

Full Length Research Paper

The effect of single and mixed treatments of lead and cadmium on soil bioavailability and yield of *Brassica napus* irrigated with sewage effluent: A potential human risk

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The effect of single and mixed treatments of Lead (Pb) and Cadmium (Cd) on soil bioavailability and uptake by *Brassica napus* was investigated in green house using soil from Golden Ridge Estate and wastewater from Pakamisa Sewage Treatment Plant, Gweru, Zimbabwe. Pb and Cd had significant effects on metal soil bioavailability and yield in single treatments. Soil bioavailable Pb in mixed treatments significantly correlated positively with uptake by *B. napus*. Yield was significantly correlated negatively with soil bioavailable Pb and Cd as well as metal uptake. Soil bioavailable Pb accounted for 49.2% (single treatment) and 6.1 % (mixed treatment) of the variation in *B. napus* yield. Pb uptake accounted for 4.63% (single treatments) and 88.77% (mixed treatments) of the variation in yield. Cd accounted for 0.38% (single treatment) and 7.98% (mixed treatments) of the variation in yield. Findings of this study indicate that *B. napus* grown in Golden Ridge Estate soil and irrigated with wastewater from Pakamisa Sewage Treatment Plant accumulates Pb and Cd to above recommended maximum limits for human consumption and is not fit for human consumption.

Key words: Lead, cadmium, soil bioavailability, *Brassica napus*.

INTRODUCTION

In Zimbabwe, urban authorities and water authorities are collaborating with peri-urban farmers to strengthen the role of peri-urban horticulture in wastewater recycling and creating sustainable food systems. A major challenge of peri-urban horticulture using treated wastewater for irrigation is the supply of safe products in this often polluted environment. Pollution by heavy metals presents a risk to the consumers as well as producers of these horticultural products (Tandi et al., 2005). Mapanda et al. (2005) indicated that vegetable gardens of the City of Harare, Zimbabwe, irrigated with wastewater cause significant heavy metal enrichment in soils. The City of Gweru started irrigating its croplands that includes vegetables at Golden Ridge Estate with wastewater in the early 1990s. The potential of Lead (Pb) and Cadmium (Cd) to accumulate in Golden Ridge Estate soils is not known. No studies have

been done to monitor heavy metals in wastewater and to quantify their uptake by crops at Golden Ridge Estate.

Pb and Cd are cumulative and indestructible toxins that can only be eliminated through excretion (Moolenaar and Lexmand, 1999). Pb and Cd accumulate in the body causing health problems that include damage to the nervous system, reduced intellectual capacity and hypertension (Staeson, 2002). Humans acquire these metals from contaminated water and food after the addition of Pb and Cd to agricultural soils and uptake by food and fodder crops (Johnson and Jones, 1995). Information on the uptake of Pb and Cd is therefore important in designing strategies for predicting uptake of the metals into the food chain.

In Zimbabwe, *Brassica napus* (rape) is the most common green leafy vegetable that is consumed in most households. It is relatively cheaper than other vegetables and is available from the vegetable markets throughout the year. The objective of this study was to investigate the effects of single and mixed Pb and Cd treatments on soil bioavailability and uptake by *B. napus*, grown using soil

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from Golden Ridge Estate and irrigated with wastewater from Pakamisa Sewage Treatment Plant.

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 *B. napus* (rape) that is sold to vendors in the City of Gweru, maize and wheat.

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 (1992). Soil texture was determined by the hydrometer method of Gee and Bauder, (1986). Soil pH was determined using a 1:5 soil suspension of 0.01 M CaCl₂ after calibrating pH meter Session 1 with pH buffers 7 and 10. Cationic exchange capacity (CEC) was determined by saturating the soil with 1M CH₃COONH₄ buffered at pH 5.2. Total organic carbon was determined using the method described by Houba et al. (1989). Treated waste water from irrigation canals at Golden Ridge Estate coming from Pakamisa Sewage Treatment Plant was collected and analyzed for Pb and Cd concentrations using the method of McGrath and Ceggara, (1992).

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 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.

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 (Pescod, 1992). 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.

B. napus growing

In each 5 L soil packed pot (5 kg), three shallow holes were drilled and *B. napus* seeds were added and covered with a thin layer of soil. In order to eliminate nutrient deficiency compound D fertilizer (N8%, P14%, and K7%) was added at a rate 700 kg/hectare on planting. The soils in the pots were then enriched with lead nitrate [Pb(NO₃)₂] and Cd(NO₃)₂.4H₂O 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 100 kg/hectare at one month intervals (Agritex, 1993). After the vegetables were established the rest of the seedlings were thinned to remain with 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 *B. napus*. 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 (1992).

Pot *B. napus* sampling and testing

Harvesting of *B. napus* 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 *B. napus* were harvested to constitute a sample per harvest. Leaves were washed with de-ionized water and oven dried at 65°C to a constant weight. Dried *B. napus* 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-ionized 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 analyzed for Pb and Cd using atomic absorption spectrometry (Shumadzu, 2007).

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 *B. napus* 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 bioavailable Pb and Cd, their uptake by *B. napus* and yield. Regression analysis was done to determine the correlation between soil bioavailable metal concentration and *B. napus* uptake and yield. All statistical analysis was performed using GENSTAT Version3.

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 (1993) ** Source: USEPA (1992) ND – Not detectable.

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 molkg⁻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 *B. napus* yield

Data on soil bioavailable Pb and Cd, *B. napus* metal uptake and corresponding yield for single and mixed treatments are given in Tables 2 and 3. From level 3 to 5 soil bioavailable Pb and Cd increased with increase in level in single treatments ($P > 0.05$). The same trend was observed in mixed treatments except in level 5 where soil bioavailable Pb and Cd was less than level 4 (Table 4). 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 *B. napus* 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 (WHO, 1993) ($P < 0.05$)]. Pb uptake by *B. napus* in level 4 and 5 was significantly lower than that in single treatments.

Treatment had significant effects on yield and soil bioavailable Pb and Cd in single treatments as well as soil bioavailable Pb in mixed treatments (Table 4).

Relationship between soil bioavailable Pb and Cd, and their uptake and yield by *B. napus*

The regression coefficients for the relationship between soil bioavailable Pb and Cd, their uptake and *B. napus* yield in single and mixed treatments are given in Tables 5

and 6. Particularly soil bioavailable Pb in mixed treatments was significantly correlated positively with uptake by *B. napus* than in single treatments. Weaker negative relations were found between *B. napus* Pb and Cd uptake and yield; soil bioavailable Pb and Cd and corresponding yield. Strong negative relations were only found between soil bioavailable Pb and *B. napus* yield ($r = -0.701$) and between *B. napus* Pb uptake and yield ($r = -0.942$). The regression models were linear and the residuals were normally distributed. Soil bioavailable Pb accounted for 49.2% of the variation in yield in single treatments (Table 5) whereas Pb uptake accounted for 88.7% of the variation in yield in mixed treatments (Table 6).

DISCUSSION

B. napus irrigated with sewage effluent as well as the control 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 *B. napus* for human consumption. Despite failure to detect Cd in soil, its uptake by *B. napus* was high. Cd might be detected in future studies corroborating Tandi et al. (2005) who reported that repeated application of sewage effluent resulted in accumulation of heavy metals in the soil to detectable levels.

Selvam and Wong (2008) found that Cd tends to accumulate higher concentrations in the roots than the shoot of *B. napus*. It is possible that in our experiments higher Cd levels were accumulated in the roots of *B. napus* than in the shoot. Our study did not determine Cd levels in roots of *B. napus*.

Soil bioavailability data indicated that Golden Ridge Estate soils have the potential to accumulate high levels of Pb and Cd over a long period of time. The increase in soil bioavailable Pb concentrations in mixed than single treatments tallies with the findings of Madyiwa et al., (2002).

B. napus accumulated Pb and Cd in clayey soils and the results were consistent with the findings of Mathe-Gasper and Anton, (2005) and Licinia et al., (2007). Cd accumulation positively correlated with Cd bioavailability in the soil corroborating findings of Selvam and Wong, (2008). Kachenko and Singh (2006) reported that broad leaf vegetables are hyper accumulators of heavy metals. Our findings suggest that the interaction of Pb and Cd in soils and possibly other factors reduce Pb uptake by *B. napus*. The observed weak positive correlations between soil metal bioavailability and corresponding metal uptake by *B. napus* was similar to the findings of Moolenaar and Lexmond, (1999). The negative correlations observed for Pb uptake and yield is in agreement with the findings of Miller (1997) who reported that Cd in soil reduced uptake of Pb in *Zea mays* L (Con). However, our results contradicted the findings of Carlson and Bazzaz (1997) who reported that uptake of Pb by plants increased as Cd

Table 2. Soil bioavailable Pb and Cd, vegetable metal uptake and corresponding yield by *B. napus* (rape) single treatments.

Treatment	Mean soil bioavailable metal (mg/kg)	Mean metal uptake (mg/kg)	Mean rape yield (g/pot)
Pb single treatments			
Control	0.83 ^a	3.3 ^a	2.0 ^a
Wastewater	1.23 ^a	3.85 ^a	2.05 ^a
Pb100	176.9 ^b	7.27 ^b	3.51 ^a
Pb200	219.5 ^b	271.5 ^b	2.83 ^a
Pb400	422.1 ^b	123.0 ^b	0.29 ^b
Cd single treatments			
Control	0 ^a	0.025 ^a	2.0 ^a
Wastewater	0 ^a	0.03 ^a	2.05 ^a
Cd3	0.37 ^a	0.06 ^a	2.05 ^a
Cd6	0.54 ^a	0.32 ^b	3.1 ^a
Cd12	1.84 ^b	0.79 ^b	3.0 ^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 rape mixed treatments.

Treatment	Mean soil bioavailable metal (mg/kg)	Mean metal uptake (mg/kg)	Mean rape yield (g/pot)
Pb mixed treatments			
Control	0.83 ^a	3.3 ^a	2.0 ^a
Wastewater	1.23 ^a	3.85 ^a	2.05 ^a
Cd3+Pb100	223.15 ^b	8.6 ^b	3.0 ^a
Cd6+Pb200	465.25 ^c	15.27 ^b	2.5 ^a
Cd12+Pb400	417.25 ^c	41.53 ^c	1.4 ^b
Cd mixed treatments			
Control	0 ^a	0.025 ^a	2.0 ^a
Wastewater	0 ^a	0.03 ^a	2.05 ^a
Cd3+Pb100	0.21 ^b	0.13 ^b	3.0 ^a
Cd6+Pb200	0.61 ^b	2.1 ^c	2.5 ^a
Cd12+Pb400	0.27 ^b	0.17 ^b	1.4 ^b

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 *B. napus* using Pb and Cd inorganic salts.

Source of variation	Single		Mixed	
	F	p	F	p
Pb				
Yield	4.31	p<0.069	2.40	p>0.186
Soil bioavailability	4.79	p<0.069	7.82	p<0.065
Cd				
Yield	30.47	p<0.002	0.78	p>0.508
Soil bioavailability	10.40	p<0.01	1.15	p>0.377

concentrations were raised in the soil. The observed high negative correlations between *B. napus* yield response to Pb uptake in mixed treatments compared to single treatments is similar to the observations of Molgorzata and Hakan (2001).

The observed decline in yield as Pb concentrations increased was due to the toxicity of the metal to *B.napus*. This suggests that repeated irrigation of Golden Ridge Estate crops with effluent over a long period of time could result in low crop yield. 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 (Selvam and Wong, 2008).

Table 5. The regression coefficients of the relationship between soil bioavailable Pb and Cd, and their uptake and yield by *B. napus* in single and mixed treatments.

	b	(mg/kg) r²(%)	n	Significance
Pb single treatments				
Soil bioavailability and uptake	0.231	0.69	7	NS
Soil bioavailability and yield	-5.848	49.20	7	p<0.05
Pb mixed treatments				
Soil bioavailability and uptake	2.019	32.89	7	p<0.05
Soil bioavailability and yield	-1.177	6.10	7	NS
Cd single treatments				
Soil bioavailability and uptake	1.559	78.39	7	p<0.01
Soil bioavailability and yield	-0.034	9.82	7	NS
Cd mixed treatments				
Soil bioavailability and uptake	-0.411	13.48	7	P<0.05
Soil bioavailability and yield	0.351	10.15	7	NS

Table 6. Relationship between Pb and Cd uptake by *B. napus* and corresponding yield in single and mixed treatments.

	b	g/pot r²(%)	n	Significance
Pb				
Single treatments	0.140	4.63	7	NS
Mixed treatments	-0.798	88.77	7	p<0.01
Cd				
Single treatments	0.035	0.38	7	NS
Mixed treatments	0.088	7.98	7	NS

Conclusion

B. napus accumulates high levels of Pb and Cd to above recommended limits and poses a hazard to humans after consumption. Contaminated Golden Ridge Estate soil and wastewater from Pakamisa Sewage Treatment Plant are not fit for irrigating *B. napus* for human consumption. Golden Ridge Estate should take action to reduce levels of Pb and Cd in the wastewater or stop selling rape to residents of the City of Gweru as this is likely to result in health problems due to the cumulative nature of Pb and Cd.

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