HEAVY METALS UPTAKE BY CHILLI PLANTS (*CAPSICUM ANNUUM* L.) PLANTED IN RICE HUSK CHAR AND COCO PEAT MEDIA

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by

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TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Cd	Cadmium
Pb	Lead
RHC	Rice husk char
CP	Coco peat
AAS	Atomic Absorption spectrophotometer
dw	Dry weight
ppm	Part per million
mg/L	Milligram per liter
FAO	Food Agriculture Organization
WHO	World Health Organization
NPK	Nitrogen, phosphorus and potassium
T	Treatment
µ	Microgram

LIST OF APPENDICES

PENGAMBILAN LOGAM BERAT OLEH TUMBUHAN CILI (*CAPSICUM ANNUUM* L.) YANG DITANAM DALAM ARANG SEKAM PADI DAN SABUT KELAPA

ABSTRAK

Kajian ini mengkaji pengambilan Kadmium (Cd^{2+}) dan Plumbum (Pb^{2+}) oleh pokok cili merah dan kesannya terhadap pertumbuhan tanaman dan kualiti buah-buahan. Dua media tanaman yang digunakan; arang sekam padi dan sabut kelapa. Eksperimen telah dijalankan dalam reka bentuk yang rawak lengkap blok (CRBD), yang terdiri daripada tiga replikasi dengan menggunakan pelbagai cili komersial (*Capsicum annuum* L.*)*. Logam yang dipilih mempunyai pelbagai kepekatan antara 10-30 ppm. Kandungan logam yang dipilih di bahagian yang berlainan, iaitu akar, pucuk dan buah-buahan telah dianalisis di tiga peringkat pertumbuhan tumbuhan (vegetatif, berbunga dan matang) menggunakan analisis penyerapan atom spektrofotometri (AAS). Keputusan menunjukkan bahawa pengumpulan logam berat dalam akar dan pucuk dalam kedua-dua media meningkat dengan ketara (p<0.05). Kepekatan kedua-dua logam melebihi had keselamatan yang telah ditetapkan oleh Pertubuhan Pemakanan dan Pertanian (FAO) Pertubuhan Kesihatan Sedunia (WHO) masing-masing, 0.05 dan 0.1 ppm untuk Cd dan Pb, manakala, dalam Akta Makanan Malaysia (1983) dan Peraturan-Peraturan Makanan (1985) 1.0 dan 2.0 ppm untuk Cd dan Pb masing-masing.Walau bagaimanapun , morfologi tumbuhan tidak terjejas oleh pengumpulan logam berat tidak termasuk ketinggian pokok. Ketinggian tumbuhan menurun dengan ketara (p<0.05) pada peringkat vegetatif dengan kehadiran Pb^{2+} dalam media sekam padi dan juga pada peringkat matang (p<0.05) apabila kilang itu telah dirawat dengan dos Cd^{2+} 30 ppm. Sebahagian daripada nutrien telah terjejas dengan penambahan Cd^{2+} dan Pb^{2+}. Sebagai contoh, dalam

kehadiran Cd^{2+}, dalam media sekam padi, Mg^{2+}, Ca^{2+}, Fe^{3+} dan Cu^{2+}, telah berkurangan dengan ketara ($p<0.05$) manakala hanya Mg^{2+} terjejas dalam buah-buahan dalam media sabut kelapa . Untuk Pb^{2+} , penurunan yang ketara ($p>0.05$) diperhatikan untuk Fe^{3+} dan Cu^{2+} dalam media sekam padi , serta Mg^{2+} dan Mn^{2+} dalam media sabut kelapa.

HEAVY METALS UPTAKE BY CHILLI PLANTS (*CAPSICUM ANNUUM* L.*)* PLANTED IN RICE HUSK CHAR AND COCO PEAT MEDIA

ABSTRACT

The current study investigated the uptake of Cadmium (Cd^{2+}) and Lead (Pb^{2+}) by chilli plant and their effect on the plant growth and fruit quality. Two planting media were used; rice husk char and coco peat. The experiment was conducted in a complete randomized block design (CRBD), comprising of three replications using a commercial chilli variety (*Capsicum annuum* L.*)*.The selected metals were employed in various concentrations ranging from 10-30 ppm.The contents of the selected metals in different parts of the plant, namely roots, shoots and fruits were analyzed at three different stages of plant growth (vegetative, flowering and maturity) using atomic absorption spectrophotometer analysis (AAS). Results showed that the accumulations of heavy metals in roots and shoots in both the growing media increased significantly ($p<0.05$). The concentrations of both metals exceeded the safety limits set by Food Agriculture Organization (FAO) and World Health Organization (WHO), 0.05 and 0.1 ppm for Cd and Pb respectively. Nevertheless, they are within the limits recommended by Malaysia Food Act. (1983) and Malaysia Food Regulation (1985) 1.0 and 2.0 ppm for Cd and Pb, resoectivly. However, the plant growth was not affected by the accumulation of heavy metals excluding plant height. The plant height significantly decreased ($p<0.05$) at vegetative stage in the presence of Pb^{+2} in rice husk char media and also at maturity stage ($p<0.05$) when the plant was treated with Cd^{2+} 30 ppm dosages. Some of the nutrients were negatively affected by the addition of Cd^{+2} and Pb^{2+}. For example, in the presence of Cd^{+2}, in rice husk char media, Mg^{2+}, Ca^{2+}, Fe^{3+} and Cu^{2+}, were significantly diminished ($p<0.05$) while only Mg^{2+} was significantly decreased ($p<0.05$) in the

fruits in coco peat media. For Pb^{+2}, a notable decrease (p>0.05) was observed for Fe^{3+} and Cu^{2+} in rice husk char media, as well as Mg^{2+} and Mn^{2+}in coco peat media.

CHAPTER 1

INTRODUCTION

1.1 Background and Rationale

Chillies have been a part of the human diet in the North and South America since 7500 BC. It is one of the major cultivated products in the Americas, domesticated in several different parts of South and Central America (Huq & Arshad, 2010; Nadeem et al*.*, 2011)**.** The first appearance of chilli was in Mexico and South America and it took several decades to reach Malaysian territory. Approximately, there are seventy types of plants cultivated in the worldwide as spices which, mostly distributed in Asia **(**Chomchalow, 1996). Among these, 20-35 spices belong to the genus Capsicum, from Solanaceae family. The genus of Capsicum include five famous spices, cultivated in different parts of the world and are well known as; *C. annuum*, *C. frutescens, C. pubescens, C. baccatum* and *C,* and *chinense* (Lee, 2007; Delelegn, 2011).

Chillies are considered to be among the most widespread cultivated vegetables in Malaysia. According to an estimate, it covers about 14,560 hectares area/year in Malaysian states including; Johor, Perak and Kelantan (Animhosnan.blogspost.com; Ariffin, 1989; Mussema, 2006). Furthermore, the most popular cultivated chilli species in Malaysia are; Capsicum *annum* L. and Capsicum *frutescens* L. They are known by different names such as; hot paper, red paper or capsicum (Idris et al., 2001). There are several common local varieties planted in Malaysia including; Kulai, MC4, MC10, MC11, MC12, CB1, CB2, CB3, CB4, Bangi, Puteh, Langkap, Tanjung and Minyak (Idris et al., 2001; Lee, 2007). Still, chilli production in Malaysia is less than the requirement for their consumption which estimated as 23,000 ton/year. Therefore, more chilli sources are required which are now either being cultivated within Malaysia at larger scale or being imported from several other countries such as India, China or Thailand (Lee, 2007). As consequence of food contamination, increasing demands has been given to the safety food by many researchers worldwide. Over the world, risks on millions of lives are known to be strongly associated with the consumption of contaminated food by many factors included; heavy metals, pesticides and toxins (Khan, et al., 2009; Suruchi & Khanna, 2011). Therefore, this project was proposed to study the uptake of some heavy metals in chilli plant and discussed in more details as shown below.

Heavy metals are defined as metals that have specific density more than 5 g/cm^3 and are available in the earth's crust at different concentrations (Kisku et al., 2011). They deposit in soil at faster rate at higher pH as well as in the presence of a high proportion of carbonates (Maria et al., 2007). Therefore, their accumulation was found in the surface layer of the soil. However, the increase in their concentrations in soil increases their absorbance by a plant, which threatens human's life (Mocanu et al., 2006).

Cadmium, Lead, Arsenic and Mercury are known as the most toxic heavy metals, they have damaging effects to the human body tissues and there is no mechanism for the removal of these metals from human body. These metals have long half-lives Gonick (2008) and are non-biodegradable substances having no known biological functions in plants and human (Wilberforce & Nwaabue, 2013). There are limited studies on the uptake of heavy metals by chilli plants at various growth stages. The most of the studies focused on the metals concentration in edible parts of vegetables. In addition, the reports which describe the heavy metals uptake by chilli plants are scarce compared with other vegetables. Also, the importance of chillies in our life and the nutritional value of chilli have led us to undertake this study.

Heavy metals can be classified into four groups According to their toxicity and importance for human and plant, as shown below:

i). Highly toxic metals include Mercury (Hg), Cadmium (Cd), and Lead (Pb).

ii). Less toxic includes Tin (Sn) and Aluminium (Al).

iii). Non significant includes Barium (Ba), Lithium (Li) and Zirconium (Zr).

iv). Significant metals includes Copper (Cu), Zinc (Zn), Iron (Fe), Manganese (Mn), Magnesium (Mg) and Chromium (Cr).

The metals compiled in the latest group (iv) if taken in higher quantity than required, may also be harmful for human health (Raikwar et al.,2008; Suruchi & Khanna, 2011). Two of the most toxic heavy metals are described in details in the following section.

a) Cadmium (Cd)

Cadmium (Cd) is considered as one of the most dangerous environmental pollutants. It is found as a mineral combined with other elements (cadmium oxide and cadmium chloride). The main sources of Cd toxicity are from mines, paints, atmospheric deposition and fertilizers (super-phosphate) (Curtis & Smith, 2002). Cd has a serious impact on the human body; it accumulates in the human body daily, the average amount of Cd accumulated in the human body at age 50 is 30 mg (Raikwar et al., 2008). Furthermore, high dose of Cd can lead to lung cancer and high blood pressure. Cd can replace calcium in the bones leading to the fragility and distortions for bones. It can also affect the immune system and causes prostate cancer, heart disease and anemia (Harmanjit, 2006).

b) Lead (Pb)

Lead (Pb) is a toxic metal at any doses. Pb accumulates in the soil surface because of its low solubility in water (Cecchi et al., 2006). Thus, it is in very low uptake by the plant. Urban soils usually contain high amounts of Pb up to 1840 mg/L (Curtis & Smith, 2002). The major sources of Pb are automobiles exhaust, batteries, fertilizers, paints, insecticides and industrial processes and their emissions (Kibria, et al., 2010). Pb is a carcinogenic substance, causes many problems when it enters into the human body including bones frailty, heart diseases, loss of memory and attention, decreased fertility in humans (Laidlaw & Taylor; Raikwar et al., 2008). Several studies have proven the relationship between blood contamination with lead and lower intelligence quotient in children (Coultre, 2001; Burke & Miller, 2011). Table 1.1 shows the recommended safety intake of heavy metals. While, table 1.2 shows Safety limits suggested by Malaysian food Act (1983) and food regulations (1985) for some heavy metals in vegetables.

Table 1.1 FAO/WHO Maximum permissible values for Cd and Pb in fruiting vegetables.

Metal	Maximum permissible values (ppm).
Cd	0.05
Pb	0.1

Source: FAO/WHO (2011).

Table 1.2 Safety limits suggested by Malaysian food Act (1983) and food regulations (1985) for some heavy metals in vegetables.

Metal	Maximum permissible values (ppm).
Cd	1.0
Pb	2.0
As	1
Hg	0.05

(Maimon et al., 2009).

1.2 Problem Statement

According to several studies and reports, heavy metals such as Pb and Cd have serious effects on the human life including effects on young children and pregnant women. Measurement of their concentrations, uptake, accumulations and evaluating their effects on the plant growth, including concentration of some nutrients in the fruits, may helps to minimize their harmful effects to the human body. Consequently, this study was proposed to investigate the uptake of some heavy metals by chilli plants.

1.3 Objectives

i). To determine the uptake and accumulation of Pb and Cd in chilli plants that inoculated artificially with certain concentrations of the same metals during various stages of plant growth.

ii). To evaluate the effect of cadmium and lead on the plant growth and nutrients concentration in the chilli fruits after possible uptake by spike metals.

iii). To compare the affect of rice husk char and coco peat media on the uptake and accumulations of Cadmium and Lead in chilli plant parts at different growth stages.

1.4 Hypothesis

i). Obtained result might be higher than permissible limits specified by FAO and WHO, 2011.

ii). The use of inorganic fertilizers could increase the heavy metals uptake by chilli plants.

iii). Chilli plants performance might be affected by the accumulation of heavy metals.

iv). Presence of heavy metals in planting media could affect the uptake and concentrations of some nutrients in chilli fruits.

CHAPTER 2

LITERATURE REVIEW

2.1 Contamination of Vegetables

Vegetables comprise about 15% of the daily diet of the Malaysian population. The high quality of vegetables production means good health for the consumers (Jipanin et al., 2001). However, the environmental pollutants may harm the quality of vegetables which ultimately can induce harmful effects on the consumer's health. The main environmental pollutants in vegetables are toxic heavy metals from agronomic sources particularly agricultural, chemical and processing operations (Suruchi *&* Khanna, 2011). Human activities may also increase the concentration of heavy metals in the environment (Ghaaran, et al., 2009; Tuan & Popova, 2013). Several studies have shown that some of the common vegetables are capable of accumulating high levels of metals from the contaminated soil (Radwan & Salama, 2006; Zahir, et al., 2009; Uwah, 2009; Pinho *&* Ladeiro, 2012; Wilberforce & Nwaabue, 2013). Many people were diseased resulting from consumption of contaminated vegetables (Ismail, et al., 2005). Table 2.1 presents a summary of heavy metals found in various vegetables in worldwide.

2.2 Effect on the Human Health

Some heavy metals are needed for living organism in low amounts such as Cu, Fe, Zn, Cr, Mg and Mn (Vailliers et al., 2010), whereas, some of them are toxic even at low concentrations such as; Pb, Hg, As and Cd (Pacyna, 1987; Ghaaran et al., 2009). Under certain conditions, these metals can act as mildly to highly toxic substance, bio-accumulating in the human bodies *via* food chain and impart pronounced effects on the normal physiological activities. Once accumulated, these are not degradable or transformed into harmless products and they have the possibility to accumulate into human body (Radwan, et al., 2006).

Table 2.1 A summary of heavy metals concentrations in various vegetables species in worldwide (ppm)

Country/City	Vegetables	Plant part	Concentrations (ppm)							Sources
			Pb	Cd	Co	Zn	Ni	Fe	Cu	
India/Kalipur	Tomato	Fruits	31.0	-	-	155.0	-	-	55.0	(Kisku, et al., 2011)
		Roots	78.0	-	-	127.0	-	-	63.0	
		Shoots	34.0	-	-	1153.0	-	-	54.0	
Libya/Musrata	Green paper	Fruits	0.47	0.07	0.34	0.024	0.19	-	2.97	(Elbegermi, et al., 2012)
	Tomato	Fruits	0.51	0.25	0.45	8.42	0.20	-	2.24	
	Spinach	Leaves	0.32	0.27	0.54	16.8	0.26	-	5.32	
Malaysia/ Sepang	Spring Onion	Leaves	0.01	0.09	-	1.20	-	0.67	0.13	(Ismail, et al., 2005)
	Brinjal	Fruits	0.002	0.01	-	0.11	-	0.15	-	
	Potato	Roots	0.007	0.01	-	0.22	-	0.47	0.09	
S. Africa/Alice	Cabbage	Leaves	-	0.26	-	14.4	-	-	0.69	(Bvenura & Afolayan, 2012)
	Onion	Fruits	-	0.21	-	89.8	-	-	9.24	
Pakistan/Karachi	Tomato	Fruits	0.53	0.24	-	12.3	0.14	7.92	3.12	(Zahir, et al., 2009)
	Neem	Fruits	1.55	0.29	-	10.8	0.14	9.96	1.83	
Poland/Wrociaw	Parsley	Leaves	0.14	0.04	-	6.88	-	-	1.58	(Danuta, et al., 2007)
	Lettuces	Leaves	0.03	0.03	-	3.70	-	-	0.36	
China/Huanggang	Pepper	Fruits	0.20	-	-	4.87	-	-	-	(Hu, et al., 2012)
	Eggplant	Fruits	0.19	-	-	3.89	-	-	-	
	Tomato	Fruits	0.12	-	-	5.14	-	-	-	
Egypt/Alexandria	Green Pepper	Fruits	0.47	0.05	-	12.5	-	-	4.53	(Radwan & Salama, 2006)
	Tomato	Fruits	0.26	0.01		7.69	-	-	1.83	
	Lettuce	Leaves	0.58	0.07	-	9.76	-	-	1.97	
	Eggplant	Fruits	0.21	0.02	-	11.5			1.41	
France/ Lille	Lettuce	Leaves	0.34	0.37	-	-	-	-	-	Christelle, et al.(2006)
	Potato	Fruits	0.02	0.09	-	-	-	-	-	

The most vulnerable people who can be affected by these metals are pregnant women and newborn (Boon & Soltanpour, 1992). They cause many problems to the human body such as malfunctioning in the nerve system, kidney and liver. Furthermore, depending on the consumed dose, heavy metals induce various types of cancers and are also responsible for low birth weight (ATSDR, 1994 a, 1999 b). Further effects of severe mental retardation in newborn children have also been noticed in some cases where the pregnant mother who ingested highly toxic amounts of heavy metals either through direct or indirect consumption of vegetables (Mahaffey et al., 1981; Coultre, 2001; Burke &Miller 2011; Suruchi & Khanna, 2011). Table 2.2 shows maximum daily intake values for Cd, Pb, Hg and Zn suggested by Food Agriculture Organization (FAO) and World Health Organization (WHO).

2.2 Safety limits for human intake of some heavy metals recommended by FAO/WHO

Heavy metal	Maximum limit/ day/ bw (µg/kg)	
	Children	Adults
Cadmium	-	0.8
Lead	0.03 to 9	0.02 to 3
Mercury (inorganic)	-	4
Zinc	-	20 mg/day

Source: FAO/WHO (2011)

Othman (2001) studied the contents of heavy metals in five types of green vegetables (amaranth, Chinese cabbage, cowpea leaves, leafy cabbage and pumpkin leaves) collected from different areas in Dar El Salaam, Tanzania. A direct positive relation between Zn and Pb levels in soils with the levels in vegetables had been reported.

A study carried out by Rosen (2010) has showed that Pb did not easily accumulate in the fruiting part of vegetables and fruit crops (corn, beans, squash, tomatoes, apples and straw). However, he has found high concentrations of heavy metals accumulated in leafy vegetables (lettuce) and on the surface of the root crops.

Nwoko and Equnobi (2002) determined the concentration of Pb in soil and vegetables in the land, which was abandoned by a battery factory in Ibadan, Nigeria. They reported that Pb was present in plant tissues. Mostly roots were found to have higher concentration of Pb than shoots.

Khairiah et al. (2002) have investigated the uptake of Cr in roots, fruits and leaves of *Brassica nigra*, *Ipomaa aquatic*, *Capsicum annuum*, *Vigna sinensis*, *Manihot esculenta* and *Ipomea batatas*. Their experiment was conducted in two agricultural areas at Sepang and Bangi, Malaysia. The obtained results showed higher concentrations of Cr in fruity and root type of vegetables compared to the leafy vegetables. Nevertheless, the result was acceptable compared with Malaysian Food Act 1983 and Ministry of Health, 1999. On the other side, both areas containing low concentrations of Cr in agriculture soils.

A study was carried out by Al-Eed et al. (2002) to estimate the concentration of some heavy metals in different spices available in local markets in Saudi Arabia, such as Pb, Ca, Co, and Se. The levels of Pb between trace to 14.30 ppm (dw), while the concentration of Cd was between 1.25 to 3.05 ppm. The level of Co was reported between Zero to 0.64 ppm, and that for Selenium was recorded from Zero to 13.3 ppm. The study showed high levels of some heavy metals and remained above the safety recommendation limits suggested by FAO and WHO, 2001.

Chiroma et al. (2003) reported the contamination of vegetables and soil by heavy metals irrigated with sewage water in Yola, Nigeria. They reported that high concentrations of the metals (Fe, Zn, Cu, Mg and Pb) in soil were accumulated in different parts of the plants. They also, indicated that Fe was found more in roots and leaves, while, Mg and Mn were highly accumulated in unwashed plants.

Khairiah et al. (2004) investigated the uptake of Zn, Pb and Cd by red chilli (*Capsicum annuum* L.) and long beans (*Vigna sinensis*). It was reported that the concentration of Zn in long beans was higher than in chilli. Nevertheless, the contents of heavy metals in their experiment were within the safety limit suggested by Malaysian Food Act (1983) and Food Regulations (1985) which are 1.0, 2.0 and 30 ppm for Cd, Pb and Zn, respectively.

A study on the uptake of heavy metals in vegetables was carried out by Kumar et al. (2007). They have investigated the concentrations of several heavy metals such as Cd, Pb, Cu, Co, Zn, Fe, and Ni in various vegetables collected from the local market of Anand town, India. High concentration of Cd was recorded in the onion, coriander and cauliflower. High concentrations of Co and Cu were recorded in cauliflower and bottle Gourd. However, Fe concentration was high in cauliflower and cucumber, while, vetches and lady finger showed high content of Ni. High concentration of Pb was observed in cauliflower and onion, while, the maximum content of Zn was recorded in cucumber and cauliflower. Conclusively, the concentration of heavy metals was within safety limits except Fe, which was due to content of iron in the soil.

Kanakaraju et al. (2007) determined the contents of Zn, Cu, Mn, Co, Pb and Fe in leafy vegetables (Kala, green mustard and white mustard) and fruit vegetables (cucumber and long bean) collected from two sites (Siburan and Beratok) at Kuching, Sarawak, Malaysia. The obtained results showed slightly higher concentrations of Pb in various vegetables and fruits from both sites (2.0, 2.3 and 2.9 ppm) than the maximum levels recommended by the Malaysian Food Act 1983, which is 1.5 ppm, while the concentration of Pb (0.1 ppm) was considerably higher than FAO/WHO guidelines (2001). Their study had proven that the heavy metals contaminations were due to atmospheric deposition in case of Pb and the water used for the irrigation, spraying methods and fertilizers for the other metals than Pb.

Kasar and Kore (2009) carried out a study on the heavy metal contents in two plants (*Capsicum annum* and *Cucumis sativus*). Their results showed that concentrations of Zn, Cu and Fe were higher in soil, whilst, Pb and Cd concentrations were higher in plants. The highest accumulations (0.13 ppm) of Pb were recorded in the roots of *Capsicum annum* and in leaves (0.15 ppm) of *Cucumis sativus*. The highest concentration of Cu (1.03 ppm) was recorded in roots of Capsicum and in leaves (0.29 ppm) of *Cucumis sativus*.

Bhat et al. (2009) investigated the concentrations of some minerals and heavy metals in 23 types of medicinal plants. The investigation was carried out in different parts of the plant's roots, leaves, barks and rhizome. Their study indicated that the important minerals and heavy metals were found in all plants with different percentage. Heavy metals were found in high levels in Vitiveria Zizinalis plant, including Ar (53.1 mg /100g), Cr (6.74 mg/100g), Co (10.2 mg/100g), Hg (3.6 mg/100g) and Ni (3.28 mg/100g). The highest level of copper was found in *Berberis aristata* (134.96 mg/100g) while the lowest level was in *Sida Cordifolia* (0.05 mg /100g).

A study was carried out by Ibrahim et al. (2012) on the uptake of heavy metals by some spices collected from a local market in Erbil, Iraq. The results were 6.1-47.0, 56-650, 6-44, 10.5-22.5, 8.5-26-5, 26-88.6, 1.25-14.6 and 0.045-1.35 ppm for the metals Zn, Fe, Cu, Cr, Co, Mn, Pb and Cd, respectively. The results were within the safety limit suggested by FAO and WHO (2001) with the exception for Pb and Cd. The concentration of Pb was high in fenugreek cinnamon, whereas, Cd in ginger. From the author's point of view the excess of heavy metals concentrations might be due the fertilizers or sewage sludge or even both that they used in their study.

2.3 Fertilizers and Their Effect on the Uptake of Heavy Metals

Fertilizers can be defined as a chemical compounds given to the plants to improve their growth and production. These are usually applied through the soil. Farmers use different kinds of fertilizers to get rich crops. Fertilizers contain essential nutrient compounds for plants to grow. These compounds are made from organic sources like the animal manure or from waste sludge. There are some of fertilizers made from mineral compounds or from compounds produced in the industry (Atafar, et al., 2010). However, fertilization can effect on the accumulation and transfer of heavy metals in soil-plant system. (Mortvedt, 1996; Zhao et al., 2010). Table 2.3 shows the contents of inorganic fertilizers suggested by the American Association of Plant Food Control Officials (AAPFCO).

Table 2.3 AAPFCO Risk-Based concentration of inorganic fertilizers.

Metals	NPK (ppm / 1% P_2O_5)	Micronutrient (ppm /1% micronutrient)
As	13	112
Cd	10	83
Co	136	2228
Pb	61	463
Hg	1	6
Mo	42	300
Ni	250	1.900
Se	26	180
Zn	420	2.900

Source: Minnesota Department of Health (2008).

Rahaman et al. (2007) studied the effect of organic and inorganic fertilizers on the uptake of Cd by rice plants. They reported that accumulation of Cd in rice plant ranged between 0.17-1.53 ppm at Bangladesh Agricultural University (BAU) site and 1.33- 2.24 ppm at Madhupur site. The total Cd uptake varied between 0, 31-8.99 ppm and 4.99-11.89 ppm in both sites, respectively. The concentration of Cd from inorganic fertilizers was more than organic fertilizers. The results also showed that there was an inverse relationship between the contents of Cd and total dry matter, where the concentration of Cd decreased with the increase of total dry matter.

Ramadan and Ashkar (2007) studied the effect of different concentrations of mineral fertilizers (NPK) and chicken manure on the distribution of heavy metals in the soil and in two varieties (GS12 and A-Lisa) of tomato crop in Banha, Egypt.

They found that the levels of Fe and Pb were low in soil in the case of planting Alisa variety compared with GS12 variety in all treatments. They also noted the presence of high concentrations of Fe and Pb in different rates of chicken manure. Mostly, the concentration of Fe was high in Alisa organs compared with GS12 under different rates of chicken manure and mineral fertilizers. The concentration of Pb was lower in Alisa organs compared with GS12. The affectivity rates of different fertilizers impacted positively on the total production of the two tomato varieties.

Mirlean et al. (2008) studied the effect of phosphate fertilizer industries on the environment. Their study investigated the concentration of mercury in soil at different depths and various distances from the phosphate fertilizer factory. The concentrations of mercury were found high in topsoil (0-5 cm). This concentration further decreased with the increase in depth (60-100 cm). Their study indicated that the industrial manufacturing of super-phosphates contaminates the environment with mercury. Table 2.4 shows the vertical distributions of mercury concentrations at varying distances from the factory.

Table 2.4 Vertical distributions of mercury concentrations (µg/kg) in soil profiles at varying distances from the factory.

Distance from factory (Km)	Sampled depth intervals (cm)			
	0-5	5-20	20-60	60-100
0.5	458 (9)	110 (6)	43 (3)	29 (3)
1.5	62 (3)	38 (3)	21 (2)	17 (2)
3.0	43 (2)	23 (2)	21 (2)	20 (3)
80.0	26 (2)	22 (2)	20 (2)	23 (2)

The value in parenthesis is the standard deviation (±SD). Source: Mirlean et al (2008).

Mousavi et al. (2010) reported that the solid waste composts, vermicomposting and inorganic fertilizers could affect the concentration of Pb and

Cd in soil and their consequent uptake by rice plant. They found that the concentration of Cd was high (14.11 ppm) in grain grown in an inorganic fertilizer. Lead accumulated mostly in the roots (71%) during 3 years application of solid waste compost and half inorganic fertilizers (20 ton /ha).

2.4 Effect of Waste Water on the Heavy Metals Uptake in Plants

Several studies have shown that vegetables, especially leafy crops grown in pollutant agriculture soils have a higher concentration of heavy metals compared with those grown in unpolluted agriculture soils (Guttormsen et al., 1995).

Davies (1978) reported that the concentration of Pb in garden soils increased with the length of habitation. Also, noted that a human activity is the main reason of agriculture soil contamination. Similar studies have also shown a clear contamination of urban soils by Pb, Cd and Zn (Preer et al., 1978; Culbard et al., 1983; Christelle et al**.,** 2006).

A study carried out by Yusuf et al. (2002) on Cu, Cd, and Ni concentrations in vegetables in industrial and residential areas. The study showed that the concentration of these metals is different in various vegetables due to the soil. Vegetables grown in industrial areas were richer in heavy metals than those grown in the residential areas due to pollution.

Somasundaram (2003) investigated the heavy metals content in leafy vegetables of sewage-irrigated area in Tamil Nadu, India, and they found that the percentage of heavy metals (Cd, Cu, Pb, Zn, and Mn) included in leafy vegetables were in high rates.

A comparative study conducted by Saif et al. (2005) on the effect of wastewater mixed with industrial effluent and well water, which used for plant irrigation collected from different places in Karachi, Pakistan. The results proved that wastewater has high concentration of Zn, Cu, Fe, and Cr, where, 36% of the analysed samples were found higher in Mn, Cd, Ni and Pb than the normal limits. High contents of Zn, Fe, Mn, Cd, Ni and Pb were observed in soil. Plants such as spinach was found had high concentrations of many heavy metals over the safety limits. Therefore, an area, which irrigated with wastewater and industrial effluent, was not safe for plant irrigation.

A study conducted by Chove et al. (2006) on two heavy metals (Pb &Cu) in two popular leafy vegetables, pumpkin leaves (*Cucurbits moschata*) and Chinese cabbage (*Brassica chinensis*) grown around the Morogoro city in Tanzania. The samples were collected from three places and irrigated with contaminated water. The amounts of heavy metals were found in the range from 0.89-1.39 ppm and 0.05-0.315 ppm for Cu and Pb, respectively.

A study carried out by Mapanda et al. (2007) on the contamination of vegetables by heavy metals using wastewater irrigation. The study showed that the concentration of some heavy metals (Cu, Zn, Ni, Pb, Cd and Cr) in vegetables in two regions in Harare, Zimbabwe, was high. Pb concentration was higher than the standard limit in 60% of the vegetables. Result also showed that the distribution of 25% of Pb in vegetables from both regions was above the FAO/WHO recommended limits.

Investigation on the uptake of some heavy metals was done by Cheraghi et al. (2009) on eight types of vegetables collected from polluted and unpolluted areas by wastewater in Iran. Results showed that higher accumulation of heavy metals such as (Cu (9-36.5 ppm), Zn (48.1-181ppm), Mn (109.1-183ppm) and Pb (8.5-30.2 ppm), was accumulated in vegetables collected from polluted areas than that collected from unpolluted area Cu (8.1-17 ppm), Zn (44.7-85.5 ppm), Mn (109.2-165 ppm) and Pb (4.3-7.3 ppm). Therefore, their investigation proved that the wastewater is the main contributor for the accumulation heavy metals in soil and then into the plants.

A study carried out by Abdullahi et al. (2009) indicated that uses of flushing water from industries can increase the uptake of heavy metals by vegetables, and they found that the concentrations of Pb and Cr in the vegetables are above the safety limits suggested by FAO/WHO, 2001.

A study done by Prabu (2009) to measure the concentrations of some heavy metals in vegetables irrigated with contaminated water (Akaki Riverweed, Ethiopia). The results showed that the rate of accumulation of Cr in all analyzed vegetables was over the recommended safety limits. Leafy vegetables were found to contain an excess concentration of Cd than other vegetables. On the other hand, the analysis of irrigation water showed higher levels of Cd, Cr, Cu, Zn, Mn, Ni and Fe than the natural elemental limits in fresh water.

A study conducted by Hu, et al. (2009) on five popular vegetable species collected from urban agriculture soils, located about 100-200 m away from lead and zinc mining and smelting plant in China. The spices were Malabar spinach (*Basella alba* L.*)*, pakchoi (*Brassica chinensis* L.), celery (*Apium graveolens* L.), amaranth (*Amaranthus spinosus* L.) and lettuce (*Lactuca sativa L*.). Two wild plants were selected as a control and were collected approximately 5 km away from the factory. The study proved that the contents of Cd and Pb in agriculture soils was above the recommended limit compared with other plants collected away from the factory (control samples). This finding means that the atmospheric particles have high levels of heavy metals, which contributed to the contamination of soil as well plants around.

Usha et al. (2011) studied the ability of sunflower plants to uptake Pb from effluent water. Plants were exposed to various concentrations of Pb (5, 10, 15, 20, 25 and 30 ppm) in effluent water. Higher concentration of Pb in roots than in leaves and stems were reported. Physical and biological parameters along with metal accumulation in plants were found to be increased with the increase in the effluent water concentration up to 15 ppm, followed by decline in higher dose of metal concentration. The study concluded that sunflower plant has a great possibility to be used as a phytoremediation agent to remove lead from polluted soil.

2.5 Effect of Heavy Metals on the Uptake of Minerals and Plant Growth

A research study conducted by Savitha (2008) on the effect of an Fe-EDTA on yield and quality of red chilli (*Capsicum annuum* L.) showed that the chilli fruits have the highest color value and oleoresin content at 0.5 percent spry of Fe-EDTA at 50 and 90 days after transplanting. It was also pointed out that the number of branches and dry matter yield were affected by combining application foliar sprays with Fe-EDTA treatments. The dry matter yield was increased under this treatment, whereas, the contents of some micronutrients (Mn, Cu and Zn) were not affected.

Gangamrutha (2008) had studied the effect of copper nutrition on the yield and quality of chilli (*Capsicum annuum* L.) in Karnataka, India. His study concluded that the combined application of $CuCl_2$ at 2.5kg/ha through soil and 0.25% spray can increase the amount of ascorbic acid, oleoresin contents and color value. The study also found that the concentrations of some elements such as Mn, Fe, Cu and Zn in sundry fruits were increased at the same application.

John et al. (2009) studied the uptake and plant performance of *Brassica juncea* L, when the plant exposed to different concentrations of Cd and Pb (150, 300, 450, 600, 750 and 900 and 150, 300, 600, 900, 1200 and 1500µM, respectively. They reported that the accumulation of Cd in the plant roots was more than accumulation of Pb. Also, they indicated that the plant growth was decreased as a result of exposure to Cd and Pb. Protein contents were also increased at lower concentrations of heavy metals. Proline was decreased at higher concentrations of heavy metals.

Aydinalp et al. (2009) investigated the effect of heavy metals (Cd, Ni, Zn, Cu, and Cr) on germination and growth of the Alfalfa plant (Mediago sativa). They have reported that Cd and Cr at 10 ppm doses are highly affected the seed germination and plant growth, while 20 ppm of both Cu and Ni is the most effective dose. There was no any significance at high concentrations of Zn on seed germination. However, the plant shoot growth was increased by 13%, 59%, 35%, and 6.6% at 5 ppm doses of Ni, Cr, Cu and Zn, respectively, while, 20-40 ppm Zn had increased the shoot growth.

Ghani et al. (2010) studied the effect of Pb on growth, chlorophyll and Pb concentration in two varieties of Maize *Zea mays* L. (Neelam and Desi). Results showed that the roots were affected negatively by Pb stress. However, the growth of maize shoots was less affected for both verities. The photochemical efficiency was affected in Neemlam varieties under high Pb concentration, whereas, there was no any effect on the Desi varieties. Pb accumulation was higher in Desi variety than

Neelam variety. The Pb accumulation in roots, shoots and leaves was increased in both varieties under Pb stress compared to control.

Abdel Fatah et al. (2010) worked on the effect of Co uptake on the nutrients and heavy metals in Lettuce Plants. The experiment was conducted in two different soils (sandy and calcareous). Co was given at different concentrations ranging from 10-20 ppm. The results concluded that a positive relation was observed between Co and macronutrients (N.P.K) in sandy soil. A similar correlation was observed between micronutrients and Co, except for Mn. However, negative correlation was recorded between Co and heavy metals (Pb, Cd and Ni). In the case of calcareous soil, positive relation was observed between Co and Pb, Cd and Ni. The dry weight was observed in negative relation with Co, while, micronutrients and chlorophyll gave a positive relation with Co treatments.

Malik et al. (2011) have studied the effect of Zn on the plant performance in two plants, i.e. rice (*Oryza Sativa*, variety–BR49) and red amaranth (*Amaranthus Sp.)*. The obtained results showed a significant impact of Zn on the growth and yield of both plants. The accumulation of Zn in both plants roots, shoots and grain was increased by increasing the doses of Zn, whereas, the fresh and dry weight of matter production and length of roots and shoots were decreased with the increase of Zn dose. The study concluded that Zn had a high impact on the growth and yield of red amaranth and rice plant.

A study carried out by Chaves *et. al.* (2011) investigated the effect of heavy metals on plant growth and their accumulation by sunflower plant. Cd was used at 10, 20, 30 and 40 mg/dm of soil, while Zn and Cu concentrations were used at 20, 40, 60 and 80 mg/dm. The obtained results showed that the plant height was reduced by the action of Cd and Zn at high concentrations. However, at high concentration of Zn, leaf area was decreased. There was no any effect of the heavy metal ions on plant dry weight. On the other hand, the uptake of heavy metals by the sunflower plant was significantly affected by the presence of these metals in the plant parts.

2.6 Nutritional Value of Vegetables and Fruits

Vegetables and fruits constitute an essential part of the human diet. They are an important source of vitamins such as vitamin C (ascorbic acid), A and E, thiamine B1, niacin B3, pyridoxine B6 and folic acid B9. Furthermore, these are considered as good sources of minerals and dietary fiber (Nurul et al., 2012) and containing 91% of vitamin C, 30% of Folic acid, 27% of vitamin B6, 15% of vitamin B3, and 17% of vitamin B1.

The vegetables and fruits provide human body about 16% of magnesium, 19% Iron and 9% of the calories. For example Nuts supply human body fatty acids, vitamin E, proteins, minerals, and Riboflavin B2, which are necessary for the growth and survival. These also act as buffering agents for acidic substances produced during the digestion process (Craig & Beck, 1999; Sarkiyayi et al., 2010). Table 2.5 shows the nutritional value of some fruits and vegetables.

Table 2.5 Constituents of vegetables and fruits that have a positive impact on human health and their sources

Constituent	Sources	Impacted human diseases
Vitamin C	Broccoli, cabbage, cantaloupe, citrus fruits, guava, kiwi fruit, leafy greens, pepper, pineapple, potato and tomato.	Cancer, cataracts, heart disease, stroke
Vitamin A (Carotenoids)	Dark-green vegetables (collards, spinach, and turnip greens), orange vegetables (Carrots, pumpkin, and sweet potato), orange-flesh fruits (apricot, cantaloupe, mango, nectarine, orange, papaya, peach, persimmon, pineapple, and tomato).	
Vitamin E	Nuts (almonds, cashew nuts, filberts, macadamias, pecans, pistachios, and walnuts).	
Flavonoid	Red, blue, and purple fruits (apple, blackberry, blueberry, cranberry, grape, nectarine, peach, plum and prune, pomegranate, raspberry, and strawberry).	
Fiber	Most fresh fruits and vegetables, nuts, cooked dry beans and peas	Diabetes, heart disease
Foliate	Dark-green leafy vegetables (spinach, mustard greens, and romaine lettuce), legumes (Cooked dry beans and peas, green peas), oranges).	Birth defects, cancer, Heart disease
Potassium	Baked potato or sweet potato, banana and plantain, cooked dry beans, cooked greens, dried fruits (apricots and prunes), winter (orange) squash	Hypertension, stroke

Source: Kader (2001)