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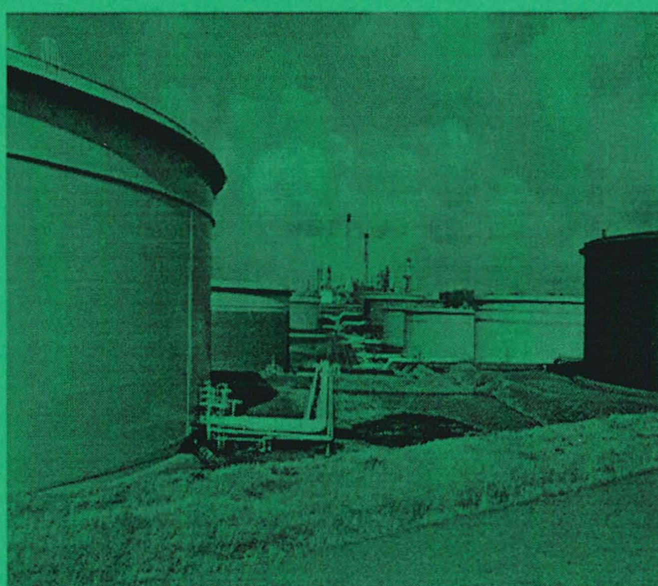
Helèn Gustafsson



Examensarbete
Handledare: John Scullion och Stig Ledin

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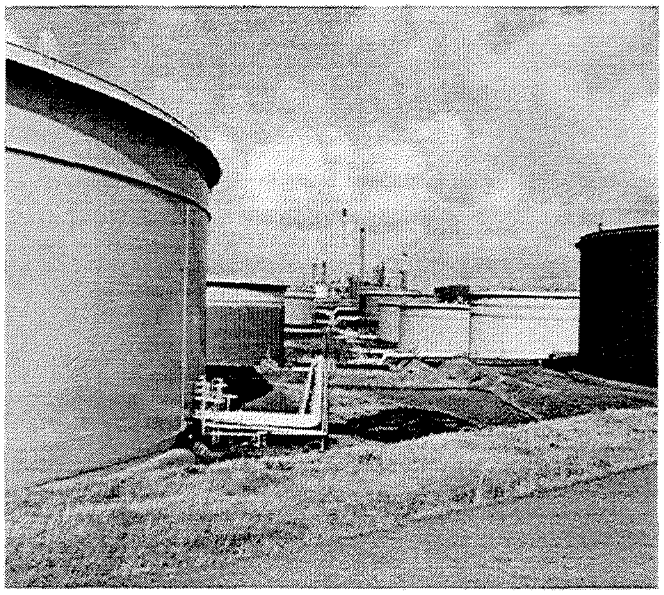

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ABSTRACT

The objectives of this thesis was to investigate if the plant *Brassica juncea* (Indian mustard) was able to extract more bioavailable toxic metals (principally nickel) from the metal and oil contaminated soil compared to *Trifolium repens* (Clover) and *Lolium perenne* (Ryegrass) and to evaluate the effects of agronomic practices (e.g. fertilizer and soil pH) on the metal uptake by the plants. The study was carried out in a pot experiment at the University of Wales, Aberystwyth. The soil used was from Elf Oil Refinery in Milford Haven.

There was no significant difference in metal (Zn and Ni) uptake between the three plants species. The average Zn concentration was between 35-67 mg/kg (dry weight) and 10-18 mg/kg (dry weight) of Ni according to Figure 4.

The average reduction with nutrients was over 30% in both Zn and Ni concentrations in the plants. Ryegrass indicated a higher response to nutrient treatment and a more uniform uptake of Zn and Ni compared to Indian mustard and Clover.

Results show that the concentrations of both Zn and Ni, with high significance ($P<0,001$) increased over time in the sulphur treated soil with an associated significant ($P<0,01$) increase in metal concentrations in the plants. Ryegrass had more than half of indian mustard and clover concentrations of zinc and also the lowest nickel uptake at 488 mg/kg dry weight compared to clover and indian mustard at respectively 916 and 1336 mg/kg dry weight. It is important to bear in mind that all plants in the sulphur treatment were dead which make these comparisons very uncertain.

However, the results in this investigation did not prove the plants to be suitable to clean up the contaminated landfarm soil. Even if calculations are made with optimal Ni concentration and optimal biomass of Indian mustard the time span of 20 years (the time Elf Oil wanted the soil to be remediated) was too short. Nothing indicated either, if the bioavailable pool of Ni will be replaced of Ni from the unavailable pool after plant uptake or not. Other clean up options which has been described in the literature review have to be considered.

Even if this work was not able to give a clear answer about metal uptake of the plants, phytoremediation may still be the viable decontamination method of the refinery soil. The ability of plants to accumulate heavy metals seems to have a great future potential and further research is necessary to validate the possibility to clean the site with phytoremediation.

REFERAT

Syftet med detta examensarbete var att undersöka ifall *Brassica juncea* (senapsart) kunde ackumulera mer toxiska metaller ur en olje- och metall förorenad mark än *Trifolium repens* (rajgräs) och *Lolium perenne* (vitklöver) samt se vilka effekter ökad näring och sänkt pH i marken hade på växterna. Studien utfördes som skalförsök i växthus på Universitetet i Wales, Aberystwyth och jorden som användes var från Elf Oil Refinery i Milford Haven, Wales.

Det var ingen signifikant skillnad mellan de tre växternas upptag av Nickel (Ni) och zink (Zn). Medelkoncentrationerna av Zn i växterna låg mellan 35-67 mg/kg (torr vikt) och 10-18 mg/kg av Ni.

Med ökad näring reducerades både Ni och Zn's koncentrationer i växterna med minst 30%. En trolig förklaring är att det skett en koncentrationsutspädning genom den ökade biomassan. Näringstillskottet gav en större respons på rajgräsets upptag jämfört med de andra och koncentrationsvariationerna var också mindre.

Resultaten visade att koncentrationerna av både Ni och Zn i jorden med svaveltillskott ökade med hög signifikans ($P<0{,}001$) över tiden. Det var även en signifikant ($P<0{,}01$) ökning av växternas metallupptag. Rajgräs tog upp hälften av Ni och Zn än vad *B. Juncea* och vitklöver gjorde och hade även den lägsta koncentrationen av Ni på 488 mg/kg (torr vikt) jämfört med vitklöver och *B. Juncea* på 916 respektive 1336 mg/kg (torr vikt). Det är dock viktigt att komma ihåg att växterna i jorden med svavel var döda vid försökets slut och gör resultatet osäkert.

Den här studien kunde inte visa att metoden för rening av mark med hjälp av växter (phytoremediation) var lämplig på raffinaderiets område. Även om beräkningar görs med optimala förhållanden på både upptag och biomassa är tidsperioden på 20 år för kort för att få ner koncentrationerna i marken.

Fler undersökningar är nödvändiga för att ta reda på om Elf Oil Refinery kan rena sin mark med phytoremediation. Med den information som finns i dag är mitt råd att överväga andra metoder. En intressant följdfråga är om den lätttillgängliga och upptagbara delen av Ni eller Zn i marken blir ersatt utav den mer svårtillgängliga, vilket är nödvändigt om marken ska renas.

Phytoremediation är verkligen en intressant metod för framtiden och kommer med mer forskning ha potential att konkurrera med andra reningsmetoder.

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1 INTRODUCTION

Past and present industrialization and waste disposal activity have created a lot of derelict and contaminated land. The Environment Agency in the UK and Wales have estimated that over 300,000 hectares of land are affected, covering between 5,000 and 20,000 "problem sites" (http://www.environment-agency.gov.uk/gwcl/LC_Back ground.htm, 2000-07-20). However, there is also an increased environmental awareness and concerns regarding the environmental impact of polluted land. This has resulted in a need to either remove the materials that contaminate or render them harmless. Historically, many sites have been cleaned up simply with removing the contaminants from one place to another place where it has minor impact. This landfill site option has been a relatively cheap and quick way of a disposal of the wastes. Today, current legislation requires high landfill taxes, the number of landfill sites is decreasing and their regulation is becoming more strict (pers. comm. Scullion,2000).

With this increasing awareness of environmental protection, increased costs of waste disposal, and changing legislation, developers and landowners have started to be more interested in both available techniques and new research for land remediation. There is also an increasing need for more land close to larger cities and there is great interest in many former industrial sites since they often can be found in relatively central, urban locations.

Many of the contaminated sites in the UK have problems with organic contaminants. The contamination of waters and soils with petroleum or petroleum products is one of the major problems. The demand for petroleum as a source of energy has resulted in an increase in world production to about 3500 million metric tons per year in 1998 (www.spi.se/olje/raoljeproduktion.htm, 2000-07-17*)*. A great part of the oil is transported over the sea because the major oil-producing countries are not the same as the major oil consuming countries and the transport results in an increased risk of oil pollution. Although the large crude oil spills following tanker accidents are of concern, bigger sources of oil pollution come from leaking storage tanks, pipelines, and wastes or run off from petroleum refineries and other industries (Crawford and Crawford, 1996).

Elf Oil has a refinery in the south of Wales which has used an area between 1974 and 1995 for the treatment (landfarming) of crude oil spills and other organic wastes. The landfarming treatment allows the waste sludge in the soil to be broken down naturally by microbial action, ultimately to carbon dioxide, water, N_2 and SO_2 etc.
However, associated with the deposition of oil are a number of potentially toxic components, in this case heavy metals. The land has therefore elevated concentrations in the soil of heavy metals (nickel is the one of most concern) in addition to different hydrocarbon compounds from the oil waste. Unlike organic compounds, metals cannot be broken down into less harmful components and therefore may stay in the soil (Alexander, 1994).

There currently exists UK government legislation that gives guidelines to the levels of these metals that can safely be allowed to accumulate in an environment. If a particular heavy metal level goes above these guidelines the company responsible may incur legal action resulting in heavy fines or suspension of their licence to use the landfarm sites.

Consequently Elf Oil is concerned to remediate their soils within these guidelines. For Nickel the guideline value is 70 mg Ni/kg soil and for zinc, 300 mg Zn/kg soil (ICRCL, 1987). Another aspect that could be of concern in the future is cleaning up of the already contaminated landfarms for a suitable after use if and when Elf Oil moves off the site or if the landfarm sites need to be developed for some other purpose. Even if the levels of heavy metals are below the government guidelines some sort of land restoration will need to take place to render the site suitable for development.

To minimize the future clean up costs ELF are interested in a remediation technique that can remove the contaminants (*in situ*) in an efficient and cost effective manner. Any new treatment should cause minimal disruptions to the physical and chemical properties of the soil. Techniques that would be interesting for the cleaning of the industrial sites are the ones that simultaneously treat and remove the contaminants, nickel, zinc and hydrocarbons from the polluted soil. A novel treatment strategy is phytoremediation which uses plants for cleaning soils by accumulating the metals and may also enhance the degradation of hydrocarbons. The metal contaminated plant tissue end up in a safe deposit after harvest.

The primary objective of this work was to investigate if it is possible to remediate this landfarm with phytoremediation in a realistic time span (20 years) and how much metals (nickel of most interest) the plants can remove in each crop.

The strategy assumes that the plants accumulate heavy metals but may also stimulate the degradation of organic compounds with their enhancement of the microbial activity in the soil and improvement of the physical and chemical properties of the contaminated soil (Aprill *et al.,* 1990).

2 AIMS AND OBJECTIVES

The aims of this thesis are:

- to investigate if a moderate-accumulating plant (*Brassica juncea*) is able to extract more bioavailable toxic metals (principally nickel) from the soil compared to non-accumulating plants (*Trifolium repens* and *Lolium perenne*)

- to evaluate the effects of addition of fertilizers and soil pH regulation on the above processes

- to investigate the effect on metal-uptake of applying EDTA-complexes

3 REVIEW OF LITERATURE

The review of literature is focused on factors influencing the contaminants behaviour in the soil. The properties of the contaminants, soil pH and organic matter often play key roles in determining success of any treatment. The influence of these factors on the availability of each contaminant found in Elf soils is discussed.

3.1 Contamination of Metals

Heavy metals that get into the environment can cause long term negative ecological effects. The metals may be enter the food chain of animals by grazing on metal contaminated plants and then may be passed on and accumulated in the human body (Gardea-Torresdey *et al.*, 1996). Metals such as As, Cd, Cr, Pb and Hg are often cited as primary contaminants of concern and are classified as Group A contaminants according to ICRCL (1987). Zn and Ni are also problematic contaminants, as in the case of Elf Oil. Both Zn and Ni belong to a Group B of contaminants, which means that the contaminants are phytotoxic but not normally hazards to human health (loc. Cit.).

By using different chemical extractants, researchers (Alloway, 1995) have found that heavy metals are associated with soil solids in four major ways in soils treated with sewage sludge (Table 1).

First, a small proportion is held in adsorbed or exchangeable forms which are available for plant uptake. *Second*, the elements are bound by the soil organic matter and by the organic material in the sludge. Organically bound elements are not readily available to plants, but can be released over a period of time. The *third* association of heavy metals in soils is with carbonates and with oxides of iron and manganese. These forms are less available to plants than either the exchangeable or organically bound forms, especially if the soils are not allowed to become too acid. The *fourth* association is commonly known as the residual form, which consists of sulfides and other very insoluble compounds that are less available to plants than any of the other forms.

Table 1. Forms of four heavy metals found in a Greenfield Sandy loam that received 45 Mg/ha sewage sludge annually for 5 years. From Brady and Weil (1996)

	Percentage of elements in each form			
Forms	Ni	Zn	Pb	Cu
Exchangeable/adsorbed	5	2	1	2
Organically bound	24	28	3	34
Carbonate/iron oxides	33	39	85	36
Residual	40	31	12	29

Nickel

Nickel is a very abundant element. It is found in all soils and is emitted from volcanos. It can naturally occur in large concentrations in ultra mafic (serpentine) soils, and is found primarily in combination with oxygen (oxides) or sulfur (sulfides). Soils polluted with Ni are rather rare compared to soils polluted with other heavy metals. High levels of Ni can occur on smelter wastes and mine spoils where oxidation of sulphides acidify the soils and solubilize nickel to toxic levels (Alloway, 1995).

Nickel has the atomic number 28 and atomic weight 58.7 u and can occur in several oxidation states. It is only Ni^{2+} that it is stable in a wider range of pH levels and redox conditions found in soils (Lepp, 1981).

Nickel in Plants

Nickel has been shown to be essential for plants. Earlier reports have shown a stimulation of germination and growth of various species by Ni (Alloway, 1995). According to Marschner (1986) Ni deficiency is unlikely to occur because of the small amount required by plants. High levels of Ni in plants can induce zinc or iron deficiency because of the competition between the ions (Marschner, 1986). The symptoms of Ni toxicity appear to be a combination of chlorosis (the Fe-deficiency induced) and foliar necrosis followed by poor growth. Other toxic symptoms include stunted growth of the roots and shoots, deformation of various plant parts, and unusual spottings on leaves and steams (Lepp, 1981). Critical toxicity levels for moderately tolerant species are more than 50 mg /kg dry weight (Marschner, 1986). Background values in the plants are 0.15 mg /kg dry wt but Ni concentration of 370 mg /kg dry wt is not unusual in vascular plants near smelter industries (Lepp, 1981).

The Effect of Nickel to Animals and Humans

Nickel seems to be required to maintain health in animals and humans. A small amount of Ni is probably essential for humans, although a lack of Ni has not been found to affect the health of humans. The ion radius of Ni^{2+} is close to those of Fe^{2+}, Mg^{2+}, Cu^{2+} and Zn^{2+}. Therefore Ni can replace them in metal-enzymes and causes disruption of metabolic pathways (Alloway, 1995). Ni has also been recorded to be toxic to microorganisms and is therefore used as a fungicide (Lepp, 1981).
The most common adverse health effect of Ni in humans is an allergic reaction, but the major source of exposure for humans of Ni is by eating food containing Ni, or by drinking water which contains small amounts of Ni (Lepp, 1981). Lung effects, including chronic bronchitis and reduced lung function, have been observed in workers who breathed large amounts of Ni. Current levels of Ni in workplace air are much lower than in the past, and today only few workers show symptoms of Ni exposure. People who are not sensitive to Ni must eat very large amounts of nickel to show adverse health effects. Workers who accidently drank water containing very high levels of nickel (100,000 times more than in normal drinking water) had stomach aches and effects to their blood and kidneys (http://www.atsdr.cdc.gov/tfacts15.html, 2000-07-20).

The Behaviour of Nickel in Soils

The +2 oxidation state is as mentioned the only stable form of nickel in the soil environment and its electronic structure favours formation of complexes with organic matter. Bioaccumulation of Ni in humus is pronounced and Ni^{2+} favours bonding to "softer" organic ligands containing nitrogen and sulphur. As the smallest of the divalent transition metal cations, Ni^{2+} fits easily into octahedral sites and co-precipitates readily into Mn and Fe oxides in soils. Chemisorption on oxides, noncrystalline aluminosilicates, and layer silicate clays is favourable above pH 6, but lower pH favours exchangeable and soluble Ni^{2+}. Because solubility decreases markedly at higher pH, mobility of Ni, rated as medium in acid soils, becomes very low in neutral and alkaline soils. Under reducing conditions, Ni^{2+} is incorporated into sulfides that restrict mobility to very low levels (McBride, 1994). So the mobility of Ni in soils is strongly dependent on pH, clay content, and the amount of organic matter. It seems like the pH is the most important factor determining the distribution of Ni between the solid and solution phases, while the other factors are of secondary importance (Alloway, 1995). Background levels in soils vary between 5 to 55 mg/kg dry wt (McBride, 1994).

Zinc

Zinc is one of the most common elements in the earth's crust. It is found in air, soil, and water. Zn combines with other elements to form Zn compounds. Common Zn compounds found at hazardous waste sites include zinc chloride, zinc oxide, zinc sulfate, and zinc sulfide. Zn toxicity in soils is usually associated with human activity such as overuse of fertilizers, pesticide, manures and industrial activities (Armour and Brennan, 1999). Rain and snow can also remove Zn dust particles from the air. The Zn compounds can thereafter move into the groundwater and into lakes, streams, and rivers. Zn has atomic number 30 and atomic weight 65.4 u and is essential for plants, animals and humans. Zn is antagonistic to Fe and Cu. The negative effects of these two metals can be neutralised with optimal additions of Zn (SNV,1976).

Zinc in Plants

Some Zn is released into the environment by natural processes, but most comes from human activities. Plants take up zinc predominantly as a divalent cation (Zn^{2+}). At higher pH, it is presumably also taken up as monovalent cation ($ZnOH^{+}$). High concentrations of other divalent cations such as Ca^{2+} inhibit zinc uptake somewhat. Zinc deficiency is widespread among plants grown in highly weathered acid soils and in calcareous soils. The low availability of zinc in calcareous soils of high pH results mainly from the adsorption of zinc to clay or $CaCO_3$. The most characteristic symptoms of Zn deficiency in dicotyledons are stunted growth and decreased leaf size (Marschner, 1986).

When the Zn supply is large, Zn toxicity can readily be induced in nontolerant plants. Toxicity effects such as inhibition of root elongation and chlorosis of young leaves are shown. Quite often, Zn induces a deficiency of both Fe and Mn since they have similar ionic radii as Zn (Ebbs and Kochian, 1997).

The critical toxicity levels of Zn in leaves of crop plants are more than 400-500 mg/kg (dry wt). Increasing soil pH by liming is the most effective procedure for decreasing both Zn content and Zn toxicity in plants (Marschner, 1986).

The Effect of Zinc to Animals and Humans

Zinc is an essential element in our diet. Too little Zn can cause health problems, but too much Zn is also harmful. Zinc is required for the activity of various types of enzymes, including RNA and DNA polymerases. Therefore, it is not surprising that Zn deficiency is associated with an impairment of carbohydrate metabolism and protein synthesis. Zn deficiency in humans can results in dwarfism, delayed sexual maturity and enlargement of the spleen. Normal intake of Zn for humans is between 10-15 mg/day. However, too much zinc can also be damaging to your health. Harmful health effects generally begin at levels from 100 to 250 mg/day intake range (SNV, 1976).

The Behaviour of Zinc in Soils

Zinc tends to occur as the sulfide mineral, sphalerite (ZnS) in rocks, but weathers to soluble Zn^{2+} ion in the oxidizing environment of soils. The +2 oxidation state is the only one possible in the soil. In acid, aerobic soils, Zn has medium mobility, held in exchangeable forms on clays and organic matter. However, at higher pH, chemisorption on oxides and aluminosilicates and complexation with humus lower the solubility of Zn^{2+} markedly. Consequently, Zn mobility in neutral soils is very low. If soils are slightly alkaline, Zn-organic complexes can become soluble and raise solubility. In soils contaminated by high levels of Zn, precipitation of Zn oxide, hydroxide, or hydroxycarbonate may limit Zn^{2+} solubility at pH 6 or higher. In the reducing environment of flooded soils, release of Zn^{2+} from dissolving Fe oxides may initially increase availability, but Zn mobility is ultimately restricted by extreme insolubility of ZnS (McBride, 1994).

Under acidic, oxidizing conditions, Zn^{2+} is one of the most soluble and mobile of the trace metal cations. It does not complex tightly with organic matter at low pH. Acid-leached soils often have Zn deficiency because of depletion of this element in the surface layer. Calcareous and alkaline soils also commonly have Zn deficiency, but the cause is low solubility (McBride, 1994). Background levels of Zn in soils are between 17 to 125 mg/kg dry wt (Alloway, 1995).

3.2 Contamination of Oil

Crude oils and petroleum products are very complex mixtures of several thousands of hydrocarbons, and many compounds contain oxygen, sulphur, and nitrogen (Calabrese and Kostecki, 1988). Once the hydrocarbons reach the soil, they can move in one or more of seven directions. They may *vaporize* into the atmosphere without chemical change, they may be *absorbed* by soils, they may move downward through the soil in liquid or solution form and be lost from the soil by *leaching*, they may undergo *chemical reactions* within or on the surface of the soil, they may be *broken down* by soil microorganisms; they may wash into streams and rivers in *surface runoff*, and they may be *taken up* by plants or soil animals and move up the food chain. The specific fate of these chemicals will be determined at least in part by their chemical structures, which is highly variable for all of the different compounds (Brady and Weil, 1996).

Oil Effects on Plants

The toxic effects of hydrocarbons on terrestrial higher plants and their use as weedkillers have been ascribed to the oil dissolving the cytoplasmic membrane, and therefore allowing cell contents to escape. The polycyclic aromatic hydrocarbon (PAH) fraction of petroleum is particularly toxic to living organisms and its persistence and genotoxicity increases as the molecular size increases up to four or five benzene rings, and the toxicological concern shifts to chronic toxicity. They are thermodynamically stable due to their large negative resonance energy, which is the reason for their persistence in the environment (Crawford and Crawford, 1996).

Oil Effects on Animals and Humans

Petroleum hydrocarbons may be toxic to microorganisms, animals and humans. Bioassays for petroleum pollution show that low concentrations (5-100 mg/l) of crude oil or petroleum fractions in water can kill or inhibit the growth of microalgae and juvenile forms of marine animals (Calabrese and Kostecki, 1988).

Several microorganisms can break down hydrocarbons. Straight chain alkenes between C6 - C12 or branched with a small number of methyl or ethyl side chains are relatively "easy" to degrade for microorganisms but the occurrence of complex branched chains make them much harder to degrade (Hornick *et al.*, 1990).

Reasons for low degradation rates of hydrocarbons can be: low temperature, oxygen depletion or nutrient deficiency together with a decreased microbial activity. The low microbial activity may be due to a lack of mineral nitrogen, because crude oil in general has a very high carbon/nitrogen ratio which can result in a microbial nutrient deficiency (Hornick *et al.*, 1990).

The Behaviour of Oil Products in Soils

The movement of petroleum in soils could be in the seven directions described in 2.4 due to the hydrocarbons own properties (Brady and Weil, 1996).
The migration is primarily controlled by the oil's viscosity but also the porosity or permeability of the soil. Almost all oils are less dense than water and tend to "float" at the interface between the water-saturated and unsaturated zones.

Central to the issue of understanding the fate of oil is its composition and chemistry. The principal properties of interest are the partitioning properties of vapor pressure (or the solubility in the air), water solubility, and the octanol water partitioning coefficients (Calabrese and Kostecki, 1988).

The effects of applied oil on soil physical properties tend in the long-term to be beneficial. Aggregation, soil porosity, water holding capacity and retention seem all to increase. Short-term, an increased hydrophobic effect may be shown with an impedance of water movement into the soil aggregates. As a consequence there is an increase of water flow between the aggregates (Hornick *et al.*, 1990).

Refinery Processes

Because crude oil is a complex mixture of hydrocarbons it requires a number of separation processes before it can yield useful products such as petrol, diesel or fuel oil. After the crude oil leaves the storage tanks on the refinery, it is heated up by furnace to about 350 ^{0}C and some of the oil vaporizes and rises up in the furnace unit. Fractions of the oil with high boiling points (such as diesel oil) condense low down while lighter fractions (gasoline) and lower boiling point rise higher. The separated fractions move then to the next stage of their refining process. To increase the yield of petrol a "cracking" process is performed on the refinery where heavier fractions breaks down further to more valuable petrol with a higher octane level. This can be done by intense heat and pressure and is promoted by addition of a chemical Ni-catalyst (Elf oil information leaflet process).

Although product recovery may be maximized, the refining of crude oil produces effluents and sludge with substantial amounts of oil and grease which makes oil refineries large contributors of oily wastes. Unfortunately other waste products such as solvents, fuel additives, plastics and heavy metal contaminants are also produced in the refinery process. This leads to a complex mixture of the refinery wastes which is both toxic and hard for the microorganisms to degrade in a landfarm area which often is used as the treatment technique for oil refineries (Hornick *et al.*, 1990).

Options for Remediation

Heavy metals are as mentioned before not unusual constituents in crude oil. Oil contaminated soils therefore often show elevated concentrations of Pb, Cd, Zn and Ni (Calabrese and Kostecki, 1988). Although they may be released in the breakdown of a metal-containing compound, they are not degradable in the same sense as carbon-based molecules (Francis, 1998).

The metal atom is not the major building block for new cellular components, and while a significant amount of carbon is released to the atmosphere as CO_2, the metal atom is not often volatilised as an organic compound can be (Crawford and Crawford, 1996).
Due to their toxicity to plants and other living organisms when present in high concentrations, the metals often complicate and limit a soil remediation process (Calabrese and Kostecki, 1988).

Soil washing is one common treatment of soils, applied in both *in situ* and *ex situ*. *In situ*, the washing is on site, or it can be as an *ex situ* option, using particle-sized separation. The underlying principle in the particle-sized separation is that the contaminants have greatest affinity to the finest particles, the silts and the clays in the soils. Therefore the biggest particles can be separated and this reduces the volume of contaminated soil together with the costs of further treatment. This enables a more expensive treatment to a smaller volume of contaminated soil. As a generalisation, coarse soils show greater potential for soil washing than predominantly silt and clay soils (Pratt, 1993).

Incineration has long been recognised as an applicable *ex situ* technology for removing toxic organics from contaminated soils. It can be divided into two main categories:
Two stage systems that use volatilisation and pyrolysis to convert the toxic compounds into the gaseous phase and then thermal combustion of the gaseous organics.
One stage systems that destroy the organics directly within the contaminated soil. End products are bottom ash which is normally returned to the original site for backfilling (if not metal concentrations are to high) or discharged to a landfill (Pratt, 1993).

A traditional option is removal of the contaminated soil and deposal in a “safe” *landfill site*. As said before, the use of landfills is decreasing because peoples increasing environmental awareness together with changing legislation with landfill taxes.
Soil cover is another option were the contaminated material is isolated with a watertight layer with a new planting bed on the top (pers. comm. Ledin and Scullion, 2000).

Bioremediation treatment is when you try *"to harness natural processes and develop techniques to accelerate these processes for the bioremediation of contaminated soils, sediments, and plumes"*. The fundamental principle is to break down the contaminants to less toxic compounds, ultimately carbon dioxide, water and biomass (Sadowsky and Turco, 1998).

3.3 Phytoremediation

Phytoremediation is the term used when you achieve the goal of bioremediation through the use of green plants and their associated processes together with natural processes where the plants act as a catalyst to clean the soil. This makes phytoremediation a solution with a relatively low level of financial and technical input and an interesting clean up technique (Salt *et al.*, 1995).

Hyperaccumulating plant species are unusually good at bioconcentrating metals in their tissues but most of the hyperaccumulators are rather small herbaceous plants growing on naturally metalliferous sites or on old mining deposits (Robinson *et al.*1997a; Brown *et al.*, 1994). The ideal plant species to remediate a contaminated soil would be one with high biomass that can both tolerate and accumulate the contaminant of interest (Wenzel *et al.*, 1998).

According to Brooks (1977) relatively few plants are able to accumulate concentrations of nickel exceeding 15 µg/g on dry weight basis, unless they are growing over ultrabasic rocks, and values above 100 µg/g are uncommon. Values exceeding 1000 µg Ni/g (0.1%) in above ground dry biomass are restricted to a group of unusual plants which Brooks defines as Ni-hyperaccumulators.

Some of the most likely plant spieces for phytoremediation are members of the Brassicaceae family. *Thlaspi caerulescens*, for instance, is a known Zn-hyperaccumulator and can tolerate as much as 40 000 mg Zn/kg dry wt in shoots. However its use in the field is limited because the plant is extremely small and slow-growing (Ebbs and Kochian, 1997). Other Brassicaceae, which are members of the mustard family show promising results for the use in remediation of metal contaminated soils. *Brassica juncea* for example, has been shown by Blaylock *et al.* (1997) to accumulate moderate levels of Se, Pb, Cd, Ni, Zn and Cu. The effectiveness of this species for phytoremediation depends much on its ability to tolerate the relatively high concentrations of available metals in the soil (Blaylock *et al.,* 1997). It can also display a higher concentration of metals in its shoots compared to its roots and have therefore a high shoot/root metal ratio compared to non-accumulator plants. This increases the metal uptake when it is time to harvest the plants (Brown *et al.,* 1994).

Work of Brown *et al.* (1994) shows that *B. juncea* produces at least 20 times more biomass than *T. caerulescens* under field conditions which gives it the potential to remove more metals from the soil. Blaylock *et. al.* (1997) agrees in their work that one key factor for successful phytoremediation is the relatively high biomass production of the plant, and report that *B. juncea* can produce 18 t/ha of biomass in approximately 2.5 month of cultivation and are therefore suitable for harvesting their plant tissue to decrease the metal concentrations in the soil.

Central to the process of phytoremediation, is the nature of the soil on which the hyperaccumulator plants are to be grown. Robinson *et al.* (1997a; 1997b; 1999) have shown that the metal concentration in the plant is proportional to the plant-available metal concentration in the soil.

A high total concentration of a given element does not necessary indicate a high concentration of its soluble form which could be removed in a phytoremediation operation. Predictions can be made of the expected metal concentration in the plants by measuring the bioavailable (soluble) metal content of the soil. $CaCl_2$ extraction is a common analysis to give some indication of bioavailable metals in the soils and will be described further in chapter 4 (Materials and Methods).

In *"the best case scenario"* for phytoremediation, the available metal in the soil should remain relatively constant, where an equilibrium situation between soluble and insoluble metals could allow replacement of the soluble fraction removed by the plant. This would go on until all the metal in the soil has been removed. In *"worse-case scenario"*, most of the metal would be matrix bound, and once the initial metal harvest has been obtained, plants could not remove more metals without soil modifications.

The success of phytoremediation is dependent on several factors. One modification may involve acidification. Robinson *et al.* (1997b) showed correlations between soil-pH and metal content in plants. Low pH increases the concentration of bioavailable metals in the soil and gives an enhancement in plant uptake of metals.

Another soil modification can be application of synthetic chelates, for example EDTA. Plants must produce sufficient biomass while accumulating high concentrations of metal. However metals must also be in a bioavailable form to the plants. It has been proposed that adding a compound such as EDTA to the soil that solubilises non-available heavy metals, would increase metal yields in the plant (Robinson *et al.,* 1997b). The research of Blaylock *et al.* (1997) indicates that the accumulation of metals (Cd, Cu, Ni and Zn) in the shoots of *B. juncea* can be enhanced through the application of EDTA to the soil. Epstein *et al.* (1999) also observed an increase in Pb uptake by *B. juncea*. EDTA is a well-known chelating agent which bonds with many metals including Ni and Zn, and is degraded in the natural environment in a few months (Robinson *et al.*, 1997b).

A soil amendment can be addition of fertilizer. The application of NPK-fertilizer gave a threefold increase of the biomass of reproductive matter of the nickel-hyperaccumulator plant *Alyssum bertolonii* compared to the unfertilised plants and did not show any" trade off" in the nickel concentration with plant biomass increase (Robinson *et al.,* 1997a).

Very few publications exist concerning phytoremediation of petroleum products, but there is increasing evidence that degradation of organic contaminants is enhanced in vegetated soil compare to nonvegetated soil (Wenzel *et al.*, 1998). However, organic contaminants can be tightly adsorbed to soil particles and resist degradation and use by microbes which make them to poor targets for phytoremediation.

The addition of organic amendments has been shown to increase microbial activity in the soil by improving its chemical and physical properties. The presence of roots has been shown to be a major source of organic matter and enhance the degradation of hydrocarbons according to Aprill and Simms (1990).

4 MATERIALS AND METHODS

4.1 Overall Approach

A pot experiment were performed over 10 weeks in a greenhouse at the University of Wales, Aberystwyth with three different species (*Brassica juncea, Trifolium repens* and *Lolium perenne*) to determine metal uptake, plant biomass, and residual soil metals. Plants were grown under modified pH (+/- S) and nutrient regimes (+/- NPK). Two side experiments were set up in addition to the main experiment and were tested across a limited range of treatment combinations. One aimed to determine possible increase in metal uptake with applications of EDTA-complexes and the other one was to determine if applications of sulphur affected plant growth and not only the pH in the soil. Treatments were applied in a factorial design. The main experiment included the three different plant species, addition of sulphur or not and use (at optimal levels) or no use of fertilizers (NPK).

Brassica juncea was chosen because it belongs to a family whose members are known to be good at tolerating elevated levels of heavy metals. *B. juncea* has been shown to accumulate moderate levels of both Ni and Zn together with Cd, Pb, Cr and Cu (Ebbs and Kochian, 1997). It was therefore interesting to compare this species to non-hyperaccumulating species.

Legumes are also relatively good at accumulating metals in plant tissue and one advantage with legumes is that legumes are able to fix N by Rhizobia. This allows them to become independent of the soil N supply. Generally, legumes such as *Trifolium repens* (clover) are poor competitors with grasses for light, water and nutrients. Grasses (such as *Lolium perenne*) have a faster growth rate and can overtop and shade the legumes if they grow together (Haynes, 1980).

Lolium perenne (ryegrass) is a fast growing, high yield perennial grass and does not require reestablishment on a yearly basis. It has a relatively large rhizoplane surface area because of its fibrous root system which is advantageous in the establishment of an active microbial population and in exploring soil. *Lolium perenne* is also more resistant to phytotoxic effects compared to other plant species (pers. comm. Scullion, 2000).

4.2 Site Description

During the 1990's Elf Oil were the seventh largest oil and gas company in the world (Brown pers. comm., 2000). Elf oil refinery has two landfarm areas, which have received petroleum refinery wastes for more than 20 years. Effluents from a number of the refinery's processes were collected in a wastewater pond and the sludges from the pond bottom were removed and transported to land treatment sites. At the sites, the sludge was spread and periodically mixed with the soil to a depth of 50 cm to aerate the soil as in usual agricultural practices.

As a nickel catalyst in the form of 10% nickel octate was used in the cracking process, the land has now elevated concentrations in the soil of nickel (Figure 1.) in addition to different hydrocarbon compounds.

According to the refinery's licence values, Elf Oil already exceeded the threshold limit for Ni (70 mg Ni/kg at soil pH 6.5) and cannot continue to apply oil waste on the two landfarm sites. However, the sites are not available to humans and animals as long as the refinery exists and the levels of contamination make it a relatively low risk site.

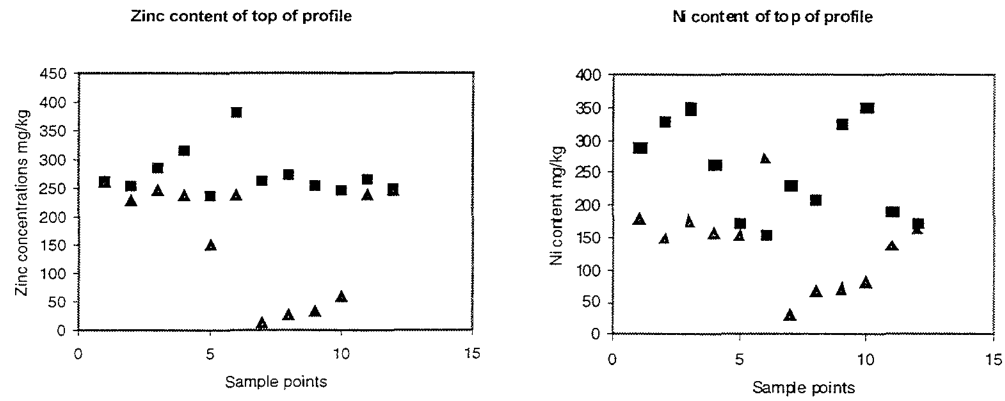


Figure 1. Total concentrations of Zn and Ni (mg/kg dry wt) in Landfarm 1 (▲) and 2 (■).

4.3 GCMS Analyses

This degradation of hydrocarbons has not been tested because of the time limitation of this study, but GCMS analyses were performed to evaluate the different organic compounds in the soil and the results are shown in Appendix A.
The suite of oil compounds showed high quantities of straight and branched chains of hydrocarbons with 10 to 15 carbons. PAH's such as pyrene and naphthalene together with phthalates were present too. Phthalates thought to emanate from the plastic in the storage bags.

4.4 Site Geology

The oil refinery is situated in the middle of arable fields and the topsoil and subsoil are a reddish brown stony clay loam with granular or fine subangular blocky structure. During the Pleistocene Period, glacial till (boulder clay) and associated sand and gravel, were deposited by ice sheets moving from the north-west to the south-east. Although these deposits have been largely removed by post-glacial erosion, some small patches still exist in some areas (Rudeforth,1984).

The Bedrock at the site belongs to the Red Marls sub-division of the Lower Old Red Sandstone. This consists mainly of mudstones and siltstones continually repeated by tight relatively steep folds whose local dip direction varies greatly. There has been no major faulting recorded by the geological surveys carried out in the site area. The annual rainfall in the area is 1100 mm (Rudeforth,1984).

4.5 Experimental Design

The soil was collected during March 2000 at the two landfarm areas on Elf Oil refinery in Milford Haven. Earlier investigation at the site determined that high nickel concentrations seemed to be associated with oil smell in the soil (Scullion pers. comm. 2000). Seven pits were dug with an oily smell associated with them. The soil was taken from the top 50 cm of the soil profile. The soil samples were placed in plastic buckets and sealed with plastic bags in room temperature.
Concentrations of nickel in earlier analysis varied across the sites and soils were therefore carefully mixed to homogenise as much as possibly. The 70 kg soil used in experiment was partially dried then sieved through a 10 mm stainless steel sieve.

4.6 Soil Analyses

Analyses of Soil pH and Moisture Content

The soil pH was measured in a 1:2.5 (10g soil:25 ml water) suspension using a glass electrode (MAFF, 1986). Moisture content was determined after 7 days by drying soils at 105 ^{0}C for 24 hours (This method is provided by the University of Wales, Soil Science Department).

Determination of the Metal Content of the Plants

Total metals in plants were extracted from approximately 0.5 grams (when possible) of oven dried (80 ^{0}C) cut up plant material. It was refluxed in 100 ml conical flask together with 15 ml concentrated nitric acid and boiled (in a fume cupboard) until no brown fumes were visible (McGrath and Cegarra, 1992). 10 ml of 0.5 M hydrochloric acid was added and the solution filtered through Whatman No. 1 filter paper into 50 ml or 100 ml volumetric flask depending on initial plant tissue amount. The samples were then diluted to 50 or 100 ml using distilled water. Concentrations Zn and Ni were determined using an atomic absorption spectrophotometer (Pye-Unicam SP9) after calibration with standards.

Determination of the Solubility of Metals in the Soils

Extractions with $CaCl_2$ were used to measure plant-available metals. The soil was air-dried at room temperature before it was mixed with 0.1 M $CaCl_2$ (20 ml to 2 g soil) and shaken for 16 hours. All extractions were filtered using Whatman 1 filterpaper and deionized water was used to bring the samples to proper solution/soil ratio (McGrath and Cegarra, 1992). Concentrations Zn and Ni were determined using an atomic absorption spectrophotometer (Pye-Unicam SP9) after calibration with standards.

4.7 Experimental Details

Pot Study

A pot study was conducted to determine if the hyper-accumulating *Brassica juncea* plants are able to extract more nickel and zinc from the soil of the refinery compared to the non-accumulating plants (*Trifolium repens* and *Lolium perenne*) with four different treatments.

Treatments in the Pot Study

Four treatment groups, sulphur or no sulphur added, and fertilizer and no fertilizer added were replicated 5 times with all three species in a factorial design which resulted in 60 pots in the main experiment.

For lowering the pH as one experimental treatment elemental sulphur was used.
To estimate the amounts of sulphur required to modify soil pH in the soil 0,1M hydrochloric acid together with 10 grams of the mixed experimental soil was used. One mole S is equivalent to two mole of hydrogen ions which gives the amount of 48 grams elemental sulphur to 10 kg soil according to Figure 2.

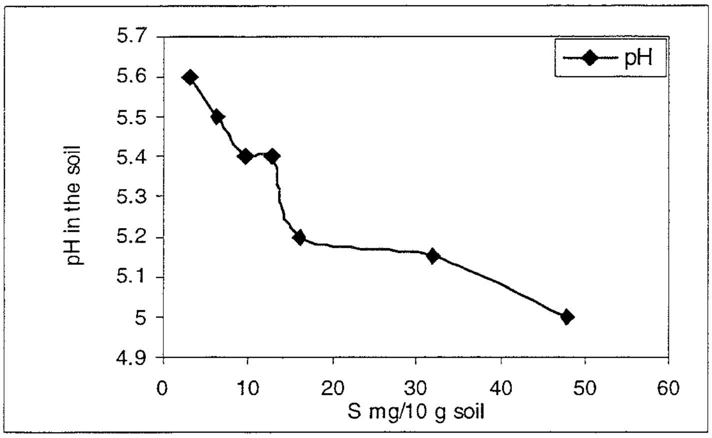


Figure 2. The amounts of sulphur equivalent required lowering the pH of final soil.

The elemental sulphur was applied with a 1mm sieve over the 2 cm thick layer of soil and then mixed together carefully.

The effect of addition of sulphur may not only be on pH in the soil, sulphur is also a nutrient for plants. To eliminate the possibility of nutrient response on the plants, additions of Na_2SO_4 were made in all the solutions used in the experiment (10 mg S/pot).

The addition of N, P and K to soil increases the plant biomass (Ebbs and Kochian, 1997) and stimulates the biodegradation of oil and individual
hydrocarbons (Alexander, 1994). With an increased plant biomass, an enhancement in metal uptake may follow (Robinson *et al.,* 1997b).

Farmer guidelines (pers. comm. Scullion, 2000) show that 200 kg N/ha and season for both ryegrass and clover is a common amount. According to this a nutrient solution with 2 g/l of 20:20:20 NPK were made up (20 mg N/pot).

Environmental Conditions in the Pot Study

The soil was placed in 12 cm diameter pots (560 g of soil/pot) and planted with seeds of the three plant species. The ryegrass (*Lolium perenne cv. Talbot BA 10915*) and the clover (*Trifolium repens cv. Menna)* were obtained from Institution Grassland and Environmental Research (IGER), Aberystwyth. The Indian mustard (*Brassica juncea ori 060)* was delivered from King & Co, Essex. The plants will from now on be called by their common name.

10 seeds of each species were placed in every pot and put into a green house for a period of 10 weeks with a controlled temperature within the limits of 15 and 25 ^{0}C. The day period was maintained to 16 hours by artificial lighting. The pots were placed in individual trays to prevent losses of amendments from leaching. The seeds were germinated in the soil and the Indian mustard was thinned out after two weeks to four in each pot because of increased growth.

Plants were grown for 14 days before the first addition of nutrients (20:20:20 NPK) was applied in solution (50 ml/pot) and then there was an addition every fortnight. The plants were watered twice a day to replace water lost through evapotranspiration. The controls were given distilled water with sulphate addition (10 mg/pot and treatment). The different treatments were arranged randomly.

Soil Amendment: EDTA

It has been proposed that adding a compound to the soil that solubilises non-available heavy metals, would increase metal yields in the plant (Robinson *et al.*, 1997b). Similar to Robinson (1997b) the complex EDTA was chosen and applied as a solution (12 mmol EDTA/kg soil) as the disodium salt. This is a possible way of increasing the metal content in the plants.

Due to practical limitations EDTA was applied with only a limited range of other treatments. The soil used was with the addition of sulphur and the hyperaccumulator species Indian mustard for the possibility of maximal metal uptake.

Fertilizer Effect of S Addition

Sulphur is one of the macronutrients that are required by plants and there may be a nutrient response when sulphur is added to lower the pH.
To eliminate this treatment response (to S and NPK) a side experiment with 9 pots with no sulphur were performed.
Three pots of each species with the soil from the main experiment (no other treatment) were used.

Nutrients (NPK) were added in the same amount as in the main experiment. Biomasses were measured in the end of the experiment to investigate possible decreases in plant growth.

Microbial Activity

If the different treatments in the main pot experiment have negative effects on microbial activity the degradation of organic compounds decreases together with decomposition of organic matter. One easy way of determine microbial activity is to measuring soil respiration in form of carbon dioxide (evolved over time). Inhibitory effects on microbial activity have been noticed in the presence of heavy metal contaminated soil (Baath, 1989). A separate experiment was set up for measuring respiration rates. Because of practical limitations only a small range of treatments were set up.

Four replicates in 6 different treatments:

- Sulphur added soil with and without nutrients
- No sulphur added in the soil with and without nutrients
- Sulphur added to the soil with EDTA and nutrients
- No sulphur added soil with EDTA and no nutrients

Glass flasks (100 ml) were loaded with 25 g of soil with a moisture content of 16.6% as in the field. Nutrients were added two times and as a NPK 20:20:20 solution (2,5 mg NPK/ml and flask) together with Na_2SO_4 (1,25 mg Na_2SO_4/ml and flask). The amount was proportional to what a crop will need for a whole season (200 kg N/ha) as in the main experiment. The treatments with no nutrients were only given sulphate (1,25 mg Na_2SO_4/ml per flask) and water. Using a pasteur pipette, 1.5 ml of the different solutions was added to each flask. All flasks were partial sealed to decrease water losses but allow gas exchange. They were incubated at 20 ^{0}C in the dark with periodic stirring and water addition to compensate for oxygen consumption and evaporative water losses. The flasks were prevented water Microbial activity was recorded after a 2-hour incubation time under which the flasks were sealed. Carbon dioxide concentrations was measured using a gas chromatograph and showed how much carbon (C) that respired under the two hours.

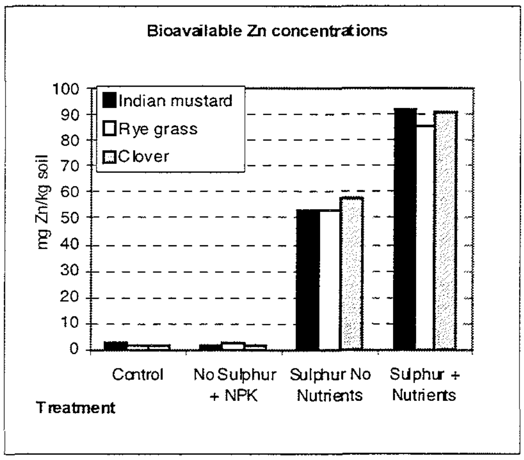
4.8 Statistics

Statistical analysis using the two-way analysis of variance procedure (ANOVA) was undertaken to interpret significant variation between different treatments and to test for interactions between them.

5 RESULTS

5.1 Bioavailable Metal Concentrations in the Soil

The ranges of $CaCl_2$ extractable metal concentrations in the soils are given in Figure 3. The measurement was made in the soil after plant harvest. Results show that the concentrations of both Zn and Ni, with high significance ($P<0,001$) increased over time in the sulphur treatment. In the nutrient treatment the results also indicate significant ($P<0,01$) enhancement of the metal concentrations of Zn and Ni.



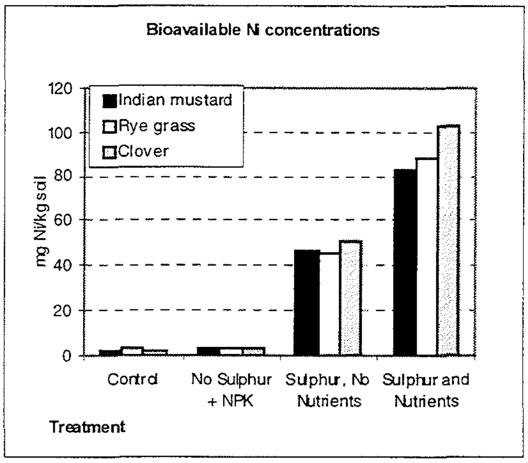


Figure 3. $CaCl_2$ extractable metal concentrations in the refinery soil.

5.2 Soil pH

The pH in the mixed refinery soil was in average 6.5 in the start of the pot experiment. During the experiment the pH declined in the pots according to Table 2. A reduction of half a pH unit was observed in no sulphur pots and a reduction of another half unit between with and without nutrient application.

Table 2. pH in the pots after harvest (mean values)

	Indian mustard	Rye grass	Clover
Sulphur and nutrients	3.7	3.8	3.7
Sulphur and no nutrients	4.1	4.1	4.1
No sulphur but nutrients	6	5.9	5.8
Control	6	6	6

5.3 Metal Concentrations in the Plants

All plants specimens contained elevated concentrations of Ni and Zn compared to normal plants growing in non-contaminated soil were background values are 0,15 mg/kg dry wt for Ni (Lepp, 1981) and 5 to 35 mg/kg dry wt for Zn (McBride, 1994).

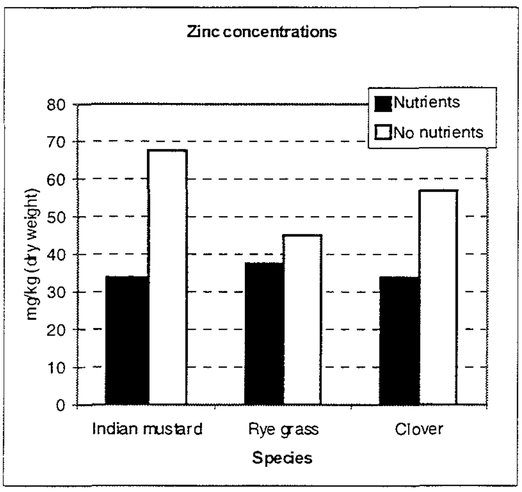
There was a great deal of variability in some of the individual metal contents. Both nickel and zinc content in the three plant species varied more in the absence of nutrient than with nutrients. The variation between individuals was despite the plants being closely controlled and having been planted in homogenous soil mixtures.

Metal Concentrations in the Plants with +/- Nutrient Treatment

Zinc
There was no significant difference in Zn uptake between the three plants species. The average Zn concentration was between 35-68 mg/kg (dry weight) depending on the nutrient treatment. There were highly significant (P< 0,001) differences in Zn concentrations with nutrient and no nutrient treatment in the non-sulphur treated soil.

Nickel
The results did not indicate a species effect in Ni uptake of the three plant species. The mean values were between 10-15 mg Ni/kg (dry weight) depending on nutrient treatment. The data demonstrated significantly (P< 0,01) lower values in the plants with nutrients compared to non-nutrients in the non-sulphur treated soil. The results are shown in Figure 4.

The average reduction of metal concentrations with nutrients was over 30% in both Zn and Ni concentrations in the plants. Ryegrass indicated higher response to nutrient treatment and a more uniform uptake of Zn and Ni compared to Indian mustard and Clover. Clover and Indian mustard had a 40 respectively 50 times bigger variance in no nutrient treatments compared to a nutrient treatment.



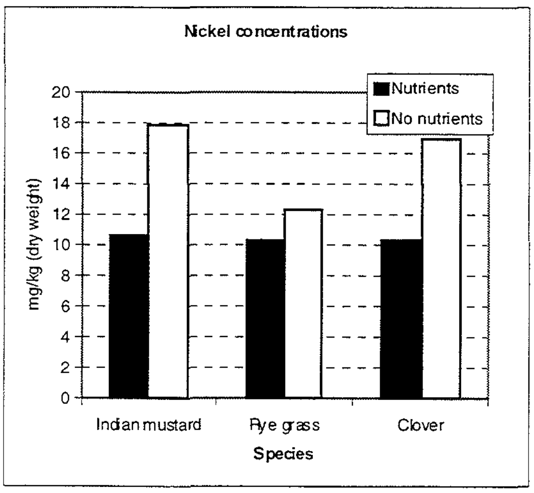


Figure 4. Mean metal concentrations in the three species, indian mustard, ryegrass and clover.

Metal Accumulation by the Plants under Sulphur Amended Conditions

Zinc
There was no significant response to nutrient treatment and no species effect. However, ryegrass had more than half of Indian mustard and clover concentrations of Zn according to Figure 5.

Nickel
Significant ($P< 0,05$) increase of Ni concentrations were caused by nutrient addition. There were very highly significant ($P< 0,001$) difference in Ni uptake between the three species. Ryegrass has the lowest metal uptake. Significant interactions ($P< 0,05$) were obtained because of the treatment response are not independent. Effect of nutrients markedly greater for clover compared to Indian mustard and ryegrass.

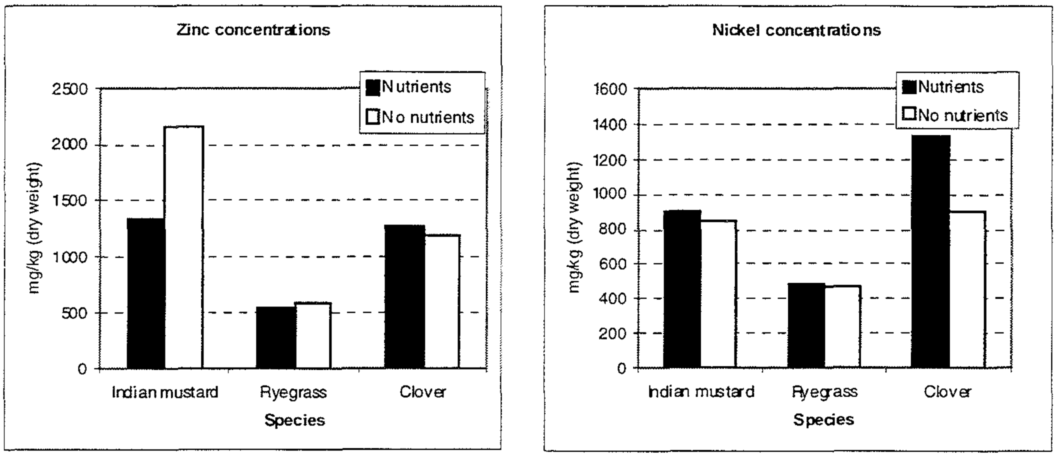


Figure 5. Total metal concentrations in the three species, indian mustard, ryegrass and clover.

The Metal Concentrations of *Brassica juncea*

Zinc
There were significantly ($P<0,05$) higher concentrations in sulphur treated plants compared to untreated.
Higher concentrations was recorded in the absence of nutrients in both the control soil and sulphur-amended soil but this was not significant.

Nickel
Here the results indicate significance ($P<0,01$) in sulphur response. The increase is from 12 mg Ni/kg to 916 mg Ni/kg (dry weight). In sulphur treated soil the nutrient addition increases the Ni uptake while in untreated (control soil) the Ni uptake decreases. Suggests interactions but there were no significant interactions between the treatments.

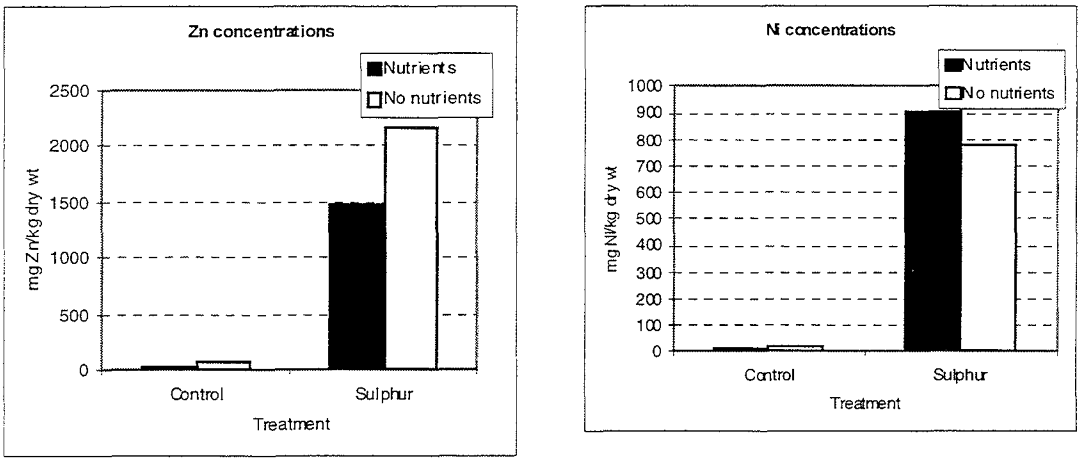


Figure 6. Enhancement of Zn and Ni concentrations in shoots of Indian mustard *(B. juncea)* grown in sulphur amended soil compared with plants grown in control soil.

<u>The Metal Concentrations of *Lolium perenne*</u>

Zinc

There were significantly higher concentrations (P<0,001) in sulphur treated compared to untreated plants. The Zn uptake increase 14 times in the sulphur treated pots which is shown in Figure 7. There were no significant nutrient response.

Nickel

Here the results show a significant (P<0,001) sulphur response as with Zn, Figure 7. The effects of nutrient treatment differ depending on the sulphur treatment as with Indian mustard above.

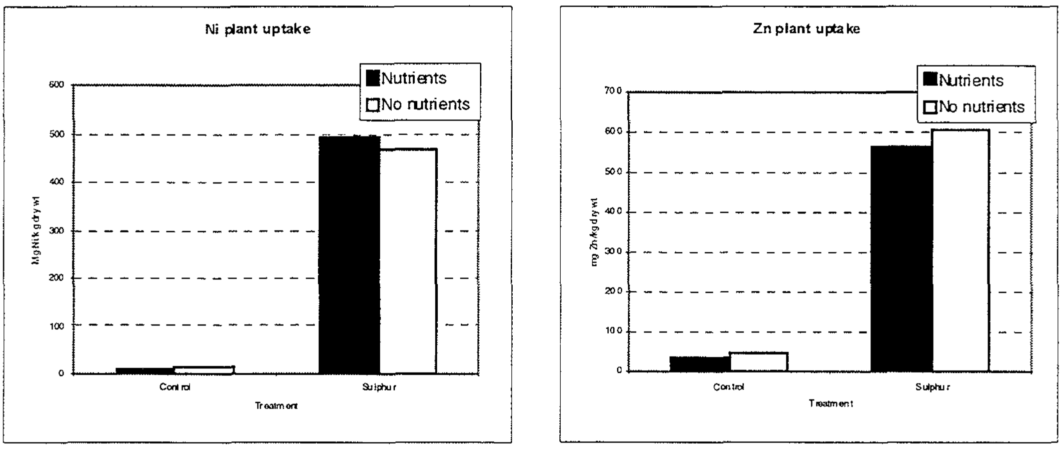


Figure 7. Enhancement of Zn and Ni concentrations in shoots of ryegrass (*Lolium perenne*) grown in sulphur amended soil compared with plants grown in control soil.

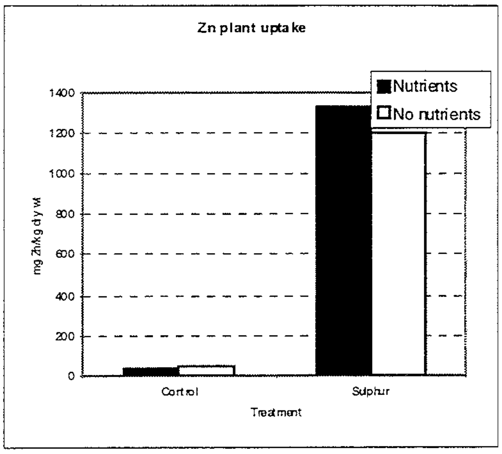
The Metal Concentrations of *Trifolium repens*

Zinc

The statistical data suggest that there is significant (P< 0,01) response in Zn uptake because of sulphur treatment as with Indian mustard and Ryegrass before.

Nickel

In Figure 8. clover shows a high significantly (P< 0,001) increase in Ni uptake due to sulphur treatment and also a significant (P< 0,05) response to nutrient treatment.
In sulphur treated soil, nutrients increase Ni uptake while nutrients decrease metal concentrations in control soils giving a significant interaction between sulphur and nutrients (P< 0,05).



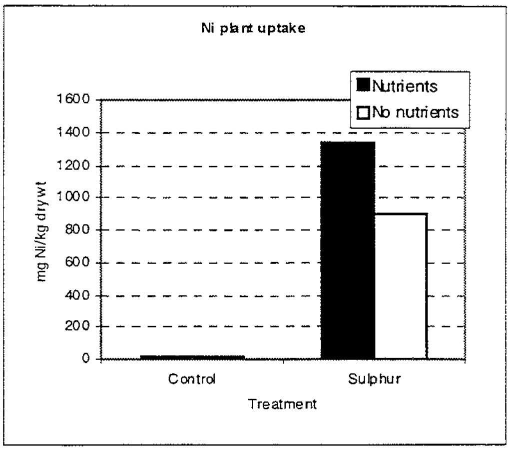


Figure 8. Enhancement of Zn and Ni concentrations in shoots of clover (*Trifolium repens*) grown in sulphur amended soil compared with plants grown in control soil.

5.4 The Biomass Production

All the plants in the sulphur treated soil were almost dead when they were harvested (only some of the un-fertilized ryegrass was still alive). The biomass of Indian mustard increased 15 times in the control soil due to fertilizer addition. Ryegrass and clover increased 12 respectively 20 times grown in the same soil according to Table 3.

Table 3. Mean values of the plant biomass production (g dry weight/pot)

Treatment	Indian mustard	Ryegrass	Clover
Sulphur+NPK	0.12	0.27	0.04
Sulphur-NPK	0.04	0.07	0.03
No sulphur+NPK	3.2	3.9	3.5
No sulphur-NPK	0.21	0.33	0.17

5.5 Soil Amendment: EDTA

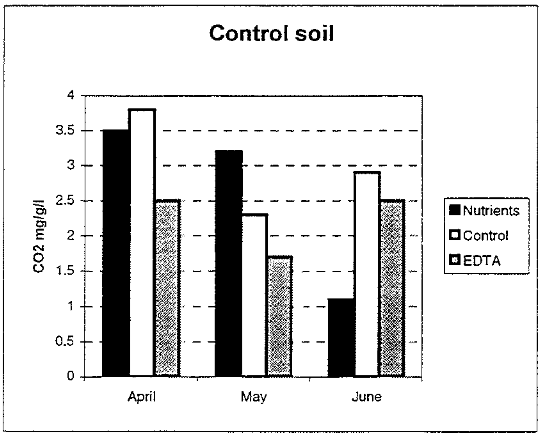
All the Indian mustard rapidly died after the addition of EDTA. The metal concentration of the dead material showed a slight increase in Ni uptake and a slight decrease in Zn uptake compared to the Indian mustard grown in sulphur amended soil.

Table 4. Mean values of metal uptake of Indian mustard in EDTA treated soil compared with plant metal uptake in sulphur treated soil

Indian mustard	Ni mg/kg dry wt	Zn mg/kg dry wt
EDTA soil	983	1235
Sulphur soil	906	1476

5.6 Carbon Dioxide Evolution

Respiration rates (or CO_2 evolved) expressed as CO_2 (mg $g^{-1}h^{-1}$) for the different treatments as in the main experiment (with or without sulphur application in the soil and with or without nutrient addition). The treatments have four replicates and four extra flasks are made with an EDTA application as in the side experiment. The CO_2-experiment averaged over the 5 week incubation period (26th April until 2nd June). The results are presentated in Figure 9.



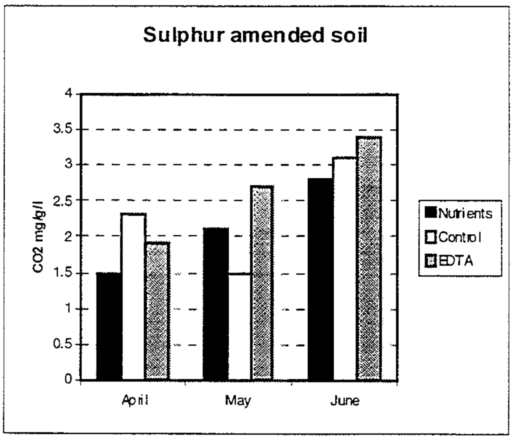


Figure 9. Respiration rates, expressed as CO_2 (mg $g^{-1}h^{-1}$).

6 DISCUSSION

6.1 General Aspects

First of all, it is important to bear in mind that all plants in the sulphur treatment were dead within the 10 weeks time. Further work about phytoremediation of the land farm soil could be directed at applying less sulphur in the soil for achieving a higher biomass of the plants with a following increase in total metal uptake. Due to the uncertainties associated with the laboratory data, these cannot fully reflect performance under field conditions and merely provide an indication of expected field effects. The fact that moisture, light and temperature differ from that in the field together with the manipulations of the soil prior to pot experiments must be suspected of creating artificial results. This investigation is also limited of the short period of time available for the experiment and experimental problem such as sample manipulation may have introduced problems. The plant tissues have been cut, and may cause inconsistencies in metal concentrations. For example, Indian mustard (with its steams, leaves and seeds) shows more variance compared to ryegrass. However, even if ryegrass has a more uniform uptake problems can arise when harvested. The plant biomass can be hard to remove when most of it grows close to the soil compared to Indian mustard.

6.2 The Effects of Sulphur Amendments

Soil

The results imply an important sulphur induced effect. The sulphur lowered the pH in the soil so toxic levels of both Zn and Ni occurred in the plants according to toxicity levels by Marschner (1986). The toxicity stressed all the plants, and experimental problem with low biomass and few replicates occurred. This resulted in uncertainties about the significance. Some of the non-significant result may have been significant if more plants had survived.
Acidification usually reduces microbial activity and Lepp (1981) recorded a 36% decrease in enzyme activity between pH 3,5 and 7. The respiration rates in this study did not indicate this relationship between a decreased microbial activity and the sulphur amended soil with low pH. Also presence of heavy metals such as Ni and Zn inhibit microbial activity in both acid and alkaline soils (Lepp, 1981).

Plants

The total metal uptake of the S treated plants was very low because of the low biomass. In the sulphur treated soil the fertilizer resulted in a larger concentrations in the plants of Zn and Ni. Possible explanations seem to be the decreased soil pH with following increase in bioavailable Zn and Ni concentrations.

At low pH a detrimental effect of Al^{3+} also occurs, which can influence and cause more damage on the already stressed plants (Marschner, 1986).

6.3 The Results in Untreated Soil

Soil

The bioavailable amounts of zinc and nickel are very low (only 1 or 2 % of total) and makes it hard for the plants to take up any Zn or Ni ions. The metals in the soil will probably never be removed by plants without any soil amendments. Even if the metals are unavailable to plants they still may be toxic to animals or humans if the soil itself is ingested (Robinson *et al.,* 1999).

Plants

The mineral fertilizer (NPK) decreased the plant concentrations with 30% of Zn and Ni in untreated soil. However, the fertilizer increased the biomass between 12 and 20 times depending on plant species. Despite the dilution effect, fertilizer treated soil gives a higher total metal uptake with fertilizer than without (See Appendix B).

6.4 Fertiliser Amendments

The mineral fertilizer (NPK) applied in this experiment decreased soil pH (eg. the nitrification of ammonium) together with partial breakdown of present organics, which generates H^+ ions. This has resulted in a significant higher value of the bioavailable concentrations of both Zn and Ni in the soil according to Figure 3 above. This resulted in an increase of Ni uptake in all three species (Figure 6-8). Ni seems to be more sensitive to the soil pH compared to Zn, which is in accordance to Alloway (1995).

6.5 Calculations of Plant Nickel Uptake

Predictions can be made for calculations about the time span to remove Ni (which is more of a contamination problem compared to Zn on Elf site) from the soil. If we assume a soil depth of 15 cm, a soil density of 1,5 g/cm^3 (gives 2000 ton soil/ha) and a concentration of Ni content of 70 mg/kg soil (mean value of all the $CaCl_2$ extractable Ni concentrations in the sulphur treated soil), the plant available pool of Ni will be 140 kg/ha. The total Ni pool will be 700 kg/ha if we assume a total Ni concentration of 350 mg/kg soil. To reach the UK guideline of Ni, 560 kg Ni/ha have to be removed. It will take over 140 years to clean the site with ryegrass even with optimal Ni uptake in the sulphur amended soil (467 mg/kg dry wt) and biomass production that may be unrealistic in the contaminated refinery soil (8 ton/ha). Similar results with the Ni uptake by clover together with optimal conditions. It is not enough with 100 years to clean the site. Indian mustard is closest to clean the site with the limit of 20 years. With an average biomass production of 18 ton/ha (Blaylock *et. al.*, 1997) and Ni uptake of 906 mg/kg dry wt it would take 34 year. This biomass is in uncontaminated soil and cannot be expected in the refinery soil. With a more realistic biomass production of 7 ton/ha, the time span to clean the soil will be 80 years.

6.6 The Effect of Chelating Agent Addition on the Metal Uptake

The strategy for increasing metal yield with applying a chelating agent to the soil was a subsidiary experiment and only applied in the sulphur treated soil. In an already hard environment for the plants because of high metal concentrations, the plants rapidly died when the EDTA increased the high available metal concentrations. Similar results has been shown by Blaylock *et al.*(1997) when Indian mustard hyper accumulated Pb to the cost of its death (which did not matter in his phytoextraction operation as dead tissue was harvested and burnt as easily as live material). In phytoremediation live material is preferable and therefore also a smaller application of EDTA (than 12 mmol EDTA/kg soil).

6.7 The Effect of Sulphur Addition to Plant Growth

The second subsidiary experiment did not indicate any increased biomass response due to sulphur applications.

6.8 Respiration Rates in Relation to Sulphur Amendments (pH)

There appears to be a weak relationship between respiration rates (or CO_2 evolved) and the sulphur treatment. Higher respiration rates are generally linked to higher pH values (Lepp, 1981). There are inconsistencies in the result which make it hard for any clear conclusions.

7 CONCLUSIONS

The unexpectedly low pH in the sulphur treated soil seems to be a result of an over estimated calculation of the amount of applied elemental sulphur. Even if the applied amount sulphur was similar to recommended application rates (pers. comm. Scullion, 2000) the expected pH-level of 5 in the sulphur treated soil went down to 4. The study does not yield a reliable answer about metal accumulation of plants as they died in the sulphur treated soil.

Sulphur treatment seems to be necessary as the content of bioavailable metals is very low in the control soil. It does not matter how many crops that will be grown on the site if the major part of the Ni (and Zn) in the untreated refinery soil is silicate bound or present in a very insoluble form.

Central for a successful phytoremediation is to achieve a high plant biomass (Blaylock *et al.*, 1997) and the result showed a more than 12 times increase of biomass with addition of fertilizer in all three plant species. This followed a decreased metal concentration, which seemed to be due to biomass dilution. However, the prediction of expected total metal uptake showed an increase and is in accordance with other authors work (Blaylock *et al.*,1997; Robinson *et al.*, 1997b).

However, the results in this investigation did not prove the plants to be suitable to clean up the landfarm soil. Even if calculations are made with optimal Ni uptake and optimal biomass of Indian mustard the time span of 20 year to clean the site was far too short. Nothing indicated either, if the bioavailable pool of Ni will be replaced of Ni from the unavailable pool after plant uptake or not. Other clean up options which has been described in the literature review have to be considered.

Despite the facts that this work was not able to give clear answer about metal uptake of the plants, phytoremediation may still be the viable decontamination method of the refinery soil. The ability of plants to accumulate heavy metals seems to have a great future potential, and further research is necessary to validate the possibility to clean the site with phytoremediation.

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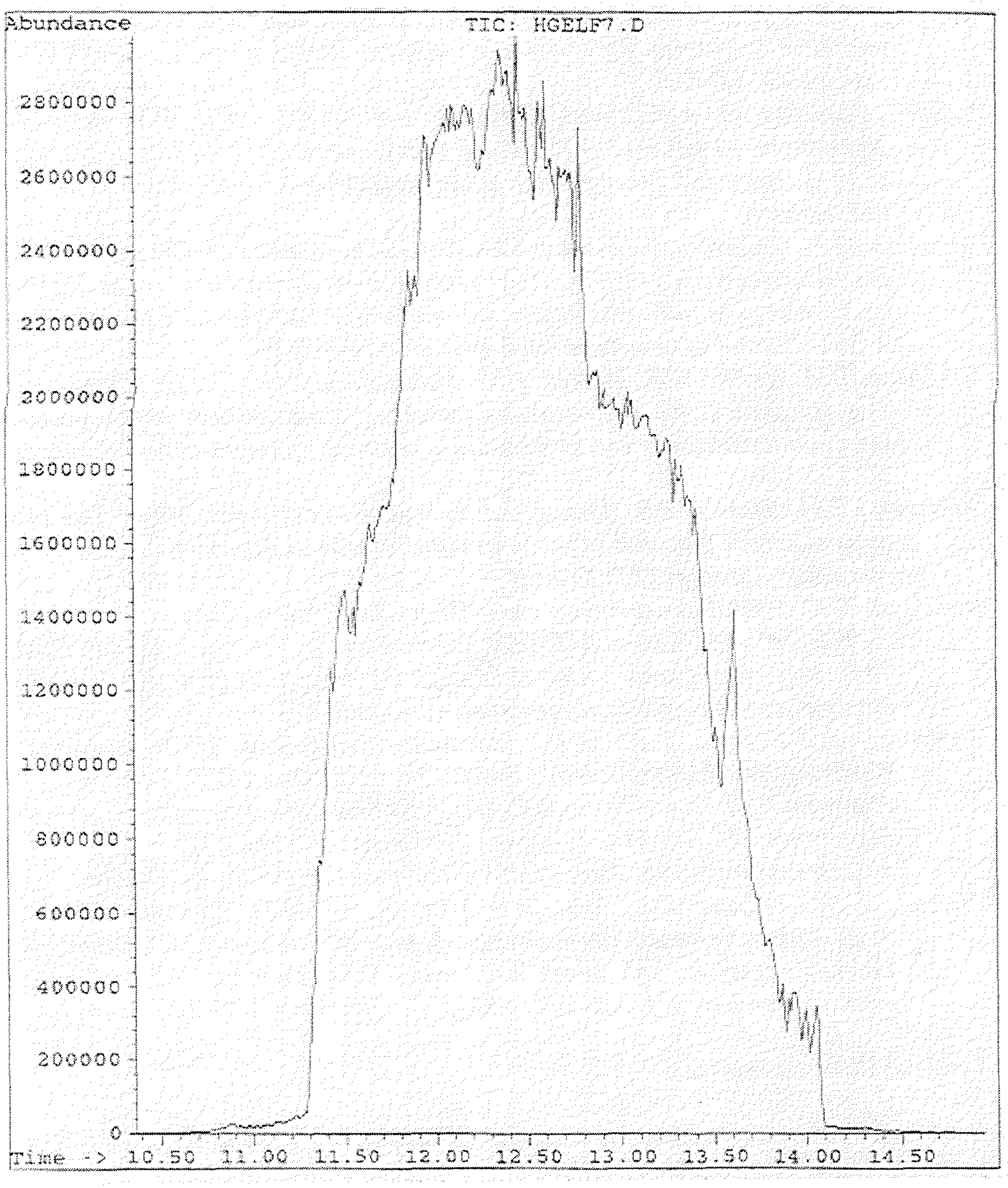
Personal Communication:

Brown, B. (2000). Environmental Manager, Elf Oil Refinery, Milford Haven, Wales.
Ledin, S. (2000). Dr, The Swedish University of Agriculture, Uppsala.
Scullion, J. (2000). Dr, The University of Wales, Aberystwyth, Wales.

9 APPENDICES

Appendix A: GCMS-result



Appendix B: Calculations of total metal uptake and years to clean the site

	Ni uptake mg/kg (dry wt)	Biomass in pot study ton/ha	Total Ni uptake g/season	Average biomass ton/ha	Total Ni uptake g/season	Years to clean the site
Control soil +NPK						
Indian mustard	11	2,9	32	22	242	2314
Ryegrass	10	3,5	35	7	70	8000
Clover	10	3,2	32	5	50	11200
Control soil -NPK						
Indian mustard	18	0,2	3,6	15	270	2074
Ryegrass	12	0,3	3,6	5	60	9333
Clover	17	0,2	3,4	3	51	10980
Sulphur soil +NPK						
Indian mustard	906	0,1	100	4	3624	155
Ryegrass	488	0,3	122	4	1952	287
Clover	1336	0,04	53	4	5344	105
Sulphur soil -NPK						
Indian mustard	916	0,04	37	3	2748	204
Ryegrass	467	0,06	28	3	1401	400
Clover	900	0,03	27	3	2700	207

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Swedish University of Agricultural Sciences
Department of Soil Sciences
Division of Agricultural Hydrotechnics
P.O. Box 7014
S-750 07 UPPSALA, SWEDEN

Tel. +46-(18) 67 11 85, +46-(18) 67 11 86