CHAPTER 3

GENERAL METHODOLOGY

3.1 Introduction

This chapter presents the general methodology that was followed in this study. It provides background information on the study area and discusses the major principles that were used in designing the study. It also outlines the major components of the study and provides a brief discussion of the methodology followed in each component of the study. The detailed methods and materials used in the study are presented in each chapter. Data analysis techniques employed in this study are presented in this chapter and mentioned in subsequent chapters for clarity of discussions.

Firle Farm was selected to be the study site because treated sewage from industrial sources is continuously disposed onto pasture grass on which cattle graze. Flood disposal and irrigation of pastures at Firle farm has been practiced for over 30 years as a form of tertiary treatment of sewage whereby grass takes up nutrients for metabolic purposes. Preliminary studies indicated that the treated sewage had high enough levels of Pb and Cd to warrant investigation in the sandy soil and pasture grass. The sandy nature of the soils was postulated to allow the metals to be readily available for uptake by grass.

3.2 Background of study area

Firle farm employs 32 farm workers and supports 3000 beef cattle. The cattle are born and bred on the farm. The cattle are sent to abattoirs for slaughter and sale of beef to the population at large. The farm workers carry out all operations including disposal of treated sewage, tend to cattle and use water from boreholes situated on the farm for domestic purposes. There were reports of cattle dying on the farm during the study. Although chemical pollution has been suspected in some cases no chemical tests were carried out to confirm the suspicions.

The pastures at Firle farm comprise star grass and kikuyu grass and both grass species are perennial. While star grass reportedly grows faster than kikuyu, the former is considered to be more resilient to droughts (interview with Farm staff, 2000). As a result, the two grasses have been grown together, in order to reduce the risk of failure of pasture. There is no data available on the application rates of sewage sludge and effluent on land at Firle farm, but based on annual sewage output and area irrigated, the application rate is about 48 megalitres/ha/year or 126-167 t solids/ha/year (Nyamangara, 1999).

3.2.1 Location of study area

Firle and Churu farms are located on the outskirts of the City of Harare. The Municipality of Harare owns Firle farm and Firle Wastewater Treatment Plant. Churu farm, which is adjacent to the Firle farm, belongs to the Government of Zimbabwe. Firle Wastewater Treatment Plant is located on the outskirts of Glen View residential area, south west of Harare City. The study area receives an average annual rainfall (over a 30 year period) of 800mm (Department of Meteorological Services, 1977).

The study site comprised two adjacent sections, one section in Churu farm and the other in Firle farm (Figure 3.1). The areas are 30 metres apart and are separated by a fence and road. The sites have a general slope of less than 3% towards a nearby river.

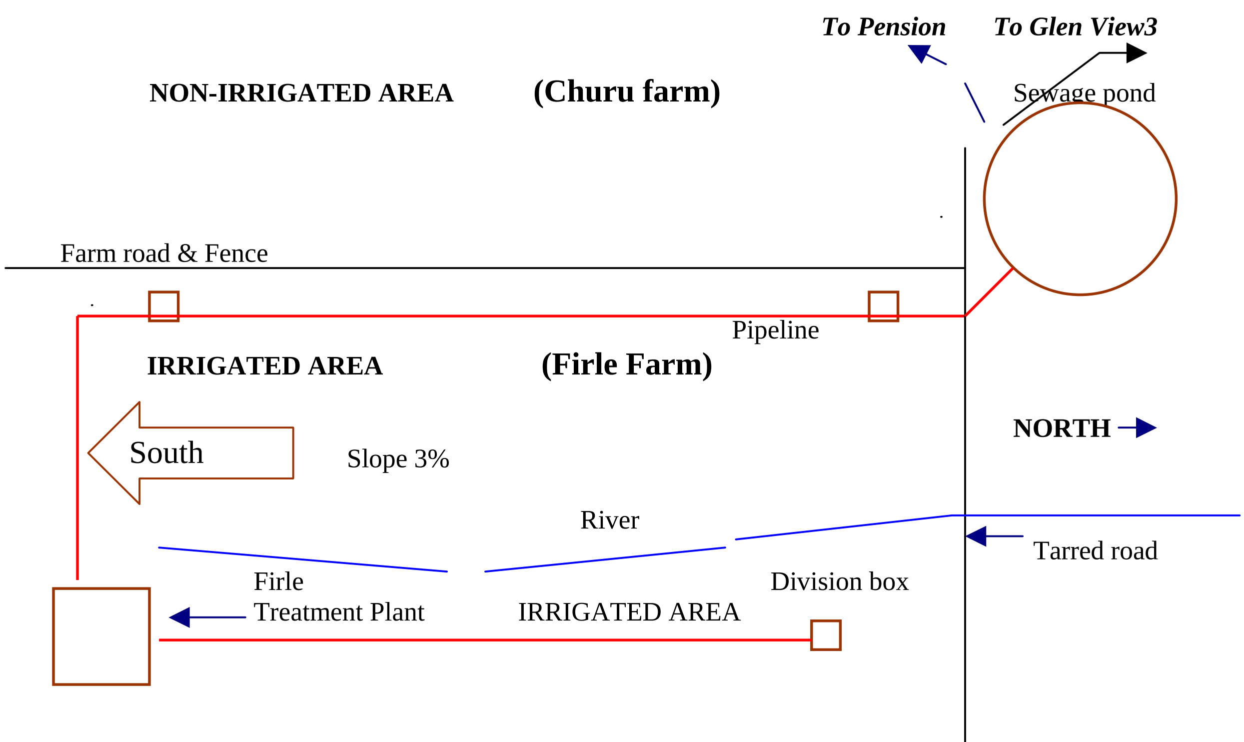

Figure 3.1: Schematic diagram of the study area

3.2.2 Sources of pollutants for study area

Firle Wastewater Treatment Plant processes both industrial and domestic effluent. The plant services the southern residential areas and many industries within the Firle catchment area. The industrial areas and the specific factories within the catchment area are listed below.

A) *Willowvale Industrial areas*

- Imponent Tanning
- Aluminium Industry
- Industrial Galvanising and Fabrication
- Tube and Pipe Industry
- Power Lines Central Africa

B) *Southern Industrial Area*

- Radiator Clinic
- Radiator and Tinning

C) *Adbernie Industrial Area*

- Edison Products

D) *Graniteside Industrial Area*

- Hardcrame Company
- Clover Electroplaters
- Mcdiarmid and Company
- Capri Corporation

E) *Msasa Industrial Area*

- Msasa Platers
- Sobhair refrigeration
- Msasa Game Skins

3.2.3 Treatment plants

Firle Wastewater Treatment Plant comprises of six units with a capacity to process 144 megalitres of effluent per day. Two of the units use the conventional wastewater biological trickling filtration system that produces low quality water (ca. 48 megalitres) used for irrigation of pastures. The other four units use the Biological Nutrient Removal System (BNRS) that produces high quality water (ca. 96 megalitres), which is discharged into the natural river system. The low quality water produced from the biological filtration system is mixed with digested sewage sludge from all the processing units to produce a slurry (3-4% solids) used to irrigating 860 hectares of pasture.

3.3 Study design

The study was divided into 3 major components: (1) baseline assessment of Pb and Cd levels in the study area, prior to subsequent detailed experiments (2) greenhouse experiments to assess the capacity of star grass to accumulate Pb and Cd and (3) experiments on Pb and Cd uptake by star grass under field conditions. The key approaches and principles that guided the design of each component of the study are presented in this section together with the study design of each component.

In baseline assessment, two approaches were followed. The first approach was to analyse past records of chemical analysis of treated sewage to confirm presence and levels of Pb and Cd in treated water disposed onto pasturelands. The second approach was to test Pb and Cd in soils of the study area to provide levels of Pb and Cd prior to carrying out components 2 and 3. Soil texture and other soil properties, such as clay content and cation exchange capacity, were determined to provide data to describe soils and assess any relationships to Pb and Cd levels in the soil. The levels obtained from analysis of past records were compared to national and international legislated limits to assess compliance. Pb and Cd levels obtained from tests were used to derive long-term levels of accumulation.

In the greenhouse experiment, the approach involved exposing star grass to a range of levels of Pb and Cd so that responses in metal content of the soil, yield of grass and metal content of grass to Pb and Cd added to the soil could be obtained. To achieve this, inorganic salts of the metals were added to the soil on which star grass was planted. Treated sewage was applied to the soil, instead of water for the following reasons: Some research findings indicate that metal uptake from soils in which inorganic salts are added do not adequately simulate uptake under field conditions. Among the reasons cited, is the absence of the effect of organic matter on availability of metals, which would occur under normal soil conditions. Several researchers indicate that organic matter in sewage substantially reduces metal availability to plants (Christensen, 1989a; Murray, 1995). However, others indicate that added inorganic metals remained largely bio-available to plants as they would be adsorbed on cation exchange sites of soil components, clay and organic matter (King and Morris, 1972; Doyle, 1978). In this experiment, inorganic metal salts and treated sewage were applied together to bring the experiment closer to field conditions where organic matter in treated sewage could affect plant availability of Pb and Cd. This design was assumed to simulate field situations where different Pb and Cd pollutant loads from the sources could find their way into raw sewage and subsequently treated sewage and onto pasturelands.

Given that the soils used in this experiment were non-polluted, a pot experiment in which star grass was grown was considered appropriate for raising levels of Pb and Cd. Notwithstanding weaknesses of pot experiments described in de Vries (1980), pots were used in this study because they allowed for controlled variation of Pb and Cd added and required less chemicals than would be the case with a field experiment in which chemicals are added to the soil. Schmidt (2003) observed that pot experiments are a low-cost approach to testing different soils, crops and combinations of different elements and they simplify measurements of relevant parameters, such as element balance, compared to field experiments. The same author noted that in many pot experiments involving plant responses to chelate addition to soils, plants stopped growing on transplanting onto the contaminated soil, hindering assessment of the effect of the chelate on growth. In this experiment therefore, the design was to establish the crop first, so that the root system would develop, then add Pb and Cd in solution to facilitate faster uptake of Pb and Cd. Considering that many researchers have reported that most Pb measured in plants in the environment is a result of direct deposition (Johannesson, 2002), this study was designed to apply inorganic Pb and Cd directly onto the soil surface to minimise chances of contaminating grass.

To improve predictions of plant responses to soil concentration, soil bio-available metal levels were used in place of total soil concentrations to develop soil-vegetative tissue metal uptake models for Pb and Cd in the greenhouse and field experiments. In addition, this study combined the concept of log-transforming soil and plant concentrations (as recommended by US

Department of Energy, 1998) and use of bio-available metal levels in soils to produce soil-vegetative tissue metal uptake models of Pb and Cd for star grass on a sandy soil.

Since there are many methods of extracting the bio-available metals from soils one method was selected and used in both the greenhouse and field experiments to ensure consistency. Ammonium acetate was selected as the extracting agent because it has been used widely over the years and the bio-available metal fraction it extracts strongly correlates with plant metal content (McGrath and Cegarra, 1992). Its suitability for extracting Cd has been confirmed (Soane and Saunder, 1959; Robinson, 1997).

In this study, de-ionised water and standard series of Pb and Cd were used for quality control on measurements of levels of Pb and Cd. No certified reference materials were used due to logistical problems in acquiring and using them in Zimbabwe. It is acknowledged that the use of appropriate certified reference materials for quality control improves comparison of the results of studies to statutory limits. It is further noted that since the matrix in reference material used for calibration is never strictly identical to that of the samples being analysed (ILAC, 1996) this variation could generate a bias in analytical results, if careful selection of the reference material is not undertaken. The bias is slight if (1) similarities between the sample and reference material is good, (2) the measuring instrument is robust enough to detect matrix differences and (3) if the samples are properly treated before analysis. Most local laboratories are yet to adopt the use of these reference materials in environmental work.

3.3.1 Baseline assessment of Pb and Cd levels in the study area

Analysis of past records on levels of Pb and Cd in treated sewage

The Municipality of Harare provided past records on chemical tests carried out on treatment of sewage. Recommended levels of Pb and Cd in treated sewage were obtained from literature. Firle Treatment works, sewage conveyance system, Firle Farm and paddocks for cattle were inspected to familiarise the author with the treatment and disposal systems.

Baseline characterisation of soils and grass in study area

The area selected for the study consisted of 2 sections: (1) one section for testing long-term accumulation of Pb and Cd and (2) another section for: (a) sampling soils for the greenhouse experiment and (b) laying out the field experiment. Each section was 1000 m^2. The first section, labelled "Irrigated area" in Figure 3.1, was located in Firle Farm and it had received sewage application for the previous 29 years. The second section, labelled "Non-irrigated area" in Figure 3.1 was located in the adjacent Churu farm and it had not received sewage application before. The design was to use previously non-polluted soil in the greenhouse experiments and locate treatments for the field experiments in the same area and soil type.

Soil samples were taken from the two areas. The soils were sampled at 10 cm depth intervals to provide data for describing soil properties and accumulation of Pb and Cd along the soil profile. It was decided that total soil metal concentrations be measured to obtain total levels of accumulation. Total soil concentrations of Pb and Cd were determined by extraction using the aqua regia method and atomic absorption spectrometry (Department of Environment, 1989). Soil texture, clay content, cation exchange capacity and pH were determined using standard methods described in detail in Chapter 4. Land slopes were also measured.

The data obtained from soil tests on the irrigated and non-irrigated areas was used to further select and demarcate two smaller portions, one from within each section, for detailed experimental work. To eliminate variations in observations induced by differences in soil properties (other than those induced by treated sewage disposal) in subsequent experiments, it was decided that the two portions for detailed experiments should have similar soil properties, particularly texture, soil depth and land slope. The areas with minimal differences in these soil properties were marked for the greenhouse and field experiments.

To validate uptake of Pb and Cd by pasture grass, the levels of the metals were tested in samples taken from mixed kikuyu and star grass pasture that was within the irrigated area at the time. The samples were cut at 5 cm off the ground. Pb and Cd in grass were extracted using hydrochloric acid (HCl) and nitric acid (HNO_3) employing the standard procedures detailed in Chapter 4. The levels of heavy metals were then determined by atomic absorption spectrometry (Department of Environment, 1989). Since the individual grasses could not be separated during sampling, the levels of the metals in the mixed grass were considered to reflect uptake by either or both grasses.

The levels of Pb and Cd in the non-irrigated soil were assumed to reflect background levels of a non-polluted soil. The levels in the irrigated area represented 29 years of pollution. Therefore the difference between the levels in the two areas was considered to be long-term accumulation. Total soil metal concentrations were also analysed to evaluate levels of correlations with concentrations in pasture grass and to justify whether there was enough evidence to carry out subsequent experiments on the basis of bio-available soil concentrations.

3.3.2 Greenhouse Pb and Cd uptake by star grass under treated sewage application

To evaluate responses of yield and metal content in grass to metal levels in soils, star grass was grown in soils with Pb and Cd levels ranging from non-toxic to toxic levels. Yield and bio-available Pb and Cd levels in soils and star grass were measured using detailed procedures presented in Chapter 5. The data obtained was considered useful in developing dose-response models for (1) soil metal content (dose) and metal content in grass (response), (2) soil metal content (dose) and yield of grass (response) and (3) metal content in grass (dose) and yield of grass (response). The purpose of the models was to enable prediction of responses based on measurement of one parameter, that is dose level.

A pot experiment was designed as described below to apply different levels of Pb and Cd. To exclude rainfall, the experiment was set up in a greenhouse. Pots were filled using soil from the non-polluted section of the study area and star grass was grown inside the pots. The soil in all pots was uniform, with respect to Pb and Cd levels.

Five treatments, each with 3 replicates, were set up to raise Pb levels in the soil. The purpose of these treatments was to establish the dose-response relationships of Pb added as a single metal. The first two treatments (a control irrigated using water and another treatment irrigated using treated sewage) did not receive inorganic Pb. The treatment irrigated with treated sewage was meant to apply the lowest level of Pb using treated sewage. In the remaining treatments, the concentration of Pb in the soil was raised by 3 levels of 300 mg/kg, 600 mg/kg and 1200 mg/kg of soil through the addition of $Pb(NO_3)_2$. Measured volumes of treated sewage were used to irrigate the treatments. Pb content was measured in the water and treated sewage that was used to irrigate star grass.

In the second set of treatments there were 7 treatments of Cd. The aim was to establish the dose-response relationships of Cd added as a single metal. The two treatments that did not receive inorganic Pb also served as treatments of Cd, since inorganic Cd was not added to the soil. The treatment irrigated with treated sewage was included to represent the lowest level of Cd addition to the soil. In the remaining Cd treatments, the concentration of soil Cd was raised by 5 levels of 10 mg/kg, 20 mg/kg, 40 mg/kg, 60 mg/kg and 80 mg/kg of soil through the addition of CdS. The volumes of irrigation were the same as in the first set. Cadmium content was measured in the water and treated sewage that was used to irrigate star grass.

The third set of treatments was intended to establish dose-response relationships of Pb and Cd when both metals were added to the soil. This set of treatments was included in the study because literature provided conflicting findings on Pb-Cd interactions in the soil. The aim was to investigate this interaction in a sandy soil and star grass because interaction was postulated to occur under field conditions. Of the five treatments set up two did not receive inorganic metal addition. Therefore the data obtained for similar treatments in the first and second sets also served the third set. The remaining treatments of combined Pb and Cd were: 300 mg/kg Pb combined with 10 mg/kg Cd, 600 mg/kg Pb combined with 20 mg/kg Cd and 1 200 mg/kg Pb combined with 40 mg/kg Cd. The treatments received the same irrigation applications as those in the first set. The levels of Pb and Cd were determined in each treatment.

The following standard analytical procedures, described in detail in relevant chapters, were used to determine Pb and Cd levels. The extraction of Pb and Cd in treated sewage, soils (for determination of total concentration) and grass utilised concentrated acids, hydrochloric acid (HCl) and nitric acid (HNO_3) to dislodge Pb and Cd from samples of sewage, soils and grass into solution. After filtration, total Pb and Cd in soils, treated sewage and grass leachates were determined using atomic absorption spectrometry (Department of Environment, 1989). Total soil Pb and Cd were determined during baseline assessment of the study area. Pb and Cd in treated sewage were determined for the greenhouse and field experiments. Bio-available Pb and Cd in the soil were extracted by 1 M ammonium acetate (CH_3COONH_4) using procedures recommended by McGrath and Cegarra (1992). After filtration of the leachate, the levels of Pb and Cd were determined by atomic absorption spectrometry.

A 210 VGP atomic absorption (absorbance range: -0.0820 to 3.200; concentration: 5 significant digits; reproducibility: <+/-5%) spectrometer was used to determine the concentrations of Pb and Cd in leachates of treated sewage, soils and grasses. Deionised water, which was used as a blank for calibrating the spectrometer, was subjected to the same extraction procedures as the samples. This procedure was undertaken to ensure that the metal being extracted had a matrix similar to the same metal in the sample. Standards of Pb and Cd were used to produce a standard linear graph of absorbance and metal concentration. Where the graph was not linear, fresh standards were prepared or the instrument was checked for problems such as lamp alignment and burner height. Once a linear graph was obtained, the metal level in the blank (which was expected to be zero) was measured and the instrument auto-zeroed (for background correction), prior to re-measuring levels of standards to re-check performance of the instrument. Measurements from the samples were then taken, while re-checking levels in standards after reading every 5 samples. Where low levels of metal concentrations were encountered, standard solutions were diluted accordingly. In this study, values of concentration were rounded off to two decimal places and those values lower than this were considered to be non-detectable for purposes of analysis. Webster (2001) noted that few measurements of soil properties are accurate to more than 3 significant figures, implying that with typical laboratory errors of 2-5%, sampling fluctuations could swell the error, making the first two figures more meaningful, thus significant.

3.3.3 Field assessment of Pb and Cd uptake

This component of the study was undertaken to evaluate levels of accumulation of Pb and Cd in star grass in response to changes in bio-available metal levels in soils under field conditions. The approach used in this part of the study was similar to that used in greenhouse experiment except that Pb and Cd levels in the treatments were raised by increasing total quantities of treated sewage applied to the soil, instead of adding inorganic Pb and Cd. It was assumed that the higher the quantity of treated sewage added to the soil, the higher the total quantity of Pb and Cd added to the soil, hence the higher the bio-available metal content of the soil.

Four treatment levels were set up on 12 field plots in the non-polluted area. One treatment level was located in the area previously irrigated in order to assess accumulation in a soil already polluted. Five levels of irrigation application were allocated to the plots randomly. The non-polluted area consisted of the control (which did not receive treated sewage application) and the following treatments: (1) Treatment 1, where half of the estimated water requirements of grass was to be applied (2) Treatment 2, where the estimated water requirements was to be satisfied (3) Treatment 3, where twice the estimated water requirement was to be provided. Treatment 4, located in the irrigated area received the same level of application as treatment 3.

The plots were prepared for border irrigation and star grass was grown in each treatment. Treated sewage was supplied to treatments 1 to 3 using a pump and a conveyance pipeline and Treatment 4 using a furrow. During irrigation the discharge of the pump was measured volumetrically, using a bucket and stop watch. The discharge in the furrow was measured using a flume. The average water application per irrigation and for the whole period was computed from discharge data for each replicate and treatment.

It was not possible to pre-determine the levels of Pb and Cd in treated sewage. Therefore the period of application of treated sewage was deliberately lengthened (11 months), to even out any variations in the concentrations of Pb and Cd between irrigation events. Samples of treated sewage were collected during each irrigation event and tested for Pb and Cd. Levels of Pb and Cd in star grass and bio-available levels in the soil from each treatment were tested on samples collected on 5 occasions during the experiment. The methods used for testing Pb and Cd in samples from the greenhouse experiments were applied in this component. Details are presented in Chapter 6.

3.4 Data analysis

The following sections present the analyses that were carried out in each component of the study. Statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS) package, SPSS 8.0 for Windows (www.spss.com, 1997), to determine normality of data inputs, means, ranges, and standard deviations of various data sets throughout the study. Techniques such as Analysis of Variance (ANOVA), correlation analysis, regression analysis and Student's *t*-tests were used to test for significance of the effect of one set of data on another and regression models developed. Significance levels were quoted at $p \leq 0.001$, $p \leq 0.01$ and $p \leq 0.05$, although Webster (2001) noted that this was a matter of choice and normally $p \leq 0.01$ and $p \leq 0.05$ would suffice. The specific areas in which different types of analysis were employed and the use of the outputs are briefly discussed below and also specified in the subsequent chapters for clarity of discussions presented.

In the first component of the study, arithmetic mean levels and ranges of Pb and Cd in raw sewage, treated effluent and digested sludge were determined. Arithmetic means, ranges and standard deviations were also determined for soil chemical properties that were cited in literature as influencing Pb and Cd levels in soils and grass. The computed mean levels in treated sewage and soil properties were compared to values and limits quoted in literature to assess similarities or differences and compliance to local and international legislated limits. The difference in the average values of Pb and Cd in the control and irrigated areas was computed to determine long-term accumulation in the soil. In grasses average metal concentrations were determined for comparison with national and international legislated values as well as for correlation with metal concentrations in soils.

Arithmetic means of levels of Pb and Cd in the soil and star grass were computed from measured levels in replicates of each treatment. The maximum level of accumulation of Pb and Cd in star grass was considered together with evidence of toxicity (damage to the plant leaves and/or stems) in grass to provide an indicator of the capacity of grass to absorb Pb and Cd. This level was compared to levels of accumulation in other grasses and plants, cited in literature, to establish whether star grass had a higher or lower capacity to accumulate Pb and Cd, relative to capacities of other plants.

In the second component of the study measured data for soil bio-available metal content and growth parameters of yield and metal content in grass, was first tested for normality and then normalized using the $\log_{10}$ function. Analysis of variance and comparison of means, were used to determine the levels of significance of (1) treatment on soil bio-available metal levels, (2) soil metal content on (a) yields and (b) content of metal in grass and (3) metal content in grass on yield.

Regression analysis on $\log_{10}$ (*variables*) was used to develop the following dose-response relationships:

(1) Pb and Cd content in star grass versus yield

(2) soil bio-available Pb and Cd concentration versus yield of star grass

(3) soil bio-available Pb and Cd concentration versus metal content in star grass

Correlation analysis was then used to select and test the strength of best-fit regression models of the variables. The best-fit regression model, whether linear or non-linear, was considered to be the regression model with the highest correlation coefficient (that is, Pearson product moment correlation coefficient, r^2 value). The correlation coefficients of the best-fit regression models were then compared to the critical values for correlation coefficients for one independent variable, in order to assess the significance of association of the variables in the regression models. After confirming the strength of the regression models, toxicity levels in grass were derived from $\log_{10}$ *grass metal content* versus $\log_{10}$ *yield* models of Pb and Cd and used to derive corresponding soil bio-available metal levels using $\log_{10}$ *soil bio-available metal concentration* versus $\log_{10}$ *metal concentration in grass* models. To test whether the regression models from single and mixed treatments were statistically different, the *t*-test for comparison of regression coefficients was used.

In the third component of this study, the levels of the metals in soil and grass samples from field plots were determined as in the second component. Regression analysis on $\log_{10}$ (*variables*) was used to develop the following dose-response relationships:

(1) soil bio-available Pb and Cd concentration versus yield of star grass
(2) soil bio-available Pb and Cd concentration versus metal content in star grass

Analytical techniques similar to those used in the second component of the study were employed in the third component of the study.