CHAPTER 6

FIELD ASSSESSMENT OF LEAD AND CADMIUM UPTAKE BY *Cynodon Nlemfuensis* UNDER REPEATED APPLICATION OF TREATED WASTEWATER

6.1 Introduction

This chapter presents a field assessment and models of accumulation of Pb and Cd in star grass under irrigation with treated sewage. Models produced under greenhouse conditions (Chapter 5) estimate that star grass can absorb more than 40 mg/kg Pb and 1 mg/kg Cd recommended for pasture, if bio-available levels in the soil are more than 106.3 mg/kg and 0.63 mg/kg, respectively. However considering that conditions for availability of the metals from the soil in the pot experiment are different from those in the field, it was decided to extend the investigation of Pb and Cd uptake to the field to reflect real life conditions and develop models appropriate for these conditions. Therefore, the purpose of this component of the study was to develop soil-vegetative metal uptake models for predicting Pb and Cd uptake in star grass under field conditions where sandy soils were subjected to continuous disposal of treated sewage. The models were postulated to be useful for estimating grass metal content and providing an indication of suitability of using star grass grown under similar conditions as pasture.

Unlike in the greenhouse experiment where the concentrations of Pb and Cd were varied using inorganic salts of Pb and Cd, in the field the concentrations of the metals varied depending on the metal content strength of influent wastewater. The strength of influent was related to industrial and commercial operations (Junkins et al, 1983). To develop models representative of field situations, it was necessary to vary quantities of Pb and Cd applied amongst different experimental units (treatments) so as to vary the levels of Pb and Cd applied. To vary the quantities of Pb and Cd applied to treatments, using incoming treated sewage, it was necessary to vary the total volumes of treated wastewater applied to the treatments over a long time. It was assumed that the quantity of Pb and Cd would vary proportionally to the quantity of treated wastewater applied. Therefore the levels of the metals in treated water used for irrigating star grass had to be determined for each irrigation event, so as to determine the quantities of the metals added to the soils over time.

6.2 Objectives

This component of the study was aimed at developing Pb and Cd uptake models for star grass on sandy soil subjected to varying quantities of treated wastewater disposal under field conditions.

The specific objectives of this chapter were to:

1. develop Pb and Cd uptake models based on soil bio-available metal content and metal content in grass under field conditions
2. estimate the allowable limit of bio-available soil Pb and Cd content for a sandy soil on which star grass pasture grows under field conditions
3. establish the effect of rate of accumulation of Pb and Cd in a sandy soil and on uptake by star grass under field conditions

6.3 Detailed methods and materials

6.3.1 Estimated irrigation requirements of star grass

In setting up the field experiment, it was important to estimate irrigation requirements of star grass so as to decide on the quantities of treated sewage to apply to the soil. The irrigation requirements were estimated using the modified Penman method described in the Food and Agricultural Organisation (FAO), Irrigation and Drainage paper number 24 together with 30-year climatic data from the nearest meteorological station to the study area. The nearest meteorological station, Belvedere in Harare is located at an altitude of 1 471 m above sea level at a latitude of 17^0 50' S and longitude of 31^0 01' E (Department of Agricultural Technical and Extension Services and Department of Meteorological Services, 1989). The area has a mean annual rainfall of 800 mm/annum and it lies in Agro-ecological Region IIA.

Table 6.1 presents the potential evapo-transpiration and estimated irrigation water requirements of star grass for a full year, covering the period January to November, during which the experiment was run. The months with excess water have theoretical negative water requirements, which are however not carried forward to the next month since that water is normally lost as run-off, deep percolation losses or evaporation. The data in Table 6.1 shows that for optimum growth, grass would require a net of 765.4 mm of irrigation per year to supplement rainfall.

Table 6.1: Estimated crop water and irrigation requirements of star grass.

Month	Potential evapo-transpiration mm 10 day periods 1	 2	 3	Monthly total (mm)	Rainfall (mm/month) Belvedere	80% dependable rainfall (mm/month)	Irrigation requirement mm/month	Mean irrigation requirement mm/day
July	26	24	28	78	2.5	2	76	2.53
Aug	34	36	40	110	3.2	2.56	107.44	3.58
Sep	46	52	52	150	10.3	8.24	141.76	4.73
Oct	56	54	56	166	37.6	30.08	135.92	4.53
Nov	50	48	44	142	93.2	74.56	67.44	2.25
Dec	42	42	38	122	190.5	152.4	-30.4	-1.01
Jan	40	40	40	120	172.5	138	-18	-0.60
Feb	38	37	35	110	178.5	142.8	-32.8	-1.09
Mar	31	35	36	102	94	75.2	26.8	0.89
Apr	33	32	36	101	40.5	32.4	68.6	2.29
May	30	25	26	81	9.5	7.6	73.4	2.45
Jun	24	24	24	72	5	4	68	2.27
Total Jan-Nov							**765.36**	

6.3.2 Experimental set-up

The field experiment was set up in the portions of Firle farm and Churu farm that had been set aside for field experiments. The area in Churu farm was located 2m down-slope of the position where soils for the greenhouse experiment were taken from. The portion in Firle farm that was selected for this experiment lay within the area that was studied during soil characterisation. The two areas were 30m apart.

It was assumed that the area not previously irrigated had a higher chance of showing marked changes in soil bio-available levels of Pb and Cd added through treated sewage during the 11-month period of the experiment, than the area that had been irrigated for 30 years. This assumption was based on the fact that the unpolluted area had less organic matter and CEC (Table 4.2 in Chapter 4) to immobilise Pb and Cd. Therefore in addition to the control, 3 treatments of Pb and Cd were set up in this area, while the fourth treatment was located in Firle farm. The fourth treatment was included in the study to investigate Pb and Cd uptake by star grass from a soil that has been receiving treated sewage for a long time.

All treatments were set on field plots measuring 10m x 10m. Each treatment had 3 replicates. The control did not receive any treated sewage application. The 3 treatments in Churu farm were planned to receive the following amounts of supplementary irrigation:

(1) treatment 1: half of the estimated water requirement
(2) treatment 2: the estimated water requirement

(3) treatment 3: twice the estimated water requirement of grass was provided
Treatment 4 had to receive the same application as treatment 3.

To provide water to the plots in Churu farm, a 150 m long, 90mm diameter polyethylene pipeline was installed from an outlet box, along the pipeline from Firle Treatment Plant to the sewage pond (Figure 3.1) and finally to the top 3 plots (X_1, Y_1 and Z_1 in Figure 6.1). Inside the outlet box (on the main pipeline) there was a 200 mm pipe outlet joined to the main pipeline through a t-piece on which there was a valve. The outlet box had outlets on 3 sides, through which treated sewage was released for disposal onto the farm by flooding.

A potable, 8 horsepower pump and petrol engine, were used to pump treated wastewater from the outlet box to the plots in Churu farm. Three hydrants were installed at the middle of the top plots, labelled X_1, Y_1 and Z_1 along the top edges of the plots. Each hydrant served four downstream plots, through a 40 m flexible hose provided to deliver water from the hydrant to the rest of the plots served by that hydrant. As an example the hydrant at X_1 served X_1 to X_4.

One outlet was used to irrigate the 3 plots of treatment 4. The plots in treatment 4 were set up side-by-side in the same manner as plots X1, Y1, and Z1. Water was supplied to treatment 4, from the outlet box through a small 10m long clay-lined earth channel that brought treated wastewater towards the plot located at the middle and directed it along the top edges of the plots at a distance of 2 m from the plots into 2 m long channels that joined the plots. The channel was run at 2 m away from the edges of the plots to minimize seepage of water from the channels into the plots. A portable flume was installed at the upper ridge of the plot during each irrigation event so as to measure the water supplied to each plot.

6.3.3 Preparation of field plots

The boundaries of the areas in which the plots were set up were marked. The areas were then ploughed and ripped. After removing plant materials, roots and debris, the areas were manually levelled in the direction perpendicular to the direction of irrigation and at a slope of 3 % in the direction of flow of irrigation water. Thus the land sloped uniformly in the direction X_1 to X_4, to ensure uniform water application (Figure 6.1).

The boundaries of the plots were marked, leaving a 2m buffer zone between any two plots. The buffer zones served four purposes. The first purpose was to minimise surface and ground water flow from one plot to the next. The second purpose was to provide soil for forming ridges around each plot so as not to disturb the area levelled inside the plot. The third purpose was to provide pathways for movement of people. The fourth purpose in the case of treatments 1 to 3 was to provide an area in which water could spill without causing direct application to a particular plot during water measurement.

III 2,3 **Z_1** III 2,1 III 2,2	II 1,3 **Y_1** II 1,1 II 1,2	I 1,3 **X_1** I 1, 1 I 1,2
III 1,3 **Z_2** III 1,1 III 1,2	C 1,3 **Y_2** C 1, 1 C 1,2	C 2,3 **X_2** C 2, 1 C 2,2
II 3,3 **Z_3** II 3,1 II 3,2	I 2,3 **Y_3** I 2, 1 I 2,2	III 3,3 **X_3** III 3, 1 III 3,2
I 3,3 **Z_4** I 3, 1 I 3,2	II 2,3 **Y_4** II 2, 1 II 2,2	C 3,3 **X_4** C 3, 1 C 3,2

C – Control; I, II, III –Treatments 1, 2 and 3 respectively; 1,1 to 3,3 – soil and grass sampling positions

Figure 6.1: Plot layout at Churu farm

Thirty centimetre high ridges (bunds) were made around each border. Each plot therefore formed an irrigation border. An irrigation border is an area that is level along one axis and slopes in the direction of flow of irrigation water. In order to increase uniformity of water application across the level sides of the border and along the direction of water flow, a small furrow was made within the border (on the inside of the top ridge) to catch the water as it came out of the flexible hose or flume. This allowed the water to fill the furrow first before over-flowing down the plot at all points along the furrow at the same time.

After preparing the plots, 5cm deep furrows spaced at 20 cm were made in the plots along the direction of flow of water, for planting star grass. Strands of star grass with several nodes on them were placed in the furrows and covered with soil. Small amounts of water were applied to the grass for a period of 3 weeks to establish it. The rainfall that fell at the time was also useful in assisting the establishment of grass. The plots in Churu farm were randomly assigned to the control and treatments 1-3 (Figure 6.1) prior to administration of the treatments.

6.3.4 Irrigation of grass

At the beginning of the experiment, the pump was tested in order to determine the point to which the gate valve had to be opened in order to provide sufficient water for irrigation and at a relatively constant head above the suction pipe. The flow was directed away from the plots during this process. During irrigation, the discharge of the pump was measured 3 times at the beginning and 3 times at the end of irrigation of each plot. A 20-litre container and a stopwatch were used for the purpose. The mean discharge was used in computations of quantities of irrigation water applied and concentrations of the metals (Appendix 3). Samples of water were also taken at the beginning and end of each irrigation event for determining metal content. Since complete irrigation required 2 days, 4 samples were collected during each irrigation event.

In order to measure the volume of water applied to treatment 4, the level of flow was set on the scale of the flume while adjusting the amount of water coming from the outlet box. During the set up, the potable flume was installed at 2 m away from the plots and the channel was breached to direct the flow away from the plots. After set up, the flume was installed at the ridge of the border and the breach was closed to direct the flow into the borders. The flow was set as close as possible to the average discharge of the pump and application time was recorded.

Irrigation of grass in treatments 1 to 4 commenced 3 weeks after planting. Eight irrigation applications were undertaken during the period of the experiment with treatment 1 receiving 25.7 m^3, treatment 2, 49.4 m^3, treatment 3, 97.8 m^3 and treatment 4, 85.9 m^3.

6.3.5 Soil sampling and testing

Soil samples were taken using soil augers from field plots on 3 occasions during the experiment. The soil was sampled from 3 points within each plot. Figure 6.1 shows the points from which the samples were collected in each plot. As an example, in plot X_1 the samples were collected at points I 1,1, I 1,2 and I 1,3. The soil samples were collected from depths of 0-10, 10-20, 20-30 and 30-40 cm using a soil auger. For each horizon the soil from the 3 sampling points was mixed to form one sample for each depth for that plot. After removing plant debris the samples were air-dried and passed through a 2mm sieve.

Soil depth and soil properties of clay content, pH and cation exchange capacity were determined for use in interpreting Pb and Cd in soils. Soil texture, from which clay content was derived, was determined using the hydrometer method (Gee and Bauder, 1986). Soil pH was determined using a 1:5 soil suspension of 0.01M $CaCl_2$. Cation exchange capacity was determined by saturating the soil with 1M CH_3COONH_4 buffered at pH 5.2. Bio-available soil concentrations were determined using procedures recommended by McGrath and Cegarra

(1992). A 1 M (CH_3COONH_4) solution was added to the soil sample and the suspension was shaken using a mechanical shaker. The suspension was the filtered, after which levels of Pb and Cd were measured on the atomic absorption spectrometer.

6.3.6 Grass sampling and testing

Grass samples were taken from each plot on 5 out of a planned 6 occasions during the field experiment. The sixth sample had to be foregone due to a limited budget. On 3 of these occasions, the samples were taken at the same time as soil samples for purposes of comparing soil and grass levels of Pb and Cd. The first crop was harvested 45 days after the start of irrigation and the re-growth samples were collected at an interval of 51 days thereafter. The grass samples were taken next to the position where soil samples were taken. Thus, from each plot 3 replicates of grass samples were tested.

The grass was cut at 5 cm height off the soil surface, washed using de-ionised water, oven dried at 65 ^{0}C, ground and sieved through a 0.1 mm sieve. The samples were then ashed at 550 ^{o}C for 16 hours and digested with 25% HCl and concentrated HNO_3. After filtration, Pb and Cd were determined using atomic absorption spectrometry. Three samples of the grass that were taken during planting were subjected to the same metal extraction procedure prior to determination of levels of Pb and Cd.

6.3.7 Sewage effluent and sludge sampling and testing

During each irrigation event, 4 samples of treated wastewater were collected for testing Pb and Cd. The levels of Pb and Cd in the mixed effluent and sludge were determined by atomic absorption spectrometry (Department of Environment, 1989) after extraction with HCl and concentrated HNO_3.

6.3.8 Data analysis

Means and standard deviations were calculated using measured data on clay content, soil pH and CEC. Analysis of variance was performed on measured values of pH, CEC and clay content of the soil profiles to determine whether the application of different volumes of treated sewage on the soil parameters was significant at 95% confidence level. Correlation analysis was used to test the strength of association of soil pH versus depth, CEC versus depth and clay content versus depth. The significance of the association was determined by comparing r^2 values with the Pearson $r^2_{critical}$ value of 0.87 ($p \leq 0.05$).

For statistical analysis and development of dose-response relationships, measured data on bio-available metal concentrations and concentration of metals in grasses was first tested for normality and then transformed to $\log_{10}$ values. To assess Pb and Cd accumulation in the soil profile, correlation analysis was used to relate soil depth and $\log_{10}$ (*metal concentration*). Analysis of variance was used to test the significance of treatment on (1) bio-available Pb and Cd and on (2) levels of the metals in grass.

To develop the best-fit models for uptake of Pb and Cd under field conditions two approaches were used to analyse the data obtained. In the first approach, the data for each of the 3 sample sets of bio-available metal levels and grass metal levels was used to draw up a model for each harvest and test its strength. This was done to assess whether any of the models of individual harvests could be representative of multiple harvests.

In the second approach, dose-response models of average bio-available soil levels and average levels of the metals star grass throughout the life of the experiment were drawn up, to assess whether they could represent multiple harvesting of grass. The assumption was that in the field, a grass crop is planted and animals continue to feed on the crop until the old crop is removed and a new crop is planted. Therefore the regular grazing of animals could be regarded as being synonymous with regular harvesting of the grass crop. To develop models representative of this situation, it was decided to analyse each harvest and each soil-sampling event as a replicate of the average situation that prevails under field conditions over a long time.

The best-fit models were tested for strength by comparing the computed correlation coefficients and the critical *t* values from the *t*-test for comparison of regression coefficients.

6.4 Results

6.4.1 Soil pH, cation exchange capacity and clay content

Table 6.2 presents data on selected soil parameters of CEC, pH and clay content. Soil pH varied from 4.9 to 5.5 in some horizons of the control to a maximum of 5.35 to 7.4 in some horizons of the previously irrigated area. There was a gradual increase in CEC with increase in the level of treatment. Treatment 4 had a very high CEC, particularly in the top horizons. Clay content decreased from the control and treatments 1 to 3 to treatment 4. Analysis of variance on data (Table 6.2) from soil profiles shows that pH, CEC and clay content increased significantly ($p \leq 0.05$) with treatment.

Table 6.2: Mean soil properties (standard deviations) and soil depth

Depth (cm)	Control			Treatment 1			Treatment 2			Treatment 3			Treatment 4		
	Har 1	Re-g3	Re-g4	Har 1	Re-g3	Re-g4	Har 1	Re-g3	Re-g4	Har 1	Re-g3	Re-g4	Har 1	Re-g3	Re-g4
PH															
0-10	5.50	5.47	4.87	5.47	5.53	5.40	5.60	5.80	5.37	6.21	5.63	6.13	5.90	5.70	7.40
	(0.53)	(0.49)	(0.29)	(0.36)	(0.70)	(0.20)	(0.76)	(0.46)	(0.40)	(0.30)	(0.31)	(1.21)	(0.31)	(0.46)	(0.10)
0-20	4.90	5.23	5.03	5.55	5.67	5.60	5.51	5.67	5.77	6.15	5.73	6.10	6.10	5.93	6.53
	(0.49)	(0.35)	(0.12)	(0.88)	(0.91)	(1.05)	(0.67)	(0.76)	(1.00)	(0.48)	(0.59)	(1.06)	(0.44)	(0.50)	(0.81)
20-30	5.33	5.07	4.93	5.35	5.60	5.63	5.56	5.73	5.40	6.33	6.07	5.25	5.43	5.40	6.90
	(0.33)	(0.51)	(0.31)	(0.25)	(0.95)	(1.57)	(0.51)	(1.10)	(0.60)	(0.99)	(1.24)	(0.35)	(0.10)	(0.14)	(0.57)
30-40	4.90	5.23	4.97	6.00	5.63	5.63	5.54	5.15	5.33	6.20	6.03	5.25	5.70	5.35	6.35
	(0.21)	(0.76)	(0.06)	(1.00)	(1.05)	(1.31)	(0.19)	(0.78)	(0.72)	(0.87)	(1.10)	(0.21)	(0.01)	(0.07)	(0.35)
Cation exchange capacity ($\text{cmol}_c\text{kg}^{-1}$)															
0-10	1.61	1.95	1.42	2.63	2.53	2.73	2.57	3.17	3.18	2.93	2.73	3.51	31.17	31.78	29.11
	(0.09)	(0.32)	(0.13)	(0.35)	(0.45)	(0.38)	(0.21)	(0.15)	(0.25)	(0.35)	(0.52)	(1.05)	(6.84)	(11.4)	(6.05)
10-20	2.07	1.83	1.81	2.67	2.2	2.47	2.53	3.00	2.52	2.53	2.47	2.47	11.37	12.2	9.56
	(0.19)	(0.23)	(0.12)	(0.55)	(0.1)	(0.32)	(0.49)	(0.14)	(0.37)	(0.25)	(0.38)	(0.38)	(4.43)	(8.17)	(2.76)
20-30	1.83	1.9	1.90	2.17	2.5	2.10	2.53	2.30	2.20	2.67	2.70	2.70	4.30	5.33	4.58
	(0.18)	(0.00)	(0.28)	(0.23)	(0.87)	(0.28)	(0.25)	(1.15)	(0.42)	(0.35)	(0.61)	(0.61)	(2.76)	(3.16)	(0.99)
30-40	1.98	1.69	1.79	2.4	2.3	2.90	2.15	2.57	2.25	2.70	3.07	1.91	-	-	
	(0.17)	(0.04)	(0.13)	(1.11)	(0.44)	(0.99)	(0.07)	(0.04)	(0.07)	(0.56)	(1.46)	(0.20)			
Clay content (%)															
0-10	4.67	5.33	4.67	3.43	4.00	5.66	4.33	4.67	5.00	5. 00	5.33	5.00	4.00	3.00	2.25
	(0.58)	(1.53)	(1.16)	(1.40)	(0.00)	(1.52)	(0.58)	(1.53)	(1.00)	(0.00)	(1.53)	(1.00)	(1.41)	(1.00)	(0.35)
10-20	5.33	6.00	4.33	2.93	5.33	6.33	5.67	7.00	6.66	6.67	7.00	6.66	4.5	2.67	2.50
	(0.58)	(2.00)	(1.16)	(0.97)	(0.58)	(1.15)	(1.53)	(1.00)	(0.58)	(0.58)	(1.00)	(0.58)	(2.12)	(0.58)	(0.71)
20-30	6.33	6.00	6.50	3.73	6.67	8.56	7.33	8.33	6.66	7.33	7.00	6.66	4.5	4.0	3.00
	(0.58	(0.00)	(2.13)	(2.84)	(2.31)	(2.52)	(2.52)	(2.52)	(1.16)	(1.16)	(1.00)	(1.50)	(3.54)	(1.00)	(0.00)
30-40	7.00	6.67	6.5	4.6	6.67	7.00	5.5	7.33	10.33	8.33	10.00	10.33	-	-	-
	(1.00)	(1.53)	(1.19)	(3.03)	(1.53)	(1.01)	(0.71)	(1.16)	(3.05)	(2.89)	(2.65)	(3.05)			

Har - Harvest 1 (i.e. first crop); Reg - re-growth; - missing values

Comparison of means of treatments showed that the pH in treatments 3 and 4 were significantly ($p \leq 0.05$) higher than in the control. The pH in treatment 4 was also significantly ($p \leq 0.05$) higher than in the rest of the treatments except treatment 3. The CEC was significantly ($p \leq 0.05$) higher in treatment 4 than in the rest of the treatments and the control. There was no significant difference in CEC in the latter. The CEC was significantly ($p \leq 0.05$) higher in treatment 4 than in the rest of the treatments and the control. There was no significant difference in CEC in the latter. Comparison of means of pH, CEC and clay content within each depth showed that there was no significant difference in pH and CEC with increase in depth, but there was a significant difference ($p \leq 0.05$) in clay content with depth. The 30-40 cm horizon had significantly ($p \leq 0.05$) clay content that the 0-10 cm horizon.

Comparing Pearson's correlation coefficient (r^2_{critical} of 0.87) to the computed correlation coefficients in Table 6.3 shows that there was no association between pH and soil depth except in treatment 3 (re-growths 3 and 4). Generally, there was no significant ($p \leq 0.05$) association between CEC and soil depth except in treatment 4 and treatments 2 and 3 (re-growth 4). In these cases CEC was negatively correlated to soil depth implying that the top soil layers had a higher CEC than lower soil horizons. Generally, clay content positively correlated with soil depth.

Table 6.3: Correlation coefficients for pH, cation exchange capacity and clay content versus soil depth

Treatment	pH			CEC			Clay content		
	Harvest 1	Re-growth 3	Re-growth 4	Harvest 1	Re-growth 3	Re-growth 4	Harvest 1	Re-growth 3	Re-growth 4
Control	-0.58	-0.68	0.37	0.48	-0.44	0.73	1.00	0.94	0.87
Treatment 1	0.63	0.53	0.84	-0.67	-0.32	0.04	0.79	0.61	0.85
Treatment 2	-0.44	-0.82	-0.30	-0.82	-0.81	-0.89	0.54	0.92	0.72
Treatment 3	0.25	0.92	-0.90	-0.44	0.64	-0.89	0.98	0.93	0.91
Treatment 4	-0.57	-0.75	-0.78	-0.96	-0.96	-0.95	0.99	0.87	0.98

6.4.2 Bio-available Pb and Cd content of soils and grass

Soil and grass Pb and Cd levels per harvest

Table 6.4 presents data on bio-available metal levels from soil samples taken at the same time as grass samples of the first crop, 3rd and 4th re-growth crops as well as concentrations in star grass obtained from the first crop, and 1st, 2nd, 3rd and 4th re-growth crops. This data is the basis on which the dose-response relationships were derived for each harvest. Details on mean bio-available metal concentrations of the soil profile along the 10 cm soil horizons are presented in Appendix 4.

Soil bio-available Pb and Cd levels

Table 6.4 shows that the maximum levels of bio-available Pb and Cd in the soil profile were 12.55 mg/kg and 0.90 mg/kg respectively. Comparison of means between treatments and among sampling events showed that treatment 4 had significantly ($p \leq 0.05$) higher levels of Pb and Cd. Treatments 2 and 3 had significantly higher levels of Pb than the control. The mean level of Cd in the control was significantly ($p \leq 0.05$) lower than the rest of the treatments. However there were no significant differences in the latter.

Grass Pb and Cd levels

In grass the maximum levels attained were 16 mg/kg Pb and 2.17 mg/kg Cd. Comparison of means showed that Cd in the 3rd and 4th re-growth crops was significantly higher ($p \leq 0.05$)

than the rest of the re-growths and the first crop. Differences in mean levels of Pb between harvests were not statistically significant.

Correlation of bio-available Pb and Cd and soil depth for each harvest

Comparing Pearson's correlation coefficient ($r^2_{critical}$ of 0.87) to the computed correlation coefficients in Table 6.5 shows that bio-available Pb was strongly correlated to depth in the control and in treatment 4. A similar trend also existed for Cd. In the control bio-available Pb showed positive correlation while in treatment 4 the association between soil depth and Pb concentration was negative. The correlation coefficients of both Pb and Cd changed from a positive trend (in the control) to a progressively negative trend with increase in treatment level.

Table 6.4: Mean soil profile bio-available metal and grass concentrations

Treatment	Mean bio-available soil profile concentration (mg/kg)			Mean grass concentration (mg/kg)				
Sample	First crop	Re-growth		First crop	Re-growth			
		3	4		1	2	3	4
				Lead (Pb)				
Control	0.389	0.339	0.67	2.56	2.89	2.67	6.0	6.33
	(0.069)	(0.059)	(0.208)	(0.694)	(0.84)	(1.53)	(3.46)	(2.08)
Treatment 1	1.19	0.733	0.72	3.33	3.44	5.33	9.33	8.00
	(0.018)	(0.227)	(0.194)	(0.333)	(0.69)	(1.46)	(2.19)	(0.67)
Treatment 2	1.34	0.947	1.3	3.67	3.89	6.00	10.56	10.11
	(0.185)	(0.528)	(1.201)	(0.882)	(0.19)	(1.67)	2.01)	(2.36)
Treatment 3	1.76	1.175	0.67	4.89	4.44	7.11	9.89	10.11
	(0.117)	(0.238)	(0.355)	(0.385)	(1.02)	(2.83)	(2.91)	(1.39)
Treatment 4	12.55	9.00	12.4	14	16.00	16.00	15.00	15.00
	(1.050)	(1.400)	(0.50)	(1.50)	(1.31)	(3.00)	(2.77)	(4.37)
				Cadmium (Cd)				
Control	0.011	0.02	0.03	0.33	0.30	0.39	0.56	0.44
	(0.007)	(0.021)	(0.006)	(0.05)	(0.28)	(0.35)	(0.51)	(0.19)
Treatment 1	0.022	0.019	0.24	0.11	0.11	1.0	1.22	1.22
	(0.03)	(0.007)	0.183)	(0.09)	(0.09)	(0.00)	(0.38)	(0.69)
Treatment 2	0.04	0.019	0.02	0.11	0.11	1.17	1.28	1.44
	0.007)	(0.028)	(0.015)	(0.100)	(0.09)	(0.29)	(0.25)	(0.51)
Treatment 3	0.065	0.033	0.03	0.11	0.33	1.06	1.67	1.44
	0.022)	(0.025)	(0.014)	(0.017)	(0.33)	(0.10)	(0.58)	(0.19)
Treatment 4	0.9	0.9	0.1	1.33	2.00	2.17	1.47	1.54
	(0.020)	(0.400)	(0.020)	(0.14)	(0.34)	(0.40)	(0.50)	(0.47)

() standard deviation

Combined data for soils and grass for experimental period

Table 6.6 presents the average bio-available levels and average grass concentrations of Pb and Cd for all samples taken over the 11-month period of the experiment. Each harvest was treated as a replicate. This data was $\log_{10}$-transformed and used to develop dose-response relationships representing the average bio-available and grass metal concentrations in this experiment.

Table 6.5: Correlation coefficients for soil depth and bio-available soil metal concentration

Treatment	Lead			Cadmium		
	Harvest 1	Re-growth 3	Re-growth 4	Harvest 1	Re-growth 3	Re-growth 4
Control	0.87	0.94	0.96	0.65	-0.87	-0.87
Treatment 1	-0.84	-0.13	0.05	0.77	0.85	-0.40
Treatment 2	0.68	0.87	0.93	0.00	0.24	-0.70
Treatment 3	-0.77	0.88	-0.40	0.00	0.24	-0.97
Treatment 4	-1.00	-0.88	-0.90	-0.99	-0.96	-0.69

The data shows a gradual increases in Pb and Cd concentrations in both soils and grasses from the control to treatment 3 and a sharp increase in treatment 4. Analysis of variance showed that there was a significant ($p \leq 0.001$) increase in the level of bio-available Pb and a significant ($p \leq 0.05$) increase in the level of Cd corresponding to each harvest with increase in treatment. Comparison of mean levels between treatments showed that there was no significant difference in bio-available Pb from the control to treatment 3. However treatment 4 had significant ($p \leq 0.05$) higher levels of bio-available Pb than all other treatments. There was no significant difference in bio-available Cd levels amongst all treatments.

Table 6.6: Average bio-available Pb and Cd levels in soils and grass (mg/kg)

Treatment	Pb concentrations		Cd concentrations	
	Soil	Grass	Soil	Grass
Control	0.466 (0.178)	4.090 (1.902)	0.020 (0.009)	0.404 (0.102)
Treatment 1	0.881 (0.267)	5.886 (2.699)	0.094 (0.009)	0.710 (0.574)
Treatment 2	1.196 (0.216)	6.846 (3.316)	0.086 (0.010)	0.822 (0.657)
Treatment 3	1.202 (0.545)	7.288 (2.675)	0.042 (0.019)	0.922 (0.681)
Treatment 4	11.317 (2.007)	15.330 (0.836)	0.633 (0.046)	1.702 (0.362)

Grass Pb and Cd levels

Analysis of variance showed that there was a significant ($p \leq 0.001$ for Pb and $p \leq 0.05$ for Cd) increase in levels in grass with treatment. Comparison of means between treatments showed that the differences in means in the control and treatments 1 to 3 were not significant. Treatment 4 had significantly ($p \leq 0.05$) higher grass levels of Pb than the rest. Treatment 4 had significantly ($p \leq 0.05$) higher levels of Cd in grass than the Control. The differences in the rest of the Cd treatments were not significant.

6.4.3 Soil bio-available Pb and Cd response to treatment

The effect of the treatment on bio-available concentrations of Pb and Cd in the soil profile is presented in Figures 6.2 and 6.3, using $\log_{10}$-transformed data from Table 6.6. Figure 6.2 presents what appears to be a general increase in bio-available Pb with increase in quantity of treated sewage applied to the soil. However analysis of variance shows that statistically, there was no significant ($p \leq 0.05$) increase in Pb with treatment.

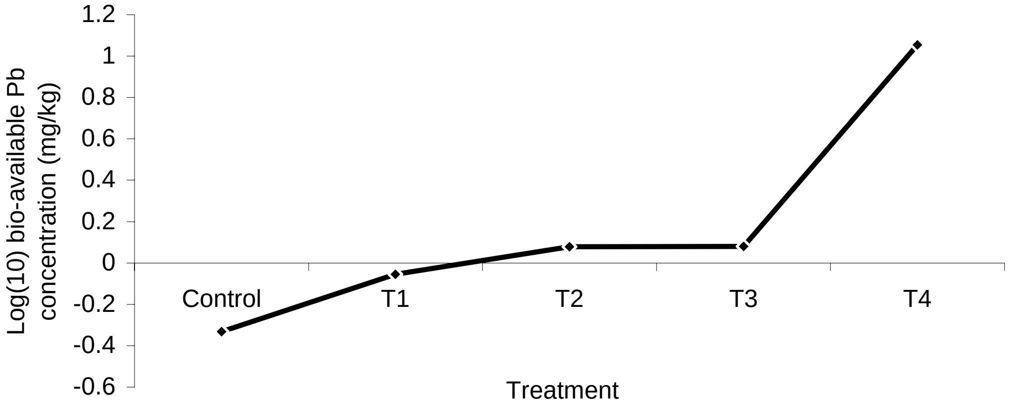

Figure 6.2 Treatment versus log(10) bio-available soil Pb concentration

Although there appears to be a general increase in Cd content of soils with increase in treatment level (Figure 6.3) analysis of variance showed that the rise in Pb levels was statistically insignificant ($p \leq 0.05$). Treatment 3 had a lower bio-available level than expected.

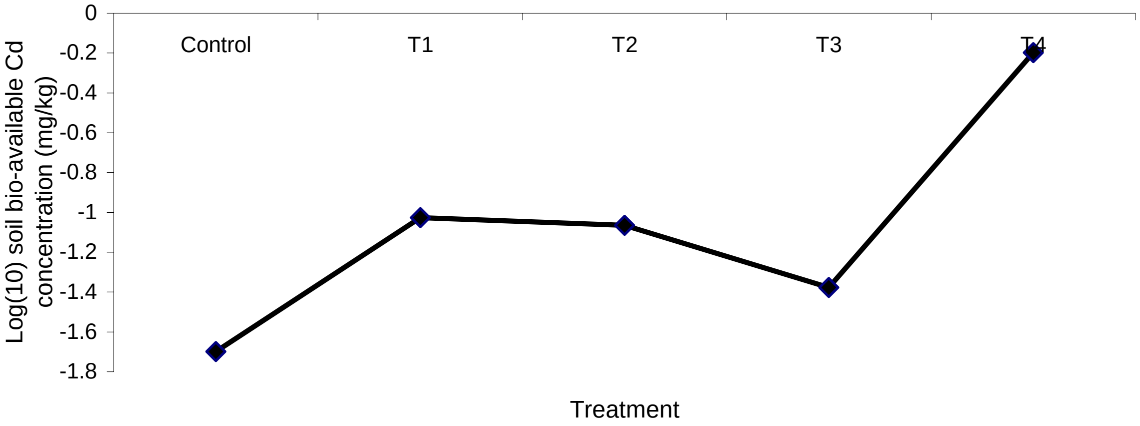

Figure 6.3: Treatment versus Log(10) bio-available soil Cd concentration

6.4.4 Grass Pb and Cd content response to treatment

Figure 6.4 shows a general increase in Pb levels in grass with increase in treatment level. Soil bio-available levels significantly ($p \leq 0.05$) increased Pb uptake by star grass. Levels of Pb increased by 375% from a minimum of 4.09 mg/kg to a maximum uptake of 15.33 mg/kg.

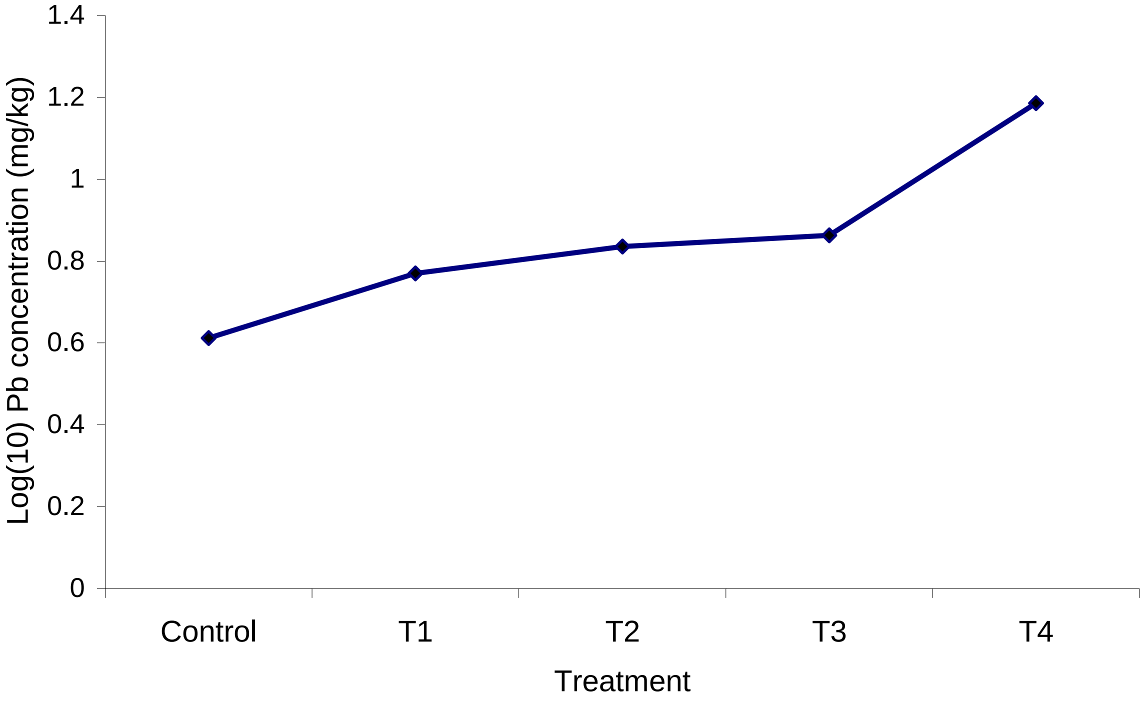

Figure 6.4 : Treatment versus Log(10) grass Pb concentration

Similarly Figure 6.5 presents a general rise in Cd concentration with increase in level of treatment. Soil bio-available levels significantly ($p \leq 0.05$) increased Cd uptake by star grass. Overall, Cd uptake increased 425% from an average of 0.40 mg/kg to an average of 1.70 mg/kg (Table 6.4). The sharp increase in Cd uptake, followed a pattern observed by Hofwegen and Veenstra (1995) where a 50% increase in total soil Cd from 0.5 mg/kg to 0.82 mg/kg led to a large increase (1200%) from 0.08 mg/kg to 1 mg/kg in brown rice.

Whereas Pb levels in grass were within the 40 mg/kg limit recommended for pasture grass, Cd levels were above the recommended 1 mg/kg maximum limit in treatments 2 to 4. According to Johannesson (2002) plant uptake of Cd ions is generally considerably higher than that of Pb ions.

6.4.5 Correlation between bio-available and grass Pb and Cd contents for each grass crop

The regression models of $\log_{10}$-transformed bio-available concentrations in soils versus $\log_{10}$-transformed concentrations in individual grass harvests are presented in Figures 6.6 and 6.7 for Pb and Cd, respectively. The models are based on data from soils and grass samples that were taken at the same time. These are referred to as Harvest 1, Re-growth 3 and Re-growth 4 in Figures 6.6 and 6.7.

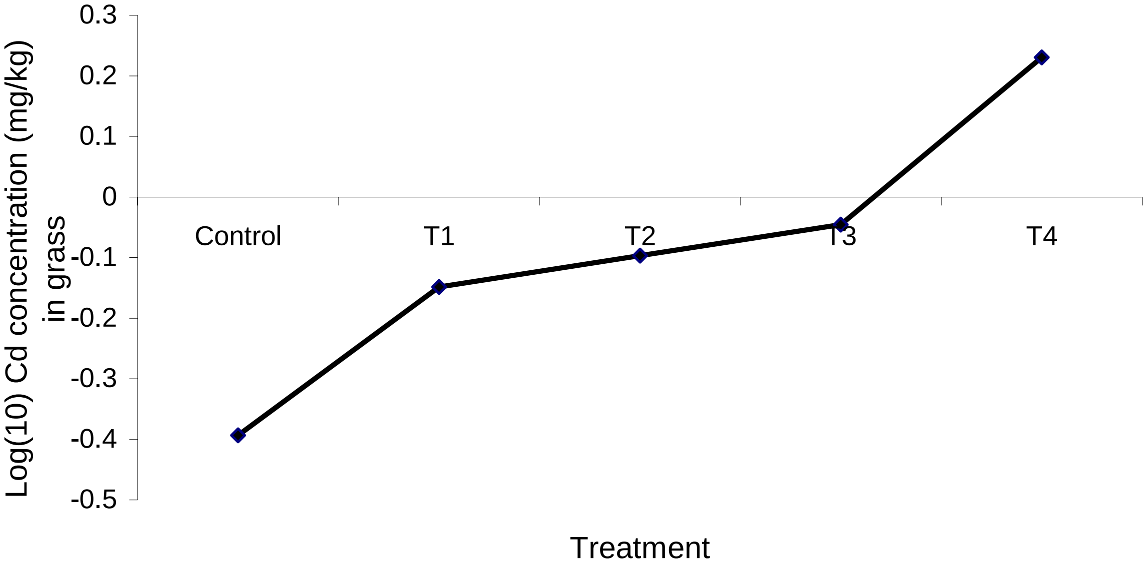

Figure 6.5: Treatment versus Log(10) grass Cd concentration

Figure 6.6 shows positive correlation between $\log_{10}$ (*bio-available Pb*) and $\log_{10}$ (*grass Pb concentration*) represented by the following models:

$y = 0.5162x + 0.5493$ ……(r^2 value = 0.96)
$y = 0.2491x + 0.9688$ ……(r^2 value = 0.86)
$y = 0.2159x + 0.9473$ ……(r^2 value = 0.72), respectively, where:

y = $\log_{10}$ grass Pb concentration (mg/kg)
x = $\log_{10}$ soil bio-available Pb concentration (mg/kg)

The correlation coefficients of 0.96, 0.86 and 0.72, confirmed that while the 3 models had strong correlation (compared to a critical r^2 value of 0.87) only the regression model for the first crop had a strong enough association of x and y to be used for predictions only in the first crop.

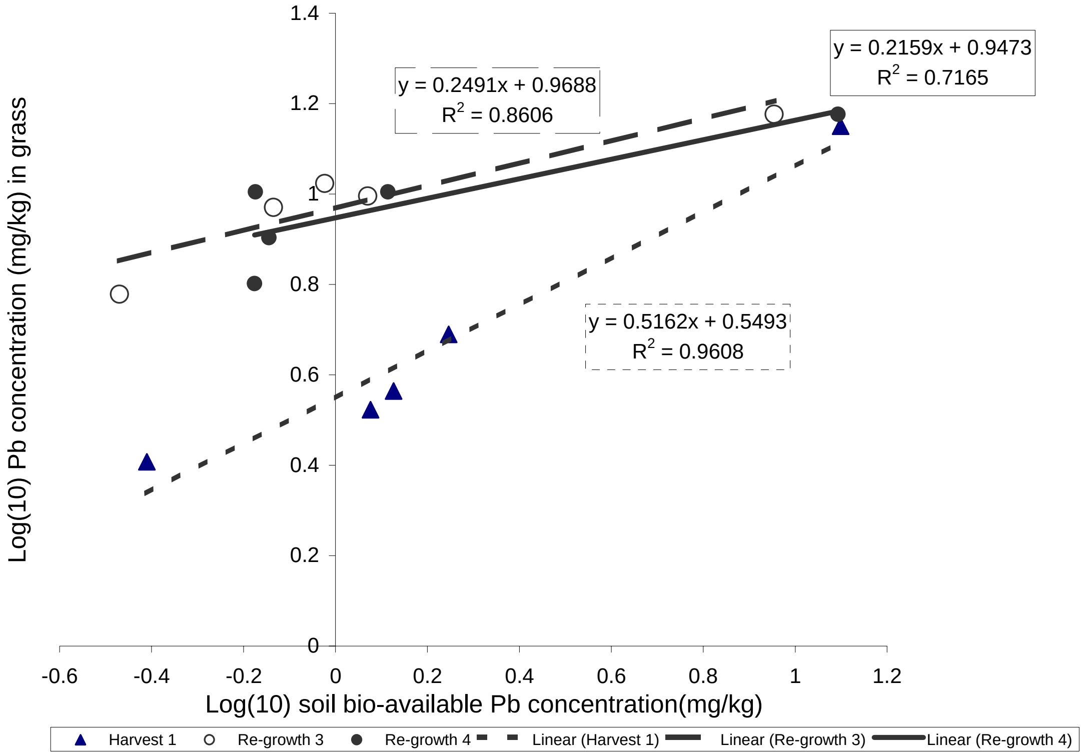

Figure 6.6: Log(10) bio-available soil Pb versus log(10) Pb level in grass

The three Pb models were tested to establish whether they were statistically different against the hypothesis that no difference existed between the slopes and no differences existed between the intercepts of the regression equations. This condition would be satisfied if $-2.306 \le t_s \le +2.306$ at 95% confidence level, t_s being the computed *t*-statistic.

Using $s^2_{(pooled)} = \{(n_1-1)s_1^2 + (n_2-1)s_2^2\}/(n_1+n_2-2)$, in which n_1 and n_2 are sample sizes for samples under comparison respectively and n_1+n_2-2 is the pooled degrees of freedom, the three equations were compared as follows:

Models $y = 0.5162x + 0.5493$ versus $y = 0.2491x + 0.9688$: t_s was 592 for slopes and 9.76 for intercepts. Models $y = 0.2491x + 0.9688$ versus $y = 0.2159x + 0.9473$: t_s was 6.93 for slopes and 10.75 for intercepts. Therefore in all the cases, the models of Pb grass content response to soil bio-available concentration, were statistically different.

The regression models relating Cd content in grass to soil bio-available concentrations (Figure 6.7) had low correlation coefficients ($r^2 = 0.59$ for the first crop, 0.20 for the third re-growth and 0.008 for the fourth re-growth compared to a critical r^2 of 0.87).

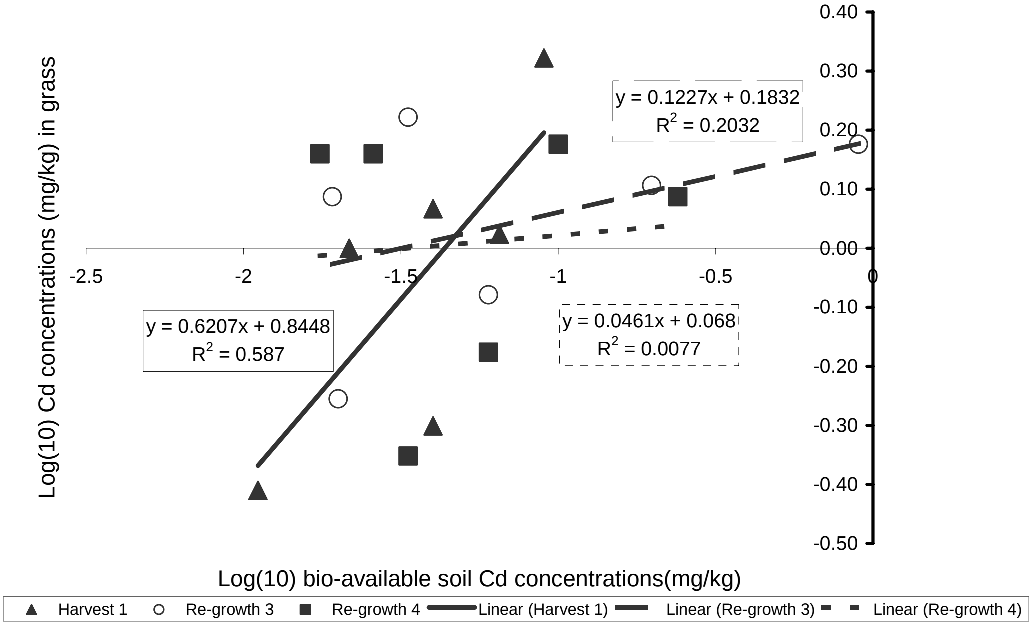

Figure 6.7: Log(10) bio-available soil Cd level versus log(10) Cd level in grass

6.4.6 Correlation between average bio-available Pb and Cd in soils and average Pb and Cd contents in grass

The best-fit regression models, Figures 6.8 and 6.9 were obtained based on $\log_{10}$ values of the concentrations in Table 6.6.

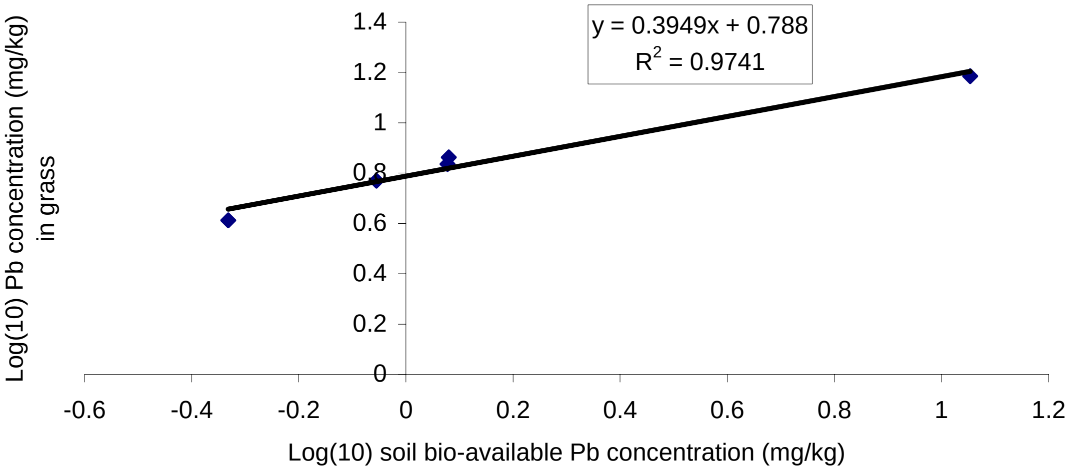

Figure 6.8: Log(10) mean bio-available soil Pb versus log(10) mean Pb level in grass

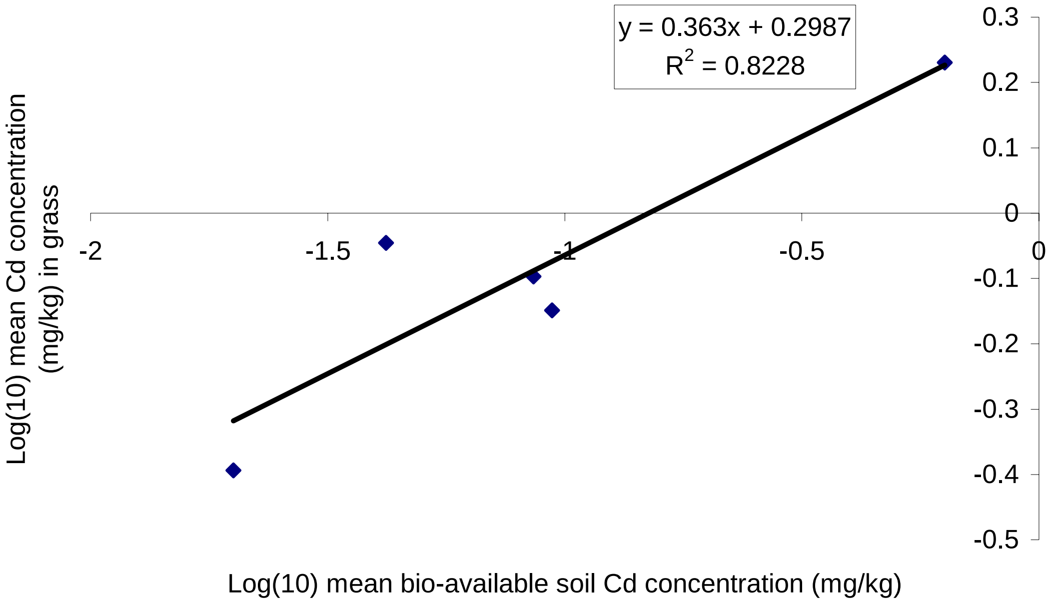

Figure 6.9 : Log(10) mean bio-available soil Cd versus log(10) mean Cd level in grass

Figure 6.8 presents a regression model that has a significantly strong correlation of r^2 of 0.97 against $r^2_{critical}$ of 0.87 ($p \leq 0.05$). Figure 6.9 presents a regression model for Cd, with a correlation of r^2 of 0.82, which is marginally weak when compared to against $r^2_{critical}$ of 0.87 ($p \leq 0.05$).

The regression models representing the average situation in which all grass crops are considered as replicates are:

$y = 0.3949x + 0.788$ for Pb,

where: $y = \log_{10}$ (*concentration of Pb in grass, mg/kg*) and x is $\log_{10}$ (*soil bio-available concentration of Pb, mg/kg*) and

$y = 0.363x + 0.2987$ for Cd

where: $y = \log_{10}$ (*concentration of Cd in grass, mg/kg*) and x is $\log_{10}$ (*soil bio-available concentration of Cd, mg/kg*).

Although the regression model for Cd was much stronger than the models for individual crop harvests, it marginally fell short of being sufficient for predicting grass concentrations on the basis of bio-available concentrations.

Using the model for Pb predicts a bio-available soil concentration of 115.2 mg/kg when the concentration of Pb in the grass is 40mg/kg. Similarly the model for Cd predicts 0.2 mg/kg when the bio-available soil concentration is 1 mg/kg. However the latter should be considered as a rough estimate.

6.4.7 Rate of metal application from treated sewage

Table 6.7 presents the quantities of treated wastewater that were applied to treatments 1-4 and the average concentrations of the metals added to the plots. The latter were computed using the volume of water applied and the concentration of the irrigation water derived from detailed information provided in Appendix 3.

Table 6.7: Quantities of treated sewage and computed average metal concentrations (standard deviation) applied to field plots

Plot number	Volume of irrigation (m^3/plot)	Mean Pb concentration applied to plots (mg/l)	Mean Cd concentration applied to plots (mg/l)
T 1.1	25.70	0.42 (0.21)	0.18 (0.07)
T 1.2	25.05	0.43 (0.19)	0.18 (0.08)
T 1.3	26.42	0.43 (0.16)	0.14 (0.06)
T 2.1	48.68	0.45 (0.18)	0.16 (0.06)
T 2.2	49.88	0.43 (0.18)	0.13 (0.07)
T 2.3	49.59	0.41 (0.19)	0.17 (0.09)
T 3.1	100.46	0.44 (0.16)	0.20 (0.07)
T 3.2	98.60	0.44 (0.16)	0.19 (0.07)
T 3.3	94.25	0.46 (0.21)	0.20 (0.06)
T 4.1	99.30	0.45 (0.15)	0.18 (0.05)
T 4.2	95.20	0.43 (0.17)	0.17 (0.07)
T 4.3	93.25	0.43 (0.14)	0.16 (0.06)

Appendix 3 shows that the concentrations of Pb and Cd ranged from undetectable levels (rounded off to zero) to 0.9 mg/l and 0.30 mg/l, respectively. From Table 6.7, the mean concentrations of Pb and Cd applied to the plots were 0.44 mg/l and 0.17 mg/l respectively. A comparison of the mean values of Pb and Cd in the table and the legislated limits (0.01 and 0.05 for Cd and 5 and 20 mg/l for Pb for long-term irrigation and short-term irrigation respectively (Table 4.1) shows that Pb levels were within legislated limits. However Cd levels were predominantly higher than the legislated limits and the mean value was 3.4 times the long-term legislated level.

Average increases in metal concentrations in soil and grass above the levels in the control are presented in Table 6.8.

Table 6.8: Average increase in profile Pb and Cd levels above levels in the control (mg/kg)

Treatment	Soil Pb concentrations		Pb concentrations in grass		Soil Cd concentrations		Cd concentrations in grass	
	Average	Increase	Average	Increase	Average	Increase	Average	Increase
Control	0.466	0.000	4.090	0.000	0.020	0.000	0.404	0.000
Treatment 1	0.881	0.415	5.890	1.800	0.094	0.074	0.710	0.306
Treatment 2	1.196	0.730	6.850	2.760	0.086	0.066	0.800	0.396
Treatment 3	1.202	0.736	7.290	3.200	0.042	0.022	0.900	0.496
Treatment 4	11.317	10.851	15.330	11.240	0.633	0.613	1.700	1.296

Average depths of irrigation: Treatment 1: 257.2 mm, Treatment 2: 493.8 mm, Treatment 3: 977.7 mm, Treatment 4: 960.2 mm

There was a progressive increase in bio-available Pb and Cd with treatment, up to 0.74 mg/kg Pb and 0.07 mg/kg Cd for treatments on previously unpolluted soil. For these treatments, maximum increases in levels of the metals in grass were 3.2 mg/kg Pb and 0.5 mg/kg Cd.

6.5 Discussion

The maximum accumulation of Pb of 15.33 mg/kg in grass was below the 40 mg/kg legislated in U.K for pasture grass. Although the concentration of Pb in treated sewage over the 30 years of disposal could not be ascertained, the 2.6 mg/l, maximum level determined from Harare City Council data, 1.2 mg/l average level for the greenhouse experiment and 0.44 mg/l average level for the field experiment, were below the recommended level of 5.0 mg/l and therefore acceptable. These levels resulted in star grass accumulating non-toxic levels of Pb. It may be concluded that treated sewage disposal practices at Firle farm do not pose a hazard to cattle through accumulation of Pb in star grass. In contrast, the increase in soil bio-available Cd concentration caused by application of treated sewage with 0.17 mg/l (17 times the legislated level for long-term irrigation) led to accumulation of Pb to levels higher than recommended.

In the field the only significant regression models for Pb were the model for the first crop, $y = 0.5162x + 0.5493$ and the model representing average conditions over an 11-month period, $y = 0.3949x + 0.788$. Therefore, the single variable regression model of Pb for the first crop was not applicable to re-growths while the latter was. Thus the model $y = 0.3949x + 0.788$, which was strongly significant ($p \leq 0.05$) could be considered for use in predicting the concentration of Pb in star grass on the basis of bio-available soil concentrations extracted using the procedures recommended by McGrath and Cegarra (1992). The use of this model could approximate field conditions where animals are grazing and therefore harvesting grass periodically, leading to new re-growths each time. The model predicts a bio-available soil concentration limit of 115.2 mg/kg for a concentration of 40 mg/kg in star grass.

In this component of the study, a significant single variable regression model for Cd based on field data for each grass harvest could not be obtained. This outcome suggests that under field conditions of this study, there were other factors that needed to be incorporated into the model to improve the Cd models in order for prediction of metal concentration in grass to be possible for each harvest.

Sample et al (1998) obtained significant model fits of ln (*total soil concentration*) and ln (*above ground plant concentrations*) after including pH and calcium (Ca). Similarly the decline in the strength of correlation for both Pb and Cd with the re-growths was probably caused by other soil factors that needed to be incorporated in the models. US Department of Energy (1998) improved single variable regression models of natural $\log_{10}$ (*above-ground plant tissue concentration of Pb*) versus total metal concentration in soils by incorporating pH in a multiple regression model for Pb. In this study an improvement in the model fit was obtained by using average values of bio-available soil concentrations from all soil samples and average grass concentrations for all grass samples harvested over a period of 11 months. Using this approach, a model in which the correlation between x and y was strong but fell marginally short of being significant was obtained. If the strength of correlation in the model $y = 0.363x + 0.2987$ would be considered high enough to permit rough predictions, then for a Cd limit of 1 mg/kg in grass the soil bio-available limit would be 0.20 mg/kg.

The general lack of clear relationship between soil pH and depth in this component of the study was similar to the findings in section 4.4.2 of chapter 4 where there was also no clear association between pH and total metal concentration. The marginal increase in the pH of treatments receiving treated sewage, above that of the control was also consistent with the findings of soil charaterisation (chapter 4). These findings were also in agreement with observations by Nyamangara and Mzezewa (1999) that indicated increases in pH of surface horizons of treatments that received sludge over treatments that did not receive from 6.8 to 8.0 respectively. The increase in pH and the stronger correlations between pH and bio-available Pb and Cd are attributed to organic matter added to the soil through treated sewage. This observation was also noted in chapter 4 and is in agreement with observations by MacGrath and Lane (1989).

The CEC of the control in this component of the study fell between 1 and 2 cmol$_c$kg^{-1}. This is consistent with the base levels of CEC obtained in the control during soil characterisation (Table 4.3). In treatments 1 to 3 CEC increased slightly to about twice the base levels in the control suggesting that the metal ions that were being added to the soil through treated sewage could be responsible for the increase. The CEC of treatment 4 had the same order of magnitude as the values presented in Table 4.3, suggesting a high association between CEC and years of application of treated sewage.

There was a general lack of clear and consistent association between soil depth and CEC in all treatments except treatment 4 and the 4th re-growths of treatments 2 and 3. Since the r^2 values of treatment 4 and 4th re-growths of treatments 2 and 3 were negative, their top soil horizons had higher CEC than the lower layers. This suggested that there was accumulation of cations in the top horizons. This argument is supported by the fact that these treatments received the highest quantity of treated sewage, and therefore had a chance of accumulating more cations from treated sewage. The gradual increase in CEC suggests an association in the amount of treated sewage added to a treatment and the CEC. The stronger correlation coefficients of CEC and bio-available Pb and Cd in treatment 4 compared to all other treatments could be attributed to the higher CEC shown in Table 6.2. The stronger negative correlation of soil depth and CEC in treatment 4 suggests that the cations including Pb and Cd were largely located in the top soil horizons where the grass roots could easily access them. The correlation of clay content and soil Pb and Cd was generally weak except for treatment 4 where clay content negatively correlated to soil Pb, possibly due to accumulation of clay in top soil layers over time.

This study offered some lessons relating to field experiments. Practical limitations, particularly pump breakdowns at the Firle Wastewater Treatment Plant, reduced the total amount of irrigation application. In this experiment, Treatments 1 to 4 received on average 257.2 mm, 493.8 mm and 977.7 mm and 960.2 mm respectively, although higher amounts could have been applied. However the results obtained after applications of these amounts suggest that the amounts of treated sewage did not limit the adequacy of the data for modelling. Furthermore, the concentrations of the metals could not be pre-determined prior to irrigation under field conditions. However measuring the metal levels in treated sewage at each irrigation event and running the field experiment for a long time circumvented this limitation. It was assumed that this would even out variation in levels of metals amongst applications to provide an average situation representative of reality.