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.

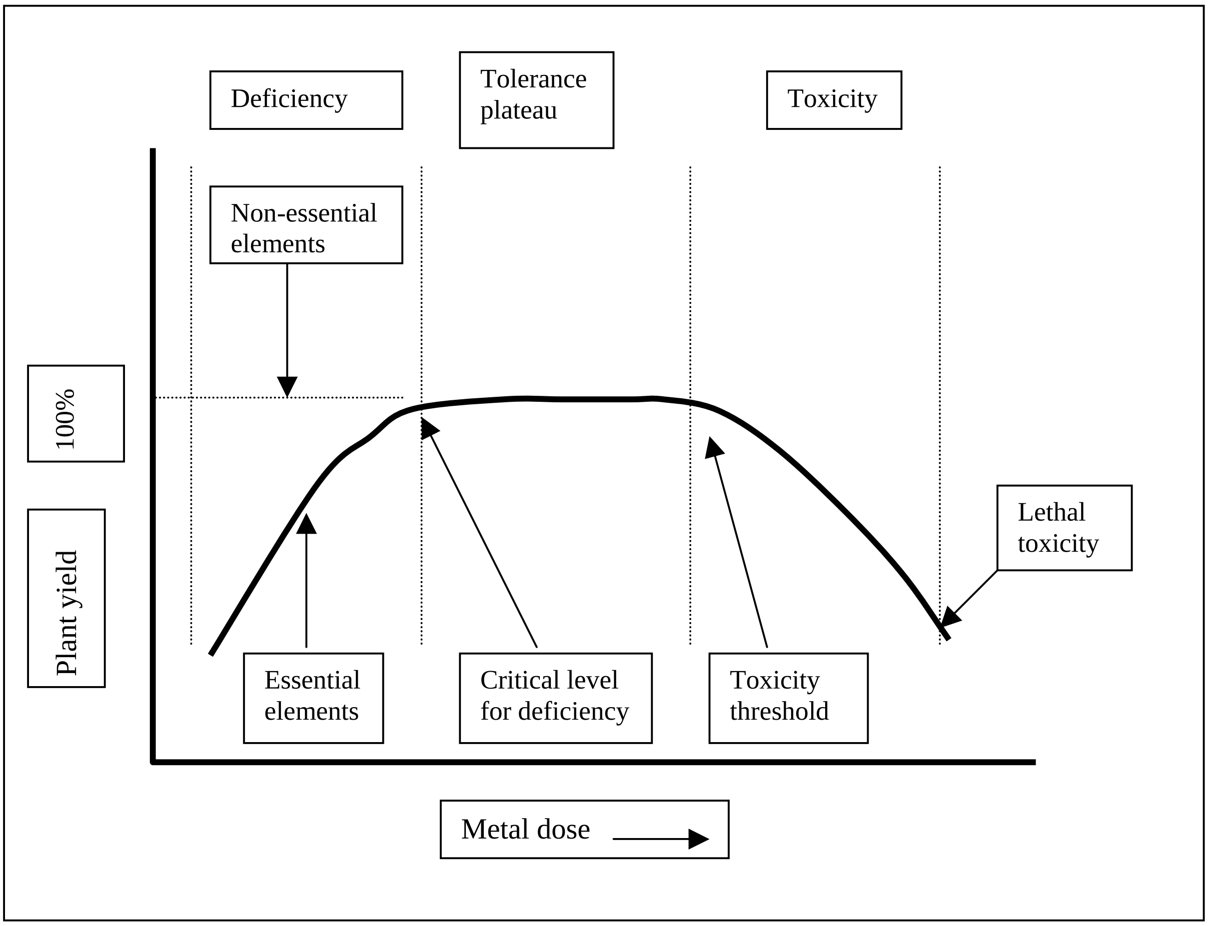

Figure 2.1: Generalised dose-response curve for nutrient metals (Adapted from Malan, 1999)

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.