REMEDIATION OF HEAVY METALS (Cd and Pb) AND PHTHALATE ESTERS IN CONTAMINATED SOIL TREATED WITH COMPOSTS

BY

LINDA LUNGA SIBALI

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DECLARATION

I hereby declare that this dissertation is the result of my own investigation and has not been submitted previously for any degree at any University, except where acknowledged.

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LINDA LUNGA. SIBALI

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ABSTRACT

The availability of heavy metals (lead and cadmium) and phthalate esters (dimethyl phthalate, diethyl phthalate and di-2-ethylhexyl phthalate) from artificially contaminated soil samples to which were added compost of different compositions, were measured weekly for 28 days. Heavy metals and phthalate esters were determined by atomic absorption spectroscopy (AAS) and gas chromatography (GC), respectively. Six different types of composts (C1-C6) were used for heavy metals whilst three different types of composts (C1, C2 and C4) were used for phthalate esters. For heavy metals, the total metal sorption in composts changed with time and the changes were dependent on type of compost and its formulation. Generally, for all the composts used lead was highest on 7th day and cadmium on 14th day. The highest rate of compost appeared to give the most efficient fixing of the metals. Compost C5 (i.e. a mixture of sawdust, tobacco waste and rock phosphate) gave the most efficient fixing of lead while compost C4 (i.e. a mixture of sawdust, tobacco waste and poultry manure) gave the most efficient fixing of cadmium. Generally, compost C4 appeared to be the most efficient of all the compost used for fixing these metals. For phthalate esters, it was observed that there was a gradual decrease in their concentrations in contaminated soil throughout the period of incubation (0-28th day) at 30 ^{0}C. Compost C2 (i.e. a mixture of sawdust, tobacco waste and pig dung) gave the most efficient fixing of all phthalate esters used.

In addition, both incubation periods of heavy metals and phthalate esters pH and EC were determined.

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ABBREVIATIONS

µg/g - microgram per gram

kg - Kilograms

ml - millilitre

g/mol - gram per mole

ppm - parts per million

mg/kg - milligram per kilogram

µm - micro gram

m - metre

mm - millimetre

Hrs - Hours

^{0}C - Degrees celcious

EC - Electical conductivity

Cd - Cadmium

Pb - Lead

DMP - Dimethyl phthalate

DEP - Diethyl phthalate

DEHP - Di- (2-ethylhexyl) phthalate

BUBE - Butyl benzoate

$CaCO_3$ - Calcium carbonate

HNO_3 - Nitric acid

$CdCl_2.H_2O$ - Cadmium chloride

PbS - Lead sulphide

PbNO$_3$ - Lead nitrate

PbBr - Lead bromide

HCL - Hollow Cathode Lamp

PVC - Poly Vinyl Chloride

ECPI - European Council for Plasticisers and Intermediates

EPA - Environmental Protection Agency

AAS - Atomic Absorption Spectroscopy

ET-AAS - Electrothermal Atomic Absorption Spectroscopy

GF-AAS - Graphite Furnace Atomic Absorption Spectroscopy

LIBS - Laser-Induced Breakdown Spectrometry

LIF - Laser-Induced Flourescence

LIS - Laser-Induced Spectrometry

XRFS - X-ray Fluorescence Spectrometry

EDXRF - energy-dispersive X-ray fluorescence

ASV - Anodic Stripping Voltammetry

SWASV - Short Wave Anodic Stripping Voltammetry

PAEs - Phthalate esters

GC - Gas chromatography

DHHS - Department of Health and human Service

OECD - Organization for Economic Co-operation and Development

OMEE - Ontario Ministry of Environment and Energy

ATSDR - Agency for Toxic Substances and Disease Registry

CCREM - Canadian Council of Resource and Environmental Ministers

CDC - Center for Disease Control

CEC - Commission of the European Communities

WHO - World Health Organisation

NIOSH - National Institute for Occupational Safety and Health

IARC - International Agency for Research on Cancer

U.S. - United States

UK - United Kingdom

Chapter 1

1.1 INTRODUCTION

Soil is the term understood by almost everyone, yet the meaning of this term may vary among different people and soil can be defined in many ways. The farmer, engineer, chemist, geologist, and layman bring different viewpoints or perspective to their concepts of soil (Brady, 1990 and Foth, 1990).

Contamination of soils by heavy metals is one of the environmental problems that the scientific community faces today. Such soils are difficult to treat because the heavy metals cannot be destroyed. Moreover, they are usually bonded with other contaminants, in particular organic ones. The presence of these organic pollutants can make the removal of metals more complicated (Mulè and Melis, 2000).

Metals and their compounds are indispensable to the safety and economy of most nations and have been key factors in liberation of modern civilization from hunger, disease and discomfort. Few, if any, of the metals known to mankind have not found some application in industry, and the number of commercial uses continues to grow with the development of modern science and technology. Inevitably, each industrial process generates wastes, which must be discharged into the environment, along with the ever-growing list of new metallic compounds. It has been estimated that the toxicity of all the metals being released annually, into the environment, exceeds the combined total toxicity of all the radioactive and organic wastes, as measured by the quantity of water needed to dilute such wastes to the drinking water standards (Nriagu and Pacyna, 1988). Pollutant metals are non-degradable and the continuing build-up of such toxins in mankind's life support system has to be of some health concern. The current levels of toxic metals in some environmental compartment may be high enough to constitute a threat to human health. Nevertheless, it is incontestable that each environmental compartment has a limited carrying capacity for metal pollution, and, with enough time, the current rates of metal inputs will become stressful to many ecosystems. (Nriagu, 1988)

During the last decades, concern about the hazards of toxic metals and other chemicals on human health and the environment has increased worldwide. In many countries effective legislative and administrative measures have been taken to reduce environmental pollution and to prevent adverse effects resulting from exposure to chemicals in the work environment and the general environment (Berlin and Van der Venne, 1987 and Zabel, 1989).

Toxic metal wastes from defense-related activities, industry, and municipal sources have routinely entered the environment through disposal in landfill sites or by accidents such as that which occurred at Chernobyl. These practices have resulted in surface contamination problem, transport to ground water, and/or bioaccumulation of radionuclides and toxic metals (Cornish et al., 1995; Cunningham et al., 1995; Riley et al., 1992). Metals such as Cd and Pb are prevalent in soils near industrial centers at concentrations up 350 µg of Cd/g (Bunzl et al., 1991; Lux et al., 1995). Toxic metals are often inhibitory to other bioremediative processes, e.g., hydrocarbons degradation (Said et al., 1991). Microbial communities are of primary importance in remediation of metal contaminated soils and represent a substantial proportion of the in situ biomass and metabolic diversity. The structure and diversity of soil microbial communities have been shown to change in soil in the presence of toxic metals (Babich et al., 1985; Frostegård et al., 1993; Frostegård et al., 1996; Pennanen et al., 1996). Microorganisms can alter metal chemistry and mobility through reduction, accumulation, mobilization, and immobilization (Avery, 1995; Beveridge, 1989; Lovley, 1994; Valentine et al., 1996).

Organic contaminants like phthalate esters are by far the most widely produced worldwide. Both ortho-phthalic and terephthalic acid may be reacted with an appropriate alcohol to produce phthalate esters which may be used as plasticisers. In practice, terephthalates are more commonly used in the USA than elsewhere. Phthalate esters are manufactured from methanol (C_1) or other alcohols up to C_{17}, although phthalates use as PVC plasticisers in generally are in the range of C_4 to C_{13} (the lower molecular weight phthalates finding use in nitrocellulose; the higher phthalates as synthetic lubricants for the automotive industries). Just over one million tons of phthalates are produced in Western Europe each year (ECPI, 2002). Plasticisers in general and phthalates in particular are currently the subject of considerable media, legislative and scientific debate. This is not new. Concerns regarding plasticisers have been raised on a variety of topics at regular intervals ever since the early 1980's. These have included carcinogenicity, environmental effects, oestrogen mimicking and most recently exposure via toys. However, any fears have repeatedly been shown to be unfounded. Plasticised PVC has been used for more than 40 years without a single known case of it having caused any ill health and the environmental effects of phthalates are known to be minimal. Academia and industry have continually worked together to address the concerns and conduct necessary research. Indeed there is more known today about phthalates than almost any other chemicals. Unfortunately, politics play increasingly important part in any debate relating to chemicals but, in the majority of cases, the authorities have adopted a scientific approach to these concerns. Indeed, it is as a consequence of the large amount of scientific research that has been carried out and also because flexible PVC makes such a valuable contribution to modern life that plasticiser usage continues to grow (ECPI, 1999).

Organic soil amendments such as compost can reduce the availability of heavy metal contaminated soil. The increased addition of compost was found to alleviate the toxic effects heavy metal have on plant health while redistributing metals to a less available form (Diener, 2002). The possible use of this kind of organic waste is under constant review in developed countries because further degradation of the environment must be prevented and because their use as organic amendment would involve important savings in the use of nitrogen, phosphorus and, to a lesser extent, potassium based inorganic fertilizer (Rodríguez, 1982). However, before they can be used, such waste should undergo a composting process to eliminate many phytotoxic substances and pathogenic microorganisms (Moreno et al., 1997).

The contaminants are digested, metabolized, and transformed into humus and inert byproducts, such as carbon dioxide, water, and salts. Compost bioremediation has proven effective in degrading or altering many types of contaminants, such as chlorinated hydrocarbons, wood preserving chemicals, solvents, heavy metals, pesticides, petroleum products and explosives. Compost used in bioremediation is referred to as "tailored" or "designed" compost in that it is specially made to treat specific contaminants at specific sites. The ultimate goal in any remediation project is to return the site to its precontamination condition, which often includes revegetation to stabilize the treated soil. In addition to reducing contaminant levels, compost provides soil conditioning and also provides nutrients to a wide variety of vegetation (U.S. EPA, 1997).

When organic matter decomposes to the point that it becomes humus, then it can be defined as humic, which is the soluble portion of humus. The greater the humification index (proportion between humified and not humified parts of a given organic substance) is, the greater the organic substance that has been transformed into humic acids. Humic acid is the collective term for humic acid and fulvic acid, which make up the main fraction of natural humic matter. Humic acids are an excellent natural and organic way to provide soils and plants with a concentrated dose of essential nutrients, vitamins and trace elements. Not only in agriculture, humic acids are also used in the industry and for environmental applications such as wastewater treatment or erosion control (http://www.humintech.com). The application of compost, which has shown to improve physical and chemical soil properties as well as plant growth, crop yield and crop quality (Mays et al., 1973; Pinamonti and Zorzi, 1996; Rodrigues et al., 1996; Smith, 1996) and to suppress soil-borne plant pathogens (Lums et al., 1986; Chen et al., 1988; Mandelbaum et al., 1988), could alleviate poor soil fertility status and increase food production (Smith and Hughes, 2002).

1.1.1 Aim and Objectives of this Study:

Aim of this study:

Evaluating several composts as a means of reducing the availability to plants of heavy metals (Pb and Cd). Heavy metals like Cd and Pb from soil are easily taken up by plants and may constitute a threat to human and animal health if such plants are eaten. Several federal and state regulation authorities list Cd and Pb amongst other elements as potentially toxic.

(ii) To investigate the fixing of phthalate esters (DMP, DEP and DEHP) in contaminated soil by compost.

Specific objectives of this study are:

- To spike the soil with known concentration of heavy metals (Pb and Cd) and phthalate esters (DMP, DEP and DEHP).
- To analyze and monitor the remediation of heavy metals and phthalate esters in soil incubated at 30 ^{0}C on a weekly bases [(0th, 7th, 14th, 21st and 28th) days] using different types and rates of composts.
- To come up with the most efficient composts for remediation of heavy metals and phthalate esters from soil which will have added advantage by adding nutritive value to the soil for plants nutrition.
- To find out which compost and at which rate (amount) is best for this remediation process according to its remediation ability and time.

This study has the potential to contribute to the environmental protection strategies, with the aim of coming up with the cheapest ways of remediating harmful organic (phthalate esters) and inorganic (heavy metals) toxins, which are found in the soils. This study will also be more beneficial to farmers because whilst remediation is taking place, there will be addition of nutrients to the soil for high crop yields.

1.2 LITERATURE REVIEW

1.2.1 Soils and Chemical Pollution

According to the Minister of Water Affairs and Forestry "We as South Africans have serious shortage of hazardous and toxic waste disposal sites and treatment facilities and alarmingly high percentage as much as 50% of such waste is being disposed indiscriminately and illegally" (Carnie, 1996a).

The impact of heavy metal contamination on agricultural soils depends not only on the types and amount of sewage sludge applied, but also on soil properties. A sewage sludge that contains large quantities of heavy metal may contribute significantly to the build-up of heavy metals in soils and subsequent accumulation in plants at low pH conditions (Al-Wabel, 1998).

The soil is a primary recipient by design or accident of a myriad of waste products and chemicals used in modern society. It has been always convenient to "throw things away", and the soil has been the recipient of most of these things. Every year millions of tons of these products from a variety of sources – industrial, domestic, and agricultural- find their way onto the world' soils. Once these materials enter the soil, they become part of biological cycles that affects all forms of life. One of the challenges facing humankind is a better understanding on how wastes affect these cycles and, in turn, the well being of all plant and animal life. Tons of organic residues and animal manures are brokendown by soil microbes each year, and large quantities of inorganic chemicals are fixed and bound tightly by soil minerals. Soil has its capacity to accommodate these chemicals, and there's a disastrous effect on environmental quality when the limits have been exceeded.

An enormous quantity of organic chemicals is manufactured every year, included are plastics and plastisizers, lubricants, and refrigerants, fuels and solvents, pesticides, and preservatives. Some are extremely toxic to humans and other life. It is imperative, therefore, that we control the release of organic chemicals and that we learn of their fates and effects once they enter the soil (Brady et al., 1991).

Table 1.1: Physical properties of some soils

Soil properties	**Soil 1**[a]		**Soil 2**[b]	
EC (dSm^{-1})	4.65		1.25	
pH	7.51		7.95	
$CaCO_3$ (%)	8.90		26.70	
O.M. (%)	0.5		0.20	
Sand (%)	90		92	
Silt (%)	5		5	
Clay (%)	5		7	
Soil texture	Sand		Sand	
Heavy metals	**c**[c]	**d**[d]	**c**	**d**
Pb (mg kg^{-1})	0.3	20.0	0.40	25.0
Cd (mg kg^{-1})	0.03	2.5	0.03	3.5

[a]Slightly calcareous soil; [b]Moderately calcareous soil; [c]Extracted by DTPA; [d]Extracted by $HNO_3 + HClO_4$

1.2.2 Complexed Chemicals

Researchers have found that some chemicals that are normally subject to microbial attack seem to be protected from such degradation when the compounds are complexed by soil organic matter or sorbed by inorganic materials. The complexation is essentially irreversible and the sorption by metals and its oxides or silicate clays is so tight that the compounds are only very slowly available. Some compounds may also be trapped between the internal structure layers of some silicate clays. The availability usually decreases as the soil-contaminant complex ages over time. Reduced availability of pollutants constrains their remediation by microorganisms. It also has some implications for regulatory policies, since pollutants so held are not likely to move into the groundwater or elsewhere in the environment (Brady et al., 1991).

1.2.3 Heavy Metals

The toxicity of inorganic contaminants released into the environment is now estimated to exceed that from organic and radioactive sources combined. A fair share of these inorganic substances end up contaminating soils. The greatest problems most likely involve cadmium and lead. To a greater or lesser degree, both these elements are toxic to humans and other animals. Cadmium is extremely poisonous; lead is moderately so in mammalian toxicity (Brady et al., 1991).

Table: 1.2 Sources of Inorganic Soil Pollutants (Cd and Pb)

Chemical	**Major uses and sources of soil contamination**	**Organisms Principally harmed**	**Human health effects**
Cadmium	Electroplating, pigments for plastics and paints, plastic stabilizers, batteries, and phosphate fertilizers	H, A, F, B, P	Heart and kidney diseases, bone embrittlement
Lead	Combustion of oil, gasoline, and coal; iron and steel production; and solder on water-pipe joints	H, A, F, B	Brain damage, convulsions

H= humans, A= animals, F= fish, B= birds, P= plants

1.2.3.1 <u>Heavy Metals In Question</u>

1.2.3.1.1 Cadmium

Cd occurs in nature largely in the mineral greenockite as the sulfide (CdS) but most of it is obtained from lead and zinc sulfide ores. Cadmium behaves very much like zinc, both favoring a combination with sulfur. Cadmium is also a common impurity in zinc used for galvanized iron pipes, and contamination in water distribution system may result from this source. According to some authors, environmental cadmium may contribute to the pathogenesis of hypertension in man. It is clear that renal tubular damage may occur after sufficient exposure to cadmium (Fairbridge, 1972)

Cd like zinc, it is readily taken up by crops from soil in which its content has been enhanced. Cadmium is therefore an element, which can readily enter mammalian food chain at dangerous level. There is no reason to doubt that Cd is generally phytotoxic at its relatively high level (Rand et al., 1976).

Minute quantities of Cd are suspected of being responsible for adverse changes in arteries of human kidney. On the other hand, there is an indication that Cd might be a dietary essential. It may also enter water as a result of industrial discharge or the deterioration of galvanized pipes (Purves, 1985).

1.2.3.1.2 Lead

Lead occurs most commonly as galena (PbS) in mixed lead zinc-deposits (Ewers and Schipköter, 1991). Two thirds of the global lead recourses exist in relatively large deposits in the U.S., Australia, C.I.S. and Canada (Ewers and Schipköter 1991). During the 1990's, Canadian mines, located mainly in the Yukon, New Brunswick, British Columbia and the Northwest Territories, have produced an average of 243 kilotonnes/year (Stanford, 1992; Keating, 1995).

The production of batteries, used predominantly in the automotive industry, comprise the largest market for refined lead followed by use of lead in glass and paint pigments, and in compounds used for PVC stabilization (OECD, 1993; Keating and Wright, 1994; Keating, 1995). Rolled and extruded lead products are used extensively in the construction industry. Although lead was once used extensively in the production of lead gasoline additives, the current demand for lead in this area is low, comprising only a small fraction of the world market (Keating, 1995). Nevertheless, the growth in the lead acid battery industry has made up for this decline in lead use (ARSDR, 1993). Lead is also used in cable sheathing (primary marine cables), alloyed materials (i.e., solder, bearings and bushings, and brass and bronze), in the production of shot and ammunition as well as in a variety of other miscellaneous products (OECD, 1993; Keating and Wright, 1994; Keating, 1995).

1.2.3.2 Environmental Fate and Behavior of (Cd and Pb) in Soils

Cadmium

Cadmium is the heavy metal of greatest concern in agricultural soils. It is not required for the growth and development of either plants or animals and can be toxic to both. Cadmium is loosely held by soil constituents and is readily available to plants. Thus, increased concentrations in soil result in increased concentrations in crops. Cadmium accumulation in plant materials varies with crop type and plant part. In general, broadleaf plants accumulate more cadmium than grasses, and plant leaves and stems accumulate more than seeds. Broad-leaved vegetables, such as lettuce and Swiss chard, accumulate more cadmium than most other plants. Consequently, Canadian guidelines and regulations have been introduced to allow only minor increases in soil cadmium. These guidelines allow waste recycling to land (such as sewage-sludge application), while maintaining the quality of food products needed for domestic and international markets (Webber, Wastewater Technology Centre, Burlington, 2001).

Lead

In nature, lead occurs mainly in the +2 valence state (Kabata-Pendias, 1992; Davies, 1995) although a stable valence state of +4 is also known to exist (Davis, 1995). Lead is rarely found in the elemental state but occurs in a variety of mineral ores of which galena (PbS) is most common (Stokinger, 1981). The crustal abundance of lead has been estimated to approximately 16mg/kg (Davies, 1995) and levels in uncontaminated soils tend to reflect the content of the parent rocks (Kabata-Pandias and Pandias, 1992). However, due to the historical dispersive uses of lead, most surface soils tend to be enriched by anthropogenic lead. A global mean concentration of 25 mg/kg in surface soils was recently estimated by Kabata-Pendias and Pendias (1992) in a review of a literature.

Lead is present in soils as predominantly the plumbous ion (Pb^{+2}), in the soil solution, as sulphates, carbonates and oxides in precipated forms, and as Pb silicates in the soil lattices (Davies, 1995). The lead compounds in contaminated soils have been reviewed by Kabata-Pendias and Pendias (1992) and Hempfill et al. (1991). The combustion of leaded gasoline produces halide salts such as PbBr, PbBrCl, Pb(OH)Br and $(PbO)_2PbBr_2$ in the exhaust particles but these are very unstable and readily convert into oxides, carbonates and sulphates (Kabata-Pendias and Pendias, 1992). Most of the lead associated with mining waste consists of PbS altered to $PbSO_4$ at the surface of the waste piles (Hemphill et al., 1991). The prevailing lead forms associated with smelter operations include the sulphide (PbS), sulphates ($PbSO_4$) and oxides (PbO and PbO_2) (Hemphill et al., 1991). The lead in pigments added to some paints may include forms such as the octate, oxide, carbonate, sulphate and chromate (Hemphill et al., 1991).

Soil is considered to be a sink for anthropogenic lead (Davies, 1995; Hill 1992; OECD, 1993) and this association can be regarded as irreversible and permanent unless removed through intervention (Stokes, 1989). Lead is persistent in soil because of its low solubility, which tends to increase with residence time (Leita and De Nobili, 1991), its strong complexing behavior with organic matter (Kabata-Pendias and Pendias, 1992), and its relative freedom from microbial degradation (Davies, 1995; OECD, 1993). Accumulating in the surface horizons, it only moves downward slowly in association with the slow downward movement of organic matter (Carelli et al., 1995). From the limited knowledge about the chemistry and speciation of lead in soil (Davies, 1995; Environment Canada, 1994), lead is reported to be mainly absorbed into clay minerals, adsorbed and co-precipated with Mn oxides and Fe and Al hydroxides, and adsorbed onto colloidal organic matter and complexed with organic moieties as well as in some soils, associated, although to a lesser extent than with the forms above, with carbonates (Davies, 1995; Kabata-Pendias and Pendias, 1992).

Lead is not degraded in the environment although some fate processes can transform certain chemical Pb species into others (ATSDR, 1993; Carelli et al., 1995). It has been suggested that biomethalation of inorganic Pb can occur but is not considered a significant mobilization process (Andre et al., 1984; Beijer and Jernelov, 1984; Walton et al., 1988; CCREM, 1987; ATSDR, 1993).

Limited leaching of Pb from soils has been reported (OECD, 1993). Mobility, however can be influenced by the soil pH and the content of humic and fulvic acids and of clay and organic material in the soil (Ewers and Schipköter, 1991; Environment Canada, 1994). Acidic conditions favor solubilization of Pb from the solid phase of soils and acidic soils tend to have a lower Pb content (Kabata-Pendias and Pendias, 1992). The possibility of solubilization of soil Pb by acid rain and its subsequent solubilization and leaching into ground water has been suggested (Jaworski et al., 1987; OMEE, 1994a). However there is no evidence supporting the theory (OECD, 1993). In addition, Nelson and Campbell (1991) reported that probably humic acid and fulvic acid interactions, rather than acidification, are the main route for Pb leaching and transport. These authors also suggest that organic matter plays the most important role in Pb fixation. The soil's cation exchange capacity (CEC), texture, clay mineralogy, redox potential, organic content and pH as well as the levels of other elements present therein (i.e., phosphorus, sulphur, nitrogen, and cadmium) can affect bioavailability (Merry et al., 1986; Panis and Lucianer, 1987; OECD, 1993). There is, however, a paucity of information about speciation and the bioavailable form of Pb in soil solutions due to analytical difficulties presented by the low concentrations of Pb in such samples. Nevertheless, it is generally agreed that in most soils, only a small proportion of the Pb is bioavailable. (Davis, 1995), and that the critical bioavailable fraction is the amount of Pb^{+2} in the soil solution (Andriano, 1986; Kabata-Pendias and Pendias, 1992). In acid soils, notably, as much as 95% of the Pb solution can be present in cationic forms (Gregson and Alloway, 1984). Furthermore, suggestion have been made that even in heavily polluted soils, especially those in higher pH, part of the Pb in soil solutions is present at high molecular weight organo-Pb complex, a form not available for plant uptake (Gregson and Alloway, 1984). Lead solubility and hence, bioavailability can be decreased through Pb precipitation as hydroxide, phosphate and carbonate (Kabata-Pendias and Pendias, 1992; OMEE, 1994a) by the addition of compost (Hirshorn, 1989), lime or phosphate fertilizers (Kabata-Pndias and Pendias, 1992).

1.2.3.3 Sources and Accumulation of Heavy Metals (Cd and Pb) In Soil

Cadmium and lead are widely distributed in the environment and are considered particularly toxic elements. Their accumulation has multiple effects on the usability and functions of soil in the eco-system.

Cadmium

Cadmium is an especially toxic heavy metal that is very mobile in soil. Even minimal amounts in soil that has a low pH or humus level will result in cadmium uptake into plants. Levels exceeding guidelines for foodstuffs or feeds can result. Most of the literature on the toxicity of cadmium relates it to smelting. For example, Belgium is the principal producer of cadmium in Europe and environmental contamination in areas near zinc smelting plants, which release cadmium into the environment, is severe. Such a severe degree of contamination would not have arisen from the ZnCdS releases. There are also areas where extensive mining operations have led to contamination particularly affecting the soil. In the UK, the highest level of soil contamination due to former mining activities has been found in the Somerset village of Shipham. "The health of Shipham residents has been extensively investigated and we consider Shipham to be a useful example to examine in regard to the possible hazards of soil contamination with cadmium" (Buchet et al., 1990; Staessen et al., 1999). An extensive study was made in 1979 of the inhabitants' health, as well as the health of volunteers living in the nearby town of North Petherton in which there was no exposure to heavy contamination with metals. The pollution was associated with old mine workings and in more recent years the soil has been disturbed by the development of pasture. Many houses and gardens are on polluted land but the Cd in the soil is tightly bound up in minerals and is not readily available for uptake by locally grown produce (Buchet et al., 1990; Staessen et al., 1999). Cadmium is used in plating metals and in manufacture of batteries. Cd is also found as constituents in specific organic pesticides and in domestic and industrial sewage sludge. Additional localized contamination of soils with metals results from ore-smelting fumes, industrial waste, and air pollution. Some of the toxic metals are being released to the environment in increasing amounts, while others (most notably lead, because of changes in gasoline formulation) are decreasing. Metals are daily ingested by humans, either through the air or through food, water and-yes-soil (Brady et al., 1991). Storm water is the largest source of Cd, contributing about 44% of Cd load, this being mainly associated with the wear of tyres, break pads and lubricating oils. Cd as an impurity in fertelizer is also a significant source of CdS (Gray and Becker, 2002)

Lead

Excluding leaded gasoline combustion, solid waste and effluent discharges are estimated to be 61.7 kilotonnes/yr and 0.6 kilotonnes/yr on average, respectively (Jaques, 1985). Given the nature of the main sources of emissions and discharges, most of the environmental impact of lead tends to be localized (Ewers and Schipkoter, 1991). Sources of lead in soil are mostly anthropogenic and reflect both present and historical inputs (Stokes 1989; Davies, 1995; Kabata and Pendias and Pendias, 1992; OECD, 1993; Wixson and Davies, 1993). High concentrations generally only occur locally and tend to decrease in a negative exponential fashion with distance from the source of emissions (Davies, 1995; Ewers and Schipköter, 1991; Wixson and Davies, 1993). However, more widespread contamination, especially from "hot spots" (Stokes, 1989) can occur via wind and water erosion (Carelli et al., 1995). Lead levels in solution in urban areas tend to be greater than those in remote or rural areas (Flegal et al., 1990) as a consequence of historical inputs from the combustion of lead gasoline and of current and past release from smelters and metal processing plants (Stokes, 1989; Davies, 1995; Wixson and Davies, 1993). Localized high concentrations of lead in soil have been attributed to wash down and peeling of leaded paint from houses, bridges and storage tanks (Schmitt et al., 1988; Krueger and Duguay, 1989; Mielke et al., 1989).

1.2.3.4 The Impact of Heavy Metals To The Environment

Irrespective of their sources, toxic elements can and do reach the soil, where they become part of the food chain: soil→plant→animal→human. Unfortunately, once the element becomes part of this cycle, they may accumulate in animal and human body tissues to toxic levels. This is especially critical for fish and other wildlife and for humans at the top of the food chain. For example, as earthworms ingest contaminated soil, the chemicals tend to concentrate in the earthworm bodies. When birds and fish eat the earthworms, the pollutant can build up to lethal levels. It has already resulted in restrictions on the use of certain fish and wild life for human consumption. Also, it has become necessary to curtail the release of these toxic elements in the form of industrial waste (Brady et al., 1991).

1. 2.3.4.1 Cadmium Poisoning

One of the early signs of Cd intoxication of the kidney is tubular proteinuria, or the excretion of low molecular weight proteins and retinal-binding proteins (Nomiyama, 1981; Frieberg et al., 1984/85). This form of protenuria, however, is not always specific for Cd and may be found in other forms of renal dysfunction. The currently known 'early warning signs' of cadmium induced cellular damage and loss of reserves (or vitality) are thus poor, making it difficult to estimate the actual number of people with sub clinical Cd poisoning.

The release of Cd from numerous non-ferrous metal mines and smelters has resulted in 9.5% of paddy soils, 3.2% of upland soils and 7.5% of orchard soils in Japan being severely contaminated with Cd (Asami, 1984). Food crops, especially rice, grown in such soils, are also contaminated with this metal. As a result, the dietary intake of Cd (about 56 μg per day) and the average Cd concentration in renal cortex (60-100 μg g^{-1}) in Japanese population (Tsuchiya, 1976; Friberg and Vahter, 1983; Piscator, 1985) currently leave little safety margin for many residents. Renal Cd concentrations close to the critical value of 200 μg g^{-1} and daily Cd intakes of 70 μg are occasionally exceeded, by many elderly multiparous women (with poor nutritional habits) who live in the contaminated areas (Friberg and Vahter, 1983). As to be expected, many elderly multiparous women with poor nutritional habits who live in the contaminated areas of Japan, and elsewhere, now show signs of renal dysfunction (Roels et al., 1981; Nogawa, 1984). Metallic models (Kjellstrom and Nordberg, 1978) suggests that daily intake of 100-180 μg Cd per day by residents in the highly polluted areas of Japan, can leave on excessive renal Cd accumulation in about 5-10% of the people so exposed. On the other hand, the intake rate of 42-69 μg per day in the unpolluted areas (Nogawa, 1984) can place 0.5-1% of the population above the critical level (200 μg litre^{-1}) over an extended period of time. An average daily intake of 32 μg Cd, which is typical of many countries (Hutton, 1982; Friberg and Vahter, 1983), can result in 0.1% of the population having Cd concentrations in the renal cortext above the 200 μg g^{-1} threshold for preteinuria (Piscator, 1985). If it is assumed that only 5-10% of human population actually ingest this amount of Cd, the number of persons likely to accumulate Cd, possibly to the stage of renal dysfunction, is estimated to be 250 000-500 000. This number pertains to the background occurrence of parasthesia, and the number actually at risk is probably considerably higher. Most of the people presently at risk from environmental Cd poisoning are believed to be in Japan and central Europe where the soils have become contaminated with this element (Asami, 1984 ; Kloke et al., 1984).

1.2.3.4.2 Lead Poisoning

Environmental exposure to low levels of lead has been associated with a wide range of metabolic disorders and neuropsychological deficits (NAS, 1980; EPA, 1986). The well known toxic (metabolic and cellular) effects of lead in asymptomatic children include (i) impairments in haeme, vitamin D and red blood cell nucleotide metabolism, (ii) perturbations of calcium homeostasis in the hepatocytes, bone cells and brain cells, and (iii) neurological damage (Rosen, 1985; Lansdown and Yule, 1986). Although many of the biochemical and neurological changes associated with lead toxic have been observed at blood lead (PbB) concentrations as low as 60 μg litre^{-1} .The threshold for possible medical intervention has been set at 250-300μg litre^{-1} (CDC, 1985). A nationwide survey in the USA done between 1976 and 1980 found that 3.5% of the children under the age of 5 had PbB levels of 300μg litre^{-1}.or more which is well above the medical intervention threshold. The total number of school children was about 16.9 million, implying that about 590 000 children in the country between the ages of 6 months and 5 years are over-exposed to lead and may be suffering form lead poisoning. There was a marked racial difference in the lead risk, with about 10.2% (or about 264 000) levels. Also, about 5% of the children aged 6-17 and 1.8 of the adult population in the 18-74 age group had PbB in excess of 300 μg litre^{-1}., the number of pre- school children at risk increase to 3.5 million, with black children accounting for about 30% of this total. For all age groups, the number of people at risk is over 26 million at the 200μg litre^{-1} PbB threshold. In recognition of the magnitude of this national health issues in children (black, white, poor, rich, city or country side), the US Environmental Protection Agency has concluded that there is a national health problem associated with exposure to environmental lead for the general population, in particular to pre-school children. The center for Disease Control in the United States now recommends that all children of 9 months to 6 years of age should be screened for lead at least once annually. The distribution of PbB concentrations in the population of Western Europe and Australia is similar to that of United States (EPA, 1986; Lansdown and Yule, 1986). On the other hand higher PbB concentrations have been reported in some urban population in developing countries. Thus extrapolation of the USA data to urban areas of other countries may not be very realistic. If the threshold concentrations for health is set at 200 μg litre^{-1} PbB, the total number of over exposed people great risk of being poisoned is estimated to be 130-200 million, assuming that only 20-30% of the human population (of 5×10^9) leaving in urban areas have the PbB distribution similar to the general population of the United States. At 300 µg litre^{-1} threshold, the number of school children (about 10% population) at greatly increased risk of being poisoned by lead is estimated to 5-7 million. Even if only a small fraction of the persons at risk experience some health impairment, the fact remains that lead poisoning must be regarded as the most prevalent public health problem in many parts of the world. The figures above are for people already over burdened by lead as determined by their PbB concentrations. On a global scale, the actual number people exposed to relevant environmental Pb levels which can results in PbB concentrations of 200 µg litre^{-1} or more, is well over 1 billion. For example, (Lenvin, 1987) has estimated the number of people in the USA exposed to drinking water with a Pb concentration above 20 µg litre^{-1} is about 42 million. Levin's estimates of the number of children, nubile women and adult males at increased risk experiencing various adverse health effects due to the lead in their drinking water should be disconcerting to some public health officials. In addition, drinking water represents only a minor route of Pb exposure for the human population in the US (Mahaffey et al.., 1982).

1.2.3.5 Standards and Guidelines for Metals in Soil

During recent years, many countries have introduced special legislation to ensure the appropriate disposal of hazardous waste. Authorities have commonly been made responsible for applying these regulations. According to the CEC (Directive on Toxic and Dangerous Waste) (78/319/EEC) the member state of EEC must ensure that toxic and dangerous waste is kept separate from other matter, that the packaging is appropriate and that the labeling indicates its composition and origin.

Environmental health standards for chemicals may be formulated either in terms of concentrations in environmental components or in terms of amounts of substances that might be taken up into the body within a period of time (WHO, 1978). Since metals in soil may be taken up by plants and thus enter the food chain, limit values for maximum tolerable metal concentrations in agricultural and garden soil were set in various countries. In the federal Republic of Germany the guideline values by Kloke(1980) are widely used for the judgment of metal concentrations in agricultural and garden soil.

Table: 1.3 Maximum admissible concentrations of Cd and Pb in compost used in soil on which compost is applied. unit: mg/kg dry weight

Metal	**CH**[a]	**I**[d]	
	Compost (mg/kg)	Compost (mg/kg)	Soil (mg/kg)
Cadmium	3	10	10
Lead	150	500	500

CH[a] =Switzerland: Ordinance and substances Hazardous to the environment (1986) issued under the Environment Protection Law (1983) and Water Protection Law (1971).
I[d] =Italy: Supplemento ordinario alla Gazetta Ufficiale No. 25, of 13. 09. 1984, Rome.

In some traditional industrial areas and near hazardous waste disposal sites the toxic contamination of soil may represent a health risk for the residents of these areas. Questions arise whether these areas can be used for residential or agricultural purposes. In order to provide a basis for administrative decisions on redevelopment measures the Dutch authorities have developed guideline values for contaminant levels in soil and ground water. The U.S. Environmental Protection Agency, e.g., promulgated a number of effluent limitation guidelines and standard pursuant to sections 301, 304, 306, and 307 of the Federal Water Control Act. These standards and guidelines are prepared by several organizations e.g., WHO (1980, 1984, 1987), ACGIH (1988), EPA (1986), NIOSH and (MAK, 1988; Henschler, 1988; Henschler and Lehnert, 1986).

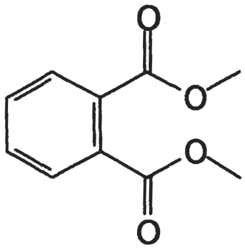
1.2.4 Phthalate Esters

1.2.4.1 Chemistry and Uses

Phthalates are a family of organic chemical compounds that have been developed in the last century. Although the various kinds of phthalates in use today have a certain similarity of appearance and structure, phthalates perform many different tasks. There is no way to complete the sentence "phthalates are…". Phthalates look like vegetable oil. They have little or no smell. They are incorporated into products that consumers use every day. About 80 percent of all the phthalates manufactured today are used as "plasticizers". That is, they make plastics flexible without sacrificing strength or durability. Their chief use is as plasticizers in vinyl, a very familiar, popular and versatile form of plastic. Vinyl (also known as PVC, or polyvinyl chloride) is ordinarily hard. But when certain phthalates are added into the vinyl manufacturing process they act as a lubricant among the long vinyl molecules, permitting them to slip and slide against one another. The result: a technological marvel that helps make our lives better in numerous ways. From construction to toy-making to medical care, flexible vinyl has helped make products that are more durable, cleaner, clearer, and economical. Not all phthalates are used as plasticizers for PVC. Different phthalates keep nail polish from chipping, make perfume linger longer, or make tool handles strong and more resistant to breaking. Others help adhesives, caulking, paint pigments and many other materials perform their jobs better. Because phthalates are so widely used, they have undergone extensive testing for possible health effects on humans or damage to the environment. Some areas of concern have been identified, which are under intense study. But in sum, the record of phthalates is excellent. Phthalates do not persist in the environment; they biodegrade readily. If they make their way into the body, they do not accumulate in animals or humans; inside the body, they break down quickly and are excreted. Most important, in their long history of beneficial service to consumers, there has never been any scientifically validated evidence that they have ever caused anyone any harm (Phthalates Information Center, 2002).

In this study only dimethyl phthalate (DMP), diethyl phthalate (DEP) and di-2-ethylhexyl phthalate (DEHP) phthalate esters were used.

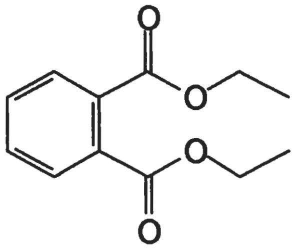
Dimethyl Phthalate (DMP)



DMP

Dimethyl phthalate is a colorless oily liquid with a slightly sweet odor (Trenton, 1986). The chemical formula for dimethyl phthalate is $C_{10}H_{10}O_4$, and the molecular weight is 194.19 g/mol. The vapour pressure for dimethyl phthalate is 4.19×10^{-3} mm Hg at 20 °C, and it has a log octanol/water partition coefficient (log K_{ow}) of 1.56. It is slightly soluble in water (Cincinnati 1987). Dimethyl phthalate has many uses, including in solid rocket propellants, plastics, and insect repellants. Acute (short-term) exposure to dimethyl phthalate, via inhalation in humans and animals, results in irritation of the eyes, nose, and throat (Bethesda, 1993). No information is available on the chronic (long-term), reproductive, developmental, or carcinogenic effects of dimethyl phthalate in humans. Animal studies have reported slight effects on growth and on the kidney from chronic oral exposure to the chemical. EPA has classified dimethyl phthalate as a Group D, not classifiable as to human carcinogenicity (U.S. Environmental Protection Agency, 1999).

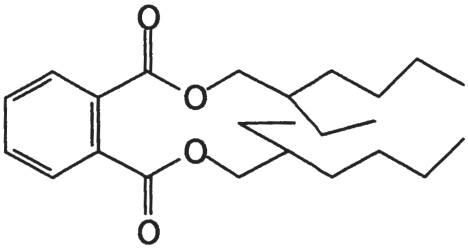
Diethyl Phthalate (DEP)



DEP

Diethyl phthalate is a colourless liquid that has a bitter, disagreeable taste. This synthetic substance is commonly used to make plastics more flexible. Products in which it is found include toothbrushes, automobile parts, tools, toys, and food packaging. Diethyl phthalate can be released fairly easily from these products, as it is not part of the chain of chemicals (polymers) that makes up the plastic. Diethyl phthalate is also used in cosmetics, insecticides, and aspirin (ATSDR, 1996).

Di-(2-ethylhexyl) Phthalate (DEHP)



DEHP

Di-2-ethylhexyl phthalate (CAS No [117-81-7]) is also known as di-octyl phthalate (DOP). In Western Europe it accounts for 45% of all plasticiser usage and as such is generally considered as the industry standard. The reason for this is that it is in the mid range of plasticiser properties. It is the phthalate ester of the alcohol 2-ethyl hexanol, which is normally manufactured by the dimerisation of butyraldehyde, the butyraldehyde itself being synthesised from propylene. The widespread sales of DEHP plasticiser are a reflection of its all around plasticising performance and its provision of adequate properties for a great many cost-effective, general purpose products. It possesses *reasonable* plasticising efficiency, fusion rate and viscosity (of great importance for plastisol applications). Some concerns have been periodically raised as to the toxicity of this material, but it can be said that these concerns are often related to the vast number of studies which have been carried out, some of which are of dubious quality (ECPI, 2002). DEHP, which is used extensively to soften PVC products, including many life-saving medical devices, PVC flooring, wire and cable sheathing and car interiors, has been recognised as being non-carcinogenic to humans by most international authorities, including the European Commission, for several years. IARC however, had previously classified it as “possibly carcinogenic to humans” based on some early research studies carried out on rodents (IARC, 2000).

1.2.4.2 Exposure of Humans to Phthalates

It has been suggested that the spreading of municipal sewage sludge on agricultural land could lead to phthalates being taken up by plants and entering the human food chain. However this subject has been comprehensively studied over the years and there is no evidence to suggest that phthalates can enter the human food chain in this way. There are two points to bear in mind: the rates at which phthalates biodegrade in sewage sludge and soils; and the uptake by plants from the ground of phthalates such as di-2-ethylhexyl phthalate (DEHP). Phthalates are subject to both aerobic and anaerobic (in the presence, or not, of oxygen respectively) biodegradation. There are several investigations, which demonstrate that when they are contained in sewage sludge they are rapidly degraded. For example, (Adams et al., 1995) one study examined garden soil containing DEHP Within 20 days 75% of phthalate levels had been degraded and after 30 days the figure was more than 90%. A major research study on the uptake by plants of DEHP was conducted in the United States in 1989 (Aranda et al., 1989). Among the plants grown and tested were four food chain crops: lettuce, carrots, chilli peppers and tall fescue (a pasture grass). The authors concluded that "...because intact DEHP was not detected in any plants, DEHP uptake by plants was of minor importance and would not limit sludge additions to soils used to grow these crops". Eating some foods packaged in plastics, especially fatty foods like milk products, fish and seafood, oils, but levels still usually quite low (ECPI, 2001). A number of packaging materials and tubings used in the production of foods and beverages are polyvinyl chloride contaminated with phthalate esters, primarily DEHP. These esters migrate from the packaging to the food or beverages. The extent of migration depends upon a number of factors such as temperature, surface area contact, lipoidal nature of food, and length of contact (Peakall, 1975).

Before we consider oral exposure, one other route must be mentioned: that arising from the use of phthalates for medical equipment, which may lead to intravenous exposure following, for instance, the storage of blood in PVC blood bags. The migration of DEHP into blood stored in this way was reported by Jaeger Rubin (1972), but migration of DEHP from medical devices had been discussed as early as 1960 (cited in Jaeger and Ruben 1972). Other medical equipment found to leach phthalates directly into humans include dentures. Lygre et al., (1993) found some phthalate ester in saliva samples of patients who had recently received new dentures.

No information is available on the chronic effects of dimethyl phthalate in humans. Animal studies have reported slight effects on growth and on the kidney from chronic oral exposure to dimethyl phthalate. EPA has not established a Reference Concentration (RfC) or a Reference Dose (RfD) for dimethyl phthalate (U.S. Environmental Protection Agency, 1999). Over the past 40 years the effect of phthalates in the environment has been extensively studied. Research demonstrates that phthalates, at current and foreseeable exposure levels, do not pose a risk to human health or to the environment (ECPI, 2001).

1.2.4.3 Reproductive effects of Phthalates

Some phthalates can interfere with normal sexual development in rodents, when given in high doses, by processes that do not appear to involve receptor interference. Sexual development in rats happens rapidly and shortly before birth. Some tests showing reproductive effects involved administering very high doses of some phthalates to pregnant rats shortly before they were to give birth. The research indicated that levels of testosterone, a male hormone key to sexual development, were significantly suppressed in the male fetuses when the mother rats were fed very high doses of some phthalates. No effects were seen at lower doses. In other words, heavy doses of the phthalate suppressed production of the male sex hormone, interrupting the maturation process. The doses that caused these effects were above those any human being would be exposed to under any realistic scenario. The Phthalate Esters Panel and the chemical industry continue to devote significant financial and human resources to further research the issue of endocrine disruption (Phthalates Information Center, 2002).

1.2.4.3 Phthalates Fate in the Soil

Phthalates have also been reported in samples taken from various compartments of the terrestrial environment, including soils, landfill sites and plants. One route of entry of phthalates into soil systems is via fallout from the atmosphere, as discussed by Thuren and Larsson (1990). Their research led to an estimation of fallout of 285 $\mu g\ m^{-2}$ DEHP per annum to Swedish soils. Another potential route of entry is via the application of sewage sludge to soil. Large volumes of sludge are disposed on to agricultural land, providing beneficial soil fertilising and conditioning properties. It is therefore of significant interest to determine whether potential toxins in the sludge might find their way into crop plants grown on this land. (Metzler, 2001).

1.2.4.4 Degradation of Phthalates in Soil

Despite the constant input and widespread distribution of phthalates into environmental systems, the rapid degradation of these chemicals is of paramount importance in preventing, in most instances, their accumulation. As a rule, biodegradation pathways are dominant in soils (Staples et al., 1997). The degradation rates of phthalates are dependent on their molecular weight; those with longer alkyl side-chains tend to have longer half-lives in a given environment. This phenomenon was observed by Shelton et al., (1984), who found DEHP to remain intact in anaerobic digester sludge, whereas the lower molecular weight DMP and DEP were completely degraded. A similar picture was reported by Ejlertsson et al., (1996); (1997), who found that more soluble phthalates were degraded in aerobic conditions, whilst less soluble esters were not. Degradation in soils of DEHP (Roslev et al. 1998) has also been reported . Roslev et al. (1998) investigated degradation of DEHP in sludge amended soil and found the rate to be dependent on the bioavailability of the chemical. Thus, although initially the half life of DEHP was 58 days, it was calculated that 40% would remain after a one year incubation, due to lack of bio availability. As might be expected, degradation in these laboratory conditions was slower at 4 ^{0}C than at 20 ^{0}C, implying that phthalate degradation in the environment will be slower in winter than in summer (Metzler, 2001).

Chapter 2

2.1 REVIEW OF ANALYTICAL METHODS

Several methods have been reported in literature for the analysis of heavy metals and phthalate esters. These methods are highly sensitive and provides excellent detection limits for measuring concentrations of heavy metals and phthalate esters from soil samples.

2.1.1 Heavy Metals

2.1.1.1 Atomic Absorption Spectroscopy (AAS)

A simple, reliable and relatively fast method has been developed to selectively separate and concentrate trace amounts of lead from soil samples for measurement by flame atomic absorption spectrometry. By the passage of aqueous soil samples through an octadecyl-bonded silica membrane disk modified by a recently synthesized bis(anthraquinone)sulfide, Pb^{2+} ions adsorb quantitatively and almost all matrix elements will pass through the disk to drain. The retained lead ions are then stripped from the disk by minimal amount of acetic acid as eluent. The proposed method permitted large enrichment factors of about 300 and higher. The limit of detection of the proposed method is 50 ng Pb^{2+} per 1000 ml. The effects of various cationic interferences on the recovery of lead in binary mixtures were studied. (Shamsipur et al., 2000). Several studies on the influence of heavy metals to the growth of vegetables have been carried out in Cuba by the Ministry of Agriculture in order to evaluate the effects resulting on the continuous application of fertilizers and other materials to the soils. The analysis of metal contents in soil and vegetable samples is often troublesome due to the low concentration levels to be determined. In the present work EDXRF, AAS and ASV methods were applied and compared for the evaluation of Cd and Pb contents in red ferralitic soil and sorghum samples. Several certified reference materials (CRM) (inorganic and organic matrixes) were analyzed in order to evaluate the performance of the analytical procedures and the bias and precision of the results. A study was performed with growing sorghum in several series of pots where different quantities of metals were added to the soil substrate. The observed correlation between the metal contents in soil and plants as well as the influence of different additions of each metal on the plant growth is also presented (Estévez Alvarez et al., 2001). Soil profiles were sampled at an altitude of about 2400 m, representing soils developed above different bedrocks. The concentration of lead in the profiles was found to be strongly dependent on the metal content in the bedrock underlying the soil and was strongly enriched in the top 10 cm. The dolomitic bedrock in the study area contains elevated lead concentrations compared with other dolomites. Dissolution of dolomite and accumulation of weathering residues during soil formation resulted in high lead concentrations throughout the soil profile. The enrichment of lead in the topsoil, however, is largely attributed to atmospheric input. The isotopic signature of the lead clearly indicates that it is mainly of natural origin and that of atmospheric deposition of anthropogenic lead contributed to about 20 to 40% of lead concentration in the topsoil on the topsoil on the bedrock with elevated lead concentrations. In the soils on bedrock with normal lead concentrations, the anthropogenic contribution is estimated to be about 75%. The HNO_3-extractable lead concentration, was determined by digesting 5g sub-samples in 50ml of 2M HNO_3 for 2hrs at 100 ^{0}C, following the procedure used by the Swiss Soil Monitoring Network (Federal Office of Environment, Forests and Landscape, 1987). The extracts were analysed by flame atomic absorption spectroscopy (AAS). Soil samples from a round robin test (Swiss Federal Research Station for Agro-ecology and Agriculture, 1989a) were included and an agreement with the certified values for lead within 15% was found. The standard deviation for three samples with 7 to 13 measurements was between 7 to 10% (Nowack et al., 2001).

2.1.1.2 Graphite Furnace Atomic Absorption Spectrometry (GF-AAS)

The lead concentrations of fruit samples were determined and the soil samples related to these fruits were also analysed for Pb after extraction with various chemical reagents. The relation between the fruit-lead and soil-extractable lead concentrations was examined in order to explain the bioavailability of lead. A linear relation was observed between the hot Na_2EDTA extraction-soluble Pb contents in the soil and the Pb concentrations in the mulberry ($R2$=0.95), strawberry and apple grown on these soils. Probable chemical forms of lead in soil were evaluated. Acceptable agreement (at least 91%) was achieved from the results with GF-AAS (Yamana et al., 2000). It was shown that the direct solid sampling graphite furnace atomic absorption spectrometry (SS-GFAAS), equipped with a transversely heated graphite atomiser and efficient deuterium background correction can be a powerful alternative for the trace analysis of heavy metals in different materials. The direct solid sampling technique has been successfully applied to the analysis of cements (Cd and Pb), calcium fluoride single crystals (Cd and Pb), barytes (Cd and Pb) and deposits of the surface of historical buildings (Pb) using the platform technique in a very wide concentration range (upper - ppm to sub - ppb range). The analytical results from the SS-GFAAS were compared with those obtained by Zeeman - GFAAS or FAAS after microwave aqua regia extraction and/or aqua regia - hydrofluoric acid digestion. In many cases good agreement was achieved with values obtained by reference methods. When using test sample masses between 0.029 to 0.756 mg an overall precision of about 10 % RSD was obtained after standard calibration using different masses of certified reference materials (CRMs) such as sediments and soils. Difficulties, which can arise in special cases - like calibration problems, relatively bad precision, poor agreement with reference method - are shown and discussed (Nowka et al., 2000).

2.1.1.3 Electrothermal Atomic Absorption Spectroscopy (ET-AAS)

A method was developed for the simultaneous bi-element determination of Cd and Pb in aqua regia digests of soils and sediments using an electrothermal atomic absorption spectrometer with a transversely heated graphite atomizer and longitudinal Zeeman-effect background correction. A fast furnace program with no pyrolysis stage or chemical modifier was used, and the total duration of the time-temperature program of the graphite furnace was 45 s. The method detection limits calculated from blank samples were 0.18 μg l−1 and 2.5 μg l−1 for Cd and Pb, respectively. The accuracy of the method was confirmed with a certified reference soil (recoveries 90–110%, within-batch RSD most often less than 5% with six replicates) and with 12 sediment samples from an international proficiency test. z-scores were calculated for the results of the sediment samples with 46 satisfactory and only 2 questionable results. Some analytical problems were encountered during analysis. Fast furnace programs were found to be suitable for the simultaneous determination of the two elements in aqua regia digests of soils and sediments (Myöhänen et al., 2002).

2.1.1.4 Laser-induced breakdown spectrometry (LIBS) combined with Laser-induced fluorescence (LIF)

The investigation of a hyphenated technique combining laser-induced breakdown spectrometry (LIBS) with laser-induced fluorescence (LIF) for the analysis of Pb and Cd in soils is described. In order to evaluate the applicability of the technique for fast in-situ analytical purposes, measurements were performed at atmospheric pressure. The plasma radiation was detected using a Paschen-Runge spectrometer equipped with photo multipliers for the simultaneous analysis of 22 different elements. The photo multiplier signals were processed by a fast gate able multichannel integrator. Calibration curves were recorded using a set of spiked soil samples. Limits of detection were derived from these curves for Cd (6 µg/g) and Pb (17 µg/g) using the LIBS signals. LIBS-LIF measurements were performed for Cd. The excitation wavelength as well as the detected fluorescence wavelength for Cd was 228.8 nm. The calibration curves based on the LIF signals showed significantly improved limits of detection of 0.3 µg/g for Cd (Hilbk-Kortenbruck et al., 2001).

2.1.1.5 Laser induced Spectroscopy (LIS)

Analysis of heavy metals in soils, sand, and sewage sludge samples has been studied by means of time-resolved optical emission spectrometry from laser-induced plasma. The instrumental setup has been developed as a fact screening detector for future application as an on-line and in situ method. Several experimental parameters had to be optimized. Various factors affecting the detection limits and the quality of the analysis have been investigated. These include aerosol production, crater formation, size effects, timing effects, laser intensities, and humidity. In order to improve reproducibility of elemental analysis, a special data analysis program has been developed. It consists of a data analysis program and of the principal component regression calibration technique, which utilizes many spectral lines of each element. A special renormalization algorithm has been tested for internal calibration. The computer program provides good calibration plots and detection limits in the 10 µg/g range, which are usually below the ecological requirements (Wisbrun et al., 1994).

2.1.1.6 Laser Excited Atomic Fluorescence Spectrometry (LEAFS)

This method focuses on the development of new instruments, and new applications of laser excited atomic fluorescence spectrometry, LEAFS, in recent years. Such developments include solid-state tunable lasers, deep UV tunable lasers, the use of charge coupled detectors (CCDs), and the applications of LEAFS for trace metal determination in various samples. The advent of diode lasers with their now somewhat improved range of wavelengths and power output, provides opportunities for research and applications in LEAFS. The further development of the coupling of second and third harmonic crystals to pulsed diode lasers shows promise for compact and robust instrumentation. There have been no recent instrumental developments that might provide more isotopic selectivity beyond the elements like uranium where the spectral isotope splitting is greater than most elements, but laser diodes could provide this due to their potential to provide an output with very narrow spectral bandwidth. The advent of optical parametric oscillator-based lasers has enabled LEAFS to be much more practical then in the past when dye lasers were used. This should be the harbinger of more applications of LEAFS to complex real sample analyses that cannot be done by other techniques for reasons of sensitivity or selectivity. Array detectors provide an additional degree of freedom by provision of more spectral information more rapidly, which should aid the study of complex samples that might produce complex background problems. The recent literature indicates that the sensitivity, selectivity and ease of method development of LEAFS is well-established, and that there are no substantial analytical disadvantages to the technique beyond the instrumental limitations associated with the single element at a time mode of operation and the complexity of the laser systems. Laser technology continues to develop rapidly, which heralds a bright future for LEAFS (Stchur et al., 2001).

2.1.1.7 X-Ray Fluorescence Spectroscopy (XRFS)

Using energy dispersive X-ray fluorescence analysis with an X-ray tube filtered with Ti, it was possible to determine the concentration at ppm level of Pb in soil treated with organic compounds of urban garbage. It was possible to observe a significant increase in the contents of Pb in the soil treated in comparison with the soil control (dos Anjos et al. 2000). Three representative soils were selected and spiked with toxic elements, namely Pb and Cd. These elements were added as nitrate salts depending on the physical properties of the soil. The main constituents of soil, the elements originally present and spiked toxic elements were determined by X-ray fluorescence spectrometry. The instrument was PHILIPS PW 2404 X-ray fluorescence spectrometer. It was established that toxic elements accumulated in the finest particle size fraction of the soil irrespective of the type of the soil, in which they are adsorbed primarily by clay minerals and soil organic matter. If particle size distribution is taken into account, it can be concluded that the amount of trace elements and toxic elements are determined by particle size distribution. That is the maximum of the particle size distribution corresponds to that of the concentration distribution of toxic elements. Another point is that pollution can also modify the original distribution of trace elements, which in turn affects plant uptake (Hartyáni et al., 2001).

2.1.1.8 Square Wave Anodic Stripping Voltammetry (SWASV)

Mercury-electroplated-iridium microelectrode array based sensors were successfully used for the simultaneous determination of Cd and Pb trace concentrations by means of square wave anodic stripping voltammetry (SWASV). These recently developed sensors consist of an array of (42×42) 6-μm-diameter iridium micro discs onto which Hg micro drops are electroplated. The Ir micro disc arrays were achieved by using micro fabrication processing techniques and Hg charge of 15mC was identified to be the optimum amount to be electroplated onto the 6-μm-diameter micro disc array. The microelectrode array based sensors were used to analyse both water certified samples covering a wide range of metal concentrations (from 1 to ~1000ppb) and soil extracts collected at different soil depths (from the surface to about 1m depth in the Rouyn-Noranda region, Que., Canada). Different physico-chemical forms of the three metals were extracted from the soil samples using barium chloride, sodium pyrophosphate and oxalic acid/ammonium oxalate solvents. On the other hand, all the Cd forms extracted from the soil are found to be ASV-electro active since a satisfactory agreement was obtained between the concentrations measured by SWASV and those determined by means of graphite furnace atomic absorption spectrometry (GF-AAS). While Pb concentration profiles in soil were found to be particularly dependent on the extractant used, Cd concentration profile exhibited the same trend for the three extractants. The differences in metal concentrations between extractants are discussed and the predominant physico-chemical metal forms are established as a function of the horizon in the soil profile. Finally, the developed SWASV sensors are shown to be successful to measure reliably metal concentrations as low as 50ppt in soil extracts with a preconcentration time of only 10min **(Silva et al., 2001).**

2.1.1.9 Anodic Stripping Voltammetry (ASV)

Binding of Cd(II) and Pb(II) to two humic acids was studied by differential pulse anodic stripping voltammetry using continuous differential equilibrium functions and linear Scatchard plots. One of the humic acids was a commercial sample from Aldrich (AHA) and the other was a sedimentary humic acid isolated from a Brazilian water reservoir (BBHA). The complexation study was performed at pH between 5 and 6 in 0.020 mol l−1 KNO_3. The complexes were considered fully labile in the range of the metal to ligand concentration ratios studied, having diffusion coefficients (DML=1.3×10−7 and 2.7×10−7 cm2 s−1, for BBHA and AHA, respectively) smaller than the free cation [DM=8×10−6 and 7×10−6 cm2 s−1 for Cd(II) and Pb(II), respectively]. Differential equilibrium functions and the discrete site model led to similar interpretations of the experimental data. Binding of Pb(II) to both HA samples was stronger than for Cd(II), with a greater heterogeneity of binding sites. This was verified by the heterogeneity parameter, Γ, obtained from the differential equilibrium functions, as well as from the number of classes of binding sites used to fit the titration data to Scatchard plots (Abate et al. 2002). The interaction of fulvic acids with Cd(II) ions has been studied by differential pulse anodic stripping voltammetry (ASV), at pH 7.0 and 0.1M KNO3. Voltammetric data obtained during the titration of mixtures of Cd(II) and fulvic acids at different concentrations have been analysed using multivariate curve resolution (MCR). The application of this method allowed the resolution of the major contributions involved in the titration experiments. Apart from free Cd(II), two more contributions related with the complexation process were detected and resolved, and their corresponding pure voltammograms and concentration profiles were estimated. Simultaneous analysis of independent voltammetric titrations using the proposed MCR methods is shown extremely recommended because it overcame some of the limitations observed in the analysis of individual titrations (Antunes et al., 2002).

2.1.1.10 Mass Spectroscopy (MS)

Mass spectrometric methods for the trace analysis of inorganic materials with their ability to provide a very sensitive multielemental analysis have been established for the determination of trace in high-purity materials (metals), in different technical samples e.g. pure chemicals in soils samples. Whereas such techniques as spark source mass spectrometry (SSMS), laser ionization mass spectrometry (LIMS), laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS), glow discharge mass spectrometry (GDMS), secondary ion mass spectrometry (SIMS) and inductively coupled plasma mass spectrometry (ICP-MS) have multi-elemental capability, other methods such as thermal ionization mass spectrometry (TIMS), accelerator mass spectrometry (AMS) and resonance ionization mass spectrometry (RIMS) have been used for sensitive mono- or oligoelemental ultratrace analysis (and precise determination of isotopic ratios) in solid samples. The limits of detection for chemical elements using these mass spectrometric techniques are in the low ngg−1 concentration range. The quantification of the analytical results of mass spectrometric methods is sometimes difficult due to a lack of matrix-fitted multielement standard reference materials (SRMs) for many soil samples. Therefore, owing to the simple quantification procedure of the aqueous solution, inductively coupled plasma mass spectrometry (ICP-MS) is being increasingly used for the characterization of solid samples after sample dissolution. ICP-MS is often combined with special sample introduction equipment (e.g. flow injection, hydride generation, high performance liquid chromatography (HPLC) or electrothermal vaporization) or an off-line matrix separation and enrichment of trace impurities (especially for characterization of high-purity materials and environmental samples) is used in order to improve the detection limits of trace elements. Furthermore, the determination of chemical elements in the trace concentration range is often difficult and can be disturbed through mass interferences of analyte ions by molecular ions at the same nominal mass. By applying double-focusing sector field mass spectrometry at the required mass resolution—by the mass spectrometric separation of molecular ions from the analyte ions—it is often possible to overcome these interference problems. Commercial instrumental equipment, the capability (detection limits, accuracy, precision) and the analytical application fields of mass spectrometric methods for the determination of trace and ultratrace elements and for surface analysis are discussed (Becker and Dietze, 1998). Attic dust and soil samples were collected from three communities in southern Nevada and Utah. The samples were analyzed for two heavy metals (Pb and Cd) by ICP-MS. Both elements were traditionally found enriched in road and house dust in urban areas and were even more highly enriched in attic dust. Lead stood out as having the highest enrichment factors (relative to both the local soil and its natural abundance) and was highly correlated with the age of the house (R2=0.87, n=8). The data suggest that undisturbed attics may act as archives of atmospheric dust, by preferentially trapping and preserving airborne particulate matter, and should be considered by researchers interested in study of past atmospheric dust and/or reconstructing exposure histories (Cizdziel and Hodge, 2000).

2.1.1.11 Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)

The determination of the total content and the leachable aliquot by aqua regia dissolution of two heavy metals (Cd and Pb) in soils was developed by microwave digestion technique combined with inductively coupled plasma atomic emission spectrometry. A complete digestion of soils was achieved by using an acid mixture of HF–HCl–HNO_3 (1:3:1); the microwave-irradiated closed vessel system used for the determination of aqua regia leachable quota, proved to be a viable alternative to the traditional reflux system. The experimental study was conducted using five BCR standard reference materials (CRM 141R 'Calcareous Loam Soil', CRM 142 'Light Sandy Soil' and CRM 143 'Sewage Sludge Amended Soil' in two laboratories with different microwave ovens and ICP-OES. Calculated recovery, repeatability, and reproducibility confirm the good performance of the procedures adopted. A Nested Design statistical analysis was carried out for both procedures and showed that the major source of variability in the analysis was due to ICP-OES measurements rather than microwave-assisted dissolution (Bettinelli et al., 2000).

2.1.2 Phthalate Esters

2.1.2.1 Gas Chromatotraphy (GC)

The phthalate ester DEHP concentrations were determined from soil sample. DEHP was extracted with organic solvent methylene chloride using gas chromatography (GC) and electron capture detector (ECD) for analysis. The clean up was done using silica gel column. The sample detection limt was 1.3 mg/kg and percent recovery was greater than 0 to 158 (http://www.atsdr.cdc.gov).

2.1.2.2 High Resolution Gas Chromatography and Mass Spectrometry (HRGC/MS)

A method was developed for coupling of an accelerated solvent extraction system (ASE) with high resolution gas chromatography (HRGC) and mass spectroscopy (MS) for the analysis of phthalate esters (PAEs) in soil samples. Organic solvent used for extraction of phthalate esters was methylene chloride and clean up was done using silica gel column. The sample detection limit was 660 µg/kg and percent recovery was from 8 to 158 (http://www.atsdr.cdc.gov).

2.2 METHODS USED IN THIS STUDY

In this study, heavy metals (Cd and Pb) were analysed using flame atomic absorption spectroscopy coupled with ambic-2 extraction method. Ambic-2 extraction method was chosen as an analytical method for determination of the extractable concentration amount of the element from soil in Alice at Phandulwazi Agricultural High School. The methods were chosen because of their high selectivity, sensitivity and reproducibility especially in the analysis of Cd, Pb and other heavy metals in soils.

Gas chromatography (GC) was also used to analyses specific phthalate esters namely DMP, DEP and DEHP. GC was used because is one of the most important tool in chemistry and also because of its simplicity, sensitivity and effectiveness in separating components of mixtures. It is widely used for quantitative and qualitative analysis of organic mixtures. The analytical extraction method used is soxlet extraction method using dichloromethane as an extracting solvent for the duration of 10 hours. The detector used was flame ionisation detector (FID).

Chapter 3

3. EXPERIMENTAL

3.1 Sampling

3.1.1 Sampling Point and Sample Collection

The sampling point for soil samples was from Phandulwazi Agricultural High School at Alice in the Eastern Cape Province of South Africa. The soil is used for cultivation of crops. Only 4 kg of soil was collected for this study. A clean spade was used to excavate a profile pit. Since this study is related to heavy metal analysis it was more favorable the soil core be taken with a tube inside the core. The sample was collected into thoroughly cleaned glass containers. The glass containers were filled so that the remained air space was as small as possible.

3.1.2 Sample Preparation for Analysis of Heavy Metals and Phthalate Esters

The soil sample was air-dried. After air-drying it was oven dried at 80 ^{0}C for 24 hrs. The dried sample was crushed with a mortar and pestle then sieved through 80mesh sieve. Appropriate amount of soil sample was treated with Ambic-2 extraction method for heavy metal analysis and with soxlet extraction method for phthalate esters analysis.

3.2 Compost

Compost was prepared with the help of Agronomy Department, Faculty of Agriculture and Environmental Sciences, University of Fort Hare.

3.2.1 Preparation of Compost

Large amounts of selected organic wastes materials such as poultry manure, sawdust/ wood shavings, tobacco wastes (tobacco sticks and dust), cabbage waste, pig dung and cattle manure (cow dung) were collected from various sources in the Border Region of the Eastern Cape. Composting was carried out in eighteen cylindrical shaped compost bins. The bins were made from wire mesh with wooden plank frames. The height and diameter of a bin were 1m and 0.5m respectively. There were six treatments, viz: pig manure, poultry manure, cabbage waste, cattle manure, poultry manure + rock phosphate and cattle manure + rock phosphate and were replicated three times. The treatments were sawdust/ wood shavings and tobacco wastes-based, forming the major components of the compost mixture due to availability and accessibility with little or no cost. These basic components consisted of 20% sawdust and 40% tobacco waste by weight of the mixture. The sawdust and wood shavings were mixed together in ratio 1:1 while, treatments consisting either pig manure or cattle manure had sawdust and wood shavings mixed at ratio 2:1. The poultry manure, pig manure, cabbage waste, and cattle manure were added, respectively as 40% of the total weight of the mixture. On the other hand, rock phosphate was added to each of the treatments with pig and poultry manures, respectively at 10% by weight of the mixture. The materials were allowed to compost for eighty seven days.

Table 3.1 Materials used to make the compost

Compost	Composition
Compost 1	Sd + Tw + Pm + Rp
Compost 2	Sd + Tw + Pd
Compost 3	Sd + Tw + Cd
Compost 4	Sd + Tw + Pm
Compost 5	Sd + Tw +Cd +Rp
Compost 6	Sd + Tw + Cb

Sd = Sawdust; Pm = Poultry manure; Pd = Pig dung; Cb = Cabbage waste; Rp = Rock phosphate; Cd = Cow dung; Tw = Tobacco waste

3.3 Spiking of Pre extracted Soil Sample

The soil sample for heavy metal analysis was spiked with 100ppm of Cd and Pb, after background analysis of these metals was done. For Phthalate Esters pre-extracted soil was spiked with 20ppm of phthalate esters (DMP, DEP and DEHP).

3.4 Treatment of Spiked Soil Sample with Compost

3.4.1 Heavy Metals

In this study, heavy metal (Cd and Pb) spiked soil sample was treated with six (Table 3.1, C1-C6) types of composts at three different rates. Rates used per compost were 10t/ha, 20t/ha and 40t/ha in each treatment. Heavy metals from the compost treated soil samples were analysed at 0th, 7th, 14th, 21st, 28th days of incubation at 30 ^{0}C.

3.4.2 Phthalate Esters

In this study, phthalate esters (DMP, DEP and DEHP) spiked soil sample was treated with three (Table 3.1; C1, C2 and C4) types of composts at three different rates. Rates used per compost were 10t/ha, 20t/ha and 40t/ha in each treatment. Phthalate esters from the compost treated soil samples were analysed at 0th, 7th, 14th, 21st, 28th days of incubation at 30 ^{0}C.

3.5 ANALYSIS OF HEAVY METALS

3.5.1 Stocks Preparation (1000 ppm)

3.5.1.1 Cadmium

2.4 g cadmium chloride, 50 ml hydrochloric acid was dissolved in 1000ml volumetric flask and filled with distilled water up to the mark.

3.5.1.2 Lead

1.6 g lead nitrate, 50 ml nitric acid were dissolved in 1000 ml volumetric flask and filled with distilled water up to the mark.

3.5.2 Ambic-2 Extraction Method

The Ambic methods are modification of the Hunter (1974) or ISFEI method introduced by Van der Merwe, Johnson and Ras (1984). The main difference between Ambic-1 and Ambic-2 is that extraction in the case of ambic-2 is based on the volume of the soil; no acid is used to clarify colored solutions; di-sodium EDTA is used instead of di-ammonium EDTA; and stirring is applied instead of reciprocal shaking. This element is suitable for the determination of micro-elements in a wide range of soils.

3.5.2.1 Ambic-2 extraction solution

0.25 mol dm^{-3} NH_4HCO_3 + 0.01 mol dm^{-3} Na_2 EDTA + 0.01 mol dm^{-3} NH_4F + Superfloc: Superfloc solution was prepared by slowly adding 5 g Superfloc N-100 to 1 dm^3 lukewarm de-ionized water while being stirred at 400 rpm. The final solution should be viscous and gel-like. 197.6 g ammonium bicarbonate, 37.2 g di-sodium EDTA and 3.7 g ammonium fluoride were dissolved in 5 dm^3 de-ionized water. 100 ml Superfloc was added, mixed well and made up to 10 dm^3. After being allowed to stand overnight, the pH was adjusted to 8.0 with concentrated ammonia solution.

3.5.2.2 Extraction

2.5 g of soil was scooped into a sample cup. 25cm^3 extraction solution was added to the soil. The mixture was stirred for 10 minutes at 400 rpm. The extract was filtered into a clean 50 ml volumetric flask using Whatman no.1 filter paper, and then analysed with Atomic Absorption Spectroscopy (AAS).

Unicam 969 (Solar System) Atomic Absorption Spectrometers (AAS) was used throughout this investigation for heavy metal analysis.

3.5.3 pH and Electron Conductivity Determination

Soil pH or soil reaction is an indication of the acidity or alkalinity of soil and is measured in pH units. Soil pH is defined as the negative logarithm of the hydrogen ion concentration. Soil electrical conductivity is a measure of ease of electron flow in the soil. The pH and EC ground and sieved soil samples were measured in a 1:10 water extract. The pH and EC were determined by adding 10ml of distilled water into 1g of dried soil sample. This procedure was repeated before and after the soil was treated with compost.

Electron conductivity (EC) and pH were measured with 660 Conductometer and 691 pH meter respectively both manufactured by Metrohm Swiss made.

Table 3.2 List of reagents (salts and solvents) and Grade/Suppliers

Reagent	**Grade/ Supplier**
NH_4HCO_3	AR/ Merck
Na_2EDTA	Associated Chemicals Enterprise (PTY) LTD.
NH_4F	AR/ Merck
HNO_3	NT Laboratories
Superfloc N-100	AR/ Merck
$Pb(NO_3)_2$	AR/ Merck
$CdCl_2.H_2O$	AR/ Merck

3.6 ANALYSIS OF PHTHALATE ESTERS

3.6.1 Preparation of standards:

A stock solution (1000mg/l) for each ester (DEP, DMP and DEHP) and an internal standard, butyl benzoate (BUBE) was prepared in 50ml volumetric flask. The required volume of each ester was calculated from the density of each ester. BUBE was used as an internal standard. The calculated volumes required to prepare the required concentration of each ester (50ml, 1000mg/l) are tabulated below:

Table 3.4 Preparation of a 1000mg/L stock solution

PHTHALATE ESTER	DENSITY	VOLUME
DMP	1.19	42 µl
DEP	1.12	44.64 µl
DEHP	0.98	51 µl
BUBE	1.01	49.5 µl

The calculated volumes were measured out using an automated micropipette and the volumetric filled up to the mark with methanol. The above volumes were pipetted into 50ml volumetric flask and filled to the mark with methanol.

A solution of each pure compound in methanol (1 µl) was injected into the GC to determine the compound's retention time. Three replicate injections were made for each ester.

3.7 Method Development for Phthalate Esters Analysis

3.7.1 Optimizing GC Conditions:

A set of gas chromatographic (GC) parameters was determined using PE-5 column (30m, 0.32mm I.D., 0.25µm film thickness). The first step was to choose a set of tentative parameters based on previous work done using other columns, size of the esters, boiling points and polarity of the esters. These parameters were used to find the elution times of the phthalate esters. Carrier gas flow rate, temperature program and detector temperature are parameters used keeping other constant to ascertain the effect of each on the elution times and resolution of peaks. By trial and error means a set parameters was developed and used.

The GC operating conditions were as follows:

Injector Temperature: 250^{0}C Detector Temperature: 300^{0}C

Oven Ramped Temperature:150^{0}C/0min., to 200^{0}C/0min. Rate: 5^{0}C/min.250^{0}C/0min.

Split Ratio: 50:1 Flow Rate: 10ml/min.

Phthalate Esters used are:

- Dimethyl phthalate (DMP)
- Diethyl phthalate (DEP)
- Di- (2-ethylhexyl) phthalate (DEHP)

All pure ester solutions were obtained from Merck-Schuchardt analytical grade and have assay greater than 98%. DMP, DEP and DEHP densities were 1.19, 1.12 and 0.98 respectively.

Internal Standard used:

- Butyl Benzoate (BUBE)
- The internal used is also having an assay greater than 98% obtained from Merck-Schuchardt analytical grade.

Solvent used:

- Methanol

Methanol, which had 99.5% minimum assay, was distilled to remove possible impurities.

Table 3.3 List of reagents (phthalates standards and solvents) used:

REAGENTS	DENSITY	GRADE	SUPPLIER
DMP	1.19	Analytical	Merck-Schuchardt
DEP	1.12	Analytical	Merck-Schuchardt
DEHP	0.98	Analytical	Merck-Schuchardt
BUBE	1.01	Analytical	Merck-Schuchardt
Methanol		HPLC	Baxter/ Burdick Jackson
Dichloromethane		HPLC	Baxter/ Burdick Jackson

3.8 Extaction Method used for Phthalate Esters Analysis

3.8.1 Soxhlet Extraction (SE)

10g of the dried, sieved and spiked pre-extracted soil sample was weighed into a pre-extracted Whatman extraction thimble then extracted for 10hrs with 120 ml dichloromethane. The extract was allowed to cool, after which it was filtered and then concentrated at 40 °C to about 2 ml on the vacuum rotary evaporator. The reduced extract was then carried through the column chromatographic clean-up process as described above.

3.8.2 Silica gel column chromatography (Clean up)

The chromatographic column (20 cm×8 mm I.D.) was slurry packed with 5.0 g of activated silica gel which was made into a slurry with about 1.2% (v/m) water-adsorbent using triply distilled water and then stirred well before use. About 0.5 ml of anhydrous sodium sulphate was placed at the top of the column to absorb any water in the sample or the solvent. The column was pre-eluted with 15 ml of petroleum spirit. Phthalates were then eluted with 2×10 ml portions of the extracting solvent. The eluate was collected, dried with anhydrous sodium sulphate and then evaporated to dryness using the Buchi vacuum rotary evaporator. The I.S. (butyl benzoate) was added and the residues were reconstituted with 2 ml of the extracting solvent for GC analysis. Then 1 μl of an extract was injected for analysis in GC.

Perkin-Elmer Autosystem XL Gas Chromatograph was used throughout this investigation for phthalate esters. The GC was fitted with a FID detector. Data acquisition and analysis were performed using Perkin-Elmer Turbochrom 4 Data Acquisition and Analysis software.

Chapter 4

4.1 RESULTS AND DISCUSSION

4.1.1 Heavy Metals

The background concentration of heavy metals was determined from six composts (C1-C6) and soil. Also pH and electrical conductivity (EC) were measured. The results are shown at table 4.1 below.

Table: 4.1 Electrical conductivity (EC), pH and background levels of Pb and Cd of composted manure and soil

Compost	pH	EC	Cd	Pb
		µS cm^{-1}	mg kg^{-1}	
Compost 1	6.92	7060	0.16	9.17
Compost 2	6.96	5210	1.20	6.13
Compost 3	8.00	6520	1.37	4.40
Compost 4	7.76	7620	0.52	5.00
Compost 5	8.48	6870	0.59	4.00
Compost 6	4.85	11950	0.67	4.20
Soil	4.85	208	0.11	7.22

The heavy metal characteristics of the six composts and the sandy loam soil shown in Table 4.1 the composts were acidic (composts 1,2 and 6) to neutral (compost 4) to alkaline (composts 3 and 5) while the soil sample was acidic. EC of water extracts varied greatly amongst composts types (5210-11950 µS cm^{-1}) while the EC for the sandy loam soil was the lowest (208 $\mu S\ cm^{-1}$). The highest values of EC were recorded for compost made mainly of plant waste (compost 6 = sawdust + tobacco waste + cabbage waste). The background levels of cadmium ranged from 0.52-1.37mg/kg in composts and 0.11 mg/kg in soil. Similarly, levels of lead in composts ranged from 4.00-9.1737 mg/kg and 7.22mg/kg in soil. These background levels of the metals in composts and in soil were within acceptable range as seen on (table 1.3).

Table 4.2 shows the pH and EC of the soil samples treated with compost after spiking with 100ppm cadmium and lead for the 21 days remediation monitoring. Generally, the pH and EC did not show any significant changes with the different soil treatments except with treatment S+(C4), 20 t/ha and S+(C6), 10t/ha where the pH dropped to <2. The very high EC values for these samples suggest contamination, the source of which is unknown.

Figures 1-12 showed the amount of metals measured in spiked soil samples after various treatments. The figures gave an indication of the degrees of metal sorption by composts. Generally, all composts showed some metal fixing properties although temporary. Also, generally, the maximum fixing of lead by composts occurs on the 7th day while the maximum fixing of cadmium occurred on the 14th day after which the metals were gradually released into the soil. For all the composts, the highest rate applied, 40t/ha appeared to give the most efficient fixing of the metals from the contaminated soil except for compost C4 where treatment 2 [i.e., Soil+(Compost) 20t/ha] gave the most efficient removal.

Compost C1 had the maximum fixing of lead on the 7th day at 40 t/ha (figure:1c) it appeared that for compost, the maximum fixing of cadmium occurred on the 14th day for all the soil treatment types (figures 2a-2c). For compost C2, the maximum fixing of lead also occurred on the 7th day at 40 t/ha (figure 4c). Compost C3 had the maximum fixing of lead on the 7th day at 40 t/ha (figure 5c) while the maximum and fixing of cadmium occurred on the 14th day at t/ha. This compost appeared to be the most efficient in fixing cadmium (of all the composts investigated) as the fixing of the cadmium was > 80 % (figure 6c). Compost C4 had the maximum fixing of lead on the 7th day at 40 t/ha (figure 7c) while it showed the maximum fixing of cadmium occurred on the 14th day at 20 t/ha (figure 8b). Compost C5 (i.e. mixture of sawdust + tobacco waste + rock phosphate) also appeared to be the most efficient for fixing lead in contaminated soil although on temporary basis. The fixing of lead was good up to the 21st day (figures 9a-9c) although it gave the best fixing (compared to all the compost studied), which was > 90 % on the 7th day at 40 t/ha (figure 9c). The maximum fixing of cadmium for compost C5 also occurred on the 14th day at 40 t/ha (figure 10). For compost C6, it appeared that the maximum fixing were observed on the 7th and 14th days with all the soil treatment types (figure 11a-11c) as no significant difference was observed on the fixing of the metal for two days. The efficiency of compost C6 in fixing lead was next to that of of compost C5. The maximum fixing of cadmium for C6 occurred on the 14th day at 40 t/ha. It appeared that the suspected contamination of C4 at 20 t/ha and C6 at 10 t/ha. The suspected contamination of C4 at 20 t/ha and C6 at 10 t/ha (table 4.2) did not affect their efficiency of fixing metals.

Generally, the trend in the composts efficiencies in fixing metals in contaminated soil were:

C5 > C6 > C4 > C3 > C2 > C1 for Pb and

C3 > C4 > C6 > C5 = C1 =C2 for cadmium

The reason for this trend is difficult to explain at this stage as there are several factors involved in the metal fixing by humus containing substances like compost.

Table 4.4 shows the C/N. Total P and Total K for each of the basic manure. The results show that each of the basic manures is very rich in nutrient. One advantage of using composts to treat soils is that it can improve soil nutrient quality.

4.1.2 Phthalate Esters

The background concentration of phthalate esters (DMP, DEP and DEHP) was determined from three composts (C1, C2 and C4) and soil.. The results are shown at table 4.3 below.

Table: 4.3 Background levels of DMP, DEP and DEHP of composted manure and soil

	Phthalate Esters (mg/kg)		
Compost	**DMP**	**DEP**	**DEHP**
Compost 1	ND	0.0001	0.0003
Compost 2	0.0001	0.0001	0.0002
Compost 4	0.0001	ND	0.0003
Soil	ND	0.0001	0.0004

ND = Not detected

The phthalate esters background concentrations of three composts and the sandy loam soil are shown in table 4.3. DMP is not detected in C1 and in soil but in compost C2 and C4 it of equal concentration amount, which is 0.0001 mg/kg. DEP was detected in composts C1, C2 and in soil showing equal concentration amounts of 0.0001 mg/kg. DEP was not detected in compost C4. DEHP was detected in all composts (C, C2 and C4) and in soil. DEHP background levels range between 0.0002-0.0003 mg/kg and 0.0004 mg/kg in soil.

Table 4.5 shows the pH and EC of the soil samples treated with compost after spiking with 20ppm of DMP, DEP and DEHP for the 28 days remediation monitoring. The observations showed that pH is increasing and EC is decreasing. This is indicating that there are continuous reactions and steady fixing of the phthalate esters.

Figures 13-21 showed the amount of phthalate esters measured in spiked soil samples after various treatments. The figures gave an indication of the degrees of phthalate esters remediation by compost. All compost used showed some phthalates remediation properties. In all the figures and treatments, phthalates concentrations are gradually decreasing day by day of analysis. For all the composts, the highest rate applied, 40 t/ha appeared to give the most efficient fixing of phthalate esters from contaminated soils.

The results show that DMP is degrading in the compost treated soil. All the compost treated soil show that the rate or treatment of the compost matters. This is due to the fact that DMP is highly degraded in all the treatments at treatment 3 which has a rate 40 t/ha and the least treated one is treatment 1 at all the treatments which is the treatment having the lowest rate which is 10 t/ha.

DEP is also showing degradation process in the compost treated soil. DEP same as DMP shows that the rate is playing a vital role in the degradation process. This is also due to the fact that the higher the rate the higher the degradation. It also appears that all DEP treatments are fixed at maximum amount on degradation on 28th day of treatment which is >80% in all the composts. Except in Figure: 4.20a-c, where treatment 1 and 2 have high degradation percent rate than treatment 3, which has the highest rate of compost.

DEHP also show degradation process in all the treatments. The rates don't seem to show any significant difference in the compost treated soil for DEHP degradation. Although the rates don't seem to play any significant role, the degradation does take place. DEHP degradation process is slower compared to those of DMP and DEP. Approximately 20 % of DEHP fixed in all the treatments.

All composts show similar efficiency of fixing phthalate esters.

Table: 4.2 Characteristics of the treated soil after spiking with 1[kjpo00ppm of Cd and Pb

Treatment	Days	pH				EC (µS/cm)			
		0th	7th	14th	21st	0th	7th	14th	21st
S+(C1), 10t/ ha		4.73	5.1	4.54	5.4	310	301	269	247
S+(C1), 20t/ ha		5.05	5.54	5.13	5.63	329	320	213	290
S+(C1), 40t/ ha		5.03	5.85	5.72	6.02	360	338	227	288
S+(C2), 10t/ ha		4.39	5.58	4.74	5.69	256	294	504	264
S+(C2), 20t/ ha		4.91	5.77	5.03	5.76	338	344	223	300
S+(C2), 40t/ ha		5.03	5.79	5.65	6.29	374	335	433	273
S+(C3), 10t/ ha		4.65	5.5	4.9	5.52	343	332	361	260
S+(C3), 20t/ ha		4.66	5.75	5.33	5.88	413	364	387	316
S+(C3), 40t/ha		5	6.09	6.57	6.5	377	394	287	332
S+(C4), 10t/ ha		4.4	5.33	4.88	5.68	265	331	265	252
S+(C4), 20t/ ha		4.54	5.69	1.48	1.89	296	391	2130	1906
S+(C4), 40t/ ha		5.02	6.08	4.26	4.41	331	428	772	852
S+(C5), 10t/ ha		4.29	5.43	5.95	5.44	259	306	180	232
S+(C5), 20t/ ha		4.47	5.5	5.39	5.62	264	324	204	264
S+(C5), 40t/ ha		4.71	6	5.86	5.98	277	376	224	316
S+(C6), 10t/ha		5.01	6.81	1.97	2.47	468	469	946	988
S+(C6), 20t/ha		4.92	5.96	6.22	6.27	330	373	198	296
S+(C6), 40t/ha		4.29	4.98	7	6.72	233	308	206	393

S = Soil Sample, C1-C6 = Different compost samples 1-6 indicating their quantities used for soil treatment for the purpose of the Heavy metals (Cd and Pb) remediation; t = tons; ha = hecta

Table: 4.5 Characteristics of treated soil after spiking with 20ppm of DMP, DEP and DEHP

Treatment	Days	pH					EC (µS/cm)				
		0th	7th	14th	21st	28th	0th	7th	14th	21st	28th
S+(C1), 10t/ ha		5.22	5.97	6.02	6.10	6.13	2743	2480	2160	1980	1660
S+(C1), 20t/ ha		5.45	6.12	6.22	6.49	6.66	3180	2710	2400	2130	1990
S+(C1), 40t/ ha		5.66	6.43	6.49	7.00	7.14	3221	2890	2670	2330	2090
S+(C2), 10t/ ha		5.26	6.50	6.88	7.12	7.19	2890	2140	2000	1870	1580
S+(C2), 20t/ ha		5.44	6.61	6.81	7.29	7.38	2670	2140	1980	1890	1630
S+(C2), 40t/ ha		5.43	6.64	6.94	7.38	751	2710	2290	2100	1990	1770
S+(C4), 10t/ ha		5.21	6.20	6.33	6.57	6.77	2420	2270	2110	1970	1810
S+(C4), 20t/ ha		5.29	6.16	6.36	6.88	7.19	3150	2360	2130	2000	1670
S+(C4), 40t/ ha		5.54	6.50	6.71	6.97	7.26	3340	2880	2220	2090	1790

S = Soil Sample; C1, C2 and C4 = Different Compost samples 1, 2 and 4 indicating their quantities used for soil treatment for the purpose of phthalate esters (DMP, DEP and DEHP) remediation; t = tons; ha = hecta.

Table: 4.4 Nutrient Composition of Organic Materials and Composts used

Organic Material	OC (g/kg)	Total N (g/kg)	C/N	Total P (g/kg)	Total K (g/kg)
Tw	389.0	18.9	21	2.2	33.3
Pd	348.1	21.4	16	9.4	19.7
Pm	331.6	28.5	12	7.8	23.4
Cd	356.2	24.5	15	4.4	20.9
Cb	361.4	23.8	15	4.9	26.3

Tw =Tobacco waste; Pd = Pig dung; Pm = Poultry manure; Cd = Cow dung; Cb = Cabbage dung

(Table 4.4 results were used in this thesis with the courtesy of Prof. Mkeni and Dr. J.A. Adediran from Faculty of Agriculture and Environmental Sciences, Soil Science Dept. U.F.H.).

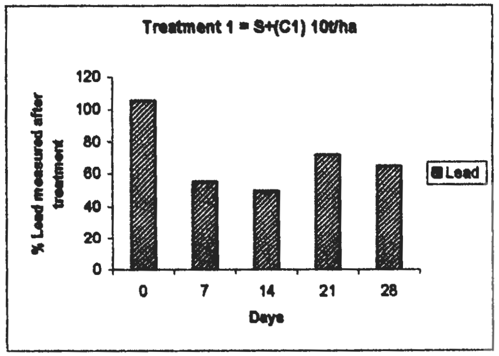


Figure: 4.1a

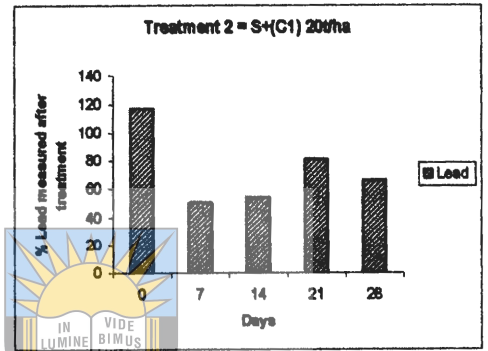


Figure: 4.1b

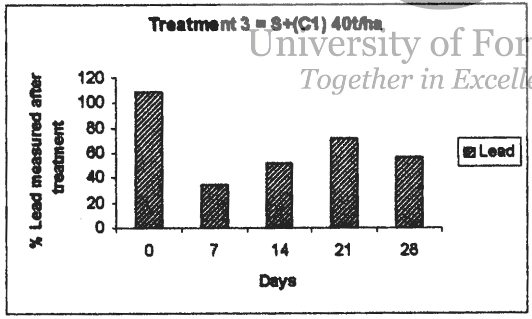
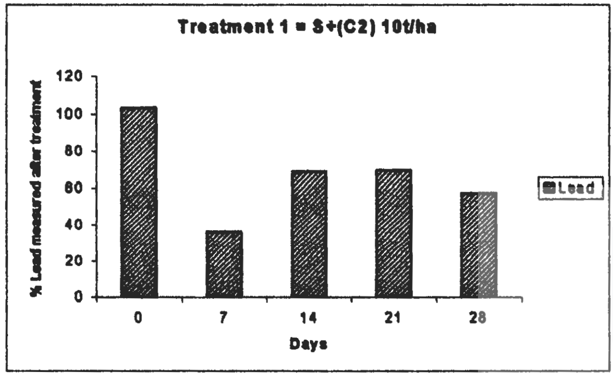


Figure: 4.1c

Figure: 4.1a-c: %Lead measured after treatment with compost C1 at different tons/hecta



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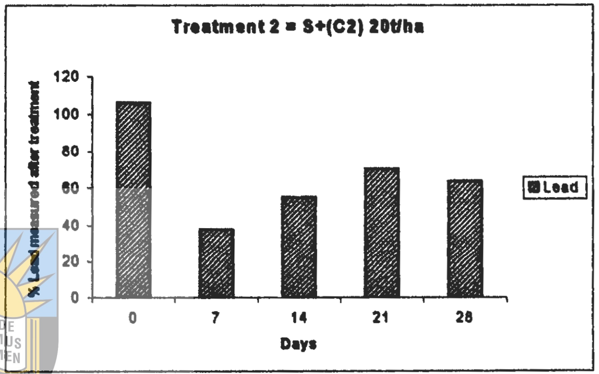


Figure: 4.3b

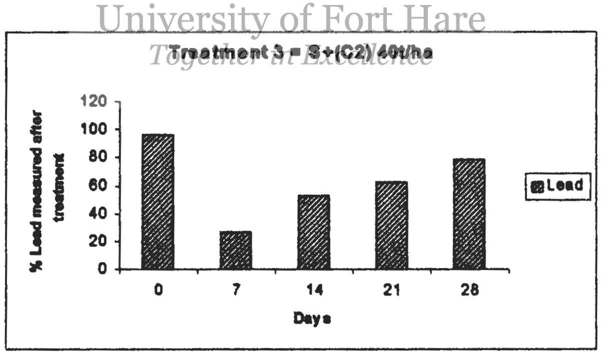
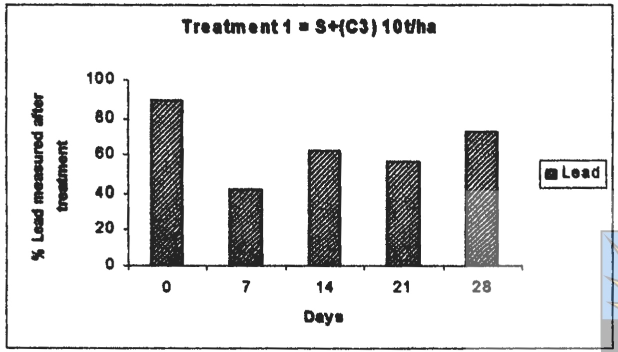


Figure: 4.3c

Figure: 4.3 a-c: % Lead measured after treatment with compost C2 at different tons/hecta



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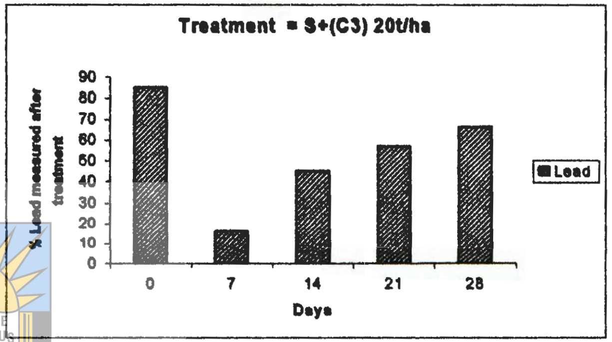


Figure: 4.5b

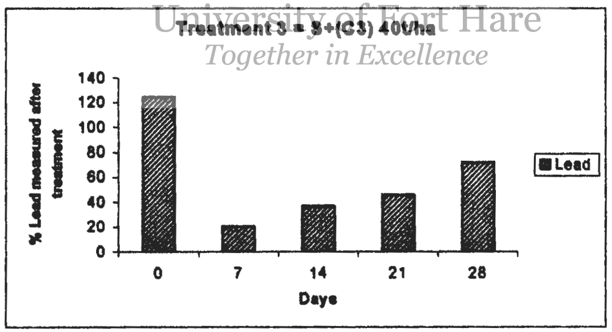


Figure: 4.5c

Figure 4.5a-c: % Lead measured after treatment with compost C3 at different tons/hecta

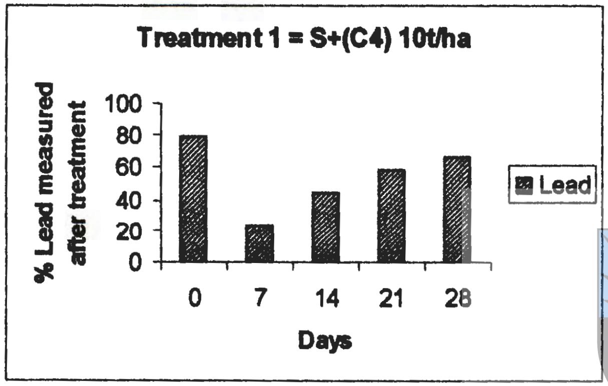


Figure: 4.7a

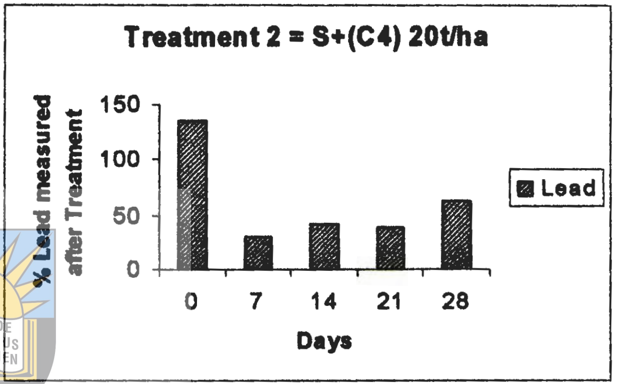


Figure: 4.7b

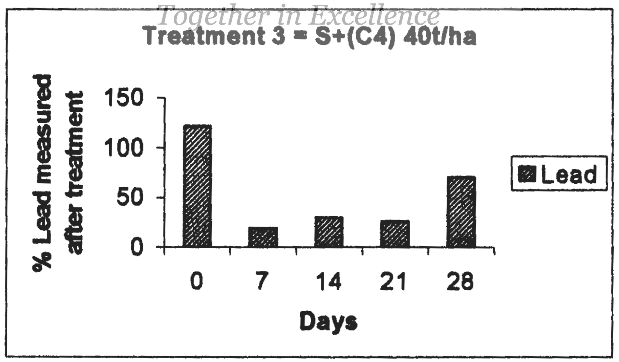


Figure: 4.7c

Figure 4.7a-c: % Lead measured after treatment with compost C4 at different tons/hecta

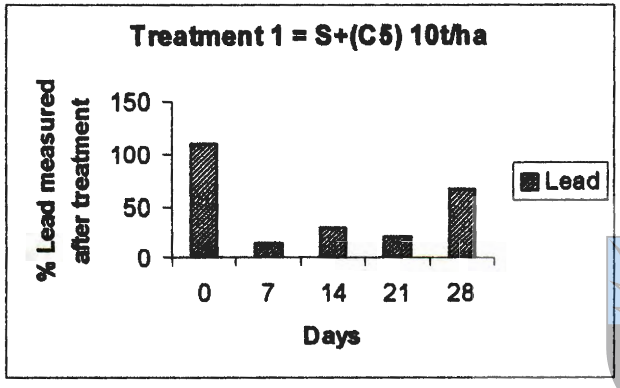


Figure: 4.9a

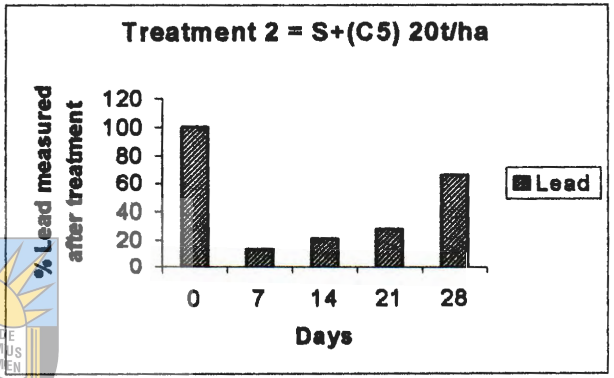


Figure: 4.9b

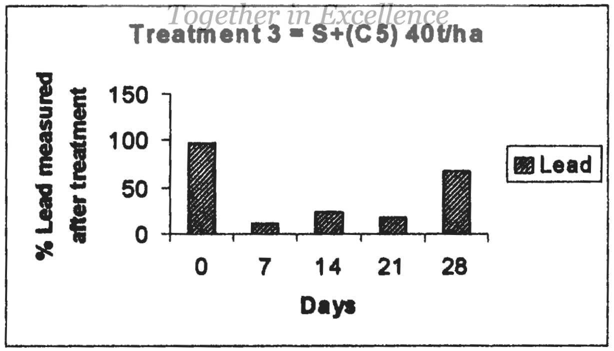


Figure: 4.9 c

Figure 4.9a-c: % Lead measured after treatment with compost C5 at different tons/hecta

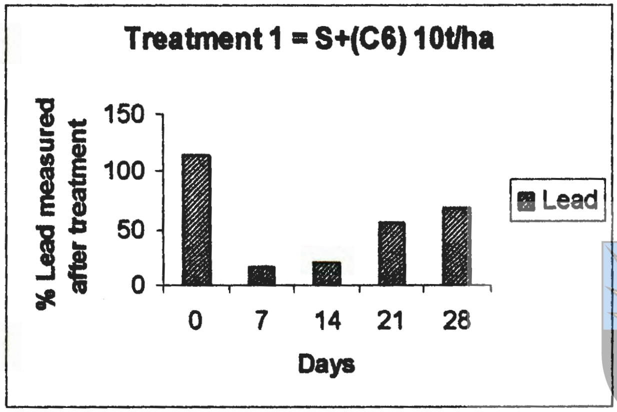
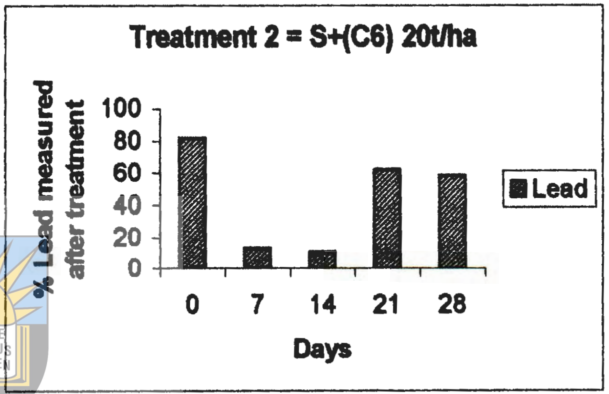


Figure: 4.11a



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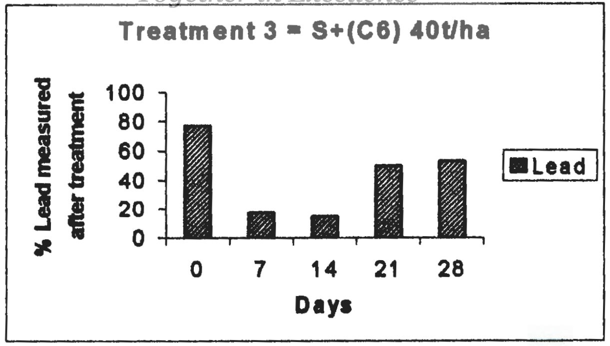


Figure: 4.11c

Figure 4.11a-c: % Lead measured after treatment with compost C5 at different tons/hecta

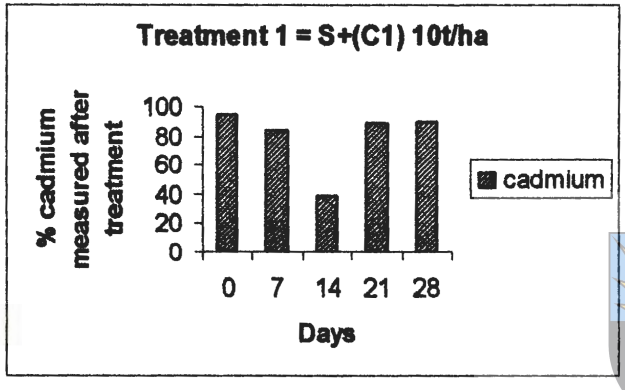


Figure: 4.2a

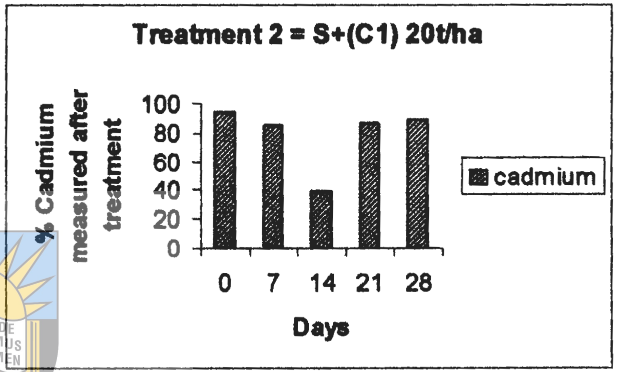


Figure: 4.2b

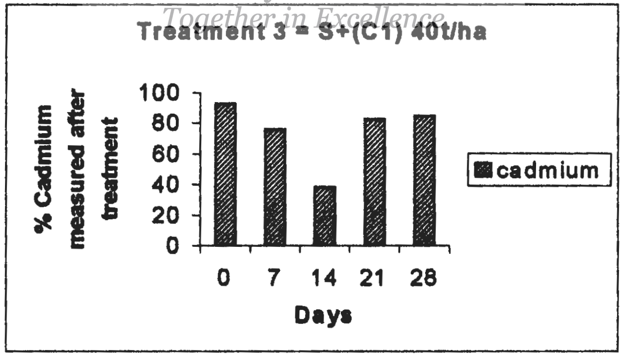
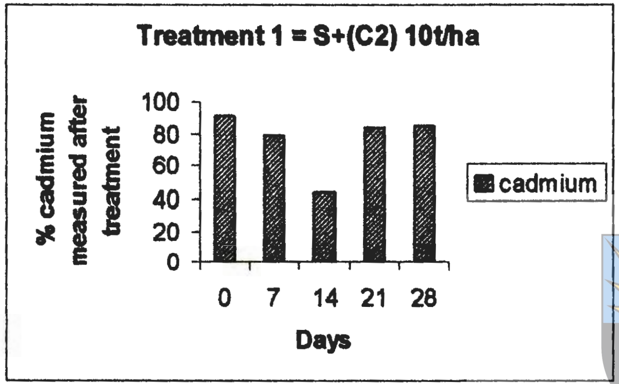


Figure: 4.2c

Figure 4.2a-c: % treatment a-c: % Cadmium measured after treatment with compost C1 at different tons/hecta



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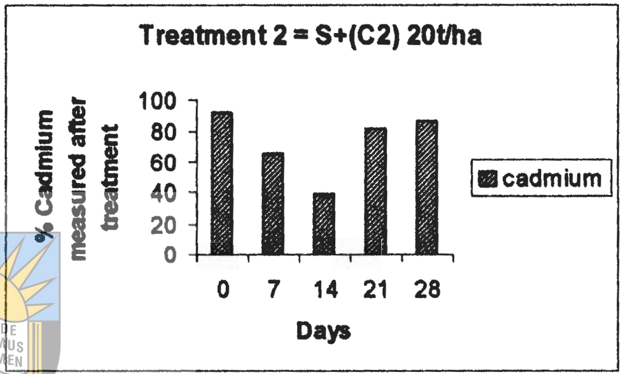

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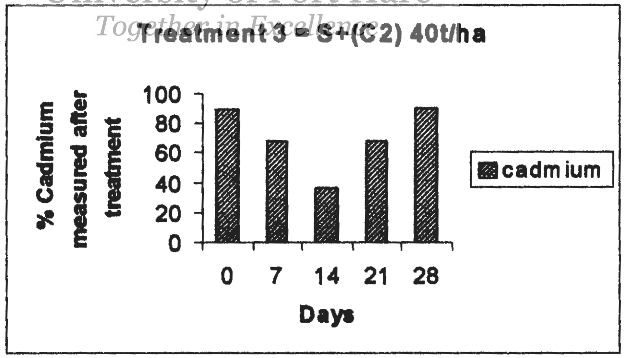
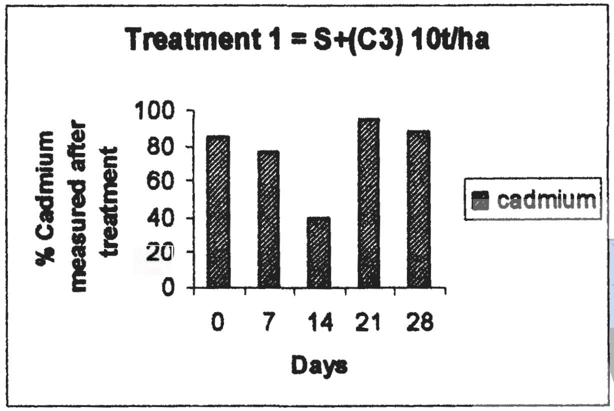


Figure: 4.4c

Figure 4.4a-c: % Cadmium measured after treatment with compoct C1 at different tons/hecta



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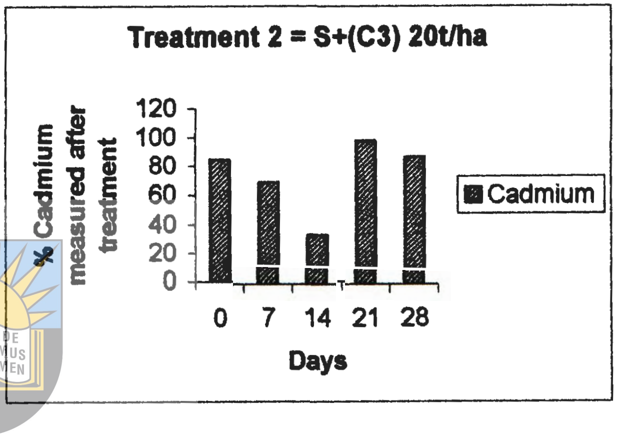


Figure: 4.6b

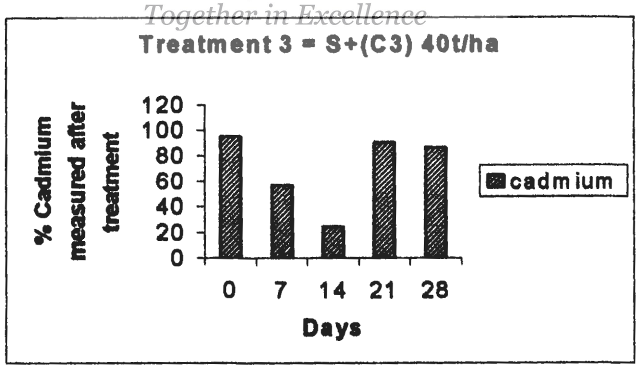


Figure: 4.6c

Figure 4.6a-c: % Cadmium measured after treatment with compost C3 at different tons/hecta

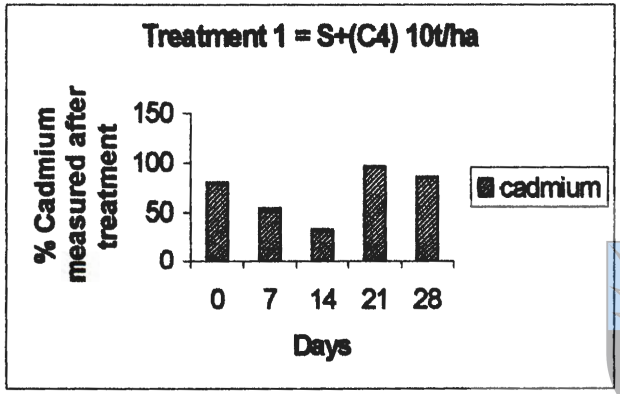
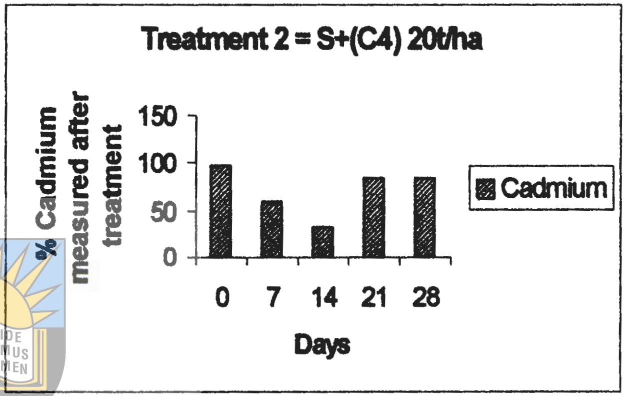


Figure: 4.8a



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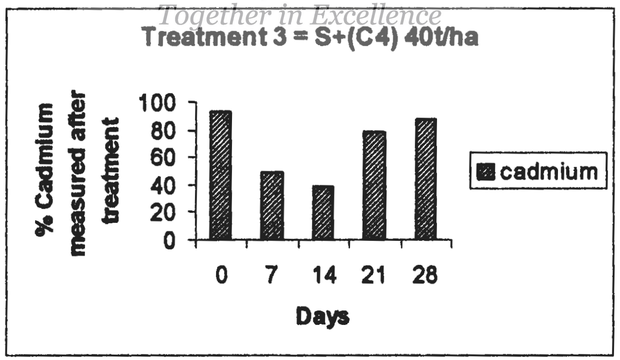


Figure: 4.8c

Figure 4.8a-c: % Cadmium measured after treatment with compost C4 at different tons/hecta

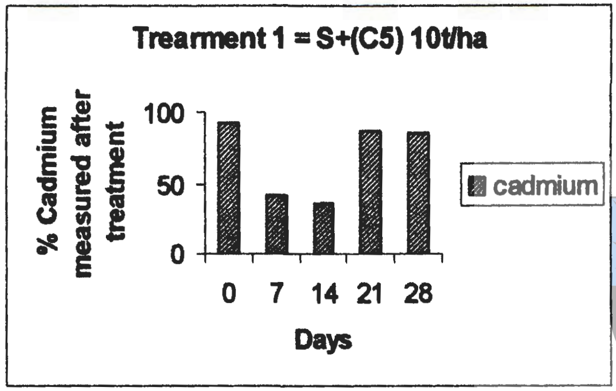


Figure: 4.10a

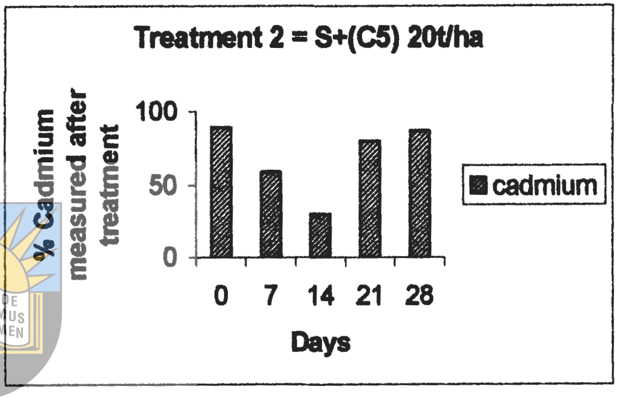


Figure: 4.10b

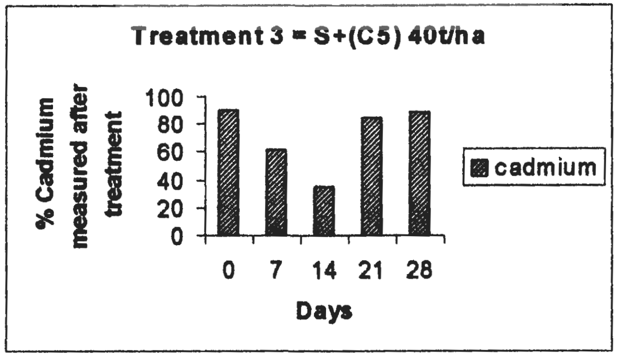


Figure: 4.10c

Figure 4.10a-c: % Cadmium measured after treatmentwith compost C5 at different tons/hecta

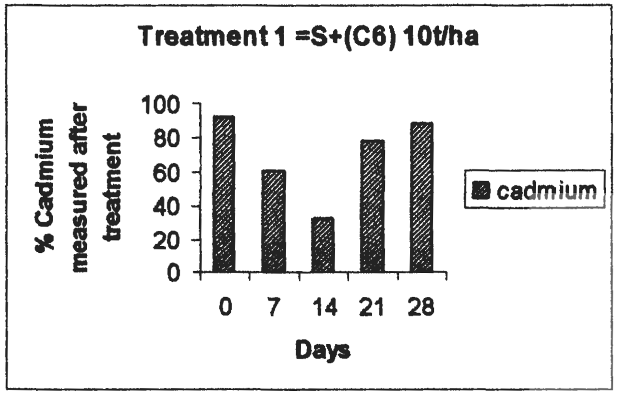


Figure: 4.12a

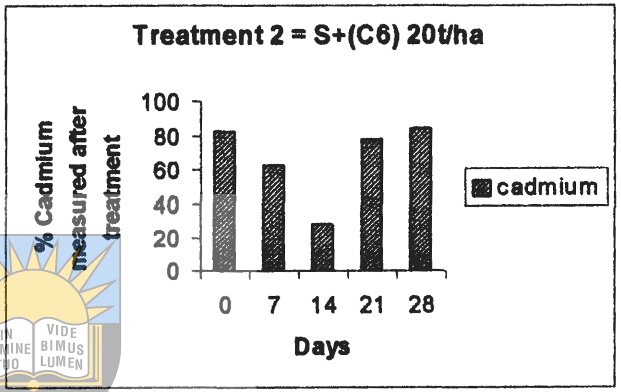


Figure: 4.12b

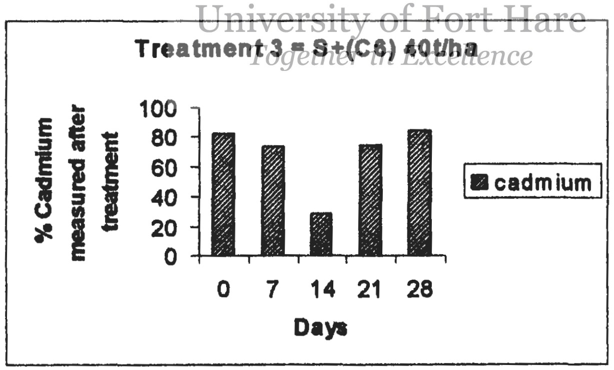


Figure: 4.12c

Figure 4.12a-c: % Cadmium measured after treatment with compost C6 at different tons/hecta

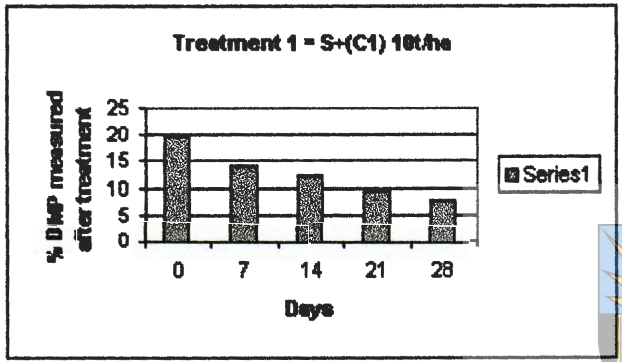


Figure 13a

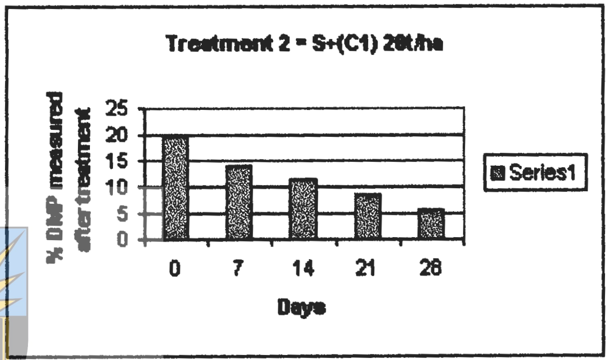


Figure 13b

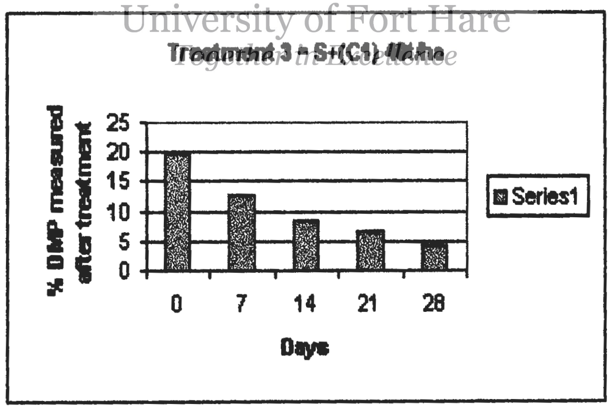


Figure 13c

Figure 13a-c: %DMP measured after treatment with compost C1 at different tons/hecta

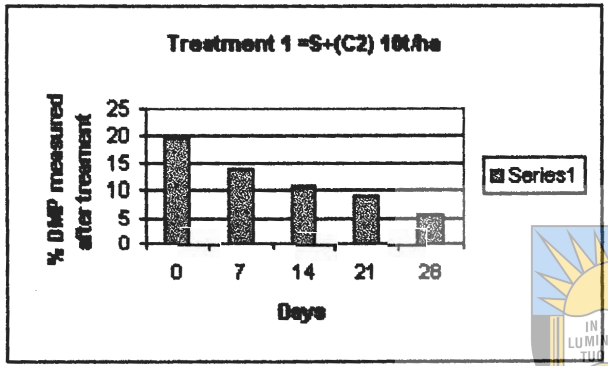


Figure: 16a

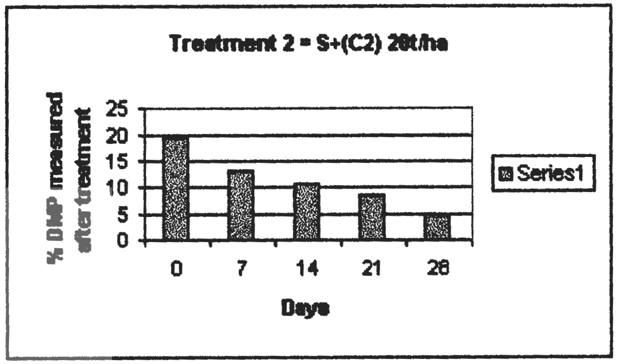


Figure: 16b

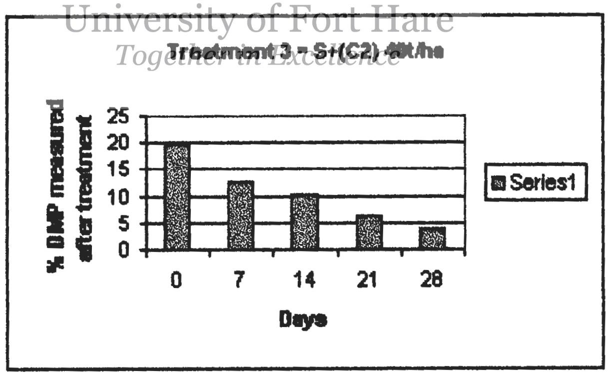


Figure: 16c

Figure 16a-c: %DMP measured after treatment with compost C2 at different tons/hecta

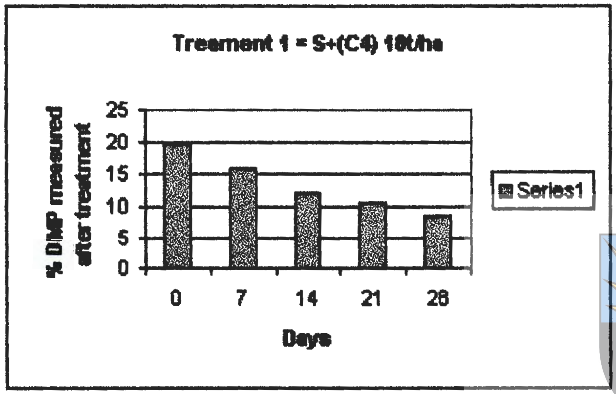

Treament 1 = S+(C4) 10t/ha

Figure: 19a

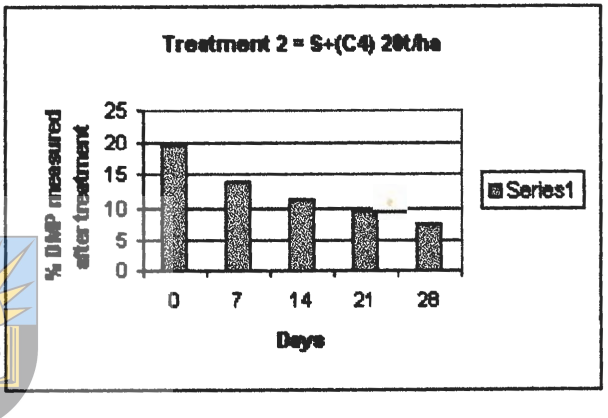

Treatment 2 = S+(C4) 20t/ha

Figure: 19b

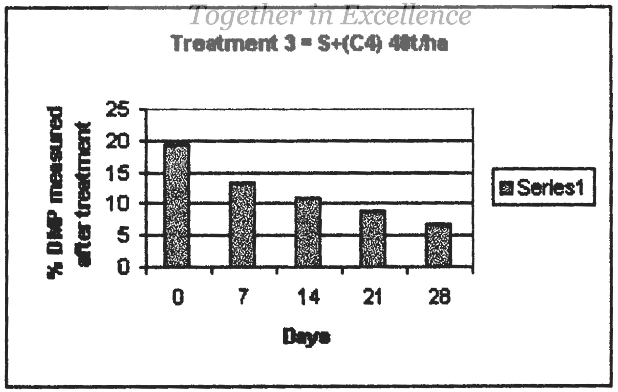

Treatment 3 = S+(C4) 40t/ha

Figure: 19c

Figure 19a-c: %DMP measured after treatment with compost C4 at different tons/hecta

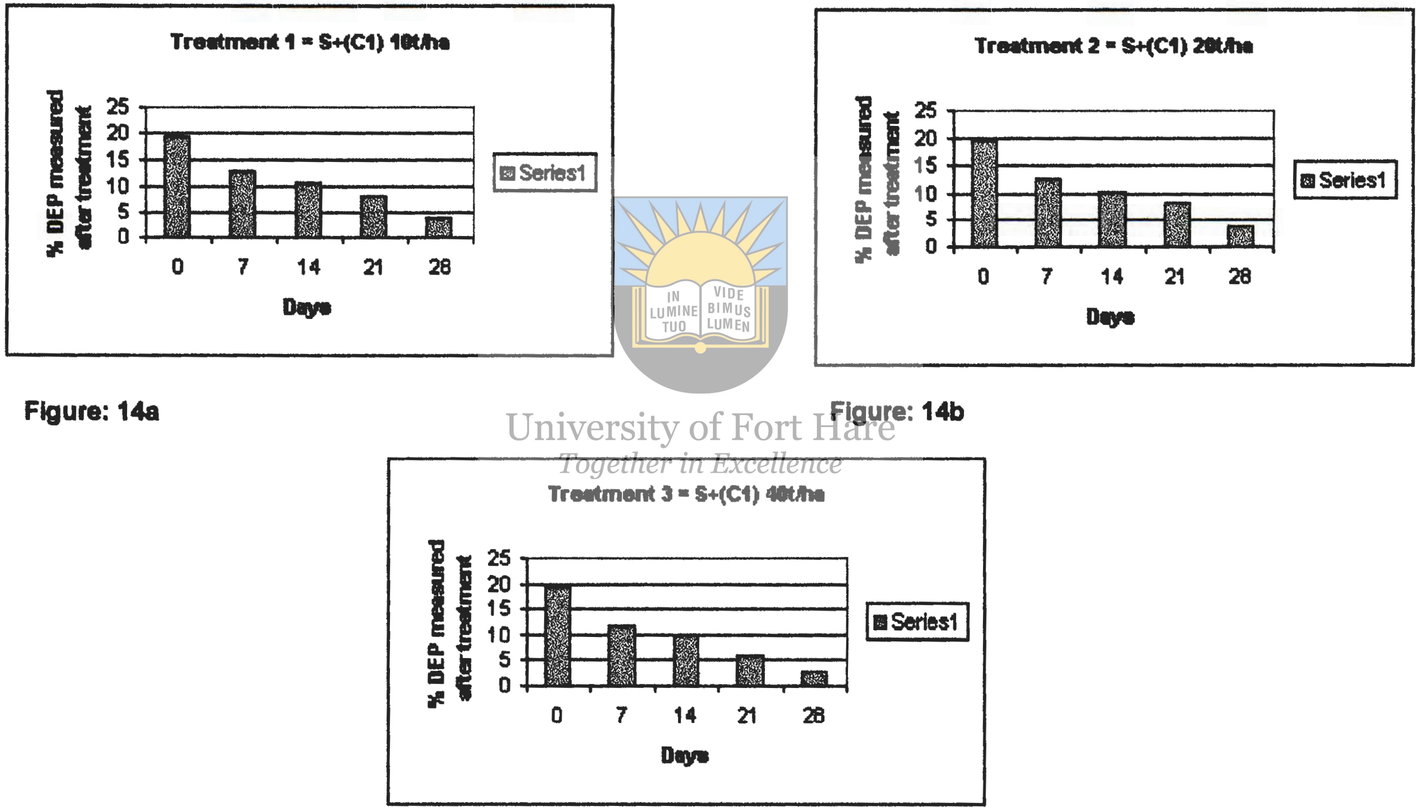


Figure: 14a

Figure: 14b

Figure: 14c

Figure 14a-c: %DEP measured after treatment with compost C1 at different tons/hecta

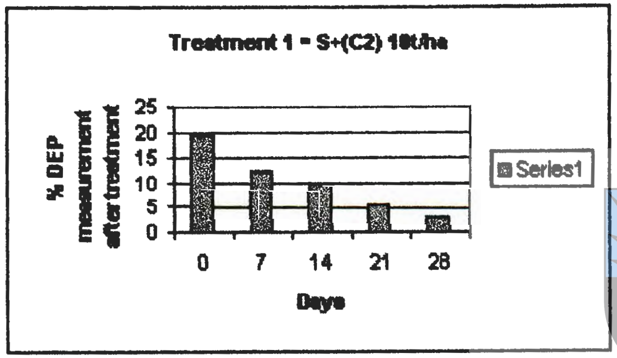


Figure: 17a

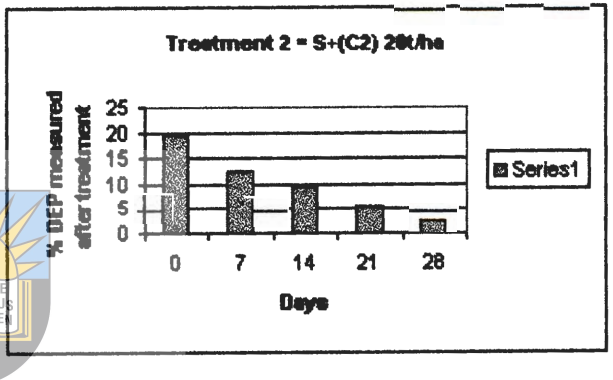


Figure: 17b

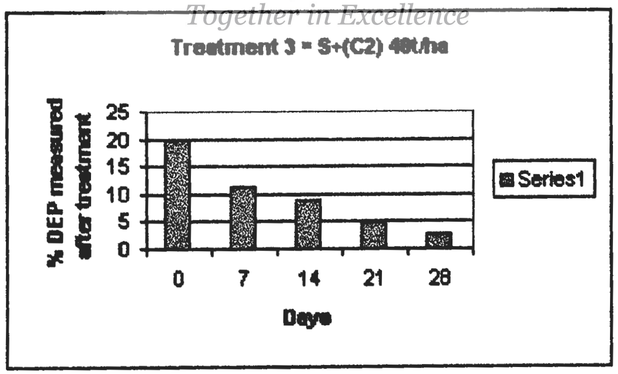


Figure: 17c

Figure 17 a-c: %DEP measured after treatment with compost C2 at different tons/hecta

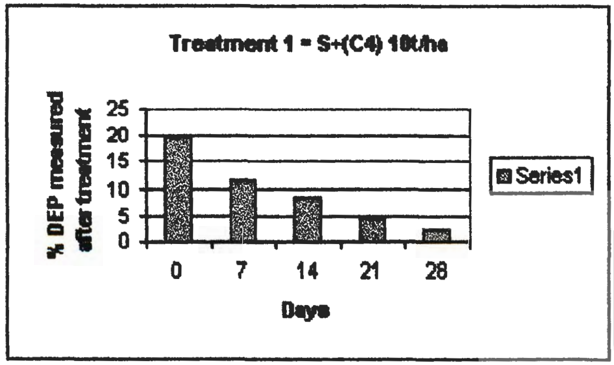
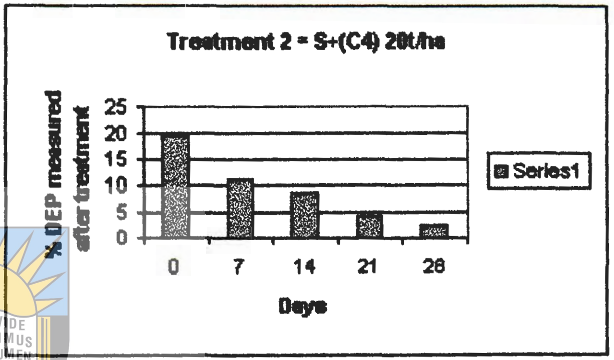


Figure: 20a



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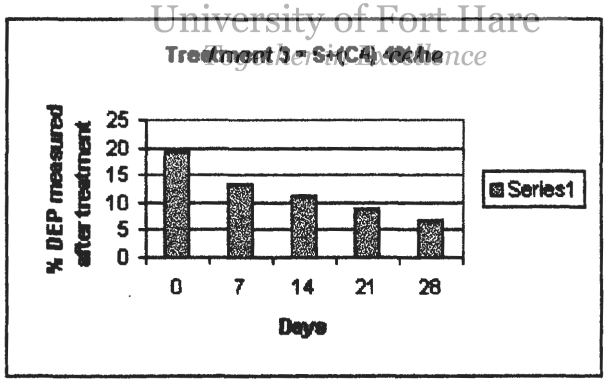


Figure: 20c

Figure 20a-c: %DEP measured after treatment with compost C4 at different tons/hecta

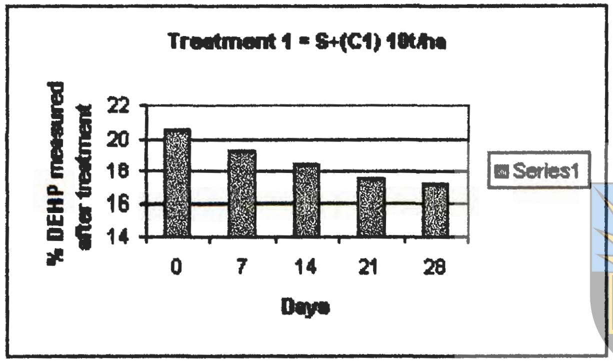


Figure: 15a

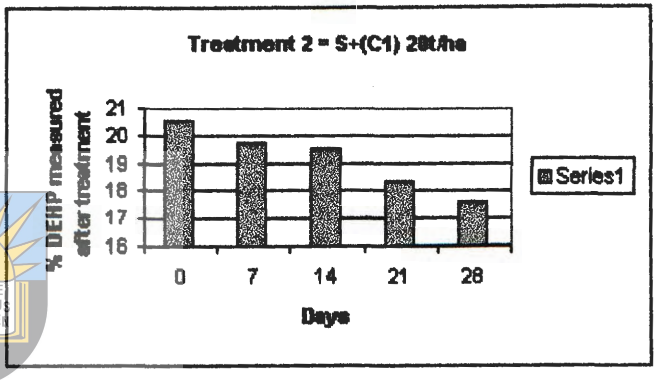


Figure: 15b

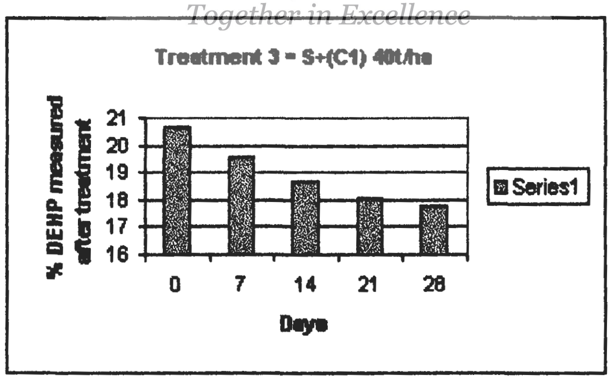


Figure: 15c

Figure 15a-c: % DEHP measured after treatment with compost C1 at different tons/hecta

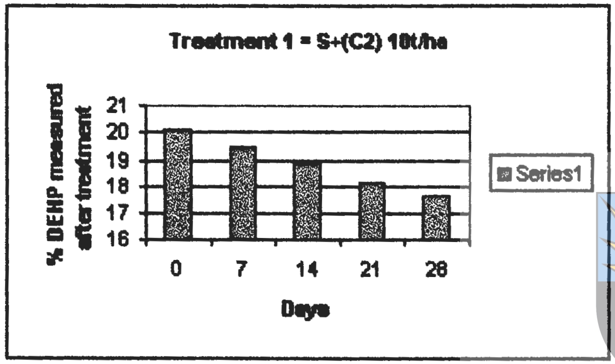
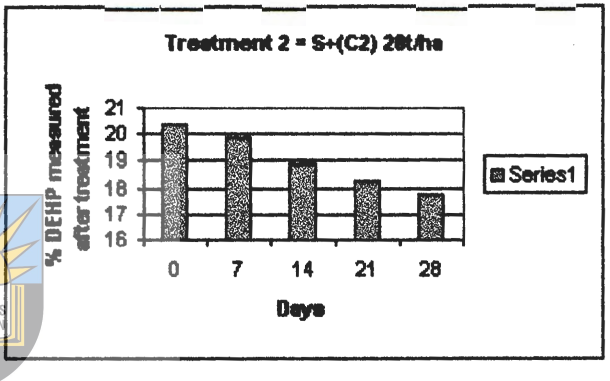


Figure: 18a



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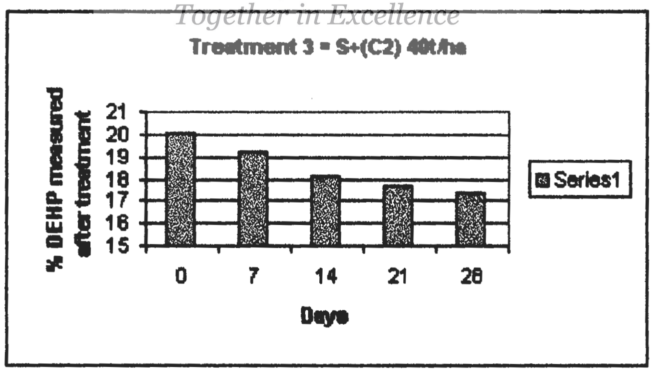

Figure: 18c

Figure 18a-c: %DEHP measured after treatment with compost C2 at different tons/ hecta

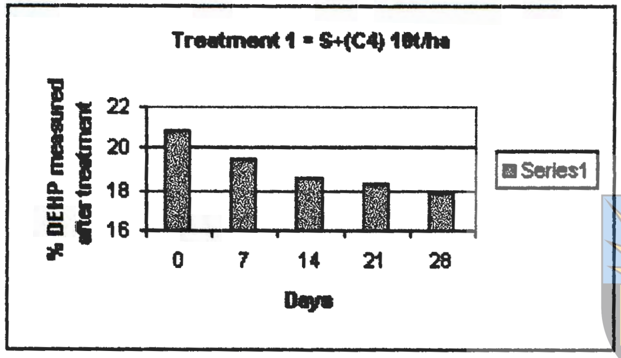

Figure: 21a

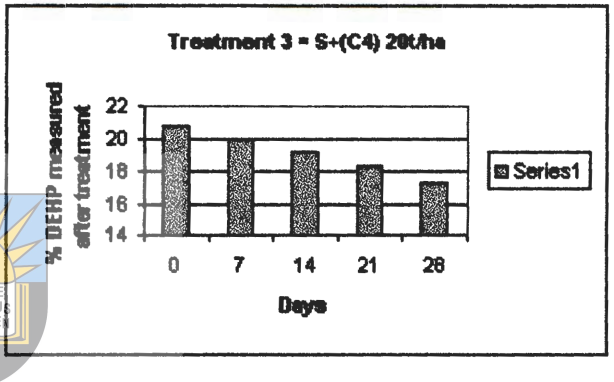

Figure: 21b

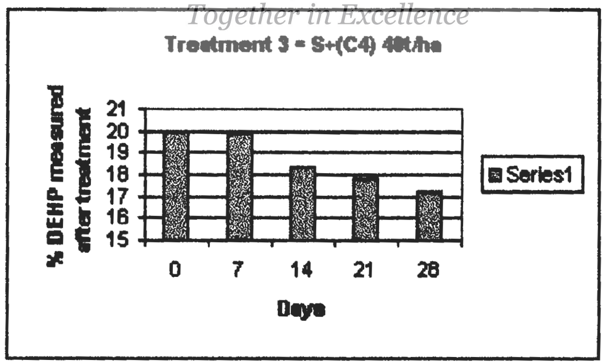

Figure: 21c

Figure 21a-c: %DEHP measured after treatment with compost C4 at different tons/hecta

Chapter 5

5.1 CONCLUSSION

Composts added to soil routinely (just before their retention times for cadmium and lead expires) might be useful in preventing the availability of lead and cadmium for plant uptake from contaminated soil.

According to the data that is obtained from phthalate esters remediation, it was shown that phthalates do degrade in soil and compost remediation effectively. This was confirmed by declines on phthalate esters concentration amounts. These phthalate esters also show that they might be different in under certain conditions because DMP and DEP were degrading almost at the same rate whilst DEHP was slowly degrading compared to the other two. This might be attributed to the fact mentioned before that the degradation rates of phthalates are dependent on their molecular weight. DEHP has higher molecular weight than DMP and DEP. It can also be concluded that compost can be used in phthalate esters soil for remediation purposes.

In addition the compost will add nutrient to soil. This will be beneficial to farmers as it will improve soil quality and leads to higher crop yields.

Chapter 6

6.1 REFERENCES:

ACGIH (American Conference of Governmental Industrial Hygienists). (1988). Documentation of the Treshold Limit Values for Chemical Substances and Physical Agents in the Work Environment, 4th Ed. ACGIH, Cincinnati, Ohio.

Adams, W.J., Biddinger, G.R., Robillard, K.A. and Gorsuch, J.W. (1995). Env. Toxicol. and Chemistry, 14,9,1569.

Adriano, D. C. (1986). Trace Elements in the Terrestrial Environment. New York: Springer-Verlag.

Alloway, B.J. (1990). Heavy Metals in Soils. Blackie, Glasgow and London.

Al-Wabel, M.I., Al-Omran, A.M., Shalaby, A.A., and Choudhary M.I. 1998. Commun. Soil Sci. Plant Anal. **29** (17 & 18): 2713-2724.

Anderson, J.M. and Ingram, J.S.I (1989). TSBF. A handbook of Methods of Analysis. CAB International, p.39.

Anderson, J.M. and Ingram, J.S.I. (1993). Tropical Soil Biology and Fertility: A handbook of Methods: CAB International. Wallington, U.K. p 35.

Aranda, J.M., O'Connor, G.A. and Eiceman, G.A. (1989). J. Environ. Qual. **18**: 45-50.

Asami, T. (1984). Pollution of soils by cadmium. In: Changing metal cycles and human health, ed. By J.O. Nriagu: 95-111.

Antunes, M.C., Simão, J.E., Duarte, A.C., Esteban, M. and Tauler, R. (2002). Analytica Chimica Acta, **459**:2:291-304.

ATSDR (Agency for Toxic Substances and Disease Registry) (1993). Toxicological Profile for Lead. Prepared for U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry, Atlanta, Georgia. TP-92/12. April 1993: 307 + appendices.

ATSDR (Agency for Toxic Substances and Disease Registry) (1996). Diethyl Phthalate, Cas No. 84-66-2, September 1996. (www.atsdr.cdc.gov/tfacts73.html).

Avery, S. V. 1995. J. Ind. Microbiol. **14:** 76-84.

Bache, B.W., in The Encyclopaedia of Soil Science, eds. Fairbridge, R.W. and Finkl, C.W. Dowden, Hutchinson and Roiss, Stroudburg, Pa (1979), 487-492.

Babich, H., and G. Stotzky. (1985). Environ. Res. **36:**111-137.

Becker, J.S. and Dietze, H.J. (1998). Spectromica Acta Part B: Atomic Spectroscopy, **53**:11:1475-1506.

Berlin, A., and van der Venne, M.T. (1987). Trace Elem. Med. **4**: 166-172.

Bethesda, M.D. (1993). U.S. Department of Health and Human Services. Hazardous Substances Data Bank (HSDB, online database). National Toxicology Information Program, National Library of Medicine.

Bettinelli, M., Beone, G.M., Spezia, S. and Baffi, C. (2000). Analytical Chimica Acta, **424**:2:289-296.

Beveridge, T. J. (1989). Annu. Rev. Microbiol. **43:**147-171.

Brady, N.C. (1990). The nature and properties of soils. 10th edition. MacMillan Publ. Co., New York, NY.

Brady, N.C. and Weil, R.R., (1999). Soil and Chemical Pollution. The Nature and Properties of Soil. 12th Edition. Prentice and Hall, Inc. Simon and Schuter, USA.

Buchet J.P., Lawerys R., Roels, H. (1990). The Lancet **336**: 699-702.

Bunzl, K., and W. Schimmack. (1991). Radiochim. Acta **54:**97-102.

Carelli, G., Sannolo, N. De Lorenzo, G., and Castellino, N. (1995). Ecosystems. In: Inorganic Lead Exposure: Metabolism and Intoxication, N. Castellino, P. Castellino and N. Sannolo (Eds). CRC Press Inc: 3-11.

Carnie, T. (1996a). We're in the Muck, says Asmal. The Mercury, 18 September.

CCREM (Canadian Council of Resource and Environmental Ministers). 1(987). Canadian Water Quality Guidelines. Prepared by the Task Force on Water Quality Guidelines of the Canadian Council of Resource and Environment Ministers. March 1987.

CDC (1985). Preventing lead poisoning in young children. A statement by the Centre for Disease Control, US Dept. of Health and Human Services, Atlanta. Georgia, January 1985.

CEC (Commission of the European Communities)-Council Directive on the Approximation of Laws, Regulations and Administrative Provisions Relating to the Classification, Packaging and Labelling of Dangerous Substances (67/ 584/ EEC).

Chen, Y. and Stevenson, F.J. (1986). in The role of Organic Matter in Modern Agriculture, eds. Chen, Y and Ainmemelech, Y. Martinus Nijhoff, Dordrecht: 195-205.

Cizdziel, J.V and Hodge, V.F. (2000). Microchemical Journal, **64**:1:85-92.

Cincinnati, O.H. (1987). U.S. Environmental Protection Agency. Health and Environmental Effects Profile for Phthalate Acid Alkyl, Aryl, and Alkyl/Aryl Esters. ECAO-CIN-P188. Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment, Office of Research and Development.

Cornish, J. E., W. C. Golberg, R. S. Levine, and J. R. Benemann. (1995). Phytoremediation of soils contaminated with toxic elements and radionuclides, p. 55-62. *In* R. E. Hinchee, J. L. Means, and D. R. Burris (ed.), Bioremediation of inorganics. Third International *In Situ* and On-Site Bioreclamation Symposium, no. 10. Battelle Press, Columbus, Ohio.

Cunningham, S. D., W. R. Berti, and J. W. Huang. (1995). Remediation of contaminated soils and sludges by green plants, p. 33-45. *In* R. E. Hinchee, J. L. Means, and D. R. Burris (ed.), Bioremediation of inorganics. Third International *In Situ* and On-Site Bioreclamation Symposium, no. 10. Battelle Press, Columbus, Ohio.

Davis, B.E. (1995). Lead. In: Heavy Metals in Soils, 2nd Edition, B.J. Alloway (Ed.) Blackie Academic and Professional, New York: 206-223.

DHHS (1982). Blood lead levels for persons 6 months-74 years of age: United States. 1976-1980. Public Health Service, National Centre for Health Statistics. US Department of Health and human Service. Hyattsville, Maryland.

Diener, R. G., Collins, A., Bearce, B., Bissonnette, G. (2002). Bioremediation of Pesticide Contaminated Media. (http://www.caf.wvu.edu/wvfes/projects.htm).

dos Anjos, M.J., Lopes, R.T., de Jesus, E.F.O., Assisc, J.T., Cesareo, R. and Barradas, C.A.A. (2000). Spectrochimica Acta Part B: Atomic Spectroscopy, **55**:7: 1187-1192.

ECPI (European Council for Plasticisers and Intermediates) (1999). The Health and Environmental Effects of Phthalates, Update Paper presented by Dr. D.F. Cadogan at PVC '99, Brighton, UK, April 1999. (http://www.ecpi.org),

ECPI (European Council for Plasticisers and Intermediates) (2001). Environmental Effects of Phthalates, June 2001. (http://www.ecpi.org).

ECPI (European Council for Plasticisers and Intermediates) (2002). Phthalate Esters, July 2002. (http://www.ecpi.org).

Ejlertsson, J., Meyerson, U. and Svensson B.H. (1996). Biodegradation 7: 345.

Ejlertsson, J., Alnervic, M., Jonsson, S. and Svesson, B.H. (1997). Environ. Sci. Technol. **31**: 2761.

Environment Canada. (1991). Review and Recommendations for Canadiàn Interim Environmental Quality Criteria for Contaminated Sites. Prepared for Canadian Council of Ministers of the Environment subcommittee on Environmental Quality Criteria for Contaminated Sites by Angus Environmental Ltd. Inland Waters Directorate, Water Quality Branch, Ottawa. Scientific Series No. 197.

EPA (US Environmenta Protection Agency). (1986). Air quality criteria document for lead, **I-IV** Environmental Criteria and Assessment Office, US Environmental Protection Agency, Research Triangle Park, North Carolina.

EPA (United States Environmental Protection Agency) (1997). Innovative Uses of Compost, Bioremediation and Pollution Prevention, October 1997. (http://www.epa.gov).

EPA (U.S. Environmental Protection Agency) (1999). *Integrated Risk Information System (IRIS) on Dimethyl Phthalate*. National Center for Environmental Assessment, Office of Research and Development, Washington, DC.

Estévez Alvarez, R., Montero, A.A., Jiménez, N.H., Muñiz,U.O., A. R. Padilla, A.R., J. Molina, J. and Quicute de Vera, S. (2001). Journal of Radioanalytical and Nuclear Chemistry, **247**:3:479-486.

Ewers, U. and Schipköter, H.W. (1991). Lead. In: Metals and Their Compounds in the environment: Occurrence, Analysis, and Biological Relevance, E. Merian (Ed.) Verlagsgesellschaft, Weinheim, New York: 971-1014.

Fairbridge, R.W. (1972). The Encyclopedia of Geochemistry and Environmental Sciences. **IVa**, Van Nostrand-Reinhold, New York.

Fatoki, O.S. and Awofolu, R.O. (2003). Journal of Chromatography A, **983,** Issue 1-2,3: 225-236

Federal Office of Environment, Forests and Landscape, (1987). Weg-leitung für die Probenahme und Analyse von Schadstoffen im Boden. Eidgenössische Drucksachen- und Materialzentrale. Nr. 730.950d. EDMZ, Bern, Switzerland.

Flegal, A.R. and Chisolm, J.J. 1991. Environ. Res. **55** (2): 199-212.

Foth, H.D. (1990). Fundamentals of Soil Science. 8th edition. Wiley and Sons, New York, NY.

Friberg, L.F. and Vahter, M. (1983). Environ. Res. **30**: 95-123.

Frieber, L. (1984/85). Cadmium and health: A toxicological and epidemiological appraisal. CRC Press, Boca Raton, Florida.

Frostegård, A., A. Tunlid, and E. Bååth. (1993). Appl. Environ. Microbiol. **59:**3605-3617.

Frostegård, A., A. Tunlid, and E. Bååth. (1996). Biochem. **28:**55-63.

Gilberto Abate, G., and Jorge, J.C., and Masini, C. (2002). Organic Geochemistry, **33**:10:1171-1182.

Gray, SR. and Becker, N.S.C. (2002). Urban Water, **4**, Issue 4: 331-346.

Gregson, S.K. and Alloway, B.J. (1984). Gel permeation chromatography studies on the speciation of lead in solutions of heavily polluted soils. J. Soil Sci. **35**: 55-61.

Henschler, D. (ed.) (1998). Gesundheitsschädliche Arbeitsstoffe. Toxikologisch-arbeitsmedizinische Begründung von MAK-Werten. VCH Verlagsgesellshaft, Weinheim-Basel-Cambridge-New York.

Hartyáni, Z., Dávid, E, Szabó, S, Szilágyi, V., Horváth, T and Hargitai Tóth, A (2001). Determination of the Trace Elements Distribution of Polluted Soils in Hungary by X-ray methods. Microchemical Journal, **67**:1-3:195-2000.

Henschler, D. and Lehnert, G. (eds.) (1986). Biologische Arbeitsplatz-Werte (BAT-Werte). Arbeitsmedizinnisch-tocologische Begründungen. VCH Verlagsgessellschaft, Weinheim-Deer-field Beach/FloridaBasel.

Hilbk-Kortenbruck, F., Noll, R., Wintjens, P., Heinz Falk, H. and Becker, C. (2001). Spectrochimica Acta Part B: Atomic Spectroscopy, **56**:6:933-945.

Hill, S.J. (1992). Lead. In: Hazardous Metals in the Environment, M. Stoepler(Ed.). Elsevier Science Publishers: 231-255.

Hirshon, S. 1989. Getting the lead out. Protect yourself, June 1989: 13-16.

Hunter, A.H. (1974). Tentative ISFEI Soil Extraction Procedure. International Soil Fertility Evaluation and Improvement Project. N.C. State University Raleigh, N.C.

Hutton, M. (1982). Cadmium in the European Community. MARC Report No. 26, Monitoring and Assessment Research Centre, Chelsea College, University of London.

IARC (International Agency for Research on Cancer) (2000). ECPI Press Releases. DEHP Confirmed as Non-Carcinogenic February 2000. (http://www.ecpi.org).

Jaques, A.P. 1985. National Inventory of Sources and Release of Lead (1982). Environmental Protection Programes Directorate, Environmental Protection Services, Environment Canada, Cat No. En 49-4/5-3. In Stokes 1989.

Jaworski, J.F., Nriagu, J., Denny, P., Hart, B.T., Lasheen, M.R. Subraminian, V., and Wong, M.H. (1987). Group report. In: Lead, Mercury, Cadmium and Arsenic in the Environment, T.C. Hutchinson and K.M. Meema (Eds). John Wiley and Sons Ltd: 3-17.

Kabata-Pendias, A. and Pendias, H. (1992). Trace Elements in Soil and Plants, 2nd Edition. CRC Press Inc., London: 365.

Keating, J. and Wright, P. (1994). Lead. In: Canadians Mineral Yearbook: Review and Outlook. Natural Resources Canada, Ottawa: 27.1-27.19.

Keating, J. (1995). Lead. In: Canadian Minerals Yearbook. Natural Resources Canada, Ottawa. July 1995.

Kjellstrom, T.E. and Nordberg, G.F. (1978). Environ. Res. **16**: 248-269.

Kloke, A. (1980). Orientierungsdaten für tolerierbare Gesmtgehalte einiger Elemente in Kulturböden (Richtwerte 1980). Mitt. VDLUFA.**1-3.**

Kloke, A., Sauerbeck, D.R. and Vetter, H. (1984). In: Changing metal cyclyes and human health, ed. By J.O. Nriagu: 113-141. Berlin, Springer-Verslag.

Krueger, J.A. and Duguay, K.M. (1989). Comparative analysis of lead in Maine urban soils. Bull. Environ. Contm. Toxicol. **42**: 547-581.

Landsdown, R and Yule, W. (1986). Lead toxicity. Baltimore, Johns Hopkins University Press.

Leita, L. and De Nobilli, M. 1991. Water-soluble fractions of heavy metals during composting of municipal solid waste. J. Environ. Qual. **20**: 73-78.

Levin, R. (1987). US Environmental Protection Agency, Report No. EPA-23-09-86-019, Washington, DC.

Lovley, D. R. 1994. Adv. Agron. **54**:175-231.

Lux, D., L. Kammerer, W. Rühm, and E. Wirth. (1995). Sci. Total Environ. **173/174:**375-384.

Mahaffey, K.R., Annest, J.L., Roberts, J. and Murphy, R.S. (1982). New England J. Med. **307**: 573-579.

MAK (1988), Maximum concentrations at the Workplace and Biological Tolerance Values for Working Materials, DFG Report No. XXIV. VCH Verlagsgesellschaft, Weinheim-Basel-Cambrigde-New York.

Merr, R.H., Tiller, K.G., and Alston, A.M. (1986). Plant Soil **95**: 255-269.

Metzler, M. (2001). The Handbook of Environmental Chemistry. **3**, Part 1: 176 Springer-Verslag, New York.

Mielke, H.W., Adams, J.L., Reagan, P.L. (1989). Environ. Chem. Health **9**(supp): 253-271. In: ATSDR 1993.

Moreno, J.L., García, C, Hernández, T. and Ayuso, M. (1997). Soil Sci. Plant Nutr., **43** no.3: 565-573.

Mulè, P. and Melis, P (2000). Commun. Soil Sci. Plant Anal., **31** (19&20):3193-3204.

Myöhänen, T., Mäntylahti, V., Koivunen, K. and Matilainen, R. (2002). Spectrochimica Acta Part B: Atomic Spectroscopy, **57**:11:1681-1688.

NAS (1980). Lead in the Human Environment. Washington, DC, US National Academy of Sciences.

Nelso, D.W. and Sommers, L.E. (1975). Proc. Of the Indiana Academy of Science **84**: 456-462.

Nelson, W.O. and Campell, P.G.C. (1991). Environ. Poll. **71**: 91-130.

NIOSH (National Institute for Occupational Safety and Health), Criteria for a Recommended Standard

- (1973) Occupational Exposure to Chromic Acid,
- (1973) Occupational Exposure to Inorganic Mercury,
- (1975) Occupational Exposure to Chromium VI,
- (1975) Occupational Exposure to Inorganic Arsenic,
- (1977) Occupational Exposure to Inorganic Nickel,
- (1978) Occupational Exposure to Inorganic Lead (Revised Criteria) Us Department of Health, Education and Welfare, Washington, D.C.

Nomiya, K. (1981). In: Cadmium in the environment. **11**, ed. By J.O. Niriagu: 643-689. New York, Wiley.

Nogawa, K. (1984). Cadmium. In: Changing Metal cycles and human health, ed. By J.O. Nriagu: 275-284. Berlin, Springer-Verslag.

Novosamsk, I., Houba, V.J.G., Eck van, R. and Vark van, W. (1983). Commun. Soil Sci. Plant Anal. **14**: 239-249.

Nowack, B., Obrecht, J.M., Schuep, M., Schulin, R., Hansmann, W. and Köppel, V (2001). J. Environ. Qual. **30**: 919-926.

Nowka, R., Mueller, H., Marr, I.L. and Ansari, T.M. (2000). Success with Some Difficult Samples. **45**: No. 3.

Nriagu, J.O. and Pacyna, J.M. (1988). Nature **333** (6169): 134-139.

Nriagu, O.J. (1988). Environ. Pollut. **50:** 139-161.

OECD (organization for Economic Co-operation and Development). (1993). Risk Reduction Monograph No.1: Lead. Background and National Expirience with Reducing Lead. Environmental Directorate, Organization for Economic Co-operation and Development, Paris: 277.

Okalebo, J.R. (1985). Kenya J. Sci. and Technol. **B6**: 129-133.

Okalebo, J.R., Gathua, K.W. and Woomer, P.L. (1993). Laboratofy Methods of Soil and Plant Analysis: A working manual.

OMEE (Ontario Ministry of Environment and Energy). (1994a). Scientific Criteria Document for Multimedia Environmental Standards Development – Lead. ISBN 0-7778-2529-5. March 1994: 162 + appendices.

Panis, P. and Lucianer, A. (1987). Genio Rurale **4**: 31-34. In: Carelli et al. 1995.

Pekall, D. (1975). "Phthalate Esters: Occurrence and biological effects", Springer-Verslag, New York.

Pennanen, T., Å. Frostegåard, H. Fritze, and E. Bååth. (1996). Appl. Environ. Microbiol. **62:** 420-428.

Phthalates Information Center (2002). What are phthalates? (www.phthalates.org).

Piscator, M. (1985). Environ. Health Perspect. **63**: 127-132.

Purves, D. (1985). Trace-Elemental Contamination of The Environment. Revised Edition: 144-262.

Rand, M.C., Greenberg, A.E. and Taras, T.M. (1976). Standard Methods for The examination of water and waste water. 14th. American Public Health Association: 179-215.

Riley, R. G., and Zachara, J. M. (1992). Chemical contaminants on DOE lands and selection of contaminant mixtures of subsurface science research. Publication DOE/ER-0547/T. U.S. Department of Energy, Washington, D.C.

Rodríguez, F.J. (1982). Normativas sorbe la aplicación de lodos residuals en agricultura an relación con los metales pesados. Avances para la Normativa en Espãna. In La Recuperación de los Recursos Sólidos, Ponencias del Coloquio-Congreso celebrado en Soria, Soria.

Roels, H.A. (1981). Environ. Res. **26**: 217-240.

Rosen, J.F. (1985). Metabolic and cellular effects of lead: a guide to low-level lead toxicity in children. In: Dietary and environmental lead: Human Health effects, ed. By K.R. Mahaffey: 157-185. Amsterdam, Elsevier.

Roslev, P., Madsen, P.L., Thyme, J.B. and Henriksen, K. (1998). Appl. Environ. Microbiol. **64**: 4711.

Said, W. A., and D. L. Lewis. (1991). Appl. Environ. Microbiol. **57:**1498-1503.

Schmitt, M.D.C., Trippler, D.L., Wachtler, J.N. (1988). Water Air Pollut. **39**: 157-168. In: ATSDR 1993.
Sham S. Goyal, (1997). J. Chromatography A, **789**:1-2:519-527.

Shamsipur, M., Raoufi, F. and Sharghi, H. (2000). Talanta, **52**:4:637 – 643.

Shelton, D.R., Boyd, S.A. and Tiedje, J.M. (1984). Environ. Sci. Technol. **18**: 93.

Silva, P.R.M., El Khakani, M.A., Chaker, M., Dufresne, A., and Courchesne, F. (2001). Sensors and Actuators B: Chemical, **76**:1-3: 250-257.

Singer, M. J. and Munns, D.N. (1987). Soils:An Introduction. Macmillan publishing company, New York.

Sposito, G. and Page, A.L. (1985). In Metal Ions in Biological Systems, **18** Circulation of Metals in the Environment, ed. Sigel, H. Marcel Dekker, New York: 287-332.

Staessen, J.A., Roels H. and Emelianov, D. (1999). Lancet 1999; **353**: 1140-44.

Staples, C.A., Perteson, D.R., Parketon, T.F. and Adams, W.J. (1997). Chemosphere **35**: 667.

Stchur, P., Yang, K.X., Hou, X., Sun, T. and Michel, R.G. (2001). Spectrochimica Acta Part B: Atomic Spectroscopy, **56**:9:1565-1592.

Stoeppler, M. (1994). Sampling and Sdample Preparation, Practical Guide for Analytical Chemists. Springer-Verlag Berlin Hedel berg, New York: 46-49.

Stokes, P.M. (1989). Environ. Goechem. Health **9**(suppl): 7-25.

Swiss Federal Research Station for Agro-ecology and Agriculture, (1989a). VSBo-Ringversuch, Auswertungsbericht. Eidgenössische Forschungsanstalt für Agrikulturchemie und Umwelthygiene FAC, Liebefeld-Bern, Switzerland.

Tekalign, T., Haque, I. And Aduayi, E.E. (1991). Soil, plant, water fertelizer, animal, manure and compost analysis manual. Plant Science Division Working Document No. 13. International Livestock Centre for Africa (ILCA), Addis Ababa, Ethiopia.

Thuren, A. and Larsson, P. (1990). Environ. Sci. Technol. **24**: 554.

Trenton, N.J. (1986). Hazardous Substance Fact Sheet on Dimethyl Phthalate. New Jersey Department of Health.

Tsuchiya, K. (Ed.) (1976). Cadmium studies in Japan. Amsterdam, Elsevier.

Valentine, N. B., H. Bolton, Jr., M. T. Kingsley, G. R. Drake, D. L. Balkwill, and A. E. Blymale. (1996). J. Ind. Microbiol. **16:**189-196.

Van Der Merve, A.J., Johnson, J.C. and Ras, L.S.K. (1984). A NH_4HCO_3-NH_4F-$(NH_4)_2$ EDTEA method for determination of extractable P, K, Mg, Cu, Fe, Mn and Zn in soils. SIRI int. Bull. B2/2.

Walkey, A. and Black, I.A. (1934). Soil Sci. **37**: 29-38.

Webber, M.D. (2001). Wastewater Technology Centre, Burlington. USA.

Wixson, B.G. and Davies, B.E. (Eds). 1993. Lead in Soil Task Force-Recommended Guidelines. Society for Environmental Geochemistry and Health. Science Reviews, Northwoo: 132.

WHO (1977). Environmental Health Criteria 3: Lead. Geneva, World Health Organization.

WHO (1978). Principles and Methods for Evaluating the Toxicity of Chemicals. Environ. Health Crit. 6.

WHO (1980). Recommended Health-based Limits in Occupation Expossure to Heavy Metals. WHO Tech. Rep. Ser. 647.

WHO (1984). Guidelines for Drinking Water Quality. **1-3**. WHO, Geneva.

WHO (1987). Regional Office for Europe, Air Quality Guidelines for Europe. WHO Regional Publications. Europian Series No. 23, Copenhagen.

Yamana, M., Dilginb, Y. and Gucer, S. (2000). Analytica Chimica Acta, **410**:1-2:119-125.

Yeomans, J.C. and Bremmer, J.M. (1989). Commun. Soil Sci. Plant, Anal., **197**: 1467-1476.

Zabel, T.F. (1989). Sci. Total Environ. **78**:187-204.

Zimdahl, R.L., and Stogerboe, R.K. (1977). Environ. Sci. Technol. **11**: 1202-1207.

http://www.atsdr.cdc.gov

http://www.esf.edu/pubprog/brochure/soilph/soilph.htm

http://www.humintech.com/001/animalfeeds/general.html

http://www.nal.usda.gov/ttic/tektran/data/000010/38/0000103864.html

APPENDIX 1

AAS instrumental parameters

Element	**Wavelength**	**Flame**	**Bandpass**	**Lamp**
Cd	228.8	Air/acetylene	0.5	HCL
Pb	283.3	Air/acetylene	0.4	HCL