THE INFLUENCE OF HEAVY METALS UPON TREE GROWTH IN SOUTH WALES FORESTS

EDWARD MORGAN

A THESIS SUBMITTED TO THE COUNCIL FOR NATIONAL ACADEMIC AWARDS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN COLLABORATION WITH THE FORESTRY COMMISSION

DEPARTMENT OF SCIENCE

THE POLYTECHNIC OF WALES

JUNE 1983

DECLARATION

This thesis has not been nor is being currently submitted for the award of any other degree or similar qualification.

Edward Morgan

EDWARD MORGAN

ACKNOWLEDGEMENTS

I would like to thank my supervisors for the helpful guidance and encouragement which they have given to me throughout this project. Especially grateful thanks are extended to Dr K. W. Burton, my director of studies, and Dr A. Roig with whom I have enjoyed working immensely. I would also like to thank Mr David Clark for his assistance in sampling, and the rest of the staff at the science department, both technical and academic, for their assistance throughout my studies. I would also like to thank all the members of the Forestry Commission, especially Mr N Danby, who have given their assistance to my project.

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ABSTRACT

This study has combined laboratory and greenhouse based experiments using sitka-spruce seedlings, with field studies to examine the effects by heavy metals upon tree growth.

Water culture techniques were used to determine the upper critical tissue concentrations for cadmium, copper, lead, nickel and zinc ions: these being the lowest concentrations at which shoot yields were affected. Concentrations of these heavy metals in the soil and foliage at the South Wales forestry sites were found to be elevated with respect to a control site in Mid Wales (Tywi). A comparison of these foliar levels with the critical tissue concentrations showed several sites to be at risk to nickel toxicity (Burton et al 1983).

The interactive effects of heavy metals, studied by means of factorial experiments in water culture, did not reveal any synergistic or antagonistic reductions in the yields of the seedlings. However, the interactive effects of these metals were found, in some cases to be additive. Several sites had foliar levels of cadmium and lead which approached their upper critical tissue concentrations. The effects upon tree growth may be even greater at these sites. These experiments also showed that heavy metals can influence the uptake into the shoots and roots of other heavy metals and nutrients.

The field situations were more closely modelled using greenhouse experiments with seedlings grown in a typical forestry soil with added heavy metals. Effects upon the growth of the seedlings, as measured by yields of shoots and roots and root lengths, were demonstrated. At soil concentrations of cadmium and lead which were similar to those found at the forestry sites, there were disturbances in the root-mycorrhizal associations indicating that tree growth may also be indirectly affected by the heavy metals in the soil.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

Table

Chapter 1

INTRODUCTION

South Wales is a heavily industrialized and urbanized region. Much of its industry was attracted to the region because of abundant coal supplies which were used in several processes requiring large quantities of energy. In the past the major industries associated with heavy metals were the smelting and refining of non-ferrous heavy metals, such as copper and nickel. These were mainly located at the mouths of the Swansea and Neath valleys. In recent times, however, most of these operations have closed down. As well as these large scale operations, concentrated mainly in the west of the region, there are and have been many other industries located throughout South Wales which are involved with metals. These include car battery factories, scrap yards and coal burning plants (Welsh Office 1975).

It is well known that all these industries can contribute substantial quantities of heavy metals to the environment as smokes and dusts. Concern over the potential damage of this industrialization led to the setting up of several investigations stretching back as far as the early nineteenth century. Reported damage to crops in the Swansea and Neath region reached its climax between 1914 and 1918 when an increase in the metal smelting resulted in great damage to both horticultural and veterinary products. In the early 1920's analytical findings showed excesses of copper, cadmium and zinc in crops and animals. The long term effects of this damage to the populations of plants are expressed as an absence of indigenous trees such as birch and

oak, and of some species of grasses (Welsh Office 1975).

An investigation of the heavy metal burdens of soils and plants in the lower Swansea and Neath valleys showed that levels of Cd, Cu, Ni and Pb were far higher than relatively non-industrial areas to the west. There was a broadly similar geographical trend for all the metals with levels falling off with increasing distance from the source. However, it was also noted that the region affected by metals seemed to extend beyond the maximum distance sampled (at 32 km) (Goodman and Roberts 1971).

A survey of atmospheric metal levels was carried out over the whole of South Wales, using moss bags as deposition gauges, to determine which areas were currently most at risk (Goodman and Smith 1975). Distribution maps were then drawn up for each metal which showed the areas subject to background levels, elevated background levels and "hotspots" of these metals in summer and winter. It was found that all three types existed over the region and that the "hotspots" are not confined to the western side of the region, historically areas of non-ferrous heavy metal smelting and refining. This suggested that a number of point sources were responsible for these "hotspots", though there would be a degree of overlap between adjacent areas.

It has also been shown that there are elevated levels of Cd, Cu, Ni and Pb in oak leaves, grasses and soils in a valley in South Wales (Rhondda Fawr) and, in particular, within the urban development area (Burton and John 1977). The fact that there are such high levels of these heavy metals within such urban areas supports the view that the motor vehicle and the burning of coal, known to release heavy metals into the atmosphere, can be primary sources of heavy metals under

certain conditions.

A report on the possible causes of poor growth of sitka-spruce (Picea sitchensis) at Margam forest stated that the soils were amongst the most infertile in the principality and that this barreness was a relatively recent phenomenon (Jones 1970). This study tried to relate the distribution of individual hazards, such as sulphur dioxide levels, salt levels and temperature variations, to the extent of tree damage. However, heavy metals were not included in this study. No one individual factor seemed to be responsible for the poor growth in this forest and it was suggested that a combination of effects by more than one of the hazards was the most likely cause.

From these various studies it can be seen that there are elevated levels of heavy metals in the atmosphere, soils and vegetation of South Wales which have on occasion been responsible for damage to crops and animals.

Forestry plantations are now extensive throughout South Wales. In general, there is a mixture of species, though sitka-spruce (Picea sitchensis) is the most widely planted. Sitka-spruce has been widely planted in South Wales because it grows relatively well on damp sites, especially high land, and it stands exposure better than any other common conifer. Commercially the timber is a first class pulpwood and readily acceptable for many building jobs. It is faster growing than the norway spruce and a very large wood volume producer (Forestry practice 1980).

The growth of trees in several of the forests in South Wales is worse than can be accounted for by edaphic and climatic conditions. This poor growth is of great concern since substantial amounts of revenue are most certainly being lost as a result.

A study of the effects of pollution upon the structure and functioning of temperate forest ecosystems led to a broad classification of the nature of relationships between the two (Smith 1974). In areas receiving relatively small amounts of air pollution, the vegetation and soil of forest ecosystems presumably function as sinks for air contaminants (Class 1). When such ecosystems are exposed to high doses of pollution, the contaminants can induce acute morbidity or mortality of specific trees, often resulting in loss of canopy within the forest (Class 3). Exposure to an intermediate level (Class 2) may adversely and more subtly affect the ecosystems by nutrient stress, by reducing the rates of photosynthesis or reproduction, increasing the susceptibility to disease or insect outbreaks, and by microbial stress (Smith 1974).

The impact of a class 3 relationship upon a forest ecosystem is often a simplification in its structure and function. It may not only result in increased morbidity and mortality, but can also bring about basic changes in hydrology, and overall stability of the ecosystems which can result in soil erosion.

As there is a lack of indigenous tree species in the lower parts of the Swansea and Neath valleys, where once there was an abundance, it seems likely that a class 3 relationship has, in the past at least, been operating in this area.

The symptoms of intermediate levels of atmospheric and soil pollutants are, in general, fairly non-specific and are often difficult to relate to particular factors. Heavy metals have been shown to be the cause of such subtle effects around a brass foundry in Sweden. Here, increases in the soil levels of copper and zinc were related to depressions in the rates of decomposition of needle litter (Ruhling and

Tyler 1973), soil enzymatic activity (Tyler 1974) and nitrogen mineralization (Tyler 1975). Poor growth may result from a combination of such factors which would seem to be capable of having little effect if examined individually.

Even though the potential magnitude of the impact of these effects is much greater than acute damage, there has been little investigation of these situations. One reason for this may be the difficulty of correlating the overall damage to tree growth with a particular factor. Situations which have been studied are those in which the problems occur as a result of sulphur dioxide pollution or when photosynthesis has been prevented because of the presence of heavy metal particulates. There seem to have been few attempts at establishing what effects the heavy metals are having when present in the tissues and not just deposited on them. Also, in the soil, the effects upon root development and the associated microbial populations have not been related to the levels of heavy metals.

As there are large expanses of forestry in South Wales where there are also elevated levels of heavy metals in the vegetation and soil, it seems likely that a class 2 situation, as defined above, may have resulted. The aim of this study is to establish what effect these heavy metals may be having upon tree growth over South Wales.

To avoid confounding effects between different species, one species was chosen for this study: the conifer sitka-spruce (Picea sitchensis). This is the most important of the species now growing in South Wales and therefore a logical choice.

The area studied is shown in Figure 1.1. This map also shows the extent of the forestry plantations in South Wales and also includes one forest in Mid Wales which was examined as a potential control site,

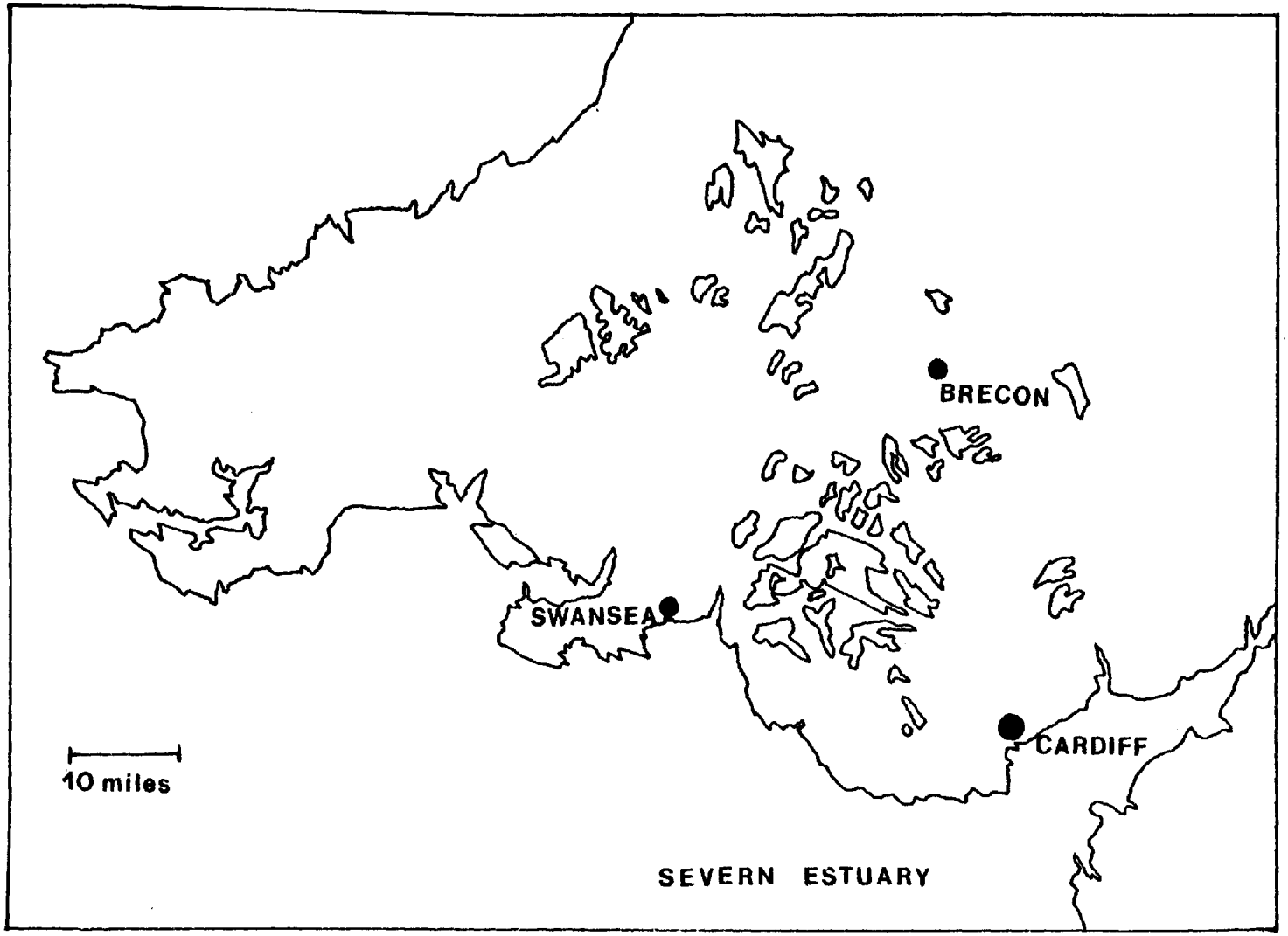


Figure 1.1 Map of South and Mid Wales showing the extent of forestry sites

since it is situated well away from large centres of industrialization and urbanization (Chapter 5).

The term "heavy metal" is extremely ambiguous, though commonly used, as here. It is difficult to define and most attempts to do so have usually found no general concurrence. It is derived from a categorization by density - which is certainly not a biologically significant variable. Most other terms have similar drawbacks in that they can be misconstrued to include metals other than those intended. Here it is proposed to study those non-ferrous metals known to be present at elevated levels in the region. This includes cadmium, copper, lead, mercury, nickel and zinc, though comparative references to others may also be made.

1.1 Literature review

In order to decide which approaches would best suit the aim of this project, a literature survey was undertaken to examine the methods used by other workers in this field. It immediately came to light that little work had been reported which examined the effects by heavy metals upon sitka-spruce, though some work had been carried out on the effects of heavy metals upon the various constituents of temperate forestry ecosystems. It was therefore necessary to look more widely at approaches adopted for studying the effect of heavy metals upon other trees and plants.

One method often used in studies of coniferous tree growth involves the assessment of the trees on site, which may then be related to the soil and foliar levels of certain elements. However, other studies have examined the effects of other factors. A review of these studies is given below.

1.1.1 Site studies

Essentially this is an empirical method since the tree growth parameters of one species are overall representations of the interactions between various climatic and edaphic factors. There are many different sites of action for the heavy metals: the soil being one example, where the availability of nutrients may be affected by alteration in the speciation. The results yielded by this method may indicate which factors have affected tree growth, even though they do not necessarily give any indication of how these effects are brought about. For example, in a study of the growth of sitka-spruce on basaltic soils in Northern Scotland (James et al 1978), the foliar levels of aluminium were found to be correlated with tree growth over 17 sites, even though the levels of soil extractable Al ($CaCl_2$) were found not to be correlated with growth. This study illustrates some of the problems with this type of approach, in that large number of sites were sampled, many soil samples (30) were taken at each site and many chemical analyses were carried out on each sample, and yet, of the soil variables, only an increasing availability of phosphate and numbers of mycorrhizae were associated with sites of better tree growth.

A study of Ponderosa and Jeffrey pines at the Sequioa and Los Padres forests in California showed that a disease induced by photochemical oxidants (mainly ozone) was associated with the onset of the smog season. It also demonstrated that within the more severely affected areas, 15-25% of the current needles showed visible symptoms of the disease (Williams 1980). This study illustrated that field observations can be very productive when the pollution is very severe in

certain forestry areas.

Several field studies have linked the presence of other atmospheric pollutants such as sulphur dioxide, and particulate material containing heavy metals emitted from point sources such as smelters, with effects upon forestry ecosystems. Accumulation of metals in the soil and forest humus layers has also taken place and has been shown to have harmed vegetative growth within 15 km of smelters (Costescu and Hutchinson 1972, Freedman and Hutchinson 1980, Hutchinson and Whitby 1974, Whitby and Hutchinson 1974). However, such effects have, in general, only been demonstrated in areas of extremely high contamination of the class 3 type (Smith 1974).

Interactive effects between the components of air pollution have been demonstrated to increase toxicity. Ozone and sulphur dioxide had additive injurious effects upon Ponderosa pine needles (Evans and Miller 1975). It has also been shown that sulphur dioxide caused a decrease in the soil pH, thereby increasing the solubility of cadmium, copper and cobalt (Hutchinson 1981). Such interactions may have serious consequences for the functioning of the ecosystems.

Many studies of other components of temperate forestry ecosystems apart from the trees themselves have been carried out. Effects upon the mycorrhizae, which are associations of fungi with the roots of higher plants, vital for the uptake of nutrients in many situations, and other microorganisms involved in nutrient cycles, have been demonstrated. However, they have not been conclusively linked with poor tree growth. It has been shown, for example, that there were fewer mycorrhizae associated with the roots of willow and poplar trees growing on copper mine tailings than associated with the roots of tree growing in other areas (Harris and Jurgensen 1977). A reduction in the mineralization of

soil nitrogen has also been linked to a build up of copper and zinc in the soils of a forestry ecosystem around a brass foundry in Sweden (Tyler 1975). The variety of these effects has demonstrated the need for field assessment as part of a comprehensive study.

Other approaches to the problem of assessing the effects of heavy metal pollution have been of a more experimental nature, using controlled environments, where effects upon seedlings grown in water culture and soil experiments have been examined. In addition to looking at how trees may be directly affected, experiments which have examined the effects of heavy metal pollution upon the various biotic and abiotic components of forestry ecosystem have been widely reported.

1.1.2 Soil experiments with heavy metals

Several studies have looked at how nitrogen and carbon mineralization may be affected by heavy metals in the soil. In general the approach adopted to examine nitrogen mineralization has been to sample a soil or litter layer, add solutions of a heavy metal salt at various concentrations, incubate, then measure the concentrations of inorganic and organic nitrogen forms (Bhuiya and Cornfield 1974, Giashuddin and Cornfield 1978, Liang and Tabatabai 1978, Spalding 1979). Usually these studies have confirmed the results of field studies which demonstrated the actions of heavy metals upon mineralization, litter respiration etc (Tyler 1974, Tyler 1975, Ruhling and Tyler 1973). However, some workers also showed that the heavy metals inhibited the microorganism populations, rather than acting directly as enzyme inhibitors.

Few studies have examined the effects by heavy metals upon tree

species grown in soil, though there are many reports of studies involving cereal and vegetable crops. In one study, however, uncontaminated microcosms containing individual oak tree seedlings (Quercus) and the soil associated with the seedling roots were lifted from the ground and removed to the laboratory. These were then treated with litter from a contaminated forest and additional dust obtained from a Pb smelter. This was performed without greatly disturbing the microcosm. Various experiments were then carried out which demonstrated the effects of the contamination upon the microcosm. These included the transport and distribution of the metals amongst the ecosystem components (Jackson et al 1978a), alterations in nutrient cycles (Jackson et al 1978b), and effects upon microbial populations (Ausmus et al 1978). However, these were not shown to have deleterious effects upon tree growth.

The above experiments examined the integrated effects of several heavy metals which were present in the contaminated litter and smelter dust. It could not, however, be shown which of the metals, and therefore the important components, were responsible for these disruptions in the microcosms. However, some studies reported in the literature have demonstrated the effects of the addition of particular heavy metals to the soil.

In an investigation of the uptake of five levels of lead (0-600 mg / kg) into eight tree species from three soil types (Rolfe 1973), it was demonstrated that lead levels in all treatment combinations were enhanced in the leaves, stems and roots of each of the eight species; higher uptake being associated with higher soil Pb levels. Two year old seedlings of each species were used. Lead uptake by the plants was reduced by approximately half when high levels of soil P (phosphate)

were present. This is interesting since it indicates a potential ameliorating effect of P for situations of Pb toxicity.

The individual and combined effects of lead and cadmium have been examined with respect to the growth of two year American sycamore seedlings (Plantanus occidentalis) (Carlson and Bazzaz 1977). Synergistic effects between Pb and Cd were demonstrated to disturb photosynthesis and reduce growth.

As stated above few studies have examined the effects of heavy metals upon trees grown in soil, though there have been many studies of effects upon other plant species. In general, these experiments were similar to those that involved tree species in that growth parameters and physiological effects within the plants were examined. However, soil is not the only medium which may be used.

1.1.3 Water and sand culture

Water and sand culture techniques have often proved of great value in determining the symptoms associated with the deficiency and toxicity of many elements. They have several advantages over soil culture techniques. Most importantly they tend to afford a greater control over growing conditions. They are also more versatile in that they may be used to examine effects which could not otherwise be studied. For example, the effects of complex species upon metal uptake may not be studied in soil since it is difficult to determine the concentrations of these complexes in the narrow zones of the root-soil interfaces. However, using water culture techniques, it is possible to measure concentrations of complex species far more easily.

No studies of the toxic effects of heavy metals upon spruce or

other tree species in water or sand culture have been reported in the literature, though the effects of deficiencies of nutrients within the solution, and optimizations of the nutrient solution, have been studied (Leyton 1952, Ingestad 1959). The exudations from various coniferous seedlings have also been collected and analysed using an aseptic water culture technique (Smith 1969). However, the techniques used with other plants have been more diverse.

One approach has been to examine the mechanisms of tolerance to heavy metals in various species which grow naturally in areas of high soil metal levels. Also the tolerance mechanisms within certain strains of species, including grasses, which have adapted to reclaimed spoil tips and other such areas, have been studied. In general, these have taken the form of physiological and biochemical investigations of the mechanisms (Antonovics et al 1971, Jones and Wilkins 1971, Mathys 1977, Wainwright and Woolhouse 1975). Other physiological and biochemical studies of heavy metals have examined the toxic effects of heavy metals at subcellular levels in the plant, thereby gaining more precise information on the exact mechanisms and sites of action (Bittell et al 1974, Miller 1973).

Water and sand culture techniques have been used to characterize the uptake of heavy metals into the roots (Cutler and Rains 1974, Dupont et al 1980). Some studies have been carried out to find out if, after adsorption by the roots, the heavy metals could accumulate in other parts of the plant including the edible vegetative and flowering structures or seeds (Jarvis et al 1976).

Many studies have been carried out to determine whether the uptake of a particular heavy metal was passive or active (Harrison et al 1979). Other studies have looked for factors which affect the uptake of these

toxic metals so that it might be controlled in some way (Iwai et al 1974).

Speciation of the heavy metals in the soil solution undoubtedly plays a vital role in determining the extent to which a metal will be taken up into the roots. Water culture has proved an ideal medium with which to gain such knowledge, since effects brought about in nutrient solutions may be characterized far more easily than in soils. In one such study, the complexation of copper by several different ligands demonstrated how structure and charge influenced the adsorption of the metal by excised barley roots (Coombes et al 1978).

Many studies of the toxic effects of heavy metals in water and sand culture have measured various growth parameters and related these to particular solution concentrations of the metals which may vary with with different conditions. However, Beckett and Davis (1977) developed a method which they then used to determine the upper critical tissue concentrations of several heavy metals in the shoots of barley, rape, lettuce and wheat (Davis and Beckett 1978): upper critical tissue concentrations being the lowest shoot concentrations at which the shoot yields of the plants are reduced by the toxic effects of the heavy metals. The main advantages of such a quantification seemed to be that these tissue concentrations were consistent over different growing conditions. This technique has also been applied to determine the interactive effects of heavy metals upon yield and uptake of other heavy metals (Beckett and Davis 1978, Davis and Carlton-Smith 1980).

Studies of the interactive effects between heavy metals have usually, though not always, been carried out in water or sand culture. The influence of one metal upon the uptake of others has often been the

subject of such studies. In such cases it is usual for this metal to be of interest because of its toxic effects (John 1978, Root et al 1978). However, there have also been studies where several toxic heavy metals were added to the nutrient solution so that the nature of the combined effects of the metals could be studied and understood (Beckett and Davis 1978, Coughtrey and Martin 1979).

1.2 Design of the investigation

Essentially this study was broken down into three related, though distinct, sections dealing with different aspects of the effects of heavy metals upon tree growth. These were the field studies at the forestry sites, and experimental work in water culture and soil.

It was decided that the project should not consist entirely of exhaustive sampling and assessment of the forestry sites, but rather a characterization of the sites so that sufficient information about the forestry sites may be gained. This could have been extended to cover more sites if required. The work involving seedlings grown using water and soil culture techniques was necessary to obtain qualitative and quantitative information on the toxic effects of heavy metals which could then be related to the field situations.

The assessment of the South Wales forestry sites consisted of obtaining measurements of tree growth parameters and of sampling the foliage and soil to determine the concentrations of certain heavy metals and nutrients.

The laboratory experiments were divided into the experiments conducted in water culture, and those using soil as a growth medium. The water culture work was further divisible into three subsections. In the first, the aims were to develop the techniques for growing sitka-spruce

seedlings in solutions doped with heavy metal salts, to gain information on the typical symptoms of toxicity due to particular heavy metals, to yield quantitative information on the upper critical tissue concentrations of the heavy metals, and to find out if and how heavy metals can alter the uptake and translocation of nutrients.

The second section of water culture work in the water culture system examined the interactive effects of heavy metals. Here a factorial experimental design was utilized to examine the consequences of interactions between the metals. The third section looked at the speciation of the heavy metals in the nutrient solutions and how this could affect the uptake and translocation of the element. Here a computer programme was utilized to predict the equilibrium speciation within the nutrient solutions. It must however be remembered that, as the plants grow and uptake of nutrients and metals occur, the speciation of the solution may change. Also certain substances, such as amino acids, fatty acids, carbohydrates and others, may be exuded by the plant roots. There may therefore be certain kinetic effects by these substances upon the speciation of the solutions according to the rates at which they are exuded.

The section of experimental work using seedlings grown in soil was necessary to simulate field conditions more closely. Here the aim was to collect information not only on the direct toxic effects of the metals upon growth, but also on the more indirect effects, such as those upon soil microorganisms, which cannot be studied in water culture.

Chapter 2

WATER CULTURE EXPERIMENTS WITH INDIVIDUAL ELEMENTS

2.1 Introduction

2.1.1 Historical

A number of laboratory experiments have been carried out using sitka-spruce (Picea sitchensis) seedlings to establish nutrient requirements under controlled conditions (Leyton 1958, White and Leaf 1958). Until Ingestad (1959) few workers used reliable conditions, as often no attention was paid to possible secondary effects such as change of pH, presence of fertilizers, altered solubility of nutrients and ion antagonism. Often the purpose of investigation into nutritional conditions was to determine how a nutrient deficiency was to be diagnosed. Only in the last decade or so has interest been aroused regarding the effect of enhanced levels of metals, both essential and non-essential, upon normal plant growth.

The study of ion deficiency in plants was the main interest and generally two different experimental approaches, using either ocular estimation of deficiency symptoms (Lundblah 1955, Wallace 1951) or foliar analysis (Goodall and Gregory 1947, Lundegarth 1951), were adopted. Disease symptoms associated with a large number of elemental deficiencies in spruce have been described (Becker-Dillinger 1940, Nemec 1938), yet in most cases the symptoms are non-specific secondary effects. Similarly the manifestations of metal toxicity in plants are relatively non-specific, providing little indication as to the

fundamental nature of the problem (Woolhouse 1980).

These manifestations are many and varied, including leaf chloroses, withering of shoots, abortion of flowers and general depression of growth. Therefore metal ion toxicity in plants may not be diagnosed by a visual examination of the tissues alone. For this reason chemical analysis of foliar and other tissues is necessary to determine the concentrations at which growth is affected. A suitable regime of experiments must be developed whereby both the individual and interactive effects of the metal ions may be examined.

To this end a suitable nutrient solution was selected for use in water culture experiments where sitka-spruce seedlings were grown in solutions supplemented with heavy metals.

The development of this nutrient solution for the growth of spruce seedlings was based upon several important considerations, which included optimization of solution pH and the form of nitrogen supplied. The effect of pH upon the rooting medium has been studied by several workers. Nemec (1938) found an optimum pH of about 5 for sitka-spruce in an experiment with liming of a nursery soil. Leyton (1952), in a water culture experiment, showed that the optimum pH ranged between 4 and 5 using both ammonium and nitrate ions as the nitrogen source. However there are some indications that if sufficient and well balanced nutrient solutions are supplied, the growth of plants may be unaffected over a wide pH range (Olsen 1958).

By using growth and elemental contents of needles as indices of the health of a plant, it was possible to develop an optimum nutrient solution for norway-spruce seedlings (Picea abies, Ingestad 1959). This nutrient solution has been used by the Forestry Commission as a standard nutrient solution for growing sitka-spruce seedlings and was used for

these experiments in water culture.

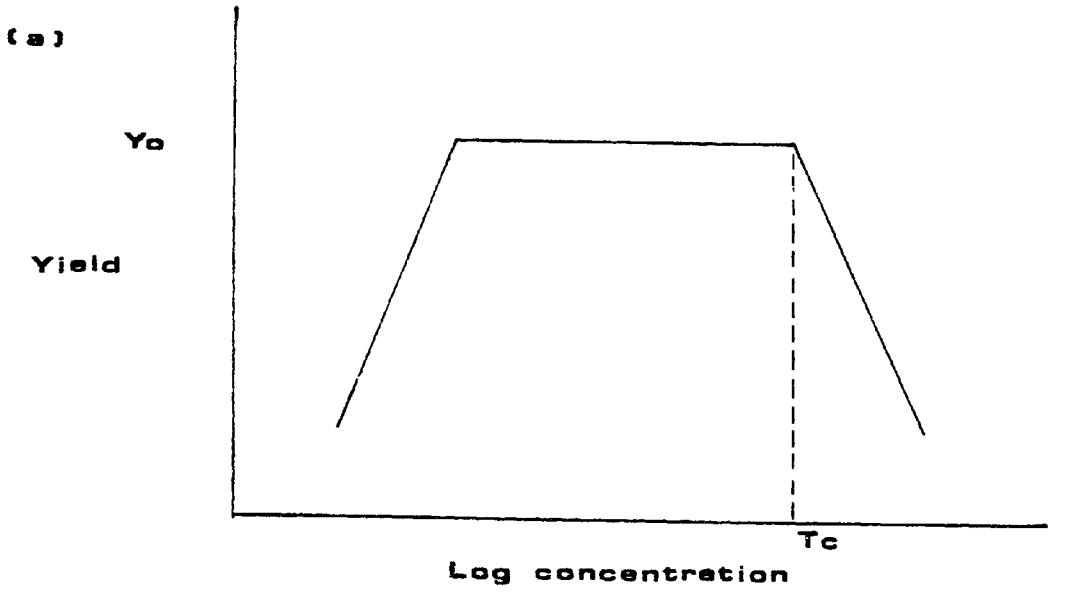
2.1.2 Diagnostic criteria for metal ion toxicity

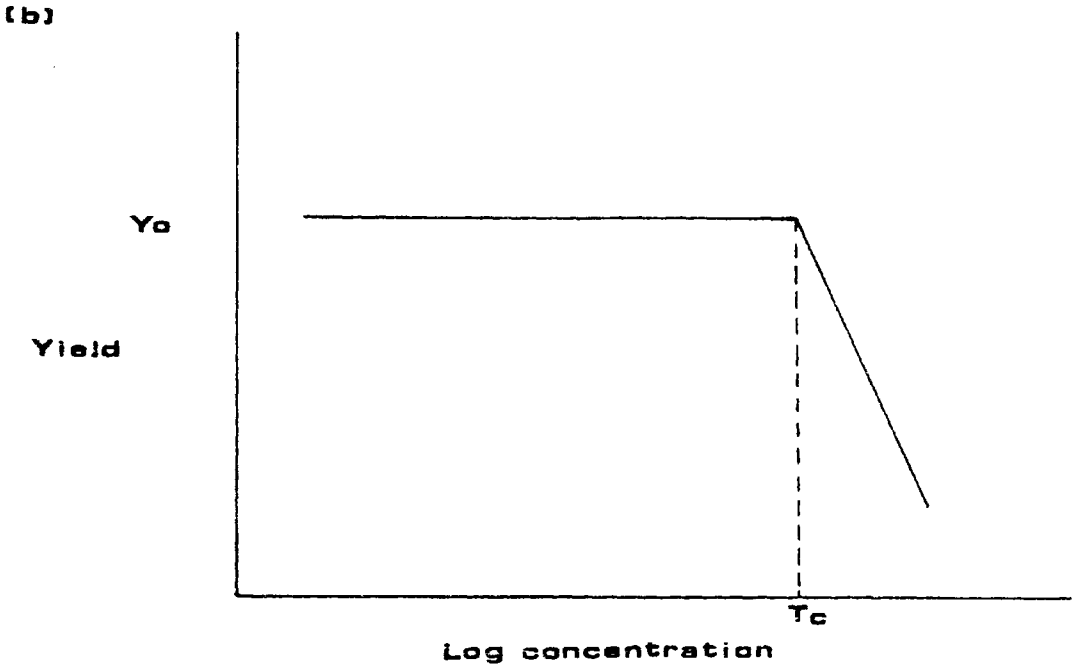
On the basis of the general principles discussed above, some initial experiments were carried out which examined the types of effects that heavy metals have upon sitka-spruce. The aim of these experiments was to establish the solution concentrations of particular heavy metals at which growth was affected over various periods of time.

Two parameters were examined initially with regard to growth; (1) yield as dry weight, (2) lengths of shoots and roots. These are, however, poor indices of toxicity since the growth of the plants which are unaffected by the heavy metals is defined by many other factors, such as the levels of nutrients and other ions, length of exposure time to metal, light and water availability. Another index which can be used is the concentration of heavy metal in solution or soil solution, but the effects of such concentrations will again depend upon these other growth defining factors. However, methods which measure total metal concentration, although subject to some sources of error, such as the inclusion of inactive species, are more specific than the methods which measure plant growth parameters alone and are, as such, likely to prove of greater value.

The relationship between the yield, in terms of dry matter of a plant, and the concentration of essential elements, either in the solution surrounding the roots or present in the plant tissue, has been described. Figure 2.1a shows such a relationship; below a certain concentration, known as the lower critical concentration, a deficiency of that element leads to a reduction in the yield of the plant. In this

Figure 2.1 Diagrammatic representation of a yield curve for (a) an essential element, (b) a non-essential element: Yo is the yield unaffected by toxicity, Tc is the upper critical concentration





case, the absence of that particular element limits the growth of the plant. Both essential and non-essential elements exhibit upper critical concentrations as shown in Figures 2.1a and 2.1b respectively. The concentration of the particular element is then at a toxic level which reduces the yield of the plant. Both types of element exhibit yield plateaux, where changes in concentration of the element do not affect the yield. The more toxic the element, the shorter the yield plateau appears.

In the study of particular methods for assessing toxicity it has been shown that the effect upon yield of a potentially harmful element depends on its concentration in the plant tissue (Beckett and Davis 1977). It was shown that the tissue concentrations of individual elements, where yield was affected as a result of the toxic concentration of the element, was relatively independent of other factors. This was established for several metals over a wide range of conditions in several plants, namely barley, lettuce, oats and rape (Beckett and Davis 1977, Davis and Beckett 1978) and a simple scheme based upon shoot analysis was devised to assess the harmful effects of accumulation of these elements in the soil.

A statistical treatment was necessary to determine the position of the split-point, that being the point below which yield is unaffected and above which it is reduced due to toxic effects. A purely (non-statistical) subjective estimation of the split-point was sometimes difficult to apply: two lines could often be drawn by eye, but in some cases the range of treatments nearly missed the yield plateau. This would tend to confuse and render any subjective estimation open to error. Therefore a statistical method was devised which made the process automatic and objective (Beckett and Davis 1977).

This statistical method has been used here to determine the upper critical concentrations of several heavy metals in sitka-spruce seedlings.

2.1.3 Experiments carried out with individual elements

The aim of these experiments was to develop quantitative techniques for the study of the effects of heavy metals and then to apply these techniques to the study of individual elements. A list of the experiments with a short introduction to each is given below.

2.1.3.1 Preliminary investigation Copper is an essential element for plants (Somner 1931), but it is also known to be toxic under certain conditions, where it generally manifests itself as a chlorosis and stunting of growth (Foy et al 1978). On the other hand, it has not been proven beyond doubt that nickel is essential for the growth of plants, though recent studies have shown it to be necessary for the functioning of certain enzyme systems (Bartha and Ordal 1965, Bertrand 1974, Dixon et al 1975). There are, however, a number of reports of nickel phytotoxicity (Brenchley 1938, Cotton 1913, Wolff 1913).

A study of the effects produced by mercury are of interest because it is an extremely toxic element at low concentrations, especially when converted to to certain organic forms.

The aim of this experiment was to establish the methods necessary to grow the sitka-spruce seedlings in solutions supplemented with heavy metals. It was also hoped that the lengths of time necessary for the effects to manifest themselves could be determined so that future experiments could be planned in a better manner.

2.1.3.2 Upper critical tissue concentrations Upper critical tissue concentrations are the concentrations in the tissues below which growth is unaffected by accumulation of the element and above which toxic effects lead to a reduction in growth. It has been demonstrated that critical tissue concentrations are relatively independent of the length of time that plants are grown, the concentration of nutrients and the availability of light and water. Thus it is a suitable index of the toxicity of the heavy metals and may be applied to sitka-spruce seedlings grown in nutrient solution.

One possible application of this information might be as an index of the risk that heavy metals pose to normal functioning and subsequent growth of the trees. If the concentrations of heavy metals in foliar tissues were determined at several sites and compared to these values it might then be possible to assess which sites were affected by the heavy metals.

The aim of this investigation was to determine the upper critical concentrations of the metals cadmium, copper, lead, nickel and zinc in the shoot tissues of the sitka-spruce seedlings.

2.1.3.3 Yields, cadmium concentrations and time This experiment was carried out with the aim of relating the length of time that the plants were grown to the uptake of cadmium and its subsequent effect upon yield.

2.1.3.4 Supplementary critical concentration experiment The application of upper critical concentrations to the problem of heavy metal concentrations is dependent upon the range of conditions over which these values hold true. This experiment was designed to define this range more fully. It was carried out with older seedlings for a longer

experimental period in solutions doped with cadmium. It was also performed with the aim of establishing the effect of cadmium on the uptake of certain nutrients from the solution.

2.2 Materials and methods

Water culture techniques were used to grow sitka-spruce seedlings in solutions doped with heavy metals. Sitka-spruce seeds were obtained from the Forestry Commission research station at Alice Holt Lodge, Surrey. These seeds were of Queen Charlotte Island origin and provenance. The seed lot was blown to remove empty and light seed in order to increase seed uniformity. The seeds were then set aside for storage. When required, a batch of seeds (usually 30 grammes) was chilled at 4°C for a few weeks. This had the effect of increasing the uniformity of the germination (Wakeman 1981). The seeds were germinated over 10-14 days in trays of commercial peat. These were covered lightly with acid-washed silver sand to prevent the growth of a fungus, known as damping off, which often attacks young seedlings.

2.2.1 Preliminary investigation

In this investigation the seeds were germinated at room temperature. After germination, when the seedlings were beginning to lose their seed cases, they were carefully extracted from the peat using a pencil or seeker to loosen the tray contents. Sixteen seedlings were inserted through small holes in cardboard pieces cut to fit on top of 250 ml glass beakers. The sides of the beakers were covered with black polythene to prevent algal growth in the nutrient solution. Quarter strength nutrient solution (whose full strength composition is shown in

Table 2.1) was poured into each beaker to within one centimetre of the brim. Each card was then placed onto a beaker in such a position that all the roots were covered with solution. The beakers were positioned on a bench approximately one metre below two fluorescent day-light tubes, which supplied light for 16 hours a day. The day to night ratio was controlled by means of a suitable time switch. A diagrammatic representation of a beaker and its seedlings is shown in Figure 2.2.

The solution composition was changed 5 days later to half strength, then to full strength after another five days. The nutrient solution was recommended by the Forestry Commission for use with sitka-spruce seedlings as one similar to Ingestads (1959) and had a pH of 6.5. At 31 days the heavy metals (Cu, Ni and Hg) were added as chloride salts to the solutions; all chemicals used were of AnalaR grade. Each metal was added separately at concentrations of 0.1, 0.5, 1, 5 and 10 mg/l; two replicates were used per concentration. The beakers were randomised with regard to their position on the bench. The seedlings were grown for another 42 days; solutions being changed weekly to avoid depletion of vital nutrients and oxygen.

At the end of the experimental period the plants were carefully dissected into shoots and roots and their lengths measured. All the shoots and roots from each replicate were then placed into separate weighed beakers, dried at 105°C overnight, left to cool in a desiccator, and then reweighed to determine their yields as dry matter. The average and standard deviation of the lengths were calculated for each replicate.

2.2.2 Upper critical tissue concentrations

The method used to grow the seedlings was the same as in the

Figure 2.2 Diagrammatic representation of a healthy sitka-spruce seedling growing in nutrient solution

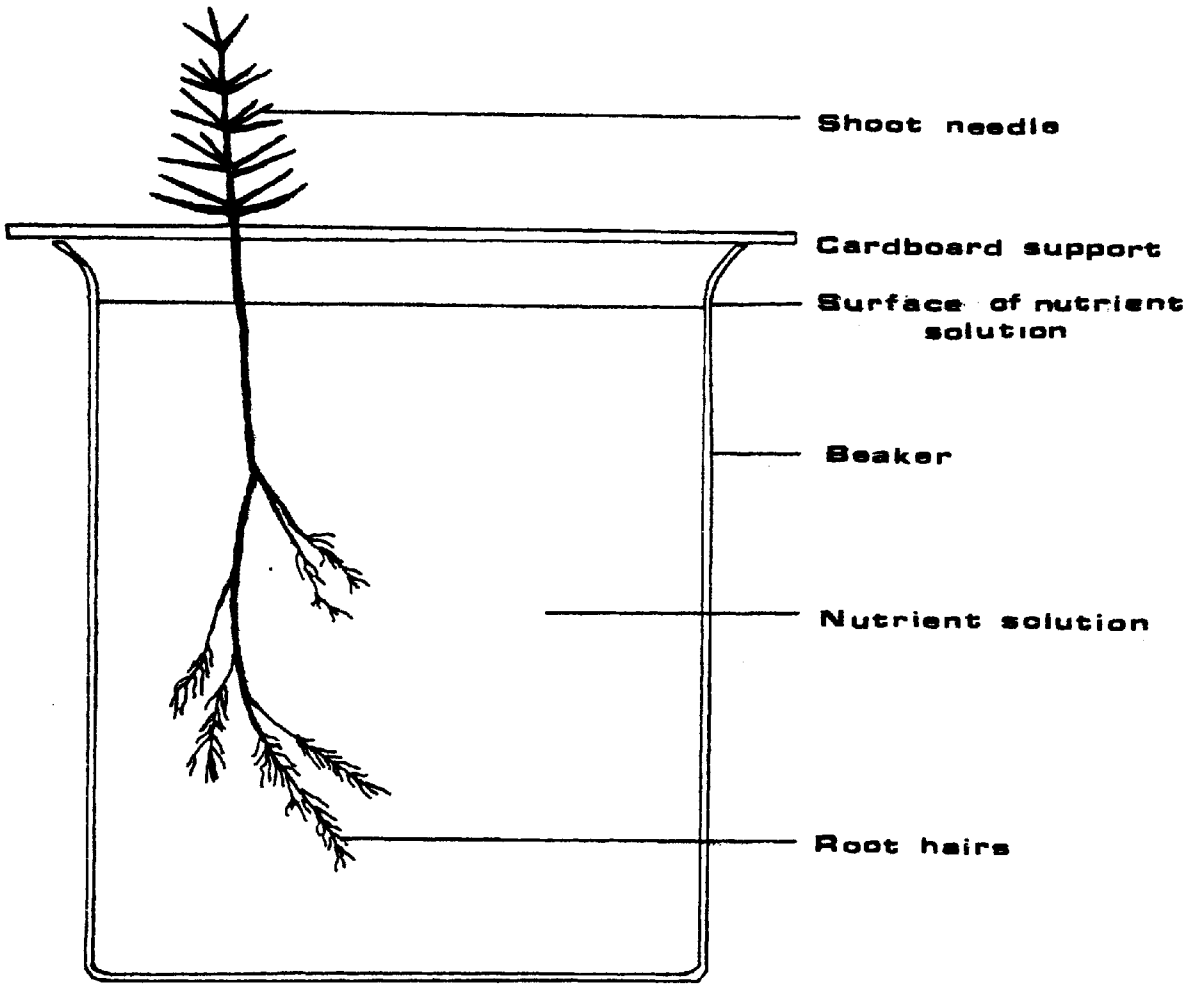


Table 2.1

Composition of nutrient solution used for preliminary water culture experiment: concentration of each element in full strength nutrient solution and stock solution

Stock solution		Full strength elemental composition	
Compound	Concentration (g/l)	Element	Concentration (mg/l)
NH_4NO_3	28.57	N	100
$NaH_2PO_4 \cdot 2H_2O$	5.03	P	10
KCl	9.50	K	50
$CaCl_2 \cdot 6H_2O$	5.48	Ca	10
$MgSO_4 \cdot 7H_2O$	8.62	Mg	8.5
$FeCl_3$	0.198	Fe	0.7
$MnSO_4 \cdot 4H_2O$	0.162	Mn	0.4
$CuSO_4 \cdot 5H_2O$	0.0118	Cu	0.03
$ZnSO_4 \cdot 7H_2O$	0.0132	Zn	0.03
H_3BO_3	0.114	B	0.2
$NaMoO_4$	0.0018	Mo	0.007

previous experiments but with several improvements in the growing conditions. The seeds were obtained in the same manner and germinated in an electrically heated propagator at 25°C. The propagator controlled the air and ground temperatures so that they did not fall below the specified value. Seedlings were carefully extracted from the peat and 20 were placed into polythene supports which fitted neatly into the recesses at the top of polythene beakers. These provided stable support for the seedlings just above the level of the nutrient solutions. The air temperature was adjusted to 20°C for the experimental period.

The nutrient solution was Ingestad's nutrient solution (Ingestad 1959), as shown in Table 2.2; its pH was 4.5. The metals were added individually to the nutrient solutions as chloride salts; their concentrations are shown in Table 2.3. Where copper and zinc were added they were omitted from the basic nutrient solutions. Three experiments were carried out for each of the heavy metals except nickel which was carried out twice.

The plants were grown for at least 42 days after addition of the metals to the solution, solutions being changed every 5 days to avoid depletion of nutrients and oxygen. At harvest they were taken out of solution and their roots washed with deionized water. The largest and smallest plants were discarded to eliminate any gross anomalies in the results. The plants were then separated into shoots and roots and the respective yields determined by drying overnight at 105 degrees centigrade in weighed beakers, reweighing the beakers after cooling in a desiccator. The average yield of shoots and roots was determined for each concentration by dividing the weight in the beaker by the number of plants.

The plants were acid digested with a 3:1 mixture of nitric and

Table 2.2

Composition of Ingestad's nutrient solution used for water culture experiments with sitka-spruce seedlings: concentration of each element in full strength and concentration of compounds used in stock solution

Stock solution		Full strength elemental composition	
Compound	Concentration (g/l)	Element	Concentration (mg/l)
NH_4NO_3	14.3	N	50
$KH_2PO_4.2H_2O$	4.4	P	10
KCl	7.13	K	50
$CaCl_2.6H_2O$	21.9	Ca	40
$MgSO_4.7H_2O$	15.4	Mg	15
$FeCl_3.6H_2O$	0.5	S	20
$MnCl_2.4H_2O$	0.06	Fe	0.93
H_3BO_3	0.1	Mn	0.17
$CuCl_2.2H_2O$	0.005	B	0.17
$ZnCl_2.2H_2O$	0.004	Zn	0.02
$Na_2MoO_4.2H_2O$	0.0007	Cu	0.02
		Mo	0.003

Table 2.3

Concentrations of metals added to the nutrient solutions for determination of upper critical tissue concentrations

Experiment	Concentration (mg/l)
Cd 1, 2, 3	0, 0.025, 0.05, 0.1, 0.25, 0.5, 0.75, 1, 2, 5, 10
Ni 1, 2	0, 0.01, 0.025, 0.05, 0.075, 0.1, 0.25, 0.5, 0.75, 1, 2.5, 5, 7.5, 10
Pb 1, 2	0.01, 0.025, 0.05, 0.075, 0.1, 0.15, 0.4, 0.5, 0.75, 1, 2.5
Cu 1, 2, 3	0.02, 0.05, 0.075, 0.1, 0.25, 0.5, 0.75, 1, 2.5, 5, 7.5, 10
Zn 1, 2, 3	0.02, 0.1, 0.25, 0.5, 0.75, 1, 1.5, 2, 5, 10, 25, 100

perchloric acids, the beakers being heated to complete the dissolution of the plant material. After cooling, the digestates were filtered through Whatman No 42 paper and diluted to 10 or 25 ml volume. The concentrations were determined by flame atomic absorption spectrophotometry using appropriate standards and blanks at the instrumental conditions shown in Table 2.4. The spectrophotometer was a Varian model 1100.

2.2.3 Yields, cadmium concentrations and time

The seeds were germinated as in the previous experiment but 15 seedlings were transferred to supports on beakers of one litre capacity. Thirty days after sowing, cadmium (chloride salt) was added to each solution at concentrations of 0, 0.05, 0.5 and 5 mg/l. During the course of the experiment duplicate beakers were harvested at 13, 28, 42, 75 and 100 days. The yields of the shoots and roots were determined by weighing the dry tissue as above and their respective concentrations and contents of cadmium determined by flame atomic absorption (Table 2.4).

2.2.4 Supplementary critical tissue concentration experiment

The method used here to determine the upper critical tissue concentration for cadmium was similar to that of the previous experiments on critical concentrations except that the cadmium was added to the full strength nutrient solution after 60 days instead of 31 days. The levels of cadmium added to the nutrient solutions were 0, 0.05, 0.5, 1 and 10 mg/l; each concentration being replicated three times. The plants were grown for a further 80 days, so that they were 140 days old when harvested. The plants were divided into shoots and roots, dried, weighed

Table 2.4

Analytical data for elements determined by flame atomic absorption spectrophotometry

Element	Wavelength (nm)	Spectral band width (nm)	Optimum working range (mg/l)	Sensitivity (mg/l)	Detection limit (mg/l)
Cadmium	228.8	0.5	0.5-2	0.011	0.006
Copper	324.7	0.2	2-8	0.004	0.003
Lead	217.0	1	5-20	0.11	0.02
Magnesium	202.5	1	5-20		
Manganese	279.5	0.2	1-4	0.024	0.003
Nickel	232.0	0.2	3-12	0.066	0.008
Potassium	404.4	0.5	200-800		
Zinc	213.9	0.2	0.4-1.6	0.009	0.02

All elements were determined using using a gas mixture of air and acetylene. The sensitivity is defined as that concentration of an element in aqueous solution which absorbs 1% of the incident radiation intensity passing through a cloud of atoms being determined. The detection limit is defined as the concentration in solution of an element which can be detected with a 95% certainty, that is, the quantity which gives a reading equal to 1.64 times the standard deviation of a series of at least 10 determinations at near blank levels.

and digested as previously, then analysed for cadmium, copper, zinc, manganese and potassium by flame atomic absorption at the instrumental conditions shown in Table 2.4.

2.3 Results

The results were divided into observations made during the course of the experiments and experimental data based upon the parameters measured. From observations made throughout the experiments it can be seen that there was no mycorrhizal infection of the roots. This concurs with the findings of Ingestad (1959) who stated that this was probably related to the method of growing the seedlings in solutions with higher nutrient concentrations than are normally present when mycorrhizal associations are prevalent.

2.3.1 Preliminary investigation

The results of this investigation have been divided into observations of the plants made during and at the end of the experiment and those results based on the parameters measured.

2.3.1.1 Observations At the two lowest concentrations of copper that were added to the solutions the shoots possessed needles that were dark green in colour with no signs of chlorosis. The main roots were very long, with an abundance of secondary roots and root hairs. At one mg/l copper some differences were noted: as well as being slightly smaller, the needles were yellowish green in colour. The roots were also smaller and darker in colour, with few secondary roots or root hairs. At 5 mg /1 copper, very few of the shoots possessed the normal green needles found in the lower concentrations and many plants were moribund. At 10

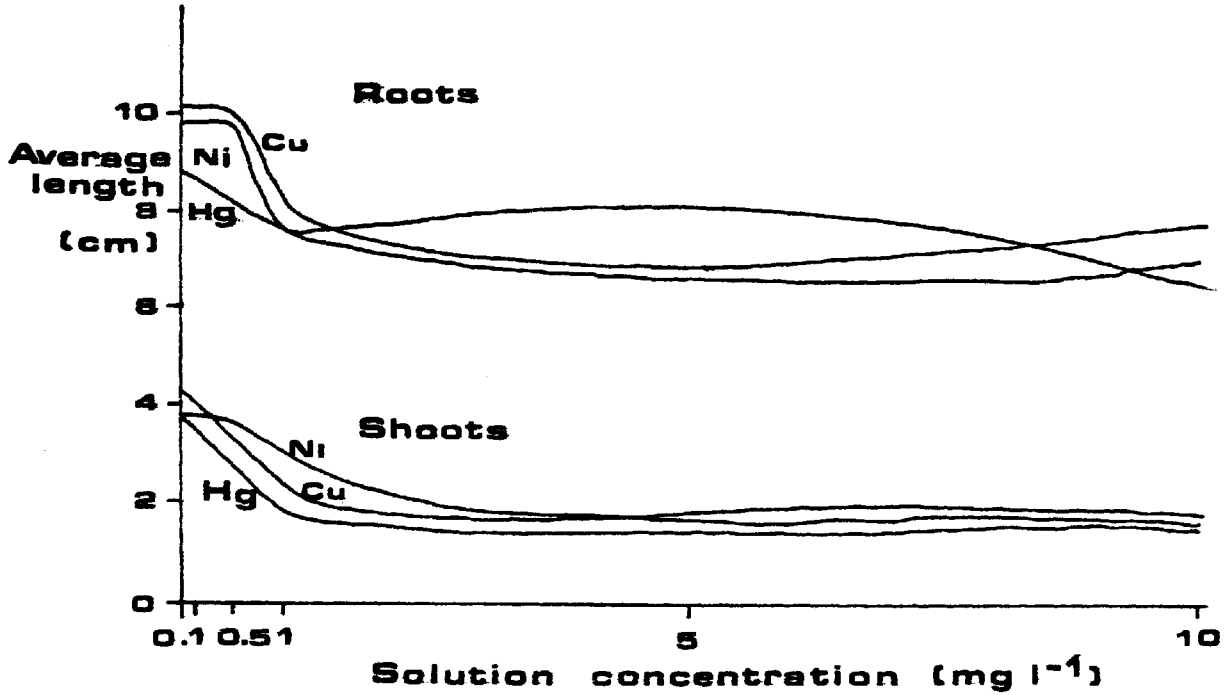
mg/l copper, all plants appeared moribund and did not seem to have grown during the experimental period. The roots again had few or no secondary roots or root hairs.

At nickel concentrations of 0.1 and 0.5 mg/l the plants appeared healthy without chlorosis in the shoots and the roots were long, branched and covered with root hairs. At one mg/l nickel several plants were apparently dead. All the roots possessed fewer secondary roots than at lower nickel concentrations. Where the solution concentration was five mg/l nickel, almost all the plants were moribund and the roots were dark and unbranched. It was interesting to note that, though nickel suppressed growth when one mg/ l nickel was added, the plants were not chlorotic.

At mercury concentrations of 0.1 and 0.5 mg/ l , though the shoots and roots were small in comparison to the plants grown at the lowest concentrations of the other metals, there were no other obvious effects. At one mg/ l mercury, the plants were smaller than at 0.5 mg / 1, possessing thin roots which branched very little. At 5 and 10 mg / l mercury the plants were progressively smaller but retained their green colour. Mercury did not seem to kill the plants at the concentrations added over the experimental period but did almost completely suppress growth.

2.3.1.2 Plant growth measurements Two of the parameters measured as indicators of metal toxicity were shoot and root length. The means of the lengths of shoots and roots were plotted against solution concentration on a linear scale and are presented in Figure 2.3. The graph shows clear differences between the metals in terms of the concentrations at which effects were produced, mercury being the most

Figure 2.3 Graph of average shoot and root lengths in preliminary experiment as functions of the solution concentrations of Cu, Ni and Hg



toxic.

The average of the replicate dry weights of the shoots and roots for each concentration were calculated and the results, plotted against solution concentration (mg/ l), are presented in Figure 2.4.

Shoot weights were not reduced at solution concentrations of copper below five mg/ l, though average root weights were slightly reduced at concentrations of one mg/ l copper. The graph of shoot and root lengths shows that one mg/ l copper was sufficient to reduce the lengths of both shoots and roots. This concurred with the observations of chlorosis at this level of copper.

Nickel seems to be at least as toxic as copper. The concentrations at which effects were first produced were similar over this time scale, though none of the effects at these levels were associated with chlorosis.

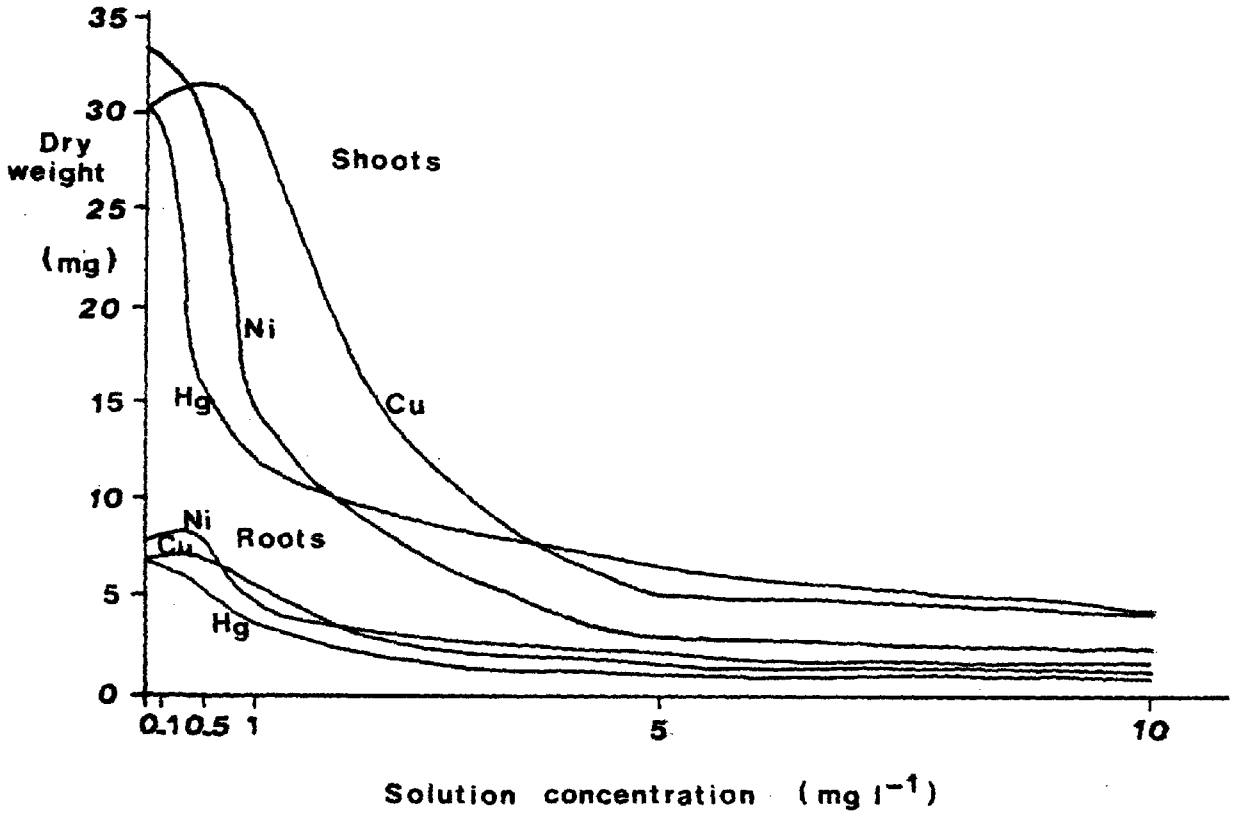
Though mercury did not kill the plants or produce symptoms of chlorosis, it can be seen that even at the lowest concentrations it reduced shoot and root lengths and dry weights.

2.3.2 Upper critical tissue concentrations

Though the aim of this experiment was to determine the upper critical levels of the heavy metals, some observations were made during the experiment.

2.3.2.1 Observations The plants grown in low concentrations of cadmium were slightly chlorotic, even though these plants seemed to be growing as well as the controls (those plants growing in solutions without added cadmium). As cadmium concentration increased, as well as a general stunting of growth, chlorosis was more apparent, culminating at the

Figure 2.4 Graph of shoot and root dry weights as functions of the solution concentrations of Cu, Ni and Hg in preliminary experiment



highest concentrations in small seedlings with shoots that were all yellow-green. The roots were also small, dark in appearance with no branching or root hairs.

Over the range of copper concentrations added, there was a gradation in the appearance of the shoots and the roots. At the lowest levels the plants appeared healthy, the shoots were dark green and possessed many whorls of needles; the roots were long, many-branched with root hairs. As concentrations increased the shoots became yellowish-green indicating some form of chlorosis; the roots were smaller with fewer branches and root hairs. These observations agreed with those of the previous experiment.

Increasing solution concentrations of lead had no visible effect upon the seedlings up to a concentration of one mg/ l lead, though there were slightly smaller roots in one experiment. At 2.5 mg/ l lead there was a slight reduction in the roots of both replicates. There was no evidence of chlorosis at any concentration.

The visible effect of increasing solution concentration of nickel was a general stunting of growth without chlorosis, as observed in the previous experiments with nickel.

The symptoms of zinc toxicity observed at the highest concentrations of zinc was a retardation of growth; plants being stunted and slightly chlorotic.

2.3.2.2 Statistical analysis Beckett and Davis (1977) developed a statistical method to determine upper critical concentrations, which is based upon the relationship between the yield, in terms of dry matter of the plant shoots, and the logarithm of the concentration of an element, either in a nutrient solution or its shoot tissue.

It has been shown that yield curves (graphs of yield plotted against concentration of an element) reduce to straight lines when concentrations are plotted in logarithmic form (Finney 1947). One line is a horizontal yield plateau and the other a sloping regression line (Figure 2.1). The two equations describing these lines are: 1. When the tissue or solution concentration (T) is less than the critical concentration (Tc) then the estimated yield (Ym) equals the mean yield of all plants in concentrations below the critical concentration (Yo).

$$Ym = Yo \quad \text{where } T<Tc$$

2. When the concentration (T) is greater than the critical level (Tc) then the estimated yield (Ym) can be calculated from the least squares regression line

$$Ym = Yo - (\log Tc - \log T).c \quad \text{where } T>Tc$$

The total error in yield $(Y - Yo)^2$ is a minimum only when Yo is the mean yield for all points with T>Tc, and $Ym = Yo - (\log Tc - \log T)c$ is the least squares regression line.

Beckett and Davis (1977) described a method of ascertaining which division of results produces the closest fit as follows;

1. List all pairs of log T and Y values in order of increasing log T;

2. Examine the graph by eye and select a value of log T that clearly lies above the split-point;

3. Perform a regression analysis on this pair of log T and Y values and all those below it on the list and record the residual mean square of the regression;

4. Calculate the mean (plateau) and variance of the remaining Y values;

5. Determine the value of log T (split-point) at the

intersection of the regression and the plateau;

6. Calculate a pooled standard error from the sum of the residual mean square (3) and the variance (4).

7. Repeat (3) to (6) with progressively one more pair of log T and Y values in the regression, and one less on the plateau, each time, up to a value of log T which clearly lies below the split-point (these interactive calculations may easily be performed by a simple computer programme);

8. Tabulate the calculated split-point (5) and pooled standard error (6) for every value of T for which the operations (3) to (6) were performed. The optimum split-point is the one for which T lies between the highest T of the corresponding regression and the next highest T that is paired with the first Y on the plateau. If more than one split-point meets this criterion select the one with the lowest standard error.

The lethal concentration (Tl), which is the concentration which would produce zero growth, was calculated by extrapolating the regression line to zero yield.

A computer programme was developed to calculate the upper critical concentrations automatically. This programme was run on a powerful programmable calculator (Hewlett-Packard HP-41C calculator with an extended memory). This extension allowed the development of the programme "CRTTC", which is listed in Appendix A.

The information required for input was correctly sequenced yield and log concentration data. The programme calculated the appropriate Log T value at the split-point, regression line (b), correlation coefficient (r), the standard error of the regression line (residual mean square), yield plateau (Yo) and its standard error (Yvr), and then added the standard errors together to give a pooled standard error (S.E). These are listed in Tables 1-13 in Appendix A for each experiment.

The upper critical and lethal tissue concentrations, Tc and Tl respectively, were determined by the above method (Burton et al 1983)

and are listed in Table 2.5 along with the corresponding yield plateaux (Yo), regression lines (b), correlation coefficients of the regression lines (r) and the pooled standard errors determined at the split-points. Although there were originally three zinc and three lead experiments, several of these did not yield Tc results so that only one and two results are given for lead and zinc respectively. In these particular experiments even the plants growing in the highest concentrations did not accumulate sufficient metal to reduce the shoot yields and therefore no values were obtained for the critical concentrations.

The yield plateaux (Yo) for individual experiments of each element were generally similar. This was probably due to the concurrent growth of the plants. The yield curves (yield plotted against log tissue concentration) for Cu, Cd, Pb, Ni and Zn are shown in Figures 2.5a-d respectively. In the cases of copper and zinc, no lower critical concentrations were exhibited, showing that even at the lowest concentrations used, the basic nutrient solution provided an adequate supply of these essential elements.

2.3.3 Yields, cadmium concentrations and time

2.3.3.1 Observations The effect of the cadmium was noticeable after 42 days; here plants grown in the higher concentrations were slightly chlorotic with few roots and root hairs. The differences between the concentrations became more marked with time. After 100 days the roots of those plants grown in 5 mg/ 1 cadmium were very dark, and smaller than at lower concentrations. At 0.05 mg/ 1 there were interesting gradations of colour in the shoots. The plants were smaller than the controls with dark green basal whorls becoming lighter further up the shoots then yellow in the top whorls. At 0.5 mg/ 1 cadmium this feature

Figure 2.5 Typical shoot yield curves for (a) Cu; (b) Cd; (c) Ni; (d) Pb

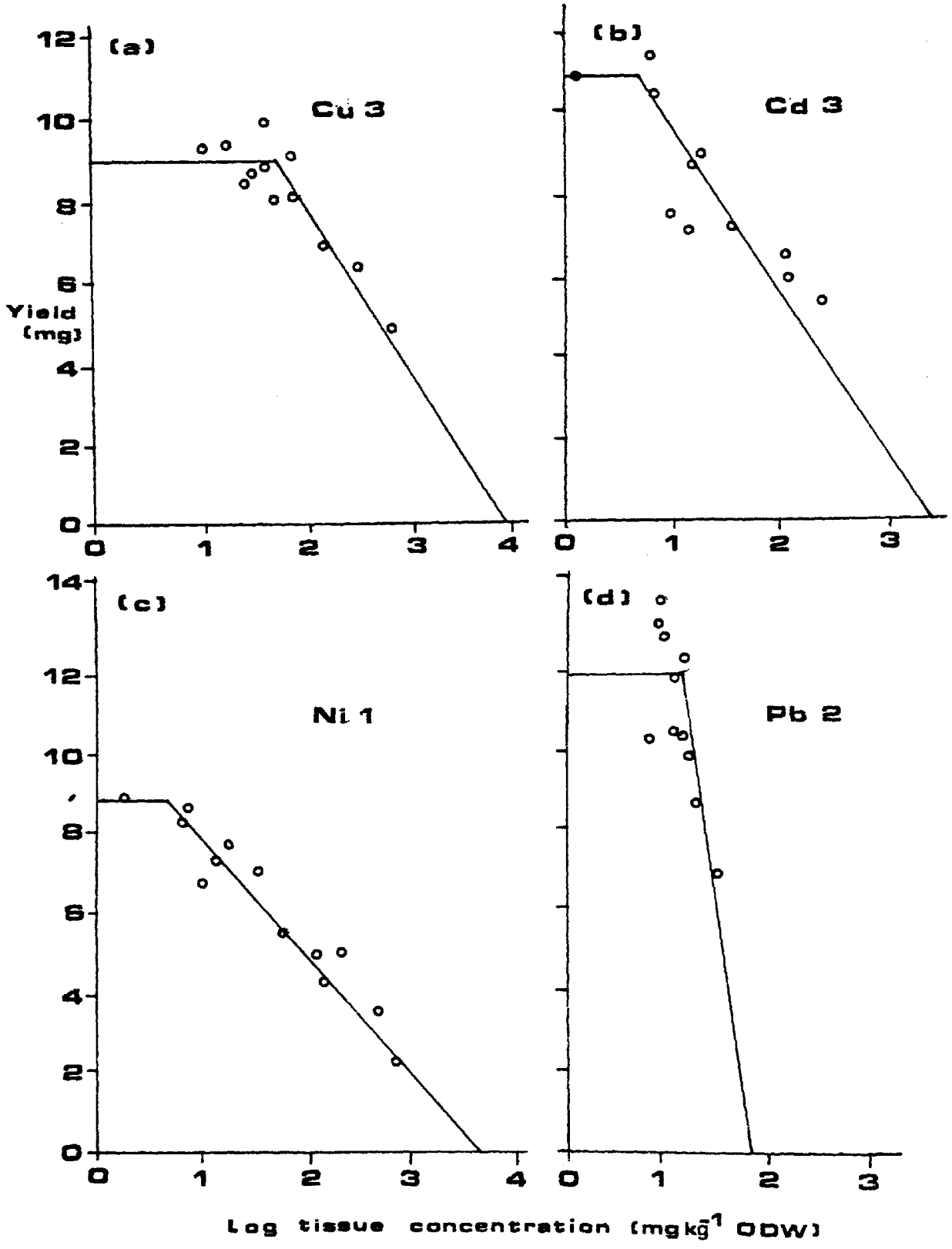


Table 2.5

Yield curve data obtained from calculations of upper critical concentrations

Exp	Critical tissue concentration Tc (mg/ kg)	Lethal tissue concentration Tl (mg/ kg)	Yield plateau Yo (mg)	Pooled Standard Error S.E (mg)	Correlation coefficient of regression line (r)
Cd 1	3.68	11881	10.77	0.666	-0.90**
Cd 2	6.22	14078	10.56	0.810	-0.90**
Cd 3	5.72	2779	10.63	1.262	-0.84**
Ni 1	5.08	5085	8.73	0.316	-0.96**
Ni 2	6.58	16002	8.05	0.369	-0.92**
Pb 1	18.44	41.69	11.95	5.84	-0.76*
Pb 2	19.20	43.29	11.62	5.28	-0.83*
Cu 1	103.63	10064	6.58	0.44	-0.92*
Cu 2	97.24	11288	6.97	0.956	-1.00**
Cu 3	61.96	7253	8.81	0.637	-0.97**
Zn 1	225.5	$4.8*10^{13}$	3.84	0.12	-0.95**

* Correlation significant $p<0.05$
** Correlation significant $p<0.01$

was still apparent, but was not as striking. At 5 mg/ 1 cadmium some of the plants were dead; these had no chlorophyll in the shoots and in some cases were necrotic.

2.3.3.2 Variations in yield with cadmium concentration and time The average results of the yields, cadmium concentrations and contents are shown for shoots and roots in Tables 2.6 and 2.7 respectively. The graph of shoot yield variation with time (Figure 2.6) confirms that there were differences in the shoot yields at 42 days as noted above. At this time there was an apparent increase in the growth rate of all the plants except those in 5 mg/ 1 cadmium.

The highest growth rate was, as expected, in the control group (0.16 mg dry weight per plant per day) and the lowest in 5 mg/ 1 cadmium (less than 0.03 mg dry weight per plant per day). At 5 mg/ 1 cadmium the plants grew very little over the 100 day period and when moribund (after 75 days) accumulated cadmium more easily (Figures 2.7 and 2.8). At 5 mg/ 1 cadmium, root concentrations varied greatly over the experimental period (Table 2.7), which was probably due to differing numbers of plants dying in each beaker.

2.3.4 Supplementary cadmium critical concentration experiment

2.3.4.1 Observations At 0 mg/ 1 cadmium (control group) the shoots were of even height, dark green with many whorls of needles. The roots were branched fairly regularly and long with abundant root hairs. At higher concentrations root development was affected and the number of root hairs was reduced; at 10 mg/ 1 Cd the shoots were almost devoid of chlorophyll.

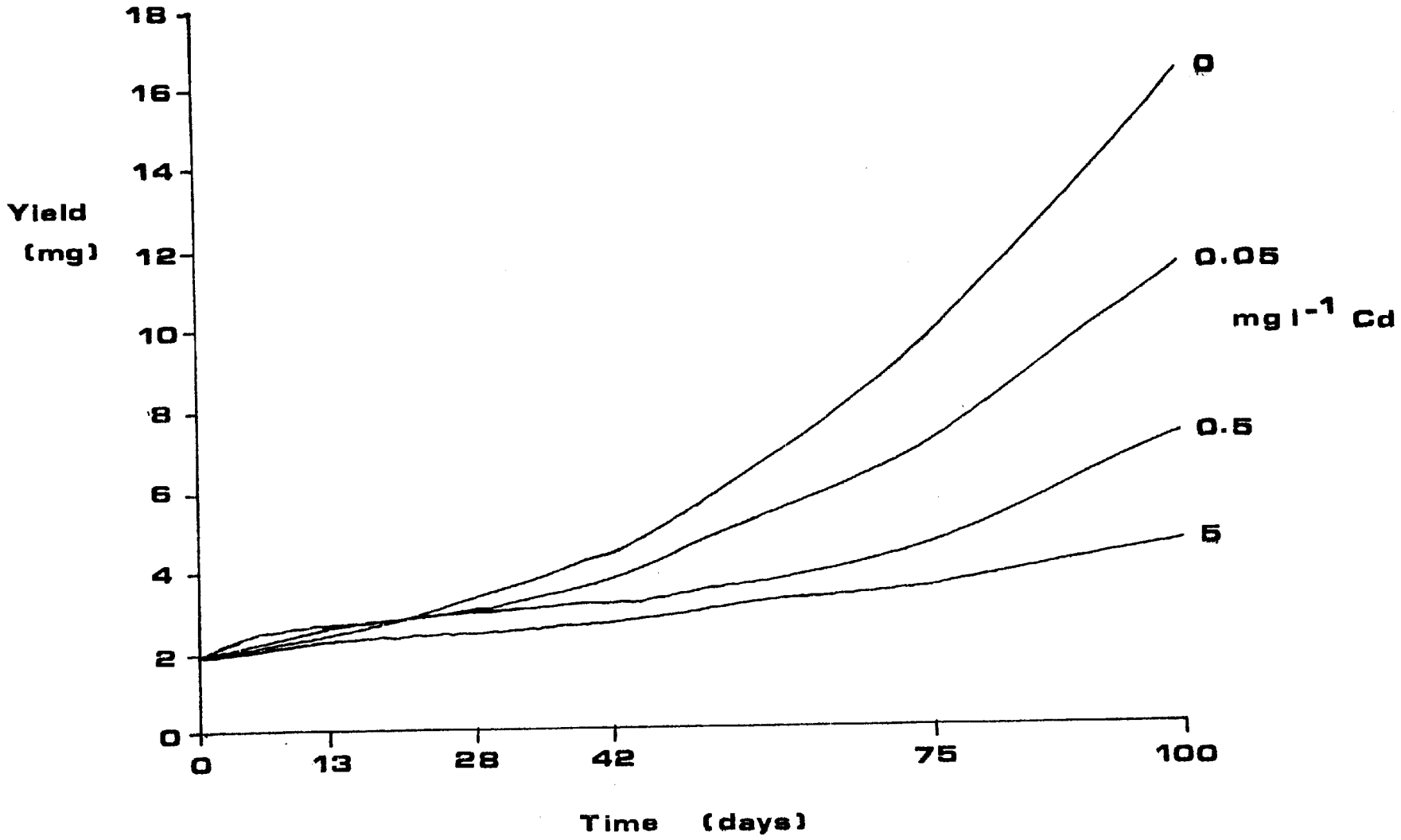


Figure 2.6 Variation in shoot yields over 100 days for sitka-spruce seedlings grown in Ingestad's solution supplemented with 0 - 5 mg l^{-1} Cd

Figure 2.7 Variations in shoot Cd content over 100 days for sitka-spruce seedlings grown in Ingestad's solution supplemented with 0 - 5 mg l^{-1} Cd

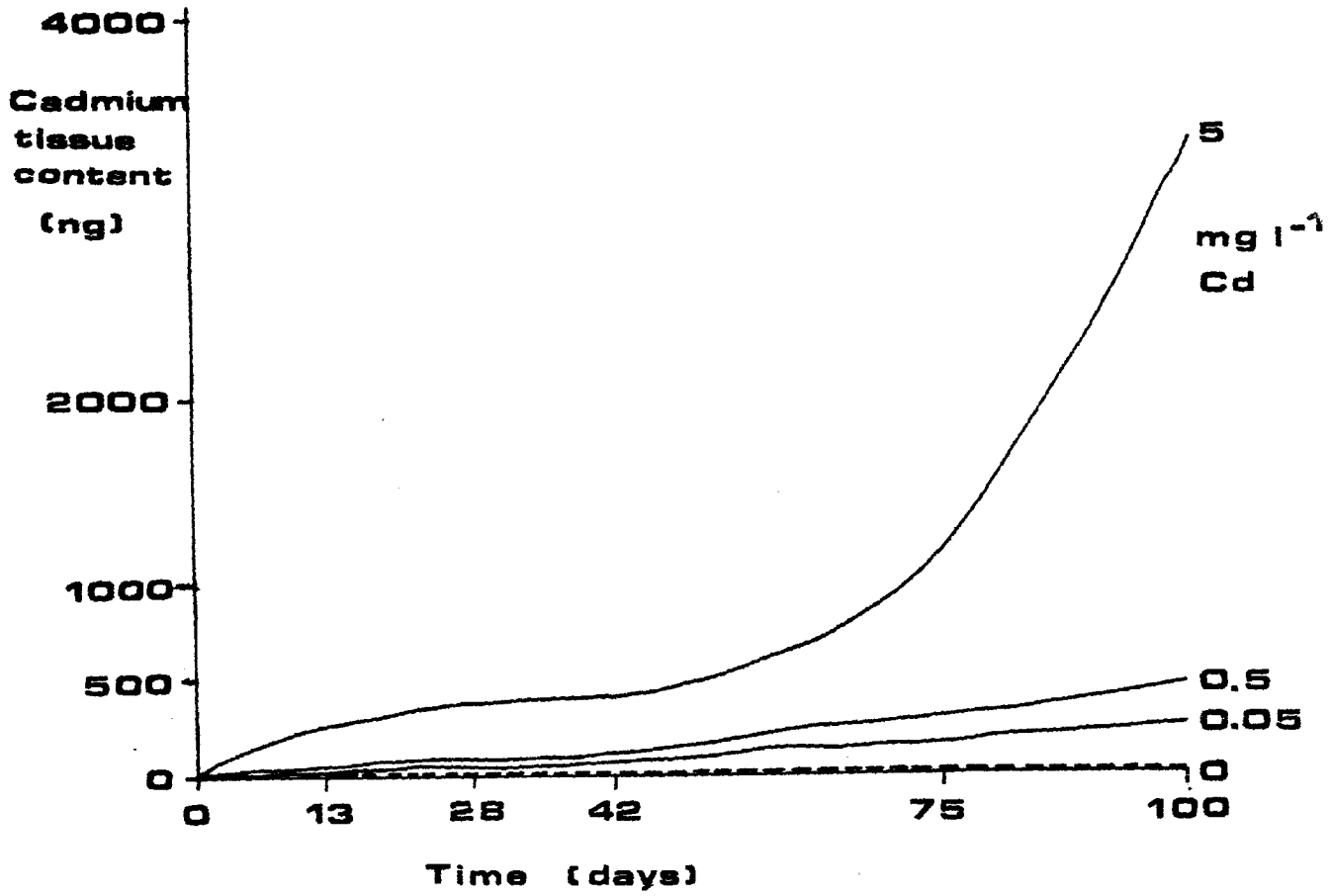


Figure 2.8 Variation in shoot Cd levels over 100 days for sitka-spruce seedlings grown in Ingestad's solution supplemented with 0 - 5 mg l^{-1} Cd

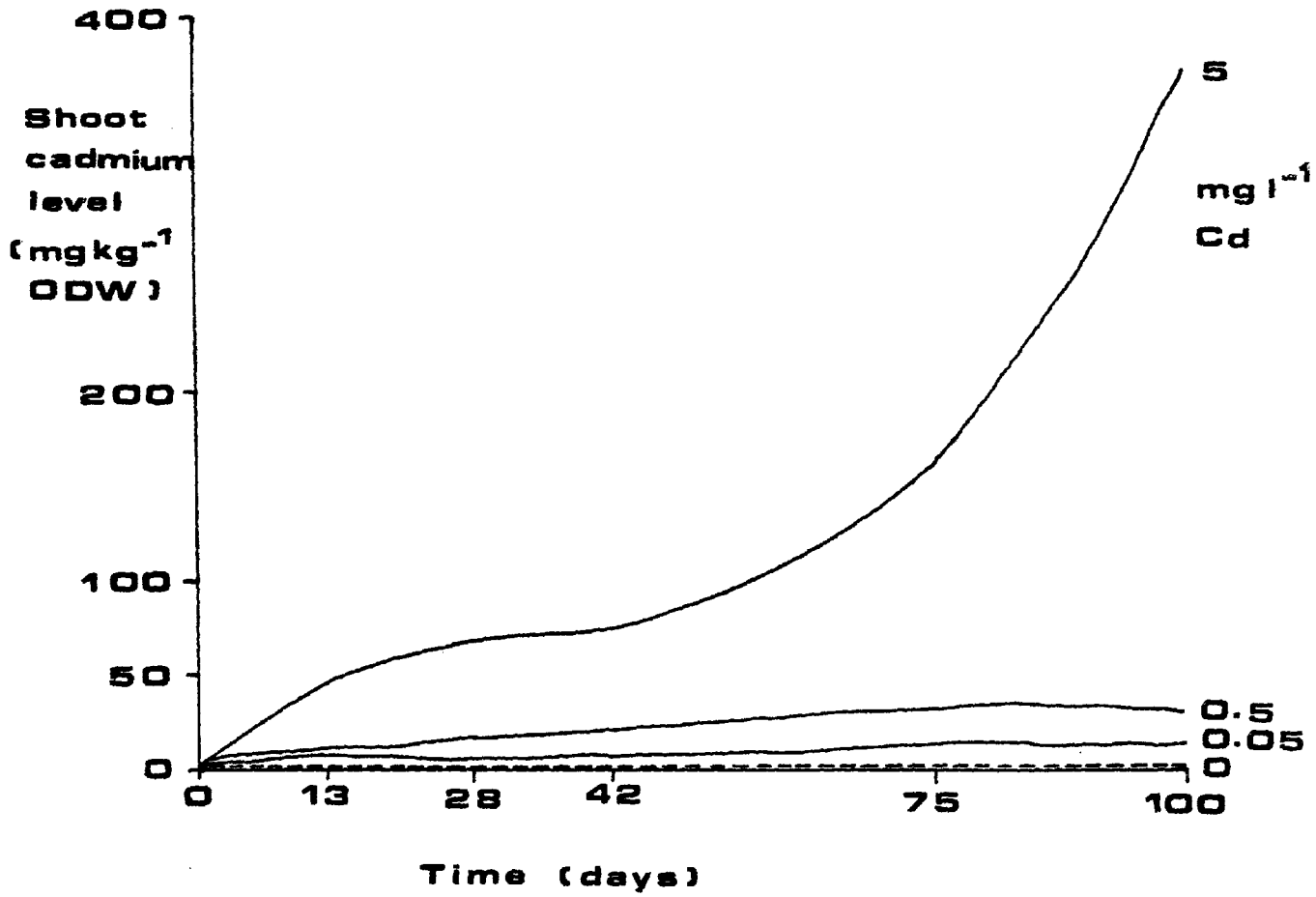


Table 2.6

Variation of shoot yields and cadmium concentrations and contents with respect to time

Time (days)	Parameter	Cadmium solution concentration (mg/ 1)			
		0	0.05	0.5	5
0	Y	1.97	1.97	1.97	1.97
	C	2.5	2.5	2.5	2.5
	TC	4.92	4.92	4.92	4.92
13	Y	2.4	2.73	2.89	2.50
	C	1.55	14.84	21.2	95.71
	TC	3.72	40.51	61.27	239.27
28	Y	3.43	3.13	3.12	2.88
	C	1.42	12.4	32.35	141.6
	TC	4.87	38.81	100.9	407.81
42	Y	4.43	3.77	3.1	2.7
	C	1	15.8	34.06	152.6
	TC	4.43	59.57	105.59	411.75
75	Y	9.75	7.15	4.65	3.55
	C	1.47	25.29	58.98	328.04
	TC	14.34	180.82	274.26	1164.54
100	Y	16.38	11.6	7.33	4.57
	C	0.5	22.8	59.21	738
	TC	8.2	264.5	434	3373

Y = Yield per shoot
C = Concentration of cadmium (mg/ kg of dry weight)
TC = Tissue content (ng/shoot)

Table 2.7

Variation of root yields and cadmium concentrations and contents with respect to time

Time (days)	Parameter	Cadmium solution concentration (mg/ 1)			
		0	0.05	0.5	5
0	Y	0.55	0.55	0.55	0.55
	C	26.5	26.5	26.5	26.5
	TC	14.6	14.6	14.6	14.6
13	Y	0.7	0.76	0.63	0.72
	C	22.1	95.8	152.7	275.8
	TC	15.47	72.81	96.20	198.6
28	Y	0.66	0.62	0.69	0.74
	C	23.0	150.0	161.8	541.3
	TC	15.18	93.45	110.8	397.9
42	Y	1.15	0.95	1.04	0.93
	C	19.6	128.0	161.3	390.0
	TC	22.62	121.6	168.2	364.3
75	Y	3.94	2.67	1.78	1.49
	C	10.9	282.8	266.9	1023
	TC	42.90	756.2	475.9	1527
100	Y	5.23	5.22	4.00	1.80
	C	12.72	218.0	257.0	559.0
	TC	66.5	1138	1028	1006

Y = Yield per root
C = Concentration of cadmium (mg/ kg of dry weight)
TC = Tissue content (ng/root)

Plant growth tended to be varied, some plants having developed more than others in the same replicate. There were also some plants which had fungus on their shoots and roots at 10 mg/ 1 cadmium. The gradation of colour from the basal to the uppermost whorls of the shoots that was noted in the previous experiment conducted over 100 days (Section 2.3.3.1), was also present at one mg/ 1 Cd in this experiment.

2.3.4.2 Statistical analysis The method of Beckett and Davis (1977) was again applied to the results of this experiment. The yields have been plotted against the log tissue concentrations (Figure 2.9). From the observations it was noted that many of the plants at 10 mg/ 1 cadmium were moribund and Figure 2.9 reveals this clearly. Beckett and Davis (1977) stated that moribund tissues tended to accumulate more of the metal than live plants and excluded these from their Tc calculations as they would give untrue reflections of the positions of the regression line and yield plateau. For this reason the three results with the highest cadmium concentrations were excluded from these calculations.

The programme "CRTTC" (see Appendix A) gave a value for the Cd upper critical tissue concentration (Tc) as 3.5 mg/ kg Cd and for the lethal tissue concentration (T1) as 292 mg/ kg Cd (Table 15, Appendix A). The correlation coefficient (r) obtained was 0.78 which is 99% significant with 10 degrees of freedom. The yield plateau (Yo), the average shoot yield of those plants unaffected by the accumulation of cadmium, was 19.4 mg per plant, which, as expected, was far greater than those of previous experiments (Burton et al 1983).

2.3.4.3 Effect upon nutrient concentrations The concentrations of cadmium and the nutrients Cu, K, Mg, Mn and Zn in the shoots and roots are listed in Table 2.8 and 2.9 respectively. Students "t" tests were

Figure 2.9 Yield curve of shoot yield plotted against log tissue concentration in supplementary experiment to determine Cd upper critical level (Cd4)

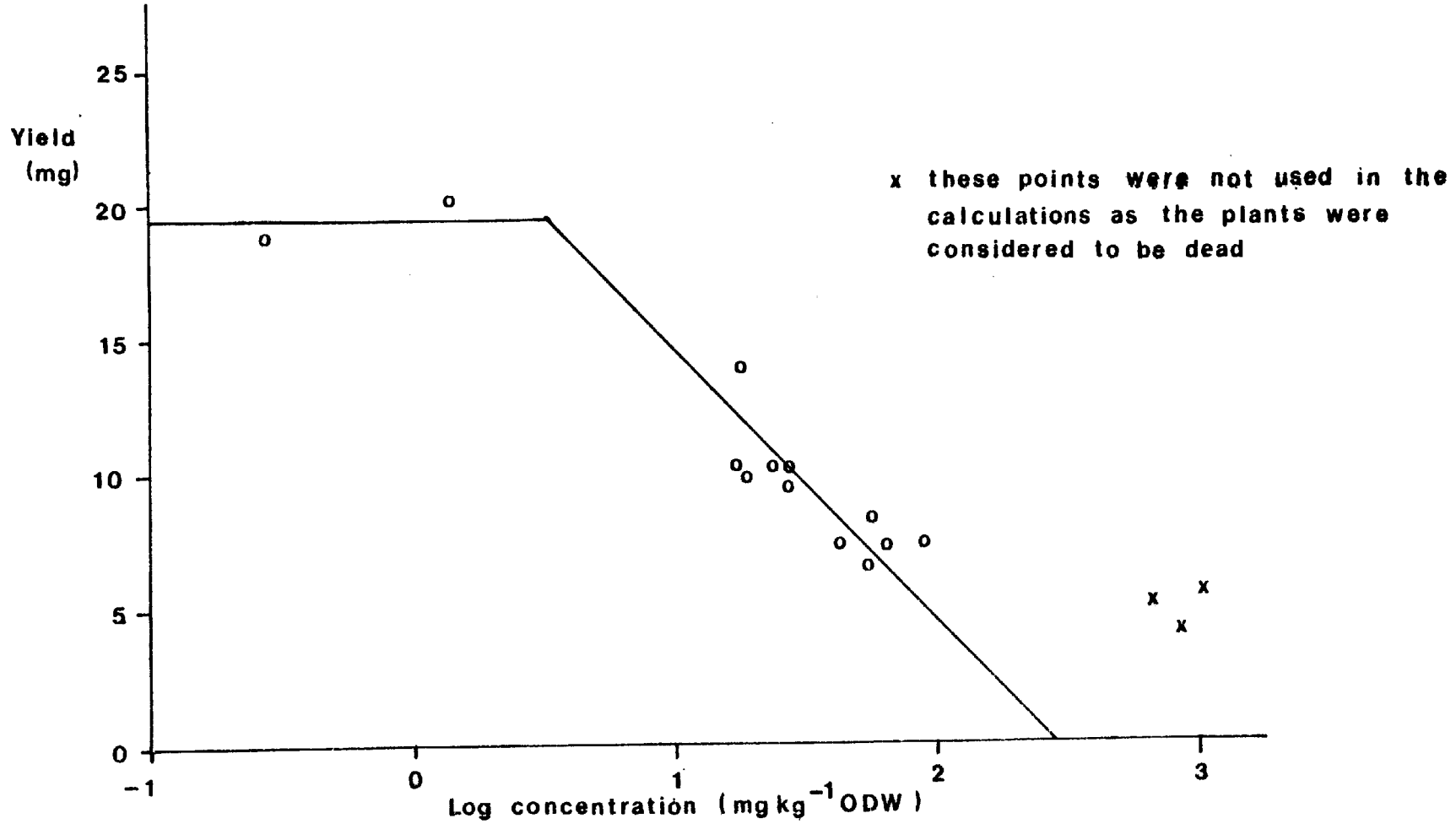


Table 2.8

Cadmium experiment Cd4; concentrations of nutrients and cadmium in shoots

Solution cadmium concentration (mg/ l)	Shoot tissue concentration (mg/ kg)					
	Cd	Cu	Mn	Zn	Mg	K
0	1.40	15.19	284.7	21.36	1538	1.01
0	0.00	12.18	238.7	23.75	1080	0.77
0	0.27	6.73	280.4	22.99	1670	0.90
0.05	17.45	15.11	162.4	27.85	1718	1.13
0.05	18.70	16.40	161.3	24.60	1289	1.14
0.05	17.79	17.63	178.3	28.90	1895	1.33
0.1	24.15	18.17	123.2	34.84	1353	1.37
0.1	26.65	18.20	116.5	27.29	1702	1.34
0.1	23.99	16.16	113.1	31.81	1578	1.25
0.5	45.22	21.74	81.5	35.33	1904	1.09
0.5	56.09	29.94	104.8	44.91	2283	1.20
0.5	58.35	23.20	104.4	37.70	2454	1.26
1	59.62	27.83	78.4	40.49	1827	1.17
1	70.62	15.50	98.1	38.74	2215	1.22
1	92.43	15.77	89.4	44.69	2149	1.32
10	696.5	33.17	116.1	61.16	1960	0.56
10	1093.0	102.46	113.0	46.11	2316	0.68
10	891.1	264.00	132.0	173.30	2528	0.69

Table 2.9

Cadmium experiment Cd4; concentrations of nutrients and cadmium in roots

Solution cadmium concentration (mg/ l)	Root tissue concentration (mg/ kg)					
	Cd	Cu	Mn	Zn	Mg	K
0	8.99	57.2	404.4	94.0	557	0.46
0	8.11	67.6	357.1	132.7	678	0.56
0	4.61	51.2	324.2	83.2	447	0.49
0.05	194.9	93.6	107.0	63.5	511	0.45
0.05	190.3	50.6	91.1	65.8	522	0.45
0.05	414.1	70.4	82.2	73.4	554	0.33
0.1	343.0	62.4	26.0	70.2	611	0.52
0.1	241.8	95.9	33.9	69.1	614	0.43
0.1	87.4	154.0	59.2	118.5	1123	0.76
0.5	322.7	79.9	32.0	87.9	457	0.45
0.5	457.3	127.2	25.0	105.0	776	0.79
0.5	291.2	118.6	33.9	144.0	570	0.54
1	347.0	91.5	30.5	114.3	604	0.52
1	448.7	104.5	27.9	134.0	763	0.53
1	403.4	117.8	26.2	130.9	594	0.31
10	1540.0	214.3	53.6	205.4	379	0.36
10	912.4	238.1	47.6	214.3	219	0.95
10	894.7	263.2	65.8	411.2	605	0.57

used to establish whether there were significant differences between the different cadmium concentrations, each concentration having three replicates (Tables 2.8 and 2.9). The calculations were made using a Hewlett-Packard HP-41C programmable calculator with a statistics module, which calculated the "t" value using the equation which tested the null hypothesis Ho: $\mu_1 - \mu_2 = d$ with $n_1 + n_2$ degrees of freedom (Eckshlager 1961).

There were significant decreases in the concentrations of manganese in the shoots ($p<0.001$) and roots ($p<0.001$) in all the replicates treated with cadmium. There were also significant increases in the zinc concentrations in the shoots ($p<0.05$) and significant increases in copper concentrations in the shoots ($p<0.05$) and roots ($p<0.05$). At 10 mg/ kg cadmium, where many of the plants were moribund, concentrations of the nutrients often varied greatly. When tested against all the lower concentrations, it was found that shoot potassium concentrations were significantly reduced ($p<0.001$) in 10 mg/ l cadmium. In the roots there were large increases in copper concentrations ($p<0.001$) and zinc concentrations ($p<0.001$) at this concentration (Table 2.9).

2.4 Discussion

A combination of the observations and experimental results reveal many important features about the effects of heavy metals upon sitka-spruce seedlings.

2.4.1 Symptoms of metal toxicity

At the lowest concentrations of nickel there were signs of stunting of shoot and root growth, and at higher concentrations, symptoms of

necrosis and chlorosis were apparent. However, it has been pointed out that in monocotyledons nickel toxicity often showed up as alternate green and light-yellow banding of the leaves, whereas in dicotyledons the symptoms were of a general chlorotic mottling (Vanselow 1966). The main toxic symptom of nickel has been described as chlorosis or yellowing of the leaves followed by necrosis; other symptoms included stunted growth of shoots and roots, deformation of various plant parts and unusual spotting on leaves and stems (Mishra and Kar 1974). As chlorosis was not a principal manifestation of nickel toxicity here, but rather a general stunting of growth, it is clear that the toxic effects of nickel are of a different nature in sitka-spruce to those produced in other plants.

In the sitka-spruce seedlings copper toxicity manifested itself as a chlorosis at low concentrations with reductions in plant growth parameters as concentrations increased. In other plants generally similar symptoms have been described (Foy et al 1978).

In sitka-spruce seedlings, cadmium toxicity seemed to be associated with chlorosis: symptoms of this were apparent in all the replicates to which cadmium had been added. There are, however, few reports of cadmium phytotoxicity manifesting itself in this manner.

The effect of lead upon sitka-spruce seedlings was stunting of growth of the shoots and roots without chlorosis. Lead phytotoxicity has been described as producing general decreases in growth parameters after lead treatment in hydroponic cultures with red maple (Davies and Barnes 1973), oats (Fiusello and Molinari 1973) and corn (Carlson et al 1975). In all these studies chlorosis was found not to be a major factor in lead toxicity and the effect of lead upon sitka-spruce seedlings was similar to those in other plant species.

Zinc produced general stunting of growth and slight chlorosis at the highest concentrations added. These symptoms are similar to those described by other workers who found that zinc phytotoxicity symptoms were similar to symptoms of phosphate toxicity (Takkar and Marr 1978). This chlorotic response was attributed to an interference in iron metabolism. Toxic symptoms of mercury were reductions in growth at very low solution concentrations without accompanying chlorotic or necrotic effects.

2.4.2 Upper critical tissue concentrations

The variation in the values of upper critical concentrations (Table 2.5) agree well with those obtained by Beckett and Davis (1977) and Davis and Beckett (1978) for other plants. Here, for example, the cadmium Tc value varied between 3.5 and 6.2 mg/ kg whereas it varied from 6 to 10 mg/ kg in other plants.

Table 2.10 lists the average upper critical values derived from all the experiments (Burton et al 1983), along with generalized values of upper critical tissue concentrations obtained from data for barley, rape, lettuce and other plants (Davis and Carlton-Smith 1980). These are included to show the relative sensitivity of sitka-spruce to the different metals.

The average Tc value for lead in sitka-spruce is 19 mg/ kg whereas the average lethal concentration is only 43 mg/ kg, which is low compared with the other metals studied. This could be explained by a sequestration and extrusion of the lead in shoots, up to a concentration of 19 mg/ kg, above which the "available" lead exerts an extremely toxic effect. This agrees with the findings of other workers who have

Table 2.10

Upper critical concentrations (Tc) in sitka-spruce and generalized average critical levels in other plants*

Element	Sitka-spruce Tc (mg/ kg)	Generalized Tc* (mg/ kg)
Cadmium	4.78	8
Nickel	5.83	11
Lead	18.82	35
Copper	87.61	20
Zinc	225.5	200

* Tentative data taken as an average from several species

reported that even when translocated, lead could be extruded from plant cells.

The average critical tissue concentration of copper in sitka-spruce is well above the generalized average (Table 2.10). The other essential element examined, zinc, also had a high average Tc value (226 mg/ kg). In fact there were two other zinc experiments which did not yield Tc values (Section 2.3.2).

Previous workers have found that the upper critical concentration of zinc in barley varied between 170 and 520 mg/ kg with an average at 390 mg/ kg (Davis and Beckett 1978). Though it was impossible to determine an accurate estimation of the zinc Tc value in the seedlings on the basis of one experiment, it seems likely that the result was of the correct order of magnitude.

The cadmium upper critical concentration was determined as 4.8 mg/ kg Cd in the sitka-spruce seedlings, which is approximately half the value determined for other plants. The interesting point to note about

the cadmium experiments is that the value determined by the experiment conducted over a longer time is consistent with the values obtained by the other experiments. Since the older plants were harvested after 140 days and others at approximately 73 days, it may be assumed that these values hold true, at least for cadmium, at different stages of development. The nickel Tc value is an average of only two results, but it must be noted that the range of treatments was appropriate (Figure 2.5c), since the Tc values obtained (Table 2.5) are very similar.

2.4.3 Relationship between cadmium concentration and time

The rate of accumulation of cadmium into apparently dead shoot tissues was far greater than in the live tissues (Figure 2.7). This clearly demonstrates that the inclusion of moribund plants in the calculations of critical tissue concentrations would have invalidated the results. If the graph of shoot cadmium concentration against time (Figure 2.8) is examined, it is apparent that the shoot concentrations did not increase between days 75 and 100 in those plants grown in solution concentrations of 0.05 and 0.5 mg/ l cadmium. This was probably due to the accelerating growth rate of the shoots (Figure 2.8), which meant that the rate of accumulation of cadmium from the roots into the shoots was insufficient to maintain the increase in the Cd concentration. This happened because the shoot yields were increasing at a greater rate than were the root yields. It is therefore difficult to interpret this effect in terms of older trees since the relative growth rates of roots and shoots would undoubtedly alter.

2.4.4 Effect of cadmium upon nutrient uptake and translocation

This experiment indicates that cadmium can have important effects

upon the uptake of macro nutrients, such as K, and micro-nutrients such as Cu, Mn and Zn alike (Section 2.3.4.3). However, many of the effects were only manifest in the highest solution concentration of cadmium (10 mg/ l) where most of the plants were moribund. It is not understood why these particular increases or decreases occurred in these tissues and in some ways it is irrelevant since it is likely that they occurred after the onset of morbidity (Beckett and Davis 1977).

The most important effect of cadmium was upon the concentration of manganese in both shoots and roots. In all replicates where cadmium had been added to the solutions there were greatly significant reductions in manganese tissue concentrations ($p<0.01$). The greatest reductions were in root concentrations which indicates that the major effect was upon the uptake into the roots as opposed to a reduction in the proportion translocated from the roots to the shoots. This effect would probably have contributed to the observed reductions in the shoot yield in this experiment (Section 2.3.4). This type of effect by cadmium has been noted by other workers who reported significant reductions in manganese concentrations in the leaves of lettuce plants (John 1976). Cadmium has also been found to depress the root uptake of manganese in bush beans by 96%, and there was also some reduction in the supply to the shoots as a result of decreased uptake into the roots (Wallace et al 1977).

The slight increases in copper concentrations noted in both shoots and roots indicates that increasing cadmium concentrations facilitated uptake by the roots of a constant supply of copper.

The positive interaction between cadmium and zinc that was found in this study has also been noted by other workers (Jones et al 1973,Turner 1973), though these reports seem to conflict with the results of other

workers who found that cadmium additions decreased zinc uptake (Koshino 1973, Saitoh et al 1973).

It is unlikely that increasing copper and zinc concentrations produced the reductions in shoot yields observed as the shoot concentrations were still well below the upper Tc values determined in previous experiments. Large synergistic interactions between copper, cadmium and zinc would have to occur if these increases in concentration were to affect seedling growth but, taken in conjunction with the decreases in the manganese levels, such interactions may have accounted for the observed effects. It is, however, difficult to separate the causes of toxicity from the effects of toxicity in such a situation.

The overall nutrient balance in different plant species is affected by many interacting factors. Those known to be of importance are; the pH of the growing medium (Wallace et al 1977), species and interspecies variations and the level of a particular nutrient. It has been noted, for example, that phosphate can affect the uptake of lead (Miller and Koeppe 1977, Rolfe 1973).

The results of this study provide further evidence of metal-metal interactions in uptake by plants. With the aim of identifying and separating these interactions, experiments with improved experimental designs, such as factorial designs, were carried out (Chapter 3). These were necessary to clarify those interactions already known, but also to elucidate some higher order interactions which have not as yet been established.

2.5 Summary and conclusions

The aim of this study was to establish the methods for growing sitka-spruce seedlings in water culture in controlled environments and

then to use these techniques to assess the effects of individual heavy metal ions added to the nutrient solution (Ingestad 1959). Several experiments were carried out which enabled descriptions of the typical symptoms of toxicity of the metals Cd, Cu, Hg, Ni, Pb and Zn. These were, in general, non-specific and usually took the form of stunted growth combined with chlorosis. The symptoms found here for Cu, Pb and Zn were similar to those described for other species, whereas those for Cd and Ni were different (Section 2.4.1).

Upper critical tissue concentrations, which are the levels at which yields are first reduced due to the toxic effects, were determined in seedling shoots by the method of Beckett and Davis (1977) for the metals Cd, Cu, Ni, Pb and Zn (Table 2.10, Burton et al 1983). These upper critical levels are independent of other growth influencing factors. This information may enable the assessment of the relative toxicities of these metals to sitka-spruce and also some estimation of the risk of metal toxicity occurring at particular forest sites.

Cadmium, nickel and lead were found to be more toxic in sitka-spruce seedlings than in other plant species (Davis and Carlton-Smith 1980) with critical values of 4.8, 5.8 and 19 mg/ kg respectively. The seedlings were, however, relatively more tolerant of copper and zinc. It was not possible to obtain upper critical tissue data for all the experiments involving zinc and lead since in several experiments the shoots did not accumulate sufficient metal to affect yields. One experiment was run over a longer period (100 days) to determine whether the Cd critical tissue level changed appreciably and the value obtained (3.5 mg/ kg Cd) agreed well with those determined with shorter experimental periods.

Effects by cadmium upon the nutrient uptake were examined and it was found that several nutrients were affected, though the most significant of these were reductions in the shoot and root uptake of manganese (Tables 2.8 and 2.9 respectively). This demonstrated the need to examine the effects of metal interactions in the seedlings.

Chapter 3

INTERACTIVE EFFECTS OF HEAVY METALS IN WATER CULTURE

3.1 Introduction

There have been few studies which examined interactions between combinations of heavy metals in higher plants. Most studies with heavy metals have concentrated on the effects of heavy metals added singly to the rooting medium. As more work on multi-element effects is published, it becomes clear that there is little reason to suppose that these interactions are less widespread or complex in plants than they are in animals (Underwood 1977).

Results of experiments which examine the additions of single heavy metals, although important to the basic understanding of the mechanism by which they affect plants, may only be relevant if the situation being modelled is one where only one metal could possibly interfere with plant function and growth. However, in many situations more than one element may have an effect. The soils and vegetation around non-ferrous metal smelters, for example, often have enhanced concentrations of copper and nickel (Hutchinson and Whitby 1974). If the effects of only the individual elements were to be studied here, then important interactive effects could be missed.

In many areas of South Wales there are enhanced levels of several heavy metals including Cd, Cu, Ni and Zn (Goodman and Roberts 1971, Burton and John 1977). This study must therefore examine the interactive effects of at least 2 or 3 of the more important metals if it is to be

comprehensive.

There are two major questions which must be answered when studying the interactive effects of elements upon plant growth (Beckett and Davis 1978):-1. How far does the presence of one of these elements in the solution round the roots of the test crop modify the uptake of other elements and their translocation to the shoots? 2. How far does the presence of one of these elements in the tissues of the shoot modify the toxicity of the other elements in the same tissues? These have been distinguished as "differential absorption" and "differential tolerance" effects (Vose 1963).

In the case of "differential tolerance", there are further questions that may be posed:- 1. How far does one element modify the critical level of another element in the tissue i.e. the concentration at which its toxicity first becomes apparent? 2. How does one element modify the toxicity of another above this critical level?

It was decided to study the interactive effects of heavy metals upon both the yield of sitka-spruce seedlings and the concentrations of heavy metals and nutrients within these plants. An experimental design which would do this efficiently was sought.

In experiments of this nature a considerable advantage is gained if the experiment can be designed so that the effect of changing one variable or factor can be assessed independently of the others. One way of achieving this is to decide on a set of values or levels for each of the factors to be studied and to carry out one or more trials of the experiment with each of the possible combinations of the factors. Here the term "factor" is used in a general sense to denote any feature of the experimental conditions which may be assigned at will from one trial to another.

Factors can be either qualitative or quantitative. For these experiments it was decided that all the factors should be quantitative since they could be arranged in order of magnitude. The values of a factor examined in an experiment are known as levels and the set of all factors employed in any given trial is called the treatment or treatment combination.

It can be shown that if the result of changing two or more factors is to be studied, then in general, the most efficient method for doing so is to use a factorial design; an efficient method being one which obtains the required information with the required degree of precision and minimum expenditure of effort. The advantages of using a factorial design for this particular experiment are:-

1. When there are no interactions, a factorial design gives the maximum efficiency in the estimation of the effects.

2. When interactions exist, their nature being unknown, a factorial design will avoid misleading conclusions and can often elucidate their nature. For example, negative significant effects reveal that the two factors involved are, to a degree, either independent or antagonistic, whereas positive significant effects show the presence of a synergism between the factors.

3. Since the effect of one factor is estimated at several levels of other factors, the conclusions are valid over a wide range of conditions.

It was therefore decided that an experiment with a 3*3*3 factorial design with cadmium, copper and nickel each at three evenly spaced levels would be adopted.

3.2 Methods

Sitka— spruce seedlings were germinated and grown in the same manner as the previous experiments in water culture (Chapter 2), except that only 15 seedlings were transferred to each beaker. When 31 days old, the three heavy metals cadmium, copper and nickel were added to the solutions as chloride salts at three levels each, making a total of 27 treatments for each experiment. Three experiments of 3*3*3 design (Davies 1979) were carried out.

The first two experiments were single replicate experiments with the same treatments, whereas the third experiment was carried out with slightly different levels with each treatment duplicated. This experiment with two replicates was carried out with one batch of seeds so that all comparisons were made under similar conditions, making it unnecessary to confound any of the higher order interactions with respect to any extraneous variation. The levels of the third experiment were chosen in light of the results of the first two experiments. The levels of the factors in each of the experiments were quantitative, that is to say evenly spaced. The lowest levels were taken as the level in basic nutrient solution. The intermediate levels were estimated as the levels of the factors that would just affect the plant growth and the highest levels were twice these (Table 3.1).

The solutions were changed every five days as previously. When 73 days old the plants were harvested and the yields per replicate determined as described in Chapter 2. In the first experiment, after acid digestion of the plant material, the solutions were analysed for Cd, Cu and Ni, but in all subsequent trials concentrations of Cd, Cu, Ni, Mg, Mn and Zn were determined by flame atomic absorption

Table 3.1

Concentrations of the elements Cd, Cu and Ni used in the factorial experiments (mg/ l)

	Experiment		
	1	2	3
Cadmium	0	0.25	0.5
Copper	0.02	5	10
Nickel	0	0.5	1.0

spectrophotometry (Chapter 2) and Ca by flame photometry.

3.3 Results

Statistical analyses of quantitative factorial designs may reveal the presence of certain significant interactive effects, but the interpretation of these interactions requires a basic understanding of the procedure involved in the calculations.

The effect of a factor is the change in response produced by a change in the level of that factor. When a factor is examined at two levels, the effect is simply the difference between the average response of all trials carried out at the first level and that of all trials at the second level. However, when the factors are present at three levels, as in this case, the effect is represented by differences or comparisons between the means corresponding to the different levels of the factors.

For these experiments, where all the factors are quantitative, there may be a functional relation between the responses and the levels of a factor. The comparisons of interest will be those giving the most information about the relation e.g. slope and curvature. The advantages

of using three levels of factors are that information is supplied on both the linear and quadratic components of the effects.

A linear component of the effect is estimated on the assumption that, over the range of the factors, the relation between the expected responses and the corresponding values of the factor is, to a sufficient degree of approximation, linear. Significant positive linear effects imply a linear increase over the range of treatments and significant negative effects imply the opposite of this.

A quadratic component may imply a maximum or minimum response at some intermediate factor combination, or at a point outside the range examined for all the factors, and may indicate a need for further experimental work at a different set of levels. In this case the regime for the calculation of the quadratic components uses a method where, if negative effects are significant, there is a tendency towards a maximum and when they are positive there is a tendency towards a minimum. This must be born in mind at all times when interpreting these analyses.

There are two types of effect: main effect and interaction. The average difference in response between the levels of a factor is the main effect of that factor. However, if the effect of a factor is different at levels of another factor, then the two factors are said to interact. The same may also be said for the interactions between three factors.

An interaction may be subdivided into its components. This may make the experiment more sensitive by revealing significant interactions which might otherwise be overlooked. If one component is large and the others are small, then the large component may be masked in the average over all the components. Significant interactions may either be positive

or negative thereby indicating the direction of the response. The linear component with respect to a factor B of the linear component of factor A is called the linear A * linear B interaction. A positive linear A * linear B interaction in these calculations indicates a tendency towards an increasing response at the highest levels of both A and B (Figure 3.1a). A negative linear A * linear B interactions indicates the opposite of this (Figure 3.1b).

There may also exist linear A * quadratic B interactions i.e. interactions between the quadratic components of linear A with respect to B. When this is positive (Figure 3.2a), there is a tendency towards a minimum response for these particular calculations. Similarly there may also be significant quadratic A * linear B interactions which may be either positive or negative depending upon the response (Figure 3.3).

Interactions between the quadratic components of both A and B indicate a rather complex situation which is extremely difficult to interpret. However, by the law of multiplication of mathematical signs, significant positive interactions between these components indicate a tendency towards a maximum response at the intermediate levels of both factors (Figure 3.4a): negative quadratic A * quadratic B indicating the opposite (Figure 3.4b).

The method used to calculate the quantitative main and interactive effects with and without replication (Davies 1979) was transformed into a calculator programme (FAC) for use with a Hewlett-Packard HP-41C calculator and is listed in Appendix B. A flow chart of the programme is shown below:

The 27 responses were entered sequentially into memory registers 0-26 for the first replicate and into registers 27-53 if analysis of a duplicate experiment was required. In the factorial experiments in

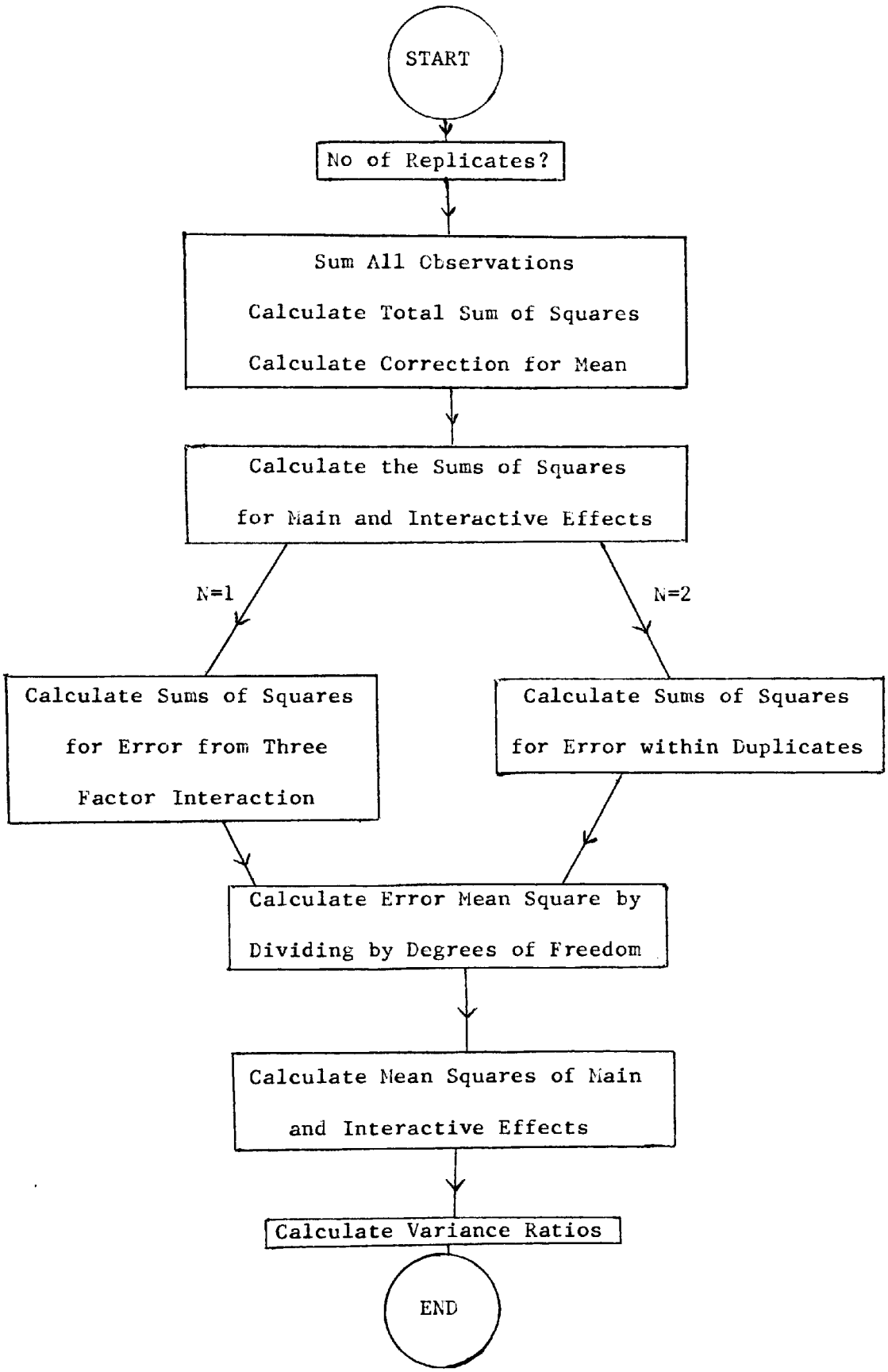

START
No of Replicates?
Sum All Observations
Calculate Total Sum of Squares
Calculate Correction for Mean
Calculate the Sums of Squares
for Main and Interactive Effects
N=1
N=2
Calculate Sums of Squares
for Error from Three
Factor Interaction
Calculate Sums of Squares
for Error within Duplicates
Calculate Error Mean Square by
Dividing by Degrees of Freedom
Calculate Mean Squares of Main
and Interactive Effects
Calculate Variance Ratios
END

Figure 3.1 Typical effects of an interaction between the linear components of factors A and B (a) positive; (b) negative

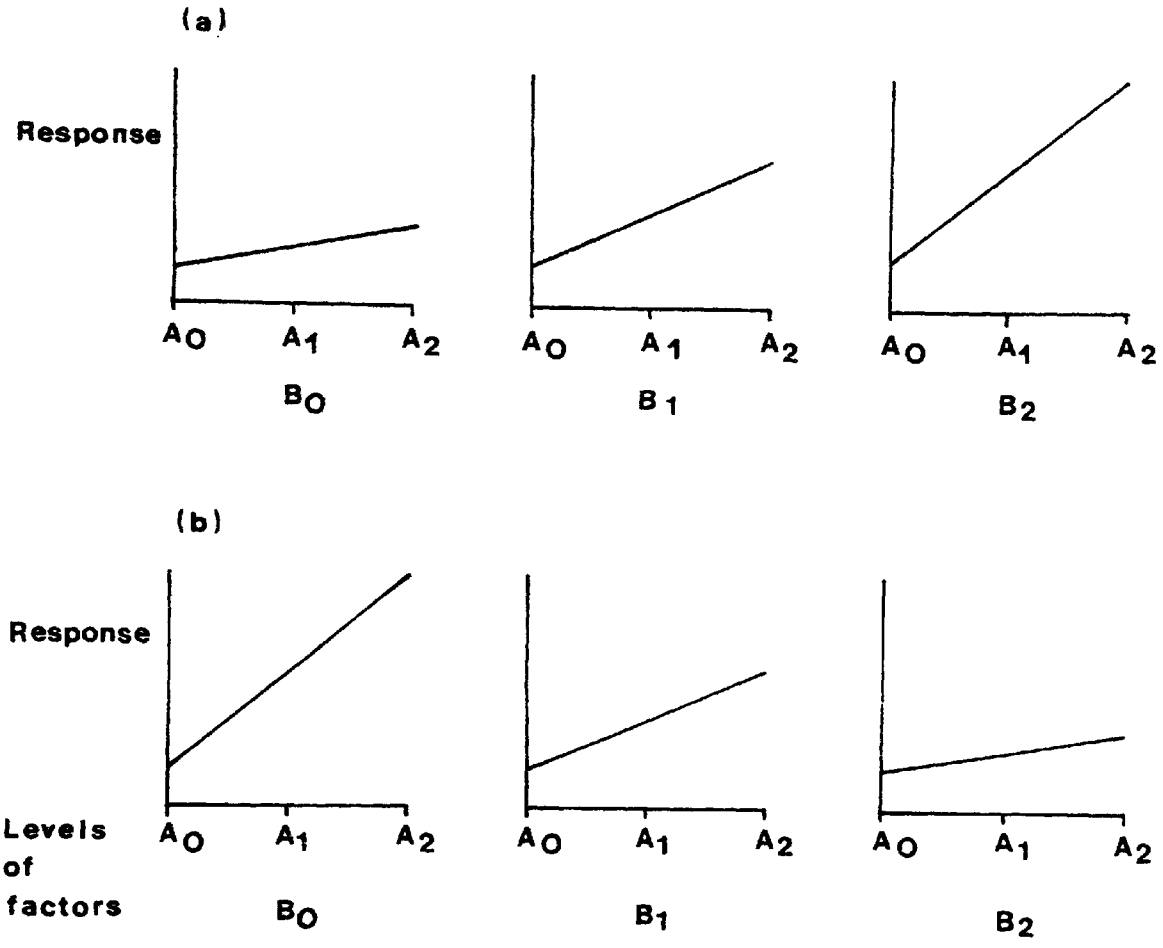


Figure 3.2 Typical effects of an interaction between the linear component of factor A and the quadratic component of factor B (a) positive; (b) negative

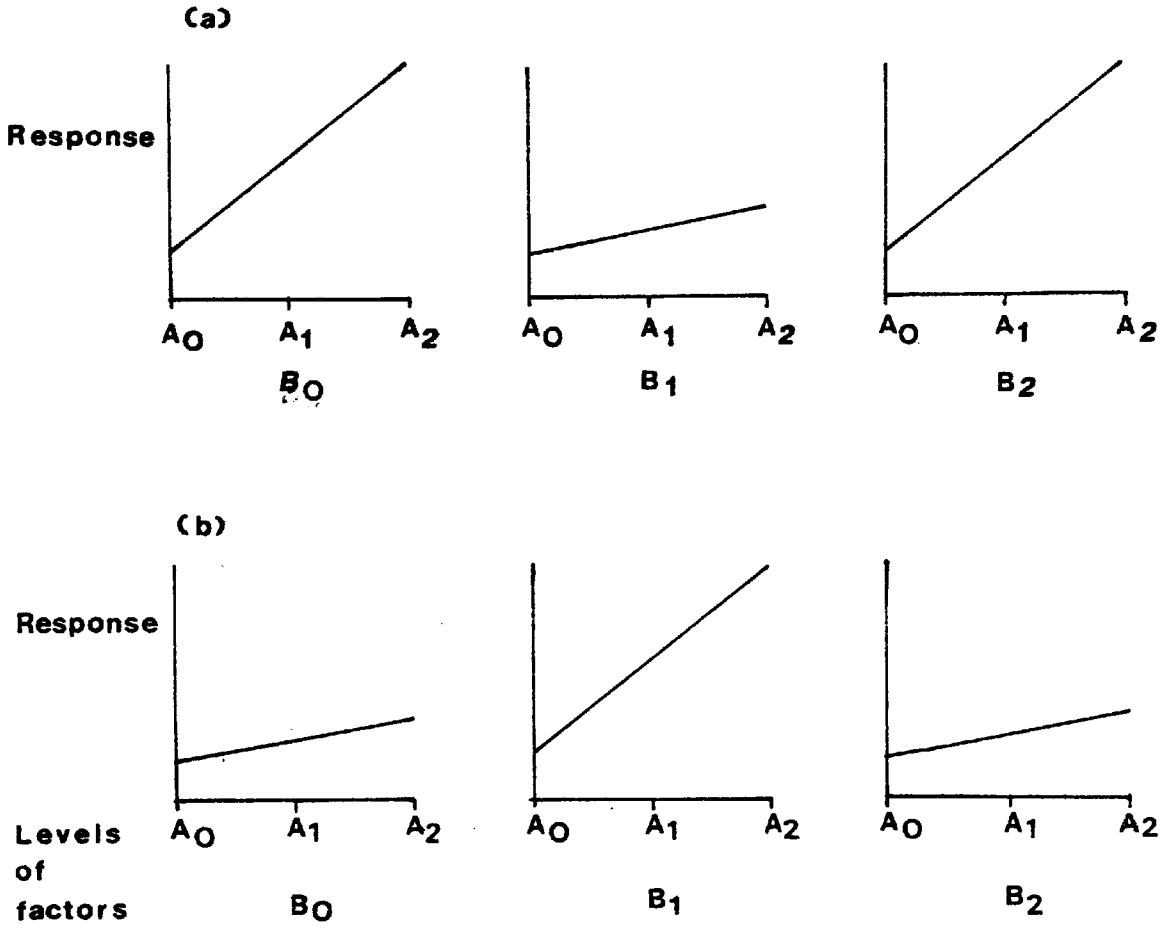


Figure 3.3 Typical effects of an interaction between the quadratic component of factor A and the linear component of factor B (a) positive; (b) negative

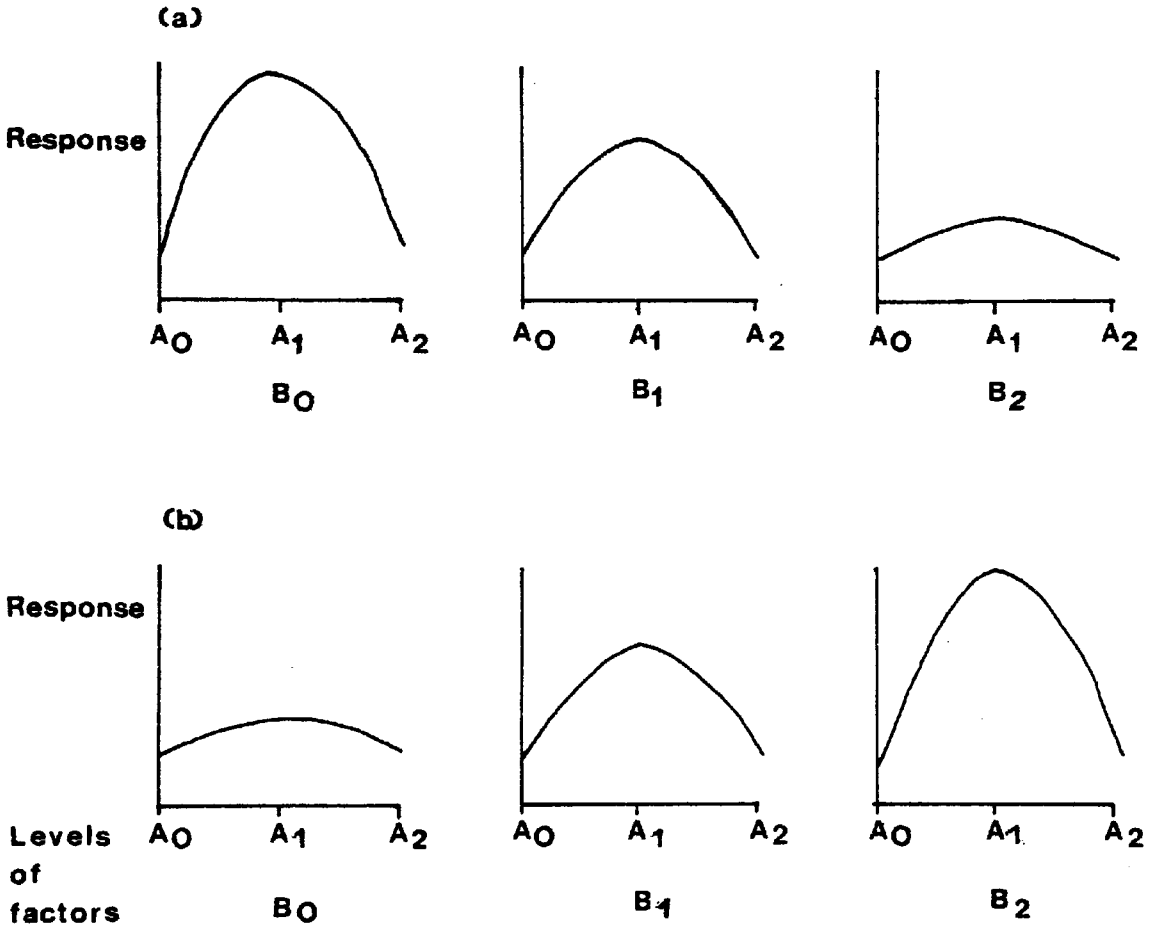
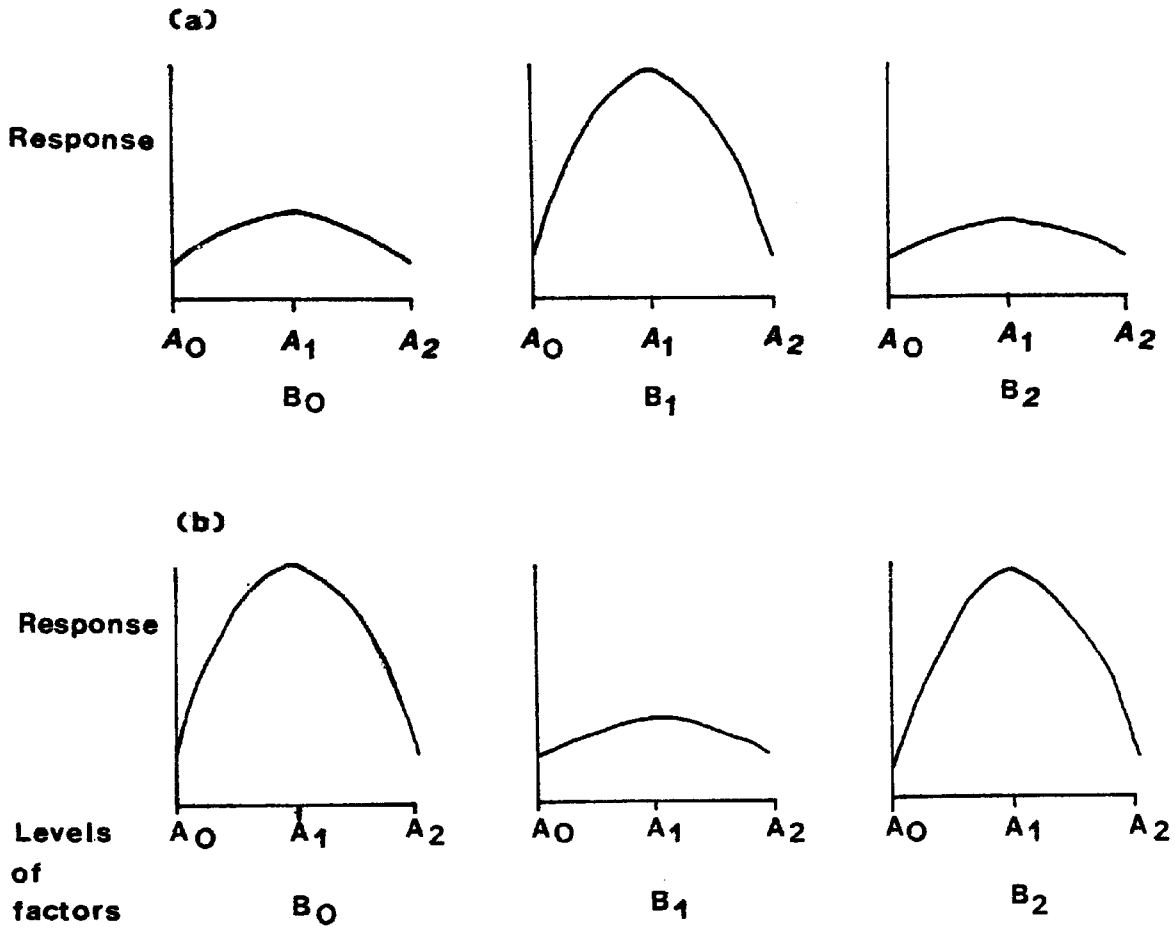


Figure 3.4 Typical effects of an interaction between the quadratic components of factors A and B (a) positive; (b) negative



which there were no replications of the treatments, an estimate of error variance based on the highest order (three factor) interaction was used. This is customary and may be justified on the grounds that the highest order effects are often negligible. In the experiment with two replicates, the error due to replication was used as the estimate of error variance freeing the degrees of freedom associated with the three factor interaction from the calculation of the error.

3.3.1 Experiment 1

The results of this experiment may be divided into those associated with the effects upon yield and those dealing with the effect upon the concentrations of heavy metal.

3.3.1.1 Yields Table 2 in Appendix B lists the total plant, shoot and root yields per replicate obtained in the first experiment arranged in the standard order. These results were entered into the calculator which produced a complete analysis of variance for each set of results. The effects upon yield (positive or negative), variance ratios and their significances are listed in Table 3.2. The addition of copper to the solutions produced linear effects (negative) upon the yields of shoots, roots and total plants. However neither Cd nor Ni was found to have affected any of the yields. There were several interesting interactions which had not been suspected before. Negative significant effects by the quadratic Cd * linear Ni interactions affected the total yields of the plants, and shoots and roots separately. There was also a positive linear Cd * quadratic Cu interaction which significantly affected the yield of the roots.

3.3.1.2 Concentrations of heavy metals The concentrations of Cd, Cu

Table 3.2

Variance ratios and significances for effects of treatments upon yields in factorial experiment 1

	Variance ratios		
	Total plant yields	Shoot yields	Root yields
Lin Cd	1.16	0.56	4.20
Quad Cd	-0.05	0.00	-0.77
Lin Cu	-89.24**	-79.22**	-102.87**
Quad Cu	-0.48	-1.16	0.53
Lin Ni	-1.47	-1.29	-1.74
Quad Ni	-1.28	-0.97	-2.27
Lin Cd* lin Cu	1.93	3.10	0.01
Lin Cd* quad Cu	0.50	0.04	5.59*
Quad Cd* lin Cu	-3.96	-3.89	-3.32
Quad Cd* quad C	0.02	0.12	-0.41
Lin Cd* lin Ni	0.59	0.52	0.70
Lin Cd* quad Ni	1.24	0.83	2.76
Quad Cd* lin Ni	-6.73*	-6.46*	-5.97*
Quad Cd* quad Ni	0.01	0.01	0.04
Lin Cu* lin Ni	0.42	0.76	-0.04
Lin Cu* quad Ni	-0.35	-0.64	0.04
Quad Cu* lin Ni	0.39	0.24	0.97
Quad Cu* quad N	0.88	0.90	0.60

* Significant, $p<0.05$.
** Highly significant, $p<0.01$.

Table 3.3

Variance ratios and significances for effects of treatments upon Cd, Cu and Ni concentrations in factorial experiment 1

	Variance ratios					
	Shoots			Roots		
	Cd	Cu	Ni	Cd	Cu	Ni
Lin Cd	57.08**	-2.95	-5.81*	232.05**	-4.67	-4.51
Quad Cd	-20.53**	-2.98	1.01	-9.03*	-0.29	1.07
Lin Cu	52.88**	290.43**	49.97**	-130.10**	-63.83**	-2.69
Quad Cu	22.76**	41.77**	6.30*	41.45**	0.59	2.96
Lin Ni	1.72	6.32*	94.55**	-0.19	4.56	20.14**
Quad Ni	0.77	-8.07*	0.16	5.27	0.00	-0.89
Lin Cd* lin Cu	9.90*	-0.93	-1.04	-91.44**	-3.03	7.51*
Lin Cd* quad Cu	-22.13**	-5.34*	-2.67	-2.69	-0.78	-3.12
Quad Cd* lin Cu	8.21*	0.54	0.54	39.08**	-0.01	-0.71
Quad Cd* quad Cu	2.86	-2.77	-0.07	5.39*	-0.71	0.97
Lin Cd* lin Ni	-0.01	-2.61	-2.29	-2.49	-0.03	-2.62
Lin Cd* quad Ni	-4.04	-1.79	-3.17	-1.96	-1.13	0.24
Quad Cd* lin Ni	0.04	3.32	0.53	0.73	0.03	0.04
Quad Cd* quad Ni	-0.92	-2.16	-2.83	-2.99	-2.36	-0.38
Lin Cu* lin Ni	1.85	5.17	35.37**	0.50	3.33	-1.74
Lin Cu* quad Ni	0.49	0.18	5.36*	-0.35	0.05	0.84
Quad Cu* lin Ni	0.03	-9.20*	0.13	0.03	-0.01	0.00
Quad Cu* quad Ni	-0.86	-1.57	0.17	-1.92	-0.22	-0.71

* Significant, $p<0.05$.
** Highly significant, $p<0.01$.

and Ni were determined in the shoots and roots of the 27 replicates and these are listed respectively in Tables 3 and 4 in Appendix B. The analyses of variance were calculated as above and the signs of the effects, variance ratios and their significances are listed in Table 3.3.

As would be expected, there were significant positive main effects upon the concentrations of Cd, Cu and Ni in shoots and roots caused by the linear components of each of these elements. There were also some significant interactions. Copper affected the shoot and root concentrations of both cadmium and nickel and was also involved in several significant interactions. There were other effects and interactions involving cadmium and nickel, but were fewer than those involving copper.

3.3.2 Experiment 2

The results of this experiment may be divided into three sections dealing with the yield, concentrations of heavy metals and nutrients.

3.3.2.1 Yields The yields of the total plants, shoots and roots per treatment are listed in standard order in Table 5, Appendix B. The analyses of variance (Table 3.4) provided some interesting comparisons with the first experiment. In the former, the only significant main effects were those involving the linear Cd and Cu components. Nickel had no significant effects upon the yields. It is interesting to note that there were no significant interactive effects in this experiment, whereas the first experiment revealed significant negative quadratic Cd * linear Ni effects upon the total yields of the plants, and shoots and

Table 3.4

Variance ratios and significances for effects of treatments upon yields in factorial experiment 2

	Variance ratios		
	Total plant yields	Shoot yields	Root yields
Lin Cd	-25.47**	-30.24**	-14.75**
Quad Cd	0.01	0.30	-1.69
Lin Cu	-80.14**	-98.53**	-59.00**
Quad Cu	-0.14	-0.50	-1.94
Lin Ni	-1.28	-0.96	-2.16
Quad Ni	-0.03	0.04	-1.32
Lin Cd* lin Cu	1.61	2.22	-0.22
Lin Cd* quad Cu	2.41	2.37	0.47
Quad Cd* lin Cu	2.90	2.13	0.01
Quad Cd* quad Cu	-1.24	-3.43	-0.58
Lin Cd* lin Ni	1.15	2.51	-0.12
Lin Cd* quad Ni	0.44	0.29	1.33
Quad Cd* lin Ni	-0.16	-0.77	0.82
Quad Cd* quad Ni	0.48	1.97	-1.57
Lin Cu* lin Ni	-2.70	-3.36	-1.57
Lin Cu* quad Ni	0.29	0.30	0.31
Quad Cu* lin Ni	-0.01	0.41	-0.16
Quad Cu* quad Ni	-3.77	-2.38	-0.09

* Significant, $p<0.05$.
** Highly significant, $p<0.01$.

Table 3.5

Variance ratios and significances for effects of treatments upon Cd, Cu and Ni concentrations in factorial experiment 2

	Variance ratios					
	Shoots			Roots		
	Cd	Cu	Ni	Cd	Cu	Ni
Lin Cd	64.58**	4.13	-1.06	27.32**	24.2**	-3.45
Quad Cd	-3.90	1.13	-0.02	-25.72**	298.00**	-0.65
Lin Cu	-11.32**	41.37**	1.58	-57.68**	1373.0**	0.81
Quad Cu	12.09**	2.86	1.35	33.03**	16.48**	4.10
Lin Ni	0.02	2.27	6.97*	-2.81	-4.69	7.36*
Quad Ni	-0.11	-0.32	0.66	0.09	-2.05	0.22
Lin Cd* lin Cu	-2.46	4.32	-0.89	-16.41**	29.54**	-0.03
Lin Cd* quad Cu	8.74*	1.77	0.26	41.94**	202.00**	-1.88
Quad Cd* lin Cu	4.87	0.45	-0.97	4.47	5.67*	-1.94
Quad Cd* quad Cu	-0.43	0.47	-0.01	-9.15*	-0.01	-0.03
Lin Cd* lin Ni	0.04	0.25	-1.75	0.31	-0.01	-3.60
Lin Cd* quad Ni	0.38	0.14	0.61	1.14	1.52	0.95
Quad Cd* lin Ni	-0.98	-0.97	-0.48	-0.24	-3.35	-0.34
Quad Cd* quad Ni	-1.30	-1.20	0.01	0.11	0.24	0.01
Lin Cu* lin Ni	1.01	1.56	1.02	0.11	0.24	0.01
Lin Cu* quad Ni	0.00	0.00	1.32	-2.44	-2.83	-0.96
Quad Cu* lin Ni	-2.58	-1.02	1.21	0.29	-7.00*	2.25
Quad Cu* quad Ni	-0.31	-0.68	0.21	0.08	-6.98*	0.11

* Significant, $p<0.05$.
** Highly significant, $p<0.01$.

roots separately.

3.3.2.2 Concentrations of heavy metals The concentrations of heavy metals in shoots (Table 6, Appendix B) and roots (Table 7, Appendix B) were determined and the analysis of variance were calculated and are listed in Table 3.5.

In the shoots, there were again linear effects upon the concentrations of the elements added and also a negative effect by Cu upon the concentration of Cd. Only one interaction was significant: this was a positive interaction between the linear Cd and quadratic Cu components which tended to decrease the Cd shoot concentrations at the intermediate level of Cu. In the roots the linear Cd, Cu and Ni components had the expected main effects. There were also several significant interactive effects, though these always involved a component of the element whose response was being determined.

3.3.2.3 Concentrations of nutrients The nutrient concentrations of the shoots and roots are listed in Tables 8 and 9, Appendix B respectively. Calculations of the main effects and interactions between the heavy metals yielded the analyses of variance for shoot nutrient concentrations (Table 3.6) and root nutrient concentrations (Table 3.7). These tables show that there were many interactions between the heavy metals which affected the nutrient concentrations in the shoots, but that only manganese was affected in the roots.

3.3.3 Experiment 3

As this experiment was conducted with two replicates, a more complex analysis of variance was performed. A calculation of the

Table 3.6

Variance ratios and significances for effects of treatments upon shoot nutrient concentrations in factorial experiment 2

	Variance ratios			
	Zn	Mg	Mn	Ca
Lin Cd	0.08	1.73	-22.30**	1.22
Quad Cd	2.65	1.78	38.00**	1.02
Lin Cu	18.29**	0.21	-1.22	-12.22**
Quad Cu	6.62*	10.92*	97.91**	5.16
Lin Ni	4.99	-0.60	-0.73	-2.17
Quad Ni	-0.27	0.10	0.34	-0.07
Lin Cd* lin Cu	0.01	6.13*	86.61**	-5.38*
Lin Cd* quad Cu	3.79	0.60	-43.14**	-0.50
Quad Cd* lin Cu	-0.31	-1.68	-53.35**	-0.01
Quad Cd* quad Cu	1.33	0.12	0.83	0.28
Lin Cd* lin Ni	0.60	0.02	0.98	-1.31
Lin Cd* quad Ni	2.66	-0.55	-2.96	0.44
Quad Cd* lin Ni	0.99	0.01	0.21	-0.13
Quad Cd* quad Ni	-0.86	0.19	0.44	-0.02
Lin Cu* lin Ni	6.16*	3.88	3.83	-0.27
Lin Cu* quad Ni	1.76	-0.01	-0.02	-0.01
Quad Cu* lin Ni	-0.98	-0.03	4.66	-3.04
Quad Cu* quad Ni	-0.23	0.00	-4.46	0.02

* Significant, $p<0.05$.
** Highly significant, $p<0.01$.

Table 3.7

Variance ratios and significances for effects of treatments upon root nutrient concentrations in factorial experiment 2

	Variance ratios			
	Zn	Mg	Mn	Ca
Lin Cd	-0.01	0.08	-69.13**	0.02
Quad Cd	-2.05	-1.02	19.78**	-2.04
Lin Cu	0.01	0.00	-53.27**	-0.15
Quad Cu	-1.82	-0.58	26.00**	2.29
Lin Ni	1.49	-0.92	-0.04	-0.11
Quad Ni	0.45	0.45	1.54	1.99
Lin Cd* lin Cu	-0.01	0.02	96.37**	0.10
Lin Cd* quad Cu	0.00	0.06	-35.41**	-4.91
Quad Cd* lin Cu	0.00	-0.13	25.42**	0.06
Quad Cd* quad Cu	4.16	3.06	6.27*	0.77
Lin Cd* lin Ni	0.00	0.01	0.33	-0.24
Lin Cd* quad Ni	-2.91	1.95	-0.29	-0.20
Quad Cd* lin Ni	0.01	0.00	2.60	-0.13
Quad Cd* quad Ni	-1.16	-0.65	-2.63	3.91
Lin Cu* lin Ni	0.00	0.24	0.69	0.00
Lin Cu* quad Ni	-3.08	4.32	-0.11	-0.10
Quad Cu* lin Ni	0.00	0.04	3.81	-1.34
Quad Cu* quad Ni	-1.07	-1.69	-0.39	-0.83

* Significant, $p<0.05$.
** Highly significant, $p<0.01$.

Table 3.8

Variance ratios and significances for effects of treatments upon yields in factorial experiment 2

	Variance ratios		
	Total plant yields	Shoot yields	Root yields
Lin Cd	-0.61	-0.65	-0.23
Quad Cd	0.12	0.15	0.01
Lin Cu	-4.22*	-5.70*	-1.61
Quad Cu	-0.23	-0.21	-1.37
Lin Ni	-0.82	-0.82	-0.69
Quad Ni	-0.03	0.00	-0.07
Lin Cd* lin Cu	1.47	1.63	-1.45
Lin Cd* quad Cu	-0.24	-0.46	0.01
Quad Cd* lin Cu	0.42	0.38	0.68
Quad Cd* quad Cu	-0.04	-0.05	-0.06
Lin Cd* lin Ni	0.00	0.00	0.09
Lin Cd* quad Ni	-1.30	-1.53	-0.66
Quad Cd* lin Ni	0.28	0.20	0.19
Quad Cd* quad Ni	0.03	0.04	0.04
Lin Cu* lin Ni	0.01	0.14	0.41
Lin Cu* quad Ni	-0.02	0.00	-0.16
Quad Cu* lin Ni	-0.26	-0.10	-0.48
Quad Cu* quad Ni	0.08	0.08	0.14
Cd* Cu* Ni	1.32	1.46	1.05

* Significant, $p<0.05$.
** Highly significant, $p<0.01$.

Table 3.9

Variance ratios and significances for effects of treatments upon shoot heavy metal concentrations in factorial experiment 3

	Variance ratios		
	Cd	Cu	Ni
Lin Cd	34.92**	-1.13	-0.40
Quad Cd	0.43	2.19	0.37
Lin Cu	-6.65*	30.97**	-11.14**
Quad Cu	0.00	-0.11	7.72*
Lin Ni	-2.29	0.06	152.23**
Quad Ni	0.18	3.01	-1.40
Lin Cd* lin Cu	-5.28*	-0.01	1.76
Lin Cd* quad Cu	0.22	1.10	-0.22
Quad Cd* lin Cu	-0.49	0.88	-0.20
Quad Cd* quad Cu	-1.01	0.00	0.47
Lin Cd* lin Ni	-2.30	0.27	0.02
Lin Cd* quad Ni	-0.71	0.44	-0.13
Quad Cd* lin Ni	0.14	-0.07	1.30
Quad Cd* quad Ni	0.03	0.12	-2.19
Lin Cu* lin Ni	0.12	0.10	-2.85
Lin Cu* quad Ni	4.76*	0.09	2.95
Quad Cu* lin Ni	0.73	1.92	3.24
Quad Cu* quad Ni	-0.97	0.00	-0.92
Cd* Cu* Ni	1.16	0.90	2.30

* Significant, $p<0.05$.
** Highly significant, $p<0.01$.

Table 3.10

Variance ratios and significances for effects of treatments upon root heavy metal concentrations in factorial experiment 3

	Variance ratios		
	Cd	Cu	Ni
Lin Cd	113.33**	1.22	-23.94**
Quad Cd	-2.54	-0.07	25.32
Lin Cu	-64.01**	257.72**	-62.40**
Quad Cu	10.71**	-0.03	38.84**
Lin Ni	-0.37	2.37	271.13**
Quad Ni	0.00	4.11	-9.03**
Lin Cd* lin Cu	-23.71**	0.75	41.59**
Lin Cd* quad Cu	4.02	-0.01	-29.84**
Quad Cd* lin Cu	1.86	-0.22	-13.69**
Quad Cd* quad Cu	-1.78	0.34	13.57**
Lin Cd* lin Ni	-0.25	-0.31	-0.28
Lin Cd* quad Ni	-0.20	-1.36	2.49
Quad Cd* lin Ni	0.41	0.55	22.95**
Quad Cd* quad Ni	1.86	0.01	-18.94**
Lin Cu* lin Ni	0.25	3.67	-8.06**
Lin Cu* quad Ni	0.34	1.31	10.89**
Quad Cu* lin Ni	1.59	3.15	48.42**
Quad Cu* quad Ni	-1.00	-0.15	-9.79**
Cd* Cu* Ni	0.40	0.48	12.25**

* Significant, $p<0.05$.
** Highly significant, $p<0.01$.

significance of the three factor interaction was possible, since the error due to the difference between the replicates was used as the experimental error, freeing the 8 degrees of freedom belonging to the three factor interaction.

3.3.3.1 Yields The total yields of plants, shoot and root yields in replicates 1 and 2 are listed in Tables 10 and 11, Appendix B. Results of the statistical analysis giving the signs of the significant effects upon yield, variance ratios and the degree of their significances are presented in Table 3.8. The important points to note about these results are the lack of main effects and interactions involving cadmium and nickel, even though their concentrations were increased with respect to the previous experiments: copper retaining its effect upon total plant and shoot yields per treatment but failing to affect root yields.

3.3.3.2 Concentrations of heavy metals Heavy metal concentrations in the shoots and roots of replicates 1 and 2 (Tables 12-15, Appendix B) were subjected to the analysis of variance as previously. The signs of the effects, variance ratios and their significances are listed as Table 3.9 (shoots) and Tables 3.10 (roots). In the shoots (Table 3.9) there were significant main effects by Cd, Cu and Ni upon their respective concentrations ($p<0.01$ in all cases). There were also some main effects by copper upon the concentrations of other metals and some interactions involving Cu which affected Cd shoot concentrations.

In the roots (Table 3.10) there were the expected significant main effects upon the concentrations of the metals actually added, and also the copper effects found in the previous experiments. No factor significantly affected the copper concentrations in the roots apart from

Table 3.11

Variance ratios and significances for effects of treatments upon shoot nutrient concentrations in factorial experiment 3

	Variance ratios			
	Zn	Mg	Mn	Ca
Lin Cd	0.26	0.01	-28.63**	0.42
Quad Cd	-2.70	-0.05	8.28**	-0.31
Lin Cu	0.00	-3.02	-40.35**	-1.44
Quad Cu	0.26	9.69**	23.47**	0.81
Lin Ni	0.26	0.36	-0.02	0.45
Quad Ni	0.09	0.14	-1.52	0.05
Lin Cd* lin Cu	-0.07	-0.22	40.38**	-2.71
Lin Cd* quad Cu	0.25	-0.77	-18.77**	-0.77
Quad Cd* lin Cu	-1.14	-0.38	-13.09**	0.58
Quad Cd* quad Cu	-3.02	0.05	2.72	0.07
Lin Cd* lin Ni	0.26	0.04	8.87**	0.17
Lin Cd* quad Ni	-0.03	0.01	-0.08	-0.27
Quad Cd* lin Ni	0.39	-0.06	-0.29	0.53
Quad Cd* quad Ni	0.29	0.41	0.52	-0.02
Lin Cu* lin Ni	0.68	0.19	5.68*	0.98
Lin Cu* quad Ni	0.23	0.26	0.28	0.04
Quad Cu* lin Ni	0.42	0.19	1.13	0.05
Quad Cu* quad Ni	-0.24	-0.06	-0.57	0.09
Cd* Cu* Ni	1.33	1.29	2.29	0.32

* Significant, $p<0.05$.
** Highly significant, $p<0.01$.

Table 3.12

Variance ratios and significances for effects of treatments upon root nutrient concentrations in factorial experiment 3

	Variance ratios			
	Zn	Mg	Mn	Ca
Lin Cd	0.32	-66.80**	-1.55	-0.19
Quad Cd	1.13	9.79**	0.05	-3.84
Lin Cu	1.39	-69.42**	-4.51*	-0.80
Quad Cu	2.78	29.04**	13.20**	1.64
Lin Ni	0.55	-17.62**	-1.06	1.15
Quad Ni	-0.15	-0.12	-0.52	0.12
Lin Cd* lin Cu	3.40	66.32**	-1.37	0.00
Lin Cd* quad Cu	1.69	-35.05**	0.03	-3.16
Quad Cd* lin Cu	0.82	-26.32**	-2.66	-0.20
Quad Cd* quad Cu	0.10	10.49**	0.02	-0.06
Lin Cd* lin Ni	0.16	20.50**	0.22	0.00
Lin Cd* quad Ni	0.26	-5.95*	0.90	-0.77
Quad Cd* lin Ni	-0.02	0.16	0.07	-0.01
Quad Cd* quad Ni	-0.04	-0.18	-0.14	-0.09
Lin Cu* lin Ni	0.53	25.87**	5.29*	4.08
Lin Cu* quad Ni	-0.02	-9.62**	-0.71	0.23
Quad Cu* lin Ni	-0.38	1.54	3.01	0.41
Quad Cu* quad Ni	-0.71	-0.64	-0.86	-0.10
Cd* Cu* Ni	0.10	8.68**	1.16	0.38

* Significant, $p<0.05$.
** Highly significant, $p<0.01$.

copper itself. Nickel however, was affected by every component except those involving quadratic Cd components in combination with a Ni component. The complex three factor Cd*Cu*Ni interaction was also significant.

3.3.3.3 Concentrations of Nutrients The shoot and root concentrations of Zn, Mg, Mn and Ca were determined and the results for both replicates are listed in Tables 16-19, Appendix B. Analyses of variance were performed as previously described and the results are shown in Tables 3.11 (shoots) and 3.12 (roots). There were few effects upon the concentrations in either shoots or roots of Zn, Mg and Ca; notable exceptions being effects by Cu upon shoot and root Mg concentrations and a positive lin Cu* lin Ni interaction which increased root Mg concentrations. There was an abundance of effects upon both shoot and root concentrations of Mn, which were also noted in the previous experiment (see Tables 3.6 and 3.7).

3.4 Discussion

In some experiments there were highly significant effects produced by some factors and combinations of components which were not present in subsequent experiments. These cannot be ignored purely because they only occurred once. Conditions, although generally similar, could have changed from experiment to experiment sufficiently to alter the results shown. As well as the changes produced as a result of altering the levels of factors in the third experiment, variations in the first two may have been produced by uncontrolled variables such as the highest temperature attained in the propagator and differences in treatment between the batches.

As a result of the above observations it was decided to interpret the effects produced in light of their significances ($p<0.05$ or 0.01), whether the effects were similar in shoots and roots, if they were found in other experiments here or reported by other workers in other species. In this manner it was hoped that some meaningful interpretation was possible.

3.4.1 Yields

Linear Cu components significantly reduced yields in all the experiments except the roots in Experiment 3. Cadmium main effects were less apparent; only in Experiment 2 did the linear Cd component reduce the seedling yields ($p<0.01$ for total plant, shoot and root yields). Nickel main effects were not found, although it was involved in several interactions with Cd in the first experiment.

When interactions can be assumed negligible, it may be inferred that the factors operate independently, and conclusions based on the significance of the main effects may be legitimately drawn (Davies 1979). On this basis it can be said that the effects of Cd and Cu upon yield are additive since in Experiment 2 they both induced very significant reductions in the total yields and yields of the shoots and roots of the seedlings.

Conditions were such that the main effects of Ni in all the experiments and Cd in Experiments 1 and 3 were not evident. Therefore, it is not possible to state whether Ni operates independently of either Cd or Cu in relation to effects upon yield. However interactive effects of the metals Cu, Ni and Zn upon the yields of young spring barley have been found, using a non-factorial experiment design, to be additive or partially additive above certain threshold levels of inactivity which

are below the critical levels of the elements (Beckett and Davis 1978). By calculating values of expected growth for combined Pb and Cd treatments from a multiplication of the reductions in growth due to separate heavy metal treatments, it was shown that there were synergistic reductions in the growth of American sycamore (Plantanus occidentalis.L). Several growth parameters, including diameter, new stem and root growth and foliage biomass, were shown to be more affected by a combination of Pb and Cd treatments than that expected from an addition of the two values (Carlson and Bazzaz 1977).

It is interesting to note that the information on these interactions, though incomplete, have been established by separate techniques and show that the effects of the heavy metals upon growth are all at least partially additive. This may prove of some help when examining the relative hazards due to the heavy metals at the forestry sites.

3.4.2 Concentrations of heavy metals

There were many effects by the elements alone and interactions which influenced the uptake of heavy metals. Main effects by the linear components of the heavy metal, which had been added, are expected and therefore of little interest. However, there were main effects by heavy metals added to the solution which influenced the concentrations of another metal.

3.4.2.1. Cadmium All significant effects of 95, 99% probability or greater which influenced the concentrations of Cd in the shoots and roots are listed according to experiment in Table 3.13. Linear components of Cd increased Cd shoot and root concentrations in all the

Table 3.13

Treatments producing significant effects upon cadmium concentrations in factorial experiments 1,2 and 3

	Exp.1	Exp.2	Exp.3
Shoots			
	+ Lin Cd	+ Lin Cd	+ Lin Cd
	- Quad Cd		
	+ Lin Cu	- Lin Cu	- Lin Cu
	+ Quad Cu	+ Quad Cu	
	+ Lin Cd*lin Cu		- Lin Cd*lin Cu
	- Lin Cd*quad Cu	+ Lin Cd*quad Cu	
	+ Quad Cd*lin Cu		
	+ Lin Cu*quad Ni		
Roots			
	+ Lin Cd	+ Lin Cd	+ Lin Cd
	- Quad Cd	- Quad Cd	
	- Lin Cu	- Lin Cu	- Lin Cu
	+ Quad Cu	+ Quad Cu	+ Quad Cu
	- Lin Cd*lin Cu	- Lin Cd*lin Cu	- Lin Cd*lin Cu
		- Lin Cd*quad Cu	
	+ Quad Cd*lin Cu		
	+ Quad Cd*quad Cu	- Quad Cd*quad Cu	

experiments. In several cases this was accompanied by a negative quadratic component which signified a tendency for the Cd concentration to increase to a maximum at the intermediate level, possibly in the form of a concentration plateau above which Cd could not be accumulated by the plants.

Table 3.13 also shows the effect that Cu had upon Cd accumulation. In all the experiments, negative linear and positive quadratic Cu components reduced the root Cd concentrations. This was also reflected in the effects of Cu upon shoot Cd levels; 2 of the 3 experiments having negative linear and positive quadratic Cu components.

This effect has been shown in many studies: plant Cd and Ni concentrations were reduced by additions of Cu (Cataldo and Wilding 1978), and Cd concentrations in lettuce leaves (Lactuca sativa) were reduced by additions of copper to the soil (Lepp 1981), although others have shown the opposite effect in corn and rye plants (Cunningham et al 1975). In that study it was noted that the pattern of interaction was more consistent if treatments causing severe phytotoxicity are deleted from consideration. In this study, nickel had no main effects nor was it involved in any interactions which influenced Cd concentrations.

The interactions which affected Cd concentrations (Table 3.13) were many and difficult to interpret precisely. The most consistent interaction was that between the linear components of Cd and Cu which reduced Cd root concentrations in all experiments. The presence of this interaction actually implies that, as Cd concentrations in the solutions were increased, the effect of copper in reducing root Cd concentrations became more important. This is essentially a competitive effect. In the shoots and roots there were several interactions which were inconsistent with regard to their effect even when the levels were kept the same, as

in Experiments 1 and 2.

It is interesting to note that, even though the effect of Cu upon Cd concentrations were significant in all experiments, in Experiment 2, where Cd main effects reduced the yields of the seedlings, there were no interactive effects involving Cd and Cu. This indicates that reductions in Cd levels brought about by the Cu treatments were either insufficient to have any effect upon its phytotoxicity or that the Cd toxicity was not totally dependent upon the total amount of Cd taken up by the roots.

3.4.2.2 Copper All significant effects of 95, 99% or greater probability which influenced plant Cu concentrations are listed, according to experiment, in Table 3.14. Copper shoot and root levels were mainly influenced by the linear Cu components, although two quadratic components (one in shoots and another in roots) were significant. Neither shoot nor root concentrations were affected by the action of Cd alone, although Cd* Cu interactions were present.

Linear and quadratic Ni components increased shoot Cu levels in Experiment 1, but did not have effects in any other experiment. This was in agreement with one report (Veltrup 1979), where it was found that root Cu accumulation into intact Barley plants (Hordeum vulgare) was increased over two hours due the presence of Ni in solution. However, others have reported no such effects upon accumulation of Cu by the roots of sycamore (Lepp and Eardley 1978). Significant interactions between Cu and Ni were determined as negative quad Cu* lin Ni in one experiment in shoots and in another in roots, which also had a negative quad Cu* quad Ni interaction. This again demonstrates the complexity of the system, which cannot be that easily interpreted.

Interactions between Cd and Cu were significant in their effects

Table 3.14

Treatments producing significant effects upon copper concentrations in factorial experiments 1,2 and 3

Exp.1	Exp.2	Exp.3
Shoots		
+ Lin Cu	+ Lin Cu	+ Lin Cu
- Quad Cu		
+ Lin Ni		
- Quad Ni		
- Lin Cd*quad Cu		
- Quad Cu*lin Ni		
Roots		
+ Lin Cu	+ Lin Cu	+ Lin Cu
	+ Quad Cu	
	+ Lin Cd*lin Cu	
	+ Lin Cd*quad Cu	
	+ Quad Cd*lin Cu	
	- Quad Cu*lin Ni	
	- Quad Cu*quad Ni	

upon root Cu concentrations in Exp.2 and also shoot levels in Exp.1. The lin Cd* lin Cu interaction was positive meaning that root Cu concentrations were increased when both were present at the highest levels. However the quad Cd* lin Cu and lin Cd* quad Cu interactions were both positive indicating the tendency of Cd at all levels to decrease the root Cu uptake (e.g. Figure 3.3a).

The involvement of Cd in determining plant Cu concentrations has been examined previously, but these results were inconsistent since it has been shown that Cd could enhance shoot Cu concentrations in lettuce tops (John 1976), had no effect on Cu uptake by metal tolerant populations of Holcus lanatus (Coughtrey and Martin 1979), and reduced Cu uptake by bushbeans (Wallace et al 1977). The inconsistency of these effects shown here and those demonstrated by others makes a meaningful interpretation very difficult.

3.4.2.3 Nickel All significant effects of 95, 99% or greater probability which influenced Ni concentrations are listed in Table 3.15. The shoot concentrations of Ni in the first experiment were affected by positive linear Cu and Ni components which also interacted to increase Ni concentrations. The linear Cu component in Exp.3 however reduced shoot and root Ni levels. The effect by Cu is therefore inconsistent and cannot easily be interpreted. Inconsistent effects by Cu have also been reported in corn (Cunningham et al 1975).

Experiment 3 yielded a multitude of significant main effects and interactions ($p<0.01$). Linear (as stated above) and quadratic Cu components reduced Ni levels in shoots and roots; reductions were also effected by the Cd components in the roots. All interactions between Cd and Cu were significant in increasing root Ni levels and all those

Table 3.15

Treatments producing significant effects upon nickel concentrations in experiments 1,2 and 3

Shoots		
Exp.1	Exp.2	Exp.3
- Lin Cd		
+ Lin Cu		- Lin Cu
+ Quad Cu		+ Quad Cu
+ Lin Ni	+ Lin Ni	+ Lin Ni
+ Lin Cu*Lin Ni		
+ Lin Cu*quad Ni		
Roots		
		- Lin Cd
		+ Quad Cd
		- Lin Cu
		+ Quad Cu
+ Lin Ni	+ Lin Ni	+ Lin Ni
		- Quad Ni
+ Lin Cd*lin Cu		+ Lin Cd*lin Cu
		- Lin Cd*quad Cu
		- Quad Cd*lin Cu
		+ Quad Cd*quad Cu
		+ Quad Cd*lin Ni
		+ Quad Cd*quad Ni
		- Lin Cu*lin Ni
		+ Lin Cu*quad Ni
		+ Quad Cu*lin Ni
		- Quad Cu*quad Ni
		Cd*Cu*Ni

between Cu and Ni were significant in reducing root Ni levels (see Table 3.15). Two interactions between Cd and Ni significantly increased root Ni concentrations, these were quad Cd* lin Ni and quad Cd* quad Ni. There was also a significant three factor interaction. The most interesting point to note about this experiment (Exp. 3) was that the interactions between two metals all either increased or decreased the root Ni concentration. Such interactions have not been reported previously.

3.4.3 Nutrient concentrations

The interactions of heavy metals have usually been studied with the aim of reducing heavy metal levels in crops. Here the effects of heavy metals upon nutrient concentrations both singly and in combination have been assessed and have brought to light several interesting effects which have not been reported before.

3.4.3.1 Zinc There were only three significant effects by the treatments upon Zn concentrations; both in Experiment 2. The shoot Zn levels were increased by the two Cu treatments ($p<0.01$ in both cases). Most results have demonstrated the opposite effect where Zn uptake was strongly inhibited by Cu (Chaudry and Loneragan 1972, Bowen 1969 and Schmid et al 1965). It has been pointed out that the situation in soil and water culture could be very different, due in particular to the presence of chelates and Cu-colloid interactions in the soil. In an examination of Zn uptake by excised barley roots, a slight stimulation by Cu was found (Veltrup 1979), and it was concluded that Zn and Cu were taken up by separate mechanisms. This conclusion is supported by the results presented here.

Table 3.16

Treatments producing significant effects upon manganese concentrations in factorial experiments 2 and 3

Exp.2	Exp.3
Shoots	
- Lin Cd	- Lin Cd
+ Quad Cd	+ Quad Cd
+ Lin Cu	- Lin Cu
	+ Quad Cu
+ Lin Cd*lin Cu	+ Lin Cd*lin Cu
- Lin Cd*quad Cu	- Lin Cd*quad Cu
- Quad Cd*lin Cu	- Quad Cd*lin Cu
Roots	
- Lin Cd	- Lin Cd
+ Quad Cd	+ Quad Cd
- Lin Cu	- Lin Cu
+ Quad Cu	+ Quad Cu
	- Lin Ni
+ Lin Cd*lin Cu	+ Lin Cd*lin Cu
- Lin Cd*quad Cu	- Lin Cd*quad Cu
+ Quad Cd*lin Cu	- Quad Cd*lin Cu
+ Quad Cd*quad Cu	+ Quad Cd*quad Cu
	+ Lin Cd*lin Ni
	- Lin Cd*quad Ni

Several groups of workers have reported antagonistic effects of Cd upon Zn uptake (Coughtrey and Martin 1979, Koshino 1973, Lagerwerff and Biersdorf 1972, Saitoh et al 1973), though no significant effects by Cd have been found here.

One positive interaction between lin Cu and lin Ni was significant in increasing the root Zn levels in both experiments, though no reports of this have been found in the literature.

3.4.3.2 Magnesium Studies of the influence of heavy metals upon Mg levels have not been reported in the literature and, although positive quadratic effects by Cu were significant in the shoots in both experiments, no linear effects were found, making any appreciation of the results difficult. However, in Experiment 3 both the linear and quadratic Cu components reduced root Mg concentrations and a lin Cu* lin Ni interaction increased root Mg levels.

3.4.3.3 Manganese Many of the different treatments affected Mn levels in Experiments 2 and 3, the significant effects being listed in Table 3.16. Both linear and quadratic Cd components significantly reduced shoot and root Mn levels in Experiments 2 and 3 ($p<0.01$ in all cases), this being in agreement with the results discussed in Chapter 2 (Section 2.4.4), and with several reports in the literature of Cd effects in other plants. Cadmium depressed the Mn uptake of bushbeans (Wallace et al 1977) and reduced Mn concentrations in tops of lettuce and roots of oats (John 1976).

In an effort to develop a biochemical diagnostic method for detecting deficiencies and excesses of certain trace elements, it has been shown that Mn deficiencies could be characterized by an increase in

activity of the enzyme peroxidase in plants grown under field conditions. It has also been found that peroxidase activity increases as soil Cd levels increase (Cottenie 1981). In light of the above results it would seem that there is a link between Cd toxicity and Mn deficiency in several plant species.

Linear and quadratic Cu components reduced the levels of Mn in the roots in both experiments and in the shoots in Exp.3. However lin Cu increased shoot Mn levels in Exp.3. Reports of interactions between Cu and Mn have not been found in the literature and it is not known exactly how extensive this effect is in other plants. There was also an unaccountable negative linear Ni effect in the roots (Exp.3).

Significant interactions between Cd and Cu were consistently present in both the shoots and roots in Experiments 2 and 3. In the shoots, the respective Cd and Cu lin* lin, lin* quad and quad* lin interactions all significantly increased Mn concentrations in both experiments (Tables 3.7 and 3.10). The presence of this interaction is difficult to interpret in view of the fact that both Cd and Cu reduced Mn levels in both shoots and roots. These interactions have not been reported previously. Other interactions present were positive lin Cu* lin Ni and negative lin Cu* quad Ni ($p<0.01$ for both cases), which increased root Mn concentrations in Experiment 3.

3.4.3.4 Calcium Two significant effects were found in Experiment 2. These were the negative lin Cu and a negative lin Cd* lin Cu interaction which have not, as far as is known, been reported elsewhere.

3.5 Conclusions

Copper and cadmium reduced the yields of sitka–spruce seedlings

though the levels of nickel added were insufficient to produce this main effect. A few interactive effects upon yields were demonstrated, albeit inconsistently. Moreover, it has been shown that the toxic effects of the elements Cd and Cu in sitka-spruce seedlings may be regarded as at least partially additive, though it was not possible to elucidate how Ni behaves in relation to Cd and Cu or vice versa.

The effect of treatments on heavy metal and nutrient concentrations in the seedlings yielded an abundance of information on interactions. The presence of significant effects may clarify the mechanisms by which these elements exert their toxic actions and modify the toxic actions of others. It was noted that some of these effects consistently agreed with the results of other workers: these were the reductions in manganese uptake by cadmium, and the reduction of cadmium uptake by copper treatments. These effects are widespread, indicating that certain overriding principles govern their behaviour.

Some significant effects were found in all experiments, though these have either not been reported elsewhere, or reports of them were conflicting. Possible reasons for these discrepancies might be that different plant species were examined or that treatments were not really comparable.

Interactions not previously demonstrated between Cd and Cu increased nickel concentrations in the roots and also increased the manganese levels in the shoots and roots, even though both cadmium and copper separately reduced manganese levels (Section 3.4.3.3). This interaction was also shown consistently to reduce shoot and root cadmium concentrations. Another interaction not previously described was that between Cu and Ni which reduced nickel levels in the roots.

Several other interactive effects were shown to be of significance

in these experiments, though other studies are not in consistent agreement with these. Other apparently significant effects were neither consistent within these experiments nor within the literature, thus indicating that they were either erroneous, perhaps as a result of bad experimental design, or that they were only present in certain circumstances where other factors may influence their presence.

It has been shown that heavy metals can play important roles in the uptake of other heavy metals and nutrients into both the shoots and roots of sitka-spruce. Many of these interactions were of a high order and have not been described previously. Such effects, which reflect the complexity of the natural laws governing metal uptake, could not have been demonstrated if non-factorial designs had been used.

Chapter 4

SPECIATION IN THE NUTRIENT SOLUTIONS

4.1 Introduction

A knowledge of the equilibrium concentrations of metal ions and complex forming species which may be present in natural conditions or in water culture solutions is essential if one wishes to know how uptake of nutrients and other chemical species and the subsequent growth of plants is affected by metal ions. A particular metal may, for example, reduce the concentration of a phosphate species that is normally predominant, and thus indirectly influence plant growth. On the other hand the metal itself may be taken up into the plant by the roots and exert there a direct effect upon plant growth.

In the water culture experiments reported in Chapters 2 and 3, the speciation of metal ions in the solutions was not considered. It is important to separate the effects by heavy metals upon the nutrient concentrations in the medium and those brought about by more direct toxic actions in the plant. This can only be done if the equilibrium distribution of species present in the nutrient solution is known. Major factors that must be taken into consideration when such predictions of speciation of nutrient solutions are made are complexation by organic and inorganic species, pH, and redox potential. In the soil, speciation of metals is more complex and is governed by additional factors such as adsorption-desorption processes, and the fact that both pH and redox potential can vary significantly according to prevailing conditions (for

example, when certain soils become waterlogged, a combination of the redox potential and water content may produce a predominant cadmium sulphide species where cadmium sulphate would otherwise be present, Iimura and Ito,1978).

It has been shown that, in the soil solution, heavy metals such as copper, zinc and lead are predominantly complexed by organic molecules. In acidic conditions the soluble fraction of heavy metals will mainly consist of species complexed by fulvic acids and simpler organic and amino acids, though under alkaline conditions humic acids, which generally form very stable complexes with heavy metals, become more soluble (Cottenie et al 1979).

It is known, however, that in the region of soil immediately surrounding the root, known as the rhizosphere, there are potentially modifying influences. As well as large numbers of bacteria and fungi, a number of organic compounds, known to have been exuded by the plant roots, are present. These include sugars, amino acids, vitamins, organic acids, nucleotides, enzymes, terpenes and many unidentified compounds (Marks and Kozlowski 1973). From coniferous tree seedlings, in particular, it has been demonstrated that amino acids, carbohydrates and organic acids are exuded (Smith 1969). Amongst those factors which are known to influence the extent and composition of the exudates are the age of the plant, temperature, and utilization by microorganisms (Marks and Kozlowski 1973).

Though the situation is extremely complex and not well understood, it is clear that these organic molecules are potentially very important in relation to the uptake of heavy metals into plants, both in soil and water media. Amino acids, in particular, are present in the region around plant roots as a result of exudation, and also because they are

the natural products of mineralization of dead organic material. It is also known that amino acids form very stable complexes with heavy metals and may therefore play an important role in their uptake.

In studies involving the uptake of copper from solutions containing various copper complexes, it has been shown that a considerable proportion of the copper absorbed by excised barley (Hordeum) roots from a copper sulphate solution, in which the predominant copper species was hexaquo copper (charge 2+), was freely exchangeable with calcium ions (here copper was present in the free space within the root). This contrasted with the uptake patterns from solutions of copper glycine (charge 0) and bisethylenediamine (charge 2+). In these cases, a greater proportion was non-exchangeable with calcium ions, and it was concluded that the charge of the complex may have governed uptake, but that the structure and possibly stability governed the subsequent binding (Coombes et al 1978).

It was the aim of this study to use the computer programme COMICS to describe the equilibrium distribution of chemical species in an Ingestad's nutrient solution (Ingestad 1959) which had been doped with several different heavy metals. Having ascertained the equilibrium distribution, it was then hoped to gain a clearer picture of the influence that complexation of the heavy metals, specifically by amino acids, has upon their uptake.

4.2 Computation of speciation distribution

The aqueous solution chemistry of metal ions plays a central and dominant role in determining the interactions between plants and metals. Metal ions in solutions tend to act as "Lewis acids", where their

behaviour is governed by their ability to accept electrons. As Lewis acids they will react with a wide range of ligands. Quantitatively, the tendency for a metal-ligand interaction to occur is measured by the equilibrium constant for the reaction. Other factors that determine how these interact include the pH of the system, where hydrogen ions may compete with the metal ion for the ligand (Morel et al 1973), and hydrolysis reactions. One important restriction in the use of stability constants is that they apply only to equilibrium situations. However, if such limitations are considered, then stability constants may be used to describe aqueous metal ion/ligand systems. When used in combination with appropriate conservation equations, they allow a complete description of the distribution of the complexes.

A computer programme COMICS (Concentrations of Metal Ions and Complexing Species) has been developed for such calculations in a multi-metal multi-ligand mixture (Perrin and Sayce 1967). The computer programme utilizes an iterative method known as the Newton-Raphson procedure (Cracken and Dorn 1964). The information that is required is the pH of the solution, the total concentrations of each metal ion and ligand and the relevant equilibrium constants (pKa values and stability constants). One drawback is that all possible species must be considered and another is the reliability of K values in the literature. Otherwise it is possible to obtain a result from the iterative method which satisfies the criteria which have been set by the algorithm, and yet the result could be erroneous, since no account has been taken of a particular species which may in fact be present. There are other problems asssociated with computational speciation, but treated carefully the method is extremely useful (Phipps 1981).

The computer programme (COMICS) deals with a maximum of 10 metals

and 10 ligands, but can handle any number of complexes that may be formed. It is a general treatment for calculating concentrations in the presence of all types of metal-complex equilibria, including formation of mixed species (such as MLL" or MLM"), hydrolysed species, such as M(OH), $M_2(OH)_3$ or M(OH)L, protonated species, such as LM(HL) or $M(HL)_2$, and polynuclear species, such as M_2L_3 or $M_2(OH)_2L_2$, where M and M" represent metal ions and L and L" are ligands (Perrin and Sayce 1967).

Although the COMICS programme is not listed here, a brief description of the iterative method is given below:

At first the computer assumes that complex formation is negligible, so that free metal concentrations are equal to the total metal concentrations: the free ligand concentrations are then calculated from the pKa values. The complex species concentrations are calculated and added to the respective free metal and ligand concentrations to give estimates of the total metal and ligand concentrations. The initial estimates of the free metal and ligand concentrations are replaced by new values obtained from these calculated total concentrations. The calculations are then repeated to obtain better values of free metal and ligand concentrations. This process is then continued until the calculated concentrations of the total metals and ligands differ from their actual values by less than a specified quantity. Within this accuracy the final values of free metal and ligand concentrations satisfy all equations for metal and ligand concentrations and are used to compute the equilibrium concentrations of all species.

4.3 Materials and methods

The composition of Ingestad's nutrient solution (Ingestad 1959) is

shown in Table 2.2 with the units of concentration as mg/ 1. The computer programme was set up with data files giving the pH of the solution and the nutrient and heavy metal concentrations in units of moles per litre. The metals studied were cadmium, copper, lead, nickel and zinc. The concentration range was 0-10 mg/ 1 for all the metals except zinc, which was 0.02-100 mg/ 1. All the metals were added as chloride salts in the water culture experiments, so the data files were also adjusted for the increases in the chloride concentrations. The concentrations of ions in the nutrient solutions were present at a level such that the ionic strength could be considered zero. The equilibrium constants used (Inczedy 1976) were therefore adjusted where necessary for zero ionic strength.

From the computer runs, it was then established which species were present in the nutrient solutions used in the determinations of the upper critical tissue concentrations (Chapter 2). It was also used to examine which species would be present in separate solutions containing 5 mg/ 1 cadmium and 20 mg/ 1 copper with various proportions of glycine, aspartic acid, alanine and leucine. The concentrations of amino acids were calculated at molar ratios of 0, 0.5, 1, 1.5, 2 and 4, amino acid to metal. These were chosen to give a range of complexed forms of the metals with amino acids.

When the data on copper speciation was examined (Table 4.1) it was seen that the percentage of copper complexed by the amino acids increases as the proportion of amino acid is increased. The complexes exist in two forms; 1:1 and 1:2 metal to amino acid. When the data on the cadmium species was examined (Table 4.2) it was seen that glycine and alanine only form substantial levels of the 1:1 complexes with

Table 4.1

Effect of addition of various proportions of four amino acids upon levels of copper complexes with amino acids (Cu:AA) and free copper species in Ingestad's solution containing 20 mg/ 1 copper; results expressed as percentages of total copper.

Amino acid	Copper species	Proportion added					
		0	0.5	1	1.5	2	4
Glycine	Free Cu ions %	90.19	44.25	13.44	1.50	0.00	0.00
	1:1 CuGly %	0.00	48.7	70.38	44.92	5.68	0.00
	1:2 CuGly %	0.00	2.13	14.67	53.4	94.32	99.97
Aspartic acid	Free Cu ions %	90.19	43.52	10.61	0.80	0.00	0.00
	1:1 CuAsp %	0.00	50.51	78.89	49.91	5.78	0.00
	1:2 CuAsp %	0.00	9.27	9.30	49.21	94.22	100.0
Alanine	Free Cu ions %	90.19	43.97	12.51	1.29	0.01	0.00
	1:1 CuAla %	0.00	49.40	72.73	46.29	5.31	0.00
	1:2 CuAla %	0.00	1.76	13.37	52.32	94.73	100.0
Leucine	Free Cu ions %	90.19	49.02	23.14	5.35	0.02	0.00
	1:1 CuLeu %	0.00	38.10	48.60	35.33	2.85	0.00
	1:2 CuLeu %	0.00	7.44	25.65	58.70	97.14	100.0

CuGly = Copper glycine complex
CuAsp = Copper aspartic acid complex
CuAla = Copper aanine complex
CuLeu = Copper leucine complex

Table 4.2

Effect of addition of various proportions of four amino acids upon levels of cadmium complexes with amino acids (Cd:AA) and free cadmium species in Ingestad's solution containing 5 mg/ l cadmium; results expressed as percentages of total cadmium.

Amino acid	Cadmium species	Proportion added					
		0	0.5	1	1.5	2	4
Glycine	Free Cd ions %	70.90	69.44	65.62	61.80	58.22	46.16
	1:1 CdGly %	0.00	2.06	7.32	12.52	17.17	32.02
	1:2 CdGly %	0.00	0.00	0.11	0.32	0.64	2.80
Aspartic acid	Free Cd ions %	70.90	65.57	46.02	32.16	23.08	8.66
	1:1 CdAsp %	0.00	7.48	34.07	51.37	60.99	67.06
	1:2 CdAsp %	0.00	0.03	1.00	3.27	6.42	20.77
Alanine	Free Cd ions %	70.90	51.41	25.10	3.96	1.14	0.27
	1:1 CdAla %	0.00	27.46	64.56	94.38	98.18	98.72
	1:2 CdAla %	0.00	0.00	0.00	0.06	0.20	0.90
Leucine	Free Cd ions %	70.90	65.24	50.20	50.20	38.36	12.68
	1:1 CdLeu %	0.00	7.88	27.64	41.42	49.73	59.72
	1:2 CdLeu %	0.00	0.09	1.52	4.47	8.32	25.37

CdGly = Cadmium glycine complex
CdAsp = Cadmium aspartic acid complex
CdAla = Cadmium alanine complex
CdLeu = Cadmium leucine complex

cadmium, whereas leucine and aspartic acid has 25 and 21 percent respectively of the total cadmium as 1:2 complexes when proportions of 4:1 amino acid to cadmium are present.

It was decided that if the effect of complexation of the metals was to be studied in relation to their uptake by the seedlings, then, for each metal, two proportions of amino acid to metal would be added to the nutrient solution. For copper it was thought that molar ratios of 1:1 and 1:2 would be likely to demonstrate the effects of uptake of 1:1 and 1:2 copper to amino acid complexes respectively, since these would be the predominant copper species in the nutrient solutions at these ratios (Table 4.1).

For cadmium the choice was not as simple since the level of complexation was not consistent for all the amino acids (Table 4.2). Proportions of 1:1.5 and 1:4 for each of the amino acids were used as they gave a range of complexation with cadmium ions. Higher proportions were not used as they may have had some effects upon the speciation of nutrients.

Sitka-spruce seedlings were obtained, germinated and grown in the manner as previously described (Chapter 2), except that 15 seedlings were placed into 100 ml beakers. Thirty one days after germination had been started the metals and amino acids were added in triplicate at the concentrations and proportions given above. The seedlings were then grown for a further seven days in these solutions, after which they were harvested, weighed, digested and their respective cadmium and copper concentrations determined as previously described (Section 2.2). This length of time was chosen with the aim of producing differences in the levels of metals without reducing the shoot and root yields as a result of the toxic effects of the metals.

4.4 Results and discussion

The results discussed below are basically divisible into two sections; those dealing with the concentrations of nutrients and heavy metals in the solutions as used for the work on upper critical concentrations and those dealing with the uptake of copper and cadmium by the seedling roots from solutions in which the metals were complexed with amino acids.

4.4.1 Speciation of the nutrient solutions

The computer printouts, which contained information on the concentrations of all the species in the nutrient solutions, are not presented here as most of the information contained in these was not pertinent to the investigation. Only the total concentrations of the metals, their speciation and the speciation of nutrients whose concentrations were influenced by the presence of these metals are included. Concentrations below 0.1 nM were considered to be insignificant and excluded from the discussion.

4.4.1.1 Cadmium Table 4.3 shows that when 0-10 mg/ 1 cadmium was added to the solution the following species were present; free cadmium ions (Cd^{2+}), cadmium chloride ($CdCl^{+}$), cadmium hydroxide (1) ($CdOH^{+}$), and sulphate ($CdSO_4$). Most of the cadmium remained in solution as the free ionic form (Cd^{2+} , approximately 70%); this and the proportion of the other cadmium species remained constant as the total concentration of cadmium increased. This is to be expected since the solution concentrations of the ions with which the metal forms the various complexes are very high in comparison to the Cd level and do not

Table 4.3

Cadmium species concentrations in Ingestad's solution supplemented with various concentrations of cadmium

Total Cd concentration		Cd^{2+}	$CdCl^{+}$	$CdSO_4$	$CdOH^{+}$
mg/ l	µM	µM	µM	µM	nM
0.01	0.089	0.063	0.0192	0.0061	----
0.02	0.178	0.127	0.0383	0.0122	0.58
0.05	0.445	0.317	0.0958	0.0305	1.45
0.075	0.667	0.476	0.1436	0.0457	2.17
0.10	0.890	0.635	0.1915	0.0610	2.90
0.25	2.22	1.583	0.4778	0.1522	7.23
0.50	4.45	3.171	0.960	0.3048	14.67
0.75	6.67	4.572	1.439	0.4568	21.63
1.00	8.90	6.337	1.925	0.6089	29.29
2.50	22.2	15.79	4.822	1.515	73.81
5.00	44.5	31.57	9.753	3.022	151.0
7.50	66.7	47.18	14.78	4.504	232.2
10.00	89.0	62.78	19.95	5.977	317.7

therefore limit the extent to which these complexes may be formed.

The distribution of the phosphate species (PO_4^{2-}) was also examined. The total concentration was 0.255 mM. When no cadmium was present the concentration of the most important free phosphate ion, $H_2PO_4^-$, was 0.237 mM. At 10 mg/ l cadmium this figure was unchanged. Other phosphate species were also unaffected by increases in the cadmium concentration, for example, the concentration of $FeHPO_4$ remained constant at all added levels of cadmium.

4.4.1.2 Copper The equilibrium concentrations of all the main copper species are listed in Table 4.4. The solutions effectively contained five copper species; these were free copper ions (Cu^{2+}), copper chloride (1) ($CuCl^+$), hydroxide (1) ($CuOH^+$), hydrogen phosphate ($CuHPO_4$), and sulphate ($CuSO_4$). Their proportions remained constant as the copper concentrations increased. Most of the copper (90%) was present in the solution as free ions. The percentages of other species were; sulphate (8.5%), chloride(1) (1.2%), hydroxide(1) (0.1%) and phosphate (0.04%). There was no alteration in the distributions of the phosphate species as copper concentratons increased.

4.4.1.3 Lead Table 4.5 shows that proportions of free lead ions (Pb^{2+}), lead chloride (1) ($PbCl^+$), chloride (2) ($PbCl_2$) and sulphate ($PbSO_4$) did not increase in accordance with the total lead concentrations. The species Pb_3PO_4 was not present at low lead concentrations, but as total concentrations increased it became more significant until it accounted for 64% of the total at 10 mg/ l lead (remembering that there are three atoms to each molecule). Most of the remaining lead was present as Pb^{2+} (26%), $PbSO_4$ (6.3%) and $PbCl^+$ (3.3%).

Table 4.4

Copper species concentrations in Ingestad's solution supplemented with various levels of copper

Total Cu concentration		Cu^{2+}	$CuSO_4$	$CuCl^+$	$CuOH^+$	$CuHPO_4$
mg/ l	µM	µM	µM	µM	nM	nM
0.02	0.315	0.284	0.0273	0.0034	0.225	----
0.05	0.788	0.710	0.0682	0.0085	0.56	----
0.075	1.18	1.063	0.1022	0.0128	0.84	----
0.10	1.58	1.423	0.1368	0.0171	1.31	----
0.25	3.94	3.548	0.3411	0.0473	2.82	1.47
0.50	7.88	7.096	0.6818	0.0858	5.64	2.94
0.75	11.8	10.63	1.020	0.1286	8.44	4.41
1.00	15.8	14.23	1.366	0.1727	11.30	5.9
2.50	39.4	35.48	3.394	0.438	28.2	14.7
5.00	78.8	70.98	6.752	0.898	56.4	29.4
7.50	118.0	106.3	10.06	1.379	84.4	44.1
10.00	158.0	142.4	13.40	1.890	113.0	59.0

Table 4.5

Lead species concentrations in Ingestad's solution with various added concentrations of lead

Total Pb concentration mg/ l	µM	Pb^{2+} µM	$Pb_3(PO_4)_2$ µM	$PbSO_4$ µM	$PbCl^+$ µM	$PbCl_2$ nM
0.025	0.121	0.089	--------	0.0214	0.0107	0.200
0.05	0.241	0.177	--------	0.0427	0.0212	0.404
0.075	0.362	0.265	--------	0.0640	0.0319	0.607
0.10	0.483	0.365	--------	0.0854	0.0425	0.810
0.25	1.210	0.878	0.0041	0.2120	0.1055	2.010
0.50	2.41	1.701	0.0296	0.4110	0.2045	3.890
0.75	3.62	2.457	0.089	0.593	0.2962	5.661
1.00	4.83	3.134	0.184	0.7562	0.3780	7.220
2.50	12.1	6.031	1.290	1.454	0.7294	13.98
5.00	24.1	8.913	3.976	2.146	1.090	21.10
7.50	36.2	10.99	7.061	2.644	1.351	26.32
10.00	48.3	12.71	10.310	3.056	1.578	31.03

The distribution of the phosphate species were affected by the increasing lead concentration (Table 4.6). As shown above, lead phosphate was the predominant lead species in solution at the higher lead levels, but it only accounted for 9% of the total phosphate concentration when 48.3 μM lead was added (remembering again that there again two molecules of phosphate to every molecule of lead phosphate). The proportions of the three other phosphate species were reduced by additions of lead to the solution. The most important of these, $H_2PO_4^{2-}$, was reduced from 92.8% of the total phosphate at 0.121 μM lead to 84.8% at 48.3 μM lead. The concentration of the next most important phosphate species, $FeHPO_4$, was also slightly reduced at the highest concentration but only accounted for a very small overall change in the phosphate speciation.

4.4.1.4 Nickel Table 4.7 shows that three nickel species were present when nickel was added to the solution in the range 0.025-10 mg/ l (0.426-170 μM). These were free nickel ions (Ni^{2+}), nickel chloride (1) ($NiCl^+$) and nickel sulphate ($NiSO_4$). Here again the proportions of the various nickel species remained constant as total nickel levels increased. Ni^{2+} ions were the predominant nickel species (91.1%), then $NiSO_4$ (8.6%), with $NiCl^+$ only accounting for a very small percentage (0.2%). Phosphate ion speciation was constant and unaffected as nickel levels increased.

4.4.1.5 Zinc Additions of zinc to the solution at concentrations varying between 0.02 and 100 mg/ 1 (0.764 uM and 15.3 mM) had no effect upon the concentrations of any species except zinc hydroxide ($ZnOH_2$). There were no Zn^{2+} ions and indeed the $Zn(OH)_2$ species was the only significant zinc species found to exist under these conditions.

Table 4.6

Concentrations of phosphate species in Ingestad's solution supplemented with lead ions; The total concentration of phosphate was 0.255 mM

Lead concentration µM	$H_2PO_4^-$ mM	$Pb_3(PO_4)_2$ µM	$FeHPO_4$ µM	HPO_4^{2-} µM	H_3PO_4 µM
0.121	0.237	-------	16.36	0.431	1.563
0.241	0.237	-------	16.36	0.431	1.563
0.362	0.237	-------	16.36	0.431	1.563
0.483	0.237	-------	16.36	0.431	1.563
1.210	0.237	0.0041	16.36	0.431	1.563
2.410	0.237	0.030	16.36	0.430	1.563
3.620	0.236	0.089	16.36	0.430	1.562
4.83	0.234	0.184	16.35	0.430	1.561
12.1	0.234	1.290	16.35	0.426	1.541
24.1	0.229	3.976	16.35	0.416	1.511
36.2	0.223	7.061	16.34	0.405	1.471
48.3	0.216	10.31	16.33	0.393	1.428

Table 4.7

Nickel species concentrations in Ingestad's solution with various added concentrations of nickel

Total nickel concentration mg/ l	µM	Ni^{2+} µM	$NiSO_4$ µM	$NiCl^+$ µM
0.025	0.426	0.388	0.037	0.659
0.05	0.852	0.776	0.075	1.317
0.075	1.28	1.166	0.112	1.979
0.10	1.70	1.548	0.149	2.629
0.25	4.26	3.880	0.373	6.609
0.50	8.52	7.761	0.746	13.26
0.75	12.8	11.66	1.119	19.93
1.00	17.0	15.49	1.486	26.55
2.50	42.6	38.82	3.711	67.87
5.00	85.2	77.68	7.381	139.3
7.50	128.0	116.8	11.03	214.6
10.00	170.0	155.1	14.57	293.0

Phosphate ion distribution was also constant as zinc levels increased.

4.4.2 Effect of amino acids upon uptake of metals

The average concentrations of copper and cadmium in the shoots and roots of each treatment were calculated from the three replicates and the results expressed in Tables 4.8 (Cu) and 4.9 (Cd). For a comparison between the control treatments and those with added amino acids one statistical method which may be used with this small number of replicates is Lords "μ" test; the difference between the mean values of the treatment and control is calculated and divided by the sum of the ranges between replicates of both treatments (Eckshlager 1961). The results of these tests are also shown in Tables 4.8 and 4.9. It was not possible to use the "t" test to distinguish between the treatments as the number of replicates per treatment was too small for an accurate estimation of the variances.

In the experiment with 20 mg/ l Cu added to the solutions there were several significant effects (Table 4.8); three in the roots and three in the shoots. An examination of the ranges of concentrations obtained both in roots and shoots, reveals that Cu levels of those plants grown in solutions with added amino acids varied greatly in comparison with the controls; this was also true of the Cd experiment (Table 4.9). Where consistent results were obtained then the effect was usually significant, even though the mean values were not very different. However the ranges were often as large as the mean values themselves.

The presence of alanine in the solution induced increases in root Cu levels and at the higher ratio of alanine to copper (2:1), the

Table 4.8

Amino acid experiment: effect of four amino acids added at proportions of 1:1 and 1:2 copper to amino acid upon the uptake of copper (solution concentration 20 mg/ l) into shoots and roots of sitka-spruce seedlings and results of statistical test (Lords "μ" test) against uptake by controls (without added amino acids).

Amino acid	Proportion of Cu to amino acid	Mean copper concentration (mg/ l)		Range of concentration (mg/ l)		Lords "μ" test statistic	
		Roots	Shoots	Roots	Shoots	Roots	Shoots
Glycine	1:1	18349	3331	8175	391	0.510	1.990**
Glycine	1:2	22922	3634	13036	3403	0.637	0.365
Aspartic acid	1:1	19454	3664	13647	1259	0.417	0.963*
Aspartic acid	1:2	27751	2468	15535	742	0.763*	0.133
Alanine	1:1	21792	2759	4567	860	0.945*	0.420
Alanine	1:2	25198	4763	11727	4306	0.781*	0.546
Leucine	1:1	4898	3007	2642	595	0.409	0.937*
Leucine	1:2	4278	2769	5592	1290	0.367	0.296
Control	----	8459	2356	4997	100	-----	-----

* significant, $p < 0.05$ ** significant, $p < 0.01$.

Table 4.9

Amino acid experiment: effect of four amino acids added at proportions of 1:1.5 and 1:4 cadmium to amino acid upon the uptake of cadmium (solution concentration 5 mg/ l Cd) into shoots and roots of sitka-spruce seedlings and results of statistical test (Lords "μ" test) against uptake by controls (without added amino acids).

Amino acid	Proportion of Cd to amino acid	Mean Cadmium concentration (mg/ l)		Range of concentration (mg l)		Lords "μ" test statistic	
		Roots	Shoots	Roots	Shoots	Roots	Shoots
Glycine	1:1.5	1085	52.2	391	28.0	0.890*	0.240
Glycine	1:4	1706	67.0	335	38.1	2.060**	0.152
Aspartic acid	1:1.5	962	85.7	965	77.6	0.364	0.305
Aspartic acid	1:4	944	59.2	394	88.7	0.664*	0.610
Alanine	1:1.5	706	110.8	348	123.2	0.311	0.392
Alanine	1:4	1880	171.6	2016	313.7	0.602	0.348
Leucine	1:1.5	769	70.9	772	15.5	0.243	0.497
Leucine	1:4	461	51.2	52	18.1	0.212	0.388
Control	----	523	60.4	240	5.66	-----	-----

* significant, $p < 0.05$ ** significant, $p < 0.01$.

increase in root copper levels was greater. If the tissue levels are compared with the levels of Cu species that should be present in the solutions (Table 4.1), it can be seen that at the lower ratio of alanine to copper (1:1), most of the Cu (73%) should have been present as the 1:1 complex (charge 1+), and that at the higher proportion (1:2) most of the Cu should have been present as the 1:2 copper alanine complex (95%). In the aspartic acid (1:2) treatment, in which the main Cu species was copper aspartic acid 1:2 complex, there were also significantly increased root Cu levels. Leucine and glycine however, did not significantly affect root Cu uptake and the mean root Cu levels in the leucine treatments were much lower than the other amino acids.

The uptake pattern of copper from solutions containing alanine and aspartic acid contrasted markedly with reports by other workers which demonstrated that the more positively charged complexes would be taken up by the roots (Coombes et al 1978). The information yielded by these experiments do not really shed any light upon nor can be interpreted in terms of root surface interactions with the complex species, though much attention was at one time given to the cation exchange property of roots (Drake 1964), since there is no evidence that ions must be adsorbed on exchange sites before entering the active uptake process (Nye and Tinker 1977).

It is also difficult to understand why alanine should have been specifically responsible for the increased uptake when other amino acids, which had similar levels of complexation (all others) and structure (glycine), did not have this effect. It is, however, difficult to distinguish between the effects of the amino acids upon uptake because of the variation between replicates and it cannot therefore be stated with any great certainty that one amino acid had a greater effect

upon the uptake of copper than others.

In the shoots there were three significant effects upon copper uptake, none of which involved alanine (1:1 or 1:2) or aspartic acid (1:2). Any increases in root Cu uptake were not therefore reflected in their transport to the shoots. Again variation between replicates may have masked effects.

The effects of amino acids upon the uptake of cadmium (5 mg/ l Cd solution concentration) are presented in Table 4.9. An initial examination of this table reveals that variations between replicates were again large and this together with the general low level of complexation (Table 4.2) may have combined to mask effects upon Cd uptake. There were no significant effects upon shoot Cd levels, though both proportions of glycine (1:1.5 and 1:4) and aspartic acid (1:4) significantly increased root Cd levels. This is interesting since glycine did not complex Cd as much as the other amino acids (Table 4.2).

4.5 Conclusions

The major effects on equilibrium speciation produced by the addition of the heavy metals, cadmium, copper and nickel, to Ingestad's solution were increases in the free ionic forms (M^{2+}) and slight increases in the sulphate (MSO_4), chloride (1) (MCl^+), and hydroxide (1) (MOH^+) species. Lead behaved slightly differently at the highest concentrations examined, lead phosphate being the main species formed. Lead was the only metal examined that significantly altered phosphate speciation. Increases in zinc solution concentrations only increased the solution levels of zinc hydroxide.

There were significant increases in the root and shoot levels of

copper brought about by additions of amino acids to the solutions (Table 4.8). Increases in shoot Cu levels could not be related to corresponding increases in root Cu levels, though other effects may have been masked by the variations between treatments. Glycine (proportions of Cd to amino acid 1:1.5 and 1:4) increased root Cd levels, as did aspartic acid (1:4), though again the large variations between replicates may have masked other effects. There were no significant effects upon shoot Cd levels.

If the effects of amino acids are to be studied in greater detail, then it is important to design the experiment so that the effects can be accurately determined with variances kept to a minimum. Though the quality of information derived from this experiment is limited, it has shown that root exudations may play important roles in the uptake of metals and subsequently affect the balances of nutrients and metals within the plants.

Chapter 5

ASSESSMENT OF FORESTRY SITES

5.1 Introduction

The project was set up with the aim of studying the effects of heavy metals upon the growth of sitka-spruce at South Wales forest sites. In accordance with this, assessments of tree growth and determinations of heavy metal and nutrient concentrations had to be carried out at the forestry sites. Though South Wales has been shown to be contaminated by heavy metals (Burton and John 1977, Goodman and Roberts 1971), it has not been clearly shown that there are elevated levels of heavy metals at the forestry sites in South Wales. A sampling programme was therefore set up with the help of the forestry commission.

The methods used in the assessment of the sites were chosen in relation to the amount of work that could possibly be performed in the time available and how this information could be integrated within the rest of the study. It was possible to sample a number of different phases which could be used in the measurement of heavy metal contamination at the forestry sites including soil, soil solution, roots, foliage of both the trees and other vegetation and the atmosphere. Within these phases there were also a number of parameters that could be measured and related to the site growth indices. Site growth could have been assessed by standard methods employed by the forestry commission and these included yield classes, production classes and five year intercepts.

The soil has certain major properties which are of great relevance to the nutrition and growth of trees, and was therefore sampled at several of the South Wales forestry sites. Total concentrations of heavy metals, as measurements of contamination, provide information as to the soil burden of heavy metals, but have little relevance to the nutrition of the plant and it was decided that some measurement of the availability of the metals from the soil would more meaningful.

Available concentrations of metals and nutrients have been correlated with some site growth indices (James et al 1978) and it was hoped that important factors in these relationships at South Wales sites could be discerned in this study. A certain amount of sampling was also necessary to categorize the type of soil upon which the trees were growing.

Foliar tissues have often been sampled because the leaf is the focal point of many plant functions and is a relatively sensitive indicator for those mineral elements that directly affect photosynthesis (Smith 1962). But in addition to this, foliar sampling has many practical advantages. For instance, foliar analysis has been put to good use in determining which nutrient was in short supply and causing the visual symptoms of bad growth in trees (Will 1965), but though it has proved its worth in these crops, it is still not a sufficiently refined method to use with certainty on established, steadily growing crops (Binns and Grayson 1967).

Many studies have correlated tissue concentrations in foliage with such factors as tree height (Leyton and Armson 1955, Hoyle and Martin 1964), site index (Morison 1970) and soil nutrient concentrations (Wells 1965, James et al 1978). These tissues are obviously very useful as diagnostic tools and were therefore sampled.

Foliar tissues have also been sampled because it was thought that these actively photosynthesising tissues would be those most similar to the shoot tissues of young seedlings. This similarity enables some comparisons to be made with the upper critical tissue concentrations and interactive effects of the heavy metals studied in Chapters 2 and 3.

Root sampling at forestry sites was also an alternative that may have provided some information on the tissue levels of the heavy metals and also about mycorrhizae. However the volume of work in any study of roots and mycorrhizae in the field is very prohibitive. There are many problems associated with the separation and identification of the roots in forest soils (Fogel 1980). In any chemical analysis of these tissues contamination from the soil would have been a major problem.

Air sampling could have yielded important information about the present levels of heavy metals in the atmosphere, but this is not of direct relevance since much of the contamination that could be affecting tree growth would have occurred in the past and this technique could not account for this. In addition to this, atmospheric levels of heavy metals in South Wales have been reported elsewhere (Welsh Office 1975). In that study, the levels of many elements, including those being studied in this project, were currently found to be elevated in summer and winter.

In any appraisal of forestry sites, as well as the choice of phases and parameters to be measured within them, some standard measures are usually taken to classify the tree growth at the sites. The most common and convenient of these is the yield class system.

Yield classes are essentially based on the division of forest stands into steps of even numbered annual increments in volume of wood

per hectare. Thus yield class 14 has a maximum mean annual increment of 14 cubic metres per hectare. In practice yield classes are usually assessed from the top height (the mean height of up to 100 trees of largest diameter at breast height, 1.3m, per hectare), since there is a good relationship between top height and total (cumulative) volume production of a stand. This relationship can be used by measuring top height, converting this to volume production and dividing by the age of the stand to derive mean annual volume increment and therefore yield class (Hamilton and Christie 1971).

Yield classes may be divided up into local or general yield classes. Local variations in the relationship between top height and volume total production do occur and these are accomodated by assigning one of three production functions (Production Classes) to a site. The effect of this may be to maintain, raise or lower the yield class figure. The top heights of at least 5 trees in a minimum area of 0.5 hectare are measured if estimates of yield classes are required. However several studies have estimated yield classes at 0.01 hectare sites (James et al 1978). Yield class values have been obtained in this study from local forest offices where normal site sizes have been used, and from reports on forestry experiments where the treatment area was greater than 0.01 hectare sites.

The South Wales forestry sites were sampled to assess how heavy metals affect forest tree growth by collecting foliage and soil at contaminated and uncontaminated sites, and establishing which of the contaminating factors were important.

5.2 Site descriptions

The first forestry sites in South and Mid Wales that were sampled

were chosen on the grounds that they were the control treatments of Forestry Commission experiments conducted at sites with soils typical of forestry plantations, and as such it was known from their records exactly how they had been treated. Later, other sites were sampled to gain a more even distribution over South Wales forests. A site at Mid Wales was sampled which again was a control treatment of an experiment. This particular site was sampled because it was known that the soil had similar properties to those sampled in South Wales, but was remote from any source of heavy metal contamination.

All the sites had sitka-spruce growing on them, but not all were sampled for both soil and foliage. A total of nine sites were sampled as shown on the map of South and Mid Wales in Figure 5.1. The names of the forests, the unique grid reference positions on ordnance survey maps 147 and 170, the dates of planting of the trees at present growing on them, their yield classes and whether they were sampled for soil or foliage are shown in Table 5.1. A total of 6 sites were sampled for soil and 8 were sampled for foliage.

All the sites had fairly organic soils, most of these being peaty gleys, though at site 1 (Cymer) the soil was a deep peat and at site 5 (Rhondda) it was an iron pan with Moor grass dominant. The peaty gley soils sampled in South Wales were of a type known as Rhondda peaty gley whereas site 9 in Mid Wales at Tywi-Dolgoch had a similar peaty gley with dominant Moor grass.

The deep peat site was a blanket bog, type 9b (Pyatt et al 1979), with many Hares-tail cotton grass (Eriophorum vaginatum) and Moor grass plants. A deep peat is defined as a peat which is deeper than 0.45m and a blanket bog is usually a deep peat which is relatively shallow (less

Figure 5.1 Map showing positions of forestry sites in South and Mid Wales which were sampled as part of this study

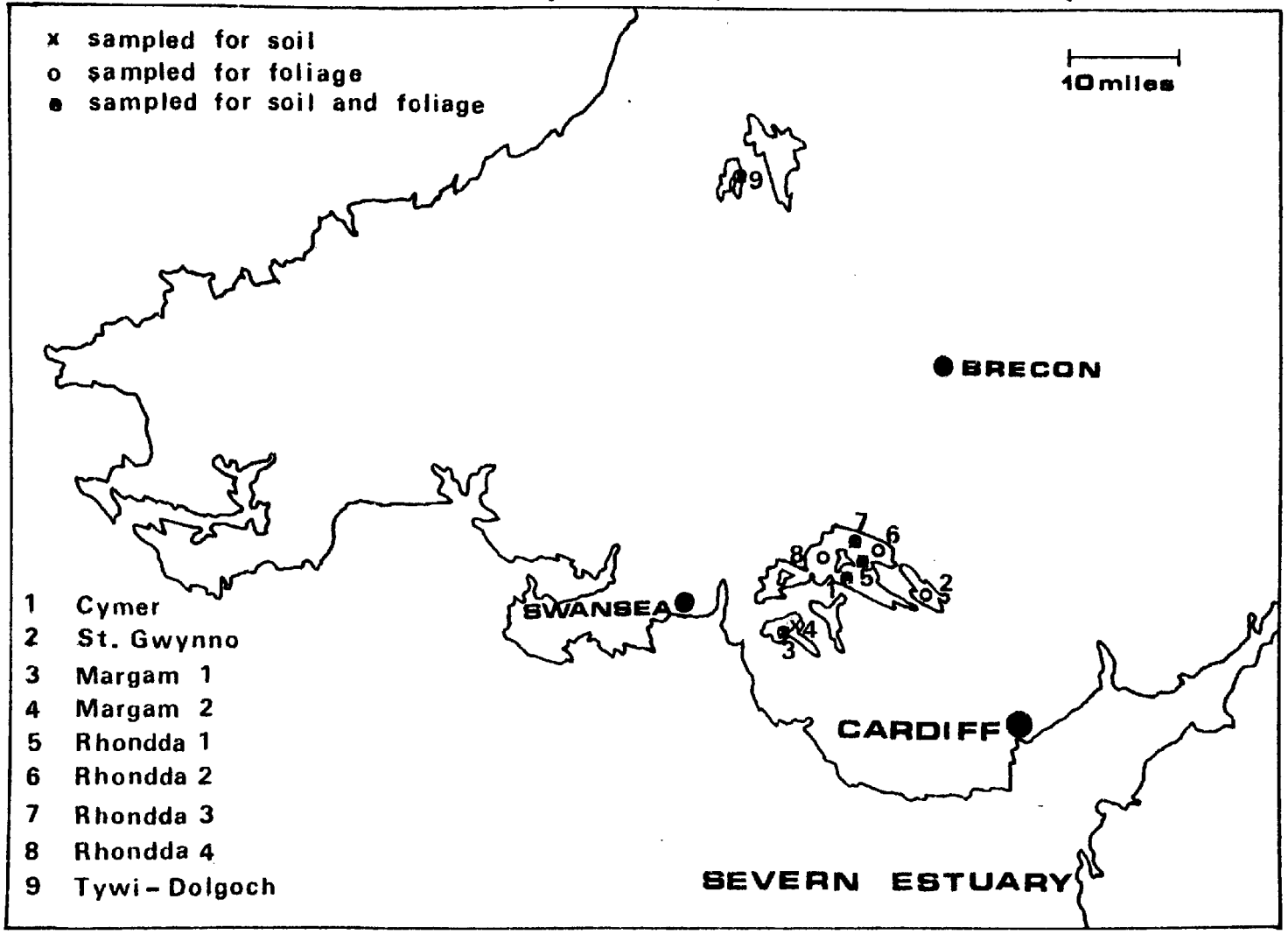


Table 5.1

General information about sampled forestry sites

Site No	Forest	Ordnance survey grid reference	Year planted	Yield Class	For what sampled
1	Cymer	ST 889 980	1974	8[3]	F and S
2	Llanwynno	ST 025 955	1970	14[3]	F
3	Margam 1	ST 825 899	1972	11-12[1]	F and S
4	Margam 2	ST 821 906	1941	14[2]	S
5	Rhondda 1	ST 909 976	1971	12[3]	F and S
6	Rhondda 2	ST 924 032	1967	6-8[1]	F
7	Rhondda 3	ST 906 020	1966	6-8[1]	F and S
8	Rhondda 4	ST 844 017			F
9	Tywi-Dolgoch	SN 784 576	1971		F and S

1 General yield class
2 Local yield class
3 Provisional general yield class
F Foliar samples
S Soil samples

than 3 m) and highly humified (Pyatt et al 1979).

Peaty gley soils are in general fairly organic or minero-organic and acidic in nature. They also often have impeded drainage or continuous flushing. This class of soil includes gley soils with either a surface peaty layer or a very dark coloured A horizon (Curtis et al 1976).

The vegetation present at particular sites is often indicative of the types of soil, and based upon this several systems of classification of peats have been proposed (Pyatt et al 1979, Anderson 1950 and Zehetmayer 1954), though other systems have been related to the chemical properties of the different types (Toleman 1973).

The vegetation at several of the sites was often dominated by purple moor grass (Molinia caerulea). This coarse perennial grass is common on heaths, bogs and moors and when dominant is present as tussocks. The hares-tail cotton grass is often found in abundance at high altitudes and generally its presence can be taken as a sign of poor peat.

A short description of the individual sites is given below.

Site 1: Cymer forest. Altitude 488m with a south easterly aspect, severely exposed. Annual rainfall is 1500mm. The position sampled was part of a forestry commission experiment. The soil is deep peat , type 9b, though both Hares-tail cotton grass and Purple moor grass were present. The soil is one metre in depth with carboniferous sandstone below. The trees were planted in 1973, spaced every two metres apart.

Site 2: Llanwynno. This site had a mixture of trees growing on it, divided up into compartments of which one had sitka-spruce (compartment 4). The altitude is 356m with an easterly aspect. The soil is a Rhondda

peaty gley.

Site 3: Margam 1. This site is also part of a forestry commission. The altitude is 280m with a northerly aspect. Annual rainfall of 1750mm. The soil is a Rhondda peaty gley with pennant sandstone below. The trees are spaced every two metres (0.0004 hectare per plant). Cross drains were dug in 1971, just prior to planting in 1972, at a depth of one metre to improve drainage. A previous crop had been harvested in 1971 at an age of between 38-44 years and had a general yield class of 11-12, and a local yield class of 9-11.

Site 4: Margam 2. This site is at an altitude of 300m with a south westerly aspect and is severely exposed. Annual rainfall 1750mm. The soil is Rhondda peaty gley with pennant sandstone beneath. The trees were planted in 1941 with spacing at two metres. They were thinned out in 1970 and again in 1974. In 1970 the site had a local yield class of 14 and a top height of 12.5m. Generally this site was a bad growth area.

Site 5: Rhondda 1. The altitude of the site is 500m. and it has a south westerly aspect with severe exposure. The annual rainfall is 2286mm. The soil is ironpan and it has a Moor grass dominated vegetation. In 1970, a year before planting, the soil was tine ploughed to a depth of 0.40 m with plough drains. When a pan is a main cause of a drainage problem and the soil is more or less impermeable then the tine plough is used to break up the iron pan and obtain some vertical movement of moisture to give immediate improvement (Forestry Commission 1978). As part of a forestry experiment it was assessed in 1976 and assigned a provisional general yield class of 12. Foliar analysis showed that the average needle weight was 2.78 mg and the percentages of dry weight of the

nutrients was as follows; nitrogen 1.84, phosphorus 0.245 and potassium 0.69. Over the whole experiment it was thought that nutrient levels were adequate.

At the beginning of 1977 a further assessment showed that though height growth was adequate, foliage was thin and diameter growth was poor and that most of the trees showed signs of "flagging", attributable to exposure. In April 1977 a further assessment was performed at which time it was thought that growth was patchy with poor yellow/green colour and that the stand was unlikely to reach canopy closure before 12-14 years due to a general lack of foliage.

Site 6: Rhondda 2. Altitude 488m with a northerly aspect. The soil is a Rhondda peaty gley under Moor grass.

Site 7: Rhondda 3. Altitude 512m with a south easterly aspect. The soil is a Rhondda peaty gley under Moor grass.

Site 8: Rhondda 4. Altitude 317m with a north westerly aspect. The soil is a Rhondda peaty gley with Moor grass.

Site 9: Twyi-Dolgoch, Forestry commision experiment reference number 63.1, experiment 26. The altitude is 490m. It is a moderately to severely exposed site. The rainfall averages 2000 millimetres per annum. When foliage samples were taken in October 1977 the average needle weight was 5.55 milligrammes with nutrient levels as percentage of dry weight; nitrogen 1.75, phosphorus 0.269 and potassium 0.97. The mean height of the trees was 110.88 centimetres. The soil is a peaty gley under Moor grass with a base of Silurian rock.

5.3 Methods

The sites have in some cases been sampled for both foliage and soil, whereas at others only foliage or soil were taken. The techniques and methods are completely separate and are dealt with in individual sections.

5.3.1 Soil techniques

In the characterization of soil properties pertinent to this investigation, there were essentially four operations that had to be considered; these were sampling, treatment of material, chemical and physical analysis and a statistical treatment of the data.

The variability of the soil properties at particular sites is an important factor in deciding the number of replicate samples to be taken. It has been shown that there was a small advantage to be gained from sampling sites of one hectare as opposed to 0.01 hectare in terms of the variability of some nutrients (Blyth and Macleod 1978), though the main advantage of larger sites in forestry work is that correlations are usually performed between nutrients and tree growth parameters that are designed for sites of at least 0.5 hectare (yield class). It has however been common practice for 0.01 hectare sites to be sampled and the results used in correlations with tree growth parameters (James et al 1978). Some of the sites were control treatments of forestry commission experiments which only allowed assessment of 0.01 hectare. A site size of 0.01 hectare was therefore chosen for all the sites. The positions of the sample sites were chosen within the boundaries of the experimental sites so that edge effects due to other treatments would be avoided.

In studies of plant nutrition or nutrient circulation an

examination of the soil from the top 0-15 centimetres has been shown to be adequate since most of the root uptake of nutrients including that of large trees occurs in this zone. This was the depth chosen for sampling.

High variability within plots causes large variations in the correlation coefficients between the tree growth and soil properties and this varies with the soil type and parameter to be measured. In the absence of a knowledge of the spatial variability at a site it is difficult to assess the significance in the differences which may be implied by the data (Ball and Williams 1968). The intensity of sampling must therefore be sufficient to bring the variation about a mean value to a statistically reliable value. Composite samples do not show the variability at a site and it has been shown that the variability in such samples can be as large as the mean values. Composite samples cannot therefore be used in a study of this nature.

It has been shown that the average number of topsoil samples required per plot to secure 95% confidence limits for a range about the mean of plus or minus 10% was 6 for total nitrogen, 9 for phosphorus and 29 for 0.5M acetic acid extractable nutrients. On this recommendation 30 samples were taken from each site in an attempt to obtain reasonably accurate representations of the chemical properties.

The samples were collected using a soil auger, extruded into polythene bags, and sealed. The contents of each bag were mixed on return to the laboratory and a pH analysis performed immediately. Several analyses were performed during the following days on the fresh material, whereas others used oven-dried material that had been ground to pass a 2 mm stainless steel sieve. The moisture contents were determined by drying overnight at 105 degrees centigrade in an oven so

that concentratons could be converted to a dry-weight basis.

5.3.1.1 Acidity as measured by pH A pH meter and electrodes were set up and left to stabilize. The meter was calibrated against two buffer solutions; one either side of the expected pH range. For each sample duplicate analyses were performed. Twenty grammes of fresh soil was placed into a 100 ml beaker to which 50 ml of deionized water was added. This was then stirred for a few minutes, and allowed to stand for a further 15 minutes (Liang and Tabatabai 1977). The electrodes were inserted into the slurry and the reading was recorded to one decimal place after needle drift had ceased. The replicates were averaged and the mean and standard deviation of the pH of the soil at each site was calculated.

5.3.1.2 Organic matter/ organic carbon The method used was loss on ignition (Allen 1974). Replicate 2 gramme oven-dry samples of soil were weighed accurately in porcelain crucibles, placed in a furnace which had previously been heated to 400 degrees centigrade and left for 4 hours. They were then removed and left to cool in a desiccator and reweighed. The percentage loss in weight of the average of the two replicates then gave an approximation of the organic matter content and a mean value and standard deviation of all the samples at each site was calculated. The percentage of organic carbon was calculated from the organic matter content by dividing by a constant 1.9. This is the normal ratio of carbon to organic matter found in peaty or peaty gley soils (Allen 1974).

5.3.1.3 Total heavy metal concentrations Approximately 2.5 g of oven-dry replicate soil samples were weighed accurately on an analytical

balance to 4 decimal places. 10 ml of 3:1 nitric: perchloric acid mixture was added, left overnight, heated to digest the remaining undigested material, left to cool and then filtered through Whatman No 42 paper. The volume was made up to 25 ml with deionized water. The solutions were analysed using a Varian model 1100 atomic absorption spectrophotometer using an air acetylene flame. Appropriate standards and blanks were prepared with each set of samples. The metals Cu, Cd, Mn, Ni, Pb and Zn were determined (Chapter 2) and the mean and standard deviations for each site calculated as previously.

5.3.1.4 Extractable heavy metal concentations For most purposes chemical extraction techniques, although essentially empirical, are used for the measurement of available nutrients in the soil. In these methods ions are displaced from adsorption sites by other replacing ions applied in great excess (Allen 1974). The normal choice of an extractant in acidic soils is an acidic extractant. Acetic acid is the most commonly used acidic extractant and has been used in this study.

Twenty five grammes of fresh peaty soil was weighed into a beaker to which 200 ml of 2.5% (0.5M) glacial acetic acid was added. The contents were stirred and left to stand for 10 minutes. The supernatant was filtered through Whatman No 42 paper into a dry bottle and a further 50 ml was added to wash the ions through the peat. Further small additions were made until a total volume of 250 ml had been leached; these additions saturated the peat and were followed by an adequate time for filtering. Several blanks were prepared with each batch of samples. The solutions were analysed for heavy metals by flame atomic absorption as described above for total heavy metals except that the standards and blanks were made up with acetic acid.

The moisture content of each of the samples was determined at the time of weighing, allowing corrections for the moisture content to be made. The results were calculated in the same manner as previously described.

5.3.1.5 Total nitrogen concentrations The nitrogen content of the peaty soils was determined for 10 duplicate samples from each site. The method employed involved a Kjeldahl digestion (Allen 1974) followed by determination of the ammonium ions using a specific ammonia gas electrode. The Kjeldahl digestion stage involved weighing 0.25 g of dried ground peaty soil into a 50 ml round bottomed Kjeldahl flask to which 2 g of a mercury catalyst was added (K_2SO_4:HgO 20:1 w/w). Three millilitres of concentrated sulphuric acid was run down the neck of each flask. The flasks were heated gently until the frothing had subsided. The heat was then gently increased and left until the digestate became a straw colour after which it was heated for a further 30 minutes. The digestate was allowed to cool and diluted to 50 ml with deionized water.

The determination of the ammonium ions by specific ion electrode was carried out as follows: The contents of the Kjeldahl flask were made up to 100 ml in a graduated cylinder. One millilitre of the solution containing sample was transferred to a 150 ml beaker containing 99 ml of 0.4 M NaOH with added NaI (15 g/l)(Orion 1978a). The sodium hydroxide converted the ammonium ions to ammonia gas to which the electrode is sensitive, whereas, the sodium iodide precipitated the mercury in the solution so that it did not interfere with the determination by the formation of mercuro-ammonia complexes. The electrode was then inserted, the solution stirred, and a reading taken when it had stabilized. The electrode was calibrated in a similar manner with an appropriate set of

ammonium standards.

5.3.1.6 Extractable ammonium-nitrogen concentrations Ten grammes of fresh soil was shaken with 100 ml of 2M KCl for one hour and filtered through Whatman No 42 paper. A 20 ml aliquot was pipetted into a 30 ml beaker and the concentration of ammonium ions determined by specific ion electrode (Banwart et al 1972). The mean and standard deviation of the 30 samples were calculated as previously described above.

5.3.1.7 Extractable nitrate-nitrogen concentrations Determination of nitrate in the soil was performed on fresh samples using an extractant (Orion 1978b) to bring the nitrate ions into solution, followed by determination with a specific nitrate electrode (Smith 1975). The extractant was a mixture of aluminium sulphate, boric acid, silver sulphate and sulphamic acid adjusted to a pH of 3 with sodium hydroxide. Fifty millilitres of this solution was stirred with 10 g of fresh soil and left to settle before determination of the nitrate. The results were calculated as nitrate-nitrogen from the 30 samples at each site as above.

5.3.2 Foliar analysis

The foliar sampling method used in this study was a standard Forestry Commission technique (Danby 1979). It has been shown that in any study the class of trees to be sampled is determined by the study objectives (Lowry and Avard 1968). It was thought that for this study the samples should be taken from six dominant trees within the 0.01 hectare sites since it is known that site classes and foliar concentrations are highly correlated in dominant trees.

The choice of the height within the crown to be sampled was made on

the basis that tissue concentrations in the upper crown samples are correlated with such factors as tree height (Leyton and Armson 1955) and soil nutrient concentrations (Wells 1965). The choice of aspect to be sampled is generally considered unimportant (Van Den Dreissche 1974), though sampling from a constant aspect may avoid confounding effects.

Foliage of the current years growth has been sampled because it has been shown that there are high correlations between their nutrient levels and availability of soil nutrients (Lavender and Carmichael 1966). Sampling usually takes place in the Autumn or Winter as it is thought that these are periods of minimal physiological change within the plant (Leyton 1958). Sampling during such a stable period has the advantage that comparisons between different stands are still valid even though times of sampling are not exactly the same.

The exact procedure followed was that samples were taken from the upper whorls of the six most dominant trees at each of the 0.01 hectare sites in October 1980, 1981 and 1982 (see Table 5.1). Six inch samples were cut from the branches with the most southerly facing aspect and sealed in polythene bags.

In the laboratory the needles were stripped from the branches and dried at 85 degrees centigrade in an oven overnight. The average needle weight, as dry matter, was determined by counting the number of needles in triplicate 0.5 g samples. The needles to be chemically analysed were ground, redried and stored in polythene bags prior to analysis (James et al 1978).

The chemical analysis was performed in three stages. The percentage of nitrogen was determined by the same method as employed for the soils using a Kjeldahl digestion and ammonia specific ion electrode (Section

5.2.1.5) except that triplicate 0.1 g samples were used. A separate digestion of triplicate 0.5 g foliar samples was performed to determine phosphorus, heavy metals and other nutrients. This digestion was the same as previously used for the digestion of soils (Section 5.2.1.3).

Phosphorus was determined as orthophosphate by a molybdenum blue method (Allen 1974) using a stannous chloride reagent (0.5 g $SnCl_2.2H_2O$ in 250 ml 2% v/v HCl) to reduce the heterophosphomolybdate complex. Acid was included in the standards at levels comparable to the samples. The absorbance was measured at 700 nm using water as a reference. Flame atomic absorption was used to determine Cd, Cu, K, Mg, Mn, Ni, Pb and Zn ion concentrations (Chapter 2) and a flame photometer (Corning 400) was used to measure Ca ion concentrations.

The average needle weight and concentrations of heavy metals and nutrients were calculated from the average of the three replicates.

5.4 Results and discussion

The information about the chemical properties of the soil samples collected from the sites are presented in Tables 5.2, 5.3 and 5.4. Table 5.2 lists those chemical properties which are generally used to describe the soils. The mean and standard deviation of 30 duplicate samples at each site (10 for total nitrogen) enable some estimation of the variability of the soil properties. The average carbon and nitrogen contents confirm that the soils were, in general, similar to each other, all being highly organic at least in the top 15 cm of the soil. The soils were all very acidic and since it is known that the optimum pH for the growth of sitka-spruce lies between 4.5 and 5.5 it can be expected that this has some effects upon growth (Leyton 1956).

The concentrations of the extractable nitrogen forms show that they

Table 5.2

Concentrations of total nitrogen, ammonium-nitrogen, nitrate-nitrogen, carbon content and pH of the site soils

Site No	Total nitrogen % ODW		Ammonium-nitrogen mg/ kg ODW		Nitrate-nitrogen mg/ kg ODW		Carbon content % ODW		pH	
	M	SD	M	SD	M	SD	M	SD	M	SD
1	0.92	0.31	128.3	53.6	14.4	5.4	17.9	11.7	3.90	0.19
3	1.27	0.24	110.4	38.9	4.3	1.7	19.4	9.7	3.92	0.23
4	1.11	0.36	62.8	32.1	12.9	4.2	32.2	10.8	3.39	0.13
5	1.06	0.30	118.8	40.4	7.5	1.5	47.5	1.3	3.70	0.16
7	1.10	0.25	106.2	50.1	7.4	1.7	45.3	1.4	3.81	0.17
9	1.10	0.22	164.4	66.4	138.7	74.2	45.3	1.8	3.30	0.16

M Mean
SD Standard deviation
ODW Of dry weight

Table 5.3

Total soil concentrations of heavy metals at forestry sites (mg/ kg of dry weight)

Site No	Cadmium		Copper		Lead		Manganese		Nickel		Zinc	
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
1	2.1	1.0	71.2	67.8	174.8	147.1	17.9	9.8	17.0	8.2	39.3	29.8
3	0.0	0.0	63.4	61.4	116.6	79.1	9.4	6.2	20.0	9.2	25.4	15.1
4	2.4	1.3	181	77.5	223.8	221.5	17.4	9.9	13.1	5.1	39.4	14.6
5	4.2	0.8	146	42.7	337.2	97.2	8.2	3.7	31.8	14.2	107.7	24.5
7	2.7	0.8	138	62.8	377.5	112.2	17.8	7.8	23.4	8.8	112.2	17.3
9	0.8	0.2	6.8	6.1	47.2	38.0	10.2	3.8	3.0	2.4	32.9	21.4

M Mean
SD Standard deviation

Table 5.4

Extractable soil concentrations of heavy metals at forestry sites (mg / kg of dry weight)

Site No	Cadmium		Copper		Lead		Manganese		Nickel		Zinc	
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
1	0.8	1.0	1.3	1.7	0.0	0.0	3.6	2.1	0.0	0.0	15.3	9.2
3	0.0	0.0	1.6	1.2	0.0	0.0	2.5	1.3	0.0	0.0	8.8	3.0
4	0.0	0.0	1.0	1.1	0.0	0.0	5.3	2.5	0.0	0.0	8.7	3.7
5	1.5	1.3	1.6	1.0	0.0	0.0	1.6	0.8	0.0	0.0	32.9	8.9
7	0.9	0.5	2.0	1.0	0.0	0.0	7.0	2.3	0.0	0.0	14.3	4.2
9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

M Mean
SD Standard deviation

were similar, except that the Tywi-Dolgoch site in Mid Wales (Site 9) had higher levels of both nitrate and ammonium concentrations than any of the other sites. An interesting point to note is that in all the soils both total nitrogen and extractable ammonium concentrations were extremely variable within the sites as well as between sites, even though the intensity of sampling was fairly high. The other properties were in general less variable.

5.4.1 Total heavy metal concentrations

The total soil concentrations of heavy metals, presented as the means of 30 duplicated samples from each site, are listed in Table 5.3. The table reveals that the South Wales sites had concentrations greater than those at the Tywi-Dolgoch site for all the metals except manganese. The actual degree of enrichment was not the same for each of these metals at the South Wales sites, since individual sites represented maxima for different metals. This would seem to suggest that different input sources were responsible for the elevation in these levels. However, heavy metal concentrations were extremely variable within the sites and some method of distinguishing between the average site values was necessary which took these variabilities into account.

The data was assessed for normality of distribution about the mean values using "Lilliefors" test (Hollander and Wolfe 1973). It was found that this data was normally distributed, therefore allowing the use of parametric statistical tests. An analysis of variance was then used to compare the mean values obtained at the Tywi-Dolgoch site (Site 9) with the mean values of the other sites using a linear comparison method (Cochran and Cox 1957). The results of these analyses showed that Tywi-

Dolgoch was significantly different at the $p<0.01$ level for all the metals except manganese.

The maximum relative degree of enhancement, using the mean values of the Tywi-Dolgoch site as a reference, was 5 for cadmium (Site 5; Rhondda 1), 25 for copper (Site 4; Margam 2), 8 for lead (Site 7; Rhondda 3), 11 for nickel (Site 5; Rhondda 1) and 3.5 for zinc (Site 7; Rhondda 3). These enhancements in soil levels are similar to those reported previously in a valley in South Wales, where enhancements in top soil compared with depth profile samples were shown to be 5 for cadmium, 10-20 for copper, 30 for lead and 15 for nickel (Burton and John 1977). The cadmium and copper figures are the same and the two nickel values are similar. Lead, however, is quite different and is probably related to the traffic incidence within the urban strip, which has been shown to be responsible for the accumulation of lead in soils as a result of the combustion of leaded petrol (Burton and John 1977, Gish and Christensen 1973).

Though these are the sites of maximum enhancements in soil levels, most of the sites also had sufficiently enhanced Cd, Cu, Pb and Ni levels to approach the above figures. Zinc however, was only substantially enhanced at 2 sites (Sites 5 and 6: Rhondda 1 and 2).

The average concentrations (mg/ kg of dry weight) normally found in soil have been calculated as 0.06 for Cd, 30 for Cu, 40 for Ni, 300 for Zn and 10 for Pb (Lindsay 1979). A comparison of the levels found in the soils in this study with these concentrations shows that there are above average levels of Cd, Cu and Pb in the soils in South Wales. Of course the nature of the soil's parent material is very important in such comparisons since it may have been enhanced in one or more element. Such comparisons are therefore highly tentative.

5.4.2 Extractable heavy metal concentrations

The extractable concentrations of heavy metals are listed in Table 5.4. This table shows that none of the heavy metals were extractable at Site 9 (Tywi-Dolgoch). It also shows that the levels of several of these metals were extremely variable in comparison to their total soil concentrations. Neither lead nor nickel was extractable from the soil at any of the sites. It is well known that lead is tightly bound to organic or colloidal materials in soil, or it may be present in a precipitated form; all of which serve to reduce the uptake of lead into plant roots by conventional processes involving soluble ionic movement (Zimdahl and Koeppe 1977). It is therefore no surprise that the treatment with what is basically a dilute solution of a weak acid did not extract the lead ions.

It has been shown that the levels of extractable lead (using 2.5% acetic acid) near the site of non-ferrous smelting in the lower Swansea valley in South Wales decreased with increasing distance (Goodman and Roberts 1971). Here there was 260 mg/ kg (ODW) extractable lead present at a distance of 1.5 km from the valley mouth in which the smelter was situated. Downwind the levels fell off rapidly towards what were considered to be background levels of about 6-9 mg/ kg (ODW). These are higher than those obtained in this study and, even though the methods of extraction were slightly different, the sites near the smelter were probably more contaminated.

Plant availability and extractability of nickel have been shown to be reduced when organic matter content and pH increased (Halstead et al 1969, Foy et al 1978) and, though the soil pH values were very low, the large amount of organic matter present may well have played an important

part in its low availability. In comparison to the extractable levels of nickel in the nearby lower Swansea and Neath valleys, these sites were relatively uncontaminated, but total soil levels were higher than background levels.

The amounts of copper extractable from the soils were fairly constant and low at all the South Wales sites. Copper, like lead and nickel, is also tightly bound by organic material (Cottenie et al 1979) and this was probably the overriding factor that determined its availability in the soils.

The extractability of cadmium and zinc in relation to total concentrations varied substantially over and within the five South Wales sites. These two elements are again subject to the same influences as other elements but it is likely that pH, as the only factor that was in favour of increasing the elements' extractability, was important. Again, extractable levels of cadmium were lower than at the most contaminated sites of the Swansea and Neath valley sites, but some of the sites studied here (Table 5.4) had levels above the level taken as background for that study (Cd 0.5 mg/ kg, Goodman and Roberts 1971).

The extractable levels of the essential manganese ion at the South Wales sites were higher than at Site 9 (Tywi-Dolgoch), though the relationship of these levels to levels in other contaminated areas and its effects are unknown.

Within the sites, where some of the metals were extractable, the variabilities about the mean values were great. It was thought that this variation would be within plus or minus 10% of the mean values of the extractable nutrients with this extracting procedure and number of samples taken at each site, but this was obviously not the case.

Problems associated with the extraction of nutrients and heavy

metals from soil have often been recognized, and attention has been called to the lack of theoretical justification in the selection of extractants (Symeonides and McRae 1977). The type of soil is an important consideration when extractants are chosen, though proposals have been made which recommended extractants for individual elements on the basis that they were suitable for a variety of soil types. (Cd: Symeonides and McRae 1977, Zn: Lindsay and Norvell 1978).

The use of these extractants may have produced data that varied less about the mean values, but would have meant more work on the analysis of the soils. However, it seems likely that most of the variation was associated with variations in the soil properties at the sites rather than those due to the extractant themselves since many of the soil properties, including those not involving extraction procedures, varied as much or even more. It is likely that the only way that would have successfully reduced these variabilities would have been to increase the intensity of sampling.

5.4.3 Foliar analysis

Table 5.5 lists the concentrations of N, P, K, Ca and Mg in the foliar samples and the average needle weights taken at the eight sites. The main value of this data lies in establishing if there are any deficiencies in the levels of the major nutrients and whether they are related to effects produced by the heavy metals.

The concentrations of the heavy metals are listed in Table 5.6, the levels of which will be discussed in later chapters in relation to the critical tissue concentrations and interactive effects which have been established in water culture (Chapters 2 and 3). This information is,

Table 5.5

Average needle weight of and concentrations of major nutrients in foliar samples taken from forestry sites (all results expressed as dry weight)

Site No	Average needle wt (mg)	Nitrogen %	Phosphorus %	Potassium %	Calcium mg/ kg	Magnesium mg/ kg
1	3.07	1.55	0.28	0.90	550	968
2	4.33	1.98	0.17	0.61	1856	991
3	3.82	1.51	0.32	1.22	720	1217
5	2.24	1.41	0.26	0.69	1692	2251
6	2.61	1.82	0.17	0.69	2670	744
7	4.35	1.88	0.15	0.44	682	734
8	4.03	1.48	0.15	0.39	625	1161
9	5.55	1.86	0.25	0.46	1556	932

Table 5.6

Concentrations of heavy metals in foliage samples taken from forestry sites (mg/ kg dry weight)

Site No	Cadmium	Copper	Lead	Manganese	Nickel	Zinc
1	2.37	8.5	9.8	408	10.5	17.2
2	1.60	3.0	7.4	240	2.9	16.8
3	2.06	8.2	10.5	395	11.0	16.3
5	3.54	11.0	10.3	166	9.0	16.3
6	3.20	5.5	9.4	194	14.8	23.4
7	0.82	5.5	6.7	160	5.8	42.6
8	1.92	5.4	5.4	996	9.4	43.2
9	0.35	2.3	3.4	65	2.5	23.3

however, important in its own right in that it establishes background and elevated concentrations in the foliar tissues of the trees. An examination of the data reveals that all the sites in South Wales had elevated foliar concentrations relative to the Mid Wales site (Tywi-Dolgoch) for all the metals except zinc.

Comparisons between contaminated and uncontaminated samples of the same species are more tenable than those between species, but no other work has been found which has established the levels in foliar samples in sitka-spruce in contaminated and uncontaminated environments, making it impossible to relate the concentrations found in this study to other situations. It is not, for example, known if the background foliar concentration of 0.35 mg/ kg (ODW) cadmium determined in this study can be related to other situations where conditions, such as soil type, may be different.

It was not possible to use methods such as multiple regression and factor analysis as has been used in other studies to determine which factors were related to the growth of sitka-spuce (James et al 1978) because of the relatively low number of samples of both soil and foliage that were taken and also because of the variability in the soil properties.

5.5 Summary

Sampling for foliage and soil was carried out at typical forestry sites on which sitka-spruce were growing in South and Mid Wales with the aim of determining the extent of contamination by heavy metals. A total of nine 0.01 hectare sites were sampled, though only six sites were sampled for soil and eight for foliage. A general assessment of each site was made from Forestry Commission records and site inspection. The

soils were characterized by extracting and analysing 30 cores taken from 0 to 15 centimetres depth at each site.

It was found that in general the sites were quite similar with regard to their main chemical and physical properties, though levels of some extractable ammonium species were lower at the South Wales sites than at the Mid Wales site (Tywi-Dolgoch, Site 9). A study of total and extractable concentrations of heavy metal concentrations (Tables 5.3 and 5.4) showed that the soils at the South Wales sites had higher levels than the Mid Wales site (Site 9), and that this site could be used as a control for comparative purposes. The extractable soil levels of several of the metals examined at some of the sites in South Wales were similar to those found in a nearby area (lower Swansea and Neath valleys), which had in the past possessed a large non-ferrous metal smelting complex (Goodman and Roberts 1971), though other sites in South Wales had lower levels of these and other metals.

Sitka-spruce foliar concentrations of cadmium, copper, lead manganese and nickel were higher at all the South Wales sites than at the Mid Wales site (Tywi-Dolgoch, Site 9). This information, as well as demonstrating that there are enhanced levels in the foliar tissues, may be useful for other work which assesses the potential impact of these metals upon growth (Chapter 7).

It was not possible to correlate site growth indices, foliar and soil levels of heavy metals. The main reason for this being the great variabilities in the soil properties within each site. A great increase in the sampling intensity would have been required to bring the standard deviation about the mean values to within reasonable levels (plus or minus 10%). These values may then have been used to correlate the different site factors.

Chapter 6

GREENHOUSE EXPERIMENT

6.1 Introduction

Previous experiments with sitka-spruce seedlings (Chapters 2 and 3) used a water-culture medium to study the effects of particular heavy metals individually or in combination. Though these studies have yielded information about the actions of heavy metals within the plants, they have not described the complete picture. The different chemical and physical properties of the soil tend to complicate the system which one is attempting to model and therefore some account must be taken of these complications when dealing with a solid rooting medium as opposed to a medium in which water is the major constituent. The more complex interactions between the heavy metals, soil, soil microorganisms and the plants have important consequences, and are studied here in relation to tree growth.

6.1.1 Influence of soil factors

The buffering capacity of solid surfaces is important in soils; soil particles act as reservoirs of metal ions which are released when the concentration of ions in the soil solution between the root and soil particle has decreased as a result of root uptake or diffusion.

Another factor influencing the absorption of heavy metals from the soil by plants is the total concentration of the metal. Many studies both in field and greenhouse experiments have shown that increasing the heavy metal levels of the soil enriched the levels of metals in

plants. However, plants growing in cadmium-enriched soils in containers in a greenhouse were found to absorb more cadmium than the same plants grown on the same soil amended with identical amounts of cadmium in the field (De Vries and Tiller 1978, Page and Chang 1978). Roots in the container grown plants would have been subject to contaminated soil exclusively, whereas in the field, roots may extend to depths below the contaminated layer. This may have accounted for the differences in the levels. Tissue concentrations have also been shown to be influenced by the cation exchange capacity of the soil (Haghiri 1974).

Synergistic and antagonistic effects by heavy metals upon the uptake of other metals from soils have been reported (Lagerwerff and Biersdorf 1972) and, even though there have been subsequent investigations in this area, the knowledge is still extremely incomplete for a number of reasons (Chapter 3). To describe more clearly these interactions in soil requires a knowledge of diffusion coefficients and of the mechanisms by which ions are taken up by plant roots. These may only be established from information on the concentrations and concentration gradients of all the ions present (Nye 1966).

Plant roots growing in soil tend to grow along pores and channels. Depending upon the size of pores, the roots will either have sufficient room to penetrate and therefore will continue growing, or alternatively have too little room for penetration, in which case the roots do not grow freely, or channels may be much larger than the roots, thereby producing a lack of contact between the root and soil (Low 1972). When these channels are large, root hairs tend to proliferate in the damp atmosphere, effectively altering the amount of metal ion taken up into the plant root by increasing the surface area. Also, into these larger

spaces, roots excrete a thicker layer of mucilage containing hydrophilic polymers across which ions and uncharged solutes can pass more easily (Brams 1969).

6.1.2 Plant-soil interactions

In the soil, plant roots can induce changes in the diffusion of solutes by the excretion of hydronium or bicarbonate ions and organic substances. Carbon dioxide may also be released from the roots as a result of respiration. Each of these compounds or elements can create changes in the concentrations of ions and solutes in which one is interested or may affect other ions, which in turn influence the levels of more important ions (Nye and Tinker 1977).

It has long been supposed that roots excrete hydronium ions at their surfaces, so rendering nutrients available or exchanging them for cations. However, it has been shown that the plants absorb more anions than cations and would be expected to excrete more hydroxyl or bicarbonate ions in order to maintain electrical neutrality within the plant (Cunningham 1964, Walker 1960). This would have the effect of making the soils around roots more alkaline, and influence the uptake of metal ions or other solutes in this manner.

Respiration causes the liberation of carbon dioxide from the plant roots, this usually diffusing away from the roots under aerobic conditions, resulting in a lowering of the pH.

It has been known for many years that organic compounds are exuded from healthy undamaged plant roots (Rovira 1962). It is normally considered that "exudates" are soluble low molecular weight compounds, though other materials are produced by the root. In the soil region immediately around a developed root (rhizosphere), the presence of these

compounds is a major characteristic.

There are both direct and indirect consequences of this, though they are difficult to separate. Primarily the effects will depend on the amounts of material exuded. Direct effects by exudates relate to the chemistry of the rhizosphere, the main possibilities being desorption of the nutrient or heavy metal ions from adsorption sites in the soil. Chelation effects may also be important since organic and amino acids are major components of the exudates and can form very stable compounds with metal ions.

These compounds are exuded in greater quantities in solid media than in a water medium (Barber and Gunn 1974, Boulter et al 1966, Hale et al 1973). This directly concerns this study since a soil medium was used for the growth of the plants. It is known that the microorganism populations in the rhizosphere are modified by root exudates and they may therefore indirectly influence solute uptake because these microorganisms can change the phase equilibria of soil nutrients and heavy metals. Bacteria and fungi present near the root surface will take up ions and modify their speciation by incorporation into their tissues. Nutrient and other metal ions may also pass more easily into the roots because of the interconnecting nature of mycorrhizae and roots (Fogel 1980).

It is known that rhizosphere bacteria and fungi decompose soil minerals and also mineralize organic matter (Nye and Tinker 1977). Any significant reduction in this activity would lead to lower levels of nutrients in the soil solution and could be expected to have serious implications for the growth and development of the trees.

6.1.3 Ecosystem parameters

The presence of these complicating factors in the soil medium essentially changes the water culture model from a simple "Solution - Metal ion - Plant root" system to a more complex "Soil - Soil solution - Microorganism - Plant root" system. With this increase in complexity comes a greater number of relevant parameters which may be used to assess the effects of pollution upon the ecosystem.

The direct effects by the heavy metals upon the vegetation of forest ecosystems have been demonstrated by many workers, most notably in the regions around non-ferrous metal smelters (Buchauer 1973, Burkitt et al 1972, Little and Martin 1972, Whitby and Hutchinson 1974). These effects are really the products of simplifications of the forest ecosystem, brought about by the contamination, to a point where the effects are almost obvious to the naked eye, but these are not the only effects. In forest ecosystems more subtle indications of contamination may occur; these being relatively non-specific, such as shifts in species composition, reductions in soil respiration, reduced reproduction and increased susceptibility to disease and insect infestation (Smith 1974).

It has been shown that nitrogen mineralization in the mor horizon of a spruce forest is severely reduced at distances up to 7-9 kilometres from a brass mill. The severity of the effect was found to be correlated with the increasing levels of copper in the soil (Tyler 1975). It was also shown that the litter decomposition at that site was retarded as a result of the copper contamination (Ruhling and Tyler 1973).

As well as effects upon those processes essentially brought about and controlled by the activity of bacteria and non-symbiotic fungi, other microorganisms can be affected by heavy metals. Populations of

mutualistic, symbiotic associations between fungi and the absorbing roots (known as mycorrhizae) of willow and poplar trees were reduced because of the presence of copper in copper mine tailings (Harris and Jurgensen 1977). As it is well known that mycorrhizae enhance the uptake and translocation of ions, particularly phosphate, into coniferous trees under many conditions in forests (Bowen 1973, Harley 1969), it is of great importance to study the potential effects that heavy metals bring about.

6.2 Materials and methods

One method of studying forest ecosystem processes in a controlled environment is to sample intact forest microcosms with complete biotic assemblages and soil profiles which are similar to those in the contaminated areas, contaminate them without disturbing the microcosms and then measure certain parameters which might be affected by the contaminants (Ausmus et al 1978, Jackson et al 1978a, 1978b). The advantages of such an approach lie in its flexibility, in that it may be used to examine the effect of one or more contaminants such as heavy metals in the absence of others such as sulphur dioxide or ozone. Another method used previously is to sample soils of various properties, add heavy metals and then transplant seedlings (Rolfe 1973). This method was adopted in order to determine which type of soil would best suit the tree species in contaminated situations.

Although the former method was preferable in that there is less disturbance of the microcosm, for the purposes of this study it was impractical since there were no uncontaminated areas near South Wales which had the correct type of soil and age of trees. The manner in which

the soils could be "artificially" contaminated was also unsuitable in that a mixing of the soils with various amounts of heavy metals would have been necessary to produce ranges of these metals before seedlings could be grown in it. It was also impractical to use already contaminated soils and grow plants in these, since it would not have been possible to vary the concentrations of individual elements sufficiently for a detailed study.

It was therefore decided to adopt a different approach, whereby an uncontaminated soil would be obtained with the same properties as those at the contaminated sites in South Wales (except that it would have lower concentations of heavy metals), which could then be "artificially" contaminated. Sitka-spruce (Picea sitchensis) seedlings could then be transplanted into it and grown for an extensive period of time under greenhouse conditions. This would allow some measure of control over climatic conditions, input and output parameters, and a comparison between the contaminated and uncontaminated situations.

6.2.1 Choice of soil

Soil samples were taken from typical forestry sites in South and Mid Wales for comparison of the major soil characteristics and total and extractable heavy metal contents. This has been dealt with more fully in earlier sections on the sampling of soils at forestry sites (Chapter 5). Most of the sites had the same soil type (Rhondda peaty gley), though the site at Cymer (grid reference ST 889 980) was a Molinia dominated blanket bog, type 9b (Pyatt et al 1979). At each site thirty samples were taken down to depths of 15 centimetres (Blyth and Macleod 1978) and broken down manually into small aggregates following the standard quartering method to homogenize the soils.

The soils were characterized by the following standard methods: acidity as measured by pH in water, extractable heavy metal levels using a 0.5 M acetic acid extractant and determination of the metals by flame atomic absorption, total heavy metal concentrations by perchloric-nitric (1:3) acid mixture digestion and flame atomic absorption, organic carbon by pyrolysis at 400°C, total nitrogen by Kjeldahl digestion and the resulting ammonium determined by specific ion electrode, ammonium-nitrogen by extraction with 2M KCl and specific ion electrode, and nitrate by extraction and specific ion electrode.

Grid reference positions and site characterization data of the sites are presented in Tables 5.1-5.4. The Mid Wales site (Tywi-Dolgoch) was generally very similar to those in South Wales except that it had no extractable and comparatively low total concentrations of heavy metals (Tables 5.3 and 5.4). These characteristics made it suitable for use as an uncontaminated soil which could be treated experimentally.

6.2.2 Soil preparation

A large volume of soil was taken from the site at Tywi-Dolgoch at depths down to 15 centimetres and broken down manually into small aggregates, as previously described in Chapter 5. The peaty soil was mixed thoroughly and 'washed' silver sand added. The silver sand was considered uncontaminated with heavy metal ions for the purposes of this experiment, since leaching with concentrated hydrochloric acid yielded no heavy metals. The addition was made to allow drainage and prevent the development of anaerobic soil conditions. Several trials were performed to find the appropriate proportion of soil to sand and it was decided that 1:1 would be suitable since it held the moisture for a few days

after watering.

The percentage of moisture in this mixture was determined after drying at 105°C overnight, so that concentrations of heavy metals added to the solution could be related to a dry weight basis. An equivalent of 400 grammes of oven-dry 1:1 mixture was placed into 5 inch garden pots and 25 ml of solution containing the chloride salt of the particular heavy metal was added at the soil concentrations (mg/ kg of dry weight) at the levels shown below. Each experiment was carried out in triplicate and four control pots were also prepared to which only 25 ml of deionized water was added.

Cadmium	0.1, 0.4, 1, 2, 4, 8 and 16
Copper	0.5, 1, 2, 4, 8, 16 and 32
Lead	5, 10, 25, 50, 100, 200 and 400

The metals and the levels to be added were chosen as such because it was believed that it was these metals at these levels that could be having important effects at the forestry sites. All salts were analytical grade reagents. Each pot was treated with only one heavy metal so that their individual effects could be studied.

After 24 hours the contents of each pot were mixed thoroughly and 48 hours after this 10 seedlings, which had been germinated 4 weeks previously, were transplanted into each pot. Prior to and during germination these seedlings were treated in exactly the same manner as described earlier (Section 2.2, Materials and methods) except that they were older when the experiment was started.

A set of pots without seedlings was prepared in duplicate in the same manner in order to follow the changes in extractability of the heavy metals with time; samples being taken at 0, 3, 17, 31, 59 and 100

days. The analytical method used was the same as that described for the determination of extractable ions in the forest soils.

6.2.3 Control of parameters during experiment

The field moisture capacity of the soil was determined by saturating a known dry weight of normally moist soil with water, leaving it to drain for 24 hours and then weighing the soil. The weight of water remaining was the field capacity of the soil. The moisture levels in each pot were kept above one third of that level during the course of the experiment by watering every few days.

The soil moisture level was controlled because of the marked effects that occur in soils and plants when they are reduced to unfavourable levels. The interpretation of the effects produced in such a situation would be extremely complicated since it would involve changes in the transport of solutes and physiological processes such as the absorbing power of the root due to reduced water potential. Contact between the root and soil may also have been reduced by shrinkage of the soil or root.

The pots were placed into a greenhouse and their positions randomized, both sets being maintained at the same conditions for 100 days. The number of live plants in each replicate as the experiment progressed were recorded in order to keep track of the rate of morbidity.

6.2.4 Treatment of plants and soils

At the end of the experiment whole plants were extracted carefully from the soil and washed to remove as much of the soil as possible,

since particles adhering to the roots would make all subsequent determinations inaccurate. They were then separated into roots and shoots for examination, measurement and the determination of concentrations of heavy metals and certain nutrients. The lengths of the main root of each plant, number of root tips and mycorrhizal development (James et al 1978) were noted and recorded.

The plants of each treatment were combined and dried at 105°C overnight in weighed glass beakers, left to cool in a desiccator and reweighed to determine the yields as dry matter. The shoots and roots of each replicate were digested with a 3:1 mixture of concentrated nitric and perchloric acids, the solutions filtered using Whatman No 42 paper and the concentratons of heavy metals, potassium and calcium determined by flame atomic absorption and flame photometry using appropriate standards and blanks as in Chapter 2.

The soils in which the plants were growing and also the control set were analysed for total concentrations of heavy metals and concentrations of extractable ammonium-nitrogen by the methods used for the field sites. The effects upon the microbial populations were not measured directly using the direct dilution plating counting technique (Parkinson et al 1971) because of problems in separating the microorganisms from the large amounts of organic material in this soil.

6.3 Results and discussion

The results can be divided into those concerning the behaviour of the metals in the soil, both with and without plants growing in them and those concerning the plants, which may be further subdivided into sections dealing with the tissue concentrations of heavy metals and nutrients, their relationships to yield and the direct effects upon root

development.

6.3.1 Behaviour of metals added to the soils

The results of monitoring the extractable heavy metals in the set of pots without plants are listed in Table 6.1. Only at the highest concentrations could the changes be followed successfully. At the two highest Cd concentrations (8 and 16 mg/ kg) the extractable levels decreased throughout the experiment, the final concentrations being about 20% of the totals. The relationship between the total and extractable Cd levels in these soils were then similar to those found at the South Wales sites.

In comparison to the other metals, copper was not very extractable. This was probably due to the ability of Cu^{2+} to form very stable complexes with organic materials (Cottenie et al 1979). Similar levels were found at the South Wales sites where there were extractable concentrations up to only 10 mg/ kg Cu out of total concentrations of several hundred mg/ kg Cu.

At the highest concentrations of lead added, 10% remained extractable after 17 days: this did not change appreciably over the rest of the 100 day period. At the South Wales forestry sites, total Pb levels varied from 100-340 mg/ kg (Table 5.3) and, though this was the range covered here, none was extractable. This suggests that either the incorporation of Pb was incomplete or that experimental conditions differed slightly. Addition of silver sand may have produced different physical and chemical properties which made the Pb ions more extractable.

The total and extractable concentrations of heavy metals in the

Table 6.1 Greenhouse experiment: changes in the extractable soil concentrations (mg/ kg ODW) of Cd, Cu and Pb through the experiment

Conc added (mg/ kg ODW)		Extractable concentration (mg/ kg ODW)				
		Time of sampling after beginning of experiment (days)				
		3	17	31	59	100
Cadmium	0.1	0.0	0.0	0.0	0.0	0.0
	0.4	0.0	0.0	0.0	0.0	0.4
	1.0	1.2	0.4	0.0	0.2	0.2
	2.0	1.6	0.8	0.0	0.0	0.0
	4.0	3.5	1.7	1.4	0.4	1.1
	8.0	7.6	2.8	3.0	1.7	1.7
	16.0	10.7	7.5	7.4	2.6	2.8
Copper	0.5	0.0	0.0	0.0	0.0	0.0
	1.0	0.0	0.0	0.5	0.0	0.0
	2.0	1.5	1.5	0.6	0.0	0.0
	4.0	2.5	2.3	0.5	0.0	0.0
	8.0	2.9	2.1	0.8	0.7	0.0
	16.0	3.6	3.3	2.0	0.3	0.7
	32.0	4.3	4.5	2.9	0.6	1.1
Lead	5.0	5.0	0.0	0.0	0.0	0.0
	10.0	5.0	0.0	0.8	0.0	1.0
	25.0	8.1	4.5	2.5	2.0	2.6
	50.0	18.3	13.1	4.8	2.0	3.3
	100.0	25.8	10.8	11.2	4.1	6.0
	200.0	71.4	23.2	25.6	12.0	10.7
	400.0	238.6	44.6	46.0	39.9	30.0

ODW = Of dry weight

Table 6.2 Greenhouse experiment: total and extractable concentrations of Cd, Cu and Pb in soil after 100 days

Concentration added (mg/ kg ODW)		Extractable concentration (mg/ kg ODW)	Total concentration (mg/ kg ODW)
Cadmium	0.1	0.00	0.54
	0.4	0.19	0.83
	1.0	0.30	1.57
	2.0	0.30	2.50
	4.0	1.03	3.93
	8.0	2.02	8.40
	16.0	3.70	17.33
Copper	0.5	0.00	4.83
	1.0	0.00	5.00
	2.0	0.00	5.67
	4.0	0.30	5.75
	8.0	0.29	9.17
	16.0	0.70	16.50
Lead	5.0	0.8	34.2
	10.0	1.3	40.0
	25.0	1.7	47.9
	50.0	2.5	70.8
	100.0	6.6	141.0
	200.0	11.7	183.4
	400.0	24.6	435.0

ODW = Of dry weight

soils in which the plants were grown are presented in Table 6.2. The extractable levels were similar to those in the set without seedlings at 100 days (Table 6.1), indicating that the changes in extractability occurred at the same rates. The total concentrations of heavy metals approximately matched the sums of concentrations in the original soil and those added if the addition of the silver sand is taken into account, though there were some slight anomalies in the Cd treatments (Table 6.2). Overall, however, there were no indications that heavy metals had been lost from the pots as a result of leaching.

6.3.2 Plant yields and elemental concentrations

In this section the effects of the treatments are discussed in relation to yields of dry matter, availability of the metals in the soil and influence upon metal uptake, and uptake of other heavy metals and the nutrients potassium and calcium.

The yields of the shoots and roots and the corresponding tissue concentrations of the heavy metals with which the soils were treated are listed in Table 6.3. This table shows that Cd and Pb concentrations increased in both shoots and roots with respect to the total concentrations in the soil, but that Cu did not.

6.3.2.1 Effects upon yield When shoot yields in terms of dry weight are plotted against the log concentrations of the heavy metals, (Finney 1947), critical tissue concentrations may be obtained using a standard technique (Beckett and Davis 1977). This method has been used previously (Chapter 2) for sitka-spruce seedlings, though the plants were grown using a water culture technique. However, for this experiment the method was not sufficiently accurate for the determination of the critical

Table 6.3 Greenhouse experiment: plant yields and tissue concentrations of Cd, Cu and Pb after 100 days

Concentration added (mg/ kg ODW)		Shoot concentration (mg/ kg ODW)	Root concentration (mg/ kg ODW)	Shoot yield (mg/plant)	Root yield (mg/plant)
Cadmium	0.0	0.0	2.0	9.96	5.32
	0.1	5.9	27.1	8.48	4.62
	0.4	7.0	24.3	6.11	4.11
	1.0	6.4	33.9	10.48	5.90
	2.0	12.9	60.6	5.80	2.89
	4.0	14.4	93.4	6.33	3.70
	8.0	17.4	134.9	4.97	3.20
	16.0	25.1	164.8	4.65	2.93
Copper	0.0	9.6	49.3	9.96	5.32
	0.5	11.6	63.9	9.70	5.29
	1.0	16.4	113.9	6.48	4.13
	2.0	18.1	116.9	10.02	4.33
	4.0	51.5	228.7	5.50	3.37
	8.0	36.0	93.6	3.37	2.44
	16.0	30.5	197.6	13.95	8.30
	32.0	10.9	150.0	9.30	3.37
Lead	0.0	1.7	67.2	9.96	5.32
	5.0	19.6	88.3	6.43	3.68
	10.0	23.0	126.8	6.58	4.35
	25.0	27.5	228.2	8.81	4.89
	50.0	41.0	283.3	9.53	5.13
	100.0	71.7	444.8	7.55	3.87
	200.0	60.5	477.2	7.00	4.10
	400.0	391.6	1220.5	4.15	2.20

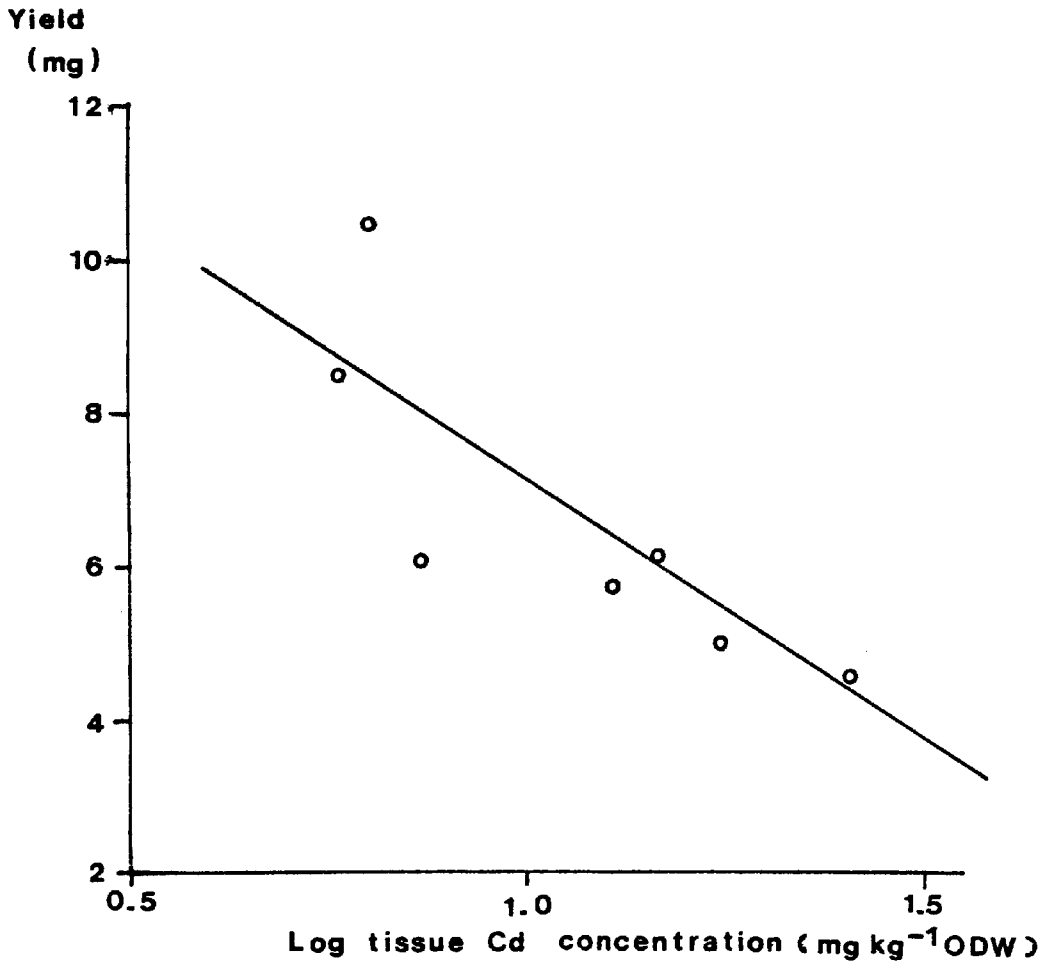
values. The data used in the calculations of these values with the programme "CRTTC" are shown in Tables 16-18 (Appendix A). The reasons for these inaccuracies may have been that the number of points in these calculations was insufficient to produce reasonable correlations and accurate representations of the regression lines below the critical points.

When all the points of the cadmium treatment were used in a least squares regression, a significant negative correlation coefficient was obtained (Figure 6.1, $b=-0.24$, $r=-0.81$, $p<0.01$ where $n=8$, Fisher and Yates 1948). The growth of the seedlings treated with Cd was increasingly affected as tissue concentrations increased. As the critical level has been established as 4.8 mg/ kg Cd for sitka-spruce seedlings (Chapter 2, Burton et al 1983) and for other plants (barley, lettuce, rape and wheat) as 8 mg/ kg Cd (Davis and Carlton-Smith 1980), this is to be expected since all the plants except those in the control set had accumulated sufficient cadmium to exceed the critical Cd level.

An upper critical lead value, which agreed well with the average value obtained in the water culture study (19 mg/ kg), was obtained using this method (18.9 mg/ kg), but the regression line at the point of intersection was not quite significantly correlated at the $p<0.05$ level. However when all the yields were plotted against the log shoot lead concentrations a significant negative correlation was obtained ($r=-0.63$, $p<0.05$, Fisher and Yates 1948), but the slope of the line ($b=-0.011$) shows that growth was only slightly affected and not sufficiently to allow a calculation of the critical shoot Pb concentration.

A significant correlation coefficient was not obtained when the shoot yields were plotted against the log shoot copper concentrations for the copper treatments. This could be expected since copper tissue

Figure 6.1 Yield curve for Cd greenhouse experiment: shoot yield plotted against log tissue Cd concentration



concentrations did not increase over the range of Cu treatments used (Section 6.3.1).

6.3.2.2 Soil availability in relation to plant uptake The extractable soil levels of the heavy metals present at the end of the experiment (Table 6.2) were plotted against the shoot and root concentrations of the metals with which the soils had been treated, and it was found that significant positive correlations existed for Cd (shoots; $b=5.53, r=0.91, p<0.001$. roots; $b=42$, $r=0.94$, $p<0.001$ Fisher and Yates 1948) and Pb (shoots; $b=14.35$, $r=0.94$, $p<0.001$. roots; $b=44.1$, $r=0.98$, $p<0.001$ Fisher and Yates 1948). There were, however, very low correlations between the extractable Cu levels and tissue Cu concentrations. As only low concentrations of Cu were extractable, it would not be expected that seedling growth would be affected.

Correlations between the extractable levels of Cd and the leaf Cd concentrations in radish (Raphanus sativus) in a range of soils have shown other extractants, such as ammonium nitrate and a mixture of ammonium acetate and acetic acid, to reflect more accurately the available levels (Symeonides and McRae 1973). The correlations obtained with acetic acid in that study were only significant at the $p<0.01$ level whereas other extractants, such as ammonium nitrate, were more significantly correlated with the amounts taken up. However, the correlations obtained in this study with an acidic peaty soil suggest that the acetic acid extractant adequately reflected the available levels when fairly wide ranges of concentrations were used.

Plant growth was affected when only 0.1 mg/ kg Cd was added to the soil, giving a total concentration of 0.54 mg/ kg (Table 6.2), where none of the metal was extractable. This in turn produced sufficient

increases in Cd concentration within the plants to significantly affect growth. Though the correlations with Cd uptake were quite good, the method was not sufficiently sensitive and other methods or extractants may give better results. Even if an extractable value could have been obtained, it remains of dubious value to state what extractable concentration of cadmium would be sufficient to produce reductions in the yield of the plants, since this may even vary widely within one particular class of soil.

6.3.2.3 Uptake of elements other than those added Tables 6.4, 6.5 and 6.6 list the shoot and root concentrations of heavy metals other than those added, and the concentrations of potassium and calcium for the cadmium, copper and lead treatments respectively. There were some significant negative correlations between the Cd and Cu treatments and the shoot concentrations of K. Root K concentrations were also negatively correlated with Pb treatments. However, these were not greatly significant ($p<0.05$ in all cases), and an examination of the data revealed that the relationships were tenuous and inconclusive. There were no significant correlations between the levels of treatments or tissue concentrations of lead or copper and the tissue concentrations of calcium or potassium.

Previous studies in water culture have demonstrated the existence of interactive effects of heavy metals which affected the uptake of nutrients and heavy metals (Chapters 2 and 3), but a more detailed experiment examining them with treatment combinations of heavy metals would have to be performed to substantiate their effects upon nutrient and heavy metal uptake from the soil.

Table 6.4 Greenhouse experiment: tissue concentrations of K, Ca, Cu and Pb in shoots and roots of sitka-spruce seedlings grown in soil amended with Cd

Cadmium added (mg/ kg ODW)	K (% ODW)		Ca (% ODW)		Cu (mg/ kg ODW)		Pb (mg/ kg ODW)	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
0.0	0.85	0.59	1841	1211	21.3	68.0	1.7	67.2
0.1	1.18	0.45	1670	1010	16.7	92.1	N/D	20.4
0.4	0.99	0.40	1519	993	19.9	105.2	N/D	88.2
1.0	1.35	0.40	1908	955	13.5	63.3	1.7	91.8
2.0	1.03	0.48	1563	636	23.5	38.8	2.4	48.9
4.0	1.04	0.49	1688	1515	15.7	50.6	N/D	62.4
8.0	0.80	0.44	1274	778	14.6	42.7	1.5	82.2
16.0	0.75	0.37	1523	852	19.1	48.3	N/D	64.2

N/D = Not detectable
ODW = Of dry weight

Table 6.5 Greenhouse experiment: tissue levels of K, Ca, Cd and Pb in shoots and roots of sitka-spruce seedlings grown in soil amended with Cu

Copper added (mg/ kg ODW)	K (% ODW)		Ca (% ODW)		Cd (mg/ kg ODW)		Pb (mg/ kg ODW)	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
0.0	0.85	0.59	1841	1211	1.5	3.0	1.7	67.2
0.5	1.05	0.47	1105	710	1.9	6.8	0.8	91.8
1.0	0.82	0.55	1641	910	1.7	9.1	1.9	34.2
2.0	1.30	0.81	1747	1154	N/D	N/D	1.9	44.0
4.0	1.21	0.79	2273	1485	N/D	N/D	N/D	N/D
8.0	0.92	0.36	1650	739	N/D	4.6	3.7	51.4
16.0	1.10	0.75	1882	1205	N/D	6.0	N/D	68.1
32.0	0.39	0.62	806	1114	N/D	5.0	2.0	55.9

N/D = Not detectable
ODW = Of dry weight

Table 6.6 Greenhouse experiment: tissue levels of K, Ca, Cd and Cu in shoots and roots of sitka-spruce seedlings grown in soil amended with Pb

Lead added (mg/ kg ODW)	K (% ODW)		Ca (% ODW)		Cd (mg/ kg ODW)		Cu (mg/ kg ODW)	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
0.0	0.55	0.59	1841	1211	N/D	4.0	9.6	49.3
5.0	1.06	0.54	1360	1087	N/D	5.4	17.0	109.0
10.0	1.10	0.66	2187	1437	N/D	N/D	41.4	77.0
25.0	0.75	0.55	1088	853	N/D	16.2	12.4	47.6
50.0	1.10	0.43	1241	709	N/D	7.1	10.4	45.2
100.0	0.98	0.43	1435	862	N/D	12.9	24.1	47.0
200.0	0.95	0.53	1608	915	N/D	N/D	26.0	133.3
400.0	0.48	0.34	1807	1137	N/D	N/D	102.4	193.2

N/D = Not detectable
ODW = Of dry weight

6.3.3 Effects upon root development

In this section the effects of the heavy metals upon the seedling root development in terms of lengths of main roots, root branching and mycorrhizal development are discussed.

6.3.3.1 Lengths of roots The average root lengths and their standard deviations were calculated for each treatment (Table 6.7). This data was then tested for normality of distribution using "Lilliefors" test (Hollander and Wolfe 1973), and it was found that all the data fitted this pattern. This allowed the use of parametric statistical tests such as "t" test and analysis of variance. The average root lengths of the control group plants were tested against the averages of all other concentrations and the combined average of root lengths for each metal using the students "t" test (Eckshlager 1961) to detect significant differences between the groups. The results of these tests are shown in Table 6.7 along with the the probabilities and degrees of freedom for each test.

The results reveal that when Cd was added to the soil to give a total concentration of 2.5 mg/ kg and an extractable concentration of 0.3 mg/ kg , there were significant reductions in root lengths. The average root length of combined Cd treatments was also significantly different from that of the control group ($p<0.01$ with 76 degrees of freedom).

Lead produced significant reductions in the root lengths at total concentrations of 48 mg/ kg when extractable concentrations remaining at the end of the experiment were 17 mg/ kg and above. There were also significant differences between the average root lengths of the combined Pb treatments and the control group. Copper did not clearly affect root

Table 6.7 Greenhouse experiment: statistical analysis of effects by Cd, Cu and Pb upon root lengths

Concentration added (mg/ kg ODW)		Average root length (cm)	Standard deviation (cm)	"t" value from test with control	Number of degrees of freedom
Cadmium	0.1	10.41	5.90	0.91	24
	0.4	9.51	6.48	1.19	24
	1.0	12.23	6.54	0.26	23
	2.0	5.33	2.41	3.22*	25
	4.0	7.17	3.90	2.61*	27
	8.0	8.27	4.18	2.42*	32
	16.0	5.7	3.09	2.58*	23
Copper	0.5	14.91	7.46	0.47	31
	1.0	8.55	3.35	1.79	25
	2.0	9.32	4.05	1.28	23
	4.0	6.93	4.94	1.49	20
	8.0	5.86	2.57	2.73*	20
	16.0	17.90	4.53	0.98	19
	32.0	6.89	3.74	2.34*	24
Lead	5.0	7.72	3.17	2.37*	27
	10.0	9.58	4.09	0.99	21
	25.0	7.70	3.89	2.50*	29
	50.0	8.14	4.95	2.10*	28
	100.0	6.58	3.27	2.26*	23
	200.0	7.11	2.38	2.11*	23
	400.0	2.95	0.64	2.09*	19
Control		13.07	6.50	-----	-----
All Cd treatments		8.16	5.21	3.31**	76
All Cu treatments		10.02	6.15	1.7	66
All Pb treatments		8.16	3.75	4.34**	68

* Significant at the $p<0.05$ level, ** Significant at the $p<0.01$ level

lengths in any of the treatments. This was also true when the average root length of the combined Cu treatments was tested against the control group. The differences between the lengths of roots in the Cd and Pb treatments and the control group were probably due to the extractable levels of these metals which remained relatively high throughout the experiment.

6.3.3.2 Mycorrhizal form and root branching The results of the analysis of the plant roots for mycorrhizae and root branching are presented for the different treatments in Tables 6.8 and 6.9. It was found that the only group that possessed any true mycorrhizae was the control group and that all other root branching was pseudo or non-mycorrhizal.

Four of the nineteen control plants had the true mycorrhizal characters of regular dichotamously branching roots, swollen with shiny smooth surfaces and whitish tips (James et al 1978). All other roots in this group and other treatments had dark irregular branching roots with no beading or swelling or were normally branched roots indicating that they were either pseudo or non-mycorrhizal (James et al 1978). These two groups were difficult to separate and it was considered unimportant since the main interest lay in the effect upon infection by the mycorrhizae.

In other studies, counts of root tips and measurements of lengths of main and lateral roots were made for successive 10 centimetre lengths, with unbranched roots being discounted, enabling a ratio of root tip to root length to be estimated (James et al 1978). Unfortunately, the seedling roots were often shorter than 10 cm, since these were the roots of seedlings and not mature trees. However, the

Table 6.8 Greenhouse experiment: effects of Cd, Cu and Pb upon the number of plants possessing mycorrhizae and branching roots

Concentration added (mg/ kg ODW)		No. of plants possesing different types of roots		
		True mycorrhizal	Pseudo-mycorrhizal	Non-branching
Cadmium	0.1	0	6	1
	0.4	0	5	2
	1.0	0	4	2
	2.0	0	5	3
	4.0	0	7	3
	8.0	0	12	3
	16.0	0	3	3
Copper	0.5	0	13	1
	1.0	0	8	1
	2.0	0	5	1
	4.0	0	3	1
	8.0	0	6	3
	16.0	0	2	0
	32.0	0	5	2
Lead	5.0	0	7	2
	10.0	0	2	2
	25.0	0	10	2
	50.0	0	10	1
	100.0	0	6	2
	200.0	0	4	2
	400.0	0	1	1
Control		4	15	0

ODW = Of dry weight

Table 6.9 Greenhouse experiment: statistical analysis of effects by Cd, Cu and Pb upon the numbers of root tips/cm of root length

Concentration added (mg/ kg ODW)		Average no of root tips/cm	Standard deviation of no/cm	"t" value from test with control	Number of degrees of freedom
Control		0.661	0.319	-----	----
Cadmium	0.1	0.799	0.226	0.94	24
	0.4	0.841	0.287	1.10	23
	1.0	0.605	0.113	0.33	22
	2.0	1.185	0.285	3.20**	23
	4.0	0.898	0.374	1.54	25
	8.0	0.830	0.390	1.27	30
	16.0	0.385	0.100	1.42	21
Copper	0.5	0.917	0.502	1.71	31
	1.0	0.867	0.476	1.26	26
	2.0	0.646	0.203	0.10	23
	4.0	0.689	0.373	0.13	21
	8.0	0.594	0.126	0.48	24
	16.0	0.645	0.243	0.07	24
	32.0	0.902	0.269	1.48	23
Lead	5.0	0.573	0.282	0.62	25
	10.0	0.426	0.290	0.95	20
	25.0	0.551	0.231	0.93	28
	50.0	0.581	0.218	0.69	28
	100.0	0.565	0.175	0.68	24
	200.0	0.443	0.129	1.28	22
	400.0!	-----	-----	----	----

** Significant at the $p<0.01$ level
! Not determined because there were few plants
ODW = Of dry weight

average number of root tips per centimetre of plant root were calculated by combining the results of the replicates for each treatment. This gave averages and standard deviations for all the treatments (Table 6.9) which were tested for normality of distribution, as above, using "Lilliefors" test. It was found that each of the treatments possessed normally distributed data and students "t" tests were then performed to test for the differences between the control group and other treatments. There were no clear effects upon the numbers of root tips by any treatment except a slight increase in number, significant at the $p<0.05$ level, by 2 mg/ kg Cd.

These tests only took into account those plants whose roots had branched, and it can be seen from Table 6.8 that many of the roots in the soils treated with heavy metals possessed non-branching roots, whereas all the plants in the control group had branching roots. It seems likely that the soil treatments of heavy metals were the cause of this.

It may therefore be concluded that the numbers of important mycorrhizal associations and the amount of root branching were reduced by Cd, Cu and Pb, and may have resulted in some disturbance of the nutrient balances within the seedlings.

6.4 Summary

Shoot and root concentrations of cadmium and lead were positively correlated with the extractable levels of these metals remaining in the soil at the end of the experiment and to the total amounts added. Copper tissue levels were not correlated with either of these. As shoot cadmium concentrations increased, there was a concomittant reduction in the

shoot yields; lead shoot concentrations were also negatively correlated with yield though the effect was less marked. Copper was found to have no effects upon shoot or root yields.

It was found that there were critical levels of cadmium and lead treatments at and above which the lengths of the seedling main roots were reduced significantly (0.3 mg/ kg Cd extractable and 2.5 mg/ kg Cd total, 1.7 mg/ kg Pb extractable and 48 mg/ kg Pb total). Copper,on the other hand did not affect the lengths of the main root at any critical treatment point. This was probably related to its unavailability in this type of soil.

The amounts of root branching and the numbers of important symbiotic mycorrhizae were significantly reduced by increases in the soil levels of cadmium and lead. It might be expected that heavy metals affect root development but the effects upon mycorrhizae may be of equal or greater importance at forestry sites with low nutrient status because of the importance of mycorrhizae in the uptake of certain nutrients such as phosphate.

Chapter 7

GENERAL DISCUSSION

The aim of this study was to assess the effects of heavy metal pollution upon tree growth in South Wales. As there are a number of species of tree that grow in South Wales, one species, the most commercially important, sitka-spruce (*Picea sitchensis*), was the subject of this project. Tree growth is very difficult to assess in relation to heavy metals because there are a number of climatic and edaphic factors quite apart from heavy metals which can influence tree growth. The forestry sites, upon which sitka-spruce grow, are obviously fairly inhomogeneous; the age of the trees, chemical and physical properties of the soils, climatic conditions and the forestry practice employed at particular sites all vary over the region studied.

It has been shown that the complexity of temperate forestry ecosystems tends to be correlated with the level of pollution in these systems (Smith 1974). At forestry sites with very high levels of pollution, there may be extreme simplifications of the ecosystem and, as a result, the factor causing this simplification is easily detected. However, at lower levels of pollution, the effects are more subtle, with changes in species composition, disease outbreaks, insect infestations, reduced productivity and reproductive capacity being the relatively non-specific expressions of this pollution (Smith 1974). In such a situation, as may exist in South Wales, the problem is first of all one of deciding if the poor growth can be attributed to pollution and if so of establishing the extent to which this is affecting tree growth.

Several of the methods used in this study have been fairly empirical since a study of one area was required and not a general study of the effects of heavy metal pollution upon plant growth at physiological or biochemical levels. Taking an overall view of the effects by heavy metals upon tree growth by sampling a large number of sites and then correlating certain variables with the tree growth was not possible because many of the soil properties varied greatly within the forestry sites and because there were difficulties in obtaining site growth parameters which could be compared over the whole study area (Chapter 5). It was therefore necessary to break down the work into areas dealing with particular aspects of effects by heavy metals and examine these experimentally (Chapter 1). The information obtained at the forestry sites, however, remains of great value since the experimental results can be related to the forestry situations.

This has been carried out by establishing the upper critical tissue concentrations of several heavy metals in water culture, thereby gaining some idea of the potential impact that certain foliar levels of heavy metals could have (Chapter 2). Other aspects of the work have examined the impact of heavy metals upon mycorrhizae and root development, though again these are potential effects and not actual effects established in field studies. Having done this, it is then necessary to relate the two studies.

The different techniques employed to grow the seedlings have, of necessity, made use of highly artificially controlled environments where several confounding factors were kept constant, though some experiments have been carried out to determine the effects of varying these (for example, the effect of the age of the seedlings upon the upper critical

values).

One problem was the age of the seedlings used in these experiments since the effects of the heavy metals may not be the same in forestry grown trees. During the development of a plant, changes occur at all functional levels within the plant. Therefore any comparison of the effects found in seedlings with trees in forestry situations must be based, as far as possible, on similar tissues. Any other comparisons should be treated tentatively. It is hoped that the techniques used here have yielded results that are not entirely dependent upon particular conditions and therefore have some general relevance to the growth of mature trees.

Though several areas of the investigation have employed techniques which examined the effects of particular metals, it was hoped to integrate these studies by looking at the effects of combinations of metals. However, the interpretation of these results is very difficult since field studies have indicated that the levels of these different metals vary substantially. However, even if they were constant, the degree of interaction would depend heavily upon environmental conditions. This indicated that the best approach to this problem was to determine the effects of important factors and to relate them to the field studies.

In doing this, the project has relied heavily upon the use of water culture techniques to grow sitka-spruce seedlings, using these as a basis for the controlled experiments in which the effects were studied. The advantages of water culture techniques over soil media are that they allow almost complete control over growing conditions and that the plants develop very quickly in the nutrient solutions. This enabled a study of the effects of heavy metals in reasonably short periods of

time. One possible disadvantage of using this technique is that the effects of heavy metals upon soil grown plants may be different. To overcome this, some experiments with soil grown plants have been carried out (Chapter 6).

The typical visual symptoms of heavy metal toxicity in sitka-spruce have been established and were shown to be relatively non-specific effects which may invariably be associated with other diseases. As such, they may have little value in field situations unless they can be related to foliar concentrations of heavy metals with mature trees.

It has been demonstrated by many workers that certain elements are more toxic to some plant species than others, though some of these differences may have been due to errors in the particular methods used. Differences in the tolerances to heavy metals occur between different classes of plant: monocotyledons having demonstrably higher tolerance indices than do dicotyledons (Cottenie 1981). Even within a particular class of plants the tolerances may vary significantly. It has been shown, for example, that the tissue Cd concentration in dicotyledonous plants, whose yields had been reduced by 25%, varied between 15 mg/ kg Cd for the field bean (Phaesolus vulgaris) and 160 mg/ kg Cd for the cabbage (Brassica oleracea) (Bingham et al 1975). Clearly, therefore it is not good enough to use an index of phytotoxicity for an element which was calculated using a species of plant different from the one of interest.

Many methods have been proposed which supposedly indicate the relative toxicities of heavy metals to plants. One method, for example, takes the form of a bioassay- tolerance index which is the mean length of the longest roots in metal solution divided by the mean length of the

longest roots in metal-free solution, expressed as a percentage (Wilkins 1957). This index, however, depends greatly upon growing conditions and also takes no account of the effect upon shoots.

Other methods determine what tissue concentrations of metals may produce 10 or 25% reductions in yield (Bingham et al 1975). However, the slope of the regression line from which these figures are calculated (the slope of the yield between the onset of toxicity and the point where there would theoretically be no growth) is subject to variation with growing conditions (Beckett and Davis 1977, Davis and Beckett 1978); these 10 or 25% values will therefore vary from situation to situation.

Upper critical tissue concentrations have many advantages over other indices of heavy metal toxicity. Values of upper critical tissue concentrations have been shown to hold true for a variety of conditions (Beckett and Davis 1977, Davis and Beckett 1978). Since shoot yields seem to be the most critically affected sites, and the critical concentrations of these heavy metals in the shoots are the most relevant concentrations of these metals (Beckett and Davis 1978), these were chosen as indices of toxicity for this study.

Though it was not completely necessary to determine the upper critical levels using water culture as the growing medium, it has mainly been used because of the advantages afforded in quick growth and control over conditions. Some attempts were made to determine upper critical tissue concentrations using soil grown plants (Chapter 6), but this was not completely successful. These experiments were set up with a relatively small number of treatments and a combination of this and the slower growth rate in soil may have led to the inconclusive results.

In studying the toxicities of heavy metals to plants grown in water

culture, it is important to establish what confounding effects the heavy metals may have upon nutrient speciation in the solutions. Calculation of the levels of all complex species in the nutrient solutions, with levels of the heavy metals set at approximately the same as used in the critical concentration experiments, was carried out using the computer programme "COMICS" to examine the possibility of such effects.

In the solutions to which Cd, Cu and Ni had been added, the most common species in the solutions were the free divalent metal ions (M^{2+}). In the case of zinc, however, the predominant species was zinc hydroxide ($Zn(OH)_2$); this was the only significant Zn species present. For lead, the lead phosphate species ($Pb_3(PO_4)_2$) became the most important of the lead species, accounting for about 30% of the lead at 2.5 mg/ l Pb (12.1 µM, the highest level actually used in the water culture experiments, Table 4.5), though it would account for 60% of the total phosphate if the concentration of Pb was increased to 10 mg/ l Pb (48.3 µM Pb, Table 4.6). It was therefore unlikely that the toxicities of the metals resulted from alterations in the speciation of the nutrients in the solutions.

7.1 Foliar and upper critical concentrations

The method of assessing tolerance to heavy metals was then applied to sitka-spruce in forestry plantations by comparing foliar and upper critical levels of the heavy metals. However, there are several problems in this approach.

Foliar levels of heavy metals are the total concentrations of heavy metals, and may be derived from several input sources. It has been shown by moss bag studies that several heavy metals are currently present at

fairly high levels in the atmosphere of South Wales, and are presumably there as a result of emission from various sources (Welsh Office 1975). These may be deposited onto the soil and taken up into the trees via the roots or impacted on the needle surfaces, where some may remain insoluble. When metals are deposited at the surface of the soil, they may accumulate there over a period of time as a result of several processes or they may move laterally and vertically in the soil layers. Any metal that is not completely insoluble may then, depending upon the plants themselves, be available for uptake into the plant roots.

Solutions present on leaf surfaces are moderately acidic, with many different metals and ligands present. On the leaf surfaces there are fixed negatively charged sites which can act as absorbers (Phipps 1981). It is therefore possible that a substantial proportion of the metal, if it is available, may be captured by the ion exchange processes at the needle surface.

The chemical speciation and the resulting biological activities of metals which have taken up either by roots or by needle surfaces may well be different, although it would be impossible to differentiate between them without sophisticated biochemical techniques. It is therefore necessary to generalize about the activities of the metals derived from the two different sources and assume that they are approximately similar since they have been subjected to similar chemical and biological processes.

One proposed method of looking at field situations relates the normal background and upper critical tissue concentrations of an element to its concentration in a tested crop, giving an index of relative hazard "I" (Beckett and Davis 1977):-

$$I = m. \log_{10} T/n$$

where n is the normal background concentration, T is the concentration of the metal at the site being assessed, and m is an expression incorporating n and the upper critical level Tc:

$$m = 1/(\log Tc - \log n)$$

Using the average upper critical tissue concentrations of sitka-spruce seedlings for the elements Cd, Cu, Ni, Pb and Zn (Table 2.10, Burton et al 1983) and the foliar levels of these metals at the control site (one selected as having normal background concentrations of these metals; Site 9: Tywi-Dolgoch, Mid Wales), then the relative hazards presented by these metals at the South Wales sites may be calculated using the foliar concentrations at these sites (Table 5.6). The values of m and n for the metals Cd, Cu, Ni, Pb and Zn are given below.

	Cd	Cu	Ni	Pb	Zn
m	0.881	0.633	2.71	1.313	1.014
n	0.35	2.3	2.5	3.4	23.3

The relative hazards presented by these metals by these metals were calculated for the seven South Wales sites at which foliar samples were taken and are listed in Table 7.1. When a value of "I" is greater than one then the toxic effects of the metal become apparent. It is not possible to compare the values of "I" determined here with those of other workers (Beckett and Davis 1977, Davis and Beckett 1978), since the situations to which these other workers have applied the relative hazards are mainly those involving agricultural crops grown on arable land. It must also be stressed, however, that these indices do not take

Table 7.1

Relative hazards (I) of toxicity due to particular heavy metals at forestry sites in South Wales assessed by means of critical tissue and background concentrations

Site	Relative hazard (I)				
	Cd	Cu	Ni	Pb	Zn
1. Cymer	0.73	0.36	1.70	0.60	0.00
2. Margam 1	0.58	0.07	0.17	0.44	0.00
3. Margam 2	0.68	0.35	1.75	0.64	0.00
5. Rhondda 1	0.89	0.43	1.51	0.63	0.00
6. Rhondda 2	0.85	0.24	2.10	0.58	0.00
7. Rhondda 3	0.33	0.24	0.99	0.39	0.27
8. Rhondda 4	0.36	0.24	1.56	0.26	0.27

account of any interactive effects, but merely give an indication of the sites which are most likely to be affected by particular metals.

An examination of this table reveals that most of the sites would seem to be at risk to nickel toxicity, the exception being site 2 (Margam 1). The foliar concentrations of nickel were above the upper critical level at all the other sites, yielding index values greater than one. Several sites also had foliar Ni levels which were above the average upper critical concentration for nickel established at 11 mg/kg for several other plant species (Table 2.10, Davis and Carlton-Smith 1980). Therefore, even allowing for an error in the value for sitka-spruce, it would seem reasonable to assume a risk of Ni phytotoxicity at these sites. Cadmium and lead may also contribute to the overall toxicity since values of I approached unity at several sites; cadmium values being generally higher than those of lead (Burton

et al 1983).

The other metals examined, copper and zinc, would seem to have no toxic effects upon tree growth if the interactive effects are negligible since the foliar levels were, at most sites, only just above the normal background concentrations and well below the upper critical levels.

7.2 Interactive effects of heavy metals upon yield

The factorial experiments carried out in water culture examined the effects of heavy metals individually and in combination. These experiments were not completely successful in that there were few effects by the individual metals upon the yields of the plants. This would probably have had some influence upon the interactive effects, though the experiments did identify many consistent effects upon nutrient and heavy metal uptake. It is difficult to relate the interactive effects of the metals to the individual hazards determined above (Section 7.1), since it is only clear from the results of the experiments examining the interactions that the effects of the metals copper and cadmium are additive.

It is not known if other effects upon yield, such as those between Cd and Ni are antagonistic, additive or synergistic in sitka-spruce. It has, however, been shown that the toxic effects of Cu, Ni and Zn are directly or at least partially additive in young spring barley, when the elements are present above certain threshold levels, which may be lower than the upper critical levels of the individual elements (Beckett and Davis 1978). It has also been shown that Pb-Cd interactions resulting in the reduction of yield of american sycamore tree can be related to the product of their tissue concentrations (Carlson and Bazzaz 1977). There

are certain similarities in these effects and, though the magnitudes of the interactions are not completely known, indicate that where the relative hazards of at least two of the heavy metals approach unity at certain sites, then these sites might have slightly higher risks of metal toxicity than could be accounted for in any appraisal of the individual hazards.

Those sites where Cd, Ni and Pb are known to present individual risks of toxicity (values of I approaching, equal to or greater than unity) could therefore be in even greater risk. In particular this could occur at Sites 1, 3, 5 and 6 (Cymer, Margam 2, Rhondda 1 and 2) where the values of I exceed one for nickel and 0.5 for cadmium and lead.

7.3 Effects upon seedlings grown in soil, in relation to forestry situations

In water culture systems, some complex effects, such as those affecting the rooting system, cannot be studied very easily. They have, however, been examined here in a greenhouse experiment, with the plants being grown in soil. In this experiment, sitka-spruce seedlings were grown in a relatively uncontaminated peaty soil, typical of South Wales forests, which had been doped with heavy metals. This experiment allowed the effects of certain metals to be related fairly directly to the forestry situations.

The extractable soil Cd levels in the 0.1 and 0.4 mg/ kg Cd treatments of this experiment (Table 6.2) were lower than the soil extractable levels of Cd found at several forestry sites (Table 5.4). A comparison of the corresponding site foliar levels of Cd (Table 5.6) with the shoot Cd levels obtained with the Cd treatments in the

greenhouse experiment (Table 6.3) shows that the foliar Cd levels at the forestry sites could have resulted mainly from root uptake if most of the tree roots were, as would be expected, present in the surface layer of the soil.

Lead behaved slightly differently in that more lead was extractable during and at the end of the experiment than was found at any forest site; shoot Pb levels in those plants treated with lead in the greenhouse experiment were consequently far higher than found at any of the sites. Site foliar Cu levels were lower than those in the shoots of the greenhouse plants, even though the extractable levels of Cu at the sites and in the greenhouse soils were similar (Section 6.3.1).

Overall these results have shown that where the relationships between the amounts of metals taken up from the experimentally treated and site soils were similar (for Cd and Cu), then significant proportions of the foliar levels of the metals may have been taken up through the roots.

There were direct relationships between the tissue levels of Cd and Pb, in both the shoots and roots of the experimentally treated plants, and their respective extractable soil levels present at the end of the experimental period, and also with the total amounts which had been added to the soils. The different effects upon plant growth produced by increasing metal concentrations within the plants were therefore related to the increasing levels of the metals in the soils.

Though it was not possible to establish upper critical concentrations of Cd, Cu and Pb in these soil grown plants, it was shown, at least for Cd, that as shoot levels increased, the corresponding shoot yields decreased; the lowest levels at which these

effects were obvious being 6-7 mg/ kg tissue Cd (Figure 6.1). This figure is slightly higher than the critical tissue concentration value determined in the water culture experiments, though the number of treatments was not sufficient to determine this value accurately. This clearly demonstrates that the effects upon yields as a result of shoot uptake can also be related back to the soil levels. The corresponding relationship was only marginally apparent for lead and could not be shown for copper.

Toxic effects by the metals Cd, Cu and Pb upon root development and mycorrhizal associations were demonstrated in the greenhouse experiment. These may be related to effects at forestry sites by examining the extractable and total levels of metals in soils of the greenhouse experiment and in forestry soils.

The lengths of the main roots were found to be reduced at extractable concentrations greater than 0.3 mg/ kg Cd, when the total concentrations were greater than 2.5 mg/ kg Cd. The extractable soil concentrations of Cd at the South Wales sites were found to vary between 0 and 1.5 mg/ kg Cd with total concentations up to 4.2 mg/ kg Cd. Even allowing for some decreases throughout the experimental period in the extractable Cd levels in the soils to which Cd had been added (Table 6.1), it is clear that the extractable Cd levels at the sites were sufficient to have affected root development.

Again, it is difficult to interpret the effect of Pb upon the length of the main roots, as determined in Section 6.3.3.1, where the average lengths of the main roots of plants growing in soils with extractable concentrations of 1.7 mg/ kg and above, were significantly reduced. One reason for this may have been that the extractable soil levels of lead were higher than found at the forestry sites (zero

concentration at all sites). There were no effects by Cu upon the average length of the main roots, even though the extractable levels in the experimental soils were above those present at the South Wales forestry sites.

There were no significant reductions of root branching of these plants in any of the treatments, but the statistical tests used here did not take into account those seedlings whose roots were completely unbranched (Table 6.8). Many seedlings grown in the soils treated with Cd and Pb only possessed a main root, whereas all the plants in the control group had branching roots. Some plants in the Cu treatments had unbranched roots, but their numbers were smaller than in the Cd and Pb treatments (Table 6.8).

Mycorrhizae, which are symbiotic fungi closely associated with the roots of many plant species, are known to be important for the growth of of coniferous trees in many situations where soil nutrient levels are not exceptionally high (Harley 1969, Bowen 1973). It was shown in the greenhouse experiment that the numbers of seedlings with roots infected with mycorrhizae were lower in all the soils treated with the heavy metals Cd, Cu and Pb, and that the mycorrhizae were only present in the control treatment. Such effects were demonstrated in the experimentally treated soils which had lower levels of extractable Cd and Cu ions than are present at some forestry sites in South Wales.

It is interesting to note that the mycorrhizae did not infect seedling roots in the copper treatments even though no other effects by Cu treatments upon the roots or shoots were demonstrated in this experiment. It seems likely that the extractable levels of Cu in the Cu treatment were not sufficient to increase the shoot and root uptake of

Cu to levels where effects, such as those shown for Cd and Pb upon shoot yields and root development, were manifest, but they were sufficiently toxic to disturb the mycorrhizae-root associations. This would seem to indicate that these associations have a lower tolerance than the plants themselves to certain extractable levels of Cu.

As this type of effect has been demonstrated previously for willow and poplar trees growing on copper mine tailings (Harris and Jurgensen 1977) and, taking into account the levels of these cadmium and copper at the forestry sites, it seems likely that these heavy metals may be affecting tree growth at some of the South Wales forestry sites. This might have been established more clearly if a suitable regime of root sampling at the forestry sites had been adopted, but there are many difficulties in separating and identifying the fine roots and mycorrhizae in field samples (Fogel 1980), and such a programme would not necessarily have yielded more conclusive results.

7.4 Effects upon uptake of heavy metals and nutrients

Using a factorial experimental design, many effects involving heavy metals, both individually and interactively, were observed in the sitka-spruce seedlings (Chapter 3). These effects reveal the mechanisms by which heavy metal elements can exert toxic actions and modify the toxic action of other elements. These experiments have shown certain effects to be consistent with different species and conditions. Other effects have been shown to be consistent only with sitka-spruce seedlings and, still further, some were inconsistent, though highly significant, and may have resulted from slightly different experimental conditions which could not be controlled, such as the highest temperature that was attainable within the propagator used for the water culture experiments.

Most significant of all the effects was that by Cd in greatly reducing the shoot and root uptake of manganese. This effect has been demonstrated in various other studies and would seem to be one of the main ways in which Cd brings about its toxic actions. It was also interesting to note that Cu treatments significantly reduced the root and shoot uptake of Cd in almost all the experiments, except for the particular experiment where Cd effects upon yield were significant (Section 3.2.2.1). This indicates that the reductions in the Cd concentrations within the seedlings brought about by the Cu treatments were either insufficient to have any effect upon Cd phytotoxicity, or that the toxic effects of Cd were related to other factors as well as the total amounts of Cd taken up into the plants.

The presence of such interactions reflects the complexity of natural laws governing metal uptake and the subsequent toxic effects, and could not have been demonstrated as effectively if non-factorial experimental designs had been used.

It is known that the uptake of heavy metals and nutrients by the shoots and roots are not governed solely by the concentrations of their respective ions in the soil solution, since the plants themselves, according to conditions, may influence this process. Natural complexing agents exuded from plant roots, such as amino acids and organic acids, can influence the speciation of metal ions in the region around the roots. An experiment was carried out to identify more clearly what kinds of effects this could have upon heavy metal uptake by the roots and shoots of sitka-spruce seedlings.

The effects upon Cd and Cu uptake by the four amino acids glycine, aspartic acid, alanine and leucine were examined. It was shown that the

uptake of Cu by both shoots and roots was higher when the Cu in the solution was complexed by certain amino acids than when copper ions alone were present in the nutrient solutions (Table 4.7). Calculation of the equilibrium concentrations of the metal species in the nutrient solution was again carried out by the computer programme "COMICS". It was not possible, however, to relate the increases in shoot Cu levels to corresponding increases in root Cu levels. One possible reason for this may have been the large variations in data within the replicates. Cadmium levels in the seedling roots were also increased with respect to uncomplexed Cd when Cd in the solution was also complexed with certain amino acids, though again large variations in the results between the replicates may have masked certain effects.

A comparison of the effects of complexation upon the uptake of metals with those demonstrated in the factorial experiments reveals that the various mechanisms governing metal uptake may be very complex. Since it is likely that the pattern of exudation would be different in conditions of nutrient stress or metal toxicity, then any change of pattern could, by modifying the levels of complexation with amino acids and other exudates, further alter the amounts of the various heavy metals taken up.

Chapter 8

CONCLUSIONS

There were several problems associated with the sampling of roots and soil at the forestry sites which effectively negated the possibility of establishing correlations between site growth parameters and soil and foliar parameters. Therefore the approach adopted was one of establishing what effects heavy metals can have upon tree growth and then comparing these with the forestry situations.

The effects of heavy metals upon shoot yield were assessed in relation to the foliar levels present at forestry sites across South Wales and it was found that tree growth may be affected at several sites. The only metal that individually seems to present a hazard is nickel: most of the sites in South Wales had foliar levels above the critical level at which the toxic effects of the metal are apparent. Lead and cadmium present lower risks individually, but, as effects upon yield seem to be at least partially additive, their effects, in combination with those of nickel, could increase the overall phytotoxicity at several forestry sites. Foliar levels of zinc and copper are elevated with respect to control sites but are well below the critical levels and do not therefore seem to present any hazard in this respect.

More complex effects by cadmium, copper and lead upon root development and mycorrhizal associations were assessed in soil experiments. It was shown that the extractable levels of Cd in the peaty forest soils of South Wales are sufficient to affect the development of

the roots, though the forestry levels of extractable copper and lead were well below the levels necessary to produce these effects.

It was also shown in these experiments that mycorrhizal associations are disturbed by low extractable levels of Cd, Cu and Pb. At the forestry sites it seems likely that levels of Cd and Cu are above the levels necessary to disturb these associations and, as a result, may be reducing the uptake of nutrients, for which mycorrhizae are so important, namely phosphate and nitrate.

Using these criteria to assess the effects of heavy metals upon the the growth of sitka-spruce trees, it has been shown that the forest sites most at risk to toxicity are those in the forests Cymer, Margam and Rhondda.

Studies of the effects of combinations of heavy metals upon yield and uptake of heavy metals and nutrients have shown that the natural laws governing interactions are complex and highlighted the need for further research in this area with better experimental designs. Statistical analysis of such experiments may then enable a more complete understanding of the influence of heavy metals upon the growth of trees.

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Appendix A

CALCULATION OF UPPER CRITICAL AND LETHAL TISSUE CONCENTRATIONS

A.1 Equations used

The slope of the regression line (b), also called regression coefficient, was calculated by means of the relationship :-

$$b = \frac{\Sigma(x).\Sigma(y) - n\Sigma(x.y)}{[\Sigma(x)^2 - n\Sigma(x^2)]} \quad \text{....(Davies and Goldsmith (1947)}$$

The correlation coefficient(r)of the regression line (b), which characterizes the degree of mutual dependance between the two variables x and y, was calculated by means of the relationship :-

$$r = \frac{n\Sigma x.y - \Sigma x \Sigma y}{[n\Sigma x^2 - (\Sigma x)^2][n\Sigma y^2 - \Sigma(y^2)]} \quad \text{....(Davies and Goldsmith (1947)}$$

The estimate of the variance (v) about the regression line was determined from an analysis of variance based upon n-2 degrees of freedom. Since two degrees of freedom were used up in calculating the regression coefficient and correlation coefficient. The sum of squares about the regression line was computed by subtracting the sum of squares due to the regression from the total sum of squares.

$$\text{Sum of squares due to the regression} = b^2(x-x)^2 = \frac{\left(\Sigma xy - \frac{\Sigma x \Sigma y}{n}\right)^2}{\Sigma(x^2) - \frac{(\Sigma x)^2}{n}}$$

$$\text{Total sum of squares} = \Sigma (y-y)^2 = \Sigma (y^2) - \frac{(\Sigma y)^2}{n}$$

The sum of squares about the regression line

$$\Sigma (y-y)^2 - b^2 \Sigma (x-x)^2$$

was then divided by n-2 degrees of freedom to give the residual mean square about the regression.

The yield plateau (Yo) was calculated as the average of those yields not used in the regression.

$$Yo = \frac{\Sigma y}{n}$$

where y is a yield obtained from one replicate, and n is the number of points not used in the regression.

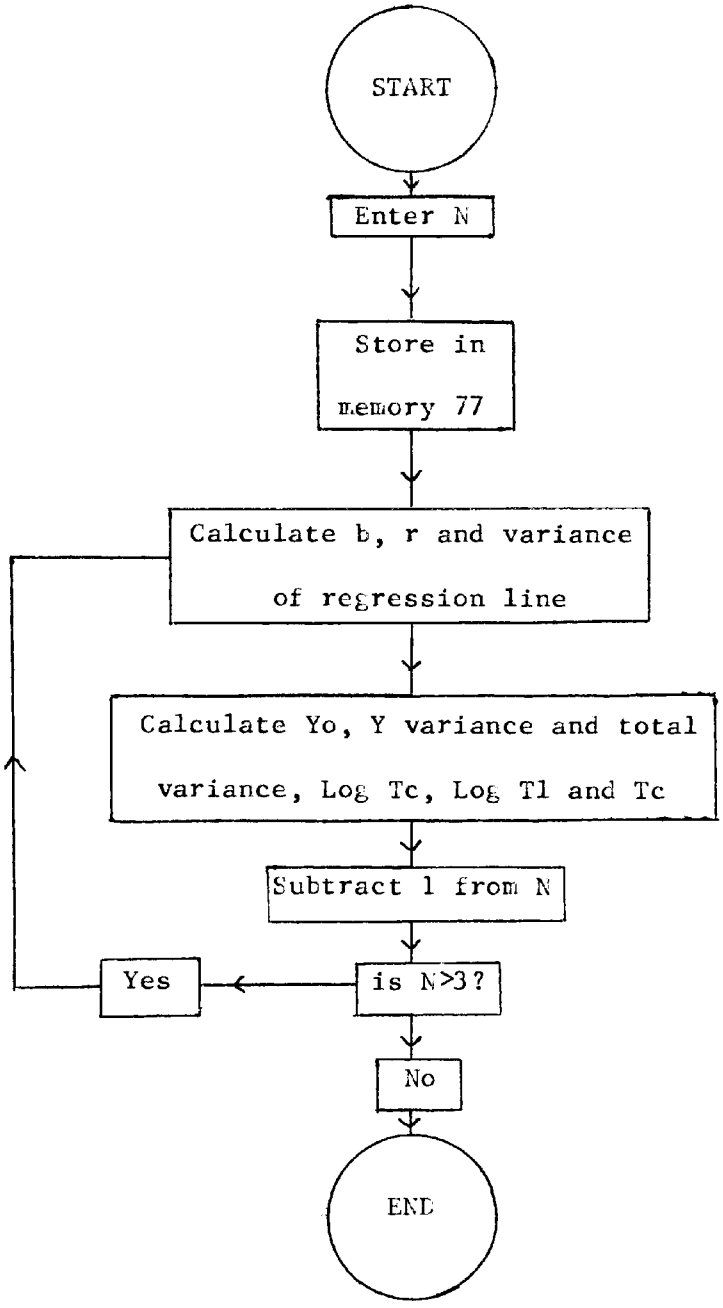
The variance about the yield plateau (VYo) was calculated as the square of the standard deviation

$$VYo = s^2 = \frac{\Sigma(x)^2 - \frac{(\Sigma x)^2}{n}}{(n-1)}$$

The logarithms of the critical and lethal concentrations, logTc and logTl, were calculated as intercepts on the regression line (b) where the yield plateau (Yo) intersected the regression line (b) and where the regression line were extrapolated to zero yield respectively.

These equations were combined into a calculator programme "CRTTC" used in conjunction with a Hewlett-Packard HP-41C programmable calculator, a flowchart and listing of which are given below.

A.2 Flowchart of calculator programme "CRTTC"



A.3 Listing of Calculator Programme "CRTTC"

Step 01	"CRTTC	Step 27	GTO 01	Step 53	STO 91
	BEEP		XEQ 00		RCL 75
	REG 71		XEQ 03		STO 92
	CL		7.006		*
	0		RCL 00		-
	STO 79		X<=Y?		RCL 71
	STO 80		GTO 02		X ↑2
	STO 83		FRC		RCL 76
	STO 86		.002		RCL 72
	1		-		*
	STO 00		STO 00		-
	"ENTER N		1		/
	PROMPT		ST+ 00		STO 81
	STO 77		CL		"b=
	500		GTO 01		ARCL X
	/		LBL 02		AVIEW
	ST+ 00		"FIN		STOP
	LBL 01		AVIEW		RCL 76
	RCL IND 00		STOP		RCL 75
	ENTER ↑		LBL 00		*
	1		RCL 71		RCL 71
	ST+ 00		STO 89		RCL 73
	CLX		RCL 73		*
	RCL IND 00		STO 90		-
	+		*		RCL 76
	ISG 00		RCL 76		RCL 72

Step 79	*
	RCL 71
	X↑ 2
	-
	RCL 76
	RCL 74
	STO 93
	*
	RCL 73
	X↑ 2
	-
	*
	SQRT
	/
	"R=
	ARCL X
	AVIEW
	STOP
	RCL 75
	RCL 71
	RCL 73
	*
	RCL 76
	/
	-
	X↑ 2
	RCL 72
	RCL 71

Step 107	X↑ 2
	RCL 76
	/
	-
	/
	RCL 74
	RCL 73
	X↑ 2
	RCL 76
	/
	-
	X<>Y
	-
	RCL 76
	2
	/
	STO 80
	"V=
	ARCL X
	STOP
	RTN
	LBL 03
	.00002
	STO 78
	CL
	RCL 77
	2
	*

Step 137	RCL 00
	1
	-
	INT
	-
	X<=0?
	GTO 04
	RCL 77
	500
	/
	ST+ 78
	RCL 00
	INT
	ST+ 78
	LBL 05
	RCL IND 78
	+
	ISG 78
	GTO 05
	MEAN
	STO 83
	"Yo
	ARCL X
	AVIEW
	STOP
	1
	RCL 76
	-

Step 165	X=0?	Step 193	RCL 93	Step 221	ARCL X
	CTO 04		*		AVIEW
	SDEV		-		STOP
	X↗ 2		/		RTN
	STO 79		STO 81		END
	LBL 04		RCL 89		
	"YVr		RCL 90		
	ARCL X		RCL 81		
	AVIEW		*		
	STOP		-		
	RCL 79		RCL 91		
	RCL 80		/		
	+		STO 82		
	STO 85		"LOG T1		
	"TOTVR		ARCL X		
	ARCL X		AVIEW		
	AVIEW		STOP		
	STOP		RCL 83		
	RCL 89		RCL 81		
	RCL 90		*		
	*		RCL 82		
	RCL 91		+		
	RCL 92		"LOG Tc		
	*		ARCL X		
	-		AVIEW		
	RCL 90		STOP		
	X↗ 2		10↗ X		
	RCL 91		"Tc=		

A.4 Data derived from the calculations:

The sets of data listed in these tables were derived from the calculations using the calculator programme "CRTTC". The yields (Y) were plotted against the logarithm of the tissue concentrations (Log T) and are listed in order of increasing Log T. In the tables the regression slopes are given as (b), the correlation coefficients are (r), the standard errors about (b) are given as (Vb), the yield plateaux as (Yo), the standard error about the yield plateaux as (YVr) and the total standard errors as (V). The logarithms of the split-point tissue concentrations are also listed (Log Ts.p.): one of these split-point concentrations being a critical concentration (Log Tc).

Table A.1

Experiment Cd 1

Y (mg)	Log T	(b)	(r)	(Bvr) (mg)	Yo (mg)	(Yvr) (mg)	(V) (mg)	Log Ts.p
10.77	0.1072	-2.37	-0.92	0.62	----	---	0.62	-----
10.65	1.0009	-2.51	-0.90	0.67	10.77	---	0.67	0.5654
8.39	1.0077	-2.24	-0.94	0.36	10.71	0.01	0.36	0.3286
8.43	1.1271	-2.32	-0.94	0.38	9.94	1.80	2.18	0.6935
9.38	1.2196	-2.36	-0.93	0.45	9.56	1.77	2.22	0.8791
8.37	1.2688	-2.11	-0.95	0.27	9.52	1.33	1.60	0.6763
8.31	1.3510	-1.98	-0.95	0.30	9.33	1.29	1.59	0.6772
6.49	1.6025	-1.65	-0.97	0.13	9.19	1.22	1.35	0.3196
6.98	1.7307	-1.89	-1.00	0.01	8.85	1.96	1.97	0.7172
6.27	2.0370							
4.29	3.1297							

Tc= 3.68 mg/ kg
Tl= 11881 mg/ kg

Table A.2

Experiment Cd 2

Y (mg)	Log T	(b)	(r)	(Evr) (mg)	Yo (mg)	(Yvr) (mg)	(V) (mg)	Log Ts.p
10.56	0.0828	-2.24	-0.90	0.81	----	---	0.81	-----
10.39	0.8357	-2.54	-0.90	0.78	10.56	---	0.78	0.7836
10.74	1.0065	-2.44	-0.88	0.86	10.48	0.02	0.87	0.8248
7.52	1.0711	-2.14	-0.88	0.64	10.56	0.03	0.67	0.6193
9.28	1.1992	-2.55	-0.97	0.22	9.80	2.34	2.56	0.9864
9.06	1.2882	-2.50	-0.96	0.27	9.70	1.81	2.08	1.0139
7.99	1.3334	-2.39	-0.95	0.34	9.59	1.51	1.85	1.0261
8.67	1.4758	-2.91	-0.99	0.14	9.36	1.63	1.76	1.3035
7.38	1.9900	-3.24	-0.97	0.22	9.28	1.46	1.67	1.4933
5.96	2.5713							
4.68	2.7687							

Tc= 5.72 mg/ kg
Tl= 2779 mg/ kg

Table A.3

Experiment Cd 3

Y (mg)	Log T	(b)	(r)	(Bvr) (mg)	Yo (mg)	(Yvr) (mg)	(V) (mg)	Log Ts.p
10.63	0.0934	-2.52	-0.86	1.19	---	---	1.19	-----
11.44	0.8280	-2.78	-0.84	1.26	10.63	---	1.26	0.7576
10.26	0.8733	-2.28	-0.84	0.81	11.04	0.33	2.14	0.4162
7.48	1.0103	-1.81	-0.83	0.53	10.78	0.36	0.90	0.2149
6.98	1.1587	-2.09	-0.86	0.51	9.95	2.96	3.47	0.6566
8.52	1.2570	-2.65	-0.98	0.13	9.36	3.99	4.12	0.959
8.66	1.2862	-2.61	-0.97	0.16	9.22	3.31	3.47	1.0104
7.33	1.5070	-2.08	-0.95	0.11	9.14	2.80	2.91	0.7869
5.88	2.1394	-2.81	-0.88	0.17	8.91	2.82	2.99	1.4055
6.44	2.1466							
5.21	2.4779							

Tc= 3.68 mg/ kg
Tl= 11881 mg/ kg

Table A.4

Experiment Cu 1

Y (mg)	Log T	(b)	(r)	(Evr) (mg)	Yo (mg)	(Yvr) (mg)	(V) (mg)	Log Ts.p
6.29	1.2271	-0.70	-0.41	0.45	---	---	0.45	-----
6.15	1.2502	-0.83	-0.45	0.48	6.29	---	0.48	1.7792
5.85	1.3210	-1.10	-0.55	0.47	6.22	0.01	0.48	1.8563
6.19	1.4512	-1.65	-0.75	0.31	6.10	0.05	0.36	1.9841
7.31	1.5176	-2.17	-0.90	0.16	6.12	0.04	0.20	2.0475
7.15	1.6225	-2.19	-0.87	0.20	6.36	0.31	0.51	1.9727
6.49	1.6647	-2.16	-0.84	0.25	6.49	0.35	0.60	1.9451
7.42	1.8081	-2.85	-0.92	0.17	6.49	0.29	0.46	2.0119
6.36	1.8755	-2.14	-0.92	0.09	6.61	0.36	0.45	1.8819
6.03	2.1041	-2.83	-0.92	0.12	6.58	0.32	0.44	2.0155
5.97	2.2809							
4.90	2.5183							

Tc= 103.6 mg/ kg
Tl= 10065 mg/ kg

Table A.5

Experiment Cu 2

Y (mg)	Log T	(b)	(r)	(Bvr) (mg)	Yo (mg)	(Yvr) (mg)	(V) (mg)	Log Ts.p
7.06	1.0294	-1.65	-0.68	0.97	---	---	0.97	-----
5.94	1.3379	-1.92	-0.70	0.99	7.06	---	0.99	1.6961
9.17	1.3456	-2.38	-0.81	0.73	6.50	0.63	1.36	1.8950
6.25	1.5123	-1.88	-0.78	0.52	7.39	2.69	3.21	1.5710
6.27	1.5776	-2.22	-0.85	0.43	7.05	2.12	2.55	1.7131
6.62	1.7493	-2.71	-0.94	0.23	6.94	1.73	1.96	1.8873
7.18	1.7587	-2.99	-0.96	0.17	6.89	1.40	1.57	1.9534
6.60	1.8741	-3.14	-0.96	0.20	6.93	1.18	1.38	1.9766
7.62	1.8908	-3.65	-1.00	0.02	6.89	1.02	1.04	2.0622
6.11	2.2409	-3.38	-1.00	0.00	6.97	0.96	0.96	1.9879
5.61	2.3917							
3.81	2.9233							

Tc= 97.24 mg/ kg
Tl= 11289 mg/ kg

Table A.6

Experiment Cu 3

Y (mg)	Log T	(b)	(r)	(Evr) (mg)	Yo (mg)	(Yvr) (mg)	(V) (mg)	Log Ts.p
9.16	1.0553	-2.54	-0.87	0.54	---	---	0.54	-----
9.37	1.2605	-2.80	-0.88	0.53	9.16	---	0.53	1.4968
8.23	1.3944	-2.86	-0.87	0.59	9.27	0.02	0.61	1.5066
8.66	1.4381	-3.23	-0.91	0.48	8.92	0.37	0.85	1.6515
8.67	1.6388	-3.59	-0.93	0.43	8.86	0.26	0.70	1.7220
9.74	1.6650	-3.72	-0.92	0.50	8.82	0.20	0.70	1.7607
7.84	1.6746	-3.30	-0.92	0.41	8.97	0.31	0.72	1.6551
8.92	1.8582	-4.0	-0.97	0.20	8.81	0.44	0.64	1.7921
8.10	1.8723	-3.52	-0.98	0.10	8.82	0.38	0.48	1.6748
6.78	2.1585	-3.35	-0.96	0.20	8.74	0.39	0.58	1.6990
6.35	2.4507							
4.76	2.7666							

Tc= 61.96 mg/ kg
Tl= 7253 mg/ kg

Table A.7

Experiment Ni 1

Y (mg)	Log T	(b)	(r)	(Evr) (mg)	Yo (mg)	(Yvr) (mg)	(V) (mg)	Log Ts.p
8.73	0.245	-2.51	-0.96	0.38	---	---	0.38	-----
8.25	0.745	-2.69	-0.96	0.35	8.73	---	0.35	0.7059
8.61	0.790	-2.72	-0.96	0.38	8.49	0.12	0.50	0.8232
6.63	0.976	-2.64	-0.94	0.42	8.53	0.06	0.48	0.8077
7.32	1.098	-3.04	-0.97	0.23	8.06	0.94	1.17	1.0846
7.71	1.196	-3.27	-0.98	0.19	7.91	0.81	1.00	1.2214
7.08	1.525	-3.39	-0.96	0.22	7.88	0.66	0.87	1.3012
5.53	1.955	-3.13	-0.93	0.25	7.76	0.64	0.89	1.3197
4.93	2.054	-2.92	-0.91	0.30	7.48	1.17	1.47	1.3715
4.19	2.075	-2.85	-0.88	0.45	7.20	1.75	2.20	1.5034
4.89	2.267	-4.48	-1.00	0.00	6.90	2.46	2.46	1.8230
3.37	2.616							
2.26	2.853							

Tc= 5.08 mg/ kg
Tl= 5085 mg/ kg

Table A.8

Experiment Ni 2

Y (mg)	Log T	(b)	(r)	(Bvr) (mg)	Yo (mg)	(Yvr) (mg)	(V) (mg)	Log Ts.p
8.15	0.279	-1.92	-0.94	0.30	---	---	0.30	-----
8.04	0.619	-2.03	-0.94	0.30	8.15	---	0.30	0.7236
7.97	0.777	-2.05	-0.93	0.33	8.10	0.01	0.33	0.7834
6.91	0.858	-2.02	-0.92	0.36	8.05	0.01	0.37	0.8184
7.40	1.092	-2.21	-0.94	0.31	7.77	0.34	0.65	1.0270
6.89	1.124	-2.21	-0.93	0.36	7.69	0.28	0.63	1.0818
6.66	1.139	-2.35	-0.93	0.37	7.56	0.33	0.70	1.2127
7.19	1.319	-2.77	-0.96	0.24	7.43	0.39	0.63	1.3922
6.38	1.743	-3.00	-0.95	0.26	7.40	0.34	0.61	1.4986
5.53	1.856	-3.03	-0.94	0.35	7.29	0.41	0.76	1.5740
6.19	1.997	-3.61	-0.98	0.20	7.11	0.68	0.88	1.7496
5.74	2.067	-3.46	-0.96	0.38	7.03	0.69	1.07	1.7645
2.79	2.793							
3.63	2.803							

Tc= 6.58 mg/ kg
Tl= 16002 mg/ kg

Table A.5

Experiment Pb 1

Y (mg)	Log T	(b)	(r)	(Bvr) (mg)	Yo (mg)	(Yvr) (mg)	(V) (mg)	Log Ts.p
12.37	0.9128	-1.24	-0.07	8.20	---	---	8.20	-----
7.09	0.9699	0.66	0.03	8.79	12.37	---	8.79	1.1430
10.65	0.9773	-3.02	-0.02	8.19	9.73	13.93	22.12	1.1660
13.23	1.0124	-3.97	-0.18	9.48	10.04	7.25	16.72	1.1860
12.49	1.0370	0.02	0.00	10.27	10.84	7.39	17.66	1.2048
5.18	1.1055	7.40	0.26	10.67	11.17	6.09	16.76	1.2441
12.51	1.1361	-7.88	-0.36	5.37	10.17	10.84	16.21	1.2704
8.36	1.2109	-2.93	-0.11	7.57	10.50	9.82	17.38	1.2888
13.66	1.2467	-24.32	-0.87	2.25	10.24	8.99	11.24	1.3456
10.37	1.3006							
9.67	1.3969							

Table A.10

Experiment Pb 2

Y (mg)	Log T	(b)	(r)	(Bvr) (mg)	Yo (mg)	(Yvr) (mg)	(V) (mg)	Log Ts.p
11.23	0.9542	-7.96	-0.60	4.29	---	---	4.29	-----
11.19	0.9562	-9.19	-0.63	4.56	11.23	---	4.56	1.1931
10.52	0.9827	-11.85	-0.68	4.53	11.21	0.00	4.54	1.2235
15.51	1.0430	-20.43	-0.89	2.14	10.98	0.16	2.31	1.2666
10.27	1.2217	-19.72	-0.74	2.57	12.11	5.23	7.80	1.2567
12.96	1.2695	-26.00	-0.81	2.37	11.74	4.60	6.97	1.2870
10.96	1.2742	-19.26	-0.76	1.91	11.95	3.93	5.84	1.2658
10.24	1.3036	-14.21	-0.56	2.54	11.81	3.41	5.95	1.3028
7.38	1.3650	-14.00	-0.58	1.30	11.61	3.23	4.53	1.5046
6.66	1.4098							
8.62	1.4468							

Tc= 18.44 mg/ kg
Tl= 41.69 mg/ kg

Table A.11

Experiment Pb 3

Y (mg)	Log T	(b)	(r)	(Evr) (mg)	Yo (mg)	(Yvr) (mg)	(V) (mg)	Log Ts.p
10.11	0.8156	-6.14	-0.55	3.36	---	---	3.36	-----
12.81	1.0265	-12.01	-0.82	1.79	10.11	---	1.79	1.2565
12.73	1.0290	-12.42	-0.79	2.03	11.46	3.63	5.66	1.1950
13.66	1.0980	-13.46	-0.76	2.31	11.88	2.35	4.66	1.1979
10.23	1.1242	-10.64	-0.64	2.38	12.33	2.36	4.74	1.1928
10.15	1.2294	-16.88	-0.77	2.02	11.91	2.65	4.67	1.2415
11.64	1.2480	-20.40	-0.83	2.07	11.61	-2.64	-4.71	1.2701
12.08	1.3002	-19.59	-0.83	3.08	11.62	2.17	5.28	1.2834
8.47	1.3056	-12.67	-0.86	1.07	11.68	1.91	2.98	1.1744
9.56	1.3336							
6.70	1.4880							

Tc= 19.20 mg/ kg
Tl= 43.29 mg/ kg

Table A.12

Experiment Zn 1

Y (mg)	Log T	(b)	(r)	(Lvr) (mg)	Yo (mg)	(Yvr) (mg)	(V) (mg)	Log Ts.p
3.49	1.8820	-0.23	-0.45	0.09	---	---	0.09	-----
4.10	1.8956	-0.28	-0.56	0.08	3.49	---	0.08	2.7581
3.46	1.9562	-0.25	-0.51	0.08	3.80	0.19	0.27	2.4375
4.27	1.9937	-0.33	-0.67	0.06	3.68	0.13	0.19	2.6773
3.62	2.0578	-0.27	-0.63	0.05	3.83	0.17	0.23	2.4514
3.91	2.0718	-0.33	-0.73	0.05	3.79	0.14	0.19	2.6060
3.63	2.1032	-0.34	-0.71	0.06	3.81	0.11	0.17	2.6421
4.28	2.1850	-0.50	-0.91	0.03	3.78	0.10	0.13	2.8320
3.80	2.5239	-0.31	-0.95	0.01	3.85	0.12	0.12	2.3530
3.67	2.8090	-0.25	-0.91	0.08	3.84	0.10	0.11	2.2560
3.44	3.3548							
3.44	3.7597							

Tc= 225.5 mg/ kg
Tl= $4.8*10^{13}$ mg/ kg

Table A.13

Experiment Zn 2

Y (mg)	Log T	(b)	(r)	(Bvr) (mg)	Yo (mg)	(Yvr) (mg)	(V) (mg)	Log Ts.p
4.03	1.9301	-0.17	-0.21	0.23	---	---	0.23	-----
3.74	1.9791	-0.17	-0.19	0.25	4.03	---	0.25	2.4678
3.31	2.0237	-0.22	-0.25	0.27	3.89	0.04	0.31	2.5586
4.42	2.0274	-0.39	-0.45	0.21	3.69	0.13	0.34	2.7705
3.76	2.0723	-0.33	-0.38	0.24	3.88	0.22	0.46	2.7098
4.02	2.1629	-0.48	-0.51	0.24	3.85	0.17	0.41	2.8312
4.13	2.3112	-0.61	-0.56	0.28	3.88	0.14	0.42	2.9114
3.97	2.4524	-0.73	-0.58	0.35	3.92	0.12	0.48	2.9850
4.92	2.6535	-1.06	-0.70	0.41	3.92	0.11	0.52	3.1080
3.66	2.7377	-0.39	-0.43	0.29	4.03	0.20	0.49	3.0470
4.03	3.3227							
3.19	3.6690							

Table A.14

Experiment Zn 3

Y (mg)	Log T	(b)	(r)	(Bvr) (mg)	Yo (mg)	(Yvr) (mg)	(V) (mg)	Log Ts.p
3.74	1.6782	-0.44	-0.42	0.30	---	---	0.30	-----
3.70	1.7718	-0.56	-0.51	0.30	3.74	---	0.30	2.4754
4.63	1.8201	-0.67	-0.59	0.28	3.72	0.00	0.28	2.5600
4.69	1.8770	-0.61	-0.53	0.32	4.02	0.28	0.59	2.4350
3.88	2.0211	-0.50	-0.44	0.34	4.19	0.30	0.64	2.4116
4.88	2.0282	-0.59	-0.48	0.39	4.13	0.24	0.63	2.5074
3.73	2.1872	-0.26	-0.25	0.33	4.25	0.29	0.62	2.5687
3.85	2.2313	-0.34	-0.28	0.43	4.18	0.28	0.71	2.6865
3.47	2.4223	-0.43	-0.28	0.64	4.14	0.25	0.89	2.8531
4.37	2.6972	-1.66	-0.73	0.62	4.06	0.27	0.89	3.0145
4.13	3.2550							
2.86	3.3544							

Table A.15

Experiment Cd 4

Y (mg)	Log T	(b)	(r)	(Evr) (mg)	Yo (mg)	(Yvr) (mg)	(V) (mg)	Log Ts.f
18.58	-0.5699	-5.50	-0.92	2.93	---	---	2.93	-----
20.26	0.1476	-7.35	-0.93	1.83	18.58	---	1.83	0.4323
10.19	1.2417	-6.12	-0.78	1.85	19.42	1.42	3.27	0.5424
9.82	1.2502	-6.60	-0.79	1.94	16.34	29.12	31.06	0.9035
14.07	1.2718	-7.62	-0.83	1.807	14.71	30.06	31.87	1.1096
10.57	1.3739	-5.31	-0.81	0.84	14.58	22.63	23.49	0.8990
10.32	1.3800	-4.69	-0.75	0.89	13.91	20.78	21.67	1.0083
9.52	1.3830	-3.52	-0.59	0.91	13.40	19.17	20.08	1.1874
7.08	1.6553	-0.68	-0.08	0.99	12.91	18.31	19.30	1.7437
9.11	1.7489	-4.06	-0.38	1.03	12.27	19.80	20.83	1.6584
6.63	1.7661	-0.28	-0.04	0.65	11.95	18.60	19.25	1.8137
8.23	1.7754	-4.49	-0.87	0.12	11.47	19.31	19.43	1.2257
7.45	1.8492							
7.32	1.9658							
5.03	2.8429	These plants were considered dead and their data						
4.33	2.9499	were not used in the calculations						
5.63	3.0386							

Tc= 3.49 mg/ kg
Tl= 292 mg/ kg

Table A.16

Greenhouse experiment cadmium treatments

Y (mg)	Log T	(b)	(r)	(Evr) (mg)	Yo (mg)	(Yvr) (mg)	(V) (mg)	Log Ts.p
8.48	0.7709	-6.90	-0.81	1.82	---	---	1.82	-----
10.48	0.8062	-7.05	-0.77	2.27	8.48	---	2.27	0.9182
6.11	0.8451	1.00	0.97	0.80	9.48	2.00	2.80	4.8700
5.80	1.1106	0.17	0.23	12.36	8.36	4.79	17.15	4.8127
6.33	1.1584	-0.38	-0.54	7.53	7.72	4.82	12.35	2.4881
4.97	1.2405							
4.65	1.3997							

Table A.17

Greenhouse experiment copper treatments

Y (mg)	Log T	(b)	(r)	(Evr) (mg)	Yo (mg)	(Yvr) (mg)	(V) (mg)	Log Ts.p
9.96	0.9823	-4.76	-0.39	10.71	---	---	10.71	-----
9.30	1.0374	-4.79	-0.35	12.85	9.96	---	12.85	1.2903
9.70	1.0645	0.77	0.58	14.96	9.63	0.22	15.18	2.8171
6.48	1.2148	-0.17	-0.13	28.02	9.65	0.11	28.13	4.5877
10.02	1.2577	-0.44	-0.38	25.40	8.86	2.59	28.03	4.2670
13.95	1.4843	-0.66	-0.58	16.72	9.09	2.21	18.94	3.8430
3.37	1.5563							
5.50	1.7118							

Table A.18

Greenhouse experiment lead treatments

Y (mg)	Log T	(b)	(r)	(Bvr) (mg)	Yo (mg)	(Yvr) (mg)	(V) (mg)	Log Ts.p
9.96	0.2304	-2.08	-0.73	2.00	---	---	2.00	-----
6.43	1.2923	-2.35	-0.60	2.37	9.96	---	2.37	1.2773
6.58	1.3617	0.87	0.80	4.86	8.20	6.23	11.11	2.8093
8.81	1.4393	-0.44	-0.37	15.41	7.66	3.98	19.39	3.5500
9.53	1.6128	-0.54	-0.47	13.33	7.95	2.99	16.32	3.6272
7.00	1.8555							
4.15	2.5928							

Appendix B

CALCULATION OF THE ANALYSIS OF VARIANCE TABLES FOR THE FACTORIAL EXPERIMENTS

The analyses of variance of the 3*3*3 factorial designs were calculated using a method by which the quantitative effects of either single or duplicate treatments could be established (p 332, Davies 1979). This method was transformed into a programme for a Hewlett-packard HP-41C programmable calculator which is listed below. The data memory allocation of the calculator was adjusted to 088 using the size function which allowed the programme to calculate the effects and variance ratios without completely destroying the data. If a single replicate experiment was analysed then the responses were entered into memory registers 0-26. If analysis of a second replicate was required then these responses were entered into registers 27-53.

In factorial experiments, such as this, there is a standard order in which the responses are set out. The standard form for a 3*3*3 factorial design is shown in Table B.1. The memory registers of the calculator into which the responses of the first replicate are placed are listed in this table. The second replicate is exactly the same but starts with register 27 in position corresponding to the A 0*B 0*C 0 treatment.

Table B.1

Standard form for a 3*3*3 table together with the calculator memory registers used for the location of responses in the programme "FAC"

		Factor C								
		C 0			C 1			C 2		
		Factor B			Factor B			Factor B		
		Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Factor A	A 0	00	03	06	01	04	07	02	05	08
	A 1	09	12	15	10	13	16	11	14	17
	A 2	18	21	24	19	22	25	20	23	26

Listing of the Programme "FAC".

Step 01	LBL "FAC	Step 27	INT	Step 53	ARCL X
	"NO OF REPS =?		RCL IND X		AVIEW
	PROMPT		ST+56		STOP
	1		X↑ 2		SF 01
	X<Y?		ST+ 57		LBL 09
	GTO 08		RCL IND 54		0
	GTO 09		RCL IND 68		STO 28
	LBL 08		+		STO 29
	0		STO IND 68		STO 32
	STO 56		1		STO 33
	STO 57		ST+ 54		.02601
	STO 58		ISG 68		STO 27
	STO 59		GTO 10		LBL 01
	STO 60		RCL 56		RCL 27
	.02601		"ΣX=		INT
	STO 68		ARCL X		RCL IND X
	27.05301		AVIEW		ST+ 28
	STO 54		STOP		X↑ 2
	LBL 10		X↑ 2		ST+ 29
	RCL 54		54		ISG 27
	INT		/		GTO 01
	RCL IND X		CHS		RCL 28
	ST+ 56		RCL 57		FS? 01
	X↑ 2		+		GTO 06
	ST+ 57		STO 68		"ΣX=
	RCL 68		"RTSS=		ARCL X

Step 79	AVIEW	Step 107	ChS	Step 135	9.01101
	STOP		STO 47		XEQ A
	LBL 06		STO 48		ST+ 34
	X↑ 2		STO 62		STO 38
	27		STO 64		ST+ 65
	/		3.00501		ST- 64
	STO 28		XEQ A		2
	ChS		STO 35		*
	RCL 29		ST+ 37		ST+ 47
	+		ST- 62		ST- 49
	FS? 01		ST+ 63		ST- 63
	XEQ D		2		12.01401
	STO 43		*		XEQ A
	FS? 01		ST+ 48		ST+ 35
	GTO 05		ST- 49		ST+ 38
	"TSS=		ST- 65		2
	ARCL X		6.00801		*
	AVIEW		XEQ A		ST- 63
	STOP		STO 36		ST- 65
	LBL 05		ST+ 37		2
	.00201		ST+ 47		*
	XEQ A		ST+ 49		ST+ 49
	STO 34		ST+ 63		15.01701
	STO 37		ST+ 64		XEQ A
	STO 46		ST+ 65		ST+ 36
	STO 49		ST- 46		ST+ 38
	STO 63		ST- 48		ST+ 64
	STO 65		ST- 62		ST+ 65

Step 163	2	Step 191	24.02601	Step 219	9
	*		XEQ A		/
	ST- 47		ST+ 36		RCL 28
	ST- 49		ST+ 39		-
	ST- 63		ST+ 46		FS? 01
	18.02001		ST+ 47		XEQ D
	XEQ A		ST+ 48		STO 34
	ST+ 34		ST+ 49		RCL 37
	STO 39		ST+ 62		X↑ 2
	ST+ 48		ST+ 63		RCL 38
	ST+ 49		ST+ 64		X↑ 2
	ST+ 62		ST+ 65		+
	ST+ 63		0		RCL 39
	ST+ 65		STO 32		X↑ 2
	ST- 46		XEQ C		+
	ST- 47		FS? 01		9
	ST- 64		XEQ D		/
	21.02301		STO 27		RCL 28
	XEQ A		0		-
	ST+ 35		STO 33		FS? 01
	ST+ 39		RCL 34		XEQ D
	ST+ 62		X↑ 2		STO 35
	ST+ 63		RCL 35		.00603
	2		X↑ 2		XEQ A
	*		+		STO 36
	ST- 48		RCL 36		STO 54
	ST- 49		X↑ 2		STO 57
	ST- 65		+		STO 67

Step 247	CHS	Step 275	ST+ 55	Step 303	ST- 66
	STO 55		ST- 57		19.02503
	STO 56		10.01603		XEQ A
	STO 66		XEQ A		ST+ 37
	1.00703		ST+ 37		2
	XEQ A		2		*
	STO 37		*		ST- 56
	2		ST- 67		ST- 57
	*		2		ST- 67
	ST+ 56		*		20.02603
	ST- 57		ST+ 57		XEQ A
	ST- 67		11.01703		ST+ 38
	2.00803		XEQ A		ST+ 54
	XEQ A		ST+ 38		ST+ 55
	STO 38		ST+ 66		ST+ 56
	ST+ 55		ST+ 67		ST+ 57
	ST+ 57		2		ST+ 66
	ST+ 66		*		ST+ 67
	ST+ 67		ST- 55		0
	ST- 54		ST- 57		STO 32
	ST- 56		18.02403		XEQ C
	9.01503		XEQ A		FS? 01
	XEQ A		ST+ 36		XEQ D
	ST+ 36		ST+ 56		STO 39
	ST- 66		ST+ 57		0
	ST+ 67		ST+ 67		STO 33
	2		ST- 54		RCL 36
	*		ST- 55		X↑ 2

Step 331	RCL 37	Step 359	ST+ 59	Step 387	XEQ A
	X↑ 2		ST+ 61		2
	+		ST- 58		*
	RCL 38		ST- 60		ST- 60
	X↑ 2		3.02109		ST-61
	+		XEQ A		8.02609
	9		2		XEQ A
	/		*		ST+ 58
	RCL 28		ST+ 59		ST+ 59
	-		ST- 61		ST+ 60
	FS? 01		4.02209		ST+ 61
	XEQ D		XEQ A		0
	STO 36		4		STO 32
	.01809		*		XEQ C
	XEQ A		ST+ 61		FS? 01
	STO 58		5.02309		XEQ D
	STO 61		XEQ A		STO 37
	CHS		2		RCL 27
	STO 59		*		RCL 35
	STO 60		ST- 59		-
	1.01909		ST 61		RCL 34
	XEQ A		6.02409		-
	2		XEQ A		STO 40
	*		ST+ 60		RCL 39
	ST+ 60		ST+ 61		RCL 35
	ST- 61		ST- 58		-
	2.02009		ST- 59		RCL 36
	XEQ A		7.02509		-

Step 415	STO 41	Step 443	STO 45	Step 471	X/ 2
	RCL 37		STO 53		18
	RCL 34		"3FMS=		/
	-		ARCL X		FS? 01
	RCL 36		AVIEW		XEQ D
	-		STOP		"Lb=
	STO 42		FS? 01		XEQ E
	RCL 43		XEQ 07		RCL 65
	RCL 40		RCL 62		XEQ F
	-		XEQ F		X↑ 2
	RCL 41		X↑ 2		54
	-		18		/
	RCL 42		/		FS? 01
	-		FS? 01		XEQ D
	RCL 34		XEQ D		"Qb=
	-		"La=		XEQ E
	RCL 35		XEQ E		RCL 66
	-		RCL 63		XEQ F
	RCL 36		XEQ F		X↑ 2
	-		X↑ 2		18
	STO 44		54		/
	"ABC=		/		FS? 01
	ARCL X		FS? 01		XEQ D
	AVIEW		XEQ D		"Lc=
	STOP		"Qa=		XEQ E
	RCL 44		XEQ E		RCL 67
	8		RCL 64		XEQ F
	/		XEQ F		X↑ 2

Step 499	54	Step 527	/	Step 555	FS? 01
	/		FS? 01		XEQ D
	FS? 01		XEQ D		"LaQb=
	XEQ D		"QaLb=		XEQ E
	"Qc=		XEQ E		RCL 56
	XEQ E		RCL 49		XEQ F
	RCL 46		XEQ F		X↑ 2
	XEQ F		X↑ 2		36
	X↑ 2		108		/
	12		/		FS? 01
	/		FS? 01		XEQ D
	FS? 01		XEQ D		"QaLc=
	XEQ D		"QaQb=		XEQ E
	"LaLb=		XEQ E		RCL 57
	XEQ E		RCL 54		XEQ F
	RCL 47		XEQ F		X↑ 2
	XEQ F		X↑ 2		108
	X↑ 2		12		/
	36		/		FS? 01
	/		FS? 01		XEQ D
	FS? 01		XEQ D		"QaQc=
	XEQ D		"LaLb=		XEQ E
	"LaLb=		XEQ E		RCL 58
	XEQ E		RCL 55		XEQ F
	RCL 48		XEQ F		X↑ 2
	XEQ F		X↑ 2		12
	X↑ 2		36		/
	36		/		FS? 01

Step 583	XEQ D	Step 611	"QbQc=	Step 639	RCL 40
	"LbLc=		XEQ E		-
	XEQ E		RCL 53		RCL 41
	RCL 59		RCL 45		-
	XEQ F		/		RCL 42
	X↑ 2		"3FVR=		-
	36		ARCL X		RCL 34
	/		AVIEW		-
	FS? 01		STOP		RCL 35
	XEQ D		CF 01		-
	"LbQc=		RTN		RCL 36
	XEQ E		CLX		-
	RCL 60		LBL E		"ESS=
	XEQ F		ARCL X		ARCL X
	X↑ 2		AVIEW		AVIEW
	36		STOP		STOP
	/		RCL 45		27
	FS? 01		/		/
	XEQ D		STOP		STO 45
	"QbLc=		RTN		"ERR=
	XEQ E		LBL D		ARCL X
	RCL 61		2		AVIEW
	XEQ F		/		STOP
	X↑ 2		RTN		RTN
	108		LBL 07		LBL C
	/		RCL 68		RCL 33
	FS? 01		RCL 44		3
	XEQ D		-		

```
Step 667  RCL 28
          -
          RTN
          LBL A
          STO 31
          0
          STO 32
          LBL 02
          RCL 31
          INT
          RCL IND X
          ST+ 32
          ISG 31
          GTO 02
          RCL 32
          X↑ 2
          ST+ 33
          RCL 32
          RTN
          LBL F
          "EFF=
          ARCL X
          AVIEW
          STOP
          RTN
          END
```

ORIGINAL DATA

The original data used by the programme to generate the effects and analyses of variance are set out below in standard order. In these tables yields are expressed in milligrammes (mg) and concentrations of heavy metals and nutrients in mg/ kg. All data relate to dry weight measurements.

Table B.2

Factorial experiment 1. Yields of plants per treatment (mg)

Total yields

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	133.6	94.6	60.3	135.5	56.2	41.1	119.5	85.7	53.1
Cd 1	137.7	98.8	61.6	125.1	113.3	53.7	122.2	74.7	40.0
Cd 2	120.1	76.9	54.0	127.5	143.5	66.2	96.4	101.7	64.3

Shoot yields

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	116.6	77.4	47.5	110.4	47.2	33.4	94.7	72.7	44.7
Cd 1	105.9	77.9	50.5	92.9	92.2	45.8	93.3	64.5	35.2
Cd 2	94.3	62.7	45.4	98.0	114.7	54.3	75.3	81.8	52.4

Root yields

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	22.0	17.4	12.8	25.1	9.0	7.9	24.8	13.0	8.4
Cd 1	31.8	19.8	11.1	32.2	21.1	7.9	28.9	10.2	4.8
Cd 2	25.8	14.2	8.6	29.5	28.8	11.9	21.1	20.1	11.9

Table B.3

Factorial experiment 1. Concentrations of heavy metals in shoots (mg/ kg)

Cadmium

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	1.00	0.60	1.05	0.56	0.65	2.00	0.70	0.80	1.00
Cd 1	20.07	15.82	86.63	20.24	14.91	90.07	16.08	40.16	145.60
Cd 2	34.46	39.87	77.10	35.71	7.63	98.99	41.50	21.45	84.49

Copper

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	16.7	220.2	1473.7	13.6	148.3	3593.0	10.6	488.3	2908.0
Cd 1	14.2	158.3	2376.2	21.5	184.4	3712.0	32.2	930.2	3693.0
Cd 2	8.3	462.5	1982.0	15.3	74.1	2947.0	10.4	337.0	1908.0

Nickel

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	1.00	2.00	0.80	29.52	112.92	139.73	77.40	91.75	268.46
Cd 1	0.50	1.00	2.00	21.53	21.69	116.38	42.80	134.42	340.91
Cd 2	0.50	1.00	0.80	27.24	11.60	98.16	53.12	49.02	178.05

Table B.4

Factorial experiment 1. Concentrations of heavy metals in roots (mg/ kg)

Cadmium

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	10.31	9.22	7.36	18.81	6.43	8.14	20.14	11.27	9.69
Cd 1	188.70	82.07	67.57	143.63	53.32	47.47	142.73	134.80	104.20
Cd 2	319.80	105.63	101.74	305.10	39.06	84.03	307.40	62.19	63.03

Copper

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	11.6	224.1	8594.0	8.0	6667.0	8861.0	141.1	4615.0	10714
Cd 1	78.6	1566.0	7207.0	124.2	426.5	7595.0	121.1	6863.0	12500
Cd 2	77.5	4225.0	930.2	67.8	1389.0	7563.0	63.1	1891.0	6723

Nickel

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	0.00	0.00	0.00	292.03	74.41	84.43	456.86	102.30	79.41
Cd 1	0.00	0.00	0.00	62.11	31.61	90.00	86.51	130.40	138.96
Cd 2	0.00	0.00	0.00	90.41	23.16	111.76	89.56	66.17	111.76

Table B.5

Factorial experiment 2. Yields of plants per treatment (mg)

Total yields

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	101.0	107.4	76.1	98.9	92.9	75.5	113.4	92.4	58.2
Cd 1	99.6	80.7	72.4	113.4	77.3	60.4	95.8	78.3	55.9
Cd 2	79.9	77.4	56.1	88.9	70.5	59.0	85.6	73.9	60.8

Shoot yields

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	80.0	85.4	59.4	75.3	68.2	56.0	68.2	73.3	44.2
Cd 1	75.4	58.4	53.6	87.2	58.9	46.5	73.5	58.7	41.6
Cd 2	58.6	58.7	44.6	69.2	52.4	44.7	65.9	56.8	49.7

Root yields

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	21.0	22.0	16.7	23.6	24.7	19.5	25.9	19.1	14.0
Cd 1	24.2	22.3	18.6	26.2	18.4	13.9	22.3	19.6	13.7
Cd 2	21.3	18.7	11.5	19.7	18.1	14.3	19.7	17.1	11.1

Table B.6

Factorial experiment 2. Concentrations of heavy metals in shoots (mg/ kg)

Cadmium

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	0.84	1.56	2.24	1.77	0.98	2.38	1.52	1.81	3.01
Cd 1	23.87	9.13	7.46	19.87	6.79	5.74	19.96	6.81	9.61
Cd 2	25.03	9.08	8.77	19.26	10.17	26.85	22.26	11.74	13.42

Copper

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	18.8	6.7	265.2	16.6	99.0	294.6	14.3	156.9	316.7
Cd 1	23.2	111.3	242.5	14.3	101.9	198.9	20.4	127.8	396.6
Cd 2	17.1	127.8	269.1	10.8	90.7	894.9	15.2	228.9	578.5

Nickel

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	3.75	5.86	5.05	39.84	11.73	14.29	43.43	20.46	226.24
Cd 1	3.98	17.12	46.64	32.11	25.47	21.51	61.22	34.07	60.10
Cd 2	5.12	8.52	6.73	14.45	15.27	33.56	34.90	35.21	40.24

Table B.7

Factorial experiment 2. Concentrations of heavy metals in roots (mg/ kg)

Cadmium

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	6.33	9.09	107.80	8.47	8.10	10.26	7.72	10.47	14.29
Cd 1	454.60	41.84	50.16	369.10	43.48	43.17	269.10	44.23	53.50
Cd 2	225.40	36.25	98.52	226.80	55.25	79.23	230.10	62.40	54.05

Copper

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	59.5	1818.0	3443.0	105.9	1569.0	3974.0	96.5	2160.0	3929.0
Cd 1	72.3	426.0	1680.0	85.9	515.3	1799.0	145.7	689.0	1460.0
Cd 2	82.2	1698.0	4565.0	88.8	1934.0	5682.0	76.1	2339.0	4730.0

Nickel

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	14.30	13.64	17.96	330.50	32.39	25.64	386.10	52.36	714.30
Cd 1	12.40	10.72	34.95	126.00	108.70	215.83	170.40	168.37	292.00
Cd 2	14.08	16.30	26.09	91.37	27.62	69.93	116.80	76.02	72.07

Table B.8

Factorial experiment 2. Concentrations of nutrients in shoots (mg/ kg)

Zinc

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	71.25	22.25	45.45	55.78	45.45	573.20	40.00	43.66	452.50
Cd 1	47.74	59.93	179.10	41.28	44.14	83.87	74.83	51.11	149.00
Cd 2	56.31	51.11	38.12	39.02	49.62	402.68	81.94	96.83	672.00

Magnesium (*1000 mg/ kg)

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	2.813	1.493	2.104	2.590	1.613	1.786	2.000	1.535	2.489
Cd 1	2.420	1.541	1.633	2.236	1.698	2.043	1.803	1.831	2.464
Cd 2	2.133	2.300	2.579	1.662	1.908	3.020	2.276	2.025	2.515

Manganese

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	250.0	117.1	166.4	259.0	110.0	133.9	222.9	102.3	158.4
Cd 1	119.4	102.7	149.3	131.9	84.9	172.0	108.8	119.3	168.3
Cd 2	136.5	153.3	157.0	137.3	124.0	156.6	121.4	140.8	171.1

Calcium (*1000 mg/ kg)

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	2.009	1.360	1.653	1.780	1.702	1.595	1.837	1.340	2.020
Cd 1	2.131	1.682	1.666	1.537	1.516	1.920	2.186	1.370	0.858
Cd 2	2.590	1.826	1.610	2.452	1.534	1.799	2.304	1.572	1.079

Table B.9

Factorial experiment 2. Concentrations of nutrients in roots (mg/ kg)

Zinc

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	123.8	86.4	293.4	156.8	85.0	138.5	119.7	157.1	485.7
Cd 1	128.1	237.7	655.9	194.7	37.0	129.5	349.8	234.7	131.4
Cd 2	122.1	70.7	226.1	96.5	127.1	153.9	111.7	122.8	117.1

Magnesium (*1000 mg/ kg)

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	1.915	1.364	1.497	1.801	1.113	1.539	1.351	1.309	1.964
Cd 1	1.756	0.673	0.807	1.731	1.223	1.619	1.570	1.148	2.007
Cd 2	1.174	2.038	1.739	1.904	1.381	1.748	1.777	1.608	1.802

Manganese

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	381.0	45.5	29.9	508.5	40.5	51.3	308.9	52.4	35.7
Cd 1	41.3	22.4	53.8	38.2	27.2	36.0	44.8	25.5	73.0
Cd 2	23.5	27.2	43.5	50.8	27.6	35.0	25.4	29.2	45.0

Calcium (*1000 mg/ kg)

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	2.552	2.032	2.138	2.648	1.810	1.831	2.413	2.340	1.914
Cd 1	2.583	2.004	1.919	2.386	2.430	5.784	2.404	1.821	2.606
Cd 2	2.516	2.429	2.330	3.173	1.972	1.874	2.721	1.567	1.613

Table B.10

Factorial experiment 3, replicate 1. Yields of plants per treatment (mg)

Total yields

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	134.7	115.7	70.1	99.4	162.8	105.4	103.9	97.7	114.1
Cd 1	97.2	94.0	129.2	142.2	102.7	70.3	103.2	112.9	109.1
Cd 2	94.4	123.6	130.9	103.7	95.8	119.5	117.7	109.3	68.4

Shoot yields

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	103.2	85.9	50.8	78.8	116.2	72.7	80.4	68.4	87.8
Cd 1	74.2	67.2	92.5	103.0	73.3	48.8	73.5	81.2	83.5
Cd 2	69.1	87.4	97.1	79.1	64.2	84.0	86.0	78.8	50.4

Root yields

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	31.5	29.8	19.3	20.6	46.6	22.7	23.5	29.3	26.3
Cd 1	23.0	26.8	36.7	39.2	29.4	21.5	29.7	31.7	25.6
Cd 2	25.3	36.2	33.8	24.6	31.6	35.5	31.7	30.5	18.0

Table B.11

Factorial experiment 3, replicate 2. Yields of plants per treatment (mg)

Total yields

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	205.0	181.3	113.8	180.2	143.9	112.8	149.5	128.1	120.6
Cd 1	145.0	127.9	116.4	156.0	138.2	143.0	132.8	169.0	125.5
Cd 2	172.6	136.1	135.4	100.3	143.0	149.4	174.2	118.8	103.9

Shoot yields

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	154.6	132.2	77.3	130.7	105.1	79.7	107.6	93.3	90.8
Cd 1	107.5	91.6	83.2	115.3	102.7	104.7	98.2	121.2	95.8
Cd 2	128.0	104.1	99.8	78.3	105.1	109.1	129.2	87.5	71.6

Root yields

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	50.4	49.1	36.5	49.5	38.8	33.1	41.9	34.8	29.8
Cd 1	37.5	36.3	33.2	40.7	35.5	38.3	34.6	47.8	29.7
Cd 2	44.6	32.0	35.6	22.0	37.9	40.3	45.0	31.3	32.3

Table B.12

Factorial experiment 3, replicate 1. Concentrations of heavy metals in shoots (mg/ kg)

Cadmium

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	0.97	2.92	10.87	2.55	0.86	2.77	1.24	5.13	2.29
Cd 1	25.67	7.46	4.34	19.47	4.11	10.23	23.18	8.65	6.01
Cd 2	34.82	19.50	9.29	30.42	12.49	8.36	25.65	7.64	31.83

Copper

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	15.8	91.7	182.1	17.5	35.0	86.0	17.1	191.9	81.2
Cd 1	13.5	70.9	43.2	12.1	30.7	43.4	15.3	32.3	74.9
Cd 2	14.5	74.4	56.6	12.6	38.9	68.5	14.5	30.1	267.9

Nickel

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	0.00	0.00	0.00	102.82	24.55	28.94	79.02	59.99	46.73
Cd 1	0.00	0.00	0.00	22.85	21.88	32.87	93.24	50.53	43.20
Cd 2	0.00	0.00	0.00	36.07	24.98	22.07	65.15	33.03	101.30

Table B.13

Factorial experiment 3, replicate 2. Concentrations of heavy metals in shoots (mg/ kg)

Cadmium

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	1.30	1.14	2.60	3.07	3.37	2.56	1.87	1.62	2.21
Cd 1	7.94	19.71	7.24	20.87	5.86	5.27	14.30	4.97	8.37
Cd 2	28.98	86.65	13.56	43.54	27.15	9.19	24.83	10.31	7.00

Copper

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	16.2	49.2	100.3	19.1	64.2	72.2	17.4	44.2	110.1
Cd 1	11.6	51.9	121.9	10.8	48.7	50.1	17.8	32.0	83.5
Cd 2	13.7	52.8	87.8	16.0	38.1	64.2	9.7	45.7	43.7

Nickel

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	0.00	0.00	0.00	63.90	3.37	26.40	49.75	43.98	47.94
Cd 1	0.00	0.00	0.00	26.91	30.21	20.10	77.41	37.98	50.66
Cd 2	0.00	0.00	0.00	58.79	27.15	19.29	64.64	46.89	25.89

Table B.14

Factorial experiment 3, replicate 1. Concentrations of heavy metals in roots (mg/ kg)

Cadmium

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	33.43	13.46	25.94	29.22	8.61	4.41	8.55	6.86	15.25
Cd 1	344.35	93.51	45.07	332.47	64.80	55.95	202.53	72.74	68.52
Cd 2	297.19	130.17	94.91	236.34	111.04	101.66	324.16	154.49	130.89

Copper

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	119.1	1201.0	2072.5	163.8	643.8	1585.9	159.6	674.1	1825.1
Cd 1	108.7	932.8	1253.4	89.3	704.8	1674.4	92.6	820.2	2031.3
Cd 2	79.1	690.6	1716.0	111.8	759.5	1464.8	78.9	1147.5	2777.8

Nickel

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	19.18	28.66	31.30	611.70	77.32	92.69	387.32	157.10	184.52
Cd 1	0.00	22.54	9.65	47.30	54.56	62.98	188.65	129.43	170.04
Cd 2	13.99	0.00	0.00	126.10	74.49	80.37	247.7	159.11	283.50

Table B.15

Factorial experiment 3, replicate 2. Concentrations of heavy metals in roots (mg/ kg)

Cadmium

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	11.94	6.13	10.99	20.26	5.18	6.07	27.19	5.78	8.42
Cd 1	64.16	66.28	57.36	246.79	64.96	51.04	181.10	77.59	70.88
Cd 2	337.18	253.75	112.64	355.41	124.33	79.60	267.30	108.91	93.13

Copper

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	104.2	529.5	1109.6	116.2	850.5	1193.4	95.5	775.9	1627.5
Cd 1	86.7	909.1	1506.0	79.9	760.6	1462.1	101.2	1610.9	1986.5
Cd 2	67.3	1563.0	1573.0	215.9	791.6	1240.7	38.3	750.8	1702.8

Nickel

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	11.98	12.30	23.40	517.15	28.45	71.12	294.80	139.45	154.46
Cd 1	22.77	16.64	25.72	63.96	87.41	67.96	205.29	153.83	180.24
Cd 2	13.54	18.88	23.99	220.59	81.87	52.51	213.38	131.09	142.51

Table B.16

Factorial experiment 3, replicate 1. Concentrations of nutrients in shoots (mg/ kg)

Zinc

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	56.31	99.97	131.77	73.74	57.61	154.55	72.78	84.96	69.05
Cd 1	358.09	37.66	90.11	337.21	161.90	119.08	68.76	99.53	151.37
Cd 2	47.58	164.66	75.44	47.94	59.07	58.67	86.64	54.53	55.22

Magnesium (*1000 mg/ kg)

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	2.335	2.108	2.147	2.804	1.315	1.937	2.698	2.147	2.014
Cd 1	2.222	2.155	1.894	1.989	2.140	2.993	2.570	2.030	2.146
Cd 2	2.876	2.073	1.549	2.235	2.070	1.843	2.310	1.631	3.029

Manganese

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	283.4	129.9	124.0	294.6	100.4	107.3	195.0	125.0	105.9
Cd 1	110.2	108.7	100.5	134.0	126.9	157.2	143.5	93.0	111.5
Cd 2	123.7	109.3	9.3	139.7	109.0	112.2	131.4	83.1	189.5

Calcium(*1000 mg/ kg)

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	2.326	3.192	3.072	2.728	3.692	2.850	2.736	1.180	2.423
Cd 1	3.232	3.367	3.470	3.696	2.799	2.725	3.061	3.401	4.186
Cd 2	8.203	2.839	1.482	4.150	1.947	1.845	3.455	1.777	2.877

Table B.17

Factorial experiment 3, replicate 2. Concentrations of nutrients in shoots (mg/ kg)

Zinc

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	34.32	32.50	45.80	39.63	27.68	39.66	76.28	24.42	33.43
Cd 1	32.93	30.38	31.94	35.08	24.65	36.22	36.05	25.04	329.64
Cd 2	29.63	41.28	98.69	77.39	31.28	56.74	77.21	317.65	35.35

Magnesium (*1000 mg/ kg)

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	1.928	1.364	1.966	2.001	1.639	1.932	1.978	1.759	1.964
Cd 1	2.036	1.658	2.021	2.057	1.598	1.761	2.250	1.455	1.925
Cd 2	1.948	1.654	1.848	2.640	1.716	1.430	1.867	1.899	1.895

Manganese

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	289.9	84.4	105.8	250.7	77.5	91.6	183.1	103.4	107.9
Cd 1	95.8	97.5	111.8	108.9	90.6	110.3	112.5	74.7	102.3
Cd 2	92.2	96.5	120.7	133.2	107.5	92.1	97.1	103.4	112.4

Calcium (*1000 mg/ kg)

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	1.520	1.097	1.294	1.454	1.332	1.506	1.441	1.608	1.597
Cd 1	1.628	1.528	1.623	1.778	1.363	1.576	1.578	1.279	1.514
Cd 2	1.445	1.393	1.453	2.043	1.427	1.100	1.509	1.543	1.676

Table B.18

Factorial experiment 3, replicate 1. Concentrations of nutrients in roots (mg/ kg)

Zinc

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	384.8	152.7	222.6	443.8	140.9	467.2	268.8	198.3	412.9
Cd 1	455.6	216.8	89.6	238.4	77.5	516.7	204.1	183.3	187.4
Cd 2	120.0	62.9	586.3	138.8	88.1	1173.9	294.8	256.7	1133.8

Magnesium (*1000 mg/ kg)

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	2.374	1.904	2.526	3.338	1.304	2.059	1.733	1.869	2.310
Cd 1	2.293	1.968	1.470	1.959	1.794	2.305	1.506	1.664	1.679
Cd 2	1.959	1.490	1.596	2.104	1.568	1.519	2.117	1.625	2.022

Manganese

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	944.3	68.9	107.3	639.2	27.9	105.9	124.5	124.0	110.2
Cd 1	134.0	126.9	157.3	143.5	93.0	111.5	108.7	100.5	123.7
Cd 2	139.7	36.0	27.4	131.4	25.4	29.6	109.3	26.3	44.6

Calcium(*1000 mg/ kg)

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	3.815	3.691	2.850	5.340	1.180	2.423	3.192	3.072	3.232
Cd 1	3.696	2.799	2.725	3.061	3.401	4.186	3.367	3.470	8.203
Cd 2	4.150	2.348	1.627	3.455	2.690	2.535	2.839	2.131	3.056

Table B.19

Factorial experiment 3, replicate 2. Concentrations of nutrients in roots (mg/ kg)

Zinc

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	205.4	77.2	90.1	117.4	162.8	114.6	78.5	380.9	110.3
Cd 1	74.2	48.9	114.2	111.8	71.3	30.8	284.7	58.2	93.7
Cd 2	65.2	161.9	127.8	292.9	60.1	67.8	50.6	121.2	78.4

Magnesium (*1000 mg/ kg)

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	1.968	1.194	1.884	1.840	1.615	1.832	1.669	1.684	1.967
Cd 1	2.753	1.447	1.770	2.188	1.537	1.848	1.870	1.481	1.973
Cd 2	2.042	1.958	1.760	2.480	1.868	1.404	1.618	1.743	1.815

Manganese

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	729.8	21.4	32.3	509.7	36.9	24.2	120.5	37.4	35.2
Cd 1	34.7	29.0	39.2	35.1	33.2	30.8	34.1	27.2	35.4
Cd 2	32.1	44.7	40.2	59.1	40.9	26.1	28.9	33.6	32.5

Calcium (*1000 mg/ kg)

	Ni 0			Ni 1			Ni 2		
	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2	Cu 0	Cu 1	Cu 2
Cd 0	2.381	1.324	1.507	2.323	1.675	1.662	1.790	1.724	1.846
Cd 1	2.933	2.755	2.259	2.580	2.113	2.219	2.168	1.778	2.189
Cd 2	2.579	2.031	1.405	2.046	1.847	1.365	1.889	2.077	1.703

Appendix C

PUBLISHED PAPER

The influence of heavy metals upon the growth of sitka-spruce in South Wales forests. 1. Upper critical and foliar concentrations

K W BURTON and E MORGAN

Department of Science, The Polytechnic of Wales, Pontypridd, S.Wales, UK

A ROIG

Departamento de Fertilidad de Suelos, Centro de Edafologia y Biologia Aplicada del Segura, CSIC, Murcia, Spain

Key words Critical level Foliar tissue Heavy metals Sitka-spruce Tree growth

Summary The upper critical concentrations of several heavy metals were determined in sitka-spruce (Picea sitchensis) following the method of Beckett and Davis (1977). The values obtained (mg/kg of dry matter) were Cd (4.8), Ni (5.8), Pb (19), Cu (88) and Zn (226). It has been shown that nickel might be present at sufficiently enhanced levels in the foliar tissues of trees in certain forest areas of South Wales to affect normal functioning and growth. The cadmium foliar levels approach but do not exceed the critical Cd level, but may possibly have some impact considering the additivity of toxic effects of heavy metals. Lead, copper and zinc would seem to present no risk as their foliar levels are well below the critical levels.

Introduction

It is now well recognized that industrial activity over a long period of time has led to an enhancement of the levels of heavy metals in the

environment. South Wales is an example of an area where there has been a steady growth in industrial and urban development since the eighteenth century; major industries being coal mining, metallurgy and petrochemicals. In such an area a major sink of heavy metals, which would be emitted into the environment in the form of smokes, dusts and leachates, is the soil, and it has been verified that there are in fact enhanced levels of heavy metals in the soils of the region.[3,8] It has also been observed, in an investigation of poor growth of sitka-spruce at Margam Forest, that the soils were amongst the most infertile in Wales, and that the barreness of the area was a relatively recent phenomenon.[10]

Forestry plantations are now extensive in South Wales and it is therefore important to establish why there is such poor growth in certain areas, if only as a guideline to future planning. This publication desribes an investigation of one possible factor influencing growth of sitka-spruce - the toxic effects of heavy metals.

Several indices have been used as methods of assessing heavy metal toxicity. In general, however, these are non-specific and dependent upon plant growth which is defined by many other factors such as the levels of nutrients and the availability of light and water. For instance, the use of total dry weight of plant or shoot length in situations of bad growth cannot, as an index, distinguish between the toxic effects of heavy metals and nutrient deficiency. One index which has been specifically related to the toxic effects of elements and found to be relatively independent of other factors is the shoot tissue concentration of the element. As the shoots have been identified as a major impact area in relation to heavy metal toxicity, it is to be

expected that the concentrations of such toxic substances in the shoots will serve as an index of the toxic effects of such elements.[2]

Essential and non-essential elements may be examined using tissue concentrations even though they produce different effects. With essential elements it is found that below a certain concentration of the element the yield of dry matter of the plant decreases; that is to say, the plant is deficient in this element. Above this deficient level is the yield plateau, where a change of concentration does not affect the yield.[1,2] At higher levels there is a critical concentration above which the yield again decreases. Non-essential elements, on the other hand, only exhibit a yield plateau, followed by a decrease in yield above an upper critical level. Thus both essential and non-essential elements exhibit an upper critical level above which yields are reduced because of toxic effects.

This paper establishes the upper critical tissue concentrations of cadmium, copper, lead, nickel and zinc in sitka-spruce and, from a comparison with foliar tissue concentrations of sitka-spruce in South Wales and Mid Wales, tries to assess whether in fact such heavy metals present a hazard.

Materials and Methods Upper critical concentrations in sitka-spruce (Picea sitchensis) were determined by growing seedlings in water culture, using nutrient solutions doped with heavy metals. The origin and provenance of the seed was Queen Charlotte Islands. The seed lot was blown to remove empty and light seeds, then sieved to remove the largest and smallest seed to increase seed uniformity. The seeds were then set aside for storage and subjected to a pretreatment process as required.

This consisted of a chilling process at 4°C, which increased the uniformity of germination.[13] The seeds were sown in a commercial peat covered with silver sand to prevent damping off and germinated at a temperature of 25°C in an electrically heated propagator. The light was supplied by universal daylight tubes positioned one metre above the trays with a day:night ratio of 16:8 hours.

After germination, seedlings were pricked out of the peat and transferred to supports (20 per support) which were suspended on 250 ml beakers covered with black polythene to prevent algal growth in the solutions. The temperature was adjusted to maintain at least 20°C in the propagator and nutrient solution added to within one centimetre of the brim. The solution composition was changed every five days from quarter to half, and finally to full strength. The solution was one developed by Ingestad (1959) for norway-spruce seedlings, though it is used as a standard nutrient solution for sitka-spruce by the Forestry Commission (see Table 1). The pH of the solution is 4.5.

Thirty one days after sowing, the heavy metals (Cd-ions, Cu-ions, Ni-ions, Pb-ions and Zn-ions) were added as chloride salts to the nutrient solutions at the concentrations shown in Table 2. Chloride salts were used since chloride ions were already present at far higher levels in the solutions and would not have interfered with the nutrient composition (Table 1). In the experiments used to determine the copper and zinc critical levels, these metals were omitted from the basic nutrient solutions: the amounts added in the treatments (Table 2) therefore represented the total amounts of these particular metals in the solutions. The seedlings were grown for at least another 42 days, the nutrient solutions being changed weekly to avoid depletion of vital

nutrients and oxygen. An additional experiment (Cd4) was carried out to ascertain whether there was any change in the cadmium critical concentration with older plants. Sixty day old seedlings in nutrient solutions were doped with cadmium, and then grown for a further 30 days.

At the end of the growing period the roots were washed with deionized water and the largest and smallest plants from each beaker discarded to eliminate any gross anomalies in the results. The plants were separated into shoots and roots, and their respective yields determined by drying overnight at 105°C in weighed beakers, and then reweighing after cooling in a desiccator. The shoots and roots were then digested with a 3:1 mixture of nitric and perchloric acids, the beakers being heated to complete dissolution of the plant material. After cooling, the digestates were filtered through Whatman No 42 paper and made up to either 10 or 25 millilitres volume. The concentrations of metals in the solutions were determined by flame atomic absorption spectrophotometry (AAS) (Varian model 1100) using appropriate blanks and standards.

Foliar samples were taken according to standard Forestry Commission procedure in late September 1980, 1981, 1982 from the upper crown of six dominant trees at each of the eight 0.01 hectare sites in South and Mid Wales (as shown in Figure 1). The sites were chosen on the basis of having similar soil characteristics; most of the sites having peaty gley soils. Such samples represent a full growing season and are therefore effectively six months old. Dominant trees were chosen since it is this class of trees which are normally used by the Forestry Commission in the assessment of growth at particular sites. Table 3 lists the grid reference positions, the ages of the trees (where available), the

provisional or general yield class (where available) as a measure of tree growth, and the altitudes and aspects of the sites. Six inch samples were taken from the branches with the most southerly facing aspect. The needles were stripped, washed in deionized water, dried at 85°C overnight, ground and redried. Duplicate 0.5 g samples were analysed for cadmium, copper, lead, nickel and zinc using the method described above.

Results and discussion

Upper critical concentrations

The relationship between yield in terms of dry matter and the toxicity of an element can be modelled by converting the concentration of the element to logarithmic form.[1] The data then reduce to intersecting straight lines;[7] one being a yield plateau, the other a regression line as shown in Figure 2. In this present study the two intersecting straight lines could often be drawn by eye, but in some cases the range of treatments nearly missed the yield plateau, tending to confuse any subjective estimation. A statistical method, developed by Beckett and Davis (1977), made the assessment of upper critical tissue concentrations both automatic and objective.

The critical tissue concentrations (Tc, Table 4) were determined from the split-points, where the yield plateaux (Yo) intersected the regression lines. The lethal tissue concentrations (Tl) (which were the concentrations obtained when the regression lines were extrapolated to zero yield), the pooled standard errors about the lines (S E) and the correlation coefficients (r)and their significances are also listed in Table 4.

The correlation coefficients were all either 95 or 99% significant; the lowest correlations being obtained with zinc and lead. Although there were originally three zinc experiments, only the results of one are given in Table 4 since the others did not yield Tc values. With these other two runs, even at the highest concentrations, accumulation of zinc was insufficient to reduce the shoot yields and thus no upper critical concentrations were obtained. The reproducibilities of the Tc values obtained for each metal (Table 4) were found to agree well with those of earlier workers.[1,5] The lower correlation for lead, which is also evident from the yield curve (Figure 2) is probably a result of the lower sensitivity of the AAS method for Pb, as there is in any case a dilution factor of approxiamately 25 from actual sample to analytical solution.

The upper critical concentration of lead is 19 mg/kg whereas the lethal concentration is only 43 mg/kg, which is low compared with the other metals studied. This could be explained by a sequestration and extrusion of the lead in shoots up to a concentration of approxiamately 19 mg/kg, above which the "available" lead exerts an extremely toxic effect. Earlier workers have observed that, once translocated, lead could be "extruded" from cells throughout the plant.[11] For cadmium, upper critical concentrations are consistent in plants of different ages and development. Other workers have also demonstrated this for cadmium and other metals in several different species over various stages of development,[1,5] though no data of this nature exists for mature trees.

Examples of the yield curves obtained for Cd, Cu, Ni, Pb and Zn are shown in Figure 2. In the cases of Cu and Zn, no lower critical concentrations were exhibited, showing that even at the lowest levels

used there was an adequate supply of these essential elements.

Table 5 lists the average upper critical concentrations for the sitka-spruce seedlings along with generalized upper critical and "background" (natural and uncontaminated) concentrations, derived from data for barley, rape, lettuce and other plants.[5] These are included to show the relative sensitivity of sitka-spruce to the different metals. The average Tc's of copper and zinc in sitka-spruce are either well above or equal to the generalized average values. However, the non-essential elements are relatively more toxic, the upper critical concentrations being approxiamately half those observed for other plants.

Foliar analysis

The foliar concentrations of heavy metals in samples collected at seven sites in South Wales and the one in Mid Wales are listed in Table 6. The Mid Wales site is well removed from urban and industrial development and represents a control site where there should only be background levels of heavy metals. Work done by the authors has shown the total soil levels of all the heavy metals and acetic acid extractable levels of certain metals at these sites to be enhanced in comparison with the control site in Mid Wales. For example, at the South Wales sites total soil concentrations of Cd of upto 3.5 mg/ kg and extractable levels upto 1.6 mg/kg were detected, whereas the total soil Cd level at the Mid Wales site was 0.8 ppm with none extractable. This indicates that soil is a likely source of the heavy metals in the foliar tissues, but it should still be recognized that there could still be an input via atmospheric deposition. This will be discussed in future papers.

A meaningful comparison of foliar tissue concentrations with upper

critical concentrations will depend upon the speciation of the heavy metals in the foliar tissues. Such a comparison is only valid if the the majority of the heavy metals entered the foliar tissues via the soil and roots.

Another possible contribution to heavy metal levels in foliar tissues is via impaction onto the needle surfaces. The speciation of the heavy metals in the impacted material may not allow their incorporation into the tissues; in other words the metals are present in an inactive form. However, the needles were washed prior to analysis: this will have removed some of the insoluble surface material. The insolubility of the material is not the only problem since speciation of the solubilized material may differ from that contributed by the roots. The two processes leading to incorporation into the tissues, however, would seem to be broadly similar since leaf surface solutions are fairly acidic, containing many ligands and metals.[12] Leaf surfaces also have fixed negatively charged ligand sites which can bind metal ions.[12]

It has been shown in experiments using combinations of metals with sycamore trees[4] that the toxic effects of certain heavy metals are additive, and not antagonistic. The levels of the individual elements in the foliar tissues may therefore be used as indices of the risk of heavy metal toxicity. However, this relies on the assumption that these critical levels are similar in mature trees, but this has not been verified since no work of this nature has been carried out. However, the aim here is to compare the toxic effects of metals in the shoots of sitka-spruce seedlings with those levels present in young actively photosynthesizing foliar tissues to determine the potential risk to

these more mature trees in field situations.

Several of the sites had concentrations of cadmium which approached the Tc value and were above the concentrations found in the control. It can also be seen that the concentrations of cadmium present exceeded the background concentrations found in other plants. Most of the sites had nickel concentrations above the Tc values of sitka-spruce, and also above the generalized average, and it is therefore possible that nickel has affected tree growth. The concentrations of lead were well below the critical values, although above the background concentrations found in sitka-spruce and other plants, and therefore lead probably has no effect upon the normal growth of trees. The essential elements, copper and zinc, have high critical concentrations which far exceeded their foliar concentrations and it is therefore unlikely that they affect tree growth.

Table 6 shows that the sites with the highest concentrations of cadmium and nickel, Rhondda 1 and 2 respectively, have also been assigned low general yield classes, which are signs of poor growth. However, using the variables detailed in Table 3 independently it could be shown that altitude is as important a factor as the levels of heavy metals are in determining the growth of the trees. The overall tree growth at any one site is more complex than this and is controlled by many factors. Therefore, correlations with the site information of Table 3 have not been attempted since there is insufficient site data on too few sites.

Even though it has not been possible here to investigate fully all the factors influencing the toxicity of heavy metals to sitka-spruce, using

upper critical concentrations, it has been shown that some heavy metals are present at sufficiently enhanced levels that they might present a risk to normal functioning in the foliar tissues. Two other major aspects of the problem of heavy metal toxicity, namely the interactive effects of heavy metals and the more indirect impact upon soils and roots such as by interference with mycorrhizal associations and nutrient uptake will be discussed in later papers.

Acknowledgement The authors wish to thank Mr Danby and Dr Binns of the Forestry Commission for their help ad advice and Mr D H C Clark for assistance in collecting the foliar samples. They are also grateful to the SERC and British Council for the financial support awarded to E Morgan and A Roig respectively.

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Table 1. Full-strength composition of the nutrient solution[7]

Compound	Concentration g/l
NH_4NO_3	14.3
$KH_2PO_4 \cdot 2H_2O$	4.4
KCl	7.13
$CaCl_2 \cdot 6H_2O$	21.9
$MgSO_4 \cdot 7H_2O$	15.4
$FeCl_3 \cdot 6H_2O$	0.5
$MnCl_2 \cdot 4H_2O$	0.06
H_3BO_3	0.1
$ZnCl_2$	0.004
$CuCl_2 \cdot 2H_2O$	0.005
$NaMoO_4 \cdot 2H_2O$	0.0007

Table 2. Concentrations of metals added to the nutrient solutions

Experiment	Concentration mg/l
Cd 1,2,3	0, 0.025, 0.05, 0.1, 0.25, 0.5, 0.75, 1, 2, 5, 10
Cd 4*	0, 0.05, 0.1, 0.5, 1, 10
Ni 1,2	0, 0.01, 0.025, 0.05, 0.075, 0.1, 0.15, 0.4, 0.5, 0.75, 1, 2.5, 5, 7.5, 10
Pb 1,2	0.01, 0.025, 0.05, 0.075, 0.1, 0.15, 0.4, 0.5, 0.75, 1, 2.5
Cu 1,2,3	0.02, 0.05, 0.075, 0.1, 0.25, 0.5, 0.75, 1, 2.5, 5, 7.5, 10
Zn 1,2,3	0.02, 0.1, 0.25, 0.5, 0.75, 1, 1.5, 2, 5, 10, 50, 100

* Each concentration replicated three times

Table 3. Site information of foliar samples

Forest site	Grid Reference	Date Planted	Provisional or General Yield Class*	Altitude m	Aspect
Cymer	ST 889980	1974	8	488	South
St Gwynno	ST 025955	1970	14	356	East
Margam	ST 825899	1972	22-24	280	North
Rhondda 1	ST 909976	1971	12	500	North-East
Rhondda 2	ST 904032	1967	6-8	488	North
Rhondda 3	ST 906020	1966	6-8	512	South-East
Rhondda 4	ST 844017			310	North-East
Tywi-Dolgoch	SN 784576	1971		490	North-East

* A yield class is an index of tree growth at a site: higher yield classes representing better tree growth

Table 4. Yield curve data derived from upper critical concentration calculations

Experiment		Critical Tissue concentration Tc mg/kg ODM	Lethal Tissue concentration Tl mg/kg ODM	Yield Plateau Yo mg	Pooled Standard Error	Correlation Coefficient r
Cadmium	1	3.68	11881	10.77	0.67	- 0.9**
"	2	6.22	14078	10.56	0.81	- 0.9**
"	3	5.72	2778	10.63	1.26	- 0.84**
"	4	3.49	292	19.42	3.27	- 0.78**
Nickel	1	5.08	5082	8.73	0.32	- 0.96**
"	2	6.58	16002	8.05	0.37	- 0.96**
Lead	1	18.44	41.69	11.95	5.84	- 0.76**
"	2	19.20	43.29	11.62	5.28	- 0.83*
Copper	1	103.63	10064	6.58	0.44	- 0.92*
"	2	97.24	11288	6.97	0.96	- 1**
"	3	61.96	7253	6.81	0.64	- 0.97**
Zinc	1	225.50	4.8 * 10	3.84	0.12	- 0.95**

ODM = Of Dry Matter

* Correlation significant at 0.05 level

** Correlation significant at 0.01 level

Table 5. Upper critical tissue concentrations (Tc) for sitka-spruce and generalized* upper critical and background concentrations[4]

Element	Sitka-spruce Tc mg/kg ODM	Generalized Tc mg/kg ODM	Generalized background concentration mg/k ODM
Cadmium	4.78	8	0.5
Nickel	5.83	11	2
Lead	18.8	35	3
Copper	87.6	20	8
Zinc	226	200	40

ODM = Of Dry Matter

* Tentative data from various sources

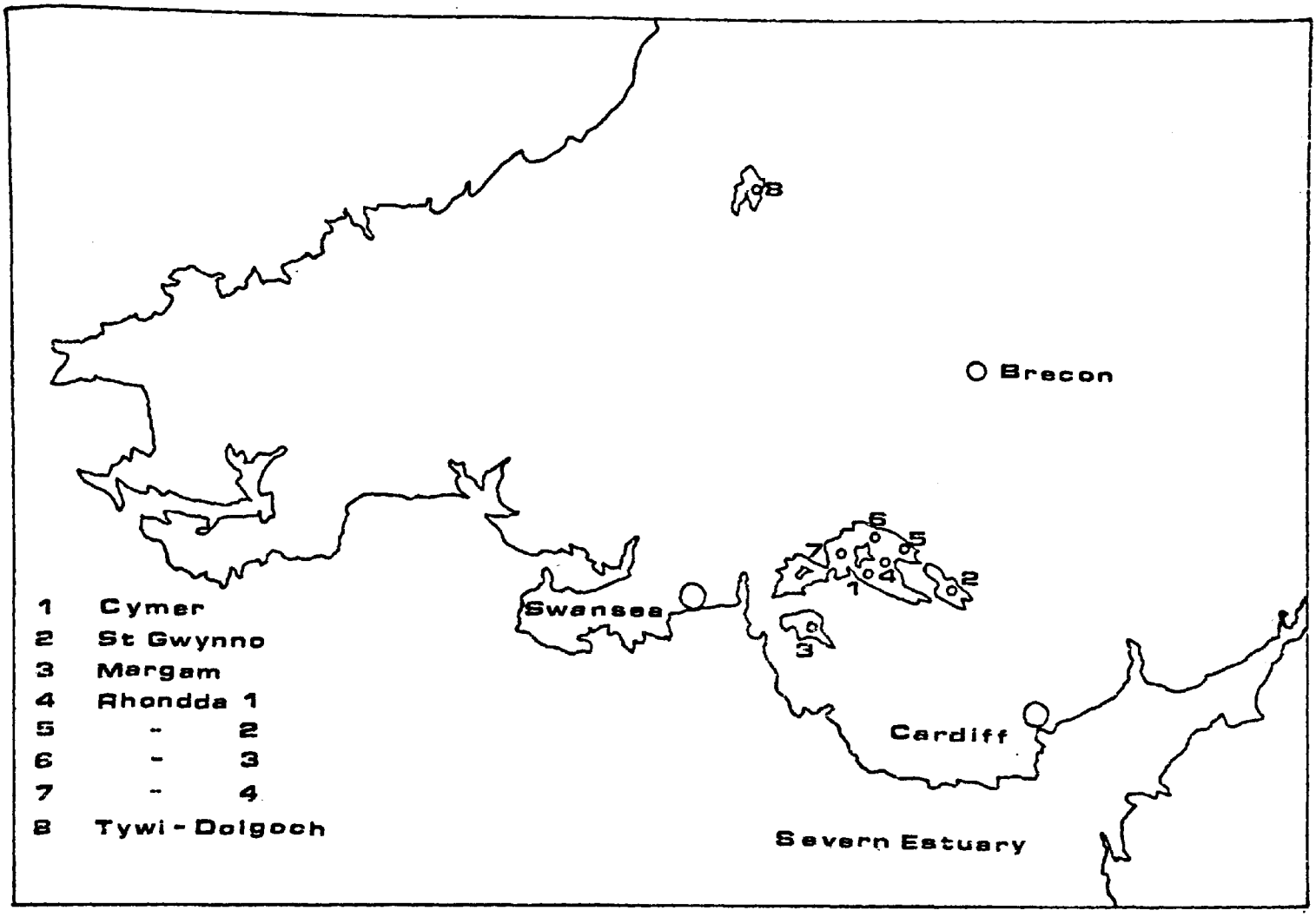
Table 6. Sitka-spruce foliar analysis for heavy metals at South Wales sites

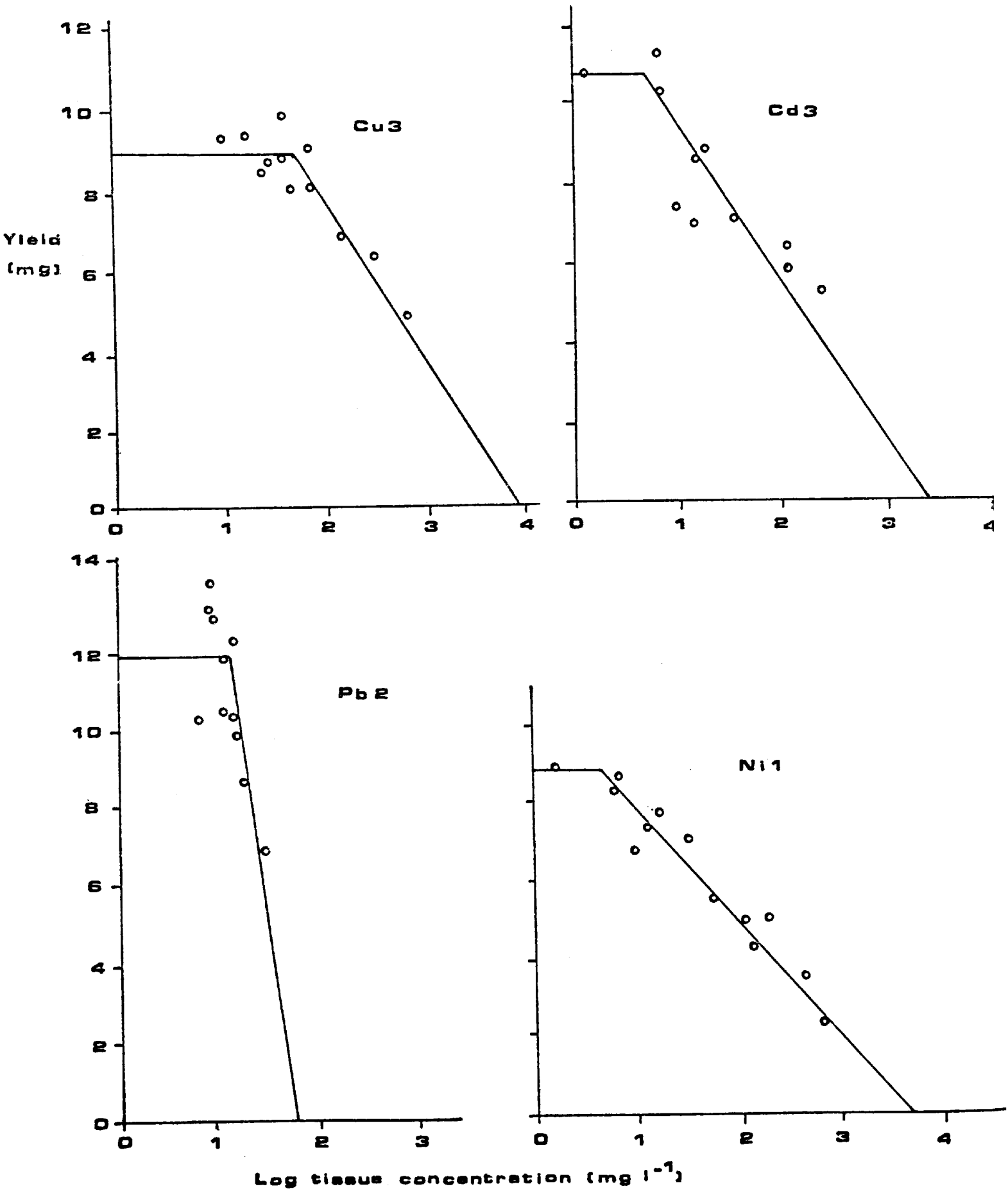
Site	Heavy Metal Concentrations mg/ kg ODM				
	Cadmium	Nickel	Lead	Copper	Zinc
Cymer	2.37	10.5	9.8	8.5	17.2
St Cwynno	1.60	2.9	7.4	3.0	16.8
Margam	2.06	11.0	10.5	8.2	21.2
Rhondda 1	3.54	9.0	10.3	11.0	16.3
Rhondda 2	3.20	14.8	9.4	5.5	23.4
Rhondda 3	0.82	5.8	6.7	5.5	43.2
Rhondda 4	1.92	9.4	5.4	5.4	43.2
Tywi-Dolgoch	0.35	2.5	3.4	2.3	23.3

ODM = Of Dry Matter

Figure 1. Map showing positions of sampled sites in South and Mid Wales

Figure 2. Typical yield curves for Cd, Cu, Ni and Pb


1 Cymer
2 St Gwynno
3 Margam
4 Rhondda 1
5 - 2
6 - 3
7 - 4
8 Tywi - Dolgoch
Brecon
Swansea
Cardiff
Severn Estuary


Cu3
Cd3
Pb2
Ni1
Yield (mg)
Log tissue concentration (mg l^{-1})