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Phytoremediation and rhizosphere manipulation using different amendments

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By

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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.

Acronyms

As	Arsenic
ATP	Adenosine triphosphate
B	Boron
BM	Bone meal
Ca	Calcium
$CaCl_2$	Calcium chloride
Ca $(NO_3)_2$	Calcium nitrate
CaTHDOP	Calcium tetra hydrogen di-orthophosphate
CaTDOP	Calcium trihydrogen diorthophosphate
CDTA	Trans-1,2-cyclohexene-diamine tetraacetic acid
Cd	Cadmium
CEC	Cation exchange capacity
Ce	Cement
Co	Cobalt
CO_3	Carbonate
Cr	Chromium
Cu	Copper
DDT	Dichlorodiphenyltrichloroethane
DOC	Dissolved organic carbon
DTPA	Diethylenetrinitrilo-pentaacetic acid
DW	Dry weight
EDTA	Ethylene diamine tetra acetic acid
EGTA	Ethylebis[oxyethylenetrinitrilo]-tetraacetic acid
Fe	Iron
G	Galena soil
g	Gram
H^+	Hydrogen ion
HA	Humic acid
ha	Hectare
HEDTA	Hydroxyethyl-ethylene-dinitrilo-triacetic acid

Hg	Mercury
K	Potassium
kg	Kilogram
K_2HPO_4	Di-potassium di-hydrogen phosphate
KNO_3	Potassium nitrate
M	Molar
mg	Milligram
Mn	Manganese
Mo	Molybdenum
muM	Micromole
µM	Micromole
Ni	Nickl
OH^-	Hydroxyl ion
ROS	Reactive oxygen species
Pb	Lead
ppm	Part per million
SBS	Stock Bardolph Soil or sewage treated soil
Se	Selenium
TF	Transfer factor
TOC	Total organic carbon
Wt	Weight
Zn	Zinc

Summary

Phytoremediation is a remediation technology which employs vegetative growth to ameliorate toxicity. In this thesis are nine chapters with different experiments undertaken to investigate and study different aspects to enhance phytoremediation mostly phytoextraction and phytostabilization.

In two pot experiments using two different crop ryegrass (*Lolium perenne*) and two flax (*Linum usitatissimum*) varieties Viola and *Elise,* ryegrass decreased the pool of heavy metals compared with bare soil using EDTA as extractant. NH_4^+ decreased the soil pH, increased EDTA-extractable Zn and increased the Zn uptake. Lime addition increased the pH and depressed Zn uptake. The pool of extractable EDTA was not changed by growing both of the flax varieties. Lime increased the EDTA-extractable Cu and Pb significantly, but decreased the Zn, and pH increased in this order $NH_4^+ < NO_3^- < NH_4^+$ +lime < NO_3^- + lime. The EDTA-extractable Cu decreased in the order NO_3^- + lime > NH_4^+ + lime > $NH_4^+ > NO_3^-$. Ammonium decreased the pH more than other treatments.

In agar using Bromocresol purple indicator NH_4^+ decreased the pH in the rhizosphere of different plants. With two different initial pH treatments (7 and 3.2) the NH_4^+ decreased the pH in the rhizosphere at high initial pH 7 and maintained the low pH at initial pH 3.2 to 4 against the buffer capacity. At different initial pHs 4, 5, 6, 7 and 8 the ammonium decreased the high pH and maintained the low pH, but NO_3^- had no effect on the pH. Ammonium increased the toxicity of Zn due to pH decreases. There was no effect of both nitrogen sources NH_4^+ or NO_3^- on rhizosphere pH when applied as a foliar application. These indicated that the NH_4^+ can decrease the pH in

the rhizosphere of plants and could play an important role in manipulation of the rhizosphere bioavailability of heavy metals. Toxicity of the three metals is Cu > Pb > Zn in this order and the crops tolerance is following this order pea > flax > barley.

An agar-Hoagland nutrient solution contaminated with two soils, sewage treated soil (SBS) and galena soil (G), was used with flax as a test crop. The ammonium treatment lowered the pH in both soils, but with galena treated greater than SBS soil, this is attributed to the buffering capacity of the SBS soil. Averaged over all the concentrations the NH_4^+ treatments resulted in higher Zn shoot content than NO_3^- treatment, while in Cu shoot content nitrate was more than ammonium. The transfer factor of lead with ammonium treatment was greater than nitrate treatments at the 0.1 and 0.25% galena and the transfer factor of the Zn and Pb more than Cu in all treatments.

At high initial pH 8 and high concentration of Zn and Cu barley grew well and this is attributed to immobilization of Zn and Cu compared with low pH 5 and 6.5 where the barley plant did not survive. Ammonium lowered the high pH 8 and caused lower biomass production of barley than nitrate. Ammonium and pH play an important role on the manipulation of the rhizosphere and can be used to decrease or increase of heavy metal accessibility and bioavailability.

The characteristics of two amendments cement and bone meal were investigated, for their effect of germination, pH, and adsorption of some heavy metals. Cement decreased barley germination at high percent of mixing with washed acid sharp sand more than 5% w/w and barley grew well with a low concentration less than 2% w/w compared with control. Bone meal depressed barley germination at 0.4 g/ 20 ml water. On the batch experiments for Zn, Cu, and Pb adsorption by cement or bone meal, the cement had greater affinity for adsorption of Zn, Cu and Pb than bone

meal. Cement and bone meal were incubated with two contaminated soils, sewage - treated soil (SBS) and galena for three months and EDTA and $CaCl_2$ were used as extractants. EDTA extracted less heavy metal from bone meal treated soils than cement treated soils, while the $CaCl_2$ more with cement than bone meal. On application of cement and bone meal amendment in pot experiment with high Zn and Cu concentrations with ammonium or nitrate the results showed that the cement amendment immobilized the Zn and Cu and the plant grew while with bone meal treatments and control the plant not survive. This indicated that the cement has greater ability to immobilize heavy metals than bone meal. Ammonium decreased the pH compared with nitrate and increased the pool of heavy metals. Also, this revealed that the EDTA is not as suitable an extractant as $CaCl_2$, which gave more extractable heavy metals with cement in the incubation experiment.

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Chapter 1

Introduction

1.1 General

Pollution in general is any change in nature that leads to contamination and consequently changes the biodiversity. Page (1997) defined pollution more precisely as any harmful or undesirable change in the physical, chemical or biological quality of air, water or soil as a result of the release of e.g. chemical, radioactivity, heat or large amount of organic matter (sewage). Usually the term is applied to changes arising from human activity although natural pollutants, e.g. volcanic dust, sea salt, are known (Lawrence *et al.*, 1998). Redistribution of heavy metals by human activity such as mining, industry and smelting, or by geochemical weathering processes, causes contamination to ecosystems, and consequently is affecting directly or indirectly human, animal and plant life.

Remediation is the process of environmental clean up of contaminated sites and the technology used to eliminate or decrease the contamination from soil, surface water, or ground water. Remediation is the action taken to clean up contamination and brings the site to a non-harmful condition. Large areas of agricultural or arable land in the world were affected by different pollutants, for example in the United Kingdom, about 50,000 to 250,000 hectares (Denner, 1992). Most of the contaminated land located in densely developed countries is due to industrial activity, nuclear energy and military use. Some of the contaminated materials are long-lived toxic chemicals : a) heavy metals, b) radioactive elements c) organic substances; all of these substances

affect human health from the present to the future (Page, 1997). In the last three decades the remediation and the protection of the environment have been big challenges in the world. Most of the methods for soil and water are highly expensive such as excavation and also soil physical, chemical properties and biological activity are affected by some remediation methods such as soil washing (Pulford and Watson, 2003).

Phytoremediation is an in situ method, which is cheap and economically attractive. The combination and integration of phytoremediation with other methods such as chemo remediation or physioremediation is a good strategy to improve the clean up of the environment and contaminated sites.

1.2 Heavy metals in soil

Heavy metal concentrations in soil range from less than 1 mg/kg to over 1000 mg/kg (Adriano, 2001). Heavy metals are present in the Earth's crust naturally in different minerals at different concentration and many of these metals are essential for cells (e.g. Cu, Fe, Mn, and Zn) (Marschner, 1995). In particular environments the mobility of heavy metals depends on the host minerals of those elements, for example quartz and feldspar minerals are parent materials more stable than ferromagnesian minerals (i.e., biotite , olivine and amphibole) .

Excessive levels of many metals can negatively affect soil quality, which leads to crop yield reduction (Marschner, 1995) and poses significant hazards to human, animal, and ecosystem health (Adriano et al., 2004). This includes the metals/metalloids, such as As, Cd, Cr, Cu, Pb Hg, Ni, Se, and Zn. Other less common metallic species such as Al, Cs, Co, Mn, Mo, Sr and U can be also considered contaminants (Marschner, 1995).

After weathering, heavy metals are either leached through the soil in solution, precipitated as other chemical compounds such as hydroxides, sulphates, phosphates, carbonates etc, or held on the surfaces of soil components such as silicate clays, hydrous oxides and humified organic matter (Davies, 1980).

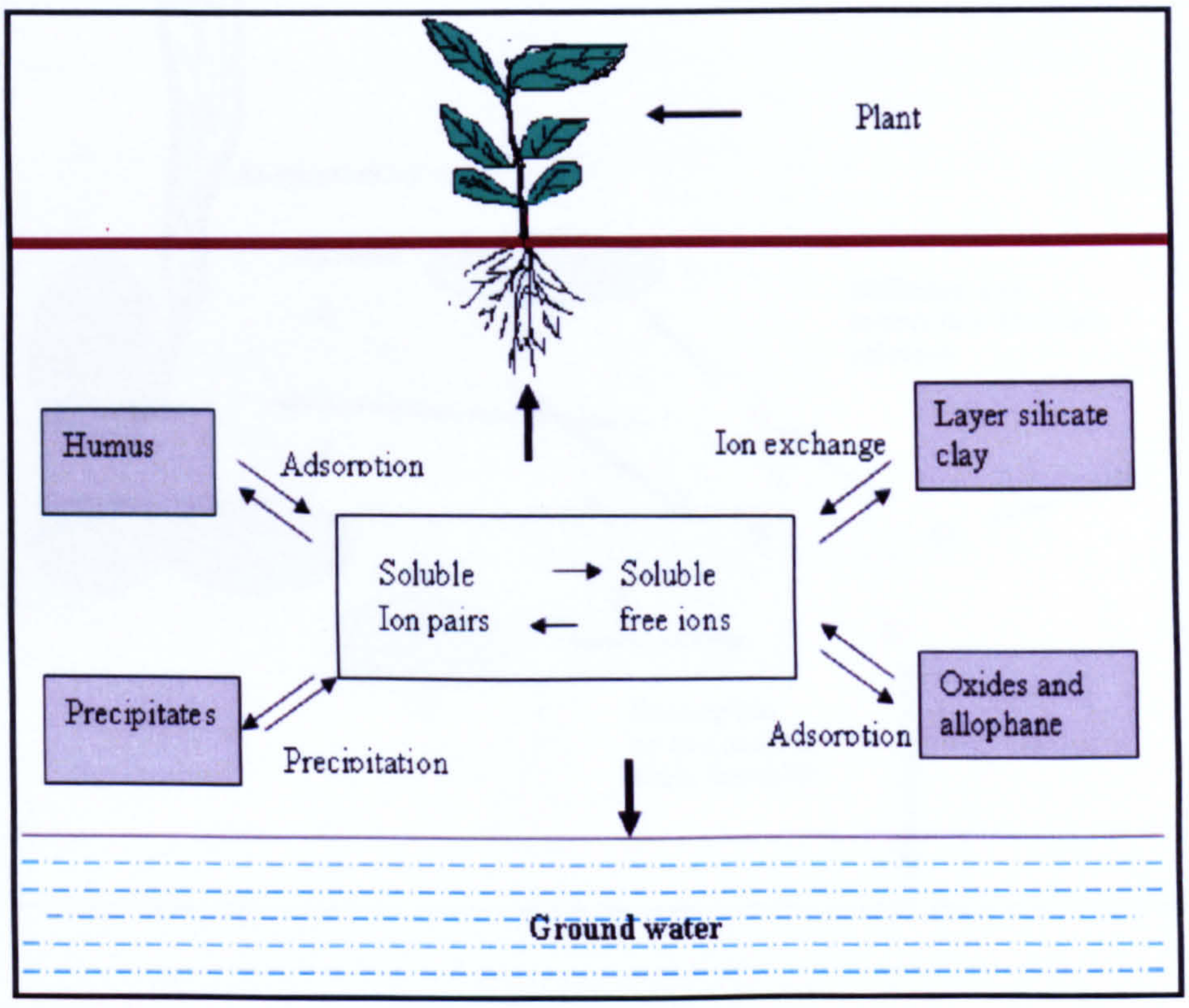

Figure 1.1 Illustrates interaction processes governing solubility, mobility and availability of metals in soils (modified from Environmental Chemistry of soil. McBride, 1994)

The heavy metals may be present in high concentration as a total amount, but it is the availability and lability of those metals that is more important to determinig their toxicity. By using the different extractants we can determine and estimate the potential toxicity, deficiency, or sufficiency to plants and animals to some extent. The extractability of the elements can be limited or controlled by these processes shown in figure 1.1 (McBride, 1994)

1.2.1 Availability of heavy metals

The movement of metals from the soil (solid phase) to the plant tops follow five steps as shown in figure 1.2.

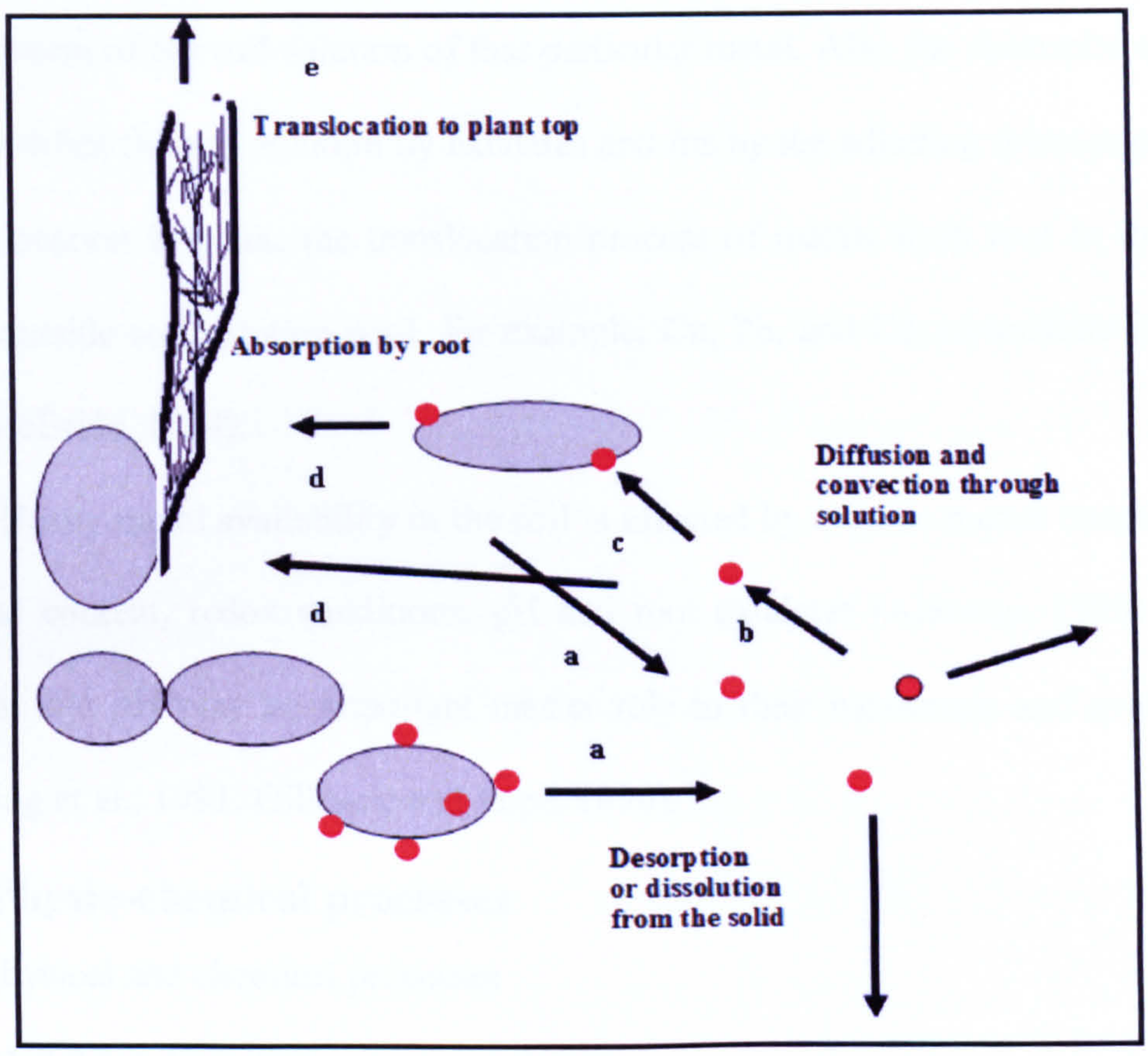

Figure 1.2 Illustrates the five steps for metal to move from soil metal to plants (Modified from environmental chemistry of soils. McBride 1994)

a) adsorption or dissolution, which depends on the solubility of the elements and the ease of desorption, b) diffusion and convection, the transfer by diffusion is very slow due to low concentrations of elements in the soil solution, which is very common. Convection is very important for non trace elements, such as Ca^{2+}, which is usually in high concentration in the solution. Evapotranspiration drives the transfer of water from the soil to the plants through the roots. c) Sorption or precipitation, after diffusion and convection, probably readsorbed or precipitated before reaching to the roots by other materials or compounds such as humus and clays, which can greatly

limit the movement of certain elements. On other hand, some elements such as Cd^{2+}, move rapidly through the soil matrix because it tends to adsorb in exchangeable form. d) Absorption by roots may be passive or active absorption, depending on the concentration of the soil solution of that particular metal. Also the rhizosphere of the plant modifies the soil solution by exudates and /or by the adhering microorganisms. e) translocation in plant, the translocation process of metals from root to the plant tops is outside soil solution pool, for example, Cu, Pb, and Cd accumulate in or on roots (McBride, 1994).

Heavy metal availability in the soil is affected by organic matter content, clay type and content, redox conditions, pH and root exudates (Alloway, 1990) Redox potential and pH play an important master role in their movement and availability (Conkling et al., 1991; Gillespie and Pope, 1990).

1.2.2 Physio-chemical processes

Is the physical and chemical processes

1.2.2.1 Adsorption

Adsorption occurs when a charged solute species, is attracted to the charged soil surface by electrostatic attraction and/or through the formation of specific bonds. Retention of charged solutes by charged surfaces are grouped into two groups. a) Specific adsorption, which involves chemical bond formation between the ions and the sorption on the soil surface. b) Non specific adsorption (ion exchange) is a process in which the charge on the ions balances the charge on the soil particles through electrostatic attraction (Bolan *et al.*, 1999).

Both soil and soil solution physiochemical characteristics determine the equilibrium of metals between solution and solid phases. The pH affects largely the concentration of metals in soil solution (Adriano, 2001) and the nature of both organic and inorganic anions (Harter and Naidu, 1995). Values of pH > 6 can decrease free

metal ion activities in soils due to increase in pH-dependent surface charge on oxides of Fe, Al and Mn, chelation by organic matter, or precipitation of metal hydroxides.

1.2.2.2 Complexation

Heavy metals can form both inorganic and organic complexes with a range of solutes in soils. The complexation of metals by organic ligands or inorganic salts affects negatively the metal adsorption by the soil and increases absorption by plants if they are complexed with chelates such as EDTA. Boekhold *et al.*(1993) found that formation of inorganic anionic complexes decreases the adsorption of Cd^{2+} by soils. Naidu et al., 1994; Oconnor et al., 1984) indicated that chloride forms a soluble complex with Cd^{2+} as $CdCl^{+}$, thereby lowering the adsorption of Cd^{2+} onto soil particles. On the other hand, Haas and Horowitz (1986) pointed out that Cd^{2+} adsorption by kaolinite was enhanced by the presence of organic matter via the formation of an adsorbed organic layer on the clay surface. This may be attributed to soil constituents that have a high affinity for metal cations because of the presence of ligands or groups that can chelate metals (Harter and Naidu, 1995). At high pH, the carboxyl, phenolic, alcoholic and carbonyl functional groups in soil organic matter dissociate, thereby increasing the affinity of ligand ions for metal cations to form complex compounds. The affinity of ligand to heavy metals to make a complex depends on the type of metal; for example the affinity for metal cations complexed by organic matter is in the following order: $Cu^{2+} > Cd^{2+} > Fe^{2+} > Pb^{2+} > Ni^{2+} > Co^{2+} > Mn^{2+} > Zn^{2+}$ (Adriano, 2001). The clay minerals are generally coated with metal oxides and by organic matter too; these coating substances provide the surface exchange of heavy metals (Davies, 1980).

Heavy metal adsorption by soil is pH dependent, a result of the surface chemistry of solid materials. Soils and sediments have a pH-dependent, or variable,

charge associated with the reaction of protons, oxide and hydroxide minerals, and with certain functional groups of humic substances (Evans, 1989; Sposito, 1984). This dependency is different with different metals, for example Cu and Pb are affected irregularly by pH change, while for Zn correlates regularly, as pH increases, availability of the Zn decreases (Tyler and Olsson, 2001).

1.2.2.3 Precipitation.

Precipitation is the predominant process of metal immobilization in alkaline soils in the presence of anions such as sulphate, carbonate, hydroxide and phosphate. The retention of heavy metals can also be induced by liming due to an increase in the pH, heavy metals precipitate as oxides or hydroxides or carbonates (Adriano, 2001), high pH can also precipitate heavy metals in the presence of sulphates or carbonate.

1.2.3 Toxicity

All the heavy metals are toxic at high concentrations, and any metal (or metalloid) is considered a ‘‘contaminant’’ if it occurs at sufficient concentration to affect the environmental or human health (McIntyre, 2004). The heavy metals that have been studied most extensively in soils are those that are essential for the nutrition of higher plants: Cu, Fe, Mn, Mo and Zn (Marschner, 1995). Some other heavy metals are not essential in organisms and their presence in organisms or plants at levels above the background could affect some of their physiological and morphological functions such as Pb. The toxicity of heavy metals depends on many factors such as availability and accessibility of the metal in the media soil solution, water and sediments, type of metal. For example, Cu toxicity is higher than Zn toxicity, and species of plant, hyperaccumulater can tolerate high concentration of specific metals and can phytoextract metals several times more than nonhyperaccumulater.

1.2.4 Extraction and total metal determination

As mentioned in section 1.2.2.2, complexation of heavy metals may be with organic carbon compounds (DOC) and/or with inorganic species, such as carbonate, chloride, sulphate, and hydroxide, or with chelating agents such as EDTA and NTA. These complexations are very useful for assessing methods of extraction to approach plant uptake or investigate heavy metal contamination in contaminated sites. Strong complexes or salts may be used for measuring bioavailable and bio-accessible fractions of heavy metals. Methods can be grouped in three categories: (1) methods for assessing metals in pore waters, including assessment of metal speciation and the activity of the free metal ion; (2) single and sequential extractions; (3) rigorous digestion procedures to determine total metal concentrations in soils or sediments. Methods for assessing metals in pore water may provide an estimate of the actually available and accessible fraction, whereas extractions and digestions may provide estimates of potentially total fractions. Also the methods can differ in relevance from metal to metal and from soil to soil. The total metal content in soil or sediment is represented in three forms; it may be in inert content inside clay minerals or sorbed or precipitated as carbonate or iron and aluminium oxide or dissolved in pore water. The metal dissolved in pore water may exist as free ion or organic complex or inorganic complex, as illustrated in figure 1.3 (Peijnenburg and Jager, 2003).

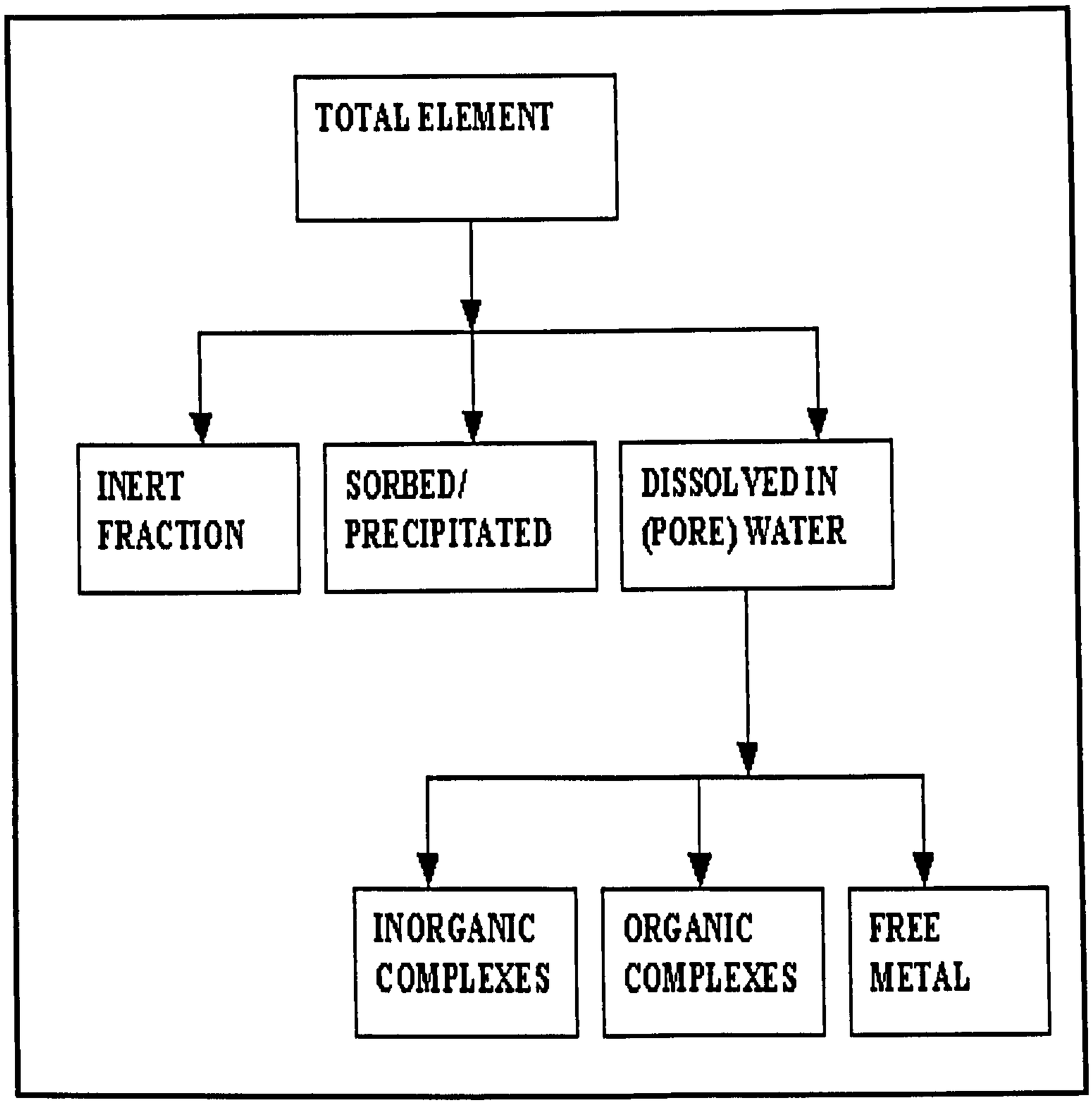

Figure 1.3 Illustrates the various metal fractions present in soil, sediment, and water matrices. Modified from Peijnenburg and Jager. 2003

1.2.5 Zinc in soil.

Zinc is considered one of the most important micronutrients for animals and plants but on the other hand is toxic if it exceeds the sufficient level. The total Zn in the normal soil is 10-300 ppm (Lindsay, 1979; Tisdale, 1985) and the heavy clay contained three times more than sand (Sposito, 1989). Zinc containing minerals in the soil are sulphide (ZnS), carbonate ($ZnCO_3$) (Smithsonite), ZnF_2O_4 (Franklinite), $ZnSO_4$ (Zincosite) and silicate $Zn(OH)_2 Si_2O_7. H_2O$ (Lindsay, 1979). Zn is also present as impurity in other minerals such as Mn and Fe oxides, these minerals have

large surface areas, which make the heavy metals more mobile compared to silicates, consequently play an important role in retaining and supplying trace elements (Alloway, 1990).

The Zn content of the soil solution depends on the original parent materials and the specific mineral which control the Zn solubility and availability. The solubility of these minerals decreases in the order $Zn(OH)_2$ (amorp) > α-$Zn(OH)_2$ > β-$Zn(OH)_2$ γ- $Zn(OH)_2$ ε-Zn(OH)2 > $ZnCO_3$ > ZnO. Both the capacity factor and intensity factor of Zn in the soil are essential to Zn availability in the long run, but the solubility and availability to the plant depends on the readily available zinc concentration in the soil solution (intensity) (Lindsay, 1979).

Adsorption of Zn by the soil inorganic and organic constituents is very important for plant nutrition and movement in the soil profile to the ground water. The adsorption of Zn depends on the soil mineral composition, clay more than sand and for specific clay mineral, vermiculite more than montmorillonite and kaolinite (Agbenin and Olojo, 2004; Daviscarter and Shuman, 1993; Shuman, 1975) and is affected positively by pH (Shuman, 1976; Taylor et al., 1995). The effect of pH at low organic matter is greater than at high organic mater (Jahiruddin et al., 1992) Al and Fe oxides increase Zn adsorption (Dang *et al.*, 1994). Liming redistributed Zn from the exchangeable fraction to less soluble fractions.

The interaction of Zn with organic matter acts in two different ways; organic matter may make the Zn more available to the plant, or binds it strongly and decrease availability to the plant and prevent percolation to the ground water, the binding ability of organic matter (Humus) to heavy metals varies; for example Cu > Ca > Mg > Zn (Zunino and Martin, 1977a; Zunino and Martin, 1977b). Other cations in the soil solution such as Cd, Cu, Mg and Ca effectively compete with Zn for adsorption sites

and therefore affect its mobility (Agbenin and Olojo, 2004; Christensen, 1984; Elzinga et al., 1999; Harter, 1992; Voegelin et al., 2001). Desorption of Zn depends on type of extractant and for example desorption by $CaCl_2$ is less than that by EDTA (Szymura *et al.*, 1993). Also CEC and amorphous oxides play important roles in Zn solubility and availability.

1.2.6 Cu in soil

Copper minerals include malachite ($Cu_2(OH)_2CO_3$) and chalcopyrite ($CuFeS_2$) and it can found naturally in sandstones. It binds with organic matter, in clay minerals and with Fe and Mn oxides (Tisdale, 1985) and is residual from anthropogenic processes such as fertilizer and pesticides and wastes (Adriano, 2001; McBride, 1994). The Cu concentration ranges from 2 – 100 ppm in rural soil (Lindsay, 1979) and the toxic level between 20 to 100 ppm (Fageria et al., 2002).

Adsorption of Cu depends on many factors, such as organic matter, clay content type of clay, Fe and Mn oxides, pH, Ca, and Cu concentration. For example copper adsorption is affected positively by presence of humic acid (Arias et al., 2002). Copper is more adsorbed in a soil which has high clay content than in soil that has less clay content. Type of clay is important; montmorillonite sorbs Cu more than kaolinite. When the Ca concentration in soil solution increases the stability of organic matter mineral complexes increases, and thus the dissolution of organic matter decreases and inhibits the release of Cu-binding organic matter. In contrast high Na concentration in soil solution increases the organic matter dispersion and increases the dissolution of organic matter, consequently releasing more Cu in the soil solution (Zhang and Xia, 2005). The adsorption of Cu by different soils depends on its concentration. At low concentration, less than 100 ppm, Cu adsorbed was 95-99% of applied Cu, while at

high concentration, 100-2000 ppm the adsorption of Cu decreased to 60 to 24% (Alva *et al.*, 2004). At pH 6.2- 7.9 adsorption was high, but decreased from 77 to 34% at pH 9.9. The leaching of Cu with DOC solution at pH 7 increased due to formation of aqueous Cu-DOC complexes (Burton et al., 2005).

1.2.7 Pb in soil

Lead is widely distributed in the world and ranks about 36th in natural abundance among elements in the Earth's crust. The most common Pb minerals are sulphides, (galena), cerussite ($PbCO_3$) and anglesite ($PbSO_4$) Also hydroxypyromorphite [$Pb_5(PO_4)_3OH$] Cloropyromorphite [$Pb_5(PO_4)_3Cl$] (Lindsay, 1979). Also other sources of lead into soil include deposition from the air, fertilizers, herbicides (Huang et al., 2005; Li, 2006; Morschel et al., 2004; Zhang et al., 2006) and the discharge of sewage sludge containing large quantities of lead and other heavy metals onto agricultural and garden soils increases contamination of the environment (Mench et al., 1992).

The typical total concentration is between 2 – 200 mg/kg soil, and some researches recorded that non contaminated soils contain less than 100 ppm Pb^{2+} and less contaminated soils between 82 – 150 mg/kg dry soil (Adriano et al., 1994). Significantly affected soils contain 400–800 mg Pb/ kg soil. Lead contaminated soil has a long history because the Pb is not taken up by plants as much as other elements and has been used by humans for many years for example, in northern Euroupe in medieval times rather than over the industrial development time (Brannvall *et al.*, 1999) and high levels of Pb^{2+} are found in A horizon of soils (Watmough et al., 2004)

Soil solution contains only about 0.005- 0.13% of the total soil Pb^{2+} and is available to the plants (Alloway, 1990). Its availability depends highly on soil constituents such as clay content, organic matter content, soil particle size, CEC, pH.

For example Pb has high affinity to bind with organic matter (Sillanpa and Jansson, 1992)). The relationship between soil pH and plant lead content is less clear (Alloway, 1990; Sillanpa and Jansson, 1992) but many researchers recorded that the availability of Pb^{2+} can be affect by root exudates, root surface area, micro organisms such as mycorrhizae and the rate of transpiration (Alloway, 1990). Addition of lime increases the adsorption and precipitation of Pb, and a competition between Pb^{2+} and other metals (Basta and Tabatabai, 1992; Geebelen et al., 2002; Geebelen et al., 2003). Furthermore the important mechanism governing the Pb^{2+} in the soil solution and bioavailability is the precipitation mechanism (Chrysafopoulou et al., 2005).

1.3 Heavy metals in plants

Heavy metals play an important role in biomolecules such enzymes, chlorophyll, proteins; but in contrast are very toxic if present in excess amount. Some of the heavy metals are necessary to the plant, which can not grow properly or normally without them, and these essential elements are Mo, Zn, B, Cl, Cu, Fe, Mn, Mo, and Zn (Marschner, 1995). Co is essential for some plants such as N-fixing legumes (Fageria et al., 2002). Table 1.1 shows the range of critical, sufficient and toxic elements in mg/kg plants and form absorbed.

Table 1.1 illustrates the insufficient, sufficient and toxic range of the element concentrations in plants mg/kg. From (Fageria et al., 2002)

		Concentration mg/kg plant		
Element	**Form absorbed**	**Insufficient**	**Sufficient**	**Toxic**
B	H_3BO_3; BO_3^-; $B_4O_7^{2-}$	< 10	10-100	50-200
Cl	Cl^-	<2000	2000-20000	> 20000
Cu	Cu^{2+}	3-5	5-20	20-100
Fe	Fe^{2+}; Fe^{3+}	<50	50-250	>1000
Mn	Mn^{2+}	10-20	20-300	300-500
Mo	MoO_4^{2-}	<0.1	0.1-0.5	10-50
Zn	Zn^{2+}	15-20	20-100	100-400
Ni	Ni^{2+}	1-5	1-5	10-100
Co	Co^{2+}	<0.2	0.2-0.5	15-50

Elevated concentrations of heavy metals in the soil surface cause serious environmental problems, including toxicity to flora and fauna. Toxicity of the metal depends on the availability and solubility in the soil solution more than the total concentration in the soil.

The bioavailable fraction of the total contaminant mass in soil and sediment is the proportion actually available to receptor organisms, including human and ecological organisms. Bioavailability refers to the potential for living organisms to take up chemicals from food (i.e., oral) or from the biotic environment (i.e., external) to the extent that the chemicals may become involved in the metabolism of the organism (National Research Council, 2003). To be available, metals have to come in contact with the plant in the presence of water (i.e., physical accessibility) and need to be in a particular form (i.e., chemical accessibility) to be able to enter a plant root. The uptake of metals and distribution in the plant organs are controlled by several factors; species-specific, metal-specific, presence of other metals, additives and amendments. In species- specific, for example the plant selectivity plays an important role in absorption of metals. In trees heavy metals have different mobility, Pb, Cr and Cu are

held in the roots while Cd, Ni and Zn are translocated into the shoots. This selectivity is very important to control of movement of heavy metals (Pulford and Watson, 2003). Some plants have special characteristics to accumulate a high concentration of heavy metals and this phenomenon occurs rarely in terrestrial plants. To date, only about 400 plant species have been identified as natural metal hyperaccumulators, representing less than 0.2% of all angiosperms (Brooks *et al.*, 1998). Threshold values of metal concentrations have been used to define metal hyperaccumulation, including 10, 000 mg /kg dry weight of shoots for Zn and Mn, 1000 mg/ kg for Co, Cu, Ni, As and Se, and 100 mg/kg for Cd. Red beet is characterized by the highest zinc accumulation, and highest Zn concentration ratios (shoots/roots): 2.8, 2.2, 2.0. (Sekara et al., 2005). Pb, Cd, Ni and Co were higher in roots than shoots and accumulation in the vegetable was in the following order potato > cauliflower > cabbage (Chatterjee and Dube, 2005). Heavy metal concentration in cotton decreased in the following order leaves > seeds > roots > stems, while in flax and hemp roots > stems > leaves > seeds. Metal-specific Cd inhibits root growth more strongly than shoot growth and more effectively than zinc ions (Angelova *et al.*, 2004) and Zn uptake by plants is greater than that of Cu. Presence of other metals can affect uptake, for example antagonistic effect of Zn on Cd for root uptake and distribution within the plant ((Jiao et al., 2004). Lime and organic amendments produce high plant biomass and low heavy metal uptakes (Clemente *et al.*, 2004).

1.3.1 Zinc in plants

Zinc is an essential nutrient and it has an important role as a metal component of enzymes or as a functional or regulatory cofactor of several enzymes. Zn deficiency can cause reduction of biomass (Marschner, 1995). Deficiency of Zn can occur in acidic or alkaline sandy to sandy loam soils, and Zn concentration in the plant ranges

from 15-20 mg/kg (deficient) and from 20-100mg/kg (sufficient) 100-400 mg/kg (toxic) (Fageria et al., 2002).

Zinc deficiency is particularly widespread, for example deficiency in wheat, leading to a severe decrease in wheat production and nutritional quality of grains (Cakmak et al., 1999; Cakmak et al., 1996; Graham et al., 1992). As in soils and plants, Zn deficiency is also a common nutritional problem in humans, especially in developing countries where diets depend on cereal-based foods and are poor in animal protein (Prasad, 1984). Foods derived from cereals are not only low in Zn, but also rich in compounds that reduce the bioavailability (utilization) of Zn to humans, such as phytic acid and fibre. Zinc availability to plants ranges from being deficient in some areas such as semiarid soils, which are high in $CaCO_3$ and pH to toxic in polluted and acid soils. The uptake and tolerance of Zn differs from plant to plant and from species to species. For example *Thlaspi caeruecens* has a five times higher Zn concentration than *Thlaspi ochroleucum* (McGrath et al., 1997). The tolerance to Zn toxicity was found to decrease in the following order: *E. camaldulensis* > *A. holosericea* > *M. leucadendra* (Reichman et al., 2001). Zn concentration can vary within a plant; for example root tissue concentrations were higher than shoot tissue concentrations (Reichman et al., 2001). Some plants are more tolerant to Zn, for example 853 ppm in *E. maculata* and 698 ppm *in E. urophylla* (Soares et al., 2001). Sequestration of Zn in the vacuole has long been considered as a cause of plant resistance (Harmens et al., 1994; Verkleij et al., 1998). Other metals like Mg, Ca and K can ameliorate Zn toxicity to (Pedler et al., 2004) and high concentration of Zn reduces Ca and Fe shoot D.M to deficit amount, in some plants such as ecotypes of *H lanatus* (Soares et al., 2001).

1.3.2 Cu in plants

Copper is considered one of the essential micronutrients (Fe, Mn, B, Zn, Cu, Mo and Cl) to plant growth because it is necessary for several enzymes involved in biological reactions. Cu is essential for plants but may be toxic too. The range of essential Cu is 3-5 mg/kg and the sufficient range is 5-20 mg/kg. The toxic range from 20-100 mg/kg plant (Fageria et al., 2002).

The bioavailability of Cu depends on its form in the soil, rather than in the total amount accumulated (Zemberyova *et al.*, 1998). Evaluating metal forms by sequential extraction techniques is considered a good tool for Cu fractionation and to assess bioavailability (Grzebisz et al., 1997; Tessier et al., 1979). Although fractionation is operationally defined, the bioavailability of copper to fauna and flora can often be closely related to the distribution of metal fraction in the soil (Schramel *et al.*, 2000). For example, exchangeable copper, which can be extracted from a soil matrix using salt, is believed to be the most important, if not the only, bio-available fraction for plant root accumulation (Sparks, 1984).

Deficient, sufficient, and toxic copper levels and complexity of the soil-plant relationship may induce changes in the properties of the soil rhizosphere, and consequently its metal speciation (Hamon et al., 1995; Jeffery and Uren, 1983; Levesque and Mathur, 1986). Cu can affect non tolerant plants by damaging plant roots and reducing the root hair proliferation, for example for Rhodes grass (*Chloris gayana Knuth.*) (Sheldon and Menzies, 2005). Also copper tends to accumulate in the root tissue with little translocated to the shoots (Marschner, 1995). The toxicity of Cu to plants is different between species; some species can tolerate high concentrations of Cu, for example *Elsholtzia splendens* can tolerate 80 ppm (Jiang *et al.*, 2004). Cu affect the weight, length, of roots more than shoots (Zheng *et al.*, 2004).

Concentrations of Cu are generally extremely low in soil solution, with more than 98% of Cu in solution bound to soluble organic matter in soils of neutral pH (Sauve *et al.*, 1997) and Cu adsorption is highly pH dependent (Tye *et al.*, 2004). Copper usually accumulated in soil surface due to high affinity for solid phase organic matter, and is therefore not readily leached (McBride *et al.*, 1997).

1.3.3 Pb in the plants

Lead content in agricultural soils is less than 100 mg/kg soil (Kabata-Pendias and Pendias, 1992). The natural and apparently safe concentration of Pb in plants ranges from 0.1 to 10 mg /kg plant (Bohn, 2001) and the allowable concentration of Pb in cereal, including Wheat (*Triticum aestivum L.*) is 0.235 mg/kg DW (European Commission, 2001) Sensitivity to Pb seems to change with age of the plant and soil lead concentration and also is different from species to species, for example (*P. satium*) is more sensitive to soil lead than carrot and radish, which in acid soil tolerate 500 mg Pb /kg soil without yield reduction (Pond, 2005). A study by Chatterjee and Dube (2005) on cauliflower, potato, and cabbage collected from fields receiving sewage sludge, recorded that the accumulation of heavy metals was higher in roots than in leaves and shoots and rate of accumulation was in the following order potato > cauliflower > cabbage.

When amendment for mobilization is added to the soil, such as EDTA, the concentration of Pb in roots, stems and leaves increases (Boonyapookana *et al.*, 2005). In contrast additions of inorganic salts such as K_2HPO_4, $CaCl_2$ and KNO_3 decrease Pb^{+2} absorption and accumulation by mung bean seedlings (Singh, 1994). In Pb-contaminated soil, the symptoms appear on vegetable young leaves as marked chlorosis, brown necrotic spots, later developed on almost the entire foliage of plants,

and aged leaves had a wilted look (Chatterjee and Dube, 2005). The Pb transfer from the soil to crop tissues is generally low. Many researchers found that the bio-concentration factor, i.e. the concentration ratio of Pb in plant tissues to Pb in soil, ranged mostly from 0.001 to 0.5, depending on plant species and environmental factors (Chamberlain, 1983).

1.4 Rhizosphere

The rhizosphere is the soil in contact with the plant roots, and a place of heterogeneity and a source of root exudates. Root exudates create a new chemical (nutrient solubility, pH, O_2, CO_2 and other organic compounds), physical (aeration and moisture) and biological (microorganisms, soil pathogens, and allelopathy) environment and the characteristics are changed or modified to give positive or negative affects (El-Shatnawi and Makhadmeh, 2001).

The rhizosphere is known as the zone of greatest interrelationship between plants and microorganisms and has the highest activity of the soil microbiota (Grayston *et al.*, 1997). It also plays an important role in the bioavailability of nutrients and metals to plants, bacteria and mycorrhizal fungi. The microbial populations are an essential part of the rhizosphere and affect the rhizosphere soil by their various activities, such as water and nutrient uptake, exudation, and biological transformations. Most mineral nutrients are taken up by the plants through the rhizosphere, where micro-organisms interact with plant products in root exudates. The root exudates consist of organic acid anions, amino acids, purines, phytosiderophores, sugars, vitamins, nucleosides, inorganic ions such asHCO_3^-, OH^-, H^+, gases (CO_2, H_2), enzymes and root border cells all of which have key direct or indirect effects on

mineral nutrient bioavailability for plant uptake and growth. Dakora and Phillips (2002) found for example Rrooibos tea (*Aspalathus Linearis L.*) plants can modify their rhizosphere by root exudates, producing OH^- and HCO_3^- to tolerate growth in acid soils. The root exudates are the wide varieties of chemicals compounds secreted into the soil by roots and include sugars, amino acids, lipids, coumarins, flavonoids, proteins, aliphatics and aromatics: these are examples of the primary substances found within the microzone and these chemical compounds can regulate the rhizosphere physically, chemically and biologically (Walker *et al.*, 2003) as well as providing lubrication of the root tip, maintenance of root-soil contact, protection of roots from desiccation, stabilization of soil micro-aggregates, and selective adsorption and storage of ions (Bengough and McKenzie, 1997; Griffin et al., 1976; Hawes et al., 2000). Walker *et al.* (2003) divided the root exudates into two groups Low-M_r compounds such as amino acids, organic acids, sugars, phenolics, and various other secondary metabolites believed to comprise the bulk of root exudates, and high-M_r exudates (polysaccharides and proteins).

Understanding of the biology, biochemistry, and genetic development of roots has considerably improved during the last decade (Benfey and Scheres, 2000; Smith and Fedoroff, 1995). In contrast, the processes mediated by roots in the rhizosphere such as the secretion of root border cells and root exudates are not yet well understood (Hawes *et al.*, 2000). Plant root exudates provide nutrition to rhizosphere microbes, thus increasing microbiological activity in the rhizosphere, which in turn stimulates plant growth (Marschner and Baumann, 2003).

In the study that was conducted by Burkland *et al.* (1995) to determine the concentrations of heavy metals, Zn, Pb, and Cd, in leachate from mine tailings using

batch and column experiments, they pointed out that some organic ligands in the rhizosphere have the capability to increase the solubility of Zn.

An investigation done by Tao *et al.* (2004) on acidification - alkalization and their effects on root-induced Cu fractionation changes within the rhizosphere of plants (legume and non-legume plants, pea, soybean maize and wheat) using contaminated calcareous soil, indicated that the changes were similar among the plant species and the effect of root exudates on Cu fractionation is complexation rather than decrease or increase in pH. They also found that the biological activity by microorganisms increased the exchangeable Cu. Furthermore Tao *et al.* (2003) illustrated that the Cu accumulated in the maize plant is more than the initial quantity of the exchangeable Cu in the soil and attributed that to increase in pH, DOC, redox potential and biological activity which resulted from root induced changes in the rhizosphere. Su, *et al.* (2004) recorded that the pH around the roots increased with distance from the root when the soil was amended with 25% sludge, and decreased with distance from the root when soil was amended with 10% sludge or without any amendment, and they attributed that to the increase in soil NH_4^+ (ammonium) concentration following the application of the 25% sewage sludge to soil.

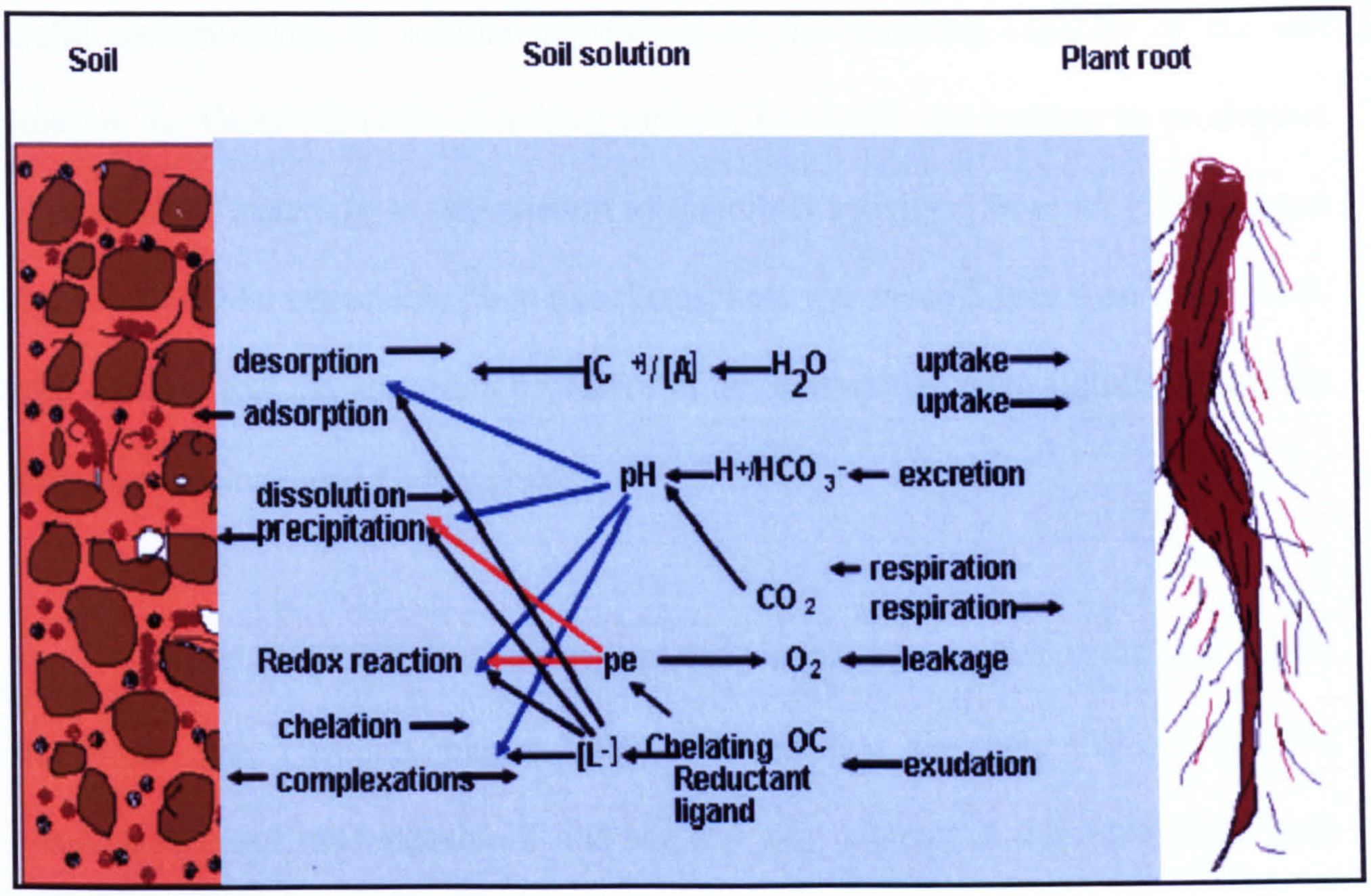

Figure 1.4. Illustrates the soil- soil solution –rhizosphere interface, showing the various processes and how it can interacts each other. OC = organic carbon; C+ = cation; A-= anion; L= ligand; pe = redox potential. Modified from Adriano et al. (2004).

Figure 1.4 illustrates the processes of heavy metals solubility in the soil and rhizosphere and the factors, which affect these processes, such as pH, pe, cations and anions present and organic carbon. The processes in the rhizosphere are excretion, respiration, leakage, exudation, uptake (which dilute the metals in the soil solution) and the chemical reactions in the soil, such as desorption, adsorption, dissolution, precipitation, redox reaction, chelation and complexation.

Organisms and plants can modify the chemistry of the soil and soil solution in the rhizosphere (Marschner, 1995) and among these diverse effects are the following. i- Movement of additional contaminants to the rhizosphere as a result of convective flow of solution to plant roots. ii) Organism- and plant-induced changes in solution chemistry that affect sorption, such as changes of pH, ionic strength, and macronutrient cation concentrations. iii- Excretion of organic ligands affecting total

metal concentrations in solution depending on the buffering capacity of the soil solution. iv- Generation of new sorbing surfaces for metals (for instance by production of dead plant material); v- Stimulation of microbial activity. Lin *et al.* (2004) found that the NH_4OAc extractable Pb in rice rhizosphere was much higher than in bulk soil, which meant that the activation processes in the rhizosphere were significant and the amount of bioavailable Pb increased.

Chiu *et al.* (2002) studied physical and chemical properties of the rhizosphere in Tsuga and Yushania plants They observed that the pH, CEC, OC, C/N, concentrations of exchangeable K and Mg and clay contents in the rhizosphere were more than in the bulk soil; Cu and Zn bioavailability depends on the pH and root exudates of the rhizosphere.

1.5 Phytoremediation

All conventional methods of physical or chemical remediation are expensive, labour intensive, and induce changes in the physical, biological and chemical properties of the soil. These methods of remediation of contaminated soils are mainly applicable to relatively small areas, not for large sites such as industrial and agro-chemically contaminated soils. Phytoremediation is the alternative method using the free solar energy pump for pollutants and contaminants.

Phytoremediation refers to the use of green plants, soil amendments and agronomic techniques to remove contain or reduce the pollutants harm (Salt et al., 1998; Cunningham and Ow, 1996; Lombi et al., 2001).

Phytoremediation of contaminated soils to meet the strategy goals should have at least one of these advantages, high plant biomass production or high containment and plant adaptive capacity to variable environments. However, to succeed they must be tolerant to most contaminants and be capable of accumulating significant concentrations of phytotoxic chemicals in their tissues. Crops having high biomass production, but not high pollutant concentration could be used over a long time period for decontamination, if the concentration of pollutants in biomass is below critical level for livestock consumption (Murillo *et al.*, 1999). Crops have an economic value to remove, contain or render harmful environmental pollutants, constitute a cheap remediation method and are environmentally non-destructive (Lasat, 2002). They provide an innovative technique to recover degraded land, remediate contaminated soils and facilitate improvement of soil structure (Brooks et al., 1998; Wenzel et al., 2003).

Plants such as hyper-accumulator plants that have adaptive mechanisms for tolerating or accumulating high metal contents in their rhizosphere can be employed in clean up of soils, sediments and water (Chen et al., 2004; Khan et al., 1998). Phytoremediation can be categorized under five major processes or groups, depending on the metal fate: (a) Phytoextraction is the removal and concentration of metals into harvestable plant parts. (b) Phytodegradation is the degradation of contaminants by plants and their associated microbes. (c) Rhizofiltration is the absorption of metals by plant roots from contaminated waters. (d) Phytostabilization is the immobilization and reduction in the mobility and bioavailability of contaminants by plant roots and their associated microbes. (e) Phytovolatilization is the volatilization of contaminants by plants from the soil into the atmosphere (Salt et al., 1998). It's a relatively slow process, and may take some years to reduce metal contents in soil to a safe and

acceptable level due to small size and slow growth of most identified metal hyperaccumulator plants (Linger et al., 2002). It must be considered as a long-term strategy (Cunningham *et al.*, 1995).

1.5.1 Phytoextraction

The term ``Phytoextraction" mainly concerns the removal of heavy metals or radionuclides from soil by means of the uptake capabilities of plants.

The phytoextraction success depends on plant yield and high metal concentrations in plant shoots (Solhi *et al.*, 2005). Phytoextraction of heavy metals and radionuclides represents one of the largest economic opportunities for phytoremediation because of the size and scope of environmental problems associated with metal-contaminated soils. Recently many researchers have considered two strategies for phytoextraction: one depends on tolerant plants such as hyperaccumalators, which accumulate high metals in their biomass, while the other strategy uses other crops which have high biomass production and low concentration of heavy metals. The lack of elements such as Zn, mentioned above in section 1.3.1 can cause a serious problem in crop production and consequently to livestock (Gupta *et al.*, 2001). Using crops for phytoremediation and by enhancing phytoextraction for these crops may satisfy the needs of humans and livestock if toxic thresholds are not exceeded.

1.5.2 Enhancing phytoremediation

Plant uptake of metals is frequently restricted by limitations of contaminant bioavailability, for example vegetation growing on heavily lead-contaminated soil or solutions has been reported to contain only 0.01 to 0.06% of shoot dry biomass as lead (Huang *et al.*, 1997), levels well below that required for efficient phytoextraction of

Pb. In order to enhance metal uptake, soil amendments with metal chelating agents such as EDTA, HEDTA, DTPA, EGTA, NTA, citrate and hydroxylamine to make metals soluble, bioavailable and absorbed by plant roots have shown promise (Blaylock et al., 1997; Lesage et al., 2005; Li, 2006). The type of chelate and its time of application are important considerations. It has also been suggested that if the plant biomass can be increased, then metal phytoextraction can be increased to more than that which the plant can take up normally (Ebbs and Kochian, 1997; Shtangeeva et al., 2004). Manipulation of the rhizosphere by NH_4^+/NO_3^-, use of plant growth regulators (PGR) such as auxins and cytokinins have shown promise to enhance phytoremediation abilities of non-hyper-accumulating plants by increasing their growth and biomass (Fuentes, 2000) and has become a topical research field in the last decade, as it is safe and potentially cheap compared to traditional remediation techniques (Glick, 2003; Lasat, 2002; Pulford and Watson, 2003; Salt et al., 1998).

1.6 Amendments

An amendment is a physical, chemical, natural or synthesized compound, which improves the physio-chemical properties of the soil against unwanted event or events, such as contamination, wind erosion or as a key for solving environmental problems of soil, sediments, water and air. Soil amendments such as fertilizer, manure, sewage sludge, or lime are used to help stabilize the area and promote plant growth. The effect of vegetation on the movement of heavy metals from contaminated soils is not fully understood. On the other hand to add the amendment to enhance phytoextraction of heavy metals (mobilization), or stabilize to prevent leaching of heavy metals to the ground water and allow plant growth in polluted sites

(immobilization), or to manipulate the rhizosphere rather than bulk soil bioavailability (manipulation) of the heavy metals is a goal and strategy for phytoremediation.

1.6.1 Mobilization

Mobilization in situ chemically enhances soil flushing by extracting solutions such as organic and inorganic acids, and complexation agents are the technologies that have been used for remediation (Grcman et al., 2001; Vulava and Seaman, 2000). For example, EDTA enhanced the phytoextraction of Pb and Zn by (*Viola baoshanensis, Vertiveria zizanioides*) more than salts $(NH_4)_2SO_4$ and NH_4NO_3 (Zhuang *et al.*, 2005). EDTA can affect the plant at high concentration (Meers et al., 2005a) and enhance the mobility of soil Cu and Pb, but not Zn and Cd (Wu et al., 2003; Wu et al., 2004). Neagoe *et al.* (2005) used *Lupinus angustifolius L. and Secale cereale L.* and recorded increase in biomass by the addition of the amendments, in the following order compost > topsoil > urea, and they attributed that to improvement of soil chemicals and physical properties. The mobilization of amendments depends on the type of the amendment and plant variety or species; for example, Meers et al. (2005b) found that the amendments EDTA or DTPA does not affect the phytoextraction by canola plants, but rather increases the liability of heavy metals to leach to ground water. Leachate concentrations persisted for more than 1 year after harvest so the application of chelate-assisted phytoextraction is limited by the risk of groundwater pollution (Wenzel *et al.*, 2003).

Solhi *et al.* (2005) investigated the effect of three amendments (manure, sulphuric acid and DTPA) on two crops, sunflower (*Helianthus annuus*) and canola (*Brassica napus*). They indicated that the manure gave higher biomass production and the sunflower had a higher extracting potential for Pb and Zn removal from polluted soil.

1.6.2 Immobilization

Addition of amendment or amendments to decrease toxicity of heavy metals and prevent their mobility to ground water is one strategy of phytoremediation. Many researchers investigated effects of different amendments on different polluted sites, for example Wasner *et al.* (2001) used lime at different rates (0, 75, 150 and 300 t/ha) and found a decrease in Mn and Fe availability, Zn and Cu unchanged, improvement of hydraulic conductivity, permeability and good root development. Triple phosphate and rock phosphate are viable for reducing availability of Pb and Zn (Ownby *et al.*, 2005). Iron oxide reduces availability of Zn, Ni and Cr (Chamon et al., 2005). The addition of amendments lime, zeolite, hydroxyapatite and iron oxide to different soils with different concentrations of Zn (0, 150, 300, 600, 1200 and 2400 mg/kg soil) showed that the amendments enhance the growth of the plants and reduce Zn toxicity (Chlopecka and Adriano, 1996). Using zeolitic material synthesized from coal fly ash for the immobilization of heavy metals (Zn, Pb, As, Cu, Sb, Co, Tl and Cd) in contaminated soils decreased the Cu, Zn, Ni, Cd and Co due to sorption of these elements by clay minerals and decreased the acidity by the presence the lime and residual NaOH (Querol *et al.*, 2006).

Heavy metals were removed from strongly metal-polluted sewage sludge by using NaOH and Na_2S or a mixture of them. The results showed that when iron and aluminium are present in the leachate, adsorption and/or co-precipitation of Pb, and Zn with $FeOH_3$ and $AlOH_3$ might occur at increasing pH conditions and the best removal efficiencies by the mixture obtained were: Pb (100%), Cu (99.7%), and Zn (99.9%) (Marchioretto *et al.*, 2005).

1.6.3 Manipulation of the rhizosphere

Manipulation of the availability of heavy metals in the rhizosphere may be enhanced by different nutrition such as nitrogen in different forms, mainly by the altering the pH in the rhizosphere. Many researchers used different plants with different sources of N-nutrition. For example strawberry plants were grown in sandy mineral soil with three different nitrogen forms $(NH_4)_2$ SO_4, $Ca(NO_3)_2$ or NH_4NO_3 to study the root induced pH and growth response. The results showed that the lowest pH value was recorded in the rhizosphere with fertilizer $(NH_4)_2SO_4$ (Sas *et al.*, 2003). As to the effect of NH_4^+ or NO_3^- as nitrogen source on the rhizosphere of ryegrass growing in two soils luvisol soil (P mainly bound with Ca) and an oxisol (where P it bound to Fe and Al), the result indicated that the NH_4^+ nutrition decrease the pH of the rhizosphere by 1.6 units and NO_3^-- N increased the pH of the rhizosphere by 0.6 units and these changes in the pH extended to 1 to 4 mm from the root surface (Gahoonia *et al.*, 1992). In solution culture pH can be controlled and manipulating by NH_4^+: NO_3^- ratio. Increasing the NH_4^+ as a source of N decreased the solution pH due to H^+ release by roots and NH_4^+ uptake (Marschner, 1995). A study conducted by Mahmood *et al.* (2005) also found that bacterial population density in the rhizosphere soil was higher under NH_4^+ than NO_3^- supplied at 100 mg/kg soil. Study by Zhang *et al.* (2004) to quantify the effects of applied N fertilizers (NH_4^+or NO_3^-) at different concentrations (0, 100 and 300 mg N/kg soil) on P uptake by winter wheat (*Triticum aestivum L.*) and on the change of soil pH in the root zone related to reductions of inorganic P fractions in the rhizosphere soil. They recorded that NH_4^+-N fertilizer resulted in a greater biomass than NO_3^--N nutrition and the soil pH around the roots decreased by 0.30 and 0.65 units, respectively. A study by Braun *et al.* (2001) on peach rhizosphere solution chemistry as influenced by addition of NH_4^+ found higher concentrations of H^+ and

Al^{3+} in the rhizosphere than in the bulk soil. Another investigation with two grass species and different concentrations of NH_4^+ supply and two different sources of P as K_2HPO_4 and rock P showed in both grasses H^+ increase with NH_4^+ increase, but for K_2HPO_4 more H^+ due to high uptake of NH_4^+ (Logan *et al.*, 2000). In a study by Brix *et al.* (2002) on the effect of root-zone acidity and nitrogen source NH_4^+ or NO_3^- on *Typha latifolia L.* growth and uptake of NH_4^+ and NO_3^- at different pHs (3.5, 5.0, 6.5 or 7) they illustrated that growth completely stopped at pH 3.5 and high uptake with NH_4^+ at pH 6.5 and with NO_3^- at pH 5 (Brix *et al.*, 2002). The H^+ releases as the result of the cations–anions uptake balance may not only be related to rhizosphere acidification but also due to root respiration in an alkaline medium (Hinsinger, 1998). Tang *et al.* (1999) illustrated that increased addition of NO_3^-, in a pot experiment with legume species, resulted in the decline of H^+ release from plant roots. Tang *et al.*, (2000) found that NO_3^- treated surface soil under clover or lupin was less significantly acidified than non-treated soil under the same vegetation. It was assumed that the observed effect was due to NO_3^- uptake within the soil surface 5 cm and a lower excess cation uptake and consequently less H^+ excretion in NO_3^- treated soils.

1.6.4 Bone meal

Bone meal is the bone of animals after grinding by a mill and is used for different purposes such as bone charcoal, which used for colour purification and absorption of heavy metals due to its high phosphate content. Cotter-Howells (1996) found that the addition of phosphate as amendment to contaminated soil increased the immobilization of heavy metals by formation of Pb – Zn phosphate. In studies by Cotter-Howells and Caporn, (1996), phosphorus amendments were used to immobilise zinc and Pb in contaminated soil. They found the potassium dihydrogen phosphate (K_2HPO_4) was more effective than other phosphorus amendments but it was more

leachable. However, bone meal is a more soluble form of phosphate than rock apatite with the potential to reduce metal solubility and it can provide a suitable, low cost, natural phosphate source for the immobilization of some toxic metals in contaminated soils. Bone meal can immobilise the heavy metals in the contaminated soils due to following reactions. 1- Formation of metal phosphates 2- Precipitation of other metal compounds in response to pH increases 3- Adsorption of metals onto the bone meal surface (Hodson *et al.*, 2000).

1.6.5 Cement

Cement was used 2000 years ago in Roman and ancient Greek times for cementing the materials in buildings and other purposes such as water reservoirs under ground. The slow reaction of lime and volcanic ash in the presence of the proper amount of water formed a hard cementing material. Portland cement is composed of the essential compounds lime (CaO), silica (SiO_2) and alumina (Al_2O_3). When the cement is mixed with water, the dicalcium silicates and the tricalcium silicates react with water molecules to form calcium silicate hydrate ($3CaO \times 2SiO_2 \times 3H_2O$) and calcium hydroxide $Ca(OH)_2$ producing high pH.

Cement can be used to achieve immobilization and degradation of contaminants simultaneously and the amendment might be dependent on the source of the cement and/or the compounds tested (Hwang *et al.*, 2005). In a study to improve surface soil structure and to prevent the crust formation which affects the seedling emergence of wheat two amendments were used in a pot experiment, Portland Cement and barnyard manure with a rate of 0, 2, 4 and 6% wt/wt of the soil samples. The result showed that seedling emergence of wheat (with the rate 6% wt/wt) was higher after the Portland cement treatment (Seker, 2003).

1.7 Aims

In phytoremediation generally three components control the toxicity of each polluted site, which is illustrated in figure 1.5 This diagram shows three circles, each circle representing one of the components, available elements in soil solution, total elements in the soil and the plant species.

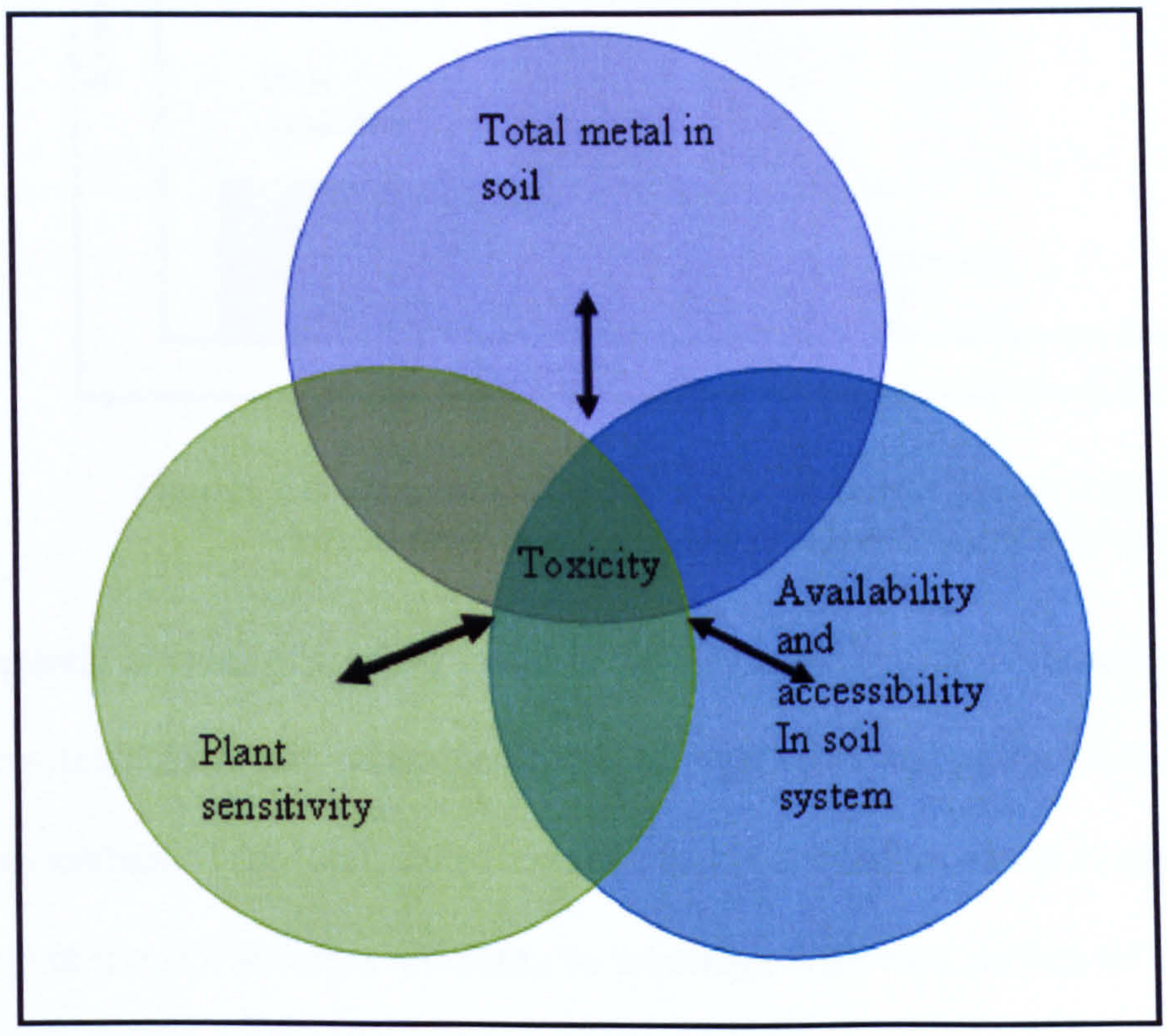

Figure 1.5 Diagram shows the relation between total, soluble metals and plant sensitivity in polluted soil.

The interaction of three of them is the triangle of the toxicity. Altering one of them or both of them is the key of solution and strategy to reduce and ameliorate polluted soil. For example if the sensitivity of the plant to the toxic heavy metals decreases, the triangle of the toxicity decreases, too.

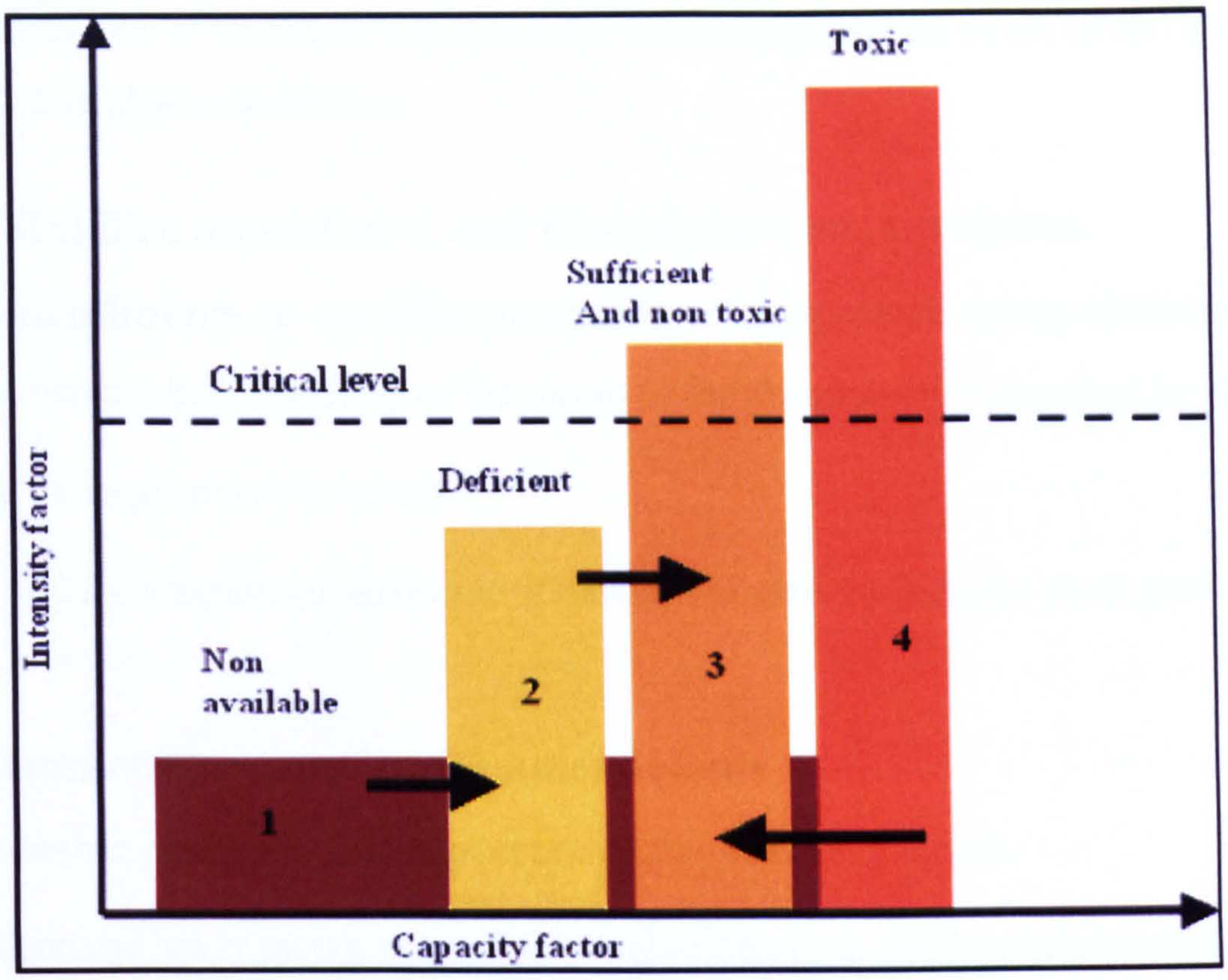

Figure 1.6 Diagram illustrates the intensity factor capacity factor and critical level of availability and solubility of elements

The total amount of a heavy metal in the soil is the source of the available element in the soil solution, and consequently to the plant roots and to the biomass of the plants. This amount of the total, called capacity factor or quantity factor is not readily soluble. Ions in the soil solution are ready to exchange with root surface of the plant, readily soluble or available (intensity factor). As the capacity factor increases the intensity factor increases due to environmental factors, management, time and human activity. The Figure 1.6 shows the relation between the capacity factor and intensity factor and the sufficient, the critical and the toxic level for the plant. Manipulation (immobilization and mobilization) can be the main key of amelioration and detoxification by phytoremediation. As mentioned above in the amendment review there are also amendments to mobilize the element or elements and others to mobilise the elements with some risk for ground water pollution and depressing plant growth.

The overall aim of the thesis was to test the folloing application of the model shown in figure 1.5 to phytoremediation.

1.7.1 Mobilize amendment and Rhizosphere manipulation

Use amendments to mobilise metals by rhizosphere manpulation

a- to increase bioavailability of the metals in the rhizosphere of the plant by altering the rhizosphere environment.

b- to add the amendment as source of nitrogen to assess affects the plant growth.

1.7.2 Immobilize metals with amendments

a- to enhance plant growth in high concentrations of heavy metals.

b- to decrease heavy metals accessibility and solubility in highly contaminated soil

Chapter 2

Material and methods

2.1 Cleaning of glassware and other items

All items, glassware (volumetric flasks, conical flasks etc....) were washed thoroughly with tap water and soaked overnight in 10% Decon 90 in deionised water. All items and glassware were taken, washed several times with tap water and three to four times with deionised water. Items and glassware were dried in a drying cabinet at 40 °C. All clean items and glassware were stored in new plastic bags and the plastic bags were closed to prevent contamination with the dust or other contaminants in the lab.

2.1.1 Tap water

Tap water is water used for first washing and rinsing any plastic or glassware, and also used for rinsing and washing roots (first washing).

2.1.2 Deionised water

Deionised water prepared by purifying the tap water with anion and cation ion exchange resin (pure lab deioniser ELGA) and the conductivity less than 0.5MΩ. Deionised water was used for irrigation of pot experiments, washing the roots several times after tap water, glassware and preparation of plant nutrient solutions.

2.2 Collection of samples

The samples from soil surface (0-20 cm) were collected from an arable field on a farm near Glasgow, Scotland, (UK grid reference NS 500657 and NS 510652). These soils were uncontaminated other than by inputs of heavy metals due to diffuse pollution resulting from its proximity to industrial areas and roads. Some properties of the soils are shown in table 2.1

2.3 Soil preparation.

About 50 kg of the fresh sample was divided into four portions and each portion spread on a plastic sheet; large stones, plants, plant roots and large impurities were removed. The samples were sieved with 4-mm stainless steel sieve then transferred to plastic bags, each one about 8-7 kg, and then stored in a cold room at 4 °C.

2.4 Characterization of soil.

2.4.1 Some physical and chemical properties

Table 2.1 shows some physical and chemical properties of the two soils.

Soil reference	Total metal mg/kg			EDTA extractable metal mg/kg			Clay%	O.M%	pH 1:2.5 H_2O
	Cu	Zn	Pb	Cu	Zn	Pb			
510652*	57	131	104	17.1	18.0	47.0	15.0	8.0	5.5
500657Ø	51	127	76.2	17.1	11.0	23.0	15.0	8.0	5.5

*soil used for flax experiment

Ø soil used for ryegrass experiment

2.4.2 Lime requirement methodology

Approximately 60 g of calcium ethanoate was dried in an oven at 105 °C for one hour, and cooled in a desiccator. Exactly 40 g of calcium ethanoate was weighed into a 1.5l beaker and about 900 ml of deionised water were added. Exactly 0.6 g MgO and 8 g 4-nitrophenol was added too. The solution was warmed in a hot plate, transferred to a 1000 ml volumetric flask the pH was adjusted by conc. HCl or MgO to 7 ± 0.1

10 g of 2-mm air dry soil, 3 replicates, were weighed into glass bottles and 25-ml of deionised water was added to each replicate. The pH was measured and recorded. 20 ml of the buffer solution were added to each replicate and shaken for 5 minutes then the pH was measured. 20 ml of buffer solution was added to the 25 ml of deionised water, and the pH was measured (Rowell, 1994).

2.4.3 Field capacity determination experiment

Two 4" flowerpots were filled with 500 g of the soil and weighed and, then each pot was put on a beaker to receive the drainage water from the pots. 200 ml of water was added to each pot; after 48 hr the volume of leachate water was measured and by the difference between the added water, and the leached water the water content at field capacity was calculated.

2.4.4 Loss on ignition (LOI %)

Six crucibles were cleaned well as in procedure (2.2.1). All crucibles were dried in oven over night at 105°C, cooled in a desiccator then each one weighed empty by 4 figure digital balance (AB 204-5 Mettler Toledo). 3-4 g of each soil accurately weighed, (three replicates) and heated overnight in an oven at 105 °C, cooled in a desiccator, weighed and heated for 6 hrs in a muffle furnace (Gallenkamp size 3) at 550 °C. All crucibles were cooled in a desiccator, weighed with the same balance and O.M was calculated as shown in table 2.2.

Table 2.2 Shows the % LOI in the experimental soils.

Soil	LOI%				
	R1	R2	R3	Average	Stdv
1	7.35	6.80	7.04	7.1	0.27
2	8.66	8.61	8.62	8.6	0.03

2.4.5 pH

Soil pH determined in soil water solution or in agar or in chemical solution with a combination glass electrode and pH meter (Mettler Delta 320) using buffer solutions of pH 4 and 7.

2.5 Germination test for pot experiment

Six clean glass Petri dishes were prepared. Three filter papers were laid on the bottom of each Petri dish. 20 seeds of flax (*Elise)* variety distributed in each Petri-dish, 3 replicates (1, 2, and 3) and 20 seeds of flax (*Viola)* variety in the Petri dishes (4, 5, and 6). Ten ml of tap water was dripped in each Petri-dish. After three days germinated seeds were counted for each Petri dish and results were recorded as shown in table 2.3.

Table 2.3 The germination percent of in two flax varieties Elise and Viola.

Variety	Germination%
Elise	92
Viola	95

2.6 Pot experiments

Soil was collected from the surface (0-20 cm) of an arable field on a farm near Glasgow, Scotland (UK grid reference NS 500657 and 510652). These soils were uncontaminated other than by inputs of heavy metals due to diffuse pollution resulting from its proximity to industrial areas and roads. Properties of the soils are shown in section 2.4.1 table 2.1. The soils were sieved in the fresh state through a 4 mm stainless steel sieve, emptied on to a large plastic sheet and mixed thoroughly. 750 g of fresh soil was put into each pot (15 cm diameter). 2.1 g of calcium carbonate was mixed thoroughly with the soil in half of the pots in each experiment (depends on the lime requirement method section 2.4.2) of the pots to increase the pH by one unit. All pots were packed to the same level to achieve the same bulk density in each. 0.5 g of perennial ryegrass (*Lolium perenne*) or 15 seeds of two flax varieties (*Elise* and *Viola*) seed was sown in the half of the pots and the rest were left as bare soil controls. All pots were placed in individual saucers, and irrigated with deionised water to field capacity by difference in weight. Thereafter, water was supplied via the saucers throughout the experimental period, except in the late stage when salts appeared on the soil surface then water was added to the soil surface.

2.7 Plant preparation for digestion

2.7.1 Above ground biomass

The plant shoots were cut about 2 cm above the surface of the soil and the fresh weight was measured. The plants were washed with deionised water to remove any soil particles adhering to the shoots. The shoots were dried with tissue paper. Each plant sample from each pot was put in a paper envelope and numbered with pot number; all samples were dried at 75°C for 72 h. Dry weights were measured with 4 figure digital balance (AB 204-5Mettler Toledo) and samples were stored for analysis.

2.7.2 Below ground biomass

The roots of each pot were gently taken from the soil, with soil material adhering to the roots and washed in a 500 ml beaker with deionised water. The roots were transferred to the stream of tap water and cleaned thoroughly. Each sample was then washed by deionised water several times, then put in a 500 ml beaker with about 250 ml of deionised water and transferred to the ultra-sonic bath (Sonicor) for about 10 minutes. If any turbidity was seen in the water the roots were cleaned and the same procedure was repeated; if not, excess water was removed and the samples put in a paper envelope and dried at 75°C for 72 hr. Dry weight was taken with 4 figure digital balance (AB 204-5Mettler Toledo).

The beakers with wet soils were transferred to a water bath (Gallenkamp England) to dry. Once all the water had evaporated, the soil was put on plastic polyethylene sheet and allowed to air dry, soil crushed and mixed, sieved with a stainless steel 2 mm sieve and stored for analysis. This sample is referred to as around rhizosphere soil. The rest of the soil in each pot was put on a polyethylene sheet, left to air dry, sieved with 2 mm stainless steel and the samples stored for analysis (bulk soil).

2.7.3 Grinding the plant material

All the plant samples shoots and roots were ground separately with a grinding mill machine (Glen Creston Ltd England) with sieve 1.5 mm diameter and after each sample all parts of the grinding machine were cleaned with a vacuum cleaning machine and compressed air from a compressor. All samples were put in plastic bags separately, ready for digestion and AAS analysis.

2.8 Aqua Regia Digestion of soil

Aqua Regia solution contains three parts 6 molar HCl to one part 69% HNO_3. About 50 g of sieved soil was ground and 1 g sample weight with 4 figure digital balance numbers in duplicate was weighed and emptied in digestion tubes. Each set of 40 had two blanks and two samples from the soil reference material (certified reference material LGC6135). Ten ml of aqua regia solution was added to each soil sample. All digestion tubes were allowed to stand overnight for about 16 h, to allow the acid to equilibrate with the soil. The digestion tubes were placed in the digestion block at a temperature of 125°C for about 3 to 4 h, until the tubes were clear of brown gas. The tubes were allowed to cool and 10 ml of deionised water added to each tube, then the digests were filtered using Whatman type 50 hardened filter papers into 50 ml volumetric flasks and made to volume. Samples were stored in polyethylene bottles at 4 °C for analysis.

2.9 AAS analysis

Table 2.4 Shows some characteristics used for metal analysis

Character	Elements		
	Cu	Zn	Pb
Lamp serial No.	B76922	B62269	B15424
Lamp current (mA)	10	10	15
Wave length (nm)	321.8	213.8	283.3
Background correction	no	yes	no
Energy	74	68	74
Fuel flow (l/min)	2.5	2.5	2.5
Air flow (l/min)	8	8	8
Top standard mg/l	3	5	20

2.10 Nitric acid digestion.

All the plant samples were dried in the oven at 75 °C and ground with a grinding machine with 1.5 mm a sieve (section 2.7.3). After the sample was mixed thoroughly, about 0.2 g was weighed and put in the bottom of a digestion tube. Each batch consisted of 36 samples, 2 blanks and 2 reference plant materials (tomato leaves 1573a). Ten ml conc. nitric acid was added to each tube, all tubes were allowed to stand overnight, and then heated in a digestion block with the temperature adjusted at 125 °C for 3 h until the solution was clear. Samples were transferred and washed into 50 ml volumetric flasks, transferred to glass vials and stored for analysis.

2.11 0.05 M NH_4 EDTA extractions

Exactly 73 g of EDTA free acid was put in a 5 litre conical flask and placed on a magnetic stirrer. Deionised water was added to approximately ¾ of the conical flask and about 40 ml NH_3 solution (36%) were added gradually to the flask. The pH was adjusted and measured to pH 7 with NH_3 solution (36%) and a pH meter electrode dipped on the solution. The solution was transferred to the 5 l volumetric flask, deionised water added to complete the volume of the flask. 50 ml were added to each 5 g soil sample into capped glass bottle No.4. All the sets of bottles were prepared in three replicates along with two blanks and two reference materials were transferred and placed on end over end shaker 30 (revolution/min) rpm for two h. Solutions were filtered with (50 Whatman) filter into glass vials for Zn, Cu, and Pb determination by AAS.

2.12 Reference material

Reference materials of any material such as soil, spoil and plants are analysed by reference laboratory or laboratories and these materials are used to assess the accuracy of all analyses The analyses of the spoil, soil and plant reference material with the same methodology are used for analysis of soil or plant in the thesis and both are illustrated in table 2.5 and 2.6.

Table 2.5 illustrates the total-aqua-regia and EDTA-extractable heavy metals, Zn, Cu and Pb content in mg/kg A) measured in standard reference soil LGC 6135 B) certified reference value.

Type of digestion or extractants	Heavy metals mg/kg reference soil Zn	Cu	Pb
A			
Aqu-regia	291	109	402
Standard error	9.5	2	6
B			
Aqu-regia	345	107	411
Uncertainty	49	5	26
A			
EDTA	268	90	242
Standard error	14.5	6	13
B			
EDTA	316	105	391
Uncertainty	41	5	16

Table 2.6 illustrate the heavy metals content in mg/kg of certified reference value for tomato plant (1573a) and along thesis sample.

Type of digestion Nitric acid	**Heavy metals mg/kg reference plant Zn**	**Cu**	**Pb**
Along thesis samples	31	5	----
standard error	0.85	0.25	----
Certified reference value	30.9	4.7	----
standard error	0.7	0.14	----

2.13 Agar-plant Experiment

2.13.1 Glass rhizobox design

To reveal the roots and the rhizosphere, a glass box for agar and quarter Hoagland solution was developed. This box consisted of two transparent glass sheets 25x25 cm sandwiched over a U-shaped glass rod 7 mm thick and sealed with silicone

sealant and the box fitted with plastic bags avoiding contact of agar media with silica gel (Figure 2.1).

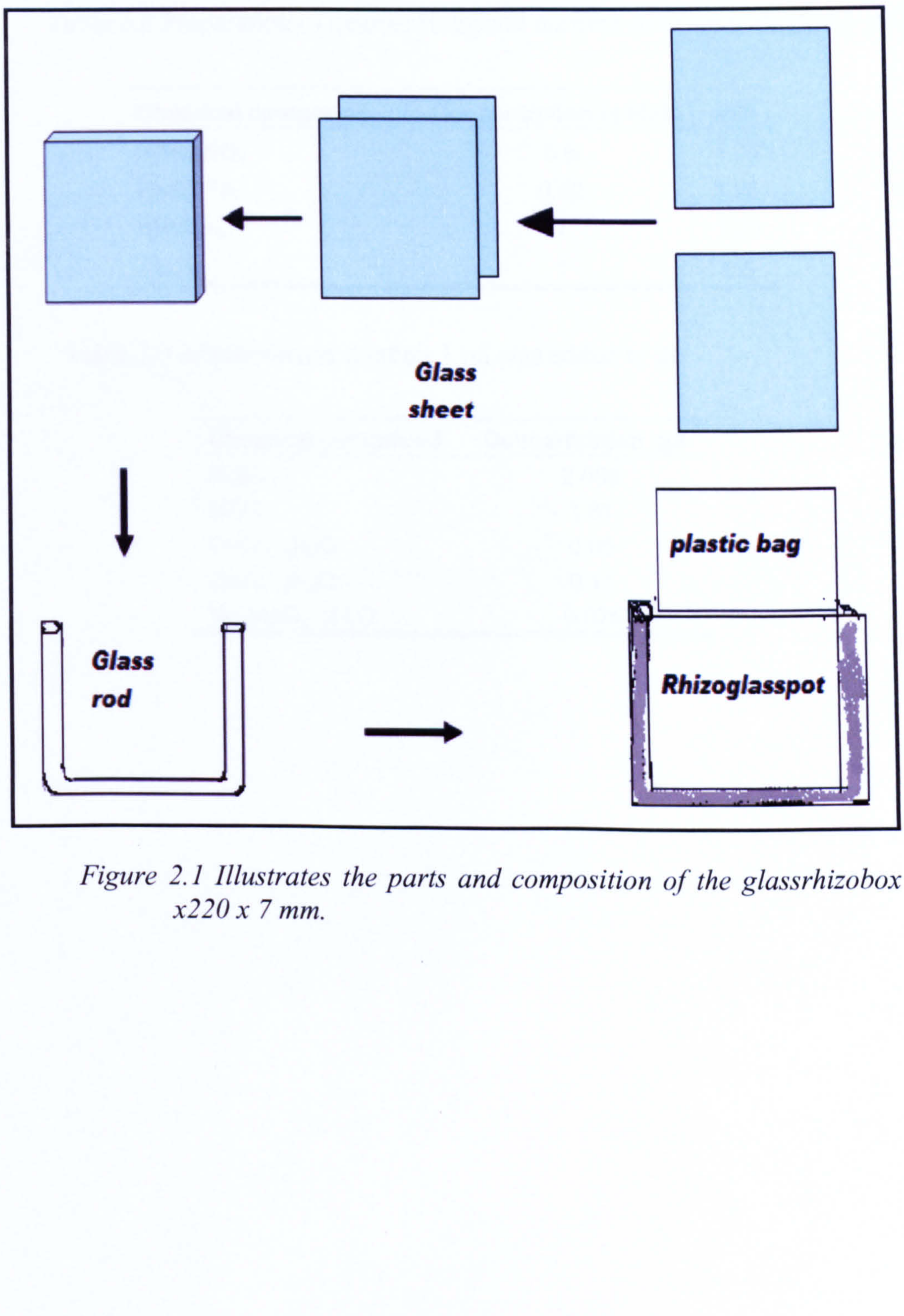

Figure 2.1 Illustrates the parts and composition of the glassrhizobox 220 x220 x 7 mm.

2.13.2 Hoagland solution

Table 2.7 Preparation of quarter Hoagland nutrient solution with KNO_3

Chemical compound	Concentration in Mol/l	ml/l
KNO_3	1	1.25
Fe-EDTA	0.02	1.25
KH_2PO_4	1	1.25
$CaCl_2$	1	0.5

Table 2.8 Preparation of quarter Hoagland nutrient solution with $(NH_4)_2SO_4$

Chemical compound	Concentration in Mol/l	ml/l
$(NH_4)_2SO_4$	0.5	1.25
Fe-EDTA	0.02	1.25
KH_2PO_4	1	1.25
$CaCl_2$	1	0.5

Table 2.9 Micronutrient mixture 1 ml was added to 2l.

Chemical compound	Concentration g/l
H_3BO_4	2.086
$MnCl_2$	1.81
$CuCl_2 .{}_4H_2O$	0.05
$ZnCl_2 .{}_2H_2O$	0.11
$Na_2MoO_4. {}_2H_2O$	0.025

2.13.3 Bromocresol purple indicator

2.13.3.1 Preparation of Bromocresol purple

Exactly 0.1 g of Bromocresol purple, sodium salt, indicator grade (company Alrich. chem. co.) dye content 90% was dissolved in 18.5 ml of 0.01 M NaOH and diluted with deionised water to 250 ml.

2.13.3.2 Calibration

About 250 ml of deionised water in 400 ml beaker with 20 drops of Bromocresol purple dye acidify with diluted HNO_3, until it became sharp yellow. With a pipette 0.01 M NaOH was added gradually and colour was changed, pH was measured and recorded (Table 2.11).

Table 2.10 shows the colour changes with different PH.

pH	4.1	5.4	5.6	5.8	6	6.3	6.4	6.5
Colour	Sharp Yellow	Yellow	Yellowish	Yellowish	Colourless	Bluish	Less purple	Purple

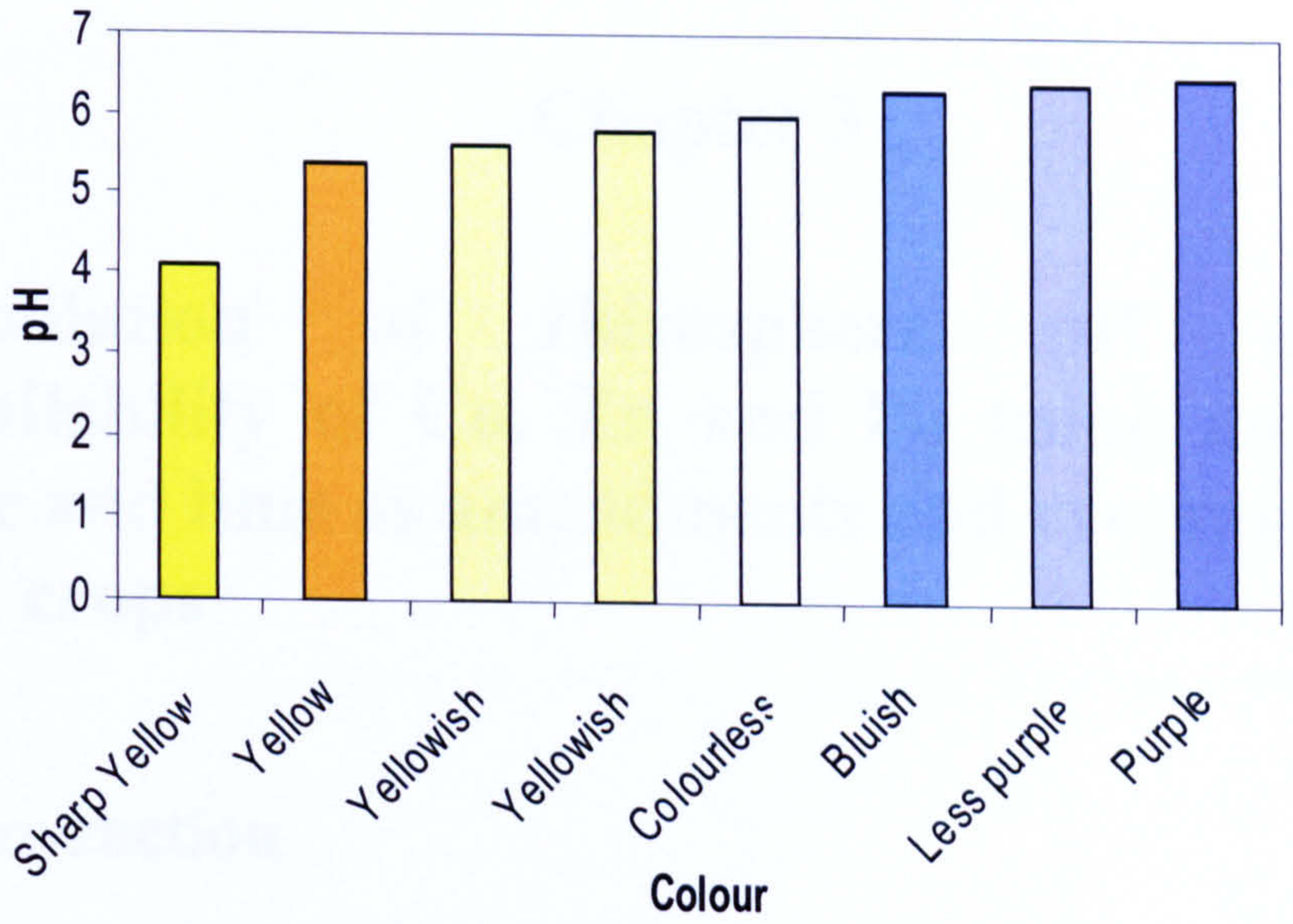

Figure 2.2 Illustrates the changes in Bromocresol purple with different pH

Chapter 3

Manipulation of rhizosphere pH and the bioavailability of Cu, Zn and Pb using ammonium, nitrate and lime as amendments and ryegrass and flax as test crops

3.1 Introduction

Heavy metals are natural constituents of the earth's crust, but the redistribution of these elements by human activities such as mining, smelting, various industrial processes and waste disposal can result in toxicity problems affecting human health, crop production, livestock production and wild fauna and flora (Alloway, 1990; Ross, 1994). Remediation technologies for heavy metal contaminated soils have been developed using a variety of physical and chemical methods; for example, excavation and disposal by landfill, *in situ* encapsulation or containment, separation of pollutants by techniques such as soil washing and electrokinesis, or stabilisation using grouts and cements (Bio-Wise, 2001). These technologies rely heavily on engineering-based techniques, tend to lack environmental sensitivity and are expensive. More recently, a group of techniques known collectively as phytoremediation have been developed. These use green plants either to extract the heavy metals from soil, or to stabilise them in a non-bioavailable form (Kumar et al., 1995; Pulford and Watson, 2003; van der Lelie et al., 2001). It has been suggested that such techniques are cost effective and are ecologically preferable to the more highly engineered solutions.

In the development of phytoextraction procedures most attention has been on changes to the total metal concentration in a soil. But recently it has been argued that it is the bioavailable fraction of the metal that is important and that a decrease in this fraction could be considered as an acceptable outcome of phytoremediation (Dickinson, 2000; Dickinson and Pulford, 2005). If the amount of metal in this fraction can be controlled then the scope for phytoremediation will be increased. There has also been concern regarding the fate of plants with high metal contents, and especially the transmission of metals into the food chain or their spread in to the wider environment. One strand of this argument concerns the type of plant grown, and whether or not it has an economic value. Hyperaccumulator plants, which can take up large amounts of metal and tolerate high concentrations in their tissues (Brooks *et al.*, 1998), have been used for phytoremediation (McGrath and Zhao, 2003), but have no economic value. The main alternative that has been suggested is the use of trees, either for bioenergy production or for their landscaping qualities (Dickinson, 2000). If the concentration of contaminants in the biomass can be maintained below a critical level for livestock consumption, crops could potentially be used for phytoremediation (Murillo *et al.*, 1999). One way to limit metal uptake would be to control the pH in the soil, which is a key parameter influencing solubility for most heavy metals.

Most plants require a soil pH in the range between 4.5 and 8.5. At the lower end of the scale, metal toxicity will become evident and at high pH, micronutrient deficiencies are likely to occur (Moraghan, 1991). Specific species have much narrower pH tolerance, but others have reasonable tolerance over a broad range of pH. Traditionally the pH of a soil for agricultural purposes is controlled by the use of lime, but at the micro scale pH can vary considerably from point to point in a soil. The rhizosphere pH is affected by root and microbial respiration, which have an acidifying

effect due to the dissolution of CO_2, and release of plant and microbial exudates, often organic acids. In addition to these effects, plant roots release ions in response to nutrient uptake in order to maintain charge balance within their cells (Hinsinger et al., 2003; Marschner, 1995). Uptake of cations results in release of H^+, while anion uptake causes release of OH^- or HCO_3^-. These ions can modify the chemical properties of the rhizosphere, either directly by affecting pH or indirectly by promoting release of sorbed ions (Gahoonia and Nielsen, 1992a; Gahoonia and Nielsen, 1992b; Gahoonia et al., 1992; Hinsinger and Gilkes, 1996). A few studies have examined the effect on heavy metal uptake by plants of changes in rhizosphere pH as a result of nitrogen fertilizer addition (Chaignon et al., 2002; Jentschke et al., 1998; Loosemore et al., 2004).

Used in combination, application of lime to control the bulk soil pH and manipulation of rhizosphere pH by use of ammonium or nitrate as a nitrogen source could allow much of the soil metal to be held in a non-bioavailable, relatively insoluble form, while sufficient metal could be released in the rhizosphere and then taken up by plant roots. This would achieve simultaneous phytostabilisation of much of the soil metal and a slow rate of phytoextraction. The aim of this study was to measure the changes in soil bioavailability and plant uptake of two essential elements, Cu and Zn, and a non essential element, Pb, by ryegrass and flax using NH_4^+ or NO_3^- as the sources of nitrogen, with and without addition of lime.

3. 2 Materials and methods

The material and methods are described in Chapter 2 (section 2.6) and the physical and chemical properties of the soils used are shown in table 2.1. When the plants had germinated, fertilizer was applied in solution at rates of 50 mg P per pot (as

potassium hydrogen phosphate) to all pots, 100 mg N per pot as potassium nitrate to 16 pots and as ammonium sulphate to the rest of the pots in the case of ryegrass (*Lolium perenne L.*). At the three leaf stage of the two flax varieties (*Viola and Elise*) 100 mg N/ pot was added as potassium nitrate for the half of the pots (4 with Viola, 4 with Elise limed, 4 with Viola, 4 with Elise non-limed and 4 bare soil limed and 4 others non -limed) and the same amount of nitrogen was added as ammonium sulphate to the other half of the pots in the same manner. All pots were arranged in randomized block design. After 6 weeks shoots were harvested 2 cm above the soil surface, and prepared for analysis as in Chapter 2 (section 2.7.1), and the root mass was prepared as Chapter 2(section 2.7.2) and digested as procedure in Chapter 2 (section 2.10). The bioavailable fraction of soil heavy metals was measured as in Chapter 2 (section 2.11).

3.3 Results and Discussion

3.3.1 Ryegrass experiment

3.3.1.1 EDTA extractable metal in soil.

The data were averaged over all treatments in order to assess the effect of plant growth on the EDTA extractable pool of metals in the soil. Growing ryegrass in this soil caused a significant decrease in the EDTA extractable Cu, Zn and Pb compared to bare soil with no plant growth (Table 3.1a), suggesting that this pool of metal was depleted by plant uptake. Chelating agents such as EDTA are commonly used as extractants to assess the bioavailability of heavy metals in soils (Manouchehri et al., 2006).

Table 3.1 Effect of a) ryegrass growth and b) liming on EDTA extractable Cu, Zn and Pb measured in pot soil following the ryegrass harvest.

Treatment	**EDTA extractable metal mg / kg soil**		
(n = 16)			
a	Cu	Zn	Pb
Ryegrass	10.5**b**	5.9**b**	21.1**b**
Bare soil	14.7**a**	10.2**a**	26.4**a**
LSD *P* <0.01	**0.67**	**0.80**	**1.60**
b			
Lime	13.1**a**	7.1**b**	23.7
No lime	12.1**b**	8.9**a**	23.9
LSD *P* <0.01	**0.70**	**0.80**	**NS**

Values in the same column in part a or b with different letters were significantly different at P < 0.01

Previous work has shown that there is a shift of metal from a potentially bioavailable pool to an immediately available pool as a result of plant growth. Bakhsh et al.(1990) showed that growth of ryegrass over 48 weeks increased $CaCl_2$-extractable Zn, considered to be immediately available to plants, but that the acetic acid, EDTA and acid oxalate-extractable pools of Zn all decreased. Tao *et al.* (2003) showed that in the rhizosphere of maize there was a shift of Cu from the carbonate, oxide and organic pools to the exchangeable pool. The exchangeable Cu, which was the source of copper taken up by the plants, initially increased over the first 25 days of cultivation, but had declined to a very low level after 100 days.

In order to assess the effect of liming, the extractable metals with or without addition of lime were averaged over all nitrogen treatments (Table 3.1b) Addition of lime caused a significant decrease in EDTA Zn, a slight but significant increase in EDTA Cu and had no effect on EDTA Pb. So while the overall bioavailability, as measured by EDTA extraction of Zn, can be controlled by liming, this is not the case with Cu and Pb, possibly because these two elements form strong complexes with humified organic matter. It is likely that soluble OM, released due to the increase in

pH caused by addition of lime, maintained higher extractable concentrations of Cu and Pb.

Table 3.2 Effect of nitrogen source and lime on EDTA extractable Cu, Zn and Pb measured in pot soil following ryegrass harvest.

Treatment (n = 8)	**pH**	**EDTA extractable metal mg / kg soil**		
		Cu	**Zn**	**Pb**
$(NH_4)_2SO_4$ + lime	6.2**b**	12.8**a**	7.6**b**	23.4**ab**
$(NH_4)_2SO_4$	5.6**d**	12.9**a**	9.9**a**	24.9**a**
KNO_3 + lime	6.7**a**	13.5**a**	6.7**c**	23.9**ab**
KNO_3	6.1**c**	11.3**b**	7.9**b**	22.9**b**
LSD *P* <0.05	**0.07**	**0.70**	**1.18**	**1.70**

Values in the same column with different letters were significantly different at P < 0.05

When the effects of both liming and the form of nitrogen used are considered separately (Table 3.2) the use of NH_4^+ as the nitrogen source resulted in significantly higher amounts of EDTA Cu, Zn and Pb in the unlimed soil, and a decrease in pH of about 0.5 of a unit, compared to use of NO_3^-. Addition of lime nullified this effect for Cu and Pb, but not for Zn, although the differential of 0.5 pH unit was maintained. The similar amounts of extractable Cu and Pb in the limed soil are regardless of nitrogen source confirms the possible role of soluble organic complexes.

3.3.1.2 Biomass yield and metal content

Table 3.3 Effect of nitrogen source and lime on fresh and dry shoot weight and root weight of ryegrass.

Treatment (n = 8)	**Weight in g**		
	Fresh shoot	Dry shoot	Dry root
$(NH_4)_2SO_4$ + lime	32.19**a**	3.48**a**	1.48**a**
$(NH_4)_2SO_4$	32.15**a**	3.48**a**	1.19**ab**
KNO_3 + lime	27.34**b**	2.80**b**	0.90**b**
KNO_3	28.64**b**	3.49**a**	1.22**ab**
LSD *P* <0.05	**2.1**	**0.27**	**0.35**

Values in the same column with different letters were significantly different at $P < 0.05$

Addition of NH_4^+ as the source of nitrogen produced a significantly higher fresh weight of grass tissue harvested than NO_3^-, and liming had no effect. However dry weight yields of grass were the same for both NH_4^+ treatments and for NO_3^- without addition of lime, but significantly lower for NO_3^- plus lime (Table 3.3). The dry weight of root tissue in the nitrate + lime treatment was significantly less than for the ammonium + lime, whereas the root yields for the unlimed treatments were the same. The combined effect of liming and the use of nitrate would result in a high pH in this soil. A value of 6.7 is reported in Table 3.2, but the actual pH at the root surface may have been even higher due to the release of OH^- and HCO_3^- ions. This may have directly affected the root and shoot yields or could have induced nutrient deficiency.

Table 3.4 Effect of nitrogen source and lime on Cu, Zn and Pb in shoot and root of ryegrass.

Treatment n = 8	**Metal content in shoot mg/kg**			**Metal content in root mg/kg**		
	Cu	Zn	Pb	Cu	Zn	Pb
$(NH_4)_2SO_4$ +lime	12.1**a**	46.2b**c**	n.d	57**d**	83.8**b**	n.d
$(NH_4)_2SO_4$	13.2**a**	71.6**a**	n.d	121.7**b**	107.6**a**	n.d
KNO_3+lime	7.5**b**	41.5**c**	n.d	111.3**c**	75.3**c**	n.d
KNO_3	5.6**c**	48.9**c**	n.d	129.3**a**	111.3**a**	n.d
L.S.D at .01	**1.25**	**6.3**		**N.S**	**N.S**	**n.d**
L.S.D. at .05				**5.1**	**10.2**	

Values in the same column with different letters were significantly different at $P < 0.05$

The concentrations of Pb in plant tissue were too low to detect. Addition of NH_4^+ caused a higher concentration of Cu and Zn in leaf tissue than use of NO_3^- as the source of nitrogen, although the effect was only marginal for Zn when lime was added (Table 3.4). When no lime was added, there was no difference in the concentrations of Cu and Zn in root tissue between the two nitrogen treatments. Liming and NO_3^- as the nitrogen source resulted in more Cu but less Zn in the root tissue compared to use of ammonium with no lime (Table 3.4), reflecting the greater bioavailability of Cu in this treatment.

Table 3.5 Effect of a) nitrogen source averaged over both lime treatments and b) lime averaged over both nitrogen treatments on Cu, Zn and Pb in shoot and root of ryegrass.

Treatment (n = 16)	**Metal content in shoot mg / kg**			**Metal content in root mg / kg**		
	Cu	Zn	Pb	Cu	Zn	Pb
a						
$(NH_4)_2SO_4$	12.6**a**	58.9**a**	nd	89.3**b**	95.7	nd
KNO_3	6.5**b**	45.2**b**	nd	120**a**	93.3	nd
LSD P <0.01	1.25	4.77		5.1	NS	
b						
Lime	9.8	43.8**b**	nd	84.1**b**	79.5**b**	nd
No lime	9.4	60.2**a**	nd	120**a**	109**a**	nd
LSD P <0.01	**NS**	**4.77**		**5.1**	**14.4**	

Values in the same column in part a or b with different letters were significantly different at P < 0.01

Averaged across both lime treatments (Table 3.5a), there was a higher concentration of Cu and Zn in leaf tissue when NH_4^+ was used compared to NO_3^-. Zn concentration in the roots was the same for both N treatments, but Cu was higher in the NO_3^- treatment. Averaged across both N treatments (Table 3.5b), there was a higher concentration of Zn in leaves and roots, but Cu in roots only, in the unlimed treatment. Liming had no effect on the Cu concentration in leaf tissue.

Table 3.6 Transfer of metals from roots to leaves (Conc leaf / conc root)

Treatment	Ratio of shoot metal conc. : root metal conc.	
	Cu	Zn
$(NH_4)_2SO_4$ + lime	0.21	0.55
$(NH_4)_2SO_4$	0.11	0.67
KNO_3 + lime	0.07	0.55
KNO_3	0.04	0.44

Movement of metals within a plant is also an important issue when considering their fate in the environment. Copper was translocated from roots to shoots to a lesser extent than Zn (Table 3.6), especially when nitrate was the source of nitrogen.

3.3.2 Flax experiment

3.3.2.1 EDTA extractable metals in soil

For the bulk pot soil (non-rhizosphere soil), there is no significant difference between either variety or bare soil in EDTA extractable Cu, Zn and Pb (Table 3.7 a) and this is attributed to a transfer of metals from less available pool to a more available pool due to plant root bioactivity, replacing metals which are depleted. These findings agreed with work of Tao *et al.* (2003) that there was shift of Cu from carbonate, organic and oxide pools to exchangeable pool in rhizosphere of maize plant. Also Romkens *et al.*, (1996)) pointed out that there is no difference between bare soil and planted soil in copper solubility in non-polluted soil without lime

In order to assess the effect of lime addition on EDTA-extractable metals, all nitrogen treatments, varieties and bare soil were averaged (Table 3.1b). Only the EDTA-extractable Cu was affected positively with lime (Table 3.7) ($P < 0.01$) and this is

attributed to release of Cu from the organic matter due to shift in pH with $CaCO_3$ (Adriano, 2001). Burton *et al.* (2005) illustrated that at pH 7 the mobility of Cu increased due to formation of aqueous Cu-DOC complexes. In contrast, when the Ca concentration in soil solution increases the stability of organic matter mineral complexes increase, and thus the dissolution of organic matter decreases and inhibits the release of Cu-binding organic mat (Zhang and Xia, 2005).

Table 3.7 Effect of a) two varieties of flax growth and b) liming on EDTA extractable Cu, Zn and Pb measured in pot soil following flax harvest.

Treatment	**EDTA extractable mg/kg soil**		
	Cu	Zn	Pb
A (n = 16)			
Elise	17.6	12.3	45.4
Viola	18.1	12.8	46.4
Bare soil	17.5	12.9	46.3
LSD p > 0.05	**NS**	**NS**	**NS**
B (n = 24)			
Lime	18.5**a**	9.3**b**	45.0**b**
No lime	16.9**b**	16.0**a**	46.8**a**
LSD p > 0.01	**1.17**	**0.6**	
LSD p > 0.05			**1.8**

Values in the same column in part a or b with different letters were significantly different.

Table 3.8- Effect of nitrogen sources with and without lime on pH and EDTA extractable Cu, Zn and Pb measured in pot soil following flax harvest.

Treatment n = 16	**pH**	**EDTA extractable mg/kg soil**		
		Cu	Zn	pb
$(NH_4)_2SO_4$ + lime	6.2**b**	17.9**b**	10.0**c**	45.7
$(NH_4)_2SO_4$	5.1**d**	17.3**c**	16.4**a**	46.9
KNO_3 + lime	6.7**a**	19.1**a**	8.7**d**	44.4
KNO_3	5.5**c**	16.6**d**	15.6**b**	46.8
L.S.D at .01 level	**0.1**	**0.6**	**0.7**	**N.S.**

Values in the same column with different letters were significantly different

In order to assess the EDTA- extractable metals in the four nitrogen treatments with and without the addition of lime, the data were averaged over varieties and bare soil (non-rhizosphere soil), (Table 3.8). Lime addition significantly decreased EDTA extractable Zn with both nitrogen sources (NH_4^+ and NO_3^-) (table 3.8) ($P < 0.05$). In the case of nitrogen sources with addition of lime the NH_4^+ had more EDTA-extractable Zn than NO_3^- and this is attributed to lowering the pH by the NH_4^+. The addition of nitrogen source as NH_4^+ can alter the pH and increase the EDTA-extractable Zn and the lime can depress the Zn availability. Availability of Zn can be controlled by the addition of lime and by the nitrogen source. The Cu availability or extractability by EDTA was increased by the lime addition with both nitrogen sources, and there was no significant difference in EDTA-extractable Pb between all treatments (Table 3.8) ($P < 0.05$).

Table 3.9 Effect of A) two varieties of flax growth and B) liming on EDTA extractable Cu, Zn and Pb measured in rhizosphere soil following harvest.

Treatment N = 16	**EDTA extractable mg/kg soil**		
	Cu	Zn	Pb
A			
Elise	15.0	13.1	38.8
Viola	14.8	12.1	38.3
LSD p > 0.05	N.S.	N.S.	N.S.
B			
Lime	18.8**a**	11.7**b**	40.4**a**
No lime	11.**b**	14.0**a**	36.7**b**
LSD p > 0.01	**1.5**	**2.2**	**N.S.**
LSD p > 0.05			**3.7**

Values in the same column in part a or b with different letters were significantly different.

There is no significant difference in EDTA-extractable metals in the rhizosphere soil between the two varieties of flax (*Elise and viola*): averaged over all other treatments (Table 3.9a). With addition of lime, the same effect of increasing the EDTA- extractable Cu ($P < 0.01$) and Pb ($P < 0.05$) occurred (Table 3.9). Calcium increases the biological activity along with rhizosphere activity and exudates, therefore enhancing the solubility of Cu and Pb from organic matter. The amount of Cu and Pb bound by organic matter is more than Zn due to their high binding affinity with organic matter. Decreasing the Zn with lime addition can be attributed to the increase of pH, which affects Zn solubility (Tyler and Olsson, 2001). Major cations in the soil solution such as Ca and Mg effectively compete with Zn for adsorption sites and therefore affect its mobility in high pH. (Christensen, 1984; Elzinga et al., 1999; Harter, 1992; Temminghoff et al., 1995; Voegelin et al., 2001). Different pHs affect the Zn solubility and bioavailability (Tyler and Olsson, 2001).

Table 3.10 Effect of nitrogen sources with and without lime on EDTA extractable Cu, Zn and Pb measured in rhizosphere soil following harvest.

Treatment n = 12	**pH**	**EDTA extractable mg/kg soil**		
		Cu	Zn	Pb
$(NH_4)_2SO_4$ + lime	6.2b	23.4a	13.0b	44.7
$(NH_4)_2SO_4$	5.1d	11.1c	16.1a	39.3
KNO_3 + lime	6.8a	14.1b	8.6c	36.2
KNO_3	5.5c	11.0c	12.0b	34.1
LSD p > 0.01	**0.1**	**2.1**	**3.1**	**N.S**

Values in the same column with different letters were significantly different

In the rhizosphere soil around the roots the NH_4^+ treatment has the highest EDTA extractable Zn and the lowest pH. Unlimed NO_3^- was more than limed NO_3^- ($P < 0.05$) (Table 3.10). Thus the uptake of NH_4^+ by the roots combined with the releasing of H^+ from the roots to the rhizosphere lowers the pH and enhances the Zn bioavailability. These findings also have been recorded by Sas *et al.* (2003). The EDTA-extractable Cu follows this order: limed NH_4^+ > limed NO_3^- > unlimed NH_4^+ = unlimed NO_3^- ($P < 0.05$) (Table 3.10), and this is attributed to the release of Cu from organic matter. EDTA- extractable Pb was not significant among all treatments ($P < 0.05$) (Table 4).

Table 3.11 Effect of nitrogen sources with and without lime on EDTA extractable Cu, Zn and Pb measured in pot soil and rhizosphere soil following flax harvest.

Treatment	Location	EDTA extractable mg/kg soil			pH
n = 8					
		Cu	Zn	Pb	
$(NH_4)_2SO_4$+lime	pot soil	17.6b	9.9b	46a	6.2b
	around root	23.4a	14.6a	45a	6.0c
$(NH_4)_2SO_4$	pot soil	17.0bc	16.3a	47a	5.1e
	around root	11.1f	16.1a	39b	5.1e
KNO_3+lime	pot soil	19.3b	8.6b	44b	6.9a
	around root	14.1d	8.7b	36bc	7.0a
KNO_3	pot soil	17.3b	15.4a	46a	5.8d
	around root	11.0f	12.0b	34c	6.1bc
L.S.D $P < 0.01$		**2**	**4.5**	**5.1**	
L.S.D $P < 0.05$					**0.1**

Values in the same column with different letters were significantly different

In the NH_4^+ with lime the EDTA-extractable Cu and Zn around the root were more than pot soil ($P < 0.01$) while the Pb was same ($P < 0.05$) (Table 3.11). Lime addition with NH_4^+ treatments had more EDTA-extractable Cu, Zn ($P < 0.01$) and Pb ($P < 0.05$) in pot soil than around the root soil. This is attributed to the effect of lime and biomass heavy metals uptake. For the NO_3^- limed and unlimed treatments, EDTA-extractable Cu, Zn ($P < 0.01$) and Pb ($P < 0.05$) were more in pot soil than around root soil (Table 3.11).

3.3.2.2Biomass yield and metal content

Table 3.12 Effect of nitrogen source and lime on fresh and dry shoot and root weight average of two varieties of flax.

Treatment n = 8	**Weight in g**		
	Fresh shoot	Dry shoot	Dry root
$(NH_4)_2SO_4$ + lime	5.8c	0.8c	0.10b
$(NH_4)_2SO_4$	15.3a	2.0a	0.30a
KNO_3 + lime	6.3c	0.8c	0.12b
KNO_3	13b	1.7b	0.31a
LSD $P < 0.05$	1.4	0.3	0.05

Values in the same column with different letters were significantly different

Figure 3.1 The effect of NH_4^+ on the right and NO_3^- on the left with same variety (Elise).

The fresh weight of shoot and dry weight of shoot with the unlimed NH_4^+ treatment was greater than all other treatments ($P < 0.05$) (Table 3.12 and Figure 3.2).

The dry weight of root with unlimed NH_4^+ and NO_3^- was more than limed NH_4^+ and NO_3^- ($P < 0.05$). The NH_4^+ lowered the pH more than other treatments for enhancing the nutrient uptake, which gives high biomass production (Table 3.12). The fresh weight of shoot and dry weight of shoot and root for unlimed NO_3^- was more than limed NO_3^-. Addition of lime depressed the biomass of the flax (Figure 3.1 and 3.3), due to pH lowering the available pool of nutrients.

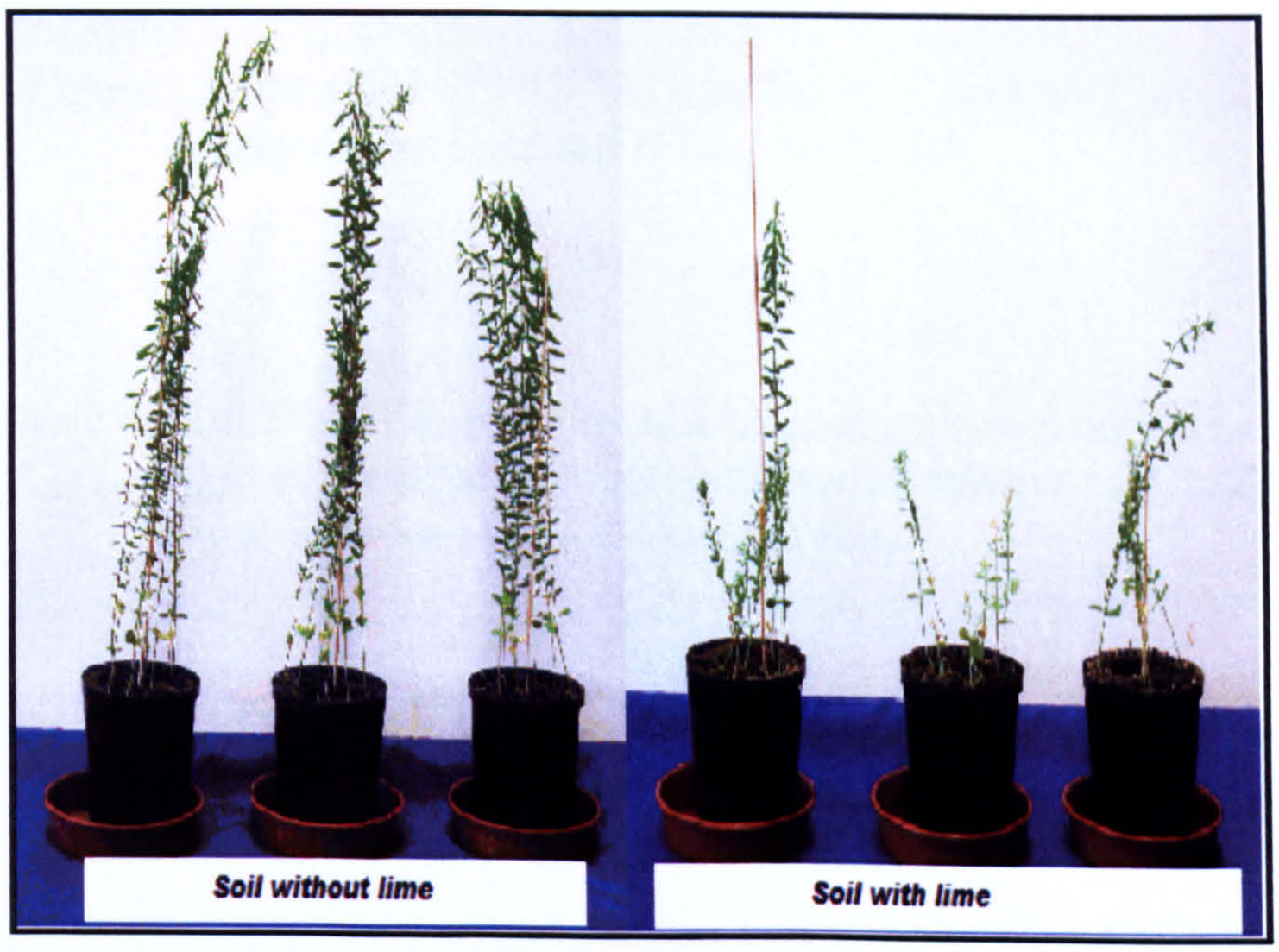

Figure 3.2 The effect of lime with NH_4^+ on the same variety Elise

Figure3.3 The effect of NH_4^+ with addition of lime on two flax varieties (Viola) on the right and (Elise) on the left.

Table 3.13 Effect of a) nitrogen source averaged over both lime treatments b) lime averaged over both nitrogen treatments on Cu, Zn and Pb in shoot and root of two varieties of flax.

Treatment n = 24	Metal content in shoot mg/kg			Metal content in root mg/kg		
	Cu	Zn	Pb	Cu	Zn	Pb
a						
$(NH_4)_2SO_4$	10.9**a**	73.9**a**	nd	43.4**b**	56.6**a**	43.1
KNO_3	6.4**b**	45.7**b**	nd	48.4.1**a**	26.8**b**	42.5
LSD p > 0.05	0.8	5.8		2.3	1.2	N.S.
b						
Lime	11.1**a**	49.2**b**	nd	52.5**a**	50.5**a**	34.8
No lime	6.3**b**	70.4**a**	nd	39.3**b**	32.8**b**	30.7
LSD p > 0.05	0.8	5.8		1.2	1.2	N.S

Values in the same column in part a or b with different letters were significantly different

Among the nitrogen sources, Cu and Zn shoot uptake is significantly higher with NH_4^+ than NO_3^- ($P < 0.05$) (Table 3.13). This is indicated that NH_4^+ plays an

important role in bioavailability of heavy metals, and is attributed to decreasing the pH in the rhizosphere, also by altering the biological activity. The Pb was not detected (the detection limit of Pb is 0.2mg/l) and this revealed that the solubility of Pb is very low. Soil solution contains only about 0.005- 0.13% of the total soil Pb^{2+} available to the plants (Alloway, 1990).

The Cu root content is significantly higher with NO_3^- than NH_4^+ and, in the case of Zn root content, with NH_{4+} more than NO_3^- ($P < 0.05$) and this attributed to less Zn being available with NO_3^- fed plants. The Pb root content showed no significant difference between the two sources of nitrogen NH_4^+ and NO_3^- ($P < 0.05$) (T able 3.13).

Addition of lime affected Zn shoot uptake negatively and Cu uptake positively ($P < 0.05$) and Pb was not detected (Table 3.13). This is attributed to increasing the pH which decreased the Zn bioavailability, but increased availability of Cu due to dissolution of organic matter. Addition of lime increased root Zn and Cu content. Pb root content was not affected by lime addition. Heavy metals content depends on the metal concentration in the soil solution and the plant varieties and also species within the plant varieties. For example, in the polluted soils where the concentration of heavy metals is high, cotton plant content of the heavy metals were decreasing in the following order: leaves > seeds > roots > stems, while the flax strongly absorbs and accumulates heavy metals compared with and hemp and cotton (Angelova *et al.*, 2004). The accumulation of pollutant elements was higher in roots than in leaves and shoots (Angelova et al., 2004; Mesquita et al., 2004).

Table 3.14 Illustrates the transfer factor from roots to shoots (concentration of metals in shoot: concentration of metals in the root).

Variety	Treatment n = 4	Shoot metal concentration: Root metal concentration	
		Cu	Zn
Elise	$(NH_4)_2SO_4$ + lime	0.2	0.8
	$(NH_4)_2SO_4$	0.3	2.9
	KNO_3 + lime	0.2	2.7
	KNO_3	0.1	1.6
Viola	$(NH_4)_2SO_4$ + lime	0.2	0.7
	$(NH_4)_2SO_4$	0.3	2.5
	KNO_3 + lime	0.2	1.3
	KNO_3	0.1	1.5

The shoot-root metal ratio transfer for Zn was greater than in Cu in all the treatments (Table 3.14). This differed from plant to plant, for example pumpkin, chicory, and red beet were characterized by the highest Zn concentration ratios (shoots/roots): 2.8, 2.2, and 2.0 (Sekara et al., 2005).

3.4 Conclusion

The conclusion can be made on the strategies of phytoremediation, which build up on phytoextraction and phytostabilization. Ryegrass changed the pool of heavy metals measured as EDTA-extractable heavy metals compared by the bare soil (no plant) suggesting that it was due to the plant uptake. In the flax experiment no difference in the pool of heavy metals was found between both flax varieties and bare soil (no plant). NH_4^+ without lime decreased the pH compared to other treatments in both experiments and increased EDTA-extractable Zn. Lime decreased EDTA extractable and shoot uptake Zn and increased EDTA-extractable Cu. NO_3^- with lime increased Cu and decreased Zn. Ammonium with lime positively affected rhizosphere EDTA-extractable heavy metals compared with pot soil. Clearly a difference exists between ryegrass and flax in heavy metal extraction with different treatments and also

between different treatments for different heavy metals. For example ammonium can maximise Zn phytoextraction and nitrate with lime can minimise Zn phytoextraction. Lime increased the Cu EDTA-extraction and plant phytoextraction. In the rhizosphere the ammonium with lime increased the EDTA-extractable heavy metals compared to bulk soil. The decrease of pH around root (Rhizosphere) with NH_4^+ or increase with NO_3^- presumably is vague and not clear due to many factors, such as a complex soil system, biological, organic matter and other physiochemical processes. In the next chapter a pure agar system is developed to investigate the effect of ammonium or nitrate on rhizosphere pH, which is the main factor to decrease or increase the accessibility and availability of heavy metals.

Chapter 4

Development of an agar search method to study the effect of NH_4^+ and NO_3^- on the rhizosphere pH of plants.

4.1 Introduction

In the previous chapter's experiments there was the effect of NH_4^+ on the rhizosphere pH but it may be that some other factors, which were mentioned in Chapter 3, section 3.4, were affected too. These experiments assess and develop a new method to control and reveal the changes in the rhizosphere pH by this technique. This system is nothing but glass transparent sheets 25 X 25 cm sandwiched on 7 mm glass rod bent to three sides of a square as in Chapter 2, section 2.13.1, and uses Bromocresol purple as indicator as in Chapter 2 section 2.13.3 and also a pH meter and glass electrode measuring in and out-rhizosphere. The roots of plants in agar system with the glass were very obvious and clear to visualize.

4.2 Material and methods

4.2.1Different plants with the same pH

Hoagland solution was prepared as in Chap. 2, section 2.13.2 in 5 litre volumetric flasks with NH_4^+ or NO_3^- as nitrogen source. Each solution was divided into 1 litre portion in 2 litre beakers and heated on hot plates at 100 °C, and agar powder (OXOID

product) was added (6.5 gm/l). When the solutions were clear they were allowed to cool to about 50 °C and drops of Bromocresol purple were added and the solution was poured in the rhizoglassboxes in plastic bags to. After 24 h when the solution was gelatinized the seeds (flax, oats and pea) were sown separately in three replicates along with control without plant. All rhizoglassboxes were transferred to a specially designed rack in the growth chamber (Figure 4.11 and 4.17), at a temperature of 20°C with 16 h fluorescent light. After 5 weeks, pH in and out of the rhizosphere in each replicate was measured with pH electrode and photographs were taken with a digital camera.

4.2.2 Two different pHs with the same plant (flax)

The procedure was as in section 4.2.1., except each solution was divided into two portions, one left as it was (pH 7) and the other portion adjusted to the pH 3.2 with 0.01 M HCl.

4.2.3 Five different pHs with the same plant (flax)

The procedure was as in section 4.2.1. Each solution was divided into five portions, each portion adjusted to the specific pH (4, 5, 6, 7 and 8) with 0.01 M HCl to lower pH or with 0.01 M NaOH to the higher pH.

4.2.4 Five different Zn concentrations with the same plant (flax)

The procedure was as in section 4.2.1. Six solutions of Zn concentration were prepared with either NH_4^+ or NO_3^- solutions with control (0, 10, 20, 0, 40, 60, 80 and 100 mg/l)

4.2.5 Foliar application of NH_4^+ or NO_3^- on plant (flax)

The procedure was as in section 4.2.1. Each solution was divided to litres and the NH_4^+ or NO_3^- solution added as a foliar spray on the flax shoots.

4.3 Result and discussion

4.3.1 Different plants with the same pH

There was a difference in pH between in the rhizosphere and out of the rhizosphere in all plants with NH_4^+ treatment in comparison with NO_3^- treatment in and out of the rhizosphere and control (Figures 4.1, 4.3, 4.4 and 4.5). There was a difference between inside and outside of the rhizosphere of the pea plant but not as much difference for flax and oats, and this is presumably due to exudates of pea more than other plants keeping the buffering capacity high, which affects the reduction of the pH by NH_4^+/H^+ exchange between the solution and roots and also out of the rhizosphere. For the NO_3^- treatments there were no differences between inside and outside of the rhizosphere for all the plants, (Figure 4.2, 4.5 and 4.6).

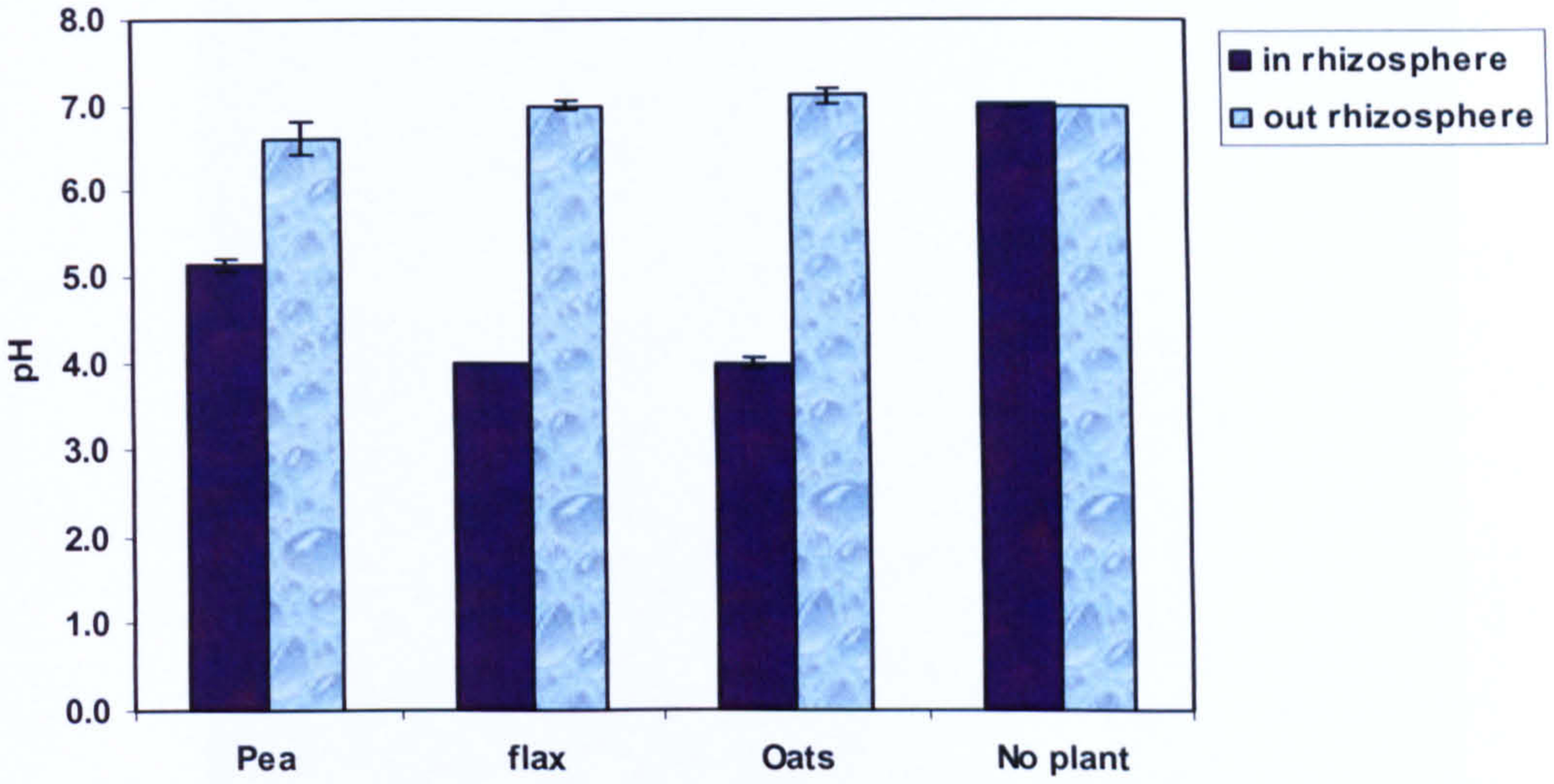

Figure 4.1 The effect of NH_4^+ on pH in and out of the rhizosphere of different plants in agar system.

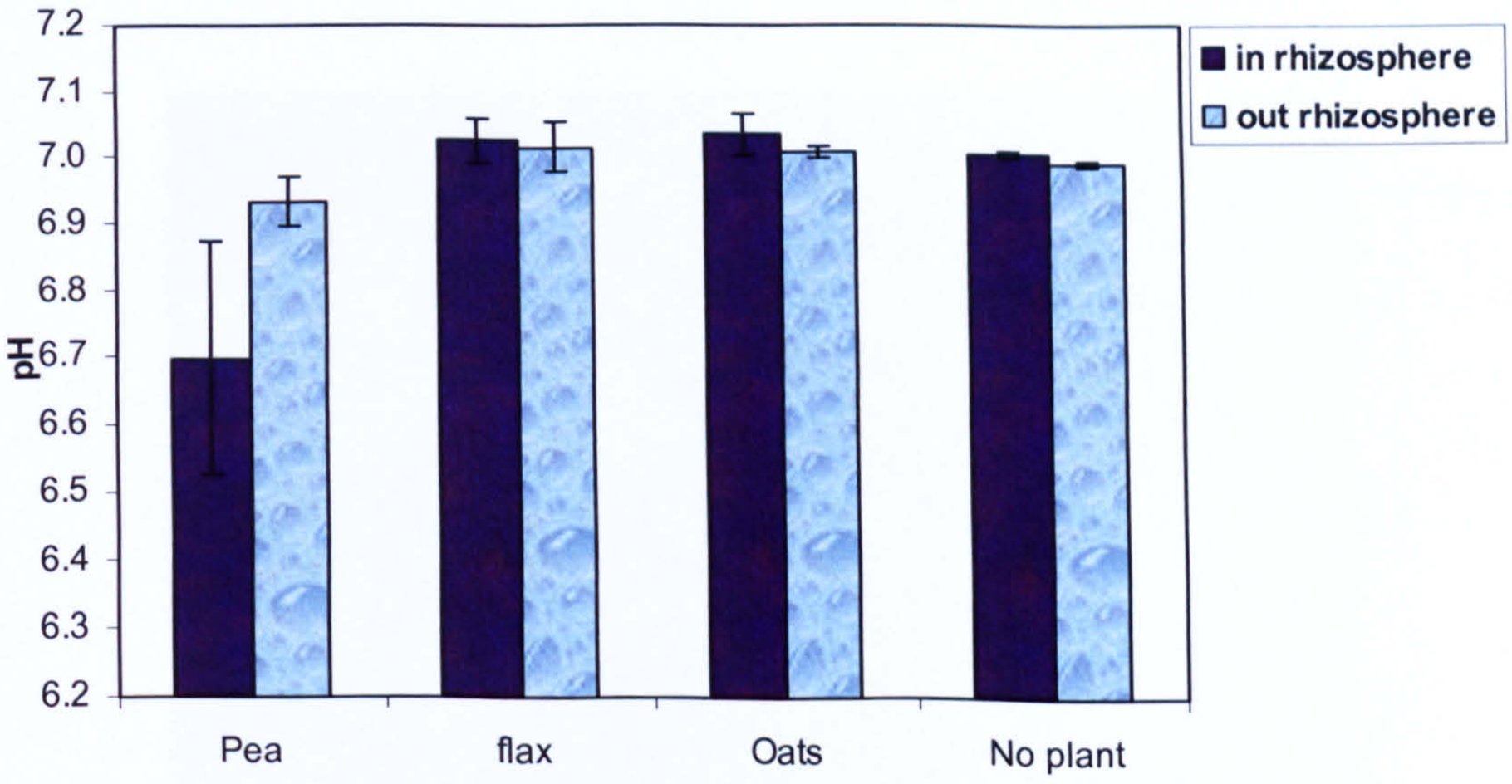

Figure 4.2 The effect of NO_3^- on pH in and out of the rhizosphere of different plants in agar system.

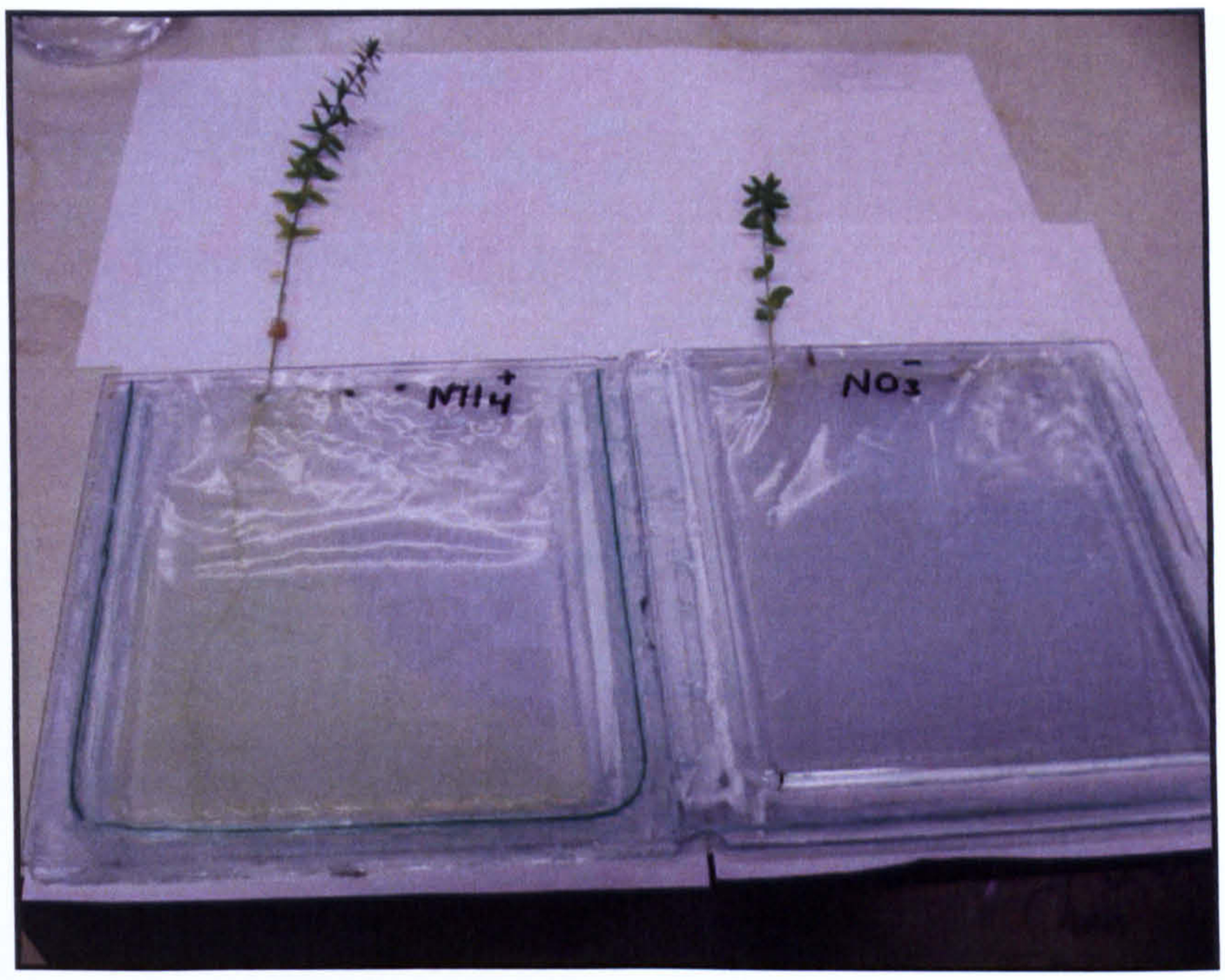

Figure 4.3 Illustrates the difference between NH_4^+ and NO_3^- to increase or decrease acidity of the flax rhizosphere using Bromocresol purple indicator.

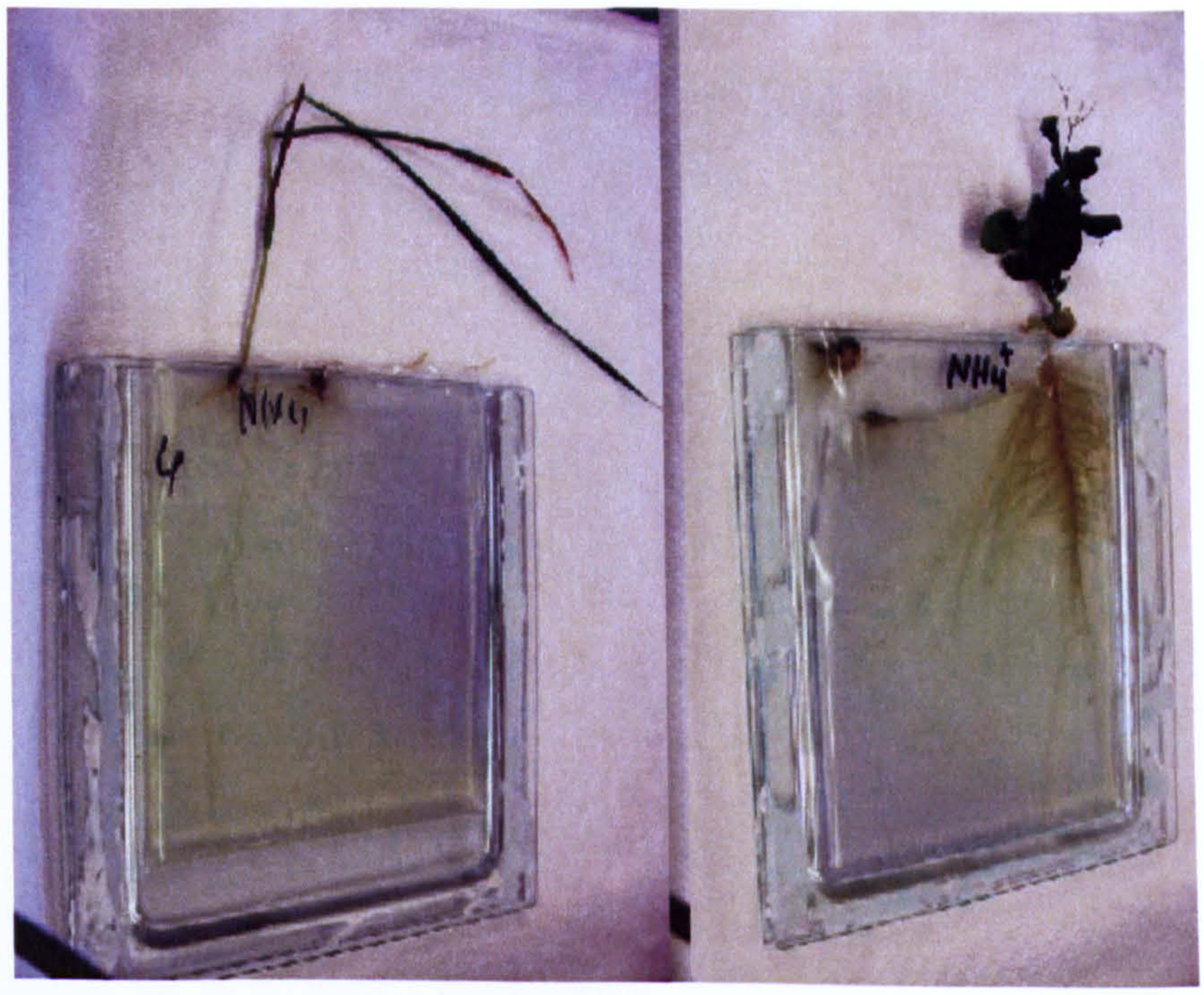

Figure 4.4 Illustrates the effect of NH_4^+ on acidity of Pea rhizosphere on the right and oats rhizosphere on the left, using Bromocresol purple indicator.

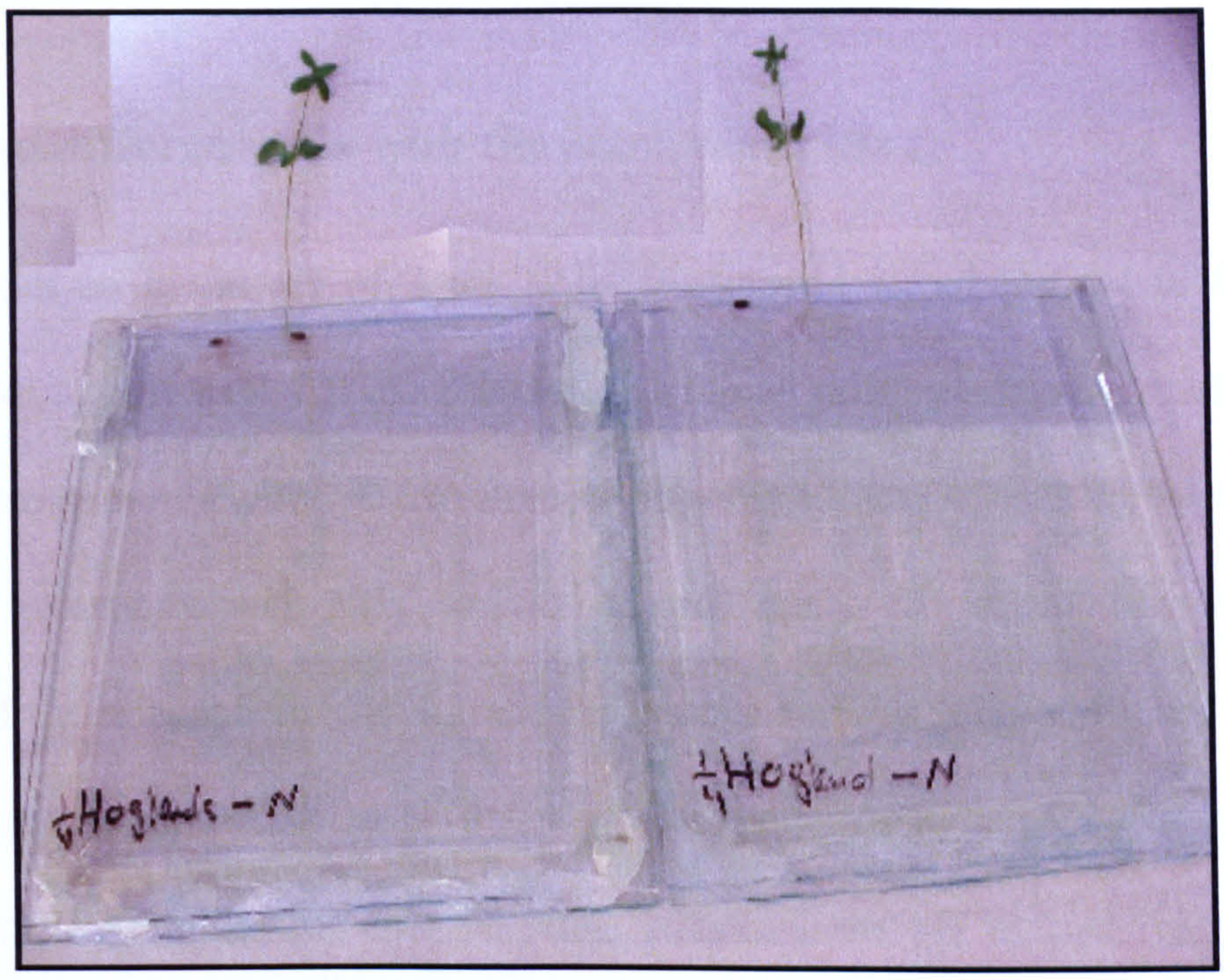

Figure 4.5 Illustrates the flax rhizosphere without addition of nitrogen, using Bromocresol purple indicator.

Figure 4.6 Illustrates the difference between NH_4^+ and NO_3^- on pH of flax rhizosphere using Bromocresol purple indicator.

4.3.2 Two different pHs with the same plant (flax)

From an initial pH of 7 the NH_4^+ treatment lowered the pH to 4.3 in the rhizosphere after 5 weeks in comparison with the all other treatments, however there was no change in pH with NO_3^- treatments (Figures 4.7 and 4.9). With the low initial pH (3.2) treatments, with NH_4^+ addition the pH in the flax rhizosphere was 4, the outside of the rhizosphere was 4.2 and the control was 4.8 (Figure 4.8 and 4.9). The NH_4^+ treatment controlled the pH level against the buffering capacity in comparison with control. At initial pH 3.2 the NO_3^- increased the pH in flax rhizosphere in comparison with control (Figure 4.8, and 4.10).

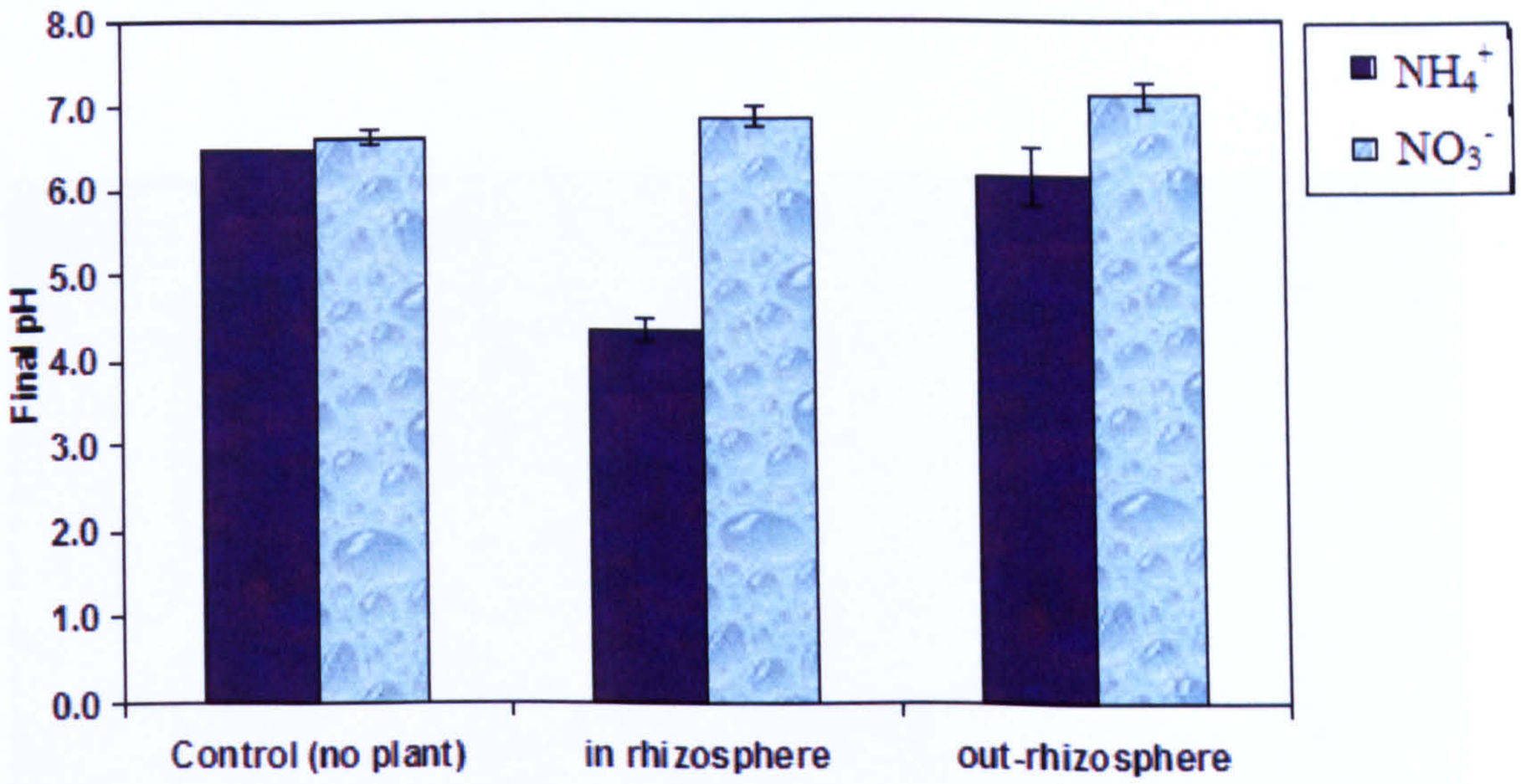

Figure 4.7 The effect of NH_4^+ or NO_3^- on pH in and out of the rhizosphere of flax and control (without plant) with initial pH 7 in rhizoglassbox in agar media

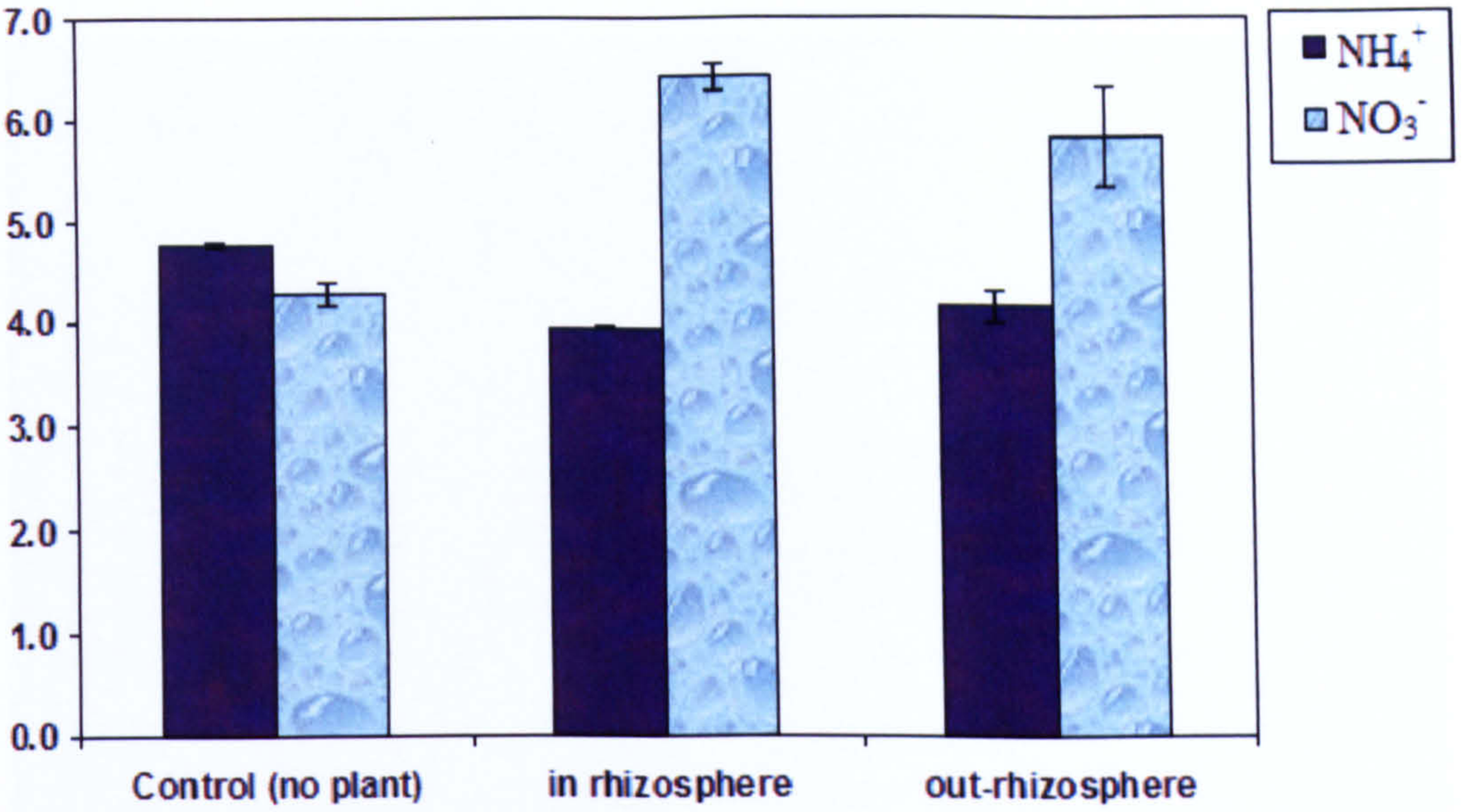

Figure 4.8 The effect of NH_4^+ or NO_3^- on pH in and out of the rhizosphere of flax and control (without plant) with initial pH 3.4 in rhizoglassbox in agar media.

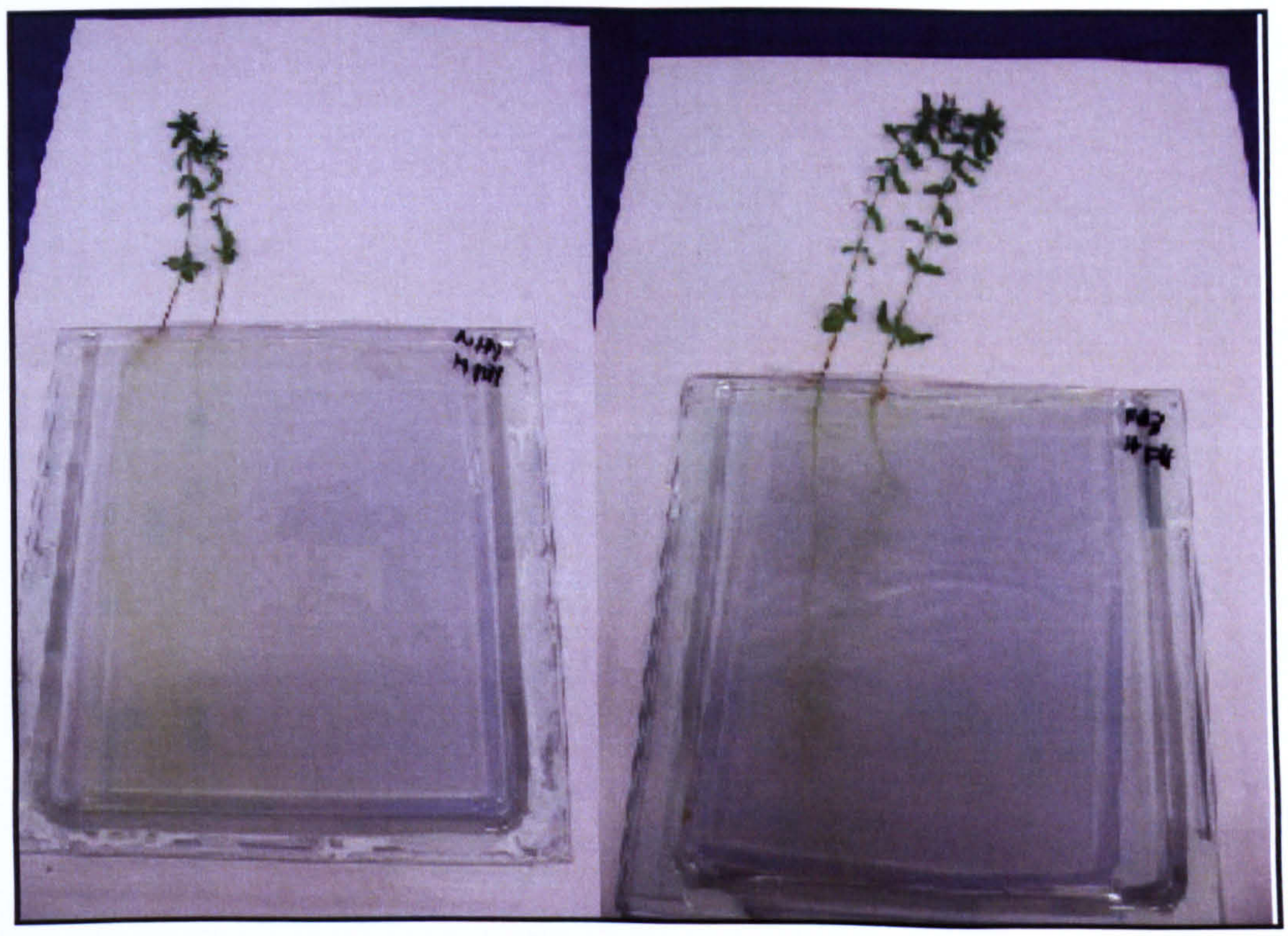

Figure 4.9 The effect of NH_4^+ or NO_3^- on flax rhizosphere at pH with Bromocresol purple indicator.

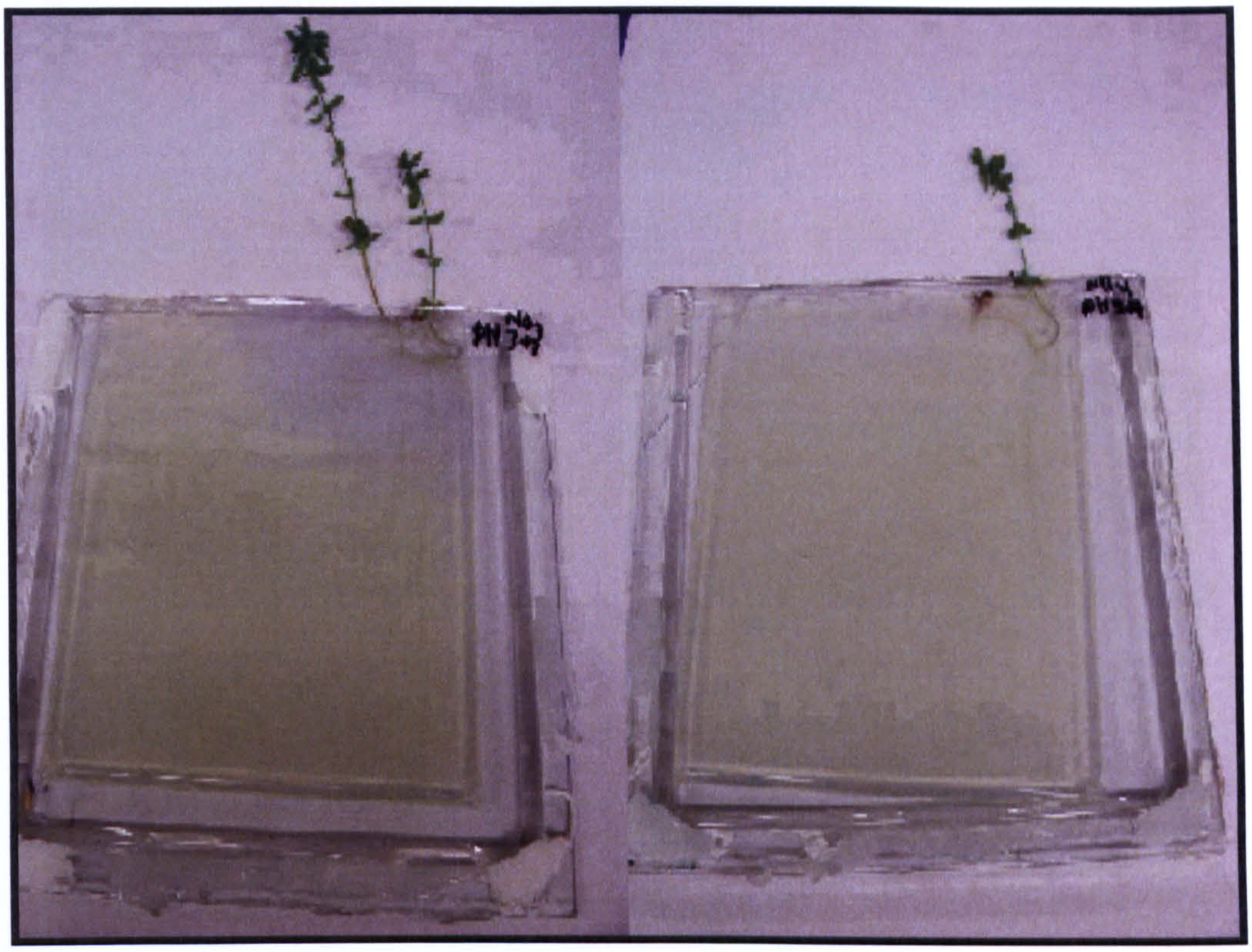

Figure 4.10 The effect of NH_4^+ or NO_3^- on flax rhizosphere at pH 3.2 with Bromocresol purple indicator.

Figure 4.11 The effect of NH_4^+ or NO_3^- on flax rhizosphere at pH 3.2 and 7 (7 = high pH and 3.2 = low pH) in agar media with Bromocresol purple indicator.

4.3.3 Five different pHs with the same plant (flax).

In the flax rhizosphere with addition of NH_4^+ the pH was decreased in all the initial pH treatments (4-8 pHs) in comparison with control and out of the rhizosphere. However, regardless of initial pH, there was no change of flax rhizosphere in pH in comparison with control and out of rhizosphere (Figures 4.12, 4.13 and 4.15).

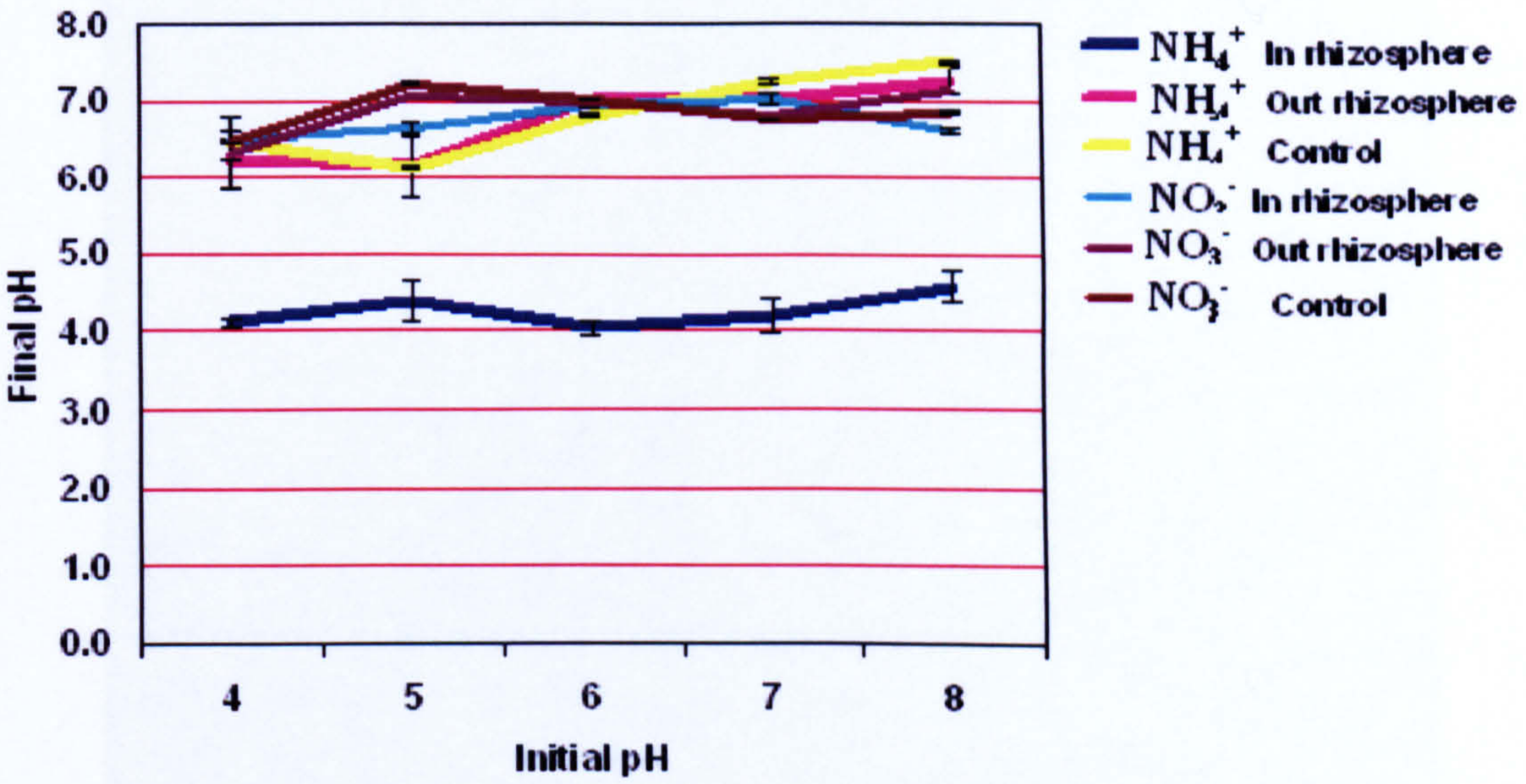

Figure 4.12 The effect of NH_4^+ or NO_3^- on pH in and out of flax rhizosphere with different initial pH's in agar media.

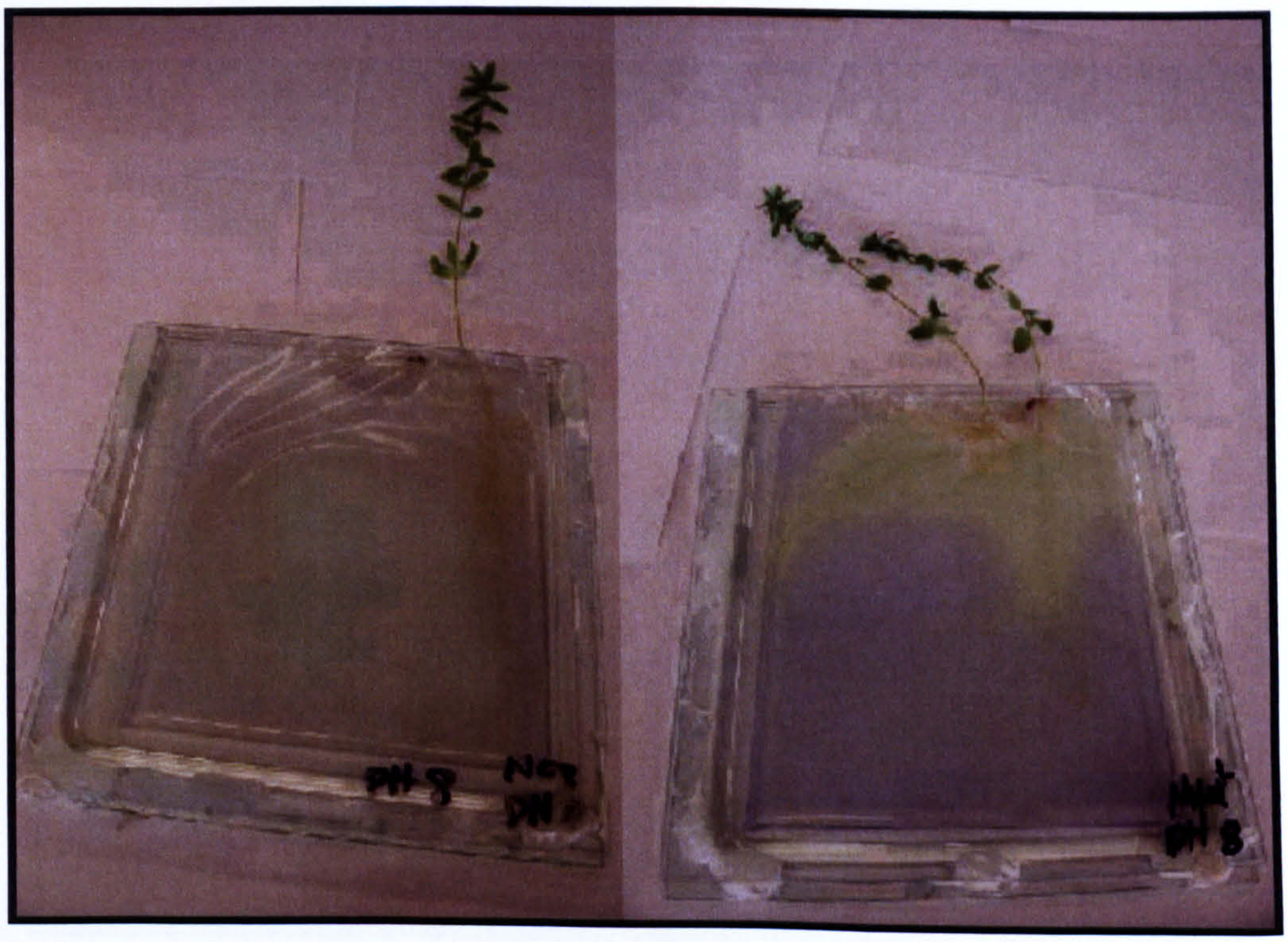

Figure 4.13 The effect of NH_4^+ or NO_3^- on flax rhizosphere at pH 7 with Bromocresol purple as indicator.

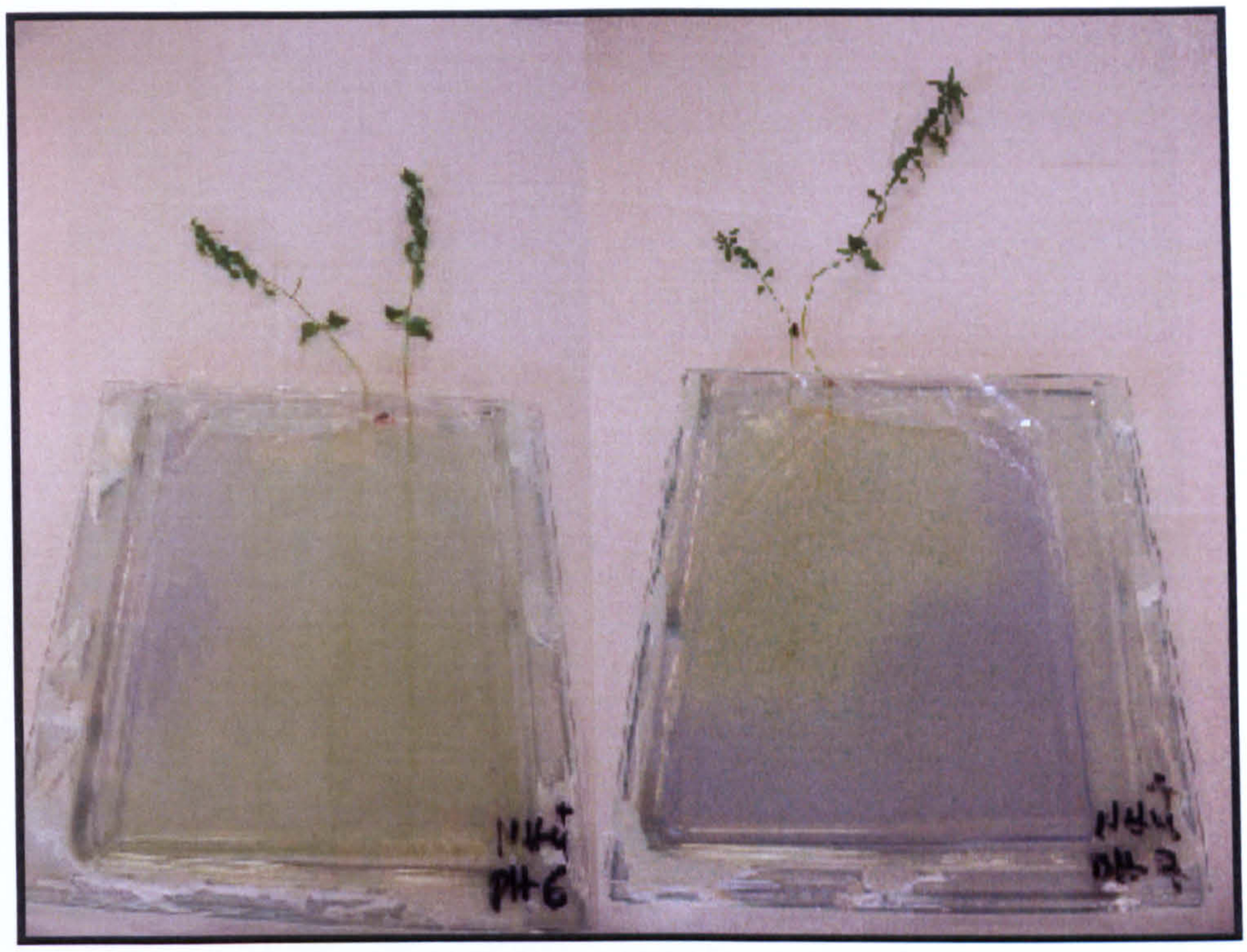

Figure 4.14 The effect of NH_4^+ or NO_3^- on flax rhizosphere at pH 7 and 6 with Bromocresol purple as indicator.

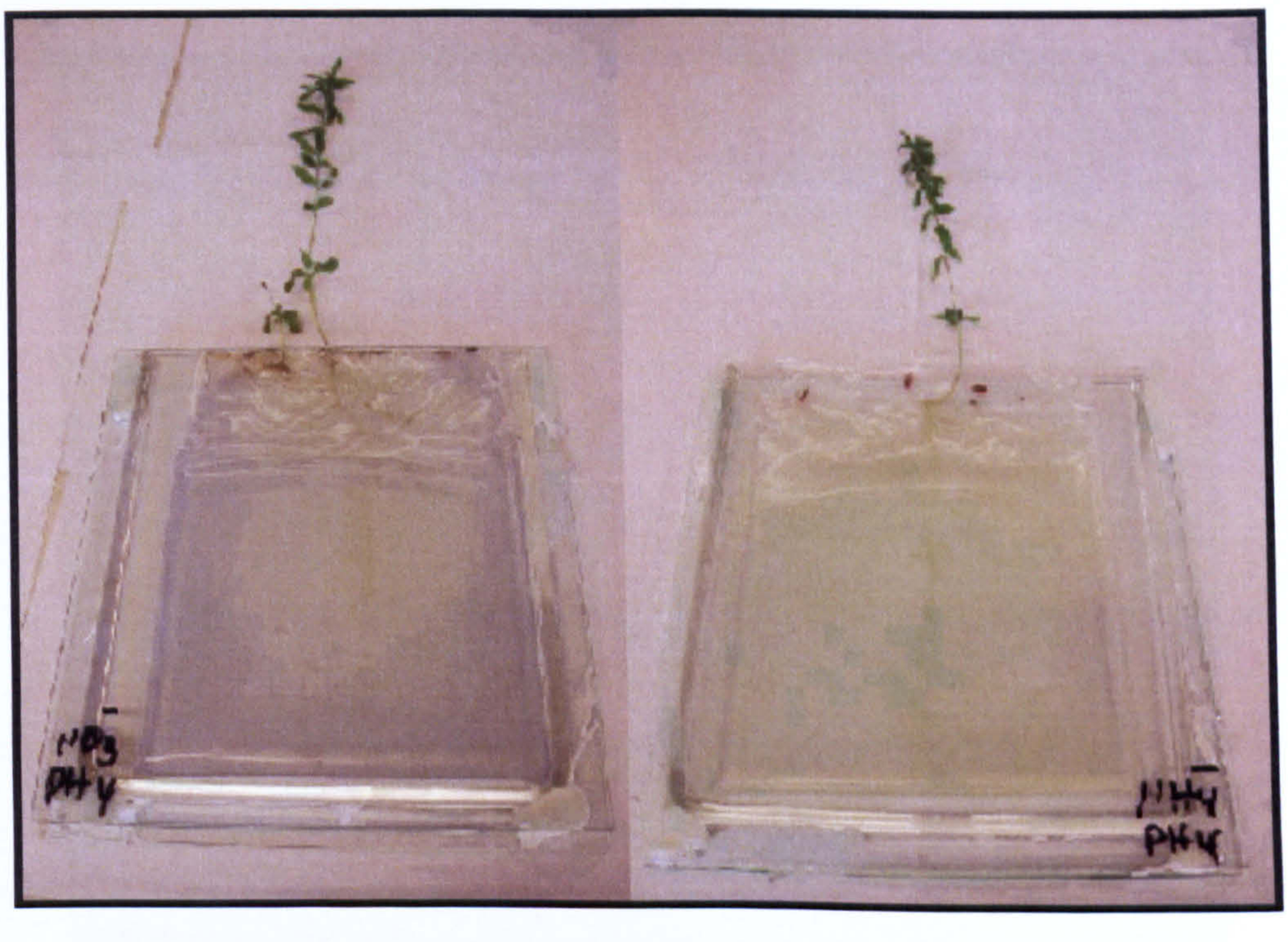

Figure 4.15 The effect of NH_4^+ or NO_3^- on flax rhizosphere at pH 4 with Bromocresol purple as indicator.

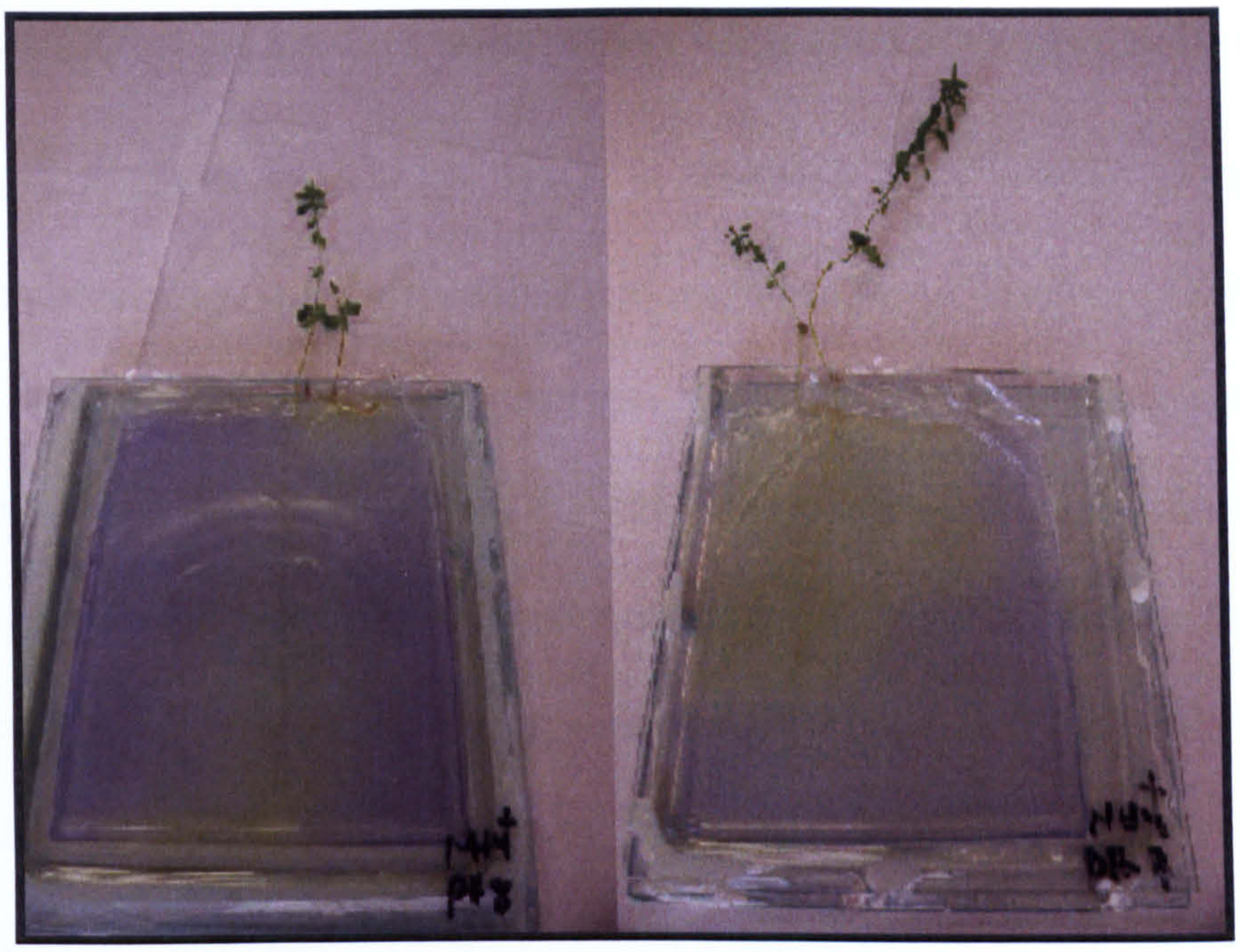

Figure 4.16 The effect of NH_4^+ or NO_3^- on flax rhizosphere at pH 7 and 8 in with Bromocresol purple as indicator.

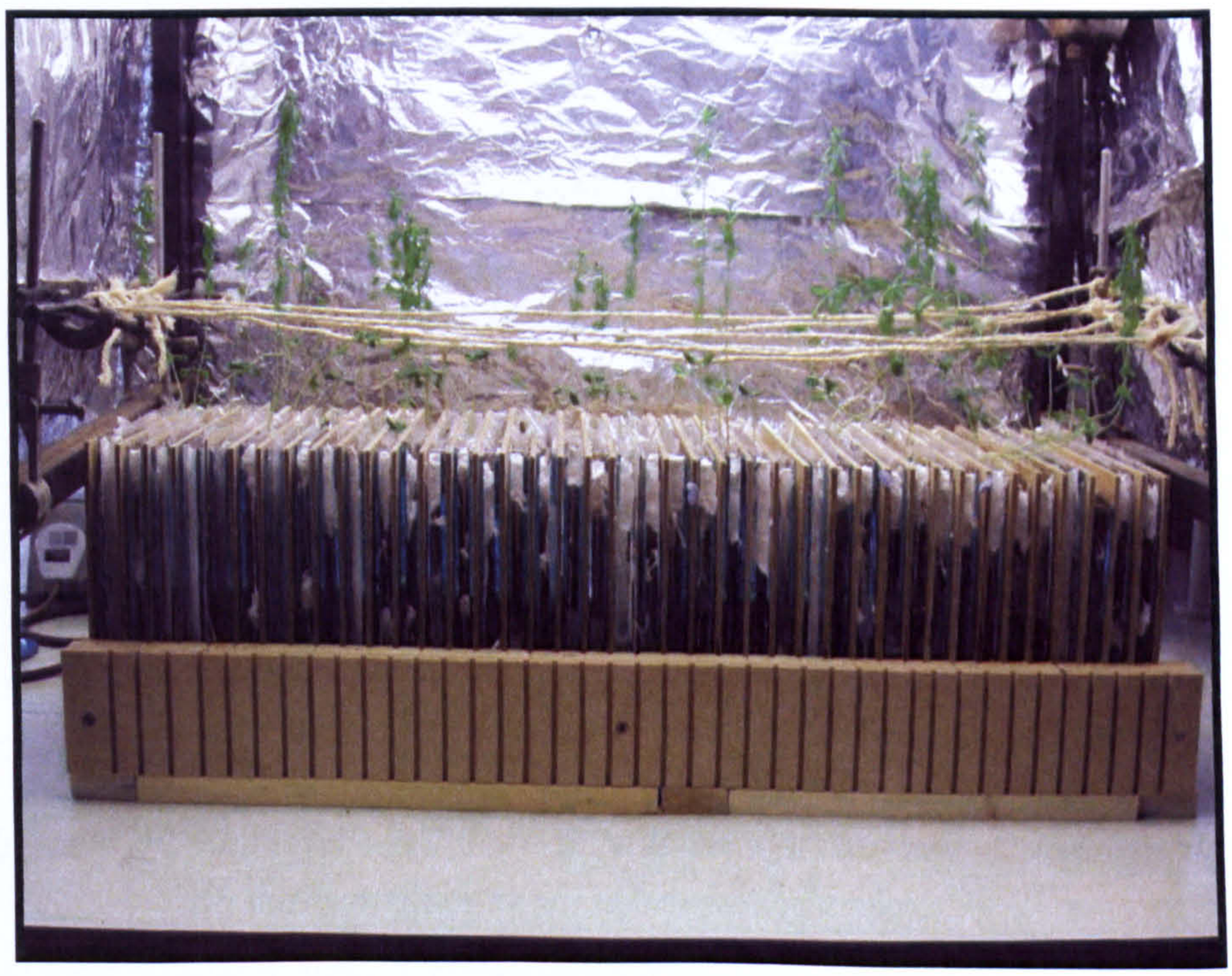

Figure 4.17 Rhizoglassboxes fitted in the wood rack and growing of flax with NH_4^+ or NO_3^-. in agar media with Bromocresol purple as indicator.

4.3.4 Five different Zn concentrations with the same plant (flax).

The aim was to assess methodology to detect the effect of NH_4^+ or NO_3^- on the pH in an indirect way, using zinc toxicity as an indicator for lowered pH. As the pH decreases the solubility of zinc increases too. There was no difference in shoot length between NH_4^+ and NO_3^- below 80 mg Zn/l but there was a significant difference at 80-100 mg Zn/l where the flax root was more affected with NH_4^+ treatment. There was a difference between NH_4^+ and NO_3^- treatments in root length. This implied that the NH_4^+ decreased the pH of the agar media and consequently increased the availability and solubility of Zn (Figure 4.18, 4.19, 4.20, 4.21and 4.22)

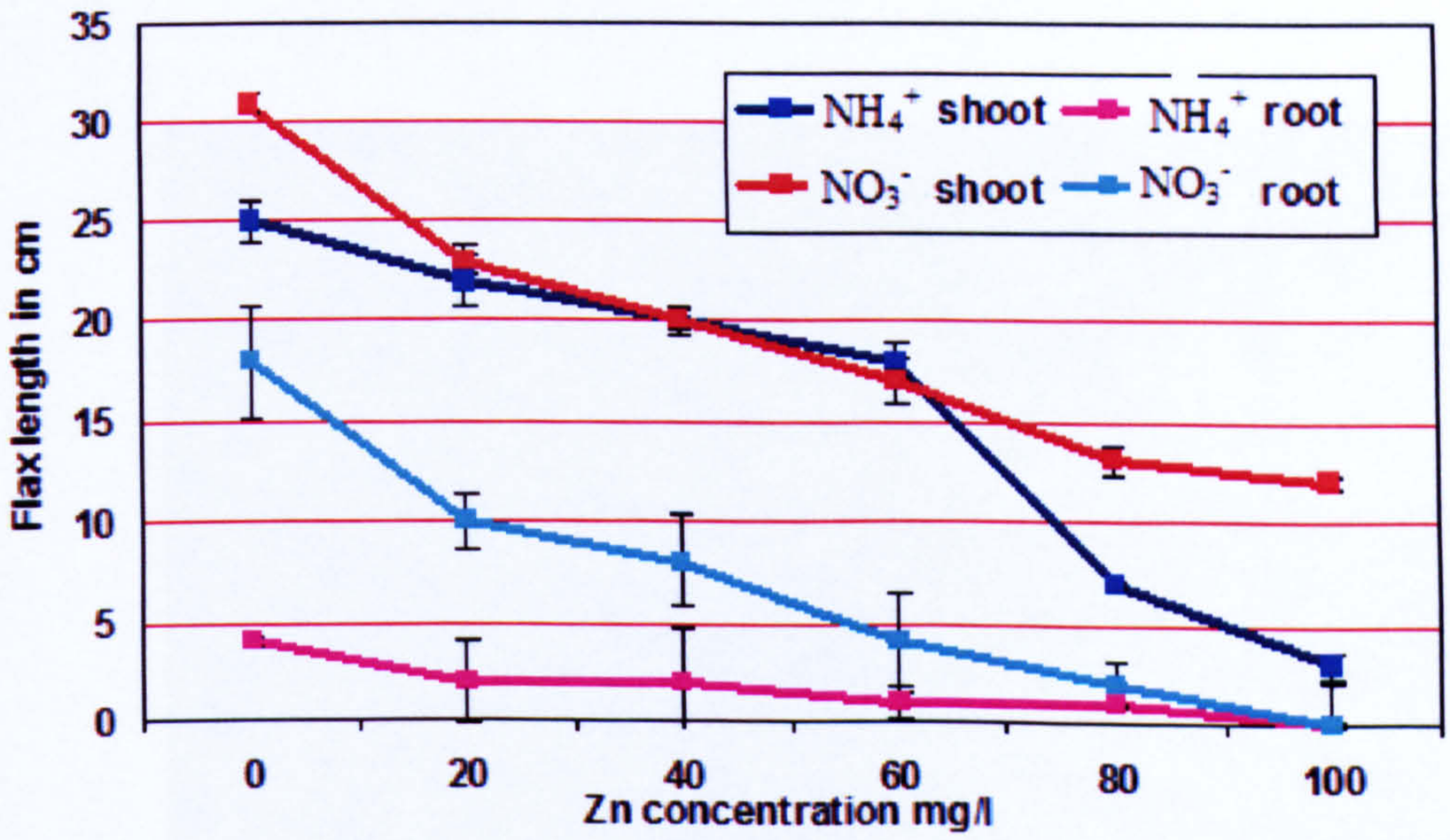

Figure 4.18 The effect of NH_4^+ or NO_3^- on the oats shoot and root by different Zn concentrations.

Figure 4.19 Shows the control with NH_4^+ or NO_3^- root growth of oats plant using Bromocresol purple.

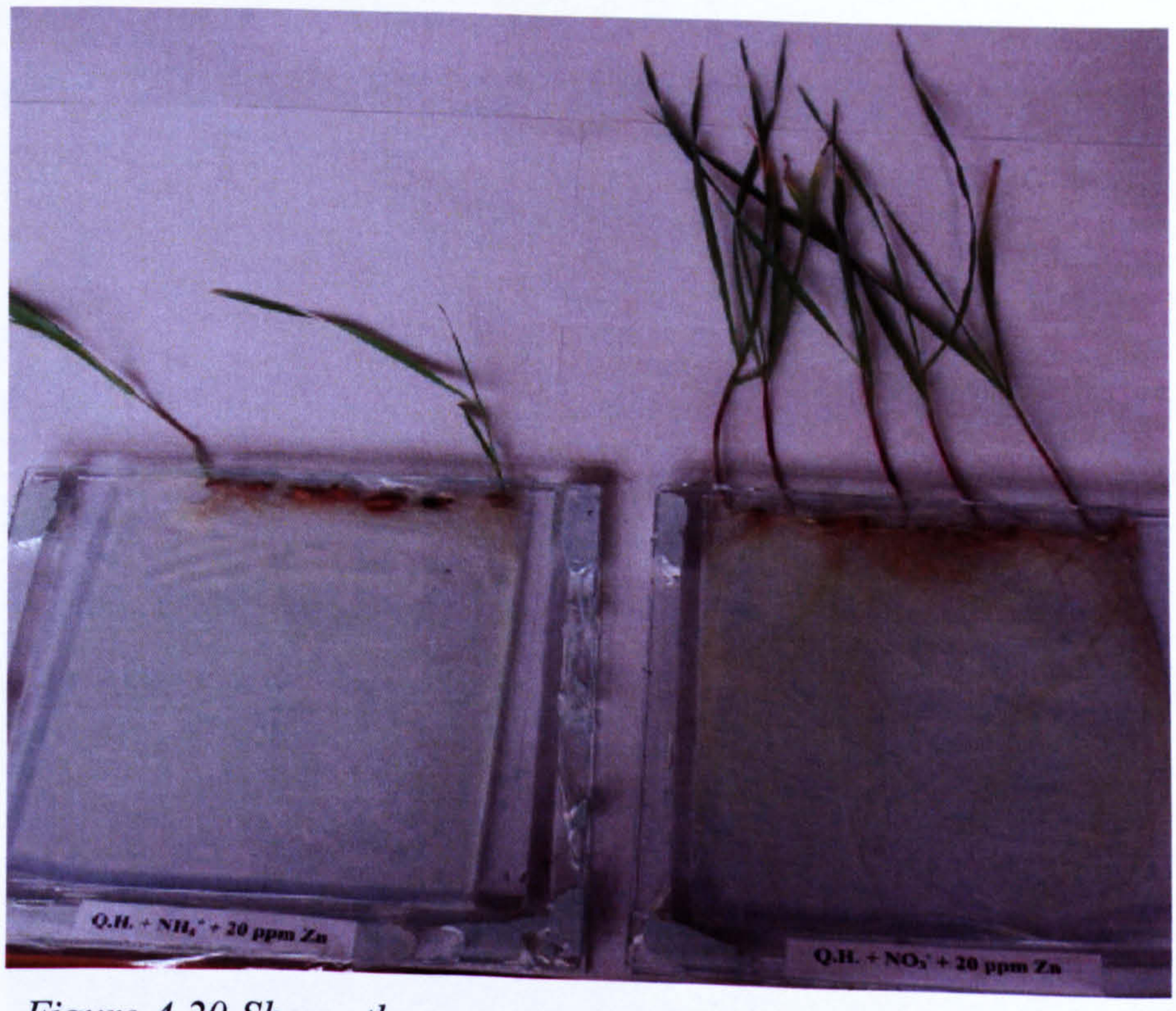

Figure 4.20 Shows the root growth of oats plant with NH_4^+ or NO_3^- and 20 mg/l Zn concentration using Bromocresol purple.

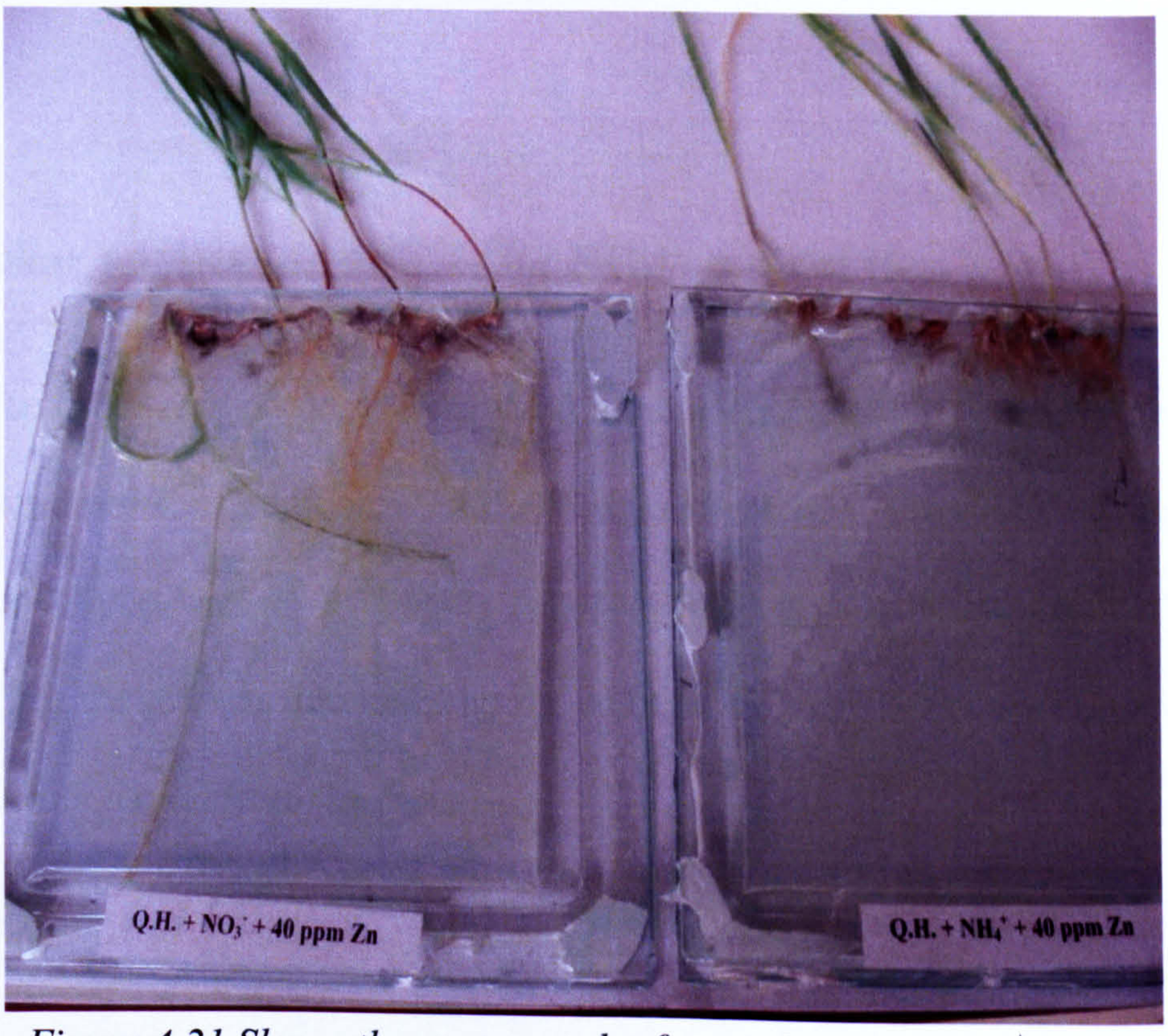

Figure 4.21 Shows the root growth of oats plant with NH_4^+ or NO_3^- and 40 mg/l Zn concentration using Bromocresol purple.

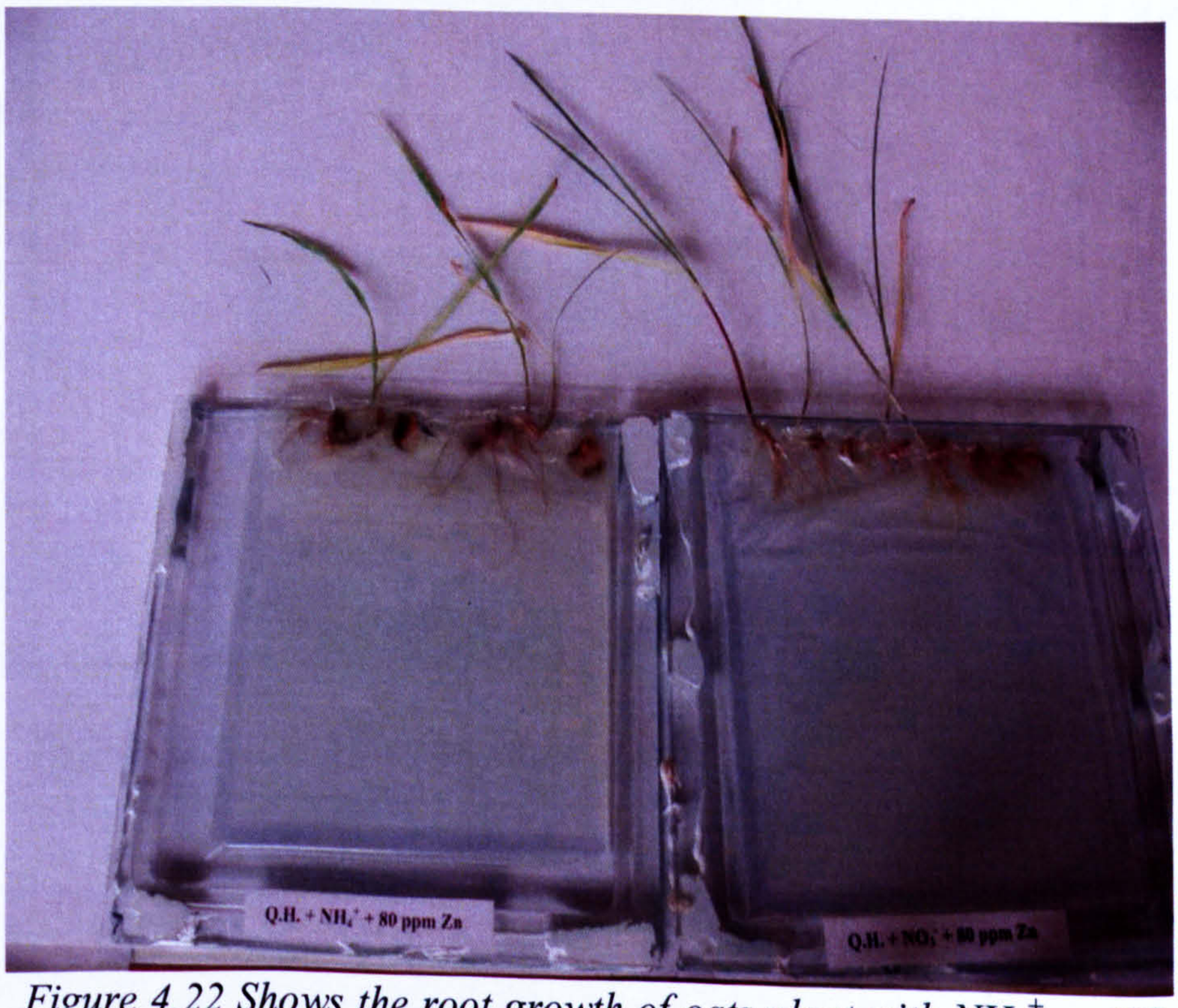

Figure 4.22 Shows the root growth of oats plant with NH_4^+ or NO_3^- and 80 mg/l Zn concentration using Bromocresol purple.

4.3.5 Foliar application of NH_4^+ or NO_3^- on plant (flax)

The other methodology followed for the addition of NH_4^+ or NO_3^- was as a foliar spray instead of addition with the Hoagland solution. There was no change in the pH and there was no difference between NH_4^+ and NO_3^- (Figure 4.23). This revealed that the pH was decreased by the addition of NH_4^+ in the rhizosphere due to exchange with the H^+ from the roots.

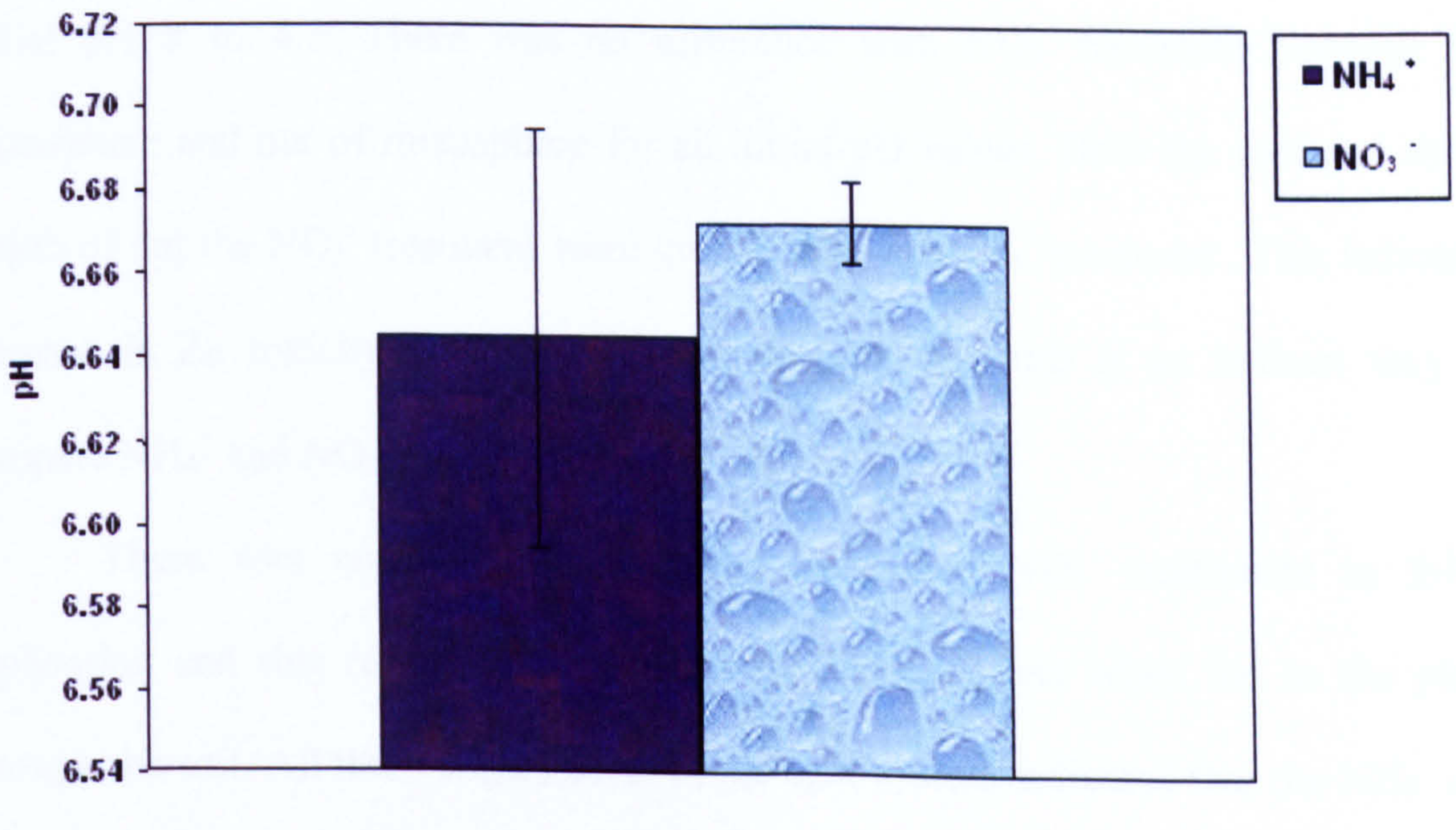

Figure 4.23 The effect of foliar application of NH_4^+ or NO_3^- on the pH of flax rhizosphere.

4.4 Conclusion

To explore the effect of NH_4^+ or NO_3^- on the increase or decrease of the pH in the rhizosphere 5 experiments were performed: different plants, two different pHs, five different pHs, different metal concentration (Zn) and different methods of NH_4^+ and NO_3^- application (foliar application). The NH_4^+ decreased the pH of the rhizosphere of all the plants in different magnitude compared with NO_3^-, control and out of rhizosphere. Bromocresol purple indicator as shown in photographs and pH measurement revealed this pH effect. The NO_3^- treatment did not affect the plants' rhizosphere as compared with out of rhizosphere and control. The NH_4^+ decreased pH in the rhizosphere at the initial pH 7 compared with out of rhizosphere, control and NO_3^- treatments and raised the initial pH 3.2 to 4.2. The NO_3- was slightly raised the pH compared with control and out-rhizosphere.

The NH_4^+ treatment maintained the pH 4.1 at initial pH 4 and decreased the initial pH 8 to 4.8. There was no difference with NO_3^- treatment between in-rhizosphere and out of rhizosphere for all initial pH values. Both the root and shoot length of oat the NO_3^- treatment were greater than for NH_4^+ treatment. This indicates increase in Zn toxicity with NH_4^+ due to low pH and this is an indirect way to compare NH_4^+ and NO_3^-.

There was no difference between NH_4^+ and NO_3^- treatments in foliar application and this revealed that NH_4^+ only had an effect when fed to the plant through the soil. All these experiments in the agar system indicated that the NH_4^+ can decrease the pH in the rhizosphere of the plants and could play an important role in manipulation of the rhizosphere in the bioavailability of heavy metals. Ammonium could be used as a control of solubility and accessibility of heavy metals by changing the rhizosphere pH. Other experiments will investigate and study the effect of

amendments with different metal concentration and plant phytoextraction; it is necessary to build that on to investigate heavy metal toxicity to some plants which will be use as a test crop in different heavy metal concentrations.

Chapter 5

Effect of different concentrations of Zn, Cu, and Pb on the seed germination of different crops

5.1 Introduction

This chapter is not going to explain deeply the physiological aspects of toxicity of these metals, which is explained elsewhere, rather the levels of concentration, which affect the germination of seeds. The aim of these experiments was to evaluate the effect of Cu, Zn and Pb toxicity on test crops, obtaining the lowest and highest concentration of these metals for crop growth.

5.2.1 Zn

A stock solution of 2000 mg/L of zinc was prepared by dissolving exactly 17.59 g of $ZnSO_4 .7H_2O$ and concentrations of Zn (0,100, 200, 400, 500, 600, and 2000 mg/l) for pea and barley and (0, 40, 100, 200, 500, 1000 and 2000 mg/l) for flax were prepared, Three replicate Petri dishes were set up with 10 seeds of pea or barley or flax and distributed in a completely randomised design on the table in the laboratory at the room temperature and covered with aluminium foil to keep them in the dark. After one week germinated seeds were counted and after 2 weeks plant shoot and root lengths were measured in cm.

5.2.2 Cu

A stock solution of Cu was prepared by dissolving exactly 15.859 gm of $CuSO_4 .5H_2O$ in water in a two litre volumetric flask and different concentrations of Cu (0, 20, 40, 60, 80, and 120 mg/l) otherwise the same procedure as described in section 5.2.1.

5.2.3 Pb

A stock solution of 2000 mg/l of lead was prepared by dissolving exactly 6.4 g of $Pb(NO_3)_2$ in a 2 litre of water in a volumetric flask and different concentrations of Pb (100, 200, 400, 500, 600, and 2000 mg/l) were used as in section 5.2.1

5.3 Result and discussion

5.3.1 Zinc

Zn concentrations affected pea germination drastically above 450 mg/l and the concentration that decreased to 50% germination of the control (D50) was 600-700 mg/l (Figure 5.1 and 5.2). The tolerance of toxicity is different from one species to the other. Pea had different sensitivity to Zn from barley. Yang *et al.* (2005) indicated that the germination success includes straightened hypocotyls and radical from the seeds. In pea only the hypocotyls were shown in (Figure 5.2) with different concentrations of Zn, but it gave good indication compared with control.

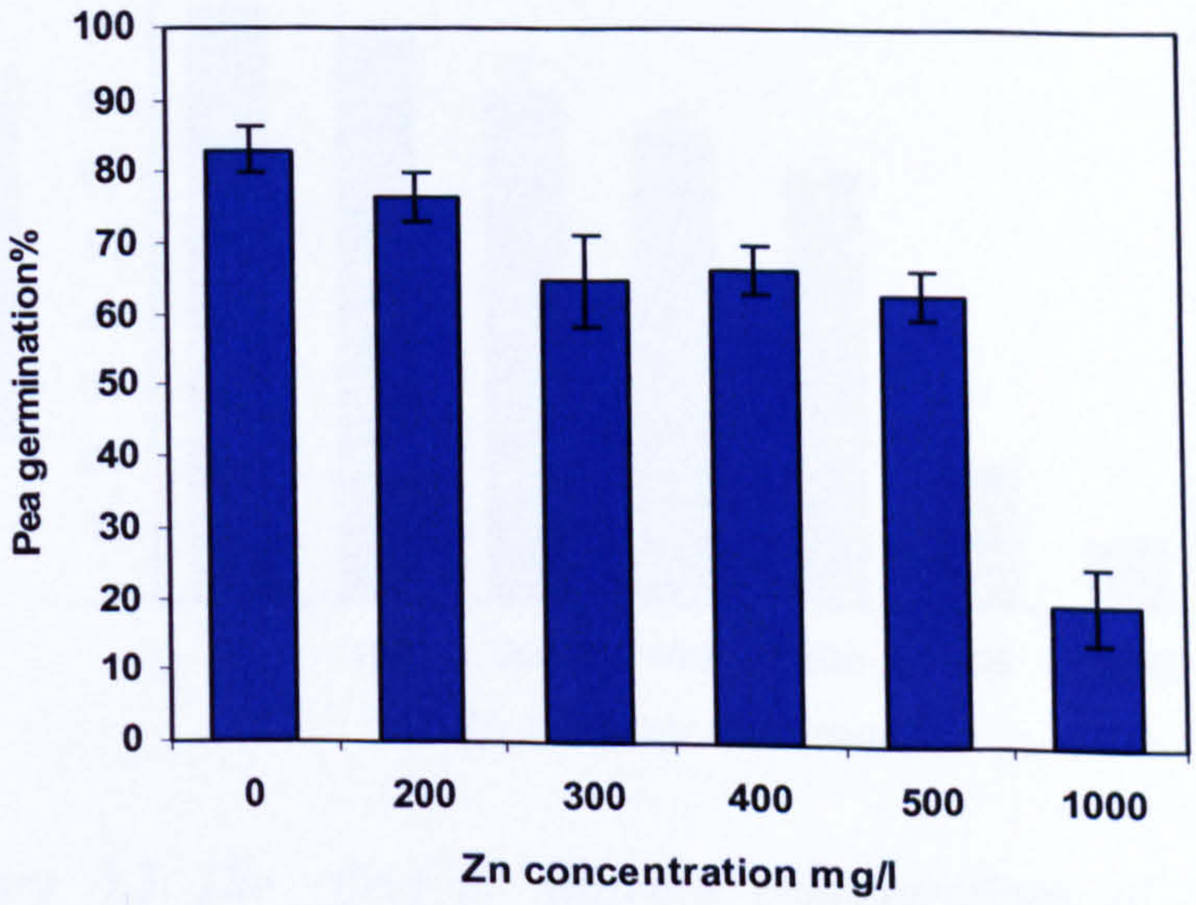

Figure 5.1 Effect of different concentrations of Zn in mg/l on germination of Pea.

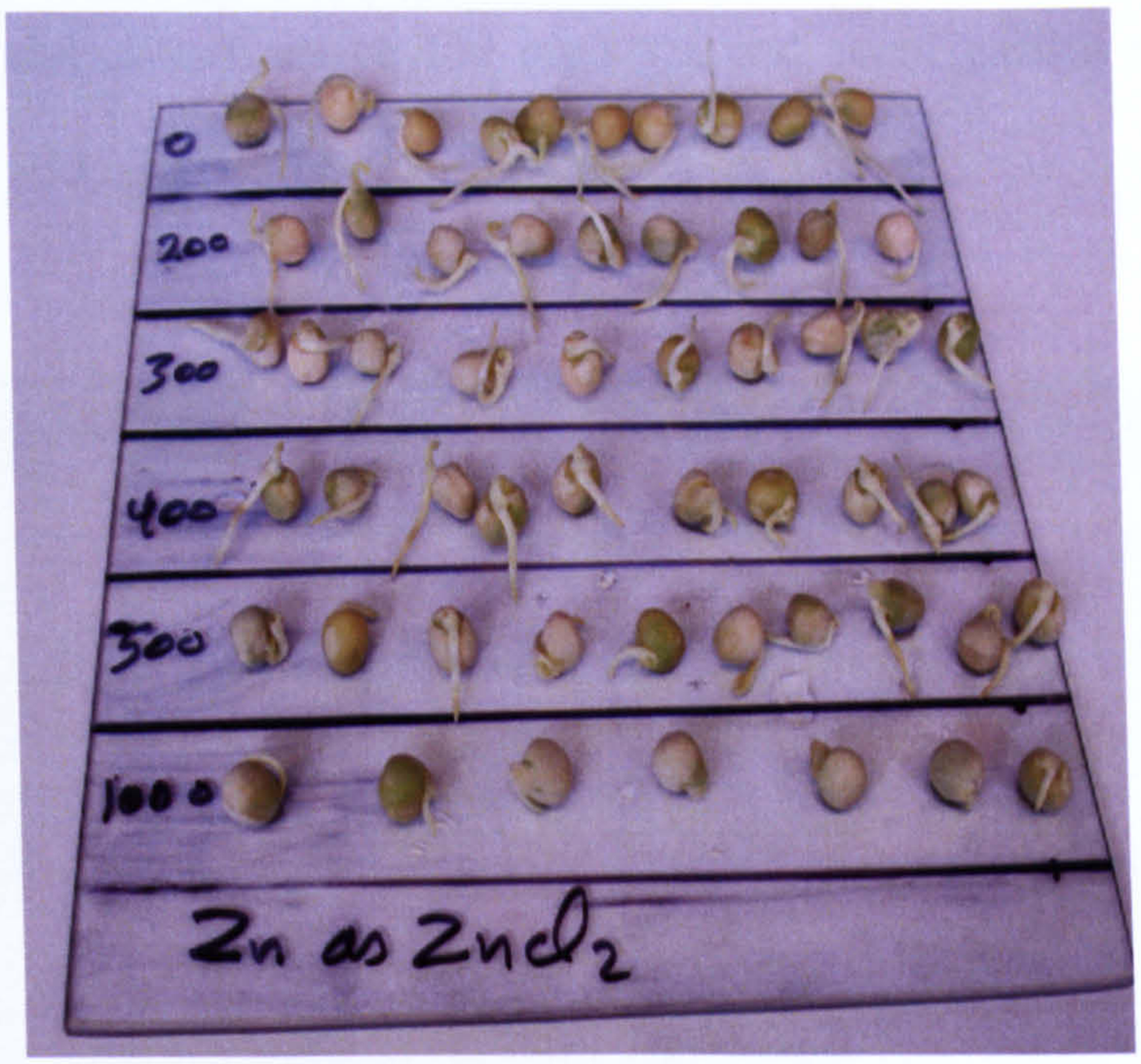

Figure 5.2 Germination of pea seeds in different concentrations of Zn in mg/l.

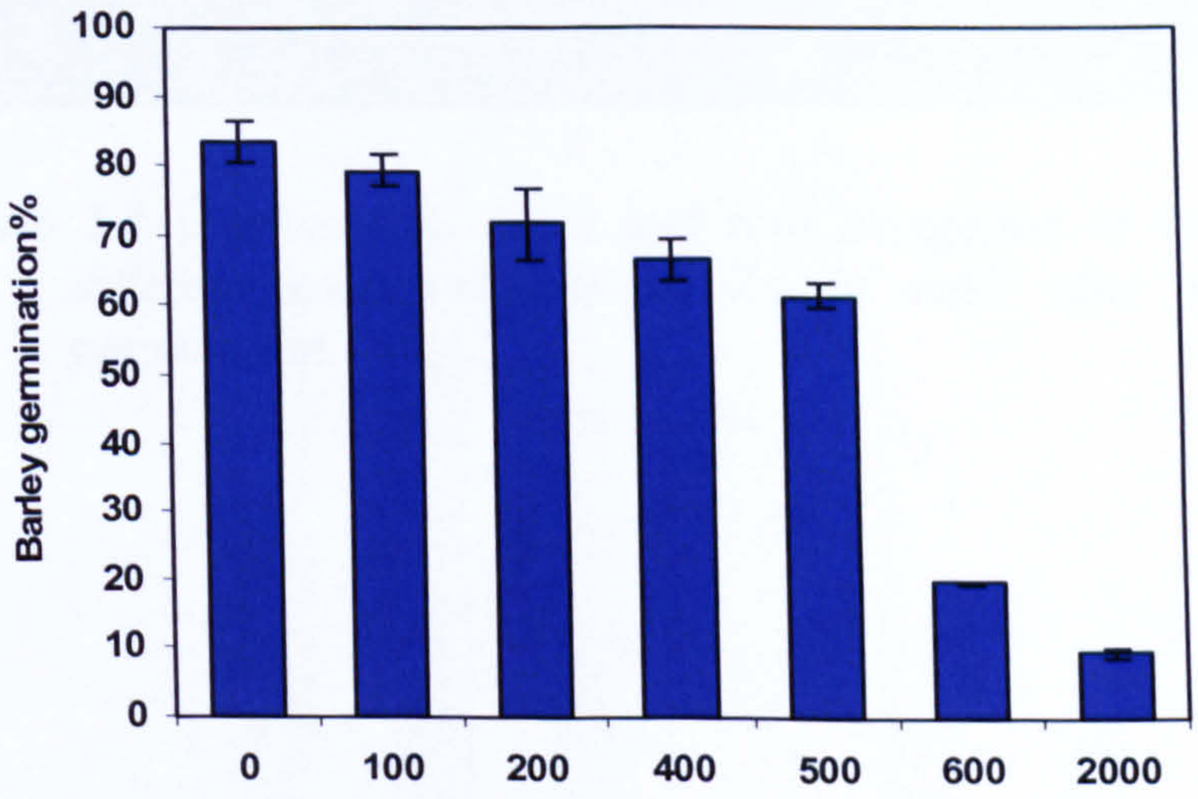

Figure 5.3 The effect of different concentrations of Zn in mg/l on germination% of barley

Germination of barley seeds decreased drastically with increasing Zn concentrations and the D50 of germination of barley seeds was at 550 mg/l (Figure 5.3 and 5.4). There was no root growth above 400 mg/l Zn, and shoot growth was drastically inhibited above 500 mg/l.

Figure 5.4 Germination, shoot and root elongation of barley seeds in different concentrations of Zn in mg/l after one week of germination.

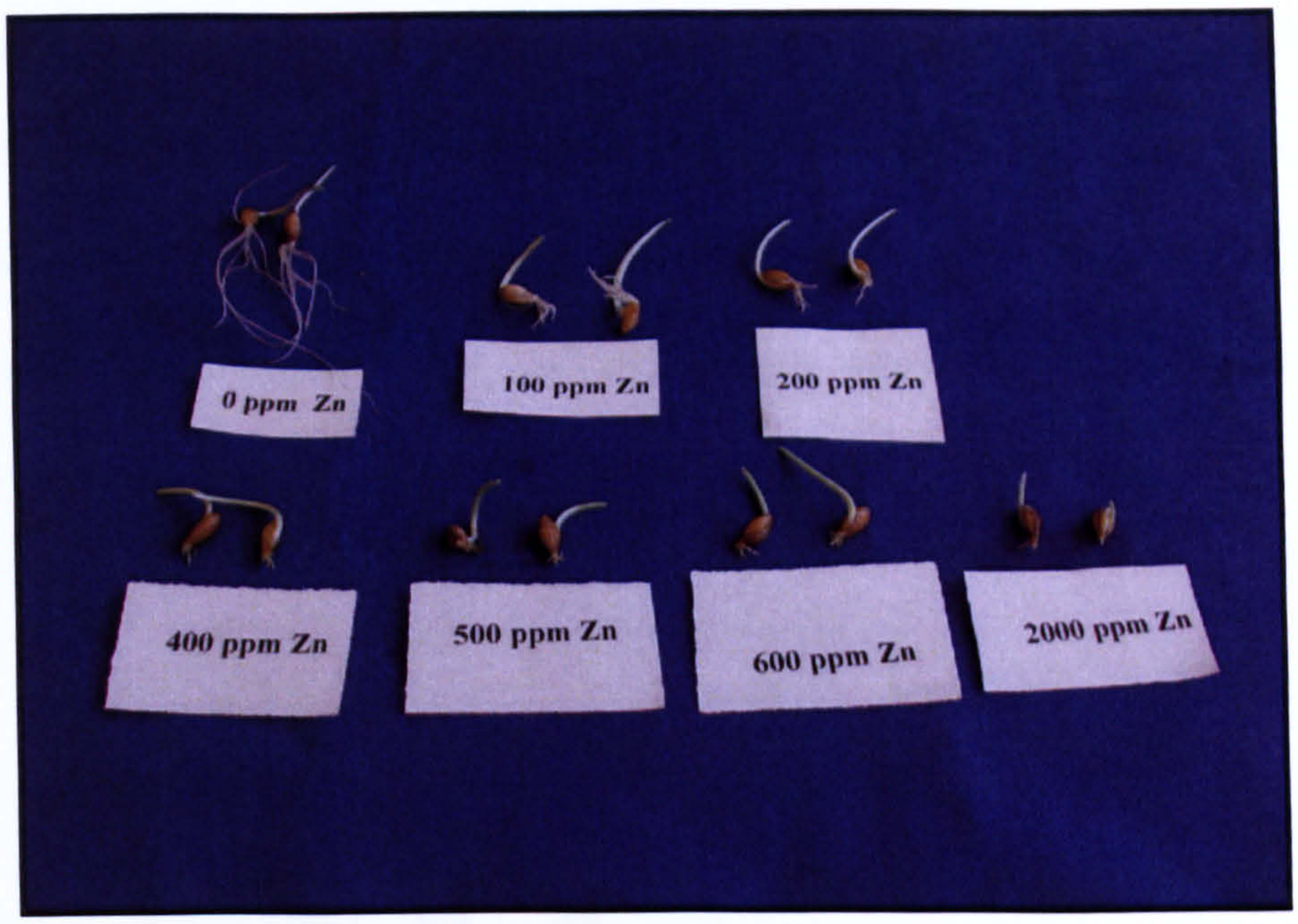

Figure 5.5 Germination, shoot and root elongation of barley seeds in different concentrations of Zn in mg/l after one week of germination.

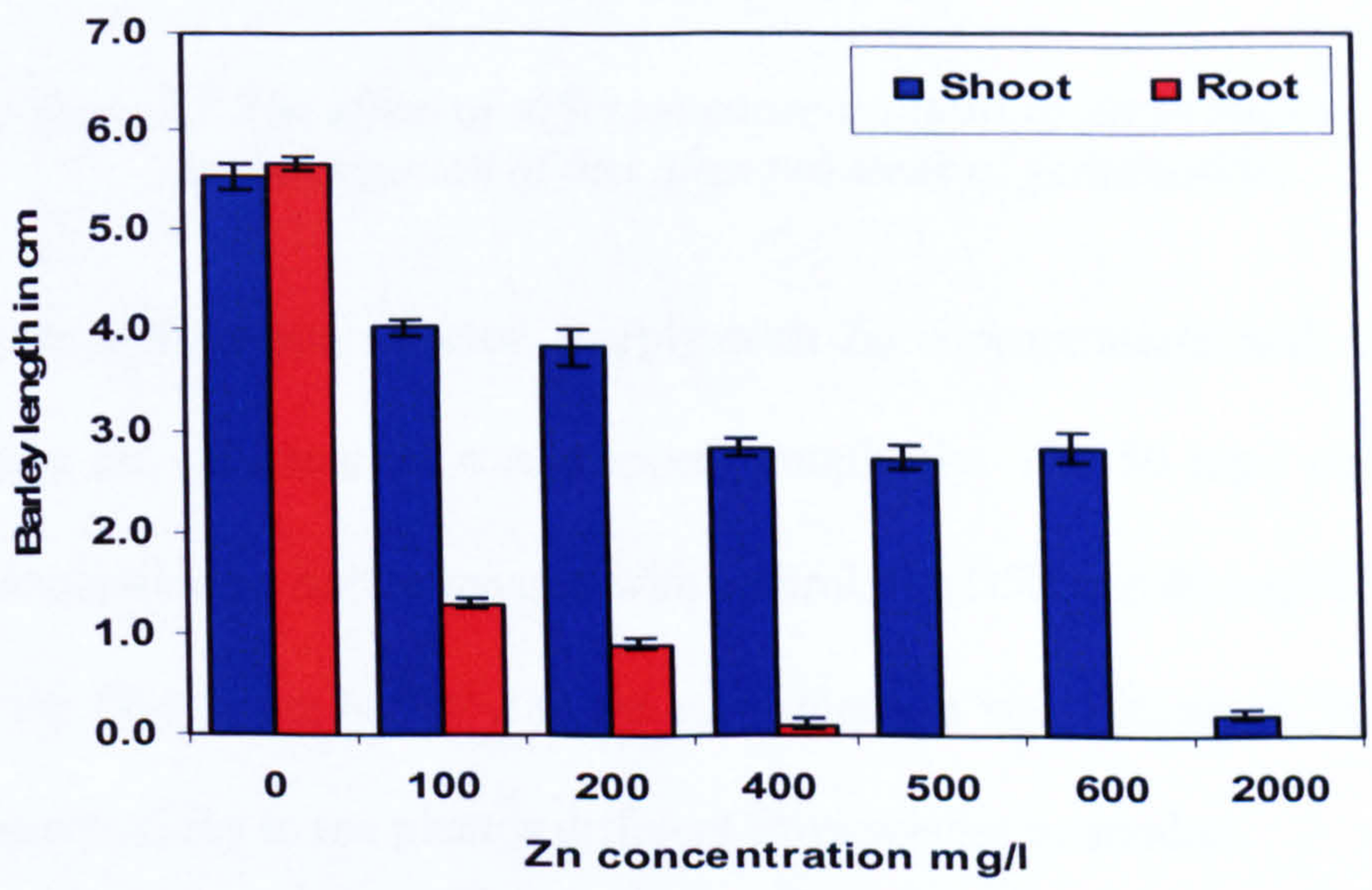

Figure 5.6 The effect of different concentrations of Zn in mg/l on shoot and root elongation of barley two weeks after germination.

Increasing zinc concentration decreased flax shoot and root growth, but the root was more affected. At 40 mg/l Zn concentration of the root length was reduced to half that of the control (R50), and above100 mg/l the root growth was completely blocked. The difference between shoot length and root length was mostly constant at all the concentrations.

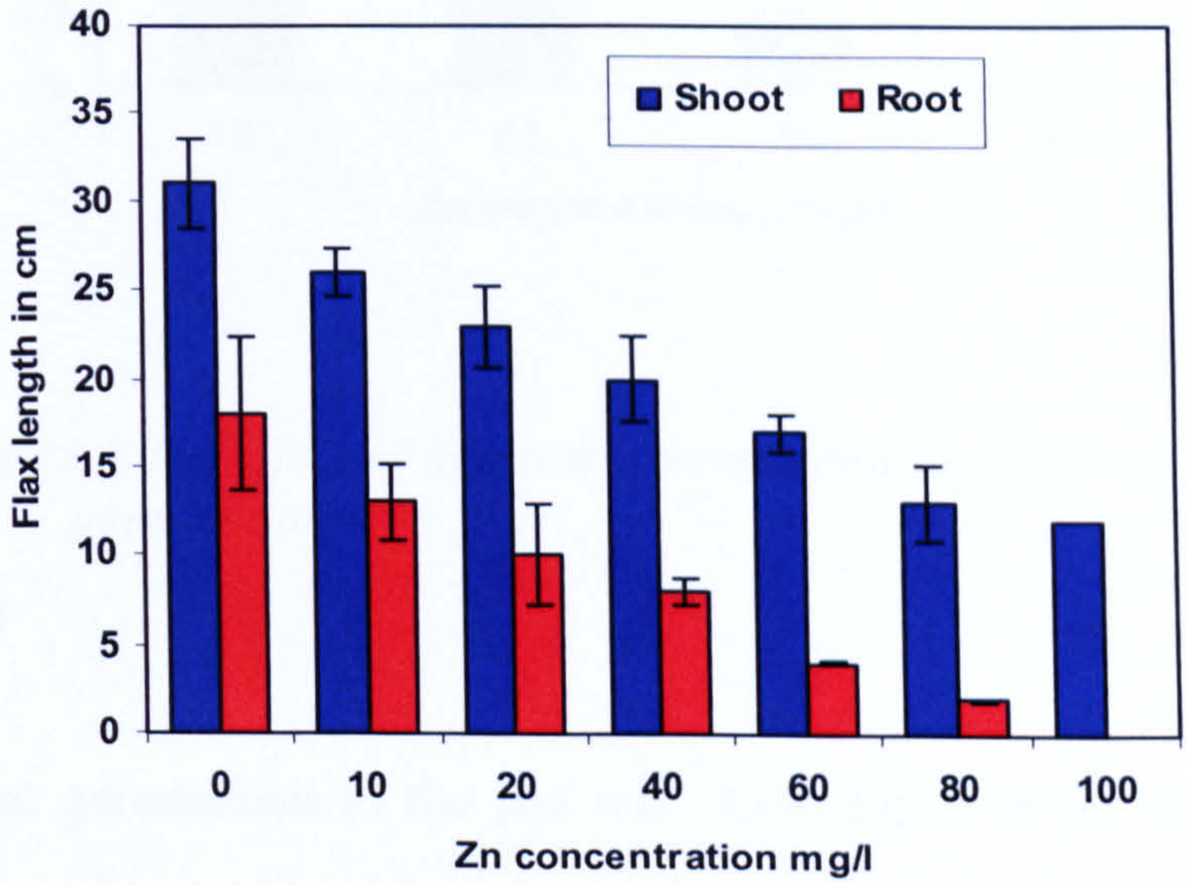

Figure 5.7 The effect of different concentrations of Zn in mg/l on shoot and root elongation of flax after two week of germination.

The flax germination was affected sharply with Zn concentrations and at 200 mg/l concentration the germination was stopped completely. The 50 mg/l concentration reduced germination by half compared with control, the D50 was 40 mg/l (Figure 5.8), and while the D50 in the barley with the same element was 500 mg /l. This revealed that the metal toxicity to the plant is different from species to another.

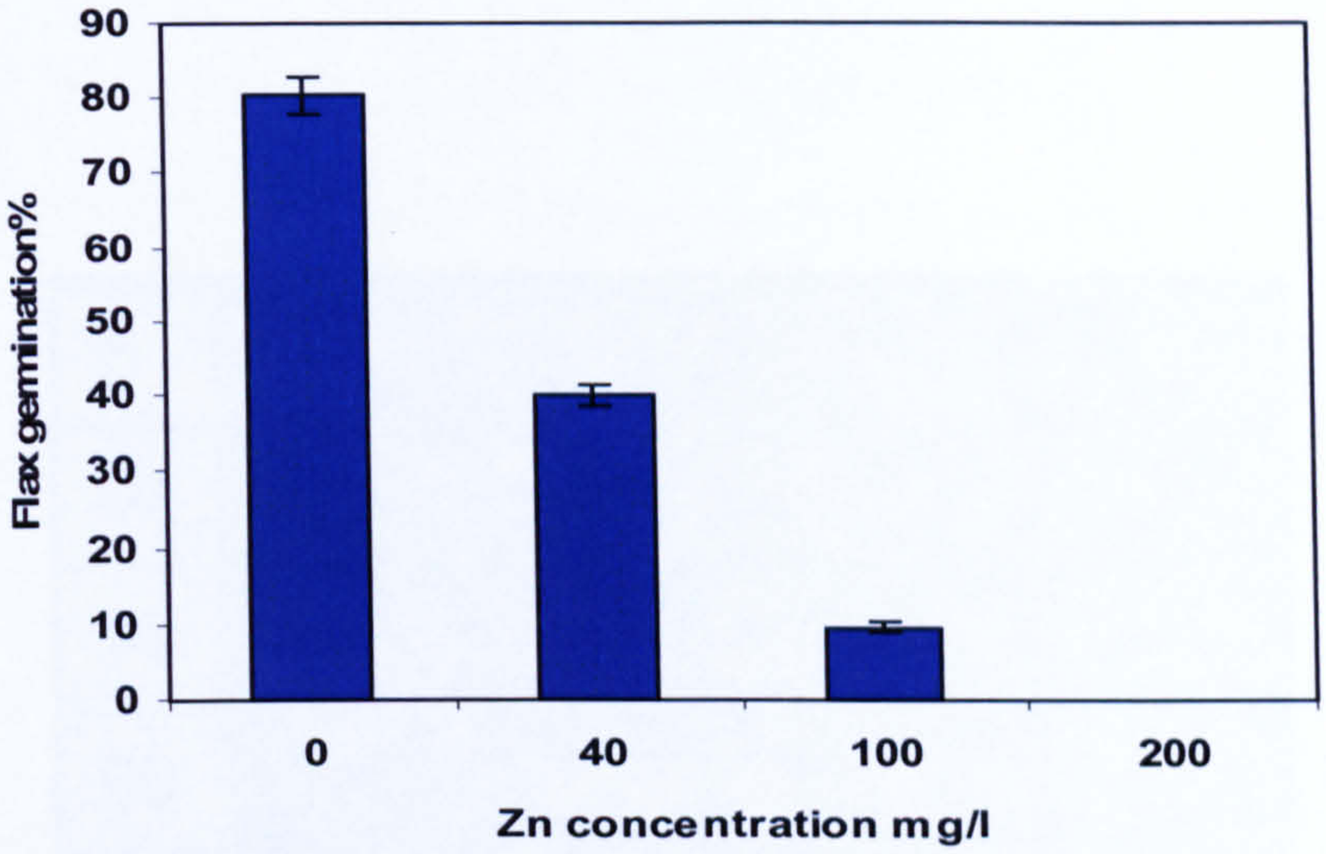

Figure 5.8 The effect of different concentrations of Zn in mg/l on flax seed germination.

5.3.2 Copper

The seed germination of the pea was drastically affected by >20 mg/l Cu concentration, D50 was a 70 mg/l and only 23% germinated at 120 mg/l (Figure 5.9 and 5.10).

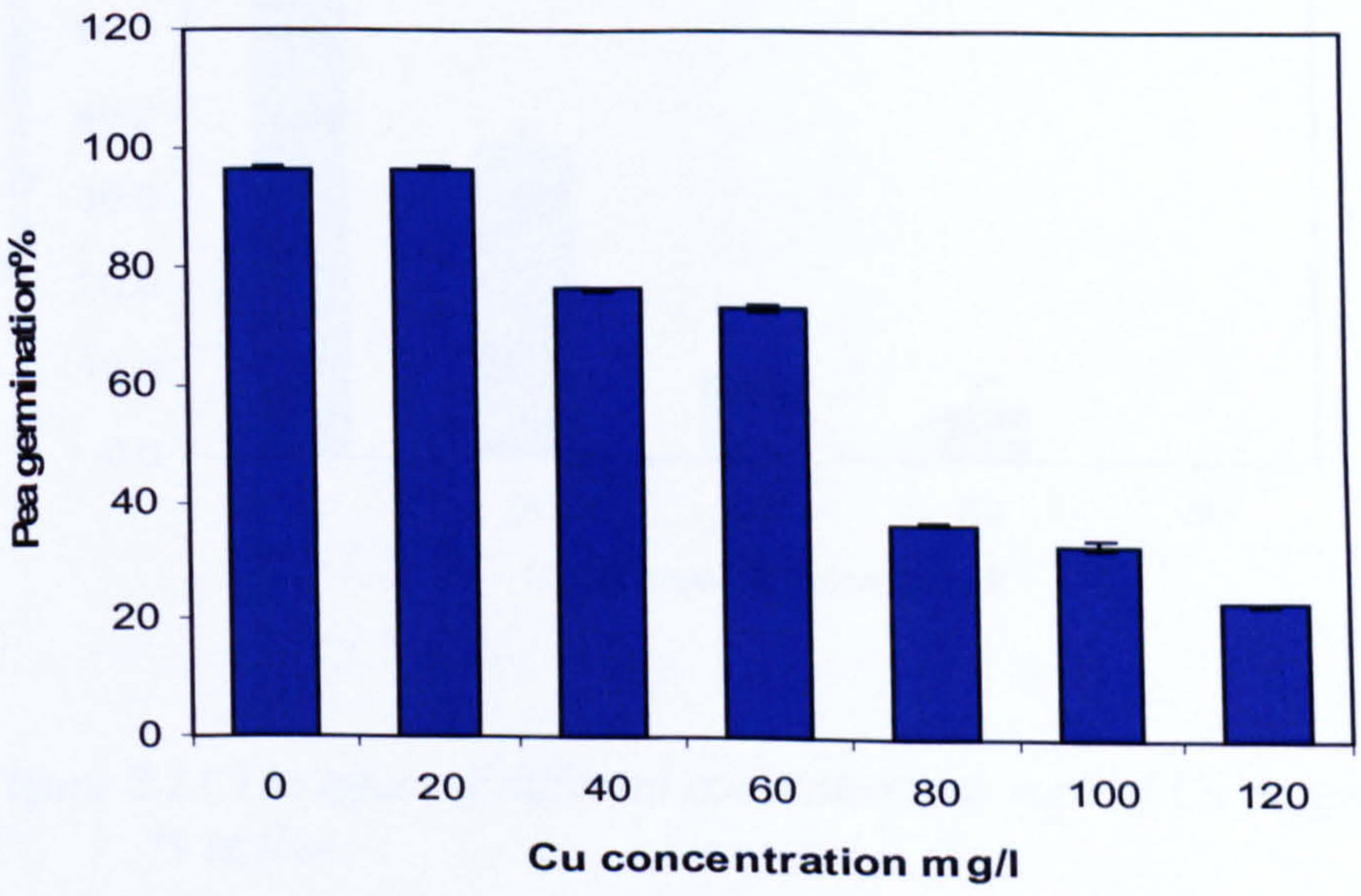

Figure 5.9 The effect of different concentrations of Cu in mg/l on germination % of pea.

Figure 5.10 Germination of pea seeds in different concentrations of Cu in mg/l.

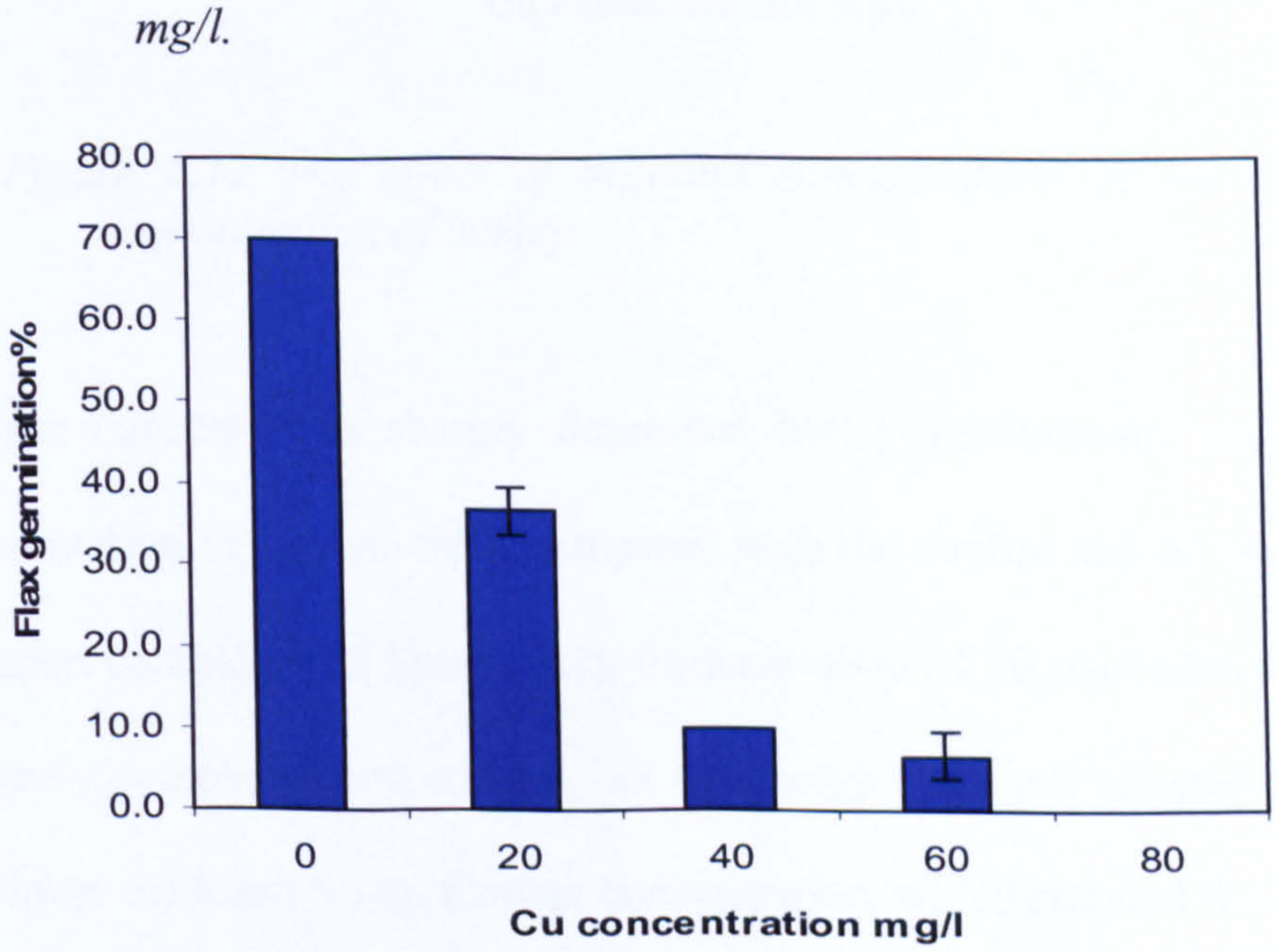

Figure 5.11 The effect of different concentrations mg/l of Cu on germination % of flax.

The flax seed germination was affected by Cu concentrations drastically and inhibited completely at 80 mg/l of Cu concentration. The reduction of seed germination of flax was D50 at 20 mg/l Cu concentration (Figure 5.11). The flax was more affected by Cu than pea. Presumably this is attributed to different seed size.

Due to small size of flax seeds, only germination was done.

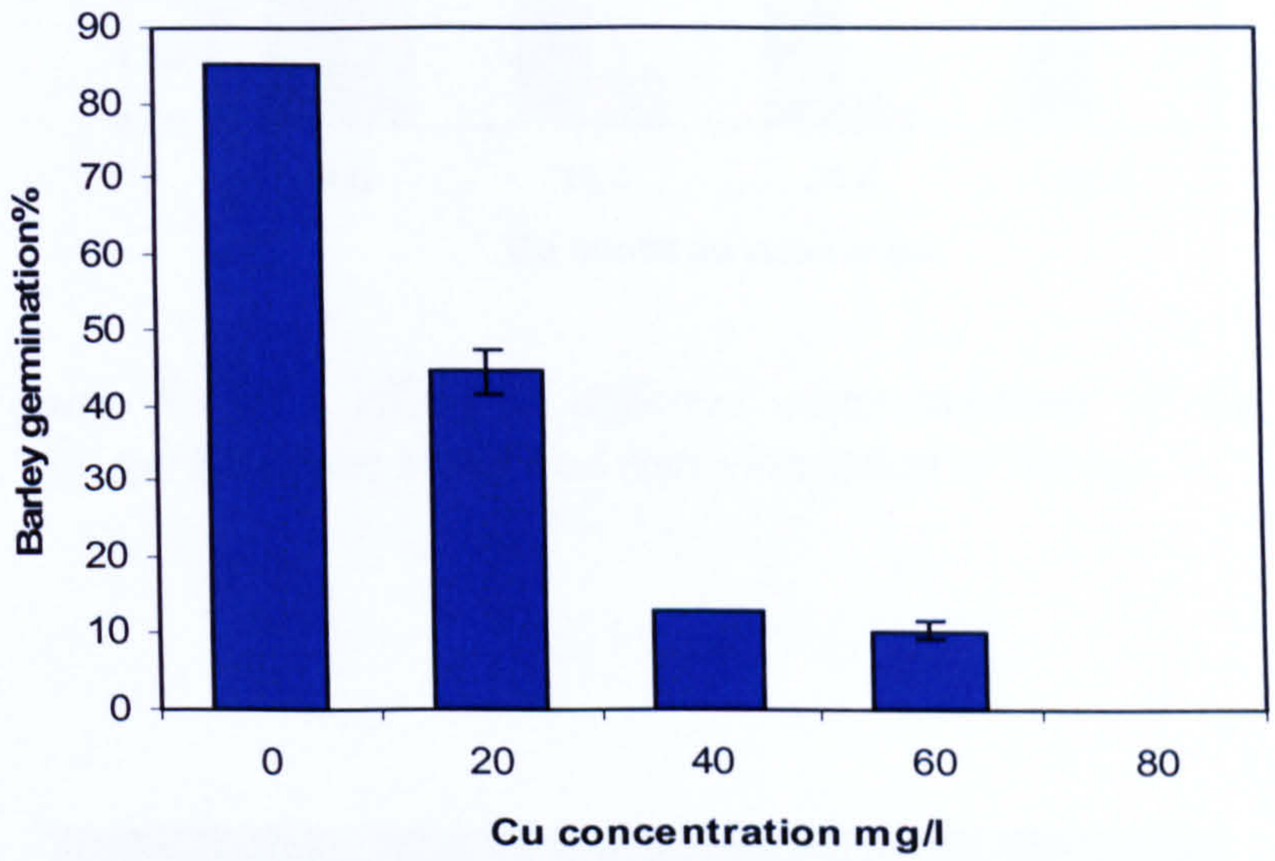

Figure 5.12 The effect of different concentrations of Cu in mg/l on germination of barley.

Copper concentration sharply depressed barley germination, only 20 mg/l reduced germination more than 50% compared with the control and 80 mg/l blocked the germination completely (Figure 5.12). Concentration of 20 mg/l affected the root growth severely compared with control, but hypocotyls were not affected as much as the roots (Figure 5.13 and 5.14). Copper concentration, which reduced the root length to 50% (R50) of the control length, was 5 mg/l and R50 was similar for shoots.

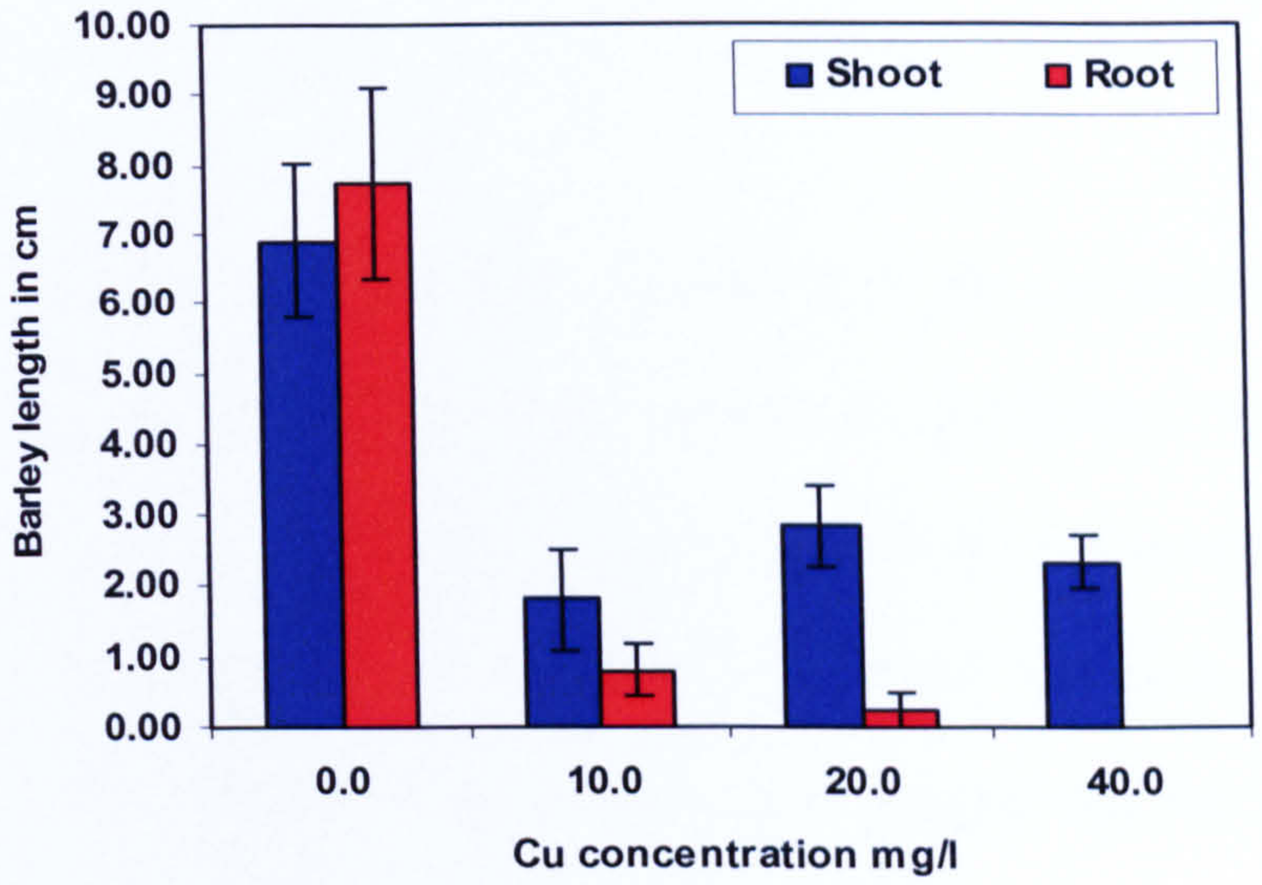

Figure 5.13The effect of different concentrations of Cu in mg/l on germination, shoot and root elongation of barley.

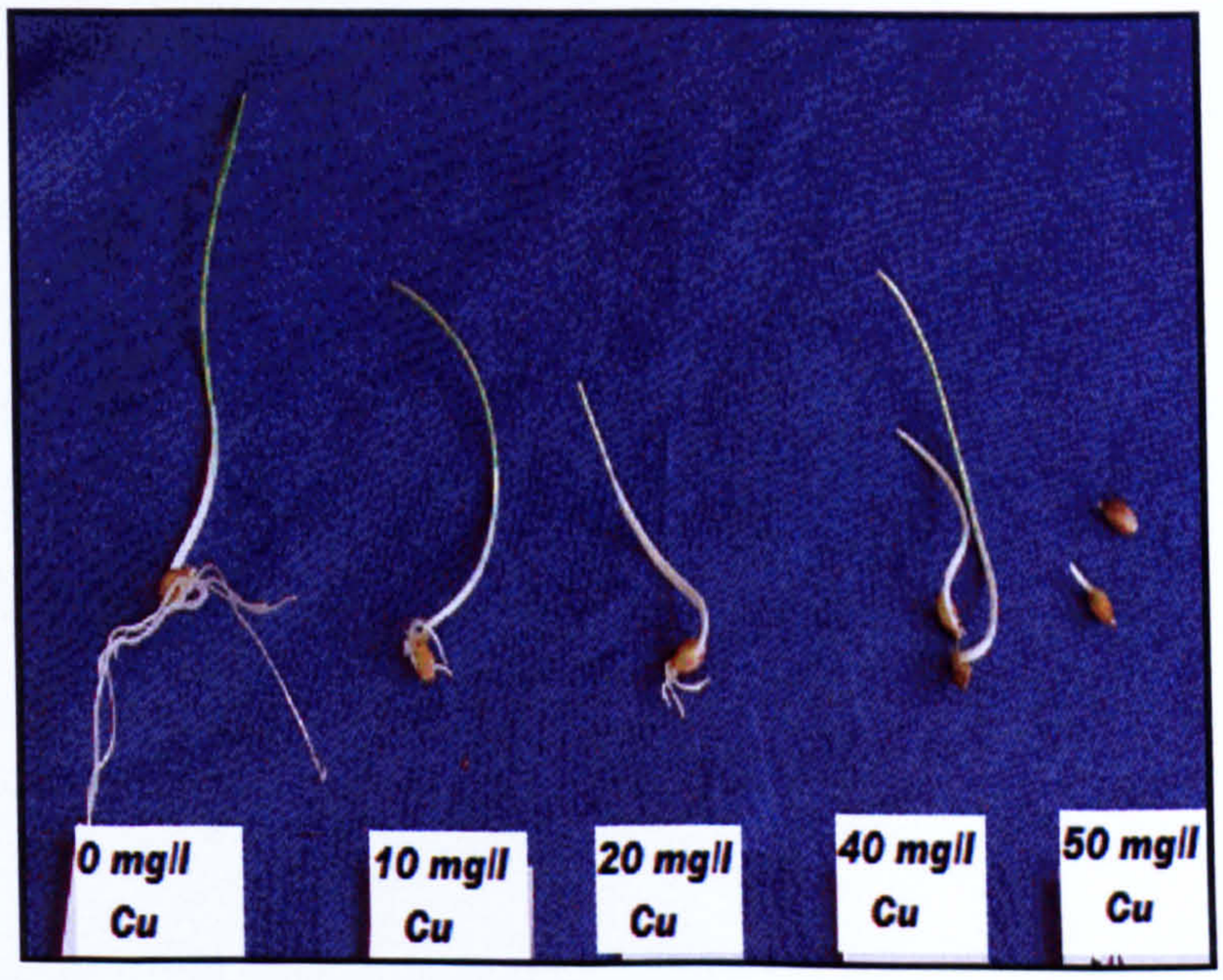

Figure 5.14 Germination, root and leaf elongation of barley in different concentrations of Cu in mg/l.

5.3.3 Lead

The lead toxicity to pea was increased with Pb concentration but not as regularly as Cu and Zn. 700 -750 mg/l decreased the germination % by 50% (D50) (Figure 5.15 and 5.16). The Pb was more toxic than Zn but not as toxic as Cu. In the pea only the hypocotyls could be compared with control, and gives a good and fast indicator for the toxicity. Some other researchers suggest that the root elongation gives a better evaluation for toxicity than seed germination and hypoctyl growth (Ye et al., 2002).

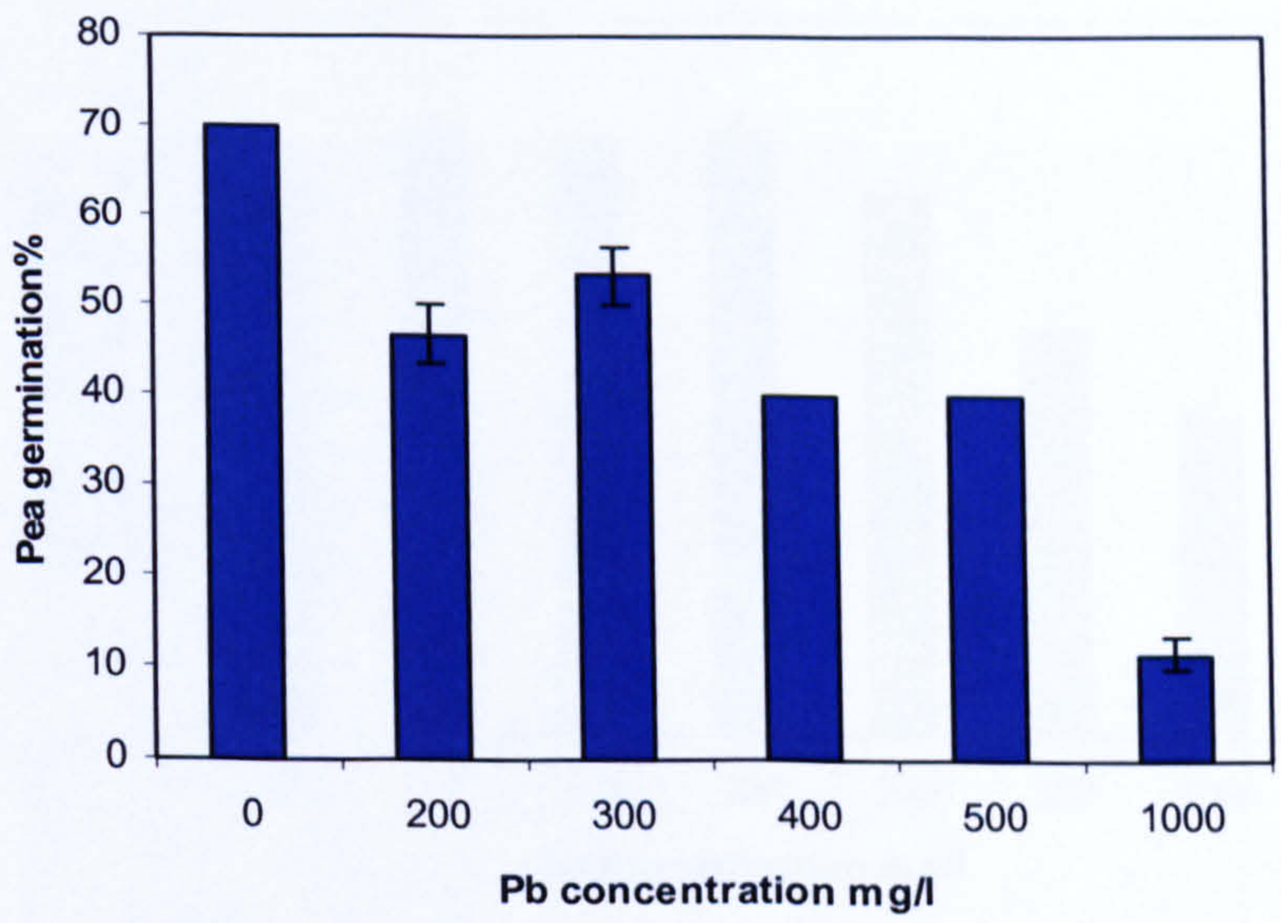

Figure 5.15 The effect of different concentrations of Pb in mg/l on germination of pea.

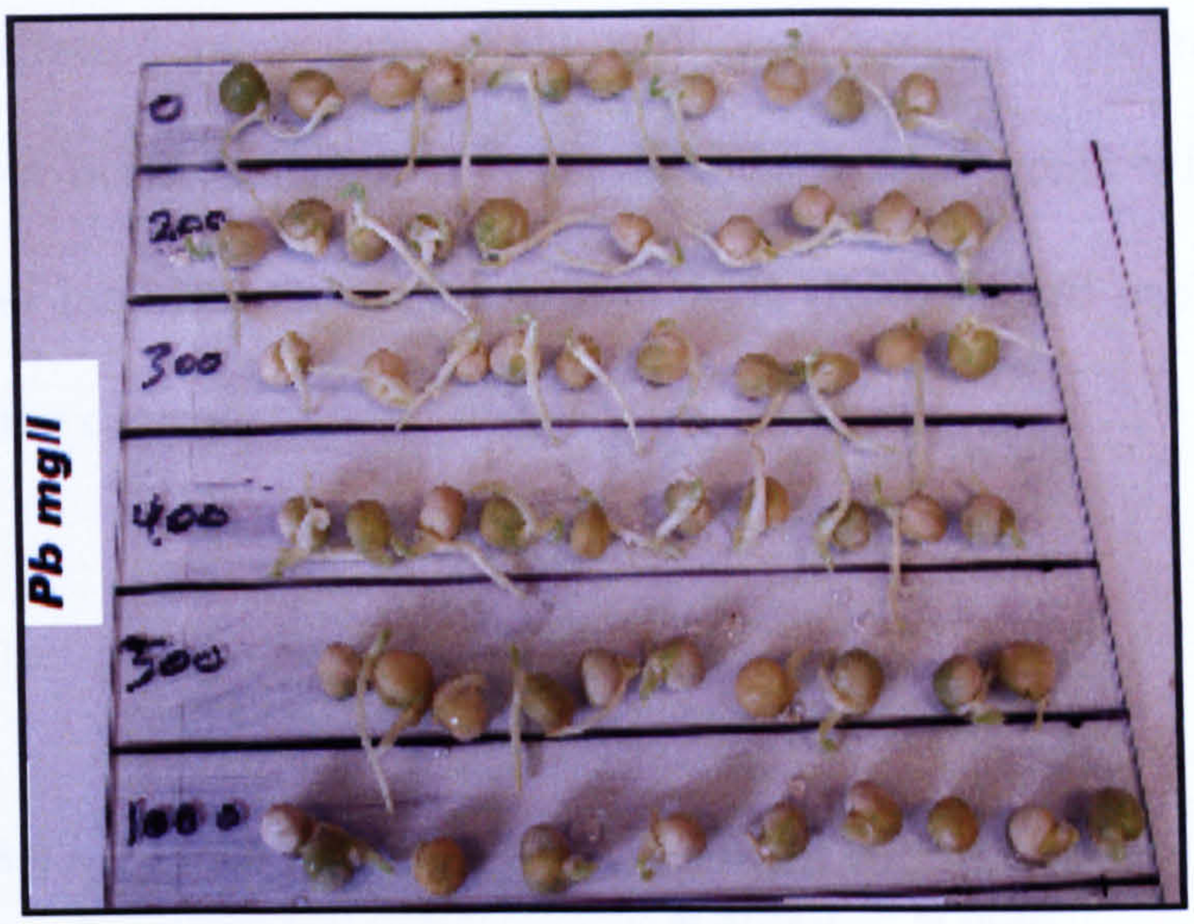

Figure 5.16 Germination of pea seeds in different concentrations of Pb in mg/l.

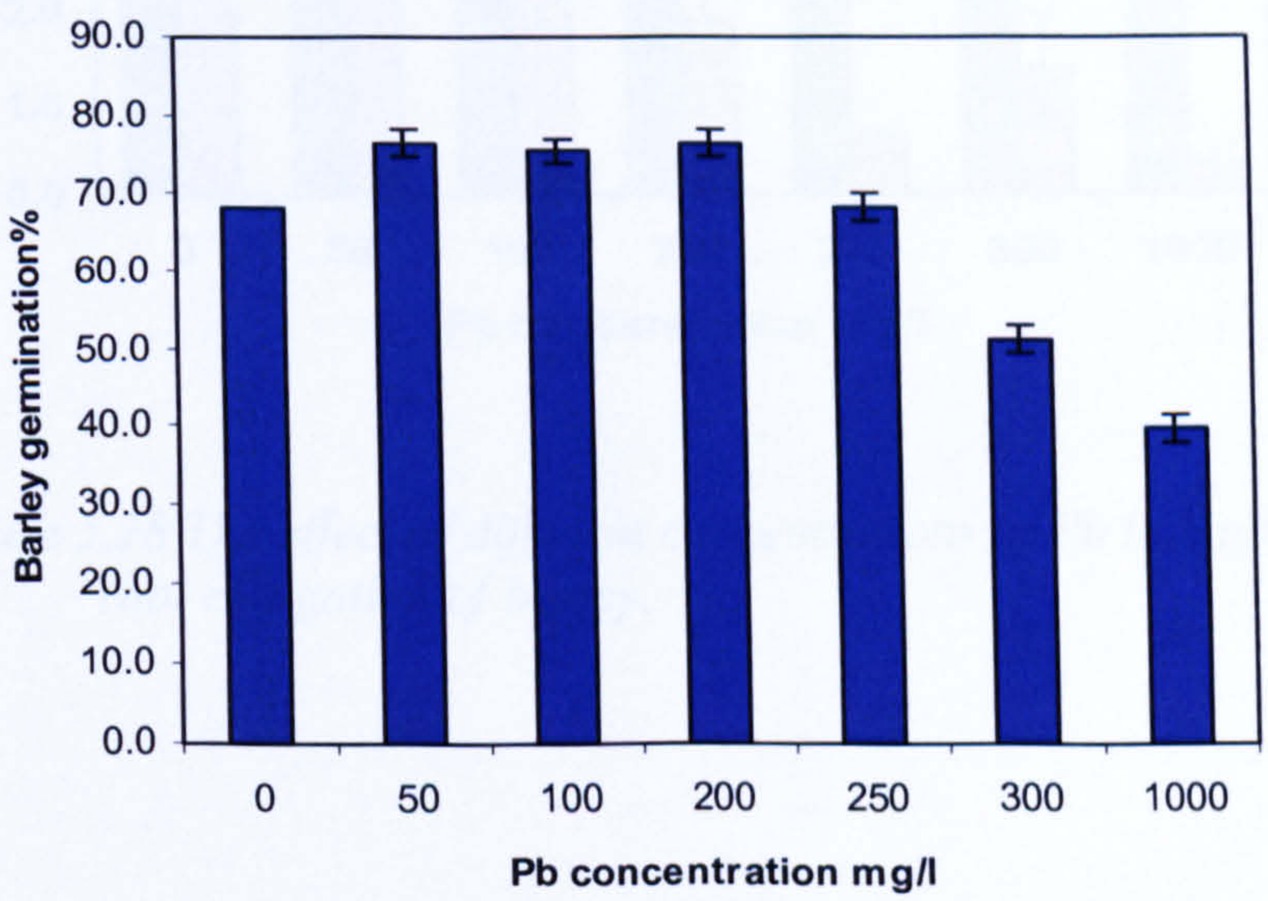

Figure 5.17 The effect of different concentrations of Pb in mg/l on germination of barley.

Effect of lead concentrations on shoot length and germination % was irregular; 1000 mg/l inhibiting a 50% of germination in comparison with control (Figure 5.17). In the assessment of phytotoxicity on the root and shoot elongation (Figure 5.18 and 5.19),

the root was affected more than shoot with lead toxicity. The radical of seed elongation was a better indicator of Pb toxicity and this agreed with Yang *et al.* (2004). The lead inhibited the root length to 50% (R50) at 150 mg/l concentration compared with the control.

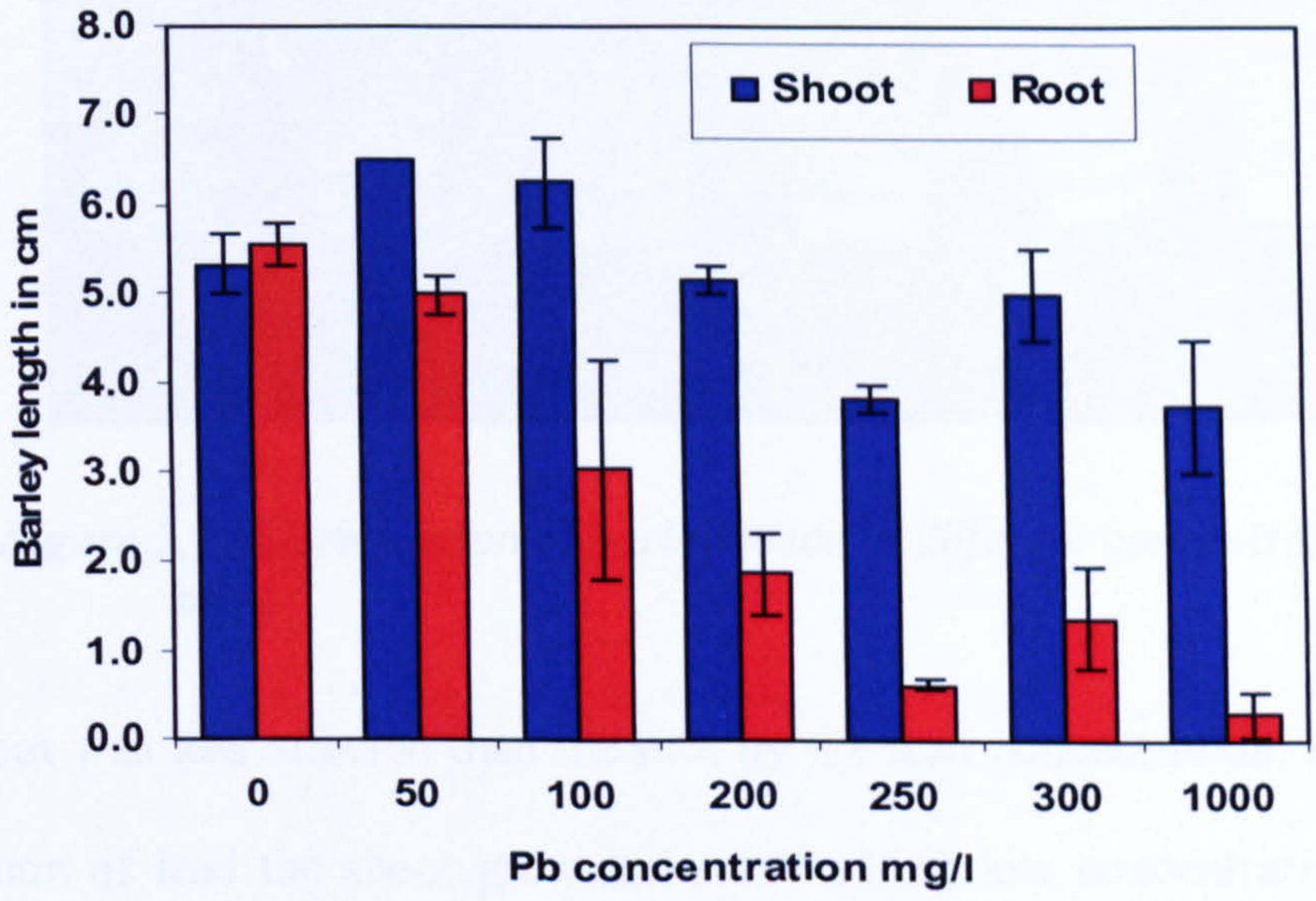

Figure 5.18 The effect of different concentrations of Pb in mg/l on shoot and root elongation of barley.

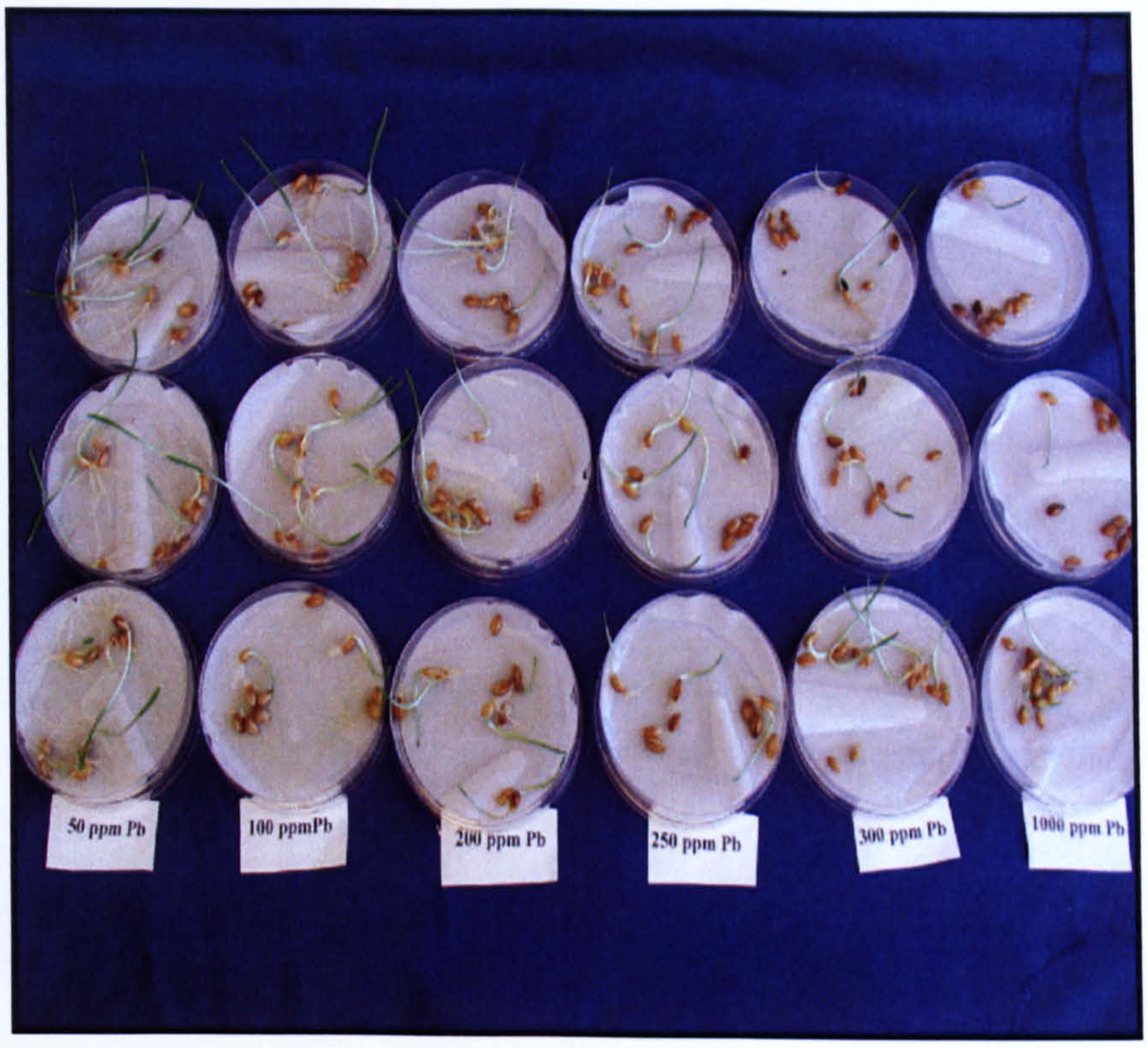

Figure 5.19 Germination of barley seeds in different concentrations of Pb in mg/l.

Barley shoot was less affected than the root by the lead concentration. At 1000 mg/l concentration of lead the shoot grew and survived. At low concentration of lead as shown in Figure 5.20, there was no effect on the germination or shoot length, but it stimulated the growth and germination of barley.

Figure 5.20 The effect of different concentrations of Pb in mg/l on germination, root and leaf elongation of barley.

Lead toxicity affected flax seed germination; 200 mg/l blocked the germination completely and the D50 was 35 mg/l concentration. This indicated that the toxicity effect is different from metal to metal and from plant to plant.

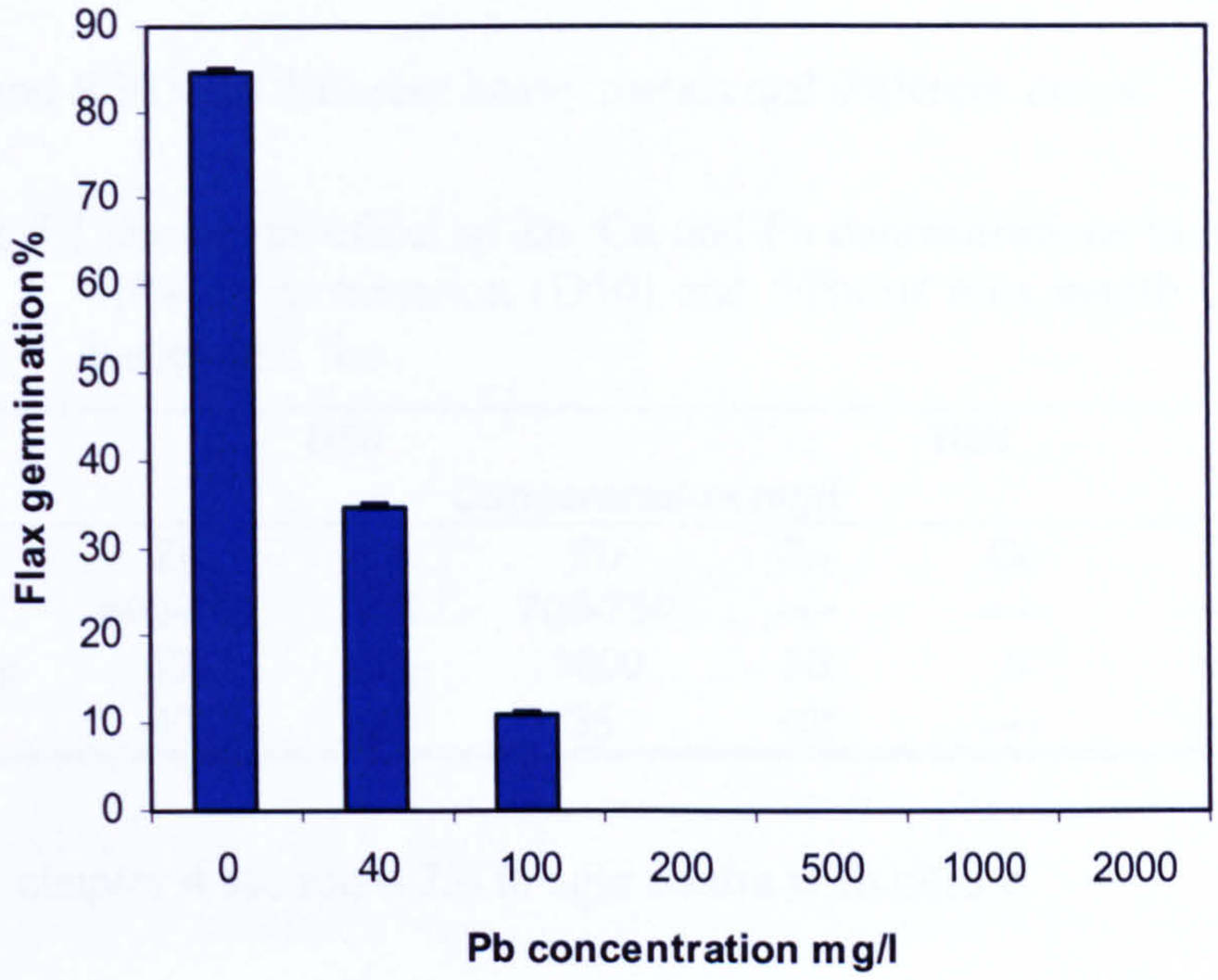

Figure 5.21 The effect of different concentrations of Pb in mg/l on germination of flax.

5.4 Conclusion

The germination was done in Petri dishes in lab conditions with different concentration of metals and in single method test (single metal concentrations). It was found that the toxicity of the metals was in the following order: Cu > Pb > Zn. The toxicity tolerance was different from plant to plant. For Cu toxicity the following order was found: pea > flax > barley. In barley, shoots were more tolerant of the toxicity than roots, especially at high concentration in both Pb and Cu. Barley shoot tolerated Cu concentration of 40 mg/l, while the roots tolerated 20 mg/l Cu concentration. Barley shoots and root can tolerate 1000 mg/l Pb. The toxicity differed from plant to plant and from species to species. Metals are different in their toxicity to the plants. A good parameter for toxicity determination was root elongation and this

agreed with Shu (2002). The more toxic heavy metal was Cu > Pb > Zn and this agreed with Wheeler (1995), for Cu and Zn. Table 5.1 shows some parameter of toxicity, D50 and R50 with different heavy metals and different crops.

Table 5.1 shows the effect of Zn, Cu and Pb concentrations in mg/l reducing 50% of germination (D50) and 50% of root length (R50) of pea, barley and flax.

Crop	D50			R50		
	Concentration mg/l					
	Zn	Cu	Pb	Zn	Cu	Pb
Pea	600-700	70	700-750	----	-----	-----
Barley	550	25	1000	50	5	150
Flax	40	20	35	40*	----	------

* results from chapter 4 section 4.2.4 in agar media with nitrate

Information on metal toxicities obtained from these experiments designated in this chapter was used to give a general idea about the concentration range, especially high concentrations, in future experiments.

Chapter 6

Effect of different pH and two sources of nitrogen (NH_4^+ and NO_3^-) on Phytoextraction of Zn and Cu in agar media using barley as a test crop.

6.1 Introduction

The pH is one of the main factors affecting the bioavailability, solubility and immobilization of heavy metals. At high pH, heavy metals precipitate as oxides, hydroxides, sulphates and carbonates (Chapter 1, section 1.2.2) and lowering the pH increases solubility. Manipulation of the rhizosphere by addition of nitrogen source as NH_4^+ or NO_3^- can decrease or increase the pH in the rhizosphere by H^+/OH^- exchange, and consequently increase or decrease the solubility and bioavailability of heavy metals in the rhizosphere. The objectives of this experiment are to assess two approaches: one is immobilization of heavy metals by increase of pH, and the second to manipulate the rhizosphere by adding NH_4^+ or NO_3^- as the nitrogen source.

6.2 Material and Methods

Quarter Hoagland solution (30 l) was prepared as in section 2.16 without nitrogen source. The solution was divided into two lots (A and B), of 15 l. From part A solutions of 120 mg Zn /l as $ZnSO_4$ (chemical grade Analar Hop.&Williams) and 60 mg Cu/l as $CuSO_4.5H_2O$ (chemical grad Analar Hop. &Williams) were prepared C.

From solution C two solutions D and E were prepared, D with NH_4^+ as $(NH_4)_2SO_4$ and E with NO_3^- as KNO_3, both of them the same as quarter Hoagland solution concentration. B solution, which has low concentration of Zn and Cu (quarter Hoagland solution), was used to prepare two solutions F and G , F with ammonium as $(NH_4)_2SO_4$ and G with nitrate as KNO_3 .The resultant four solutions (D, E, F and G) were divided into three parts each of 2l and pH adjusted to 5, 6.5 or 8. For pH 5 and 6 pH was adjusted by 0.01M HCl or 0.01M NaOH and for pH 8 pH was adjusted by Ca $(OH)_2$. All beakers were placed on a hot plate with a magnetic stirrer and agar (6.5 g/l) was added to each beaker. When solutions became clear, each treatment was poured into four 500 ml beakers; from each treatment four replicates were made. When agar was gelatinized, 15 barley seeds were seeded in each beaker. Figure 6.1 shows the layout of the experiment. After 6 weeks barley plants were harvested and prepared for analysis (chapter, 2, section, 2.6) and Cu and Zn were determined by AAS (chapter, 2, section, 2.8) and the data statistically analysed.

Block I

NH_4^+ pH 5 H Zn &Cu	NH_4^+ pH 6.5 H Zn &Cu	NH_4^+ pH 8 H Zn &Cu
NO_3^- pH 5 H Zn &Cu	NO_3^- pH 6.5 H Zn&Cu	NO_3^- pH 8 H Zn &Cu
NH_4^+ pH 5 Low Zn&Cu	NH_4^+ pH 6.5 Low Zn &Cu	NH_4^+ pH 8 Low Zn &Cu
NO_3^- pH 5 Low Zn &Cu	NO_3^- pH 6.5 Low Zn &Cu	NO_3^- pH 8 Low Zn &Cu

Block II

NH_4^+ pH 5 H Zn &Cu	NH_4^+ pH 6.5 H Zn &Cu	NH_4^+ pH 8 H Zn &Cu
NO_3^- pH 5 H Zn &Cu	NO_3^- pH 6.5 H Zn&Cu	NO_3^- pH 8 H Zn &Cu
NH_4^+ pH 5 Low Zn&Cu	NH_4^+ pH 6.5 Low Zn &Cu	NH_4^+ pH 8 Low Zn &Cu
NO_3^- pH 5 Low Zn &Cu	NO_3^- pH 6.5 Low Zn &Cu	NO_3^- pH 8 Low Zn &Cu

Block III

NH_4^+ pH 5 H Zn &Cu	NH_4^+ pH 6.5 H Zn &Cu	NH_4^+ pH 8 H Zn &Cu
NO_3^- pH 5 H Zn &Cu	NO_3^- pH 6.5 H Zn&Cu	NO_3^- pH 8 H Zn &Cu
NH_4^+ pH 5 Low Zn&Cu	NH_4^+ pH 6.5 Low Zn &Cu	NH_4^+ pH 8 Low Zn &Cu
NO_3^- pH 5 Low Zn &Cu	NO_3^- pH 6.5 Low Zn &Cu	NO_3^- pH 8 Low Zn &Cu

Block IV

NH_4^+ pH 5 H Zn &Cu	NH_4^+ pH 6.5 H Zn &Cu	NH_4^+ pH 8 H Zn &Cu
NO_3^- pH 5 H Zn &Cu	NO_3^- pH 6.5 H Zn&Cu	NO_3^- pH 8 H Zn &Cu
NH_4^+ pH 5 Low Zn&Cu	NH_4^+ pH 6.5 Low Zn &Cu	NH_4^+ pH 8 Low Zn &Cu
NO_3^- pH 5 Low Zn &Cu	NO_3^- pH 6.5 Low Zn &Cu	NO_3^- pH 8 Low Zn &Cu

Figure 6.1 Shows the layout of the experiment (there was also randomisation of treatments within each block).

6.3 Result and discussion

6.3.1Biomass

Barley grew in high concentration of Zn and Cu only at high pH (8), but did not grow at low pH (5, 6.5) (Figure 6.2 and 6.3). Barley with nitrate was better than with ammonium (Figure 6.4 and 6.5) and this is attributed to Zn and Cu toxicity due to lowering of pH. For the low concentration of Zn and Cu there was no difference in shoot length, fresh shoot, dry shoot and dry root weight, between the two sources of nitrogen at all initial pHs ($P < 0.05$) (Table, 6.1, 6.2,). Root length and final pH for the nitrate treatment were greater than those for the ammonium ($P < 0.05$) (Table, 6.1). This is attributed to low pH of ammonium treatment, which affects the root more than the shoot.

In the high Zn and Cu concentration treatments, shoots and roots appeared only in the pH 8 treatments, with NO_3^- and NH_4^+. The NO_3^- final pH was higher than NH_4^+ pH in all treatments, in dry shoots there is no difference between the initial pH's with NO_3^- treatment, but the dry root weight for the NH_4^+ and NO_3^- with initial pH 8 were greater than other treatments ($P< 0.05$) (Table 6.1 and 6.2).

Table 6.1 Effect of different pH and two sources of nitrogen NH_4^+ or NO_3^- on shoot length, root length of barley and final pH in low and high concentration of Zn and Cu.

Zn and Cu Concentration	N source	Initial pH	Length in cm Shoot	Root	final pH
	NH_4^+	5	23.5b	8.3b	4.0c
Low		6.5	27.2ab	5.3b	4.0c
		8	26.8ab	9.3 b	4.3b
	NO_3^-	5	28.4ab	16.8a	6.5a
		6.5	30.1a	23.0a	6.5a
		8	30.2a	20.5a	6.5a
	NH_4^+	5	0.0	0.0	3.0d
High		6.5	0.0	0.0	3.8c
		8	14.6c	3.0b	6.3a
	NO_3^-	5	0.0	0.0	5.0b
		6.5	0.0	0.0	5.0 b
		8	22.3b	4.1b	7.0a
LSD	$P < 0.05$		7.0	8.8	0.9

The same letters in the same columns are not significantly different.

Table 6.2 Effect of different pH and two sources of nitrogen NH_4^+ or NO_3^- on shoot fresh and dry weight, and dry root weight of barley and final pH in low and high concentration of Zn and Cu.

Zn and Cu Concentration	N source	Initial pH	Weight in g Fresh shoot	Dry shoot	Dry root	final pH
	NH_4^+	5	0.8bc	0.11a	0.04b	4c
Low		6.5	1.1abc	0.14a	0.07a	4c
		8	1.5ab	0.17a	0.04b	4.3bc
	NO_3^-	5	1.8a	0.18a	0.07a	6.5a
		6.5	1.7a	0.16a	0.07a	6.5a
		8	1.9a	0.19a	0.1a	6.5a
	NH_4^+	5	0	0	0	3d
High		6.5	0	0	0	3.8cd
		8	0.5c	0.11a	0	6.3a
	NO_3^-	5	0	0	0	5b
		6.5	0	0	0	5b
		8	1.0	0.15a	0.1a	7a
LSD	$P < 0.05$		0.82	0.11	0.06	0.9

The same letters in the same columns are not significantly different.

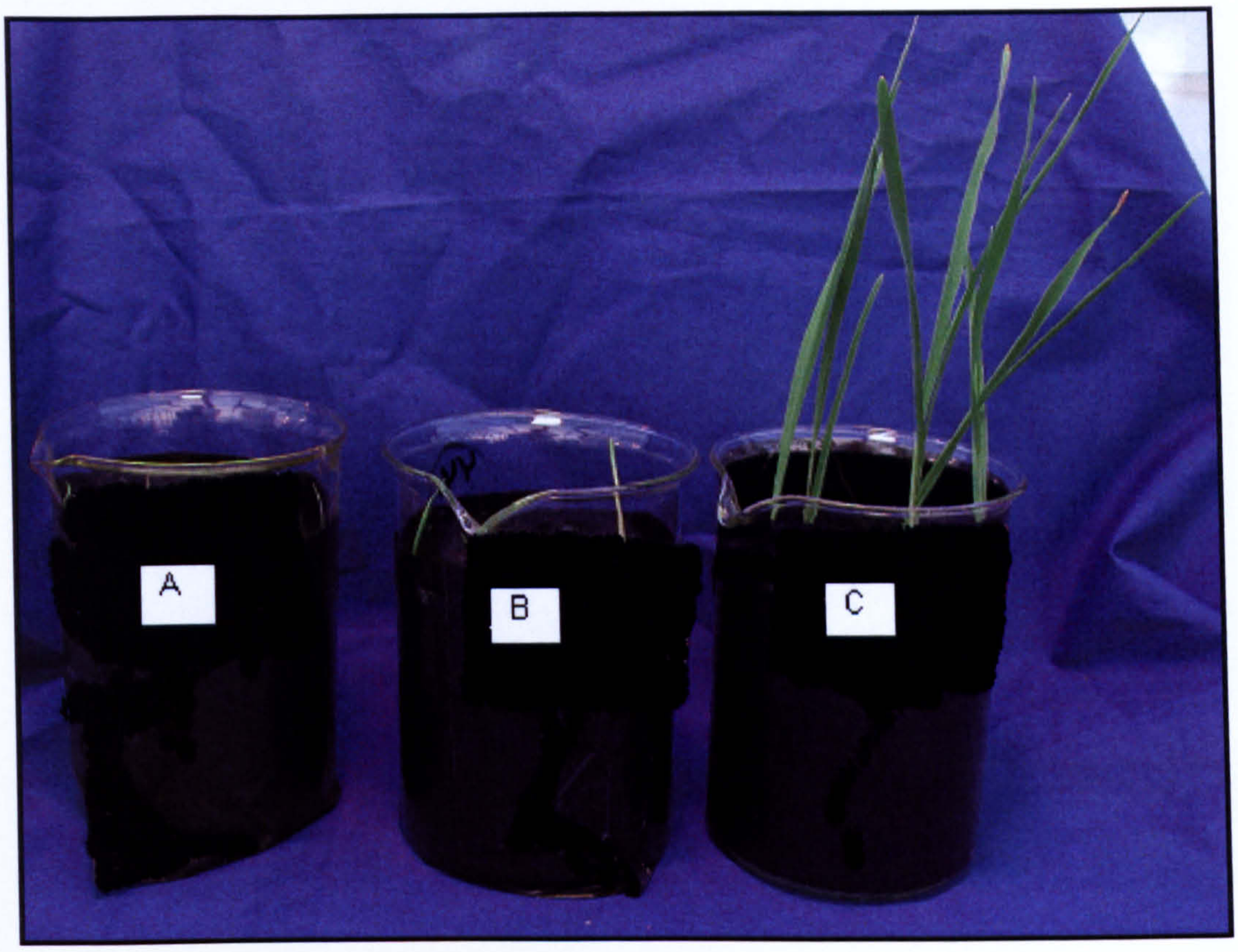

Figure 6.2 The effect of high concentration of Zn and Cu with NO_3^- at different pH(A = pH 5; B = pH 6; C = pH 8).

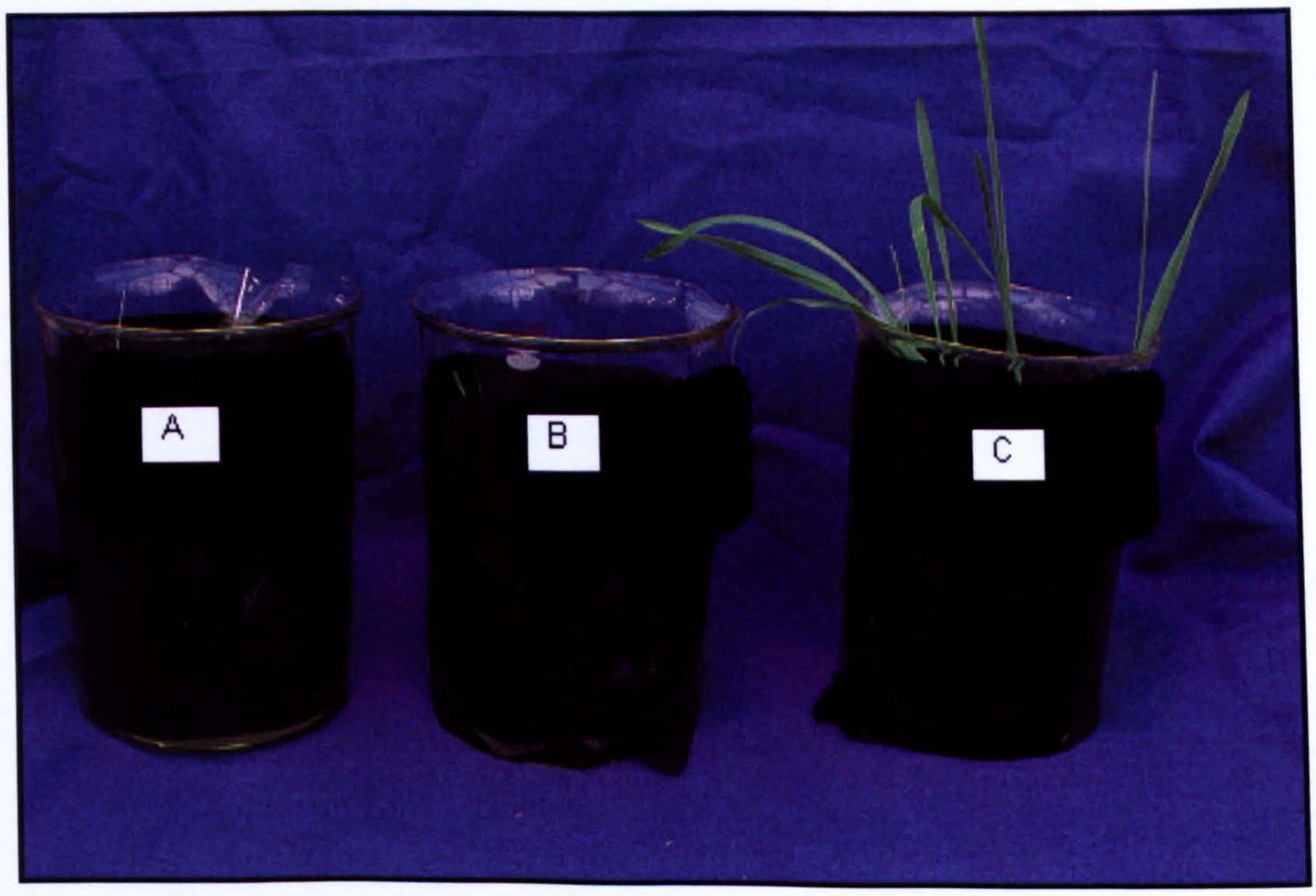

Figure 6.3 The effect of high concentration of Zn and Cu with NH_4^+ at different pH(A = pH 5; B = pH 6; C = pH 8).

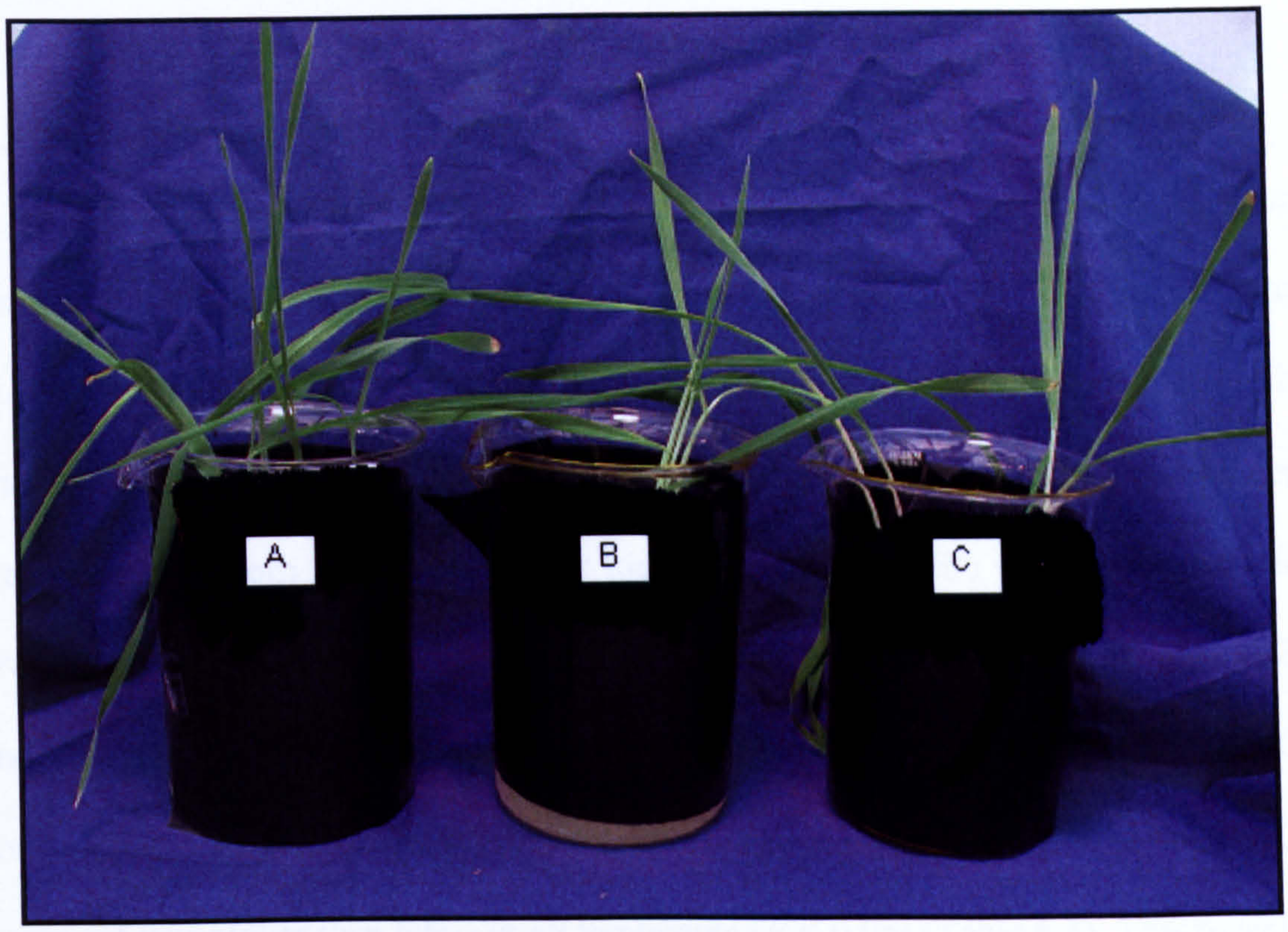

Figure 6.4 The effect of low concentration of Zn and Cu with NO_3^- at different pH (A = pH 5; B = pH 6; C = pH 8).

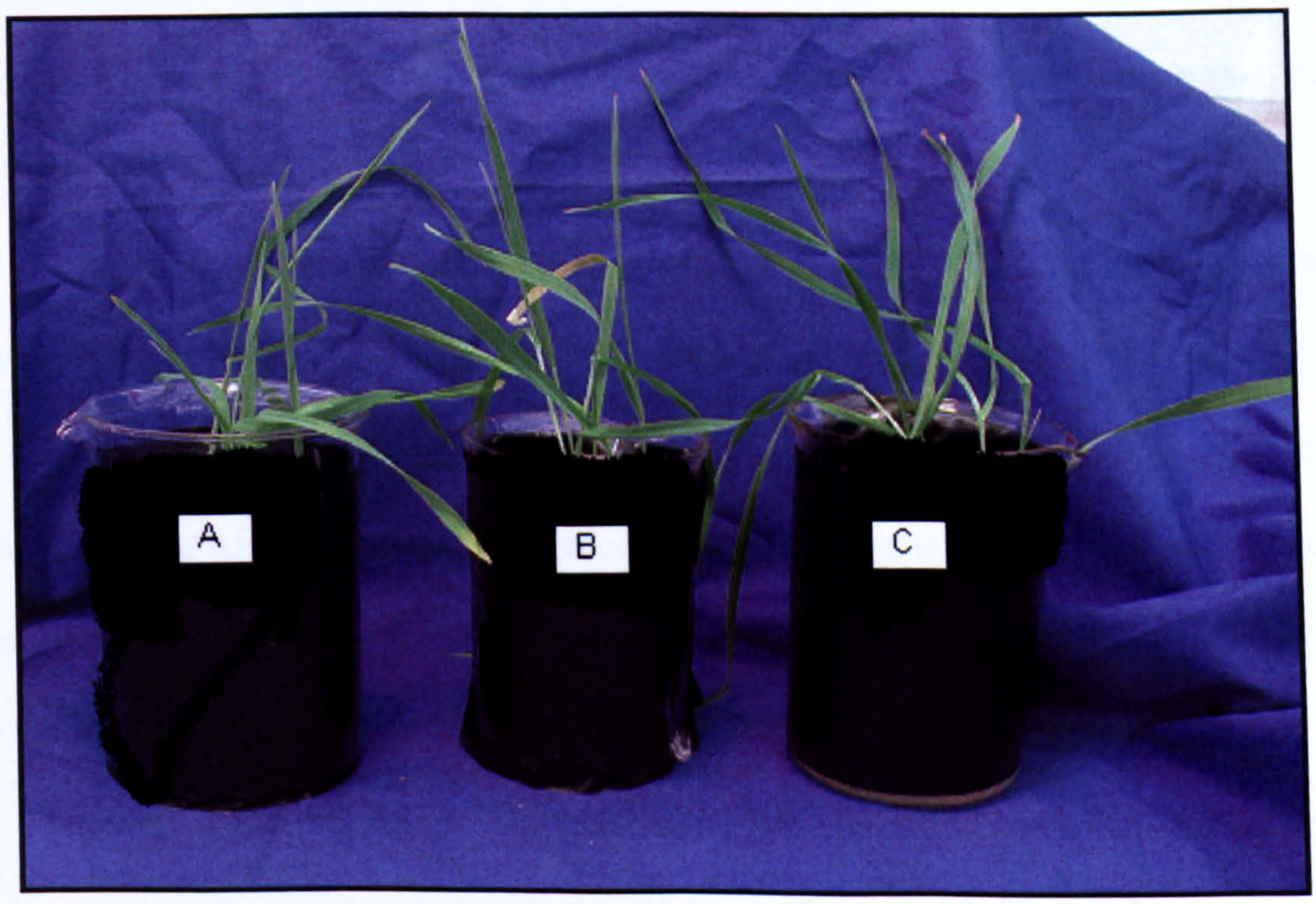

Figure 6.5 The effect of low concentration of Zn and Cu with NH_4^+ at different pH (A = pH 5; B = pH 6; C = pH 8).

6.3.2 Metal content

There was no difference betweezinn NH_4^+ and NO_3^- treatments in Zn shoot content at the high concentration of Zn and Cu at initial pH 8. This may be attributed to decrease of pH with ammonium, which increases the toxicity. Consequently the zinc uptake was reduced due to higher pH in the case of nitrate. The Zn shoot content was greater for both NH_4^+ and NO_3^- at high concentration with initial pH 8 than for low zinc concentration ($P < 0.05$) (Table 6.3). This is attributed to availability of Zn and Cu at high concentrations of Zn and Cu. Barley plants at high Zn and Cu concentrations at pH 5 and 6.5 did not survive (Figure 6.1 and 6.2). The pH with NO_3^- treatment was higher than NH_4^+ in all treatments ($P < 0.05$) (Table 6.3). This revealed the exchange between H^+ root and NH_4^+nutrient. At low concentrations of Zn and Cu there was no difference in Zn shoot uptake between ammonium at initial pH (5 and 8) and nitrate at initial pH 5 and the Zn shoot content with these treatments was greater than with nitrate with initial pH8 and 6.5 treatments. This is attributed to the final pH of these treatments.

At the high Zn and Cu concentrations, the Cu shoot content at initial pH 8 for the ammonium treatment was greater than for the nitrate treatment. This is attributed to availability of Cu at high concentration and lowering the pH by ammonium. At low concentrations of Zn and Cu the Cu shoot content with (ammonium with initial pH8) and (nitrate with initial pH 5) was greater than all other treatments. This is attributed to the lowering of the pH to 4.5 by ammonium (Table 6.3) ($P < 0.05$)

The Cu was not detected in the shoot at low concentrations of Cu and Zn with ammonium with initial pH 5 and 6.5 (Table 6.3). This may be attributed to the formation of ammonium Cu complex at low pH and low Cu concentration.

In roots at low Zn and Cu concentrations NH_4^+ treatment resulted in a greater Zn concentration than NO_3^- treatment for all initial pH's (Table 6.3). This is attributed to the NH_4^+ causing a decrease in the pH, which enhances the solubility of Zn. At high Zn and Cu concentrations at initial pH 8, the concentration of Zn roots with NO_3^- treatment was greater than NH_4^+ treatment. The root Zn concentrations in high Zn and Cu concentrations at initial pH 8 for both nitrogen NH_4^+ and NO_3^- were higher than in the low concentration at all initial pHs (Table 6.3). The ammonium affected the Zn in the low concentration by lowering the pH to 4. In high concentration and pH 8, ammonium lowered the pH to 6. Also, the buffering capacity of the agar nutrient solution played an important role, but is not as much as NH_{4+} for lowering the pH.

29 Table 6.3 The effect of different pH and two sources of nitrogen NH_4^+ or NO_3^- on heavy metal content of shoot and root of barley and final in low and high concentration.

Concent. Zn and Cu	Treatments	Initial pH	Shoot metal content mg/kg Zn	Shoot metal content mg/kg Cu	Root metal* content Zn	Root metal* content Cu	pH
Low	NH_4^+	5	29.6ab	nd	51	29.2	4.2c
		6.5	23.2bc	nd	40.2	31.7	4c
		8	36.7a	12.1a	62.9	71.9	4.5c
	NO_3^-	5	30.8a	12.6a	35	72	6.4a
		6.5	18.1c	10.6b	33.3	51.1	6.5a
		8	23bc	10.6b	24.3	68.6	6.7a
LSD	***P* < 0.05**		**6.9**	**1.95**	-------	-------	------
High	NH_4^+	8	403.5	34.3a	2092.3	1719.3	6.3b
	NO_3^-	8	429.7	22.9b	2189.2	1098.3	7a
LSD	***P* < 0.05**		**N.S**	**5**	------	-------	**0.6**

The same letters in the same columns are not differently significant.

* the dry matter of root of replicates being too small, all the replicates were collected together for analysis.

The transfer factor of Zn was more than the transfer factor of Cu; and the transfer factor at low metal concentration was more than that at high concentration. This is

attributed to in high concentration leading to more absorption by the roots than by the shoots, and the heavy metals may accumulate in the free space of the roots.

Table 6.4 The effect of different pH and two sources of nitrogen NH_4^+ or NO_3^- on transfer factor of Zn and Cu (heavy metal content of shoot/ heavy metal content of root).

Concentration of Zn and Cu	Nitrogen Source	Initial pH	Transfer factor	
			Zn	Cu
Low	NH_4^+	5	0.6	0
		6.5	0.6	0
		8	0.6	0.2
Low	NO_3^-	5	0.9	0.2
		6.5	0.5	0.2
		8	0.9	0.2
High	NH_4^+	8	0.2	0.2
	NO_3^-	8	0.2	0.2

6.4 Conclusion

The pH, source of nitrogen and the type of heavy metal and its concentration were the main factors, which affected phytoextraction. The increasing of the pH on polluted site can decrease the solubility and accessibility of heavy metals and enhance the plant growth. Ammonium can reduce the pH and increase the heavy metal phytoextraction. In the next experiment the agar media is contaminated with different high metal soils, one is galena (G) soil that contains high Pb and the other sewage treated soil, which contain Zn and Cu (SBS).

Chapter 7

Manipulation of flax rhizosphere to availability of heavy metals in different concentrations added as soil constituents with NH_4^+ or NO_3^- in agar system.

7.1 Introduction

Some researchers have suggested that there are three factors or mechanisms by which the nitrogen affects the pH a) nitrification/denitrification reactions b) displacement of H^+/OH^- adsorbed c) release or and uptake of protons by roots in response to NH_4^+ or /and NO_3^-. Of all these three mechanisms, only mechanism c) is associated with the plant rhizosphere and is more effective due to the limited volume of soil (Marschner, 1995; Marschner and Romheld, 1994). In this experiment only the OH^- or H^+ exchange is relevant because it is in an agar system, which was sterilized and without microbial activity. The aim of this chapter is to test the hypothesis that uptake of Zn, Cu and Pb from two soils can be manpulated by the altering the flax rhizosphere using two nitrogen sources (NH_4^+ and NO_3^-).

7.2 Material and methods

Quarter Hoagland solution (30 l) was prepared without any nitrogen source (A). From solution A two solutions were prepared, B with NH_4^+ as $(NH_4)_2SO_4$ and C with NO_3^- as KNO_3. From solution B or C, thirty-two 500 ml beakers were each filled with 400 ml. All of them were placed on the hot plate with a magnetic stirrer, and 6.5 g of agar was added to each beaker. When the solution of each beaker was clear, the treatments of SBS or G as a source of heavy metals were added and mixed thoroughly. The treatments were 0, 0.1, 0.25 and 1% w/w SBS or G soil and some physical and chemical properties are illustrated in Table 7.1. Figure 7.1 illustrates the treatments of the experiment. After 24 h the agar solution was gelatinized, and 15 seeds of linseed were sown in each beaker. Each beaker was covered with black polyethylene bags to protect the roots from the light. All the treatments were randomly distributed in completely block randomised design. After 8 weeks, the plants were harvested, shoot length, shoot weight, root length, and root weight were determined. The roots were washed several times with tap water and put in beakers and laid in an ultrasonic bath for 15 minutes, then washed again with deionised water several times. Shoots and roots were allowed to dry in the oven at 80 °C for 72 h, then ground and prepared for chemical analysis and the data were statistically analysed with GLM with MINITAB.

Block I

NH_4^+ 0.0% SBS	NH_4^+ 0.1% SBS	NH_4^+ 0.25% SBS	NH_4^+ 1% SBS
NH_4^+ 0.0% G	NH_4^+ 0.1% G	NH_4^+ 0.25% G	NH_4^+ 1% G
NO_3^- 0.0% SBS	NO_3^- 0.1% SBS	NO_3^- 0.25% SBS	NO_3^- 1% SBS
NO_3^- 0.0% G	NO_3^- 0.1% G	NO_3^- 0.25% G	NO_3^- 1% G

Block II

NH_4^+ 0.0% SBS	NH_4^+ 0.1% SBS	NH_4^+ 0.25% SBS	NH_4^+ 1% SBS
NH_4^+ 0.0% G	NH_4^+ 0.1% G	NH_4^+ 0.25% G	NH_4^+ 1% G
NO_3^- 0.0% SBS	NO_3^- 0.1% SBS	NO_3^- 0.25% SBS	NO_3^- 1% SBS
NO_3^- 0.0% G	NO_3^- 0.1% G	NO_3^- 0.25% G	NO_3^- 1% G

Block III

NH_4^+ 0.0% SBS	NH_4^+ 0.1% SBS	NH_4^+ 0.25% SBS	NH_4^+ 1% SBS
NH_4^+ 0.0% G	NH_4^+ 0.1% G	NH_4^+ 0.25% G	NH_4^+ 1% G
NO_3^- 0.0% SBS	NO_3^- 0.1% SBS	NO_3^- 0.25% SBS	NO_3^- 1% SBS
NO_3^- 0.0% G	NO_3^- 0.1% G	NO_3^- 0.25% G	NO_3^- 1% G

Block IV

NH_4^+ 0.0% SBS	NH_4^+ 0.1% SBS	NH_4^+ 0.25% SBS	NH_4^+ 1% SBS
NH_4^+ 0.0% G	NH_4^+ 0.1% G	NH_4^+ 0.25% G	NH_4^+ 1% G
NO_3^- 0.0% SBS	NO_3^- 0.1% SBS	NO_3^- 0.25% SBS	NO_3^- 1% SBS
NO_3^- 0.0% G	NO_3^- 0.1% G	NO_3^- 0.25% G	NO_3^- 1% G

Figure 7.1 Layout of the experiment with different treatments (all treatments randomized in each block).

Table 7.1 Total, EDTA-extractable metals and pH in mg/kg soil for galena (G) and Stock Barldolph soils (SBS).

Soil	Total metals in mg/kg			EDTA- extractable metals			pH 1:5
	mg/kg			mg/kg			H_2O
	Zn	Pb	Cu	Zn	Pb	Cu	
G	25486	64591	3307	997	38370	79	6
SBS	2137	680	799	800	194	365	7

7.3 Result and discussion

7.3.1 Effect of manipulation of rhizosphere on flax biomass

The NO_3^- treated soil had a significantly higher pH than NH_4^+ treated soil ($P<$ 0.05), averaged across both soils and all soil concentrations (Table 7.2) and in all other treatments (Table 7.4). This is attributed to H^+/ NH_4^+ exchange between the roots and the soil, which decreased the pH in the rhizosphere. On average of both soils and concentrations, the shoot length, root length, fresh shoot, dry shoot and dry root weight with nitrate were greater than ammonium. This is attributed to the lowering of the pH by ammonium. On average combining of both nitrogen sources and all concentrations the SBS soil treated agar media was greater than galena in all above parameters. This attributed to buffering capacity of the SBS soil (Table 7.2 and Figure 7.3 and 7.3) ($P < 0.05$).

Table 7.2 Effect of a) nitrogen source averaged over both concentration and soil and b) soil averaged over both concentration and nitrogen source on pH, shoot and root length in cm and dry weight in g.

Treatment	pH	Length in cm		Weight in g		
		shoot	root	Fresh shoot	Dry shoot	Dry root
a) N (n = 32)						
NH_4^+	4.7b	16.8 b	8.0 b	1.8b	0.33 b	0.08 b
NO_3^-	6.9a	17.5 a	11.4 a	2a	0.36 a	0.10 a
LSD P < 0.05	**0.1**	**0.7**	**0.34**	**0.13**	**0.01**	**0.008**
b) soils (n = 32)						
SBS	6.0 a	20.5 a	13.8 a	2.2a	0.43 a	0.11 a
G	5.6 b	13.8 b	5.3 b	1.6b	0.26 b	0.07 b
LSD P < 0.05	**0.12**	**0.72**	**0.34**	0.13	**0.01**	**0.008**

Values in the same column in part a or b with different letters were significantly different

SBS = Stock Bardolph Soil
G = Galena

There was no significant difference between NH_4^+ and NO_3^- on shoot length with 0.1, 0.25 and 1% concentrations in the same soil. In shoot length the ammonium with different concentrations of SBS soil was significantly greater than control, but there was no difference in the case of galena soil (Table 7.3, Figure 7.2). In shoot length the nitrate with different concentrations of galena was less than control (Table 7.3 and Figure 7.5), but there was no difference in the case of SBS soil ($P < 0.05$) (Table 7.6). Shoot length and fresh shoot weight were greater, the NO_3^- control (0% SBS or galena) than for the NH_4^- control (Table 7.3 and Figure 7.2). This indicated that the ammonium decreased the pH with control and galena soil more than with SBS soil, which is attributed to the high buffering capacity of the SBS soil and low buffering capacity of the galena soil. Flax was more affected by chlorosis with (NO_3^- + G) treatments than (NH_4^+ + G) treatments (Figure 7.4, 7.5, 7.9 and 7.10). This may be

attributed to the low pH, which occurred with ammonium and made the Zn more available to compete with Pb and Cu, consequently reducing their effects.

Table 33 Table 7.3 Effect of NH_4^+ or NO_3^- with addition different concentrations of SBS or G on pH, shoot and root length in cm and dry weight in g.

Treatment	Concentration	pH	Length in cm		Weight in g		
			shoot	root	Fresh shoot	Dry shoot	Dry root
NH_4^+	0% SBS	3.9 c	13b	4.7 ef	1.5c	0.3c	0.1b
	0.1% SBS	5.2 b	22 a	13.2 c	2.4a	0.4b	0.1b
	0.25% SBS	5.4 b	23 a	12.2 cd	2.7a	0.5a	0.1b
	1% SBS	5.9 b	22 a	15.1 b	2.4a	0.5a	0.1b
NH_4^+	0% G	3.9 c	13 b	5. 4e	1.5c	0.2d	0.1b
	0.1% G	3.9 c	14 b	7.2 e	1.3c	0.3c	0.1b
	0.25% G	4.4 c	15 b	4.9 d	1.4c	0.2d	0.1b
	1% G	5.3 b	12 b	2.2 e	1.1c	0.2d	0.1b
NO_3^-	0% SBS	6.9 a	20 a	11.5 d	2.9a	0.4b	0.1b
	0.1% SBS	6.5 a	21 a	18 a	2.2b	0.1e	0.2 a
	0.25% SBS	6.8 a	21 a	18 a	1.9b	0.4b	0.1b
	1% SBS	6.7 a	22 a	18.2 a	1.9b	0.4b	0.1b
NO_3^-	0% G	7.1 a	20a	5.7 e	2.6a	0.5a	0.1b
	0.1% G	6.8 a	11bc	7 e	2.5a	0.4b	0.1b
	0.25% G	7.1 a	13 bc	6.5 e	1.4c	0.2d	0.1b
	1% G	7.1 a	11 bc	3.5ef	1.1c	0.5a	0.1b
LSD P < 0.05		0.6	3.7	1.7	0.7	0.05	0.04

Values in the same column with different letters were significantly different

Dry weight of shoot with NH_4^+ with 0.25 and 1% SBS and NO_3^- with G 1% and 0% was more than with other treatments (Table 7.3) ($P > 0.05$).). Root length and pH, NO_3^- with 0.1, 0.25 and 1% of SBS were greater than those with NH_4^+ with 0.1, 0.25 and 1% of SBS ($P < 0.05$). This may be attributed to the toxicity of Cu, which discoloured the roots. Dry weight of root with (NO_3^- + SBS) at 0.1% concentration was significantly greater than with all other treatments ($P > 0.05$) (Table 7.3).

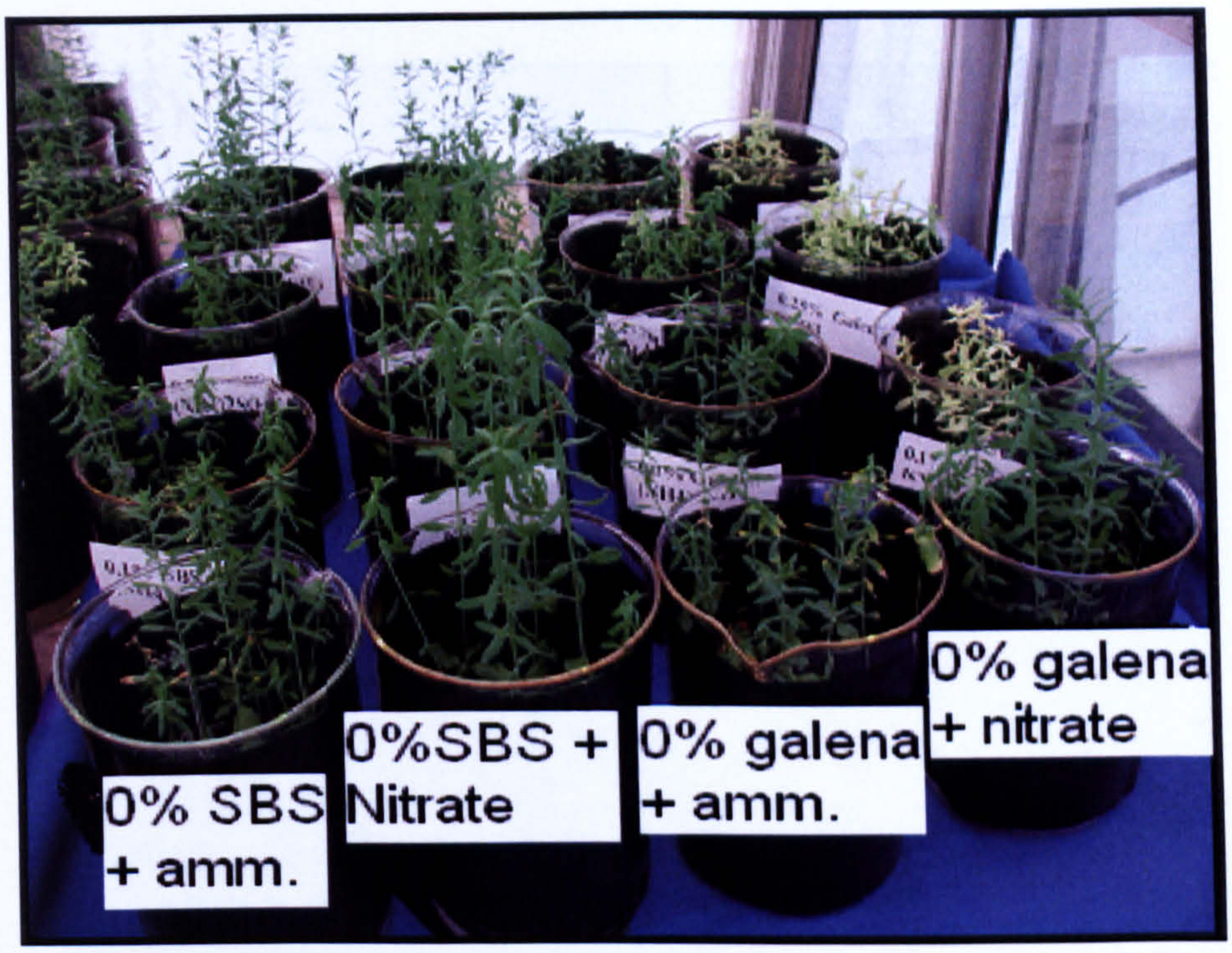

Figure 7.2 The effect of NO_3^- or NH_4^+ with different soil, SBS or galena concentrations 0.0, 0.1, 0.25 and 1% (0% front row) and (1% rear row) in agar system on flax growth.

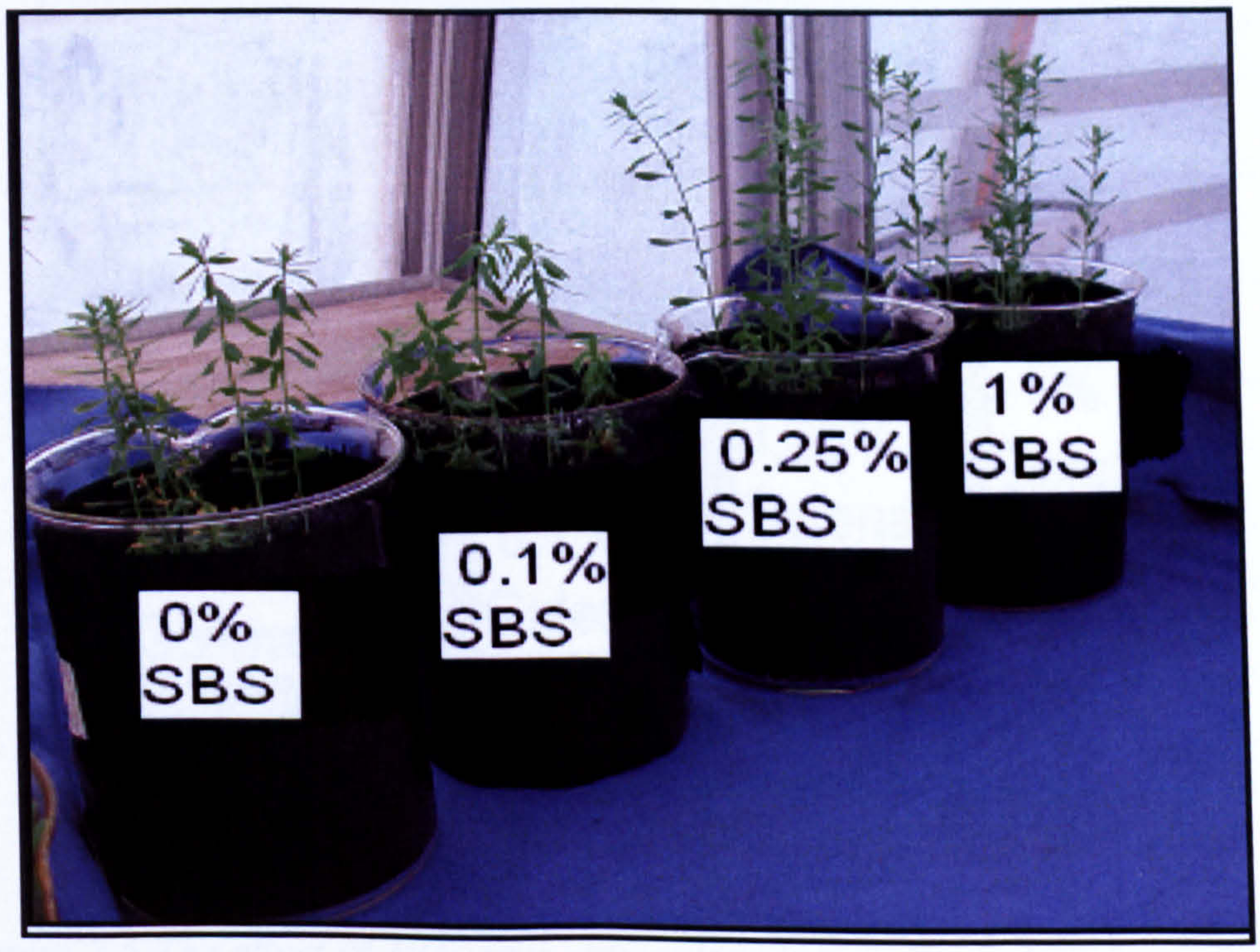

Figure 7.3 The effect of NH_4^+ with different SBS concentrations 0.0, 0.1, 0.25 and 1% in agar system on flax growth.

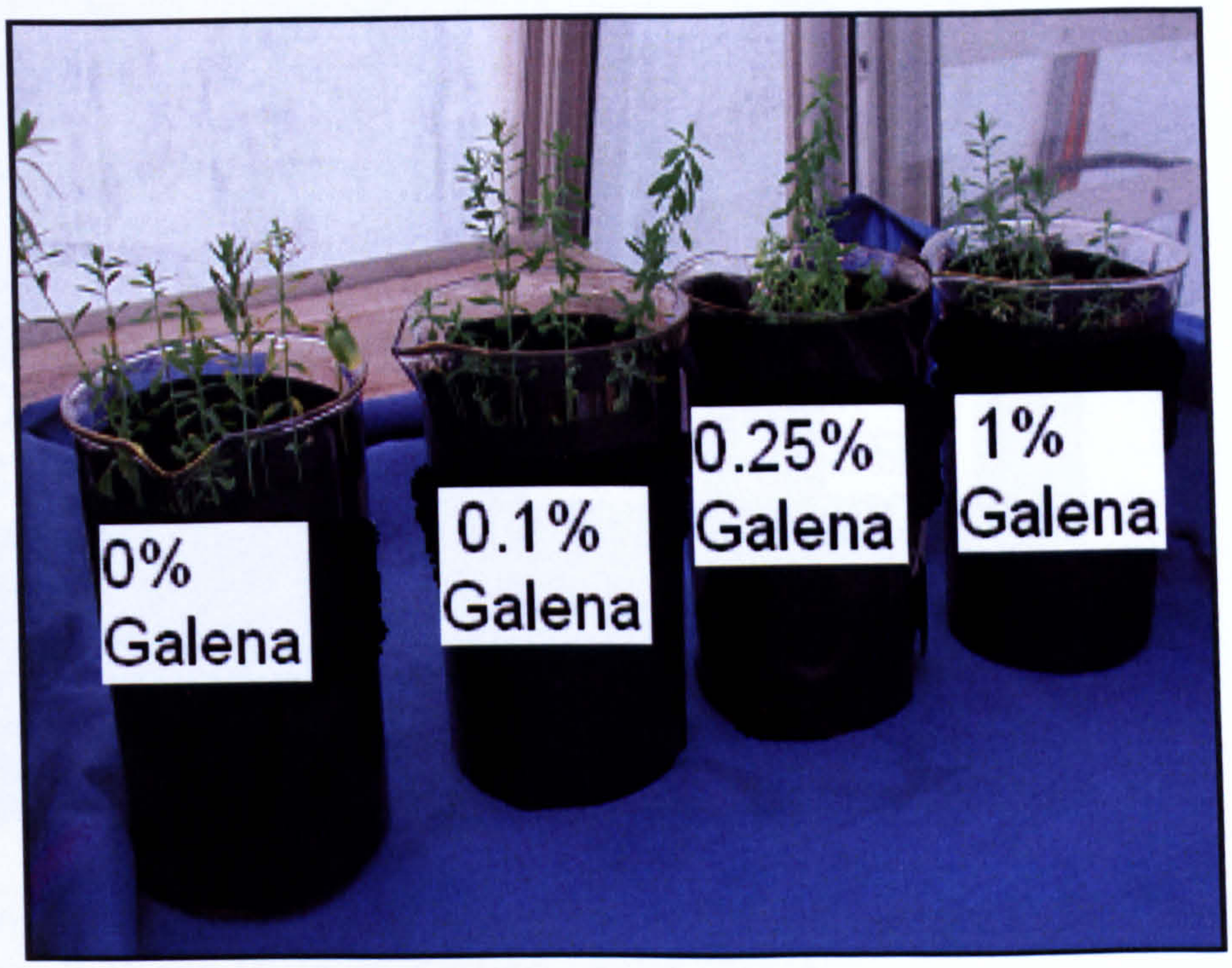

Figure 7.4 The effect of NH_4^+ with different galena concentrations 0.0, 0.1, 0.25 and 1% in agar system on flax growth.

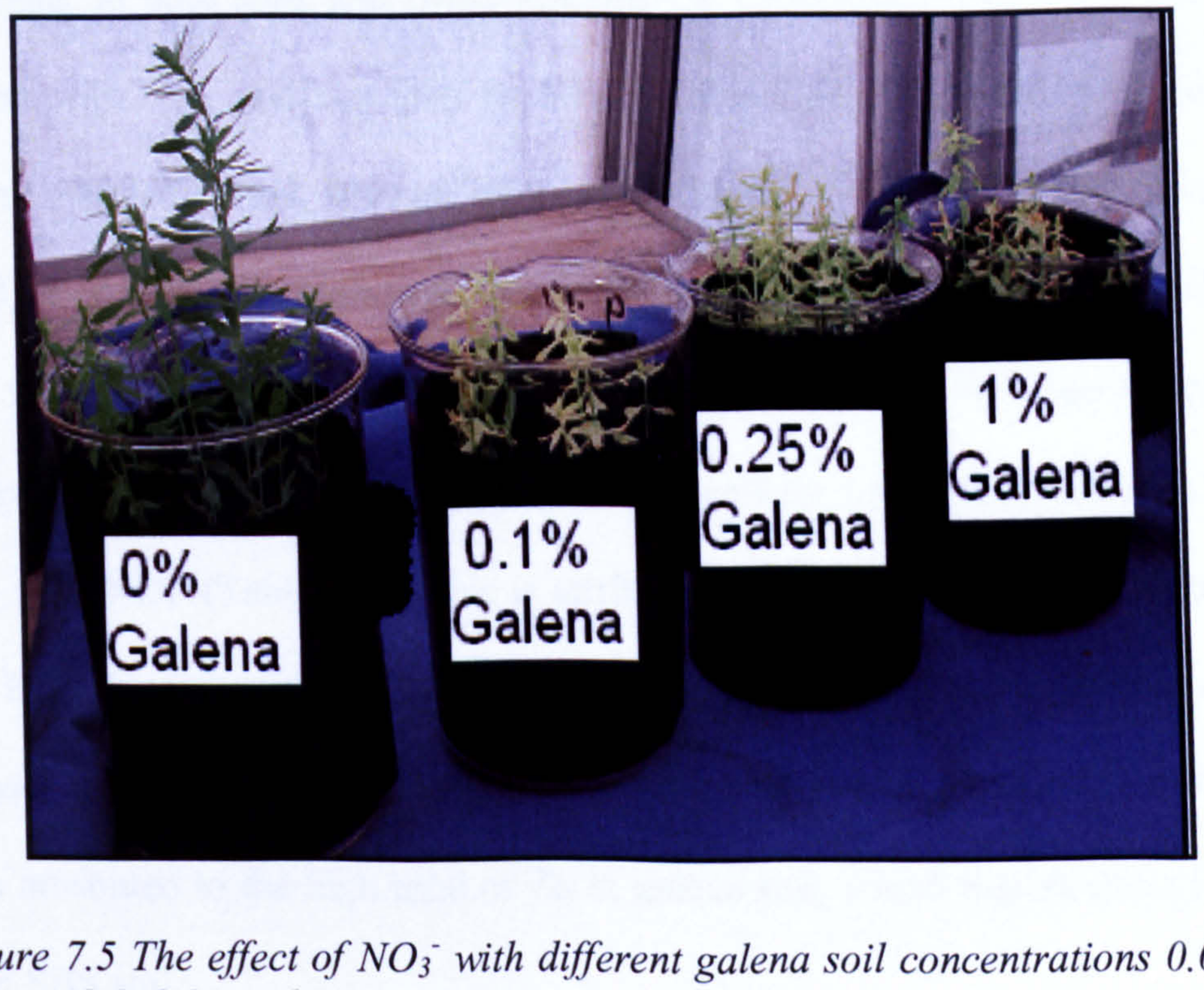

Figure 7.5 The effect of NO_3^- with different galena soil concentrations 0.0, 0.1, 0.25 and 1% in agar system on flax growth.

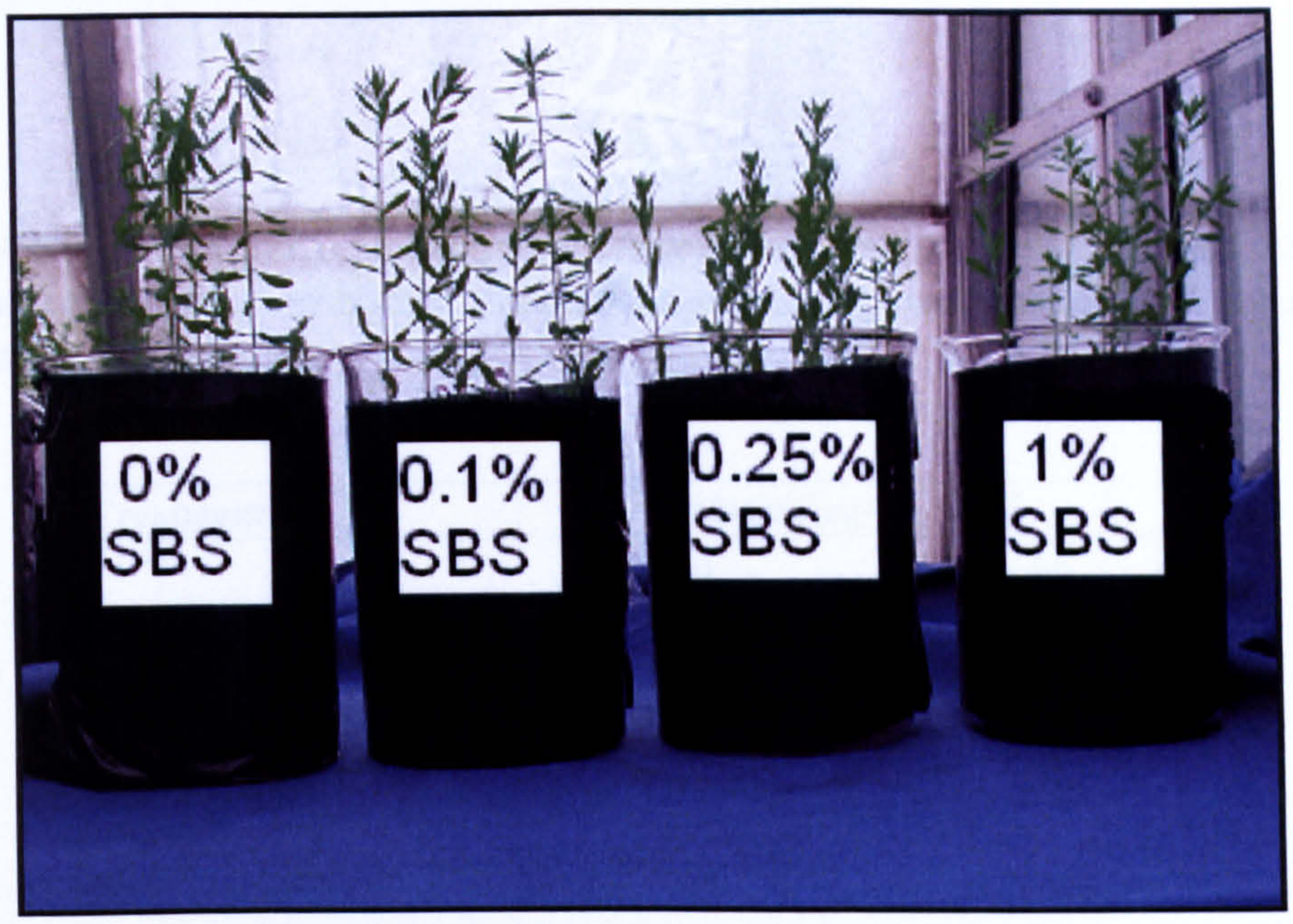

Figure 7.6 The effect of NO_3^- with different SBS soil concentrations 0.0, 0.1, 0.25 and 1% in agar system on flax growth.

7.3.2Effect of manipulation of rhizosphere on flax metal content

On average, in both soils and concentrations the concentration of Cu in the shoot was higher with NO_3^- addition than for NH_4^+ (Table 7.4). This is attributed to the Cu not being affected by pH, because of enhanced solubility of organic matter in SBS (Chapter 3). For soils, Cu uptake averaged over both nitrogen sources and all soil concentrations was significantly higher with galena than with SBS. Averaged over the two soils and four soil concentrations flax shoot Zn content was higher with NH_4^+ treatment than with NO_3^- (Table 7.4). This is attributed to decreasing the pH by the proton release from the roots to balance NH_4^+ and this agreed with (Tyler and Olsson, 2001). Flax shoot Zn content with galena soil was significantly higher than with SBS soil and this is attributed to the high total of Zn in galena soil, which was an about13 time more than SBS soil.

Table 7.4 Effect of a) nitrogen source averaged over both concentration and soil and b) soil averaged over both concentration and nitrogen source on shoot Cu and Zn content in mg/kg.

Treatment	Metals mg/ kg Shoot	
	Cu	Zn
a (n=32		
NH_4^+	5.2b	155.9a
NO_3^-	7.2a	151.7b
LSD P < 0.05	**0.3**	**2.25**
b (n=32)		
SBS	5.5	27.5b
G	6.9	280.1a
LSD P < 0.05	**0.30**	**2.3**

Values in the same column in part a or b with different letters were significantly different

Averaged over both concentrations, the NH_4^+ treatment caused greater ($P <$ 0.01) Pb uptake by flax than the NO_3^- treatment (Table 7.5) and this revealed the effect of NH_4^+ by decreasing the pH of the rhizosphere, which increased the Pb uptake. There is a positive correlation between Pb sorption and the pH in the soil solution (Basta and Tabatabai, 1992). The soil concentrations 0.1% and 0.25% resulted in high Pb concentrations in shoots than 1% concentration, suggesting that lead toxicity at 1% concentration reduced the biomass, and decreased shoot metal concentration, as illustrated in Table 7.5 and Figures 7.13, 7.4 which show chlorosis, necrosis and dwarf appearance.

Table 7.5 Effect of a) nitrogen source averaged over concentration and b) concentrations averaged over nitrogen source (G. soil) on shoot Pb content in mg/kg.

Treatment	Pb mg/kg
a (n=12)	
NH_4^+	93.9a
NO_3^-	62.9b
LSD P < 0.01	**19**
b (n=8)	
0.1%	99.9a
0.25%	105.6a
1%	29.6b
LSD P < 0.01	**23.1**

Values in the same column in part a or b with different letters were significantly different

In 0.1% concentration of SBS there was no significant difference between the two sources of nitrogen in shoot Cu content, however, ammonium treatment was more than nitrate in shoot Zn content ($P < 0.05$) (Table 7.6). This revealed that the Zn was strongly affected by the pH, pH 5.2 with ammonium and 6.5 with nitrate treatments.

Table 7.6 Effect of NH_4^+ or NO_3^- with addition of different concentration of SBS or G on metal content mg/kg shoot and root.

Treatment	Concentration	Metals mg/kg shoot			Metals mg/kg root*		
		Cu	Zn	Pb	Cu	Zn	Pb
NH_4^+	0% SBS	nd	18.4g	nd	29	47	nd
	0.1% SBS	10.5b	37.9f	nd	134	128	nd
	0.25% SBS	5.6d	29.0fg	nd	235	263	38
	1% SBS	8.4c	39.9f	nd	166	192	nd
NH_4^+	0% G	nd	17.7g	nd	42	217	nd
	0.1% G	2.3e	126.9d	42.7	34	443	190
	0.25% G	8.0c	308.0c	140.0a	47	1026	716
	1% G	6.9c	669.1b	100.8b	63	1052	737
NO_3^-	0% SBS	nd	12.0h	nd	51	56	nd
	0.1% SBS	12.1b	24.5g	nd	99	134	nd
	0.25% SBS	4.9d	25.2g	nd	235	232	23
	1% SBS	9.1c	33.2fg	nd	214	201	nd
NO_3^-	0% G	nd	12.5h	nd	27	23	nd
	0.1% G	10.7b	96.6e	16.6d	117	602	98
	0.25% G	15.0a	308.5c	71.3c	53	778	513
	1% G	5.6d	711.2a	99.0b	59	991	3163
LSD P < 0.05		1.5	11.4	5.4	-----	-----	------

Values in the same column with different letters were significantly different.

* the dry matter of the root replicate was too small to allow replication for metal analysis.

Table 7.7 Effect of NH_4^+ or NO_3^- with addition of different concentration of (SBS) or (G) on metal on transfer factor

Treatment	Concentration	Transfer factor (TF) Metals (C. shoot/C. root)		
		Cu	Zn	Pb
NH_4^+	0% SBS	0	0.4	0
	0.1% SBS	0.1	0.3	0
	0.25% SBS	0.0	0.1	0
	1% SBS	0.1	0.2	0
NH_4^+	0% G	0.0	0.1	0
	0.1% G	0.1	0.3	0.2
	0.25% G	0.2	0.3	0.2
	1% G	0.1	0.6	0.1
NO_3^-	0% SBS	0.0	0.2	0.0
	0.1% SBS	0.1	0.2	0.0
	0.25% SBS	0.0	0.1	0.0
	1% SBS	0.0	0.2	0.0
NO_3^-	0% G	0.0	0.5	0.0
	0.1% G	0.1	0.2	0.2
	0.25% G	0.3	0.4	0.1
	1% G	0.1	0.7	0.0

The transfer factor was different from treatment to other and in Zn was greater than Cu and lead. This is attributed to the metal accessibility, plant metal requirement.

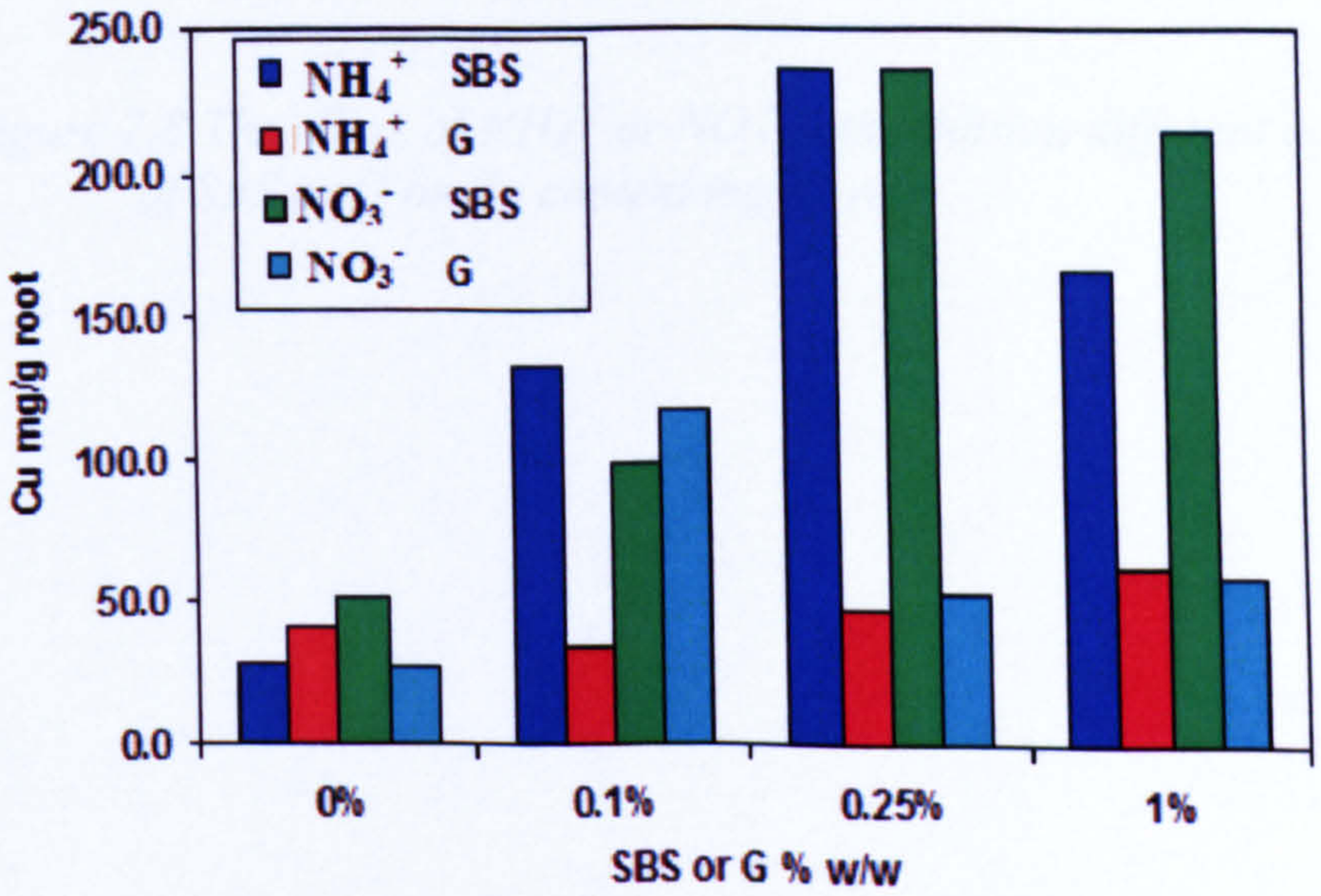

Figure 7.7 The effect of NH_4^+ or NO_3^- with addition of different concentration of SBS or G on Cu content mg/kg root.

The root Cu content, with NO_3^- + SBS treatment was greater than NH_4^+ + SBS at 1% (Table 7.6 and Figure 7.7). This was attributed to the NH_4^+ enhancing the availability of Cu leading to the toxicity of Cu at high SBS concentrations. The root Zn content with both nitrogen sources with galena soil treatments was greater than SBS soil (Figure 7.8).

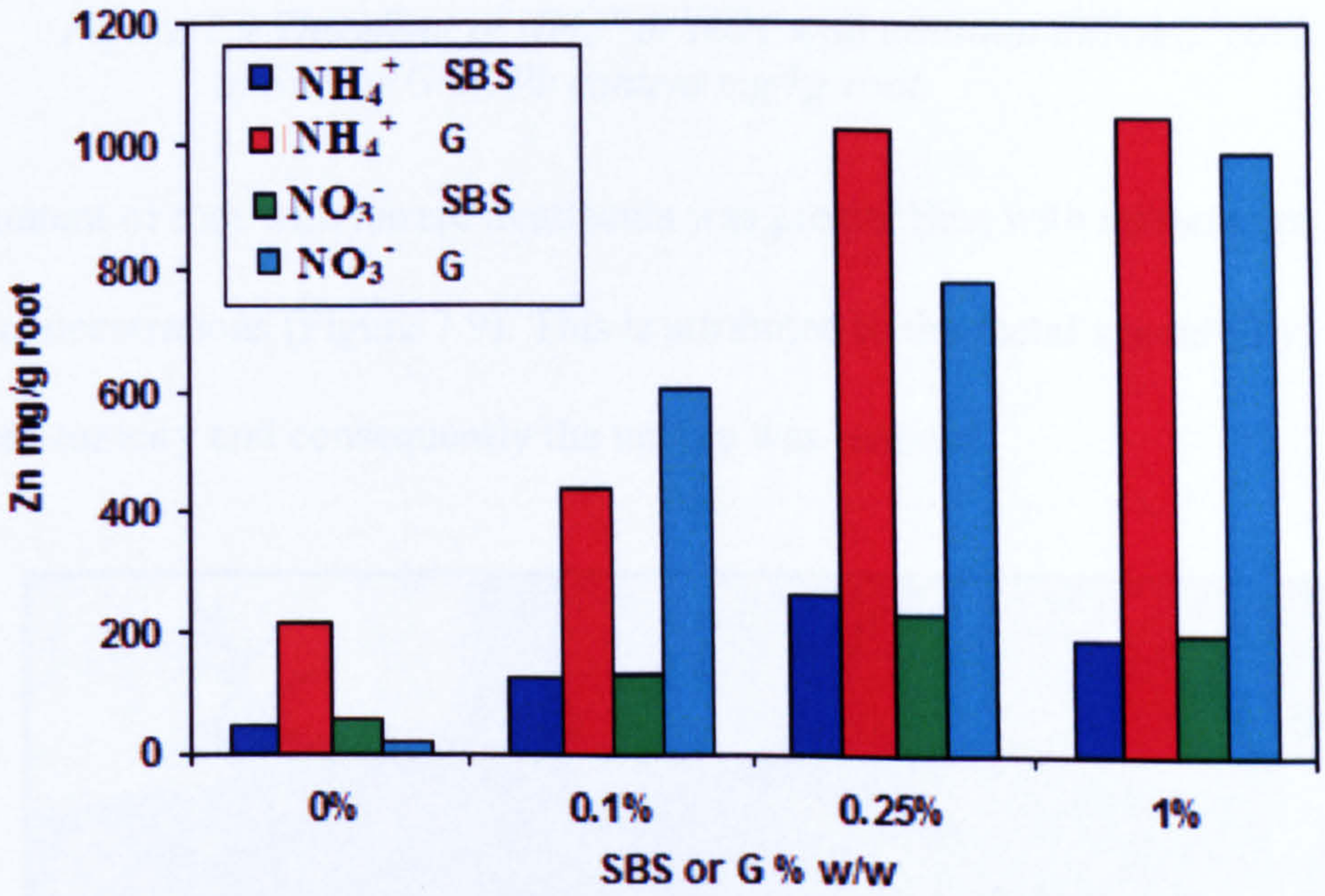

Figure 7.8 The effect of NH_4^+ or NO_3^- with addition different concentration of SBS or G on Zn content mg/kg root.

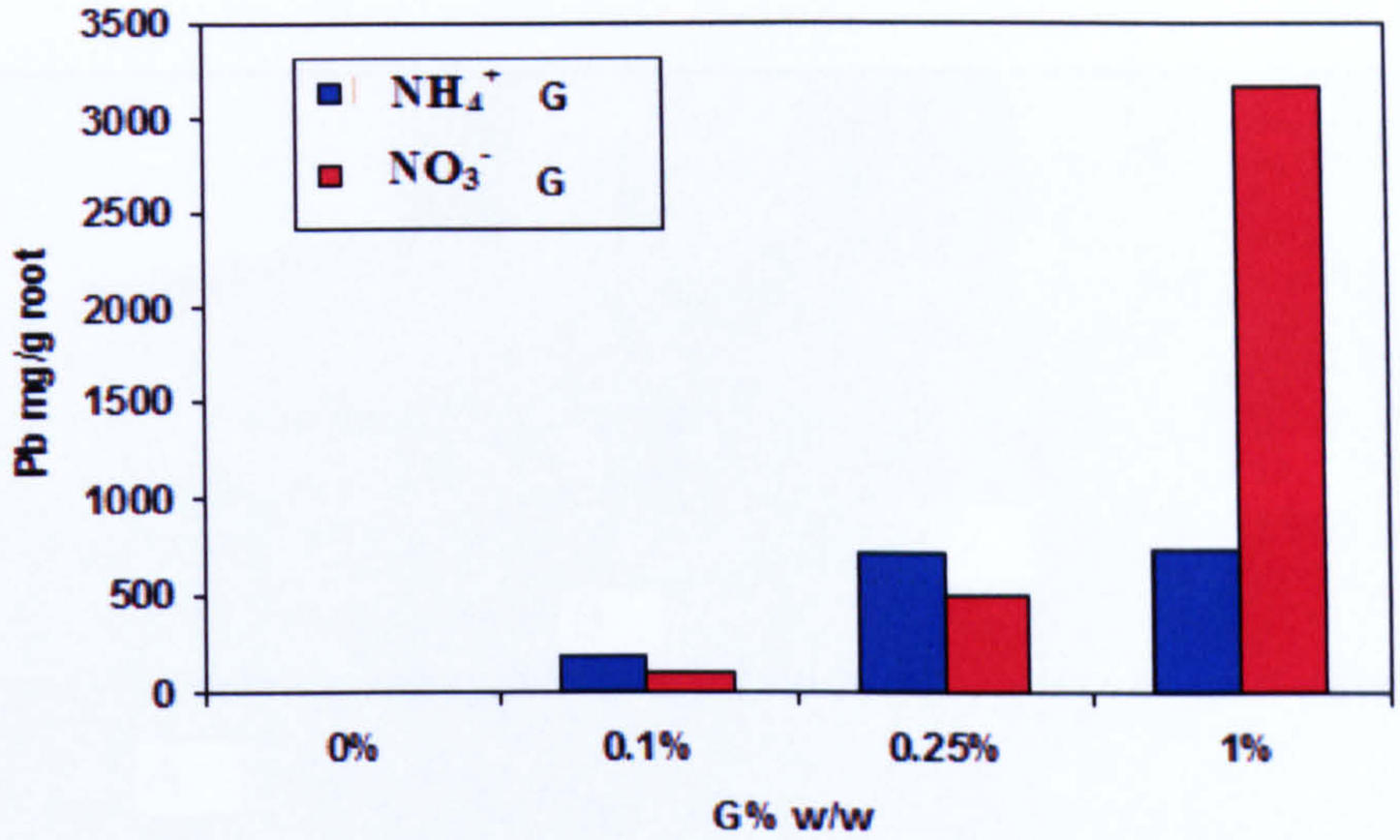

Figure 7.9 The effect of NH_4^+ or NO_3^- with addition different concentration of SBS or G on Pb content mg/kg root.

Lead content of root with nitrate treatments was greater than with ammonium at 0.1% galena concentrations (Figure 7.9). This is attributed to the metal's solubility, which enhanced toxicity and consequently the uptake was reduced.

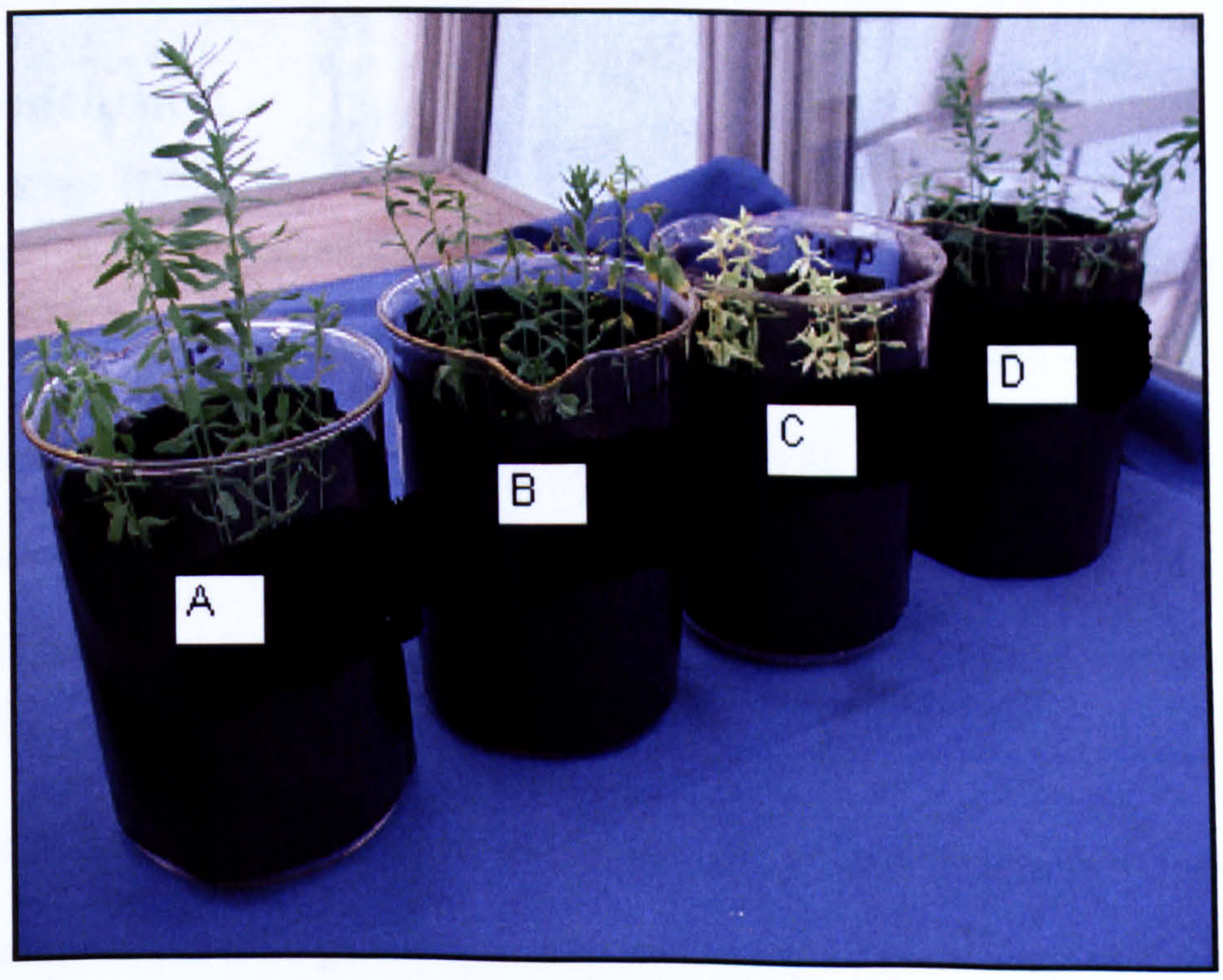

Figure 7.10 The effect of NO_3^- or NH_4^+ with two different galena soil concentrations 0.0 and 0.1% on flax growth in agar system (A = NO_3^- with 0% galena; B = NH_4^+ with 0% galena; C = NO_3^- with 0.1% galena; D = NH_4^+ with 0.1% galena).

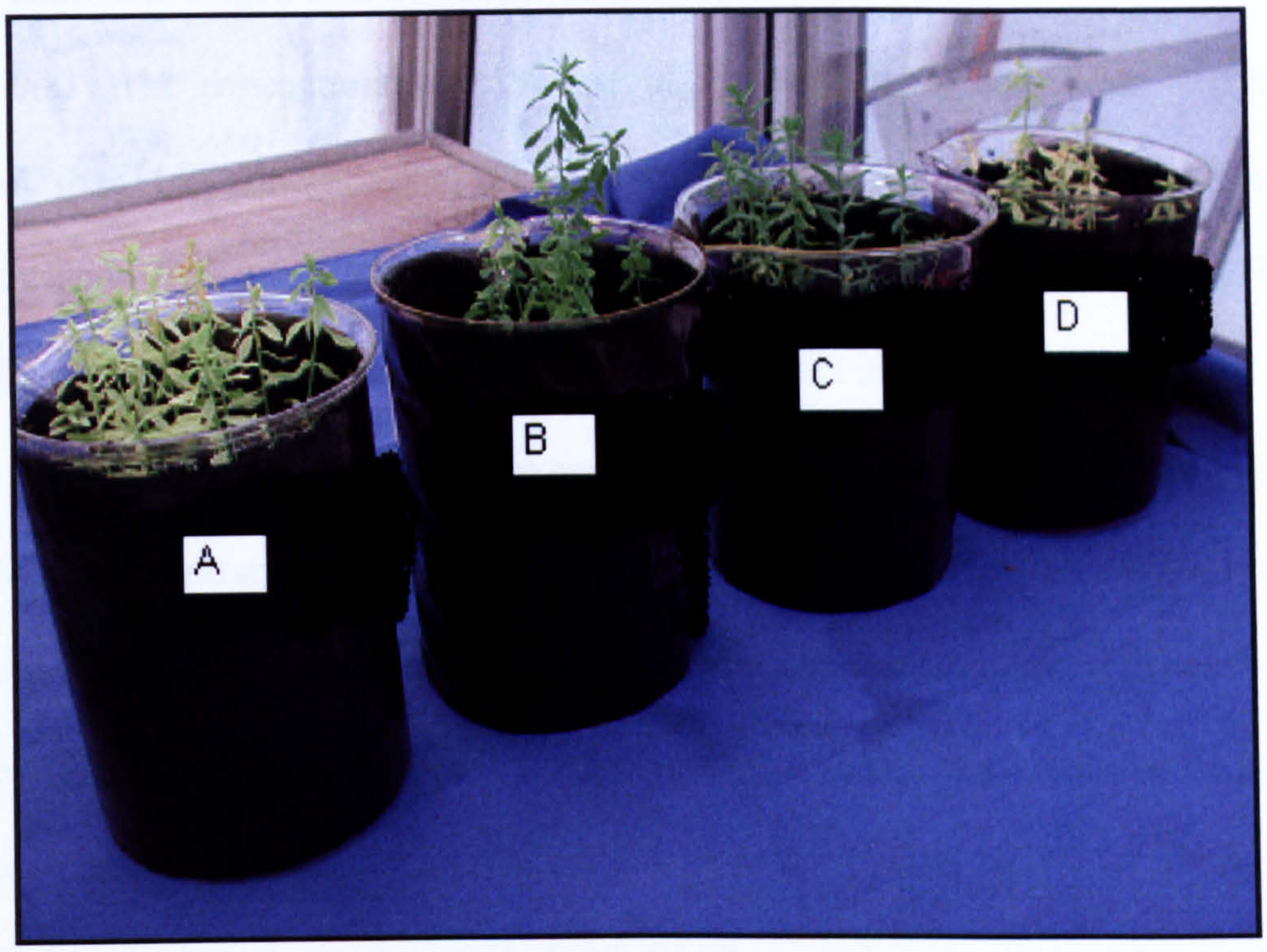

Figure 7.11 The effect of NO_3^- or NH_4^+ with two different galena soil concentrations 0.25 and 1% on flax growth in agar system (A = NO_3^- with 0.25% galena; B = NH_4^+ with 0.25% galena; C = NO_3^- with 1% galena; D = NH_4^+ with 1% galena).

7.4 Conclusion

pH was affected significantly by nitrogen source NH_4^+ in both soils and concentrations . In length and dry weight of root and pH, NO_3^- with 0.1, 0.25 and 1% of SBS concentrations were greater than NH_4^+ with 0.1, 0.25 and 1% of SBS concentrations ($P < 0.05$). In root length and pH, NO_3^- with 0.1, 0.25 and 1% of G concentrations were greater than NH_4^+ with 0.1, 0.25 and 1% of G concentrations ($P < 0.05$).

Cu shoot content in all averaged nitrogen sources and concentrations was significantly higher with galena than SBS due to higher availability of Cu in galena than SBS, which is high in organic matter. Flax shoot Zn content was higher with NH_4^+ treatment than NO_3^- (in an average of two soils and four concentrations). Lead

shoot content averaged over both concentrations, with the NH_4^+ was higher than NO_3^- ($P < 0.01$) The concentrations 0.1% and 0.25% were more significant in Pb root content than 1% concentration. This is attributed to the toxicity of lead at last concentration. The root Cu content with NH_4^+ + SBS treatment was more than NO_3^- + SBS in low concentration and was less than NO_3^- + SBS in high concentration.

There was no difference in Pb root content between NH_4^+ and NO_3^- on 0.1 and 0.25% G concentrations, but there was a difference with 1% G concentration, this attributed to ammonium enhancing shoot uptake more than nitrate (Table 7.6 and Table 7.7). From this experiment it is concluded that the flax plant can be used in phytoremediation assessment in moderate to high toxic sites, source of nitrogen can manipulate the flax rhizosphere and play a significant role in heavy metal extraction.

Chapter 8

Characterization of Amendments, cement and bone meal.

8.1 Introduction

From the previous chapter it can be seen that the toxicity of heavy metals affects the biomass production and consequently the phytoextraction of heavy metals. To develop a new amendment, the characteristics of this amendment are very important, especially information about effects on plant germination, adsorption of heavy metals and pH. This chapter investigates some essential characteristics of cement amendment and bone meal.

8.2 Material and methods

8.2.1 pH value of the amendments

Exactly 0.025, 0.05, 0.1, and 0.15 g of cement, bone meal were put separately into 100 ml glass bottles and 30 ml of deionised water was added to each bottle and all were shaken by hand periodically for 30 minutes and the pH was recorded for each sample.

Exactly 10 g of 1mm washed sharp sand or soil (UK grid reference NS 510652) were mixed thoroughly with 0.05 g of cement separately. All samples were transferred to 100 ml glass bottles and 20 ml of deionised water was poured in each glass bottle. All the bottles were shaken for 10 minutes and pH was measured for each.

8.2.2 The adsorption of Cu, Zn and Pb by the amendments cement and bone meal.

Stock solutions of 1000 mg/l of Cu, Zn and Pb were prepared separately by dissolving exactly 7.854 g of $CuSO_4 .5H_2O$, 8.795 g of $ZnSO_4 .7H_2O$ and 3.197g of Lead (II) nitrate 99% (Avocado L.T.D) (solution A, B and C respectively). From solutions A, B and C the concentrations (100, 250, 300, 600, and 1000 mg Cu/l), (100, 150, 300, 400, and 500 mg Zn/l) and (30, 60, 120, 240, and 300 mg Pb/l) were prepared.

One g of cement or bone meal was put into glass bottles and three replicates were used. Fifty ml of each concentration were poured into three replicates separately. All bottles were transferred to the shaker at 32 r.p.m. (Revolution per minute) for 1 hr. The samples were filtered into clean glass bottles using hardened filter paper No. 50 and Cu, Zn and Pb concentrations in mg/l at equilibrium were determined by the AAS. The results were calculated and adsorption of Cu by the two amendments was determined.

8.2.3 Effect of cement and bone meal on heavy metals immobilization after three months of incubation

A pot experiment was carried out to investigate the effect of cement or bone meal using two different soils, Galena soil and SBS soil mixed with 1 mm sharp sand, 2 and 4% w/w of cement or bone meal mixed thoroughly with G + sharp sand or SBS soil, along with 0% (control) All mixed samples were emptied in plastic pots separately. The result was 45 pots as shown in Figure 8.1 All pots were completely randomized and watered with deionised water; after 12 weeks, all samples were air

dried and EDTA-extractable and 0.01M $CaCl_2$ extractable heavy metals Zn, Cu and Pb were analysed with AAS. The results were statistically analysed with GLM MINITAP.

8.2.4 Effect of cement and bone meal on barley germination

Four concentrations of cement (2, 5, 10 and 15 % w/w in acid washed sand soil along with a zero cement (control) were used. 10-15 of barley seeds were seeded in pots containing 80 g of sand/cement mixing and the moisture content was kept at field capacity. Four concentrations of bone meal (0, 0.1, 0.2, 0.3 and 0.4 gm 20 ml/Petri dish) were used; 10-15 seeds of barley seeds were seeded in each Petri dish.

Block I

0% cement SBS	0% BM SBS	2% cement galena
0% cement galena	2% cement SBS	2% BM SBS
0% BM galena	2% BM galena	4% cement galena
4% cement SBS	4% BM galena	4% BM SBS

Block II

0% cement SBS	0% BM SBS	2% cement galena
0% cement galena	2% cement SBS	2% BM SBS
0% BM galena	2% BM galena	4% cement galena
4% cement SBS	4% BM galena	4% BM SBS

Block III

0% cement SBS	0% BM SBS	2% cement galena
0% cement galena	2% cement SBS	2% BM SBS
0% BM galena	2% BM galena	4% cement galena
4% cement SBS	4% BM galena	4% BM SBS

Block IV

0% cement SBS	0% BM SBS	2% cement galena
0% cement galena	2% cement SBS	2% BM SBS
0% BM galena	2% BM galena	4% cement galena
4% cement SBS	4% BM galena	4% BM SBS

Figure 8.1 shows the layout of the cement and bone meal incubation experiment (treatments randomized in each block).

8.3 Result and discussion

8.3.1pH assessment

Cement pH was very high without mixing, around 12 (Table 8.2). When the cement is mixed with water, the dicalcium silicates and the tricalcium silicates react with water molecules to form calcium silicate hydrate ($3CaO \times 2SiO_2 \times 3H_2O$) and calcium hydroxide Ca $(OH)_2$ producing high pH, and it was little affected by the quantity of the cement. The pH of bone meal was near to neutral (pH 6.5). The pH of cement was very alkaline compared with bone meal.

Table 8.1 The effect of different weight of cement or bone meal on pH in 30 ml.

Weight in g	pH	
	Cement	Bon meal
0.025	11.87	6.48
0.05	12	6.73
0.1	12.2	6.93
0.15	12.25	6.95

The pH is one of the main factors which affects the adsorption, and consequently immobilization of heavy metals. Cement has a high pH (more than 12). When cement was mixed with soil A, the pH of mixture of cement with sharp sand (Table 8.2) was not as affected as soil B and this difference is attributed to the buffering capacity of soil B. The toxicity of heavy metals occurs mostly in acid soils and this gives the opportunity to use the cement as amendment in acid toxic site soils.

Table 8.2 The effect of cement (Ce) on pH of two different soils A) acid washed sand and B) (UK grid reference NS 510652).

Treatment	pH	
	Average	Stand. error
0.5%Ce of A soil	11.7	0.11
0.5% Ce of B soil	8.7	0.04

8.3.3 Adsorption assessment

The Cu adsorption by cement followed the H-isotherm curve, which suggested that the cement has more affinity for adsorbing Cu or Cu is precipitated (Table 8.4 and Figure 8.2). Cement adsorbed more Cu than bone meal (Table 8.3, 8.4, Figure 8.2 and 8.3)

Table 8.3 Illustrate the Cu concentration mg/l at equilibrium and Cu adsorbed in mg/g cement.

Initial concentrations Cu mg/l	Cu concentration mg/l at equilibrium	Cu mg/g Cement
100	0	5.21
250	0	12.5
300	0	14.63
600	0	32.75
1000	0.2	47.41

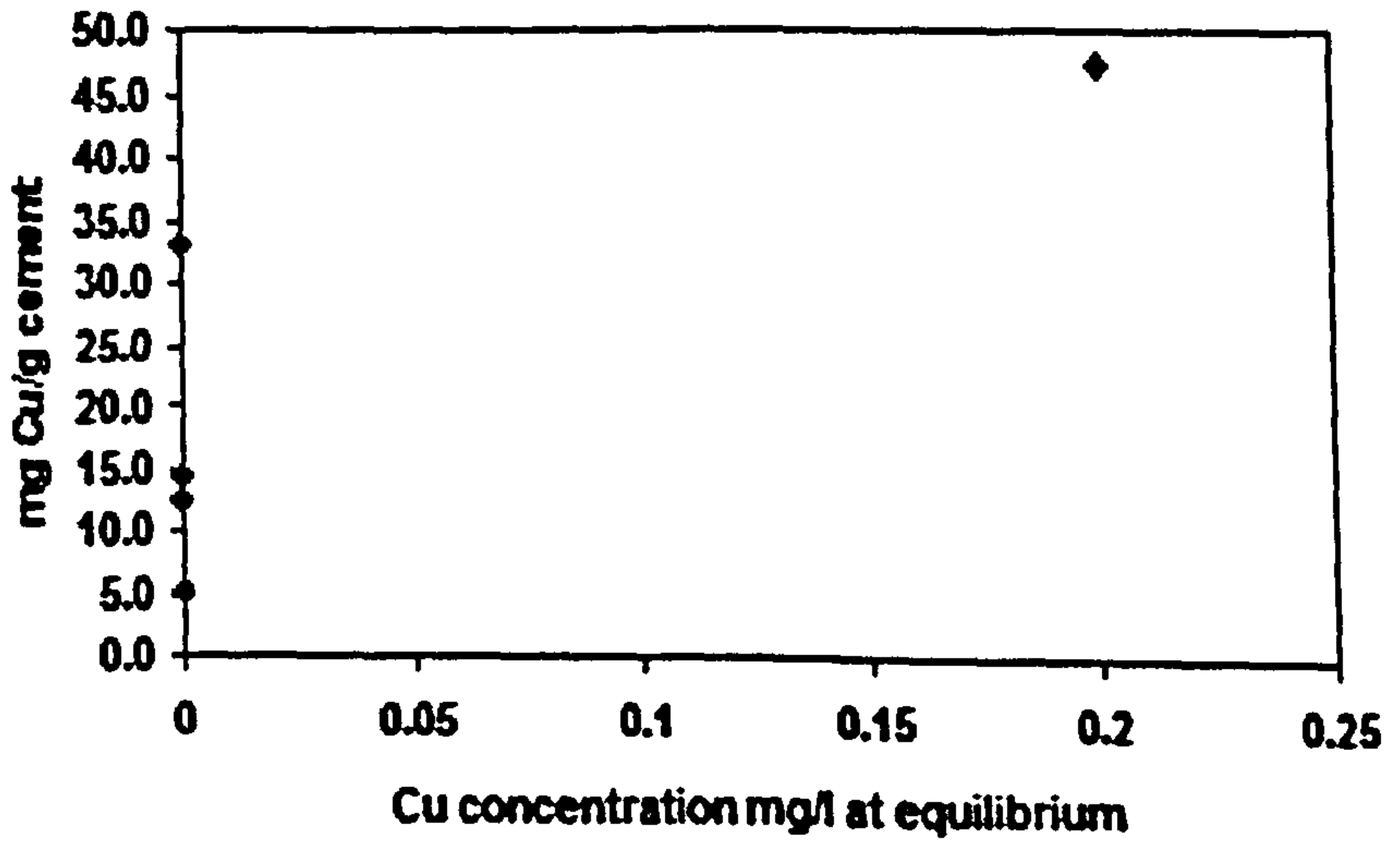

Figure 8.2 Illustrates the Cu concentrations mg/l at equilibrium and Cu adsorbed in mg/g cement.

Table 8.4 Illustrates the Cu concentrations mg/l at equilibrium and Cu adsorbed in mg/g bone meal.

Initial concentrations Cu mg/l	Cu concentration mg/l at equilibrium	Cu mg/g bone meal
100	7.35	1.4
250	32.1	1.9
300	103	1.1
600	195	2.3
1000	247	3.7

Cu adsorption by bone meal was irregular at low concentration, and bone meal had less affinity for Cu adsorption than cement. This may be attributed to the higher surface area and high pH of the cement and the irregularity may be attributed to the hydrophobia of fats in the bone meal to the water, which gave irregular adsorption.

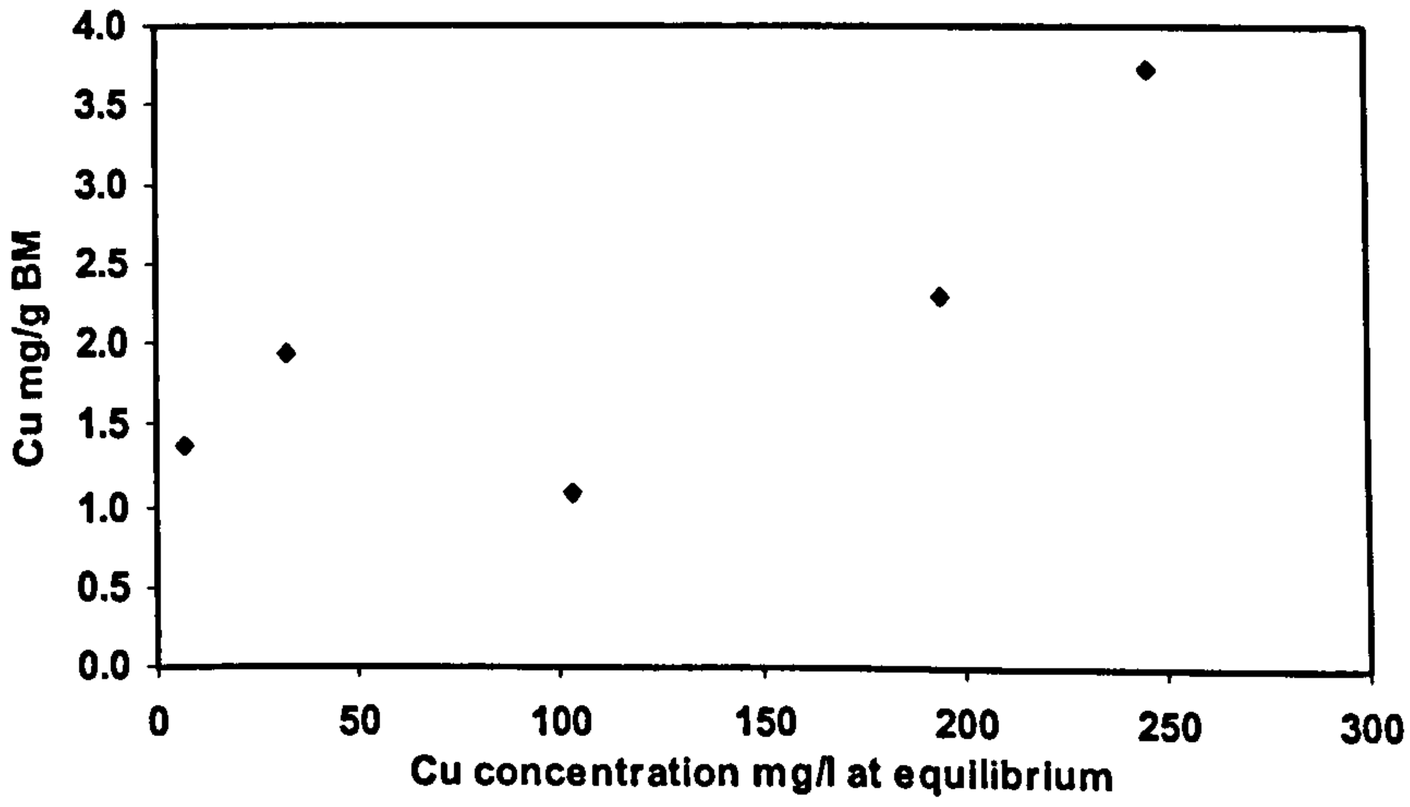

Figure 8.3 Illustrates the Cu concentrations mg/l at equilibrium and Cu adsorbed in mg/g bone meal.

Table 8.5 Illustrates the Zn concentration mg/l at equilibrium and Zn adsorbed in mg/g cement.

Initial concentrations Zn mg/l	Zn concentration mg/l at equilibrium	Zn mg/g Cement
100	0.11	5.6
150	0.14	8.2
300	0.16	17.7
400	0.16	22
500	0.24	27.1

The Zn adsorption by the cement followed S-curve isotherm, and may be due to the precipitation of the Zn at high pH and high concentration (Table 8.6, and Figure 8.4)

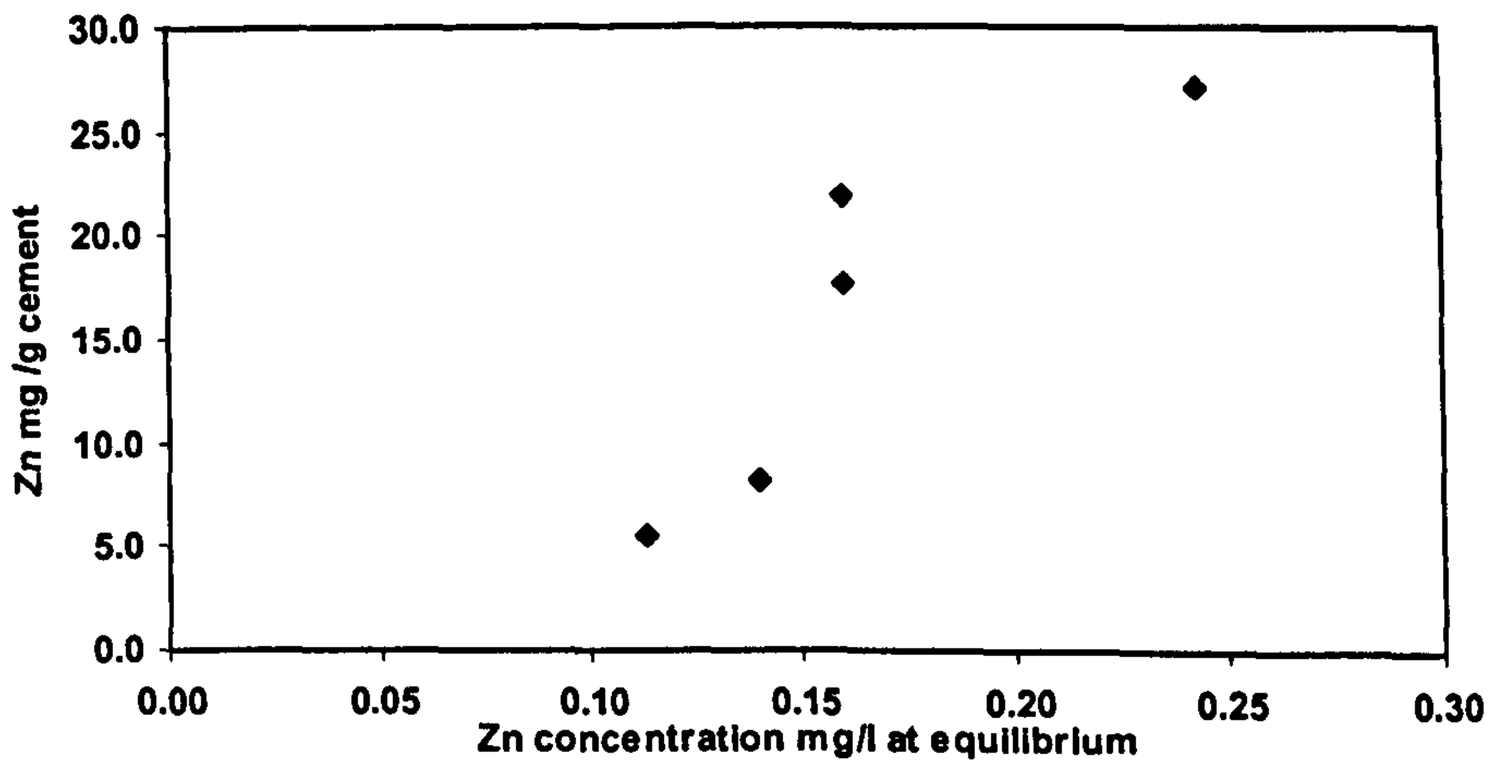

Figure 8.4 Illustrates the Zn concentrations mg/l at equilibrium and Zn adsorbed in mg/g cement.

Zn adsorption by the bone meal was unlike any normal isotherm curve shape; the adsorption was irregular. (Table 8.7 and Figure 8.11)

Table 8.6 Illustrate the Zn concentration mg/l at equilibrium and Zn adsorbed in mg/g bone meal.

Initial concentrations mg/l	Zn concentration mg/l at equilibrium	Zn mg/g bone meal
100	30.3	4.1
150	95	3.3
300	309.7	2.6
400	363	2.6
500	483	2.7

The adsorption of Zn by the bone meal did not follow any isotherm curve. Bone meal had higher affinity in low concentration of Zn than high concentration and this is indicated by low Zn adsorbed by bone meal, and also there was no precipitation at high concentration due to the pH. (Table 8.6 and Figure 8.5)

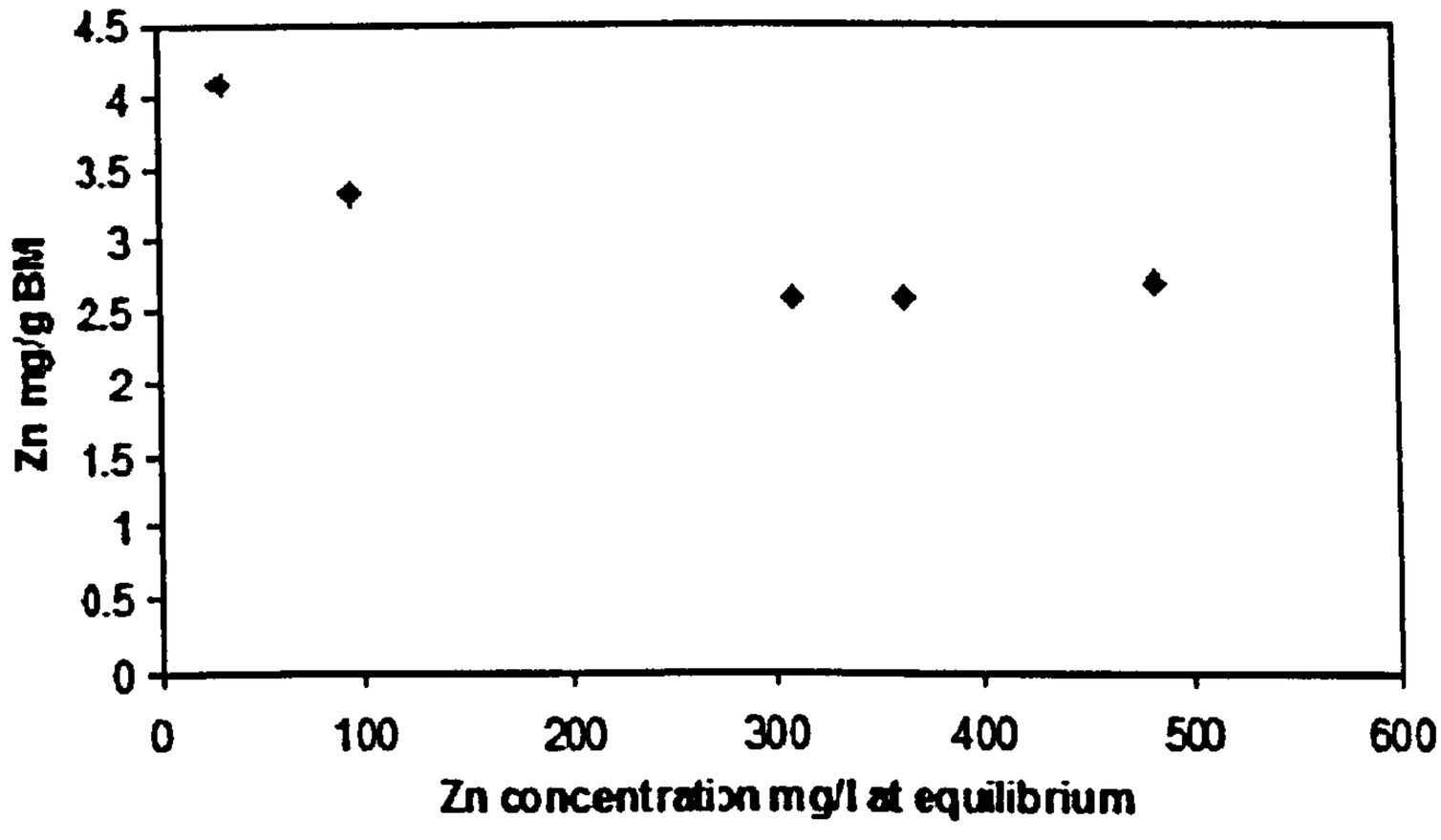

Figure 8.5 Illustrates the Zn concentrations mg/l at equilibrium and Zn adsorbed in mg/g bone meal.

The adsorption of Pb by cement followed the C-curve isotherm. Adsorptive concentration increased with the adsorption increase, and the surface of adsorbate increased. The C-isotherm is characterized by initial slope, which is independent of adsorptive concentration to the maximum. (Table 8.7 and Figure 8.6)

Table 8.7 Illustrate the Pb concentrations mg/l at equilibrium and Pb adsorbed in mg/g cement.

Initial concentrations Pb mg/l	Pb concentration mg/l at equilibrium	Pb mg/g Cement
30	0.11	1.3
60	0.13	3
120	0.16	6.8
240	0.21	11.6
300	0.23	14.2

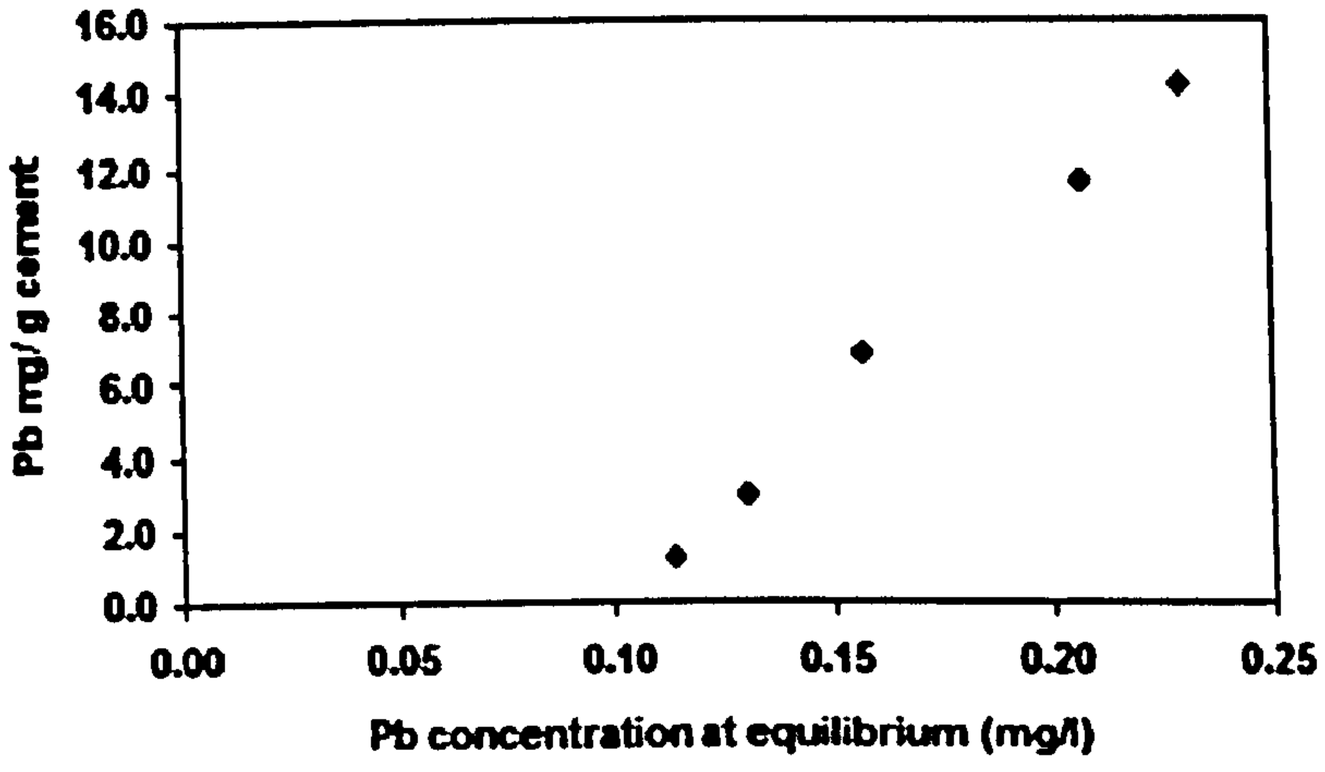

Figure 8.6 Illustrates the Pb concentrations mg/l at equilibrium and Pb adsorbed in mg/g cement.

Lead adsorption by bone meal increased in low concentrations and at high concentration constant the maximum was 16 mg/g bone meal.

Table 8.8 Illustrate the Pb concentration mg/l at equilibrium and Pb adsorbed in mg/g bone meal.

Initial concentrations Pb mg/l	Pb concentration mg/l at equilibrium	Pb mg/g bone meal
30	3.7	1
60	0.5	7
120	0.9	12
240	8.4	16
300	52.8	16

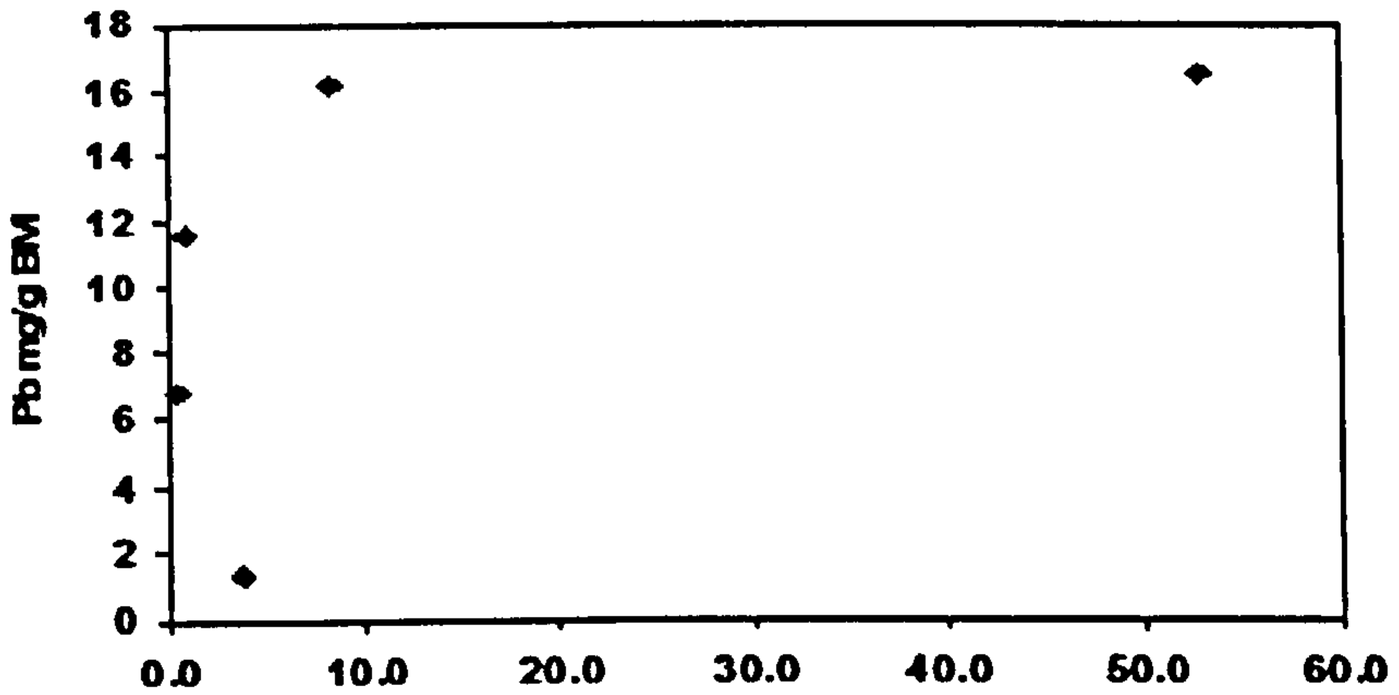

Figure 8.7 Illustrates the Pb concentration mg/l at equilibrium and Pb adsorbed in mg/g bone meal.

The adsorption of Pb by bone meal followed the H-curve isotherm character. At high concentration there was no change in the adsorption (Table 8.8 and Figure 8.7).

8.3.4 Amendment incubation

To assess the availability and solubility of heavy metals in both soils with both amendments at different concentration, two methods of extraction were used, EDTA extract and 0.01M $CaCl_2$.

Table 8.9 Effect of a) two amendments averaged over both two soils and three concentrations b) two soils averaged over both two amendments and three concentrations on EDTA-extractable and 0.01M $CaCl_2$-extractable metals after three months of incubation.

Treatments	EDTA-extractable metals			0.05M $CaCl_2$-extractable metals			pH
a (n = 12)	Cu	Zn	Pb	Cu	Zn	Pb	
Cement	248a	496	2924 a	2	7 b	58	9 a
B.M	101b	509	2483 b	2	15 a	68	7 b
LSD P< .05	113	N.S	328	N.S	4	N.S	1
b (n = 12)							
G	15 b	131 b	5378 a	1 b	18 a	127 a	8
SBS	333 a	874 a	30 b	3 a	5 b	0	8
LSD P< .05	68	28	328	0.7	4	46	N.S

Values in the same column in part a or b with different letters were significantly different

The EDTA extractable Cu from cement treatment averaged in both soils and concentrations was more than B.M amendment ($P < 0.05$) (Table 8.11). There was no significant difference between two amendments in amount of Cu extractable with 0.01M $CaCl_2$, while this was less than EDTA-extractable Cu. This is attributed to the EDTA being a stronger extractant than 0.01 M $CaCl_2$ in extractability of heavy metals. There was no significant difference between the two amendments in the EDTA-extractable Zn. The 0.01 M $CaCl_2$- extractable Zn in B.M treatment was significantly higher than cement amendment. The cement immobilizes the Zn more than B.M but in the Pb no difference was remarked ($P < 0.05$).

Table 8.10 Effect of two amendments on EDTA-extractable and 0.01M $CaCl_2$- extractable heavy metals after three months of incubation.

Treatments	EDTA-extractable metals			0.05M $CaCl_2$-extractable metals			pH
a (n = 4)	Cu	Zn	Pb	Cu	Zn	Pb	
0% Ce+G	7d	109c	5515a	nd	32	350a	6.1d
2% Ce+G	25d	146c	5318b	nd	nd	nd	11.1a
4% Ce+G	38d	182c	6635a	nd	nd	nd	12.0a
0% Ce+SBS	489a	922a	29c	3	7	nd	7.1c
2% Ce+SBS	465b	823b	24c	4	nd	nd	9.2b
4% Ce+SBS	463b	792b	24c	4	nd	nd	9.1b
0% BM+G	7d	105c	5485a	nd	37	316a	6.2
2% BM+G	7d	117c	4750b	nd	19	62b	6.1b
4% BM+G	8d	126c	4563b	nd	17	32b	6.2a
0% BM+SBS	481a	878a	32c	2	8	nd	7.1c
2% BM+SBS	53c	923a	35c	4	6	nd	7.2c
4% BM+SBS	50c	904a	35c	5	5	nd	7.1c
LSD $P < 0.05$	22	80	1175	1	6	39	0.9

Values in the same column in part a or b with different letters were significantly different

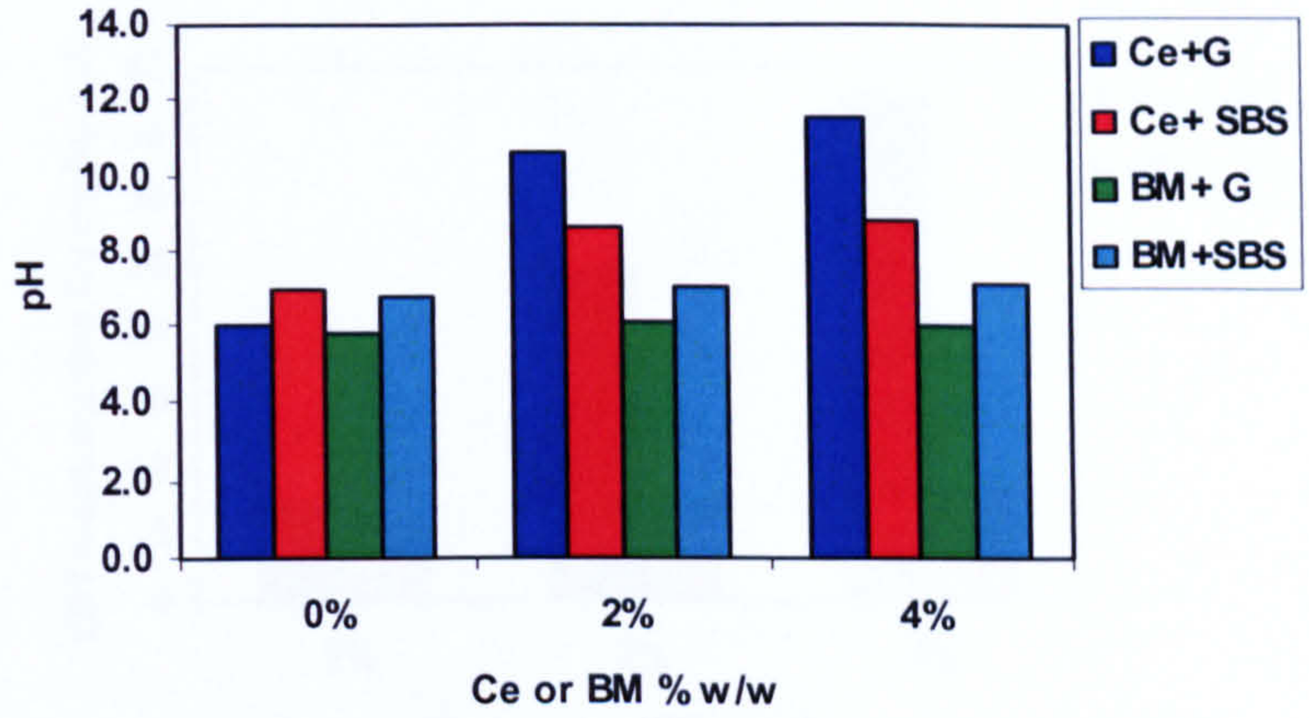

Figure 8.8 The effect of cement or BM on the pH of two soils SBS and G after three months of incubation.

In EDTA-extractable Cu 0% Ce+G < 2% Ce +G = 4% ce+G ($P < 0.05$) and in the 0.01 M $CaCl_2$-extractable Cu was not detected (Table 8.12 and Figure 8.8) this indicated that the cement amendment adsorbed the Cu from the soil and the Cu precipitated at high pH (Figure1). Cu 0% Ce+SBS > 2% Ce + SBS = 4% Ce+ SBS ($P < 0.05$) in EDTA extractable Cu, the cement amendment reduced the EDTA-extractable Cu and there was no difference between the 2% and 4% addition. In SBS soil the B.M amendment decreased EDTA extractable Cu more than cement amendment in both concentrations (2% and 4%) ($P < 0.05$) (Figure 8.9) and this may be attributed to the organic matter content of the soil, high pH in cement amendment may solubilise the organic matter and release the Cu higher than B.M amendment.

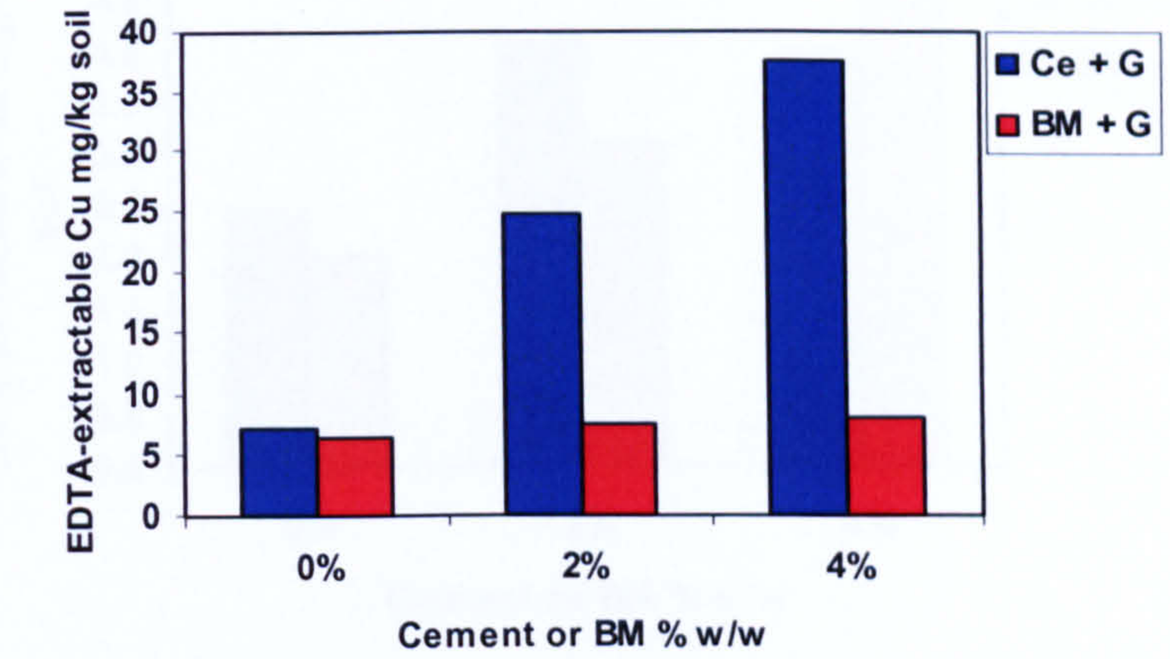

Figure 8.9 The effect of cement or BM on EDTA-extractable Cu of G soil after three months of incubation.

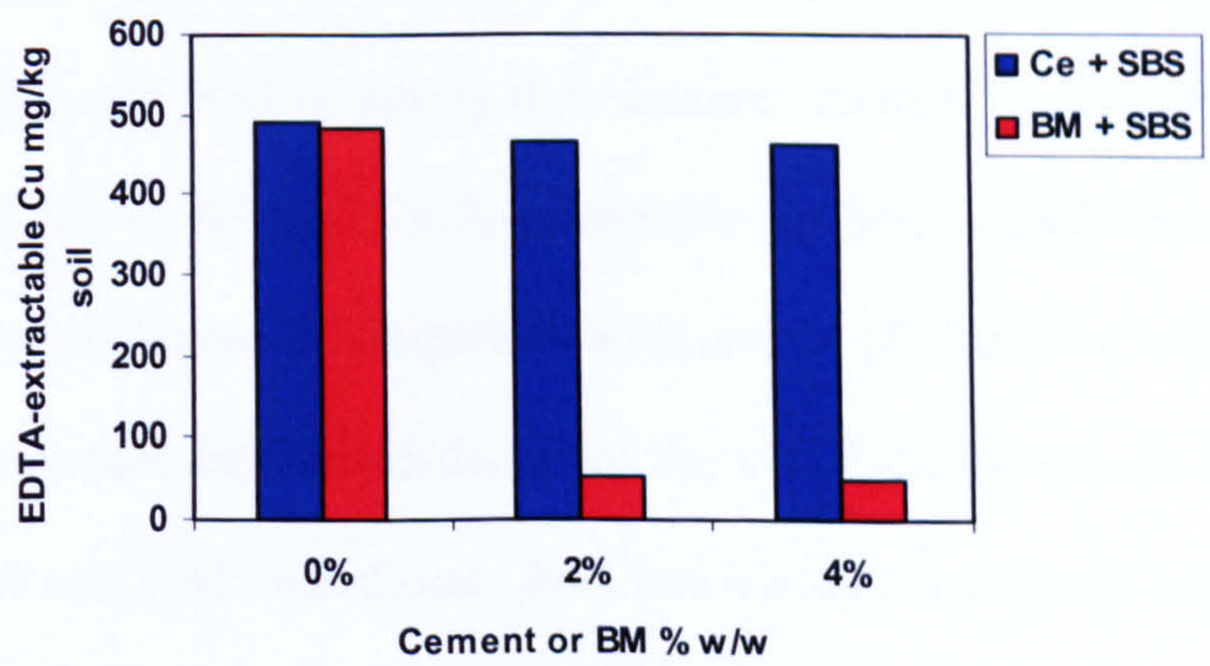

Figure 8.10 The effect of cement or BM on EDTA-extractable Cu of SBS soil after three months of incubation.

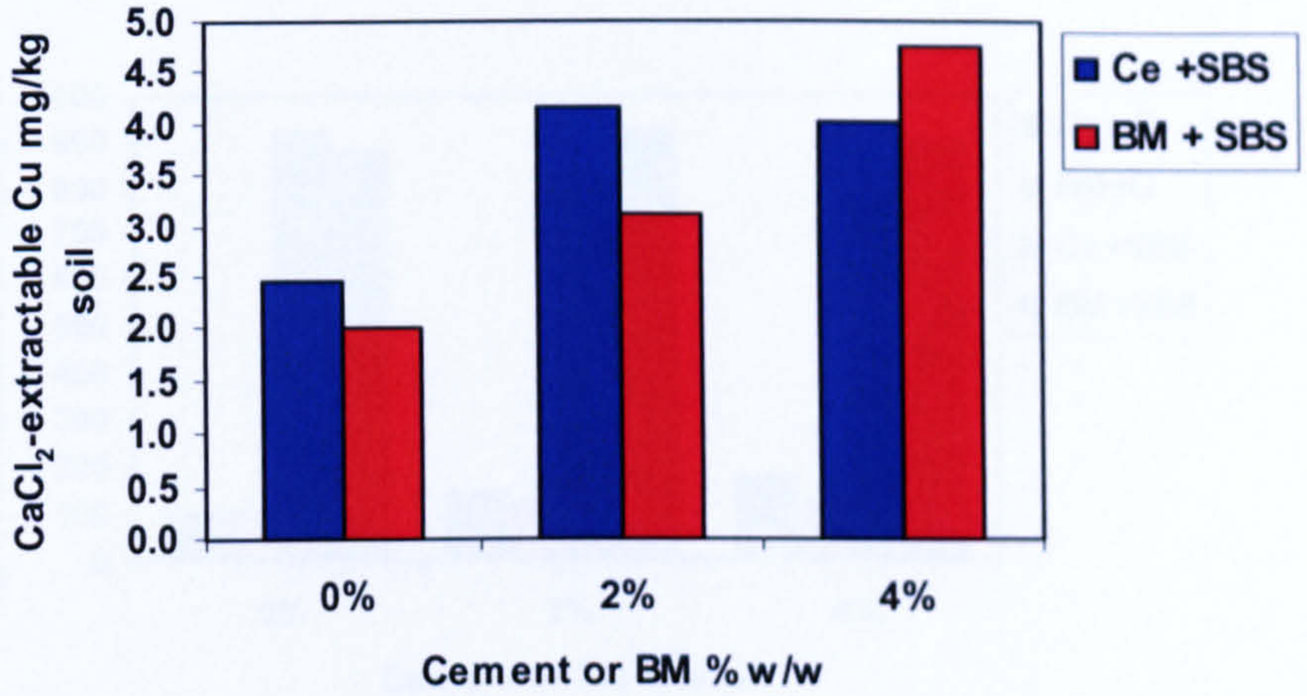

Figure 8.11 The effect of cement or BM on $CaCl_2$-extractable Cu of SBS soil after three months of incubation.

There was a difference between cement and BM treatment in 4 % treatment in 0.01 M $CaCl_2$ extractable Cu (Table 8.12 and Figure11)

In EDTA-extractable Zn in G soil there was no difference between the two amendments cement and B.M or among their concentrations ($P < 0.05$) (Table 8.12 and Figure 8.12), but in 0.01 M $CaCl_2$-extractable Zn both amendment with their concentrations were effective in comparison with control ($P < 0.05$) (Table 8.12 and Figure 8.13). The cement amendment decreased the EDTA-extractable Zn in SBS soil more than control and B.M amendment. Both amendments decreased 0.01 M $CaCl_2$ extractable Zn in comparison with control ($P < 0.05$) (Table 8.12 and Figure 8.13).

In EDTA-extractable Pb there was no difference between both amendments and their concentrations, However both amendments decrease 0.01 M $CaCl_2$-extractable Pb comparison with control and cement amendment was decrease Pb more significantly than BM amendment in G soil ($P < 0.05$) (Table 8.12 and Figure 8.14).

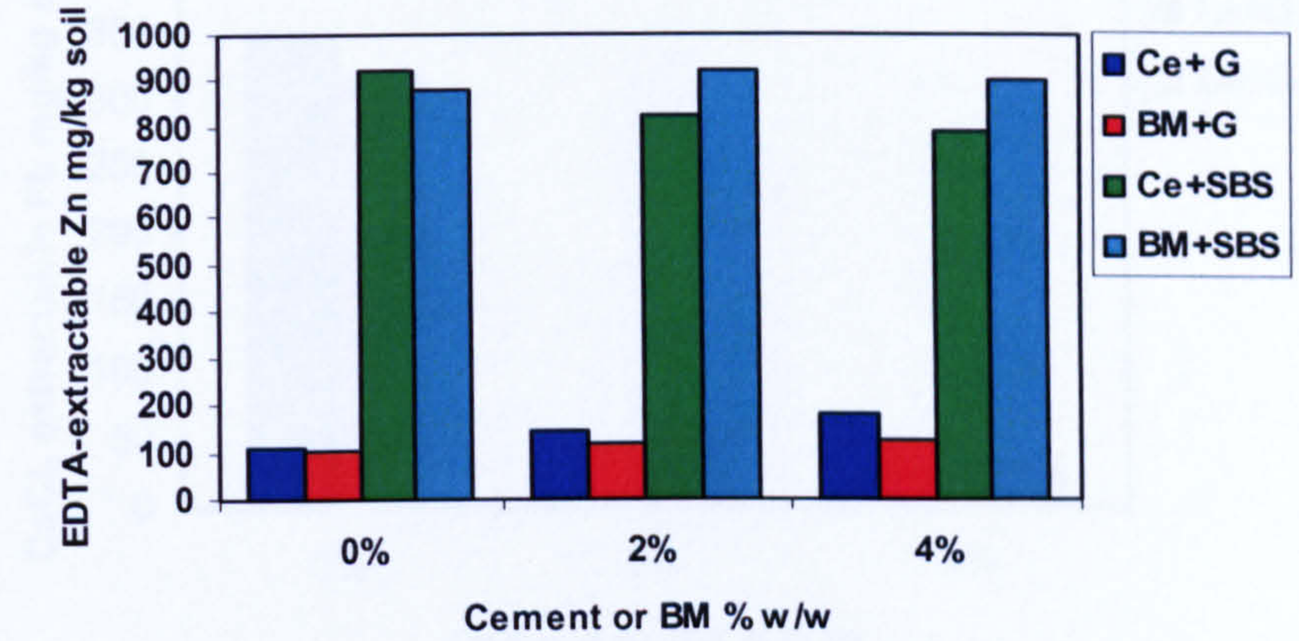

Figure 8.12 The effect of cement or BM on EDTA-extractable Zn of SBS and G soil after three months of incubation.

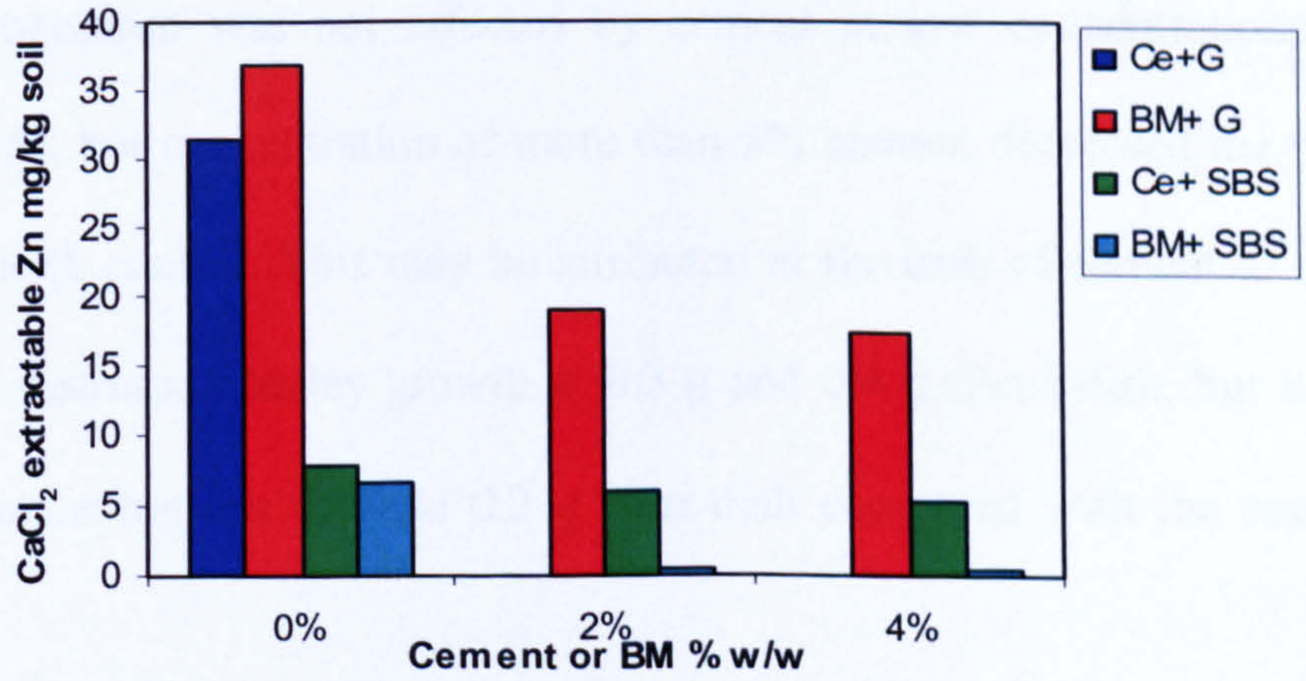

Figure 8.13 The effect of cement or BM on $CaCl_2$-extractable Zn of SBS and G soil after three months of incubation.

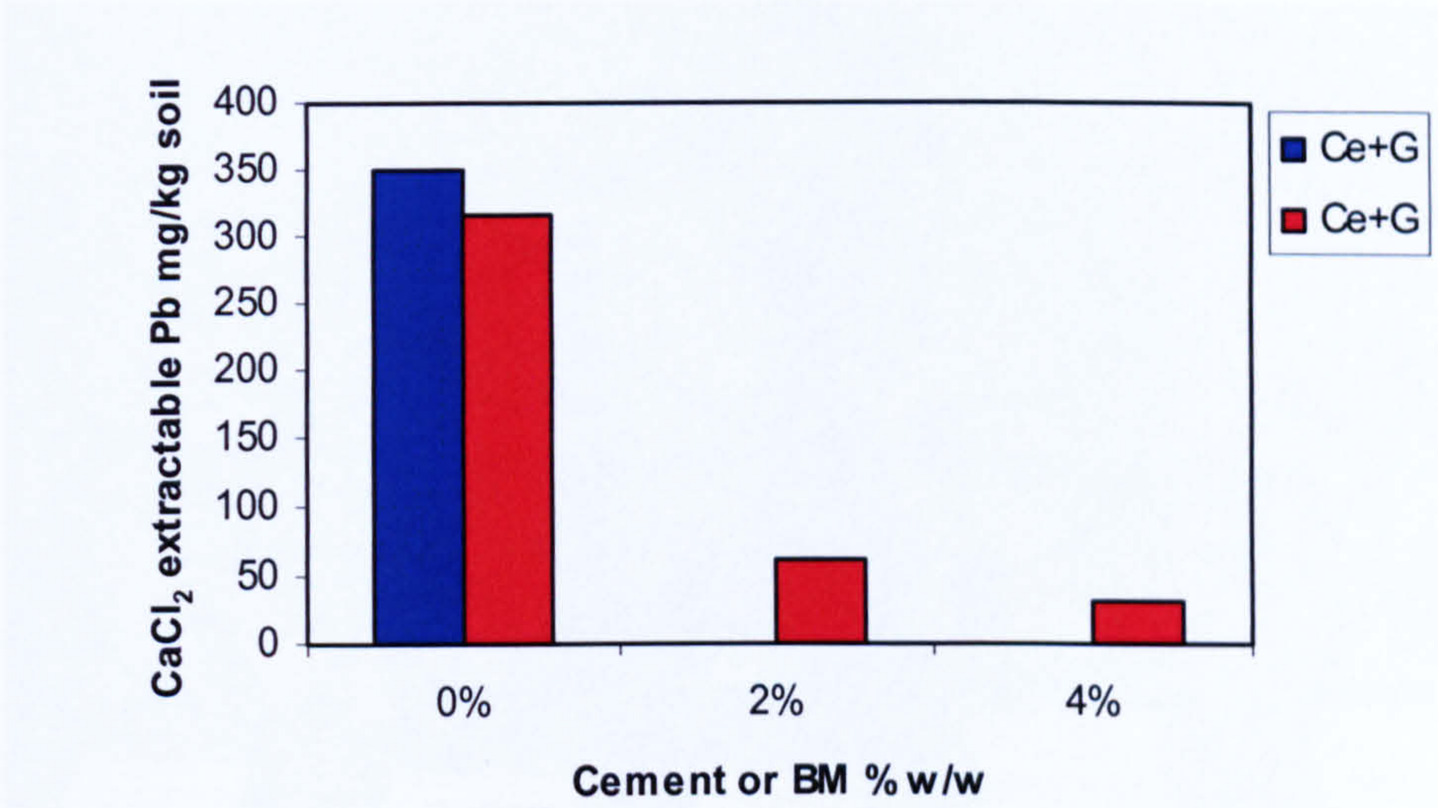

Figure 8.14 The effect of cement or BM on $CaCl_2$-extractable Pb of G soil after three months of incubation.

8.3.4 Barley germination in different concentrations of cement and bone meal

Barley germination was not affected by cement in low concentrations 2 and 5% (Figure 8.15), but concentration of more than 5% cement decreased the plant growth compared with control. This may be attributed in the case of cement to the high pH. Bone meal decreased barley growth at 0.3 g and 0.4 g /Petri dish, but had no effect with the concentrations 0.1 and 0.2 g/Petri dish compared with the control (Figure 8.16).

Figure 8.13 Illustrate the germination of barley seeds, root and leaf elongation in different concentrations of cement % w/w soil

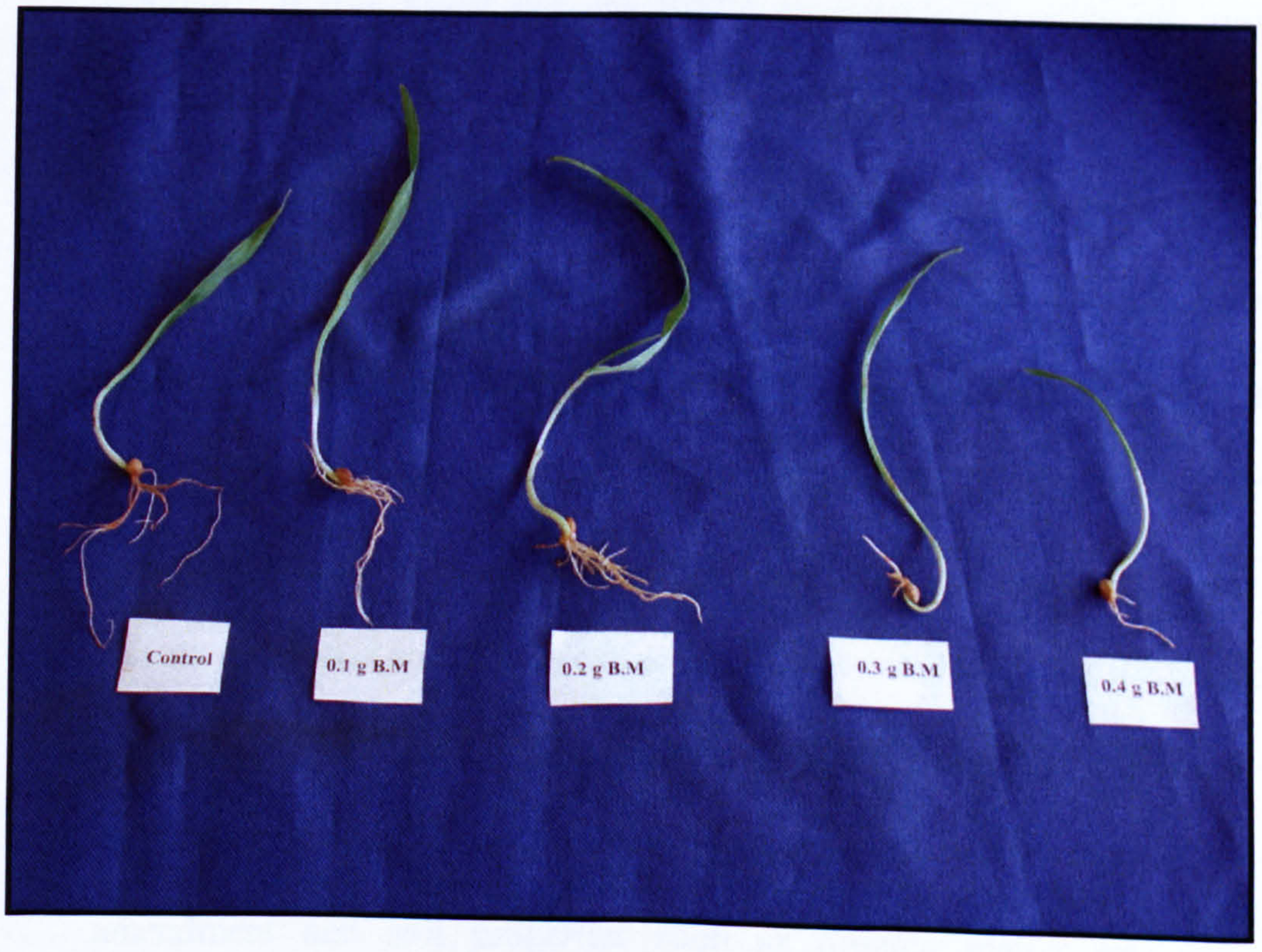

Figure 8.14 Illustrate the germination of barley seeds, root and leaf elongation in different concentrations of bone meal in g/20 ml deionised water/ Petri dish.

8.4 Conclusion

The cement amendment in general has more affinity to adsorb or precipitate heavy metals than bone meal. Cement also can be used as lime to increase the pH of acid soils. The amendments cement and bone meal, differ in their affinity to different metals, and the affinity of those amendments follows this order: for Cu and Zn cement > bone meal and for Pb cement = bone meal.

A pot experiment was conducted over three months incubation with cement or bone meal (BM) with two different soils, one high in lead content and the other sludge treated soil (SBS). The available or soluble metals were assessed by two methods using chelating agent EDTA and dilute salt ($CaCl_2$) solution. It was found that the EDTA-extractable Cu averaged over all concentrations and the two soils with the cement was more than that with BM but was not different with $CaCl_2$ extraction. There was no difference between cement and BM in EDTA-extractable Zn on average over all concentrations and two soils, but their effect upon cement gave more $CaCl_2$-extractable Zn than BM. For EDTA-extractable Pb averaged over all concentrations and two soils BM gave a lower value than cement and there was no difference with $CaCl_2$ extraction. It was concluded that the extraction for assessing the availability and accessibility depends on the extractant used and immobilization of heavy metals depends on the type and quantity of the amendment and soil properties such as organic matter and pH. The technology of both, controlling the mobility and availability of heavy metals by amendments and manipulation of the rhizosphere to change a particular area, but not all the soil, is the master key and strategy of phytoremediation.

The cement amendment can be used in the toxic sites to elevate or render the toxicity effect. In the next chapter barley is used as a test crop to study the effect of cement amendment compared with bone meal and control, with high Zn and Cu concentrations.

Chapter 9

Effect of different concentrations of amendments cement and bone meal on immobilization of Cu and Zn and manipulation of the rhizosphere with NH_4^+ or NO_3^- on bioavailability and solubility of Cu and Zn using barley as a test crop.

9.1 Introduction

Phytoremediation is a cost effective and environmental friendly technique, but there is particular challenge on highly toxic sites, which inhibit growth of plants and reduce the biomass production and consequently lower phytoextraction. The integration between amendments and plants was the task of the experiments described in this chapter. The main goal of this research is to investigate the novel amendment cement and compare it with bone meal, and additionally to manipulate the barley rhizosphere by two nitrogen fertilizer sources. Pot experiment using sand soil polluted by Cu and Zn and mixed with different rates of cement or bone meal was used. All pots were sown with barley (*Hordium Vulgare* L) and two sources of nitrogen fertilizers NH_4^+ as $(NH_4)_2SO_4$ or NO_3^- as KNO_3 were added

9.2 Materials and methods

Sharp sand 1mm in diameter was artificially contaminated with 60 mg/kg soil Cu as $CuSO_4$ and 60 mg/kg soil Zn as $ZnSO_4$. The solution of both salts of metals was mixed thoroughly with the soil. The soil allowed to air dry and mixed several times thoroughly. 650 gm of polluted soil were mixed with cement or BM in four different percent in weight basis 0%, 0.5%, 1% and 1.5%, each treatment was replicated four times. The result was 2 nitrogen sources x 2 amendment x4 percent x 4 replicates = 64 treatments (16 treatments x 4 blocks).

All pots were put in saucers. Barley seeds were seeded on 24/05/05. The pots were irrigated by deionised water from the surface, after 10 days plants were thinned to 8 plants per pot. The nitrogen, 34 mg/pot, in the same rates, as either NH_4^+ or NO_3^- was added. Phosphorus, 16mg/pot, was added after seeding. Other micronutrients in the same amount in quarter Hoagland solution were added to all pots at the two to three leaf stage of barley plant. After 9 weeks, length of shoots and roots were measured. All plants were harvested, fresh weight of shoots were taken. Shoots and roots were allowed to dry in the oven at 72 °C for72 hr and weighed with electronic 4 digital balance. Samples of roots and shoots were ground by machine and prepared for chemical analysis. Soil samples, bulk and around the roots, were air dried and pH in bulk and around soil roots, Cu and Zn contents in shoots and roots, EDTA- extractable metals and 0.01 M $CaCl_2$ Cu and Zn soil were determined with AAS and the data were recorded and statistically analysed with MINITAB (GLM).

Block I

NO_3^- 0% cement	NH_4^+ 0% cement	NH_4^+ 0.5% cement	NO_3^- 1% B.M
NO_3^- 0.5% cement	NO_3^- 0% B.M	NH_4^+ 1% cement	NH_4^+ 0.5 B.M
NO_3^- 1% cement	NO_3^- 0.5% B.M	NH_4^+ 1% B.M	NH_4^+ 0% B.M

Block II

NO_3^- 0% cement	NH_4^+ 0% cement	NH_4^+ 0.5% cement	NO_3^- 1% B.M
NO_3^- 0.5% cement	NO_3^- 0% B.M	NH_4^+ 1% cement	NH_4^+ 0.5 B.M
NO_3^- 1% cement	NO_3^- 0.5% B.M	NH_4^+ 1% B.M	NH_4^+ 0% B.M

Block III

NO_3^- 0% cement	NH_4^+ 0% cement	NH_4^+ 0.5% cement	NO_3^- 1% B.M
NO_3^- 0.5% cement	NO_3^- 0% B.M	NH_4^+ 1% cement	NH_4^+ 0.5 B.M
NO_3^- 1% cement	NO_3^- 0.5% B.M	NH_4^+ 1% B.M	NH_4^+ 0% B.M

Block IV

NO_3^- 0% cement	NH_4^+ 0% cement	NH_4^+ 0.5% cement	NO_3^- 1% B.M
NO_3^- 0.5% cement	NO_3^- 0% B.M	NH_4^+ 1% cement	NH_4^+ 0.5 B.M
NO_3^- 1% cement	NO_3^- 0.5% B.M	NH_4^+ 1% B.M	NH_4^+ 0% B.M

Figure 9.1 Illustrates the layout of the experiment (randomization in each lot).

9.3 Results and discussion

9.3.1EDTA and $CaCl_2$ extractable metals

To assess the availability and solubility of heavy metals two extracting agents were used; EDTA chelate and dilute salt 0.01 M $CaCl_2$. Over both amendments, cement and B.M, NH_4^+ increased the EDTA extractable Cu and 0.01 M $CaCl_2$ extractable Zn in the bulk soil compared to NO_3^- ($P < 0.05$) (Table 9.1).

Table 9.1 Effect of a) nitrogen source averaged over cement, BM and three concentrations and b) amendment averaged over both two nitrogen sources and three concentrations on EDTA and $CaCl_2$ extractable Cu and Zn in mg/kg soil post harvest of barley.

Treatment (n=24)	EDTA-extractable mg/kg Bulk soil		$CaCl_2$-extractable mg/kg Bulk soil	
	Cu	Zn	Cu	Zn
a)				
NH_4^+	45.4a	52.4	10.4	18.7a
NO_3^-	42.5b	50.8	10	14.3b
LSD $P < 0.05$	1.5	NS	NS	1.3
b)				
Cement	44..6	52.2	8.4b	13b
B.M	43.4	51.0	12a	20a
LSD $P < 0.05$	NS	NS	0.7	1.3

Values in the same column in part a or b with different letters were significantly different

There was no significant difference between amendments for EDTA-extractable Cu and Zn, but in 0.01 M $CaCl_2$ extractable Cu and Zn was higher for bone meal than cement ($P < 0.05$) and this is attributed to cement immobilizing the heavy metals more than B.M. The EDTA chelate was stronger than salt 0.01M$CaCl_2$ for heavy metal extraction. Ethylendiaminetetraacetate EDTA, which increases the solubilization of poorly available metals in soils, followed by a large accumulation of metal complexes in biomass (Blaylock et al., 1997; Sarret et al., 2001).

Table 9.2 Effect of a) nitrogen source averaged over both three concentrations and b) concentrations averaged over both two nitrogen sources on EDTA and $CaCl_2$ extractable Cu and Zn in mg/kg and pH around roots soil post harvest of barley.

Treatment	EDTA-extractable mg/kg		$CaCl_2$-extractable mg/kg		pH 1:5 H_2O
	Cu	Zn	Cu	Zn	
a (n = 12)					
NH_4^+	20.5	26.5	2.2b	1.1	8.4b
NO_3^-	22.1	27.4	2.8a	1.5	9.0a
LSD P < 0.05	**N.S**	**N.S**	**0.40**	**N.S**	**0.16**
b (n = 8)					
0.5%	23.4	35.0	2.4	1.0	8.4
1%	19.6	24.2	2.4	1.0	8.8
1.5%	21.0	21.8	2.7	1.1	8.9
LSD P < 0.05	**3.5**	**3.62**	**N.S**	**N.S**	**0.24**
c (n = 4)					
0.5%Ce + NH_4^+	21.8	34.3	2.2	1.2	8.0b
1%Ce + NH_4^+	18.8	23.8	2.2	1.11	8.6a
1.5%Ce + NH_4^+	21.0	21.5	2.20	1.03	8.7a
0.5%Ce + NO_3^+	24.9	35.6	2.54	0.8	8.9a
1%Ce + NO_{3+}	20.5	24.6	2.52	1.0	9.0a
1.5%Ce + NO_3^+	21.0	22.1	3.2	1.2	9.1a
LSD P < 0.05	**N.S**	**N.S**	**N.S**	**N.S**	**0.42**

Values in the same column in part a or b with different letters were significantly different

Over the three concentrations there was no significant difference in EDTA-extractable Cu and Zn between the two sources of nitrogen NH_4^+ and NO_3^-. In both average concentrations, the NO_3^- had more Cu in 0.01 M $CaCl_2$ and pH with NO_3. higher than NH_4^+ treatment ($P < 0.05$) (Table 9.2) and this indicated the increase in acidity by the NH_4^+ more than NO_3^-. There was no germination in the control (0% concentration) in both sources of nitrogen (Figure 9.6 and 9.9) due to the toxicity of Zn and Cu. Also all B.M concentrations treatment harmed and affected plant growth and no plants survived to the end of the experiment (Figure 9.8, 9.10 and 9.16). There was no difference between NH_4^+ and NO_3^- with all cement concentrations in both heavy

metals Cu and Zn with both extracting agents. However, there was a significant difference between ammonium and nitrate with different concentrations in the following order: NO_3^- + 0.5% cement > NH_4^+ +0.5% cement, NO_3^- + 1% cement > NH_4^+ + 0.5% cement and NO_3^- + 1% cement > NH_4^+ + 0.5% cement. The NH_4^+ increased the acidity on average over all concentrations, or as interaction with each concentration individually, more than NO_3^- ($P < 0.05$) (Table 9.2) and this agreed with previous results (Chapter 3). The cement amendment was superior compared with B.M amendment. All the plants amended with B.M died. This probably due to biological toxicity of B.M. Toxicity germination test of B.M on barley seeds 0.4 g of B.M affect the germination shoot and root length (Chapter 8 section 8.3.1 Figure 8.3) and this may need further research.

Table 9.3 Effect of a) nitrogen source averaged over three concentrations and two locations b) locations averaged over both two nitrogen sources and three concentrations on EDTA and $CaCl_2$ extractable Cu and Zn in mg/kg and pH around roots and bulk soil post harvest of barley.

Treatment	EDTA-extractable mg/kg		$CaCl_2$-extractable mg/kg		pH
	Around roots and bulk soil mg/kg				1:5 H_2O
	Cu	Zn	Cu	Zn	
a (n=24)					
NH_4^+	31.9	36.9b	1.7b	4	9.1
NO_3^-	33	40.5a	2.1a	0.6	9.3
LSD P < 0.05	**NS**	**2.06**	**0.3**	**0.52**	**0.1**
b (n=24)					
around	21.3	27b	2.5a	1b	8.7b
bulk	43.5	50.5a	1.3b	3.5a	9.7a
LSD P < 0.05	**4.1**	**2.06**	**0.31**	**0.52**	**0.1**
c (n=16)					
0.50%	30.9b	40.6a	2.3a	5.7a	8.7b
1%	32.3ab	38.6a	1.7b	0.5b	9.4a
1.50%	34.1a	37b	1.8b	0.6b	9.5a
LSD P < 0.05	**3.2**	**3.04**	**0.46**	**0.76**	**0.15**

Values in the same column in part a or b with different letters were significantly different

Averaged over all of the concentrations of cement, and in the around roots and bulk soils, nitrate resulted in a higher soil pH, EDTA Zn, $CaCl_2$-Zn and $CaCl_2$-Cu than ammonium ($p < 0.05$) (Table 9.3). Over nitrogen treatments and both the root and the bulk soil, the concentration 0.5% cement was significantly higher in both heavy metals and both methods of extractants than 1.5% except EDTA-extractable Cu. This is attributed to high immobilization at high concentration due to adsorption or precipitation by the cement amendment, and in the case of Cu may be due to organic matter dissolution and strong extractant EDTA.

On average over all the concentrations and the nitrogen sources, the EDTA-extractable Cu and Zn in bulk soil were higher than rhizosphere soil. This is attributed an exhausted pool of the heavy metals in the rhizosphere more than bulk soil. In 0.05 M $CaCl_2$-extractable Cu rhizosphere was higher than bulk soil and this is attributed the Cu being more available in the low pH and absence of organic matter. The 0.05 M $CaCl_2$ –extractable Zn in rhizosphere (around root) was less than bulk soil and this is attributed to the changed pool of Zn in rhizosphere soil and the plant requirement of

Zn more than Cu. Both heavy metals with both extractants EDTA and 0.01 M $CaCl_2$ the 0.5 cement concentration was higher than high concentration 1.5% and this is due to the immobilization of Zn and Cu.

9.3.2Biomass

On average over all amendments and concentrations, the nitrate treatment was significantly higher than ammonium in fresh, dry shoot and dry root ($P < 0.05$) (Table 9.4 and Figure 9.7 and 9.8) and there was no significant difference in shoot length. The cement amendment was significantly higher than B.M in shoot length, shoot fresh weight, dry shoot weight and dry root weight ($P < 0.05$) (Table 9.4 Figure 9.6, 9.7, 9.8 and 9.9).

Table 9.4 Effect of a) nitrogen source averaged over cement, BM and three concentrations and b) amendment averaged over both two nitrogen sources and three concentrations on shoot length, fresh shoot, dry shoot and dry root of barley in g.

Treatment	Shoot Length	Weight in g		
	In cm	Fresh shoot	Dry shoot	Dry root
A (n = 16)				
NH_4^+	14.6	1.3b	0.4b	0.09b
NO_3^-	15	1.6a	0.5a	0.17a
LSD $P < 0.05$	**NS**	**0.2**	**0.06**	**0.03**
B (n = 16)				
Cement	26.4a	2.4a	0.7a	0.25a
BM	3.2b	0.5b	0.1b	0.01b
LSD $P < 0.05$	**1.25**	**0.2**	**0.06**	**0.03**

Values in the same column in part a or b with different letters were significantly different

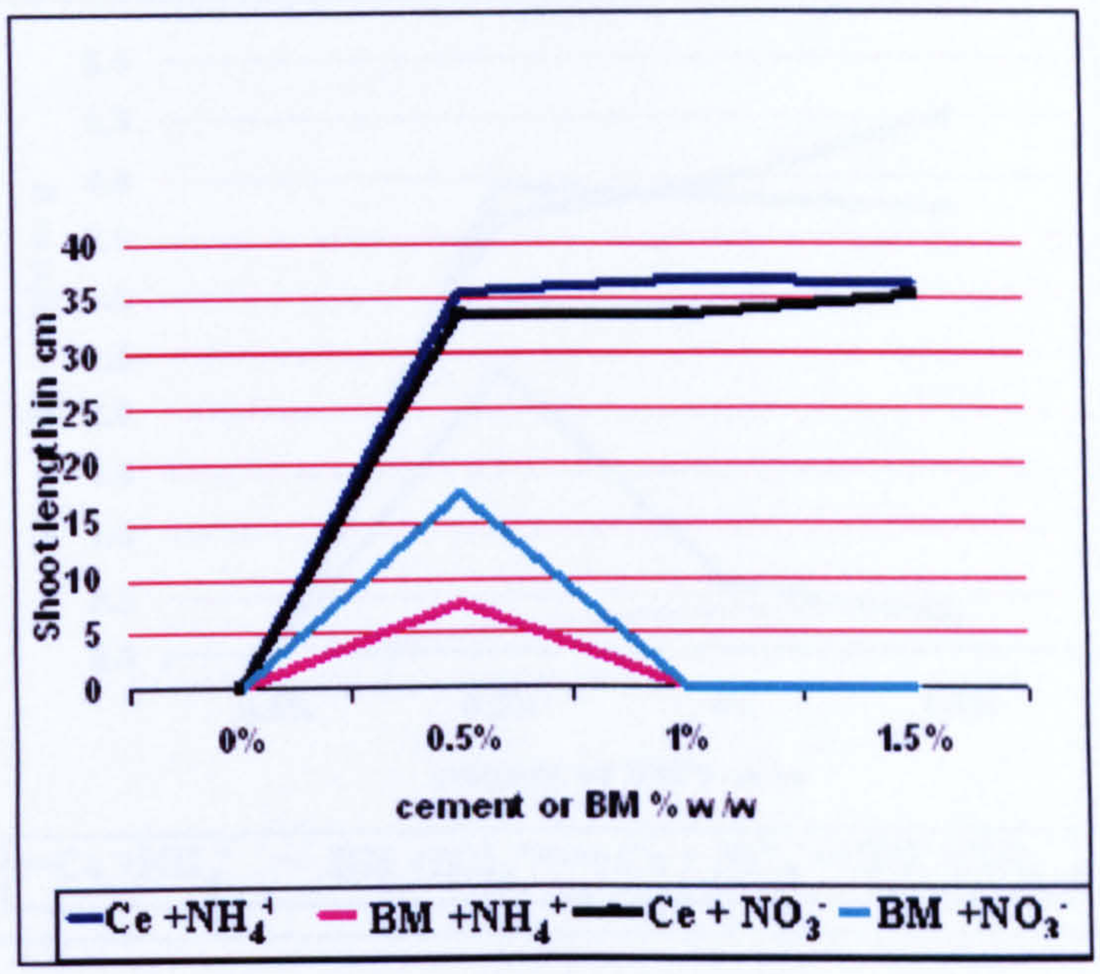

Figure 9.2 The effect of two amendments and two source of nitrogen NH_4^+ or NO_3^- on shoot length in cm.

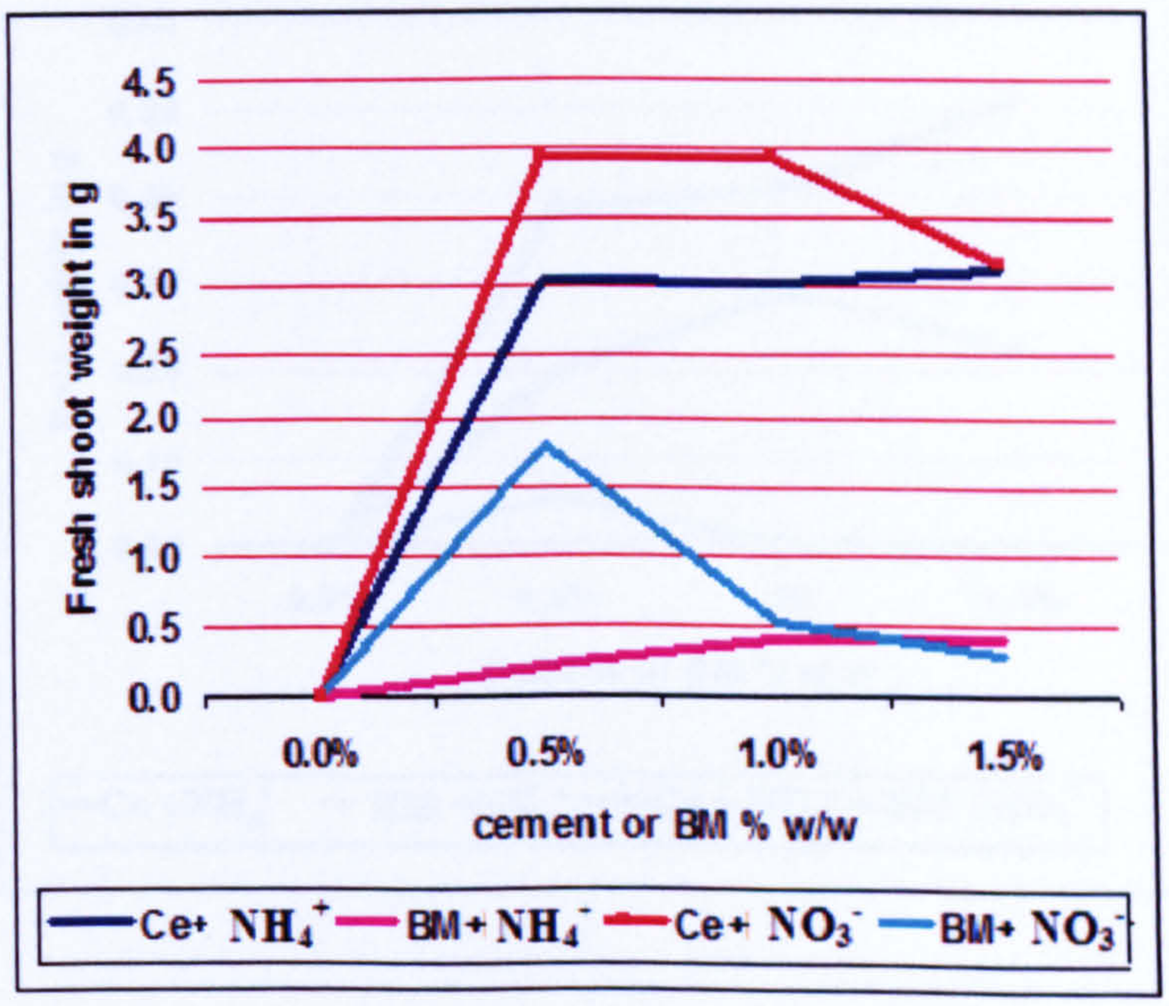

Figure 9.3The effect of two amendments and two source of nitrogen NH_4^+ or NO_3^- on fresh shoot weight in g.

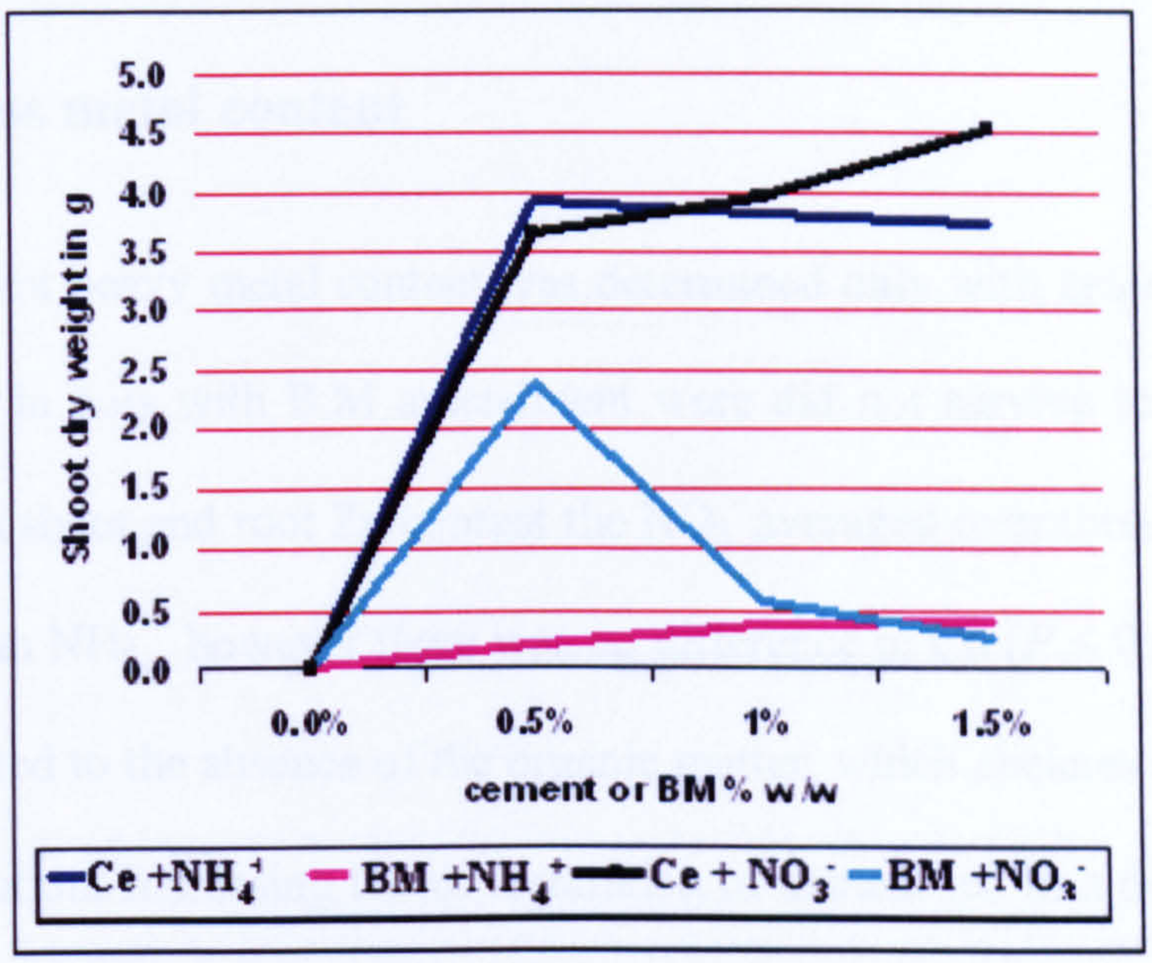

Figure 9.4The effect of two amendments and two sources of nitrogen NH_4^+ or NO_3^- on shoot dry weight in g.

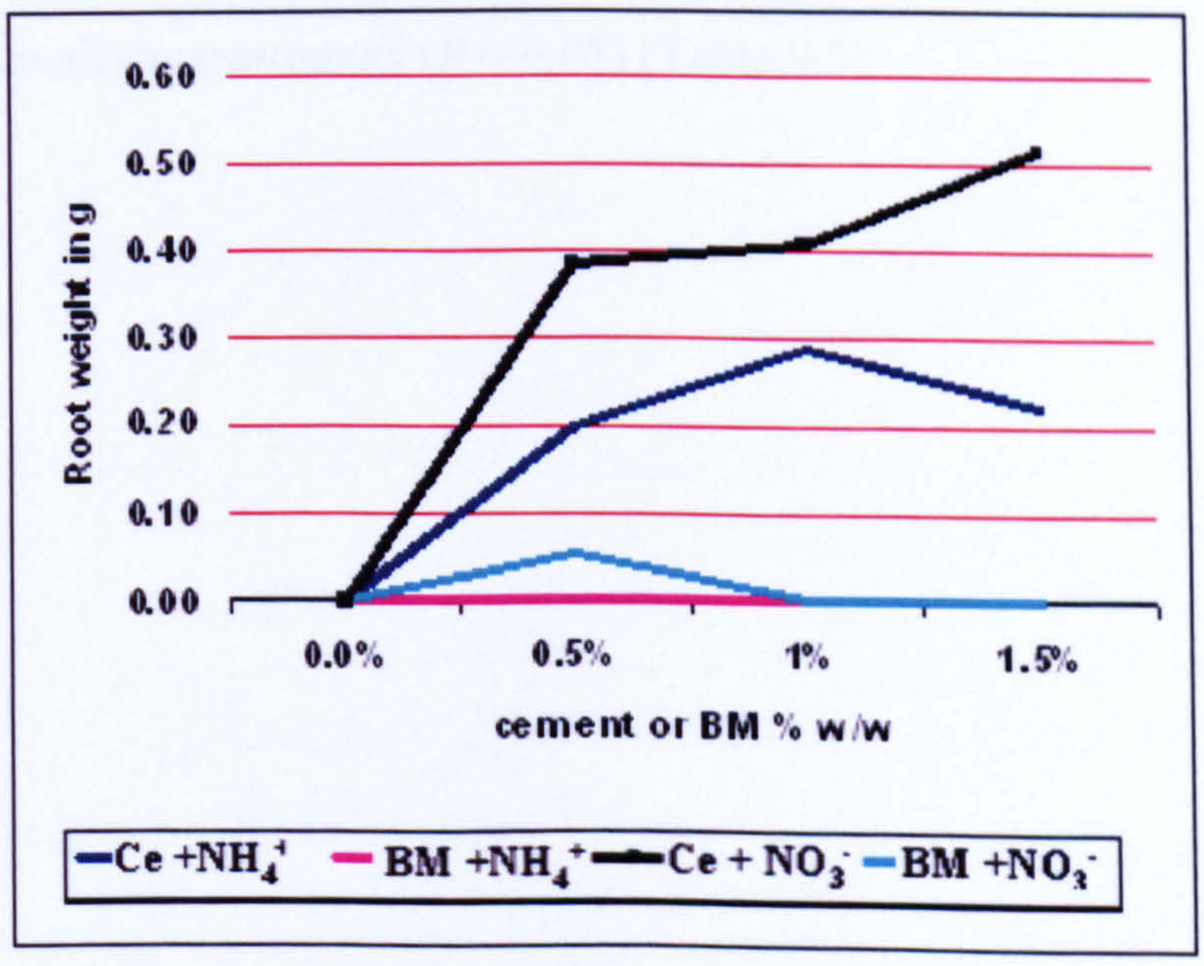

Figure 9.5The effect of two amendments and two sources of nitrogen NH_4^+ or NO_3^- on root dry weight in g

9.3.3 Biomass metal content

The plant heavy metal content was determined only with cement amendment. All the plants in pots with B.M amendment were did not survive to the end of the experiment. In shoot and root Zn content the NO_3^- averaged over three concentrations was higher than NH_4^+, however there was no difference in Cu ($P < 0.05$) (Table 9.5). This is attributed to the absence of the organic matter, which chelates and releases the Cu in soil solution. Increasing the concentration of cement led to a decrease in metal content in both shoots and roots (Table 9.5). In the nitrogen source and cement interaction, the Zn shoot and root content with nitrate was higher than ammonium at the same concentration. There was no difference in shoot Cu content and root Cu content between all the treatments ($P < 0.05$) (Table 9.5)

Table 9.5 Effect of a) nitrogen source averaged over three concentrations and b) concentrations averaged over both two nitrogen sources on metal content Cu and Zn mg/kg of shoot and root of barley

Treatment	Metal content in shoot mg/kg		Metal content in root mg/kg	
	Cu	Zn	Cu	Zn
a (n = 12)				
NH_4^+	72.9	99.4b	1053.1	76.7b
NO_3^-	69.7	153.8a	1523.4	108.5a
LSD P < 0.05	**N.S**	**17.5**	**N.S**	**12.8**
b (n = 8)				
0.50%	92.4a	135.6a	2400.8a	127.9a
0.25%	60.4b	136.8a	765.9b	87.7b
1%	61.3b	107.3b	698.0b	62.2c
LSD P < 0.05	**20.0**	**2.4**	**1306.0**	**19.0**
c (n = 4)				
0.5%Ce + NH_4^+	89.8	101.1b	1633.3	101.7b
1%Ce + NH_4^+	67.5	98.3b	748.3	72.3c
1.5%Ce + NH_4^+	61.4	98.9b	777.8	56.3c
0.5%Ce + NO_3^+	95.0	170.2a	3168.3	154.2a
1%Ce + NO_{3+}	53.2	175.3a	783.5	103.2b
1.5%Ce + NO_3^+	61.1	115.8b	618.3	68.2c
LSD P < 0.05	**N.S**	**45.8**	**N.S**	**33.4**

Values in the same column in part a or b with different letters were significantly different

The transfer factor (shoot metal -to-root metal ratio) was increased with cement concentration for both Cu and Zn and in both nitrogen sources (Table 9.6). This is attributed to solubility and bioavailability of the metals, more soluble and bioavailable more accumulated in the root and less available less accumulated in the root. The transfer factor of Cu was less than the transfer factor of Zn.

Table 9.6 Effect of different concentrations of cement amendment on transfer factor of Cu and Zn in shoot and root of barley.

Treatment	Transfer factor	
	Cu	Zn
0.5%Ce + NH_4^+	0.06	1
1%Ce + NH_4^+	0.01	1.4
1.5%Ce + NH_4^+	0.08	1.8
0.5%Ce + NO_3^-	0.03	1.1
1%Ce + NO_3^-	0.07	1.7
1.5%Ce + NO_3^-	0.1	1.7

Figure 9.6 Shows different concentrations 0%, 0.5%, 1% and 1.5% of cement amendment from the left to the right with same nitrogen source NH_4^+ on detoxification of Zn and Cu.

Figure 9.7 The effect of cement amendment 0.5% on detoxification of Zn and Cu with two treatments of nitrogen sources NH_4^+ on the right side and NO_3^- on the left side using barley as a test crop.

Figure 9.8 Shows bone meal on the left side and the cement amendment on the right side with the same concentration 1.5% and same nitrogen source NH_4^+ on detoxification of Zn and Cu using barley as a test crop.

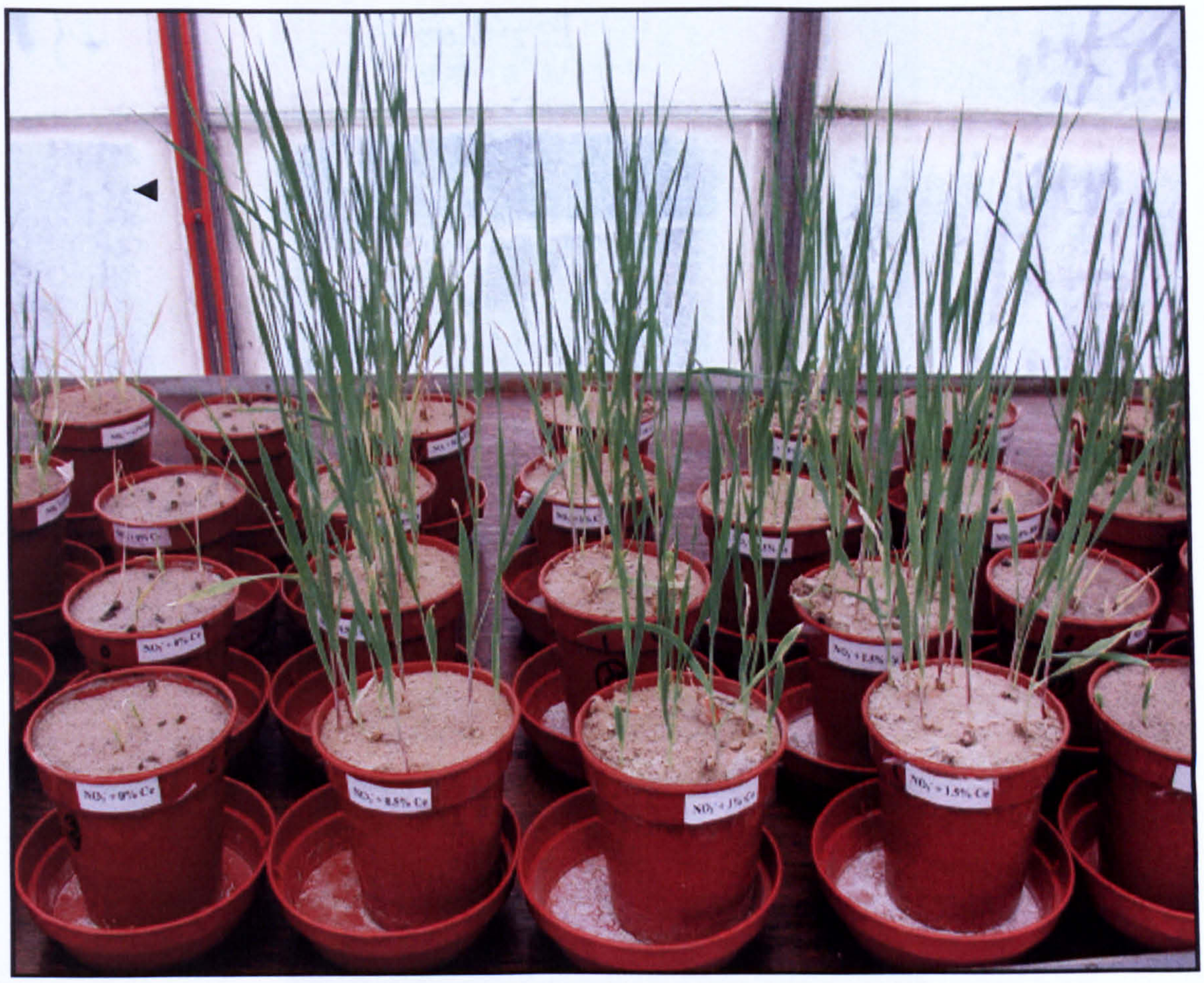

Figure 9.9 Shows different concentrations 0%, 0.5%, 1% and 1.5% cement amendment from the left to the right with same nitrogen source NO_3^- on detoxification of Zn and Cu using barley as a test crop.

Figure 9.10 Shows different concentrations 0.5%, 1% and 1.5% bone meal amendment from the right to the left with same nitrogen source NO_3^- on detoxification of Zn and Cu using barley as a test crop.

Figure 9.11Shows cement on the right and bone meal on the left in the same concentration 1.5% and same nitrogen source NO_3 on detoxification of Zn and Cu using barley as a test crop.

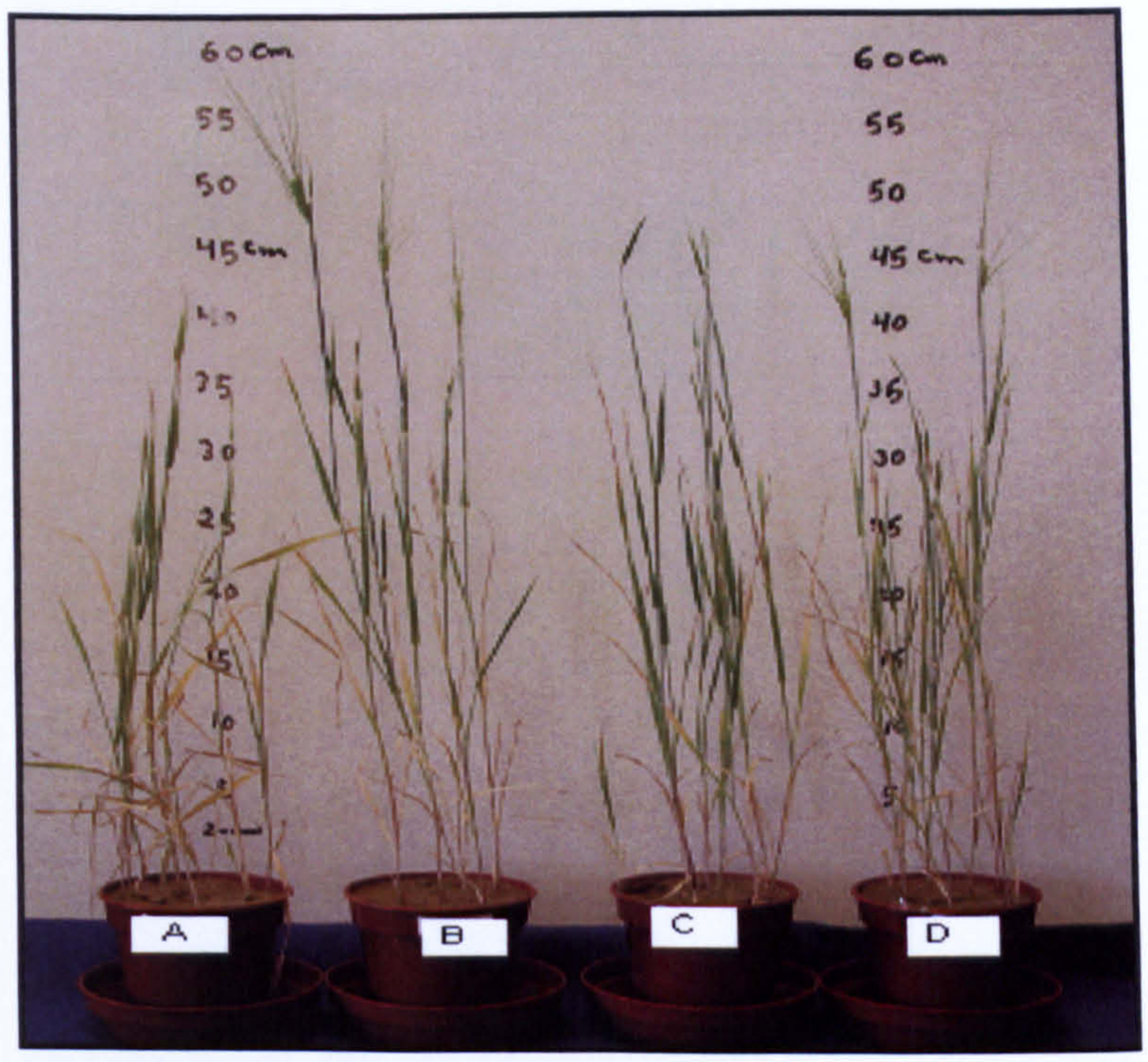

Figure 9.12 Shows two nitrogen sources NO_3 and NH_4^+ with two concentrations 0.5% and 1% of cement amendment on detoxification of Zn and Cu for barley growth, A=(0.5% cement + NO_3^-); B = (0.5% cement + NH_4^+); C = (1% cement + NO_3^-); D = (1% cement + NH_4^+).

Figure 9.13Shows different concentrations 0%, 0.5%, 1% and 1.5% cement amendment from the left to the right with same nitrogen source NO_3^- on stabilization of Zn and Cu using barley as a test crop.

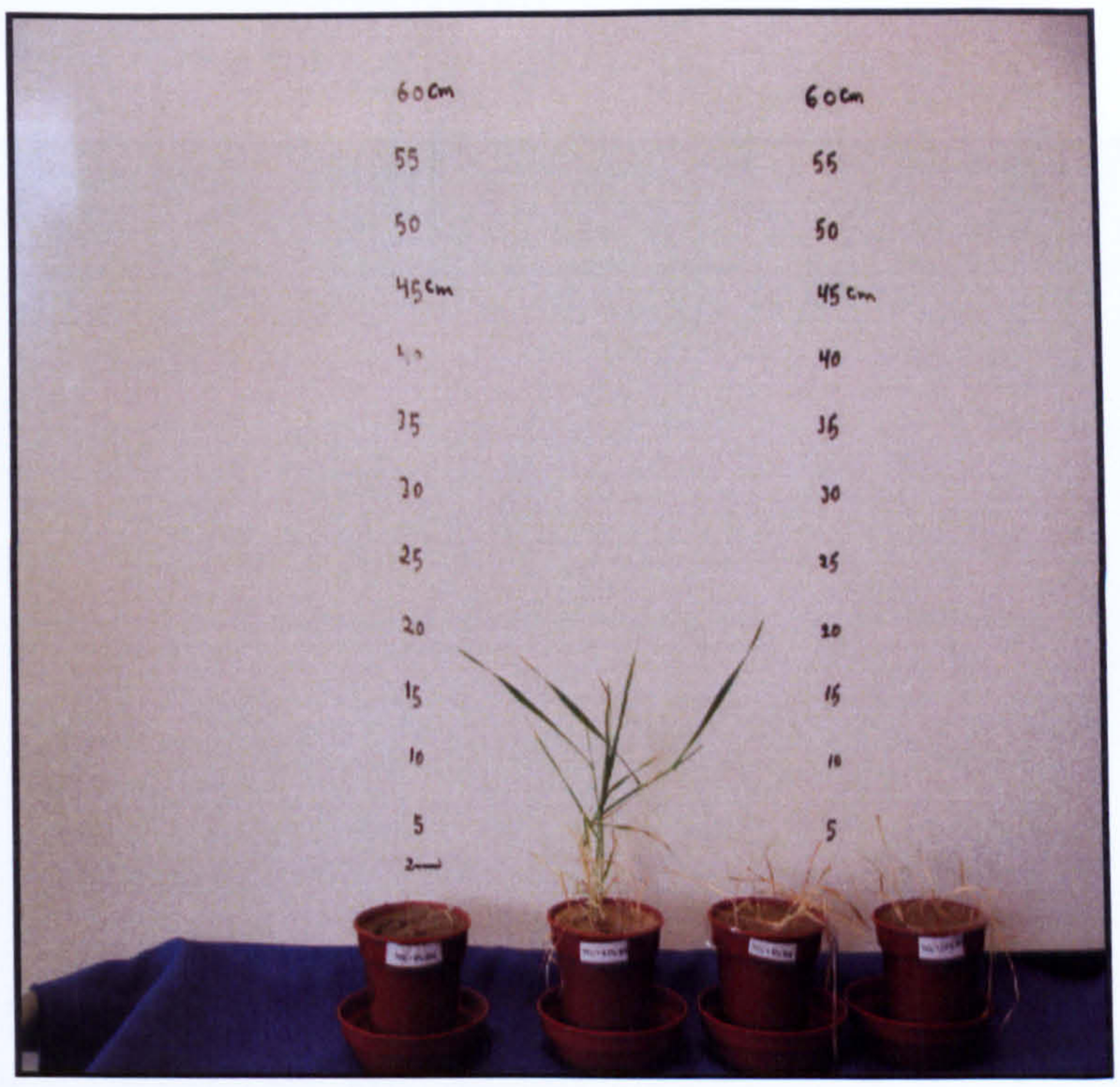

Figure 9.14 Shows different concentrations 0%, 0.5%, 1% and 1.5% bone meal amendment from the left to the right with same nitrogen source NO_3^- on stabilization of Zn and Cu using barley as a test crop.

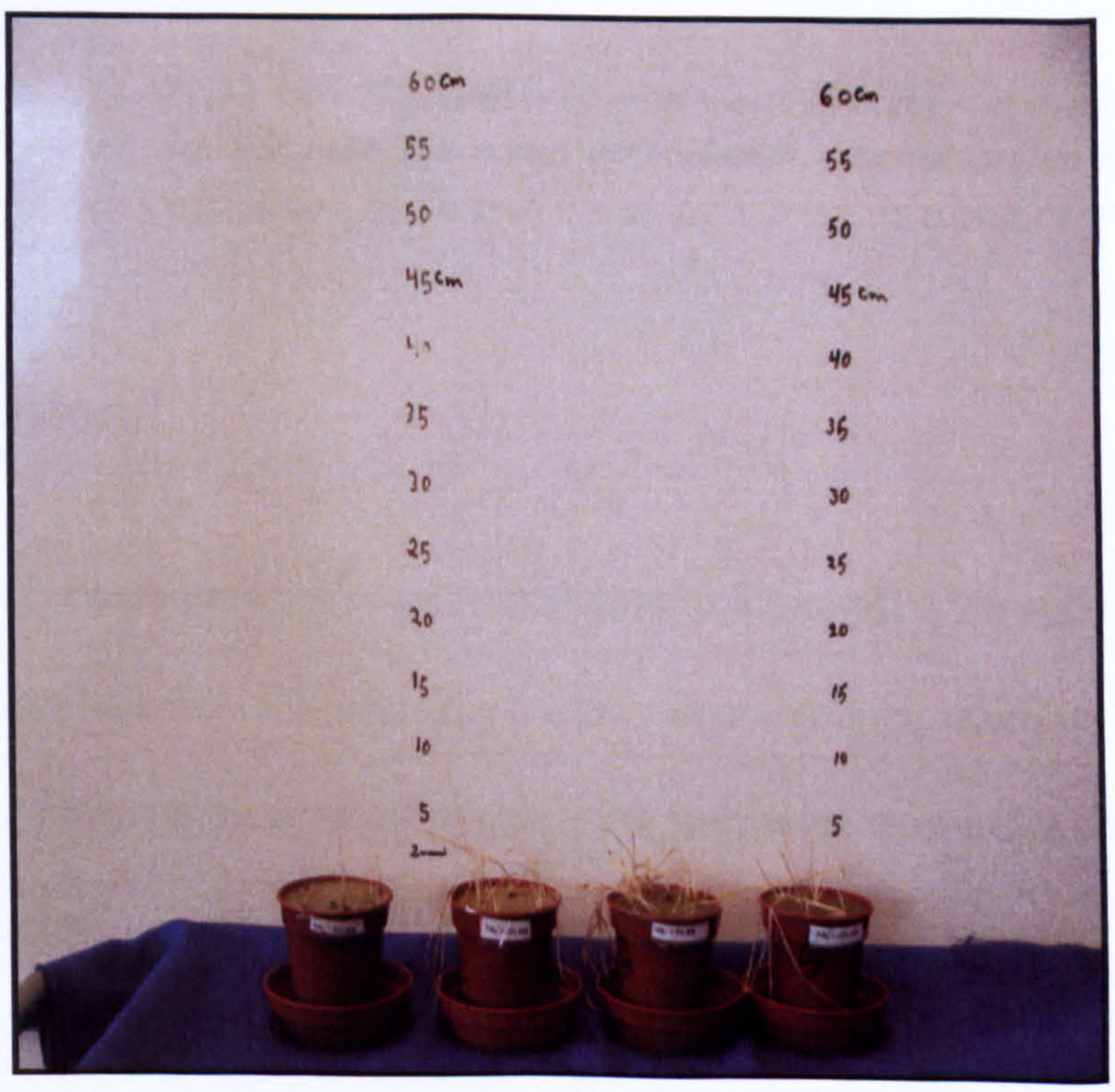

Figure 9.15 Shows different concentrations 0%, 0.5%, 1% and 1.5% bone meal amendment from the left to the right with same nitrogen source NH_4^+ on stabilization of Zn and Cu using barley as a test crop.

Figure 9.16 Shows two different nitrogen sources NH_4^+ on the right and NO_3^- on the left with the same amendment cement oncentration1.5% on detoxification of Zn and Cu using barley as a test crop.

9.4 Conclusion

Overall, cement shows promise as an amendment to immobilise Zn and Cu in soil, making them less harmful. The amount of zinc, but not copper, taken up by plants can be varied using ammonium or nitrate as nitrogen sources to manipulate concentrations in the rhizosphere.

Chapter 10

Discussion

Phytoremediation is a way to utilise solar free energy. It is cost effective and environmentally friendly, and is built on two main strategies: one is phytoextraction and the other is phytostabilization. Many researchers are concentrating on hyperaccumulators (metallophytes), which accumulate 10 to 100 times more heavy metals than non- metallophytes and contain the contaminant in their biomass. This leads to other problems: the need to extract the heavy metals again by different ways such as incineration; low biomass production; restriction to specific environments and exhaustion of nutrients, especially N and P, in a soil. However, to assess more efficient phytoremediation, the use of crops and other plants, which have high biomass production have been suggested possibly with addition of soil amendments. Any amendment has to be more effective and reasonable.

In this thesis many experiments were undertaken using different crops, and a novel amendment integrated all of these to give new aspects and strategies for using phytoremediation. The manipulation of the rhizosphere by perennial ryegrass (*Lolium perenne*) with two sources of nitrogen gave a good indication of the prospects for changing the acidity with ammonium. Consequently, the pool of heavy metals in cultivated soil decreased compared with non-cultivated soil (bulk soil). The ryegrass altered the total heavy metals in the soil and it can be used for phytoextraxtion. Lime addition decreased the available pool of Zn and increased the Cu and Pb solubility to some extent. Also the flax crop can be used for phytoextraction of heavy metals. Flax is a non- edible crop and is used for industrial material such as painting and ground

coverage of houses. Altering the flax rhizosphere by ammonium or nitrate with and without addition of lime to enhance the bioavailability of heavy metals was investigated. Ammonium changes the rhizosphere pH compared with bulk soil and nitrate treatment, consequently the heavy metal pool in the rhizosphere changed. However, the addition of lime decreased the Zn availability with ammonium compared to that without addition. Using lime and different nitrogen sources can manipulate the rhizosphere of plants to enhance the phytoextraction without risk of accessibility of heavy metals outside the rhizosphere and with less leaching hazard. A new system to reveal the changes in the rhizosphere pH, a rhizoglassbox was developed, with agar medium and Bromocresol purple as pH indicator. The ammonium decreased the rhizosphere pH of the plants compared with out-rhizosphere and nitrate treatment. Ammonium altered the rhizosphere pH of different plants to different degrees, while the nitrate did not affect the rhizosphere pH. When the ammonium or nitrate was added as foliar spray on flax shoots, there was no change in the rhizosphere pH. The ammonium plays an important role in the decrease of the pH, consequently increasing the available pool of metals and enhancing phytoextraction. The ability of the crops to tolerate heavy metal toxicity differs with species and this depends on the plant mechanism for detoxification of heavy metals. From monitoring of the toxicity of some heavy metals and their effects on different plants, seed germination experiments were assessed and shoot and root length were measured. The results showed that the Cu had greater affect on seed germination than Zn and Pb. Flax was more sensitive to the toxicity than pea. Barley had different D50 from other crops.

An agar medium was used to assess the manipulation of the rhizosphere at low and high toxicity level of metals along with ammonium or nitrate. The results

showed that ammonium acidified the rhizosphere compared with nitrate and control; this leads to the plant toxicity at the high metal concentration.

Ammonium positively affected phytoextraction of heavy metals. With different initial pH 5, 6.5 and 8 with high and low Zn and Cu concentrations, the results showed that the high pH decreased the solubility and bioavailability of heavy metals and the plant grew in high pH while at pH 5 and 6.5 at high Zn and Cu concentrations the plants did not survive. Ammonium lowered the rhizosphere pH from pH 8 to around pH 5. The toxicity of heavy metals depends on the three main factors: total metal in the soil, available pool and plant species. Interaction together gives the possibility of toxicity for the plant 10.1. Reducing toxicity requires altering one of these factors, for example to change the available and accessible metal to an unavailable form, but to provide sufficient amounts to the plants. Also by reducing the toxicity, biomass of plants is increased, and the metal removal from the toxic site increased. Using cement as amendment for plants to ameliorate toxicity is not mentioned in literature. Some of the cement characteristics were investigated and compared with bone meal. The cement pH was very high compared with bone meal, and the cement pH was decreased by mixing with soil, which has high buffering capacity, but was not changed with sand, which has very low buffering capacity. Cement has greater affinity to adsorb or precipitate heavy metals compared with bone meal. Three months of incubation with two soils high in metal content and two methods of extraction, EDTA and low concentration salt (0.01 M $CaCl_2$) were assessed. The cement gave more heavy metal extracted by EDTA compared with bone meal and low heavy metal extraction with 0.01 M $CaCl_2$ extraction. Cement decreased barley growth at high concentration. Cement immobilized the heavy metals Zn and Cu and enhanced barley growth while the bone meal affected the plant growth severely. All the plants treated with bone

meal did not complete the growth cycle. Thus cement shows potential as an amendment to immobilize heavy metals in soil. The same is true of ammonium as this nitrogen source could result in a lowering of pH in the rhizosphere to allow sufficient metal to be taken up to satisfy plant requirements. Thus a combination of cement treatment and ammonium fertilizer addition could achieve stabilisation of the heavy metals in soil to allow crop growth, but would limit phytoextraction of metals.

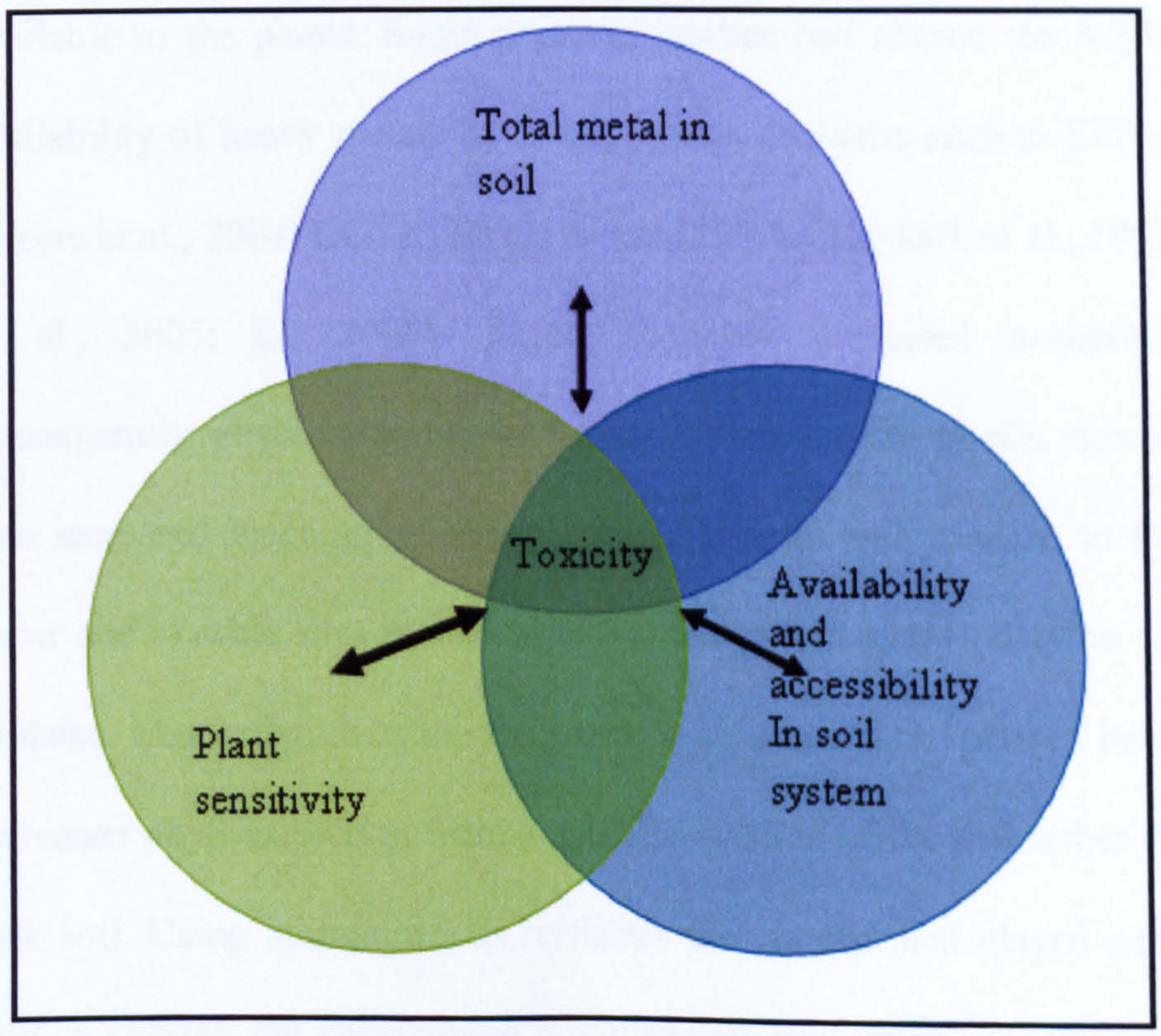

Figure 10.1 Diagram shows the relation between total, soluble metals and plant sensitivity in polluted soil.

From 10.1 three components are control the toxicity to the plants in polluted soils. These components are: 1) total metal in the soil, which is affected by many factors, for example precipitation and the composition of the soil parent material 2) availability and accessibility of heavy metals in the soil solution or interface phase 3) sensitivity of the plant to heavy metals, ranging from hyperaccumulator to sensitive crops. Also in figure 10.2 illustrates the aims of the thesis experiments, which were generally to increase accessibility of metals from the unavailable pool to a pool, sufficient in magnitude, available to the plants. Some previous studies had altered the solubility and availability of heavy metals by flushing with chelators such as EDTA, EDDS (Meers et al., 2004) EGTA, HEDTA and DTBA (Bleylock et al., 1997; Lesage et al., 2005; Li, 2006). These chelators increased accessibility and consequently phytoextraction of heavy metals by the plants, however there was increased leaching of heavy metals through soil solution to the ground water and to other sites by lateral movement. Plant growth may be affected by chelates. Manipulation of the rhizosphere by altering the pool of heavy metals increased phytoextraction from a specific volume of the soil rather than in the bulk soil. Using ammonium as fertilizer and amendment played an important role in altering the rhizosphere environment especially pH. The pH was the key to changing the pool of most of the heavy metals from unavailable to available. Consequently the phytoextraction of heavy metals is increased. The pH can change the population of mycorrhizae and microorganisms such as bacteria in the soil especially in the rhizosphere, which contains several times more than the bulk soil. Manipulation of rhizosphere by fertilizer amendments as a source of nitrogen and decreasing the pH had previously been described.

When $(NH_4)_2SO_4$, NH_4NO_3 and $Ca(NO_3)_2$ were used as fertilizer, it was found that NH_4^+ lowered the pH in the rhizosphere more than the other two fertilizers (Sas et al., 2003). Brix et al. (2002) studying the effect of NH_4^+ or NO_3^- on *Typha latifolia* at different pHs found high metal content and optimum growth with NH_4^+ at 6.5 pH. The result from chapter 3 illustrated the effect of NH_4^+ in lowering the pH especially in the rhizosphere while the pH not affected by nitrate. The ammonium displaced heavy metals from the unavailable to the available pool. In terms of the model shown in figure 1.6 (chapter 1) the metal was shifted in the direction of sufficient amount as in hypotheses 1-3. This gives maximal plant biomass and consequently more heavy metal phytoextraction. Alerting the unavailable pool by aggressive amendments such as EDTA or acids will affect the plants and increase the toxicity triangle area for the plants (Figures 10.1 and 10.2). Addition of lime is effective and can manipulate the soil pH, base saturation in acid soils and improve physical characteristic of the soils to permit root penetration through a greater volume of soil. Copper availability was increased by the lime addition and Zn was decreased. In contrast lime addition at different rates as mentioned in chapter 1 section 1.6.2 decreased Mn and Fe accessibility and left availability of Cu and Zn unchanged (Wasner et al., 2001). Ownby et al., (2005) indicated that rock phosphate was viable for reducing Pb and Zn availability. Toxicity of Zn was reduced by lime addition (Chlopecka and Adrrano, 1996) in agreement with the findings of chapter 3 and with the hypothesis presented in figure 10.1 that the triangle of toxicity is diminished by reducing the available pool of heavy metal and displacing heavy metal from more accessible and toxic to less accessible and sufficient. Different plants

have different sensitivity to different heavy metals. The available pool of heavy metals differed between bulk soil and rhizosphere with ryegrass but not in the case of flax. The plants differed in germination, shoot length and root length as the triangle of toxicity decreased and increased depending on the plant's sensitivity (Figure 10.1 and chapter 5). Immobilization of heavy metals decreased the triangle of the toxicity (Figure 10.1) and shifted the toxicity state to sufficient availability of heavy metals from No 4 to 3 figure10.2.

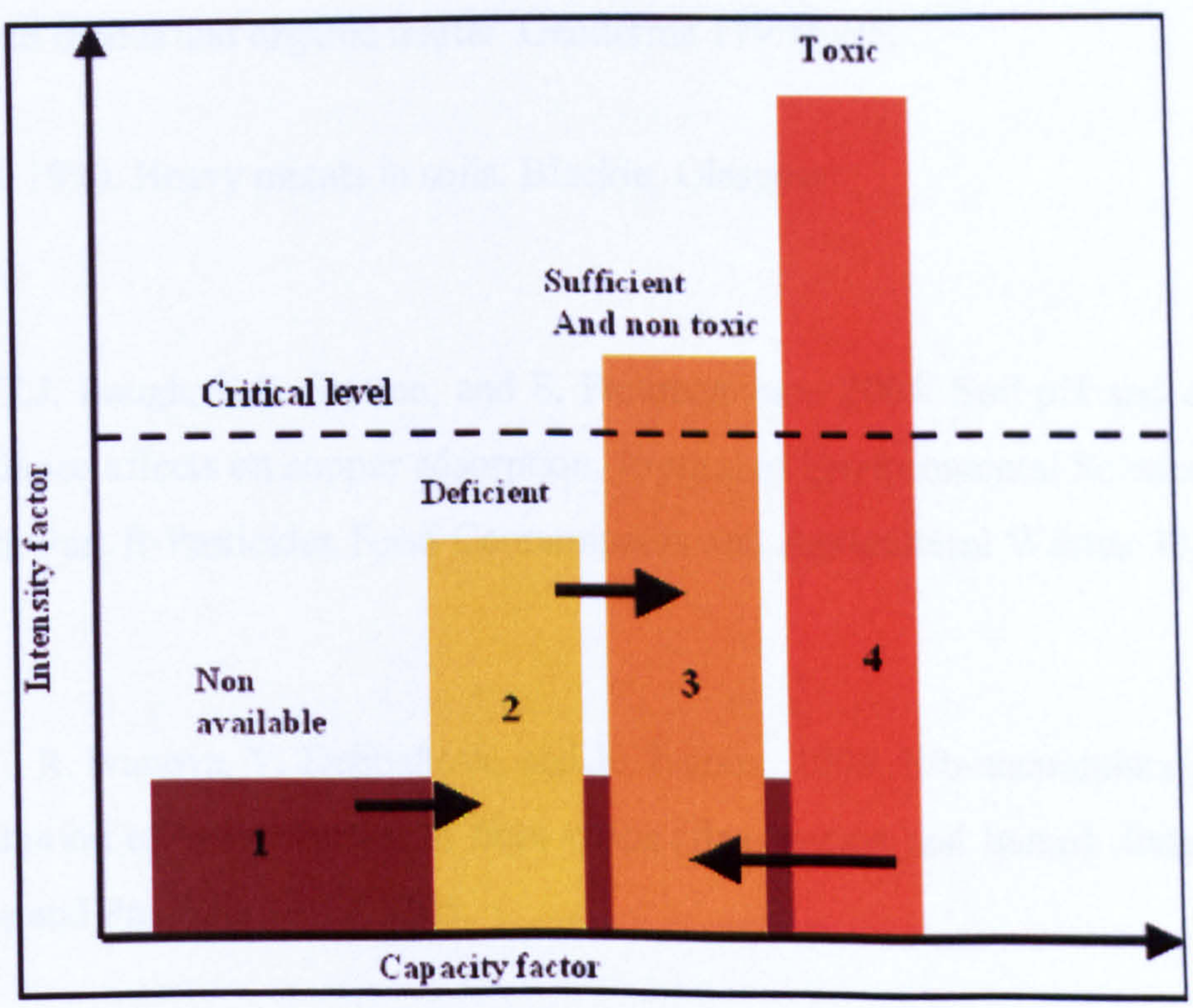

Figure 10.2 Diagram illustrates the intensity factor capacity factor and critical level of availability and solubility of elements.

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