BIOACCUMULATION OF HEAVY METALS AND BIOCHEMICAL CHANGES INDUCED IN VEGETABLES IRRIGATED BY WASTE WATER AT BUDDHA NULLAH AGRICULTURAL SITES (PUNJAB)

A

Thesis

Submitted to

LOVELY PROFESSIONAL UNIVERSITY

Transforming Education Transforming India

for the award of

DOCTOR OF PHILOSOPHY (Ph.D.)
in
(Botany)

By

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PUNJAB

2020

DECLARATION

I hereby declare that the thesis entitled, **“Bioaccumulation of Heavy Metals and Biochemical Changes Induced in Vegetables Irrigated by Waste Water at Buddha Nullah Agricultural Sites (Punjab)”**submitted for Degree of Ph.D. in Botany to Department of Botany, Lovely Professional University is entirely original work and all ideas and references have been duly acknowledged. The research work has not been formed the basis for the award of any other degree.

Jagdev Singh

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CERTIFICATE

This is to certify that Mr. **Jagdev Singh** has completed the **Ph.D. Botany** titled, "**Bioaccumulation of Heavy Metals and Biochemical Changes Induced in Vegetables Irrigated by Waste Water at Buddha Nullah Agricultural Sites (Punjab)**" under my guidance and supervision. To the best of my knowledge, the present work is the result of his 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.

Signature of Supervisor
Dr. Anand Mohan
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Dedicated to

My

Mother

Late Sardarni Rajinder Kaur

ABSTRACT

This research work was conducted to evaluate the bioaccumulation of heavy metals in different types of vegetables (root, leafy, flower and fruit) irrigated with heavy - metal - rich waste water of Buddha Nullah, Ludhiana, a highly polluted riverine passing through the city carrying sewage as well as heavy - metal - rich industrial effluents. An experimental site along the bank of Buddha Nullah was selected for the study where vegetable crops were grown and irrigated with the heavy - metal - laden waste water of this nullah. Another area 4 kms away from the Buddha Nullah was selected as a control site where the same vegetables were raised, but were irrigated with normal borewell water. Physiochemical parameters and amount of heavy metals in water and soil samples from both the sites were analyzed. Some of the parameters like COD, BOD, EC, TSS, TDS and heavy metals (Arsenic, Cadmium, Chromium and Lead) were found to be much higher in the waste-water samples collected from the Buddha Nullah site in comparison to that of borewell water site. These parameters were found to be higher than prescribed limits of FAO, 1985; Pescod, WHO, 2003; Indian Standard (Awasthi, 2000) and European Union Standards (EU, 2002). Similarly the physiochemical analysis of soil samples revealed significant higher values of EC and heavy metals (As, Cd, Cr and Pb) in waste-water irrigated site samples as compared to that of borewell irrigated site samples. The mean values of heavy metals (As, Cr and Pb) in soil samples were found to be within the limits prescribed by European Union (2002) except Cd which was detected to be higher than the prescribed limits. The enrichment factor (EF) of all the heavy metals (As, Cr, Cd and Pb) in the soil samples collected from waste water site was found to be greater than 1.5 which points to the heavy metal accumulation in the soil owing to the irrigation with the waste water of Buddha Nullah. The enrichment factor analyzed was found to be in the descending order: Cr > Pb > Cd > As. Bioaccumulation factor (BAF) of As was detected to be less than one (<1) in all the vegetables studied but the mean content of Arsenic in the edible portions of all the vegetables were found to be significantly higher in waste-water irrigated vegetable samples as compared to borewell water irrigated samples. Similarly bioaccumulation factor (BAF) of Cd and Cr was calculated to be greater than one (>1) in all the vegetables studied except *Lycopersicum esculentum*. Bioaccumulation factor of lead (Pb) was found to be

greater than one (>1) in root (*Raphanus sativus*) and leafy (*Spinacia oleracea*) vegetables but less than one (<1) in flower (*Brassica oleracea*) and fruit (*Lycopersicum esculentum*) vegetables. Bioaccumulation factor (BAF) greater than one (>1) indicates that the consumption of these vegetables can pose serious health risks to the consumers. Similarly, the waste-water treated seeds showed significantly lower percentage of germination, seedling growth and higher relative toxicity as compared to that of borewell water treated ones in all the vegetables studied. Seed vigour was recorded to be significantly higher in borewell treated seeds in comparison to that of waste water treated seeds. Biochemical parameters like chlorophyll a, chlorophyll b, total chlorophyll, and starch content were analyzed to be decreased and that of proteins, reducing sugars, non-reducing sugars, and total sugars level increased in all the waste-water irrigated vegetable samples as compared to that of borewell water irrigated ones. The amount of non-enzymatic antioxidants like proline, total phenols, flavonoids, carotenoids and ascorbic acid was found to be significantly higher in waste-water irrigated vegetables as compared to the borewell water irrigated ones. The activity of enzymatic antioxidants like catalase, peroxidase, superoxide dismutase, glutathione reductase and ascorbate peroxidase was also found to be greatly enhanced in vegetables irrigated with waste water as compared to that of borewell water irrigated ones. This phenomenon can be attributed to the activation of defence mechanism against the oxidative stress induced by the heavy metal bioaccumulation in the cells. No chromosomal aberrations were noted in the cytological studies carried out under high magnification 60X of Zeiss fluorescence microscopy in root tip cells of waste-water-irrigated and borewell water irrigated vegetable samples. The production of vegetables irrigated with waste water was found to be higher as compared to the vegetables irrigated with borewell water. This might be due to the presence of higher amounts of essential nutrients like nitrate, sulphate and phosphate in the waste water of Buddha Nullah.

Health survey revealed that the population consuming the vegetables irrigated with waste water of Buddha Nullah suffers more with the heavy metals related diseases (skin allergy, cancer, mental illness, respiratory diseases and premature births etc.) than the population consuming borewell irrigated vegetables. A comparative analysis revealed that the females were affected more than the males. The bioaccumulation of all the heavy metals studied in all the vegetables was significantly higher than the permissible levels and the consumption of these vegetables can pose serious health risks to the consumers. The study recommends a restriction on cultivation of edible crops, especially root and broad leaved vegetables (*Raphanus sativus* and *Spinacia oleracea*) along the banks of Buddha Nullah. The consumers must be made aware of the health risks associated with the consumption of such heavy metal rich vegetables. The farmers are advised not to grow vegetables along the banks of Buddha Nullah but instead opt for alternative cropping systems like floriculture and agroforestry.

ACKNOWLEDGEMENT

My deep sense of gratitude and profound thanks to my research supervisor **Dr. Anand Mohan**, Associate Professor, Department of Biosciences and Bioengineering, Lovely professional University, for his kind guidance, motivation, support, cooperation and suggestion of this topic for research. His sincere guidance and encouragement inspired me to successfully complete my research work. I also express my sincere thanks to **Dr. Joydeep Dutta**, Head, Department of Botany for his support and advice at every step of this research work.

I take this opportunity to express heartfelt thanks to **Mr. Ashok Mittal** (Chancellor), **Mrs. Rashmi Mittal** (Pro-Chancellor), **Dr. Rameshwar S. Kanwar** (Vice Chancellor), **Dr. Loviraj Gupta** (Executive Dean), **Dr. Monica Gulati** (Registrar) LPU for their motivation and support along with providing an opportunity to work in such a renowned university. I would also like to acknowledge **Dr. Neeta Raj Sharma** (Associate Dean), School of Bioengineering and Biosciences, LPU, for her motivation, support and valuable suggestions throughout this research work. I am cordially grateful to **Dr. Rekha** for her moral support and kind suggestions. I also convey my heartiest thanks to **Dr. Ramesh Thakur**, **Dr. Devendra Pandey, Dr. Ashish Vyas, Dr Anupam Tiwari, Dr Dhriti Kapoor, Dr. Madhuri Girdhar, Dr. Nupur** and **Dr. Dheeraj Nim** for their inspiration, advice and suggestions.

The present work could not have been completed without the encouragement, assistance and cooperation given to me from time to time by my research colleagues **Mr. Murthy, Mrs. Mittu, Mrs. Namrata, Ms. Shaista Manzoor, Mrs. Narinderjeet and Ms. Kamaljit Kaur**. I am really privileged to have these Ph.D. scholars as my batchmates.

I also want to express my appreciation to lab assistants **Mr. Aman Bhatti, and Mr. Kuldip Singh** for providing the required chemicals and equipment at all stages of this research work.

I wish to express my heartfelt regard to my better half **Mrs. Varinder Kaur** for her co-operation, never-ending support, encouragement and patience from the beginning to the completion of this work. It would have been extremely difficult for me to complete this work without her support at every step. I am thankful for her unrelenting encouragement and dedication, despite the countless sacrifices she made for me. Above all, I am highly indebted to my dearest younger daughter, **Simranpreet Kaur Natt** not only for her constant help, getting expertise for computational work, but also for the utmost support and immense proficiency to prepare this manuscript.

My deep thanks to all those people, whose names are not mentioned, but they directly or indirectly helped me to complete this research work. I would like to pay my thanks to the laboratory assistance provided by seed store, soil and water testing laboratory, multi-element analysis laboratory, Punjab Agricultural University, Ludhiana for procurement of authentic seeds and analysis of samples. Special thanks to **Dr. Amritpal Singh Satyamaan** (retd. Professor, Department of Mathematics, Statistics and Physics, **Punjab Agricultural University**) for his help and guidance in statistical analysis and interpretation of the data obtained.

Lastly but above all, I bow my head in gratitude to **WAHEGURU** with whose grace and blessings I have been able to infuse stability, dedication, management, patience and sincerity into my work throughout the project.

Well Equipped laboratory assistance and opportunity to carry out this research work provided by **Lovely Professional University** is gratefully acknowledged.

(Jagdev Singh)

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PICTURES

LIST OF ANNEXURES

LIST OF ABBREVIATIONS

As	-	Arsenic
BAF	-	Bio accumulation factor
BOD	-	Biological oxygen demand
BTT	-	Bioaccumulation, persistence, toxicity
BW	-	Borewell water
Cd	-	Cadmium
Cr	-	Chromium
COD	-	Chemical oxygen demand
Cu	-	Copper
EC	-	Electrical conductivity
EF	-	Enrichment factor
EU	-	European union
FAO	-	Food and agriculture organization
fw	-	Fresh weight
H_2O_2	-	Hydrogen peroxide
HRI	-	Health risk index
ICID	-	Commission on irrigation and drainage
Fe	-	Iron
Mn	-	Manganese
Ni	-	Nickel
NO^-	-	Nitric oxide
O_2.	-	Singlet oxygen
OH^+	-	Hydroxal ion
$ONOO^-$	-	Peroxinitrite
Pb	-	Lead
pH	-	Hydrogen ion concentration
ROS	-	Reactive oxygen species
-SH	-	Sulphahydral group
TDS	-	Total dissolved solids
TSS	-	Total soluble solids
WHO	-	World Health Organization
WW	-	Waste water
Zn	-	Zinc

1.0 INTRODUCTION

This chapter presents a brief account of importance of vegetables, advantages and disadvantages of waste water irrigation, bioaccumulation of heavy metals, biochemical changes induced and probable health hazards linked with ingestion of vegetables cultivated at heavy metal contaminated sites

Foods provide nourishment and energy which is the basic requirement of all the living organisms for growth and development. Green plants which are cultivated and used as food are known as food crops. The chief food crops can be divided into three main groups i.e. cereals, vegetables and fruits. Those herbaceous plants, the vegetative edible portions (root, stem, leaf or fruit) of which are consumed raw, cooked or preserved in different ways are commonly referred to as vegetables (Encyclopedia Britannica, 1969).Vegetables are known to be the powerhouses of nutrition. Nutrition is a step-wise set of coherent metabolic processes by which the food is ingested, digested and converted into energy required for structural and functional development of the body. Low vegetable intake makes the diet unbalanced which is the cause of many chronic diseases and deficiency disorders. Although the exact mechanism by which the vegetable consumption reduces the risk of diseases has yet not fully been explored but still they are considered as low cost medicines for curing a wide range of dangerous diseases. Nowadays, all physicians, nutritionists, dieticians and fitness trainers strongly recommend increasing the amount of raw vegetables in daily diet to stay fit. Vegetables are easily available and most affordable source of phytonutrients like vitamins, minerals, antioxidants, dietary fiber, carotenoids, flavonoids and some other phenolic compounds which are essential components of various metabolic activities leading to production of energy required for body activities and hence considered an important part of healthy and balanced diet.

Vegetables apart from botanical classification are classified into different groups based on culture, life cycle, plant part used or the family to which they belong. They are broadly classified into leafy, root, salad, tuberous, cucurbits and solanaceous vegetables. Leafy vegetables are direct seeded crops in which leaves are the edible portions, for example, spinach, coriander and *Amaranthus* etc. These are rich in fiber, antioxidants, vitamin A, Zn, thiamine and deficient in cholesterol and fatty acids

(Bhat et al., 2014). Leafy green vegetables keep the body hydrated as they contain more water which contributes towards healthy hair and skin (Settaluri et al., 2015). Lettuce, celery, parsley, chicory etc. are known as salad vegetables and are an adequate source of carotenoids, flavonoids, antioxidants and folic acid (Meyer et al., 2006; Dias, 2012). Radish, carrot, turnip etc. belong to the group of root vegetables and are a rich source of water and minerals. Carrots provide a special combination of three important flavonoids (quercetin, muteolin and kempferal) which are known to mitigate many dangerous diseases (Simon and Goldman, 2007). Cucurbits include cucumber, melon gourd, and pumpkin which are enriched with tocopherols, carotenoids and vitamin C (Dhillon et al., 2012). Tomato, chillies, bell peppers and egg plants belong to the group solanaceous vegetables. Tomato is the richest source of phytochemicals like carotene, lycopene, phytolene and neurosporene. Eggplant and bell peppers are an excellent source of carotenoids, flavonoids, and phenolic compounds especially chlorogenic acid, caffeic acid, flavonoid and niacin (Dias, 2012). Different type of beans like french beans, clustered beans, broad beans and cowpea are rich in dietary fiber and isoflavonoids which aid in smooth working of digestive system (Trinidad et al., 2010 and Misra, 2012). Tuberous vegetables include potato, sweet potato and *Colocasia etc.* are a good source of amino acids and carbohydrates (Dias, 2012). Okra is an independent group of vegetables rich in calcium, phosphorous, potassium, sodium, zinc, vitamin A, B_5, B_6, B_9 and amino acids (Amano, 2018). The presence of these nutritive constituents have a positive influence on human health as they help in reducing the risk of life threatening diseases like cancer, muscular dystrophy and meloblastic anemia (Herrera et al., 2009). Almost all the vegetables are quit low in sugars and fats, which is why increasing their amount in daily diet is important for weight loss, decrease cholesterol and sugar levels in the body, which reduces the risk of fatal diseases like heart failure and diabetes. Gluten-free *Brassica oleracea* (cauliflower) has recently been found to be a very good alternative diet for celiac people (Thomas R, 2016). Stuffed raw vegetables are the only nutritious component in the junk and filler fast foods like pizza and burgers which are very popular amongst today’s generation. To prevent ever increasing risk of chronic diseases especially diabetes, cardiac arrest and cancer amongst the population in developing countries, the World Health Organization (WHO) specifies including at least 400 g of vegetables in daily diet (WHO, 2003; WHO, 2015). Majority of people are deprived of balanced diet across the globe and there is a large gap between the recommended and actual intake of vegetables, especially in developing Asian and African countries. Nutrients and health benefits associated with them are represented in a tabulated form below:

Table 1.1: Impact of non-nutritive and nutritive components of vegetables on human health (Kader et al., 2001)

Components	Vegetables	Health benefits
Vitamins, Minerals and Fiber		
Retinol	Tomato, nectarine, cantaloupe, carrots, dark green vegetables,	Prevents nyctalopia and cataract
Ascorbic acid	Leafy vegetables	Helps in prevention of stroke, scurvy and strengthening of immune system
Tocopherol	Spinach, coriander and pea	Healthy hair and skin
Phytomenadione	Leafy greens, green onions, cabbage	Reduces the risk of diabetes, cancer and heart disease
Vitamin B complex	Lentils, mustard, broccoli, solanaceous vegetables	Prevents premature births and cardiac failure
Iron (Fe)	Leafy vegetables (spinach)	Prevents anemia, osteoporosis and helps in controlling blood pressure
Calcium (Ca)	Rutabaga, cauliflower, pumpkin, raisins, cooked vegetables	Strengthening of bones and teeth, reduction of osteoporosis and maintenance of blood pressure
Magnesium (Mg)	Okra, spinach, lentils	Helps in proper functioning of nervous and immune system
Potassium (K)	Squash, cantaloupe, cooked greens	Boosts immune system, controls hypertension and prevents arteriosclerosis

Fiber	All root, leafy and fruit vegetables	Proper functioning of digestive system
Phenolic compounds		
Proanthocyanin-anthocyaninins	Root and solanaceous vegetables	Prevents cancer, heart disease,, cataract, controls hypertension
Flavan-3-ols	Leafy vegetables, melon gourd	Averts platelet aggregation and cancer
Flavanones	Cucurbits, brassica	Reduces the risk of cancer
Flavones	Carrots, tomatoes	Mitigates cancer, allergies and heart disease
Carotenoids		
Lycopene	Tomato, spinach, lettuce,	Prevents tumour growth, male infertility, cancer and heart disease
α carotene	Broccoli, cabbage, carrots	
β carotene	Potato, leafy greens, swiss chard, broccoli, cantaloupe	Reduces the risk of tumour
Monoterpenes sulfur compounds	Garlic, onions, mustard greens, broccoli	Controls cancer, cholesterol, hypertension and diabetes
Xanthophylls	Summer squash, turnip, sweet corn, spinach, okra, cantaloupe	Prevents muscular degeneration

1.1 Annual production and state wise contribution towards vegetable crops in India

After China, India ranks second in vegetable production in the world. India has witnessed voluminous increase in vegetable production and consumption over the last few years i.e. from 58.5 million tons in 1991-1992 to 175 million tons in 2016 - 2017 The vegetable crops have shown a significant contribution of 60% in annual horticultural crop production over the last 5 years. The area under vegetable cultivation in India has also increased many folds and now it is estimated to be 10.3

million hectares (Horticultural statistics at a glance, 2018). Population explosion, education, nutritional and health awareness among the people are main reasons of day -by-day increase in demand, consumption and production of vegetables. Bihar, Uttarakhand, Madhya Pradesh and West Bengal are the major contributing states towards total vegetable production in India. The following figures represent overall and statewise production of vegetables in India.

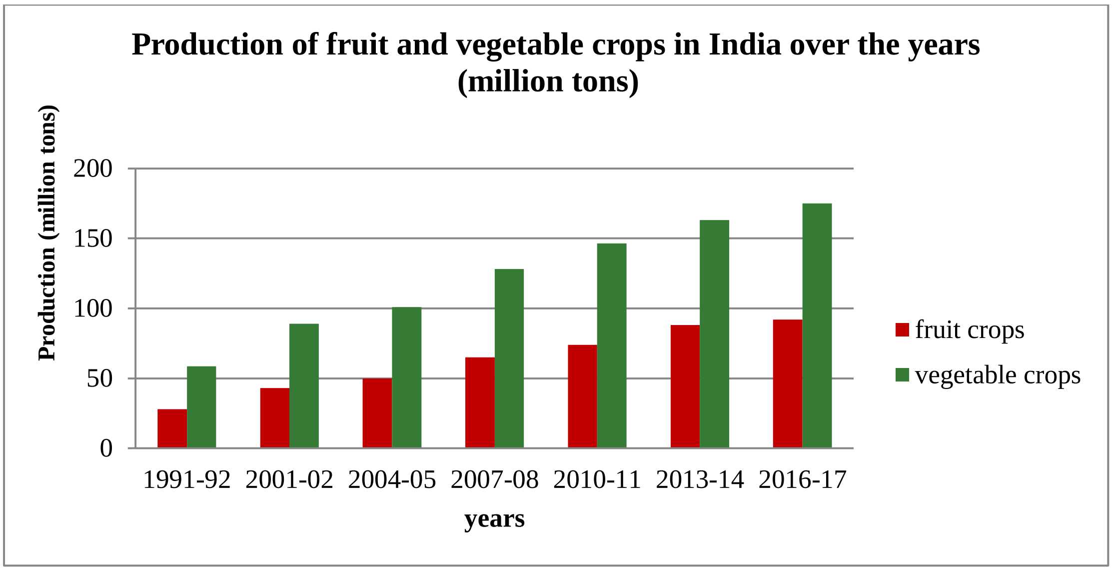


Fig 1.1: Production of fruit and vegetable crops in India over the years /million tons (Horticultural statistics at a glance, 2018)

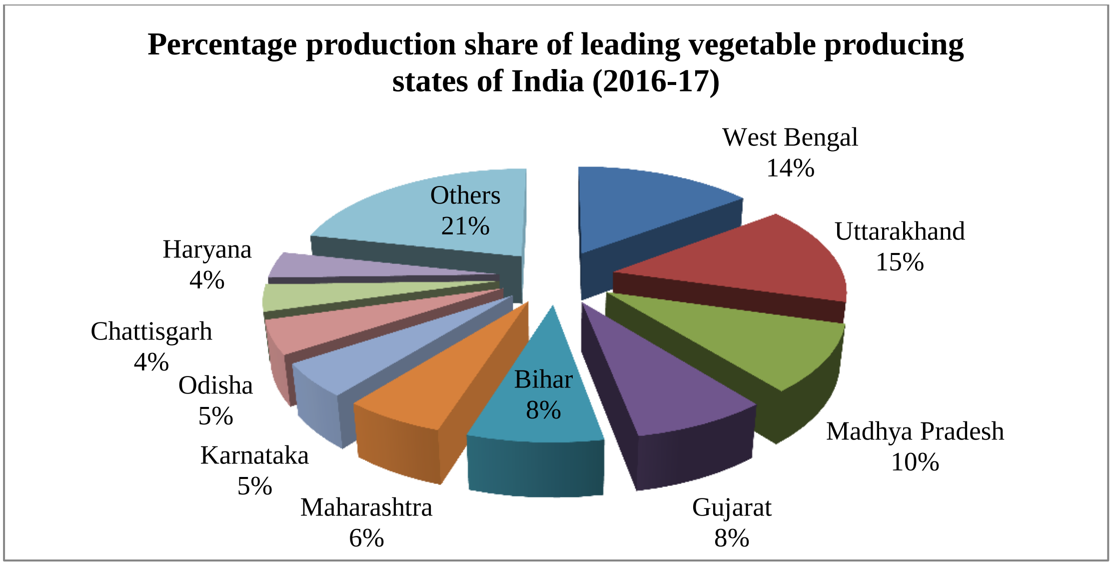


Fig 1.2: State wise production of vegetables in India (Horticultural statistics at a glance, 2018).

1.2 Importance of waste water irrigation

Irrigation is the most important and basic aspect of agricultural practices. Generally fresh water from various sources like rain, streams, rivers, canals and ground water is used for watering the crops in rural areas but waste water is also extensively used for agricultural practices by marginal farmers in the peri-urban areas worldwide. Keeping in view the fast depleting water resources, the reuse of waste water agricultural irrigation is the need of the hour in order to compensate for other water sources for drinking purpose. Decrease in pressure on fresh water resources especially in areas experiencing water shortage is one of the most recognized benefits of waste water reuse of agriculture and its contribution towards food safety (Corcoran et al., 2010). Avoided cost of extracting ground water is another major benefit of waste water reuse for irrigation resulting in reducing the energy required to pump ground water which accounts about 65% of the total cost of irrigation activities (Cruz, 2009). The natural richness of micro and macronutrients in waste water relieves the farmers of heavy expenses incurred on expensive chemical fertilizers (Dreshsel et al., 2010 and Winpenny et al., 2013) and it has proven to increase crop yield (Moscoso, 2017). Waste water reuse also helps in preserving ground water reservoirs needed for drinking purpose because it helps in recharging these sources with high quality water (Moscoso and Egocheaga, 2002). Use of waste water for irrigation may provide economic benefits to the marginal farmers engaged in agricultural practices along the banks of such waste water carrying nullahs but presence of large amount of biological pathogens and toxic chemical pollutants like heavy metals renders long term application of this practice harmful for human health. Waste water irrigation enriches the soil with toxic heavy metals which then get transferred to the edible crops (especially vegetables) grown in such polluted areas. Heavy metals are exceedingly persistent and non-biodegradable so their accumulation in edible crops creates serious health problems among the consumers. The advantages as well as potential risks associated with waste water irrigation are listed below:

Table 1.2: Advantages, disadvantages and probable health risks associated with waste water reuse for irrigation (Kretschmer et al., 2002)

Advantages	Disadvantages	Risks
• Decrease in pressure on fresh water resources • Avoided cost of extracting ground water for irrigation • Savings on chemical fertilizers expenses to be realized • Improved crop yield • Maintenance of natural nutrient cycle • Ensures food safety • Prevention of pollution of fresh water resources • Potential source of macro and micro nutrients • Reduction in eutrophication of water bodies • Availability throughout the year • Boon for marginal farmers	• Biological pathogens- 1. Bacteria 2. Helminthes 3. Protozoans 4. Schistosome • Chemical pollutants- 1. Heavy metals 2. Hydrocarbons 3. Pesticides • Physiochemical parameters of soil- 1. Change in pH 2. Sodification 3. Nitrification 4. Bioaccumulation of heavy metals	• Probable risk to human health by pathogenic microorganisms • Probable harm to human health by heavy metals entry into food crops • Probable physiochemical alteration in soil due to heavy metal and salt accumulation • Potential harm to the agricultural crops

1.3 Universal scenario of waste water reuse for irrigation

Approximately 20 million hectares of land worldwide is irrigated by waste or partially treated waste water by 200 million farmers of which the majority belongs to developing Asian countries (Hussain et al., 2001 and Qadir et al., 2007). Ensink et al (2004) estimated that 32,500 hectares of agricultural land in Vietnam, China, Mexico and 30,600 hectares of agricultural land in Pakistan was irrigated by waste water.

Only 10 % of the total 280 million cubic meters waste water produced in Ghana is used for irrigation of 4600 hectares of agricultural crops (Agodzo et al., 2003). In Mexico about 5 lakh hectares of land is alone under waste water irrigation in (Scott et al., 2000). Not only the developing but even developed countries of Europe, Australia, and America were reported to use the waste water for irrigation of edible crops (Marsalek et al., 2002). Substantial use has been documented in countries like Israel, Kuwait and Saudi Arabia who are facing water scarcity but some high rainfall countries like Japan with annual precipitation of 1714 mm are also indulged in this practice. Waste water reuse is increasingly becoming an important aspect of water resource planning worldwide. Globally the estimated area under waste water irrigation is increasing in an accelerated way. Only 94 million hectares of land was irrigated using waste water in 1950s which increased to 299 million hectares in 2010 according to the reports by International Commission on Irrigation and Drainage (ICID, 1950). Diagrammatic representation of the area under waste water irrigation in different countries is given below:

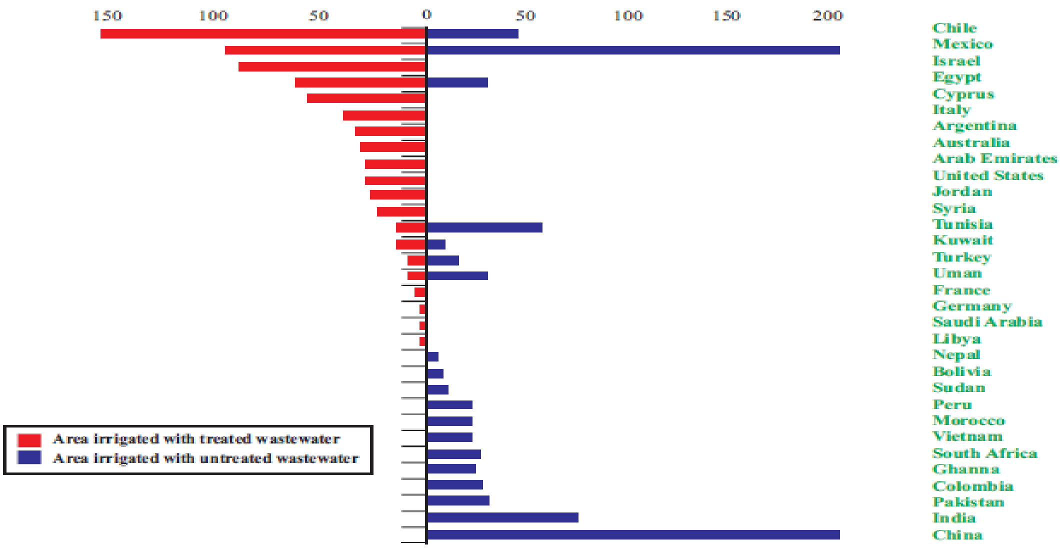


Fig 1.3: Country wise area under waste water irrigation in agriculture (thousand ha); Source: Jimenez and Asano (2008)

1.4 Indian scenario of waste water reuse for irrigation

In urban areas of India, about 5 million litres of waste water was generated per day in 1947 which increased to an estimated 30 billion litres per day in 1997 (Winrock

International India, 2007) and is still increasing day by day. An estimated 73,000 hectares of agricultural land used to be irrigated with waste water in India in early 90s (Strauss and Blumenthal, 1990). Approximately 40,000 hectares of land is irrigated by waste water along the banks of Musi River which collects the sewage and industrial disposal of Hyderabad city in Andhra Pradesh (Buechler and Mekala, 2003). According to Sawhney (2004) the pollution control board has set the standards for industrial effluents but not for sewage waste water even though it contributes towards 90% of total waste water volume in India. The untreated waste water produced from major cities like Ahmedabad, Bangalore, Hyderabad, Kolkata, Mumbai and New Delhi was directly used for irrigation of vegetable, cereal, flower and fodder crops (Winrock International India, 2007). Along the banks of Musi River in Hyderabad, about 21,000 hectares of paddy crop and 1000 hectares of vegetable crops were irrigated with mixed waste and fresh water (Mekala, 2006). Thousands acres of wheat crop was extensively irrigated with waste water around Ahmedabad and Kanpur cities of India (Winrock International India, 2007). Approximately 12000 farmers were reported to irrigate 1700 hectares of land to grow different types of vegetables throughout the year in areas around Keshopur, New Delhi (Winrock International India, 2007).

1.5 Suitability of waste water for irrigation

Despite of the fact that reuse of waste water for crop irrigation is a medieval practice and has evolved with the history of mankind but it has never met the methods and quality standards of reuse. Physiochemical parameters like pH, biochemical oxygen demand, chemical oxygen demand and presence of some specific salts (dissolved and suspended), ions of various elements and toxic pollutants like arsenic, cadmium, chromium and lead etc. determine the suitability of water for irrigation. Higher amounts of such components in the water make it unsuitable for irrigation. Soil and vegetable crops irrigated with such water get enriched with higher amounts of these toxic components which prove to be fatal for soil, plant and human health. Waste water generally carries significantly higher amounts of these pollutants especially metalloids and toxic heavy metals viz. As, Cd, Cr and Pb which get bio-accumulated in the edible crops raised along the banks of such waste water carrying nullahs.

Unplanned industrialization, anthropogenic activities and increase in population are the reasons behind increased production of pollutant - rich waste water and their entry into food chain and environment (Guadarrama-Brito and Fernandes, 2015). According to Marschner (2012) only few representatives of heavy metal group like Cr^{3+}, Cu, Fe, Mn, Mg and Zn are considered essential as they play an important part in certain metabolic activities in plants and animals but most of the heavy metals and some metalloids like Cd, Cr, Pb and As are grouped as non-essential because they have no part to play in any of the metabolic activities of living organisms. Gall et al (2015) reported these metals to be of deleterious nature and extremely harmful to the living organisms. Agency for toxic substances and disease registry has included these toxic heavy metals amongst 20 most perilous substances on this earth (Khalid et al., 2017). Heavy metals are very toxic and hazardous pollutants because of their three characteristics: bioaccumulation, persistence, and toxicity (BPT).Waste water rich in such toxic heavy metals is rendered unfit for irrigation of edible crops like vegetables.

1.6 Effect of waste water irrigation on soil and vegetables

Continuous and long term irrigation has a drastic effect on texture, concentration, composition and stability of organic matter in the soil (Levy et al., 2014). It also increases the amount of nitrogen and phosphate which helps in increasing the crop yield but relatively large amounts have a negative effect on growth and development of the plant and also affect the biodegradation of organic compounds (Oke, 1966 and Ramirez et al., 2012). Richness of waste water in soluble salts induces soil salinization which acts as a limiting factor in the absorption capacity of other nutrients by plants (Baccaro, 2006). Soil degradation is further increased by the addition of toxic heavy metals such as As, Cr, Cd, and Pb which are abundant in the waste water used for irrigation. The soil enriched with these toxic heavy metals can limit fertility and phytotoxic potential with a consequent effect on plant growth and development (Becerra et al., 2015). Other physiochemical parameters of soil such as texture, pH, and electrical conductivity also play an important role in accumulation and bioavailability of these heavy metals (Bixio and Wintgens, 2006).

Due to their short life cycle, perishable nature, nearby markets and higher prices, the marginal farmers prefer to grow vegetable crops in vicinity of metropolitan cities along the banks of waste water nullahs. Vegetables raised in such soils uptake and bio-accumulate large amounts of heavy metals in their edible portions which can adversely affect the human health. Uptake, mobility and accumulation ability of these heavy metals widely differs in vegetable species even among varieties and cultivators of the same species (Säumel et al., 2012). The amount of Cd accumulation in vegetable species was found to be in the following descending order: solanaceous > root > melon > legume vegetables (Zhou et al., 2016). Similarly the leafy vegetables are reported to bioaccumulate maximum amounts of these toxic heavy metals as compared to fruit vegetables (Chetan and Patel, 2015 and Zhou et al., 2016). Heavy metals badly affect the growth and development of plants as well as the health of consumers by altering the structure and biochemical activities at cellular level (Jaishankar et al., 2014).

1.7 Mechanism of action of heavy metals at cellular level

Disruption of metabolic functions in living organisms by heavy metals is done in two ways, firstly by their bioaccumulation in the cell vacuole and secondly, by displacement of the essential nutrients from their original place thereby hindering the biochemical activities. Although the exact mechanism by which the heavy metals act is still uncertain but they are known to be responsible for elevated levels of reactive oxygen species (ROS) like OH^-, $O_2^{\cdot -}$, $^1O_2$, $RO^\cdot$, $ROO^\cdot$, LOOH and H_2O_2 in the cell thus inducing oxidative stress. They react with sulfhydryl (-SH) enzyme system involved in cellular energy production and their subsequent inhibition (Csuros and Csuros, 2002; Nimse and Pal, 2015). They also react with glutathione replacing H atoms from -SH groups onto adjacent glutathione molecules which deactivate them for further reactions. To cope up with the oxidative stress induced, the plant starts synthesizing higher amounts of secondary metabolites and show an increased activity of antioxidant enzymes. Induction of oxidative stress and production of antioxidants at cellular level is represented in the figure given below:

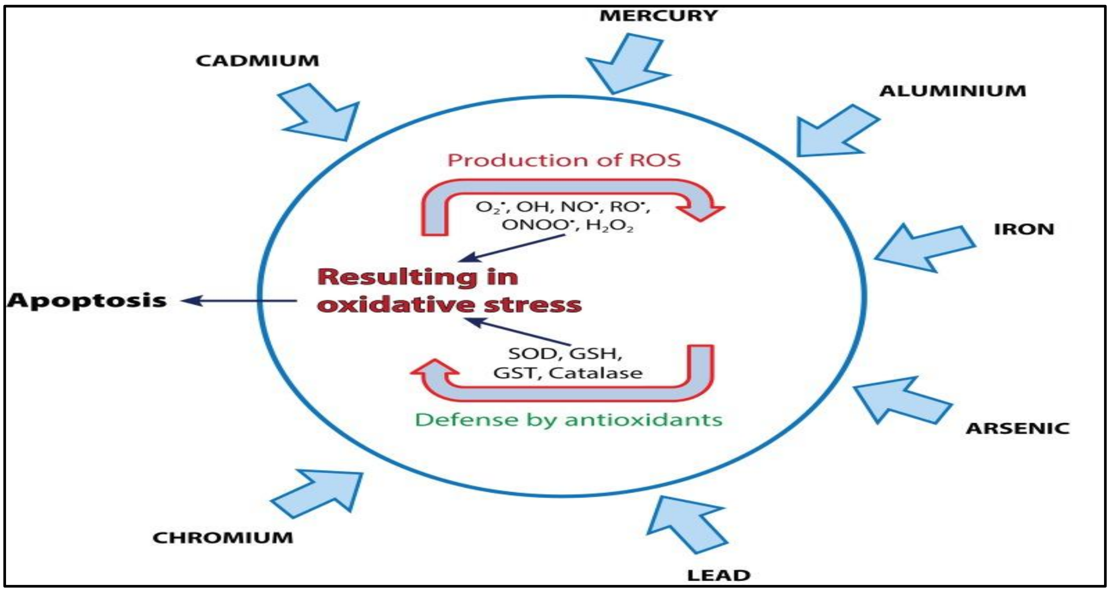


Fig 1.4: Induction of oxidative stress by heavy metals and antioxidants defence mechanism at cellular level (Jaishankar et al., 2014)

1.8 Effect of waste water irrigation on biochemical parameters of vegetables

Although some of the heavy metals (Cu, Zn, Ni, Mg and Mn) are essential for cellular metabolism and act as a cofactor for many enzymes but many heavy metals have been reported to be toxic in nature and induce oxidative stress in plants and subsequent alteration in their physiological and biochemical metabolism leading to an adverse effect on their growth and development. A remarkable increase in some secondary metabolites like proline, phenols, ascorbic acid, carotenoids, flavonoids and total soluble sugars has been reported in vegetables under heavy metal stress (Damera et al., 2015; Emamverdian et al., 2015 ; Zafar et al., 2016). Increase in the amount of these compounds suggests that they are playing an important role in protection of plant against heavy metal induced oxidative stress acting as antioxidants (Guo et al., 2005). The activity of various antioxidant enzymes has also been reported to be increased in vegetables irrigated with waste water as a defence mechanism against oxidative stress induced by heavy metal uptake (Kachout et al., 2009). Similarly the amount of the photosynthetic pigments (chlorophyll a, chlorophyll b), proteins and total carbohydrates was found to be decreased in vegetables irrigated with heavy - metal - rich waste water (Dubey and Singh, 1999). Oxidative stress induced by heavy metals and metalloids is the main cause of growth reduction in plants (Supalkova et al., 2007). Various non-essential heavy metals are known to disrupt the process of photosynthesis, seed germination and nitrogen fixation in plants by altering the activity of the enzymes like carboxylase and nitrate reductase (Najeeb et al., 2011). Waste water irrigation has been found to increase the enrichment factor (EF) and bioaccumulation factor (BAF) of heavy metals in vegetable crops (Gupta et al., 2010). The toxicity symptoms induced in vegetables irrigated by waste water are represented in the table given below:

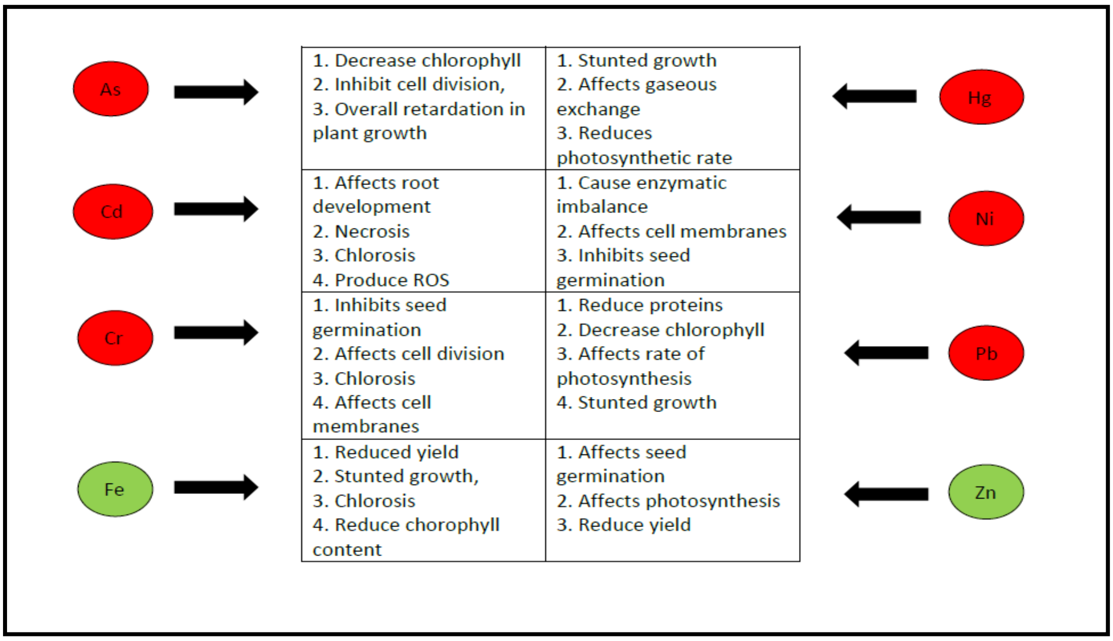


Fig 1.5: Toxicity symptoms of some heavy metals in plants (Kumar and Aery, 2016)

1.9 Effect of waste-water-irrigated vegetables on human health

Minamata and Itai-itai diseases in Japan which were caused by release of untreated industrial waste water rich in mercury and cadmium in water channels and their subsequent entry into human system are important examples of how the entry of toxic metals in the food chain affects the human health. Serious human health issues like malnutrition, mental retardation, gastrointestinal problems and weak immune mechanism have been directly related to ingestion of heavy metal contaminated vegetables (Turkadogan et al., 2003; Gress et al., 2015 ; El-Kadi et al., 2018). Certain heavy metals like lead and cadmium are supposed to be the cause of intra uterine growth retardation (Rai, 2018a). Lead contaminated vegetables have been reported to be the main cause of neurological and cardiovascular problems among children (Trichopolous, 1997; Al-saleh et al., 2017). Lead and cadmium contamination adversely affect human health and also cause other complications like hypertension, lung cancer, dysfunction of renal and nervous system (Zhou et al., 2016). Excessive levels of As in vegetables is found to be the main cause of dermal problems, cancer and respiratory complications (Lin et al., 2013; Hu et al., 2013 ; Islam et al., 2017). Chromium exists in different ionic forms of which Cr^{6+} is most dangerous among all other ionic forms and is suspected to be the main cause of lung cancer (Park et al., 2004). High level of cadmium contamination causes post-menopausal breast cancer (Hiroaki et al., 2014). Obiora et al (2016) reported that consumption of lead and manganese contaminated vegetables causes Dementia and Alzheimer among the regular consumers. Cui et al (2005) surveyed and reported that people who ingested metal contaminated vegetables were found to be suffering more from a serious renal dysfunction than the people consuming non contaminated ones. Different indices including bioaccumulation factor (TF), daily intake of metals (DIM) and health risk index (HRI) are used to determine the level of health risk associated by consumption of heavy - metal - rich waste water. Therefore various national and international organizations like World Health Organization (WHO) have set safe limits for the heavy metals in soil and food crops (Chary et al., 2008 and Qishlaqi et al., 2008). Major source and critical health risks associated with heavy metals has been represented in the following table:

Table 1.3: Important non-essential heavy metals/metalloids and health implications

Heavy metals/ metalloids	Major Source	Medium/ route of exposure	Permissible levels (mg/l)	Critical health risks
As	Fossil fuels, pesticides, smelting, industrial waste water	Contaminated food	0.02	Melanosis, encephalopathy, depression, hyperkeratosis, Bronchitis, hepatomegaly

Cd	Pesticide, refining, welding, plastic, industrial waste water	Contaminated water and food stuffs	0.06	Itai-itai, kidney damage, cancer, hypertension, and weight loss
Cr	Dye industry, sewage waste water, electroplating	Food crops	-	Renal dysfunction, hemolysis, pulmonary fibrosis and lung cancer
Pb	Paint, mining, batteries	Food crops	0.1	Mental retardation, anemia, anorexia malaise, gastrointestinal damage and brain damage

1.10 Nature and genesis of research problem

Ludhiana is the largest, prosperous, affluent and most polluted city of Punjab. Being one of the most industrialized towns of north India, is also referred to as the Manchester of India. Thousands of large, medium and small scale industries in the field of pesticides, distilleries, pharmaceuticals, woollen, cotton, synthetic yarn, steel, auto parts, rubber, dyeing and cycle manufacturing are operational in Ludhiana. Urbanization and industrialization have caused a negative impact on the environment in terms of degradation and pollution of Buddha Nullah which is a seasonal water stream that passes through this thickly populated city and is a carrier of sewage and industrial effluents. Total discharge of nutrient rich sewage waste water in Buddha Nullah is estimated to be 500 million litres per day. Furthermore about 16 million litres of untreated industrial effluents laden with toxic heavy metals, cyanide, harmful pesticides and toxic organic compounds is also thrown directly into the Buddha Nullah on a daily basis.

The pollution level of Buddha Nullah and its surroundings have reached such a dangerous point that several residents, Pollution Control Board and National Green

Tribunal filed common writ petition in Punjab and Haryana High Court to make a comprehensive plan and strategy to make Buddha Nullah, Ludhiana, free of negative impacts of unplanned and rapid industrial growth. The court has directed the Punjab government to actively monitor the protection and preservation of not only Buddha Nullah but also the health issues faced by residents of Ludhiana . Recently a project of 650 crores has been approved by the state government for rejuvenation of highly polluted Buddha Nullah (ENS, 2020)

There is evidence of progressive biological and chemical pollution of soil on both banks of Buddha Nullah up to 200 meters. An extensive survey conducted revealed that about 85% farmers cultivate only vegetable crops in fields along the banks of this nullah and frequently use its nutrient and heavy - metal - rich waste water for irrigation of these vegetable crops. Vegetables grown in these fields are consumed by large number of local residents which can pose serious health problems to the consumers.

Review of literature indicates bioaccumulation of heavy metals in vegetables irrigated both with municipal waste water and effluent - rich industrial waste water. Buddha Nullah receives significantly higher amounts of nutrient rich sewage and heavy - metal - rich industrial waste water in almost equal amounts. So four vegetables belonging to different group i.e. *Raphanus sativus* (root), *Spinacia oleracea* (leafy), *Brassica oleracea* (flower) and *Lycopersicum esculentum* (fruit) were selected to determine the bio-accumulation of four non-essential toxic metals i.e. Arsenic (As) Cadmium (Cd),Chromium (Cr), and Lead (Pb) irrigated with waste water of Buddha Nullah and to compare with the bioaccumulation of same heavy metals in the same vegetables grown away from Buddha Nullah and irrigated with borewell water. Bioaccumulation level of heavy metals in vegetables grown on both the sites shall also be compared with the permissible levels to assess their suitability for human consumption and their effect on qualitative and quantitative production of these vegetables. This analysis will provide useful information about principle sources, distribution and fate of these heavy metals (As, Cd, Cr and Pb) in the environment and their bioaccumulation in food chain, fresh food supply challenges and approaches to eliminate or reduce the level of contamination. On the basis of bioaccumulation of heavy metals in the vegetables studied, the outcome of present research work will also help to make recommendations and suggestions to the authorities and farmers regarding types of vegetables to be raised and not to be raised on such contaminated sites and the health risk associated with the consumption of these vegetables. From review of literature, the following research gap was observed in the previous studies:

1.11 Research gap

There is no baseline data available for vegetable contamination due to poor quality irrigation/waste water irrigation available in our country. This research focuses to fill the gap available in such area and the toxicological levels of heavy metals when applied to human health through vegetables.

1.12 Research hypothesis

1) Large number of vegetables growing in agricultural lands are irrigated by waste water, i.e. water from sewage and industry. This water contains pollutants and heavy metals much above the permissible limit. These pollutants could be absorbed by growing vegetables and accumulate in their tissues and ultimately be passed on to the human body which could have profound effect on health and cause many diseases and disorders in human population.

2) By analyzing the samples of all the vegetables for the amount of heavy metals absorbed and the effect of these heavy metals on various biochemical contents like chlorophyll, proteins, sugars, starch etc., of these vegetables, this research will study the vegetables which absorb the minimum amounts (permissible limits) of heavy metals and are recommended to be grown in peri-urban areas where mode of irrigation is only the waste water. This will provide an understanding of the reuse of waste water in a better way and will also be beneficial for both the producers (marginal farmers) as well as the consumers (human population).

Null hypothesis: It was assumed that waste water and borewell water irrigation have the same effect on bioaccumulation of heavy metals and biochemical changes induced in the vegetables.

1.13 Brief objectives of the research

1. Survey and collection of vegetable samples from contaminated cropping area of Buddha Nullah (Ludhiana) and control area irrigated by borewell water.

2. Analysis of toxic metals (As, Cd, Cr and Pb) absorbed by the vegetables.

3. Analysis of physiochemical parameters of waste water (COD, BOD, nitrate, phosphate and heavy metal contents).

4. Analysis of metabolic parameters like chlorophyll, other pigments, starch, sugars and proteins of vegetable plant parts.

5. Comparative analysis of contamination of heavy metals in different type of vegetables (root, leafy, flower and fruit) for all the parameters.

2.0 REVIEW OF LITERATURE

This chapter deals with a brief account of scientific thoughts and the findings of previous researchers in their studies related to the present research work.

2.1 Implications of waste water irrigation

The waste water reuses have both beneficial and harmful implications. The beneficial implications include food security, employment opportunities, natural recycling of nutrients and reliable supply of water for irrigation. Hussain et al (2001) suggested that waste water can have an impact on property values as the waste-water-irrigated land in Haroonabaad district of Pakistan has a higher value than the land irrigated with canal or borewell water because of free availability of nutrient rich waste water throughout the crop season. According to Shahid et al (2015) long term and continuous use of waste water for irrigation resulted in reduction of crop productivity. On the other hand, Khalid et al (2017) reported that the long term practice of waste water for irrigation tends to increases the accumulation of harmful pollutants in the soil which change the soil structure, crop production and human health. Waste water also contains microbial pathogens, eggs and cysts of worms, chemical pollutants like heavy metals and metalloids which pose serious risks to human health and environment though their entry into the food chain. Reuse of waste water for irrigation purposes is a common practice worldwide (Ensink, 2004: Jaramillo, 2017 ; Li et al., 2017)

2.2 Effect of industrial effluents and sewage on physiochemical properties of waste water

Kansal (1994) conducted a comparative study of different industrial effluents and observed the content of heavy metals in effluents of different industries in the following order: sugar mill effluents > tannery effluents > electroplating industry effluents. Brar et al (2002) reported that sewage water increases the heavy metal content of industrial waste water. Amount of heavy metal arsenic, zinc, copper, aluminum, nickel, manganese, ferric, chromium was found to be increased 4, 31, 42, 50, 52, 98, 150, 1400 times respectively when municipal waste water was mixed with the effluents of a textile mill. Anna and Sridhar (2002) reported that amount of heavy metals in waste water differ remarkably, depending upon the source from which they originated. Khuranaand Singh (2012) analyzed the COD, BOD, total dissolved solids, pH, nitrogen, phosphorous, potassium, Electrical Conductivity, calcium carbonate in waste water of an industrial town Sangrur, Punjab, India and reported a significant increase in all the parameters studied. Akan and Coworkers (2007) also reported that the effluent of different industries differ in amount of the same pollutant. Dheri and others (2007) studied that effluents of different types of industries flushed in sewage drainage further elevate the level of heavy metals. Therefore for environmental protection and sustainable crop production, the use of waste water after proper dilution is a must before its use for irrigation. Gupta et al (2010) studied the physiochemical parameters of waste water used for irrigation of vegetables in the fields of Durgapur, West Bengal and reported a significant increase in EC, TDS, TSS, COD and BOD content in the waste water as compared to that of normal borewell water. Similarly Alghobar et al (2014) analyzed the physiochemical parameters of sewage waste water and reported a significant increase in parameters like pH, COD, BOD, EC, TSS, TDS, and metal ions. In a similar experiment Rolly (2014) studied the physiochemical parameters of sewage, waste water in Karnataka and reported higher levels of EC, total hardness, sulphate and chlorides above the Indian standard limits.

2.3 Heavy metal content in waste water

Dheri et al (2007) analyzed the heavy metal content in sewage waste water and reported a significant increase in the various heavy metals studied in waste water samples as compared to that of normal ground water samples. Gupta et al (2008) essayed the presence of heavy metals (Cd, Cr, Cu and Pb) in the sewage waste water of Titagarh, West Bengal frequently used for irrigation purposes. Similarly Singh et al (2012) reported the presence of elevated amounts of heavy metals used for irrigation in peri-urban areas of Nagpur, India. Chopra and Pathak (2012) analyzed the waste water of Bindal river, Dehradun and reported high concentration of heavy metals. Mahakalkar et al (2013) analyzed the waste water of Nag river and reported higher concentrations of Fe, Mn, Zn and Pb above the permissible levels. Lone et al (2013) in their analysis of sewage water reported a significantly higher amount of Pb and Cd.

2.4 Impact of heavy metal enriched waste water irrigation on physiochemical properties of soil

2.4.1 pH

Review of literature showed that different researchers had different observations regarding continuous and long term application of waste water on soil pH. Hayes et al (1990) reported no significant difference observed in pH of soils treated with waste water and normal borewell water. Osaighovo et al, (2006) attributed increase in soil pH to presence of Ca^{+2} and Mg^{+2} ions. Saravanamoorthy and Kumar (2007) revealed that continuous use of effluent - rich waste water from a textile mill for 55 years increased the surface soil pH by 0.4 units. El-Hady (2007) noted the effect of industrial and domestic waste water in Egypt and reported an increase of 0.5 units in surface soil which was significantly higher as compared to normal soil samples. Morguan et al (2009) confirmed an increase in soil pH with waste water irrigation.

2.4.2 Electrical conductivity

Nawaz et al (2006) in their physiochemical studies of soil samples in Pakistan reported a significant decrease in electrical conductivity of the soil samples continuously irrigated with waste water. Rusan et al (2007) evaluated EC value in soil irrigated for a long time (10 years) with contaminated water and found a significant increase in electrical conductivity. Morugan et al (2009) studied the effect of salt accumulation by sewage waste water irrigation on Electrical Conductivity in calcareous soils of Spain and found a slight increase in electrical conductivity and sodium content.

2.4.3 Soil organic carbon

Yadav et al (2002) analyzed the samples of soil irrigated with domestic sewage water for 36 years and reported a significant increase in soil organic carbon from 1.24 to 1.78%. Gupta and Mitra (2002) reported an increase in soil organic carbon from 0.90 to 0.37% in sewage water irrigation for 52-60 years in soils of Calcutta, India. Therefore, long term waste water is useful for carbon sequestration in soil and an important soil quality sustaining practice. Rattan et al (2005) studied that soil organic carbon, an important indicator of soil quality and reported 38-79% increase in organic carbon content in soil irrigated with waste water as compared to that of normal soil.

2.4.4 Calcium carbonate and soil micro-nutrients

Hayes et al (1990) reported 440 times increase in total nitrogen content in waste-water-irrigated soils. Gupta and Mitra (2002) in their study on soils irrigated with sewage waste water reported an increase in total nitrogen, total phosphorous and total calcium content in the waste-water-irrigated soils as compared to normal soils. El-Arvy and Elbordiny (2006) conducted a similar study in Egypt and found 1.42 % decrease in surface soil calcium carbonate content in soil samples irrigated with waste water. El-Hady (2007) compared the calcium carbonate of surface soil in both waste water and ground water irrigated soils in Egypt and found a considerable increase in calcium carbonate content in soils irrigated with waste water. Russan et al (2007) studied the effect of long term (10 years) municipal waste water irrigation on available nitrogen, phosphorous and potassium accumulation in soil and reported a significant increase in all the parameters.

2.4.5 Heavy metal content

Schirado et al (1986) reported elevated levels of extractable diethlene triamine penta acetic acid, nickel, cobalt and cadmium in 0 to 15 centimeters layer soil irrigated with untreated waste-water-irrigated soil as compared to the lower layers. Yadav et al (2002) reported that soil analyzed after 35 years of regular domestic sewage water irrigation was found to have a significant accumulation of heavy metals in 0-30 cm soil layer. Gupta et al (2012) described that long term use of sewage water in soils of Calcutta, India showed 6.6 fold increase in total ferric, copper, manganese, cadmium, zinc, nickel, lead, cobalt and chromium concentration in comparison to that of normal soil. Rattan et al (2005) reported a significant increase in ferric, copper, cadmium, manganese, nickel, chromium and zinc in surface soil irrigated with contaminated water as compared to that of normal one. The accumulation of heavy metal in surface soil may be the result of adsorption reaction of negatively charged soil colloids for these cationic heavy metals.

Rattan et al (2005) reported that sewage water irrigation for 20 years showed a significant increase in lead-29%, nickel-63%, ferric 170%, copper-170%, zinc 208%

in soil as compared to normal water irrigated ones, but manganese content was found to be depleted by 31%. Lin et al (2008) reported a long term in-situ accumulation of nickel and chromium in soil profile of a large scale effluent recharge after 22 years of waste water usage in coastal plain of Israel.

2.5 Heavy metal bioaccumulation in vegetables irrigated by waste water

Vegetables are the main crops which are extensively grown in urban areas in the fields along the banks of waste water channels and are frequently irrigated with nutrient rich sewage and toxic heavy metal loaded waste water. These toxic heavy metals are absorbed by the roots along with other nutrients and get accumulated in the edible parts of the plant. Nutrients contribute towards better growth of plants but the heavy metals alter the biochemical activities leading to cellular stress. Studies done by of most of the researchers confirm that the bioaccumulation of these metals varies from species to species. Leafy vegetables have been found to be hyper accumulators followed by root vegetables as compared to other all other vegetables (Zhou et al., 2016).

Sharma and Kansal (1986) studied the bioaccumulation of some of toxic and nontoxic heavy metals in samples of leafy vegetables and fodder crops like spinach, coriander and barseem collected from the fields where the main source of irrigation was sewage waste water. The results of the study revealed that these leafy crops showed high accumulation of nontoxic or essential metals like zinc, manganese, copper, iron and toxic metals like cadmium. Spinach was found to be a higher accumulator of all the heavy metals studied, as compared to the other two crops.

Stalikas et al (1997) made a comparative analysis of heavy metal uptake by different types of vegetables irrigated with industrial cum sewage waste water and reported significantly higher amount of cobalt, arsenic, chromium, cadmium, copper, ferric, manganese, lead, nickel, zinc and selenium uptake in spinach as compared to the other vegetables. The samples were collected and analyzed from two different sites of agricultural lands in Greece.

Fytianos et al (2001) analyzed the bioaccumulation of lead, nickel, cobalt, copper, cadmium, manganese and zinc in root and leafy vegetable samples collected from an industrial area in northern Greece and found higher accumulation in leafy vegetables like spinach and lettuce as compared to bioaccumulation of these metals in root vegetables. Both the vegetables, spinach and lettuce, accumulated significantly higher amounts of cadmium as compared to all other heavy metals analyzed.

Alam et al (2003) analyzed the samples of some root and leafy vegetables collected from the fields in Jessor district of Bangladesh where waste water was used for irrigation of these vegetable crops and compared the bioaccumulation of some heavy metals like Cd,Cu, Zn and Pb in both types of vegetable samples collected from waste-water-irrigated sites. The samples collected from the contaminated sites were found to be higher accumulators of heavy metals as compared to the samples collected from the other site where irrigation was done with normal borewell water. Comparative analysis showed a higher accumulation of lead in *Colocasia esculenta* (root vegetable), Zn in *Moringa oleifera* (leafy vegetable) and Cu in other root vegetables analyzed.

Lone and coworkers (2003) in their experiments to find the impact of waste water irrigation on bioaccumulation of toxic elements and micronutrients in vegetables found a significantly higher bioaccumulation of cadmium, lead, nickel, chromium and micronutrients zinc, ferric, copper, manganese in okra (*Abelmoschus esculentus* L.) and spinach (*Spinacia oleracea*). But level of bioaccumulation was different in crops grown in spring and winter season leading to the fact that temperature might be playing an important role in uptake of metals in plants.

Temmerman and Hoenig (2004) analyzed cadmium and lead accumulation in spinach (*Spinacia oleracea*), lettuce (*Beta vulgaris*), *Cichorium endivia*, *Valerianella locusta* and lamb lettuce grown in waste water of a smelter. Leafy vegetable spinach showed maximum accumulation of cadmium whereas lead was found to be maximum in lamb lettuce. It ensures the fact that uptake of different toxic metals differ in different species. Tandi et al (2004) compared the uptake of zinc and copper in *Brassica juncea* and lettuce grown in fields which were irrigated with sewage sludge and effluent - rich in heavy metal content since a long time. The results of the present study revealed that lettuce bio-accumulated seven and three times whereas mustard rape fourteen and two times higher amounts of both the metals respectively as compared to the level of the same metals in samples of same vegetables collected from the control site.

Eriyamremu et al (2005) evaluated the impact of refineries and petrochemical natural gas processing industrial waste water rich in cadmium and lead on different vegetables like *Vernonia amygdalina* (bitter leaf), *Talininium triangular* (Ceylon spinach), *Amaranthus hybridus* (amaranthus) and *Telgaria accidentalis* fluted pumpkin. The maximum uptake of cadmium and lead was reported to be 0.035 and 0.49 mg per kg in *Amaranthus hybridus* and *Telgaria accidentalis* respectively. Both of these showed a higher tendency to accumulate lead and cadmium as compared to that of the other vegetables studied.

Saraswat et al (2005) studied the bioaccumulation of some essential micronutrients in wastewater irrigated root and leafy vegetables and found higher amount of micronutrients (zinc, manganese, iron and copper) in waste-water irrigated vegetables such as radish, cauliflower, broad bean and okra as compared to borewell irrigated water.

Demirezen and Aksoy (2006) analyzed the samples of lettuce, parsley, onion, *Phaseolus esculentum* (bean), eggplant, *Cucurbita pepo* (pumpkin) and okra collected from peri - urban areas of Keyseri (Turkey) irrigated with municipal, domestic and industrial discharges to estimate the amount of cadmium, lead, copper, nickel and zinc uptake by these vegetables. Amount of all the heavy metals except zinc was found to be higher in the vegetables grown in urban areas of Keyseri as compared to the samples collected from the rural area. Kachenko and Singh (2006) conducted a comparative study and analyzed the samples of some leafy vegetables collected from 3 smelter contaminated sites across South Wales, Australia and found the cadmium bioaccumulation to be in the following order: mint > *Spinacia* > lettuce > leak > cabbage. Mint and spinach accumulated cadmium in very high concentrations above the codex alimentarius commission’s limits.

Pandey (2006) studied cobalt, nickel, zinc and copper bioaccumulation in radish and spinach irrigated with waste water contaminated with industrial effluents of an electroplating industry rich in heavy metals. The samples were analyzed after 40 days and a significantly higher uptake of the metals analyzed was recorded in the samples of spinach as compared to that of radish. The results of this study confirmed the leafy vegetables are hyper accumulators of the heavy metals as compared to the other type of vegetables. Singh and Kumar (2006) also conducted a comparative study and analyzed the samples from five different agricultural sites around New Delhi where contaminated water was used for agricultural practices since a long time. The results revealed that the amounts of cadmium, lead and zinc were much higher than the specified limits of WHO in both okra and spinach samples analyzed.

Sharma and co-workers (2007) carried out a field study to detect the seasonal effect on heavy metal bioaccumulation in the plants. The experimental area selected were fields around Varanasi where the use of waste water for irrigation is a common practice among the marginal farmers. Samples of *Beta vulgaris* raised during summer and winter seasons were collected and analyzed for cadmium, zinc, lead, copper, chromium, manganese and nickel uptake. Analysis revealed that concentration of all the metals was much higher in the edible parts of *Beta vulgaris* in both summer and winter seasons.

Rai and Tripathi (2008) also conducted a similar study and vegetable samples were collected from four sites in Lohta village of Varanasi India irrigated with contaminated water received from the discharge of diesel locomotive works. Samples of vegetables turnip, radish and cabbage were found to be carrying high amounts of toxic waste elements in their edible portions as compared to the other vegetables studied.

Gupta and others (2008) monitored the bioaccumulation of toxic elements by the vegetables grown in the fields of Titagarh, West Bengal where waste water was used for irrigation of the vegetable crops. After analysis the concentrations of chromium, cadmium, nickel, zinc and lead were found to be beyond the safe limits in all the vegetable samples analyzed. The estimated amounts of contamination were found to be so high that vegetables were rendered unfit for human consumption. Farooq et al (2008) collected the samples of different vegetables grown around an industrial area in Pakistan and analyzed the presence of lead, copper, chromium, zinc and cadmium in these vegetables samples. The study suggested that these vegetables can be consumed by the residents of the area because the uptake of the toxic metal level was within the permissible limits in spinach, coriander, lettuce, cabbage, cadin and cauliflower grown in that area. A similar study was conducted by Srinivas et al (2009) and the bioaccumulation of lead, nickel, zinc and copper was found to be within specified limits in the vegetable samples collected from an industrial area of Vishakhapatnam.

Gupta et al (2010) conducted a comparative study to estimate the amount of bioaccumulation of different metals in different types of vegetables grown in vicinity of Durgapur city in West Bengal. Vegetables were frequently irrigated by water contaminated with industrial effluents. Analysis revealed maximum uptake of iron followed by lead, manganese and cobalt in decreasing order. Maximum bioaccumulation of above mentioned metals was observed in *Raphanus* and minimum in *Colocasia* roots. Overall maximum amount of cadmium was detected in all the vegetables analyzed. Huong et al (2010) conducted a trial in Vietnam to estimate the impact of contaminated water irrigation and toxic metal uptake by the vegetables and reported the level of chromium, cadmium and lead to be exceeding the Vietnamese standards in all the vegetables studied. Bioaccumulation of lead was found to be much higher as compared to the other metal uptake. Jagtap et al (2010) collected and analyzed the samples of eleven different types of vegetables grown and raised in a contaminated urban area to ascertain the bioaccumulation of toxic trace elements and found that different types of vegetables uptake different amount of toxic metals even when grown in similar environmental conditions. Maximum accumulation of zinc was detected in carrot, nickel in radish, cadmium in spinach and chromium in bitter gourd. Leafy vegetable spinach was found to be hyper accumulator.

Ramesh and Murthy (2012) observed the metal bioaccumulation of heavy metals by the vegetable grown in an area receiving large amounts of industrial effluents in Bangalore, Karnataka, India. The studies revealed that *Beta vulgaris* samples obtained from three out of four sites were detected to have the level of copper, zinc, lead, chromium, and manganese above the permissible levels specified Out of all the heavy metals studied, chromium uptake was found to be much higher (70.70 ppm and 127.27 ppm) in *Beta vulgaris* and *Coriandrum sativum* respectively. Gosh and co-workers (2012) investigated the uptake of heavy metals by some species of root vegetables (radish, potato, carrot, turnip) and observed that out of all the vegetables analyzed, carrots uptook cadmium, chromium, nickel and lead in significantly higher amounts as compared to other root vegetables studied. The bioaccumulation of chromium and nickel was found to be much higher than the other heavy metals in all the vegetables analyzed.

Labhade (2013) collected the samples of vegetables grown in industrial area of Nashik, Maharashtra, India and reported that 35% of the vegetable samples collected from industrial area indicated higher concentration of lead and 25% samples had higher concentration of arsenic. Copper accumulation was detected to be to be below permissible levels. Comparative analysis of accumulation of heavy metals in coriander, spinach, onion, cauliflower, brinjal, tomato, cucumber, potato and carrot revealed that cadmium concentration was analyzed to be much higher than permissible levels in onion, coriander, cauliflower and spinach. The same vegetable samples grown away from the industrial area were found to be less contaminated. Gebrekidan et al (2013) evaluated the bioaccumulation of eight essential and nonessential heavy metals in the vegetables irrigated with waste water richly contaminated with tannery effluents in north Ethiopia and reported the bioaccumulation of metals by onion to be in the following descending: order lead > nickel > chromium > cadmium > cobalt whereas the accumulation of essential heavy metals was much more and in the following descending order ferric > manganese > zinc > copper. No Edible portions of the plant were found to accumulate more amounts of the heavy metals as compared to the other parts of the plant.

Mohammed and Jimoh (2014) studied the amount of heavy metal bioaccumulation by a leafy vegetable (lettuce) grown in farmlands near Kaduna metropolis, Nigeria, where waste water is frequently used for irrigation. It was observed that amount of zinc and iron uptake was significantly higher in lettuce. The bioaccumulation of other metals was observed to be in following descending order: cadmium > copper > lead > ferric > zinc. Parvin et al (2014) analyzed the bioaccumulation of three nonessential and highly toxic metals in five different species of vegetables. The vegetable samples were collected from a highly contaminated industrial area in Chittagong, Bangladesh, and found the levels of chromium and copper accumulation to be within the specified limits but the concentration of lead was found to be very high in leafy vegetables sweet gourd and jute leaves.

Mohod (2015) studied the heavy metal bioaccumulation in spinach and tomatoes grown near Amba Nullah of Amravati city, India and found the concentration of lead and cadmium to be much higher in both the vegetables as compared to the accumulation of zinc and copper which were within the safe limits Choudhary (2015) in their study analyzed the samples of seven vegetables such as potato, red Amaranthus, spinach, carrot, cabbage, tomato and brinjal collected from different waste-water irrigated locations of Pakshi, Bangladesh, and found the bioaccumulation of zinc, ferric, arsenic and lead to be beyond permissible limits in all the vegetables as compared to other heavy metals analyzed.

Roy and Gupta (2016) analyzed the vegetable samples taken from the waste-water irrigated fields near Asansol, West Bengal and reported very high accumulation of cadmium in samples of spinach, cauliflower, pea, carrot and brinjal as compared to borewell irrigated samples. Lead, manganese and zinc was up to permissible levels but chromium and copper in waste-water irrigated samples was observed to be much higher. A high enrichment factor of cadmium was found in spinach, copper in brinjal, manganese in cauliflower and that of manganese and zinc in carrot and pea respectively.

Sharma et al (2016) analyzed the leafy and other vegetable samples grown along Tung Dhab, an industrial-effluent-rich runnel near Amritsar and found the trend of heavy metal bioaccumulation of heavy metals to be in the following order: iron > cobalt > copper > cadmium > lead. Lead concentration was observed to be above the permissible levels in all the samples analyzed. Leafy vegetable spinach showed maximum bioaccumulation of all the metals as compared to fruit and bulb vegetables. Zhou et al (2016) studied twenty two species of six vegetables grown in heavy metal contaminated fields in Suxian district of China and reported that melon vegetables uptake minimum amount and leafy vegetables maximum amount of cadmium, copper, zinc and arsenic. Root and solanaceous vegetables were found to absorb the average amounts of all the heavy metals analyzed.

Table 2.1: Concentrations of heavy metals in vegetable edible parts (mg/kg fw) (Zhou et al., 2016)

Vegetable Type	Vegetable Species	Pb (mg/kg)	Cd (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	As (mg/kg)
Root	White radish	0.270	0.011	0.167	4.690	0.099
	Carrot	0.233	0.023	0.227	1.591	0.188
	Sweet potato	0.613	0.135	0.015	4.674	0.448
Stalk	White caitai	0.785	0.239	0.456	24.23	0.225
	Red caitai	0.939	0.176	0.478	20.95	0.396
Solanaceous	Eggplant	0.429	0.289	0.937	2.786	0.072
	Tomato	0.078	0.028	0.468	1.419	0.014
Melon	Bitter gourd	0.061	0.002	0.505	2.769	0.061
	Cucumber	0.004	0.004	0.284	1.206	0.039
	Pumpkin	0.121	0.005	0.647	2.883	0.073
Leafy and Legume	Cabbage	0.671	0.036	0.314	9.926	0.211
	Spinach	0.971	0.513	0.966	20.81	0.310
	Lettuce	1.162	0.460	0.775	11.79	0.660
	Kidney bean	0.033	0.010	1.310	5.669	0.050
Tolerance limits		0.383	0.161	0.810	10.159	0.207

Kumari et al (2016) collected and analyzed the samples of cauliflower, spinach and tomato grown along the banks and irrigated with the waste water of Buddha Nullah and found the bioaccumulation of chromium, zinc, copper, cadmium, nickel and lead in all the vegetable samples collected from Buddha Nullah site significantly higher as compared to the samples collected from a control site away from Buddha Nullah. Lead accumulation was noted to be maximum and that of cadmium to be minimum. Leafy vegetable spinach accumulated maximum amount of almost all the heavy metals studied.

Rao et al (2017) evaluated the bioaccumulation of toxic metals in tomato and chillies grown in an industrial area of Jagdelpur, Chittagong, and found the concentration of copper, cadmium, iron, and lead to be above the permissible level in both the vegetable samples analyzed. Detection of heavy metals was recorded within 0.2-0.75 mg/kg in both the vegetables analyzed.

Ahmed et al (2019) carried out a comparative study of bioaccumulation of heavy metals in vegetables irrigated in a farmland adjacent to multi-industrial zone of Ghazipur, Bangladesh in both wet and dry season. Red amaranthus, spinach, pumpkin and bottle gourd were found to bio-accumulate lesser amount of chromium, copper, zinc, cadmium and arsenic in wet than in dry season leading to the fact that climatic changes do have a marked effect on heavy metal uptake by plants. Ugya et al (2019) analyzed the bioaccumulation of metals in vegetables irrigated with waste water of Kaduna refinery and phytochemicals, Nigeria and reported bioaccumulation of lead and mercury in *Solanum melanogata, Cumunis sativus, Phaseolus vulgaris, Spinacia oleracea, Allium cepa, Lactuca sativa, Dacus carrota, Lycopersicum esculentum* and *Piper nigrum* much higher than the specified limits. Out of the five heavy metals (mercury, cadmium, silver, chromium and lead), *Solanum melanogata* was found to be the highest accumulator of cadmium (4.8mg/kg).

2.6 Heavy metal characteristics, toxicity and mechanism of action

The group of elements having physical properties viz. malleability, luster, high electric conductance, and above all, always ready to lose electrons from their outer orbit to form cations are collectively grouped as heavy metals The group of elements have an atomic density greater than 6 g / cm^3 and adversely affect the environment and living organisms (Jarup (2003). The heavy metals are ubiquitous in earth's crust, therefore their available concentration in water and soil fluctuates (Alloway, 1995). Presence of small amounts of these heavy metals in environment, especially water and soil, is harmless to the living organism. However, population explosion and haphazard industrialization led to an enormous increase in the level of these toxic substances beyond critical concentrations in those areas which were once clean (Blaylock, 2000). Bioaccumulation and biomagnification of these elements lead to a variety of lethal effects through food chain in the living organisms when released into the environment (Manohar et al., 2006).

Some of the major environmental pollutants such as copper, cadmium, chromium, lead, arsenic and zinc are present in large quantities in areas where anthropogenic activities are high (United states environmental protection agency, 1997). These heavy metals have caused severe toxicity around the world and there are documented cases of different elements that caused lethal effects. Hui et al (2014) reported severe pollution suffered by many cities of China and metal polluted area to be greater than 1 million km^2. Another report is of Rio Haina, Dominican Republic, which is the site of of former automobile battery recycling smelter and the residents of this area still suffer from extensive lead poisoning. Ranipet, a town in Tamil Nadu, India, which is contaminated by azo dyes and chromium released by tannery industries, affected nearly 3.5 million people (Jadia and Fulekar, 2009).Toxic metals like arsenic, cadmium, chromium, lead, zinc and mercury have been reported as main contaminants of groundwater responsible for cancer in the Malwa region of Punjab (Kaur et al., 2017). Sewage and industrial waste water used for irrigation purposes in peri - urban areas of Ludhiana, Punjab is highly contaminated with toxic metals like arsenic, cadmium, chromium, nickel and lead (Dheri et al, 2007). Sewage water containing cadmium has been reported to be used for irrigation purposes in Jalandhar (Sikka et al., 2009). A Russian city, Norilsk accounts for the worlds' largest heavy metal smeltering complex which releases more than 4 million tons of copper, cadmium, nickel, arsenic, lead, zinc and selenium annually (WHO, 2007).

Heterogeneous groups are formed by heavy metals due to their varied chemical and biological properties. Most of these metals viz. zinc, cobalt, lead, arsenic, chromium and mercury are extensively toxic in their soluble and elemental form (Pickering and Owen, 1997; Yu et al., 2016).

2.6.1 Arsenic (As)

Albert Magnus (1250 A.D.) discovered a prominently toxic and carcinogenic metalloid known as arsenic (As) with atomic no. 33 which belongs to group V of the periodic table. It is found in organic as well as inorganic forms in the natural environment, especially in the plants (Mcbride, 2013). Its inorganic forms (arsenate and arsenide) are more toxic to the living organisms. It is generally found in the form of sulphides, oxides or as a salt of copper, calcium, and iron (Singh et al., 2007).

Mechanism of toxicity: In human body arsenic gets bio transformed into highly harmful methylated arsenic compounds such as mono-methyl arsenic and dimethyl arsenic acid which are capable of posing serious chronic health risks. Presence of dimethyl arsenic acid in urine is an indication of chronic arsenic exposure. Mono-methyl arsenic acid (III) becomes a permanent intermediate product of the cell and can never be excreted out of the body. As compared to all other arsenicals, it is highly toxic and is the major cause of arsenic induced carcinogenesis in the body (Singh et al., 2007).

2.6.2 Cadmium (Cd)

As compared to any other heavy metal, cadmium is considered to be 2.5 times more toxic (Kabata Pendias, 2001). Environmental cadmium exposure is comparatively higher in Japan and China than in any other country of the world (Khan et al., 2010). Natural resources of cadmium are fire, forest, volcanoes and transport of soil particles whereas anthropogenic sources are mining, agricultural activities, cadmium containing sludge disposal, metal and ore processing units (Irvin et al., 1997). It predominantly enters into food chain due to its high rate of soil plant transfer factor (Satarug et al., 2011). Cadmium mainly enters into human body through food and it has been ranked 7^{th} in the list of top ten most hazardous substances (Department of Health and Human Services Agency for Toxic Substances and Disease Registry 2007). Cadmium, if absorbed or ingested, may cause persistent poisoning because its half-life is 25 to 30 (Uraguchi and Fugiwara, 2013). It interrupts calcium metabolism which can cause hypercalciuria leading to formation of kidney stones and softening of bones (Nogawa, 1981).

Mechanism of toxicity: Being non-essential and a highly toxic metal, cadmium is well known for its lethal effects on enzyme activity of the cells. It also causes nutritional deficiency in plants by inducing oxidative stress (Irfan et al., 2013). The exact mechanism by which it hinders the cellular functions is still not clear but it is known to have definite adverse effects on cellular metabolism (Patrick, 2003). When cadmium binds to cysteine rich proteins, its concentration increases 3000 folds. Hepatotoxicity and nephrotoxicity is caused by the formation of cadmium cysteine metallothionein complex. The deficiency of iron in the body is supposed to be caused by the capability of cadmium to bind with cysteine rich proteins (Castagnetto et al., 2002). Cadmium inhibits the free radical scavenging activity of metallothioneins when it replaces zinc because of their same oxidative state.

2.6.3 Chromium (Cr)

Chromium is ranked 7th most abundant heavy metal on earth (Mohanty and Kumarpatra 2013). It occurs naturally in anthropogenic activities like pigment oxidation, electroplating, tanning, wood preservation and metallurgy. Industrial effluents are responsible for chromium pollution in natural environment which adversely affect ecological and biological species (Ghani, 2011). Drastic increase in concentration of chromium in soil is attributed to the discharge of industrial wastes (Bielicka et al., 2005). Chromium exists naturally in six oxidation states (Cr^{2+} to Cr^{6+}) but its tri and hexa forms are considered to be most abundant in environment and most toxic to humans, animals and plants (Rodriguez et al., 2007; Mohanty and Kumarpatra, 2013). Cr^{3+} is easily oxidized to Cr^{6+} which is highly soluble in water and is extremely toxic in nature (Cervantes et al., 2001). The underground water in Tokyo was reported to contain Cr^{6+} 2000 times higher than the safe limits for drinking water in August, 1975. The soil and vegetable system is also disturbed with the use of chromium rich waste water for irrigation purposes (Duan et al., 2010).

Mechanism of toxicity: Hexavalent chromium penetrates the cell membrane more actively as compared to Cr^{3+}.The reactions between Cr^{6+} and biological reducing agents results in production of H_2O_2, hydroxyl radicals and superoxide ions which damage proteins and nucleic acids (Stohs and Bagchi, 1995). Cr^{6+} also is considered to be highly toxic due to its mutagenic properties (Dayan and Paine, 2001)

2.6.4 Lead (Pb)

Lead is a bright silvery metal, slightly bluish, but begins to tarnish when it comes in contact with the air. Accumulation of this toxic metal in environment is attributed to unplanned industrial activities. Fossil fuel, ammunition, mining and vehicle exhausts are the main sources of lead contamination in the environment (Martin and Griswold, 2009). Lead contaminated water bodies are the main route of lead entry into the food chain and ultimately into human body through food and drinking water (Goyer, 1990).

Mechanism of toxicity: Lead is a non-essential heavy metal which causes cell toxicity by producing oxidative stress in the cell. Lead increases the level of ROS which leads to decrease in amount of antioxidants which damage cell membranes and cell organelles (Mathew et al., 2011). Production of high ROS due to Pb eventually leads to lipid peroxidation since the free radicals collect electrons from lipid molecules present inside the cell membranes (Wadhwa et al., 2012) Lead toxicity also affects the ionic mechanism by replacing essential ions ultimately disturbing all the biochemical processes such as protein synthesis, enzyme regulation, release of neurotransmitters, intra and inter cellular signalling leading to apoptosis. Even in very small concentrations (picomolar) lead can substitute calcium affecting the activity of protein kinase which regulates memory storage and neural excitation (Flora et al., 2008).

2.7 Impact of heavy-metal-rich waste water on seed invigouration and early seedling growth

Dixit et al (1986) carried out an experiment to study the short and long time treatment of waste water on seed germination of rice seeds. One set of seed was soaked in waste water for 15-20 hours and then transferred to normal water and the second set was given continuous treatment of different concentrations of waste water. A significant decrease in germination percentage was reported in both the sets of seeds. Maximum 62% germination was observed in 15 hours treatment but only 8% in the seed given continuous treatment of 100% concentration of effluent- rich waste water of a cardboard factory.

Srivastva (1991) evaluated the effect of chloro alkali plant and paper mill effluents on seed germination of *Raphanus sativus* and *Allium cepa* treated with different concentrations of waste water and noted a significant increase in germination percentage and seed vigour of seeds treated with lower concentration of effluent - rich waste water (10%) in both radish and onion. Emergence and growth of secondary roots was also reported to be reduced drastically with the application of 100% concentration of effluents in both the vegetables. Low concentrations of waste water were observed to be beneficial as for as seed germination is concerned.

Gomathy and Oblisami (1992) estimated the influence of a pulp and paper mill effluent - rich waste water on seed germination index of some tree species. Seeds were soaked in different dilutions of the effluents for 24 hours and the germination percentage was reported to be significantly affected at 70% dilution. Seed vigour index and root length of the seedlings of all the tree species viz. neem, pungam and tamarind was reported to be reduced considerably. Lower amounts of effluent - rich waste water (25% and 50%) had no negative effect on percentage of seed germination.

Karunyal et al (1994) compared the outcome of application of 25%, 50%, 75% and 100% dilutions of waste water carrying tannery effluent on seed germination of *Acacia holoserica, Leucaena leucociphala* and *Oryza sativa* and it was concluded that the seed germination was not significantly affected by the application of 25% and 50% dilutions whereas it was strongly prevented by 70% and 100% of tannery - effluent - rich waste water. Mishra and Bera (1995) undertook a similar investigation to determine the consequences of waste water treatment of a crude tannery - effluent - rich waste water on seed germination in wheat cultivar Kalyan Sona and reported a stimulating effect up to 50% concentration but a significant phytotoxic effect with increase in effluent concentrations on percentage of seed germination, seedling growth as well as seed vigour index.

Sharma and Singh (1999) studied the effect of application of different concentrations of effluents of a rubber factory and sewage water rich in different kinds of heavy metals, especially copper and zinc, on seed germination and seedling growth of two inbreeds of *Vigna mungo* viz. PU30 and T9. Studies further revealed that sewage water significantly reduced both germination and seedling length as compared to the application of rubber factory effluents confirming municipal waste water to be more toxic in nature than the rubber factory effluents.

Neelofur et al (2001) conducted a study under controlled conditions to measure the effect of lead and copper chloride applications on seed germination of some vegetables and reported that the seed germination in *Spinacia oleracea* and *Lycopersicum esculentum* was significantly reduced with the treatment of copper and lead chloride at all concentrations applied. Maximum inhibition was reported at higher concentration (150 ppm) treatment of both the metals on seed germination of both the vegetables studied.

Simillarly Ramana and others (2002) undertook an investigation under controlled laboratory conditions to identify the effect of various concentrations (5% to 100%) of distillery-effluent-rich waste water on seed germination of some vegetables like tomato, cucumber, onion, and bottle gourd. Application of distillery effluents at low concentration (10%) significantly increased the seed germination (84 %) as compared to that of non-treated seeds in which it was found to be 63% only in all the vegetable seeds studied except tomato. At higher concentration (75% and 100%) seed germination was completely inhibited irrespective of vegetable species. Among the five vegetables studied, waste water had maximum inhibitory effect on onion, minimum on tomato.

Pandey (2004) determined the effect of an industrial effluent of an electroplating industry loaded with essential and non-essential heavy metals like zinc, nickel, chromium and cadmium on seed germination and seedling growth of a maize cultivar GK3014. A significant reduction in both the germination and growth of seedlings was observed in the seeds treated with industrial-effluent loaded waste water. Toxic effect of the effluents was observed to be significantly reduced after the dilution of the effluents was reduced to 50%.

Yadav and Meenakshi (2007) assessed the toxic effect of surgical-effluent-rich waste water on seed germination and seedling growth of two vegetables, *Raphanus sativus* and *Hibiscus esculentum.* The percentage of seed germination and seedling growth in both the vegetables studied was found to be significantly reduced with the application of higher concentrations of surgical effluents. Root growth was observed to be more affected as compared to shoot growth. Singh and others (2007) tested the after effects of water contaminated with the effluents of a fertilizer factory on seed germination and seedling growth of *Cicer arietinum* and observed a significant negative effect on percentage of germination and rate of seedling growth with gradual increase in concentrations of effluents in the water. Maximum seed germination and seedling growth was reported at 25%, but the inhibitory effect increased proportionately alongwith increase in effluent concentration in the water.

Garg and Kaushik (2008) studied the impact of both treated and untreated effluent - rich waste water of a textile mill on seed germination of two cultivars of sorghum by applying different concentrations (0, 6. 25, 12.50, 25, 50, 75 and 100%) for 24 hours.

No significant negative effect of effluents on germination percentage at lower concentration (6.25%) application was observed but a significant reduction of seed germination was reported as the concentrations increased above 50%, and at 100% effluents concentration the seed germination was reported to be completely inhibited.

Farooqi and coworkers (2009) in their study monitored the effect of cadmium and lead on germination and seedling growth of *Albizia lebbeck* under laboratory conditions. Seeds were given treatments with different concentrations of cadmium and lead and it was noted that cadmium applications had a more adverse effect on both percentage of germination and elongation of both root and shoot, than the lead treatment.

In an another study, Ling et al (2010) treated four different species of *Brassica* with 0.1, 0.2, 0.4, 0.8, 1.6, 3.2mM concentrations of $HgCl_2$ solution and observed a great reduction in coleorrhiza and coleoptile length with increasing concentrations of mercuric chloride. Seeds of *Brassica oleracea* were found to be more sensitive and that of *Brassica campestris* to be most resistant against stress induced by mercury. The effect was found to be more prominent on seedling growth than that on seed germination.

Wins and Manavalan (2010) explored the effect of waste water rich in effluents of a textile mill, on germination and seedling growth of *Vigna mungo* L. and reported that lower concentration of waste water were favorable for germination, seedling growth but with gradual increase in effluent concentration, a significant negative effect on these parameters was noted. The best response was observed at 25% concentration. Beyond 25% effluent a significant decrease in seedling growth was observed. It was concluded that with proper dilutions, the textile mill waste water can be used for irrigation.

Khan et al (2011) also observed the germination and seedling growth of three legumes i.e., pea, lentil and gram soaked in different concentrations of effluents of another textile mill and reported that low concentrations of textile water have no marked positive effect, but the concentration above 50% greatly reduced the germination percentage and dry weight of the seedlings. Pea seeds were found to be more affected as compared to gram and lentil by the textile - effluent - rich waste water application.

Dash (2012) analyzed the influence of sewage water of Gangua nullah (Bhubaneshwar, Odisha) on efficiency of seed germination and growth of seedlings in wheat and rice, and noticed a significant increase in seedling length and seed vigour index up to 50% dilution of the sewage waste water. But a significant reduction in seedling growth and seed vigour index was observed in seeds treated when the percentage of the effluents increased above 50%.

Abraham and others (2013) evaluated their impact of Cu, Pb, and Cd on seed germination of *Arachis hypogea* L. Seeds were soaked in different concentrations of these metals and reported that these heavy metals significantly decreased the seed germination. Increased concentration of Cd to 75mg/ l and 100 mg/ l greatly reduced the germination in groundnut. Pb and Cu both at 75 mg/l and 100 mg/ l also had an adverse effect but a little bit less as compared to that of Cd concentrations. Sinha and Paul (2013) undertook an investigation to assess the effect of different dilutions of domestic sewage water on germination and seedling growth of *Cicer arietinum* and *Pisum sativum* under controlled laboratory conditions and reported that the concentrations higher than 20% drastically inhibited the seed germination and root elongation in both the vegetables studied. In a similar study Khaleel et al (2013) estimated the effect of waste water of dairy effluents on seed germination of *Abelmoschus esculentus* and found that highly diluted applications of the effluents up to 20% had a favorable effect on percentage of seed germination but treatments of higher concentration of effluents have a significant inhibitory effect. Bautista et al (2013) analyzed the effect of different concentrations of chromium and cadmium on seed germination and root elongation in spinach, swiss chard and lettuce and reported that higher concentrations of cadmium significantly reduced the seed germination to 46%, 97% and 8% in swiss chard, lettuce and spinach respectively. A similar effect was observed in elongation of roots and it was observed to be 57%, 89% and 56% in the same order at 50 µ/l. Chromium was less effective as compared to cadmium, and its effect was 29%, 6% and 34% in comparison to that of control. Hassan et al (2013) tested the effect of waste water loaded with effluents of a dyeing unit on seedling growth and seed germination percentage of *Lablab niger* var. typicus and reported that effluents neutralization with normal water showed maximum seed germination (100%) but the effluent treatment after a standard limit of 20% showed negative effect on both the parameters studied.

Islam et al (2015) while studying the effect of different industrial effluents on five different leafy vegetables reported that the germination percentage of Indian spinach was observed to be 76% and 79% when treated with dyeing and pharmaceutical effluents respectively. In kangkong, jute and stem amaranthus, germination percentage was reported to be 28%, 80% and 84% under dyeing effluent treatment and 85%, 79% and 84% for pharmaceutical effluent treatment. The waste water of different industries influenced the seed germination of same crop differently. Beverage effluents have been reported to have minimum toxic effect as compared to the other industrial effluents on seed germination and seedling growth of all the leafy vegetables studied. Divya et al (2015) investigated the impact of sewage waste water on seed germination and vigour index of some monocot and dicot seeds of *Vigna radiata*, *Cajanus cajan* and *Oryza sativa*. Maximum germination was reported in treatments of 10 to 30% dilutions. At 10% dilution treatment, highest seedling growth was reported in paddy seeds as compared to other crop seed studied. Zhi and co-workers (2015) determined the effect of six heavy metals i.e., copper, mercury, chromium, cadmium, zinc and nickel on seed germination and seedling growth *Eruca sativa*. Only nickel at higher concentration was reported to have a significant negative effect on seed germination in *Eruca sativa* whereas nickel and zinc at lower concentrations were reported to increase the seed germination as well as root and shoot length.

Lalge and others (2017) analyzed the ill effects of waste water on seed germination phytotoxicity of some hemp cultivars (*Cannabis sativa*) and reported that lower concentrations of waste water from Kutchynki landfill do not have any significant effect on seed germination except 100% application of landfill waste water. Seeds treated with dilutions upto 50% showed an increased germination percentage as compared to control in all cultivars except cultivar epsilon-68 and santica-27. Vaithuiyanathan and Sundaramoorthy (2017) measured the impact of effluents released by a sugar mill on the seedling growth and development of African marigold and found highest fresh and dry weight of seedlings (3.59 g and 0.420 mg per seedling) at 10% application and lowest fresh and dry weight (760 mg and 0.047 mg per seedling) at 100% sugar-mill-effluent application. Similarly highest shoot and root length (29.10 cm and 6.80 cm per seedling) at 10% treatment and (9.40 cm and 1.83 cm per seedling) were measured at 100% concentration of mill effluents

Rahman and co-workers (2018) explored the phytotoxic effect of waste water rich in synthetic dye effluents on germination percentage and initial growth of red Amaranthus. The seeds were treated with different concentrations of the effluent -rich waste water of a loom dyeing industry in Belkuch (Bangladesh).The results of this study revealed that the seed germination was maximum (98.3%) at 5% dilution treatment but a gradual decrease in germination was observed as the concentration of effluents were increased. Maximum toxicity was reported at 100% treatment.

2.8 Impact of heavy metals on anatomical features of vegetables

Vazquez et al (1992) performed anatomical studies to find out the impact of different concentrations of Cd on histo-anatomy in roots of *Phaseolus vulgaris* L. var. contender and observed a significant decrease in cortical parenchymatous cells, increase in number of cells in pericycle, reduction in vascular cylinder cell differentiation, differentiation in plastid structure, cellular ingrowths in hypodermal tissue, abundant silver grains in outer cortex tissue of root after 120 hours of Cd application.

Maleci et al (2001) evaluated the effect of Cr^{+3} uptakes by *Calendula arvensis* and reported a significant reduction in enlargement of the meristamatic cells and badly damaged epidermal cells in the root tips of treated plants as compared to non-treated ones. Overall reduction in size of the root, stem and leaf of the plant was observed under chromium treatment.

Sandalio and others (2001) compared the after effects of different concentration of cadmium on growth and ultrastructure of pea (*Pisum sativum*). In this study also, the plants raised in controlled environment were treated with different concentrations of cadmium chloride (0 to 50 µM). The ultrastructure of leaves under 50 µM treatment showed a significant increase in intercellular spaces, an enlarged size of mesophyll cells and highly distorted chloroplast structure in the leaves as compared to lower concentrations applied.

Sridhar et al (2005) observed the outcome of different concentrations of cadmium (Cd) and zinc (Zn) treatments on structural changes induced in *Brassica juncea* seedlings and observed some abnormal depositions along with breakdown of parenchymatous cells in root and stem meristamatic tissue. Cell structural changes were reported to be more prominent in root and stem tips and had no effect on leaves. Cadmium toxicity was observed to be more as compared to that of zinc.

Vollenweider et al (2006) exposed stem cuttings of *Salix vimina* for thirteen weeks in hydroponic solution having different concentrations of cadmium chloride (0 to 200 µM/ L) and observed an increase in thickness of collenchyma cell wall, induced cell injury to conducting phloem and tannin plugging of xylem. Higher concentration of cadmium leads to the senescence of mesophyll tissue in the leaves of *Salix vimina.*

Sridhar and co-workers (2007) assessed the effects of cadmium and zinc treatment on structural changes caused in Barley and reported a gradual change in leaf structure with bioaccumulation of these heavy metals in the cells. Leaf epidermis was reported to be more compact and mesophyll cells were seen with thickened cell walls. A significant decrease in cell size and intercellular spaces with increased metal concentration was observed. Effect of cadmium concentrations on anatomical changes was more pronounced as compared to that of zinc.

Sridhar and colleagues (2011) explored the after effects of chromium and arsenic uptake by *Pteris vittata* on its cell structure. The potted plants were treated with different concentrations of chromium and arsenic for 21 days. Chromium accumulated mainly in the roots whereas, arsenic accumulated in the shoots of the plant. Microscopic studies revealed dehydration of cells resulting in collapse leaves and cellular breakdown of root parenchyma in cadmium treated plants. Anatomical features of leaf, stem or roots in the plants which were exposed to different concentrations of arsenic were not affected to that extent as that of cadmium treated ones. The results revealed a profound effect of cadmium on cellular degradation than that of arsenic in fern plants.

Mangabeira and co-workers (2011) tried to find the role of chromium accumulation on anatomical features of four macrophytes grown in nutritive solution containing 0.25 and 50 m/L $CrCl_3.6H_2O$ and reported severe cell structure disturbances and changes in shape of cell organelles. Significant alterations were detected in shape of chloroplast and nuclei in two species *Alternantheria philoxeroides* and *Borreria scabiosoides* out of four macrophytes treated with 50mg/ l concentration as compared to 0.25/l mg. Najeeb and others (2011) examined the Cd induced effects in ultrastructure of *Juncus effusus* and its remediation through exogenous application of citric acid. On the basis of microscopic observations it was reported that Cd bioaccumulation damaged root cell through cytoplasmic shrinkage and the exogenous application of citric acid significantly ameliorated the ill effects induced by the cadmium treatment and greatly helped in restoring the shape of the cells.

Gomes et al (2011) while studying the effect of lead, cadmium, zinc and copper contaminated soil on *Brachiaria decumbens* cell structure observed high lignin depositions in the cell walls of xylem as well as in endodermis in root and leaves of the plant. The cell wall of xylem tissue was found to be highly thick as compared to the plants grown in normal soil. Higher concentration (15%) of heavy metals in the soil significantly increased the thickness of both adaxial and abaxial epidermis but reduced the thickness of leaf blade.

Bini et al (2012) reported large intercellular spaces in leaf parenchyma of *Taraxachum officinale* grown in heavy metal contaminated soil of a mine site in Italy as compared to the plants grown in the soils away from the mine site. Heavy metal contamination induced significant alterations in internal membranes of mitochondria. Non-significant differences were also observed in compartmentalization of thylakoids of the chloroplasts.

Farzadafar and Zarinkarmar (2012) evaluated the effects of calcium application on cadmium toxicity in young seedlings ultrastructure of *Matricaria chamomilla* and found that the cadmium treated plants showed great abnormalities in anatomical features induced by cadmium stress. A significant increase was detected in the cell size of epidermis and parenchyma in pericycle of the root. Calcium application significantly alleviated the toxicity induced by cadmium. Moreover, it was observed that application of different concentrations of calcium chloride significantly restored the cell size of aerenchymatous tissue. Ge et al (2012) analyzed the effect of various concentrations of Cd (50 to 100 µM) applied for 40 days to the stem cuttings of two species of *Populus* and noted various changes like invagination of plasma membrane, fused vesicles forming multi vesicular bodies, presence of dense granules in cytoplasm and cell wall, reduction in number of endoplasmic reticulum, and dictyosomes, occurrence of plasmolysis and a significant expansion of mitochondria in root tips of both the cultivars of *Populus* induced under cadmium stress as compared to non-treated plant of the same species.

Salah and Omar (2013) recorded the changes occurred in anatomical features in developing roots in *Zea mays* cv. Gira 2 under different concentrations of copper and cadmium application. Both copper and cadmium treatments lead to decrease in cross sectional area as well as diameter of the root central cylinder. Highest decrease was reported in roots under 150µmol/L treatment. The toxic effect of individual applications of both the heavy metals was more pronounced as compared to the combined effect. Gupta and Chakravarti (2013) determined the amount of Cd, Pb and Hg accumulation and their effect on anatomical changes induced in mangrove system and reported greater bioaccumulation of these metals in roots than in the shoot system. Bioaccumulation level of these heavy metals in the plant tissue was observed to be in the following descending order Cd > Pb > Hg. Stem and root samples studied after 100 days of treatment showed marked deformity in the vascular bundles of stem under Hg as compared to that of Cd stress in *Bruguiera sexangula*.

Al-saadi et al (2013) screened two species of *Potamogeton* L. (*Potamogeton crispus and Potamogeton perfoliatus*) exposed to different concentrations (0, 5 to 15 mg/l) of silver and copper metal treatment and reported a significant reduction in size and thickness of leaf blade, widened intercellular spaces in cortical parenchyma and reduction in vascular bundles of stem in both the species under treatment as compared to non treated ones. The epidermal cells were found to be sunken and deformed. A significant decrease in diameter of stele in roots and size of chloroplasts in leaves was also observed in Zn treated plants in comparison to that of non-treated ones. Li et al (2014) carried an investigation to determine the effect of Cd^{+2} on the root anatomical structure of 4 rice genotypes (Mainliner lines Vixiang B and E 2B, restorer lines R 892 and Mianhu 725) and reported that high Cd^{+2} stress induced distortions in epidermis and black spots were also spotted in cortex of both maintainer and restorer lines. Restorer lines were found to be less affected as compared to the maintainer lines.

Kouhi et al (2016) undertook an investigation to determine changes induced in anatomical features of *Brassica napa* under long term application of zinc and found that Zn treatments significantly decreased the number of the cells in cortex and vascular bundles. Atabayeva and others (2016) performed an experiment to see the anatomical changes induced by the application of different concentrations of copper (0.25 and 0.5 Nm) in leaves and roots of five wheat varieties grown in hydroponic solution. Results revealed that all the wheat varieties developed strong abnormalities in their cellular structure under 0.5 Nm treatment. The thickness of epidermis (both upper and lower) and vascular bundle tissues was significantly decreased under Cu stress. Similarly a significant decrease in endodermis thickness was also observed.

Dey and Mondal (2016) explored the effect of various concentrations of chromium, lead and manganese on internal structure of *Cicer arietinum* and reported the ruptured hexagonal structure of cells in leaf, root and stems alongwith swelling of guard cells induced under chromium treatment. A distinct change in ultrastructure of xylem and phloem structure was also observed with the increase in concentrations of hexavalent chromium from 20 ppm to 100 ppm.

Sahar et al (2016) observed the after effects of mercury and lead bioaccumulation on ultrastructure of *Vallisneria* (a submerged macrophyte) and found a large number of changes in the anatomical features of *Vallisneria.* A significant reduction in the thickness of leaf blade, reduced size of epidermal tissue, change in shape of epidermis, reduction in cell size of parenchymatous tissue and reduced number of vascular bundles.

Edita et al (2017) observed the effect of various abiotic stresses like salinity and heavy metals on root anatomy of *Zea mays* and reported that under heavy metal stress, the roots develop casparian bands and a significant increase in suberin lamellae in exodermis as compared to tissue of endodermis in the roots of *Zea mays* to reduce the percolation of toxic metals through cell membranes. Akcin et al (2018) carried out a study to understand the effect of toxicity of chromium on anatomical features of *Triticum aestivum* Cvr “Ekiz” and noted that plants treated with 0.3 and 0.4 mol chromium concentration showed an increase in diameter of parenchyma and decrease in thickness of sclerenchyma in root tissue. The thickness of phloem, xylem and mesophyll tissue was also reported to be significantly decreased under chromium stress in the leaves.

2.9 Effect of heavy metal contaminated waste water on biochemical parameters of the vegetables

Smilde (1981) monitored the impact of different concentrations of chromium, copper, nickel, lead and zinc in the soil and their influence on biochemical parameters of some vegetables. It was observed that even a low dose of 100 mg/kg presence of these metals have a drastic decline in in both fresh and dry weight of all the vegetables studied. The effect was noted to be more pronounced in leafy vegetable spinach and lettuce as compared to the other vegetables studied.

Vassilev (1995) carried out an experiment to analyze the influence of cadmium stress on barley cultivars. 3 mg/l and 5mg/l of cadmium concentrations were applied to two 15 day old barley cultivars (Hemus and Obzor) and observed a significant decrease in rate of photosynthesis in both the cultivars especially in the functional activity of photosystem 2. Total chlorophyll content was observed to be significantly reduced under 6 mg/l treatment of cadmium. The response of cadmium stress was more pronounced in cultivar Hemus in comparison to that of Obzor.

Gopal et al (2003) worked for estimation of changes induced in metabolism of *Lycopersicum esculentum* cv Pusa Ruby under the application of different concentrations of cobalt sulphate ranging from 0.05, 0.1, 0.2, 0.4 to 0.5 mM to the 40 day old plants. Leaf necrosis was reported to be the first symptom that was visible with 0.5 mM of cobalt treatment. Significant reduction in content of proteins, chlorophyll, nucleic acids, starch and almost all the nutrients were other after effects induced by cadmium treatment.

Singh and Tewari (2003) evaluated the biochemical changes induced by cadmium toxicity in *Brassica juncea* L. with the application of different concentrations of cadmium, 100, 250 and 500 to the plants raised in pots. 100 to 250 ppm cadmium application improved growth of the plant but higher concentration (500 ppm) caused a significant suppression in growth. Excessive levels of cadmium also decreased the concentration of soluble proteins and chlorophyll, but increased the ratio of chlorophyll a/b.

Sinha et al (2006) applied different concentrations of waste water rich in tannery effluents to two different varieties of ADT-37 and ADT-39 of *Oryza sativa* (ADT-37, ADT-39*)* and LR-47 *Cajanus cajan* (LR-47) cultivated in Ambur, Tamil Nadu. Different dilutions of tannery effluents (25, 50, 100%) were applied to the plants. Application of concentration 25% significantly increased root length, shoot length, total biomass, photosynthetic pigments, proteins and free amino acids in the seedlings of all varieties while 50% concentration reduced all the parameters studied and at 100% effluent concentration, the growth was completely retarded.

Guo et al (2007) undertook a study to estimate the toxicities of copper, cadmium and aluminum separately and in combinations to identify their interactions in two cultivars of barley seedlings. After five weeks of the treatment, estimation of plant growth, metal accumulation, total soluble proteins and sugar contents was done. The outcome of the study revealed that at low pH (4.5) accumulation of aluminum, cadmium and copper was very high which led to significant decrease in the soluble proteins. The combination of aluminum + cadmium + copper had different interactions on both the genotypes studied. Synergistic effect of these metals was seen in Shang 70-119 but an antagonistic response was observed in Gebeina cultivar of barley.

Saravanamoorthy and Kumari (2007) performed an experiment in which they applied different concentrations of waste water of a textile mill in Karur district of Tamil Nadu and studied its effect on biochemical and morphological characteristics of two varieties of peanut (*Arachis hypogea* L.) and found that low concentrations of textile effluents increased chlorophyll a and b content in both the varieties of peanut TMV-10 and JL-24. The response was observed to be more prominent in TMV-10 as compared to JL-24 variety of peanut.

John et al (2008) conducted a study to assess the deleterious effect of heavy metal toxicity on the biochemical parameters of duckweed (*Lemna polyrhiza*). The plants were given treatment of different concentrations of lead and cadmium and it was observed that application of low concentrations of both the heavy metals showed an increase in protein and sugar content but higher concentration (>30 mg/l) resulted in drastic reduction of both protein and sugar content.

Dube et al (2009) undertook an investigation to detect the toxic effect of chromium bioaccumulation and its influence on some biochemical parameters of root vegetable carrot (*Dacus carrota* L). Different concentrations of chromium (0.1, 0.5 Nm) were added to the basal nutrient solution. After 40 days of application, toxicity symptoms like stunted growth and decreased leaf size were observed. Similarly chlorophyll, iron and phosphorous content was also found to be significantly reduced in leaves of plant studied.

Bamniya et al (2010) conducted a trial to evaluate the after effects of waste water of Ayad River in Udaipur on morphological and biochemical parameters of *Brassica juncea* and *Spinacia oleracea*. The study was conducted in laboratory by raising the plants in pots and irrigating them with the waste water collected from the river. The results of the study revealed a significant decrease in total chlorophyll, carbohydrates, proteins, amino acids and sugar contents in both the vegetables irrigated with waste water. Gupta et al (2010) analyzed the impact of heavy metal contaminated water of an industrial city Durgapur, West Bengal, on the biochemical parameters of three different type of vegetables and reported low levels of chlorophyll, protein, amino acid content but high level of total sugars in all the vegetables *(Raphanus sativus*, *Brassica nigra* and *Colocacia esculentum*) irrigated with waste water as compared to control. A significant difference in protein content in root and shoot samples was also observed.

Singh and Aggrawal (2010) conducted a study in suburban areas of Varanasi to analyze the effect of heavy metal (Cd, Cu, Pb, Cr, Cd) containing waste water (Dinapur sewage treatment plant) irrigation on physiological, morphological and biochemical parameters of a leafy vegetable grown in that area and reported a significant decrease in total chlorophyll and protein content as well as an increase in number and size of leaves in leafy vegetable *Beta vulgaris* samples irrigated with waste water as compared to samples irrigated with normal ground water. Silva et al (2010) studied the impact of application of different amendments of tannery sludge compost on growth and biochemical responses in *Capsicum annuum*. The amendment rates concentrations 0, 25, 50, 75 and 100% were applied and it was observed that the application of 25 % amendment increased the number of leaves, fruits, stem length, total dry weight and chlorophyll content; but a significant decrease in above parameters was recorded at higher concentrations (75%, 100%).

Chaves et al (2011) studied the after effects of zinc, copper and cadmium treatment on plant growth of *Helianthus annuus.* Different concentrations of cadmium, zinc and copper (0, 10, 20, 30 and 40 and 80 mg /l) were added to the soil. After 50 days of treatment it was observed that higher concentration of both cadmium and zinc treatments resulted in stunted growth of the plants. A drastic decrease in biomass, chlorophyll and protein content was also observed in plants treated with higher concentrations of zinc.

Priya and Chellaram (2012) treated some of the vegetable crops with different dilutions (25, 50, 75 and 100%) of textile effluents and analyzed its impact on the biochemical parameters. The vegetable crops selected for experiment were tomato, ladyfinger, cucumber, fenugreek and gogu (Deccan hemp). The effluents used were collected from a textile mill in Tamil Nadu. In this study, the leaves of the effluent treated plants showed drastic alterations in the carbohydrate, protein and nucleic acid contents in all the vegetables studied as compared to control. The amount of all these parameters was significantly low at all the concentration above 50% in all the effluent treated vegetables as compared to the control.

Hassanein et al (2013) studied the effect of heavy metal contaminated soil on growth, yield and physiological responses of turnip and lettuce. These vegetables were irrigated with industrial wastewater of El-Amia drain, Egypt. The magnitude of Cd in soil after long term irrigation with wastewater was detected to be significantly higher than permissible level (3mg/kg). Both turnip and lettuce exhibited decrease in leaf area, fresh and total biomass. Inhibition of turnip and lettuce growth was reported to be 57.6%, 68.5%, 31%, 81.9%, 57% and 71%, 14.6%, 33%, 19.45%, 50%, in the samples collected from 1 km to 19 km area around El-Amia drain. Moreover, number of seeds /plant were also found to be significantly reduced with increasing soil contamination. The highest reduction 44.1% in turnip and 55.5% in case of lettuce was observed as compared to the samples of normal crops. Junaid et al (2013) observed the growth and biochemical parameters of *Medicago sativa*, *Brassica juncea* and *Cicer arietinum* under lead, cadmium and copper stress and reported significantly low content protein and DNA in all the three vegetables analyzed. The content of protein was found to be lowest in chickpea (*Cicer arietinum*) and maximum reduction DNA content was noted in *Brassica juncea*. Mangal et al (2013) monitored the impact of different concentrations of Cd and Zn on biochemical contents of *Abelmoschus esculentus* and *Cyamopsis tetragonoloba*. The results of the study revealed a drastic decline in total biomass, chlorophyll and total proteins in both the vegetables treated with zinc and cadmium beyond 100 ppm in comparison to same biochemical parameters observed in control.

Murugalakshmikumari and Ramasubramanian (2014) studied the growth and biochemical characteristics of *Abelmoschus esculentus* L. treated with different concentrations of lead accetate. The toxicity of various concentrations of lead acetate (2 mM, 4 mM and 6 mM) were applied and it was observed that concentrations beyond 4 mM significantly decreased the growth and photosynthetic pigment content such as chlorophyll and carotenoids. Kapoor et al (2014) conducted an experiment to estimate the effect of different concentrations of cadmium (0, 0.2, 0.4 and 0.6.) on biochemical contents in *Brassica juncea*. The young plants were treated with different concentrations of cadmium continuously for 30 days. Analysis of biochemical parameters showed a significant increase in plant hormones viz. papaverine, cadaverine and jasmonic acid levels with enhancement in dose of Cd but the same treatments had an adverse effect on chlorophyll content which was found to be significantly reduced.

Pallavi and Joseph (2014) evaluated the effect of sewage irrigation on biochemical parameters of *Amaranthus tricolour*. Samples were collected from the peri-urban areas of Bandra contaminated with considerable amount of cadmium and lead due to long term irrigation with sewage water. The studies revealed an increase in protein, vitamin C and iron content in the samples collected from the contaminated area as compared to the samples collected from the control site. Nutritive value of waste -water-irrigated plants was found to be higher than that of control ones.

Alia et al (2015) investigated the influence of metal toxicity in *Spinacia oleracea* grown in controlled conditions. Spinach grown in soil treated with different concentrations of Cd, Pb and Zn was analyzed for biochemical parameters after 40 days of treatment. Heavy metal accumulation was found to have an adverse effect on all the biochemical parameters studied. Higher concentration of cadmium significantly reduced the amount of protein, fiber and moisture content by 31, 29 and 33% respectively. Similarly, at high dose of Pb, the decrease was observed to be 23, 22 and 29 % respectively and higher dose of Zn decreased the contents by 16,16 and 20% respectively. Toxic effect of mixture of Pb and Cd was found to be more pronounced than their individual effect. Damera et al (2015) applied different concentrations of nickel 16 ppm, cadmium 10 ppm and chromium 20 ppm to *Vigna radiata* and the analysis of biochemical parameters revealed a significant decrease in amount of carbohydrates, proteins, starch, reducing sugars, non-reducing sugars, DNA and RNA content of the plant treated with heavy metals as compared to that of control.

Tiwari et al (2017) evaluated the impact of different concentrations of cadmium (0, 25, 50, 75, 100 µm of $CdCl_2$) on morphological and biochemical changes induced in gram seedlings (*Cicer arietinum*) and observed marked reduction in number of leaves, shoots and decrease in fresh weight of seedlings at higher concentrations of cadmium chloride as compared to control. An increase was observed in soluble proteins attributed to the activation of genes responsible for the synthesis of specific proteins produced under stress to protect the vital set of cellular proteins which helps in maintenance of plant cells structure. It was reported that synthesis of specific proteins is necessary for hardening of cell walls to protect the entry of heavy metals.

2.10 Effect of heavy metals on antioxidant activity of vegetables

Plants develop their own mechanism of resistance against the ill effects of heavy metals by synthesizing some compounds, increasing or decreasing the activity of some enzymes and nucleic acids to detoxify the negative effects of heavy metals. Morphological and anatomical changes like thickness of cuticle, formation of trichomes and mycorrhizal association are the basic barriers raised by the plants to stop the entry of heavy metals in the cell. Trichomes help in storage and detoxification of heavy metal by secreting some secondary metabolites (Lee et al., 2002; Hauser, 2014). When heavy metals enter the cell after breaking these biophysical barriers, then to cope up with the toxicity of metals, cellular defence mechanism starts the synthesis of some secondary metabolites like glutathione, phenols, proline, histidine, mugineic acids and some hormones like ethylene, salicylic and jasmonic acid (Sharma and Dietz, 2006; Viehweger, 2014). Higher accumulation of heavy metals overcome above mentioned strategies and enter into cell and disturb the cellular redox system leading to production of reactive oxygen species (Mourato et al., 2012).

To save the cell from poisonous effect of reactive oxygen species, plant triggers its antioxidant mechanism and increases the activity of enzymes catalase, super oxidase dismutase, peroxidase, guaiacol peroxidase, glutathione reductase, and ascorbate peroxidase; and also increases the synthesis of some other non-enzymatic antioxidants like tocopherols, alkaloids, tannins, lignin, phenolic compounds, ascorbic acid. carotenoids and proline content which act as scavengers of oxygen reactive species (Michalak, 2006 ; Sharma et al., 2012). Secondary metabolites act as chelators, and detoxify the ill effects of heavy metals but their production, mechanism of action depends upon the amount and type of heavy metals absorbed and the tolerance limits of the plants (Solanki and Dhankhar, 2011).

El-Beltagi et al (2001) performed an experiment to analyse the effect caused by the application of different concentrations of cadmium and $CaCl_2$ (0 to 50µM concentrations) on antioxidant activity of *Pisum sativum* and reported a significant increase in lipid peroxidation and the activity of both catalase and superoxide dismutase enzymes. Guaiacol peroxidase activity was reduced to a lesser extent and there was no effect of calcium chloride treatment on the activity of enzyme glutathione reductase. A significant increase in the activity of SOD was observed at higher concentration of cadmium. Hameed et al (2001) determined the effect of lead and copper chloride treatments and production of phenolic compounds in fruit and leafy vegetables and observed an increase in accumulation of phenols in the seedlings of *Lycopersicum esculentum* and *Spinacia oleracea* when treated with different concentrations of lead and copper. Maximum production of phenols was recorded in plants treated with 150 ppm lead as compared to same concentration of copper treatment and control samples.

Kachout et al (2009) evaluated the effect of heavy metal bioaccumulation on antioxidant defence system in the leaves of two species of Atriplex (*Atriplex hortensis* and *Atriplex rosea*) raised in polluted soils contaminated with metals Zn, Pb, Ni and Cu. The antioxidant response was observed under 25, 50, 75 and 100% metal stress. Heavy metal stress significantly increased the activity of both catalase and glutathione reductase enzymes but diminished the activity of superoxide dismutase and ascorbate peroxidase.

Gupta and coworkers (2010) analyzed the samples of different vegetables irrigated with heavy-metal-rich waste water of Tamla Nullah, Durgapur, West Bengal, to quantify bioaccumulation and antioxidant response induced by heavy metals uptake in the waste water by plants. It was observed that there was a significant increase in amount of ascorbic acid and slight increase in phenol level in *Colocasia*, *Brassica* and *Raphanus* samples irrigated with contaminated waste water in comparison to normal water irrigated ones. Singh and Aggarwal (2010) carried out an experimental study to explore the impact of heavy-metal-rich waste water irrigation and its effect on antioxidant metabolites in *Beta vulgaris* L. variety All Green and reported a significantly higher accumulation of secondary metabolites like thiol, phenols, proline, carotenoids and ascorbic acid in samples irrigated with contaminated water in comparison to the control ones. Overall content of all the antioxidant metabolites and antioxidant enzyme activity studied was significantly higher in the samples irrigated with heavy-metal-rich waste water as compared to that of control ones. Damera and colleagues (2015) performed an experiment under controlled conditions to evaluate the impact of various concentrations of nickel, cadmium, chromium supplemented with 1% solution of calcium hydroxide in *Vigna radiata.* A significant increase in phenols content with increasing concentrations of heavy metals was noted in the leaves of *Vigna radiata* in comparison to that of control.

Gohari et al (2012) analyzed the outcome of different concentrations of Pb application on proline content in different plant parts of mustard and reported a significant increase in concentration of proline in roots of *Brassica napa* when exposed to increasing concentration of lead from 100 to 400 µm. Accumulation of proline was observed to be more in root as compared to the aerial parts of the plant.

Hassanein et al (2013) evaluated the impact of industrial effluent-rich waste water of El-Amia drain in Alexandria, Egypt on antioxidant responses of some leafy and root vegetables. Both the evaluated vegetables, turnip and lettuce had high concentration of non-enzymatic antioxidants like phenols, flavonoids and poly-amines (spermidine and putrescine) as compared to the samples of same vegetables irrigated with normal borewell water. Nadgórska-Socha et al (2013) explored the effect of some selected heavy metals cadmium, copper, zinc, lead and nickel on the antioxidant response of *Vicia faba* grown in monometallic contaminated soil for two months and found high level of proline content as well as a significant increase in guaiacol peroxidase, peroxidase and catalase activity in the leaves in plants grown in heavy metal contaminated soil.

Pallavi and Joseph (2014) studied the effect of heavy metals cadmium, copper and lead contaminated waste water irrigation on vitamin C (ascorbic acid) contents in *Amaranthus tricolour,* a widely consumed leafy vegetable in Tamil Nadu, and observed significantly higher amount of ascorbic acid in waste-water-irrigated *Amaranthus tricolour* and comparative low amounts of ascorbic acid were detected in the samples obtained from another site where irrigation was done by normal ground water. Kapoor et al (2014) measured the influence of cadmium stress induced 30 days after the treatment in *Brassica juncea* and analyzed the antioxidant level produced by the plant in response to the cadmium induced stress. The results revealed a significant increase of secondary metabolites like total phenols and flavonoids. The activity of all the antioxidant enzymes viz. superoxide dismutase, polyphenol oxidase, glutathione peroxidase, glutathione transferase was found to be significantly higher than that of control.

Andrianos et al (2016) tested the effect of heavy-metal-contaminated waste water irrigation water on carotenoids content in *Solanum tuberosum* and *Daucus carota* cultivated in greenhouse. The plants were grown and irrigated with different concentrations of Cr VI and Ni II. High level of carotenoids, lutein, beta carotene and activity of peroxidase and catalase was detected in carrot and potato irrigated with water rich in both the heavy metals as compared to the normal samples collected from

Greece and other European countries. Zafar et al (2016) undertook a study to investigate the effect of various concentrations (50 to 100%) of domestic sewage wastewater of Pakpattan a metropolitan city of Pakistan, on antioxidant activity and secondary metabolites content in tomato (*Lycopersicum esculentum*), okra (*Abelmoschus esculentus*) and pumpkin (*Cucurbita pepo*) A significant increase in activity of all the antioxidant enzymes catalase, peroxidase, superoxide dismutase and ascorbate peroxidase was observed in all the vegetables studied. An increase in amount of secondary metabolites (malondialdehyde, phenols and flavonoids) was also noted in vegetable samples irrigated with 100 percent sewage water.

Aria et al (2017) screened the effect of cadmium on antioxidant activity in *Vetiveria zizanioides* grown in pots and treated with different concentrations of cadmium chloride (20, 40 and 60 mg/l) and reported a significant increase in the activity of glutathione reductase, polyphenol oxidase, ascorbate peroxidase and guaiacol peroxidase in the leaves and that of peroxidase and catalase in root of the plants.

Abdulaal et al (2017) analyzed the impact of sewage water irrigation on antioxidant response in some date cultivars. Samples of fresh date fruit of three cultivars of *Phoenix dactylifera* (Ajwa, Anbara and Safawi) were collected from Saudi Arabia. Higher amounts of secondary metabolites i.e. total phenols and flavonoid) content was recorded in waste-water-irrigated cultivars. Similarly, the activity of antioxidant enzymes peroxidase, polyphenol oxidase and glutathione-s- transferase was found to be significantly higher in waste-water-irrigated cultivars as compared to control samples.

2.11 Phytochelatins

Chen et al (2008) studied the antioxidant responses against cadmium bioaccumulation in *Brassica chinensis* and reported that cadmium stress resulted in increased enzymatic synthesis of phytochelatins coupled with increased activity of overall antioxidant system in *Brassica chinensis* which lead to an effective detoxification of cadmium. Kortba et al (1999) performed a tissue culture experiment to detect the formation of heavy metal binding proteins and peptides under cadmium, zinc and copper stress in different cultivars of *Rauwolfia serpentine* and concluded that Cd^{+2} ions were more effective stimulator of phytochelatin production than Cu^{2+} and Zn^{+2} in several cultivars of *Rauwolfia* studied.

Yurekli and Kucukbay (2003) conducted a comparative study to detect the bioaccumulation of cadmium and production of phytochelatins in root and leaves of *Helianthus annuus*. The results revealed that the roots bio-accumulated more amount of Cd as compared to leaves and a corresponding increased levels of phytochelatins were produced and the amount produced was observed to be two times higher in roots as compared to the leaves in sunflower exposed to cadmium intoxication.

Fidalgo et al (2013) evaluated the antioxidant response induced by different concentrations of copper in *Solanum nigrum* and reported an increased production of significantly higher amount of phytochelatins in roots of *Solanum nigrum* treated with 200 µmol of copper which resulted in immobilization of copper and its preclusion from moving towards the shoot. Szalai et al (2013) determined the influence of salicylic acid on phytochelatin synthesis during cadmium stress in *Zea mays* and found that treatment of maize plant with cadmium followed by salicylic acid decreased the amount of phtytochelatins in roots but a significant increase in phytochelatin synthase activity was recorded in the leaves.

Batista et al (2014) monitored the impact of long term arsenic stress to identify and quantify the bioaccumulation and translocation of arsenic in various cultivars of rice and relative amount of phytochelatins produced. Higher amount of arsenic was detected in root as compared to the shoot of the plant. The outcome of this study revealed that a long term exposure to arsenic resulted in high level of As-Pc (arsenic phytochelatin) in roots which reduced the transport of arsenic from roots to the aerial parts of the plant.

2.12 Metallochelatins

These are the small binding proteins rich in cysteine that are found in mammals, eukaryotes and plants and are synthesized by mRNA translocation. Along with other metabolic functions, they also act as ROS scavengers. First metallochelatin was extracted from equine kidney in 1957 and was named by Margoshes and Vallee (1957).

Xia and coworkers (2012) evaluated the role played by heavy metal bioaccumulation in production of metallochelatins in some transgenic species of tobacco and reported the presence a special metallochelatin (EHMTI metallothione) which not only increased the tolerance but also decreased the synthesis of hydrogen peroxide, and also increased the activity of peroxidase in roots of transgenic tobacco to cope up with the stress induced by metal accumulation.

Pagani et al (2012) explored the effect of cadmium and zinc treatment on metallochelatins, production in Soya bean. Four different isoforms of metallochelatins (MT 1, MT 2, MT 3 and MT 4) were detected in the samples analyzed. Out of four types of metallochelatins MT1, MT2 and MT3 were found to be involved in detoxification of deleterious effects of cadmium in soybean whereas MT 4 exhibited Zn binding characteristics.

Grennan (2011) analyzed the effect of copper and zinc application and antioxidant produced in *Arabidopsis*. After analysis of the treated samples, six isoforms of metallochelatins were detected namely (MT1, MT2, MT3, MT4a and MT4b). The isoforms MT 1,MT 2 and MT3 were reported to be responsible for copper chelation whereas MT 4a and 4b act as Zn binder in *Arabidopsis*. In a similar study, Garcia-Hernundez et al, (1998) also measured the metallochelatin production in response to copper treatment in some mutants of *Arabidopsis* and detected the presence of MT1 and MT2 in the leaves of the plant studied. It was further revealed the MT1 and MT2 have distinct but overlapping expression. MT1 was found to play a more important role in detoxification of copper in veins of leaves in some mutants of *Arabidopsis* than MT2.

Table 2.2: Role of essential & non-essential heavy metals in plant system (Sandeep et al., 2018)

Metals	Functions
Cobalt (Co)	Constituent of vitamin B_{12} and propionate expansion of leaf disc
Copper (Cu)	Electron donor in photosystem 1
Nickel (Ni)	Component of urease enzyme
Manganese (Mn)	Involved in oxidation of carbohydrate as catalyst
Molybdenum (Mo)	Nitrate reduction and biological nitrogen fixation

Zinc (Zn)	Component of carbonic anhydrase, RNA polymerase - maintain the integrity of ribosome
Arsenic (As) Cadmium (Cd) Chromium (Cr) Lead (Pb)	Production of ROS, high concentration of secondary metabolites, increased concentration activity of antioxidant enzymes, lipid peroxidation, oxidative stress, alteration in membrane structure and transport, DNA strand breakage, genotoxicity and growth retardation

2.13 Potential risk of heavy metals to human health due to daily consumption of vegetables

Vegetables irrigated with waste water uptake have higher concentration of heavy metals from soil which get accumulated in the plant tissues. Consumption of these heavy metals is the main route of human exposure to heavy metals. According to Sobha et al (2007), heavy metals get accumulated in the soft tissue and cannot be metabolized by the body. Khan et al (2008) reported that chronic level intake of toxic heavy metals has a hazardous impact which becomes perceptible only after several years of exposure. Different indices are used to determine the toxicological effect and health risk associated with intake of contaminated vegetables.

Table 2.3: Health hazards from dietary intake of heavy metals and metalloids contaminated vegetables (Rai et al., 2019)

S. No.	Name	Sources	Toxicity Limits	Health implications	References
1	Arsenic (As)	Fossils fuels, contaminated, groundwater, thermal power wastewater	$\geq$50 µg (blood)	Keratosis, nausea, cancer, peripheral vascular disease, high mortality rate	Smith et al (2000), Mazumder (2008), Martin & Griss Wold (2009), Islam et al (2017)
2.	Cadmium (Cd)	Alloys, batteries, sewage	(food): 0.01 mg/kg/day	Hyper calcicularia,	Jarup (2003), Henson &

		sludge		fragile bones, renal dysfunction	Chedese (2004)
3.	Chromium (Cr)	Dye and electroplating industry, sewage wastewater	Not specified	Lung cancer & pulmonary fibrosis, chromosomal aberrations, and alteration in replication and transcription	O'Brien et al (2001)
4.	Copper (Cu)	Industrial waste water	Food: 10 mg/kg/day	Affects renal metabolic functions	Hough et al (2004)
5.	Lead (Pb)	Crude petrol, mining & smelting, thermal, paint, power plants waste water	Food: 25 μg/dl (mg/kg/day) blood ≤70 μ/dL	Hypertension, hallucination & vertigo, dyslexia birth defects	Teo et al (1997), Martin & Griss wold (2009), El-kadi & Abdul Wahabb (2018)
6.	Mercury (Hg)	Thermal power, chemical/chlor-alkali industries waste water	10 μg/L (in whole blood)	Proteinuria allergy, kidney and lung damage	Heaton et al (2003), Abdul Wahabb (2018)
7.	Nickel (Ni)	Ni-Cd batteries, wastewater	Food; 5 mg/kg/day	Affects renal functioning	Hough et al, (2004)
8.	Zinc (Zn)	Irrigation with contaminated waste water (industrial & sewage)	Food: 59.3 mg/kg/day	Diseases related to respiratory system	Hough et al (2004)

Tripathi et al (1997) collected the samples of vegetables green gram and Amaranthus from different suburbs in Bombay during 1991-94 and calculated the health hazard coefficients of leafy vegetables having higher concentrations of lead and cadmium and found them to be < 1 and stated that the consumption of these vegetables do not pose any health risk to the consumers.

Tandi et al (2004) reported toxicological implications for poor population ingesting long time vegetables like lettuce and mustard rape irrigated with zinc and copper contaminated industrial waste water. 75 % of zinc toxicity was through lettuce and 25% was through mustard rape consumption. Copper was analyzed to show 40% toxicity in Hazare, Zimbabwe. The study concluded that zinc toxicity was more through consumption of leafy vegetables.

Hough et al (2004) evaluated risk assessment of zinc, nickel, cadmium and copper among the populations consuming food crops grown in industrial regions of UK. The HI index was found to be >1 which showed a potential health risk especially in infants. The percentage HI associated with different heavy metals is observed to be in the following order: lead-40% > cadmium-30% > copper-14% > nickel-10%.

Rattan et al (2005) studied THQ in Indian rape, spinach and gobhi sarson. Maximum THQ value was detected for zinc, copper and nickel to be 0.155, 0.015 and 0.502 for spinach, and 0.068, 0.21,0.442 for gobhi sarson respectively. The THQ values for all the metals in both the vegetables was found to be below one, so the consumption of these vegetables is not going to pose any health implications to the consumers.

Eriyamremu et al (2005) conducted a survey and analyzed the HI associated with vegetable consumers in Nigeria delta where population consume vegetables grown in heavy metal contaminated area. The heavy metal analysis of these vegetables was done in both wet and dry season and the data revealed that the vegetables were highly contaminated with lead and cadmium. The limits of heavy metals were slightly higher than that obtained in United Kingdom. The HI range was from 1.4 to 3.8 in wet and 2.6 to 4.2 in the dry season. The values obtained indicated that consumption of these vegetables can cause serious health problem to the consumers which was further authenticated by Holden and Malamud (2006).

Kachenko and Singh (2006) collected and analyzed vegetable samples from different contaminated sites in Australia. These samples were analyzed for heavy metal content and were reported to have 63% more concentration of lead and cadmium than the recommended levels in Australia. Leafy vegetables were found to have higher amounts of these metals as compared to other vegetable so daily intake of the leafy vegetables was found to pose serious health implications related with lead and cadmium intoxication. Pasquini (2006) studied the vegetable samples collected in Jos, Nigeria where these were irrigated with waste water contaminated with zinc, manganese, copper, nickel, cadmium, lead, iron. Presence of cadmium in the waste water was detected to be 10 times higher than the safe limits specified by WHO. Iron and lead concentrations were 20 to 40 times higher in lettuce. The health hazard coefficient of these leafy vegetables was found to be >1, so these vegetables were rendered unfit for human consumption. Muchuweti et al (2006) analyzed the bioaccumulation of toxic metals in food crops (maize and Tsunga leaves) cultivated in soils amended with sewage sludge in Harare, Zimbabwe. 13.68 mg/kg cadmium was detected in leaves of Tsunga which was found to be eighty times higher than the EU permissible levels of 0.2mg/kg. Similarly copper accumulation was detected which was five times higher than EU standard limits of 20 mg /kg. Lead concentration was detected to be twenty times higher than the permissible levels of 0.3mg/kg and the zinc concentration was found to be 211 mg/kg which was reported to be four times greater than the permissible value i.e., 50 mg/kg. Similarly some other vegetable and food crop samples collected from this area also had significantly higher amounts of these metals than the safe limits specified. Tsunga leaves were consumed in large amount by the urban population, so they were found to be dangerously exposed to heavy metal related health risk factors.

Zheng et al (2007) evaluated THQ among children and adults on the basis of daily intake of five vegetables grown on heavy metal contaminated sites and calculated it for adults to be 1.322, 574.3, 301.4, 526.3 292.5 µg and 1.029, 446.8, 234.5, 4095 227.6 µg for adults and children respectively. THQ and TTHQs of these vegetables for adults and children was found to be ranging from 5.79-9.90 and 7.6-13.0 respectively which indicates severe health risks to the inhabitants residing within 500-1000 meters as compared to the consumers living away from the site.

Qishlaqi et al (2008) worked out for the estimation of toxicity of waste water irrigation on food crops cultivated along the banks of Khoshk river in Iran. Vegetable samples were collected from two different sites of the river sites and a site away from the river as control. Phyto availability contents of different heavy metals was determined and compared to the control site. Leafy vegetables, spinach and lettuce had higher contamination of cadmium (HQ>1) which was reported to pose serious health hazards to the consumers.

Zhuang and coworkers (2009) carried out an investigation on the effect of copper, zinc, lead and cadmium in soil and crops grown in four villages around Dabaoshan, Southern China. The accumulation of all the heavy metals analyzed (zinc, lead and cadmium) was detected to exceeded maximum limits in all the vegetables studied. Maximum contamination was found in leafy vegetables and rice. EDI and THQ of cadmium and lead calculated on daily intake basis was much greater than permissible limits posing a health risk to the consumers.

Khan et al (2010) investigated the bioaccumulation of metals by vegetables and health risk posed to the consumers. The vegetable samples were collected from a heavy metal contaminated area in Gilgit (Pakistan) and were analyzed for heavy metals accumulation. The concentrations of copper, nickel, lead and zinc were found to be significantly higher in edible parts of spinach (*Spinacia oleracea),* mint (*Mentha sylvestris),* sarson *(Brassica campestris*) and dwarf mallow (*Malva neglecta*). The HRI values were found to be within the safe limits (<1) for all the metals studied except lead. Therefore consumption of these vegetables was declared to be safe for human consumption.

Li and colleagues (2013) monitored the health risks associated with consumption of vegetables grown in non-ferrous mine sites in Tongling mine area, China. Eight different kinds of vegetables analyzed showed the THQ for arsenic, nickel, copper, lead, cobalt and zinc in order of 17.92, 1.01, 10.14, 0.73, 0.21, and 1.93. So arsenic and copper were found to be major risk contributors for inhabitants of the area, since THQ of arsenic and copper contributed 56.10% and 31.70%, of the total THQ value calculated for all the vegetables on the basis of daily consumption by the consumers.

Xu et al (2013) collected vegetable and soil samples from a mine area in China and evaluated the bioavailability of heavy metals in the vegetable. High levels of metals (Cu, Zn, Pb and Cd) were detected in soil samples and corresponding levels in the vegetables. Uptake trend for metals was observed to be in the order: cadmium > zinc > copper > lead. Vegetables belonging to family *Astraceae* were found to accumulate more amounts of heavy metals than the vegetables belonging to family *Liliaceae*. The total target coefficient (THQ) value was found to be greater than 1 suggesting that trace metals could pose lethal health risks to the consumers.

Seid-Mohammadi et al (2014) estimated bioaccumulation of heavy metals in sixty different type of vegetables grown in contaminated areas of Hamadan (Iran) and their daily intake values. Parsley, tarragon, sweet basil and leek samples irrigated with contaminated waste water were found to have 6.24, 1.57 and 0.15 mg/kg of lead, cadmium and chromium in parsley, tarragon and leek samples respectively. Daily dietary intake of metals was detected to be 0.004, 0.008 and 0.5 mg/day for lead, chromium and cadmium which were held responsible for the heavy metal related health implications in infants of that area.

Zhou and others (2016) after analyzing the bioaccumulation of heavy metals in different groups of vegetables reported that leafy vegetables tend to bio-accumulate highest, and melon vegetables lowest amount of heavy metals in comparison to root and solanaceous vegetables studied. Total hazard coefficient (THQ) of these vegetables was calculated to be 4.12 for adults and 5.14 in case of children. The results of the study concluded that children were more susceptible to adverse health risks posed by consumption of these vegetables than the adults.

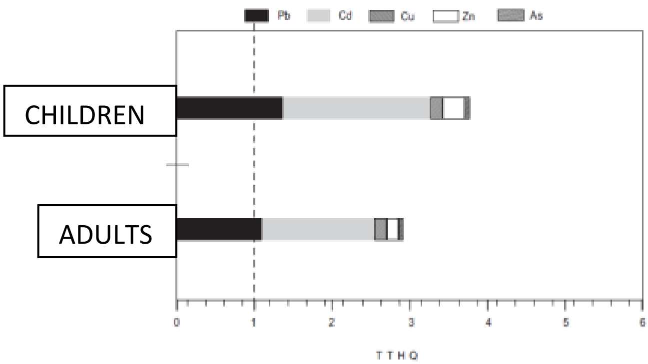


Fig 2.1: Total diet health quotient (THQ) of each metal in children and adults (Zhou et al., 2016)

Liang et al (2017) reported higher accumulation of lead, copper, chromium, cadmium, nickel and arsenic in vegetables and paddy crops grown in polluted areas of Guangdon, Southern China and found significantly higher level of all the metals except arsenic in all the vegetables studied. Chromium uptake was in order cowpea > okra > capsicum > eggplant. Leafy vegetables were found to be hyper accumulators. The HQ values for chromium, arsenic, cadmium and lead were found to be more than allowable limits. The total hazard index value of chromium was calculated to be 26.6 which can cause carcinogenic effect. Similarly, total cancer risk value calculated for arsenic, chromium, cadmium and nickel was found to be 3.4×10^{-3} which exceeds the allowable limits. Mohajer and coworkers (2014) analyzed the level of cadmium, lead and cobalt in the agricultural soils of suburban areas of Isfahan (an industrial city in central Iran). The presence of cadmium in soil was reported to be 0.8, lead 50, and cobalt 2 mg/kg respectively. The researchers related 24771 cases of cancer patients (most of them from gastrointestinal cancer) to the consumption of heavy-metal-rich vegetables grown in that contaminated area. This study was conducted for three years from 2006 to 2009. 52.8% of cancer patients were reported to be male and 47.2% females aged 59.8±3.6 years. Different workers surveyed the health risks associated with waste water irrigation in the food crops, a summary of such surveys done is represented in the following table.

Table 2.4: Various surveys conducted to assess the health risks associated with consumption of waste-water-irrigated vegetables (Dickin et al., 2016)

Survey (cross sectional)	Country	Data source	Ailments	Contamination pathways	References
327 children from a wastewater irrigation area, and 110 from control communities	Morocco	Hair samples	Health risks linked to heavy mental (Pb and Cd) consumption	Children living near irrigated areas, food	Lekouch et al (1999)

735 individuals (children and adults) from a farming population	Mexico	Blood samples	Health impacts linked to Pb exposure	Crop consumption	Cifuentes et al (2000b)
636 adults engaged in agricultural work, 108 from control community	Vietnam	Dermatological examination	Itching and chapped skin, light ulcer; dermatitis	Wastewater irrigated vegetable cultivation	Trang et al (2007c)
236 farmers from two communes, one using wastewater and another using river, rain and well water	Vietnam	Dermatological examination	Dermatitis and fungal growth	Farmers practicing aquatic plant culture	Anh et al (2007)
Samples were collected from residents in the study region of varying ages and compared	India	Samples of soils, vegetables, urine, blood, and livestock milk	Irritation of skin with black reactions possibly linked to heavy metal consumption (Zn, Cr, Cu, Ni, Co and	Consumption of contaminated food (vegetables and milk)	Chary et al (2008)

with control participants residing in the campus area			Pb)		
650 adults in 2000 households in 5 villages (using different water sources)	Cambodia	Dermatological examination	Dermatitis and fungal growth	Farmers practicing aquatic plant culture	Anh et al (2009)

Islam et al (2018) worked out for estimation of potential risks associated with human health and consumption of vegetables grown in a particular area of Bangladesh where irrigation of these vegetables was done with waste-water rich in heavy metals. Bioaccumulation of arsenic, copper, nickel, cadmium, chromium and lead in the vegetables was recorded to be significantly higher than the prescribed limits. Consumption of these metal contaminated vegetables was reported to be carcinogenic to the consumers.

Kacholi and Sahu (2018) studied the accumulation of toxic metals in water, soil and vegetables of Dar es Salaam in Tanzania. The vegetables analyzed were *Ipomoea batata*, *Amaranthus hybridus*, *Abelmoschus esculentus* and *Solanum melongena*. All the vegetables were found to carry the concentrations of metals in the following order Fe > Zn > Pb > Cu. *Ipomoea batata* was reported to have the highest concentration of heavy metals followed by *Amaranthus hybridus* > *Abelmoschus esculentus* > *Solanum melongena*. The average daily intake of lead by populations of that area was calculated to be 63 mg per person/ day which was manifold higher than level of 0.32mg/person/day endorsed by FAO/WHO (2003). HQ of lead in all three species of *Ipomoea* exceeds unity (>1), signifying serious health risk associated with the consumption of these vegetables.

Baghaie and Fereydoni (2019) in their survey to assess the probable threat of heavy metals on health among populations of Iran observed that out of 45 vegetables examined for heavy metal content and their daily dietary intake, high HQ of lead was related to cabbage and basil, cadmium and arsenic to lettuce and lowest HQ was correlated with coriander. THQ for non-carcinogenic diseases was above the standard limits and found to be much higher in females than that in males.

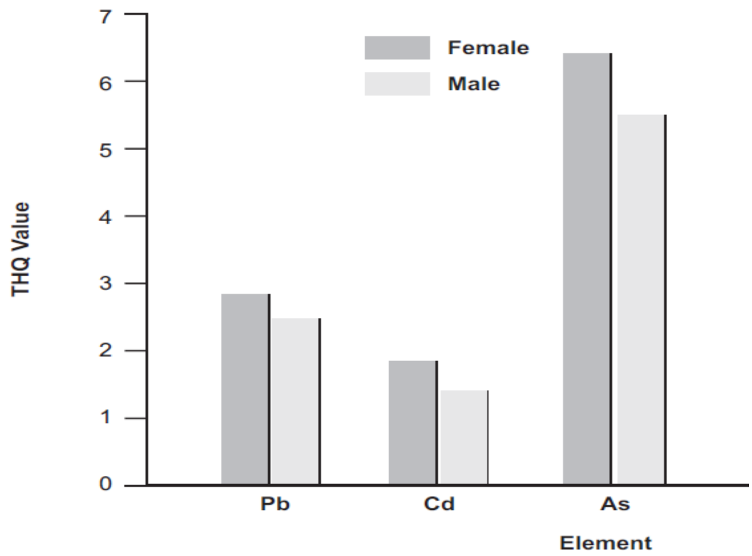


Fig 2.2: THQ values according to gender (Baghaie and Fereydoni, 2019)

3.0 MATERIAL AND METHODS

This chapter deals with the methods, techniques and equipments used for estimation of physiological and biochemical parameters of soil, water and vegetable studies and also the statistical methods applied to analyze and interpret the data obtained.

3.1 Experimental Area

Prior to finalizing the experimental area, an extensive survey of the agricultural fields along the banks of Buddha Nullah and nearby fields was done, where vegetables are cultivated as a main crop by the local farmers. Buddha Nullah is a 38 kilometer long water rivulet which runs parallel to sutlej on the south for a fairly large course in ludhiana (14 kilometer) and joins the Sutlej on north west corner. It has become the sullage/sewage and industrial effluent carrier for one of the most populated and industrial cities of India, Ludhiana. After survey, an appropriate area near Mota Singh Nagar was selected as the waste water irrigation site for experimentation where 35 acres of land was used to grow different types of vegetables and the waste water of Buddha Nullah was used for irrigational purposes. Another site water was selected as control site for the present research work 4 kilometers away from Buddha Nullah, where the same vegetables were grown and irrigated with normal borewell water.

Control site **waste-water site**

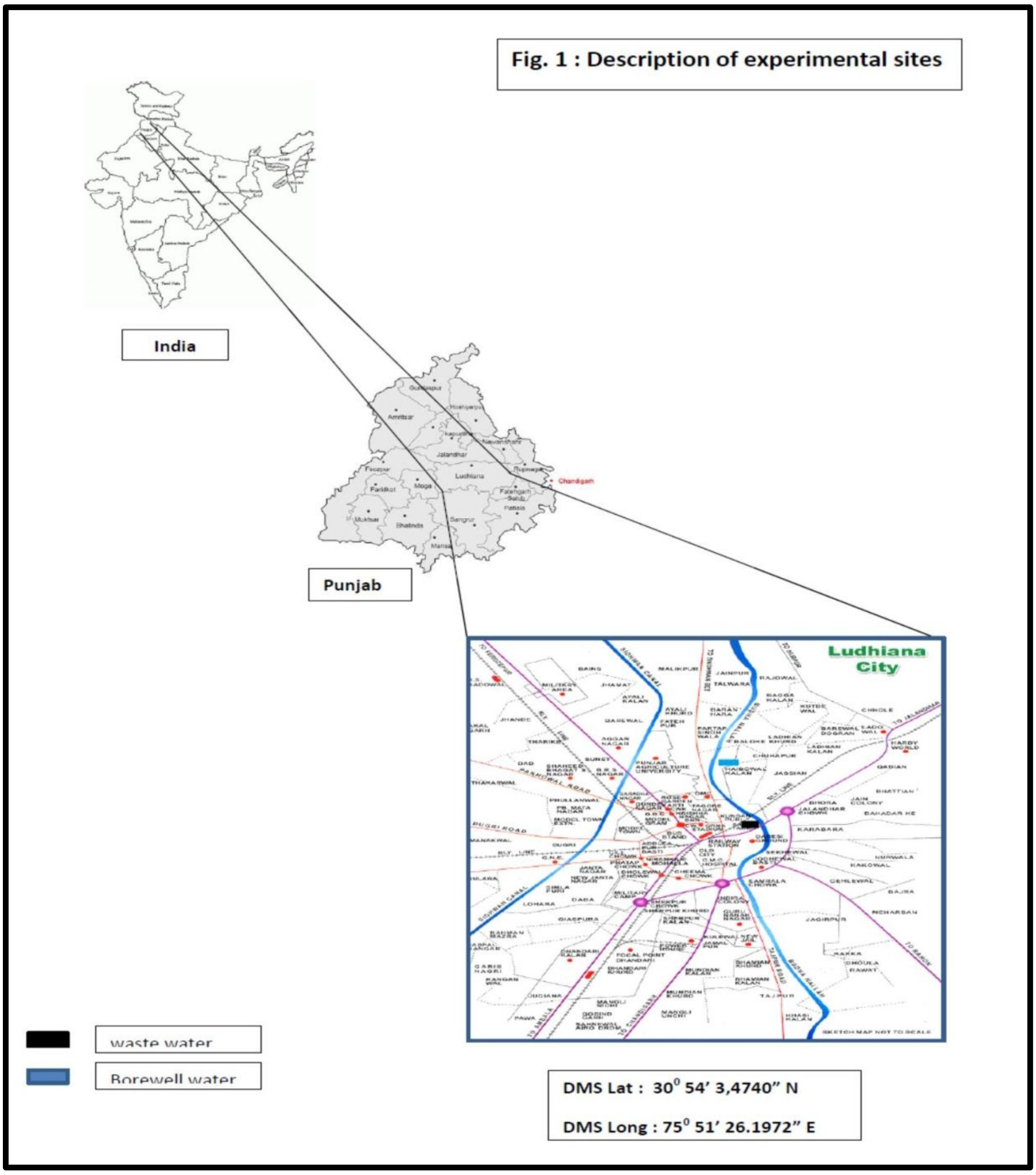
Picture 3.1: Borewell water - irrigated and waste-water-irrigated sites

Authentic seeds of the selected vegetables (*Raphanus sativus* and *Spinacia oleracea*) and 40 day old seedlings of *Brassica oleracea* and *Lycopersicum esculentum* were procured from seed store and nursery of Punjab Agricutural University, Ludhiana to raise these vegetable crops on three equal sized plots (25 feet×50 feet) on both the experimental sites simultaneously. Vegetables were sown, transplanted, grown and irrigated as per the required agricutural practices recommended by Punjab Agricultural University. The vegetables grown on waste water site were regularly irrigated with waste water of Buddha Nullah and that of control site with normal borewell water. Same varieties of all the vegetables selected for experimentation were cultivated on both the sites at the same time and under same climatic conditions. Water, soil and vegetable samples were collected from both the sites at the same time for further analysis. A brief account of agronomical practices adopted to raise the vegetable crops selected for this reseach has been summarized in the table given below:

Table 3.1 : Duration and irrigation frequency of vegetables May 2017-April 2019

Vegetable	Time of sowing	Time of maturity/sample taken	Distance between rows and plants	Irrigation	Total no. Of irrigations
Raphanus sativus	Last week of May 2017, 2018	Second week of June 2017,2018 (35 days)	45cm×7.5cm	After every 5 days	7
Spinacia oleracea	Second week of May 2017, 2018	Second week of June 2017,2018 (30 days)	20cm×5cm	After every 5 days	6
Brassica oleracea	Last week of September 2017, 2018	First week of January 2018,2019 (100 days)	45cm×30cm	After every 8 days	12
Lycopersicum esculentum	Last week of November 2017, 2018	First week of March 2018,2019 (120 days)	75cm×30cm	After every 8 days	15

The current work deals with study of bioaccumulation of heavy metals and their effect on biochemical parameters of different types of vegetables (Root, Leafy, Flower and Fruit)



Picture 3.2: Aerial view of the experimental area

Buddha Nullah passes though the metropolitan and industrial city Ludhiana (30.900965^0 N; 75.857277^0 E) which has a humid, subtropical climate with average annual rainfall of 899 mm and the temperature varies from 10^oC to 40^oC throughout the year.

3.2 Experiments done under objective no. 1

3.2.1 Collection and preservation of samples in triplicate of water, soil and vegetables

Samples of waste water and borewell water, soil and vegetables were collected randomly in triplicate from both waste water (Buddha Nullah) and borewell water (control) experimental sites.

3.2.1.1 Water samples

Grab and integrated method given by Keith (1996) was used to collect the samples from both the sites. Autoclaved and labelled glass bottles and plastic bottles made of fluorinated polymers were used for collection of samples. The samples were immediately taken to the laboratory for analysis and stored at $4^{o}C$ for further analysis. Water samples from both the sites were collected during the month of May and December (2018, 2019).

3.2.1.2 Soil samples

From both the sites, soil samples were collected from a depth of approximately 10-20cms by using *khurpa* and were properly sealed in labelled fresh and sterile zip - lock bags and brought back to laboratory in ice containers and kept at $4^{o}C$ for further analysis. The samples were ground to powdery form, air dried and sieved by using 2 mm sieve to manually remove the other contaminants before analysis. Soil samples were collected from both the sites during the month of May and December (2018, 2019).

3.2.1.3 Vegetable samples

Vegetable samples from both the waste water and control experimental sites were randomly collected in triplicate and were properly sealed in labelled fresh and sterile zip - lock bags and brought back to the laboratory in ice containers and kept at $4^{o}C$ for further analysis. Vegetable samples were collected from both the sites at the same time during both the years of study.

3.3 Experiments done under objective no. 2

3.3.1 Heavy metals (Walsh, 1955)

The heavy metal (As, Cd, Cr and Pb) analysis of the water, soil and vegetable samples was carried out at Department of Soil Sciences, Punjab Agricultural University, Ludhiana. Wet digestion method using Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) was followed for analysis (Model: 240FSAA Make: Agilent Technologies).

3.4 Experiments done under objective no. 3

3.4.1 Physio-chemical analysis of Waste & Borewell Water

3.4.1.1 Color (CPCB, 2012:7)

Principle: When light is passed through pure water, the water exhibits a light blue colour but the presence of organic matter changes the colour.

Apparatus: Glass comparator

Procedure: The colour of water samples (waste water and borewell water) was determined by visually comparing it with that of glass comparator.

3.4.1.2 Temperature (APHA, 1998)

Principle: Temperature is measured with a glass thermometer, either alcohol/toluene filled or mercury filled, with 0.1^0C graduations.

Apparatus: Mercury thermometer

Procedure: Temperature of water sample was measured in the field. A mercury thermometer was immersed in surface water for 1 minute and temperature reading was recorded.

3.4.1.3 pH (APHA, 1998)

Principle: Acidity or basicity character of a solution at a given temperature is indicated by hydrogen ion concentration and the pH is expressed as a negative logarithm of this hydrogen ion concentration. Its values from 0 - 7 represent acidic and from 7-14 indicate the alkaline nature of the solution. At pH 7, solution is considered to be neutral.

Apparatus: pH - Potentiometer

Procedure: Before measuring pH value of water samples, the electrodes of potentiometer were rinsed with distilled water and then were calibrated using standard buffer solution and blotted dry. The water samples were stirred and the pH values were noted by immersing the electrodes in it

Calculation: Measurement of pH was obtained directly from the instrument.

3.4.1.4 Electrical conductivity (APHA, 1998)

Principle: The presence of ionized substances in water determines the capacity of water to carry an electric current. It is a practical estimation of dissolved mineral content in water. The amount of ionized substances present in water is directly proportional to its current carrying capacity.

Apparatus: EC measuring instrument.

Procedure: First of all using a standard KCl solution, the conductivity of conductivity meter was standardized and after that the Electrical Conductivity of both borewell and waste water samples was measured by this conductance measuring instrument. Results were expressed in mhos/cm. EC was measured at 25°C.

3.4.1.5 Biochemical oxygen demand (APHA, 1998)

The BOD of the water samples was determined by incubating the samples at 20^oC for 5 days.

Principle: This test measures the oxygen utilized for biochemical degradation of organic material (Carbonaceous demand) and oxidation of inorganic material such as sulphides and ferrous ions during a specified incubation period of 5 days at 20°C.

Reagents

1. Phosphate buffer: It was prepared by dissolving 3.5 g K_2HPO_4, 335 g of $Na_2HPO_47H_2O$, 21.5 g of K_2HPO_4 and 1.7 g NH_4Cl in 1000 ml of distilled water.
2. Manganous sulphate solution: It was prepared by dissolving 220 g of $MgSO_4$ in 1000 ml of distilled water.
3. Calcium chloride solution: It was prepared by dissolving 27.5 g of $CaCl_2$

(anhydrous) in 1000 ml of distilled water.

4. Ferric chloride solution: It was prepared by dissolving 0.25 g of ferric chloride in 1000 ml of distilled water.
5. Sodium sulphate solution: It was prepared by dissolving 1.575 g of sodium thiosulphate in 1 litre of boiling distilled water.
6. Alkaline iodide sodium azide solution: It was prepared by dissolving 700 g of KOH and 135 g of NaCl in 1000 ml of boiling distilled water.
7. Starch indicator: It was prepared by dissolving 2 g of starch in 100 ml of hot distilled water.

Procedure: 10 ml of sample was pipetted in BOD bottles of 300 ml capacity. 2 sets of BOD bottles were prepared. 2 ml of manganous sulphate solution was added and immediately after, 2ml of alkaline iodide sodium azide reagent was added. The mixture was allowed to settle and 2 ml of conc. H_2SO_4 was added. One set was placed in the BOD incubator and allowed to incubate for 5 days at 20^{o}C. Standard sodium thiosulphate solution was used for titration until yellow colour disappeared. 1 ml of starch solution was added and titrated till the disappearance of blue colour. The volume of sodium thiosulphate used was noted.

Calculation: The BOD was calculated using the formula given below:

$$\text{BOD (mg/l)} = \frac{(D_1 - D_2)}{P}$$

Where

D_1 = Initial DO in sample (mg/l)

D_2 = DO after 5 days of incubation (mg/l)

P = Decimal fraction of the sample used.

3.4.1.6 Chemical Oxygen Demand (APHA, 1998)

Principle: This test determines the oxygen requirement equivalent of organic matter that is susceptible to oxidation with the help of strong chemical oxidant.

Reagents

1. Potassium dichromate solution (standard): It was prepared by dissolving 12.259 g of $K_2Cr_2O_7$ in 1 L of distilled water.
2. H_2SO_4 reagent: It was prepared by dissolving 10 g silver sulphate in 1 L of conc. H_2SO_4 and allowed to stand for 48 hours before use.
3. Ferrous ammonium sulphate solution standard (FAS): It was prepared by dissolving 98 g of ferrous ammonium sulphate (0.25 N) in 400 ml of distilled water. 20 ml of con. H_2SO_4 was added and resultant solution was diluted to 1000 ml.
4. Ferroin indicator: It was prepared by dissolving 695 mg of ferrous sulphate and 1.485 g of phenolphthalein monohydrate in 100 ml of distilled water.
5. Potassium hydrogen phthalate (KHP): It was prepared by dissolving 425 mg of KHP in 100 ml of distilled water.

Procedure: Some glass beads were placed in the reflex flasks containing 50 ml of sample diluted to 100 ml. 1 g of mercuric sulphate was added into it. After adding 5 ml of H_2SO_4, the solution was stirred to properly dissolve mercuric sulphate. Reflux flasks were cooled and 25 ml of 0.025 N K_2CrO_7 was slowly added into it. 70 ml of silver sulphate-sulphuric acid solution was added into it. The flask were heated and refluxed for 2 hours. Acidic solution was diluted to 300 ml. Before titration 10 drops of ferroin indicator were added and the excess of dichromate was titrated using 0.25N ferrous ammonium sulphate solution till the end point i.e., changes in colour to reddish-brown. Same procedure was adopted and blank samples were refluxed using the distilled water.

Calculation

COD (mg/l) = $(A-B) \times N \times 8000 / S$

A = amount in ml of FAS utilized for blank

B = amount in ml of FAS utilized for sample

N = FAS normality

$8000 = 1000 \times$ Millieq. wt. of O_2

S = ml sample used

FAS = Ferrous Ammonium Sulphate

3.4.1.7 Total suspended solids (APHA, 1998)

Principle: A well-mixed sample is filtered through a weighed standard glass fibre filter paper and the residue retained on the filter was dried to a constant temperature at 103 to 105^0C. The increase in weight of the filter represents the total suspended solids.

Procedure: Filter papers were first dried in an oven at 103 to 105^oC for 1 hour and then were cooled in desiccators. Next the filter papers weights were taken. After this, filter papers were fit into suction apparatus and 200 ml samples were passed through these. Then the papers were oven dried again at 103 to 105^0C for 1 hour, cooled in desiccators and weighed.

Calculation

$$\text{mg total suspended solids l}^{-1} = \frac{(A - B) \times 1000}{\text{Amount of sample taken}}$$

where,

A = weight of filter paper + dried residue (mg)

B = initial weight of filter paper (mg)

3.4.1.8 Total dissolved solids (APHA, 1998)

Principle: A well-mixed sample is filtered through a standard glass fibre filter paper, and the filtrate is evaporated to dryness in a weighed dish and dried at 180°C until constant weight has been arrived. The increase in dish weight represents the total dissolved solids.

Procedure: 100 ml of filtrate of samples during analysis of TSS were placed onto previously weighed evaporating dish and evaporated to dryness in a drying oven for at least 1 hour at 180 ± 2°C, cooled in desiccators and weighed.

Calculation

Total dissolved solids mg l^{-1} = $\frac{(A - B) \times 1000}{\text{ml of sample taken}}$

where,

A = weight of dried residue + dish (mg)

B = weight of dish before use (mg)

3.4.1.9 Nitrate (APHA, 1998)

Principle: The amount of nitrate is determined by measuring the absorbance of a sample containing 1 ml of HCl (1 N) at 220 nm. The concentration was calculated from graph of standard nitrate solution range 1-11 mg/l.

Reagents

1. Phenol disulphonic acid (PDA): took 25 gm of phenol (white) in a beaker and 150 ml of conc. sulfuric acid was added to it, and the resultant was heated for two hours.

2. Ammonium hydroxide (Concentrated)

Procedure: Nitrate was determined using a UV spectrophotometer. The water samples were neutralized to pH 7 and 2 ml of Phenol disulphonic acid reagent was added to it. By adding 10 ml of conc. NH_4OH the volume of solution was made up to 100 ml. Intensity of colour was red at 410 nm. The Nitrate was calculated against standard calibration.

3.4.1.10 Sulphate (SO_4^-)

The sulphate content was estimated by the turbidity metric method suggested by APHA (1998).

Principle: Sulphate ions get precipitated with $BaCl_2$ in acetic acid medium giving rise to crystals of $BaSO_4$ and absorbance of light is measured by a photometer.

Reagents

1. Buffer solution: It was prepared by dissolving 30 gm of Magnesium chloride, 5 gm Sodium acetate, 1 gm Potassium nitrate and 20 ml acetic acid in 1000 ml of distilled water.

2. Standard sulphate solution: It was prepared by dissolving 0.1479 gm anhydrous sodium sulphate in 1000 ml of distilled water.

Procedure: Water sample (50 ml) was diluted to 100 ml by adding distilled water and transferred to a 250 ml Erlenmeyer flask. After adding buffer solution and constantly stirring the flask, a spatula of $BaCl_2$ crystals was added. Turbidity of the suspension was measured by a photometer and the amount of sulphate was calculated by comparing against a standard curve.

Calculation

$MgSO_4$ (mg/l) = {1000 × $MgSO_4$}/ S

S = volume of sample used

3.4.1.11 Total Phosphate (PO_4^{3-})

Estimation of Phosphate was carried out using stannous chloride method suggested by APHA (1998).

Principle: Ammonium molybdate reacts with orthophosphate to form molybdophosphoric acid which is further reduced to molybdate by reacting with stannous chloride. The appearance of blue colour of this reaction can be measured at 690 or 880 nm.

Reagents

1. Ammonium molybdate solution: It was prepared by dissolving 25 gm of ammonium molybdate in 175 ml of distilled water. 280 ml of conc. Sulfuric acid was added to 400 ml of distilled water and allowed to cool. Both the solutions were mixed and final volume was raised to 1000 ml by adding distilled water.

2. Stannous chloride solution: It was prepared by dissolving 2.5 gm of stannous chloride in 100 ml of glycerol and heating it in a water bath.

Procedure: 100 ml of sample was taken in a Nessler tube. 4 ml of ammonium molybdate was added into it followed by 0.5 ml of stannous chloride and mixed well and allowed to settle for 10 minutes. The intensity of colour was measured at 690 nm immediately after.

Calculation: The quantity of phosphate was measuring by comparing it with a standard calibration curve.

3.4.1.12 Sodium (APHA, 1998)

Principle: Trace amount of sodium can be determined by flame emission photometry at 589 nm. Sample is passed through a gas flame under carefully controlled, reproducible excitation conditions. The sodium resonant spectral line at 589 nm is isolated by interference filters or by light-dispersing devices such as prisms or gratings. Emission light intensity is measured by a phototube, photomultiplier or photodiode.

Apparatus: Flame photometer (Systronics, Flame photometer 128)

Reagents

1. Stock sodium solution (1.00 ml = 1.00 mg Na)
2. Standard sodium solution

Procedure: Flame emission photometer was calibrated with different sodium standard solutions. The sample was then passed through the flame and sodium concentration was recorded from the instrument. Sample was diluted when required.

3.4.1.13 Chloride (APHA, 1998)

Principle: This method determines the chloride ion of a solution by titration with $AgNO_3$. As the $AgNO_3$ is slowly added, a precipitate of AgCl is formed. The end point of titration occurs when all the chloride ions are precipitated.

Reagents

1. Potassium chromate indicator: It was prepared by dissolving 50 gm of potassium dichromate in distilled water. Silver nitrate was added till the formation of red coloured precipitate. It was kept for 12 hrs, filtered and diluted to 1000 ml.
2. Silver nitrate: It was prepared by dissolving 2.39 gm of silver nitrate in 1000 ml of distilled water.
3. Sodium chloride (0.0141 N): It was prepared by dissolving dried 824.1 mg NaCl in 1000 ml of distilled water.

Procedure: Argentometric method was used to determine the amount of chloride. The pH of 50 ml water sample was adjusted to 7.5 followed by addition of 1 ml $K_2Cr_2O_7$. This mixture was titrated against a standard solution of $AgNO_3$ standardized against standard NaCl till precipitation of pale red colour. The amount of $AgNO_3$ used was noted. A blank solution was also titrated with $AgNO_3$ and amount of $AgNO_3$ used was noted.

Calculation

Cl^- mg/l = (A-B) × N ×35.45 × 1000 / S

Where,

A = amount of $AgNO_3$ used for sample

B = amount of $AgNO_3$ used for blank, and

N = Normality of $AgNO_3$ solution

S = Volume of sample

3.4.1.14 Potassium (APHA, 1998)

Principle: Trace amounts of potassium can be determined in either a direct-reading or internal-standard type of flame photometer at a wavelength of 766.5 nm.

Apparatus: Flame photometer (Systronics, Flame photometer 128)

Reagents

1. Stock potassium solution (1 ml = 1 mg K)

2. Standard potassium solution

Procedure: Flame emission photometer was calibrated with different potassium standard solutions. Then the sample was passed through flame and potassium concentration was recorded from the instrument. Sample was diluted when required.

3.4.1.15 Total Hardness (APHA, 1998)

Principle: It is not a specific constituent of water but the characteristics of water altered by the presence of polyvalent ions, especially those of calcium and magnesium.

Procedure: 50 ml of water sample was buffered and after adding 5 drops of the dye (Eriochrome black T). Then solution was titrated till the appearance of wine red colour. The volume of EDTA used was represented as A. Similarly the amount of EDTA used was determined using blank reagent and the amount used was represented as B. Amount of EDTA used for sample represented as C was determined by subtracting amount B from A.

Calculation

Total hardness due to $CaCO_3$ was calculated by the following formula:

$CaCO_3$ (mg/l) = $C \times D \times 1000 / S$

Where,

C = EDTA volume required for sample

D = $CaCO_3$ mg equivalent to 1 ml EDTA titrant

S = Volume of sample taken

3.4.2 Physio-chemical analysis of the soil samples:

3.4.2.1 pH (Jackson, 1973)

Principle: pH of soil determines its acidity or alkalinity. pH > 7 shows that the soil is alkaline and Ph < 7 shows that it is acidic in nature.

Apparatus: pH potentiometer.

Procedure: 30g of soil sample was dissolved in the same amount of distilled water. The slurry of the solution was made by continuous stirring and allowed to rest for 1 hour to stabilize the temperature and pH. By using the standard phosphate buffer (pH 7), the electrodes of pH meter were standardized before determining the pH of soil slurry. Then the electrodes were put in the soil slurry after stirring it for a while and the pH was recorded.

3.4.2.2 Electrical conductivity (Richards, 1954)

Principle: The amount of soluble salts present in soil is proportional to the conductivity of electric current passed through it and it depends upon the ions of salts present in the water.

Apparatus: Conducting meter.

Procedure: The soil suspension was prepared by using soil and water in ratio of 1:2. After continuous stirring for a while, the samples were allowed to rest overnight to obtain a clear solution. The electric current was passed through the suspension by using a conducting meter and EC of the soil sample was measured and represented as decisiemens per meter (dSm^{-1}).

3.4.2.3 Trace elements (Lindsay and Norvel, 1978)

Reagents

DPTA: was prepared by dissolving 0.005M DPTA and 0.01M calcium chloride in 0.1 tri ethanol buffer (pH 7.3).

Procedure: 10 gm of the soil sample was extracted with 20 ml of diethylenetriamine. The resultant solution was used for detection of toxic trace elements like Zn, Fe, Cu and Mn using ICAP-AES.

3.4.2.4 Heavy metals (Walsh, 1955)

Atomic absorption spectrophotometric method was used for the analysis of heavy metals. Preparation of aqua regia was done by mixing perchloric acid ($HClO_4$) and concentrated nitric acid (HNO_3) in ratio 1:4 v/v. 0.5 g of soil sample was taken and 15 ml of aqua regia was added to it and mixed well. The mixture was digested in a digestion chamber till the colour disappeared. The mixture was further diluted to 30 ml and filtered. The resultant solution was cooled and the final volume was raised to 50 ml by adding distilled water and transferred to a volumetric flask (50ml). This solution was analyzed to detect the amount of heavy metals using ICP-AES.

3.5 Experiments done under objective no. 4

3.5.1 Estimation of biochemical parameters

3.5.1.1 Seed germination

Procedure: The seeds of radish, spinach, tomato and cauliflower were procured from the seed store of Punjab Agricultural University, Ludhiana. The seeds were thoroughly washed with distilled water and surface sterilized by using 0.1% mercuric chloride solution. 25 seeds were taken in different petri-dishes with double layered filter paper and soaked in 100 ml of waste and borewell water separately for 12 hours at 25±1° C. The germination percentage, root and shoot length was recorded at 24, 36, 72 and 96 hours. The treatments were taken in triplicates.

Calculations

Following formulas were used to determine the seed germination parameters.

1. Germination percentage **(Ruan et al; 2002):**

$GP = N_g / N_1 \times 1000$

Where, GP = germination percentage

N_g = No. of seeds germinated

N_1 = Total no. of seeds set for test

2. Relative toxicity **(Chapagain, 1991)**

$T_r = B - W / B \times 100$

Where, T_r is relative toxicity percentage

B = Germination percentage in control at particular hour of incubation

W=Germination percentage in waste water treated seeds at same hour of incubation

3. Vigour Index **(Abdel-baki and Anderson, 1973)**

$V_1 = P \times L$

Where, V_1 = Vigour Index

P = Germination percentage

L = Seedling length

4. Fresh weight (FW): Fresh weight was calculated by randomly selecting 10 seedlings from each treatment and weight measured by weighing balance and weight per plant was calculated.

5. Dry weight (DW): the seedlings used for fresh weight were dried in hot air oven at 65°C for 24 hours and the dry weight was measured.

3.5.1.2 Enzyme activity

Five oxidoreductase enzymes were selected for this study viz. superoxide dismutase, catalase, peroxidase, glutathione reductase, ascorbate peroxidase and their estimation was done from roots, leaves, flower, and fruits of the plant according to the standard protocols as under.

3.5.1.2.1 Cell-free extracts preparation

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

3.5.1.2.2 Catalase (CAT)

The enzyme activity was assayed according to the method given by **Sinha (1972).**

Procedure: To prepare the reaction mixture, 0.5 ml of 0.2 M phosphate buffer (pH 7) was taken and 0.1 ml of properly diluted enzyme extract was added. Then by adding 0.4 ml of 0.2 M H_2O_2, the mixture was incubated at 37^oC for 5 minutes. After adding 3 ml of solution (5% w/v mixture of glacial acetic acid and potassium dichromate in ratio 3:1 v/v), the tubes were kept in boiling water bath. In one of the test tubes, enzyme extract was added after the reaction stopped this tube was labeled as control. The absorbance of the control and test samples was measured at 570 nm after cooling the tubes. Absorbance of test samples was subtracted from that of the control samples in order to determine residual amount of H_2O_2. The quantity of enzyme required for oxidation of 1mM of H_2O_2 per minute is termed as 1 unit of enzyme activity under assay conditions.

Calculations

Amount of half oxygen liberated from peroxide is referred to as 1 unit of enzyme activity.

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

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

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

3.5.1.2.3: Peroxidase (POX)

Guaiacol peroxidation method given by **Britton and Maehly (1955)** was used to determine the activity of peroxidase.

Procedure: Preparation of reaction mixture was done by taking 100 µl of enzyme extract by adding 8 nM of guaiacol and 10 nM of buffer solution (pH 7) in it. The initiation of enzyme activity was done by addition of 2.75 nM of hydrogen peroxide. Using a uv spectrophotometer, the absorbance was recorded at 470 nm and increase in absorbance indicating the formation of tetraguaiacol. Change in absorbance rate was observed to calculate the peroxidase activity and it was expressed as unit/mg of protein.

Calculations

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

Where, Extinction coefficient = 6.39×10^{-3} mM^{-1} cm^{-1}

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

3.5.1.2.4 Glutathione reductase (GR)

The activity of enzyme glutathione was determined by the method suggested by **Halliwell and Foyer (1978).**

Procedure: The reaction contained 2 ml of 0.1M buffer solution (pH 7), 5 ml of glutathione (oxidized), followed by the addition of 0.1 ml of enzyme extract and 0.2 nM NADPH. Oxidation of NADPH led to decrease in absorbance which was measured at 340 nm. Using same procedure oxidation of NADPH in enzyme extract free solution was also measured and subtracted from the value of oxidized NADPH. 1.0 µM of NADPH oxidized/minute is referred to as 1 unit of enzyme activity.

Calculations

Following formula was used to calculate the unit enzyme activity.

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

Where, Extinction coefficient = 6.22×10^{-3} mM^{-1} cm^{-1}

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

3.5.1.2.5 Superoxide dismutase (SOD)

Superoxide dismutase activity was determined according to the method given by **Kono (1978).**

Principle: This method works on the principle that superoxide radicals have an inhibitory effect on the reduction of nitro blue tetrazolium which are generated by hydroxylamine hydrochloride autoxidation.

Procedure: 500µl nitro blue tetrazolium, 100µl triton-X-100 and 1.3 ml of sodium carbonate buffer were taken in test cuvettes. Initiation of the reaction was done by adding 100µl hydroxyl amine hydrochloride. 70µl of the enzyme extract was added after 2 minutes and the absorbance was read at 540 nm. The percent inhibition in the rate of reduction in nitro blue tetrazolium was recorded.

Calculations

To calculate the enzyme activity, the following formula was used,

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

3.5.1.2.6 Ascorbate peroxidase (APX)

Ascorbate peroxidase activity was determined by the method suggested by **Nakano and Asada (1981).**

Procedure: 2.25 ml of buffer solution (pH 7) was taken and mixed with 0.2 ml of 0.5 mM of ascorbate (ascorbic acid). To this 2.7 ml of reaction mixture, 0.05 ml of enzyme extract was added and the reaction was initiated by adding 0.1mM H_2O_2. Using a spectrophotometer, the absorbance was read at 290 nm. By observing the decrease in absorbance, the oxidation of ascorbic acid was determined. 1 mole of ascorbic acid oxidized per minute at 290 nm represents 1 unit of enzyme activity.

Calculations

Quantity of enzyme needed to oxidize 1 μM of ascorbate min^{-1} g^{-1} fw is defined as one unit of enzyme activity.

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

Extinction coefficient = 2.8 × mM^{-1} cm^{-1}

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

3.5.1.2.7 Ascorbic acid

Measurement of total ascorbic acid was carried out according to the procedure given by **Davis and Masten (1991)**

Procedure: 5 mg of fresh sample was extracted by adding 1% phosphate citrate buffer in a chilled pestle and mortar. After homogenizing the mixture, the centrifugation was done for 5 minutes at 4^oC and 14000 rpm. In the resultant supernatant, after adding 1.72 nm of 2,6-l -DCPIP the absorbance was read at 518 nm.

3.5.1.2.8 Total Flavonoids

Method given by **Marinova et al (2005)** was followed to estimate the total flavonoids in the samples.

Procedure: 1 ml of sample extract was diluted to 4 ml by addition of distilled water.

After few seconds, 0.3 ml of sodium nitrite (5%) was added, followed by addition of 0.3 ml NH_4Cl (10%). Mixture was stirred for 5 minutes and then 2 ml of sodium hydroxide (1M) was added. By adding 2.4 ml of distilled water, the total volume was raised to 10 ml. Vortex mixture was used to mix the solution thoroughly. Absorbance was then read at 510 nM and results were expressed as mg rutin/gm sample weight.

3.5.1.2.9 Proline (Bates et al., 1973)

Principle: Proline is a basic amino acid in plants and it performs a major role during stress conditions. Many investigators have reported several-fold increase in the proline content under abiotic and physiological stress conditions, though the molecular mechanism has not yet been established for the increased level of proline. During selective extraction with aqueous sulphosalicylic acid, proteins are precipitated as a complex. Other interfering materials are also presumably removed by absorption to the protein-sulphosalicylic acid complex. The extracted proline is made to react with ninhydrin in acidic conditions (pH 1.0) to form red coloured chromophore complex, which is measured at 520 nm.

Apparatus

1. Spectrophotometer (Perkin Elmer, Lambda 35 UV/VIS Spectrometer)

2. Centrifuge (Remi, model: R – 24)

Reagents:

1. Acid ninhydrin

2. 3% aqueous sulphosalicylic acid

3. Glacial acetic acid

4. Toluene

5. L–proline

Procedure: 50 ml of fresh weight aliquots were mixed with 1 ml ethanol::water (40::60 v/v). The resulting mixture was left overnight at $4^{o}C$ and then centrifuged at 14000 g for 5 minutes. The mixture was stored at $-20^{o}C$. In a known dilution, ninhydrin % (v/v) and acetic acid glacial 60% v/v were added followed by ethanol

20% v/v. The tube was sealed and heated at 95^oC for 20 minutes, cooled and centrifuged at 2500 rpm for 1 minute. 100µl of the mixture was taken and the absorbance was read at 520 nm. The proline content was estimated by comparing with a standard curve.

3.5.1.2.10 Total Phenols (Marinova et al., 2005)

Principle: Transfer of electrons in alkaline medium from phenolic compounds form a blue chromophore constituted by phosphotungestic/phosphomolybdenum complex where a maximum absorption depends on the concentration of phenolic compounds.

Procedure: 0.5g of dried sample was dissolved in 50 ml of methanol. 10µl of sample extract was taken in a test tube and 2.5 ml of 10-fold diluted Folin-Ciocalteu reagent was added into it. After waiting for 5 minutes, 2.5 ml of 7 % sodium carbonate solution was added. Mixture was allowed to incubate for 1 hour at room temperature. The measurement of absorbance of the reaction mixture was recorded at 725 nm. Amount of total phenols was calculated by comparing against a standard curve using the following formula:

$$C = C1 \times V/N$$

Where, C = Total content of phenol

C1 = Concentration of gallic acid established

V = Volume of extract from calibration curve

N = Weight of the plant extract in grams

3.5.1.2.11 Carotenoids (Lichtenthaler et al., 1983)

100 mg of fresh sample was taken and ground in 10 ml of 80 % acetone using a chilled pestle and mortar. The resultant was centrifuged at 3000 rpm for 10 minutes. The pellet was discarded and supernatant was taken in a test tube. The supernatant volume was raised up to 10 ml and O.D. was read at 480 nm using a UV spectrophotometer.

Calculations

The amount of Carotenoids was calculated using the following formula

Total amount (mg/100g) = 4 × O.D. values × total volume of the sample

3.5.1.3 Total Proteins

To determine the total protein content, method given by **Lowry et al (1951)** was followed.

Principle: The amino acid tyrosine and tryptophan present in proteins reduces Folin-Ciocalteu to blue colour.

Using a chilled pestle and mortar, 1g of sample was homogenized by adding 3 ml of 100 mM potassium phosphate buffer (pH). Centrifugation of the homogenate was done for 20 minutes at 4^{o}C and 1500 rpm. The supernatant of this solution was taken for estimation of proteins.

Reagents:

1. a: It was prepared by dissolving 2% of Na_2CO_3 in 0.1N NaOH

2. b: It was prepared by adding 0.35% of $CuSO_4$ in 1% $KNaC_4H_40_6.4H_20$

3. c: Reagents a and b were mixed in ratio 50:1

4. d: Folin-Ciocalteu reagent

Stock solution: 50 ml Bovine serum was dissolved in 50ml of distilled water to prepare the stock solution for standard curve

Procedure: Equal amounts of standard and sample solution (0.1 ml) was poured in a series of test tubes and the final volume was raised to 1 ml by addition of distilled water. Only distilled water was used as blank. 5 ml of reagent c was added in each test tube and after proper mixing, the solution was allowed to rest. After 10 minutes, 0.5 ml of reagent 'd' was added and the solution was kept at room temperature in dark for half an hour. As soon as the blue colour appeared, the absorbance was observed at 660 nm.

Calculations: A graph representing concentrations versus absorbance was plotted by using a standard solution of protein. The protein content of sample was calculated from the standard curve and represented as mg/g fresh weight.

3.5.1.4 Estimation of Chlorophyll

To estimate the chlorophyll content, method suggested by **Withman et al (1971)** was followed.

Principle: Total chlorophyll is extracted with 80% acetone and the absorption at 652 nm is read in a spectrophotometer. Using the absorption co-efficient, the amount of chlorophyll is calculated.

Procedure: 5g of fresh, clean leaves were taken and homogenized in 10ml acetone solution (prepared by mixing 10ml of 0.1N NaOH in 90ml of acetone) using a chilled pestle and mortar in a dark room, to prepare a fine slurry of the sample. The supernatant was put in test tubes and kept in refrigerator for 2 hours and ground again for centrifugation at 5000 rpm for 20 minutes. After that, 80% aqueous acetone was added to make the final volume 20 ml and the absorbance was noted at 646 nm and 663 nm.

Calculations: Amount of chlorophyll a and b were calculated according to the equation given by Lichtenthaler et al, (2001)

$$Chl_a\ (mg/ml) = A_{663.2} \times 12.25 - A_{646.8} \times 2.79$$

$$Chl_b\ (mg/ml) = A_{646.8} \times 21.50 - A_{663.2} \times 5.10$$

Where: $A_{646.8}$ = absorbance at wavelength 646.8

$A_{663.2}$ = absorbance at wavelength 663.2

3.5.1.5 Total chlorophyll

Following formula given by Withman et al (1971) was used to calculate the amount of total chlorophyll.

$$\text{Total chlorophyll: } Chl\ (a+b) = (19.54 \times A_{645}) + (8.29 \times A_{663})$$

3.5.1.6 Total Sugars (DuBois, 1956)

Principle: When phenols react with hydroxylmethylfurfural and furfural, coloured products are produced which are read at 490 nm.

Reagents

1. Phenol solution (5%): 5g of pure crystalline phenol was dissolved in 100ml of distilled water to prepare this solution

2. Conc. H_2SO_4 (analytical grade)

3. Standard glucose curve

Procedure: 0.5 ml of distilled water was added into 0.5 ml of sample extract to raise the final volume to 1 ml. After adding 5 ml of 5% phenol solution, the mixture was stirred using vortex mixture. The solution was allowed to cool after adding 5 ml of conc. H_2SO_4. The absorbance of resultant colour was read at 490 nm and O.D. was compared with the standard curve.

Calculations:

$$\text{Total Sugar content } (\mu g/ml) = \frac{\text{OD of sample} \times \text{Concentration of the standard}}{\text{OD of Standard}}$$

Sugar concentration was expressed as µg/ml which was converted into percentage using the following formula:

100 µg/ml = 1% sugar content

3.5.1.7 Reducing Sugars

Reducing sugars were estimated by Nelson-Somogyi method **(Syed et al., 2007).**

Principle: Reducing sugars convert copper into cuprous oxide which gives a blue colour dye which can be measured by spectrophotometer.

Reagents

1. Copper A: The solution was prepared by mixing anhydrous $NaSO_4$, 25g of anhydrous Na_2CO_3, 25 g of sodium potassium tartrate and 20 g of $NaHCO_3$, in distilled water and the final volume was raised to 1000 ml.

2. Copper B: The solution was prepared by dissolving 15 g of $CuSO_4$, in 100 ml of distilled water and few drops of conc. H_2SO_4 were added.

3. Copper C: The solution was prepared by mixing reagents A and B in 25: 1 ratio.

4. Nelson's reagent: It was prepared by dissolving 25g of ammonium molybdate in 450 ml of distilled water, 25 ml of concentrated H_2SO_4, followed by addition of 25 ml solution of disodium arsenate (prepared by dissolving 3 g of sodium arsenate in 25 ml of distilled water). Amber bottles were used to store the solution.

Procedure: In a test tube, 0.5ml of sample extract was taken and 0.5 ml of distilled water was added into it. After that, 1 ml freshly prepared copper c solution was added and the tube was kept for 20 minutes in a water bath and allowed to cool for 20 minutes. One ml of Nelson’s reagent was added and the final volume was adjusted to 12.5 ml using the measuring cylinder and the optic density was read at 510 nm. Amount of reducing sugars was calculated by comparing the values with that of standard curve. Reducing sugars concentration was expressed as µg/ml which was converted into percentage.

Calculations

$$\text{Reducing Sugar Content } (\mu g/ml) = \frac{\text{OD of sample} \times \text{Conc. of Standard} \times \text{Volume made (12.5 ml)}}{\text{OD of Standard} \times \text{Volume taken (0.5 ml)}}$$

3.5.1.8 Non-reducing sugars

The amount of reducing sugars was subtracted from total sugars to calculate the quantity of non-reducing sugars.

Non-reducing sugars = Total sugars - Reducing sugars

3.5.1.9 Starch (Clegg, 1956)

Starch is an important polysaccharide. It is the storage form of carbohydrate in plants abundantly found in roots, tubers, stems, fruits and cereals. Starch, which is composed of several glucose molecules, is a mixture of two types of components namely amylose and amylopectin. Starch is hydrolyzed into simple sugars by dilute acids and the quantity of simple sugars is measured colorimetrically.

Principle: The sample is treated with 80% alcohol to remove sugars and then starch is extracted with perchloric acid. In hot acidic medium, starch is hydrolyzed to glucose and dehydrated to hydroxymethylfurfural. This compound forms a green coloured product with anthrone.

Reagents

1. Anthrone: Dissolve 200 mg anthrone in 100 ml of ice-cold 95% sulphuric acid
2. 80% ethanol
3. 52% perchloric acid
4. Standard Glucose solution: Stock – 100 mg in 100ml water. Working Standard – 10ml of stock solution diluted to 100 ml with water.

Procedure

1. 0.1 to 0.5g of the sample was homogenized in hot 80% ethanol to remove sugars. It was centrifuged and the residue was retained. The residue was washed repeatedly with hot 80% ethanol till the washing did not give colour with anthrone reagent. The residue was dried well over a water bath.
2. To the residue, 5.0ml of water and 6.5ml of 52% perchloric acid were added.
3. Extract was kept at 0°C for 20min. It was centrifuged and the supernatant was saved.
4. The extraction was repeated using fresh perchloric acid. It was then centrifuged and the supernatant pooled and made up to 100ml.
5. Pipetted out 0.1 or 0.2 ml of the supernatant and made up the volume to 1 ml with water.
6. The standards were prepared by taking 0.2, 0.4, 0.6, 0.8 and 1ml in each tube with water.
7. 4 ml of anthrone reagent was added to each tube.
8. Heated for eight minutes in a boiling water bath.
9. Cooled rapidly and read the intensity of green to dark green colour at 630 nm.

Calculation

Glucose content in the sample was found out by comparing it with the standard curve. The value obtained was multiplied by a factor 0.9 to arrive at the starch content.

3.5.1.10 Enrichment factor (EF)

Enrichment factor (EF) of heavy metals in the soils was calculated using method given by **Kisku et al (2000)** to determine the degree of pollution soil irrigated with heavy metals containing wastewater (treated) with respect to soil irrigated with the groundwater (control) site.

$$EF = \frac{\text{Concentration of metals in soil at contaminated site}}{\text{Concentration of metals in soil at uncontaminated site}}$$

3.5.1.11 Bioaccumulation factor (BAF)

The bioaccumulation factor was calculated according to the method given by **Sajjad et al (2009)** who defined it as the relative tendency of a metal to be accumulated by a particular species of plant. Bioaccumulation factor (BAF) was calculated to understand the extent of risk and associated hazard due to wastewater irrigation and consequent heavy metals accumulation in edible portion of test vegetables (Cui et al., 2004). The BAF's of Pb, Cr, Cd and As were calculated by the equation below:

$$BAF = \frac{\text{Amount of metal present in the vegetables}}{\text{Amount of metal present in the soil}}$$

The ratio greater than 1 (>1) denotes higher accumulation of metals in plant parts than soil **(Barman et al., 2000).** However, according to **Sajjad et al (2009)** if the transfer coefficient of a metal is greater than 0.50, the plant will have a greater chance of the metal contamination by anthropogenic activities. If the ratio is greater than 1 (>1), the plants have accumulated elements, the ratios around 1 indicate that the plants are not influenced by the elements, and ratios less than 1 (<1) show that plants exclude the elements from the uptake **(Olowoyo et al., 2010).**

3.5.1.12 Different health risk assessment indices

Hazard Quotient $HQ = [W_{plant}] \times \frac{[M_{plant}]}{RfD \times B}$

HQ < 1 is safe, whereas HQ $\geq$ 1 could pose a health risk **(Chary et al., 2008)**

In the preceding equation, W_{plant} is the dry weight of the contaminated plant material consumed (mg/day), M_{plant} is the concentration of the metal in the vegetable(s) (mg/kg), RfD is the food reference dose (the maximum acceptable oral dose of a toxic substance) of the metal (mg/day), and B is the human body mass (kg).

Daily dietary intake (DDI) = $\frac{X \times Y \times Z}{B}$

DDI reflects the daily amount of a metal consumed by an individual. Whereas X indicates the metal concentration of a given vegetable, Y is the dry weight of the vegetable, Z is the approximate daily intake and B is the average body mass of consumers.

Daily intake of metal (DIM) = $\frac{C_{metal} \times C_{factor} \times D_{food}}{B\ \text{average weight}}$

Where C_{metal} is the heavy metal concentration in plants (mg/kg), C_{factor} is the conversion factor (typically 0.085 to convert fresh vegetable weight to dry weight), D food is the daily intake of vegetables, and B average weight is the average weight of the consumers **(Rattan et al., 2005; Oves et al., 2012)**

$$HRI = \frac{DIM}{RfD}$$

HRI <1 is safe, whereas, HRI $\geq$ 1 could pose a health risk over multiple metals (Oves et al, 2012) HRI can be calculated using DIM, with RfD being the food reference dose.

3.6 Experiments done under objective no. 5

3.6.1 Statistical analysis

Statistical analysis of data gathered from all the parameters studied was done to calculate mean, standard deviation and significance level by applying different statistical tests [Karl Pearson's correlation test, Student's t-test for comparison of means and one way analysis of variance (ANOVA 1)]. For this purpose, software SPSS (version 18) was used. Significance of results was considered at $p \leq 0.05$. The results are shown as Mean ± SD of the values.

3.7 Health survey (Holyk, 2008)

Awareness and health status of farmers, their families and population consuming vegetables grown on both contaminated (Buddha Nullah) and non-contaminated (borewell) sites of the study area was surveyed.

A survey was done to evaluate the health risk posed and awareness among the population of Ludhiana about the ill consequences of consuming vegetables grown in such a polluted area of the city. Total 400 people were evaluated for this purpose. Randomly 200 people (100 males and 100 females) belonging to different levels of society consuming vegetables grown on each site were selected. Questionnaire method was used to evaluate the awareness and health status of persons selected for survey. A proforma comprising following questions was printed in three languages English, Punjabi and Hindi and used for this survey

- ✓ Name of the consumer
- ✓ Residential area
- ✓ Tick the appropriate answer

1. How many times do you consume cooked vegetables?

   (a) Once a week (b) twice a week (c) thrice a week (d) more than that

2. How many times do you consume raw vegetables (salad)?

   (a) Once a week (b) twice a week (c) thrice a week (d) more than that

3. What is the frequency of consuming seasonal root vegetables (Carrot, radish, potato etc.)?

   (a) Once a week (b) twice a week (c) thrice a week (d) more than that

4. Frequency of consuming leafy vegetables (Spinach, Cabbage etc.)

   (a) Once a week (b) twice a week (c) thrice a week (c) more than that

5. Frequency of consuming fruit vegetables (tomato, brinjal, bitter gourd etc.).

   (a) Once a week (b) twice a week (c) thrice a week (d) more than that

6. From where do you purchase the vegetables you eat?

   (a) Sabzi Mandi (b) Vendors (c) Direct from the fields (d) Fields Near Buddha Nullah (e) Grow yourself

7. Has any member of your family ever suffered from, or is suffering from any of the following diseases?

   (a) Cancer (b) Skin allergy (c) Mental Illness (d) Deformity (e) Kidney diseases

   (f) Lungs diseases (g) Any other (Specify)

8. From where you purchase the vegetables for sale to the consumer (Question to the Vendors) ?

   (a) Sabzi Mandi (b) Direct from the fields (c) Fields near Buddha Nullah

   (d) Grow yourself

9. Health status of the farmers and their family members who grow vegetables away from Buddha Nullah (Control Area).Does any member of the family suffer from any of the following diseases?

   (a) Cancer (b) Skin allergy (c) Mental Illness (d) Deformity (e) Kidney diseases

   (f) Lungs diseases (g) Any other (Specify)

10. Health status of the farmers and their family member who grow vegetables along the banks of Buddha Nullah. Does any member of the family suffer from any of the following diseases?

   (a) Cancer (b) Skin allergy (c) Mental Illness (d) Deformity (e) Kidney diseases

   (f) Lungs diseases (g) Any other (Specify)

11. Are you aware of any disease which is caused by eating vegetables?

Yes	No

Surveyed by
Jagdev Singh
Research Scholar
(41600081)
Lovely Professional University (Phagwara)

Signature
(Consumer)
Mobile (Optional)

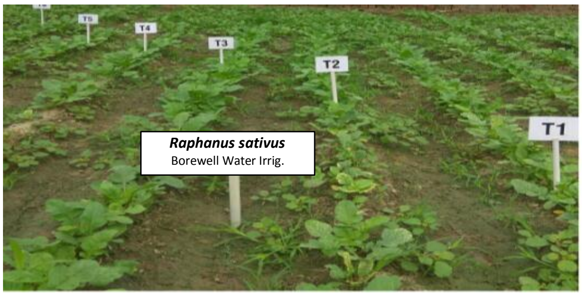
4.0 RESULTS AND DISCUSSION

This section represents the outcome of the present study and emphasizes the cause and after effects of the findings obtained with respect to the nature of irrigation water, soil and vegetables cultivated. The results obtained have also been compared with the findings of previous researchers in this field. Due emphasis on health risk assessment of populations consuming these vegetables is also given in this chapter.

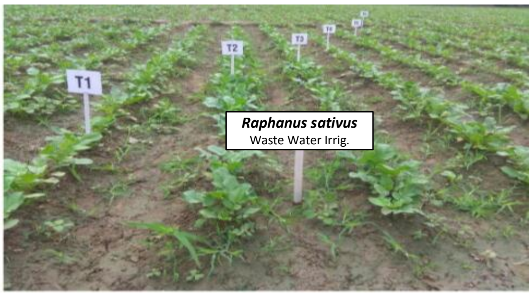
4.1 General description of the experimental area

Prior to finalizing the experimental area, an extensive survey of fields and farmers growing vegetables and using waste water for irrigation along the banks of Buddha Nullah and nearby fields was done to select the appropriate sites to carry out the present research work. Farmers prefer to grow edible crops, especially vegetables, in the fields along the banks of Buddha Nullah, a 38 km (water course) which runs parallel to Sutlej on the south for a fairly large course in Ludhiana (14 kilometers) and joins Sutlej on its north-west corner. It has become the sullage/sewage and industrial effluent carrier for one of the most populated and industrial cities of India, Ludhiana. After an extensive survey of the agricultural fields along the banks of Buddha Nullah, the area near Mota Singh Nagar was selected for experimentation where 35 acres of land was being used for growing different types of vegetables and irrigation was done using waste water of Buddha Nullah. The control site was selected 4 kilometers away from Buddha Nullah where the same vegetables were grown but are irrigated with normal borewell water.

Three plots of size 25 feet×50 feet were taken on lease to grow the vegetables on both the sites. Authentic seeds of *Raphanus sativus*, *Spinacia oleracea* and 40 day old seedlings of *Brassica oleracea* and *Lycopersicum esculentum* were procured from seed store and nurseries of Punjab Agricultural University, Ludhiana. Vegetables were sown, transplanted, grown and irrigated as per the required agricultural practices approved by Punjab Agricultural University, Ludhiana. The vegetables grown on Buddha Nullah site were regularly irrigated with waste water and those of control site with normal borewell water. Same varieties of vegetables selected for experimentation were sown, raised, and irrigated on both the sites at same time and under same environmental conditions. The samples were taken from both the sites at the same time for analysis of various parameters to be studied.



Raphanus sativus **BW**



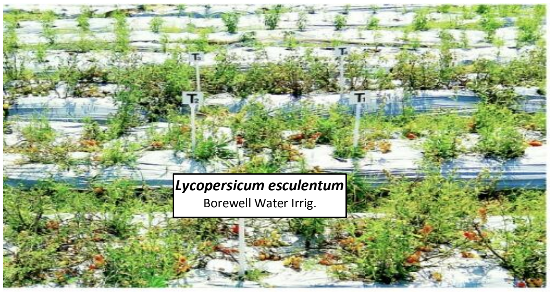
Raphanus sativus **WW**

Brassica oleracea **BW**

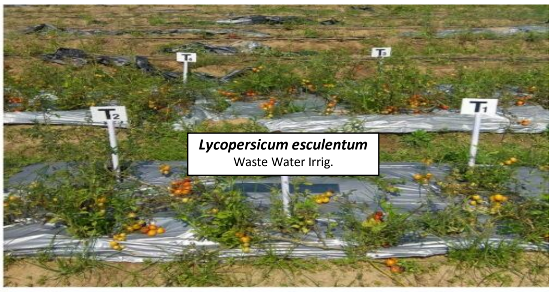
Brassica oleracea **WW**

Spinacia oleracea **BW**

Spinacia oleracea **WW**



Lycopersicum esculentum **BW**



Lycopersicum esculentum **WW**

Picture 4.1: Vegetables growing on experimental sites BW (borewell site), WW (waste water site)

Table 4.1: Showing the details of the vegetables cultivated on waste water and control site

Sr. No.	Common name	Botanical name	Variety	Edible part	Family
1	Radish	*Raphanus sativus*	Punjab safed mooli (2015)	Root (fleshy)	Brassicaceae
2	Spinach	*Spinacia oleracea*	Punjab green (1990)	Leaf	Amaranthaceae
3	Cauliflower	*Brassica oleracea*	Pusa snowball-1 (1994)	Flower (inflorescence)	Brassicaceae
4	Tomato	*Lycopersicum esculentum*	Punjab red cherry (2015)	Fruit	Solanaceae

4.2 Macro and micro climate of experimental area

4.2.1 Macro climate

Ludhiana is influenced by the local steppe climate. The climate is classified as BSh (Hot-semi arid steppe) according to the Köppen-Geiger climate classification system given by Kottek et al (2006). The average annual temperature and rainfall during experimentation was recorded to be 24.30^{O}C and 726 mm respectively. The weather of the experimental area in gross is hot with relative humidity 60%. The summer lasts from March to mid-June and cold weather begins from mid-November and persists up to the end of February

Mean temperature (^{0}C) and rainfall (mm) were observed during the experimental period in the growing season of vegetable crops (*Raphanus sativus*, *Spinacia oleracea, Brassica oleracea* and *Lycopersicum esculentum*)

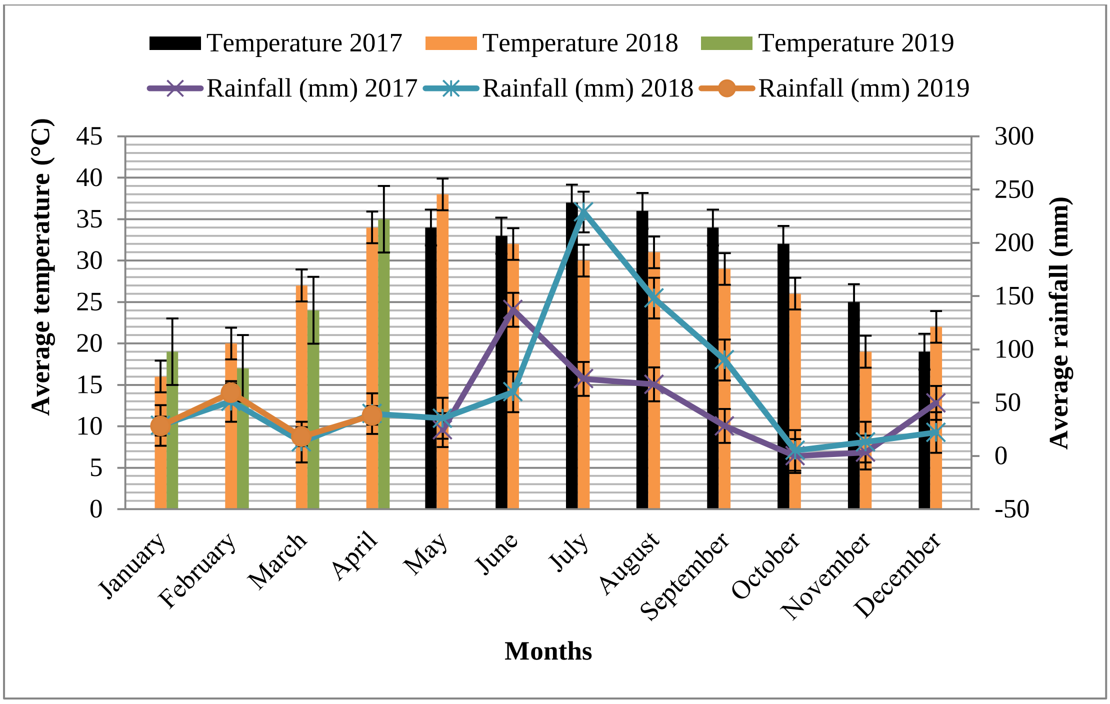


Fig 4.1: Microclimate of experimental area (May 2017-April 2019) ($p \leq 0.5$; Error bar= ±SD)

4.3 Effect of waste water irrigation on vegetables

This section of the thesis deals with the outcome of physiochemical parameters of water and soil, amount of heavy metals in water, soil and vegetable samples alongwith the biochemical changes induced in vegetables as a result of bioaccumulation of heavy metals and the survey report of health risks associated with consumption of these heavy-metal-laden vegetables among the consumers. Comparative analysis of results obtained from both the sites (waste and borewell) to evaluate the impact of waste water irrigation on bioaccumulation of heavy metals and biochemical changes induced in vegetables has also been reported in this chapter. The suitability of water for irrigation and vegetables for consumption has also been assessed by comparing the results obtained with permissible standards set by different health associations (FAO, 1985; Pescod, 1992; WHO, 1992; European union standards, 2002 and Indian standards Awasthi, 2000). To evaluate the trend of heavy metal bioaccumulation by vegetables, their effect on biochemical parameters of these vegetables and health risks associated with consumption of these heavy-metal-rich vegetables, different indices were used and these factors are discussed in detail. The discussion has been done on the basis of database obtained and average calculated for two consecutive years of study (May 2017 to April 2019).

4.4 Physiochemical analysis of waste and borewell water used for irrigation

Crop quality, quantity and soil composition are greatly affected by the quality of water used for irrigation. Water contains salts, minerals, and various pollutants but their level differs depending on the source from which the water is obtained. The high concentration of these salts, minerals and some toxic pollutants like heavy metals present in the irrigation water changes the soil composition and the pollutants are also absorbed by the plants cultivated in such soils. In the present study, the impact of water irrigation on vegetable crops from two extremely different sources has been exploited. The first source was heavy-metal-loaded waste water of Buddha Nullah and the second source was normal groundwater obtained from a 400 feet deep borewell. All the vegetables selected for study were grown on two different sites selected for experimentation. The first one was the waste water site where the vegetables were irrigated with the heavy-metal-rich waste water of Buddha Nullah and the second one was the control site where the same vegetables were raised but irrigated with normal borewell water. Presence of essential compounds in waste water makes it beneficial for crop production but presence of toxic pollutants in it drastically affects the physiochemical structure of the soil. Toxic pollutants, especially the heavy metals, get easily transferred and bio-accumulated in the edible crops such as vegetables grown in such soils which poses serious health risks among the consumers of such vegetables (Arora et al., 2008).

4.4.1 pH

pH is the assessment of concentration of hydrogen ions (H^+) in a liquid which determines alkalinity or acidity of the water. If its value is greater than 7 ($pH > 7.0$) the water is considered basic in nature and if its value is less than 7 ($pH < 7.0$), it is acidic in nature. The average pH of borewell water ranged from 7.30-7.35 in summer season and 7.65-7.75 in winter season and that of waste water of Buddha Nullah was within the range 7.65-7.75 and 7.70-7.80 in both the summer and winter season respectively. The pH values obtained correspond to the similar values reported for waste water by previous researchers (Dheri et al., 2007; Singh et al., 2009 and

Bamniya et al., 2010). The pH values of waste water and borewell water were found to be within the range of suitability of water used for irrigation. The alkaline nature of waste water can be attributed to the presence of various heavy metal ions and other pollutants contributed by sewage and industrial effluents. The pH values obtained for borewell and waste water were found to be within the prescribed limits. The values obtained in the present study are in coherence with results previously obtained by various researchers (Gupta et al., 2012; Ullah et al., 2012 and Bao et al., 2014).

4.4.2 Biochemical Oxygen Demand (BOD)

The amount of oxygen required by various microorganisms to degrade the organic matter present in waste water under anaerobic conditions is known as Biochemical Oxygen Demand. The mean BOD values of waste water in summer and winter seasons of both the experimental years correspond to 390.65±7.35 mg/l and 368±3.00 mg/l respectively. These values were found to be higher than the standard permissible limits set for irrigation water (FAO, 1985; Pescod, 1992) which suggested that waste water of Buddha Nullah is highly rich in biological contaminants. The average BOD values for borewell water in both the experimental years were detected to be 0 and 0.035±0.025 in summer and winter seasons respectively which suggests that the borewell water contains very small amount of biological contaminants. The average BOD level of waste water of Buddha Nullah was found to be greater than that of the borewell water used for irrigation. Similar trend of BOD in waste water and ground water was also observed by Singh et al (2009), Bharose et al (2013) and Alghobar et al (2014).

4.4.3 Chemical Oxygen Demand (COD)

The amount of oxygen required to oxidize the organic matter in the water is denoted as chemical oxygen demand or COD and is a swift determination of organic loading in the water. The average COD in borewell water was found to be BDL (below detection limits) in both the years of study in both summer and winter season. The amount of COD in waste water was estimated to be 179.80±6.80 mg/l and 161.30±5.30 mg/l in summer and winter seasons of both the experimental years. A slight increase in COD level was observed in summer as compared to the winter season which might be due to high rate of water evaporation during summer season as compared to that of the winter season. The COD values of waste water exceed the prescribed limits by WHO (2003). Similar higher values of COD in waste water of different industrial set ups used for irrigation have also been previously observed by Gupta et al (2010), Bamniya et al (2010) and Rolli (2014).

4.4.4 Electrical Conductivity (EC)

EC represents the ionic concentration of water which is measured by the capacity of water to conduct current and signifies the softness and hardness of the water. The EC of borewell water was analyzed to be 281.65±0.65 µmhos/cm and 272.65±14.65 µmhos/cm and that of waste water was found to be 3012±12.00 µmhos/cm and 2892±4.00 µmhos/cm in summer and winter seasons of both the experimental years respectively. The average EC values in waste water samples were many times greater than that of borewell water samples. High EC values of waste water were attributed to the presence of pollutants and some other elements and their ions contributed by industrial effluents and municipal sewage. The long term irrigation with such a high EC in waste water can cause soil salinity at Buddha Nullah agricultural sites in near future. The EC values of waste water exceeded the prescribed standards and rendered this water unfit for irrigation as per the Punjab Agricultural University specifications. Comparatively higher amounts of EC in waste water as observed in the present study also correlates with the similar results obtained in their studies by Amin-Gyampo et al (2012), Ghosh et al (2012) and Alghobar et al (2014). On the basis of EC values obtained in borewell water samples, it was recommended fit for irrigation purposes (Sadashivaiah et al., 2008).

4.4.5 Total Dissolved Solids (TDS)

The quantity of dissolved salts greatly affects the quality of water used for irrigation. On the basis of the amount of TDS, the irrigation water has been classified into classes I to V. The water in which the value of TDS is less than 175 mg/l is considered as excellent and greater than 2100 mg/l is considered to be unsuitable for irrigation (Scofield, 1935). The average amount of TDS obtained in the borewell water was 123.65±1.35 mg/l, 144.50±3.50 mg/l and 401.50±8.25 mg/l, 422.00±7.00 mg/l in waste water samples in both summer and winter seasons during first and second year of study respectively The average value of TDS in both borewell and waste water was found to be slightly higher in the winter season as compared to the summer season which might be due to the difference in rate of evaporation and flow of water during winters. The TDS value of waste water was found to be comparatively much higher than that of borewell water. This can be attributed to the fact that higher level of various heavy metals prevails in this industrial effluent-rich waste water. The mean values of TDS for borewell and waste water were found to be within the prescribed safe limits of quality standards set for irrigation (FAO, 1985; WHO, 2003). Higher amount of TDS in waste water as compared to normal ground water has also been reported by previous researchers (Islam and Shamsad, 2009; Joshi et al., 2009; Bamniya et al., 2010).

4.4.6 Total Suspended Solids (TSS)

Dry weight of suspended particles that are not dissolved in a sample of water that can be filtered by a filteration apparatus are known as TSS. The amount of total suspended solids is generally higher in surface and runoff waters as compared to the ground water because of extra addition of organic matter and clay particles from different sources. The average amount of total suspended solids was found to be almost negligible i.e., 0.20±0.01mg/l and 0.25±0.05 mg/l in borewell water but comparatively higher amounts of 296.50±0.50 mg/l and 298.15±4.85 mg/l in waste water samples in summer and winter during both the experimental years of study respectively. The results obtained are in accordance with the values obtained by previous researchers (Rahmani, 2007; Naaz and Pandey, 2010; Gupta et al., 2010). The value of TDS less than 100 mg/l is considered to be a tolerable limit for irrigation water, so the results of waste water TDS obtained in the present study make it unsuitable for irrigation because the obtained values are more than double of 100 mg/l.

4.4.7 Nitrate (NO_3^-)

According to Salisbury and Ross (1992) nitrogen is the main element which is most essential for plant growth and development. Nitrogen in the form of nitrate is beneficial for crop production but its higher concentration can also be the cause of growth retardation. The amount of nitrate in borewell water was found to be 0.05±0.005mg/l, 0.05±0.02 mg/l and in waste water to be 68.70±4.10 mg/l, 71.7±5.10

mg/l in the summer and winter seasons of both the study years respectively. The nitrate content in waste water was found to be much higher than that of borewell and greater than the prescribed limits also (FAO, 1985). Water analysis done by Gupta et al (2008) and Bharose et al (2013) also detected similar trend of nitrate in waste water and ground water used for irrigation in their studies.

4.4.8 Sulphate (SO_4^{-2})

Sulphate is another essential macronutrient which affects growth and availability of phosphorous to the plants. In the present study, the mean amount of sulphate was observed to be 16.50±1.50 mg/l and 16.85±0.15 mg/l in borewell water and 28.4±1.20 mg/l and 24.75±0.65 mg/l in waste water samples collected and analyzed during summer and winter seasons of both the years of study respectively. The amount of sulphate was found to be considerably higher in waste water as compared to that of the borewell water samples, but the quantity was found to be within the prescribed limits (FAO, 1985). Almost identical results were observed by Bharose et al (2013) in their analysis of waste water and ground water used for irrigation.

4.4.9 Total Phosphate (TP)

Phosphate is also an important macronutrient which helps in increasing crop production and soil fertility. In water, the phosphate is found in different forms like bound phosphate, condensed phosphate and ortho phosphate. The average amount of total phosphate in borewell water was detected to be 0.63±0.04 mg/l and 0.59±0.15 mg/l as compared to 4.75±0.15 mg/l and 4.35±0.05 mg/l in waste water samples during both the years of study in summer and winter seasons respectively. The average amount of total phosphate was found to be much higher in waste water as compared to that of borewell water samples but within the suggested limits (FAO, 1985). Higher amount of total phosphate detected in waste water in the present study corresponds with the values obtained by Gupta et al (2008) and Gupta et al (2010) in similar studies.

4.4.10 Sodium (Na^+)

The presence of sodium ions in water plays an important role in the determination of its suitability for irrigation and is generally associated with the problem of salinity in the soils. The borewell water samples analyzed had average amount of 52.40±0.70 mg/l and 48.75±0.75 mg/l as compared to the amount of 90.60±2.00 mg/l and 85.25±0.025 mg/l of sodium ion in waste water samples taken in the summer and winter seasons of both the consecutive years of study respectively. The results of the present study revealed a higher amount of sodium in waste water samples which was perhaps contributed by the presence of industrial effluents. Similar results have also been reported in similar studies conducted earlier by previous researchers (Bharose et al., 2013; Alghobar et al., 2014). The quantity of sodium ions in borewell was detected to be within the safe limits but that of waste water to be higher than the safe limits (FAO, 1985).

4.4.11 Chloride (Cl^-)

Chloride is another ion which plays an important role in determination of water suitability for drinking and irrigational purposes. The water containing chloride ions greater than 350 mg/l is considered to be harmful for irrigation. The average amount of 25.40±0.70 mg/l and 24.45±0.15 mg/l of chloride ions was obtained in the borewell water and 59.60±4.30 mg/l and 58.60±3.00 mg/l in the waste water samples during winter and summer seasons of both the consecutive years of study respectively. Elevated amounts of chloride concentration were detected in waste water as compared to that of borewell water samples but the concentration in both the waters used for irrigation were found to be within the safe limits (FAO, 1985). The findings are in accordance with the results already obtained by Gupta et al (2008) and Bharose et al (2013) in a similar study.

4.4.12 Potassium (K^+)

Potassium ions are also essential for plant biochemical activities because they play an important role in osmosis and transfer of nutrients from cell to cell. In the present study, the mean amounts of potassium was found to be 4.50±0.70 mg/l and 4.25±0.05 mg/l in borewell and 3.30±0.10 mg/l and 2.75±0.05 mg/l of potassium ions in waste water samples during summer and winter seasons, both in first and second year of study respectively. The average amount of potassium ions was found to be less in waste water as compared to that of borewell water. Identical results have also been previously reported by Roy and Gupta (2016).

4.4.13 Total Hardness (TH)

Total hardness in irrigation water was determined by the presence of both calcium and magnesium ions in it. In this study, the average amount of total hardness was analyzed to be 138.50±2.60 mg/l and 110.00±2.30 mg/l in borewell water samples and 77.00 ±1.90 mg/l and 74.50±1.50 mg/l in waste water during summer and winter seasons of both the years of study respectively. The amount of total hardness was considerably higher in borewell water as compared to that of waste water used for irrigation which renders the multivalent ionic strength of borewell water higher than waste water and it was attributed to the quantum flow of industrial and sewage surface water. Similar values of hardness in waste water of Durgapur, West Bengal were obtained by Gupta et al (2008) in a similar study.

Table 4.2: Physiochemical characteristics (mean) of water samples of waste water irrigation site

Name	May 2017	December 2017	May 2018	December 2018
Temperature	36.30±1.60	20.40±1.80	34.50±2.10	19.50±1.60
pH	7.90±0.20	7.70±0.30	7.50±0.10	7.80±0.40
COD	383.30±3.60	365.00±2.90	398.00±3.00	371.00±2.60
BOD	186.60±1.90	166.60±1.80	173.00±1.60	156.00±1.50
EC	3022.00±6.60	2888.00±6.20	2918.00±5.00	2846.00±6.90
TSS	296.00±3.20	293.30±2.80	297.00±2.80	303.00±3.00
TDS	409.80±2.80	415.00±3.10	393.00±3.60	429.00±3.40
NO_3^-	64.60±1.60	66.60±1.20	72.80±1.00	76.80±0.90
SO_4^-	27.20±0.90	24.10±1.20	29.06±0.60	25.40±0.40
Total phosphate	4.60±0.320	4.30±0.10	4.90±0.20	4.40±0.30
Na^+	88.60±1.60	85.50±1.20	92.60±2.60	85.00±1.60
Cl^-	55.30±1.20	55.60±1.00	63.90±0.90	61.60±0.70
K^+	3.40±0.10	2.80±0.20	3.20±0.30	2.70±0.20
CaH	92.00±1.80	85.00±2.00	100.50±3.20	87.30±1.90
MgH	52.30±1.60	40.70±1.80	65.20±2.00	64.50±1.50

All the values are expressed in mg/l except temperature in ^oC and EC in µmhos/cm (significant at $p \leq 0.05$)

Table 4.3: Showing Correlation matrix between different variables: Waste water

Variable	Temperature	pH	COD	BOD	EC	TSS	TDS	NO_3^-	SO_4^-	TP	Na^+	Cl^-	K^+	CaH	MgH
Temperature	1.000														
pH	-0.194	1.000													
COD	0.729**	-0.591*	1.000												
BOD	0.451	0.237	0.416	1.000											
EC	0.552	0.559	0.237	0.648*	1.000										
TSS	0.122	-0.306	0.200	0.092	-0.069	1.000									
TDS	-0.498	-0.606*	-0.756**	-0.022	0.073	0.100	1.000								
NO3-	0.196	-0.703*	0.533	-0.027	-0.365	0.614*	-0.454	1.000							
SO4-	0.821**	-0.546	0.886**	0.241	0.305	0.026	-0.743**	0.436	1.000						
TP	0.733**	-0.723**	0.945**	0.208	0.076	0.145	-0.819**	0.614*	0.951**	1.000					
Na+	0.396	-0.665*	0.457	0.279	-0.160	0.239	-0.354	0.291	0.448	0.527	1.000				
Cl-	0.356	-0.589*	0.704*	-0.199	-0.174	0.153	-0.636*	0.462	0.552	0.682*	0.027	1.000			
K+	0.637*	-0.094	0.594*	0.398	0.565	0.518	-0.036	0.362	0.565	0.496	0.038	0.379	1.000		
CaH	0.591*	-0.700*	0.880**	0.477	0.099	0.465	-0.579*	0.628*	0.736**	0.836**	0.736**	0.451	0.530	1.000	
MgH	0.708	-0.770**	0.862**	0.225	-0.035	0.215	-0.674*	0.462	0.801**	0.893**	0.749**	0.633*	0.400	0.857**	1.000

**:significant at $p \leq 0.01$, *:significant at $p \leq 0.05$

Table 4.4: Physiochemical characteristics (mean) of water samples of borewell water irrigation site

Name	May 2017	December 2017	May 2018	December 2018
Temperature	23.60±1.60	20.40±1.20	24.20±1.20	19.10±1.60
pH	7.30±0.20	7.20±0.30	7.30±0.40	7.40±0.30
COD	0.00.00	0.01±0.001	0.00.00	0.00.00
BOD	0.00.00	0.00.00	0.00.00	0.00...00
EC	282.30±3.20	287.00±2.70	281.00±2.30	258.00±1.98
TSS	0.00.00	0.53±0.002	0.40±0.01	0.00.00
TDS	125.00±2.50	148.00±1.90	122.30±3.20	141.00±2.90
NO_3^-	0.08±0.001	0.07±0.002	0.07±0..01	0.03±0.002
SO_4^-	15.60±1.50	16.70±1.30	16.70±1.60	17.00±1.80
Total phosphate	0.67±0.01	0.56±0.02	0.59±0.03	0.54±0.02
Na^+	51.70±2.30	49.50±1.80	53.10±1.30	48.00±1.90
Cl^-	24.70±1.60	24.60±1.39	26.10±0.80	24.30±0.90
K^+	5.20±0.40	4.20±0.80	3.80±0.11	4.30±0.20
CaH	118.00±1.60	102.00±2.80	111.00±3.20	103.00±2.80
MgH	65.00±3.50	57.60±2.90	60.40±1.60	63.60±1.30

All the values are expressed in mg/l except temperature in ^oC and EC in µmhos/cm (significant at $p \leq 0.05$)

Table 4.5: Showing Correlation matrix between different variables: Borewell water

Variable	Temperature	pH	COD	BOD	EC	TSS	TDS	NO_3^-	SO_4^-	TP	Na^+	Cl^-	K^+	CaH	MgH
Temperature	**1.000**														
pH	**0.165**	**1.000**													
COD	**-0.468**	**-0.580***	**1.000**												
BOD	**0.000**	**0.000**	**0.000**	**1.000**											
EC	**0.300**	**-0.367**	**0.070**	**0.000**	**1.000**										
TSS	**-0.148**	**-0.693***	**0.709****	**0.000**	**0.308**	**1.000**									
TDS	**-0.828****	**-0.266**	**0.293**	**0.000**	**-0.197**	**0.213**	**1.000**								
NO3-	**0.627***	**-0.179**	**-0.351**	**0.000**	**0.698***	**-0.055**	**-0.411**	**1.000**							
SO4-	**-0.514**	**0.012**	**0.012**	**0.000**	**-0.466**	**-0.076**	**0.407**	**-0.481**	**1.000**						
TP	**0.955****	**0.040**	**-0.325**	**0.000**	**0.379**	**-0.069**	**-0.818****	**0.688***	**-0.680***	**1.000**					
Na+	**0.393**	**-0.556**	**0.185**	**0.000**	**0.079**	**0.093**	**-0.288**	**0.319**	**-0.184**	**0.440**	**1.000**				
Cl-	**0.391**	**0.165**	**-0.410**	**0.000**	**0.153**	**0.015**	**-0.439**	**0.261**	**-0.040**	**0.269**	**0.002**	**1.000**			
K+	**0.135**	**0.155**	**-0.105**	**0.000**	**-0.011**	**-0.186**	**0.006**	**0.185**	**-0.557**	**0.317**	**-0.141**	**-0.556**	**1.000**		
CaH	**0.495**	**-0.313**	**-0.072**	**0.000**	**0.000**	**-0.014**	**-0.415**	**0.246**	**-0.450**	**0.613***	**0.620***	**-0.083**	**0.368**	**1.000**	
MgH	**0.201**	**0.696***	**-0.474**	**0.000**	**-0.143**	**-0.456**	**-0.370**	**-0.127**	**-0.244**	**0.198**	**-0.484**	**0.284**	**0.296**	**0.131**	**1.000**

**:significant at $p \leq 0.01$, *:significant at $p \leq 0.05$

4.4.14 Heavy metals

Heavy metals are considered to be the most important limiting factors in use of waste water agricultural irrigation. The presence of heavy metals in Buddha Nullah is attributed to the addition of industrial effluents without any treatment (Bashir et al., 2006). According to Gupta et al (2008) the concentration of heavy metals in waste water depends upon the type of industrial effluents. Buddha Nullah waste water receives the effluents from thousands of different types of large, medium and small scale industries. Continuous use of heavy-metal-rich waste water for irrigation contaminates the irrigated soil and cultivated crops with these toxic heavy metals (Nolan et al., 2003).

4.4.14.1 Arsenic (As)

In the present study, the amount of arsenic content in the waste water of Buddha Nullah was found to be many folds higher than the borewell water in both the summer and winter season of the study years. The average content of arsenic was detected to be 0.001±0.0001 mg/l and below detection limit (BDL) in borewell and 0.98±0.05 mg/l and 0.92±0.04 mg/l in waste water during summer and winter seasons in both the years of study respectively. The amount of arsenic was found to be slightly higher in waste water samples during the summer season as compared to the winter season. This can be attributed to the high temperature and seasonal change in flow of water in Buddha Nullah. The amount of arsenic in waste water was detected to be much higher than the borewell water as per the Indian standard limits prescribed for agricultural irrigation water (Awasthi, 2000). The results obtained have been corroborated with the results of previous researchers who detected higher amounts of arsenic in waste water as compared to ground water (Feizi, 2001 and Singh et al., 2012).

4.4.14.2 Cadmium (Cd)

The other metal analyzed in water used for irrigation of vegetables on both the sites was cadmium. The average amount of cadmium in waste water was detected to be 3.56±0.31 mg/l and 3.30±0.10 mg/l during summer and winter seasons during both the years of study. The results obtained showed higher amount of cadmium in waste water than that of borewell water which was found to be 0.002±0.0001 mg/l and

0.002±0.0005 mg/l during summer and winter seasons of both the years of study. The values of cadmium in waste water were found to be significantly higher than that of safe Indian standard values prescribed for the water used for agricultural irrigation. Similar results have previously been reported by Dheri et al (2007) and Singh et al (2012) in which a high level of cadmium was reported in waste water as compared to ground water.

4.4.14.3 Chromium (Cr)

The samples of borewell water contained 0.04±0.002 mg/l and 0.05±0.001 mg/l of chromium and a comparatively higher amount 6.72±0.08 mg/l and 6.46±0.15 mg/l was detected in waste water samples of Buddha Nullah during summer and winter seasons of both the years of study respectively. The amount of chromium was detected to be much higher in the waste water samples as compared to the borewell water samples in both the seasons. The amount of chromium was found to be less than the prescribed limits by Pescod (1992) and Awasthi (2000) in borewell water but many times higher than the prescribed limits in waste water of Buddha Nullah. The higher amount of chromium in Buddha Nullah water can be attributed to the effluents contributed by electroplating units of various cycle manufacturing industries situated in Ludhiana. Similar results have already been reported by some researchers in earlier studies in which significantly higher level of chromium was detected in waste water used for irrigation (Dheri et al., 2007; Chopra and Pathak, 2012).

4.4.14.4 Lead (Pb)

The amount of lead in the waste water of Buddha Nullah were analyzed to be 2.26±0.07 mg/l and 2.17±0.03 mg/l during summer and winter seasons respectively during both the years of study which was found to be significantly higher than that of borewell water which was detected to be 0.20±0.01 mg/l and 0.18±0.01 mg/l in same seasons. A slight decrease in the amount of lead was detected in both borewell and waste water samples during winter as compared to that of summer season which can be attributed to the slow activity of industrial units in winters as compared to the summers. The amount of lead during both the seasons in borewell water was found to be within, but that of waste water much higher than the prescribed Indian standard limits (Awasthi, 2000). Previous workers have also obtained similar results when they analyzed the waste water and compared it with the amount of lead in ground water (Feizi, 2001; Dheri et al., 2007, and Singh et al., 2012).

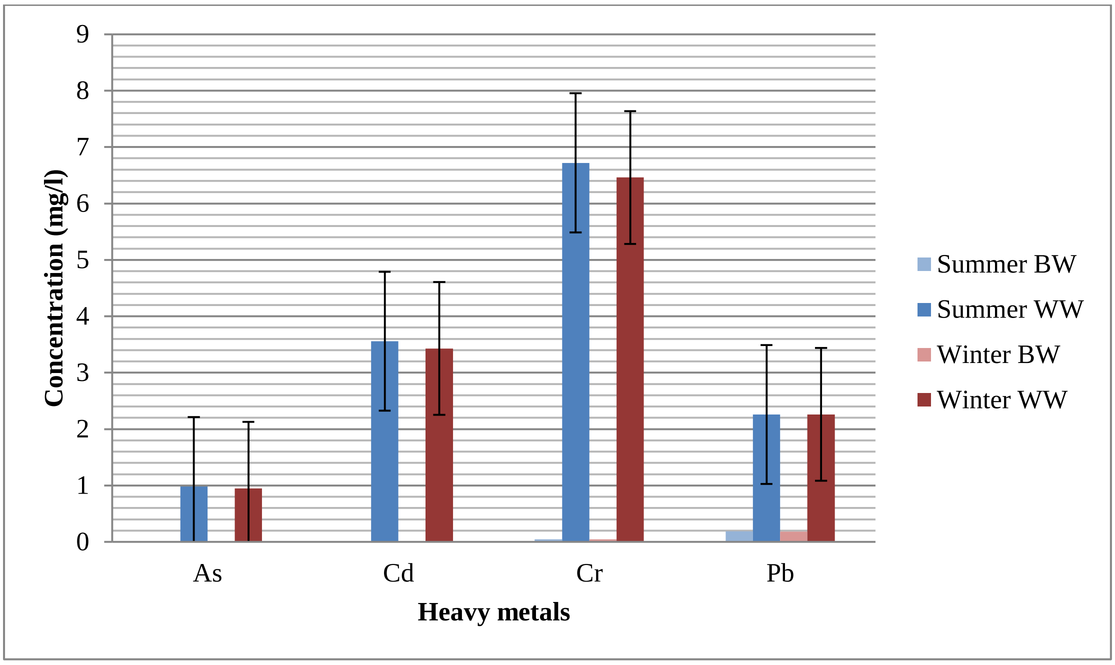


Fig 4.2: Concentration of heavy metals (mean mg/l) in borewell and waste water in summer and winter season of the experimental years of study ($p \leq 0.05$; Error bar= ±SD)

Table 4.6: Standards for heavy metals in water (mg/l)

Standards	**As**	**Cd**	**Cr**	**Pb**
Indian standard	0.01	0.003	0.05	0.01
EU standard	0.10	0.01	0.05	0.10

India standard (Awasthi, 2000)

EU: European Union standards (2002)

4.5 Analysis of physiochemical characteristics of waste and borewell water irrigated soil

4.5.1 Soil texture

The relative percentage of silt, sand and clay determines the texture of soil which is considered to be an important characteristic of soil which helps in determining the suitability of soil for crop cultivation. According to Sherene (2010) the presence of clay particles greatly affects the mobility of metals in the soil. The soil samples of both waste water and borewell water irrigated soil samples experimental sites were found to be sandy loam type. The percentage of sand, silt and clay was analyzed to be 50%, 30% and 20% in borewell irrigated and 45%, 40% and 15% respectively in waste-water-irrigated soil samples. The texture of waste-water-site soil samples was found to be a little bit different from the earlier studies done by Azad et al (1987) who reported higher concentration of clay content in waste-water-irrigated soils. This can be attributed to the fact that long term and continuous use of waste water for irrigation significantly alters the physiochemical properties of soil.

4.5.2 pH

pH is defined as negative logarithm of hydronium ion concentration. The values of pH less than 7 make the soil acidic whereas greater than 7 make it alkaline in nature. The pH values of borewell water irrigated soil were found to be 7.20±0.10 and 7.17±0.07 as compared to 7.25±0.01 and 7.35±0.01 in waste water irrigation soil samples in summer and winter seasons during first and second year of study respectively. The pH value of both the waste water and borewell water irrigated soils does not differ significantly. Sherene (2010) observed that at lower pH value, the soil solution enhances the mobilization of metals. In this study, the lower range of pH in waste-water-irrigated soils might be helping in higher translocation of heavy metals in the vegetables cultivated there.

4.5.3 Electrical conductivity (EC)

Mohammed and Mazahreh (2003) in their studies concluded that the soils irrigated with waste water were found to be having high level of Electrical Conductivity. Identical results were found in the present study where the soil samples collected from borewell water irrigated sites were found to be having EC 608.00±5.70 µmhos/cm and 571.50±4.50 µmhos/cm in both the summer and winter season and comparatively higher amounts 1838.50±3.50 µmhos/cm and 1840.50±5.50 µmhos/cm of EC were detected in waste-water-irrigated soil samples. The EC in summer season was found to be little bit higher than the winter season which can be attributed to high rate of evaporation as reported in previous studies by Ghanbari et al (2007).

4.5.4 Ferric (Fe)

Ferric is another essential micronutrient required for growth and development of plants. Iron acts as a cofactor of various enzymes, constituent of some important plant pigments, assists in nitrate reduction and is essential for the synthesis of chlorophyll. The amount of ferric was found to be 16.15±0.11mg/kg and 15.94±0.20 mg/kg in both the summer and winter season of experimental years in borewell irrigated soils whereas the mean values of iron were found to be 25.23±0.27 mg/kg and 24.82±0.11mg/kg in waste-water-irrigated soils. Waste water samples were found to contain significantly higher amounts of iron which can be attributed to the presence of higher amounts of industrial effluents in the waste water of Buddha Nullah. Similar results were obtained by Dheri et al (2007).

4.5.5 Copper (Cu)

Copper is also an essential micronutrient which plays an important role in some enzymatic activities of the plant cell. The average amount of copper in borewell water irrigated soils was detected to be 3.18±0.20 mg/kg and 2.99±0.05 mg/kg in the summer and winter seasons of both the years of study respectively. The waste-water-irrigated soil samples were found to contain the mean amount of 4.20±0.30 mg/kg and 3.79±0.11 mg/kg at the same time of study. Waste-water-irrigated soil samples showed comparatively higher average amount of copper as detected in soil samples collected from borewell irrigated site. Mahmood and Malik (2014) reported the presence of significantly higher amount of copper in waste-water-irrigated soil as compared to ground water irrigated soils. Hence the results obtained in the present study are in accordance with the previous studies done.

4.5.6 Zinc (Zn)

Zinc is that micronutrient which is required in very small quantities but plays an important role in some of the biochemical reactions taking place in the plant cell. The mean content of zinc was detected to be 4.13±0.13 mg/kg and 3.94±0.60 mg/kg in borewell irrigated soils and that of 5.16±0.13 mg/kg and 4.66±0.33 mg/kg in waste-water-irrigated soil samples during summer and winter seasons respectively in both the years of study. The results obtained in this study are in accordance with the results reported in the previous studies performed by Sinha et al (2006) and Lente et al (2012).

4.5.7 Manganese (Mn)

Manganese is another important micronutrient required for plant growth and development which acts as cofactor of various enzymes. The average amount of manganese was detected to be 4.76±0.10 mg/kg and 4.06±0.10 mg/kg in borewell irrigated soil samples and that of 10.06±0.15 mg/kg and 9.13±0.43 mg/kg in the waste-water-irrigated soil samples during summer and winter seasons of both the study years respectively. The soil samples collected from waste water site were found to have higher amount of manganese as compared to the soil samples collected from the borewell site. The results of the present study were in coherence with the previous findings in which significantly higher amounts of manganese were reported in waste-water-irrigated soils (Sinha et al., 2006; Gupta et al., 2012; Mahmood and Malik, 2014).

Table 4.7: Physiochemical characteristics (mean) of soil samples of waste water irrigation site

Variable	May 2017	May 2018	Average	December 2017	December 2018	Average
pH	7.35±0.30	7.33±0.20	7.34±0.20	7.40±0.30	7.10±0.50	7.25±0.10
EC	1835.±6.50	1842±5.80	1838.5±6.00	1799±6.20	1810±4.80	1804±5.50
Fe	25.50±2.20	24.96±1.60	25.23±2.00	24.90±2.00	24.76±0.20	24.82±0.20
Mn	9.93±0.70	10.20±0.90	10.06±0.80	8.70±0.50	9.56±0.15	9.13±0.43
Cu	4.50±0.60	3.90±0.80	4.20±0.70	3.90±0.30	3.68±0.50	3.79±0.40
Zn	5.30±0.30	5.03±0.60	5.16±0.40	5.00±0.20	4.33±0.40	4.66±0.33

Table 4.8: Correlation matrix between different variables: waste water irrigation site soil

Variable	pH	EC	Fe	Mn	Cu	Zn
pH	1.000					
EC	0.425	1.000				
Fe	0.203	0.005	1.000			
Mn	0.304	0.637*	0.312	1.000		
Cu	0.345	0.130	0.711**	0.329	1.000	
Zn	0.034	-0.221	0.647**	0.226	0.632*	1.000

**:significant at p≤0.01, *:significant at p≤0.05

Table 4.9: Physiochemical characteristics (mean) of soil samples of borewell water irrigation site

Variable	May 2017	May 2018	Average	December 2017	December 2018	Average
pH	7.10±0.50	7.30±0.30	7.20±0.20	7.25±0.60	7.10±0.80	7.17±0.70
EC	595±4.20	605±7.00	600±5.50	567±4.00	576±0.50	571.±4.50
Fe	16.40±010	16.26±0.1	16.15±0.1	15.95±0.3	15.91±0.4	15.94±0.2
Mn	4.66±0.30	4.87±0.1	4.76±0.20	3.96±0.10	4.16±0.40	4.06±0.30
Cu	3.16±0.10	3.20±0.30	3.18±0.20	3.00±0.60	2.50±0.40	2.99±0.50
Zn	4.00±0.20	4.26±0.03	4.13±0.30	3.88±0.70	4.00±0.50	3.94±0.60

Values of EC expressed in mhos cm^{-1} and micronutrients in mg/kg

Table 4.10: Correlation matrix between different variables: borewell water irrigation site soil

Variable	pH	EC	Fe	Mn	Cu	Zn
pH	1.000					
EC	-0.004	1.000				
Fe	0.405	0.586*	1.000			
Mn	0.118	0.893**	00739**	1.000		
Cu	0.289	0.502	0.583*	0.676*	1.000	
Zn	0.292	0.820**	0.723**	0.776**	0.442	1.000

**:significant at p≤0.01, *:significant at p≤0.05

4.5.8 Heavy metals

Presence of heavy metals deteriorates the soil health and the source of these toxic metals are anthropogenic activities. Heavy metals in soil are available in two different forms-immobile or bioavailable, and mobile forms. These forms are always in a dynamic state and are greatly affected by the other physiochemical characteristics of soil like texture, pH and Electrical Conductivity. The amount of these toxic metals in soil also determines the uptake and entry of these toxic metals into the food chain via food crops like vegetables. In the present study the amount of some toxic metals in the soil samples collected from both the experimental sites (waste water and borewell water) were analyzed. The results were compared within and with standard limits set for soil.

4.5.8.1 Arsenic (As)

Mean amount of arsenic was analyzed to be 5.67±0.24 mg/kg in summer and 5.51±0.28 mg/kg in soil samples collected during winter from borewell irrigated site and 9.81±0.20 mg/kg and 9.17±0.14 mg/kg was detected in soil samples collected from waste water site during summer and winter season of consecutive years of study respectively. The amount of arsenic in waste-water-irrigated soil was found to be much higher than that of borewell irrigated soil samples. Higher amounts of arsenic in waste-water-irrigated soil samples directly points towards its regular and long term irrigation with arsenic rich waste water of Buddha Nullah. Similar results were previously obtained in their study by Gupta et al (2010).

4.5.8.2 Cadmium (Cd)

Average amount of cadmium in waste water soil samples was found to be 17.25±0.27 mg/kg and 16.89±0.32 mg/kg which is more than double in amount as compared to mean amount of 7.67±0.44 mg/kg and 7.20±0.38 mg/kg detected in the borewell water soil samples in the summer and winter season during both the first and second years of study respectively. The amount of cadmium in both waste-water-irrigated and borewell irrigated soil samples was found to be much higher than the prescribed limit set by EU and Indian Standards.. Almost identical results were noticed by Gupta et al (2010), Ghosh et al (2012) and Mahmood and Malik (2014) who reported a higher level of cadmium content in waste-water-irrigated soils as compared to normal groundwater irrigated soils.

4.5.8.3 Chromium (Cr)

The soil samples collected from borewell irrigated soils exhibited the average content of chromium to be 9.75±0.11 mg/kg and 9.37±0.34 mg/kg in summer and winter seasons of both the years of study respectively. The soil samples analyzed from the waste-water-irrigated site were found to contain 24.96±0.75 mg/kg and 24.75±0.35 mg/kg of chromium in summer and winter seasons of both the first and second year of study respectively. The amount of chromium in waste-water-irrigated soil samples was found to be much higher than that of borewell irrigated soil samples. The results of the present study are in accordance with observations of earlier studies carried out by Sinha et al (2006), Ghosh et al (2012) and Mahmood and Malik (2014) who reported waste-water-irrigated soils having elevated amounts of chromium as compared to normal water irrigated soils.

4.5.8.4 Lead (Pb)

Lead was another toxic metal evaluated in the present study. The analysis of waste-water-irrigated soil samples reveals the mean amount of lead to be 19.55±0.36 mg/kg and 19.49±0.77mg/kg as compared to that of 9.00±0.20 mg/kg and 7.780±0.36 mg/kg in borewell irrigated soil samples in both summer and winter seasons of the consecutive years of study respectively. Almost identical results were noticed by Gupta et al (2010), Lente et al (2012) and Mahmood and Malik (2014) who reported higher enrichment of lead in waste-water-irrigated soils as compared to that of borewell irrigated soils.

Table 4.11: Standards for heavy metals in soil (mg/kg)

Standards	As	Cd	Cr	Pb
Indian standard	5.0	3-6	--	250-500
EU standard	--	3.0	150	50-300

India standard (Awasthi, 2000)

EU: European Union standards (2002)

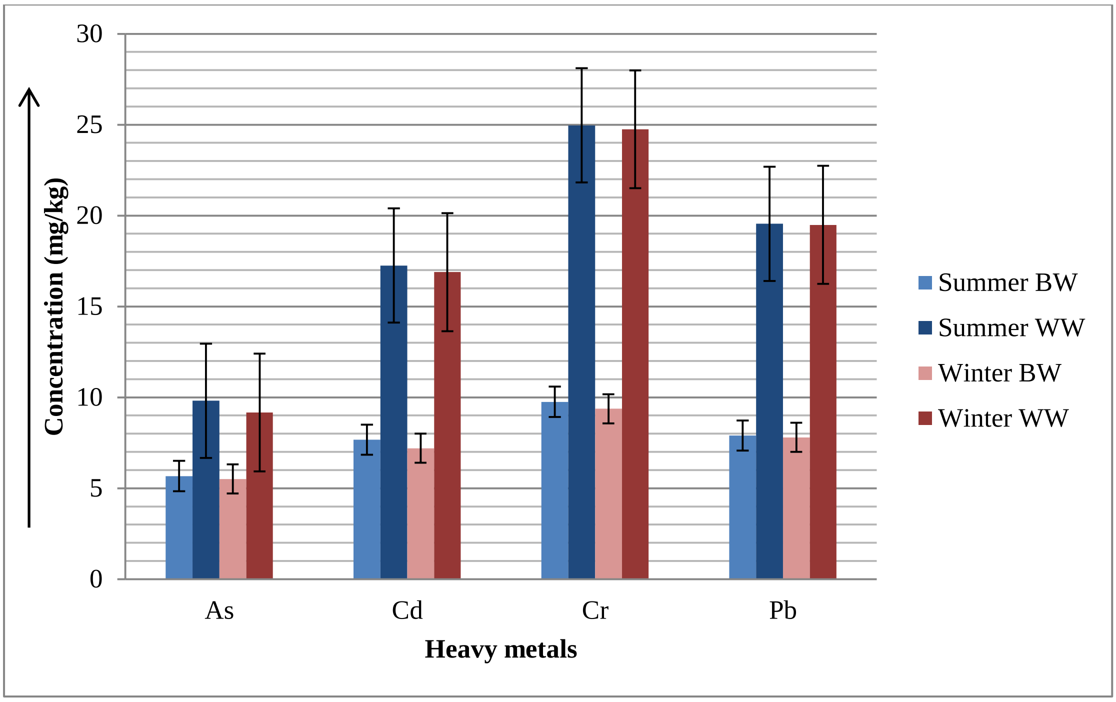


Fig. 4.3: Heavy metal concentration (average) in borewell (BW) and waste water (WW) irrigated soil samples in summer and winter season of the experimental years ($p \leq 0.05$; Error bar= ±SD)

4.6 Enrichment factor (EF) of heavy metals in soil irrigated with waste water

Heavy metal enrichment factor was calculated by dividing the average amount of each heavy metal present in waste-water-irrigated soil by the average amount of same heavy metal present in the soil samples of borewell water site taken as control (Kisku et al., 2000). Wide range of same heavy metal content was observed in the soil samples collected both from the waste water and control site. Average enrichment factor of all the heavy metals analyzed in summer season of experimental years was found in the following ascending order: As (1.73) < Cd (2.24) < Pb (2.47) < Cr (2.56). A similar trend, As (1.66) < Cd (2.34) < Pb (2.49) < Cr (2.64) was witnessed in the winter season of both the years of study. A non-significant difference in the enrichment factor of all the heavy metals As, Cd, Cr and Pb was observed in both the summer and winter seasons of study. The enrichment factor of As in waste-water-irrigated soil was found to be minimum and that of Cr to be maximum. The enrichment factor values of heavy metals ranging from 0.5-1.5 indicates their entry into the soil via natural processes whereas the values greater than 1.5 suggests the entry due to one or other anthropogenic activity. The enrichment factor of all the heavy metals analyzed in the present study was detected to be greater than 1.5 (>1.5) which suggests that their entry into the soil can be strongly correlated with the irrigation by waste water of Buddha Nullah. Identical results of enrichment factor in the waste-water-irrigated soils has also been reported in their studies by previous researchers (Misra and Tripathi, 2008; Singh et al., 2009; Gupta et al., 2010; Lente et al., 2014).

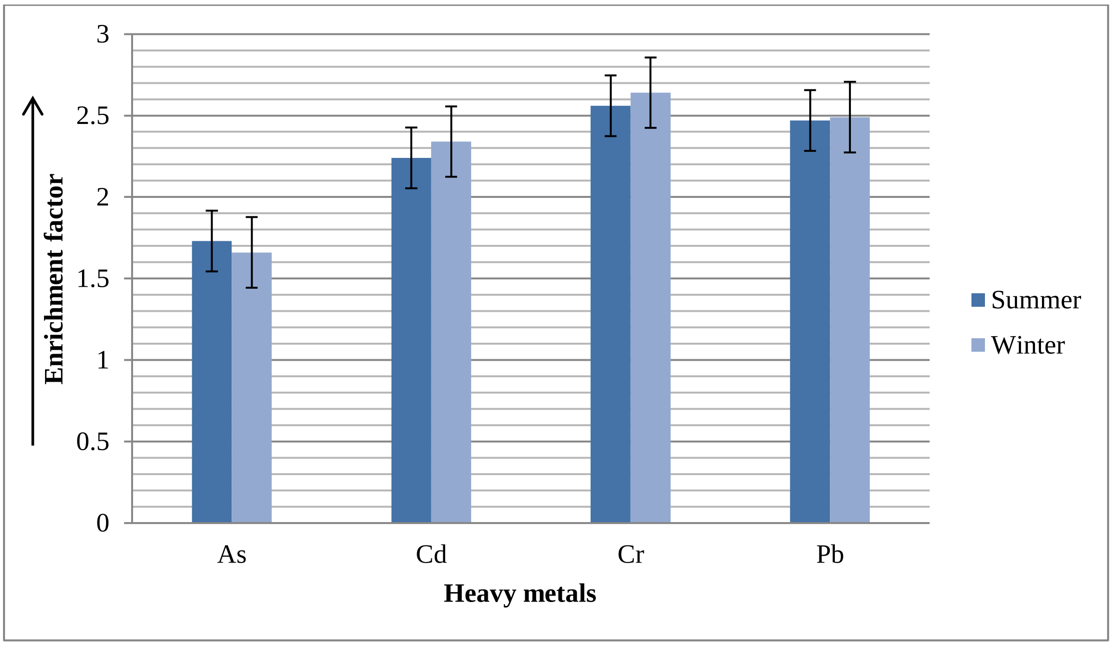


Fig 4.4: Enrichment factor (EF) of heavy metals in waste-water-irrigated soil samples of Buddha Nullah site ($p \leq 0.05$; Error bar= ±SD)

4.7 Bioaccumulation factor (BAF) of heavy metals in vegetables from soil

Bioaccumulation factor (BAF) is an important index used to determine the uptake and accumulation capacity of any metal by plant from the soil in which it is cultivated. It was determined by dividing the mean amount of metal present in plant dry weight to the amount of the same metal present in dried soil samples (Osu and Ogoko, 2014). It also determines the amount of heavy metal entering into the human beings through the food chain. The BAF of metals differs from species to species for the same metal and also from place to place (Amin et al., 2013).

4.7.1 Arsenic (As)

In this study, the mean bioaccumulation factor for As in the borewell water irrigated vegetable samples was found to be 0.25 in *Raphanus sativus*, 0.21 in *Spinacia oleracea*, 0.10 in *Brassica oleracea* and 0.05 in *Lycopersicum esculentum* in the first year of study and 0.21, 0.19, 0.10 and 0.05 in *Raphanus sativus*, *Spinacia oleracea*, *Brassica oleracea* and *Lycopersicum esculentum* respectively in the second year of study. The bioaccumulation factor followed the ascending order. *Lycopersicum esculentum* < *Brassica oleracea* < *Spinacia oleracea* < *Raphanus sativus* in vegetable samples during both the years of study. Similarly, the average bioaccumulation factor of arsenic was found to be 0.71, 0.59, 0.55 and 0.42 in the first and 0.67, 0.57, 0.50 and 0.40 in the second year of study in *Raphanus sativus, Spinacia oleracea*, *Brassica oleracea* and *Lycopersicum esculentum* respectively in wastewater irrigated vegetable samples. The bioaccumulation factor of arsenic followed the same order as in vegetables irrigated with borewell water but it was found to be much higher in waste-water-irrigated vegetable samples than that of borewell irrigated vegetables. The BAF of As was found to be less than 1 (< 1) in all the vegetable samples collected from both the borewell and waste-water-irrigated sites in both the first and second year of study.

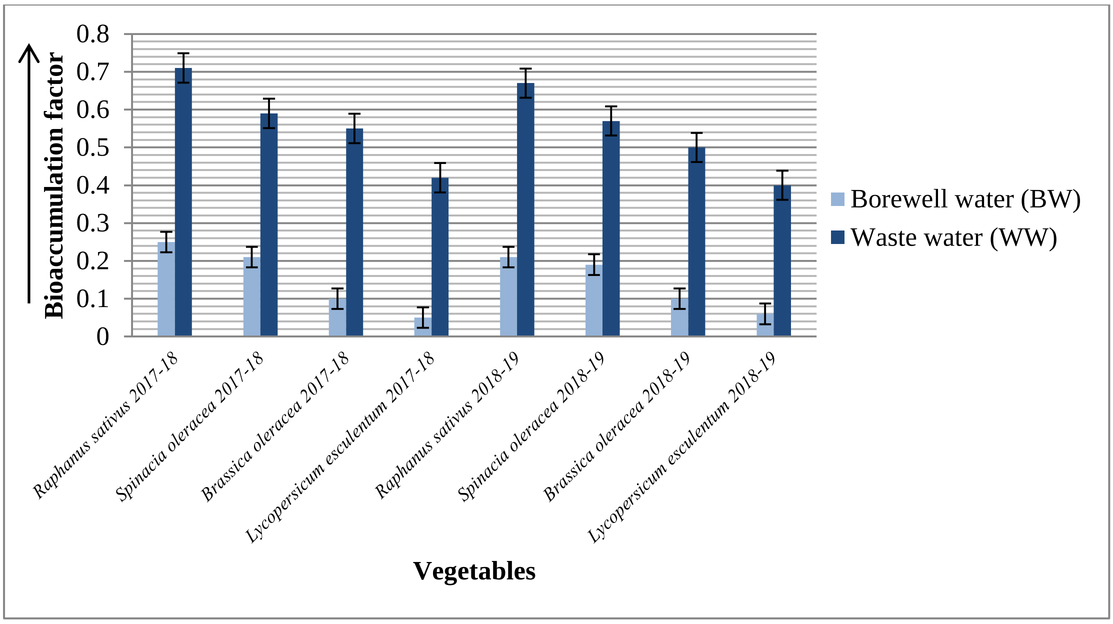


Fig 4.5: Bioaccumulation factor (BAF) of As in borewell and waste-water-irrigated vegetables ($p \leq 0.05$; Error bar= ±SD)

4.7.2 Cadmium (Cd)

The average bioaccumulation factor for Cd in waste-water-irrigated vegetable samples was found to be 1.36, 1.35, 1.00 and 0.58 in the first and 2.28, 2.21, 1.00 and 0.52 in the second year of study in *Raphanus sativus*, *Spinacia oleracea*, *Brassica oleracea* and *Lycopersicum esculentum* respectively. The bioaccumulation factor followed the ascending order, *Lycopersicum esculentum* < *Brassica oleracea* < *Spinacia oleracea* < *Raphanus sativus* in vegetable samples during both the years of study. Similarly the mean bioaccumulation factor was found to be 0.70, 0.85, 0.60 and 0.57 in the first year and 0.63, 0.72, 0.54 and 0.51 in the second year of study in *Raphanus sativus, Spinacia oleracea, Brassica oleracea* and *Lycopersicum esculentum* respectively in borewell irrigated vegetable samples. The bioaccumulation factor of Cd was recorded to be maximum in *Spinacia oleracea* but it was comparatively lower in borewell-water-irrigated vegetable samples than that of waste-water-irrigated ones.

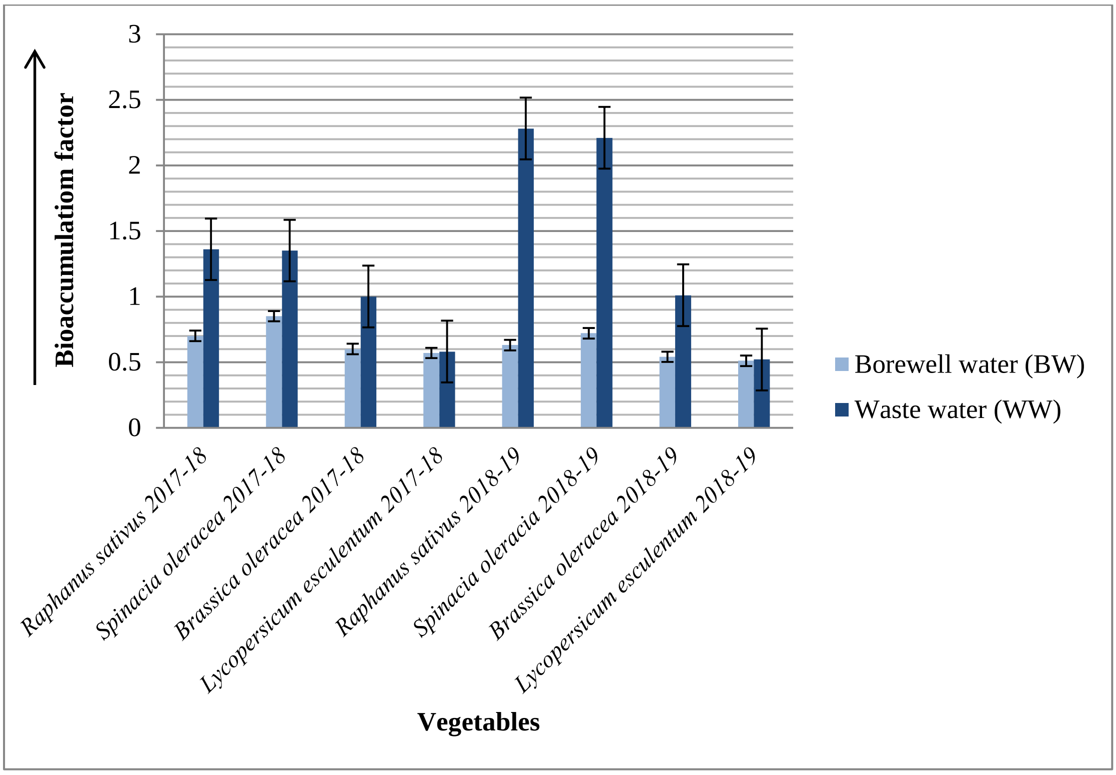


Fig 4.6: Bioaccumulation factor (BAF) of Cd in borewell and waste-water-irrigated vegetables ($p \leq 0.05$; Error bar= ±SD)

4.7.3 Chromium (Cr)

Average bioaccumulation factor for chromium in waste-water-irrigated vegetable samples was found to be 1.90 in *Raphanus sativus*, 1.85 for *Spinacia oleracea*, 1.00 in *Brassica oleracea* and 0.52 in *Lycopersicum esculentum* in the first year of study and 1.76, 1.82, 1.00 and 0.49 in *Raphanus sativus*, *Spinacia oleracea*, *Brassica oleracea* and *Lycopersicum esculentum* respectively in the second year of study. The mean bioaccumulation factor was in ascending order, *Lycopersicum esculentum* < *Brassica oleracea* < *Spinacia oleracea* < *Raphanus sativus* in vegetable samples during both the years of study. Similarly the average bioaccumulation factor was found to be 0.71, 0.59, 0.55, 0.42 and 0.67, 0.57, 0.50, 0.40 in *Raphanus sativus, Spinacia oleracea, Brassica oleracea* and *Lycopersicum esculentum* in borewell irrigated vegetable samples in the first and second year of study respectively. The bioaccumulation factor of Cr followed the same order as that of waste water irrigated vegetable samples but it was found to be much lower in borewell water irrigated samples than that of waste-water-irrigated ones in all the vegetables.

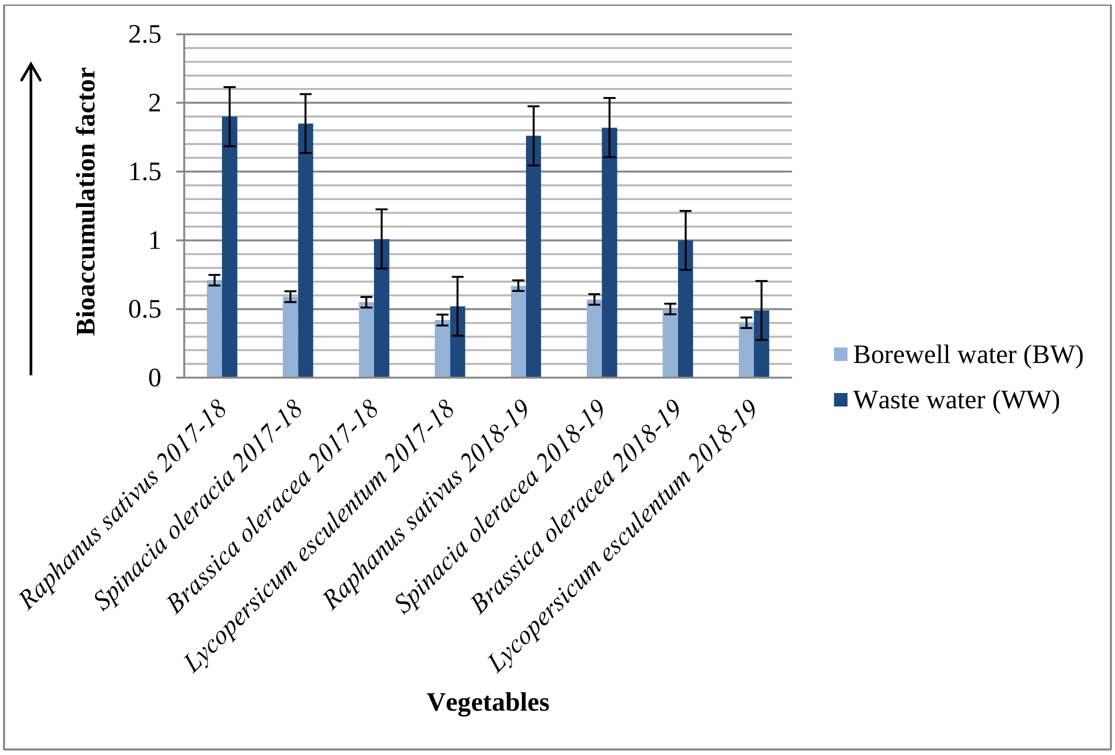


Fig 4.7: Bioaccumulation factor (BAF) of Cr in borewell and waste-water-irrigated vegetables ($p \leq 0.05$; Error bar= ±SD)

4.7.4 Lead (Pb)

In the present study, the average bioaccumulation factor for Pb in waste-water-irrigated vegetable samples was calculated to be 1.30, 1.08, 0.84, 0.52 and 1.05, 1.05, 0.74, 0.40 in *Raphanus sativus*, *Spinacia oleracea*, *Brassica oleracea* and *Lycopersicum esculentum* respectively in both the first and second year of study. The bioaccumulation factor was in the following ascending order, *Lycopersicum esculentum* < *Brassica oleracea* < *Spinacia oleracea* < *Raphanus sativus* in vegetable samples during both the years of study. Similarly the average bioaccumulation factor was found to be 0.79, 0.75, 0.57 0.57 in the first and 0.72, 0.69, 0.55, 0.48 in the second year of study in *Raphanus sativus*, *Spinacia oleracea*, *Brassica oleracea* and *Lycopersicum esculentum* respectively in borewell irrigated vegetable samples. The bioaccumulation factor of Pb followed the same order as in vegetables irrigated with waste water but the bioaccumulation factor was observed to be much higher in waste water irrigated vegetable samples than that of borewell irrigated ones.

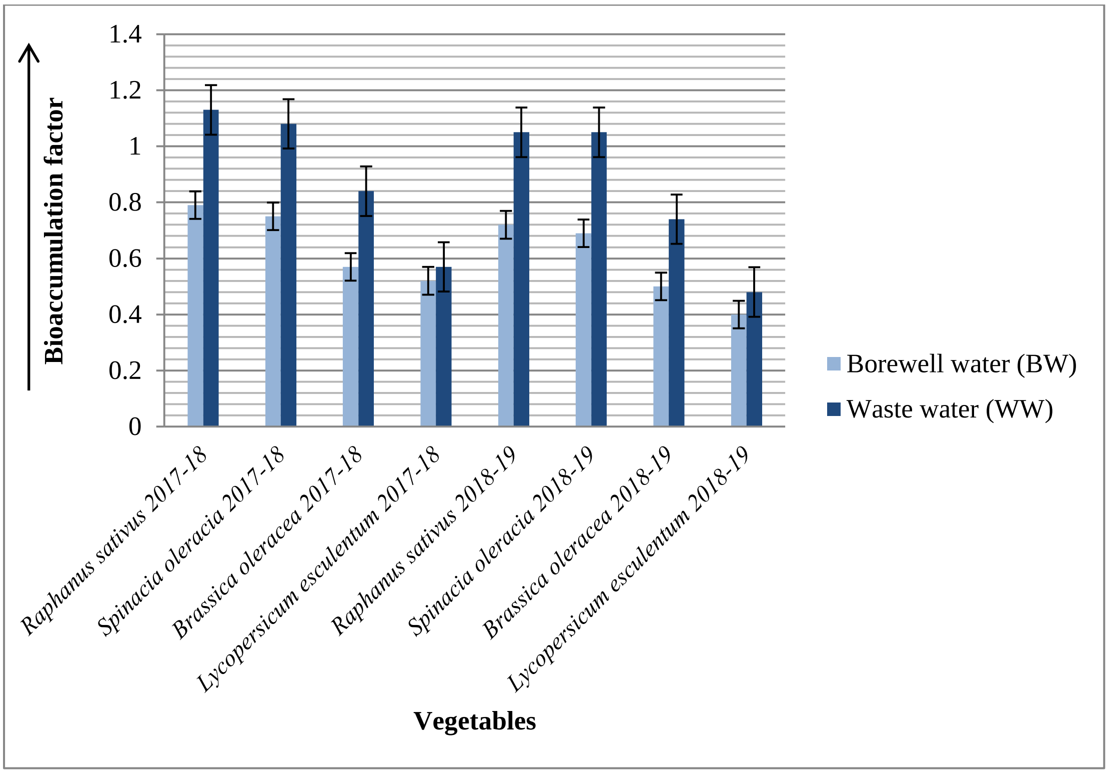


Fig 4.8: Bioaccumulation factor (BAF) of Pb in borewell and waste-water-irrigated vegetables ($p \leq 0.05$; Error bar= ±SD)

The highest bioaccumulation factor of all the metals was observed in in *Raphanus sativus* and lowest in *Lycopersicum esculentum*. The bioaccumulation factor of arsenic in all the vegetable samples collected from borewell irrigated site and waste-water-irrigated site was found to be less than 1 (<1). Similarly, the bioaccumulation factor of lead was less than 1 (<1) in *Brassica oleracea* and *Lycopersicum esculentum*. Bioaccumulation factor of Cd, Cr and Pb in *Raphanus sativus* and *Spinacia oleracea* was found to be greater than 1 (>1) in both the years of study. The bioaccumulation factor of Cd and Cr was also detected to be greater than 1 (>1) in *Brassica oleracea* also. The bioaccumulation of all the heavy metals was found to be less than 1 (<1) in *Lycopersicum esculentum.*

The bioaccumulation factor values greater than 1 (> 1) suggest that the vegetables are hyperaccumulators and the consumption of such vegetables is harmful for human health. Previous studies done by Singh et al (2010), Gupta et al (2010), Mohammed and Jimoh (2014), Roy and Gupta (2016) and Ahmed et al (2019) also reported the bioaccumulation factor to be greater than 1 (>1) in vegetables irrigated by textile effluents, municipal waste water and water rich in heavy metals. The results obtained in the present study were in coherence with the above mentioned studies.

4.8 Heavy metals bioaccumulation in vegetables

The amount (mean ± sd) of arsenic, cadmium, chromium and lead in the edible parts of the vegetables grown at borewell water irrigation site and waste-water-irrigation site (Buddha Nullah site).

4.8.1 Arsenic (As)

In the present study, the average amount of arsenic in borewell irrigated vegetables was found to be 0.02±0.001 mg/kg and 0.02±0.001 mg/kg in *Raphanus sativus*, 0.002±0.0005 mg/kg and 0.002±0.001 mg/kg in *Spinacia oleracea*, and below detectable limits (BDL) in *Brassica oleracea* and *Lycopersicum esculentum* in the first and second year of study respectively. Similarly, the mean amount of arsenic in waste-water-irrigated vegetables was detected to be 2.50±0.10 mg/kg and 2.20±0.10 mg/kg in *Raphanus sativus*, 2.10±0.10 mg/kg and 2.10±0.10 mg/kg in *Spinacia oleracea*, 0.95±0.50 mg/kg and 0.96±0.28 mg/kg in *Brassica oleracea*, 0.50±0.10

mg/kg and 0.60±0.10 mg/kg in *Lycopersicum esculentum* in the first and second year of study respectively. The critical difference CD (5%) in radish was 0.160 but the actual differences between the means of arsenic in borewell and waste-water-irrigated was much higher than this value which suggests a significant increase in bioaccumulation of arsenic by waste water irrigation in *Raphanus sativus*. Similarly, on the basis of critical difference and the actual differences between the mean values of arsenic in *Spinacia oleracea*, *Brassica oleracea* and *Lycopersicum esculentum*, there was a significant higher bioaccumulation of arsenic detected in waste-water-irrigated vegetable samples as compared to that of borewell water irrigated ones. There was no significant difference in bioaccumulation of arsenic in the first and second year of analysis but the amount of arsenic in all the vegetables studied exceeded the limits prescribed by WHO/FAO (2007), EU standards(2002) and Indian standards (Awasthi, 2000). The higher uptake of arsenic has also been reported in waste-water-irrigated spinach (0.91 mg/kg) by Biswas et al (2012). Similarly Rehman et al (2016) reported the presence of arsenic from 0.03 to 1.38 mg/kg in some vegetables irrigated with waste water in an industrial area in Pakistan.

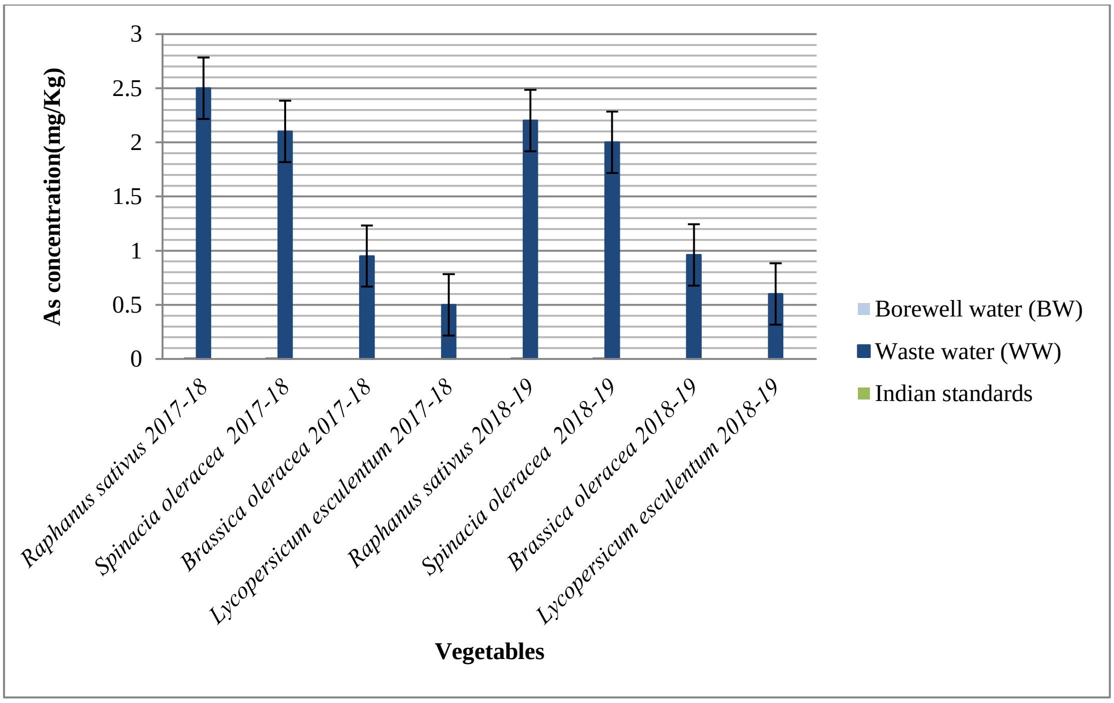


Fig 4.9: Mean bioaccumulation of Arsenic (As) in BW and WW irrigated vegetables ($p \leq 0.05$; Error bar= ±SD)

The results of the present study show comparatively higher accumulation of arsenic in root vegetable *Raphanus sativus*. Higher amount of arsenic bioaccumulation in *Raphanus sativus* irrigated with waste water in Bangladesh has also been reported by Ahmed et al (2019). The results of the present study are in coherence with the above mentioned study.

4.8.2 Cadmium (Cd)

In the present study, the average amount of cadmium in waste-water-irrigated vegetables was found to be 23.20±0.10 mg/kg and 22.85±0.18 mg/kg, in *Raphanus sativus*, 22.90±0.10 mg/kg and 22.20±0.10 mg/kg in *Spinacia oleracea*, 16.70±0.10 mg/kg and 16.86±0.15 mg/kg in *Brassica oleracea* and 9.76±0.15 mg/kg and 9.10±0.10 mg/kg in *Lycopersicum esculentum* both in the first and second years of study respectively. Similarly, the mean amount of cadmium in borewell water irrigated vegetables was detected to be 5.60±0.43 mg/kg and 5.16±0.15 mg/kg in *Raphanus sativus*, 6.20±0.10 mg/kg and 5.90±0.10 mg/kg in *Spinacia oleracea*, 4.13±0.15 mg/kg and 4.13±0.05 mg/kg in *Brassica oleracea* and 3.95±0.05 mg/kg and 3.91±0.07 mg/kg in *Lycopersicum esculentum* both in the first and second years of study respectively. The difference in the mean values of uptake by all the vegetables irrigated by borewell water and waste water exceeds the critical difference between the means obtained at 5% level of significance which suggests that the amount of cadmium bioaccumulation in waste-water-irrigated vegetables was significantly higher than the borewell irrigated vegetables. Maximum bioaccumulation of 23.20±0.10 mg/kg was detected in root vegetable *Raphanus sativus* and minimum amount of 9.10±0.10 mg/kg was detected in fruit vegetable *Lycopersicum esculentum* in both the years of study. There was not much difference in cadmium bioaccumulation in first and second years of study but the amount of cadmium in all the vegetables studied was found to be much higher than the prescribed limits (WHO/FAO 2007; EU standards, 2002; Awasthi, 2000). The results obtained in the present study are in accordance with the results obtained by previous researchers who reported higher amounts of cadmium in waste-water-irrigated vegetables. Gupta et al (2008) reported the bioaccumulation of cadmium ranging from 10.37 to 17.79 mg/kg in vegetable samples collected from a waste-water-irrigated site in Titagarh, Bangladesh. Similarly, Chopra and Pathak (2012) also reported the higher bioaccumulation of cadmium (16.89±4.12 mg/kg in *Brassica oleracea* and 19.18±3.87mg/kg in *Raphanus sativus*) irrigated by waste water of Bindal river in Dehradun. The results of the present study are also authenticated by Chaoua et al (2019) who reported 12.03 mg/kg of cadmium in *Vicia faba* irrigated in heavy-metal-rich waste water as compared to 2.3 mg / kg in borewell irrigated samples of *Vicia faba*.

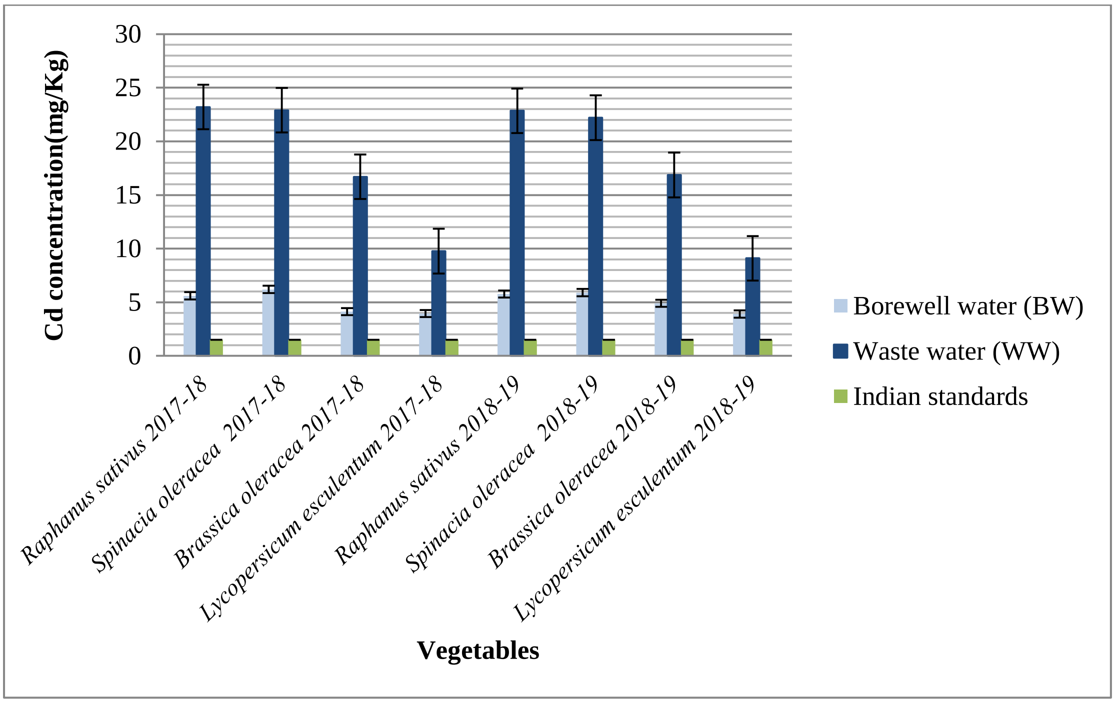


Fig 4.10: Mean bioaccumulation of Cadmium (Cd) in BW and WW irrigated vegetables (p≤0.05; Error bar= ±SD)

4.8.3 Chromium (Cr)

In this study, the average amount of chromium in borewell irrigated vegetables was analyzed to be 6.06±0.15 mg/kg and 5.76±0.15 mg/kg in *Raphanus sativus*, 5.90±0.10 mg/kg and 5.90±0.10 mg/kg in *Spinacia oleracea*, 5.00±0.10 mg/kg and 4.90±0.05 mg/kg in *Brassica oleracea* and 3.80±0.20 mg/kg and 3.91±0.07mg/kg in *Lycopersicum esculentum*, both in the first and second years of study respectively. Similarly, the mean amount of chromium in waste-water-irrigated vegetables was detected to be 46.0±0.10 and 45.50±0.10 in *Raphanus sativus*, 44.80±0.20 mg/kg and 44.20±0.10 mg/kg in *Spinacia oleracea*, 24.76±0.15 mg/kg and 24.50±0.1 mg/kg in

Brassica oleracea, 12.70±0.10 mg/kg and 12.50±0.57 mg/kg in *Lycopersicum esculentum*, both in the first and second year of study respectively. The actual difference in the mean values of uptake by all the vegetables irrigated by borewell water and waste water exceeded the critical difference between the means at 5% level of significance which suggests that the amount of chromium bioaccumulation was significantly higher in waste-water-irrigated vegetables than the borewell irrigated ones. Maximum bioaccumulation value of 46±0.10mg/kg was detected in root vegetable *Raphanus sativus* and minimum amount of 12.50±0.10 mg/kg in fruit vegetable *Lycopersicum esculentum* in both the years of study. There was no significant difference in cadmium bioaccumulation in first and second year of the study but the amount of cadmium in all the vegetables studied exceeded the prescribed limits (WHO, FAO 2007; EU standards, 2002; Awasthi, 2000). The values obtained in the present study were a little bit less but similar to the values detected in cauliflower (86.83 mg/kg), spinach (96.30 mg/kg) and radish (78.02 mg/kg) irrigated by waste water as reported by Gupta et al (2010).

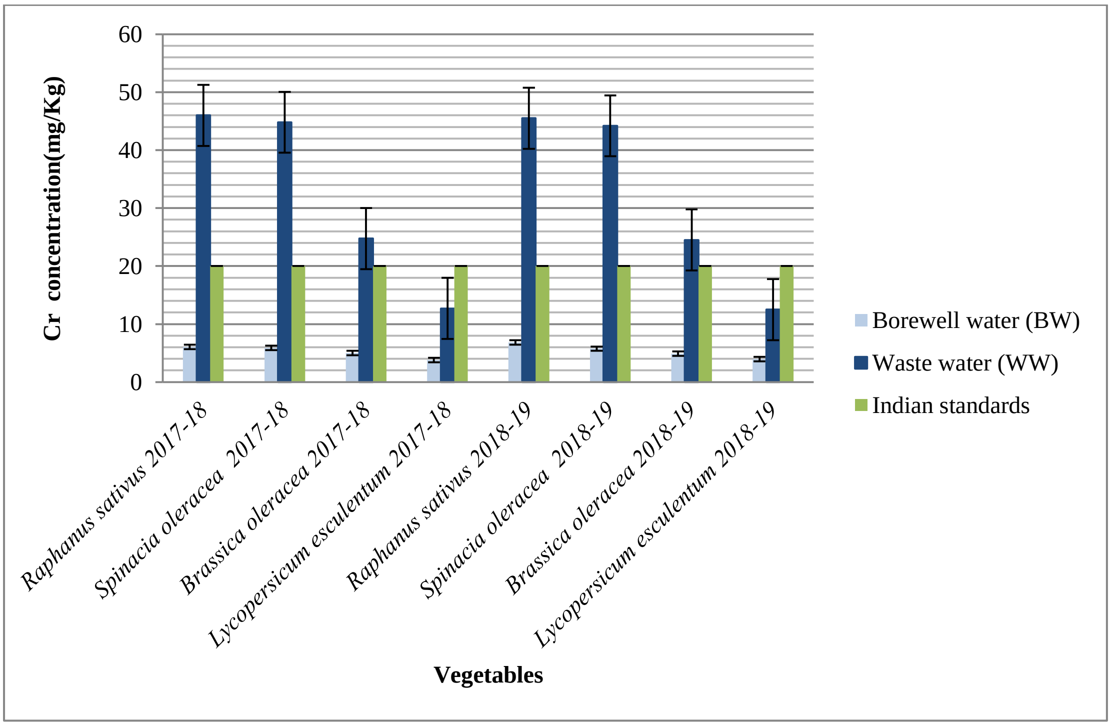


Fig 4.11: Mean bioaccumulation of Chromium (Cr) in BW and WW irrigated vegetables (p≤0.05; Error bar= ±SD)

The results of the present study are in agreement with the previous results obtained by Chopra and Pathak (2012) who reported higher accumulation of chromium (105±4.30 mg/kg) in root vegetable *Raphanus sativus* and 85.73±9.89 mg/kg in *Brassica oleracea* irrigated by waste water of Bindal river, Dehradun. The values of chromium bioaccumulation in the present study were higher than the values reported by Ugya (2019) in vegetables (0.5-2.6 mg/kg) irrigated by refinery effluents containing waste water in Nigeria.

4.8.4 Lead (Pb)

In the present study, the average amount of lead in waste-water-irrigated vegetables was found to be 21.80±0.10 mg/kg and 21.10±0.10 mg/kg in *Raphanus sativus*, 20.90±0.10 mg/kg and 20.03±0.05 mg/kg in *Spinacia oleracea*, 15.86±0.15 mg/kg and 15.00±0.10 mg/kg in *Brassica oleracea*, 9.80±0.02 mg/kg and 9.20±0.01 mg/kg in *Lycopersicum esculentum*, both in the first and second years of study respectively. Similarly, the mean amount of lead in borewell water irrigated vegetables was detected to be 6.10±0.10 mg/kg and 5.85±0.05 mg/kg in *Raphanus sativus*, 5.78±0.07 mg/kg and 5.60±0.10 mg/kg in *Spinacia oleracea*, 4.73±0.15 mg/kg and 4.40±0.10 mg/kg in *Brassica oleracea*, 4.10±0.05 mg/kg and 3.90±0.01 mg/kg in *Lycopersicum esculentum*, both in the first and second years of study respectively. The actual difference in the mean values of uptake by all the *vegetables* irrigated by borewell water and waste water exceeded the critical difference in the mean values obtained at 5% level of significance which suggests that the amount of lead bioaccumulation was significantly higher in waste-water-irrigated vegetables than the borewell irrigated ones. Maximum bioaccumulation of 21.80±0.01 mg/kg was detected in root vegetable *Raphanus sativus* and minimum value of 3.90±0.01 mg/kg in fruit vegetable *Lycopersicum esculentum* in both the years of study. There was no significant difference in lead bioaccumulation in first and second years of study but the amount of lead in all the vegetables studied exceeded the prescribed limits (WHO, FAO, 2007; EU standards, 2002 and Indian standards (Awasthi, 2000). The results of the present study are similar to the results obtained by Gupta et al (2010) in which higher amounts of lead in vegetables lettuce (34.94 mg/kg), radish (56.30 mg/kg), spinach (49.70 mg/kg), cauliflower (31.50 mg/kg) was reported by waste water irrigation as compared to groundwater irrigated samples of same vegetables. The values of lead bioaccumulation in *Lycopersicum esculentum* in the present study was less than in comparison to amount of 14.15 mg/kg as reported (Ahmed and Goni, 2010) in the tomatoes irrigated by waste water of an industrial area in Dhaka, Bangladesh. The results of the present study were similar to the results obtained by Rao et al (2017) who detected higher amount of lead bioaccumulation *in Lycopersicum esculentum* irrigated with industrial waste water.

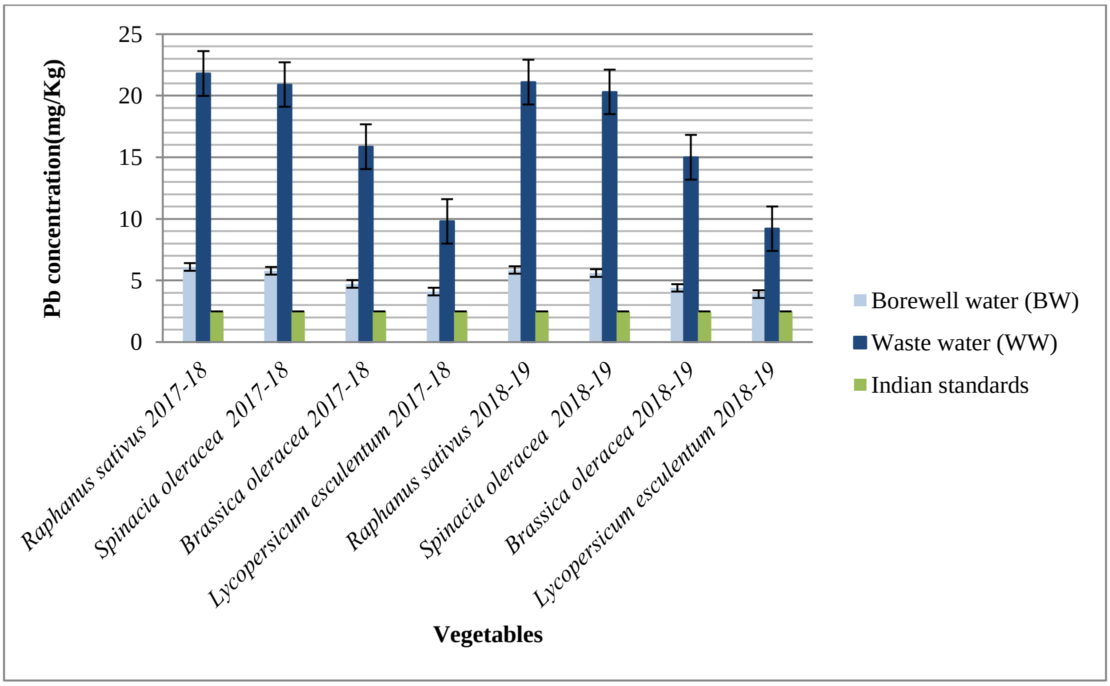


Fig 4.12: Mean bioaccumulation of lead (Pb) in BW and WW irrigated vegetables ($p \leq 0.05$; Error bar= ±SD)

Table 4.12 : Showing Standards for heavy metals in vegetables (mg/Kg)

Standards	**As**	**Cd**	**Cr**	**Pb**
Indian standard	--	1.50	20	2.50
EU standard	--	0.20	--	5.00
WHO/FAO/2007	0.05	0.20	0.50	0.30

Indian standard (Awasthi, 2000)

EU: European Union standards (2002)

WHO/FAO: World Health Organization/Food and Agricultural Organization (2007)

On the basis of results obtained in the present study it was concluded that vegetables raised in the waste water of Buddha Nullah tend to bio-accumulate higher amounts of all the heavy metals studied. Different vegetables tend to bio-accumulate the same heavy metals in different amounts. The bioaccumulation of all the metals was observed to be in the following descending order: root vegetables > leafy vegetables > flower vegetables > fruit vegetables. The trend of heavy metal bioaccumulation in vegetables was found to be in the following descending order: chromium > cadmium > lead > arsenic. The present study does not agree with the previous study on heavy metal bioaccumulation in waste-water-irrigated vegetables done by Zhou et al (2016) who reported that leafy vegetables bio-accumulate maximum amount of heavy metals as compared to the other vegetables. In the present study root vegetable *Raphanus sativus* was found to bioaccumulate maximum amount of all the heavy metals studied which can be attributed to the fact that root vegetables are in constant touch with contaminated soil and contain a lot of water The bioaccumulation of heavy metals detected in the present study was found to be comparatively less than reported by some other workers at some other waste-water-irrigated sites which can be attributed to the fact that continuous cultivation of hyper accumulator vegetables helps in phytoremediation of soil along the banks of Buddha Nullah. The present study also agrees with the previous studied by Szatanik-Kloc (2004) and Karami et al (2011) who reported that absorption of heavy metals is affected by soil type of plants, plant part and plant species and even the variety of the same species.

4.9 Biochemical changes induced in vegetables irrigated with waste water

The amount of basic biochemical constituents like total proteins, carbohydrates, total chlorophyll, starch, reducing and non-reducing sugars, secondary metabolites, non enzymatic antioxidants and the activity of enzymatic oxidants varies from species to species in plants. Bioaccumulation of heavy metals in different parts of the plants due to waste water irrigation induces physiological stress and subsequent alterations in their biochemical composition. In the present work, effect of heavy metal bioaccumulation on biochemical changes induced in the following biochemical parameters of the vegetables was evaluated.

4.9.1 Total chlorophyll (mg/g)

The green photosynthetic pigment chlorophyll is a vital constituent of photosynthetic system which helps to harness the sunlight to produce glucose which is the main source of energy for growth and development of the plant. Basically, chlorophyll is present in chloroplasts and is composed of two parts: chlorophyll a and chlorophyll b (Yakar and Bilge, 1987; Carter, 1996). In this study the mean amount of total chlorophyll in borewell irrigated samples of *Raphanus sativus* was detected to be 0.205±0.03 mg/g and 0.200±0.04 mg/g as compared to 0.185±0.05 mg/g and 0.184±0.02 mg/g in waste-water-irrigated samples during the first and second years of study respectively. In *Spinacia oleracea*, the average amount of total chlorophyll was found to be 0.214±0.07 mg/g and 0.212±0.03 mg/g in borewell irrigated but comparative lower amount of 0.194±0.04 mg/g and 0.193±0.04 mg/g in the waste-water-irrigated samples during first and second years of study respectively. Waste-water-irrigated samples of *Brassica oleracea* showed 0.181±0.02 mg/g and 0.180±0.04 mg/g as compared to 0.197±0.04 mg/g and 0.199±0.03 mg/g of average total chlorophyll in borewell water irrigated samples in first and second years of study respectively. The mean total amount of chlorophyll was analyzed to be 0.181±0.04 mg/g and 0.184±0.05 mg/g in borewell irrigated and that of 0.163±0.02 mg/g and 0.164±0.03 mg/g in waste-water-irrigated samples of *Lycopersicum esculentum* during first and second years of study respectively. The borewell irrigated samples of all the vegetables were found to have relatively higher mean amount of total chlorophyll content as compared to the waste-water-irrigated samples analyzed at ($p<0.05$).

Gupta et al (2009) reported a drastic decrease in total chlorophyll content in *Brassica nigra* and *Colocasia esculenta* irrigated with waste water. In another study, Bahmniya et al (2010) also reported a significant decrease in chlorophyll a and chlorophyll b content in *Brassica oleracea* and *Spinacia oleracea* irrigated with waste water of Ayad river in Udaipur. Singh and Aggrawal (2010) reported an increase in chlorophyll content of *Beta vulgaris* irrigated with untreated industrial waste water. Khaleel et al (2013) also obtained the similar results in which waste-water-irrigated *Abelmoshus esculentum* was found to have a significantly low amount of chlorophyll content. In a recent study done by Kapoor et al (2014) and Maity et al (2019), a significant decrease in chlorophyll content was detected in *Brassica juncea* and *Cicer arietinum* irrigated with waste water. Higher concentration of waste water has been reported to cause a deleterious effect on synthesis of chlorophyll content particularly that of chlorophyll a (Khan et al., 2011). Toxic metals have been found to cause decrease in chlorophyll content in vascular plants due to breakdown or inhibition in synthesis of photosynthetic pigment (Monni et al., 2001 and Patsikka et al., 2002). The outcome of above mentioned studies confirms the results obtained in the present study in which a drastic decrease in chlorophyll a, chlorophyll b and total chlorophyll content was noted in the vegetables irrigated with the waste water of Buddha Nullah as compared to that of borewell water irrigated ones.

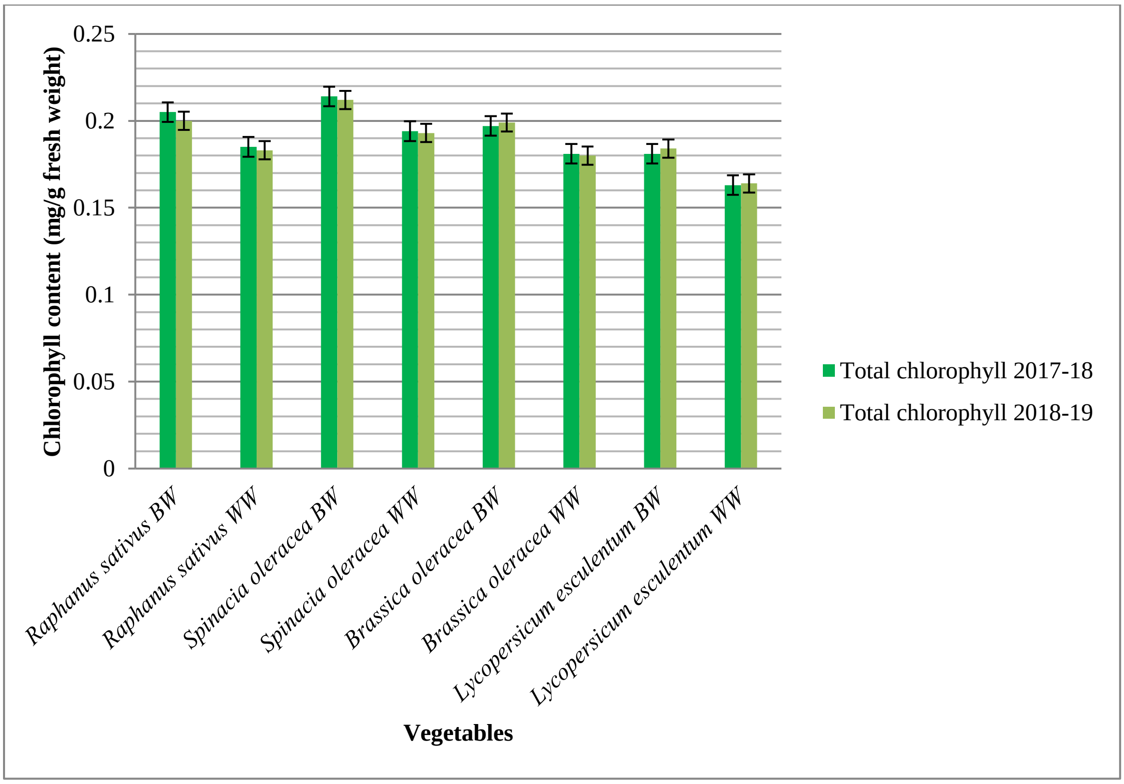


Fig 4.13: Mean amount of total chlorophyll content in waste and borewell irrigated vegetables ($p \leq 0.05$; Error bar= ±SD)

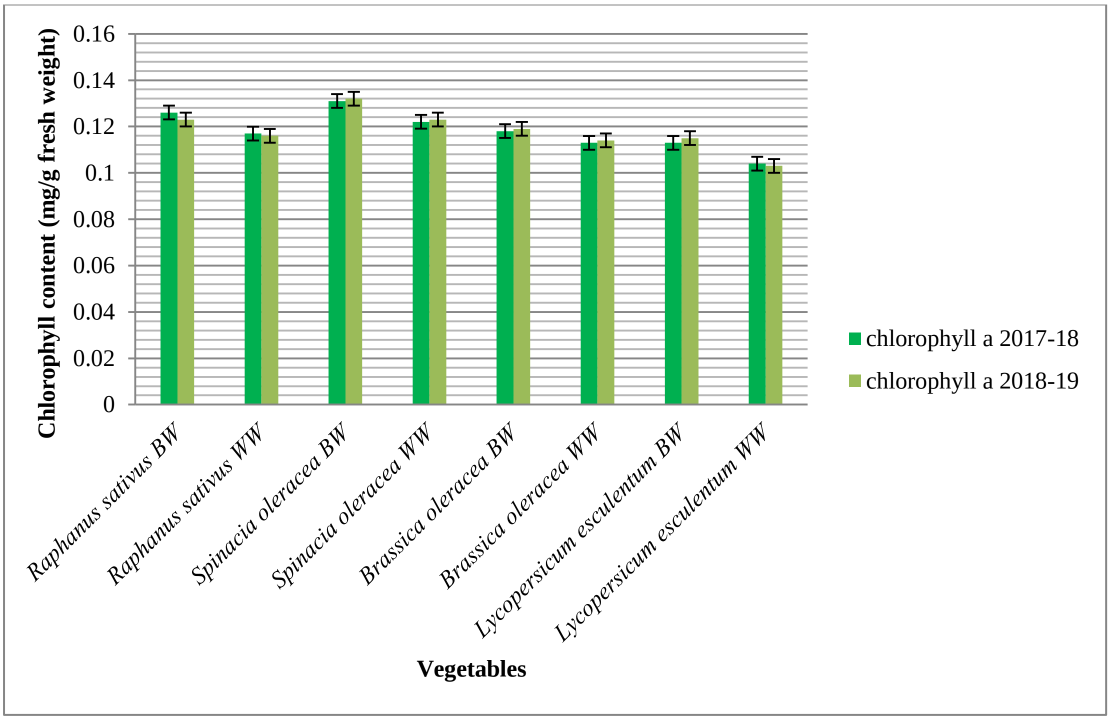


Fig 4.14: Mean amount of chlorophyll a content in waste and borewell irrigated vegetables ($p \leq 0.05$; Error bar= ±SD)

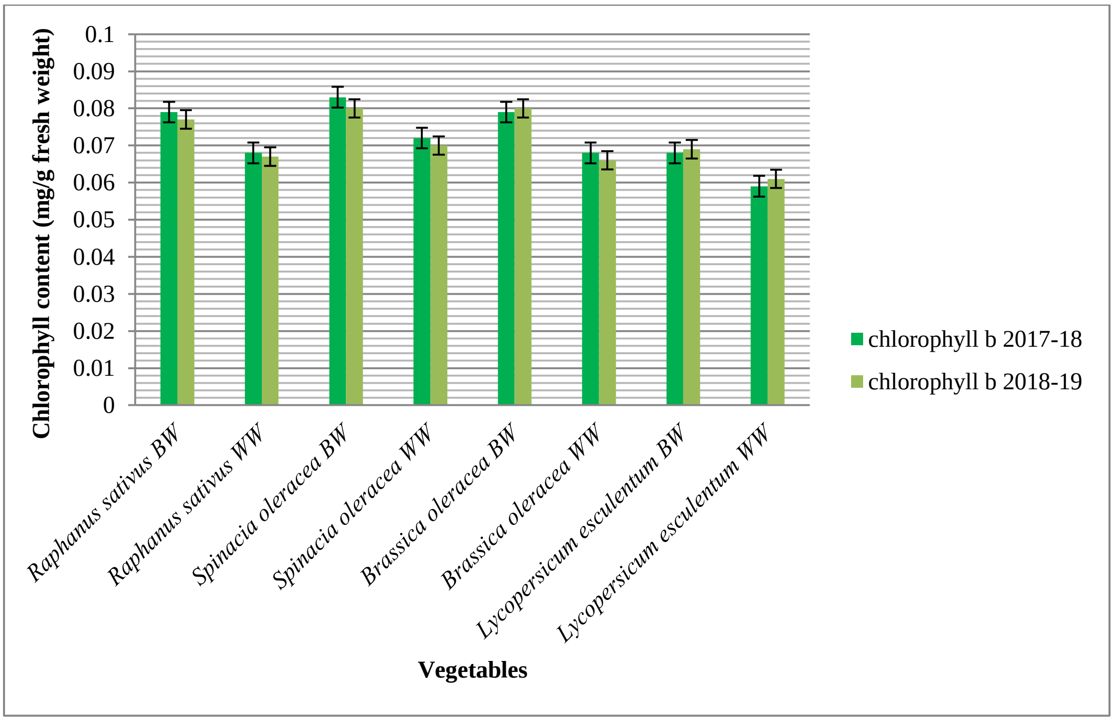


Fig 4.15: Mean amount of chlorophyll b content in waste and borewell irrigated vegetables ($p \leq 0.05$; Error bar= ±SD)

4.9.2 Starch (mg/g)

Starch is another major photosynthetic product manufactured from excess glucose and acts as a reserve energy product in the form of granules stored in chloroplasts, fruit and seed of the plants. Normal growth of plants is controlled by the starch content when the plant is under some kind of environmental stress conditions (Gibon et al., 2009). The average amount of starch content was observed to be higher in the borewell water irrigated samples of all the vegetables studied as compared to that of waste-water-irrigated ones. The waste-water-irrigated vegetable samples of *Raphanus sativus* were detected to have 1.320±0.02 mg/g and 1.329±0.03 mg/g and borewell water irrigated samples having 1.655±0.03 mg/g and 1.649±0.02 mg/g of starch content in both the first and second years of study respectively. In *Spinacia oleracea*, the mean amount of starch was found to be 1.420±0.16 mg/g and 1.431±0.04 mg/g in borewell irrigated as compared to that of 1.290±0.04 mg/g, and 1.300±0.03 mg/g in waste-water-irrigated samples during first and second years of study respectively. Waste-water-irrigated samples of *Brassica oleracea* showed 1.325±0.22 mg/g and 1.320±0.16 mg/g as compared to 1.521±0.18 mg/g and 1.516±0.17 mg/g of average starch content in borewell water irrigated samples both in first and second years of study respectively. Similarly, the mean content of starch was analyzed to be 1.328±0.31 mg/g and 1.322±0.2 mg/g in borewell in comparison to that of 1.103±0.19 mg/g and 1.109±0.3 mg/g in waste-water-irrigated samples of *Lycopersicum esculentum* during both the first and second years of study respectively. The borewell irrigated samples of all the vegetables were found to have comparatively higher amount of starch as of that in the waste-water-irrigated samples analyzed at $p<0.05$. Almost identical results were found by Damera et al (2015) who reported a significant decrease in starch content in *Vigna radiata* grown under heavy metal stress. The results were also found in accordance with the results obtained by Das et al (2016) who observed a significant lowering of starch content in *Oryza sativa* irrigated with heavy - metal - rich waste water and this decrease was attributed to an increase in activity of starch phosphorylase which is directly related with decrease in starch content (Dubey and Singh, 1999).

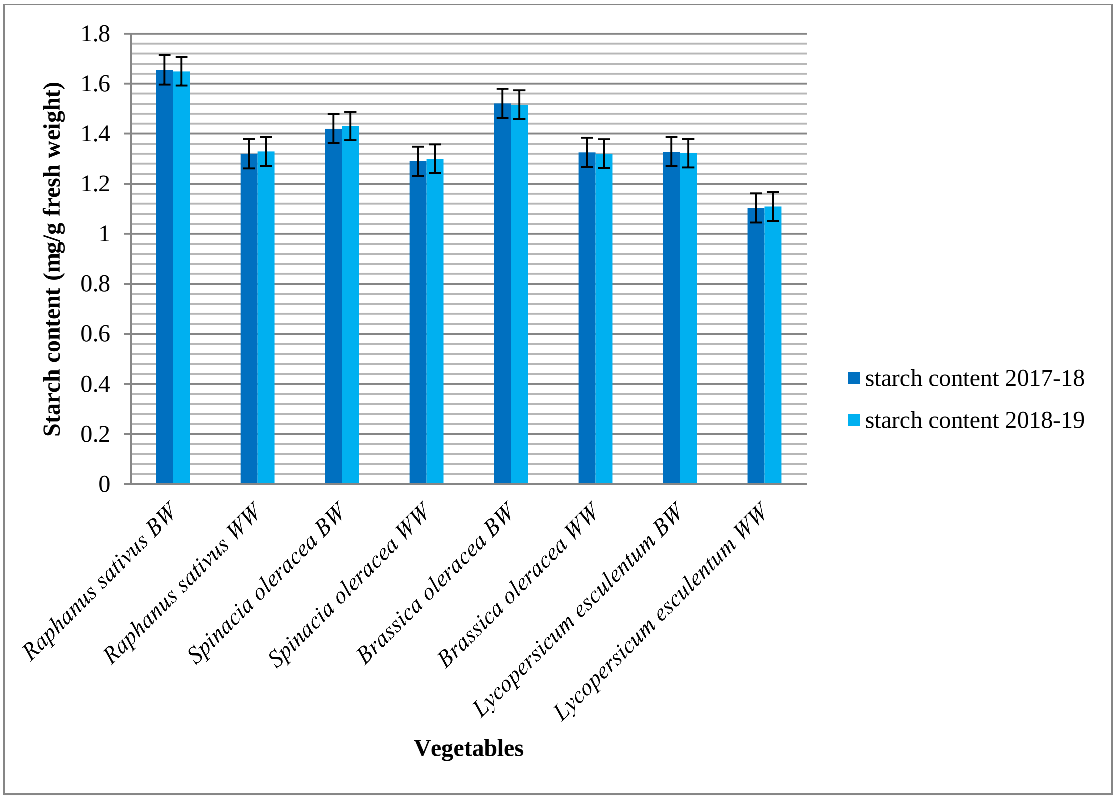


Fig 4.16: Mean amount of starch content in waste and borewell irrigated vegetables ($p \leq 0.05$; Error bar= ±SD)

4.9.3 Proteins (mg/g)

Proteins are a complex constituent of amino acids which play an important role in structural and functional activities of the plant cell. Proteins are known to facilitate intercellular membrane transport and electron transport involving energy generating reactions. They have a limited life span and are constantly translocated from mRNA for proper growth and development of the plant (Damera et al., 2015). During oxidative stress, the amount of proteins decreases due to denaturation and fragmentation (John et al., 2009) and increases due to synthesis of metalloproteins and metal binding proteins (Pal et al., 2006 and Nayek et al., 2010). The results obtained in the present study revealed an increase in average protein content in the edible portions of all the vegetables irrigated with waste water of Buddha Nullah as compared to that of borewell irrigated vegetables in both the years of study. Waste-water-irrigated samples of *Raphanus sativus* was found to have 1.30±0.20 mg/g and 1.32±0.30 mg/g as compared to 1.20±0.30 mg/g and 1.25±0.10 mg/g of average protein content in borewell irrigated root samples in both first and second years of study respectively. A mean amount of 3.28±0.30 mg/g and 3.35±0.20 mg/g as compared to that of 3.15±0.40 mg/g and 3.25±0.10 mg/g of average protein content was detected in waste water and borewell water respectively during first and second years of study in *Spinacia oleracea*. Similarly the amount of protein in borewell irrigated samples of *Brassica oleracea* were observed to be 2.75±0.40 mg/g and 2.70±0.20 mg/g in comparison to that of 2.80±0.30 mg/g and 2.78±0.40 mg/g in waste-water-irrigated samples during both the first and second years of study respectively. Analysis of waste-water-irrigated samples of *Lycopersicum esculentum* showed mean protein content to be 2.20±0.30 mg/g and 2.28±0.20 mg/g as compared to that of 2.10±0.40 mg/g and 2.20±0.30 mg/g in borewell water irrigated samples both in the first and second years of analysis respectively.

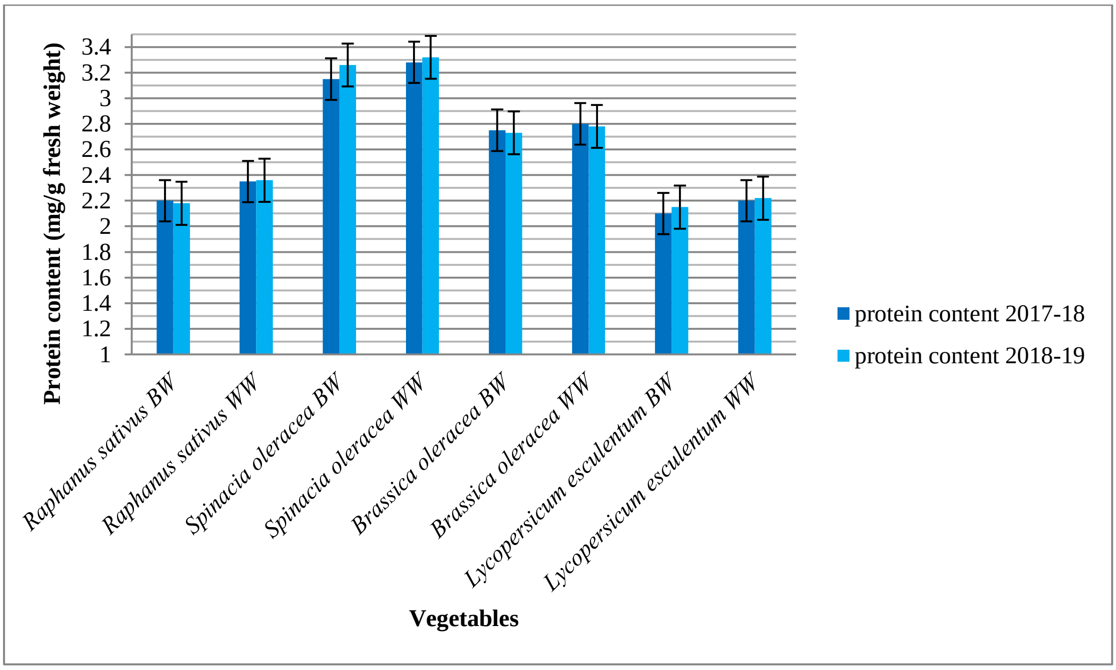


Fig 4.17: Mean amount of protein content in waste and borewell irrigated vegetables ($p \leq 0.05$; Error bar= ±SD)

The results of total protein content obtained in the present study has been found to be in agreement with the results of a similar study conducted by Gupta et al (2010) in which high content of protein was reported in waste-water-irrigated *Raphanus sativus, Colocasia* and *Spinacia oleracea*. A similar study was conducted by Ghani et al (2013) who detected significant increase of protein content in sewage water irrigated pumpkin samples in comparison to the canal water irrigated ones. Another study conducted by Swati and Yenagi (2015) also reported an increase in protein content in coriander, methi, palak and brinjal irrigated with waste water. The outcome of the present study was in agreement with the work done by above said scientists.

4.9.4 Sugar content

Sugars are the basic energy biomolecules required for growth, development and biochemical activities of the plants. The soluble carbohydrates known as sugars also help in providing protection to plant under stress conditions by maintaining and balancing the osmotic pressure in cytosol. They play an important role in protecting the cell against oxygen reactive species by maintaining membrane integrity and stabilization of proteins (Bohnert et al., 1996 and Ashraf, 2009). Sugars are divided into two groups: those having a free aldehyde or ketone group are called reducing sugars and others without the -OH group are referred to as non-reducing sugars.

4.9.4.1 Reducing sugars (mg/g)

The average amount of reducing sugars in *Raphanus sativus* was detected to be 2.320±0.04 mg/g and 2.280± 0.03 mg/g in borewell water as compared to that of 2.761±0.04 mg/g and 2.755±0.02 mg/g in waste-water-irrigated samples in both the first and second years of study. The mean amount of 2.650±0.03 mg/g and 2.700±0.10 mg/g as compared to that of 2.250±0.05 mg/g and 2.200±0.03 mg/g of reducing sugars was detected in waste water and borewell water irrigated *Spinacia oleracea* during both the first and second years of study respectively. Similarly, the amount of reducing sugars in borewell irrigated samples of *Brassica oleracea* were analyzed to be 2.187±0.02 mg/g and 2.220±0.3 mg/g in comparison to that of 2.325±0.05 mg/g and 2.365±0.10 mg/g in waste-water-irrigated samples, both during the first and second years of study respectively. Analysis of waste-water-irrigated samples of *Lycopersicum esculentum* showed the mean amount of reducing sugars to be 2.210±0.20 mg/g and 2.230±0.04 mg/g as compared to 1.875±0.30 mg/g and 1.910±0.30 mg/g in borewell water irrigated samples during both the first and second years of analysis respectively. The waste-water-irrigated samples of all the vegetables studied were found to have higher amount of reducing sugars in their edible portions at $p<0.05$ which confirmed that reducing sugars play an important role to ameliorate the oxidative stress induced by the bioaccumulation of heavy metals.

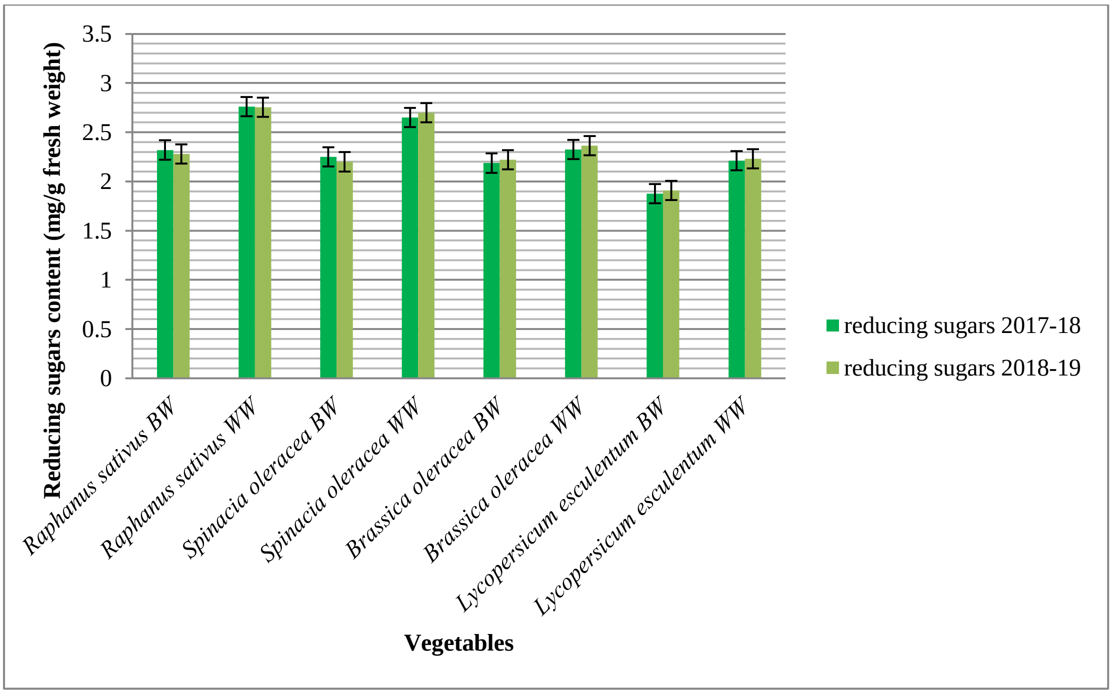


Fig 4.18: Mean amount of reducing sugar content in waste and borewell irrigated vegetables ($p \leq 0.05$; Error bar= ±SD)

4.9.4.2 Non-reducing sugars (mg/g)

The average amount of non-reducing sugars in *Raphanus sativus* was detected to be 0.430±0.03 mg/g and 0.428±0.01 mg/g in borewell as compared to that of 0.503±0.04 mg/g and 0.505±0.02 mg/g in waste-water-irrigated samples in both the first and second years of study respectively. The mean amount of 0.480±0.01 mg/g and 0.492±0.02 mg/g as compared to 0.425±0.02 mg/g and 0.422±0.02 mg/g of non-reducing sugars was detected in waste water and borewell water irrigated *Spinacia* oleracea during both the first and second year of study respectively .in . Similarly, the average amount of non-reducing sugars in borewell irrigated samples of *Brassica oleracea* obtained was 0.470±0.03 mg/g and 0.465±0.01 mg/g as equated to 0.510±0.04 mg/g and 0.500±0.02 mg/g in waste-water-irrigated samples during both the first and second years of study respectively. Analysis of waste-water-irrigated samples of *Lycopersicum esculentum* showed the mean amount of non-reducing sugars to be 0.465±0.01 mg/g and 0.460±0.03 mg/g as compared to 0.422±0.03 mg/g and 0.419±0.02 mg/g in borewell water irrigated samples both during first and second years of sample analysis respectively. The waste-water-irrigated vegetables were found to have higher amount of non-reducing sugars at $p<0.05$ in their edible portions which showed that non-reducing sugars also play an important role to counteract the oxidative stress induced by the bioaccumulation of heavy metals.

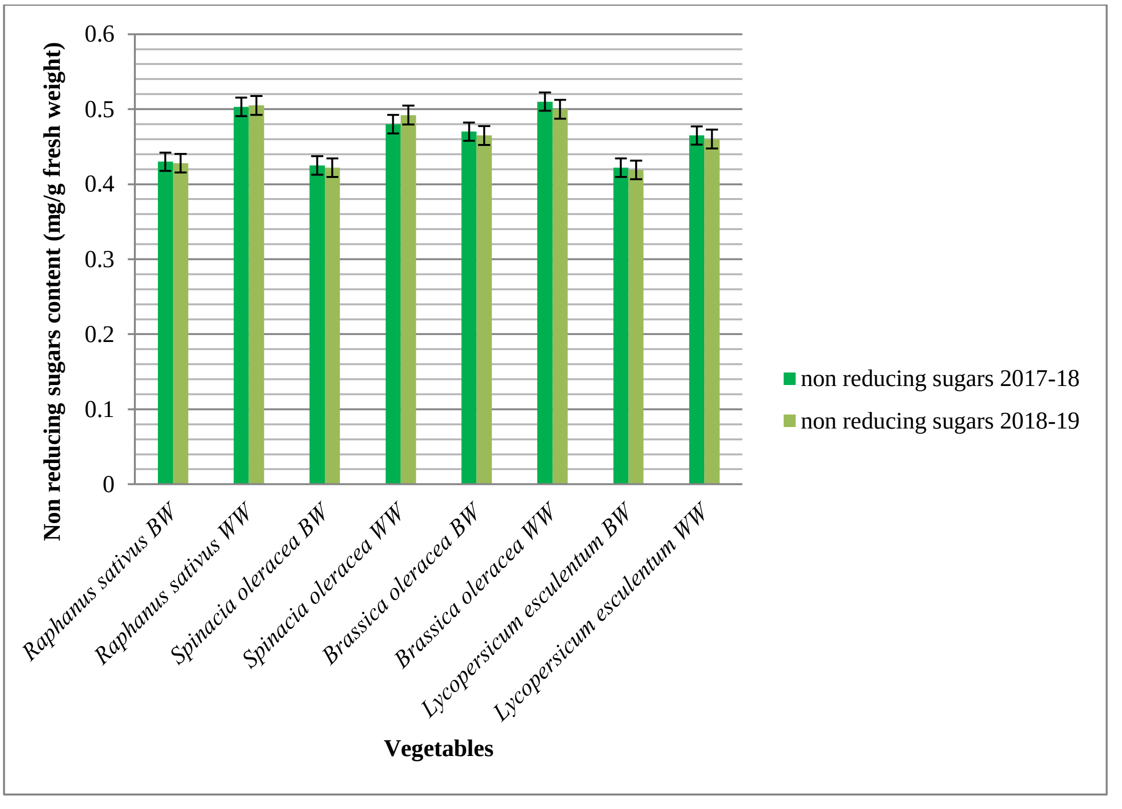


Fig 4.19: Mean amount of non-reducing sugar content in waste and borewell irrigated vegetables ($p\leq0.05$; Error bar= ±SD)

4.9.4 Total sugars (mg/g)

The average amount of total sugars in *Raphanus sativus* was detected to be 2.807±0.40 mg/g and 2.762±0.30 mg/g in borewell as equated to 3.264±0.30 mg/g and 3.255±0.20 mg/g in waste-water-irrigated samples in both the first and second years of study. The mean content 3.13±0.30 mg/g and 3.192±0.40 mg/g as compared to 2.702±0.40 mg/g and 2.65±0.20 mg/g of total sugars was detected in waste water and borewell water irrigated *Spinacia oleracea* both during the first and second years of study respectively. Similarly, the mean amount of total sugars in borewell irrigated samples of *Brassica oleracea* was observed to be 2.657±0.10 mg/g and 2.685±0.20 mg/g as compared to that of 2.824±0.40 mg/g and 2.862±0.10 mg/g in waste-water-irrigated samples both during the first and second years of study respectively. Analysis of waste-water-irrigated samples of *Lycopersicum esculentum* showed an average amount of total sugars to be 2.665±0.20 mg/g and 2.69±0.30 mg/g as compared to 2.297±0.30 mg/g and 2.329±0.20 mg/g in borewell water irrigated samples during both the first and second years of sample analysis respectively. The waste-water-irrigated vegetables were found to contain comparatively higher amount of total sugars in their edible portions at $p<0.05$ which shows that total sugars play an important role to neutralize the oxidative stress induced by the bioaccumulation of heavy metals.

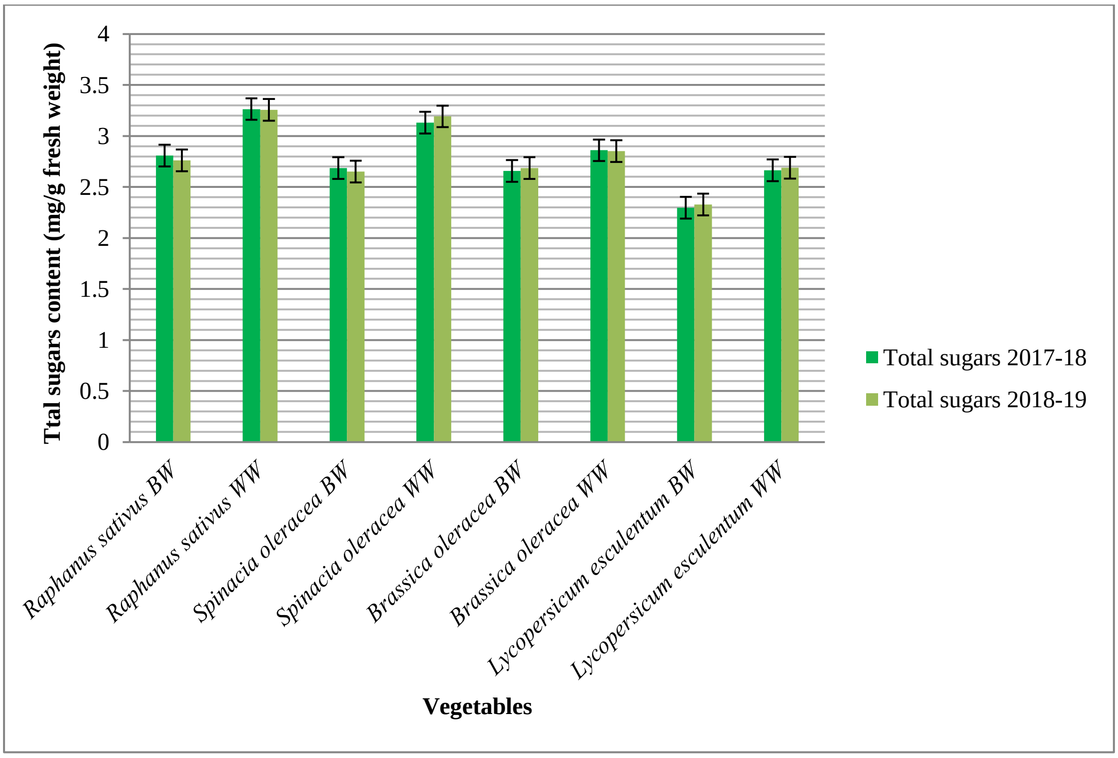


Fig 4.20: Mean amount of total sugar content in waste and borewell irrigated vegetables ($p \leq 0.05$; Error bar= ±SD)

Gupta et al (2010) reported a significant increase in sugar content in waste-water-irrigated vegetables to nullify the oxidative stress induced by the heavy metals present in the waste water. Similar results have also been obtained in another study conducted by Swati and Yenagi (2015) who observed a significant increase in reducing, non-reducing and total sugar content in sewage water irrigated leafy and fruit vegetables like palak, methi, coriander, brinjal and tomato. The results of our study are also in coherence with the recent study done by Maity et al (2019) who reported a significant increase in reducing sugars content in *Brassica juncea* and *Cicer arietinum* irrigated with waste water. The results obtained in the present study also indicated that sugar signaling might also be one of the plant defence responses in plants under oxidative stress.

4.10 Antioxidant activity in vegetables irrigated with borewell water and waste water of Buddha Nullah

4.10.1 Non enzymatic antioxidants

4.10.1.1 Ascorbic acid (mg/100g)

Ascorbic acid (vitamin C) is an important nutrient as well as the substrate of some antioxidant enzymes like APX produced during various types of environmental stresses faced by the plants (Guo et al., 2005). In the present study the average amount of ascorbic acid was detected to be 45.00±0.12 mg/100g, 46.50±0.10 mg/100g and 55.00±0.18 mg/100g, 56.00±0.12 mg/100g in the borewell and waste water irrigated *Raphanus sativus* during both the first and second years of study respectively. Similarly, in *Spinacia oleracea*, the borewell irrigated plants were found to bio-accumulate the mean amount of 42.00±0.70mg /100 gm and 42.50±0.60 mg/100g of ascorbic acid as compared to an increased mean amount of ascorbic acid 50.00±0.12 mg/100g and 51.50±0.10 mg/100g in the waste-water-irrigated samples during both the first and second years of study respectively. *Brassica oleracea* was found to have an average amount of 30±0.40 mg/100g, 30.50±0.50 mg/100 and 38.00±0.20 mg/100g, 39.20±0.30 mg/100g in borewell and waste- water irrigated samples in both the the first and second years of study respectively. The waste-water-irrigated samples of *Lycopersicum esculentum* were found to contain 44.00±0.80 mg/100 and 43.90±0.30 mg/100g of ascorbic acid as compared to 35.00±0.30 mg/100g and 34.60±0.40 mg/100g in borewell irrigated samples during both the first and second years of the study respectively. There was no significant difference of ascorbic acid amount in borewell and waste water treated samples in both first and second years of study but a remarkable increase of ascorbic acid content was detected at ($p<0.05$) in waste-water-irrigated samples as compared to borewell water samples of all the vegetables analyzed.

Singh and Aggarwal (2007) also reported a significant increase in ascorbic acid content in *Beta vulgaris* irrigated with heavy-metal-rich waste water. Gupta et al (2010) detected a marginal increase in ascorbic acid content in waste-water-irrigated samples of *Raphanus sativus*, *Brassica nigra* and *Colocasia esculentum*. Pallavi and Joseph (2014) obtained similar results in which significantly higher amount of ascorbic acid was observed in waste-water-irrigated samples of *Amaranthus tricolour* as compared to the normal ground water irrigated ones. The present study agrees with the results reported by the various researchers mentioned above.

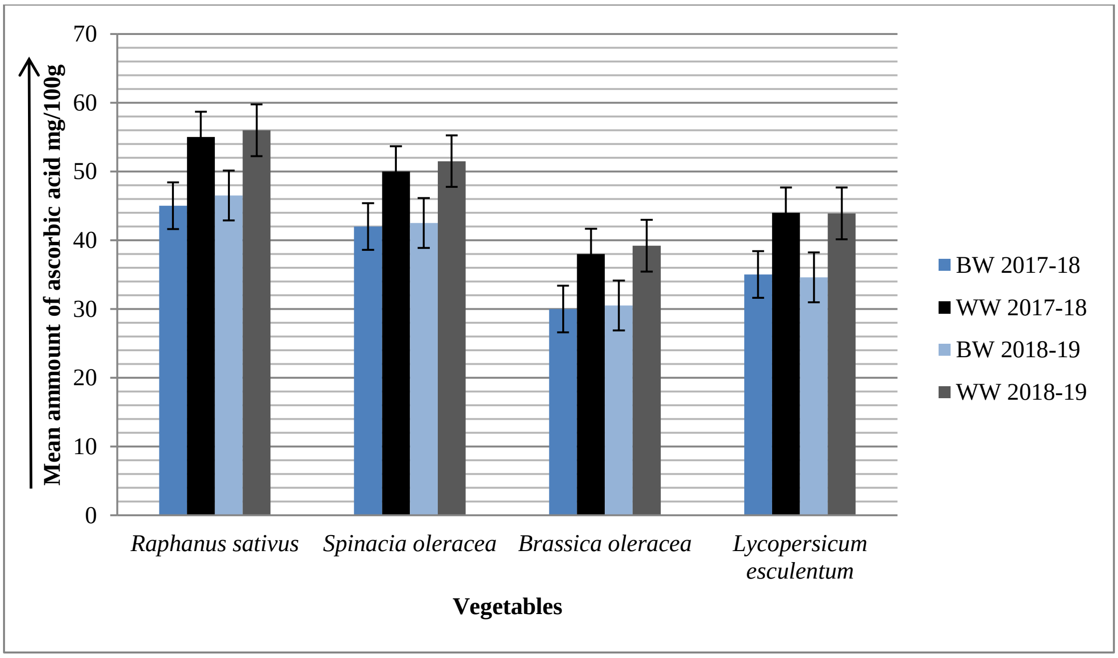


Fig 4.21: Mean amount of ascorbic acid in waste water and borewell water irrigated vegetables (p≤0.05; Error bar= ±SD)

4.10.1.2 Total phenols (mg/ g)

Michalak (2006) reported an induction in phenolic metabolism in plants under heavy metal stress. In the present study, the mean amount of total phenols was detected to be 35.00±0.70 mg/100g and 36.00±0.80 mg/ g in borewell water and that of 44.00 ±0.12 mg/g and 43.80±0.14 mg/g in the waste-water-irrigated samples of *Raphanus sativus* during both the first and second years of study respectively. Similarly in *Spinacia oleracea* the total phenolic content was detected to be 45.00±0.80 mg/g and 44.80±0.60 mg/g in borewell water but comparatively increased amount of

58.00±0.70 mg/g and 57.40±0.80 mg/g in the waste-water-irrigated samples during both the first and second years of study respectively. *Brassica oleracea* was found to have 32.00±0.90 mg/g, 31.60±0.60 mg/g and 40.50±2.00 mg/g, 42.00±0.30 mg/g mean content of total phenols in borewell and waste water irrigated samples both in the first and second years of study respectively. The waste-water-irrigated samples of *Lycopersicum esculentum* were found to contain 44.00±0.60 and 44.60±0.40 mg/g of total phenol as compared to 37.00±0.70 mg/g and 37.80±0.80 mg/g in borewell water irrigated samples during first and second years of the study. A remarkable difference in total phenolic content was observed at ($p<0.05$) in all the vegetable samples analyzed. Significant increase was noted in total phenol content in all the vegetables irrigated with waste water as compared to that of borewell irrigated ones.

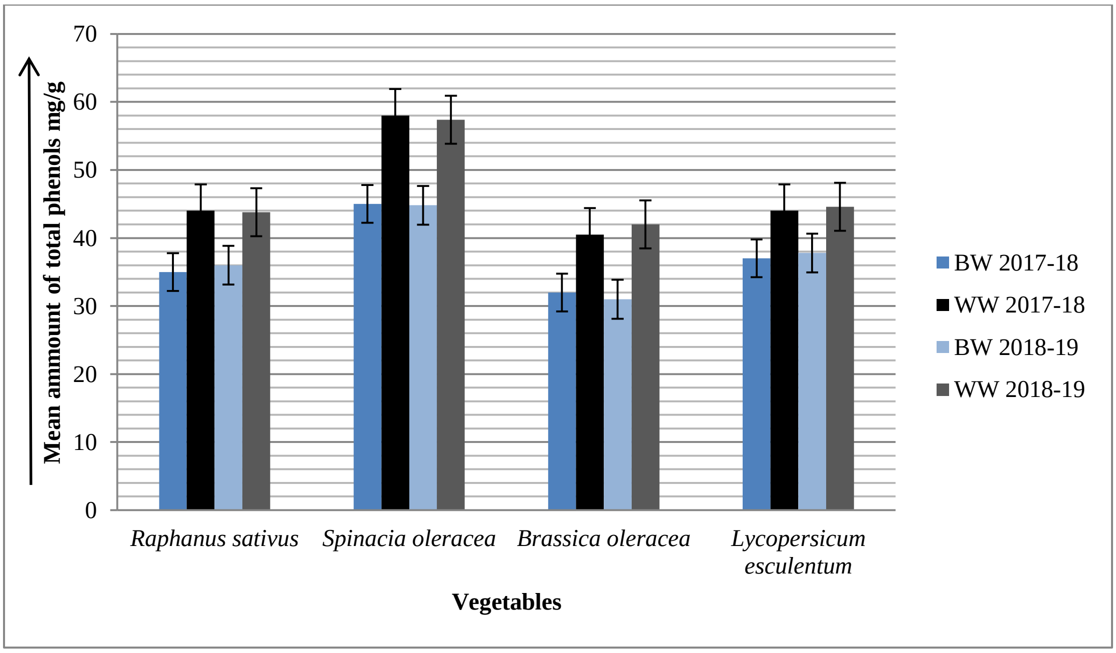


Fig 4.22: Mean amount of total phenols in waste water and borewell water irrigated vegetables ($p\leq0.05$; Error bar= ±SD)

Similar results have been obtained in the previous work done by various workers on antioxidant activity induced in vegetables by waste water irrigation. Diaz et al (2001) reported an increase in total phenolic content in wheat under nickel and aluminum stress. Similarly Hassanein and coworkers (2013) reported a significant increase in phenolic content in *Brassica napa* and *Lectucea sativa* irrigated with cadmium and lead - rich waste water. Kapoor et al (2014) also detected high amounts of phenolic content in *Brassica juncea* under cadmium stress. Higher phenolic content in *Nepholepis biserrata* grown under heavy metal stress was also reported by Manan et al (2015). In an another study Zafar et al (2016) also found significant higher amounts of total phenols in 100% waste-water-irrigated *Solanum tuberosum, Lycopersicum esculentum, Abelmoschus esculentum* and *Cucurbita pepo*. The results obtained in the present study are coherent with the results obtained by the above mentioned previous studies done on bioaccumulation of phenolic content under heavy metal stress.

4.10.1.3 Flavonoids (mg/ g)

Flavonoids are another group of secondary metabolites which are known to protect the cell membranes of plant cell under various biotic and abiotic stresses. Even low level of heavy metal stress results in increased level of flavonoids (Fini et al., 2011). In the present study, the mean amount of flavonoids was detected to be 2.00±0.02 mg/g and 2.10±0.03 mg/g and 3.95±0.04 mg/g, 4.10±0.03 mg/g in the borewell water and waste water treated *Raphanus sativus* during first and second years of study respectively. Similarly in *Spinacia oleracea,* average flavonoid content of 2.50±0.07 mg/g and 2.70±0.03 mg/g in the borewell water as compared to 4.20±0.04 mg/g and 4.25±0.02 mg/g in the waste-water-irrigated samples during first and second years of study respectively. *Brassica oleracea* was found to have 3.50±0.04 mg/g and 3.4±0.03 mg/g and 4.95±0.01 mg/g and 4.90±0.02 mg/g in borewell water and waste water treated samples in the first and second years of study respectively. The waste-water-irrigated samples of *Lycopersicum esculentum* were found to contain 4.80±0.04 mg/g and 4.70±0.07 mg/g of flavonoids as compared to 2.50±0.08 mg/g and 2.60±0.06 mg/g in borewell water irrigated samples during both the first and second years of the study respectively. A significantly remarkable higher level of flavonoids was detected at ($p<0.05$) in waste water as compared to that of borewell water irrigated samples in all the vegetables analyzed.

Singh and Aggrawal (2007) reported an increase in flavonoid content in waste-water-irrigated *Beta vulgaris*. Chandra et al (2014) observed higher amount of flavonoids in *Beta vulgaris*, *Brassica oleracea* and *Oscimum basilicum* grown in waste-water-irrigated fields. Similar results have also been reported by Manan et al (2015) and Zafar et al (2016) in which higher level of flavonoids have been detected in

Nephrolepis, Lycopersicum and Pumpkin grown in heavy metal contaminated soils by long term and continuous irrigation of waste water. The results obtained in the present study correspond with the outcome of above mentioned research.

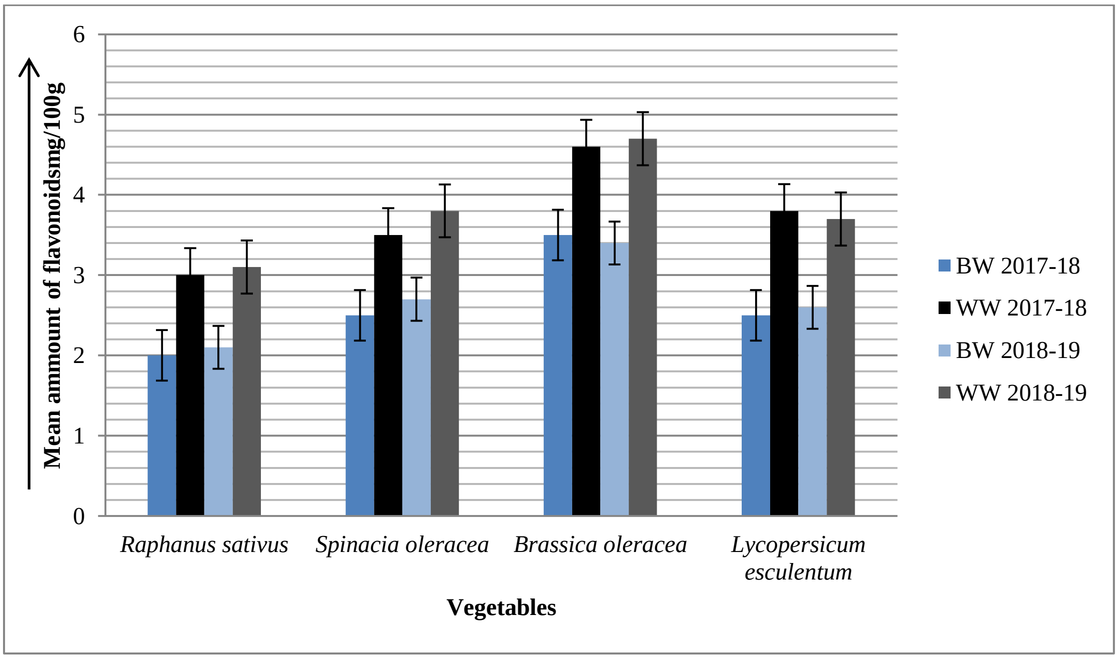


Fig 4.23: Mean amount of flavonoids in waste water and borewell water irrigated vegetables ($p \leq 0.05$; Error bar= ±SD)

4.10.1.4 Carotenoids (mg/100g)

Carotenoids are an important part of pigments associated with photosynthesis which are produced in large amounts during oxidative stress and work as an antioxidant by inducing some changes in the properties of photosynthetic system related with xanthophyll cycle (Dariusz et al., 2011). The results obtained in the present study reveal the mean amount of carotenoids to be 32.50±0.90 mg/100g, 32.30±1.00 mg/100g and 24.10±0.80 mg/100g, 23.95±0.10 mg/100g in the waste-water and borewell water irrigated *Raphanus sativus* during both the first and second years of study respectively. Similarly in *Spinacia oleracea*, the average amount of carotenoids was analyzed to be 39.70±0.70 mg/100g and 40±0.40 mg/100g in waste-water and comparatively low mean amount of 28.75±0.80 mg/100g and 28.90±0.60 mg/100g in the borewell water irrigated samples during both the first and second years of study respectively. *Brassica oleracea* was found to have 38.90±1.20 mg/100g and

39.00±0.90 mg/100g in waste-water-irrigated samples as compared to that of 27.80±1.50 mg/100g and 28.00±0.80 mg/100g in borewell water irrigated samples during both the first and second years of study respectively. The waste-water-irrigated samples of *Lycopersicum esculentum* were found to contain 50.12±0.60 mg/100g and 49.95±0.80 mg/100g of carotenoid as compared to that of 38.50±0.70 and 38.00±0.40 in borewell water irrigated samples during both the first and second years of the study respectively. Waste-water-irrigated samples of all the vegetables studied were found to have higher amounts of carotenoid as compared to borewell irrigated samples of the same vegetables

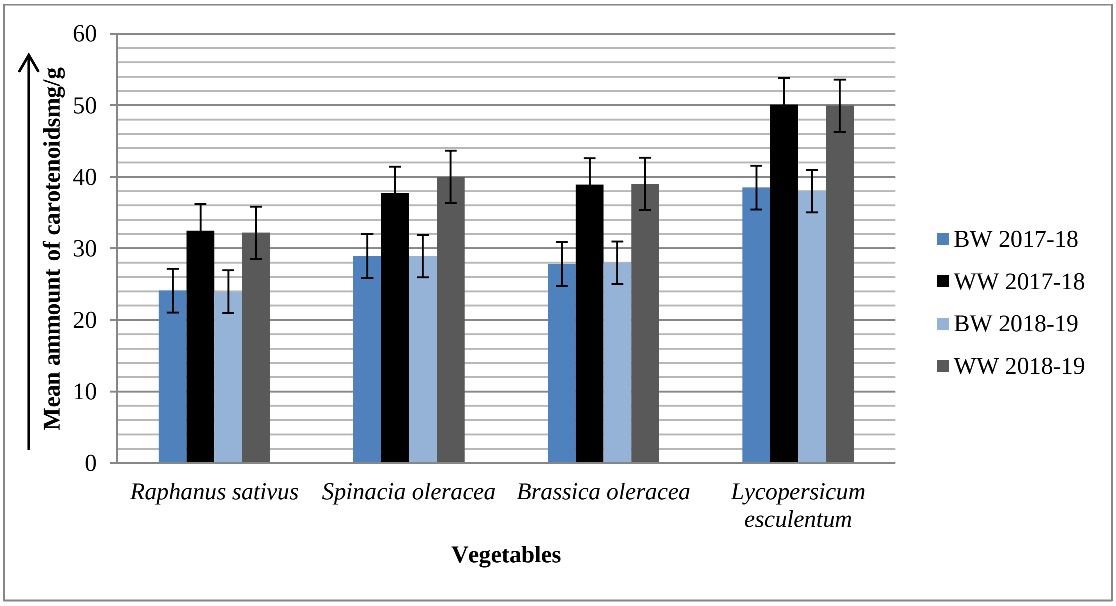


Fig 4.24: Mean amount of carotenoids in waste water and borewell water irrigated vegetables ($p \leq 0.05$; Error bar= ±SD)

There are several studies in which a significant increase of carotenoids in waste-water-irrigated vegetables was observed. Singh and Aggarwal (2010) found a significant increase in amount of carotenoids in *Beta vulgaris* irrigated with waste water. A similar study was conducted by Bahmniya et al (2010) who reported an increase in carotenoid content in *Spinacia oleracea* and *Brassica oleracea* irrigated with heavy-metal- rich waste water. A significant increase of carotenoid content was also observed in spinach irrigated with industrial waste water by Saini et al (2014). In another study done by Andrianos et al (2016) a significant increase in lutein and β carotene was detected in chromium and nickel-rich waste-water-irrigated root vegetables *Dacus carota* and *Solanum tuberosum*. The results of the present study have been confirmed by the aforementioned studies done by various researchers.

4.10.1.5 Proline (mg/g)

Accumulation of proline content in plants is an indicator that the plant is under one or the other environmental stress. Proline acts as an osmolyte and plays a protective role in maintaining the osmotic potential of the cell especially under the stress induced by heavy metal bioaccumulation (Alia and Saradhi, 1991). Even the exogenous application of proline has been reported to reduce the toxic effect of heavy metals in plants (Mohammadrezkhani et al., 2019). The results obtained in the present study revealed an average amount of proline to be 2.56±0.01 mg/g, 2.58±0.02 mg/g and 1.25±0.02 mg/g, 1.20.02 mg/g in the waste and borewell water irrigated *Raphanus sativus* during both the first and second years of study respectively. Similarly, in *Spinacia oleracea* the waste treated plants were found to contain 2.45±0.03 mg/g in the first and 2.48±0.04 mg/g of average proline content in the second year of study in waste-water-irrigated, but a lower amount of 1.20±0.01 mg/g and 1.18±0.02 mg/g in the borewell water irrigated samples during both the first and second years of study respectively. *Brassica oleracea* was found to have 2.80±0.04 mg/g and 2.85±0.03 mg/g of proline content in waste-water irrigated samples and 1.29±0.02 mg/g and 1.35±0.01 mg/g in borewell water irrigated samples both in both the first and second years of study respectively. The waste-water-irrigated samples of *Lycopersicum esculentum* were found to contain 3.45±0.03 mg/g and 3.48±0.01 mg/g of proline as compared to that of 2.85±0.01 mg/g and 2.91±0.02 mg/g in borewell irrigated samples during both the first and second years of the study respectively. A remarkable difference was detected in proline carotenoid content at ($p<0.05$) in borewell and waste-water-irrigated samples in all the vegetables analyzed. Waste-water-irrigated samples of all the vegetables studied were found to have much higher levels of proline content as compared to borewell irrigated ones.

Yang et al (2009) also reported a significant increase in proline content in the seedlings of maize under oxidative stress. Higher levels of proline were also detected in *Vicia faba* under different treatments of zinc, copper and nickel in experimental studies carried out by Nadgórska-Socha et al (2013). In another study, performed by

Li et al (2013) zinc treatment was found to induce higher accumulation of proline content in the root and leaves of wheat as compared to the plants free of zinc stress. Similarly, Damera et al (2015) reported higher level of proline content in *Vigna radiata* raised in heavy metal contaminated soils. All these findings were in agreement with the present study where high accumulation of proline content was observed in various vegetables grown under heavy metal stress induced by irrigation with heavy-metal-rich waste water.

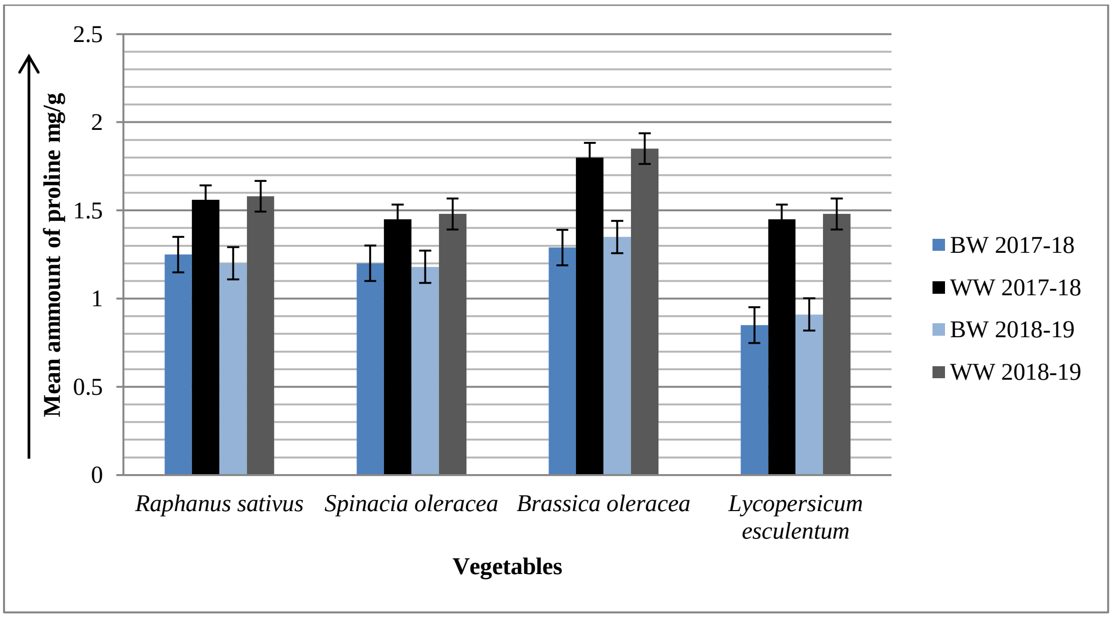


Fig 4.25: Mean amount of proline in waste water and borewell water irrigated vegetables (p≤0.05; Error bar= ±SD)

4.10.2 Effect of waste water irrigation on enzymatic antioxidants

Bioaccumulation of heavy metals are known to induce oxidative stress in plants by the production of superoxide radicals, singlet oxygen, hydroxal ions and hydrogen peroxide which leads to lipid peroxidation disruption in the structural and functional activity of cell membranes and apoptosis of the plant cell. To cope up with this oxidative stress the natural antioxidant activity of the plant is triggered which leads to an increase in the activity of some antioxidant enzyme. In the present study the effect of heavy metal bioaccumulation in vegetables irrigated with waste on the activity of following antioxidant enzymes was measured and compared with the same in the vegetables irrigated with normal borewell water.

4.10.2.1 Catalase activity (U/mg fresh weight protein)

Catalase (CAT) belongs to the group of oxidoreductase enzymes which are known to be produced in plants against heavy metal stress and are considered to convert toxic oxygen reactive species like H_2O_2 produced during stress conditions into oxygen and water (Lin et al., 2000). In this study, the activity of catalase enzyme was found to be highly increased in all the vegetables irrigated with waste water of Buddha Nullah as compared to the activity of this enzyme in the same vegetables irrigated with borewell water. The mean activity of catalase in the root vegetables *Raphanus sativus* was found to be 1.235±0.003 U/mg and 1.225±0.002 U/mg in waste-water-irrigated, and that of 0.595±0.002 U/mg and 0.589±0.001 U/mg in the borewell water irrigated samples during both the first and second years of study respectively. Similarly, the average unit activity of catalase was recorded to be 0.791±0.003 U/mg and 0.785±0.003 U/mg in borewell water as compared to that of 1.402±0.004 U/mg and 1.415±0.005 U/mg in waste-water-irrigated samples of *Spinacia oleracea* during both the first and second years of study respectively. Mean unit activity of catalase in *Brassica oleracea* was observed to be 1.395±0.002 U/mg and 1.390±0.003 U/mg as compared to that of 0.685±0.003 U/mg and 0.690±0.001 U/mg in waste- water and borewell water irrigated samples in both the first and second years of study respectively. The activity of catalase enzyme was found to be 1.275±0.002 U/mg and 1.270±0.003 U/mg in waste -water but that of 0.488±0.003 U/mg and 0.479±0.001 U/mg in borewell water irrigated samples of *Lycopersicum esculentum* during both the first and second years of study respectively. The results of the present study were found to be in accordance with the previous studies performed on activity of catalase in plants under heavy metal stress. Pandey et al (2009) reported an increase in activity of catalase enzyme under heavy metal stress in *Spinacia oleracea*. Similarly Chamseddine et al (2009) observed an increase in catalase activity in *Lycopersicum esculentum* irrigated with waste water. In another study Kachout et al (2009) also detected higher amount of catalase activity in *Artiplex hornensis* and *Artiplex rosea*. An increase in activity of catalase in potato and carrot irrigated by waste water was recently reported by Andrianos et al (2016) and in pumpkin, okra and tomato by Zafar et al (2016).

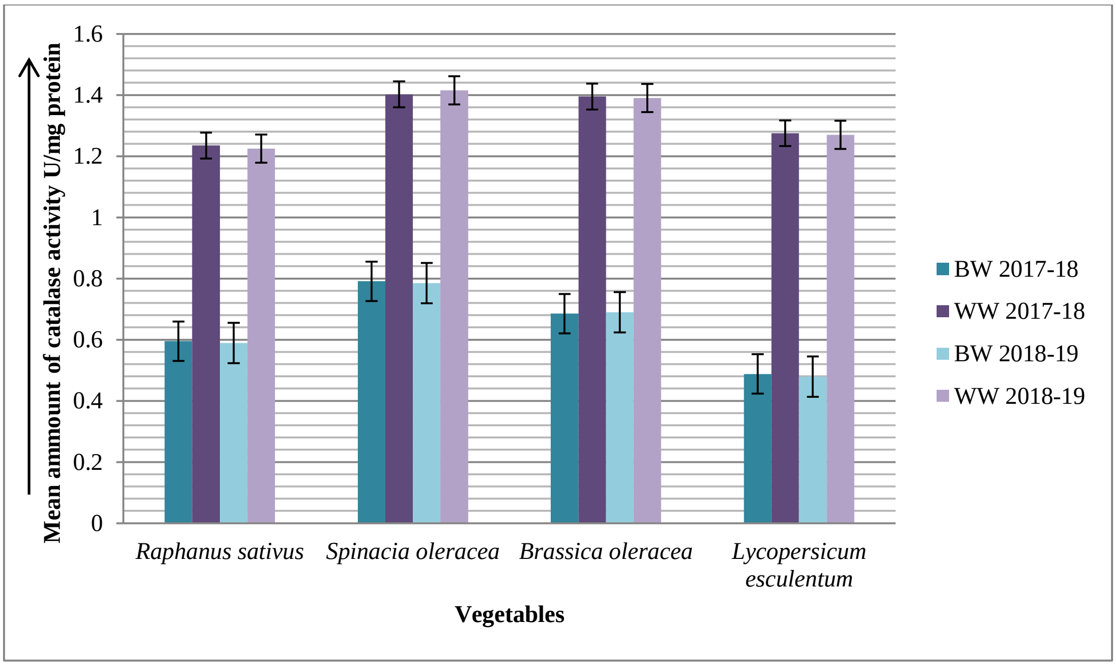


Fig 4.26: Mean amount of catalase activity in waste water and borewell water irrigated vegetables ($p \leq 0.05$; Error bar= ±SD)

4.10.2.2 Peroxidase activity (U/mg fresh weight protein)

Peroxidase is another antioxidant enzyme known to neutralize the toxic effect of oxygen reactive species under heavy metal stress by altering the electron transport chain and induction of fat peroxidation leading to apoptosis of cells in vegetables (Benavides et al., 2005; Chen et al., 2007 and Mishra et al., 2009). In our study, the activity of peroxidase enzyme was found to be highly increased in all the vegetables irrigated with waste water of Buddha Nullah as compared to the activity of this enzyme in the same vegetables irrigated with borewell water. The activity of peroxidase in root vegetables *Raphanus sativus* was found to be 1.425±0.003 U/mg and 1.435±0.003 U/mg in waste-water-irrigated, in comparison to that of 0.685±0.002 U/mg, and 0.680±0.003 U/mg in the borewell water irrigated samples during both the first and second years of study respectively. Similarly, the activity of peroxidase was recorded to be 0.632±0.002 U/mg and 6.640±0.003 U/mg in borewell and that of 1.625±0.002 U/mg and 1.632±0.002 U/mg in waste-water-irrigated samples of *Spinacia oleracea* during both the first and second years of study respectively. Unit activity of peroxidase in *Brassica oleracea* was observed to be 1.590±0.001 U/mg and 1.585±0.001 U/mg as compared to that of 0.645±0.001U/mg and 0.630±0.002 U/mg in waste-water and borewell water irrigated samples during both the first and second years of study respectively. The activity of peroxidase enzyme was recorded to be 1.470±0.003 U/mg and 1.420±0.004 U/mg in waste water and that of 0.475±0.002 U/mg and 0.465±0.004 U/mg in borewell water irrigated samples of *Lycopersicum esculentum* during both the first and second years of study respectively. A similar study was conducted by Weckx and Clijsters (1996) who reported an increase in peroxidase activity in *Phaseolus vulgaris* under different concentrations of cadmium, copper and zinc stress. Another study carried out by Pallavi and Joseph (2014) also reported a significant increase in peroxidase activity in *Amaranthus tricolour* treated with sewage waste water. Damera et al (2015) also reported a significant increase in peroxidase activity in *Vigna radiata* under Cd, Cr and Ni stress. A recent study done by Aria et al (2017) also suggested an increase in the activity of peroxidase enzyme in *Vetiveria zizanioides* irrigated with heavy-metal-laden waste water. The results obtained in the present study can be related to the results reported by above mentioned researchers.

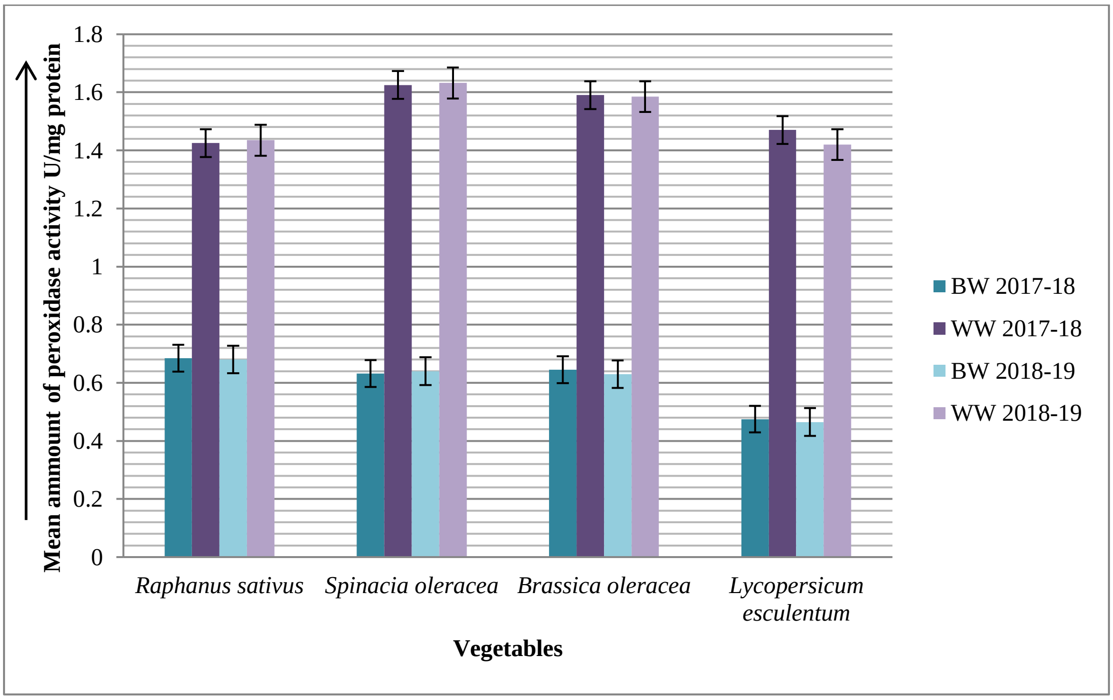


Fig 4.27: Mean amount of peroxidase activity in waste water and borewell water irrigated vegetables (p≤0.05; Error bar= ±SD)

4.10.2.3 Superoxide dismutase activity (U/mg fresh weight protein)

Superoxide dismutase is the first antioxidant enzyme which gets activated by the plants when the concentration of superoxide radicals increases heavily in the cell due to oxidative stress and SOD is known to convert toxic superoxide radicals into hydrogen peroxide which is converted into water by other oxidative enzymes like catalase and peroxidases (Zhang et al., 2009). The mean unit activity of SOD in root vegetables *Raphanus sativus* was found to be 1.950±0.001 U/mg and 1.895±0.004 U/mg in waste water as compared to that of 0.520±0.002 U/mg and 0.530±0.003 U/mg in the borewell water irrigated samples during both the first and second years of study respectively. Similarly the average unit activity of SOD was recorded to be 0.595±0.002 U/mg and 0.585±0.005 U/mg in borewell as well as 1.395±0.001 U/mg and 1.385±0.003 U/mg in waste-water-irrigated samples of *Spinacia oleracea* during both the first and second years of study respectively. Mean unit activity of SOD in *Brassica oleracea* was observed to be 1.285±0.003 U/mg and 1.270±0.002 U/mg as compared to that of 0.410±0.002 U/mg and 0.399±0.003 U/mg in waste water and borewell water irrigated samples in both the first and second years of study respectively. The average unit activity of SOD enzyme was found to be 1.185±0.002 U/mg and 1.175±0.001 U/mg in waste water and that of 0.385±0.002 U/mg and 0.372±0.001 U/mg in borewell water irrigated samples of *Lycopersicum esculentum* during both the first and second years of study respectively. Increased unit activity of SOD in *Lathyrus sativus* under lead and copper stress has already been reported by Estrella et al (2009). In the same study, a significant correlation in chlorophyll content and SOD activity was reported which is also consistent with the results of the present study. In another study conducted by Kapoor et al (2014), a significant increase in activity of SOD in *Brassica juncea* under Cd stress was reported. Similarly, the results of a recent study performed by Zafar et al (2016) also suggested an increase in superoxide dismutase activity in waste-water-irrigated vegetables like pumpkin, tomato and okra. Similar results as obtained by the above cited biologists has been obtained in the present study.

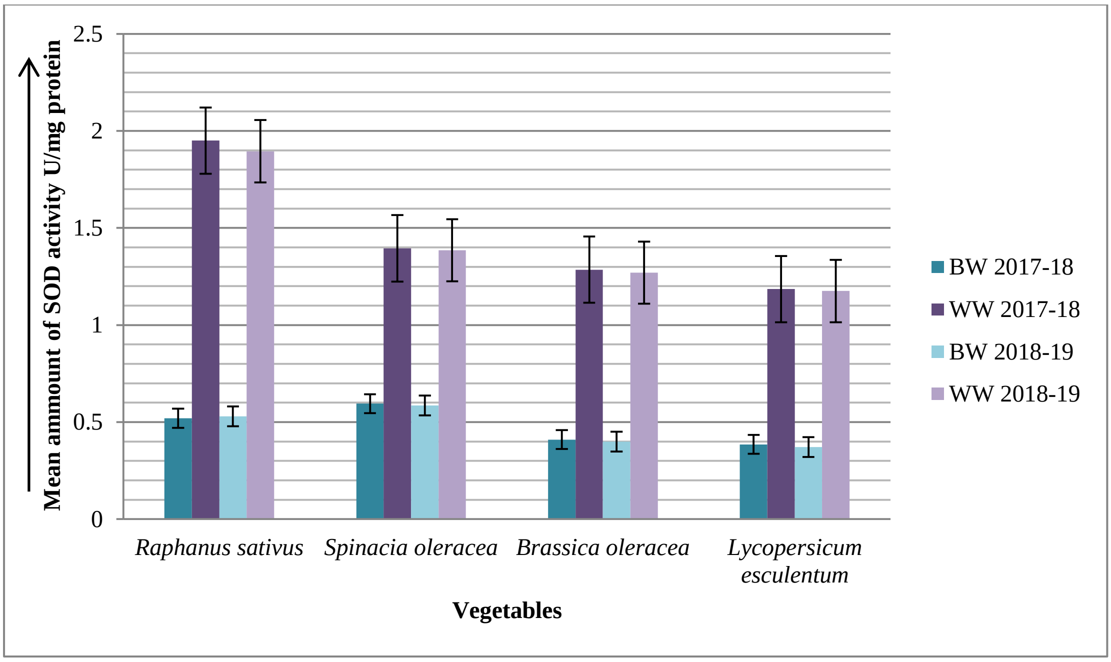


Fig 4.28: Mean amount of SOD activity in waste water and borewell water irrigated vegetables ($p \leq 0.05$; Error bar= ±SD)

4.10.2.4 Ascorbate peroxidase activity (U/mg fresh weight protein)

APX is another enzyme which is more efficient in destruction of H_2O_2 under heavy metal stress than catalase. This can be attributed to the presence of APX through the cell and its activity level is enhanced with the increased level of ascorbic acid in the cell under heavy metal stress and it acts as a reductant (Sasaki-Sekimoto et al., 2005). The mean unit activity of APX in root vegetable *Raphanus sativus* was found to be 1.235±0.004 U/mg and 1.267±0.004 U/mg in waste-water-irrigated, in comparison to that of 0.535±0.002 U/mg and 0.547±0.003 U/mg in the borewell water irrigated samples during both the first and second years of study respectively. Similarly, the average unit activity of APX was recorded to be 0.590±0.003 U/mg and 0.580±0.004 U/mg in borewell water as to that of 1.352±0.002 U/mg and 0.335±0.002 U/mg in waste-water-irrigated samples of *Spinacia oleracea* during both the first and second years of study respectively. Average unit activity of APX in *Brassica oleracea* was observed to be 1.295±0.003 U/mg and 1.310±0.002 U/mg and 0.520±0.003 U/mg and 0.530±0.003 U/mg in waste water and borewell water irrigated samples during both the first and second years of study respectively. The mean unit activity of APX enzyme was found to be 1.230±0.003 U/mg and 1.242±0.001 U/mg in waste water and that of 0.495±0.002 U/mg and 0.499±0.004 U/mg in borewell water irrigated samples of *Lycopersicum esculentum* during both the first and second years of study respectively.

Zhang et al (2015) also conducted a similar study in which a significant increase in APX activity was observed in the roots of *Ricinus communis* under cadmium stress. In another study carried out by Kachout et al (2009) higher activity of APX in *Artiplex hortensis* was observed. Aria et al (2017) also reported similar outcome in their study in which the *Vetiveria zizanioides* grown in cadmium rich soil was found to be having an increased activity of APX in the leaves of this plant. The results obtained in the present study are in coherence with findings of above mentioned studies.

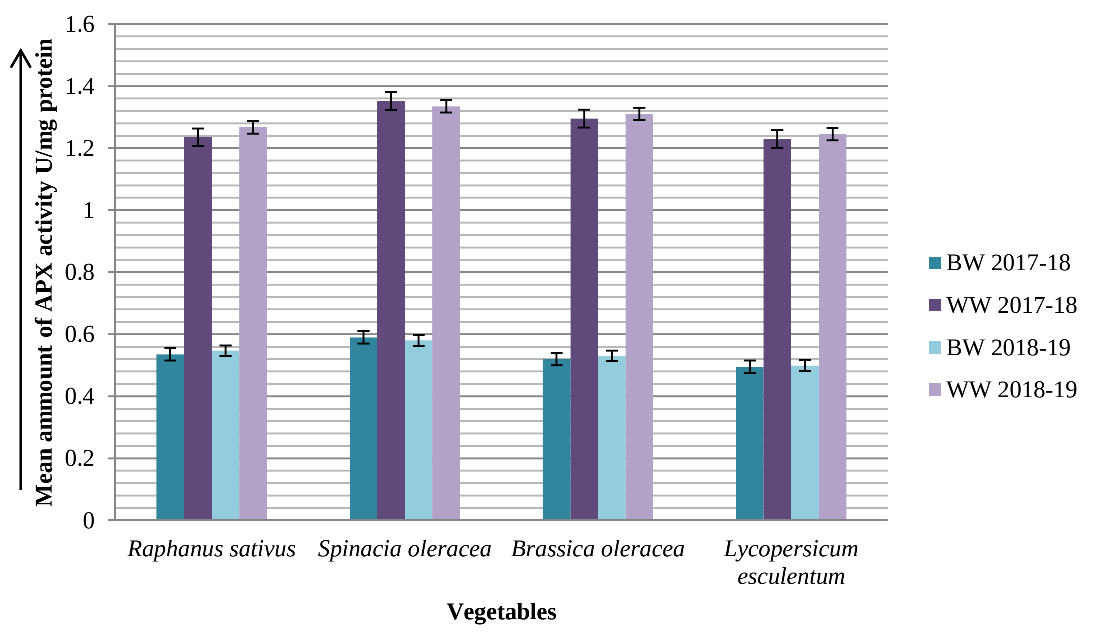


Fig 4.29: Mean amount of APX activity in waste water and borewell water irrigated vegetables ($p \leq 0.05$; Error bar= ±SD)

4.10.2.5 Glutathione reductase activity (U/mg fresh weight protein)

Glutathione reductase is known to reduce the toxic effect of singlet oxygen and superoxide radicals by preserving the $NADP^+$/NADPH ratio and the proper functioning of electron transport chain of photosynthetic system. The increase in glutathione reductase activity can also be due to excessive cellular consumption of reduced glutathione (GSH) under heavy metal stress (Asgher et al., 2017).

Glutathione reductase is known to play an important role in reduction of glutathione by oxidation of NADPH to $NADP^+$ (Foyer and Shigeoka, 2010). The average unit activity of glutathione reductase in root vegetable *Raphanus sativus* was found to be 0.095±0.001 U/mg and 0.090±0.002 U/mg in waste-water-irrigated and 0.058±0.002 U/mg and 0.052±0.003 U/mg in the borewell water irrigated samples during both the first and second years of study respectively. Similarly, the mean unit activity of glutathione reductase was recorded to be 0.050±0.001 U/mg and 0.055±0.001 U/mg in borewell as compared to that of 0.085±0.003 U/mg and 0.083±0.002 U/mg in waste-water-irrigated samples of *Spinacia oleracea* during both the first and second years of study respectively. Average unit activity of glutathione reductase in *Brassica oleracea* was observed to be 0.075±0.003 U/mg and 0.080±0.003 U/mg as to that of 0.048±0.002 U/mg and 0.046±0.003 U/mg in waste water and borewell water irrigated samples during both the first and second years of study respectively. The mean unit activity of glutathione reductase enzyme was detected to be 0.0690±0.003 and 0.0680±0.003 U/mg in waste water as compared to that of 0.0390±0.002 U/mg and 0.043±0.004 U/mg in borewell water irrigated samples of *Lycopersicum esculentum* during both the first and second years of study respectively.

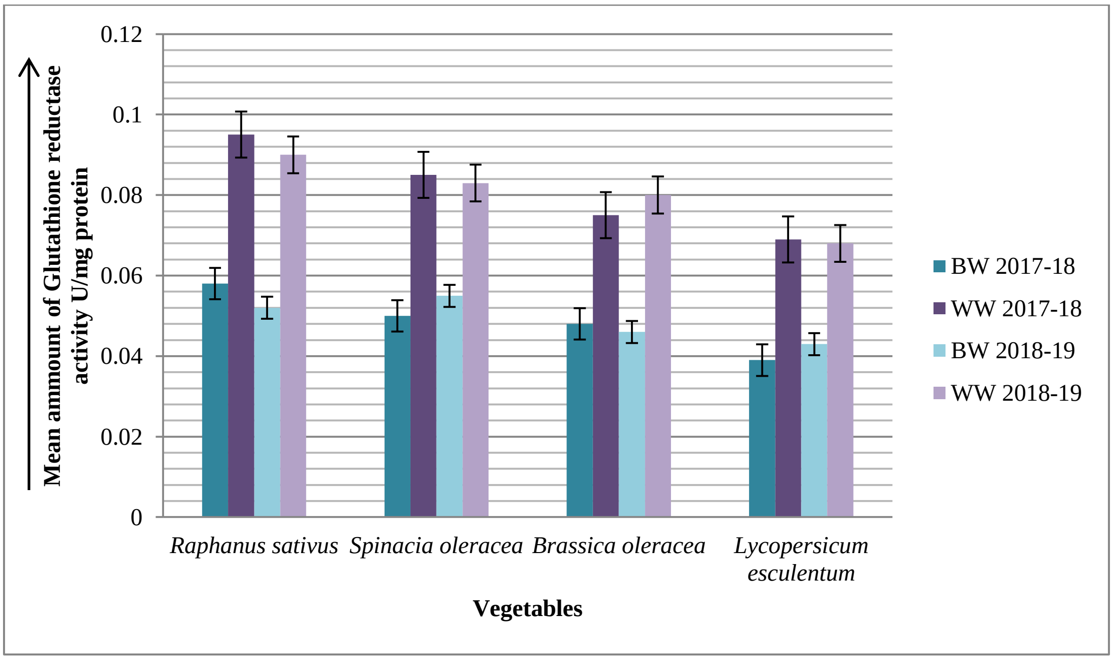


Fig 4.30: Showing mean amount of Glutathione reductase activity in waste water and borewell water irrigated vegetables (p≤0.05; Error bar= ±SD)

A similar study was conducted by Laspina et al (2005) in which an increase in activity of glutathione reductase was observed in *Helianthus annus* under cadmium stress. Almost similar results were obtained by Nehnevajova et al (2012) in *Helianthus annus* under zinc stress. In a recent study conducted by Aria et al (2017) a significant increase in glutathione reductase activity has been reported in *Vetiveria zizanioides* under cadmium stress. The results obtained in the present study were found to be in coherence with the results obtained by previous workers in their studies related to this field.

4.11 Effect of waste water on seed germination parameters of the vegetables

Seed germination is that initial and critical stage of plant development which determines the future plant health and is a critical test of probable crop productivity. Keeping in mind such prospective findings, the present study was undertaken to determine the effect of waste water as well as borewell water treatment on seed germination, seed invigouration, seedling growth and development of all the vegetables selected for the present research work.

Waste water **Borewell water**

Picture 4.2: Effect of waste water and borewell water treatment on seed germination of vegetables (*Raphanus sativus*)

4.11.1 Seed germination (%) In the present study the average percentage of seed germination was recorded to be 55%, 73.3%, 90% in borewell treated and 28.33%, 53.33% and 63.33% in waste water treated seeds of *Raphanus sativus* at 72, 96 and 120 hours respectively. Similarly, the mean percentage of seed germination in *Spinacia oleracea* was observed to be 43.3%, 58.33%, 78.33% in borewell water treated and 26.66%, 41.66% and 56.66% in waste water treatment in observations taken at the same hours respectively. Seeds of *Brassica oleracea* showed average percentage of 51.66%, 61.66%, 83.33% in borewell and 28.33%, 48.33% and 61.66% in waste water treatment at the same hours of observation respectively. The mean percentage of germination recorded in borewell and waste water treated seeds of *Lycopersicum esculentum* was 40%, 53.33%, 71.66% and 20%, 48.33 % and 53.33% respectively at the same hours of observation. Critical difference (CD) among the average values of percentage of seed germination at p<5% was observed to be 5.33 in *Raphanus sativus*, 2.85 in *Spinacia oleracea*, 2.85 in *Brassica oleracea* and 3.68 in *Lycopersicum esculentum*. The actual difference in mean values of borewell water and waste water treated was much higher than the critical values which showed the significant difference of seed germination in borewell and waste water treated seeds of all the vegetable seeds studied.

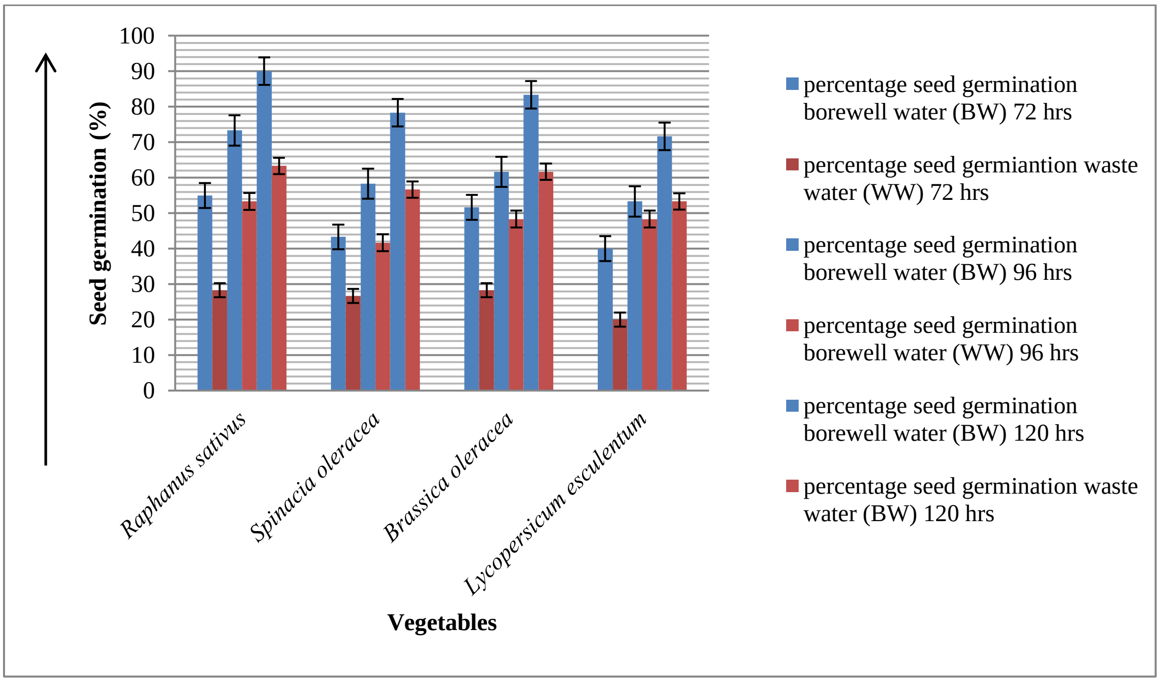


Fig 4.31: Percentage seed germination in borewell and waste water treated seeds of the vegetables ($p \leq 0.05$; Error bar= ±SD)

The results of the present study are coherent with the observations reported in previous studies by Dixit (2003), Soundarrajan and Pitchai et al (2007), Rahman et al (2018), Sharma et al (2019) and Nana et al (2019) who reported a significant decrease in germination percentage of vegetables seeds treated with different types of industrial and municipal waste water. The order of relative toxicity of waste water was observed in the following descending order *Raphanus sativus* > *Brassica oleracea* > *Spinacia oleracea* > *Lycopersicum esculentum*. The suppression of germination by waste water can be attributed to the induction of stress conditions by high TSS which increased the salinity and absorption of solutes (Kannan and Upreti, 2008) and heavy metals which reduced the availability of soluble carbohydrates by inhibiting the activity of some enzymes (Sharma and Dubey, 2005).

4.11.2 Root length (mm)

The mean root length measured in borewell water treated seeds in *Raphanus sativus* was 14.33±0.57 mm, 25.33±0.57 mm, 30.66±1.5 mm and 8.66±0.57 mm, 10.66±0.5 mm, 18.33±1.52 mm in the waste water treatment seeds at 72, 96 and 120 hours respectively. Similarly, the average root length of 4.00±1.00 mm, 6.66±0.57 mm, 10.10±1.00 mm and 6.00±1.00 mm, 10.66±1.52 mm and 14.00±2 mm was observed in waste water and borewell water treated seeds at 72, 96 and 120 hours in *Spinacia oleracea*. In *Brassica oleracea*, the average root length in borewell treated seeds was found to be 11.33±0.57 mm, 23.33±1.52 mm, 29.33±1.15 mm and in waste water treated seeds to be 6.00±1.00 mm, 10.00±1.00 mm, 15.33±0.57 mm at the same hours. The waste water treated seeds of *Lycopersicum esculentum* showed mean root length to be 3.00±1.00 mm, 5.00±1.00 mm, 10±1.00 mm and borewell treated that of 5.66±1.52 mm, 9±1.00 mm and 15.66±1.5 mm at 72, 96 and 120 hours respectively. Comparing the critical difference between the means obtained and the actual difference observed, it was concluded that waste water treatment greatly reduced the root length in seedlings of all the vegetables studied. Almost identical results were noticed by Farooqi et al (2009), Khaleel et al (2013), Bautista (2013), Divya et al (2015) and Maity et al (2019) who reported a significant decrease in growth in waste water treated seeds of both monocot and dicot seeds when treated with different concentrations of cadmium, lead, chromium and waste water from different industrial sources. The results obtained can be attributed to more rapid accumulation of heavy metals in roots due to osmotic stress induced by waste water salinity and heavy metals (Shaukat et al., 1999 and Parida; Das, 2005).

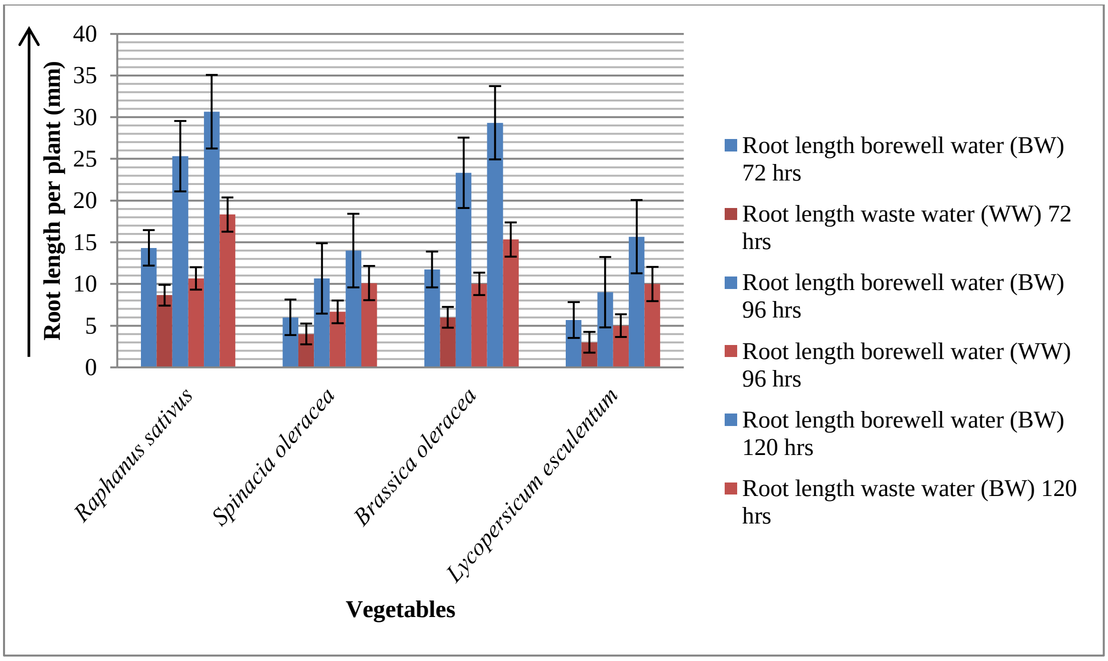


Fig 4.32: Mean root length (millimeters) in borewell and waste water treated seeds of the vegetables (p≤0.05; Error bar= ±SD)

4.11.3 Shoot length (mm)

The mean shoot length recorded in borewell water treated seeds in *Raphanus sativus* was recorded to be 18.66±1.15 mm, 40±2.00 mm, 46.66±1.15 mm and 10.00±1.00 mm, 15±4.35 mm, 26±2.00 mm in the waste water treatment seeds at 72, 96 and 120 hours respectively. Similarly, the average shoot length of 6.33±0.57 mm, 11±1.00 mm, 20.33±1.52 mm and 9±1.00 mm, 16±1.00 mm, 32±2.00 mm was observed in waste water and borewell treated seeds respectively at the same hours in *Spinacia oleracea*. In *Brassica oleracea,* the mean shoot length in borewell treated seeds was found to be 20.33±1.52 mm, 41.00±1.00 mm, 48.00±1.63 mm and 9.00±1.00 mm, 14.33±0.1.52 mm, 22.66±1.52 mm was recorded in waste water treated seeds at 72, 96 and 120 hours. Waste water treated seeds of *Lycopersicum esculentum* showed average shoot length 3.33±0.57 mm, 8.33±1.52 mm, 17.00±1.00 mm and 7.33±1.15 mm, 11.66±1.52 mm and 26.33±1.52 mm in both waste water and borewell treated seeds respectively at 72, 96 and 120 hours. Statistical analysis revealed the actual difference means was much higher than the critical difference of mean values from which it was concluded that waste water significantly reduced the shoot length in the seedlings of all the vegetables studied. Similar results have also been reported in the previous studies done by Farooqi et al (2009), Khaleel et al (2013), Bautista et al (2013), Divya et al (2015) and Maity et al (2019) who reported a significant decrease in waste water treated seeds of both monocot and dicot plants when treated with different concentrations of heavy metals like cadmium, lead, chromium and waste water from different industrial sources. The results obtained can be attributed to more rapid accumulation of heavy metals in shoots due to osmotic stress induced by waste water salinity and heavy metals (Shaukat et al., 1999 and Parida and Das 2005). In the present study, it was observed that waste water treatment had a pronounced effect on reduction of root length as compared to that of shoot length in all the vegetable seeds studied. This behavior can be attributed to the greater and rapid accumulation of heavy metals in roots than in shoots and the faster detoxification of heavy metals in shoots than in roots or the greater heavy metal ion toxicity in roots, because of their continuous and direct touch with waste water (Dubey and Dwivedi, 1987) and higher production of certain phenolic compounds which affect membrane permeability in roots as compared to shoots (Khan et al., 1999; Hameed et al., 2001).

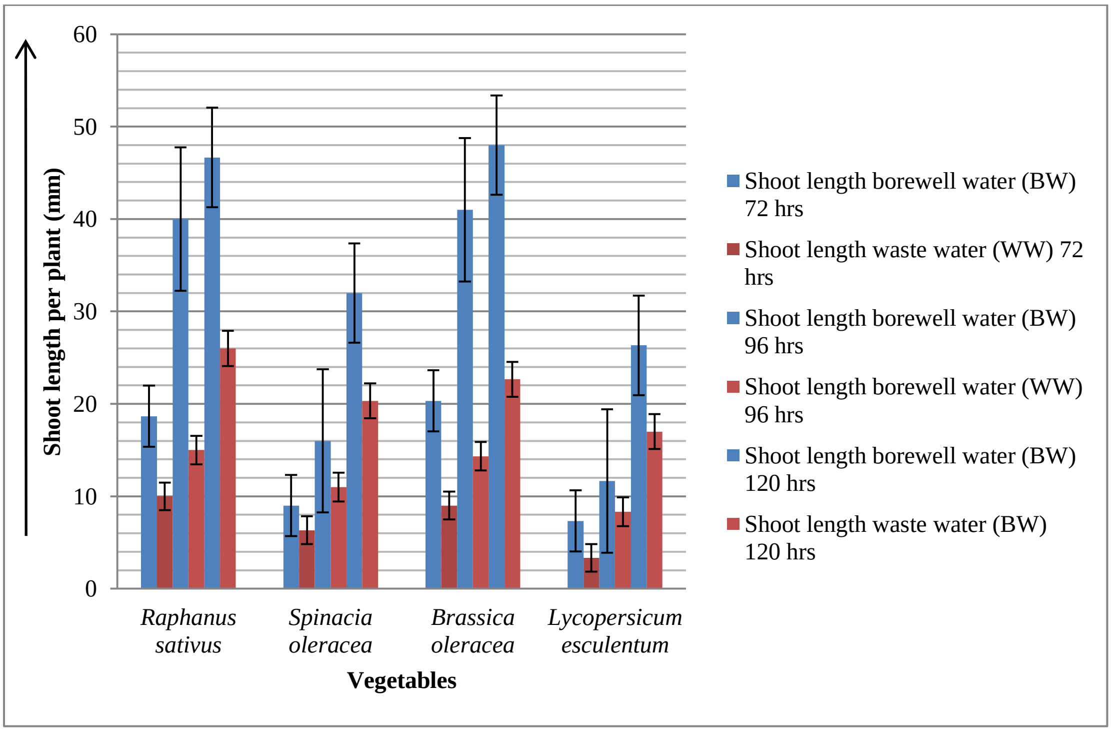


Fig 4.33: Average Shoot length (millimeters) in borewell and waste water treated seeds of the vegetables ($p \leq 0.05$; Error bar= ±SD)

4.11.4 Number of secondary roots /seedling

The average number of secondary roots observed in borewell water treated seeds in *Raphanus sativus* was observed to be 3.00±0.00, 4.66±0.57, 7.66±0.57 and 1.33±0.57, 2.00±0.00, 3.66±0.57 in the waste water treated seeds at 72, 96 and 120 hours respectively. Similarly, the mean number of secondary roots was found to be 1.33±0.57, 3.00±0.00, 4.66±1.57 and 2.66±0.57, 4.66±0.57, 6.33±0.57 in waste water and borewell water treated seed respectively at the same hours in *Spinacia oleracea*. In *Brassica oleracea*, the average number of secondary roots in borewell water treated seeds was found to be 2.33±0.57, 3.33±0.57, 5.66±0.57 and in waste water treated seeds to be 1.00±0.00, 2.66±0.57, 4.66±0.57 at 72, 96 and 120 hours. Similarly, the waste water treated seeds of *Lycopersicum esculentum* showed mean number of secondary roots to be 1.66±0.57, 2.66±0.57, 3.00±1.00 and 3.00±0.00, 5±1.00, 5.33±1.50 in both waste water and borewell water treated seeds respectively at the same hours of observation. Statistical analysis of the data revealed that the actual difference in the means was much higher than the critical difference calculated between the means in all the vegetable seed samples analyzed, from which it was concluded that waste water significantly reduced the emergence of secondary roots.

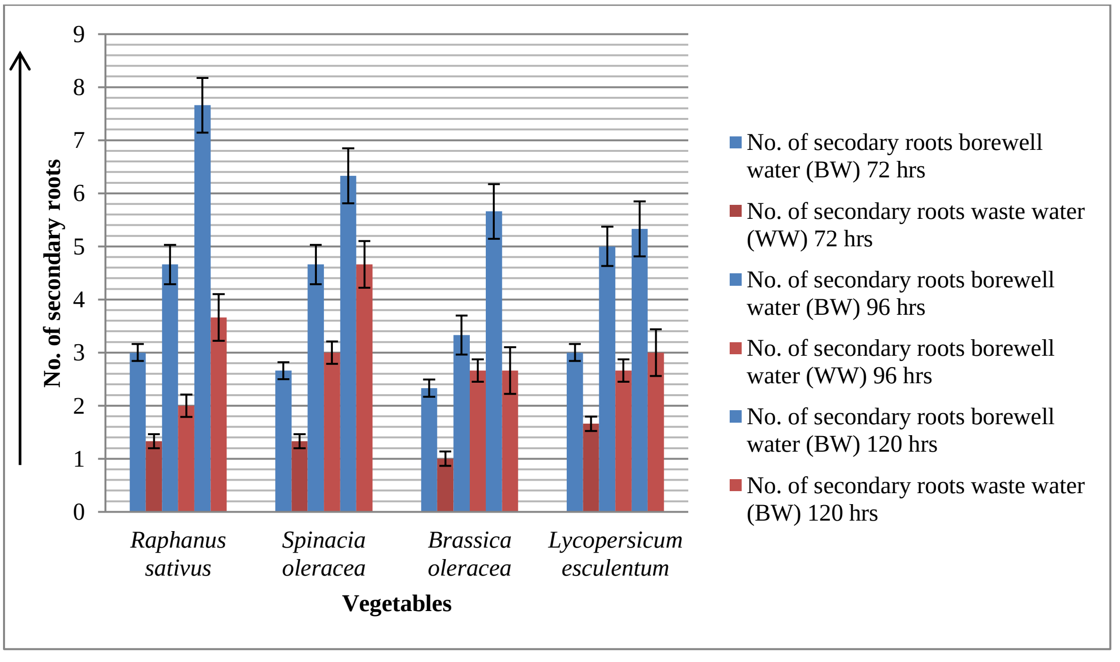


Fig 4.34: Average No. of secondary roots in borewell and waste water treated seeds of the vegetables (p≤0.05; Error bar= ±SD)

The results of the present study are coherent with the results obtained in previous studies done by Augusthy and Sherin (2001) who reported reduction in number of secondary roots of waste water treated seeds in a *Vigna radiata.* Sharma et al (2016) also reported a significant reduction in number of roots per plant in tomato and brinjal seeds when treated with 100% textile mill effluents. The results of the present study point towards the fact that heavy metals present in waste water significantly suppress the divisional activity of meristematic cells (Goldbold and Kettner, 1991; Sharifah and Hishashi, 1992).

4.11.5 Total length of seedlings (mm)

The total seedling length was obtained by adding average length of root and shoot obtained at all the hours of observation in all the vegetables samples. The seedling length measured in borewell water treated seeds in *Raphanus sativus* was found to be 32.90 mm, 65.30 mm, 75.20 mm and 18.60 mm, 25.60 mm, 44.30 mm in the waste water treated seeds at 72, 96 and 120 hours respectively.

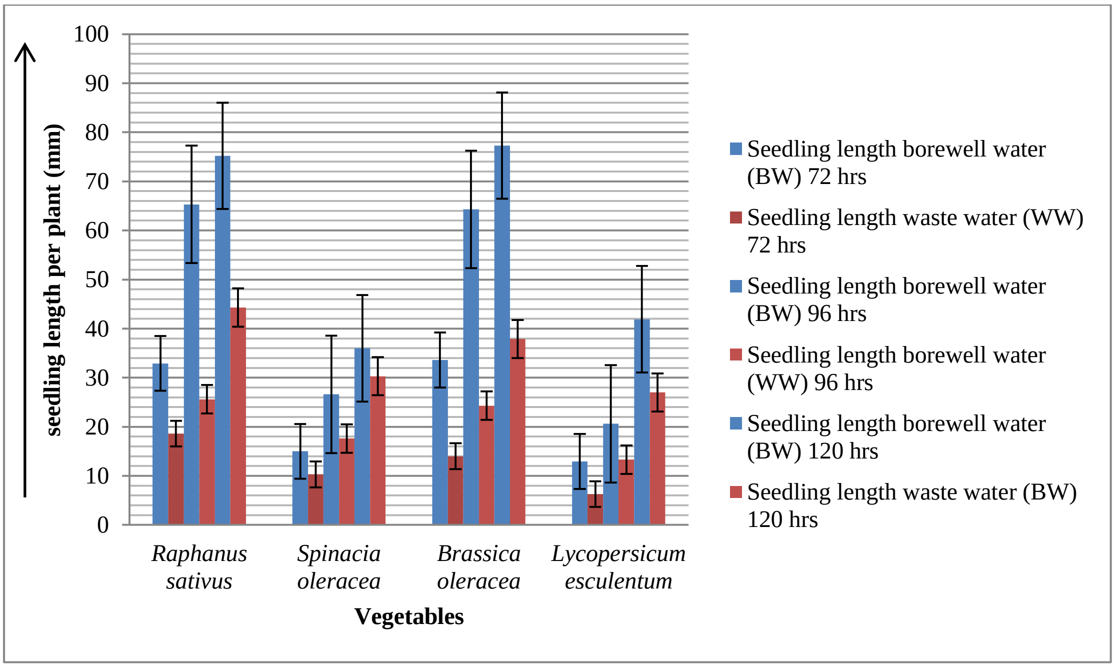


Fig 4.35: Average seedling length (mm) in borewell and waste water treated seeds of the vegetables ($p \leq 0.05$; Error bar= ±SD)

Similarly, the mean seedling length of 10.30 mm, 17.60 mm, 30.30 mm and 15.00 mm, 26.60 mm, 36.00 mm was observed in waste water and borewell treated seeds respectively at the same hours in *Spinacia oleracea*. In *Brassica oleracea*, the average seedling length in borewell treated seeds was recorded to be 33.60 mm, 64.30 mm, 77.30 mm and 14.00 mm, 24.30 mm, 37.90 mm in the waste water treated seeds at 72, 96 and 120 hours respectively. Similarly, the waste water treated seeds of *Lycopersicum esculentum* showed mean seedling length of 6.30 mm, 13.30 mm, 27.00 mm and 10.29 mm, 20.60 mm, 41.90 mm in both the waste water and borewell treated seeds respectively at the same hours of observation. The total seedling length in waste water treated seeds was much lower than the borewell treated seeds at ($p<0.05$). The results obtained in the present study substantiate the reports given by Li et al (2005), Dash (2012) and Rahman et al (2018) who reported a significant reduction in seedling length in *Vigna ambacensis, Arabidopsis thaliana* under heavy metal stress and in rice, wheat and red amaranthus under the impact of domestic waste water and dye effluents.

4.11.6 Relative toxicity (%)

The relative toxicity of waste and borewell water treatment on the seed germination was calculated by the formula given by Chapagain (1991) and the mean values of relative toxicity were calculated to be 48.54 %, 27.28 % and 29.66 % in waste water treated seeds of *Raphanus sativus* at 72, 96 and 120 hours. Similarly, the average relative toxicity of waste water treated seeds in *Spinacia oleracea* was found to be 38.56 %, 28.64 % and 27.61 % at the same hours of observations. In *Brassica oleracea*, the mean relative toxicity was calculated to be 45.15%, 21.59% and 26.05% at 72, 96 and 120 hours. Similarly, the average relative toxicity in waste water treated seeds of *Lycopersicum esculentum* was detected to be 50 %, 19.38 % and 25.55 % at the same hours respectively. There was no significant difference in the relative toxicity in *Spinacia oleracea* and *Brassica* oleracea but a significant difference was observed in *Raphanus sativus* and *Lycopersicum esculentum*. The results of the present study are in accordance with the previous studies in which a significant increase in relative toxicity percentage of lady’s finger and red amaranthus seeds treated with dye effluents as observed by David and Rajan (2015) and Rahman et al (2018). The difference between the relative toxicity in different vegetables can be attributed to the fact that seed coats play an important role in the entry and uptake of heavy metals present in the waste water (Seregin and Kozhevnikova, 2005 and Li et al., 2005).

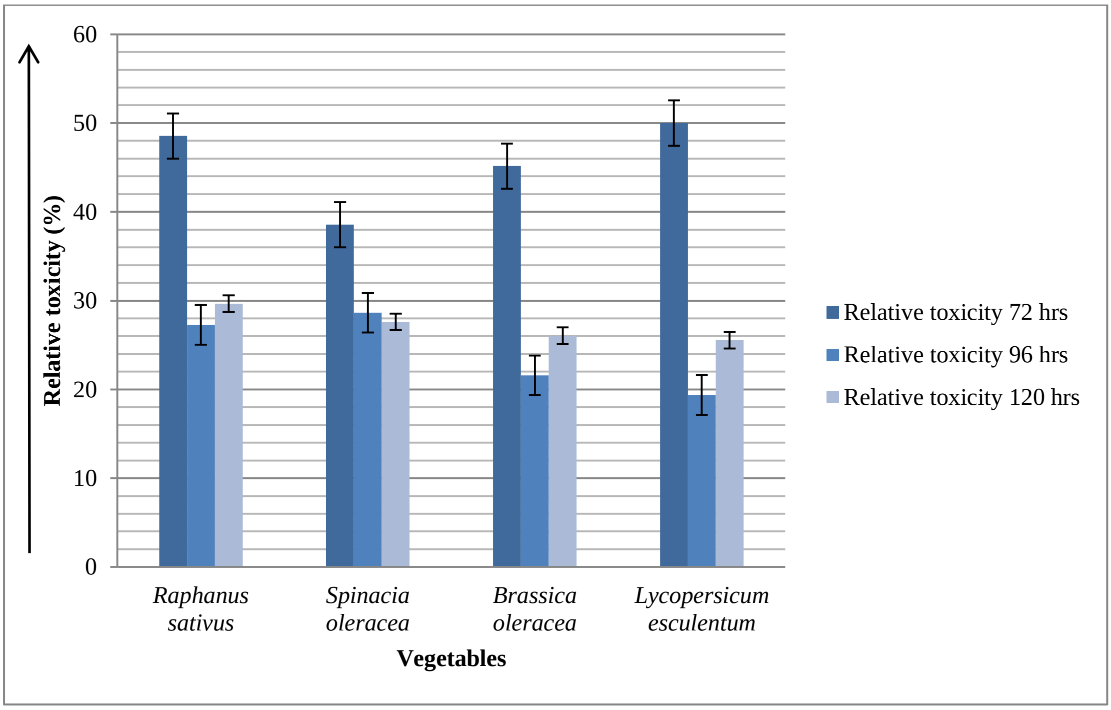


Fig 4.36: Showing relative toxicity (%) in borewell and waste water treated seeds of the vegetables ($p \leq 0.05$; Error bar= ±SD)

4.11.7 Seed vigour index

Seed vigour index was calculated by multiplying the mean values of germination percentage and seedling length (cm) in borewell and waste water treated seeds according to the method given by Abdul-Baki and Anderson (1973). The average seed vigour index in borewell water treated seeds in *Raphanus sativus* was calculated to be 180.95, 478.60, 676.80 and 53.30, 63.30, 278.52 in the waste water treated seeds at 72, 96 and 120 hours respectively. Similarly, the mean seed vigour index values of 27.39, 73.21, 171.49 and 58.33, 78.33, 281.88 were observed in waste water and borewell treated seeds respectively at the same hours in *Spinacia oleracea*. In *Brassica oleracea*, the average seed vigour index in borewell treated seeds was found to be 61.66, 83.33, 645.57 and that of 48.33, 61.66, 233.46 was recorded in waste water treated seeds at 72, 96 and 120 hours respectively. The seeds of *Lycopersicum esculentum* showed mean seed vigour index of 38.33, 53.33, 143.91 and 53.33, 71.66, 300.00 in both waste water and borewell water treated seeds respectively at the same hours of observation. Seed vigour index was found to be greatly reduced by the application of waste water. The results of the present study were authenticated by the previous studies done by Dash (2012) and Rahman et al (2018) who reported the reduction of seed vigour index in rice, wheat and red amaranthus seeds treated with domestic waste water and synthetic dye effluents.

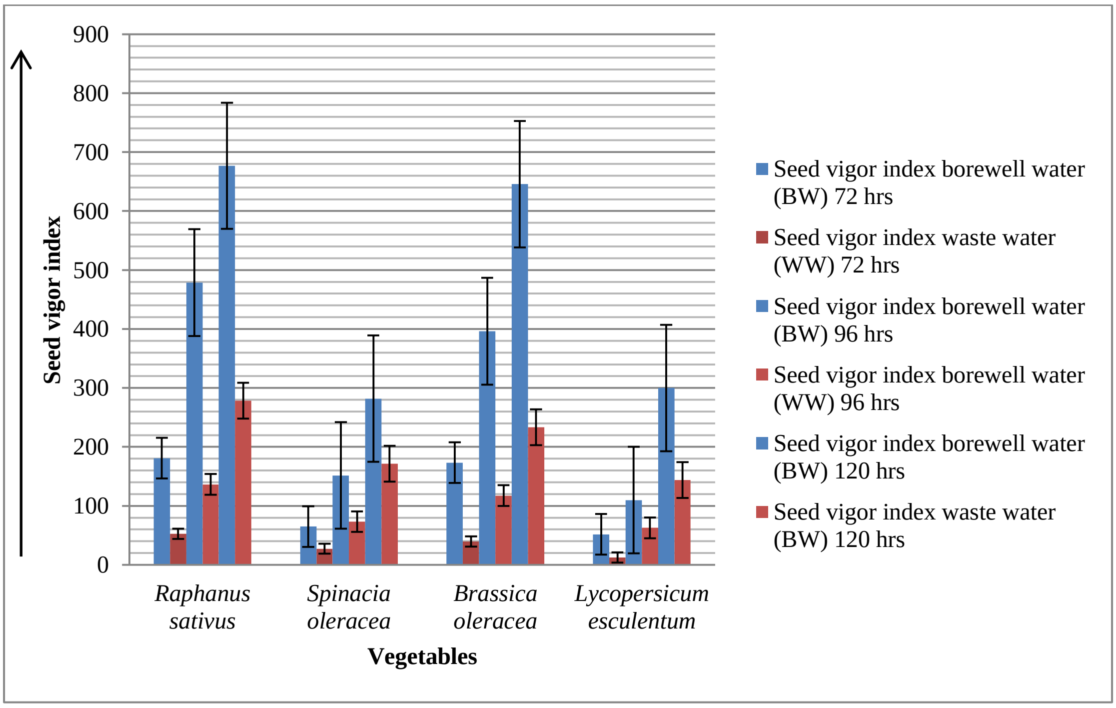
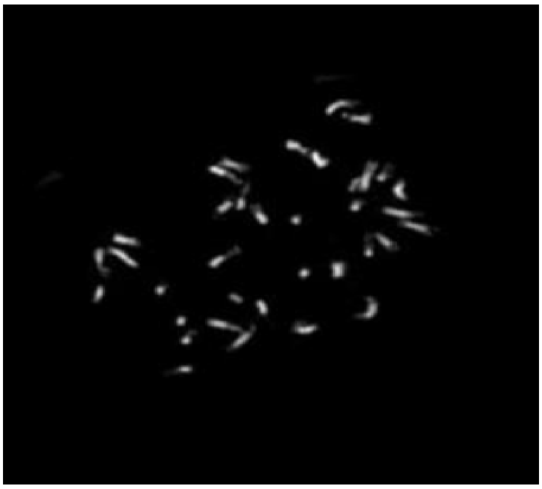


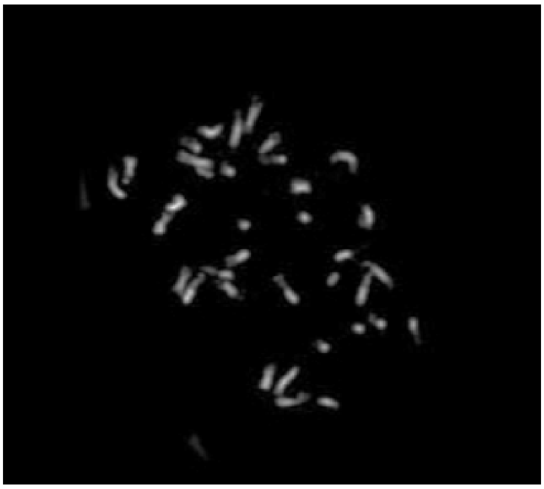
Fig 4.37: Seed vigour index in borewell and waste water treated seeds of the vegetables ($p \leq 0.05$; Error bar= ±SD)

4.12 Cytological studies

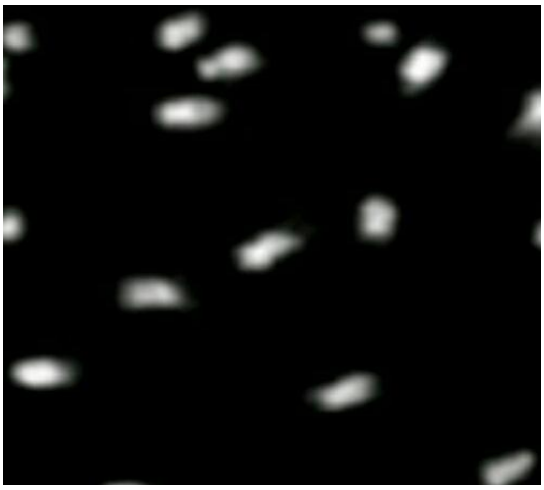
Effect of some heavy metals like lead, chromium, mercury and cadmium on chromosomal morphology has previously been reported (Ruposhev, 1976). To verify the effect of heavy metals present in the waste water on chromosomal aberrations, a mitotic study of root tip was performed and the slides were observed under zeiss inflorescence microscope 60x image. The number of chromosomes in waste-water-irrigated as well as borewell water irrigated *Raphanus sativus* and *Brassica oleracea* was found to be same (2n=18). Similarly, the root tip of *Spinacia oleracea* was also found to have same number of chromosomes (2n=12) in waste-water-irrigated as well as borewell water irrigated samples. The number of chromosomes (2n=24) was also observed in both waste water and borewell water irrigated samples of *Lycopersicum* *esculentum*. No additions, deletions or translocations were observed in the morphology of chromosomes studied in all the vegetable samples irrigated with waste water and borewell water. Aslam et al (2014) reported chromosomal aberrations under high cadmium treatment in *Hordeum vulgare*. Similarly, Kumar and Srivastava (2015) also reported chromosomal abnormalities like scattering, stickiness and laggard bridges etc. in *Vicia faba* treated with different concentrations of lead and cadmium. The results of the present study do not agree with some of the previous studies conducted by the above mentioned researches. This might be due to the toxicity of heavy metals nullified by the increased levels of antioxidants in the waste-water-irrigated vegetables.



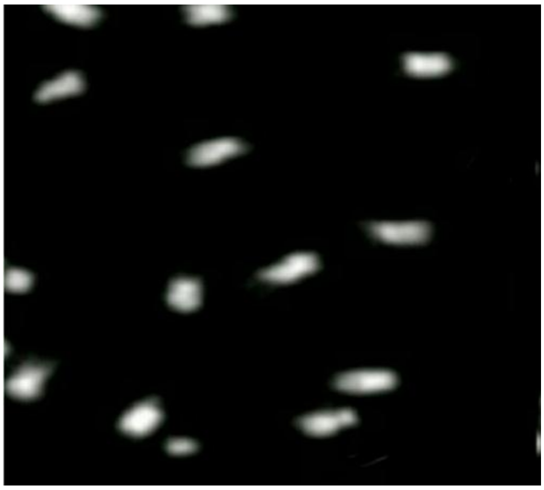
***Raphanus sativus* BW**



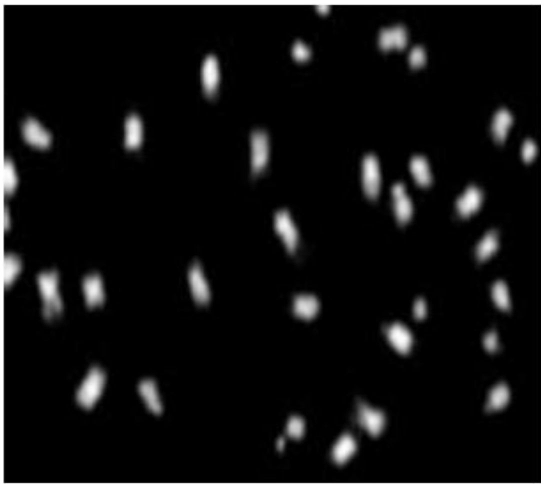
***Raphanus sativus* WW**



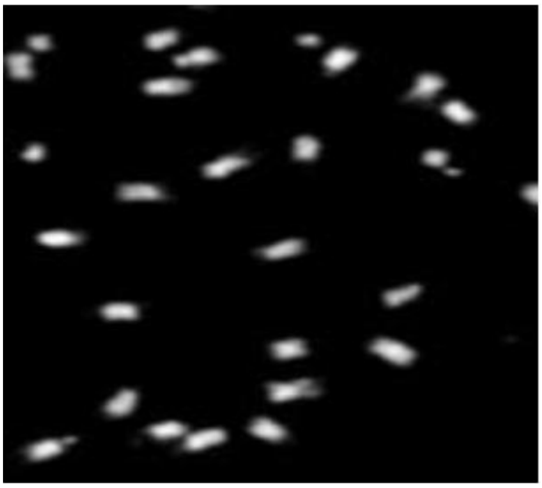
***Spinacia oleracea* BW**



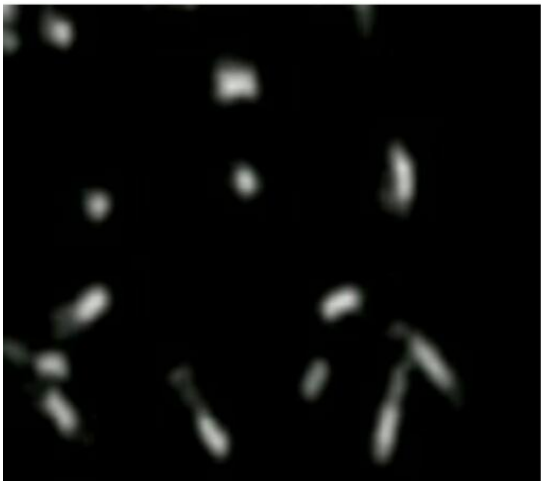
***Spinacia oleracea* WW**



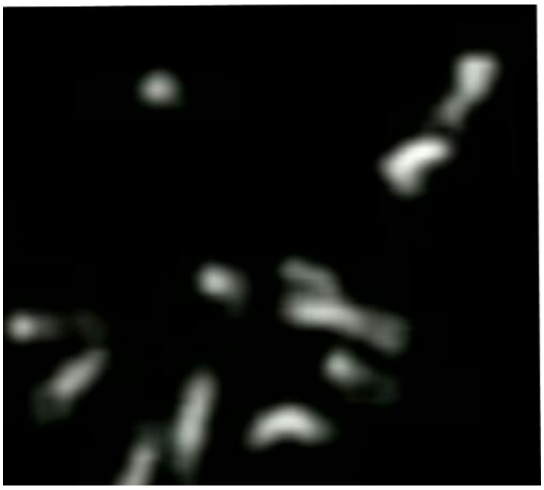
***Brassica oleracea* BW**



***Brassica oleracea* WW**



***Lycopersicum esculentum* BW**



***Lycopersicum esculentum* WW**

Picture 4.3: Zeiss fluorescence microscope 60x image of the cytological slides (mitosis)

4.13 Effect of irrigation water quality on fresh and dry weight of edible portions of the vegetables

It has already been confirmed in previous studies that use of waste water had a significant impact on crop productions (Rusan et al., 2007; Bauder et al., 2014). The results of the present study indicated a significant increase in both fresh and dry weight of the edible parts (fleshy roots of *Raphanus sativus*, leaves of *Spinacia oleracea*, curd of *Brassica oleracea* and fruit of *Lycopersicum esculentum*) in waste-water-irrigated samples of the vegetables as compared to that of borewell irrigated ones. Mean fresh root weight of waste-water-irrigated *Raphanus sativus* samples was found to be 182.00±2.80 g/plant and 183.50±1.80 g/plant as compared to that of 177.00±1.40 g/plant and 179.00±1.60 g/plant in borewell water irrigated ones. during both the first and second years of study respectively. Average total fresh leaf weight per plant was detected to be 55.40±2.10 g/ plant and 56.60±1.30 g/plant in borewell water irrigated as equated to 58.30±2.30 g/ plant, and 59.00±1.60 g/plant in waste-water-irrigated samples of *Spinacia oleracea* during both the first and second years of study respectively. Similarly, the mean amount of fresh weight curd/plant in *Brassica oleracea* was noted to be 276.50±2.06 g/ plant and 277.50±1.80 g/plant in waste-water-irrigated as compared to that of 270.00±2.30 g/ plant and 272.00±1.80 g/plant in borewell irrigated samples during both the first and second years of study respectively. In *Lycopersicum esculentum*, the average fruit fresh weight (single fruit) was calculated to be 105.75±1.80 g/ fruit and 106.30±2.00 g/ fruit in waste-water-irrigated as compared to that of 99.2±1.10 g/ fruit and 98.8±1.30 g/fruit in borewell water irrigated samples during both the first and second years of study respectively.

A significant difference was also detected in moisture content in edible portions of all the vegetables studied. The borewell water irrigated vegetables were found to have significantly higher moisture content as compared to the waste-water-irrigated vegetable samples. The average moisture content in *Raphanus sativus* (per fleshy root) irrigated with waste water was detected to be 90.54% and 90.25% as equated to 91.50% and 91.56% in borewell water irrigated samples during both the first and second years of study respectively.

Similarly, the average moisture content in leaves/plant in *Spinacia oleracea* was calculated to be 91.87%, and 91.89% in borewell water irrigated as compared to that of 89.70% and 90.90% in waste-water-irrigated during both the first and second years of study respectively. The moisture content (per curd) in *Brassica oleracea* was calculated to be 92.62% and 92.72% in borewell water irrigated and that of 90.02% and 90.96% in waste-water-irrigated samples. Average moisture content (per fruit) was found to be 90.63% and 90.68% in waste-water-irrigated as equated to 91.73% and 91.80% in borewell irrigated samples of *Lycopersicum esculentum*.

***Raphanus sativus* BW**

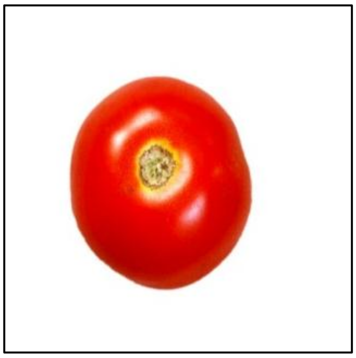
***Raphanus sativus* WW**

***Spinacia oleracea* BW**

***Spinacia oleracea* WW**

***Brassica oleracea* BW**

***Brassica oleracea* WW**



***Lycopersicum esculentum* BW**

***Lycopersicum esculentum* WW**

Picture 4.4 : Edible portions of the vegetables irrigated with borewell water (BW) and waste water (WW)

Table 4.13: Proximate composition of borewell and waste-water-irrigated vegetables (Edible portions)

	2017-18			2018-19		
Name of the vegetable	**Fresh weight (g/plant)**	**Dry weight (g/plant)**	**Moisture content (%)**	**Fresh weight (g/plant)**	**Dry weight (g/plant)**	**Moisture content (%)**
Raphanus sativus BW	177.00±1.40	14.90±0.96	91.58	179.00±1.60	15.10±0.50	91.56
Raphanus sativus WW	182.00±2.80	17.20±0.86	90.54	183.50±1.80	17.90±0.60	90.24
Spinacia oleracea BW	54.00±2.10	4.50±0.20	91.87	56.60±1.30	4.60±0.30	91.89
Spinacia oleracea WW	58.30±2.30	6.00±0.50	89.70	59.00±1.60	5.90±0.20	90.00
Brassica oleracea BW	270.00±2.30	19.90±1.60	92.62	272.00±1.80	19.70±1.20	92.75
Brassica oleracea WW	276.50±2.06	25.10±1.20	90.92	277.50±1.80	24.90±1.30	91.02
Lycopersicum esculentum BW	99.20±1.10	8.20±1.00	91.73	98.80±1.30	8.10±1.20	91.80
Lycopersicum esculentum WW	105.75±1.80	9.90±1.50	90.63	106.30±2.00	9.72±1.30	90.68

BW= Borewell water irrigated WW= Waste-water-irrigated (Mean + SD of 15 values $p<0.05$)

An increase in biomass of *Beta vulgaris* irrigated with waste water has previously been reported by Singh and Aggrawal (2010). Similarly, Madhvi et al (2014) also reported an increase in weight and leaf area of spinach grown in an industrial area and irrigated with the waste water. A similar study was conducted by Mzini and Winter (2015) in which they detected a significant increase in yield of Spinach and onion irrigated with waste water. Application of organic fertilizers was found to significantly increase the curd content of *Brassica oleracea* variety Botrytis grown under protective structure (Farahzaty and Hassan, 2013). The results obtained in the present study are similar to the results obtained by above mentioned studies carried out by previous researchers. Increase in production of vegetables irrigated with waste water can be attributed to the fact that waste water of Buddha Nullah is also highly enriched with essential macro and micro nutrients like nitrate, phosphate, sulphate, iron, manganese, copper, and magnesium which were responsible for better growth and development of these vegetables and hence an increase in quantity wise production as compared to the borewell water irrigated ones.

4.14 A survey of health risk among populations exposed to consumption of heavy metal rich vegetables

A health survey was conducted to have an overall view of the health risk associated with the consumption of vegetables irrigated with heavy - metal - rich waste water of Buddha Nullah and normal borewell water among the populations of Ludhiana. The survey conducted was based on questionnaire method given by Holyk, (2008) and on the basis of information gained from the consumers, following conclusion was drawn about the health risks associated with the consumption of vegetables irrigated with the waste water of Buddha Nullah.

4.14.1 Consumption of raw vegetables (per week)

In the survey done on male population residing along the banks of Buddha Nullah, it was observed that out of 50 males studied, 6% consumed raw vegetables irrigated with waste water twice, 50% thrice and 44% more than three times a week. The trend of consumption of raw vegetables in female population was, 8% were found to be consuming raw vegetables once, 16% twice, 26% thrice and 50% more than three times a week. Similarly, out of 50 male members of the population consuming vegetables irrigated by borewell water, 6% were found to be consuming raw vegetables thrice a week and 94% more than thrice a week. Out of 50 females surveyed, 2% were found to consume raw vegetables once, 6% twice, 8% thrice and 84% more than thrice a week. Overall consumption in male and female populations consuming raw vegetables irrigated with waste water of Buddha Nullah was significantly lower than the male and female populations consuming borewell irrigated raw vegetables.

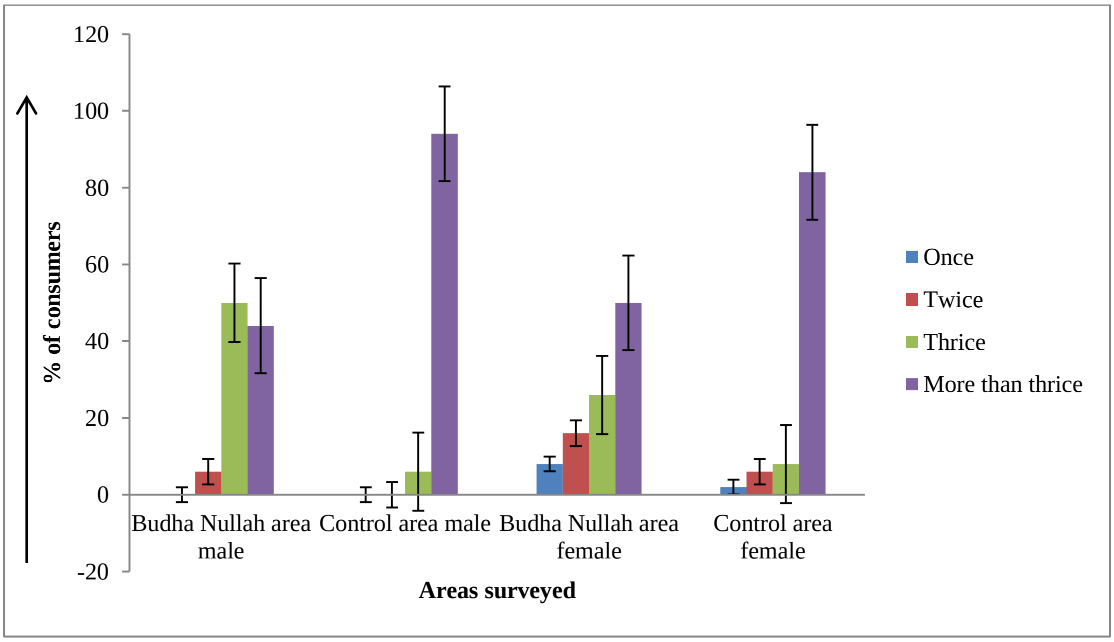


Fig 4.38: Mean % consumption of raw vegetables per week ($p \leq 0.05$; Error bar= ±SD)

4.14.2 Consumption of cooked vegetables (per week)

In the survey done on male population residing along the banks of Buddha Nullah, it was observed that out of 50 males studied, 4% consumed cooked vegetables irrigated with waste water twice, 44% thrice and 52% more than three times a week. Out of 50 females, 18% were found to be consuming twice, 36% thrice and 46% more than thrice a week. Similarly, out of 50 male members of the population consuming vegetables irrigated by borewell water, 20% were found to be consuming thrice a week and 80% more than thrice a week, and out of 50 females surveyed 2% were found to be consuming cooked vegetables twice, 8% thrice and 90% more than thrice a week. Overall consumption of cooked vegetables irrigated with waste water in male and female populations was much lower than the male and female populations consuming borewell irrigated vegetables.

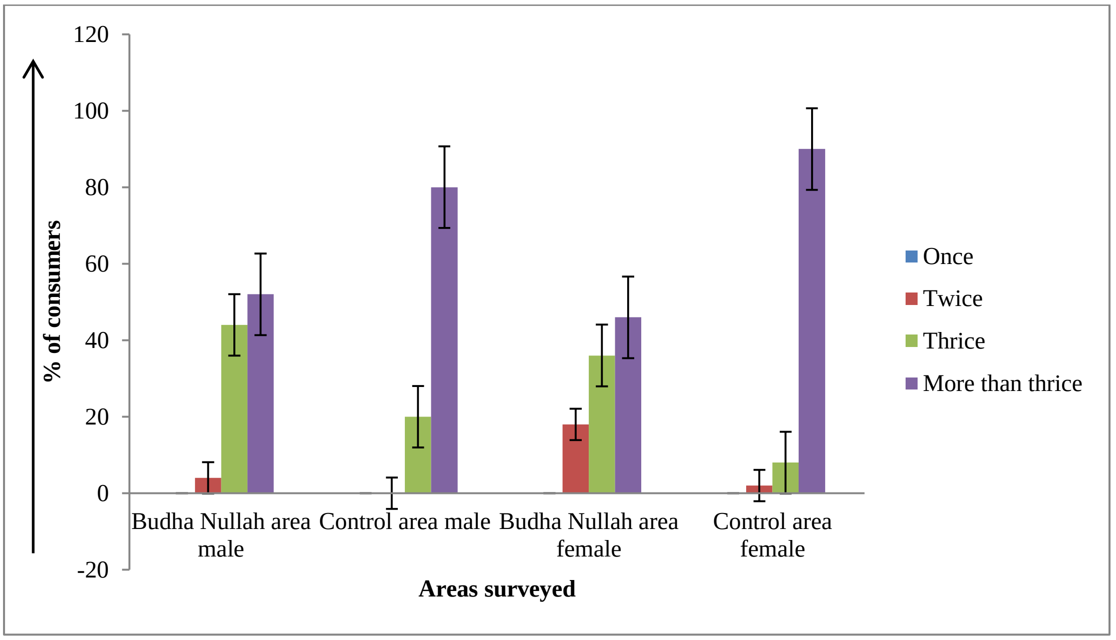


Fig 4.39: Consumption of cooked vegetables per week ($p \leq 0.05$; Error bar= ±SD)

4.14.3 Prevalence of heavy metal related diseases among the population

Previous studied have reported that consumption of heavy metal laced vegetables are the basic cause of hyperpigmentation, nervous damage (Cöl et al., 1999), respiratory irritation, lung disease, testicular and prostate cancer, renal failure, convulsions, coma (ATSDR, 2000), DNA damage (O'brien et al., 2001), leukemia (Yedjou and Tchounwou, 2007), genetic defects, carcinogenic ulcers (Kherici et al., 2009 ; Shefali et al., 2019). According to the results obtained from survey report, it was observed that 68% of male and 78% of female population consuming waste-water-irrigated vegetables and 36 % male and 40% female population consuming vegetables irrigated with borewell water were found to be suffering from any of the heavy metal related diseases (cancer, renal failure, cardiac arrest, premature birth, mental illness or allergy). The present survey revealed that prevalence of heavy metal related diseases were very much higher in both male and female populations consuming waste-water-irrigated vegetables as compared to the male and female populations consuming vegetables irrigated with normal borewell water.

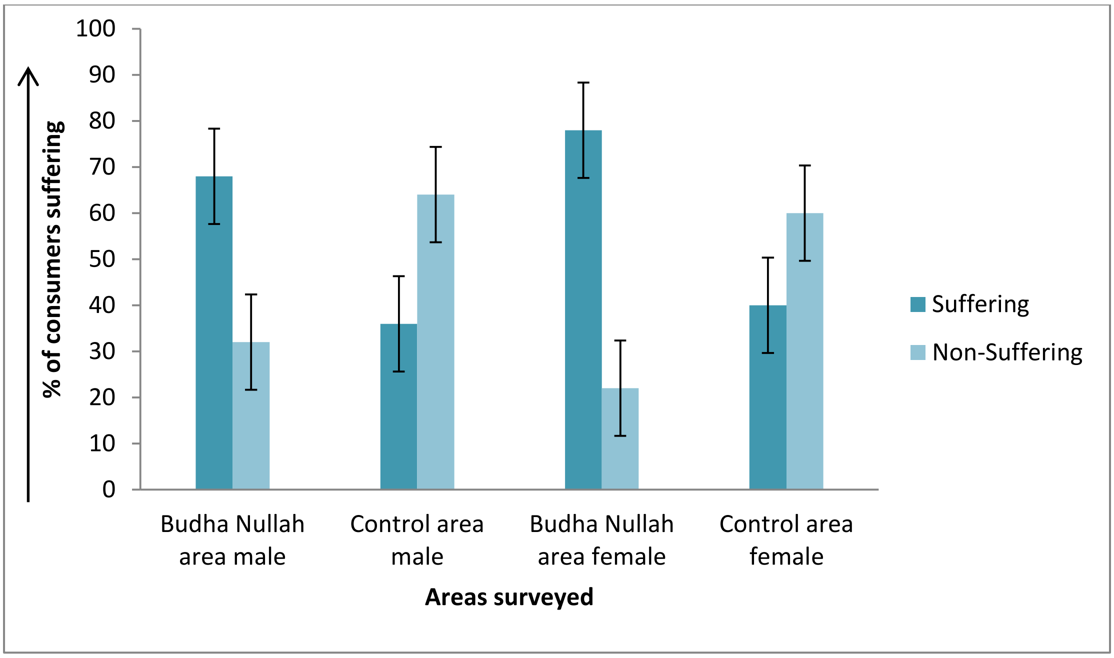


Fig 4.40: Population surveyed suffering (%) from any of heavy metal related diseases ($p \leq 0.05$; Error bar= ±SD)

4.14.4 Prevalence of heavy metal diseases among the farmers and their families

The results of survey revealed that 72 % of male and 80 % of female members of the

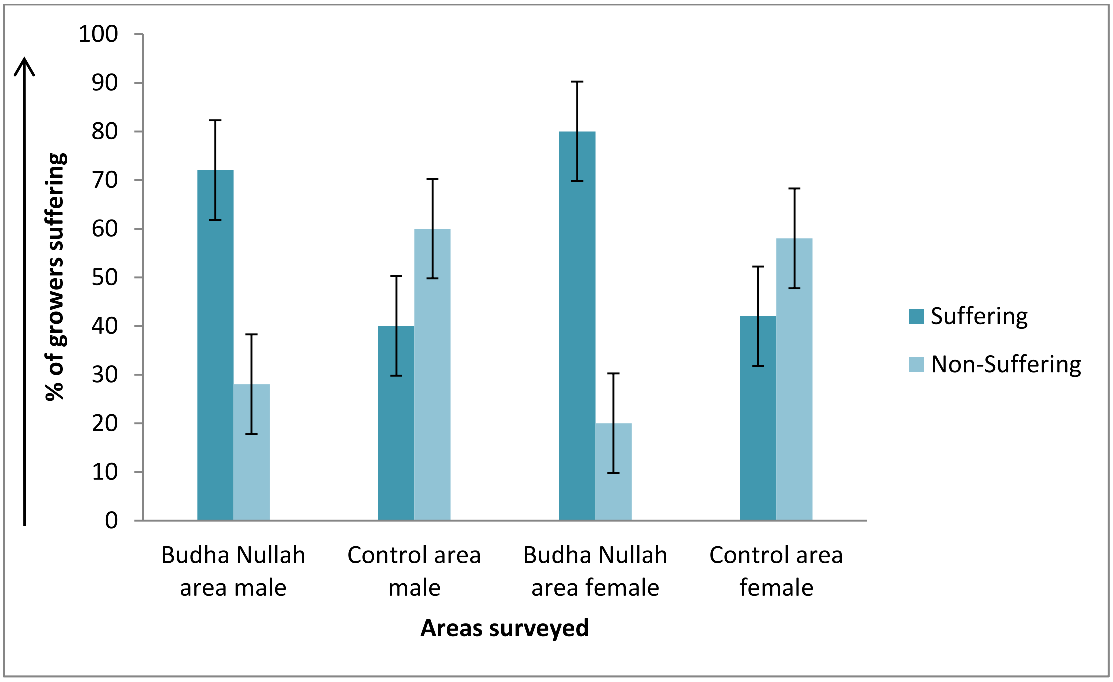


Fig 4.41: Population of farmers and their families suffering (%) from heavy metal related diseases ($p \leq 0.05$; Error bar= ±SD)

farmers and their families involved in waste water irrigation were suffering from heavy metal related diseases as compared to 40% of male and 42% of female members of the farmers involved in cultivation of vegetables irrigated with borewell water. The prevalence of heavy metal related diseases was found to be much higher in the farmers and their families using waste water for irrigation as compared to the farmers and their families using borewell water for irrigation of vegetable crops. Being constantly in touch with waste water and consumption of heavy-metal-rich vegetables might be the reason behind this difference of disease prevalence observed.

4.14.5 Awareness of heavy metal related diseases among the populations

Only 10 % of males and 7% of females of the population consuming vegetables irrigated with the waste water of Buddha Nullah and only 15% of male and 13% of female population consuming vegetables irrigated with borewell water were aware of only one disease (cysticercosis) caused by the consumption of vegetables irrigated with waste water of Buddha Nullah. Not even a single person out of the whole population surveyed was aware of the fact that heavy metals gets bioaccumulated by vegetables and can cause serious health risks.

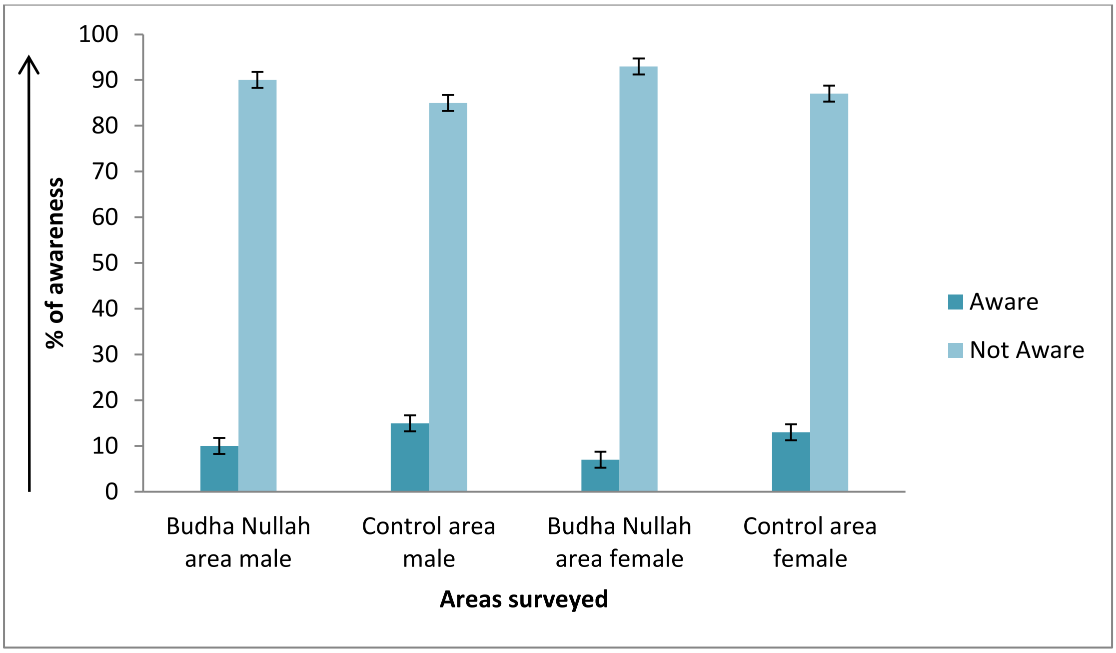


Fig 4.42: Percentage of awareness of heavy metal related diseases among the population surveyed ($p \leq 0.05$; Error bar= ±SD)

The fact was very surprising that even the well-educated persons were not aware of the other diseases related to the consumption of vegetables grown along the banks and irrigated with one of the most polluted water channel i.e. Buddha Nullah, of their city.

4.14.6 Comparative prevalence of heavy metal related diseases among the population surveyed

The present study revealed that the prevalence of heavy metal related diseases among the suffering populations consuming heavy metal contaminated vegetables irrigated by the waste water of Buddha Nullah was in the following order: Skin allergy (30%) > cancer (21%) > lungs diseases (19%) > mental illness (16%) > kidney diseases (9%) > premature births (4%) > others (1%) and in the descending order: mental illness (35%) > cancer (18%) > lungs (18%) > skin allergy (16%) > kidney diseases (8%) > premature births (3%) > others (2%) among the populations consuming borewell irrigated vegetables. The percent prevalence of the same heavy metal related disease was found to be different among both the populations surveyed.

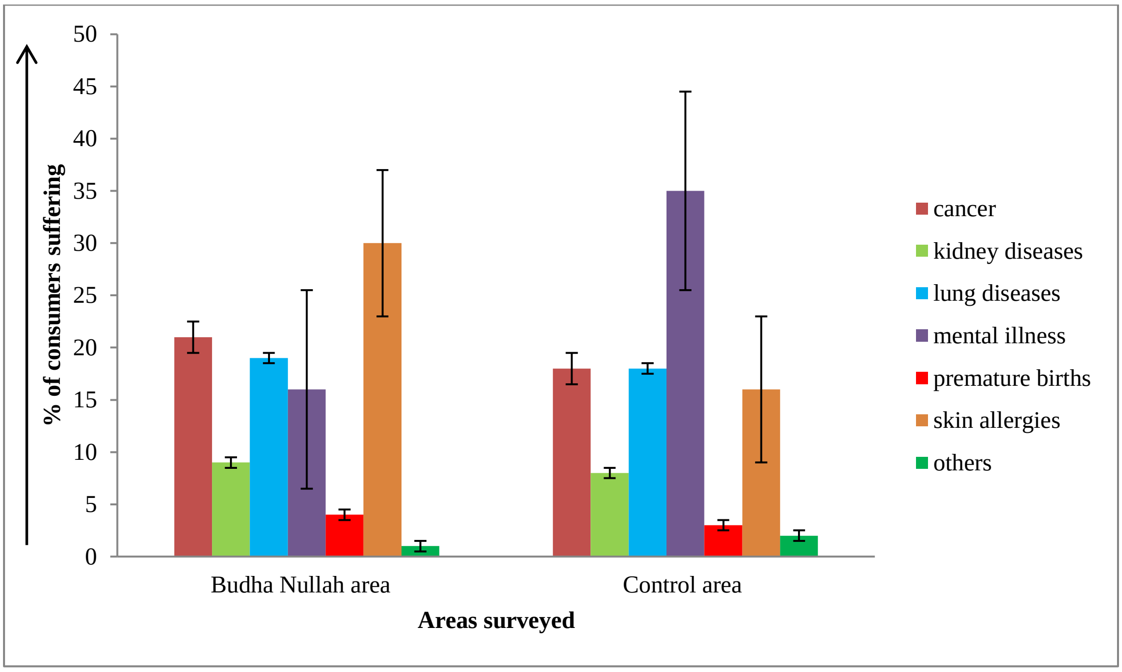


Fig 4.43: Variation in prevalence of heavy metal related diseases among the population (Error bar= ±SD)

Similar surveys have been carried out earlier among the populations raising and consuming waste-water-irrigated vegetables. Lekouch et al (1999) surveyed 327 children consuming heavy-metal-rich vegetables and 110 from the control area. The hair samples analyzed revealed the presence of 16.50±4.50, 14.80±4.50 µg lead in girls and 12.50±3.50, 2.50±0.50 µg of cadmium and lead in the boys surveyed. Cifuentes et al (2000b) analyzed the blood samples of 735 individuals to detect the effect of heavy- metal-rich crop consumption in Mexico and found the amount of lead exceeding 10 µg/l in 23% of the population as the main cause of skin related heavy metal diseases. Trang et al (2007c) surveyed the farmers attached with waste water agriculture in Hanoi and found 44.4% higher rate of dermatitis in farmers related with waste water agriculture as compared to the control site ones. Anh et al (2007, 2009) also surveyed 650 house members above 15 years related with waste water agricultural practices and found only 6.10% of the populations suffering from dermatitis. Chary et al (2008) surveyed the populations consuming vegetables irrigated by Musi river (India) and reported the high level of lead, zinc, chromium and nickel in vegetables to be the cause of skin irritation among the populations surveyed. Mohammed and Jimoh (2014) surveyed that consumption of heavy metal laced lettuce lead to the brain damage, tumour and miscarriage in Kaduna metropolis in Nigeria. Zhou et al (2016) reported the prevalence of heavy metal related diseases in children as compared to the adults consuming heavy-metal-rich leafy vegetables in Suxian district of China. Similarly, Kheirabadi et al (2016) reported that heavy-metal -rich vegetables irrigated by waste water in Hamedan province of Iran induced non carcinogenic diseases among the consumers. The results obtained in the present survey were similar to the results obtained by above mentioned researchers in their surveys conducted among the consumers, farmers and their families related with waste water irrigation of food crops in the different parts of the world.

The outcome of present study is eyebrow-raising because there is a significant difference of prevalence of heavy metal related diseases among the populations consuming vegetables irrigated with waste and normal borewell water. The use of untreated waste water is directly related to the entry of toxic pollutants into the food chain leading to serious health risks among the consumers. It was surprising to note that most of the consumers were unaware of what heavy metals are and what health risks they can pose through their entry into the food chain. Health department must create awareness among the populations about the consequences of consumption of vegetables raised in such polluted areas. The marginal farmers using waste water for irrigation of vegetables should be advised not to grow food crops in such contaminated areas but should rather opt for the other short life-cycle and high value crops like flower crops. The government authorities and pollution control board should implement the laws strictly so that the industrial set-ups and municipal corporation must treat the water before releasing-into such tributaries of rivers and other water bodies. It is the right of a common man to raise voice against such malpractices which directly or indirectly pose a negative impact on human health and create awareness among the consumers and the growers, only then the positive results can be achieved.

5.0 SUMMARY

Due to rapid increase in population and industrialization, the natural water resources are under enormous pressure. Industrial and municipal waste-water-production is increasing day - by - day. Industrial effluents contain large amount of toxic pollutants like heavy metals and these pollutant rich effluents are drained in water bodies without any treatment. This heavy-metal-rich waste water is frequently used for irrigation purposes by the peri-urban farmers due to its free and continuous availability throughout the year. These farmers tend to grow the crops with short life - span and the vegetables are preferred to be grown because of nearby markets. The heavy metals are non-biodegradable, persistent, carcinogenic and mutagenic in nature. The plants irrigated with heavy-metal-rich waste water tend to bioaccumulate these metals and pose a serious health risk to the consumers. The present research work was carried out to determine the bioaccumulation of heavy metals in the vegetables frequently irrigated with the heavy-metal-rich waste water of Buddha Nullah, Ludhiana, a tributary of the river Sutlej carrying sewage and heavy-metal- loaded effluents, and their effect on various biochemical activities and potential risk posed by heavy metals on human health.

5.1 Monitoring of heavy metals at the source point

In the present study, water, soil and vegetable samples of 4 different types of vegetables: *Raphanus sativus* (root), *Spinacia oleracea* (leafy), *Brassica oleracea* (flower) and *Lycopersicum esculentum* (fruit) were collected from the fields along the banks of Buddha Nullah, Ludhiana where the crops are regularly irrigated with the heavy metal and nutrient rich waste water. Similarly, the water, soil and vegetable samples (as control) were collected from another site about 4 kms away from Buddha Nullah where the irrigation was done with the normal borewell water. The water and soil samples from both the sites were analyzed for their physical parameters and heavy metal content for 2 years continuously. The vegetable samples from both the sites were analyzed to check the amount of heavy metal (As, Cd, Cr and Pb) bioaccumulation and their effect on biochemical parameters, antioxidant activity, yield and risk posed to the consumers.

5.2 Physiochemical analysis of borewell and waste water used for irrigation

The average mean data of physiochemical parameters of borewell water and waste water used for irrigation purposes revealed a significant increase of COD, BOD, EC, TSS, TDS and NO_3^- component in waste-water-irrigated samples as compared to that of borewell water used for irrigation. Similarly, the amount of As, Cd, Cr and Pb was detected to be manifold higher in waste water samples as compared to that of borewell water samples and the concentration of all these heavy metals was upto As (0.95± 0.02 mg/l), Cd (3.43±0.20 mg/l), Cr (6.59±0.90 mg/l) and Pb (2.21±0.16 mg/l) which was found to be significantly higher than the permissible levels.

5.3 Physiochemical analysis of soil samples from borewell water and waste water irrigation sites

Physiochemical analysis of soil samples revealed a significant increase in Electrical Conductivity of waste-water-irrigated soil sample 1839.5±4.6 mhos/cm as compared to the borewell water site soil sample which was detected to be 289.15±4.9 mhos/cm. Similarly, the level of As (9.49±0.20 mg/kg), Cd (17.07±0.26 mg/kg), Cr (24.85±0.58 mg/kg) and Pb (19.5±0.60 mg/kg) was also recorded to be significantly higher than the borewell water irrigated sites which showed the amount of these heavy metals to be As (5.59±0.26 mg/kg), Cd (7.43±0.41 mg/kg), Cr (9.56±0.22 mg/kg), Pb (7.84±0.28 mg/kg).

5.4 Enrichment factor of soil and bioaccumulation factor of vegetable samples

The enrichment factor of all the heavy metal (As, Cd, Cr and Pb) in soil samples collected and analyzed from waste water irrigation site was found to be greater than 1.5 which indicates that bioaccumulation of these heavy metals in soil was positively correlated with the waste water irrigation. The minimum enrichment factor 1.69 was observed for As and maximum 2.60 for Cr.

Similarly, the bioaccumulation factor detected in vegetable samples collected from waste water irrigation site was observed to be different for different type of vegetables. Bioaccumulation factor for As was detected to be less than 1 but for Cd and Cr the bioaccumulation factor was observed to be greater than 1 in all the vegetables except *Lycopersicum esculentum*. Again the bioaccumulation factor for Pb was detected to be greater than 1 in *Raphanus sativus* and *Spinacia oleracea* but less than 1 in *Brassica oleracea* and *Lycopersicum esculentum* which revealed that root and leafy vegetables were hyper-accumulators.

5.5 Effect of waste water on seed germination and seedling growth of vegetable seeds

The waste water treatment had a significant effect on seed germination and growth of all the vegetables studied. A significant reduction in seed germination percentage was observed in the seeds treated with waste water as compared to that of borewell treated seeds. Similarly, all other parameters like root length, shoot length, total seedling length, relative toxicity was found to be significantly higher in waste water seeds as compared to control. Similarly, seed vigour index was significantly reduced with the application of waste water in comparison to the borewell water treated seed.

5.6 Effect of borewell water and waste water irrigation on biochemical parameters of vegetables

A significant effect of waste water irrigation was noticed in the amount and activity of all the biochemical parameters like total chlorophyll, protein, carbohydrates, starch, sugars, secondary metabolites and antioxidants: enzymatic and non-enzymatic of all the vegetables studied. Total chlorophyll, carbohydrates, starch was found to be significantly decreased whereas the amount of proteins, reducing, non-reducing, total sugars, was increased in vegetables irrigated with waste water. A significant increase was observed in the amount of non-enzymatic antioxidant compounds like ascorbic acid, proline, flavonoids, carotenoids, total phenols and activity of enzymatic antioxidant enzymes like catalase, peroxidase, glutathione reductase, ascorbate peroxidase, superoxide dismutase in the edible portion and leaves of all the vegetables studied. The production and activity of these antioxidants can be attributed to the defence mechanism adopted by the plants under oxidative stress induced by accumulation of heavy metals in the cell.

5.7 Health survey

The survey conducted to evaluate the prevalence of heavy metal related diseases among the populations authenticated that these diseases are more common in the populations consuming vegetables irrigated with waste water of Buddha Nullah as compared to the population consuming vegetables irrigated with normal borewell water. The present study revealed that the prevalence of heavy metal related diseases in the population consuming heavy metal contaminated vegetables grown under irrigation of waste water of Buddha Nullah was in the order: Skin allergy (30%) > lungs diseases (20%) > cancer (20%) > mental illness (16%) > kidney diseases (9%) > premature births (4%) > others (1%) and in the descending order: mental illness (35%) > cancer (18%) > lungs (18%) > skin allergy (16%) > kidney diseases (8%) > premature births (3%) > others (2%) among the population consuming borewell irrigated vegetables. The percent prevalence of the same heavy metal related disease was found to be different in both the populations surveyed.

5.8 Conclusion

It is very clear from the present study that the waste water of Buddha Nullah is highly contaminated with heavy metals and it enriches the soil with heavy metals when irrigated with this waste water for long time. These heavy metals get bio-accumulated in the edible parts of the vegetables raised in such heavy metal rich soils. Higher levels of these heavy metals negatively affects the health of vegetable plants as well of the consumers by altering the normal biochemical reactions Leafy and root vegetables have been found to be hyper-accumulators as compared to the other vegetables studied. Taking into consideration the health risks associated with consumption of these vegetables, it is suggested that cultivation of vegetables especially leafy and root vegetables should be restricted at such polluted sites.. Cultivation of edible vegetables along the banks of Buddha Nullah is only recommended if the toxic heavy metals are removed from its highly contaminated waste water by suitable pre-treatment methods before its use for irrigation purpose. Awareness about metal toxicity and carefully monitoring the discharge of treated industrial effluents of should be created among the common people.

Future scope of work

Bioaccumulation of heavy metals in vegetables is of great concern due to its potential impact on plant and human health. The research gap identified in this study provides insight on opportunities for future work that will contribute to harvesting the benefits of waste water in changing environmental and social context, while mitigating risk to public health.

5.9 Recommendations

1. The farmers are advised to opt for non-food crops like floriculture instead of food crops due to its high demand, short life cycle and better returns. Cultivation of flower crops like Marigold (*Tagetes erecta*) will be a better option for farmers involved in agricultural practices at such polluted sites.

2. Agroforestry is another option in which fast growing and hyper-accumulator mutant varieties of *Populus*, *Smilax* and *Eucalyptus* can be grown along the banks of such waste water carrying nullahs.

3. Mixed use of waste and borewell water for irrigation will help in reducing the negative effect of waste water.

4. Drip and furrow irrigation methods instead of flood irrigation should be adopted for crops being cultivated along the banks of Buddha Nullah. These will help to reduce the amount of waste-water, hence reducing the amount of pollutants.

5. Mulching technique should be adopted to raise vegetable at such contaminated site. It will reduce the rate of evaporation and will act as a barrier between crops and pollutants especially the biological pathogens

6. Riparian buffers should be grown atleast upto 100 meters on both the sides of Buddha Nullah which will help to provide an aesthetic look as well as phytoremediation of heavy metals.

7. Awareness among the consumers regarding the consequences of consuming edible crops grown at such polluted sites, strict implementation of laws laid by Pollution Control Board and introduction of environmental education in school to university curriculum is the need of the hour.

8. Waste-water is one of the future opportunities. So, it is necessary to communicate the beneficial as well as the negative impact of this practice, and different low-cost strategies that contribute to the decision making process and

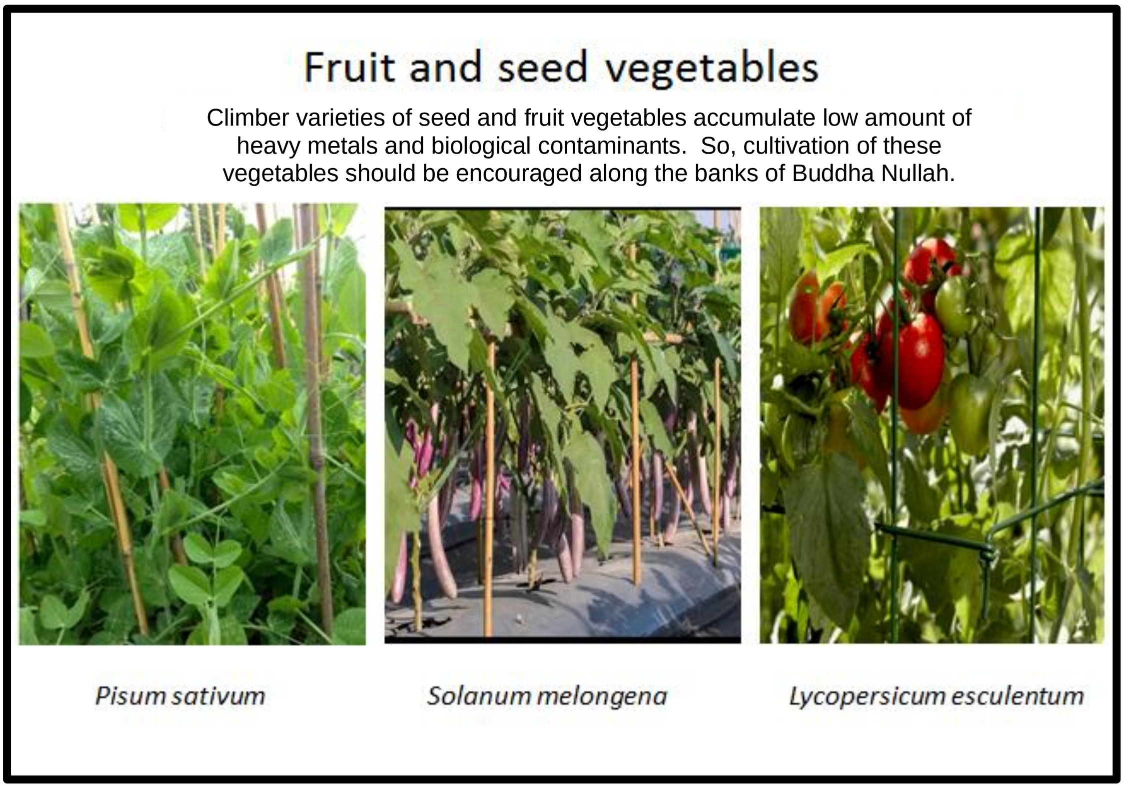
favor the adequate use of waste-water in agriculture.

9. Guru Angad Dev Ji, second of the Sikh Gurus, said:

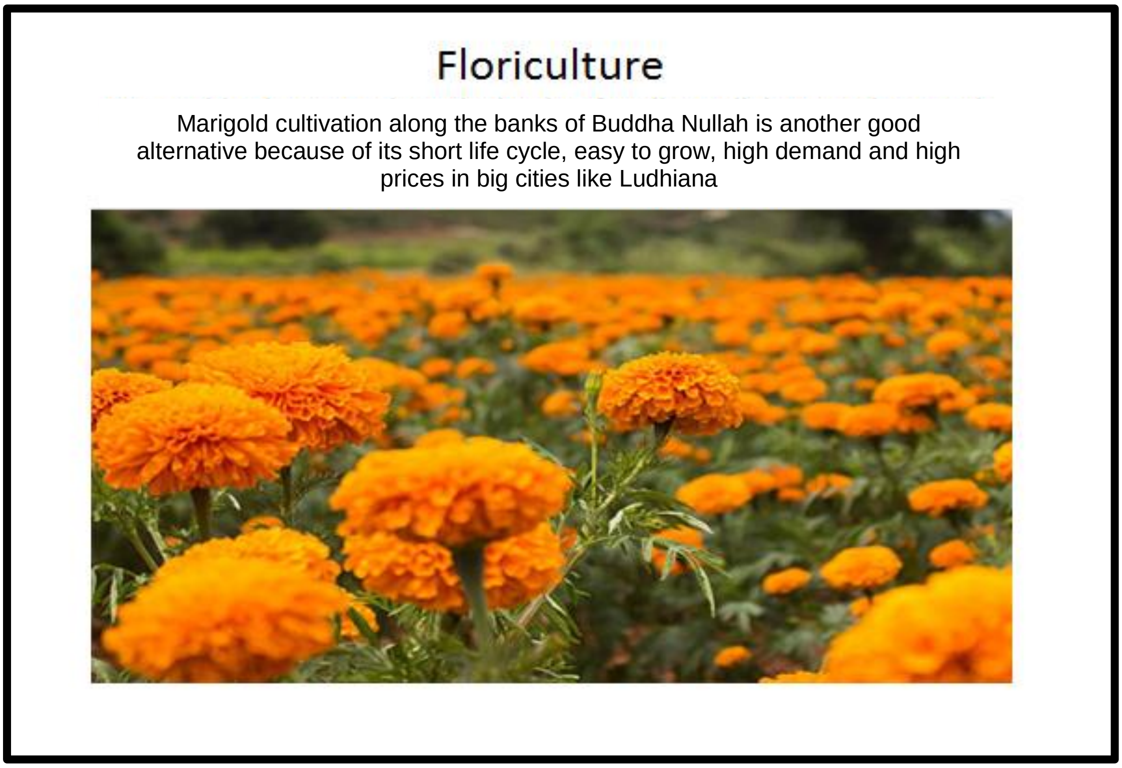
   "Pavan Guru Paani Pita, Mata Dharat Mahat"

   Air is the Guru, Water is the Father and the Earth is the Great Mother

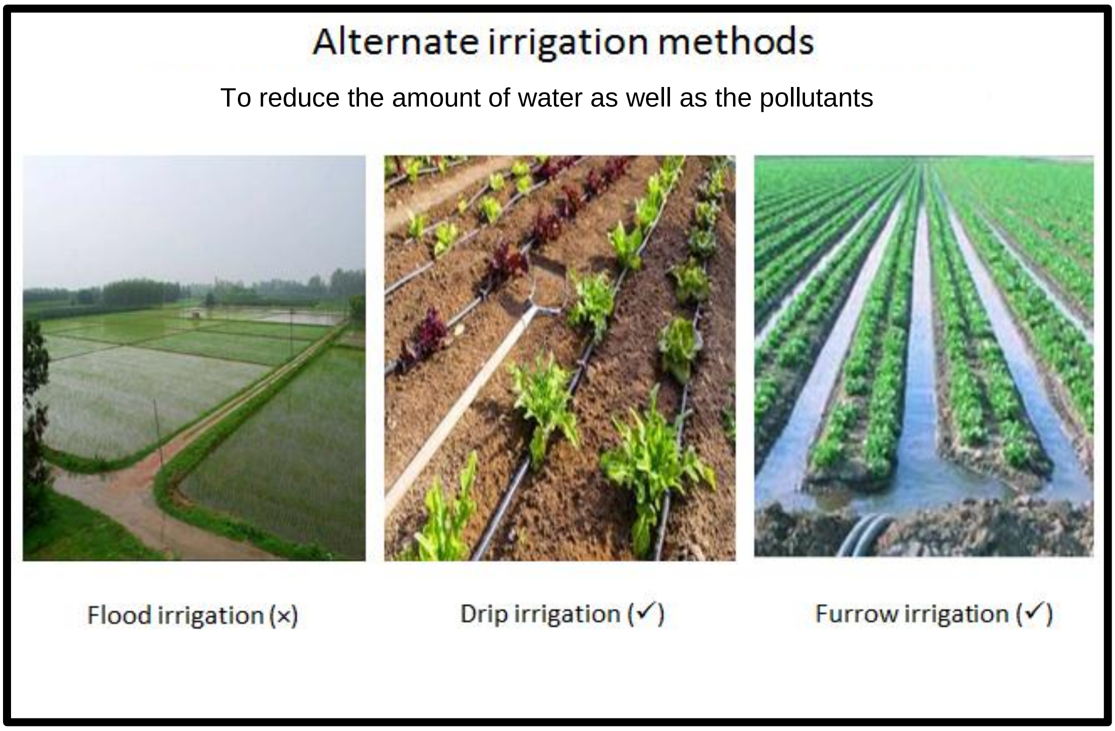
   This philosophy needs to be incorporated in life so that all human beings can take care of the environment and lead a healthy and happy life.



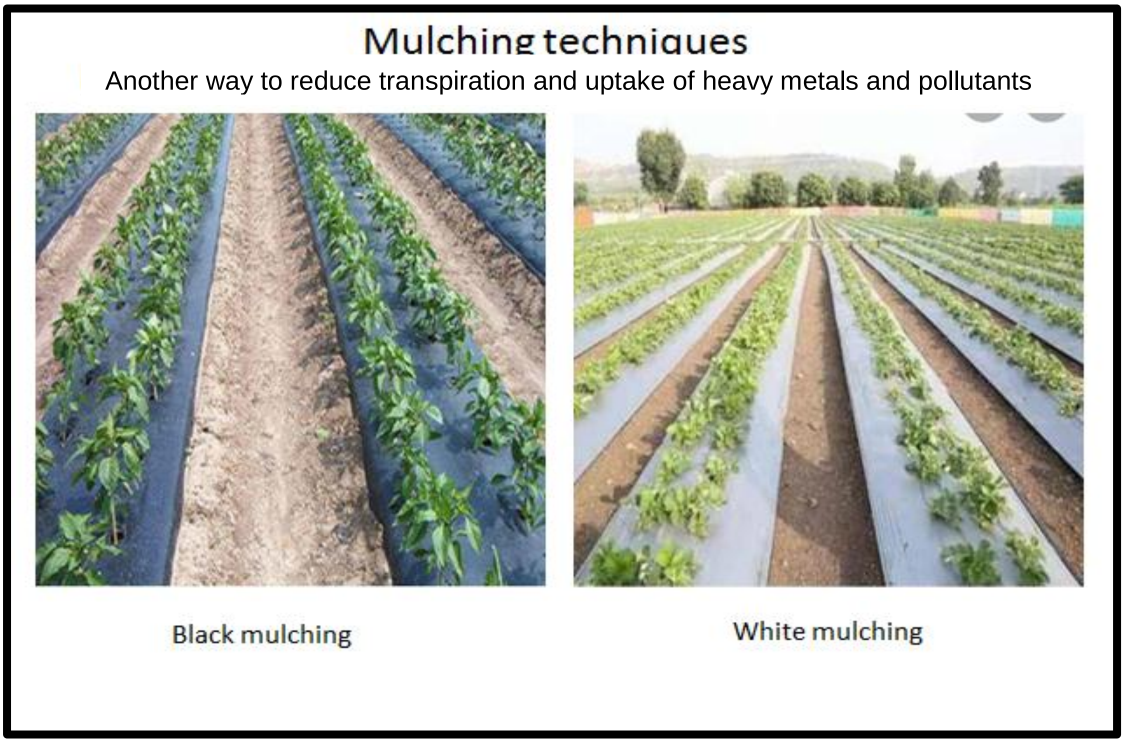
Picture 4.5: Fruits and seed vegetables



Picture 4.6 Marigold crop



Picture 4.7 Irrigation methods



Picture 4.8 Mulching techniques

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ANNEXURE 1

1. Effect of waste water irrigation on antioxidant metabolites in some selected vegetables: International Journal of Research and Analytical Reviews: December 2018, Volume 5, Issue 4.
2. Effect of heavy metal contaminated waste water irrigation on enzymatic and non-enzymatic antioxidants in some selected vegetables- Journal of Physics: Conference Series: 1531 (2020) 012093
3. Veggies near Buddha Nullah not safe : Study – Ludhiana Tribune – April 22, 2019 pp 1
4. Study finds high metal concentration in vegetable crops near Buddha Nullah – Hindustan Times – April 23, 2019 pp 2
5. These veggies are 'slow posion' - The Indian Express - July 31, 2019 pp5
6. A staggering research study conducted by LPU faculty Dr Mohan & Research scholar Jagdev Singh – Happenings LPU – August 7, 2019

ANNEXURE 2

1. ISSWM-2017 (Innovative Strategies for Sustainable Water Management) International conference held from 17.11.2017-18.11.2017 at Lovely Professional University, Phagwara– oral presentation
2. 106th Indian Science Congress Association held from 03.01.2019-07.01.2019 at Lovely Professional university, Phagwara– participation
3. International Conference on Chemical Constellation Cheminar-2019 held at Dr B R Ambedkar National Institute of Technology, Jalandhar from 0ctober12-October 13, 2019- poster presentation.
4. Recent Advances in Fundamental and Applied Sciences (RAFAS -2019)-held on November5-6, 2019 at Lovely Professional university, Phagwara Oral presentation
5. Author Workshop on How to Write Manuscripts Grant Proposals (Human Resource Development Center-LPU) held on October 12, 2018- Participation.

ANNEXURE 3

- Comparative study of contamination of vegetables irrigated by the sewage and industrial waste water.
- Contamination Chances of Vegetables Cultivated at Heavy Metal Contaminated and Waste Water Irrigation Sites in Some Developing Asian Countries
- Effect of waste water of Buddha Nullah, an irrigation riverine on seed germination and seedling growth of 4 vegetables
- A survey of health risk among populations exposed to waste water use in agriculture
- Bioaccumulation of heavy metals and biochemical changes induced in *Spinacia oleracea* and *Brassica oleracea* irrigated with waste water of Buddha Nullah Ludhiana, Punjab.