CHAPTER 5

ASSSESSMENT OF LEAD AND CADMIUM UPTAKE BY *CYNODON NLEMFUENSIS* UNDER REPEATED APPLICATION OF TREATED WASTEWATER

5.1 Introduction

This chapter describes responses in yield and metal content of star grass to increasing concentrations of Pb and Cd added to a sandy soil as single and mixed inorganic metals in combination with treated wastewater irrigation. This component of the study was intended to determine whether star grass was a high accumulator of Pb or Cd or both and if so, the level of the metal in a sandy soil and star grass, at which toxicity occurs. Therefore it sought to determine the capacity of star grass to accumulate Pb and Cd under conditions where the concentrations of single metals in the soil were raised and under conditions where the concentrations of both metals in the soil were raised. In the latter case, the investigation was focussed on determining interactions of Pb and Cd, in a sandy soil and in star grass because these were unknown.

This chapter also presents soil-vegetative tissue metal uptake models that were developed for predicting grass response to increases in Pb and Cd in the soil. Measured bio-available metal levels in the soil, metal content in grass and yield of grass were the key inputs in the models. The models were used to estimate: (1) the critical metal levels in the soil at which the yield of grass declined (2) the toxicity level in grass (3) and also the critical bio-available metal content of the soil at which the maximum recommended metal content of grass was reached.

The following assumptions were made in setting up the experiment for this component of the study. Firstly, it was assumed that increasing the concentrations of Pb and Cd in the soil would lead to: (1) increasingly higher metal uptake by grass and (2) negative effect on the yield at some threshold concentrations of the metals in grass. It was assumed, therefore, that the parameters of yield, soil metal content of grass and soil bio-available concentration would form bi-variate relationships such that the responses of dependent parameters to independent parameters could be predicted. Secondly, repeated application of treated sewage to soils amended with inorganic Pb and Cd was assumed to simulate field conditions in which treated wastewater containing occasional high doses of Pb and Cd would be disposed of on soils. The situation where single metals were added to the soil was assumed to relate to occasions when high loads of either metal would be released into the treatment system, while the situation in which mixed metals were added to the soil was assumed to represent high doses of both metals.

5.2 Objectives

The specific objectives of this component of the study were: (1) to determine the extent to which star grass accumulates Pb and Cd, (2) to develop yield and metal uptake response models in relation to bio-available soil concentrations, (3) to estimate the critical grass metal content (toxic level) at which Pb and Cd reduce yield of grass and the corresponding critical bio-available levels in the soil, (4) to estimate soil Pb and Cd levels corresponding to maximum recommended levels of Pb and Cd in pasture grass and (5) to determine interactions of Pb and Cd in a sandy soil and star grass subjected to co-presence of high levels of inorganic metals.

5.3 Detailed methods and materials

5.3.1 Experimental set-up

The experiment was carried out in a greenhouse at the University of Zimbabwe, using soils taken from Churu farm, previously uncontaminated grass from Domboshava farm and low quality treated effluent and sludge discharged from the Firle Treatment Plant. The grass from Domboshava was tested for Pb and Cd and found to have undetected levels of the two metals. Two approaches were used in the greenhouse experiment. The first approach was to investigate the effect of each metal on levels in the soil and grass by elevating each metal in the soil using its inorganic salt. This produced two sets of treatments, one for Pb and the other for Cd. The second approach was to investigate interactions of the two metals in the soil and grass by elevating their levels using combinations of the two salts in each treatment. Since the soil concentrations of Pb and Cd at which the yield of star grass would be affected were not known, guidelines on maximum permissible total soil concentrations were used to decide on concentrations to add to the soil.

In single Pb treatments, five levels of Pb treatment were experimented with. The first level was the control. It did not receive inorganic Pb. The second level (considered as the lowest level of Pb addition) did not receive inorganic Pb but it received Pb through addition of sewage. The treatment was denoted E&S and is referred likewise in the text of subsequent sections and ES in graphs. The third level was pegged at the same level as the maximum acceptable total soil concentration (300 mg/kg) from literature. The fourth level received double the maximum acceptable level while the last level received 4 times the acceptable Pb level.

In single Cd treatments, seven levels of Cd were adopted to increase reliability of the results, given that the CdS (which was the only Cd salt available in the country at the time) that was used in the experiment was known to have low solubility. The first level was the control. It did not receive inorganic Cd. The second level (considered as the lowest level of Cd addition) did not receive inorganic Cd but it received Cd through addition of sewage. The control and E&S treatments mentioned under single Pb treatments also served as part of single Cd treatments. The added inorganic Cd levels were pegged at 10, 20, 40, 60 and 80 times the maximum acceptable total concentration in the soil (1 mg/kg).

In mixed treatments, inorganic Pb and Cd were mixed in 3 more treatments to mimic field conditions where Pb and Cd would be present together in sewage as confirmed by the metal data from the City of Harare. Inorganic Pb and Cd were mixed in the order 300mg/kg Pb combined with 10 mg/kg Cd up to 1 200 mg/kg combined with 40 mg/kg. The 3 lower levels of inorganic Cd applied in single treatments were used in the combinations because, though still very high, they were relatively closer to levels detected at Firle farm than the higher levels. The control and E&S treatments mentioned above also served as part of mixed Pb and Cd treatments.

Soil from the control site at Churu farm was excavated, mixed several times into one large heap and passed through a 10mm sieve to remove large stones and grass debris. The soil was excavated from the same area (described in chapter 4) where samples were taken and tested for heavy metals. It was then packed into 79-litre pots. The packed pots were laid out randomly (Appendix 2) inside a greenhouse to exclude rainfall from interfering with irrigation applications. The randomised block design layout of the pots had one replicate of each treatment in every one of the 3 blocks.

5.3.2 Grass establishment

In each pot, seven 15cm long stems of uncontaminated star grass, each with a node, were planted. In order to eliminate nutrient deficiency, single applications of super phosphate ($Ca(H_2PO_4)_2 + CaSO_4$), potassium sulphate (K_2SO_4) and ammonium nitrate (NH_4NO_3) were added to each pot at 600, 100 and 100 kg/ha respectively. Water was applied to the grass for a period of 3 weeks to establish the crop. The treatments were then allocated to the pots at random, after which the soil was enriched with $Pb(NO_3)_2$ and CdS.

5.3.3 Soil treatment and irrigation application

The single Pb treatments received 198.99, 397.98 and 663.3g of $Pb(NO_3)_2$ so as to elevate the soil concentration by 300, 600 and 1 200 mg/kg Pb respectively. The treatments were referred to as Pb_{300}, Pb_{600} and Pb_{1200} respectively. Cadmium single treatments received 1.29, 2.58, 5.16, 7.74, 10.32 g of CdS, thereby elevating the soil concentration by 10, 20, 40, 60 and 80 mg/kg Cd respectively. These were denoted Cd_{10}, Cd_{20}, Cd_{40}, Cd_{60} and Cd_{80}. Combined Pb and Cd treatments received 300 mg/kg Pb and 10 mg/kg Cd, 600 mg/kg Pb and 20 mg/kg Cd and 1200 mg/kg Pb and 40 mg/kg Cd, and they were denoted $Pb_{300}Cd_{10}$, $Pb_{600}Cd_{20}$ and $Pb_{1200}Cd_{40}$. The combined treatments were elevated using 300 mg/kg Pb combined with 10 mg/kg Cd, 600 mg/kg Pb combined with 20 mg/kg Cd and 1200 mg/kg combined with 40 mg/kg Cd. The treatments were denoted $Pb_{300}Cd_{10}$, $Pb_{600}Cd_{20}$ and $Pb_{1200}Cd_{40}$.

The metal treatments were added to the pots in a single dose 3 weeks after planting the grasses. The inorganic chemicals were shaken in water and added to the soil surface together with an irrigation application of 2.5 l of treated sewage and 2.5 l of water. Two squeeze bottles, one for Pb and another for Cd, were used to apply Pb and Cd treatments directly onto the soil surface and minimize chances of contamination of the grass shoot and inside walls of the pots. The order of applying the metals was low concentrations to high concentrations. After each treatment, the bottles were rinsed using de-ionised water, before applying the next treatment.

Once established, 5.5 l of irrigation water (20 mm net depth) was applied per pot every 3.5 days in order to meet a peak water duty of 5.5 mm/day at an estimated 70% water application efficiency. A total of 63.5 l (324 mm) of water were applied to each of the 3 control pots while 23.5 l (120 mm) of treated effluent and sludge and 40 l (204 mm) were applied to each of the remaining 36 pots over a period of 45 days.

Irrigation water was applied to the soil using a 5 l plastic container (graduated at 0.5 l intervals for the purpose) via a 32 mm diameter flexible hose. During application, the detachable hose covered the mouth of the container. It was used to direct the application onto the soil surface and minimize human contact with treated sewage. The container was rinsed with water after irrigation of all pots.

After harvesting the first crop, the re-growth was irrigated with the same quantity of water over a 45-day period. The 45-day period before harvest was adopted following Miller's

(1974) observation that most decomposition of organic matter in sludge occurred within one month of adding sludge to soils. It was assumed that most of the organic matter added to the pots would have decomposed and released cations, including Pb and Cd into soil solution.

5.3.4 Soil sampling and testing

Soil samples were taken from the pots at depths of 0-10, 10-20, 20-30 and 30-40 cm, using a soil auger, one day after harvesting the grass re-growth. After removing plant debris the samples were air-dried and passed through a 2 mm sieve. 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. No soil samples were taken at the time of harvesting the first crop to avoid disturbing the soil.

5.3.5 Grass sampling and testing

Two grass harvests were made from each pot. The first crop was harvested 45 days after soil enrichment with Pb and Cd and the re-growth 45 days after the first harvest. All the grass in each pot was harvested to constitute a sample per harvest. The grass was cut at 5 cm height off the soil surface, washed using de-ionised water, oven dried at 65 ^{0}C to constant weight. After oven-drying above-ground tissue the yields of grass were measured by weighing and samples were taken for metal testing. The samples were ground and sieved through a 0.1 mm sieve, 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. The sample of the grass that was taken during planting was subjected to the same metal extraction process prior to determination of levels of Pb and Cd using atomic absorption spectrometry.

5.3.6 Sewage effluent and sludge collection and testing

Effluent and sludge for irrigation and metal content testing was collected seven times at the point of direct disposal onto the field. The levels of Pb and Cd in effluent and sludge were determined by atomic absorption spectrometry (Department of Environment, 1989) after extraction with HCl and concentrated HNO_3.

5.3.7 Data analysis

The data obtained in this experiment was quantitative continuous data (that is data that has any of the infinitely real numbers). There were two groups of variables identified, one associated with Pb and the other associated with Cd. In each group (1) soil bio-available concentration, (2) grass metal level and (3) yields of grass were the key variables for this analysis. All the variables involved were random continuous variables. Random continuous variables are observations or measurements that can assume any of the infinitely many non-negative real numbers (such as 0.1, 0.01 etc) and tend to vary in a haphazard manner due to natural variation. Amongst these variables, soil bio-available metal concentration was considered to be an independent/predictor/explanatory variable to grass metal content and yield. Furthermore, metal content in grass was considered as a predictor variable to yield. Given that the distribution of uptake of metals from the different soil horizons was not known, the average soil profile concentration was used. Sample et al (1998) ignored metal distribution within soil profiles in models to validate metal uptake by earthworms and assumed that the data they used represented average profile concentrations and still obtained significant regressions.

In single metal treatments of Pb and Cd the bi-variate relationship was considered to be appropriate for analysing relationships among the continuous variables. A bi-variate relationship is one in which two different continuous random variables have an association. In mixed treatments, the multi-variate approach was considered appropriate for analysing the influence of each of Pb and Cd on grass yield. Although there were 3 sources of total Pb and Cd, namely background metals in soils, metals added through treated sewage and inorganic metals added to soils, it was considered unnecessary to investigate the contribution of each source since all the sources contributed to the bio-available soil metal levels measured.

Measured data on bio-available metal content of soils, metal content of grass and yields of grass were tested for normality first. Since the data were not normally distributed, they were transformed to $\log_{10}$, to normalise them, before analyses to determine correlation coefficients and levels of significance of treatments on soil and grass metal contents and yields. The Analysis of Variance (ANOVA) was used to test the significance of differences in metal levels and yields amongst treatments. Soil bio-available metal levels were compared to the levels of the metals in the re-growth since soils and grasses were sampled at the same time.

Regression techniques are generally used to draw up relationships between variables and to estimate parameters in the regression function (model). Simple regression was therefore used to estimate and predict the responses of yield and metal concentrations to bio-available soil concentrations and the response of yield to level of metal content in grass. Correlation is a method used to measure the degree of linearity of a relationship between random variables, which have a joint distribution. The statistic for measuring correlation is the product moment correlation coefficient or simply correlation. It is also called Pearson's product moment correlation and is denoted r^2. Correlation and regression are important tools that show the degree of relationship between two variables (Canhao and Keogh, 2001). Two continuous random variables are correlated if the variables are related (associated) in such a way that the value of one is indicative of the value of the other. Simple correlation was used to measure the degree of strength of relationships between any two variables.

The different parameters (variables) were plotted against each other to provide the best-fit regression lines. The parameters in the regression lines (such as slopes and intercepts) were crosschecked using the Method of Least Squares (Canhao and Keogh, 2001). The standard errors of each of the parameters were estimated, so as to establish the confidence intervals of the parameters. The following equations were used for the purpose:

Confidence interval for a parameter = parameter estimate + t(s.e. $_{\text{parameter}}$)…equation 4

Where: s.e = Mean Square value of the error (MSE)

t = the 97.5th percentile of a t-distribution with n-2 degrees of freedom

MSE = s^2_{xy}= 1/(n-2) *{SS_{yy}-SP_{xy})^2/SS_{xx}….equation 5

Where n = sample size

SS_{yy} = sum of squares of *y*

SS_{xx} = sum of squares of *x*

SS_{xy} = sum of products of x and *y*

5.4 Results

5.4.1 Bio-available Pb and Cd content of soils

Table 5.1 shows a general increase in mean bio-available levels of Pb and Cd with increase in the level of treatment. Bio-available Pb increased from 2 mg/kg in the control to a maximum of 343.7 mg/kg and Cd increased from 0.06 mg/kg to 0.47 mg/kg in single treatments. In single metal treatments, analysis of variance showed that treatment of the soil with inorganic Pb and Cd combined with applications of a mixture of sludge and effluent significantly increased the mean bio-available Pb ($p \leq 0.001$) and Cd ($p \leq 0.05$) content of the soil profile. Comparison of means of treatments showed that soil levels in Pb_{300}, Pb_{600} and Pb_{1200} were significantly ($p \leq 0.1$) different and higher than in the control and E&S. Differences in the later were insignificant.

There was a significant difference ($p \leq 0.1$) between the mean levels of Cd_{60} and Cd_{80} on one hand and the rest of the treatments on the other. The variation amongst the latter was insignificant. Mean Pb levels in mixed treatments showed trends similar to those in single treatments. The mean level of Cd in $Pb_{1200}Cd_{40}$ was significantly ($p \leq 0.01$) higher than in the rest of the treatments.

5.4.2 Extraction capacity of star grass

Table 5.1 shows a general increase in uptake of Pb and Cd with increase in bio-available soil metal concentration. There was a general decline in metal uptake from the first grass crop to the re-growth. This component of the study found that the maximum uptake of Pb and Cd by star were 4 592 mg/kg (13.66 kg/ha) and 17.67 mg/kg (0.13 kg/kg) Cd, respectively. The maximum uptake of Pb was recorded in the treatment that received the highest level of added Pb in the first crop of the single treatments. In contrast, the highest recorded uptake of Cd (17.67 mg/kg) occurred in the re-growth of the mixed treatments. In single Cd treatments, the highest recorded uptake of Cd was only 8.67 mg/kg (0.09 kg/ha), about half of the uptake in mixed treatments.

In combined treatments, the highest uptake of Pb of 1 681.33 mg/kg that occurred in $Pb_{1200}Cd_{40}$ in the first crop was far lower than the 4 592 mg/kg registered in the single Pb treatments. This uptake of Pb was accompanied by a relatively higher uptake of Cd of 16 mg/kg Cd in the first crop.

5.4.3 Grass metal content response to bio-available soil metal content in single metal treatments

Since soil samples were not taken at the time of harvesting the first crop of grass, it was assumed that a better relationship between bio-available soil concentrations and levels of metal in grass could be obtained using data for the re-growth crop. Soil samples were taken for testing at the same time as grass samples of the re-growth crop.

Table 5.1: Soil metal and grass concentrations, yields and metal extraction levels

Treatment	Mean bio-available soil profile metal concentration (mg/kg)	Mean grass concentration (mg/kg)		Mean grass yield (g/pot)	
		First crop	Re-growth	First crop	Re-growth
Lead single treatments					
Control	2.01 (0.21)	11.33 (4.65)	5.33 (3.54)	222.69 (9.19)	77.01 (13.29)
E&S	1.23 (0.08)	12.02 (3.54)	5.33 (1.41)	198.78 (26.41)	190.10 (20.18)
Pb_{300}	84.64 (0.16)	140. 03 (10.11)	14.33 (2.2.1)	250.03 (33.20)	245.3 (35.29)
Pb_{600}	180.84 (8.23)	288.01 (4.24)	36.66 (1.41)	225.40 (14.92)	235.26 (0.49)
Pb_{1200}	343.7 (15.43)	4 592 (155.89)	315.65 (47.34)	58.43 (1.91)	219.27 (13.68)
Cadmium single treatments					
Control	Nd	1.50 (0.04)	Nd	222.73 (7.51)	77.04 (12.63)
E&S	0.060 (0.02)	1.67 (0.57)	Nd	198.78 (17.21)	190.1 (16.35)
Cd10	0.026 (0.06)	2.33 (1.15)	1.67 (1.14)	256.72 (26.46)	227.71 (15.19)
Cd20	0.050 (0.023)	1.67 (0.57)	2.67 (1.15)	245.87 (21.01)	214.89 (9.76)
Cd40	0.068 (0.014)	3.67 (1.08)	3.00 (0.95)	230.88 (9.03)	239.57 (23.56)
Cd60	0.469 (0.051)	4.67 (1.52)	4.00 (1.00)	265.42 (1.35)	228.98 (13.27)
Cd80	0.200 (0.16)	8.67 (2.14)	5.67 (2.08)	200.83 (17.44)	198.34 (7.77)
Combined Pb and Cd treatments					
Lead					
Control	2.01 (0.21)	11.33 (4.65)	5.33 (3.54)	222.69 (9.19)	77.01 (13.29
E&S	1.23 (0.08)	12.02 (3.54)	5.33 (1.41)	198.78 (26.41)	190.10 (20.18)
Pb300Cd10	131.06 (1.72)	56.67 (16.56)	8.67 (4.49)	224.94 (31.76)	220.76 (30.19)
Pb600Cd20	237.09 (22.43)	307.00 (23.94)	43.67 (10.21)	160.10 (4.31)	218.93 (10.39)
Pb1200Cd40	382.00 (24.99)	1681.3 (193.85)	93.67 (16.16)	48.3 (31.22)	147.39 (24.08)
Cadmium					
Control	Nd	1.67 (0.04)	Nd	222.73 (7.51)	77.01 (13.29)
E&S	0.060 (0.02)	1.67 (0.57)	Nd	198.78 (26.41)	190.10 (20.18)
Pb300Cd10	0.025 (0.020)	4.33 (2.31)	3.01 (0.07)	224.92 (30.87)	220.76 (22.57)
Pb600Cd20	0.071 (0.054)	10.67 (1.53)	9.03 (3.11)	160.12 (4.31)	218.89 (10.39)
Pb1200Cd40	0.200 (0.012)	16.00 (1.73)	17.67 (2.51)	48.28 (41.22)	147.4 (34.08)

Nd: not detectable
() standard deviation

Relationship of bio-available and grass Pb content in single Pb treatments

Overall, the levels of Pb in star grass increased significantly with increase in soil bio-available Pb ($p \leq 0.001$ in the re-growth). Treatments that received inorganic Pb recorded a significant ($p \leq 0.001$) increase in accumulation of Pb in grass beyond accumulations in the E&S treatment and the control. There was no significant difference in Pb uptake between the control and E&S treatment in both crops. Therefore the elevation of soil Pb levels by 300 mg/kg, 600 mg/kg and 1 200mg/kg significantly increased Pb uptake, well above the levels taken up by the grass in the control and E&S treatment in both crops.

The relationship between soil bio-available Pb and grass Pb content is presented graphically in Figure 5.1. The best-fit trend line for Pb fitted a linear relationship with regression model shown in the figure. The correlation between $\log_{10}$ (*soil bio-available Pb*) and $\log_{10}$ (*grass Pb concentrations in the re-growth*) fitted a computed Pearson's r^2 value of 0.87 and a trend-line r^2 value of 0.75 against $r^2_{critical}$ = 0.87.

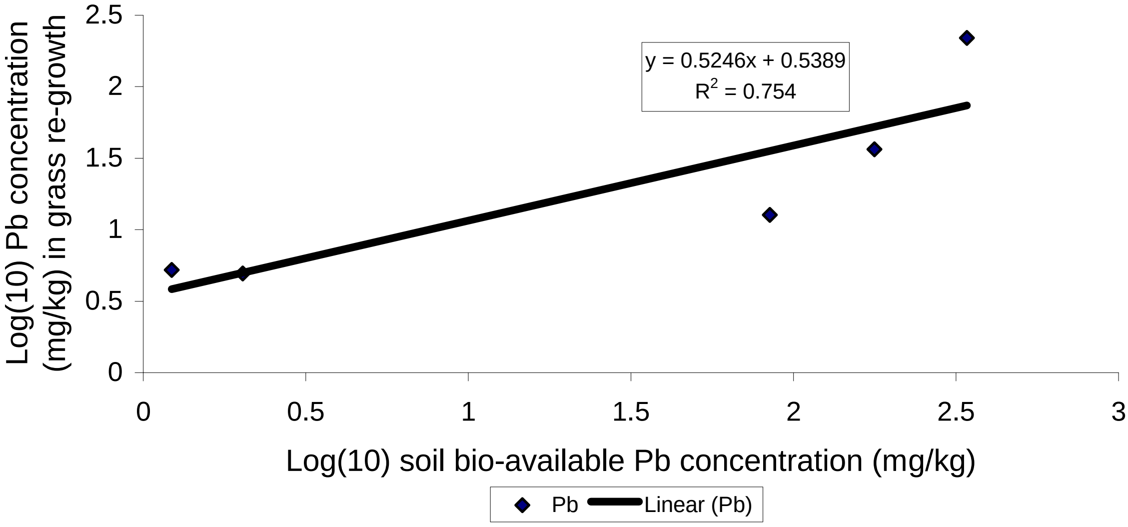

Figure 5.1: Log(10) soil bio-available level versus log(10) Pb level in grass in single treatments

Relationship of bio-available and grass Cd content in single Cd treatments

Cadmium content of grass re-growth increased significantly ($p \leq 0.001$) with increases in soil bio-available levels. There was a strong correlation between $\log_{10}$ (*soil bio-available Cd*) and $\log_{10}$ (*grass Cd concentrations in the re-growth*) with the computed Pearson's r^2 being 0.88 and r^2 trend-line being 0.78 against an $r^2_{critical}$ of 0.75. The trend-line fitted a linear relationship with the regression model shown in figure 5.2.

5.4.4 Yield response to Pb and Cd content of grass in single metal treatments

Yield response to Pb content of grass in single metal treatments

Figure 5.3 presents dose-response relationships between metal level in star grass and yields for the 2 harvests. The figure shows that there was an initial increase in yield followed by a gradual decline at higher concentrations of Pb. In the first crop, analysis of variance showed that yields of grass were significantly ($p \leq 0.001$) reduced due to the increase in the level of Pb in the grass. Comparison of means showed that this decrease was most significant ($p \leq 0.01$) in

Pb_{1200} compared to the rest of the treatments.

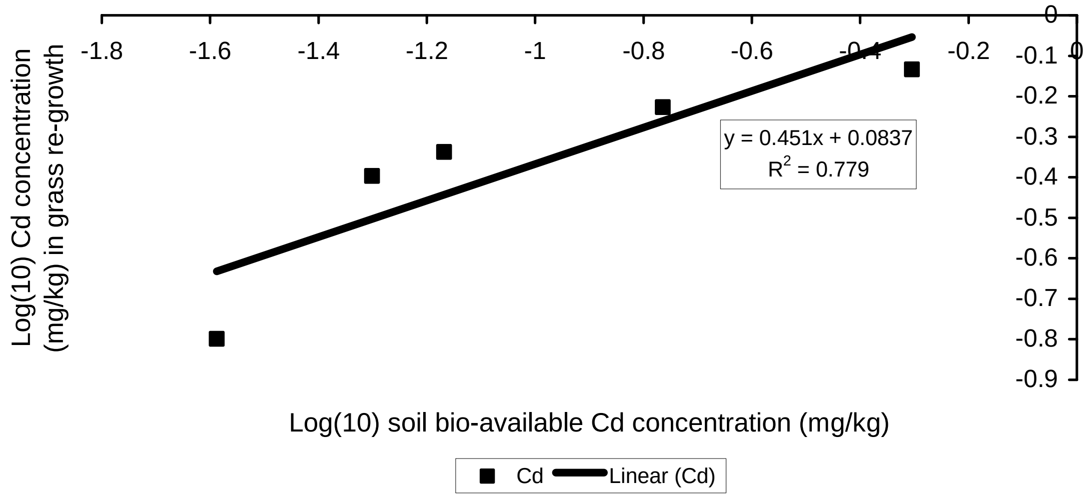

Figure 5.2: Log(10) bio-available Cd levels versus log(10) Cd levels in grass in single treatments

The differences in mean yields in the latter were insignificant. In the re-growth, yields also significantly ($p \leq 0.01$) declined as Pb content in grass increased. Comparison of means showed that the re-growth of the control had a significantly ($p \leq 0.01$) lower yield than the rest of the treatments. The differences between the mean yields of the first crop and re-growth in treatments E&S, Pb_{300} and Pb_{600} were not significant. However, the yield from control of the re-growth declined by 65.4% compared to the yield from the control of the first crop while the yield of the re-growth increased by 275.5% compared to that of the first crop in treatment Pb_{1200}.

The best-fit regression model (Figure 5.3), is a non-linear (curvilinear) model, that provides for drawing tangents at the points where the yields started to drop to locate the critical concentrations of Pb in grass at which metal content starts to reduce yield. This point is the critical toxicity limit or toxicity threshold referred to in Figure 2.1, and discussed in detail in section 5.4.8. The Pb content strongly correlated with the yield of the first crop (computed Pearson's r^2 = -0.74 and r^2 = - 0.99 for the trend-line, while $r^2_{critical}$ = 0.87) and weakly with the re-growth (computed Pearson r^2 = 0.52 and r^2 = 0.55 for the trend-line).

Yield response to Cd content of grass in single metal treatments

Cadmium content in grass did not significantly affect the yields of the first crop but it significantly ($p \leq 0.001$) reduced the yield of the re-growth. Analysis of variance amongst treatments that received inorganic Cd showed that the yield of Cd_{80} significantly declined in both crops as a result of increase in the content of Cd in grass. Comparison of means showed that in both crops, Cd_{80} was significantly lower ($p \leq 0.01$) than in the rest of the treatments. In the re-growth, the control had a significantly ($p \leq 0.05$) lower yield than the rest of the treatments while Cd_{40} and Cd_{60} had significantly higher yields than other treatments. There was a 65% reduction in yield of the control from the first crop to the re-growth.

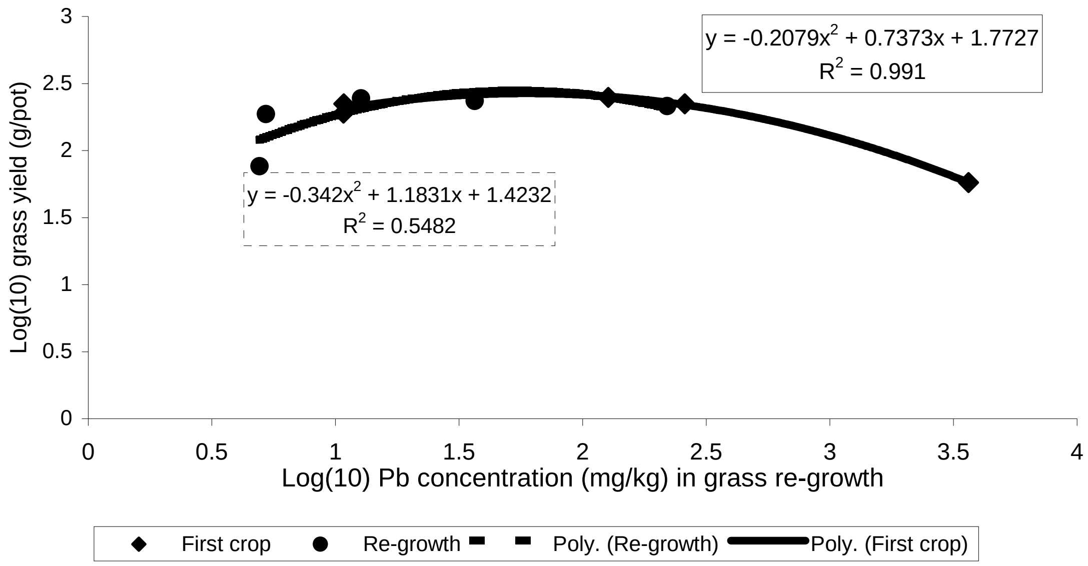

Figure 5.3: Log(10) Pb level (mg/kg) in grass versus Log(10) grass yield (g/pot) in Pb single treatments

A weak correlation (computed Pearson's $r^2 = 0.25$ and $r^2 = 0.20$ for trend-line, $r^2_{critical} = 0.75$) existed between the Cd content in grass and the yield of the first crop but there was a slightly stronger, but overall weak correlation (computed Pearson's $r^2 = 0.54$ and $r^2 = 0.51$ for trend-line, $r^2_{critical} = 0.75$) in the re-growth. Figure 5.4 presents a curving dose-response relationship of Cd concentration in grass and yield of grass. The threshold concentration of Cd in grass is located at the point (discussed in detail in section 5.4.8) of the curvature representing the highest yield. This is also the point at which yield starts to decline.

Yield response to soil bio-available Pb and Cd concentrations in single metal treatments

The soil bio-available levels of Pb and Cd were weakly correlated ($r^2 = 0.26$ for Pb and 0.47 for Cd) to the yields of the re-growths.

5.4.5 Interactions of Pb and Cd in mixed treatments

In order to analyse interactions of Pb and Cd, data from corresponding single and mixed treatments of Pb and Cd were included in the following analysis of yield response to combined Pb and Cd, bio-available metal level against treatment, levels in grass against treatment and bio-available metal level against metal level in grass.

Yield response to combined Pb and Cd

Analysis of variance for the effect of two independent variables on yield showed that combined Pb and Cd simultaneously and significantly ($p \leq 0.001$) reduced the yield of the first crop but had no significant effect on the yield of the re-growth.

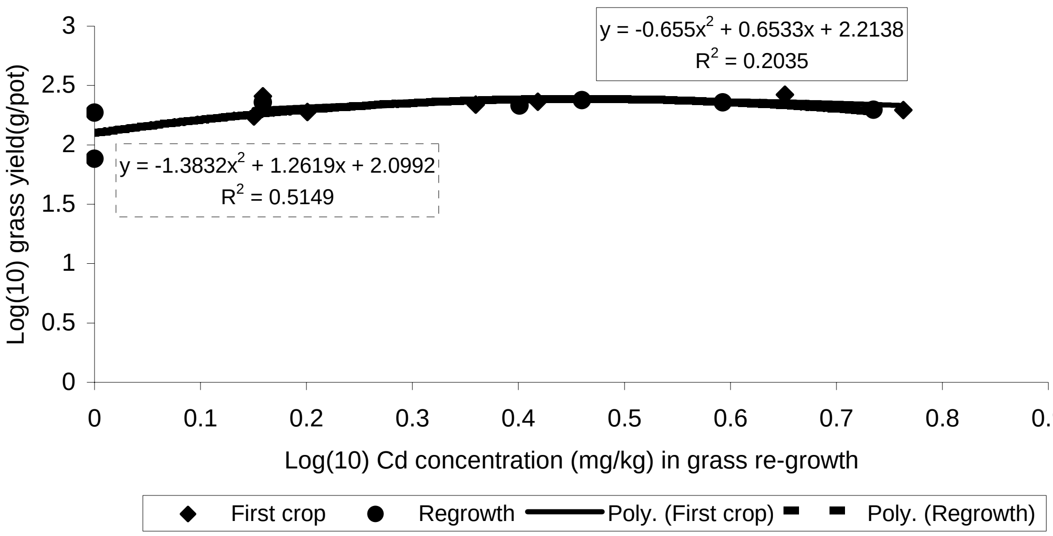

Figure 5.4: Log(10) Cd level (mg/kg) in grass versus log(10) yield of grass (g/pot) in single Cd treatments

Mixed Pb versus single Pb

Figure 5.5 presents the relationship between $\log_{10}$ (*soil bio-available Pb levels*) in single and mixed treatments and level of treatment. There was a significant ($p \leq 0.05$) increase in bio-available levels of Pb due to treatment in both the mixed treatments and single treatments that received the same Pb dose levels as mixed treatments. There was no statistical difference ($p \leq 0.05$) in the mean bio-available levels of Pb in mixed treatments compared to single treatments.

Figure 5.6 presents the relationship between $\log_{10}$ (*Pb levels*) in single and mixed treatments in grass and the level of treatment. There was no significant difference ($p \leq 0.05$) in Pb uptake between single and mixed treatments in the first crop and the re-growth that received the same level of Pb enrichment.

Figure 5.7 presents the relationship of bio-available Pb levels and Pb levels in grass re-growth. Unlike in single Pb treatments, the correlation between $\log_{10}$ (*soil bio-available Pb*) and $\log_{10}$ (*Pb concentrations in grass in the re-growth*) in mixed treatments was marginally weak (computed Pearson's $r^2 = 0.84$ r^2 trend-line = 0.70 and $r^2_{critical} = 0.87$).

Mixed Cd versus single Cd

Figure 5.8 suggests a gradual increase in bio-available soil Cd level with increase in the level of soil enrichment. However statistically, there was no increase ($p \leq 0.05$) in Cd in single treatments but there was a significant ($p \leq 0.05$) increase in bio-available Cd in mixed Pb and Cd treatments.

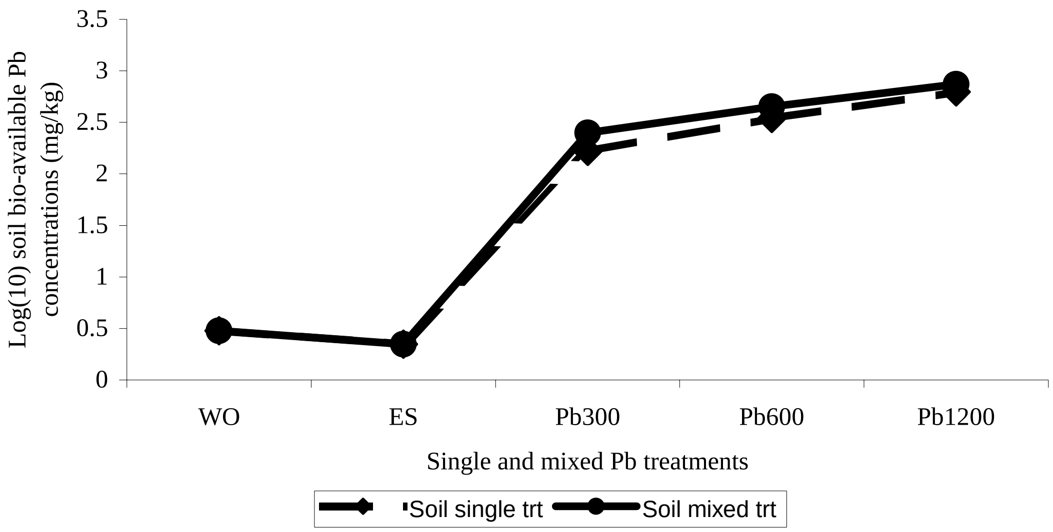

Figure 5.5: Effect of treatment on bio-available levels of Pb in single and mixed treatments

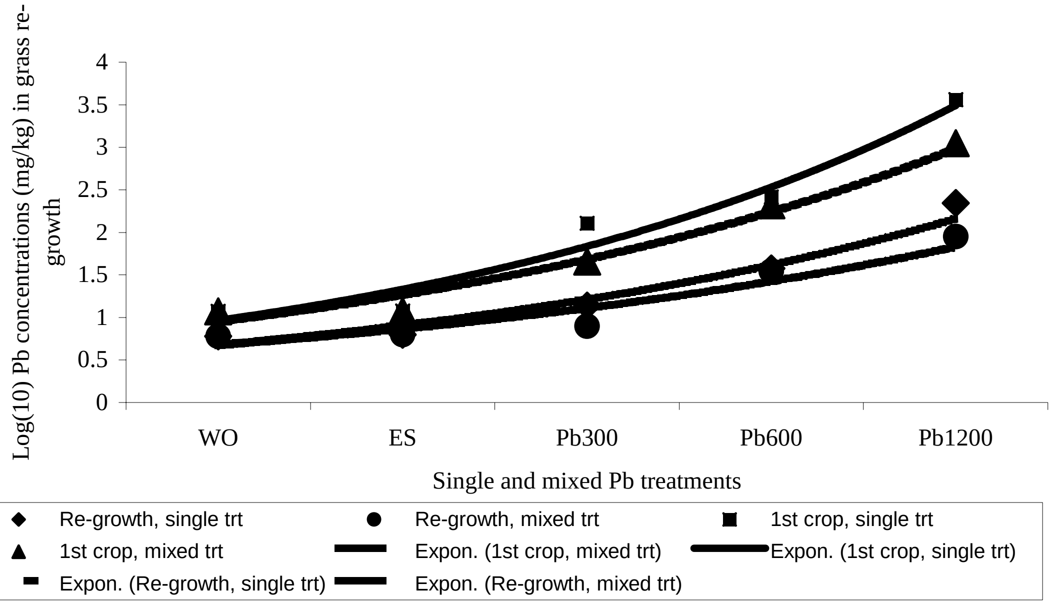

Figure 5.6: Effect of treatment on levels of Pb in grass in single and mixed treatments

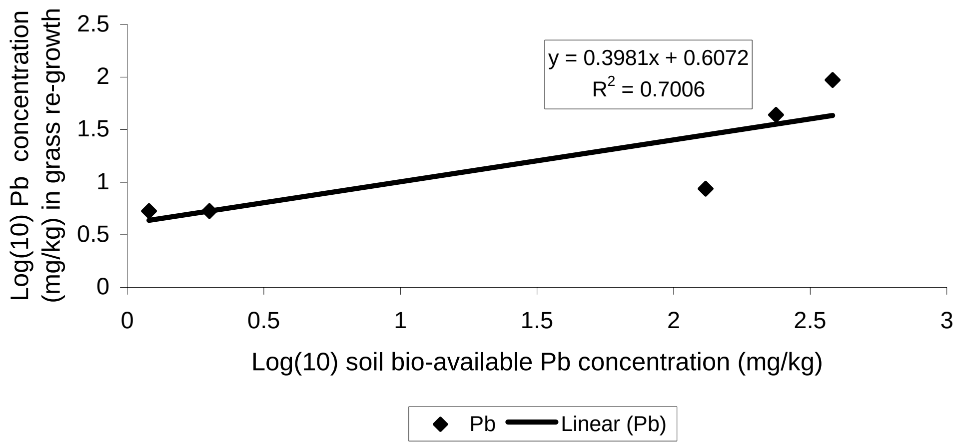

Figure 5.7: Log(10) bio-available soil Pb levels (mg/kg) versus log(10) Pb levels in grass re-growth (mg/kg) in mixed treatments

There was a significant ($p \leq 0.05$) increase in Cd level in grass with increase in soil enrichment level (Figure 5.9) in both single and mixed treatments and the two grasses.

Mixed treatments had significantly ($p \leq 0.05$) higher Cd levels than single treatments for the same doses of Cd. This is confirmed by the increasing divergence in levels of Cd in single treatments compared to mixed treatments (Figure 5.9), as treatment level increased.

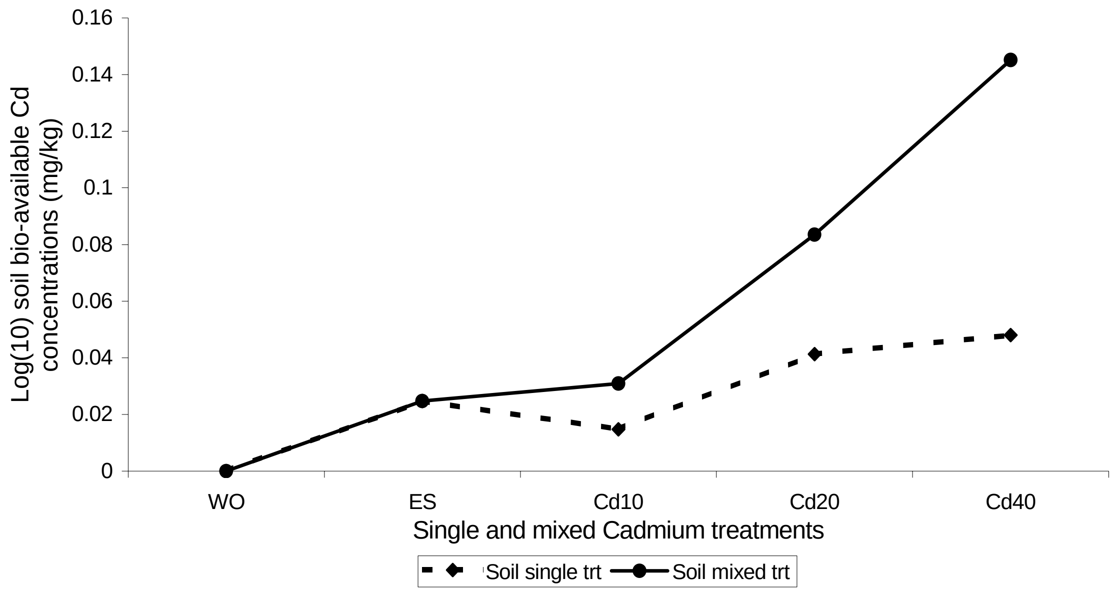

Figure 5.8: Effect of treatment on bio-available levels of Cd in single and mixed treatments

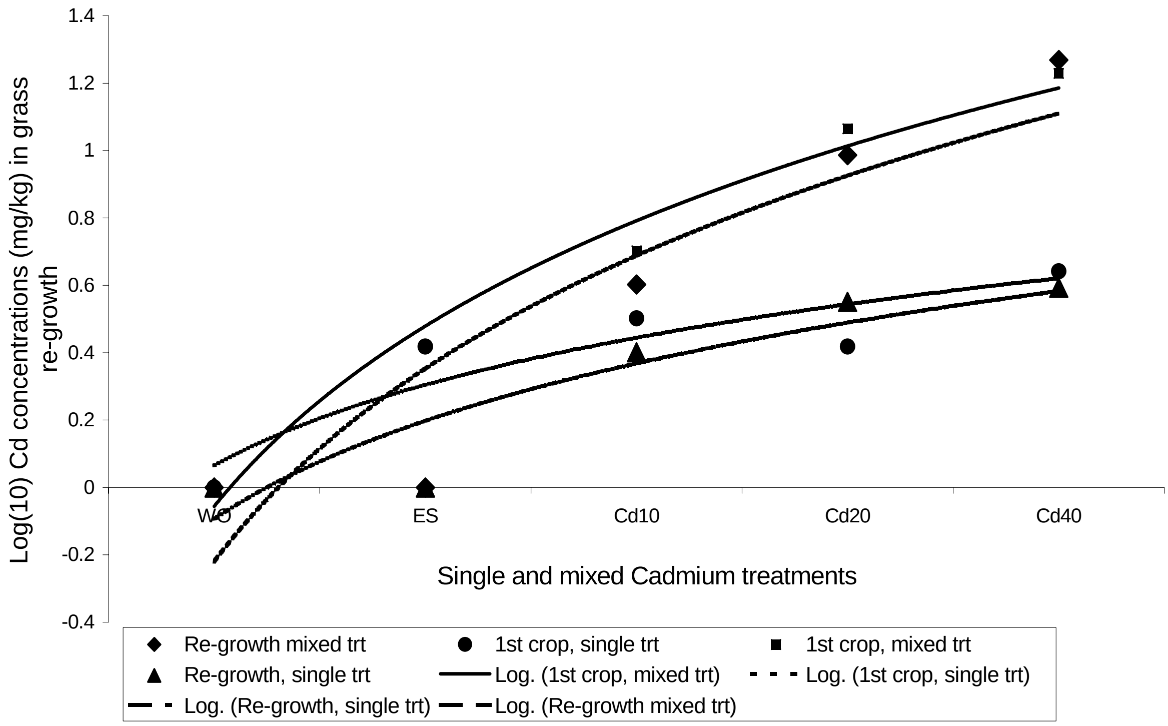

Figure 5.9: Effect of treatment on bio-available Cd levels in grass in single and mixed treatments

Figure 5.10 presents the regression model for $\log_{10}$ (*soil bio-available concentration*) versus $\log_{10}$ (*Cd concentrations in the re-growth*) for the points where Cd was detectable. The plot shows that there was a stronger correlation between $\log_{10}$ (*soil bio-available Cd*) and $\log_{10}$

(*grass Cd concentrations in the re-growth*) in mixed treatments than in corresponding single treatments (computed Pearson's $r^2 = 0.99$, r^2 trend-line = 0.98 and $r^2_{critical} = 0.75$).

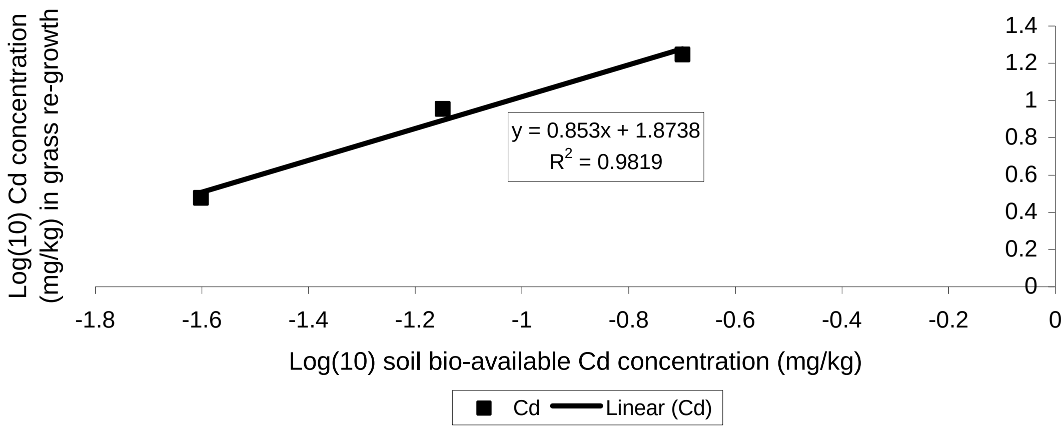

Figure 5.10: Log(10) bio-available Cd soil levels (mg/kg) versus log(10) Cd levels in grass re-growth in mixed treatments

5.4.6 Correlation of Pb and Cd in grass

Figure 5.11 presents the relative uptake of Pb and Cd in the re-growths of the single and mixed metal treatments. Correlation coefficients presented in this figure show that Pb and Cd uptake by the grass re-growths were more strongly correlated in mixed treatments than in single treatments (Pearson's $r^2 = 0.87$ and 0.76 for the mixed and single treatments, respectively). The regression coefficient increased from 0.39 in single treatments to 1.01 in mixed treatments signifying that the rate of uptake of Cd was higher, due to co-presence of Pb and Cd in mixed treatments.

5.4.7 Yield response to combined Pb and Cd

Combined Pb and Cd significantly ($p \leq 0.001$) reduced the yield of the first crop but not that of the re-growth. Analysis of variance showed that grass content of Pb and Cd simultaneously and significantly reduced the yield of the first crop ($P \leq 0.001$) but the effect was weaker on the re-growth. This is confirmed (Figure 5.12) by the strong correlation of the first crop ($r^2 = 0.99$ for Pb and 0.89 for Cd) and weaker correlation of the re-growth ($r^2 = 0.45$ for Pb and 0.51 for Cd). The models for the re-growth were considered weak due to the poor correlation between the yield and the metal content in grass.

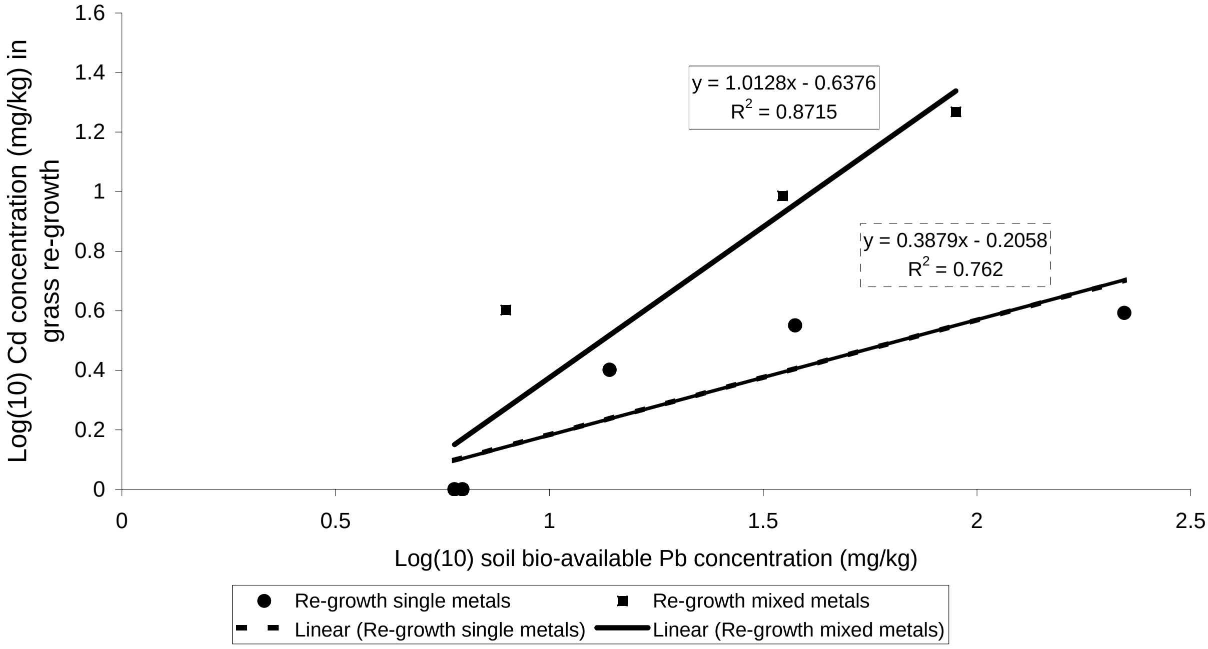

Figure 5.11: Correlation of metal contents of Pb and Cd in grass in single and mixed treatments

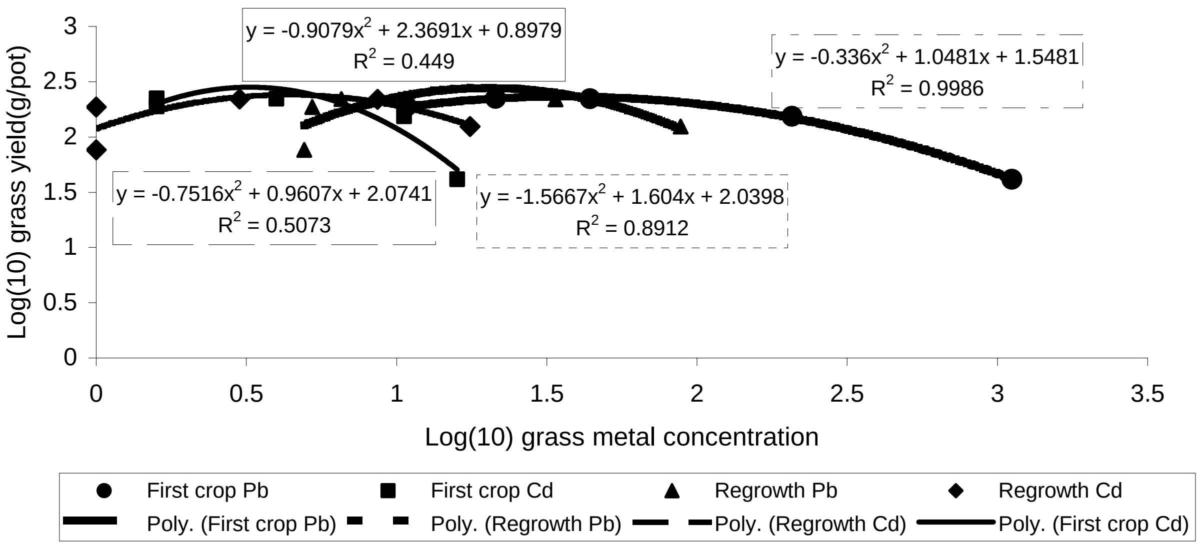

Figure 5.12: Yield response to concentrations of Pb and Cd in mixed Pb and Cd treatments

5.4.8 Yield, grass and soil metal content models and critical limits of Pb and Cd

Grass metal content versus grass yield models were used specifically to estimate toxic levels of metals in grass. For this purpose, data from single metal treatments were used. Figure 6.3 presents the following non-linear models of yield versus grass Pb concentrations:

$y = -0.2079x^2 + 0.7373x + 1.7727$ for the first crop and

$y = -0.342x^2 + 1.1831x + 1.4232$ for the re-growth.

The concentration of Pb at the peak yield (also the point when toxicity starts to reduce the yield) was obtained by equating the first derivative of $\log_{10}$ (*grass yield*) (y) with respect to $\log_{10}$ (*Pb concentration*) (x) to zero. This point occurred when x was 1.77 (equivalent to 58.88 mg/kg) and 1.73 (equivalent to 53.70 mg/kg) respectively. Substituting the values of x into the respective models gave predicted maximum yields of 266.69 and 279.25 g/pot respectively or an average of 272.97 g/pot for both crops.

Similarly, using the yield versus grass Cd content models of $y = -1.38819x^2 + 1.2609x + 2.0992$ and $y = -0.655x^2 + 0.6533x + 2.2138$ derived from Figure 5.4, the maximum yields occurred when x was 0.454 (equivalent to 2.84 mg/kg) and 0.499 (equivalent to 3.16 mg/kg) respectively for the first and second crops. The computed peak yields of 243.22 g/pot and 238.23 g/pot for the first crop and re-growth respectively, were close to each other in value, despite the fact that both models were not significant.

The model for metal uptake response to soil bio-available concentration in single Pb treatments was:

$y = 0.5246x + 0.5389$, where:

y = response variable = $\log_{10}$ (*grass Pb concentration, mg/kg*)
0.5246 = slope = regression co-efficient
x = $\log_{10}$ (*soil bio-available Pb concentration, mg/kg*)
0.5389 = regression intercept (y-intercept which tells the value of the dependent variable when the independent variable is zero).

Since grass uptake of Pb and Cd was a central aspect of this research, it was necessary to subject grass uptake models to further analysis to assess the strengths of the models. The following section presents the analysis of the strengths of these models for the re-growth crops. The mean square value of error used to compute standard errors for the slope, intercept and *y* value was 0.194. Using this value, the standard errors for the slope and intercept of this regression line were calculated to be 0.192 and 0.337 respectively. The confidence interval of *y* forms a confidence band on either side of the yield response regression line, since *y* values have different distributions at various values of *x*. The standard error for obtaining the confidence interval of *y* at any point along the regression line was obtained using the equation shown below:

Standard error of $y = sqrt\{S^2_{xy}[1/n + (x_i - mean\ x)^2/SS_{xx}]$….equation 6

Substituting the critical value of *y* of 1.73 (equivalent to 53.70 mg/kg metal concentration for the grass re-growth) in the regression model (Figure 5.1) of $y = 0.5246x + 0.5389$ gave an *x* value of 2.27, equivalent to a critical soil bio-available metal level of 186.21 mg/kg for Pb. Substituting the critical value of *x* of 2.27 and a mean *x* value (mean bio-available soil concentration of all treatments) of 1.42 in equation 6 gave a standard error of 0.207 in *y* at the critical point. This translates to a confidence interval of *y* of 0.42 (or 2.63 mg/kg) at that point (using a *t* of 2.03 at 95% confidence level). Therefore based on this model, the grass Pb concentration at which yield started to decrease was 53.70 ± 2.63 mg/kg and the soil bio-available concentration of Pb at that point was 186.21 mg/kg.

A similar calculation using single Cd treatments and the resulting model for Cd produced the following. Substituting the critical value of *y* of 0.499 (equivalent to 3.16 mg/kg content in grass) in the model, $y = 0.451x + 0.0837$, gave a soil bio-available metal level (*x*) of 0.92 (equivalent to 8.33 mg/kg of Cd). Substituting a value of 0.92 and a mean for all treatments of -1.051 gave a standard error of y of 0.11 at the critical point. Using a *t*-value of 2.014, the confidence interval of *y* (at 95% confidence level) was 0.22 (or 1.67 mg/kg). Therefore based on this model, the grass Cd concentration at which yield started to decrease was 3.16 ± 1.67 mg/kg Cd, while the bio-available soil Cd concentration was estimated to be 8.33 mg/kg.

The model for metal content response to soil bio-available soil concentration in combined treatments was $y = 0.3981x + 0.6072$ for Pb and $y = 0.853x + 1.8738$ for Cd. It was important to test whether the regression equations from single and mixed treatments were statistically different. The *t*-test for comparison of regression coefficients and the *y*-intercept was employed using the following equations:

$t_s = (M_1 - M_2)/s.e_{difference\ (slope)}$ ………..Equation 7

$t_s = (S_1 - S_2)/s.e_{difference\ (intercept)}$ ....Equation 8

where:

t_s is the t statistic for comparison of means given with the general equation t_s = difference between two means divided by the standard error of the difference

M_1 - is the slope of the model for single treatments

M_2 - is the slope of the model for mixed treatments

S_1 - is the y-intercept for single metal treatments

S_2 - is the y-intercept for mixed metal treatments

$s.e_{difference}$ = standard error of the difference between two means given as:

$s.e_{difference} = \mathrm{sqrt}\ (s^2_{(pooled)} * \{1/n_1 + 1/n_2\})$.....equation 9, where:

$s^2_{(pooled)} = \{(n_1-1)s_1^2 + (n_2-1)s_2^2\}/(n_1+n_2 - 2)$, in which n_1 and n_2 are sample sizes for single and mixed metals respectively and $n_1+n_2 - 2$ is the pooled degrees of freedom

It was hypothesised that no difference existed between the slopes of the regression equation of the single Pb treatment and the mixed Pb treatments. This hypothesis would stand, if $-2.306 < t_s < +2.306$ at 95% level of significance (two tail). The pooled degrees of freedom were 8. Calculations of $s.e_{difference}$ for the slope gave a value of 0.02 and $s.e_{difference}$ for intercepts gave 0.06 for the regression models of single Pb and mixed Pb.

Using equation 7, the t_s for the slope was calculated to be 6.3. Similarly t_s for the intercept was calculated to be - 1.14. Therefore the null hypothesis was rejected for the slopes but accepted for the intercepts. This outcome implied that there was a statistical difference between the slopes but there was no statistical difference between the intercepts of the regression equations of single and mixed Pb treatments. Therefore the regression equations predicted statistically the same concentration of Pb when $\log_{10}$ (*soil bio-available concentration*) was zero but different values elsewhere along the regression line.

To compare the regression relationships between single and mixed treatment, the null hypothesis, that no difference in slopes and intercepts of regression equations of the two existed, was adopted. Using an $s.e_{difference}$ of 0.003 for the slope and 0.004 for the intercept gave a t_s for the slope of 139.0 and a t_s for the intercept of -453.8. Since $t_{critical}$ for 10 degrees of freedom was ± 2.228 at 95% level of significance (two tailed), the null hypothesis was rejected in both cases. Therefore there was a difference in the regression coefficients and intercepts, hence regression equations. This confirms the positive impact of co-presence of Pb on uptake of Cd in mixed treatments. The impact is also confirmed by the increase in the regression coefficient from 0.39 to 1.01 (Figure 5.11) associated with relative Pb and Cd uptake from single treatments to mixed treatments.

5.4.9 Pb and Cd levels in effluent and sludge mixture

The overall mean concentration of Pb in treated sewage during the study period was 1.2 mg/l, comprising means of 1.32 mg/l for treated sewage applied to the first crop and 1.04 mg/l for treated sewage applied the re-growth (Table 5.2).

Table 5.2: Pb concentrations in samples of treated effluent and sludge mixture

Irrigation event	1	2	3	4	5	6	Total	Mean (mg/l)
First crop								
Volume applied/pot (l)	5.5	5	3	5	5		23.5	
Concentration (mg/l)	0.6	1.96	1.96	2.26	0.16			1.32
Quantity of Pb (mg)	3.30	9.80	5.88	11.3	0.80		31.1	
Second crop								
Volume applied/pot (l)	5.5	0.9	5.5	2.1	5.5	4	23.5	
Concentration (mg/l)	0.04	0.04	0.12	0.12	2.46	2.46		1.04
Quantity of Pb (mg)	0.22	0.04	0.66	0.25	13.5	9.84	24.5	

The levels exceeded the limit of 0.5 mg/l recommended for irrigation water (Table 4.1) but were below the maximum limit of 5.0 mg/l recommended for irrigation water (Ayers and Westcot, 1985). Cd levels in treated wastewater and Pb and Cd in samples taken from water used to supplement effluent and sludge mixture application were not detectable.

5.5 Discussion

5.5.1 Extraction capacity of star grass

This component of the study established that not only did star grass take up both Pb and Cd as does *C. dactylon* (Jonnalagadda et al, 2002), one of the few *Cynodon* grasses studied so far, but it did so in large quantities after exposure to high concentrations in soils. The study also showed that star grass could be ranked as one of the strongest accumulators of Pb among grasses. It extracted 4 592 mg/kg, from sandy soil that had a total soil concentration of approximately 1 200 mg/kg, equivalent to 343.7 mg/kg soil bio-available concentration of in this experiment. This extraction capacity was comparable to hyper-accumulating grasses like *Lolium perenne* (rye grass). Rye grass clippings from grass grown on a silt loam with a total

Pb soil concentration of 10006 mg/kg extracted 5 390 mg/kg while those from the same soil with a total Pb soil concentration of 5006 mg/kg extracted 2280 mg/kg (US Department of Energy, 1998). However the low yield at high concentrations limited its capacity for phyto-extraction. Baker, at al (2000) noted that hyper-accumulators should be high yielding.

Since it has been established that strong Pb hyper-accumulating plants such as Ipomea take up larger quantities of Pb than star grass, the results of this component of the study suggested that star grass could be classified as a medium Pb extractor. Ipomea accumulated 15 000 mg/kg in shoot tissue and 20 000 mg/kg in root tissue at Pb solutions of 500 mg/l and 1 000 mg/l respectively (Rhyne and Gosh, 2002). The fact that growth of the grass was severely retarded at 343.7mg/kg bio-available metal concentration suggested that the uptake of 4 592 mg/kg was close to the maximum uptake capacity of star grass. It should be noted that the high uptake of Pb by grass is chemically induced (due to addition of high levels of readily available Pb) and should not be confused with natural hyper-accumulation reported in most literature, quoting plants exposed to contaminated soil without artificially increasing bio-availability of Pb. McGrath et al (2002) noted that Pb hyper-accumulation is rare primarily because Pb is very insoluble in the soil, but Pb hyper-accumulates in plant shoots once its solubility is enhanced with synthetic chelates, such as EDTA.

The maximum uptake of 8.67 mg/kg Cd in single treatments and 17.67 mg/kg in mixed treatments suggests that star grass is a relatively small Cd hyper-accumulator when compared to hyper-accumulating plants such as maize. Maize accumulated 116.5 mg/kg at 125 mg/kg total soil concentration on a clay loam amended with $Cd(NO_3)_2$, (US Department of Energy, 1998). Notwithstanding the relatively small solubility of CdS that was used to enrich the soil in this component of the study, maximum uptake of Cd was significantly higher than the uptake capacities of other grasses reported in the database of bio-accumulators (US Department of Energy, 1998). The absence of clear signs of growth retardation in Cd treatments in this experiment suggested that the maximum extraction capacity of star grass was significantly higher than 17.67 mg/kg, implying that star grass was a strong accumulator of Cd among grasses.

Since the results of this study confirmed star grass has relatively good Pb and Cd extracting capacity they also implied the incompatibility of growing the grass for pasture on highly contaminated soils.

5.5.2 Grass yield response to Pb and Cd

The initial increase in the yield of star grass as Pb and Cd uptake increased was unusual, given that Pb and Cd are not plant nutrients. However, it could partly be attributed to uptake of sufficient vital elements (nutrients) available, largely from added fertilizers and treated sewage. Since Pb and Cd are non-essential (Johannesson, 2002; Elson and Haas, 2003) elements, the initial increase in yield cannot be attributed to them. The increase in uptake of Pb and Cd could be associated with a general increase in uptake of nutrients associated with higher growth. Polette et al (1997) postulated that the mechanisms that allow uptake of nutrients by plants could facilitate uptake of heavy metals, since the latter are generally indistinguishable from nutrients. These mechanisms are not yet well understood (Moolenar and Lexmond, 1999) although it is reported that Pb^{2+} may proxy for Ca^{2+} (Johannesson (2002) and Cd may be taken up in place of Zn (Elson and Haas, 2003). The weak correlations in the models suggest that factors other than Pb concentration influence the yield.

Yields obtained at low concentrations of Pb and Cd suggest accumulation of the metals at non-toxic levels. According to Clarkson (1986), accumulation of a heavy metal in tissue does not necessarily imply toxicity because inactive or storage depots in the plant are formed in the case of some metals. The decrease in yield with increasing uptake of Pb and Cd could be attributed to toxicity of the metals possibly due to substitution of vital nutrients and their metabolic functions because of the relative abundance of bio-available Pb and Cd compared to other ions in the soil. The drop in the yield of the control, from the first crop to the second crop, could be attributed to nutrient deficiency, while the increases in yields of the maximum treatments of Pb and Cd from the first crop to the second crop could be attributed to reduced toxicity resulting from the immobilization of the two metals by organic matter.

5.5.3 Metal uptake models and critical metal limits

Using the models generated in this study, the toxicity levels of Pb and Cd in grass and the corresponding bio-available levels in a sandy soil were estimated using data from the re-growth crop. The critical Pb and Cd concentrations in star grass at peak growth were computed to be 53.7 mg/kg and 3.2 mg/kg respectively. The corresponding critical bio-available metal levels were 186.2 and 8.3 mg/kg respectively. These limits refer to metal contents at which toxicity started to reduce yield and not the metal content limit desirable for field grazing by animals. The latter would necessitate taking account of the levels of metals taken up by animals through soil consumption during grazing, in addition to the levels in the grass. However the critical limits are considered applicable where the grass is cut and fed to animals directly.

Considering that the prescribed Pb and Cd limits in pasture grass are 40 mg/kg and 1 mg/kg, respectively (UK. Statutory Instrument No. 1412, 1995), this component of the study found the critical metal limits of star grass at peak growth to be far in excess of the prescribed limits. Substituting the logarithm of the prescribed grass Pb metal concentration of 40 mg/kg into the soil-grass concentration model gives a corresponding bio-available limit of 106.32 mg/kg. Similarly, for a limit of 1 mg/kg in grass the corresponding soil bio-available metal limit would be 0.65 mg/kg.

The study also found that star grass appeared healthy at the critical Cd concentrations and even beyond, making it difficult to recognize toxicity without testing for the metals. The lack of visible signs of toxicity at peak productivity implies that animals may graze on highly contaminated pastures that do not show obvious signs of pollution. The findings suggest that bio-available Pb and Cd limits would have to be managed at below 106 mg/kg Pb and 0.65 mg/kg Cd, respectively, so that the limits in pasture do not exceed the UK Statutory Instrument No. 1412 (1995) limits.

The relatively higher uptake of Pb and Cd in the first crop compared to the re-growth in single metal treatments suggested a corresponding higher bio-available concentration in the former. Although it was not possible to compare the bio-available metal concentrations of the soil for each crop because of the absence of the bio-available data corresponding to the first crop, this potential reduction in soil bio-available concentration could partly be attributed to the increase in organic matter and equilibration of the metals with the soil. Bak and Jensen (1998) attributed variations in bio-availability of metals, a common phenomenon in soils, to the existence of different binding sites that control sorption and de-sorption processes in the soil. The addition of organic matter through wastewater application could have increased binding sites thereby reducing bio-available metals in the soil and hence reducing uptake levels in the re-growth. It is also acknowledged that though necessary precautions were taken to minimise contamination of grasses during application of inorganic metals, any contamination that would have occurred unnoticed would have had the effect of increasing the levels of Pb and Cd in the first crop compared to the re-growth and it would be difficult to quantify and differentiate from uptake.

The bio-available Pb in the effluent and sludge treatment was lower than that in the control, despite addition of Pb through effluent and sludge. The apparent reduction in background bio-available Pb could be attributed to the sorption of Pb onto unsaturated binding sites of organic matter, leaving less in solution than in the control. However despite the differences in bio-available Pb between the control and effluent and sludge treatment, grass uptake remained similar in each harvest. According to Moolenar and Lexmond (1999), actual plant uptake in soil-crop ecosystems, not only depends on soil concentrations but also on the distribution of a chemical element in relation to other chemical species in the soil (also known as speciation) and mechanisms for root entry and translocation to aerial parts of the plant.

The absence of a significant difference in bio-available Pb levels between single and mixed treatments, that received the same dose of metal combined with treated wastewater, suggests that Cd did not influence the bio-available level of Pb in soils. The insignificance of the differences in Pb levels between the single metal and the mixed Pb and Cd treatments in star grass suggests that Cd does not influence uptake of Pb by star grass. This finding is in agreement with what Carlson and Rolfe (1979) found out in rye and fescue but contradicts the finding by Miller (1977) that Cd in the soil reduced uptake of Pb in *Zea mays* L. (corn). It also contradicts the finding by Carlson and Bazzaz (1977) that the uptake of Pb by plants (American sycamore in their case) increased due to raised concentrations of Cd.

The increase in grass levels of Cd in mixed treatments beyond the levels in single treatments and the strong correlation between Pb and Cd levels in the mixed treatments suggested increasing accumulation of Cd in star grass due to the co-presence of Pb. This finding is consistent with what Carlson and Rolfe (1979) found in rye and fescue and Miller et al (1977) found in corn. These findings on Pb and Cd therefore confirmed that different plant species accumulated different metal species to different levels. This component of the study, therefore established that co-presence of Pb and Cd did not affect the levels of Pb in the sandy soil and star grass but caused an increase in bio-available Cd in soils and Cd levels in star grass.

Regression of $\log_{10}$ (*concentrations of the chemical in grass*) versus the $\log_{10}$ (*yield of grass*) produced non-linear model fits with r^2 values that showed varying degrees of association depending on the metal and the crop (whether first crop or re-growth). In general the levels of association of $\log_{10}$ (*yield*) and $\log_{10}$ (*grass metal concentration*) in single treatments of Pb were stronger in the first crop compared to the re-growth, while the reverse was true for single Cd treatments. The higher r^2 value of 0.99 for the regression $y = -0.2079x^2 + 0.737x + 1.7727$ (first crop) compared to 0.55 in the regression $y = -0.342x^2 + 1.1831x + 1.4232$ (re-growth) suggested a much stronger association in the first crop and a weaker relationship in the re-growth. When compared against an $r^2_{critical}$ value of 0.87 at $p \leq 0.05$, the regression of the first crop was significant while that of the re-growth was not. The stronger correlation in the first crop could be attributed to the higher levels of Pb that the grass absorbed.

A different trend to that of Pb was obtained for Cd single treatments where the regression of Cd of $y = -1.3832x^2 + 1.2619x + 2.0992$ for the re-growth was stronger ($r^2 = 0.51$) than that of the first crop $y = -0.655x^2 + 0.6533x + 2.2138$ ($r^2 = 0.20$). However both regression models were not significant when compared with $r^2_{critical}$ of 0.75 at $p \leq 0.05$. Therefore generally, the association of yield and Cd content in grass became stronger in the single treatments from the first crop to the re-growth. In the mixed Pb treatments, where the regression relationship of the first crop was $y = - 0.336x^2 + 1.048x + 1.548$ ($r^2 = 1.0$) and that of the re-growth was $y = - 0.908x^2 + 2.369x + 0.898$ ($r^2 = 0.45$), the trend in which the re-growth was more weakly correlated in the re-growth persisted. The regression model of the first crop was significant ($p \leq 0.05$).

A comparison of correlation coefficients of single and mixed treatments of the same metal showed that the regression model of the single Pb treatments had the same level of association as the regression model of the mixed Pb treatments. In the mixed treatments of Cd, the first crop had a much stronger and significant ($p \leq 0.05$) regression model $y = -1.557x^2 + 1.604x + 2.040$ ($r^2 = 0.89$) compared to the re-growth regression model of $y = - 0.752x^2 + - 0.961x + 2.074$ ($r^2 = 0.51$). This suggests influence of co-presence of Pb and Cd on yield in the mixed treatments, particularly as it relates to Cd. The intercepts of the regression models for each of the metals were close in value to each other.

Regression of $\log_{10}$ (*bio-available chemical concentration in the soil*) versus $\log_{10}$ (*chemical concentrations in grass*) produced linear model fits with positive slopes and r^2 values. A comparison of r^2 values of regression models of $\log_{10}$ (*metal content in the re-growth*) and $\log_{10}$ (*bio-available metal concentration*) in the soil to $r^2_{critical}$ of 0.87 for Pb and 0.75 for Cd (at $p \leq 0.05$) showed that the regression model for Pb in the single treatment and regression models of Cd in single and mixed treatments were significant ($p \leq 0.05$). In addition, the regression model for the single treatments of Pb $y = 0.525x + 0.539$ (trend-line $r^2 = 0.75$) was stronger than the model for the mixed Pb treatments, $y = 0.398x + 0.607$ (trend-line $r^2 = 0.70$). The reverse was true for the single and mixed Cd treatments, where $y = 0.451x + 0.084$ in single Cd treatments and $y = 0.853x + 1.874$ in mixed treatments had significantly ($p \leq 0.05$) high r^2 values of 0.78 and 0.98, respectively, compared to an $r^2_{critical}$ value of 0.75.

The fact that the slopes of the regression models of single and mixed Pb treatments were statistically different and the intercepts were statistically the same, suggests that the two regression models were statistically different but closely associated. The higher r^2 value in mixed Cd treatments compared to single Cd treatments was consistent with the higher slope of the mixed Cd regression model of 1.01 compared to the slope of 0.39 for the model of the single Cd treatment. The higher slope implied a 2.6-fold increase in the rate of uptake of Cd in mixed treatment compared with single treatments. This indicates the influence of co-presence of Pb on Cd. The regression models of Pb in single and mixed treatment indicate that the concentration of Pb in grass increased at rates of 0.52 and 0.40 times (respectively) the level of Pb in the soil. Similar models for Cd suggested that the concentration in grass increased by 0.45 and 0.85 times the bio-available levels in single and mixed treatments respectively.

5.5.4 Implications of findings

This component of the study provided answers relating to the capacity of star grass to take up high levels of Pb and Cd. However it was not clear if such levels could be reached under field conditions where levels of Pb and Cd in treated sewage were much lower than the levels added through inorganic salts and treated sewage. This component also confirmed that the uptake of the metals could be described using significant models of $\log_{10}$-transformed variables of measured parameters, allowing for the estimation of toxicity levels in soils and grass and threshold bio-available levels that would have to be maintained so as not to exceed allowable metal levels in star grass. Given that the conditions under which the threshold allowable limits were derived were different from field conditions, these estimates had to be re-confirmed through a field experiment. Therefore the logical step was to take the study further and investigate uptake of Pb and Cd under field conditions.