CHAPTER SIX

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PLANT UPTAKE OF HEAVY METALS

6.1 Introduction

Heavy metals provided by soils can be absorbed into the cells of the plant root, and then translocated into various other tissues of the plant by the similar mechanisms that allow plant required nutrients to be uptaken. The availability of heavy metals in soil is usually dependent on lithogenic and pedogenic factors (Narwal et al., 1999). The ability of plants to sustain high levels of heavy metals varied among plant species. Lettuce (*Lactuce sativa)* is among the leafy vegetables that have been extensively used as bio-indicators in soil pollution monitoring studies, due to their ability to adsorb and tolerate heavy metals (Kastori 1992 and Kulli et al., 1999). This chapter presents and discusses the results of greenhouse experiments conducted to study Cd, Pb, Ni and Zn uptake by lettuce grown on metals-amended soils as detailed in 3.5.

6.2 Cadmium

Cadmium content of lettuce grown on soils incubated with four Cd amendment levels (0,5,10 and 20-mmol/kg soils) for 3, 6, 9, 12 and 15 months was estimated at the end of each growing season through acid digestion followed by analyses by Inductively Coupled Plasma Mass Spectrometer (ICP-MS).

6.2.1 The effect of Cd amendment level on the plant uptake

In the first growing season the plant Cd concentrations increased significantly in proportion to the increasing level of Cd amendment with mean values ranging from 0.005 to 1.065 μg/g dry weight (DW) as the Cd amendment level increases from 0 to 20 mmol/kg. (Table 6.1).

Table 6.1: Cadmium concentration in lettuce (μg/g) as influence by amendment level and residence time

Cadmium level (mmol/kg soil	Incubation time (months)					Contrasts
	3	6	9	12	15	
0	0.005	0.004	0.005	0.006	0.006	n.s
5	03.170	3.183	03.19	03.23	03.248	*
10	12.376	12.399	12.48	12.54	12.627	*
20	21.065	21.206	21.27	21.73	22.123	*
Contrasts	***	***	***	***	***	

*n.s= Non significant, *= Significant at 5%, *** Highly Significant at 5%,*

The trend of the plant Cd concentration in the other successive harvestings is similar to the first one The results are comparable to that obtained by Gray et al., (1999) and Jinadasa et al., (1997) who recorded a range from 0.273 to 4.495 μg/g DW Cd, 0.11 to 6.37 mg/kg uptake by lettuce grown in high-level cadmium amended soils respectively. The results are also in agreement with the findings of Seaker, (1991); Sterrett et al., (1996); Misra et al., (1994) and Logan et al., (1997), who reported an increasing uptake of cadmium by lettuce grown in soils treated with sewage sludge containing high concentrations of cadmium.

6.2.2 The effect of residence time on the Cd plant uptake

The effect of residence time on plant Cd concentration was also investigated. However plant Cd concentrations slightly increased in proportion to the incubation time with mean values ranging from 0.005 to 2.123 μg/g DW. The greatest Cd concentration (2.123 μg/g DW) was found in lettuce grown in soil incubated with 20 mmol/kg Cd for 15months. Simple regression analysis indicated that the relative uptake of Cd by lettuce grown in the control soils showed a non significant relationship with the incubation time, while the plant uptake is positively correlated with the length of incubation time (R^2 = 0.974, 0.959 and 0.891) when the amendment level increases 5,10 and 20mmol/kg respectively.

Plants grown in soils received 10 mmol/kg recorded 0.376 μg/g in the first harvest with regular significant increase (p=0.05) varied from 6.1%, 21.8%, 43.1% and 66.8% as the incubation time increases from 6, 9, 12 and 15 month respectively. The highest percentage of increase was reported in lettuce grown in the 20mmol/kg amended soils for three month which is 1.065 μg/g followed by 13.2%, 16.1%, 62.2% and 99.3 % increase with the increasing length of incubation from 6 to 15 month. The result is in agreement with the findings of Sloan et al., (1997); and Logan, (1997). In contrast with this result Canet et al., (1998) reported significant decrease of Cd and levels in lettuce leaves after a seven years experiment. The significant enhancement in the Cd concentration in lettuce may be attributed to processes that govern the balance between accumulation and solubilization of Cd in soil that allow more Cd to be released in the soil solution through time (Ainsworth et al., 1994; Schultz et al., 1987; McLaren et al., 1986).

6.2.3 Regression models for Cd bioavailability as related to total content and chemical species of Cd in the soil

Simple regression analysis of the cadmium data revealed high positive correlations between plant concentration and total soil Cd (R^2=0.928) (Figure 6.1). Similarly positive correlation was found between plant uptake and the percentage of exchangeable Cd (R^2=0.879). The plant uptake of Cd decreases as the percentages of complexed and acid soluble Cd increases (R^2=0.885 and 0.773) respectively.

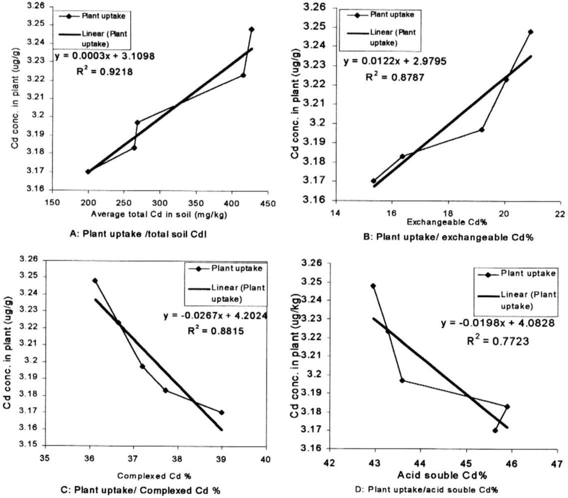

Figure 6.1: Regression models for Cd bioavailability as related to total soil Cd and % of chemical species of Cd

The second part of the result is supported by the fact that the co-precipitated (acid soluble fraction) is not an important immediate source of plant available metals because the equilibrium between this pool and the exchangeable pool is normally established only very slowly (Viets, 1962). In agreement with the first part of the result Jinadasa et al., (1997) reported a significantly positive correlation between Cd levels in vegetables and soils in Australia. Roca and Pomares (1991) reported significant positive correlation between the soil exchangeable Cd with metal content in lettuce grown in sewage sludge amended soils. Further agreements with these results is found in the findings of Sloan et al., (1997); Miner et al., (1997); Lorenz et al., (1997; Singh et al., (1995) and Logan et al., (1997). In contrast with this, Arnesen and Singh (1998) reported insignificant relationship between plant uptake and the exchangeable Cd.

6.3 Lead

Table 6.2 shows the concentrations of lead in lettuce grown on soils that received four levels of lead amendment (0, 5,10 and 120 mmol/kg soil) for 3, 6, 9, 12 and 15 months. At the end of each growing season lead concentration in plants was determined through acid digestion followed by analyses by ICP-MS.

6.3.1 The effect of Pb amendment level on the plant uptake

In the five harvests Pb concentration is greatly increased in proportion to the increasing level of Pb amendment with mean values ranging from 0.350, 0.352, 0.351, 0.324 and 0.324 to 96.450, 97.354, 97.548, 97.897 and 97.988μg/g DW as the Pb amendment level increases from 0 to 20 mmol/kg.

Table 6.2: Lead concentration in lettuce (µg/g) as influence by amendment level and residence time

Lead level (mmol/kg soil	Incubation time (months)					Contrasts
	3	6	9	12	15	
0	0.35	0.35	0.32	0.35	0.32	n.s
5	33.26	33.32	33.82	34.12	34.54	*
10	48.80	50.23	50.68	50.97	51.49	*
20	96.45	97.35	97.55	97.89	97.99	*
Contrasts	***	***	***	***	**	

*n.s= Non significant, *= Significant at 5%, *** Highly Significant at 5%,*

In agreement to this result Ylaranta (1995); Preer et al., (1995;); Nogales et al., (1997) and Rooney *et al*., (1999) reported positive correlation for Pb concentration in plants grown in soil with high content of lead. Further support for this result was found in the findings of McLean et al., (1987); Seaker, (1991); Bierman and Rosen (1994); Misra et al., (1994); Sterrett et al., (1996) and Logan et al., (1997), who reported an increasing uptake of Pb by lettuce grown in soils treated with an increasing rate of sewage sludge containing high concentrations of lead.

6.3.2 The effect of residence time on the Pb plant uptake

Lead concentrations in lettuce grown in control treatment insignificantly varied among the different harvesting times, while the other treatments showed a continuous significant elevation, which also varied from incubation time to the other (Table 6.2) Lettuce grown in 5 mmol/kg treated soils recorded 33.26 µg/g after the shortest incubation time, while steady elevation in the plant Pb concentration was recorded as the incubation time increases ((R^2 = 0.964). In the 10 mmol/kg treatment Pb concentration in plants was 48.8 µg/g after three-month incubation followed by slight increase with the increasing length of incubation from 6, 9, 12 and 15 months the recorded elevation percentages are 2.9%, 3.9%, 4.4% and 5.3% respectively in a linear model (R^2 = 0.892). Plants grown in soils that received the highest level of Pb amendment

(20mmol/kg) recorded the highest lead uptake (96.45 μg/g) after three months incubation then after, a 0.9%, 1.1%, 1.5 % and 1.6% increase was reported with 6, 9, 12 and 15 month incubation time respectively in a linear regression model (R^2= 0.868). Positive correlations between the uptake of Pb and the residence time were also proved by Logan et al., (1997) and Ylaranta, (1996) for lettuce grown in lead polluted soils for three years.

6.3.3 Regression models for Pb bioavailability as related to chemical species of Pb in the soil

Linear regression analysis of the lead concentration in plants showed a positive correlation between plant concentration of Pb and total soil Pb (R^2 =0.860). High positive correlation was found between plant uptake and the percentage of exchangeable Pb (R^2= 0.966) Figure 6.2. Plant uptake of Pb negatively correlated to the percentage of complexed Pb (R^2= 0.6484). On the other there is no significant correlation between acid soluble Pb and plant concentration (R^2= 0.6085), which may be attributed to the Pb being strongly absorbed soil particles, and is therefore largely unavailable to plants (Rooney et al., 1999). Comparing lettuce uptake to soil content of Pb Solan et al., (1997) have reported significant R^2 for Pb concentration in the lettuce leaves as a function of total metal concentration. Results are also in agreement with the findings of Sterrett et al., (1996) and Rooney et al., 1999).

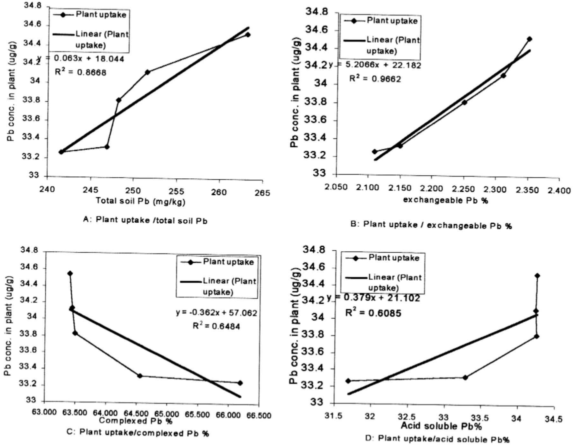

Figure 6.2: Regression models for Pb bioavailability as related to total soil Pb and the chemical forms of Pb

6.4 Nickel

Table 6.3 presents the average concentrations of nickel in lettuce grown on soils incubated with four levels of nickel (0, 5,10 and 120 mmol/kg soil) for 3, 6, 9, 12 and 15 months as determined by ICP-MS.

6.4.1 The effect of Ni amendment level on the plant uptake

As expected, in each of the five growing seasons the plant Ni concentrations significantly increases in proportion to the increasing level of Ni amendment with mean values ranging from 0.015,to 90.869µg/g DW (Table 6.3).

Table 6.3: Nickel concentration in lettuce (µg/g) as influence by amendment level and residence time

Nickel level (mmol/kg soil	Incubation time (months)					Contrasts
	3	6	9	12	15	
0	0.02	0.02	0.02	0.02	0.02	n.s
5	53.320	54.365	54.968	55.365	56.254	*
10	62.820	63.324	63.865	64.346	66.364	*
20	88.350	88.897	90.325	90.568	90.869	n.s
Contrasts	***	***	***	***	***	

*n.s= Non significant, *= Significant at 5%, *** Highly Significant at 5%,*

The significant increase in plant uptake of Ni is due to with the mobility of Ni is in the soils and can easily translocated up to other plant parts. Nogales et al., (1997) reported high levels of Ni in plants grown in soils that received higher rates of nickel nitrate amendment. Further agreements with this result were found in the findings of McLean et al.,. (1987); Roca and Pomares (1991); Chaney et al., (1994); Sloan et al., (1997); Zdenek, (1996); Moreno et al., (1997); and Logan et al., (1997) in which they reported increasing uptakes of Ni by lettuce grown in soils treated with increasing rates of sewage sludge containing high nickel concentrations.

6.4.2 The effect of residence time on the Ni plant uptake

The relative uptake of Ni by lettuce was similar in plants grown in the control soils over the 15 months incubation, while the plant uptake of Ni is positively correlated with the length of incubation time when the amendment level increases from 5 to 10 mmo/kg soil. Although the residence time imposes non-significant effect on the Ni concentration in lettuce grown in soil incubated with 20 mmol/kg, the greatest concentration of plant Ni (90.87 μg/g DW) was found after 15months (Table 6.5). Plants grown in soils received 5mmol Ni/kg soil recorded varied Ni concentrations started by 53.32 μg/g, in the 3 months harvesting followed by 2.0 %, 3.1 %, 5.5 % and 5.4 % increase as the incubation time was 6, 9, 12 and 15 month respectively. The 10 mmol/kg treated soils recorded 62.82 μg/g with regular increase which recorded as 0.8%, 1.7% 5.6 and 5.6% as the incubation time increases from 6, 9, 12 and 15 month respectively. The percentages of increase reported for Ni concentration in lettuce grown in the 20mmol/kg amended soils are 0.6 %, 2.2 %, 2.9 % and 2.8 % as length of incubation increases from 6 to 15 month. Similar to these results significant increase in Ni uptake by lettuce was observed in soils treated with sewage sludge containing high nickel concentrations over long residence time (Logan *et al*., 1997; Tu and Tu, 1996; Brallier *et al*., 1996 and Chaney *et al*., 1998). On the other hand Singh *et al*., (1995) reported decreasing uptake of Ni after three years of application

6.4.3 Regression models for Ni bioavailability as related to total content and chemical species of Ni in the soil

Figure 6.3 presents the simple regression analysis of the nickel concentration in plant, the total soil Ni and Ni speciation data revealed a high positive correlation between plant uptake and total soil (R^2= 0.944). Similarly a significant positive correlation was found between plant concentration and the exchangeable Ni % (R^2 =0.919 respectively). The exchangeable fraction as reported by Tu and Tu (1996) and Narwal et al., (1999), is the most significant indicator for harmful effect of Ni in soils. Significant negative correlations were found between plant uptake and the percentages of complexed and acid soluble Ni (R^2= 0.989 and 0.7645) respectively. The results are supported by the facts that the, exchangeable and the complexed pools are in a reversible equilibrium with one another (Viets, 1962) and the co-precipitated (acid soluble fraction) is not an important immediate source of plant available metals (Soon and Bates (1982). The result is also in agreement with the findings of Arnesen and Singh (1998).

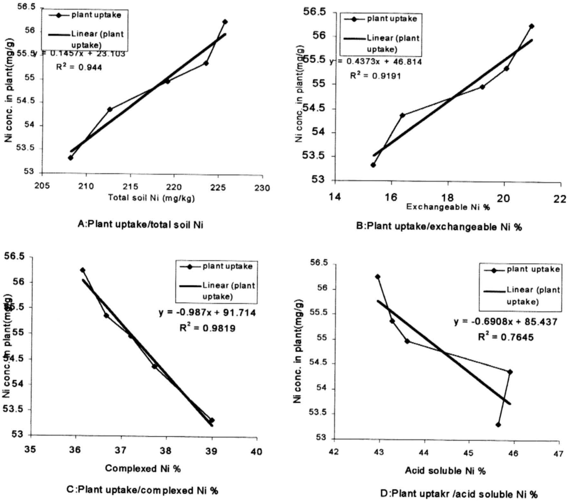

Figure 6.3: Regression models for Ni bioavailability as related to total soil Ni and the chemical forms

6.5 Zinc

Lettuce grown on soils received four levels of zinc (0, 5,10 and 120 mmol/kg soil) for incubation time of 3, 6, 9, 12 and 15 months was analyzed for total Zn concentration (Table 6.4)

6.5.1 The effect of Zn amendment level on the plant uptake

Similar to pervious metals Zn concentrations in lettuce significantly increases in proportion to the increasing level of Zn amendment in the five growing seasons with mean values ranging from 30.230 to 117.234μg/g DW (Table 6.4).

Table 6.4 Zinc concentration in lettuce (μg/g) as influence by amendment level and residence time

Zinc level (mmol/kg soil	Incubation time (months)					Contrasts
	3	6	9	12	15	
0	30.230	30.210	30.230	30.220	30.220	n.s
5	64.280	64.895	65.895	66.231	67.534	*
10	82.570	83.215	83.879	84.953	84.998	*
20	112.320	112.867	114.231	114.978	117.234	*
Contrasts	***	***	***	***	***	

*n.s= Non significant, *= Significant at 5%, *** Highly Significant at 5%,*

The significant increase in Zn levels in plants can be attributed to Zn being very mobile and even small concentrations in the soils can easily be taken up by plant roots and readily translocated to the upper part of the plant (Srivastava et al., 1993; Robson, 1993 and Lorenz et al., 1997). In agreement with the results Herawati et al., (1998) reported increasing levels of Zn in plants grown in polluted soils in Japan and Indonesia.

Further support was found in the findings of McLean *et al.*, (1987); Seaker, E.M, (1991); Moreno *et al.*, (1997); Logan *et al.*, (1997); Merrington *et al.*, (1997); and Ylaranta (1996), who reported increasing Zn uptakes levels by lettuce grown in sewage sludge soils treated with increasing rates of containing high nickel concentrations.

6.5.2 The effect of residence time on the Zn plant uptake

Zinc concentrations in lettuce grown in control treatment vary very little between the different harvesting times, while the other treatments showed a continuous slight elevation, which also varied among the incubation periods. Lettuce grown in 5 mmol/kg treated soils recorded 30.230 µg/g after the shortest incubation time (3 months), while steady elevation of Zn concentration in the plant were recorded as 1.0%, 2.5%, 3.0% and 5.1% as the incubation time increases from 6, 9, 12 and 15 months respectively.

In the 10 mmol/kg treatment, Zn concentration in lettuce was 64.280 µg/g after three-months incubation followed, by slight increase with the increasing length of incubation time from 6 to 15 months. The recorded elevation percentages are 0.8%, 1.6 %, 2.9% and 2.9% respectively. Plants grown in soils that received the highest level of Zn amendment (20 mmol/kg) recorded the highest Zn uptake (112.32 µg/g) after three months incubation then after, a 0.5%, 1.7%, 2.4 %and 4.4 % increase was reported with 6, 9, 12 and 15 month incubation time respectively (R^2= 0.847). Significant increase of plant uptake of Zn was also reported in the findings of Sloan *et al.*, (1997); Logan, (1997) Canet *et al.*, (1998); Smit *et al.*, (1998) and Merrington and Madden (2000). In contrast with the result Singh *et al.*, (1995) reported decreasing uptake of Zn after three years residence time.

6.5.3 Regression models for Zn bioavailability as related to chemical forms of Zn in the soil

Simple regression analysis of the zinc data showed that high positive correlations between plant concentration of Zn and total soil, percentages of exchangeable and complexed Zn in the soil (R^2=0.936, 0.924 and 0.883) respectively (. The positive correlation of the bioavaiable Zn and the complexed fraction is supported by the fact established by many researchers stating that, due to it's large size, the complexed metal pool is the most significant source for plant uptake of metal cations (Soon and Bates 1982 and Viets 1962).

A negative correlation was found between plant uptake and the acid soluble fraction of Zn (R^2= 0.876), which is supported by the fact that the co-precipitated form is not an important immediate source of plant available metals because the equilibrium between this fraction and the others established only very slowly. The positive correlation between the exchangeable Zn fraction and plant uptake is in agreement with the findings of Roca and Pomares (1991); Solan et al., (1997; Miner et al., (1997; Arnesen and Singh (1998); Murthy, (1982); Ma and Uren (1996); Singh et al., (1995) and Robert et al., (1995, who confirmed the significant importance of the exchangeable Zn pool in marinating the amount available for plant uptake.

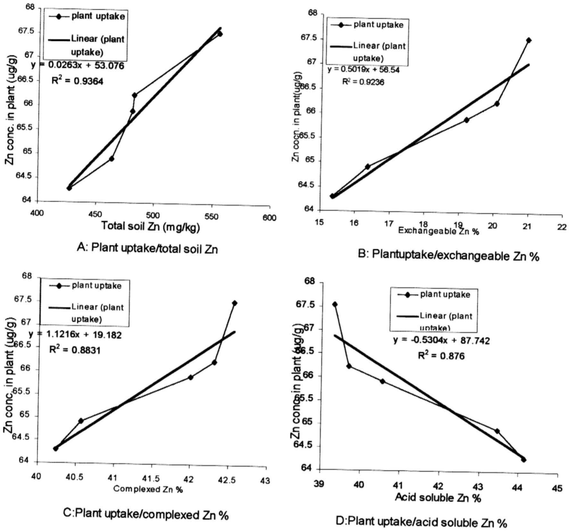

Figure 6.4: Regression models for Zn bioavailability as related to total soil Zn and the different chemical forms of Zn

6.6 Comparative investigations on the plant uptake of cadmium, lead, nickel and zinc

The variations in plant concentrations of Cd, Pb, Ni and Zn of lettuce grown on soils incubated with the metal amendment at four levels (0, 5, 10 and 20 mmol/kg) for 3 and 15 months are presented in figures 6.5 and 6.6. The plant uptake of Cd, Pb, Ni and Zn by lettuce showed a significant relationship with both level of amendment and residence time. The results show that after 3-month incubation the relative concentrations of four metals were significantly increased by higher application rates. The increase percentage is varied among the metals. The relative bioavailability of the applied heavy metals is in this order Zn > Ni> Pb>Cd. The result is in partial agreement with Sloan *et al.*, (1997) and Moreno *et al.*, (1997 who studied Long-term effects of heavy metal amendments and reported the metals bioavailability in this order Cd and Zn > Ni> Pb.

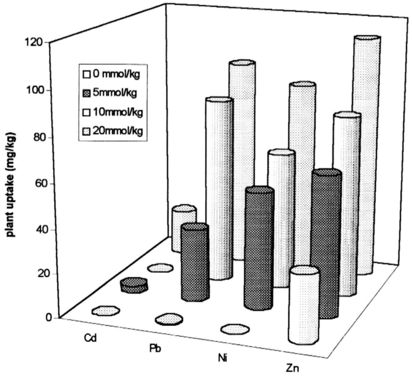

Figure 6.5: Plant uptake of heavy metals after 3-month incubation

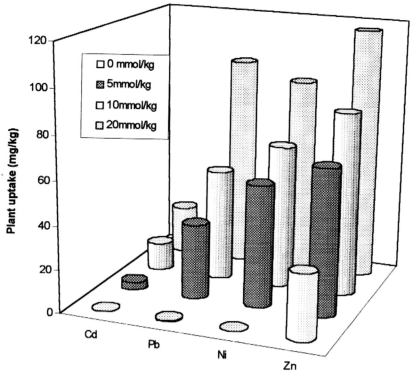

Figure 6.6: Plant uptake of heavy metals after 15-month incubation

Zinc, nickel, lead cadmium concentration in plants grown in 15-month incubated soils follow the same trend as in the previous growing seasons. The plant uptake of Cd, Pb, Ni and Zn by lettuce showed a significant relationship with both level of amendment and residence time. The results show that after 15-months incubation the relative concentrations of Zn and Pb were significantly increased by higher application rates. To a lesser extent the amendment level influenced the concentration of Ni and Cd in lettuce. The relative bioavailability of the applied heavy metals was Zn > Ni> Pb>Cd. The result is in a partial agreement with Roca and Pomares (1991) findings in which they reported the bioavailability of Zn > Ni> Cd > Pb. Sloan et al., (1997) and Moreno et al., (1997) who reported the metals bioavailability in this order Cd and Zn > Ni> Pb.

As in the findings reported by Phillips and Chapple (1995); Misra et al., (1994); Merrington and Alloway. (1994); Nemeth et al., (1993), among the metals studied, Zn and Ni showed the highest bioavailability index in terms of the metals concentrations in the harvested lettuce. Cd is least bioavaiable metal based on its concentration in plant tissues that could be due to the lower mobility of Cd. In this sense Adriano (1986) reported that Cd is generally immobile in polluted soil profiles. Also Chang et al., (1984); Dowdy et al., (1991) and Williams et al., (1987) observed slow migration of Cd in soils after sewage sludge application Nemeth *et al.*, (1993) reported that Ni is the most available metal, Zn and Cd were also desorbed more easily than Pb and therefore they are more bioavaiable to plants. Phillips and Chapple (1995) showed that the potential mobility and biological availability of the metals in soils is in this order Zn >Cd> Pb. It is generally believed that Pb have little mobility within plants so once it is taken up by plant it tend to accumulate in the roots while Cd, Zn and Ni are easily transported up to the edible parts (Kabata and Pendias 1992). The variations in plant concentrations of Cd, Pb, Ni and Zn of lettuce grown on soils amended with the lower and highest rates (5and 120 mmol/kg soil) for 3, 6, 9, 12 and 15 months are presented in figures 6.7 and 6.8.

The influence of residence time on the plant uptake is significantly varied among the studied metals. The correlation coefficients of Cd, Pb, Ni and Zn concentrations in lettuce grown in 5 mmol/kg amended soils and residence time are R^2 =0.974, 0.964, 0.657 and 0.654 respectively. R^2 for Cd, Pb, Ni and Zn concentrations in plants grown in 10 mmol/kg metal amended soils are 0.891, 0.868, 0.9137 and 0.947 respectively. Plant uptake and residence time correlations for Ni and Zn were low compared with literature values.

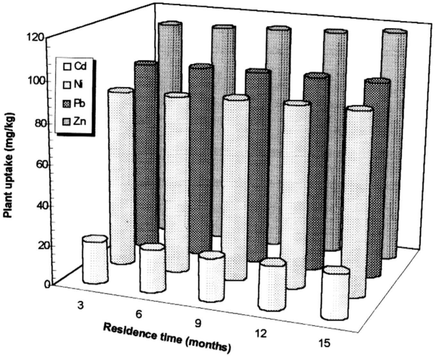

Figure 6.7: Plant uptake from 5 mmol/kg-amended soils as affected by residence time

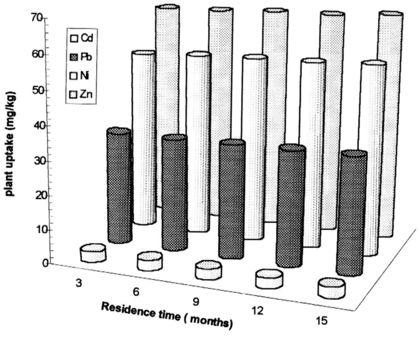

Figure 6.8: Plant uptake from 40 mmol/kg-amended soils as affected by residence time

The rest of the result is in agreement with Sloan *et al*., (1997), who concluded that the concentrations of Cd, Ni, and Zn in above ground lettuce tissue were significantly increased after by 15 years of biosolids applications. In contrast to this result Singh *et al*., (1995), reported that the lettuce uptake of Cd, Ni and Zn decreased with increasing the length of incubation time (3 years). Logan *et al*., (1997) reported that lettuce Zn concentrations increased significantly after the first 2 years of sludge application, while Ni and Pb levels were low compared to the Zn. Furthermore Logan *et al*., (1997) observed after 3 years experiment that the uptake of the metals by lettuce in the order: Zn > Ni > Cd > Pb. Brallier *et al*., (1996) stated that the relative plant uptake of Cd, Ni and were reduced after long-term experiments (16 years).