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THE USE OF ORGANIC MATERIALS AS AMENDMENTS IN THE REMEDIATION OF SOILS CONTAMINATED BY LEAD, COPPER AND ZINC

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Summary

The effectiveness of using amendments in metal stabilization was determined, and their potential for remediation of contaminated soil was evaluated. A combined approach of evaluating soil and plant metal availability as well as microbial respiration was used as an indication of effectiveness of metal immobilization. This was done by sorption studies, incubation experiments in contaminated media, and greenhouse pot experiments.

Batch sorption studies were conducted to evaluate the ability of bone meal, composts, peat, coir and wood bark to sorb Pb, Cu and Zn. Single sorption carried out over a metal concentration range of 0.1 mmol l^{-1} to 5 mmol l^{-1} using 0.001M and 0.1M $Ca(NO_3)_2$ as background electrolytes showed very high sorption of Pb, Cu and Zn by the amendments, but high background salt led to a reduction in the amount of metal sorbed by all amendments. Of the amendments tested, coir, compost and wood bark were most effective. Sorption was evaluated by applying the Langmuir equation, and maximum sorption values were calculated for all amendments. Theoretical maximum sorption of Pb was 87 mg g^{-1} (0.42 mmol g^{-1}) by coir and green waste compost; Cu was 30 mg g^{-1} (0.47 mmol g^{-1}) by green waste and general compost, while maximum Zn sorption was 14 mg g^{-1} (0.21 mmol g^{-1}) in composts , closely followed by 13 mg g^{-1} (0.19 mmol g^{-1}) in coir.

Sorption in mixed metal solutions of Pb, Cu and Zn was evaluated in a background salt of 0.001M $Ca(NO_3)_2$ only, either at equimolar concentrations of 0.1 mmol l^{-1}, equimolar concentrations of 1 mmol l^{-1} , or combinations of metals at either concentration. Metal sorption was reduced in the presence of other metals when compared with sorption in single metal solution. Pb sorption in equimolar solution of 0.1 mmol l^{-1} was approximately 50% of that in single solution, Cu was 35%, while Zn was 40% if wood bark was not considered (wood bark sorption of Zn in low equimolar metal was not different from that in single Zn solution).

Two separate incubation experiments were conducted to evaluate the effect of amendments on microbial respiration in metal contaminated media. First, the effect of metals on microbial respiration was evaluated in metal spiked amendments over a ten week period using metal solutions of Pb, Cu and Zn as contaminants. Pb, Cu and Zn toxicity led to an inhibition in CO_2 evolved in all amendments, as addition of any amount of Pb, Cu or Zn led to a decrease in amount of CO_2 evolved when compared with the non-contaminated amendments. The effect of increasing metal toxicity on CO_2 evolution was reflected best in coir, where inhibition increased with an increase in metal concentration. In other contaminated amendments however, the inhibition was highly pronounced once there was metal contamination regardless of the metal concentration.

In a second incubation study, the ability of amendments to enhance respiration in field contaminated soil was evaluated over a ten week period. CO_2 evolution in contaminated soil was enhanced by the addition of all amendments, increasing as amount of amendments increased. Cumulative CO_2-C evolved in non-amended contaminated soil was 93 mg CO_2-C kg soil^{-1} over the entire 10 week period, whereas, amending with only 1% wastes increased this to a cumulative of approximately 250 mg CO_2-C kg soil^{-1} in coir, peat and wood bark. There was variation in microbial responses in the different amendments, which may have been due to their varying ability in the supply of substrate carbon to microorganisms.

Two separate pot experiments were carried out in metal contaminated soil. In the first experiment, mine waste and sewage sludge treated soil were used as contaminants to 'spike' sharp sand at 2.5% and 25% of dry sand weight respectively. This 'made soil' was then amended with bone meal, compost, peat, coir and phosphate as 10% of 'made soil' weight, and used to grow ryegrass over a 16 week period. Addition of amendments led to increased biomass yield when compared to the non-amended soil, and biomass yield in non amended soil was higher in the sewage treated soil than in the soils treated by mine waste. Bone meal and coir led to the highest root and shoot yield.

In this first pot experiment, the effect of amendments on soil and plant metal concentrations was not clear. While there was significant reduction in soil Pb with the addition of amendments in mine waste spiked soil, there was no significant effect of amendments on available soil Cu and Zn, except by peat which led to higher soil available Cu and Zn. In the sewage sludge treated soil, there was no significant effect of amendments on available soil Pb, Cu or Zn. The inorganic source of mine waste led to greater Zn phytotoxicity than the organic sewage sludge treated soil, and leaf metal concentrations in all amended soils remained very high, reflecting the very toxic nature of the contaminants used. Nevertheless, there was significant reduction in leaf Pb by all the amendments, and leaf Cu was significantly reduced by compost, peat and coir when compared to the non-amended soil. Leaf uptake of Zn was however increased by all amendments except coir, which was not significantly different from the non-amended soil.

In the second greenhouse study, a contaminated field soil was used in pot experiments. This soil was selected for two main reasons: one, the Pb, Cu and Zn concentrations were much lower than in the soil used for the first pot experiment and second, it was naturally contaminated by long term deposition of mine waste. This soil was diluted by sand on an equal dry weight basis, before being used. Amendments green waste compost, peat, coir and wood bark were then added at zero, 1%, 10% and 20% and left to stabilize for four weeks before it was sown with ryegrass. There was increased biomass yield with addition of amendments, and EDTA extractable Pb, Cu and Zn was significantly reduced in amended soil. Leaf and root metal concentration was also significantly reduced by the application of amendments, although 20% peat led to the solubility of Zn, thus increasing extractable soil Zn and plant tissue Zn concentrations.

The main aim of this research was to stabilize metals in soil by the addition of amendments, and this was achieved. In all the different experiments, coir, composts and wood bark stood out as amendments which were consistent in sorbing Pb, Cu and Zn, reducing soil and plant available metals, and enhancing microbial respiration. Peat and bone meal sorption of metals was also high and comparable with results obtained elsewhere, but peat led to the solubility of Cu and Zn, especially at high rates of application.

List of abbreviations

mg g^{-1}	milligram per gram
mg l^{-1}	milligram per litre
µg g^{-1}	microgram per gram
mmol l^{-1}	millimole per litre
mmol g^{-1}	millimole per gram
g t^{-1}	gram per tonne
cmol K kg^{-1}	centimole potassium per kilogram
kg N ha^{-1} yr^{-1}	kilogram nitrogen per hectare per year
mg CO_2-C g^{-1} $week^{-1}$	milligram carbon dioxide-carbon per gram per week
ppm	parts per million
LSD	least significant difference
DOM	dissolved organic matter
FEPA	Federal Environmental Protection Agency (Nigeria)
FWR	Foundation for Water Research
UNEP	United Nations Environment Programme
USEPA	United States Environmental Protection Agency

List of terms

amendment	material added to the soil to modify it physically or chemically
anthropogenic	due to man's activities
batch sorption	sorption experiments evaluating metal binding over a range of concentrations using a sorbent, often entailing agitation of sorbent with solution, followed by filtering or centrifuging
bioavailable metal	metal ions present in a form which can be taken up by plants or microorganisms into their tissue cells, usually extractable by weak acids or salts
equimolar	equal metal concentration in moles per litre
'made soil'	growth medium deliberately created using soil or sand as the main component
'metal spiked'	addition of a small quantity of metal in solution or solid form in order to induce toxicity
metal toxicity	high metal concentration, to the extent of causing harm
nutrient	substance which supplies essential elements to improve plant growth
organic	of a natural, degradable source
plant biomass	plant leaves, stems and roots
remediation	to reclaim or cause to recover from contamination
sorption	to adhere or bind to a surface
total metal concentration	sum of metal bound to various fractions of soil or to any other material which can be extracted by acid digestion
waste	by-product of industrial or agricultural activity

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Dedication

To Ike
And our three 'angels'
Kelly, Chibu, and Giftie-D

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Author's Declaration

Except where specific reference is made to other sources, the work presented here is the original work of the author. It has not been submitted, in part or in whole, for any other degree. Some of the results may have been published elsewhere.

Nwachukwu

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Chapter 1.

Introduction

1.1 Rationale

Metal contamination of soils is widespread across the globe as a direct consequence of atmospheric and industrial pollution, and the clean up of such soils is a difficult task (Diels *et al.*, 2002; Voegelin *et al.*, 2003). Soil remediation is an urgent need in developing countries as they face rapid industrialization, and the challenge becomes the achievement of effective remediation through techniques which are inexpensive. This research was initiated as a direct response to the escalating problems of industrial soil contamination in Nigeria, and three approaches were taken to evaluate agricultural materials as a viable option to clean up metal contaminated soil. The capacity of the amendments to immobilise metal was determined; their effect on plant growth and metal uptake was evaluated, and the effect of metal on microbial breakdown of the amendments was also evaluated.

1.2 The contaminated environment

According to UNESCO (1986), the environment encompasses every living and non living object, the interactions between those objects, and the products of their interactions. This of course includes the atmosphere, bodies of water, the soil, and all the organisms that live in them. Heavy metals have been known to adversely affect soil physical and chemical properties, plant growth, and soil microbial population (Gibbs *et al.*, 2006; Jasper, 1994; Tiller, 1998). In the 1990 Environmental Protection Act of the UK, contaminated land is defined as "*any land which appears to be in such a condition, by reason of substances in, on or under the land that significant harm is being caused, or there is a significant possibility of such harm being caused: or pollution of controlled waters is being, or is likely to be, caused*"(DEFRA, 2006).

Soil is primarily considered as a 'source' of nutrients for plant growth, but it also acts as a 'sink' for the removal of contaminants from industrial and agricultural waste materials (Basta *et al.*, 2005; Bolan *et al.*, 2003). The soil is however, a finite and non renewable resource (Lal & Stewart, 1992). Heavy metals are highly persistent in soil, and their residence time can run into thousands of years. Unlike organic pollutants which are susceptible to biological degradation, a heavy metal will not be degraded into a harmless product, and many are considered as persistent toxins (Lee *et al.*, 2004; Sparks, 2003). These metals can however, be immobilised in contaminated soils, and made unavailable for plant uptake, thus remediating such soils (Nachtegaal *et al.*, 2005; Usman *et al.*, 2006).

Heavy metals, also referred to as potentially toxic elements, are a loose classification of a group of elements with an atomic density greater than 6 g cm^{-3} (Alloway, 1990a) .Some of these elements like iron, copper, zinc and manganese, are essential for living organisms, but over a certain 'threshold concentration', they become toxic (Oudeh *et al.*, 2002; Rout & Das, 2003). Others like lead and cadmium are non essential and are toxic even in trace amounts (Tuzen, 2003). Whether metals act as essential nutrients or toxic elements therefore depends not only on their biological role, but often also on their concentration (Gibbs *et al.*, 2006; Staunton, 2002).

1.3 Pollution, or contamination?

Pollution is widely accepted to be " *..the introduction by man into the environment of substances or energy liable to cause hazards to human health, harm to living resources and ecological systems, damage to structures or amenity, or interference with legitimate uses of the environment*" (Holdgate, 1979). The terms 'pollution' and 'contamination' are often used interchangeably, with the tendency being to use the term 'contamination' where inputs do not appear to cause obvious harmful effects, and the term 'pollution' in situations where toxicity has occurred (Alloway, 1990b). Pollution, simply put therefore, can be regarded as a poisoning of the environment; the harm that results because of the presence of substances where they would not normally be found, or where such substances are present in larger than normal quantities. Nevertheless, whether harm is caused or not, the generally accepted convention for soils is to use the term 'contamination' for any situation in which there has been an increase in the concentration of any substance (Alloway, 1990b) . Thus, contamination shall be used throughout this thesis.

1.4 Natural occurrence of metals in soils

Trace metals often form part of the parent material of many soils, and as such, many occur naturally in soils. On weathering, many of these metals are released into the soil system, where some may go into solution and be consequently taken up by plants. Under natural conditions, only a small fraction is present in plant available form, but in some soils developed from metal-rich parent materials, labile forms may be as high as 30 to 60% of the total (Karczewska *et al.*, 1998). The distribution of heavy metals in soil profiles is however, continuously altered due to the natural turnover in the rock-soil-plant system (Kabala & Singh, 2001).

The point at which any single element is considered toxic is subject to many controlling factors like soil texture, particle size, pH, and clay content (Cao *et al.*, 2004; Rooney *et al.*, 2006). Due to inherent differences in physico-chemical properties, soils differ in their ability to bind metals. Plants also differ in their tolerance of metals. While some are hyperaccumulators, others are adversely affected by the lowest trace of certain metals. Tolerance may even vary between varieties of the same plant (Gimmler *et al.*, 2002; Song *et al.*, 2004). The critical soil concentration of metals is regarded as the range above which toxicity is considered to be possible, while that in plants is the level above which toxicity effects are possible, and which values are likely to cause a ten percent depression in yield (Kabata-Pendias & Pendias, 1992). 'Normal' soil concentrations in soils and plants are presented in Table1.1.

Table 1.1 Typical concentrations of some trace metals in soils and plants

Element	Normal range in soils(total) ug g^{-1} dry wt	Toxic soil concs(total) ug g^{-1} dry wt	Normal range in plants ug g^{-1} fresh wt	Toxic concs in plants ug g^{-1} fresh wt
Cr	5-1000	75-100	0.03-15	5-30
Mn	200-2000	1500-3000	15-1000	300-500
Co	1-70	25-30	0.05-0.5	15-30
Ni	10-1000	100	0.02-5	10-100
Cu	2-100	60-125	4-15	20-100
Zn	10-300	70-400	8-400	100-400
Cd	0.01-7	3-8	0.2-0.8	5-30
Sn	<5	50	0.2-6.8	60
Hg	0.02-0.2	0.3-5	0.005-0.5	1-3
Pb	2-200	10-400	0.1-10	30-300

(concs – concentrations)
Sources: Adapted from Bohn *et. al.*(1985); Kabata-Pendias and Pendias(1992)

1.5 Diffuse and point source contamination

As relates to origin and eventual scope of contamination, soils can become contaminated in either of two ways, a point source, or diffuse contamination. A point source of contamination comes from a single, easily identifiable origin, enters the environment at a specific site, and its effects are usually restricted to a relatively small area (De Wilde, 2007; Pulford & Flowers, 2006). Potential point sources of contamination include effluent discharges from sewage treatment works, power stations, landfill sites, fish farms, oil spillage via a pipeline into the surrounding soil, pesticide spillage from a sprayer, etc. It is easier to prevent a repeat of a point source contamination because, having been identified, immediate remediative action can be taken such as a localised clean up or a longer term investment in facilities for clean-up.

A diffuse contamination on the other hand arises where substances are dispersed over a large area as a result of activities such as enrichment of rivers through leaching of fertilisers or through erosion, urban development, forestry, gas effluents from industries, or even liquid wastes from factories which have contact with surrounding soil in discharge channels as it travels over large distances (FWR, 2005; Pulford & Flowers, 2006).

It is however difficult to partition contamination as strictly single or diffuse, because the origin of some contamination may be a single point, while the eventual deposition or fall out may cover a very wide area. For example, a mine waste may be heaped in a tip at a specific location, but with run off and dispersion of fine particles by wind, rivers and large land areas far from the tip may become contaminated (Farmer *et al.*, 1997). Acid mine drainage may rise up through soil horizons leaching out heavy metals, thus transporting them to locations far from the contamination source (Bertocchi *et al.*, 2006). Another situation may be a power station emitting gases like sulphur dioxide or nitrous oxide into the air. The gas emission is quickly dispersed, oftentimes covering a whole town. Although these are both point sources, the eventual impact will be over a wide area as diffuse contamination.

1.6 Sources of Soil Contamination

Soil contamination may arise from either natural or anthropogenic causes. The UK Environment Agency has estimated that there are around 20,000 contaminated sites in England and Wales, while Scotland has 171 sites covering 1,186 ha (Environment Agency, 2004; SEPA, 2006). The total number of contaminated sites in Europe is up to 300,000, while the United States EPA estimates there are over 450,000 brownfield sites in the USA (USEPA, 2001). The presence of soil and water contamination in Canada and Australia has also long been established (Li *et al.*, 2006). There is inadequate data on the extent of contamination in developing countries, but a rapid industrial development, coupled with a lack of enforcement of environmental legislation has brought water and soil contamination to alarming levels in those regions (Olajire *et al.*, 2003). Globally, the main sources of soil contamination however are industrial activity, atmospheric deposition, and agricultural practices (Lu *et al.*, 2005).

1.6.1 Agricultural Practices

Contamination of soils by metal-containing substances can result from local farm management practices. Ma and Rao (1997) found toxic levels of Pb contamination in soils which had received pesticide $PbHAsO_4$. High Cu content has been reported in orchard and vineyard soils following repeated and long-term use of $CuSO_4$ as pesticide (Chaignon *et al.*, 2003). The regular use of copper fungicides to control downy mildew has also led to long term accumulation of Cu in vineyard soils, becoming potentially bioavailable to plants and microorganisms (Brun *et al.*, 2001). It has also been found that the contamination of soils arose from the long term use of phosphate fertilizers which contained high levels of Cd.

Biosolids are a source of nutrients like N and P which enhance plant growth and act to improve soil physical and microbiological properties (USEPA, 1995). However, increased levels of Pb and Cu have also been found in soils fertilized with sewage sludge (Garrido *et al.*, 2005; Oudeh *et al.*, 2002). For instance, soils in south eastern Nigeria which had been subjected to a 40 year application of sewage sludge, were found to be very high in Pb concentrations up to a depth of 160cm (Udom *et al.*, 2004). Where water tables are shallow, this would constitute a risk of groundwater contamination. It has been reported that heavy metals are present in UK sewage sludge as a result of road run-off and industrial inputs to urban wastewater collection system, and that municipal sewage sludge generally contains higher concentrations of Cd, Cu or Pb (Rate *et al.*, 2004). In Spain, soils which had been amended by sewage sludge for more than 10 years were found to be higher in EDTA-extractable Cd, Cu, Mn and Zn than those which were fertilized with conventional fertilizers (Kidd *et al.*, 2007). What is more, such effects may last for decades, even after application of sewage sludge has stopped (McGrath *et al.*, 2000).

In recent years, the introduction of limits on metal supplementation of pig feed, tighter effluent standards to reduce metal concentration in sewage sludge, and the banning of pesticides containing mercury and arsenic has served to reduce the input of metals into soil from those sources (Gendebien *et al.*, 2001). In the UK, sewage sludge and livestock manures have been identified as significant sources of metal addition to agricultural soil (Nicholson *et al*, 2003). Despite improvements in sludge quality however, industrial sources still input up to 50% of the total metal in sewage sludge, while the most widely recognised contamination of inorganic fertilisers is known to be from Cd present in rock phosphate. An overview of the input of heavy metals to agricultural land in England and Wales is presented in Table 1.2. Heavy metal input rates on an individual or local field basis were mainly from sewage sludge application, although atmospheric deposition was an important source in terms of quantity on a national scale.

Table 1.2 Heavy metal addition rates (g ha^{-1} yr^{-1}) to agricultural land in England and Wales

Source	Zn	Cu	Ni	Pb	Cd	Cr	As	Hg
Atmospheric deposition	221	57	16	54	1.9	7.5	3.1	1.0
Sewage sludge	4557	3210	335	1256	19	926	34	13
Livestock manures[a]								
Dairy cattle slurry	1063	281	38	44	1.9	35	13.8	0.2
Beef cattle slurry	1214	321	43	50	2.1	40	15.7	0.2
Pig slurry	2321	1679	50	29	1.4	24	7.5	0.1
Cattle FYM	718	168	28	27	2.7	20	11.9	0.2
Pig FYM	2120	1488	48	27	2.0	22	8.7	0.1
Layer manure	2734	422	47	42	6.1	27	2.2	0.1
Broiler litter	1142	175	20	18	2.6	11	1.9	0.1
Inorganic fertilisers								
Nitrogen	2.2	1.6	0.2	0.7	0.1	0.5	0.1	<0.1
Phosphate	34	4.9	3.3	0.5	1.6	17	1.1	<0.1
Potash	0.5	0.4	0.1	0.2	0.0	0.1	0.0	<0.1
Lime	53	12	25	10	1.4	29	0.0	0.0
Irrigation water	39	16	1.6	0.8	0.1	0.1	1.2	nd
Paper sludge	1380	1270	102	45	12.5	150	nd	0.5

nd, No data; FYM- farm yard manure

[a] Rate of metal addition assuming an application rate equivalent to 250 kg N ha^{-1} yr^{-1}

Source: Nicholson *et. al.*(2003)

1.6.2 *Industrial Manufacture Processes*

Industrial and bio-industrial activities lead to soil contamination (Olderman, 1994), and industrial by products and effluents which find their way into the atmosphere, surface waters, soil or groundwater are often high in contaminant metals (Garrido *et al.*, 2005; Ramsey, 2005). In the U.K for example, some 16,500ha of land are regarded as 'hardcore' brownfield sites, contamination of which arose from their industrial legacy (English Patmerships, 2003). Several industries including electroplating, metal finishing, tannery, chemical and battery manufacturing, textile, paper, rubber and paint produce large volumes of waste which are high in heavy metals (Matos & Arruda, 2003).

The use of arsenic based chemicals as wood preservatives in Sweden has caused widespread metal contamination in soils due to raw material handling, spills, deposition of sludge, and leaching from piles of treated wood by rain water. In some areas in Portugal, liquid effluents from fertilizer and chemical industries have polluted the soil with heavy metals such as Pb, Zn and Cd and other pollutants for more than 40 years in some areas (Oliveira & Pampulha, 2006). Gas or dust effluents from some factories may persist in the atmosphere, leading to a permanent 'foggy' appearance over a general area, and these effluents inevitably find their way into the soil environment. Particles of these effluents eventually settle on plant surfaces, or get washed into the soil, where they have been known to lead to a reduction in yield (Seema *et al.*, 1996).

It has been reported that metal levels in soils and plants around industrial estates were significantly higher that the background concentrations obtained at sites remote from the vicinity of the factory (Adeyeye *et al.*, 2005; Fakayode & Onianwa, 2002). Where present in high levels in soil, industrial effluents have been known to be very toxic to crops. For instance, it has been found that exposure of sunflower to wastewater of fertilizer, detergents and oils factories, led to inhibition of radicle growth, shoot growth, chloropyll content, and transpiration rate (Gadallah, 1996). The physico-chemical analyses of the wastewaters revealed that metal contents were above the limits required for irrigating agricultural land.

1.6.3 Mining activities

The most devastating source of heavy metal contamination in modern times is from industrial activities linked to mining (ADB, 2001; Basta & McGowen, 2004). Mining produces large quantities of waste in the form of tailings which lead to very high metal contamination in soils in the vicinity of mining sites (Alloway, 1990a). Also, mining activities often cause great spills which lead to high concentrations of heavy metals in the soil (Razo, 2004). Emissions of the element being mined may be dispersed over the surrounding area, many of which contain toxic elements. Following deaths of horses in near a former Pb/Zn smelter in Austria, investigations showed that some died due to Pb intoxication (Friesl *et al.*, 2006). Blood and urine samples of humans and livestock in the area also revealed elevated metal concentrations. Other effects of mining waste on the environment are contamination of groundwater by dissolved pollutants, and the acidification of soil (Schreck, 1998).

Instances abound in many countries where mining activities have led to high levels of contamination in the soils and river sediments of those areas. In Romania, areas around all metal smelting complexes were found to be heavily contaminated by Cd, Cu, Pb and Zn (Alloway, 1990a). The uranium and thallium levels in tailing samples from an old tin mine in Nigeria was found to be 100 times above the background levels in non contaminated soils (Ibeanu, 2002), while metal contamination of soil and plants in the vicinities of gold mines in China and Nigeria were much higher than in unmined areas (Salami *et al.*, 2003; Zabowski *et al.*, 2001). Soils near a slate quarry in India were also found to be very high in heavy metals (Haritash *et al.*, 2007). In addition to the huge scale of contamination from mining activities, the impact also persists over a long period of time, usually long after mining operations may have ceased (MacKenzie & Pulford, 2002).Waste from a from a former lead-zinc mine remains a significant source of soil and river contamination in

Tyndrum, Scotland and Farmer *et. al* (1997) found that this pollution persisted even after operations had been discontinued for more than 80 years.

1.7 Factors controlling sorption of metals in soil

The sorption of metals in soil is dependent on a number of factors which determine both the rate of sorption, and the strength or tenacity with which metals are bound in soil. Clay content, pH, CEC, organic matter content, ionic strength of the soil solution, as well as the presence of other metals all have an impact on sorption of metals in soil.

1.7.1 pH

The pH of a soil or medium is the most important factor influencing the behaviour of heavy metals (Alloway, 1995; Cao *et al.*, 2004; Pulford, 2007). It has a big influence on the solubility of metals in soil, and low pH has been shown to enhance solubility of copper (Lin *et al.*, 2003). On the other hand, an increase in pH or alkalinization of soil may cause metals to form insoluble compounds as hydroxides, carbonates, sulphates or phosphates (Paulose *et al.*, 2007; Staunton, 2002).

It has been reported that concentrations of heavy metals in alkaline vertisols in Turkey were low, compared to non-alkaline sites examined (Aydinalp & Cresser, 2003). At pH above 6.0, most metal ions were totally fixed or precipitated, confirming that metals become less bioavailable under alkaline conditions. The removal efficiency of metals in soil has been found to be optimal at a pH between 4 – 5.5 (Lee *et al.*, 1997). At pH <4, the removal of Pb is negligible, while at high pH values, precipitation of Pb occurs (Walker *et al.*, 2003).

1.7.2 Clay content and soil texture

Clay minerals are products of rock weathering, and the clay fraction of soil is made up of fine grained silicate minerals of diameter less than 2µm. The sorption capabilities of clay result from their large surface area and a permanent surface negative charge, which make it possible for clay to attract and hold cations such as heavy metals (Alloway, 1995; Pulford, 2007). In soil, clay minerals usually have humic colloids and hydrous oxide precipitates linked to them, and this combined organo-mineral-colloidal complex further enhances the binding of metal ions in clayey soils (Alloway, 1995). Consequently, sorption of metals has been found to be greater in soils which are fine textured, especially those which are richer in clays, than in sandy soils (Bradham *et al.*, 2006; Rooney *et al.*, 2006). Shuman, (1999) found that sorption of Zn on to organic wastes was higher in finer textured soil when compared to a sandy one.

1.7.3 Cation Exchange Capacity

The cation exchange capacity (CEC) of a soil is '*a measure of its ability to hold cations at negative sites*' (Pulford, 2007), and it is expressed as centimoles of monovalent charge. In addition to determining cation retention and selectivity, CEC controls most of the other soil chemical properties (Bortoluzzi *et al.*, 2006). It is also a function of the prevalent cations in the soil, as their ability to be displaced by metal ions will lead to a reduction in the concentration of such metal ions in solution. In mineral soils, the CEC can range from a few, to 60 $\text{cmols}_c\ \text{kg}^{-1}$, but in organic soils, it may exceed 200 $\text{cmols}_c\ \text{kg}^{-1}$(Alloway, 1995). The cation exchange capacity of a soil is one of the most important factors which determine the bioavailability of metals to plants (Liphadzi & Kirkham, 2006).

1.7.4 Organic matter

In soil, organic matter has important direct and indirect effects on metal immobilisation (Staunton, 2002; Wang & Staunton, 2006). Direct effects may be through reduction in bulk density, increasing aggregate stability and water holding capacity (Tejada *et al.*, 2007); or by interaction due to their sorption properties, whereby metals become sorbed on surfaces of organic matter (Staunton, 2002). In addition, organo-mineral complexes may be formed in soil which lead to modification of mineral surface components, causing indirect effects (Sauve *et al.*, 2000). Much of the study on effect of organic matter on metal sorption has been on the direct effects such as sorption and complexation. The possible ways in which organic matter can influence the fate of metals in soil can be summarised by the following processes:

$M + L \longleftrightarrow ML$.................complexation, or

$M + H^+ \longleftrightarrow MH^+$ .... modification of pH, or

$M + e^- \longleftrightarrow M^-$ .... Oxido–reduction reaction;

Where M= Metal; and L= ligand

Source :Staunton, (2002)

It is well established that free metals, which account for the toxic, bioavailable fraction, are controlled by binding on to natural organic matter (Covelo *et al.*, 2004). Organic materials provide a large number of sorption sites for metals from which they may be difficult to displace. Organic compounds like fulvic acids contain a large proportion of hydroxyl and carboxylic groups which have been found to be very effective in forming bonds with metals (Adriano, 2001; Basta *et al.*, 2005; Brown *et al.*, 2000). When metals react with carboxylic and phenolic acid groups on organic matter, and more than one bond is formed between the organic molecule and the metal ion, the complex is called a chelate (Pulford & Flowers, 2006). The formation of metal chelates reduces solubility of metals in soil.

1.7.5 Ionic strength of the soil solution

Ionic strength of the background solution is another identified factor which controls the sorption of heavy metals in soil. Results have shown that high Ca and Mg concentrations in solution induced a significant decrease in Pb removal by cocoa shells (Meunier *et al.*, 2003). Reduction in metal removal by tree bark has also been observed, when Na^+ ions were high in solution, thus competing with the metals for sorption sites (Villaescusa *et al.*, 2000). In another study, it was reported that a ten fold increase in $CaCl_2$ as salt matrix led to about 75% reduction in the sorption of cadmium (Escrig & Morell, 1998). Although the relative strength of binding for monovalent cations has been found to be $Cs^+ > Rb^+ > K^+ > Na^+ > Li^+$, high ionic concentrations of an individual cation could dominate these preferences, and change the order of binding (Pulford, 2007).

1.7.6 Metal interaction effects

Studies on heavy metal mobility have shown that sorption is a competitive process between metals in solution and those sorbed on soil surfaces (Veeresh *et al.*, 2003). It is well recognized that in soils, multi-element contamination occurs more frequently than monometal contamination (Antoniadis *et al.*, 2007), as several metals are released simultaneously into the environment, and a competitive effect from other metals has been known to affect the sorption of any single metal in soil (Agbenin & Olojo, 2004; Leitao, 2003).

For example, in a laboratory study of two soils in a column experiment, a significant reduction in sorption of Ni was observed when Zn and Cu were also present (Leitao, 2003). In another study, Co^{2+} and Ni^{2+} competed with each other for sorption sites in soil (Harter, 1992). It is well documented that Cu^{2+} has a stronger affinity for organics than Zn, thus Cu will be selectively sorbed onto surfaces over Zn when both metals are present in soil. Other studies have also found that Cu sorption decreased when Zn^{2+} and Cd^{2+} were present in very high concentrations (Gomes *et al.*, 2001; McBride *et al.*, 1997).

1.8 Soil chemistry of metals

The solubility of metals is the main parameter causing their transfer into the hydrosphere and biosphere (Francois *et al.*, 2004). But it is the physico-chemical properties of any given soil that determine its sorption and desorption characteristics, and consequently, the behaviour of metals in such soils (During *et al.*, 2003).

Heavy metals in soil are to a large extent sorbed on to soil particles. The state, transformations and fate of metals in soil is complex. It is constantly changing, and dependent on a myriad of factors. Sorbed metals can desorb into soil water, thus move into plants or lower soil horizons and groundwater (Alumaa *et al.*, 2002). Conditions of water logging, the soil pH, organic matter content, chemical form of the metals in soil, and the types of plants and animals in the system are all major players in determining the eventual fate of toxic metals in soil (Ross).

Metals in the soil environment may exist as:

- Free metal ions and soluble metal complexes in the soil solution
- Metal ions occupying exchangeable sites and specifically sorbed on inorganic soil fractions

Metals bound to the organic matter. The more stable sources are metals bound to the organic fraction of soil and the long-term bioavailability of metals is determined by the re-supply from more stable sources to the mobile pool

- Precipitated or insoluble compounds, or
- Metals in the structure of silicate minerals

Adapted from Basta *et. al* (2005); Roberts *et. al.* (2003).

The bioavailable fraction is composed of free ions and those bound on exchangeable sites, while those bound to organic matter can also be rendered available on decomposition (Yin *et al.*, 2002). Bioavailability of metals in soil is said to be determined by an 'intensity' and a 'capacity' factor (Zhang & Young, 2006). While intensity has been attributed to the concentration in soil pore solution, the capacity is associated with the solid phase pool, which replenishes the solution. A consideration of both the intensity and capacity are therefore equally important for predicting metal bioavailablity.

1.8.1 Species and state of potentially toxic elements in soil

The bioavailability of a heavy metal critically depends upon the chemical form in which it is present in the soil. Chemical speciation of metals is the process of identifying and quantifying the different forms or phases in which they are present (Davidson *et al.*, 1994), and it is fundamental to understanding metal behaviour in soil. In addition, metals are toxic only when they occur in certain states. For example, chromium, as Cr^{6+}, is highly toxic to plants, whereas when it occurs as Cr^{3+} it is not. Oxidised iron, as Fe^{3+} is non toxic to plants. However, the reduced form, Fe^{2+} which commonly occurs in water logged conditions is phytotoxic. Thus rice grown in paddy soils has been known to suffer from iron toxicity.

1.8.2 Lead

Lead is neither essential nor beneficial for plants or animals (Davies, 1995). In uncontaminated soils, Pb is present in concentrations less than 20 mg kg^{-1}, though this may be higher in soils where there have been anthropogenic emissions over many years. Pb has a very low lability, and it may form insoluble, highly stable complexes with organic matter (Usman *et al.*, 2006). As a result of its low solubility and resistance to microbial degradation, lead has a longer residence time in the environment than other elements.

Sources of Pb in the environment include paints, pesticide production, Pb smelting and refining, and Pb acid battery breaking (Paff & Bosilovich, 1995). Fumes from car exhausts have been a major source of Pb contamination in urban areas, while contamination from sewage sludge is the most common arising from agricultural practices (Antoniadis & Alloway, 2003). In Nigeria, studies have revealed a high level of lead content in soils and vegetation near highways as a result of emissions from car exhausts (Awofolu, 2005; Olajire *et al.*, 2003).

1.8.3 Copper

Copper is commonly present in the soil as bound to organic matter, and it is an essential micronutrient required in chloroplast reactions, electron transport, carbohydrate metabolism and protein synthesis (Kiekens, 1995; Lin *et al.*, 2003). Available copper in soils is held mainly as cation ie.Cu^{2+} on surfaces of clay minerals or in association with organic matter. Due to its high affinity for organic matter, copper availability decreases as organic matter in soil increases. Increasing soil pH also increases the amount of copper held by clay and organic matter (Alva *et al.*, 2004). Most Cu in soil is in unavailable form, with values in agricultural soils ranging from 2 to 100 mg kg^{-1} (Baker & Senft, 1995). In addition to variations due to the wide possible range of copper in soil, the changing composition of soil and soil solution leads to differences in the amount of Cu that can be extracted as bioavailable (Wang & Staunton, 2006). Cu^{2+} absorption by plant roots is a function of the soil solution activity of Cu^{2+}. It has been shown that Cu in its ionic state is the highly bioavailable fraction, which is potentially very toxic (Cheng & Allen, 2005; McBride, 2001).

1.8.4 Zinc

Total zinc content in soils is largely dependent on the composition of the parent rock materials, and except for mined areas, the Zn concentration in most soils are considered as background values (Kabata-Pendias & Pendias, 1992). Mean total Zn contents vary from 40 mg kg^{-1} in acid rocks, to 100 mg kg^{-1} in basaltic rocks, but the average in most soils is 50 mg kg^{-1}. The burning of coal, other fossil fuels and the smelting of non ferrous metals are the major Zn sources contributing to air pollution. Sewage sludges also contain an appreciable amount of zinc, and uncontrolled use of sewage sludge on agricultural land often leads to an accumulation of Zn which poses toxicity risks for plants (McGrath *et al.*, 2000).

Zinc is an essential element for humans, animals and higher plants, and its deficiency always results in the impairment of a function. The element is required for respiration enzyme activators, and the synthesis of plant growth hormones (Kiekens, 1995). Zinc deficiency in humans leads to severe growth depression, skin lesions and sexual immaturity, and in plants, its deficiency causes stunting of shoot growth and a 'rosetting' of leaves (Kiekens, 1995). However, toxic levels of Zn in plants also leads to stunting of shoots, curling and rolling of leaf tips and chlorosis (Ye *et al.*, 1997).

1.9 The impact of heavy metals on soil- plant systems

Soil physico-chemical and biological properties are affected by the presence of heavy metals, especially when levels are toxic. It has been documented that soils polluted by industrial effluents or wastes tend to exhibit a reduced porosity, leading to compaction, (Udom, 2001). Loredo *et. al.* (2003), observed that such soils have poor hydraulic conductivity, with poor water percolation, leading to runoff, high erosion rate and leaching of salts (Hernandez *et. al.*, 2007). It is generally not possible to predict plant uptake based on soil levels alone, because plant uptake depends on several other factors.

Plant species play an important role in the uptake of metals from the soil, and metal tolerance occurs in some species more than others. This tolerance may result from either metal exclusion or metal accumulation (Ross & Kaye, 1994). Some plant species effectively prevent the transfer or metal ions from the soil solution into their roots, and the excluders may be found growing in highly contaminated soil without a commensurate uptake of the metals (Ross & Kaye, 1994). Yet other plants actively accumulate metals in their tissues with no apparent deleterious effect on them, and such plants are called hyperaccumulators (Boyd, 1998; Brooks, 1998)

1.9.1 The impact of metals on plant growth, nutrient uptake and yield

Soil-plant interactions with regard to nutrient uptake or metal accumulation in plant tissue are very complex (Oudeh *et al.*, 2002). While the actual concentration of metal within the plant is a direct effect of the mobility, bioavailablity and toxicity of the metal in question, the plant biomass and proportions of different soil humus fractions has an indirect effect (Mathe- Gaspar *et al.*, 2005). Plant uptake of heavy metals causes accumulation in plant tissues, leading to phytotoxicity and a possible change in the plant community (Gimmler *et al.*, 2002). Cu accumulates in plant roots, damaging such roots, which generally leads to stunted growth in plants, and eventual death (McBride, 2001). As a direct result of poor root development, plant uptake of other essential nutrients may be hindered.

1.9.2 Metals and microbial activity

The microbial biomass is an important component of soil organic matter and an important agent in soil organic matter breakdown; the degradation of pollutants, and the recycling of plant nutrients (Kizilkaya *et al.*, 2004). Many microbial processes therefore play vital roles which determine soil fertility and which are essential for the long-term sustainability of agricultural systems (Oliveira & Pampulha, 2006). Soil biota and the processes they regulate can become affected when stress factors such as extremes of pH, temperature, or metal contamination are introduced into the environment (Oliveira & Pampulha, 2006). Much evidence suggests that microorganisms are far more sensitive to heavy metal stress than higher soil animals or plants growing on the same soils (Giller *et al.*, 1998). Changes in a microbial community can be directly linked to changes in soil quality (Breure, 2005; vanBeelen & Doelman, 1997), and soil biochemical characteristics are more dynamic and often more sensitive than physical or chemical soil properties (Friedel *et al.*, 2000). Thus, due to their function and ubiquitous presence, micro organisms can act as indicators of metal pollution (Yang *et al.*, 2006).

Elevated levels of heavy metals in soils have been shown to have adverse effects on the population size and overall activity of the soil microbial communities (Chander *et al.*, 2001; Stuczynski *et al.*, 2003). Properties such as microbial biomass, specific respiration rate, and potential metabolic activity tend to be adversely affected by metal toxicity (Gibbs *et al.*, 2006; Kelly *et al.*, 2003). For instance, the ability of bacteria to decompose leaf litter or complex substrates is significantly reduced by the presence of heavy metals (Burkhardt *et al.*, 1993; Kelly *et al.*, 2003).This is because microbial enzyme activity, as well as C and N mineralization are adversely affected by heavy metal toxicity (Aka & Darici, 2004; Kao *et al.*, 2006).

The impacts of pollutants can be extremely diverse, ranging from direct effects on soil microorganisms, to indirect effects which determine biochemical processes in soils. Thus, during decomposition processes, metals bound to organic matter may not necessarily exert a direct effect on micro – organisms (Post & Beeby, 1996). Soil microbial biomasses in high- metal contaminated soils have been found to be consistently lower than in uncontaminated or low metal soils (Barajas-Aceves, 2005). On the other hand, 'moderate' metal levels in soil may have no significant effect, or it may have a mild stimulatory effect on microbial processes (Khan & Scullion, 2002; Mathe- Gaspar *et al.*, 2005). Other studies have shown that time plays a very crucial role, as some media require to change their behaviour from enhancing, to one of inhibiting the microbial respiration (Montserrat *et al.*, 2006).

1.9.3 Metal contaminated food crops and human health

Accumulation of metals in the environment not only has deleterious effects on the ecosystem functions, but the metals constitute a health risk to animals and human beings (Matos & Arruda, 2003; Sun *et al.*, 2001). Heavy metal bioavailability in soil has been linked to risks concerning human food contamination through metal uptake by plants grown as crops or pastures (Antoniadis *et al.*, 2007). This may lead to potentially toxic elements entering the human food chain, with potential adverse effects (Rate *et al.*, 2004). In general, when crops are grown in contaminated soil, most heavy metals pose a negligible risk of accumulating to toxic levels in agricultural food crops. However, an element like cadmium is of great concern, as its transfer from soils to the edible portions of food crops is significantly greater than for other elements (McLaughlin *et al.*, 1996). Findings show that consumption of rice grown in Cd contaminated soil could pose a serious risk to human health. The element has relatively low toxicity to plants, but has been identified as a potent toxin to livestock and humans. Results from (Wang *et al.*, 2003), showed that up to 24% of total metal in plant biomass was concentrated in the grain of rice, grown in a cadmium-chromium-zinc contaminated paddy soil. There have also been reports of bone diseases caused by cadmium toxicity, from feeding on rice irrigated with water which had been contaminated from mining and smelting (Comis, 1996; Graham & Farmer, 2007).

Occurrence of lead in food crops is uncommon, and toxicity from Pb contaminated soil usually occurs from direct ingestion (Mielke, 1999). Symptoms of lead poisoning include nerve damage, increased blood pressure, reproductive disorders, mental retardation in children, and hyperactivity (Schwab *et al.*, 2005). However, it has also been shown that occupational exposure to Pb poses a very high risk of Pb poisoning, as auto mechanics and workers in dry cell battery factories were found with elevated Pb concentrations in their blood and urine (Adeniyi & Anetor, 1999; Amusan & Adeniyi, 2005). Lead is considered to be a risk to human health when soil concentrations exceed 400-500mg Pb kg^{-1} (USEPA, 2001). It was however found that the Pb contents in cassava tubers in the vicinity of a gold mine were much higher than the FAO/WHO food guidline of 0.3μg g^{-1}(FA0/WHO, 1976; Salami *et al.*, 2003). Given that cassava is a staple food crop in Nigeria, as in many other parts of West Africa, long term consumption of such contaminated food may prove hazardous.

Copper toxicity is known to cause stomach and intestinal distress, liver and kidney damage (GardeaTorresdey *et al.*, 1996). Movement of Cu into aerial parts of a plant is often minimal, but the element tends to accumulate in plant roots. Thus consumption of root crops grown in contaminated soil may pose a very serious threat. Zinc is one of the most soluble and mobile of the trace metal cations, thus transportation from soil to root to shoots of plants is very high. And because it is easily assimilated by plants, it can be highly phytotoxic (Rout & Das, 2003).

1.10 Cleaning up heavy metal contaminated soil

Contamination involves the transfer of heavy metals from a source, to a receptor which then becomes contaminated via a pathway (Pulford & Flowers, 2006). A successful clean up or remediation programme is one which can break the link between that source of contamination, and the receptor. Pollutant linkage is well illustrated by the model in Figure 1.1.

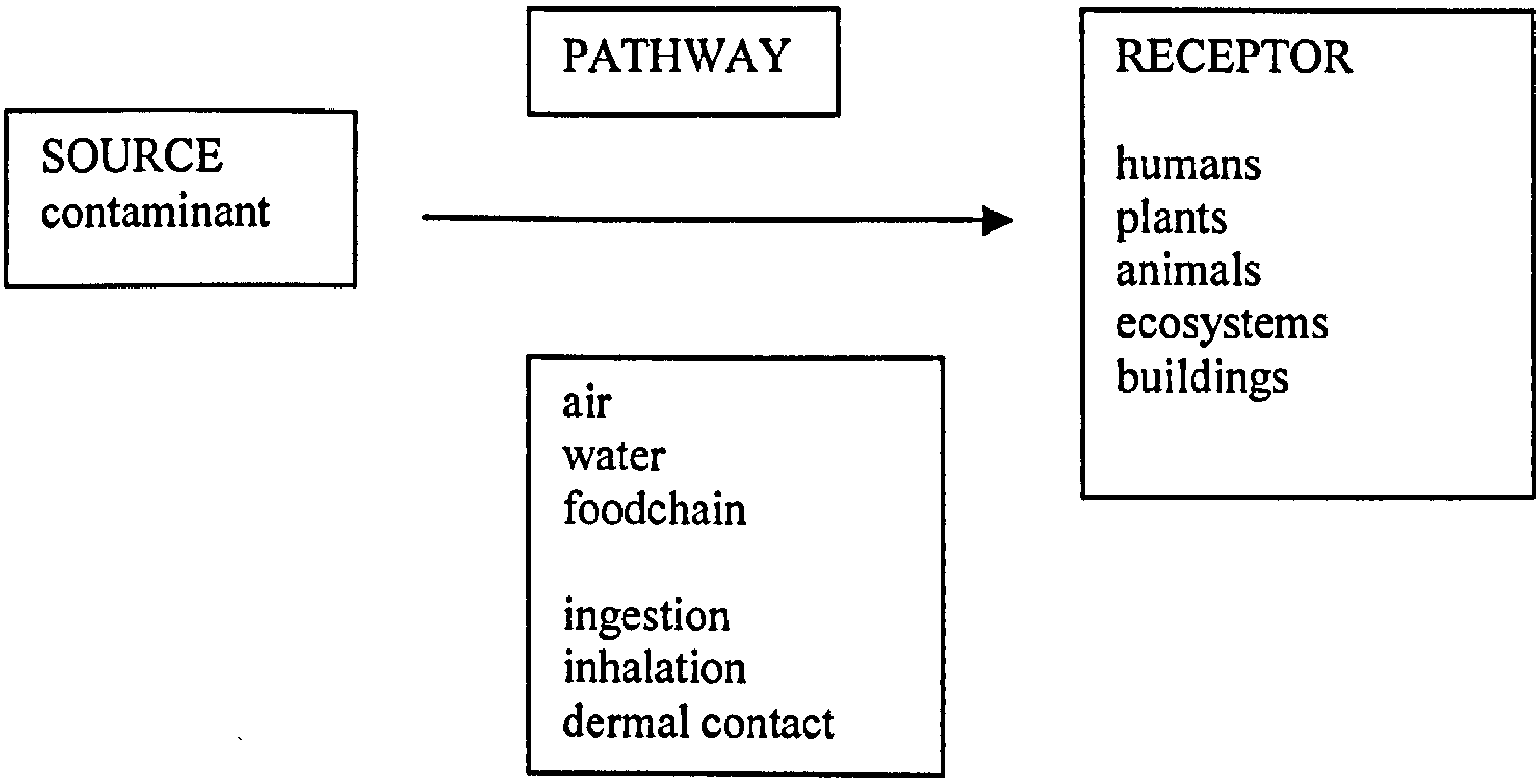


Figure 1.1 Pollutant linkage model (Pulford & Flowers, 2006)

1.10.1 Remediation techniques

Remediation of contaminated soils results in a recovery of microbial populations and activities, as well as re-vegetation of contaminated sites. Two major approaches have been used in the bid to clean up metal contaminated soils. These are either on – site (*in situ*), or away from the contamination site (*ex situ*). *Ex situ* methods often comprise **removal,** while **extraction** and **stabilization** are often used as *in situ* methods in remediating contaminated soil.

- **Removal.** This involves soil excavations, where the contaminated soil is dug up and moved away from the site. Clean, non- contaminated soil is then spread over the area. This radical approach, though often effective, is very expensive, involving the use of sophisticated, heavy machinery. When a large area of land is contaminated, the method becomes impracticable (Brown *et al.*, 2004). The 'dig and dump' practice also creates a new problem of metal contamination at the dump site. This practice has declined greatly in recent times, as a result of heavy taxes payable on landfills, and a shortage of suitable sites for 'dumping'.

- **Extraction** One method of extraction is through the use of hyperaccumulator plants to extract metals from contaminated soil. This method poses some constraints to effective *in situ* reclamation of soils because such plants often accumulate only one or two elements, they are often low in biomass yield, and their extraction efficiency diminishes with subsequent yields (Perez-de-Mora *et al.*, 2006). Soil washing with chemicals like $CaCl_2$ to leach out metal contaminants is another extraction technique which has been used to remediate contaminated soil. Soil washing works on the principle that contamination forms a surface coating on particles which can be removed by washing, flocculation and centrifuge processes. The technique is also expensive and the pH of the treated soil may be modified, which in turn has a direct effect on microbial populations. Through extraction, the delicate balance between different soil elements may be thrown into disarray and soil nutrient status depleted (Makino *et al.*, 2007). In addition, the procedure may generate additional risks by producing secondary wastes (Friesl *et al.*, 2003; Hettiarachchi *et al.*, 2001).

- **Stabilization.** Reducing heavy metal bioavailability without removing the metals from contaminated soils is the least ecologically invasive, and least expensive technique of soil remediation. Stabilization involves the use of natural materials to immobilise metals in soil, thus rendering them non- bioavailable (Meunier *et al.*, 2003; Usman *et al.*, 2006). It has been proven to be one of the most successful *in situ* methods for the immobilisation of heavy metals, and it effectively reduces toxicity to flora, fauna and public health. Apart from minimising social and ecological disturbance, stabilization is applicable to large contaminated areas, and the treatment can be sustained over a long period of time.

Innovative in situ technologies which require low inputs and cost tend to meet with social acceptance, while effectively bringing about soil remediation (Lombi *et al.*, 2004; Tiller, 1998). These desired qualities can be met by the use of cheap and abundantly available organic materials and wastes (Basta & McGowen, 2004; Bolan *et al.*, 2003). A sorbent material can be deemed as low cost '*if it requires little processing, is abundant in nature, or is a by-product or waste material from another industry*' (Bailey *et al.*, 1999). In the long term, they can be used to effectively decontaminate the soil, yet at a minimal disruption to balances in the soil environment.

1.10.2 Use of agricultural materials in cleaning up metal contaminated soil

The goal of using amendments as an *in situ* remediation technology is to achieve maximum reduction in bioavailability of metals. Several chemical and organic materials have been considered and tested for their effectiveness in immobilising heavy metals in soils. These include chemical fertilizers, agricultural limestone, mineral rock phosphate, and a wide range of agricultural waste products (Tiller, 1998). For instance, the application of 5% (w/w) red mud to clean silica sand reduced the extractability of Pb by nearly 90%, and that of Cu and Zn by more than 90% (Phillips, 1998). Addition of gypsum and phosphatic rock was effective in increasing the metal retention capacity of a contaminated soil, such that mobility of Cd, Cu and Pb was reduced (Garrido *et al.*, 2006). However, phosphate amendments were found to be generally more effective for immobilization of Pb, but less for that of Cu and Zn (Cao *et al.*, 2003). It has been suggested that in addition to phosphates, the bioavailability of soil Pb can also be reduced by using high rate of addition of oxide minerals and composts (Brown *et al.*, 2004; van Herwijnen *et al.*, 2006).

Organic materials act by re-distributing heavy metals from soluble and exchangeable forms, to fractions associated with organic matter (Quek *et al.*, 1998; Tordoff *et al.*, 2000).

Some organic materials which have been previously used in sorption studies include sago waste, saw dust, cocoa and peanut shells, oat, wheat and rice straw, sugarcane pulp, crab shells, and different types of biomass like bacteria, fungi, organic manures and sewage sludge (Bolan *et al.*, 2005; Bolan *et al.*, 2003; Chamarthy *et al.*, 2001). Remarkable success has also been achieved in cleaning up polluted water with peat (Brown *et al.*, 2000; Wilson *et al.*, 2003) and charcoal (Wilson *et al.*, 2003). Other studies have included the use of bone meal, compost, peat, coir, and tree bark as soil additives to immobilize the metals (Diels *et al.*, 2002). Composts of organic household waste, green waste compost, woodchips, bark chips, peat, cocoa shells, different types of animal manure, spent mushroom, and bone meal have also been used.

It has been shown that plant tissue concentrations of metals can be reduced by the addition of natural materials to contaminated soil. The Pb concentration in shoot tissue of wheat plants was effectively reduced by the application of phosphate fertilizers (Usman *et al.*, 2006), while in another study, addition of K_2HPO_4 to a contaminated soil both lowered the bioavailability of soil Zn as well as achieving a reduction in soybean tissue (Pierzynski & Schwab, 1993). Workers like Laperche, *et al.* (1997) reported that the addition of apatite to Pb-polluted soil led to a decrease of the Pb concentrations in plant shoots as a result of the formation of pyromorphite in the soil, and association of P with Pb in the roots. In another study, addition of zeolite significantly decreased shoot concentrations of Cu and Ni.

1.11 Selected agricultural materials

Seven agricultural materials were used at different stages in researching for this thesis, namely: bone meal, general purpose compost, peat, coir, wood bark, green waste compost, and phosphate. Composts, coir and wood bark were chosen because they are inexpensive and readily available in Nigeria; enhance soil structure, CEC and microbial activity; and they leave no toxic residue in the soil. Peat was included to serve as a reference to previous works, while bone meal and phosphate were chosen as inorganic sources that had been found effective in metal sorption.

Highlights on the individual properties of the materials are discussed in the following sections.

1.11.1 Bone meal

Much of bone meal is sold as a feed additive but with a content of about 27% total phosphate, it is also widely used in horticulture (Hall, 1998). Basically constituted of calcium phosphate, it has great affinity for Pb in solution, and a ratio of 1 bone meal :50 soil has been found to be effective in treating contaminated soil Zn, Ni, Cu and Pb (Hodson *et al.*, 2001). By forming metal bearing phosphates, bone meal is able to immobilise metals in soils (Hodson *et al.*, 2001).

1.11.2 Composts

Transforming organic wastes such as sewage sludge, industrial or green waste, and animal manures into compost has become a popular practice, reducing the need for chemical fertilizers. The practice of composting has also reduced the amounts of wastes dumped in landfill sites (van Herwijnen *et al.*, 2006; Whittle, 2002). Research has shown that the composting process greatly changes the composition of the dissolved organic matter (DOM) from organic waste, and DOM plays a fundamental role in immobilisation and transport of pollutants (Vaca-Paulin *et al.*, 2006). An increase in humin, humic acid and fulvic acid has been recorded in soil after addition of mature compost, and because humic acids released from composts are very resistant to degradation, their effects on metal mobility are likely to be long lasting (Adani *et al.*, 2007; Chien *et al.*, 2006). Addition of composts to metal-contaminated soils improves soil quality, by enhancing soil physical, chemical and biological properties (de Mora *et al.*, 2005). Compost is considered to be an environmentally safe, and relatively cheap amendment, which stimulates soil microbial activity and crop growth (Pascual *et al.*, 1997).

1.11.3 Peat

Peat is the first stage of coal formation, and it has been investigated as a sorbent for cleaning up contaminated wastewater. Inexpensive and in abundant supply, peat possesses several characteristics that make it effective in the removal of dissolved metal pollutants. It is a complex material with lignin and cellulose as major constituents, and these constituents bear functional groups like alcohols, aldehydes, ketones, carboxylic, and phenolic acid groups which are known to be involved in chemical bonding (Brown *et al.*, 2001; Qin *et al.*, 2006). The bonding nature of these functional groups enhances the ability of peat to bind heavy metals. Several studies have confirmed the natural ability of peat bogs to retain heavy metals (Brown *et al.*, 2000; Glooschenko & Capoblanco, 1982), and the actual mechanism by which metals are sorbed on to peat are said to be both complexation and ion exchange (Chen *et al.*, 1990). Those mechanisms are in turn dependent on conditions like pH, ionic strength, peat type and concentration. Peat is a very effective sink for carbon, thus it plays a very prominent role and is highly valuable in the light of escalating global carbon emissions. Therefore, there may be the need to make careful environmental considerations in the use of peat as a soil amendment, which would entail disturbing established peat cores.

1.11.4 Coir

This is a coarse fibre obtained from tissues surrounding the seed of the coconut palm, and it is generated in the separation process of the fibre from the coconut husk (Namasivayam & Sangreetha, 2006). With an annual production of about 7.5 million tons world wide, it is abundantly available (Gopal & Gupta, 2001), and is widely used as a peat free gardening material, because it makes an ideal rooting medium for young seedlings and cuttings. Coir has a high water holding capacity and an ability to retain nutrients against leaching (Vgrove, 2005). It contains lignin and cellulose contents of 70 and 30 percent respectively (Pino *et al.*, 2006) , coupled with an organic matter content of more than 95%. The cellulose and lignin are biopolymers which are associated with the removal of heavy metals (Pino *et al.*, 2006), and coir has been found to be very effective in removing up to 97% of Pb and Zn from solution (Conrad & Hansen, 2007).

1.11.5 Tree bark

The term 'bark' is used more often in a non technical context, and it refers to all tissues external to the vascular cambium (Trockenbrodt, 1990). It is available as a by-product of the timber industry, and it is high in tannin content (Vazquez *et al.*, 1994). It has been shown that the tree bark has higher ionic exchange capability than the wood from the same tree, and that aromatic hydroxyl groups in the bark are those mainly responsible for metal removal or sorption (Montes *et al.*, 2003). Martin-Dupont *et. al* (2006) found that tree barks with higher content of tannin and lignin gave the maximum sorption of Cr, Cu, Ni, Pb and Zn, and that it was the carboxylic acids and hydroxyl fractions of the tannins and lignins that was responsible for metal sorption.

1.11.6 Phosphate

Phosphate occurs naturally as rock phosphate and it has been shown that Pb phosphates can form rapidly when adequate Pb and phosphate are present in solution. In a study by Hettiarachchi *et. al* (2001), it was found that increasing the amount of P added in a Pb contaminated soil led to a highly significant reduction in bioavailable Pb.

1.12 Stabilization processes

Different terminology has been used by different authors to describe processes involved with the immobilization of metals, and this can be very confusing. Sorption has been identified as the most important process controlling the behaviour and bioavailability of metals in soils (Hettiarachchi *et al.*, 2000). Miretzsky *et. al* (2005) describes heavy metal sorption in terms of two basic mechanisms: specific sorption (surface complexation) and non-specific sorption (ion exchange). Sorption is a process which regulates partitioning of trace elements between solution and solid phases. During sorption, a material is used to bind molecules of heavy metals, thus decreasing the metals' bioavailability. Sorption involves the accumulation of a substance at an interface between the solid surface and the bathing solution

Stabilization of metals in soil, or the mechanisms by which metal is retained often involve specific adsorption, complexation, ion exchange, or precipitation processes. The different processes of metal stabilization is summarised in Figure 1.2. In some situations, a combination of more than one type of process takes place during metal immobilization.

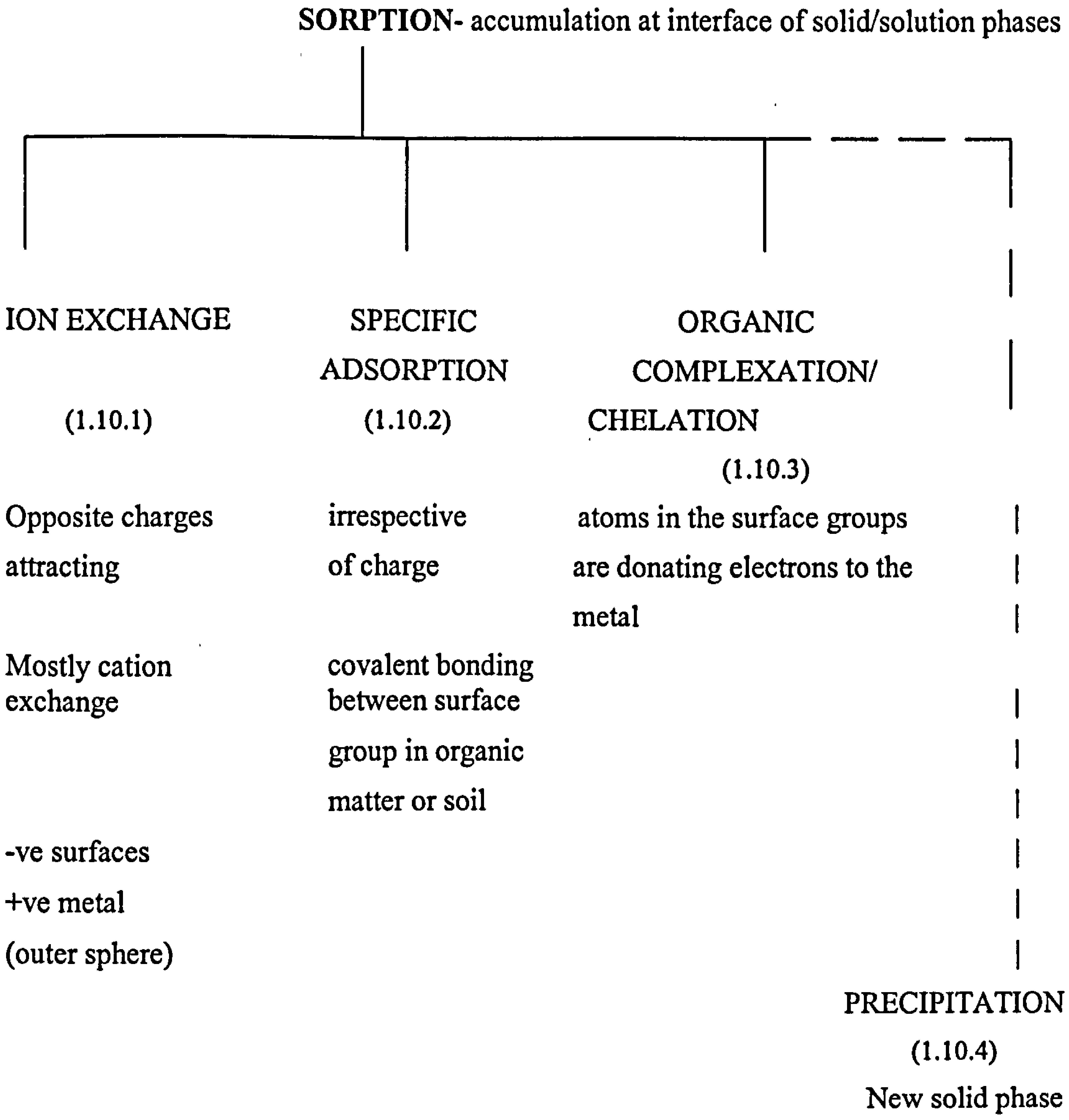


Figure 1.2 Components of the sorption process

1.12.1 Ion exchange

Ion exchange is 'the process where ions held at a charged surface by coulombic bonding are exchangeable with other ions of the same charge in solution in contact with the surface' (Pulford, 2007). In soils, cation exchange is the reaction of major importance, although some anion exchange may occur. Thus K^+ may replace Na^+ (or vice versa), when both are present in solution. Soil surfaces on which cation exchange can occur in soils are clay surfaces, humified organic matter and hydrous oxides (Alloway, 1995). The more highly charged ions are held at exchange surfaces in preference to lesser charged ones, thus Al^{3+} will be held more strongly than Ca^{2+}, and this in turn will be held more strongly than a monovalent cation like Na^{+} (Pulford, 2007).

1.12.2 Specific adsorption- (surface)

Sposito & Page (1985) suggests that specific sorption reactions can be considered as metal ions reacting with surface functional groups on clays or oxides. In effect, the sorbed ions replace a surface OH or H_2O grouping and become covalently bonded to the surface. This process is also known as chemisorption or ligand exchange (Pulford, 2007).

1.12.3 Complexation

Humic compounds with reactive groups such as hydroxyl, carboxyl, amine or sulfide groups can react with metal cations to form organometallic complexes, thus immobilising the metal (Alloway, 1995; Pulford, 2007). Metal ions in solution are surrounded by water molecules and complexes are formed when these molecules are replaced by another molecule called a ligand. Pairs of electrons are donated to the metal by electronegative atoms in the organic molecule to form a coordinate bond. If more than one bond is formed, the resulting complex is called a chelate, which is more stable than when a single bond is formed.

1.12.4 Precipitation

This is not a surface process, but occurs when a metal reacts with an anion to form a new and insoluble compound, thus taking that metal out of solution and rendering it unavailable for plant uptake. Precipitation often occurs with the formation of metal-phosphate complexes (Pulford, 2007). Other insoluble compounds which may be formed are hydroxides, carbonates or sulphates (Paulose *et al.*, 2007; Staunton, 2002).

It is quite difficult to separate the different types of processes, all of which may be occurring simultaneously (Lair *et al.*, 2007). The interest in this research was to remove metal ions from soil solution which may involve any of these processes, therefore in this thesis, the term sorption is used to describe all immobilisation of metals.

1.13 Soil and water pollution in Nigeria

Nigeria is located in the tropical zone of West Africa, with a land area of 923, 770 km^2, and like other developing countries, the country has seen increased pressure on the environment due to urbanisation, high rate of population growth and industrialisation. With the rapid growth in the Nigerian industrial sector, contamination of soil and groundwater from the non- oil sector has reached alarming levels in some places (UNEP, 2003), and the nation's Federal Environmental Protection Agency (FEPA) has long declared that soil and water pollution has reached critically high levels (FEPA, 1991).

With an international reputation for massive levels of oil pollution, it is not unexpected that much focus and funding had been on remediation of soils contaminated by crude oil (Amadi *et al.*, 1996; Ayotamuno *et al.*, 2006). In the past two decades however, research attention has been drawn to non-oil soil contamination, and several works have been done all over the country on soils contaminated by industrial activities, improper waste disposal and urbanisation (Akinola & Ekiyoyo, 2006; Oluwatosin *et al.*, 2005; Pasquini, 2006).

Unlike petroleum pollution which is generally restricted to the oil producing Niger Delta, large factories are situated all over the country, thus posing a threat over a wider area.

Nigeria has well structured environmental protection agencies, both at the federal and state levels (FEPA, 1991). However, as is common to most developing countries, there are no well established systems for waste management, nor is there an enforcement of legislations relating to toxic materials generated from different sectors (Anoliefo *et al.*, 2001; Nnorom & Osibanjo, 2006). For example, inappropriate disposal of used dry battery cells has seen an increase in Cd and Pb concentrations in emissions from 269 g t^{-1} in 1980 to 1062 g t^{-1} in 1998 (Nnorom & Osibanjo, 2006; Orisakwe *et al.*, 2004). Automobile waste dumps have led to elevated levels of heavy metals, constituting a serious threat to both surface and groundwater in some parts of Nigeria (Fakayode & Owolabi, 2003; Iwegbue *et al.*, 2006a).

Past mining activities such as coal, tin, and gold mining also introduced toxic metals into the soil and water environment in the country (Ibeanu, 2002; Salami *et al.*, 2003), a pollution legacy which is still a problem in the areas concerned. Industrialisation has brought economic development, but major environmental problems like air pollution, surface and groundwater contamination and soil contamination have also arisen from industrialisation in Nigeria (Anukam, 2003; Bako *et al.*, 2005). The major local industries responsible for on-going soil and water contamination include wood and pulp, paint and food, textiles, plastics, pharmaceuticals, brewing, distillery, iron and steel, and mining (Anukam, 2003).

Metal pollution has led to losses in agricultural production, and deleterious effects on human health have been reported (Awofolu, 2005; Olajire *et al.*, 2003; Oyedele *et al.*, 1995). High levels of Cd, Cr, Pb and even Hg have been reported in leafy vegetables contaminated by effluents and gaseous emissions from industries (Akinola & Ekiyoyo, 2006; Bakare *et al.*, 2004). The common practice of using dumpsites for cultivation and the application of ashed refuse as fertiliser has further increased the risk to human health, as crops produced in this way has been found to contain considerable amounts of heavy metals (Okoronkwo *et al.*, 2005; Pasquini & Alexander, 2004). Tree barks have also been found to be contaminated by metals through continuous exposure to fumes from car exhausts and open incineration of refuse (Ideriah *et al.*, 2006; Kakulu, 2003). The ultimate goal of this research is for future applications in Nigeria, as part of the on-going endeavour to find lasting solutions to the increasing pollution of soils from the non-oil industrial sector.

1.14 Aims and objectives of the research

1.14.1 Aim

The overall aim of the research was to evaluate the effectiveness of six agricultural wastes in the sorption of Pb, Cu and Zn, in order assess the potential of these materials in the remediation of metal contaminated soil.

1.14.2 Objectives

- To evaluate the capacity of the amendments to bind metals in solution
- To evaluate the effect of amendments on microbial respiration under metal toxicity
- To evaluate the ability of amendments to immobilise metals in contaminated soil
- To determine the effect of amendments on plant uptake of metals

Table 1.3 Some sources of soil and water pollution in Nigeria, and their geographical impact

Nature of the problem	Area affected	Chemicals/Pollutants
Hazardous waste treatment/disposal	Major cities	Obsolete pesticides, PCBs, heavy metals, asbestos waste, dry cell batteries, by-products from dyeing, painting
Air pollution	Urban locations and oil producing areas	Particulates, oxides, sulphur, carbon
Groundwater pollution	National	Leachates from municipal and industrial dumpsites, pesticide/chemical run offs, fertilizers, oil, heavy metals, mine tailings
Drinking water contamination	National	Trihalomethanes, heavy metals, pesticides, fertlizers, PAHs, PCBs
Marine pollution	Coastal areas	Oil spills, fertilizer, pesticides, heavy metals
Pollution of inland waterways	National	Heavy metals, fertilizers, pesticides, organic/inorganic pollutants, industrial discharges
Soil contamination	National	Empty chemical containers/packaging, pharmaceuticals, heavy metals, pesticides, acid rain, oil, industrial effluents

Source: Adapted from WHO/UNEP (1997)

Chapter 2

Materials and Methods

All the glassware and plastic-ware used during the research was cleaned by soaking overnight and washing in a 2-5% solution of Decon 90 chemical cleaner, rinsed three times with tap water, followed by rinsing three times in deionised water.

2.1 Characterisation of amendments used in the study

Table 2.1 Properties of the amendments

Amendments	pH (water)	% OM	% total N	CEC cmol K kg^{-1}	ug g^{-1} (total) Pb	Cu	Zn	% moisture
Bone meal	6.9	32	3.71	19.2	61	23	188	11
Coir	5.6	93	0.58	119.8	28	33	56	11
GP compost	6.4	77	0.89	62.3	30	23	49	68
GW compost	7.4	24	1.11	63.9	176	102	201	36
Peat	3.6	95	1.01	196.6	23	12	22	71
Phosphate	2.8	9	0.12	32.9	43	33	252	7
Wood bark	4.9	95	0.51	148.7	28	25	90	12

GP- general purpose; GW- green waste; OM- organic matter; CEC- cation exchange capacity

pH was read on a Mettler Delta 320 combination glass-reference electrode, organic matter determination was by loss on ignition at 450°C, total N by the Micro Kjeldahl digestion with ammonium measured by Techincon Autoanalyzer II, and CEC determination was by potassium replacement method using a Corning Flame Photometer 410. Moisture content of amendments was determined by drying to constant weight at 70°C over 12 hours, while total Pb, Cu and Zn was extracted in nitric acid.

All the amendments were purchased from Dagg's Garden Shop,Glasgow.

- **Bone meal-** The bone meal used was packaged by Daggs' as product 10304, indicated as containing total N of 3.5% and 20% P_2O_5. The original manufacturer was not stated

- **General purpose compost-** Peat free, 100% organic multipurpose compost that was manufactured from recycled materials and organic fertilisers (Gem Natural Earth Organic range, Gem Gardening, Accrington BB5 3NY).

- **Peat-** Sphagnum moss peat, an entirely organic material with good water retaining properties and a high air filled porosity (Humax Garden Peat, L&P Peat Ltd, Carlisle).

- **Coir-** Compacted dry blocks imported by Chempak, Nazeing, Essex EN9 2BB, the original source/manufacturer is unkown.

- **Wood bark-** a fine grade garden bark from Zeneca U. K.

- **Phosphate-** repackaged by Dagg's as product number 20204, this material was sold as 'superphosphates of lime', stated to have a P_2O_5 content of 18%, but information on the original manufacturer was not stated.

- **Green waste compost-** Dundee compost made by local residents from mown lawns, pruned trees and other green sources in their gardens

2.1.1 Preparation of amendments

All amendments were sieved to pass through a 2 mm sieve to ensure uniform particle size. For the sorption experiments, the amendments were air dried for 24 hours in the laboratory before use. For the incubation studies and greenhouse experiments however, amendments were used in the moist, naturally occurring form, based on an oven dry basis having previously determined their moisture content.

2.1.2 Buffering capacity of amendments

The ability of the amendments to withstand pH changes with increasing acidity was evaluated (Figure 2.1).

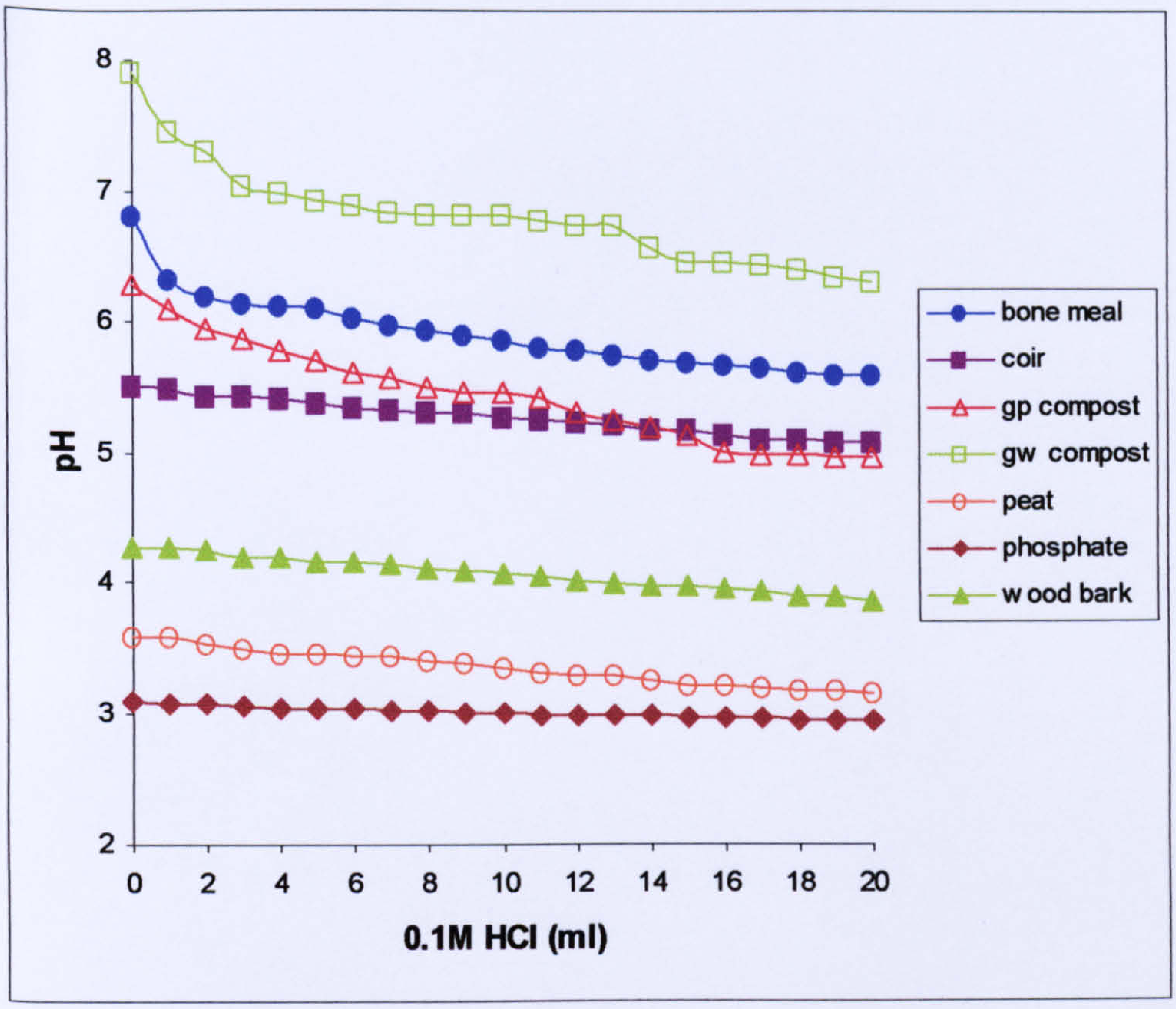


Figure 2.1 Buffering capacity of amendments in acid

Initial amendment pH was determined in deionised water as 1:10 ratio (5 g amendment in 50 ml deionised water) in order to allow for the swelling of coir which left no supernatant solution at a ratio of 1:5. The mixture was titrated with 0.01M HCl and pH readings taken at intervals of HCl addition of 1ml each, to a total of 20ml acid.

2.2 Preparation of metal solutions

ANALAR grade reagents were used throughout, and deionised water was used to prepare metal solutions unless otherwise stated. A specific mass of each metal salt was weighed to make a stock solution, which was subsequently diluted to make the concentrations required. Typically, a 10 mmol l^{-1} stock solution was made up for each metal in a 1 litre volumetric flask, and then diluted to give a range of 0.1 to 5 mmol l^{-1}. Metal solution concentrations were made in mmol l^{-1} in order to have a uniform reference point, and to achieve the same range in the given unit for all the metals used. Metal salts used in the preparation of metal solutions in the study were $PbNO_3$, $CuCl_2.2H_2O$ and $ZnSO_4.7H_2O$. The molecular name, chemical formula, and concentration of metal salts are shown in Table 2.2.

Table 2.2 Metal salts and electrolyte used in the study

Molecular name	Chemical formula of salt	1 g mol^{-1}	Weight salt (g) required to prepare
Lead (II) nitrate	$Pb(NO_3)_2$	Pb- 207.2	1 mol l^{-1} Pb = 331.2
Cupric chloride	$CuCl_2.2H_2O$	Cu- 63.55	1 mol l^{-1} Cu = 170.48
Zinc sulphate	$ZnSO_4.7H_2O$	Zn-65.39	1 mol l^{-1} Zn = 287.55
Calcium nitrate	$Ca(NO_3)_2$	Salt-164.09	*1M $Ca(NO_3)_2$ = 218.79

*salt used was 75% $Ca(NO_3)_2$.

2.3 General procedure for sorption

0.1, 0.2, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0 and 5.0 mmol l^{-1} of Pb, Cu and Zn was used to obtain sorption over a range of metal concentrations. 50 ml of metal solution was added to 0.1 g of samples weighed into 100 ml screw top glass jars in triplicate using a four figure open balance. The mixture was then agitated on an 'end over end' shaker for one hour at room temperature. Samples were immediately filtered through Whatman no2 paper, and stored at 3 - 4^0C until they were analysed using the AAS. 50ml of each metal concentration (without any amendment) was also shaken for one hour, filtered and then analysed as the initial metal concentrations.

Amount of metal sorbed was calculated as

$$X = \frac{(C_0 - C) \text{ x final volume(ml)}}{\text{weight of amendment(g)}}$$

where X = µg metal sorbed per g of amendment; C_0 = total metal concentration in solution before sorption(mg l^{-1}); and C = equilibrium metal concentration (mg l^{-1}).

Scatter points were plotted as final metal concentration in solution after sorption (C) against the amount of metal sorbed (X) to obtain a sorption isotherm, and Langmuir isotherms were plotted as C/X against C in order to assess the linearity of sorption for each element by each amendment. The two isotherms which are widely used to describe the sorption behaviour of metals in soils are the equations by Langmuir and Freundlich. Although the two equations were originally formulated from the modelling of sorption of gases, both bring into use the mass action law, and both models are similar at low concentrations (Alloway, 1995; Sposito, 1989).

The standard procedure outlined in Section 2.3 was applied in determining optimal time of shaking as a single point approach. It was also applied for producing sorption isotherms at varying metal concentration to get the capacity of amendments; varying background salt concentration; and finally, as single point method to evaluate metal competitive effects on sorption.

Scatter points were plotted as amount of metal sorbed (y axis) against equilibrium metal concentration (x axis) to obtain a sorption isotherm.

The Langmuir equation:

$x/x_m = Kc/(1 + Kc)$ Equation 1.1

was applied to data, where

c= equilibrium solution concentration(mg l^{-1} or mmol l^{-1}); x= the amount of ion sorbed(mg g-1 or mmol g-1); x_m= the maximum amount of ion sorbed; and K= a constant related to bonding

The equation is often used in its linearized form

$c/x = c/x_m + 1/Kx_m$ Equation 1.2

Sorption maxima (x_m) was calculated as the inverse of the slope of the Langmuir equation.

Sorption curves were obtained by obtaining a fitted line using the Least Squares deviation method, where

$$x_{\text{fitted}} = \frac{x_m\,(bC)}{(1 + bC)}$$

Where x_m is the maximum amount sorbed (mmol g^{-1}), and b is the Langmuir sorption constant (g L^{-1})and C is the equilibrium/observed solution concentration. In order to assess the linearity of sorption for each metal by individual amendments.

A theoretical maximum, rather than actual sorption makes for better comparison of the capacities of different amendments. It also provides information on the relative performance of any single amendment when compared with its maximum potential. This percentage performance is often subject to factors controlling sorption, as discussed in Section 1.9. Knowledge of the theoretical maximum also indicates the extent to which variable factors like concentration of background salt, or the presence of other metals affect the amount of metal sorbed. Care should be exercised in using the Langmuir equation to interpret data because in practice, the application of the equation is not always valid, as a straight line relationship may not be found. It is however widely used to obtain a value for x_m.

2.3.1 Effect of time on metal sorption

This was set up as a 5x6x3 completely randomised design. Five amendments- bone meal, general purpose compost, peat, coir and wood bark, were agitated at six shaking times- 15, 30, 60 and 120, 720 and1440 minutes. Each treatment was replicated three times, and a single concentration of 0.5mmol l^{-1} of each metal was used in the study, made up in 0.001M $Ca(NO_3)_2$. 50ml of metal solution (without any amendment) was also shaken for 15, 30, 60 and 120, 720 and 1440 minutes, filtered and then analysed as the initial metal concentrations. In order to minimise error arising from time difference in adding metal solutions to samples, and the time spent placing on and removing from the shaker, the experiment was carried out in batches of 15 samples only.

Using a four figure balance, 0.1g of air dry amendment was weighed into 100ml soft bottles, and 50 mls of metal solution measured into beakers, using an automatic dispenser which was regularly checked for accuracy of volume after every ten samples dispensed. Handling only 15 samples at a time, the metal solutions were rapidly tipped on to the amendments, taken for shaking on an end over end shaker for the specified times at room temperature, then immediately filtered through Whatman no2 paper, and stored at 3 - 4°C until they were analysed on the AAS. Dispensing time per batch was 30 seconds at onset and again while pouring into funnels after shaking. Thus the difference in contact time between the first and last sample handled was no more than 1 full minute. Loading and off-loading from the shaker took approximately two minutes. Isotherms were plotted of effect of contact time on the sorption of Pb, Cu and Zn.

2.3.2 Single Metal Sorption

Single solutions of Pb, Cu and Zn were made up using metal salts listed in Table 2.3. 10 mmol l^{-1} solutions of each metal were prepared in background $Ca(NO_3)_2$, then diluted to give 0.1, 0.2, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0 and 5.0 mmol l^{-1} respectively. The electrolyte 0.001M $Ca(NO_3)_2$ alone was used as the zero metal solution. 50ml of each metal concentration was added to 0.1g of each amendment, and then treated as described in Section 2.3.1 above. Amendments used were bone meal, coir, general purpose compost, green waste compost, peat and wood bark. The experiment was therefore set up as a 6x10x3 factorial; namely six amendments, ten metal concentrations and three replications.

2.3.3 Ionic strength of background salt

Single sorption of Pb, Cu and Zn was carried out in 0.1M $Ca(NO_3)_2$ using the method discussed in Section 2.3.2. The experiment was also set up as a 6x10x3 factorial, namely 6 amendments, 10 metal concentrations, and 3 replications. The amount of metal sorbed in 0.001M $Ca(NO_3)_2$ and 0.1M $Ca(NO_3)_2$ were then compared, and the effect of salt concentration on sorption determined statistically.

2.3.4 *Competitive (multi- metal) sorption*

Amendments evaluated for competitive sorption were bone meal, coir, general purpose compost, peat and wood bark. Metal concentrations 0.1mmol l^{-1} (low) and 1 mmol l^{-1} (high) were used in single point competitive sorption of multi-metal solutions of Pb, Cu and Zn. Multi-metal solution comprised four combinations, namely:

low equimolar solution of 0.1 mmol l^{-1} Pb, Cu and Zn (0.1:0.1:0.1 mmol l^{-1}) ...(1)
high equimolar solution of 1 mmol l^{-1} Pb, Cu and Zn (1:1:1 mmol l^{-1}) ....(2)
1 low metal + two high competing metals (0.1:1:1 mmol l^{-1}) ....(3)
1 high metal + two low competing metals (1:0.1:0.1 mmol l^{-1}) ....(4)

All multi-metal solutions were made up in 0.001M $Ca(NO_3)_2$, using the metal salts listed in Table 2.3. The experiment was set up as a 5 x 4 x 3 factorial; namely 5 amendments, 4 multi-metal solutions and three replications. Sorption was carried out as discussed in Section 2.3.1.

2.4 Measurement of metals

Metals can be measured in soils and wastes by the addition of a matrix which may displace the metal ion from its sorption site, thus forcing it into solution where it can then be measured. Other methods involve the digestion of the soil or amendment in acid, effectively removing every organic fraction, and the metals remain in the acid matrix where they can then be measured. The choice of extractant depends on the fraction of metal to be measured, whether total or available. Weak salts or acids are generally used to extract the bioavailable fraction of metals, while strong acids are used to extract the total metal content in soils or other media. Soils were air dried, and sieved to pass through a 2mm sieve, and stored in plastic bags until ready for analysis.

2.4.1 Total metal content

Total metal content was determined in soils, wastes, as well as in leaf and root tissue of ryegrass grown in contaminated soil.

2.4.2 Soils and amendments

Extraction of soils and amendments for total metal content was done using a 3:1 hydrochloric acid / nitric acid solution known as an aqua regia digest. The solution contained three parts 6MHCl to one part 69% HNO_3. An exact weight between 0.25 and 1.00g of material was weighed on a four-figure balance in triplicate. Samples were weighed into a scoop, which had been lined with aluminium foil, and with the aid of a long handle, the material was carefully placed at the bottom of block digestion tubes. 10ml of aqua regia was added to each sample, and the tubes were then allowed to stand overnight to allow the acid to equilibrate with the sample. The tubes were then placed in the digestion block and the temperature set at 125°C. Blank samples made up of aqua regia alone were also digested with each batch. The extraction unit in the fume cupboard was switched on to remove NO_2 gas evolved, and during the process of digestion, the digestion block ran for at least three hours, until the tubes were clear of brown NO_2 gas. The tubes were allowed to cool, before 10 mls of deionised water was added to each tube, and the digests then filtered, with washings, using Whatman No 50 hardened filter paper into 50 ml volumetric flasks. The solutions were then made up to volume with deionised water, and stored at 3 - 4°C until ready for measurement.

2.4.3 Plant tissue

Plant tissue was dried in an oven set between 70 – 80°C for two days until all samples were completely dry. The dry material was then ground using a bench top hammer mill in the fume cupboard, fitted with a 1. mesh size to ensure fine grinding of the material. Between samples, the grinder was cleaned using a compressed air gun and a vacuum cleaner, which was very effective in removing all the dust from each previous sample, thus preventing contamination. The ground material was then stored in self-sealing bags until ready for digestion.

In analysing ryegrass leaves and roots for Pb, Cu and Zn, exact weights between 0.25 and 0.5 g of each sample were weighed in triplicate using a four figure balance. Weighed samples were placed in digestion tubes by attaching a long handle to the scoop, so that samples were deposited right at the bottom of the tube. 10 mls of 69% nitric acid was added to each sample using an automatic pipette, and all samples were left for at least 12 hours to allow the acid to equilibrate with the plant material. Samples were then digested on a digestion block at 120°C for 3 hours until all fumes had stopped and the samples were clear. Blank samples made up of nitric acid alone were also digested with each batch. After cooling, 10 ml of deionised water was added to each sample, and then filtered through Whatman 50 hardened paper into 50 ml flasks. These were then made up to volume with deionised water, and extracts stored at 3 to 4°C until metal concentrations were read using an atomic absorption spectrophotometer.

2.4.4 Extractable metal content

Bioavailable Pb, Cu and Zn content of the soils and amendments was extracted in water and 0.05M ammonium ethylenediamimetetraacetate (EDTA).

2.4.4.1 Water

Water extraction of contaminated soils was used to measure the most readily available metal fraction. 5g of soil was weighed out in triplicate, into 100 ml glass jars with screw caps, and 50mls of deionised water was added. The samples were agitated for one hour using an end-over-end shaker. Samples were then filtered through a Whatman no 40 filter paper into plastic bottles, and stored at 3 - 4^{0}C until the samples were read on an AAS.

2.4.4.2 EDTA

0.05M EDTA was prepared by dissolving 14.6g of ethylene di-amine tetra acetic acid in approximately 950 ml of deionised water, to which 8 ml of ammonia solution was added. Then, by carefully adding a few drops of ammonia solution at a time, the pH of the solution was gradually raised to 7.0 (in the rare event that the pH exceeded 7.0, a few drops of M nitric acid was used to lower it back to 7.0). 5g of air- dried soil was weighed into 100 ml glass bottles, and 50 ml of 0.05 ammonium EDTA was added at room temperature. The solution was then shaken, filtered and stored as in section 2.5.2.1, until Pb, Cu and Zn were determined on the AAS.

2.5 Atomic Absorption Spectroscopy

There are five basic components of an atomic absorption instrument, namely: a light source which emits the spectrum of the element of interest; the flame, which is an absorption cell in which atoms of the sample are produced; a monochromator; a detector which measures the light intensity; and a display which shows the reading after it has been processed by the instrument electronics. The light source, which is a hollow cathode lamp is specific to each element, has a specified current, and operates optimally at a given wavelength. The process of atomic absorption is illustrated as

Light energy + ground state atom $\longrightarrow$ excited state atom

As the number of atoms in the light path increases, the amount of light absorbed also increases. Thus, by measuring the amount of light absorbed, the concentration of metal ions in the extract can be determined. The AAS used was a Perkin Elmer 1100B, and the conditions under which the metals were determined are shown in Table 2.3.

Table 2.3 Standard AAS conditions for the measurement of Pb, Cu and Zn

Element	Wavelength (nm)	Energy	Sensitivity check (mg l^{-1})	Linear range (mg l^{-1})
Pb	283.3	73-75	20	20
Cu	327.4	72-74	5	5
Zn	213.9	65-68	1	1

*The flame was air at 8.0, and acetylene at 2.5

2.5.1 Preparation of AAS standards

Analar grade metal standard solutions of 1000 ppm were used in all cases. The standard solutions were nitrates of Pb, Cu and Zn in 0.5 mol l^{-1} nitric acid as manufactured. To maintain the purity of the standard solutions, pipettes were not inserted into their bottles at any time. Rather, a small volume of each standard solution was poured into a 50 ml beaker, from which 10 ml was measured with a bulb pipette into a 100 ml volumetric flask, and made up to the mark with deionised water, to prepare 100 ppm stock solutions.

2.5.1.1 Single metal standards

From the 100 ppm stock solution, a series of standards were then prepared to calibrate the AAS before samples were analysed for metal concentrations. In each case, the set of standards was prepared in the same matrix that was used to extract the samples. For example, to prepare a 10 ppm standard of Pb for EDTA analysis, 10 ml of the 100 ppm Pb stock was measured with a bulb pipette into a 100 ml volumetric flask, then made up to the mark with 0.05M ammonium ethylene diaminetetraacetate. Where extractions had been done in $Ca(NO_3)_2$, 10 ml was measured from the 100 ppm Pb stock into a 100 ml volumetric flask, then made up to the mark with the appropriate concentration of $Ca(NO_3)_2$.

2.5.1.2 Multi-metal standards

Seven extraction matrices were used throughout the research, namely deionised water; 0.05M EDTA; 0.05M $CaCl_2$; 0.001M $Ca(NO_3)_2$; 0.1M $Ca(NO_3)_2$; aqua regia and nitric acid. Five concentration standards were used for each metal, and so due to the large number of matrices, standards comprised mixed metals. For example, Pb standards were 5, 10, 15, 20 and 25 ppm; while Cu and Zn were 1, 2, 3, 4 and 5 ppm respectively. This combination of metal concentrations was made up in one background at a time (i.e. standards in EDTA, standards in nitric acid etc) and is presented in Table 2.4.

Table 2.4 Composition of multi metal standards

Standard	Pb (ppm)	Cu (ppm)	Zn (ppm)
1	5	1	1
2	10	2	2
3	15	3	3
4	20	4	4
5	25	5	5

2.5.1.3 Standards in acid matrix

Preparation of AAS standards for acid extracted samples was slightly different. For standards in aqua regia, 5, 10, 15, 20 and 25mlof the 100 ppm stock solution of Pb, and 1, 2, 3, 4 and 5 ml of the Cu or Zn stock solution was measured with a bulb pipette into a 100 ml volumetric flask, to which 30 ml of deionised water was first added. 20 ml of aqua regia was then added to this, before the solution was made up to the mark with deionised water. For standards made up in nitric acid, the procedure was the same as for aqua regia, except for Pb, which was not first made up to 100 ppm stock solution, but measured directly from the 1000 ppm standard solutions. To obtain 5, 10, 15, 20 and 25 ppm Pb for example, 0.5, 1.0, 1.5, 2.0, and 2.5ml of the 1000 ppm Pb standard was measured with specific graduated pipettes for each volume, 30ml of deionised water was added, after which 20 ml of nitric acid was also added. The solutions were then made up to volume with deionised water.

2.6 Standard procedure for measuring metals on the AAS

To measure a specific element on the AAS, the appropriate lamp was inserted, the equipment programmed with information on the concentration of the standards, and the number of replications required per measurement. A blank which is made of the extracting matrix only was then used to 'zero' the equipment, after which the AAS was calibrated with the standards already prepared. One of the concentrations of standard was selected as the 're-slope', which essentially is a check to ensure continued accuracy of AAS readings.

2.6.1.1 Measurement of detection limit

The detection limit (DL) considers both the signal strength of absorbance, and the baseline noise or interferences from other sources, and it is the lowest concentration which can be clearly differentiated from zero. The AAS was calibrated as usual, and 15 ppm Pb, 2 ppm Cu and 3 ppm Zn standards were selected separately, and each was read 10 times. (Between each reading, the instrument was 'zeroed' with a blank, and re-sloped to minimise drift in the readings). A mean of the standard deviations for the 10 readings was obtained, and the detection limit calculated as

DL = 3 x means of standard deviation of ten readings;

Detection limit for Pb was found to be 0.23 mg l^{-1}, that of Cu was 0.02 mg l^{-1}, while that of Zn was found to be 0.05 mg l^{-1}.

2.7 Pot experiment in manually contaminated soils (2004)

This first pot experiment was carried out from June – September, 2004 (summer). Two contaminants were used, one of inorganic, and the other of organic source. The first was mine waste, a high Pb – Zn waste, which was obtained from waste tips very close to an old Pb mine at Tyndrum, Scotland. The other contaminant was 'sewage sludge treated soil', an organic source from Stoke Bardolph, Scotland, which was high in Cu, though lower in Pb and Zn than the mine waste. The contaminants were air dried, crushed and sieved to pass a 0.5mm sieve before being used.

Some properties of the contaminants are presented in Table 2.5.

Table 2.5 pH and total metal content of contaminants and 'made soil' before crop growth

	pH	CEC cmol K kg^{-1}	Pb ($ug\ g^{-1}$)	Cu ($ug\ g^{-1}$)	Zn ($ug\ g^{-1}$)
Contaminant					
Mine waste	5.9	7.7	65593(14)	2947(3)	25083(3)
Sewage sludge treated soil	6.9	60.1	1024(16)	895(6)	2302(5)
Made soil					
Mine waste treated	6.4	na	5247(31)	103(20)	2083(29)
Sewage soil treated	6.9	na	249(14)	171(19)	1979(10)

pH and metal values (RSD%) n=10; CEC (n=5);(na- not available)

2.7.1 Preparation of manually contaminated soils

Sharp sand was used as the major growth medium, while mine waste and sewage sludge treated soil were used as contaminants to 'spike' the sharp sand. The sand was Silvaperl 'washed and lime free horticultural grade' sharp sand (William Sinclair Horticulture Ltd, Lincoln LN6 7AH). Mine waste was added at the rate of 2.5%, i.e, 10 g mine waste to 400g sand, while sewage sludge treated soil was added at the rate of 25%, i.e, 100 g to 400g sand. Pb contamination from the mine waste was much higher than that from sewage sludge treated soil, while Cu contamination was higher from the sewage treated soil. However, the amount of Zn contamination from both sources was approximately the same. Amendments were added at the rate of 10%, and thoroughly mixed with the sand. The made soil was placed in 3½ in pots, moistened by adding 80 ml of deionised water, and then left to incubate for three days. All pots were covered to prevent drying out.

After three days, each pot was sown with 1g of rye grass seed (*Lolium perenne*) equivalent to approximately 450 seeds and watered with 10 ml of water twice daily. Concurrently a second experiment with all 12 treatments, but without any rye grass, was set up. These unplanted pots received every treatment that those sown with ryegrass received. The unplanted pots were to help monitor the natural changes, which could occur in availability of heavy metals over time, and they were assessed for the same soil parameters of the cropped pots. The pots were kept on bench tops in the greenhouse under natural lighting, and the weekly temperature was monitored with a 'Thermatag' digital thermometer. Mean daily temperatures in the greenhouse ranged from 22°C to 31°C while mean night temperatures were between 9°C and 12°C.

2.7.2 Setup of manually contaminated pot experiment

There were a total of twelve treatments, presented in Table 2.6

All treatments were added to 400g of sharp sand

Table 2.6 Treatments in the manually contaminated pot experiment

Treatment	composition	weight of components (g)	
		mine waste	amendment
1	G + 0	10	0
2	G + BM	10	40
3	G + CMP	10	40
4	G + PT	10	40
5	G + CR	10	40
6	G + P	10	40
		sewage sludge soil	amendment
7	SB + 0	100	0
8	SB + BM	100	40
9	SB + CMP	100	40
10	SB + PT	100	40
11	SB + CR	100	40
12	SB + P	100	40

G- mine waste; SB- sewage sludge treated soil; BM- bone meal; CMP- general purpose compost; PT- peat; CR- coir, 0- zero or non-amended soil

Each treatment was replicated four times, making a total number of 48 pots in the experiment.

2.7.3 Crop management in manually contaminated soils

Once plants had germinated, wetting was carried out by placing water in the saucers under each pot. Quarter- strength modified Hoagland's nutrient solution was applied to all the plants at 3 weeks and again at 6 weeks to ensure continued growth and prevent yellowing. The composition per 10 litres of ¼ strength Hoagland's solution was as recommended by Watson et. al. (2003). At four weeks after planting, visual observations were made of the crops and photographs taken, but leaves were not harvested at that time to allow enough tissue build up which will be sufficient for analysis.

2.7.4 Metal determination in manually contaminated soils

Total metal analysis of mine waste treated and sewage sludge treated soils, being the contaminants used in the greenhouse, was done by aqua regia digestion as described in section 2.5.1.1. At the end of the experiment, EDTA and water extraction of greenhouse soils was done as outlined in Sections 2.4.4.1 and 2.4.4.2 respectively.

2.7.5 Biomass yield in manually contaminated soils

Ryegrass was harvested twice, first after 8 weeks of growth, and again after a further 8 weeks of growth (i.e at 16 weeks). The aerial parts were cut just above soil level with a pair of scissors, which were wiped after each use. Roots were carefully extracted from soil and washed copiously with tap water followed by deionised water. Fresh tissue samples were weighed immediately on an open pan balance, and then dried in the oven at 70°C for two days to obtain the dry weight.

2.7.6 *Plant tissue analysis of ryegrass grown in manually contaminated soil*

Plant tissue was analysed for metals as discussed in Section 2.5.1.2. The bioaccumulation factor (BF) of heavy metals (HM), also known as the bioavailability index, was calculated as a ratio of metal content in shoots to the acid extractable concentration in soil (An *et al.*, 2004; Kidd *et al.*, 2007).

BF = mg HM (kg plant leaves)$^{-1}$ x100 /total content HM (mg kg soil^{-1})

2.8 Pot experiment in field contaminated soil (2006)

The second pot experiment was carried out in the greenhouse from May-July 2006 (summer), using natural daylight alone. One single contaminant soil was used while amendment levels ranged from zero to 1, 10 and 20%.

2.8.1 *Amendments added to field contaminated soil*

The metal levels in the contaminant sources used in the first year's study, especially the mine waste were very high, and this made it difficult to see clear effects of amendments on soil metal levels and eventual plant uptake. A decision was therefore taken to use lower concentrations of metals in a second pot experiment. Also, due to the inconsistencies in results obtained from the use of bone meal and phosphate in the first experiment, they were not included in the second pot experiment. Amendments used in the 2006 greenhouse experiment were green waste compost, peat, coir, and wood bark. All amendments were used in the moist, naturally occurring form, though the quantities used were calculated on oven dry basis.

2.8.2 Sampling of field contaminated soil

Contaminated field soil was used in this second pot experiment. Bulk soil was collected from Tyndrum, Scotland (MacKenzie & Pulford, 2002). The location sampled was about 60m down slope from an abandoned Pb mine in which production had stopped for over 80 years. Bulk soil was collected at up to 15 cm depth from a waste tip and then refrigerated within hours of collection to minimise changes in microbial activity.

2.8.3 Preparation of field contaminated soil

In the laboratory, the bulk soil was thoroughly mixed and reconstituted by mixing all bags together and the soil was stored moist in four bags at 3 to 4°C until ready for use. Ten samples were taken from each bag and total metals determined in aqua regia, while the available content was determined by EDTA extraction. Total metal in bulk soil was 4875 $\mu g\ g^{-1}$ Pb (RSD 10.3% , n=40); 280 $\mu g\ g^{-1}$ Cu (RSD 13.5% , n=40); and 3330 $\mu g\ g^{-1}$ Zn (RSD 6.5%, n=40).

Some lumps found in the bulk soil were analysed by aqua regia extraction and found to be very high in Pb (5964 $\mu g\ g^{-1}$), indicating they were pockets of mine waste. The entire bulk sample was then sieved through a 4 mm sieve to remove these clods. Ten random samples were again taken from each of the four bags of sieved soil, and the sieved soil analysed again for total Pb, Cu and Zn. Upon sieving out the clods, the average Pb content per bag had been reduced to 3870 $ug\ g^{-1}$ (1.6 % RSD, n=10). Cu and Zn in the sieved soil were 358 and 4253 $\mu g\ g^{-1}$, respectively (6.0 and 17.1% RSD, n=10).

In order to further reduce the level of toxicity in the soil, the Tyndrum soil was mixed with sharp sand in a 1:1 ratio; working on one bag of soil at a time. The sand used was moist, and sieved to pass a 2 mm sieve. Equal weights of Tyndrum soil and sand were determined on oven dry basis, and mixed thoroughly to achieve a uniform bulk sample.

Ten samples were again taken from each of the (1:1 mix) bags and digested in aqua regia for total Pb, Cu and Zn. The four bags of bulk 'made soils' were stored in a cold room at 3°C until ready for use. This 'made soil' was used in the greenhouse pot experiment. The total Pb, Cu and Zn in the 'made soil' before the addition of amendments is presented in Table 2.7.

Table 2.7 Total Pb, Cu and Zn in final field contaminated soil used for the 2006 pot experiment

		Metal concentration ($\mu g\ g^{-1}$)					
Bag	Amendment	Pb	RSD(%)	Cu	RSD(%)	Zn	RSD(%)
1	Compost	2402	(18.9)	117	(32.3)	1874	(26.5)
2	Peat	2218	(15.2)	132	(24.0)	1737	(35.5)
3	Coir	2265	(20.5)	135	(28.4)	1788	(12.1)
4	Wood bark	2236	(19.6)	140	(21.6)	1892	(24.1)

All values are means of 10 replications

2.8.4 Set up of field contaminated soil experiment

To minimise variability among replicates of the same treatment, all treatments of a given amendment were made up from a single bag of soil. Thus, soil bag 1 was treated with green waste compost, bag 2 with peat etc. 300g of soil (oven dry basis) was used in each pot, to which 0%, 1%, 10% or 20% of amendment (weighed on oven dry basis) was added, and then mixed thoroughly. Each treatment was replicated six times, making a total of 78 pots sizes of pots ($3^1/_2$ in, 4in and 5in) were used, to accommodate the differences in final soil volume, which arose from variability in the bulk of the amendments used.

Each pot was watered with deionised water to 70% moisture and left to incubate for four weeks before planting to allow for stabilisation in the soil due to addition of the amendments. Pots were watered twice each week and at 2 weeks of incubation, each pot was tipped out and re- mixed to prevent the samples from going anaerobic. The pots were tipped and re- mixed again at 4 weeks, before planting with rye grass. Five replications were planted with rye grass, while the sixth was unplanted. The greenhouse layout is presented in Figure 2.1.

The pots were kept on bench tops in the greenhouse under natural lighting, and the weekly temperature was monitored with a 'Thermatag' digital thermometer. Mean daily temperatures inside the greenhouse ranged from 22°C to 40°C and mean night temperatures were between 9°C and 14°C.

REPLICATES I				II				III			
12	8	9		10	3	7		5	0	10	
1	6	4	5	6	11	1	12	12	8	2	7
11	0	3		8	2	4		6	4	9	
7	10	2		5	0	9		1	3	11	

IV				V				UNPLANTED POTS			
4	2	5		1	7	11					
3	7	12	1	8	3	6	6				
11	9	6		4	10	2					
0	8	10		9	12	5					

Figure 2.2 Layout of greenhouse 2006 experiment

Treatments 0- no amendment; 1-GW compost 1%; 2-GW compost 10%; 3- GW compost 20%; 4- Peat 1%; 5- Peat 10%; 6- Peat 20%; 7- Coir 1%; 8- Coir 10%% 9- Coir 20%; 10- Wood bark 1 %; 11-Wood bark 10% 12-Wood bark 20%

2.8.5 *Metal determination in field contaminated soil*

Soil samples were taken from the unplanted pots after four weeks of incubation, to determine EDTA extractable Pb, Cu and Zn in the soil at the time of planting. At the end of the growth period, both the planted and unplanted pot soils were also analysed for metals by EDTA extraction as described in sections 2.4.4.2. (page 50).

2.8.6 *Biomass yield in field contaminated soil*

Ryegrass was harvested once, after a total of six weeks of growth in contaminated soil. The aerial parts were cut just above soil level with a pair of scissors which were wiped after each use. Roots were carefully extracted from soil and washed copiously with tap water followed by deionised water. Fresh tissue samples were weighed immediately on an open pan balance to two decimal places, dried in the oven at 70°C for two days and the dry weight was also measured.

2.8.7 *Tissue analysis of plants grown in field contaminated soil*

Plant tissue was analysed for metals as discussed in Section 2.5.1.2. The bioavailability factor (BF) was calculated by the formula stated in Section 2.7.6. Transport factor (TF) was calculated as the ratio of the metal content in plant leaves to that in the roots (Dahmani-Muller *et al.*, 2000; Kidd *et al.*, 2007).

2.9 Incubation Study

Two incubation studies were set up to evaluate the effect of metal toxicity on microbial respiration. The first study evaluated the effect of metal on microbial respiration in amendments, while the second study evaluated the effect of added amendments on microbial respiration in contaminated soil. Each study lasted an incubation period of 10 weeks.

2.9.1 *Microbial respiration in metal spiked amendments*

Fresh amendments equivalent to 20 g oven dry weight was weighed into 500 ml jars, then brought to 50% moisture saturation by adding in single metal solution. Metal salts used were the same as in the sorption experiment (Table 2.2, page 42), and concentrations were zero (deionised water only), 5, 10 and 50 mg g^{-1}, respectively, applied in solution. Each sample was thoroughly mixed manually, sealed and left for two weeks as a conditioning incubation to allow microbial activity to stabilize before any readings were taken (Barajas-Aceves, 2005). Thus, the CO_2 evolved during the second week of incubation was used as the basal respiration value because, after that period, samples reach a relatively constant rate of CO_2 production (Moscatelli *et al.*, 2005).

After two weeks, 10 ml of 1M NaOH solutions was placed in small jars on top of the moist amendments and the bigger jar sealed. Into a jar with no amendment was placed 10 ml NaOH to serve as the blank. After one week, the NaOH was taken out, 5 ml $BaCl_2$ added, and titrated with 1M HCl to determine the amount of CO_2 released into the NaOH solutions. 0.1% phenolphthalein was used as the indicator. Each incubation experiment was set up as a 4x4 completely randomised design without replications, since CO_2 measurement was cumulative- i.e., four amendments and 4 concentrations per each metal. The available metal content of the contaminated amendments were determined at the end of 10 weeks of incubation

2.9.1.1 Evaluating inhibition effect of metals on microbial respiration in metal spiked amendments

The CO_2 release per week was then measured as

Total CO_2 – C evolved ($mg\ kg^{-1}$) = {[(blank titre mls – amendment titre mls)*6] * 1000}/ Amendment weight (g)

After the NaOH was taken out and titrated, jars of treated amendments were left open for an hour to let in fresh air, and the moisture of the material brought back to 50% saturation with deionised water where needed. A fresh NaOH solution was then placed into each jar, and sealed. The incubation period was ten weeks for each experiment, and cumulative CO_2 release was calculated each week. Inhibition effect of metals on CO_2 evolved was calculated as:

% inhibition = (1 – B/A) *100

where A = cumulative CO_2 evolved in non contaminated amendment, and

B = cumulative CO_2 evolved in contaminated amendment

2.9.2 *Microbial respiration in field contaminated soil*

The field contaminated soil was the same as that used in the second greenhouse experiment, to which amendments green waste compost, peat, coir and wood bark were added as 0, 1, 10 and 20% as discussed in Section 2.8.4(page 60). 100g of amended soil was weighed into 500 ml screw top jars, and allowed to pre-incubate for four weeks before readings were taken. Amount of CO_2 evolved was then measured as in the first incubation study.

2.9.2.1 Evaluating the ability of amendments to enhance microbial respiration in field contaminated soil

Amount of CO_2 evolved in contaminated soil was enhanced by the addition of amendments, and this enhancement was measured as

% enhancement = (1-C/D) * 100

Where C= cumulative CO_2 evolved in non amended soil, and

D= cumulative CO_2 evolved in amended soil

2.9.3 Certified Reference Materials

Certified reference materials (CRMs) were included in each batch of soil and plant tissue analysis in order to verify the data obtained for the experimental material. The CRMs used were

Soil: LGC 6135- (Soil Hackney Brick Works soil)

Plant tissue: CRM 1573a Tomato leaves (National Institute of Standard Technology)

CRM 1575 Pine needles (National Bureau of Standards).

The certified measurents and the values obtained in this research through acid digestion of the CRMs are presented in Table 2.8

Table 2.8 Total concentrations of Pb, Cu and Zn in reference materials

		Pb (ug g^{-1})	Cu (ug g^{-1})	Zn(ug g^{-1})
LGC 6135	certified	385-437	102-112	296-394
	measured	417-590	106-124	279-308
Tomato leaves	certified	-	4.56-4.84	30.2-31.6
	measured	-	4.21-4.40	35.2-37.3
Pine needles	certified	10.3-11.3	2.7-3.3	-
	Measured	10.1-15.2	1.9-2.1	-

2.9.4 Statistical Analyses

All statistical analyses were carried out with the programme MINITAB version for windows. The data were analysed using a general linear model of ANOVA, considering the treatments as the independent variable. Where F test was significant, Tukey's test at $p < (0.05)$ was used to determine LSD for the comparison of means. Correlation was by Pearson's coefficients at $p < 0.05$.

Chapter 3

Sorption of metals

3.1 Introduction

Batch experiments are effective methods of assessing metal binding and desorption kinetics at the laboratory level (Temminghoff *et al.*, 1997), because, through them, a wide range of possible field situation scenarios can be simulated by modifying the factors which affect metal sorption. Because the sorption behaviour of metals differ, such studies are very useful in testing possible soil remediation or stabilization treatments specific to individual metals. Batch studies take into account the initial metal concentration, the weight of the adsorptive material, the reaction time, and the adsorptivity of other metals present in solution. Thus, it is effective in evaluating the performance of adsorptive materials, assessing the sorption of any single metal, or the sorption of that same metal when in competition with other metals (Morera *et al.*, 2001; Voegelin *et al.*, 2003).

Sorption isotherm analysis is a useful technique to study the immobilisation of metals in soils, as it presents the relation between the content of sorbed metals and their concentration in solution. Isotherms also provide information on soil retention capacity as well as the strength by which the metal being investigated is held onto the soil (Morera *et al.*, 2001). At the first stage of the sorption process, a rapid equilibrium is established between sorbed metal ions on the amendment material and the un- sorbed metal ions in solution. This equilibrium can be represented by sorption isotherms (Villaescusa *et al.*, 2000). Sorption isotherms have been used to investigate the nature of various types of sorption phenomena, as they provide useful models for physical sorption, as well as chemisorption processes. It is worth noting however, that metal retention does not provide evidence of the actual mechanism of an sorption process. '*Adherence of data to a proposed* *equation does not prove that the assumed (or corresponding) sorption mechanism is actually occurring. It does, however, establish a platform (albeit often temporary) that allows the process of scientific inquiry to proceed'* (Schulthess & Dey, 1996). Given that there are a number of possible mechanisms by which metals may be immobilised in soil, it is difficult to differentiate between metal retention by precipitation, or retention by sorption (Sposito, 1989).

The sorption of metals, either from single (non-competitive) or multi metal (competitive) solutions, is very important in determining metal mobility within the soil, metal uptake by plants, and the capacity of amendments to immobilise the contaminants (Markiewiez-Patkowska *et al.*, 2005). The Langmuir equation has been used in this study because of the simplicity of the mass balance equation which it is based on, and this equation assists with the determination of a value for the maximum quantity of metal being sorbed per mass of adsorbent (Ho & Mackay, 2000; Wilson, 2002).

There are three main sections in this chapter. First, the amendments were exposed to Pb, Cu and Zn solutions at differing contact times as a single point sorption, using only one metal concentration. Following on from this, the sorption of a wide range of concentrations of each single metal was examined under two background salt concentrations. Finally, the sorption of individual metals in multi-metal systems was examined. Sorption of the metals was examined by the method explained in Chapter 2 (Section 2.3; page 42).

3.1.1 Aim of the experiment

The aim was to assess the potential of bone meal, coir, compost, green waste compost, peat and wood bark as metal immobilisers in real-world contaminated soils.

3.2 Effect of time on metal sorption

3.2.1 Objective

To determine the effect of contact time between metal and amendment on the capacity of amendments to sorb Pb, Cu and Zn

3.2.2 Method

Single Pb, Cu and Zn at 0.5 mmol l^{-1} was shaken with 0.1g of amendment at varying contact times, and the equilibrium concentration determined. The method is fully discussed in Section 2.3.1 (page 44). Capacities were calculated by application of the Langmuir equation

3.2.3 Results and discussion

Figures 3.1 to 3.3 show the level of sorption for each metal at various times of shaking. In most of the amendments except for bone meal sorption of Pb, more than 95% of Pb and Cu sorbed had occurred by 60 minutes of agitation (Figures 3.1 and 3.2). There was no change in the amount of Zn sorbed beyond 60 minutes of shaking (Figure 3.3).

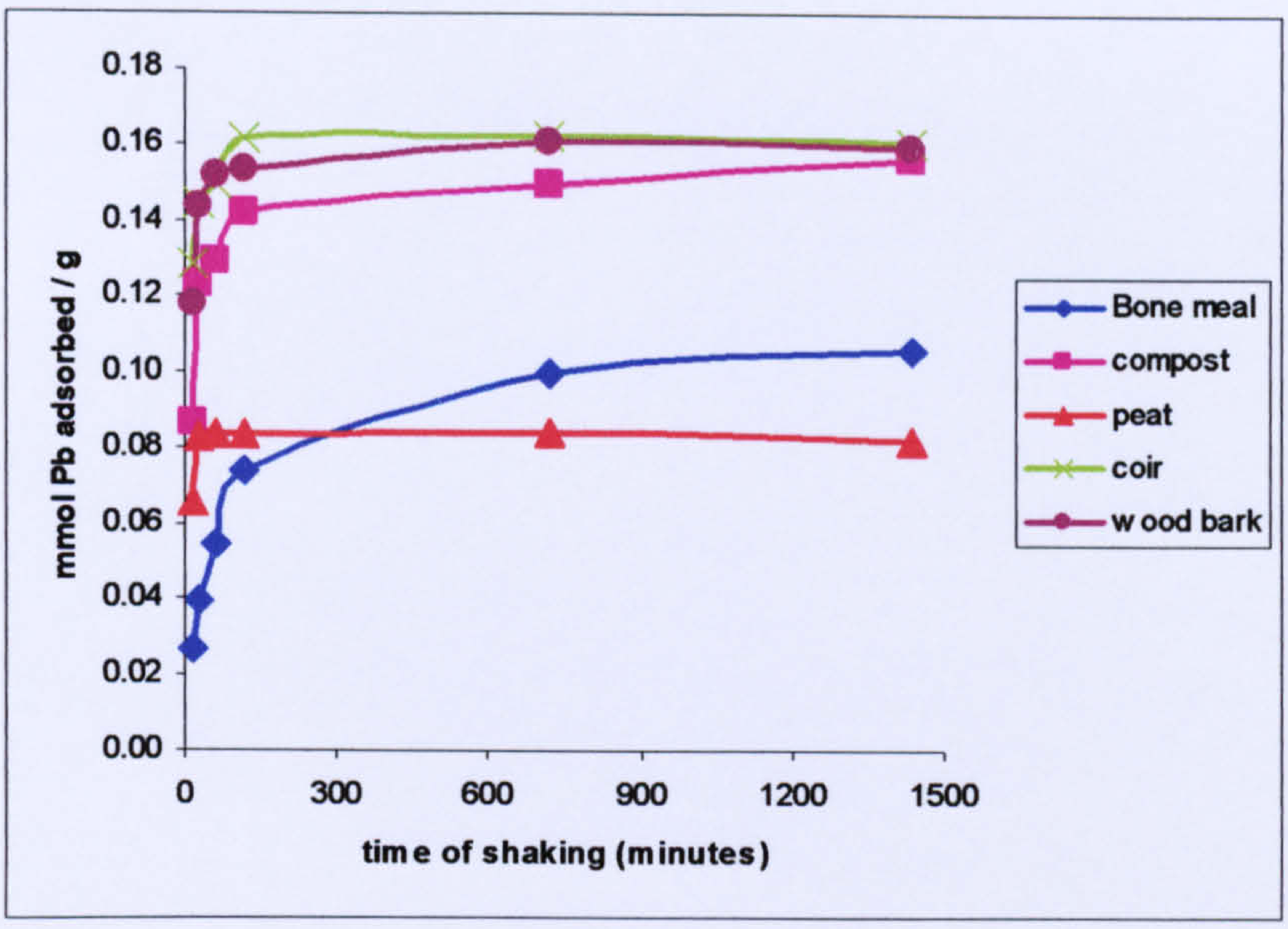


Figure 3.1 Effect of contact time on sorption of 0.5 mmol l $^{-1}$ Pb (values are means of three replications)

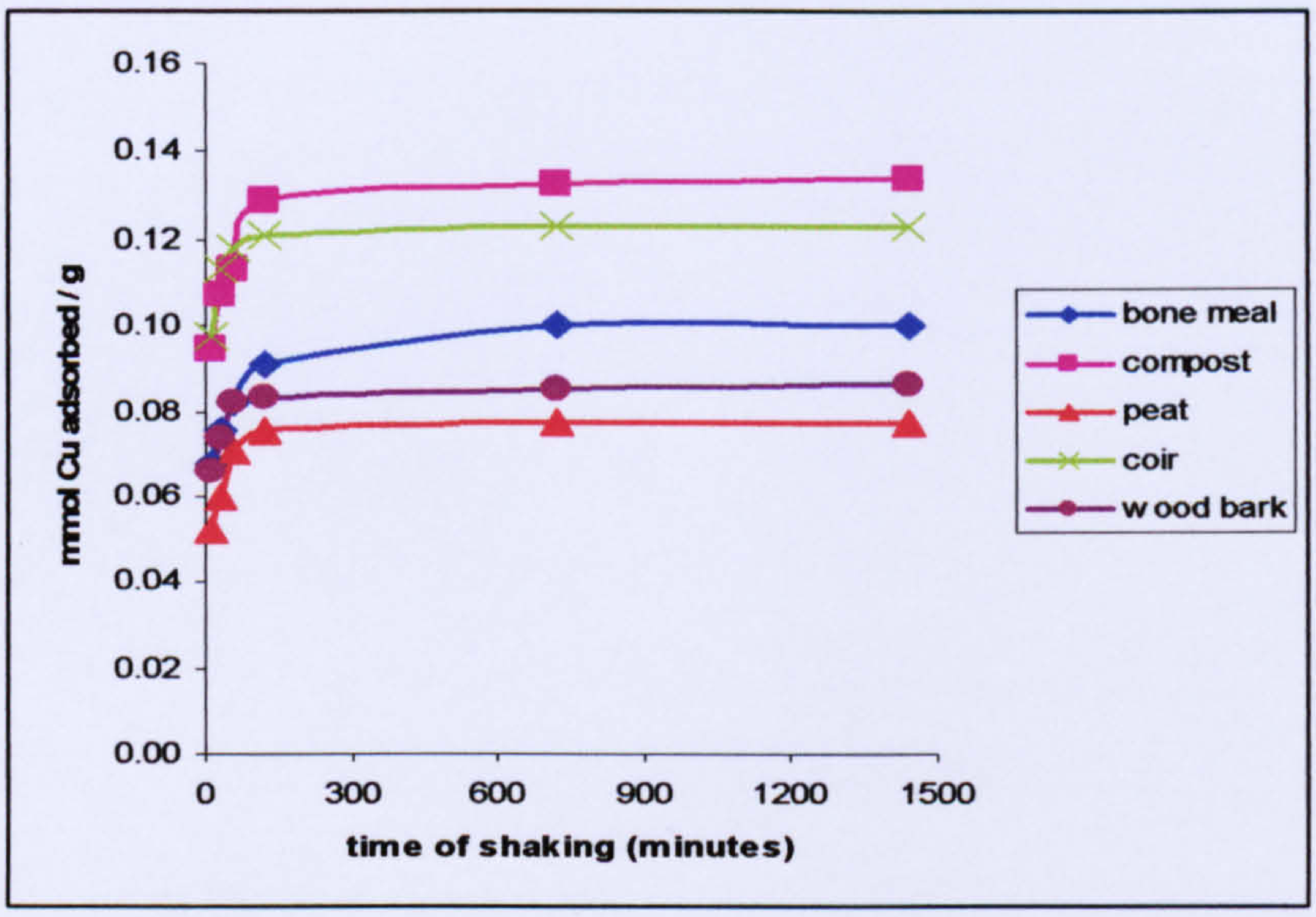


Figure 3.2 Effect of contact time on sorption of 0.5 mmol l $^{-1}$ Cu (values are means of three replications)

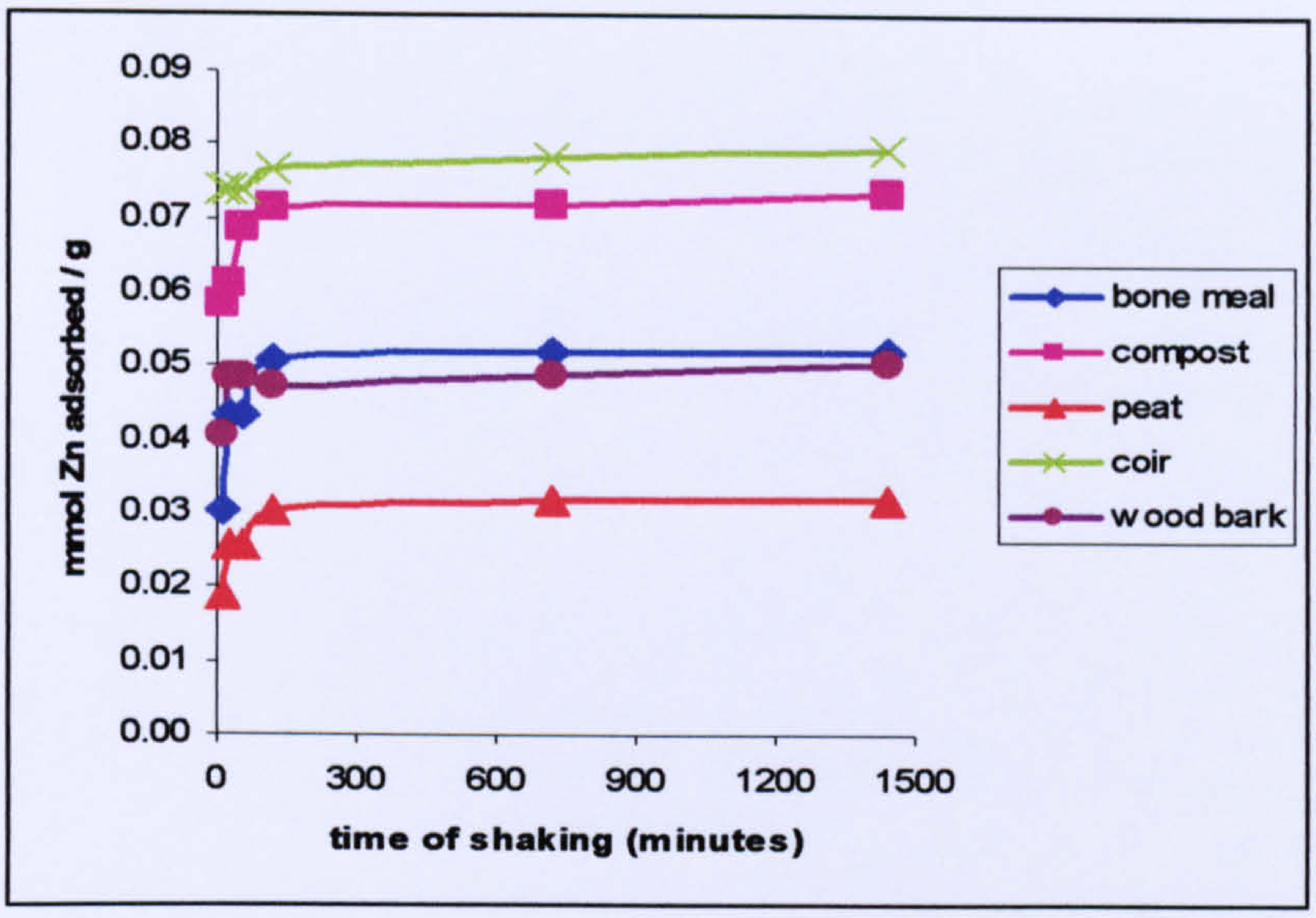


Figure 3.3 Effect of contact time on sorption of 0.5 mmol l $^{-1}$ Zn (values are means of three replications)

An optimal shaking time of 60 minutes confirmed the work of Brown *et. al* (2000) and Ucer *et. al.*(2006), even though a further 20% of Pb was sorbed by bone meal when the time of shaking increased beyond six hours. This may be due to the high pH of bone meal, which, at 6.9, was higher than that of the other amendments tested. It has been stated that the number of sites on an adsorbent which can be occupied by metal ions is a function of the pH, and longer equilibration time is required at higher pH values (Axe & Anderson, 1998). Most kinetic studies with geomedia tend to be limited to some initial '*straight line*' segment of $C_{M,\,ads}$ versus time isotherm (Harter, 1991), and the importance of reaction time beyond an initial period of 1 to 8 hours is controversial. While some investigators find relatively large time dependence, others report little or no time dependence of sorption beyond an initial period. For example, Markiewicz-Patkowska *et. al.* (2005) actually found that more than 80% of total amount of metal was removed within the first 5 minutes.

Based on the results shown in Figures 3.1 to 3.3, a time of shaking of one hour was subsequently used in all the batch studies on metal sorption in this thesis.

3.3 Ionic Strength of background salt

3.3.1 Objective

To determine the effect of background salt concentrations on the sorption of Pb, Cu and Zn by amendments bone meal, coir, compost, green waste compost, peat and wood bark

3.3.2 Method

Single Pb, Cu and Zn metal solutions were made up in 0.001M or 0.1M $Ca(NO_3)_2$. Batch sorption was then carried out in both background electrolytes. $Ca(NO_3)_2$ was selected as the background salt for this study because both Ca^{2+} and NO_3^- are dominant ions in soil solution. The method used is fully discussed in Chapter 2 (Section 2.3.3; page 45).

3.3.3 Results and Discussion

With all amendments, there was marked reduction in metal sorption at higher background salt concentration. The sorption of Pb, Cu and Zn in two concentrations of $Ca(NO_3)_2$ are shown in Figures 3.4 to 3.9. At metal concentrations below 0.5 mmol l^{-1}, sorption was similar in both salt concentrations. As the applied metal concentrations increased beyond 0.5 mmol l^{-1} however, a higher $Ca(NO_3)_2$ concentration led to a significant reduction in amount of metal sorbed.

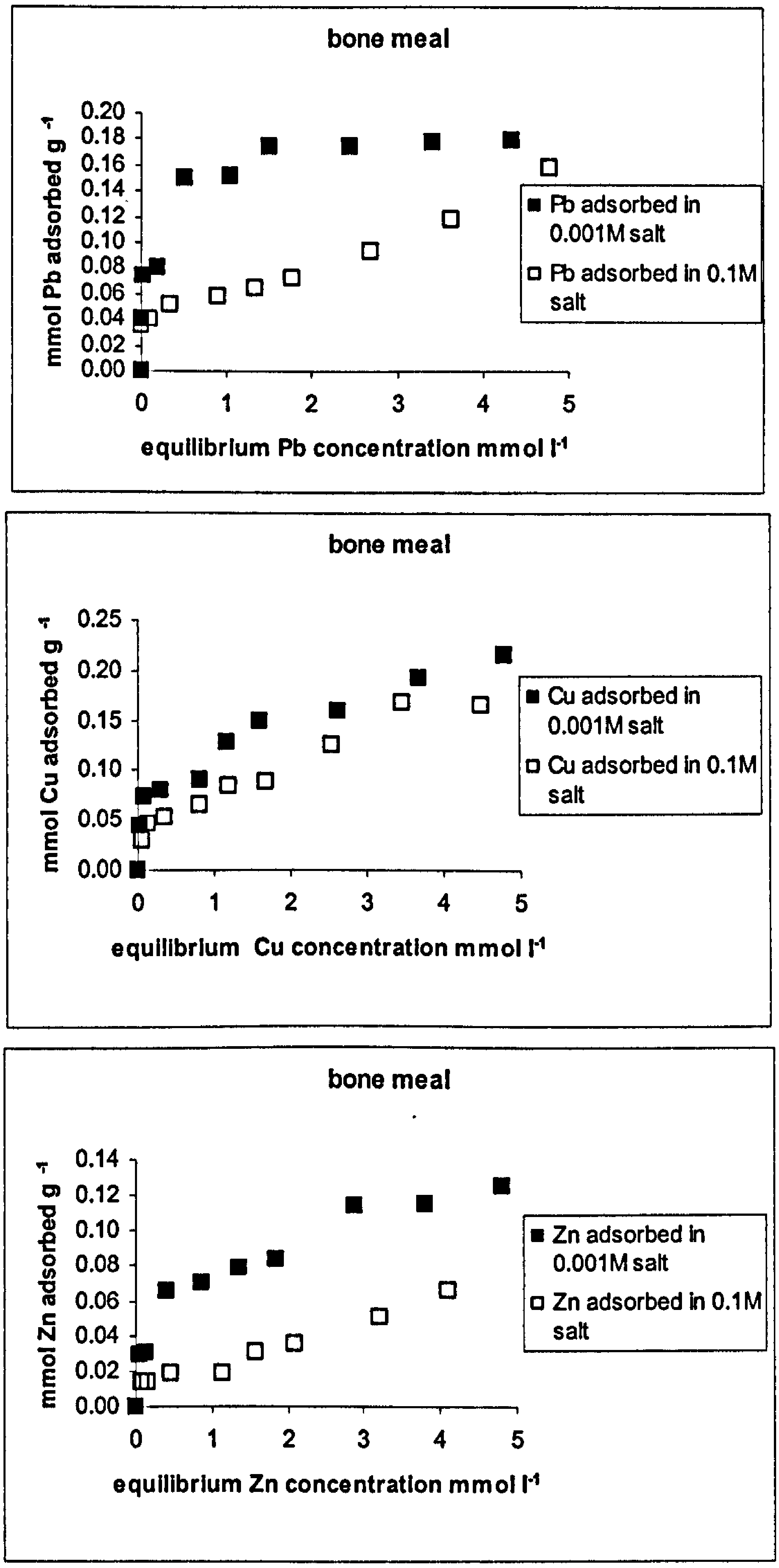
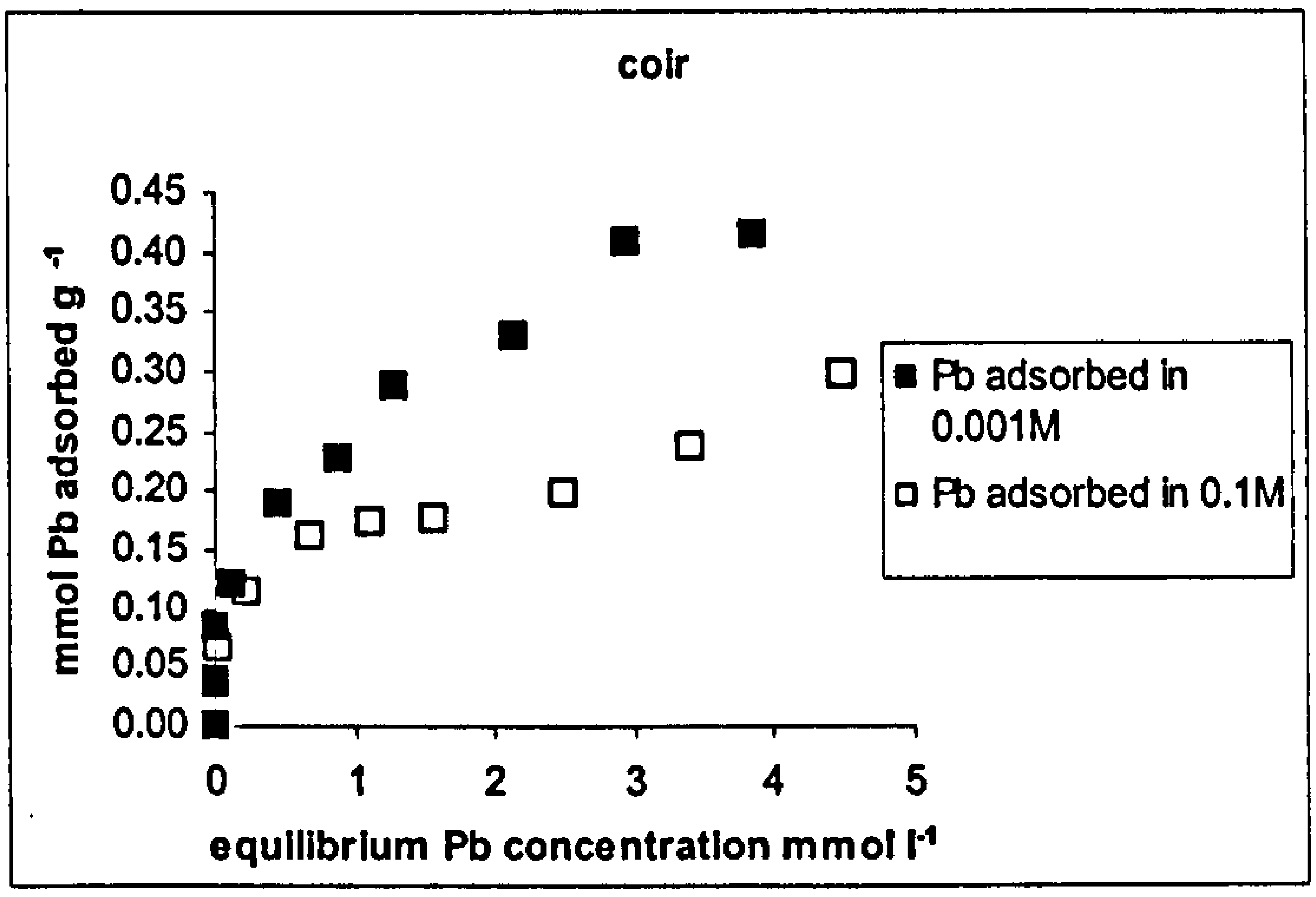
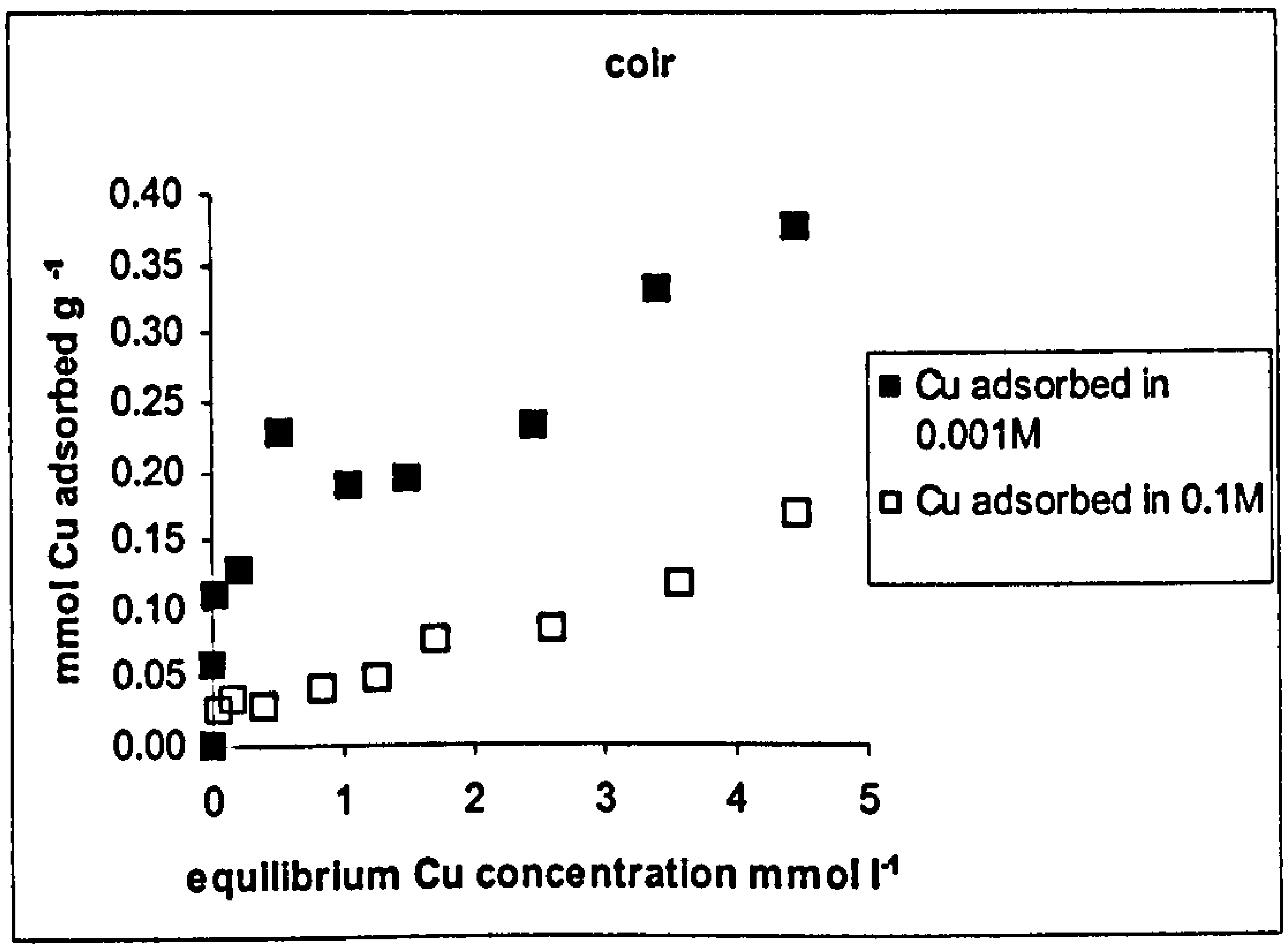


Figure 3.4 Effect of background $Ca(NO_3)_2$ concentration on sorption by bone meal (values are means of three replications)





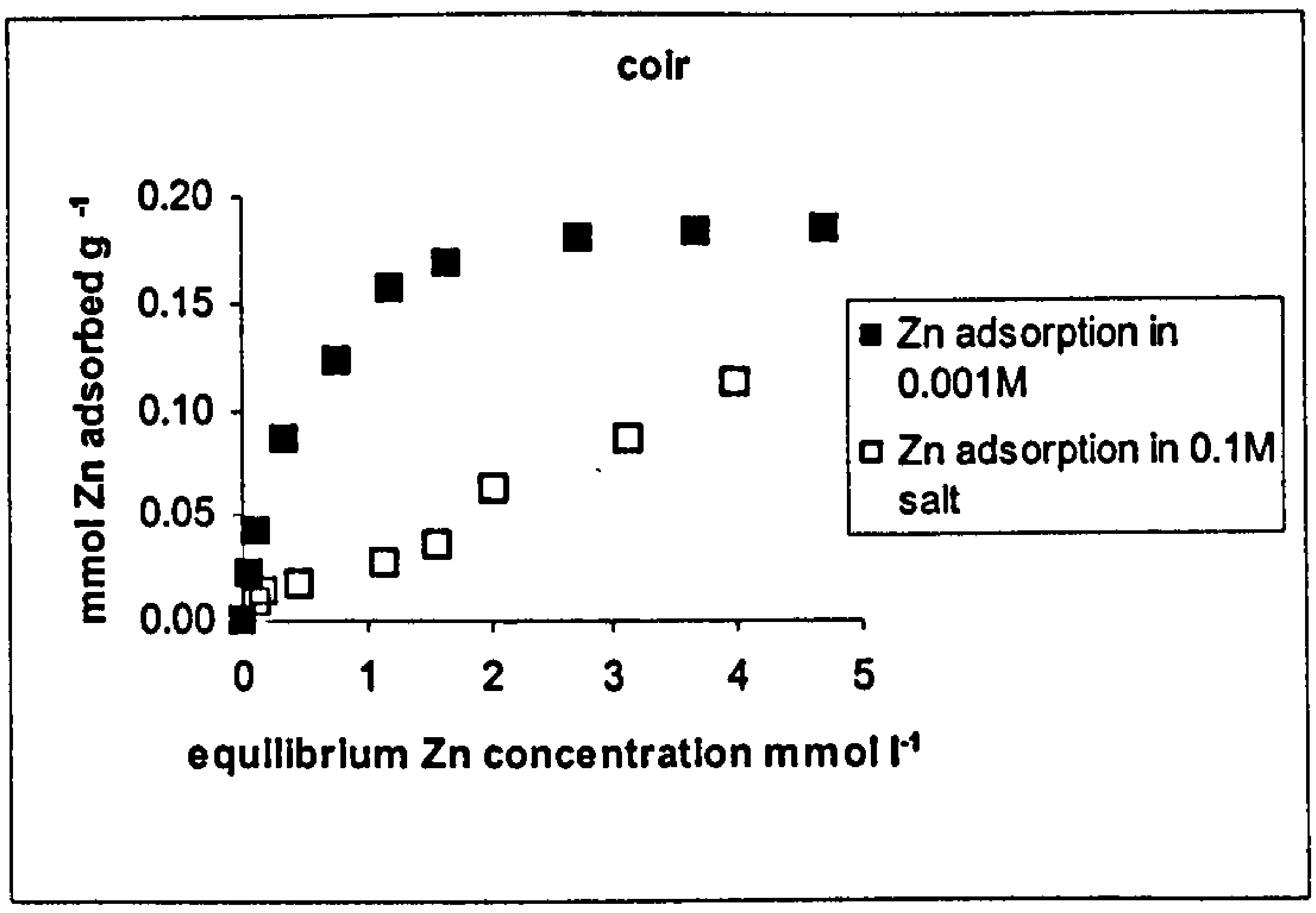
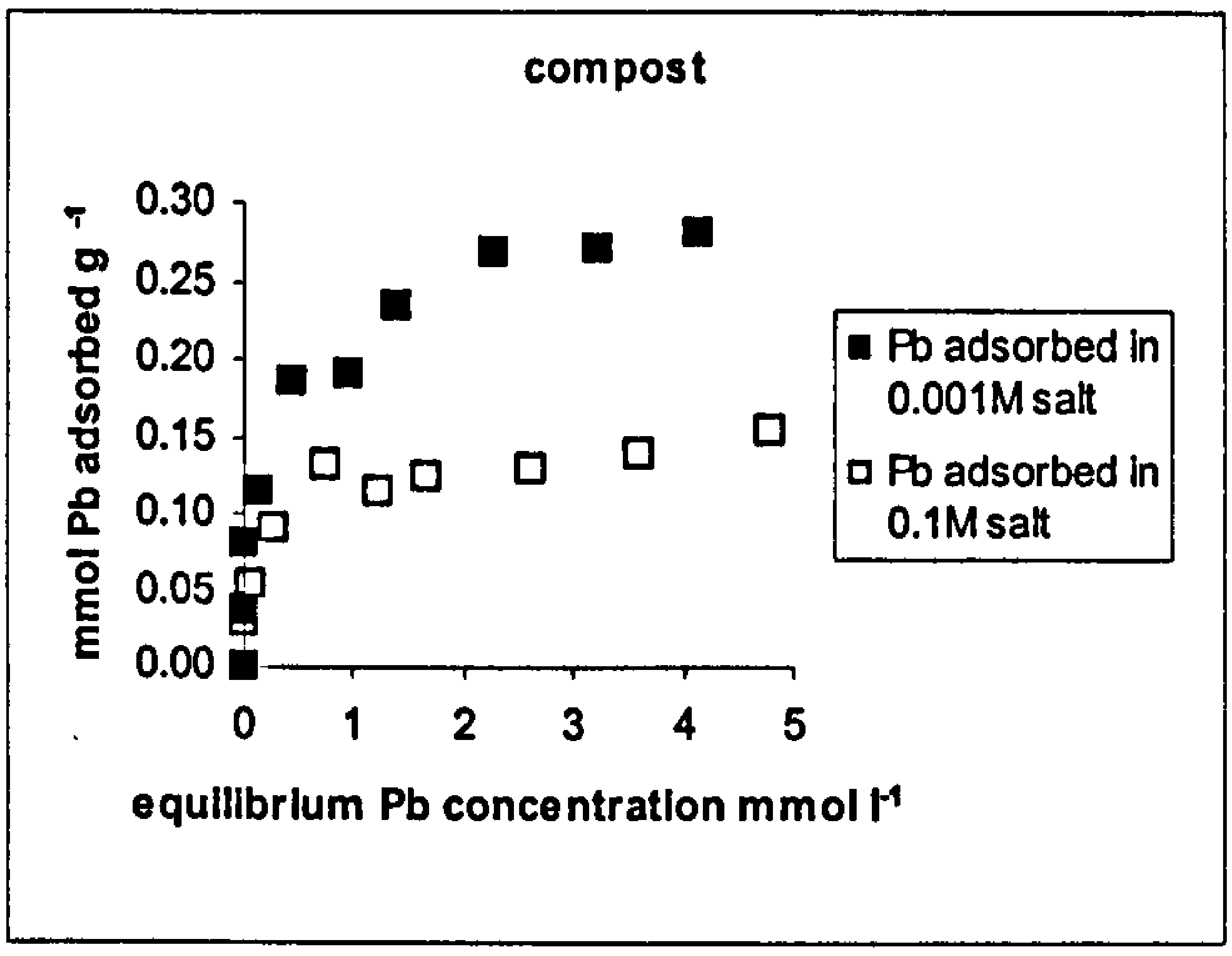
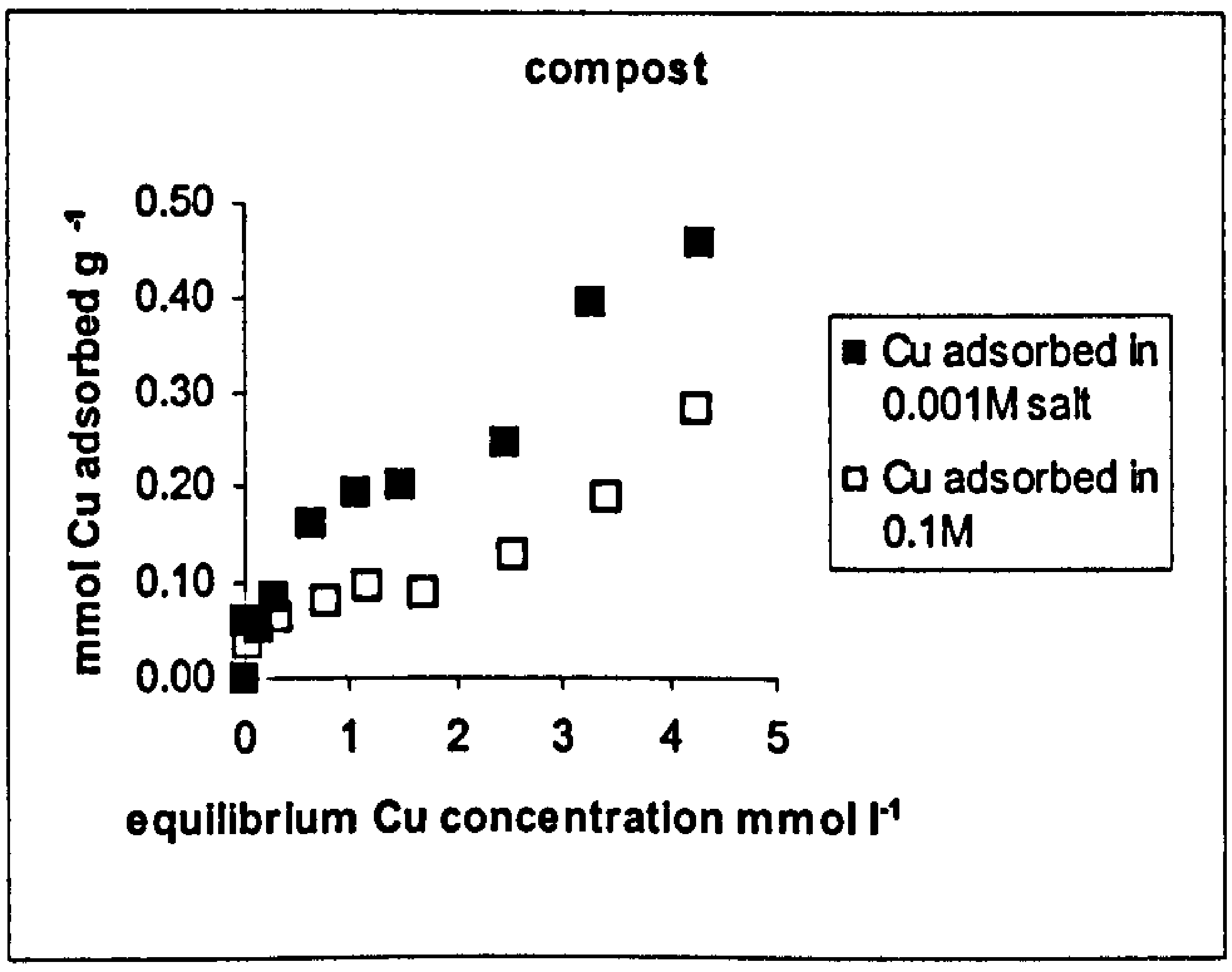


Figure 3.5 Effect of background $Ca(NO_3)_2$ concentration on sorption by coir

(values are means of three replications)





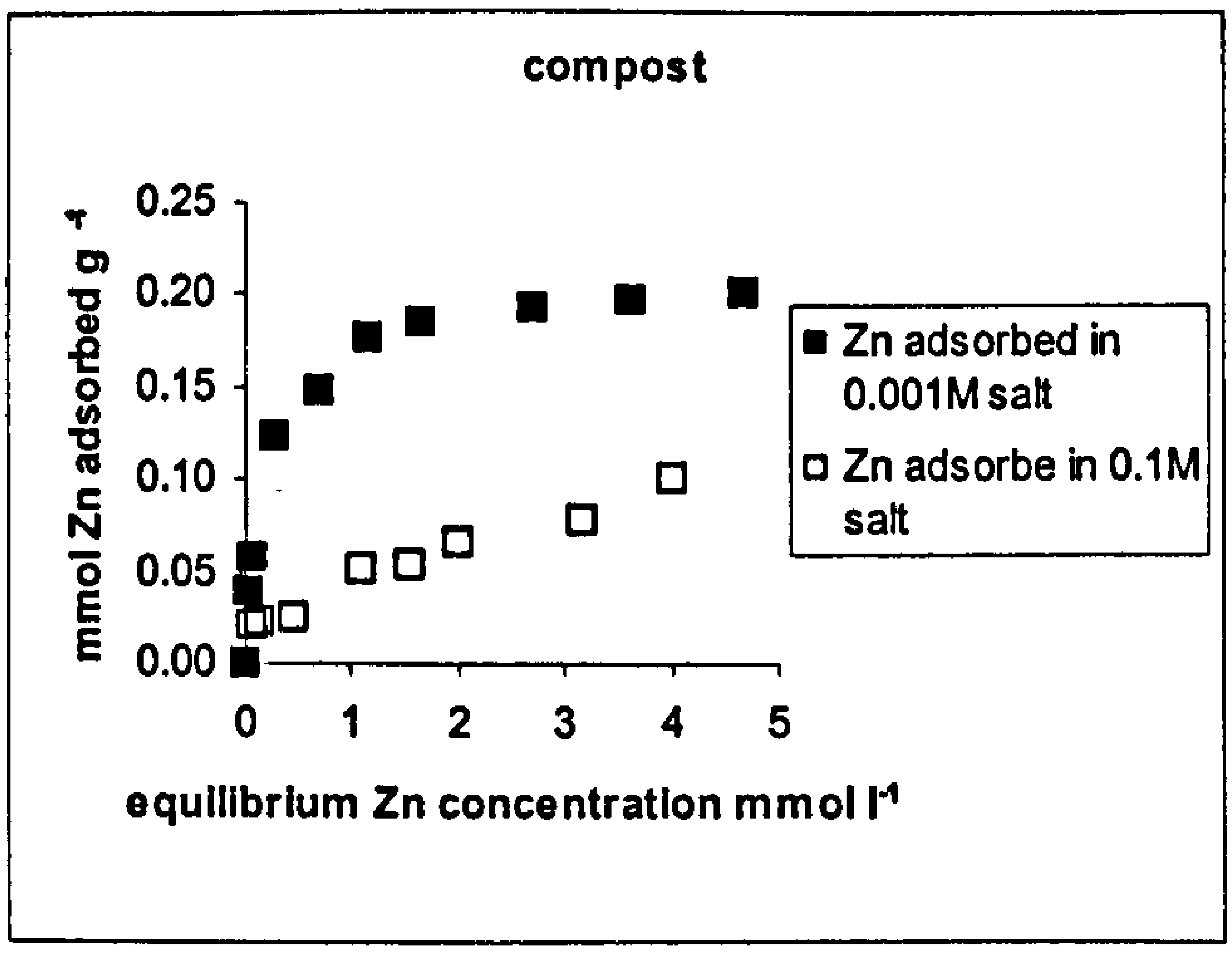
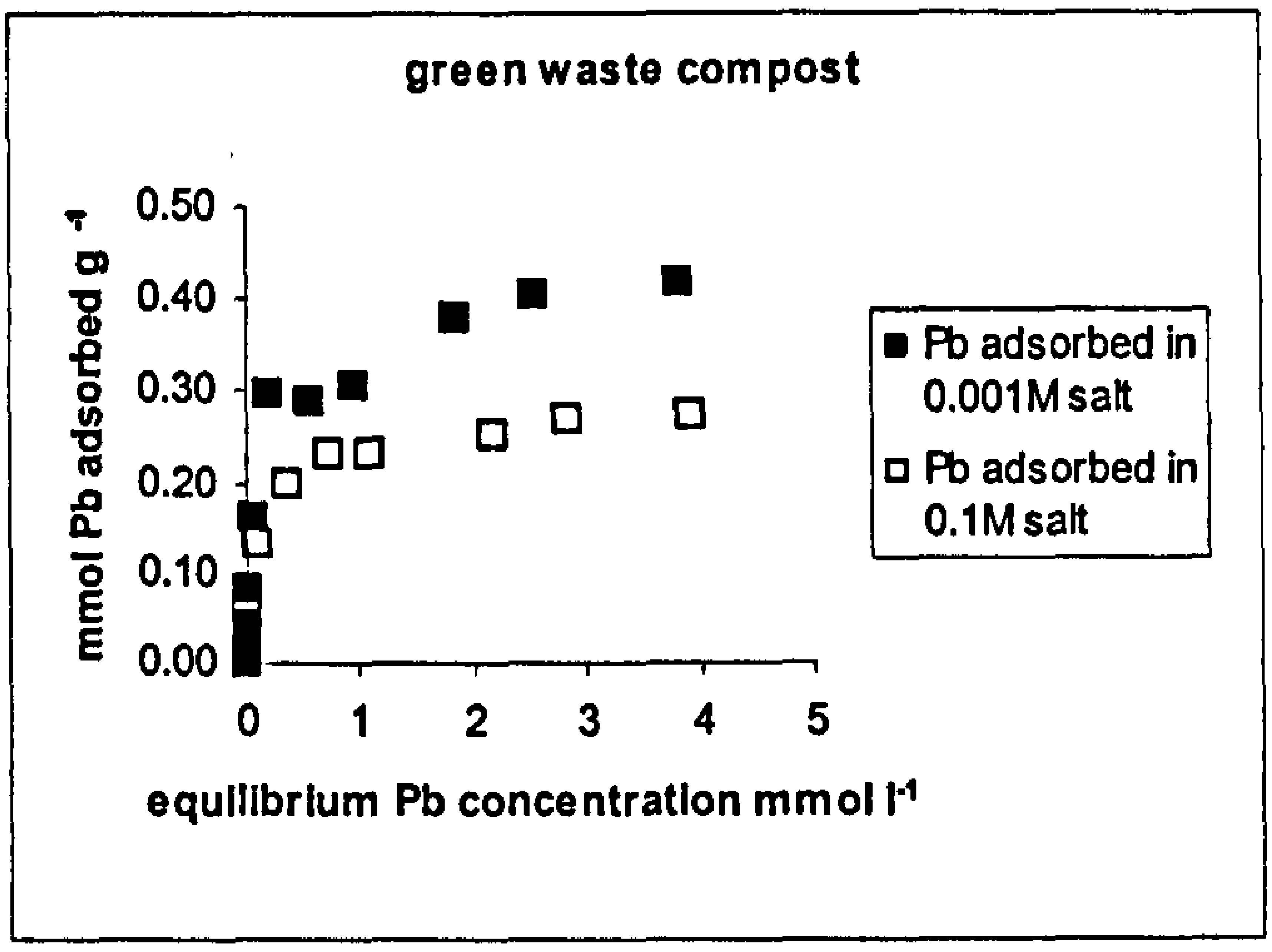
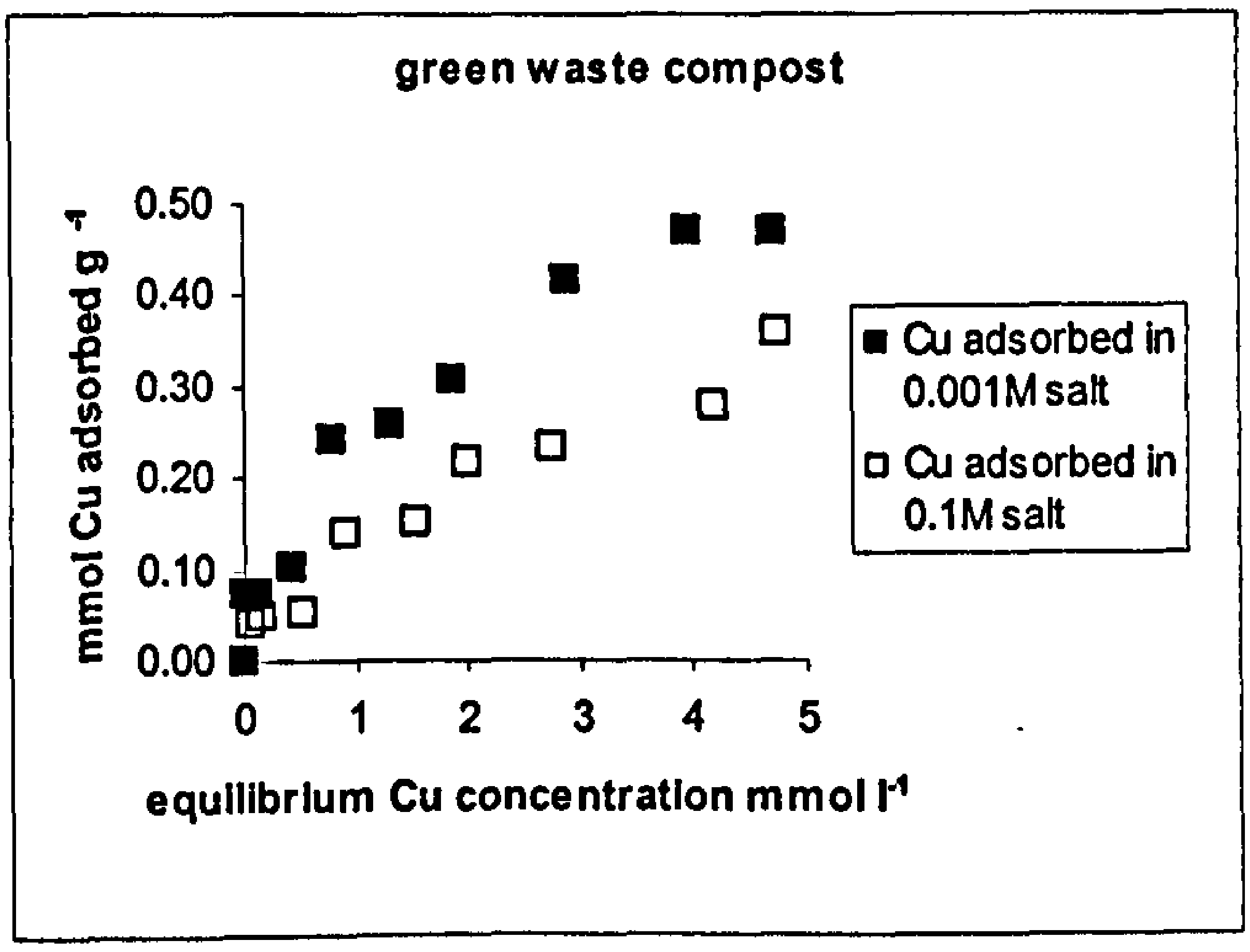


Figure 3.6 Effect of background $Ca(NO_3)_2$ concentration on sorption by compost

(values are means of three replications)





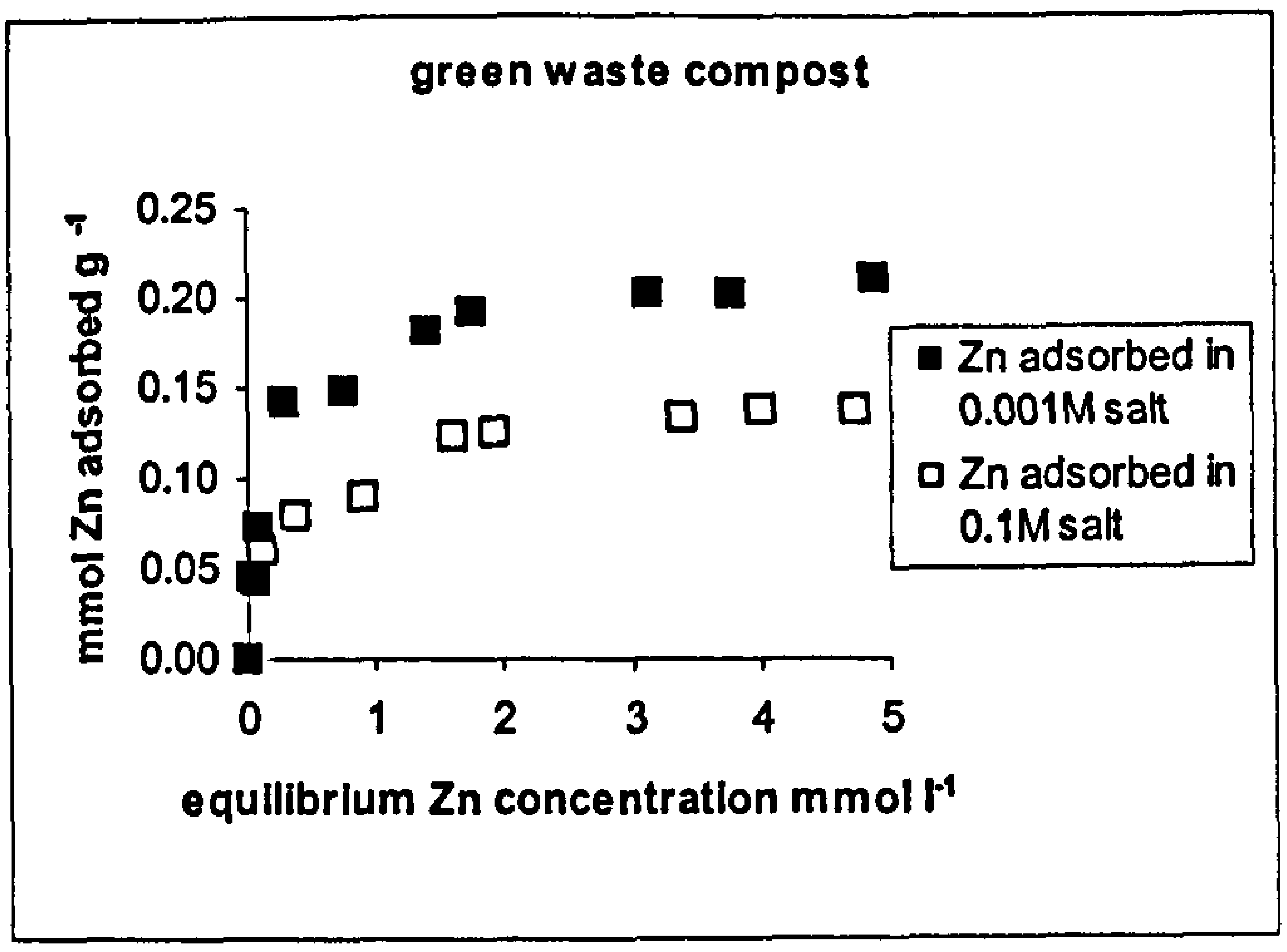
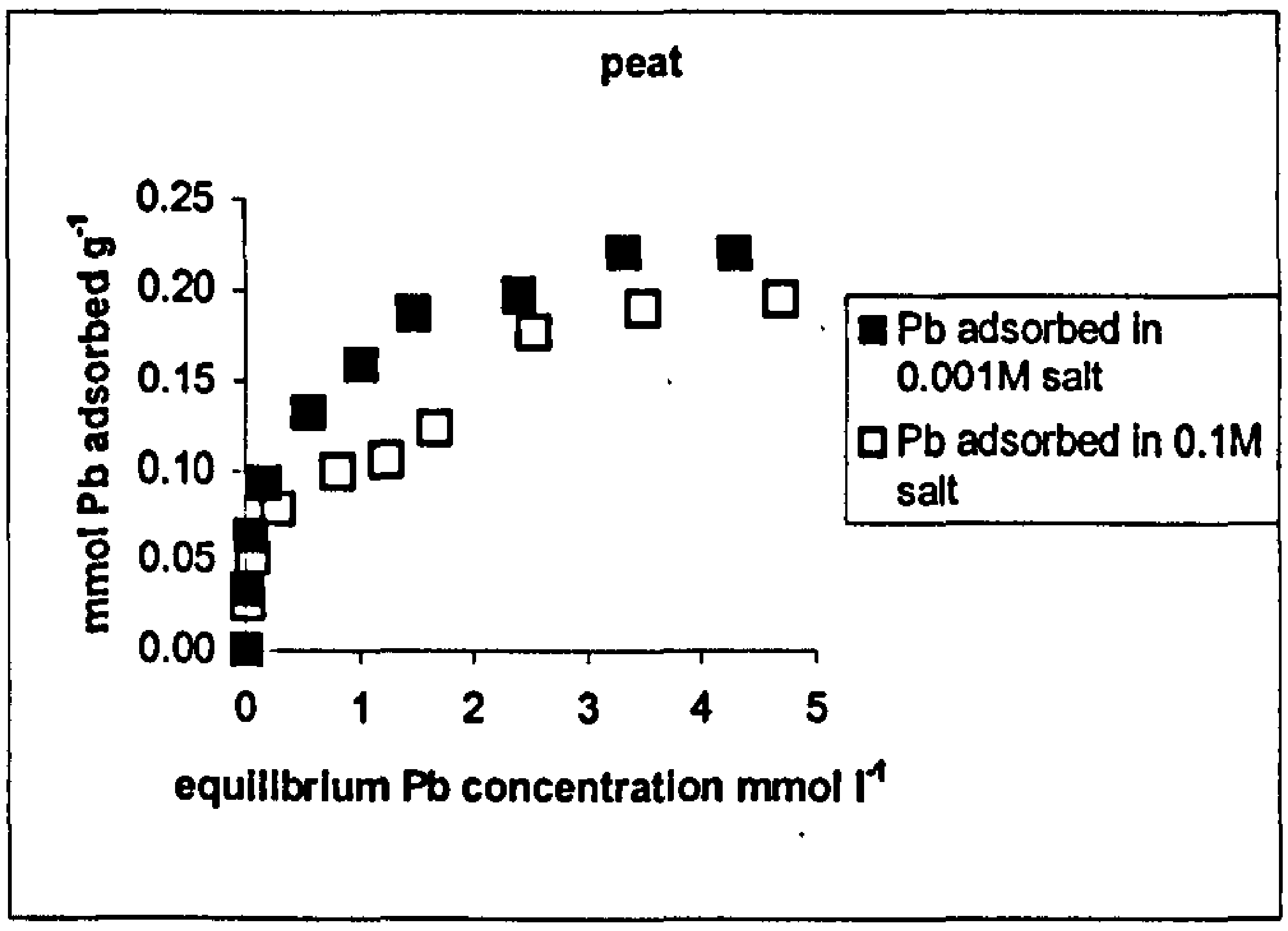
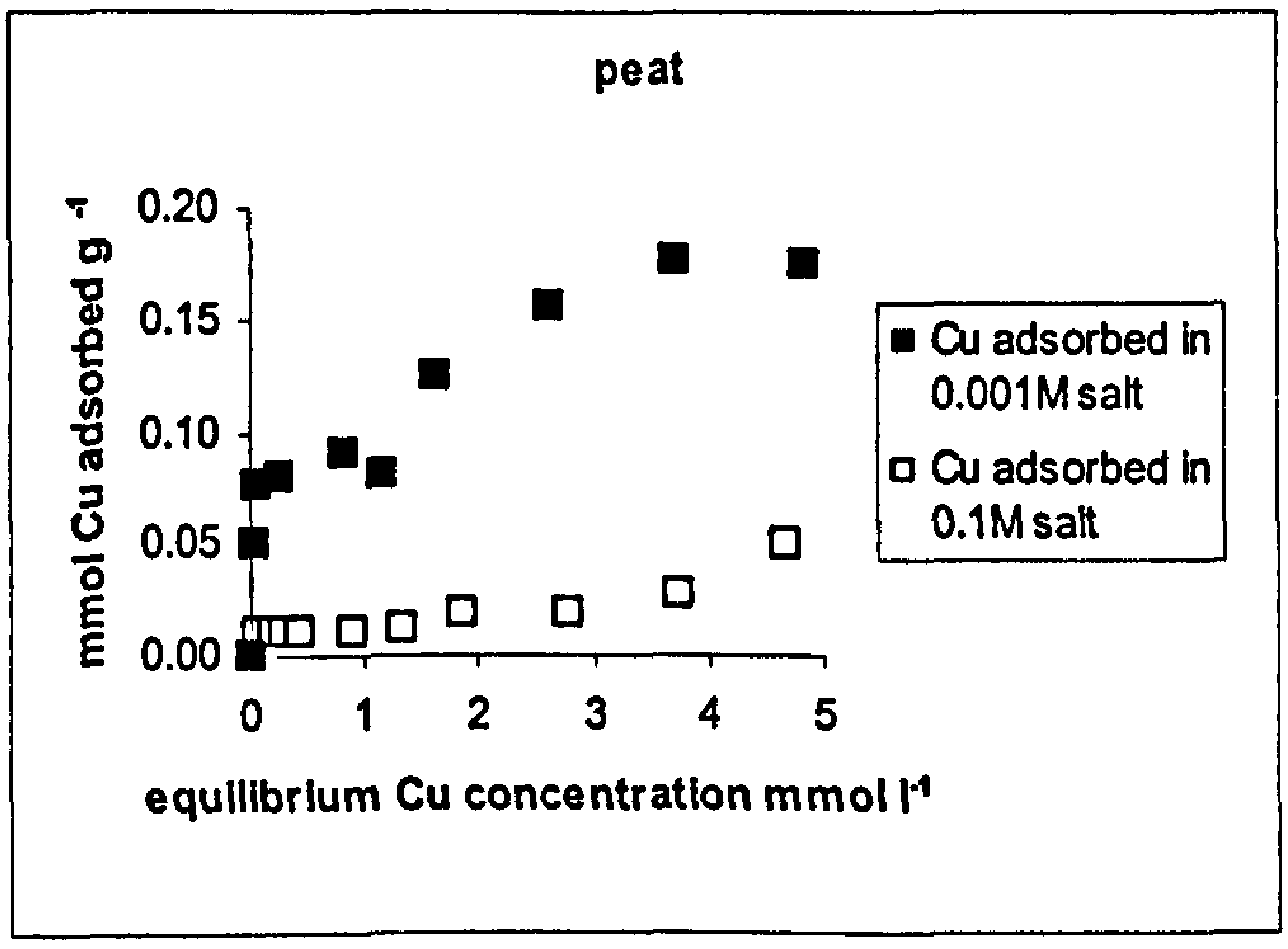


Figure 3.7 Effect of background Ca(NO$_3$)$_2$ concentration on sorption by green waste compost (values are means of three replications)





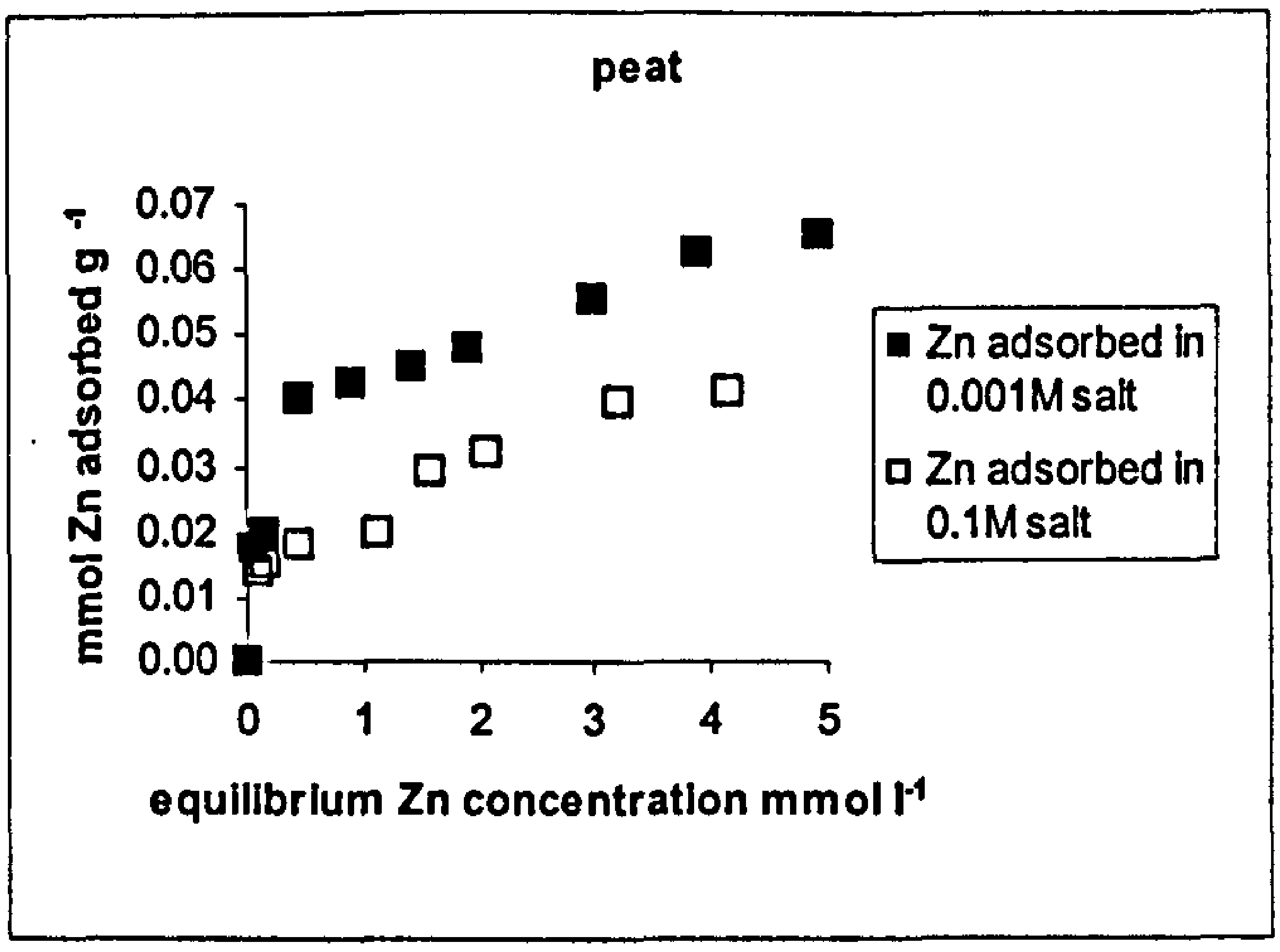
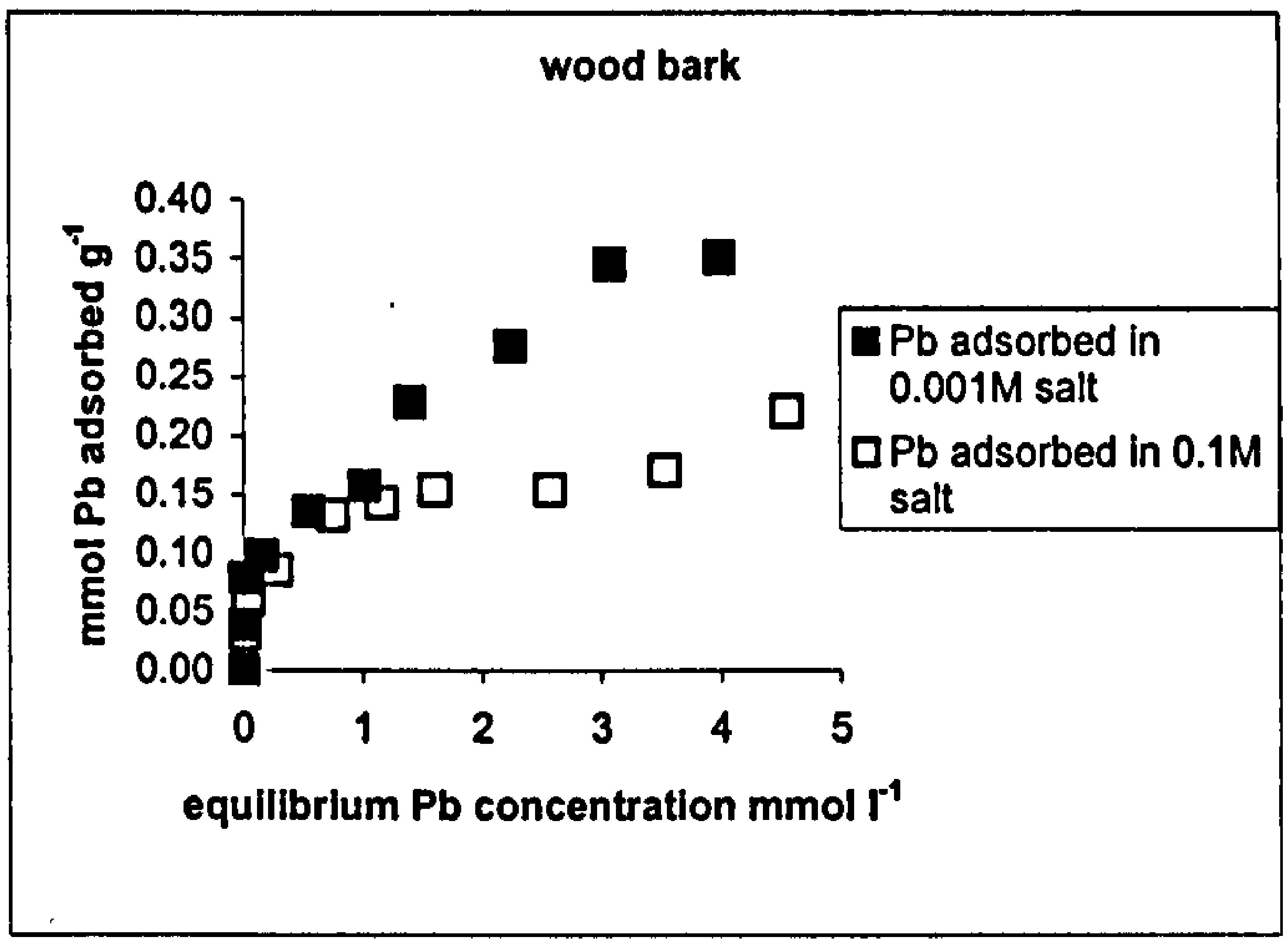
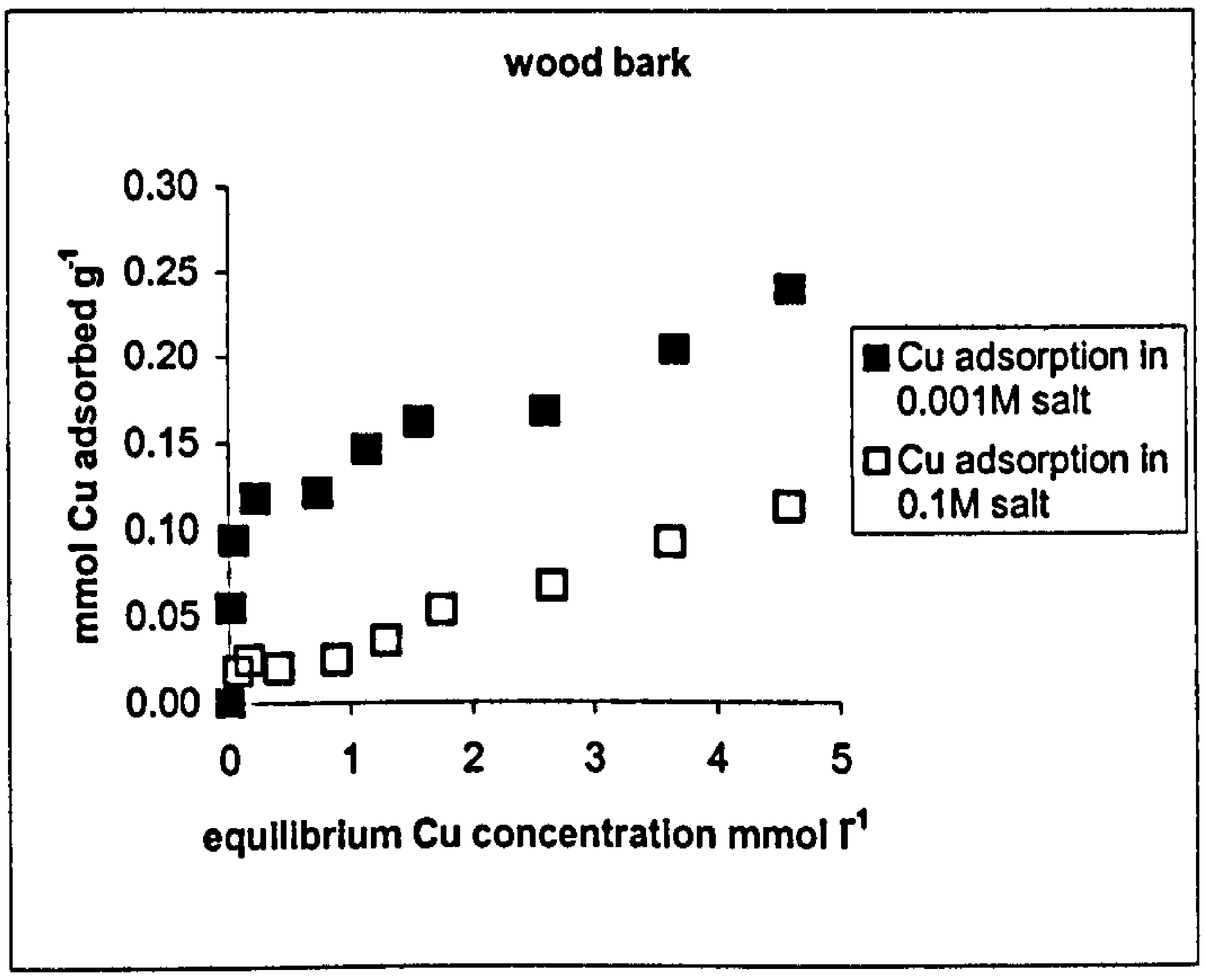


Figure 3.8 Effect of background $Ca(NO_3)_2$ concentration on sorption by peat (values are means of three replications)





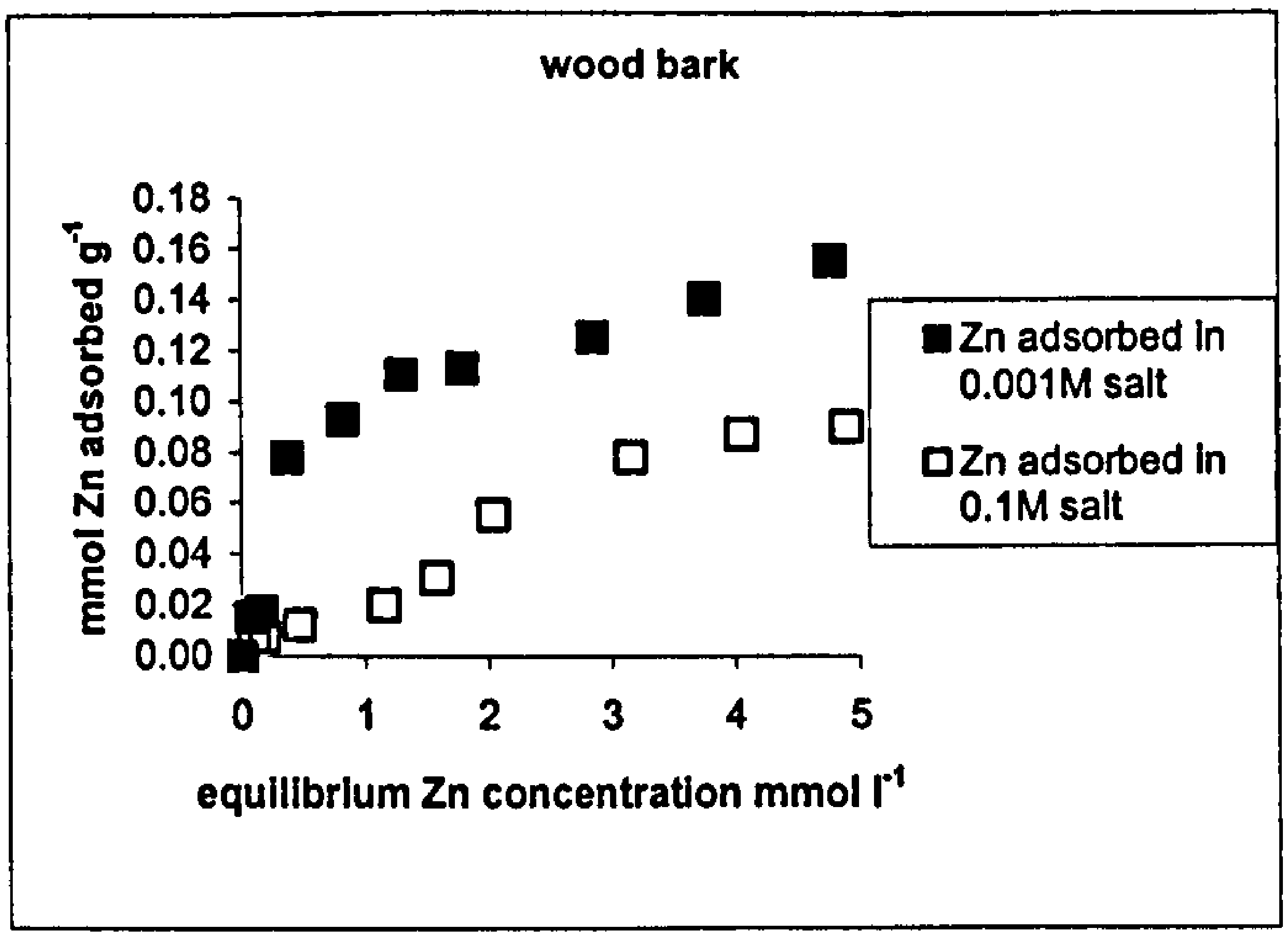


Figure 3.9 Effect of background Ca(NO$_3$)$_2$ concentration on sorption by wood bark (values are means of three replications)

As indicated by the very steep slope of the isotherms, the amendments demonstrated a high affinity for Pb, Cu and Zn at concentrations up to 1 mmol l^{-1} in low background salt. In high salt background however, the rapid sorption was only at concentrations below 0.5 mmol l^{-1}. Generally, the pattern of sorption was the same in both salt concentrations, as seen by the shape of the isotherms.

The results obtained in the study show that background salt concentration plays a very major role in metal sorption, and an overview of Pb, Cu and Zn sorption in both concentrations of calcium nitrate is presented in Figure 3.10. Data represent sorption of the highest metal concentration (5 mmol l^{-1}) only. There was significant reduction in Pb sorption by compost, coir, wood bark and green waste compost in higher background salt. However, bone meal and peat sorption of Pb was not significantly different in either background salt concentration (Figure 3.10). There was also great reduction in Cu and Zn sorption by all amendments due to higher background salt. A single exception was peat, in which sorption of Zn in either salt concentration was not significantly different.

In both background salt concentrations, capacity of amendments to adsorb Pb was in the order (highest to lowest) coir>wood bark> composts> peat> bone meal, while for Cu and Zn sorption it was composts> coir > wood bark> bone meal > peat, especially at high metal concentrations.

The inverse relationship observed between background salt concentration and the amount of metal sorbed confirms the work of Meunier *et. al.* (2003). Many soils have naturally occurring Ca^{2+}, Mg^{2+} and Na^{+} ions in soil solution, and sorption is said to be sensitive to changes in ionic strength of these salts.

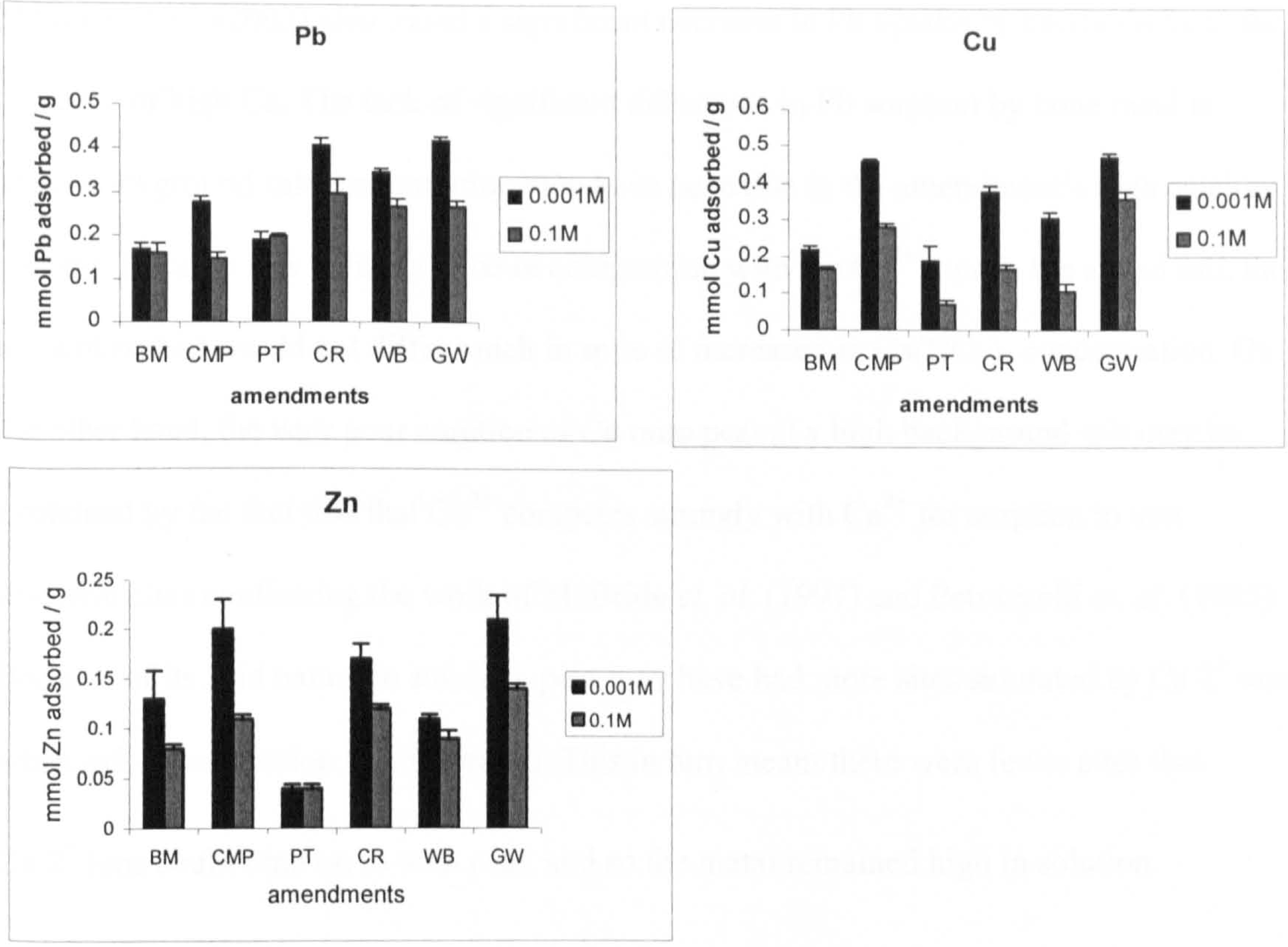


Figure 3.10. Comparative effect of $Ca(NO_3)_2$ concentration on the capacity of amendments to adsorb Pb, Cu and Zn (error bars are ± standard deviation of three replications; BM- bone meal, CMP- compost, PT- peat, CR- coir, WB- wood bark, GW- green waste compost).

These salts have been known to be important in inhibiting sorption of metal ions like Cd^{2+}, Zn^{2+} and Pb^{2+} (Ross, 1994), and such reduction in metal sorption is said to be due to competing Na, Ca and Mg ions (Azizian & Nelson, 1998; Casagrande *et al.*, 2004). The background salt concentration of soils may therefore be an important factor to consider in effective metal sorption by organic wastes, as it could reduce metal sorption in cation rich soils, especially calcareous ones.

The lowering effect of a hundred fold concentration of calcium nitrate on metal sorption is in agreement with previous works. Escrig and Morell (1998), also found that a ten fold increase in concentration of salt matrix led to 77% reduction in Cd sorption.

Meunier *et. al.* (2003) also found a significant decrease in Pb uptake by cocoa shells in the presence of high Ca. The lack of significant difference in Pb sorption by bone meal in either background salt concentration may have been due to the amendment's high calcium content. Because it is unlikely to be in competition with the Ca^{2+} ions in the added salt, the amount sorbed would not differ much in spite of increased in $Ca(NO_3)_2$ concentration. On the other hand, the very poor sorption of Cu onto peat at a high background salt may be explained by the fact that that Cu^{2+} competes strongly with Ca^{2+} for sorption to non selective sites confirming the work of McBride *et. al.* (1997) and Petruzzelli *et. al.* (1985). Because of its acid nature in solution, peat may have had more sites saturated by Ca 2^+ ions when salt concentration was increased. This in turn meant there were fewer sites that Cu 2^+ ions could bind on to with peat, and so the metal remained high in solution.

Lower Zn sorption in 0.1M $Ca(NO_3)_2$ also confirms the work of Agbenin and Olojo (2004) who found that the sorption of Cu and Zn was inversely related to Ca and Mg concentration in soil solution. Villaescusa *et. al.* (2000) had found that there was a drastic reduction in the amount of Cu and Ni removed by tree bark when NaCl concentration was increased at a constant pH. Having established a negative effect of background salt concentration on metal sorption, only results of sorption in 0.001M $Ca(NO_3)_2$ are presented for the rest of this chapter.

3.4 Single metal sorption

3.4.1 Objective

To determine the capacity of selected agricultural materials in binding Pb, Cu and Zn in single metal solutions

3.4.2 Methodology

Pb, Cu and Zn were made up as single solutions of each metal, in a background salt of 0.001M $Ca(NO_3)_2$. Batch sorption of the metals in solution was then carried out with bone meal, composts, peat, coir and wood bark, and the equilibrium metal concentration in solution was determined. The method is discussed fully in Section 2.3.2(page 45).

3.4.3 Results and Discussion

Table 3.1 Langmuir values from single metal sorption batch experiments

Amendment	X max mg g^{-1}	X max mmol g^{-1}	R^2	K_L
Pb				
Bone meal	36.49	0.18	0.9976	12.77
Coir	87.23	0.42	0.9573	3.35
Compost	58.94	0.28	0.9902	6.04
Green waste compost	86.46	0.42	0.9924	8.91
Peat	47.78	0.23	0.9886	4.32
Wood bark	75.34	0.36	0.9068	2.17
Cu				
Bone meal	13.77	0.22	0.9468	2.07
Coir	22.33	0.35	0.8957	2.49
Compost	29.80	0.47	0.7864	1.00
Green waste compost	30.17	0.48	0.9147	1.39
Peat	11.82	0.19	0.9252	2.13
Wood bark	17.06	0.27	0.8879	2.17
Zn				
Bone meal	8.40	0.13	0.9630	2.19
Coir	12.49	0.19	0.9926	3.26
Compost	13.41	0.21	0.9981	6.08
Green waste compost	13.89	0.21	0.9975	6.42
Peat	4.22	0.07	0.9788	2.70
Wood bark	11.04	0.17	0.9537	1.43

K_L is a constant related to binding, and is calculated as slope/intercept of the Langmuir equation

Table 3.2.Comparative effectiveness of amendments to adsorb single Pb, Cu and Zn at varying metal concentrations

	Amount Pb added mmol l $^{-1}$								
	0.1	0.2	0.5	1	1.5	2	3	4	5
	Amount Pb sorbed mmol l $^{-1}$								
Bone meal	0.04a	0.08b	0.08a	0.15b	0.15a	0.17a	0.17a	0.18a	0.17a
Coir	0.04a	0.08b	0.12c	0.19c	0.23c	0.29d	0.33d	0.41e	0.41e
Compost	0.04a	0.08b	0.11bc	0.18bc	0.19b	0.23c	0.27c	0.27c	0.28c
Green waste cmp	0.04a	0.08b	0.16d	0.30d	0.29d	0.31d	0.38e	0.40e	0.42e
Peat	0.03a	0.06a	0.09a	0.13a	0.16a	0.19b	0.20b	0.22b	0.19b
Wood bark	0.04a	0.08b	0.10b	0.14ab	0.16a	0.23c	0.28c	0.35d	0.35d
LSD = 0.02									

	Amount Cu added mmol l $^{-1}$								
	0.1	0.2	0.5	1	1.5	2	3	4	5
	Amount Cu sorbed mmol l $^{-1}$								
Bone meal	0.04a	0.07a	0.08a	0.09a	0.13ba	0.15a	0.16a	0.19a	0.22b
Coir	0.06a	0.11b	0.13b	0.19c	0.23d	0.19b	0.23b	0.33b	0.38d
Compost	0.06a	0.05a	0.09a	0.16bc	0.19c	0.20bc	0.25b	0.39c	0.46e
Green waste cmp	0.07a	0.08a	0.10a	0.24d	0.29e	0.31d	0.32c	0.41c	0.45e
Peat	0.05a	0.08a	0.09a	0.08a	0.08a	0.13a	0.16a	0.18a	0.18a
Wood bark	0.06a	0.09b	0.12b	0.15b	0.12b	0.16ab	0.17a	0.20a	0.31c
LSD = 0.04									

	Amount Zn added mmol l $^{-1}$								
	0.1	0.2	0.5	1	1.5	2	3	4	5
Amendment	Amount Zn sorbed mmol l $^{-1}$								
Bone meal	0.03a	0.03a	0.07a	0.07b	0.08b	0.08b	0.11b	0.11b	0.13b
Coir	0.02a	0.04ab	0.09a	0.12c	0.16d	0.17d	0.18c	0.18d	0.19d
Compost	0.04a	0.06b	0.12c	0.15d	0.18d	0.18d	0.19c	0.20d	0.20d
Green waste cmp	0.05a	0.07b	0.14c	0.15d	0.18d	0.19d	0.20c	0.20d	0.21d
Peat	0.02a	0.02a	0.04b	0.04a	0.05a	0.05a	0.06a	0.06a	0.07a
Wood bark	0.02a	0.02a	0.08a	0.09b	0.11c	0.11c	0.13b	0.14c	0.16c
LSD = 0.03									

(Values within a single column followed by the same letter are not significantly different at $p < 0.05$)

Sorption of Pb, Cu and Zn up to an initial concentration of approximately 3 mmol per litre, showed a very steep slope, indicating a very strong affinity of the materials for the metals. Above this concentration, uptake generally became constant, as indicated by the plateau or levelling of the curve from that point on (Figures 3.11 – 3.13). The amendments were significantly different with regard to the amount of Pb sorbed, especially at concentrations above 0.5 mmol l^{-1} (see Table 3.2, page 83). Isotherm curves comparing the effectiveness of the six amendments in adsorbing Pb, Cu and Zn are shown in Figures 3.6a to 3.8b. Lines denoted as 'fitted' were calculated by applying the least squares deviation method to the data. Even at a concentration as low as 0.2 mmol l^{-1}Pb, the amount of Pb sorbed by green waste compost, coir and wood bark was significantly higher than that by the other three amendments (Table 3.2).

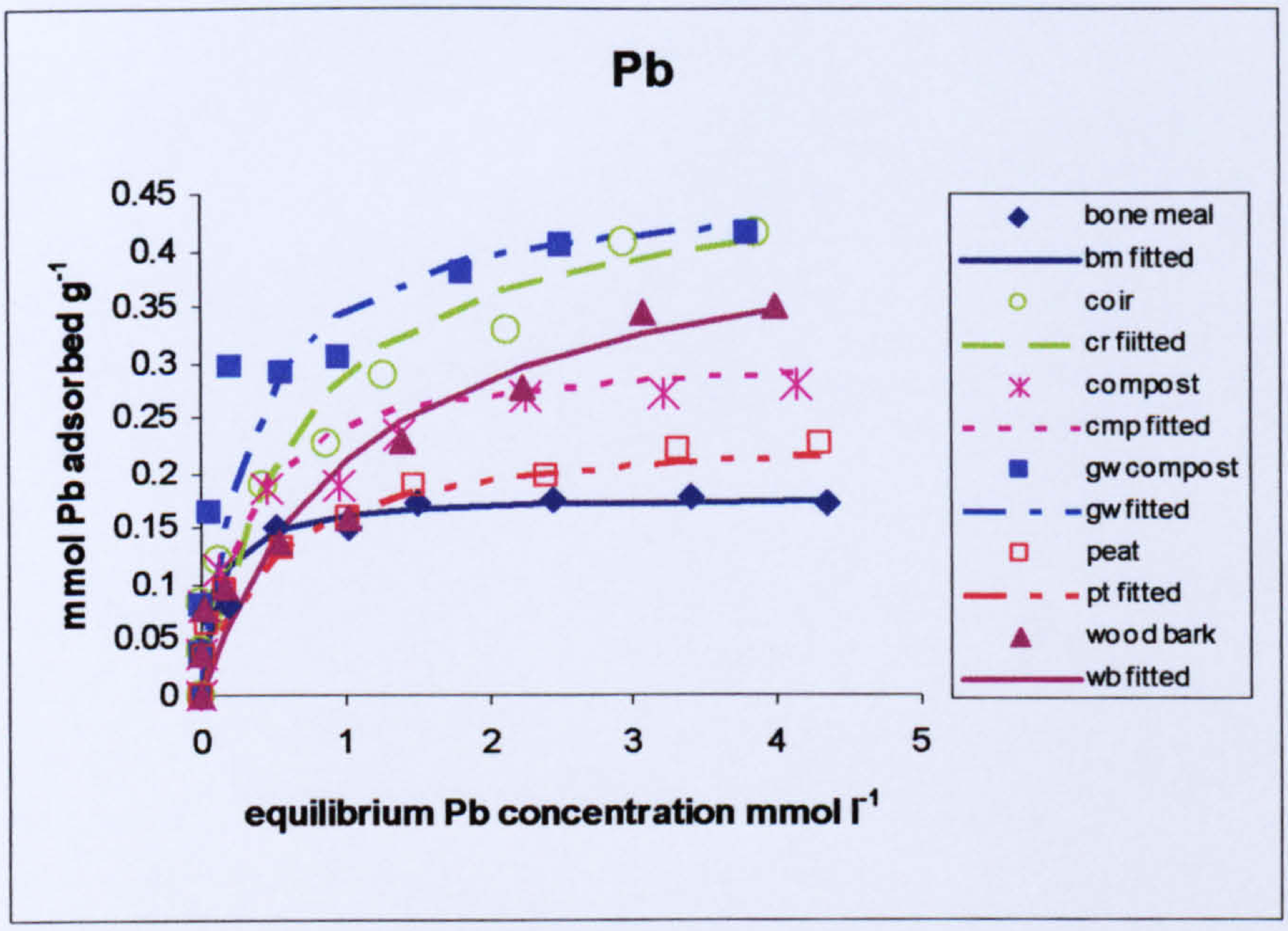


Figure 3.11 Comparative sorption of Pb by selected wastes (Values are means of three replications. Symbols are experimental data and lines are fitted from least squares deviation method)

The capacity for Pb sorption was clearly in the order Green waste compost~Coir>Wood bark>Compost>Bone meal>Peat. The lines derived by application of the Langmuir equation to the data for Pb sorption were generally linear, with $R^2 > 0.90$ (Figures 3.12 and 3.13).

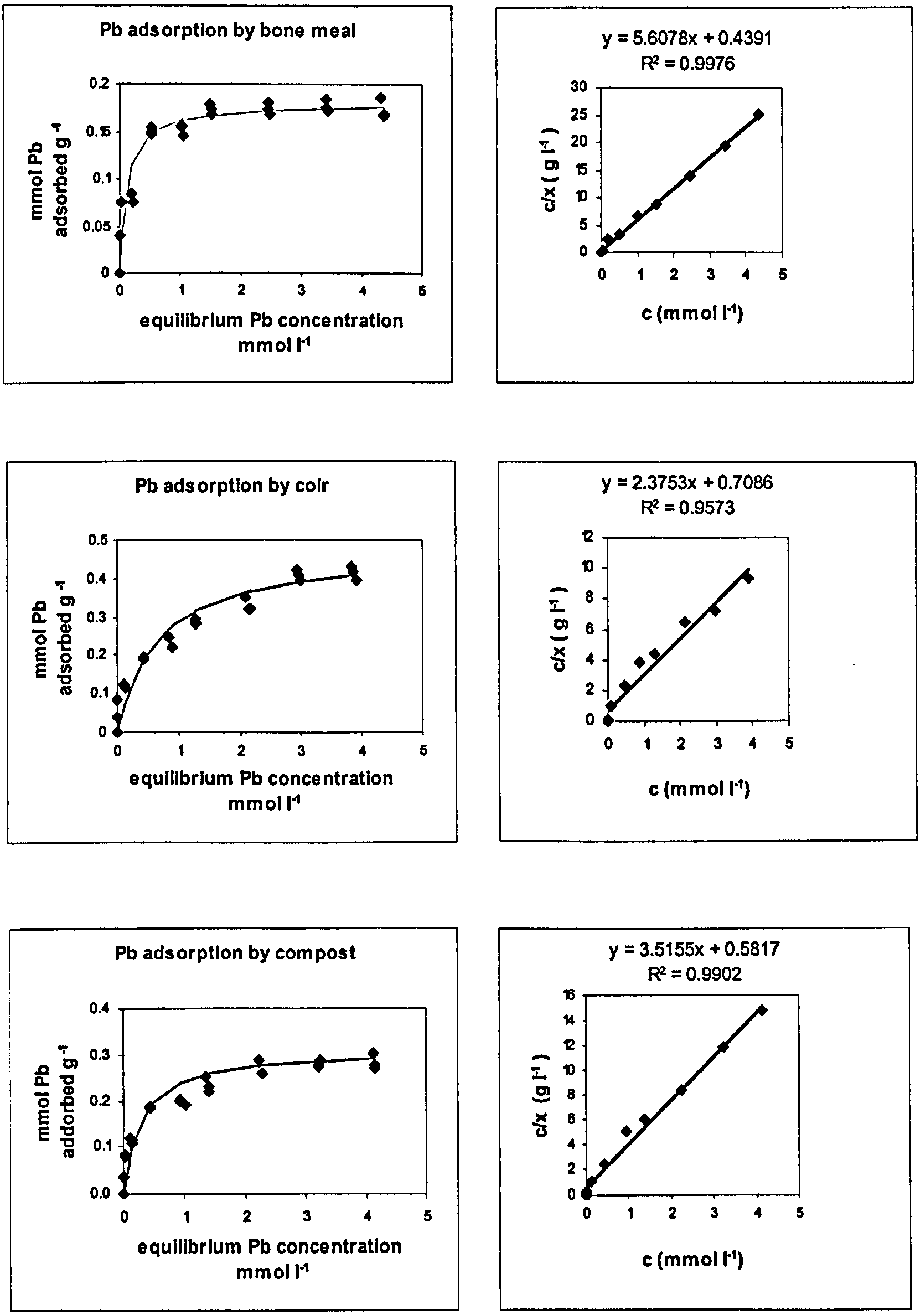


Figure 3.12 Isotherms and Langmuir equations of single Pb sorption by bone meal, coir and compost

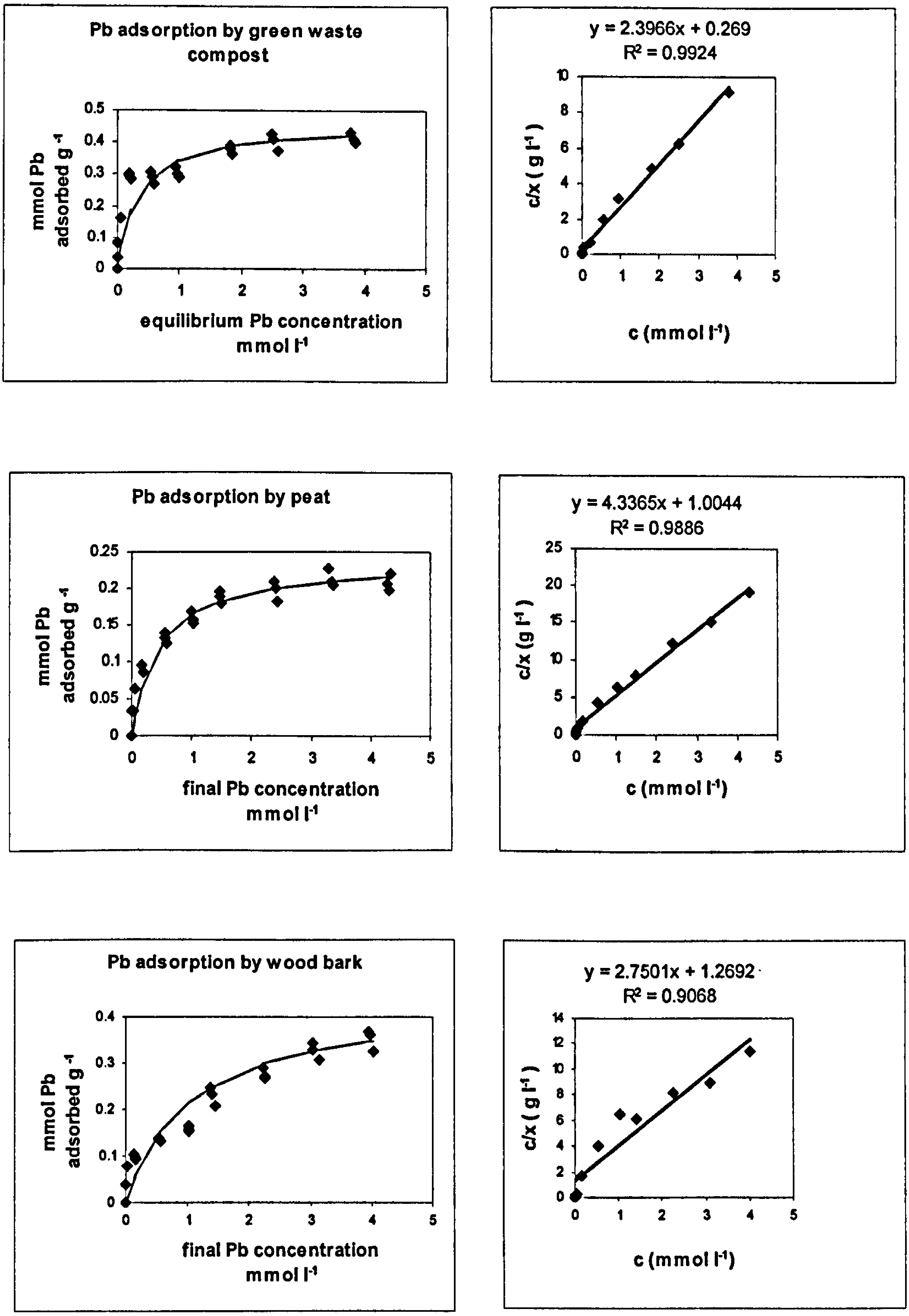


Figure 3.13 Isotherms and Langmuir equations of single Pb sorption by green waste compost, peat and wood bark

The effectiveness of the two types of compost and coir in adsorbing copper was significantly higher than that by bone meal and wood bark, while peat was the least effective in adsorbing Cu when compared with the other amendments (Table 3.2; Figure 3.16). From the sorption isotherms, peat appeared to have reached its saturation limit for Cu, adsorbing only 0.18 mmol g^{-1} from a solution of 5 mmol l^{-1}. Compost, on the other hand, sorbed 0.46 mmol g^{-1} Cu at the same concentration. Cu sorption was in the order compost~ green waste compost>coir>wood bark>bone meal~peat.

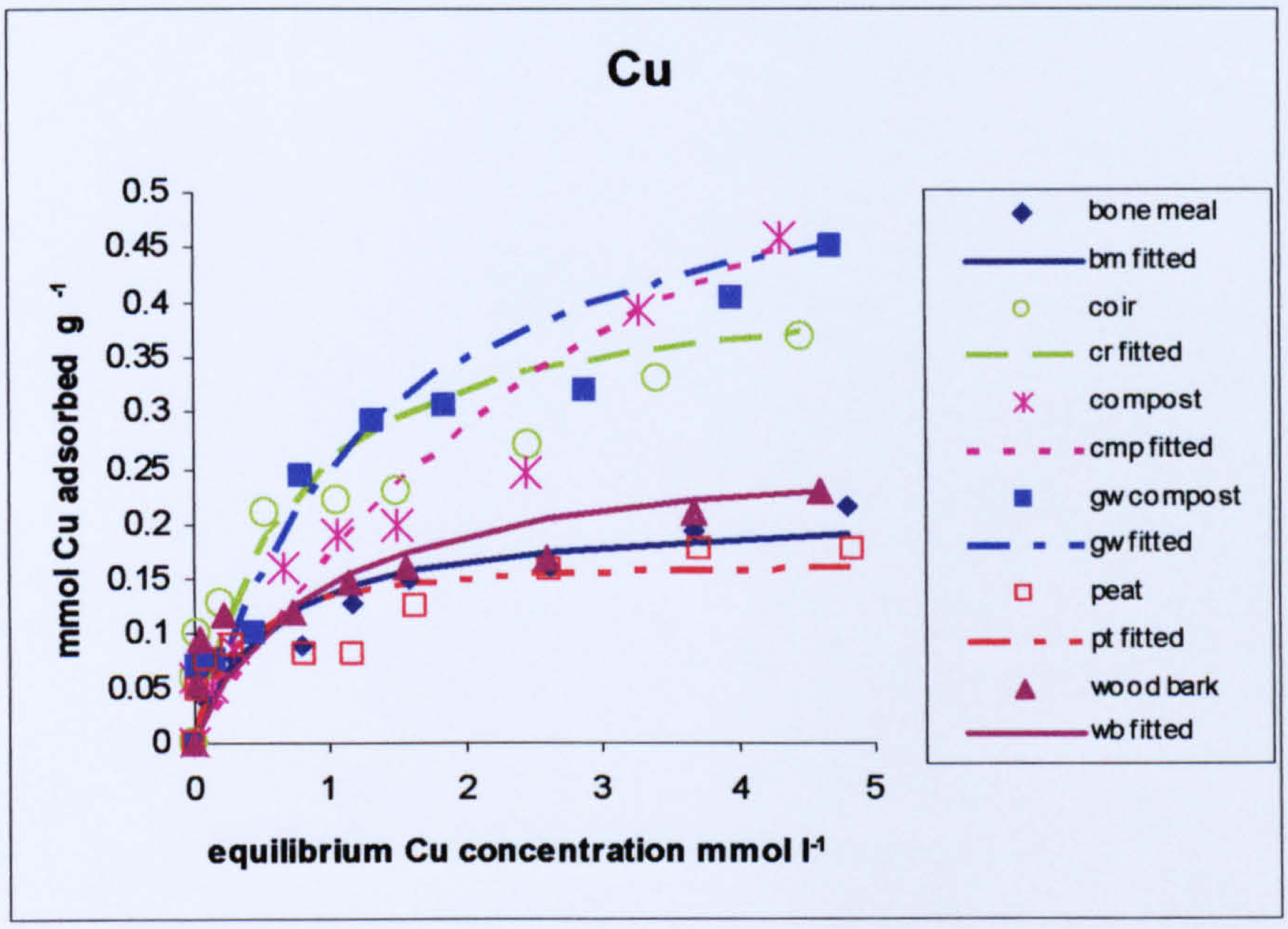


Figure 3.14 Comparative sorption of Cu by amendments (Values are means of three replications. Symbols are experimental data, and lines are fitted from least squares deviation method

Most of the amendments were very well suited to the Langmuir equation. Bone meal, green waste compost and peat sorption of Cu were strongly linear ($R^2 > 0.90$), while coir and wood bark sorption of Cu was marginally linear at an approximate R^2 value of 0.89. Compost sorption of Cu was however not linear (R^2 0.78), although it was the most effective in adsorbing Cu, ranking as high as green waste compost (Figures 3.15 and 3.16).

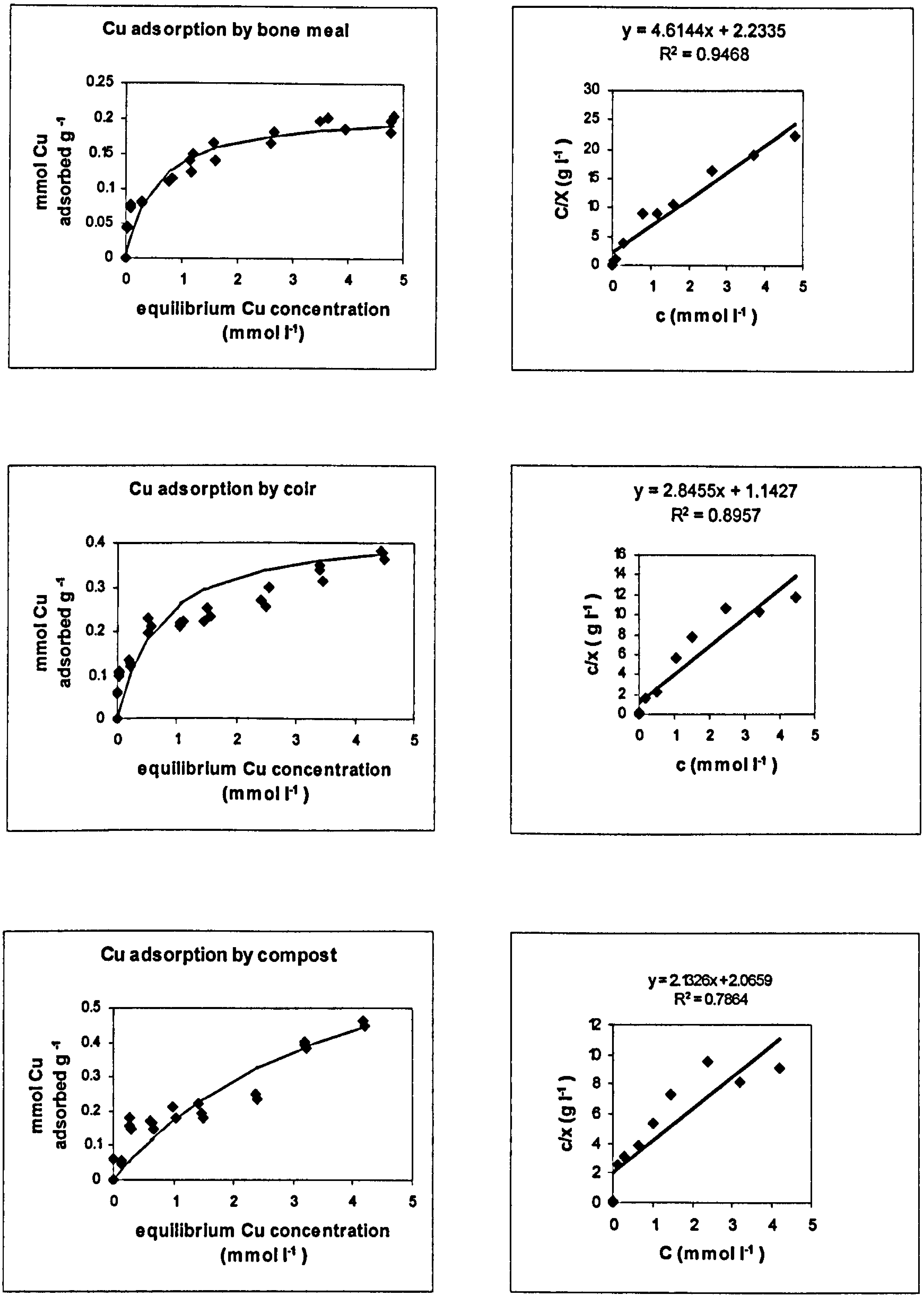


Figure 3.15 Isotherms and Langmuir equations of single Cu sorption by bone meal, coir and compost

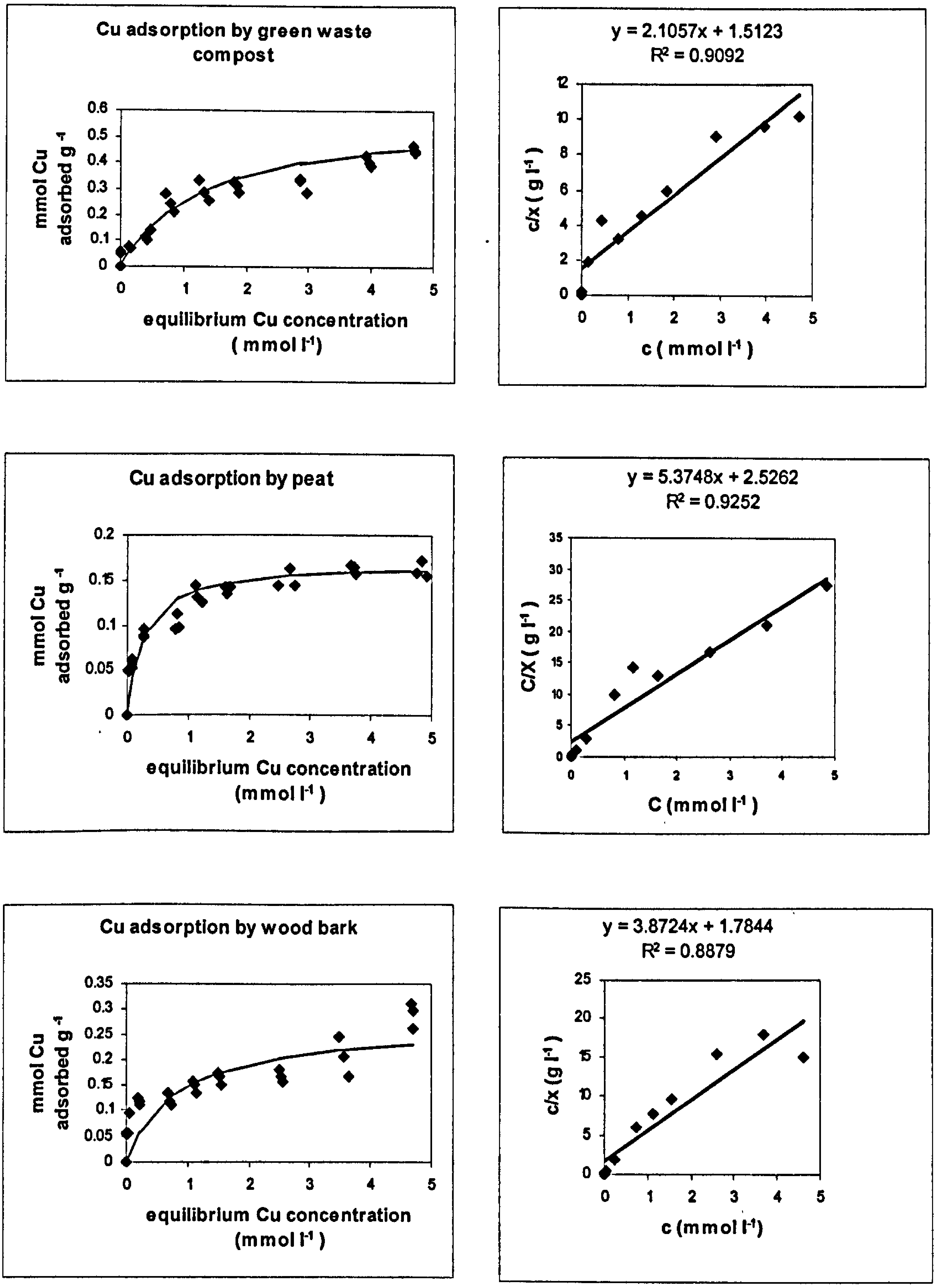


Figure 3.16 Isotherms and Langmuir equations of single Cu sorption by green waste compost, peat and wood bark

The pattern of Cu sorption assumed an 'inverted S-shape, in most amendments, displaying an initial plateau effect between 1 and 2 mmol l $^{-1}$, then continuing to rise as the Cu concentration increased towards 5 mmol $^{-1}$. This indicates a two stage sorption for Cu, which is most likely caused by fast initial chemical reactions on readily available surface sites on the amendments, while the slower reaction may be due to diffusion to internal sites, which tend to have lower affinity for metal ions, resulting in the slower rate.

The two types of compost were significantly higher in zinc sorption than the other adsorbents, and the trend of Zn sorption was thus compost~green waste compost>coir>wood bark>bone meal>peat (Figure 3.17). Again, Zn sorption was lowest in peat across all the concentrations tested. The low pH of peat may have been responsible for its low ability to adsorb Cu and Zn when compared to the other amendments, as those two elements are poorly sorbed under acid conditions

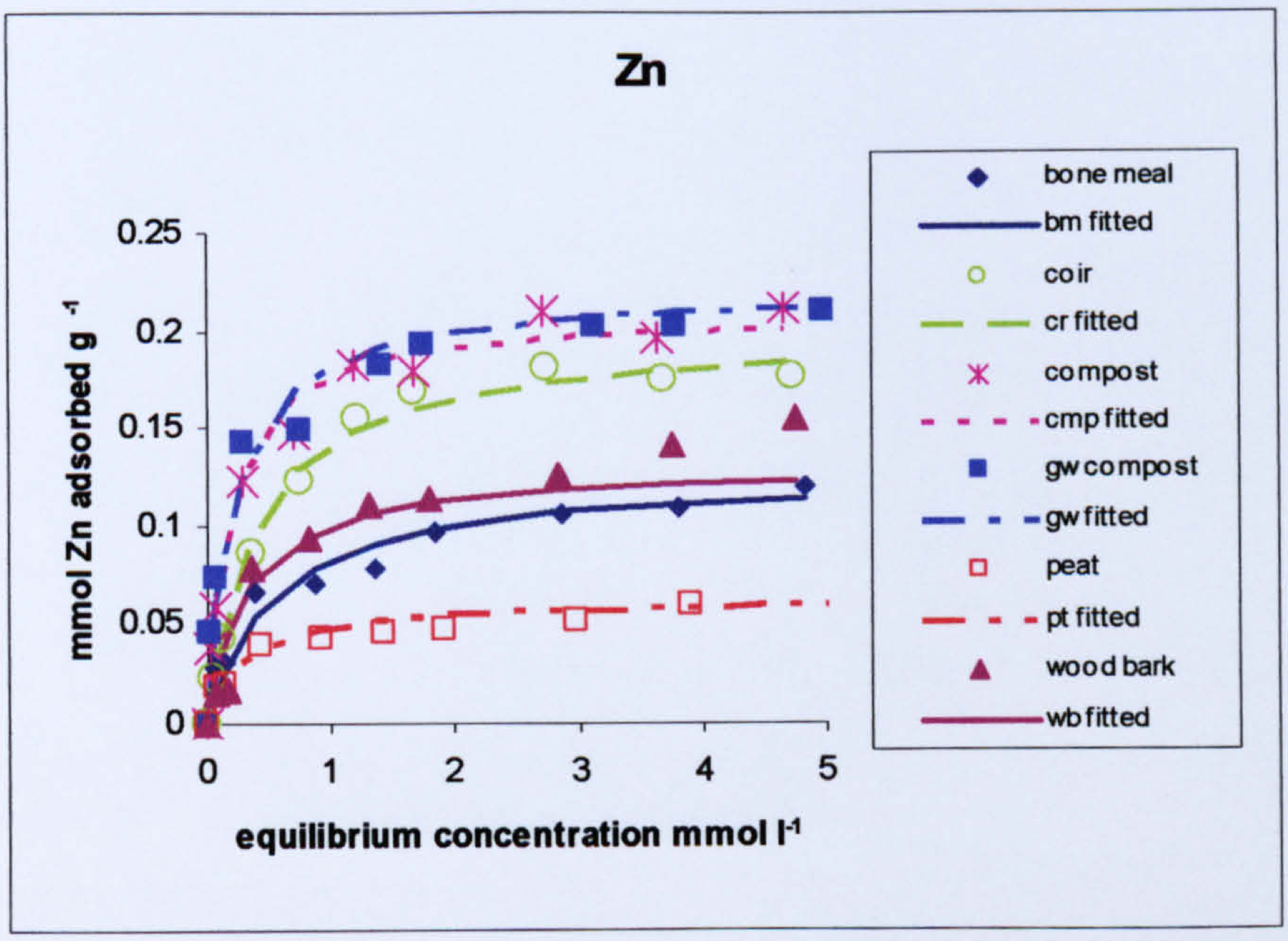


Figure 3.17 Comparative sorption of Zn by selected wastes (Values are means of three replications. Symbols are experimental data and lines are least squares deviation fitting)

The lines derived by application of the Langmuir equation to the data for Zn sorption were all strongly linear, with $R^2 > 0.95$ (Figures 3.18 and 3.19).

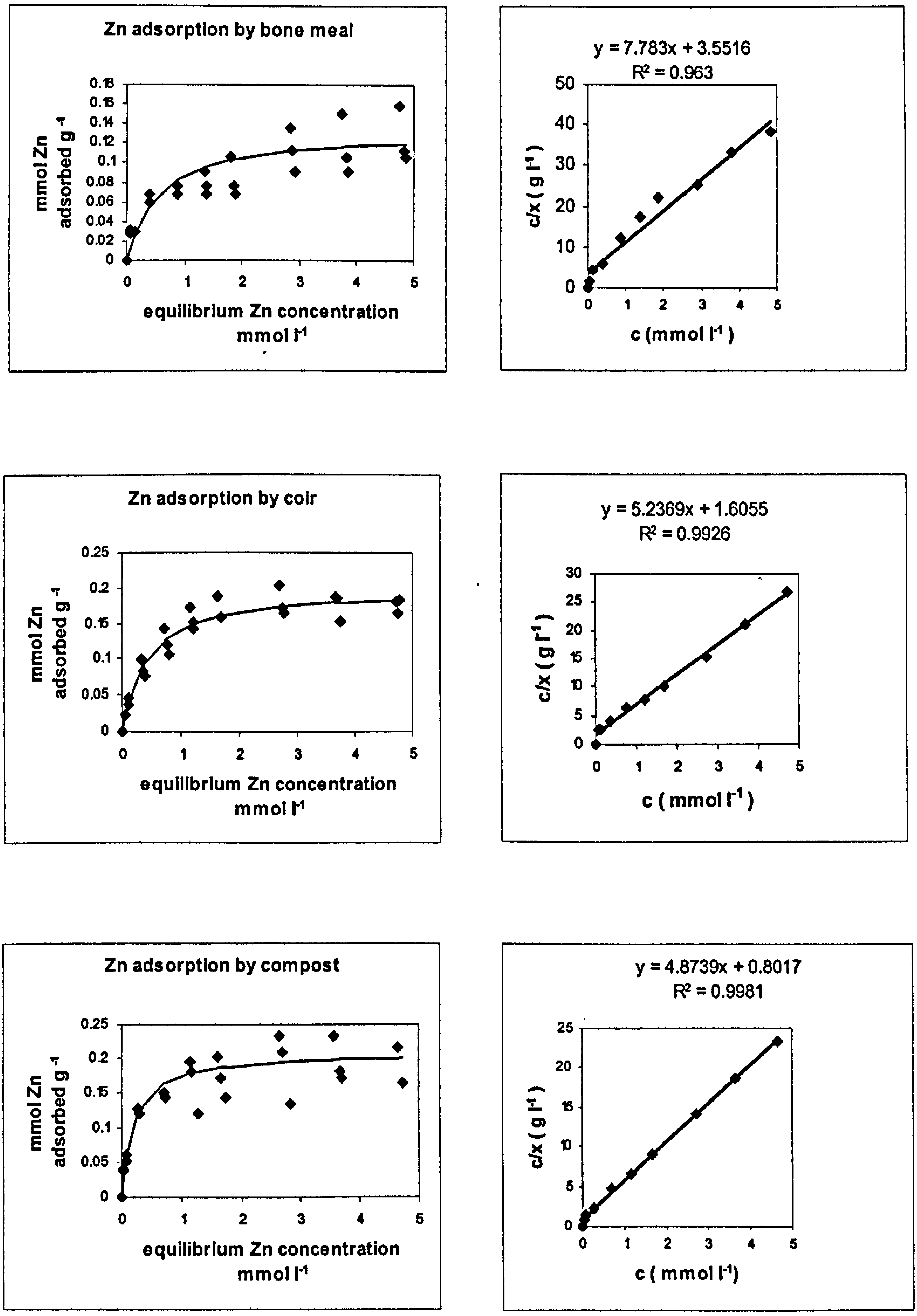


Figure 3.18 Isotherms and Langmuir equations of single Zn sorption by bone meal, coir and compost

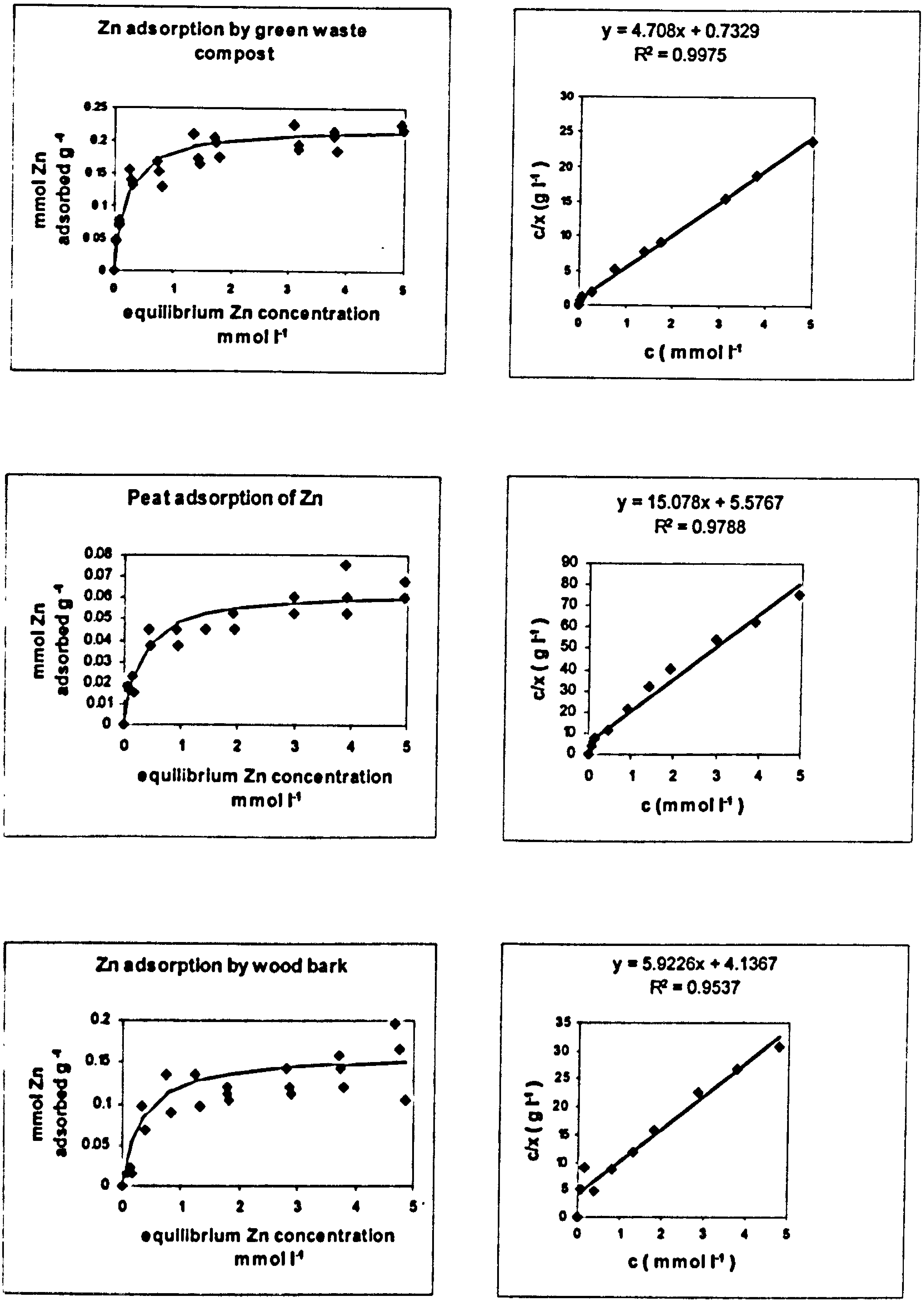


Figure 3.19 Isotherms and Langmuir equations of single Zn sorption by green waste compost, peat and wood bark

Research has shown that the composting process brings about a great change in the composition of the dissolved organic matter (Zhou & Wong, 2001), and that Cu has been found to have a very high affinity for DOM. In addition, studies have shown that there is generally an increase in DOM at higher pH (Yin *et al.*, 2002). At 7.4, the pH of the green waste compost used in the study is very high, and the amendment may have required a longer contact time with the metal to attain its optimum capacity for adorption (Axe & Anderson, 1998).

Compost sorption of Cu in the study compares very favourably with a proven effective sorbent like bone charcoal, which was found to adsorb an actual maximum of 0.45 mmol Cu g^{-1} by Wilson *et. al.* (2003). The non-linear Langmuir isotherms of Cu for some amendments follows the trend observed by Ben–Shalom *et al* (2005), who found strongly non- linear Cu sorption isotherms of Cu with chitosans, while Bradl (2004) also observed a two stage sorption for Cu.

Like Cu, Zn sorption is very sensitive to acid conditions (Miretzky *et al.*, 2005), and peat addition has been known to cause a lowering of pH in the soil thus leading to an increase in extractable metals in soil, and also decreasing the sorption of metals like Cd, Cu, Zn and Ni in the soil (Arnesen & Singh, 1999; Narwal & Singh, 1998). It has also been argued that at lower pHs, the carboxyl groups which act as bonding sites on organic materials, retain their protons, thus reducing their probability of binding to any positively charged ions like the metals (Matos & Arruda, 2003). This is because at lower pH, H^+ ions are sorbed to the surface of the amendments added, resulting in a net positive charge. This inhibits sorption of the positively charged metal ions. As the pH increases, OH^- concentration increases, and there will be a consequent increase in metal sorption (Jenne, 1998).

The single metal sorption capacity of amendments in this study, were comparable with those used elsewhere, as shown in Table 3.3.

Table 3.3 The ability of some sorbents to adsorb Pb, Cu and Zn

Metal	Adsorbent	Xmax (mg g^{-1})	Reference
Lead	Coir	46.64	Quek *et. al.*,(1998)
	Tea leaves	78.7	Tan and Khan, 1988
	Bone charcoal	80	Wilson, (2002)
	Cocoa shell	32.6	Meunier, (2003)
	Wheat bran	79.37	Ozer, (2007)
Copper	Oil palm fibres	2.0	Low et al.,1993
	Sphagnum moss peat	16.4	Ho, (1996)
	Peat biomass	25.4	Ma and Tobin, (2003)
	Modified coconut coir	227	Baes *et. al*, (1996)
	Unwashed bone charcoal	26.5	Wilson, (2002)
Zinc	Bone charcoal	25.5	Wilson, (2002)
	Modified peanut shell	20.3	Chamarthy *et. al*, (2001)
	Vermicompost	28.4	Matos& Arruda, (2003)

3.5 Competitive (multi metal sorption)

3.5.1 *Objective*

To evaluate the effect of competing metals on the individual sorption of Pb, Cu or Zn in solution

3.5.2 *Method*

Multi-metal solutions comprising Pb, Cu and Zn was made up from metal salts, all in background 0.001M $Ca(NO_3)_2$. Batch sorption was then carried out using bone meal, compost, peat, coir and wood bark as adsorbents. The method is fully discussed in Section 2.3.4; page 46.

3.5.3 *Results and discussion*

Results for metal sorption in two equimolar solutions of the three metals are presented in Table 3.4. When all three metals were present in solution in equal concentration at 0.1 mmol g^{-1} or 1 mmol g^{-1}, there was no significant difference in the capacity of the amendments to adsorb Pb. Cu sorption in equimolar mixed metal solution however showed significant differences in the capacity of the various amendments, with coir and green waste compost being significantly most effective in adsorbing Cu, while bone meal was least effective. Zn sorption in equimolar solution showed bone meal and peat to be least effective in adsorbing Zn when all metals were 0.1 mmol g^{-1}.

Table 3.4 **The effect of equimolar concentrations of competing metals on the capacity of amendments to adsorb Pb, Cu and Zn**

	Amount of metal sorbed mmol g^{-1}	
	equimolar solution of 0.1mmol l^{-1}	equimolar solution of 1 mmol l^{-1}
Pb		
Bone meal	0.023c	0.077d
Coir	0.023c	0.074d
Compost	0.022c	0.071d
GW compost	0.022c	0.075d
Peat	0.022c	0.069d
Wood bark	0.022c	0.074d
Cu		
Bone meal	0.011b	0.031a
Coir	0.017bc	0.069d
Compost	0.019bc	0.059c
GW compost	0.021c	0.072d
Peat	0.015c	0.043bc
Wood bark	0.019c	0.043bc
Zn		
Bone meal	0.011a	0.037b
Coir	0.013b	0.043b
Compost	0.022c	0.039b
GW compost	0.025c	0.042b
Peat	0.003a	0.025a
Wood bark	0.018bc	0.034a

LSD = 0.009 at $p < 0.05$

(Comparisons of individual metals only. Values within a single column which are followed by the same letter are not significantly different at $p < 0.05$)

The sorption of Pb, Cu or Zn in multi-metal solution was significantly lower than that in single metal solution, suggesting a competition among the different metals for limited binding sites on the amendments. In the multi-metal solution made up of equimolar concentrations of Pb, Cu and Zn, the amount of Pb sorbed was approximately 60% of that in a single element solution of 0.1 mmol l^{-1}Pb (Table 3.5). Cu sorption from the multi metal solution was approximately 30% of that in single metal solution. The effect of equal concentrations of Pb and Cu on Zn sorption was widely varied. While peat sorption was down to only 16% of that achieved in single Zn solution, wood bark still sorbed 95% when compared to Zn sorption in single solution.

Table 3.5 Competitive effects of Pb, Cu and Zn on amount of metal sorbed in equimolar solution of 0.1mmol l^{-1} (ratio 1:1:1)

	% of single sorption		
Amendment	Pb	Cu	Zn
Bone meal	57	25	38
Compost	58	32	58
Peat	66	30	16
Coir	58	32	58
Wood bark	57	34	95

Data are mean values of three replications

Results fo the amount of sorption when all three metals were present in equal concentrations of 0.1 mmol l^{-1}, and a ratio 1metal:10 fold concentration of the other two metals are presented in (Figure 3.20).

Individual sorption of either Pb or Cu by all the amendments was reduced in 0.1 mmol l^{-1} equimolar solution containing the three metals, when compared to sorption in single metal solution. With Zn sorption in equimolar solution of 0.1 mmol l^{-1}, the same reduction was observed, except in wood bark, where Zn sorption in equimolar solution of 0.1 mmol l^{-1} was not affected by the presence of the two other metals.

The competitive effect of other metals was more evident when competing metals were at higher concentration but the third metal was kept at the initial lower concentration of 0.1 mmol l^{-1}. In multi metal solution where 0.1 mmol l^{-1} of Pb was combined with 1 mmol l^{-1} of Cu and Zn, Pb sorption by amendments was approximately 50% of that in single solution. Sorption by bone meal in this instance was lower still, being only 35% (Table 3.6). The strongest effect from higher concentrations of competing metal ions was observed on Zn, as the presence of Pb and Cu led to the amount of Zn sorbed being between 14 to 33% of that sorbed in single Zn solution (Table 3.6).

Table 3.6 Competitive effects of Pb, Cu and Zn on amount of metal sorbed in 0.1mmol l^{-1} of one metal, and 1 mmol $^{-1}$ of the other two metals (ratio 1:10:10)

	% of single sorption		
Amendment	Pb	Cu	Zn
Bone meal	35	31	14
Compost	54	36	33
Peat	53	37	25
Coir	51	36	20
Wood bark	49	38	33

Data are mean values of three replications. (competitive Pb sorption is at 0.1 mmol l^{-1} Pb + 1 mmol l^{-1} Cu and Zn; competitive Cu sorption is at 1 mmol l^{-1} Pb + 0.1 mmol l^{-1} Cu +1 mmol l^{-1} Zn etc)

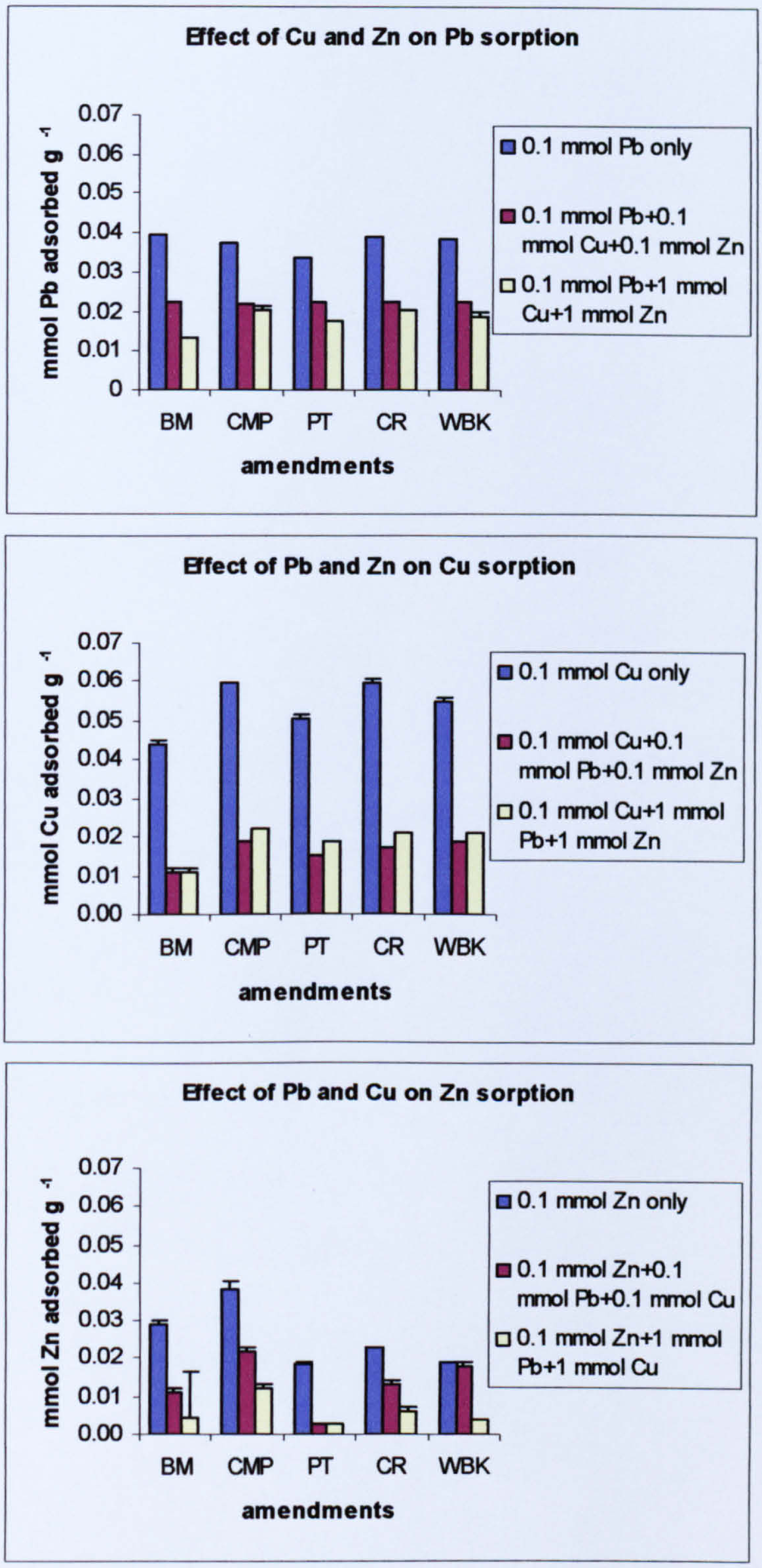


Figure 3.20 Effect of competing metals on the sorption of 0.1 mmol l^{-1} Pb, Cu and Zn

(BM- bone meal; CMP- compost; PT- peat; WBK- wood bark. Values are means standard deviation, n=3)

The sorption ability of bone meal was most affected by competing metals, as it presented the greatest reduction in the amount of Pb, Cu and Zn sorbed when compared to its capacity for the metals in single solution. No data are presented for green waste compost in competitive sorption.

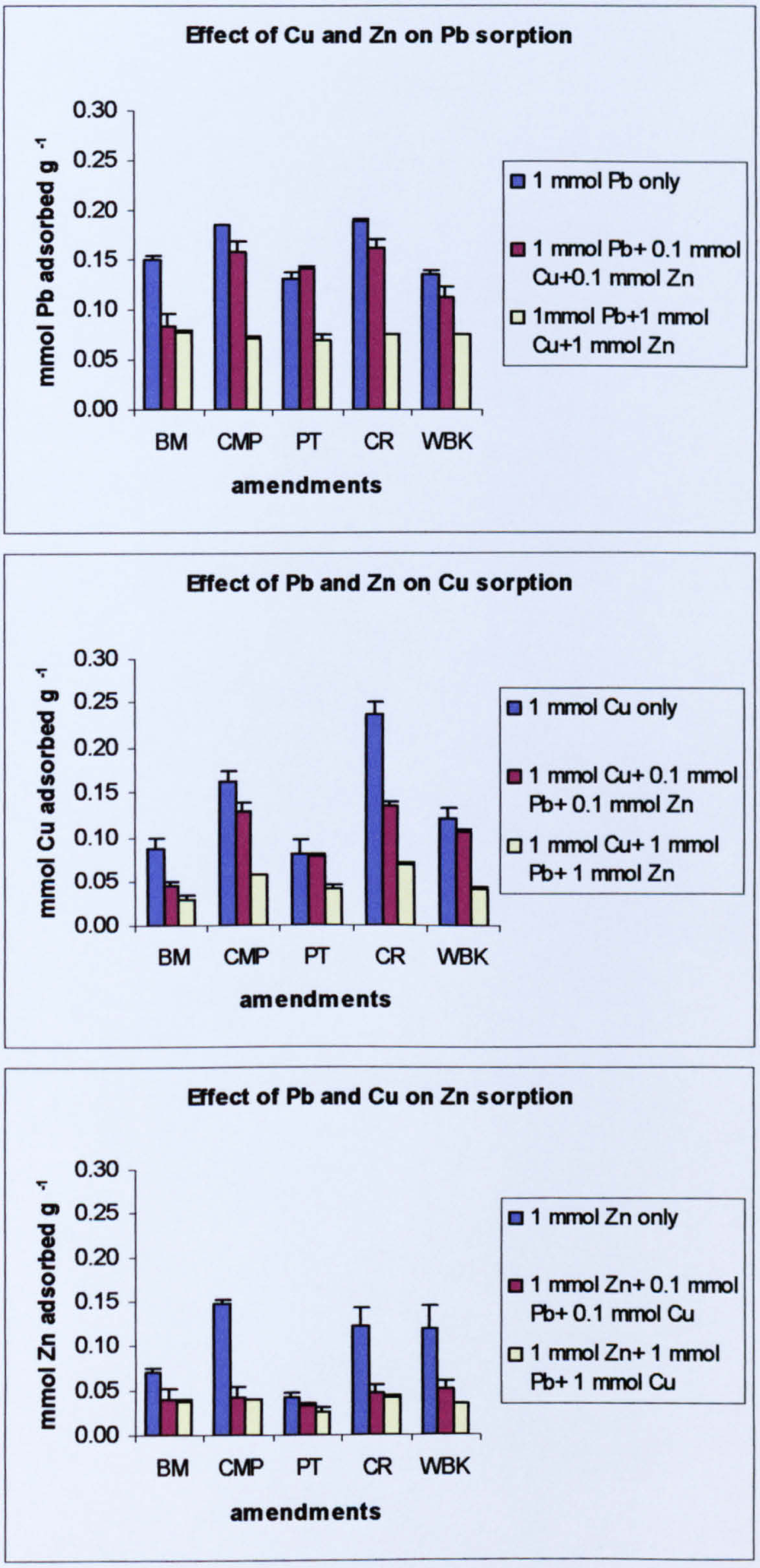


Figure 3.21 Effect of competing metals on the sorption of 1 mmol l^{-1} Pb, Cu and Zn.
(BM- bone meal; CMP- compost; PT- peat; WBK- wood bark. Values are means standard deviation, n=3)

Figure 3.21 shows the amount of individual metal sorbed in a mixed metal solution of equimolar concentration of 1 mmol l^{-1}, and also, the results obtained when only one metal was increased 10 fold, while the other two were kept at 0.1 mmol l^{-1}. From the results presented in Figures 3.20 and 3.21, a higher concentration of two out of the three metals further aggravated the effect on the metal at lower concentration, as there was greater reduction in the sorption of the metal at lower concentration when compared to its sorption in equimolar solution.

A reduction in sorption due to competing metals confirms the work of Qin *et. al* (2006). They found that compared to single solution systems, the sorption of Pb on peat was reduced by 30.8% in Pb-Cd-Cu system, and that Cu sorption was reduced by 49.7%. Antoniadis *et. al.*, (2007) also found that Zn sorption on to clay was reduced by 18% in the presence of Cu. The more pronounced effect of competing metals at higher equimolar concentration may be explained by the fact that at low metal concentrations, the abundant sorption sites on the amendments may not be fully saturated, whereas, at higher concentrations, metals with greater affinity would be preferentially sorbed than those with a lower affinity (Antoniadis *et al.*, 2007; Christophi & Axe, 2000).

The fact that Zn was most adversely affected by the presence of Pb and Cu, confirms the works of Leitao (2003), Arias *et. al.* (2006), and Ucer *et. al* (2006), that selectivity of metals is in the order of Pb > Cu > Zn sorption (see Table 3.4, page 96). In equimolar solution of Cu and Zn, Arias *et. al.* (2006) also found that Cu sorption was consistently greater than that of Zn. It has been suggested that the selectivity of metals in single solution may be explained by different ionic radius sizes of the metal ions studied, as , in decreasing order, the ionic radius of Pb >Cu>Zn (Sposito, 1989). The general consensus is that, for the single metal solution, ions with higher ionic radii are preferentially sorbed (Emsley, 1991; Tarley & Arruda, 2004).

Other researchers have contended that the amount of metal sorbed in single metal solutions may be due to differences in electro-negativity of metals , reporting a stronger attraction with elements of higher the electro-negativity (Matos & Arruda, 2003). This is especially true when comparing metals of widely varying electronegativity values, but may not be applicable in the case of Pb, Cu and Zn, all with a similar electronegativity value of approximately 1.7 (Helmenstine, 2007). Additional metal properties which have also been cited as playing a role in metal sorption are the ionic potential, first hydrolysis constant and softness parameter (Agbenin & Olojo, 2004). The influence of other factors including the type of adsorbent and experimental conditions may also lead to the occurrence of affinity sequences which are different from those predicted based on metal properties alone (Vaca-Paulin *et al.*, 2006).

In this study, higher concentrations of Pb and Cu led to highly significant reduction in amount of Zn sorbed when Zn was maintained at a lower concentration, confirming the work of Arias *et. al.* (2006). They observed that increasing the concentration of Cu while maintaining Zn at a lower concentration led to a drastic reduction in the sorption of Zn, when compared to the equimolar solution. As observed by Chamarthy *et.al* (2001), and Cao.*et al* (2004), Pb sorption was found to be least affected in a multi metal solution of Pb, Cu and Zn, while Zn was most affected by competing effects. The binding strength of the three metals was in the order Pb > Cu > Zn, even in the multi – metal system, especially at high multi-metal equimolar solutions (Table 3.4, page 96). This confirms that Pb and Cu are more strongly retained compared to Zn (Fontes & Gomes, 2003; Sag *et al.*, 2000).

3.6 Conclusion

A contact time of one hour led to more than 95% of metal sorption, and the six amendments evaluated were effective in binding Pb, Cu and Zn, with similar capacities at metal concentrations below 0.5 mmol l^{-1} (see Table 3.2, page 83) At higher metal concentrations however, there were significant differences in their capacities for metal sorption.

An increase in background salt concentration led to a significant decrease in amount of metal sorbed by all the amendments, except bone meal. Contrary to expectation, the capacity of bone meal to sorb Pb was lower in comparison to that of the other amendments used in the study. Most amendments appeared to have reached their saturation capacity when exposed to metal concentrations of 5 mmol l^{-1} (see Table 3.1, page 82), judging by the closeness of the actual sorption value to that of the theoretical maximum. The amount of metal sorbed by all six amendments is comparative with the results obtained elsewhere (Table 3.2, page 83), and where higher maximum sorption was obtained in other works, the data were often on modified amendments. By using the amendments in their natural form without washing or modification of the pH in this research, a true representation of their potential capacities was obtained.

The presence of other metals reduced the sorption of any single metal, even when the competing metals were in very low concentrations. Taking the performance with individual amendments into account, the amendments varied in their effectiveness to adsorb Pb, Cu and Zn. Composts, coir, and wood bark showed greater ability to bind metals than bone meal and peat, and the sorption of metals in the study generally adhered to the Langmuir model.

Chapter 4

Microbial respiration in metal contaminated media

4.1 Introduction

'*Microbes play an important role in the biogeochemical cycling of heavy metals and also in cleaning up or remediation of metal contaminated environments*' (Perelomov & Kandeler, 2006). Two of the most important microbial processes that take place in the soil are the carbon and nitrogen cycles. The amount of CO_2 evolved is a very reliable indicator of the effect of metal contamination on microbial activity (Rost *et al.*, 2001; Yang *et al.*, 2006). While microbial respiration has been found to suffer the greatest reduction by metal contamination, other parameters like biomass may be reduced only slightly (Rost *et al.*, 2001).

Microbial respiration is an effective measure of the rate of carbon mineralization, since about 70% of C added to the soil is lost as carbon dioxide mainly as a product of microbial respiration (Machulla *et al.*, 2004; Pulford, 2007; Usman *et al.*, 2004). Burkhardt et. al (1993) found that the ability of bacteria to decompose complex substrates was significantly reduced by the presence of heavy metals. Using microbial respiration as an indicator, the effect of metals on decomposition of organic matter can therefore be effectively evaluated. Due to their very sensitive response, microbiological properties of soil provide immediate and accurate information on small changes taking place in the soil (Ros *et al.*, 2003). After only one day of incubation at low Pb pollution level, a 50% reduction in microbial enzyme activity was observed by Tejada et. al (2007). Thus, the monitoring of soil contamination can be carried out by microbiological as well as chemical methods (Montserrat *et al.*, 2006).

Incubation periods which have been used to assess the effect of metal toxicity on microorganisms cover a very wide range. Some studies have been as few as 20 days (Perelomov & Kandeler, 2006); to as much as 300 days (Cotrufo *et al.*, 1995). The response of microorganisms to metals in spiked soil has been found to differ greatly from that of their responses to field contaminated soils (Lock & Janssen, 2001). As with plant studies, metal effects on microorganisms through fresh spiking tend to be higher than that from aged field contaminated soils. This often leads to a large discrepancy in responses obtained at similar concentration levels but where mode of contamination differs (Smolders *et al.*, 2003).

This chapter is in three main sections. First, the effect of added Pb, Cu and Zn in solution at different degrees of contamination on CO_2 evolution by the amendments was measured over 10 weeks. Second, the effect of adding different amounts of each amendment (1%, 10% and 20%) to a contaminated soil on the CO_2 evolution over 10 weeks was measured. Third, using selective extraction (water), the available Pb, Cu and Zn in contaminated amendments was determined at the end of the incubation period.

4.1.1 Aim

The aim of this study was to evaluate the effectiveness of organic materials in alleviating inhibitory effects of metals on microbial respiration.

4.2 Effect of metal toxicity on microbial respiration in metal contaminated amendments

4.2.1 *Objective*

To determine the effect of metal toxicity on CO_2 evolved in amendments which had been contaminated by single metal solutions of Pb, Cu or Zn.

4.2.2 *Methodology*

Single metal solutions of Pb, Cu or Zn was added to green waste compost, peat, coir and wood bark, a process described as metal 'spiking' of the amendments. The resulting mixtures were incubated over a period of ten weeks each. Metal concentrations used were 5, 10 and 50 mg g^{-1}. The method is fully discussed in Chapter 2 (Section 2.9.1, page 63)

4.2.3 *Results and discussion*

The quantity of CO_2 evolved in all the amendments was adversely affected by the presence of Pb, Cu and Zn (Figures 4.1 to 4.3). After 10 weeks of incubation, cumulative CO_2 in Pb contaminated amendments was in the order wood bark>peat>coir>green waste compost, at the highest level of 50 mg Pb g^{-1}. For amendments contaminated at the highest level of 50 mg g^{-1} Cu, cumulative CO_2 after 10 weeks was in the order coir>wood bark>peat>green waste compost, while in amendments contaminated with 50 mg Zn g^{-1}, it was in the order wood bark>coir~peat>green waste compost.

There was a reduction in evolution of CO_2 within a single treatment over time, in the contaminated amendments. Therefore, evolution declined as time passed from week 1 to week 2, being lowest at week 10 (Figures 4.1 to 4.3). There was also reduction in weekly CO_2 evolved in non-contaminated amendments over time.

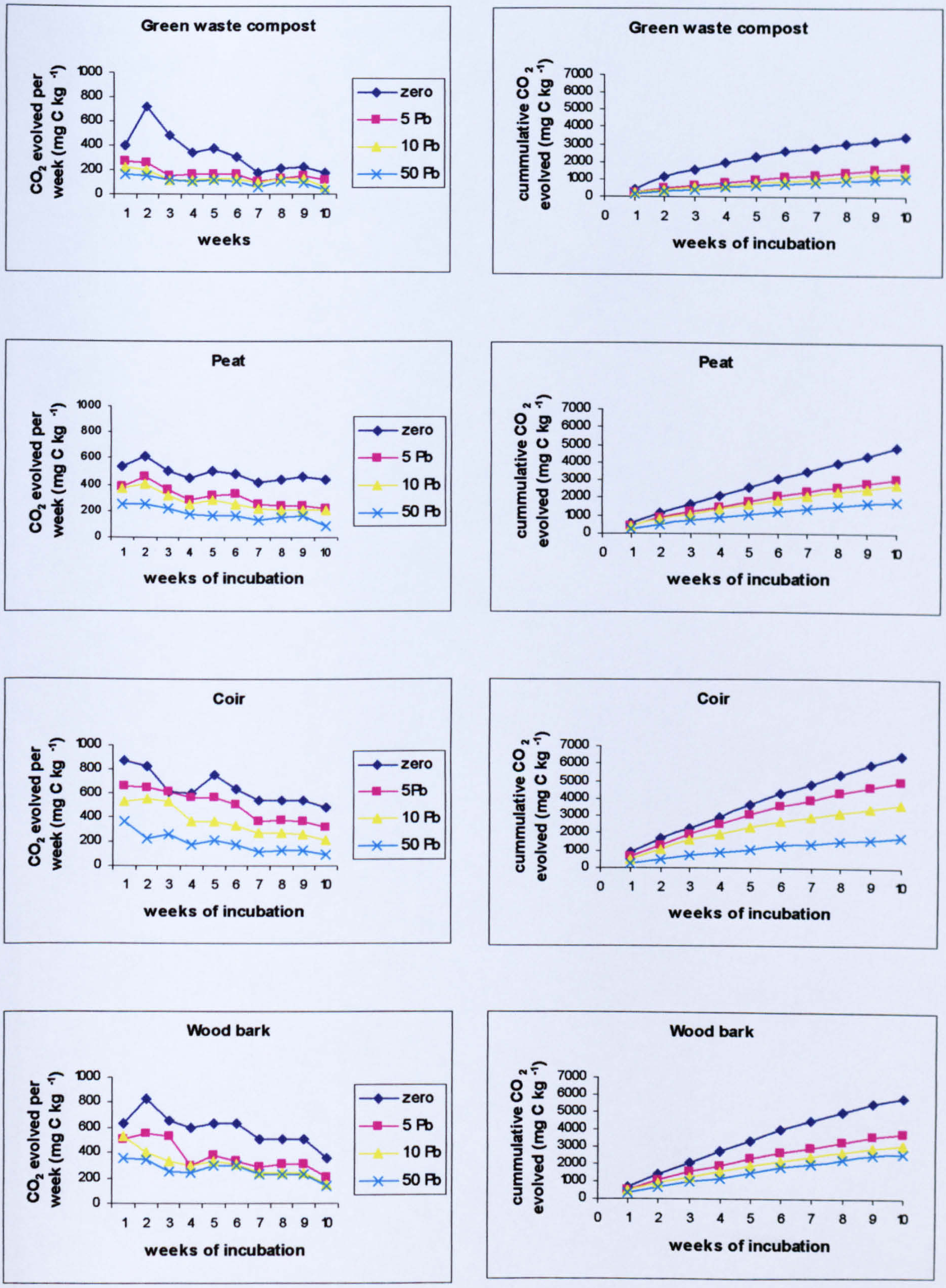


Figure 4.1 Effect of Pb on microbial respiration in contaminated amendments. (Zero - non-contaminated; 5, 10 and 50-mg g^{-1})

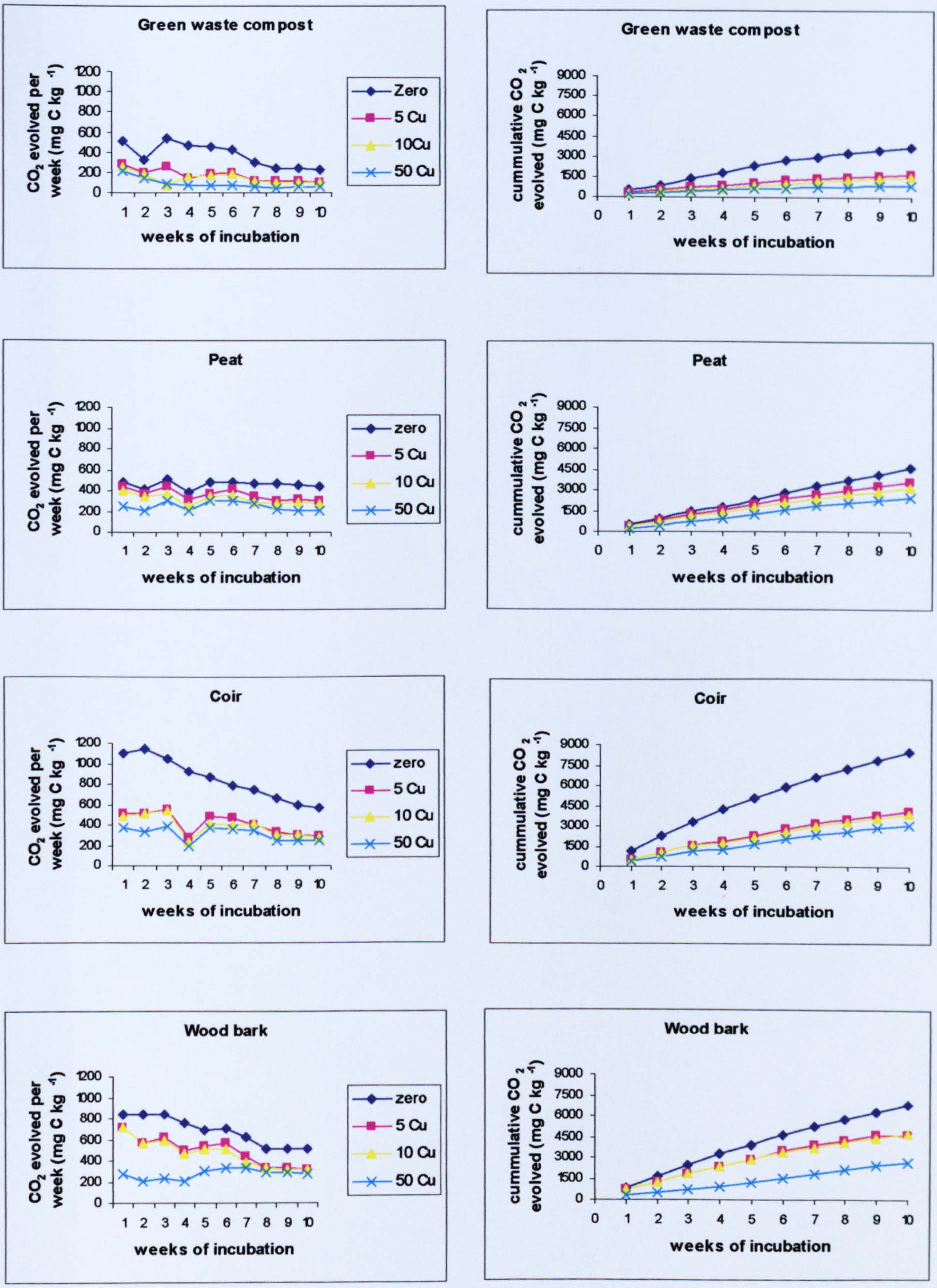


Figure 4.2 Effect of Cu on microbial respiration in contaminated amendments. (Zero - non-contaminated, 5, 10 and 50-mg g^{-1})

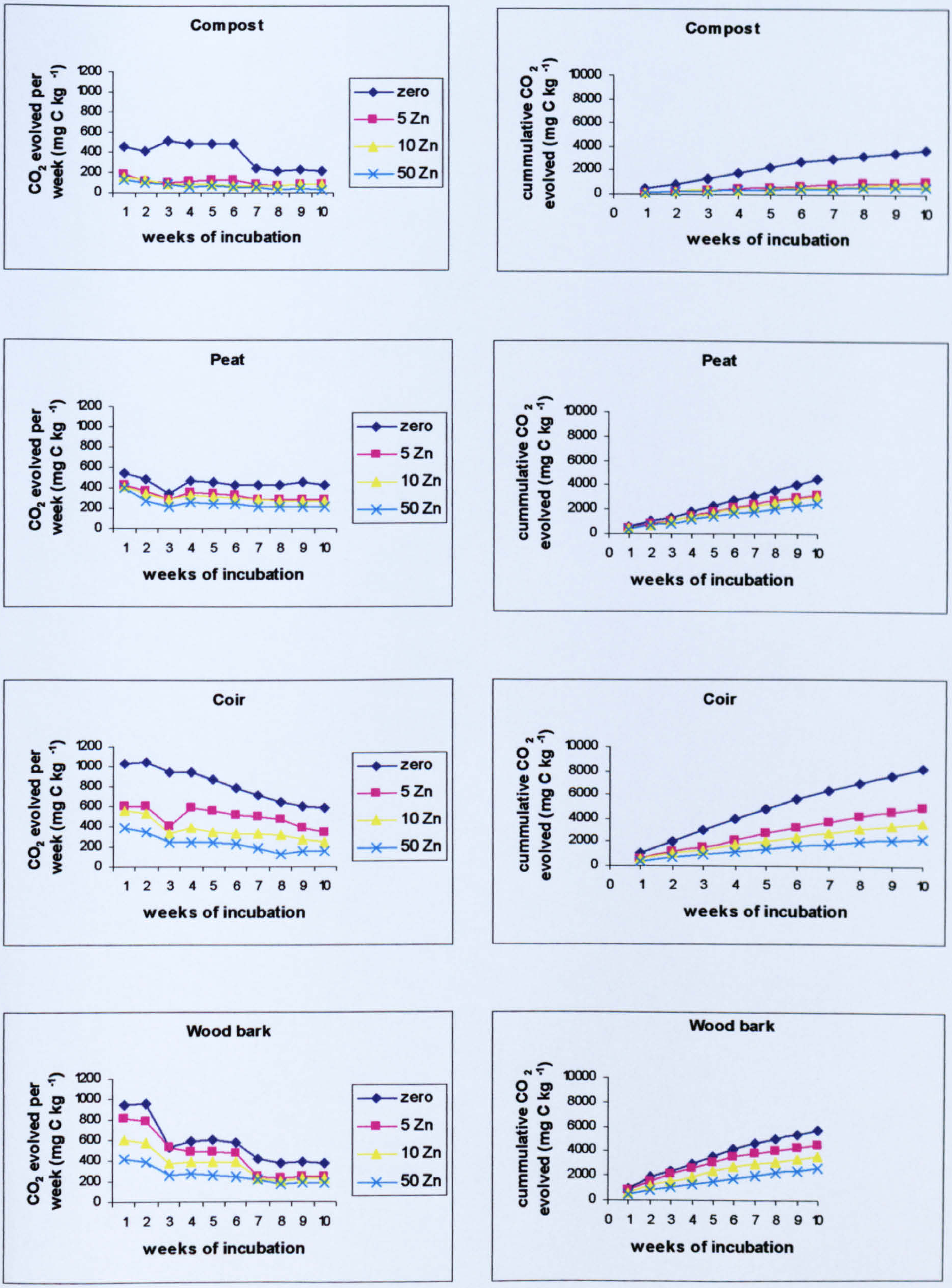


Figure 4.3 Effect of Zn on microbial respiration in contaminated amendments. (Zero - non-contaminated, 5, 10 and 50-mg g^{-1})

4.2.3.1 Mean weekly respiration in contaminated amendments

In the last five weeks of incubation, there was a generally continuous reduction in the evolution of CO_2 in all three incubation experiments. Thus, weeks 6-10 were used to estimate the weekly CO_2 evolved, giving an estimation of the microbial respiration value. At that stage, amendments had reached a relatively constant/linear rate of CO_2 production (Moscatelli *et al.*, 2005). The mean weekly CO_2 production is presented in Tables 4.1 to 4.3.

Table 4.1 Mean weekly rate of CO_2 evolved in Pb contaminated amendments

mg g^{-1} Pb applied	Amount CO_2 evolved (mg C kg^{-1} week^{-1})			
	GW Compost	Peat	Coir	Wood bark
0	203aB	441bC	528cD	480bC
5	131aA	240bB	356cC	288bB
10	111aA	213bAB	254bB	222bAB
50	77aA	140bA	123bA	207bA

LSD row= 69; column=80

Data within a single row or column which are followed by the same letter are not significantly different at $p<0.05$ (a,b, c,(row)- comparison of amendments; A, B, C- (column) comparison of metal concentration).

Table 4.2 Mean weekly rate of CO_2 evolved in Cu contaminated amendments

mg g^{-1} Cu applied	Amount CO_2 evolved (mg C kg^{-1} week^{-1})			
	GW Compost	Peat	Coir	Wood bark
0	249aB	464bC	642dB	531cB
5	117aA	317bB	330bA	335bA
10	108aA	278bAB	321cA	288bcA
50	56aA	227bA	273bA	291bA

LSD row=54; column=63

Data within a single row or column which are followed by the same letter are not significantly different at $p<0.05$ (a,b, c,(row)- comparison of amendments; A, B, C- (column) comparison of metal concentration).

Table 4.3 Mean weekly rate of CO_2 evolved in Zn contaminated amendments

	Amount CO_2 evolved (mg C kg^{-1} $week^{-1}$)			
mg g^{-1} Zn applied	GW Compost	Peat	Coir	Wood bark
0	221aB	443bB	635cD	389bB
5	86aA	285bA	425cC	239bA
10	83aA	273bA	290bB	216bA
50	41aA	210bA	152bA	182bA
LSD row =81; column=94				

Data within a single row or column which are followed by the same letter are not significantly different at $p<0.05$ (a,b, c,(row)- comparison of amendments; A, B, C- (column) comparison of metal concentration).

- Row comparisons

In each of the separate metal incubations, CO_2 evolution in the non-contaminated amendments was generally in the order coir>wood bark ≥peat>green waste compost. Under metal contamination, and comparing amendments contaminated with *similar metal concentrations*, the respiration in green waste compost was significantly lower for all metals, while CO_2 evolved in the coir, peat and wood bark was not significantly different from each other at Pb and Zn concentrations above 5 mg g^{-1}. (Tables 4.1 to 4.3), and not significantly different at Cu at 50 mg g^{-1}. Thus, as metal concentrations increased, differences between amendments became less significant.

- Column comparisons

Any addition of all three metals significantly reduced CO_2 evolution in all amendments when compared to the non-contaminated ones (Tables 4.1 to 4.3). The effect of increasing metal concentrations on CO_2 evolution is summarised in Table 4.4.

Table 4.4 Metal concentrations which led to significant differences in CO_2 evolved in contaminated amendments

	GW compost	Peat	Coir	Wood bark
Pb	any	>10	step	>10
Cu	any	>10	any	any
Zn	any	any	step	any

(GW- green waste; any- any metal concentration; ≡- stepwise increase in toxicity as metal concentration increased; >10- > 10 mg g^{-1} metal)

For green waste compost, peat and wood bark, the amount of metal added had little effect, as in many cases, the amount of CO_2 evolved by varying metal concentrations were not significantly different. For example with Pb, CO_2 evolved by contaminated peat at 5 and 10 mg g^{-1} was not significantly different, but 50 mg g^{-1} was significantly different from 5 and 10 mg g^{-1}. In wood bark, the amount of CO_2 evolved at 10 and 50 mg g^{-1} Pb were not significantly different from each other, while being different only from the lowest concentration of 5 mg g^{-1}. Coir in all cases showed more stepwise effect of contamination, with CO_2 evolved becoming less as amount of metal increased. This suggests that coir is inactivating the metal.

The very drastic reduction observed in CO_2 evolved in non-contaminated amendments as incubation period progressed can be explained by a reduction in energy source. It has been shown that, even where microbes become adjusted to a metal contaminated environment, CO_2 evolution may initially proceed unaffected, but that this would later decrease due to a decline in energy source (Kao *et al.*, 2006).

A clear reduction in amount of CO_2 evolved in the presence of Pb, Cu and Zn, even at the lowest concentration of 5 mg g^{-1} confirms that basal respiration rate, and consequently other metabolic activities tend to be adversely affected by metals (Gibbs *et al.*, 2006; Kelly *et al.*, 2003). It has been suggested that there is a resistance to decomposition of organic material with high metal concentrations (Boucher *et al.*, 2005; Hattori, 1992; Hattori, 1996). This may be explained by the fact that, under a situation of increasing metal stress, more energy is required to meet the demands of microorganisms (Khan & Joergensen, 2006).

4.2.3.2 Evaluating the amount of inhibition of microbial respiration in metal 'spiked' amendments

The inhibition effect of metals on CO_2 evolved in metal spiked amendments is presented in Tables 4.2 to Table 4.4, and was calculated as

% inhibition = (1-B/A) *100

where A = cumulative CO_2 evolved in non-metal treated amendment, and

B = cumulative CO_2 evolved in metal treated amendment

Thus, % inhibition is in comparison to respiration in individual non contaminated amendments.

Inhibition of microbial respiration by metals did not follow a predictable pattern. At low concentrations of Pb, the inhibitory effect was least in coir, but at high concentration of 50 mg Pb g^{-1}, there was no significant difference in inhibition in peat, compost or coir (Table 4.5). Inhibition in wood bark was significantly lower at this high concentration than in the other amendments.

Table 4.5 Effect of Pb toxicity on microbial respiration in contaminated wastes

		% inhibition by Pb		
	Week	5mg g^{-1} Pb	10 mg g^{-1} Pb	50 mg g^{-1} Pb
GW Compost	1	57.9d	66.4d	72.9c
	5	55.4d	64.6d	70.9bc
	10	50.9d	60.5d	69.3bc
Peat	1	27ab	34.2ab	55.9a
	5	30.9b	39.1abc	59.9ab
	10	36.1bc	43.5ab	63.3ab
Coir	1	16.9a	30.5a	69.5bc
	5	16.8a	38.1ab	70.3bc
	10	22.1ab	42.7b	72.5c
Wood bark	1	24.8ab	40.4b	54.6a
	5	33.2c	44.5b	54.7a
	10	35.5c	47.6b	55.5a

LSD = 11.4 (Values followed by the same letter within a single column are not significantly different at $p<0.05$; GW-green waste)

The inhibitory effect of Cu on microbial respiration is presented in Table 4.6. Inhibition in peat was significantly the least at all concentrations of Cu, after 10 weeks of incubation. At 5 mg Cu g^{-1}, inhibition was highest in compost, but at 50 mg Cu g^{-1} inhibition was equally high in compost and coir after 10 weeks (Table 4.6).

Table 4.6 Effect of Cu toxicity on microbial respiration in contaminated wastes

		% inhibition by Cu		
Amendment	Week	5mg g^{-1}Cu	10mg g^{-1}Cu	50mg mg g^{-1}Cu
GW Compost	1	46.7c	63.0b	68.5bc
	5	54.1c	63.0b	75.7c
	10	54.0c	61.3b	76.2c
Peat	1	11.7a	20.2a	45.7a
	5	14.7a	24.5a	42.9a
	10	21.4a	30.8a	46.1a
Coir	1	52.3c	53.6b	66.8bc
	5	52.6c	55.9b	65.6bc
	10	51.2c	53.9b	62.9b
Wood bark	1	24.4b	25.0a	71.4b
	5	25.0b	27.9a	66.7b
	10	32.3b	30.8a	60.0b

LSD = 10.7 (Values followed by the same letter within a single column are not significantly different at $p<0.05$; GW- green waste).

Table 4.7 Effect of Zn toxicity on microbial respiration in contaminated wastes

		% inhibition by Zn		
Amendment	Weeks	5 mg g^{-1} Zn	10 mg g^{-1} Zn	50 mg g^{-1} Zn
GW Compost	1	73.9d	77.2d	80.4d
	5	73.5d	79.9d	83.6d
	10	70.4d	75.7d	83d
Peat	1	19.8ab	23.1a	35.2a
	5	21.5ab	26.5a	40.3a
	10	27.1b	31.1ab	45.2a
Coir	1	47c	55c	67.8c
	5	42.1c	56.c8	70.1c
	10	39.4c	56.1c	71.9c
Wood bark	1	13ab	36.4b	56.2b
	5	15.4ab	35.1b	56.3b
	10	21.7ab	37.6b	55.4b

LSD = 10.4 (Values followed by the same letter within a single column are not significantly different at $p<0.05$; GW- green waste).

In Zn contaminated amendments, inhibition was highest in compost, and equally least in peat and coir at a low 5 mg Zn g-1 (Table 4.7). At 50 mg Zn g-1 however, % inhibition was in the order (highest to lowest) green waste compost>coir>wood bark>peat.

At 5 mg g^{-1} metal concentration, inhibition to microbial respiration was similar when comparing the four amendments. At the highest concentration of 50 mg g^{-1} however, there was a clear separating out of the amount of inhibition taking place in each amendment. Thus the ability of the amendments to sustain microbial respiration in the presence of toxicity was significantly different at high Cu and Zn concentrations, but not at low metal concentrations. From the results also, inhibition within a single treatment (i.e same amendment / same metal / same concentration) was generally unchanged within the incubation period, being the same at the onset, midway, and at the end of the experiment after 10 weeks of the incubation (Tables 4.5 to 4.7).

4.2.3.3 Correlation between organic carbon, CEC, and microbial respiration in metal contaminated amendments

In order to determine the relationship between organic carbon content of amendment, the cation exchange capacity, and the concentration of metal on the amount of inhibition in metal contaminated amendments, correlations between these parameters was determined using Pearson's correlation in Minitab statistical package. Data for the entire incubation period of ten weeks was used, and the results are presented in Table 4.5.

Table 4.8 Correlations between organic carbon, CEC, metal concentration, and inhibition of microbial respiration

	% organic carbon	CEC	metal concentration
Inhibition by Pb	-0.480*	-0.374 ns	0.779***
Inhibition by Cu	-0.543**	-0.739***	0.556**
Inhibition by Zn	-0.749***	-0.825***	0.471*

(*, **, and *** represent significant Pearson's correlation $p < 0.05$, 0.01 and 0.001 respectively).

There was significant negative correlation between the organic carbon content of each amendment and the amount of inhibition by all three metals (Table 4.8). There was also significant negative correlation between an amendment's cation exchange capacity, and the inhibition of CO_2 evolved when contaminated by Cu and Zn. This indicates that the higher the organic carbon content or CEC of the amendment, the lower the inhibitory effect of metals on respiration within the concentrations tested in the study. As expected, there was significant positive correlation between metal concentration and the amount of inhibition by Pb, Cu and Zn on all amendments, with the relationship being stronger for Pb>Cu>Zn.

It has been established that responses of microorganisms to metal contamination do not always lead to a reduction in amount of CO_2 evolved (Kao *et al.*, 2006), especially at low metal concentrations. Such discrepancies may be explained by the fact that microorganisms differ in their sensitivity to metal toxicity (Kizilkaya *et al.*, 2004).

It is possible that at similar lower metal concentrations or short-term exposure to metals, the microorganisms may compensate by a higher C turnover (Giller *et al.*, 1998), and as such, lead to a gradual change in viability. At much higher concentrations however, heavy metals cause immediate death (Vasquez-Murrieta *et al.*, 2006), and the discrepancy at such high metal concentrations is often due to a community shift, in which case the tolerance of the dominant microbial group will determine the respiration (Giller *et al.*, 1998; Insam *et al.*, 1996).

The combined effect of the acidity of the peat, with an increase in metals, led to a further constraint on respiration, confirming the findings of (Ramsey, 2005). It has been found that respiration rate is negatively correlated with Zn, Pb, Cu and Cd content (Dai *et al.*, 2004). The inhibitory effect of metals on CO_2 production may have been due to a direct inhibition of other related microbial processes, or decrease in substrate availability (Vasquez-Murrieta *et al.*, 2006). Thus, even though the toxic effect of metals leads to inhibition in microbial processes, the presence of metals appears to slow down the breakdown of the substrate, thus stabilising the amendments.

4.3 Effect of amendments on microbial respiration in field contaminated soil

4.3.1 Objectives

- To determine CO_2 evolved by microorganisms in field contaminated soil
- To determine the effect of added amendments on CO_2 evolved in field contaminated soil

4.3.2 Methodology

Bulk field soil, which was already contaminated by long term deposition of mine waste, was used for this second incubation experiment. Amendments green waste compost, peat, coir and wood bark were added to this soil at rates of zero, 1%, 10%, and 20%. The incubation was for 10 weeks, and amount of CO_2 evolved was determined each week, as discussed in Chapter 2, Section 2.9.2. (page 64).

4.3.3 Results and discussion

Basal respiration in non-amended soil was very low, totalling only 93 mg C kg^{-1} soil over the entire incubation period of ten weeks (Figure 4.4). Due to the very low organic matter content of the soil, it would offer very limited energy source to the microorganisms. As would be expected, there was significant increase in CO_2 evolved once the soil was amended with wastes (Figure 4.4). As was observed in the metal 'spiked' amendments in Section 4.1, the cumulative CO_2 evolved was highest in coir and wood bark amended soil. Respiration in green waste compost amended soil was least. The green waste compost was a fine textured material which appeared to be already in an advanced stage of decomposition before it was added to the contaminated soil, and as such, microbial breakdown and consequently respiration, may have been greatly hampered in the soils amended with this material.

In the study, it was found that amending with 20% wastes led to a greater enhancement of CO_2 evolved in soil than the amending of soil with only 1% amendment. In all soils, weekly CO_2 evolved became generally lower as time elapsed, with rate at week 10 being lower than week 9, and so on (Figure 4.4). An exception was in soils which had been amended with 20% coir. The weekly amount of CO_2 evolved did not start to decline until after week 6. This reflects the high organic carbon content of coir, and also the availability of this carbon for mineralization over a longer period.

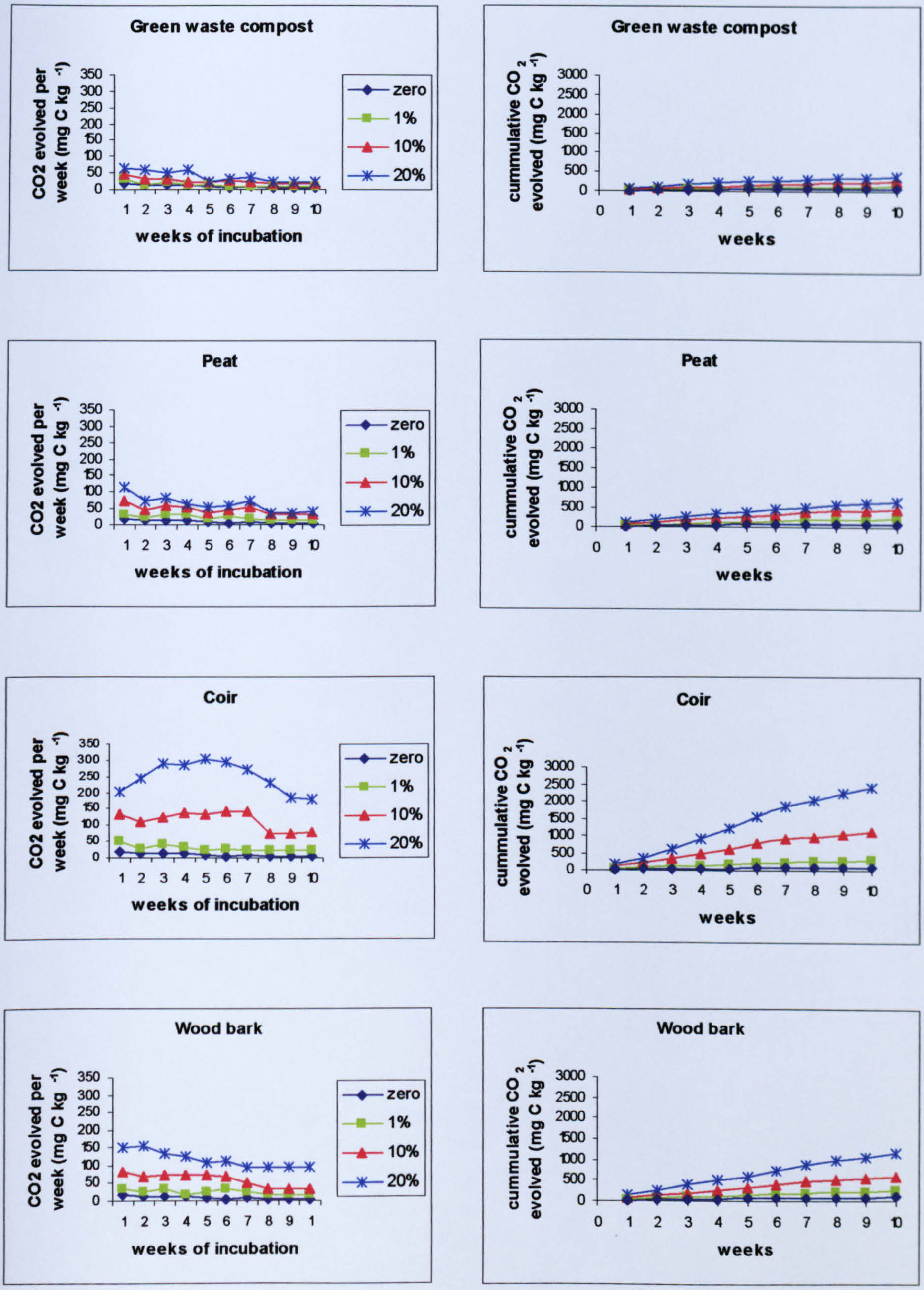


Figure 4.4 Effect of amendments on microbial respiration in contaminated soil. (Zero-non amended soil; amendments at 1, 10 and 20% of soil weight).

4.3.3.1 Evaluating the ability of added amendments to enhance microbial respiration in contaminated soil

The ability of green waste compost, peat, coir and wood bark to enhance CO_2 evolution in contaminated soil is presented in Table 4.9, and was calculated as

% enhancement = (1-C/D) * 100

Where C= cumulative CO_2 evolved in non amended soil, and

D= cumulative CO_2 evolved in amended soil

Table 4.9 Effect of amendments on enhancing microbial respiration in contaminated soil

Amendment rate		1%	10%	20%
	Week		% enhancement	
Compost	1	45.5b	60.0a	72.7a
	5	32.4a	61.7a	76.3a
	10	32.6a	63.5a	76.2a
Peat	1	40.0b	75.0b	84.2b
	5	56.6c	78.3b	84.6b
	10	56.9c	80.1bc	85.4b
Coir	1	64.7d	86.7cd	90.3bc
	5	65.2d	91.0d	95.6c
	10	67.7d	91.8d	96.2c
Wood bark	1	50.0bc	78.6b	88.2b
	5	59.6c	82.7bc	90.6b
	10	63.5cd	83.8c	92.1b

LSD = 6.8 (Values within a single column followed by the same letter are not significantly different at $p < 0.05$)

Results from the study showed that the addition of amendments led to an enhancement of microbial respiration in the contaminated soil (Table 4.9). At 1% level of amendment, there was no significant difference between enhancement by compost, peat or wood bark after the first week of incubation. At this 1% rate of amendment, the ability of compost in enhancing respiration declined as the incubation period grew longer, while coir and wood bark remained the same from the beginning to the end of the incubation period. At 10 and 20% amendment levels, percent enhancement within individual materials did not differ significantly at any time during the incubation period. Comparing the different amendments however, enhancement was in the order coir > wood bark = peat > green waste compost.

Results obtained by Tejada *et. al* (2007), indicated that the addition of organic matter to Pb contaminated soil decreased the extent to which soil enzymatic activities were inhibited. Other workers have also observed an increase in microbial activities after compost and wood chips was added to soils polluted with Cu and Ni (Kiikkila, 2002). Addition of organic wastes is known to lead to an increase in the carbon and nutrient status of the soil (Usman *et al.*, 2004). It has been established that higher doses of waste material provide a larger amount of organic matter to be degraded, thus delaying the appearance of metal toxicity and in addition, the organic amendments bind the metal, decreasing its availability (Montserrat *et al.*, 2006). This would explain why adding 20% amendment led to the greatest enhancement of microbial respiration. The enhancement of respiration by the addition of any amendment, would of course presuppose that the amendments themselves do not contain any potentially toxic elements which may inhibit microbial activity.

In the two separate incubation experiments, one with metal spiked amendments, and the other with contaminated soil, green waste compost performed poorest when compared with the other amendments. It is noted that it was lower in percent organic carbon than the other amendments, as shown in Chapter 2, Section 2.1.1.

4.4 Extractable metals in contaminated amendments at the end of the incubation period

4.4.1 *Objective*

To determine the bioavailability of Pb, Cu and Zn in contaminated amendments to microorganisms

4.4.2 *Methodology*

Water extraction was used to determine metal content in 'spiked' amendments, because metals leached with water approximate metal forms which are bioavailable to microorganisms in soil (Cela & Sumner, 2003; Montserrat *et al.*, 2006; Wilke *et al.*, 2005). The method is discussed fully in Chapter 2, Section 2.4.4.1 (page 49).

4.4.3 *Results and discussion*

The amount of Pb, Cu and Zn extracted from amendments is presented in Table 4.10. After ten weeks of incubation with metal solution, there were no significant differences between the amount of Pb extracted from different amendments at any of the three concentrations applied. There was also no significant difference in amount of water extractable Cu at 5 and 10 mg g^{-1}, but at 50 mg g^{-1} Cu, there were significant differences in amount of Cu extracted from the four amendments, with water extraction in peat being significantly higher that the other amendments. As with extractable Pb and Cu, there were no significant differences in extractable Zn at 5 and 10 mg g^{-1}, but at 50 mg g^{-1}, extractable Zn in peat was significantly higher than the concentration in the three other amendments.

Table 4.10 Water extractable Pb, Cu and Zn in contaminated amendments at the end of ten weeks of incubation

Initial metal applied (mg g-1)	Compost	Peat	Coir	Wood bark
	Amount extracted mg g-1			
		Pb		
0	nd	nd	nd	nd
5	0.03a	0.12a	0.06a	0.05a
10	0.04a	1.07a	0.09a	0.05a
50	7.34a	8.76a	6.05a	7.62a
		Cu		
0	nd	nd	nd	nd
5	0.04a	1.41a	0.15a	0.11a
10	0.52a	6.86a	1.14a	1.04a
50	20.44a	33.5c	23.81b	19.04a
		Zn		
0	nd	nd	nd	nd
5	0.16a	0.55a	0.12a	0.13a
10	0.52a	1.81a	0.36a	0.45a
50	6.55a	9.92b	4.18a	5.75a

LSD = 2.39. Values are means of three replications. Comparison is among amendments in individual metals only, so data in a row which are followed by the same letter are not significantly different at $p<0.05$

The non significant difference in amount of Pb extracted may have been due to the insoluble nature of Pb in water. The differences obtained among amendments for Cu and Zn however confirms once more the comparative ability of the amendments to bind metals as reported in Chapters Three and Five, with peat being the least effective when compared to the other amendments. Water extraction shows this significant difference in the metal binding ability of various amendments at applied concentration of 50mg g^{-1} Cu and Zn only (Table 4.10).

4.5 Conclusion

The first incubation experiment showed that the breakdown of amendments was inhibited by metal contamination, as the presence of metals led to a reduction in amount of CO_2 evolved in all amendments and there was a decrease in weekly CO_2 emission in contaminated amendments as the incubation period grew longer. There was also reduction in CO_2 evolved in non-contaminated amendments over time, indicating a limiting/constraint on the available substrate carbon. In the second incubation experiment, addition of amendments to contaminated soil enhanced respiration. It was observed that the greater the amount of amendment added, the higher the level of enhancement as measured by the amount of CO_2 evolved relative to the non-amended soil.

In both incubation experiments, coir and wood bark allowed for more CO_2 evolution than peat, while the green waste compost was poorest in CO_2 production. The amount of inhibition caused by metals on CO_2 evolution is negatively correlated with organic carbon content of the amendments and their cation exchange capacity. Thus, the higher the organic carbon content and CEC of an amendment, the less the amount of inhibition on microbial respiration in the presence of Pb, Cu and Zn. Results from the water extraction of Pb, Cu and Zn show the ability of amendments to bind the three metals in the same order as reported in Chapters 3 and 5, to be coir~compost>wood bark>peat.

Chapter 5

Effects of amendments on metal immobilisation and plant uptake in contaminated soil

5.1 Introduction

Plants are "*the major selective accumulators of all types of inorganic nutrients, including metals, on which other life forms depend*" (Ross & Kaye, 1994). Some plants may be able to restrict metal uptake, and consequent translocation of such metals within the plant. It is however impossible to completely exclude potentially toxic elements, as plants growing in non-contaminated soils have been known to contain some traces of metals in their tissues. Metal contaminated crops from contaminated soils are therefore possible hazards for the food chain (Friesl *et al.*, 2006).

It is well noted that total soil content is not an appropriate index for measuring metal bioavailability (Mantovani *et al.*, 2004; Wang *et al.*, 2003). While total concentrations may be very high, only the bioavailable fraction can be taken up by plant roots, and this is usually much lower than the total concentration. The bioavailability of soil metals is thus very crucial, whether from an agricultural or environmental stand point. The uptake of metals by plants growing in contaminated soils can be restricted by the addition of amendments to the soil, and such amendments have been known to have direct effects on the retention of metals in the soil, as well as the amount of metals taken up by plants (Besnard *et al.*, 2001).

There is no single method in the literature which is universally acceptable in predicting metal bioavailability (Sauve *et al.*, 2000). Nevertheless, growing crops in a glasshouse under controlled conditions has been used prior to full scale field studies. These studies evaluate the immobilisation of toxic metals in soil, the uptake of such metals by plants (Ginocchio *et al.*, 2006), and they eliminate costly future mistakes (Song *et al.*, 2004). Variable inputs are better controlled in the greenhouse and pot experiments also simulate field situations better than growing plants in hydroponic systems. Because the effects of soil properties on metal sorption and plant uptake can be assessed, pot experiments allow for a more realistic evaluation of the potentials for remediation

Selection of an appropriate test crop is important in pot experiments with contaminated soils. Ryegrass has been widely used as a test crop in remediation studies, as it tends to have a capacity to tolerate significant amounts of heavy metals in soil (Betts, 1997; Hao *et al.*, 2003). The plant has the ability to produce enough biomass yield in a relatively short period, providing enough for both shoot and root analysis, and the rooting system spreads to explore the whole pot, thus enabling the plant to access metal within the entire growth medium.

This chapter is in six main sections. First, growth and biomass yield of plants were assessed in contaminant spiked soils. The 'spiked soil' comprised sand to which mine waste and sewage treated soil were separately added at 2.5% and 25% of sand weight respectively. The effects of amendments on plant and soil metal concentrations were then evaluated in the spiked soil. Following on from these, plant growth, plant metal uptake and soil metal were evaluated in an industrially contaminated soil. This second soil was collected in bulk from the vicinity of an old Pb mine, where it was already contaminated through long term deposition of mine waste. Two other sections briefly reported changes in pH of contaminated soil due to the addition of amendments.

5.1.1 Aim

To reduce the bioavailable concentrations of Pb, Cu and Zn in contaminated soil and their consequent uptake by plants.

5.2 Plant growth in manually contaminated soil

5.2.1 Objective

To evaluate the effect of amendments on biomass yield of ryegrass (*Lolium perenne*) in soil which had been 'spiked' with contaminants high in Pb, Cu and Zn

5.2.2 Methodology

A pot experiments was set up in the spring/summer of 2004 using Pb/Zn mine waste and a sewage sludge treated soil as contaminants. Amendments used were bone meal, general purpose compost, peat, coir and phosphate. The methodology is discussed fully in Chapter 2 (Section 2.7.5, page 56).

5.2.3 Results and discussion

Ryegrass seeds germinated after four to five days of planting, and the dry biomass yield is presented in Tables 5.1 and 5.2. There were two harvests of ryegrass shoot, first after 8 weeks of growth, and the second harvest was after a further 8 weeks. There was only one root harvest, and that was after the total of 16 weeks of growth.

Addition of bone meal, compost and coir to mine waste contaminated soil led to significantly higher shoot yield in the first harvest than obtained for the non-amended soil, and the effect of these amendments was clearly accentuated at the second harvest (Table 5.1). For bone meal and coir, the effect on shoot yield can be linked to high root yield as well, whereas root yield in compost treated soil was not significantly higher than peat or phosphate. The sewage sludge treated soil had better yield with no amendment when compared to non-amended mine waste contaminated soil, and this may be explained by the fact that sewage would be adding in nutrients to the soil (Table 5.2). Biomass yield differences due to application of amendments was less clear in sewage sludge contaminated soil, but was more evident for mine waste contaminated soil.

Table 5.1 Biomass yield of ryegrass in soil 'spiked' with mine waste

	Shoot yield (g/pot)		Root yield(g/pot)
Amendment	1st harvest (8 weeks)	2nd harvest (16 weeks)	(16 weeks)
Zero	0.66 ± 0.15a	0.37 ± 0.08a	1.72 ± 0.33a
Bone meal	2.99 ± 0.29d	1.29 ± 0.22b	6.78 ± 1.21c
Compost	0.96 ± 0.10b	1.00 ± 0.07b	3.81 ± 0.75b
Peat	0.83 ± 0.06ab	0.48 ± 0.05a	3.58 ± 0.48b
Coir	2.07 ± 0.23c	1.08 ± 0.16b	8.28 ± 1.83c
Phosphate	0.62 ± 0.11a	0.61 ± 0.18a	2.23 ± 0.41ab
LSD $p < 0.05$	0.24	0.36	2.05

Means ± SD (n=4) Values within a single column followed by the same letter are not significantly different at $p < 0.05$

Table 5.2 Biomass yield of ryegrass in soil 'spiked' with sewage soil

	Shoot yield(g/pot)		Root yield(g/pot)
Amendment	1st harvest (8 weeks)	2nd harvest (16 weeks)	(16 weeks)
Zero	1.97 ± 0.11a	1.27 ± 0.16a	4.68 ± 0.88a
Bone meal	5.07 ± 0.41c	2.2 ± 0.23d	11.72 ± 2.85bc
Compost	2.02 ± 0.08a	1.56 ± 0.18b	8.03 ± 1.14ab
Peat	2.37 ± 0.31ab	1.66 ± 0.22b	9.22 ± 1.37b
Coir	2.51 ± 0.27b	1.47 ± 0.41a	14.72 ± 2.08c
Phosphate	2.78 ± 0.38b	1.87 ± 0.38c	7.72 ± 1.49a
LSD $p < 0.05$	0.41	0.29	4.13

Means ± SD (n=4) Values within a single column followed by the same letter are not significantly different at $p < 0.05$

Mine waste caused more symptoms of metal toxicity in ryegrass than sewage treated soil when neither soil was amended with wastes (Figure 5.1).

Figure 5.1 Ryegrass in non-amended metal 'spiked' soil after four weeks of growth (G= mine waste/mine waste, SB= sewage treated soil)

Figure 5.2 Effect of amendments on ryegrass growth in mine waste 'spiked' soil

There were clear differences between the appearance and biomass of ryegrass grown in the two types of soil. Plants in mine waste contaminated soil were stunted with scanty leaves which had a reddish, burnt appearance, except those amended with bone meal(Figure 5.2).

Figure 5.3 Effect of amendments on ryegrass growth in sewage 'spiked' soil

Appearance of plants growing in the sewage spiked soil exhibited a lesser degree of metal toxicity, and the plants were more luscious, with greater biomass (Figure 5.3).

Addition of amendments led to a significant increase in biomass yield in both the mine waste and sewage spiked soil when compared to that of the non amended soil. It was observed that all the treatments led to crops having higher root yield than shoot yields. It has been noted that plants growing in contaminated soil tend to have greater root:shoot biomass ratio. It is regarded that this is highly representative of the environmental stress that is encountered by such plants, and a survival strategy which allows the plant, through the greater root surface, to have more access to absorption of water and nutrients(Chiu *et al.*, 2006; Ke *et al.*, 2007).

5.3 Metal uptake by Ryegrass in manually contaminated soil

5.3.1 Objective

To evaluate the effects of amendments on plant tissue concentrations of Pb, Cu and Zn in plants grown in soils which had been 'spiked' with mine waste and sewage sludge treated soil

5.3.2 Methodology

Plant tissue Pb, Cu and Zn was determined in ryegrass leaves after 8 weeks of growth, and the method is fully discussed in Chapter 2, Section 2.7.6(page 57)

5.3.3 Results and discussion

5.3.3.1 First harvest in manually contaminated soil (8 weeks)

Generally, application of amendments to the mine waste spiked soil led to a decrease in uptake of Pb and Zn. However, not all the amendments were consistent in reducing leaf metal concentrations, as Cu uptake was only reduced in coir and peat treated soils. Bone meal significantly increased the uptake of Pb, while peat had significantly enhanced plant uptake of Zn (Figure5.4). The enhanced uptake of Pb could be directly linked to an increase in root biomass from the application of bone meal, which will definitely lead to increased uptake and higher metal concentration in plant tissue. Peat on the other hand would have led to a greater solubility and eventual uptake of Zn due to its very acidic pH.

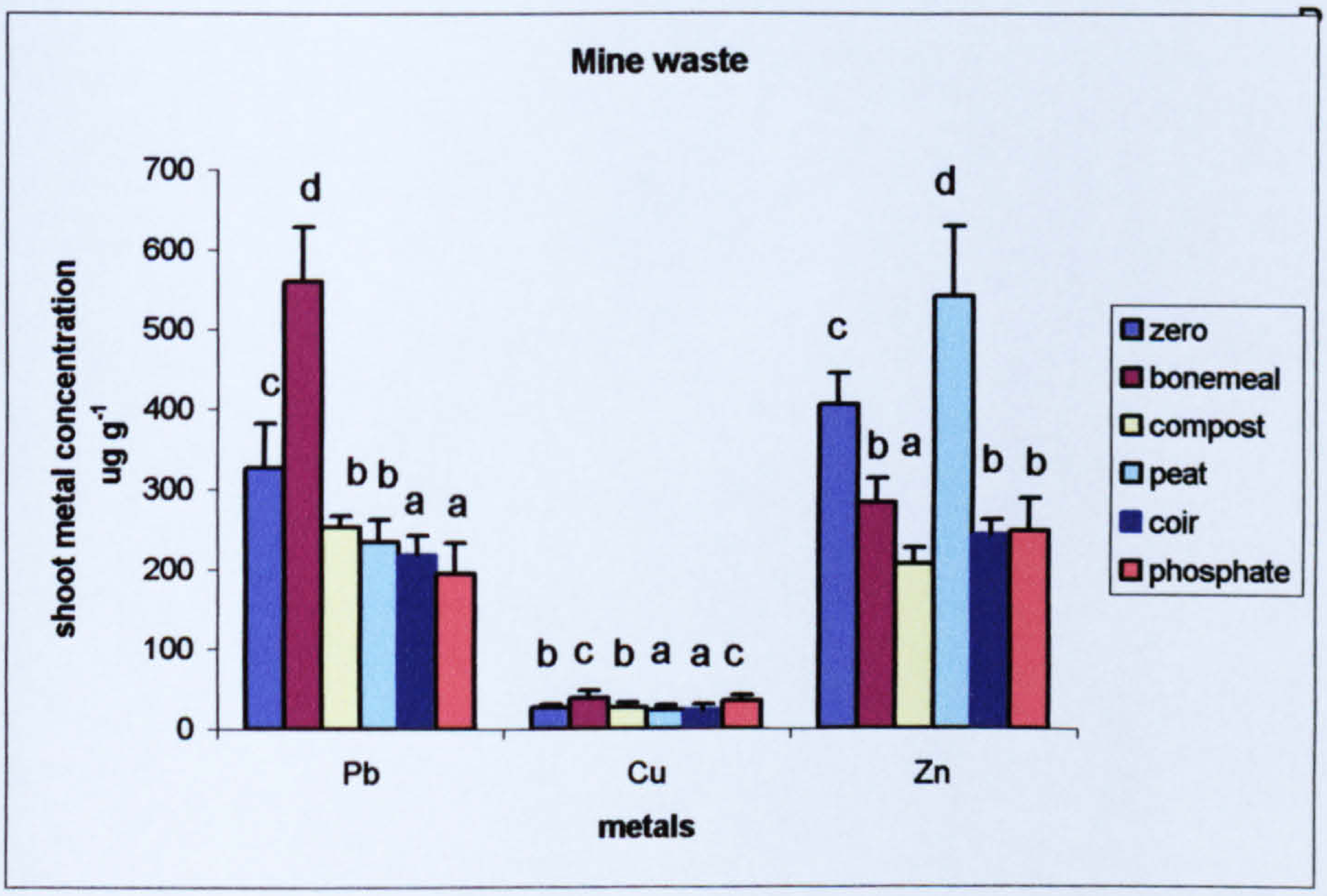


Figure 5.4 Total Pb, Cu and Zn in ryegrass leaves after 8 weeks of growth (first harvest) in mine waste 'spiked' soil (error bars represent ± standard deviation of four replications)

In the sewage sludge treated soil, addition of amendments also made a significant difference to the amount of metal taken up into ryegrass shoots. There was a significant reduction in leaf Pb, except by bone meal and phosphate (Figure5.5). Cu uptake was reduced by compost, peat and coir, but Zn uptake was enhanced by the addition of all amendments except compost, and this may be explained by the presence of easily dissolved organic fraction of the sewage soil. Zn uptake was not significantly different from that of the non – amended soil, reflecting a very high level of Zn in the soil which masked the effect of amendments at this stage in the pot experiment.

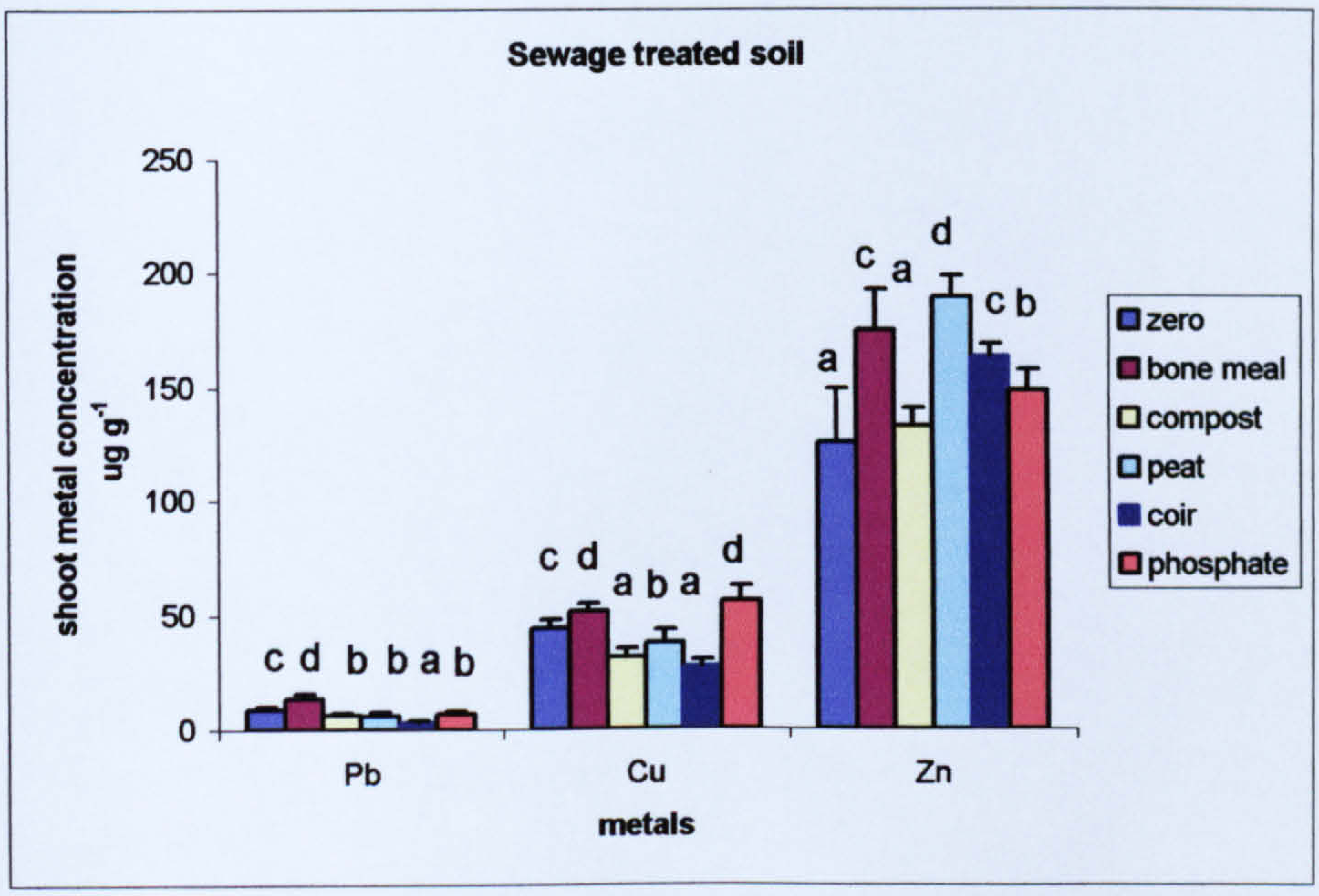


Figure 5.5 Total Pb, Cu and Zn in ryegrass leaves after 8 weeks of growth (first harvest) in sewage 'spiked' soil (error bars represent ± standard deviation of four replications)

A clearer effect of adsorbents on Pb uptake could be seen in sewage contaminated soil (Figure 5.5). The soil, with a much lower Pb concentration, showed clearly that coir had been most effective in preventing plant uptake of Pb, whereas bone meal and peat seemed to have enhanced the uptake of Cu. The concentration of Pb, Cu and Zn found in the second harvest of leaves were higher than those analysed in the first harvest (data not shown). Plants treated with bone meal manifested high levels of Pb in the leaves, both in the first and second harvest. Analysis of the bone meal used had actually shown it to contain a considerable amount of Pb and Zn (Chapter 2, Section 2.1, page 38).

5.3.3.2 Second harvest in manually contaminated soil (16 weeks)

There was very high variability in plant tissue concentrations in the second harvest, and there appeared to be an increase in metal uptake more than in the first harvest (Figures 5.6 and 5.7). In mine waste contaminated soil, addition of amendments did not lead to a reduction in shoot Pb concentration in ryegrass, as uptake in peat, coir and phosphate amended soils were not significantly different from that in the non amended soil (Figure 5.6). Cu uptake was however, significantly reduced by amending with bone meal, compost and peat, while coir and phosphate did not have significant effect.

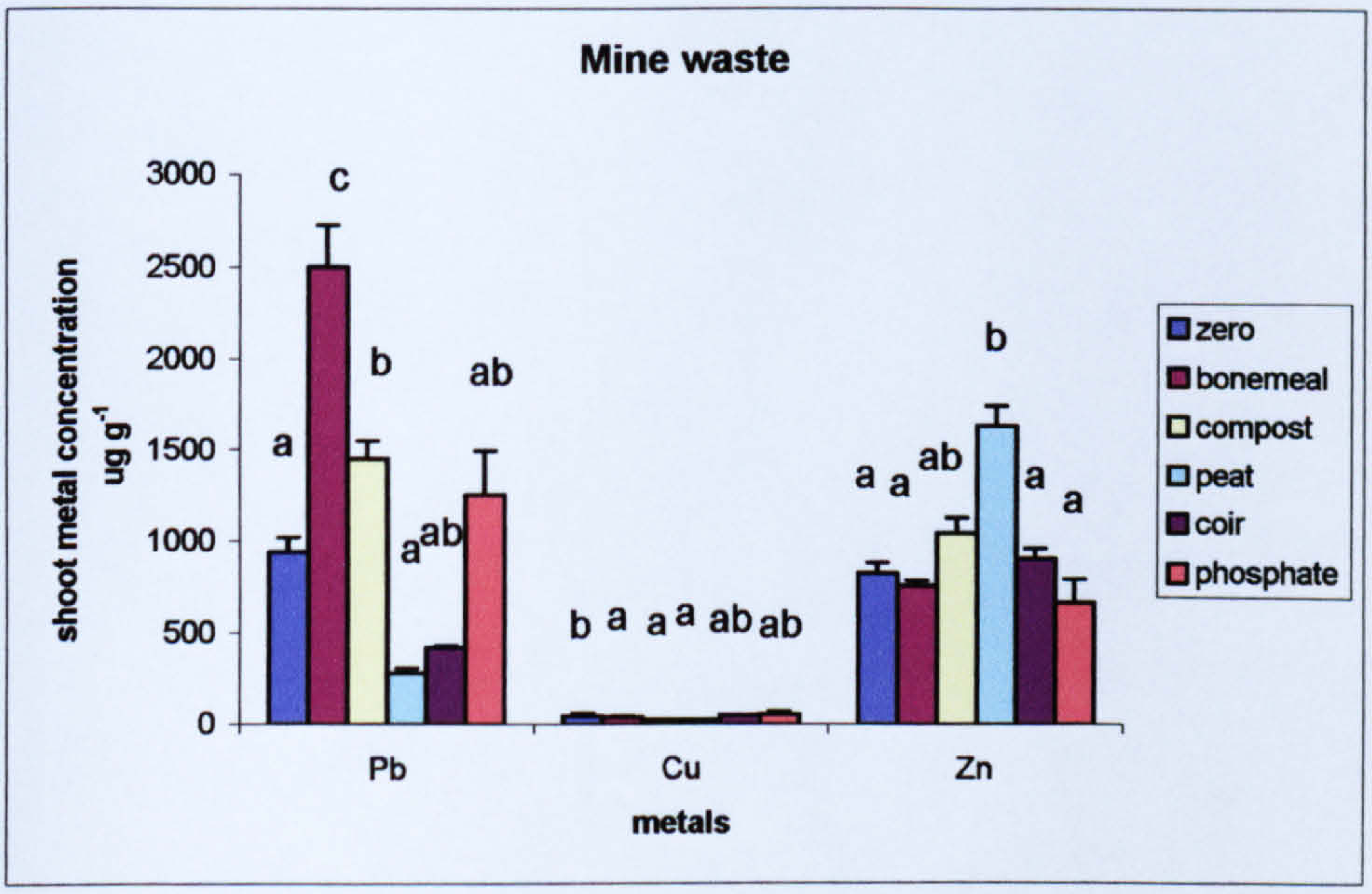


Figure 5.6 Total Pb, Cu and Zn in ryegrass leaves after 16 weeks of growth (second harvest) in mine waste 'spiked' soil (error bars represent ± standard deviation of four replications)

Generally, shoot Zn concentration was also not significantly affected by addition of amendments, except in the case of peat where Zn uptake was significantly increased over that in the non amended soil (Figure 5.6). Again, this would likely have been due to an enhanced solubility of Zn by the addition of peat.

In the second harvest, the effect of amendments on metal uptake by ryegrass in sewage sludge treated soil was evident, as addition of amendments generally led to a significant reduction in metal uptake (Figure 5.7). Shoot Pb was significantly reduced by the amending with peat, coir and phosphate, while bone meal and compost were not significantly different from the non-amended soil.

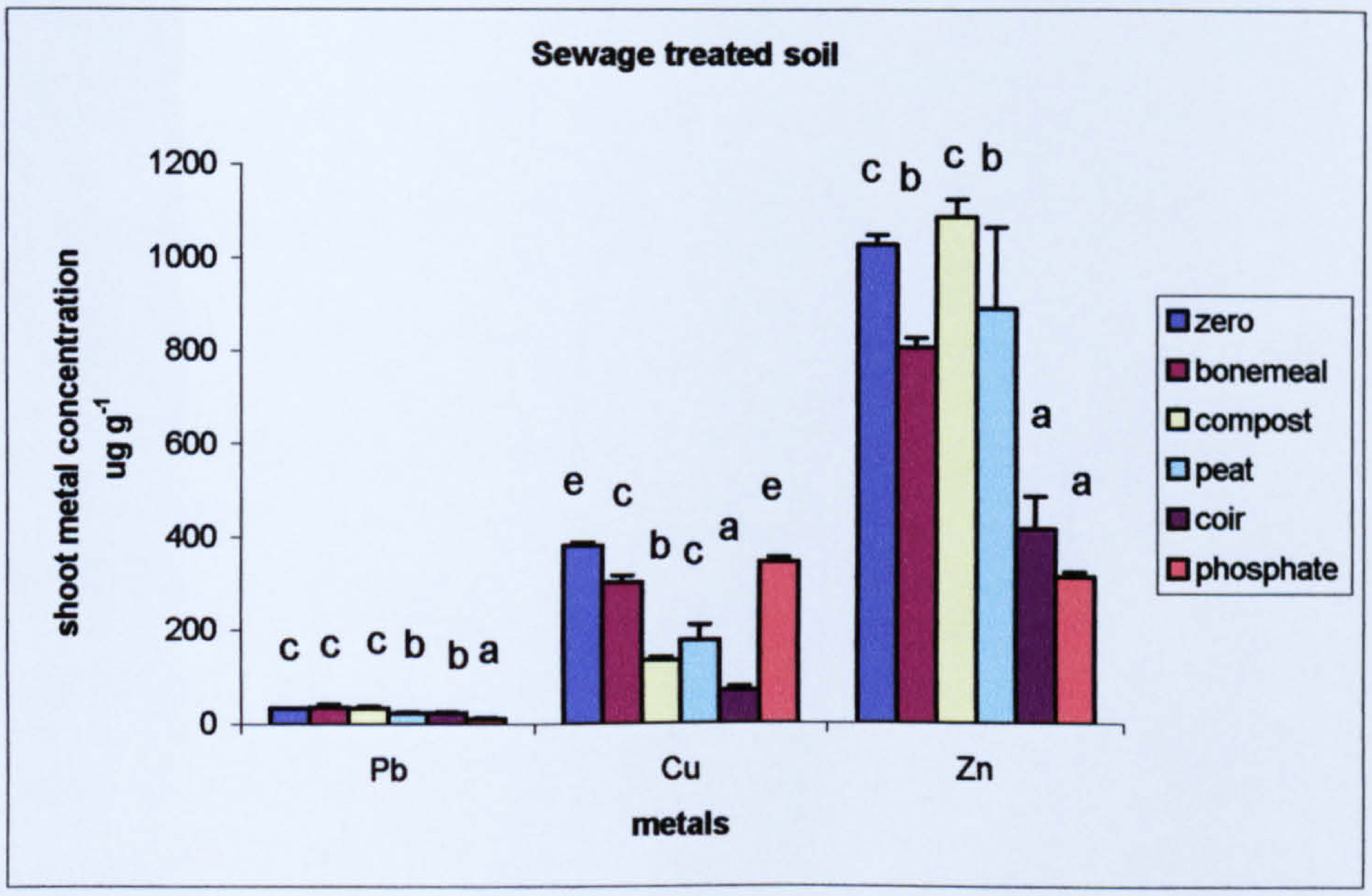


Figure 5.7 Total Pb, Cu and Zn in ryegrass leaves after 16 weeks of growth (second harvest) in sewage 'spiked' soil (error bars represent ± standard deviation of four replications)

Cu uptake was significantly reduced by all the amendments in the sewage spiked soil except phosphate which was not significantly different from the non amended soil, while Zn uptake was significantly reduced by all the amendments except peat, which again was not different from the non amended soil (Figure 5.7). The lower initial metal concentrations in the sewage sludge contaminated soil would account for the clearer effect of amendments observed in it when compared to that in mine waste contaminated soil, and a stability in the 'soil' may have been achieved quicker after the first 8 weeks.

Overall, the inconsistent effect of amendments may have been due to the fact that both contamination sources used were extremely high in the three metals, and so initial concentrations in soil were very high. The water extractable form of Pb was detectable in the mine waste (data not shown), in spite of the fact that Pb and its complexes are usually very low in solubility. In addition, both contaminants were highly variable in themselves. Ten random replications of the mine waste showed that Pb ranged from 54070 $\mu g\ g^{-1}$ to 89200 $\mu g\ g^{-1}$ while in sewage soil, Pb varied from 604$\mu g\ g^{-1}$ to 961$\mu g\ g^{-1}$. Initial Cu and Zn were also variable in both contaminant soils.

The fact that some of the amendments tended to increase extractable metals confirms the work of Perez-de-Mora *et. al* (2006), as other research has also shown that reduced plant uptake of metals is not consistently observed with soil amendments (McBride & Martinez, 2000; Rate *et al.*, 2004). These amendments provide a large sink for metal sorption, and often there could be an initial reduction in metals in solution. But later, there may be solubility and release of strongly bound metals, which then get transferred into soil solution. The resulting effect is that this sink could get saturated, and thus become ineffective (Ge *et al.*, 2000; McBride & Martinez, 2000).

Higher metal concentration in the shoots of the second harvest confirm the findings of Karaca (2004). An enhancement of Cu uptake after addition of amendments has been attributed to an increase in Cu solubility through organic matter chelation (Clemente *et al.*, 2006), while enhanced Zn uptake is said to be due to the re-release of Zn which was complexed with organic matter during the first phase of an experiment (Madrid *et al.*, 2006).

It is known that evaluation of Zn concentrations in plant leaves is not straightforward, because foliar concentrations are highly variable, even at baseline concentrations (De Vos *et al.*, 2005). Oudeh *et al.*(2002) observed that increasing applied metal rates caused higher concentrations and accumulation of foliar Zn only, and that metal accumulation by plants in response to increased applied metal concentrations was not consistent.

5.4 Comparison of amendments, mine waste and sewage sludge soil as a source of leaf Pb, Cu and Zn

5.4.1 Objective

To compare the relative contributions of initial metal present in amendments, mine waste and the sewage sludge treated soil to the total metal taken up into ryegrass leaves.

5.4.2 Methodology

Non-metal contaminated sharp sand was amended with bone meal, compost, peat, coir and phosphate, and was used to grow ryegrass plants over a period of eight weeks. These soils also received Hoagland solution to help sustain plant growth, and the leaf metal after harvest was determined as in Chapter 2, Section 2.4.3(page 48). The leaf metal concentrations in this non-contaminated, but amended soil was then compared with leaf metal obtained in zero amended mine waste treated soil, and that in zero amended sewage sludge treated soil. The results are presented as stacked columns, which show actual data comparison of leaf metal due to amendments alone(amendments + sand), mine waste treated sand alone, and sewage sludge treated sand alone.

5.4.3 *Results and discussion*

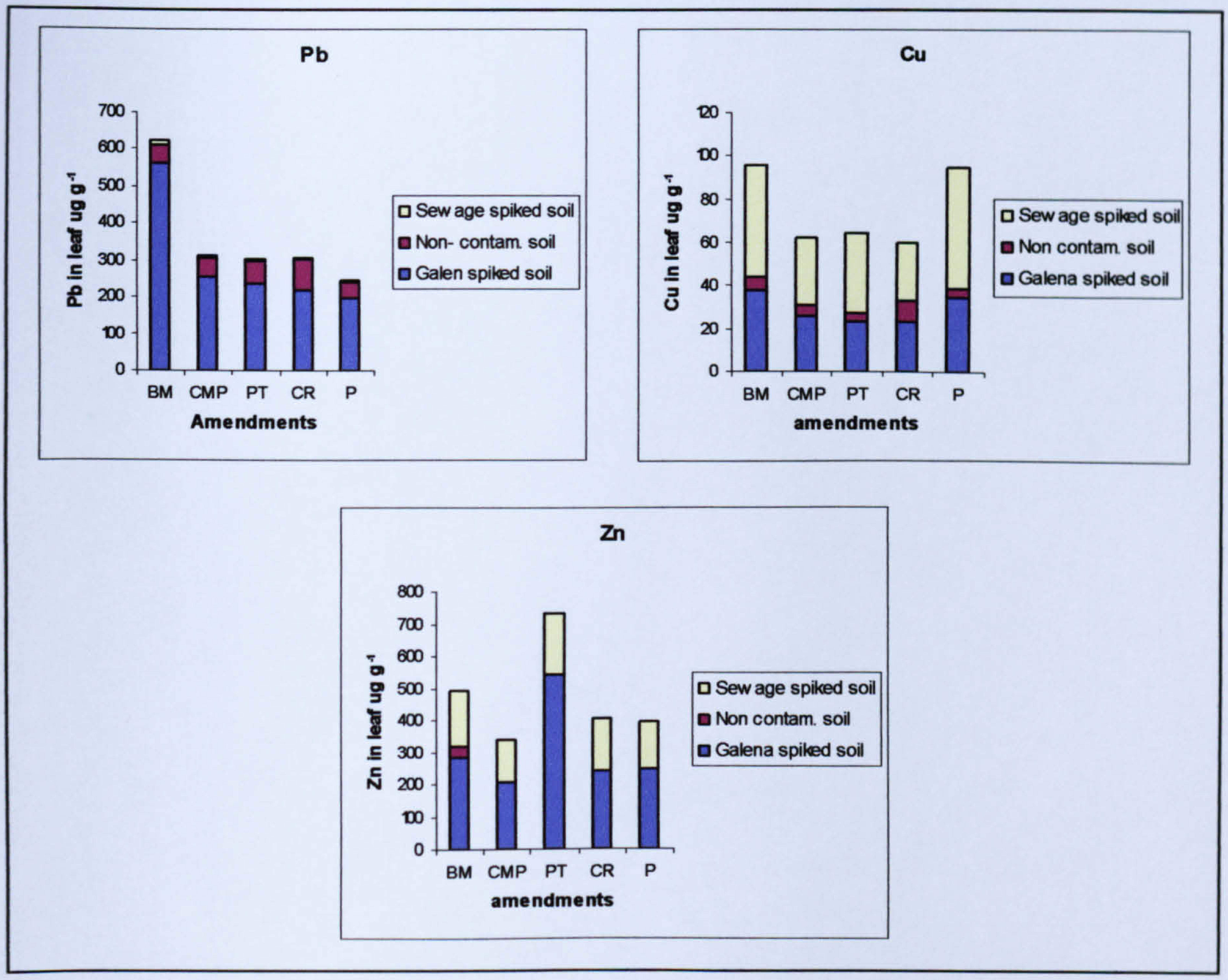


Figure 5.8 Comparative contributions of amendments and contaminants to the uptake of Pb, Cu and Zn by ryegrass after eight weeks of growth (galena- mine waste)

When compared with uptake in the 'spiked' soils it was observed that the contribution of metals from the amendments to overall metal uptake was negligible (Figure 5.8). The low concentrations of Pb, Cu and Zn in the amendments was however available to the ryegrass plants, confirming that it is impossible to completely exclude metal uptake by plants, even when such plants are growing in non contaminated soil (Friesl et al., 2006). Although Zn concentrations in both the mine waste treated and sewage sludge treated soils were approximately the same at 250 mg g-1 as reported in Chapter 2 (Section 2.7, page), plant uptake of Zn was much higher in the mine waste treated soil (Figure 5.6). This indicates that Zn was more readily bioavailable from the inorganic mine waste, but its immobilisation was greater in the organic sewage treated soil.

5.5 .Metal levels in 'spiked' soil as affected by addition of amendments

5.5.1 Objective

To evaluate the ability of amendments to immobilise Pb, Cu and Zn in soils which had been contaminated by wastes

5.5.2 Methodology

The method is discussed in Chapter 2, Section 2.7.4 (page 56).

5.5.3 Results and Discussion

The effects of amendments on the pH of mine waste and sewage spiked soils is presented in Figure 5.7.

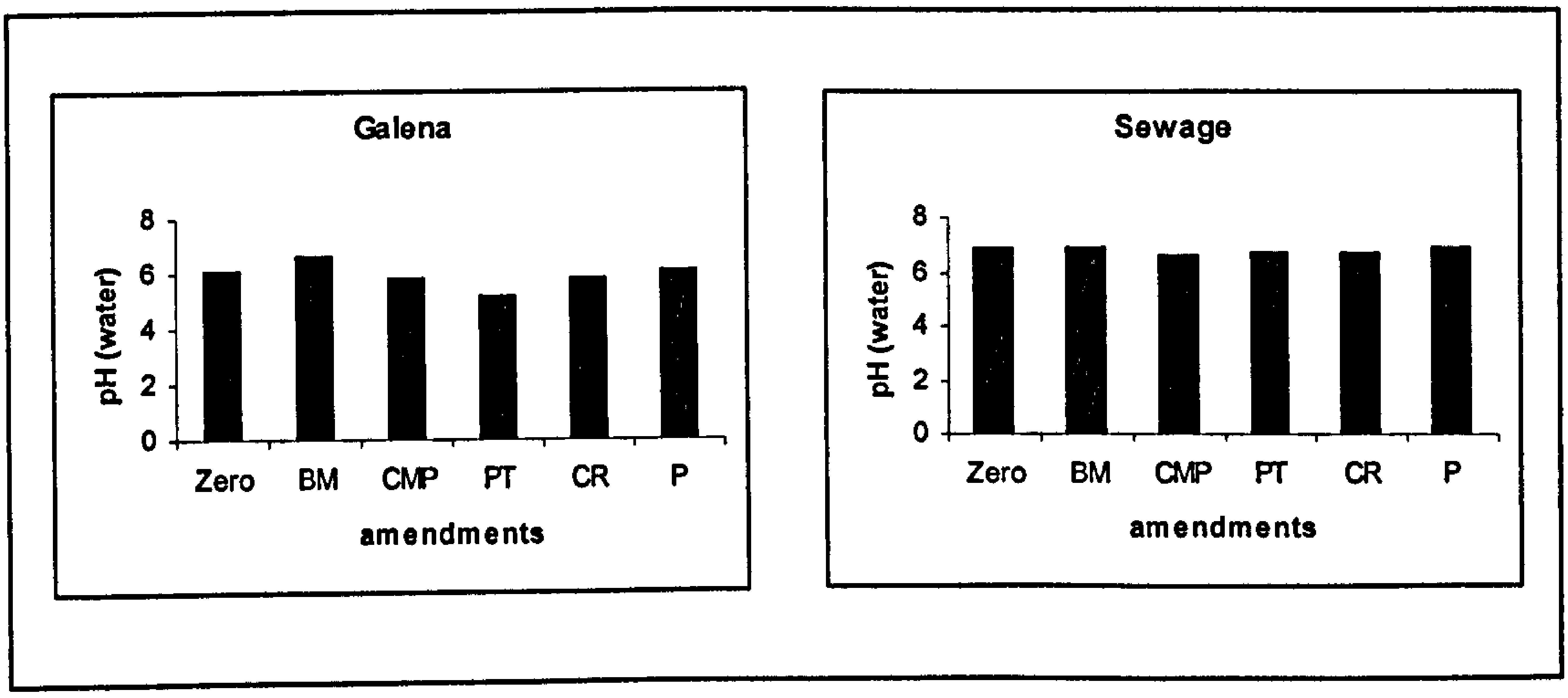


Figure 5.9 Effect of amendment on pH of contaminant 'spiked' soil (zero- non-amended soil; BM- bone meal; CMP- compost; PT- peat; CR- coir; P-phosphate)

There were marginal reductions in pH by compost and coir, while bone meal and phosphate led to a slight increase. There was virtually no difference in pH of the sewage spiked soil, when amended soils were compared with the non-amended one. In spite of the very acidic pH of the peat, it was only able to reduce the soil pH by 0.2 units. This is due to the high buffering capacity of the sewage to acidity, as reported in Chapter 2, (Section 2.7.7).

Results of EDTA extraction of spiked soils is presented in Table 5.3.

Table 5.3 EDTA extractable metals in manually contaminated mine waste spiked soils after 16 weeks of treatment (non-planted pots)

Treatment	ug g^{-1} Pb	ug g^{-1} Cu	ug g^{-1} Zn
Zero	6137.8 ± 652b	9.1 ± 0.2ab	55.9 ± 11a
Bone meal	5996.9 ± 390b	7.9 ± 0.6a	46.8 ± 3.2a
Compost	5705.3 ± 173a	10.5 ± 0.7b	50.4 ± 3.0a
Peat	6652.8 ± 407b	25.5 ± 1.3c	189.2 ± 8.5c
Coir	5034.4 ± 397a	9.9 ± 0.7b	56.2 ± 2.8a
Phosphate	5778.8 ± 338b	7.2 ± 0.8a	79.0 ± 2.6b
LSD =	744.2	1.4	9.1

Data are means ± SD (n=3). Values within a column followed by the same letter are not significantly different at $p < 0.05$.

Pots treated with coir had the lowest amount of EDTA extractable Pb at the end of the experiment, although its effect on Cu and Zn was not consistent. There was no significant reduction of Cu and Zn by any of the amendments and this may be due to the initial high metal concentrations in the mine waste spiked soil. In the sewage spiked soil, there was no significant effect of any of the amendments on extractable metals. Peat appeared to actually cause a marginal increase in the solubility of Cu and Zn in the mine waste spiked soil, which probably was a direct consequence of the low pH of the material (Table 5.4).

Table 5.4 EDTA extractable metals in sewage spiked soils after 16 weeks of treatment (non-planted pots).

Treatment	ug g $^{-1}$ Pb	ug g $^{-1}$ Cu	ug g $^{-1}$ Zn
Zero	118.4 ± 2.7	163.8 ± 12.7	353.9 ± 33.6
Bone meal	110.9 ± 7.4	154.4 ± 19.0	336.9 ± 24.3
Compost	114.8 ± 4.4	155.4 ± 7.5	354.9 ± 10.7
Peat	113.5 ± 3.6	150.0 ± 3.5	343.4 ± 3.3
Coir	116.9 ± 6.3	154.9 ± 12.2	356.5 ± 19.1
Phosphate	118.2 ± 2.9	158.3 ± 1.9	360.7 ± 9.9

*Analysis of variance of data did not result in a significant F value, so there are no significant differences between treatments.

A positive correlation was observed between extractable Zn in soil and that in the shoot of shoots of ryegrass in the mine waste spiked soil (Table 5.5).

Table 5.5 Correlations between extractable metals in soil (EDTA) and concentrations in leaves of ryegrass.

	Mine waste		Sewage	
Soil vs Shoot	correlation	p	correlation	p
Pb	0.218	0.306NS	-0.154	0.474NS
Cu	-0.406	0.059NS	0.25	0.238NS
Zn	0.77	0.000***	-0.138	0.521NS

n=48, and Pearson's correlation at $p<0.05$

There was however no significant correlation between soil and shoot levels of Pb, or between shoot and root levels of Cu. Results in this study show that EDTA was able to predict the phytoavailability of Zn in the amended soils, confirming the work of Paulose *et.al.* (2007) and Oliveira *et. al.* (2005).

5.5.4 Bioavailability factor in metal spiked soil

The bioavailability factor (BF) of heavy metals, also known as the bioavailability index, was calculated as $BF = mg\ HM\ (kg\ plant\ leaves)^{-1}\ x100\ /total\ content\ HM\ (mg\ kg\ soil^{-1})$ (An *et al.*, 2004; Kidd *et al.*, 2007). The effect of amendments on bioavailability was determined as discussed in Chapter 2, Section 2.8.5, and the results are presented in Table 5.6.

Table 5.6 Effect of amendments on bioavailablity of Pb, Cu and Zn in manually contaminated soil

	Bioavailability factor (BF)		
	Pb	Cu	Zn
Mine waste			
Zero	19.0 ± 3.3f	34.1 ± 5.2ab	56.7 ± 5.5d
Bone meal	32.9 ± 4.0g	49.0 ± 12.1d	40.0 ± 4.3bc
Compost	16.2 ± 0.9e	36.9 ± 8.3bc	31.8 ± 3.0a
Peat	15.0 ± 1.7de	33.0 ± 7.5ab	83.3 ± 13.4e
Coir	13.9 ± 1.6d	33.3 ± 8.6ab	37.3 ± 2.9ab
Phosphate	11.5 ± 2.2c	44.8 ± 9.5d	34.9 ± 5.9ab
Sewage sludge treated soil			
Zero	6.7 ± 0.8b	27.6 ± 2.2a	29.4 ± 5.5a
Bone meal	10.4 ± 1.5c	32.4 ± 2.2ab	41.2 ± 4.2bc
Compost	5.1 ± 0.8ab	21.1 ± 2.6a	33.5 ± 2.0a
Peat	4.9 ± 1.3ab	25.4 ± 4.0a	47.8 ± 2.4c
Coir	2.8 ± 0.6a	18.4 ± 2.0a	41.2 ± 1.3bc
Phosphate	5.1 ± 0.9ab	35.2 ± 4.2b	34.9 ± 2.2ab
LSD =	2.6	9.7	7.9

Means ± SD (n = 4). Values within a column followed by the same letter are not significantly different at $p<0.05$

There was higher bioaccumulation of Pb by plants in the mine waste spiked soil, a direct reflection of its much higher Pb concentration than the sewage soil. Mine waste used in the study is a very high Pb/Zn waste. With Cu however, there was no difference in bioavailability of the metal in both types of spiked soils, even the non amended ones. Amending with bone meal and phosphate however led to higher bioavailability of Cu in the mine waste spiked than the sewage soil.

Amending with peat led to high Zn accumulation in both types of spiked soils, which may have been caused by the acidic nature of the amendment. Of the three metals, Zn had the highest bioavailability factor (Table 5.6). Again, this was a reflection of not only the very high concentration of Zn in the mine waste, but also of the mobile nature of the element, which leads to high bioaccumulation in shoots, the higher the soil levels. Inorganic sources of metal tend to be more readily taken up by plants as was observed with the mine waste, than sludge-derived metals as in the sewage spiked soil, because sludge derived metals are generally organically bound, and less available for plant uptake (Kidd *et al.*, 2007).

The effect of bone meal and lime phosphate to adsorb metals in spiked soil was unclear. It has been suggested that the removal mechanism of bone meal is not only a sorption effect, but also a type of ion exchange reaction, or even precipitation (Danny *et al.*, 2004). Results from the mine waste spiked soil agree with work by Brown *et. al.* (2004); Hettiarachchi *et. al.* (2001) and Usman *et. al.* (2006) which show that phosphate is highly effective in limiting bioavailability of Pb in soils However, the unclear results observed for Cu and Zn confirms the findings of (Brown *et al.*, 2005; Friesl *et al.*, 2006). The time for artificial 'spike metals' to react with amendments was limited, probably resulting in insufficient time for the organic materials to decompose (Ginting *et al.*, 2004; Lock & Janssen, 2001; Lu *et al.*, 2005). The amount of phosphate used in amending the spiked soils may have been inadequate to provide a suitable P : Pb ratio, especially since mine waste is very high in Pb (Schwab *et al.*, 2006).

5.6 Plant growth in field contaminated soil

5.6.1 Objective

To evaluate the effect of amendments on biomass yield of ryegrass in soil which was contaminated by long term deposition of mine waste

5.6.2 Methodology

Bulk soil collected from the vicinity of an old Pb/Zn mine was diluted with equal parts of sharp sand, and then used in a pot experiment for six weeks of plant growth. Measures were taken to minimise the wide variability observed in the first pot experiment. Mixing of soil at several points before and after application of amendments and allowing amended soil to stabilize over a longer period before planting was the first step taken to achieve this.

The use of bone meal and lime phosphate was discontinued due to their inconsistent performance; multiple rates of amendment were used, and the number of replications in the pot experiment was increased. The amendments used were green waste compost, peat, coir and wood bark, and the methodology is fully discussed in Chapter 2, Section 2.8.6 (page 52).

5.6.3 Results and discussion

In the first two weeks of growth, there was no visible difference between the appearance of ryegrass in non-amended soils and those which received only 1% of amendment, and there was no indication of metal toxicity (Figure5.8). Addition of wastes at 10 and 20% led to enhanced biomass yield over the non amended soil (pictures not shown). From three weeks of growth however, ryegrass grown in the 'zero' amended soil exhibited metal phytotoxicity symptoms such as leaf chlorosis and stunted growth, in spite of receiving Hoagland's nutrient solution like all the other treatments.

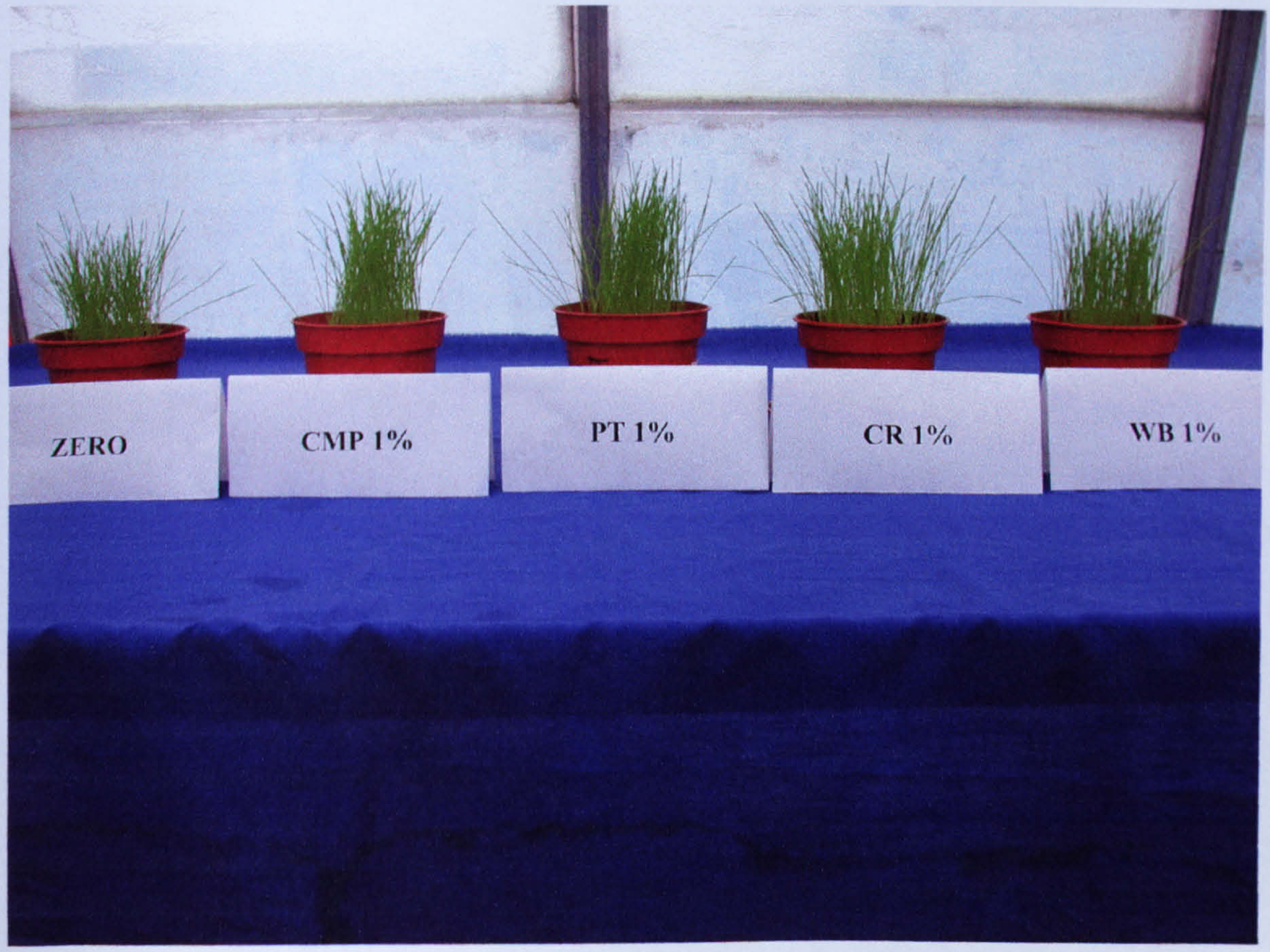


Figure 5.10 Ryegrass in field contaminated soil after two weeks of growth (Zero- non amended soil, CMP- compost, PT- peat, CR- coir, and WB- wood bark).

By four weeks of growth, the effect of the amendments on biomass yield and appearance had become apparent (Figure5.9).

The effect of added amendments was most pronounced at 20% rates, where there was a marked increase in biomass of ryegrass over the zero amendment.

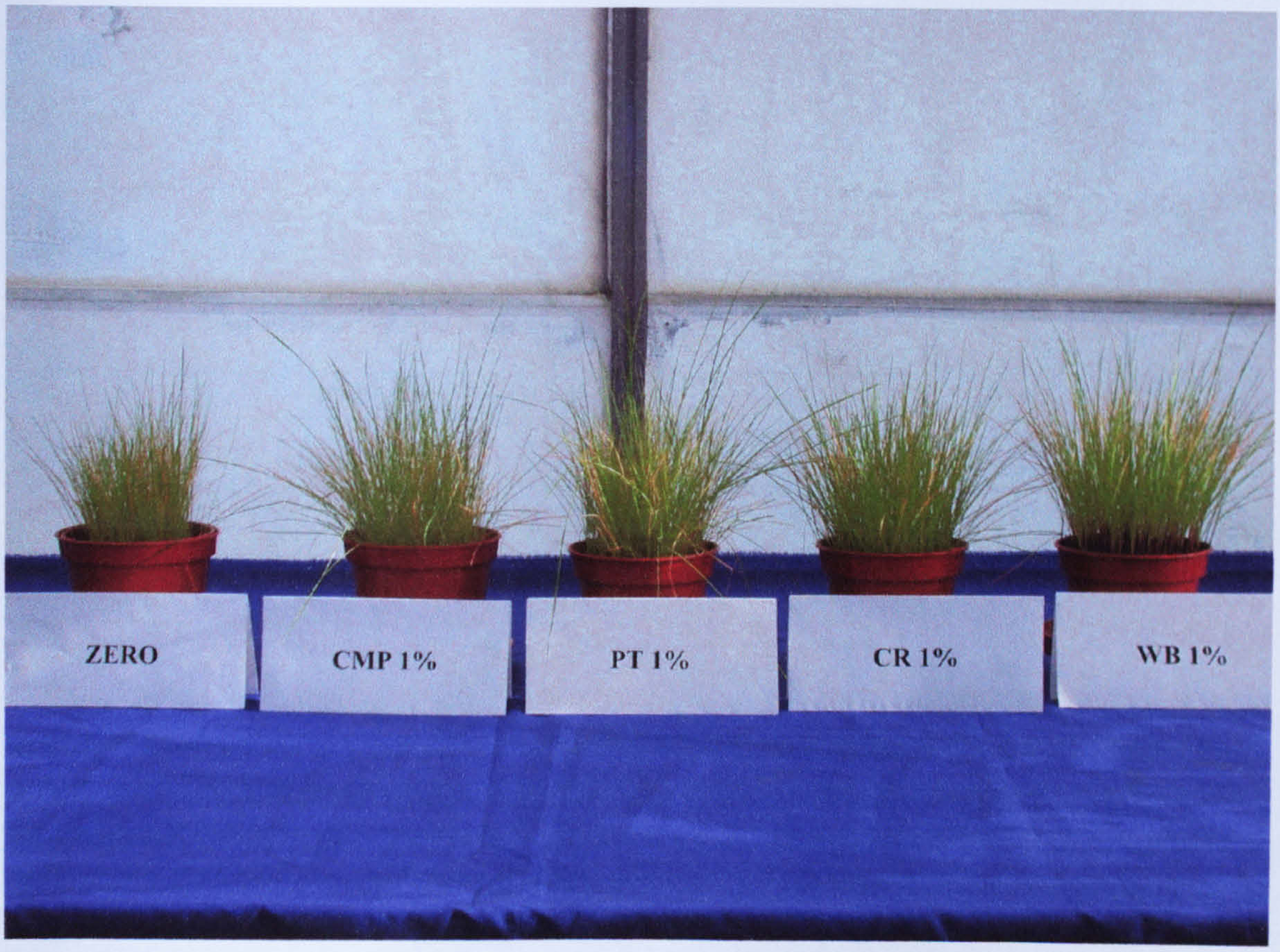


Figure 5.11 Effect of 10% amendments on ryegrass growth in field contaminated soil at four weeks (Zero- non amended soil, CMP- compost, PT- peat, CR- coir, and WB- wood bark)

Green waste compost has been known to improve crop growth due to the readily available N content, but coir is not known to possess any nutrient value. Thus the enhanced growth of ryegrass in coir treated soil would be largely due to the ability of the waste to immobilise metals in soil, thus reducing plant bioavailability. Plants receiving 20% green waste compost exhibited the highest amount of foliage, closely followed by that treated with 20% coir (Figure 5.10).

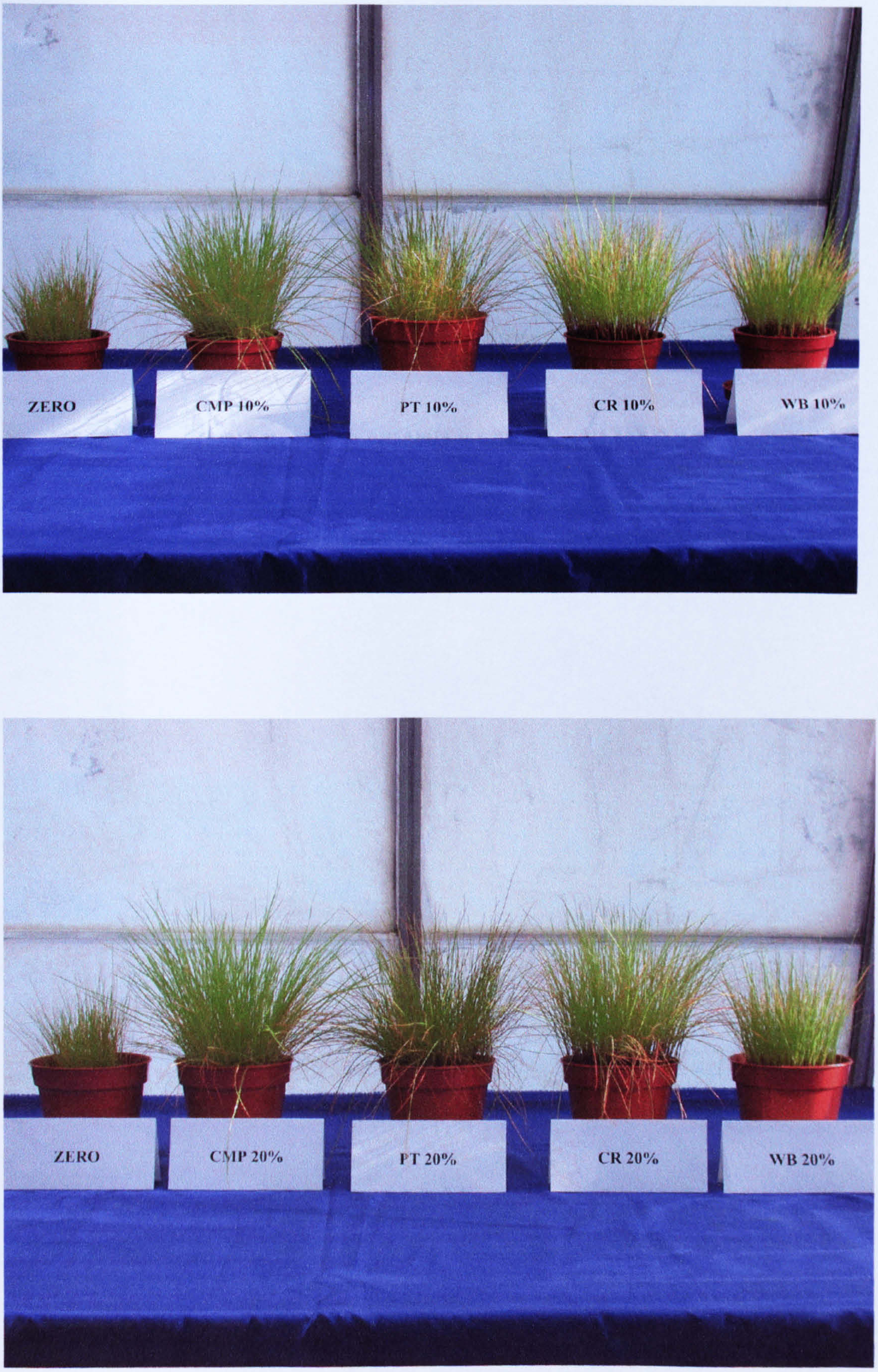


Figure 5.12 Effect of 10% and 20% amendments on ryegrass growth in field contaminated soil at four weeks (Zero- non amended soil, CMP- compost, PT- peat, CR- coir, and WB- wood bark)

In addition to the physical appearance of crops, shoot and root yield of ryegrass was significantly enhanced by the addition of amendments to the contaminated soil (Table 5.7).

Table 5.7 Shoot and root yield of ryegrass in field contaminated soil after six weeks of growth

Treatment	shoot yield (g pot-1)	root yield (g pot-1)
Zero	0.70 ± 0.04a	0.64 ± 0.08a
Compost 1%	0.93 ± 0.03b	0.89 ± 0.06b
Compost 10%	1.98 ± 0.12c	2.37 ± 0.27d
Compost 20%	3.03 ± 0.13f	3.17 ± 0.46e
Peat 1%	0.84 ± 0.07a	0.78 ± 0.12a
Peat 10%	1.85 ± 0.11c	0.97 ± 0.07b
Peat 20%	2.74 ± 0.12e	2.48 ± 0.24d
Coir 1%	1.03 ± 0.06b	1.11 ± 0.13bc
Coir 10%	1.19 ± 0.10bc	1.24 ± 0.15c
Coir 20%	2.54 ± 0.30d	2.44 ± 0.18d
Wood bark 1%	0.71 ± 0.02a	0.79 ± 0.06a
Wood bark 10%	0.85 ± 0.03a	1.19 ± 0.09bc
Wood bark 20%	0.76 ± 0.07a	1.09 ± 0.09ab
LSD <0.05	0.15	0.24

Means ± SD (n=5) Values followed by the same letter are not significantly different at $p<0.05$

The general trend observed was that, the higher the amount of amendment, the higher the biomass yield. Biomass yield in wood bark treated soils was however not significantly different from that in the non-amended soil.

The highest shoot and root yield was as a result of amending with 20% compost, followed by 20% peat, and then 20% coir. All pots received Hoagland solution to sustain growth. Shoot yield was in the order compost>peat>coir>wood bark, while root yield was in the order compost>peat~coir>wood bark.

5.7 Metal uptake by Ryegrass grown in field contaminated soil

5.7.1 Objective

To evaluate the effects of amendments on plant tissue concentrations of Pb, Cu and Zn in ryegrass grown in soil which was contaminated by long term deposition of mine waste

5.7.2 Methodology

The method is discussed in Chapter 2 (Section 2.8.7, page 62).

5.7.3 Results and Discussion

Amending soils with the selected materials led to a significant reduction in the uptake of Pb into ryegrass leaves, when compared with the non amended soil (Figure5.11). For example, leaf Pb of 55 ug g^{-1} in non-amended soil was approximately 40 ug g^{-1} when amendments were added at only 1%. This reduced further to an average of 30 ug g^{-1} with 10% amendments, and about 20 ug g^{-1} when 20% amendments were added. The lowest leaf metal of 12 ug g^{-1} was obtained by amending with 20% compost. Cu concentration in leaves was significantly reduced by the addition of 20% compost only, otherwise, uptake increased with the addition of all other amendments.

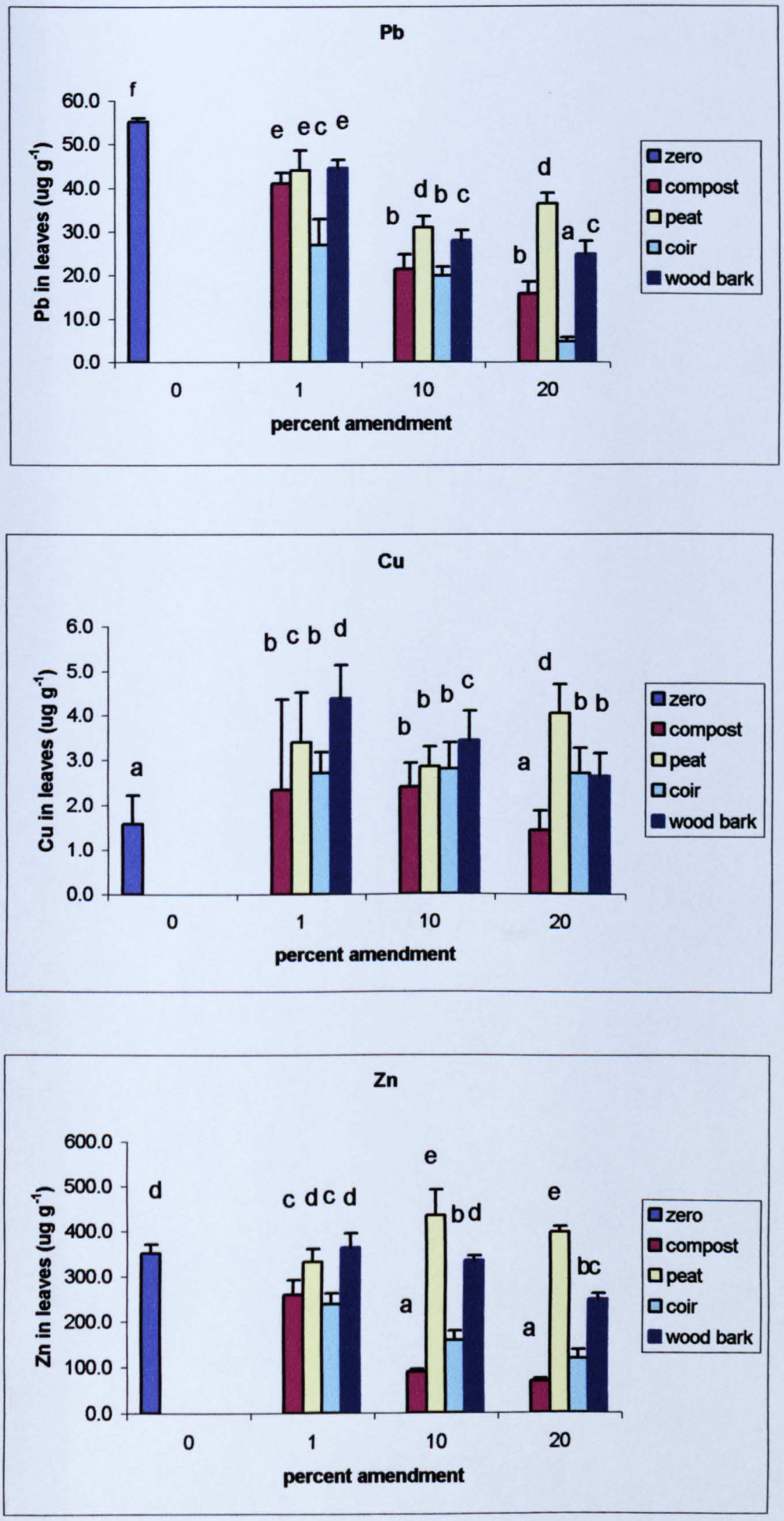


Figure 5.13 Effects of amendments on ryegrass leaf metal concentrations after six weeks of growth in field contaminated soil (Error bars are ± SD of five replicates, LSD $p<0.05$, zero- non amended soil)

Application of green waste compost and peat at 1% led a a significant reduction in Zn uptake by ryegrass leaves, while peat and wood bark effects were not significantly different from the non amended soil (Figure 5.11). With the addition of 10% amendments, green waste compost and coir led to significantly lower uptake of Zn into ryegrass leaves, while wood bark was not significantly different from the non amended soil. At 20% rate of application, green waste compost, coir and wood bark led to a reduction in leaf Zn which was significantly lower than leaf uptake in the non-amended soil. Peat however enhanced the leaf uptake of Zn when applied above a rate of 1% (Figure 5.11).

Analysis of ryegrass roots showed that root concentrations of Pb were also significantly lower in amended soils than the non amended one (Figure 5.12). For example, Pb concentrations in ryegrass roots in non-amended soils had an average of 2748 $\mu g\ g^{-1}$, but this was reduced to an average of 2481 $\mu g\ g^{-1}$ with the application of 1% green waste compost, coir and wood bark. Peat at 1% led to a marginal increase in Pb uptake by ryegrass roots. At 10% rate of application, there was a significant reduction in root Pb by all the amendments, ranging from 839 $\mu g\ g^{-1}$ by wood bark to 1452 $\mu g\ g^{-1}$ root Pb when 10% peat was applied. With 20% amendments, green waste compost, coir and wood bark led to a further significant reduction in root Pb. Peat effect at 20% was however not significantly different from root Pb at 10% peat. The greatest reduction in root Pb was due to an application of 20% wood bark, resulting in only 520 $\mu g\ g^{-1}$ Pb in ryegrass roots.

Applying amendments at all rates led to a highly significant reduction root Cu when compared with the non amended soil, being an average of only 60 $\mu g\ g^{-1}$, compared with 250 $\mu g\ g^{-1}$ in non-amended soil. Root Zn concentration was also signigicantly reduced by the application of amendments, especially at 10% and 20% (Figure 5.12).

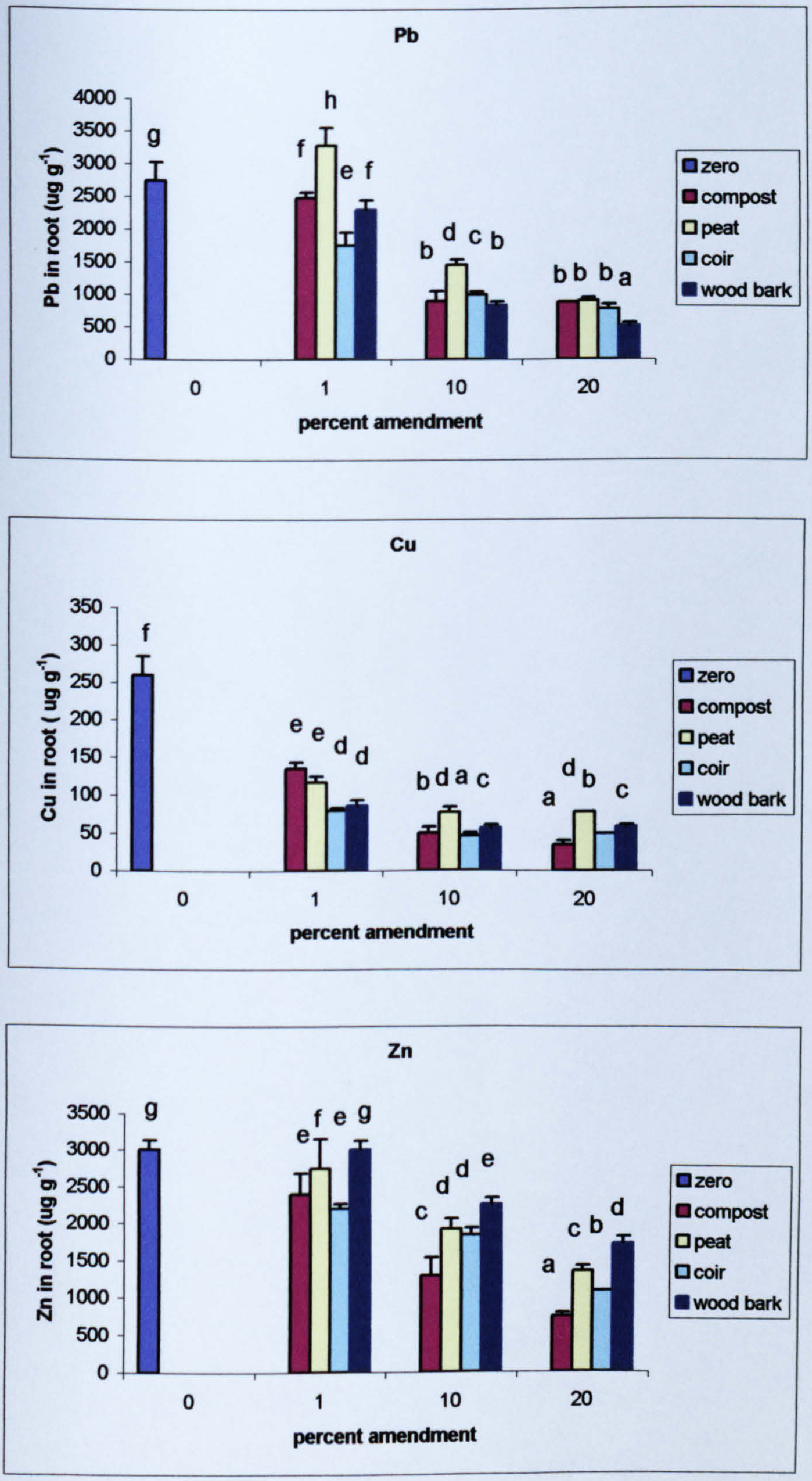


Figure 5.14 Effects of amendments on ryegrass root metal concentrations after six weeks of growth in field contaminated soil (Error bars are ± SD of five replicates, LSD $p<0.05$, zero- non amended soil)

Cu concentrations in the roots of plants also reduced from 260 µg g^{-1} in the non-amended soil to 35 µg g^{-1} in plants which received 20% compost. This agrees with the findings of Ruttens *et. al.*(2006), who found that compost additions to an industrially contaminated soil reduced metal uptake in grasses. It has been inferred that the effectiveness of amendments in reducing Zn in plants is directly related to the ability of the amendment to retain it in soil (Paulose *et al.*, 2007). Thus, an enhanced uptake of metals into roots of plants may be observed by high applications of amendments like peat.

5.7.4 Bioavailability factor in field contaminated soil

The bioavailability factor (BF) of heavy metals (HM) in industrially contaminated soil was calculated as defined in Section 5.5.4 (Sposito & Page) and is presented in Table 5.8.

Table 5.8 Effect of amendments on bioavailablity of Pb, Cu and Zn to ryegrass in field contaminated soil

	Bioavailability factor (BF)		
Treatment	Pb	Cu	Zn
None	1.17e	2.00g	1.81e
Compost 1%	1.06d	1.19f	1.46d
Compost 10%	0.42b	0.49b	0.83b
Compost 20%	0.45b	0.36a	0.53a
Peat 1%	1.29f	0.92e	1.79e
Peat 10%	0.73c	0.67cd	1.49d
Peat 20%	0.51b	0.7cd	1.21c
Coir1%	0.80c	0.62c	1.38d
Coir 10%	0.50b	0.40b	1.23c
Coir 20%	0.42b	0.46b	0.81b
Wood bark 1%	1.06d	0.66c	1.78e
Wood bark 10%	0.43b	0.48b	1.50d
Wood bark 20%	0.29a	0.53bc	1.26c
LSD $p<0.05$ =	0.11	0.10	0.14

Means (n=5). Data within a column followed by the same letter are not significantly different at $p<0.05$, none- no amendment).

Bioavailability factor (BF) for Pb, Cu and Zn was lower in amended soil than those to which no wastes had been added, and the bioavailablity decreased as amendment rates increased from 1% to 20%. This was despite the fact that the addition of some amendments enhanced soil metal concentrations. A single exception was Pb, which had a higher BF at 1% peat than in the non-amended soil.

Results showed that mobility of Pb and Cu from root to shoot was marginal (Table 5.9). The large differences between root and leaf concentrations of Pb and that of Cuindicate an important restriction of the internal transport of the metals from the roots towards the aerial parts (Dahmani-Muller *et al.*, 2000). This type of metal immobilisation in root cells is emphasised by the M_L/M_R quotient. Otherwise known as the transport factor, it was calculated as the ratio of the metal content in plant leaves to that in the roots (Dahmani-Muller *et al.*, 2000; Kidd *et al.*, 2007), and is presented in Table 5.9.

Table 5.9 Mean leaf : root metal concentration ratio in ryegrass(field soil)

	M_L/M_R ratio		
Treatment	Pb	Cu	Zn
None	0.02	0.01	0.12
Compost 1%	0.02	0.02	0.11
Compost 10%	0.02	0.05	0.07
Compost 20%	0.02	0.04	0.09
Peat 1%	0.02	0.03	0.12
Peat 10%	0.02	0.04	0.23
Peat 20%	0.04	0.05	0.29
Coir1%	0.02	0.03	0.11
Coir 10%	0.02	0.06	0.09
Coir 20%	0.01	0.06	0.11
Wood bark 1%	0.02	0.05	0.14
Wood bark 10%	0.03	0.06	0.15
Wood bark 20%	0.05	0.04	0.14

(Values are means of five replications, none- no amendment)

Of the three metals, Zn had the highest metal leaf:metal root ratio, a reflection of the mobile nature of the metal, and this follows the trend in studies by Brun *et. al* (2001) and McBride (2001).

Translocation of Cu from root to shoot was low (Figure 5.13). This is typical of the ability of Cu to accumulate in roots without a proportional transport into aerial parts (Kidd *et al.*, 2007; MacFarlane & Burchett, 2002). According to Song *et al* (2004), there appears to be an apparent strong physiological control over translocation of Cu from roots to shoots.

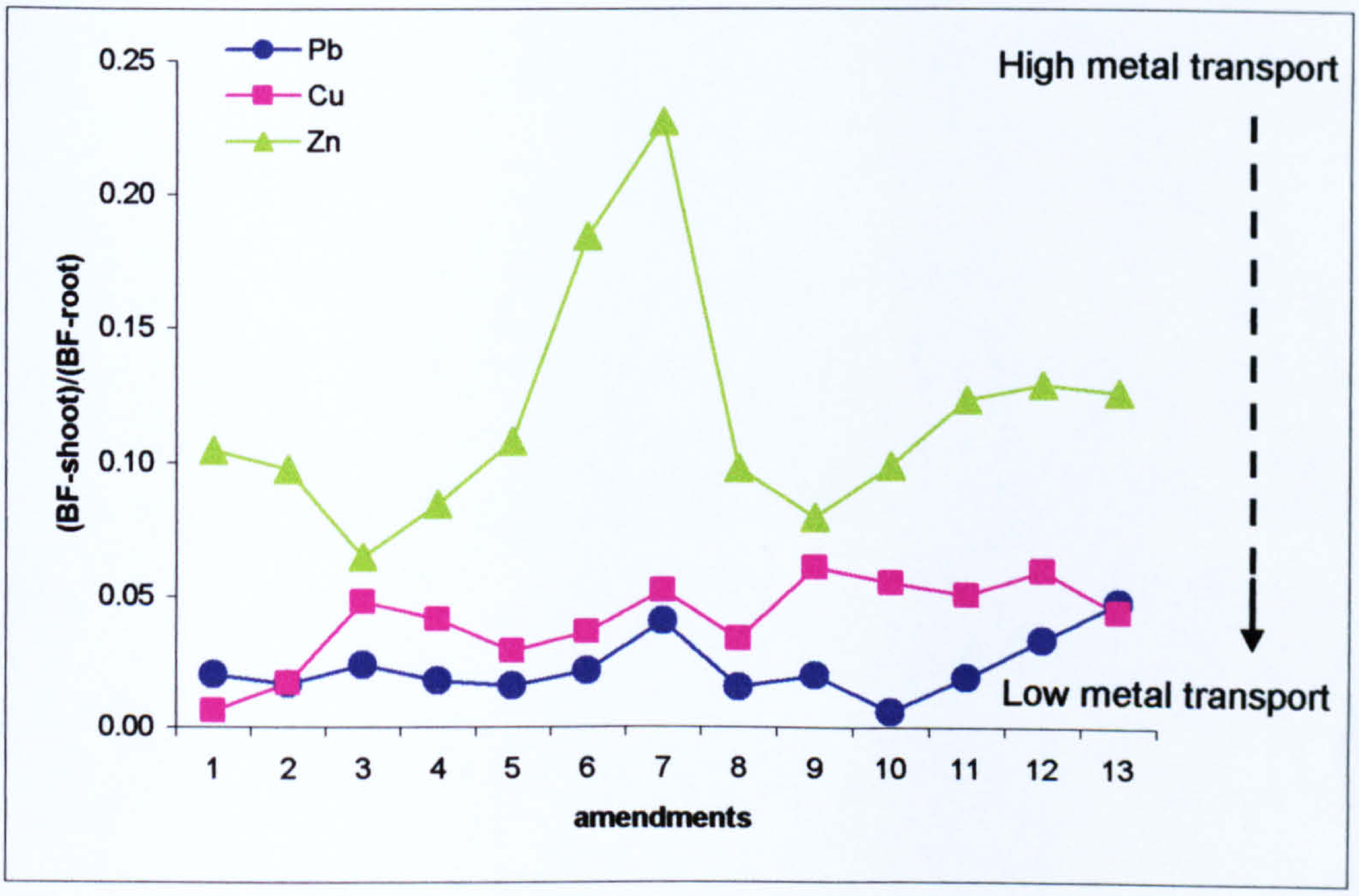


Figure 5.15 A ratio of metal bioavailability in shoot to that in root of ryegrass. (1=zero; 2-4= compost; 5-7= peat; 8-10 = coir; 11-13 = wood bark) Total growth period is six weeks, and for each amendment, there are three rates: 1%, 10%, and 20%

It has been suggested that in relating extractable soil metals to the concentrations in roots and shoots of plants, an approach should be adopted which bears in mind that that the metal concentration in the soil solution is related to the total metal content of the soil, and also that the metal content of the shoots is related to the metal content of the roots (Kalis et al., 2007). The latter holds true for mobile elements like Zn, where it has been shown that its shoot content depends linearly on the root Zn concentration (Kalis et al., 2007; MacFarlane & Burchett, 2002).

5.8 Metal levels in field contaminated soil as affected by the addition of amendments

5.8.1 Objective

To evaluate the ability of amendments to bind Pb, Cu and Zn in a field soil which had been contaminated by long term deposition of waste through mining activities

5.8.2 Methodology

The methodology is fully discussed in Chapter 2 (Section 2.8.5, page 62).

5.8.3 Results and discussion

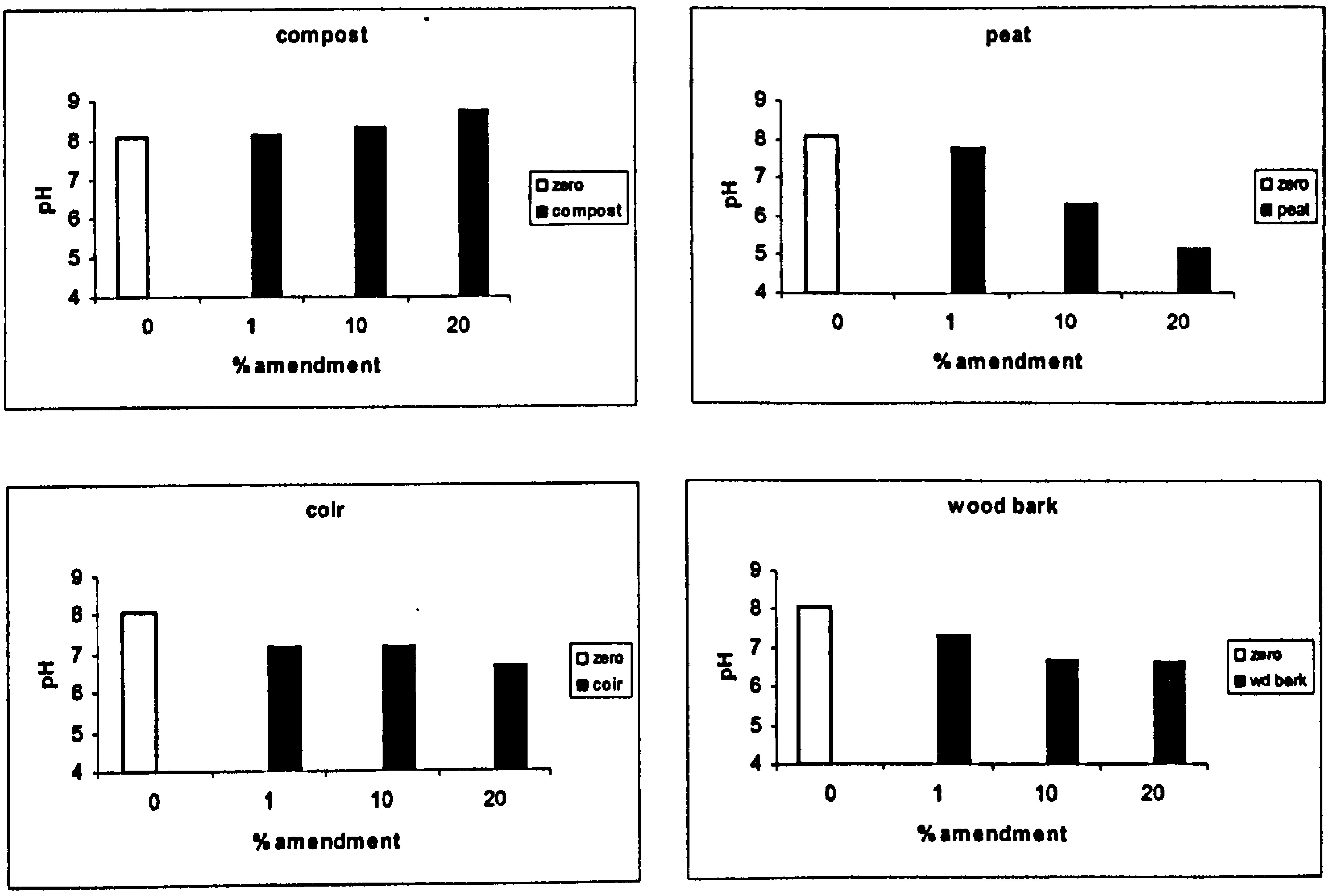


Figure 5.16. Effect of amendment on pH of field contaminated soil

When organic materials are used as amendments they affect the pH of the soil (Walker *et al.*, 2004). Addition of amendments generally led to a lowering of soil pH (Figure5.14), with peat having the greatest lowering effect, as observed by Hodson *et al* (2001).

Acidic soil conditions favour solubility of metals and restrict their ability to complex with organic matter (Lu *et al.*, 2005). Peat reduced the pH of soil by more than 3 units, while coir and wood bark reduced pH by more than one unit each. This effect on pH confirms the low buffering capacity of the soil to acid, as reported in Chapter 2 (Section 2.8.9). On the other hand, compost led to a marginal increase in soil pH by less than 1unit, the amendment itself being of higher pH than that of the soil (Figure 5.14).

Four weeks after adding in the amendments, most values of extractable Pb and Zn were significantly lower in amended soil than concentrations in the non amended soil, with greater reductions being observed the higher the amount of amendment added to the soil (Table 5.10). There was no significant difference between 1% compost, all amounts of coir, or the non amended soil in their ability to bind Cu. However, the addition of peat, coir, wood bark, and more than 1% compost led to an increase in the amount of extractable Cu above that in the non amended soil.

Table 5.10 EDTA extractable metals in amended soils after four weeks of treatment (pre-planting)

Amendment		ug g^{-1}	
	Pb	Cu	Zn
Zero	1530 ± 177c	11.3 ± 0.7a	657 ± 53c
Compost 1%	1554 ± 182c	11.3 ± 0.8a	481 ± 15a
Compost 10%	1458 ± 88bc	12.9 ± 1.2b	486 ± 68a
Compost 20%	1256 ± 48a	15.3 ± 0.1d	458 ± 14a
Peat 1%	1312 ± 91a	12.7 ± 0.5b	457 ± 8a
Peat 10%	1403 ± 121b	15.0 ± 0.6d	627 ± 82b
Peat 20%	1364 ± 55b	15.5 ± 1.4d	695 ± 14c
Coir 1%	1354 ± 122b	12.0 ± 0.6a	548 ± 25a
Coir 10%	1143 ± 168a	11.2 ± 0.9a	497 ± 52a
Coir 20%	1148 ± 20a	12.2 ± 1.1a	490 ± 50a
Wood bark 1%	1480 ± 84b	14.2 ± 0.3cd	535 ± 24a
Wood bark 10%	1307 ± 123ab	13.3 ± 0.4bc	566 ± 27a
Wood bark 20%	1138 ± 43a	13.3 ± 0.9bc	569 ± 54b
LSD	204	1.4	76

Mean ± SD (n = 5). (Values within a column followed by the same letter are not significantly different at $p < 0.05$, zero- non amended soil)

After ten weeks, soils which had received 10 and 20% levels of the amendments but had not been planted with ryegrass were significantly lower in extractable Pb than the non amended soil (Table 5.11). By amending with 20% coir, extractable Pb and Cu was reduced from 1725 ug g^{-1} to 1033 ug g^{-1}; and from 13.6 ug g^{-1} to 10.6 ug g^{-1}, respectively.

Table 5.11 EDTA extractable metals in amended soils after 10 weeks of treatment (non – planted pots)

Amendment		ug g^{-1}	
	Pb	Cu	Zn
Zero	1725 ± 79d	13.6 ± 0.3b	590 ± 11b
Compost 1%	1452± ± 34c	13.2 ± 0.3b	544 ± 19b
Compost 10%	1380 ± 103bc	16.4 ± 0.6d	540 ± 16b
Compost 20%	1363 ± 175bc	16.5 ± 0.8e	463 ± 35a
Peat 1%	1647 ± 41d	16.2 ± 0.8d	516 ± 55b
Peat 10%	1541 ± 157c	17.2 ± 0.5e	669 ± 31c
Peat 20%	1317 ± 6bc	14.9 ± 0.4c	691 ± 106c
Coir 1%	1514 ± 42c	13.9 ± 1.6bc	558 ± 19b
Coir 10%	1397 ± 75bc	13.3 ± 0.6b	548 ± 17b
Coir 20%	1033 ± 56a	10.7 ± 0.5a	441 ± 15a
Wood bark 1%	1590 ± 18d	15.2 ± 0.6d	555 ± 14b
Wood bark 10%	1291 ± 72b	14.2 ± 0.4b	557 ± 9b
Wood bark 20%	1149 ± 30a	14.0 ± 0.1b	557 ± 12b
LSD $p < 0.05$	135.7	1.2	63.7

Mean ± SD (n = 5). (Values within a column followed by the same letter are not significantly different at $p < 0.05$, zero- non amended soil)

Extractable Zn was also significantly reduced by amending soils with 20% coir, but there was no significant effect with wood bark, compared with Zn levels in the non-amended soil. At 20% coir extractable Zn was 441 ug g^{-1}, compared to 590 ug g^{-1} in the non amended soil. All amendment with peat led to significantly higher concentrations of extractable Cu, and that of Zn at 10 and 20% levels. This may have been due to a lowered pH which arose from the addition of peat.

Soils which had been sown with ryegrass were also analysed for extractable metals after harvest, which made a total of ten weeks of amendment (four weeks pre-planting, plus six weeks of plant growth). The results are presented in (Table 5.12), and they show that extractable Pb in all amended soils was significantly lower than the non-amended one.

Table 5.12 EDTA extractable metals in amended soils after six weeks of ryegrass growth

Amendment	Pb ug g-1	Cu	Zn
Zero	1520 ± 52	12.8 ± 0.2e	533 ± 10f
Compost 1%	1345 ± 28e	12.4 ± 0.3e	492 ± 11e
Compost 10%	1219 ± 21d	13.2 ± 0.4e	451 ± 11c
Compost 20%	960 ± 88b	12.9 ± 0.4e	320 ± 18a
Peat 1%	1221 ± 23d	13.9 ± 0.6f	474 ± 28d
Peat 10%	1053 ± 77c	14.3 ± 0.5g	526 ± 23f
Peat 20%	1075 ± 10c	11.6 ± 0.3d	548 ± 22f
Coir 1%	1185 ± 24d	11.2 ± 1.0d	481 ± 10e
Coir 10%	1013 ± 41b	10.0 ± 0.5c	461 ± 12d
Coir 20%	888 ± 38a	8.2 ± 0.3a	343 ± 9b
Wood bark 1%	1406 ± 23f	12.7 ± 0.4e	496 ± 6e
Wood bark 10%	1104 ± 44c	11.5 ± 0.2d	481 ± 6e
Wood bark 20%	936 ± 20b	10.8 ± 0.3b	423 ± 8c
LSD $p < 0.05$	55	0.6	19

Mean ± SD (n = 5). (Values within a column followed by the same letter are not significantly different at $p < 0.05$, zero- non amended soil)

As in the soils which were not planted with crops, addition of 20% amendments led to the greatest reduction of extractable Cu in contaminated soil. All levels of coir amendment led to extractable Cu concentrations which were significantly lower than the non-amended soil. pH thresholds have been identified for Cu (5.5), and Zn (6.2), below which the solubility of these metals increase markedly (Oudeh *et al.*, 2002). Immobilization of Zn in soils which had supported plant growth was most effective at amendments levels greater than 1%, except with peat, which resulted in higher extractable Zn, the higher the amount of the amendment added to the soil. Unlike compost, coir and wood bark which generally reduced the EDTA extractable metal concentrations, the acidic nature of peat tends to increase metal solubility in soils (Clemente *et al.*, 2007; Romero *et al.*, 2005). This would in turn enhance metal uptake by plants when using the material as soil amendment.

For non planted pots, an initial reduction in extractable metals after four weeks of amendment was not seen after a further 6 weeks, agreeing with the findings of (Perez-de-Mora *et al.*, 2006) (Tables 5.10 and 5.11). While it is well established that metals are readily bound on to natural organic matter, others have reported an increased solubility of Cu with the addition of high organic matter levels (Covelo *et al.*, 2004). It is known that organic amendments may promote metal mobility if complexes formed with soluble components of the amendments are more soluble than the initial status of the metal (Madrid *et al.*, 2006). .

EDTA-extractable metal concentrations in the industrially contaminated soil were significantly correlated with shoot and total plant tissue concentrations of Pb and Zn, but not with Cu. The results are presented in Table 5.13.

Table 5.13 Correlations between EDTA extractable metals in soil and concentrations in plant tissue of ryegrass.

	shoot		shoot + root	
Soil vs Shoot	correlation	p	correlation	p
Pb	0.698	0.000***	0.736	0.000***
Cu	0.025	0.843NS	-0.149	0.235NS
Zn	0.657	0.000***	0.357	0.003***

n=65, and Pearson's correlation at $p<0.05$

Correlation results obtained in this study were similar to the findings of Walker *et al* (2003), who found highly significant correlations between Pb and Zn concentrations in plant tissue and DTPA -extractable concentrations in soil. The lack of agreement between plant tissue and EDTA extractable soil Cu concentrations could be due to the fact that EDTA is likely to extract Cu associated with organic matter as well (Kidd *et al.*, 2007). EDTA has been known to solubilise about 80% of the total soil metal (Liphadzi & Kirkham, 2006), thus EDTA extractions often result in very high metal concentration values. It has been suggested that $CaCl_2$ be used as well as EDTA in assessing bioavailable soil metal concentrations, as some elements like Cu appear to show better correlation between plant tissue and soil values when $CaCl_2$ is used (Kabata-Pendias, 2004; Oudeh *et al.*, 2002). Other workers have found high correlations between NH_4NO_3- extractable Pb and Cu in soil and their concentrations in plant tissue (Usman *et al.*, 2006).

5.9 Conclusion

- Addition of amendments led to a general reduction in extractable Pb and Zn in field contaminated soil, thus rendering the metals less available for uptake. However, high levels of peat seemed to enhance solubility of Cu and Zn in soil. Also, shoot and root levels of all three metals were significantly reduced by the addition of amendments, especially at 20% rates of the materials. The uptake of metals appeared to be influenced by the origin of the contamination, as metals in sewage treated soil (organic), was less available for uptake than that in the mine waste (inorganic). The type of contamination was also a factor, as manually spiked soils showed more variability, thus did not respond to amendments in the same way as field contaminated field soils.

- The initial metal concentrations of metals in manually spiked and field contaminated soil, and plants grown on them were above toxic levels as stated in Chapter 1 (Section1.2, Table 1). Addition of amendments to the spiked soils did not lower metal concentrations below toxicity thresholds, because of the very high metal levels in the contaminants used. In the field contaminated soil however, the effect of amendments to reduce soil and plant metal concentrations was more evident.
- Reduced plant uptake of metals by plants was not a consistent parameter to evaluate the effectiveness of amendments to immobilise metals. Results indicate that EDTA could successfully predict the phytoavailability of Pb and Zn, whereas it was not suitable for Cu, thus the use of weaker extractants like $CaCl_2$ becomes very pertinent
- While addition of amendments can help prevent uptake of metals into plant roots, translocation within the plant system could not be predicted from available soil levels alone.
- Root concentration of metals was much higher than shoot levels, and they were a better reflection of soil metal concentrations. Addition of all the amendments significantly reduced the root levels of Pb, Cu and Zn, more than root metals in the non-amended soil. Uptake into the aerial parts of the plant however, varied from metal to metal, as a direct effect of differences in lability.

Chapter 6
General Conclusions

6.1 Research findings

- Organic amendments were very effective in immobilizing lead, copper and zinc, and this effectiveness was consistent under single and multi-metal systems, and in varying background salt concentration. The amendments were able to reduce the availability of Pb, Cu and Zn in contaminated soil and in soil solution, and generally, metal sorption from contaminated soil increased as the amount of amendments increased from 1% to 20% of soil weight. This research has shown therefore that the use of organic amendments is a viable option in the remediation of metal contaminated soil.

- Application of amendments to contaminated soil also reduced metal concentrations in plant tissue, thus, amending contaminated soil with organic materials is a very effective way of reducing metal uptake by plants. One major way of fulfilling the objectives of soil metal stabilization is the restoration of vegetation, and this was achieved in this study. There was a consistent increase in biomass yield upon the addition of amendments, and this is more noteworthy given the fact that some of the amendments like coir and wood bark did not have any nutrient values.

- The type and source of the contaminant has a direct bearing on metal toxicity, as Pb, Cu and Zn in contaminants of different origin were not equally mobile or bioavailable. Metals from mine waste (inorganic) were more readily available for plant uptake, while those from the sewage treated soil (organic) were less readily taken up by ryegrass. The complex and dynamic nature of the soil-plant systems requires in depth consideration of the specific soils and the amendments involved in any remediation research, in order to make correct interpretation of data from bioavailability studies. Differences observed in plant uptake from 'metal spiked soil' and that from the field soil confirm that metal sorption patterns differ between laboratory contaminated and field contaminated soils. Application of the amendments used in this research led to a clear reduction of metal extractability and uptake regardless of type of contaminant or mode of contamination, thus indicating their potential for cleaning up a wide range of metal contaminated soils.

- Addition of amendments reduced effect of metal toxicity on microorganisms, as the amount of CO_2 evolved in contaminated soil was enhanced by the addition of amendments. The effect of metals on CO_2 evolution in contaminated amendments and soil as seen in this research, show that soil microbial processes are very useful indicators of pollutant heavy metal stress on the health of soils. Remediation of contaminated soils therefore results not only in a re-vegetation of contaminated sites, but also in recovery of microbial populations and activities. All the amendments used in this study were able to improve soil quality, which, among other things, is to sustain biological productivity, and promote plant growth.

6.2 Constraints to the use of organic amendments in metal sorption

There are constraints to the use of amendments in remediating contaminated soil, but these constraints can largely be overcome

- Some amendments can potentially mobilize metals, as was seen with high rates of peat in this research. Due to the numerous changes which take place in the soil chemistry due to the presence of the amendments used, side effects like solubility and changes in soil pH may arise. The increase in soil and plant Cu and Zn observed with the application of peat in this research appears to be directly linked to its low pH (3.9). Care should be taken therefore in selecting amendments which would lead to minimal possible side effects.

- Periodic applications of amendments may be required to sustain immobilization of metals in the long term, since the effect of the amendments may reduce over time, thus posing a challenge for regular amendment supply. However, this constraint may be overcome through a combined application of organic materials and inorganic ones like zeolites, lime or iron sources to help stabilize the organic materials for longer periods of time.

- The choice of amendments should must be based on careful environmental considerations. For example, peat is now designated as a protected material because its maintenance as a carbon sink has become more paramount in view of increasing global CO_2 emissions

- Soil remediation through the use of amendments may be slow and require several years if the level of contamination or land area is very large. Rapid re-vegetation of contaminated soil can however be achieved by the addition of amendments, and this is very pertinent where large areas are affected, like those contaminated by mining activities

6.3 Challenges to soil remediation research in Nigeria

There is no doubt that the use of agricultural wastes instead of synthetic additives has great potential in a developing country like Nigeria where most farmers operate at the subsistence level. However, challenges which prevail at present would need to be addressed to successfully tackle the Nigerian soil contamination situation.

- The most urgent task is the need to enforce existing legislation on waste management and disposal, and there is the need for government to accord as much funding to tackle industrial waste contamination as has been done for crude oil pollution

- Constant monitoring of the levels of metals is required to safeguard farmlands, fish ponds and water bodies around facilities most prone to contamination because metal build up is a gradual process (Iwegbue *et al.*, 2006b). This regular monitoring is lacking at the moment

- Many agricultural wastes have alternative uses in Nigeria, and there may be economic considerations as to whether to commit such agricultural wastes to soil remediation purposes. For example both coir and tree bark are used as cooking fuel, while bone meal is in high demand as a component of poultry feed. Other wastes like cassava peel, corn husk, and cowpea husk which have been identified as potentially effective as soil amendments are used as livestock feed. Thus, amendment materials could become so sought after that their economic value increases such that they can no longer be affordable as wastes.

6.4 Future research

- In the immediate future, a 'metal mapping' of known contaminated soils in South eastern Nigeria. This will entail total soil metal analyses to identify both the species and concentration of metals in those soils, and a detailed documentation of findings

- Establishing single season field trials to evaluate the bioavailable fraction of metals in contaminated soils prior to any application of amendments, especially in locations currently being cultivated by local farmers. Results from such will help to relate metal concentrations in plant tissue to acceptable levels for food crops, and also to the prevailing soil properties and local climate

- A detailed characterization of agricultural wastes which have already been tried in soil remediation studies in Nigeria, and an identification of other potentially effective ones which shall also be evaluated for characteristics vital to effective sorption like the organic carbon content, the CEC and the pH, with much emphasis on those materials which do not have major alternative uses

- Testing of amendments on widely varying soil types initially on a pilot scale in the greenhouse. Nigeria has five climatic zones, with differing soil types and physico chemical properties, ranging from heavy clays to loose sands, and to very acidic soils in coastal areas to highly calcareous ones in regions with limestone deposits. Soils are rich in nutrients, but because of the tropical climate soil organic matter is low (Nwachukwu, 2002). Thus, there are no peat soils, and the natural sorption metals by soils would not depend on inherent organic matter content

- In the long term, field trials spanning several years to determine the effectiveness of amendments over time. In soil stabilization techniques, because metals remain on site they may be potentially re-mobilised as agricultural wastes degrade. In Nigeria, some materials like cowpea husk, rice bran, maize cob and husk, saw dust have been tried as amendments for metal immobilization in the short term The-long term trials will monitor the effectiveness of metal sorption, as well as the rate of remobilization of sorbed metals.

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