MODELLING LEAD AND CADMIUM UPTAKE BY STAR GRASS UNDER IRRIGATION WITH TREATED WASTEWATER

BY

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ABSTRACT

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Supervisor	:	Professor C. F. Schutte
Department	:	Chemical Engineering
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This study was conducted to investigate the capacity of *Cynodon nlemfuensis* (star grass) to accumulate lead (Pb) and cadmium (Cd) and develop metal uptake models for sandy soils receiving treated sewage from domestic and industrial sources. The study area comprised a non-polluted area and an adjacent area that received treated sewage from Harare's Firle Wastewater Treatment Plant for over 30 years.

Measured soil properties, total Pb and Cd in soils and grass and past records of Pb and Cd in treated sewage were analysed. Growing grass in a greenhouse in pots with previously non-polluted soils amended by single and mixed Pb and Cd salts and irrigated with treated sewage tested the uptake capacity of star grass. Yields, soil bio-available and grass Pb and Cd levels were measured and used to develop models for estimating critical soil and grass concentrations at which productivity declines. In the field, star grass grown in 10m x 10m plots in the non-irrigated and irrigated areas, received varying amounts of treated sewage over 11 months. Soil bio-available and grass metal contents were measured and used to develop field-based models to predict Pb and Cd content in star grass.

Star grass had a high Pb and Cd extraction capacity, making it unsuitable for pasture if grown on polluted soils. Correlation between total Pb and Cd in soils and grass was insignificant ($p<0.05$). Logarithm-based models of $\log_{10}$ *bio-available soil levels* and $\log_{10}$ *grass metal levels* provided the best-fit regression models for Pb and Cd predictions in grass. Toxicity levels of Pb and Cd that were derived for star grass from pot-based models were higher than levels recommended for pasture. Toxicity occurred without visible signs on grass, making it difficult to recognise toxicity without testing. The field-based uptake models predicted safe bio-available limits for pasture on sandy soils. The co-presence of Pb and Cd resulted in increased Cd uptake but did not significantly affect Pb uptake. Star grass can accumulate more than 1 mg/kg of Cd at total soil Cd levels of less than 1 mg/kg, suggesting that the soil limit may be too high for a sandy soil.

Key words: Modelling Pb and Cd; *Cynodon nlemfuensis*; Sandy soil; Treated sewage

EXECUTIVE SUMMARY

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Supervisor	:	Professor C. F. Schutte
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This study was conducted to investigate the capacity of *Cynodon nlemfuensis* (star grass) to accumulate lead (Pb) and cadmium (Cd) from a sandy soil irrigated with treated sewage. It also aimed to develop soil-vegetative tissue uptake models for predicting Pb and Cd levels in star grass using measured soil concentrations.

By growing star grass in pots with sandy soils amended using different levels of single and mixed inorganic salts of Pb and Cd and applying treated sewage, this study established that star grass is a high accumulator of Pb and Cd. It also established that the co-presence of Pb and Cd in the soil leads to increased uptake of Cd but does not significantly affect uptake of Pb by star grass. Star grass accumulated 8 times and 18 times the maximum levels of 40 mg/kg Pb and 1 mg/kg Cd recommended for pasture (United Kingdom Statutory Instrument No. 1412, 1995), respectively. The co-presence of Pb and Cd led to a 2.6-fold increase in uptake of Cd but did not significantly affect Pb bio-available soil levels and uptake by star grass.

Using the pot experiment, this study established that soil bio-available metal levels significantly ($p \leq 0.05$) correlate with plant metal levels through logarithm-based single-factor linear regression models of $\log_{10}$ (*above-ground tissue metal concentrations*) versus $\log_{10}$ (*soil bio-available metal concentrations*). The models predict toxicity in star grass to occur at 53.7 mg/kg Pb and 3.2 mg/kg Cd, corresponding to soil bio-available levels of 186.2 mg/kg Pb and 8.3 mg/kg Cd. Since toxicity occurred at metal levels higher than recommended for pasture without visible signs showing, the study recommends that visual signs of toxicity should not be used to decide when to stop grazing animals. Regular monitoring of bio-available levels of Pb and Cd is recommended.

In the field experiment where Pb and Cd levels in field plots were varied among treatments by applying different quantities of treated sewage, this study produced a significant ($p \leq 0.05$) model: $\log_{10}$ (*above-ground tissue Pb concentration*) = 0.3949$\log_{10}$ (*soil bio-available Pb concentration*) + 0.7880 for Pb and a strong (but marginally insignificant) model: $\log_{10}$ (*above-ground tissue Cd concentration*) = 0.363$\log_{10}$ (*soil bio-available Cd concentration*) + 0.2987 for Cd. The models predict that, to maintain Pb and Cd levels in star grass below recommended limits, soil bio-available levels should not exceed 115.2 mg/kg Pb and 0.20 mg/kg Cd. Therefore this study recommends management of soil bio-available Pb and Cd in sandy soils below 115.2 mg/kg and 0.20 mg/kg respectively, to ensure that star grass pasture is safe for animal consumption. The field-based models are considered suitable where animals graze regularly, facilitating re-growth of star grass over time.

Other results from this study suggest that the recommended limit of 1 mg/kg total Cd in soils may be too high for sandy soils under repeated disposal of treated sewage. In this study, some samples of mixed kikuyu and star grass from a sandy soil exposed to 29 years of treated sewage disposal tested up to 1.2 mg/kg despite the soil having a total Cd of 0.65 mg/kg.

THESIS CONTRIBUTION TO KNOWLEDGE

A comparison of the capacity of the *Cynodon nlemfuensis* (star grass) to accumulate Pb and Cd, obtained from this study, and that of other plants contributes vital information towards the search for hyper-accumulators. By absorbing 4 592 mg/kg Pb, star grass ranks as a strong Pb accumulator among grasses, considering that hyper-accumulating grasses such as *Lolium perenne* (rye grass) accumulated 5 390 mg/kg Pb (US Department of Energy, 1998). However overall, star grass ranks as a medium accumulator of Pb when compared to hyper-accumulating plants such as Ipomea which accumulated 15 000 mg/kg in shoot tissue (Rhyne and Gosh, 2002). Given that grasses within a species have similar uptake characteristics (McDonald et al., 1995), these findings suggest that the *Cynodon* species of grasses has uptake capacities close to 4 592 mg/kg, accompanied by very low yields. This implies that the *Cynodon* species may be a medium Pb extractor whose use in phyto-remedying polluted soils may be limited.

Prior to this study, Pb and Cd uptake characteristics that are critical to the growth and monitoring of suitability of star grass pasture, growing on soils polluted with Pb and Cd were not known. No known models were available for estimating Pb and Cd levels in star grass growing on sandy soils on which treated sewage is disposed. This study contributed to the development of soil-plant metal uptake models by combining the use of bio-available concentrations in soils and the concept of log-transforming soil and metal concentrations in grass to produce single-factor regression models for estimating Pb and Cd levels in grass based on bio-available soil levels. Using the models, the study estimates that toxicity of Pb and Cd in star grass occurs at 53.7 mg/kg Pb and 3.2 mg/kg Cd corresponding to critical soil bio-available levels (extracted using 1 M ammonium acetate) of 186.2 mg/kg Pb and 8.3 mg/kg Cd.

Furthermore, the study provides an indication of the critical levels of soil concentrations that should not be exceeded in order to ensure that levels in star grass are below recommended maximum levels. Using regression models:

(1) $\log_{10}$ (*above-ground tissue Pb concentration*) = $0.3949\log_{10}$ (*soil bio-available Pb concentration*) + 0.7880

(2) and $\log_{10}$ (*above-ground tissue Cd concentration*) = $0.363\log_{10}$ (*soil bio-available Cd concentration*) + 0.2987,

developed under field conditions, the study estimated that soil bio-available levels should be maintained below 115.2 mg/kg Pb and 0.20 mg/kg Cd to ensure compliance of star grass metal content with recommended limits of 40 mg/kg Pb and 1 g/kg Cd (United Kingdom Statutory Instrument No. 1412, 1995) for pasture grass.

Literature presents what appears to be conflicting evidence on the influence of Pb on Cd and *vice versa* on uptake by plants. By assessing the effect of the co-presence of Pb and Cd in the soil on uptake of the metals by star grass, this study contributes towards increasing available information on interactions of the metals in plants. This study found that the addition of Pb and Cd to the soil increased uptake of Cd 2.6-fold over uptake observed with single metals added to the soil, while uptake of Pb was not affected significantly in star grass. Therefore available information on interactions of Pb and Cd may not be conflicting but an indication of different uptake characteristics of plants. It may also be argued that besides reducing Cd levels in treated sewage, reduction of Pb levels can contribute towards reducing uptake of Cd.

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CHAPTER 1

INTRODUCTION

1.1 Health concerns of Pb and Cd in humans and the environment

Lead (Pb) and cadmium (Cd) are toxic metals whose contribution to environmental contamination is becoming a serious concern as they enter the air, food and water in increasingly significant amounts fed by continuous mining and use of metals (Elson and Haas, 2003). Besides Pb and Cd, other metals, such as zinc (Zn), copper (Cu), nickel (Ni), chromium (Cr), iron (Fe), silver (Ag) and mercury (Hg) are of great concern to the environment and human health. Pb and Cd are cumulative toxins that are indestructible and can only be eliminated through excretion (Moolenar and Lexmond, 1999). When they accumulate in the human body, Pb and Cd may cause health problems that include damage to the central nervous system and reduced intellectual capabilities (Wildlife, 2000) and hypertension (Staessen, 2002).

The major pathways of exposure to Pb and Cd in the non-smoking human population are: food and water for Pb and food via the addition of cadmium to agricultural soils and uptake by food and fodder crops, in the case of Cd (Scottish Executive Environment and Rural Affairs Department, 2002). Plants can take up Pb and Cd in high concentrations from the soil (Bazzaz, 1977; Johnston and Hones, 1995; Khan and Frankland, 1983) and hence provide a major pathway to the human food chain. Thus, a good understanding of uptake of Pb and Cd is critical in designing strategies for predicting uptake of the metals into the food chain.

1.2 Metal pollution from wastewater

Wastewater disposal on soils is a major source of metals to plants. The use of wastewater for irrigation is justified on the need to dispose of the water, utilize the scarce water resource, take advantage of the high nutrient content of wastewater and reduce the need for commercial fertilizers (Bayer et al, 1972). It is also a low cost method for sanitary disposal of municipal wastewater. However, disposal of wastewater on land has been widely reported to increase soil metal content, because wastewater contains heavy metals from domestic and industrial sources. Department for Environment, Food and Rural Affairs (DEFRA) and Environmental Agency (2002) noted that disposal of sewage sludge to land increased Cd concentration in soils. Janeic et al (1995) noted that Cd poses the greatest concern with respect to land application of sewage because ingestion of plants that contain large concentrations of the metal by humans and animals may result in Cd accumulation in livers and kidneys. Treated sewage is therefore a potential source of soil contamination that increases the possibility of uptake of Pb and Cd by plants that grow on the soils on which it is disposed.

In Zimbabwe, municipal wastewater is used for irrigation in many peri-urban areas and the practice is expected to increase with the expansion of the existing and creation of new urban centers. One of Harare's largest treatment plants, Firle Wastewater Treatment Plant, processes sewage coming from industrial and domestic sources and disposes mixed treated effluent and sludge on pasturelands at Firle farm. The pastureland consists of sandy soil on which mixed *Cynodon nlemfuensis* (star grass) and *Pennisetum clandestium Chiov*. (kikuyu grass) pasture is irrigated. Firle farm employs 32 farm workers and supports 3 000 beef cattle that are born and bred on the farm. The farm workers and animals may be subjected to hazards emanating from exposure to Pb and Cd. In addition, any hazards that may exist could spread wider, since the population at large consumes beef from animals bred on Firle farm.

1.3 Paucity of data on accumulation of Pb and Cd in star grass

Disposing treated sewage on pastures started over 30 years ago at Firle farm. It was considered to be a cheap method for secondary treatment of wastewater, unfit to be discharged directly into natural watercourses. Although the potential of Pb and Cd to accumulate in soil is known, their accumulation in soils has not been monitored at Firle farm. While there has been limited and inconsistent monitoring of heavy metal content in treated sewage no attempt has been made to quantify Pb and Cd uptake by grass or animals at Firle farm to ascertain compliance of metal content of grass with acceptable levels for grazing pastures. Therefore the health hazards posed by heavy metals to animals that feed on the grasses are not well documented.

To date, only a few short-term studies on the impact of sewage sludge disposal on soils have been carried out in Zimbabwe. One such study by Nyamangara and Mzezewa (1999) investigated the long-term effect of sewage sludge application on Pb, Zn, Cu and Ni levels in a clay loam soil. The study, which was carried out at Crowborough Sewage Treatment Works (one of Harare’s treatment works) concluded that sewage sludge significantly increased the levels of Pb, Zn, Cu and Ni in the soil. The results of the study raised questions regarding the potential uptake of large amounts of metals by pasture grass.

Although uptake of some heavy metals by grasses such as, *Lolium perenne* (rye grass), *Pennisetum purpureur* (elephant grass), *Agrotis stolonifera* (red top) and *Medicago sativa* (alfalfa) has been studied, limited research has been conducted on the genus *Cynodon* to which star grass belongs. No known study has determined Pb and Cd uptake characteristics of star grass. The absence of studies on Pb and Cd in star grass pastures represents a gaping hole in vital scientific information, considering that the grass is a widespread pasture grass in East, Central and Southern Africa and is grown in Zimbabwe using wastewater which potentially contains high levels of Pb and Cd.

The paucity of data on heavy metal pollution is not unique to Zimbabwe, but spread across the developing world. World Health Organisation (WHO) Working Group on Cd, (http://www.icsu-scope.org/cdmeeting/cdwgreport.htm) noted that while the developed world is more concerned about food quality and public health, the developing world and tropical areas in particular face persistent challenges of malnutrition and food security that take precedence over food quality and public health. It further confirmed that tropical areas have relatively few data on Cd accumulation in tropical soils and crops despite them covering a large part of the globe in which two thirds of the world's population lives. Such data would be important for both local public health and international trade. Recognition of the potential hazards caused by heavy metals and the need to protect the environment has resulted in greater investment into research, legislating and enforcing permissible limits of the metals by developed countries. This recognition has spread to developing countries and hence many scientists have called for more research on heavy metal pollution in the developing world.

1.4 Challenges in modelling plant metal uptake from soils

Researchers face many challenges in generating data and analysing it to develop tools for use in minimising environmental and health hazards associated with heavy metals. Many countries worldwide have legislated maximum permissible heavy metal levels (guideline values) in soils, some plants, irrigation water and food for human consumption. However legislated metal limits differ from one country to another, depending on the context in which they were developed. DEFRA and Environmental Agency (2002) stated that soil guideline values may differ from one country to another depending on the conceptual models behind the guidelines, reasons why the assessment criteria were developed, management context, legislation, policy and differences in site conditions, such as soil pH and soil type. Therefore a generic heavy metal permissible limit in soils may not be applicable to all countries and situations.

The soil-plant pathway has attracted research attention since it is a major contributor to transmission of metal pollutants to animals and humans. Efforts have been made to develop soil-plant tissue metal uptake models for predicting plant metal concentrations. Soil-plant tissue uptake models have been used in soil-plant nutrient analysis for a long time. The models have been extended to heavy metal analysis in soil-plant systems and used for predicting levels of pollutant metals in plants on the basis of metal levels in soils. Soil metal levels and plant metal content are central to the development of these models. Factors that affect these two parameters have to be taken into account in developing soil-plant tissue uptake models.

1.4.1 Soil metal concentrations and soil sampling depth

Total soil metal concentrations are widely used in the soil-plant tissue metal uptake models. One major advantage of their use is that standard methods of measuring total metal concentrations in soils are available. However the challenge is that total metal concentrations are increasingly being regarded as inadequate for predicting plant metal content and for public health assessments (Bak and Jensen, 1998). Like-wise, soil-vegetative tissue metal uptake factors (Baes et al 1984) vary with total metal concentrations and can over- or under-predict concentrations of some metals in plants, because they are based on total metal concentration (US Department of Energy, 1998).

The depth of soil from which soils are sampled to determine soil concentrations may introduce errors in relating concentrations of metals in soils and plants because concentrations vary with soil depth. According to the US Department of Energy (1998), the depth interval at which various plants in different environments obtain water and nutrients and the relative biomass of feeder roots at different depths are unknown. Therefore the challenge is what depth one should use in modelling so that the concentrations of metals in that depth reflects uptake of metals by a particular plant.

Soil-plant tissue metal uptake models have been developed from existing data measured at different sites across the world. This approach has presented challenges to modelling plant uptake. US Department of Energy (1998) noted that non-uniformity of soil sampling depth, scarcity of data and variations in methods used to measure soil metal concentration presented constraints to modelling soil-plant metal uptake.

Suggestions have been made to overcome some of the challenges. Bio-available (also known as plant available) levels of metals have been reported to correlate better with plant metal concentration. However, the absence of an agreed standard method for measuring bio-available metal levels in soils (http://www.icsu-scope.org/cdmeeting/cdwgreport.htm) has constrained their use. Another suggestion to improving soil-plant tissue metal uptake models is to incorporate factors that influence availability of metals to plants, such as pH (Jesper and Jensen, 1998). This approach has received considerable attention and although models were developed, site-specific experiments were encouraged (Sample, 1998) to obtain site-specific data for developing models.

1.4.2 Differences in uptake characteristics of plants

Research has also shown that different plant species and cultivars have different metal uptake characteristics and capacities (Kabata-Pendias, 2001). Furthermore, different organs (leaves, fruit, roots, stem) of the same plant have different metal uptake capacities. Therefore uptake characteristics of a particular plant or its organs can only be known if an experiment is carried out on a particular element. The absence of any known studies on Pb and Cd uptake by star grass implies that soil-star grass uptake and growth characteristics, such as metal uptake and yield response, are not known. In addition, the critical metal uptake levels of star grass, such as toxicity levels, are not known and cannot be extrapolated from other grasses that have been studied so far. Therefore, uptake of large quantities of Pb and/or Cd by animals grazing on the treated sewage irrigated star grass pastures could not be ruled out on the basis of available information.

1.4.3 Influence of uptake by other metals

Besides plant species and soil metal concentration, other chemicals in the soil influence uptake of metals by plants (Moolenar and Lexmond, 1999). Other chemicals present in a soil may interact with a particular metal causing an increase or reduction of uptake of the metal by a plant. Khan and Frankland (1983), Miller (1977), Carlson and Rolfe (1979) and others found different and sometimes conflicting results on the influence of Pb on Cd and *vice versa*, where the two metals co-existed in the soil. Preliminary indications were that Pb and Cd were present in treated sewage disposed on Firle farm. Therefore interactions of the two metals could not be ruled out.

In view of the preceding arguments, a long-term study was considered necessary to determine uptake of Pb and Cd by star grass growing on a sandy soil on which treated sewage is disposed of. The following objectives were formulated to investigate the issues.

1.5 Objectives of study

The general objective of this study was to establish the effect of irrigating pastures with a mixture of sewage effluent and sludge from bio-filtration plants on contamination of pasture grass by Pb and Cd. The specific objectives were:

1) To determine the long-term Pb and Cd accumulation in soils subjected to sewage effluent and sludge mixture application

2) To evaluate changes in pasture grass yield level, response to Pb and Cd concentrations and toxicity levels in grass under effluent and sewage sludge mixture application in combination with different levels of added heavy metals

3) To determine Pb and Cd accumulation in pasture grass under effluent and sewage sludge mixture application

4) To determine the maximum level of Pb and Cd concentrations in sewage effluent and sludge mixture that would allow optimisation of yield of grass and prevent heavy metal loading from exceeding acceptable limits

1.6 Scope of study

This study postulated that if star grass was exposed to very high levels of Pb and Cd, then cattle could accumulate high levels of the metals in their body organs through consumption of grass. This could lead to humans also accumulating high levels of the metals through consumption of meat from those animals. To contribute to this wide area of research, this study focused on accumulation of Pb and Cd in soils and star grass. In addition, it focused on developing soil-vegetative metal uptake models that could be used to predict metal uptake in grass using measured soil bio-available metal levels. Within this scope, the study was limited to the main areas of focus described below.

A literature review was undertaken to gather detailed information on Pb and Cd hazards in the environment and to identify gaps for research. This study is therefore based on the gaps identified. The study was conducted on one study site only since the limited financial resources only allowed a limited number of expensive chemical analyses. Recommended levels of the metals in soils and pasture grass where extracted from literature.

Data from chemical tests carried out by the City of Harare on the treatment process were incorporated in the study to save on time and costs of obtaining similar data within the context of this study. Interviews and visits to the treatment plant were undertaken to familiarize the author with the sewage treatment and disposal systems.

A greenhouse pot experiment where soils and grasses were subjected to high levels of Pb and Cd was undertaken to assess uptake of the metals and be able to define toxicity. This was complemented by a field experiment meant to assess uptake under real life conditions and determine what levels of Pb and Cd could be allowed in the soil to ensure that grass did not exceed recommended levels. In the greenhouse experiment uptake was assessed on single added Pb and Cd and the two metals combined. The latter was intended to investigate interactions of Pb and Cd in soils and grasses. One method of extracting bio-available soil metal levels was selected and used throughout the study to ensure consistency.

Since numerous soil and plant factors affect metal accumulation in soils and plants, only selected soil factors, like soil pH, cation exchange capacity (CEC), clay content and organic matter were investigated to assist in the interpretation of Pb and Cd uptake by grass. This implies that other important factors such as plant physiology and interaction of Pb and Cd with other chemical species like calcium (Ca) and zinc (Zn) were excluded from the study in order to make it focused.

The focus of this study was to relate soil metal content and metal content in organs of star grass that were consumed mostly by cattle. In this study, these organs were taken to be above-ground tissue of grass although it is acknowledged that animals sometimes consume roots and even soils as they graze. This study defined above-ground plant tissue as all plant tissue (stems and leaves) 5 cm above the ground on the assumption that cattle cut grass at 5 cm above the ground as they graze. This is the plant tissue in which growth parameters of yield and metal content were measured for use in modelling. The study therefore excluded below-ground organs such as roots.

1.7 Organisation of thesis

This thesis is organized into 8 chapters. Chapter 2 consists of a literature review that brings out gaps in knowledge that motivated this study. Chapter 3 presents the 'General Methodology' of the study, in which the overall study design and components of the study are discussed. In this chapter, the approaches and methods used in each component of the study are discussed at a general level. Detailed methods and materials are presented in each component to facilitate a better understanding of the link between the detailed methods, the results and discussions for that particular component of the study. Each component of the study constitutes a chapter, from Chapter 4 to Chapter 6. An overall discussion is presented in Chapter 7 while the conclusions and recommendations are presented in Chapter 8.

CHAPTER 2

LITERATURE REVIEW

2.1 Essential and non- essential heavy metals for plants

Heavy metals are elements with a high relative atomic mass. They occur naturally in the earth's crust. The term "heavy metal" is used extensively in literature to refer to metals with atomic numbers greater than 20 and is also associated with toxicity or pollution. According to Malan (1999), the term is vague as some authors use it to refer to second and third row transitional metals, others to all transitional metals while many use it to refer to metals not normally found in biological tissue but are harmful. In this study the term heavy metal refers to metals that have atomic numbers greater than 20 and may be harmful to plants and/or animals. These metals include Fe, Zn, Ni, Cr, Cu, As, Hg, Pb and Cd. Pb and Cd have been chosen for investigation in this study because they pose a much higher risk to the human food chain than the rest. They enter the food chain and more easily accumulate to levels that cause health problems to animals and humans.

Heavy metals such as Fe, Cu and Zn are essential for plant growth as they participate in oxidation, electron transfer and various enzyme reactions (Polette et al, 1997). Others like Pb and Cd are not known to have any metabolic roles in plants and animals and are therefore non-essential (Johannesson, 2002; Elson and Haas, 2003). In general, essential elements may be defined as metals that are necessary for a plant to complete its life cycle (Welch and Cary, 1987). Non-essential elements are metals with no known role in plant metabolism. Although recent findings indicate that Cd may be essential to certain mushrooms (Johannessson, 2002) the metal is still considered non-essential since its biological functions in plants are still not known. Polette et al (1997) postulated that the mechanisms that allow uptake of nutrients by plants could also facilitate uptake of heavy metals, as the latter are generally indistinguishable from nutrients.

2.2 Sources of Pb and Cd

The major sources of heavy metals to the environment are direct deposition from mining and industrial processes, atmospheric deposition from combustion processes and wastewater from mining activities, industrial and domestic processes. The primary production and recycling of Pb (which occurs in over 50 countries in the world) contributes to a total annual production of 6 million tonnes while that of Cd is estimated at 19 000 tonnes per year (Johannesson, 2002). Heavy metals are emitted into the atmosphere as vapour or particulates (dust) or both from combustion processes (power generation, road transport), industrial sources (iron and steel industry, non-ferrous metal industry) and waste incineration (Scottish Executive Environmental and Rural Affairs Department, 2002). From these atmospheric emissions heavy metals are then deposited onto the environment.

2.2.1 Lead

Pb is a mineral found deep within the earth and mined together with silver deposits (Elson and Haas, 2003). It exists in nature as sulphate ($PbSO_4$), carbonate ($PbCO_3$) and sulphide (PbS), which constitute the principal ore of Pb, known as galena. Impurities in the ore include Ag and gold (Au). Pb ore produces oxides when heated.

Lead is a raw material in the manufacture of tetraethyl lead ($Pb(C_2H_5)_4$), the additive in leaded gasoline. It is used in the production of lead acid storage batteries, pigments and chemicals, solder, other alloys and cables. It therefore becomes part of industrial waste from these industrial activities. WHO (1993) stated that Pb is present in tap water primarily from household plumping systems containing Pb in pipes, solder, fittings or service connections to homes. This makes domestic waste a major source of Pb. The dissolved amount depends on several factors including pH, temperature and water hardness. Wastewater consists of domestic and industrial waste that is treated and may be disposed onto lands, including pasturelands. In the process, treated wastewater may become a major source of Pb on pasturelands.

Scottish Executive Environmental and Rural Affairs Department (2002) noted that average human daily Pb intake for adults in the United Kingdom (UK) is estimated at 1.6 µg from air, 20 µg from drinking water and 28 µg from food. Food therefore constitutes a significant proportion of the daily intake of human beings. Subhuti (2001) stated that meat was among the top three main dietary sources of lead. The other two were grasses (mainly grains, such as rice) and common vegetables. The same author noted that the two plants were particularly vulnerable to taking up Pb deposited in the top layers of the soil due to their shallow rooting depths.

2.2.2 Cadmium

Cadmium is present in the earth's crust at an average of 0.2 mg/kg and usually occurs in association with Zn, Pb and copper sulphide ore bodies. Cadmium is used in the steel and plastics industries and is released to the environment through wastewater (WHO, 1993). The main sources of Cd in the environment are due to:

(1) air emission from Zn, Pb and copper smelters and industries involved in manufacturing alloys, paints, batteries and plastics
(2) wastewater from mining
(3) agricultural use of sludge and fertilisers containing Cd
(4) burning of fossil fuels
(5) deterioration of galvanised materials and Cd-plated containers

Wastewater has been reported as a major source of Cd, although the metal is often not detected in sludges (Lisk, 1972). Doyle (1978) reported Cd accumulations of over 1 mg/kg in the soil, following high rates of application of sludges over a long time. The same author also reported accumulation of 100mg/kg under furrow irrigation with sludge in some extreme cases.

The average daily intake for humans is estimated at 0.15 µg from the air and 1 µg from water, while smoking a 20-cigarette pack can lead to inhalation of around 2-4 µg of Cd (Scottish Executive Environmental and Rural Affairs Department, 2002). Johnston and Jones (1995) noted that plant-based foodstuffs were the largest source of dietary Cd and that the relative contribution of soil Cd content in plants was important but largely unresolved.

2.3 Treated wastewater as source of Pb and Cd

Treated waste material from sewage treatment plants is disposed on land as effluent or liquid sludge or dried sludge. Research has noted that most chemical pollutants are held by the organic fraction of treated sewage, that is the sludge and not the effluent. Primary sludge constitutes particulate organic material and secondary sludge consists mostly of micro-organisms. However, WHO (1989) reported that conventional treatment processes, such as the activated sludge and the bio-filtration systems have little effect on removing chemical contaminants from wastewater. This suggests that chemical contaminants may also be present in treated effluent. Junkins et al (1983) explains that during wastewater treatment, soluble (dissolved) and insoluble suspended materials are adsorbed into microorganism cells where they are broken down (digested). Digestion includes synthesis (reproduction of more cells) and oxidation (formation of carbon dioxide (CO_2), water (H_2O) and energy. Junkins et al (1983) described the process of activated sludge using the following equations:

Conversion of organic matter

$$\text{Organic matter} + O_2 \xrightarrow[\text{Enzyme}]{\text{Bacteria}} CO_2 + H_2O + \text{Energy} \quad \text{.................. equation 1}$$

Reproduction of new cells

$$\text{Organic matter} + P + NH_3 + O_2 \xrightarrow[\text{Energy}]{\text{Bacteria}} \text{Protoplasm (new cell)} + CO_2 + H_2O \quad \text{... equation 2}$$

Degradation of other cells

$$\text{Protoplasm} + O_2 \xrightarrow[\text{Enzyme}]{\text{Bacteria}} CO_2 + H_2O + (NH_3 \text{ or } NO_3) + \text{Energy} \quad \text{.......equation 3}$$

As the microorganisms die, they break open, making nutrients and heavy metals available to other microorganisms.

Liquid digested sludge differs from air-dried sludge in that during anaerobic digestion, much of the organic nitrogen present in the sludge is mineralised to ammonia, thereby bringing the ammonium ion into solution. When added to the soil, ammonium ions may volatilise, or become adsorbed onto clay minerals or organic matter, absorbed by plants or nitrified (Doyle 1978). The mineralisation process also releases metals, including Pb and Cd into solution, allowing for their adsorption onto clay minerals, hydroxides or uptake by plants.

The rate of decomposition of digested sludge was found to depend on soil moisture and texture and most decomposition took place within one month of addition of sludge to soils (Miller, 1974). This therefore suggests that liquid sludge has a higher proportion of readily available metals in solution than dried sludge. As the soil dries after addition of liquid sludge, decomposition decreases thereby reducing metal availability. Joffe (1955) attributed the decrease in mineralisation upon drying of the soil to the retardation of microbial activity. King and Morris (1972) reported decreases in soil pH and increase in cations available for plant uptake in a sandy clay loam due to the application of liquid sludge to land. The same authors also noted that large applications of sludge to soils have also been reported to create anaerobic soil conditions that increase mineralisation of organic matter present in the sludge as well as lower soil pH.

Birley and Lock (2001) noted that nearly all Cd ions applied through irrigation water are found in the topsoil due to strong sorption. However it has been observed that after filling all available attachment sites, the soil particles gradually decrease the sorption rate (Christensen 1989a). Murray (2003) noted that metal behaviour in sewage sludge amended soils and plant uptake is difficult to generalise because it strongly depends on nature of metal, sludge, soil properties and crop.

2.4 Chemistry of Pb and Cd

2.4.1 Lead

Lead has an atomic number of 82 and atomic mass of 207. It is the heaviest non-radio-active metal that naturally occurs in substantial quantities in the earth's crust (Subhuti, 2001). Pb is the most common among the heavy metals and its most abundant isotope is ^{208}Pb. Other stable isotopes also exist. Lead has two oxidation states, Pb^{2+} and Pb^{4+}. Pb^{2+} dominates environmental chemistry. There is great similarity in the ionic sizes of Pb^{2+} and Ca^{2+}, such that Pb^{2+} may proxy for Ca^{2+} (Johannesson, 2002).

Plants differ widely in their ability to absorb, accumulate and tolerate Pb (Johannesson 2002). Availability of Pb for plant uptake depends on total Pb in the soil, pH and organic matter. Although organic matter immobilises lead, the metal becomes more available as decomposition takes place. Doyle (1978) observed that Pb immobilisation from organic matter was comparatively less than that of Cd.

2.4.2 Cadmium

Cadmium, a group IIb metal in the periodic table, is a mineral mined as part of Zn deposits (Elson and Haas, 2003, Department for Environment, Food and Rural Affairs and Environmental Agency, 2002). It is a relatively rare metal that is 67th in order of abundance. Cd has an estimated half-life of between 15 and 1100 years implying that it is a long-term problem (Johannesson, 2002). It occurs as an impurity in phosphate fertilizers, with which it is applied to agricultural land. Cadmium is also added to agricultural land through treated sewage. Its most common form in soils is the free Cd^{2+} (Department for Environment, Food and Rural Affairs and Environmental Agency, 2002).

The chemistry of Cd in water is similar to that of Zn and to a lesser extent to Cu. Cadmium interacts strongly with Zn due to chemical similarity between the two metals (Department for

Environment, Food and Rural Affairs and Environmental Agency, 2002). Of all toxic metals released in large quantities into the environment Cd is generally regarded as the one most likely to accumulate in the human food chain (Johannesson, 2002). Adequate Zn intake tends to provide partial protection against the toxic effects of Cd (Elson and Haas, 2003). The presence of other metals may result in either synergistic or antagonistic interactions. The presence of Cd and Hg may result in reduced toxic effects of both metals, while interaction of Cu and Cd leads to a five-fold increase in the toxicity of each metal.

The toxicity of Cd in water is dependent upon the water's hardness and chemical speciation, which is influenced by pH, water temperature, ligands and co-existing metal cations present in water. All these factors influence uptake and bio-concentration of cadmium by aquatic organisms. In soils, Cd tends to be more mobile than many other heavy metals (Department for Environment, Food and Rural Affairs and Environmental Agency, 2002) and its adsorption has been shown to depend strongly on soil pH and to a lesser degree on hydrous oxide and organic matter (Alloway, 1995).

Most of the Cd found in water up to pH 9.0 is in the divalent cation form (Cd^{2+}). Cd is highly soluble under acidic conditions, but its solubility decreases above pH 9.0 due to the formation of cadmium hydroxide ($Cd(OH)_2$). The presence of organic matter lowers the toxicity of Cd as the metal is adsorbed onto exchange sites of organic matter (Doyle, 1978). Cd strongly binds to sulphydryl (-SH) groups hence the pronounced tendency of Cd to bio-accumulate in the food chain (Zambezi River Authority, 2001; www.agius.com/hew/resource/toxicol.htm). Cadmium accumulates in the kidneys and has a long biological life in humans of 10-35 years (WHO 1993).

2.5 Metal contamination and toxicity

All metals, including essential elements tend to be toxic to organisms at certain levels (Breckle, 1991) with essential elements tending to be toxic at high concentrations while non-essential elements are toxic at relatively low concentrations. Any addition of a contaminant to the soil is considered as contamination until it reaches a critical concentration when the buffering capacity of the soil, that is its capacity to delay adverse effects, is exceeded. At this point contamination becomes pollution (Moolenar and Lexmond, 1999). Pollution is the malfunctioning of the soil due to abundant presence or availability of metals.

Figure 2.1 illustrates a generalised model of dose-response for plants exposed to nutrient metals. When plants receive increasing input levels of essential elements like Cu and Zn the yield increases as metal dose increases. The supply and uptake reach a lower critical limit where deficiency is eliminated. At this point the yield reaches a maximum. As the supply increases beyond this limit, luxury consumption occurs and further increases in metal content does not affect the crop or its yield within a range of metal doses, referred to as the tolerance plateau in Figure 2.1.

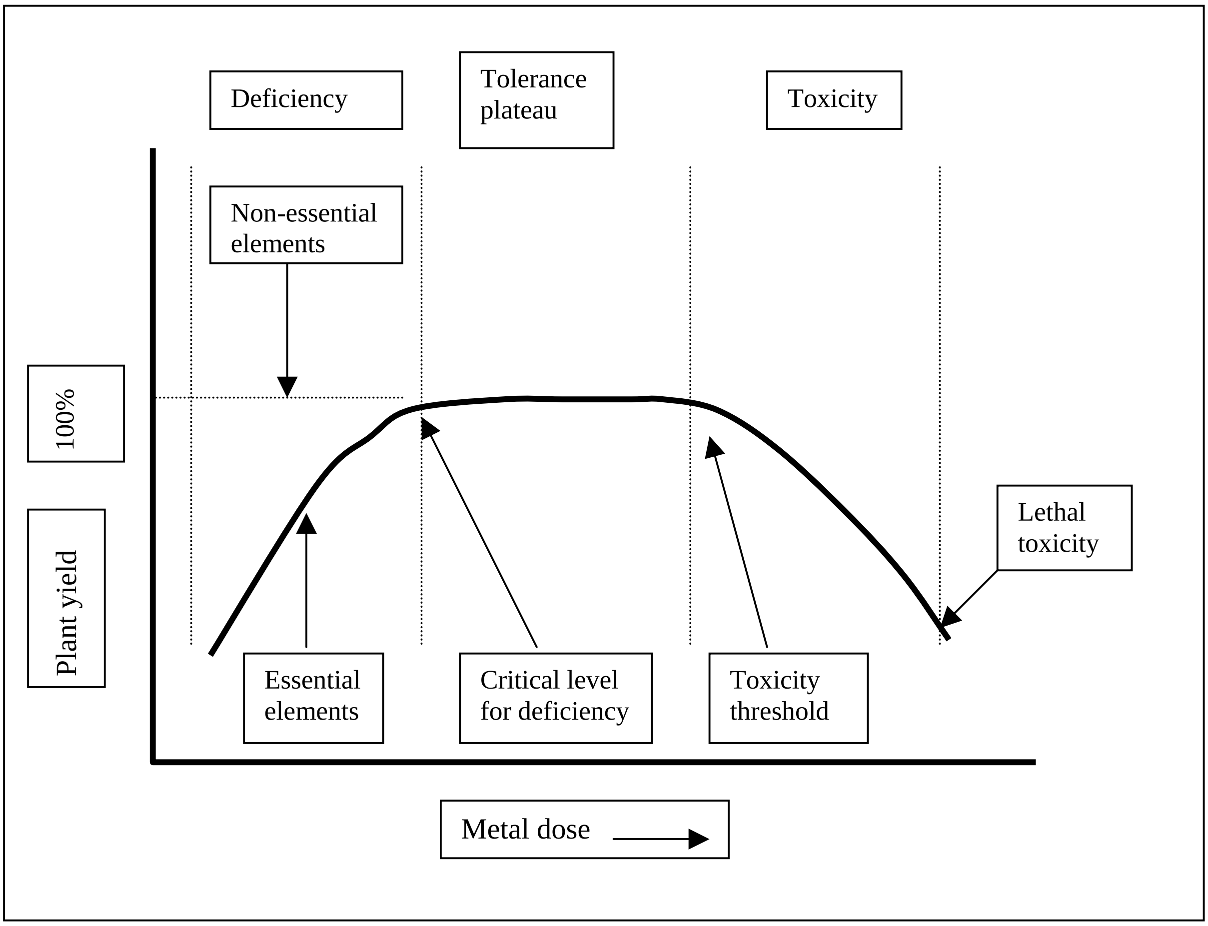

Figure 2.1: Generalised dose-response curve for nutrient metals (Adapted from Malan, 1999)

Luxury consumption (also known as tolerance) occurs when inactive complexes or storage depots are formed in the case of certain metals (Clarkson, 1986) and the metals are deposited there without toxicity occurring. Increasing metal dose beyond the upper limit of tolerance induces adverse effects on soil flora and fauna and hence biological activity. This upper limit represents the toxic level of the metal at which excessive uptake, whether of essential elements like Cu and Zn or non-essential elements like Pb and Cd, results in adverse effects on soil biota and plants as well as on mammals, birds and human consumers through the food chain (Moolenar and Lexmond, 1999). It is not known at what level Pb and Cd become toxic to star grass. If the dose-response curves of Pb and Cd in star grass are similar to Figure 2.1 and the toxic level is higher than the level recommended for pasture grass, then animals could graze on what appears to be healthy grass but would be exposed to metal hazard.

2.5.1 Lead

Lead is a bio-accumulative general poison. Birley and Lock (2001) note that industrial pollutants, including Pb, may contaminate peri-urban crops and poison consumers. They note that Pb can contaminate crops leading to neurological damage in humans. However, Johannesson (2002) noted that the concentration of Pb in the soil had to be a minimum of 87 mg/kg before any effects on basic soil processes, such as microbial activity could be observed. The same author states that uptake and accumulation of lead in tissue differed a lot between species.

The toxicity of Pb is reduced by water hardiness. Ayers and Westcot (1985) recommend a maximum of 5.0 mg/l Pb in irrigation water.

2.5.2 Cadmium

Cadmium is readily transported from the soil to the upper parts of the plants (Mengel and Kirkby, 1982). Its transfer from soils to edible plant parts of agricultural crops is significantly greater than for other heavy metals except Zn (Moolenar and Lexmond, 1999). Many studies have shown that Cd concentration in crops is positively correlated to the content in soils (IWMI, 1999).

The soil factors that play a direct role in controlling cadmium uptake by plants are the soil type, through its CEC, organic matter and pH. Haghiri (1974) reported that organic matter retained Cd through its cation exchange property. Doyle (1978) concluded that Cd adsorbed by organic matter remained largely available for plant uptake and cadmium added as salts to sludge, would possibly not exist in organic form when added to soils but would supplement a reservoir of available Cd by being adsorbed on cation exchange sites of the soil components, clay and organic matter.

John (1972) found the bonding energy for Cd to decrease in the following order: organic matter>heavy clay>silt loam> sand. This suggests that compared to the mineral fraction of the soil, Cd availability in sandy soils would be predominantly controlled by organic matter. Doyle (1978) suggested that in low CEC soils or in soils that receive large amounts of sludge, a portion of the Cd in the sludge would be leached while the rest would first fill the retention capacity of the soils (that is all exchange and adsorption sites) and then solubilise to become available for plant uptake or leach.

World Health Organisation (WHO) Working Groups on Cd (http://www.icsu-scope.org/cdmeeting/cdwgreport.htm (2000)) observed that compared with temperate soils where data is much more available on Cd, tropical soils generally had:

(1) lower levels of organic matter
(2) lower pH
(3) higher variability in clay minerals and oxyhydroxides
(4) exposure to higher temperatures and fluctuations in soil moisture

These factors make Cd accumulation in tropical soils and crops less predictable on the basis of the information already available and generated in the temperate regions of the earth. The WHO groups recommended further investigation of Cd in tropical agro-ecosystems.

2.6 Bio-availability of heavy metals

The term bioavailability may differ among various research disciplines. Most literature refers to bioavailability as the fraction of the total metal content that can be taken up by plants. This fraction depends on total soil metal content, soil texture as influenced by the cation exchange capacity, organic matter and pH. However the same term is also used to refer to availability of metals to humans and animals from different food crops and sources as influenced by physiological and nutritional factors (WHO Working Groups on Cd (http://www.icsu-scope.org/cdmeeting/cdwgreport.htm)). Proponents of this definition prefer to use the term phyto-availability to refer to metal availability to plants. In this study the term bioavailability is used to refer to plant availability.

Plant availability of micronutrients in soils is related to the total amount of micronutrients in various solid forms in equilibrium with the amount in the soil solution as dictated by the rate at which the solution phase is renewed. Chaney (1988) noted that metals exist as a variety of chemical species in a dynamic equilibrium governed by soil physical, chemical and biological properties such that only a fraction of the soil metal is readily available for plant uptake. The bulk of the metal fraction is in insoluble compounds unavailable for transport to the roots (Lasat, 2000). Tandon (1993) stated that nutrients in the soil existed in several forms notably (a) water soluble (b) exchangeable (c) specifically adsorbed, chelated or complexed (d) secondary clay minerals or oxides and primary minerals. The first three forms are thought to be important in supplying micronutrients for plant growth.

The WHO Working Groups on Cd observed that the mass balance approach in determining Cd pollution would indicate long term trends in Cd levels of the soil but would not be adequate in assessing the risk. Bio-available Cd would be more applicable but labour and cost intensive. However they noted the following constraints in the use of bio-available data:

(1) lack of generally applicable method for determining readily available Cd in soils
(2) changes in bio-available Cd with soil pH, clay content, organic matter, chloride concentration and total Cd and therefore the need to interpret crop uptake risk, taking these into account
(3) policy makers are reluctant to change from use of total concentration to bio-available concentration until there is consistency on measuring of bio-available concentrations.

2.7 Lead and cadmium health hazards to humans

Evidence of negative effects of Pb and Cd on human health has been widely reported. Lead levels in the human body have increased over time and it is estimated that the human body can take up 1-2 mg daily up to a total content of 125-200mg, a level that is 500-1000 times more than the levels detected in bones of very old human skeletons (Elson and Haas, 2003). Subhuti (2001) noted that the WHO tolerable daily uptake of 0.2 mg/day has not yet been attained in many parts of the world. According to Elson and Haas (2003), Cd increases in content with age and is estimated to peak at 40mg in the human body at 50 years of age. WHO (1993) set the maximum human intake of Cd and Pb at 1µg/kg and 3.5 µg/kg of body weight per day, respectively.

In human beings, Staessen (2002) found 2-4% variance of urinary Cd (indicating mild renal dysfunction and alterations in Zn and Cu homeostasis) related to consumption of vegetables grown on an acidic sandy soil with 4.86 mg/kg total soil Cd and 2.43 mg/kg Cd in celery. Lead poisoning in young children may cause permanent damage to the central nervous system and reduce intellectual capabilities (Wildlife, 2000; WHO, 1993). It also causes high blood pressure and hypertension in adults (Staessen, 2002). Though not very clear, Pb toxicity to humans emanates from its interference with functions performed by essential elements such as Ca, Fe, Cu and Zn in various enzymes (Elson and Haas 2003). It accumulates in the skeleton, making the bones weaker (WHO, 1993; Elson and Hass, 2003). Placental transfer of Pb occurs during gestation and throughout development (WHO, 1993). Young children may lose up to 2 intelligence quotient (IQ) points for a rise in blood levels of Pb from 10 to 20 µg/dl (Scottish Executive Environmental and Rural Affairs Department, 2002).

2.8 Plants as soil cleaners and pathway of Pb and Cd to food chain

Plant uptake of Pb and Cd makes plants potential cleaners of contaminated soils but also major sources of contamination for animals and human beings, if the plant is consumed. Plants that tolerate relatively high concentrations of potentially hazardous metals are more desirable for use in de-contaminating soils but create a greater risk to their consumers compared to those that are more sensitive (Moolenar and Lexmond, 1999).

The removal of toxic elements from contaminated soils using plants, also known as phyto-remediation, is among the most growing and exciting challenges for environmental research and problem solving. Chaney et al (1997) categorises phyto-remediation into phyto-extraction (use of plants to remove contaminants from soils), phyto-volatilisation (use of plants to make volatile chemical species of soil elements), rhizo-filtration (use of plant roots to remove contaminants from flowing water) and phyto-stabilisation (use of plants to transform soil metals to less toxic forms, without removing the metal from the soil). About 400 metal accumulating plants that take up high concentrations of heavy metals have been reported in literature (Hoover, 2002; Salt and Kramer, 2000, McGrath et al, 2002). Several phyto-remediation research studies are placing emphasis on the search for hyper-accumulating plants, that can be used to de-contaminate soils in sites polluted by heavy metals from industrial, mining and agricultural operations around the world.

Phyto-remediation can be cost effective in low- or medium-contaminated soils and does not adversely affect soil fertility (Cunningham and Ow, 1996). However, there are limitations in the use of plants for phyto-remediation (Cunningham et al, 1995; Cunningham and Ow, 1996; Chaney et al, 1997). These include contamination of vegetation and food plants, difficulty of establishing vegetation on contaminated sites and slow growth and small biomass of metal hyper-accumulators. Plants used for phyto-remediation should be fast growing and be able to accumulate large quantities of metal contaminants in their shoot (Cunningham and Ow, 1996).

Lasat (2002) defined hyper-accumulators as plant species that are capable of accumulating metals at levels 100-fold greater than those typically measured in shoots of common accumulator plants. The same author noted that hyper-accumulators will concentrate more than 10 mg/kg Hg; 100 mg/kg Cd; 1000 mg/kg Co, Cr and Pb, levels that are way beyond limits for animal and human consumption. Baker et al (2000) concluded that *Thlaspi caerulescens* is the only known species to hyper-accumulate Cd in shoots. However, the mechanism of Cd uptake in this hyper-accumulator plant is still not completely understood. It is often assumed that Cd, and other heavy metals without a biological function, are taken up by transporters for essential elements because of a lack of specificity.

The plant pathway is therefore a major source of concern since most Cd consumed by humans is obtained from the soil via food crops, while most Pb contamination through food crops is obtained from surface pollution of crops (Elson and Haas, 2003). Johnston and Jones (1995) noted that plant-based foodstuffs were the largest source of dietary Cd. They also noted that the relationship between Cd in soils and Cd content in plants was important but largely unresolved.

2.9 Treated sewage as source of Pb and Cd hazard to grazing animals via plants

Secondary treatment of treated sewage through disposal on soils utilizes the soil as an absorbent of contaminants and by default plants as contaminant extractors from soils. The main concern of the public, regarding forage and other crops grown on sewage sludge amended land is the potential uptake of trace elements by plants (Seaker, 1991). The other concern regarding toxicity to animals grazing on pasturelands is the potential ingestion of these elements from the soil under sewage sludge irrigation. Ingestion of large quantities of soil by grazing animals is a rule rather than an exception (Fleming, 1986).

Concentrations of Pb and Cd were found to be higher in the liver and kidneys of animals (Birley and Lock, 2001) exposed to the pollutants than those that were not. Roberts et al (1994) reported that at soil Cd concentrations lower than the recommended sewage sludge directive limit of 1 mg/kg (EEC, 1986) for use of sewage sludge in agriculture, grazing livestock were found to accumulate Cd in their livers and kidneys, causing restrictions in the growth of these body organs. Wilkinson et al (2001) reported a significant increase from 0.03 mg/kg to 2.57 mg/kg in the concentration of Cd in kidneys of lambs grazing on sewage sludge-treated pasture compared to untreated pastures in the U.K.

FAO (1992) noted that the potential accumulation of certain toxic metals in plants and their intake through eating of crops irrigated with contaminated water must be carefully assessed. Plants can take up high levels of heavy metals until the levels become injurious. Although DWAF (1996) reported that Pb and Cd interfere with metabolic processes, thereby affecting plant growth and crop yields, the possibility of the reduction in yield coupled with high increases in metal concentration in pasturelands going unnoticed cannot be ruled out. Where that happens cattle would continue to graze on pastures with high levels of metal concentration posing a hazard.

2.10 Potential of grasses to accumulate Pb and Cd

Research shows that some grasses have a potential to be hyper-accumulators of heavy metals. If such grasses were grown as pasture subjected to high concentrations of Pb and Cd, then they would provide high levels of heavy metals to the human being through animals that graze on the pastures. Gawronski et al (2002) who studied 21 varieties of grass (generas: *Festuca, Agrotis and Lolium*) concluded that *Agropyron repens L. Gramineae* (quack grass) was most promising for phyto-remediation purposes as its high biomass of 50 t/ha led to the uptake of 20 kg of Pb from the soil.

The maximum recommended limit of Pb in grass on which animals feed is 40 mg/kg (United Kingdom Statutory Instrument No. 1412, 1995), equivalent to 2 kg/ha for 50t/ha of grass. These findings cannot, however, be directly translated to other grasses since the mineral content of pasture is very variable depending on the species, stage of growth, soil type, cultivation conditions and fertilizer application (McDonald et al, 1995).

Limited studies have been conducted on *Cynodon* grasses to date. *Cynodon dactylon* (couch grass) reportedly accumulated high levels of Pb, after being grown on derelict mine dumps with soil Pb of 340 mg/kg in eastern Zimbabwe (Jonnalagadda et al, 2002). C*ynodon nlemfuesis* grown on a soil with a total concentration of 15 mg/kg of Pb accumulated 0.1-2.0 mg/kg Pb and 0.2-0.5 mg/kg Cd (Simunyu et al, 2002). Although these studies confirm heavy metal uptake by *Cynodon* grasses, they do not clarify the extent of pollutant uptake by the grasses. According to Birley and Lock (2001), research is required to clarify the extent of pollutant uptake by plants and the severity of adverse effects attributed to pollutant uptake.

2.11 *Cynodon nlemfuensis*

Cynodon nlemfuensis Vanderyst, also known as star grass, is a tropical and sub-tropical stoloniferous perennial grass that originated in East and Central Africa (from Ethiopia, Sudan and Democratic Republic of Congo) and was introduced to other parts of the tropics as a fodder grass (Hanna, 1992). Star grass is established by vegetative propagation and resists weed infestation once established. It is a variable species with mainly two varieties, Var. *nlemfuensis* and Var. *robustus*. The grass is used as forage grass and as a cover crop for erosion control (http://www.fao.org/WAICENT/FAOINFO/AGRICULT/AGP/AGPC/doc/pasture/Mainmenu.htm).

Star grass grows well where temperatures do not fall below -4^0C, the pH is 5-8 and rainfall is 500-2000 mm per year. The grass is harvested for hay or silage when it is 30-40 cm tall or after every 4-6 weeks growth (Hanna, 1992). Despite the widespread nature of this pasture grass in Eastern and Central Africa, there is no evidence from literature that Pb and Cd uptake characteristics of the grass have been studied.

2.12 Reliability of standard permissible toxic metal guidelines

Guidelines on heavy metal pollution in soils have been produced and are widely used for legislating against soil contamination as well as in ecological risk assessments. However, their widespread adoption has been questioned on the grounds that they vary depending on the country and purpose of origin. According to DEFRA and Environmental Agency (2002), soil guideline values may differ from one country to another depending on the conceptual models behind each set of guidelines, reasons why the assessment criteria was developed, management context, legislation, policy and differences in site conditions such as soil pH and soil type. The differences imply that soil guideline values determined in one country may not be directly applicable in another country. Furthermore, total metal levels in soils are considered unreliable in predicting plant uptake since research has shown weak correlations between total metal content of soils and plant metal content. The following observations confirm the disparities in international guidelines on permissible total soil concentrations and their weaknesses in predicting plant metal content.

FAO (1992) states that the maximum permissible concentrations of Pb and Cd in a soil under grass should not exceed 300 mg/kg and 3-5 mg/kg respectively for soil samples taken within a depth of 7.5 cm and with a soil pH above 5. Birley and Lock (2001) suggested a Pb limit of 150 mg/kg while Ross et al (1992) suggested 100 mg/kg. The USEPA Soil Screening Level (SSL) for Cd for plant uptake that is 24 mg/kg (and 78 mg/kg for human beings) is based on a total daily intake (TDI) averaging 1 µg/kg body weight/day over the first 30 years of human life (USEPA (1996). On the other hand, the Soil Code (MAFF, 1998) reported a maximum permissible soil Cd concentration of 3 mg/kg. This value relates to application of sewage sludge to agricultural land and is intended to protect human and animal health from consumption of arable crops. However DEFRA and Environmental Agency (2002) report that although plant uptake is specifically considered and precautionary advice is given for low pH soils in the Cd limit of 3 mg/kg, there is little information available about the conceptual models implicit in the value. The Dutch Integrated Intervention Value for all land uses, quoted as 12 mg/kg for soil Cd and 34.9 mg/kg for human beings over a life time, is derived from pathways that include direct ingestion of soil, consumption of crops and inhalation of dust from the soils. On the other hand, Australians indicated that they had problems with Cd in animals at Cd concentrations of less than 2 mg/kg. This scenario indicates that it may not be technically sound to transpose guidelines from one area to another (DEFRA and Environmental Agency (2002).

The soil guidelines, based on total metal concentrations, are increasingly regarded as insufficient for predicting plant metal content, especially for health risk assessments. They do not take into account the differences in bio-availability and hence toxicity in different soil types and the fact that plants do not assimilate metals from bulk concentrations in the soil (Bak and Jensen, 1998). The maximum permissible concentrations of heavy metals in surface soils amended with sewage sludge have also been based on total soil concentrations (Department of Environment, 1989) and are prone to the same shortfalls.

2.13 Reliability of guidelines on loading rates for wastewater on soils

The reliability of existing guidelines on loading rates from wastewater on soils deserves scrutiny. According to Pescod (1987b), wastewater treatment through disposal on soils requires specific loading rates that depend on; (a) nature of soil with loading rates increasing in the order clay to gravel (b) nature of sludge effluent where the more dilute the wastewater the higher the loading can be (c) climatic conditions where loading rates in dry and hot climates can be higher than in wet and cold climates and (d) crops grown where loading can be higher for less sensitive crops than sensitive crops. Murray (2003) noted that permitted agricultural loadings of toxic metals from sewage sludge are typically regulated using the soil criterion of total metal loadings or concentrations in soils and cautioned that generalised assumptions on behaviour of sludge-borne metals in soil-crop systems may under-estimate or over-estimate risks. The author further argues that in the absence of a basic understanding of metal behaviour in each specific situation, a more precautionary approach to toxic metal addition to soils is warranted. The following analysis highlights some grey areas in generalised recommendations to toxic metal addition to soils.

The generalised relationships between loading rates, nature of soil and type of wastewater presented in Table 2.1 do not specify the metal species in the sewage, the levels of the metals and the type of crop for each dosing regime. Although a reduction in dosage would reduce metal deposition the safe levels would not necessarily be guaranteed. According to Birley and Lock (2001), further research is needed to establish the safe heavy metal content of sewage waste since the risk posed by heavy metals (Cd, Cr, Cu, Pb, Zn and Hg) will depend on their dilution and uptake pathways. The same authors noted that some heavy metals might precipitate in sludge such that their concentrations in treated wastewater may be very low. In order to derive acceptable heavy metal loadings rates, it is necessary to determine intake through consumption of plants grown in contaminated soils.

Table 2.1: Sewage type, loading rates and soil type (Source: Chatterjee, 1987)

Nature of soil	Type of sewage	Dosing (litres/ hectare / day
Loams, clay	Primary treated, diluted	430 000
Sandy loam	Raw	190 000
Alluvial loam	Raw, diluted	245 000
Alluvial loam	Raw	100 000
Loams	Diluted	115 000
Sandy	Raw	170 000
Clay, loam	Raw, diluted	150 000
Clay	Raw, diluted	90 000

Other guidelines have been developed to address the need to specify the metal and its permissible level in soils. Table 2.2 presents examples of such guidelines, for areas that receive sewage application. However, the permissible limits presented in the table may differ from limits prescribed in other sources for the same reasons presented in section 2.12. As an example, while the table presents 0.033 kg/ha/year as the maximum permissible annual application rate of Cd from sludge to agricultural land, the EU recommends 0.15 kg/ha/year.

Besides being different, the guidelines do not specify the soil type and the organic content on which the Cd sorption depends (Christensen 1989b). In addition, they do not provide information on the types of crops or soils concerned. Other factors also affect metal sorption and uptake. Birley and Lock (2001) noted that evaporation, which is high in arid areas, increases salt concentration and should therefore increase plant uptake of Cd in semi-arid areas. This therefore limits widespread applicability of the guidelines.

Table 2.2: German standards for heavy metals in soil and sludge (Pescod et al., 1985)

Element	Maximum permissible levels in soil (mg/kg dry soil)	Maximum annual application rate (kg/ha/year)
Cd	3	0.033
Cr	100	2.0
Cu	100	2.0
Ni	50	0.33
Pb	100	2.0
Zn	300	5.0

Guidelines specifying metal content in sewage have been produced but have also differed depending on the country of origin. According to Johannesson (2002), the maximum allowable concentrations of Cd in sewage sludge in some countries are; Denmark (0.8 mg/kg), Finland (1.5 mg/kg), Sweden (2.0 mg/kg) and USA (8.5 mg/kg) and the differences in the accepted levels resulted from the different risk management approaches adopted in each country.

Pescod (1992) provided generalised guidelines of metal pollutant levels in wastewater (Table 2.3) that take into account the type of plant, type of metal and some soil parameters, such as pH. However, other important soil characteristics that have a bearing on plant availability, such as bioavailability were not addressed hence the same authors recommended assessments of the potential for toxicity under local conditions. Metcaff (1992) noted that Cd could accumulate in plants to levels that are toxic to humans and animals but are not toxic to plants.

Table 2.3: Recommended maximum concentrations of trace elements in irrigation waters (adapted from Pescod, 1992)

Element	Recommended maximum concentration in water (mg/l)	Remarks
As (arsenic)	0.1	Toxicity to plants varies widely, ranging from 12mg/l for Sudan grass to less than 0.05 mg/l for rice
Cd	0.01	Toxicity to beans, beets, and turnips at concentrations as low as 0.1 mg/l in nutrient solutions. Conservative limits recommended because of its potential for accumulation in plants and soils to concentrations that may be harmful to humans
Cr	0.10	Not generally recognised as essential growth element. Conservative limits recommended because of lack of knowledge on toxicity
Cu	0.2	Toxic to a number of plants at 0.1 to 1.0 mg/l in nutrient solution
Fe	5.0	Not toxic to plants in aerated soils but can contribute to soil acidification and reduced availability of phosphorus and molybdenum
Mn	0.20	Toxic to a number of crops at a few tenths of mg/l to a few mg/l, but only in acid soils.
Ni	0.20	Toxic to a number of plants at 0.5 to 1.0 mg/l; reduced toxicity at neutral or alkaline pH
Pb	5.0	Can inhibit plant cell growth at very high concentrations
Se (selenium)	0.02	Toxic to plants at concentrations as low as 0.025 mg/l and toxic to livestock. Forage is grown in soils with relatively high levels of added selenium. An essential element for animals but in very low concentrations
Sn (tin)	-	Effectively excluded by plants. Specific tolerance unknown.
Zn	2.0	Toxic to many plants at widely varying concentrations; reduced toxicity at pH>6.0 and in fine-textured soils

2.14 On-land sewage disposal methods

There are different methods of disposal of effluent through irrigation. Some of the common methods used worldwide are broad surface irrigation or flooding, sub-irrigation and ridge and furrow irrigation. Broad surface irrigation involves the discharge of treated effluent to flow overland onto the pastures or cultivated land. Sub-irrigation involves the use of surface drains to distribute the sludge mixture onto the land. The mixture is allowed to stand in the drains until it percolates and is subsequently collected by sub-surface drains. In the ridge and furrow methods, the sludge mixture is distributed using furrows. Crops may be grown on the ridges in such a manner that they do not have direct contact with the effluent.

2.15 Influence of plant and other chemical species on metal uptake

Plants and other chemical species influence uptake of metals by plants. Therefore, if bio-available metal concentrations are used to improve reliability of critical limits, they would have to be related to the plant species since uptake of Pb and Cd were observed to vary with plant species (Haghiri, 1973; US Department of Energy, 1998). An example of the effect of plant species on metal uptake is the wide range of the values of the transfer coefficient (metal concentration in tissue above ground divided by total concentration in the soil) of 1-10 for Cd and 0.01 - 0.1 for Pb (Johanneson, 2002). Alloway (1995) noted that due to numerous factors, soil to plant transfer coefficients were not precise but indicative of accumulation differences.

A further complication is that root uptake also differs depending on the plant species and other elements in the soil. 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 plant parts. Bak and Jensen (1998) noted that while ecotoxicity tests were often conducted on single metals, toxic responses to a mixture of metals could be antagonistic, synergistic or additive. Several observations were made on this aspect.

Khan and Frankland (1983) observed that when Pb or Cd caused phytotoxicity Zn levels in radish plants were close to deficient values. Cadmium and Pb have been shown to interact positively or negatively in some plant species. Miller (1977) observed that accumulation of Cd was increased by the addition of Pb while Cd in the soil reduced uptake of Pb in *Zea mays* L. (corn). Similarly lead was observed to increase uptake of Cd in rye and fescue (Carlson and Rolfe, 1979). The addition of both Pb and Cd increased the levels of both metals in

American sycamore over uptake observed with single metals added (Carlson and Bazzaz, 1977). On the other hand, Miles and Parker (1979) found low level and inconsistent synergistic and antagonistic effects among Pb, Cd and other heavy metals in uptake by bluestem and black-eyed Susan.

The preceding observations, suggest that the level of interaction between Pb and Cd also depends on the plant species. The interaction of these metals in star grass is not known. It would however be beneficial if the interaction reduces plant uptake but detrimental if it increases uptake.

2.16 Models for heavy metal content prediction

Researchers face many challenges in producing models that could be used to predict the hazard of metal pollution. One major approach to studying heavy metal accumulation is the mass balance approach in which inputs and outputs within the systems (area, field, region or country) being polluted are determined and modelled for prediction of metal concentration within environmental compartments. The mass balance approach produces long-term trends in contamination and they incorporate total metal concentration in soils. However there are major gaps in knowledge that militate against achieving mass balance calculations. It is difficult to quantify outputs, such as aerial metal deposition, leaching, runoff and erosion and the inadequacy of total soil metal concentrations in interpreting crop uptake risk.

The other approach involves setting legal criteria for limiting metal concentrations in the food chain, in particular soil, crops and water. The major advantage of this system over the mass balance approach is that the information required can be obtained by measurement and may be available at local level. The challenge in this approach however lies in that the limits of metals in soils are based on total metal concentration, which is regarded as being insufficient in interpreting crop uptake risk. The following sections present examples on these approaches.

2.16.1 Mass balance approach

Some effort currently underway towards modelling heavy metal pollution is called dynamic modelling. Scottish Executive Environmental and Rural Affairs Department (2002) noted that dynamic modelling of heavy metal pollutant concentrations in soils, was intended to predict how heavy metal concentrations change over time in response to a given deposition scenario. However the detailed information required, such as current and historic deposition, underlying geology, acidification status and rates of metal processing through soil layers on site or catchment area is mostly unavailable. WHO Working Groups on Cd made similar observations. The working groups on Cd (http://www.icsu-scope.org/cdmeeting/cdwgreport.htm) noted that while it is possible to obtain values of inputs such as aerial deposition, Cd in fertilisers and sewage sludge, there was a lack of knowledge on the role Cd inputs play in entry into the food chain, lack of global and regional input data, gaps in output information (leaching, runoff and erosion losses) and information on soil-plant interactions that cause wide variations on Cd uptake. Scottish Executive Environmental and Rural Affairs Department (2002) acknowledged the limitations in dynamic modelling by suggesting that there is a need to develop models for predicting the bio-available concentrations of metals in soils.

2.16.2 Use of soil-plant system models for metal prediction

The development of the simple model of soil-vegetative tissue uptake factors (Baes et al. 1984) often used for predicting plant metal concentration in health and ecological risk assessments provides a basis on which plant metal content may be predicted from soil concentrations. The Baes uptake factor is the ratio between the concentration of a chemical in a plant and its total concentration in the soil. It is however known that uptake relationships between soils and plants are considered to be valid only within a narrow range of chemical concentrations in the relatively nontoxic range (Carson and Bazzaz 1977). This implies that the uptake factors vary with total soil concentration (U.S. Department of Energy, 1998) and could therefore lead to over-prediction or under-prediction of concentrations of some metals in plants.

Another model, the Contamination Land Exposure Assessment (CLEA) model, developed between 1992 and 1997 by the late Professor Colin Ferguson using soil-to-plant concentration factors (as natural logarithms) for vegetable gardens, led to the development of soil guideline values for Cd (Department for Environment, Food and Rural Affairs and the Environmental Agency, 2002). The soil guideline values are used as screening tools or indicators that a given soil concentration might present a health risk to users. They are used as a basis for recommending further investigation and/or remediation.

Though the CLEA model takes into account different pathways of Cd such as ingestion from the soil, soil plant uptake and inhalation, it reportedly over-estimates plant uptake in soils where the pH is 6.5 and below. This happens because effects such as solution saturation, ionic competition and plant tolerance to Cd were not considered in the model. Where pH is less than 6.5, it is recommended that the bio-availability of Cd and plant Cd be determined on a site-specific basis (Department for Environment, Food and Rural Affairs and the Environmental Agency, 2002).

The preceding arguments suggest that the standard guidelines based on total permissible metal levels in the soil may not be reliable for predicting plant metal content. In fact bioavailability of metals has to be taken into account where the food chain is concerned since plant uptake parallels bio-available fractions of metals in soils (Alloway, 1990). It is the bio-available fraction of metals that poses a toxicological or environmental risk (Singh, 2002). Birley and Lock (2001) also concurred and suggested that the tentative acceptable total concentrations of various inorganic compounds in the soil should be regarded as first approximations requiring further research, focused on determining uptake by plants grown in contaminated soils, as a means of deriving acceptable heavy metal accumulation in the soil. The use of bioavailable metal concentration to predict plant metal content from soil metal content is undermined by the lack of consensus on a generally acceptable method to determine soil metal levels. This causes policy makers to be reluctant to change from the use of total metal concentrations to bio-available metal concentrations as indicators of contamination (http://www.icsu-scope.org/cdmeeting/cdwgreport.htm).

Other researchers have made an effort to incorporate soil parameters that affect metal availability in soils, such as pH, to improve accuracy of soil-plant metal concentration models. On the strength of significant regressions for the uptake of inorganic elements by earthworms using log-transformed concentrations that were obtained by Sample et al (1998a), the US Department of Energy (1998) recommended the use of log-transformed total soil and plant concentrations in regression models for predicting plant metal concentrations. After Alsop et al (1996) demonstrated that Baes factors under-predicted or over-predicted uptake of Pb in oats, Sample (1998) concluded that non-linear models, based on single-variables of metal concentrations in plants versus total metal concentrations in soils or multi-variable regression of metal concentrations in plants versus total metal concentrations in soils and pH, were generally more useful and therefore recommended for risk assessments.

Sample et al (1998) demonstrated, using data from field studies, greenhouse studies and pot studies on various soil types and types of plants, that log-transforming soil and plant concentrations could result in statistically significant relationships that could be used to estimate plant metal concentrations from soil concentrations, including those of Pb and Cd. They however stated that if samples from specific sites are used to develop site-specific uptake relationships, such type of data could provide more precise and accurate estimates of concentrations of chemicals in plants compared to their models. It appears that the major weakness in their models was that the data they used from the different sources in the world was not standardized, and in some cases scarce and varied. However they considered the use of the logarithmic function in the models to be better than the use of soil and plant concentrations in the Baes factor model.

2.17 Metal uptake in sewage amended soils

According to Murray (1995), short-term field experiments have shown that adsorptive properties of sludge prevent excessive uptake of many metals into crops largely due to added organic matter that complexes metals. However based on data from old sludge sites, the same author also noted that this protection can not be considered to be permanent or effective for all toxic metals. There is also a chance that this protection may not be the same where treated sewage is added continuously to soils. The level of uptake of heavy metals by plants growing in sewage-amended soils will depend on the bio-available levels of the metal in the soil (Nyamangara and Mzezewa, 1999). The bio-available levels in turn depend on the type of soil, organic matter content, pH, other chemical species present in the soil and heavy metal loading on the soil, among other factors (Johannesson, 2002; Scottish Executive Environmental and Rural Affairs Department, 2002).

Jesper and Jensen (1998) noted two approaches to increase reliability of critical metal limits in determining critical loads. These were; (1) relating critical soil metal limits to parameters controlling concentrations in soils, such as pH, soil texture, CEC and organic matter or (2) using critical limits for soil solution as a basis for deriving critical metal concentrations in soils. The former approach is the one that was utilized by Sample (1998) in developing multi-variable regression models, based on logarithm functions and incorporating other soil parameters, such as pH. The latter approach advocates for the use of bio-available metal concentrations in studies to predict plant concentration from soils amended by sewage. Overall, the preceding arguments suggest the use of bio-available soil metal concentrations instead of total metal concentrations in the prediction of hazard of heavy metals.

2.18 Review of methods of measuring bio-available metal concentrations

The problems associated with the use of bio-available metal concentrations in setting guidelines is that there is no agreed standard method of metal extraction from the soil that scientists would accept as reflecting root uptake. Readily available heavy metals are estimated by first extracting the metal from the soil into soil solution. The dissolved elements in the extract are then measured by atomic absorption spectrometry. There are many methods considered appropriate for extracting bio-available heavy metals. These methods range from the use of chelating compounds, such as Diethylene Triamine Penta Acetic Acid (DPTA) (Lindsay and Norvell, 1978); diethylene triamine-pentaacetic acid-triethanolamine (DPTA-TEA) (Lindsay and Norvell, 1978) and ethylene-diamine-tetraacetic acid (EDTA) to non-chelating compounds such as ammonium acetate (Soane and Saunder, 1959, Ernest, 1974, Robinson, 1997); calcium chloride (Murray et al, 2003) and water. Each method of soil extraction provides its own value of bio-available metal content in a given soil, depending on the relative strength of the extracting agent to solubilise the metal in the soil. According to Murray et al (2003), the relative ability of mild and aggressive metal extracting agents to assess metal bio-availability in soils has rarely been compared.

In general, chelating agents have been used widely for assessing readily available micronutrients since they combine with free metal ions in solution and ions on exchangeable sites to form soluble complexes. Chelating agents induce desorption of the metals, including Pb and Cd, from soil solids thereby increasing the metal content in soil solution and ultimately plant uptake (Chen and Hong, 1995; Baylock et al, 1998; Anderson et al, 1999). Diethylene Triamine Penta Acetic Acid (DPTA), is a mild chelating agent that was found to be useful for separating soils into deficient and non-deficient categories for Zn, Cu, Mn and Fe and was therefore considered appropriate for estimating bio-available metals (Lindsay and Noverll, 1978). The same authors noted that the DPTA micronutrient extraction method correlates well with crop response to Zn and Cu and is considered suitable for monitoring Pb, Cd, and Ni in soils receiving sludge applications. However, O'Connor (1988) observes the anomaly that very high DPTA-extractable metals may be harmless to the plant and correlations between DPTA-extracted metals and plant concentrations may not be significant enough to predict plant levels based on soil levels. The author concludes that such correlations may require consideration of pH to be significant and recommends limiting its use to the original purpose described by Lindsay and Norvell (1978). On the other hand, EDTA is a relatively strong chelating agent that has great potential for use in phyto-extraction of major contaminants through its ability to chelate with metal ions bound by the soil, thereby bringing them into solution. The addition of EDTA to the soil increased accumulation of Pb in maize (Baylock et al, 1998) because EDTA stimulated release of Pb from the soil into soil solution. Kirkham (2000) noted that EDTA mobilised Pb associated with the ion exchange and carbonate fractions. Cunningham et al (1997) and Lasat (2002) suggested that EDTA might increase the risk of spreading contamination and groundwater pollution, due to the high solubility of the Pb-chelate complex.

Other researchers prefer the use of some inorganic compounds instead of chelates, on the grounds that chelating agents may extract more than the plant available fraction of metals in the soil. Murray and Evans (2002) recommended the use of 0.01 M $CaCl_2$ in predicting plant availability after they found strong correlation between brome grass metal content and $CaCl_2$-extracted Cu, Ni, Zn and Cd from a sludge amended soil. Murray et al (2003) proceeded to recommend dilute $CaCl_2$ as a universal soil extractant for estimating trace metal availability to crops based on findings of linear regression analysis of heavy metals (including Pb and Cd) they undertook to relate concentrations of heavy metals in *Trifolium pratense* L. (red clover) and in fine and coarse textured soils amended by heavy application of sewage sludge.

Ammonium acetate, an inorganic compound, has been used widely over the years due to the strong correlation of soil bio-available metal it extracts from soils and plant metal content (McGrath and Cegarra, 1992). Soane and Saunder (1959) found strong correlation between bio-available Ni and Cd content of soils and plant metal content. Robinson (1997) recommended I M ammonium acetate as the most suitable method for estimating bio-availability after obtaining good correlation between bio-available Zn and Cd and plant metal contents. On the basis of the fore-going evidence, it is important to specify the method one selects to extract bio-available metals from the soil and also avoid using methods that may encourage spreading of soil contamination.

2.19 Review of some findings of pot and field methods for determining metal uptake

Different results on uptake of heavy metals are obtained depending on whether plants are grown in pots or field and the method of growing the crop. de Vries (1980) states that results from pot experiments in greenhouses can not be extrapolated to field conditions due to differences in environmental conditions under which the two are conducted. Results are affected by pot size, growing conditions in the greenhouse (micro-climate), watering and fertilisation regimes, all of which affect the yields and chemical composition of the plant differently from what happens in the field.

In general, experiments in which plants are grown in pots tend to give comparatively higher levels of contaminants than field experiments. Schmidt (2003) noted that heavy metals recorded in pot experiments are generally higher than those recorded under field conditions due in part to the higher efficiency of soil amendments in pots and the fact that plant roots explore potted soil intensely and are therefore always in contact with the soil amendments. Kayser at al (2000) obtained three times more Cd in tobacco (*Nicotiana tabacum* L.) and seven times more Cd in Indian mustard in pot experiments compared to field experiments. Pot studies allow for testing appropriate concentration levels, simplify measurements of relevant parameters, including leaching and are useful for designing field experiments (Schmidt, 2003). The same author suggested that when pot experiments lead to field experiments, transplants should be avoided and instead the plant should be germinated in the contaminated soil. Wu at al (1999) found that Pb concentrations resulting from transplanted corn were 45-fold the concentration in the control compared to 6-fold the concentration in the control, obtained for corn germinated in the contaminated soil. This outcome suggested that Pb uptake differed if seedlings were transplanted or germinated directly.

Contrasting results were found when soil amendments were added in a single dose compared to several doses. Grcman et al (2001) observed a 105-fold increase in Pb accumulation in cabbage compared to a 44-fold increase after adding the same amount of EDTA as a single dose and four doses, respectively. Conversely, Puschenreiter et al (2001) observed a 18-fold increase in Pb accumulation in corn compared to 8-fold increase for multiple application and single dose, respectively, of the same quantity of EDTA.

2.20 Review of sewage treatment systems in Zimbabwe

There are 139 wastewater treatment plants in Zimbabwe and of these 101 are waste stabilization ponds (Hungerbuehler, 1997). Others use the biological trickling filtration system, also known as bio-filtration system and biological nutrient removal activated sludge treatment systems. Water from wastewater stabilization pond systems and biological trickling systems is considered unfit for disposal into river systems or dams and therefore is used to irrigate pasturelands. There is, however, a tendency by local authorities to invest more into biological nutrient removal systems. Although this system is expensive to run it produces better quality effluent that is considered safe for disposal into river systems and lakes/dams so that such water may be recycled.

The use of wastewater effluent from wastewater stabilization pond and bio-filtration systems for irrigating pastures is a common practice among local authorities in Zimbabwe. Although Zimbabwe has over 30 years experience in irrigating pastures using wastewater, no detailed study has been carried out to determine the possible effects of the practice on the health of the farm workers, those living near the farms or those who consume the beef from animals bred on the pastures.

Zimbabwe has relevant legal instruments in the Zimbabwe Water Act (1999) and a legal supervising authority, the Zimbabwe National Water Authority (ZINWA) for controlling pollution by wastewater effluents but these regulations are not being fully enforced (Magadza, 2003). Each polluter is required to have a permit and is responsible for carrying out environmental audits for submission to the Pollution Control Unit of ZINWA (Zimbabwe Water Act, 1999). The Pollution Control Unit is responsible for enforcing regulations and as such it carries out inspection whenever and wherever it deems necessary. Earlier on the Public Health (Effluent) Regulations (1972) that were a part of the Rhodesia Water Act (1977) prohibited the use of raw or undigested sewage sludge and effluent waters on land used for agricultural purposes. It further stated that no effluent liquid (discharged from sewage works) could be used for irrigation and no digested sludge could be used for agricultural purposes without the prior permission from the appropriate authority.

Municipal wastewater management is the responsibility of local authorities. The authorities have laboratories that monitor the quality of effluent discharged from wastewater treatment plants. Municipal authorities in Zimbabwe take steps to minimise environmental problems emanating from the use of wastewater for irrigation. Besides the treatment of wastewater, the measures include prohibiting dairy farming on wastewater-irrigated pasturelands for fear of milk contamination and discouraging horticultural crop production using wastewater. City Council and beef abattoirs check for pathogens and helminths, in beef from animals that are bred on pastures irrigated using treated wastewater. However, they do not check for levels of chemical pollutants. This implies that metals would not be detected prior to beef being consumed by humans. In general, the wastewater treatment systems are fraught with problems that compromise operational efficiency, hence the need to investigate the impact they have on the environment.

Extreme contamination of soils with sewage sludge has been demonstrated around Harare, since the early 1970's and much of the research has been concentrated on the Crowborough farm, one of the farms that receive effluent and sludge application (Mangwayana, 1995). Although efforts are made to remove heavy metals from the effluent, they could still find their way to the pastures since liquid digested sludge is often mixed with effluent and disposed on pastures. Even where only effluent waters were disposed on pasturelands, the concentration of heavy metal in the soil were still high. Mangwayana (1995) reported 1.6 mg/kg total soil Cd. The fate of Pb and Cd is unknown at Firle Wastewater Treatment Works (one of Harare and Zimbabwe's largest treatment plants) where low quality effluent produced from the biological filtration system is mixed with liquid digested sewage sludge (to produce a slurry of 3-4% solids) that is used for irrigating 860 hectares of pasture at Firle Farm (Nyamangara, 1999).

Considering that only a few short-term studies have been done to date, it was not possible to rule out the risk posed to animals and humans by Pb and Cd through waste disposal on pasturelands. It is on the strength of this background that this study was considered necessary.

2.21 Problem statement and hypotheses

2.21.1 Problem statement

The discussion in the previous sections of this study raised a number of important issues that motivated this study. In Zimbabwe, pasturelands consisting of star grass have been grown on sandy soils on which treated sewage has been disposed for over 30 years. Despite the knowledge that sewage from domestic and industrial sources in cities contains potentially harmful heavy metals, there has not been any meaningful monitoring of heavy metal content in the treated sewage and soils. Furthermore, there has not been any attempt to quantify Pb and Cd uptake by grass or animals to ascertain compliance of metal content of grass with acceptable levels for grazing pastures, despite the detection of the two metals in treated sewage.

It has been noted from the literature review that transposing permissible metal content guidelines from one country to another could lead to inaccurate predictions of the pollution hazard. Even though most developing countries have adopted guidelines developed elsewhere, the use of permissible total soil metal concentration has come under scrutiny as research has shown that total soil metal concentrations do not predict metal content in plants accurately enough for health assessments. Bio-available metal concentrations have of late been considered as being more accurate, but limited in use because of lack of consensus on the method of measurement.

One of the largest treatment plants in Zimbabwe, Fire Wastewater Treatment Plant disposes of treated sewage on sandy soils with star grass pasture. The fact that bio-availability and hence toxicity of heavy metals is higher in sandy soils than clayey soils (Bak and Jensen, 1998) suggests the possibility of higher plant uptake of heavy metals where a sandy soil is involved. Given that different plant species have different uptake capacities (Johannesson, 2002), the absence of any known studies on uptake of Pb and Cd by star grass implies that soil-star grass uptake characteristics, including critical metal uptake levels are not known and cannot be extrapolated from other grasses that have been studied. The absence of any such study also implies that there is no model that could be used to predict metal content in plants on the basis of metal content in soils. This scenario denies municipalities vital information required for developing soil and star grass management practices and policies for sewage disposal on pasturelands.

Since bio-available metal levels in soils depend on plant, soil properties and climate, among other factors, local conditions have a bearing on acceptable limits of heavy metals. It has also been noted that those who developed models for soil-plant ecosystems encourage site-specific studies due to perceived deficiencies in the models. Therefore this study aimed to:

- determine the extent of Pb and Cd accumulation in sandy soils and star grass under irrigation with treated sewage,
- produce dose-response models for predicting metal concentrations in star grass using bio-available soil metal concentrations in sandy soils

Such information was postulated to permit the estimation of appropriate levels of the metals that can be allowed in a sandy soil and star grass, so that the production of star grass could be optimized, while minimizing potential heavy metal accumulation in beef animals that graze on such pastures.

2.21.2 Potential benefits of study

The formulating of objectives and hypotheses for this study was based on whether the answers from the study (if obtained) would be useful and to whom? It was considered that if answers were to be obtained, a number of stakeholders would be expected to benefit from the findings. At local level, consumers of beef or milk from animals bred on wastewater disposed pastures and farm workers would be in a position to, either allay fears of chemical pollution or improve wastewater monitoring system to reduce chances of pollution. Harare City Council would use the findings to identify chemical pollution hazards associated with its current wastewater management practices and institute mitigatory measures to safeguard the health of the residents. The benefit would also be expected to extend to other local authorities in Zimbabwe and potentially SADC region through dissemination of the findings. The study seeks to address and link well with pertinent issues in the region, notably environmental and health issues and integrated water management. Within this context, it also complements efforts being made by the Institute of Water and Sanitation Development in developing water quality monitoring standards in Zimbabwe and scientists studying phyto-remediation worldwide.

2.21.3 Hypotheses

This study was intended to test the following hypothesis:

(i) Star grass takes up Pb and Cd and it is a high accumulator of both metals

(ii) The disposal of treated mixed effluent and sludge emanating from industrial and domestic sources onto sandy soils increases bio-available Pb and Cd in the soil

(iii) Star grass grown on sandy soils on which treated wastewater from industrial sources is disposed of can take up Pb and Cd to levels that present a Pb and Cd hazard to grazing cattle.

(iv) Bio-available Pb and Cd in sandy soils and Pb and Cd concentrations in star grass form a relationship that can be used to predict metal concentration in the grass.

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.

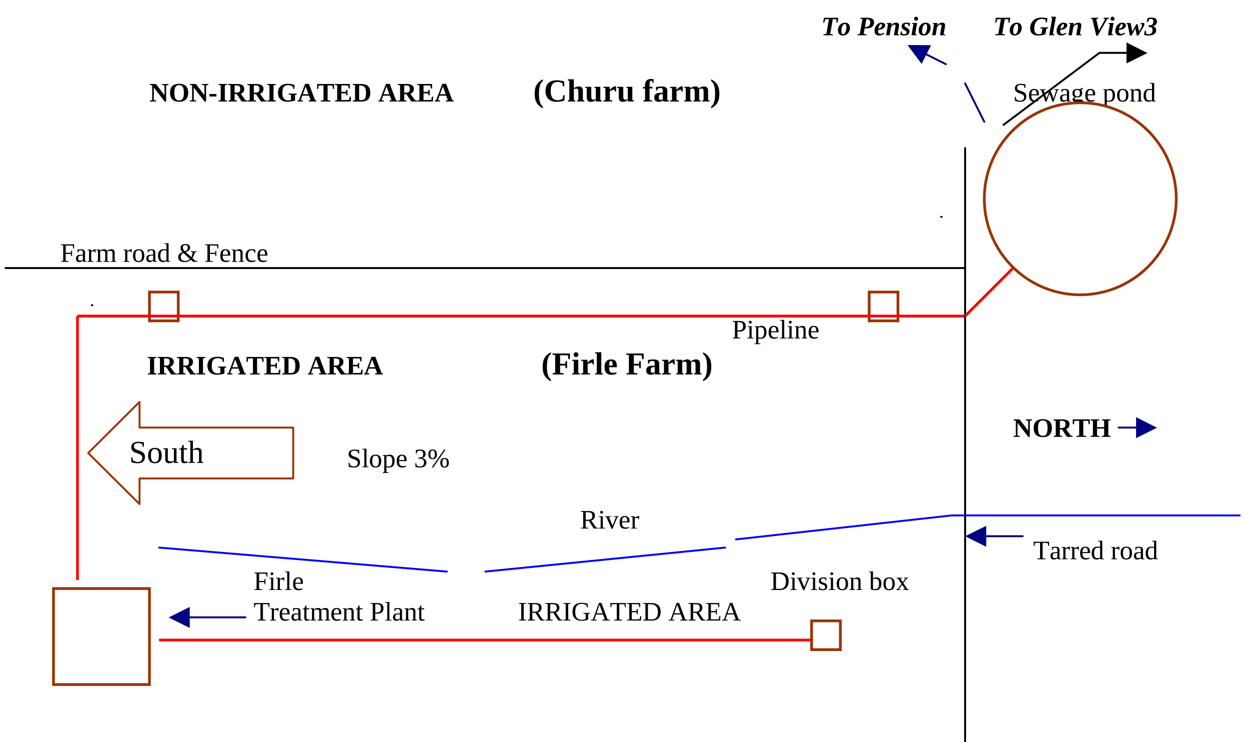

Figure 3.1: Schematic diagram of the study area

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.

CHAPTER 4

BASELINE ASSESSMENT OF LEAD AND CADMIUM LEVELS IN STUDY AREA

4.1 Introduction

This chapter describes a baseline assessment of Pb and Cd levels in treated sewage, soils and existing pasture as well as soil properties of the study area where detailed experiments described in chapters 5 and 6 were eventually carried out. The purpose of the assessment was to establish characteristics of the area and use them to select portions of the area suitable for detailed experiments on Pb and Cd. This chapter also provides an assessment of the long-term total levels of accumulation of Pb and Cd in the soils, using chemical tests on soil samples from the irrigated area and non-irrigated area.

4.2 Objectives

The specific objectives of this component of the study were:

(1) to assess Pb and Cd levels in treated sewage using past chemical records
(2) to determine long-term accumulation and distribution of Pb and Cd in a sandy soil after 29 years of treated sewage disposal
(3) to establish chemical characteristics of the soil in the study area
(4) to determine the presence and levels of Pb and Cd in mixed star and kikuyu pasture grown in the area of disposal of treated sewage
(5) to investigate relationships between total concentrations of Pb and Cd in the soil and metal content in mixed star and kikuyu grass pasture.

4.3 Detailed methods and materials

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

Available past records of chemical tests on heavy metals covered a short period of time (1991 to 1994), during which the City of Harare carried out heavy metal tests. Data on Pb and Cd levels in raw sewage, treated effluent and digested sludge were extracted and analysed statistically using the SPSS 8.0 for Windows (www.spss.com, 1997) computer package to obtain averages and ranges that were compared with legislated limits.

4.3.2 Baseline assessment of chemical characteristics of study area

Soil sampling and testing

Soil samples were taken at least 2 months after the last irrigation. The samples were taken from portions of the control and treatment areas. Each of the portions was 200 m long and 100 m wide. The portions were sub-divided into 4 plots that were 100m long and 50 m wide. From each plot, 4 auger samples located on a grid of 6.25 m x 12.5 m were taken and corresponding horizons mixed to make a composite sample. Each horizon was 10 cm in depth. The maximum soil depth was 50 cm. Areas that had evidence of localized ponding, such as irrigation furrows were avoided. Surface litter was removed prior to soil sampling using augers. Plant debris was removed from soil samples, before they were air-dried and passed through a 0.15 mm sieve.

Soil texture was determined using the hydrometer method (Gee and Bauder, 1986). Calgon (37.5g sodium hexametaphosphate ($Na_6O_{18}P_6$) and 7.94g sodium carbonate (Na_2CO_3) dissolved in 1000 ml of water) was used to disperse the soil fractions in the soil sample. The dispersed suspension was passed through a 180-µm sieve and collected in a measuring cylinder to separate the sand from the clay and silt. The weight of the sand was measured after oven-drying it overnight. A brass plunger was used to stir the suspension. A hydrometer was used to take measurements of clay and silt after 60 seconds of inserting the plunger into the suspension. Two hours from the commencement of sedimentation, a reading representing the clay content, was taken using the hydrometer. Temperatures were measured in both cases. The hydrometer readings were corrected for temperature by adding or subtracting 0.3 units for every degree above or below 20^0C, respectively. The percentage silt plus clay and clay content were corrected for hygroscopic moisture to obtain the percentages of silt and clay.

Soil pH was determined using a 1:5 soil suspension of 0.01M $CaCl_2$. A standard buffer solution (pH 7.0) of dry potassium di-hydrogen orthophosphate (KH_2PO_4) and di-sodium hydrogen orthophosphate (Na_2HPO_4) was used to calibrate the pH meter. Records of pH were made when reading on the pH meter stabilised for at least 0.1 units per 30 seconds.

Cation exchange capacity (CEC) was determined by saturating the soil with 1M CH_3COONH_4 buffered at pH 5.2. A mechanical vacuum extractor (Model LT-800-8, Concept Engineering, Inc, Nebraska, III, USA) was used for extracting exchangeable bases. Excess ammonium was washed out using ethanol (CH_2CH_3OH) and the adsorbed ammonium was determined by steam distillation and titration with sulphuric acid (H_2SO_4) in a distillation unit, Kjeltec Auto 1030 Analyser. A standard sample (Harare 5E.2) was included in every 48 samples for quality control. Where the value of the check sample was outside the expected range the whole batch was re-done.

Total heavy metals in soil were extracted with aqua regia (1:3 conc. HNO_3 and conc. HCl) and heated under reflux. After filtration, the extract was diluted with 2M HNO_3 and Pb and Cd were determined on the atomic absorption spectrometer.

Organic carbon (C) was determined by the modified Walkley and Black method (Houba et al., 1989) with additional heat applied under reflux (at 130^0C). Excess Potassium dichromate ($K_2Cr_2O_7$) was used to oxidise organic C. The excess dichromate was then titrated using ferrous ammonium sulphate ($Fe(NH_4)_2(SO4)_2$) to determine the amount used in oxidising organic C. A conversion factor of 1.33 was applied to organic C to determine total organic C assuming 75% recovery (Houba et al., 1989). A Total Organic Carbon (TOC) analyser was not available, hence the method described was used.

Sampling and testing of grass

Grass samples from the irrigated area were taken from each plot to determine the metal content. One sample, consisting of 10 sub-samples was taken from each of the 4 plots in the sewage sludge treatment. The samples were taken on a grid of 6.25 m x 10 m, within each plot.

The grass was cut at 5cm height off the ground. No grass samples were taken from the control area because the sparse and mixed grasses were different from those grown under irrigation. It was therefore not possible to compare heavy metal plant uptake between the control and the sewage sludge treatment. The samples were washed using deionised water and oven dried at 65 ^{0}C to constant weight before being 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.

To analyse the measured data, arithmetic means and standard deviations were calculated on clay content, pH, CEC, organic carbon, Pb and Cd for each soil horizon. Data from tests of Pb and Cd on grass was subjected to the same analysis for which means and ranges are presented. Analysis of variance was used to determine the significance of differences in means of various data. Simple correlation was used to measure the degree of association between any two soil properties, since it was important to investigate whether soil properties like CEC, organic matter, heavy metal content and soil depth were associated. Pearson's correlation coefficients (r^2 values) were, therefore computed for pairs of variables, including soil depth to determine trends in the relationships of the variables and likely implications on uptake and modelling of uptake of Pb and Cd.

4.4 Results

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

Firle Wastewater Treatment Plant utilises two types of sewage treatment technologies, namely biological trickling filtration plants and biological nutrient removal activated sludge plants. Appendix 1 summarises the treatment processes in the two technologies at Firle Treatment Works. The effluent from biological trickling filtration plants is mixed with sludge (shown as liquid digested sludge or farm compost material for farmland and humus sludge in Appendix 1) and directed to irrigate pastures. This approach serves two purposes, disposal of sludge without the need for landfill and secondary treatment of effluent through the soil, where the seepage is expected to be of acceptable quality before it gets into Manyame River. Sometimes liquid sludge on its own or effluent on its own is disposed on pastures.

Heavy metals in treated sewage

Table 4.1 presents results of an analysis of Pb and Cd levels and their prescribed limits according to the Zimbabwe Water (Wastewater and Effluent Disposal) Regulation (Zimbabwe Statutory Instrument 274, 2000). These regulations were derived from international guidelines, particularly the average recommended maximum limits that were based on United States Environmental Protection Agency guidelines for wastewater reuse enshrined in US EPA (1992) and also included in the table.

Table 4.1 shows the presence of Pb and Cd in both the effluent and digested sludge over the 4-year period. The average concentrations of the metals in effluent were within the acceptable limits for short-term irrigation. However the highest levels were above maximum recommended levels in both effluent and digested sludge.

The average levels of Pb of 2.6 mg/l for digested sludge and 0.20 mg/l for effluent were below the local legislated limits for both short and long-term irrigation and US EPA (1992) limits. However the highest concentration of Pb (5.03 mg/l) was marginally higher than the recommended long-term level in sludge.

The concentration of Cd in the effluent and digested sludge was not detectable in some samples, hence it was within acceptable limits. However, the upper levels of Cd in digested sludge (0.5 mg/l) and effluent (0.03 mg/l) were 50 and 3 times the local recommended level of 0.01 mg/l, for long-term irrigation, respectively. The upper level of Cd in sludge was 10 times the level recommended for short-term irrigation. This indicates a wide range in metal content of both effluent and sludge.

Table 4.1: Average (range) concentration (mg/l) of heavy metals in samples of digested sewage sludge and effluent (Source: Harare City Council file records, 1991-1994)

Metal	Digested sewage sludge	Effluent	Current local legislated levels in wastewater suitable for irrigation*		Recommended maximum concentration **
			Long term	Short term	
Pb	2.6 (0.13-5.03)	0.20 (0.05-0.48)	5	20	5
Cd	ND (ND - 0.5)	ND (ND - 0.03)	0.01	0.05	0.01

ND - not detectable
*Zimbabwe Statutory Instrument 274 (2000).
** Source : United States Environmental Protection Agency (US EPA), 1992.

4.4.2 Chemical characteristics of study area

Type of soil, soil pH, cation exchange capacity and organic carbon

Table 4.2 presents selected soil properties of clay content, soil pH, organic carbon and CEC. The control and treatment were situated on predominantly granitic sandy soil classified as Haplic Arenosol.

Table 4.2: Selected properties of a sandy soil in the irrigated and control areas

Sampled area	Soil depth (cm)	Clay content (%)	Soil pH ($CaCl_2$)	Organic carbon (%)	Cation exchange capacity ($cmol_c kg^{-1}$)
Treatment	0-10	<3	5.4 (0.5)	3.3 (0.3)	29.3 (11.2)
	10-20	5.7 (4.7)	5.5 (0.6)	1.7 (0.5)	8.1 (7.9)
	20-30	8.5 (4.4)	5.9 (0.8)	0.9 (0.6)	4.6 (3.5)
	30-40	7.3 (0.6)	5.4 (0.2)	1.1 (0.2)	2.9 (1.0)
	40-50	6.0 (1.4)	5.5 (0.1)	1.0 (0.5)	3.2 (1.7)
Control	0-10	6.3 (0.6)	4.6 (0.8)	1.3 (0.1)	1.6 (0.9)
	10-20	6.0 (1.0)	4.4 (1.1)	0.9 (0.1)	1.5 (0.8)
	20-30	5.0 (1.0)	4.6 (1.1)	1.0 (0.2)	1.4 (1.0)
	30-40	5.7 (0.6)	4.5 (1.0)	0.9 (0.2)	1.4 (0.7)
	40-50	10.0 (0.0)	4.9 (1.7)	0.7 (0.0)	1.3 (0.5)

() standard deviation

The soil consisted of a sandy top layer (0-30 cm) overlying a sandy-loam subsoil (30-50 cm). It was generally acidic with the pH of the sewage sludge and effluent irrigated soils being on average 1 pH unit higher than the corresponding horizon in the control. The very low CEC and organic C levels were consistent with sandy soils. However addition of sewage sludge and effluent resulted in a 2.6-fold and 15.5-fold increase in organic carbon and CEC, respectively in the 0-20 cm soil layers, when compared to corresponding horizons in the control.

Total concentrations of Pb and Cd in soils

Table 4.3 presents the average total concentrations of Pb and Cd in the soil profile of the irrigated and the control areas for every 10cm soil depth. Throughout the soil profiles of both the control and the irrigated areas, Pb and Cd were present. The average levels of Pb and Cd within the 0-50 cm profile of the control were 18.4 mg/kg Pb and 0.40 mg/kg Cd, respectively. The average levels of the metals in the area of disposal were 55.5 mg/kg Pb and 0.65 mg/kg Cd. Therefore the level of accumulation over the 29 years were 37.1 mg/kg Pb and 0.25 mg/kg Cd.

The mean profile concentrations of Pb of 55.46 mg/kg and Cd, 0.65 mg/kg were 3.0 and 1.6 times the mean profile levels in the control, respectively. Accumulation of the two metals predominantly occurred within the 0-20 cm horizons, particularly in the 0-10cm horizons. In the 0-10 cm horizon of the irrigated area, Pb and Cd were 11.6 and 3.2 times the levels in the 0-10 cm horizon of the control, respectively. Otherwise the background levels in the 30-50 cm horizons of the control were largely similar to the levels in the area of disposal.

Analysis of variance showed that metal levels in the treatment were significantly ($p \leq 0.01$) higher than in the control. The mean levels of the metals in the top 0-20 cm in the irrigated area were significantly ($p \leq 0.05$) higher than the levels in the 20-50 cm and also significantly higher ($p \leq 0.05$) than the levels in the 0-20 cm of the control.

Table 4.3: Average total soil metal concentrations in horizons of soil profile of irrigated and control areas

Sampled area	Soil depth (cm)	Total metal concentration (mg/kg)	
		Pb	Cd
Treatment	0-10	186.31 (120.92)	1.26 (0.35)
	10-20	33.32 (26.61)	0.75 (0.24)
	20-30	22.03 (5.70)	0.47 (0.11)
	30-40	18.71 (5.01)	0.39 (0.03)
	40-50	17.00 (9.92)	0.40 (0.25)
Control	0-10	16.00 (1.63)	0.40 (0.14)
	10-20	24.00 (13.47)	0.35 (0.09)
	20-30	19.00 (7.39)	0.49 (0.13)
	30-40	15.50 (1.00)	0.45 (0.19)
	40-50	17.33 (1.15)	0.33 (0.20)

() standard deviation

Comparison of mean levels of metals in the 20-50 cm soil horizons showed that there were no significant differences within the horizons in both the control and the treatment. Total metal concentrations of the soil horizons correlated well with depth in the irrigated areas (r^2 = -0.76 for Pb and 0.89 for Cd) and poorly in the control (r^2 = -0.27 for Pb and -0.09 for Cd).

Correlation of soil parameters and total metal concentrations

The pH correlated poorly with depth since r^2 values for the irrigated area and control area were 0.08 and 0.59 respectively compared to an r^2 critical value of 0.87. The correlation of the pH of the soil and total metal concentration along the soil horizons was weak in both the irrigated and the control areas for both Pb (r^2 = -0.44 in both cases) and Cd (r^2 = 0.35 in the irrigated area and 0.30 in the control area).

The clay content of the soils correlated strongly with total concentrations of both Pb and Cd in the irrigated area (r^2 = -0.85 for Pb and -0.87 for Cd) but relatively weakly in Pb of the control (r^2 = 0.05). In the case of Cd correlation was strong in both cases (r^2 = -0.87 in the irrigated area and 0.75 in the control area).

Cation exchange capacity correlated strongly with total concentrations in the irrigated area (r^2 = 0.99 for Pb and 0.97 for Cd). It however correlated weakly with total concentrations in the control (r^2 = 0.12 for Pb and 0.04 for Cd). Organic C strongly correlated with total metal concentrations in the irrigated area (r^2 = 0.97 for both Pb and Cd) but weakly with total metal concentrations in the control (r^2 = 0.25 for Pb and 0.34 for Cd).

Concentrations of Pb and Cd in soils and grasses

Table 4.4 presents the average total Pb and Cd concentrations in the 0-20 cm soil horizon and in grass from sub-sampling areas within the irrigated area. Mixed star and kikuyu grasses took up Pb and Cd. Cadmium was not detected in some samples. Using the Student' t-test showed that there was no significant difference in mean soil levels of Pb and Cd on sub-sampling points 1 and 4 and in levels of Cd in the corresponding grasses. However soil Pb levels at these points were significantly ($p \leq 0.05$) higher than on sub-sampling points 2 and 3 and the level for sub-sampling point 3 was significantly ($p \leq 0.05$) higher than at sub-sampling point 2. The Pb level of grass at sampling point 1 was significantly ($p \leq 0.05$) higher than on sub-sampling points 2 to 4. Sub-sampling point 2 had a significantly ($p \leq 0.05$) higher Cd level than the rest.

There was very weak correlation between total soil concentration of Pb and levels in the grass (r^2 = 0.39). Similarly, weak correlation coefficients were obtained using $\log_{10}$-transformed total soil concentration and $\log_{10}$-transformed concentration of the metals in grass.

Table 4.4: Average total metal levels (mg/kg) in 0-20 cm soil depth and mixed grass

Sub-sampling points	Pb		Cd	
	Soil	Grass	Soil	Grass
1	148.65 (15.32)	1.50 (0.04)	0.66 (0.09)	ND
2	17.0 (11.31)	1.01 (0.02)	1.19 (0.06)	1.2 (0.03)
3	88.91 (8.22)	ND	1.21 (0.11)	0.17 (0.02)
4	167.54 (17.68)	1.0 (0.03)	0.95 (0.08)	ND

() standard deviation

4.5 DISCUSSION

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

A comparison of mean levels of Pb and Cd in the treated wastewaters and legislated limits, suggested that no hazard should be expected from the two metals. However, the upper limits of Cd in treated sludge and effluent were 50 and 3 times the local legislated level of 0.01 mg/l, respectively. The limitations in the data, described below and the probability that higher levels of the metals could be discharged into the treatment system and onto the pasturelands, implied that Pb and Cd hazard could not be ruled out on the basis of the data analysed in this component of the study. This notion is supported by the fact that the upper levels of the metals in treated water were very high, particularly in the case of Cd.

The data used in this analysis was scanty. Considering that on-the-spot or grab samples were taken once every two months, it was not possible to determine the distribution of the levels within each 2 month period, let alone the variation within the intervening period or within a production cycle. Junkins et al (1983) recommended hourly sampling programs during some production cycles and non-production cycles in order to capture variations in organic loading, including detection of peak organic loads. Therefore it was not possible to ascertain the distribution of the concentrations of the metals with respect to the upper or lower values, on the basis of the data obtained from the City of Harare. If the systems tended to operate close to the upper limit, the scenario presented considerable Pb and Cd hazard. Hofwegen and Veenstra (1995) noted that a 50% increase in total soil Cd from 0.5 mg/kg to 0.82 mg/kg caused a large increase in Cd content in brown rice from 0.08 to 1 mg/kg (1200%).

The wide range of metal concentrations in both the effluent and digested sludge (Table 4.1) implied large variations in levels of pollutants in raw sewage from the sources. This variation could be a result of varying levels of dilution of the pollutants from sources or increases and decreases of activities causing pollution at the sources or intermittent high levels of pollution. Besides dilution, most variation in pollutant concentrations could be attributed to industrial pollution since domestic pollution would not be expected to vary so widely. The variability in strength and character of influent wastewater (which can cause organic shock loads to biological treatment systems) is experienced by most treatment systems and is related to industrial and commercial operation schedules, which tend to vary (Junkins et al 1983).

There was insufficient disposal data on treated sewage application. The lack of data on the proportions of mixes of sludge and effluent implied that the actual concentrations of the metals in the mixture of effluent and sludge were unknown. The levels shown in Table 4.1 however suggest that the concentration of the mixed effluent and sludge disposed on pastures was somewhere in between the concentrations of the two. This implied that based on the 1991-1994 data, treated sewage disposed on pastures had 0.20-2.60 mg/l Pb and <0.0-0.5 mg/l Cd, leaving open the possibility of irrigation water having a concentration that could be higher than recommended levels.

The fact that Harare City Council sometimes irrigated the land using effluent only or sludge only or a mixture of the two also added uncertainty regarding heavy metal loading on the soils. In addition the lack of proper irrigation schedules implied that some areas could receive more water than others, suggesting that some areas could be exposed to higher metal concentrations from the irrigation water if their irrigation coincided with a period when high loads were received by the treatment plant.

4.5.2 Pb and Cd accumulation in soils

Variations of total soil metal concentrations between irrigated and control areas

The higher levels of total Pb and Cd obtained in the soil profile of the irrigated area indicate accumulation when compared with levels in profile of the control area. The average background level of Pb of 18.4 mg/kg in the control area and the average soil profile Pb level of 55.5 mg/kg in the irrigated area indicate accumulation of 37.1 mg/kg (1.3 mg/kg/year) in the soil over 29 years. Both soils fell within the 10 mg/kg to 70 mg/kg background levels of Pb for normal soils (Johannesson, 2002). Accumulation in the top soil layers translates to an average increase of 5.7 mg/kg per year in the top 10 cm and 0.3 mg/kg per year in the 10-20 cm depth.

The mean profile levels of Cd of 0.65 mg/kg obtained in the irrigated area against a background level of 0.40 mg/kg in the control area indicates accumulation of 0.25 mg/kg in 29 years or 0.01 mg/kg/year in the irrigated area. Cadmium has been reported to be less than 1 mg/kg (Alloway, 1995), 0.2 mg/kg (WHO, 1993) and 0.1-0.4 mg/kg (Johannesson, 2002) in normal soils.

Therefore the level obtained in this study was in agreement with levels quoted in these references. McGrath and Loveland (1992) observed total Cd concentrations of 0.2 to 1.7 mg/kg in agricultural soils in England and Wales, while Alloway (1995) reported up to 100 mg/kg Cd in agricultural soils subjected to high application of phosphate fertilisers.

The preceding arguments confirm that the observed levels of all metals in the control area were the base levels for an unpolluted soil. The fact that the two areas were just adjacent eliminates the possibility of other sources of pollution, such as air pollution, being responsible for the increase in metal concentration in the soils. Therefore the metals that were identified in treated sewage led to a rise in the levels of the metals in the sandy soil.

The higher pH in the irrigated area (Table 4.3) was attributed to the alkalisation effect of basic cations contained in sewage sludge and effluent. While some variations in metal levels are expected due to inherent differences in soil chemistry between one sampling point and another, the large variation in metal levels between sampling points was attributed to poor water application and distribution associated with the flood disposal system.

In this study, the total metal concentrations of the Pb and Cd were strongly and positively correlated to clay content, CEC and organic matter, but weakly correlated to pH in the sewage-irrigated soil. In contrast the total metal concentrations of Pb and Cd in the control were generally weakly correlated to clay content, CEC and organic matter but more strongly correlated to pH. This confirms the influence of organic matter on soil parameters, including total metal content of soils and suggests that high levels of organic matter is associated with high levels of total metal content and vice versa. Since organic matter is largely retained in the topsoil (Birley and Lock, 2001), its correlation with CEC and total metal content along the soil profile suggests that where a soil receives treated sewage, the extent of exposure of plant roots to metal ions depends on level of organic matter and distribution of plant roots along the soil profile. This has implications on plant metal uptake as discussed below.

Distribution of metal concentrations along soil depth

There was a large variation of total metal concentration with depth. The top 20cm of the soil and in particular the top 10cm had relatively higher metal concentrations than the lower horizons in the irrigated area. Since Pb and Cd levels within the 30-50 cm horizons of the control and the area of disposal were similar and the 0-20 cm horizons of the irrigated area had much higher levels than the control, it can be argued that the metals were largely immobile.

Considering that the top 20cm had far less clay than the lower horizons, the high CEC could be attributed to the high organic matter content of these layers, rather than the clay content. The results suggest that organic matter held Pb and Cd in the top layers, making them immobile, and thereby confirming their high affinity to organic matter (McGrath and Lane, 1989). This is also confirmed by the high correlation between the metal levels and organic C (r^2 = 0.97). This outcome suggests that the metals accumulated in the top horizons where grass roots were expected to grow.

The variation of total metal concentration with depth presents a potentially large source of error in relating soil concentrations to acceptable limits and to plant concentrations as well as in modeling soil-plant uptake. This is so if one considers that the depth interval at which various plants in different environments obtain water and nutrients and the relative density of feeder roots at different depths are unknown (US Department of Energy, 1998). When the average profile content of Pb of 55.5 mg/kg is compared with 87 mg/kg stated by Johannesson (2002) as the lower limit at which basic soil processes, such as microbial activity, are affected, Pb problems would not be expected. However the opposite is true if the 109.8 mg/kg in the 0-20 cm depth is considered. Similarly, the average level of Cd of 1.0 mg/kg in the 0-20cm horizon would be regarded as being too high for a soil, considering that it is equal to the recommended sludge directive limit of 1 mg/kg (EEC, 1986) for use of sewage in agriculture. However, the average soil profile concentration of 0.65 mg/kg would be considered more acceptable.

Total soil concentration versus recommended guidelines

The maximum permissible concentrations of Pb in soil under grass, stated by different authorities are: 300 mg/kg (Department of Environment, 1989), 100 mg/kg (Ross et al., 1992) and 150 mg/kg Birley (2001). A comparison of the results shown in Table 4.3 and these maximum permissible limits suggests that the levels obtained in this study would be acceptable according to Department of Environment (1989) but unacceptable according to Ross et al (1992).

The disparities in the guidelines quoted here and other international literature emanate from the different climatic, soil and crop conditions under which they were determined. Indeed most of these guidelines do not specify the soil type and the organic content on which the heavy metal sorption depends (Christensen 1989b). In addition they often do not specify the type of crop or soil depth they apply to, both of which are important factors when considering the possibility of plant uptake of the metals.

Heavy metal content in mixed grasses

Table 4.4 shows that the mixture of kikuyu and star grasses accumulated large quantities of Cd and small amounts Pb compared to recommended limits. The average Pb level of 1.2 mg/kg in grass was below the 10mg/kg tolerance level for agronomic crops (Seaker, 1991) and 40 mg/kg recommended for pasture grass (U.K Statutory Instrument No. 1412, 1995). However, the fact that Cd uptake varied from non-detectable to a level of 1.2 mg/kg indicates a potential for the pasture grass to take up levels beyond the 1 mg/kg recommended for pasture for grazing animals (U.K Statutory Instrument No. 1412, 1995).

Correlation of total soil metal content and metal content in mixed grasses

A comparison of mean total soil concentration of Pb and concentration of Pb in grass shows that there was a weak correlation between the two (r^2 = -0.03–0.4). This can partly be explained by the fact plant uptake of metals is normally related to the bio-available metal concentration in the soil (Nyamangara and Mzezewa, 1999). Organic matter, pH and CEC are the most important factors that control the availability of heavy metals in the soils (Forbes et al, 1976).

4.5.3 Implications of findings

The findings of this component of the study could not be used to confirm or rule out the hazard Pb and Cd contamination in soils and grasses due to a number of reasons. Although the average level of Pb in the grass was relatively low (1.2 mg/kg), this did not necessarily confirm low uptake of the metal by star or kikuyu grasses since the assessment was done on mixed kikuyu and star grass. Similarly, although grass accumulated up to 1.2 mg/kg of Cd this did not confirm high uptake by star grass or kikuyu grasses for the same reason. The concentrations of Pb and Cd relative to their total levels in the soil and levels in the grass left questions relating to whether they interacted in the soil leading to increased or reduced uptake of one or the other or both. Bak and Jensen (1998) noted that uptake of metals by plants could be antagonistic, synergistic or additive.

The strong correlations between total soil concentration of Pb and Cd and soil properties of CEC, clay content and organic matter confirms findings of previous research work on soils amended with wastewater or sludge. While correlation provides an idea of the pattern distribution of the metals within the soil profile the lack of information on distribution of plant roots within the profile complicates selection of the soil depth on which to relate soil concentrations and plant concentrations of metals.

The accumulation of up to 1.2 mg/kg Cd in mixed kikuyu and star grasses against a total soil concentration of Cd of 0.65 mg/kg (35% less than 1 mg/kg recommended) confirmed the risk of relying on total metal concentration for purposes of predicting hazard to animals. Roberts et al (1994) reported restricted growth in livers and kidneys of animals grazing on pasture exposed to total soil Cd concentrations lower than the recommended sludge directive limit of 1 mg/kg (EEC, 1986). The high Cd content of grass in this part of the study confirmed Bak and Jensen (1998)'s observations that plants did not assimilate metals in direct proportion to total soil concentrations. The weak correlation coefficients between total soil metal content and metal content of grass were also consistent with Bak and Jensen (1998)'s observations. Indeed, Carson and Bazzaz (1977) noted that plant uptake relationships to total soil concentrations were only valid within a narrow range of chemical concentrations in the relatively non-toxic range. The data in this component of the study was not sufficient to define such a range for the sandy soil and mixed kikuyu and star grasses.

These findings suggested that the use of bio-available metal concentrations in the soil levels of metal uptake by plants should be accorded more attention in research. Highiri (1973) and US Department of Energy (1998) noted that if bio-available soil metal concentrations were to be used to improve reliability of critical metal limits in soils, they would have to be related to the plant species, since plant uptake of Pb and Cd were observed to vary with plant species.

Given the unanswered questions that emanated from the findings of this component of the study, it was considered logical to run an experiment under controlled conditions, using one of the grasses, star grass, to clarify the issues.

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.

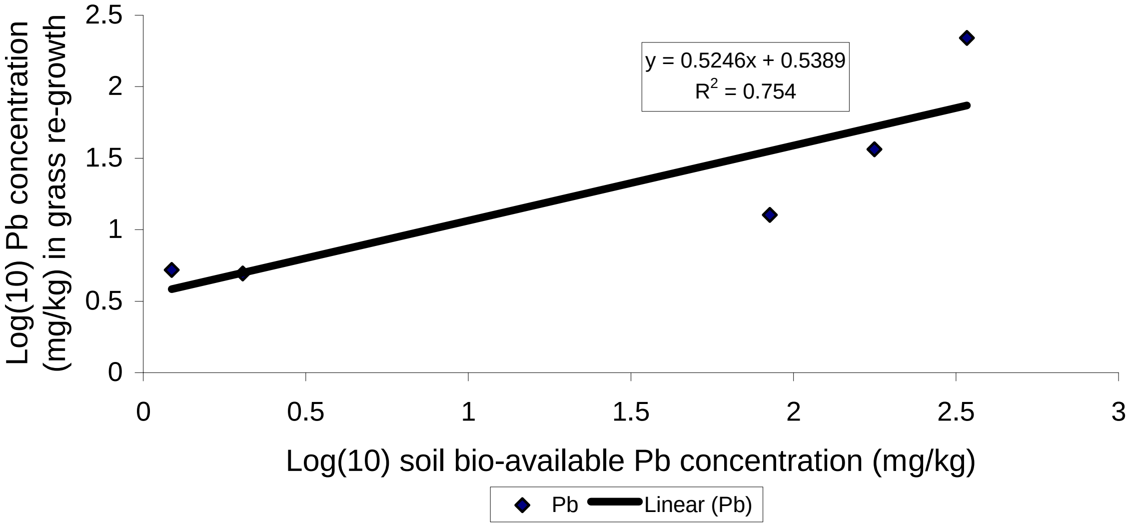

Figure 5.1: Log(10) soil bio-available level versus log(10) Pb level in grass in single treatments

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.

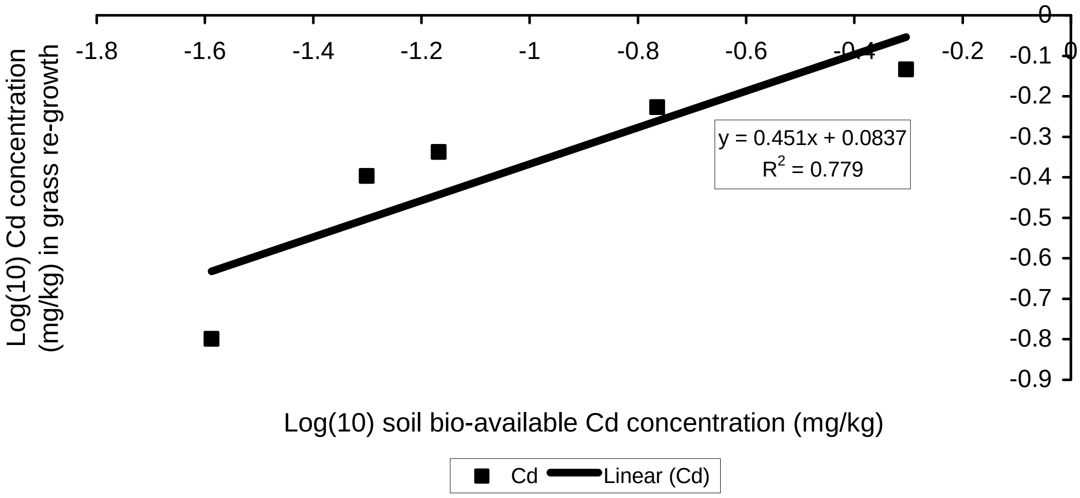

Figure 5.2: Log(10) bio-available Cd levels versus log(10) Cd levels in grass in single treatments

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.

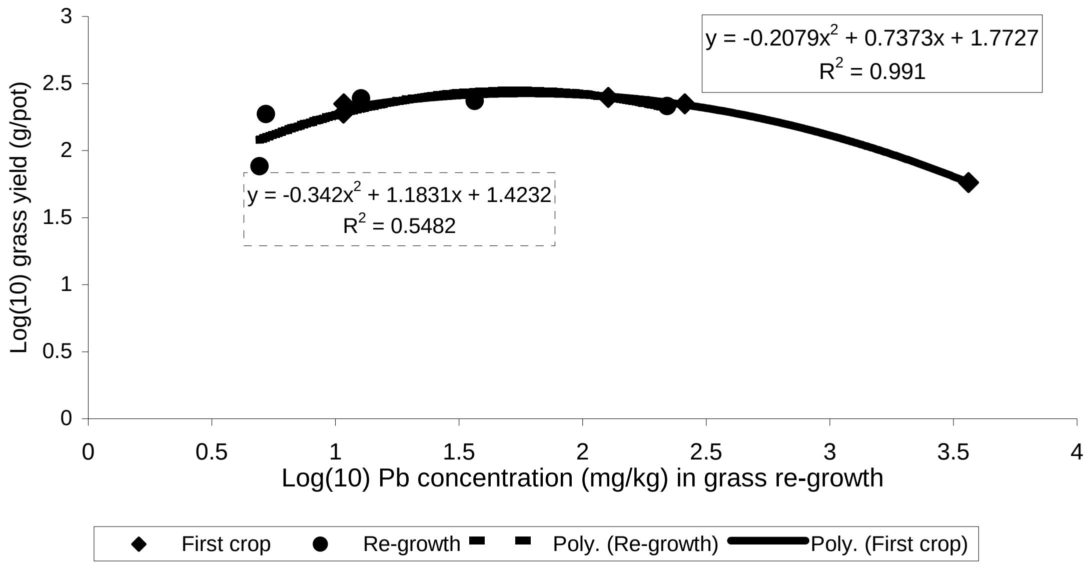

Figure 5.3: Log(10) Pb level (mg/kg) in grass versus Log(10) grass yield (g/pot) in Pb single treatments

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.

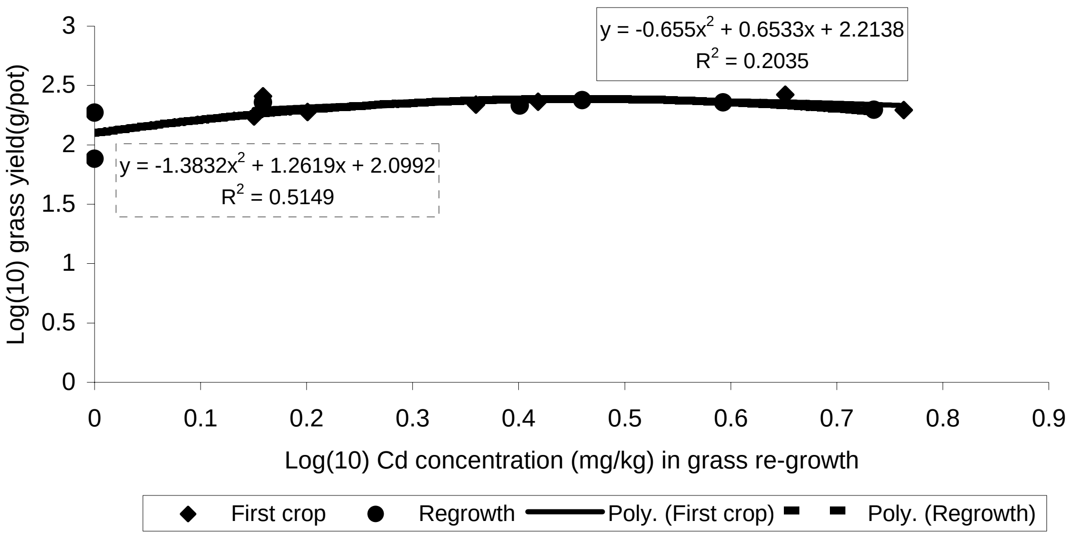

Figure 5.4: Log(10) Cd level (mg/kg) in grass versus log(10) yield of grass (g/pot) in single Cd treatments

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.

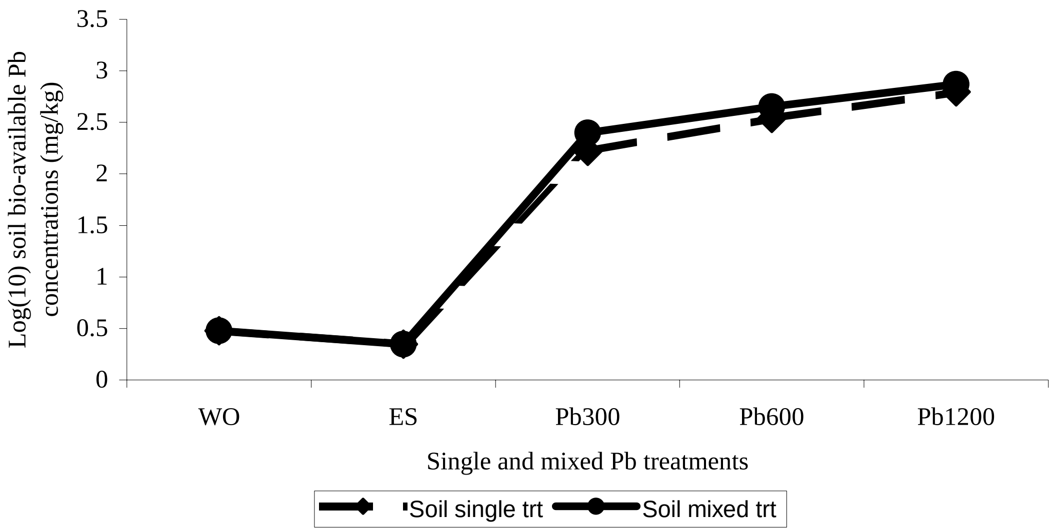

Figure 5.5: Effect of treatment on bio-available levels of Pb in single and mixed treatments

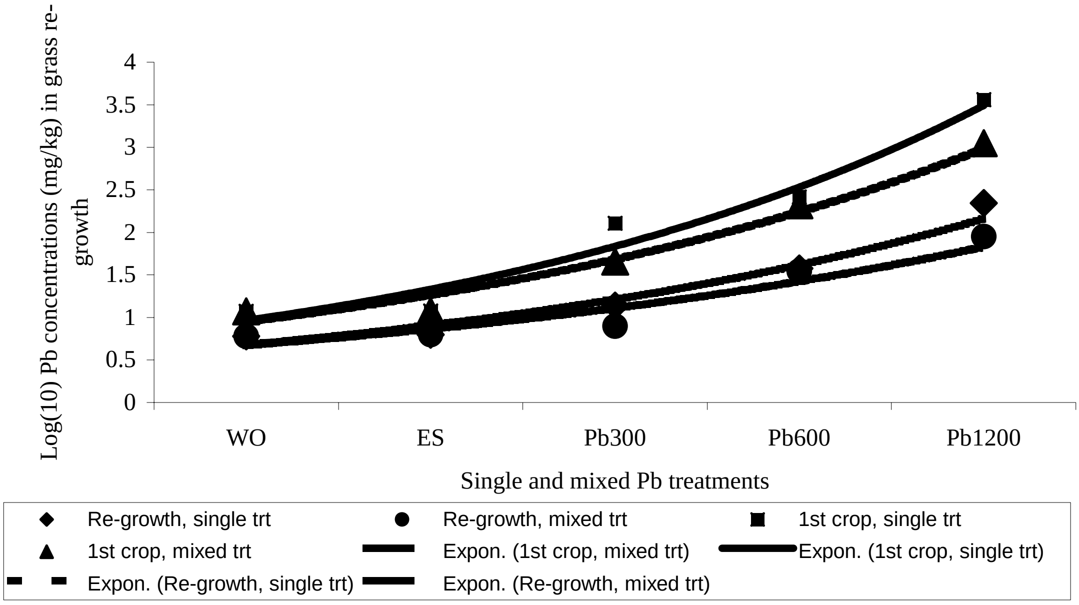

Figure 5.6: Effect of treatment on levels of Pb in grass in single and mixed treatments

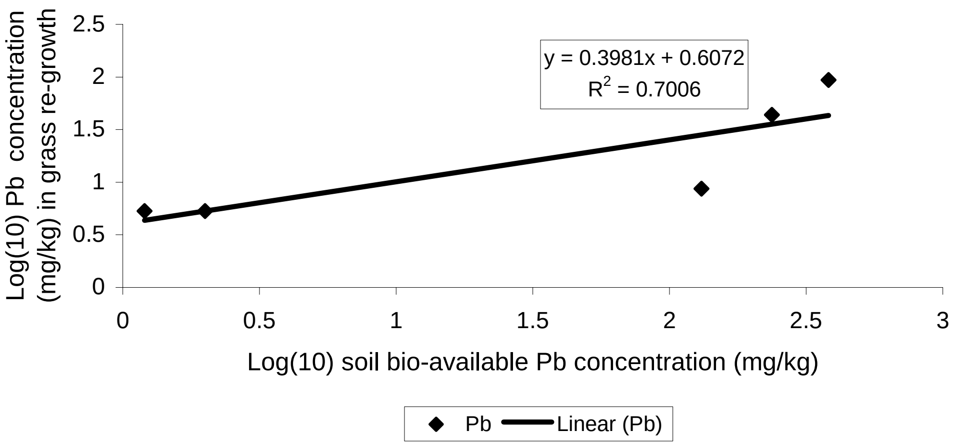

Figure 5.7: Log(10) bio-available soil Pb levels (mg/kg) versus log(10) Pb levels in grass re-growth (mg/kg) in mixed treatments

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.

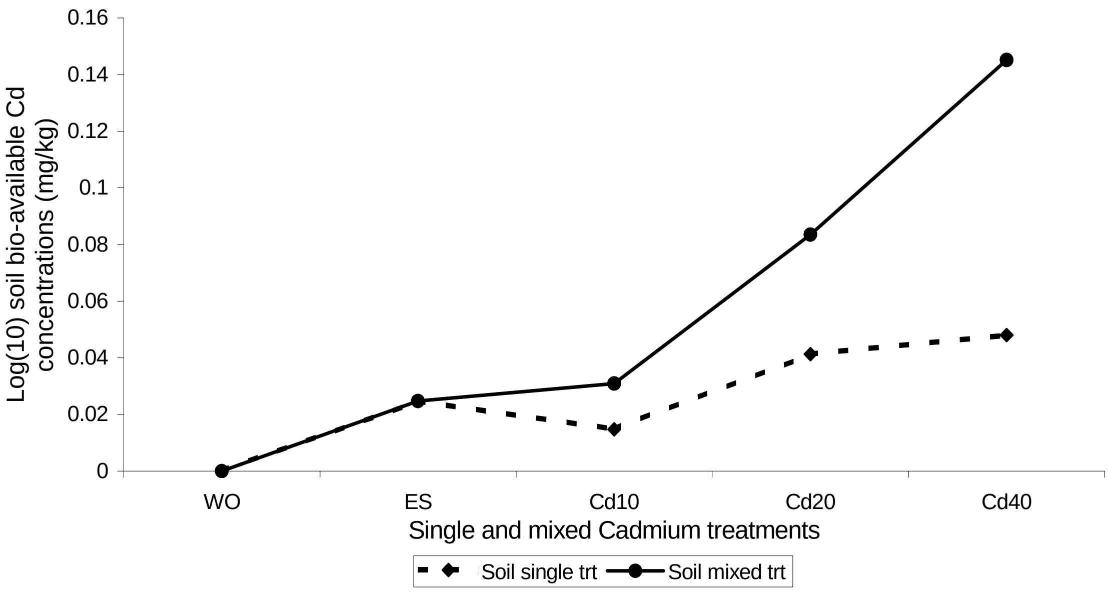

Figure 5.8: Effect of treatment on bio-available levels of Cd in single and mixed treatments

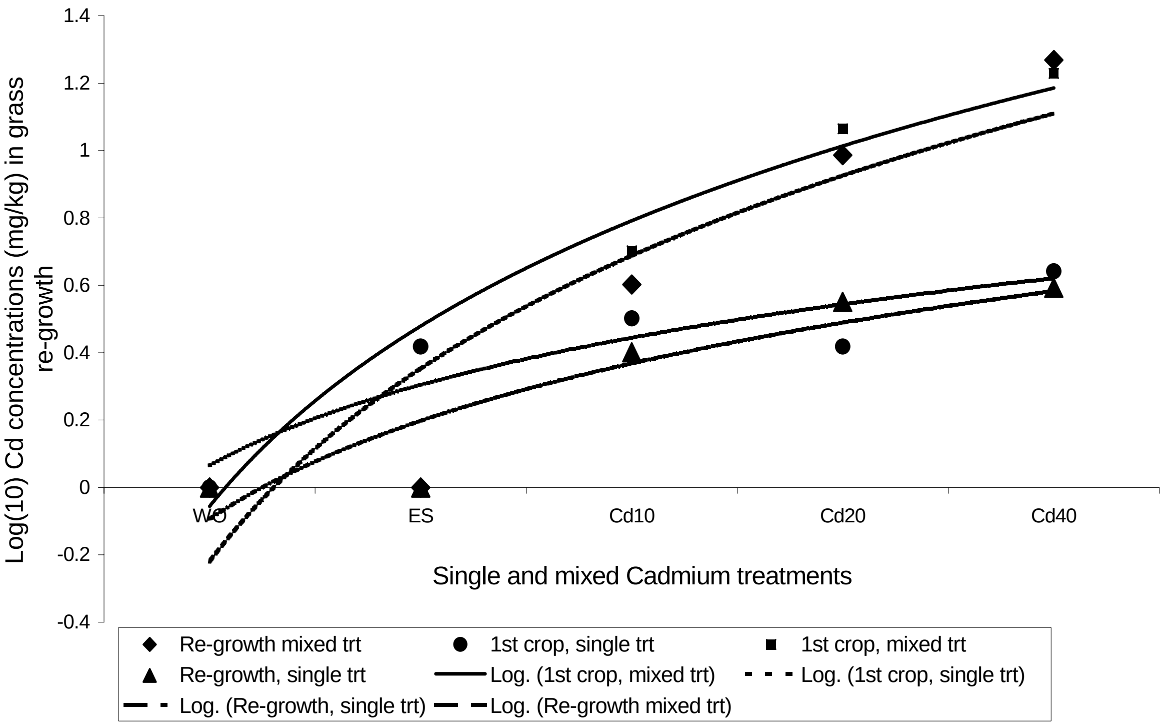

Figure 5.9: Effect of treatment on bio-available Cd levels in grass in single and mixed treatments

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).

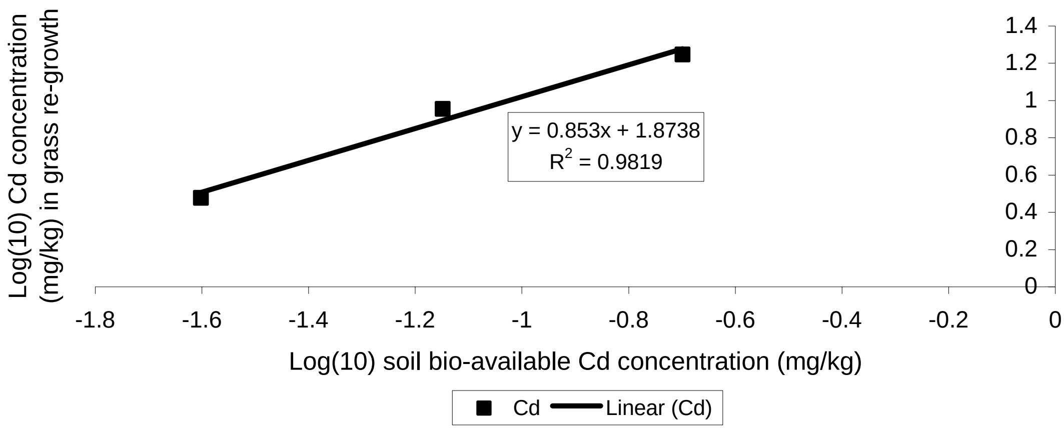

Figure 5.10: Log(10) bio-available Cd soil levels (mg/kg) versus log(10) Cd levels in grass re-growth in mixed treatments

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.

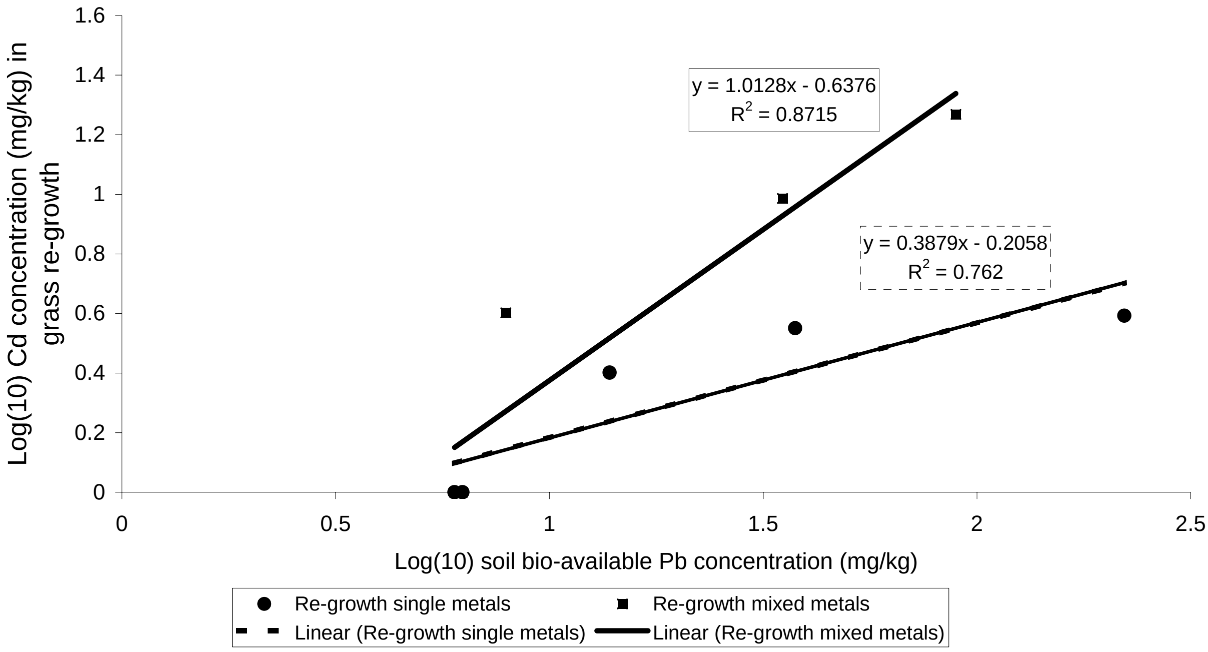

Figure 5.11: Correlation of metal contents of Pb and Cd in grass in single and mixed treatments

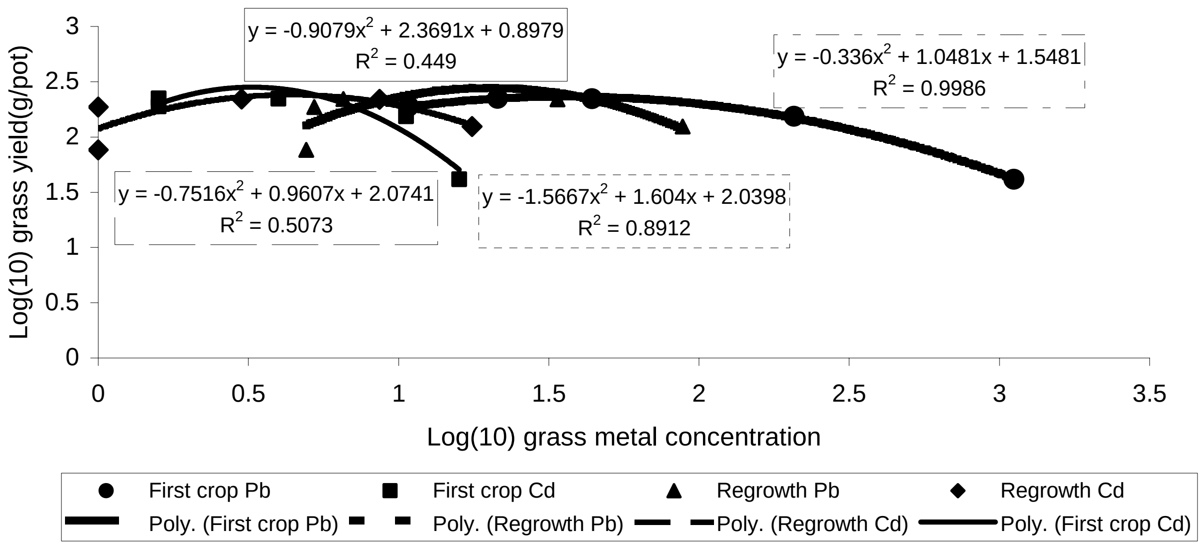

Figure 5.12: Yield response to concentrations of Pb and Cd in mixed Pb and Cd treatments

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:

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

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

$s^2_{(pooled)} = \{(n_1\text{-}1)s_1^2 + (n_2\text{-}1)s_2^2\}/(n_1+n_2 - 2)$, 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.

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 ($cmol_c kg^{-1}$)															
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.

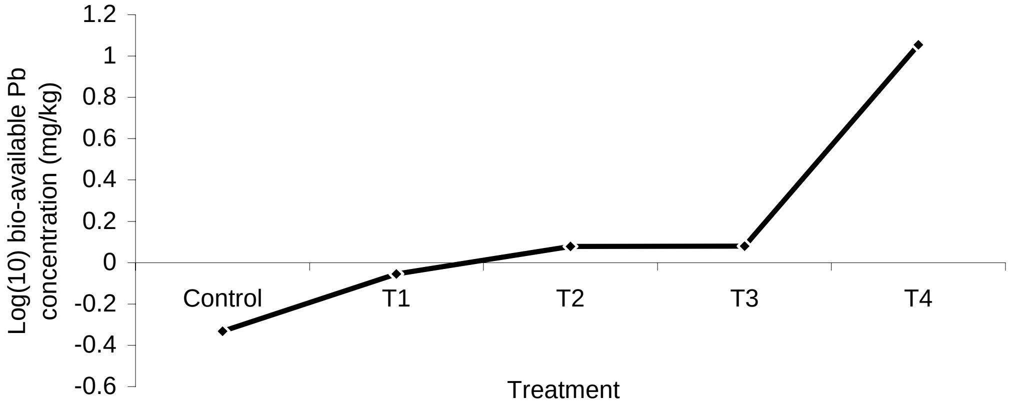

Figure 6.2 Treatment versus log(10) bio-available soil Pb concentration

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.

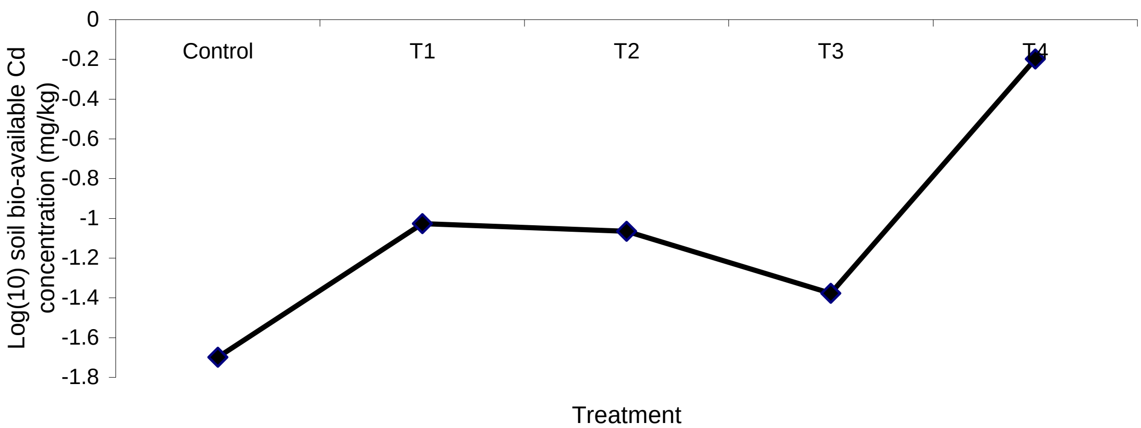

Figure 6.3: Treatment versus Log(10) bio-available soil Cd concentration

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.

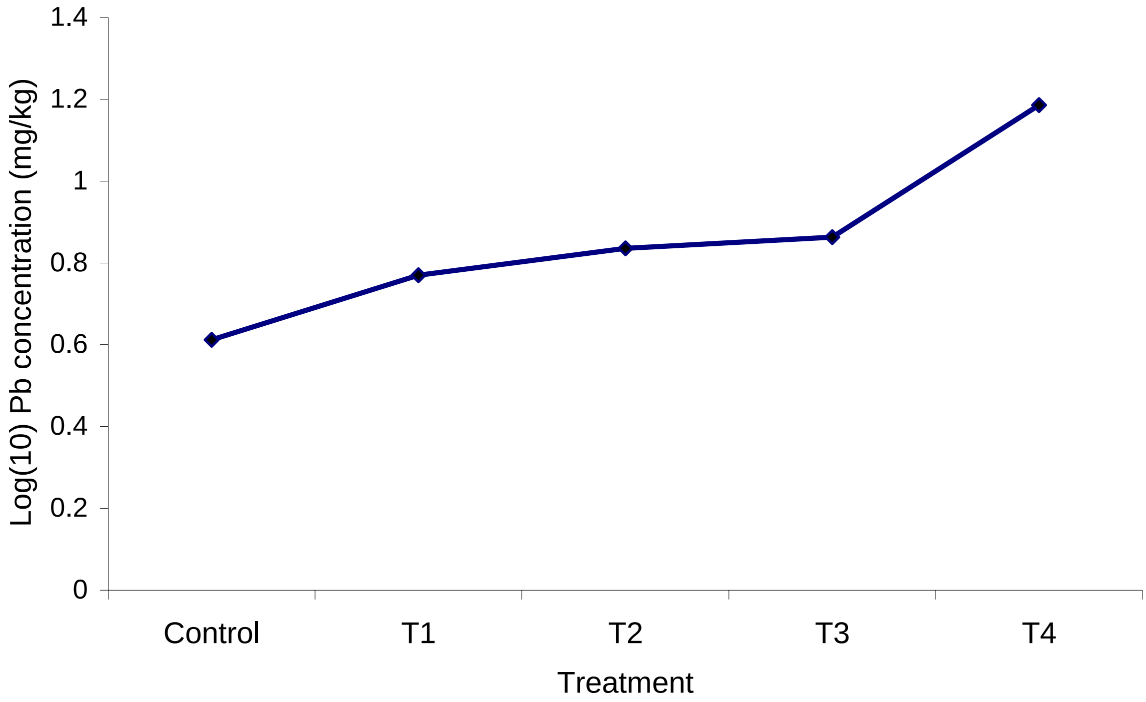

Figure 6.4 : Treatment versus Log(10) grass Pb concentration

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.

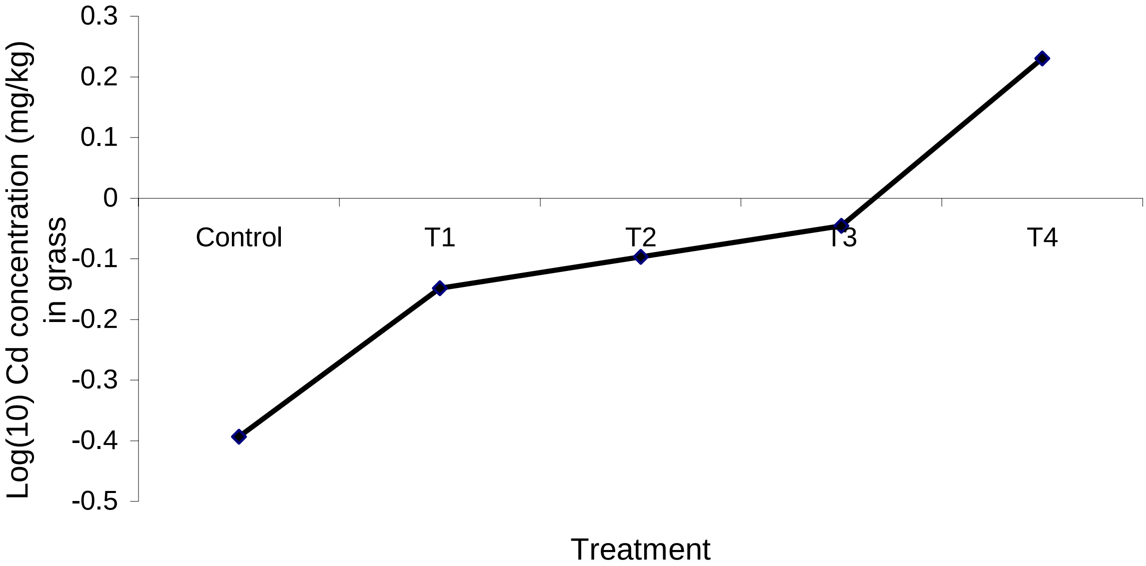

Figure 6.5: Treatment versus Log(10) grass Cd concentration

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.

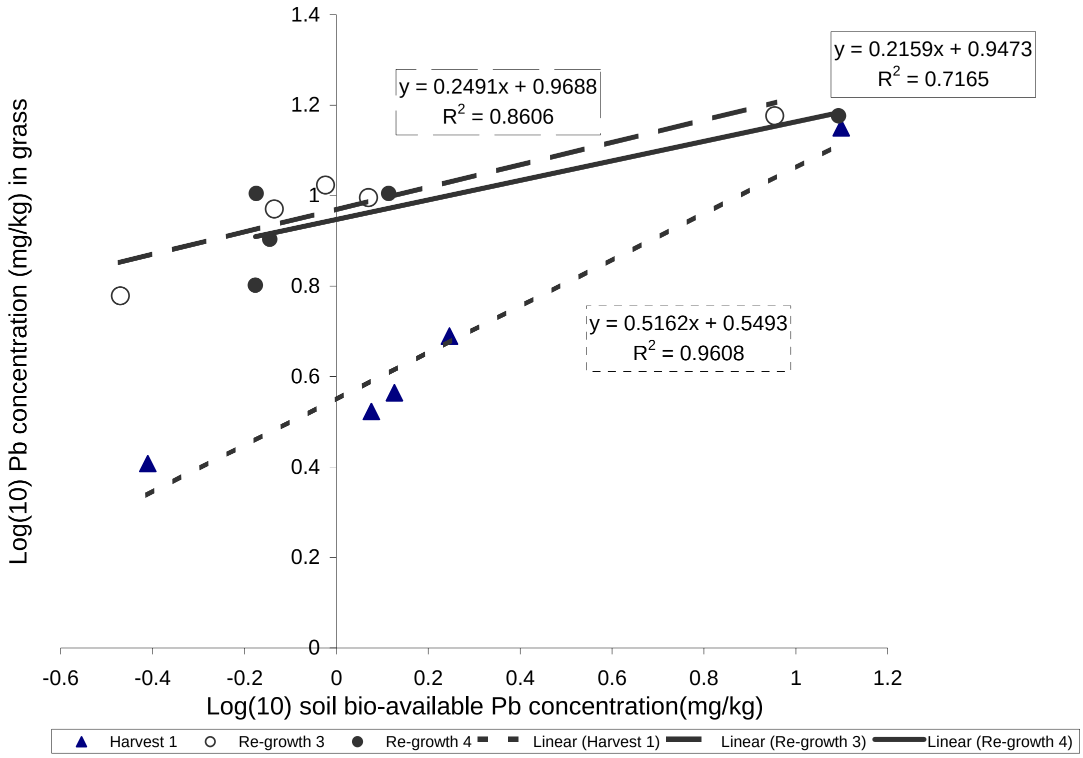

Figure 6.6: Log(10) bio-available soil Pb versus log(10) Pb level in grass

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 $-2.306 \leq t_s \leq +2.306$ 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).

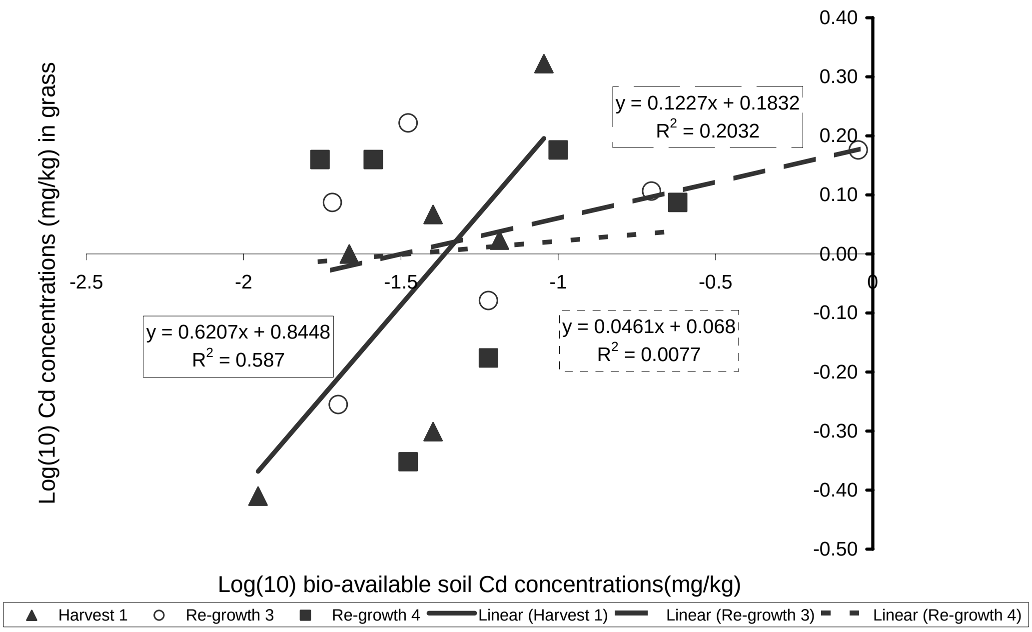

Figure 6.7: Log(10) bio-available soil Cd level versus log(10) Cd level in grass

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.

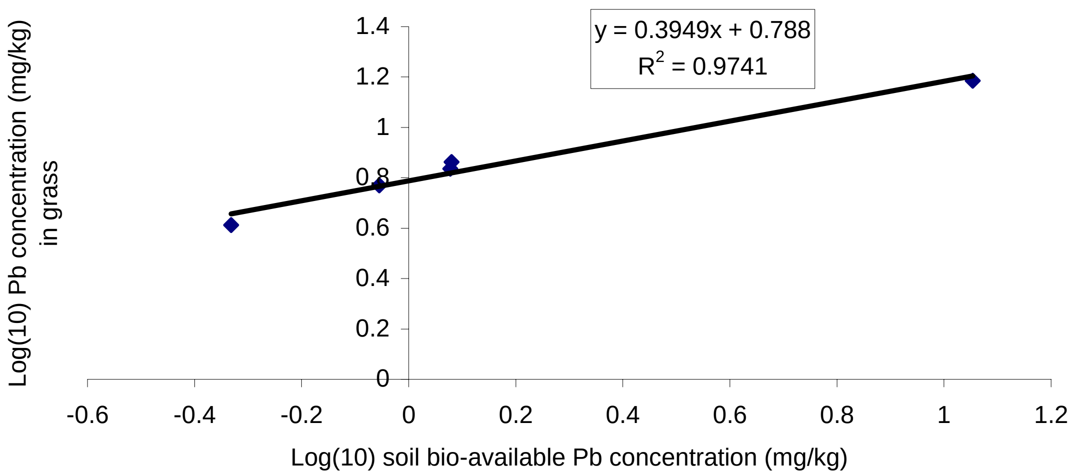

Figure 6.8: Log(10) mean bio-available soil Pb versus log(10) mean Pb level in grass

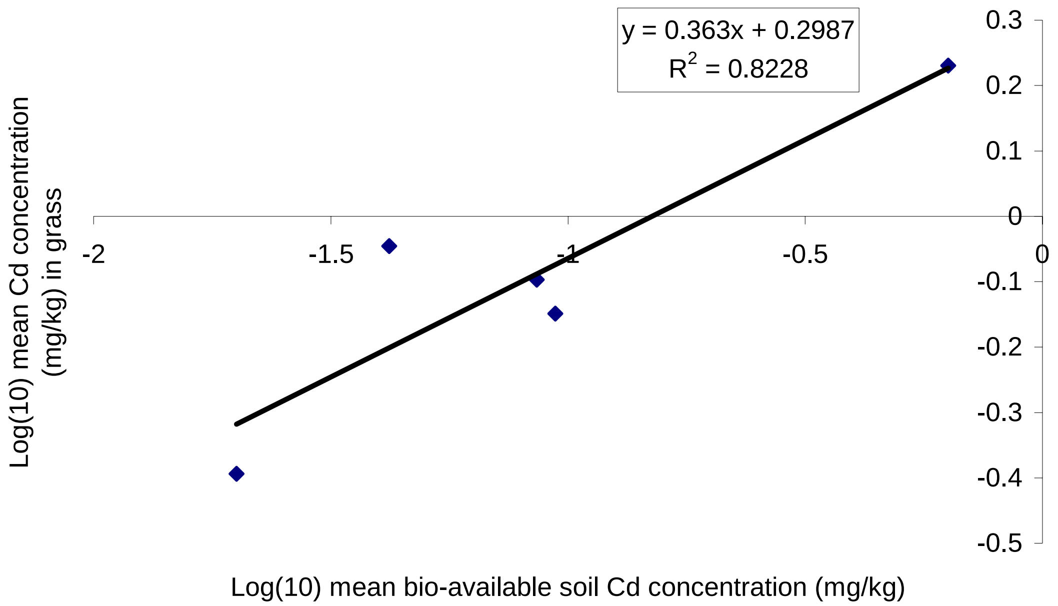

Figure 6.9 : Log(10) mean bio-available soil Cd versus log(10) mean Cd level in grass

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.

CHAPTER 7

GENERAL DISCUSSION

This chapter discusses the results obtained from all the components of the study, bearing in mind the main areas in which the study was focused. The major findings are as follows.

7.1 Long-term Pb and Cd accumulation in soils

The study found that after 29 years of disposal of treated sewage, Pb and Cd accumulation in the sandy soils occurred predominantly in the top 20 cm, particularly the top 10 cm of the soil. This is confirmed by the large difference in metal accumulation between the top and lower layers of the soil and similarity of metal levels in lower layers of irrigated and non-irrigated soils. This outcome was largely expected since organic matter, which concentrates in the top soil, has a high affinity for metals (McGrath and Lane, 1989).

At a total soil metal concentration of 186.3 mg/kg in the top 0-10 cm horizon and 33.3 mg/kg in the 10-20 cm horizon, long-term accumulation of Pb was largely below the recommended limit of 300 mg/kg in all horizons, suggesting that Pb was unlikely to be a hazard. This is confirmed by the fact that after 29 years of disposal of treated sewage on soils, mixed kikuyu and star grasses only absorbed a maximum of 1.5 mg/kg Pb. In addition, results from the field experiment show that star grass accumulated 15.33 mg/kg Pb, a figure lower than 40 mg/kg recommended for pasture grass and 53.70 mg/kg toxic limit obtained from the pot experiment. At an average annual accumulation of 5.7 mg/kg and sewage disposal regime similar to the previous 29 years, it would take another 20 years for Pb in the top 10 cm depth to reach the 300 mg/kg total Pb limit. This implies a longer period of disposal.

At 1.26 mg/kg in the 0-10 cm horizon and 0.75 mg/kg in the 10-20 cm horizon, total Cd exceeded the recommended 1 mg/kg in the top 10cm and was just below the limit in the 10-20 cm depth. This outcome suggested that Cd hazard was likely and is confirmed by uptake of up to 1.2 g/kg by mixed star and kikuyu grasses and 1.70 mg/kg by star grass in the field experiment.

The variation of metal levels with soil depth observed on total concentrations was also observed on bio-available levels in pots. The same trend was observed in the field. In all cases, this trend is attributed to the high affinity of organic matter for metals (McGrath and Lane, 1989). The variation of bio-available metal concentration with soil depth presents a potentially large source of error in relating soil concentrations to plant concentrations and in modelling soil-plant uptake. The depth interval at which various plants in different environments obtain water and nutrients and the relative biomass of feeder roots at different depths are unknown (US Department of Energy, 1998). Therefore, although the full 40 cm profile was assumed to be the depth from which grass roots took up nutrients as well as Pb and Cd in this study, this area may need further investigations.

7.2 Capacity of star grass to absorb Pb and Cd

A major feature of this study was to establish the capacity of star grass to take up Pb and Cd, given that the grass is grown for pasture and is irrigated using treated wastewater. In this respect the study found star grass to be a medium Pb and Cd extractor among plants, in general, and a high accumulator among grasses. In the pot experiment, it was capable of taking up as much as 4 592 mg/kg Pb and 316 mg Pb in the first crop and re-growth respectively and 16 mg/kg Cd and 18 mg/kg Cd in the first crop and re-growth respectively, in aerial plant parts. Severely retardation of growth at 4 592 mg/kg implies that this level was close to the maximum uptake capacity of star grass. Given that grasses within a species are known to have similar uptake characteristics (McDonald, 1995) the findings suggest that the *Cynodon* species of grasses possibly had a maximum Pb uptake capacity close to 4 592 mg/kg, implying that the *Cynodon* species may be a medium extractor of Pb. Phyto-extractors should combine high yields and high metal uptake (Baker et al, 2000). The absence of clear signs of growth retardation in Cd treatments in the greenhouse experiment suggests that the maximum extraction capacity of star grass was above 18 mg/kg attained in the experiment.

It should be noted that star grass was exposed to highly soluble Pb. High uptake of Pb is generally limited by insolubility of Pb in soils and hyper-accumulation occurs in contaminated soils when bio-availability is improved by chelates (McGrath et al, 2002). However, any unintentional contact between the grass in the first crop and added Pb during application of inorganic Pb may also have contributed to the large differences in levels between the first crop and re-growth in pot experiments. Using the results from re-growths, of from the pot study, star grass took up 8-fold and 18-fold the levels of 40 mg/kg and 1 mg/kg Cd recommended for pasture grass (United Kingdom Statutory Instrument No. 1412, 1995).

The lack of Pb and Cd toxicity signs at toxic concentrations suggests that animals could continue grazing on grass with Cd concentrations higher than the maximum limit of 1 mg/kg, unless the grass is tested or the soil is tested for bio-availability of the metal. This and the high extraction capacity of star grass imply that growing the grass for pasture in Pb and Cd polluted soils should be discouraged.

Despite total soil Cd being 0.65 mg/kg (35% lower than the 1 mg/kg maximum limit recommended for soils on which pasture grows), mixed kikuyu and star grasses accumulated up to 1.2 mg/kg Cd (20% more than the recommended limit in pasture grass). This outcome confirms the risk of relying on total soil metal concentrations when predicting hazard to animals. It also re-affirms the need to use bio-available soil metal levels instead of total concentrations in predictions. In the field experiment, star grass accumulated up to 1.70 mg/kg Cd, against a bio-available level of 0.63 mg/kg Cd (Table 6.4). Both results suggest that the limit of 1 mg/kg total Cd in the soil may be too high for star grass growing on a sandy soil under conditions of repeated application of treated sewage.

7.3 Yield responses to increasing bio-available Pb and Cd

Another key feature of this study was to examine relationships between soil bio-available levels and yield so as to predict the yield on the basis of soil bio-available concentration. The study found that the strength of this relationship was insignificant at 95% confidence level. There was very weak correlation between bio-available Pb and Cd and yield of star grass. Therefore, under the conditions of this study, $\log_{10}$ (*yield of above ground tissue*) versus $\log_{10}$ (*bio-available soil concentration*) of Pb and Cd models were not significant enough for accurate prediction of yield on the basis of soil bio-available concentrations. This implies that there were other factors not incorporated into these models, such as nutrients, that had influence on yield.

7.4 Yield-metal uptake models for Pb and Cd and toxic limits in grass

Single factor regression models for yield versus metal content in grass, based on $\log_{10}$ (*yield of above ground tissue*) and $\log_{10}$ (*metal concentration*) of Pb and Cd in the greenhouse experiment, were largely not significant ($p \leq 0.05$). Since Pb and Cd do not have known roles in metabolism, their effect below the toxic level is not clear but may be one of the reasons why the models are weak. Despite that, the models provide points where yield starts to decline allowing for estimation of the toxicity limit or toxicity threshold. The study estimated through dose-response models, that the toxic levels of Pb and Cd in star grass are 53.7 mg/kg and 3.2 mg/kg, respectively. It is further noted that these toxic levels of star grass are way above the recommended levels of Pb and Cd of 40 mg/kg and 1 mg/kg for pasture grass, respectively.

7.5 Soil bio-available-grass metal uptake models and critical metal limits

The development of soil-vegetative tissue metal uptake models on the basis of above-ground star grass tissue and soil bio-available metal concentration was another major feature of this study. Earlier findings confirmed that total soil metal levels were poorly correlated to metal concentrations in star grass. The best-fit models for soil bio-available and grass metal content data fitted into $\log_{10}$ function. Data from the greenhouse and field experiments fitted into this model, despite differences between the conditions under which the two experiments were conducted. Sample et al (1998) obtained significant model fits of ln (*total soil concentration*) and ln (*above ground plant concentrations*) using data from experiments carried out around the world.

The significant single variable regression models of $\log_{10}$ (*above-ground grass tissue Pb concentration*) = 0.525$\log_{10}$ (*bio-available soil Pb concentration*) + 0.539 and $\log_{10}$ (*above-ground grass tissue Cd concentration*) = 0.451$\log_{10}$ (*bio-available soil Cd concentration*) + 0.087 produced using single metals are considered suitable where single metals are added to the soil and for estimating toxicity limits. Bak and Jensen (1998) noted that eco-toxicity tests were often conducted on single metals. Therefore these models are not appropriate under field conditions, where other metals are also present in normal concentrations in the soil.

Under field conditions, the significant model $\log_{10}$ (*above-ground grass tissue Pb concentration*) = 0.395$\log_{10}$ (*bio-available soil Pb concentration*) + 0.788, representing the average situation over 11 months, predicts the bio-available Pb level in soils to be 115.2 mg/kg for the recommended Pb limit in grass of 40 mg/kg. The model developed in the greenhouse predicts a bio-available Pb of 106.3 mg/kg for a limit of 40 mg/kg. This happens to be close to the value predicted in the field. Although the differences may be explained by the different conditions under which the 2 models were developed, the closeness of the two figures may be a result of the lack of influence of other metals on uptake of Pb, under experimental conditions

Under field conditions, none of the single variable regression models for Cd for individual crops was significant at 95% confidence level. This suggests that the numerous soil factors present under field conditions could have distorted the single variable relationship for each harvest. In that case, a multiple regression model could be more appropriate for Cd in the first crop and subsequent re-growths. The strength of the regression model for Cd under field conditions was vastly improved by treating each harvest as a replicate, with the resulting model falling just short of being significant. The model developed was $\log_{10}$ (*above-ground grass tissues concentration*) = 0.363$\log_{10}$ (*bio-available soil concentration*) + 0.2987. This model predicts a soil bio-available Cd limit of 0.20 mg/kg as the concentration that would cause an accumulation of 1 mg/kg Cd in grass. This figure is different from the 0.65 mg/kg bio-available Cd predicted by the model $\log_{10}$ (*above-ground grass tissues concentration*) = 0.451$\log_{10}$ (*bio-available soil concentration*) + 0.087, produced under greenhouse conditions. The differences are partly attributed to the different conditions under which the two models were developed and inadequate strength of the field-based model.

In general, it should be noted that under field conditions of this study, there were other factors that needed to be incorporated into the models to improve strength of Cd models. This may serve to explain the decline in the strength of correlation of both Pb and Cd with re-growths. US Department of Energy (1998) improved single variable regression models of natural $\log_{10}$ (*above-ground plant tissue concentration*) of Pb versus $\log_{10}$ (*total metal concentration in soils*) by incorporating pH in multiple regression models for Pb. Under the experimental conditions, the model, $\log_{10}$ (*above-ground grass tissues concentration*) = 0.363$\log_{10}$ (*bio-available soil concentration*) + 0.2987, though not significant could be considered as indicative of the probable relationship between soil bio-available Cd and concentration of the metal in star grass under field conditions.

7.6 Co-presence of Pb and Cd

The results of this study suggest that co-presence of Pb and Cd does not significantly affect the levels of Pb in the sandy soil and star grass, a finding that is in agreement with what Carlson and Rolfe (1979) found in rye and fescue. The evidence is that there were no significant differences in the uptake of Pb in single and mixed treatments in the greenhouse experiment. The closeness in the predictions of bio-available Pb levels for a grass Pb content of 40 mg/kg between pot-based and field-based models is also consistent with this observation. Co-presence of Pb and Cd caused a 2.6 increase in the rate of uptake of Cd levels in star grass above the uptake in single treatments in the greenhouse experiment. It is therefore postulated that, besides the high levels of Cd in the treated sewage, co-presence of Cd and Pb contributed to the high uptake of Cd under field conditions.

7.7 Appropriate Pb and Cd levels in effluent and digested sludge

One of the objectives of this study was to determine appropriate levels of Pb and Cd in treated sewage to apply on pasturelands. Using the data obtained in this study it was not possible to determine appropriate levels in treated sewage since the levels of metals in the wastewater could not be varied. However some indications were derived from the data obtained in the study.

The levels of Pb and Cd in treated sewage fluctuated considerably. Pb in treated sewage was below legislated levels. The average Pb levels of 2.6 mg/l in digested sludge and 0.2 mg/l in effluent (Table 4.1), 1.2 mg/l determined during the greenhouse experiment and 0.4 mg/l determined during field experiment were all below the limit of 5.0 mg/l recommended for irrigation water (Ayers and Westcot, 1985). Therefore the low levels of Pb accumulation in grasses can partly be attributed the low levels in treated sewage.

In contrast to Pb, the average Cd level of 0.17 mg/kg in treated sewage applied during the field experiment, was above legislated limits, while the levels applied in the greenhouse experiment were generally below. The excessive Cd levels in treated sewage amounting to 0.17 mg/l (17 times the recommended long-term limit of 0.01 mg/l) appears to have been the determinant factor in causing high accumulation of Cd in grass. Therefore the uptake of Cd to levels higher than the recommended limits within a period of only 160 days after planting grass can be partly attributed to the high concentrations in treated sewage during the field experiment. This outcome suggests that the use of volume-based loading rates for deciding the application rate on a sandy soils and star grass would be of limited applicability unless the concentration of the metal is known. It also implies that even in the presence of organic matter, which is expected to immobilise Cd, bio-availability of Cd is still high. Doyle (1978) noted that Cd adsorbed by organic matter largely remained available for plant uptake. Mengel and Kirkby (1982) observed that Cd is readily transported to upper parts of plants leading to high uptake by plants.

CHAPTER 8

CONCLUSIONS AND RECOMMENDATIONS

8.1 Main conclusions

The main conclusions from this study with reference to the main objectives (Section 1.2) are as follows:

Objective: To determine Pb and Cd accumulation, toxicity levels and yield of pasture grass under treated sewage application.

1. The study found that star grass is a high accumulator of both Pb and Cd. In this study, it accumulated 8 times the recommended level of 40 mg/kg Pb (United Kingdom Statutory Instrument No. 1412, 1995) and 18 times the recommended level of 1 mg/kg Cd (United Kingdom Statutory Instrument No. 1412, 1995) under conditions of high levels of added inorganic metals combined with repeated applications of treated sewage in the soil. Therefore growing star grass in a sandy soil for pasture under conditions of high levels of Pb and Cd application is not advisable.

2. Using models produced in this study, the toxicity levels of Pb and Cd in grass were established to be 53.7 mg/kg and 3.2 mg/kg, respectively. These levels corresponded to soil bio-available concentrations of Pb and Cd of 186.2 mg/kg and 8.3 mg/kg, respectively. The toxicity levels in grass are higher than the levels recommended in pasture grass. By absorbing more than the recommended limits of Pb and Cd at the threshold toxicity levels without showing visible signs of toxicity, star grass poses a risk to animals if bio-available soil metal levels are not regularly measured. This risk arises because animals may be grazed on star grass with Pb and Cd levels higher than recommended (if the grass or soil is not tested), since the point at which toxicity starts coincides with the highest level of productivity/yield.

3. Co-presence of Pb and Cd did not significantly affect uptake of Pb. It however caused a 260% increase in the rate of uptake of Cd by star grass subjected to high metal doses of mixed inorganic Pb and Cd compared to high doses of single inorganic Cd combined with treated sewage application. It is postulated that this effect of Pb on Cd also occurred under field conditions, leading to high uptake of Cd. Therefore besides reducing Cd levels, reduction of Pb levels in treated sewage may reduce uptake of Cd by star grass.

4. The study established that there was some correlation between yield of star grass and bio-available metal concentration. Though not statistically significant, the regression models of $\log_{10}$ (*above-ground tissue*) versus $\log_{10}$ (*soil bio-available metal concentration*) were sufficient to derive toxicity levels of Pb and Cd in grass.

Objective: To determine Pb and Cd accumulation in pasture grass under effluent and sewage sludge mixture application.

5. Using the models produced in this study, the critical bio-available levels of Pb and Cd at which metal uptake by star grass would not exceed recommended levels of 40 mg/kg Pb and 1 mg/kg Cd, were estimated to be 115.2 mg/kg and 0.20 mg/kg, respectively. The models:

$\log_{10}$ (*above-ground grass tissue Pb concentration*) = 0.3949 $\log_{10}$ (*bio-available soil Pb concentration*) + 0.788

and

$\log_{10}$ (*above-ground grass tissue Cd concentration*) = 0.363 $\log_{10}$ (*bio-available soil Cd concentration*) + 0.2987,

produced for predicting metal content in grass based on bio-available metal content in soils, are considered to be representative of the situation where grazing animals continue to graze on pasture thereby causing new re-growths. Though still to be field tested, these models could form a basis for estimating accumulation of Pb and Cd in grass on the basis of bio-available soil concentrations, at least as indicators of potential hazard to be validated by detailed tests on plant tissue.

This study recommends the management of bio-available Pb and Cd below 115.2 mg/kg and 0.20 mg/kg, respectively, in order to avoid accumulating critical pasture levels of 40 mg/kg Pb and 1 mg/kg Cd in star grass. This would ensure that pasture is safe for animal consumption.

6. Under conditions of repeated applications of treated sewage the recommended maximum total concentration of Cd of 1 mg/kg (sewage sludge directive limit for use of sewage sludge in agriculture (EEC, 1986) may be too high for a sandy soil. Uptake of Cd beyond the recommended 1 mg/kg in mixed kikuyu and star grass pasture occurred despite the soil having an average total soil Cd concentration of 0.65 mg/kg, a value lower than the recommended 1 mg/kg. Absorption of 1.70 mg/kg by star grass against a soil bio-available concentration of 0.63 mg/kg under field conditions pointed to a similar conclusion.

7. The application of treated sewage at 17 times the recommended long-term concentration of 0.01 mg/l Cd caused star grass to accumulate Cd to levels beyond 1mg/kg, within a short period of 5 months. Therefore where concentrations of Cd in treated sewage are high, short-term accumulation of the metal in a sandy soil and star grass are important to consider.

Objective: To determine long-term Pb and Cd accumulation in soils subjected to treated sewage application.

8. Long-term accumulation of Pb and Cd occurred predominantly in the 0-20 cm depth of the sandy soil. The lower horizons of the irrigated soil had metal levels similar to background levels in non-contaminated soil. This pattern of accumulation of Pb and Cd suggests uncertainty in modelling soil-plant uptake since the depth from which the roots took up nutrients and Pb and Cd cannot be ascertained.

9. After 29 years of continuous disposal of treated sewage, with Pb concentrations of between 0.40 mg/l and 2.6 mg/l as determined in this study, total Pb was below the recommended level of 300 mg/kg for a soil. Pb accumulated in the sandy soil at an annual average of 5.7 mg/kg total concentration in the 0-10 cm depth and 0.3 mg/kg total concentration in the 10-20 cm depth of the soil. These concentrations in treated sewage caused a maximum accumulation in pasture grass of 40% of the recommended limit of 40 mg/kg in pasture. Therefore, the long-term accumulation of Pb from repeated application of Pb at less than 2.6 mg/l did not constitute a threat to the sandy soil, star grass and animals grazing on the pastures.

10. At a total soil concentration of 1.26 mg/kg in the 0-10 cm soil horizon and 0.75 mg/kg in the 10-20 cm horizon after 30 years of treated sewage disposal, total soil Cd in the top 10 cm was above the 1 mg/kg total Cd limit recommended for growing food crops. The annual accumulation rate of total Cd was 0.03 mg/kg in the top 10 cm and 0.01 mg/kg in the 10-20 cm layer. Since mixed kikuyu and star grass absorbed up to 1.2 mg/kg Cd at these soil levels, Cd poses a threat to the soil, star grass and animals grazing on the

pastures. The study recommends that under conditions of repeated applications of treated sewage with high levels of Cd, long-term limits of total Cd on sandy soils should be set lower than 1 mg/kg.

11. There was weak correlation between total Pb and Cd and the levels of the metals in mixed kikuyu and star grass, such that metal levels in grass could not be predicted on the basis of total soil metal concentrations.

Objective: To determine appropriate levels of Pb and Cd concentrations in sewage effluent and sludge mixture that would optimise yield of grass and minimise heavy metals in beef animals.

12. The study noted that although it was not possible to determine the actual appropriate levels under the conditions of the experiment where treated sewage had varying levels of metals when it was disposed onto pasturelands, the Pb level of 2.6 mg/l and below, did not present a hazard to star grass and animals. However, Cd presented a hazard at total soil concentrations lower than 1 mg/kg in the soil. The hazard, as measured by the concentration in the plant was evident after only 5 months of repeated application of treated sewage with a concentration 17 times the recommended rate.

8.2 Recommendations

The main findings of this study provide scope for further research in related areas suggested below:

1. There is a lack of knowledge on the depth from which grasses absorb metals. Given that the level of accumulation of Pb and Cd is related to soil depth and soil concentrations are inputs into soil-plant uptake models, a study to establish the depth of uptake would provide information on the depth to consider in relating metal content in grass and soil bio-available metal content.

2. Further research on uptake of the metals by re-growths of grass under the same conditions and multiple variable analysis of uptake could improve regression models established in this study. The models could consider incorporating pH or other chemicals species, especially where interaction of the metal under study with other chemical species is anticipated.

3. There is scope to carry out similar research on Pb and Cd hazard in other soil types, species of grass and other plants like agricultural crops, since these have not yet been studied locally.

4. A study in which accumulation of Pb and Cd in animal organs are investigated and related to those in grass and possibly soils would assist policy makers to draw up management practices and policies on risk assessment of Pb and Cd.

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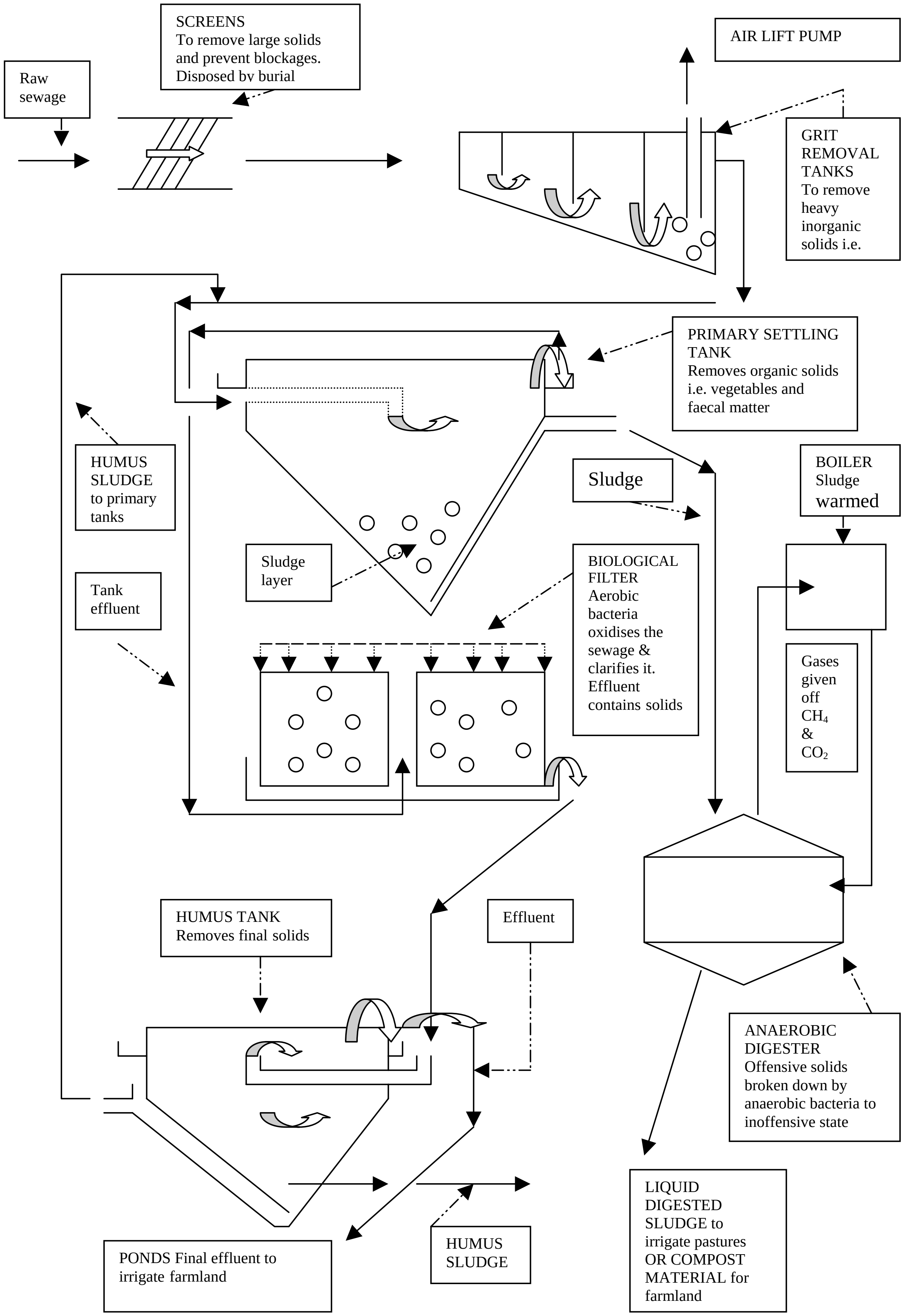
Appendix 1: Sewage treatment processes at Firle Wastewater treatment Plant



Types of treatment technologies used at Firle Wastewater Treatment Plant

Firle Wastewater Treatment Plant utilises two types of sewage treatment technologies, namely biological trickling filtration plants and biological nutrient removal activated sludge plants. Sewage treatment or wastewater processing normally comprises unit operations and processes that provide various levels of treatment. The processes are commonly referred to as preliminary, primary, secondary and tertiary. The term preliminary and/or primary refers to physical processes, where coarse suspended materials are removed. Secondary treatment refers to chemical and biological processes whereas tertiary treatment is a combination of the three. The diagram shown above presents the treatment processes in the two technologies at Firle Treatment Works and these are briefly described below.

In primary treatment a portion of suspended solids and organic matter is removed using physical operations such as screening and sedimentation. As such effluent from primary treatment normally has a considerable amount of organic matter and a relatively high BOD. Conventional secondary treatment is targeted at the removal of biodegradable organic materials and suspended solids. It includes biological treatment by activated sludge, fixed-film reactors and sedimentation. Tertiary treatment is a level of treatment that goes beyond conventional treatment to remove constituents of concern including increased amounts of organic matter and solids, toxic compounds and nutrients.

Biological trickling filtration system

In trickling filter treatment (shown as biological filter in diagram), raw sewage is directed to screens where large objects and grit are removed using grit removal tanks, before the sewage flows to primary settling tanks. In the tanks the sewage is separated into settled sewage and primary sewage. The settled sewage then flows to bio-filters where the trickling effluent goes to secondary sedimentation tanks in which secondary sludge (humus) is removed. The effluent from the secondary sedimentation tanks then goes to maturation tanks/ponds. This effluent does not meet Zimbabwe’s effluent discharge standards, therefore it cannot be discharged into rivers. Instead, in the case of Firle Treatment Works it is mixed with sludge and directed to irrigated pastures.

Biological nutrient removal sludge activated system

In the biological nutrient removal activated sludge treatment system, the preliminary stage comprises screening and grit removal. The primary stage physically separates primary sludge and effluent. After that the sludge is sent to digesters (shown as anaerobic digesters in diagram) and the settled sewage is directed to the activated sludge aeration tank.

Bacteria in the aerated tank use up most phosphates and nitrates in the sewage. Once the sludge has settled in final settling tanks (clarifiers) some of it is sent out as waste activated sludge while the other is returned to the head of the aeration basin to enrich the incoming sewage with degrading bacteria. The effluent from the biological nutrient removal treatment plants generally meets the Zimbabwe effluent standards and is therefore discharged into Manyame river

Appendix 2: Randomised block design layout of pots in greenhouse

Block 3		Block 2		Block 1	
Water		Cd_{80}		Cd_{10}	
Cd_{20}	Cd_{10}	Cd_{20}	Cd_{20} + Pb_{600}	Pb_{1200}	Cd_{80}
Pb_{1200}	Cd_{80}	Effluent & sludge	Cd_{10}	Cd_{40}	Effluent & sludge
Cd_{10} + Pb_{300}	Cd_{60}	Water	Pb_{1200}	Water	Pb_{300}
Pb_{300}	Cd_{20} + Pb_{600}	Cd_{40} + Pb_{1200}	Pb_{300}	Cd_{10} + Pb_{300}	Cd_{20} + Pb_{600}
Pb_{600}	Cd_{40} + Pb_{1200}	Cd_{60}	Cd_{10} + Pb_{300}	Cd_{20}	Cd_{60}
Cd_{40}	Effluent & sludge	Cd_{40}	Pb_{600}	Pb_{600}	Cd_{40} + Pb_{1200}

Appendix 3: Quantities of treated sewage and metals applied to field plots

Irrigation event	Plot number	Volume of irrigation (m^3/plot)	Pb applied to plots		Cd applied to plots	
			(mg/l)	(mg)	(mg/l)	(mg)
1	T 1.1	3.32	0.3	995.40	0.1	331.80
2	T 1.1	2.53	0	0.00	0.3	760.09
3	T 1.1	3.30	0.1	329.82	0.10	329.82
4	T 1.1	3.32	0.40	1326.06	0.30	994.54
5	T 1.1	3.33	0.9	2993.14	0.2	665.14
6	T 1.1	3.30	0.6	1982.50	0.1	330.42
7	T 1.1	3.30	0.55	1812.26	0.15	494.25
8	T 1.1	3.31	0.4	1323.25	0.2	661.62
Mean		**25.70**	**0.42**	**10762.43**	**0.18**	**4567.69**
1	T 1.2	3.32	0.3	996.35	0.1	332.12
2	T 1.2	3.36	0	0.00	0.3	1008.01
3	T 1.2	1.79	0.25	448.63	0.05	89.73
4	T 1.2	3.36	0.40	1344.67	0.30	1008.50
5	T 1.2	3.31	0.9	2982.35	0.2	662.74
6	T 1.2	3.29	0.6	1976.93	0.1	329.49
7	T 1.2	3.29	0.55	1807.04	0.15	492.83
8	T 1.2	3.32	0.4	1326.87	0.2	663.43
Mean		**25.05**	**0.43**	**10882.84**	**0.18**	**4586.85**
1	T 1.3	1.61	0.3	0.00	0.1	0.00
2	T 1.3	3.29	0.25	986.46	0.05	328.82
3	T 1.3	3.29	0.40	822.20	0.30	164.44
4	T 1.3	3.30	0.9	1319.05	0.2	989.29
5	T 1.3	3.31	0.55	2977.83	0.15	661.74
6	T 1.3	3.29	0.55	1807.71	0.15	493.01
7	T 1.3	3.30	0.25	1814.96	0.05	494.99
8	T 1.3	3.32	0.4	830.98	0.2	166.20
9	T 1.3	1.72		687.66		343.83
Mean		**26.42**	**0.43**	**11246.84**	**0.14**	**3642.31**
1	T 2.1	3.33	0.3	998.15	0.1	332.72
2	T 2.1	5.78	0.1	578.14	0.10	578.14
3	T 2.1	6.57	0.40	2629.66	0.30	1972.24
4	T 2.1	6.63	0.9	5970.30	0.2	1326.73
5	T 2.1	6.60	0.55	3630.35	0.15	990.10
6	T 2.1	6.57	0.55	3614.36	0.15	985.73
7	T 2.1	6.58	0.25	1645.28	0.05	329.06
8	T 2.1	6.61	0.4	2643.62	0.2	1321.81
Mean		**48.68**	**0.45**	**21709.86**	**0.16**	**7836.53**

Appendix 3: cont'd

Irrigation number	Plot number	Volume of irrigation (m^3/plot)	Pb applied to plots		Cd applied to plots	
			(mg/l)	(mg)	(mg/l)	(mg)
1	T 2.2	6.60	0.3	1980.56	0.1	660.19
2	T 2.2	6.57	0.25	1643.35	0.05	328.67
3	T 2.2	0.90	0.2	179.71	0.05	44.93
4	T 2.2	6.60	0.40	2639.77	0.30	1979.83
5	T 2.2	6.62	0.9	5957.68	0.2	1323.93
6	T 2.2	6.59	0.55	3623.49	0.15	988.23
7	T 2.2	6.60	0.55	3630.43	0.15	990.12
8	T 2.2	6.56	0.25	1638.88	0.05	327.78
Mean		**49.88**	**0.43**	**21293.87**	**0.13**	**6643.66**
1	T 2.3	6.61	0	0.00	0.3	1982.94
2	T 2.3	6.58	0.25	1645.02	0.05	329.00
3	T 2.3	6.62	0.40	2646.93	0.30	1985.20
4	T 2.3	6.61	0.9	5952.30	0.2	1322.73
5	T 2.3	3.35	0.6	2011.04	0.1	335.17
6	T 2.3	6.57	0.45	2958.68	0.1	657.49
7	T 2.3	6.66	0.25	1664.10	0.05	332.82
8	T 2.3	6.59	0.5	3295.39	0.2	1318.16
Mean		**49.59**	**0.41**	**20173.46**	**0.17**	**8263.51**
1	T 3.1	13.27	0.3	3979.97	0.1	1326.66
2	T 3.1	13.17	0	0.00	0.3	3950.85
3	T 3.1	13.15	0.40	5259.65	0.30	3944.74
4	T 3.1	13.15	0.9	11838.99	0.2	2630.89
5	T 3.1	8.83	0.6	5297.54	0.1	882.92
6	T 3.1	12.47	0.45	5612.36	0.1	1247.19
7	T 3.1	13.21	0.45	5943.74	0.25	3302.08
8	T 3.1	13.21	0.5	6605.56	0.2	2642.23
Mean		**100.46**	**0.44**	**44537.81**	**0.20**	**19927.55**
1	T 3.2	13.23	0.3	3969.85	0.1	1323.28
2	T 3.2	6.26	0	0.00	0.3	1878.03
3	T 3.2	13.19	0.9	11871.46	0.2	2638.10
4	T 3.2	13.15	0.40	5259.05	0.30	3944.29
5	T 3.2	13.20	0.3	3959.38	0.1	1319.79
6	T 3.2	13.22	0.45	5947.58	0.1	1321.68
7	T 3.2	13.18	0.45	5931.27	0.25	3295.15
8	T 3.2	13.17	0.5	6586.33	0.2	2634.53
Mean		**98.60**	**0.44**	**43524.93**	**0.19**	**18354.87**
1	T 3.3	13.17	0	0.00	0.3	3949.55
2	T 3.3	8.82	0.40	3526.35	0.30	2644.77
3	T 3.3	5.43	0.9	4888.54	0.2	1086.34
4	T 3.3	13.16	0.9	11845.62	0.2	2632.36
5	T 3.3	14.17	0.3	4249.63	0.1	1416.54
6	T 3.3	13.17	0.45	5924.35	0.1	1316.52
7	T 3.3	13.18	0.45	5932.16	0.25	3295.65
8	T 3.3	13.17	0.5	6583.36	0.2	2633.34
Mean		**94.25**	**0.46**	**42950.01**	**0.20**	**18975.08**

Appendix 4: Mean soil bio-available concentrations (standard deviations), mg/kg and soil depth

Depth	Control			Treatment 1			Treatment 2			Treatment 3			Treatment 4		
	Har 1	Re-g3	Re-g 4	Har 1	Re-g3	Re-g 4	Har 1	Re-g3	Re-g 4	Har 1	Re-g3	Re-g 4	Har 1	Re-g3	Re-g 4
								Lead							
0-10	0.37	0.23	0.50	1.28	0.80	0.80	1.31	0.90	0.16	1.82	1.00	0.47	17.92	16.91	19.30
	(0.12)	(0.12)	(0.20)	(0.26)	(0.46)	(0.35)	(0.09)	(0.60)	(0.06)	(0.34)	(0.26)	(0.12)	(2.86)	(2.12)	(1.19)
10-20	0.40	0.28	0.60	1.34	0.83	0.60	1.30	0.87	0.75	1.85	1.10	0.93	7.18	8.65	12.71
	(0.17)	(0.13)	(0.15)	(0.43)	(0..51)	(0.46)	(0.24)	(0.49)	(0.07)	(0.16)	(0.44)	(0.75)	(2.59)	(3.41)	(2.66)
20-30	0.40	0.50	0.90	1.21	0.43	0.73	1.39	1.00	1.30	1.82	1.33	0.90	-	4.79	8.45
	(0.10	(0.10)	(0.31)	(0.37)	(0.23)	(0.23)	(0.29)	(0.46)	(0.96)	(0.33)	(0.23)	(1.14)	-	(0.97)	(2.21)
30-40	-	-	-	0.94	0.87	0.77	1.35	1.35	1.27	1.56	1.27	0.05		5.6	9.30
				(0.21)	(0.46)	(0.15)	(0.15)	(0.21)	(1.25)	(0.61)	(0.31)	(0.07)		(2.33)	(1.78)
								Cadmium							
0-10	0.01	0.04	0.04	0.02	0.01	0.12	0.04	0.04	0.06	0.07	0.02	0.03	1.2	1.2	0.15
	(0.00)	(0.03)	(0.03)	(0.00)	(0.01)	(0.06)	(0.00)	(0.04)	(0.05)	(0.02)	(0.01)	(0.02)	(0.01)	(0.04)	(0.03)
10-20	0.01	0.01	0.03	0.02	0.01	0.13	0.04	0.04	0.00	0.07	0.05	0.03	1.0	0.93	0.07
	(0.00)	(0.01)	(0.02)	(0.00)	(0.01)	(0.07)	(0.00)	(0.03)	(0.00)	(0.01)	(0.05)	(0.02)	(0.05)	(0.45)	(0.01)
20-30	0.02	0.01	0.03	0.02	0.02	0.13	0.04	0.69	0.01	0.07	0.02	0.02	0.80	0.77	0.1
	(0.02)	(0.01)	(0.01)	(0.00)	(0.01)	(0.06)	(0.01	(0.14)	(0.01)	(0.02)	(0.02)	(0.01)	(0.04)	(0.13)	(0.03)
30-40	0.01	0.02	0.01	0.03	0.03	0.11	0.04	0.03	0.01	0.07	0.04	0.02	0.70	0.72	0.07
	(0.00)	(0.01)	(0.00)	(0.01)	(0.02)	(0.01)	(0.02)	(0.02)	(0.01)	(0.03)	(0.01)	(0.01)	(0.03)	(0.32)	(0.02)

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