Soil	Leaves	Roots	Soil	Leaves	Roots	Soil
Control	ND	ND	ND	ND	ND	ND	ND	ND	ND
C+ Cd 50	21.4±0.887^{b}	28.6±1.004^{b}	189.2±3.229^{i}	34.8±3.098^{c}	43.9±2.956^{d}	154.8±2.452^{l}	76.6±2.345^{g}	111.9±0.986^{k}	58.8±1.934^{e}
C + Cd 100	59.8±1.883^{e}	69.1±1.034^{f}	354.8±2.945^{n}	89.6±1.256^{h}	94.9±0.973^{h}	301.4±1.835^{n}	148.9±3.923^{l}	176.4±0.778^{k}	162.3±1.564^{l}
Cd 50 +All B	32.7±0.848^{c}	38.55±1.060^{c}	163.7±3.394^{i}	45.25±1.343^{d}	58.45±1.909^{e}	142.8±2.333^{l}	91.3±2.687^{h}	130.8±2.333^{l}	26.7±4.525^{b}
Cd100+All B	77.35±8.555^{g}	80.6±1.188^{h}	337.7±4.879^{m}	105.7±6.505^{i}	110.3±2.828^{j}	277±2.969^{o}	164±4.454^{j}	191.6±0.989^{k}	131.3±4.527^{j}
Cd50 +All M	47.3±1.414^{d}	45.5±0.848^{d}	153.6±2.404^{i}	47.65±1.626^{d}	62.1±3.111^{f}	136.2±2.966^{l}	95.65±1.767^{h}	131.3±2.050^{l}	21.15±1.484^{b}
Cd100+AllM	92.15±1.343^{h}	76.7±1.979^{g}	322±1.979^{n}	113.2±3.040^{j}	119.7±0.848^{k}	259.9±1.626^{o}	174±2.050^{j}	206.3±2.545^{p}	107.1±5.656^{i}
Cd50+Const.	56.7±1.979^{e}	59.7±1.997^{e}	132.4±1.979^{i}	51.65±3.606^{e}	65.3±1.414^{f}	129.8±1.484^{l}	100.2±1.272^{l}	137.3±1.272^{l}	9.5±2.121^{a}
Cd100+Const	95.5±0.989^{h}	87.3±1.414^{h}	312.3±1.414^{n}	116.9±3.676^{j}	124.4±2.757^{l}	253.5±3.323^{p}	198±1.555^{l}	211.5±1.555^{o}	82.3±1.131^{h}

Mean ± SD (n=3). Different small alphabets (a-o) indicate statistically significant difference at $P \leq 0.05$ by Turkey's test. (**C**: control, **Cd 50**: cadmium 50 mgkg^{-1}, **Cd 100**: cadmium 100 mgkg^{-1}, **All B:** all bacterial isolates (1-9), **All M**: All mycorrhiza cultures (1-3), **Const:** microbial consortium (All bacteria + all mycorrhiza).

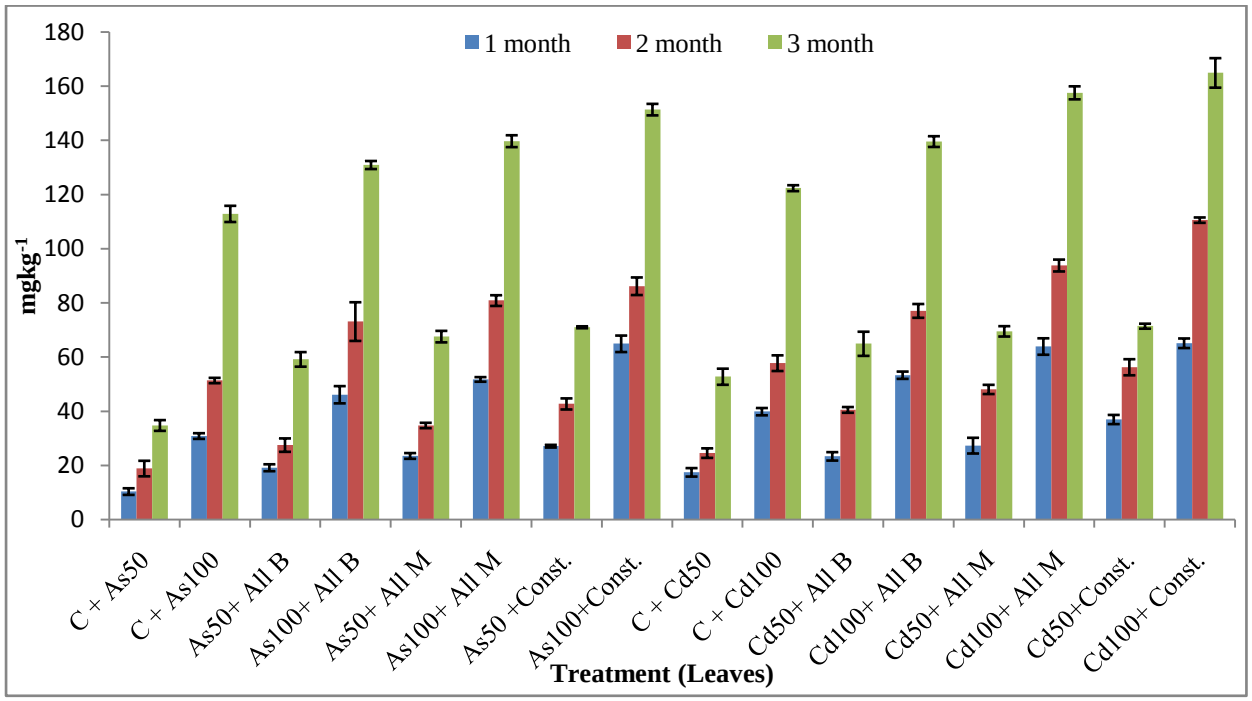

Figure 66: Effect of rhizobacteria, mycorrhiza and microbial consortium on heavy metal accumulation (As and Cd 50 and 100 mgkg^{-1}) by leaves of *Ricinus communis* (mgkg^{-1}).

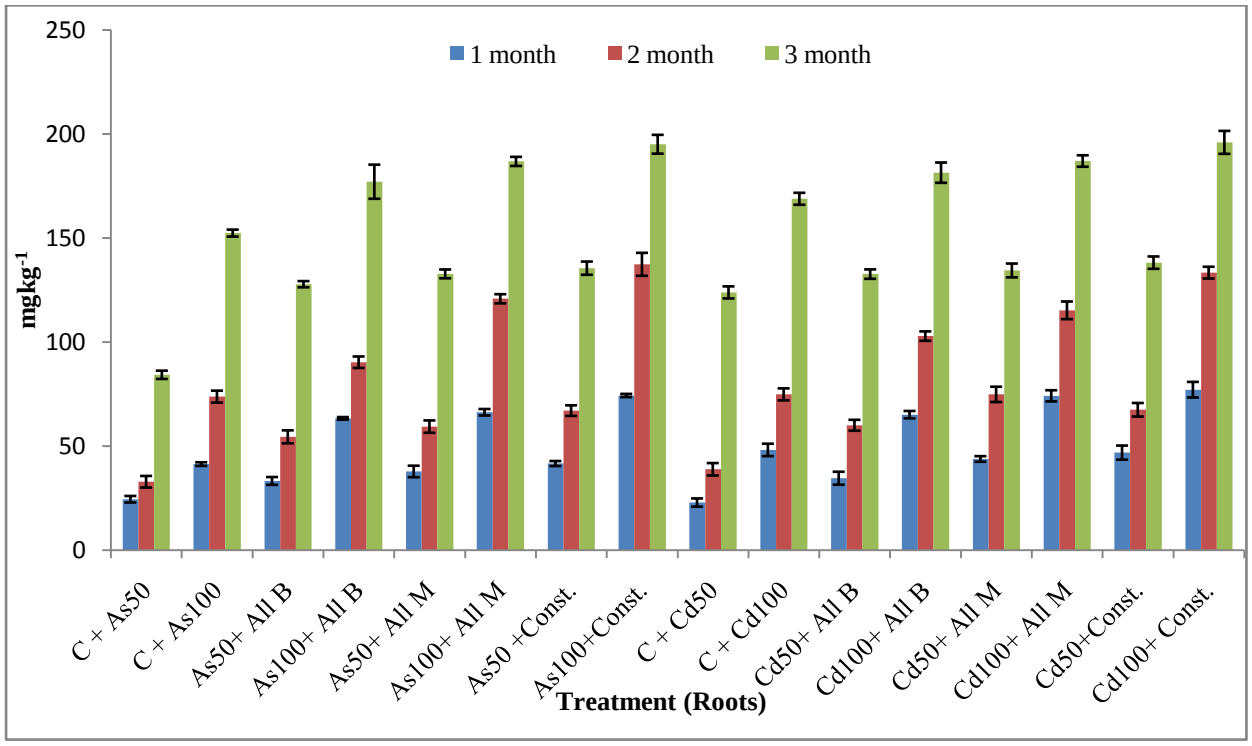

Figure 67: Effect of rhizobacteria, mycorrhiza and consortium on heavy metal accumulation (As and Cd 50 and 100 mgkg^{-1}) by roots of *Ricinus communis* (mgkg^{-1}).

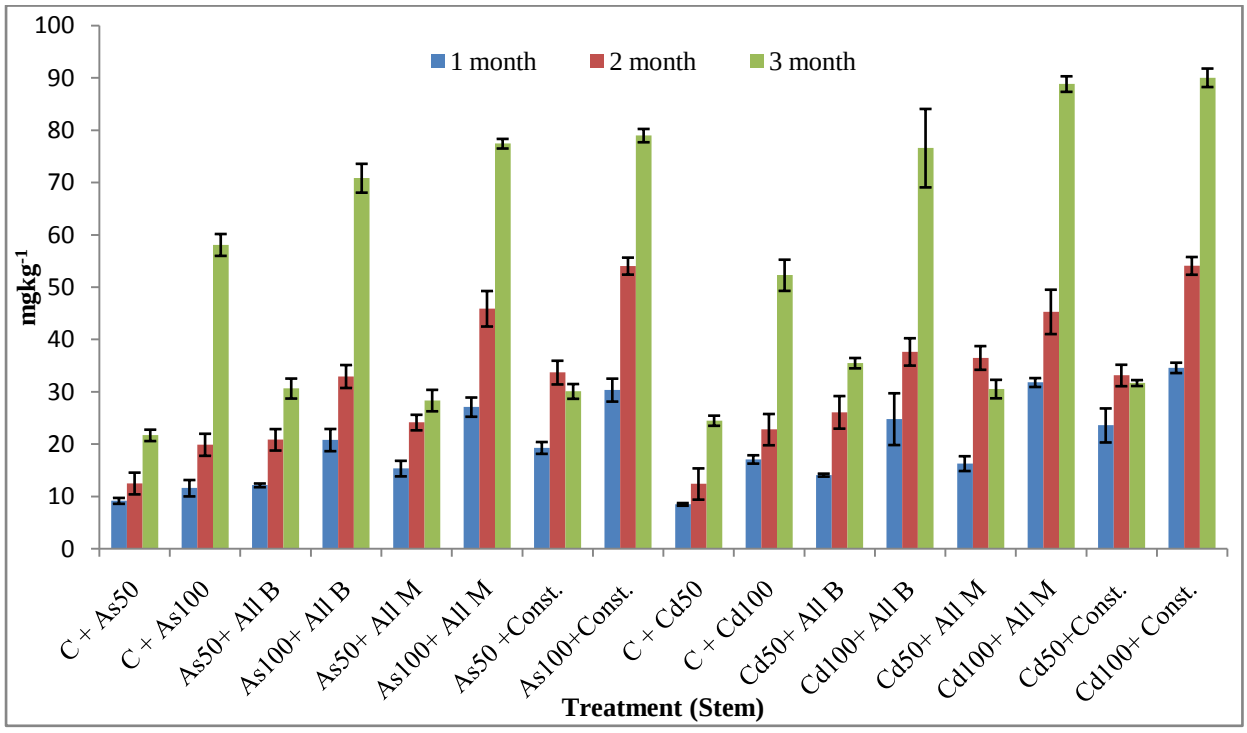

Figure 68: Effect of rhizobacteria, mycorrhiza and microbial consortium on heavy metal accumulation (As and Cd 50 and 100 mgkg^{-1}) by the stem of *Ricinus communis* (mgkg^{-1}).

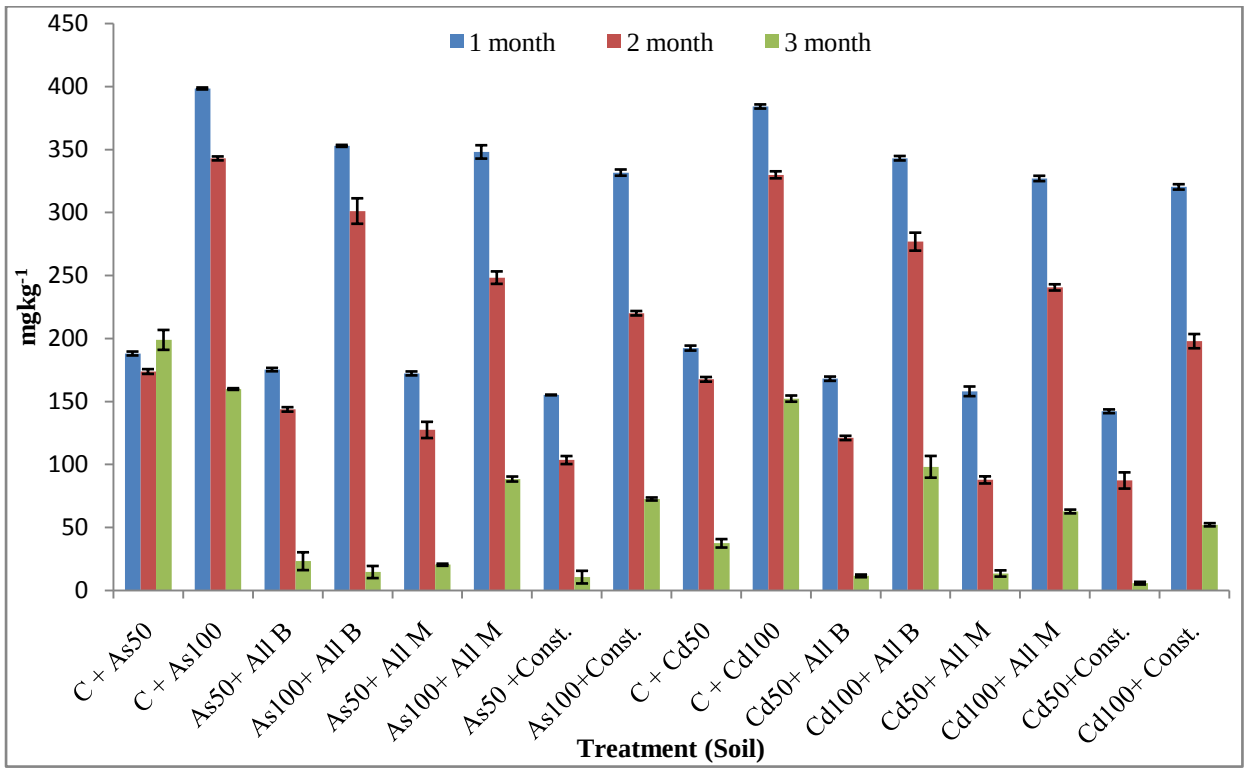

Figure 69: Amount of heavy metals (As and Cd 50 and 100 mgkg^{-1}) left in the soil after accumulation in different parts of *Ricinus communis*.

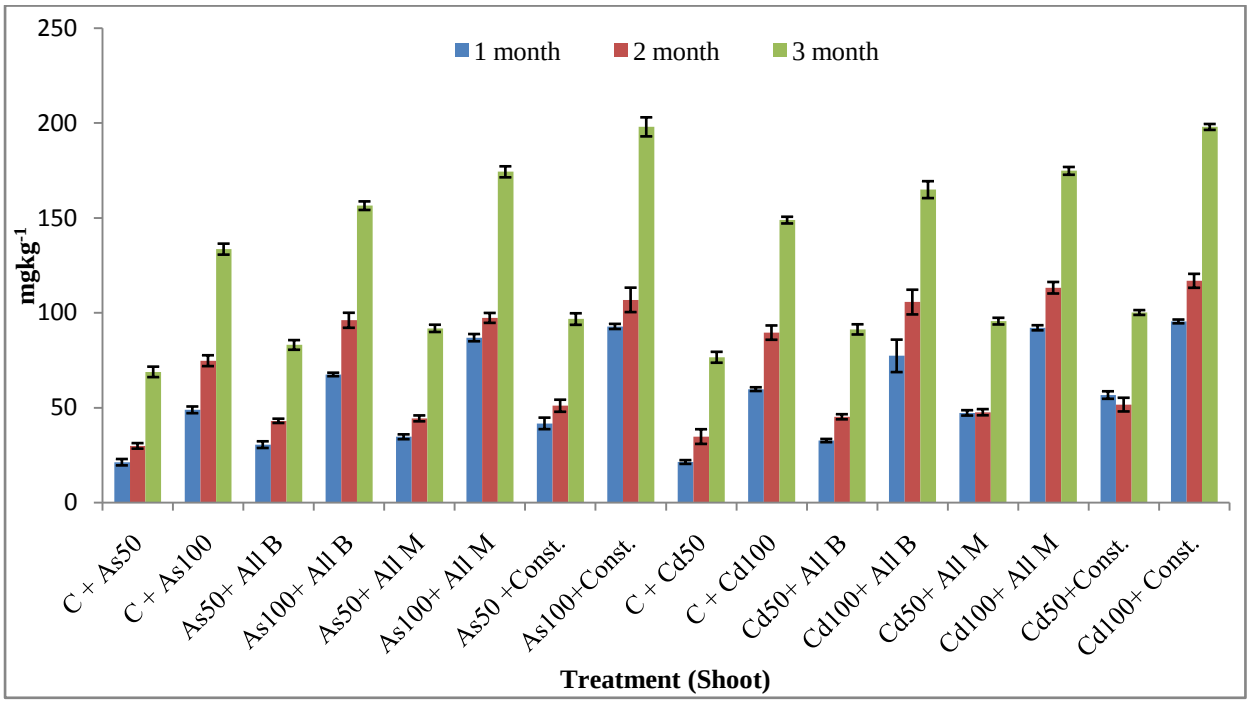

Figure 70: Effect of rhizobacteria, mycorrhiza and microbial consortium on heavy metal accumulation (As and Cd 50 and 100 mgkg^{-1}) by shoots of *Canna indica* (mg kg^{-1}).

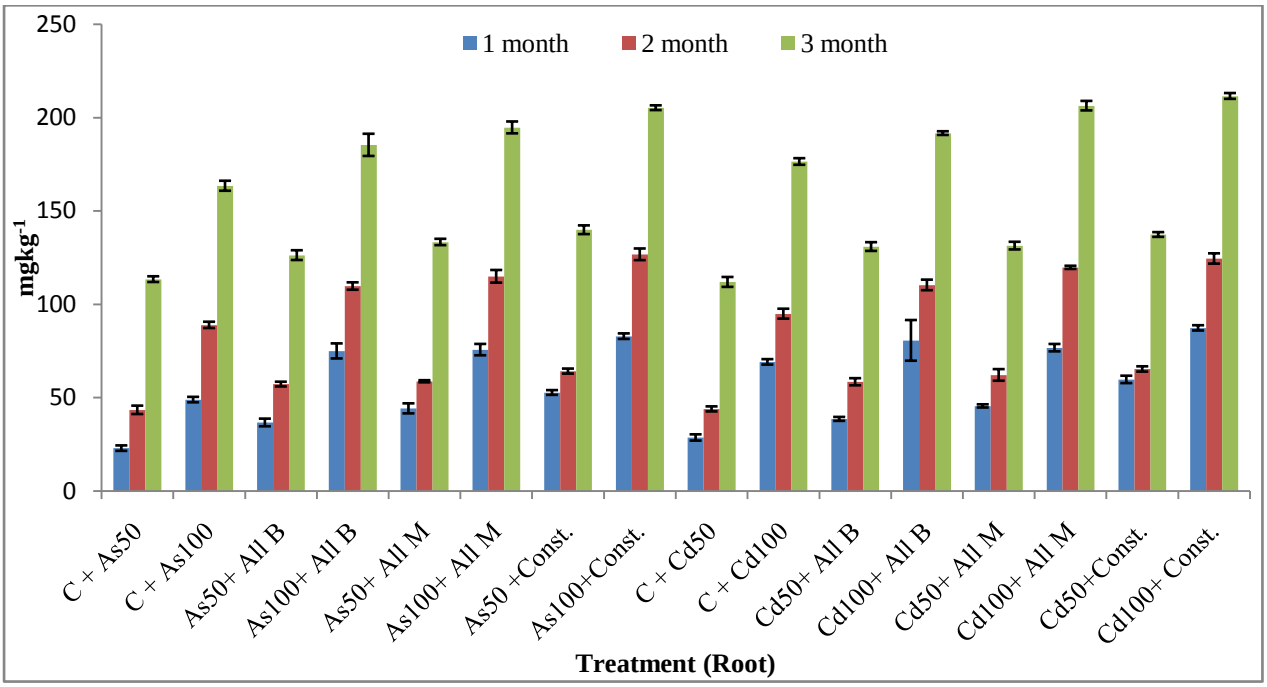

Figure 71: Effect of rhizobacteria, mycorrhiza and microbial consortium on heavy metal accumulation (As and Cd 50 and 100 mgkg^{-1}) by roots of *Canna indica* (mgkg^{-1}).

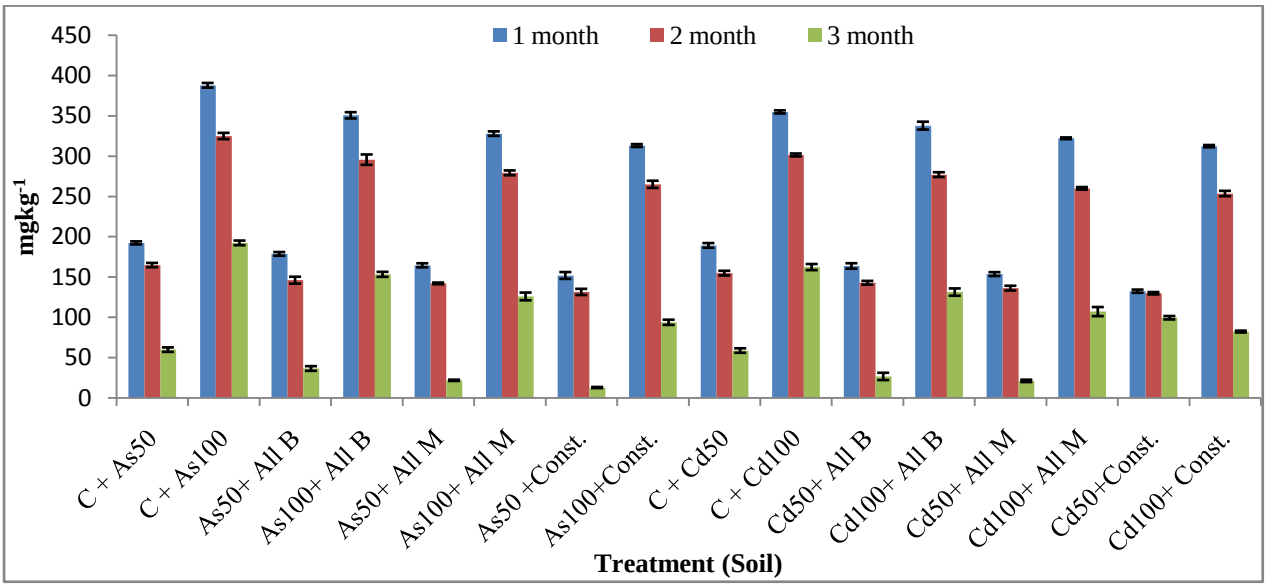

Figure 72: Amount of heavy metals (As and Cd 50 and 100 mgkg^{-1}) left in the soil after accumulation in different parts of *Canna indica.*

6.18.2 Bio-concentration factor (BCF) ofarsenic and cadmium (50 and 100 mgkg^{-1}) in *Ricinus communis* and *Canna indica* plants inoculated with rhizobacteria, mycorrhiza and microbial consortium.

Biological concentration factor (BCF) or enrichment factor (EF), is defined as the ratio of metal in plant parts (roots, stem, leaves) to the concentration of metal in soil. It is used to measure the effectiveness of plants in concentrating metals into its biomass. BCF values greater than 1 had been used to assess the phytoextraction and phytostabilization potential of plant species with respect to heavy metals in soil. Root/soil ratio (bio-concentration factor- BCF) was used to refine the plant species for phytostabilization of contaminated soils. Regarding the bio-concentration ratios, for the majority of treatments in 1st and 2nd month, BCF obtained was less than 1 (<1). BCF values of arsenic and cadmium vary accordingly with time and concentration.Overall range of BCF was observed to be 0.18-12.7, which shows a good phytoextraction potential of plants as the experimentation period increases. With time, accumulation of heavy metals (As and Cd) in the roots increases and in soil decreases, therefore, after 3rd-month sampling maximum BCF values were observed in both the plants. Maximum BCF (root/soil) ratio was seen in both the plants (*R. communis* and *C. indica*) inoculated with cadmium (50 mgkg^{-1}) and microbial consortium. In *R. communis* and *C. indica* inoculated with arsenic (50 mgkg^{-1}) and microbial consortium after the 3rd month of sampling, BCF values were found to be 8.72 and 7.77 respectively. Similarly in Cd-treated plants values were obtained to be 12.71 and 8.37 in *R. communis* and *C. indica* (Cd 50 mgkg^{-1} and microbial consortium) plants after the 3rd month of sampling.

After microbial consortium, BCF values were more in mycorrhiza (6.47, 2.11 and 9.91, 2.98) inoculated plants followed by rhizobacteria (5.51, 2.96 and 11.3 and 1.84) under arsenic and

cadmium (50 and 100 $mgkg^{-1}$) stress respectively.Whereas, BCF values of both the metals decreases with increasing concentration (100 $mgkg^{-1}$) in all the 3 months of the study period. Minimum BCF values were observed in the 1st month, because of less accumulation of heavy metals in the roots and more content in soil, followed by 2nd and 3rd month. Maximum accumulation was noticed in the case of cadmium as compared to arsenic, which is clearly indicated by the BCF values. Also, *R. communis* showed more significant ($p \leq 0.05$) accumulation of metals (As and Cd) in comparison to *C. indica* during 2nd and 3rd month in all the treatments, but in a 1st month more accumulation was seen in *C. indica* in As and Cd treatments in all the plants. In 2nd month, pots of *C. indica* inoculated with arsenic and rhizobacteria showed more BCF values than *R. communis* whereas, overall BCF values were observed to be maximum in *R. communis* (table 49 and50).

From the results obtained, it is clearly indicated that there was a significant increase in the bioconcentration factor of arsenic and cadmium in all the inoculated pots of both the plants with respect to time which is related to the phytostabilizing capability of the plants and to accumulate and stabilize metals in their roots, for further transferring them into the aerial parts of the plants. According to an experiment conducted by B. Balbanova *et al* (2015), the BCF values of different heavy metals were found to be less than 1 in *R. acetosa, S. oleracea*and *U. Dioica* showed but only for Cd BCF values more than 1. Hence these plants can accumulate maximum Cd from the soil in their roots. V. Subhashini *et al* (2014) conducted an experiment on phytoremediation potential of *C. indica* on Pb, Ni, Zn, Cd and Cr contaminated soil and the results showed *C. indica* is a potent accumulator of lead (BCF-3.64), nickel (BCF-3.26), zinc (BCF- 5.68), cadmium (BCF- 94.61) and chromium (BCF- 3.56), with maximum BCF values in Cd that shows maximum accumulation of Cd in the roots than other metals. A similar study was conducted by N. Cule *et al* (2016) on phytoremediation potential of *C. indica* L. in lead-contaminated water. The results depicted higher BCF in the below-ground biomass (roots) as compared to aboveground biomass (shoots). Hence, this can be explained in the context of good Pb accumulating capacity of *C. indica* in hydroponic conditions as well. Even, *R. communis* showed a significant increase in BCF of Cd (14.36) and Pb (6.48) in a study conducted by Niu *et al* (2017) in ahydroponic condition. Also, BCF values were observed to be increasing with respect to time and decreasing with increasing concentration of Cd that was found to be in accordance with the present study. Also in fly ash disposal sites, BCF values were found to be more than 1 in *R. communis* plants that showed the metal accumulation potential of castor plant (V.C Pandey, 2013). The BCFs of *R. communis* genotypes for DDTs varied from less to more than 1, which confirmed that castor plant possesses an exceptional capability for accumulating DDTs mainly in roots grown in contaminated soils. All these findings were in agreement with the present study.

Table 49: Effect of rhizobacteria, mycorrhiza and microbial consortium on biological concentration factor (BCF) of *Ricinus communis* and *Canna indica* inoculated with arsenic (50 and 100 mgkg^{-1}).

Time duration →	1st month		2nd month		3rd month	
Treatment ↓	*R. communis*	*C. indica*	*R. communis*	*C. indica*	*R. communis*	*C. indica*
Control	ND	ND	ND	ND	ND	ND
C + As 50	0.130±0.005^{b}	0.119±0.005^{a}	0.189±0.045^{c}	0.263±0.005^{d}	0.423±0.007^{f}	1.893±0.034^{i}
C + As 100	0.103±0.007^{a}	0.145±0.023^{b}	0.215±0.034^{d}	0.273±0.007^{d}	0.953±0.044^{h}	0.849±0.003^{h}
As 50+AllB	0.189±0.004^{c}	0.205 ± 0.007^{d}	0.379±0.012^{e}	0.391 ± 0.004^{e}	5.510±0.005^{l}	3.447 ± 0.010^{k}
As100+AllB	0.179±0.001^{c}	0.213 ± 0.007^{d}	0.300±0.005^{e}	0.369 ± 0.004^{e}	2.966±0.016^{j}	1.209 ± 0.012^{i}
As50 + All M	0.219±0.011^{d}	0.268 ± 0.010^{d}	0.466±0.012^{f}	0.413 ± 0.002^{f}	6.478±0.008^{m}	6.086 ± 0.007^{m}
As100+All M	0.190±0.002^{c}	0.230 ± 0.005^{d}	0.433±0.004^{f}	0.408 ± 0.006^{f}	2.110±0.004^{j}	1.552 ± 0.006^{i}
As 50 +Const.	0.268±0.004^{d}	0.346 ± 0.004^{e}	0.647±0.009^{g}	0.489 ± 0.006^{f}	8.720±0.012^{o}	7.775 ± 0.009^{n}
As100+Const.	0.224±0.001^{d}	0.264 ± 0.002^{d}	0.622±0.021^{g}	0.477 ± 0.005^{f}	3.685±0.009^{k}	2.185± 0.002^{j}

Mean ± SD (n=3). Different small alphabets (a-o) indicate statistically significant difference at P ≤ 0.05 by Turkey's test. (**C**: control, **As 50**: arsenic 50 mgkg^{-1}, **As 100**: arsenic 100 mgkg^{-1}, **All B:** all bacterial isolates (1-9), **All M**: All mycorrhiza cultures (1-3), **Const:** microbial consortium (All bacteria + all mycorrhiza), **ND**: not detected.

Table 50: Effect of rhizobacteria, mycorrhiza and microbial consortium on biological concentration factor (BCF) of *Ricinus communis* and *Canna indica* inoculated with cadmium (50 and 100 mgkg^{-1}).

Time duration →	1st month		2nd month		3rd month	
Treatment ↓	*R. communis*	*C. indica*	*R. communis*	*C. indica*	*R. communis*	*C. indica*
Control	ND	ND	ND	ND	ND	ND
C + Cd 50	0.119±0.023^{a}	0.151±0.004^{a}	0.232±0.009^{b}	0.283±0.034^{b}	3.304±0.006^{j}	1.903±0.004^{h}
C + Cd 100	0.125±0.012^{a}	0.194±0.006^{a}	0.227±0.005^{b}	0.314±0.055^{c}	1.108±0.034^{h}	1.086±0.007^{h}
Cd 50+AllB	0.205±0.012^{b}	0.235 ±0.004^{b}	0.496±0.010^{d}	0.443 ±0.007^{d}	11.39±0.009^{m}	4.898 ±0.009^{d}
Cd 100+AllB	0.189±0.003^{a}	0.238 ±0.021^{b}	0.372±0.004^{c}	0.398± 0.005^{c}	1.847±0.009^{h}	1.459 ±0.002^{h}
Cd 50 +All M	0.277±0.005^{b}	0.296 ±0.003^{b}	0.853±0.014^{g}	0.455 ±0.012^{d}	9.911±0.013^{l}	6.208 ±0.008^{e}
Cd 100+AllM	0.226±0.005^{b}	0.238 ±0.003^{b}	0.480±0.008^{d}	0.460 ±0.002^{d}	2.980±0.004^{i}	1.926 ±0.004^{h}
Cd 50 +Const.	0.329±0.013^{c}	0.450 ±0.007^{d}	0.772±0.012^{f}	0.503 ±0.005^{e}	12.71±0.012^{n}	8.379 ±0.004^{k}
Cd100+Const.	0.240±0.007^{b}	0.279 ±0.002^{b}	0.674±0.006^{e}	0.491 ±0.004^{d}	3.754±0.009^{j}	2.569 ±0.002^{i}

Mean ± SD (n=3). Different small alphabets (a-f) indicate statistically significant difference at P ≤ 0.05 by Turkey's test. (**C**: control, **Cd 50**: cadmium 50 mgkg^{-1}, **Cd 100**: cadmium 100 mgkg^{-1}, **All B:** all bacterial isolates (1-9), **All M**: All mycorrhiza cultures (1-3), **Const:**microbial consortium (All bacteria + all mycorrhiza), **ND**: not detected.

6.18.3 Bio-accumulation Factor (BAF) of arsenic and cadmium (50 and 100 $mgkg^{-1}$) in *Ricinus communis* and *Canna indica* plants inoculated with rhizobacteria, mycorrhiza and microbial consortium.

Bio-accumulation factor is defined as the metal concentration in shoots to the metal concentration in soil. As BCF, BAF values more than 1 shows effective phytoextraction potential of plants. The bio-accumulation factor (BAF) of the studied plants (*R. communis* and *C. indica*) species were found to be in lower range for almost all the treatments in 1st and 2nd month for both the analyzed metals (As and Cd), but was found to be more than 1 after the 3rd month. This shows the accumulation capacity of metals in the aerial parts of the plants with respect to time hence BAF also increases. Like BCF, BAF values of arsenic and cadmium were maximum in microbial consortium inoculated *R. communis* and *C. indica* plants. BAF values decrease with increasing metal concentration whereas increases with increase in the time period.

Here, maximum BAF values were seen in cadmium (50 and 100 $mgkg^{-1}$) inoculated *R. communis* (11.63 and 4.88) and *C. indica* plants (10.7 and 2.405) respectively. Whereas BAF values for rhizobacteria (3.87, 3.38 and 8.62, 2.62) and mycorrhiza (4.68, 2.45 and 7.38, 3.92) inoculated plants with arsenic and cadmium (50 and 100 $mgkg^{-1}$) were found to be more than 1 respectively (table 51-52). This shows that with an increase in plant height and time, accumulation of heavy metals in the shoots of the plant's increases, which further enhances the BAF values of plants under heavy metal stress. Moreover, a significant reduction in the BAF values was observed in the case of *C. indica* in all inoculated plants in contrast to *R. communis* in all the 3 months of experimentation period. This signifies the potential of *R. communis* as a good hyperaccumulator than *C. indica* for arsenic and cadmium. Maximum accumulation was observed in the arsenic treatments with microbial consortium at lower concentration (50 $mgkg^{-1}$) during the course of study. Hence at a lower concentration of arsenic and cadmium, more BAF was witnessed that demonstrate the higher accumulation of metal than a lower concentration by both the *R. communis* and *C. indica* plants. Present work was found to be in coherence with B. Balbanova *et al* (2015), where BAF of different heavy metals was found to be less than 1 in *R. acetosa, S. oleracea* and *U. Diocia* but maximum BAC for As and Cd were obtained in the plants collected from urban areas. Even in another experiment conducted by S. Wang *et al* (2016), BCF values of Cd and Zn were reported to be less than 1 for Cu but more than 1 for Cd and Zn in *R. communis*.

This shows the higher phytoremediation potential of Cd and Zn contaminated soils but lower uptake of Cu from the soil and accumulation in aerial parts. E.M. Eid *et al* (2016) also reported higher BCF (>1) for some heavy metals by different wild species of plants in a sewage dump site.

Table 51: Effect of rhizobacteria, mycorrhiza and microbial consortium on biological accumulation factor (BAF) of *Ricinus communis* and *Canna indica* inoculated with arsenic (50 and 100 mgkg^{-1}).

Time duration →	1st month		2nd month		3rd month	
Treatment ↓	*R. communis*	*C. indica*	*R. communis*	*C. indica*	*R. communis*	*C. indica*
Control	ND	ND	ND	ND	ND	ND
C + As 50	1.104±0.012^{h}	0.110±0.008^{a}	0.180±0.023^{a}	0.181±0.003^{a}	0.571±0.032^{e}	1.150±0.021^{h}
C + As 100	0.106±0.003^{a}	0.126±0.005^{a}	0.207±0.011^{b}	0.230±0.005^{b}	1.069±0.009^{g}	0.693±0.004^{f}
As 50 + All B	0.178±0.007^{a}	0.170 ± 0.007^{a}	0.336±0.001^{c}	0.295 ± 0.004^{b}	3.872±0.021^{k}	2.273 ±0.009^{j}
As 100 + All B	0.189±0.014^{a}	0.192 ± 0.002^{a}	0.352±0.015^{c}	0.325 ± 0.007^{c}	3.380±0.004^{k}	1.022 ±0.004^{g}
As 50+All M	0.225±0.004^{b}	0.210 ± 0.004^{b}	0.462±0.009^{d}	0.312 ± 0.006^{c}	4.681±0.007^{l}	4.203 ±0.007^{l}
As 100 +All M	0.226±0.006^{b}	0.265 ± 0.003^{b}	0.511±0.010^{e}	0.348 ± 0.004^{c}	2.454±0.002^{j}	1.392 ±0.005^{i}
As 50 + Const.	0.299±0.002^{b}	0.274 ± 0.012^{b}	0.738±0.001^{g}	0.389 ±0.012^{c}	9.497±0.003^{n}	7.5 ± 0.012^{m}
As100+Const	0.287±0.001^{b}	0.296 ± 0.002^{b}	0.637±0.002^{f}	0.404 ± 0.012^{d}	3.972±0.002^{k}	2.11 ± 0.009^{j}

Mean ± SD (n=3). Different small alphabets (a-n) indicate statistically significant difference at P ≤ 0.05 by Turkey's test. (**C**: control, **As 50**: arsenic 50 mgkg^{-1}, **As 100**: arsenic 100 mgkg^{-1}, **All B:** all bacterial isolates (1-9), **All M**: All mycorrhiza cultures (1-3), **Const:**microbial consortium (All bacteria + all mycorrhiza), **ND**: not detected).

Table 52: Effect of rhizobacteria, mycorrhiza and microbial consortium on biological accumulation factor (BAF) of *Ricinus communis* and *Canna indica* inoculated with cadmium (50 and 100 mgkg^{-1}).

Time duration →	1st month		2nd month		3rd month	
Treatment ↓	*R. communis*	*C. indica*	*R. communis*	*C. indica*	*R. communis*	*C. indica*
Control	ND	ND	ND	ND	ND	ND
C + Cd 50	0.135±0.003^{a}	0.113±0.006^{a}	0.220±0.001^{b}	0.224±0.011^{b}	2.061±0.002^{i}	1.302±0.022^{h}
C + Cd 100	0.148±0.031^{a}	0.168±0.006^{a}	0.244±0.003^{b}	0.297±0.004^{b}	1.147±0.005^{h}	0.917±0.008^{g}
Cd 50 + All B	0.223±0.004^{b}	0.199 ±0.003^{a}	0.550±0.007^{e}	0.316 ± 0.004^{c}	8.622±0.021^{m}	3.419 ±0.010^{j}
Cd 100 + All B	0.227±0.012^{b}	0.229 ±0.016^{b}	0.415±0.009^{d}	0.381 ± 0.013^{c}	2.627±0.018^{i}	1.249 ±0.009^{h}
Cd 50 + All	0.276±0.016^{b}	0.307 ±0.005^{c}	0.963±0.015^{g}	0.349 ± 0.006^{c}	7.387±0.131^{l}	4.522 ±0.007^{k}
Cd 100 +All M	0.292±0.004^{b}	0.286 ±0.002^{b}	0.579±0.004^{e}	0.435 ± 0.006^{d}	3.927±0.007^{j}	1.624 ±0.004^{h}
Cd 50 + Const.	0.426±0.005^{d}	0.428 ±0.007^{d}	1.024±0.021^{h}	0.397 ± 0.014^{c}	11.63±0.012^{o}	10.7 ±0.004^{n}
Cd100+Const	0.311±0.002^{c}	0.305 ±0.002^{c}	0.829±0.002^{f}	0.461 ± 0.007^{d}	4.88±0.007k	2.405 ±0.003^{i}

Mean ± SD (n=3). Different small alphabets (a-f) indicate statistically significant difference at P ≤ 0.05 by Turkey's test. (**C**: control, **Cd 50**: cadmium 50 mg kg^{-1}, **Cd 100**: cadmium 100 mg kg^{-1}, **All B:** all bacterial isolates (1-9), **All M**: All mycorrhiza cultures (1-3), **Const:** microbial consortium (All bacteria + all mycorrhiza), **ND**: not detected.

6.18.4 Translocation factor (TF) ofarsenic and cadmium (50 and 100 mgkg^{-1}) in *Ricinus communis* and *Canna indica* plants inoculated with rhizobacteria, mycorrhiza and microbial consortium.

Translocation factor (TF) was described as the ratio of heavy metals in plant shoot to that in plant roots. Translocation factor values were in the order of rhizobacteria<mycorrhiza <microbial consortium in both the arsenic and cadmium inoculated plants of *R. communis* and *C. indica*. Incompatible TF values were obtained as in BAF and BCF. *R. communis* showed more TF values in all the treated plants under arsenic and cadmium stress than *C. indica*. Here, TF values increase with an increase in concentration and decrease with time in all the treatments. After 3rd month, minimum TF values in both the plants inoculated with As and Cd were observed. Maximum TF values were noticed in 1st month of sampling in the plants inoculated with microbial consortium (1.11, 1.28 and 1.296, 1.293) followed by mycorrhiza (1.03, 1.18 and 0.99, 1.29) and rhizobacteria (0.94, 1.05 and 1.08, 1.19) inoculated plants in both *R. communis* and *C. indica* under arsenic and cadmium stress respectively (table 53-54). Maximum TF values were obtained for cadmium treatment in all the pots which shows more translocation of cadmium to aerial parts of both the plants from roots. Significant decrease was seen after 3rd month of sampling in all the inoculated plants, where higher TF content was found in microbial consortium (0.74, 1.18 and 0.76, 1.30) followed by mycorrhiza (0.72, 1.39 and 0.74, 1.31) and rhizobacteria (0.70, 1.11 and 0.75, 1.19) showing variable results in arsenic and cadmium (50 and 100 mgkg^{-1}) inoculated plants respectively. Even in *C. indica*, higher TF values were observed in microbial consortium inoculated plants followed by mycorrhizaand rhizobacteria under arsenic and cadmium (50 and 100 mgkg^{-1}) stress. After 3rd month of sampling TF values for all the inoculated plants of *C. indica* was discovered to be less than 1 as compared to that in *R. communis* as shown in Table 53 and 54. Higher root to shoot translocation of arsenic and cadmium demonstrate that these plants (*R. communis* and *C. indica*) have essential characteristics to be used for phytoextraction of these metals.

B. Balbanova *et al* (2015) suggested the potential of *R. acetosa*, *S. oleracea* and *U. Diocia* in translocation of various heavy metals from root to shoot with TF values > 1. Some studies also depicted TF <1 which shows that metals are stabilized in the roots of *R. communis* and not in the shoots (Pandey, 2013). Many other researchers have reported the potential of *R. communis* to grow in deliberately heavy metal polluted soil in order to uptake cadmium (Baudh and Singh, 2012; Huang*et al*.,2011), Cu (Andreazza *et al*., 2013) and Zn (Lu and He, 2005). On the other hand, some other reports suggested the lower metal concentration in shoots of *R. communis* (Olivares *et al*., 2013; de Abreu *et al*., 2012).

Table 53: Effect of rhizobacteria, mycorrhiza and microbial consortium on Translocation factor (TF) of *Ricinus communis* and *Canna indica* inoculated with arsenic (50 and 100 mgkg^{-1}).

Time duration →	1st month		2nd month		3rd month	
Treatment ↓	*R. communis*	*C. indica*	*R. communis*	*C. indica*	*R. communis*	*C. indica*
Control	ND	ND	ND	ND	ND	ND
C + As 50	0.799 ± 0.012^{c}	0.930 ± 0.004^{e}	0.954 ± 0.034^{e}	0.181 ± 0.065^{a}	0.670 ± 0.114^{b}	1.150 ± 0.044^{g}
C + As 100	1.026 ± 0.078^{f}	0.126 ± 0.067^{a}	0.966 ± 0.123^{e}	0.230 ± 0.067^{b}	1.122 ± 0.034^{g}	0.694 ± 0.026^{b}
As 50 + All B	0.941 ± 0.013^{e}	0.833 ± 0.001^{d}	0.889 ± 0.058^{d}	0.753 ± 0.002^{c}	0.702 ± 0.028^{c}	0.658 ± 0.033^{b}
As 100 + All B	1.057 ± 0.094^{f}	0.902 ± 0.060^{e}	1.116 ± 0.147^{g}	0.876 ± 0.052^{d}	1.111 ± 0.112^{g}	0.844 ± 0.038^{d}
As 50 + All M	1.03 ± 0.086^{f}	0.785 ± 0.019^{c}	0.992 ± 0.008^{e}	0.728 ± 0.043^{c}	0.722 ± 0.012^{c}	0.688 ± 0.023^{b}
As 100 + All M	1.185 ± 0.012^{g}	1.151 ± 0.070^{g}	1.049 ± 0.023^{f}	0.847 ± 0.047^{d}	1.395 ± 0.322^{i}	0.893 ± 0.002^{d}
As 50 + Const.	1.115 ± 0.048^{g}	0.791 ± 0.038^{c}	1.139 ± 0.040^{g}	0.795 ± 0.032^{c}	0.746 ± 0.004^{c}	0.691 ± 0.003^{b}
As 100 + Const.	1.281 ± 0.024^{h}	1.12 ± 0.004^{g}	1.013 ± 0.041^{f}	0.854 ± 0.046^{d}	1.180 ± 0.031^{g}	0.964 ± 0.030^{e}

Mean ± SD (n=3). Different small alphabets (a-i) indicate statistically significant difference at $P \leq 0.05$ by Turkey's test. (**C**: control, **As 50**: arsenic 50 mgkg^{-1}, **As 100**: arsenic 100 mgkg^{-1}, **All B:** all bacterial isolates (1-9), **All M**: All mycorrhiza cultures (1-3), **Const:** microbial consortium (All bacteria + all mycorrhiza), **ND**: not detected).

Table 54: Effect of rhizobacteria, mycorrhiza and microbial consortium on Translocation factor (TF) of *Ricinus communis* and *Canna indica* inoculated with cadmium (50 and 100 mgkg^{-1}).

Time duration →	1st month		2nd month		3rd month	
Treatment ↓	*R. communis*	*C. indica*	*R. communis*	*C. indica*	*R. communis*	*C. indica*
Control	ND	ND	ND	ND	ND	ND
C + Cd 50	1.135 ± 0.145^{f}	0.748 ± 0.023^{b}	0.951 ± 0.023^{d}	0.792 ± 0.056^{b}	0.623 ± 0.045^{a}	0.684 ± 0.009^{a}
C + Cd 100	1.182 ± 0.134^{f}	0.865 ± 0.034^{c}	1.076 ± 0.104^{e}	0.944 ± 0.045^{d}	1.034 ± 0.067^{e}	0.844 ± 0.034^{c}
Cd 50 + All B	1.086 ± 0.060^{e}	0.848 ± 0.045^{c}	1.111 ± 0.014^{f}	0.775 ± 0.048^{b}	0.756 ± 0.028^{b}	0.697 ± 0.008^{a}
Cd 100 + All B	1.198 ± 0.063^{f}	0.975 ± 0.238^{d}	1.117 ± 0.022^{f}	0.959 ± 0.082^{d}	1.192 ± 0.084^{f}	0.860 ± 0.190^{c}
Cd 50 + All M	0.997 ± 0.128^{d}	1.039 ± 0.011^{e}	1.131 ± 0.108^{f}	0.768 ± 0.064^{b}	0.744 ± 0.045^{b}	0.728 ± 0.024^{b}
Cd 100 + All M	1.291 ± 0.076^{g}	1.201 ± 0.013^{g}	1.207 ± 0.062^{g}	0.945 ± 0.031^{d}	1.317 ± 0.040^{h}	0.847 ± 0.020^{c}
Cd 50 + Const.	1.296 ± 0.127^{g}	0.950 ± 0.064^{c}	1.324 ± 0.010^{h}	0.791 ± 0.071^{b}	0.769 ± 0.038^{b}	0.729 ± 0.002^{b}
Cd 100 +Const.	1.293 ± 0.053^{g}	1.093 ± 0.006^{e}	1.231 ± 0.025^{g}	0.939 ± 0.008^{d}	1.301 ± 0.018^{h}	0.934 ± 0.002^{d}

Mean ± SD (n=3). Different small alphabets (a-f) indicate statistically significant difference at $P \leq 0.05$ by Turkey's test. (**C**: control, **Cd 50**: cadmium 50 mgkg^{-1}, **Cd 100**: cadmium 100 mgkg^{-1}, **All B:** all bacterial isolates (1-9), **All M**: All mycorrhiza cultures (1-3), **Const:** microbial consortium (All bacteria + all mycorrhiza), **ND**: not detected)

6.18.5 Tolerance index (TI) of *Ricinus communis* and *Canna indica* inoculated with arsenic and cadmium (50 and 100 mgkg^{-1}) along with rhizobacteria, mycorrhiza and microbial consortium.

Tolerance index is defined as the ratio of the biomass of inoculated plants (with heavy metal) to that in the control plants (without heavy metal). TI values more than 1 depicts a net increase in biomass and suggest that the plant has evolved tolerance and is a good hyperaccumulator whereas, TI values lower than 1 indicate a net decrease in biomass which shows that plant suffered a stress due to heavy metal pollution. TI value equal to 1 indicates no difference relative to non-heavy metal treated plants, hence plant is unaffected by metal stress.

Overall TI for all the inoculated plants of *R. communis* and *C. indica* were observed to be 0.398-3.377. Under less arsenic stress (As 50 mgkg^{-1}), TI of *R. communis* plants was found to be maximum in mycorrhiza (2.92) inoculated plants followed by microbial consortium (1.409) and rhizobacteria (0.882) inoculated plants after 1st month whereas under higher arsenic stress (As 100 mg kg^{-1}) different pattern of TI values were 3.57 (microbial consortium), 2.88 (mycorrhiza) and 0.39 (rhizobacteria) observed in *R. communis.* But TI significantly increased after a 2nd month in mycorrhiza inoculated plants in comparision to other treatments, where maximum TI value was found in the microbialconsortium (3.37) inoculated plants with 100 mgkg^{-1} of arsenic following mycorrhiza (3.40) and rhizobacteria (0.36) implanted *R. communis* pots. Non significant decrease was observed in the TI values of rhizobacteria and microbial consortium inoculated plants after 2nd month as compared to mycorrhiza after 1st month.

Also, after 3rd month, variant results were seen in which increase was observed only in rhizobacteria (0.87 and 0.38) inoculated plants under arsenic stress (50 and 100 mgkg^{-1}) and microbial consortium (1.65) inoculated plants (As 50 mgkg^{-1}) as compared to mycorrhiza (2.44 and 3.19) inoculated plants (As 50 and 100 mgkg $^{-1}$) respectively (table 55). Hence we can conclude that, tolerance index (TI) showed variations in the values with respect to time and concentration in all the three months of study. Significant trend was observed in all the 3 months for TI values in *R. communis* plant inoculated with mycorrhiza, rhizobacteria and microbial consortium. But overall maximum TI values were observed in mycorrhiza inoculated plants during 3 months of experimentation.

Similarly, TI values in *C. indica* under As stress were observed during all the 3 months. After 1st month of sampling, maximum TI values were observed in mycorrhiza (1.50 and 0.90) inoculated plants (As 50 and 100 mgkg^{-1}) whereas after 2nd month in microbial consortium (1.18 and 1.02) inoculated plants (As 50 and 100 mgkg^{-1}) and simultaneously after 3rd month again in mycorrhiza (1.0 and 1.07) inoculated *C. indica* plants (Table 55). In *C. indica* plants, dissimilar TI values were noticed like in *R. communis* with respect to time and concentration. But at the

end, most of the treatments showed the TI values > 1, which denoted the good hyperaccumulation capacity of both the plants to accumulate heavy metals and good potential in phytoremediating polluted soils.

Under cadmium stress (50 and 100 $mgkg^{-1}$) both the plants showed TI values more than one in almost all the treatments during 3 months of study (table 56). During 1^{st} month, maximum TI value was observed in mycorrhiza inoculated plants of *R. communis* (2.97 and 1.87) and *C. indica* (1.18 and 1.06) with 50 and 100 $mgkg^{-1}$ Cd, after 2^{nd} month maximum TI values in *R. communis* (3.51 and 2.90) and *C. indica* (1.12 and 1.03) were observed in mycorrhiza and microbial consortium inoculated plants (50 and 100 $mgkg^{-1}$ Cd) respectively. After a 3^{rd} month the trend changed from higher TI values in mycorrhizainoculated plants (2.79 and 1.78) in *R. communis* to higher TI values in microbial consortium inoculated plants (1.05 and 0.92) in *C. indica* under 50 and 100 $mgkg^{-1}$ Cd treatment. Overall results showed that *R. communis* possessed more TI values ranging from 0.36-3.37 under As stress and 0.70- 3.51 under Cd stress in all the treated plants as compared to *C. indica* where TI values ranged from 0.91-1.50 under As stress and 0.41-1.21 under Cd stress as shown in table 56.H. Diwan *et al* (2010) reported a significant increase in accumulation of Cu in *Brassica juncea* (Indian mustard) along with higher tolerance index (>1) that showed the Cr hyperaccumulation potential of Indian mustard. Inversely *B. napus* (Canola) and *Raphanus sativa* (Raddish), showed tolerance index < 1 which demonstrated the lower tolerating capabilities (Cu, Ni and Pb) of these plants against heavy metals (L. Marchiol *et al*., 2004).

Belouchrani *et al* (2016) also investigated the tolerance index of Canola (*B. napus* L.) under Zn stress. The results revealed that with an increase in time, tolerance index of canola increases but inversely, with increasing concentration of Zn, TI decreases (<1). Another study conducted by Wang *et al* (2014) on three varieties of *Salix integra*was cultivated hydroponically to evaluate its heavy metal (Pb) accumulation potential. Tolerance index of all the three species varies accordingly to the heavy metal accumulation potential of *Salix* plant. Mostly all the three varieties showed TI values < 1 and were sensitive to increasing Pb concentration. Therefore, all these studies were found to be in agreement with the present work where, *R. communis* showed more TI values as compared to *C. indica.* Hence, the present study can suggest the phytoremediation potential of *R. communis* for intensely heavy metal contaminated sites.

Table 55: Effect of rhizobacteria, mycorrhiza and microbial consortium on Tolerance index (TI) of *Ricinus communis* and *Canna indicia* inoculated with arsenic (50 and 100 mgkg^{-1}).

Time duration →	1st month		2nd month		3rd month	
Treatment ↓	*R. communis*	*C. indica*	*R. communis*	*C. indica*	*R. communis*	*C. indica*
Control	ND	ND	ND	ND	ND	ND
C + As 50	0.891 ± 0.078^{d}	0.543 ± 0.123^{c}	0.453 ± 0.102^{b}	0.621 ± 0.034^{c}	0.448 ± 0.334^{b}	0.445 ± 0.007^{b}
C + As 100	0.451 ± 1.234^{b}	0.493 ± 0.081^{b}	0.512 ± 0.034^{c}	0.543 ± 0.023^{c}	0.504 ± 0.981^{c}	0.418 ± 0.008^{b}
As 50 + All B	0.882 ± 0.084^{c}	1.203 ± 0.170^{h}	0.749 ± 0.103^{c}	1.125 ± 0.013^{g}	0.879 ± 0.331^{c}	1.095 ± 0.006^{f}
As 100 + All B	0.398 ± 0.015^{a}	0.914 ± 0.010^{d}	0.365 ± 0.031^{a}	1.092 ± 0.015^{f}	0.381 ± 0.047^{a}	1.078 ± 0.004^{f}
As 50 + All M	2.928 ± 1.386^{m}	1.505 ± 0.090^{k}	3.364 ± 0.733^{n}	1.001 ± 0.017^{e}	2.446 ± 0.531^{m}	1.003 ± 0.011^{e}
As 100 + All M	2.884 ± 1.474^{n}	0.908 ± 0.023^{d}	3.409 ± 0.696^{n}	1.203 ± 0.009^{h}	3.198 ± 1.068^{n}	1.072 ± 0.006^{f}
As 50 + Const.	1.409 ± 0.150^{j}	0.904 ± 0.003^{d}	1.359 ± 0.220^{i}	1.188 ± 0.008^{g}	1.655 ± 0.930^{l}	1.063 ± 0.009^{f}
As 100 + Const.	3.527 ± 1.460^{n}	0.954 ± 0.009^{d}	3.377 ± 1.673^{n}	1.028 ± 0.002^{e}	2.208 ± 1.115^{m}	0.918 ± 0.007^{d}

Mean ± SD (n=3). Different small alphabets (a-n) indicate statistically significant difference at $P \leq 0.05$ by Turkey's test. (**C**: control, **As 50**: arsenic 50 mgkg^{-1}, **As 100**: arsenic 100 mgkg^{-1}, **All B:** all bacterial isolates (1-9), **All M**: All mycorrhiza cultures (1-3), **Const:** microbial consortium (All bacteria + all mycorrhiza), **ND:** not detected).

Table 56: Effect of rhizobacteria, mycorrhiza and microbial consortium on Tolerance index (TI) of *Ricinus communis* and *Canna indica* inoculated with cadmium (50 and 100 mgkg^{-1}).

Time duration →	1st month		2nd month		3rd month	
Treatment ↓	*R. communis*	*C. indica*	*R. communis*	*C. indica*	*R. communis*	*C. indica*
Control	ND	ND	ND	ND	ND	ND
C + Cd 50	0.913 ± 0.089^{g}	0.345 ± 0.067^{a}	0.921 ± 0.078^{g}	0.469 ± 0.006^{b}	0.864 ± 0.671^{f}	0.412 ± 0.005^{b}
C + Cd 100	0.623 ± 0.009^{d}	0.521 ± 0.023^{c}	0.684 ± 0.056^{a}	0.581 ± 0.023^{c}	0.723 ± 0.234^{e}	0.566 ± 0.004^{c}
Cd50 + All B	1.953 ± 0.057^{n}	0.418 ± 0.060^{b}	1.531 ± 0.539^{l}	0.646 ± 0.007^{d}	1.603 ± 0.586^{l}	0.841 ± 0.001^{f}
Cd100 + All B	0.765 ± 0.008^{e}	0.413 ± 0.004^{b}	0.709 ± 0.070^{e}	0.728 ± 0.009^{e}	0.846 ± 0.390^{f}	0.805 ± 0.002^{f}
Cd 50 + All M	2.973 ± 1.468^{p}	1.180 ± 0.025^{i}	3.515 ± 0.663^{q}	0.834 ± 0.006^{f}	2.797 ± 0.817^{p}	0.917 ± 0.010^{g}
Cd 100 + All M	1.870 ± 0.947^{n}	1.068 ± 0.016^{h}	2.903 ± 0.536^{p}	1.212 ± 0.012^{j}	1.789 ± 0.545^{m}	1.111 ± 0.005^{i}
Cd 50 + Const.	2.365 ± 0.783^{p}	0.965 ± 0.013^{g}	2.160 ± 1.074^{o}	1.123 ± 0.014^{i}	1.922 ± 0.935^{n}	1.057 ± 0.004^{h}
Cd100 + Const.	1.090 ± 0.261^{h}	1.019 ± 0.046^{h}	1.058 ± 0.306^{h}	1.033 ± 0.001^{h}	1.369 ± 0.670^{k}	0.926 ± 0.009^{g}

Mean ± SD (n=3). Different small alphabets (a-q) indicate statistically significant difference at $P \leq 0.05$ by Turkey's test. (**C**: control, **Cd 50**: cadmium 50 mgkg^{-1}, **Cd 100**: cadmium 100 mgkg^{-1}, **All B:** all bacterial isolates (1-9), **All M**: All mycorrhiza cultures (1-3), **Const:** microbial consortium (All bacteria + all mycorrhiza), **ND:** not detected).

6.18.6 Phytoextraction capacity (PC) of *Ricinus communis* and *Canna indica* inoculated with50 and 100 mgkg^{-1}of arsenic and cadmium along with rhizobacteria, mycorrhiza and microbial consortium.

Phytoextraction capacity/ potential is described as the ratio of total biomass (total DW of the plant) to the total heavy metal accumulated (in all parts of the plant). Phytoextraction capacity of a plant should be more than 1 to prove it as a good hyper-accumulator. Therefore, form the present study we have observed variant results during 3 months of course study in both the plants (*R. communis* and *C. indica*) under heavy metal stress (As and Cd 50 and100 mgkg^{-1}).

After 1st and 2nd month of sampling, *R. communis* showed maximum PC values than *C. indica* in all the As and Cd (50 and100 mgkg^{-1}) inoculated plants (mycorrhiza, rhizobacteria and microbial consortium) where maximum PC values were observed in microbial consortium (1.98 and 6.71) inoculated *R. communis* plants (As 100 mgkg^{-1}) followed by mycorrhiza (1.21 and 4.14) and rhizobacteria (0.56 and 2.02) in 1stand 2nd months respectively as compared to control (0.284 and 0.843) plants. But after 3rd month, PC values were found to be higher in mycorrhiza inoculated plants of *R. communis* 9.28 and 10.5, with PC values in microbial consortium inoculated plants as 8.63 and 10.5 and least PC values in rhizobacteria inoculated plants 7.51 and 2.96 (As 50 and 100 mgkg^{-1}) respectively. A similar range of PC values was seen in *C. indica* plant inoculated with rhizobacteria, mycorrhiza and microbial consortium (As 50 and 100 mgkg^{-1}). Although, PC values were found to be less in *C. indica* inoculated plants in comparison to *R. communis*, both the PC values were found to be increasing in the order as, microbial consortium (0.14, 0.04; 2.27, 1.73 and 12.2, 9.80) > mycorrhiza (0.11, 0.05; 1.8 ,1.76 and 10.4, 9.78) > rhizobacteria (0.13, 0.05; 1.96, 1.69 and 10.5, 9.08) in As 50 and 100 mgkg^{-1} treatments after 1st , 2ndand 3rd months respectively as compared to control (0.10, 0.05;1.20, 1.16 and 7.15, 4.81) after 1st, 2nd and 3rd month respectively as shown in table 57.

In pots (*R. communis* and *C. indica*) under Cd stress (50 and 100 mgkg^{-1}) also showed an almost similar trend in PC values during all the 3 months of study. More PC values were observed in some treatments as compared to As treated pots (*R. communis* and *C. indica*). In *R. communis* plants, maximum PC values were observed in the microbial consortium (1.71 and 0.66) inoculated plants after 1st month of sampling (Cd 50 and 100 mgkg^{-1}) followed by mycorrhiza (1.51 and 0.92) and rhizobacteria (1.40 and 0.59) respectively. Whereas in a 2nd month maximum PC values were observed in mycorrhiza (5.50 and 3.76) inoculated *R. communis* plants (Cd 50 and 100 mgkg^{-1}) with least PC values in rhizobacteria inoculated plants (4.89 and 2.03) respectively.

By the end of 3rd month, PC values again shifted from higher to lower in order of microbial consortium (14.7 and 6.93) > mycorrhiza (10.7 and 6.52) > rhizobacteria (10.4 and 6.35) in Cd 50 and 100 mgkg^{-1}inoculated *R. communis* plants respectively. But in *C. indica*, a similar trend was

observed for all the 3 months, where microbial consortium inoculated *C. indica* plants showed maximum PC values than mycorrhiza and rhizobacteria inoculated plants as shown in table 58. 1st, 2nd and 3rd month PC values for microbial consortium inoculated *C. indica* plants under Cd stress (50 and 100 mgkg^{-1}) were observed to be 0.10, 0.06; 1.79, 1.89 and 9.18, 9.76 respectively. Hence, the values of phytoextraction capacity (PC) shows that maximum potential to phytoremediate heavy metals from soil depends on the plant's biomass and the heavy metals accumulated in the parts of the plants. Here, a significant difference ($p \leq 0.05$) in PC values was observed after a 3rd month in *C. indica* and *R. communis* to be proven as good hyperaccumulators. Therefore, PC values for both the plants (>1) showed their good phytoremediation potential after 2nd and 3rd month.

Table 57: Effect of rhizobacteria, mycorrhiza and microbial consortium on phytoextraction capacity of *Ricinus communis* and *Canna indica* inoculated with arsenic (50 and 100 mgkg^{-1}).

Time duration →	1st month		2nd month		3rd month	
Treatment ↓	*R. communis*	*C. indica*	*R. communis*	*C. indica*	*R. communis*	*C. indica*
Control	ND	ND	ND	ND	ND	ND
C + As 50	0.34 ± 0.023^{d}	0.16 ± 0.023^{b}	1.04 ± 0.452^{g}	1.28 ± 0.045^{h}	4.41 ± 0.342^{n}	7.15 ± 0.871^{p}
C + As 100	0.28 ± 0.041^{c}	0.05 ± 0.022^{a}	0.84 ± 0.234^{f}	1.16 ± 0.231^{h}	2.14 ± 0.321^{k}	4.81 ± 0.312^{n}
As 50 + All B	0.56 ± 0.014^{e}	0.13 ± 0.012^{b}	2.02 ± 0.477^{k}	1.96 ± 0.032^{j}	7.51 ± 0.233^{p}	10.5 ± 0.612^{g}
As 100 + All B	0.25 ± 0.005^{c}	0.05 ± 0.019^{a}	0.95 ± 0.305^{f}	1.69 ± 0.029^{j}	2.96 ± 0.621^{l}	9.08 ± 0.312^{f}
As 50 + All M	1.31 ± 0.008^{h}	0.11 ± 0.051^{b}	3.90 ± 0.273^{m}	1.81 ± 0.134^{j}	9.28 ± 0.570^{r}	10.5 ± 0.639^{g}
As 100 + All M	1.21 ± 0.051^{h}	0.05 ± 0.021^{a}	4.14 ± 0.084^{n}	1.76 ± 0.219^{i}	10.5 ± 0.627^{s}	9.78 ± 0.913^{f}
As 50 + Const.	0.87 ± 0.214^{f}	0.14 ± 0.013^{b}	3.03 ± 0.948^{m}	2.27 ± 0.343^{k}	8.63 ± 1.017^{q}	12.2 ± 1.111^{t}
As 100 +Const.	1.98 ± 0.126^{j}	0.04 ± 0.017^{a}	6.71 ± 0.023^{o}	1.78 ± 0.124^{i}	10.5 ± 0.415^{g}	9.80 ± 0.891^{f}

Mean ± SD (n=3). Different small alphabets (a-s) indicate statistically significant difference at $P \leq 0.05$ by Turkey's test. (**C**: control, **As 50**: arsenic 50 mgkg^{-1}, **As 100**: arsenic 100 mgkg^{-1}, **All B:** all bacterial isolates (1-9), **All M**: All mycorrhiza cultures (1-3), **Const:** microbial consortium (All bacteria + all mycorrhiza)

Table 58: Effect of rhizobacteria, mycorrhiza and microbial consortium on phytoextraction capacity of *Ricinus communis* and *Canna indica* inoculated with cadmium (50 and 100 mgkg^{-1}).

Time duration →	1st month		2nd month		3rd month	
Treatment ↓	*R.communis*	*C. indica*	*R. communis*	*C. indica*	*R. communis*	*C. indica*
Control	ND	ND	ND	ND	ND	ND
C + Cd 50	1.17 ± 0.034^{c}	0.08 ± 0.034^{a}	2.32 ± 0.045^{i}	1.06 ± 0.022^{c}	5.64 ± 0.231^{m}	3.21 ± 0.112^{k}
C + Cd 100	0.61 ± 0.007^{b}	0.04 ± 0.023^{a}	1.82 ± 0.233^{d}	1.31 ± 0.455^{g}	4.38 ± 0.112^{l}	4.18 ± 0.894^{l}
Cd 50 + All B	1.40 ± 0.079^{c}	0.09 ± 0.013^{a}	4.89 ± 0.226^{l}	1.14 ± 0.131^{c}	10.4 ± 0.214^{q}	6.23 ± 0.699^{n}
Cd 100 + AllB	0.54 ± 0.038^{b}	0.05 ± 0.031^{a}	2.03 ± 0.438^{i}	1.77 ± 0.093^{d}	6.35 ± 1.064^{n}	9.43 ± 1.121^{p}
Cd 50 + All M	1.51 ± 0.041^{c}	0.09 ± 0.034^{a}	4.85 ± 0.041^{l}	1.50 ± 0.912^{e}	10.7 ± 0.060^{r}	7.32 ± 0.749^{o}
Cd 100 +AllM	0.92 ± 0.010^{b}	0.05 ± 0.019^{a}	3.76 ± 1.435^{k}	1.77 ± 1.011^{f}	6.52 ± 0.109^{n}	9.68 ± 0.981^{p}
Cd 50 +Const.	1.71 ± 0.013^{d}	0.10 ± 0.018^{b}	5.50 ± 0.352^{m}	1.79 ± 0.453^{g}	14.7 ± 0.295^{s}	9.48 ± 0.743^{p}
Cd100+Const.	0.66 ± 0.062^{b}	0.06 ± 0.034^{a}	2.38 ± 0.459^{j}	1.89 ± 0.281^{h}	6.93 ± 0.050^{n}	9.76 ± 0.812^{p}

Mean ± SD (n=3). Different small alphabets (a-s) indicate statistically significant difference at $P \leq 0.05$ by Turkey's test. (**C**: control, **Cd 50**: cadmium 50 mgkg^{-1}, **Cd 100**: cadmium 100 mgkg^{-1}, **All B:** all bacterial isolates (1-9), **All M**: All mycorrhiza cultures (1-3), **Const:** microbial consortium (All bacteria + all mycorrhiza)

CHAPTER 7
SUMMARY & CONCLUSIONS

SUMMARY AND CONCLUSIONS

The present study was designed to evaluate the potential of native microbes and wild species of plants found in the heavy metals contaminated land of Jalandhar region (Focal point, Industrial area). As these heavy metals are responsible for posing negative consequences for both environmental and human health, therefore, these toxic elements should be dealt with an eco-friendly technique using the native plants i.e Phytoremediation. One major strategy of phytoremediation is focused on hyperaccumulating plants that can accumulate/concentrate abundant heavy metals (contaminants) in the above and below ground parts. Hence, phytoextraction is considered one of the most important and explored approaches of phytoremediation. In the vicinity of Jalandhar city, various industries are present that are consuming different raw material consisting heavy metals and then disposing of off their treated/untreated effluents in the nearby water bodies, which is further used for agriculture purpose. Therefore, the present study is focussed on the ability of native microorganisms and plants to eradicate/detoxify these potent contaminants (heavy metals) from the soil.

Monitoring of inorganic contaminants (heavy metals) and other co-pollutants at the source point: For this study, soil samples were collected from the fields nearby some of the industries in the focal point, industrial area, Jalandhar (Punjab) and analysed for the physico-chemical parameters along with heavy metal content in them. After physico-chemical and heavy metal analysis, four sites were selected that showed maximum heavy metal content out of which two metals were found in abundance as compared to BIS standards and normal field soil.

Studies on natural populations of plants and microbes dominating the contaminated site: Two plant species were selected based on the higher biomass production and hyperaccumulating features, namely *Ricinus communis* and *Canna indica.* A total of 32 bacterial species and three arbuscular mycorrhizal species were isolated from the above-mentioned soil samples by different methods. Arbuscular mycorrhizal species were isolated from the soil samples by two different methods i.e wet sieving and decanting method and sucrose density gradient centrifugation with further quantifying them along with exploring their root colonization.

The selected AM fungal species were: *Glomus hoi, Glomus claroideum* and *Acaulospora kentinensis*, which were procured in bulk amount from Centre of Mycorrhizal research (CMR), The Energy Institute (TERI), New Delhi. These AM fungal cultures were further inoculated with host plant (*Sorghum bicolor*) in order to multiply them for further use in large-scale experimentation. Rhizobacterial species were isolated from the soil samples collected from polluted sites by two methods *viz.* Serial dilution and media enrichment methods. Out of these cultures, only 9 bacterial isolates were selected based on their minimum inhibitory concentration (2000 ppm). And were

further characterised by 16S rRNA technique and sequences were submitted to NCBI database for accession numbers. These selected bacterial cultures were then analysed for their biocompatibility or synergistic effects.

Developing a novel phytoremediation system of major toxicants: Selected native wild plant species (*Ricinus communis* and *Canna indica*) were inoculated with two different concentrations *viz*, 50 and 100 $mgkg^{-1}$ of arsenic and cadmium along with all rhizobacteria, all mycorrhiza and microbial consortium separately in pots triplicates to analyse various physiological and chemical parameters. Seeds of *R. communis* were procured from and were treated prior to sowing with sodium hypochlorite solution to obtain speedy growth of plants. Similarly, *C. indica* plants were collected from the same site and their roots and rhizomes were separated, washed and dried, prior to sowing in the pots. The experiment was set up for almost 90 days (3 months) for analysis of variant parameters including enzymatic activities, phytochemicals (total phenolic and flavonoid) content, physical parameters, wet weight/dry weight, photosynthetic pigments, heavy metal uptake and other phytoremediation factors (biological concentration factor, biological accumulation factor, translocation factor, tolerance index and phytoextraction capacity).

Determination of antioxidant enzymatic activity related to various plant parts in presence of heavy metals: Initially, prior to determine enzymatic activity, protein content was found to be increased significantly in first two months of time period and tremendously decreased after 3rd month with respect to control (without microbial inoculation) in all the inoculated plants of *R. communis* and *C. indica.* Protein content varied in all the treatments with respect to time and was found to be maximum in most of the plants (*R. communis* and *C. indica*) inoculated with microbial consortium and cadmium. Comparably, the enzyme activities (Catalase, ascorbate peroxidase, glutathione reductase and guaiacol peroxidase) also manifested uneven results in both the plants with respect to time and concentration. Maximum catalase (CAT) activity was possessed by leaves and root of both the plants inoculated with all mycorrhiza followed by the consortium and all rhizobacteria under arsenic stress but was vice versa under cadmium stress where CAT activity was found to be in the order of rhizobacteria < mycorrhiza <microbial consortium. CAT activity in leaves and roots of both the plants was found to be increased with time and decreased with concentration. Similarly, ascorbate peroxidises (APX) in both the leaves and roots of *R. communis* and *C. indica* showed enhanced activity with time and decreased activity with increasing heavy metal concentration. A significant increase in APX activity in all the leaves and roots of both the plants treated with arsenic and cadmium along with microbial consortium was observed where a significant increase was seen in roots in comparison to leaves of both the plants. Identical results were obtained for glutathione reductase (GR) that displayed more activity in roots than leaves regarding time and concentration under As and Cd stress in both the plants inoculated with the

microbial consortium. Also in guaiacol peroxidase (GPX), maximum activity was shown by roots than leaves of both the plants inoculated with the microbial consortium, following mycorrhiza and rhizobacteria in 1st and 2nd month but decreased significantly after a 3rd month in both leaves and roots of both the plants in all the treatments.

Heavy metals effect on phytochemicals and DPPH percentage scavenging activity in plants: After the phytochemical screening of both the plants, total phenolic and flavonoid content was found to be maximum in leaves than roots and stem of microbial consortium treated *R. communis* and leaves of *C. indica*. Total phenolic content increased after a 3rd month in all the treatments as compared to control (without treatment) and least TPC content was noticed in the control plants inoculated with only two concentrations of heavy metals. Also, TPC increased significantly with increasing concentration of As and Cd. An almost identical trend was observed for total flavonoid content (TFC) in both the plants where maximal flavonoid content was found in leaves of microbial consortium inoculated both plants in 1st two months but decreased significantly after a 3rd month in comparison to control (without treatment). Elevated TPC and TFC were noticed in leaves of both the plants inoculated with Cd at 100 mg kg^{-1}. DPPH scavenging activity also showed variable results with maximum percentage scavenging activity in roots of *R. communis* and *C. indica* inoculated with As and consortium. With increasing concentration of heavy metals, no significant decrease in percentage scavenging activity was observed but significant difference was found in percentage scavenging activity in leaves and roots of *R. communis* and *C. indica* under As and Cd stress with time in contrast to control. Further, quantitative and qualitative estimation of phenols and flavonoids was conducted by HPTLC method that resulted in variable values of TPC and TFC in leaves, roots and stem of *R. communis* and Leaves and roots of *C. indica*.

Determination of physiological, photosynthetic and phytoremediation parametersof various plant parts in presence of heavy metals: Physical parameters including plants height, wet weight/dry weight along with photosynthetic parameters (Chlorophyll a, chlorophyll b, total chlorophyll and Carotenoid content) were also affected adversely with two concentrations of heavy metals (As and Cd 50 and 100 mg kg^{-1}) as compared to control (without treatment). No significant difference was observed in the height of both the plants with increasing concentrations of As and Cd. Similarly, according to the height of both the plant's wet weight and dry weight of roots, stem and leaves of *R. communis* and *C. indica* varied with respect to time and concentration. Whereas, control plants (without treatment) manifested more wet weight and dry weight along with height. Overall more height, wet weight and dry weight were observed in plants inoculated with microbial consortium under arsenic stress.

Photosynthetic pigments (Chl a, Chl b, Chl $_{a+b}$, Chl $_{x+c}$) showed a significant decrease in chlorophyll content with increasing concentration and time in contrast to control. Overall maximum

photosynthetic pigments were found in pots of both the plants inoculated with microbial consortium along with cadmium. Also, photosynthetic pigments value dominated in *R. communis* than *C. indica* and were found to be least in control plants inoculated with only As and Cd. After analysing all these activities, both the plants were subjected to heavy metal analysis in different parts and soil. The results depicted the enhanced accumulation rate with time and concentration in all the treated pots, with maximum accumulation in pots of both the plants inoculated with the microbial consortium and cadmium (100 $mgkg^{-1}$) in comparison to arsenic and control pots (only treated with As and Cd). The sequence in which higher accumulation was observed in both the plants inoculated with: microbial consortium > mycorrhiza > rhizobacteria along with cadmium. Roots of both *R. communis* and *C. indica* accumulated maximum Cd and As with increasing time period as compared to leaves. Conversely, the content of heavy metals (As and Cd) in soil decreased with time in all the treated plants. After detecting the heavy metal accumulation in plant parts, various phytoremediation parameters were calculated and hyperaccumulating potential of both the plants was determined based on the values >1. Hence, BCF, BAF, TF, TI and PC supported the phytoextraction potential of *R. communis* and *C. indica* in contaminated land. Almost all these factors exhibited values more than 1 in all the treatments after 3 months of experimentation period that suggest the good hyperaccumulating capabilities of both the plants to accumulate heavy metals in their below and above ground parts by reducing the levels of contaminants in the soil.

From this research work, it is concluded that in comparison to all the rhizobacterial and mycorrhizal cultures, the microbial consortium was proved to be effective in increasing the hyperaccumulating/phytoextraction potential of *R. communis* and *C. indica* under heavy metal stress (As and Cd). Moreover, the microbialconsortium also enhanced the scavenging activity along with enzymatic activities in parts of both the plants that result in reducing the toxic effects of heavy metals in the contaminated soil. Also, the results suggest that both the studied plants along with microbial consortium own inflated ability to detoxify the ROS produced in response to heavy metal stress throughout its development and growth stages by depicting higher activities of antioxidant enzymes.

Hence, *Ricinus communis* and *Canna indica* plants along with microbial consortium manifested their phytoremediation potential for novel phytoremediation system of As and Cd metals from polluted soil. In future, detailed studies at molecular levels are required to find the exact mechanism behind the neutralization effect by plants and native microbes, the role of other enzymes to increase the efficacy of phytoremediation and various other biochemical pathways for reducing heavy metal toxicity in soil along with increasing the phytoextraction potential of native plant species.

CHAPTER 8

BIBLIOGRAPHY

BIBLIOGRAPHY

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