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The influence of heavy metals upon the growth of sitka-spruce in South Wales forests

I. Upper critical and foliar concentrations

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Summary The upper critical concentrations of several heavy metals were determined in sitka-spruce (*Picea sitchensis*) following the method of Beckett and Davis.[1] The values obtained (mg kg^{-1} 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 barrenness 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 describes 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.[1] 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[9] 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.

Table 1. Full-strength composition of the nutrient solution[7]

Compound	Concentration, g per litre
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
$MnCl_2\cdot 4H_20$	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

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 80 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

Table 2. Concentrations of metals added to the nutrient solutions

Experiment	Concentration mg l^{-1}
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

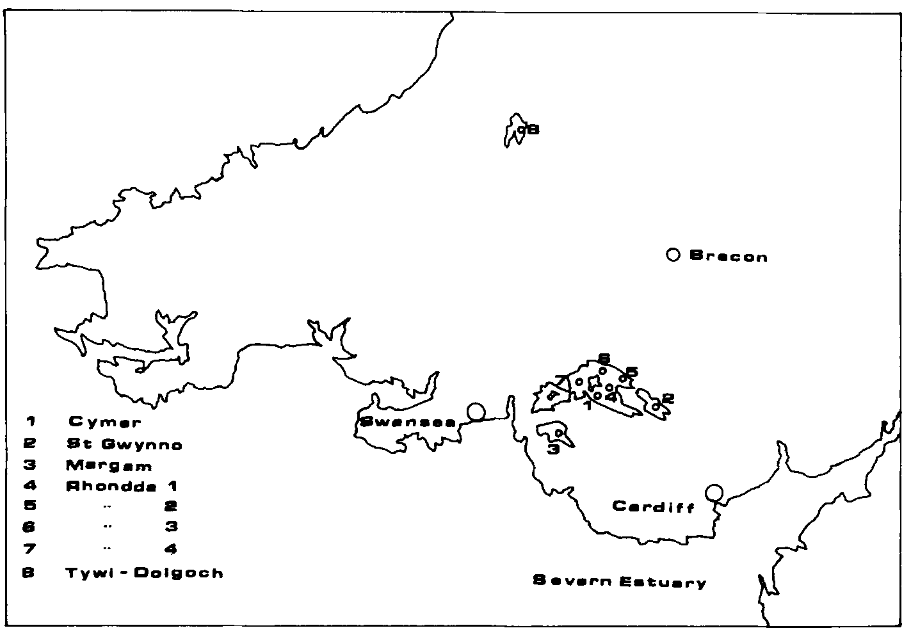

Fig. 1. Map showing positions of sampled sites in South and Mid Wales.

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 Fig. 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,[1] made the assessment of upper critical tissue concentrations both automatic and objective.

The critical tissue concentrations (T_C, Table 4) were determined from the split-points, where the yield plateau (Y_O) intersected the regression line. The lethal tissue concentrations (T_L) (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 T_C 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 T_C 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

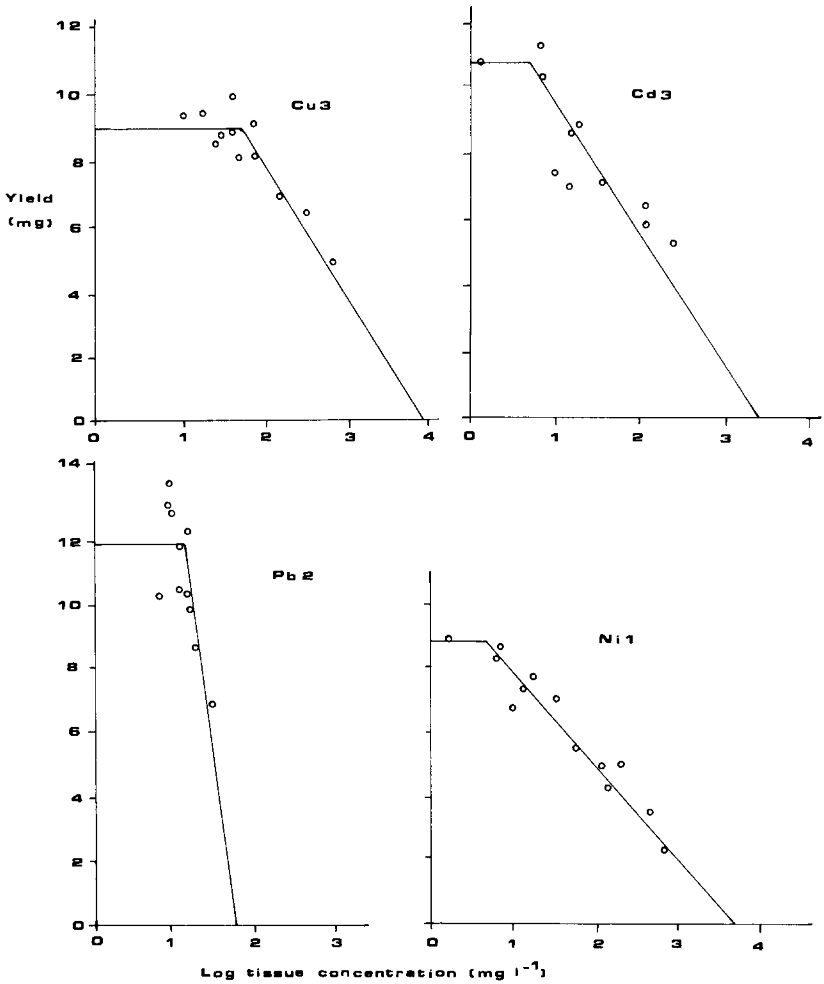

Fig. 2. Typical yield curves for Cd, Cu, Ni and Pb.

from the yield curve (Fig. 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 approximately 25 from actual sample to analytical solution.

The upper critical concentration of lead is 19 mg kg^{-1} whereas the lethal concentration is only 43 mg kg^{-1}, 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 approximately 19 mg kg^{-1}, 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

Table 4. Yield curve data derived from upper critical concentration calculations

Experiment	Critical tissue concentration T_C mg kg^{-1}ODM	Lethal tissue concentration T_L mg kg^{-1}ODM	Yield plateau Y_O mg	Pooled standard error	Correlation coefficient r
Cadmium 1	3.68	11881	10.77	0.67	−0.9**
Cadmium 2	6.22	14078	10.56	0.81	−0.9**
Cadmium 3	5.72	2778	10.63	1.26	−0.84**
Cadmium 4	3.49	292	19.42	3.27	−0.78**
Nickel 1	5.08	5082	8.73	0.32	−0.96**
Nickel 2	6.58	16002	8.05	0.37	−0.96**
Lead 1	18.44	41.69	11.95	5.84	−0.76**
Lead 2	19.20	43.29	11.62	5.28	−0.83*
Copper 1	103.63	10064	6.58	0.44	−0.92*
Copper 2	97.24	11288	6.97	0.96	−1**
Copper 3	61.96	7253	6.81	0.64	−0.97**
Zinc 1	225.50	4.8* 10^{13}	3.84	0.12	−0.95**

ODM = Of Dry Matter

* Correlation significant at 0.05 level

** Correlation significant at 0.01 level

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 T_C'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 approximately 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

Table 5. Upper critical tissue concentrations (Tc) for sitka-spruce and generalized upper critical and background concentrations[4]

Element	Sitka-spruce Tc mg kg^{-1}ODM	Generalized Tc mg kg^{-1}ODM	Generalized background concentration mg kg^{-1}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

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^{-1} and extractable levels upto 1.6 mg kg^{-1} 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

Table 6. Sitka-spruce foliar analysis for heavy metals at South Wales sites

Site	Heavy metal concentrations, mg kg^{-1}ODM				
	Cadmium	Nickel	Lead	Copper	Zinc
Cymer	2.37	10.5	9.8	8.5	17.2
St Gwynno	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

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 T_C 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 T_C 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.

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