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# Growth alterations in Scots pine seedlings grown in metal-polluted forest soil: implications for restorative forest management

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## Summary

Heavy metals originating from smelting activity have deteriorated forest ecosystems in many places. The threat to forests does not cease even when the smelting activity ends, due to the pool of metal cations absorbed into soils. When reforestation is planned in such areas, it is vital to know if seedlings survive in those conditions. We investigated the effects of nickel and copper added to soil on the growth of Scots pine seedlings. Root growth showed the strongest response, Ni being more harmful than Cu. The reduction in the green biomass produced during the experiment was nearly the same as that in root biomass, Cu being more harmful. Copper toxicity in the aboveground parts is not direct, however, since Cu is not transported to the foliage or stem in toxic amounts. The results show that afforestation may be possible even in areas polluted by smelting activity for decades.

## 1. Introduction

Of the areas burdened by anthropogenic pollution, those around metal smelters are among the most complex due to the deposition of metals particles and sulphur in different forms. Typically, the impacts of emissions from such facilities are limited to the immediate vicinity. There are places, however, where extensive, long-term metal smelting activities have caused forest death and decline in forest productivity covering several thousands of hectares. Among the most intensively studied areas are those around the smelters in Sudbury in Canada (HUTCHINSON and WHITBY, 1974) and on the Kola Peninsula in northwest Russia (TIKKANEN and NIEMELÄ, 1995; NÖJD and REAMS, 1996; RAUTIO and HUTTUNEN, 2003).

For instance, in the Monchegorsk area (on Kola, Russia), the smelting activity has lasted for over half a century, emitting vast amounts of $SO_2$ and metals (mainly Cu and Ni). Sulphur and metals depositing on vegetation have not only direct effects, such as acute $SO_2$-caused leaf damage, but also indirect effects via contaminated soil. Especially metals in areas where deposition has lasted for decades and metal supplementation in experimental studies have been found to deteriorate soils by replacing nutrients, damaging mycorrhizae, slowing down litter decomposition, etc. (DIXON and BUSCHENA, 1988; BÅÅTH, 1989; DEROME and LINDROOS, 1998; FRITZE et al., 1996; TIKKANEN and NIEMELÄ, 1995).

In this study, we simulated a situation where emissions have been successfully cut down and the only threat to forest health comes from the metal-contaminated soil. Furthermore, we imitated the common afforestation method where the soil is ploughed and tree seedlings are planted in mineral soil. In such conditions, we grew Scots pine seedlings in copper and nickel treated soil in factorial experiment for one growing season and measured the alterations in seedling growth. We expected Ni to have more deleterious effect on aboveground parts, due to restricted transport of Cu to shoots (STONE and TIMMER, 1975; HEALE and ORMROD, 1982; RAUTIO et al. 2004), but in roots the role and the strength of the impact of these metals have been contradictory in many of the previous studies.

## 2. Material and methods

### 2.1. Experimental design

A factorial experiment was set up on an experimental field of the Botanical Gardens of the University of Oulu during the summer (early June - late September) 1997. Four-year-old nursery-grown bare-rooted mycorrhizal Scots pine (*Pinus sylvestris* L.) seedlings were grown in mineral forest soil treated with elevated levels of copper and nickel. Soil originated from dry heath forest (*Vaccinium* site type), pH 4.2 (0.01M $CaCl_2$-extract), LOI (loss of ignition) % 1.2, Cu 1.8 and Ni 0.0 mg/kg dry soil (ammonium acetate + EDTA, buffered to pH 4.65, extractable). Cu and Ni (as sulphates: $CuSO_4 x 5H_2O$ and $NiSO_4 x 6H_2O$) were dissolved into water and mixed in the soil with cement mixer before the seedlings were planted. The roots of the seedlings were rinsed with water before planting in order to remove attached organic soil. The initial biomass (total fresh mass) of the seedlings was measured before planting. The seedlings were grown in pots containing 0.75 litre of mineral forest soil. Cu and Ni were added to the soil, both in four concentrations. The amounts of Cu added were 0, 25, 40 and 50 mg in kg of dry soil and the respective Ni quantities were 0, 5, 15 and 25 mg $kg^{-1}$. All doses of one element were administered together with all doses of the other, resulting in a 4x4 factorial experiment. Soil concentrations of Cu and Ni (ammonium acetate + EDTA-extractable fraction) in the end of the experiment are reported in the Tab. 1 (see RAUTIO, 2000; RAUTIO et al., 2004 for details in soil analyses). 20 seedlings were grown in each treatment combination and of these ten seedlings per treatment were randomly selected for this study, resulting in a total of 160 seedlings. The remaining 10 seedlings were used to analyse possible stress indications (morphometric and gluthathione levels) in needle cells (KUKKOLA et al., 2000), element concentrations in roots and aboveground parts (RAUTIO et al., 2004) and levels of phenolic compounds and photosynthates (ROITTO et al., 2005). The seedlings were grown outside and watered (with tap water) only in case of long (several days) dry periods.

### 2.2. Measured parameters

We terminated the experiment at the turn of September-October 1997, when growth was ceased. At this time the mean daily temperature was around 5 °C and night frosts had begun (the thermal growth season ended on 4 Oct. 1997 according to the Finnish Meteorological Institute (1997a, b)). The seedlings were pulled up with roots and the roots were washed with water. The seedlings were cut into the following parts: root, current year's needles (1997, i.e. C-needles), previous year's needles (1996, i.e. C+1-needles) and wood (= all aboveground woody material). Some of the seedlings still had some two-year-old needles (from summer 1995) left, but since most of the seedlings had already shed these needles and only few senescent remained, these needles were ignored in the subsequent computations.

Apical shoot growth was measured from the main stem at the end of the experiment as a measure of the growth in length of the current year's (1997) annual shoot. The total dry masses (measured after at

**Tab. 1:** Soil concentrations of Cu and Ni (mg/kg dry wt) in the end of the experiment in the different Cu and Ni addition levels (mg/kg dry soil) according to RAUTIO et al. (2004).

| Addition | | Concentrations | |
|---|---|---|---|
| Cu | Ni | Cu | Ni |
| 0 | 0 | 2.9 | 0.0 |
| 0 | 5 | 1.8 | 4.4 |
| 0 | 15 | 0.8 | 12.5 |
| 0 | 25 | 1.8 | 23.2 |
| 25 | 0 | 16.4 | 0.3 |
| 25 | 5 | 15.9 | 1.9 |
| 25 | 15 | 14.8 | 8.6 |
| 25 | 25 | 16.4 | 16.3 |
| 40 | 0 | 25.1 | 0.0 |
| 40 | 5 | 25.7 | 3.0 |
| 40 | 15 | 25.7 | 9.2 |
| 40 | 25 | 23.5 | 14.5 |
| 50 | 0 | 26.8 | 0.0 |
| 50 | 5 | 32.6 | 2.9 |
| 50 | 15 | 28.1 | 6.4 |
| 50 | 25 | 30.1 | 12.8 |

least two days at 60 °C) of all current (C-needles) and previous year's needles (C+1-needles), shoots (aboveground woody part) and roots were recorded. The root/shoot ratio was computed as the mass of roots divided by the sum of the mass of all needles (formed during the summers 1996 and 1997) and the aboveground wood. The average mass of current needles (needle pairs) was evaluated from a sample of ten needle pairs from each seedling, and the total number of current needle pairs was estimated by dividing the total mass of current needles by the average mass of current needles (needle pairs). The proportion of the total mass of current needles out of the total aboveground biomass was computed as a percentage.

### 2.3. Data analysis

The data was analysed by means of two-way factorial ANOVA with the Cu and Ni levels as fixed factors. Initial biomass ($\log_{10}$ transformed) was used as a covariate. In the cases of proportional variable (root / shoot ratio and the percentage of current needle mass out of the aboveground biomass), the variation explained by the initial biomass was not removed from the variables because the initial biomass did not explain (significantly) the variation in these proportional variables.

Instead of a "normal" multiple comparison procedure (16 treatments would result in 120 pairwise comparisons), polynomial contrasts were applied to the data (CHEW, 1976; STEEL et al., 1997). This procedure offers (at least) two advantages over a traditional multiple comparisons procedure. First, polynomial contrasts give more detailed information of the effects and interrelationships among the factors used in the experiments (BAKER, 1980). Secondly, in professor Chew's (1976) words: "In comparing the effects of, say, 10 ... and 40 ppm (mg $kg^{-1}$) of a certain chemical, if the linear and/or quadratic regression of response on concentration is significant, then no multiple comparison procedure is necessary. All concentrations are significantly different in their effects."

Since there are four treatment levels (= 3 df) in both factors, up to a third-degree polynomial (linear [straight line: $y=a+bx$], quadratic [U-shape: $y=a+bx+cx^2$] and cubic [S-shape: $y=a+bx+cx^2+dx^3$]) term could be fitted to both factors. In the case of no interaction between the main effects (Cu and Ni levels), polynomial contrasts were applied to both factors separately (Tab. 2). In the case of significant interaction – indicating, for example, that the effect of the Cu levels depend on the level of Ni – polynomial contrasts were obtained separately for Cu at each Ni level and vice versa (Tab. 3) (cf. CHEW, 1976; BAKER, 1980; STEEL et al., 1997). Only the contrast (linear, quadratic or cubic) that explained best (had the highest sum of squares) the trend is reported for each case in Tab. 2 and 3.

## 3. Results

### 3.1. Root biomass production

Of the variables measured here, root mass showed the strongest response to added metals (Fig. 1 and Tab. 3). In the most high-dose treatments root mass was only about half of that in the control (Cu 0 / Ni 0 mg $kg^{-1}$) treatment (Fig. 1). Ni seemed to have a stronger impact than Cu, though the effect of Ni varied somewhat at the different Cu levels, the linear trend being the most significant (Tab. 3). Cu levels had a significant effect on the roots only at a higher Ni levels: at Ni addition 15 mg $kg^{-1}$ Cu caused a curvilinear response, whereas at Ni 25 the response was linear (Tab. 3). The role of supplemental Ni in root growth was also clearly visible in the root/shoot ratio. The response to supplemental Cu was much weaker (Fig. 2 and Tab. 3).

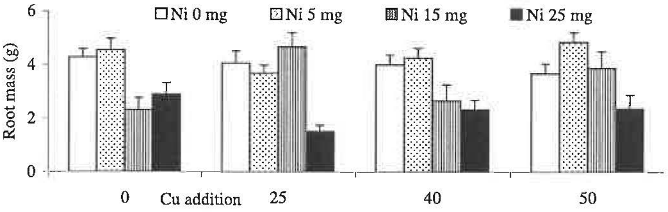

**Fig. 1:** Total dry mass of roots (grams, mean + 1 S.E.). The Cu supplementation levels (0, 25, 40 and 50 mg/kg dry soil) are shown on the x-axis and the Ni supplementation levels (0, 5, 15 and 25 mg/kg dry soil) are denoted by legends.

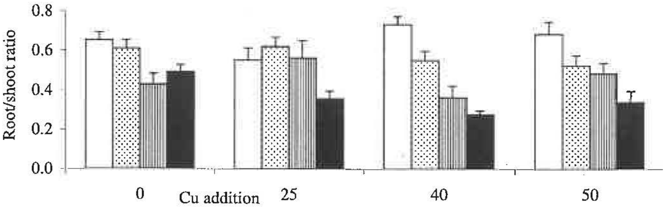

**Fig. 2:** Root / shoot ratio. See Fig 1. for legends.

### 3.2. Aboveground biomass production

The length of the current summer's (1997) apical shoots at the end of the experiment was in the most severe cases more 25% lower than in control seedlings (Fig 3 and Tab. 2). This effect was mainly due to Cu since Ni did not have any statistically significant effect on shoot growth. In contrast to the current apical shoot, the total green biomass produced during the experiment (C-needles) was affected by both Cu and Ni addition. In the case of Cu, the linear trend explained most of the variation (Tab. 2), whereas in the case of Ni, low levels seemed to somewhat enhance the biomass production, while higher supplemental doses reduced it (Fig. 4, Tab. 2).

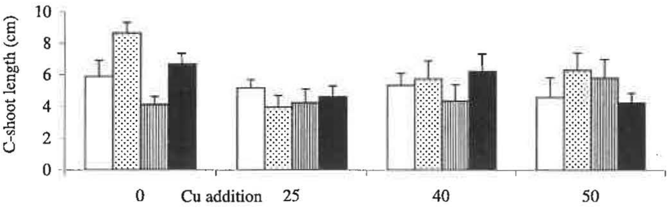

**Fig. 3:** Apical shoot length (current summer's growth, cm) at the end of the experiment. See Fig 1. for legends.

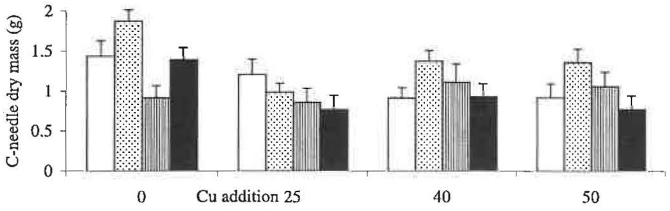

**Fig. 4:** Total dry mass (grams) of all current-year needles formed during the experiment. See Fig 1. for legends.

Both the current shoots' length and the current needles' biomass were affected by the supplementation levels of Cu and Ni more or less independently of the other element; no significant interaction was observed (Tab. 2). In the other measured growth parameters, the supplementation levels of one metal (Cu or Ni) clearly influenced the kind of effect the other metal had on the measured parameter. The number of needle pairs formed during the experiment responded to Cu added together with Ni. The effect of metal additions on the number of formed needles was not, however, unambiguous but varied depending on the combination. For example, Cu addition alone had no effect but Ni added alone resulted in a cubic response: the number of formed needles was generally highest at low and high Ni addition levels (Fig. 5, Tab. 3). Neither of the added metals when added alone affected the weight of individual needles formed during the experiment (i.e. C-needles). Moderate additions seemed to even increase the growth slightly, shown as quadratic trends (Fig. 6, Tab. 3). The amount of green biomass formed during the experiment per total aboveground biomass was affected mainly negatively by Cu but low Ni levels seemed even to enhance this ratio (Fig. 7, Tab. 3).

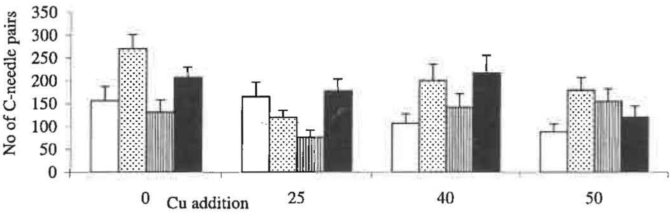

**Fig. 5:** Estimated number of current-year needle pairs formed during the experiment. See Fig 1. for legends.

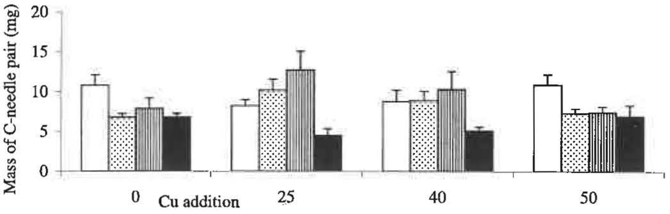

**Fig. 6:** Average mass of current-year needle pairs (milligrams). See Fig 1. for legends.

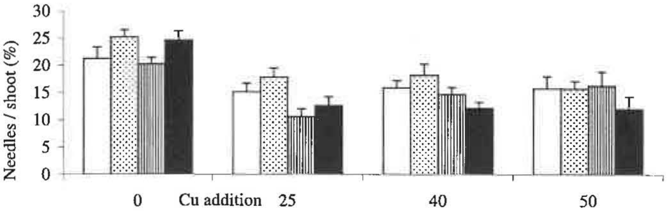

**Fig. 7:** Proportion (%) of the mass of needles formed during the experiment out of the total aboveground mass. See Fig 1. for legends.

## 4. Discussion

### 4.1. Root biomass production

Ni was much more deleterious for root growth than Cu. In consistency with the present data Hale et al. (1985) have previously reported Ni to be more injurious than Cu (in tomato), especially for root growth. Wong and Bradshaw (1982), on the other hand, found Cu to be more toxic for root growth than Ni (in *Lolium perenne*). Here, copper administered alone did not have a significant effect on root growth even at the highest level of supplementation (50 mg kg$^{-1}$), whereas Ni supplementation alone clearly decreased root mass. The above trend can also be seen in the root/shoot ratio, though the linear decrease of root biomass compared to aboveground biomass (=decrease in the root/shoot ratio) was stronger when both elements were present in high concentrations: the decrease in the root/shoot ratio in relation to the Ni addition levels was most marked at the two highest Cu levels, and Cu caused a linear decrease in the root/shoot ratio only at the highest Ni level. Also Hale et al. (1985) observed decrease in root biomass in relation to shoot biomass in Ni-treated tomatoes, but in their study this trend was no longer visible when the Cu dose was increased. Burton et al. (1986), then again, did not find any effect of Ni addition either alone or in combination with Cu (or Cd) on the root or shoot growth of Sitka spruce. In both of the above studies (Hale et al., 1985; Burton et al., 1986), the Ni and Cu doses of supplementation - given in nutrient solutions - were quite low compared to the doses used here. Lozano and Morrison (1982), on the other hand, found the root mass of white spruce to decrease in relation to Cu supplementation, and this decrease was larger when 10 mg kg$^{-1}$ of Cu was added together with 10 mg kg$^{-1}$ of Ni (in nutrient solution) compared to treatment with 10 mg kg$^{-1}$ of Cu and 1 mg kg$^{-1}$ of Ni.

The mechanisms behind the decreased root growth might be diverse. Heale and Ormrod (1982), for example, noticed lateral roots to be stunted and thickened due to Ni and Cu administration – alone or in combination. We noticed overall browning of roots in some metal treatments: in Cu50Ni25-treatment, for example, no light fine root tips were observed, whereas over 75% of the root tips in the controls were healthy (light) (Kukkola et al., 2000). Cu has been found to inhibit cell elongation in roots (Barcelo and Poschen-

**Tab. 2:** Results of the analysis of variance (SS = sum of squares, df = degrees of freedom, F-values and $r^2$ = coefficient for determination of the whole model) of the total dry mass (DM) of C-needles, apical shoot length and percentage of the total dry mass of C-needles out of the total aboveground dry mass. Significant terms have been partitioned further into linear, quadratic and cubic terms (see Material and methods for details). Significance symbols after F-values: *** = $p<0.001$, ** = $p<0.01$, * = $p<0.05$, ° = $0.1 < p > 0.05$, ns = $p>0.1$.

| | | DM of C-needles | | Apical shoot length | | DM of C-needles / shoot | |
|---|---|---|---|---|---|---|---|
| Source | df | SS | F | SS | F | SS | F |
| Initial mass (log) | 1 | 14.31 | 79.1*** | 45.66 | 5.7* | - | - |
| Cu level | 3 | 9.36 | 17.2*** | 89.34 | 3.7* | 1988.35 | 21.82*** |
| | 1 | | Linear | | Linear | | Linear |
| Ni level | 3 | 3.32 | 6.1*** | 38.49 | 1.6ns | 395.58 | 4.34** |
| | 1 | | Cubic | | | | Cubic |
| Cu x Ni | 9 | 1.78 | 1.1ns | 97.23 | 1.4ns | 377.87 | 1.38ns |
| Error | 143 | 25.87 | | 1143.4 | | 4373.95[#] | |
| $r^2$ | | 0.54 | | 0.19 | | 0.39 | |

[#] Error df = 144

**Tab. 3:** Results of the analysis of variance of the total root dry mass, total (estimated) number of needle pairs, average mass of needle pair and root/shoot ratio. Significant terms have been further partitioned into linear, quadratic and cubic terms (see Material and methods for details). See Tab. 2 for symbols.

| | | Root mass | | No. of needle pairs | | Needle pair mass | | Root / shoot ratio | |
|---|---|---|---|---|---|---|---|---|---|
| Source | df | SS | F | SS | F | SS | F | SS | F |
| Initial mass (log) | 1 | 224.2 | 386.6*** | 122697 | 19.1*** | 185.0 | 12.3*** | - | - |
| Cu level | 3 | 18.0 | 10.3*** | 144348 | 7.5*** | 26.4 | 0.6ns | 0.09 | 1.1ns |
| Ni level | 3 | 76.4 | 43.9*** | 123856 | 6.4*** | 368.2 | 8.1*** | 1.94 | 23.95*** |
| Cu x Ni | 9 | 17.7 | 3.4*** | 140232 | 2.4* | 294.5 | 2.2* | 0.61 | 2.5* |
| Cu in Ni 0 ppm | 3 | 2.7 | 1.6ns | 30702 | 1.6ns | 59.2 | 1.3ns | 0.18 | 2.2° |
| | 1 | | | | | | | | Cubic |
| Cu in Ni 5 ppm | 3 | 2.7 | 1.6ns | 123307 | 6.4*** | 79.2 | 1.7ns | 0.06 | 0.7ns |
| | 1 | | | | Linear | | | | |
| Cu in Ni 15 ppm | 3 | 12.3 | 7.1*** | 58883 | 3.1* | 140 | 3.1* | 0.21 | 2.6° |
| | 1 | | Cubic | | Guadratic | | Guadratic | | Cubic |
| Cu in Ni 25 ppm | 3 | 17.9 | 10.3*** | 71687 | 3.7* | 42.6 | 0.9ns | 0.25 | 3.1* |
| | 1 | | Linear | | Linear | | | | Linear |
| Ni in Cu 0 ppm | 3 | 7.0 | 4.0** | 83948 | 4.4** | 95.7 | 2.1ns | 0.32 | 4.0** |
| | 1 | | Linear | | Cubic | | | | Linear |
| Ni in Cu 25 ppm | 3 | 13.5 | 7.8*** | 102806 | 5.3** | 247.2 | 5.5** | 0.39 | 4.8** |
| | 1 | | Linear | | Guadratic | | Guadratic | | Linear |
| Ni in Cu 40 ppm | 3 | 43.2 | 24.8*** | 53544 | 2.8* | 172.8 | 3.8* | 1.23 | 15.2*** |
| | 1 | | Linear | | Cubic | | Linear | | Linear |
| Ni in Cu 50 ppm | 3 | 30.3 | 17.4*** | 23790 | 1.2ns | 147.1 | 3.2* | 0.6 | 7.4*** |
| | 1 | | Linear | | | | Linear | | Linear |
| Error | 143 | 82.6 | | 918460 | | 2157.9 | | 3.9[#] | |
| $r^2$ | | 0.81 | | 0.37 | | 0.29 | | 0.40 | |

[#] Error df = 144

RIEDER, 1990; ARDUINI et al., 1995), but the actual mechanisms of Cu- or Ni-induced inhibition of root growth are poorly known processes (KAHLE, 1993). DONCHEVA (1998) suggested that Cu caused decreased root growth by affecting root meristem cell proliferation. Ultrastructural investigation (of maize roots) by DONCHEVA (1998) revealed disturbances in plasmalemma, endoplasmic reticulum and mitochondrial membranes. In the case of Ni, L'Huillier et al. (1996) concluded that the Ni-induced growth reduction in maize was mainly due to depressed mitotic activity in root meristem. In addition, heavy metals have been shown to cause oxidative stress in plants, which, in turn, triggers defence reactions, such as mobilization of enzymatic and non-enzymatic antioxidant reserves (e.g. peroxidase and glutathione) (CLIJSTERS et al., 1999; GUPTA et al., 1999). However, studies on peroxidase activity, for example, have yielded contradictory results. We did not observe significantly different peroxidase activities in the roots of Ni- or Cu-treated vs. control seedlings (KUKKOLA et al., 2000). TARVAINEN et al. (1991), in turn, noticed elevated peroxidase activity due to nickel treatment in laboratory. ROITTO et al. (1998) sprayed Scots pines with low concentrations of Cu and Ni (with or without simulated acid rain) and observed no significant differences in peroxidase activities due to the metal (or acid rain) treatments in roots or in current-year needles, whereas PANDOLFINI et al. (1992) detected increased peroxidase activity in shoots and roots of wheat (*Triticum aestivum* L.) grown in perlite containing elevated levels of Ni.

### 4.2. Aboveground biomass production

Even though Cu seemed to have an effect on the aboveground biomass produced during the experiment, this was not due to the effect of Cu *in situ*, since only a small proportion of the added Cu was transported to needles or shoots (RAUTIO, 2000; RAUTIO et al., 2004). This is in consistent with HEALE and ORMROD (1982), who noticed that the Cu levels of *Pinus resinosa* needles were unaffected by the Cu levels in the nutrient solutions given (see also MARSCHNER, 1995). Stone and TIMMER (1975) reported that, in eastern hemlock (*Tsuga canadensis*), root Cu increased linearly with the extractable soil content, whereas foliar Cu was unaffected. Here (RAUTIO et al., 2004), Cu concentrations were below 5 mg kg$^{-1}$ in C-needles and around 13 mg kg$^{-1}$ in shoots in the most high-dose Cu treatment (Cu 50 / Ni 0 mg kg$^{-1}$ soil). The critical toxicity level of copper in leaves is above 20-30 mg kg$^{-1}$ in dry matter, but since the transport to shoots and leaves is highly restricted, the use of concentrations in aboveground tissues as indicators of Cu toxicity has been questioned (STONE and TIMMER, 1975; MARSCHNER, 1995). In addition to the low Cu levels, we did not find indications of Cu stress in needles; no significant differences in morphometric measurements or (total or oxidised) glutathione levels were observed in comparison to the controls (KUKKOLA et al., 2000).

Ni has much easier access to the aboveground parts than Cu. Here, the Ni concentrations in current needles at the highest Ni supplementation level (25 mg kg$^{-1}$) rose to over 30 mg kg$^{-1}$, which is sixfold compared to the foliar Cu concentration at the highest Cu dose (less than 5 mg kg$^{-1}$ in the 50 mg kg$^{-1}$ addition level) (RAUTIO et al., 2004). Regardless of this, Ni did not have an effect on shoot length, and the total green biomass produced during the experiment was affected much more by the Cu addition. Accordingly GABBRIELLI et al. (1999) reported the shoot length of pea (*Pisum sativum*) to be unaffected by Ni toxicity, even though biomass production in shoots (and roots) was affected.

The length of the current apical shoot and the number of current summer's needle pairs are affected mostly by factors (mainly temperature) prevailing during the previous growing season, when the terminal buds containing the needle and shoot primordia are formed. Still, such factors as toxic levels of metals may have an effect on how many of the needle primordia develop to fully-grown needles and also on the developing shoot meristems. Nevertheless, a better indicator of growth retardation is the total mass of needles formed during the experiment, but even here the total number of needles naturally affects the total mass of all needles. Consequently, we assume the average mass of needles (here needle pairs) to be the most reliable parameter – of those used here – to describe the possible toxic symptoms caused by metals to the green biomass formed during the experiment. Ni explained most of the variation in the average mass of needles, more than the CuxNi interaction, which was also significant. Even when the length of the stem was only slightly affected by the metal treatments here, growth reductions could be expected in the future growing periods. This is because the most active photosynthetic biomass (current needles) was reduced and the new shoot growth during the next growing season is largely dependent on the assimilates supplied by 1-year-old (previous year's) needles (FISCHER and HÖLL, 1991). Because 2- and 3-year-old needles also have an important role in supplying assimilates to the growing shoot, it is probable that a continuous metal stress would have a more pronounced effect on the (total) assimilating biomass - and consequently on shoot growth - than was seen here.

Both Cu and Ni are considered essential micronutrients for plants (MARSCHNER, 1995), which may explain why both Cu and Ni seem to enhance current needles' mass when administered in low concentrations together. The decrease in the average needle mass at higher metal supplementation levels is presumably due to various reasons. One symptom commonly noticed in connection with metal toxicity is the failure of the photosynthetic apparatus. The physiological mechanisms behind the inhibitory effect that heavy metals have on photosynthesis are, however, complex (CLIJSTERS et al., 1999). Chlorophyll content, for example, has been noticed to be reduced by Cu (GALLEGO et al., 1996; CISCATO et al., 1997). The results of L'HUILLIER et al. (1996) with Ni on maize, in turn, support the hypothesis by GREGER et al. (1991), who suggested that heavy metals do not affect photosynthesis so much *per se*, but rather reduce carbohydrate transport, which indirectly also affects roots. These observations get support from the present experiment since ROITTO et al. (2005) observed, using material from this experiment, that Ni increased sucrose concentration in the needles, indicating disturbances in carbohydrate transport. Indirect effects may come via roots as well. Metal damage to roots may result in moisture stress (e.g. PATTERSON and OLSON, 1983), which has an effect on, for example, photosynthesis. The seedlings with the most high-dose treatment (Ni25 Cu50) were also noticed to have damage in the photosynthetic machinery, including light-coloured thylakoids. Swollen thylakoids and granulated stroma were seen in the present material in all treatments (KUKKOLA et al., 2000). These symptoms resemble those detected in Scots pine needles around copper-nickel smelters on the Kola Peninsula (KUKKOLA et al., 1997).

## 5. Conclusions

Our results show that, in a situation where curtailment of sulphur and metal emissions has been successfully accomplished, afforestation may be possible in metal-polluted areas exposed to heavy metal deposition for decades, such as those around the Monchegorsk smelter complex. In this study, we used Cu and Ni supplementation levels that exceeded, when at their highest concentrations, those detected in the mineral soil in the environs of the Monchegorsk smelter complex (RAUTIO and HUTTUNEN, 2003). Still, the seedlings used in this experiment survived in all treatment categories, though damage (KUKKOLA et al., 2000) and growth reduction were evident in the most high-dose treatments. In the organic layer in the vicinity of Monchegorsk the Cu and Ni levels are much higher than those used in this study, but since their concentrations in the mineral layer have remained rather low (RAUTIO and HUTTUNEN, 2003), indicating strong absorption in organic material, the metal pool in the organic layer is not likely to pose an acute threat to seedling survival. Here, we followed the growth of the seedlings for only one growing season, and it is likely that growth retardation and mortality would have been greater over a longer period due to, for instance, frost damage. Nevertheless, the highest metal levels used here exceeded the metal levels detected, for example, in the surroundings of Monchegorsk to that extent that we assume afforestation to be possible even in quite seriously polluted forest damage areas, presuming that the direct threat by atmospheric deposition of sulphur and heavy metals is drastically reduced.

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