American-Eurasian J. Agric. & Environ. Sci., 6 (5): 526-531, 2009
ISSN 1818-6769

The Effect of Single and Mixed Treatments of Lead and Cadmium on Soil Bioavailability, Uptake and Yield of *Lactuca sativa* Irrigated with Sewage Effluent under Green House Conditions

D.Z. Moyo and C. Chimbira

Department of Biological Sciences, Midlands State University, P.Bag 9055, Gweru, Zimbabwe

Abstract: A greenhouse experiment was conducted to determine the effect of single Pb (100, 200 and 400 mg/kg soil) and Cd (3, 6 and 12mg/kg soil) and mixed treatments of Pb and Cd using treated wastewater from Pakamisa sewage treatment plant and soil from Golden Ridge Estate on soil bioavailability, *Lactuca sativa* uptake and yield. Results showed that treatment had significant effects on yield (>80% reduction) in Pb single treatments as well as soil bioavailable Pb and Cd in single treatments. A strong positive relationship (r=0.70) was observed between soil bioavailable Cd and its uptake by lettuce in single treatments whereas a strong negative relationship was observed between soil bioavailable Pb and lettuce yield (r=-0.8) in single treatments and between lettuce Pb uptake and Pb yield (r=-0.576). Findings of the study indicated that irrigation of Golden Ridge Estate with treated wastewater from Pakamisa sewage treatment plant is likely to have long term effects on soil bioavailable Pb and Cd as well as yield of grown vegetables and be a potential risk for consumers of these vegetables.

Key words: Lactuca sativa · Lead · Cadmium · Soil bioavailability

INTRODUCTION

Due to urbanisation and population growth, the problem of water shortage is becoming more serious especially in arid and semi-arid regions. In addition, the rapid increases in urbanisation and population result in the generation of more wastewater. With serious food shortages in developing countries the use of wastewater is becoming an attractive way of increasing food production. In Zimbabwe most cities are experiencing severe challenges in the provision of clean water for domestic use as well as proper sewage disposal.

In recent years peri-urban agriculture which provides food and employment to city dwellers in Zimbabwe has been hit by water shortages resulting in city authorities using sewage effluent to irrigate the crops. The advantage of the sewage effluent to peri-urban farmers is its high quantities of nutrients such as phosphorus and nitrates [1]. However, the wastewater is also contaminated with toxic heavy metals such as Lead and Cadmium [2]. Heavy metals pose a health risk to consumers of contaminated food products [3,4]. Golden Ridge Estate in the city of Gweru in Zimbabwe irrigates horticulture products such as *Lactuca sativa* (lettuce) using wastewater from domestic and industrial areas. The amounts of heavy metals in this water or their potential to accumulate in soil, their uptake by lettuce or effects on yield have not been investigated.

The aim of this study was to investigate the potential of *Lactuca sativa* irrigated with sewage effluent in the uptake of Lead and Cadmium using single and mixed metal treatments under greenhouse conditions.

MATERIALS AND METHODS

Golden Ridge Estate: Golden Ridge Estate is located 10 km north of the City of Gweru, Zimbabwe. The Estate is under irrigation with treated sewage effluent from Pakamisa Sewage Treatment Plant. Major crops cultivated are vegetables such as *Brassica napus* (rape) and *Lactuca sativa* that are sold to vendors in the City of Gweru, maize and wheat.

Pakamisa Sewage Treatment Plant: Pakamisa Sewage Treatment Plant processes both industrial and domestic effluent from the City of Gweru. The industries include a radiator clinic, panel beaters and spray painters, alloy processing plants, motor vehicle garages and battery

Corresponding Author: D.Z. Moyo, Department of Biological Sciences, Midlands State University, P.Bag 9055, Gweru, Zimbabwe

manufacturers. The plant uses the conventional wastewater biological trickling filtration system and ponds. The system produces low quality water that is used to irrigate crops at Golden Ridge Estate. This is considered to be a cheap and profitable way for secondary treatment of wasterwater that is unfit to be discharged directly into natural watercourses.

Soil and Wastewater Characterization: Soil from Golden Ridge Estate was collected in 10 L buckets and transported to the laboratory for analysis and use in greenhouse experiments. The soil was tested for Pb and Cd concentrations according to the method of McGrath and Ceggara [5]. Soil texture was determined by the hydrometer method of Gee and Bauder [6]. Soil pH was determined using a 1:5 soil suspension of 0.01 M $CaCl_2$ after calibrating pH meter Sension 1 with pH buffers 7 and 10. Cationic exchange capacity (CEC) was determined by saturating the soil with 1M CH_3COONH_4 buffered at pH 5.2. Total organic carbon was determined using the method described by Houba *et al.* [7]. Treated wastewater from irrigation canals at Golden Ridge Estate coming from Pakamisa Sewage Treatment Plant was collected and analysed for Pb and Cd concentrations using the method of McGrath and Ceggara [5].

Green House Experiments: The experiments were carried out in a green house at Midlands State University from September to November 2007. Soil from Golden Ridge Estate and treated wastewater from Pakamisa Sewage Treatment Plant were used in the metal treatment experiments.

Single Metal Treatment: Five treatment levels each of Pb and Cd were used. The first level was a control without inorganic Pb and Cd and used drinking tap water from the university. The second level only received treated wastewater and no inorganic metals. The third level used the maximum permissible Pb concentration of 100 mg/kg of soil or maximum acceptable Cd of 3 mg/kg of soil [8]. The fourth level doubled the maximum acceptable concentration of the metals while the fifth level received four times the acceptable metal concentration in the soil. Each level was replicated three times.

Mixed Metal Treatments: Five treatment levels of Pb and Cd were used. The first and second levels were the same as those of single treatments. The third level had inorganic Pb and Cd mixed in proportions of 100 mg/kg Pb and 3 mg/kg Cd. The fourth level was a combination of 200 mg/kg Pb and 6 mg/kg Cd. The fifth level was a combination of 400 mg /kg Pb and 12 mg/kg Cd.

***L. Sativa* Growing:** In each 5 L soil packed pot (5 kg), three shallow holes were drilled and *Lactuca sativa* seeds were added and covered with a thin layer of soil. In order to eliminate nutrient deficiency compound D fertilizer (N8%, P14%, K7%) was added at a rate 700kg/hectare at planting. The soils in the pots were then enriched with lead nitrate $(Pb(NO_3)_2$ and $Cd(NO_3)_2.4H_2O$ the inorganic sources of Pb and Cd, respectively according to the relevant treatment level. Ammonium nitrate (N34.5%) fertilizer was added to all the pots irrespective of treatment level at a rate of 100kg/hectare at one month intervals [9]. After the vegetables were established the rest of the seedlings were thinned to remain five plants per pot that were almost the same size. Two litres of treated wastewater were applied per pot for levels 2 to 5 while level 1 received tap water three days per week. Plants were allowed to grow for a period of two and a half months.

Pots Soil Sampling and Testing: Soil samples were taken from pots to a depth of 20 cm using a soil auger after harvesting *L.* sativa. Plant debris was removed from the soil by sieving with a 2 mm sieve. Bioavailable Pb and Cd concentrations were determined using the procedure of McGrath and Ceggara [5].

Pot *L. Sativa* Sampling and Testing: Harvesting of *L. sativa* was made from each pot two and a half months after soil enrichment with inorganic salts of Pb and Cd. All above ground consumable leafy parts of *L. sativa* were harvested to constitute a sample per harvest. Leaves were washed with de-ionised water and oven dried at 65°C to a constant weight. Dried *L. sativa* was weighed to determine yield before analysis for Pb and Cd concentrations. The samples were ground, air dried and placed in conical flasks. They were wetted with water and 25 ml of nitric acid was added and gently heated to start the reaction and then cooled. Ten milliliters of perchloric acid was added to each sample and gently heated to concentrate it. If the mixture became too dark, 2-3 ml of nitric acid was added with continuous heating. After the mixture had turned yellow or colourless, decomposition was complete. The mixture was left to cool and 2 ml of hydrochloric acid was added. De-ionised water was used to prepare fixed volumes of measurement solution. Processed blank test solutions were prepared in the same way as the samples. Samples were analysed for Pb and Cd using atomic absorption spectrometry [10].

Data Analysis: One-sample t-tests were used to compare mean levels of Pb and Cd in the soils and treated sewage effluent to the standard permissible levels. Data on bioavailable metal concentration in soil, metal uptake by *L. sativa* and yield were tested for normality and then transformed using the formula Y= ln (X). ANOVA was used to test the significance of treatment on biovailable Pb and Cd, their uptake by *L. sativa* and yield. Regression analysis was done to determine the correlation between soil bioavailable metal concentration and *L. sativa* uptake and yield. All statistical analysis was performed using GENSTAT Version3.

RESULTS

Soil Characterization: Golden Ridge Estate soil had clay content of 78%, soil pH 5.52, organic carbon 1.7% and cationic exchange capacity of 39.8 $\text{mol}_c\text{kg}^{-1}$.

Pb and Cd Concentrations in Soil and Wastewater: Data on Pb and Cd concentrations in soil and wastewater are given in Table 1. Pb and Cd levels were below the maximum permissible limits.

Bioavailable Pb and Cd in Pot Soils, Metal Uptake and *L. Sativa* Yield: Data on soil bioavailable Pb and Cd, L sativa metal uptake and corresponding yield for single and mixed treatments are given in Tables 2 and 3. From level 3 to 4 soil bioavailable Pb and Cd were increased with increasing level of mixed treatments ($P>0.05$). In level 5 soil bioavailable Pb and Cd were decreased (Table 3). The same trend was observed in single treatments except in level 5 where soil bioavailable Cd was more than level 4 (Table 2). *L. sativa* accumulated higher amounts of Pb and Cd in Level 5 in mixed treatments and there was a corresponding reduction in yield. This reduction in yield was also observed in Level 5 Pb single treatments but not in Cd treatments. In levels 1 and 2 little Pb in soil was accumulated in higher proportion in plants than in level 3. The uptake of Pb and Cd by L. sativa in levels 1 and 2 was significantly higher than the recommended human maximum consumption limits (Pb 3.5 µg/kg and Cd 1 µg/kg [11] ($P<0.05$).

Treatment had significant effects on yield in Pb single treatments as well as on soil bioavailable Pb and Cd in single treatments (Table 3).

Table 1: Levels of Pb and Cd in Golden Ridge Estate soil and wastewater

Metal	Pb	Cd
Soil metal concentration (mg/kg)	0.83	ND
Recommended maximum level (mg/kg)*	100	3
Treated wastewater (mg/L)	1.29	ND
Recommended wastewater maximum level for irrigation (mg/L)**	5	0.01

*Source: WHO [11] ** Source: USEPA [12] ND – Not detectable

Table 2: Soil bioavailable Pb and Cd, vegetable metal uptake and corresponding yield by *L. sativa* (lettuce) single treatments

Treatment	Mean soil bioavailabe Metal (mg/kg)	Mean metal uptake (mg/kg)	Mean lettuce yield (g/pot)
Pb treatments			
Control	0.47^a	2.97^a	2.05^a
Wastewater	0.60^a	3.16^a	2.64^a
Pb 100	62.03^b	13.4^b	2.11^a
Pb 200	221.61^c	14.82^b	0.46^b
Pb 400	210.4^c	15.33^b	0.23^b
Cd treatments			
Control	0^a	0.02^a	2.05^a
Wastewater	0^a	0.04^a	2.64^a
Cd 3	0.37^b	0.263^b	2.99^a
Cd 6	1.56^c	2.61^c	2.54^a
Cd 12	1.76^c	1.88^c	2.03^a

Columns with different superscripts are significantly different ($P<0.05$)

Table 3: Soil bioavailable Pb and Cd, vegetable metal uptake and corresponding yield by *L. sativa* (lettuce) mixed treatments

Treatment	Mean soil bioavailabe Metal (mg/kg)	Mean metal uptake (mg/kg)	Mean lettuce yield (g/pot)
Pb treatments			
Control	0.47^{a}	2.97^{a}	2.05^{a}
Wastewater	0.60^{a}	3.16^{a}	2.64^{a}
Pb 100+Cd 3	102.93^{b}	9.73^{b}	2.06^{a}
Pb 200+ Cd 6	185.46^{b}	66.6^{c}	0.70^{a}
Pb 400+ Cd 12	67.09^{b}	265.97^{c}	1.82^{a}
Cd treatments			
Control	0^{a}	0.02^{a}	2.05^{a}
Wastewater	0^{a}	0.04^{a}	2.64^{a}
Cd 3 + Pb 100	0.20^{b}	0.016^{a}	2.06^{a}
Cd 6 + Pb 200	0.27^{b}	0.015^{a}	0.70^{a}
Cd 12 + Pb 400	0.09^{a}	0.46^{b}	1.82^{a}

Columns with different superscripts are significantly different ($P<0.05$)

Table 4: Analysis of variance results of effect of single and mixed treatments on soil bioavailability, uptake and yield of *L. sativa* using Pb and Cd inorganic salts/

	Single		Mixed	
Source of variation	F	p	F	p
Pb				
Yield	6.36	P<0.08	1.45	P>0.294
Uptake	0.08	P>0.792	1.22	P>0.360
Soil bioavailability	6.36	P<0.083	2.97	P>0.1
Cd				
Yield	0.02	P>0.897	1.16	P>0.342
Uptake	5.65	P<0.055	3.36	P>0.105
Soil bioavailability	5.43	P<0.045	1.81	P>0.243

Table 5: Regression coefficients of the relationship between soil bioavailabe metal and its uptake and lettuce yield

	B	(mg/kg) r^{2}(%)	N	Significance
Pb single treatments				
Soil bioavailability and uptake	0.080	4	7	NS
Soil bioavailability and yield	-0.882	64.3	7	P<0.05
Uptake and yield	-0.512	33.2	7	P<0.05
Pb mixed treatments				
Soil bioavailability and uptake	-0.623	42.5	7	P<0.05
Soil bioavailability and yield	0.0178	1.2	7	NS
Uptake and yield	-0.133	10.8	7	NS
Cd single treatments				
Soil bioavailability and uptake	0.990	48.5	7	P<0.05
Soil bioavailability and yield	-0.0279	10.1	7	NS
Uptake and yield	-0.115	6.7	7	NS
Cd mixed treatments				
Soil bioavailability and uptake	0.393	8.6	7	NS
Soil bioavailability and yield	-2.604	67.4	7	P<0/05
Uptake and yield	-1.381	34.5	7	P<0.05

Relationship Between Soil Bioavailable Pb and Cd and Lettuce Pb and Cd Uptake: There was a weak positive correlation between soil bioavailable Pb and its uptake by lettuce (r = 0.21) in single treatments. 4.5% of the variation in lettuce Pb uptake was associated with soil bioavailability of Pb ($r^2 = 0.045$). However, there was a strong positive correlation between soil bioavailable Cd and its uptake by lettuce (r = 0.70). 49.5% of the variation in lettuce Cd uptake was associated with Cd soil bioavailability ($r^2 = 0.495$). In mixed treatments there was a negative relationship between soil bioavailable Pb and its uptake by lettuce (r=-0.57) whereas a positive relationship was found in Cd experiments (r=0.29).

Relationship Between Soil Bioavailable Pb and Cd and Lettuce Yield: There was a strong negative correlation between soil bioavailable Pb and lettuce yield (r = -0.8) in single treatments. Soil bioavailable Pb accounted for 64% (r^2=0.64) of the variation in lettuce yield . The same trend was observed in soil bioavailable Cd versus lettuce yield in mixed treatments of Cd and Pb. However, a weak negative correlation was observed between soil bioavailable Cd and lettuce yield in single treatments. The same trend was observed for soil bioavailable Cd and lettuce yield in mixed treatments.

Relationship Between Lettuce Pb and Cd Uptake and Yield: A negative relationship was observed between lettuce Pb uptake and yield (r=-0.576) as well as between Cd uptake and yield (r=-0.258) in single treatments. The same trend was observed in mixed treatments.

The regression coefficients of the relationship between soil bioavailable metal and its uptake and lettuce yield are given in Table 5.

DISCUSSION

The accumulation of Cd by lettuce without reduction in yield is similar to the findings of Chen *et al.* [13] who reported that as much as 20 mg of Cd per kilogram of dry biomass was accumulated without much adverse effects on growth.

The increase in bioavailable Pb up to level 4 and a decrease in level 5 was due to a higher uptake of Pb by lettuce and this was particularly evident in mixed metal treatments (Table3). This decrease corresponded to an increased uptake of Pb and corroborates findings of Malgorzata and Hakan [14] who reported that the potential of lettuce to accumulate Pb and Cd is enhanced if the two elements appear in combination. This also corroborates findings of Carson and Bazzaz [15] who reported that uptake of Pb by plants increased as Cd concentrations were raised in the soil.

The observed reduction in yield in Pb single treatments could be attributed to toxicity of Pb to lettuce at concentrations of 200 mg/kg of soil and above in single treatments. This suggests that repeated irrigation of Golden Ridge Estate crops with effluent over a long period of time could result in low crop yield. However, in mixed treatments of Pb and Cd the reduction in yield was not significant. The absence of decline in yield in Cd single treatments (Table 2) could be attributed to the fact that Cd accumulates in roots than in the shoot and the reduction in dry weight is seen in the root but not the shoot [16] *L. sativa* irrigated with sewage effluent as well as the control (levels 1 and 2) took up Pb and Cd concentrations that are above the maximum permissible limits for human consumption. Therefore the soil in Golden Ridge Estate and the wastewater from Pakamisa sewage treatment plant are not fit for irrigating *L. sativas* for human consumption. Despite failure to detect Cd in soil, its uptake by *L. sativa* was high. Cd might be detected in future studies corroborating Tandi *et al.* [2] who reported that repeated application of sewage effluent resulted in accumulation of heavy metals in the soil to detectable levels.

REFERENCES

1. Nyamangara, J. and J. Muzezewa, 1999. The effect of long-term sewage sludge application on Zn, Cu, Ni and Pb levels in clay loam soil under pasture grass in Zimbabwe. Agriculture, Ecosystems and Environment, 73: 199-204.
2. Tandi, N.K., J. Nyamangara and C. Bangira, 2005. Environmental and potential effects of growing leafy vegetables on soil irrigated using sewage sludge and effluent: A case of Zn and Cu. J. Environmental Science and Health, 39: 461-471.
3. Goyer, R.A., 1990. Lead toxicity: from overt to sub-clinical to subtle health effects. Environmental Medicine, 74: 377-389.
4. Nordberg, G.F., 2004. Cadmium and health in the 21st century- historical remarks and trends for the future. Biometals, 17: 485-489.
5. McGrath, S.P. and J. Cegarra, 1992. Chemical extractability of heavy metals during and after long-term application of sewage sludge to soil. J. Soil Sci., 43: 313-321.

6. Gee, G.W. and J.W. Bauder, 1986. Particle size analysis. Methods of soil analysis. Ed. Klute, A. American Society of Agronomy, Madison W. I. USA.
7. Houba, V.J.G., J.J. Van dre Lee, I. Novozamsky and I. Waling, 1989. Soil and plant analysis. Part 5. Wagenigen Agricultural University. Netherlands, pp: 4-10.
8. Pescod, M.D., 1992. Wastewater treatment and use in agriculture. Food and Agricultural Organisation (FAO) Irrigation and drainage paper 47. Rome Italy: FAO.
9. Agritex, 1993. Farm Management Handbook Department of Agricultural Technical and Extension Services. Harare. Zimbabwe.
10. Shimadzu Cook Handbook, 2007. Atomic Absorption Spectrophotometry cookbook. Shimadzu Corporation. Kyoto. Japan.
11. World Health Organisation (WHO), 1993. Guidelines for drinking water quality. 2nd Ed. Vol 1. Recommendations. WHO. Geneva.
12. USEPA (United States Environmental Protection Agency), 1996. Soil Screening Guidance. Technical Background Document. EPA/540/R95/128.
13. Chen, W., L. Li, A. Chang, L. Wu, S. Kwon and R. Bottoms, 2009. Cadmium uptake by lettuce in fields treated with Cadmium-Spiked phosphorus fertilizers. Communications in Soil Science and Plant Analysis, 40(7-8): 1124-1137.
14. Malgorzata, M. and A. Hakan, 2001. Influence of lead and cadmium on growth, heavy metal uptake and nutrient concentration of three lettuce cultivars grown in hydroponics culture. Communications in Soil Science and Plant Analysis, 32: 571-583.
15. Carson, R.W. and F.A. Bazzaz, 1997. Growth of rye grass and fescue as affected by lead-cadmium - fertilizer interaction. J. Environmental Quality.
16. Selvam, A. and J.W.C. Wong, 2008. Phytochelation and synthesis and Cadmium uptake by Brassica napus. Environmental Technol., 29(7): 765-773.