ANALYSIS OF PHYTOREMEDIATION CAPABILITY OF WEEDS AND FODDER PLANTS OF KALA SANGHIAN DRAIN, DISTRICT KAPURTHALA FOR HEAVY METAL EXPOSURE AND SUITABILITY FOR CONSUMPTION AFTER EXPOSURE.

A Thesis

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in

(BOTANY)

By

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DECLARATION

I hereby declare that the thesis entitled**," Analysis of phytoremediation capability of weeds and fodder plants of Kala Sanghian drain, district Kapurthala for heavy metal exposure and suitability for consumption after exposure"** submitted for Ph.D. Botany Degree to School of Bioengineering and Biosciences, 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 award of any other degree.

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CERTIFICATE

This is to certify that **Mrs. Narinderjit Kaur** has completed the Ph.D. Botany titled**, "Analysis of phytoremediation capability of weeds and fodder plants of Kala Sanghian drain, district Kapurthala for heavy metal exposure and suitability for consumption after exposure "** under my guidance and supervision. To the best of my knowledge, the present work is the result of her original investigation and study. No part of this thesis has ever been submitted for any other degree or diploma. The thesis is fit for the submission for the partial fulfillment of the condition for the award of degree of Ph.D. in Botany.

Dr. Anand Mohan

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THESIS

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ABSTRACT

The present work analyses the phytoremediation ability of weeds and fodder plants growing in Kala Sanghian Drain region by exposure of heavy metals in pot experiments using different concentrations of metals and further checking the suitability of milk of grazing animals in that area for human consumption. Present work had been divided into three parts. In the first part effect of different concentrations of heavy metals on various morphological, biochemical and enzymatic parameters were studied on selected weeds. In the second part heavy metal uptake in the roots of selected plants were observed and in the third part effect of heavy metals on milk of grazing animals of studied area were analyzed by comparing with standard milk samples. Metal accumulation was seen in grazing animals of Kala Sanghian drain and milk was taken from dairy surrounding those regions.

Effect of heavy metals on various parameters of the selected plants: In the present study, three plant species (*Achyranthes aspera, Eruca sativa, Cyperus iria*) were selected for finding out their phytoremediation capacity. Two heavy metals, i.e., Chromium and Lead were used by taking salt of Chromium as Potassium Dichromate and of Lead as Lead Nitrate. The various concentrations of Chromium were 0 mg/Kg, 50 mg/Kg, 100 mg/Kg, 150 mg/Kg, 200 mg/Kg, 250 mg/Kg, 300 mg/Kg, 350mg/Kg and 400 mg/Kg of Chromium. Different concentrations for Lead were 0 mg/Kg, 100 mg/Kg, 200 mg/Kg, 300 mg/Kg, 400 mg/Kg, 500 mg/Kg, 600 mg/Kg, 700 mg/Kg, 800 mg/Kg and 900 mg/Kg Lead. However, no plant growth was observed at higher concentrations of Pb and Cr (400 mg/Kg of Cr and 900 mg/Kg of Pb). Therefore, these concentrations were not taken into consideration in tables and graphs. During the study, various parameters that were studied include morphological parameters (root length, shoot length, fresh weight, dry weight and moisture content), biochemical parameters (chlorophyll a, chlorophyll b and total chlorophyll) and antioxidant enzymes (CAT, POD). The results obtained on morphological parameters of selected plants showed the significant decrease in growth parameters with increase in concentration of both heavy metals at $p > 0.0001$. The results obtained on biochemical parameters also showed significant decrease ($p>0.0001$) with increase in concentration of both heavy metals. Antioxidant enzymes are the category of enzymes that can be produced against oxidative stress caused by various environmental factors. They are produced in plants as defence mechanisms to protect them from various stresses. In the present research

work, effect of two antioxidant enzymes, i.e., catalase and guaiacol peroxidase enzymes were studied under heavy metal stress. It resulted in significant increase in the specific activity of both antioxidant enzymes with increase in concentration of both heavy metals up to a certain limit. After that, decrease was observed in the amount of enzyme activity at a very high concentration.

Statistical analyses were carried out using one-way ANOVA (Graph Pad Prism version 8.4.1) and multiple comparisons was done by Tukey's multiple comparison test. All the results were compared with previous studies showing same trend.

Uptake of heavy metals in the roots of selected plants: Plants can accumulate heavy metals in their various parts through phytoextraction mechanism and thus can be used as hyper accumulator species in various heavy metals contaminated soils. In the present study, uptake of heavy metals in the roots of selected plants were analyzed using atomic absorption spectrophotometer (AAS) and it was found that *Cyperus iria* accumulated more chromium (Cr) followed by *Eruca sativa* and then *Achyranthes aspera* also *Cyperus iria* contained more lead (Pb) in its roots followed by *Achyranthes aspera* and then *Eruca sativa.* Therefore, from the study it is concluded that *Cyperus iria* can be used for phytoremediation of chromium and lead contaminated areas.

Metal accumulation in the milk of grazing animals: Metal content in milk are of particular concern as milk is largely consumed by infants and children and it is observed in other studies that metal concentration could vary with season, age and breed, dairy period and many other factors. In this part, metal accumulation in the milk of grazing animals was done by inductively coupled plasma mass spectrophotometer (ICPMS) and then compared with standard milk samples to find out their accumulation in the milk sample. The study concluded that amount of chromium and lead were less than permissible values and generally milk is safe to consume in the studied area.

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Narinderjit Kaur

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CHAPTER- 1

INTRODUCTION

Environmental pollution in the form of soil, air and water is increasing day by day. Different type of pollutants are responsible for causing environmental pollution. Heavy metal pollution is one such pollution caused by heavy metals. Metals that are denser than water by 4g/cm^3 are called heavy metals (Nagajyoti *et al*., 2010). In the periodic table, these metals are given position among transition metals having atomic mass between 63-200.59 g and specific gravity greater than 4 (Kennish, 1992). The important toxic metals are As, Cd, Cr, Hg, Pb, Zn, Cr etc. that cause environmental pollution (Raskin *et al*., 1997; Lasat, 2000). Plants as nutrients use some of these metals and some have no known function. In reality, metals are the components of the soil as these are present in less amount in the rocks. However, when their amount in the soil becomes high then these metals become toxic heavy metals, which are also non - biodegradable. They have very long life span. Therefore, they persist in the environment for longer time and cause pollution of mostly soil and water.

Heavy metal pollution has spread all around the world. It costs much threat to the life of all living organisms including human beings. Main root cause of this pollution is increasing urbanization, industrialization, change in land ore patterns and distribution and increase in population. Heavy metals enter in the atmosphere by human and nature's activities. Nature's resources include breaking of rocks, soil removal and volcanic activities and man - made sources are use of metals in agricultural tools and fertilizers, mining industry, smelting and electroplating industry, paint and varnishing industry etc. (Ali *et al*., 2013; Dixit *et al*., 2015). Some of them play no role in plant and animal life. They include As, Pb, Cd and Hg (Rai *et al*., 2019). United States Environment Protection Agency and others have added them in the list of harmful substances (Khalid *et al*., 2017; Rai, 2018; ATSDR, 2007). Some of the heavy metals are important components of the biological system including Cu, Fe and Zn. These metals become toxic if their amount is more than prescribed limits.

Heavy metals being persistent in nature are accumulated in soils and plants. Although some of them are required in small quantity to the plant as micronutrient, but if their concentration increase beyond threshold value, they become deleterious to them and produce various deficiency symptoms like necrosis, chlorosis, stunted growth and may be even death in severe cases. Toxic effects of heavy metals may occur due to

attachment of metals with protein side chains, causing the structural disruption or their working may get impaired leading to appearance of above given symptoms in plants (Sharma *et al*., 1997; Vajpayee *et al*., 2000; Hall, 2002).

Heavy metal stress led to generation of formation of ROS causing oxidative stress in plants. Plants eliminate this stress by producing different type of antioxidant enzymes like SOD, POD, CAT, GR etc. SOD and CAT scavenge free radicals like O_2^- into hydrogen peroxide (H_2O_2) which is further broken down to H_2O by APOX and GR. Thus, antioxidant defense system plays an important role to relieve the plants from heavy metal stress and to boost up their resistance power.

Content of heavy metals are also documented in various food crops and fodder plants and from them in domestic animals and human beings through food chain. Food crops and weeds growing on polluted sites are playing major role in transfer of heavy metals through uptake by roots from soil and transferred to higher trophic level through food chain.

Pollution by these heavy metals is a greater problem all around the world. To solve this problem, different workers use different methods. These are – physical methods, chemical methods and biological methods. Brief description of these methods are given in the coming paragraphs.

1.1 Physical method: Sedimentation, Screening, Filtration.

1.2 Chemical methods: Chemical precipitation, Coagulation, Ion exchange, Adsorption, Neutralization, Membrane separation.

1**.2.1 Chemical precipitation**- In this method different chemicals are used for removing metals that form insoluble precipitates with them and then removed by sedimentation and clear water is separated from waste water by decantation method. In this method, chemicals mostly used are hydroxide [as Ca (OH_2) and Na (OH)] as precipitant for removal of Cu and Cr heavy metals (Mirbagheri and Husseini, 2008) and sulphides in the form of thiol ligand for removal of Cu and Cd (Matlock *et al*., 2001)

1.2.2 Ion exchange –Different types of resins are used for heavy metal removal. They may be artificial or natural resins. For example Purolite C 100 resin for Pb separation by Badawy *et al*., (2009), INDION 225H resin for Cu removal by Thakare and Jana, (2015), AMBERJET 1200Na resin for Ni and Pb by Zewail and Yousef, (2015).

1.2.3 Coagulation and Flocculation – In coagulation process, smaller soluble or insoluble particles combine to form larger aggregates called coagulants that are used for heavy metal removal. Some of the coagulants are Aluminium sulphate, Magnesium chloride, Polyaluminium chloride etc. (Renault *et al*., 2009). In flocculation technique, flocs are used for heavy metal removal like PAC, sodium dodecyl sulfate, poly ferric sulfate etc. (Fuand Wang, 2011).

1.2.4 Membrane Filtration – These techniques vary depending upon the type of membrane. It further includes various techniques like RO, ultra-filtration and nano filtration. In RO (reverse osmosis) technique, Mohsen – Nia *et al* (2007) had removed copper and nickel from wastewater by Na_2 EDTA and Ozaki *et al* (2002) had removed Cu, Ni and Cr by aromatic polyamide (ES20) membrane. In ultrafiltration, low pressure is used to remove dissolved and colloidal materials by trapping heavy metals in outer part micelle formed by anionic surfactants. This process removes Cd and Zn from wastewater by Landaburu- Aguirre *et al*., 2010. Nano filtration is a method with size of pores of membrane between RO and ultra-filtration technique. Mehdipour *et al* (2015) had removed Pb from wastewater by using this technique.

1.2.5 Adsorption – This technique is more advantageous than the previous ones as it has high efficiency of metal removal, less cost, less sludge production. Adsorption is a surface phenomenon that is different from absorption. Different type of adsorbents applied for excluding toxic metals are - activated carbon (Sardella *et al*., 2015), zeolite, waste of agriculture, sunflower (Jain *et al*., 2010), pith of coconut (Suksabye and Thiravetyan, 2012), marine algae, plant biomass, bacterial and fungal bio sorbent etc. for bio sorption. Some of the drawbacks of all these techniques are - high cost, wastage of energy, less separation, need phase change, large sludge production, health problems, not environmental friendly, less efficiency etc. So these are less preferred for heavy metal removal. Preferred methods are biological methods such as bioremediation and phytoremediation.

1.3 Biological methods: Bioremediation and phytoremediation.

1.3.1 Bioremediation – Conventional methods of soil decontamination like land filling and digging up or incineration are not very useful as one has to find new areas for land filling every time. Instead of these the use of living microorganisms like bacteria, fungi etc. to remove or reduce heavy metals from polluted sites is called bioremediation. By this process, organic matter present in the waste is broken down into less toxic form by

the microbes but these microbes mostly work under aerobic conditions. (Colberg and Young, 1995) and some of the contaminants may not be degraded by microbial attacks. Thus, phytoremediation as a branch of bioremediation came into existence in the last two decades. It is also called green technology that uses different types of plants to decontaminate the polluted site. It is of less cost, ecofriendly and much effective to treat heavy metal pollution.

Type of bioremediation process depend upon factors like the soil microbe types, nature and contaminant type, cost of technique and the environmental conditions prevailing in the area (Smith *et al*., 2015). Two approaches are used for bioremediation technique: in - situ and ex – situ approach. In in - situ approach, the pollutants are treated at the original site without disturbing the structure of soil, and without digging up of earth. Various in-situ techniques are bioventing, bio slurping, biosparging and phytoremediation. In ex-situ methods of bio-remediation, the pollutant is excavated from the polluted site and transported to another site for making it harmless, thus disturbing the structure and properties of the soil. These are mostly costlier than in-situ techniques of bioremediation. These include bio pole, bioreactor, land farming etc.

1.3.2 Phytoremediation – It consist of two words –'phyto' - means plant and 'remedian'- means to remove or clean.

There are five main types of phytoremediation. **Rhizofiltration**- In this technique, both aquatic and terrestrial plants are used to filter the contaminants from water sources and their concentration in plants roots. This technique is mainly used for Cu, Zn, Pb, Cd and Ni heavy metals released by the industries or agricultural practices (USPA 2000). **Phytostabilsation** – It means to stabilize the movement and availability of the contaminant in the soil, so that contaminant cannot leach into underground water table and to other non-contaminated areas. **Phytoextraction** – Among all types, the most commonly used method to decontaminate the soils without disturbing their properties is the phytoextraction method. In this method, the contaminants from soil are transferred into the harvestable parts of plant. For the effectiveness of this technique, two approaches are used. (a) Continuous phytoextraction - by plant itself. (b) Chelate assisted phytoextraction - phytoextraction by binding metal chelators to enhance the metal uptake (Salt *et al*., 1997). Plants that have the ability to extract and accumulate greater amount of heavy metals from soil into their roots and then to shoots are called hyper accumulator plants. **Phytodegradation**- It is the process of breakdown of

complex organic compounds into simple forms and then to introduce them into their cells and tissues to be used later on. **Phytovolatilization** – In this process, contaminants taken from soil along with water enter into the leaves, from where they are transpired in volatile form into the atmosphere but the drawback of this method is that contaminant can recycle back into the soil along with the rain, for example Hg (Henry, 2000).

Some factors to be kept in mind while considering a plant to be used as phytoremediator are – the type of root system, amount of biomass produced, plant's adaptability to prevailing conditions, toxic effects of metals on the growing rate of plant and time needed by it to remediate the pollutant from the polluted area. For effectiveness of the process, native plants should be used. By phytomining process, some useful heavy metals can be recovered from soil and can be used for various purposes such as use of selenium containing substances for bio fortification (Wu *et al*., 2015)

For the removal of heavy metals, various crop plants were used by different researchers but as metals can accumulate in their harvestable parts; these metals can enter into the human body leading to some of the problems. To prevent these problems, wild weeds are used for phytoremediation purpose due to their high biomass production and storage of vast amount of heavy metals in their parts.

So in the present work, some of the wild weeds and fodder plants were used for their phytoremediation ability. These are *Cyperus iria, Achyranthes aspera* and fodder plant *Eruca sativa*.

Two metals Chromium as potassium dichromate and Lead as lead nitrate were used as heavy metals in the present study to find their amount accumulated in the selected wild plants for the present study. Some of their characters, sources of occurrence in environment and importance are given ahead.

1.4 CHROMIUM: Chromium is an element with symbol 'Cr' and atomic number 24. It is present in sixth group of the modern periodic table. It is shiny transition metal. Chromium is the 3rd hardest element after Diamond and Boron. The melting point of chromium is about 1907˚ C. Atomic mass of chromium is 51.996. It has many oxidation states i.e. from zero to six but most common are chromium (III) and chromium (VI). It is obtained from its ore chromite ($FeOCr_2O_3$). Main chromium producing countries are South Africa, Kazakhstan, India and Turkey (USGS, 2017). Hexavalent chromium is more carcinogenic than trivalent chromium. It can enter the

human body via soil, water, air and by eating contaminated food. It occurs in the form of halides, oxides and sulfides. Cr is released from environment and human made sources. Natural sources are - volcanic eruptions and rocks. Manmade sources are - various industries like textile, leather tanning, electroplating, mining, agricultural equipment etc. (Moncur, 2005). It is a toxic heavy metal. It can be used as a salt of chromate or dichromate.

1.4.1 Importance: Hexavalent chromium is commonly used as oxidizing agent in steel production industry, Cr plating, in coloring and pigments industry, in preserving wood, treatment of cooling tower water. It may also be used as anti-corrosive in paints, primers and other surface coatings. Hexavalent chromium causes toxic effects on respiratory system, liver, skin and eyes of human beings. It can enter in water by weathering of rocks containing chromium or from industrial discharge. In soil, it can enter by dumping of industrial waste or from rock sediments. According to Hossner (1996), 0.5 to 5 mg/ml of Cr can produce toxicity symptoms in plant in solution form and 5 to 100 mg/g of its concentration is toxic for plants if present in soil.

1.5 LEAD: It is an element with symbol 'Pb' and its atomic number is 82. It is in solid form at room temperature. It is also a heavy metal, soft and malleable with melting point 327.5° C. Its color is silver with bluish tinge when freshly cut, changing to dull grey color on exposure to air. Its atomic mass is 207.2. Its most common oxidation state is +2. The main countries producing Pb are Australia, China, Ireland, Mexico, Russia and USA (USGS, 2017).

1.5.1 Importance: Lead is used in lead-acid batteries, for bullet making, in scuba-diving weight belts (Krestovinikoff and Halls, 2006), in statues and sculptures, as architectural metal, in roofing material, cladding, flashing, in gutters, (Weatherings to Parapets, 2017), in gasoline, in paint, in plumbing, in pipes, in ceramics, in solders, in cosmetics etc. Lead is a toxic and poisonous heavy metal that causes severe damage to living beings. It enters the human body through inhalation, ingestion or skin absorption. It can cause much damage to brain and kidney leading to death in some cases. Lead is rare in nature. It is usually found in ore form along with zinc and silver. It occurs in small amount in ores such as Glena, anglesite and cerussite.

In India, fodder plants, agricultural crops and some wild weeds are cultivated or naturally grow along various wastewater drains. In addition, municipal water is used to irrigate the crops that contain high amount of different heavy metals. In the present study, Kala Sanghian drain was taken into consideration.

1.6 About Kala Sanghian: It is a village in Kapurthala. Average rainfall is about 70 cm. Kala Sanghian is located 19 km towards South from district headquarter Kapurthala. It lies in the mid of Nakodar and Kapurthala. Kapurthala is one of the historical and smallest district of Punjab in terms of area and population. It is divided into two non-contagious parts –one is Kapurthala - Sultanpur Lodhi portion that lies between 31° 07' and 31° 22' North to 75°40'and 75°55'East.

Kala Sanghian drain is the most polluted drain in the area starting from Himmatpur village and ending in Malsian village joining with Chitti bein and finally falling into Sutlej river. The drain carries waste from leather industry, focal point and surgical complex. There are more than 200 electroplating units in Jalandhar and most of which do not have waste treatment plants, so untreated waste containing toxic chrome effluents are discharged into this drain resulting in its pollution. Large population of the area is suffering from various diseases like skin rashes, eye, stomach and respiratory problems and even cancer due to consumption of polluted ground water. To decontaminate the soils and wastewater of the area, the present study was undertaken by using the phytoremediation capacity of some of the wild weeds and fodder plants with the following objectives.

- Screening and selection of weeds and fodder plants of Kala Sanghian area for phytoremediation capability.
- Carrying out of pot culture experiments with differential exposure of chromium and lead heavy metals.
- Analysis of morphological, biochemical and reproductive parameters after exposure of heavy metals.
- Analysis of phytoremediation capability along with statistical analysis and selection of appropriate weed.
- Analysis of effect on grazing animal milk quality through consumption of metal absorbing fodder plants.

CHAPTER- 2

REVIEW OF LITERATURE

Environmental pollution can occur due to industrialization and extraction of natural resources in large scale, and it is responsible for degradation of environmental health. Among all kinds of pollution, heavy metals are major contributors to the environmental pollution (Nedel Koska and Doran, 2000). The metal present in soil can easily enter into food chain through plants and crops and also risky for humans, animals, plants and whole environment (Farouk *et al*., 2011).

2.1 Definition of weeds:

Most plants used as phytoremediator of contaminated soils or waters produce low biomass, used for eating purpose and may not tolerate the different agro climatic conditions. To tackle with these limitations of plants, scientists are showing interest towards the use of wild weeds for heavy metal removal. They can grow in any type of environmental conditions without the use of synthetic fertilizers. If grown in heavy metal polluted sites, they produce large biomass without much damage. They also grow very fast and are easy to harvest. Many researchers have done work on wild weeds and studied their heavy metal accumulation nature (Eddy & Ekop, 2007; Khankhane and Varshney, 2008; Sanghamitra *et al*., 2011; Karitha & Jegadeesan, 2014; Hammami *et al*., 2015; Singh *et al*., 2016; Kassaye *et al*., 2017; Subha & Srinivas, 2017; Subhashini *et al*., 2017).

Different workers define weeds differently. Anything that does not require much care for growth may be regarded as weed. Weeds are the plants that are undesirable in a particular place or the plant growing where it is not wanted. Due to their interference with the desirable plants, they are mostly removed from the fields or open areas. Some of the characteristics of weeds are- abundant seed production, rapid population, long time survival, high adaptability, capacity to grow in human disturbed places etc. Weeds cause many problems. They compete with cultivated crops for light, water and space. They reduce crop yield, serve as host for crop diseases.

Although weeds are regarded as unwanted plants, still they possess many beneficial properties in different fields such as medicine, organic farming, phytoremediation, food, fodder etc. There are some more advantages of weeds as well for example: they help in proper maintenance of soil, add organic matter, provide germ

plasm, give chances for employment to people, food for wildlife and can be consumed by human beings.

2.1.1 General characters of selected weeds and fodder plants for present study-

2.1.1.1 General characters of *Cyperus iria*: It is commonly called rice flat sedge and belongs to Cyperaceae family found in various parts of the world. It is annual to perennial herb having fibrous and yellowish red roots, about 10-70 cm tall. Stem is tufted and angled, leaves are green, rough to touch having reddish brown leaf sheath, inflorescence is simple to compound and fruit is dark brown to black in color (Holm *et al*.,1997). It was first described by Linnaeus (1753). The genus *Cyperus* contain about 300 species (Lye, 1981). Reproduction occurs by seeds. Seeds can germinate between 40-50˚ C temperature on the surface of soil. Chromosome number vary from n=56 to n = 64 (Bir *et al*., 1992). It shows kranz type leaf anatomy, thus a type of C_4 plant (Lin *et al*., 1982). Many species are used by humans for example *C. papyrus* of Africa for making sleeping mats, *C. textilis* for making mats in India, *C. esculentus* tubers as vegetables, *C. articulatus* used as spice and in cosmetic industry (Atala, 2012) and some are grown as ornamental plants.

2.1.1.2 General characteristics of *Achyranthes aspera*: It is a genus of medicinal and ornamental plants in family Amaranthaceae. The genus includes many species but among these, two are common species from medicinal point of view, these are *Achyranthes aspera* and *A. bidenta*. *Achyranthes aspera* is a common introduced weed in tropical areas. It is also annual to perennial herb about 1- 2m tall. Leaves are opposite and simple in arrangement and round in shape. Stem is hard at the base; inflorescence is terminal and axillary with many flowers having numerous seeds that are oval, small with persistent bracts. Fruit is capsule, orange to reddish brown in color (Smith, 1981). It mostly occurs in wastelands, roadsides, railway tracks, etc. It has many medicinal properties such as anti-inflammatory (Girach *et al*., 1992), anti-cancerous (Chakraborty *et al*., 2002), anti-fungal properties (Sharma *et al*., 2011). The flowering and fruiting time is between September to April.

2.1.1.3 General characteristics of *Eruca sativa*: It is a genus of flowering plants belonging to family Brassicaecae. The genus includes about five species. *Eruca sativa* is an annual herb with common name True Rocket or Rocket Salad. It is up to 10-100 cm tall possessing true roots, branched stem and compound leaves. Flowers are bisexual, yellowish white in color, fruit is siliqua and seeds are yellowish brown to

reddish with oval to round shape (Garg and Sharma, 2014). It is a winter crop with a growing period from end of September to March. It is also considered as weed plant (Al-Shehbaz, 1985). Plant can grow from seeds or through tissue culture technique (Bianco, 1995) *Eruca* species are used as oil seeds, as fodder plants and as vegetable crops.

2.2 Heavy metals

Heavy metals are a part of the environment from the very beginning of earth, but overuse of resources and anthropogenic activities involving combustion of fossil fuels, industrial discharge and solid waste discharge has mostly concentrated these metals in the nature (Sharma *et al*., 2016). Although, uses of heavy metals and their harmful effects are known for more than centuries, but now a day, there is much increase in their use and further release into the environment at much higher rate (Järup, 2003; Inoue, 2013).

Heavy metals are defined variously. Some of these definitions are based on the following aspects**:**

- Physical aspects:
- Reactions to the chemicals:
- Harmful effects on living organisms:

Physical aspects are based on density of heavy metals. Various levels of densities are proposed in different times and metals above those levels are said to be heavy metals. Previously metals with density 7 g/cm^3 or above it was considered toxic but some toxic metals like arsenic are not included in this category. Recently, metals having density higher than 3.5 g/cm^3 are considered heavy metals (Duffus, 2002; Hodson, 2004; Appenroth, 2010).

Based on chemical reactivity, those metals are known as heavy metals, which are highly reactive. These metals have the capacity to form bonding with Sulphur containing substances and thus become toxic. Toxic results can occur due to replacement of essential metals with these heavy metals in the metabolic reactions. Toxic effects were confirmed by some researchers with the replacement of Zn^{2+} metal with Cd^{2+} in some of the biological reactions (Babula *et al*., 2008; Appenroth, 2010; Luque-Garcia *et al*., 2011).

Many studies show that heavy metals cause toxicity to living organisms (Appenroth, 2010; Nagajyoti *et al*., 2010; Saha and Panwar, 2014). An experiment was done on *Lemna minor* to study the growth parameters of plant under various heavy metals stresses. Based on this study, the trend of toxicity of metals to *Lemna minor* was $Ag^{+} > Cd^{2+} > Hg^{2+} > Cu^{2+} > Ni^{2+} > Zn^{2+} > Co^{6+} > Cr^{6+} > As^{3+} > As^{5+}$ (Appenroth, 2010).

Heavy metals enter the environment through weathering of rocks, volcanic eruptions and flooding in natural way but human activities are mainly responsible for releasing harmful waste into the biosphere, thus adding more and more of toxic metals in the environment. It was also confirmed by some scientists that certain chemical reactions like redox reactions, acidic and basic reactions and change in value of pH of soil and water are responsible for increasing the amount of toxic metals like Cr, As etc. in the environment by converting them to more soluble form (Hashim *et al*., 2011; Varalakshmi and Ganeshamurthy, 2012).

Heavy metals when released in environment move along biotic and abiotic factors and attach to the air-borne particles through their natural path such as dust release or volcanic activities (Csavina *et al*., 2012). On water bodies, pollutants are added from air borne particles, industrial and domestic discharge and agricultural run-off. Drinking water sources contain high concentration of lead and Cd in the whole world. Soil is also not free from toxicity of harmful metals. It acts like a dustbin where waste can be disposed of in large amount. From soil, metals could also leach to underground water and via wind into atmosphere. (Tomáš *et al*., 2012; Aziz *et al*., 2015). Below given factors are responsible for transferring heavy metals along different components of environment:

- Exchange of ions in different components of environment.
- Precipitation.
- Adsorption and desorption.
- Through mobility or immobilization of biotic factors.
- By plants directly through roots from soil.

The distribution of metals among various biotic and abiotic components is shown in figure 2.1.

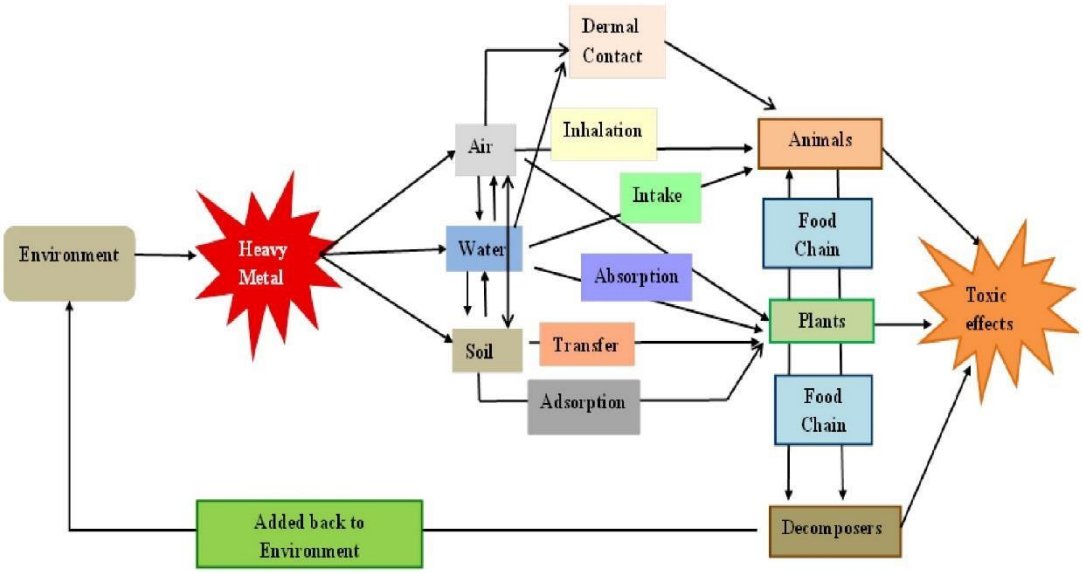


Fig 2.1: Distribution of metals among various biotic and abiotic components of environment.

2.3 Heavy metals in soil

Soil act as main means for the nourishment of life. It acts as a sink for nutrients and provides a medium for the completion of various biogeochemical cycles. As discussed earlier, heavy metals are added to soil through natural weathering of rocks, discharge of polluted waste and wastewater on soil, deposition from atmosphere etc. Many researchers reported the occurrence of heavy metals in agricultural soils across the world (Zhuang *et al*., 2009; Iqbal *et al*., 2011; Varalakshmi and Ganeshamurthy, 2012). Iqbal *et al* (2011) reported buildup of heavy metals like Ni, Cd, Cu, Cr and Co in the samples of soil taken from agricultural lands of Pakistani Punjab irrigated with wastewater. In Dar Es Salaam City of Tanzania, the amount of Pb, chromium and copper in some samples of soil was reported to be 22.85 mg/Kg, 502.33 mg/Kg and 21.073 mg/Kg respectively (Mwegoha and Kihampa, 2010). In addition, it was reported that the content of heavy metal was highest in the top layer and decreased downwards. An experiment done at Zimbabwe to know the effects of long term watering the maize plants with wastewater. The results showed that the amount of some of heavy metals such as Cu, Zn, Cd, Ni and Cr in soils was more than the permissible limits (Masona *et al*., 2011). In another study carried out in Bangalore, India, it was found that the soil getting sewage water has maximum concentration of chromium followed by lead, nickel and cadmium (Varalakshmi and Ganeshamurthy, 2012). In a flyash

contaminated site near thermal power plant in Uttar Pradesh, India concentration of cadmium was found to be maximum followed by Fe, Ni and Pb (Singh *et al*., 2010b). In Baoding City, China the sewage water irrigated soil had much higher concentration of zinc (153.77 mg/Kg), lead (38.35 mg/Kg), copper (35.06 mg/Kg), nickel (29.81 mg/Kg) and cadmium (0.22 mg/Kg) than the control soil (Xue *et al*., 2012). In another study done around sulfuric acid factory of Western Guangdong Province, China, Liu *et al* (2012) reported significant heavy metal contamination (Tl, Pb, Cu, Zn, and Ni) of agricultural soil. Copper content in agricultural soils near Narora Atomic Power Station, India was 27.95 mg/Kg (Singh *et al*., 2014). Li *et al* (2019) reviewed the sources of contamination of soils with heavy metals, their harmful effects and the various techniques that can be used to decontaminate heavy metals.

Heavy metals are taken up by plants through various openings like tip of roots, different junctions of roots, cuts and area around the root. Other ways for the entrance of metals into the plant tissues were found to be upper protective layer of epidermis, stomata and openings of the leaf tips called hydathodes (Peralta-Videa *et al*., 2009; Guala *et al*., 2010). The amount of metal absorbed by the plant depends upon some factors like – the properties of soil (physical, chemical etc.), number of various openings, number of branches on root and their length and area of the leaf. Upward movement of metals occur through xylem and phloem after their absorbance by different parts of the plant. After entering the plant system, metal produce toxic symptoms that depend upon some factors like –

- Size: It means if the size of metal is small; the rate of penetration is more.
- Concentration: It means if metal is concentrated more, it can produce more effect.
- Chemistry of ions: Some metallic ions are more reactive than others are. For e.g. metal ions like, $Hg^{2+,}$ Cd^{2+} and Pb^{2+} are more attractive towards those amino acids that contain sulphur in their structure.

2.4 Heavy metal content in fodder plants, milk, meat and other dairy products and their effect on human health:

Heavy metal content in forage, animal body, milk, meat and dairy products have been studied by many researchers all around the world. Food crops are mostly grown in soil and soil get contaminated with heavy metals from atmosphere deposits, domestic animal manure, use of pesticides and fungicides, watering the crops with polluted water,

excessive use of fertilizers etc. (Chary *et al*., 2008; Mansour *et al*., 2009; Gall *et al*., 2015; Woldetsadik *et al*., 2017). Li *et al* (2017a, 2017b) grew lettuce, amaranth, spinach, cowpea and rice in Hg contaminated soil and confirmed that these crops contain metals that were harmful for human life. Ahmad *et al* (2009) and Sobukola *et al* (2010) studied high amount of Pb in some forage species. A study was done in Egypt to check the occurrence of some metals like Cd, Pb, Cu, Ni, Al and Hg in milk of cows and buffaloes feeding on reed and barseem plants and results showed that heavy metal content in reed plants was higher as compared to barseem. Rozso *et al* (2003) also found large amount of Pb in fodder and roughage grown in agricultural areas. Some metals were detected by Mor (2005) in fodder grass in high amount than in straw. In one of the study done in Andhra Pradesh by Devasena *et al* (2012) confirmed that dry forage like paddy and groundnut straw contain high amount of Cr (< 1.6 mg/Kg) than green forage. A study was done by Sathyamoorthy *et al* (2016) to observe the amount of Pb, Cd, As and Hg in cattle meat and fodder samples and the results confirmed that all the given heavy metals were more than permissible amount in meat samples and amount of Cd, As and Hg were more than required in fodder samples. Ahmad *et al* (2013) studied that soils and fodder plants contaminated with heavy metals like cadmium, chromium and lead could be a possible risk to livestock. They collected samples during different seasons and from different locations. The results showed that location and season have different effect on soil- metal concentration and soil in summer had higher concentration of Pb, Cd and Cr than winter season soil.

Malik *et al* (2017) conducted a study to assess the heavy metal content in fodder crops irrigated with Hudiara drain in Pakistani Punjab. Along with it, they also checked heavy metal content of milk of cattle's that graze on those drain contaminated fodder crops. The results confirmed that drain water was polluted with Cd, Cr, Ni, Fe, Cu and Mn. All the ten samples of fodder crops studied were contaminated with all heavy metals and their concentration were more than recommended dose that was a serious matter of concern

Shen *et al* (2018) studied destructive effects of some heavy metals on health of human beings and animal (sheep) near zinc smelter area of China. The amount of copper, cadmium, lead and zinc in contaminated pastures were higher in irrigated water, soils and forage than in healthy pastures. Concentrations of Cd and Pb were 177.82 and 16.61 times higher in fodder crops than control. Both metals were also in high amount in the tissues of sheep feeding on contaminated forages. Thus, the study concluded that

sheep, food and grain were seriously harmed by heavy metals in studied area that was a riskier factor for human health.

Amount of four heavy metals i.e. Cd, Cr, Pb and Ni were studied in three fodder plants i.e. barseem, sorghum and maize and milk samples from cattle fed on these fodder samples by Farid and Baloch (2012) in Faisalabad, Pakistan. Sources of heavy metals were untreated city effluent. The result of the study confirmed the increased concentration of all the heavy meal in soil, fodder and milk samples above the permissible limits. Similar kind of study was done by Malik *et al* (2017) to assess the heavy metal content in fodder crops irrigated with drain wastewater in Pakistani Punjab. Ten different sites were surveyed during the study and various heavy metals were detected in fodder crops above the recommended dose. The concentration of Ni, Cd, Cr, Mn, Fe and Cu were maximum in fodder crops of Hudiara drain water. In addition, the milk samples collected from cattle grazing on the fodder crops contained high amount of various heavy metals. Bhatti *et al* (2016) studied heavy metal content in barseem (*Trifolium alexandrinum*) that is mostly used as fodder crop in Punjab area. Heavy metals studied include Cr, Cu, Co, Cd and Pb. Among all the studied heavy metals, only Cr amount was found above permissible levels in barseem. Sathiamoorthy *et al* (1997) studied the content of few heavy metals in some medicinal and fodder plant of Negev desert and found that certain plant species accumulated higher level of those metals as compared to their amount in soil.

Food crops grown in green houses under controlled environmental conditions are also not completely free from contaminants. Liu *et al* (2011a, b) reported that vegetables grown in green houses contain more concentration of Cd than those grown in open fields. Table 2.1 and 2.2 shows the heavy metal profile of fodder and meat samples.

Table 2.1: Heavy metal profile of fodder as cited by various authors:

Heavy Metal (ppm)					**Area**	**Author(s)**
Cu	**Zn**	**Cr**	**Pb**	**Cd**		
43-251	19-50	-	2.4-145	0.5-10	Industrial area, India	Sidhu *et al*., 1994
-	-	-	2.4-14.5	0.50-10	Bangalore, India	Gowda *et al*., 2003

-	-	-	0.76-6.62	0.17-0.73	Grass in Thailand	Parkpian *et al*., 2003
-	-	-	1.60-2.94	0.025-0.19	Fodder in Thailand	
49.13	36.89	0.8	-	-	Sewage water, Aligarh, UP India	Paul *et al.*, 2005
			0.008-0.04	0.002-0.003	North West Province of South Africa	Dzoma *et al.*, 2010
-	-	0.16-0.42	-	-	Chittoor Dt of Andhra Pradesh, India	Devasena *et al*., 2012

Table 2.2: Heavy metal profile of meat of dairy cows as cited by various authors:

Heavy metals (ppm)					**Meat**	**Organ**	**Area**	**Author(s)**
Cu	**Zn**	**Cr**	**Pb**	**Cd**				
93.24	58.49	-	2.18	0.42	Beef	Liver	Markets of Lahore	Mariam *et al*., 2004
318.52	56.26	-	4.25	0.41	Mutton			
6.91	54.53	-	3.15	0.49	Chicken			
5.42	46.18	-	2.02	0.909	Beef	Kidney		
6.40	51.38	-	3.85	0.45	Mutton			
81.51	66.26	-	2.19	0.33	Beef	Lean meat		
5.01	65.82	-	4.25	0.37	Mutton			
12.86	28.52	-	3.1	0.31	Chicken			
0.87	4.24	0.43	0.25	0.22	Beef	Liver	Nigeria	Akan *et al*., 2010
0.54	2.34	0.76	0.16	0.76	Mutton			
1.02	6.23	1.22	1.34	0.44	Chevon			
1.44	3.11	065	0.22	0.27	Chicken			
0.54	3.87	0.32	0.15	0.17	Beef	Kidney		
0.34	1.76	0.65	0.08	0.34	Mutton			
0.62	4.76	0.85	1.22	0.39	Chevon			

0.44	2.23	0.27	0.16	0.16	Chicken			
-	-	-	0.47	0.16		Liver		
-	-	-	5.5	0.87	Beef	Kidney	Indonesia	Harlia and Balia, 2010
-	-	-	0.21	0.02		Meat Balls		
1.99	-	3.62	0.08	0.08	Beef	Liver	Southern Nigeria	Iwegbue, 2008

2.5 Heavy metal contamination of food crops and impact on human health:

Food crops are grown worldwide to meet the feeding needs of increasing population. Also with huge population explosion, agricultural lands and land use patterns keep on changing. To meet the needs and with less agricultural land, crops are grown along waste water drains, waste lands and are also irrigated with industrial waste waters or treated effluents to increase the production quantitatively but not concerning about quality of food crops thus leading to serious health impacts on consuming them.

Various developing and underdeveloped countries are facing heavy metal contamination of food crops. In India, various research groups have worked on this topic and verified the results that watering crops with untreated water contaminate the crops with heavy metals and thus effecting human health through entrance by food chain (Rai & Tripathi, 2008; Ghosh *et al*., 2012; Garg *et al*., 2014; Saha *et al*., 2015; Chabukdhara *et al*., 2016). Ali *et al* (2019) reviewed the toxicological impacts of heavy metals, environmental disturbance related to them and their accumulation in living things. Trophic transfer of toxic metals through food chain with special reference to fish, rice and tobacco were also discussed. A review article was presented by Rai *et al* (2019) discussing about heavy metal content in food crops. In the paper, they discussed about heavy metal contamination in soil to food crops and to humans. Translocation mechanism of heavy metals and their remediation techniques were also analyzed. In China also, having highly populated country, waste water is used for irrigation purpose. As a result, a survey was done on food safety and health and result revealed that 10% of Chinese habitat is effected with some toxic metals such as Cu, Hg, Cd, Cr and Ni. Similar kind of studies were done in Bangladesh (Sultana *et al*., 2017), Pakistan (Khan *et al*., 2010), Hong Kong (Hu *et al*., 2013), Argentina (Rodriguez *et al*., 2014). In most

of the developed countries like America, England and other European countries, heavy metal content has been reported in processed/ packed foodstuffs that is also a serious matter of concern (Gonzalez – Martin *et al*., 2018). Various researchers reported that intake of food crops contaminated with various heavy metals cause health related problems in human beings such as gastrointestinal problems, mental retardation, suppressed immune system, malnutrition (Hu *et al*., 2013; Khan *et al*., 2008b; Dickin *et al*., 2016). Health impacts of some heavy metals are provided in table 2.3.

Table 2.3: Showing Health Impacts of Some of the Heavy Metals

Metal	**Effect**	**Reference**
Pb	Mental growth causing heart problems, Impact on nervous system, Less IQ in children & infants	Zhou *et al*., 2016; Al-Selah *et al*., 2017; United Nations, 1998.
Pb & Cd	Bone fracture, kidney dysfunction, hypertension and diseases of liver, lungs & immune system	Jarup 2003; Zhou *et al*., 2016; Zhuang *et al*., 2009; El-Kady & Abdul-Wahhab, 2018
As	Cancer, dernal and respiratory problems, effect reproductive and immune system, diseases of cardiovascular and gastrointestinal system	Chiou *et al*., 1995; Hu *et al*., 2013; Lin *et al*., 2013; Zhou *et al*., 2016; El-Kady and Abdul Wahhab, 2018
Zn	Impact on Immune system, respiratory problems	Hough *et al*., 2004; Zhou *et al*., 2016
Cu	Liver damage and gastrointestinal problems, Kidney damage	Gaetke and Chow, 2003; Rehman *et al*., 2014; Zhou *et al*., 2016; USEPA, 2002a
Cr (VI)	Lung cancer	Park *et al*., 2004; Liu *et al*., 2013
Hg	Pink disease, minamata disease, kidney & lung damage, loss of senses, ringing in ears, less field of vision	Jarup 2003; El-Kady and Abdul-Wahhab., 2018; Ogola *et al*., 2002; USEPA, 2002a

Ni	Effect on kidney functioning, respiratory & kidney disease by smoking having nickel carbonyl compound, obstruct sperm formation	Hough *et al*., 2004; Srivastava & Gupta, 1996
Cd	Kidney damage, pulmonary emphysema & osteoporosis	Salt *et al*., 1997; Bhattacharyya *et al*., 1988

2.6 Heavy Metals in Milk

Milk and its products act as major constituents of the daily diet in India as well as whole world. These milk products contain most of the nutrients, so they can act as source of nourishment for people of all ages especially children. Milk is a good source of protein, calcium and different vitamins. Children and elderly people mostly consume milk and milk products. Therefore, they are most important culprits of heavy metal exposure.

Large number of studies were done in different countries to show the content of different heavy metals in milk and dairy products. Zirarti *et al* (2018) wrote a review article on presence of heavy metal toxicity in milk and products made of it in the world around. Different researchers used different methods to analyze metal content in milk but most common among all methods was flame atomic absorption spectrometer as it is cheap and gave results quickly. They also reviewed that certain factors like season, area, amount of forage and technique used for heavy metal detection also contribute to metal pollution. Harlia *et al* (2018) did an experiment to verify the safety of milk and milk products from contamination by heavy metals. They collected 30 samples each of fresh, pasteurized and dodel milk and tested them for cadmium and lead metals. They confirmed that about 83 and 90% fresh milk samples were contaminated with Pb and Cd respectively, followed by pasteurized (67% contamination by Pb) milk and dodel milk (60 % contamination by Pb and Cd). Garba *et al* (2018) did a research on heavy metal content in milk of cows in Borno, Nigeria. The study was conducted to determine the amount of heavy metals – Pb, Cu, Cr, Co, Cd, Zn, Ni, Fe, Mn and Ag in fresh milk samples. Among all the metals detected, the highest concentration (0.365- 10.688 µg/g) was of Fe followed by Mn (0.047- 1.965 µg/g) and Pb (0.168- 1.394 µg/g). From the study, they concluded that although the amount of studied metals was not harmful but bioaccumulation of Pb and Cd through food chain could be a cause of concern. Meshref

et al (2014) examined the content of some heavy metals and trace elements like Zn, Cd, Fe, Cu and Pb in milk and milk products and the possible health risks to human beings through their consumption in Egypt. They collected total 77 milk samples from different milk sources and found that concentration of Pb was more than recommended limits (0.02mg/Kg). Kabir *et al* (2017) did a research on 50 cow's milk for presence of some heavy metals in Bangladesh. Their study concluded that concentration of metal were increased with increase in the age of cow. They also concluded that Cd, Cr, As and Hg were the toxic metals present in cow's milk and can cause greater harm to human health through consumption of metal contaminated milk. Anneta (2012) reported that processed milk contain high content of heavy metals than raw milk and this report was further confirmed by Kazi *et al* (2009) who found Pb in large amount in processed milk from shops. Many workers found the path of entrance of heavy metals in milk via animal food, use of dirty water for irrigation or closeness of industrial plant near animal farms (Cai *et al*., 2009; Iftikhar *et al*., 2014). Zain *et al* (2016) reported the entrance pathway of toxic metals into milk through milk utensils, at the time of milk processing, through irrigation by contaminated water, through feeding of cattle and their surrounding area. Maximum tolerable limits for Pb and Cd in milk samples were recommended by different regulatory authorities. These limits are:

Pb in milk = 0.02mg/ml (By Codex Alimentarius Commission, 2015; European Union, 2006) Cd in milk = 0.01 mg/ml (by FAO/WHO Codex Alimentarius Commission, 1999)

In India, Pb in milk = 0.02 and 0.1 mg/ml (by FSSAI, 2011). Heavy metal content in milk is given main importance than other food products because milk is consumed by people at every age group, especially children and elderly people (FAO, 2017). Chandrakar *et al* (2018) reviewed heavy metal content in Indian milk and its ill effects on human health. Their study also concluded that food chain contamination act as primary pathway for entry of heavy metals into animal body and then into milk and milk products and finally into human body through drinking this contaminated milk as it is of primary importance to infants and elderly people as discussed above also. The researchers also suggested some remedial measures to tackle this problem such as use of metal free water and food for animals and proper treatment of industrial wastewater before its discharge. Table 2.4 shows the heavy metal content in milk samples in India.

Table 2.4: Showing Heavy Metal Content in Milk Samples in India

Sr. No.	Area Studied	Type of Milk	Metal Range	References
1	Hyderabad	Buffalo Milk	Pb- 0.22 Zn- 3.96	Shailaga *et al*., 2014
2	Tamil Nadu	Goat Milk	As -0.082-0.56 Cd -0.030-0.016 Pb -0.064-0.052 Ni- 0.069-0.067	Dhanalakshmi *et al*., 2013
3	Maharashtra	Buffalo Milk	Zn -0.111-7.23 Pb -0.065-0.137 Cr -0.014-1.606 N_i -0.04-0.749	Nirgude *et al*., 2015
4	Vadodara	Milk & Milk Products, Branded Milk	Cd -1.51 Cd -0.23	Chandorkar *et al*., 2013
5	Delhi	Branded Milk	Zn -2.28	Raina *et al*., 2013
6	Bombay	Cow Milk	Zn - 0.496- 0.786 Cr - 0.013 - 0.175 Hg - 0.015 - 0.023 Pb - 0.139 - 5.904	Zodape *et al*., 2012
7	Jharkhand	Cow Milk	Fe -0.2-13.2 Mn -0.17-0.59 Ni -0.31-0.63	Giri *et al*., 2011

Pilarczyk *et al* (2013) did a comparative study on two breeds of cow from an organic farm and concluded that Simmental cow's milk contained lower concentration of cadmium and lead as compared to the milk of Hostein – Friesian cows and concluded that irrespective of the same environment, the variations found in heavy metal content in milk was the result of variation in metabolic background of the two breeds.

2.7 Heavy metals in water

Entry of heavy metals into water sources occur through various industrial activities like electroplating, smelting manufacturing industries, untreated domestic sewage etc.

Mandour and Azab (2011) identified steel, plastic and battery industry as major sources of heavy metals in drinking water. It was also reported in one of the study that if water remain in the coolers for many days, it could contain many harmful metals like Al, Fe, Pb, Zn, Ni, Mn etc. (Alabdula'aly and Khan 2009). Chowdhury *et al* (2016) reviewed the presence of heavy metals and their consequences on drinking water in developing countries. In the paper, they emphasized kind of research occurring in these countries to study metal content in drinking water and type of human health impacts associated with them. Factors responsible for entry of heavy metals into water were also discussed in detail. In Nigeria, 32.7% of Cd and 36.7 % of Pb was found in drinking water of wells that was much higher than values given by WHO (2011). In a study conducted on As in Bangladesh, Chakraborti *et al* (2010) reported that most of drinking water in the district contain arsenic in water above the guideline limits provided by WHO. They also reported that tube wells in hilly areas did not contain any arsenic but plain areas including coastal regions were much contaminated with it. In India also, many researchers reported different heavy metals in water sources (Dutta, 2013; Chakrabarty and Sharma, 2011; Chennaiaha *et al*., 2014; Berisha and Goessler, 2013; Krishna *et al*., 2009). Table 2.5 shows some of the sources of metal contamination in drinking water in some of the countries.

Table 2.5: Showing Sources of Metal Contamination in Drinking Water

Metal	**Studied Area**	**Contaminator Source**	**Reference**
As, Cu, Fe, Pb, Zn	Italy	Leaking from pipes distributing water to homes	Tamasi & Cini, 2004
Ca, Cu, Pb, Cr, Zn	Dipriz stream in Turkey	Coal fired power plant	Demirak *et al*., 2006
Pb, Cd, Cr, Cu, Ni	Many island in Greece	Anthropogenic activities	Karavoltsus *et al*., 2008
Fe, Ni, Pb, Zn	Industrial Area, Hyderabad, India	Industrial activities	Krishna *et al*., 2009
Pb, Cd, Ni, Cu, Cr	Dhaka, Bangladesh	Industrial waste, pesticides, tannery effluents	Ahmad *et al*., 2010

Cd, Mn, Pb	Assam, India	Geogenic contamination	Chakrabarty & Sharma, 2011
As, Cr, Se	Makkah, Saudi Arabia	Geological features around studied area	Khdary & Gassim, 2014

According to IARC (2016), As and Cd are important human carcinogens. Many researchers studied the health impacts of As, Cd and Pb (Fu *et al*., 2013; ATSRD, 2015). Removal of harmful metals from polluted water sources was mostly done by using different types of adsorbents by various researchers.

2.8 Different terms used for uptake of heavy metals from soil and their translocation to upper parts of soil:

Translocation factor (TF): It is calculated as - concentration of metal in shoot as compared to that in the root.

TF = C shoot/ C root mg/ Kg

If TF > 1, then it is said that effective translocation has occurred.

Bioaccumulation factor (BAF): It can be computed as concentration of toxic metal in the shoot compared to that present in the soil.

BAF = C shoot/ C soil mg/ Kg.

If BAF > 1, then the plant is regarded as hyper accumulator and if BAF < 1, then the given plant species is regarded as excluder (Cluis, 2004; Rezvani and Zaefarian, 2011).

Metal Extraction Ratio (MER): It is computed to check the ability of plant species that how much amount of metal can be immobilized in that species and to determine its suitability for phytoremediation after accumulating metal.

MER is calculated as – metal concentration in the above ground part of plant multiplied by mass of that part to metal concentration in the soil multiplied by mass of soil root zone and whole multiplied by 100.

MER = C plant (mg/ Kg) x M plant (g)/ C soil (mg/Kg) x M root zone (g) X 100

Here, C is metal concentration of above ground part of plant and M is mass of that part of plant.

2.9 Heavy metal uptake by plants:

Heavy metal like cadmium, chromium, copper, lead, mercury and zinc are natural constituents of soil (Lasat, 2000). Metals such as Zn, Cu, Mn, Ni, and Co act as micronutrients for plant growth while metals like Cd, Pb, As and Hg have no known biological function (Gaur and Adholeya, 2004).

Heavy metal polluted soils mainly contain Cr, Cu, Pb and Hg (Marques 2009). *Thlaspi caerulescens* is the plant that can accumulate up to 26000 mg/Kg of zinc and up to 22 percentage of cadmium from polluted areas (Brown *et al*., 1995; Kochian, 1996). Henry (2000) reported up to 500 mg/l Pb uptake by *Brassica juncea*.

Metal hyper-accumulators are interesting tools to study the phytoremediation technology to detoxify metals from soils (Rai, 2012). Salt *et al* (1995) found the role of Ni tolerance and transport by *Thlaspi goesingense*, using plant biomass production, protoplast viability assays and by checking uptake of Ni into roots and shoots. They concluded that Ni tolerance play important role in generating the hyper accumulating potential in hydroponically cultured *T. goesingense.*

Tolerance power of five weed plants i.e. *Cassia tora, Ipomoea carnea, Datura inoxia, Lantana camara* and *Phragmytes karka* for chromium heavy metal by two species of *Brassica* plant was studied by Ghosh *et al* (2005). From the study, it was observed that *P. karka* could tolerate greater level of chromium than other weeds and *I. carnea* had maximum capacity of extracting chromium from soil as compared to other weed plants. Mustafa and Hayder (2021) gave a review article showing the phytoremediation potential of some aquatic weed plants like *Salvinia molesta* and *Pistia stratiotes* to remediate waste waters in Malaysia.

The scientists had investigated metal hyper accumulators as those plants that have the capacity to absorb maximum amount of metals from soil and then gathering them in their upper harvestable parts (Chaney *et al*., 1983). Greatest content of heavy metals was found in *Lactuca serriola, Chenopodium album*, *Artemisia vulgaris* and *Atriplex nitens* plant species.

Al-Zahrani and Abdulrahaman (2014) documented the phytoremediation potential of five native plant species i.e. *Dipterygium glaucum, Indigo feraspiniflora, Salsola kali, Suaeda aegyptiaca and Zygophyllum album* growing at industrial area in Jeddah City for accumulation of Al, Ba, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn heavy metals and found that *Z. album* to be best to remediate polluted soils.

Metal uptake and its storage in different plant parts depend upon interaction between metals present in soil. Hyper accumulating plants possess perfect mechanism for detoxification of these accumulated metals like repair of cells, chelate formation among metals, biotransformation etc. (Salt *et al*., 1998). Carbohydrates like malate, oxalate, citrate etc. are the main charge balancing anions present in the photosynthetic tissues. Some of them were connected with much of metal accumulation in plants (Gabbrielli *et al*., 1997; Homer *et al*., 1995). The tripeptide glutathione (GSH) is produced by gamma-glutamylcysteine synthetase (γ-ECS) and glutathione synthetase (GS). Higher GSH production is considered an important factor to increase cellular defense against oxidation stress.

Since GSH is precursor of phytochelatin (PC), over expression of γ-ECS or GS leads to high phytochelatin accumulation under metal stress conditions (Verbruggen *et al*., 2009).

To overcome metal stress, plants have evolved different mechanisms such as synthesis of the S-rich metal chelator gluthione (GSH) and phytochelatins (Hall, 2002, Gasic and Korban, 2007). GSH is the most abundant low molecular weight thiol compound in plants that plays the main role in protecting plants from environment stresses. Different ions cross the plasma membrane of the cell through secondary transporters. Once inside the plant, the insoluble metals from carbonates or phosphates precipitate them in living and non- living (simplistic or apoplastic) compartments (Raskin *et al*., 1998)

Tangahu *et al*. (2011) reviewed the metal uptake mechanisms by plants and showed various factors affecting their uptake (figure 2.2).

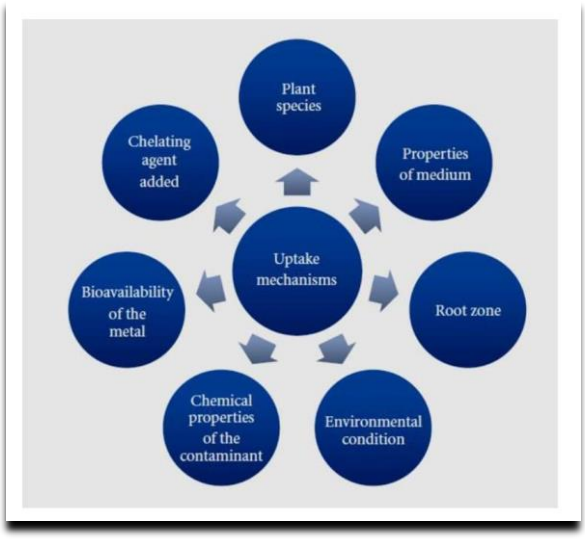


Fig. 2.2: Factors affecting the uptake of heavy metals by plants (Source from Tangahu *et al*. 2011)

The metal uptake is affected by characteristics of the plant species involved and the prevailing environmental conditions (Burken and Schnoor, 1996). In an experimental study by Traunfeld and Clement (2001), pH adjustment of soil from 6.5 to 7.0 with lime can reduce lead uptake by plants. Growth substances and root length are affected by temperature variations. Amount of metal taken up by plants depends on the availability of the metal in the water stage, its holding time and its association with other substances present in the water. Plants affect the soil by lowering its pH thus affecting the availability of the metals to the plant itself (Fritioff and Greger, 2003), enhancing their accessibility by the addition of chelating agents (Ginneken *et al*., 2007). It was observed by Seuntjens *et al* that accessibility of metals also depend upon release of certain organic acids by the plant roots. (Seuntjens *et al*., 2004).

2.9.1 Toxic effects of heavy metals on seed and seedling growth:

It was observed that high amount of metals in the soil effect seed germination and growth of seedlings. Most harmful effects of toxic metals on growth of seeds and their germination were observed in the form of reduced root – shoot length and dry weight (Wang *et al*., 2003), loss of nutrients, oxidation stress etc. Toxic effects of Cd, Hg, Pb and Cu on seed germination and their growth in *Arabidopsis* was studied by Li *et al* (2005). The study showed that metals had much toxicity on seedling growth than seed germination. A laboratory experiment was done by Pokorska – Niewiada *et al* (2018) to study the toxicity of cadmium, lead, mercury, chromium, nickel and zinc on seed germination of six species of plants that are important source of food for humans. Among these seeds, cress, rye and barley were found to be mostly effected by studied metals. Four varieties of *Phaseolus acontifolius* Jacq. were studied by Monalisa (2009) to find the toxicity of five toxic metals i.e. Zn, Cd, Cu, Ni and Pb on seed germination & seedling growth. The results showed that with increasing level of toxicity of metals, there was decrease in percentage seed germination, root – shoot length, fresh and dry weight with most toxic effects shown by Cd among all the 5 metals studied.

Many workers studied toxic effects of Cr on seed germination reduction. Peralta *et al* (2001) in their study on *Medicago sativa* cv. Malone found 23% reduction in seed germination in contaminated soils. Jain *et al* (2000) studied 32 – 52% reduction in germination of sugarcane bud at 20 ppm and 80 ppm chromium. Parr & Taylor (1982)

found 48% reduction in seed germination on using 500ppm of hexavalent chromium in *Phaseolus vulgaris.* Zeid (2001) reported that increase in activity of protease enzyme on treatment with Cr could be a possible reason for reduction in seed germination percentage. Pb metal also inhibit seed germination and its toxic effects had been studied in many plants such as *Pinus halepensis, Hordeum vulgare, Zea mays, Spartina alterniflora, Oryza sativa* etc. (Islam *et al*., 2008). As reported by Zeid (2001), that increase in protease enzyme could reduce percentage of seed germinating ability. Similar observations were given by Sanger *et al* (2008) that seed germination may be inhibited by interaction of lead with protease and amylase enzyme. Wozny *et al* (1982) and Verma & Dubey (2003) had confirmed the decreased percentage of seed germinating ability and growth of seedlings in *Lupinus* and rice respectively. Li *et al* (2005) also studied the toxic effects of some metals like mercury, copper, cadmium and lead on sprouting of seeds and growth of seedling in *Arabidopsis* plant and found that heavy metals cause more toxicity on growth of seedling than their germination. Same results were shown by Srinivas *et al* (2013) by growing three plant species i.e. *Coccinia indica, Mentha viridis* and *Trigonella foenum- graecum* in Ni and Pb heavy metals and reported that high concentration of Ni and Pb up to 300 and 500 ppm respectively resulted in much reduced seedling growth.

2.9.2 Toxic effect on photosynthetic activity of plants:

Several researchers studied the bad effects of toxic metals on photosynthetic activity of the plants. Reduction in total chlorophyll content was studied by Sheoran *et al* (1990) in pigeon pea by cadmium and nickel strain, by Singh & Tewari (2003) in *Brassica juncea* by cadmium stress, by Ouzounidou (1993) in *Silene compacta* and *Thlasppi ochrolucum* by copper anxiety, by Siedlecka and Krupa (1996) in *Zea mays* and *Acer rubrum* by different heavy metals.

Toxic effects of Pb on photosynthetic activity were studied by many researchers viz. Stefanov *et al*., 1995; Rebechini & Hanzely, 1974; Ahmad & Tajmir, 1993; Vodnik *et al*., 1999 and Sersen *et al*., 1998. Harmful effects of Pb include reduction in photosynthetic activity due to obstruction in activities of Calvin cycle enzymes or less formation of chlorophyll (Stefanov *et al*., 1995), changes in structure of chloroplast with leaf cells showing less number of grana and stroma in *Ceratophyllum demersum* (Rebechini & Hanzely, 1974), causing inhibition in uptake of Mg and Fe element that are essential for chlorophyll synthesis (Ahmad & Tajmir, 1993), more toxicity of Pb on

chlorophyll b than on chlorophyll a (Vodnik *et al*., 1999). Drazkiewicz (1994) reported that chlorophyll structure destruction in Pb treated plants occur due to enhanced activity of chlorophyllase. Increase in amount of chlorophyll in PSII or LHCII at low dose of Pb in thylakoids of cucumber and poplar plants were studied by Savari & Coworkers (2002), but at 50mM of Pb concentration, there was much reduction in seedling chlorophyll. Nath and Ali (2018) wrote a review paper in which they discussed the harmful effects of Pb on plants. Various parameters such as growth retardation, less photosynthesis, root blackening, hormonal imbalance and membrane deformation of the plants were reduced due to Pb toxicity.

Pb is mostly accumulated in dicots than monocot roots (Huang & Cunningham, 1996); also, roots contain more amount of Pb accumulation than shoots as root endodermis act as barrier in between them.

2.10 Heavy Metal Remediation Technology:

Today, the remediation of pollution affected by heavy metals in soil, water and sludge is a major challenge. There are different methods available like physical, chemical and biological methods. Stabilization, soil washing, soil flushing, electro kinetics, encapsulation, solidification and vapour extraction are the various chemical techniques which are costly that can make the soil infertile (Marques, 2009). Biological methods like phytoremediation, uses plants to remove the contaminants especially heavy metals from soil, which encourage the establishment of plants on contaminated soil. It is concluded that phytoremediation is cheaper and ecosystem - friendly technique as compared to other methods. These techniques are discussed below.

2.10.1 Physical Remediation:

Soil replacement and thermal desorption are the physical remediation methods. Soil replacement involves replacing the contaminated soil with new soil. The latter method involves the heating of contaminated soil to make the contaminant volatile and then collecting the heavy metal. However, soil replacement is expensive and only suitable for smaller area that were polluted severely (Zhou, 2004). In addition, the other method is expensive and uses expensive devices and takes longer desorption time.

2.10.2 Chemical Remediation: It includes following sub methods:

a) **Chemical Fixation**- This method involves the addition of reagents and materials into the polluted soil and making heavy metals to form insoluble matters that arrest the migration of heavy metals into water and plant. Bolan

et al (2003) reported that chemical fixation method is useful when the contaminant concentration is less in the contaminated site.

b) Vitrify Technology- It is based on heating the contaminated soil for about 1400-2000°C, to make the organic matter to volatize. Fu JH (2008) reported that this technology was very complex that need high energy to melt and is expensive, which reduces its application as remediation.

2.10.3 Biological Remediation:

This method includes microbial remediation and Phytoremediation.

a) **Microbial Remediation-** Microbes cannot destroy the heavy metals but can prevent their mobility and transformation i.e. immobilizing through changing the heavy metals in respect of their physical and chemical properties by oxidation – reduction reaction, intracellular accumulation and precipitation. Decontamination by microbes is useful in mining sites, industrial waste dumpsite, in sewage sludge detoxification etc. as reported by Bosecker (2001). Remediation by microbes has certain disadvantages as it depends on some factors like temperature, oxygen, moisture, pH that are not same throughout whereas microbes can act only in specific conditions (Jones, 1997).

b) **Phytoremediation-** Phytoremediation is a green technology that uses living green plants for remediating pollutants or contaminants (Mc Cutcheon and Schnoor, 2004).

2.11 Mechanism of Phytoremediation:

Phytoremediation takes advantage of plant root systems for its unique and uptake capabilities with the help of entire plant parts through bioaccumulation, translocation and contaminant degradation/ storage ability mechanism. Phytoremediation avoids the polluted media to be excavated and transported thus reducing the spread of contaminants to other areas and can decontaminate more than one type of pollutant at same place.

Plants can detoxify the contaminated sites by several mechanisms. The root system acts as an important platform for uptake of contaminants and it also provides greater surface area for absorption and accumulation of water, nutrients with other nonessential contaminants (Raskin and Ensley, 2000).

There are three main types of phytoremediation namely, phytostabilization, phytoextraction and phytovolatilization.

2.11.1 Phytostabilization:

This technique is known as in-place activation. Some plant species can control the mobility of contaminants present in water and soil by absorption, accumulation and adsorption in the root zone of the plant. By fixing the toxic heavy metals through the process of precipitation, adsorption and reduction, plants reduce the migration of contaminants, prevents leaching, run off, reduces soil erosion and thus prevent the contaminant entering in to the food chain and ground water. (Mendz and Majer, 2008). This technique is mainly used in the process of re - formation of vegetation cover where otherwise no plant growth could occur because of high metal concentration. Metals like Cd, As, Cr, Cu, Zn etc. can be removed using this technique. Effectiveness of this technique over other soil remediation techniques is that problem of disposal of hazardous waste and biomass is not required (Zhang *et al*., 2009). Trigueros *et al* (2012) reported in their study that *Nerium oleander* has shown phytostabilization of Pb by accumulating 26 mg/Kg of Pb in root than the leaves that accumulated 2 mg/Kg of Pb.

2.11.2 Phytoextraction:

This method involves the adsorption of heavy metals by plant's root system, translocating, and accumulating at the above ground parts of plants (Salt *et al*., 1995). Plants with high potential of removing metal from contaminated soils are called as hyper accumulator plant species (Wuana *et al*., 2010). These plant species are capable of accumulating more than 100 times of heavy metals than other plants. Nickel, Copper and Zinc are mostly removed by this method from contaminated areas. This method is cost effective when compared to other conventional methods and the contaminant can be eradicated permanently from the soil. It is also concluded that the amount of waste can be reduced up to 95% and can also be recycled and reused from the plant matter in some cases (EPA, 2000).

2.11.3 Phytovolatilization:

In this method, heavy metals are converted into volatile or gaseous form and thus rendering them harmless using root-secreted matters. This mechanism is suitable for pollutant found in water and soil. Mercury can be removed by this method. This technology is only applicable for volatile organic compounds and inorganic chemicals having volatile properties. The advantage of this method is transforming the volatile

contaminant into less toxic form. However, the use of this technology is limited because the contaminant need to be volatile.

Many other researchers classified phytoremediation technology into various other types that are depicted in figure 2.3. Figure also shows the heavy metal uptake mechanism by various processes of phytoremediation.

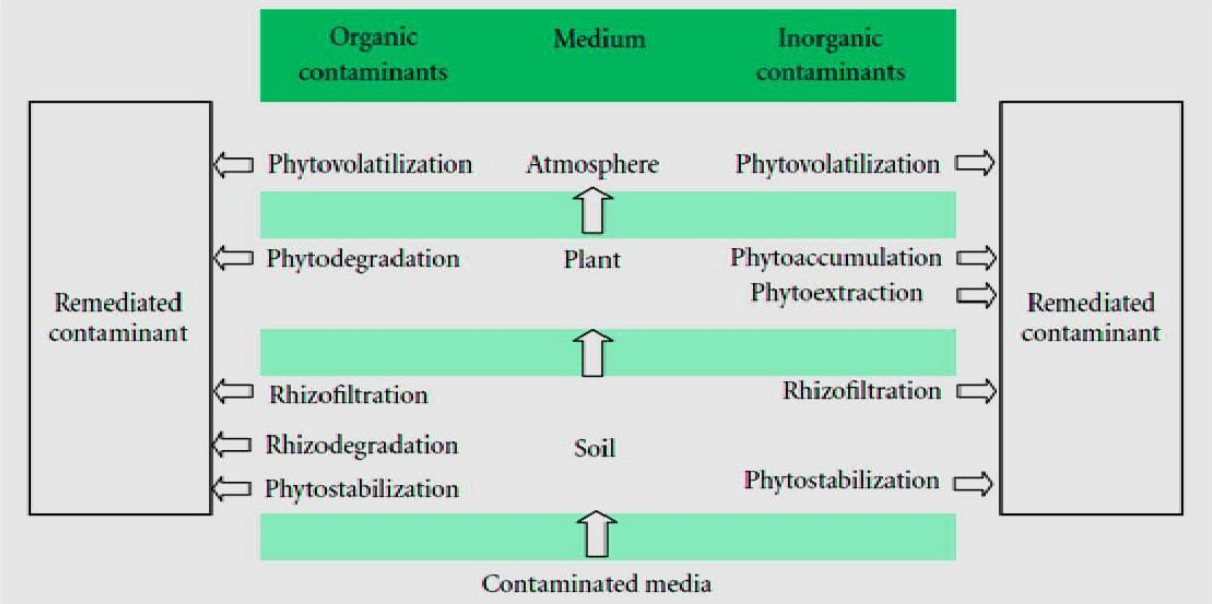


Fig. 2.3: Showing metal uptake mechanism in phytoremediation technique (Source: ITRC, 2009)

Figure 2.3 shows that inorganic contaminants can be removed by phytostabilization and phytoextraction and organic contaminants can be reduced by phytodegradation, rhizofiltration, and rhizodegradation [USEPA, 2000; Prasad and Freitas, 2003]. Different scientists further defined these types briefly as given in the coming paragraphs.

a) Phytoextraction – By this technique, some tolerant plant species can extract and translocate pollutants to their above ground harvestable parts, so that the pollutant could be properly disposed of from that area (Kramer, 2005).

b) Phytostabilization – Here plants can stabilize polluted area in order to prevent soil erosion and leaking of contaminants into the soil. Plants that are suitable for this technique should contain enormous root system and should be tolerant to the toxic metals.

c) Phytoimmobilisation- In this method, some of the soil factors are modified to reduce the mobility and bioaccessability of pollutants.

d) Phytovolatilization – Here plants are used to volatilize pollutants. Plants extract volatile harmful compounds from soil and transform them to a gas that can be released via transpiration process. (Raskin *et al*., 1997; Ghosh and Singh, 2005a).

e) Phytodegradation – This method is used to transform some of the organic toxins to simple substances to be stored in the plant tissue later on (Ghosh and Singh, 2005b).

Tangahu *et al* (2011) reviewed the relationship between different phytoremediation techniques that is shown in figure 2.4.

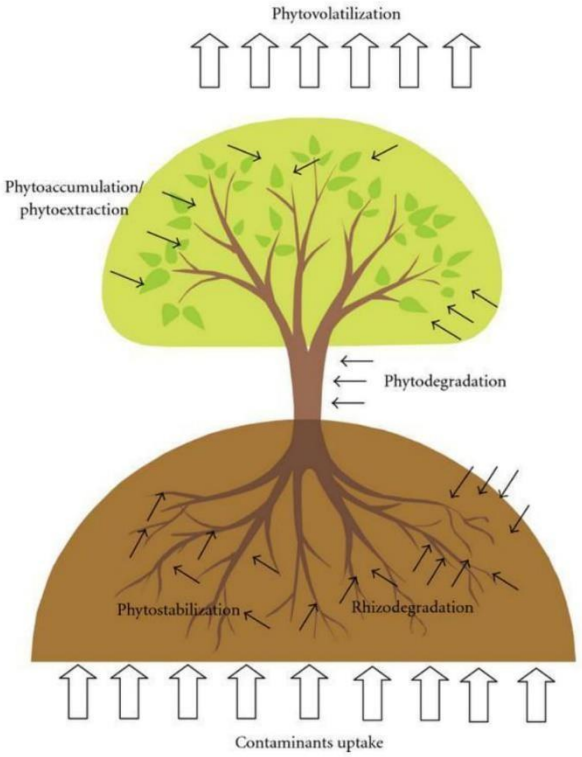


Fig. 2.4: Relationship between various mechanisms of phytoremediation (Source: Tangahu *et al*., 2011)

Phytoextraction process can extract metal and organic toxins directly from soil and then translocating them to aboveground parts of the plant and this is successful when hyper- accumulating plants are used. According to Brooks (1998), the hyper accumulator plants can concentrate metals 100 times higher as compared to normal non – accumulating plants. Among the best-studied hyper-accumulators (< 0.2% angiosperms), *Thlaspi caerulescens, Brassica juncea, B. oleracea*, *Berkeya coddii* and

Thlaspi goesingense can be mentioned. Wu *et al* (2010) & Mudgal *et al* (2010) observed that around 400 plant species could act as metal hyper-accumulator plant.

Zhuang *et al* (2007) investigated Phytoremediation potential of some plants like *Vertiveria zizanioides*, *Dianthus chinensis* and some species of *Rumex* for Pb, Zn, and Cd heavy metals. *Zea mays*, *Sorghum bicolor*, *Helianthus annuus* were found phytoremediator of Cr heavy metal (Abou- Shanab *et al*., 2004). Greater phytoextraction power of *Chenopodium album* (chulai) was seen for Fe, Mn, Zn, Cr, Pb, Ni and Cd as reported by Gupta and Sinha (2007). *Typha angustifolia* showed efficient phytoremediation potential 0for Cr, Pb, Cu, Ni, Cd and Zn (Demirezen and Aksoy, 2004). Even the aquatic weed like *Eichhornia crassipes* is reported to be potential phytoremediator for heavy metals in the polluted water bodies. (Tiwari *et al*., 2007). Plant species belonging to euphorbiaceae, lamiaceae, brassicaceae, fabaceae, asteraceae and scrophulariaceae are the main phytoremediators families of heavy metals (Salt *et al*., 1998; Dushenkov 2003).

Kumar *et al* (1995) did investigations on high biomass producing fast growing species of genera *Brassica* such as Indian mustard (*B. juncea*), black mustard (*B. nigra*), turnip (*B. campestris*), rape (*B. napus*), Kale (*B. oleracea*) and found that *B. juncea* could accumulate nickel, lead, chromium, zinc and copper metals in their stems in high amount as compared to other varieties.

Phytoremediation is found to be the best-suited method to clear and separate the pollutants from soil without changing its fertility and structural pattern (Ghosh and Singh, 2005). Among various methods of phytoremediation, technique of phytoextraction is most advantageous because soil erosion and leaking of contaminants into the soil are very less (Jadia and Fulekar, 2009). Accessibility and mobile nature of heavy metal in soil and then in the plant is major factor that affect the phytoextraction potential.

Biological Concentration Factor (BCF), Biological Accumulation Coefficient (BAC) and Translocation factor (TF) can be used to know the effectiveness of phytoremediation technology. BCF is calculated as metal concentration ratio of plant roots to soil (Yoon *et al*., 2006). Biological Accumulation Coefficient (BAC) is computed as heavy metal ratio in shoots and soil (Li *et al*., 2007; Cui *et al*., 2007). The TF shows the ability of a plant to capture metals in its upper removable parts and is

calculated to find the efficiency of phytoremediation process. TF is computed as the ratio of metal concentrated in the shoots as compared to its amount in the roots (Chakroun *et al*., 2010). Value of TF more than 1 indicates that metal has been transported from roots to the shoot part (Jamil *et al*., 2009).

An important factor that help in selecting the phytoremediating plant species is that how much amount of heavy toxic metal is removed by that plant species. The efficiency of phytoextraction by a given species depends on two key factors viz- biomass and metal accumulation capacity (Blaylock *et al*., 1997). Metal hyper-accumulators and non-accumulator species have been documented for their selection as remediator species. Small size and slow growth rate of hyper accumulator plants could lead to their less use. This loss could be compensated by non-accumulator species producing high biomass but with low capacity for metal uptake (Ebbs *et al*., 1997). However, some places contain too much metal contamination that no toxicity symptoms are visible in plant species but biomass loss is very high. The plant species may accumulate large value of one metal but may be sensitive to other metal making it less useful for metal decontamination work (Hinesly *et al*., 1978). As studied by Chaney *et al* (1999) that several maize inbred lines can accumulate high levels of Cd, but are of no use to remediate soils at the normal ratio of Zn and Cd.

Plant biomass, mass of soil and bio concentration factor are the three important characteristics that tells about the ability of a plant species to phytoremediate the contaminate (Zhao *et al*., 2003). Success of phytoremediation process depends upon agronomic practices that are used at particular area. Chaney *et al* (1999) discussed the effectiveness of evolving agronomic practices. These agronomic practices were developed with an agenda to promote the ability of some non-accumulator plants for metal phytoextraction. It was observed by Chaney *et al* that plant increases their living matter when phosphorus is applied as fertilizer to them but it can hinder the uptake of other essential metals from soil (Chaney *et al*., 2000).

Brassica juncea L. has higher ability to remediate Cd, Pb and Zn from aquatic areas having maximum concentration of 50 microgram/milliliter of given metals. Therefore, it can be raised in such environment that are contaminated with toxic heavy metals like cadmium, lead and zinc afterwards harvesting and burning could be done to recover the metals or can be disposed of with safety.

Abdullah *et al*., 2013, investigated use of sunflower for Cd contaminated soil. Sheoran *et al* (2012) investigated phytoremediation of metal contaminated mining sites. Meriem *et al* (2013) studied phytoremediation of polluted water using roots of aquatic plant *Phragmites australis* with metallic trace elements

Swapna *et al* (2014) studied the accumulation pattern of some toxic heavy metals like Fe, Cr, Al, Cd, Hg, Cu, Zn, Pb and Ni in *Chromolaena* plant species and found greater variations in the amount of metal accretion and dispersal in different plant parts. Maximum amount of various studied metals was found to be accumulated in the root as compared to upper parts of the plant.

Phytoremediation as one of the environmental safe solution to metal contaminated water and assessment of plant removal ability was confirmed by Jatin *et al* (2013). G. Saxena *et al* (2019) presented a review article on phytoremediation of metal contaminated land in which they provided a detailed study of both conventional and advanced approaches to phytoremediation process, their capability to low down heavy metals from environment and acceptance of this technique by people for rectifying metal polluted land. Kamaruzzaman *et al* (2011) did experimentation on accumulation and distribution of Lead and Copper in *Avicennia marina* and *Rhizophora apiculata*. Mahdavi *et al* (2012) investigated Pb and Cd accumulation in *Avicennia marina*. Karimi (2013) carried out an experimentation to study the potential of alfalfa (*Medicago sativa*) and sorghum (*Sorghum bicolor*) for phytoremediation of soil contaminated with chromium. The study revealed that both are effective accumulator of chromium, but the potential of alfalfa was more pronounced than sorghum for uptake of chromium metal from polluted soils.

Mojiri (2012) investigated ability of *Typha domingensis* to rectify heavy metals from municipal wastewater by phytoremediation process. These results showed that most metal removal from wastewater by *Typha domingensis* was after 48 h and in order of Fe>Mn>Zn>Ni>Cd. Singh *et al* (2011) studied the removal of lead heavy metal from wastewater by the use of some aquatic plants.

Khan and Gaikwad (2013) investigated phytoremediation potential of *Brassica juncea* L. with reference to chemical atrazine. Iqbal M *et al* (2012) investigated effect of EDTA chemical and metal application on heavy metal absorbance and expression of genes in different species of *Brassica*. Kumar *et al* (2011) investigated the effect of

EDTA on increasing the phytoextraction of lead by Indian mustard (*Brassica juncea*). Sharifi *et al* (2014) studied the effect of dimercaprol on phytoremediation of Lead by *Zea mays*, *Helianthus annuus* and *Sinapis arvensis*.

The advantages of phytoremediation technology are i) It is aesthetically sound, solar energy based technology (ii) very less environmental disruption (iii) it preserves top soil due to in situ nature (iv) it is most useful at sites with shallow, low level contaminants (v) useful in broad range of contaminants (vi) it is cheaper than conventional methods. The only disadvantage of this technique is that it is time-consuming process and generally takes much time to clean up an area and disposal of metal rich plant material is also problematic.

Identification of plants for removing heavy metals by phytoremediation process is still at its starting phase. Keeping in mind the occurrence of heavy metals in the soils of Kala Sanghian drain area of Kapurthala, the present problem was taken up to find out the plants acceptable for remediation of those soils.

2.12 Phytoremediation in World Scenario:

Phytoremediation technique is known as environment friendly proposal for remediation of polluted environment. This green approach is widely applicable for decontamination of broad range of organic and inorganic contaminants (Mwegoha, 2008). Contaminated water treatment using plants was proposed 300 years back (Hartman, 1975). Interest in phytoremediation process mainly focusses towards finding of metals for hyper accumulator plant species. Baumann (1885) reported *Viola calaminaria* and *Thlaspi caerulescens* as first plant species that accumulated metal in their leaves. Extensive experimentation has been implemented to realize the biological mechanism of phytoextraction of metals. Naturally occurring and native plant species are mostly responsible for accumulating high metal and made the phytoremediation process interesting. In recent times, hyper accumulator plant species are given maximum attention because of their efficiency on accumulating heavy metal 100 fold greater than common non accumulator plants. It was reported that hyper accumulators have the capacity to concentrate more than 10,000 ppm of Ni and Zn,1000 ppm of Co, Cr, Cu, 100 ppm of Cd and 10 ppm of Hg. Reeves and Baker (1999) have reported that 400 plant species from 45 plant families have potential of hyper accumulation capacity.

Chinmayee *et al* (2012) worked on a weed *Amaranthus spinosus* to find out its ability to remediate some of the heavy metals from the contaminated soils. BCF and TF were more than one showing that *Amaranthus spinosus* is good agent for heavy metal accumulation. Singh *et al* (2016) did an experimentation to check the ability of *Solanum nigrum, Euphorbia hirta, Amaranthus hybridus* and *Xanthium strumarium* weeds as phytoremediators against Cd, Pd and Ni contaminated lands and found that studied weeds grow well in contaminated soils and produced more antioxidant enzymes and are good for restoring contaminated areas. Subhashini *et al* (2017) did pot culture experimentation using three weed plant species i.e. *Acalypha indica, Abutilon indicum* and *Physalis minima* for their ability to accumulate Pb, Ni, Cd and Cr from contaminated soils. The results showed that *A. indica* accumulated Pb, Ni and Cr, *A. indicum* accumulated Cr and *P. minima* accumulated Pb and Cr. So all weeds were recommended as good agents for cleanup of polluted lands. Wang *et al* (2010) worked on *Cyperus rotundus* to find its ability for remediation of diesel-contaminated wetland in Shanghai, China by doing open-air pot experimentation. Concentration of diesel taken 1000, 5000, 10000, 15000 and 20000 mg/Kg of soil. Results showed that antioxidant enzyme activity of CAT and AAO was at peak on 15000 and 10000 mg/Kg soil respectively. There was decrease in chlorophyll content and protein. They declared that the weed has the potential to restore diesel-affected land. Hammami *et al* (2015) conducted an experimentation to detoxify Cd contaminated soils by the use of some weed species- *Portulaca oleracea, Solanum nigrum*, *Abutilon theophrasti* and *Taraxacum officinale*. The results showed that. *T. officinale* and *S. nigrum* have the ability to detoxify Cd from soils. Demario *et al* (2019) studied the removal of some metals such as Pb, Cr, Al, As, Cd, Cu etc. and certain nutrients by *Sagittaria montevidensis* from contaminated areas of Brazil. Results showed that studied plant had the ability to accommodate maximum amount of metals in the roots and act as food agent for rectifying polluted soils. In another study, the phytoremediation ability of *Calendula officinalis* for Cd contaminated soils with the use of three chelating agents- EDTA, citric acid and tartaric acid was done. The results confirmed that plant grow well in all Cd containing soils with decreased dry weight and increased antioxidant enzyme activity. Therefore, they regarded *C. officinalis* as Cd hyper accumulator plant with BCF (1.3-2.90) and TF (1.28-1.58) value greater than one. It is also concluded that citric acid as a chelating agent can increase the phytoremediation ability of *C. officinalis*. Yan *et al* (2020) gave a review article in which they focused on the stretegies

that can be applied to improve the efficiency of techniques of phytostabilization and phytoextraction like genetic engineering etc. Girdhar *et al* (2014) did pot and hydroponic experimentation to know the heavy metal removing capacity of three weed plants i.e. *Cannabis sativa, Chenopodium album* and *Solanum nigrum* by using 5, 10, 50, 100, 200, 300 and 350-ppm concentration of Cr, Cu, Cd, Ni and Pb of heavy metals. Morphological parameters like shoot length, number of branches, leaf area and pollen fertility were studied during the study. Results showed that toxicity on morphological parameters increased with increased dosage of metals and it also lead to the formation of sterile pollen grains. They also confirmed the hyper accumulator nature of *C. sativa* for Cr metal by hydroponic experimentation.

2.13 Phytoremediation in India:

Pollution, land degradation, destruction of wild life, erosion of genetic resources and industrial waste disposal are major environmental problems in India. It was observed that soil degradation was very less in silvi pastoral system where trees and grass were grown together and under this system, *Prosopis juliflora* and *Leptochloa fusca* were maintained to restore alkaline soil. In Pariyej reservoir wetland, a study was conducted on *Nelumbo nucifera*, an aquatic plant by Kumar *et al* (2008). They reported that the plant could accumulate greater content of some of the toxic metals like lead, zinc, copper, cadmium, nickel etc. Subha and Srinivas (2017) selected eight weed species to check their phytoremediation potential in Benthic sludge lake, Hyderabad. These were *Euphorbia geniculata, Amaranthus viridis, Ricinus communis, Polygonum glabrum, Ipomea carnea, Parthenium hysterophorus Cyperus alopecuroides* and *Eucalyptus globulus.* Heavy metals used were Cu, Fe, As, Cd, Cr, Zn, Ni, and Pb. Phytoremediation ability was calculated in the form of BCF. Results showed that *I. carnea* could accumulate high amount of As and Pb, *E. geniculata* was good for Cd and Cr, *E. globulus* for Cu, Fe and Zn and *P. glabrum* for Ni. Karitha and Jegadeesan (2014) done a study on three weed species- *Trianthema portulocastrum, Saccharum spontaneum* and *Ipomea carnea* to remediate soil Hg and Cd. They observed that *I. carnea* and *T. portulocastrum* could accumulate Cd in high concentration in their plant parts. Singla and Singh (2018) from Jalalabad, Punjab, studied phytoremediation capacity of one weed *Chenopodium album* from two different sites (one irrigated with polluted water and other with underground water). They observed that metal

accumulation ability of weed growing in polluted area was more than the other one growing in underground-irrigated water.

2.13.1 Phytoremediation potential of different plant species:

In two different studies done by Rossini Oliva *et al* (2009) reported that *Erica andevalensis* had the potential to accumulate Pb and Zn heavy metals in their root epidermal tissues when grown in highly polluted areas (Rossini Oliva *et al*., 2009; 2012, Zinc concentration tolerance was up to 1500 µM).

Mandal *et al* (2014) carried out the extensive literature review of phytoremediation in India. His survey concluded that heavy metal originates through geogenic sources as in Arsenic confined to West Bengal and Bangladesh, man – made sources as in industrialization residues, agricultural activities, sewage discharge and sludge used for various crops and pesticides such as copper oxychloride and Bordeaux mixture as a growth enhancer in piggery and poultry farms.

The review done by Mandal *et al* (2014) brought out the Indian scenario of phytoremediation as:

1) *Salix acmophylla* could be a bio-surveillance agent for Cu, Ni, Pb in soil and water bodies and mainly suitable for phytoremediation of metal contamination from lakes and soils.

2) The hyper accumulation process of toxic metals by plants include – metal transportation over the plasma membrane of root cells → loading of xylem and its transformation → detoxification and detachment of metals at the cellular levels of whole plant. More than 400 plant species have been documented that act as hyper accumulator plant species.

3) Phytoremediation potential of various weed species such as *Ludwigia parviflora, Enhydra sp., Eleusine indica, Filmbristylis sp., Ageratum conyzoides, Croton sparsiflorus, Lantana camara, Vitis trifolia, Asteracantha longifolia* conducted for arsenic contaminated soil showed the increased accumulation of arsenic by the shoots in soils loaded with 2-14 mg/ Kg of arsenic and had greater potentiality to act as hyper accumulator plants for this metal.

4) Two successive harvests with DAP as fertilizer emerged as the promising management strategy for amelioration of arsenic contaminated soil

of West Bengal through phytoextraction using *Pteris vittata* and found to be useful for growing rice resulting in decreased As content in rice grain of <1 ppm with average improvement in rice grain yield of 8% after one growth cycle.

5) Five species of *Brassica* namely, *Brassica juncea* cv. Pusa Bold, *Brassica campestris* cv. Pusa Gold, *Brassica carinata* cv. DLSC-1, *Brassica napus* cv. Early napus, *Brassica nigra* cv. IC-247 were tested for their hyper accumulation potential of Zn, Cu, Pb, and Ni and Cd heavy metals. It was concluded that *Brassica carinata* cv. DLSC-1could decrease the metal load by 15% for Zn, 12% for Pb and 11% for Ni from naturally contaminated soil of urban Delhi.

6) For Ni contaminated soil, Castor (*Ricinus communis* L.) was reported to accumulate large amount of Ni and could be used as a hyper accumulator plant.

7) Phytoremediation of Cr was tested for cleanup of Cr contaminated silt loamy and sandy soils by fenugreek (*Trigonella foenumgraecum* L.), spinach (*Spinacia oleracea* L.), and rye (*Brassica campestris* L.) and showed that the family Cruciferae (raya) was the most tolerant to Cr toxicity, followed by Chenopodiaceae (spinach) and Fabaceae family (fenugreek).

8) *Jasminum auriculatum* was found to be tolerant up to 1000µg / gm of Cr in Cr contaminated soils than *Crossandra infundibuliformis* and *Jasminum sambac* which were found sensitive at this concentration of Cr.

9) *Phragmites karka, Bacopa monnieri* and *Scirpus lacustris* were grown in cannery discharge and mud having 23 mg/ l and 214 mg/Kg Cr respectively and significant reduction was observed in Cr concentrations. *Ocimum tenuiflorum* L. (tulsi) could withstand Cr stress and protect the above plant parts from phytotoxicity of Cr by modifying various metabolic reactions.

10) Phytoremediation potential of three grasses i. e. *Cymbopogan martinii, Cymbapogan flexuosus* and *Vetiveria zizanoides* was documented for Cd toxicity and it was observed that *Vetiveria zizanoides* had the capability for detoxification of cadmium-polluted lands.

11) Marigold and tuberous plants possess the potential of Cd hyper accumulation and these two crops could be grown for rectifying cadmium - contaminated soils with less to medium level of contaminants. *Chrysanthemum* could be used for phytostabilization of Cd- contaminated soils.

Brassica juncea was reported to be tolerant to heavy metal having much growth with large biomass, but its oil production has been reduced because of Cd toxicity (Bhuiyan *et al*., 2011). Hernandez *et al* (2008) confirmed that *Brassica* species have high accumulation ability for zinc than lead and cadmium. Ten cultivars of *Brassica juncea* were explored by Qadir *et al* (2004) and it was observed that among these ten cultivars, few had a high tolerance for Cd metal up to 2000 µM metal concentration.

Nouairi *et al* (2006) compared *Brassica juncea* and *Brassica napus* for their Cd uptake potential and reported that *Brassica juncea* was able to accumulate Cd in shoots under hydroponic solution. Seth *et al* (2012) also confirmed the report of Nouairi *et al* (2006) that *Brassica juncea* was enough for its heavy metal uptake like Cd, Cr, Cu and Pb. Podar *et al* (2004) reported in his experiment that heavy metal uptake under homogenous conditions may affect the toxicant quantity for its uptake capacity by *Brassica* plant. Many other studies reported in *Brassica* species showed that heavy metal accumulation using chelating agents tend to increase the remediation potential and root to shoot translocation factor (Neilson and Rajakaruna, 2015). In one of the study done, it was documented that addition of citric acid and Sodium Nitrilo tri acetic acid (NTA) to heavy metal contaminated soil have increased the heavy metal uptake especially Cd by *Brassica juncea* plant. Clemente *et al* (2005) studied the role of *Brassica juncea* for heavy metal contaminated site like Zn, Cu and Pb by using cow dung and compost as amendments and without them and reported that heavy metal uptake depends on soil pH even though there was plant growth regulators present.

It was concluded by Adediram *et al*, that addition of bacteria inoculums had increased the heavy metal uptake capacity of *Brassica juncea* especially for Zn metal (Adediram *et al*., 2015). Abduallah *et al* (2015) concluded in his research that *Brassica napus* could remediate 44 to 67% of Pb from sewage sludge.

Suitability of Alfalfa plant for heavy metal uptake in co-contaminated soil was tested by Agnello *et al* (2014). They observed poor translocation of metal and after 15 days of pot culture experiments, root and shoot living matter of the plant was less with 100% mortality. They also concluded that soil nutrient

plays an important part in plant growth than the contaminants obstructing the growth of plant. Research done by Ding and Luo (2005), Zhang *et al* (2013) and Ouvrard *et al* (2011) before Agnello *et al* (2014) had reported that Alfalfa plant was suitable for co-contaminated soil for phytoremediation capability.

Tahra *et al* (2013) inferred that biological accumulation of heavy metals by alfalfa from farm soil and industrial area in Oman had shown the results in the order of heavy metal uptake as iron > aluminium > nickel > zinc > chromium > copper > copper > lead and interesting part of their study was that lead had translocated much in edible shoot than edible root which was otherwise recorded as least concentration.

2.14 **Issue on Problem**:

From the review, it is clear that weedy plants have potential to accumulate various heavy metals. Use of native weedy plant species is gaining significant and greater importance. Weed plant species are mostly focused on the phytoextraction techniques due to their greater adaptability to local environmental conditions (Dang *et al*., 2011; Wei *et al*., 2008; Wei *et al*., 2009).

Hence, the objective of present study was to find out some of the weed and fodder plants of Kala Sanghian drain, Kapurthala for their phytoremediation potential for heavy metal uptake and their suitability for consumption after exposure with the following research gaps.

- The weeds of given area have not been studied for their phytoremediation capability (*Cyperus iria, Achyranthes aspera, Eruca sativa*).
- Assessment of toxicity of these weeds after heavy metal exposure and suitability as cattle fodder has not been carried out.
- Assessment of medicinal value after exposure of heavy metals has not been assessed earlier and is a burning topic of herbal medicinal research.

CHAPTER- 3

HYPOTHESIS

Phytoremediation is a very fast emerging, cleanup and environment friendly technology. Being utilized at a larger scale as a heavy metal remediation technique due to its low cost as compared to other remediation techniques. Various weeds and crop plants are utilized for their phytoremediation ability in various parts of the world, as they are non- edible as well as can produce high biomass irrespective of the accumulation of toxic metals in their plant parts.

The present study investigated the phytoremediation potential of three plant species that are regarded as of no use to most farmers. The experimentation was carried out in pot culture in field conditions and heavy metal content in the roots were analyzed by Atomic Absorption Spectrophotometer. Finally, milk samples were collected from local vendors of that area and were checked for their high contamination with heavy metals.

If the selected weeds and fodder plants accumulate heavy metals in their roots or other parts, then they can be employed as good hyper accumulator plants in various polluted areas of Punjab for their phytoremediation capacity. The milk contamination has to be analyzed for any possible public health hazard.

CHAPTER 4

OBJECTIVES

- Screening and selection of weeds and fodder plants of Kala Sanghian area for phytoremediation capability.
- Carrying out of pot culture experiments with differential exposure of chromium and lead heavy metals.
- Analysis of morphological, biochemical and reproductive parameters after exposure of heavy metals.
- Analysis of phytoremediation capability along with statistical analysis and selections of appropriate weed.
- Analysis of effect on grazing animal milk quality through consumption of metal absorbing fodder plants.

CHAPTER 5

METHODS AND MATERIALS

Table. 5.1 List of chemicals used during research study:

Sr. No	Chemical Name	Company
1.	Acetone	Loba Chemie
2.	Sodium Carbonate	Loba Chemie
3.	Sodium Hydroxide	Loba Chemie
4.	Sodium Potassium Tartrate	Loba Chemie
5.	Phosphate Buffer	Loba Chemie
6.	Copper Sulphate	Loba Chemie
7.	Folin -ciocalteau	Loba Chemie
8.	BSA	Loba Chemie
9.	Hydrogen Peroxide	Loba Chemie
10.	Guaiacol	Loba Chemie
11.	Nitric Acid	Loba Chemie
12.	$HClO_4$	Loba Chemie

5.1 Objective (1st) - Screening and selection of weeds and fodder plants of Kala Sanghian area for phytoremediation capability.

Under this objective, screening of large number of weed plants of the study area was done for their phytoremediation ability by visiting the area. From these screened plant species, then selection of some weeds and fodder plants for the present study were selected. The selected plant species were- *Cyeprus iria* (Umbrella ridge or Rice flour sedge), *Eruca sativa* (Tara mira) and *Achyranthes aspera* (Puth kanda).

5.2 Objective (2nd) - Carrying out of pot culture experiments with differential exposure of chromium and lead heavy metals.

Under this objective, pot culture experimentation was done under natural field conditions using two heavy metals. Heavy metals used in the present study were:

1. Cr

2. Pb

The salts of heavy metals used for metal treatments were:

1. Chromium [Cr (VI)] = $K_2Cr_2O_7$

2. Lead [Pb (II)] = $Pb(NO_3)_2$

The different concentrations of Pb used were – 100, 200, 300, 400, 500, 600, 700, 800 and 900 mg/Kg. The different concentrations of Cr used were – 50, 100, 150, 200, 250, 300, 350 and 400 mg/Kg, but no growth was observed at 900 mg/Kg of Pb and 400 mg/Kg of Cr concentration. Therefore, these concentrations were not shown in graphs and tables. Control was also observed for comparison purpose. Along with this, physicochemical analysis of the soil (soil texture, pH and alkalinity) was also done and the principle and procedure of various instrumentation used for the present study were provided.

5.2.1 Analysis of Soil:

5.2.1.1 Physicochemical parameters of soil:

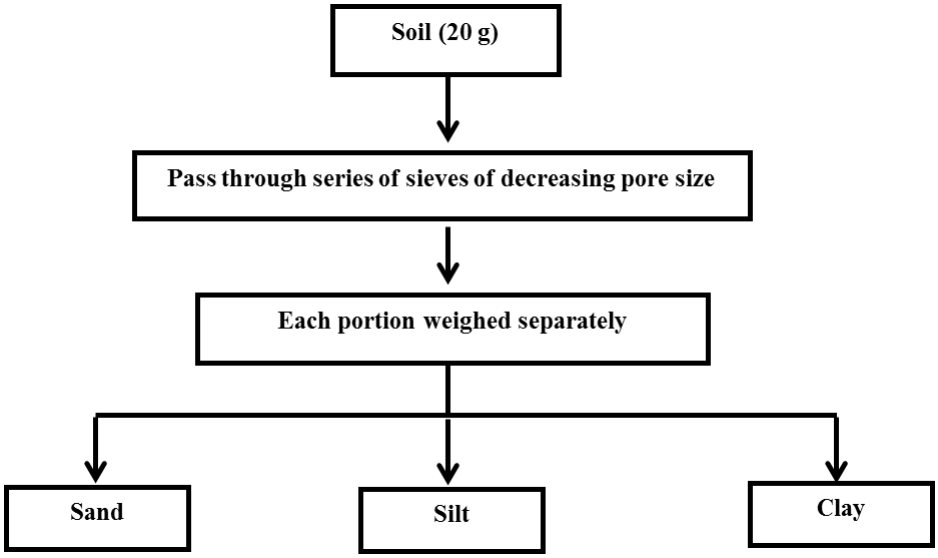
Soil was collected from the experimental area up to depth of 20 – 25 cm in an area of 10 x10 cm and its various physical and chemical attributes such as soil texture, soil pH, and presence of selected heavy metals content were tested by the methods given by Trivedy *et al* (1987) and Chand *et al* (2011) with slight change.

5.2.1.2 Texture of Soil:

Soil texture has main role to play in retention of nutrients in soil and water holding capacity. This property of soil is determined based on relative composition of various particle sizes. The soil particles of different sizes are grouped together and the relative percentage of each group gives an information about soil texture. Based on size, different particles are grouped as:

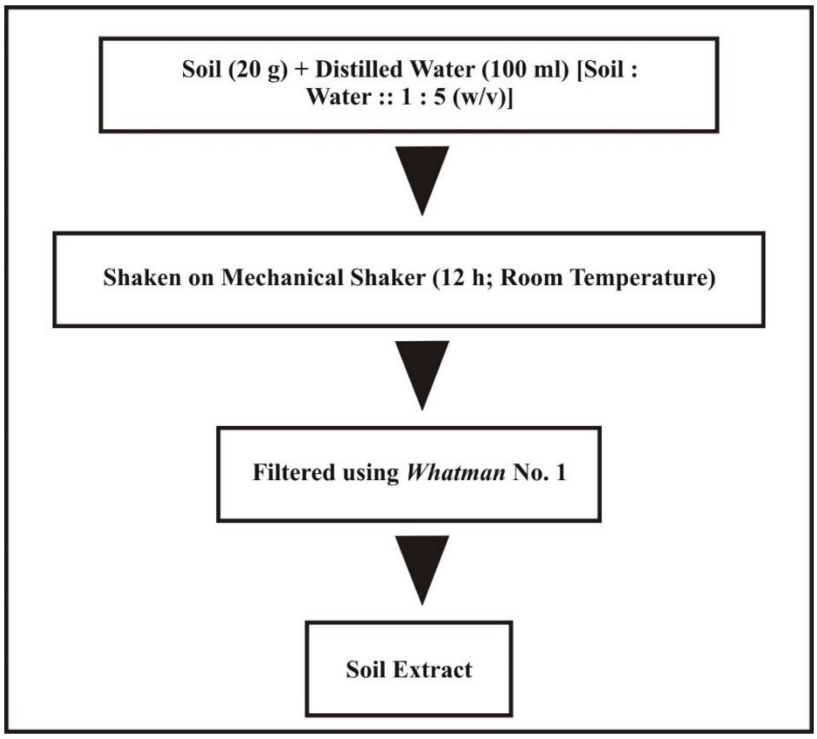
- Sand: 0.5 – 2.00 mm, • Silt 0.002 – 0.5 mm • Clay: < .002 mm.

- Procedure:



Preparation of soil extracts

Soil extracts were prepared by below given procedure to carry out various physicochemical analysis.



5.2.1.3 pH of Soil:

pH is the logarithmic measure of hydrogen ion concentration and gives information about the absorbed H and OH ions. It is the measure of acidity and alkalinity of soil extract. pH determines the chemical nature of soil. Metal mobility and its availability in soil is largely dependent upon the soil pH. pH of soil extract was measured using pH meter.

5.2.2 Instrumentation used:

Following instrumentation was used in the present study to perform biochemical studies.

5.2.2.1 Spectrophotometer:

Most of the biochemical parameters were carried out using the technique of spectrophotometry (UV-Visible PC Based Double Beam Spectrophotometer), based on Lambert– Beer's law. According to this law, the intensity of monochromatic light absorbed by a substance is directly related to the amount of the substance in the solution. It is usually represented as:

$A=\varepsilon CL$

Where

A is absorbance with no units

ε is the molar absorbance coefficient having 'L/mol/cm' unit.

L is the length of the path followed by the sample and it is 1 cm in this case when the cuvette contain sample to be measured.

C is the concentration of the test compound in solution, expressed in mol/L

5.2.2.2 Atomic Absorption Spectrophotometer:

Metal uptake studies were carried out using atomic absorption spectrophotometer. A light beam through hollow cathode lamp is directed to monochromators and detector through the flame. The amount of light captured by the atomized element in the flame is detected by the detector and absorbance is recorded. Each element absorbs a specific wavelength; therefore, a source lamp of that particular element is used.

5.2.2.2.1 Principle of Atomic Absorption Spectrophotometer:

Atomic Absorption Spectrophotometer is based on Beer-Lambert law where it is stated that absorption of light while travelling through a solution is dependent on the concentration. In addition, a particular metal absorbs a light of specific wavelength and this leads to excitation of metal from ground state to excited state.

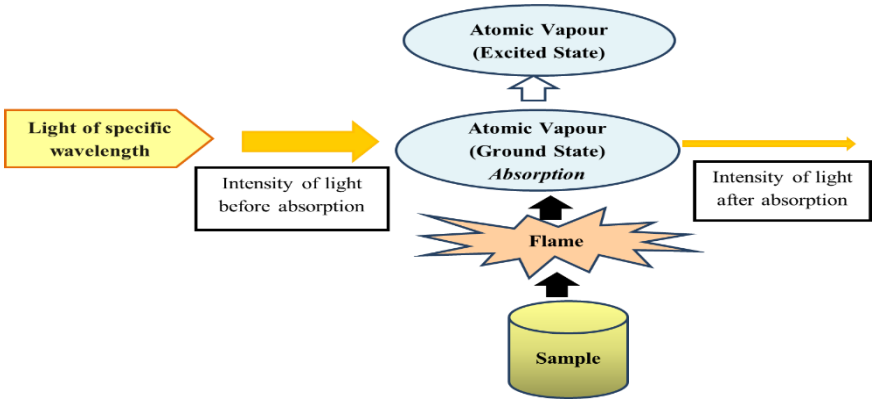
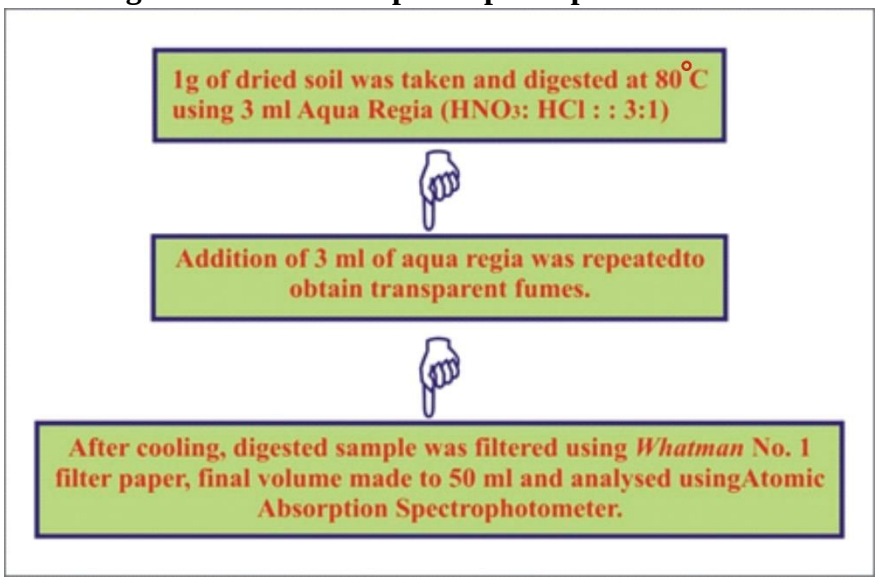


Fig 5.1: Atomic absorption spectrophotometer.



5.3 Objective (3rd) - Analysis of morphological, biochemical and enzyme activity parameters after exposure of heavy metals.

Under this objective, first harvesting of plant sample was done and then various morphological (root length, shoot length, fresh and dry weight, moisture content etc.), biochemical (chlorophyll *a*, chlorophyll *b*, total chlorophyll and protein content) and enzymatic parameters (Catalase and Guaiacol Peroxidase) were studied in detail for all the selected plants by using standard protocols (Arnon, 1949 for chlorophyll estimation; Lowry *et al*, 1951 for protein; Aebi 1984 for catalase and Putter 1974 for POD).

5.3.1 Harvesting of plant samples:

Harvesting of plant samples was done two times- one at 40 days and another at 80 days after sowing. The samples collected were studied for various morphological, biochemical and antioxidant enzyme activities.

5.3.1.1 Morphological parameters:

The observation of various growth parameters in the form of shoot length, root length, fresh weight, dry weight and moisture content were noted down at regular intervals.

5.3.1.1.1 Root length (cm): Root length was measured by using standard centimeter scale at regular intervals.

5.3.1.1.2 Shoot length (cm): It was measured by using a standard centimeters scale at regular intervals.

5.3.1.1.3 Fresh Weight and Dry Weight (gm)

Five seedlings were taken for the measurement of fresh weight (FW) on specified days. These weighed seedlings were then dried in oven at 60±2ºC for overnight for calculating dry weight. For weighing, digital balance of readability 0.001 g was used.

5.3.1.1.4 Moisture Content (percentage %)

Moisture content (MC) was also calculated from fresh weight and dry weights of seedlings using the formula given below. The FW and DW taken for these calculations were same as mentioned elsewhere.

$$\text{Moisture Content}(\%) = \frac{\text{Fresh Weight} - \text{Dry Weight}}{\text{Fresh Weight}} \times 100$$

5.3.1.2 Biochemical Analysis of Plant Material:

Plants grown in pot cultures were evaluated for the effects of different treatments of metals on plant pigments (chlorophyll *a*, *b* and total chlorophyll) and content of protein. For this study, firstly enzyme extract was prepared by homogenizing known weight of fresh plant material in phosphate buffer using pastle and mortar. Then centrifugation was done and supernatant was used for the estimation of various biochemical activities viz: total protein content, total chlorophyll.

5.3.1.2.1 Chlorophyll estimation

Amount of chlorophyll was calculated by the method of Arnon (1949) in the given plant material.

Sr. No.	Chemicals and reagents	Concentration used
1	Acetone	80% in 50mM Phosphate buffer (pH 7.0)

The photosynthetic pigments are soluble in acetone and chlorophyll a shows maximum absorbance of visible light at 663 nm, whereas, chlorophyll b shows maximum absorbance of visible light at 645 nm.

Procedure

Under ice-cold conditions, crushing of 1 g of sample with four milliliters of 80% acetone was done and the stuffed material was centrifuged for 20 minutes at 13000 rpm at 4ºC temperature. Absorbance of supernatant was noted down at wavelength of 645 and 663 nm.

Amount of chlorophyll *a*, chlorophyll *b* and total chlorophyll were computed by using the given formulae and then amount of chlorophyll in plant material was demonstrated as mg/g FW.

$$\text{Total Chlorophyll} = [(\text{Absorbance}_{645} \text{ x } 20.2) + (\text{Absorbance}_{663} \text{ x } 8.03)] \frac{V}{1000x\ W}$$

$$\text{Chlorophyll } a = [(\text{Absorbance}_{663} \text{ x } 12.7) - (\text{Absorbance}_{645} \text{ x } 2.69)] \frac{V}{1000x\ W}$$

$$\text{Chlorophyll } b = [(\text{Absorbance}_{645} \text{ x } 22.9) - (\text{Absorbance}_{663} \text{ x } 4.68)] \frac{V}{1000x\ W}$$

Where

A645 = Absorbance at 645 nm

A663 = Absorbance at 663 nm

V = Volume of plant extract W = Fresh Weight

5.3.1.2.2 Protein estimation:

Estimation of protein in the plant material was done by using the Lowry *et al* (1951) method with slight modifications.

Sr. No.	Reagents Used
1	Reagent A – 2.0 % Sodium Carbonate in 0.1N Sodium Hydroxide
2	Reagent B – 0.5% of Copper Sulphate in 1 % Potassium Sodium Tartrate
3	Reagent C – Freshly prepared 50 ml of Reagent A and 1.0 ml of Reagent B at the time of use
4	Reagent D – Folin-ciocalteau (FC) reagent
5	Standard protein solution (stock) – 50 mg of bovine serum albumin (BSA) dissolved in distilled water and the final volume was made to 50ml. Standard stock solutions were diluted to prepare working standard solutions of different concentrations.

Principle

In Lowry's method, phosphomolybdate and phosphotungstate (present in Folin-ciocalteu reagent) react with tyrosine and tryptophan, (the amino acids present in proteins) under alkaline conditions to form blue coloured product, which can be measured spectrophotometrically.

Procedure

Crushing of 1 gm of plant material with pestle and mortar was done by using 3 ml of potassium phosphate buffer (50mM) at 7.0 pH in too much cold condition and then centrifugation was done at 13,500 rpm for 20-25 minutes at temperature of 4ºC. 100ml of supernatant was mixed with 900ml of distilled water and then 5 ml of reagent C was added. The test tube with 1 ml distilled water served as blank. This mixture was mixed well and allowed to stand for 10 minutes followed by the addition of 500ml of reagent D. The reaction mixture was mixed thoroughly and incubated at room temperature for 30 minutes in dark conditions. The optical density of the blue colour was measured at 660 nm. A standard curve of protein solution representing concentration vs. absorbance was plotted and a linear regression equation was obtained

which was used to calculate the protein content of the samples, which was expressed as mg/g Fresh Weight.

5.3.1.3 Antioxidant enzymatic activity:

Preparation of plant extracts:

One-gram fresh plant samples were weighed and crushed in 3ml of extractant buffer using pestle and mortar under ice-cold conditions. The stuffed material was centrifuged for 20 minutes at 13000 rpm and 4ºC. The clear liquid overlying material was collected for investigation of enzymatic activity. The extracting buffer having 50mM of Pot. Phosphate buffer at 7.0 pH was used for estimating the enzymatic activities (i.e. CAT and POD).

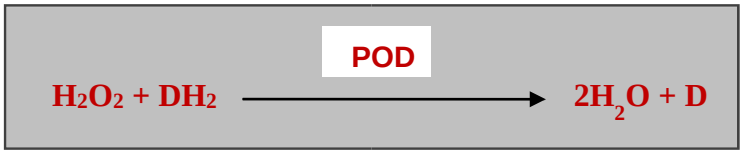
5.3.1.3.1 Guaiacol Peroxidase (POD, EC. 1.11.1.7)

Activity of POD was calculated by using the technique developed by Putter (1974) with slight modifications.

Sr. No.	Chemicals and Reagents	Concentrations used
1	Potassium Phosphate Buffer	50mM; pH 7.0
2	Guaiacol solution	20.0mM
3	Hydrogen Peroxide (H_2O_2)	12.4mM

Principle

The dehydrogenation of a wide range of organic compounds for example phenols, hydroquinones, aromatic amines, pyrogallol, guaiacol and o-cresol is carried out by peroxidases. Guaiacol peroxidase catalyses the reduction of hydrogen peroxide in the presence of guaiacol substrate that is converted to tetra-guaiacol.



One mole of guaiacol is oxidized by one mole of hydrogen peroxide to form possibly more than one compound, which are called as guaiacol dehydrogenation product

(GDHP). The rate of formation of GDHP is used to find out the activity of guaiacol peroxidase.

Procedure:

In a cuvette, 50mM of phosphate buffer + 20mM of guaiacol solution + 12.4 mM of hydrogen peroxide + 60 µl of enzyme extract was added to make 3ml of reaction mixture. Release of oxygen from guaiacol and GDHP formation was observed at wavelength 436nm for one minute at interval of five seconds and the temperature was set at 25ºC. Formation of one micro molar of GDHP/ minute/gm FW is known as one unit of enzyme activity and was by the following given formulas.

Calculation:

$$\text{Unit activity(U/min/g Fresh Weight)} = \frac{\text{Change in absorbance/min} \times \text{Total volume (ml)}}{\varepsilon \times \text{Volume of sample used(ml)} \times \text{Fresh Weight of sample}}$$

Where, ε represents extinction coefficient = 26.6mM^{-1}cm^{-1}

$$\text{Specific activity(UA/g Protein)} = \frac{\text{Unit activity(U/min/g Fresh Weight)}}{\text{Protein content(mg/g Fresh Weight)}}$$

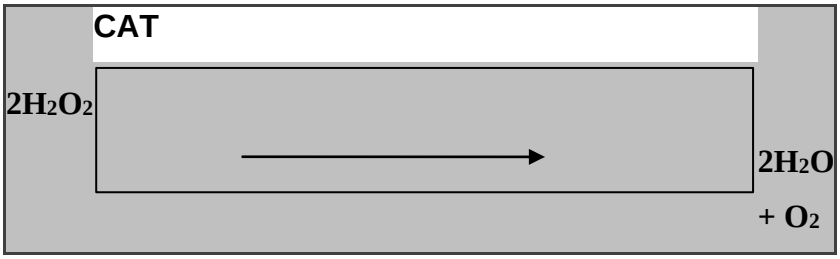
5.3.1.3.2 <u>Catalase (CAT, EC 1.11.1.6):</u>

The method of Aebi (1984) was used for estimating the activity of enzyme catalase with slight changes.

Sr. No.	Chemicals and reagents	Concentration used
1	Potassium Phosphate Buffer	50mM; pH 7.0
2	Hydrogen peroxide (H_2O_2)	15.5mM

Principle

The catalase enzyme catalyzes the breakdown of H_2O_2 to form H_2O and O_2.



The catalase activity can either be calculated by finding out approximate breakdown of hydrogen peroxide or liberation of O_2. The breakdown of hydrogen peroxide (H_2O_2) leads to the decline in extinction per unit time at 240 nm. For that reason, the difference in extinction per unit time finds out catalase activity.

Procedure:

To prepare three ml of reaction mixture, 50mM of pot. Phosphate buffer + 15.5 mM of H_2O_2 + 60 micro liter of enzyme extract was used. Due to breakdown of H_2O_2, reduction in absorbance was observed at 240nm for one minute at set temperature of 25°C. One unit of enzyme activity was interpreted as the quantity of enzyme requirement to release half amount of H_2O_2 and this unit activity was calculated by using the following given formulas.

Calculation

$$\text{Unit activity(U/min/g Fresh Weight)} = \frac{\text{Change in Unit activity(U/min/g Fresh Weight)}}{\varepsilon \times \text{Volume of sample used(ml)} \times \text{Fresh Weight of sample}}$$

Where, ε represents extinction coefficient = 39.4mM^{-1}cm^{-1}

$$\text{Specific activity(UA/g Protein)} = \frac{\text{Unit activity(U/min/g Fresh Weight)}}{\text{Protein content(mg/g Fresh Weight)}}$$

5.4 Objective (4th) - Analysis of phytoremediation capability along with statistical analysis and selection of appropriate weed.

Under this objective, first heavy metal analysis was done using standard procedure as given by Allen *et al* (1976) with slight modifications and then statistical

analysis was done using one-way analysis of variance (one-way ANOVA) to find the quantity of metal digested by the particular species of plant and to know whether it was capable of rectifying polluted area from selected metals or not.

5.4.1 Statistical data observation:

All the calculations were done in triplicates. Calculation of mean and standard deviation was also carried out for all the values. One-way ANOVA was done to analyze the data statistically by using the method developed by Bailey (1995). P-values $\leq$ 0.0001 were considered significant for comparison purpose.

5.4.2 Analysis of metals (Cr and Pb):

Plants with metal treatment, after harvesting were analyzed for metal uptake. The procedure adopted for this was as follows:

5.4.2.1 Digestion of samples:

The plant samples that were collected samples then dried at normal temperature. The dried samples (1 g) were digested in H_2SO_4: HNO_3: $HClO_4$ (1:5:1) by following the method suggested by Allen *et al* (1976). After digestion, samples were strained through Whatman filter paper (No.1) using double distilled water and final volume was made up to 50 ml.

5.4.2.2. Elemental analysis:

The uptake of Cr (VI) and Pb in the roots of all the selected plants were studied using atomic absorption spectrophotometer.

Sample Preparation:

The dried plant samples were digested by the method given by Allen *et al* (1976). The samples were weighed (0.4 g) and put in digestion beakers. The digestion mixture consisted of nitric acid (HNO_3) and perchloric acid ($HClO_4$) in the ratio of 2:1. In the digestion beaker, 5 ml of digestion mixture was initially added and heated. The addition of acid mixture was continued until the solution of digested plant sample became clear. After the digestion was complete, the final volume of the sample was made up to 15 ml with DW and strained with Whatman No. 1 filter paper. The samples were then stored in glass vials for further use.

Calculations:

Let the concentration of the analyte observed = x ppm (*i.e.* x mg of Cr (VI) in 1000 ml of solution)

Let the final volume of the sample prepared after digestion = y ml

$$\text{Therefore, y ml of solution contains} = \frac{xy}{1000} \text{ mg of Cr(VI)}$$

Let y ml of solution be prepared from w g of plant material, then

$$\text{1 g of plant material contains} = \frac{xy}{1000w} \text{ mg/g of Cr(VI)}$$

The same procedure and calculations were repeated for analysis of Lead.

5.5 Objective (5th) - Analysis of effect on grazing animal milk quality through consumption of metal absorbing fodder plants.

Under this objective, wet digestion method was done by atomic absorption spectrophotometric analysis of prepared milk samples for analyzing milk quality of dairy farms that fed on metal containing fodder. Then comparison was done to observe the amount of Cr and Pb heavy metals in standard milk samples.

CHAPTER- 6

RESULTS AND DISCUSSION

6.1 Study Area:

The present study was undertaken by selecting some of the weeds and fodder plants (*Cyperus iria, Achyranthes aspera* and *Eruca sativa*) growing along the banks of Kala Sanghian drain and fields near to it.

The village Kala Sanghian is located along Jalandhar – Kapurthala road. Kala Sanghian drain is directly receiving large amount of wastewater effluent from leather tanning and sports complex Jalandhar and the effluent is very rich in various heavy metals. This drain then lead to Chittibein and finally to river Sutlej. Most villagers use this heavy metal rich polluted water for irrigating their crops. These metals leach into the soil surface and then move into human body when they eat these affected crops. Therefore, this drain has become a serious problem. Large number of studies are being carried out time to time for the treatment of this wastewater by various methods. In the present study, phytoremediation potential of some of the weed plants of the area was worked out. (Fig 6.1). The present study was undertaken in a village of Banga area of S. B. S. Nagar, Punjab. The average annual rainfall in the area is 924 cm with average temperature of 35- 40°C. Texture of the soil is loamy sand with pH value between 8.0 to 7.8 (Punjab Soils).

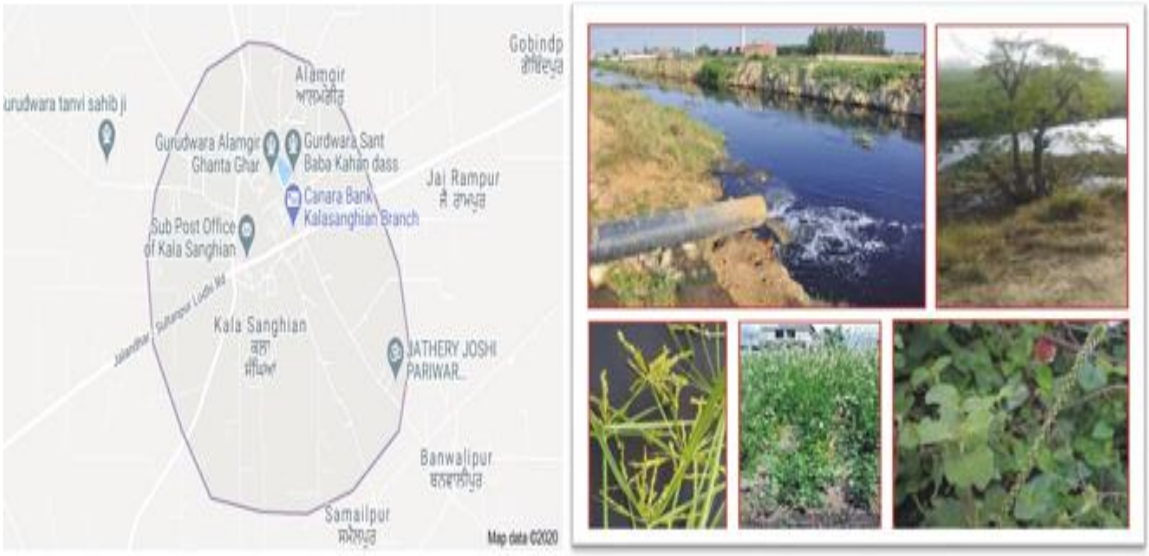
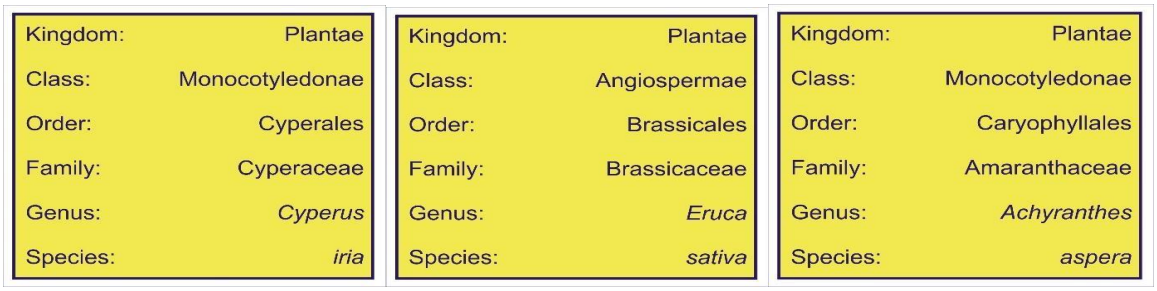


Fig 6.1: Showing study area and plant species (*C. iria*, *E. sativa* and *A. aspera*)



6.2 Raising of plant material:

Pot culture experiments were carried out under natural field conditions in one of the village of Banga area S. B. S. Nagar, Punjab. Healthy seeds were sterilized with 0.02 % mercuric chloride solution ($HgCl_2$) for few minutes and then washed with distilled water for several times. Then seeds were grown in plastic pots of 10 cm height and 12 cm width pre filled with 4Kg of grounded, sieved soil free from heavy metals (especially Chromium and Lead). Pots were left in the field and watered daily. The research work was carried out from July 2017 to September 2019. During this period, the seeds of selected plants were grown in their different growing seasons with similar potting pattern followed everytime and studies on their morphological, biochemical, antioxidant enzyme activities were done. Analysis of metal deposition in plant roots was also done. Along with this, chromium (VI) and lead (II) accumulation in the milk of grazing animals (cattle) was also analyzed.

Studies carried out on different weeds and fodder plants pertaining to various morphological parameters, biochemical parameters and enzymatic parameters showed that increase in the concentration of both heavy metals Cr (VI) and Pb (II) resulted in less growth, decreased value of chlorophyll and increased activity of antioxidant enzymes to some extent. The observations made on different growth stages (40 and 80 days) of selected weeds and fodder plants were presented below.

STUDIES ON CYPERUS IRIA PLANT

6.3 Toxic effects of Cr (VI) on *Cyperus iria*:

6.3.1 Morphological characteristics:

In this part, root length, shoot length, fresh weight, dry weight and moisture content were studied for 40 and 80 days old plants and the effects of Cr (VI) were analyzed.

6.3.1.1 Root length: Cr (VI) caused less growth in root length of potted plants. In comparison to control, 350 mg/Kg Cr (VI) caused 58.7% and 64.1% decrease in root length in 40 and 80 days old plants respectively. No growth was observed at 400 mg/Kg Cr concentration. The F-ratio for one-way ANOVA for all the growth stages were significant, thus showing the remarkable effect of the treatments. (Table 6.1, fig 6.2, 6.3)

Table 6.1: Cr (VI) effect on root length (cm) of *Cyperus iria* plants during different growth stages.

Root length of *Cyperus iria* plants at 40 and 80 days		
Concentration Cr (VI) (mg/Kg)	**40 Days**	**80 days**
0	4.24 ± 0.0306	5.10 ± 0.0306
50	3.88 ± 0.0306	4.12 ± 0.0252****
100	3.38 ± 0.0306**	3.53 ± 0.0252****
150	3.12 ± 0.0208***	3.25 ± 0.0300****
200	2.86 ± 0.0306****	3.23 ± 0.0252****
250	2.52 ± 0.0208****	2.91 ± 0.0300****
300	1.94 ± 0.0200****	2.03 ± 0.0200****
350	1.75 ± 0.0153****	1.83 ± 0.0200****
F-ratio (df 7,16)	**61.07****	**4984****
HSD	**0.59**	**0.07**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

40 days **80 days**

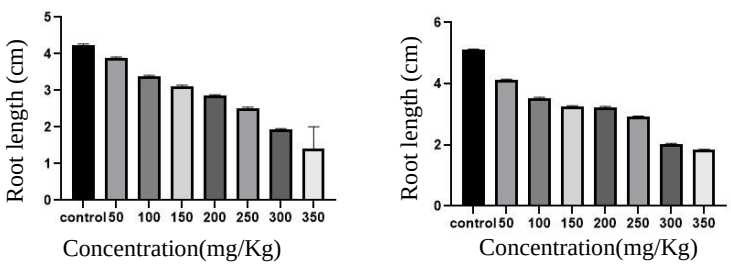


Fig. 6.2, 6.3: Effect of metal stress of Cr (VI) on root length (cm) of 40 and 80 days old *C. iria* plants.

6.3.1.2 Shoot length: Shoot length also get reduced with increasing concentration of Cr (VI) in comparison to control. Maximum reduction was noted at 350 mg/Kg Cr (VI) concentration at 40 days old plants by 62.5%. In 80 days old plants, there is slight increase in shoot length at 50 mg/Kg Cr (VI) concentration by 6.6% and maximum reduction was observed at 350 mg/Kg Cr (VI) concentration by 46.3%. Then growth get arrested. The F-ratio was found to be significant (Table-6.2, fig-6.4, 6.5).

Table 6.2: Cr (VI) effect on shoot length (cm) of *Cyperus iria* plants during different growth stages**.**

Shoot length of *Cyperus iria* plants at 40 and 80 days		
Concentration Cr (VI) (mg/Kg)	**40 Days**	**80 days**
0	10.52 ± 0.0321	18.34 ± 0.0306
50	8.80 ± 0.0306*****	19.56 ± 0.0191****
100	9.95 ± 0.0400****	17.16 ± 0.0153****
150	8.82 ± 0.0300****	15.22 ± 0.0451****
200	8.44 ± 0.0404****	14.76 ± 0.0416****
250	7.95 ± 0.0306****	14.32 ± 0.0945****
300	6.60 ± 0.0907****	13.63 ± 0.0400****
350	3.94 ± 0.0300****	9.85± 0.0451****
F-ratio (df 7,16)	**6361******	**12345******
HSD	**0.12**	**0.13**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

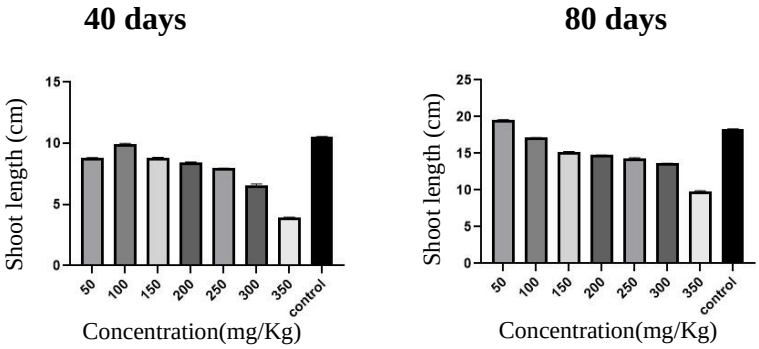


Fig. 6.4, 6.5: Effect of metal stress of Cr (VI) on shoot length (cm) of 40 and 80 days old *C. iria* plants.

6.3.1.3 Fresh weight: Toxic effects of Cr (VI) were also noticed on fresh weight of the plant. Reduction of 84.4% and 86.5% was observed at 350 mg/Kg Cr (VI) in 40 and 80 days old plants respectively. The F-ratio for one way ANOVA for all growth stages was found to be significant (Table 6.3, Fig 6.6, 6.7).

Table 6.3: Cr (VI) effect on fresh weight (gm) of *Cyperus iria* plants during different growth stages.

Fresh weight of *Cyperus iria* plants at 40 and 80 days		
Concentration Cr (VI) (mg/Kg)	**40 Days**	**80 days**
0	31.47 ± 0.89	27.54 ± 1.09
50	22.71 ± 1.96****	19.36 ± 0.46****
100	19.54 ± 0.72****	17.19 ± 0.77****
150	16.45 ± 0.77****	14.32 ± 0.59****
250	11.28 ± 1.10****	10.91 ± 0.53****
300	9.26 ± 0.45****	7.91 ± 0.89****
350	4.89 ± 0.96****	3.71 ± 0.50****
F-ratio (df 7, 16)	**174.3******	**331.8******
HSD	**3.14**	**1.986**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

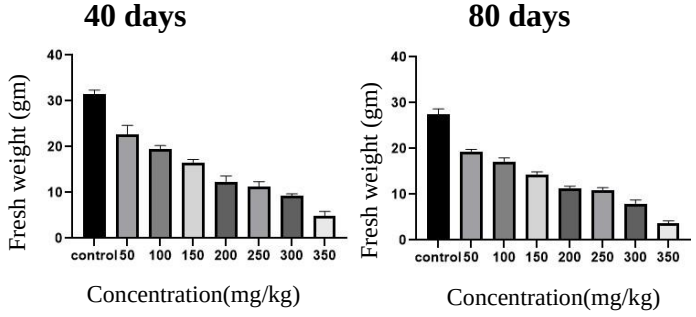


Fig. 6.6, 6.7: Effect of metal stress of Cr (VI) on fresh weight (gm) of 40 and 80 days old *C. iria* plants.

6.3.1.4 Dry weight: Dry weight also get decreased by 86.9% and 90.8% in 40 and 80 days old plants as compared to control plants. The F-ratio for one way ANOVA for all growth stages was found to be significant. (Table 6.4, Fig 6.8, 6.9)

Table 6.4: Cr (VI) effect on dry weight (gm) of *Cyperus iria* plants during different growth stages.

Dry weight of *Cyperus iria* plants at 40 and 80 days		
Concentration Cr (VI) (mg/Kg)	**40 Days**	**80 days**
0	23.14 ± 0.85	19.38 ± 0.56
50	14.86 ± 1.01****	12.24 ± 0.78****
100	11.39 ± 0.52****	9.44 ± 0.57****
150	10.22 ± 0.68****	7.85 ± 0.51****
200	6.90 ± 0.66****	5.56 ±0.31****
250	6.37 ± 0.55****	4.45 ± 0.42****
300	4.29 ± 0.68****	2.53 ± 0.34****
350	3.03 ± 0.70****	1.77 ± 0.41****
F-ratio (df 7,16)	**249.4******	**396.1******
HSD	**2.04**	**1.43**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

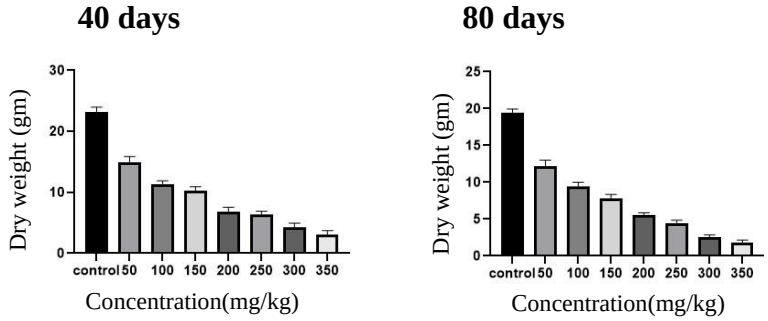


Fig. 6.8, 6.9: Effect of metal stress of Cr (VI) on dry weight (gm) of 40 and 80 days old *C. iria* plants.

6.3.1.5 Moisture content: Increase in moisture content of 102.6% and 129.7% was observed at 300mg/Kg Cr (VI) in comparison to plants taken as control. The F-ratio for all growth stages was found to be significant. (Table 6.5 Fig 6.10, 6.11)

Table 6.5: Cr (VI) effect on moisture content of *Cyperus iria* plants during different growth stages.

Moisture content of *Cyperus iria* plants at 40 and 80 days

Concentration Cr (VI) (mg/Kg)	40 Days	80 days
0	26.41 ±3.98	29.56 ± 2.71
50	34.11 ± 8.70	36.76 ± 3.82
100	41.66 ± 2.79	45.12 ± 0.95*
150	37.77 ± 5.24	45.07 ± 4.87*
200	43.74 ± 1.45	50.61 ± 2.53**
250	43.26 ± 6.00	59.14 ± 3.91****
300	53.53 ± 8.36	67.92 ± 4.48****
350	36.62 ± 16.83	52.03 ± 11.18**
F-ratio (df 7,16)	**0.7545**	**16.34****
HSD	**32.53**	**14.63**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

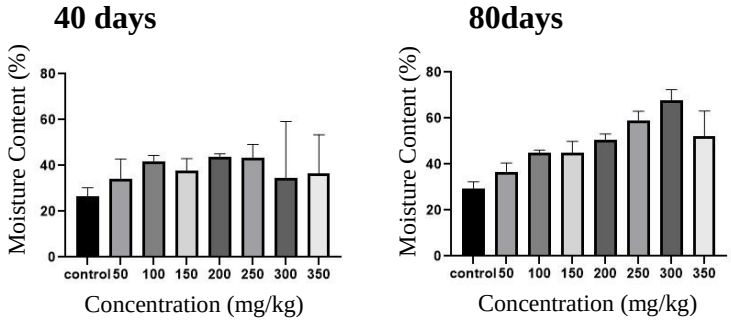


Fig. 6.10, 6.11: Effect of Cr (VI) metal stress on moisture content of 40 and 80 days old *C. iria* plants.

6.3.2 Biochemical Parameters

6.3.2.1 Chlorophyll *a* – Decrease in the amount of chlorophyll *a* was observed in *Cyperus iria* plants when grown in different treatments of Cr. A significant decrease of 88.6% and 90.3% was observed in leaves of 40 and 80 days old plants at 300 mg/Kg Cr (VI) concentration. The F-ratio was found to be significant for all treatments. (Table 6.6, fig 6.12, 6.13)

Table 6.6: Cr (VI) effect on content of chlorophyll *a* (mg/g FW) in the leaves of *C. iria* plants during different growth stages.

Chlorophyll *a* value in the leaves of *C. iria* plants at 40 and 80 days		
Concentration Cr (VI) (mg/Kg)	**40 Days**	**80 days**
0	0.3417 ± 0.0031	0.2740 ± 0.0131
50	0.2813 ± 0.0035****	0.1747 ± 0.0091

100	0.2453 ± 0.0060****	0.1460 ± 0.0174
150	0.2609 ± 0.0150****	0.1622 ± 0.0057
200	0.1800 ± 0.0026****	0.0936 ± 0.0056
250	0.0819 ± 0.0060****	0.0699 ± 0.0048
300	0.0646 ± 0.0016****	0.0535 ± 0.0020
350	0.0388 ± 0.0035****	0.0264 ± 0.0040
F-ratio (df 7,16)	**905.6**	**1.418**
HSD	**0.074**	**0.395**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

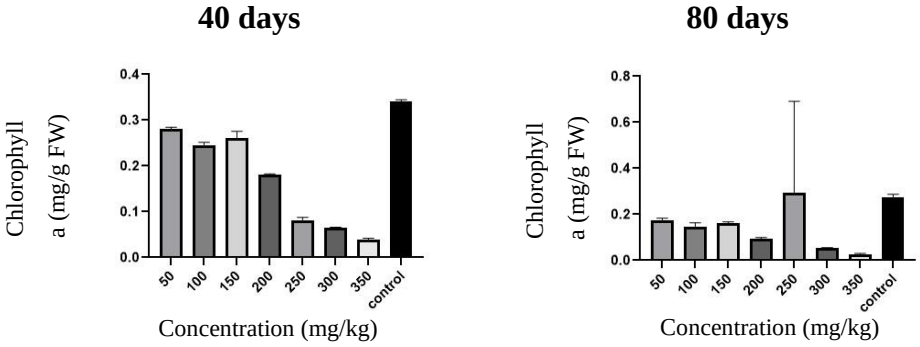


Fig. 6.12, 6.13: Effect of Cr (VI) metal on chlorophyll a (mg/g FW) in the leaves of 40 and 80 days old *C. iria* plants.

6.3.2.2 Chlorophyll *b*- Chlorophyll *b* content in the leaves also showed reduction when grown in the increasing concentration of Cr (VI). The reduction of 89.2% and 88.6% was observed in 40 and 80 days old plants respectively. Significance of F-ratio showed the effect of treatment at all stages (Table 6.7, fig 6.14, 6.15).

Table 6.7: Cr (VI) effect on content of chlorophyll *b* (mg/g FW) in the leaves of *C. iria* plants during different growth stages.

Chlorophyll *b* value in the leaves of *C. iria* plants at 40 and 80 days		
Concentration Cr (VI) (mg/Kg)	**40 Days**	**80 days**
0	0.2763 ± 0.0086	0.1820 ± 0.0056
50	0.2213 ± 0.0143****	0.0947 ± 0.0015****
100	0.1947 ± 0.0075****	0.0907 ± 0.0015****
150	0.2333 ± 0.0087**	0.0827 ± 0.005****
200	0.0820 ± 0.0135****	0.0683 ± 0.0040****
250	0.0680 ± 0.0106****	0.0547 ± 0.0015****
300	0.0507 ± 0.0067****	0.0443 ± 0.0031****
350	0.0297 ± 0.0038****	0.0207 ± 0.0068****
F-ratio (df 7,16)	**294.1**	**410.2**

HSD	**0.01**	**0.746**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

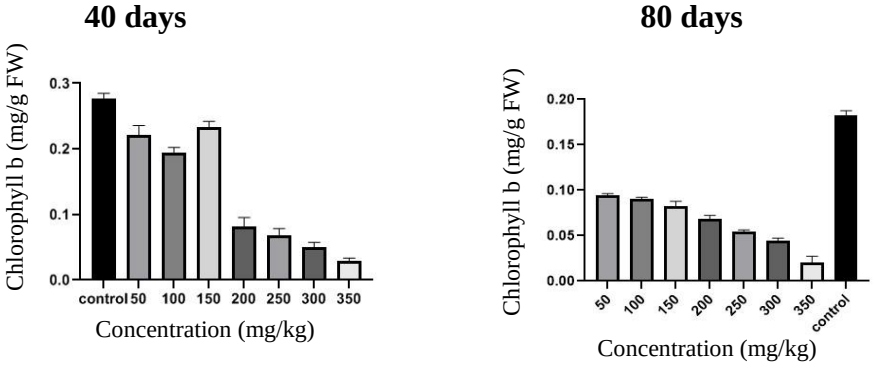


Fig. 6.14, 6.15: Effect of Cr (VI) metal on chlorophyll b (mg/g FW) in 40 and 80 days old *C. iria* plants.

6.3.2.3 Total chlorophyll-The leaves of *Cyperus iria* plants showed reduced content of total chlorophyll when grown in soil containing different chromium treatments. In comparison to control, 350 mg/Kg Cr (VI) treatment caused a reduction of around 89% in both 40 and 80 days old plants respectively. No further growth was observed at higher Cr concentration. The F- ratio for all treatments was found to be significant. (Table 6.8, fig 6.16, 6.17)

Table 6.8: Cr (VI) effect on total chlorophyll content (mg/g FW) in the leaves of *C. iria* plants during different growth stages.

Total chlorophyll value in the leaves of *C. iria* plants at 40 and 80 days		
Concentration Cr (VI) (mg/Kg)	**40 Days**	**80 days**
0	0.6180 ± 0.0056	0.4560 ± 0.0078
50	0.5027 ± 0.0110****	0.2693 ± 0.0106****
100	0.4400 ± 0.0135****	0.2367 ± 0.0164****
150	0.4943 ± 0.0224****	0.2448 ± 0.0085****
200	0.2620 ± 0.0151****	0.1620 ± 0.0089****
250	0.1499 ± 0.0085****	0.1246 ± 0.0037****
300	0.1152 ± 0.0052****	0.0978 ± 0.0049****
350	0.0685 ± 0.0054****	0.0471 ± 0.0069****
F-ratio (df 7,16)	**872.1****	**576****
HSD	**0.0346**	**0.013**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

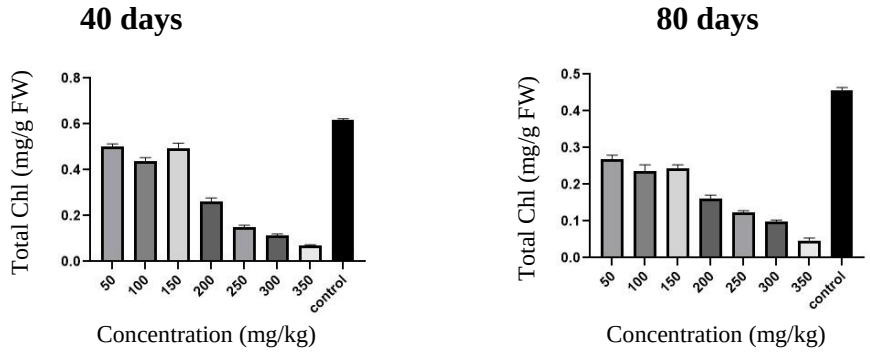


Fig. 6.16, 6.17: Effect of Cr (VI) metal on total chlorophyll content (mg/g FW) in 40 and 80 days old *C. iria* plants.

6.3.3 Protein content and Antioxidant Enzymes.

6.3.3.1 Protein content: Reduction was observed in the protein content with rise in chromium application. Much reduction was observed at 350 mg/Kg Cr (VI) concentration with 73.7% and 96.8% in 40 and 80 days old plants. After this, no growth was seen. Significance of F ratio was implied at all treatments. (Table 6.9 fig 6.18, 6.19).

Table 6.9: Cr (VI) effect on protein content (mg/g FW) in *C. iria* plants during different growth stages.

Protein value in the leaves of *C. iria* plants at 40 and 80 days		
Concentration Cr (VI) (mg/Kg)	**40 Days**	**80 days**
0	4.99 ± 0.40	2.52 ± 0.05
50	3.84 ± 0.12****	2.22 ± 0.04
100	3.36 ± 0.04****	1.81 ± 0.03**
150	2.85 ± 0.04****	1.53 ± 0.04***
200	2.56±0.08****	1.41±0.02****
250	1.83 ± 0.04****	1.27 ± 0.03****
300	1.61 ± 0.05****	0.73 ± 0.55****
350	1.31 ± 0.05****	0.08 ± 0.01****
F-ratio (df 7,16)	**198.8****	**47.21****
HSD	**0.431**	**0.558**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

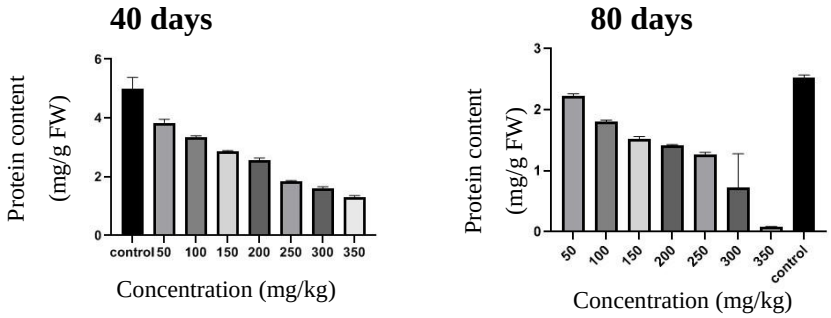


Fig. 6.18, 6.19: Effect of Cr (VI) metal on protein content (mg/g FW) in 40 and 80 days old *C. iria* plants.

6.3.3.2 Catalase (CAT) – The activity of antioxidants enzyme CAT get enhanced with increasing value of chromium. Maximum increase was observed at 300 mg/Kg concentration of 410.9% in 40-day-old plants and maximum increase of 382.4% in 200 mg/Kg concentration of Cr (VI) in 80 days old plants as compared to control plants. Then decreases in enzyme activity was noticed at high values with very less difference in their values. F ratio value was also found to be significant for all stages of treatment. (Table 6.10, fig 6.20, 6.21).

Table 6.10: Cr (VI) effect on specific activity of CAT (UA/g protein) in the leaves of *C. iria* plants during different growth stages.

Specific activity of catalase in *C. iria* plants at 40 and 80 days		
Concentration Cr (VI) (mg/Kg)	**40 Days**	**80 days**
0	7.40 ± 0.24	5.23 ± 0.1
50	12.21 ± 0.96	10.87 ± 0.32****
100	20.53 ± 0.74*	16.24 ± 0.36****
150	23.42 ± 0.34****	20.01 ± 0.15****
200	28.87 ± 0.40****	25.23 ± 0.12**
250	34.72 ± 0.51****	23.30 ± 0.18****
300	37.81 ± 0.83****	18.99 ± 0.25****
350	35.07 ± 0.26****	17.40 ± 0.26****
F-ratio (df 7,16)	**39.49****	**856.4****
HSD	**2.641**	**0.511**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

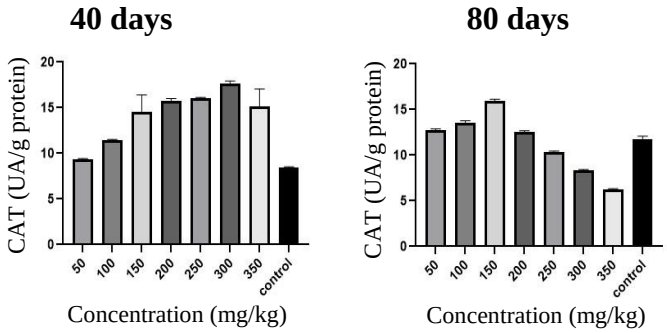


Fig. 6.20, 6.21: Effect of Cr (VI) metal on specific activity of CAT (UA/g protein) in 40 and 80 days old *C. iria* plants.

6.3.3.3 Guaiacol Peroxidase (POD). The activity of POD was observed to get increase with rise in amount of hexavalent chromium and maximum increase was found at 300 mg/Kg conc with 107.6% in 40 days old plants. In 80 days old plants there is increase of 36% at 150 mg/Kg Cr (VI). Then reduction in the activity of POD was observed and maximum reduction of 46.6% was noticed at 350mg/Kg Cr in comparison to control plants. No growth was seen after that. F ratio was also found to be significant (Table 6.11, fig 6.22, 6.23).

Table 6.11: Cr (VI) effect on specific activity of POD (UA/g protein) in the leaves of *C. iria* plants during different growth stages.

POD Specific activity in *C. iria* plants at 40 and 80 days		
Concentration Cr (VI) (mg/Kg)	**40 Days**	**80 days**
0	8.42 ± 0.11	11.74 ± 0.34
50	9.35 ± 0.08****	12.77 ± 0.12
100	11.43 ± 0.11****	13.55 ± 0.23**
150	13.59 ± 0.13****	15.97 ± 0.18****
200	15.74 ± 0.27****	12.52 ± 0.17
250	16.06 ± 0.09****	10.36 ± 0.10*
300	17.48 ± 0.16****	8.37 ± 0.07****
350	14.11± 0.22****	6.26 ± 0.06****
F-ratio (df 7, 16)	**1261******	**127******
HSD	**0.45**	**1.26**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

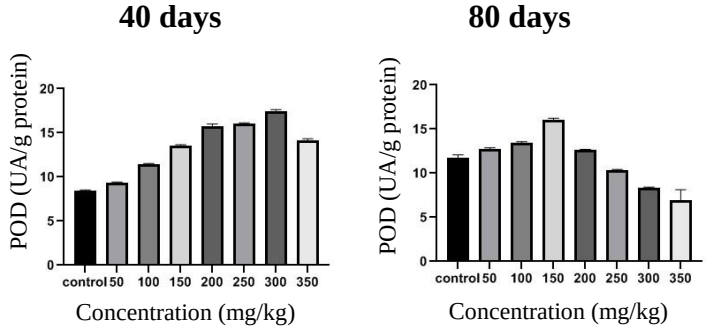


Fig. 6.22, 6.23: Effect of Cr (VI) metal on specific activity of POD (UA/g protein) in 40 and 80 days old *C. iria* plants.

6.4 Toxic effects of Pb (II) on *Cyperus iria*:

6.4.1Morphological characters:

6.4.1.1 Root length: Pb (II) caused a much drop in length of root of potted plants. In contrast to control, firstly 100 mg/Kg Pb (II) first caused a non-significant increase of 3.5% and then caused significant 78% decrease in root length in 40 days old plants. In 80 days old plants we had almost similar variation with 16.7% increase in root length at 200 mg/Kg Pb (II) and then 78% decrease at 800 mg/Kg Pb (II). The F-ratio for one-way ANOVA for all the growth stages were significant, thus showing the remarkable effect of the study. (Table 6.12, fig 6.24, 6.25). Plants were not able to grow at further higher Pb (II) concentration (900 mg/Kg) in all the parameters studied. Therefore, this concentration was not taken into consideration in the tables and graphs.

Table 6.12: Pb (II) effect on root length (cm) of *Cyperus iria* plants during different growth stages.

Root length of *Cyperus iria* plants at 40 and 80 days		
Concentration Pb (II) (mg/Kg)	**40 Days**	**80 days**
0	5.10 ± 0.0306	5.48 ± 0.1518
100	5.28 ± 0.1528	6.29 ± 0.0764*
200	3.79 ± 0.2401****	6.40 ± 0.1735*
300	3.25 ± 0.0300****	5.36 ± 0.1752
400	3.23 ± 0.0252****	4.74 ± 0.3398
500	2.91 ± 0.0300****	3.66 ± 0.5205****
600	2.03 ± 0.0200****	2.30 ± 0.3508****
700	1.83 ± 0.0200****	1.60 ± 0.2458****
800	1.12 ± 0.0265****	1.28 ± 0.0656****
F-ratio (df 8,18)	**627.9******	**160.7******
HSD	**0.278**	**0.776**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

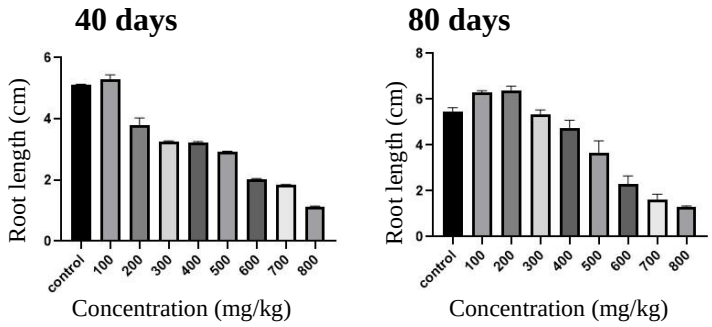


Fig. 6.24, 6.25: Effect of metal stress of Pb (II) on root length (cm) of 40 and 80 days old *C. iria* plants.

6.4.1.2 Shoot length: Shoot length also get reduced with rise in amount of Pb (II) in contrast to control plants. First, a slight increase 15.8% and 12.2% in length of 40 and 80 days old plants was found at 200 mg/Kg Pb (II). Then maximum reduction was observed at 800mg/Kg Pb (II) concentration by 55.6% and 62.5% in 40 and 80 days old plants respectively. The F-ratio was found to be significant (Table-6.13, fig-6.26, 6.27)

Table 6.13: Effect of Pb (II) on shoot length (cm) of *Cyperus iria* plants during different growth stages.

Shoot length of *Cyperus iria* plants at 40 and 80 days		
Concentration Pb (II) (mg/Kg)	**40 Days**	**80 days**
0	25.45 ± 0.1311	32.47 ± 0.1833
100	27.64 ± 0.2003****	35.27 ± 0.2563****
200	29.48 ± 0.1600****	36.45 ± 0.2400****
300	25.72 ± 0.0929	31.61 ± 0.2506
400	22.88 ± 0.2413****	26.01 ± 0.2848****
500	19.90 ± 0.2291****	22.49 ± 0.1804****
600	17.59 ± 0.1539****	18.51 ± 0.2358****
700	15.47 ± 0.1531****	15.88 ± 0.1801****
800	11.29 ± 0.2542****	12.17 ± 0.9157****
F-ratio (df 8,18)	**3167******	**1686******
HSD	**0.534**	**1.07**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

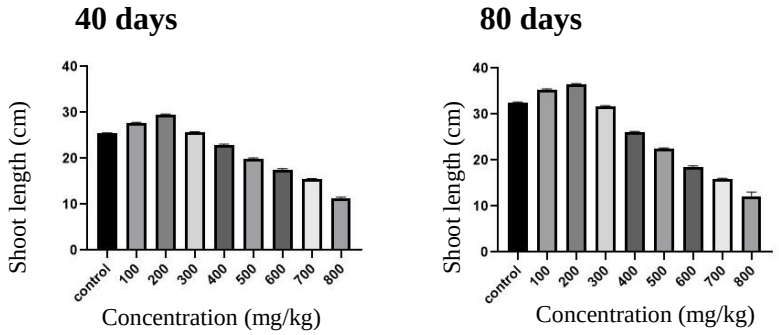


Fig 6.26, 6.27: Effect of metal stress of Pb (II) on shoot length (cm) of 40 and 80 days old *C. iria* plants.

6.4.1.3 Fresh weight –Toxic effects of Pb (II) were also noticed on fresh weight of the plant. Reduction of 49.4% and 84.7% was observed at 800 mg/Kg Pb (II) in 40 and 80 days old plants respectively. No growth was observed after that. The f-ratio for one way ANOVA for all growth stages was found to be significant. (Table 6.14, Fig 6.28, 6.29).

Table 6.14: Effect of Pb (II) on fresh weight (gm) of *Cyperus iria* plants during different growth stages.

Fresh weight of *Cyperus iria* plants at 40 and 80 days		
Concentration Pb (II) (mg/Kg)	**40 Days**	**80 days**
0	29.01 ± 0.58	22.56 ± 0.20
100	27.39 ± 0.18	21.25 ± 0.33*
200	25.27 ± 0.18	17.19 ± 0.77****
300	23.57 ± 0.27	14.39 ± 0.60****
400	19.39 ± 0.11***	11.43 ± 0.54****
500	15.42 ± 0.28**	9.42 ± 0.23****
600	11.62 ± 0.16***	6.72 ± 0.28****
700	7.59 ± 0.24****	4.39 ± 0.09****
800	5.97 ± 0.20****	3.45 ± 0.22****
F-ratio (df 8,18)	**18.90******	**838.3******
HSD	**9.98**	**1.20**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

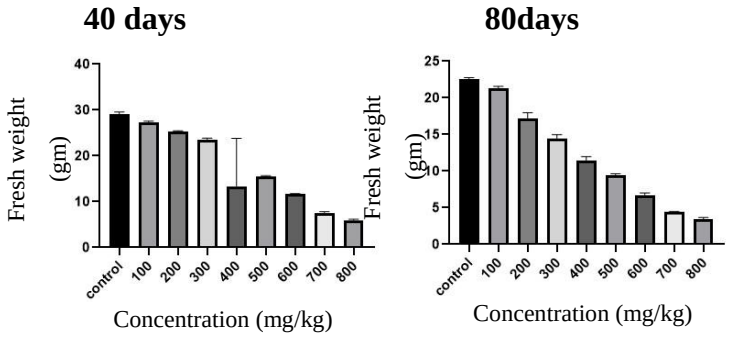


Fig. 6.28, 6.29: Effect of metal stress of Pb (II) on fresh weight (gm) of 40 and 80 days old *C. iria* plants.

6.4.1.4 Dry weight - Dry weight also get decreased by 82.6% and 96.6% at 800 mg/Kg Pb (II) in 40 and 80 days old plants as compared to control plants. The F-ratio for one way ANOVA for all growth stages was found to be significant. (Table 6.15, Fig 6.30, 6.31).

Table 6.15: Effect of Pb (II) on dry weight (gm) of *Cyperus iria* plants during different growth stages.

Dry weight of *Cyperus iria* plants at 40 and 80 days		
Concentration Pb (II) (mg/Kg)	**40 Days**	**80 days**
0	16.46 ± 0.23	13.63 ± 0.25
100	14.58 ± 0.33****	11.81 ± 0.34***
200	11.31 ± 0.38****	9.23 ± 0.12****
300	9.50 ± 0.18****	6.34 ± 0.12****
400	7.38 ± 0.17****	4.32 ± 0.12****
500	6.68 ± 0.29****	2.59 ± 0.16****
600	4.67 ± 0.28****	1.26 ± 0.07****
700	3.55 ± 0.24****	0.59 ± 0.16****
800	2.86 ± 0.08****	0.46 ± 0.17****
F-ratio (df 8,18)	**1062******	**504.5******
HSD	**0.729**	**1.095**

Data shown as mean ± S.D of triplicates Tukey's multiple comparison test (n=24). Significant at ****$p \leq 0.0001$, ***$p \leq 0.001$, **$p \leq 0.01$, *$p \leq 0.05$.

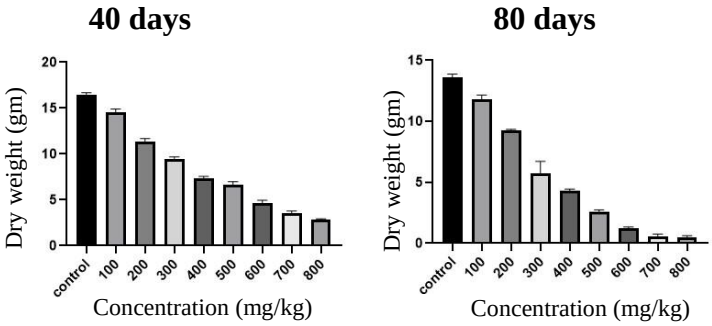


Fig. 6.30, 6.31: Effect of metal stress of Pb (II) on dry weight (gm) of 40 and 80 days old *C. iria* plants.

6.4.1.5 Moisture content –Increase in moisture content of 43.1% and 119.4% was noticed at 800 mg/Kg Pb (II) concentration in contrast to control plants. F-ratio for all growth stages was found to be significant. (Table 6.16, fig 6.32, 6.33)

Table 6.16: Effect of Pb (II) on moisture content of *Cyperus iria* plants during different growth stages**.**

Moisture content of *Cyperus iria* plants at 40 and 80 days		
Concentration Pb (II) (mg/Kg)	**40 Days**	**80 days**
0	43.27 ± 0.74	39.56 ± 1.
100	46.75 ± 0.87	44.40 ± 1.72
200	55.26 ± 1.18****	46.25 ± 2.4
300	59.71 ± 0.54****	55.84 ± 2.65****
400	61.94 ± 1.05****	62.17 ± 1.12****
500	56.68 ± 1.88****	72.50 ± 2.3****
600	59.77 ± 2.92****	81.32 ± 0.30****
700	53.31 ± 1.73****	86.52 ± 3.95****
800	52.09 ± 0.23****	86.82 ± 4.17****
F-ratio (df 8,18)	**34.08******	166.3****
HSD	**5.23**	**7.156**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at****$p \leq 0.0001$, ***$p \leq 0.001$, **$p \leq 0.01$, *$p \leq 0.05$.

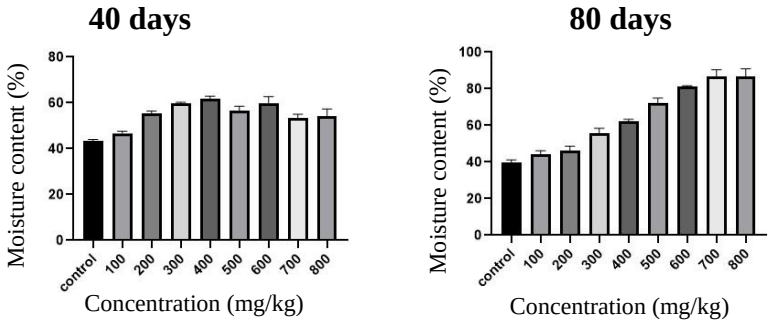


Fig. 6.32, 6.33: Effect of Pb (II) metal stress on moisture content of 40 and 80 days old *C. iria* plants.

6.4.2 Biochemical Parmeters

6.4.2.1 Chlorophyll *a* –Amount of chlorophyll *a* also get decreased in *Cyperus iria* leaves brought up in different treatments of Pb (II). Firstly, there is increase of 16.5% at 200 mg/Kg of Pb (II) amount in 40 days old plants and very non – significant increase at 100 mg/Kg of Pb (II) amount in 80 days old plants. A significant decrease of 47.3% and 90% was observed in 40 and 80 days old plants at 800 mg/Kg Pb (II) concentration. The F-ratio was found to be significant for all treatments. (Table 6.17, fig 6.34, 6.35)

Table 6.17: Effect of Pb (II) on chlorophyll a content (mg/g FW) in the leaves of *C. iria* plants during different growth stages**.**

Chlorophyll *a* content in the leaves of *C. iria* plants at 40 and 80 days		
Concentration Pb (II) (mg/Kg)	**40 Days**	**80 days**
0	0.6543 ± 0.0075	0.6720 ± 0.0175
100	0.7527 ± 0.0242	0.6857 ± 0.0500
200	0.7613 ± 0.0186	0.5497 ± 0.0110****
300	0.5857 ± 0.0114	0.4407 ± 0.0137****
400	0.6293 ± 0.0045	0.3693 ± 0.0137****
500	0.5500 ± 0.0298	0.2757 ± 0.0200****
600	0.4657 ± 0.0085	0.0780 ± 0.0220****
700	0.3685 ± 0.0085****	0.0667 ± 0.0055****
800	**0.3443 ± 0.0226*** **	**0.0573 ± 0.0057**** **
F-ratio (df 8,18)	**18.54**** **	**405**** **
HSD	**0.196**	**0.062**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

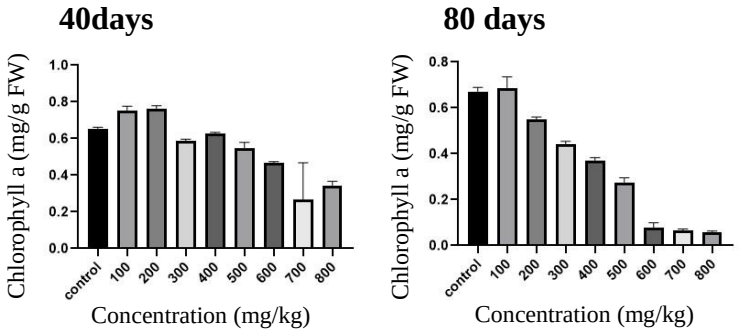


Fig. 6.34, 6.35: Effect of Pb (II) metal on chlorophyll *a* (mg/g FW) in 40 and 80 days old *C. iria* plants.

6.4.2.2 Chlorophyll *b*- Chlorophyll *b* content in the leaves also showed reduction when grown in the rising amount of Pb (II). First, increase of 18.8% at 200 mg/Kg of Pb (II) concentration and 57.5% decrease at 800 mg/Kg concentration in 40 days old plants was noticed. The reduction of 80.4% was observed in 80 days old plants. Significance of F-ratio showed the effect of treatment at all stages (Table 6.18, fig 6.36, 6.37).

Table 6.18: Effect of Pb (II) on content of chlorophyll b (mg/g FW) in *C. iria* plants during different growth stages.

Chlorophyll b content in *C. iria* plants at 40 and 80 days		
Concentration Pb (II) (mg/Kg)	**40 Days**	**80 days**
0	0.4847 ± 0.0110	0.4317 ± 0.0081
100	0.5547 ± 0.0093****	0.4157 ± 0.0075
200	0.5763 ± 0.0073****	0.3907 ± 0.0051***
300	0.4300 ± 0.0082***	0.3547 ± 0.0093****
400	0.3733 ± 0.0080****	0.3263 ± 0.0097****
500	0.3437 ± 0.0190****	0.1873 ± 0.0055****
600	0.2863 ± 0.0042****	0.1443 ± 0.0080****
700	0.2533 ± 0.0093****	0.1287 ± 0.0075****
800	**0.2060 ± 0.011****	**0.0843 ± 0.0093****
F-ratio (df 8,18)	**478.2****	**889.7****
HSD	**0.029**	**0.028**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

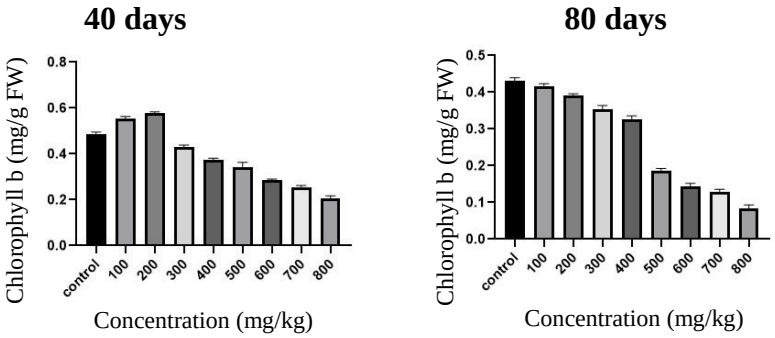


Fig. 6.36, 6.37: Effect of Pb (II) metal on chlorophyll *b* (mg/g FW) in 40 and 80 days old *C. iria* plants.

6.4.2.3 Total chlorophyll- The leaves of *Cyperus iria* plants showed reduced content of total chlorophyll when grown in soil containing different concentration of Pb (II). As compared to control plants, firstly, 40 days old plants showed 17.3% increase at 200 mg/Kg Pb (II) and then there was decrease of 51.65% and 87.1% at 800 mg/Kg Pb (II) in both 40 and 80 days old plants respectively. The F-ratio for all treatments were found to be significant. (Table 6.19, fig 6.38, 6.39)

Table 6.19: Pb (II) effect on content of total chlorophyll (mg/g FW) in the leaves of *C. iria* plants during different growth stages**.**

Content of total chlorophyll in the leaves of *C. iria* plants at 40 and 80 days		
Concentration Pb (II) (mg/Kg)	**40 Days**	**80 days**
0	1.1390 ± 0.0046	1.1037 ± 0.0097
100	1.3073 ± 0.0268	1.1013 ± 0.0466
200	1.3377 ± 0.0114	0.9403 ± 0.0121****
300	1.0157 ± 0.0168	0.7953 ± 0.0045****
400	1.0027 ± 0.0125	0.6957 ± 0.0127****
500	0.8937 ± 0.0486**	0.4630 ± 0.0229 ****
600	0.7520 ± 0.98****	0.2223 ± 0.0217****
700	0.6218 ± 0.0246****	0.1953 ± 0.0068****
800	**0.5503 ± 0.0314****	**0.1417 ± 0.0081****
F-ratio (df 8,18)	**54.69****	**1090****
HSD	**0.198**	**0.057**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

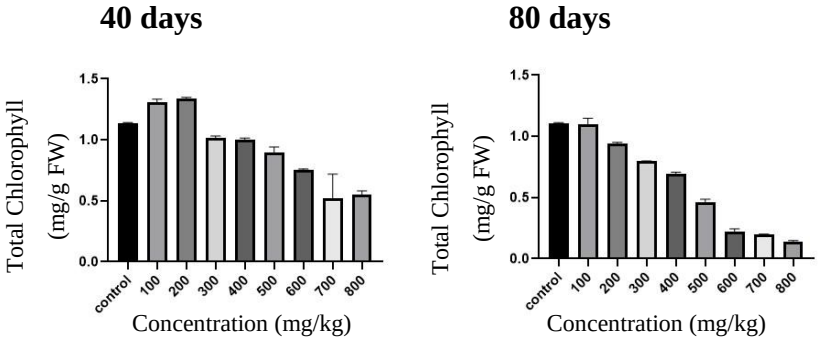


Fig. 6.38, 6.39: Effect of Pb (II) metal on total chlorophyll (mg/g FW) in 40 and 80 days old *C. iria* plants.

6.4.3 Protein content and Antioxidant Enzymes.

6.4.3.1 Protein content. Reduction was observed in the protein content with rising amount of Pb (II) treatment. Much reduction was noticed at 800 mg/Kg Pb (II) concentration with 77.9% and 81.7% in 40 and 80 days old plants. No plant growth was seen at higher concentration. Significance of F- ratio was implied at all treatments. (Table 6.20 fig 6.40, 6.41).

Table 6.20: Pb (II) effect on content of protein (mg/g FW) in the leaves of *C. iria* plants during different growth stages.

Amount of protein in *C. iria* plants at 40 and 80 days		
Concentration Pb (II) (mg/Kg)	**40 Days**	**80 days**
0	6.13 ± 0.24	7.12 ± 0.14
100	5.48 ± 0.14***	6.26 ± 0.08**
200	5.18 ± 0.06****	4.78 ± 0.10****
300	4.50 ± 0.14****	4.30 ± 0.08****
400	3.74 ± 0.12****	3.26 ± 0.07****
500	2.69 ± 0.13****	2.49 ± 0.17****
600	2.35 ± 0.08****	1.77 ± 0.06****
700	1.79 ± 0.14****	1.34 ± 0.10****
800	**1.35 ± 0.09****	**1.30 ± 0.50****
F-ratio (df 8,18)	**492.3****	**334.8****
HSD	**0.386**	**0.578**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

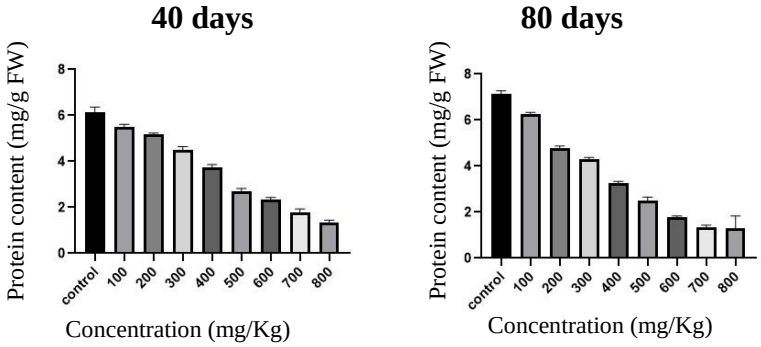


Fig. 6.40, 6.41: Effect of Pb (II) metal on protein content (mg/g FW) in 40 and 80 days old *C. iria* plants.

6.4.3.2 Catalase (CAT) – The specific activity of antioxidants enzyme catalase get enhanced with rise in value of Pb (II). Maximum increase was observed at 600 mg/Kg concentration of 269.5% in 40 days mature plants and highest increase of 314.1% in same concentration of Pb (II) in 80 days mature plants in contrast to control plants. Then drop in enzyme activity was noticed up to 800 mg/Kg Pb (II) and then no plant growth was seen at 900mg/Kg Pb (II). The F- ratio value was also found to be significant for all stages of treatment (Table 6.21, fig 6.42, 6.43).

Table 6.21: Pb (II) effect on specific activity of CAT (UA/g protein) in the leaves of *C. iria* plants during different growth stages.

CAT Specific activity in the leaves of *C. iria* plants at 40 and 80 days		
Concentration Pb (II) (mg/Kg)	**40 Days**	**80 days**
0	10.23 ± 0.11	7.57 ± 0.21
100	13.05 ± 0.55****	10.87 ± 0.32****
200	18.48 ± 0.14****	16.24 ± 0.36****
300	23.42 ± 0.34****	20.01 ± 0.15****
400	28.87 ± 0.40****	25.23 ± 0.12****
500	36.31 ± 0.21****	27.51 ± 0.06****
600	37.81 ± 0.83****	31.35 ± 0.07****
700	33.34 ± 0.13****	24.61 ± 0.14****
800	**26.65 ± 0.09****	**20.43 ± 0.15****
F-ratio (df 8,18)	**1915****	**4009****
HSD	**1.122**	**0.608**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

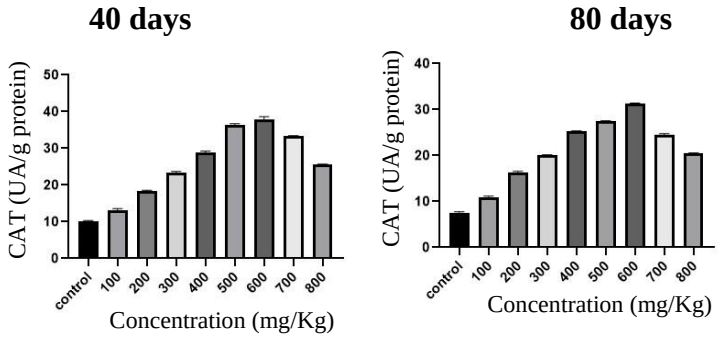


Fig. 6.42, 6.43: Effect of Pb (II) metal on specific activity of CAT (UA/g protein) in 40 and 80 days old *C. iria* plants.

6.4.3.3 Guaiacol Peroxidase (POD). The activity of POD was noticed to get increase with rising amount of Pb (II) in addition to it maximum increase was found at 600 mg/Kg concentration with 100.6% in 40 days old plants. In 80 days old plants there was increase of 67.4% at same concentration of Pb (II). Then decrease was observed in enzyme activity. F ratio was also found to be significant (Table 6.22, fig 6.44, 6.45)

Table 6.22: Pb (II) effect on specific activity of POD (UA/g protein) in the leaves of *C. iria* plants during different growth stages.

Specific activity of POD in the leaves of *C. iria* plants at 40 and 80 days		
Concentration Pb (II) (mg/Kg)	**40 Days**	**80 days**
0	16.45 ± 0.13	11.74 ± 0.34
100	18.51 ± 0.17****	12.77 ± 0.12****
200	23.56 ± 0.23****	13.55 ± 0.23****
300	24.79 ± 0.35****	15.97 ± 0.18****
400	28.37 ± 0.11****	18.52 ± 0.17****
500	29.52 ± 0.11****	19.15 ± 0.10****
600	33.01 ± 0.77****	19.66 ± 0.10****
700	31.40 ± 0.15****	16.36 ± 0.08****
800	**28.71 ± 0.07****	**13.50 ± 0.14****
F-ratio (df 8,18)	**1010****	**803.9****
HSD	**0.886**	**0.516**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

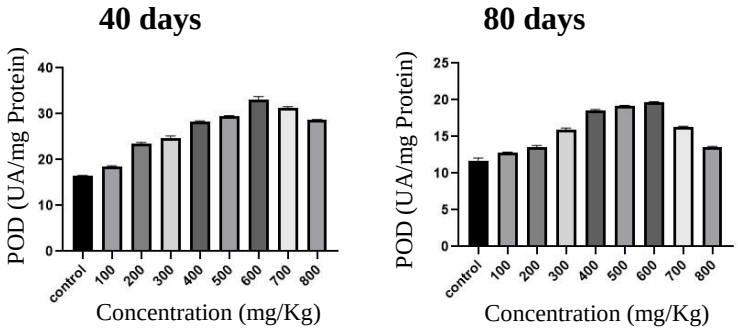


Fig. 6.44, 6.45: Effect of Pb (II) metal on specific activity of POD (UA/g protein) in 40 and 80 days old *C. iria* plants.

STUDIES ON ERUCA SATIVA PLANT

6.5 Toxic effects of Cr (VI) on *Eruca sativa*:

6.5.1 Morphological characters:

6.5.1.1 Root length: Cr (VI) caused a much drop in length of root of potted plants. In comparison to control, 350 mg/kg Cr (VI) caused around 50% decrease in root length in 40 and 80 days old plants. The F-ratio for one-way ANOVA for all the growth stages were significant, thus showing the significance of the treatments. (Table 6.23, fig 6.46, 6.47).

Table 6.23: Cr (VI) effect on root length (cm) of *Eruca sativa* plants during different growth stages.

Root length of *Eruca sativa* plants at 40 and 80 days		
Concentration Cr (VI) (mg/Kg)	**40 Days**	**80 days**
0	6.36 ± 0..5590	7.29 ± 0.3672
50	6.35 ± 0.5252	7.27 ± 0.1457
100	7.05 ± 0.1950	7.19 ± 0.1815
150	6.66 ± 0.3799	6.58 ± 0.2558
200	4.97 ± 0.2954**	5.71 ± 0.4503**
250	4.35 ± 0.1955****	5.15 ± 0.3279****
300	3.84 ± 0.2250****	4.10 ± 0.1604****
350	3.03 ± 0.1900****	3.64 ± 0.2587****
F-ratio (df 7,16)	**53.86****	**77.66****
HSD	**0.99**	**0.809**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

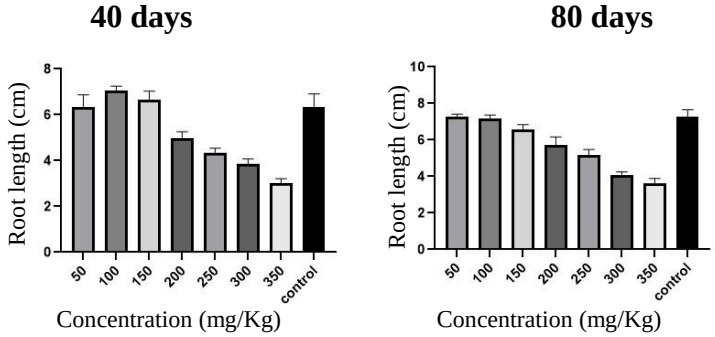


Fig. 6.46, 6.47: Effect of metal stress of Cr (VI) on root length (cm) of 40 and 80 days old *E. sativa* plants.

6.5.1.2 Shoot length: Shoot length got a varied observation at different amount of Cr (VI) in comparison to control plants. Firstly, increase of 39.9 % and 18.5% in length of 40 and 80 days old plants was found at 100 mg/Kg Cr (VI). Then reduction was observed at 350mg/Kg Cr (VI) concentration by 37.9% and 47.9% in 40 and 80 days old plants respectively. The F- ratio was found to be significant (Table 6.24, fig 6.48, 6.49).

Table 6.24: Cr (VI) effect on shoot length (cm) of *E. sativa* plants during different growth stages.

Shoot length of *E. sativa* plants at 40 and 80 days		
Concentration Cr (VI) (mg/Kg)	**40 Days**	**80 days**
0	17.60 ± 0.6874	25.93 ± 0.3329
50	20.61 ± 0.4412****	28.17 ± 0.2754
100	24.63 ± 0.2255****	30.74 ± 0.4375
150	20.29 ± 0.9914***	26.08 ± 0.5686
200	17.10 ± 0.6062	23.74 ± 0.3306
250	14.90 ± 0.2784***	20.27 ± 0.2754
300	12.58 ± 0.2159****	15.96 ± 0.7171
350	10.92 ± 0.2219****	13.49± 0.1779
F-ratio (df 7,16)	**218.1****	**2.559**
HSD	**1.495**	**15.94**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

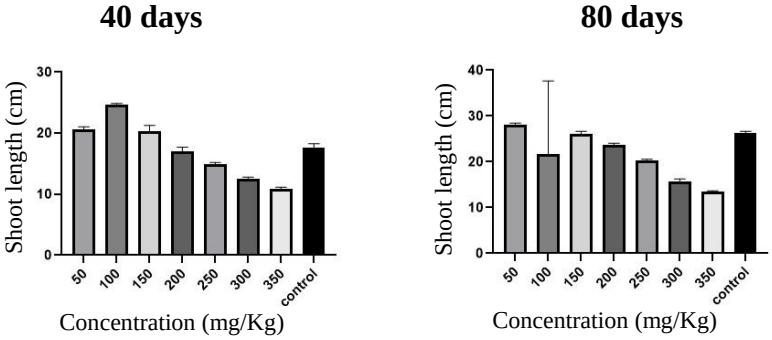


Fig 6.48, 6.49: Effect of metal stress of Cr (VI) on shoot length (cm) of 40 and 80 days old *E. sativa* plants.

6.5.1.3 Fresh weight –Toxic effects of Cr (VI) were also noticed on fresh weight of the plant. Reduction of 68.8% and 76.3% was observed at 350 mg/Kg Cr (VI) in 40 and 80 days old plants respectively. The F-ratio for one way ANOVA for all growth stages was found to be significant (Table 6.25, Fig 6.50, 6.51).

Table 6.25: Cr (VI) effect on fresh weight (gm) of *E. sativa* plants during different growth stages.

Fresh weight of *E. sativa* plants at 40 and 80 days		
Concentration Cr (VI) (mg/Kg)	**40 Days**	**80 days**
0	36.08 ± 0.28	27.60 ± 1.09
50	33.66 ± 0.22****	24.18 ± 0.35****
100	34.11 ± 0.41***	25.82 ± 0.25*
150	28.74 ± 0.45****	18.90 ± 0.84****
200	23.62 ± 0.17****	15.66 ± 0.20****
250	20.54 ± 0.53****	12.58 ± 0.21****
300	15.76 ± 0.45****	7.91 ± 0.89****
350	11.23 ± 0.63 ****	6.52 ± 0.28****
F-ratio (df 7,16)	**1343******	**522.2******
HSD	**1.226**	**1.73**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

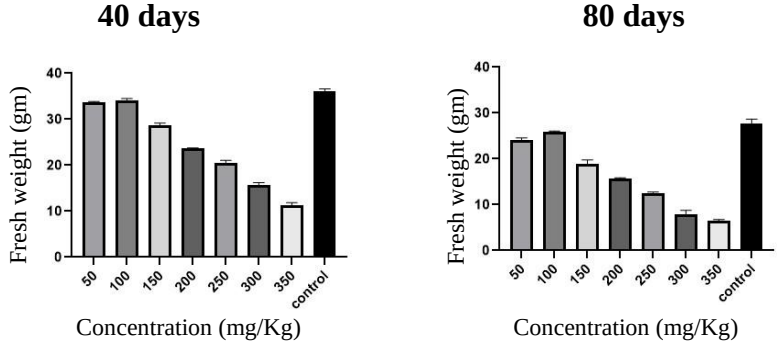


Fig. 6.50, 6.51: Effect of Cr (VI) metal on fresh weight (gm) of 40 and 80 days old *E. sativa* plants.

6.5.1.4 Dry Weight- Dry weight also get decreased by 69.9% and 79.7% at 350 mg/Kg Cr (VI) in 40 and 80 days old plants as compared to control plants. The F-ratio for one way ANOVA for all growth stages was found to be significant. (Table 6.26, Fig 6.52, 6.53).

Table 6.26: Cr (VI) effect on dry weight (gm) of *E. sativa* plants during different growth stages.

Dry weight of *E. sativa* plants at 40 and 80 days		
Concentration Cr (VI) (mg/Kg)	**40 Days**	**80 days**
0	27.28 ± 0.45	21.50 ± 0.50
50	24.41 ± 0.28****	19.24 ± 0.51****
100	25.88 ± 0.18***	20.63 ± 0.35
150	21.71 ± 0.27****	16.17 ± 0.26****
200	18.55 ± 0.22****	12.04 ±0.41****
250	16.25 ± 0.33****	9.84 ± 0.45****
300	12.52 ± 0.25****	6.52 ± 0.23****
350	8.21 ± 0.38****	4.36 ± 0.38****
F-ratio (df 7,16)	**1472******	**820******
HSD	**0.859**	**1.12**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

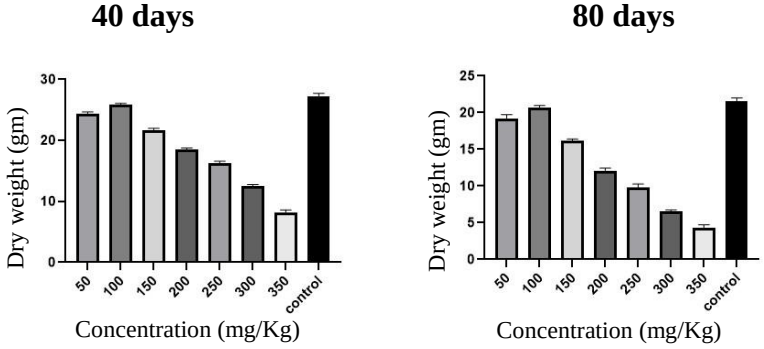


Fig. 6.52, 6.53: Effect of Cr (VI) metal on dry weight (gm) of 40 and 80 days old *E. sativa* plants.

6.5.1.5 Moisture content –Increase in moisture content of 12.6% at 50 mg/Kg of Cr (VI) in 40 days old plants and of 49.5% was observed at 350 mg/Kg Cr (VI)

concentration in 80 days old plants as compared to control plants. The F-ratio for all growth stages was found to be significant. (Table 6.27, fig 6.54, 6.55)

Table 6.27: Cr (VI) effect on moisture content of *E. sativa* plants during different growth stages.

Moisture content of *E. sativa* plants at 40 and 80 days		
Concentration Cr (VI) (mg/Kg)	**40 Days**	**80 days**
0	24.38 ±0.66	22.05 ± 2.65
50	27.47 ± 0.39	20.45 ± 2.65
100	24.11 ± 1.14	20.10 ± 1.15
150	24.44 ± 2.11	14.36 ± 3.58
200	21.47 ± 0.40	23.13 ± 2.84
250	20.85 ± 1.69	21.75 ± 4.26
300	20.55 ± 1.73*	17.03 ± 7.81
350	26.89 ± 0.84	32.98 ± 6.85
F-ratio (df 7,16)	**12.78******	**4.487**
HSD	**3.59**	**12.62**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

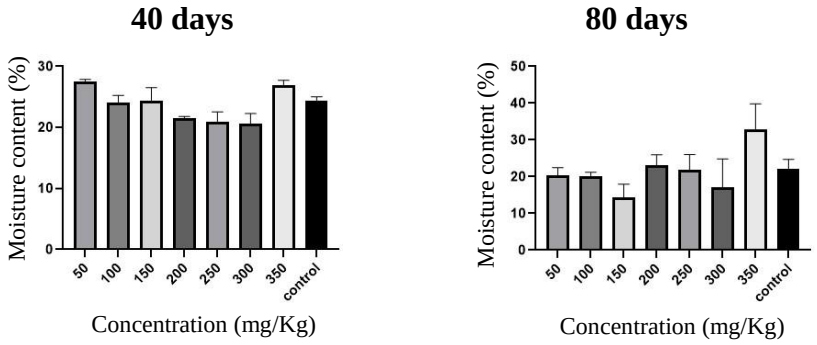


Fig. 6.54, 6.55: Effect of Cr (VI) metal on moisture content of 40 and 80 days old *E. sativa* plants.

6.5.2 Biochemical Parameters

6.5.2.1 Chlorophyll *a* –Amount of chlorophyll *a* also get decreased in *E. sativa* plants grown in different treatments of hexavalent chromium. Firstly, there was increase of 3.35% at 50 mg/Kg of Cr (VI) concentration in 40 days old plants and 5.3% increase at 50 mg/Kg of Cr (VI) concentration in 80 days old plants. A significant decrease of 68.5% and 69.3% was observed in 40 and 80 days mature plants at 350 mg/Kg Cr (VI)

concentration. The F-ratio was found to be significant for all treatments. (Table 6.28, fig 6.56, 6.57).

Table 6.28: Cr (VI) effect on chlorophyll a content (mg/g FW) in the leaves of *E. sativa* plants during different growth stages.

Chlorophyll a content in the leaves of *E. sativa* plants at 40 and 80 days		
Concentration Cr (VI) (mg/Kg)	**40 Days**	**80 days**
0	2.4290 ± 0.1181	1.8567 ± 0.0799
50	2.5110 ± 0.1546	1.9557 ± 0.0717
100	2.1883 ± 0.0386	1.6320 ± 0.9468
150	1.8023 ± 0.0587****	1.6270 ± 0.8335
200	1.5397 ± 0.0806****	1.1670 ± 0.1223
250	1.3240 ± 0.0995****	0.9240 ± 0.0685
300	0.9223 ± 0.0416****	0.6383 ± 0.0960
350	0.7640 ± 0.0601****	0.5683 ± 0.0646*
F-ratio (df 7, 16)	**154******	**3.811******
HSD	**0.264**	**1.276**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

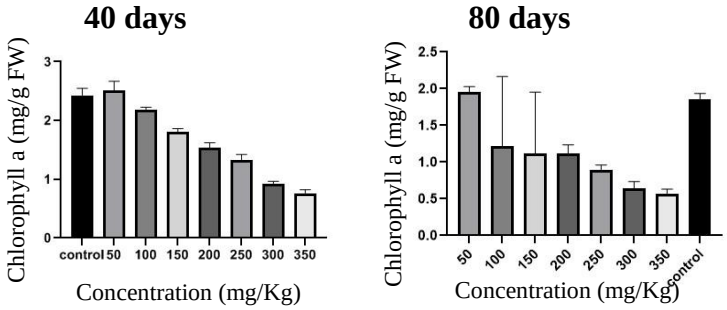


Fig. 6.56, 6.57: Effect of Cr (VI) metal on chlorophyll a (mg/g FW) in the leaves of 40 and 80 days old *E. sativa* plants.

6.5.2.2 Chlorophyll *b*- Chlorophyll *b* content in the leaves also showed reduction when grown in the increasing concentration of Cr (VI). First, the increase of 4.73% and 11% at 50 mg/Kg of Cr (VI) concentration was observed in 40 and 80 days old plants respectively. Then reduction of 73.7% and 97.6% was observed at 350 mg/Kg of Cr (VI) in 40 and 80 days old plants respectively. Significance of F-ratio showed the effect of treatment at all stages. (Table 6.29, fig 6.58, 6.59).

Table 6.29: Cr (VI) effect on content of chlorophyll b (mg/g FW) in the leaves of *E. sativa* plants during different growth stages.

Chlorophyll b content in *E. sativa* plants at 40 and 80 days		
Concentration Cr (VI) (mg/Kg)	**40 Days**	**80 days**
0	1.8043 ± 0.0172	1.4293 ± 0.0548
50	1.8897 ± 0.0303	1.5867 ± 0.0321
100	1.5953 ± 0.0630	1.3987 ± 0.0235
150	1.2973 ± 0.0087	1.3790 ± 0.3265
200	1.0923 ± 0.0055	0.0915 ± 0.0041****
250	0.7061 ± 0.5316**	0.0547 ± 0.0015****
300	0.7733 ± 0.0637***	0.0443 ± 0.0031****
350	0.4734 ± 0.3487***	0.0343 ± 0.0085****
F-ratio (df 7,16)	**13.87******	**120.3******
HSD	**0.745**	**0.334**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

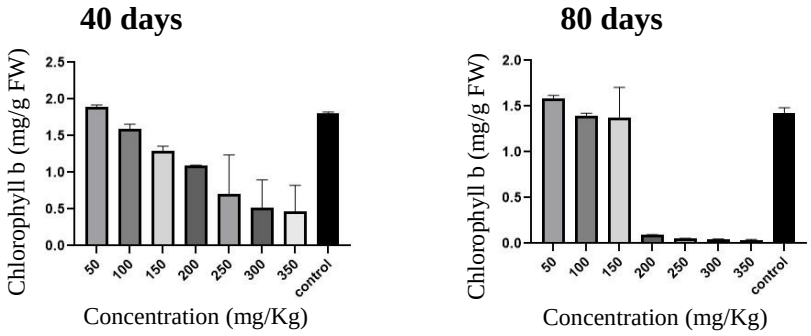


Fig. 6.58, 6.59: Effect of Cr (VI) metal on chlorophyll *a* (mg/g FW) in 40 and 80 days old *E. sativa* plants.

6.5.2.3 Total chlorophyll- The leaves of *E. sativa* plants showed reduced content of total chlorophyll when grown in soil containing different amount of hexavalent chromium. In comparison to control, firstly 40 and 80 days old plants showed 3.9% and 7.8% increase at 50 mg/Kg Cr (VI) respectively and then there was decrease of 70.7% and 81.6% at 350 mg/kg Cr (VI) in both 40 and 80 days old plants respectively. The F-ratio for all treatments was found to be significant. (Table 6.30, fig 6.60, 6.61)

Table 6.30: Cr (VI) effect on content of total chlorophyll (mg/g FW) in the leaves of *E. sativa* plants during different growth stages.

Total chlorophyll content in *E. sativa* plants at 40 and 80 days		
Concentration Cr (VI) (mg/Kg)	**40 Days**	**80 days**
0	4.233 ± 0.1188	3.2860 ± 0.0520
50	4.4007 ± 0.1245	3.5423 ± 0.0405

100	3.7837 ± 0.0928	2.6177 ± 0.9662
150	3.0997 ± 0.0585*	2.4997 ± 1.0279
200	2.6320 ± 0.0862**	1.2055 ± 0.1208**
250	2.0301 ± 0.6201****	0.9473 ± 0.0670***
300	1.6957 ± 0.0248****	0.6827 ± 0.0945***
350	1.2374 ± 0.3795****	0.6027 ± 0.0563***
F-ratio (df 7,16)	**34.70****	**17****
HSD	**1.03**	**1.42**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

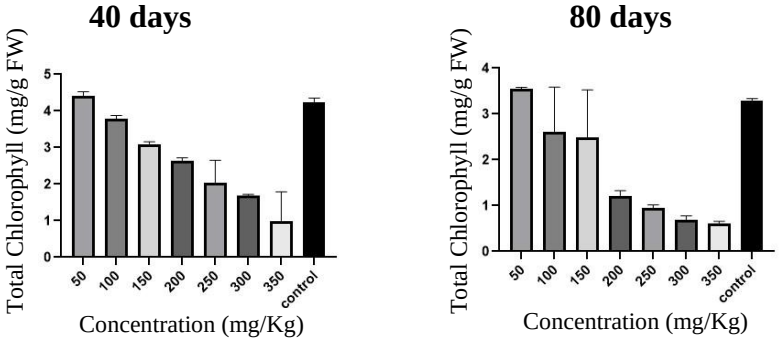


Fig. 6.60, 6.61: Effect of Cr (VI) metal on total chlorophyll (mg/g FW) in 40 and 80 days old *E. sativa* plants.

6.5.3 Protein Content and Antioxidant Enzymes.

6.5.3.1 Protein Content: Reduction was observed in the protein content with rising amount of hexavalent chromium treatment. Much reduction was observed at 350 mg/Kg Cr (VI) concentration with 73.7% and 96.8% in 40 and 80 days old plants. No plant growth was seen at higher concentration of Cr. Significance of F- ratio was implied at all treatments. (Table 6.31 fig 6.62, 6.63).

Table 6.31: Cr (VI) effect on protein content (mg/g FW) in the leaves of *E. sativa* plants during different growth stages.

Content of protein in *E. sativa* plants at 40 and 80 days		
Concentration Cr (VI) (mg/Kg)	**40 Days**	**80 days**
0	4.99 ± 0.40	2.52 ± 0.05
50	3.84 ± 0.12****	2.22 ± 0.04
100	3.36 ± 0.04****	1.81 ± 0.03*
150	2.85 ± 0.04****	1.53 ± 0.04**
200	2.56 ± 0.08****	1.41 ± 0.02***
250	1.83 ± 0.04****	1.27 ± 0.03****
300	1.61 ± 0.05****	0.73 ± 0.55****

350	1.31 ± 0.05****	0.08 ± 0.01****
F-ratio (df 7,16)	**198.8****	**41.79****
HSD	**0.431**	**0.58**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

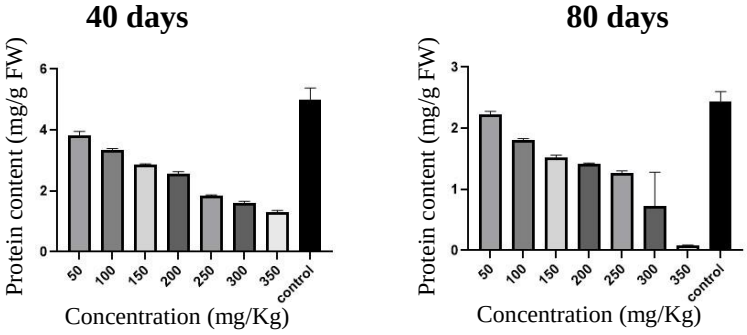


Fig. 6.62, 6.63: Effect of Cr (VI) metal on protein content (mg/g FW) in 40 and 80 days old *E. sativa* plants.

6.5.3.2 Catalase (CAT) – The activity of antioxidants enzyme CAT get enhanced with rise in value of hexavalent chromium. Maximum increase was found at 300 mg/Kg concentration of 214% in 40 days old plants and maximum increase of 199.6% in 200 mg/Kg concentration of Cr (VI) in 80 days old plants as compared to control plants. Then decrease in specific activity of enzyme was observed at higher concentrations until the growth get arrested at 900 mg/kg of Cr. The F-ratio value was also found to be significant for all stages of treatment. (Table 6.32, fig 6.64, 6.65).

Table 6.32: Cr (VI) effect on specific activity of CAT (UA/g protein) in the leaves of *E. sativa* plants during different growth stages.

Specific activity of CAT in *E. sativa* plants at 40 and 80 days		
Concentration Cr (VI) (mg/Kg)	**40 Days**	**80 days**
0	10.63 ± 0.45	8.42 ± 0.38
50	15.22 ± 0.48	10.87 ± 0.32****
100	20.22 ± 0.46**	16.24 ± 0.36****
150	23.42 ± 0.34****	19.71 ± 0.39****
200	28.87 ± 0.40****	25.23 ± 0.12****
250	35.21 ± 0.39****	23.30 ± 0.18****
300	33.38 ± 0.16****	22.82 ± 0.29****
350	30.88 ± 0.33****	20.87 ± 0.23****
F-ratio (df 7,16)	**79.05****	**1265****
HSD	**5.19**	**0.838**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

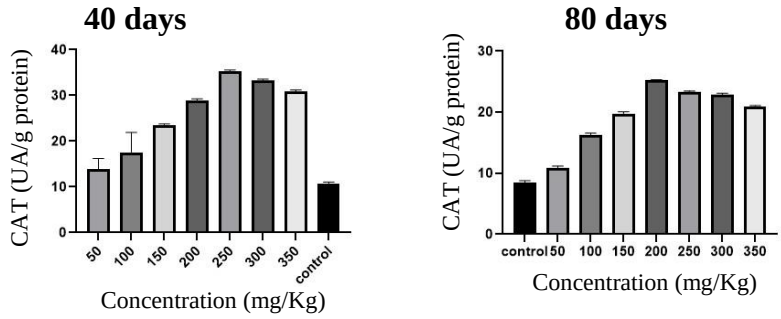


Fig. 6.64, 6.65: Effect of Cr (VI) metal on specific activity of CAT (UA/g protein) in 40 and 80 days mature *E. sativa* plants.

6.5.3.3 Guaiacol Peroxidase (POD). The activity of POD was observed to get increase with rising value of hexavalent chromium and maximum increase was found at 200 mg/Kg concentration with 58.2% in 40 days old plants. In 80 days old plants there is increase of 125.2% at 250 mg/Kg concentration of Cr (VI). After that, decrease in POD activity was observed. F- ratio was also found to be significant. (Table 6.33, fig 6.66, 6.67)

Table 6.33: Cr (VI) effect on specific activity of POD (UA/g protein) in the leaves of *E. sativa* plants during different growth stages.

Specific activity of POD in *E. sativa* plants at 40 and 80 days		
Concentration Cr (VI) (mg/Kg)	**40 Days**	**80 days**
0	13.99 ± 0.27	12.78 ± 0.40
50	16.57 ± 0.32****	14.61 ± 0.29****
100	19.79 ± 0.45****	18.19 ± 0.22****
150	23.72 ± 0.33****	20.56 ± 0.28****
200	25.92 ± 0.29****	25.45 ± 0.18****
250	24.45 ± 0.24****	28.79 ± 0.39****
300	22.56 ± 0.25****	26.92 ± 0.18****
350	21.00± 0..47****	25.99± 0.27****
F-ratio (df 7,16)	**1310******	**437******
HSD	**0.814**	**0.954**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

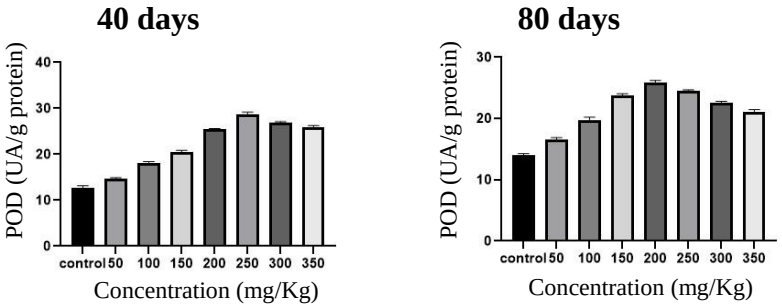


Fig. 6.66, 6.67: Effect of Cr (VI) metal on specific activity of POD (UA/g protein) in 40 and 80 days old *E. sativa* plants.

6.6 Toxic effects of Pb (II) on *Eruca sativa*:

6.6.1 Morphological characters:

6.6.1.1 Root length: Pb (II) caused a much drop in length of root of potted plants. In contrast to plants taken as control, firstly 200 mg/Kg Pb (II) caused 20.2% and 26.7% increase in root length in 40 and 80 days old plants respectively. Then 800 mg/Kg Pb (II) caused 51.1% and 39.7% decrease in root length in 40 and 80 days old plants respectively. The F-ratio for one-way ANOVA for all the growth stages were significant, thus showing the significance of the test. (Table 6.34, fig 6.68, 6.69)

Table 6.34: Pb (II) effect on root length (cm) of *E. sativa* plants during different growth stages.

Root length of *E. sativa* plants at 40 and 80 days		
Concentration Pb (II) (mg/Kg)	**40 Days**	**80 days**
0	6.36 ± 0.1629	6.85 ± 0.3225
100	7.30 ± 0.1845**	8.34 ± 0.2850****
200	7.65 ± 0.1845***	8.68 ± 0.1193****
300	6.47 ± 0.1656	7.43 ± 0.0945****
400	5.49 ± 0.3449*	6.05 ± 0.1750****
500	4.05 ± 0.5311****	5.47 ± 0.1457****
600	3.90 ± 0.0200****	5.15 ± 0.2500****
700	3.45 ± 0.1701****	4.63 ± 0.1587****
800	3.11 ± 0.1405****	4.13 ± 0.2021****
F-ratio (df 8,18)	**131.5****	**181.9****
HSD	**0.745**	**0.593**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ****$p \leq 0.0001$, ***$p \leq 0.001$, **$p \leq 0.01$, *$p \leq 0.05$.

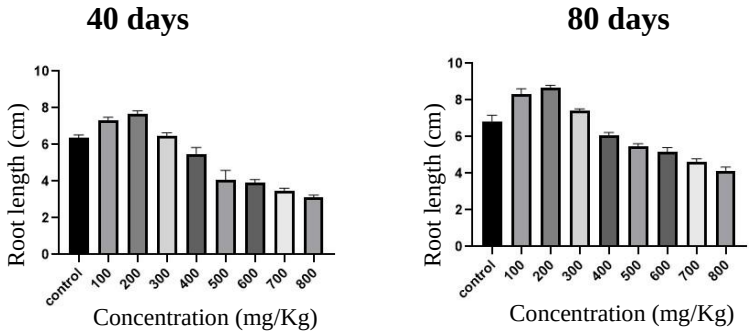


Fig. 6.68, 6.69: Effect of metal stress of Pb (II) on root length (cm) of 40 and 80 days old *E. sativa* plants.

6.6.1.2 Shoot length: Decline in length of shoot was also observed with rise in value of Pb (II) in comparison to plants taken as control. Firstly, a slight increase of 15.8 % in shoot length of 40 days old plants was found at 200 mg/Kg Pb (II). Then reduction was observed at 800 mg/Kg Pb (II) concentration by 50.8% and 59.2% in 40 and 80 days old plants respectively. The F-ratio was found to be significant (Table 6.35, fig-6.70, 6.71).

Table 6.35: Pb (II) effect on shoot length (cm) of *E. sativa* plants during different growth stages.

Shoot length of *E. sativa* plants at 40 and 80 days		
Concentration Pb (II) (mg/Kg)	**40 Days**	**80 days**
0	25.45 ± 0.1311	35.45 ± 0.3905
100	27.64 ± 0.2003****	34.87 ± 0.1750
200	29.48 ± 0.1600****	30.43 ± 0.2155****
300	25.32 ± 0.6424	28.81 ± 0.1721****
400	22.76 ± 0.3525****	26.01 ± 0.2848****
500	18.77 ± 0.4583****	22.49 ± 0.1804****
600	16.59 ± 0.1539****	17.18 ± 0.3591****
700	15.54 ± 0.2203****	16.54 ± 0.4924****
800	12.50 ± 0.2193****	14.44 ± 0.2152****
F-ratio (df 8,18)	**999.1****	**2144****
HSD	**0.929**	**0.8476**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

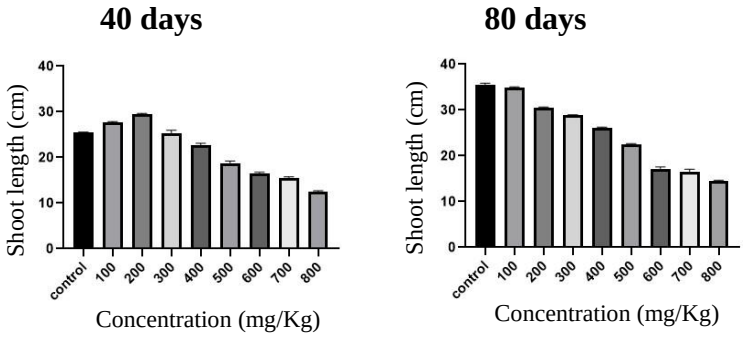


Fig 6.70, 6.71: Effect of metal stress of Pb (II) on shoot length (cm) of 40 and 80 days old *E. sativa* plants.

6.6.1.3 Fresh weight – Toxic effects of Pb (II) were also noticed on fresh weight of the plant. Reduction of 83.2% and 85.2% was observed at 800 mg/Kg Pb (II) in 40 and 80 days old plants respectively. No further decrease in growth was observed after that at higher concentration. The F-ratio for one-way ANOVA for all growth stages was found to be significant. (Table 6.36, Fig 6.72, 6.73).

Table 6.36: Pb (II) effect on fresh weight (gm) of *E. sativa* plants during different growth stages.

Fresh weight of *E. sativa* plants at 40 and 80 days		
Concentration Pb (II) (mg/Kg)	**40 Days**	**80 days**
0	29.65 ± 0.77	25.40 ± 0.25
100	27.39 ± 0.18	21.25 ± 0.33****
200	25.23 ± 0.23	17.27 ± 0.64****
300	23.82 ± 0.17*	14.86 ± 0.31****
400	20.21 ± 0.76**	11.10 ± 0.27****
500	15.79 ± 0.41****	9.42 ± 0.23****
600	10.64 ± 0.33****	6.76 ± 0.30****
700	7.59 ± 0.24****	5.41 ± 0..38****
800	4.97 ± 0.46****	3.76± 0.14****
F-ratio (df 8, 18)	**88.21******	**1429******
HSD	**4.816**	**0.97**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

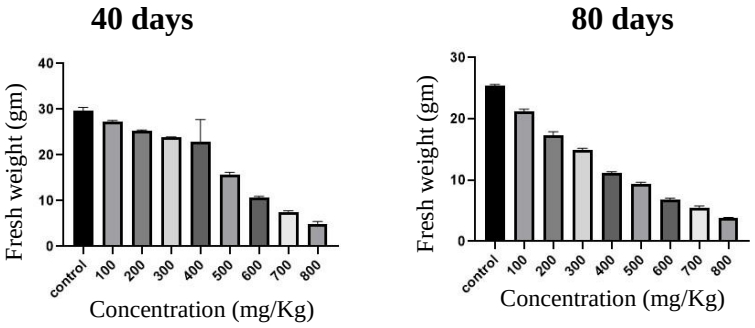


Fig. 6.72, 6.73: Effect of metal stress of Pb (II) on fresh weight (gm) of 40 and 80 days old *E. sativa* plants.

6.6.1.4 Dry weight- Dry weight get decreased by 85.6% and 81.5% at 800 mg/Kg Pb (II) in 40 and 80 days old plants as compared to control plants. The F-ratio for one-way ANOVA for all growth stages was found to be significant. (Table 6.37, Fig 6.74, 6.75).

Table 6.37: Pb (II) effect on dry weight (gm) of *E. sativa* plants during different growth stages.

Dry weight of *E. sativa* plants at 40 and 80 days		
Concentration Pb (II) (mg/Kg)	**40 Days**	**80 days**
0	21.70 ± 0.22	14.65 ± 0.20
100	17.54 ± 0.22****	11.81 ± 0.34****
200	14.80 ± 0.25****	10.57 ± 0.23****
300	11.86 ± 0.24****	8.84 ± 0.25****
400	9.09 ± 0.58****	6.32 ± 0.45****
500	6.65 ± 0.34****	4.51 ± 0.29****
600	5.33 ± 0.37****	4.06 ± 0.24****
700	3.55 ± 0.24****	3.21 ± 0.24****
800	3.11 ± 0.23****	2.70 ± 0.22****
F-ratio (df 8, 18)	**1276****	**676.6****
HSD	**0.906**	**0.809**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

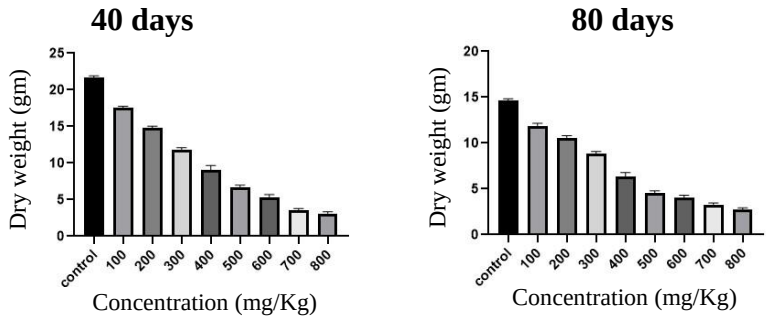


Fig. 6.74, 6.75: Effect of Pb (II) metal on dry weight (gm) of 40 and 80 days old *E. sativa* plants.

6.6.1.5 Moisture content – Increase in moisture content of 116.1% at 500 mg/Kg of Pb (II) in 40 days old plants and an increase of 23% was observed at 500 mg/Kg Pb (II) concentration in 80 day old plants and then decrease of 33.5% at 800 mg/Kg of Pb (II) concentration in 80 days old plants as compared to control plants. The F-ratio for all growth stages was found to be significant. (Table 6.38, fig 6.76, 6.77)

Table 6.38: Pb (II) effect on moisture content of *E. sativa* plants during different growth stages.

Moisture content of *E. sativa* plants at 40 and 80 days		
Concentration Pb (II) (mg/Kg)	**40 Days**	**80 days**
0	26.78 ± 2.60	42.33 ± 1.34
100	35.95 ± 1.08****	44.41± 1.71
200	41.33 ± 1.36****	38.69 ± 3.56
300	50.19 ± 0.64****	40.54 ± 1.81
400	55.04 ± 1.55****	42.96 ± 5.45
500	57.87 ± 1.85****	52.08 ± 4.15
600	49.95 ± 2.18****	39.82 ± 5.14
700	53.31 ± 1.73****	40.65 ± 0.53
800	**37.39 ± 1.56****	**28.15 ± 4.08****
F-ratio (df 8, 18)	**112.3****	**9.495****
HSD	**4.88**	**10.04**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

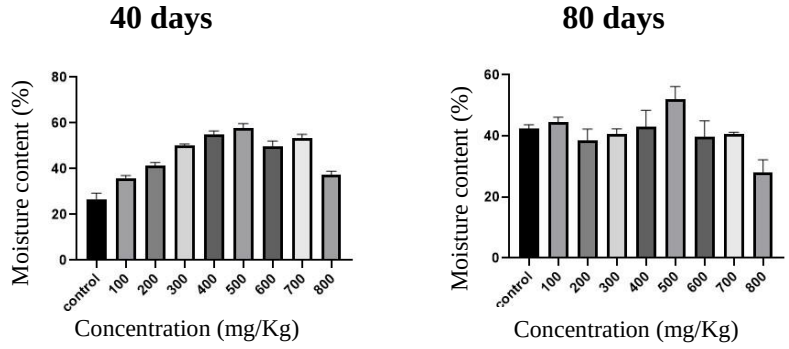


Fig. 6.76, 6.77: Effect of Pb (II) metal stress on moisture content of 40 and 80 days old *E. sativa* plants.

6.6.2 Biochemical Parameters

6.6.2.1 Chlorophyll *a* –Amount of chlorophyll *a* also get decreased in the leaves of *E. sativa* plants grown in different treatments of Pb (II). A significant decrease of 59.7% and 70.3% was observed in 40 and 80 days mature plant leaves at 800 mg/Kg Pb (II) concentration. The F-ratio was found to be significant for all treatments. (Table 6.39, fig 6.78, 6.79)

Table 6.39: Pb (II) effect on chlorophyll a content (mg/g FW) in *E. sativa* plant leaves during different growth stages.

Chlorophyll *a* content in *E. sativa* plants at 40 and 80 days		
Concentration Pb (II) (mg/Kg)	**40 Days**	**80 days**
0	3.5963 ± 0.1558	3.2013± 0.0663
100	3.3030 ± 0.0527*	3.1547 ± 0.0748
200	3.1310 ± 0.0345**	3.0843 ± 0.0342
300	2.8553 ± 0.1016****	2.5563 ± 0.0888****
400	2.6450 ± 0.0731****	2.2220 ± 0.0501****
500	2.4407 ± 0.0751****	1.8417 ± 0.0778****
600	2.1897 ± 0.0230****	1.6333 ± 0.0561****
700	1.8667 ± 0.0905****	1.3237 ± 0.0665****
800	1.4487± 0.1018****	0.9510 ± 0.0259****
F-ratio (df 8,18)	**188.5******	**526.9******
HSD	**0.2510**	**0.18**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

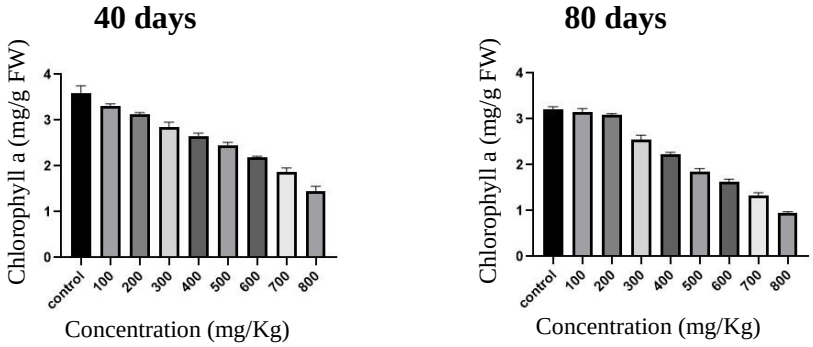


Fig. 6.78, 6.79: Effect of Pb (II) metal on chlorophyll *a* (mg/g FW) in 40 and 80 days old *E. sativa* plants.

6.6.2.2 Chlorophyll *b*- Chlorophyll *b* content in the leaves also showed reduction with rise in the value of Pb (II) metal. Reduction of 78% and 87.1% was observed at 800

mg/Kg of Pb (II) in 40 and 80 days old plants respectively. Significance of F-ratio showed the effect of treatment at all stages (Table 6.40, fig 6.80, 6.81).

Table 6.40: Pb (II) effect on chlorophyll b content (mg/g FW) in *E. sativa* plant leaves during different growth stages.

Chlorophyll b content in *E. sativa* plants at 40 and 80 days		
Concentration Pb (II) (mg/Kg)	**40 Days**	**80 days**
0	2.6883 ± 0.831	2.4940 ± 0.0252
100	2.3723 ± 0.0826***	2.1627 ± 0.0683****
200	2.1453 ± 0.0455****	1.8563 ± 0.0835****
300	2.0433 ± 0.0429****	1.6097 ± 0.0566****
400	1.8413 ± 0.1008****	1.3900 ± 0.0718****
500	1.6033 ± 0.0284****	1.1050 ± 0.0252****
600	0.9427± 0.0611****	0.6663 ± 0.0742****
700	0.7043 ± 0.0172****	0.5610 ± 0.0641****
800	0.5907 ± 0.0.338****	0.3210± 0.0390****
F-ratio (df 8,18)	**454.9******	**469.1******
HSD	**0.174**	**0.171**

Data shown as mean ± S.D of three replicates Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

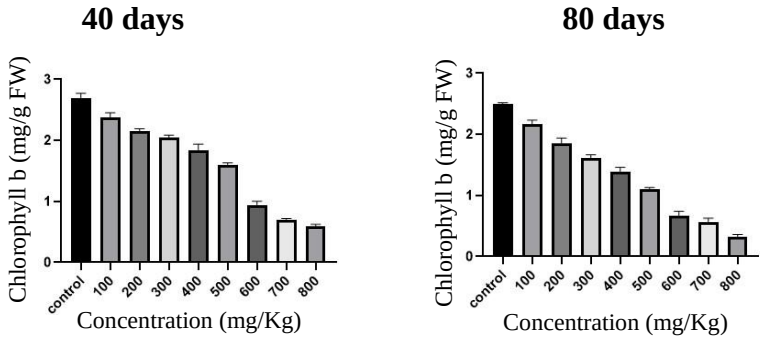


Fig. 6.80, 6.81: Effect of Pb (II) metal on chlorophyll b content (mg/g FW) in 40 and 80 days old *E. sativa* plants.

6.6.2.3 Total chlorophyll- The leaves of *E. sativa* plants showed reduced content of total chlorophyll when grown in soil containing different concentration of Pb (II). As compared to control plants, there was decrease of 67.5% and 77.9% at 800 mg/Kg Pb (II) in both 40 and 80 days old plants respectively. No growth was seen after that. The F- ratio for all treatments were found to be significant. (Table 6.41, fig 6.82, 6.83).

Table 6.41: Pb (II) effect on total chlorophyll content (mg/g FW) in the leaves of *E. sativa* plants during different growth stages.

Total chlorophyll content in *E. sativa* plants at 40 and 80 days		
Concentration Pb (II) (mg/Kg)	**40 Days**	**80 days**
0	6.2847 ± 0.1711	5.7547 ± 0.0582
100	5.6753 ± 0.0571****	5.2710 ± 0.0544**
200	5.2763 ± 0.0208****	4.9407 ± 0.0730****
300	4.8987± 0.0992****	4.1660 ± 0.0531****
400	4.4863 ± 0.1725****	3.6120 ± 0. 1042****
500	4.0440 ± 0.0497****	2.9467 ± 0.0811****
600	3.1323 ± 0.0487****	2.2997 ± 0.1212****
700	2.5710 ± 0.0825****	1.8847 ± 0.1221****
800	2.0393 ± 0.0960****	1.2720 ± 0.0637****
F-ratio (df 8, 18)	**603.1******	**801.4******
HSD	**0.286**	**0.275**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

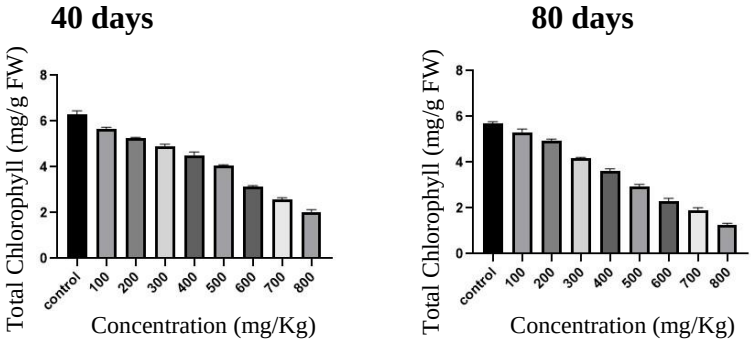


Fig. 6.82, 6.83: Effect of Pb (II) metal on total chlorophyll (mg/g FW) in 40 and 80 days old *E. sativa* plants.

6.6.3 Protein content and Antioxidant Enzymes.

6.6.3.1 Protein content: Reduction in the content of protein was noticed with rising level of Pb (II) treatment. Much reduction was noticed at 800 mg/Kg Pb (II) concentration with 73.2% and 80.2% in 40 and 80 days old plants. Significance of F-ratio was implied at all treatments. (Table 6.42 fig 6.84, 6.85).

Table 6.42: Pb (II) effect on protein (mg/g FW) in the leaves of *E. sativa* plants during different growth stages.

Content of protein in *E. sativa* plants at 40 and 80 days		
Concentration Pb (II) (mg/Kg)	**40 Days**	**80 days**
0	8.48 ± 0.53	6.57 ± 0.35
100	7.20 ± 0.25**	6.43 ± 0.21
200	6.88 ± 0.42***	4.78 ± 0.10****
300	5.13 ± 0.57****	4.41 ± 0.23****
400	4.86 ± 0.24****	3.41 ± 0.30****
500	3.58 ± 0.27****	2.71 ± 0.28****
600	3.19 ± 0.18****	2.63 ± 0.25****
700	2.61 ± 0.16****	1.95 ± 0.30****
800	2.27 ± 0.28****	1.30 ± 0.53****
F-ratio (df 8,18)	**118.5******	**115.4******
HSD	**1.003**	**0.867**

Data shown as mean ± S.D of triplicates Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

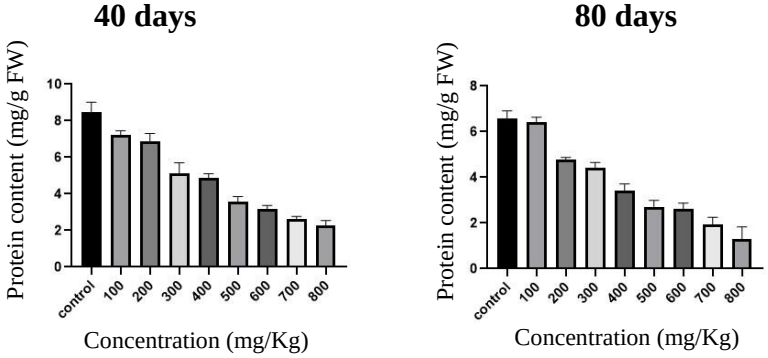


Fig. 6.84, 6.85: Effect of Pb (II) metal on content of protein (mg/g FW) in 40 and 80 days old *E. sativa* plants.

6.6.3.2 Catalase (CAT): The activity of antioxidants enzyme CAT gets raised with increase in amount of Pb (II). 222.9% increase was noticed at 700 mg/Kg concentration of Pb in 40 days old plants and 177.1% increase at 600 mg/Kg concentration of Pb (II) in 80 days' mature plants in contrast to control. Then decrease in enzyme activity was observed until no growth above 800 mg/Kg Pb concentration. The F-ratio value was also found to be significant for all stages of treatment. (Table 6.43, fig 6.86, 6.87).

Table 6.43: Pb (II)) effect on specific activity of CAT (UA/g protein) in the leaves of *E. sativa* plant leaves during different growth stages.

Specific activity of CAT in *E. sativa* plants at 40 and 80 days		
Concentration Pb (II) (mg/Kg)	**40 Days**	**80 days**
0	12.36 ± 0.34	11.25 ± 0.34
100	15.58 ± 0.31****	16.46 ± 0.23****

200	18.48 ± 0.14****	18.11 ± 0.17****
300	23.42 ± 0.34****	20.08 ± 0.19****
400	28.87 ± 0.40****	25.23 ± 0.12****
500	36.31 ± 0.21****	27.67 ± 0.29****
600	37.81 ± 0.83****	31.18 ± 0.30****
700	39.91 ± 0.40****	26.19 ± 0.39****
800	25.86 ± 0.28****	22.43 ± 0.15****
F-ratio (df 8,18)	**1808****	**1751****
HSD	**1.169**	**0.735**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

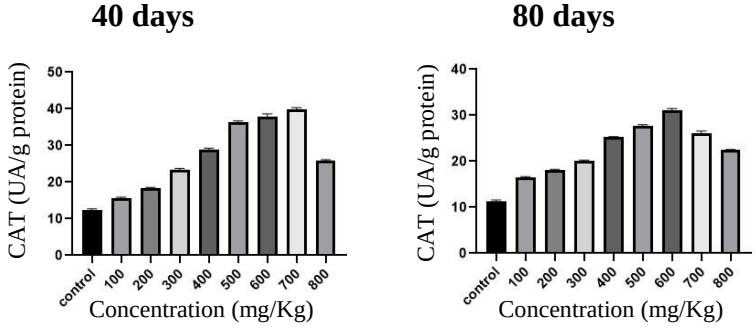


Fig. 6.86, 6.87: Effect of Pb (II) metal on specific activity of CAT (UA/g protein) in 40 and 80 days old *E. sativa* plants.

6.6.3.3 Guaiacol Peroxidase (POD): The activity of POD was noticed to get enhanced with rise in value of Pb (II) with maximum increase found at 700 mg/Kg concentration with 59.2% in 40 days old plants. In 80 days old plants there was increase of 47.2% at 700 mg/Kg concentration of Pb (II). Decrease was observed after that and no growth was found after 800 mg/Kg of Pb. F ratio was also found to be significant. (Table 6.44, fig 6.88, 6.89)

Table 6.44: Pb (II) effect on specific activity of POD (UA/g protein) in *E. sativa* plant leaves during different growth stages.

Specific activity of POD in *E. sativa* plants at 40 and 80 days		
Concentration Pb (II) (mg/Kg)	**40 Days**	**80 days**
0	25.43 ± 0.42	25.64 ± 0.19
100	29.54 ± 0.36	29.84 ± 0.19****
200	32.63 ± 0.16**	30.55 ± 0.23****
300	34.25± 0.36***	31.49 ± 0.26****
400	35.47 ± 0.16***	33.24 ± 0.28****
500	37.31 ± 0.42****	34.53 ± 0.24****

600	37.35 ± 0.21****	35.60 ± 0.26****
700	40.49 ± 0.23****	37.76 ± 0.27****
800	35.20 ± 0.47***	32.63 ± 0.22****
F-ratio (df 8,18)	**21.22****	**644****
HSD	**5.359**	**0.688**

Data shown as mean ± S.D of triplicates Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

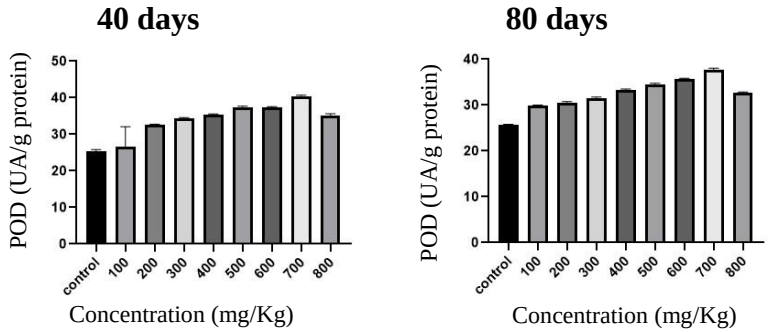


Fig. 6.88, 6.89: Effect of Pb (II) metal on specific activity of POD (UA/mg protein) in 40 and 80 days old *E. sativa* plants.

STUDIES ON ACHYRANTHUS ASPERA PLANT

6.7 Toxic effects of Cr (VI) on *Achyranthes aspera*:

6.7.1 Morphological characters:

6.7.1.1 Root length: Cr (VI) caused a much fall in length of root of potted plants. In comparison to control, 300 mg/Kg Cr (VI) caused 49.2% and 46.3% decrease in root length in 40 and 80 days old plants respectively. Not much decrease was observed at higher concentrations. The F-ratio for one-way ANOVA for all the growth stages were significant (Table 6.45, fig: 6.90, 6.91)

Table 6.45: Cr (VI) effect on root length (cm) of *A. aspera* plants during different growth stages.

Root length of *A. aspera* plants at 40 and 80 days		
Concentration Cr (VI) (mg/Kg)	**40 Days**	**80 days**
0	5.44 ± 0.1060	7.47 ± 0.1808
50	5.68 ± 0.1750	6.80 ± 0.1323***
100	5.13 ± 0.1258	6.46 ± 0.0961****
150	4.65 ± 0.0961***	5.70 ± 0.1557****
200	4.40 ± 0.1500*****	5.10 ± 0.1000****
250	3.41 ± 0.2386****	4.53 ± 0.1501****

300	2.76 ± 0.1652****	4.01 ± 0.0902****
F-ratio (df 6, 14)	**139.1******	**267.6******
HSD	**0.439**	**0.371**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

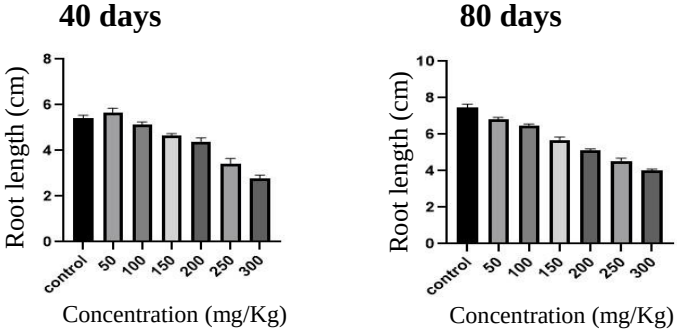


Fig. 6.90, 6.91: Effect of metal stress of Cr (VI) on root length (cm) of 40 and 80 days old *A. aspera* plants.

6.7.1.2 Shoot length: Shoot length get reduced with rise in concentration of hexavalent chromium in contrast to control. Reduction was noticed at 300 mg/Kg Cr concentration by 49.2% and 46.3% in 40 and 80 days old plants respectively. The F-ratio was found to be significant (Table 6.46, fig 6.92, 6.93)

Table 6.46: Cr (VI) effect on shoot length (cm) of *A. aspera* plants during different growth stages.

Shoot length of *A. aspera* plants at 40 and 80 days		
Concentration Cr (VI) (mg/Kg)	**40 Days**	**80 days**
0	9.59 ± 0.1518	16.59 ± 0.2120
50	8.80 ± 0.0306***	15.41 ± 0.2228****
100	8.50 ± 0.1387****	13.91 ± 0.2013****
150	7.94 ± 0.2163****	12.57 ± 0.1447****
200	7.66 ± 0.1601****	10.89 ± 0.1290****
250	7.45 ± 0.1050****	10.48 ± 0.1277****
300	6.60 ± 0.0907****	10.20 ± 0.1500****
F-ratio (df 6,14)	**149.3******	**633.2******
HSD	**0.387**	**0.4845**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test

(n=24). Significant at****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

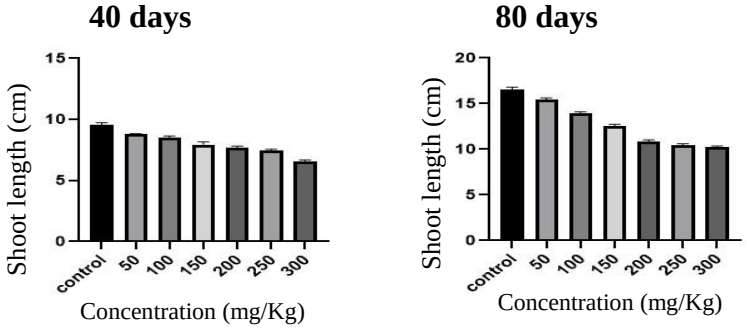


Fig. 6.92, 6.93: Effect of metal stress of Cr (VI) on shoot length (cm) of 40 and 80 days old *A. aspera* plants.

6.7.1.3 Fresh weight –Toxic effects of Cr (VI) were also observed on fresh weight of the plant. Firstly, a slight non- significant increase in weight was found at 100 mg/Kg of Cr (VI) of 4.9% in 40 days old plants and at 50 mg/Kg of Cr (VI) of 5.6% in 80 days old plants. Reduction of 47.6% and 47% was observed at 300 mg/Kg Cr (VI) in 40 and 80 days old plants respectively. The F-ratio for one-way ANOVA for all growth stages was found to be significant. (Table 6.47, Fig 6.94, 6.95).

Table 6.47: Cr (VI) effect on fresh weight (gm) of *A. aspera* plants during different growth stages.

Fresh weight of *A. aspera* plants at 40 and 80 days		
Concentration Cr (VI) (mg/Kg)	**40 Days**	**80 days**
0	16.50 ± 0.15	18.30 ± 1.59
50	16.91 ± 0.15	19.34 ± 0.19**
100	17.32 ± 0.20	19.15 ± 0.23*
150	14.58 ± 0.30****	16.55 ± 0.26****
200	11.97 ± 0.48****	13.90 ± 0.15****
250	9.77 ± 0.33****	11.19 ± 0.25****
300	8.64 ± 0.28****	9.69 ± 0.26****
F-ratio (df 6, 14)	**392.2******	**500.7******
HSD	**0.868**	**0.845**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

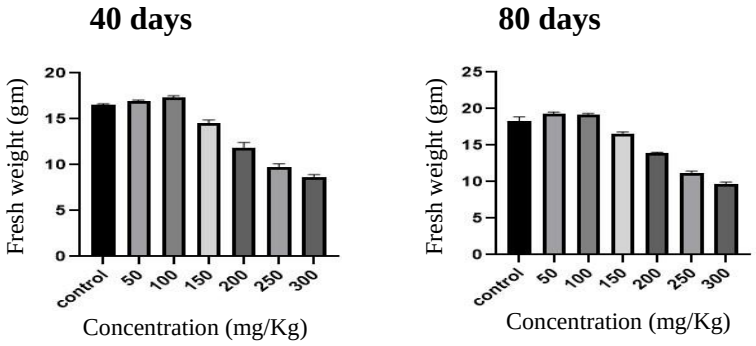


Fig. 6.94, 6.95: Effect of metal stress of Cr (VI) on fresh weight (gm) of 40 and 80 days old *A. aspera* plants.

6.7.1.4 Dry weight- Firstly a slight increase in weight was found at 100 mg/Kg of Cr (VI) of 17% in 40 days old plants and at 50 mg/Kg of Cr (VI) of 4.8% in 80 days old plants. Dry weight gets decreased by 65.9% and 58% at 300 mg/Kg Cr (VI) in 40 and 80 days old plants as compared to control plants. No growth was observed after that. The F-ratio for one-way ANOVA for all growth stages was found to be significant. (Table 6.48, Fig 6.96, 6.97).

Table 6.48: Cr (VI) effect on dry weight (gm) of *A. aspera* plants during different growth stages.

Dry weight of *A. aspera* plants at 40 and 80 days		
Concentration Cr (VI) (mg/Kg)	**40 Days**	**80 days**
0	12.61 ± 0.16	15.70 ± 0.16
50	13.58 ± 0.29	16.46 ± 0.18
100	14.76 ± 0.27****	15.92 ± 0.19
150	10.58 ± 0.23***	13.38 ± 0.40
200	7.46 ± 0.12****	10.69 ±0.39
250	6.37 ± 0.55****	8.57 ± 0.19*
300	4.29 ± 0.68****	6.59 ± 0.13**
F-ratio (df 6,14)	**341.2******	**8.597******
HSD	**1.04**	**5.96**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

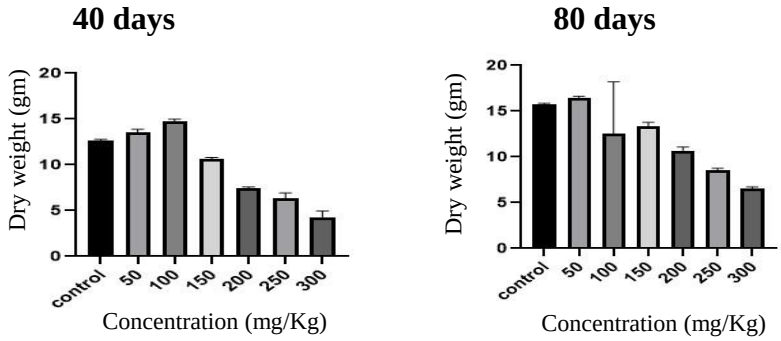


Fig. 6.96, 6.97: Effect of Cr (VI) metal on dry weight (gm) of 40 and 80 days mature *A. aspera* plants.

6.7.1.5 Moisture content –Decrease in moisture content of 37% at 100 mg/Kg of Cr (VI) and then increase of 114.1% at 300 mg/Kg Cr (VI) concentration in 40 days and increase of 126.3% at 300 mg/Kg of Cr (VI) concentration in 80 days old plants as compared to control plants was found. The F-ratio for all growth stages was found to be significant. (Table 6.49, fig 6.98, 6.99)

Table 6.49: Effect of Cr (VI) on moisture content of *A. aspera* plants during different growth stages.

Moisture content of *A. aspera* plants at 40 and 80 days		
Concentration Cr (VI) (mg/Kg)	**40 Days**	**80 days**
0	23.59 ± 1.54	14.14 ± 2.46
50	19.71 ± 1.18	14.88 ± 1.69
100	14.78 ± 0.93	16.82 ± 1.92
150	27.41 ± 3.12	19.13 ± 3.63
200	35.56 ± 3.36	23.05 ± 3.58*
250	34.68 ± 7.11	23.41 ± .37**
300	50.54 ± 6.38	32.00 ± 2.81****
F-ratio (df 6,14)	**1.885**	**17.23****
HSD	**31.58**	**7.11**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

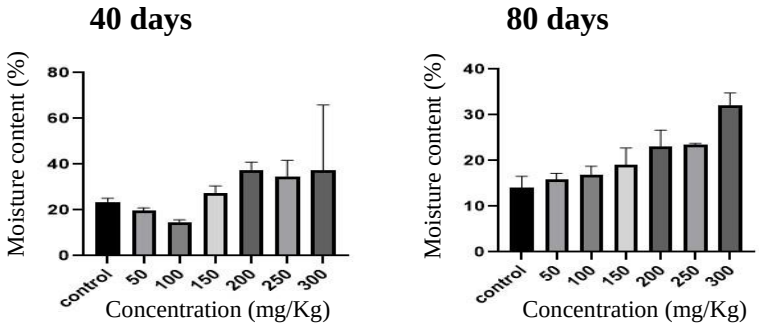


Fig. 6.98, 6.99: Effect of Cr (VI) metal on moisture content of 40 and 80 days mature *A. aspera* plants.

6.7.2 Biochemical Parameters

6.7.2.1 Chlorophyll *a* –Amount of chlorophyll *a* decreased in the leaves of *A. aspera* plants grown in different treatments of Cr (VI). A significant decrease of 61.3% and 90.4% was observed in 40 and 80 days old plant leaves at 300 mg/Kg Cr (VI) concentration. Cr concentration higher than 300 mg/Kg did not cause any change in chlorophyll value, thus not taken into consideration. The F-ratio was found to be significant for all treatments. (Table 6.50, fig 6.100, 6.101)

Table 6.50: Cr (VI) effect on content of chlorophyll *a* (mg/g FW) in the leaves of *A. aspera* plants during different growth stages.

Chlorophyll a content in the leaves of *A. aspera* plants at 40 and 80 days		
Concentration Cr (VI) (mg/Kg)	**40 Days**	**80 days**
0	1.8263 ± 0.0249	0.9140 ± 0.0400
50	1.7553 ± 0.0386	0.7993 ± 0.0208***
100	1.3930 ± 0.0282****	0.6750 ± 0.0170****
150	1.0837 ± 0.0115****	0.4680 ± 0.0210****
200	1.0713 ± 0.0132****	0.2920 ± 0.0178****
250	0.9543 ± 0.0319****	0.1680 ± 0.0139****
300	0.7063 ± 0.0518****	0.0870 ± 0.0114****
F-ratio (df 6,14)	**532.1****	**627.5****
HSD	**0.087**	**0.061**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

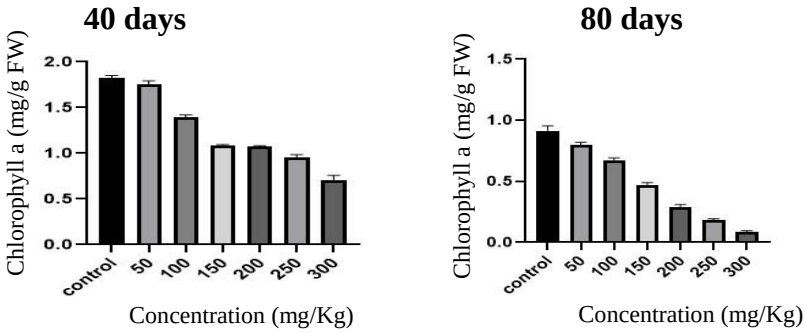


Fig. 6.100, 6.101: Effect of Cr (VI) metal on chlorophyll a (mg/g FW) in 40 and 80 days mature *A. aspera* plants.

6.7.2.2 Chlorophyll *b*- Chlorophyll *b* content in the leaves also showed reduction when plants were grown in rising level of hexavalent chromium. The reduction of 93.6% and 93.3% was observed at 300 mg/Kg of Cr (VI) in 40 and 80 days old plants respectively. Significance of F-ratio showed the effect of treatment at all stages (Table 6.51, fig 6.102, 6.103).

Table 6.51: Cr (VI) effect on chlorophyll b content (mg/g FW) in the leaves of *A. aspera* plants during different growth stages.

Chlorophyll b content in the leaves of *A. aspera* plants at 40 and 80 days		
Concentration Cr (VI) (mg/Kg)	**40 Days**	**80 days**
0	0.7970 ± 0.0404	0.6560 ± 0.0281
50	0.7563 ± 0.0302	0.4200 ± 0.0406****
100	0.6960 ± 0.0191***	0.2580 ± 0.0301****
150	0.2333 ± 0.0087****	0.0921 ± 0.0050****
200	0.0820 ± 0.0135****	0.0683 ± 0.0040****
250	0.0680 ± 0.0106****	0.0547 ± 0.0015****
300	0.0507 ± 0.0067****	0.0433 ± 0.0021****
F-ratio (df 6,14)	**769.2****	**339.7****
HSD	**0.06044**	**0.0610**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

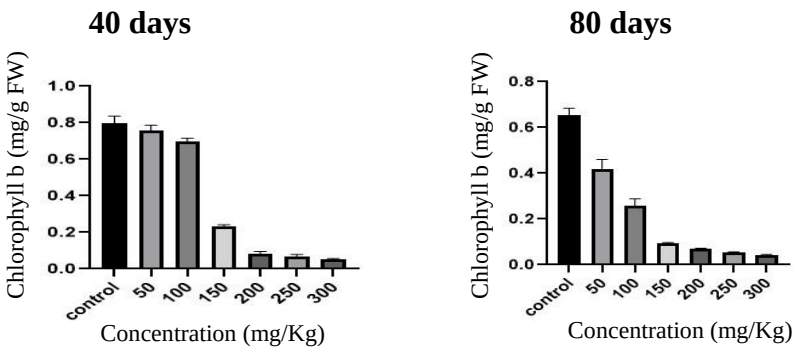


Fig. 6.102, 6.103: Effect of Cr (VI) metal on chlorophyll *b* (mg/g FW) in 40 and 80 days mature *A. aspera* plants.

6.7.2.3 Total chlorophyll- The leaves of *A. aspera* plants showed reduced content of total chlorophyll when grown in soil containing different amount of hexavalent chromium. In contrast to control, there was decrease of 71% and 91.8% at 300 mg/Kg Cr (VI) in both 40 and 80 days old plants respectively. The F-ratio for all treatments was found to be significant. (Table 6.52, fig 6.104, 6.105)

Table 6.52: Cr (VI) effect on content of total chlorophyll (mg/g FW) in the leaves of *A. aspera* plants during different growth stages**.**

Total chlorophyll content in the leaves of *A. aspera* plants at 40 and 80 days		
Concentration Cr (VI) (mg/Kg)	**40 Days**	**80 days**
0	2.6233 ± 0.0155	1.5700 ± 0.0680
50	2.5117 ± 0.0670*	1.2193 ± 0.0564****
100	2.0890 ± 0.0473****	0.9330 ± 0.0227****
150	1.3170 ± 0.0185****	0.5601 ± 1.0240****
200	1.1533 ± 0.0143****	0.3603 ± 0.0146****
250	1.0223 ± 0.0339****	0.2350 ± 0.0131****
300	0.7570 ± 0.0462****	0.1280 ± 0.0096****
F-ratio (df 6, 14)	**1111******	**658.7******
HSD	**0.108**	**0.1005**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

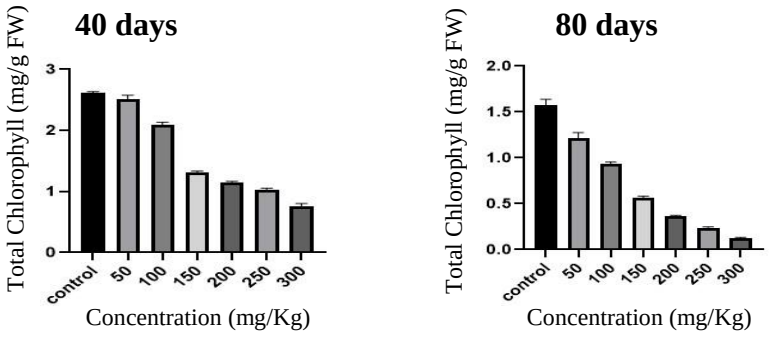


Fig. 6.104, 6.105: Effect of Cr (VI) metal on total chlorophyll (mg/g FW) in the leaves of 40 and 80 days mature *A. aspera* plants.

6.7.3 Protein content and Antioxidant Enzymes.

6.7.3.1 Protein Content: Reduction was observed in the protein content with rising amount of hexavalent chromium treatment. Much reduction was observed at 300 mg/Kg Cr (VI) concentration with 67.7% and 77.9% in 40 and 80 days old plants. No further growth was observed after that. Significance of F-ratio was implied at all treatments. (Table 6.53, fig 6.106, 6.107).

Table 6.53: Cr (VI) effect on protein content (mg/g FW) in the leaves of *A. aspera* plants during different growth stages.

Content of protein in the leaves of *A. aspera* plants at 40 and 80 days		
Concentration Cr (VI) (mg/Kg)	**40 Days**	**80 days**
0	4.99 ± 0.40	3.31 ± 0.41
50	4.40 ± 0.30	2.22 ± 0.04**
100	4.06 ± 0.09	1.81 ± 0.03****
150	3.75 ± 0.12	1.53 ± 0.04****
200	1.80 ± 0.42	1.36 ± 0.07****
250	1.83 ± 0.04	1.27 ± 0.03****
300	1.61 ± 0.05	0.73 ± 0.55****
F-ratio (df 4, 14)	**94.25**	**30.09**
HSD	**0.535**	**0.727**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

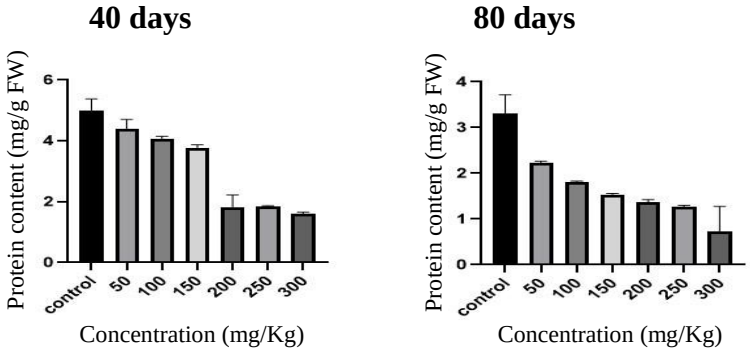


Fig. 6.106, 6.107: Effect of Cr (VI) metal on protein content (mg/g FW) in 40 and 80 days mature *A. aspera* plant leaves.

6.7.3.2 Catalase (CAT) – Antioxidants enzyme activity of catalase get enhanced with rise in level of Cr (VI). Maximum increase was observed at 300 mg/Kg concentration of 178.5% in 40 days old plants and maximum increase of 140% in 250 mg/Kg

concentration of Cr (VI) in 80 days old plants as compared to control plants. The F-ratio value was also found to be significant for all stages of treatment. (Table 6.54, fig 6.108, 6.109).

Table 6.54: Cr (VI) effect on specific activity of CAT (UA/g protein) in *A. aspera* plant leaves during different growth stages**.**

CAT Specific activity in *A. aspera* plants at 40 and 80 days		
Concentration Cr (VI) (mg/Kg)	**40 Days**	**80 days**
0	6.47 ± 0.17	8.54 ± 0.18
50	7.56 ± 0.20***	10.87 ± 0.32
100	7.86 ± 0.12****	13.56 ± 0.17
150	10.03 ± 0.20****	15.85 ± 0.33
200	12.86 ± 0.20****	19.65 ± 0.08
250	17.59 ± 0.22****	20.50 ± 0.21
300	18.02 ± 0.25****	18.99 ± 0.25
F-ratio (df 6, 14)	**1781******	**1.016**
HSD	**0.5477**	**1183.5**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

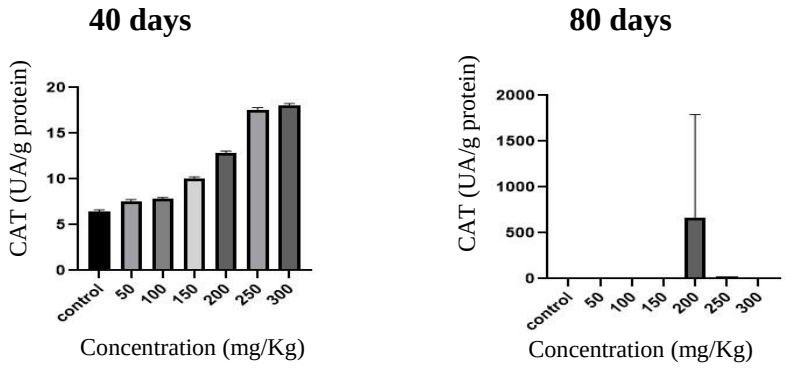


Fig. 6.108, 6.109: Effect of Cr (VI) metal on specific activity of CAT (UA/g protein) in 40 and 80 days old *A. aspera* plant leaves.

6.7.3.3 Guaiacol Peroxidase (POD). The activity of POD was observed to get increase with rise in value of hexavalent chromium and maximum increase was found at 300 mg/Kg concentration with 112.7% in 40 days old plants. In 80 days mature plants,

increase of 149.3% at 300 mg/kg of Cr (VI) was seen. Significance of F-ratio was observed at all stages of treatment. (Table 6.55, fig 6.110, 6.111)

Table 6.55: Cr (VI) effect on specific activity of POD (UA/g protein) in *A. aspera* plant leaves during different growth stages.

POD Specific activity in *A. aspera* plants at 40 and 80 days		
Concentration Cr (VI) (mg/Kg)	**40 Days**	**80 days**
0	10.27 ± 0.28	7.66 ± 0.10
50	12.66 ± 0.20****	11.62 ± 0.13*
100	16.01 ± 0.36****	12.77 ± 0.22**
150	18.65 ± 0.29****	16.24 ± 0.10****
200	20.38 ± 0.13****	17.40 ± 0.43****
250	21.42 ± 0.27****	17.78 ± 2.44****
300	21.85 ± 0.16****	19.10 ± 1.08****
F-ratio (df 6, 14)	**953.1****	**24.63****
HSD	**0.704**	**5.063**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

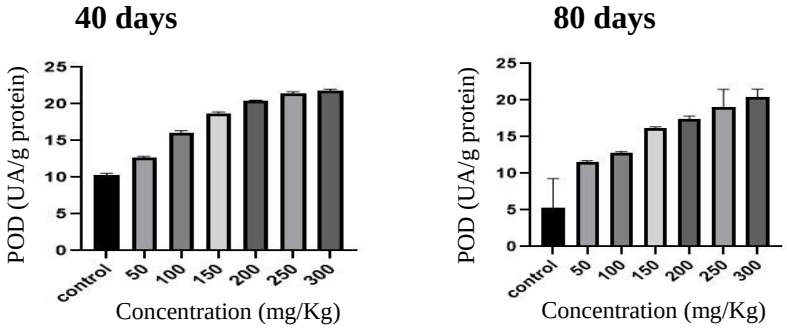


Fig. 6.110, 6.111: Effect of Cr (VI) metal on specific activity of POD (UA/g protein) in 40 and 80 days mature *A. aspera* plant leaves.

6.8 Toxic effects of Pb (II) on *Achyranthes aspera*:

6.8.1 Morphological characters:

6.8.1.1 Root length: Pb (II) caused a much fall in the length of root of potted plants. In comparison to control, 800 mg/Kg Pb (II) caused 55.5% and 50.9% decrease in root length in 40 and 80 days old plants respectively. The F-ratio for one-way ANOVA for all the growth stages were significant, thus showing the significance of the test. (Table 6.56, figures 6.112, 6.113)

Table 6.56: Pb (II) effect on root length (cm) of *A. aspera* plants during different growth stages.

Root length of *A. aspera* plants at 40 and 80 days		
Concentration Pb (II) (mg/Kg)	**40 Days**	**80 days**
0	6.64 ± 0.1850	7.36 ± 0.1856
100	6.36 ± 0.1500	6.86 ± 0.1290**
200	5.99 ± 0.0603**	6.27 ± 0.0764****
300	5.36 ± 0.1752****	5.74 ± 0.2183****
400	4.87 ± 0.2359****	5.23 ± 0.0681****
500	4.29 ± 0.0850****	5.04 ± 0.0808****
600	3.64 ± 0.3050****	4.59 ± 0.1290****
700	3.32 ± 0.1137****	3.97 ± 0.1607****
800	2.95 ± 0.0833****	3.61± 0.1531****
F-ratio (df 8,18)	**187.1******	**239.8******
HSD	**0.492**	**0.404**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

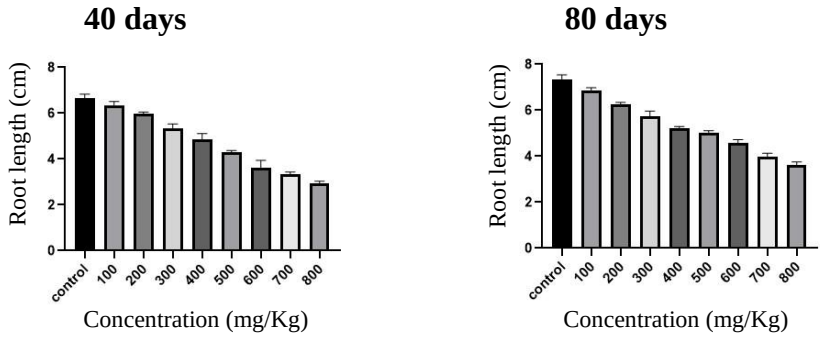


Fig. 6.112, 6.113: Effect of metal stress of Pb (II) on root length (cm) of 40 and 80 days old *A. aspera* plants.

6.8.1.2 Shoot length: Shoot length get reduced with rise in value of Pb (II) in comparison to plants taken as control. Reduction was observed at 800 mg/Kg Pb (II) concentration by 55.5% and 50.9% in 40 and 80 days old plants respectively. The F-ratio was found to be significant (Table 6.57, fig 6.114, 6.115)**.**

Table 6.57: Pb (II) effect on shoot length (cm) of *A. aspera* plants during different growth stages.

Shoot length of *A. aspera* plants at 40 and 80 days

Concentration Pb (II) (mg/Kg)	40 Days	80 days
0	21.45 ± 0.1311	25.72 ± 0.2413
100	20.64 ± 0.2003****	25.51 ± 0.1600
200	19.15 ± 0.3523****	24.56 ± 0.1960
300	16.75 ± 0.1320****	22.60 ± 0.2359
400	16.42 ± 0.2524****	22.03 ± 0.1041
500	15.74 ± 0.1856****	17.66 ± 0.3504
600	14.59 ± 0.1539****	16.51 ± 0.2358
700	13.58 ± 0.2066****	15.88 ± 0.1801
800	11.29 ± 0.2542****	13.61 ± 0.1955
F-ratio (df 8,18)	**316.6****	**2.630***
HSD	**0.923**	**13.35**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

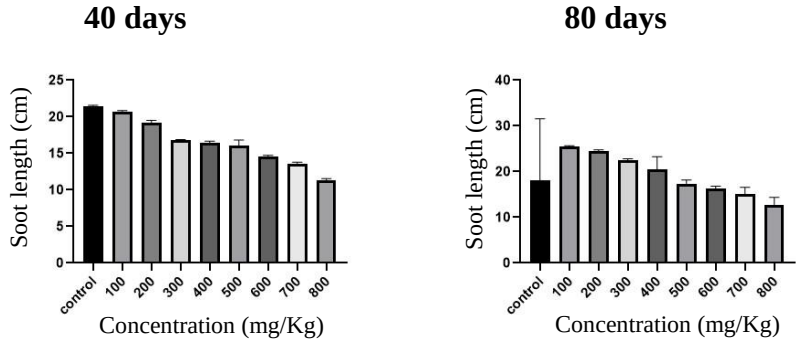


Fig. 6.114, 6.115: Effect of metal stress of Pb (II) on shoot length (cm) of 40 and 80 days *A. aspera* plants.

6.8.1.3 Fresh weight – Toxic effects of Pb (II) were also noticed on fresh weight of the plant. Reduction of 53.8% and 67% was observed at 800 mg/Kg Pb (II) in 40 and 80 days old plants respectively. The F-ratio for one-way ANOVA for all growth stages was found to be significant. (Table 6.58, Fig 6.116, 6.117).

Table 6.58: Pb (II) effect on fresh weight (gm) of *A. aspera* plants during different growth stages.

Fresh weight of *A. aspera* plants at 40 and 80 days		
Concentration Pb (II) (mg/Kg)	**40 Days**	**80 days**
0	20.58 ± 0.20	19.56 ± 0.19
100	19.48 ± 0.19	17.92 ± 0.09**
200	18.58 ± 0.14	17.19 ± 0.77****
300	16.84 ± 0.11***	14.39 ± 0.60****
400	15.75 ± 0.08****	12.57 ± 0.24****
500	15.42 ± 0.28****	10.87 ± 0.33****

600	13.78 ± 0.15****	9.98 ± 0.13****
700	9.98 ± 2.29****	8.46 ± 0..18****
800	9.50 ± 0.22****	6.45± 0.22****
F-ratio (df 8,18)	**74.67******	**437.4******
HSD	**2.24**	**1.074**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

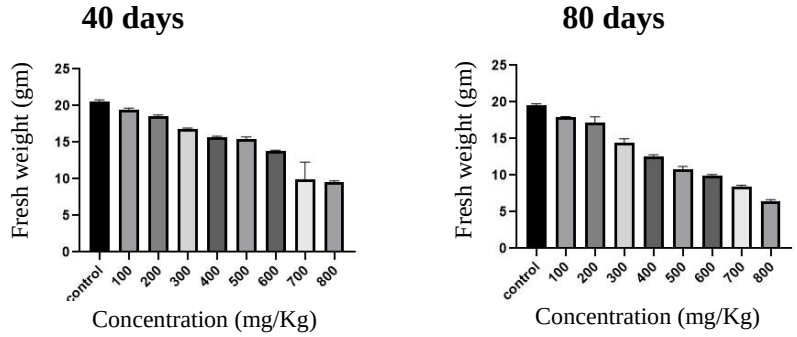


Fig. 6.116, 6.117: Effect of metal stress of Pb (II) on fresh weight (gm) of 40 and 80 days old *A. aspera* plants.

6.8.1.4 Dry weight- Dry weight get decreased by 59.3% and 73.2% at 800 mg/Kg Pb (II) in 40 and 80 days old plants in comparison to control. No growth was noticed after that showing harmful effect of metal stress. The F-ratio for one-way ANOVA for all growth stages was found to be significant. (Table 6.59, Fig 6.118, 6.119).

Table 6.59: Pb (II) effect on dry weight (gm) of *A. aspera* plants during different growth stages.

Dry weight of *A. aspera* plants at 40 and 80 days		
Concentration Pb (II) (mg/Kg)	**40 Days**	**80 days**
0	16.46 ± 0.23	14.54 ± 0.15
100	14.58 ± 0.33****	12.79 ± 0.13
200	12.31 ± 0.38****	10.68± 0.24****
300	10.61 ± 0.11****	9.71 ± 0.09****
400	9.45 ± 0.19****	6.43 ± 0.12****
500	8.71 ± 0.25****	5.72 ± 0.25****
600	8.23 ± 0.09****	5.25 ± 0.11****
700	7.54 ± 0.23****	4.64 ± 0.23****
800	6.70 ± 0.29****	3.89 ± 0.15****
F-ratio (df 8,18)	**530.4******	**122.3******
HSD	**0.714**	**1.76**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

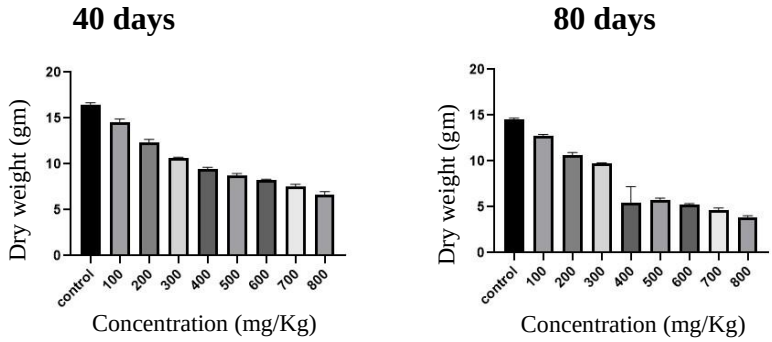


Fig. 6.118, 6.119: Effect of Pb (II) metal on dry weight (gm) of 40 and 80 days mature *A. aspera* plants.

6.8.1.5 Moisture content –Increase of 117.2% at 500 mg/Kg Pb (II) concentration in 40 days and increase of 90% at 400 mg/Kg of Pb (II) concentration in 80 days old plants as compared to control plants was found. The F-ratio for all growth stages was found to be significant. (Table 6.60, fig 6.120, 6.121)

Table 6.60: Pb (II) effect on moisture content of *A. aspera* plants during different growth stages.

Moisture content of *A. aspera* plants at 40 and 80 days		
Concentration Pb (II) (mg/Kg)	**40 Days**	**80 days**
0	20.02 ± 0.42	25.69 ± 0.15
100	25.15 ± 1.13	28.64± 0.96
200	33.77 ± 2.35	37.81 ± 2.02****
300	37.01 ± 0.34	32.40 ± 3.54**
400	39.97 ± 1.24	48.85 ± 1.36****
500	43.49 ± 1.63	47.40 ± 2.13****
600	40.29 ± 0.68	47.40 ± .49****
700	21.64 ± 18.41	45.13 ± 1.77****
800	29.55 ± 1.60	39.70 ± 1.52****
F-ratio (df 8,18)	**2.270****	**68.96****
HSD	**21.8**	**5.189**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at****$p \leq 0.0001$, ***$p \leq 0.001$, **$p \leq 0.01$, *$p \leq 0.05$.

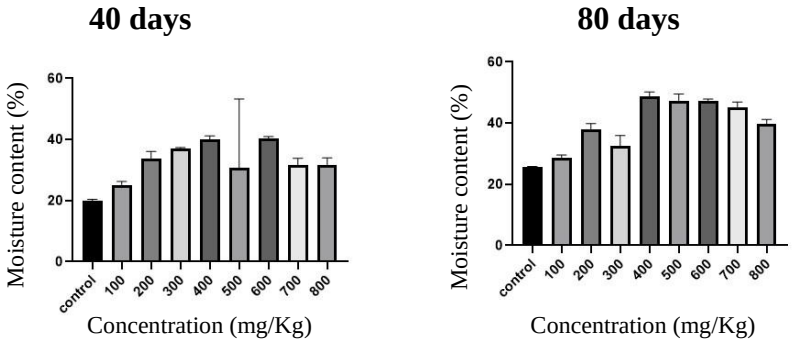


Fig. 6.120, 6.121: Effect of Pb (II) metal on moisture content of 40 and 80 days old *A. aspera* plants.

6.8.2 Biochemical Parameters

6.8.2.1 Chlorophyll *a* –Amount of chlorophyll *an* also get decreased in *A. aspera* plant leaves growing in different treatments of Pb (II). A significant decrease of 87% and 95.6% was observed in 40 and 80 days' mature plants at 800 mg/Kg Pb (II) concentration. The F-ratio was found to be significant for all treatments. (Table 6.61, fig 6.122, 6.123)

Table 6.61: Pb (II) effect on chlorophyll a content (mg/g FW) in the leaves of *A. aspera* plants during different growth stages.

Chlorophyll a content in *A. aspera* plants at 40 and 80 days		
Concentration Pb (II) (mg/Kg)	**40 Days**	**80 days**
0	2.6543 ± 0.0075	1.9387± 0.0440
100	1.8517 ± 0.1046****	1.5657 ± 0.1263****
200	1.5613 ± 0.1728****	1.3497 ± 0.0981****
300	1.18705 ± 0.0938****	0.9140 ± 0.0764****
400	0.9293 ± 0.0045****	0.76933 ± 0.0137****
500	0.7500 ± 0.0298****	0.54263 ± 0.0616****
600	0.4657 ± 0.0085****	05363 ± 0.0540****
700	0.3837 ± 0.0025****	0.1533 ± 0.0235****
800	0.3443± 0.0226****	0.0840 ± 0.0104****
F-ratio (df 8,18)	**147.5**	**265.4**
HSD	**0.3198**	**0.1919**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

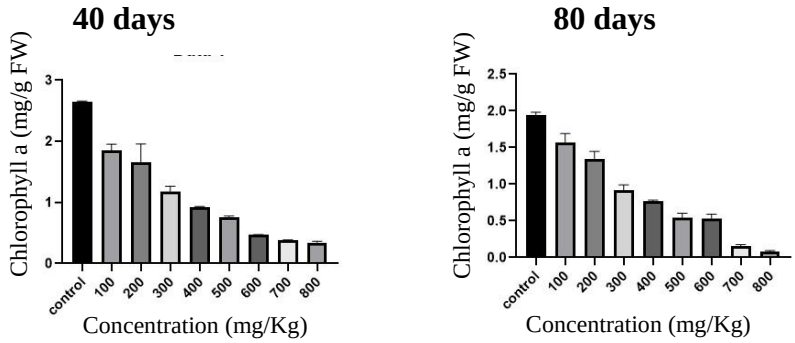


Fig. 6.122, 6.123: Effect of Pb (II) metal on chlorophyll *a* (mg/g FW) in 40 and 80 days old *A. aspera* plant leaves.

6.8.2.2 Chlorophyll *b*- Chlorophyll *b* content in the leaves also showed reduction when grown in the higher levels of lead. The reduction of 87.3% and 94.8% was observed at 800 mg/Kg of Pb (II) in 40 and 80 days old plants respectively. Significance of F-ratio showed the effect of treatment at all stages (Table 6.62, fig 6.124, 6.125).

Table 6.62: Pb (II) effect on chlorophyll b content (mg/g FW) in the leaves of *A. aspera* plants during different growth stages.

Chlorophyll b content in *A. aspera* plants at 40 and 80 days		
Concentration Pb (II) (mg/Kg)	**40 Days**	**80 days**
0	1.9180 ± 0.0509	1.4317 ± 0.0081
100	1.5880 ± 0.0667****	1.2340 ± 0.0393****
200	1.3753 ± 0.1029****	0.9573 ± 0.0568****
300	1.1300 ± 0.0947****	0.8157 ± 0.0673****
400	0.7563± 0.0300****	1.3900 ± 0.0718****
500	0.4970 ± 0.0318****	0.6633 ± 0.0190****
600	0.2863± 0.0042****	0.3907 ± 0.0258****
700	0.2533 ± 0.0093****	0.3093 ± 0.0115****
800	0.2427 ± 0.0462****	0.0740± 0.0053****
F-ratio (df 8,18)	**354.1******	**591******
HSD	**0.167**	**0.099**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

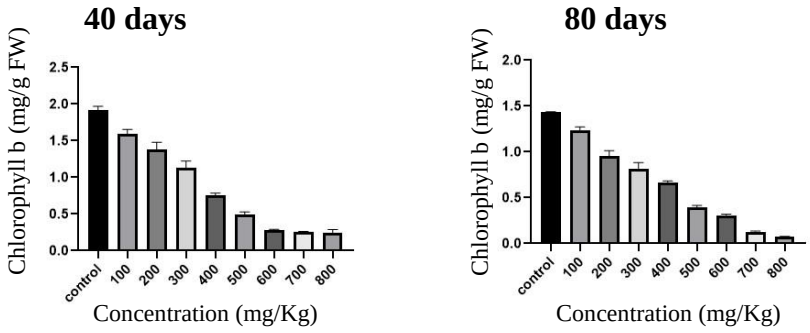


Fig. 6.124, 6.125: Effect of Pb (II) metal on chlorophyll *b* (mg/g FW) in 40 and 80 days old *A. aspera* plant leaves.

6.8.2.3 Total chlorophyll- The leaves of *A. aspera* plants showed reduced content of total chlorophyll when raised in different levels of Pb (II). In comparison to control, there was decrease of 87.1% and 95.3% at 800 mg/Kg Pb (II) in both 40 and 80 days old plants respectively. The F-ratio for all treatments were found to be significant. (Table 6.63, fig 6.126, 6.127)

Table 6.63: Pb (II) effect on content of total chlorophyll (mg/g FW) in the leaves of *A. aspera* plants during different growth stages**.**

Total chlorophyll content in *A. aspera* plants at 40 and 80 days		
Concentration Pb (II) (mg/Kg)	**40 Days**	**80 days**
0	4.5723 ± 0.0570	3.3703 ± 0.509
100	3.4397 ± 0.0692****	2.7997 ± 0.1196****
200	2.9367 ± 0.2592****	2.3070 ± 0.1123****
300	2.3005± 0.1740****	1.7297 ± 0.0489****
400	1.6857 ± 0.0317****	1.4327 ± 0.0158****
500	1.2470 ± 0.0030****	0.9330 ± 0.6399****
600	0.7520 ± 0.0098****	.8457 ± 0.0481****
700	0.6370 ± 0.0070****	0.2820 ± 0.0165****
800	0.5870 ± 0.0505****	0.1580 ± 0.0112****
F-ratio (df 8,18)	**486******	**918.2********
HSD	**0.313**	**0.1809**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

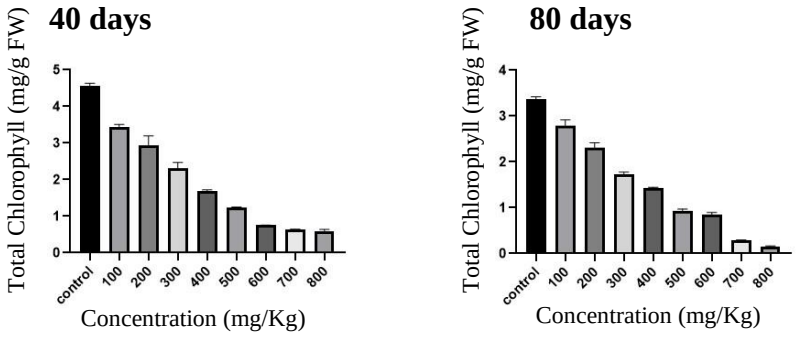


Fig. 6.126, 6.127: Effect of Pb (II) metal on total chlorophyll (mg/g FW) in 40 and 80 days old *A. aspera* plant leaves.

6.8.3 Protein content and Antioxidant Enzymes.

6.8.3.1 Protein content: Reduction was observed in the protein content with rising amount of lead. Much reduction was observed at 800 mg/Kg Pb (II) concentration with 65.8% and 81% in 40 and 80 days old plants. No significant reduction in protein content was observed at further higher concentration of Pb. Significance of F- ratio was implied at all treatments. (Table 6.64 fig 6.128, 6.129).

Table 6.64: Pb (II) effect on protein content (mg/g FW) in *A. aspera* plant leaves during different growth stages**.**

Content of protein in of *A. aspera* plants at 40 and 80 days		
Concentration Pb (II) (mg/Kg)	**40 Days**	**80 days**
0	8.46 ± 0.20	7.12 ± 0.14
100	7.66± 0.11**	6.26± 0.08*
200	6.60 ± 0.28****	5.75 ± 0.14****
300	5.48 ± 0.25****	4.50 ± 0.33****
400	4.73 ± 0.15****	3.88 ± 0.22****
500	4.31 ± 0.17****	2.64 ± 0.19****
600	3.76 ± 0.19****	1.77 ± 0.06****
700	3.45 ± 0.23****	1.57 ± 0.31****
800	2.89 ± 0.15****	1.35 ± 0.48****
F-ratio (df 8,18)	**291.6******	**224.8******
HSD	**0.56**	**0.718**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

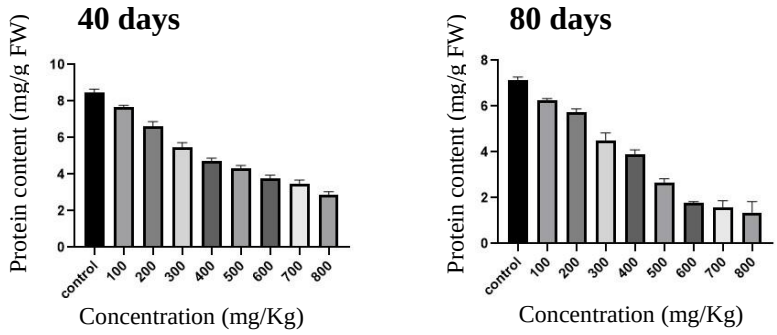


Fig. 6.128, 6.129: Effect of Pb (II) metal on protein content (mg/g FW) in 40 and 80 days old *A. aspera* plants.

6.8.3.2 Catalase (CAT) – The activity of antioxidant enzyme CAT gets increased with increase in amount of Pb (II). Much rise was noticed at 700 mg/Kg concentration of 285.6% in 40 days' mature plants and enhancement of 32% at 700mg/Kg concentration of Pb (II) in 80 days' mature plants in comparison to plants taken as control. At higher concentration of Pb, harmful effects were observed in the form of no plant growth. The F- ratio value was also found to be significant for all stages of treatment. (Table 6.65, fig 6.130, 6.131).

Table 6.65: Pb (II) effect on specific activity of CAT (UA/g protein) in *A. aspera* plant leaves during different growth stages.

CAT Specific activity in *A. aspera* plants at 40 and 80 days		
Concentration Pb (II) (mg/Kg)	**40 Days**	**80 days**
0	10.46 ± 0.28	8.24 ± 0.57
100	13.05 ± 0.55****	11.24 ± 0.56
200	18.48 ± 0.14****	16.24 ± 0.36**
300	23.42 ± 0.34****	20.01 ± 0.15****
400	28.87 ± 0.40****	25.23 ± 0.12****
500	36.31 ± 0.21****	27.51 ± 0.06****
600	37.81 ± 0.83****	31.35 ± 0.07****
700	40.34 ± 0.13****	34.94 ± 0.48****
800	35.21 ± 0.05****	30.82 ± 6.22****
F-ratio (df 8,18)	**1944******	**56.83******
HSD	**1.26**	**6.01**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at****$p \leq 0.0001$, ***$p \leq 0.001$, **$p \leq 0.01$, *$p \leq 0.05$.

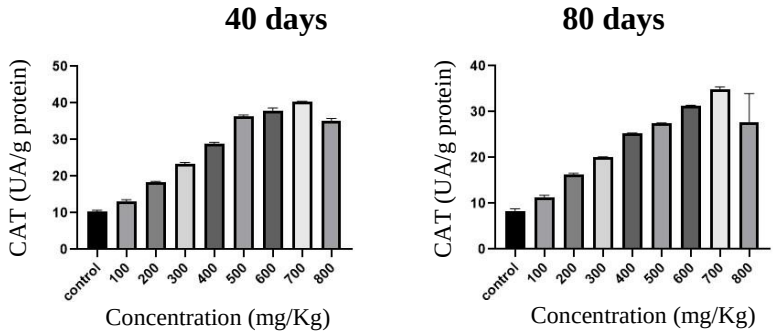


Fig. 6.130, 6.131: Effect of Pb (II) metal on specific activity of CAT (UA/g protein) in 40 and 80 days old *A. aspera* plant leaves.

6.8.3.3 Guaiacol Peroxidase (POD) - The activity of POD was noticed to get increase with rise in value of Pb (II) with maximum increas found at 700 mg/Kg concentration with 111.1% in 40 days old plants. In 80 days old plants, there was increase of 76.6% at 600 mg/Kg concentration of Pb (II). F-ratio was also found to be significant. (Table 6.66, fig 6.132, 6.133).

Table 6.66: Pb (II) effect on specific activity of POD (UA/g protein) in *A. aspera* plants during different growth stages**.**

POD Specific activity in *A. aspera* plants at 40 and 80 days		
Concentration Pb (II) (mg/Kg)	**40 Days**	**80 days**
0	16.45 ± 0.13	16.76 ± 0.26
100	18.51 ± 0.17***	19.30 ± 0.40****
200	23.56 ± 0.23****	20.79 ± 0.23****
300	24.79± 0.35****	22.21 ± 0.44****
400	28.37 ± 0.11****	25.62 ± 0.25****
500	30.18 ± 0.67****	28.67 ± 0.27****
600	33.01 ± 0.77****	29.60 ± 0.17****
700	34.73 ± 0.70****	28.62 ± 0.19****
800	28.91 ± 0.35****	26.08 ± 0.19****
F-ratio (df 8,18)	**553.6******	**799.5******
HSD	**1.308**	**0.798**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

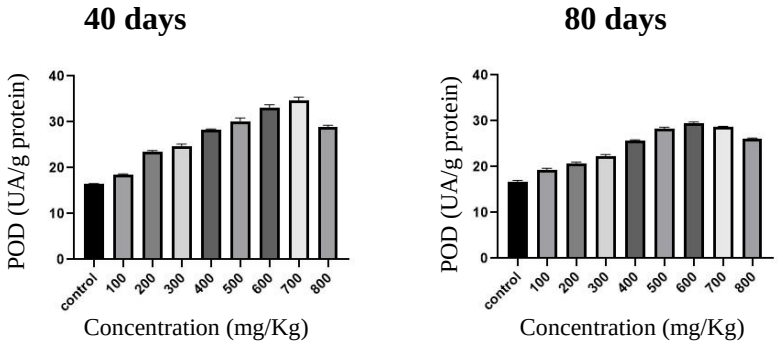


Fig. 6.132, 6.133: Effect of Pb (II) metal on specific activity of POD (UA/g protein) in 40 and 80 days old *A. aspera* plants.

6.9: Effect of metal uptake (Cr and Pb) in the roots of *C. iria*, *E. sativa* and *A. aspera*:

6.9.1 Cr (VI) uptake effect in *Cyperus iria* plant roots:

Metal absorbing capacity of the roots of *C. iria* plant get increased with rise in value of hexavalent chromium and much increase of 1.36% was found at 350 mg/Kg chromium in plants that were 40 days mature and in 80 days' mature plants there was increase of 1.64% at 350 mg/Kg of chromium (VI) in contrast to plants taken as control plants. F-ratio was also found to be significant (Table 6.67, fig 6.134, 6.135).

Table 6.67: Cr (VI) uptake effect in *Cyperus iria* plant roots during different growth stages (mg/g DW).

Cr(VI) uptake in *Cyperus iria* plant roots at 40 and 80 days		
Concentration Cr (VI) (mg/g)	**40 Days**	**80 days**
0	0	0
50	0.0867 ± .0252*	0.2833 ± 0.0306****
100	0.1500 ± .0300***	0.4267 ± 0.0451****
150	0.2800 ± .0200****	0.6100± 0.0361****
200	0.5800 ± .0458****	1.2500± 0.0361****
250	0.9300 ± .0361 ****	1.4400 ± 0.0361****
300	1.2067 ± .0252****	1.5300± 0.0300****
350	1.3467 ± .0351****	1.6400 ± 0.0458****
F-ratio (df 7,16)	**932****	**992.8****
HSD	**0.084**	**0.099**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

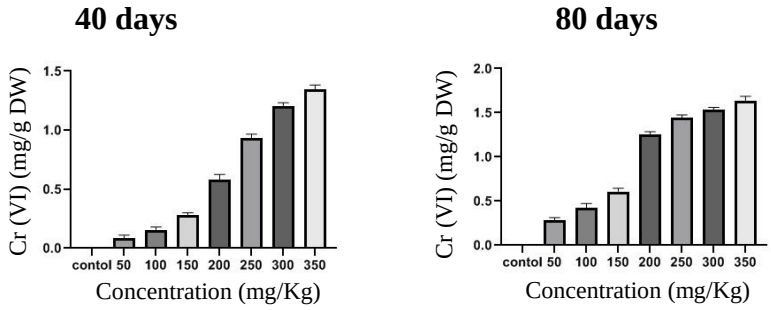


Fig. 6.134, 6.135: Effect of Cr (VI) uptake in 40 and 80 days old *C. iria* plant roots.

6.9.2 Cr (VI) uptake effect in *Eruca sativa* plant roots:

Uptake of metal in the *Eruca sativa* roots get increased with rise in level of hexavalent chromium with much rise of 1.16% found at 350 mg/Kg concentration in plants that were 40 days mature. In 80 days old plants there was increase of 1.28% at 350 mg/Kg Cr (VI). F-ratio showed its significance at all stages of growth (Table 6.68, fig 6.136, 6.137)

Table 6.68: Cr (VI) uptake effect in *E. sativa* plant roots during different growth stages (mg/g DW).

Cr(VI) uptake in *E. sativa* plant roots at 40 and 80 days		
Concentration Cr (VI) (mg/g)	**40 Days**	**80 days**
0	0	0
50	0.0667±0.0404*	0.0867±0.404
100	0.1300±0.0458****	0.2833±0.0751****
150	0.2833±0.0503****	0.5800±0.0557****
200	0.4533±0.0451****	0.7567±0.0351****
250	0.6133±0.0651****	0.9367±0.0513****
300	0.8300±0.0500****	1.2067±0.0404****
350	1.1600±0.0400****	1.2867±0.0551****
F-ratio (df 7,16)	**237.9******	**306.1******
HSD	**0.128**	**0.137**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

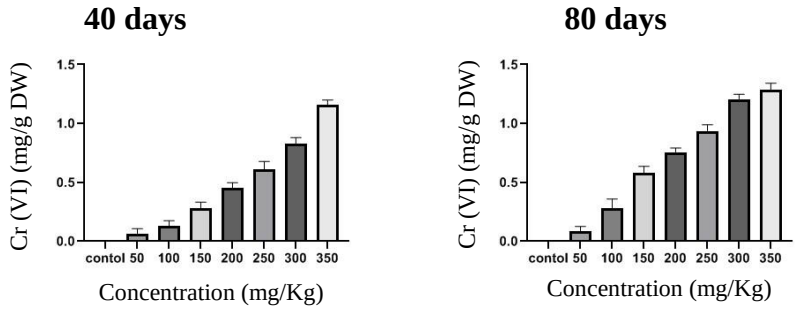


Fig. 6.136, 6.137: Effect of Cr (VI) uptake in 40 and 80 days old *E. sativa* plant roots.

6.9.3 Cr (VI) uptake effect in *A. aspera* plant roots:

Metal uptake in the roots get enhanced with rise in level of hexavalent chromium with much increase of 0.38% found at 300 mg/Kg of Cr in 40 days old plants. In plants that were 80 days mature, there was increase of 0.46% at 300 mg/Kg chromium. Increase that observed was non-significant. (Table 6.69, fig 6.138, 6.139).

Table 6.69: Cr (VI) uptake effect in *A. aspera* plant roots during different growth stages (mg/g DW).

Cr (VI) uptake in *A. aspera* plant roots at 40 and 80 days		
Concentration Cr (VI) (mg/Kg)	**40 Days**	**80 days**
0	0	0
50	Nd	0.1100±0.0265*
100	0.0567±0.0252	0.1533±0.0351***
150	0.0833±.0306	0.1833±0.0252****
200	0.1233±.0404	0.2300±0.0300****
250	0.2333±0.0451	0.2800±0.0400****
300	0.3867±0.0351	0.4633±0.0451****
F-ratio (df 7,16)	**55.27******	**62.68******
HSD	**0.089**	**0.089**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at****$p \leq 0.0001$, ***$p \leq 0.001$, **$p \leq 0.01$, *$p \leq 0.05$.

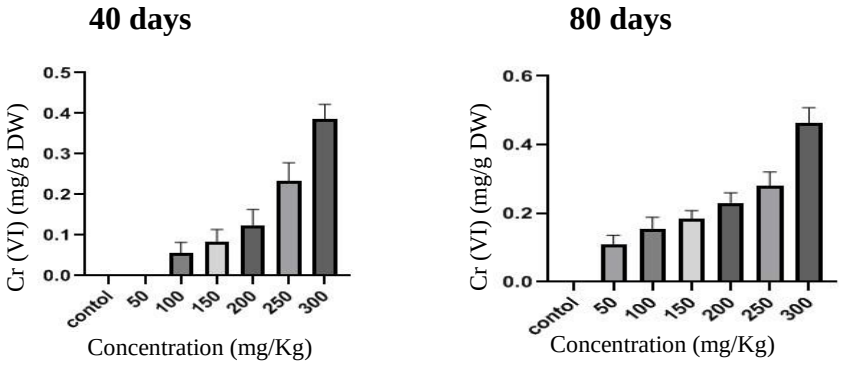


Fig. 6.138, 6.139: Effect of Cr (VI) metal uptake in the roots of 40 and 80 days old *A. aspera* plants**.**

6.9.4 Effect of Pb (II) uptake in the roots of *Cyperus iria* plant:

Absorption of metal in the plant roots get enhanced with rise in value of Pb (II) and maximum increase was found at 800 mg/Kg concentration with1.88% in 40 days' mature plants. In plants that were 80 days mature, there was increase of 1.96% at 800 mg/Kg concentration of Pb (II). F-ratio was also found to be significant. (Table 6.70, fig 6.140, 6.141).

Table 6.70: Pb (II) uptake effect in *Cyperus iria* plant roots during different growth stages (mg/g DW).

Pb(II) uptake in *Cyperus iria* plant roots at 40 and 80 days		
Concentration Pb (II) (mg/g)	**40 Days**	**80 days**
0	0	0
100	0.44±0.05****	0.68±0.04****
200	0.60±0.04****	0.78±0.04****
300	0.74±0.06****	0.92±0.04****
400	0.95±0.03****	1.17±0.03****
500	1.22±0.05****	1.36±0.03****
600	1.43±0.05****	1.56±0.05****
700	1.84±0.05****	1.91±0.04****
800	1.88±0.02****	1.96±0.04****
F-ratio (df 8,18)	**797.2******	**1008******
HSD	**0.11**	**0.094**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

40 days **80 days**

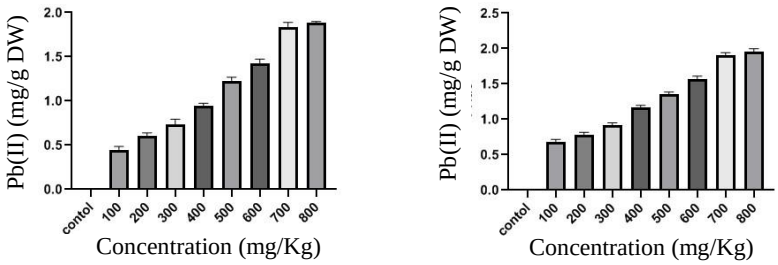


Fig. 6.140, 6.141: Effect of Pb (II) metal uptake in the roots of 40 and 80 days old *C. iria* plants.

6.9.5 Effect of Pb (II) uptake in the roots of *Eruca sativa* plant:

With rise in value of Pb (II), the metal absorbing potential of plant roots also get enhanced with maximum increase found at 800 mg/Kg concentration of 1.27% in 40 days' mature plants. In plants with 80 days' maturity, there was increase of 1.61% at 800 mg/Kg concentration of Pb (II). F-ratio was also found to be significant (Table 6.71, fig 6.142, 6.143).

Table 6.71: Pb (II) uptake effect in *E. sativa* plant roots during different growth stages (mg/g DW).

Pb(II) uptake *E. sativa* plant roots at 40 and 80 days		
Concentration Pb (II) (mg/g)	**40 Days**	**80 days**
0	0	0
100	0.11±0.03	0.17±0.05*
200	0.16±0.04	0.26±0.05***
300	0.25±0.04**	0.53±0.04****
400	0.33±0.06****	0.90±0.11****
500	0.56±0.06****	1.24±0.05****
600	0.79±0.06****	1.37±0.05****
700	1.05±0.12****	1.52±0.05****
800	1.27±0.06****	1.61±0.05****
F-ratio (df 8,18)	**176.3******	**375.7******
HSD	**0.166**	**0.159**

Data shown as mean ± S.D of triplicates using Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

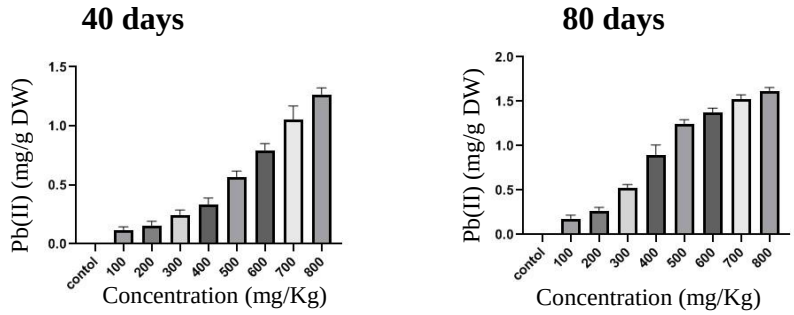


Fig. 6.142, 6.143: Effect of Pb (II) metal uptake in the roots of 40 and 80 days old *E. sativa* plants.

6.9.6 Effect of Pb (II) uptake in the roots of *A. aspera* plant:

With rise in value of Pb (II), the metal absorbing potential of plant roots also get enhanced with maximum increase found at 800 mg/Kg concentration of 1.19% in 40 days' mature plants. In plants with 80 days' maturity, there was increase of 1.94% at 800 mg/Kg concentration of Pb (II). F-ratio was also found to be significant (Table 6.72, fig 6.144, 6.145).

Table 6.72: Pb (II) uptake effect of in *A. aspera* plant roots during different growth stages (mg/g DW).

Pb(II) uptake in *A. aspera* plant roots at 40 and 80 days		
Concentration Pb (II) (mg/Kg)	**40 Days**	**80 days**
0	0	0
100	0.10±0.05	0.21±0.04***
200	0.18±0.05**	0.30±0.04****
300	0.23±0.05***	0.46±0.04****
400	0.36±0.06****	0.57±0.05****
500	0.53±0.07****	0.86±0.05****
600	0.88±0.06****	1.06±0.05****
700	1.17±0.04****	1.24±0.04****
800	1.19±0.05****	1.52±0.04****
F-ratio (df 8,18)	**271.7******	**467.2******
HSD	**0.137**	**0.118**

Data shown as mean ± S.D of triplicates Tukey's multiple comparison test (n=24). Significant at ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05.

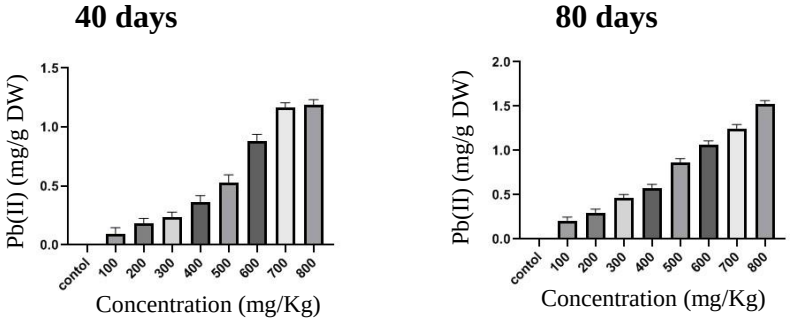


Fig. 6.144, 6.145: Effect of Pb (II) metal uptake in the roots of 40 and 80 days old *A. aspera* plants.

6.10: Presence of heavy metals in milk of grazing animals:

People of all ages especially infant and children consume milk and milk products. Existence of heavy metals in milk can act as direct and indirect indicator of healthy status of milk and degree of environmental pollution respectively. Food chain act as an important component for metal accumulation in plants growing in polluted areas. From plants, these metals can be transferred into body of animals grazing in polluted areas, especially in cattle (Miranda *et al*., 2005). Finally, entry of noxious metals occurs into human body via ingestion of milk as well as milk products (Cai *et al*., 2009). The maximal suggested dose of lead in milk by Codex Alimentarius Commission (2015) and EC (no.1881) European Union (2006) is 0.02mg/ml. According to FSSAI (2011), this limit for lead in milk is 0.02 and 0.1mg/ml. Daily nutritional intake level of chromium in milk is 1.61 mg/Kg (Buachoon, 2004) and its permissible value as given by Oliver (1997) is 0.2mg/day

For analysis of milk samples, milk was collected from cattle farmers residing near the area to be studied. Instantly after milking, sample collection was done. Milk sample were taken in triplicate and mean value was taken for examining the presence of metals by microwave digestion method. The dilution was done with 1% HCl solution and metal determination was carried out by inductively coupled plama mass spectrophotometer equipped with auto sampler iCAP RQ from Saif labs PU, Chandigarh was used to find the value of heavy metals in the milk samples.

6.11 Conclusion about Cr:

Our results showed that use of chromium in studied plants lead to remarkable fall in all the morphological parameters on increasing the concentration of chromium. **(Table 6.1-6.5, 6.23-6.27, and 6.45-6.49)**. Similar type of results was also obtained earlier by

other workers like Akinci and Akinci 2010; Nath et. al., 2009; Aziz Eman et. al., 2007; Tandon and Gupta 2002; Chatterjee and Chatterjee, 2000, Hunter and Vergnano, 1953, Daniels et.al., 1972 etc. to name a few. Reduction in dry matter with rise in the value of Cr in all the plants was seen. Nath *et al* (2008) and Vajpayee *et al* (2001) also observed fall in dry weight in controlled conditions. It was reported by Zurayk *et al* (2001) that interconnection of saltness and hexavalent chromium resulted unremarkable reduction in dry biomass accommodation of *Portulaca oleracea*. Chatterjee and Chatterjee (2000) also found fall in dry matter value of cauliflower with excess concentration of chromium. Sihag and Joshi (2018) also observed decrease in fresh and dry weight in *Sorghum bicolor* (L.) with increase in chromium concentration.

Our study also found decrease in the amount of photosynthetic pigments (chlorophyll a, b and total chlorophyll) with rise in value of Cr (VI) in all the studied plant species. **(Table 6.6-6.8, 6.28-6.30, and 6.50-6.52)** Chatterjee and Chatterjee (2000), Singh *et al* (2006), Nath *et al* (2008), Zengin and Munzuroglu (2006), Elloumi *et al* (2007), Bishnoi *et al* (1993), Joshi *et al* (1999), Diwan *et al* (2012) and Sihag and Joshi (2018) also observed decrease in photosynthetic pigments in cauliflower, paddy, *Vigna sinensis* L. *Savix Hassk* , sunflower, almond, pea (*Pisum sativum* L.), legumes, Indian mustard and *Sorghum bicolor* (L.) respectively with increasing chromium concentration.

Reduction in chlorophyll value in the leaves may occur because of altered chlorophyll structure or increased activity of chlorophyllase enzyme. Inhibition of delta-amino le-vulenic acid dehydratase activity by the use of chromium could also result in breakdown of chlorophyll structure (Vajpayee *et al*., 2000). Another reason for drop in chlorophyll level under Cr stress could occur because of deactivation of enzymes involved in the process of chlorophyll formation.

In the present work, content of soluble protein had been found to get reduced with increase in amount of chromium in all the studied plants. **(Table 6.9, 6.31, 6.53)** Singh and Sinha (2005) also observed fall in its content in *Brassica juncea* plant growing in tannery waste containing different heavy metals. Sumanlata (1995), Mehta (1996), Bhardwaj (1998) and Sihag and Joshi (2018) observed decrease in protein content in guar, sunflower and sorghum respectively with increase in concentration of heavy metal chromium. Percolation of nitrogen from plant might have caused the decrease in protein content in leaves by Cr (VI) application or it may be due to enzyme degradation responsible for nitrogen uptake by plants.

The present study showed an increased level of antioxidant enzymes (catalase and peroxidase) with increase in concentration of Cr heavy metal (up to 300 mg/Kg) and then decline in specific activity of enzymes were seen. **(Table 6.10-6.11, 6.32-6.33, and 6.54-6.55)** The results were in equality with the earlier work of Nath *et al* (2009) where he found rise in the catalase activity of *Phaseolus mungo* Roxb. with increasing value of chromium heavy metal and tannery discharge. Jain *et al* (2000) also observed decline in catalase activity with increase in chromium concentration. Decrease in specific activity of the enzyme may be attributed to exchange of iron from active sites with other metals or because of its less amount in the leaves. Plants can activate their antioxidant enzyme cascade to detoxify harmful metals (Prasad 1998; Shanker et. al., 2003). Samantaray *et al* (1999) used these antioxidants as enzyme markers to discover mung bean varieties that could tolerate chromium metal.

Generation of superoxide radicals by chromium application are responsible for increase in the activity of antioxidant enzymes. It is observed by Shanker *et al* (2005) that fall in the level of enzyme activity at higher chromium amount might be due to negative effect of chromium on them.

6.12 Conclusion about Pb:

Adverse effects of lead include much hindrance of root growth, yellowing of leaves, hampering of photosynthesis and activity of enzymes (Sharma and Dubey, 2005). Our results revealed that on application of Pb (II) in studied plants, there was significant decrease in all the morphological parameters on increasing the concentration of lead. **(Table 6.12-6.16, 6.34-6.38, and 6.56-6.60)** Çimrin *et al* (2007) also observed decrease in fresh and dry weight and root /shoot length of *Pisum sativam and Zea mays*. In tomato seedlings also root shoot length and fresh biomass was reduced with rise in amount of lead heavy metal. Tandy *et al* (2006) and Guo *et al* (2007) also noted fall in root and shoot length with lead heavy metal. Same results were obtained by some other researchers showing reduction in morphological parameters in *Paspalum distichum* and *Cynodon dactylon* (Shua *et al*., 2002), *Ipomoea aquatic* (Gothberg *et al*., 2004), *Phaseolus vulgaris* (Haider *et al*., 2006). Present study also observed fall in the amount of photosynthetic pigments (chlorophyll a, b and total chlorophyll) with rise in level of Pb (II) in all the three plants. **(Table 6.17-6.19, 6.39-6.41, and 6.61-6.63)**. Sheetal *et al* (2016) also showed same type of results in their study. They revealed that biomass, rate of photosynthesis and pigment content get decreased in comparison to plants taken as

control in mustard var. Pusa Jaikisan as the amount of heavy metal content increased. Nagajyoti *et al* (2008) also observed decrease in total chlorophyll content in groundnut by toxic metal discharge from the industries. Our results for reduction in the value of chlorophyll were also in conformity with the results of Siedlecka and Krupa (1996) in *Zea mays*, Zengin, Munzuruglu, (2006) in sunflower, Elloumi *et al* (2007) in almond etc. Fall in total chlorophyll content in two varieties of wheat with Cd and Pb metals by 50% (Gerek 79) and 70 % (Bolal 2973) was observed by Oncel *et al* (2000). Reduced chlorophyll content in green gram had been reported by Pandey and Pathak (2006) due to nickel stress. Dubey and Pandey (2011) also reported reduced photosynthetic pigments in black gram due to nickel stress. Rebechini *et al* (1974) observed changes in structure of chloroplast in an acquatic plant *Ceratophyllum demersum* when treated with lead. Drazkiewicz *et al* (1994) observed increase in chlorophyllase enzyme activity that resulted in increased chlorohyll degradation in lead treated plants. In addition, chlorophyll synthesis is inhibited by reduced accumulation of Mg and Fe. Bohner *et al* (1980) stated that drop in the value of chlorophyll might be due to inhibition of electron transport system.

In the present work, content of soluble protein had been found to fall with increase in value of chromium in all the studied plants. **(Table 6.20, 6.42, 6.64** Johna *et al* (2008) observed that with increasing value of cadmium and lead in *Lemna polyrrhiza* L., content of protein start decreasing. Decrease in protein content in *Brassica juncea* L. by 95% by Cd (900 μM) and 44% by Pb (1500 μM) at the time of flowering was reported by John *et al* (2009). Palma *et al* (2002) found that decrease in content of protein in *L. polyrrhiza* might be due to increased stress condition that resulted in increase in activity of protease enzyme resulting in breakdown of protein. Samantary (2002) also reported inhibition of protein synthesis by heavy metals. Costa and Spitz (1997) also noted fall in the value of soluble protein in presence of metal stress in *Lupinus albus.* In wheat also, Tiwari *et al* (2013) found decreased carbohydrate and protein content with increase in Pb concentration. Gardea Torresday *et al* (2004) and Romero-Puertas *et al* (2007) reported that Cd, As and Pb can reduce protein content by interfering in the absorption of magnesium and potassium ions. Increase in protein breakdown or prohibition of rubisco activity may also be responsible for decrease in protein content (Muthuchelian *et al.*, 2001; Siedlecka and Krupa, 1996).

The present study noted a rise in the level of antioxidant enzymes (catalase and peroxidase) with increase in concentration of Pb heavy metal (up to 700 mg/kg) and

then decline in specific activity of enzymes was seen. **(Table 6.21-622, 6.43-6.44, and 6.65-6.66)** Decrease in activity of catalase enzyme could occur due to its less formation or its deactivation by reactive oxygen species observed at much high value of lead (Verma *et al*., 2003). Levine *et al* (1989) noted that hampering of enzyme activity by lead resulted from interaction of lead with enzyme –SH groups. Gwozdz *et al* (1997) also noted enhanced antioxidative enzyme value at low level of metal and then decrease at increased metal level.

6.13 Metal toxicity in roots:

In the present study, heavy metal (Cr and Pb) uptake was studied in roots of all the three plant species by atomic absorption spectrophotometer **(Table 6.67-6.72).** Harmful effects of metal can occur due to uptake of metal beyond a certain limit by different plant parts. Content of heavy metal in roots was found to be more than stems and leaves (Zhao and Duo, 2015). Also, root act as barrier for upward movement of metal into another part of plant. (Liu *et al*., 2009).

6.14 Metal content in milk:

Our results concluded that amount of both the heavy metals were found to be within permissible limits i.e. 1.98 ppb (or μg/l) and 26.18 ppb (or μg/l) respectively. The maximum permissible limits for chromium is given as 0.02 mg/l (Oliver, 1997) and for lead is 0.02 and 0.1 mg/ml (FSSAI, 2011). The lead content in all the three samples was lower than the amount that was reported earlier in literature (Tripathi *et al*., 1999; Licata *et al*., 2004). When compared with standard milk samples from some parts of India, it was found that lead content in standard milk was 2.28 μg/l (Tripathi *et al*., 1999) from a study done in Mumbai. In another study on cow milk of different brands in Mumbai by Zodape *et al* (2012), it was observed that range of chromium in different brands was between 0.013-0.175 μg/l and that of lead was 0.139-5.904 μg/l. Toxicity of metal is dependent on various elements like its entry in body, the stage of metal, level of absorption, etc. (Mertz, 1986). The presence of toxic metals in milk more than the admissible levels was observed mainly from the developing countries because of the unclean state of processing, contaminated food and water used for animal drinking and the ignorance of the persons indulged in dairy industry.

CHAPTER-7

SUMMARY AND CONCLUSION

- Fall in the value of all growth parameters with rise in metal dosage at all stages of growth was observed. Both metals showed reduction in root /shoot length, fresh and dry matter in all the selected plants in contrast to plants taken as control.
- When compared to control plants, there was decrease in chlorophyll a, chlorophyll b and total chlorophyll content at all stages of growth. This may be due to degradation of chlorophyll pigments due to much toxicity of Cr and Pb.
- Amount of protein also showed reduction with rise in value of both heavy metals in all the three plant species. This may be due to breakdown of protein structure by metal stress that increases the activity of protease enzyme.
- CAT activity is mostly found in peroxisomes of green leaves. It is an important enzyme against oxidative stress in plants (Foyer and Noctor, 2000). Peroxidases also catalyze oxidoreduction between H_2O_2 and various reductants. Both enzymes are capable of eliminating H_2O_2 and result in release of reactive oxygen species.
- Specific activity of CAT was estimated to check the H_2O_2 reduction potential of the various plants. It was observed the activity of catalase enzyme get enhanced in presence of metal stress in comparison to plants kept as control plants. Maximum increase was observed at 700 mg/Kg concentration of lead and 300 mg/Kg concentration of chromium and after that decrease was observed.
- POD activity was also found to increase under increasing concentration of both heavy metals when compared to control plants. Further, at very high concentration of metals, reduction in POD activity was observed.
- All the results obtained were in conformity with previous studies on various weeds and crop plants.
- Accumulation of Cr and Pb in the roots of selected plant species was done by AAS and it was revealed that *C. iria* plants accumulated more Cr followed by *E. sativa* and *A. aspera*. Accumulation of Pb in the roots of *C. iria* was more followed by *A. aspera* and least accumulation in *E. sativa*.

  Conclusion- Thus from the study, it was clear that from all the three selected plant species, *Cyperus iria* could be used for decontamination of polluted areas.

Our results concluded that amount of both the heavy metals were found to be within permissible limits i.e. 1.98 ppb (or μg/l) and 26.18 ppb (or μg/l) respectively. The maximum permissible limits for chromium is given as 0.02 mg/l (Oliver, 1997) and for lead is 0.02 and 0.1 mg/ml (FSSAI, 2011).

It is recommended that although the heavy metal values were within safe limits, but these are present in high dosage. So in future, milk should be checked for their presence before consumption as milk is mostly consumed by human society especially by children and elderly people so that they may not develop diseases by consuming such poisonous milk.

Few points that need attention for further research in future:

- Need to boost up the agricultural procedures in order to enhance the phytoremediating ability of plants.
- Attention is needed to explore contaminated area with an aim to find out genetic makeup of cultivated or fierce plants that can be used for rectifying the polluted areas.
- Need to find out such plants that can be grown turn by turn so that metal extraction level can be encouraged.

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List of Publications

Narinderjit Kaur, Madhuri Girdhar and Anand Mohan (2020). Toxic effects of hexavalent chromium on physiological and biochemical parameters of *Cyperus iria* (rice flatsedge) – a weed plant. *Plant Cell Biotechnology and Molecular Biology*. 21(3 & 4):67–73. (Published: 17 February 2020).

Narinderjit Kaur. International Conference on Biosciences and Biotechnology (ICBB-2019) on the theme of "Science for Community" held on 4-5th November 2019 at Lovely Faculty of Technology and Sciences, LPU. (**Paper Presented**)

Narinderjit Kaur. "National Conference on Plant Sciences: Network in Health and Environment" held on 30-31st October at Khalsa College Amritsar. (**Poster Presented**)

Narinderjit Kaur. "International Conference on Innovative Strategies for Sustainable Water Management" held on 17-18th November 2017 at School of Bioengineering and Biosciences, LPU. (**Poster Presented**)