HUMAN HEALTH RISKS ASSOCIATED WITH HEAVY METAL-CONTAMINATED SOIL AND FOOD PLANTS IRRIGATED WITH WASTEWATER

By

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Thesis submitted to Department of Environmental Sciences University of Peshawar in partial fulfillment of the requirements for the degree of Doctor of Philosophy

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DEDICATION

I lovingly dedicate this thesis to my caring mother, Mrs. Raham Din (Late) who supported my each step of the way.

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All blessing are unto Almighty Allah who enables me to complete this work. I consider it my utmost to express gratitude to the prophet Muhammad (S.A.W.). I feel great pleasure and honour to acknowledge my deep sense of gratitude from the core of my heart to my supervisors Dr. Sardar khan, Associate Professor in Department of Environmental Sciences , University of Peshawar and Dr. Shafiq-Ur- Rehman, Professor in Department of Environmental Sciences, University of Peshawar. Their guidance, immaculate supervision, kind and keen interest inspired self confidence in me. Further my cordial thanks go to Dr. Ihsanullah, Head of Plant Sciences Department and Mr. Yousaf, Senor Scientist in the Department of Plant Nutrition for their graciously assistance and guidance during the laboratory work in the Nuclear Institute of Food and Agriculture. Similarly I would like to thanks Mr. Hassan Laboratory Assistance, Department of Plants Breeding and Genetics in Agriculture University Peshawar, Mr. Gul Hassan, Laboratory Assistance, Department of environmental Sciences, University of Peshawar and Mr. Tariq Laboratory Assistance in the Department of Geology, University of Peshawar who's never hesitated to assist me at any stage in the completion of my laboratory works for research. Sincere and deepest thanks to Dr. Ayesha S. Abdullah, Dean and Assistance Professor in the Peshawar Medical College, Mrs. Salma Khalid and Miss. Hafsa Habib, Dr. Naveed Sharif, Dr. Sheikh Ibqar Azeem, Dr. Shamilah Hafizi, and Shandana Ikram for their moral and refreshing support. Thanks to Tariq Aziz for his help in composing thesis.

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Abstract

Long-term wastewater irrigation is not only a way of contaminations of soil with heavy metals (HMs) but also accumulates HMs in food crops. Samples of both soil and vegetables were collected from wastewater irrigated sites of Peshawar, Pakistan in winter and summer seasons for analysis of HMs concentrations in them and to investigate the health risk of associated with HMs contaminated vegetable. The mean concentrations (mg/kg dry weight) of selected HMs like Cu, Pb, Cd, Ni, Zn, Mn, Cu, Co and Hg in soil irrigated with wastewater in winter, summer seasons and preference soil were 56.9, 37.01 and 13, 61.28, 62.1 and 10.4, 7.68, 8.9 and 1.2, 100.78, 106.64 and 23, 159.55, 193.4 and 12, 341.33, 387.7 and 14, 40.54, 41.1 and 24, 61.64, 88.2 and 15 and 459.455, 93.1 and 23 mg/kg, respectively

Similarly the mean concentrations (mg/kg dry weight) of studied HMs like Cu, Pb, Cd, Ni, Zn, Co, Cr, Hg, Mn and Fe in the vegetables grown in wastewater irrigated soil in winter, summer season and preference soil were 26.8, 24.69 and 13 63.7, 44.7 and 11.45, 8.3, 13.98 and 3, 38.31, 34.and 32.4, 64.1.128.1, and 33, 25, 21.77 and 11, 20.7, 57.45 and 31.68, 421.76, 497.55 and 100.01, 41.1, 32.26 and 17 and 576.85, 632.3 and 300 respectively.

The concentrations of studied HMs were significantly higher in both soil and vegetables samples collected in summer season than their concentrations in winter's samples. The concentrations of Pb, Cd, Ni and Cr exceeded their respective permissible limits set by WHO/FAO. Transfer factor (TF) values of selected HMs from soil to vegetables and the target hazard quotients (THQs) to the possible health risks to local population through the food chain were also calculated. The mean values of root transfer factor (RCFs) ranged from 0.3-1.4, 0.2-1.4, 0.4-3.7, 0.4-1.1, 0.3-0.6, 0.004-0.18, 1-1.8, 0.12-1.5 and 0.4-1.08 for Pb, Cu, Cd, Ni, Zn, Mn, Co, Cr and Hg, respectively in vegetables of study area.. Similarly, values of shoot transfer (SCFs) for Pb, Cu, Cd, Ni, Zn, Mn, Co, Cr and Hg ranged from 0.2-1.4, 0.2-4, 0.3-4.7, 0.2-0.8, 0.47-2.46, 0.02-0.45, 0-1.2, 0.34-2.2 and 0.5-2.01, respectively in the samples of vegetables. Values of fruit transfer (FCFs) were 0.3-1.1, 0.2-2.4, 0.8-5.7, 0.2-0.6, 0.31-0.69, 0.002-0.14, 0.4-1.6, 0.3-1.4 and 0.6-0.8 for Pb,

Cu, Cd, Ni, Zn, Mn, Co, Cr and Hg, respectively. The dietary intake of metals (DIM) values of Pb, Cd, Ni, Zn, Mn, Cr and Cu by children were ranged from 0.001-0.019, 0.001-0.005, 0.01-0.02, 0.013-0.038, 0.01-0.02, 0.003-0.018, 0.001-0.011, respectively. Similarly the values of DIM for Pb, Cd, Ni, Zn, Mn, Cr and Cu by adults via consumption of vegetables were 0.01-0.023, 0.002-0.006, 0.001-0.03, 0.014-0.042, 0-0.13, 0.002-0.032 and 0.001-0.013, respectively. The DIM values of Pb and Cd were significantly ($p<0.05$) higher as compared to respective reference dose (RfD). However, the DIM values of Zn, Cu and Mn were significantly ($p<0.05$) lower than their respective RfD. The THQ of Pb, Cd, Ni, Zn, Mn, Cr and Cu ranged from 0.668-5.055, 1.362-5.609, 0.36-1.23, 0.044-0.126, 0.08-3.46, 0.002-0.019 and 0.025-0.281, respectively for children. Similarly, the THQ ranged from 0.744-5.586, 1.517-6.248, 0.049-0.119, 0.12-3.86, 0.002-0.021 and 0.028-0.313 due to Pb, Cd, Ni, Zn, Mn, Cr and Cu, respectively for adults. Greenhouse pot experiments using spinach (*Spinacia oleracea*) coriander (*Coriandrum saivum***)**, purslane (*Portulaca oleracea**)*** as representative vegetables were conducted to assess the up take pattern of three different doses of Cd, Pb, Zn, Cd/Pb, Cd/Zn and Pb/Zn The highest dose of Cd, Pb and Zn in single form and in mixture (Cd/Pb, Cd/Zn and Pb/Zn) significantly reduced the growth *S. oleracea,* C. *saivum* and *P. oleracea* seedlings. Similarly, in case of full grown plants of *S. oleracea* and *C. saivum* the highest doses of Cd, Pb, Zn, Cd/Pb, Cd/Zn and Pb/Zn reduced the growth In case of all *S. oleracea*, *C. saivum* and *P. oleracea* seedlings the concentrations of Cd, Pb and Zn were positively correlated with their concentrations in soil. The mixture of HMs such as Cd/Pb, Cd/Zn and Pb/Zn the metals behaved antagonistically, which were reflected in the growth parameters of selected plants. In case of full grown plants of *S. oleracea* and *C. saivum* the Cd, Pb and Zn concentrations in soil and roots of plants were positively correlated. Similarly, the concentrations of Cd, Pb and Zn in the root and shoot of full grown plants of *S. oleracea* and *C. saivum* were positively correlated. Total protein content, fiber and moisture contents of S. *oleracea, C. saivum* significantly reduced with increasing concentrations of Cd, Pb and Zn alone and also their mixture (Cd/Pb, Cd/Zn and Pb/Zn) in plant tissue. The impacts of mixture of Cd, Pb and Zn on total protein, fiber and moisture content were more sever than the impacts of individual Cd, Pb and Zn but less than the sum of impacts of individual HMs. Similarly, the increasing concentrations of selected HMs reduced the up take of Na, K, Ca, Fe, Mg, Mn and Cu by *S. oleracea* and *C. saivum.*

It was concluded from this study that soils of Peshawar are rich with HMs because of wastewater irrigation. The plants grown in these soils contaminated with HMs. The HMs adversely impacted the growth parameters of plants, change the up take of important metals by plants and reduced moisture contents, total protein and fiber. The consumption of vegetables in the wastewater irrigated areas in a Peshawar causes health risks to the residents.

Chapter:-1 Introduction

Rapid urbanization and industrialization along with improper environmental planning are not only responsible for shortage of irrigation water but also for the discharge of wastewater. Water bodies are continuously and gradually polluted by natural and manmade activities. Organic matter of plants and animals, industrial and sewage effluent, surface washing, agrochemicals and treatment work's wastes are responsible for polluting water bodies (Lokeshwari et al., 2006; Forslund et al., 2010). To cope with the problem of water shortage in irrigation sector, wastewater has been used as a non-conventional resource from the past few decades (Chary et al., 2007). Long-term wastewater irrigation is not only causing accumulation of HMs in soil but also contaminating food crops with HMs (Boularbah et al., 2006). The sources of HMs are industrial effluent, agriculture waste, and domestic sewage (Bing et al., 2011). According to Khan et al, (2008a) in metropolitan areas of Beijing, China, both the industrial and domestic sewage are treated biologically and then used for irrigation purpose. Even after biological treatment, the wastewater contained beneficial nutrients, organic and inorganic pollutants (Chen et al., 2005).

Surface soil receives HMs such as Cd, Zn, Cr, Ni, Pb and Mn from wastewater irrigation. Due to continues wastewater irrigation, the capacity of soil to hold the HMs decreases and finally the soil releases the HMs to groundwater and/or the soil solution available for plant uptake. Excessive accumulation of HMs in agricultural soils through wastewater irrigation may not only result in soil contamination (Zhao et al., 2010), but also leads to elevated HMs uptake by crops and thus affect food quality and safety (Muchuweti et al., 2006). Vegetables cultivated in wastewater-irrigated soils are responsible to take up HMs in large enough quantities to cause human health risks. Under specific condition, the HMs accumulate in the soil to the levels, which are toxic and can lead to arise ecological consequences (Wang et al., 2005). Wastewater irrigation is responsible for food chain contamination with heavy metals (Chung et al., 2011), which is one of the important pathways of entering the toxic HMs into human body. Even the low concentration of HMs in the children's blood can affect mental development.

Climate, atmospheric deposition, the nature of the soil where plants grow, degree of maturity of the plant at the time of harvesting and irrigation with wastewater are the factors which effect contamination of plants with HMs (Muchuweti et al., 2006).

The fresh water available for irrigation in Pakistan is not enough for the needs of irrigation sector. However, the farmers use wastewater as an alternative for irrigation purpose (Sadiq et al., 2005). There is no proper management and disposal of effluent in the whole country including its capital (Islamabad) that lack proper management and disposal of industrial effluents of its two industrial estates (Abbas et al., 2007). Rapid increase in population and lack of proper management and disposal of effluents of tanneries and textile industries are responsible for contamination of soil in Pakistan (Khan, 2001). Unplanned urbanization and transportation are other two factors responsible for the number and amount of toxicants in the environment of Pakistan (Younas et al., 1998). Soil contaminated with HMs is now becoming an environmental and health issue in Pakistan. Previously many studies have shown the presence of HMs even in the blood and other human body parts of Pakistanis. The mean Pb level in the blood of the school children of Peshawar was 0.212 mg/L (Zahoorullah et al., 1994).

Peshawar's environment has suffered tremendously due to various factors including overpopulation, Afghan influx and unplanned growth of the city, poor regulatory framework, transportation and industrialization. Some of the important industries in Peshawar are leather, textile, marble, match, steel, pharmaceutical, paper, sugar, glazed pottery, embroidery carpets and wood works. Leather and textile industries are present in neighborhood of the city. These industries are the main sources of contamination of the environment with HMs (Sadia et al., 2006).

Hayatabad industrial estate contributes to the contamination of environment because of its untreated disposal the industrial effluents. The effluent released by Hayatabad industrial estates continuously enters the water bodies of Peshawar City and accumulate HMs in the soil which then make their way to human bodies through contaminated food chain.

Recently, a large number of articles have been published on wastewater irrigated soil contaminated with HMs (Mapand et al., 2005, Khan et al., 2008ab). However, it is necessary for a better understanding to study HMs accumulation in the soil ecosystem, the adverse impacts of HMs on soil and plant quality, and human health in detailed. A well published data is not available for Peshawar City related to food chain contamination with heavy metals present in irrigation water. The accumulation of heavy metals in wastewater irrigated soils and their entering to food chain and related human health risks need more extensive research. The present study was conducted to achieve the following objectives.

1.2 Objectives

- To assess the extent of HMs contamination in wastewater-irrigated soil and grown crops.
- To determine the variation of concentration of different HMs in the soil of different parts of Peshawar city.
- To find out relationship between the concentrations of HMs in soil and plants.
- To estimate the health risks of HMs through consumption of vegetables.
- To investigate the uptake of HMs in crop plants.
- To find out the availability of metals to plants and their accumulation in the food chain.
- To quantify the toxicicological impact of HMs on the vegetation irrigated with wastewater.

Chapter:-2 Background study

1. Introduction

Heavy metals (HMs) are released to the environment from both natural and anthropogenic sources. Major anthropogenic sources like industries, agriculture, mining, transport and domestic sewage discharge HMs to the environment and lead to geoaccumulation, bioaccumulation and biomagnifications (Sharma et al., 2006; Gazso, 2001). Rapid urbanization and industrialization along with improper environmental planning and management are responsible for the discharge of wastewater and shortage of irrigation water. Agriculture sector needs more water than any other sector and nearly the whole world is facing fresh water shortage. In order to cope with the problem of water shortage in irrigation sector, wastewater has been used as a non-conventional resource from the past few decades (Chary et al., 2007). In suburban areas, the use of industrial or municipal wastewater for irrigation purposes is a common practice in many parts of the world (Singh et al., 2004). Farmers as well as municipalities always appreciate the use of wastewater for irrigation purpose because it is an alternative of fresh water for farmers and a way of disposal for municipalities. Water bodies are continuously and gradually polluted with HMs (Lokeshwari et al., 2006).

Long-term wastewater irrigation is not only causing accumulation of HMs in soil but also contaminating food crops with HMs (Boularbah et al., 2006; Mapanda et al., 2005). According to Khan et al. (2008a) metropolitan areas of Beijing, China, both the industrial and domestic sewage are treated biologically and then used for irrigation purpose. Even after biological treatment, the wastewater contains beneficial nutrients, organic and inorganic pollutants like HMs (Chen et al., 2005). Irrigation with wastewater from industries and urban areas is an important source of HMs for the agriculture soils (Cui et al., 2005). HMs reach soil through irrigation, get adsorbed and retain in the form of organic and inorganic soil alloids (Bride, 1986). Plants uptake the HMs from soil out of which some are good for their bioligical functions like metabolic process and enzymatic reactions (Adrian, 1986). HMs are components of enzymes, helping in protein synthesis and iron balance of the cell (Kosolapov et al., 2004). Both the deficiency and excess of HMs can cause serious disorders in ecological system. Excessive uptake of

HMs by plants is neither good for plants nor for human beings who are the plants consumers. They cause phytotoxicity in plants and health problems in human (Haq et al., 2003).

As mentioned earlier, soil receives HMs such as cadmium (Cd), zinc (Zn), chromium (Cr), nickel (Ni), lead (Pb) and manganese (Mn) from wastewater irrigation. Due to continued and long term use of wastewater for irrigation the capacity of soil to hold the HMs decreases and finally the soil releases the HMs to groundwater and/or the soil solution available for plant uptake. Excessive accumulation of HMs in agricultural soil through wastewater irrigation may not only result in soil contamination, but also leads to elevated HMs uptake by crops and thus affect food quality and safety (Muchuweti et al., 2006). Furthermore, vegetables cultivated in wastewater-irrigated soil take up HMs in large quantities are causing human health problems (Khan et al., 2008b). Under specific condition the HMs accumulate in the soil to the levels, which are toxic and can lead to ecological consequences (Wang et al., 2005).

HMs enter human body through different ways but the food chain contamination is one of the important pathways which contributes 90% of HMs as compare to other routes like inhalation and dermal contact (Loutfy et al., 2006). Figure:-1 shows the route of transmission of HMs to human body.

The lifetime exposure to HMs is a pressing environmental health risk which was not properly investigated in the past (Suk et al., 2002). The accumulation of HMs in the human bodies can cause serious health problems. Chronic exposure to certain HMs reduces lung function (Rastogi et al., 1991; Tager et al., 2005), is responsible for asthma, emphysema and lung cancer (Kuo et al., 2006). HMs bind to proteins and inhibit enzymes like enzymes of mitochondria (Rossi et al., 1993; Goering, 1993). Those metals in the human body interfere with many voltage and ligand-gated ionic channels which cause many neurotoxic effects (Garza et al., 2006). Metals specially Mg, Ni and As can cause tachycardia, increased blood pressure and anaemia which is due to inhibitory effect on haematopoiesis (Huang and Ghio, 2006). In the past, numerous research works were conducted by different researchers to highlight the wastewater irrigation and its adverse impact on environment and human health (Khan et al., 2008ab; Chary et al., 2007). However, the main objective of this study was to summarize the research work about the wastewater irrigation, food crop contamination with HMs and health problems.

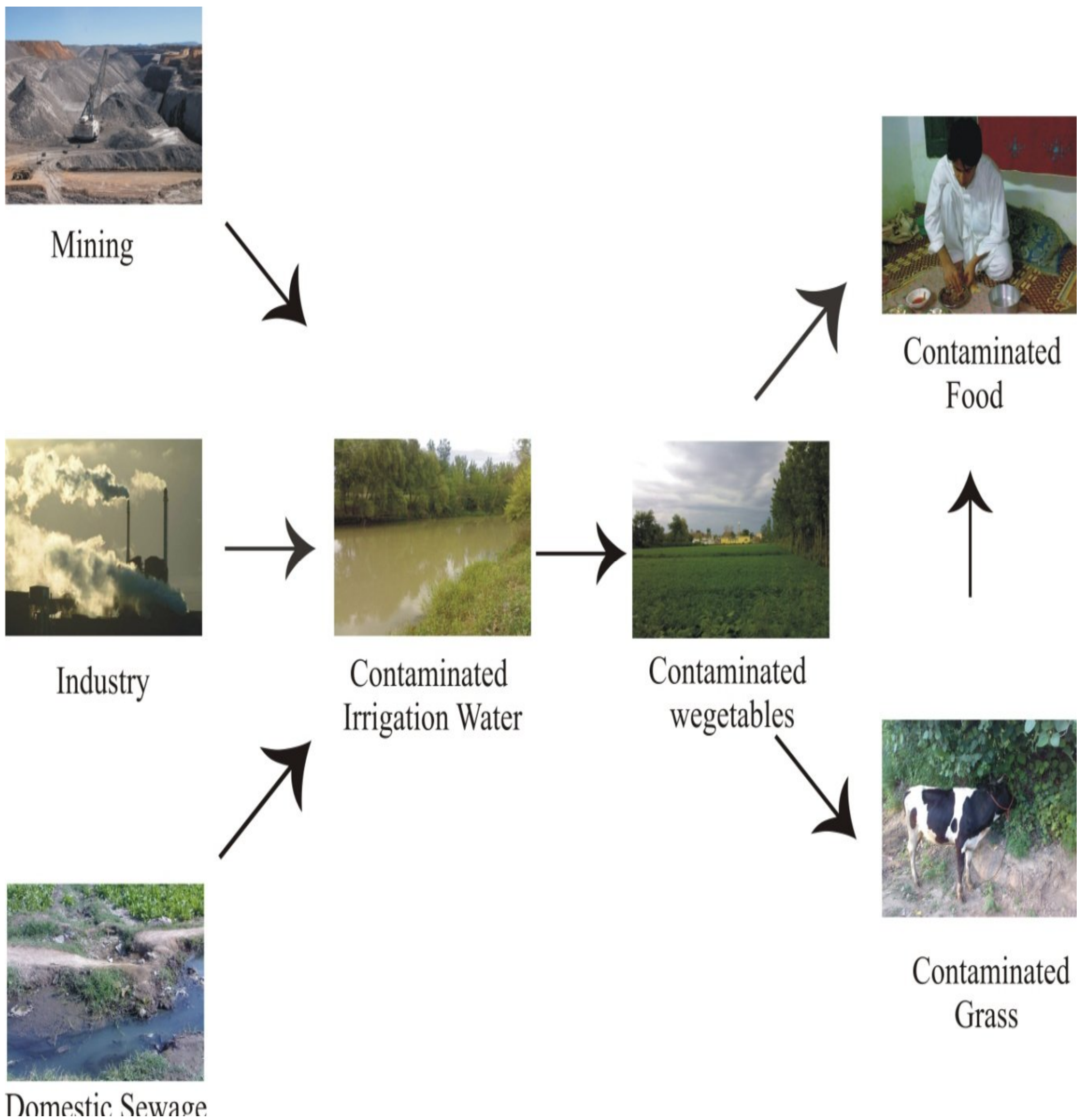

Fig 1. Food chain contaminated with HMs through wastewater irrigation

2. Free availability of fertilizers

Commercial fertilizers are expensive while majority of the farmers in developing countries are poor and can not afford the cost of these fertilizers. Industrial and domestic sewage irrigation recovers the deficiency of some of the nutrients (Parveen et al., 2006). The use of sewage for irrigation is inexpensive, easy and logical practice (Dursun et al., 2005), increases crop yields and reduces cost of production because crops irrigated with wastewater need less inorganic nutrients in the form of fertilizers (Ahmad et al., 2006). Sewage can be a good replacement of commercial fertilizers for crop production because it contains plants nutrients and organic matter (Dursun et al., 2005). Wastewater irrigation provides nutrients to plants in the required amount or even more than the required amount.

Wastewater irrigation is considered a good source of organic matter and plant nutrients but among all the necessary things for plants the most important are nitrogen (N) and phosphorus (P) (Shah and Raizullah, 2003). The availability of N increases cations capacity of soil, which as a result increase the capacity of soil to retain nutrients (Epstien et al., 1976). Furthermore, sewage is also a good source of micronutrients such as Fe, Zn, Mn, Cu (Chen and Stevenson, 1986). The nutrients are inorganic raw materials necessary for the growth and development of plants and required in certain quantities (Parveen et al., 2006). The nutrients increase plant productivity (Bansal, 1992). Some nutrients like N are important in carbohydrates utilization, P is important in energy transformation and K in enzymes activation, protein synthesis and osmotic regulation (Samuel, 1985). Mohammad and Athamneh, (2004) reported that the uptake of N is higher in wastewater irrigated plants than the plants that get nutrients from commercial fertilizers like diammonium phosphate (DAP) Plants uptake 79.2 mg/plant of N in case of fertilizer treatment while 577.8 mg/plant of N in case of application of 80 t/ha of wastewater.

Wastewater irrigation increases plants biomass and productivity due to improved soil fertility, increase soil aeration, permeability, water retention, water infiltration, better aggregation and fewer surfaces crusting. A positive correlation exists among micronutrients content and soil physio-chemical properties (Singh and Mongina 1993; Goswami and Darmaker, 2002). In the developing world countries like Pakistan and some developed countries are also using a mixture of municipal and industrial wastewater for crop production (Nazif et al., 2006).

3. Positive impacts of wastewater irrigation on plants

The use of wastewater for irrigation is a common practice nowadays. These practices are responsible for the improvement of physical, chemical and biological properties (Parkpian et al., 2003) of soil and finally improve the fertility index of soil and ultimately increase the production per unit area. As mentioned earlier, the wastewater contains both macro and micro nutrients so its application significantly increase the germination rate of seeds and, increase the growth of seedling and improve the yield of crops. According to Qasim et al, (2000) 75% and 100% sewage mixture is good for maize plants. The application of the wastewater increases N uptake by plants. The application of 160 t/ha of wastewater increased the N uptake by lettuce plants from 1.93% (control treatment) to 3.7% and K showed increase uptake only in treatment with 20 t/ha (Mohammad and Athamneh., 2004). Similarly the application of wastewater has increased the value of the fresh and dry weight of lettuce. Burun et al, (2006) reported that with increasing rate of wastewater application to soil (3-25 t/d) the barley grain yield and nutrient contents of barley were increased. Dursun et al, (2005) in a experiment on seedling of pepper observed the maximum values of plant growth parameters like germination rate, germination period, hypocotyls length, cotyledon width, shoot and root length, shoot and root fresh weight, shoot and root dry weight, stem diameter and leaf per seedling at treatment with 75% of wastewater. Similarly number of productive tiller, grain/ spike, weight of 1000 grain, grain yield, straw yield, plant height, spike length, leaf area (cm^2) of wheat plants with the treatment of 40 t/ha and 80 t/ha of wastewater showed maximum values (Jamil et al.,2006).

The applications of wastewater has significantly increased growth parameters of bean seedlings like root and shoot length, root and shoot fresh weight, root and shoot dry weight (Zeid and Abou El Ghate 2007). Spinach growers get maximum yield with the application of wastewater having a large amount of organic matter (Ahmad et al., 2006). The application of wastewater enhances leafy vegetables yield in short run and according to Ahmad et al, (2006) 1% increase in the use of wastewater is responsible for 23% increase in spinach yield. Wastewater containing HMs, which are essential for the activities of living things but as the concentration increases up to a certain limit create toxicity in plants (Gulfaraz et al., 2003). The metals like Fe, Mn and Co are essential metals and are required for many enzymes for the normal functions of body (Gulfaraz et al., 2003).

4. Sources of HMs in irrigation water.

4.1. Mining

Mining and processing are the important factors for environmental contamination with HMs (Navarro et al., 2008; Gabler and Schneider 2000; Marszalek and Wasik 2000)**,** and has resulted in disturbances in aquatic and terrestrial ecosystem (Malinovsky, et al., 2002). Physical disturbance of surrounding landscape, effluent from acid mine transported into near by rivers, emitted dust and spilled mine tailing are the factors for the contamination of environment from the mine area (Zhuang et al., 2009). Mining activity is considered as one of the factor of contamination of water with HMs (Prasad and Bose, 2001) and surrounding agriculture soil accumulates excessive amount of HMs (Pruvot et al., 2006)**.** The adverse impact of mining activities depends on many factors like mine type and size of the operation. Mining operation first changes the topography of land and then become a factor for the change of hydrogeologic conditions (Bell et al., 2000). Generally the impacts of the mining on soil, plants and on human decreases with increasing distance from the mine (Zhuang et al., 2009).

HMs pollution of mine area causes health problems in the local inhabitants (Kachenko and Singh, 2006) such as high blood Pb in the children (Pruvot et al., 2006; Bosso and Enzweiler, 2008). Certain regions or villages around Dabaoshan in China mine have been termed endemic cancer regions because esophageal cancers, liver cancer, and other cancers were reported frequently in humans and poultry, with a mortality rate approaching to 56% in humans because of high contamination of soil, sediments and plants with HMs in the surrounding areas (Zhuang et al., 2009). The Aras river in Iran which is the largest river of the country is responsible for the supply of water to tens of cities and villages, irrigation to a large piece of land, fish husbandry and to industrial areas. The concentrations of HMs like Cd, Cr, Cu, Fe, Mn, Mo, Pb and Zn were (not detectable for Cd), (0.39- 0.41 mg/l), (0.51- 1.03 mg/l, (0.62- 1.5 mg/l), (0.38- 0.63 mg/l), (0.22- 1.21mg/l) (ND- 0.01mg/l), (1.2- 1.5mg/l) respectively, in the surface water in the vicinity of Sungun River (Bidhendi et al., 2007).

4.2. Domestic sewage

Table:1 shows the concentration of HMs in the domestic sewage in many countries of the world. Growing urbanization along with other environmental problems created huge urban runoff in the last decades. The discharge of sewage and runoff to the water bodies and soil is a major threat for environment worldwide (Shinya and Tsuruho, 2003). Urban runoff is a source of pollution to river and coastal oceans and consists of pollutants which appear as risk not only for plants and animals but also for humans (Nabizadeh et al., 2005). The threat of this risk is increasing with increasing sources of pollution and accumulation of pollutants over a longer period of time (Molly, 2002). Chemicals from homes and offices, automobiles, industrial discharges, erosion from construction sites are the main sources of contamination of the existing drainage. Repeated application of this water for irrigation can build up the concentration of HMs to a level which is toxic for plants and humans (Kakar et al., 2006).

Table-2.1 Concentrations of HMs (mg/l) in domestic sewage used for irrigation

Area	Zn	As	Cd	Pb	Cu	Cr	Ni	Fe	Mn	Co	References
Tehran city, Iran	0.638	ND*	0.04	0.097	0.035	ND	ND	ND	ND	ND	Nabizadeh et al. (2005)
Quetta, Pakistan	0.08-0.21	ND	ND	0.05-0.12	0.03-0.07		0.09-0.18	0.55-1	0.14- 0.23		Kakar et al. (2006)
Rawalpindi, Pakistan	0.5-0.83	ND	0.021-0.031	0.24-0.84	0.37-0.62	0.39-0.45	ND	ND	ND	ND	Chattah et al. (2005)
Abotabad, Pakistan	0.43-0.62	ND	0.012-0.034	0.25-0.36	0.21-0.49	0.38-0.44	ND	ND	ND	ND	Chattah et al. (2005)
Attok Pakistan	0.46-2	ND	0.024-0.46	0.59-0.73	0.62-0.84	0.27-0.41	ND	ND	ND	ND	Chattah et al. (2005)
Pandu, Peshawar, Pakistan	0.06	ND	0.26	0.75	0.99		0.11	ND	ND	ND	Perveen et al. (2006)
Well water Zob- Ahan steel industrial complex, Iran	0.08-0.21	ND	ND	0.06-0.13	0.025-0.125	0.125-0.25	0.055-0.175	0.07-19.8	ND	0.14-0.20	Rahmani and Rezaei (2007)
Surface water Zob- Ahan steel industrial complex, Iran	2	ND	0.1	1	1	0.5	2	3	ND	1	Rahmani and Rezaei (2007)
Alaro River Ibadan, Nigeria	ND	ND		0.14	0.0923	0.021	0.03	ND	0.456	ND	Fakayode (2005)
Drainage ditches Florida, USA	121	18.67	70.50	5.53	63.7	2.05	3.05			1.1	Minglui et al. (2004)
Wastewater Jaipur City, India	0.68 - 60.84	ND			0.05-1.0		0.01-0.07	0.1-0.4	0.1-0.4	ND	Singh and Chandel (2006)
Karnaphuli River estuary, Bangladesh	1.112	ND	0.09	0.675	0.891	0.512	0.891	28.1	0.983	ND	Das et al. (2002)
Luxor, upper Egypt	0.03	ND	ND	ND	traces	ND	ND	0.036	0.012	ND	Hussein et al. (2004)
Gangetic plain, India	785	ND	154.5	ND	317.7	35.3	47.17	ND	186	ND	Singh and Agrawal (2007)
Westren Delhi, India	0.061	ND	0.00153	0.033	0.029	ND	0.049	1.464	0.064	ND	Rattan et al. (2005)

ND* not detected

4.3. Industries

Table: 2 show the concentration of HMs in the effluent of many industries**.** Industrial revolution, which is a source of providing basic facilities to human on one hand but generating many problems for man and its environment on the other hand. The effluent of industries consists of HMs the discharge of which to water bodies not only contaminated water bodies but also the soil. HMs contamination not only degrades the quality of our natural resources but also adversely impacts agriculture production. The industrial sector discharges effluent to water bodies like rivers, canals and drainage system (Haq et al., 2005). The municipality considers these local water bodies as a natural and easily available sink for untreated wastewater from industries The discharge of untreated industrial effluent in the surface drains contaminate surface water bodies and then contaminate the soil and plants (Haq et al., 2005). The process of contamination of ground water and soil with HMs by industries has increased nowadays Industrial wastewater is also a threat towards the groundwater quality

Water pollution is a problem for the environment but especially for the people who live in developing countries (Rehman et al., 2008). Rattan et al. (2005) reported that the HMs like Zn, Fe, Ni, Mn, and Cu in the sewage effluent of Keshipur effluent irrigation scheme had much higher concentration as compared to local groundwater. Similarly Rehman et al. (2008) conducted a study to analyze the concentration of HMs in the drained water of three main industrial estates of Pakistan (Peshawar, Gujranwala and Hattar Haripur). The wastewater of these industries was highly contaminated with HMs, especially Pb and As. These effluents have been used for irrigation since last fifty years.

Table-2.2 Concentrations of HMs (mg/l) in the industrial effluents

Area	Zn	As	Cd	Pb	Cu	Cr	Ni	Fe	Mn	Co	References
Korangi area Karachi	0.005-5.5	ND	0.004-2.4	0.05-2.25	0.005-1.91	0.004-5.62	0.02-5.35	(0.04-5.58)	0.01-1.97	ND	Saif et al. (2005)
Marble industries	0.06	ND	0.056	3.20	ND	0.46	0.03	0.03	ND	ND	Khan et al. (2002)
Match industries	0.06	ND	0.056	3.20	ND	0.46	0.03	0.03	ND	ND	Khan et al. (2002)
Steel industries	0.05	ND	0.07	1.49	ND	0.03	2.02	0.02	ND	ND	Khan et al. (2002)
Pharmaceutical industries	0.01	ND	0.03	0.7	ND	0.03	2.05	0.08	ND	ND	Khan et al. (2002)
Textile mill(Islamabad, Pakistan)	4.69	0.8	1.7	0.76	4.4	1.15	3.5	5.46	1.94	2.57	Gulfaraz et al. (2003)
Textile mill (Rawalpindi , Pakistan)	5.1	0.6	2.7	1.56	7.1	1.95	2.6	5.06	1.24	2.17	Gulfaraz et al. (2003)
Refinery (Islamabad, Pakistan)	1.57	0.19	409	5.13	3.77	1.15	2.2	3.7	0.53	0.15	Gulfaraz et al. (2003)
Refinery (Rawalpindi , Pakistan)	2.51	0.69	3.4	6.11	2.79	0.159	3.201	2.12	1.11	0.19	Gulfaraz et al. (2003)
Hydrogenated oil (Islamabad, Pakistan)	.19	ND	0.157	0.011	1.09	0.12	6.2	3.64	0.156	0.21	Gulfaraz et al. (2003)
Hydrogenated oil (Rawalpindi , Pakistan)	0.496	ND	2.15	0.18	2.18	0.1	5	4.6	0.17	0.11	Gulfaraz et al. (2003)
Biomass power plant	6.73	ND	0.028	0.108	ND	0.14	0.041	0.05	0.36	0.311	Nagajyoti et al. (2008)

5. Food chain contamination with HMs.

5.1. Soil contamination with HMs

Table: 3 summarize the HMs concentration in the soil irrigated with wastewater. Wastewater contains not only HMs but also organic matter necessary for plants and soil ecosystem. Soil organic matter consists of two fractions i.e the humus fraction (humic acid, fulvic acid, humin) and the particulate fraction (Maldonado et al., 2008). Moreover, the application of wastewater significantly increases the HMs in soil (Samaras and Kallianou., 2000). Soil organic matter increases with long term wastewater irrigation because of the carbon present in wastewater (Jamil et al., 2006). The decomposition of organic matter in the soil is also responsible for the release of N content in plant available form after several years of wastewater application (Hemandez et al., 2002) and also increases the amount of soil P, Mohammad and Athamneh, (2004) observed an increase of 7.66 mg/kg to 77.13mg/kg of N with the addition of 160 t/ha of wastewater. Soil pH is responsible for the availability of HMs, involved in the control of soil reaction with HMs and precipitation and dissolution of mineral in the soil (Chaudri et al., 2001). The application of wastewater to irrigated soil decreases soil pH (Jamil et al., 2006; Mohammad and Athamneh, 2004; Chang et al., 1990; Karthikeyan et al., 2005; McLaren et al, 2004; Maldonado et al., 2008). The decrease in soil pH, which is actually the generation of acidity, was due to the mineralization of wastewater (Welch and Lund., 1989). The application of wastewater is also responsible for the decrease of electric conductivity (EC) of soil. The application of 160 t/ha of wastewater decrease soil EC from 0.508 ds/m to 0.49ds/m (Mohammad and Athamneh., 2004). High salinity can affect the mobility of HMs (Maldonado et al., 2008).

The mobility and bioavailability of HMs in the soil depends upon the physical and chemical properties of soil. The process like adsorption, precipitation reactions are the factors controlling the mobility of HMs in the soil (Li et al., 2003). Similarly El-Demerdashe et al. (1995) reported that long term addition of wastewater to soil accumulated HMs to a level which is toxic to some plants. Similar findings were observed in study conducted by Jarausch et al, (2000). The wastewater application to soil increase the level of HMs in the soil which is positively correlated with soil organic matter and negatively correlated with soil pH **(**Jarausch et al., 2000)**.** HMs soon after the entrance to the soil get adsorbed by the solid fraction (Surita et al., 2007). The presence, potential toxicity and mobility of HMs in the soil depends upon the saturation of specific sites of adsorbent, the crystalinity of absorbent, the morphology of absorbent and soil pH (Puls et al., 1992) The presence of HMs in soil is not only responsible for the toxicity of plants and animals but also for stopping the essential biochemical processes which further change the balance of ecosystem. Accumulation of HMs in the soil decrease N fixation process and decomposition of organic matter which is further responsible for the change of natural nutrient cycles (Baath, 1989). The presence of microorganisms is very important for many process like assimilation, nitrogen fixation and degradation of organic matter for the use of plants (Brookes, 1995), which can be adversely affected by HMs in soil **(**Stuczynskiel et al., 2003; Nwuche and Ugodi, 2008).

Table- 2.3 Concentrations of HMs (mg/kg) in soil irrigated with different wastewater

Area	Zn	As	Cd	Pb	Hg	Cu	Cr	Ni	Fe	Mn	Co	References
D. I. Khan , Pakistan	16	ND	ND	40	ND	15	ND		15.5	21		Jamil et al. (2006)
Chihuahua, Mexico	ND	ND	4.48	155.83	ND	51.36	ND	10.74	ND	ND	ND	Maldonado et al. (2008)
Dalsung copper-tungsten mine, Southeast Korea.	419	ND	4.4	1,028	ND	1,953	ND	ND	ND	ND	ND	Jung (2008)
Imo State, southeast Nigeria (Dry season)	ND	ND	6.2	71.9	0.02	ND	4.7	6.5	ND	ND	ND	Onweremadu et al. (2007)
Imo State, southeast Nigeria (Rainy season)	ND	ND	2.9	51.9	0/01	ND	2.2	1.9	ND	ND	ND	Onweremadu et al. (2007)
Central Jordan	146.94	ND	4.98	62.17	1.81	ND	83.93	ND	ND	ND	ND	Banat et al. (2005)
Guiyang area of Chenzhou, Hunan Province, China	508.6	44.6	7.53	348.3	ND	356	ND	ND	ND	ND	ND	Guo-li et al. (2008)
Turkey	12.1	ND	5.9	80	ND	20	ND	22	ND	171	48	Turkdogan et al. (2002)
Yellow River, China	224.86	446.64	7.43	223.22	ND	132.82	47.07	ND	ND	ND	ND	Yu et al. (2006)
Yangzhong, China	98.1	10.2	0.3	35.7	0.2	33.9	77.2	38.5	ND	ND	ND	Huang et al. (2007)
Northwest Spain	85.4		0.15	24.8	0.45	15.7	24.1	8.4	ND	ND	ND	Lopez-Mosquera et al. (2000)
Southern Italy	150	ND	ND	100	ND	120	150	120	ND	ND	20	Adamo et al. (2002)
Coimbatore, Tamil Nadu, India	397	ND	0.07	175.5	ND	157	114	171.4	ND	ND	ND	Malarkodi et al. (2007)
Belgrade, Serbia	268.4	ND	8.90	350	ND	122.29	70.23	123.7	ND	641	25.59	Grzetic et al. (2008)
Nilohi, Delhi, India	7.31	ND	0.20	1.19	ND	4.91	ND	1.19	20.1	3.29	ND	Rattan et al. (2005)
Mundka, Delhi, India	3.68	ND	0.11	2.60	ND	4.39	ND	0.58	50.2	7.46	ND	Rattan et al. (2005)

5.2. HMs in plants

Table: 4 summarize the HMs concentration in the plants irrigated with wastewater.

The uptake of HMs by plants from the soil depends on the soil properties like temperature, amount of nutrients, organic matter and soil pH (Akan et al., 2009). The accumulation of HMs in plants body is positively correlated with the soil organic matter (Chen et al., 1990) and negatively correlated with soil alkalinity (Akan et al., 2009). The uptake of HMs by plants is also positively correlated with wastewater irrigation (Sharma et al., 2007). According to Akan et al. (2009) the uptake of HMs by plants was higher in dry season than rainy season because of wastewater irrigation. The accumulation of HMs in plants not only depends on their availability for plants but also depends on plant species as well (Punz and Seighardt, 1993). Vetches can accumulate HMs like Co, Cu and Fe many times than their level in the soil (Kumar et al., 2009). The order of accumulation of Zn and Cd in vegetables was Green pepper >spinach >dill>mint>celery>eggplant>cress, Radish leaves >Cress>Radish roots>Dill>Spinach>Eggplant respectively (Bigdeli and Seilsepour., 2008).

The concentrations of HMs within the same plant are different n different plant organs.

The concentration of HMs in different organs of plants are in the order leaf>stem>root>tuber>seed (Santamaria. et al, 1999). Vegetables accumulate high amount of HMs in roots and leaves (Ademoroti, 1996). Leaves contain higher content of HMs (Akan et al., 2009).

Kumar et al. (2009) found high mobility rate of HMs from soil to roots but little from roots to other plant organs. Rate of mobility of different HMs are different. Rates of mobility of Cd, Pb, Fe, Zn, Co, Ni and Cu are 1.61, 1.54, 1.21, 1.19, 1.17, 0.99 and 0.90 respectively(Kumar et al., (2009). The transfer potential of HMs among different plants systems i.e soil-root, root – stem and stem-leaves are different. The rate of transport of HMs in different plant systems are in order of stem-leaves >soil-root > root-stem (Kumar et al., (2009).

Table-2.4 Concentrations of HMs (mg/kg) in plants

Area	Zn	As	Cd	Pb	Cu	Cr	Ni	Fe	Mn	References
Wheat (Triticum indicum)	2.452	1.942	1.918	3.496	5.403	ND	5.403	ND	ND	Gulfaraz et al. (2003)
Barley(Hordium vulgare)	8.4	ND	ND	ND	7.500	ND	ND	7	1.3	Burun et al. (2006)
Effluent irrigated vegetables	78.8	ND	7.4	64	103.2	22.5	88	638.8	973.3	Haq et al. (2005)
Tube well irrigated vegetables	32.4	ND	1.8	12.4	19.50	4.2	14.8	172.5	111.8	Haq et al. (2005)
vegetables irrigated with dairy-industry sludge	25.3	ND	0.1	0.37	5.000	1.74	2.45	ND	ND	Lopez-Mosquera et al. (2000)
Wheat (Triticum indicum)	49.6	ND	ND	ND	51.6	ND	10.1	122	53	Rattan et al. (2005)
Spinach(spinecia oleracea)	77.1	ND	ND	ND	20.6	ND	18.4	711	39.3	Rattan et al. (2005)
Maize (Zea mays)	78.8	ND	ND	ND	14.9	ND	16.5	531	26	Rattan et al. (2005)
Wheat(Triticum indicum)	65.3	ND	ND	ND	9.39	ND	20	404	15.3	Rattan et al. (2005)
Cauliflower (Brassica oleracea)	46.7	ND	ND	ND	10.8	ND	14.4	328	31.8	Rattan et al. (2005)
Cucumber (Cucurbeta indica)	79.4	ND	ND	ND	19.3	ND	21.5	932	19.9	Rattan et al. (2005)
Parsley(Petroselinum crispum)	85.78	ND	11.54	7.44	ND	ND	ND	ND	ND	Eslami et al. (2007)
Leek (Allium cepa)	72.12	ND	8.34	10.21	ND	ND	ND	ND	ND	Eslami et al. (2007)
Radish (Raphanus sativs)	107.5	ND	7.64	19.15	ND	ND	ND	ND	ND	Eslami et al. (2007)
Wheat(Triticum indicum)	ND	ND	0.93	3.06	ND	3.1	8.9	ND	ND	Chatha et al. (2002)
Wheat (Triticum indicum)	65.3	ND	ND	ND	9.39	ND	20	404	15.3	Rattan et al. (2005)
Spinach(spinecia oleracea)	77.1	ND	ND	ND	20.6	ND	13.2	711	39.3	Rattan et al. (2005)
Maize (Zea mays)	78.8	ND	ND	ND	14.9	ND	18.3	531	26	Rattan et al. (2005)

5.3. Human health risks associated with HMs

Some HMs like Cu, Zn and Co are essential for metabolic activities of animals and plants at some concentration but others like Cd and Pb is toxic to plants, animals and humans even at low concentration. The excessive accumulation of HMs in soil and plants pose serious health risks to human (Granero and Domingo, 2002).

The product of average concentration of HMs in the food item and the consumption rate of that food item is the daily intake of the HMs by human (Santos et al., 2004; Yu et al., 2006). The average consumption rate of vegetable is 200 g/person.d in Huludan city (Rattan et al.,2005) The dietary intakes of Hg, Pb, Cd, Zn and Cu in Huludan city, China for adult are 2.135×10^{-3} mg/d, 8.15×10^{-2} mg/d, 4.19×10^{-2} mg/d, 12.26 mg/d, 2.73 mg/d respectively, while for children are 9.71×10^{-4} mg/d, 4.33×10^{-2} mg/d, 2.02×10^{-2} mg/d, 7.25 mg/d, and 1.437 mg/d. The daily intake of HMs through vegetables in Baiyin, China is 0.05-0.16 mg/d, 0.99-2.2 mg/d 3, 2.15-5.1 mg/d, 6.29-13.16 mg/d,0.10-0.22 mg/d for Cd, Pb, Cu, Zn and As respectively (Yu et al., 2006). The daily intake of for Pb, Cd, Cu and Zn through vegetables is 30 µg/ g, 4.67 µg/ g, 0.45 µg/ g and 1.58 µg/ g for Pb, Cd, Cu and Zn in Alexandria, Egypt (Mohamed et al., 2006). The consumption rate of vegetables in the area around the Dabaoshan mine, China is 274g/d and the intake of Cu, Zn, Pb and Cd through these vegetables is 327 µg/ g , 2357 µg/ g , 47 µg/ g and 59 µg/ g respectively(Zhuang et al., 2009). The daily consumption of rice and vegetables in Fujian Province, southeast China is 0.5kg and 0.5kg respectively, while the concentration of As in rice and vegetables is 0.20mg/kg and 0.17mg/kg respectively so the total intake of As through rice and vegetables is .10mg and 0.09mg respectively (Huang et al., 2006). The combined intake is 0.19 (Huang et al., 2006), which is higher than the permissible limit i.e 0.14mg for As (Tripathi et al., 1997)

The ratio of determined dose of pollutant to its reference dose is target hazard quotient (THQ) (Zhuang et al., 2009), if its value is less than 1, then will not produce apparent adverse impacts (Zhuang et al., 2009). Zheng et al., (2007) calculated target hazard quotients (THQs) for individual HM for individual vegetables specie with THQ = $C \times (E_F\ E_D\ F_{IR}) / (W_{AB}\ T_A\ RfD) \times 10^{-3}$ where E_F is the exposure frequency (365 days/year), E_D is the exposure duration (70years). F_{IR} is the food ingestion rate (g/person/day). C is the metal concentration in food (μg/g). WAB is the average body weight and TA is the averaging exposure time (365 days/year number of exposure years). The reference doses used for Pb, Cd, Zn and Cu as 4 μg/kg/d, 1 μg/kg/ d, 300 μg/kg/d, 40 μg/kg/d respectively. Rattan et al, (2005) assessed the risk to human health (Hazard Quotient, HQ) for the intake of HMs like Zn, Cu and Ni through vegetable with the formula HQgv = (add/RfD), where HQgv is hazard quotient to human from the consumption of green leafy vegetables, add is the average daily dose (mg metal/kg body weight/day) and RfD the reference dose. The reference doses used for Zn, Ni and Cu as 0.30 mg/kg bw/day, 0.02 mg/kg bw/day and 0.5 mg/kg bw/day respectively

Zhuang et al, (2009) calculated THQ with THQ = (EFr × ED × FI × Mc)/ (RfD× Bw × AT) $\times 10^{-3}$ where EFr is exposure frequency, ED is exposure duration, FI is food ingestion (g /person/d); MC is metal concentration in food (μg /g) RfDo is the oral reference dose (mg/ kg/ d); BW is the average body weight. AT is averaging time for noncarcinogens (365 days /year−1× number of exposure years, assuming 70 years in this study). The values for HQgv for Cu are 0.004 to 0.021, 0.008 to 0.015 and 0.004 to 0.014 for gobhi, spinach and Indian rape respectively in western Delhi, India. The values for HQgv for Zn are 0.040 to 0.068, 0.035 to 0.152 and 0.027 to 0.053 for gobhi, spinach and Indian rape respectively western Delhi, India. The values for HQgv for Ni are 0.027 to 0.442, 0.046 to 0.502 and 0.016 to 0.429 for gobhi, spinach and Indian rape respectively western Delhi, India. Total diet THQs of each metal (TDHQ) for Hg, Pb, Cd, Zn and Cu were 0.053, 0.364, 0.749, 0.731 and 1.220 respectively for adult in Huludan city, China, while for children was 0.042, 0.331, 0.618,0.739 and 1.099 (Zheng et al., 2007). Total THQ (TTHQ) of HMs for individual vegetables is the sum of the THQ of each metal Zheng et al., 2007). TTHQ of HMs (Hg, Pb, Cd, Zn and Cu) for vegetables in Huludao city, China is 0.963 and 0.531 for adult and children respectively (Zheng et al., 2007).

The presence of one pollutant can increase or decrease the adverse impacts of other pollutant (Harrison and Chirgawi., 1989). With the assumption that they only produce additive impacts THQs can be summed up to produce hazard index (HI) for the combination of different HMs. HI is 3.119 and 2.828 for the adult and children in Huludao city, China through diet (Zheng et al., 2007). The relative contribution of vegetable in HI were 22.2% and 18.7% for adult and children respectively Huludao city, China (Zheng et al., 2007).

Chapter:-3 Spatiotemporal variation in heavy metal concentrations in vegetables grown on wastewater irrigated soils and their estimated intake and health risk

1 Introduction

In the whole world especially in 3rd world's countries the unconventional growth of population has caused a serious food shortage, which combine with repeated droughts in recent years increase the consumption of fresh water resources in most of the countries in arid and semi-arid climate regions of the world and as a result it pressurized the available fresh water resources. Long-term wastewater irrigation is not only causing the accumulation of heavy metals (HMs) in soil ecosystem but also contaminating food crops (Boularbah et al., 2006). Wastewater irrigation is known for its contribution of HM contents into soil ecosystem (Mapanda et al., 2005). Under specific condition, the HMs accumulate in the soil to the levels, which are toxic and can lead to ecological consequences (Wang et al., 2005). HMs enter to human body through many pathways but the food chain contamination is one of the important routs which contributes 90% of HMs as compared to other routes like inhalation and dermal contact (Loutfy et al., 2006).
Heavy metals are toxic for plants and can reduce their yield, affect leaf and root growth and inhibit enzymatic activities (Zeng et al., 2008). The excessive accumulation of HMs in soil and vegetables pose serious health risk to human (Granero and Domingo, 2002). The presence of HMs in the soil ecosystem not only dangerous to plants and animals but also have adverse impacts on ecosystem itself. The lifetime exposure to the mixture of chemical like HMs is a pressing environmental health problem which was not properly investigated in the past (Suk et al., 2002). HMs increase the risk of stone formation nephrocalcinosis (Jarup, 2003) and renal cancer (Boffetta et al., 1993). Similarly, HMs increase the excretion of low molecular weight proteins which is a reason of tubular dysfunction, which further causes the reduction in the rate of glomerular filtration (Boffetta et al., 1993). Potential health risks of pollutants are mainly divided into carcinogenic and non-carcinogenic risks. Health risk can be estimated by many methods (Zheng et al., 2007) like target hazard quotient (THQ), a ratio of the estimated dose of a contaminant to the reference dose (RfD) (Zhuang et al., 2009 ; Zheng et al., 2007). However, Chien et al, (2002), Wang et al, (2005) and Zheng et al, (2007) have used THQ

for health risk assessment. For an expose population, if the dose of a HM is greater than its reference dose (RfD) then the value for THQ will be greater than 1, it means that the expose population will experience health risks (Zheng et al., 2007).THQ only provide an indication of risk level of certain population due to pollutants rather than providing quantitative estimation (Zheng et al., 2007; Wang et al., 2005). In the environment HMs are in mix form, so the study of single HM on health risks is not the true representative of the health risks of mixture of HMs. To assess the potential risks of mixture of HMs another approach can be used, which is called hazard index (HI) (Zheng et al., 2007).

On the average, 60% of wastewaters in United States are reused (Harury, 1997), 432 m cubic meters wastewater was treated in the state of California, 260 m cubic meters water in the state of California used for agriculture sector (Pescod 1992). In Japan (1996) more than 13 million cubic meters treated wastewater were used for irrigation activities (Taniyama and Adachi, 1999). Morocco, Jordan, Pakistan, Oman and Saudi Arabia are the countries are using wastewater for irrigation practices first among the countries of Middle East (Arabi et al., 1996).

Pakistan has semi-arid to arid climate and sever fresh water shortage. The farmers of Pakistan found wastewater as a source of irrigation and this practice increases its scope continuously almost in all cities of the country (Mukhtar et al., 2003). The use of urban wastewater in agriculture sector has become a widespread practice.

The present study was conducted to assess the soil pollution (HMs) load in Peshawar, Khyber Pakhtunkhwa in two seasons (summer and winter), investigate the HM uptake rate by vegetables and calculate THQs and HI of HMs for evaluating the non-carcinogenic health risks from individual metal and combined HMs due to daily consumption of vegetables from wastewater irrigated fields.

2 Materials and methods

2.1 Study area

Peshawar is the provincial capital of Khyber Pukhtoonkhwa (KPK), Pakistan. The city lies at the edge of the Khyber pass with coordinate 34^{o} N and 71.30^{0} E and 347 m above from the sea level. The total area of Peshawar City is 2257 km^2. The Peshawar valley is covered with consolidated deposits of silt, sands and gravel of recent geological times. The flood plains are the areas between Kabul River and Budni Nala. The meander flood plain extends from Warsak in the Northwest towards Southeast in the upper Northern half of the district.

Peshawar's environment has suffered tremendously due to various factors including overpopulation, Afghan influx and unplanned growth of the city, poor regulatory framework, transportation and industrialization. Some of the important industries in Peshawar are leather, textile, marble, match, steel, pharmaceutical, paper, sugar, glazed pottery, embroidery carpets and wood works. Leather and textile industries are present in neighborhood of the city. These industries are the main sources of contamination of the environment with HMs (Sadia et al., 2006).

2.2 Sampling

2.2.1 Soil Sampling

Two types of soil samples were collected i.e. wastewater irrigated and reference soil. Reference soil was collected from the site irrigated with clean water. In both cases, soil samples were randomly selected and taken from the upper horizon (0-25 cm) (Khan et al., 2010). Soil samples were dried, crushed and passed through 2 mm mash sieve. The samples were sealed in Kraft paper envelops and stored until analysis.

2.2.2 Vegetables Sampling

Vegetable samples, as given in Table 3.1 were collected from the same site where soil samples were collected. Plants samples were divided into two parts roots and shoots, while some cases into fruits as well. Plant samples were washed with deionized water to remove the visible soil and then dried at 80°C till constant weight achieved. The samples were separately crush through steel grinder and stored for further analyses.

Table 3. 1 List of field collected vegetables

English Name	Local Name	Botanical Name
Brinjal	Bengin	*Solanum melongena*
Okra	Bendi	*Abelmoschus esculentus*
Ridge gourd	Tori	*Luffa acutangula*
Spinach	Palak	*Spinacia oleracea*
Purslane	Kolfe ka sag	*Portulaca oleracea*
Green Amaranth	Chleri ka sag	*Amaranthus viridis*
Tomato	Tamater	*Solanum lycopetsicum*
Bitter melon	Karela	*Momordica charantia*
Yam	Kachalo	*Colocasia esculanta*
Coriander	Dhnya	*Coriandrum sativum*
Turnip	Shalgum	*Brassica rapa*
Radish	Molli	*Raphonus sativus*

2.3 Sample extraction and analysis

2.3.1 Soil sample extraction and analysis

The soil pH and electric conductivity was measured with H_2O (1:2.5 ratio, dry wt/v) using related electrodes. The soil organic matter (SMO) was calculated using the modified Walkley and Black procedure (Khan et al., 2010). Dried soil sample (0.5 g) was taken into acid washed and dried digestion tube. Acid mixture (15 ml) of HNO_3, $HClO_4$, and H_2SO_4 (5:1:10) was added and then the samples were left overnight. On the next day the digestion tubes were placed on a digestion block at a temperature of 80^0C for 1 hr and then raised to160-180^0C till clear solution obtained. The complete digested solutions were cooled, filtered into acid washed volumetric flasks and diluted to 50 ml with double deionized water. The concentrations of HMs in the digested solutions were measured using atomic absorption spectrometer (Analyst 700 of PerkElmer) in the Geochemistry Laboratory, National Center of Excellence in Geology, University of Peshawar.

2.3.2 Vegetable sample extraction and analysis

Dried plant sample (0.5 g) was taken into acid washed and dried digestion tube. Acid mixture (15 ml) of HNO_3, $HClO_4$, and H_2SO_4 (5:1:10) was added and the samples were left overnight. On the next day the digestion tubes were placed on a digestion block at a temperature of 80^0C for 1 hr and then temperature increased to120-130^0C till clear solution obtained. After complete digestion, the samples were filtered into acid washed volumetric flasks and diluted to 50 ml with double deionized water. The concentrations of HMs in the plant extracts were measured using atomic absorption spectrometer (Analyst 700 of PerkElmer) in the Geochemistry Laboratory, National Center of Excellence in Geology, University of Peshawar.

2.4 Data analysis

2.4.1 Root and shoot transfer factor

To find out bioaccumulation rates, the root transfer factor (RCF) and shoot transfer factor (SCF) were calculated as used by Khan et al. (2008b).

$RCF = C_{root} / C_{soil}$

$SCF = C_{shoot} / C_{soil}$

Where C_{roots}, C_{shoot} and C_{soil} are the concentration of root, shoot and soil, respectively.

2.4.2 Dietary intake of metals (DIM)

The average DIM of HMs for adults and children were calculated on the basis of following formula

$DIM = (C_{metal}\ C_{factor}\ D_{food\ intake})/W_{average\ body\ weight}$

Where C_{metal} is the metal concentration ($mgkg^{-1}$) in vegetables, C_{factor} is the conversion factor (0.085) for fresh vegetables weight into dry vegetables weight. D_{food}

$_{intake}$ is the daily intake of vegetables for adult (245 g person^{-1}day^{-1}) and children (170 g person^{-1}day^{-1}) in Peshawar, while $W_{average\ body\ weight}$ is the body weight of adult (72 kg) and children (32 kg).

2.4.3 Risk assessment

Target hazard quotient was determined with the methods mentioned by Chien et al. (2002) with the help of following mentioned equation

$$THQ = (E_F\ E_D\ F_{IR}\ C)/(RfD\ W_{AB}\ T_A) \times 10^{-3}$$

E_F is the exposure frequency (365 days year^{-1}), E_D is the exposure duration, F_{IR} is the food ingestion rate (g person^{-1}day^{-1}fresh weight) C is the metal concentration in food (µg g^{-1}), RfD is the oral reference dose (mg kg^{-1} day^{-1}), W_{AB} is the average body weight and T_A is the average exposure time (365 days year^{-1} number of exposure years).

2.5 Statistical Analysis

Statistical analysis were done using SPSS (11.5) Statistical software. The measurements were expressed in term of range and average with standard deviation; also the figure is represented with the average values. Microsoft excel software (Microsoft 2007) was used to evaluate the results graphically. ANOVA and cluster analysis (CA) statistical techniques were used determine significant differences.

3 Results

3.1 Physio-chemical properties of soil

Table 3.2 summarizes the physio-chemical properties of soil of study area collected during summer and winter seasons**.** The average pH values were 6.2, 6.0 and 6.5 for soil irrigated with wastewater in winter and summer seasons and referenced soil, respectively. The values of electric conductivity were 0.5, 0.7, and 0.55 mS cm^{-1} for soil in winter, summer and reference site, respectively. Similarly, the SOM in the soil irrigated with wastewater was 1.96 mg kg^{-1} in winter, while 1.6 mg kg^{-1} in summer and 2 mg kg^{-1} in referenced soil.

A great variation in the concentrations of HMs was found between summer and winter samplings and also between the different sites. The concentrations of HMs in the soils collected in summer were higher than the winter samples. The average concentrations of Cu in the soils irrigated with wastewater in summer and winter seasons were 56.9 mg kg^{-1} and 37.01mgkg^{-1}, respectively, while 13 mgkg^{-1} in reference soil. Similarly, Pb concentrations were 61.28, 62.1 and 10.4 mg kg^{-1} in wastewater irrigated soil collected in winter and summer seasons and reference soil, respectively. Furthermore, the average concentrations of Cd, Ni, Zn, Mn, Cu, Co and Hg were 7.68, 100.78, 159.55, 341.33, 40.54, 61.64 and 459.45 mg kg^{-1}, respectively in winter wastewater-irrigated soils, while 8.9, 106.64, 193.4, 387.7, 41.1, 88.2, 593.1 mg kg^{-1}, respectively in summer wastewater irrigated soils. However, the average concentrations of all HMs were significantly (P=0.05) higher in wastewater irrigated soils as compared to reference soil. The concentrations of Cd, Ni and Hg in the soil samples exceeded the UK regulation, 1989; SEPA 1995 and EU 2000 limits. However, the concentrations of Cu, Pb, Zn and Cr were lower than the regulation, 1989; SEPA 1995 and EU 2000 limits

Table 3.2 Initial physio-chemical properties of soil collected from study areas in winter, summer and reference soil

Parameters	Winter		Summer		Reference				
	Range	Average (std)	Range	Average (std)	Range	Average (std)			
Ph	6.1-6.3	6.2 (0.2)	5.9-6.1	6 (0.08)	6.6-6.8	6.5 (0.1)			
EC (mS/cm)	0.14-1.46	0.5 (0.2)	0.6-0.72	0.7 (0.04)	0.54-0.56	0.55 (0.03)			
O. Matter	1-4.4.5	1.96 (1.3)	1.14-2	1.6 (0.31)	0.9-1.1	1 (0.1)			
Soilparticle (%)									
>1mm	8.9-24.2	12.7 (5.3)	36.6-60.9	50 (10.04)	39.7-40.8	40 (1.5)			
1mm-250µm	40.6-56.8	48.2 (5.7)	29.8-51.4	38 (9.55)	40.5-41.8	41 (1.6)			
250µm-64µm	31.8-48.5	37.6 (5.7)	8.4-14.5	11 (2.86)	28-32	30 (2)			
64µm-20µm	0.5-2.55	1.6 (0.7)	0.9-2.16	1.03 (0.88)	1.1-1.8	1.7 (0.04)	Permissible limits		
HMs							uk	SEPA	EU
Cu	27.2-51.4	37.01 (7.51)	32.3-104	56.9 (27.6)	11-14	13 (1.2)	80	100	140
Pb	10.6-110.3	61.28 (32.68)	21.8-94.6	62.1 (31.16)	9.5-11	10.4 (2)	300	350	300
Cd	4.65-9	7.68 (2.81)	2.5-13.8	8.9 (5.42)	1-1.3	1.2 (0.01)	3	0.6	3
Ni	80.1-117.5	100.8 (13.63)	102-110	106.64 (3.7)	22-25	23 (2)	50	60	75
Zn	82-235	159.5 (51.04)	160.6-241.9	193.4 (32.1)	10-14	12 (2)	200	300	300
Mn	246.3-412.4	341.3 (56.52)	334.3-420.4	387.7 (36.7)	12-16	14 (3)	NA	NA	NA
Co	38.3-45.5	40.5 (2.43)	40-43.4	41.1 (1.6)	23-25	24 (1.8)	NA	NA	NA
Cr	26.3-86.3	61.6 (18.95)	72.4-100.4	88.2 (12.04)	13-16	15 (2.2)	400	250	150
Hg	246-846	459.4 (221.8)	414.8-838.1	593.1 (178)	21-24	23 (2.3)	1	NA	NA

3.2 Heavy metals in plant tissues

The concentrations of selected HMs in the edible parts of vegetables irrigated with wastewater during summer and winter seasons and reference site are given in Table-3.3. The average concentrations of some HMs such as Cu, Cd, Zn, Cr, Fe and Hg in summer vegetables were higher as compared to winter. Similarly, the average concentrations of HMs in the vegetables grown on wastewater irrigated soils were significantly (P=0.01) higher as compared to reference soil grown vegetables.

The HM concentrations in vegetables irrigated with wastewater were in order of summer grown vegetables > winter grown vegetables > reference soil grown vegetables (Fig. 3.1).

Fig 3.2 represents the comparison of Cu concentrations in different vegetables species like *Solanum melongena, Abelmoschus esculentus, Luffa acutangula, Spinacia oleracea, Amaranthus viridis, Solanum lycopersicum, Momordica charantia, Colocasia esculanta, Brassica rapa and Raphonus sativus* with its permissible limit set by WHO/FAO. Cu concentration in all the selected vegetables samples were with in the permissible limit. The concentrations of Pb in all the selected vegetables samples were significantly above the permissible limits set by WHO/FAO except *P. oleracea*.(Fig 3.3). Similarly the concentrations of Cd, Ni and Cr were exceeded the permissible limits of FAO/WHO in all the selected vegetables while that of Mn were significantly below the limit (Fig. 3.4, 3.5, 3.6 and 3.7). The trend of HMs bioaccumulation in selected vegetables species was *A. viridis* > *R. sativus* > *S. lycopersicum* > *B. rapa* > *P. oleracea* > *L. acutangula* > *A. esculentus* > *S. oleracea* > *A. sativum* > *M. charantia* > *S. melongena* > *C. sativum* (Fig. 3.8).

The average concentrations of Cu in the vegetables species irrigated with wastewater in summer and winter seasons were 26.8 mg/kg and 24.69 mg/kg, respectively while, in referenced vegetables was 13 mg/kg.

Similarly the average concentration of other HMs like Cu, Pb, Cd, Ni, Zn, Co, Cr, Hg, Mn and Fe in the vegetables grown in wastewater irrigated soil in winter, summer season and preference soil were 63.7, 44.7 and 11.45, 8.3, 13.98 and 3, 38.31, 34.and 32.4, 64.1.128.1, and 33, 25, 21.77 and 11, 20.7, 57.45 and 31.68, 421.76, 497.55 and 100.01, 41.1, 32.26 and 17 and 576.85, 632.3 and 300 respectively.

Table 3.3 HMs concentrations (mg/kg dry weight) in edible parts of vegetables irrigated with wastewater in winter and summer seasons and referenced vegetables

HMs	Winter		Summer		Referenced		Permissible limits
	Range	Average	Range	Average	Range	Average	WHO/FAO
Cu	3.7-39.7	24.69 (11.6)	10.3-42.1	26.8 (10.4)	12-15	13 (2)	73
Pb	33.3-91.3	63.7 (15.2)	3.6-82.1	44.7 (24.13)	11-12	11.45 (0.13)	0.3
Cd	1.5-13.2	8.3 (3.2)	5.4-25.4	13.98 (5.72)	2-5	3 (1.2)	0.1
Ni	16.8-44.8	38.31(20)	15.1-60	34.1 (12.86)	30-33	32.4 (1.4)	67
Zn	44.6-84.6	64.1(15.5)	107-167.4	128.12 (21.97)	32-40	33 (4)	100
Mn	25-60.4	41.1(10.66)	9.3-64.6	32.26 (19)	15-20	17 (3.2)	500
Co	12.5-39.4	25.1(12.32)	11-40.9	21.77 (12.74)	10-12	11 (1.4)	NA
Cr	5.1-27.7	20.7(9.15)	20.8-106.2	57.45 (34.06)	30-32	31.68 (3)	2.3
Fe	235-830	576.85(158.44)	424.6-1236	632.3 (267.79)	220-345	300 (48)	425.5
Hg	205.7-632.3	421.76 (172.31)	380.4-739.7	497.55 (124.92)	100-103	100.01 (2.03)	0.03

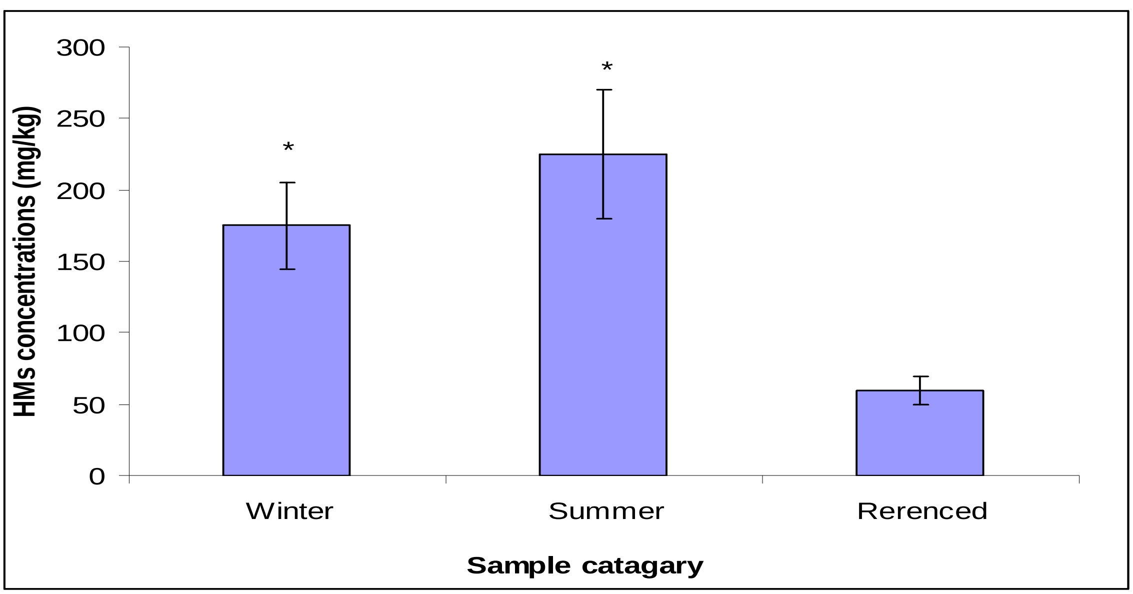

Fig. 3.1. Comparison of average total HMs in all vegetables collected in winter, summer from wastewater irrigated soils and reference site. Asterisks indicate significant difference (P=0.01).

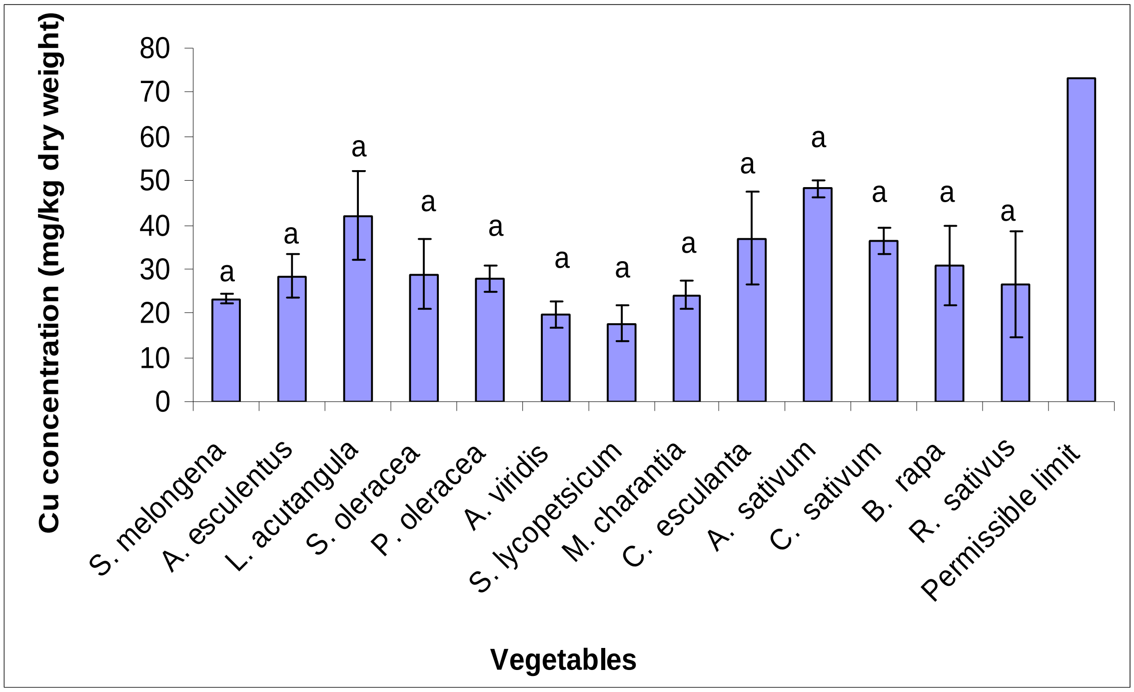

Fig 3.2 Comparison of Cu concentration in vegetables. The error bars indicate the standard deviation while (a) indicate significant decrease from permissible limit

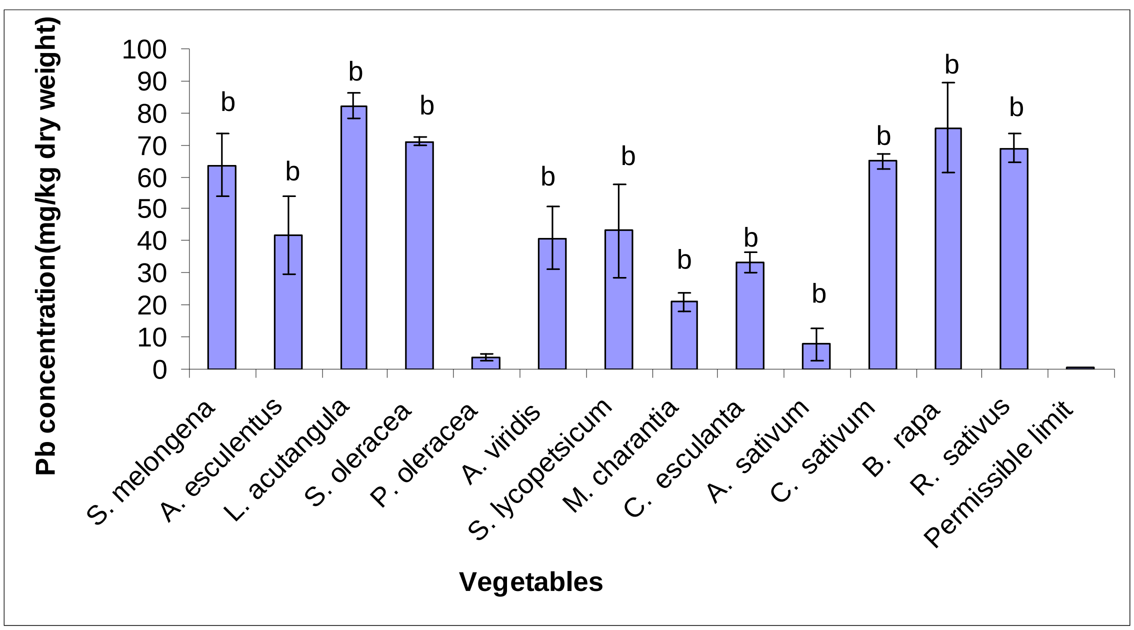

Fig 3.3 Comparison of Pb concentration in vegetables. The error bars indicate the standard deviation while (b) indicate significant increase from permissible limit

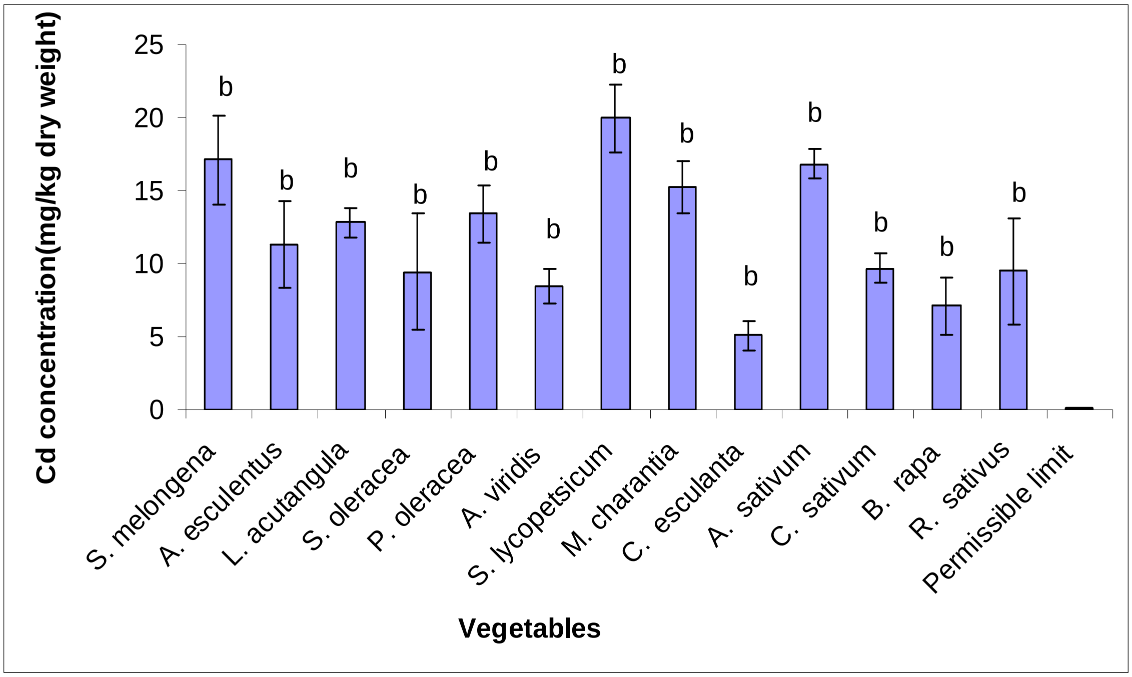

Fig 3.4 Comparison of Cd concentration in vegetables . The error bars indicate the standard deviation while (b) indicate significant increase from permissible limit

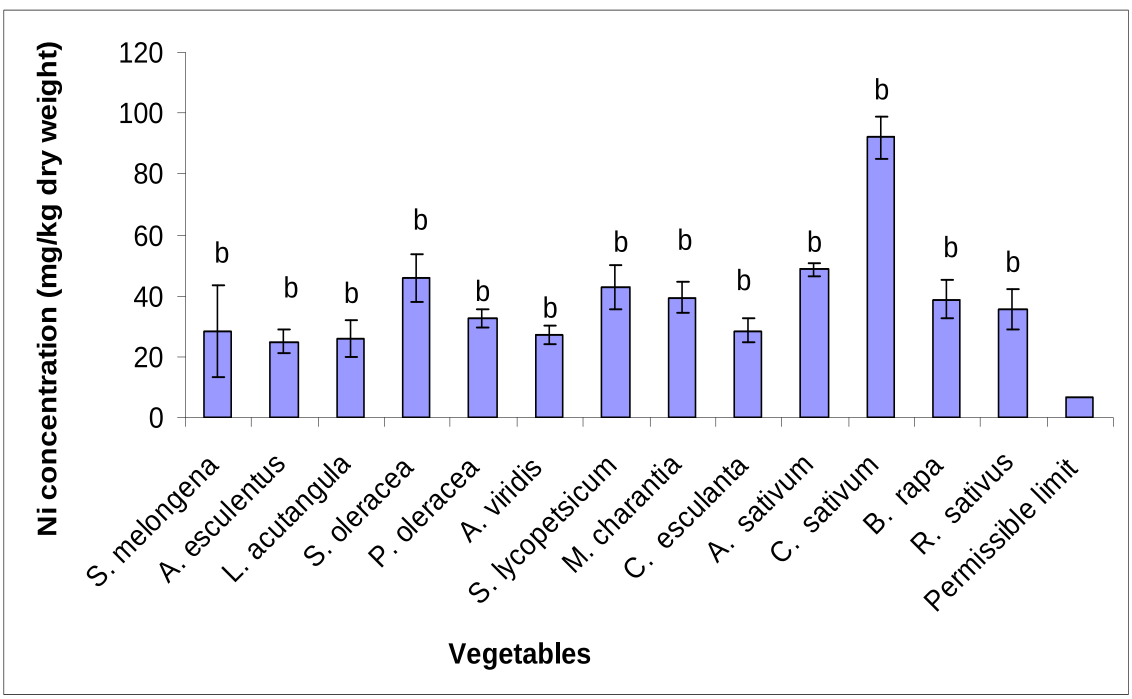

Fig 3.5 Comparison of Ni concentration in vegetables**.** The error bars indicate the standard deviation while (b) indicate significant increase from permissible limit

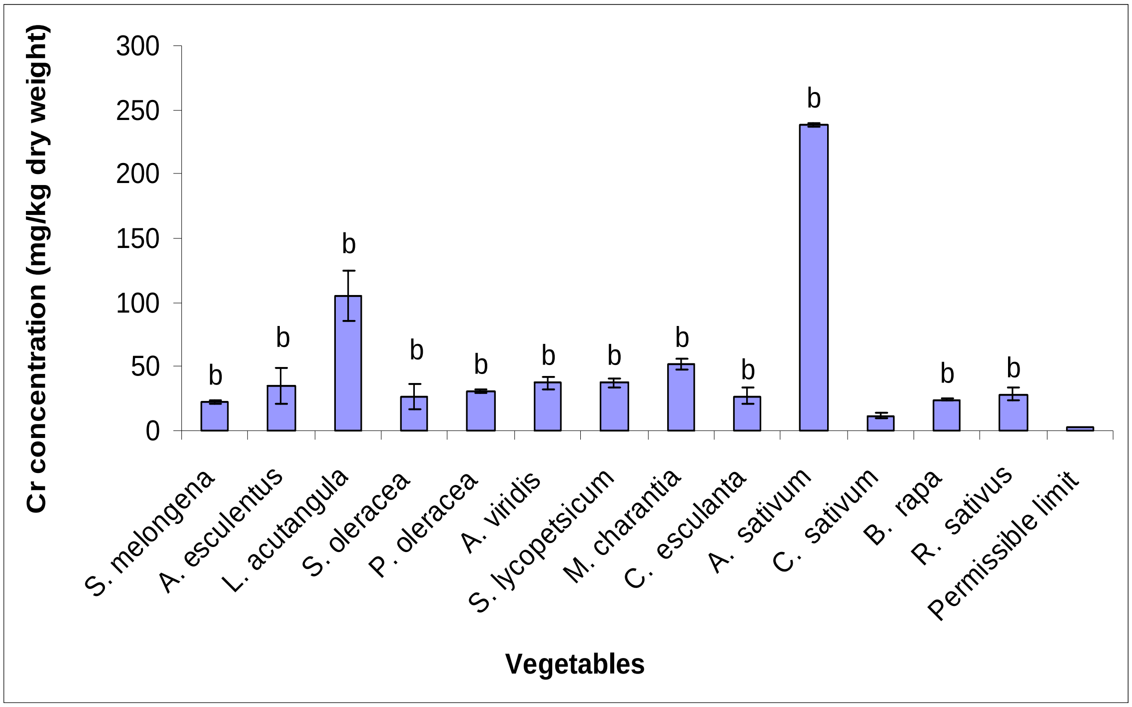

Fig 3.6 Comparison of Cr concentration in vegetables . The error bars indicate the standard deviation while (b) indicate significant increase from permissible limit

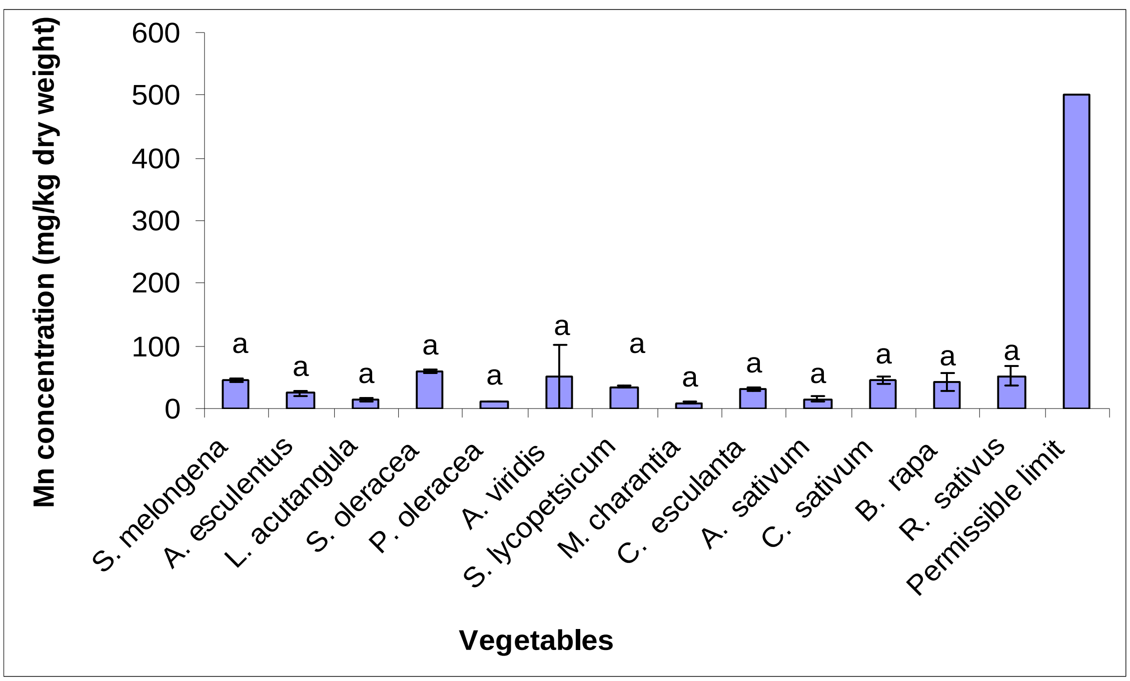

Fig 3.7 Comparison of Mn concentration in vegetables. The error bars indicate the standard deviation while (a) indicate significant decrease from permissible limit

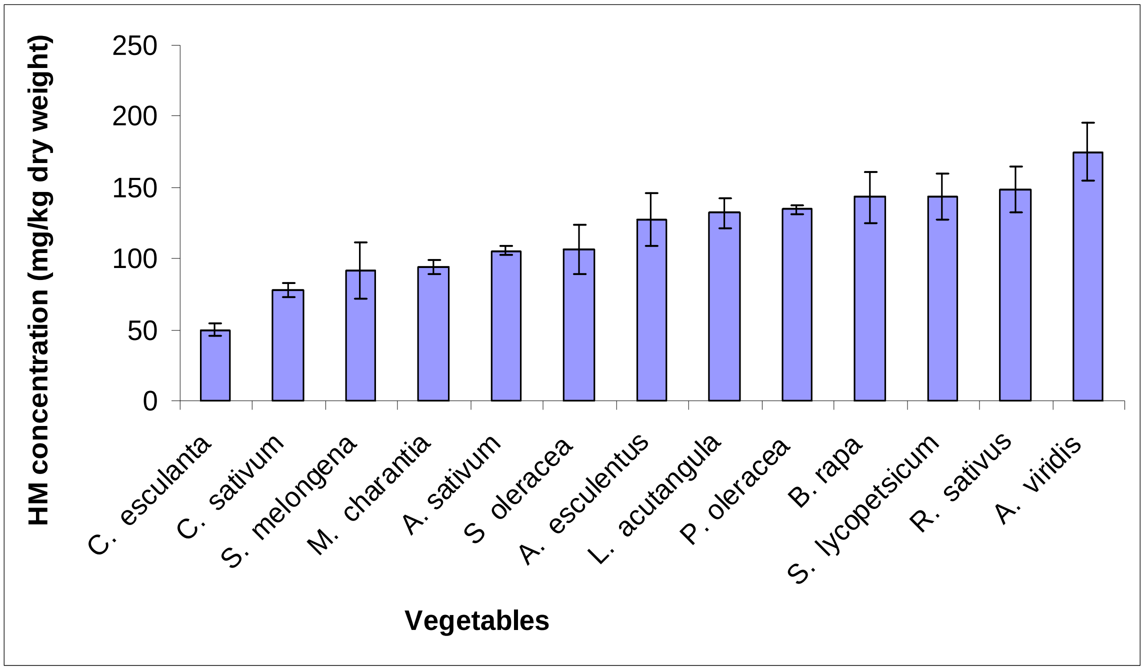

Fig 3.8 Comparison of HM concentration in vegetables. The error bars indicate the standard deviation

3.3 Root, shoot and fruit transfer factor

Table 3.4 summarizes the RCFs, SCFs and FCFs values for selected HMs of vegetables grown in wastewater irrigated soil in study area. The RCFs values were ranged from 0.3-1.4, 0.2-1.4, 0.4-5.3, 0.4-1.1, 0.3-0.6, 0.004-0.18, 0.36-1.8, 0.12-1.5 and 0.39-1.08 for Pb, Cu, Cd, Ni, Zn, Mn, Co, Cr and Hg, respectively in selected vegetables of study area. Similarly the SCFs values for Pb, Cu, Cd, Ni, Zn, Mn, Co, Cr and Hg, were ranged from 0.2-1.4, 0.2-4, 0.3-4.7, 0.2-1.4, 0.45-2.46, 0.02-0.45, 0-1.5, 0.34-2.2 and 0.39-2.01, respectively in the vegetables. The FCFs values were ranged from 0.23-1.1, 0.2-2.4, 0.8-5.7, 0.2-0.6, 0.31-0.69, 0.002-0.14, 0.4-1.6, 0.3-1.4 and 0.5-0.9 for Pb, Cu, Cd, Ni, Zn, Mn, Co, Cr and Hg, respectively in the wastewater irrigated vegetables of Peshawar. The highest RCFs value for HMs (average of Pb, Cu, Cd, Ni, Zn, Mn, Co, Cr and Hg) were found in *M. charantia* followed by *S. lycopersicum*, *A. viridis*, *L. acutangula*, *S. oleracea*, *A. esculentus*, *S melongena*, *B. rapa*, *R. sativus* and *C. esculanta*, respectively (Fig 3.9). The trend of SCFs values for vegetables was *S. oleracea* > *A. esculentus* > *S. lycopersicum* > *L. acutangula* > *A. viridis* > *B. rapa* > *R. sativus* > *C. esculanta* > *M. charantia*> *S. melongena* (Fig 3.10). The FCFs values were highest for *S. lycopersicum* followed by *L. acutangula*, *M. charantia*, *S. melongena* and *A.s esculentus* (Fig 3.11). RCFs values of vegetables (average of RCFs of *M. charantia S. lycopersicum*, *A. viridis*, *L. acutangula*, *S. oleracea*, *A. esculentus*, *S. melongena*, *B. rapa*, *R. sativus* and *C. esculanta*) were highest for Cd (2) followed by Co(1.186), Mn (0.888), Cu (0.74), Pb(0.71), Hg (0.65), Ni (0.64) Cr (0.546) and Zn (0.505) (Fig 3.12). The SCFs values of vegetables show a trend of Cd >Cu > Hg > Co > Zn > Cr > Pb > Ni > Mn (Fig 3.13). The FCFs values of vegetables were highest for Cd followed by Cr, Co, Cu, Pb, Hg, Zn, Ni, and Mn (Fig 3.14).

Table 3.4 HMs transfer factor from soil to root, shoot and fruit of vegetables irrigated with wastewater

Vegetables species		Pb	Cu	Cd	Ni	Zn	Mn	Co	Cr	Hg
S. melongena	RCFs	1.00	0.2	0.4	0.4	0.50	0.118	1.70	0.50	0.60
	SCFs	0.20	0.2	1.0	0.7	0.47	0.150	000	1.30	0.50
	FCFs	1.10	0.2	1.8	0.2	0.69	0.140	1.60	0.30	0.50
A. esculentus	RCFs	0.90	0.3	1.0	0.7	0.60	0.150	1.20	0.30	0.80
	SCFs	1.00	0.4	0.3	0.7	2.46	0.450	1.10	2.20	1.00
	FCFs	1.00	0.6	0.8	0.3	0.60	0.060	0.80	1.30	0.90
L. acutangula	RCFs	0.80	0.6	1.7	0.6	0.60	0.080	1.60	0.50	0.80
	SCFs	0.60	0.9	3.1	0.5	0.63	0.140	1.50	0.50	0.60
	FCFs	1.10	2.4	2.1	0.5	0.31	0.020	0.40	1.40	0.60
S. oleracea	RCFs	1.40	1.4	0.6	0.8	0.50	0.180	1.00	0.40	0.50
	SCFs	1.40	4.0	0.9	0.8	0.59	0.170	1.00	1.20	1.30
A. viridis	RCFs	0.60	0.8	3.7	0.7	0.60	0.060	1.80	ND	0.50
	SCFs	0.60	0.8	2.2	1.4	0.74	0.120	0.60	ND	0.90
S. lycopersicum	RCFs	0.30	0.6	5.3	0.7	0.60	0.010	1.10	1.00	0.40
	SCFs	0.40	0.7	4.7	0.4	0.67	0.030	1.20	0.40	0.80
	FCFs	0.30	0.3	5.7	0.6	0.61	0.006	1.20	0.90	0.80
M. charanti)	RCFs	0.70	1.0	3.5	0.5	0.60	0.004	1.10	1.50	ND
	SCFs	0.60	0.2	1.4	0.4	0.73	0.020	1.00	0.40	ND
	FCFs	0.23	0.5	4.3	0.6	0.68	0.002	0.40	0.70	ND
C. esculanta	RCFs	0.30	0.9	1.1	0.5	0.30	0.080	0.36	0.38	0.39
	SCFs	0.70	1.0	1.7	0.3	0.45	0.100	0.37	0.36	0.39
B. rapa	RCFs	0.80	0.7	0.8	0.4	0.40	0.096	1.00	0.12	1.08
	SCFs	0.40	0.6	2.0	0.2	0.64	0.180	0.72	0.34	2.01
R. sativus	RCFs	0.30	0.9	ND	1.1	0.35	0.090	1.00	0.21	0.79
	SCFs	0.60	0.9	2.0	0.8	0.54	0.120	0.53	0.35	0.94

ND Not detected

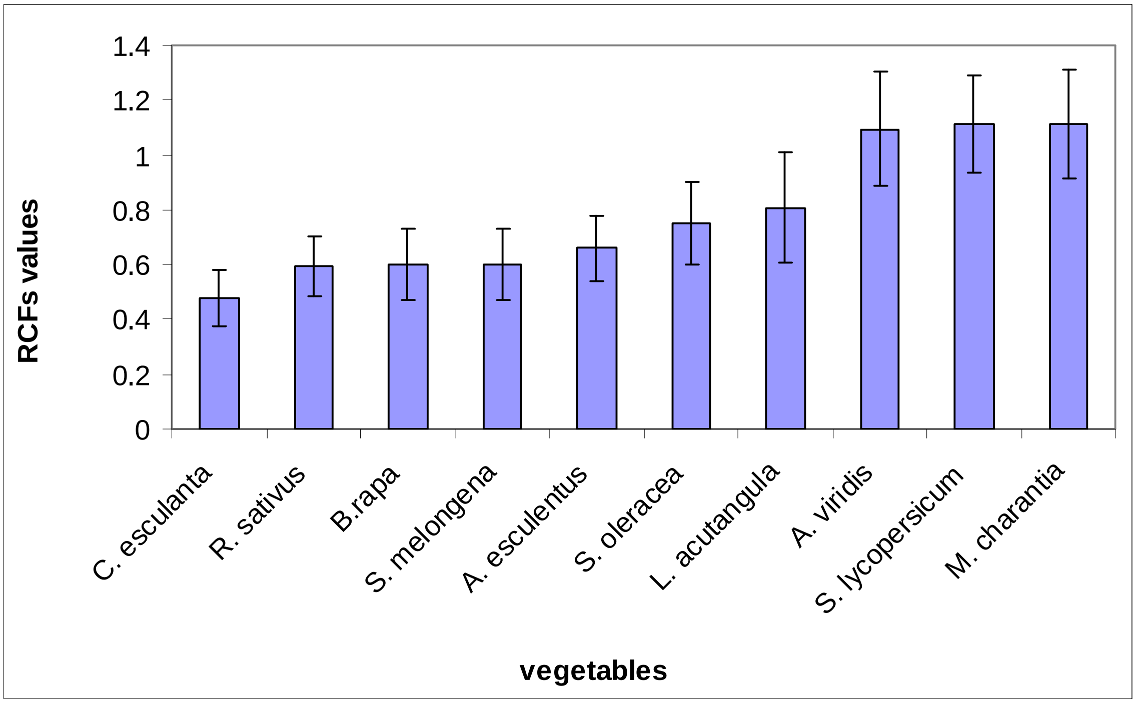

Fig 3.9 Comparison of RCFs values of HMs for studied vegetables. The error bars indicate the standard deviation

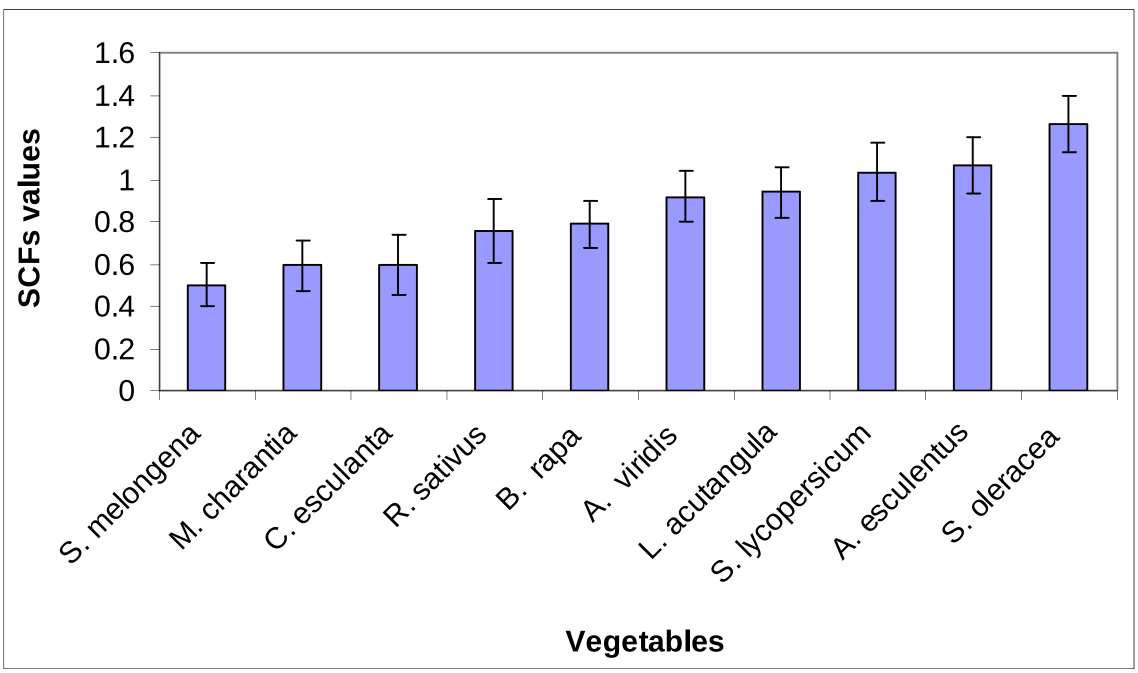

Fig 3.10 Comparison of SCFs values of HMs for studied vegetables The error bars indicate the standard deviation

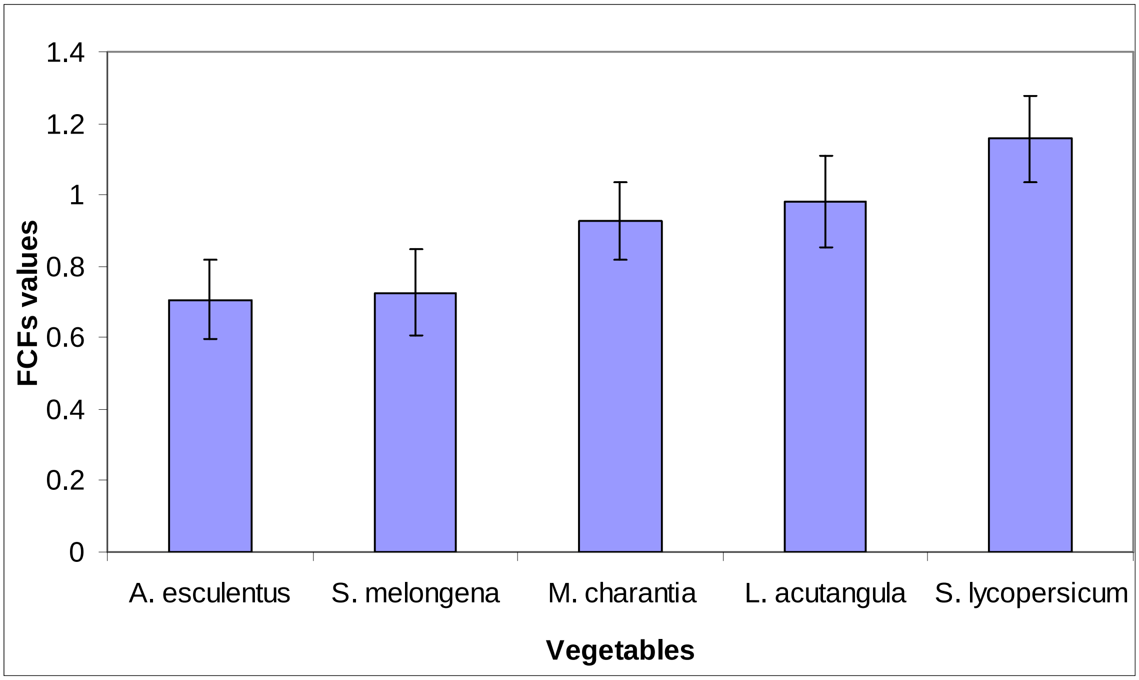

Fig 3.11 Comparison of FCFs values of HMs for studied vegetables The error bars indicate the standard deviation

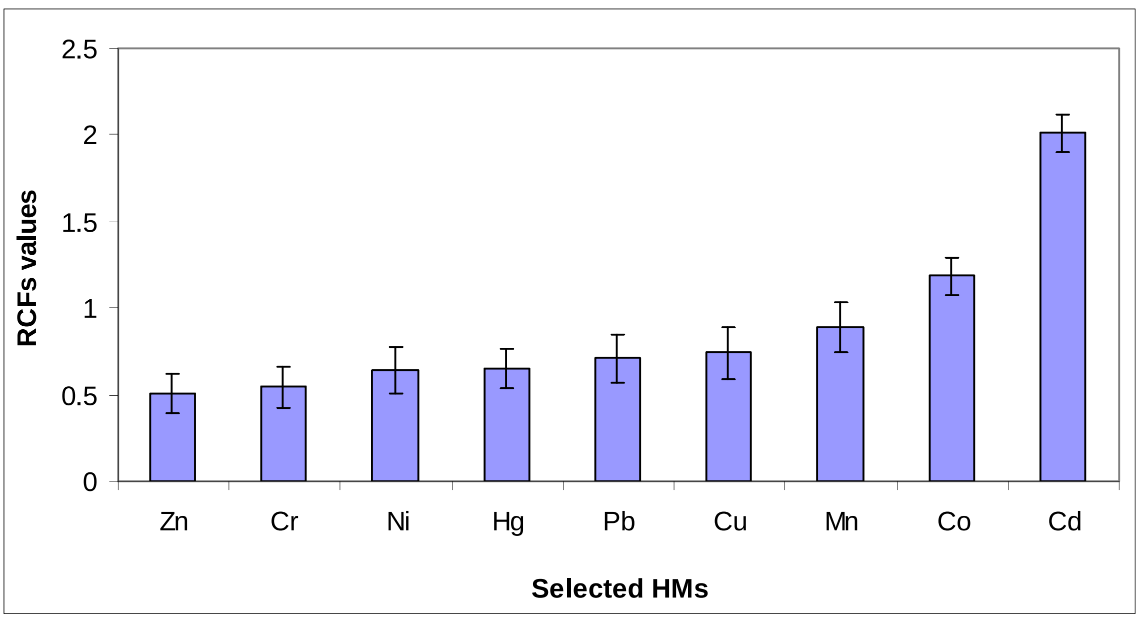

Fig 3.12 Comparison of RCFs values of HMs for studied vegetables The error bars indicate the standard deviation

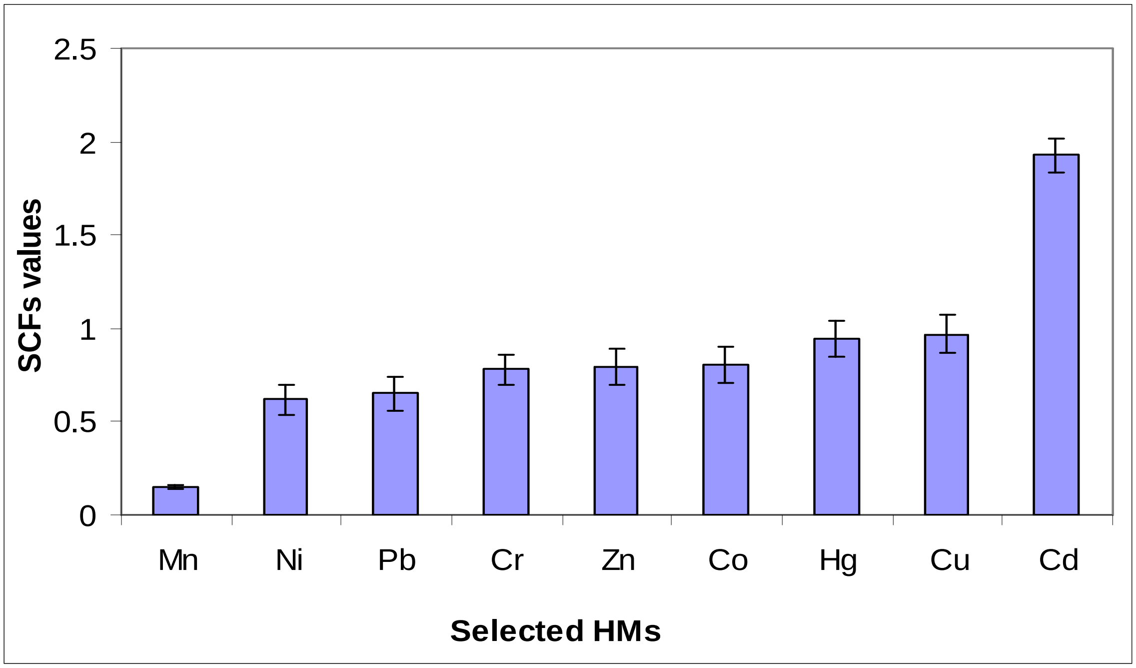

Fig 3.13 comparison of SCFs values of HMs for studied vegetables The error bars indicate the standard deviation

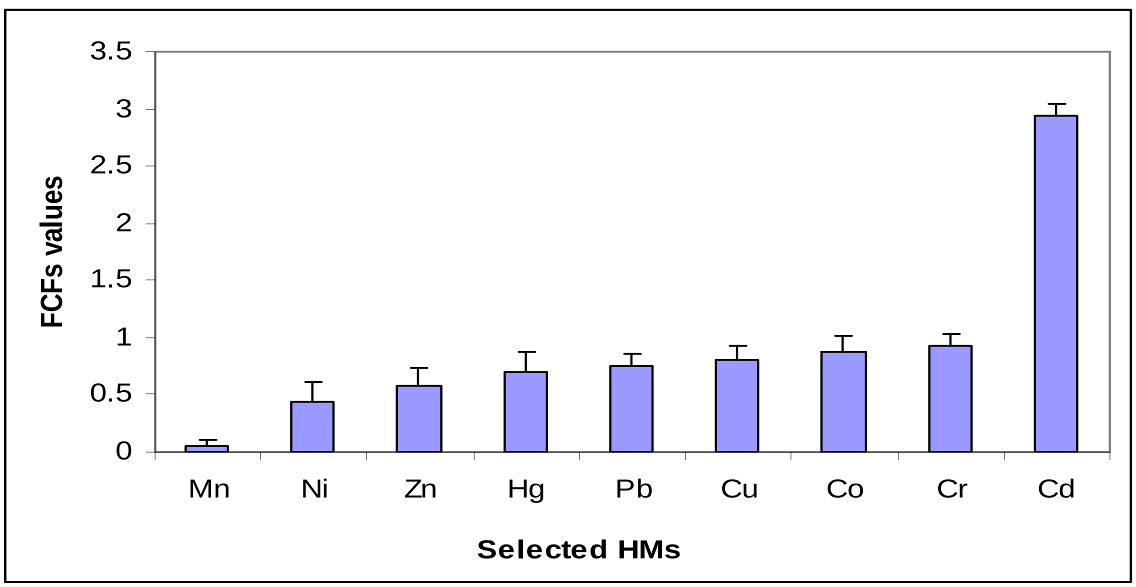

Fig 3.14 Comparison of FCFs values of HMs for studied vegetables The error bars indicate the standard deviation

3.4 Dietary intake of HMs

Table 3.5 summarizes the dietary intake of HMs via vegetables by both children and adults, respectively. The dietary intake values of Pb, Cd, Ni, Zn, Mn, Cr and Cu by children were ranged from 0.001-0.02, 0.001-0.005, 0.01-0.02, 0.013-0.038, 0.01-0.11, 0.003-0.018, and 0.001-0.011, respectively. Similarly, the values of dietary intake of Pb, Cd, Ni, Zn, Mn, Cr and Cu by adults via vegetables were ranged 0.01-0.023, 0.002-0.006, 0.01-0.03, 0.015-0.042, 0-0.13, 0.003-0.032 and 0.001-0.013, respectively. The DIM values of Pb and Cd were significantly ($p<0.05$) higher as compared to respective RfD (0.004 for Pb and 0.001 for Cd) both for children and adult (Table 3.4). The DIM values of Zn, Cu and Mn were significantly ($p<0.05$) lower than their RfD (Table 3.4). In case of Mn, the DIM values via Raphonus sativus was significantly ($p<0.05$) higher than its RfD value. The DIM values of HMs (the sum of DIM values of Pb, Cd, Zn, Cu and Mn) for Children via vegetables showed a trend of *R. sativus* > *S. oleracea* > *A. viridis* > *S. lycopersicum* > *A. esculentus* > *M. charantia* > *C. sativum* > *S. melongena* > *L. acutangula* > *B. rapa* > *C. esculanta* (Figures 3.15) and for adults the order were *R. sativus* > *M. charantia* > *C. sativum* > *S. lycopersicum* > *S. oleracea* > *A. viridis* > *B. rapa* > *A. esculentus* > *S. melongena* > *L. acutangula* > *C. esculanta* (Figure 3.16).

Table 3.5 Dietary intake of HMs by children and adults via consumption of vegetables

		Pb	Cd	Ni	Zn	Mn	Cr	Cu
R. sativus	Children	0.019*	0.002*	0.01	0.014	0.11*	0.008	0.011*
	Adults	0.021*	0.002*	0.01	0.016	0.13*	0.009	0.013*
C. esculanta	Children	0.009*	0.001*	0.01	0.013	0.01	0.007	0.001
	Adults	0.010*	0.002*	0.01	0.015	0.01	0.008	0.001
S. oleracea	Children	0.019*	0.003*	0.01	0.032	0.02	0.007	0.008
	Adults	0.021*	0.003*	0.01	0.036	0.02	0.008	0.009
L. acutangula	Children	0.011*	0.002*	0.01	0.032	0.01	0.018	0.004
	Adults	0.021*	0.004*	0.01	0.036	0	0.032	0.012*
S. lycopersicum	Children	0.002*	0.005*	0.01	0.028	0.01	0.006	0.003
	Adults	0.013*	0.006*	0.01	0.031	0.01	0.02	0.005
S. melongena	Children	0.016*	0.003*	0.01	0.030	0.01	0.007	0.006
	Adults	0.022*	0.006*	0.01	0.033	0.01	0.007	0.007
A. esculentus	Children	0.001*	0.001	0.01	0.028	0.01	0.008	0.007
	Adults	0.017*	0.003*	0.01	0.031	0.01	0.008	0.008
	Children	0.011*	0.002*	0.01	0.032	0.01	0.01	0.005
P. oleracea	Adults	0.015*	0.004*	0.01	0.036	0	0.009	0.008
A. viridis	Children	0.017*	0.001	0.02	0.038	0.01	0.014	0.006
	Adults	0.021*	0.003*	0.02	0.042	0.02	0.011	0.006
M. charanti)	Children	0.020*	0.003*	0.01	0.031	0.01	0.003	0.001
	Adults	0.023*	0.006*	0.01	0.035	0	0.015	0.007
	Children	0.003*	0.002*	0.02	0.018	0.01	0.003	0.008
C. sativum	Adults	0.023*	0.003*	0.03*	0.020	0.01	0.003	0.011*
B. rapa	Children	0.019*	0.002*	0.01	0.018	0.11*	0.008	0.011*
	Adults	0.021*	0.002*	0.01	0.016	0.13*	0.009	0.013*
RfD(mg/kg bw/day)		0.004	0.001	0.02	0.30	0.033	1.5	0.04

* Above the respective reference dose

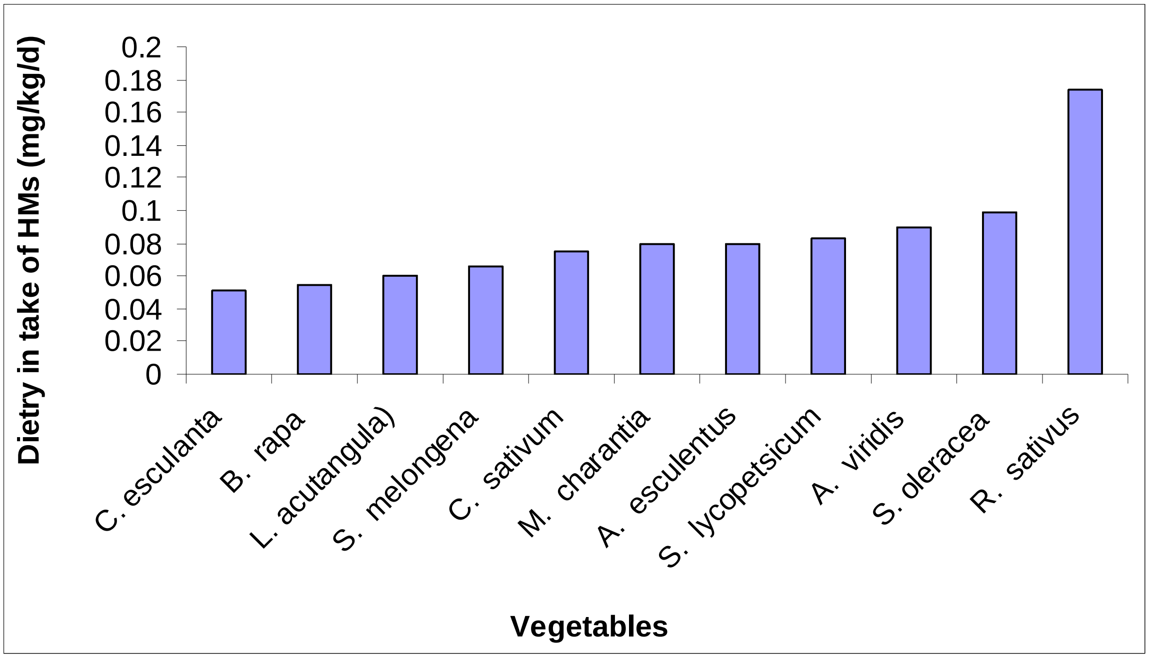

Fig 3.15 Comparison of dietary in take of HMs (sum of DIM of Pb, Cd, Zn, Cu and Mn) by children via different vegetables species

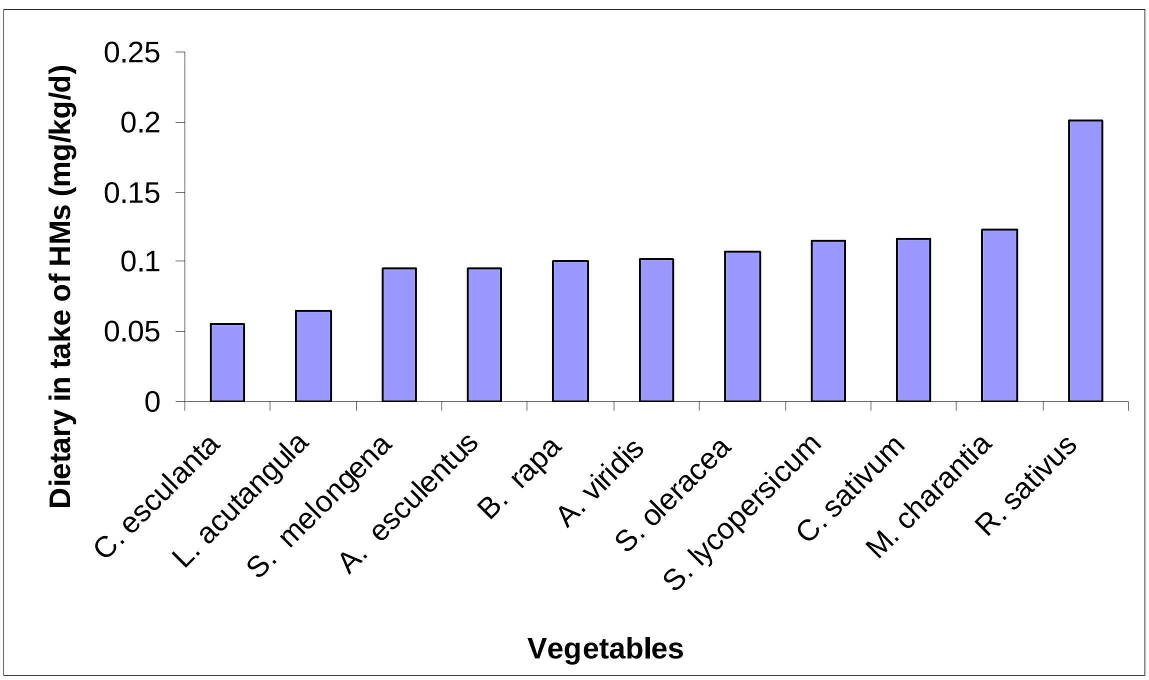

Fig 3.16 Comparison of dietary in take of HMs (sum of DIM of Pb, Cd, Zn, Cu and Mn) by adults via different vegetables species

3.5 Health risks

The values of THQs of studied HMs through consumption of vegetables grown with wastewater in study area for children and adults are listed in Table 3.6. The THQ values of Pb, Cd, Ni, Zn, Mn, Cr and Cu were ranged from 0.7-5, 1.3-5.6, 0.36-1.23, 0.044-0.126, 0.08-3.46, 0.002-0.019 and 0.025-0.281, respectively for children. Similarly, the THQ values were ranged from 0.7-5.6, 1.5-6.2, 0.042-1.08, 0.049-0.14, 0.08-3.86, 0.002-0.021 and 0.028-0.313 due to Pb, Cd, Ni, Zn, Mn, Cr and Cu, respectively for adults. Total THQ for children through consumption of *R. sativus, C. esculanta, S. oleracea, L. acutangula, S. lycopersicum, S. melongena, A. esculentus, A. viridis, M. charantia, C. sativum* and *B. rapa* were 11, 4.3, 8.6, 8.8, 9.3, 11.3, 7.5, 6.6, 10.8, 9.5 and 3.4, respectively. Similarly, the consumption of *R. sativus, C. esculanta, S. oleracea, L. acutangula, S. lycopersicum, S. melongena, A. esculentus, A. viridis, M. charantia, C. sativum* and *B. rapa* by adults are responsible for TTHQ of 12.2, 4.8, 9.6, 9.8, 10.3, 12.6, 8.3, 7.4, 12.0, 10.6 and 3.8, respectively. The relative contribution of Pb, Cd, Ni, Zn, Mn, Cr and Cu to TTHQ were 44.9%, 37.7%, 7.3%, 1.0%, 6.9%, 0.1% and 2.1% for adults and 44.9%, 37.6%, 7.3%, 1.0%, 6.9%, 0.08% and 2.1% for children, respectively (Table 3.7)

Table 3.6 THQ of HMs by children and adults via consumption of vegetables

		Pb	Cd	Ni	Zn	Mn	Cr	Cu	TTHQ
R. sativus	Children	4.7*	2.0*	0.49	0.100	3.46*	0.005	0.281	11.0
	Adults	5.2*	2.2*	0.54	0.053	3.86*	0.006	0.313	12.2
C. aesculanta	Children	2.2*	1.3*	0.38	0.044	0.25	0.005	0.025	4.30
	Adults	2.5*	1.5*	0.42	0.049	0.27	0.005	0.028	4.80
S. oleracea	Children	4.7*	2.5*	0.61	0.107	0.47	0.005	0.192	8.60
	Adults	5.3*	2.8*	0.68	0.119	0.53	0.005	0.214	9.60
L. acutangula	Children	4.7*	3.3*	0.38	0.107	0.11	0.019	0.274	8.80
	Adults	5.2*	3.6*	0.43	0.119	0.12	0.021	0.305	9.80
S. lycopersicum	Children	2.9*	5.3*	0.56	0.093	0.28	0.012	0.103	9.30
	Adults	3.2*	5.9*	0.62	0.104	0.32	0.013	0.115	10.3
S. melongena	Children	5.0*	5.1*	0.64	0.100	0.36	0.004	0.154	11.3
	Adults	5.6*	5.6*	0.71	0.111	0.41	0.005	0.171	12.6
A. esculentus	Children	3.9*	2.7*	0.36	0.092	0.20	0.005	0.187	7.50
	Adults	4.4*	3.0*	0.40	0.102	0.22	0.005	0.208	8.30
A. viridis	Children	2.7*	2.3*	0.97	0.126	0.41	0.007	0.130	6.60
	Adults	3.0*	2.5*	1.08*	0.140	0.46	0.007	0.145	7.40
M. charanti	Children	4.3*	5.6*	0.53	0.104	0.08	0.009	0.160	10.8
	Adults	4.8*	6.2*	0.59	0.116	0.08	0.010	0.179	12.0
C. sativum	Children	5.0*	2.6*	1.23*	0.059	0.36	0.002	0.242	9.50
	Adults	5.6*	2.9*	1.37*	0.066	0.40	0.002	0.269	10.6
B. rapa	Children	0.7	1.6*	0.56	0.060	0.32	0.002	0.194	3.40
	Adults	0.7	1.8*	0.62	0.066	0.36	0.002	0.216	3.80

* Above 1

Table 3.7 Relative contribution of HMs in TTHQ

HMs	THQ(%) for adults	THQ(%) for children
Pb	44.9	44.9
Cd	37.7	37.6
Ni	7.30	7.30
Zn	1.00	1.00
Mn	6.90	6.90
Cr	0.10	0.08
Cu	2.10	2.10

4 Discussion

The results of the present study showed that long term application of wastewater led to contamination of soil and edible parts of vegetables with HMs. The concentrations of HMs showed spatial and temporal variations both in soil and vegetable species. The variations in HMs concentrations are due to many reasons like variations in sources of HMs, their quantity in wastewater for irrigation, soil pH, organic content, temperature, electric conductivity, moisture, evapotranspiration and frequency of irrigation and intervals. The application of wastewater leads to change physicochemical characteristics of soil and HMs concentrations (Khan et al., 2008a). The mobility and bioavailability of HMs in the soil depends upon the physical and chemical properties of soil. The application of wastewater decreases soil pH (Jamil et al., 2006; Maldonado et al., 2008) and increase soil organic matter because of the carbon present in wastewater (Jamil et al., 2006). The results showed that wastewater application dropped soil pH by 0.5 and 0.2 units in summer and winter seasons as compared to reference soil. Organic matter in soil irrigated with wastewater was more than two fold higher as compared to reference soil. Furthermore, the wastewater application has also increased the level of HMs in the soil which is positively correlated with soil organic matter and negatively correlated with soil pH **(**Jarausch et al., 2000)**.** Previously, the accumulation of HMs in soil treated with high doses of wastewater has also reported by Jan et al., (2010).

In the study area, soil contamination with HMs was mainly due to wastewater irrigation and HM concentrations in wastewater-irrigated soils were significantly higher ($P<0.05$) than the reference soils. In the present study, the concentration of Zn (159.55mg /kg) was higher than those reported by Jamil et al., (2006) in D. I. Khan, Pakistan and by Turkdogan et al (2002) in Turkey, while lower than those reported by Rattan et al, (2005) in India, by Yu et al, (2006) in Yellow River, China and by Jung, (2008) in Southeast Korea. However, some HM concentrations such as Cr, Cu, Ni and Pb were lower than the concentrations reported in the literature (Jamil et al., 2006; Turkdogan et al., 2002; Jung, 2008; Onweremadu et al., 2007). Furthermore, the concentrations of Cd, Pb and Mn were inconsistent with the findings reported in the past (Maldonado et al., 2008; Turkdogan et al., 2002; Onweremadu et al., 2007; Malarkodi et al., 2007). This variation and inconsistency of HM concentrations in the soil of the study are with previous studies may be due to differences in characteristics of soil and physiology of plants (Sharma et al., 2007).

Interestingly, a significant variation was found in HM concentrations between summer and winter seasons. The concentrations of HMs in the soil irrigated with wastewater in summer were significantly higher ($P<0.05$) than the soil irrigated with wastewater in winter season. The changes in concentrations of HMs between the seasons may be correlated with temperature variation and several other factors like irrigation interval and quantity of water used, pH, organic matter, soil moisture, bioavailability of nutrients and metals. Jan et al., (2010).observed a negative relationship between the adsorption of Pb with soil pH and EC, while a positive relationship with temperature. In this study, the pH of the soil-irrigated with wastewater in summer was 0.3 lower than the pH of soil irrigated with wastewater in winter. Organic matter in soil, irrigated with wastewater in summer was 0.36% more than the soil irrigated with wastewater in winter. In summer, the decomposition rate of organic matter is relatively high which release HMs in to soil solutions (McGrath et al., 1994). The lower value of pH and higher values of electro conductivity and organic matter of the soil irrigated with wastewater in summer can be responsible for high concentrations of HMs in the soil collected in summer. In summer, high temperature causes low moisture contents in soil which further leads to high frequency of irrigation. This high frequency of irrigation can be increased the concentrations of HMs in concerned soil because the HMs in soil are positive correlated with the concentrations of HMs in water for irrigation.

In this study, the concentrations of HMs in vegetable species grown in summer were significantly ($P<0.05$) higher than their concentrations in winter vegetables. This great variation in HM concentrations is caused by the differences of pH, organic matter, physiology of the different season plants, temperature and humidity. The soil physiochemical properties affect the uptake of HMs and their bioavailability also depends on soil pH because at low pH metals solubility in soil solution is increased (Oluyemi et al., 2009). The results of our study showed that the soil pH was lower in summer wastewater irrigated soil as compared to winter which could be accelerated the HM uptake by summer season vegetables. Similarly, the presence of organic matter increases the uptake of HMs like Zn by wheat plant (Rupa et al., 2003; Chen and Aviad., 1990). The high ambient temperature and low humidity in summer season are responsible for high transpiration rates which may be one of the reasons of higher uptake of HMs during summer season as compared to winter season (Ingwersen and Streck, 2005).

Results of the present and previous studies (Sharma et al., 2007 ; Muchuweti et al., 2006; Khan et al., 2008 ab ; Boularbah et al., 2006) demonstrated that the plants irrigated with wastewater contaminated with HMs. All the studied vegetable species grown on wastewater irrigated soil during both seasons were contaminated with Cu, Pb, Cd, Ni, Zn, Mn, Co, Cr, Fe and Hg and mostly exceeded their respective permissible limits set by SEPA and WHO, except Mn which was within its permissible limits. In this study, the concentrations of Cr, Cd, Ni, Pb and Zn in vegetable samples were more than the concentrations reported previously (Khan et al., 2008b; Jan et al., 2010; Eslami et al., 2007; Chatha et al., 2002), while Cu, Ni, and Zn concentrations were lower than those reported in the literature (Jan et al., 2010; Rattan et al., 2005; Burun et al., 2006; Haq et al., 2005). In case of Fe and Mn concentrations, our results were in agreement with the study of Rattan et al, (2005).

The transfer factor of HMs from soil to plant is an important component of human exposure to metals through food chain. In order to investigate THQ for selected HMs it is important to assess soil to plant transfer factor of HMs and its daily intake through food chain. RCFs, SCFs and FCFs varied widely for plant species. The RCFs values were higher for Cd, Co, Mn and Cu, while lower for Hg, Ni, Cr and Zn. Similarly, the SCFs values were higher for Cd, Cu, Hg, Co and lower for Mn, NI, Pb and Cr. Furthermore, the high FCFs values were found for Cd, Cr, Co, Cu and lower for Mn, Ni, Zn and Hg. The results indicated that the values of transfer factors for Cd, Cr, Cu, Ni, Pb and Zn were many folds higher than their values reported by Khan et al, (2008b) The values of transfer factors for Zn, Cu, Ni and Mn were lower and for Cd, Pb, Cr were higher as compared to the values mentioned by Jan et al, (2010). The values of metals transfer factors for Hg, Pb, Cd, Zn and Cu found by Yang et al, (2009) were lower than the values mentioned in our results. The differences in transfer factor values may be due to different soil properties, season, temperature, plant maturity and transpiration rate.

The DIM values of Pb and Cd were significantly ($p<0.05$) higher as compared to respective RfD (0.004 for Pb and 0.001 for Cd) both for children and adult (Table 3.4). The DIM values of Zn, Cu and Mn are significantly ($p<0.05$) lower than their RfD (Table 3.4). In case of Mn, the DIM values via Raphonus sativus was significantly ($p<0.05$) higher than its RfD value. In the study area the vegetable were sold in the local market and the people consume vegetable on daily basis so the average total metal concentrations were used to calculate THQ values both for children and adults. Data indicate that the values of THQ for Cd and Pb were > 1 for almost all vegetable species. These vegetable species poses possible health risks, while safe with respect to other HMs like Ni, Zn, Cr and Cu. The relative contribution of Pb is more as compare to other HMs in the total THQ while, Cr contributing on minimum. The results of this study suggest that the consumption of vegetable grown on wastewater contaminated soil is a health risk because the values of THQ are >1.

Chapter 4: HMs toxicities on spinach (*Spinacia oleracea*) seedling

1. Introduction

Many HMs like Cd and Pb are nonessential and toxic for plant growth (Zhiqiang et al., 2009), but plant takes them up rapidly when present in growing medium like soil (Fusconi et al., 2006). Cd is phytotoxic in nature, it not only inhibit the growth parameters of plants but also cause death of plant (Toppi and Gabbrielli, 1999). Moreever Cd badly affects the process of respiration and photosynthesis and decreases water and nutrient uptake by plants (Kuo and Kao, 2004). Cd reduces root growth because of the reduction of the rate of new cell production (Liu et al., 2004), inhibits the activities of antioxidative enzymes of plants (Correa et al., 2006) and induces oxidative stress in cells (Sandalio et al., 2001).

Similarly, Pb causes retardation of plant growth and inhibition of seed germination (Iqbal and Shazia, 2004), also have significant negative impacts on seedling bio mass, root length and shoot length (Juseph et al., 2002). Like Cd, Pb adversely affects the process of photosynthesis, respiration and metabolism of plants (Paolacci et al., 1997). HMs like Zn, which is one of the essential elements for many physiological processes, but its higher concentration, makes it toxic (Baccio et al., 2005). Zn significantly changes mitotic activity (Rout and Das, 2003), disturbs membrane integrity and permeability (Stoyanova and Doncheva, 2002) and finally kills the cell of plants (Chang et al., 2005). Zn causes necrosis of shoot (Mahmood et al, 2005) and inhibits root development (Lingua et al., 2008). Cd, Pb and Zn reduce the uptake of essential elements like Mn, Fe, K, Mg and Ca in plants (Wu et al., 2007). This reduction causes toxicity in plants in term of reduction in plant weight and length.

Seedling and seed germination stage of plant life are sensitive to environmental factors such as HMs pollution so many researchers consider this stage is good for analysis of change in plant growth parameters under the influence of HMs (Abedin and Meharg, 2002). A mixture of HMs are present in the soil, the presence of one pollutant can increase or decrease the adverse impacts of other pollutant. In the past, the effects of the single HM on plants were investigated by many researchers (He et al., 2008; Yang et al., 2009). The joint affects of HMs were investigated by few researchers (Farooqi et al., 2009; Hauang et al., 2009). However, the study of combine toxicity of Cd/Pb, Cd/Zn and Pb/Zn on plants seedlings needs more attentions by researchers through the world. The present study was undertaken to investigate the toxicological effects of Cd, Pb and Zn alone and in mixture forms (Cd/Pb, Cd/Zn and Pb/Zn) on seedlings biomass (shoot and root fresh and dry weights), seedling lengths (shoot and root lengths), cell size, shoot diameter, number of leaves, mitotic index and HMs accumulation in *S. oleracea* seedling.

2. Methodology

2.1 Soil preparation

Table 1 summarizes the basic properties of soil used for this experiment. Soil was collected from an uncontaminated site of Peshawar, Pakistan. The soil was air dried and passed through a 5 mm sieve. The soil was spiked (mg/kg) with Pb (Pb1= 300, Pb2= 400 and Pb3= 500), Cd (Cd1= 0.5, Cd2= 1 and Cd 1.5), Zn (Zn1=250, Zn2= 500, Zn3= 700), Cd/Pb (Cd1/Pb1= 0.5/300, Cd2/Pb2=1/400, Cd3/Pb3= 1.5/500), Cd/Zn(Cd1/Zn1= 0.5/250, Cd2/Zn2=1/500, Cd3/Zn3=1.5/700) and Pb/Zn(Pb1/Zn1= 300/250, Pb2/Zn2= 400/500, Pb3/Zn3= 500/700). The soil after spiking with HMs was thoroughly mixed, air dried and passed through a 5 mm sieve. The plastic pots which were filled with 500 g of HMs spiked soil and four replicates were prepared for each treatment.

Table 4.1Basic properties of soil used for this study

Parameter	Values
pH	7.7
Organic matter (%)	1.6
EC (dS/m)	6.71
Soil particle size (%)	
< 20 µm	7.6
20-62 µm	66
62-250 µm	25.7
250 µm – 1mm	0.577

2.2 Seedling preparation

The seeds of *S. oleracea* were disinfected with 30% (w/w) H_2O_2 solution for 10 min, followed by thorough washing with deionized water. The disinfected seeds were germinated in petri dishes inside the folds of wet filter papers at 28±1 ^{0}C. After four days uniform seedlings (4) were cultivated in each plastic pot. This experiment was performed in a greenhouse with day temperature of 25 ± 4^{0}C and night temperature of 19 ± 3^{0}C. The plants were under sun light of 12 h and relative humidity of 65 ± 2%. The seedlings were irrigated with deionized water and pots re-randomized on regular basis so that each plant get same amount of light and temperature. After 2 weeks of cultivation, the seedlings were harvested and washed with deionized water to remove soil particles.

2.3 Recording growth parameters

Root and shoot fresh weights, root and shoot lengths and diameter were recorded with the help of electronic balance, ruler and screw gage respectively. The plants were dried in oven at 80 ^{0}C for 3 days and dry weights were recorded.

2.4 Cell size

The leaves cell size of *S. oleracea* seedlings were recorded with microscope with a micro scale on its eye piece.

2.5 Mitotic index

Fifteen root tips of germinated roots of each treatment were fixed in a mixture of ethanol-acetic acid (3:1) for 24 h, hydrolysed in 1M HCl for 10 min and stained in 2% aceto-orcine. The root tips then were cut off in a drop of 45% acetic acid, macerated and squashed (Sharma and Sharma, 1980). The prepared slides were carefully observed under light microscope and the mitotic index (MI) were calculated with the formula

MI (%) = (Number of dividing cells/Total number of cells) × 100

2.6. HMs analysis

Dried plants were ground and 0.5 g weighted and took into acid washed, dry digestion tube. Acid mixture (15 ml) of HNO_3, $HClO_4$, and H_2SO_4 (5:1:10) was added and the samples were left overnight. On the next day the digestion tubes were placed on a digestion block at a temperature of 80^0C for 1 h and then raised to120-130^0C till clear solution obtain. After complete digestion the transparent solutions were filtered into acid washed volumetric flasks and make the volume 50 ml with double deionized water, The concentrations of HMs in the digested solutions were measured using atomic absorption spectrometer (Analyst 700 PerkElmer) in the laboratory of National Center of Excellence in Geology, university of Peshawar, Pakistan.

2.7. Statistical analysis

The data were statistically analyzed using statistical packages SPSS 17. The data were analyzed by one way of variance (ANOVA) to confirm the variability and validity of results. Duncan's multiple range test (DMRT) was performed to determine significance between the treatments with a significance level of $p < 0.05$. Linear regression analysis was also performed to establish the relationships between the HM concentrations in the plants tissues and the HM concentrations in the soil.

3.0 Results

3.1 Effects of HMs on plant fresh and dry weight

Figures 4.1, 4.2, 4.3 and 4.4 present the toxicological effects of Cd, Pb, Zn individually and in mixture on plant biomass (shoot and root fresh and dry weights). HM concentrations had a significant ($p<0.05$) adverse impacts on the biomass of *S. oleracea* seedlings (Table 4.2). At the highest application rate of Cd the shoot and root fresh and dry weight were decreased by 49.2%, 50%, 38% and 41%, respectively (Table 4.3) as compared to control, respectively. Similarly, at the highest dose of Pb shoot and root fresh and dry weight decreased by 44%, 45%, 30.8% and 35% (Table 4.3) as compared to control, respectively. The addition of high dose of Zn decreased shoot and root fresh and dry weight by 40%, 47.5%, 29.7% and 32% (Table 4.3) as compared to control, respectively.

The reductions in shoot and root fresh and dry weight were 60%, 68%, 61% and 63%, respectively as compared to control, under the influence highest dose of Cd/Pb. Similarly, at the highest dose of Cd/Zn, shoot and root fresh and dry weight decreased by 57%, 62.5%, 35% and 64.3%, respectively (Table 4.3) as compared to control. The addition of high dose of Pb/Zn decreased shoot and root fresh and dry weight by 32%, 35%, 33% and 57.1% (Table 4.3) as compared to control, respectively.

With both Cd and Pb added to soil the toxicity of seedlings biomass was further increased but was less than the sum of the toxicity with both Cd and Pb alone. The toxicity in the biomass of seedlings under the influence of combine Cd/Zn was less than the toxicity due to Zn and more than the Cd toxicity alone. The combine toxicity of Zn/Pb was less than toxicity of Zn and Pb alone on spinach biomass. The dry weights of both shoot and root were more affected than their fresh weights of spinach seedlings. The root fresh weigh and dry weight were affected more than the shoot fresh and dry weights.

Table 4.2. ANOVA analysis of growth parameters of S. *oleracea*

Parameters		Sum of Squares	df	Mean Square	F	Sig.
leaves	Between Groups	103.112	18	5.728	7.307	.000
	Within Groups	44.688	57	.784		
	Total	147.799	75			
Shoot length	Between Groups	28.843	18	1.602	48.103	.000
	Within Groups	1.899	57	.033		
	Total	30.742	75			
Shoot fresh weight	Between Groups	47.566	18	2.643	191.880	.000
	Within Groups	.785	57	.014		
	Total	48.351	75			
Shoot dry	Between Groups	10282.455	18	571.248	6897.875	.000
	Within Groups	4.720	57	.083		
	Total	10287.176	75			
Shoot diameter	Between Groups	14.357	18	.798	77.544	.000
	Within Groups	.586	57	.010		
	Total	14.943	75			
Root length	Between Groups	148.518	18	8.251	171.959	.000
	Within Groups	2.735	57	.048		
	Total	151.253	75			
Root fresh weight	Between Groups	.827	18	.046	14.388	.000
	Within Groups	.182	57	.003		
	Total	1.010	75			
Root dry weight	Between Groups	4.358	18	.242	5.140	.000
	Within Groups	2.685	57	.047		
	Total	7.043	75			
Cell size	Between Groups	63.277	18	3.515	21.396	.000
	Within Groups	9.365	57	.164		
	Total	72.642	75			
Mitotic index	Between Groups	13784.621	18	765.812	373.192	.000
	Within Groups	116.968	57	2.052		
	Total	13901.589	75			

Table 4.3 Comparison of reduction of growth parameters (%) of different HM treatments than the control treatment of *S. oleracea* seedlings

Treatments	Seedlings biomass				Length				No of leave	MI
	Shoot fresh weigh	Root fresh weight	Shoot dry weight	Root dry weight	Shoot length	Root length	Shoot diameter	Cell size		
Cd 1	26.9	29	21.5	25.0	25.9	34.2	3.00	20.0	25.0	7.30
Cd2	48.8	49	35.0	36.0	26.7	37.8	6.30	30.0	31.2	18.9
Cd3	49.2	50	38.0	41.0	29.3	56.9	7.80	45.0	50.0	22.7
Pb 1	26.0	28	16.9	19.0	19.0	13.8	2.80	20.0	21.8	6.40
Pb 2	39.0	32	29.0	31.0	24.1	23.1	5.80	26.0	29.9	15.0
Pb 3	44.0	45	30.8	35.0	25.0	46.2	7.10	42.5	41.0	20.3
Zn 1	25.0	27.5	12.0	15.0	14.9	11.3	2.30	18.0	19.0	8.10
Zn 2	37.0	37.5	27.0	30.9	19.2	16.5	4.50	21.0	25.0	13.0
Zn 3	40.0	47.5	29.7	32.0	22.0	31.8	5.10	35.0	37.5	18.9
Cd1/Pb 1	50.0	55.0	38.0	40.0	37.9	41.8	3.90	22.0	43.7	8.00
Cd2/Pb 2	55.0	59.0	51.0	55.7	48.3	57.3	9.20	33.0	50.0	24.7
Cd3/Pb 3	60.0	68.0	61.0	63.0	55.2	66.9	10.2	65.5	62.5	27.0
Cd1/Zn 1	48.0	50.0	34.0	35.7	27.5	10.0	7.10	46.0	37.5	7.80
Cd2/Zn 2	50.2	52.5	34.9	50	30.0	12.0	8.10	52.0	43.7	18.5
Cd3/Zn 3	57.0	62.5	35.0	64.3	36.9	40.0	9.10	58.0	50.0	24.1
Zn1/Pb 1	26.6	30.0	23.0	25.7	20.0	36.0	4.30	32.5	25.0	9.80
Zn2/Pb 2	28.1	34.0	30.7	32.9	22.0	40.0	7.10	45.0	31.2	13.3
Zn3/Pb 3	32.0	35.0	33.0	57.1	23.8	58.0	8.10	55.0	37.5	16.6

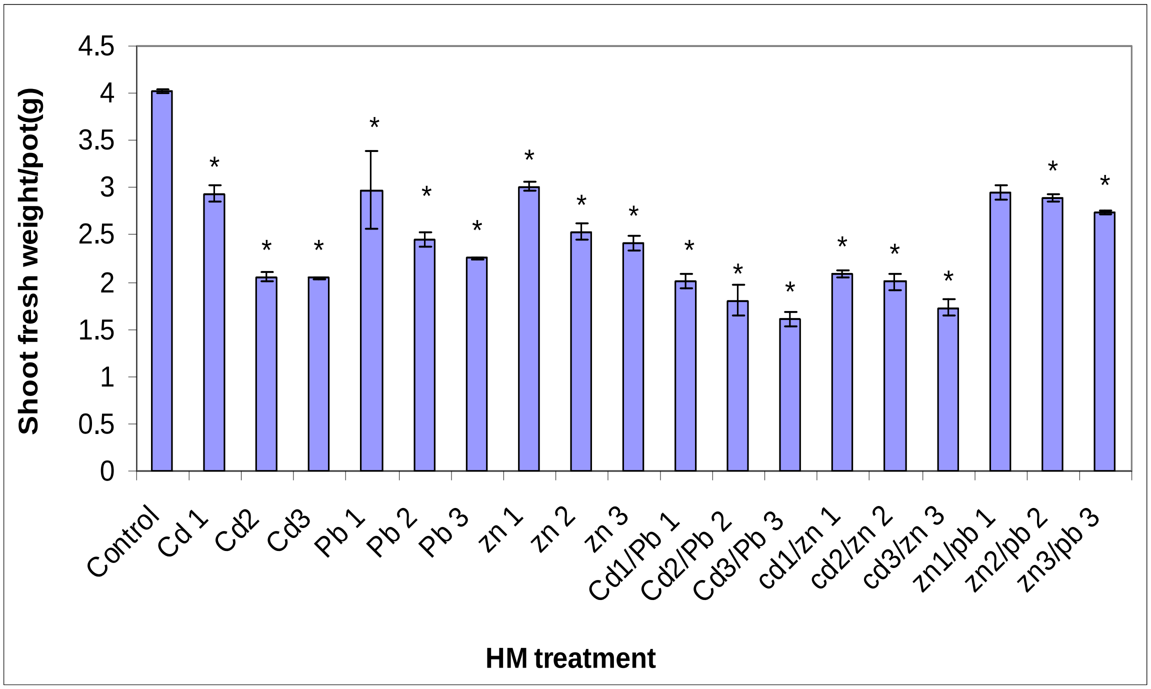

Fig 4.1 Effects of different concentrations of HMs on shoot fresh weight. The error bars indicate the standard deviation, while asterisks (*) indicate significant ($p<0.05$) change from control

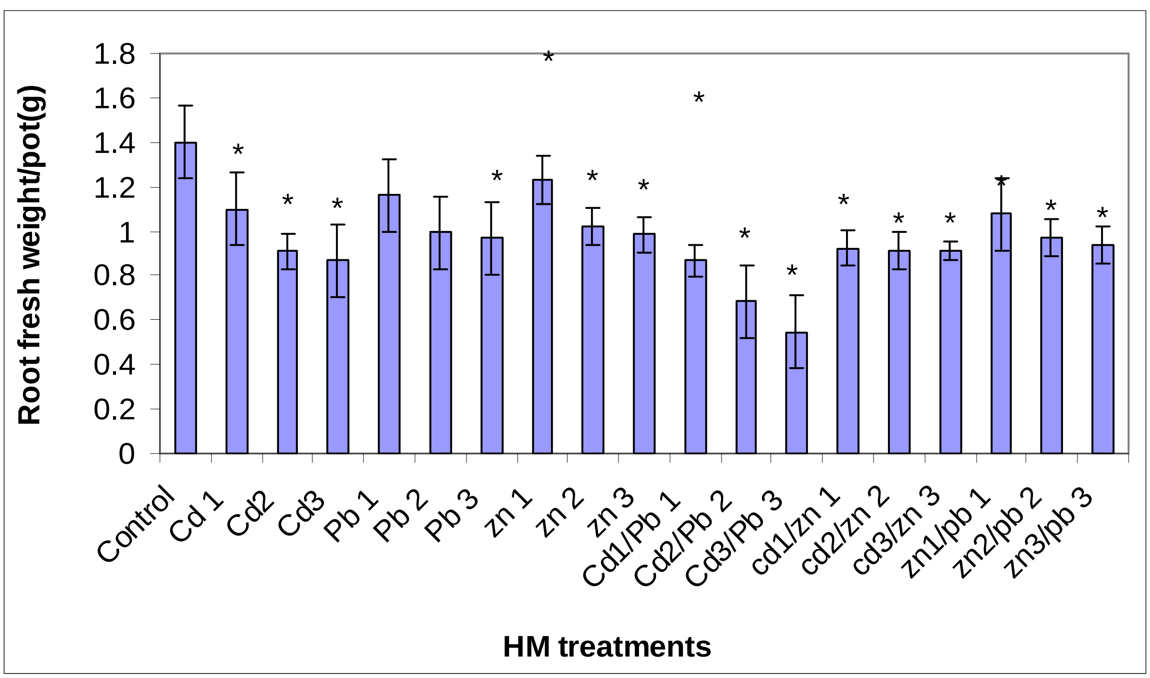

Fig 4.2 Effects of different concentrations of HMs on root fresh weight. The error bars indicate the standard deviation, while asterisks (*) indicate significant ($p<0.05$) change from control

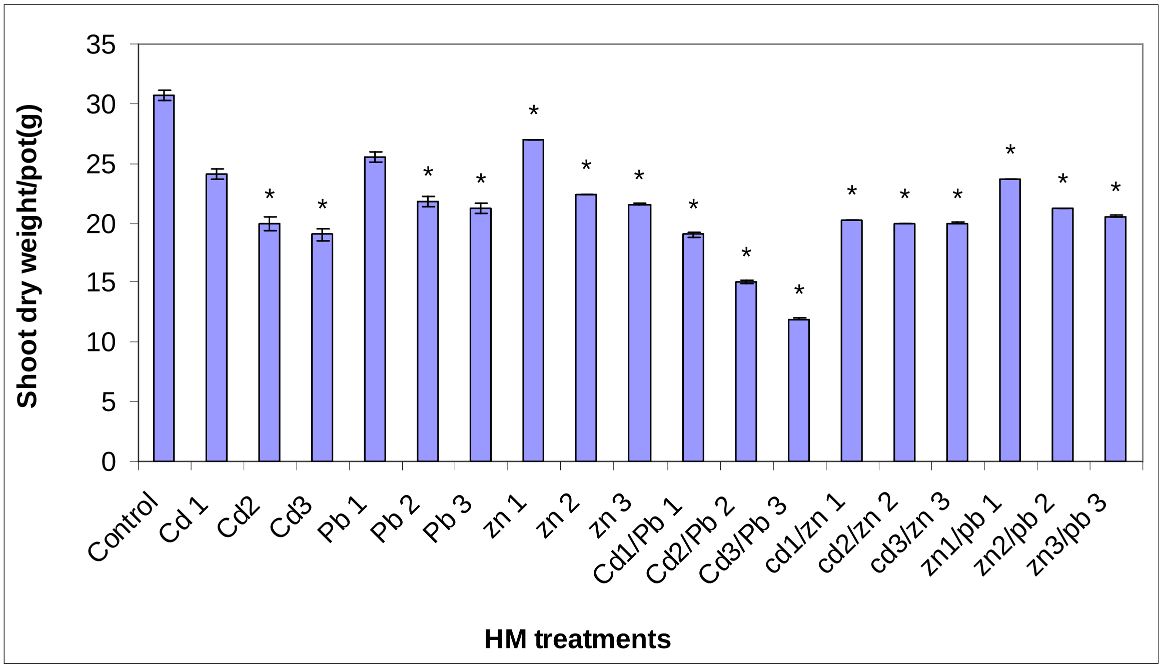

Fig 4.3 Effects of different concentrations of HMs on shoot dry weight. The error bars indicate the standard deviation, while asterisks (*) indicate significant ($p<0.05$) change from control

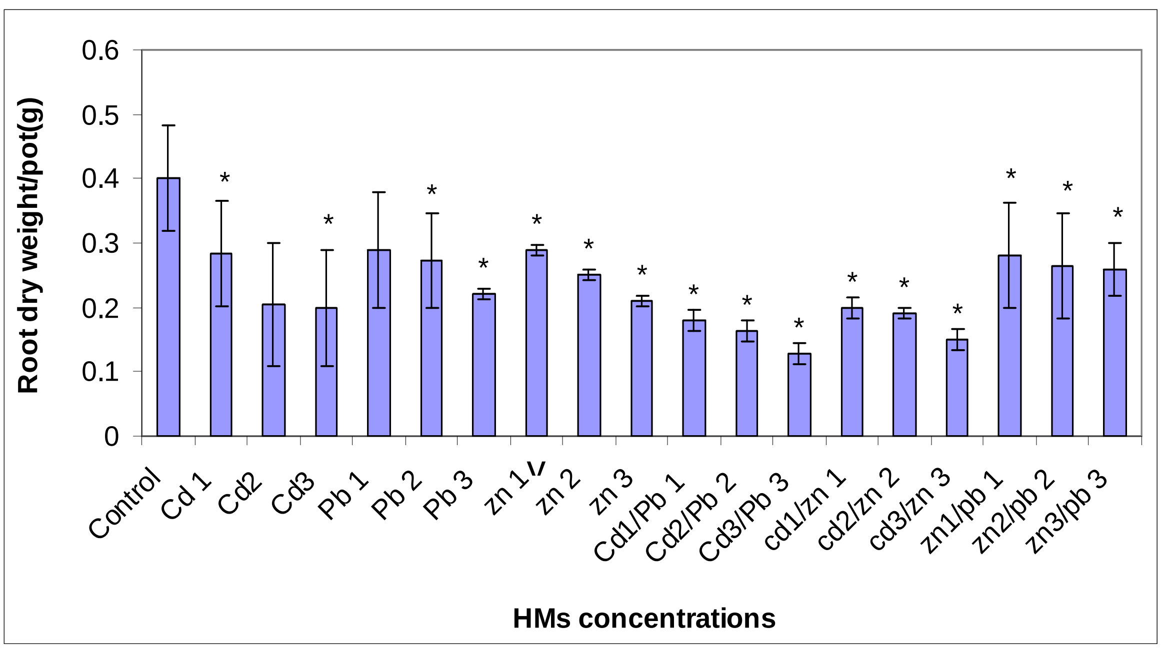

Fig 4.4 Effects of different concentrations of HMs on root dry weight. The error bars indicate the standard deviation, while asterisks (*) indicate significant ($p<0.05$) change from control

3.2. HMs effects on Shoot and root lengths

The data in table 4.2 summarizes that different concentrations of Cd, Pb, Zn, Cd/Pb, Cd/Zn and Pb/Zn had a significant ($p<0.05$) toxicological impacts on shoot and root lengths of *S. oleracea* seedlings. The increasing concentrations of HMs in the soil resulted a decrease in the shoot and root lengths of spinach seedlings (Fig. 4.5, 4.6). At the highest dose of Cd the shoot and root lengths decreased by 29.3% and 56.9%, respectively (Table 4.3) as compared to control. Similarly, at the highest dose of Pb the shoot and root lengths were decreased by 25.0% and 46.2%, respectively (Table 4.3) as compared to control. At the highest dose of Zn the shoot and root lengths were decreased by 22% and 31.6%, respectively (Table 4.3) as compared to control.

However, Cd and Pb spiked soil further decreased both shoot and root lengths. The reduction in the shoot and root length were 55.2% and 66.9%, respectively in comparison with the control under the influence of highest dose of Cd/Pb. The combine toxicity of Cd/Zn in the shoot and root lengths was 36.9% and 40%, respectively as compared with the control. The reduction in shoot and root lengths were 23.8% and 58%, respectively under the combine toxicity of Pb and Zn. In general the root lengths were more affected than shoot lengths.

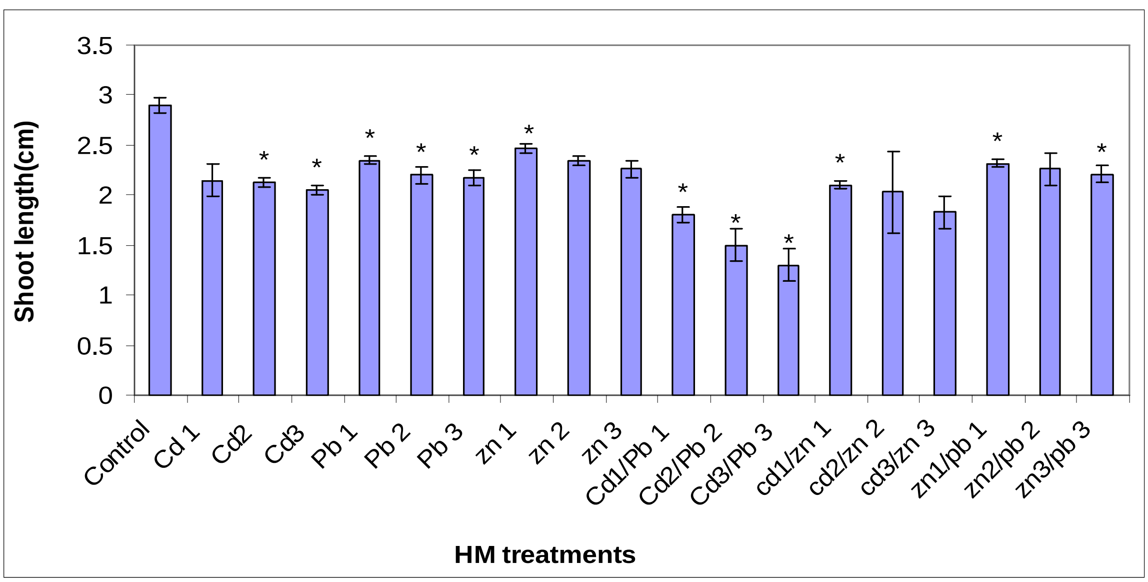

Fig 4.5 Effects of different concentrations of HMs on shoot length. The error bars indicate the standard deviation, while asterisks (*) indicate significant ($p<0.05$) change from control

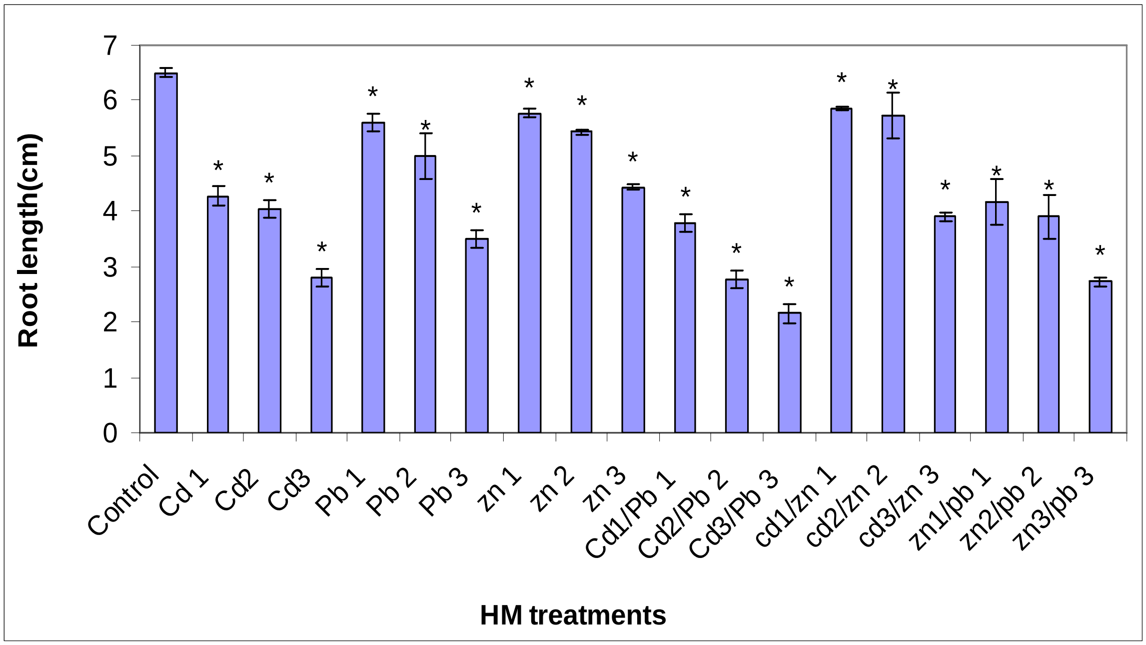

Fig 4.6 Effects of different concentrations of HMs on root length. The error bars indicate the standard deviation, while asterisks (*) indicate significant ($p<0.05$) change from control

3.3. HMs effects on shoot diameter of *S. oleracea* seedlings

Table 4.2 summarizes the effects of HMs on shoot diameter of selected plant seedlings. Different concentrations of Cd, Pb, Zn, Cd/Pb, Cd/Zn and Pb/Zn had a significant ($p<0.05$) toxicological impacts on shoot diameter of *S. oleracea* seedlings. The increasing concentrations of HMs in the soil resulted a decrease in the shoot diameter of *S. oleracea* seedlings (Fig. 4.7). similarly, the reduction in shoot diameter was 7.8%, 7.1%, 5.1%, 10.2%, 9.1% and 8.1%, respectively under the influence of highest doses of Cd, Pb, Zn, Cd/Pb. Cd/Zn and Pb/Zn (Table 4.3) as compared to control.

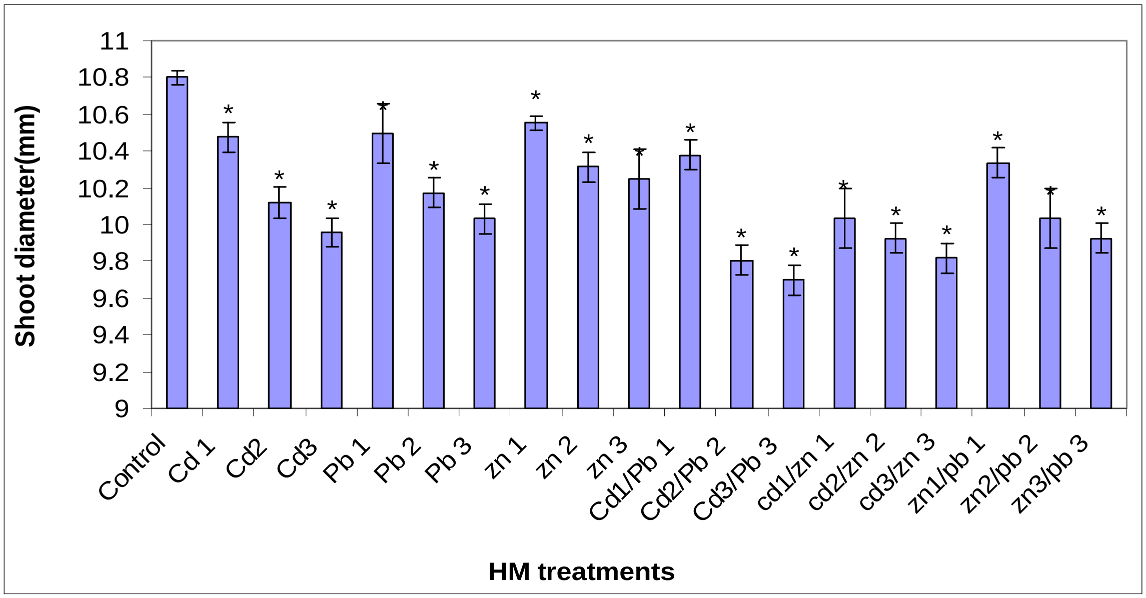

Fig 4.7 Effects of different concentrations of HMs on shoot diameter. The error bars indicate the standard deviation, while asterisks (*) indicate significant ($p<0.05$) change from control

3.4. HMs effects on Cell Size, No of Leaves and Mitotic Index (MI)

The increasing concentrations of HMs in the soil resulted a decrease in the cell size, number of leaves and root MI of *S. oleracea* seedlings (Fig. 4.8, 4.9, 4.10). The reduction in cell size, number of leaves and root mitotic index of *S. oleracea* seedlings was significant ($p<0.05$). At the highest dose of Cd, the cell size, number of leaves and MI showed a reduction of 45%, 50% and 22.7%, respectively (Table 4.3) as compared to control. Similarly, at the highest dose of Pb, the cell size, number of leaves and MI showed a reduction of 42.5%, 41% and 20.3%, respectively (Table 4.3) as compared to control. At the highest dose of Zn, the cell size, number of leaves and MI showed a reduction of 35%, 37.5% and 18.9%, respectively (Table 4.3) as compared to control. The reduction in the cell size, number of leaves and MI were 65.5%, 62.5%, and 27%, respectively as compared to control under the influence highest dose of Cd/Pb. In Cd/Pb spiked soil, cell size, number of leaves and MI were further decreased but the decrease was less than the sum of reduction due to both Cd and Pb alone. The reduction in the cell size, number of leaves and MI were 58%, 50%, and 24.1%, respectively as compared to control under the influence highest dose of Cd/Zn. The reduction in the cell size, number of leaves and MI were 55%, 37.5%, and 16.6%, respectively as compared to control under the influence highest dose of Cd/Zn.

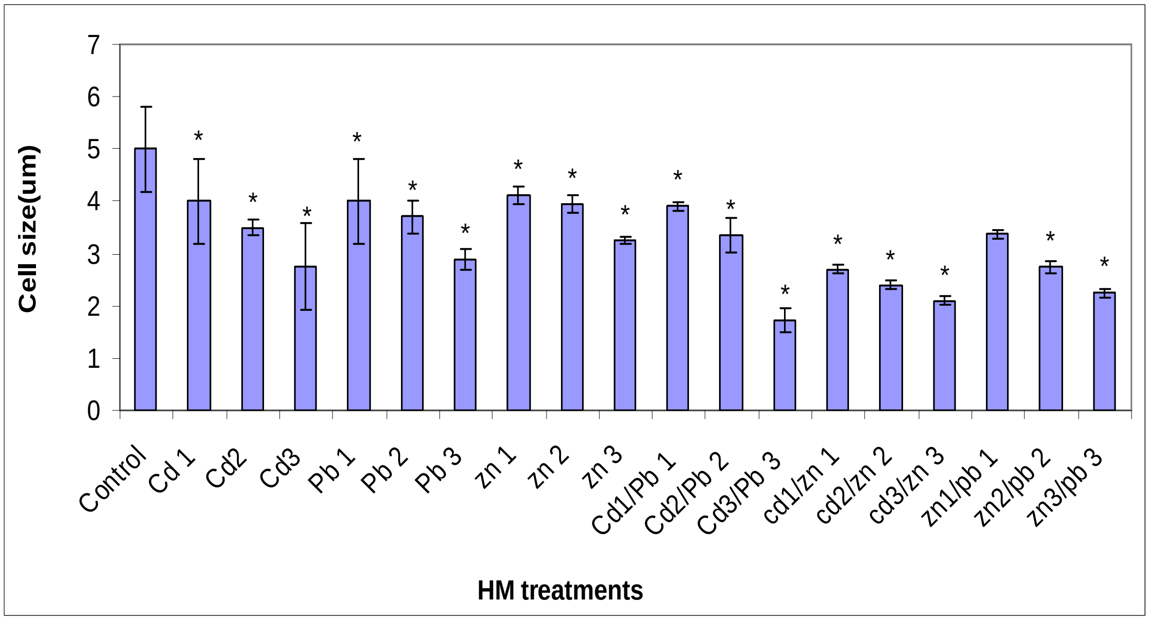

Fig 4.8 Effects of different concentrations of HMs on cell size. The error bars indicate the standard deviation, while asterisks (*) indicate significant ($p<0.05$) change from control

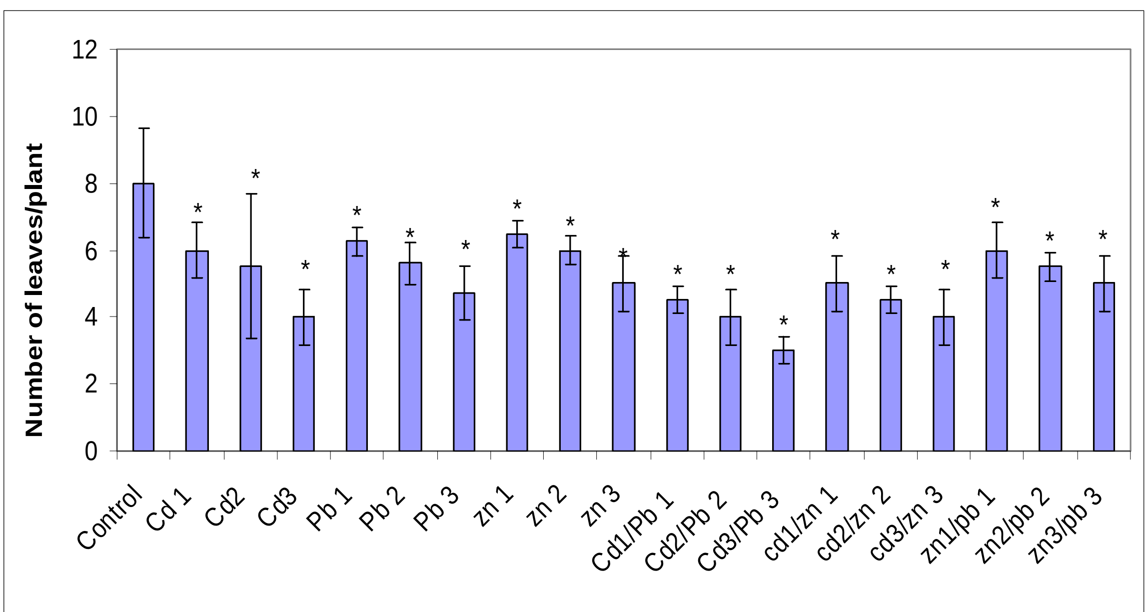

Fig 4.9 Effects of different concentrations of HMs on number of leaves. The error bars indicate the standard deviation, while asterisks (*) indicate significant ($p<0.05$) change from control

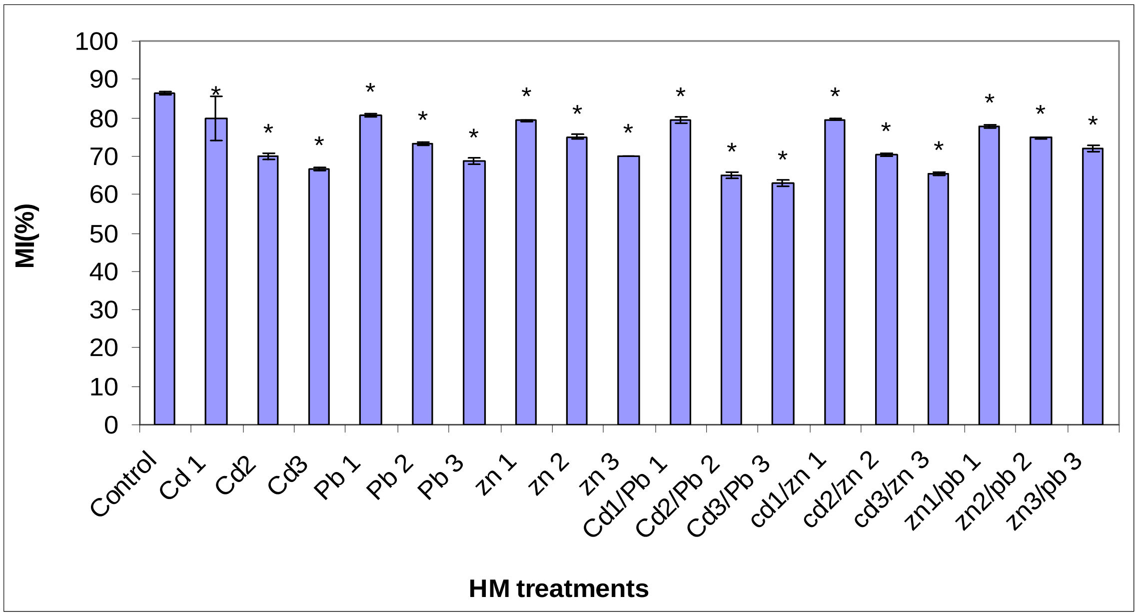

Fig 4.10 Effects of different concentrations of HMs on MI. The error bars indicate the standard deviation, while asterisks (*) indicate significant ($p<0.05$) change from control

3.5. Up take of HM

The relationships between the Cd concentrations in soil and in seedling tissue are given in figure 4.11. All three relationships ie between the single Cd concentration in soil and seedlings (Fig 4.11-a), between Cd/Pb in soil and in seedlings (Fig 4.11-b) and between Cd/Zn in soil and in seedlings (Fig 4.11-c) show that with increasing concentrations of Cd in soil whether in single form or in mixture its content in the seedlings increased. Seedlings were accumulated Cd 3440%, 1760% and 323% of its content in the soil spiked with single Cd, Cd/Pb and Cd/Zn, respectively (Fig. 4.14), Similarly, the concentrations of Pb in seedlings showed increase with increasing concentration in the soil. The regression analysis showed positive relationships between the concentrations of Pb in soil and seedlings in all three treatments ie single Pb, Pb/Cd and Pb/Zn (Fig 4.12-a, Fig 4.12-b, Fig 4.12-c). Seedlings were accumulated Pb 35.025%, 30.65% and 10.12% of its content in the soil spiked with single Pb, Pb/Cd and Pb/Zn, respectively (Fig. 4.14), The regression analysis showed strong positive relationship between the Zn contents in soil and seedlings in single Zn, Zn/Cd and Zn/Pb treatments (Fig 4.13-a, Fig 4.13-b, Fig 4.13-c). Zn was accumulated by seedlings 27.54%, 26.9% and 24.8% from the soil in single Zn, Zn/Cd and Zn/Pb, respectively.

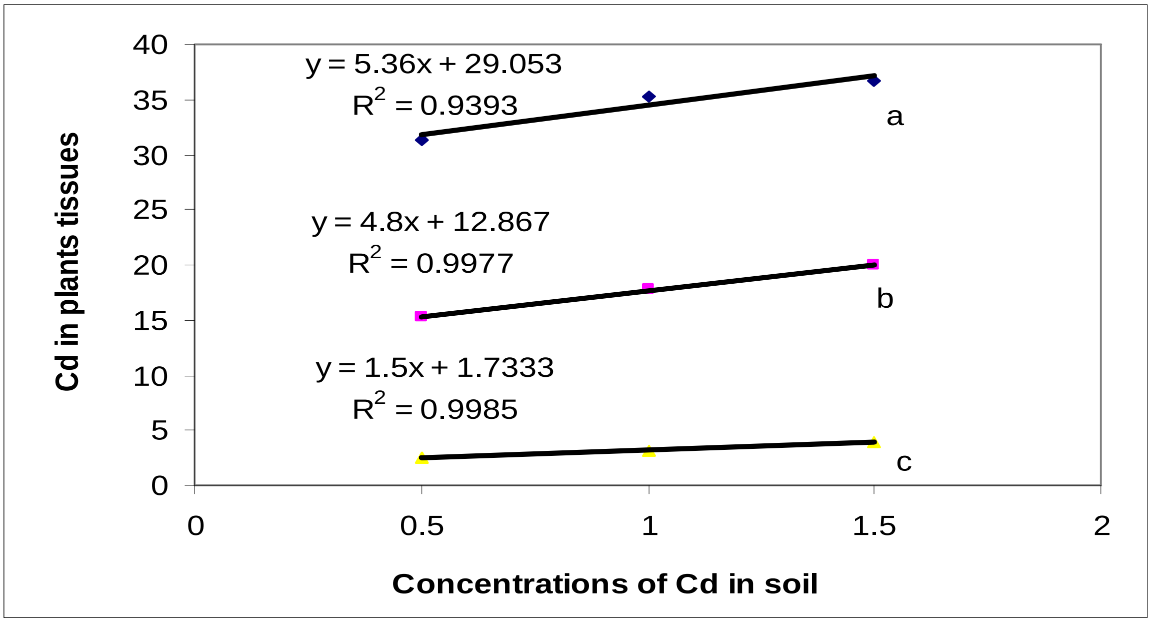

Figure 4. 11. Linear regressions model for Cd concentrations in soil and seedlings tissues on dry weight basis. (a) relationship between soil Cd and seedlings in single Cd treatments. (b) Relationship between soil Cd content and seedlings in Cd/Pb treatments. (c) Relationship between soil Cd content and seedlings in Cd/Zn treatments.

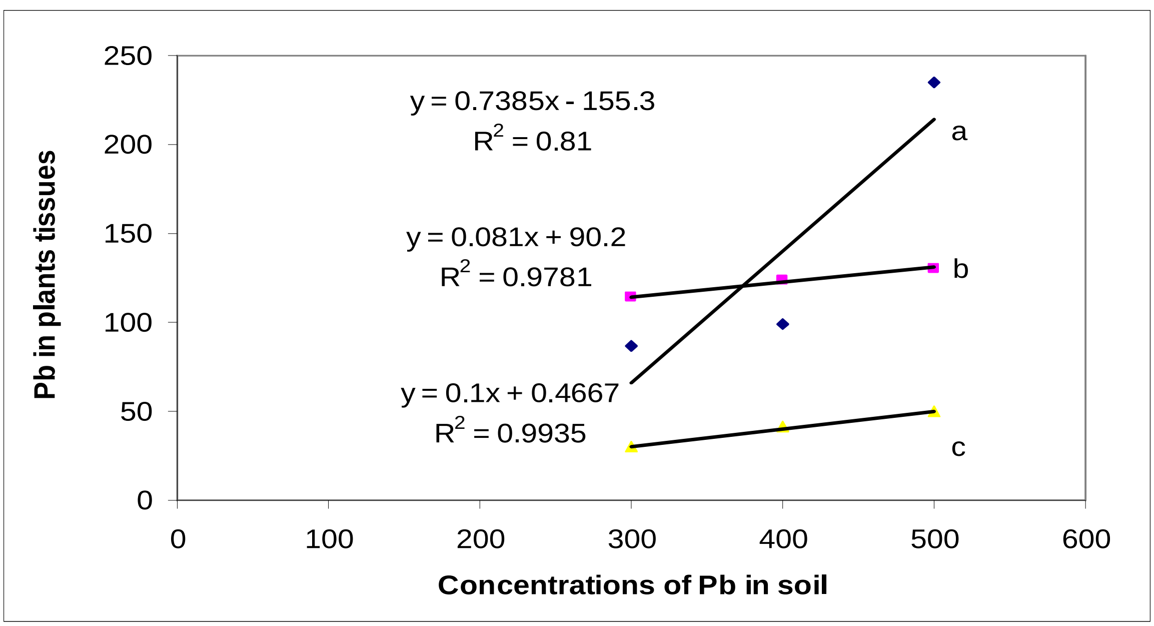

Fig 4.12 Linear regressions model for Pb concentrations in soil and seedlings tissues on dry weight basis. (a) relationship between soil Pb and seedlings in single Pb treatments. (b) Relationship between soil Pb content and seedlings in Cd/Pb treatments. (c) Relationship between soil Pb content and seedlings in Pb /Zn treatments

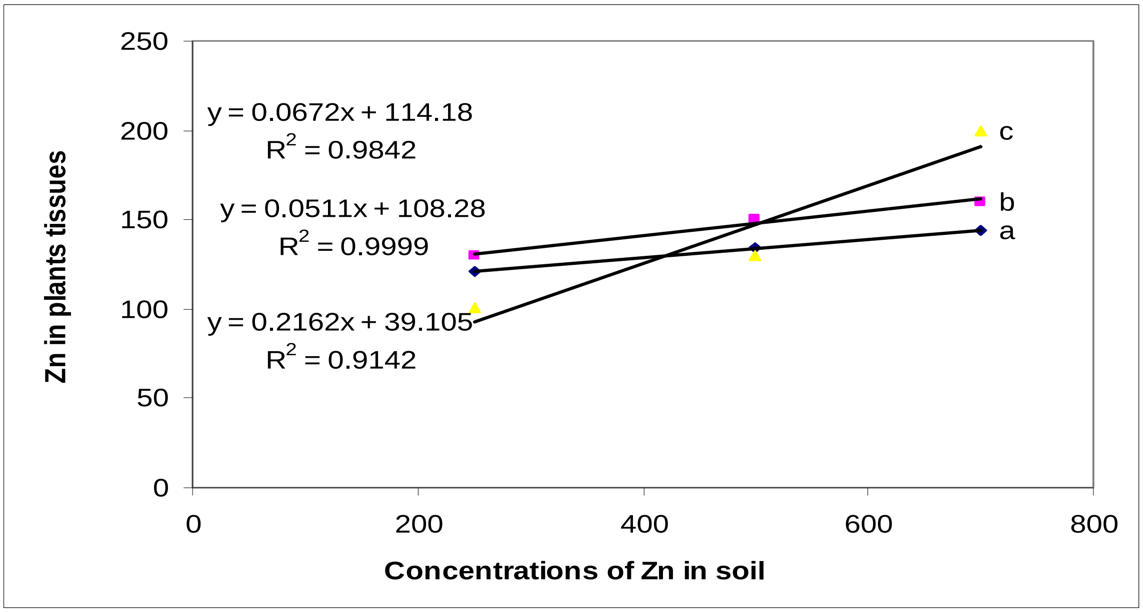

Fig 4.13 Linear regressions model for Zn concentrations in soil and seedlings tissues on dry weight basis. (a) relationship between soil Zn content and seedlings in single Zn treatments. (b) Relationship between soil Zn and seedlings in Zn /Pb treatments. (c) Relationship between soil Zn content and seedlings in Cd/Zn treatments

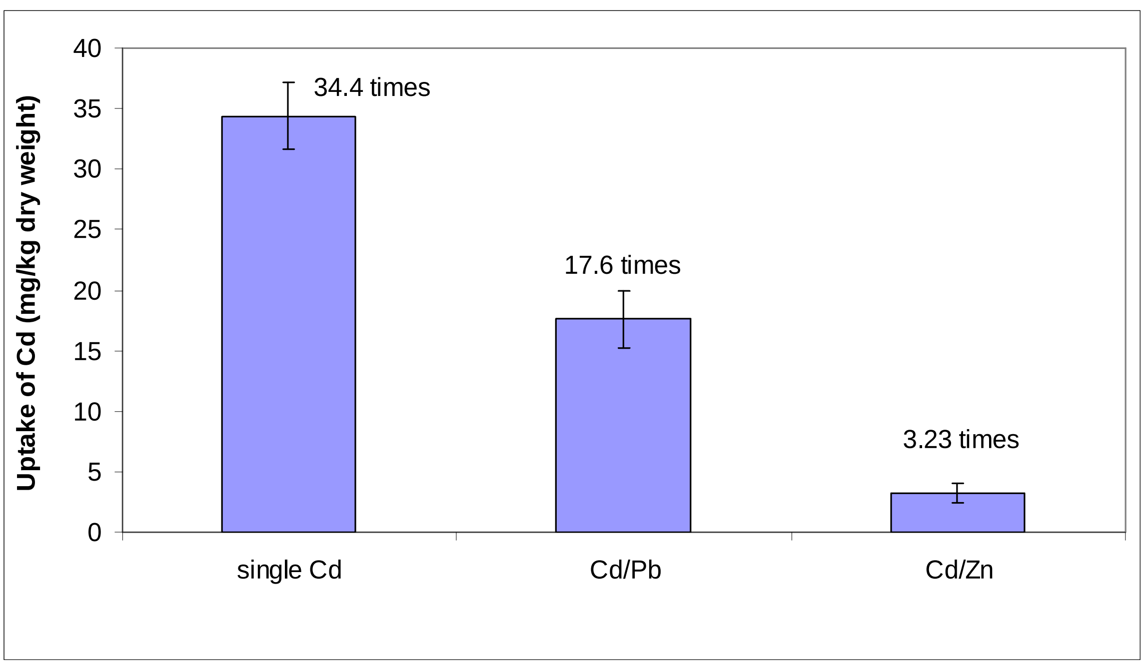

Fig 4.14 Comparison of Cd up take from different treatments. The error bars indicate the standard deviation

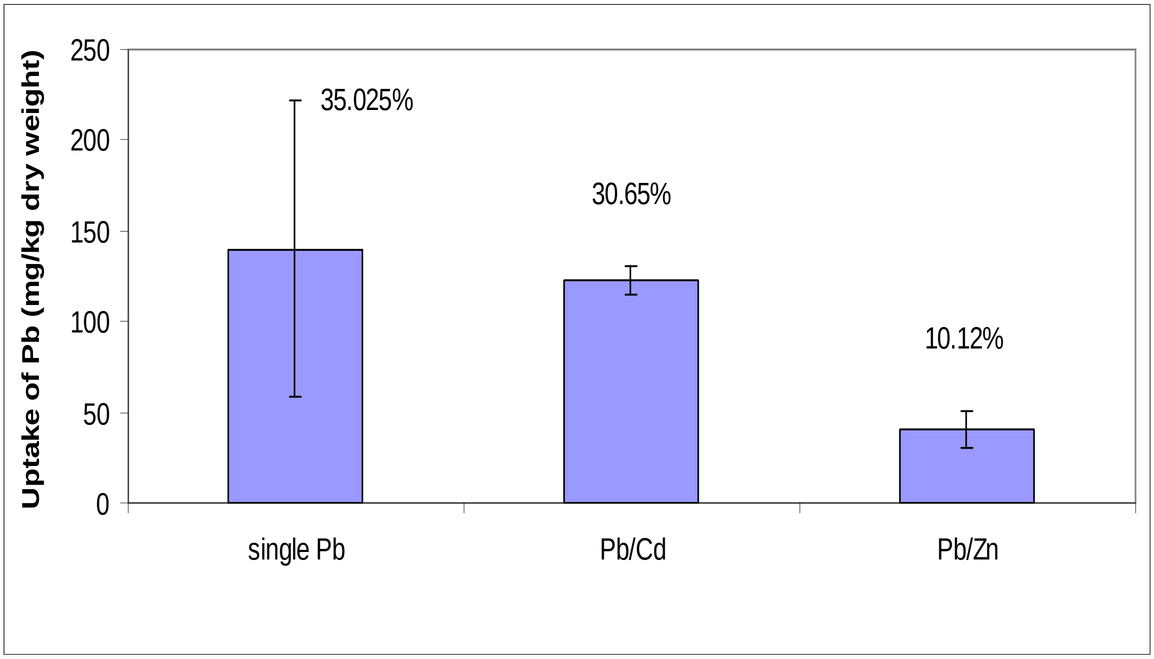

Fig 4.15 Comparison of Pb up take from different treatments. The error bars indicate the standard deviation

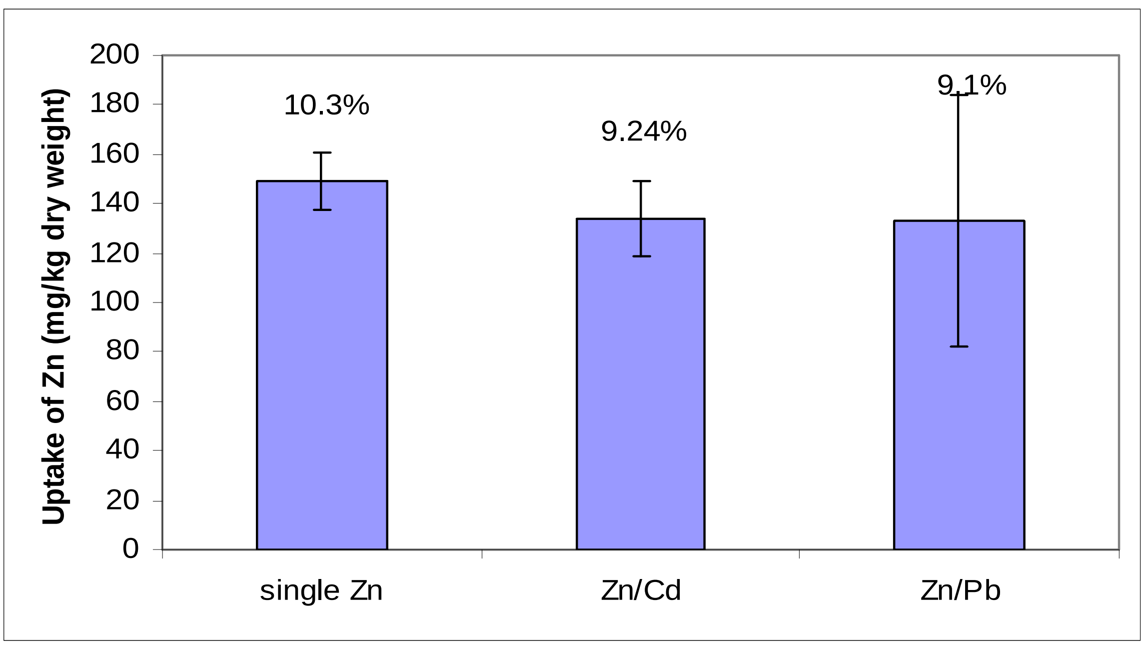

Fig 4.16 Comparison of Zn up take from different treatments. The error bars indicate the standard deviation

4.0, Discussion

Results of the present and previous studies (Khan et al., 2008; Devi et al., 2007; An et al., 2004; Sun et al., 2008; Nedjimi and Daoud, 2009; Farooqi et al., 2009;Lingua et al., 2008) present that plants grow on HMs contaminated soil contain high concentrations of HMs in their tissues. HMs affect plants adversely because of their toxicity in plant growth, reduction in plant yield and inhibition of enzymatic activities (Zeng et al., 2008).

Cd is one of the HMs that causes phytotoxicity, inhibited plant growth and the toxicity increase with increasing its concentration in soil. In the present study, increasing concentrations of Cd has significantly ($p<0.05$) reduced shoot and root fresh and dry weights. The present results are agreed with the results of previous studies. An et al, (2004) observed drop in shoot and root growth in *Cucumis sativus* with increasing concentrations of Cd in its growing medium. Increasing concentrations of Cd in growing medium decreases shoot and root biomass as observed by Shukla et al. (2003). Similarly, Devi et al, (2007) also observed reduction of root and shoot dry weights in rice with increasing concentrations of Cd. However, the results of the present study are inconsistent with the results of few studies on plant seedlings. Vogel-Mikus et al, (2006) have not observed any toxicity in maize and sunflower with increasing concentrations of Cd. Similarly Lin et al, (2007) observed that Cd concentrations from 0.3 to 33 mg/kg enforced the growth of wheat seedlings.

Increasing concentrations of Cd in soil produced significant ($p<0.05$) toxicity in *S. oleracea* seedlings in terms of shoot and root lengths. Cd reduced shoot length up to 29 %, while reduced root length up to 72%. The present results of root and shoot lengths are consistent with the findings of Shukla et al, (2003) who found a reduction in root and shoot lengths with increasing concentrations of Cd in growing mediums of plants. Similarly Liu et al, (2004) reported that reduction of formation of new cells under the influence of Cd leads reduction in shoot and root lengths.

Other growth parameters like number of leaves, cell size, shoot diameter and MI were significantly ($p<0.05$) affected with increasing concentrations of Cd in soil ecosystem. The toxicity in term of shoot diameter was not severe as compared to other parameters. In case of cell size and MI, Cd was toxic for *S. oleracea* seedlings. It reduced the cell size up to 50% from its normal size in control treatment, while reduced MI up to 22%. Jun-yu et al, (2008) also observed reduction of MI in rice with increasing concentrations of Cd. Cd causes cellular damage like swelling of mitochondria, cracking of epidermis, rough endoplasmic membrane (Jin et al., 2008), which may be the reason of reduction in cell size.

S. oleracea seedlings produced toxicity with increasing concentrations of Pb in terms of all growth parameters. Increasing concentrations of Pb significantly ($p<0.05$) reduced seedling biomass, seedling length, cell size, number of leaves, seedling diameter and MI of *S. oleracea* seedlings. The results of the present study consistent with results of previous studies. Increasing concentrations of Pb in growing medium dropped the growth of shoot and root in *Cucumis sativus* (An et al., 2004). Farooqi et al, (2009) found a decrease in seedling dry weight of *A. lebbeck* with increasing concentrations of Pb. Liu et al, (2004) reported that reduction of formation of new cells under the influence of Pb leads a reduction in shoot and root lengths. Increasing concentrations of Pb adversely effect seedling length in *Avicennia marina* (MacFarlane and Burchett, 2002). The toxicity of Pb in *S. oleracea* seedling in the form of reduction in shoot and root fresh and dry weights is less than the Cd. Zayed and Amin, (2002) observed a decrease in dry weights of *T. Alexendrium* with increasing up take of Pb.

Zn is an essential element for plants, but its excess can significantly damage plants (Wang et al., 2009). This study showed that increasing concentrations of Zn were responsible for increase toxicity in seedlings. Shoot and root fresh and dry weights were reduced with increasing Zn concentrations. Zn reduced plant biomass because of creation of deficiency of macronutrients such is P (Marschner, 1995). Mahmood et al, (2005) found a reduction in growth of corn with increasing concentrations of Zn. Zn has also significantly reduced MI in *S. oleracea* seedling and these result are agreed with those reported by Rout and Das, (2003).

Metals in the soil are always present in the form of mixture (Spurgeon et al., 1994) Bioaccumulation of one HM in the mixture inhibits or enforce the accumulation of other HM (Peralta-Videa et al., 2002). *S. oleracea* seedling accumulated Cd from its treatments of our experiment in the order of single Cd (3440%) > Cd in Cd/Pb (1760%). Similarly the up take of Pb was in order of Pb (35.025%) > Pb in Cd/Pb (30.85%). The up take pattern of Cd and Pb in Cd/Pb showed that both Cd and Pb affect the up take of each other in antagonistic way. The growth parameters of spinach seedling showed the dose response for Cd and Pb i.e with increasing concentrations of these metals in the plant tissues increased the adverse impacts on growth of plant. HMs in mixture behaved differently than individual HMs due to their interactions with each other. The up take pattern of both Cd and Pb were reflected in the growth parameters of seedlings. The toxicities of Cd and Pb in form of Cd/Pb in terms of al most all growth parameters were more than the individual toxicities of Cd and Pb but less than their additive toxicities. An et al, (2004) produced the same result in studying *Cucumis sativus.* Zhiqiang et al, (2009) found additional effects on biomass, root and shoot lengths of Chinese cabbage with increasing concentrations of Cd along with Pb. On the other hand, Luan et al, (2008) found no response in shoot growth with the combine addition of Cd/Pb in soybean. Roots of *S. oleracea* seedlings were mostly affected with combine Cd/Pb as compared to shoot (Table 4.3). Combine addition of HMs affects mostly root of plants because HMs accumulate mostly in root and then a small amount is transfer to shoot (An et al., 2004).

The combine toxicity of Cd/Zn showed different pattern in spinach seedlings. The order of toxicity in terms of shoot fresh weight was Zn > Cd/Zn >Cd. In case of single toxicity in terms of shoot fresh weight the Zn was more toxic than Cd. The combine toxicity is less than Zn because Cd reduces Zn up take (Weihong et al., 2009).

The combine toxicity of Cd/Zn was more than the toxicity of single Zn and Cd in terms of seedling biomass, root length, cell size and mitotic index. The results are consistent with the study of Weihong et al., (2009) and Xu *et al*., (2006)**.** In some cases like shoot fresh weight, cell size and MI the combine toxicity was less than the toxicity due to Zn alone. The reason may be the reduction in up take of Zn by Cd, while in case of shoot length both Zn alone and Cd/Zn have positive impact. Like Cd/Pb and Cd/Zn, the co-presence of Zn and Pb also resulted the changes in the up take of them. In case of *S. a oleracea* seedlings the up take of both Zn and Pb was affected in their combine form. The up take of Pb was in order of Pb (35.025%) > Pb in Zn/Pb (10.12%). Similarly the up take of Zn was in order of Zn (27.5%) > Zn in Zn /Pb (24.8%). The up take pattern of Zn and Pb in Cd/Pb showed that both Zn and Pb affect the up take of each other in antagonistic way the results are agreed with MacFarlane and Burchett, (2002). Pb reduced the Zn up take by 9.8% on the other hand Zn reduced the up take of Pb by 71.1%. The up take pattern is reflected in the growth parameters of *S. oleracea* seedlings. In case of *S. oleracea* seedlings the toxicities due to Zn and Pb in all its three treatments were in order of Zn (45.2% average reduction of all growth parameters from control treatment) > Pb (39.4% average reduction of all growth parameters from control treatment) > Zn/Pb (35% average reduction of all growth parameters from control treatment). The reduction of growth due to Pb was 39% and due to Zn was 45%. The combine toxicity should be 84% but the actual toxicity observed was 35%, even lower than the single toxicities. The difference of expected and actual combine toxicities was due to the antagonistic affects of Zn and Pb on the up take of each other.

Chapter: 5 HMs toxicities on coriander (*Coriandrum saivum*) seedlings

1. Introduction

The vegetables grow with wastewater contain HMs, which are not only dangerous to vegetables but also to animals and human beings as well (Murtaza et al., 2003; Khan et al., 2008b). Under specific condition the HMs accumulate in the soil to the levels, which are toxic and can lead ecological consequences (Wang et al., 2005). HMs enter to human body through many ways but The food chain contamination is one of the important pathways which contributes 90% HMs as compare to other routes like inhalation and dermal contact (Loutfy et al., 2006).

HMs are toxic for plants they can reduce plant yield, affect leaf and root growth and inhibit enzymatic activities (Zeng et al., 2008). Many HMs like Cd and Pb are non essential for plant growth. Cd is responsible for reduction in weight of plant (Kosobrukhov, 2004), reduces chlorophyll content and thus negatively impacted photosynthesis (Kambhampati et al., 2005) and respiration in plants (Liang et al., 2005). Furthermore, Cd induces changes in plants at all biochemical, physical and genetic levels, which are responsible for reduction in the growth of plants (Nouairi et al., 2006a), leaf chlorosis, and leaf and root necroses (Baryla et al., 2001). Similarly, reduces root growth because of the reduction of the rate of new cell production (Liu et al., 2004).

Like Cd, Pb also adversely affects the photosynthesis in plants by reducing chlorophyll content due to reducing the uptake of chlorophyll essential elements like Mg and Fe (Bruzynski, 1987). Pb has significant negative impacts on seedling dry mass, root and shoot length ((Juseph et al., 2002), adversely affects the process of respiration and metabolism of plants (Paolacci et al., 1997). The study of the effects of individual HMs in control environment provide us useful information about HMs, but these information can not reflect the real exposure of plants to HMs in natural environment. The natural environment contains a mixture of different HMs. (Feron and Groten, 2002). The effects of individual HMs on plants are different from the join effects of mixture of HMs (Zhou et al., 2006). The combine effects of mixture of HMs depend on the components of mixture (Jensen and Sverdrup, 2002).Many researchers like He et al, (2008) and Yang et al, (2009) studied the impacts of individual HMs on plants, but few researchers gave attention to the impacts of combinations of HMs on plants. However, the study of combine toxicity of Cd/Pb, Cd/Zn and Pb/Zn on plants seedling needs attentions of researchers. The present study was undertaken to understand the toxicological effects of Cd, Pb and Zn alone and in mixture (Cd/Pb, Cd/Zn and Pb/Zn) in terms of seedling biomass (shoot and root fresh and dry weights), seedling lengths (shoot and root lengths), cell size, shoot diameter, number of leaves, mitotic index and HMs accumulation in *C. saivum* seedling.

2. Methodology

Soil and *C. saivum* seedlings were prepared with same methods discussed in chapter 4. The same soil was used and the same treatments were prepared. Similarly, plant growth parameters, cell size, and MI were measured using the methods mentioned in chapter 4. HMs were extracted and analyzed using acids mixture. The detailed procedure is given in chapter 4. The data were statistically analyzed using statistical packages SPSS 17. The detailed procedure is given in chapter 4.

3. Results

3.1. Effects of HMs on shoot and root fresh and dry weight.

Figures 5.1, 5.2, 3.5 and 5.4 present the toxicological effects of Cd, Pb, Zn, Cd/Pb, Cd/Zn and Pb/Zn on seedling biomass (shoot and root fresh and dry weights) of C. *saivum*. HM concentrations were significantly ($p<0.05$) affected the seedling biomass of C. *saivum*. Shoot and root fresh and dry weights reduced to 67.1%, 52.6%, 58.8% and 53.6%, respectively as compared to control (Table 5.2) at the highest dose of Cd. Similarly at the highest dose of Pb shoot and root fresh and dry weights were 78.1%, 53.6% 61.8%, and 69.4%, respectively as compared to control (Table 5.2). The addition of high dose of Zn decreased shoot and root fresh and dry weights to 93.4%, 63.7%, 67.4% and 70.7%, respectively as compared to control (Table 5.2).

Shoot and root fresh and dry weights were 65.8%, 49.5%, 58.1% and 44.9%, respectively as compared to control under the influenced of highest dose of Cd/Pb.

Similarly, at the highest dose of Cd/Zn shoot and root fresh and dry weights were 69.3%, 59.8% 65.5%, and 60.2%, respectively (Table 5.2) as compared to control.. The addition of high dose of Pb/Zn decreased shoot and root fresh and dry weight to 77.7%, 75.8%, 70.1% and 68.1%, respectively (Table 5.2) as compared to control. The combine effects of Cd, Pb and Zn on C. *saivum* were more toxic than their individual effects.

The dry weights of both shoot and root were more affected than fresh weights of C. *saivum* seedlings. The root fresh and dry weights were affected more than the shoot fresh and dry weights like *S. oleracea* seedling mentioned in chapter # 4.

Table 5.1 ANOVA analysis of growth parameters of *C. saivum*

		Sum of Squares	df	Mean Square	F	Sig.
Number of leaves	Between Groups	27.434	18	1.524	5.036	.000
	Within Groups	17.250	57	.303		
	Total	44.684	75			
Shoot dry weight	Between Groups	.422	18	.023	10.997	.000
	Within Groups	.122	57	.002		
	Total	.544	75			
Shoot fresh weight	Between Groups	84.068	18	4.670	219.320	.000
	Within Groups	1.214	57	.021		
	Total	85.282	75			
Shoot length	Between Groups	227.382	18	12.632	5.426	.000
	Within Groups	130.378	56	2.328		
	Total	357.759	74			
Shoot diameter	Between Groups	1.217	18	.068	5.236	.000
	Within Groups	.736	57	.013		
	Total	1.953	75			
Root dry weight	Between Groups	.547	18	.030	17.053	.000
	Within Groups	.102	57	.002		
	Total	.649	75			
Root fresh weight	Between Groups	.636	18	.035	44.610	.000
	Within Groups	.045	57	.001		
	Total	.681	75			
Root length	Between Groups	42.064	18	2.337	25.991	.000
	Within Groups	5.125	57	.090		
	Total	47.189	75			
Cell size	Between Groups	10.401	18	.578	1.288	.230
	Within Groups	25.568	57	.449		
	Total	35.969	75			
Mitotic index	Between Groups	7683.338	18	426.852	7.369	.000
	Within Groups	3301.922	57	57.928		
	Total	10985.259	75			

Table 5.2 Comparison of growth parameters of HM treatments with the control of *C. saivum* seedlings

Treatments	Seedlings biomass				Length				No of leave	MI
	Shoot fresh weigh	Root fresh weight	Shoot dry weight	Root dry weight	Shoot length	Root length	Shoot diameter	Cell size		
Cd 1	94.6	73.7	91.2	67.6	87.5	76.4	85.9	76.2	82.5	92.8
Cd2	83.2	70.5	64.7	58.4	76.0	66.9	63.8	61.9	75.0	91.6
Cd3	67.1	52.6	58.8	53.6	70.0	60.7	60.7	52.9	70.0	89.6
Pb 1	95.2	89.5	93.2	92.5	92.5	88.1	95.2	85.7	97.5	94.6
Pb 2	90.5	63.2	67.9	86.7	91.7	81.4	69.3	81.0	85.0	93.6
Pb 3	78.1	53.6	61.8	69.4	87.5	79.7	67.6	76.7	80.0	78.7
Zn 1	99.0	90.7	95.4	98.1	95.0	88.0	99.7	87.4	95.0	98.1
Zn 2	94.6	81.6	69.9	62.4	91.0	87.1	96.9	76.7	92.5	96.6
Zn 3	93.4	63.7	67.4	70.7	88.5	82.0	83.1	66.7	82.5	91.2
Cd1/Pb 1	70.1	62.1	84.7	63.4	76.5	69.5	80.3	66.7	67.5	85.5
Cd2/Pb 2	66.8	56.3	61.0	52.5	73.5	66.9	76.9	57.1	62.5	75.1
Cd3/Pb 3	65.8	49.5	58.1	44.9	67.2	53.6	63.1	38.1	50.0	72.0
Cd1/Zn 1	86.3	72.1	76.6	69.1	86.2	75.6	82.3	76.7	80.0	91.6
Cd2/Zn 2	74.0	61.6	70.1	65.9	82.5	71.4	72.6	57.1	62.5	89.0
Cd3/Zn 3	69.3	59.8	65.5	60.2	74.7	61.5	62.4	45.7	57.5	87.2
Zn1/Pb 1	90.9	86.3	79.9	76.0	88.5	80.5	85.9	66.7	90.0	93.4
Zn2/Pb 2	82.4	71.1	74.1	70.3	85.0	79.7	74.3	61.9	75.0	86.7
Zn3/Pb 3	77.7	75.8	70.1	68.1	72.5	68.0	68.4	52.1	65.0	77.3

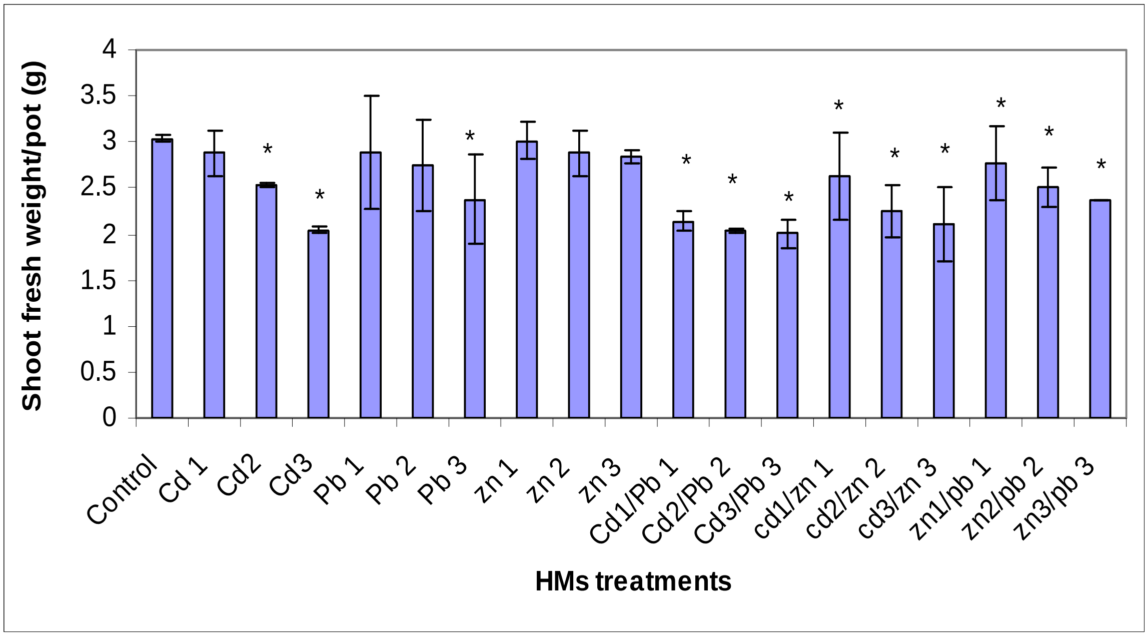

Fig 5.1 Effects of different HM treatments on shoot fresh weight. The error bars indicate the standard deviation, while asterisks (*) indicate significant ($p<0.05$) change from control

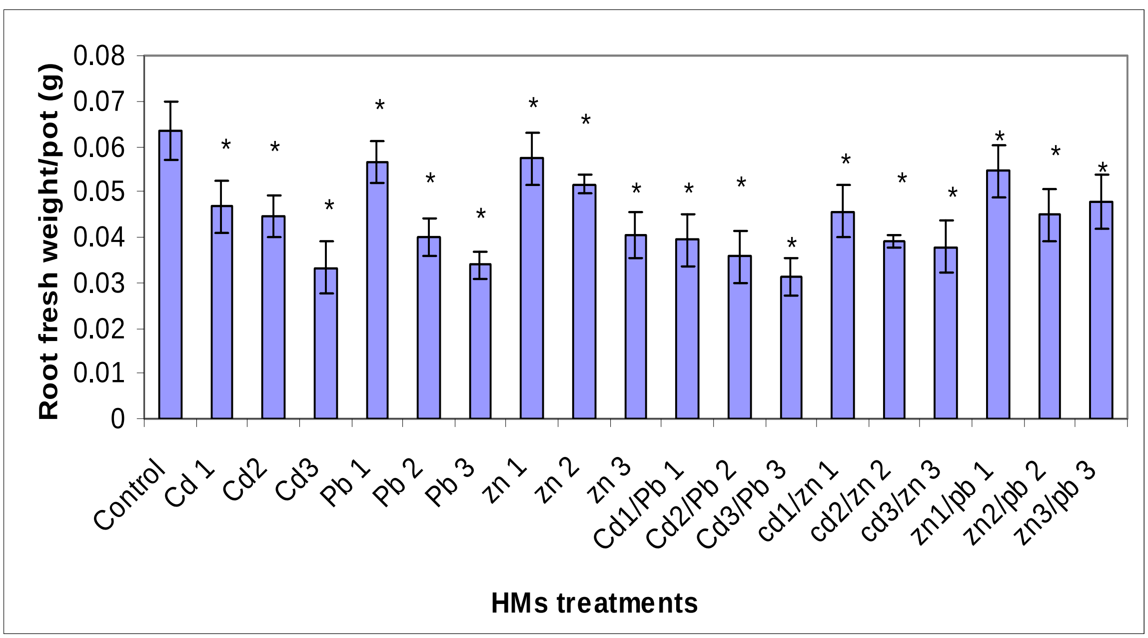

Fig 5.2 Effects of different HM treatments on root fresh weight. The error bars indicate the standard deviation, while asterisks (*) indicate significant ($p<0.05$) change from control

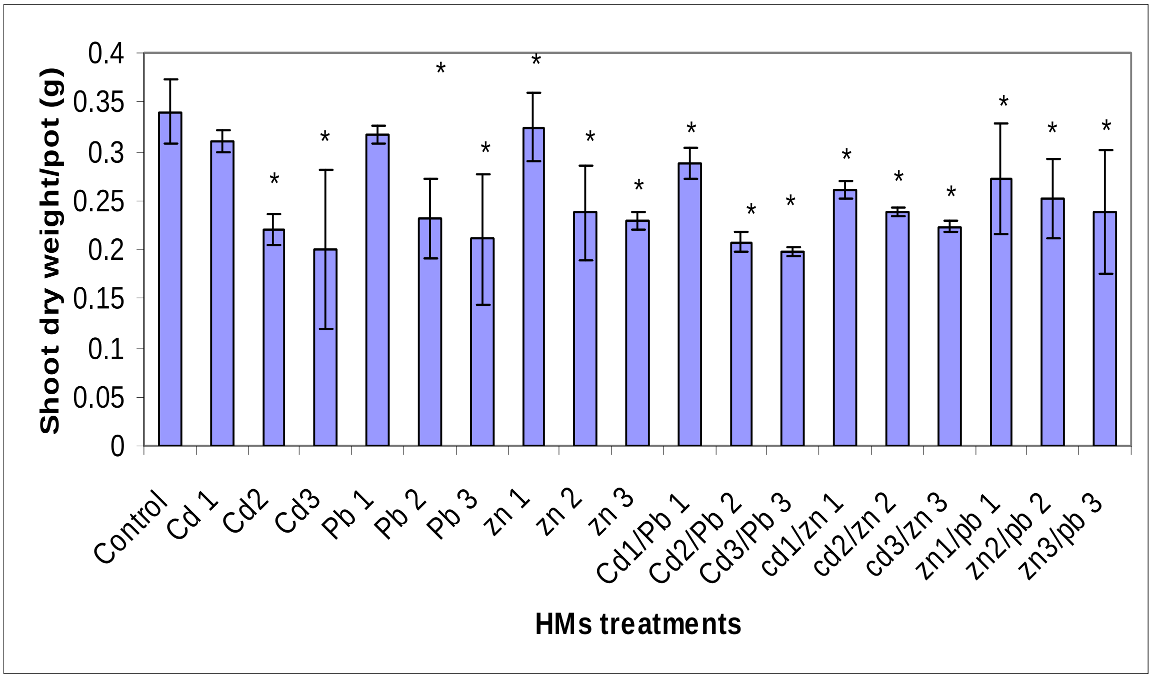

Fig 5.3 Effects of different HM treatments on shoot dry weight. The error bars indicate the standard deviation, while asterisks (*) indicate significant ($p<0.05$) change from control

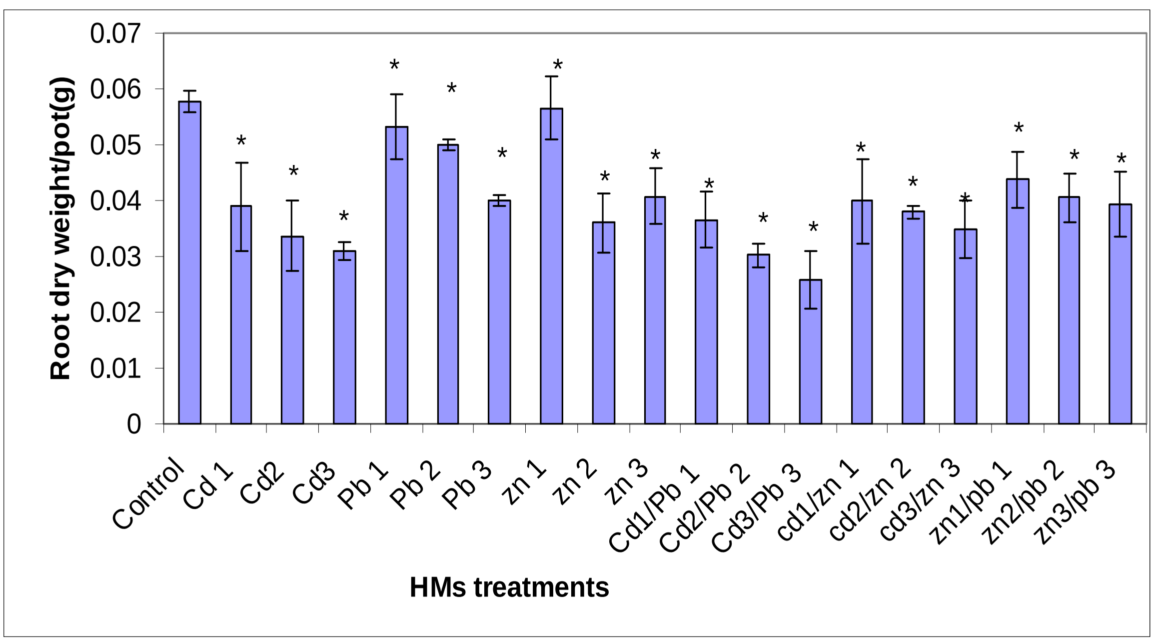

Fig 5.4 Effects of different HM treatments on root dry weight. The error bars indicate the standard deviation, while asterisks (*) indicate significant ($p<0.05$) change from control

3.2. HMs effects on shoot and root lengths

Table 5.1 summarizes the toxicological impacts of different concentrations of Cd, Pb, Zn, Cd/Pb, Cd/Zn and Pb/Zn on shoot and root lengths of *C. saivum* seedlings. The data indicates that increasing concentrations of HMs significantly ($p<0.05$) affected the growth parameters of *C. saivum* seedlings as compared to control. The increasing concentrations of HMs resulted decrease in the shoot and root lengths of *C. saivum* seedlings (Fig. 5.5, 5.6). The highest dose of Cd was reduced shoot and root lengths to 70% and 60.7%, respectively (Table 5.2) as compared to control. Similarly, at the highest dose of Pb the shoot and root lengths were reduced to 87.5% and 79.7%, respectively (Table 5.2) as compared to control. At the highest dose of Zn the shoot lengths were 88.5%, 82% (Table 5.2) as compared to control. Shoot and root lengths were 67.25% and 53.6%, respectively as compared to control under the influence of highest dose of Cd/Pb.

Similarly, at the highest dose of Cd/Zn shoot and root lengths were 74.7% and 61.5%, respectively (Table 5.2) as compared to control. The addition of high dose of Pb/Zn decreased shoot and root lengths to 72.5% and 68%, respectively (Table 5.2) as compared to control. In general, the root lengths were more affected than shoot lengths. The impact of HMs on shoot and root lengths was in order of Cd < Pb < Cd/Pb. Zn was positively affected the shoot lengths of selected plant.

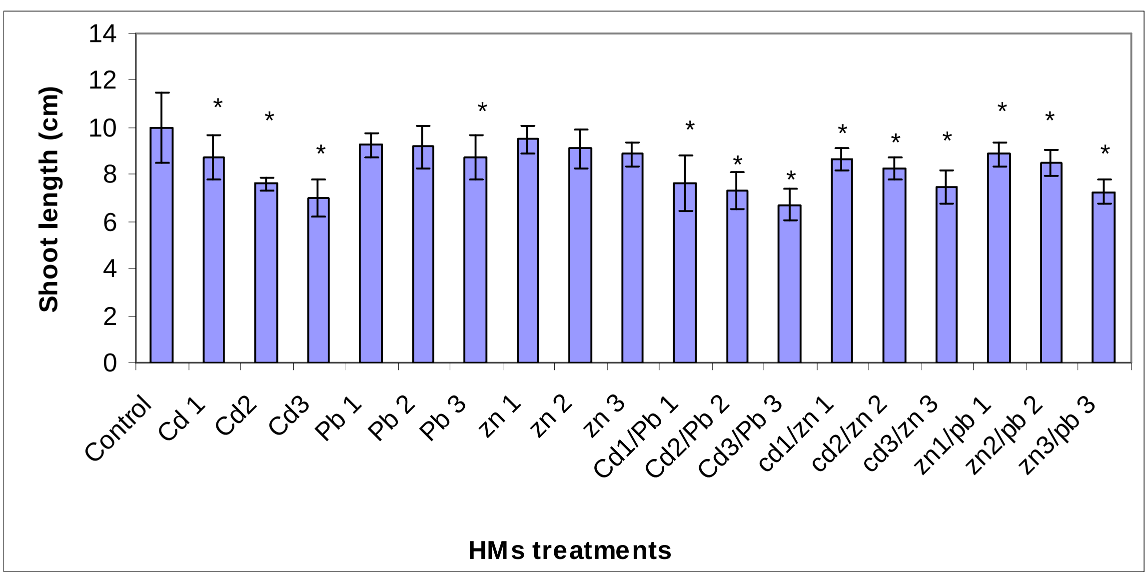

Fig 5.5 Effects of different concentrations of HMs on shoot length. The error bars indicate the standard deviation, while asterisks (*) indicate significant ($p<0.05$) change from control

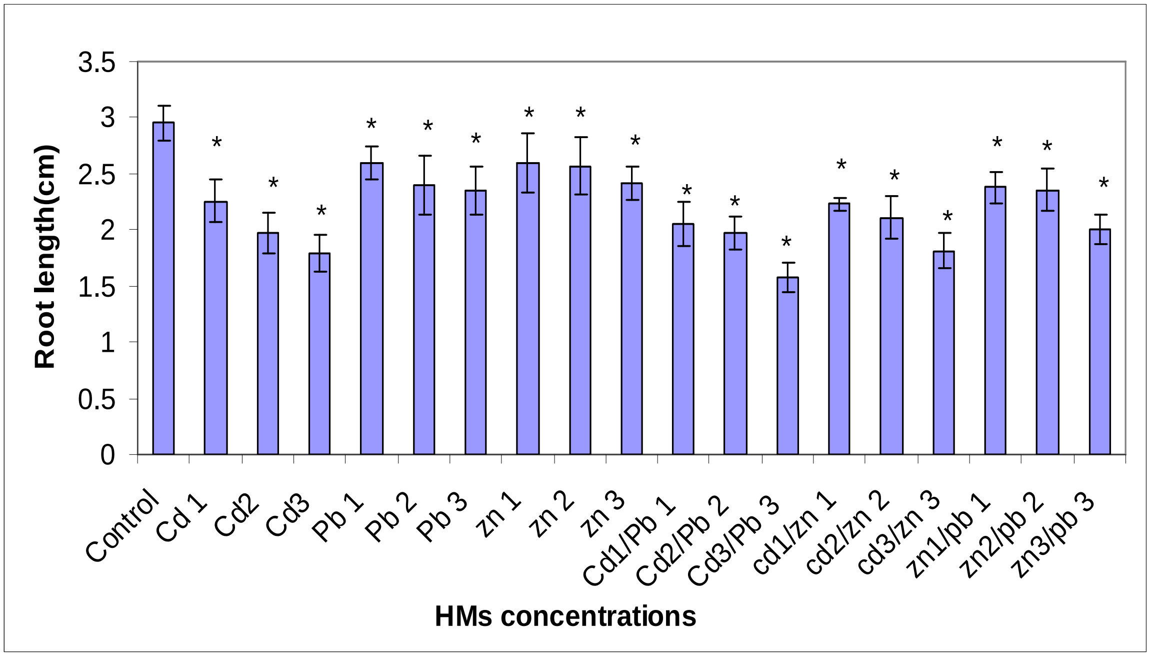

Fig 5.6 Effects of different concentrations of HMs on root length. The error bars indicate the standard deviation, while asterisks (*) indicate significant (p<0.05) change from control

3.3. HMs effects on shoot diameter

The data in Table 5.1 summarizes the affects of different concentrations of Cd, Pb, Zn, Cd/Pb, Cd/Zn and Pb/Zn on shoot diameter of *C. saivum* and found that the effects were significant (p<0.05). The increasing concentrations of HMs in the soil resulted a decrease in the shoot diameter of *C. saivum* seedlings (Fig. 5.7). Shoot diameters were 60.7%, 67.6%, 83.1%, 63.1%, 62.4% and 68.4%, as compared to control under the influence of highest dose of Cd, Pb, Zn Cd/Pb, Cd/Zn and Pb/Zn respectively.

The combine toxicities of Cd, Pb and Zn observed were more than the toxicities produced by individual HMs. The toxicological effects of Cd/Pb more severe than the effects of individual Cd and Pb, on the other hand the effects of combine Cd/Zn on shoot diameter were more than the effects of single Cd but less than single Zn. In case of Pb/Zn, the toxicity was significantly (p<0.05) less as compared to their individual toxicities.

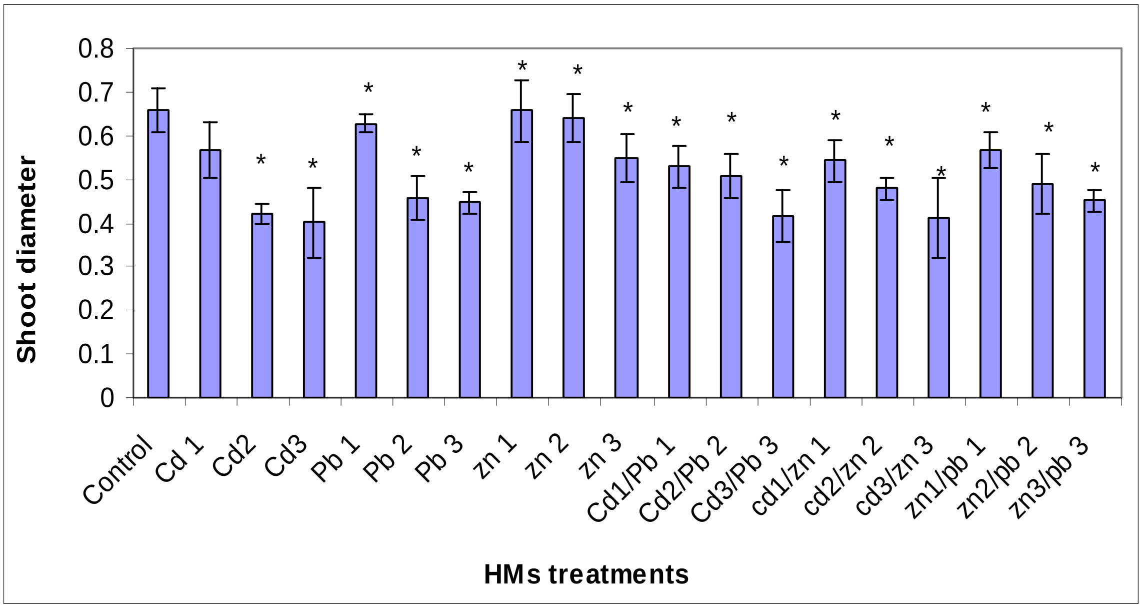

Fig 5.7 Effects of different concentrations of HMs on shoot diameter. The error bars indicate the standard deviation, while asterisks (*) indicate significant ($p<0.05$) change from control

3.4. HMs effects on cell size, number of leaves and mitotic index

The increasing concentrations of HMs resulted a decrease in the cell size, number of leaves and root mitotic index of *C. saivum* seedlings (Fig. 5.8, 5.9, 5.10). The reduction in cell size, number of leaves and root mitotic index of *C. saivum* seedlings is significant ($p<0.05$) (Table 5.2). At the highest dose of Cd the cell size, number of leaves and MI were 52.9%, 70% and 89.6%, respectively (Table 5.2) as compared to control. Similarly, at the highest dose of Pb the cell size, number of leaves and MI showed a reduction of 76.7%, 80% and 78.7%, respectively (Table 5.2) as compared to control. At the highest dose of Zn the cell size, number of leaves and MI showed a reduction of 66.7%, 82.5% and 91.2%, respectively (Table 5.2) as compared to control. The reduction in the cell size, number of leaves and MI were 38.1%, 50%, and 72%, respectively as compared to control under the influence of highest dose of Cd/Pb. Cell size, number of leaves and mitotic index were 45.7%, 57.5%, and 87.2%, respectively as compared to control under the influence of highest dose of Cd/Zn. Similarly, at highest dose of Pb/Zn cell size, number of leaves and MI were 52.1%, 60.5%, and 77.3%, respectively.

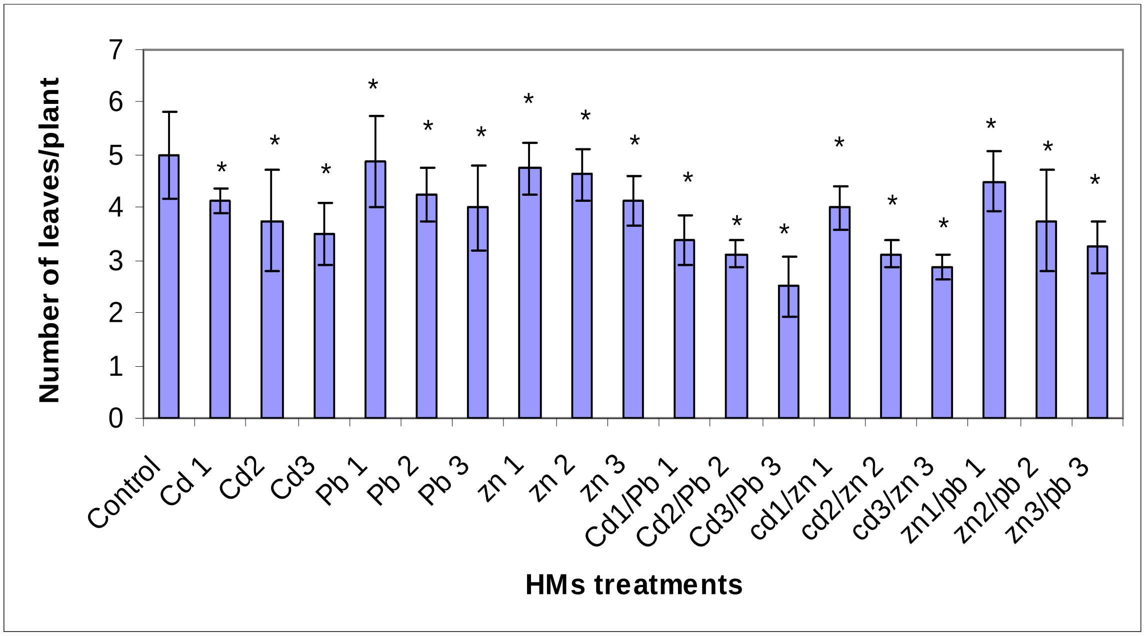

Fig 5.8 Effects of different concentrations of HMs on number of leaves. The error bars indicate the standard deviation, while asterisks (*) indicate significant ($p<0.05$) change from control

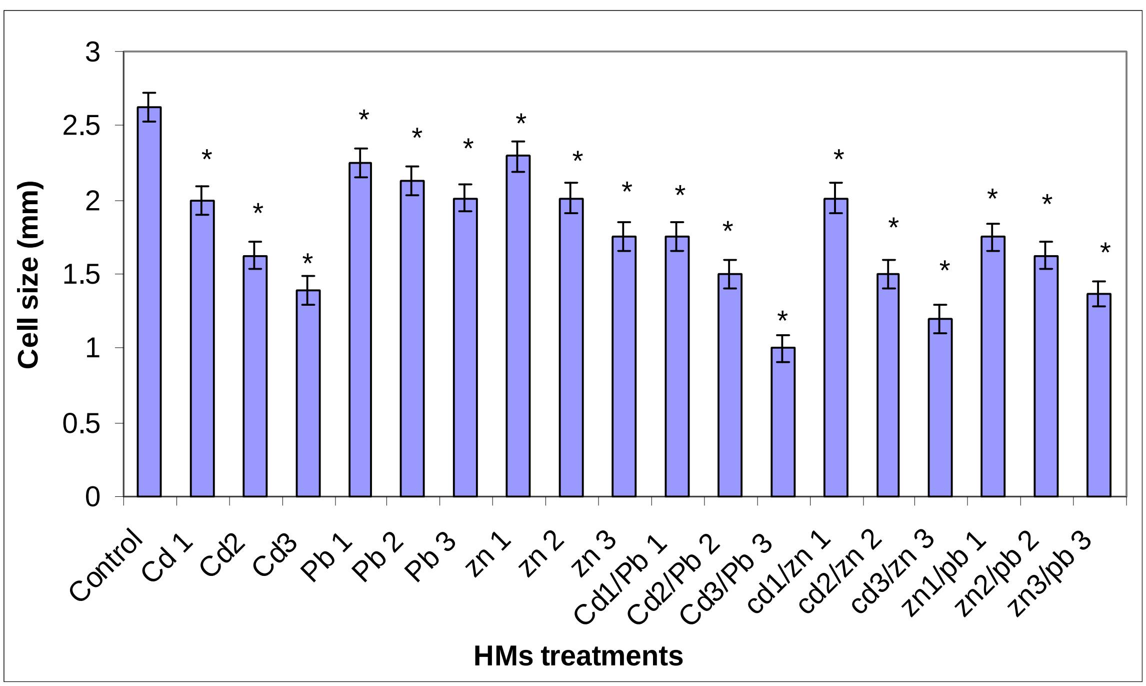

Fig 5.9 Effects of different concentrations of HMs on cell size. The error bars indicate the standard deviation, while asterisks (*) indicate significant ($p<0.05$) change from control

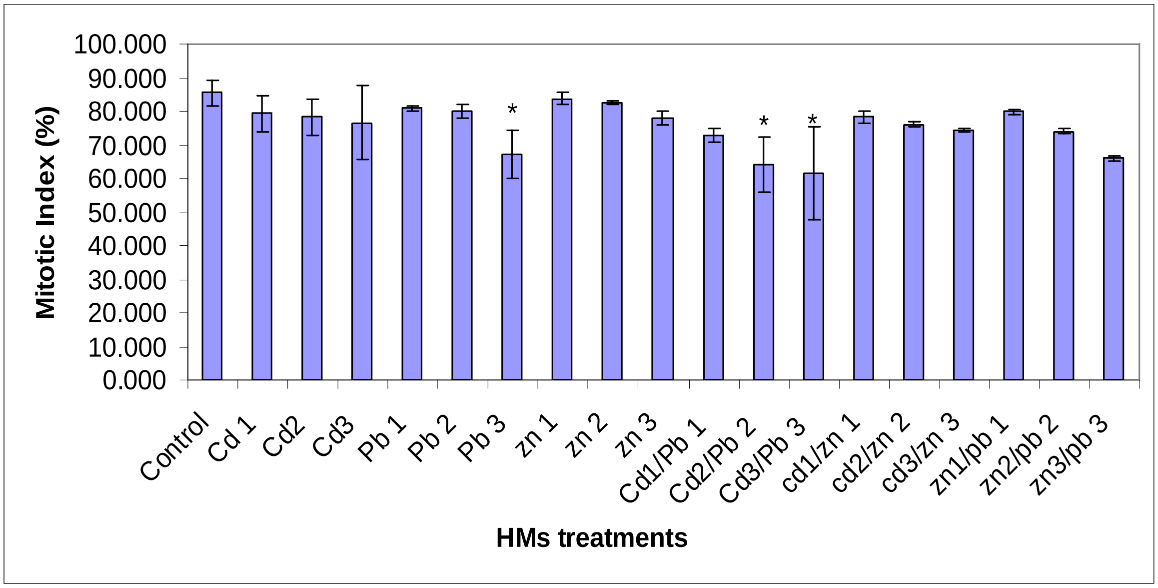

Fig 5.10 Effects of different concentrations of HMs on mitotic index The error bars indicate the standard deviation, while asterisks (*) indicate significant ($p<0.05$) change from control

3.5. Up take of HM

Fig 5.11 presents the relationships between the Cd concentrations in soil and in seedling tissue. All three relationships ie between the single Cd concentration in soil and seedlings (Fig 5.11(a), between Cd/Pb in soil and in seedlings (Fig 5.11 (b) and between Cd/Zn in soil and in seedlings (Fig 5.11 (c) shows that with increasing concentrations of Cd in soil whether in single form or in mixture its content in the seedling increasing. Seedlings 3470%, 1870% and 483% accumulated Cd from single Cd, Cd/Pb and Cd/Zn treatments, (Fig 5.13) respectively. Similarly, the concentrations of Pb in seedlings showed increase with increase its concentration in the soil. The regression analysis showed positive relationships between the concentrations of Pb in soil and in seedlings in all three treatments ie single Pb, Pb/Cd and Pb/Zn treatments (Fig 5.11 a, b, c). Fig 5.14 presents that Pb accumulated in the seedlings were 30.8%, 26.5% and 10.2% of its content in the soil of Single Pb, Pb/Cd and Pb/Zn treatments, respectively. The regression analysis showed strong positive relationship between the Zn concentration in soil and seedlings in single Zn, Zn/Cd and Zn/Pb treatments (Fig 5.12 a, b, c). Seedlings accumulated Zn 31.19%, 27.1% and 29% of Zn concentration in the soil in single Zn, Zn/Cd and Zn/Pb treatments, respectively.

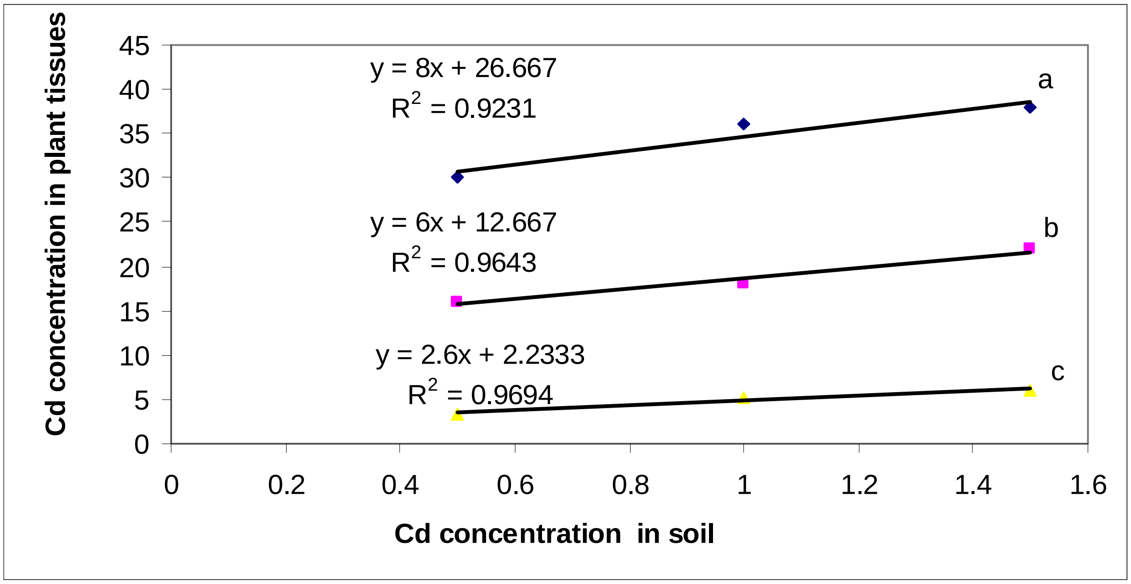

Fig. 5.11. Linear regressions model for Cd concentrations in soil and seedlings tissues on dry weight basis. (a) relationship between soil Cd content and seedlings in single Cd treatments. (b) Relationship between soil Cd content and seedlings in Cd/Pb treatments. (c) Relationship between soil Cd content and seedlings in Cd/Zn treatments

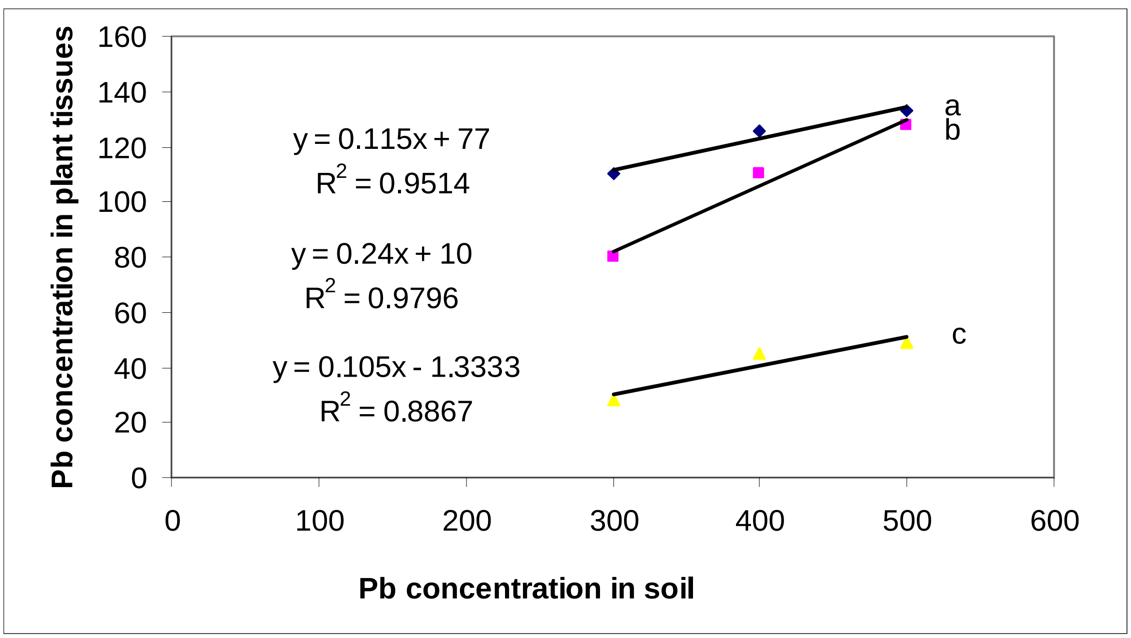

Fig 5.12. Linear regressions model for Pb concentrations in soil and seedlings tissues on dry weight basis. (a) relationship between soil Pb content and seedlings in single Pb treatments. (b) Relationship between soil Pb content and seedlings in Cd/Pb treatments. (c) Relationship between soil Pb content and seedlings in Pb /Zn treatments

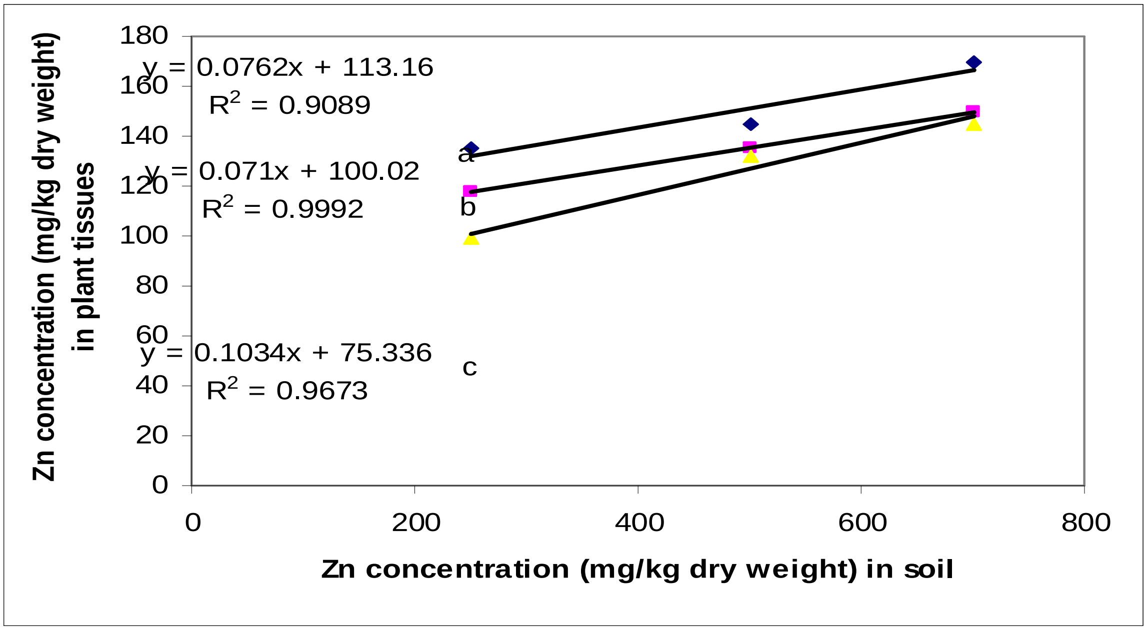

Fig 5.13. Linear regressions model for Zn concentrations in soil and seedlings tissues on dry weight basis. (a) relationship between soil Zn content and seedlings in single Zn treatments. (b) Relationship between soil Zn content and seedlings in Zn/Cd treatments. (c) Relationship between soil Zn content and seedlings in Pb/Zn treatments

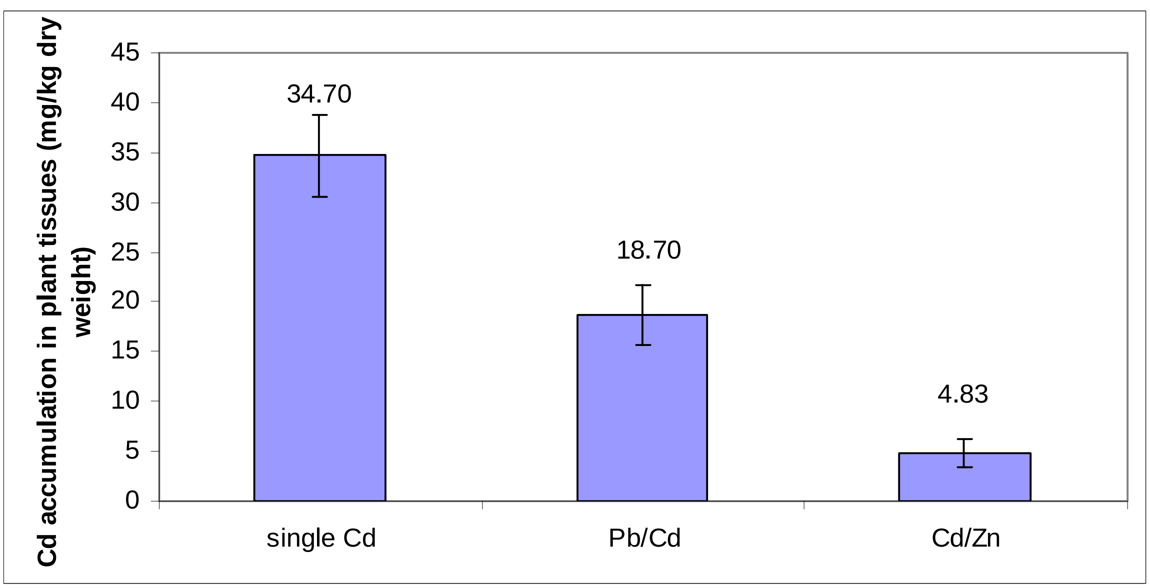

Fig 5.14 Comparison of Cd up take from different HMs spiked soil. The error bars indicate the standard deviation

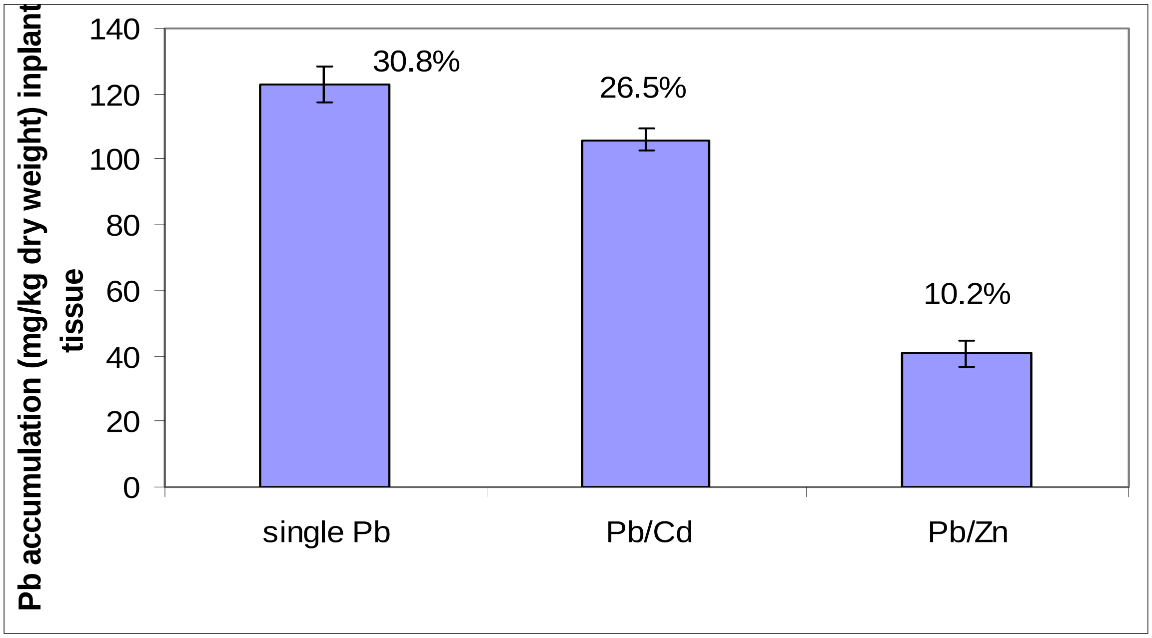

Fig 5.15 Comparison of Pb up take from different HMs spiked soil. The error bars indicate the standard deviation

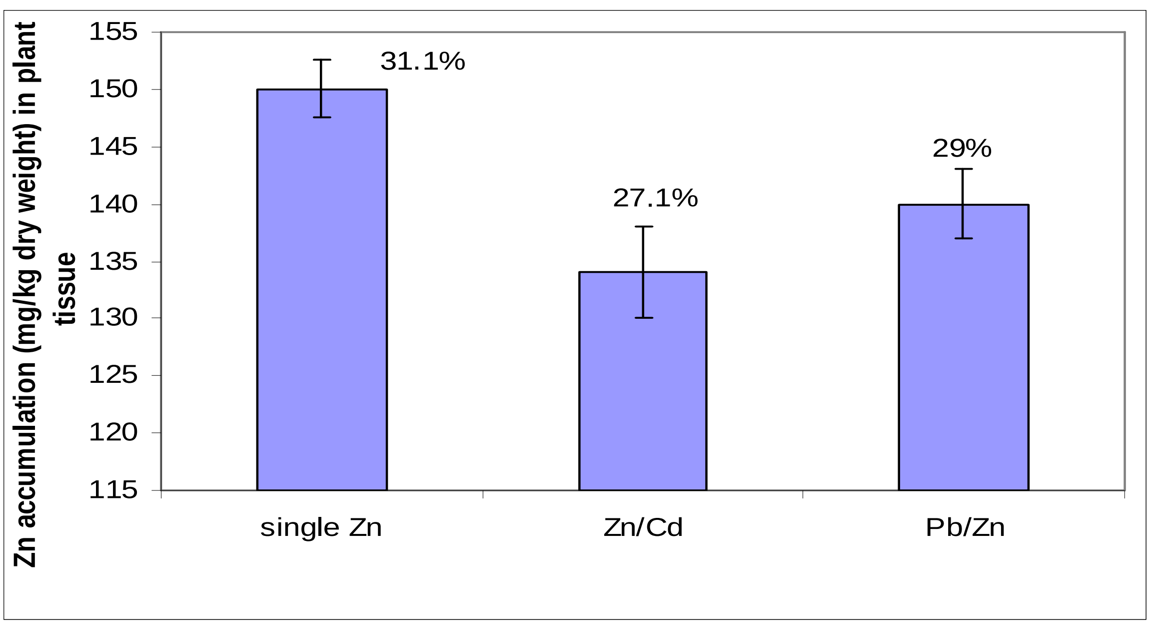

Fig 5.16. Comparison of Zn up take from different HMs spiked soil. The error bars indicate the standard deviation

4.0. Discussion

The present and previous studies (Khan et al., 2008ab; Devi et al., 2007; An et al., 2004; Sun et al., 2008; Nedjimi and Daoud, 2009; Farooqi et al., 2009; Lingua et al., 2008) indicated that HMs are plants toxicant and affect plants badly by reduction plant growth, yield and in inhibition enzymatic activities.

Some of the HMs like Cd have no known physiological activity (Lasat, 2002), but inhibits plant growth and its toxicity increase with increasing Cd concentrations in growing medium (soil). In the present study, increasing concentrations of Cd were significantly ($p<0.05$) reduced shoot and root fresh and dry weights, root and shoot lengths. The results of this study are agreed with results of previous studies and our study on *S. oleracea* seedling as mentioned in chapter- 4. Cd causes reduction in growth rates of plants (Larbi et al., 2002). Auda et al, (2010) found reduction in root and shoot dry weights and lengths of carrot under the influence of concentration of Cd in growing medium. Similarly, Farooqi et a;, (2009) observed reduction in root and shoot lengths, root and shoot dry weights and seed germination in wheat and *Albizia lebbeck* under the impacts of Cd. The results of Fei-bo et al, (2006), Wu and Zhang, (2002a) are inconsistent with the results of presents study.

Present study found that Cd toxicity was more sever in case of root length. Root was more sensitive as compared to shoot because it is the part of plant which comes in contact with toxicant first. Increasing concentrations of Cd are responsible for reduction in shoot and root lengths (Shukla et al., 2003). Liu et al, (2004) reported that reduction in formation of new cells under the influence of Pb and Cd leads to reduce both shoot and root lengths.

Other growth parameters of plant like number of leaves, shoot diameter and MI were adversely affected with increasing concentrations of Cd in soil. Jun-yu et al, (2008) observed a reduction in MI of rice plant with increasing concentrations of Cd.

C. saivum seedlings produced toxicity with increasing concentrations of Pb in terms of all growth parameters. Increasing concentrations of Pb significantly ($p<0.05$) reduced seedling biomass, seedling length, number of leaves, seedling diameter and mitotic index of *C. saivum* seedlings. The results of the present study consistent with results of previous studies. Increasing concentrations of Pb in growing medium drop growth of shoot and root in Cucumis sativus (An et al., 2004). Farooqi et al, (2009) also found decrease in seedling dry weight of *A. lebbeck* with increasing concentrations of Pb. Liu et al, (2004) reported that reduction in formation of new cells under the influence of Pb leads reduction in shoot and root lengths. Increasing concentrations of Pb adversely effect seedling length in *A. marina* (MacFarlane and Burchett, 2002). The toxicity of Pb in *C. saivum* seedling in the form of reduction in shoot and root fresh and dry weights is less than the Cd. Zayed and Amin, (2002) observed a reduction in dry weights of *Trifolium Alexendrium* with increasing concentrations of Pb.

Zn is an essential element for plants, but its excess can significantly damage plants (Wang et al., 2009). This study showed that increasing concentrations of Zn were responsible for increase in toxicity in seedlings of selected plant. Shoot and root fresh and dry weights were reduced with increasing Zn concentrations. Zn reduces plant biomass because of creation of deficiency of macronutrients such is P (Marschner, 1995). (Mahmood et al, 2005) found a reduction in growth of corn with increasing concentrations of Zn. Zn has significantly reduced MI in *C. saivum* a seedling, the result is agreed with (Rout and Das, 2003).

As discussed in detailed in chapter- 4, for *S. oleracea* seedlings that HMs in mixture form interact each other. Just like *S. oleracea* seedlings, *C. saivum* seedlings also showed antagonistic effects on the up take of Cd and Pb in mixture form. *C. saivum* seedlings accumulated Cd from its treatments of our experiment in the order of Cd (3470% of its soil content) > Cd in Cd/Pb (1870%). Similarly *C. saivum* accumulated Pb from its treatments of our experiment in the order of Pb (30.8% of its soil content) > Pb in Cd/Pb (26.5%). *C. saivum* r seedlings accumulated more Cd than *S. oleracea* seedlings in case of all three treatments of Cd (3440% of its soil content for *S. oleracea* and

3470% for *C. saivum*), Cd in Cd/Pb (1760% and 1870%) and Cd in Cd/Zn (323% and 483%). While, *C. saivum* accumulated less Pb than *S. oleracea* seedlings. The up take pattern of both Cd and Pb are reflected in the growth parameters of *C. saivum* seedlings just like *S. oleracea* seedlings. The toxicities of Cd and Pb in form of Cd/Pb in terms of al most all growth parameters were more than the individual toxicities of Cd and Pb, but less than their additive toxicities. In case of *C. saivum* seedlings the toxicities due to Cd and Pb in all its three treatments were in order of Cd/Pb (40.9% of control treatment average of all growth parameters) > Cd (58.9%) > Pb (66.2%). The reduction of growth due to Cd was 41.1% and due to Pb was 33.8%. The combine toxicity should be 74.9% but the actual toxicity observed was 59.1%. The difference of expected and actual combine toxicities is due to the antagonistic affects of Cd and Pb on the up take of each other. Other studies like Kalavrouziotis et al, (2009), Zhiqiang et al, (2009) and (An et al., 2004) produced same results. The roots of *C. saivum* seedlings were more affected with combine toxicities of Cd and Pb just like the *S. oleracea* seedlings in our study and *C. sativus* studied by An et al, (2004).

Both Zn and Cd compete with each other because of their similar chemical and physical properties (Auda et al., 2010). In *C. saivum* seedlings the up take of Cd of was in order of Cd (3470%) > Cd in Cd/Zn (483%) and the up take of Zn was in order of Zn (31.1% of its soil content) > Zn in Zn /Cd (27.2%). Zn reduced the Cd up take by 86.1% on the other hand Cd reduced the up take of Zn by 12.6%. the results are consists with results of (Weihong et al., 2009) who observed a reduction in the up take of Cd in the presence of Zn and Sharma et al, (1999), who observed reduction in the up take of Zn in the presence of Cd. The up take pattern of Cd and Zn was reflected in the growth parameters of *C. saivum* seedlings. The toxicities due to Cd and Zn in all its three treatments were in order of Cd/Zn (47.1% average reduction of all growth parameters from control treatment) > Cd (41.1% average reduction of all growth parameters from control treatment) > Zn (29.8% average reduction of all growth parameters from control treatment). The reduction of growth due to Cd was 41.1% and due to Zn was 29.8%. The combine toxicity should be 70.9% but the actual toxicity observed was 47.1%. The difference of expected and actual combine toxicities is due to the antagonistic affects of

Cd and Zn on the up take of each other. Auda et al, (2010) found a reduction in the inhibitory effects of Cd on carrot growth and dry matter with addition of Zn. Similarly Sharma et al, (2007) observed synergistic interaction of Zn with Cd at its low concentrations while antagonistic at high Zn concentrations.

Just like other HMs Pb ands Zn also interact with each other if present in mixture form. In case of *C. saivum* seedlings both of Pb and Zn antagonistically affected the up take of each other, which consistence with the results of MacFarlane and Burchett, (2002). MacFarlane and Burchett, (2002) observed that the accumulation of Zn enhances the accumulation of Pb in leaves and vice versa. The up take of Pb was in order of Pb (30.8%) > Pb in Zn/Pb (10.2%), similarly the up take of Zn was in order of Zn (31.1% of its soil content) > Zn in Zn /Pb (29%). The up take pattern of Zn and Pb in Zn /Pb showed that both Zn and Pb affect the up take of each other in antagonistic way. Pb reduced the Zn up take by 6.8% on the other hand Zn reduced the up take of Pb by 66.8%. The up take pattern is reflected in the growth parameters of *C. saivum* seedlings. In case of *C. saivum* seedlings the toxicities due to Zn and Pb in all its three treatments were in order of Zn/Pb (56.7% average reduction of all growth parameters from control treatment) > Pb (33.8% average reduction of all growth parameters from control treatment) > Zn (29.2% average reduction of all growth parameters from control treatment). The reduction of growth due to Pb was 33.8% and due to Zn was 29.2%. The combine toxicity should be 63% but the actual toxicity observed was 56.7%. The difference of expected and actual combine toxicities is due to the antagonistic affects of Zn and Pb on the up take of each other. Present results of our study are consistent with the results of Boedeker et al, (1993).

Chapter: 6 HMs toxicities on Purslane (*Portulaca oleracea)* seedling

1.0. Introduction

HMs accumulates in soil not only disturbed the normal functioning of soil ecosystem but also disturb the development of plants (Khan et al., 2008a). HMs are plant toxicant, they can reduce plant yield, affect leaf and root growth and inhibit enzymatic activities (Zeng et al., 2008).

Cd decreases the growth of plants (An et al., 2004), decreases plant biomass and length (Shukla et al., 2003; Devi et al., 2007), reduces the formation of new cells (Liu et al., 2004) and reduces mitotic index (Jun-yu et al., 2008). Cd which, is toxic HM decreases nitrogen concentration in plants which further negatively affects photosynthesis, protein and carbohydrates formation and uptake of essential elements like Cu, Mn, Mg and K and affect plant yield (Auda et al., 2010). Similarly Pb, which is nonessential element, produces toxicity in plants. Pb has significant negative impacts on seedling dry mass, root and shoots length (Juseph et al., 2002). Pb adversely affects the process of photosynthesis, respiration and metabolism of plants (Paolacci et al., 1997).

Pb reduces plant weight (Farooqi et al, (2009), plant length (MacFarlane and Burchett, 2002) and formation of new cells (Liu et al., 2004). Zn is an essential element for plants, but its excess can significantly damage plants (Wang et al., 2009). Zn causes necrosis of plant shoot (Mahmood et al, 2005) and inhibits root development (Lingua et al., 2008).

According to Abedin and Mehrage, (2002) both seedling and seed germination stage are important for the study of impacts of HMs. Environment is the complex of mixture of elements. HMs has antagonistic or synergistic relationship among them. So to study the impacts of HMs on plants it is necessary to study them in combine form. In the past the effects of the single HMs on plants were investigated by many researchers (He et al., 2008; Yang et al., 2009). The joint affects of HMs were investigated by few researchers like (Farooqi et al., 2009; Hauang et al., 2009; An et al., 2004), but the study of combine toxicity of Cd/Pb, Cd/Zn and Pb/Zn on *P. Oleracae* seedling needs attentions of researchers.

The present study was undertaken to investigate the toxicological effects of individual Cd, Pb and Zn and their mixtures (Cd/Pb, Cd/Zn and Pb/Zn) in terms of seedling biomass (shoot and root fresh and dry weights), seedling lengths (shoot and root lengths), cell size, shoot diameter, number of leaves and mitotic index in *P. Oleracae* seedlings.

2.0. Methodology

Soil and *P. Oleracae* seedlings were prepared with same methods discussed in chapter 4. The same soil was used and the same treatments were prepared. Similarly, plant growth parameters, cell size, and MI were measured using the methods mentioned in chapter 4. HMs were extracted and analyzed using acids mixture. The detailed procedure is given in chapter 4. The data were statistically analyzed using statistical packages SPSS 17. The detailed procedure is given in chapter 4.

3.0. Results

3.1. Effects of HMs on shoot and root fresh and dry weights.

Figures 6.1, 6.2, 6.3 and 6.4 presents the toxicological effects of individual Cd, Pb and Zn and in combination on seedling biomass (shoot and root fresh and dry weights). The pursolane seedling were significantly ($p<0.05$) effected with increasing concentrations of individual Cd, Pb and Zn and also their combinations. Shoot and root fresh and dry weight reduced by 42.3%, 38.1%, 34.9%, 39.2%, (Table 6.2) from control respectively at the highest dose of Cd. Similarly at the highest dose of Pb shoot and root fresh and dry weights were reduced 38.1%, 33.1%, 32.6%, and 38.3%, (Table 6.2) from control respectively. The addition of high dose of Zn decreased shoot and root fresh and dry weights 37.5%, 29.4%, 24.7%, 37.3%, (Table 6.2) from control respectively. The combine toxicity of Cd/Pb reduced shoot and root fresh and dry weights 45.2%, 40.0%, 40.4%, 49.17%,from control respectively.

Shoot and root fresh weight and shoot and root dry weight were reduced 43.6%, 40.0%, 36.6%, 40.0%, respectively in comparison with the control under the influence highest dose of Cd/Zn. Similarly at the highest dose of Pb/Zn shoot and root fresh and dry weights were reduced by 47.8%, 40.3%, 34.2%, 39.2%, (Table 6.2) as compared to control respectively. The toxicities of Cd/Pb, Cd/Zn and Pb/Zn were much more than the toxicities produced by individual Cd, Pb and Zn.

The dry weights of both shoot and root were more affected than their fresh weights of pursolane seedlings like the seedlings of *S. oleracea* and *C. saivum* mentioned in chapters 4 and 5 respectively. The shoot fresh and dry weights were affected more than the root fresh and dry weights.

Table 6.1. ANOVA analysis of growth parameters of *P. oleracea*

		Sum of Squares	df	Mean Square	F	Sig.
leaves	Between Groups	135.421	18	7.523	2.504	.004
	Within Groups	171.250	57	3.004		
	Total	306.671	75			
Shoot length	Between Groups	277.672	18	15.426	3.736	.000
	Within Groups	235.385	57	4.130		
	Total	513.057	75			
Shoot fresh weight	Between Groups	30200.132	18	1677.785	353.218	.000
	Within Groups	270.750	57	4.750		
	Total	30470.882	75			
Shoot dry	Between Groups	133.882	18	7.438	71.284	.000
	Within Groups	5.947	57	.104		
	Total	139.830	75			
Shoot diameter	Between Groups	6.806	18	.378	2.158	.015
	Within Groups	9.989	57	.175		
	Total	16.795	75			
Root length	Between Groups	47.090	18	2.616	2.669	.003
	Within Groups	55.872	57	.980		
	Total	102.962	75			
Root fresh weight	Between Groups	89.793	18	4.988	425.984	.000
	Within Groups	.668	57	.012		
	Total	90.460	75			
Root dry weight	Between Groups	17.177	18	.954	24.283	.000
	Within Groups	2.240	57	.039		
	Total	19.417	75			
Cell size	Between Groups	14616.152	18	812.008	5.968	.000
	Within Groups	7755.884	57	136.068		
	Total	22372.037	75			
Mitotic index	Between Groups	135.421	18	7.523	2.504	.004
	Within Groups	171.250	57	3.004		
	Total	306.671	75			

Table 6.2 Comparison of reduction of growth parameters of HM treatments from control treatment of *P. oleracea* seedlings

Treatments	Seedlings biomass				Length				
	Shoot fresh weigh	Root fresh weight	Shoot dry weight	Root dry weight	Shoot length	Root length	Shoot diameter	No of leave	MI
Cd 1	13.1	25.6	18.2	20.0	22.6	25.0	18.4	34.1	7.10
Cd2	33.1	30.6	24.2	25.8	23.5	29.2	19.6	40.9	11.7
Cd3	42.3	38.1	34.9	39.2	24.4	29.2	24.9	45.5	13.5
Pb 1	10.0	23.1	14.0	18.0	17.9	21.3	16.0	25.9	4.20
Pb 2	15.0	25.6	16.3	23.5	19.8	25.8	17.4	27.5	6.60
Pb 3	38.1	33.1	32.6	38.3	22.9	27.2	19.5	29.5	9.60
Zn 1	9.00	20.3	12.1	16.7	10.2	19.6	13.6	22.3	4.40
Zn 2	14.0	23.5	14.5	20.7	19.0	24.6	15.1	23.4	5.70
Zn 3	37.5	29.4	24.7	37.3	21.4	25.0	17.5	28.6	9.30
Cd1/Pb 1	15.0	30.0	20.6	25.3	23.1	27.3	20.8	36.9	11.9
Cd2/Pb 2	35.0	33.3	27.3	27.8	27.6	37.9	23.3	45.5	12.5
Cd3/Pb 3	45.2	40.0	40.4	49.17	30.2	39.1	27.4	47.3	15.1
Cd1/Zn 1	15.4	26.9	35.5	40.5	23.5	26.1	19.3	36.4	9.50
Cd2/Zn 2	34.6	33.0	26.3	27.6	27.4	32.1	23.1	43.6	13.9
Cd3/Zn 3	43.6	40.0	36.6	40.0	27.3	32.4	26.6	47.9	15.1
Zn1/Pb 1	11.2	24.6	16.7	20.0	21.8	25.9	20.8	28.6	6.60
Zn2/Pb 2	17.2	27.0	18.9	27.3	31.2	27.3	20.0	28.2	7.40
Zn3/Pb 3	47.8	40.3	34.2	39.2	29.0	30.4	23.4	31.8	10.5

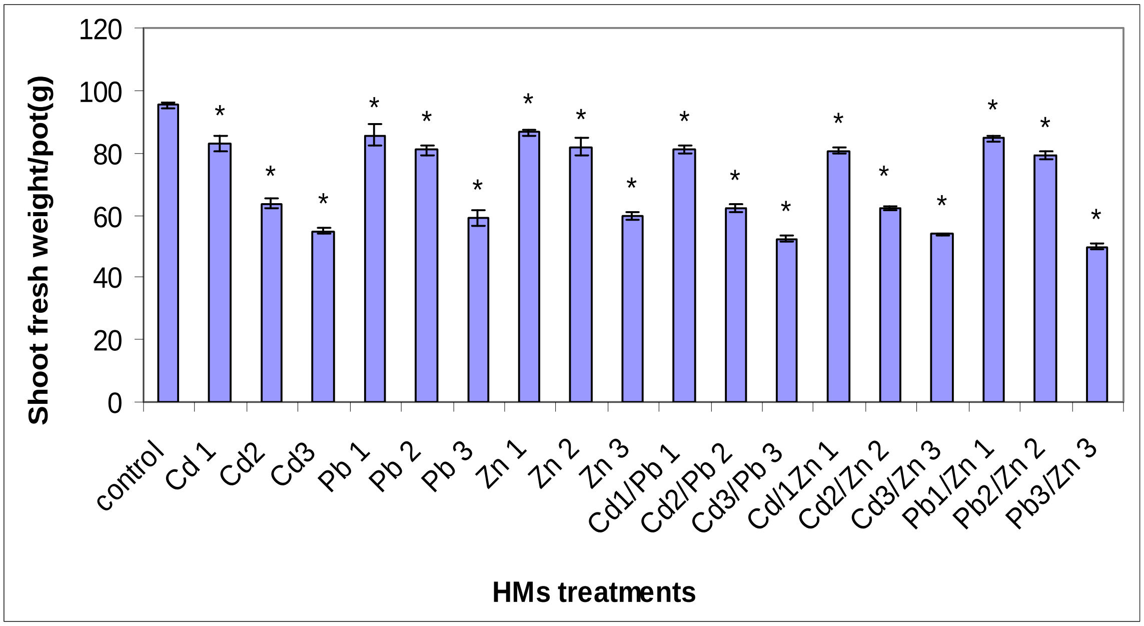

Fig 6.1 Effects of different concentrations of HMs on shoot fresh weight. The error bars indicate the standard deviation, while asterisks (*) indicate significant ($p<0.05$) change from control

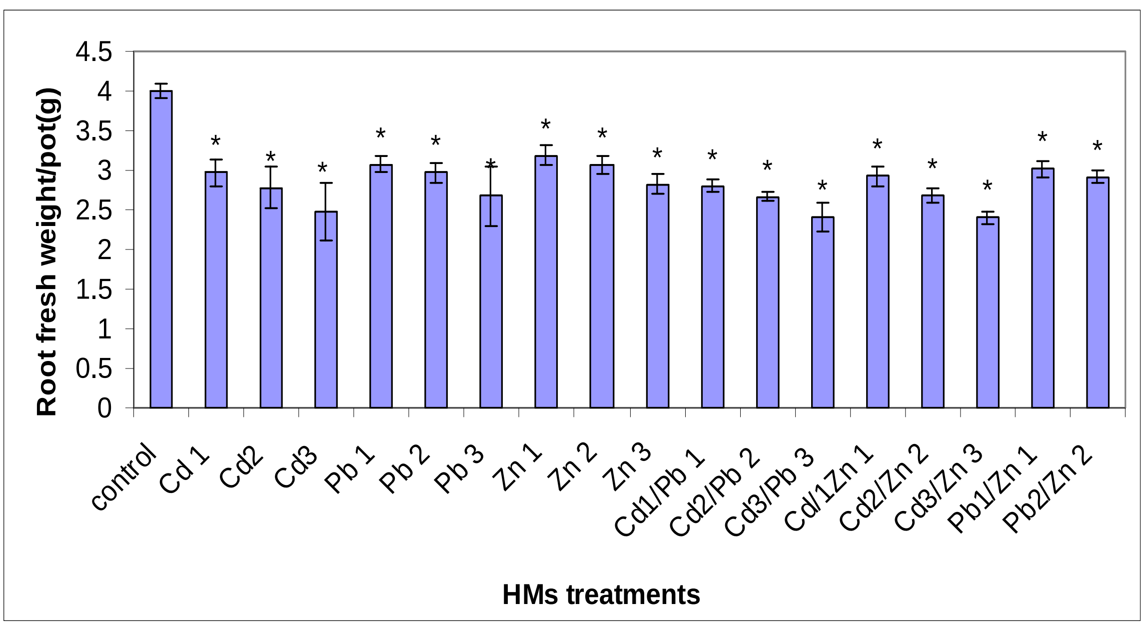

Fig 6.2 Effects of different concentrations of HMs on root fresh weight. The error bars indicate the standard deviation, while asterisks (*) indicate significant ($p<0.05$) change from control

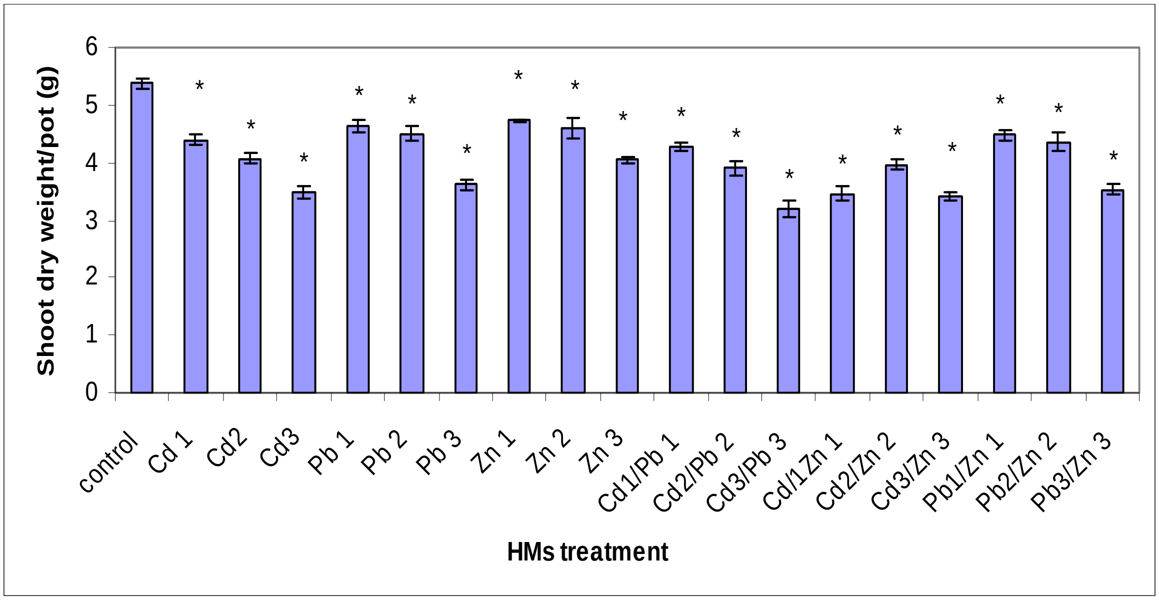

Fig 6.3 Effects of different concentrations of HMs on shoot dry weight The error bars indicate the standard deviation, while asterisks (*) indicate significant (p<0.05) change from control

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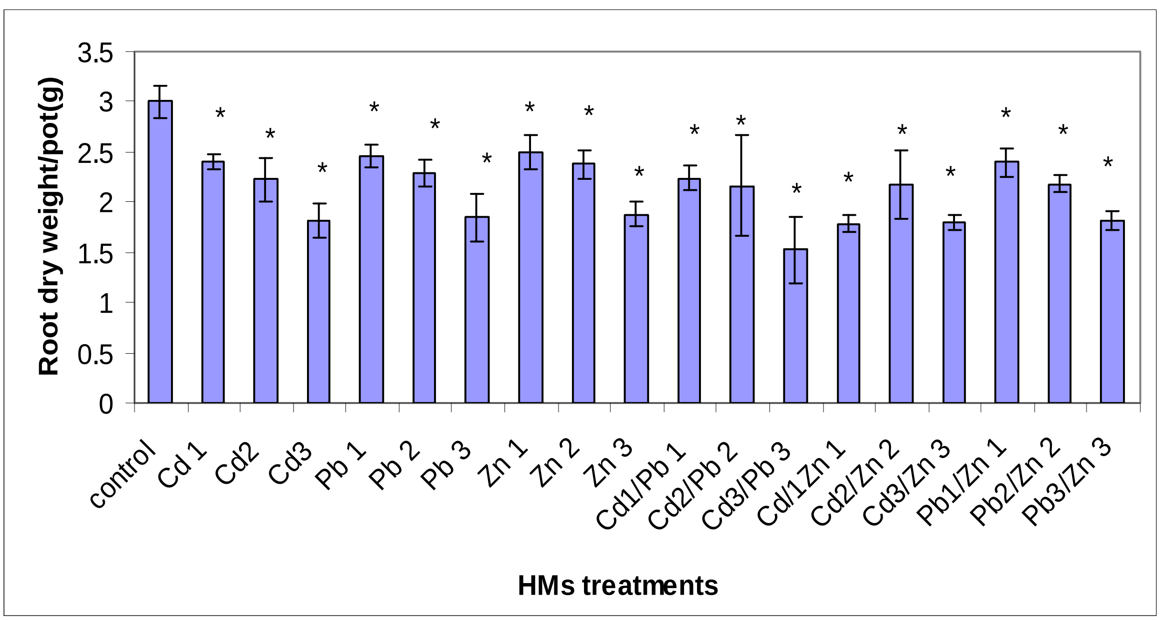

Fig 6.4 Effects of different concentrations of HMs on root dry weight. The error bars indicate the standard deviation, while asterisks (*) indicate significant (p<0.05) change from control

3.2. HMs effects on Shoot and root lengths

Data in table 6.2 summarizes that Cd, Pb, Zn, Cd/Pb, Cd/Zn and Pb/Zn had a significant ($p<0.05$) toxicological impacts on shoot and root lengths of *P. oleracea* seedlings. The increasing concentrations of HMs resulted decrease in the shoot and root lengths of *P. oleracea* seedlings (Fig. 6.5, 6.6). The highest dose of Cd reduced shoot and root lengths by 24.4% and 29.2%, respectively (Table 6.2) as compared to control. Similarly at the highest dose of Pb the shoot and root lengths were reduced to 22.9% and 27.2%, respectively (Table 6.2) as compared to control. At the highest dose of Zn the shoot and root lengths were reduced by 21.4% and 25.0%, respectively (Table 6.2) as compared to control. Shoot and root lengths were reduced by 30.2% and 39.1%, respectively as compared to control under the influence of highest dose of Cd/Pb.

Similarly at the highest dose of Cd/Zn shoot and root lengths were reduced by 27.3% and 32.4%, respectively, (Table 6.2) as compared to control. The addition of highest dose of Pb/Zn reduced shoots and root lengths by 29.0% and 30.4%, respectively (Table 6.2) as compared to control.

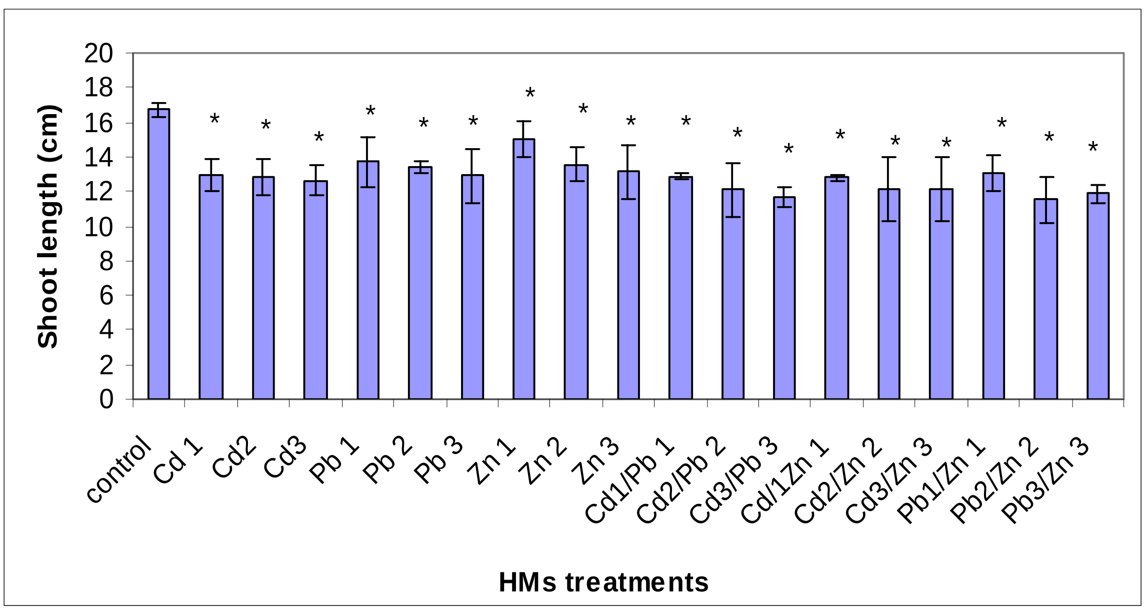

Fig 6.5 Effects of different concentrations of HMs on shoot length. The error bars indicate the standard deviation, while asterisks (*) indicate significant ($p<0.05$) change from control

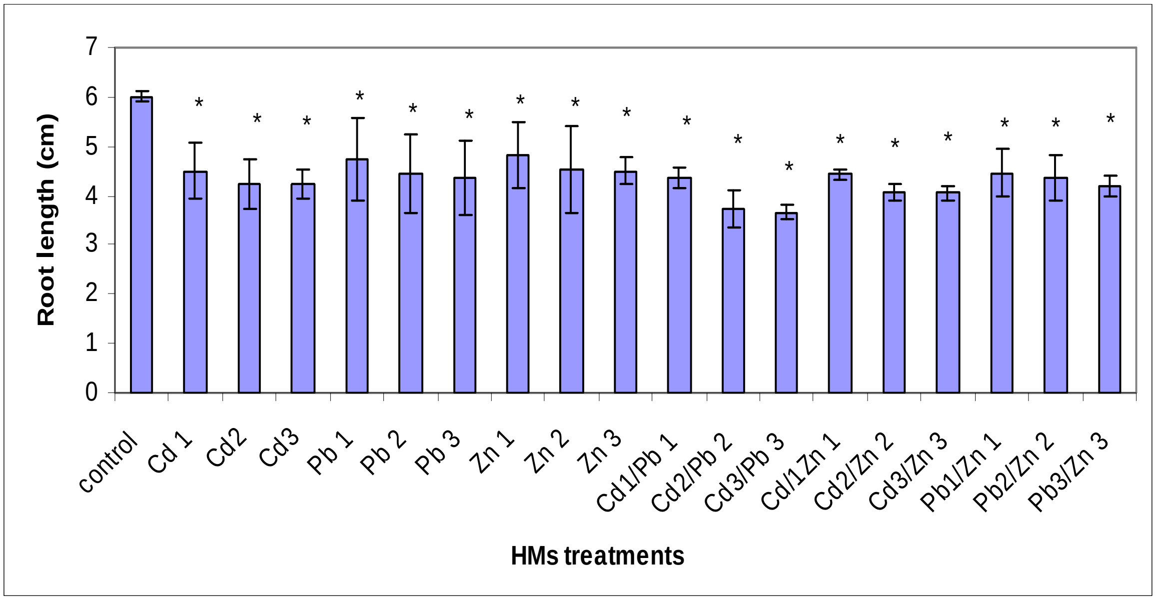

Fig 6.6 Effects of different concentrations of HMs on root length. The error bars indicate the standard deviation, while asterisks (*) indicate significant (p<0.05) change from control

3.3. HMs effects on shoot diameter

The date in Table 6.2 summarizes that different concentrations of Cd, Pb, Zn, Cd/Pb, Cd/Zn and Pb/Zn had a significant (p<0.05) toxicological impacts on shoot diameter of *P. oleracea* seedlings. The increasing concentrations of HMs in the soil resulted a decrease in the shoot diameter of *P. Oleracae* seedlings (Fig. 6.7). Shoot diameters were reduced by 24.9% , 19.5, 17.5, 27.4, 26.6 and 23.4%, as compared to control under the influence of highest doses of Cd, Pb, Zn, Cd/Pb, Cd/Zn and Pb/Zn respectively. In all three treatments of combine toxicity (Cd/Pb, Cd/Zn. Pb/Zn) the shoot diameters were more affected as compare to their single forms (Cd, Pb, Zn)..

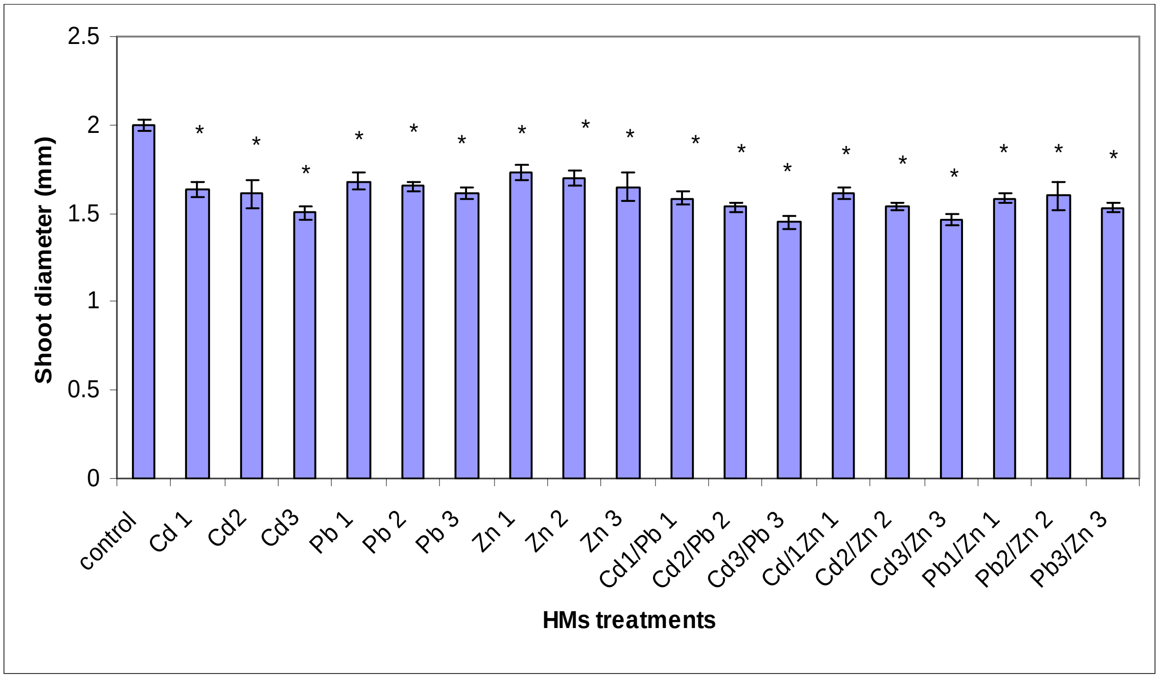

Fig 6.7. Effects of different concentrations of HMs on shoot diameter The error bars indicate the standard deviation, while asterisks (*) indicate significant (p<0.05) change from control

3.4. HMs effects on number of leaves mitotic index

The increasing concentrations of HMs resulted a significant (p<0.05) decrease in the cell size, number of leaves and root mitotic index of *P. Oleracae* seedlings (Fig. 6.8, 6.9). The reduction in number of leaves and root mitotic index of *P. Oleracae* seedlings is significant (p<0.05) (Table 6.1). At the highest dose of Cd the number of leaves and MI were 45.5% and 13.5%, respectively (Table 6.2) as compared to control. Similarly At the highest dose of Pb the number of leaves and MI showed a reduction of 29.5% and 9.6%, respectively (Table 6.2) as compared to control.. At the highest dose of Zn the number of leaves and MI showed a reduction of 28.6% and 9.3%,respectively (Table 6.2) as compared to control.. The reduction in number of leaves and MI were 47.3% and 15.1%,respectively as compared to control under the influence of highest dose of Cd/Pb. Number of leaves and MI were 47.9% and 15.1%,respectively as compared to control under the influence of highest dose of Cd/Zn. Similarly at highest dose of Pb/Zn, number of leaves and MI were 31.8% and 10.5%, respectively as compared to control.

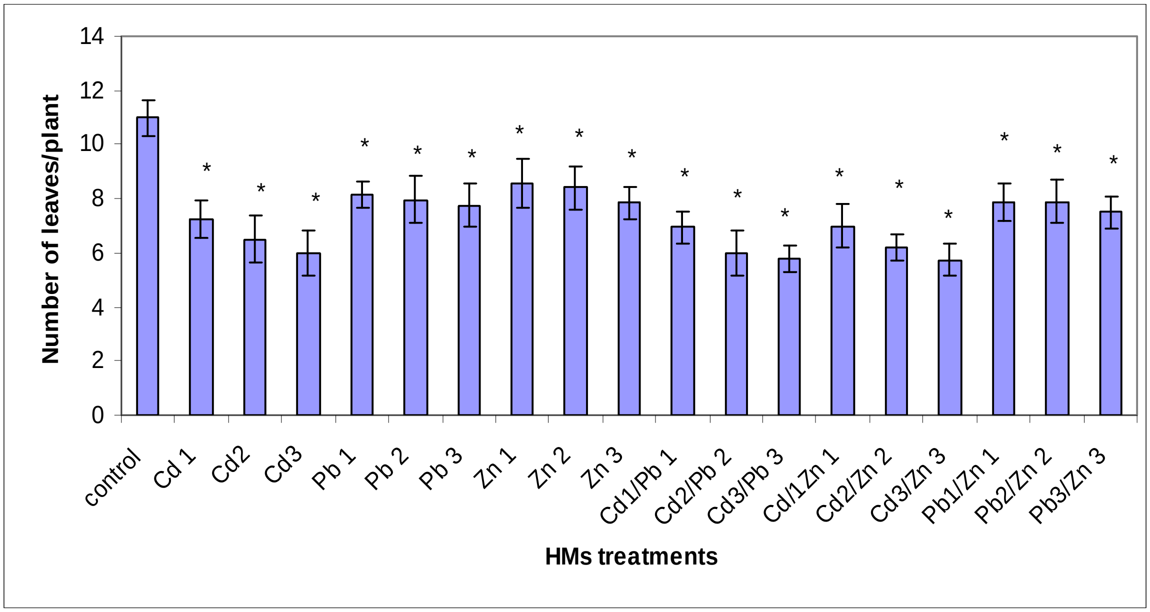

Fig 6.8 Effects of different concentrations of HMs on number of leaves. The error bars indicate the standard deviation while asterisks (*) indicate significant ($p<0.05$) change from control

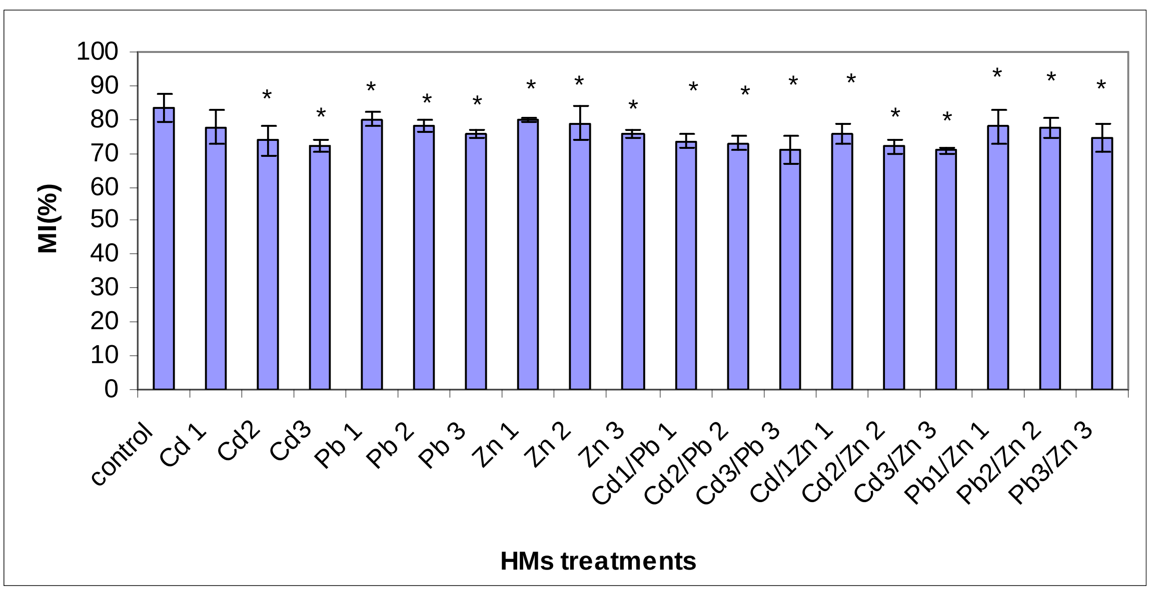

Fig 6.9 Effects of different concentrations of HMs on MI. The error bars indicate the standard deviation while asterisks (*) indicate significant ($p<0.05$) change from control

3.5. Up take of HM

The relationships between the Cd concentrations in soil and in seedling tissue are given in Fig 6.10. All three relationships ie between the single Cd concentration in soil and seedlings (Fig 6.10(a), between Cd/Pb in soil and in seedlings (Fig 6.10(b) and between Cd/Zn in soil and in seedlings (Fig 6.10 (c), show that with increasing concentrations of Cd in soil whether in single form or in mixture its concentration in the seedling increasing. The selected seedlings accumulated Cd, 42.3, 16.1 and 10.1 times of its concentration in the soil spiked with single Cd, Cd/Pb and Cd/Zn treatments, respectively (Fig 6.13). Similarly, the concentrations of Pb in seedlings showed increase with increase its concentration in the soil. The regression analysis showed positive relationships between the concentrations of Pb in soil and in seedlings in all three treatments ie single Pb, Pb/Cd and Pb/Zn. (Fig 6.11 a, b, c). Fig 6.14 presents that seedlings accumulated Pb 35%, 31% and 10.1% of its concentration in the soil of Single Pb, Pb/Cd and Pb/Zn treatments, respectively. The regression analysis showed strong positive relationship between the Zn concentration in soil and seedlings in single Zn, Zn/Cd and Zn/Pb treatments (Fig 6.12 a, b, c). *P. Oleracae* seedlings accumulated 27.5%, 26.9% and 26.8%, of Zn concentration in the soil in single Zn, Zn/Cd and Zn/Pb treatments respectively (Fig 6.15).

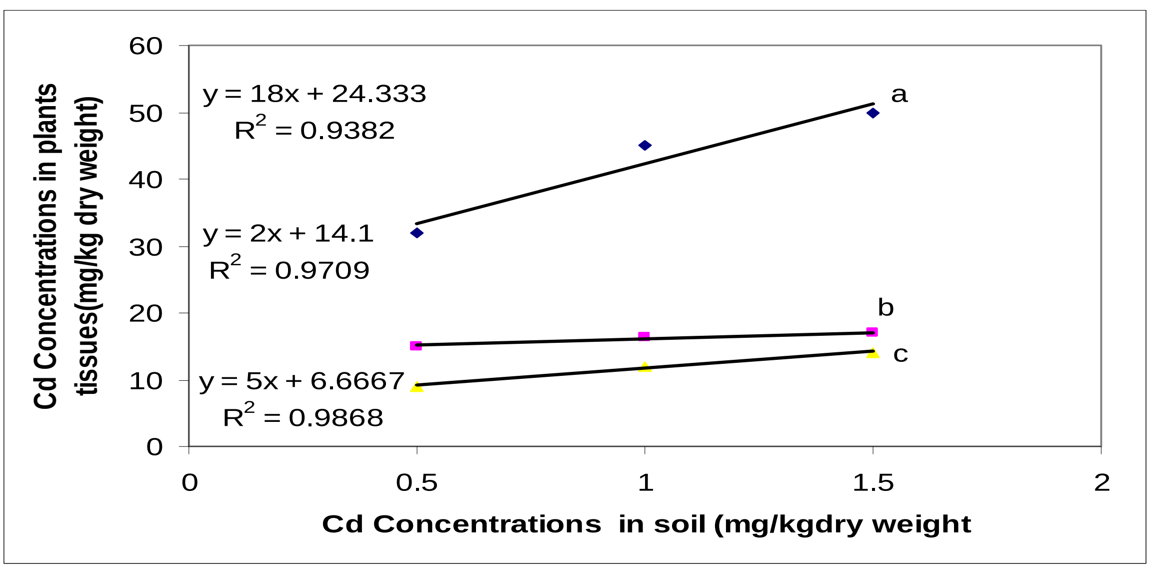

Fig. 6.10. Linear regressions model for Cd concentrations in soil and seedlings tissues on dry weight basis. (a) relationship between soil Cd content and seedlings in single Cd treatments. (b) Relationship between soil Cd content and seedlings in Cd/Pb treatments. (c) Relationship between soil Cd content and seedlings in Cd/Zn treatments.

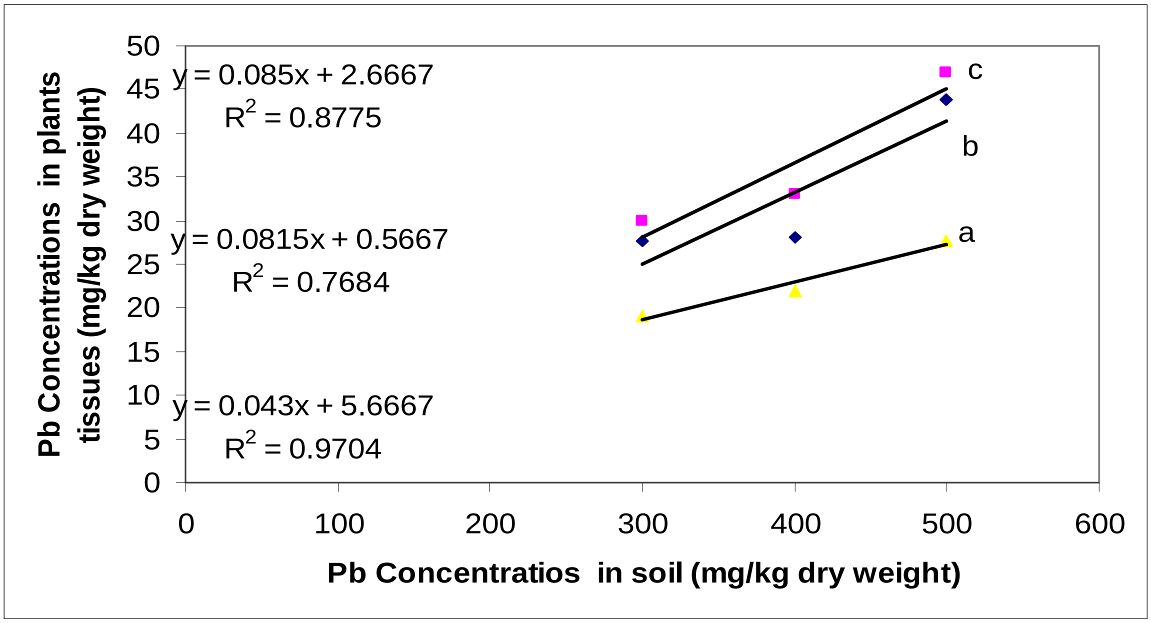

Fig 6.11. Linear regressions model for Pb concentrations in soil and seedlings tissues on dry weight basis. (a) relationship between soil Pb content and seedlings in Pb/Zn treatments. (b) Relationship between soil Pb content and seedlings in Cd/Pb treatments. (c) Relationship between soil Pb content and seedlings in Single Pb treatments

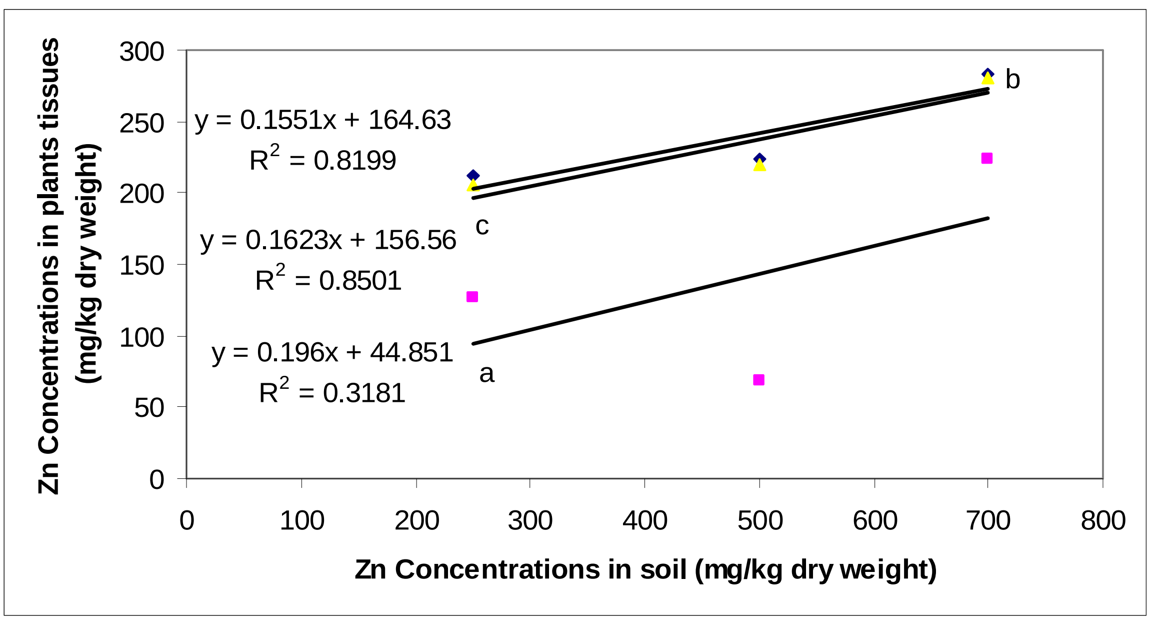

Fig 6.12 Linear regressions model for Zn concentrations in soil and seedlings tissues on dry weight basis. (a) relationship between soil Zn content and seedlings in Zn/Cd treatments. (b) Relationship between soil Zn content and seedlings in Zn/Pb treatments.(c) Relationship between soil Zn content and seedlings in single Zn treatments

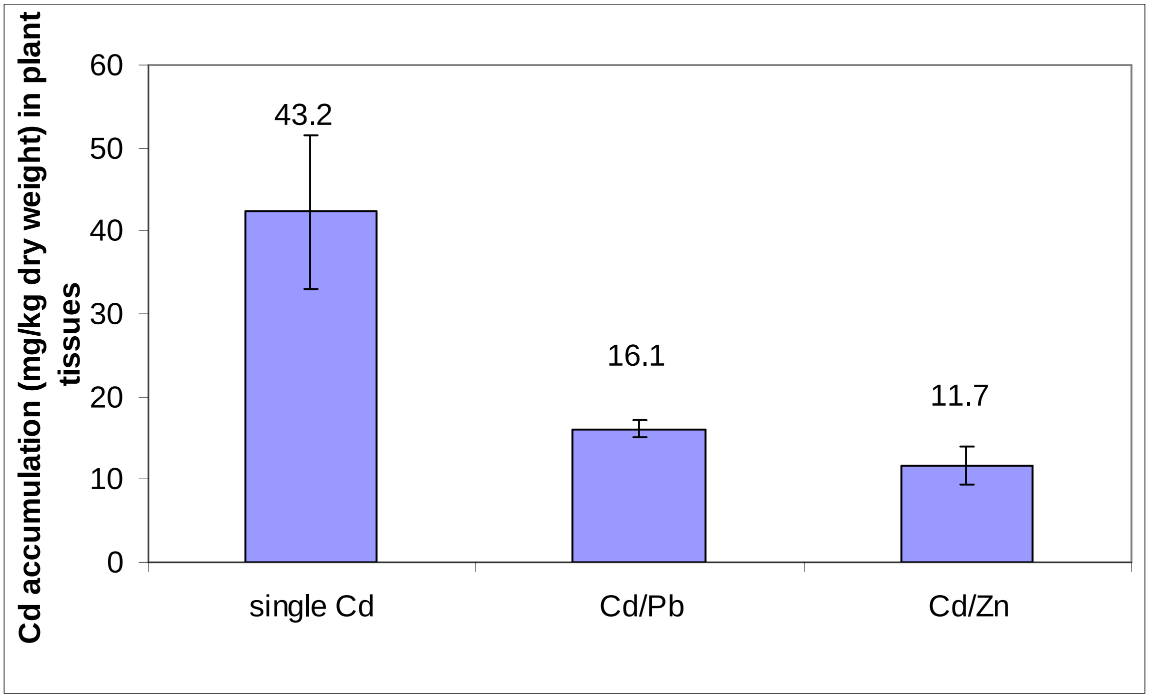

Fig 6.13 Comparison of Cd up take of from different treatments. The error bars indicate the standard deviation

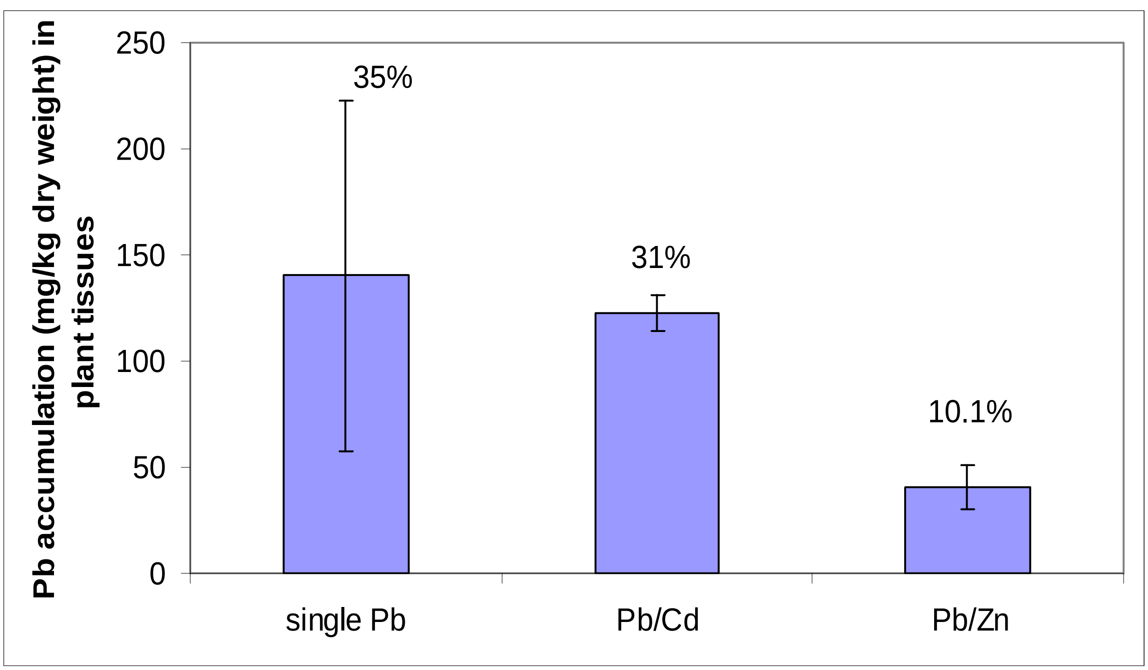

Fig 6.14 Comparison of Pb up take of from different treatments. The error bars indicate the standard deviation

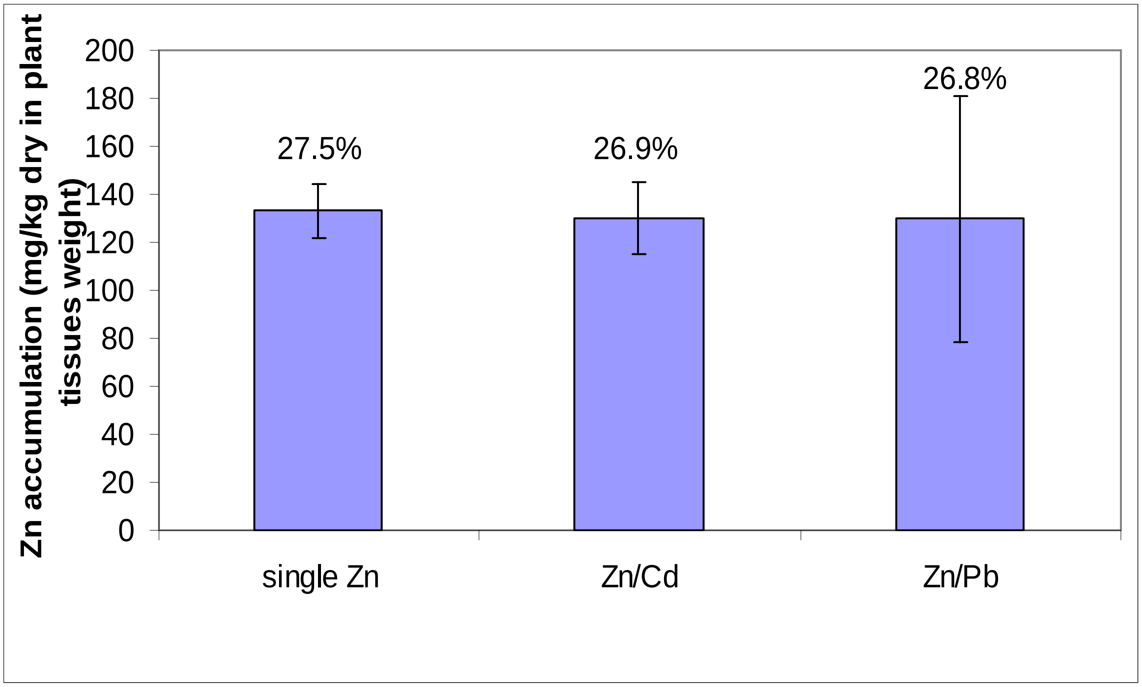

Fig 6.15 Comparison of Zn up take of from different treatments. The error bars indicate the standard deviation

4.0 Discussion

Plants have the ability to accumulate HMs in their body tissues when grow on HMs contaminated soil (Khan et al., 2008b). HMs are plants toxicant and affect plants badly. Zeng et al, (2008) reported that HMs can reduce plant yield, affect leaf and root growth and inhibit enzymatic activities

Cd inhibited plant growth and the toxicity increased with increasing concentration in soil. In the present study, increasing concentrations of Cd significantly ($p<0.05$) reduced shoot and root fresh and dry weights. The results of present study are agreed with the results of previous studies and our studies on *S. oleracea,* and *C. saivum* seedlings mentioned in chapters: 4 and 5, respectively. Shute and Macfie, (2006), Auda et al, (2010), Xiao-li and Shu-zhen, (2007) observed a reduction in biomass and lengths of soybean, carrot and wheat, respectively. On the other hand Fei-bo et al, (2006), Wu and Zhang, (2002a) and Vogel-Mikus et al, (2006) have not observed any toxicity in plants under the influence of Cd

Other growth parameters like number of leaves shoot diameter and mitotic index were adversely affected with increasing concentrations of Cd. Jun-yu et al, (2008) observed a reduction of mitotic index in rice plant with increasing concentrations of Cd. The increasing concentration of Cd is responsible for change the rate of photosynthesis. This reduces the synthesis of carbohydrates. Protein and finally decreases the growth of plants (Auda et al., 2010)

Increasing concentrations of Pb significantly ($p<0.05$) reduced biomass, length, number of leaves, diameter and mitotic index of *P. Oleracae* seedlings. The results of the present study are consistent with the results of previous studies. Increasing concentrations of Pb in growing medium dropped growth of shoot and root in *C. sativus* (An et al., 2004). Farooqi et al, (2009) found a decrease in seedlings dry weight of A. lebbeck with increasing concentrations of Pb. Increasing concentrations of Pb adversely effected seedling length in ***A.*** *marina* (MacFarlane and Burchett, 2002). The toxicity of Pb in *P. Oleracae* seedling in the form of reduction in shoots and root fresh and dry weights was less than the Cd. It may be due to high up take of Cd as compared to Pb. Seedling accumulated 432% Cd in their tissues grown in 1mg/kg Cd spiked soil. While, accumulate only 35% of the soil Pb. Zayed and Amin, (2002) observed a reduction in dry weights of *T.Alexendrium* with increasing concentration of Pb

Zn is an essential element for plants, but its excess can significantly damage plants (Wang et al., 2009). This study showed that increasing concentrations of Zn are responsible for increase toxicity in seedlings. Shoot and root fresh and dry weights were reduced with increasing Zn concentrations. Zn reduced plant biomass because of creation deficiency of macronutrients such is P (Marschner, 1995). Mahmood et al, (2005) found a reduction in growth of corn with increasing concentrations of Zn. Zn significantly reduced mitotic index in *P. Oleracae* seedlings. The results are agreed with Rout and Das, (2003).

Cd, Pb and Zn reduce the uptake of elements in plants. Cd decreases uptake of Mn, Fe, K, Mg, Ca and Zn (Wu et al., 2007; Larbi at al., 2002; Dong et al., 2006) and finally change the nutrient concentration and composition in plants (Zhang et al., 2002). Excess of Zn decreases the uptake of elements like Mg, Mn, Cu and Fe in plants (Wang et al., 2009). These elements are very important for plant biological process like protein synthesis, chlorophyll (Wang et al., 2009). There reduction causes toxicity in plants in term of reduction in plant weight and length.

The combine toxicities of HMs depend on their mobility from soil to root and then root to shoot (An et al., 2004). As mentioned in detailed in chapter: 4 for *S. oleracea* seedlings and chapter: 5 for *C. saivum* seedlings that the toxicities of individual Cd and Pb are different than toxicities of their mixture. The presence of Pb in growing medium affects the up take of Cd by plants and vice versa. In *P. Oleracae* seedlings the up take of Cd was in order of Cd (4320%) > Cd in Cd/Pb (1610%) and the up take of Pb was in order of Pb (35%) > Pb in Pb/Cd (31%). Pb reduced the Cd up take by 62.7%, on the other hand Cd reduced the up take of Pb by 88.6%. The up take pattern was reflected in the growth parameters of *P. Oleracae* the results are agreed with the results our studies on *S. oleracea* seedlings and *C. saivum* seedlings and the studies of (Zhiqiang et al., 2009), (Kalavrouziotis et al., 2009). In case of *P. Oleracae* seedlings the toxicities due to Cd and Pb in all its three treatments were in order of Cd/Pb (44.7% average reduction of all growth parameters from control treatment) > Cd (36.9% average reduction of all growth parameters from control treatment) > Pb (27% average reduction of all growth parameters from control treatment). The reduction in growth due to Cd was 36.9% and due to Pb was 27%. The combine toxicity should be 63.9% but the actual toxicity observed was 44.8%. The difference in expected and actual toxicities was due to the antagonistic affects of Cd and Pb on the up take of each other.

Zn and Cd are usually found together in ores because of same physical and chemical properties. Just like the findings of our studies for *S. oleracea* and *C. saivum* seedlings and Hart et al, (2002), Köleli et al, (2004) and Sharma et al, (1999) the presence of Cd in growing medium reduces the up take of Zn by plants and vice versa. In case of *P. Oleracae* seedlings the up take of Cd was in order of Cd (4320%) > Cd in Cd/Zn (1170%) and the up take of Zn was in order of Zn (27.5%) > Zn in Zn /Cd (26.9% of its soil content). Zn reduced the Cd up take by 72.9% on the other hand Cd reduced the up take of Zn by 2.54%.The up take pattern of Cd and Zn is reflected in the growth parameters of *P. Oleracae* seedlings. The toxicities due to Cd and Zn in all its three treatments were in order of Cd/Zn (40.5% average reduction of all growth parameters from control treatment) > Cd (36.9% average reduction of all growth parameters from control treatment) > Zn (30.8% average reduction of all growth parameters from control treatment). The reduction in growth due to Cd was 36.9% and due to Zn was 30.8%. The combine toxicity should be 67.7%, but the actual toxicity observed was 40.5%. The difference in expected and actual combine toxicities was due to the antagonistic affects of Cd and Zn on the up take of each other, which were discussed in detailed for *S. oleracea* and *C. saivum* seedlings. Weihong et al, (2009) for *V. zizanioides* and Xu *et al*, (2006) for ryegrass produced the same results.

The co-existence of Zn and Pb like other HMs also result the change in the up take of them. As discussed in detailed for *S. oleracea* and *C. saivum* seedlings Zn and Pb interact each other when present together. The up take of Pb was in order of Pb (35%) > Pb in Zn/Pb (10.1%). Similarly, the up take of Zn was in order of Zn (27.5%) > Zn in Zn /Pb (26.8%). The up take pattern of Zn and Pb in Zn /Pb showed that both Zn and Pb affected the up take of each other in antagonistic way. Pb reduced the Zn up take by 2.5%, on the other hand Zn reduced the up take of Pb by 71%. The up take pattern was reflected in growth parameters. In case of *P. Oleracae* seedlings the toxicities due to Zn and Pb in all its three treatments were in order of Zn/Pb (42.2% average reduction of all growth parameters from control treatment) > Zn (30.8% average reduction of all growth parameters from control treatment) > Pb (27% average reduction of all growth parameters from control treatment). The reduction in growth due to Pb was 27% and due to Zn was 30.8%. The combine toxicity should be 57.8%, but the actual toxicity observed was 42.2%. The difference in expected and actual combine toxicities was due to antagonistic affects of Zn and Pb on the up take of each other.

Chapter: - 7 Impacts of HMs on spinach (Spinacia oleracea) nutritional values

1. Introduction

HMs accumulates in soil not only disturbed the normal functioning of soil ecosystem but also disturb the development of plants (Khan et al., 2008ab). HMs affects plants adversely. HMs are toxic for plants they reduce plant yield, affect leaf and root growth and inhibit enzymatic activities (Zeng et al., 2008). Many HMs like Cd and Pb are nonessential and toxic for plants growth (Zhiqiang et al., 2009), but plants take them up rapidly when present in growing medium (Fusconi et al., 2006).

Cd is Phytotoxic in nature, it not only inhibits the growth parameters of plants but also causes death of plant (Toppi and Gabbrielli, 1999). Cd badly affects the process of respiration and photosynthesis and decrease water and nutrient uptake by plants (Kuo and Kao, 2004), decreases the growth of plants (An et al., 2004) and decreases plant biomass and length (Shukla et al., 2003; Devi et al., 2007). Cd which, is toxic HM decreases nitrogen concentration in plants which further negatively affects photosynthesis, protein and carbohydrates formation and uptake of essential elements like Cu, Mn, Mg and K and affect plant yield (Auda et al., 2010). Cd is responsible for reduction of chlorophyll, sugar and protein, phenol and alters enzyme activity which leads to reduce plant yield.
Pb reduces plant weight (Farooqi et al, 2009), plant length (MacFarlane and Burchett, 2002) and formation of new cells (Liu et al., 2004). Pb has significant negative impacts on seedlings dry mass, root and shoot lengths (Juseph et al., 2002). Pb adversely affects the process of photosynthesis, respiration and metabolism of plants (Paolacci et al., 1997). Pb adversely impacts photosynthesis in plants by reducing chlorophyll content due to reducing the uptake of chlorophyll’s essential elements like Mg and Fe (Bruzynski, 1987). Pb is responsible for reducing photosynthesis in plants because of infected chloroplast, changing essential enzymatic process for photosynthesis and disturbing the closing of stomata (Sharma and Dubey, 2005). Zn is an essential element for plants, but its excess can significantly damage plants (Wang et al., 2009). Its excess is very toxic for plants, inhibits plant growth and is responsible for chlorosis in leaves (Lopez-Mill et al., 2009). According to Wang et al, (2009), reduced chlorophyll content is responsible for

chlorosis in leaves. Zn damage root and also kills cells (Wang et al., 2009). Zn is responsible for reduction in weight of plant (Kosobrukhov, 2004), reduces chlorophyll content and thus negatively impacts photosynthesis in plants (Kambhampati et al., 2005). Elements like Mn, Mg, Cu and Fe are very important for physiological process of plants but increasing concentrations of HMs reduce their uptake by plants. Mn involves in splitting water molecules necessary for photosynthesis, Mg is important for chlorophyll, Cu, Fe and Mn are important for synthesis of chlorophyll (Wang et al., 2009).

Elements like Cu, Mn, Mg and Fe reduce the toxicity of HMs like Zn by reducing uptake of Zn (Jiang et al., 2007), the deficiency of these metals are dangerous for plants like deficiency of Mg is responsible for cholorosis in plant leaves (Jiang et al., 2007) and induce oxidative stress (Tewari et al., 2006). The protein levels of *E. crassipes* treated with different concentrations of heavy metals decreased gradually as the metal concentrations increased (Jiang et al., 2007)

2.0. Methodology

Data in table 7.1 summarizes the basic properties of soil used for this experiment. Soil was collected from an uncontaminated site of Peshawar, Pakistan. The soil was prepared by the method used for *S. oleracea* seedling as mentioned in chapter- 4. The plastic pots which were filled with 1000g of spiked soil and four replicates were prepared for each sample. The seeds of *S. oleracea* were disinfected in 30% (w/w) H_2O_2 solution for 10 min, followed by thorough washing with deionized water. The disinfected seeds were germinated in Petri dishes inside the folds of wet filter papers 28±1 ^{0}C. After four days uniform seedlings (4) were cultivated in each plastic pot. This experiment was performing in a greenhouse with day temperature of 25 ± 4^{0}C and night temperature of 19 ± 3^{0}C. Plants were under sun light of 12 h and relative humidity of 65 ± 2%. plants were irrigated with deionized water. Pots were re-randomized on regular basis so that each plant get same amount of light and temperature. After 6 weeks of cultivation the plants were harvested and washed with deionized water to remove soil particles. Plants root and shoot were separated. Plant growth parameters, cell size, and MI were measured using the methods mentioned in chapter 4. HMs were extracted and analyzed using acids mixture. The detailed procedure is given in chapter- 4.

Table 7.1 Basic properties of soil used for this study

Parameter	Values
pH	6.7
Organic matter (%)	2
EC(dS/m)	2.1
Soil particle size (%)	
< 20 µm	8.15
20-62 µm	47.3
62-250 µm	44
250 µm – 1mm	0.34

2.1. Moisture, fiber and protein contents

Weighted an empty china dish alone and then weighted again with the sample. Placed the dish in oven at 100^0C for 4 hrs. Repeated the process for another 2 hrs. Calculated the moisture contents with following formula

$$\text{Moisture (\%)} = \frac{\text{Weight of fresh sample} - \text{Weight of sample after drying}}{\text{Weight of sample}} \times 100$$

Took 2 g dry sample with petroleum ether to remove the crude fat. Took the residue to flask along with 200 ml boiling 0.255 N H_2SO_4. Connected with the condenser and boil for 30 minutes. Filtered the contents of the flask through linen cloth in fluted funnel. Washed the residue with distil water till washings were free from acid (perform barium chloride test). Transferred the residue to the digestion, flask again with the help of boiling 0.313 N NaOH solution and the made the volume 200 ml. connected the flask with reflux condenser and boil for 30 min. Filtered hot through Gooch crucible prepared with an asbestos mat and washed thoroughly with boiling water and then with 15 ml ethyl alcohol. Dried the crucible's contents at 110^0C, cooled and weighted denoted as (W1). Kept the crucible in muffle furnace at 500^0C to 550^0C over night and weighted again and denoted as (W2). Calculated the fiber content with the help of following formula.

$$\text{Crude fiber (\%)} = \frac{W_1 - W_2}{\text{Weight of sample}} \times 100$$

Dried 5 g samples of plants and digested with 2-3 g of digestion mixture (copper sulphate: potassium sulphate: ferrous sulphate:: 5:94:1) and 25 mL concentrated sulphuric acid on a burner in inclined position them boiled briskly for 2 hours. After complete digestion when the appearance of solution was transparent the cool solutions were diluted to 50 ml with double deionized water, filterd into acid washed volumetric flasks added 10 mL strong alkali till alkaline. Distilled into 25 mL 4% boric acid solution using methyl red as an indicator and titrated with N/10 H_2SO_4 solution. Calculated protein contents with following formula

Percent N2 x 6.25 = per cent protein

2.2. Statistical analysis

The data were statistically analyzed using statistical packages SPSS 17. The data were analyzed by one way of variance (ANOVA) to confirm the variability and validity of results, Duncan's multiple range test (DMRT) was performed to determine significance between treatments with a significance level of $p < 0.05$. Linear regression analysis was performed to establish the relationships between the HMs concentrations in the plan6s tissue and the HMs concentrations in the soil.

3.0. Results

3.1 HMs effects on plant biomass

Figures 7.1, 7.2, 7.3 and 7.4 present the toxicological effects of individual Cd, Pb and Zn and their mixture on the biomass (shoot and root fresh and dry weights) of *S. oleracea*. HMs concentration had a significance ($p<0.05$) adverse impacts on biomass of *S. oleracea*. At the highest dose of Cd the shoot and root fresh and dry weights were decreased by 25.3%, 42.4%, 10.1% and 35.1%, respectively (Table 7.2) as compared to control. Similarly, at the highest dose of Pb shoot and root fresh and dry weights were decreased by 24.7%, 47.1%, 7.6% and 28.1%, respectively (Table 7.2) as compared to control. The addition of high dose of Zn decreased shoot and root fresh and dry weights by 23.0%, 43.5%, 5.7% and 14.4%, respectively (Table 7.2) as compared to control.

The reductions in shoot and root fresh and dry weights were 30.1%, 78.8%, 13.9% and 42.1%, respectively as compared to control, under the influence of highest dose of Cd/Pb. Similarly, at the highest dose of Cd/Zn shoot and root fresh and dry weights decreased by 228.7%, 58.8%, 12.2% and 43.2%, respectively (Table 7.2) as compared to control. The addition of high dose of Pb/Zn decreased shoot and root fresh and dry weights by 26.5%, 54.7%, 11.4% and 32.1%, respectively (Table 7.2) as compared to control.

With both Cd and Pb added to soil the toxicity of seedling biomass was further increase but, was less than sum of the toxicity with both Cd and Pb alone. The toxicity in the biomass of seedlings under the influence of combine Cd/Zn was less than the toxicity due to Zn and more than the Cd toxicity. The combine toxicity of Zn/Pb was less than the toxicities due to individual Zn and Pb on *S. oleracea* biomass. The dry weights of both shoot and root were more affected than their fresh weights of *S. oleracea* seedlings. The root fresh and dry weights were affected more than the shoot fresh and dry weights.

Table 7.2 Reduction (%) in growth parameters of *S. oleracea* due to different HMs from control

Treatments	Shoot fresh weigh	Root fresh weight	Shoot dry weight	Root dry weight	Shoot length	Root length
Cd 1	7.00	23.5	2.50	21.1	8.20	65.9
Cd2	18.6	27.1	6.30	28.1	13.1	70.7
Cd3	25.3	42.4	10.10	35.1	18.0	72.7
Pb 1	5.40	20.0	1.90	9.00	6.60	18.8
Pb 2	18.3	21.2	5.90	14.0	11.5	33.3
Pb 3	24.7	47.1	7.60	28.1	13.0	38.8
Zn 1	3.00	18.9	1.80	8.10	4.60	16.0
Zn 2	17.7	20.5	3.80	10.1	4.90	20.8
Zn 3	23.0	43.5	5.70	14.4	3.00	25.7
Cd1/Pb 1	10.5	30.9	3.50	28.1	10.1	66.1
Cd2/Pb 2	27.4	32.5	12.1	35.1	14.3	75.9
Cd3/Pb 3	30.1	78.8	13.9	42.1	20.9	78.0
Cd1/Zn 1	6.80	25.9	3.00	25.1	6.40	66.1
Cd2/Zn 2	19.2	29.4	10.6	32.1	18.0	77.6
Cd3/Zn 3	28.7	58.8	12.2	43.2	23.0	61.0
Zn1/Pb 1	6.50	23.5	2.50	15.1	2.00	21.5
Zn2/Pb 2	20.4	27.1	9.70	17.1	6.20	37.6
Zn3/Pb 3	26.5	54.7	11.4	32.1	14.1	41.2

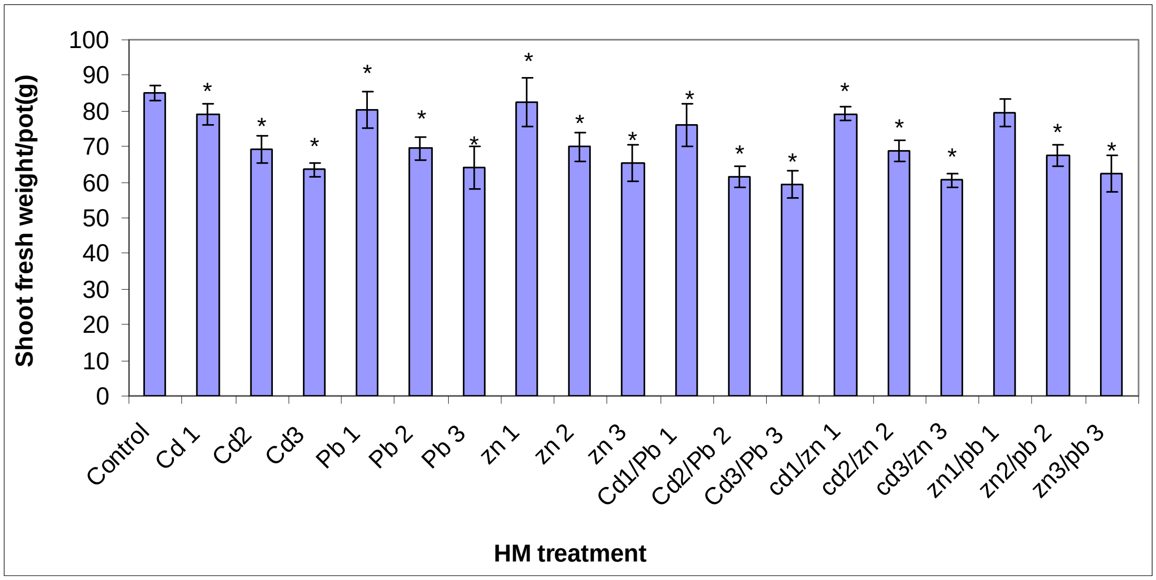

Fig 7.1 Effects of different concentrations of HMs on shoot fresh weight. The error bars indicate the standard deviation, while asterisks (*) indicate significant ($p<0.05$) change from control

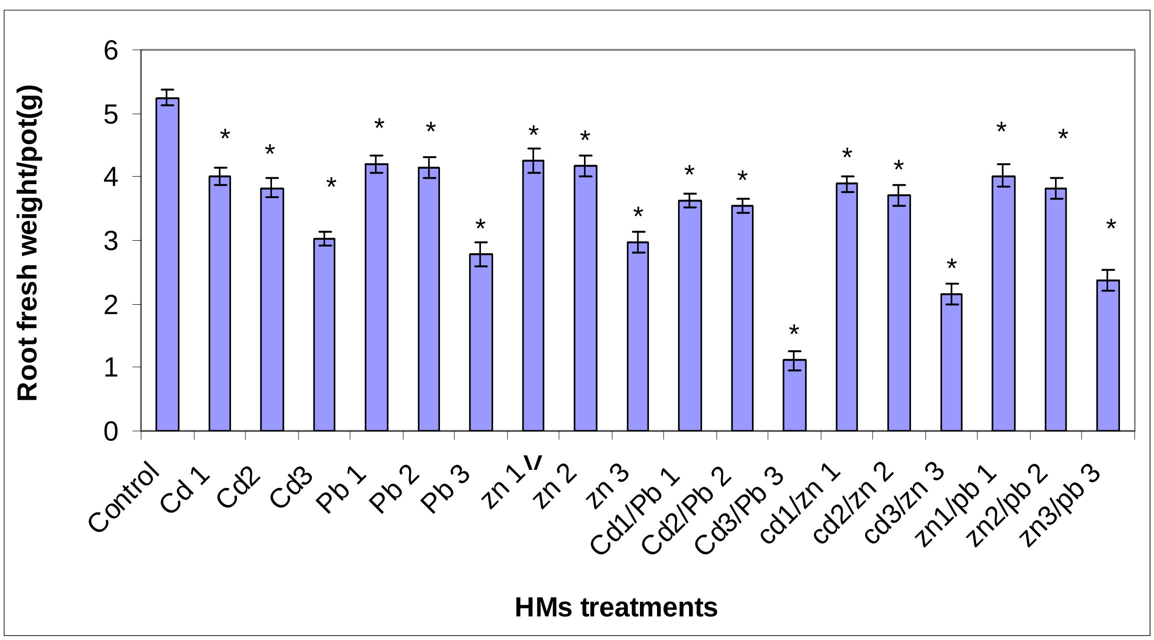

Fig 7.2 Effects of different concentrations of HMs on root fresh weight. The error bars indicate the standard deviation, while asterisks (*) indicate significant ($p<0.05$) change from control

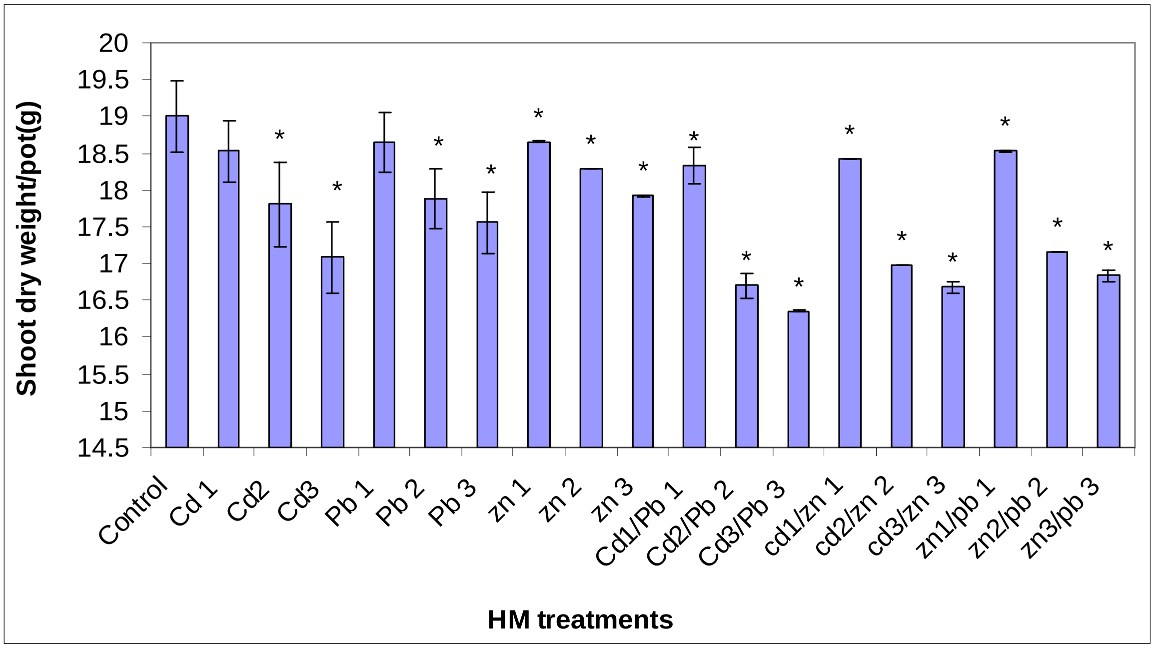

Fig 7.3 Effects of different concentrations of HMs on shoot dry weight. The error bars indicate the standard deviation, while asterisks (*) indicate significant ($p<0.05$) change from control

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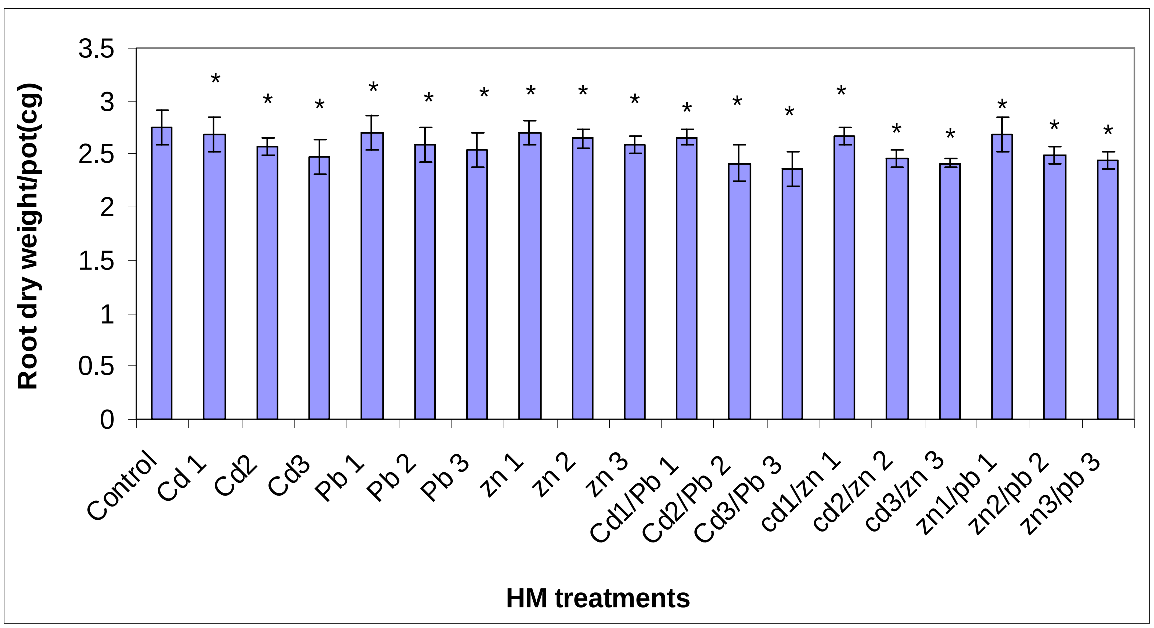

Fig 7.4 Effects of different concentrations of HMs on root dry weight. The error bars indicate the standard deviation, while asterisks (*) indicate significant ($p<0.05$) change from control

3.2. HMs effects on plant length

Shoot and root lengths of *S. oleracea* were negatively significantly ($p<0.05$) effected with addition of individual Cd, Pb and Zn and their mixture. The increasing concentrations of HMs in the soil resulted a decrease in the shoot and root lengths of *S. oleracea* plant (Fig. 7.5, 7.6). At the highest dose of Cd the shoot and root lengths were decreased by 18% and 72.7%, respectively (Table 7.2) as compared to control. Similarly, at the highest dose of Pb the shoot and root lengths were decreased by 13% and 38.8%, respectively (Table 7.2) as compared to control. At the highest dose of Zn the shoot and root lengths were decreased by 3.00% and 25.7&, respectively (Table 7.2) as compared to control. The reduction in the shoot and root lengths were 20.9%and 78%, respectively as compare to control, under the influence of highest dose of Cd/Pb.

The combine toxicity of Pb/Zn in the shoot and root lengths were 23% and 61%, respectively as compare to control. The reduction in shoot and root lengths were 14.1% and 41.2% , respectively under the influence of combine effects of Pb and Zn.
In general the root lengths were more affected than shoot lengths.

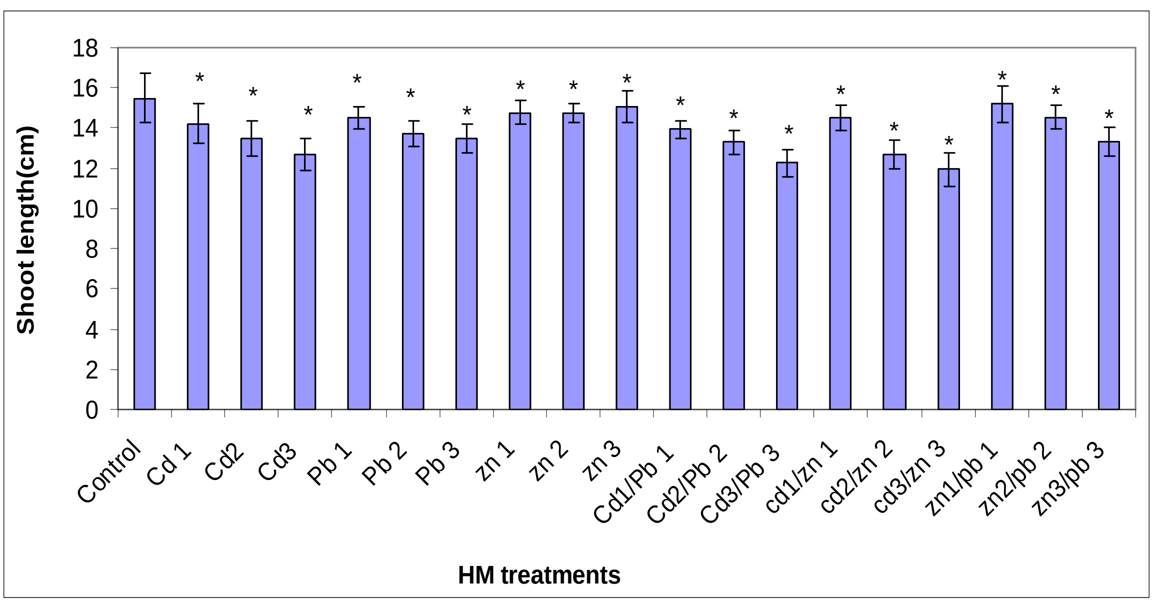

Fig 7.5 Effects of different concentrations of HMs on shoot length. The error bars indicate the standard deviation, while asterisks (*) indicate significant ($p<0.05$) change from control

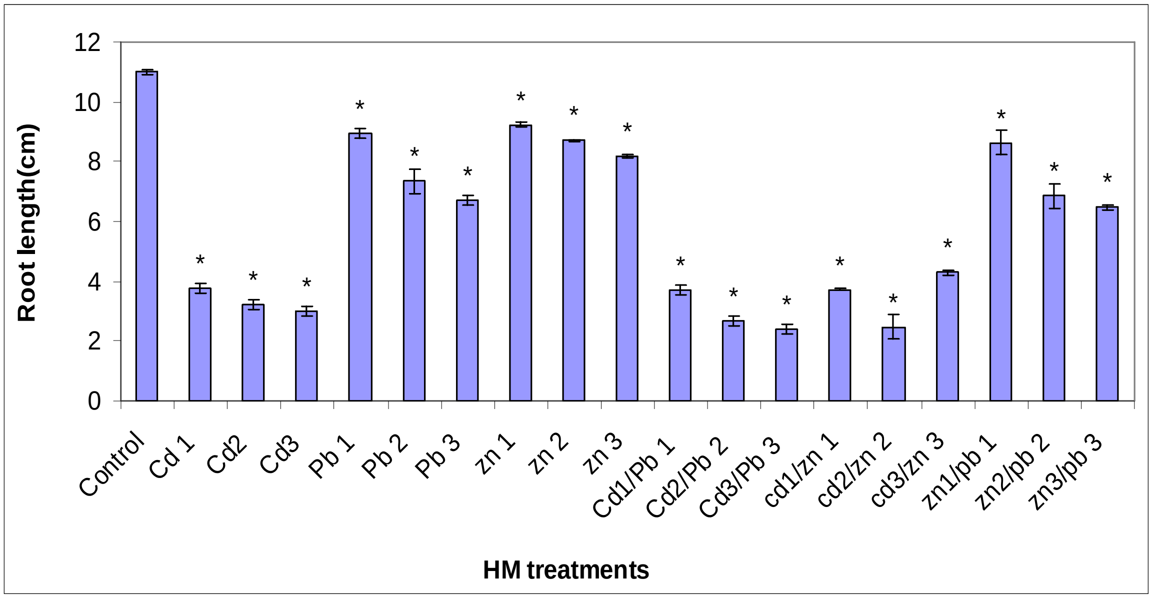

Fig 7.6 Effects of different concentrations of HMs on root length. The error bars indicate the standard deviation, while asterisks (*) indicate significant ($p<0.05$) change from control

3.3 HMs effects on total protein, fiber and moisture contents

Figures 7.7, 7.8 and 7.9 present the toxicological effects of Cd, Pb, Zn, Cd/Pb, Cd/Zn and Pb/Zn on total protein, fiber and moisture contents of *S. oleracea*. HMs concentration had a significant ($p<0.05$) adverse impacts on protein, fiber and moisture of *S. oleracea*. At the highest dose of Cd total protein, fiber and moisture contents of *S. oleracea* decreased by 31%, 28.7% and 32.9%, respectively (Table 7.3) as compared to control. Similarly, at the highest dose of Pb total protein, fiber and moisture contents decreased by 23%, 21.7% and 28.7%, respectively (Table 7.3) as compared to control. The addition of high dose of Zn decreased total protein, fiber and moisture contents by 15.9%, 15.7% and 20.4%, respectively (Table 7.3) as compared to control.

The reductions in total protein, fiber and moisture contents were 35.7%, 36.7% and 43%, respectively as compared to control, under the influence highest dose of Cd/Pb. Similarly, at the highest dose of Cd/Zn total protein, fiber and moisture contents were decreased by 26.6%, 25.7% and 30.7%, respectively (Table 7.3) as compared to control. The addition of high dose of Pb/Zn decreased total protein, fiber and moisture contents by 26 %, 27.7% and 40.3%, respectively (Table 7.3) as compared to control. The effects of mixture of Cd and Pb on total protein, fiber, Na, K, Ca, Fe and Mn and are more toxic than their individual effects. While in case of moisture contents the combine toxicity of Cd and Pb are less sever than the toxicity due to Pb and more than single Cd. The toxicity due to mixture of Cd and Pb in case of Ca and Mg contents of *S. oleracea* was less sever than their individual forms. The combine toxicity due to Cd and Zn is less severe than the toxicity due to Cd and more than the toxicity due to Zn in all most all nutrients contents of *S. oleracea*. The toxicity due to Pb/Zn was severe than the toxicity due Pb and Zn alone in case of nutrients contents.

Table 7.3 Reduction (%) in nutrients of *S. oleracea* with different concentrations of HMs in comparison with control.

Treatments	Total protein	Fiber	Moisture	Na	K	Ca	Fe	Mg	Mn	Cu
Cd 1	11.3	13.7	19.6	29.7	18.7	19.8	14.0	27.0	15.0	11.3
Cd2	19.0	18.3	22.1	46.0	21.0	31.2	18.2	49.3	22.0	13.8
Cd3	31.0	28.7	32.9	58.7	23.5	42.3	20.7	67.0	31.7	21.6
Pb 1	8.30	11.5	13.7	28.0	16.0	13.2	12.0	24.9	12.3	09.7
Pb 2	9.70	14.0	20.7	33.0	18.7	17.0	17.7	31.7	16.7	12.0
Pb 3	23.0	21.70	28.7	41.7	20.3	26.8	19.3	66.2	27.7	16.4
Zn 1	8.20	7.70	10.7	18.3	13.3	12.4	11.3	17.7	11.3	08.2
Zn 2	10.5	9.70	18.7	24.7	17.0	15.6	14.3	24.9	13.0	11.4
Zn 3	15.1	15.7	20.4	26.3	25.0	18.3	14.3	43.6	20.3	15.4
Cd1/Pb1	23.7	24.7	27.0	56.3	27.7	31.4	20.0	39.7	34.0	17.4
Cd2/Pb2	37.3	31.0	30.7	65.0	30.3	44.6	23.7	41.0	49.7	30.7
Cd3/ Pb3	35.7	36.7	43.0	67.3	31.0	54.7	29.3	45.7	52.3	34.3
Cd1/Zn1	17.0	15.3	21.4	38.0	20.0	22.7	15.7	44.0	45.2	14.8
Cd2/Zn2	23.0	23.7	29.0	46.0	28.0	40.8	19.7	48.7	50.9	16.2
Cd3/ Zn3	26.6	25.7	30.7	60.3	31.0	53.3	22.3	52.3	57.7	27.7
Pb1/Zn1	10.4	19.3	27.7	30.7	18.0	19.0	13.7	45.7	35.0	10.3
Pb2/Zn2	12.4	22.3	32.7	36.7	25.0	29.3	16.0	49.3	39.0	13.7
Pb3/Zn3	26.0	27.7	40.3	39.7	28.0	35.7	19.7	56.7	41.1	22.5

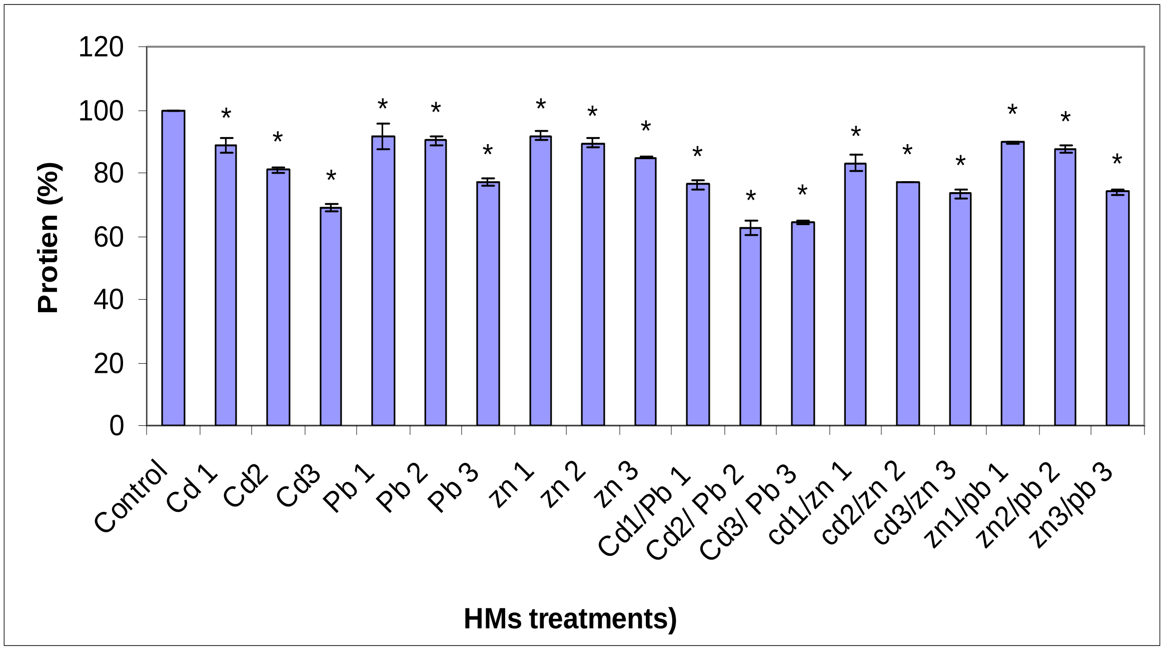

Fig 7.7 Comparison of total protein content with control under the influence of different concentrations of HMs. The error bars indicate the standard deviation, while asterisks (*) indicate significant ($p<0.05$) change from control

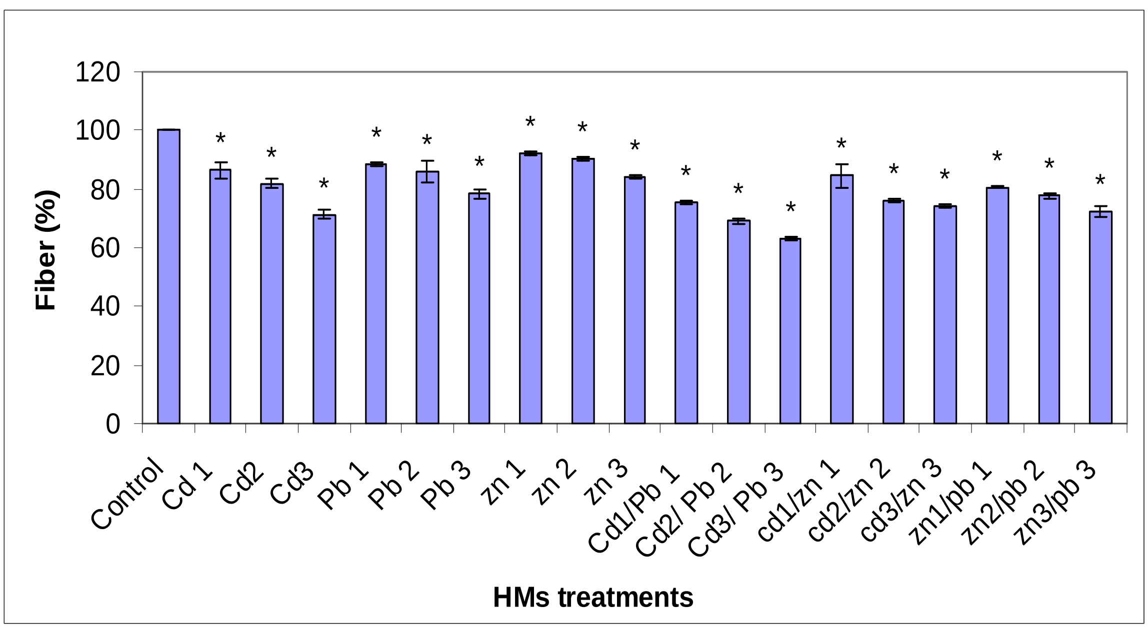

Fig 7.8 Comparison of fiber content with control under the influence of different concentrations of HMs. The error bars indicate the standard deviation, while asterisks (*) indicate significant($p<0.05$) change from control

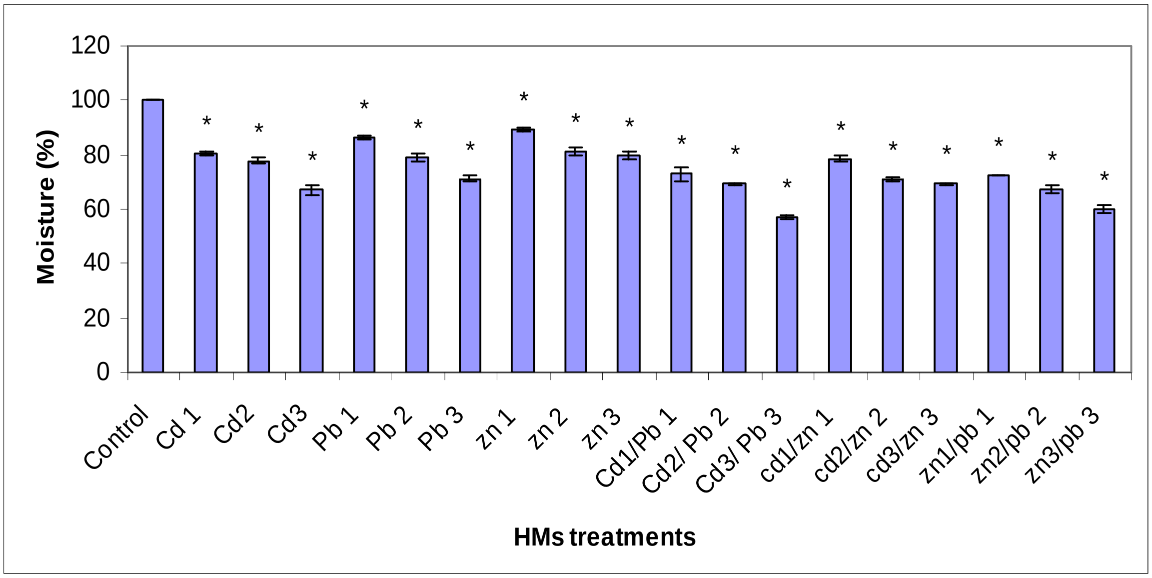

Fig 7.9 Comparison of moisture content with control under the influence of different concentrations of HMs. The error bars indicate the standard deviation, while asterisks (*) indicate significant ($p<0.05$) change from control

3.4 HMs effects on metals up take of minerals

The increasing concentrations of HMs resulted a decrease in Na, K, Ca, Fe, Mg, Mn and Cu in *S. oleracea* (Figures 7.10, 7.11, 7.12, 7.13, 7.14, 7.15, 7.16). The reduction in concentrations Na, K, Ca, Fe, Mg, Mn and Cu of *S. oleracea* was significant ($p<0.05$). At the highest dose of Cd, the concentrations of Na, K, Ca, Fe, Mg, Mn and Cu were reduced to 58.7%, 23.5% and 42.3%, 20.7%, 67%, 31.7% and 21.6, respectively (Table 3.2) as compared to control. Similarly At the highest dose of the concentrations of Pb, the concentrations of Na, K, Ca, Fe, Mg, Mn and Cu showed a reduction of 41.3%, 20.3%, 26.8%, 19.3%, 66.2%, 27.7 and 16.4%, respectively (Table 7.3) compared to control. At the highest dose of Zn, the concentrations of Na, K, Ca, Fe, Mg, Mn and Cu showed a reduction of 26.3%, 25%, 18.3%, 43.6%, 20.3% and 15.4%, respectively (Table 7.3) compared to control. The reduction in concentrations of Na, K, Ca, Fe, Mg, Mn and Cu were 67.3%, 31%, 54.7%, 29.3%, 45.7%, 52.3 and 34.3%, respectively as compared to control under the influence highest dose of Cd/Pb. The concentrations of Na, K, Ca, Fe, Mg, Mn and Cu were 60.3%, 31%, 53.3%, 22.3%, 52.3, 57.7% and 29.7%, respectively as compared to control under the influence highest dose of Cd/Zn. Similarly, at highest dose of the concentrations of Pb/Zn Na, K, Ca, Fe, Mg, Mn and Cu were 39.7%, 28%, 35.7%, 19.7%, 56.7%, 41.1 and 22.5%, respectively as compared to control.

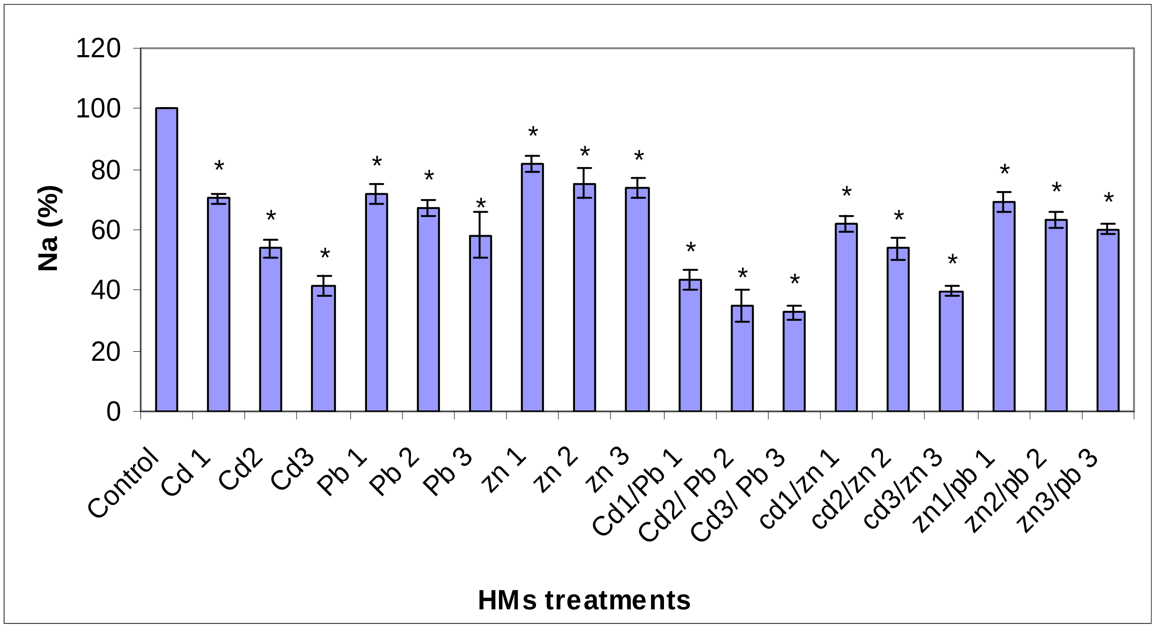

Fig 7.10 Comparison of Sodium (Na) with control under the influence of different concentrations of HMs. The error bars indicate the standard deviation while asterisks (*) indicate significant change from control

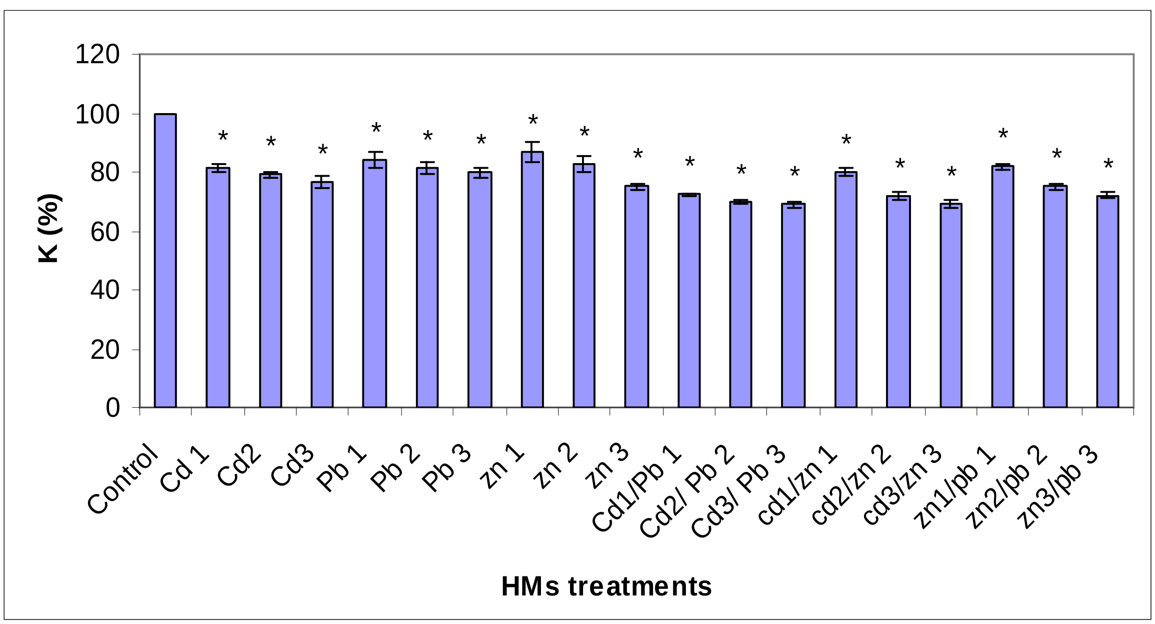

Fig 7.11 Comparison of Potassium (K) with control under the influence of different concentrations of HMs. The error bars indicate the standard deviation while asterisks (*) indicate significant change from control

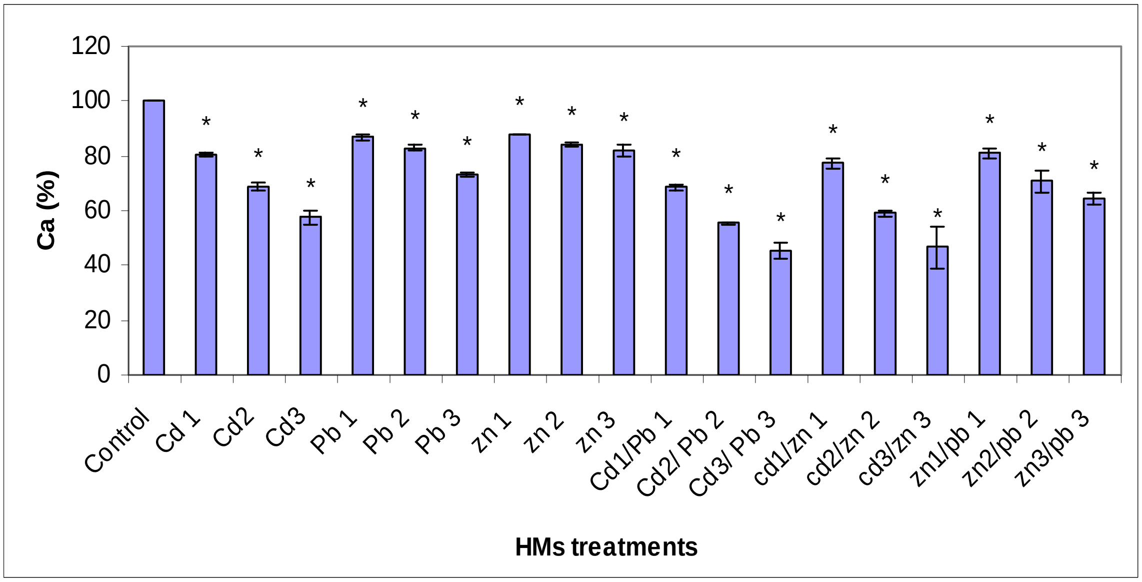

Fig 7.12 Comparison of Calcium (Ca) with control under the influence of different concentrations of HMs. The error bars indicate the standard deviation while asterisks (*) indicate significant change from control

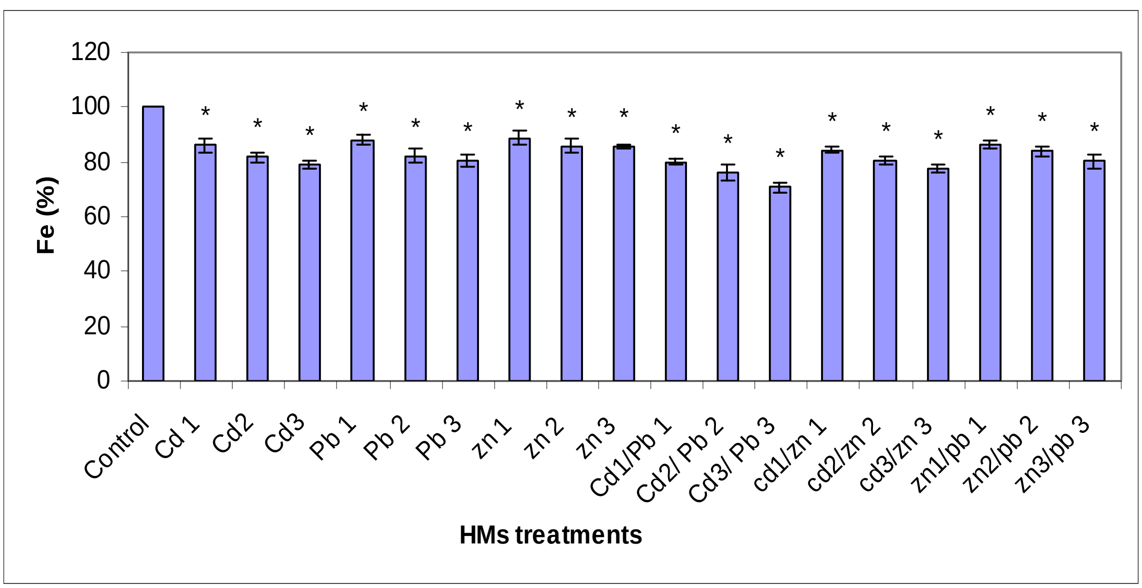

Fig 7.13 Comparison of Iron (Fe) with control under the influence of different concentrations of HMs. The error bars indicate the standard deviation while asterisks (*) indicate significant change from control

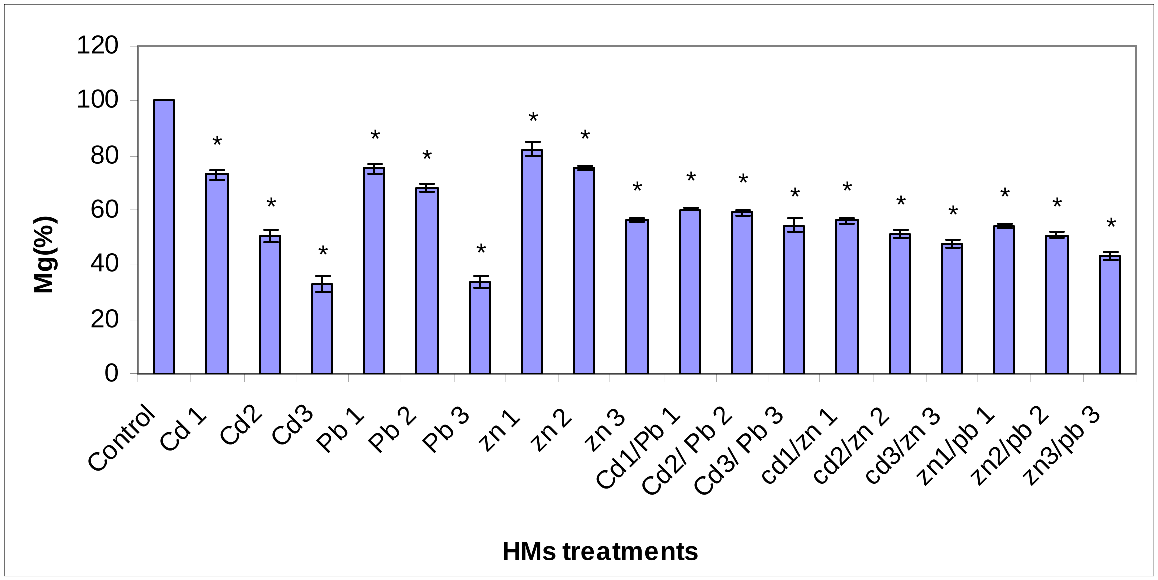

Fig 7.14 Comparison of magnesium (Mg) with control under the influence of different concentrations of HMs. The error bars indicate the standard deviation while asterisks (*) indicate significant change from control

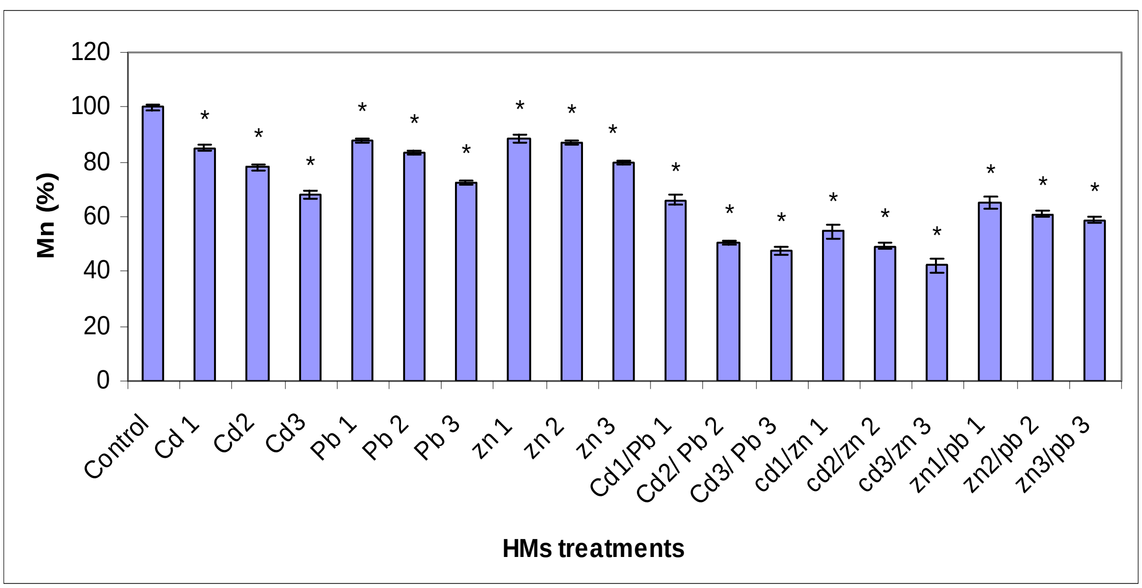

Fig 7.15 Comparison of manganese (Mn) with control under the influence of different concentrations of HMs. The error bars indicate the standard deviation while asterisks (*) indicate significant change from control

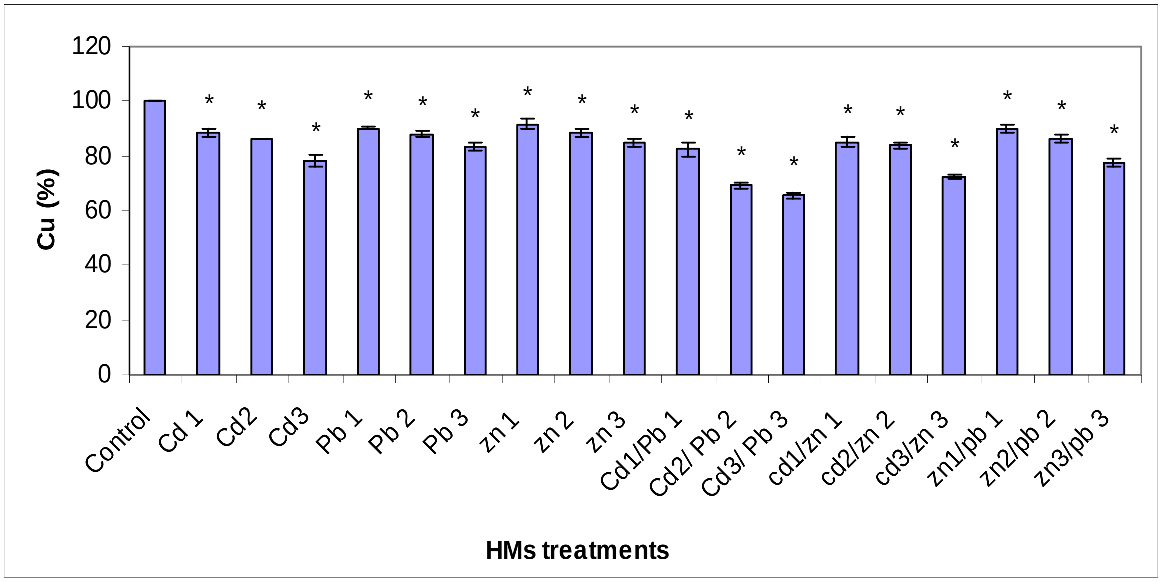

Fig 7.16 Comparison of Copper (Cu) with control under the influence of different concentrations of HMs. The error bars indicate the standard deviation while asterisks (*) indicate significant change from control

3.5. Up take of HM

The relationships between the Cd concentrations in soil and in root tissues are given in fig 7.17. All three relationships ie between the single Cd concentration in soil ant seedlings (Fig 7.17-a), between Cd/Pb in soil and in root (Fig 7.17-b) and between Cd/Zn in soil and in root (Fig 7.17-c) show that with increasing concentrations of Cd in soil whether in single form or in mixture its content in the root increasing. Root accumulated Cd is in order of single Cd > Cd/Pb > Cd/Zn (Fig. 7.21) Similarly, Figures 7.18, 7.19, 7.20 present the relationship between the Cd concentrations in the root of *S. oleracea* and shoot in single Cd, Cd/Pb and Cd/Zn treatments. All the relationships are positive. The increasing concentrations of Cd in root, increase concentration in shoot as well.

The concentrations of Pb in root showed an increase with increasing concentration in the soil. The regression analysis showed positive relationships between the concentrations of Pb in soil and in root in all three treatments ie single Pb, Pb/Cd and Pb/Zn treatments (Fig 7.22-a, Fig 7.22-b, Fig 7.22-c). fig 7.26 presents that Pb accumulates in the root is in order of Single Pb > Pb/Cd > Pb/Zn. Similarly, figures 7.23, 7.24, 7.25 present the relationship between the Pb concentrations in the root of *S.*

oleracea and shoot in single Pb, Cd/Pb and Pb/Zn treatments. All the relationships are positive. The increasing concentrations of Pb in root, increase concentration in shoot as well

The regression analysis showed strong positive relationship between the Zn concentrations in soil and root in single Zn, Zn/Cd and Zn/Pb treatments (Fig 7.27-a, Fig 7.27-b, Fig 7.27-c). root accumulated Zn is in order of single Zn > Zn/Cd > Zn/Pb (Fig. 7. 31). Figures 7.28, 7.29 and 7.30 present the relationship between the Zn concentrations in the root of *S. oleracea* and shoot in single Zn, Cd/Zn and Pb/Zn treatments. All the relationships are positive. The increasing concentrations of Zn in root, increase concentration in shoot as well.

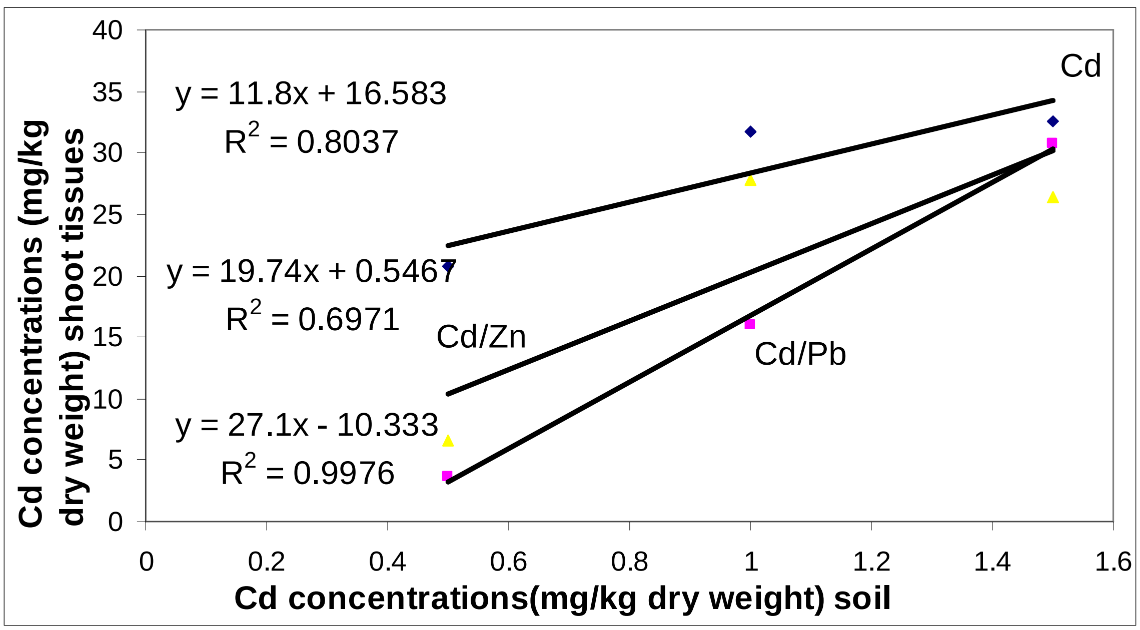

Fig. 7.17 Linear regressions model for Cd concentrations in soil and root tissues on dry weight basis. (a) Relationship between soil Cd content and root in single Cd treatments. (b) Relationship between soil Cd content and root in Cd/Pb treatments. (c) Relationship between soil Cd content and root in Cd/Zn treatments

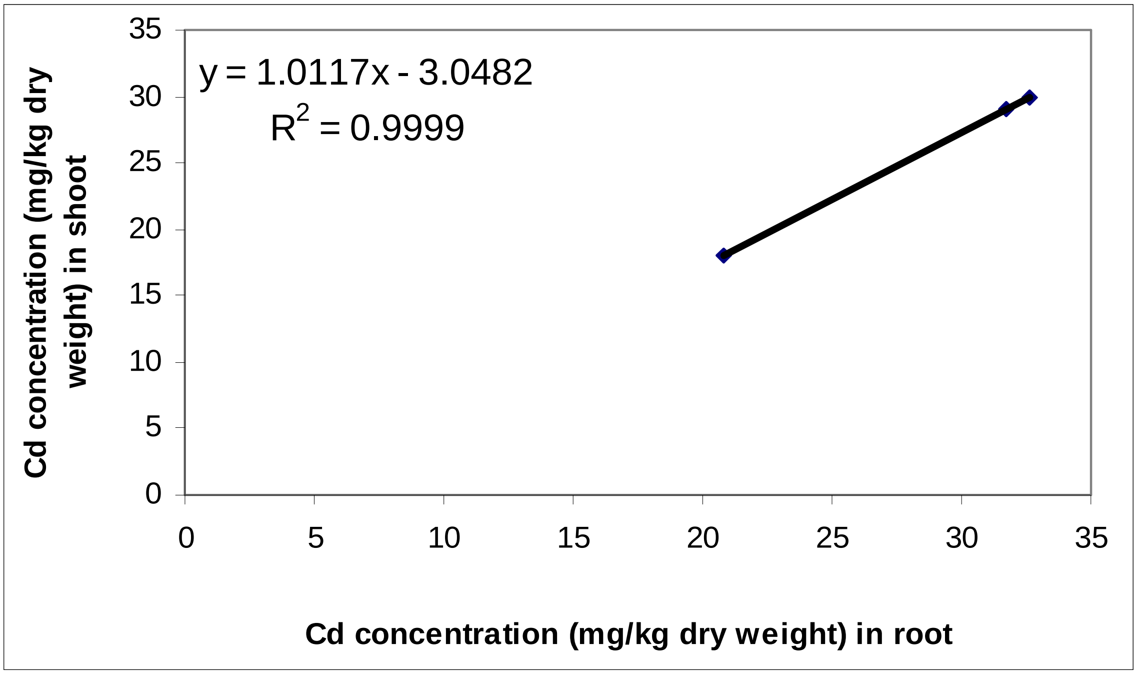

Fig. 7.18 Linear regressions model for Cd concentrations in root and shoot in single Cd treatment.

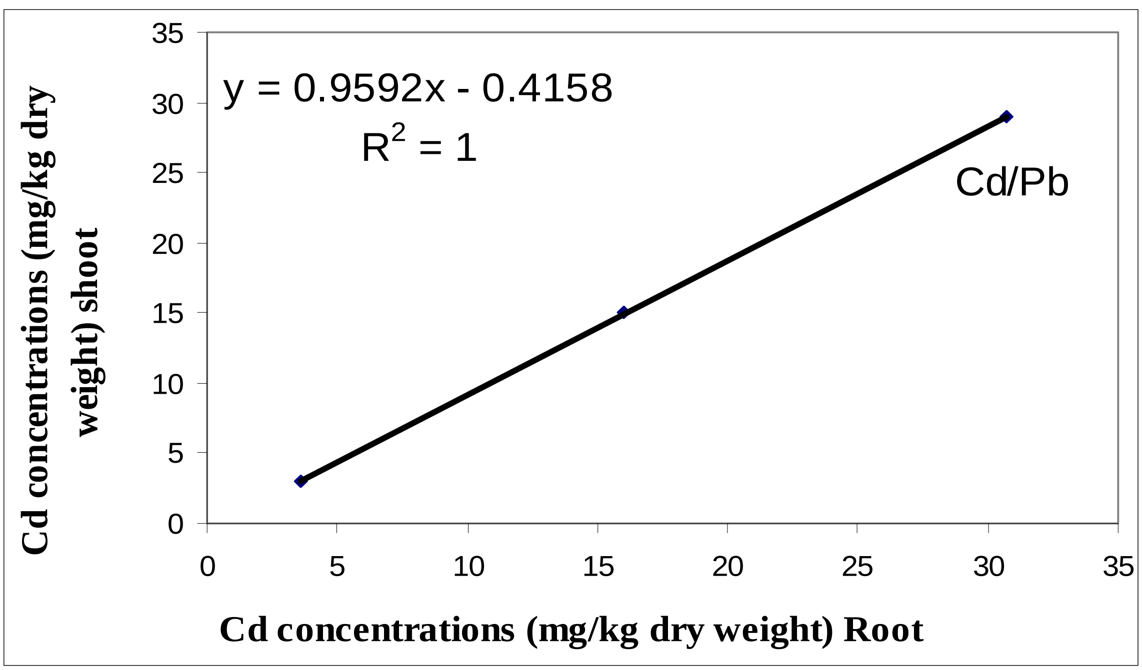

Fig. 7.19 Linear regressions model for Cd concentrations in root and shoot in Cd/Pb treatment.

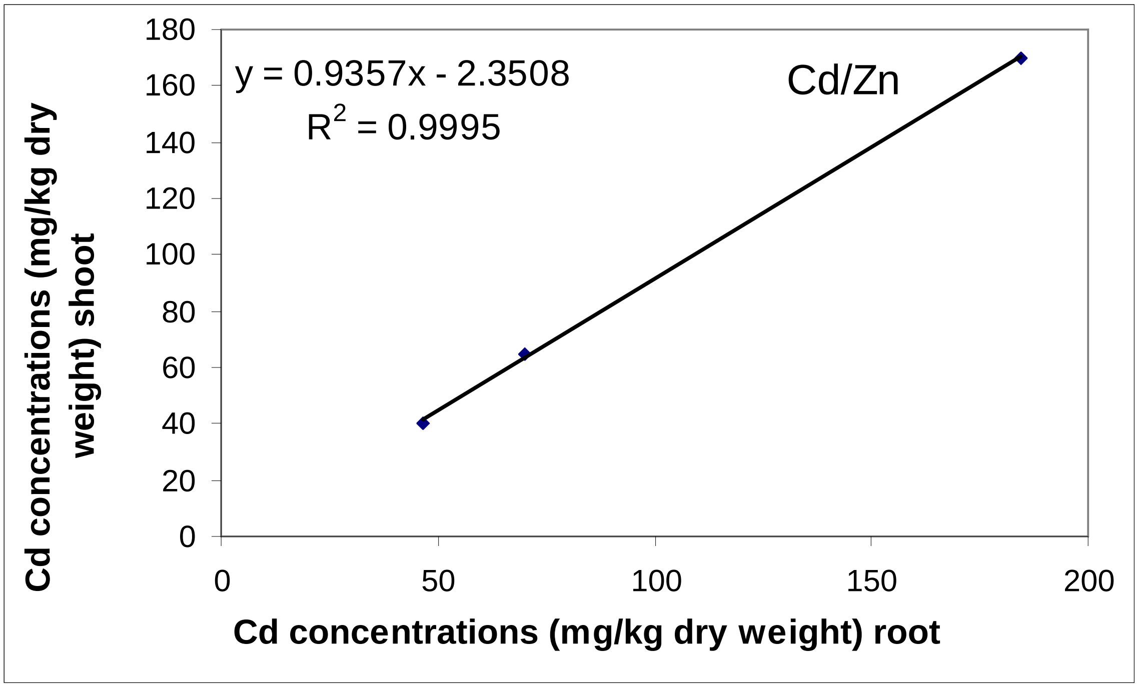

Fig. 7.20 Linear regressions model for Cd concentrations in root and shoot in Cd/Zn treatment.

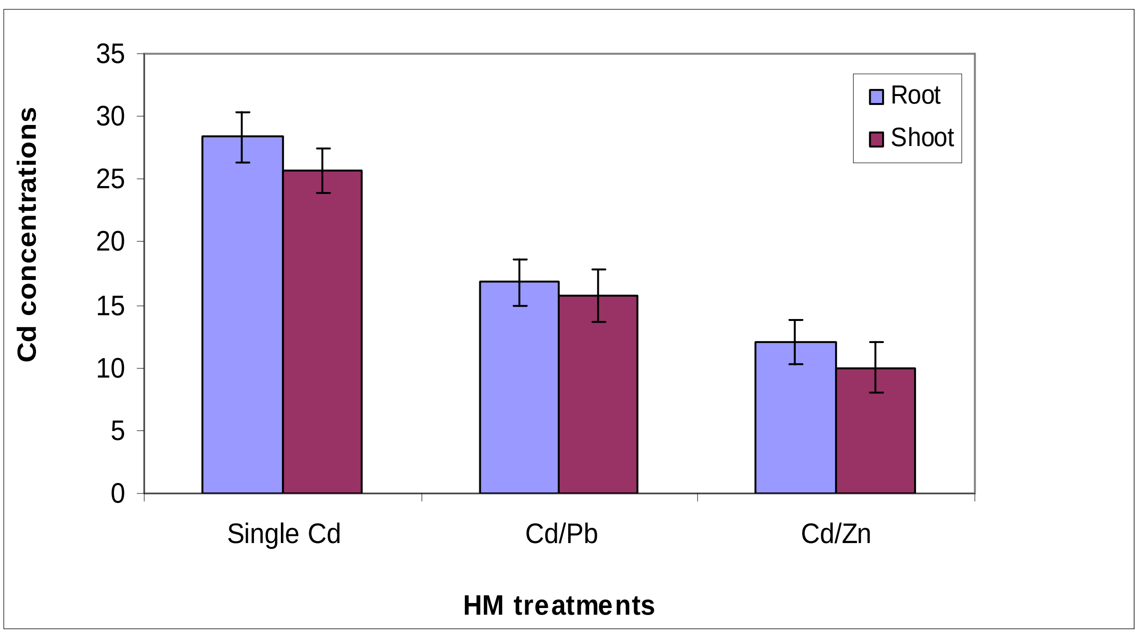

Fig 7.21 Comparison of Cd up take from different treatments. The error bars indicate the standard deviation

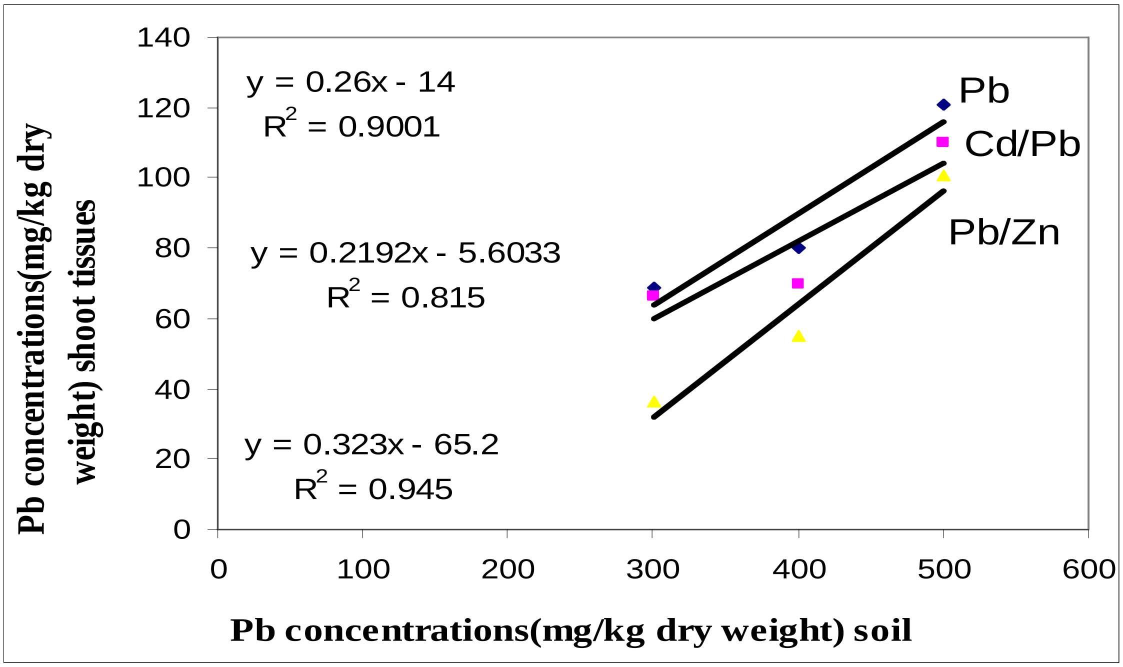

Fig. 7.22 Linear regressions model for Pb concentrations in soil and root tissues on dry weight basis. (a) Relationship between soil Pb content and root in single Pb treatments. (b) Relationship between soil Pb content and root in Cd/Pb treatments. (c) Relationship between soil Pb content and root in Cd/Zn treatments

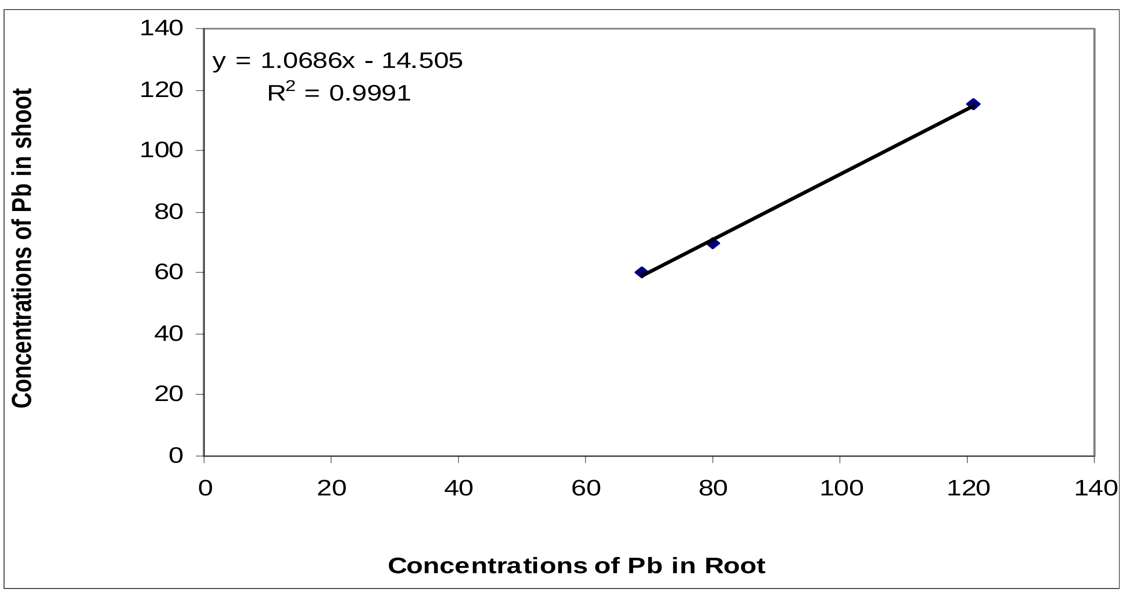

Fig. 7.23 Linear regressions model for Pb concentrations in root and shoot in single Pb treatments

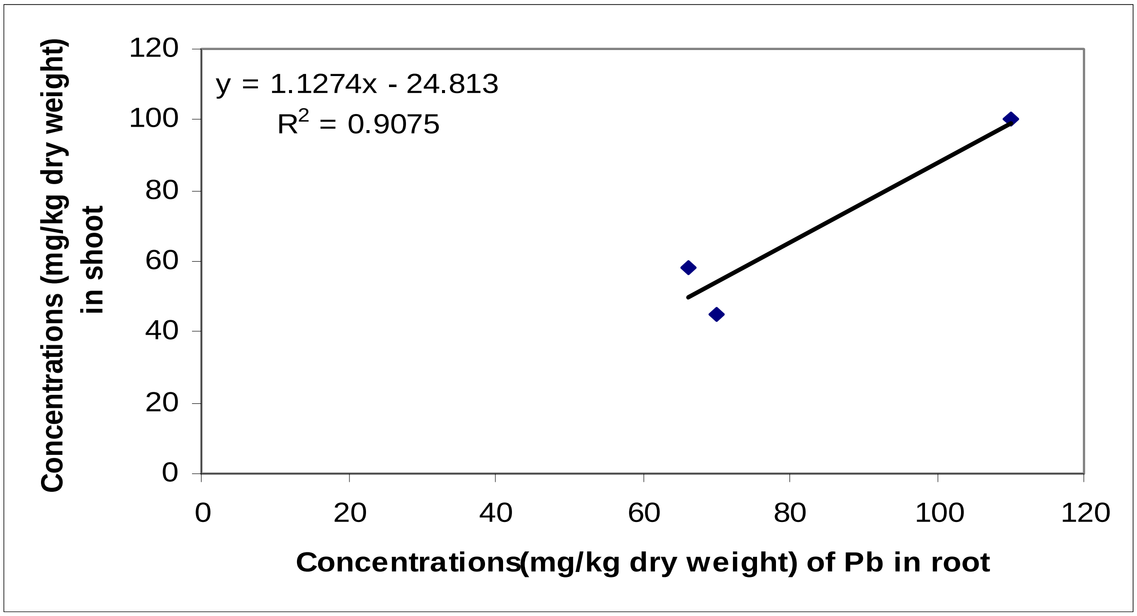

Fig. 7.24 Linear regressions model for Pb concentrations in root and shoot in Pb/Cd treatment

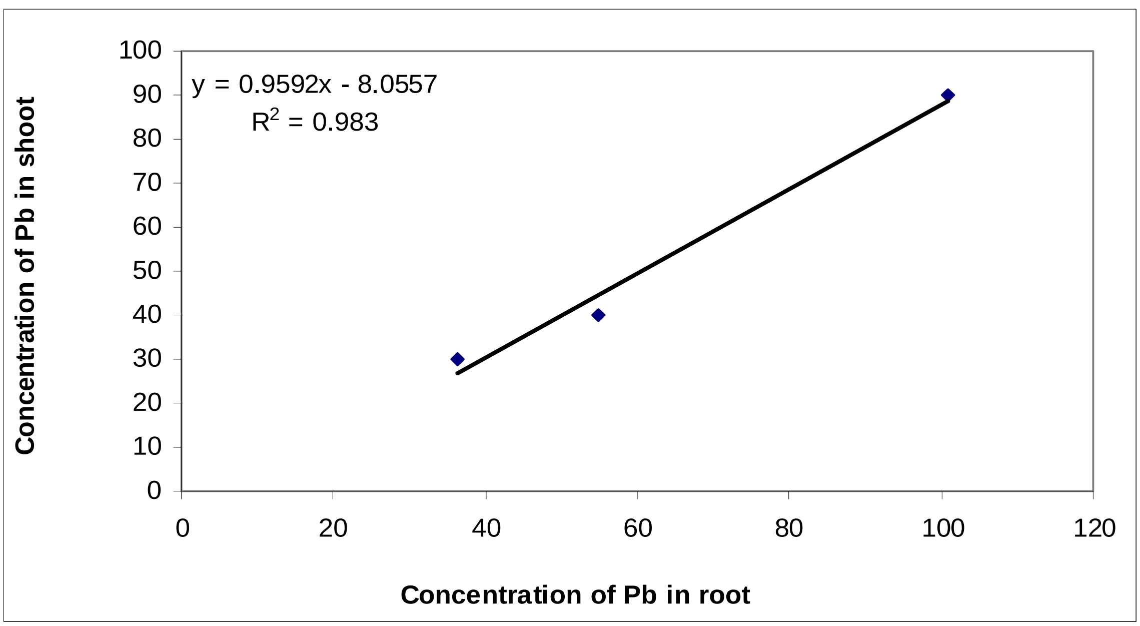

Fig. 7.25 Linear regressions model for Pb concentrations in root and shoot in Pb/Zn treatment

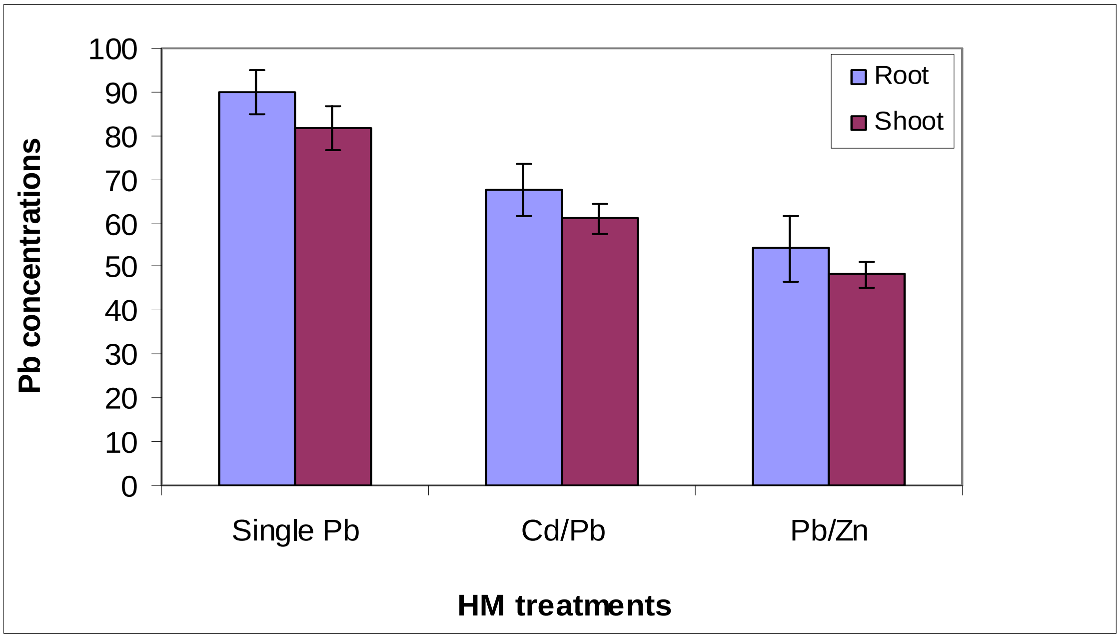

Fig 7.26 Comparison of Pb up take of different treatments. The error bars indicate the standard deviation

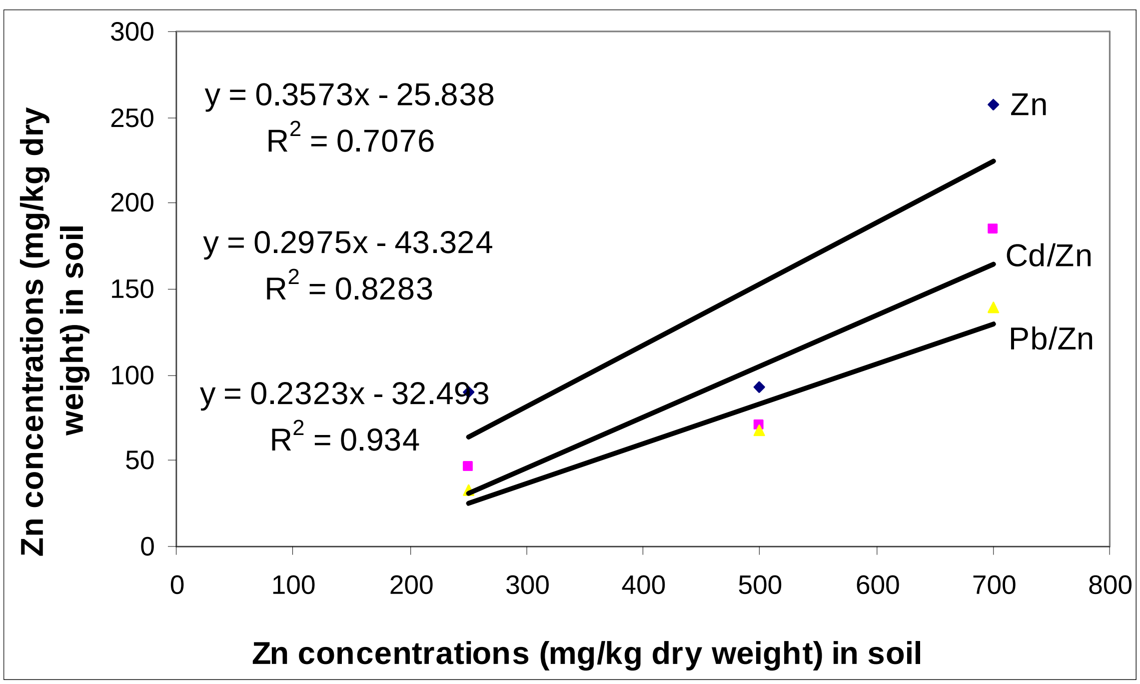

Fig. 7.27 Linear regressions model for Zn concentrations in soil and root tissues on dry weight basis. (a) Relationship between soil Zn content and root in single Zn treatments. (b) Relationship between soil Zn content and root in Cd/Pb treatments. (c) Relationship between soil Zn content and root in Cd/Zn treatments

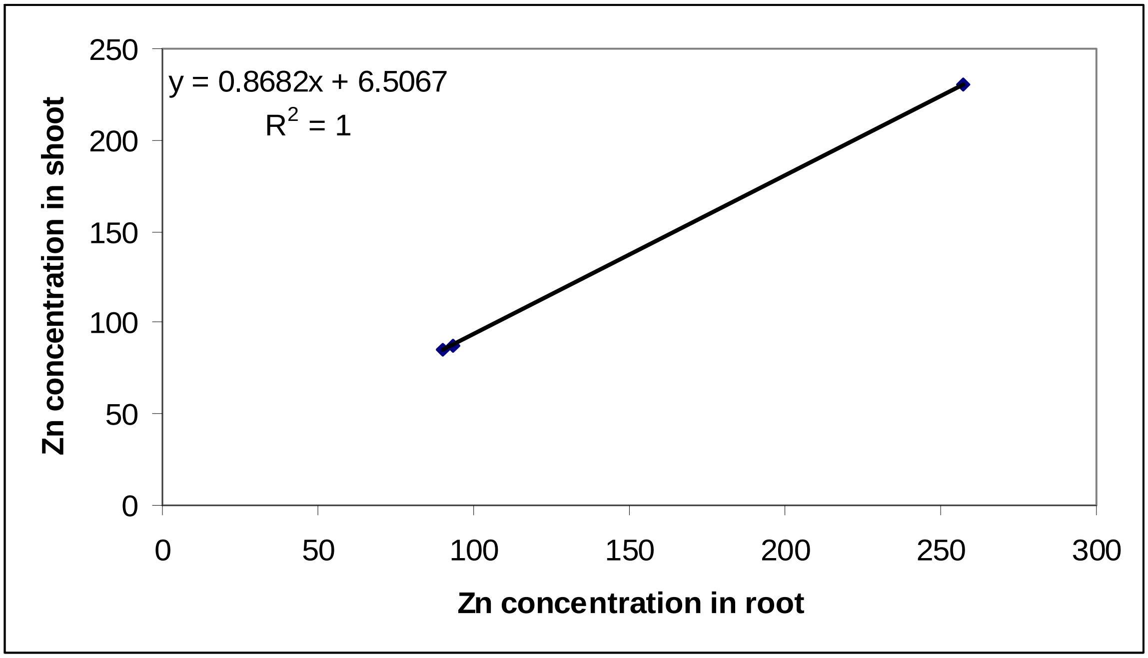

Fig. 7.28 Linear regressions model for Zn concentrations in root and shoot in single Zn treatment

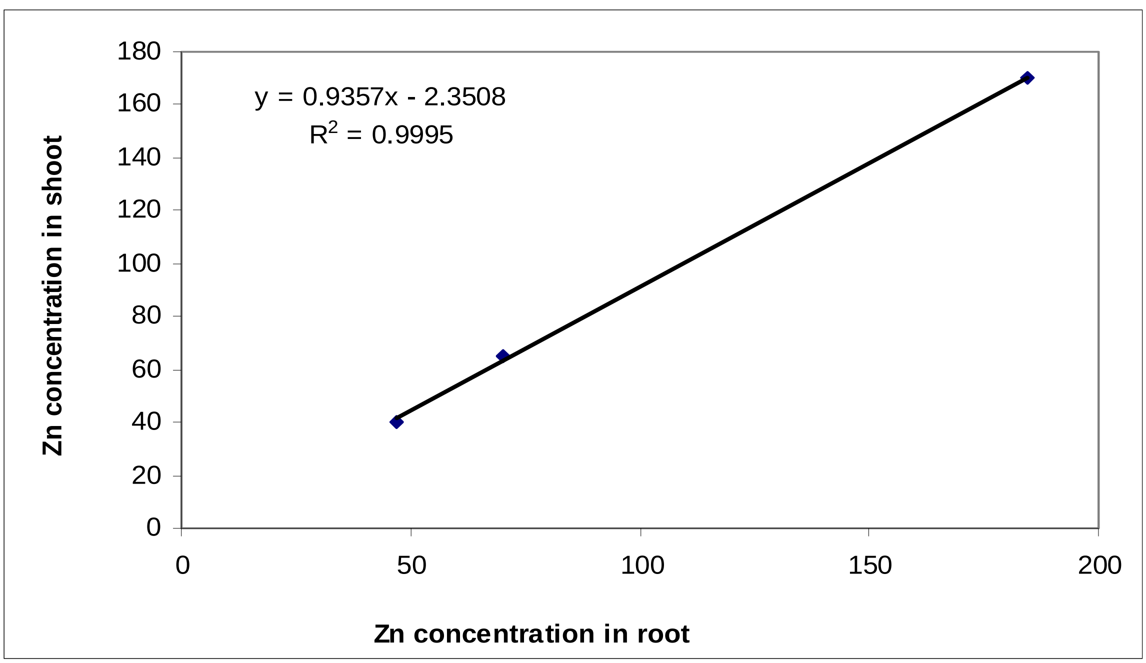

Fig. 7.29 Linear regressions model for Zn concentrations in root and shoot in Cd/Zn treatment

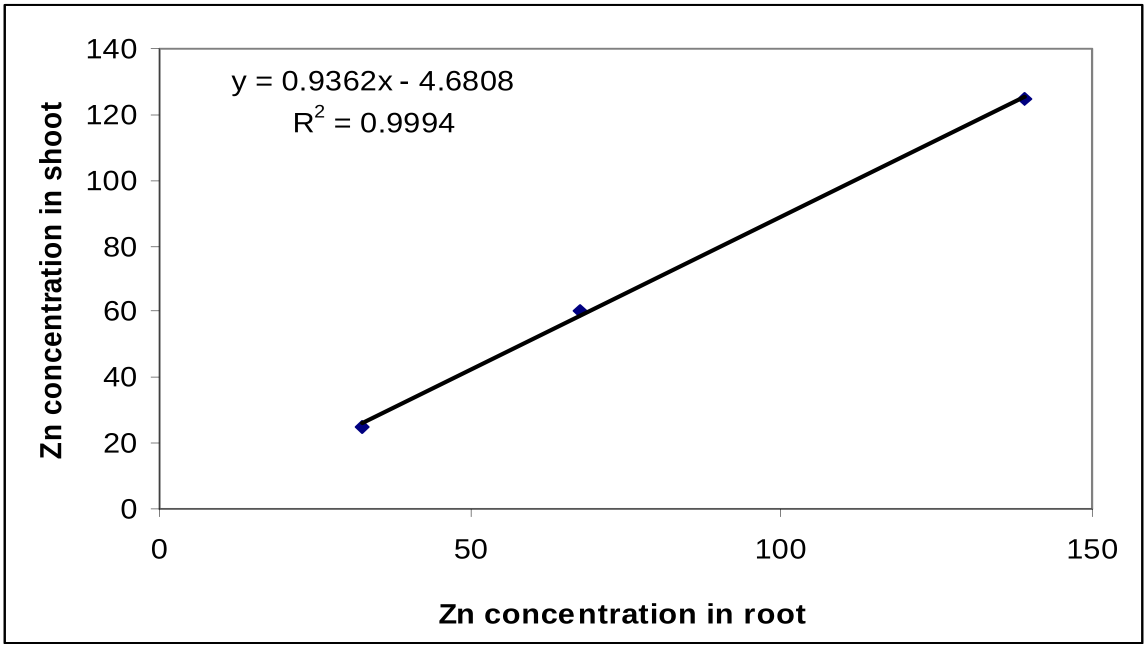

Fig. 7.30 Linear regressions model for Zn concentrations in root and shoot in Pb/Zn treatment

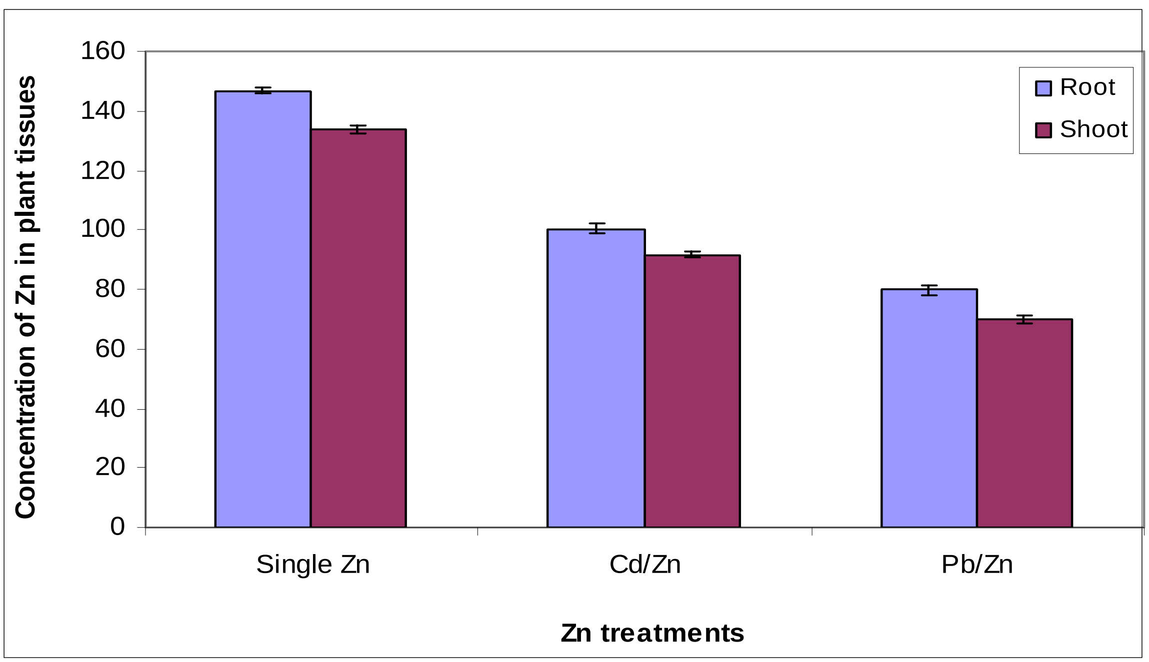

Fig 7.31 Comparison of Zn up take of different treatments. The error bars indicate the standard deviation

4.0, Discussion

Plants grow on HMs contaminated soil, accumulate HMs in their body tissues (Sun et al., 2008; Khan et al., 2008ab; Lingua et al., 2008).HMs are toxic for plants they can reduce plant yield, affect leaf and root growth and inhibit enzymatic activities (Zeng et al., 2008).

Cd inhibited plant growth and the toxicity increase with increasing Cd concentration in soil. In the present study, increasing concentrations of Cd significantly ($p<0.05$) reduced shoot and root fresh and dry weights. The results are agreed with results of (An et al., 2004; Shukla et al., 2003; Devi et al., 2007). The results of the present study are not consistent with the results of (Vogel-Mikus et al., 2006; Lin et al., 2007). Increasing concentrations of Cd in soil produced toxicity in plant biomass and plant lengths of *S. oleracea* which is an agreement with the study of Shukla et al, (2003). Cd toxicity was more severe in root in terms of both biomass and length. Root is more sensitive than shoot because it is the part of plant which comes in contact with toxicant first. Liu et al, (2004) reported that reduction of formation of new cells under the influence of Pb and Cd leads reduction in shoot and root lengths.

S. oleracea produced toxicity with increasing concentrations of Pb in terms of plant biomass and length. Increasing concentrations of Pb significantly ($p<0.05$) reduced plant biomass, and length. An et al, (2004) and Farooqi et al, (2009) observed drop in shoot and root growth of *C. sativus, A. lebbeck* with Increasing concentrations of Pb in growing medium. Zn is an essential element for plants, but its excess can significantly damage plants (Wang et al., 2009). This study showed that increasing concentrations of Zn are responsible for increase toxicity in *S. oleracea*. Shoot and root fresh and dry weights were reduced with increasing concentrations Zn. Zn reduces plant biomass because of creation of deficiency of macronutrients such is P (Marschner, 1995). Mahmood et al, (2005) found reduction in growth of corn with increasing concentrations of Zn.

The combine toxicity of Cd/Pb in terms of biomass and length is severe than the toxicity of Cd and Pb alone in *S. oleracea*. Combine toxicity of Cd/Pb is sever than toxicity of single Cd and Pb but is less than the additive toxicity of the two HMs alone. The up take results showed that in combination with Pb, Cd decreased the up take of Pb and Pb decreased the up take of Cd. So although both Cd and Pb are toxic but as they decrease the up take of each other so the toxicity is not the additive of both toxicities. Kalavrouziotis et al, (2009) produced the same results in *Broccoli* at low Cd concentrations, while An et al, (2004) produced the same result in studying *C. Sativus*. Combine toxicity of Cd/Zn is sever than toxicity of single Cd and Zn but is less than the additive of the toxicity of the two HMs alone The combine toxicity is not the additive sum of both the toxicities because Zn and Cd both reduces the up take of each other by plants (Weihong et al., 2009).

The combine toxicity of Cd/Zn is more than the toxicity of single Zn and Cd in terms of biomass plant length. The result is consistent with the study Xu et al, (2006)**.** Combine toxicity of Pb/Zn in terms of all biomass and length is more than the single toxicities but, less than additive sum of the single toxicities of both Zn and Pb. The reason of reduction toxicological effects of Pb/Zn is the synergetic affects of Zn and Pb. Pb decreases the up take of Zn and Zn reduces the up take of Pb.

The increasing concentrations of Cd, Pb and Zn decrease total protein contents in plants. The results of this study showed a drop in the total protein content in plant shoot with increasing concentrations of Cd, Pb and Zn alone and also their mixture (Cd/Pb, Cd/Zn and Pb/Zn).The results are consistent with the results of previous studies (Auda et al., 2010; Wang et al., 2009; Kastori et al., 1992). Auda et al, (2010) and Zengin and Munzuroglu, (2006) found a reduction in protein content of carrot, sunflower with increasing concentration of Cd in growing medium, respectively. High dose of Pb like1500 µM is responsible for 77% reduction in protein content in *B. juncea.*

Khudsar et el, (2004), John et al, (2009) and Wang et al, (2009) found a reduction in content of protein in alga and rapeseed, respectively with increasing concentration of

Zn. Many reasons are responsible for drop of protein content with HMs, the drop may be due to accelerating the degradation of protein with increasing protease activity (Palma et al., 2002), disturbance of nitrogen metabolism in presence of HMs like Cd and Pb. The protease activity increases in stress conditions (John et al., 2009) like presence of HMs in growing medium. According to Odjegba and Fasidi, (2006), HMs like Cd and Pb disturb the nitrogen metabolism, which further decreases the synthesis of protein. HMs like Cd is responsible for reduction in the process of photosynthesis which reduces the synthesis of protein (Auda et al., 2010).

The results of present study showed that increasing concentrations of HMs resulted decrease in Na, K, Ca, Fe, Mg, Mn and Cu in *S. oleracea* (Figures 7.10, 7.11, 7.12, 7.13, 7.14, 7.15, 7.16). The results are consistent with the previous results. Excess of Zn decreased the uptake of elements like Mg, Mn, Cu and Fe in plants (Wang et al., 2009), The increasing concentration of Cd interferes with other elements like K, Ca and Mg, disturbed their distribution in plants parts and also decreases their contents in plants tissues (Auda et al., 2009). Wu et al, (2007) found a decrease in Mn concentration in barley plants with increasing concentration of Cd. Cd decreases the uptake of Fe (Larbi at al., 2002) and K, Mg and Ca (Dong et al., 2006). Toxicity in terms of reduction uptake rates of essential elements by Cd is severe than the toxicity due to Pb and Zn. There are two reasons of this one Cd is more toxic second Cd highly accumulates in plants especially in root. Salt et al, (1995) found bioaccumulation coefficients of Cd of up to 1100 in shoot and 6700 in root at 0.1µg/ml in soil. The reduction in the up take of elements is more in Cd/Pb treatments in comparison their alone treatments, but is less than additive sum of the toxicities. The reason may be to reduction in the uptake of both HMs. Similarly, the combine toxicities in terms of reduction metals up take in Cd/Zn treatments is less sever than the toxicity due to Cd but, more than the toxicity due to Zn. Zn reduces Cd uptake (Auda et al.,2010) so the toxicity also reduced. Similarly, the combine toxicity due to Pb/Zn is less than the toxicity due to Pb alone.

The high concentrations of Cd, Pb and Zn were in root, the results are agreed with previous studies. López-Millán et al, (2009) found highest concentrations of Cd in tomato root. Jiang and Wang, (2008) observed 10 times Zn in root of *P. australis.* Similarly, Vogel-Mikus et al, (2005) observed highest concentration of Pb in roots of plant. Roots show high concentrations of HMs as compare to other plant parts because HMs come in contact with root of plant first (Breckle, 1991)

Chapter:- 8 Impacts of HMs on coriander (*Coriandrum saivum*) nutritional values

1. Introduction

Vegetables grown in wastewater-irrigated soils are responsible to take up HMs in large enough quantities to cause human health problems. Under specific condition the HMs accumulate in the soil to the levels, which are toxic and can lead to arise ecological consequences (Wang et al., 2005). HMs accumulates in soil not only disturbed the normal functioning of soil ecosystem but also disturb the development of plants (Khan et al., 2008a). HMs affects plants adversely. HMs are toxic for plants they can reduce plant yield, affect leaf and root growth and inhibit enzymatic activities (Zeng et al., 2008).

Cd decreases the growth of plants (An et al., 2004), decreases plant biomass and length (Shukla et al., 2003; Devi et al., 2007), reduces the formation of new cells (Liu et al., 2004) and reduces mitotic index (Jun-yu et al., 2008). Cd which is toxic HM decreases nitrogen concentration in plants which further negatively affects photosynthesis, protein and carbohydrates formation and uptake of essential elements like Cu, Mn, Mg and K and affect plant yield (Auda et al., 2010). Cd is responsible for reduction of chlorophyll, sugar, protein and phenol and alters enzyme activity which leads to reduce plant yield.

Cd, Pb and Zn reduce the uptake of element in plants. Cd decrease uptake of Mn, Fe, K, Mg, Ca and Zn (Wu et al., 2007; Larbi at al., 2002; Dong et al., 2006) and finally change the nutrient concentration and composition in plants (Zhang et al., 2002). Excess of Zn decreased the uptake of elements like Mg, Mn, Cu and Fe in plants (Wang et al., 2009). These elements are very important for plant biological process like protein synthesis, chlorophyll (Wang et al., 2009).

Pb is not essential for plants but, plants easily absorb and accumulate Pb in their body tissues. Pb is most toxic for plants and causes many morphological and physiological changes in plants (Kambhampati et al., 2005). Pb has significant negative impacts on plant dry mass, root and shoots length (Juseph et al., 2002). Pb adversely affects the process of photosynthesis, respiration and metabolism of plants (Paolacci et al., 1997). It is responsible for reduction in weight of plant (Kosobrukhov, 2004), reduces chlorophyll content and thus negatively impacted photosynthesis in plants (Kambhampati et al., 2005). Pb adversely impact photosynthesis in plants by reducing chlorophyll content due to reducing the uptake of chlorophyll's essential elements like Mg and Fe (Bruzynski, 1987) Pb is responsible for reducing photosynthesis in plants because of infected chloroplast, changing essential enzymatic process for photosynthesis and disturbing the closing of stomata (Sharma and Dubey, 2005). Pb accumulates in root by binding itself to cell wall, organic acid and polysaccharides (Harmens et al., 1994). Pb is immobile in root due to which the amount of Pb in shoot is always less than it amount in root. Root is more sensitive towards HMs pollution. It may be due to direct toxicity of HMs in terms of inhibition root elongation and/or lowering concentration of important elements (Malik et al., 1992).

Although Zn is essential element for many physiological process of plant but, become toxic with its increasing concentration (Baccio et al., 2005). Its excess is very toxic for plant, it inhibit plant growth and is responsible for chlorosis in leaves (Lopez-Millan. et al., 2009). According to Wang et al, (2009) Zn reduced chlorophyll content and is responsible for chlorosis in leaves. Zn damage root and also kills cell (Wang et al., 2009). Elements like Mn, Mg, Cu and Fe are very important for physiological process of plants but increasing concentrations of HMs reduce their uptake by plants. Mn involves in splitting water molecules necessary for photosynthesis, Mg is important for chlorophyll, Cu, Fe and Mn are important for synthesis of chlorophyll (Wang et al., 2009). Elements like Cu, Mn, Mg and Fe reduce the toxicity of HMs (Jiang et al., 2007), the deficiency of these metals are dangerous for plants like deficiency of Mg is responsible for cholorosis in plant leaves (Jiang et al., 2007) and induce oxidative stress (Tewari et al., 2006). The protein levels of *E. crassipes* treated with different concentrations of HMs decreased gradually as the metal concentrations increased (Jiang et al., 2007).

2.0. Methodology

Soil and C. *saivum* plants were prepared with same methods in chapter-7. The same soil was used and the same treatments were prepared. Similarly, plant growth parameters, cell size, and MI were measured using the methods mentioned in chapter-7. Metals were extracted and analyzed using acids mixture. The detailed procedure is given in chapter 4. Total protein, fiber and moisture contents in C. *saivum* were analyzed by the methods mentioned in chapter-7. The data were statistically analyzed using statistical packages SPSS 17. The detailed are given in chapter-4

3.0 Results

3.1 HMs effects on plant biomass

Figures 8.1, 8.2, 8.3 and 8.4 present the toxicological effects of Cd, Pb, Zn, Cd/Pb, Cd/Zn and Pb/Zn on the biomass (shoot and root fresh and dry weights) of *C. saivum*. HMs concentration had a significant ($p<0.05$) adverse impacts on biomass of *C. saivum*. At the highest dose of Cd the shoot and root fresh and dry weights were decreased by 27.2%, 21.3%, 29.3% and 46.4%, respectively (Table 8.1) as compared to control. Similarly, at the highest dose of Pb shoot and root fresh and dry weights were decreased by 21.0%, 19.3%, 4.7% and 30.6%, respectively (Table 8.1) compared to control. The addition of high dose of Zn decreased shoot and root fresh dry weights by 9.9%, 15.1%, 13.9% and 29.3%, respectively (Table 8.1) compared to control.

The reductions in shoot and root fresh dry weights were 29.6%, 23.4%, 35.8% and 75.1%, respectively as compare to control, under the influence highest dose of Cd/Pb. Similarly, at the highest dose of Cd/Zn shoot and root fresh and dry weights were decreased by 23.5%, 20.0%, 30.1% and 57.8%, respectively (Table 8.1) as compared to control. The addition of high dose of Pb/Zn decreased shoot and root fresh and dry weights by 19.8%, 19.0%, 20.4% and 26.9%, respectively (Table 8.1) as compared to control.

The toxicity due to mixture of Cd and Pb was more severe than their individual toxicities but was less than the sum of toxicity due to individual Cd and Pb. The toxicological impacts on the biomass of *C. saivum* under the influence of mixture of Cd/Zn was less than the toxicity due to Zn and more than the Cd toxicity. The combine toxicity of Zn and Pb was less than their individual toxicity. The dry weights of both shoot and root were more affected than their fresh weights of *C. saivum*. The root fresh and dry weights were affected more than the shoot fresh and dry weights.

Table 8.1 Reduction (%) in growth parameters of *C. saivum* plant due to different HMs from control

Treatments	Shoot fresh weigh	Root fresh weight	Shoot dry weight	Root dry weight	Shoot length	Root length
Cd 1	7.4	4.8	7.4	32.4	17.2	27.3
Cd2	16.0	11.0	14.7	41.6	35.9	33.3
Cd3	27.2	21.3	29.3	46.4	45.7	48.5
Pb 1	3.7	2.7	6.6	7.5	12.5	24.2
Pb 2	13.6	8.9	13.9	13.3	29.7	30.3
Pb 3	21.0	19.3	4.7	30.6	37.8	45.5
Zn 1	1.2	1.8	6.0	1.9	5.6	17.3
Zn 2	3.2	3.0	13.1	7.6	14.4	22.4
Zn 3	9.9	15.1	13.9	29.3	17.5	37.6
Cd1/Pb 1	12.3	6.8	11.5	56.6	28.1	30.3
Cd2/Pb 2	18.5	13.0	20.4	67.5	45.3	36.4
Cd3/Pb 3	29.6	23.4	35.8	75.1	49.4	54.5
Cd1/Zn 1	4.9	3.4	7.8	31.6	21.9	28.5
Cd2/Zn 2	8.6	4.7	13.9	49.1	27.5	47.6
Cd3/Zn 3	23.5	20.0	30.1	57.8	34.1	50.6
Zn1/Pb 1	3.1	5.5	7.4	14.0	15.0	25.5
Zn2/Pb 2	12.3	9.6	18.0	19.7	22.2	37.6
Zn3/Pb 3	19.8	19.0	20.4	26.9	30.9	44.2

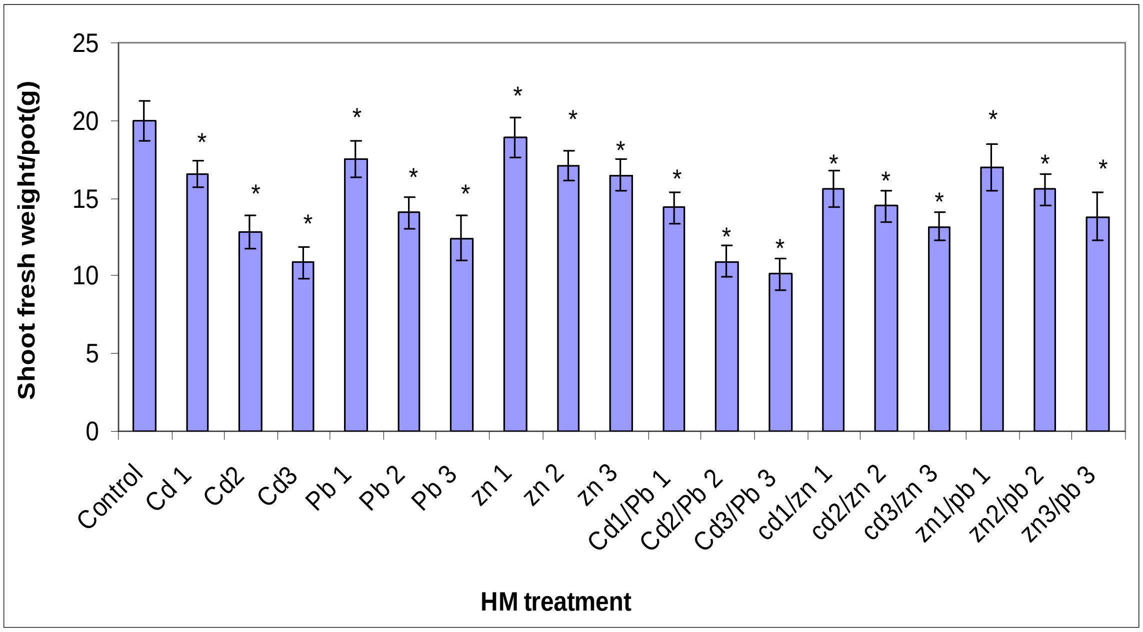

Fig 8.1 Effects of different concentrations of HMs on shoot fresh weight. The error bars indicate the standard deviation. The error bars indicate the standard deviation while asterisks (*) indicate significant change from control

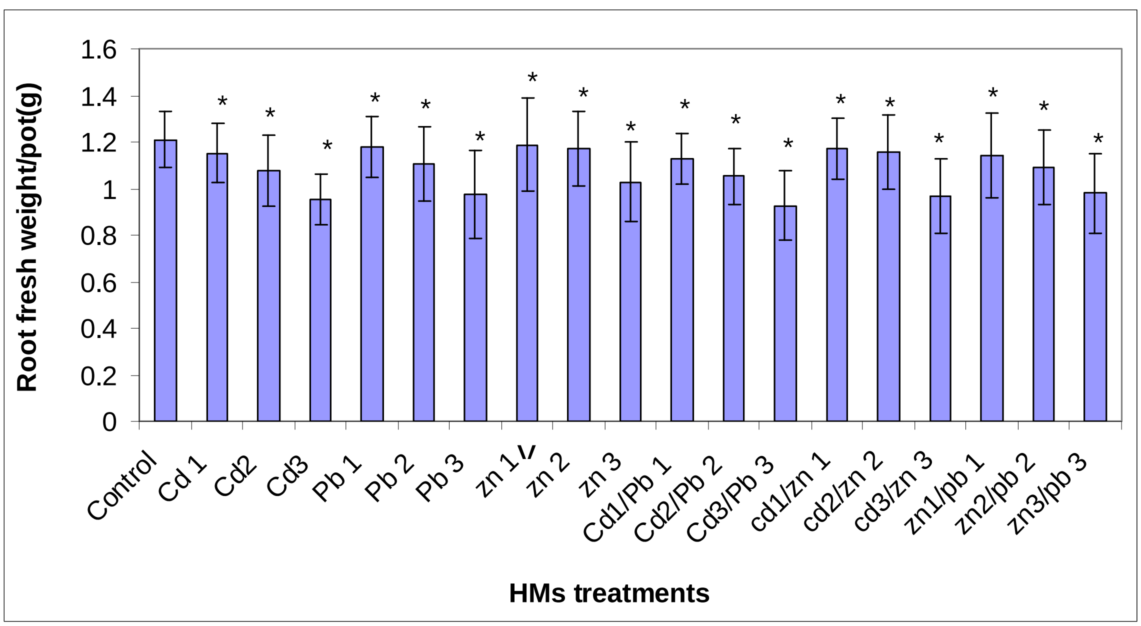

Fig 8.2 Effects of different concentrations of HMs on root fresh weight. The error bars indicate the standard deviation. The error bars indicate the standard deviation while asterisks (*) indicate significant change from control

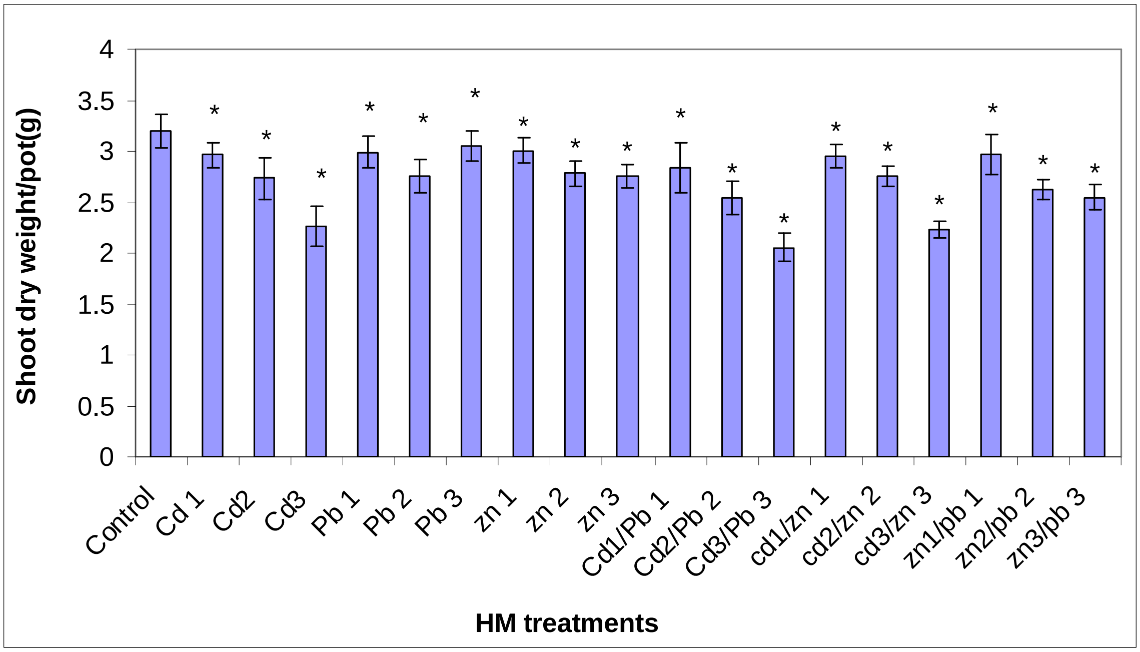

Fig 8.3 Effects of different concentrations of HMs on shoot dry weight. The error bars indicate the standard deviation. The error bars indicate the standard deviation while asterisks (*) indicate significant change from control

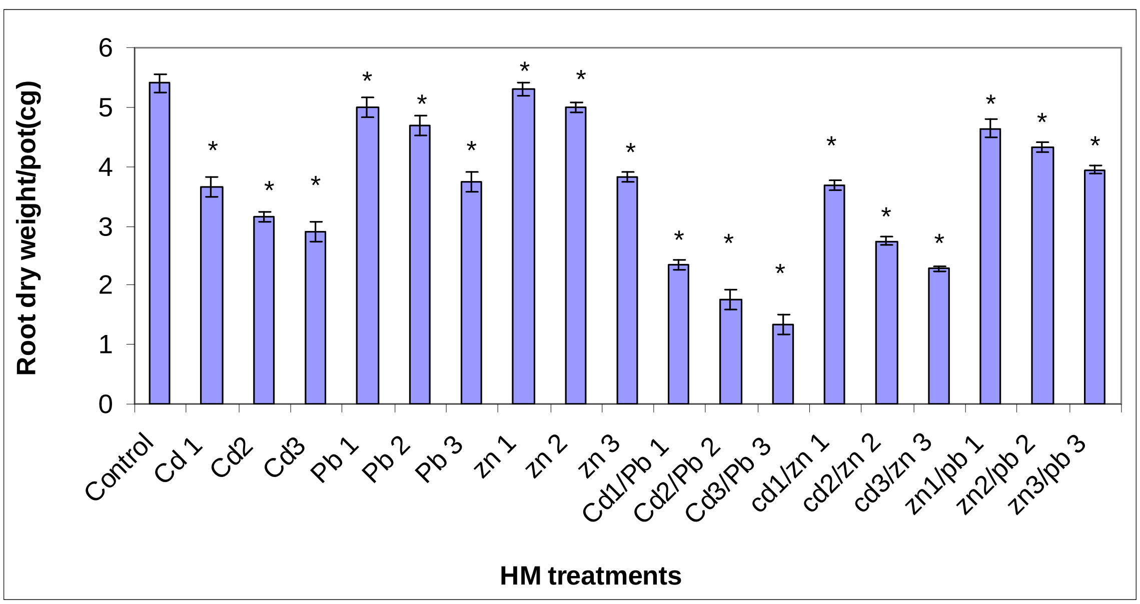

Fig 8.4 Effects of different concentrations of HMs on root dry weight. The error bars indicate the standard deviation. The error bars indicate the standard deviation while asterisks (*) indicate significant change from control

3.2. HMs effects on plant length

Different concentrations of Cd, Pb, Zn, Cd/Pb, Cd/Zn and Pb/Zn had a significant ($p<0.05$) toxicological impacts on shoot and root lengths of *C. saivum*. The increasing concentrations of HMs in the soil resulted a decrease in the shoot and root lengths of *C. saivum* (Fig. 8.5, 8.6). At the highest dose of Cd the shoot and root lengths were decreased by 45.7% and 48.5%, respectively (Table 8.1) as compared to control. Similarly, at the highest dose of Pb the shoot and root lengths were decreased by 37.8% and 45.5%, respectively (Table 8.1) as compared to control. At the highest dose of Zn the shoot and root lengths were decreased by 17.5% and 37.6%, respectively (Table 8.1) as compared to control. The plants grown in soil spiked with mixture of Cd and Pb have shorter root and shoot as compare to the plants with contaminated with individual Cd and Pb. The reduction in the shoot and root lengths were 49.4% and 54.5%, respectively as compare to control, under the influence of highest dose of Cd/Pb.

The combine toxicities of Cd/Zn in the shoot and root lengths were 34.1% and 50.6%, respectively as compare to control. The reduction in shoot and root lengths were 30.9% and 44.2%, respectively under the influence of combine effects of Pb and Zn. In general the root lengths were more affected than shoot lengths just like *S. oleracea* plant.

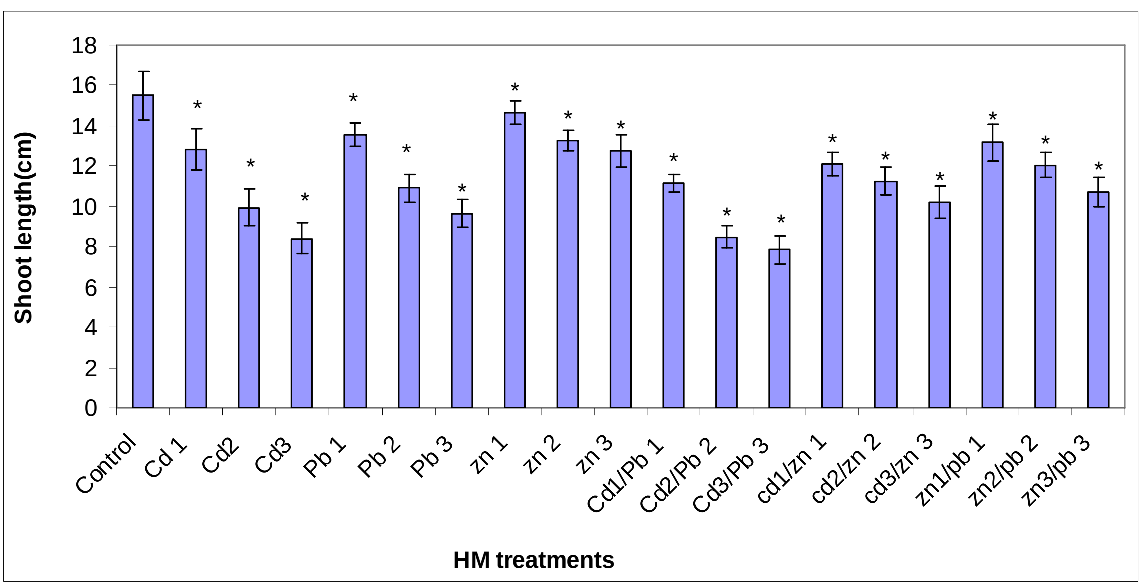

Fig 8.5 Effects of different concentrations of HMs on shoot length. The error bars indicate the standard deviation. The error bars indicate the standard deviation while asterisks (*) indicate significant change from control

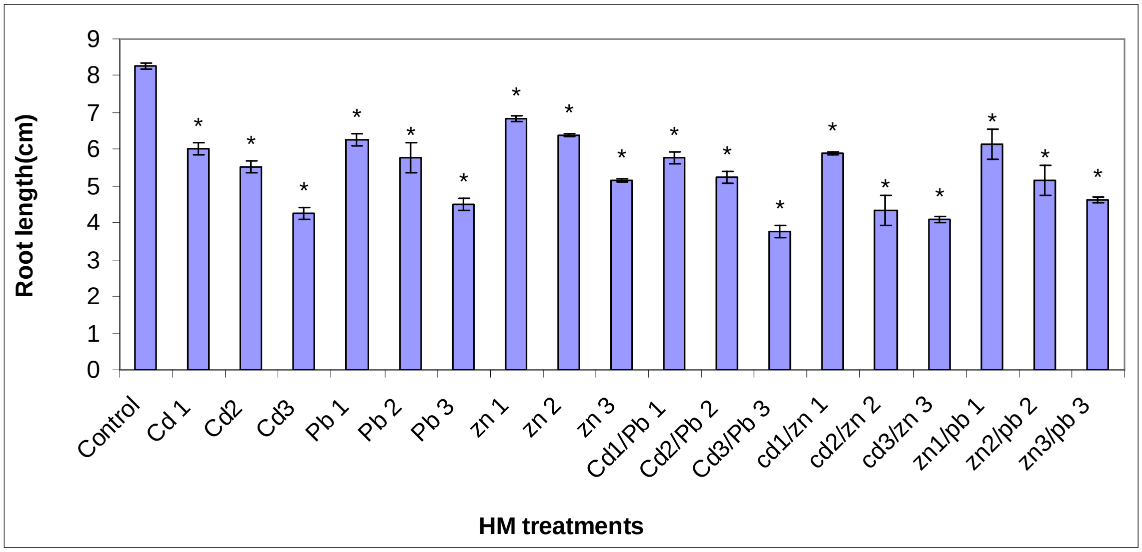

Fig 8.6 Effects of different concentrations of HMs on root length. The error bars indicate the standard deviation. The error bars indicate the standard deviation while asterisks (*) indicate significant change from control

3.3 HMs effects total protein, fiber and moisture contents

The toxicological effects of Cd, Pb, Zn, Cd/Pb, Cd/Zn and Pb/Zn on total protein , fiber and moisture contents of *C. saivum* are given in figures 8.7, 8.8 and 8.9. HMs concentration had a significant ($p<0.05$) adverse impacts on protein, fiber and moisture. At the highest dose of Cd total protein, fiber and moisture contents of *C. saivum* decreased by 16%, 31.7% and 21.7%, respectively (Table 8.2) as compared to control. Similarly, the highest dose of Pb total protein, fiber and moisture contents decreased by 11%, 28.3% and 21.3%, respectively (Table 8.2) compared to control. The addition of highest dose of Zn decreased total protein, fiber and moisture contents by 9.7%, 15.7% and 20.4%, respectively (Table 8.2) compared to control.

The reductions in total protein, fiber and moisture contents were 10.7%, 36.7% and 29.3%, respectively as compare to control, under the influence highest dose of Cd/Pb. Similarly, at the highest dose of Cd/Zn total protein, fiber and moisture contents decreased by 8.7%, 25.7% and 25.7%, respectively (Table 8.2) as compare to control.

The addition of highest dose of Pb/Zn decreased total protein, fiber and moisture contents by 7 %, 24.7% and 22.3%, responsibility (Table 8.2) as compare to control.

The toxicity in the biomass of seedlings under the influence of mixture of Cd/Zn was more than the toxicity due to Zn and less than the Cd toxicity. The toxicity due to mixture of Zn and Pb was more than due to individual Zn and Pb.

Table 8.2 Reduction (%) in nutrients of *C. saivum* r with different concentrations of HMs as compare to control.

Treatments	Total protein	Fiber	Moisture	Na	K	Ca	Fe	Mg	Mn	Cu
Cd 1	5.30	17.7	12.3	17.1	18.7	13.7	17.1	15.3	18.3	21.2
Cd2	8.00	20.7	15.3	25.5	21.0	17.7	26.8	19.0	24.3	30.3
Cd3	16.0	31.7	21.7	29.3	23.5	23.7	29.3	22.3	37.3	43.3
Pb 1	4.30	13.3	11.3	15.3	13.4	12.7	14.8	13.0	16.1	18.3
Pb 2	6.00	15.3	14.7	21.5	18.7	15.3	16.7	16.7	21.3	25.4
Pb 3	11.0	28.3	21.3	23.0	20.3	20.3	21.1	20.0	35.7	32.3
Zn 1	2.70	7.70	10.7	12.4	11.3	9.50	13.9	11.3	14.3	15.7
Zn 2	4.00	11.7	18.7	18.5	15.0	12.3	15.3	13.7	17.2	18.0
Zn 3	9.70	15.7	20.4	20.4	19.8	19.3	18.3	19.7	22.3	32.7
Cd1/Pb1	6.70	24.7	17.0	20.0	25.7	18.3	23.3	22.8	33.2	37.3
Cd2/Pb2	7.30	31.0	21.3	25.1	29.3	29.3	36.7	30.3	39.3	40.5
Cd3/ Pb3	10.7	36.7	29.3	30.5	31.4	34.3	40.3	35.7	44.3	46.6
Cd1/Zn1	6.00	15.3	15.4	18.3	18.9	12.3	20.1	18.7	27.0	33.7
Cd2/Zn2	6.40	23.7	20.0	21.7	26.3	19.3	29.3	26.7	37.3	37.1
Cd3/ Zn3	8.70	25.7	25.7	25.4	28.8	27.3	35.3	28.7	40.3	43.3
Pb1/Zn1	5.50	13.3	12.7	16.0	16.5	10.3	18.3	16.0	22.1	29.3
Pb2/Zn2	6.00	22.3	18.7	19.0	23.4	16.3	24.3	23.7	35.7	35.1
Pb3/Zn3	7.00	24.7	22.3	23.7	27.4	22.3	33.1	26.7	37.3	40.3

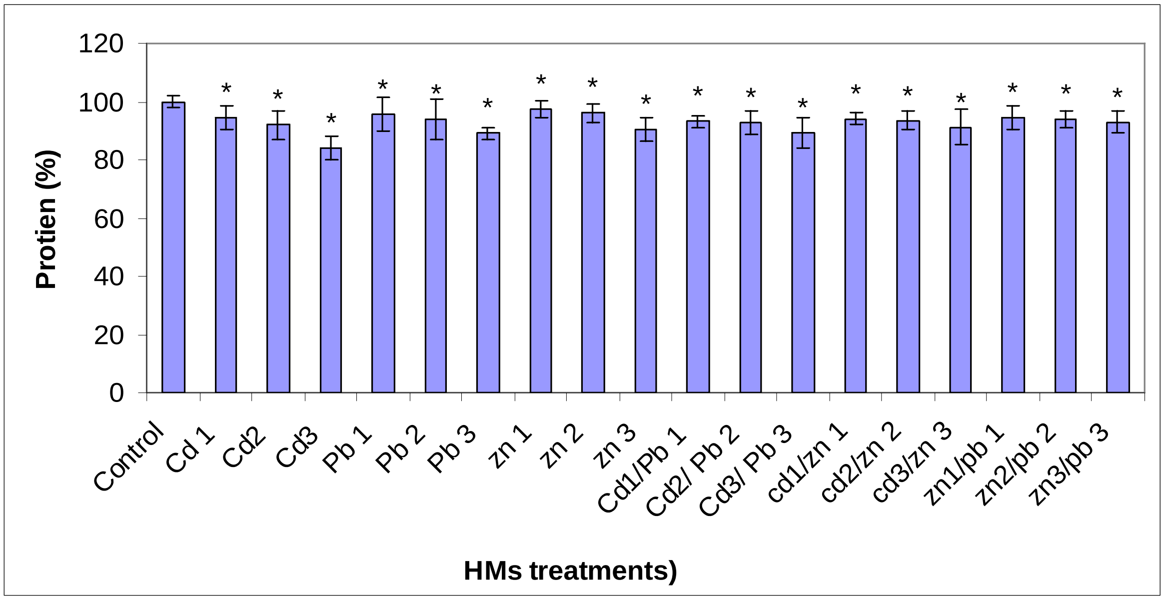

Fig 8.7 Comparison of total protein content with control under the influence of different concentrations of HMs. The error bars indicate the standard deviation. The error bars indicate the standard deviation while asterisks (*) indicate significant change from control

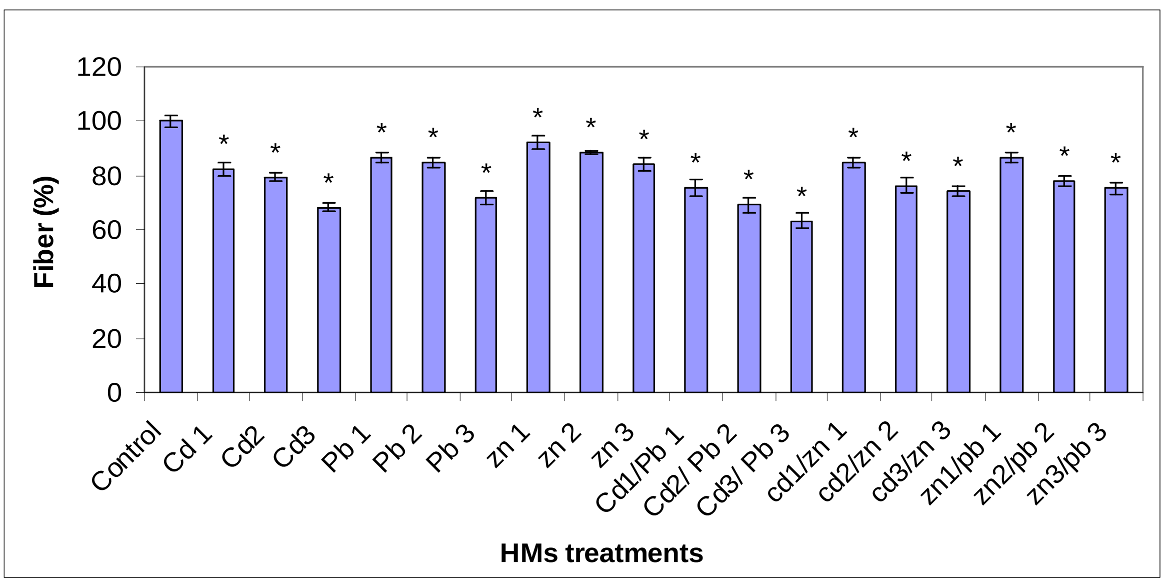

Fig 8.8 Comparison of fiber content with control under the influence of different concentrations of HMs. The error bars indicate the standard deviation. The error bars indicate the standard deviation while asterisks (*) indicate significant change from control

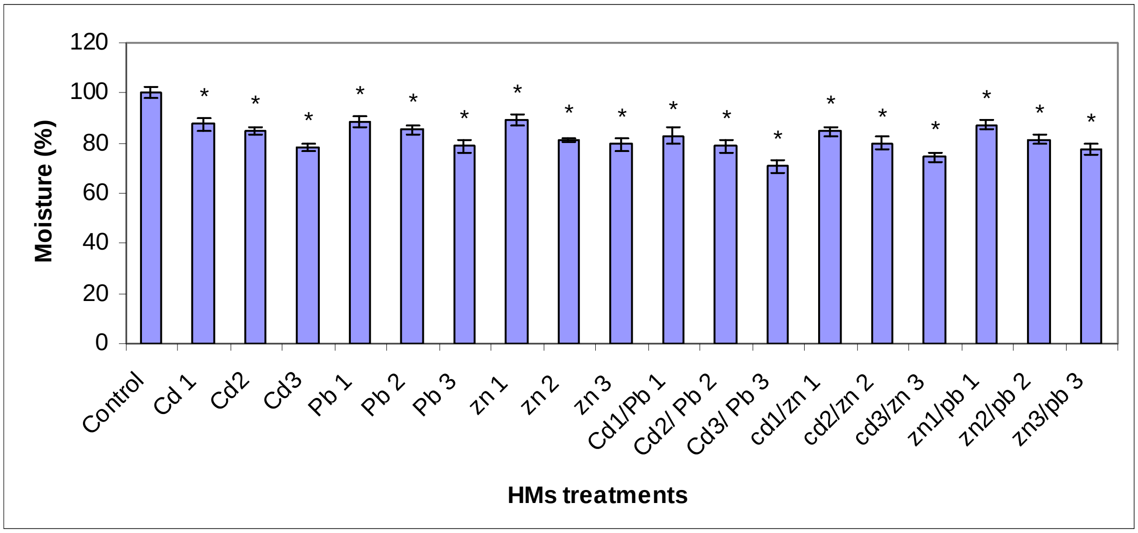

Fig 8.9 Comparison of moisture content with control under the influence of different concentrations of HMs. The error bars indicate the standard deviation. The error bars indicate the standard deviation while asterisks (*) indicate significant change from control

3.3 HMs effects on metals up take

The increasing concentrations of HMs resulted decrease in Na, K, Ca, Fe, Mg, Mn and Cu in *C. saivum* (Figures 8.10, 8.11, 8.12, 8.13, 8.14, 8.15, 8.16). The reduction in Na, K, Ca, Fe, Mg, Mn and Cu of *C. saivum* is significant ($p<0.05$). At the highest dose of Cd, Na, K, Ca, Fe, Mg, Mn and Cu reduced 29.3%, 23.5%, 23.7%, 29.3%, 22.3%, 37.3% and 43.3% (Table 8.2) as compared to control respectively. Similarly At the highest dose of Pb the reduction in concentrations of Na, K, Ca, Fe, Mg, Mn and Cu were 23%, 20.3%, 20.3%, 21.1%, 20%, 35.7 and 32.3 (Table 8.2) as compared to control respectively. At the highest dose of Zn, the uptake of Na, K, Ca, Fe, Mg, Mn and Cu showed a reduction of 20.4%, 19.8%, 19.3%, 18.3%, 19.7%, 22.3% and 32.7% (Table 8.2) as compared to control respectively. The reduction in Na, K, Ca, Fe, Mg, Mn and Cu were 30.5%, 31.4%, 34.3%, 40.3%, 35.7%, 44.3% and 46.6 respectively in comparison with the control under the influence highest dose of Cd/Pb. Na, K, Ca, Fe, Mg, Mn and Cu were 25.4%, 28.8%, 27.3%, 35.3%, 28.7%, 40.3% and 43.3% respectively in comparison with the control under the influence highest dose of Cd/Zn. Similarly, at highest dose of Pb/Zn, Na, K, Ca, Fe, Mg, Mn and Cu were 23.7%, 27.4%, 22.3%, 33.1%, 26.7%, 37.3% and 40.3%, respectively.

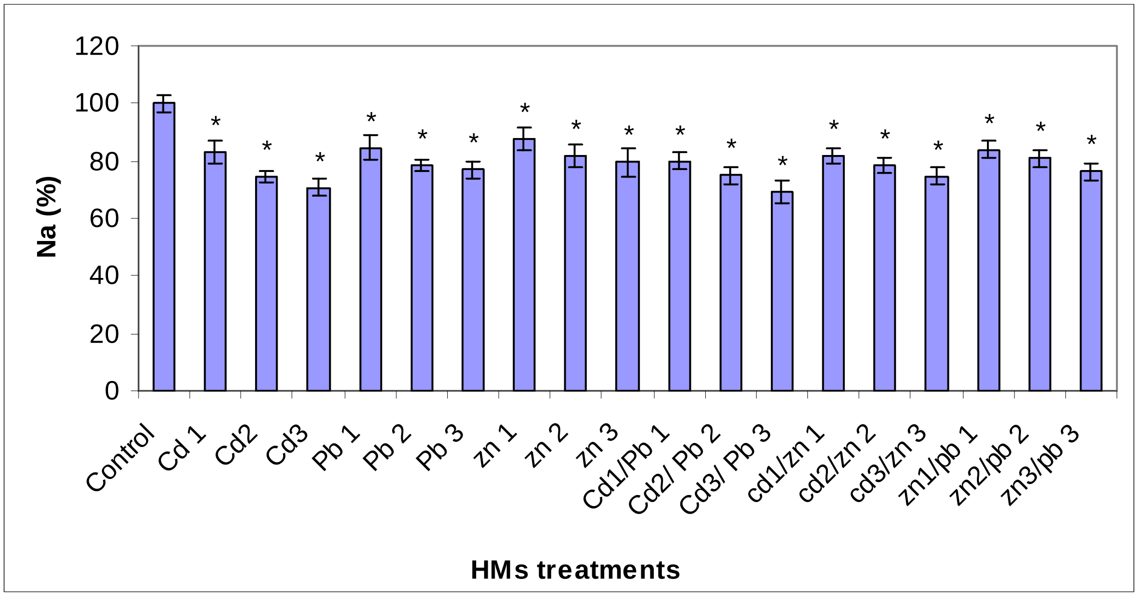

Fig 8.10 Comparison of Sodium (Na) with control under the influence of different concentrations of HMs. The error bars indicate the standard deviation. The error bars indicate the standard deviation while asterisks (*) indicate significant change from control

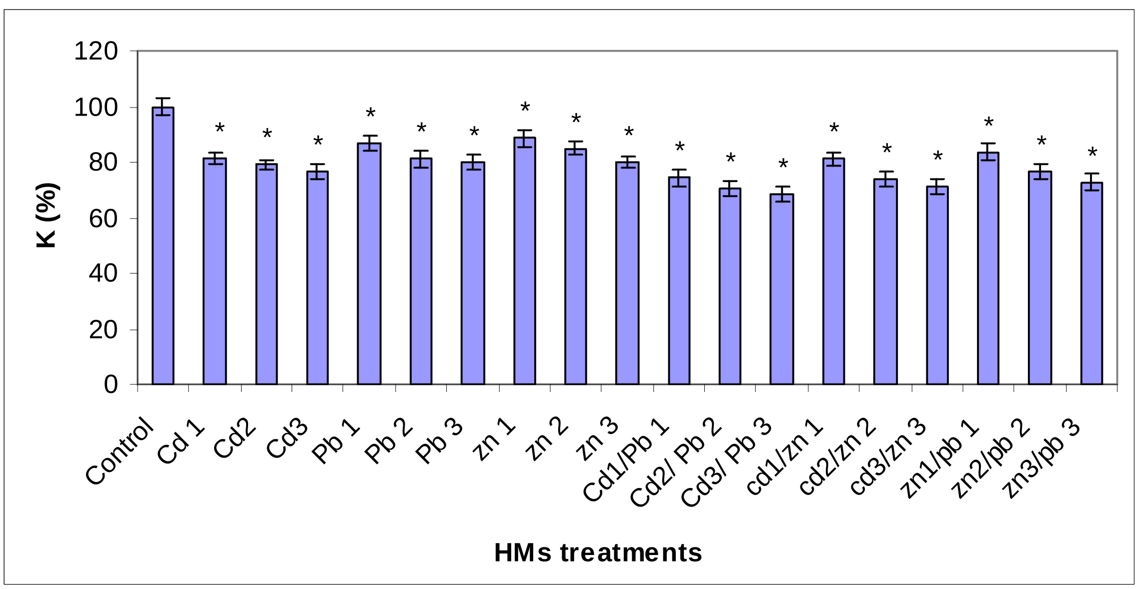

Fig 8.11 Comparison of Potassium (K) with control under the influence of different concentrations of HMs. The error bars indicate the standard deviation. The error bars indicate the standard deviation while asterisks (*) indicate significant change from control

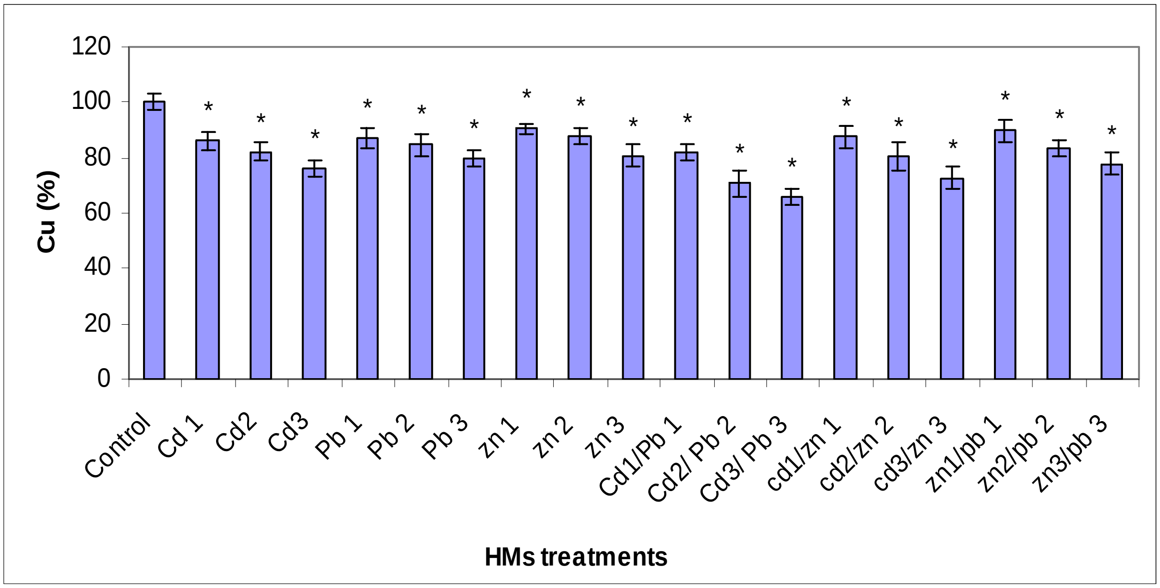

Fig 8.12 Comparison of Calcium (Ca) with control under the influence of different concentrations of HMs. The error bars indicate the standard deviation. The error bars indicate the standard deviation while asterisks (*) indicate significant change from control

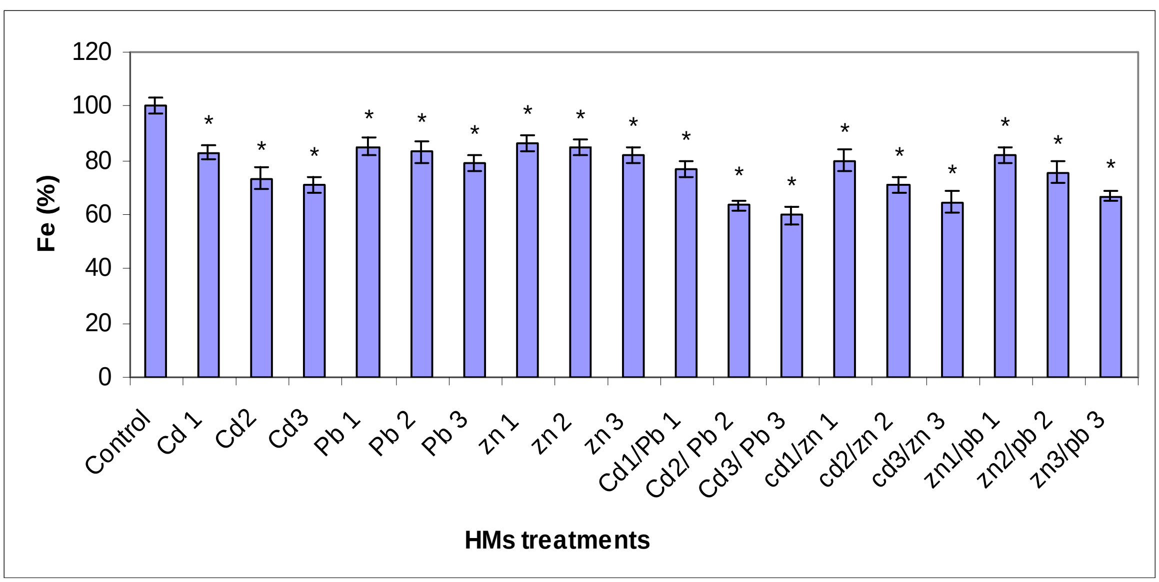

Fig 8.13 Comparison of Iron (Fe) with control under the influence of different concentrations of HMs. The error bars indicate the standard deviation. The error bars indicate the standard deviation while asterisks (*) indicate significant change from control

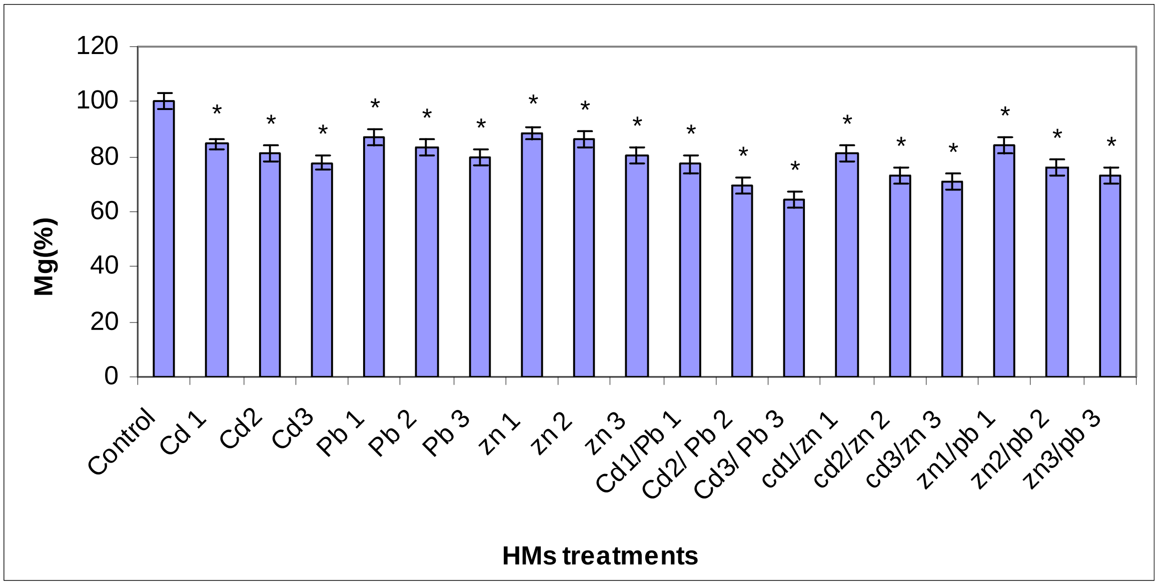

Fig 8.14 Comparison of magnesium (Mg) with control under the influence of different concentrations of HMs. The error bars indicate the standard deviation. The error bars indicate the standard deviation while asterisks (*) indicate significant change from control

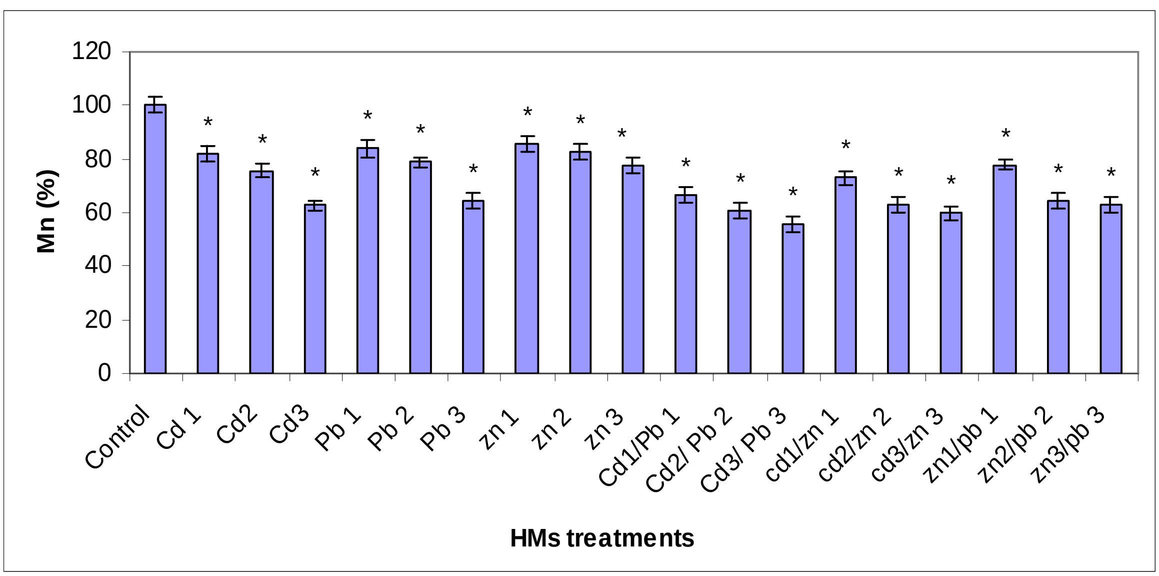

Fig 8.15 Comparison of manganese (Mn) with control under the influence of different concentrations of HMs. The error bars indicate the standard deviation. The error bars indicate the standard deviation while asterisks (*) indicate significant change from control

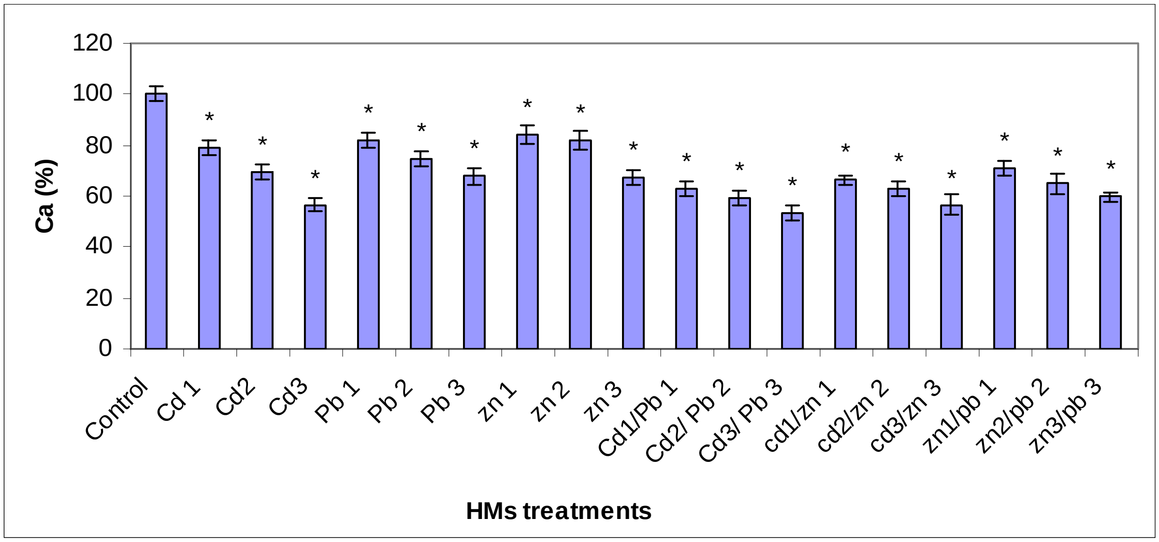

Fig 8.16 Comparison of Copper (Cu) with control under the influence of different concentrations of HMs. The error bars indicate the standard deviation. The error bars indicate the standard deviation while asterisks (*) indicate significant change from control

3.5. Up take of HM

The relationships between the Cd concentrations in soil and in root tissues are given in given in fig 8.17. All three relationships ie between the single Cd concentration in soil and seedlings (Fig 8.17-a), between Cd/Pb in soil and in root (Fig 8.17-b) and between Cd/Zn in soil and in root (Fig 8.17-c) show that with increasing concentrations of Cd in soil whether in single form or in mixture its concentrations in the root increasing. Cd accumulated in the root is in order of single Cd > Cd/Pb > Cd/Zn (Fig. 8.21). Similarly, figures 8.18, 8.19, 8.20 present the relationship between the Cd concentrations in the root of *C. saivum* and shoot in Cd, Cd/Pb and Cd/Zn treatments. All the relationships are positive. The increasing concentrations of Cd in root, increase concentration in shoot as well.

Similarly, the concentrations of Pb in root showed increase with increasing concentration in the soil. The regression analysis showed positive relationships between the concentrations of Pb in soil and in root in all three cases ie single Pb, Pb/Cd and Pb/Zn treatments (Fig 8.22-a, 8.22-b, 8.22-c). Figure 8.26 presents that Pb accumulated in root is in order of Single Pb > Pb/Cd > Pb/Zn. Similarly, Figures 7.23, 7.24, 7.25

present the relationship between the Pb concentrations in the root of spinach and shoot in single Pb, Cd/Pb and Pb/Zn treatments. All the relationships are positive. The increasing concentrations of Cd in root, increase concentration in shoot as well

The regression analysis showed strong positive relationship between the Zn concentrations in soil and root in single Zn, Zn/Cd and Zn/Pb treatments (Fig 8.27-a, Fig 8.27-b, Fig 8.27-c). Zn accumulated in root is in order of single Zn > Zn/Cd > Zn/Pb (Fig. 8. 31). Figures 8.28, 8.29 and 8.30 present the relationship between the Zn concentrations in the root of *C. saivum* and shoot in single Zn, Cd/Zn and Pb/Zn treatments. All the relationships are positive. The increasing concentrations of Cd in root, increase concentration in shoot as well.

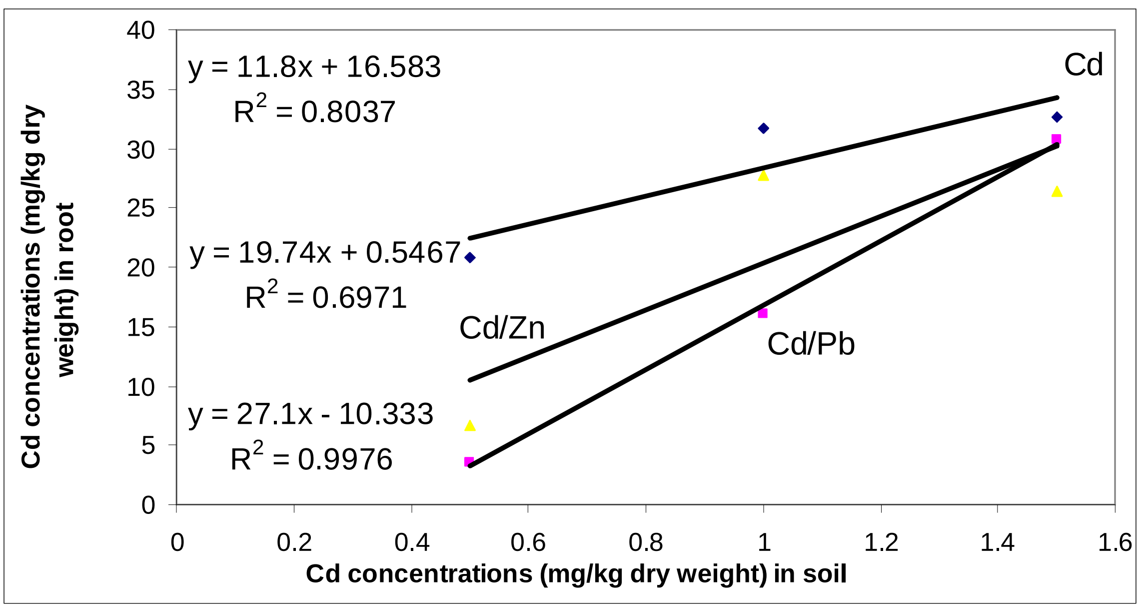

Fig. 8.17 Linear regressions model for Cd concentrations in soil and root tissues on dry weight basis. (a) Relationship of single Cd content in soil and root. (b) Relationship between soil and root Cd content in Cd/Pb treatments. (c) Relationship between soil and root Cd content in Cd/Zn treatments

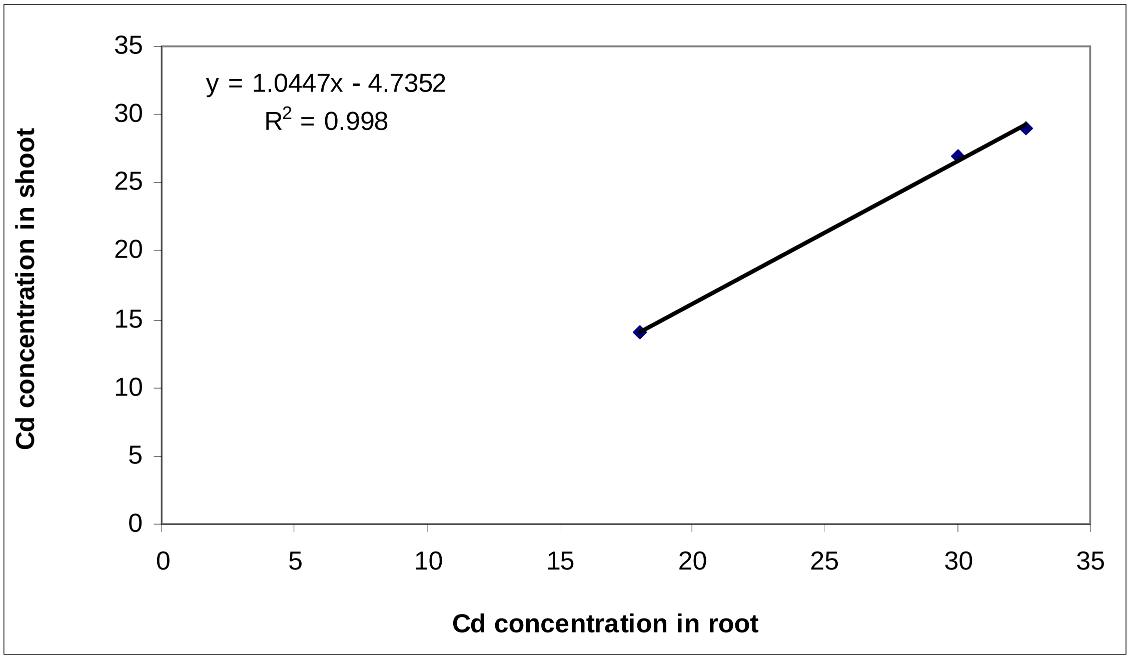

Fig. 8.18 Linear regressions model for Cd concentrations in root and shoot on dry weight basis in single Cd treatment.

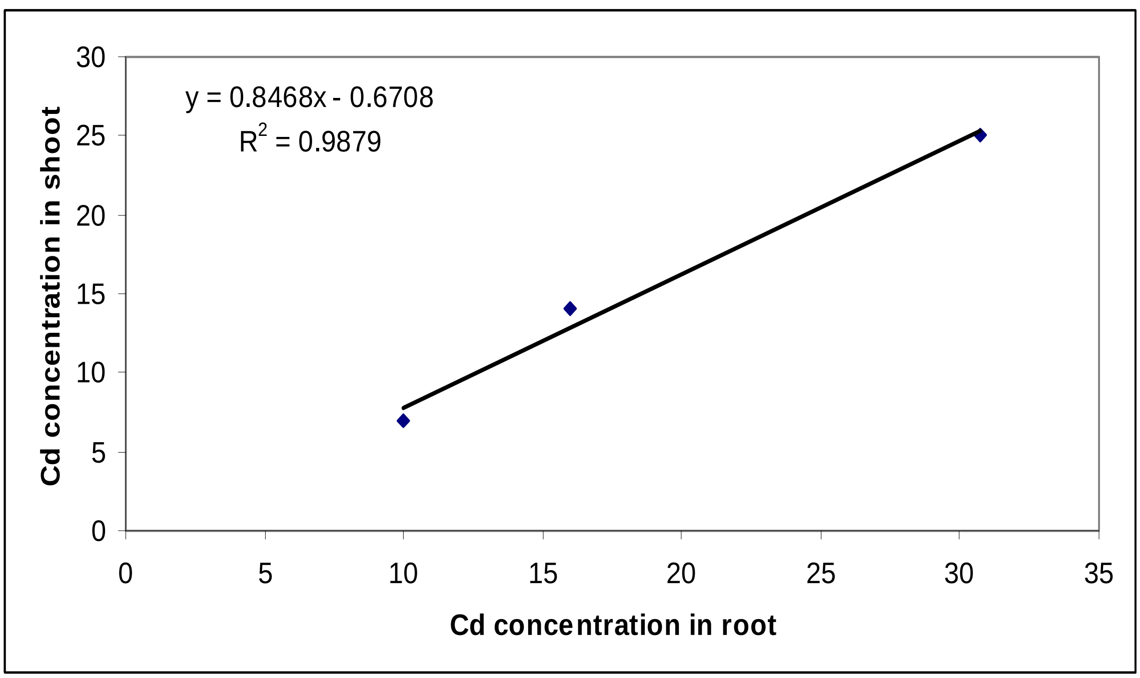

Fig. 8.19 Linear regressions model for Cd concentrations in root and shoot on dry weight basis in Cd/Pb treatment.

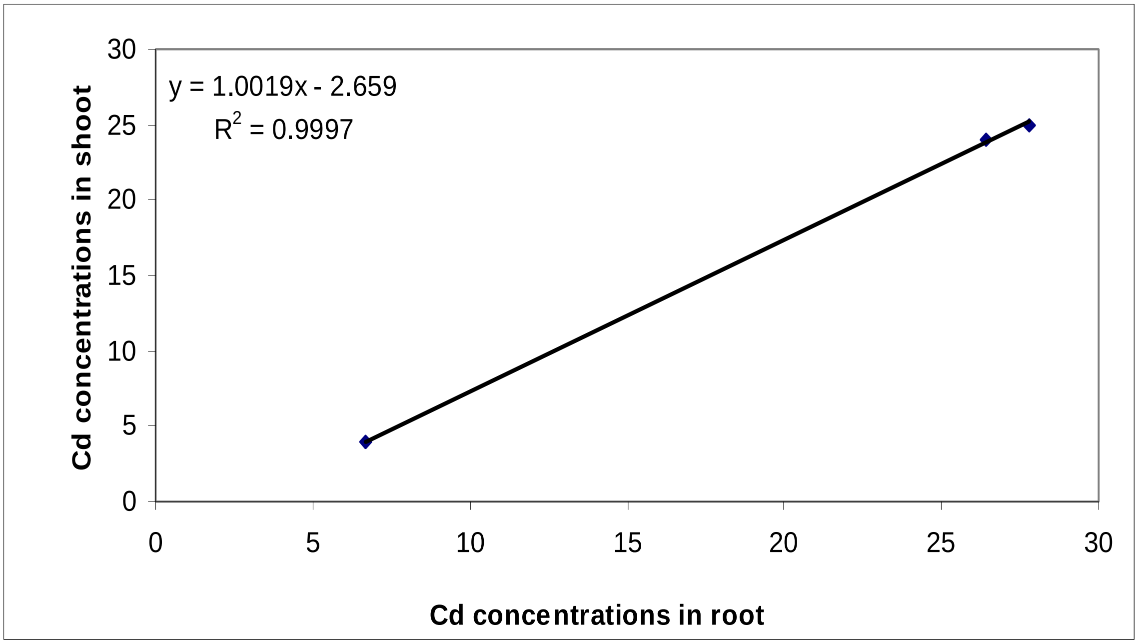

Fig. 8.20 Linear regressions model for Cd concentrations in root and shoot on dry weight basis in Cd/Zn treatment.

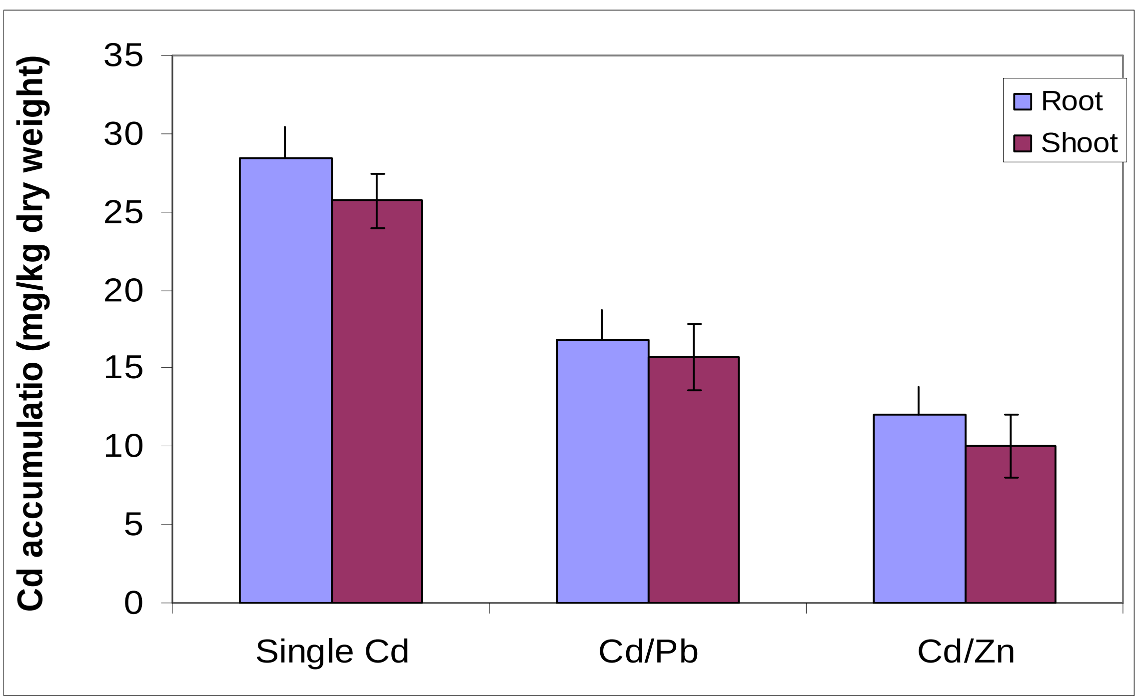

Fig 8.21 Comparison of up take of Cd from different treatments. The error bars indicate the standard deviation

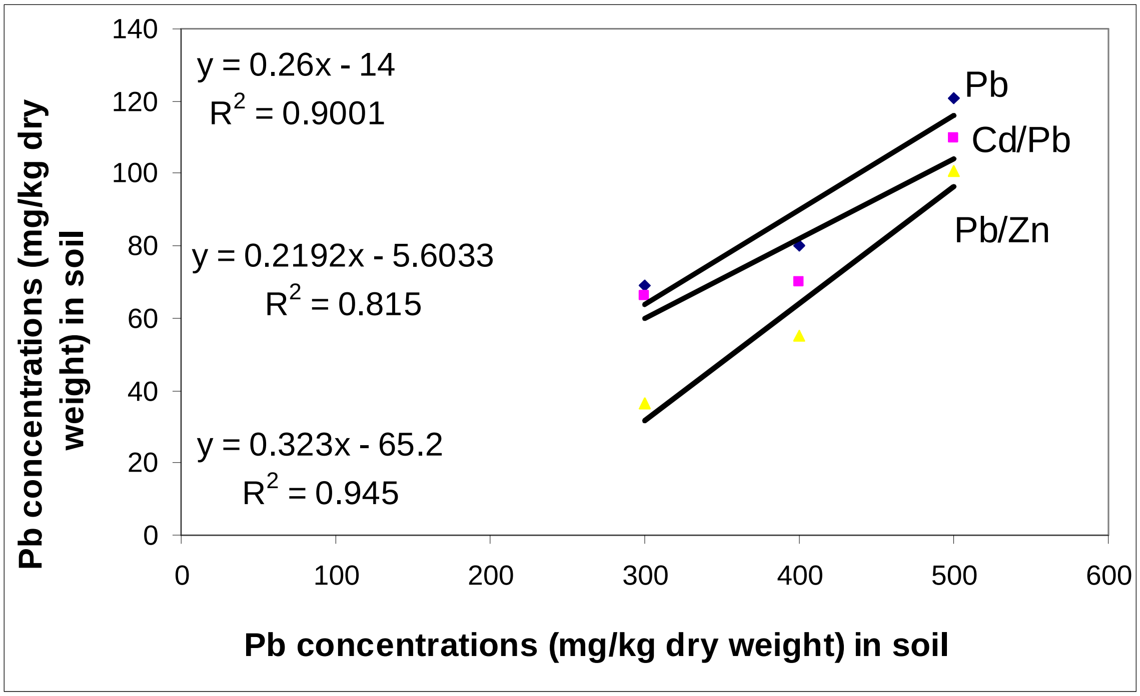

Fig. 8.22 Linear regressions model for Pb concentrations in soil and root tissues on dry weight basis. (a) Relationship between soil Pb content and root in single Pb treatments. (b) Relationship between soil Pb content and root in Cd/Pb treatments. (c) Relationship between soil Pb content and root in Cd/Zn treatments

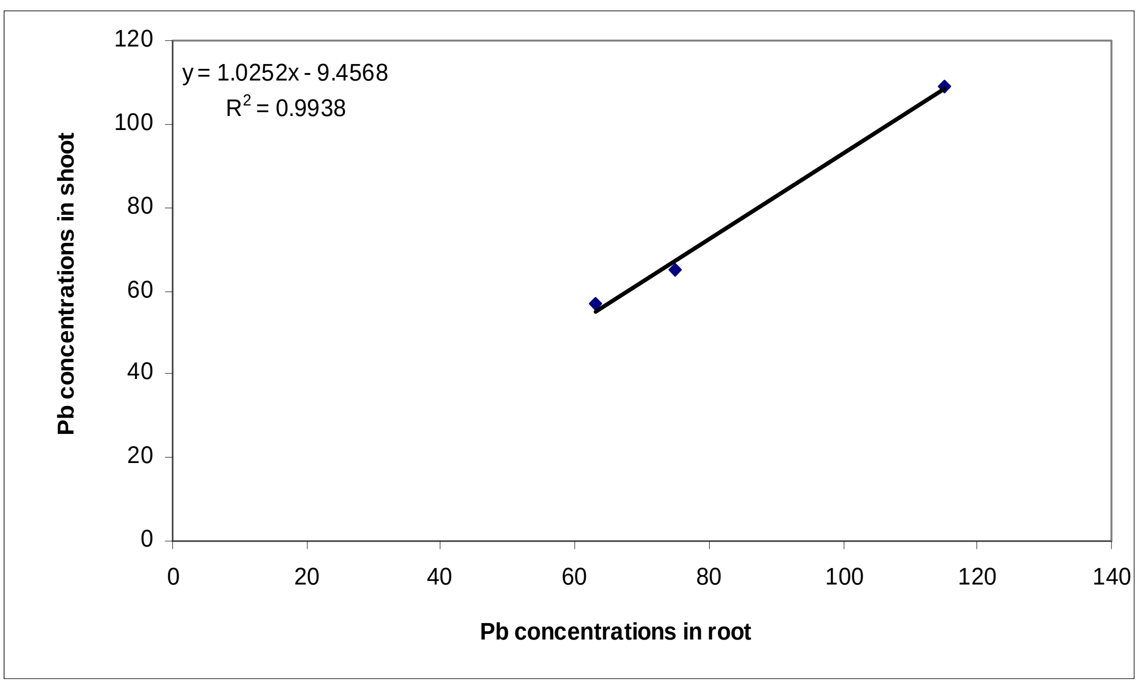

Fig. 8.23 Linear regressions model for Pb concentrations in root and shoot on dry weight basis in single Pb treatments

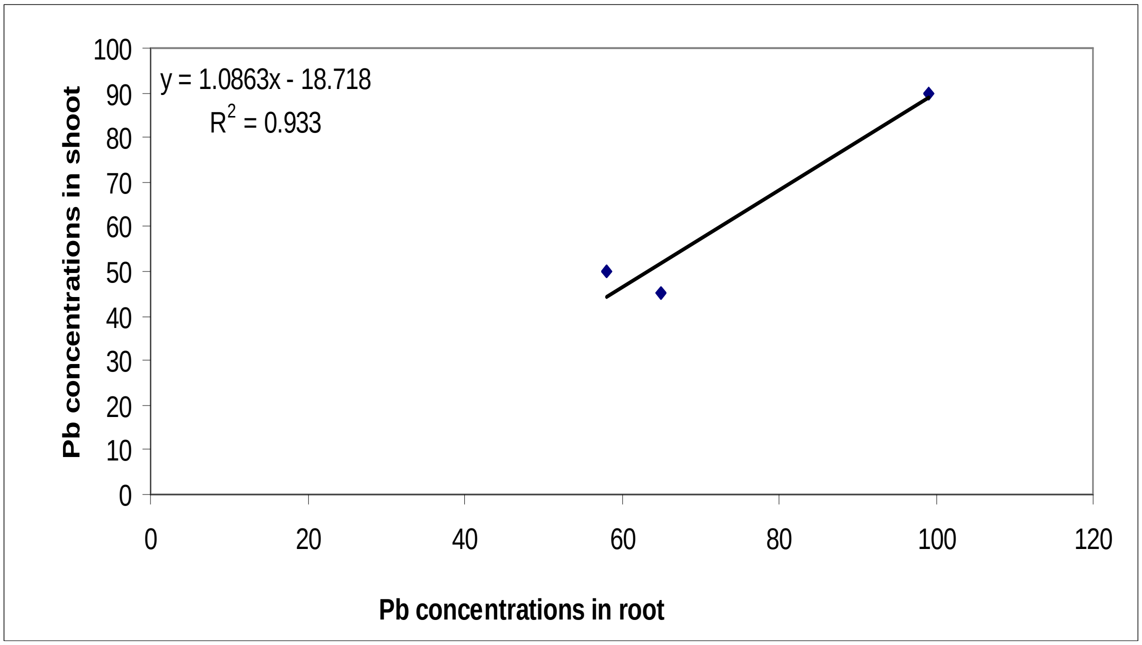

Fig. 8.24 Linear regressions model for Pb concentrations in root and shoot on dry weight basis in Pb/Cd treatment

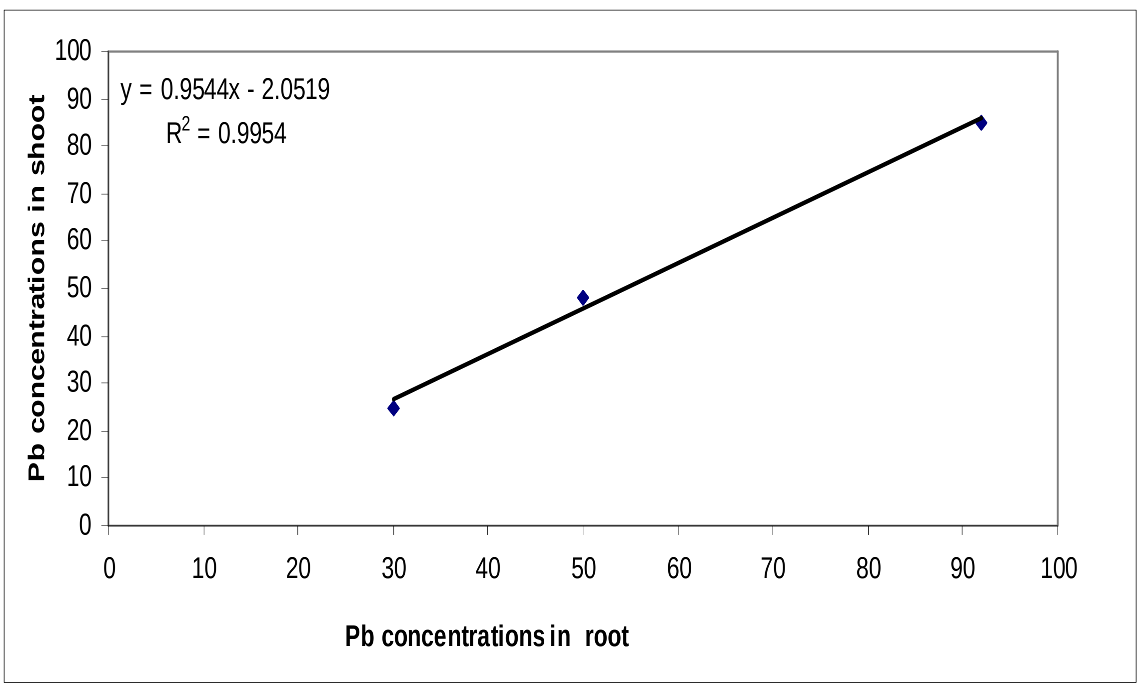

Fig. 8.25 Linear regressions model for Pb concentrations in root and shoot on dry weight basis in Pb/Zn treatment

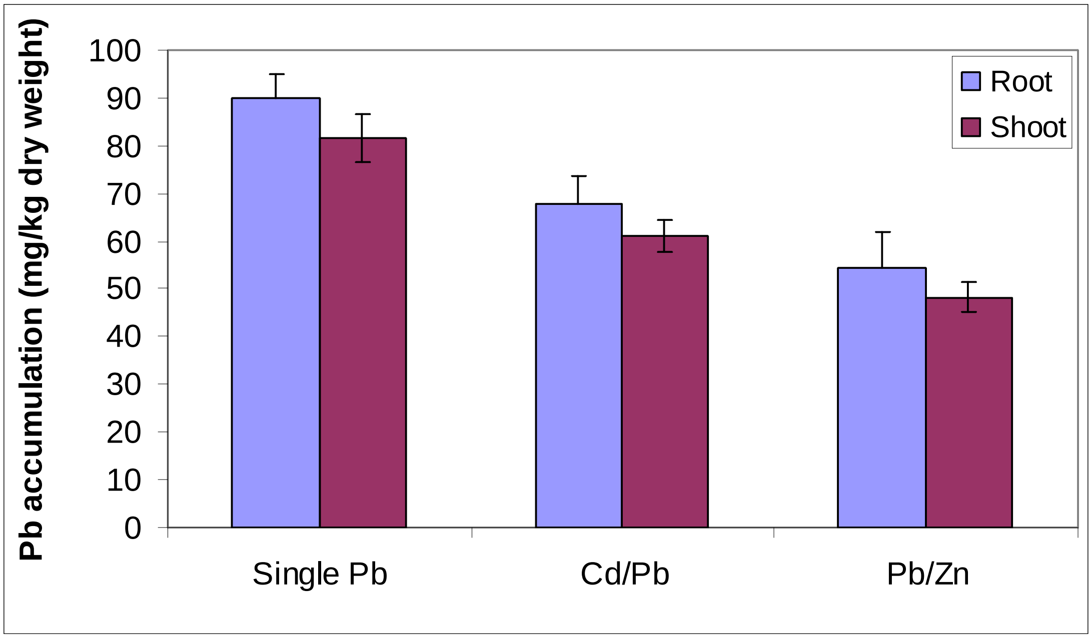

Fig 8.26 Comparison of Pb up take from different treatments. The error bars indicate the standard deviation

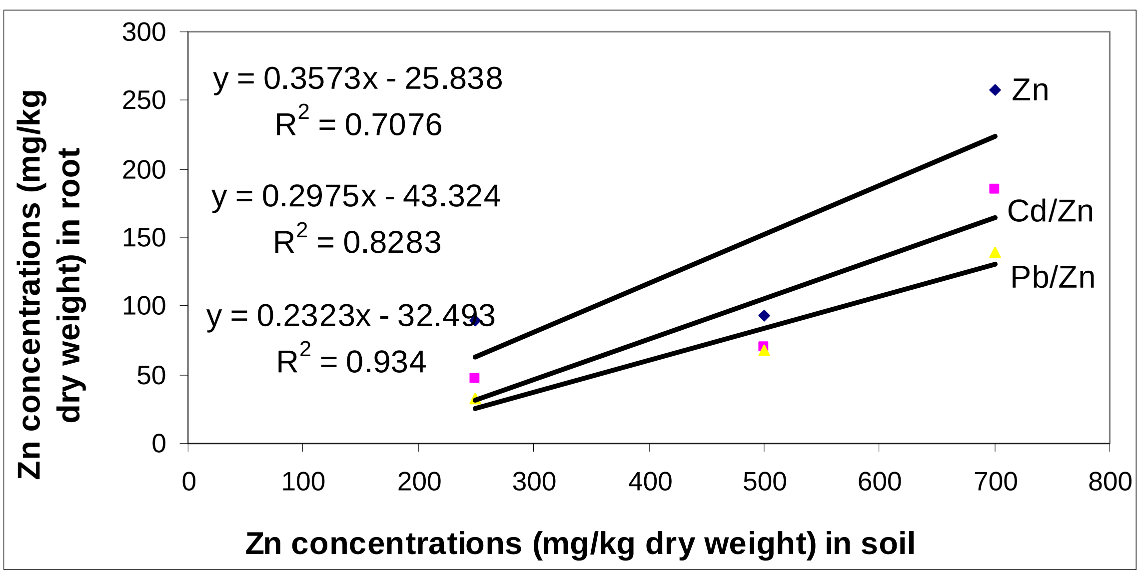

Fig. 8.27 Linear regressions model for Zn concentrations in soil and root tissues on dry weight basis. (a) Relationship between soil and root Zn content in single Zn treatments. (b) Relationship between soil and root Zn content in Cd/Pb treatments. (c) Relationship between soil and root Zn content in Cd/Zn treatments

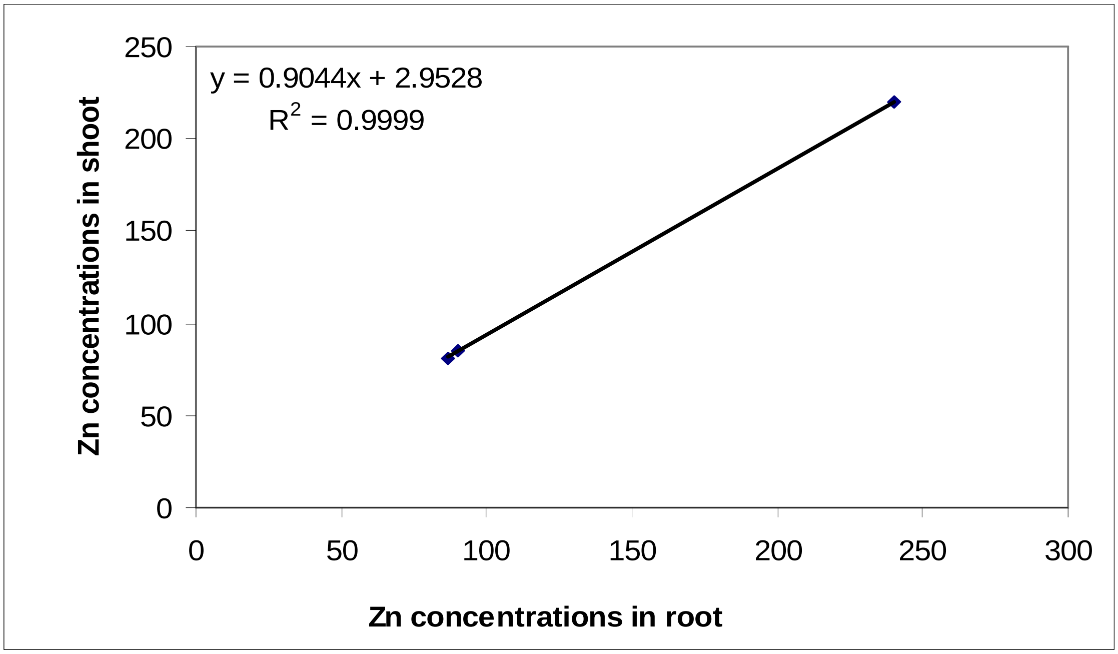

Fig. 8.28 Linear regressions model for Zn concentrations in root and shoot on dry weight basis in single Zn treatment

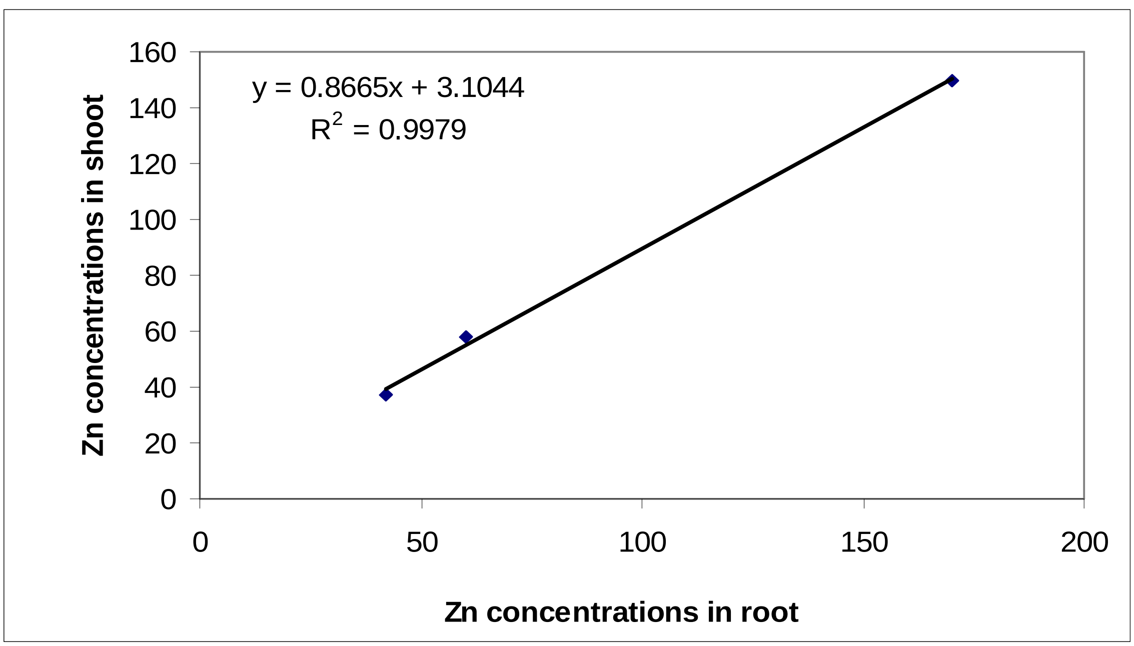

Fig. 8.29 Linear regressions model for Zn concentrations in root and shoot on dry weight basis in Cd/Zn treatment

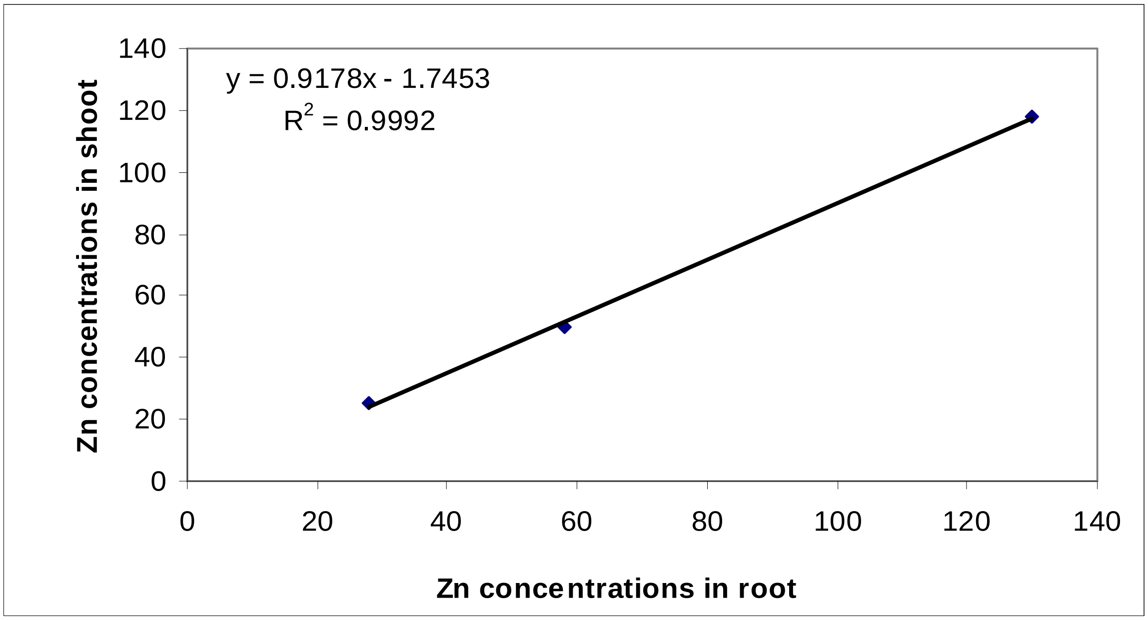

Fig. 8.30 Linear regressions model for Zn concentrations in root and shoot on dry weight basis in Pb/Zn treatment

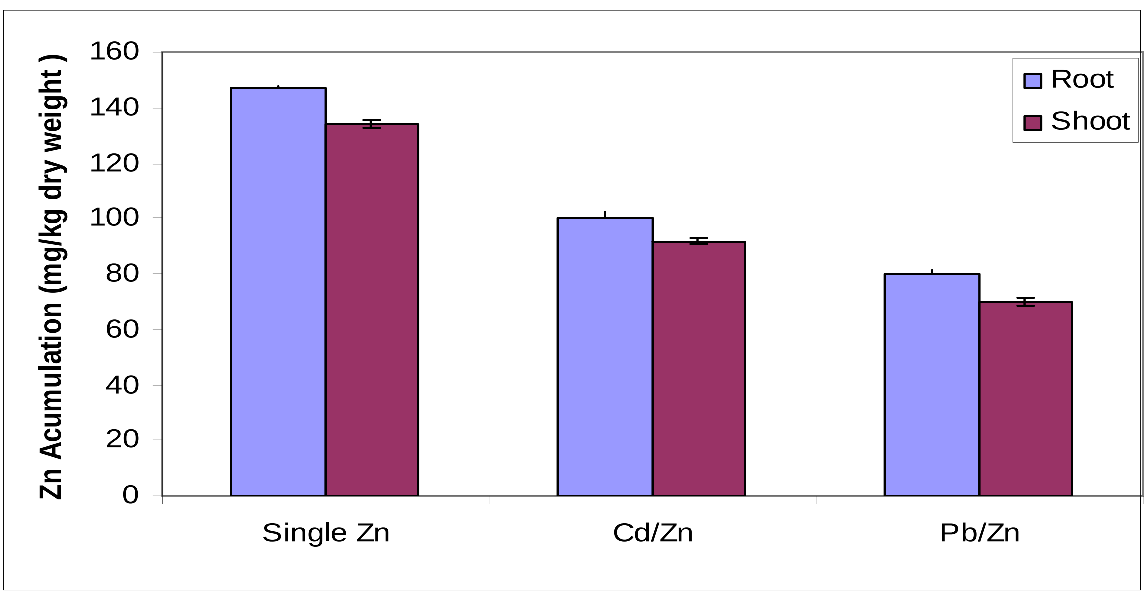

Fig 8.31 Comparison of Zn up take from different treatments. The error bars indicate the standard deviation

Discussion

HMs are toxic for plants they can reduce plant yield, affect leaf and root growth and inhibit enzymatic activities (Zeng et al., 2008). The results of many studies like (Sun et al., 2008; Khan et al., 2008; Lingua et al., 2008) showed that plants grow on contaminated soil accumulate HMs in their body tissues many times more than the acceptable limits.

Cd, which is non essential HM, inhibited plant growth and the toxicity increases with increasing Cd concentration in soil. In the present study, increasing concentrations of Cd significantly ($p<0.05$) reduced shoot and root fresh and dry weights, which showed consistency with the results of our studies on *S. oleracea*, *C. saivum*, *P. oleracea* seedlings and *S. oleracea* mature plant and the results of other studies by (An et al., 2004; Shukla et al., 2003; Devi et al., 2007). Increasing concentrations of Cd in soil produced toxicity in plant biomass and plant lengths of *C. saivum* which is an agreement with the study of Shukla et al, (2003). Just like *S. oleracea*, *C. saivum* plant also produced more toxicity in root, which is discussed in detailed for *S. oleracea* plant in chapter- 7. *S. oleracea* also produced a dose response for Pb in terms of plant biomass and length. Increasing concentrations of Pb significantly ($p<0.05$) reduced plant biomass, and length. An et al, (2004) and Farooqi et al, (2009) observed drop in shoot and root growth of *C. sativus, A. lebbeck* with increasing concentrations of Pb in growing medium. Zn is an essential element for plants, but its excess can significantly damage plants (Wang et al., 2009). Shoot and root fresh and dry weights of *C. saivum* were reduced with increasing Zn concentrations. Zn reduced plant biomass because of creation of deficiency of macronutrients such is P (Marschner, 1995). Mahmood et al, (2005) found a reduction in growth of corn with increasing concentrations of Zn. As discussed in detailed for *S. oleracea*, *C. saivum*, *P. oleracea* seedlings and *S. oleracea* mature plant that HMs in mixture form interact each other. In case of *C. saivum* plants Cd and Pb showed antagonistic effects on the up take of each other in mixture form. *C. saivum* root accumulated Cd from its treatments of our experiment in the order of Cd (2680%) > Cd in Cd/Pb (1890%), similarly accumulated Pb from its treatments of our experiment in the order of Pb (22.5%) > Pb in Cd/Pb (16.7%). Cd in the absence of Pb accumulated 2680%

Cd while in the presence of Pb accumulated 1890%. The 29.5% difference is actually due to the interaction of Pb with the up take of Cd. Similarly Pb accumulated by *C. saivum* root from Cd/Pb 25.8% less than its single form. The mobility of Cd and Pb from root to shoot also effected in mixture form. In case of single Cd, 87% of Cd from root transferred to shoot while in case of Cd/Pb 81% transferred to shoot. Pb in case of its single form 91% transferred to shoot from the root and 90% in case of Cd/Pb. The up take pattern of both Cd and Pb are reflected in the growth parameters of *C. saivum* just like *S. oleracea*. The toxicities of Cd and Pb in form of Cd/Pb in terms of al most all growth parameters are more than the toxicities due to individual Cd and Pb but less than their additive toxicities. In case of *C. saivum* the toxicities due to Cd and Pb in all its three treatments were in order of Cd/Pb (38% reduction from control treatment average of all growth parameters) > Cd (29.6% reduction from control treatment average of all growth parameters) > Pb (23% reduction from control treatment average of all growth parameters). The reduction of growth due to Cd was 29.6% and due to Pb was 23%. The combine toxicity should be 52.6% but the actual toxicity observed was 38%. The difference of expected and actual combine toxicities is due to the antagonistic affects of Cd and Pb on the up take of each other.

Auda et al, (2010) observed that due to similar chemical and physical properties, Zn and Cd compete with each other. In *C. saivum* the accumulation of Cd in root was in order of Cd (2680%) > Cd in Cd/Zn (1080%) and the accumulation of Zn in root was in order of Zn (36.1%) > Zn in Zn /Cd (25.1%). Zn reduced the Cd accumulation by 60% on the other hand Cd reduced the up take of Zn by 30%. Similarly the co-existence of Cd/Zn also changes the transfer of each other from root to shoot. The presence of Pb reduced the accumulation of Cd in shoot by 22% and Cd reduced the accumulation in shoot by 0.6%. The results are consists with results of (Weihong et al., 2009). The up take pattern of Cd and Zn is reflected in the growth parameters of *C. saivum*. The toxicities due to Cd and Zn in all its three treatments were in order of Cd/Zn (37.3% average reduction of all growth parameters from control treatment) > Cd (29.6% average reduction of all growth parameters from control treatment) > Zn (24.7% average reduction of all growth parameters from control treatment). The reduction of growth due to Cd was 29.6% and due to Zn was 24.7%. The combine toxicity should be 54.3% but the actual toxicity observed was 37.3%. The difference of expected and actual combine toxicities is due to the antagonistic affects of Cd and Zn on the up take of each other. The results are agreed with the results of our studies on *S. oleracea*, *C. saivum*, *P. oleracea* seedlings and *S. oleracea* mature plants, Auda et al, (2010) and Sharma and Agrawal, (2006).

The accumulation of Pb in root of *C. saivum* was in order of Pb (90%) > Pb in Pb/Zn (54.3%) and the accumulation of Zn in root was in order of Zn (146.8% of its soil content) > Zn in Zn /Pb (79.8%). Zn reduced the Pb accumulation by 39.7% on the other hand Cd, reduced the up take of Zn by 46.7%. The accumulation of Pb in shoot of *C. saivum* was in order of Pb (81.7%) > Pb in Pb/Zn (48.3%) and the accumulation of Zn in shoot was in order of Zn (134% of) > Zn in Zn /Pb (70%). Zn reduced the Pb accumulation by 40% on the other hand Pb, reduced the up take of Zn by 48%. The results are consists with results of (Weihong et al., 2009). The up take pattern of Pb and Zn is reflected in the growth parameters of *C. saivum*. The toxicities due to Pb and Zn in all its three treatments were in order of Pb/Zn (38.7% average reduction of all growth parameters from control treatment) > Zn (24.7% average reduction of all growth parameters from control treatment) > Pb (23.1% average reduction of all growth parameters from control treatment). The reduction of growth due to Pb was 23.1% and due to Zn was 24.7%. The combine toxicity should be 47.8% but the actual toxicity observed was 38.7%. The difference of expected and actual combine toxicities is due to the antagonistic affects of Pb and Zn on the up take of each other.

Chapter:-9 Conclusion

Long term irrigation of soil with wastewater accumulated elevated level of HMs in the soil. The concentrations of all selected HMs in the soil in summer season were significantly higher than their concentrations in winter season. The vegetables grown with wastewater irrigated soil in both season were contaminated with HMs but their concentrations in summer season were significantly higher than their concentrations in winter seasons. The THQ values for Pb and Cd were more than 1 while for Ni, Zn, Mn, Cr and Cu were less than 1. Furthermore, the TTHQ vales were many times higher than 1 which showed that consumption of vegetable grown in the study areas pose health risks.

The contamination of plants with HMs produces toxicities in them. Increasing concentrations of Cd, Pb and Zn in single and mixture forms produced significant ($p<0.05$) toxicity in *S. oleracea, C. saivum*, *P. oleracea* seedlings and *S. oleracea* and *C. saivum* mature plants in terms of shoot and root lengths, shoot and root biomass, number of leaves, MI, cell size and shoot diameter. *S. oleracea, C. saivum*, *P. oleracea* seedlings and *S. oleracea* and *C. saivum* mature plants showed the dose response for Cd, Pb and Zn i.e. with increasing concentrations of these metals in the plant tissues increased the adverse impacts on growth of plant.

The toxicity of Cd in *S. oleracea, C. saivum*, *P. oleracea* seedlings and *S. oleracea* and *C. saivum* mature plants were sever than the toxicities produced by Pb and Zn..

Bioaccumulation of one HM in the mixture, inhibit or enforce the accumulation of other HM. The up take pattern of Cd and Pb in Cd/Pb showed that both Cd and Pb affect the up take of each other in antagonistic way. HMs in mixture behaved differently than individual HMs due to their interactions with each other. The up take pattern of both Cd and Pb were reflected in the growth parameters of *S. oleracea, C. saivum*, *P. oleracea* seedlings and *S. oleracea* and *C. saivum* mature plants. The toxicities produced in *S.*

oleracea, C. saivum, *P. oleracea* seedlings and *S. oleracea* and *C. saivum* mature plants by Cd and Pb in form of Cd/Pb in terms of al most all growth parameters were more than the toxicities due to individual Cd and Pb but less than their additive toxicities. The difference in toxicity due to individual Cd and Pb and their mixture form (Cd/Pb) is due to the antagonistic affects of Cd and Pb on the up take of each other

The up take pattern of Cd and Zn in Cd/Zn showed that both Cd and Zn affect the up take of each other in antagonistic way. The up take pattern of both Cd and Zn were reflected in the growth parameters of *S. oleracea, C. saivum*, *P. oleracea* seedlings and *S. oleracea* and *C. saivum* mature plants. Similarly, Pb and Zn effected the up take of each other in antagonistic way in case of *S. oleracea, C. saivum*, *P. oleracea* seedlings and *S. oleracea* and *C. saivum* mature plants. The up take pattern of Pb and Zn were reflected in the growth parameters of S. *oleracea, C. saivum*, *P. oleracea* seedlings and *S. oleracea* and *C. saivum* mature plants.

Total protein content, fiber and moisture contents of *S. oleracea, C. saivum* reduced with increasing concentrations of Cd, Pb and Zn alone and also their mixture (Cd/Pb, Cd/Zn and Pb/Zn) in plant tissue. The impacts of mixture of Cd, Pb and Zn on total protein content, fiber and moisture were more sever than the impacts of individual Cd, Pb and Zn but less than the sum of impacts of individual HMs. Similarly, the increasing concentrations of selected HMs reduced the up take of Na, K, Ca, Fe, Mg, Mn and Cu by *S. oleracea* and *C. saivum.*

Present study found that Cd toxicity was more sever than toxicities due to Pb and Zn in case of root than shoot. Root was more sensitive as compared to shoot because it is the part of plant which comes in contact with toxicant first.

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