http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 7 (8): 1438-1442, 2004
ISSN 1028-8880

The Uptake of Heavy Metals by Fruit Type Vegetables Grown in Selected Agricultural Areas

[1]J. Khairiah, [2]M.K. Zalifah, [1]Y.H. Yin and [2]A. Aminah
[1]Environmental Sciences Program, School of Environmental Sciences and Natural Resources,
Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia
[2]Food Science Program, School of Chemical Sciences and Food Technology,
Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia

Abstract: A study on the uptake of heavy metals by fruit vegetables from agricultural areas was conducted at Agrotek, Sepang and Bangi, Selangor. The objective of the study was to determine the uptake of heavy metals by chilies (*Capsicum annum*) and long beans (*Vigna sinensis*) from the soil. Heavy metals studied were Pb, Cd and Zn. Wet digestion method was used for heavy metals analysis in the vegetables. Heavy metals from soil samples were extracted by sequential extraction method, which extract heavy metals from easily and freely leachable fraction (EFLE), acid reduction (AR), organic oxidation (OO) and resistant fraction (RR). The results of the study showed that the level of Zn content in long beans (*Vigna sinensis*) was higher than in chilies (*Capsicum annum*). However the concentrations of heavy metals studied in these vegetables were still below the maximum level allowed by the Malaysian Food Act (1983) and Food Regulations (1985) for Pb (2.00 mg kg^{-1}), Cd (1.00 mg kg^{-1}) and Zn (30.00 mg kg^{-1}).

Key words: Heavy metals, vegetables, soil, accumulation, fraction

INTRODUCTION

The metal contamination in superficial soils and vegetation is derived largely from several anthropogenic activities such as mining operations, industrial process emissions and transportation[1]. Heavy metals are mobile and easily taken up by plants in the environment. Plants have shown to have great ability to accumulate metals from the environment. Metals uptake by plants may pose risks to human health when vegetables are grown on or near contaminated areas. The health risks will depend on the chemical composition of the waste material, its physical characteristics, the vegetables cultivated and the consumption rate. Vegetables are highly recommended foods for humans and large amounts are consumed as part of their daily diets[2]. Malaysian dietary guidelines recommend that adult eat five servings of fruits daily to maintain good health[3]. Thus accumulation of heavy metals in the edible parts of vegetables represents a direct pathway for their incorporation into the human food chain[4].

Heavy metals occur in the soil in soluble form and in the combined state[5]. However, only soluble, exchangeable and chelated metal species in the soils are the mobile and hence more available forms for plants[1]. The mobility of trace elements is a very important factor in determining the availability and solubility of heavy metals in the soil and soil solution. The mobility of trace elements is determined by their specification in the soil and this speciation is in turn dependent on parameters such as organic matter and mineralogical composition, pH and Eh of the soil[6]. Mobility of heavy metals is also related to their immobilization in the solid soil[7].

Metals accumulation in plant depends on plant species, growth stages, types of soil and metals, soil conditions, weather and environment[8-10]. In soils, heavy metals may be found in one or more of the following forms namely a) dissolved (in soil solution), b) exchangeable (in organic and inorganic components), c) as structural components of the lattices of soil minerals and d) insoluble precipitates with other soil components. The first two forms are available to the plants while the other two are potentially available in the long term[7].

In order to assess the availability of metals in soil for plant absorption, the chemical forms must be determined. Sequential extraction procedures are useful to determine the geochemical partitioning of heavy metals in the soil[11-13]. The aim of this study was to determine the potential uptake of heavy metals by fruit vegetables and speciation of heavy metals from agricultural areas.

Corresponding Author: Zalifah Mohd. Kasim, Food Science Program, School of Chemical Sciences and Food Technology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia Tel: 603 89292991 2963 Fax: 603 89293232 E-mail: iffa@pkrisc.cc.ukm.my

MATERIALS AND METHODS

The study was carried out at two agricultural areas namely Sepang and Bangi, Selangor Darul Ehsan. Vegetables chosen for the study were long beans (*Vigna sinensis*) and chilies (*Capsicum annum*). Random sampling of vegetables and soil were done twice at three sampling sites for each study area and experiments were run replicated three times. Soil samples were taken at 1-30 cm depth.

Vegetable samples were washed with distilled water, carefully dried with tissue paper and weighed immediately after collection before being dried in the oven at 70°C for 24 h. Samples were weighed and ground in a mortar followed by wet digestion with HNO_3: $HCLO_4$ (2:1) in the conical flask for 2-3 h on a sand bath[14]. Some 10 mL of hydrochloric acid (HCl) was added to solute inorganic and oxides salt. Digested samples were filtered with 0.45 μm pore size cellulose nitrate membrane filter paper (Millipore) and made up to 100 mL with distilled water. Heavy metals concentration was determined by atomic absorption spectrometry (AAS) (Perkin Elmer model 1100B).

Soil samples were air dried in the laboratory environment before being ground with mortar and sieved to pass through 250 μm mash size. Heavy metals were extracted using sequential extraction which provided four different fractions namely: easily leachable and ion exchange fraction (EFLE), acid reducible fraction (AR), organic oxidation fraction (OO) and resistant fraction (RR)[11]. Ten gram of soil samples were added with 50 mL of 1.0 M NH_4CH_3OO (pH 7) to extract metals from easily leachable metals from the soil. Samples were shaken for 1½ h and centrifuged for ½ h at 3000 rpm before being filtered with 0.45 μm pore size filter paper (Milipore) and brought to 50 mL volume with distilled water. Samples were washed by adding 50 mL distilled water, followed by shaking and centrifuging in the same way for every fraction in the sequential extraction.

The metals from acid reducible fraction were extracted by adding 50 mL of 0.25 M $NH_2OH.HCl$ (pH 2). The organic oxidation fraction was produced by using 50 mL of 1.0 M NH_4CH_3OO after oxidation with 30% H_2O_2 in a water bath for 1-1 ½ h. The rest of the samples were digested using HNO_3 : $HClO_4$ on sand bath at 100°C until the samples became whitish in colour. Concentration of metals in the soil samples was determined by atomic absorption spectrometry (Perkin Elmer model 1100B). All analysis were replicated three times. Carbon organic analysis was determined by using method by Walkey and Black[15].

RESULTS AND DISCUSSION

The study showed that the concentration of metals in long beans (*Vigna sinesis*) and chilies (*Capsicum annum*) were in sequence for Sepang and Bangi areas were Zn>Pb>Cd for both vegetables (Fig. 1). The study of the metals in the soil from long bean and chili indicated that Pb and Cd levels were higher in Sepang from easily leachable and ion exchange fractions whereas Zn content showed the same concentration. In the acid reduction fraction, heavy metals content studied were higher in samples Bangi (Table 1).

The metals from easily leachable and ion exchangeable fractions represent metals that are mobile in the soil, including those metals that are leachable and potentially bioavailable to plants. The higher concentration of heavy metals in the available form corresponds directly to higher accumulation in the plant.

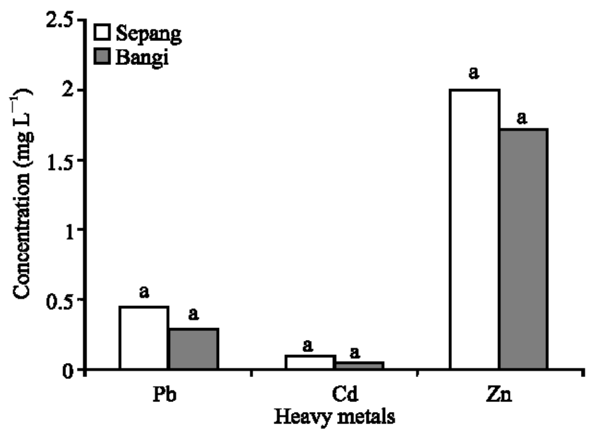

Fig. 1: Average concentration of heavy metals in long beans

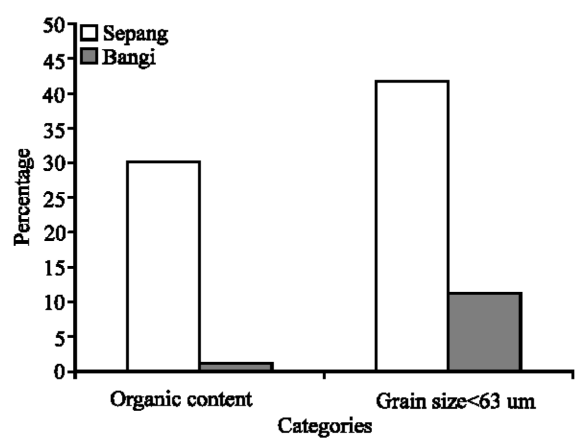

Fig. 2: Average organic content and grain size for long beans in sepang and bangi soils

Table 1: Average of heavy metals contents in the soil (mg kg^{-1}) (n=6)

	EFLE		AR		OO		RR	
	Sepang	Bangi	Sepang	Bangi	Sepang	Bangi	Sepang	Bangi
Long beans								
Pb	0.56^{a}_{D}	0.19^{b}_{D}	0.34^{b}_{D}	0.39^{ab}_{D}	4.17^{b}_{C}	6.16^{a}_{C}	10.97^{b}_{B}	15.63^{ab}_{A}
Cd	0.11^{a}_{DE}	0.03^{b}_{E}	0.04^{b}_{E}	0.12^{a}_{DE}	0.27^{b}_{BC}	0.22^{b}_{CD}	0.84^{a}_{A}	0.83^{a}_{A}
Zn	0.97^{a}_{EF}	0.95^{a}_{EF}	0.25^{b}_{F}	0.25^{b}_{F}	10.63^{c}_{B}	17.13^{a}_{A}	3.64^{b}_{DE}	7.61^{a}_{C}
Chili								
Pb	0.60^{a}_{D}	0.22^{b}_{D}	0.14^{c}_{D}	0.45^{a}_{D}	6.92^{a}_{C}	5.33^{ab}_{C}	16.33^{ab}_{A}	16.98^{a}_{A}
Cd	0.09^{a}_{DE}	0.03^{b}_{E}	0.03^{b}_{E}	0.12^{a}_{DE}	0.37^{a}_{B}	0.20^{b}_{CD}	0.73^{a}_{A}	0.83^{a}_{A}
Zn	0.73^{a}_{EF}	0.69^{a}_{EF}	0.15^{b}_{F}	4.37^{a}_{D}	12.57^{bc}_{B}	15.84^{ab}_{A}	3.62^{b}_{DE}	9.83^{a}_{BC}

Note: Different capital letters-refers to significant difference ($p<0.05$) among the different fraction for both location. Different small letters-refers to significant difference ($p<0.05$) between different location in the same fraction

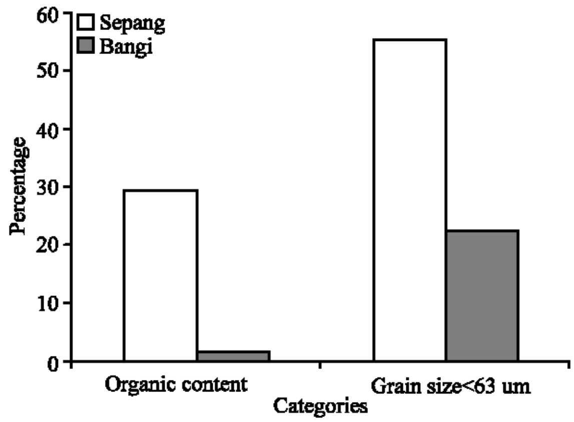

Fig. 3: Average organic content and grain size for chilies in sepang and bangi soil

Hence this fraction may indicate the potentially risky forms of metals. Metals extracted from the other fractions were strongly trapped in the soil and could not be taken up easily by plants. Maiz *et al.*[16] reported that Cd, Zn, Mn and Pb were loosely bonded and easily extracted from the soil in the easily leachable and ion exchange fraction.

Heavy metals in both long beans and chilies from the same area indicated the same sequence of metals content. This suggested that the uptake of metals by fruity vegetables from the same species were the same regardless of the area in which they which they were grown. This finding also agreed with Xian[17] that the metals concentrations in the cabbage studied were in the same order of metals as in the soil[17]. The uptake of metals by plants depended on the availability of metals from the soil and, the amount of metals in plants would reveal the availability of metals in soil to the plants.

Heavy metals levels in the plant are determined not only by the concentration in soils, but also by physicochemical properties of soil[18]. Heavy metals found in this study are generally at the same level or even lower than those reported for vegetables consumed in several countries and their levels in species grown in other agricultural and industrial areas are also still bellow the level allowed by the Malaysian Food Act[19].

Copper is one of the micronutrients required by plants for metabolism[20,21]. Lead and cadmium are toxic metals that have no clear function in plant metabolism[22]. Lead was reported to interfere with the electron flow at the water splitting site in photosynthesis in tomato, spinach[23] and *Nostoc muscorum*[24]. A large proportion of cadmium was known to accumulate in root tissues, especially when plants were grown on contaminated soil[25].

The uptake of metals were reciprocal to levels in the soil[26]. Cadmium bioaccumulation and distribution within plants were strongly affected by both soil type and plant cultivars/line[27]. The Cd concentration was highest in grain/seed of durum wheat (*Triticum turgidum* var *durum* L.) The Cd accumulation in their study produced the distribution pattern that corresponded to the metal-organic complex bound Cd found in the soil. Plants grown in the Zn and Cu contaminated soils also accumulated a great proportion of metals in their roots.

The results of this study also showed that the content of Pb and Zn was high in organic oxidation fraction in the soil taken from the long bean farm in Bangi. Lead and cadmium from the Sepang soils and Zn from the soils of the chilies farm in Bangi were extracted in large amounts from the organic oxidation fraction. Pb and Zn from soils of the long bean farm in Bangi were higher than those taken from Sepang, while Cd content was discovered to be the same for both areas. The concentrations of all metals in the chilies farms were found to be similar in both study areas except for Zn, which was higher in Bangi (Table 1). The percentage of organic content and grain size in the Sepang soil was seen to be higher while soil pH was higher in the Bangi site (Fig. 2 and 3).

Cadmium was highest in exchangeable phase (51%)[16]. Zn was mainly associated with oxide fraction (54%) and followed by exchangeable fraction (13%). Pb was mainly in the fraction bound to Fe-Mn oxides from acid reduction fraction. Many previous researchers have reported the association of Mn and Pb to Fe-Mn oxides.

Study of heavy metals from Zn smelting process found that a high percentage of Zn (43%) and Pb (46%) were bounded to organic matter[17,28]. Heavy metals tend to form complexes with organic matter in the soil (humic and fulvic acids) and affinity of each metal towards organic matter is different. Organic matter plays an important role not only in forming complexes, but also in retaining heavy metals in an easily exchangeable form. These two properties affect each heavy metal differently[7]. According to Jardao[29] the degree of binding of metals with organic matter was seen as follows Cu>Zn>Pb> Cd.

The role of Fe, Al and Mn oxides and hydroxides in heavy metals is well established, their ability to do this being inversely proportional to their degree of crystallization[30]. Studies done by previous researchers indicated that heavy metals in the other forms such as Fe-Mn oxides and organic compounds were very low in solubility and high stability for biological activity would not have direct bearings on their uptake by plants[17,31]. Metals trapped in the silicate clay minerals were extracted in the resistant fraction. Metals in this fraction were not easily available to plants[11].

The result of this study also indicated that potential bioavailability of heavy metals in the soil was strongly influenced by chemical forms of metals in the soil such as whether it is chelated with organic matters, oxides and hydroxides form and trapped in the silicate clay.

The heavy metals contents in long beans (*Vigna sinensis*) and chilies (*Capsicum annum*) studied were generally low and still below the maximum level allowed by the Malaysian Food Act[19] for Pb (2.00 mg kg^{-1}), Cd (1.00 mg kg^{-1}) and Zn (30.00 mg kg^{-1}). The exchangeable form of metals studied were very low indicating that under the present conditions, the availability of these metals to plant would be minimal. Most of the metals were found in the resistant fraction.

ACKNOWLEDGMENTS

The authors show great appreciation to Universiti Kebangsaan Malaysia for funding ST/8/2001 grant to ensure the success of this research and to Encik Awangku Jalaludin Awang Besar for the help in sample collection and metal analysis.

REFERENCES

1. Arambarri, R., R. Garcia and E. Millan, 1999. Relationships between metal contents in soil and grass contaminated by different anthropogenic sources. Toxicol. Environ. Chem., 72: 221-231.
2. Cobb, G.P., K. Sands, M. Waters, B.G. Wixson and E. Dorward-King, 2000. Accumulation of heavy metals by vegetables grown in mine wastes. Environ. Toxic. Chem., 19: 600-607.
3. Anonymous, 1999. Malaysian Dietary Guidelines. National Coordinating Committee on Food and Nutrition, Ministry of Health, Kuala Lumpur, Malaysia.
4. Florijin, P.J., 1993. Differential distribution of cadmium in lettuce (*Lactucasative* L.) and maize (*Zea mays* L.). Den Haag: Cip-Gegevens Koninklijke Bibliotheek.
5. Bloemen, M.L., B. Markert and H. Leith, 1995. The distribution of Cd, Cu, Pb and Zn in top soils of Osnabruk in relation to land use. Sci. Total Environ., 166: 137-148.
6. Boisson, I., M. Mench, J. Vangronsveld, P. Ruttens and T. De Koe, 1999. Immobilization of trace metals and arsenic by different soil additives; evaluation by means of chemical extraction. Commun. Soil Sci. Plant Anal., 30: 365-387.
7. Zalidis., G., N. Barbayirinis and M.T. Matsi, 1999. Forms and distribution of heavy metals in soils of the Axios Delta of Northen Greece. Commun. Soil. Sci. Plant Anal., 30: 817-827.
8. Chang, A.C., A.L. Page, J.E. Warneke and E. Grgurevic, 1984. Sequential extraction of soil heavy metals following a sludge application. J. Environ. Qual., 1: 33-38.
9. Domergue, F.L. and J.C. Vedy, 1992. Mobility of heavy metals in soil profiles. Intern. Environ. Chem., 46: 13-23.
10. Petruzzelli, G., 1989. Recycling wastes in agriculture: heavy metals bioavailability. Agric. Ecosyst. Environ., 27: 493-503.
11. Badri, M.A., 1984. A study of comparative behavior of selected heavy metals in polluted and unpolluted estuarine and coastal sediments. Ph.D Thesis, University of Lancaster.
12. Howard, J.L. and S. Jianing, 1996. Sequential extraction analysis of heavy metals using a chelating agent (NTA) to counteract resorption. Environ. Pollut., 91: 89-96.
13. Tessier, A., P.C.G. Camphell and M. Bisson, 1979. Sequential extraction procedure for the speciation of trace metals. Analyt. Chem., 51: 844-851.
14. AOAC., 1984. Official Method of Analysis 14th Edn. Sidney Williams. Association of Official Chemists, Inc. Virginia, USA.
15. Walkey, A. and I.A. Black, 1934. An examination of the Degtjareff method of determining soil organic matter and a proposal modification of the chromic acid titration method. Soil Sci., 37: 1.

16. Maiz, I., M.V. Esnaola and E. Millan, 1997. Evaluation of heavy metal availability in contaminated soils by short sequential extraction procedure. Sci. Total Environ., 206: 107-115.
17. Xian, X., 1989. Effects of chemical forms of cadmium, zinc and lead in polluted soils on their uptake by cabbage plants. J. Plant Soil., 113: 257-264.
18. Page, A.L., F.T. Bingham and A.C. Chang, 1981. Cadmium. In Effects of Trace Metals on Plants Function. London : Lepp. Applied Sci. Pub. London.
19. Malaysian Food Act, 1983. Food Act 1983 (Act 281) and Regulations. Malaysia: The Commissioner of Law Revision.
20. Hopkins, W.G., 1999. Introduction to Plant Physiology. 2nd Edn. New York, John Wiley and Sons.
21. Berg, L.R., 1977. Introductory Botany: Plants, People and the Environment. New York, Saunders College Publishing.
22. Michalak, E. and M. Wierzbicka, 1988. Differences in lead tolerance between *Allium cepa* plants developing from seeds and bulbs. J. Plant Soil., 199: 251-260.
23. Prasad, S.M., J.B. Singh, L.C. Rai and H.D. Kumar, 1991. Metal-induced inhibition of photosynthesis electron transport chain of cyanobacterium *Nostoc muscorum*. FEMS Microbiol. Lett., 82: 95-100.
24. Rai, L.C., B. Tyagi and N. Malick, 1996. Alternation in photosynthetic characteristics of *Anabaena doliolum* following exposure to UVB and Pb. Photochem. Photobiol., 64: 658-663.
25. Kabata-Pendias, A. and H.K. Pendias, 1992. Trace Elements in Soils and Plants. 2nd Edn. Ann arbor: CRC Press.
26. McGrath, S.P., F.J. Zhao, S.J. Dunham, A.R. Crosland and K. Coleman, 2000. Long-term changes in the extractability and bioavailability of zinc and cadmium after sludge application. J. Environ. Qual., 29: 875-883.
27. Cieslinki, G., K.C.J. Van Rees, P.M. Huang, L.M. Kozak, H.P.W. Rostad and D.R. Knott, 1996. Cadmium uptake and bioaccumulation in selected cultivars of durum wheat and flax as affected by soil type. J. Plant Soil, 182: 115-124.
28. Xian, X. and G. Shokohifard, 1989. Effects of pH on chemical forms and plant availablity of cadmium, zinc and lead in polluted soils. Water, Air Soil Pollut., 47: 265-273.
29. Jardao, C.P. and G. Nickless, 1989. Chemical associations of Zn, Cd, Pb and Cu in soils and sediments determined by the sequential extraction technique. Environ. Technol. Lett., 10: 743-752.
30. Chao, T.T., 1972. Selective dissolution of manganese oxides from soils and sediments with acidified hydroxylamine hydrochloride. Soil Sci. Soc. Am. Proc., 36: 764-768.
31. Tuin, B.J.W., 1989. Extraction of heavy metals from contaminated clay soils. Ph.D Thesis, University of Technology. Netherlands.