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

II. Greenhouse experiments

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Summary Experiments on sitka-spruce seedlings grown in acidic peaty gley soils under greenhouse conditions, where the soils where doped with increasing amounts of Cd, Cu and Pb up to maximum levels of metal added of 16 ppm, 32 ppm and 400 ppm respectively, showed that the levels of Cd and Pb in shoots and roots increased with increasing levels in the soil, whereas levels of copper appeared to be independent. The addition of these three metals to the soils did not influence the uptake of other heavy metals, or of the nutrients potassium or calcium. Increases in the shoot cadmium levels significantly reduced the yields of the plant shoots. However, the plant yields were only affected by the highest level of lead that was added to the soil (400 ppm Pb) and unaffected by all the copper treatments (0–32 ppm Cu in the soil).

The lengths of the sitka-spruce roots were reduced when cadmium and lead levels in the soil exceeded certain threshold concentrations (2.5 ppm total Cd, where 0.3 ppm was extractable with 0.5 M acetic acid; and 48 ppm total Pb, where 1.7 ppm was extractable). However, root lengths were not reduced by copper. This was probably related to the fact that copper appears to be relatively unavailable in the type of soil used, as only 1.1 ppm Cu was extractable from a total of 32 ppm Cu added. Root branching was apparently reduced by increases in the soil levels of cadmium, copper and lead. The roots of some control plants had symbiotic mycorrhizal associations (4 out of 19 plants), whereas the roots of all the plants grown in the soils with added heavy metals did not develop these.

Introduction

It is well recognized that forest ecosystems have been disturbed as a result of contamination by heavy metals, there being many reports of significant effects upon individual components of these ecosystems which, in general, represent manifestations of serious breakdowns in their structure. This contamination has often resulted from industrial activities of different kinds which are remote from the sites affected. It has been shown, for example, that accumulation of lead in the soil of a northern hardwood forest ecosystem is 305 g ha^{-1} yr^{-1} and, that at this current rate, the doubling time of lead accumulation in humus is 30 years[2,3].

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Effects of contamination by heavy metals upon the vegetation in the areas in the immediate vicinity of metal smelters and mining areas are very well documented[6, 7, 20, 28], though remoter areas may be affected in a more subtle manner. As well as these direct effects upon plant growth, certain soil processes have been disturbed. For instance, nitrogen mineralization and other processes controlled by soil micro-organisms have been seriously affected by heavy metals[22, 27]. This has led to significant increases in the depths of litter layers in forests in some areas. It has also been shown that the development of mycorrhizae on the roots of Popular and Willow trees grown in iron and copper mine tailings was inhibited in the presence of copper[17]. Such disturbances in these relationships may have serious consequences for growth.

A classification of contaminated temperate forest ecosystems and the type of impact produced by pollution at such sites has been proposed[25], and it has been shown that in a typical class 3 situation gross simplications of the ecosystems may occur because of adverse effects of pollutants, such as the complete disruption of vital soil processes. However, in a class 2 situation, these effects are of a more subtle nature. Typical impacts could be reduced productivity, shifts in species composition and increased secondary effects such as insect out-breaks or disease epidemics, these being non-specific phenomena which are difficult to relate directly to specific pollutants. In class 1 situations, the forest acts only as a sink for the heavy metals and they are accumulated without harmful effects.

South Wales is heavily industrialized, with major industries such as coal mining and petrochemicals being possible sources of heavy metal contamination. The emission of heavy metals has led to an enhancement in the soil levels of heavy metals in the region[8,16] and it seems possible that heavy metals may be having a detrimental effect upon the environment[9]. It has been observed, in an investigation of the poor growth of sikta-spruce at Margam forest, that the soils were amongst the most infertile in Wales and that this barrenness was a recent phenomenon[19]. Since forestry plantations are now extensive in South Wales, and it is known that in general there is poor tree growth, it is important to establish the reasons for this.

In a previous study, sitka-spruce seedlings were grown in water culture to determine the upper critical foliar levels of several heavy metals[9]. These critical levels are the levels of the metals in shoot tissue above which the shoot yields are reduced[3]. Using these values it has been shown that tree growth may be affected by accumulation of heavy metals in the foliar tissues[9]. Although this information is vital to the assessment of the impact of heavy metals, more complex interaction

tween soil, soil microorganisms and plant roots have important ɔnsequences. Several factors may influence the behaviour of the heavy .etals in the peaty soils of South Wales. These are acidity, organic ;atter, drainage, the presence of mycorrhizae and other microorganisms, nd only by examining the interactions between heavy metals and these actors can a more complete picture of the situation be gained.

The aim of this work was to grow sitka-spruce seedlings in a peaty oil, typical of South Wales sites, supplemented with lead, copper and :admium, and thus to examine the influence of these metals upon the ·ield of dry matter, mycorrhizal associations and accumulation of ıeavy metals by their tissues.

ıaterials and methods

Soil samples were taken from typical forestry sites in South and Mid Wales. Six forestry sites ·ere sampled in total, with five from South Wales and one (a control site) from the rural and :latively non-industrialised area of Tywi in Mid Wales. These forests were the same as those ımpled in previous work by the authors, though the actual sites were not all identical[9]. The ımples were analysed in order to compare the major soil characteristics and to measure the ɔtal and extractable heavy metal concentrations. The sites had similar soil types, most being eaty gley soils, though the site at Cymer was a Molinia dominated deep peat, type 9b[21]. At ıch site thirty samples were taken from 0–15 centimeters deep[4] and broken down manually ıto small aggregates, using the standard quartering method to homogenize the peats.

The soils were characterized by measuring the following standard parameters using fresh and ·en dried soils (105°C for 16 hours): acidity as measured by pH in water (fresh soil)[1], extract- ·le heavy metal levels (fresh soil, 0.5*M* acetic acid, the concentrations of the metals being ·termined by flame atomic absorption)[1], total heavy metal concentrations after a perchloric- tric (1:3) acid mixture digestion and flame atomic absorption (oven-dried soil)[1], organic rbon by pyrolysis at 400°C (oven-dried soil)[1], total nitrogen by Kjeldahl digestion and the sulting ammonium determined by specific ion electrode (oven-dried soil)[5], ammonium-nitrogen · extraction with 2M potassium chloride and specific ion electrode (fresh soil)[2], and nitrate by .traction and specific ion electrode (fresh soil)[24].

Grid reference positions and data characterizing the sites are shown in Table 1. The site in id Wales (Tywi-Dolgoch) was very similar to those of South Wales except that it had no tractable and comparatively low total concentrations of heavy metals. These characteristics ıde it suitable for use as an uncontaminated soil in an experiment to study the effects of ·reasing levels of heavy metals upon sitka-spruce seedlings (*Picea sitchensis*) grown in a soil ɔical of those encountered in South Wales. A composite soil sample was therefore taken from : forestry site which was sufficient in bulk for the purposes of the experiment.

The soil was mixed thoroughly and washed quartz sand (0.1–1.0 mm particle size) added to w drainage and to prevent anaerobic soil conditions developing, the proportion 1:1 being ·opriate for this. Sufficient quantities of the fresh soil-sand mixture were placed into 5 inch ·ter flat bottomed conical plastic garden pots (equivalent to 400 grammes of an oven-dry ·e per pot), and 25 ml portions of solutions containing the chloride salt of the heavy ·l were added to give the soil concentrations (parts per million as dry weight: ppm i.e. mg) shown below. Each experiment was carried out in triplicate and four control pots were prepared to which only 25 ml of deionized water was added.

Cadmium (Cd^{2+})	0.1, 0.4, 1, 2, 4, 8 and 16
Copper (Cu^{2+})	0.5, 1, 2, 4, 8, 16 and 32
Lead (Pb^{2+})	5, 10, 25, 50, 100, 200 and 400

Table 1. Chemical analysis of the forest soils

Soil parameters	Forest sites and grid reference positions					
Heavy metal levels (ppm)	Cymer ST889980	Margam 1 ST821906	Margam 2 ST825899	Rhondda 1 ST909976	Rhonnda 3 ST906020	Tywi-Dolgoch SN784576
Cd_T	2.1	0.0	2.4	4.2	2.7	0.8
Cd_E	0.8	0.0	0.0	1.5	0.9	0.0
Cu_T	71.2	63.4	181.0	146.0	138.0	6.8
Cu_E	1.3	1.6	1.0	1.6	2.0	0.0
Ni_T	17.0	20.0	13.1	31.8	23.4	3.0
Ni_E	0.0	0.0	0.0	0.0	0.0	0.0
Pb_T	175	117	224	337	378	47
Pb_E	0.0	0.0	0.0	0.0	0.0	0.0
Zn_T	39.3	25.4	39.4	107.8	112.2	32.9
Zn_E	15.3	8.8	8.7	32.9	14.3	0.0
pH	3.90	3.92	3.39	3.70	3.81	3.30
Carbon content (%)	17.9	19.4	32.2	47.5	45.3	45.3
Total nitrogen (%)	0.92	1.27	1.11	1.06	1.10	1.10
Extractable ammonium-nitrogen (ppm)	128.3	110.4	62.8	111.8	106.2	164.4
Extractable nitrate-nitrogen (ppm)	14.4	4.3	12.9	7.5	7.4	138.7

ODW = Of dry weight. RDW = related to dry weight

After 24 hours the contents of each pot were mixed throughly and, 48 hours after this, 1(seedlings, which had been germinated 4 weeks previously, were transplanted into each pot Each pot was treated with one element so that its particular effects could be studied. A set o: pots without seedlings was also prepared to follow the changes in extractability of the heav metals through the course of the experiment; samples being taken at 0, 3, 17, 31, 59 and 10(days.

The field moisture capacity of the soil was determined and the moisture level in each pc was kept above one third of this during the course of the experiment. The pots were placed in greenhouse and their positions randomized, both sets being maintained at the same conditior for 100 days.

At the end of the experiment whole plants were extracted carefully from the soil, washe and separated into roots and shoots. The lengths of the main root of each plant and the exte of their branching and mycorrhizal development were recorded. The plants of each treatme were combined and dried at 105°C for 16 hours, weighed to determine the yields, and digest with a 3:1 mixture of nitric and perchloric acids. The drying temperature of 105° C was used a compromise between the loss of certain volatile components and the release of water fro tightly bound forms in the plant tissue which may only be removed by increasing t temperature[1]. The solutions were filtered and the heavy metal and nutrient concentratio determined by flame atomic absorption and flame photometry using appropriate standards a blanks. The soils in which the plants were growing were analysed for total and extractal concentrations of heavy metals by the methods used above.

able 2. Changes in the extractable soil concentrations (ppm ODW) of Cd, Cu and Pb in a peaty oil taken from Tywi forest, Mid Wales

		Extractable concentration (ppm)*				
onc added pm ODW)		Time of sampling after beginning of experiment (days)				
		3	17	31	59	100
admium	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
opper	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
ead	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

DW = Of dry weight
DW = Extracted from fresh soils, but values quoted are related to soils dried at 105°C for 16 urs

esults and discussion

ils

The results of monitoring the extractable heavy metals in the set of ts without seedlings are shown in Table 2. Only at the higher concentions could the changes be followed successfully. For the two highest concentrations (8 and 16 ppm) the extractable levels decreased oughout the experiment, the final concentrations being about 20% he totals. The proportion of extractable to total Cd concentrations then similar to that found at the South Wales sites.

opper was not easily extracted from the soil, probably owing to its ty to form very stable complexes with organic materials and thus only changes in the highest treatments could be followed ssfully[10]. Similar proportions were extractable at the South Wales when comparable total concentrations were determined, only 10

Table 3. Total and extractable concentrations of Cd, Cu and Pb after 100 days growth in a peaty soil taken from Tywi forest, Mid Wales

Concentration added (ppm ODW)		Extractable concentration (ppm RDW)	Total concentration (ppm 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	0.0	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. RDW = related to dry weight

ppm out of total concentrations of several hundred ppm Cu being in an extractable form.

At the highest concentrations of lead, approximately 10% of the amount added remained extractable after 17 days (Table 2). On the other hand, total Pb levels at the South Wales sites varied from 100–340 ppm and, though this was the range covered here, none was extractable (Table 1). This suggests that perhaps the kinetics of the uptake of Pb by the soil are rather slow, and therefore incomplete over a 100 day period. There appears to be a trend of decreasing extractable Pb over a 100 day period, but this is masked at the lower Pb levels because of the inherent imprecision of the determination.

The total and extractable concentrations of Cd, Cu and Pb in the soils in which the plants were grown are presented in Table 3. The extractable levels were similar to those in the set without plants at 100 days (Table 2), indicating that the changes in extractability had occurred at the same rate. The total concentrations of heavy metals also approximately matched the sums of the amounts in the original soil and the extra quantities added, if the addition of the quartz sand is taken into account, though there were some slight anomalies in the Cd treatment (Table 3). Overall, however, there were no indications that heavy metals had been lost from the pots as a result of leaching.

Table 4. Plant yields and tissue concentrations of Cd, Cu and Pb after 100 days growth in a peaty soil taken from Tywi forest, Mid Wales

Concentration added (ppm ODW)		Shoot concentration (ppm ODW)*	Root concentration (ppm 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

*Plant material dried at 105°C for 16 hours, ODW = Of dry weight

Plant yields and elemental concentrations

The yields of the shoots and roots and the corresponding tissue concentrations of heavy metals with which the soils were treated are listed in Table 4. The concentrations of heavy metals and nutrients in deceased plants are quite different from those observed in living plants. Also, one of the principal aims of this investigation was to assess how a build up of heavy metals in living plants can influence their behaviour. Therefore, only plants which survived the treatments of heavy metals over the 100 day period were used in the above determinations. The surviving plants were all of the same seed lot and origin and had been treated to ensure homogeneity among the seedlings[9]. Therefore, it would seem reasonable to be able to make comparisons between surviving plants on a per plant basis in different treatments even though the numbers per pot may be different.

Table 4 shows that Cd and Pb concentrations in both shoots and roots increased steadily with increasing total concentrations in the soil, but

that Cu did not fit this pattern. It was not possible to determine upper critical tissue values for Cd, Cu and Pb in these experiments using the method of Beckett and Davis[3], the most likely reason for this being that the number of treatments was too small for an accurate determination of the position of the split-points, and therefore of the upper critical tissue concentration values.

However, in the Cd experiment, the shoot yields were reduced by increases in the log shoot heavy metal concentration ($r = -0.81$, $p < 0.01$ where $n = 8$[14]). As the Cd critical level has been established as 4.8 ppm Cd for sitka-spruce seedlings[9], and for other plants (an average of the values determined for barley, lettuce, rape and wheat) as 8 ppm[11], this behaviour is to be expected, since almost all the plants except those in the control treatment had accumulated sufficient Cd to exceed the critical level. There was also a significant correlation between the shoot yields and the levels of Pb ($r = -0.63$, $p < 0.05$ where $n = 8$[14]) showing that seedling growth had been affected. However, in the case of copper no such correlation was observed.

The extractable soil levels of the heavy metals present at the end of the experiment (Table 3) were plotted against the shoot and root concentrations of the metals with which they had been treated, and it was found that significant positive correlations existed for Cd (shoots; $r = 0.91$, $p < 0.001$. roots; $r = 0.94$, $p < 0.001$[14]) and Pb (shoots; $r = 0.94$ $p < 0.001$. roots; $r = 0.98$, $p < 0.001$[14]). It was also observed that copper did not affect the yield of the seedlings and there was no apparent correlation between extractable Cu levels and tissue Cu concentrations.

Correlations between the extractable levels of Cd and the shoot Cd concentrations in a range of soils have shown extractants other than acetic acid to give a more accurate assessment of available levels[26], but the correlations obtained for this acidic peaty gley soil tend to suggest that the use of acetic acid adequately reflects the available levels when fairly wide ranges of concentrations were used. However, plant growth was affected when only 0.1 ppm Cd was added to the soil, giving a total concentration of 0.54 ppm (Table 3). There were, in this case significant increases in the Cd content of the plants even though none of the metal was apparently extractable. This therefore suggests that, at these low levels of Cd in this type of soil, the extraction method is not really sensitive enough to detect such small quantities of available metal. This is a function, not only of the extracting solvent, but also of the final method of determining the Cd *i.e.* flame AAS. It was found that the uptake of Cd, Cu and Pb did not have any significant influence upon the shoot and root concentrations of other heavy metals, or of potassium and calcium.

le 5. Statistical analysis of the effects by Cd, Cu and Pb treatments upon root lengths of lings grown in soil from Tywi forest, Mid Wales

centration ed m ODW)		Average root length (cm)	Standard deviation (cm)	't' value from test with control	Number of degrees of freedom
dmium	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
opper	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
ead	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
ontrol		13.07	6.50	–	–
ll Cd treatments		8.16	5.21	3.31**	76
ll Cu treatments		10.02	6.15	1.7	66
ll Pb treatments		8.16	3.75	4.34**	68

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

fects upon roots

Lengths of roots. The average root lengths and their standard viations were calculated for each treatment (Table 5). The average ot lengths of the control group plants were tested against the averages all other treatments and the combined average of root lengths for h metal using students 't' test[12] to detect significant differences ween the groups. The results of these tests are shown in Table 5 ng with the probabilities and number of degrees of freedom for each. s shows that when Cd was added to the soil to give a total concen- ion of 2.5 ppm Cd and an extractable concentration of 0.3 ppm, e were significant reductions in root lengths. The average root th of the combined treatments was also significantly different to of the control group ($p < 0.01$ with 76 degrees of freedom).

ead produced significant reductions in the root lengths at total

Table 6. Effects of the Cd, Cu and Pb treatments upon the number of plants possessing mycorrhizae and branching roots of sitka-spruce seedlings grown in a peaty gley soil from Tywi forest, Mid Wales

Concentration added (ppm ODW)		No. of plants possessing 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

concentrations of 48 ppm and extractable concentrations of 17 ppm and above. There were also significant differences between the average root lengths of the combined treatments and the control group. Copper did not clearly affect root lengths at any particular level, not even when the average lengths of the plants roots for the Cu treatments were tested against the control group. The differences between the lengths of roots in the Cd and Pb treatments and those of the control group were probably due to the extractability of these metals, which was high throughout the experiment. However, the extractable and total concentrations of Cd were similar to the South Wales forestry sites and this effect may therefore have some consequences for tree growth.

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 Table 6. It was found that the only group that possessed any true mycorrhizae was the control group and that all other root branching was associated with pseudo-mycorrhizae.

Four of the nineteen control plants had the true mycorrhizal characters of regular dichotamously branching roots, swollen with shiny smooth surfaces and whitish tips. It is well known that symbiotic mycorrhizal associations are necessary for nutrient uptake in coniferous trees[15] and as their numbers were reduced by Cd, Cu and Pb, the nutrient balances of the plants may have been disturbed.

All other roots in this group and other treatments had dark irregular branching roots with no beading or swelling. All the roots of the control group had branching roots whereas in the other groups many plants did not (Table 6), and it seems that the heavy metals were the most likely cause of this.

Conclusions

The shoot yields of the seedlings were affected by additions of Cd to the soil, though not by the Cu treatments, nor by all but the highest levels of Pb. There were also many effects upon the roots again by Cd and Pb, but not by Cu, including decreases in root lengths and branching. The number of mycorrhizae associations with the roots, on the other hand, were reduced by the treatments of all the metals.

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