The Effects of Heavy Metals on the Germination and Radicle Growth of Some Forest Plants of Northern Minnesota

A report for the

Minnesota Environmental Quality Board

Regional Coppper-Nickel Study

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Abstract

The effects of copper, nickel, and cobalt on the germination and radicle growth of *Betula papyrifera*, *Lonicera tatarica*, *Picea glauca*, *P. mariana*, *Pinus banksiana*, *P. resinosa*, and *P. strobus* were studied under laboratory conditions. Seedlings were grown on filter paper, mineral soil, organic soil, and tailings. Reduction in radicle length was used as an index of heavy metal toxicity.

There were no effects on the germination of any species over the range of concentrations tested. There was an inverse relationship between radicle length and metal concentration. The seedlings grown on tailings did not develop symptoms of copper, nickel, or cobalt toxicity.

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Introduction

Background

The Ely-Hoyt Lakes region of the Duluth Complex in northern Minnesota has been shown to contain potentially recoverable quantities of copper and nickel sulfide ores (Bonnichsen 1974). The Minnesota Environmental Quality Board's Regional Copper-Nickel Study is a multifaceted program designed to assess the potential impacts of mining and smelting on the natural and social environments of the area.

Several investigations have demonstrated the serious impacts of acid rain and heavy metal pollution in mining and smelting regions (see Jordan 1975, Whitby & Hutchinson 1974, and Whitby et al. 1976 for reviews of pertinent studies). These ecological impacts include damage to existing vegetation, changes in soils composition, inhibition of revegetation of denuded areas, and disruption of aquatic ecosystems. The inhibition of revegetation has been shown to persist for up to 50 years after the cessation of smelting (Thomas 1965).

Whitby (1974) used a bio-assay technique to evaluate the impacts of heavy metal contamination of soils around the mining and smelting works at Sudbury, Ontario. This study uses a modification of that technique to determine some of the impacts of heavy metal contamination of northern Minnesota soils.

Objectives

The primary objectives of this study were:

1) to study the effects of known concentrations of heavy metals on the germination and radicle growth of plants native to the area;

2) to compare radicle growth of seedlings on mineral and organic soils containing various amounts of heavy metals;

3) to rank the plants according to their sensitivity to each metal;

4) to determine if seedlings germinated on tailings developed symptoms of heavy metal toxicity; and

5) to determine the degree of heavy metal binding in two northern Minnesota soils. The methods used to achieve these objectives included a survey of the literature, testing the germination of seeds, measuring radicle growth of seedlings, and analyzing soil extracts for heavy metal content.

The results of this study should help assess some of the impacts of heavy metal contamination of forest ecosystems and aid in the selection of species for revegetation of disturbed areas.

Materials and Methods

Literature review

The literature review gathered existing information on the effects of various heavy metals on plants, the impacts of heavy metal contamination on forest ecosystems, and the procedures for determining the effects of heavy metals on the germination and radicle elongation of seedlings. The literature review was not exhaustive. Relevant publications are listed in the bibliography.

Seeds

Table 1 lists the plant species that were tested for sensitivity to heavy metals. With the exception of paper birch, all seeds were from sources in northern Minnesota. The white pine seeds had been treated with a bird and rodent repellant (Arasan 42-S). All other seeds were untreated. Arasan 42-S is Dupont's brand of thiram. Some studies (Demerrit & Hocker 1970, Dobbs 1971) report a reduction in germination as a result of thiram seed treatments. The treatment apparently did not affect germination in this study. The seeds were stored at approximately 4° C until used in the various germination tests.

Sample germination tests were conducted with each species. Green ash, green alder, and speckled alder had low germination levels.
Cold soaking did not improve germination. There was not enough time to use conventional stratification techniques so these species were not used in the radicle growth tests.

Soils

One of the goals of this research was to compare the effects of heavy metal contamination on mineral and organic soils. Both soil types occur in the Study Area. Table 2 compares the naturally occurring copper, nickel, and cobalt content of the soils used in this research with average values for other soils.

TABLE 1. Data on Seeds Used in Heavy Metal Experiments

Common name	Scientific name[1]	Year collected	Collection location
Green alder	*Alnus crispa* (Ait.) Pursh	1977	MN DNR Region II
Speckled alder	*Alnus rugosa* (Du Roi) Spreng.	1977	MN DNR Region II
Paper birch	*Betula papyrifera* Marsh	?	New York
Green ash	*Fraxinus pennsylvanica* Marsh	1974	MN DNR Region II
Honeysuckle	*Lonicera tatarica* L.	1975	MN DNR Region I
White spruce	*Picea glauca* (Moench) Voss	1975	MN DNR Region II
Black spruce	*Picea mariana* (Mill) BSP	1976	MN DNR Region II
Jack pine	*Pinus banksiana* Lamb.	1976	MN DNR Region II
Red pine	*Pinus resinosa* Ait	1975	MN DNR Region II
White pine	*Pinus strobus* L.	1975	MN DNR Region II

[1]The botanical nomenclature in this report follows that of Fernald (1950).

Table 2. Copper, nickel, and cobalt content of soils.

	Copper	Nickel	Cobalt
Normal Range (ppm)	2-100[a]	10-1,000[a]	1-40[a]
Mean value for oven dry soils (ppm)	20[a]	40[a]	8[a]
Mesaba Series - mineral 0-5 cm. depth (ppm)	21.2[b] / 3.7	31.7[b] / 3.2	8.0[b,c] / —
Moose Lake Series - organic 0-5 cm. depth (ppm)	12.5[b] / 1.1	9.0[b] / 1.0	3.2[b] / 1.0

[a]From Bowen (1966).

[b]Mean / Std. deviation. Data from Copper Nickel Study analyses.

[c]One sample - no standard deviation.

The organic soil was collected from the NE¼, NE¼, Sec. 31, T. 61 N.- R. 10 W. It is a Moose Lake series Typic Borohemist.

The mineral soil belongs to the Mesaba series and was collected from the NW¼, Sec. 24, T. 61 N. - R. 12 W. It is a Typic Dystrochrept (coarse loamy mixed).

Bulk soil samples were collected from the upper 10 cm. of the soil profiles (after removal of the L, F, and H horizons). The samples were transported to the lab in plastic bags for further treatment. In the lab the organic soil samples were spread on trays to air dry. Soils were used in air dry rather than standard oven dry form because the heat used in oven drying may permanently alter the heavy metal binding capacity of soils (Hesse 1971). When the soil was dry,the larger roots and fibrous materials were removed manually. The remainder was ground in a Wiley mill without a screen. This produced a somewhat homogenous mixture suitable for use in the germination and extract experiments. The mineral soil samples were combined and air dried. The soil was then sieved using a 2 mm mesh plastic screen to remove roots and pebbles.

Metals

Copper, nickel, and cobalt were used in the germination and radicle growth experiments because they commonly occur in sulfide ores (Bonnichsen 1974), and they are toxic to plants at relatively low concentrations (Bowen 1966). The heavy metals were added to the soils as ions in aqueous solution. Reagent grade sulfate or nitrate salts ($CuSO_4 \cdot 5H_2O$, $Ni(NO_3)_2 \cdot 6H_2O$, and

$Co(NO_3)_2 \cdot 6H_2O$) were used to prepare stock solutions of 1,000, 2,000, and 5,000 ppm. All the metal solutions were slightly acidic (pH 5 to 6).

Tailings

One potential problem associated with copper-nickel development in Minnesota will be tailings disposal. Observations of seedlings germinated on tailings were made to see if signs of heavy metal toxicity developed. The rate of radicle growth on tailings was compared to that of seedlings grown on mineral soil.

Thirteen different tailings samples were tested. They were differentiated on the basis of particle size, extraction process, and origin of the ore sample from which they were prepared. Appendix table A-1 lists the identifying characteristics of each sample.

Laboratory materials and procedures

Several precautions were required to avoid heavy metal contamination of soils and solutions. All glassware and lab utensils were washed with detergent, rinsed with 0.5 N hydrochloric acid, and final rinsed with deionized distilled water. Disposable polystyrene petri dishes and acid washed (Whatman # 541) filter paper were used in the germination and growth tests. Solutions were prepared with deionized distilled water.

Adsorption of the heavy metals on glass, plastic and paper surfaces presented some problems in determining actual (available) heavy metal concentrations. Analyses of stock solutions and extracts from filter paper saturated with heavy metal solutions were performed to determine the magnitude of the adsorption problem.

Greenhouse conditions

All seeds tested for germination and radicle growth were placed on saturated filter paper in petri dishes. Saturated conditions were maintained by adding deionized distilled water as necessary. The petri dishes were kept on greenhouse benches until germination appeared to be complete or until the seedlings had grown too large for the dishes.

Standard germination test conditions require adequate moisture, aeration, and a light/temperature regime of eight hours of light at 30^{o} C (86^{o} F) and 16 hours of darkness at 20^{o} C (68^{o} F) (USDA 1974). It was not possible to maintain standard conditions in the greenhouse, but the variation in environmental conditions probably had little impact on the germination and growth of seedlings when compared to factors such as seed size, species, substrate, and metal content. Also all seeds of a given treatment group were exposed to the same conditions. The temperature in the greenhouse ranged from 20^{o} C (68^{o} F) to 29^{o} C (85^{o} F) during the experiments. To avoid excessive temperatures within the petri dishes, the greenhouse bench was shaded with opaque plastic sheets. No attempt was made to artificially control photoperiod.

After the germination and growth period, the seedlings were removed from the greenhouse and stored at approximately 4^{o} C (38^{o} F) until the radicle length of all seedlings of a given treatment could be measured.

Preparation of saturated soil pastes

A plant's ability to obtain water and minerals from the soil solution depends in part on the relative amount of water in the soil (Brady 1974). Thus it was necessary to keep the supply of water and metal ions available to the seedlings as uniform as possible during the experiment. This was accomplished by maintaining saturated conditions in the soil and filter paper.

The use of saturated soil pastes also allowed the collection of soil extracts that could be analyzed to determine the water soluble (available) heavy metal content of the soil.

The method used to prepare saturated soil pastes was an adaptation of the method described by Hesse (1971). The basic procedure was to add water and/or metal solutions to dry soil until saturation is achieved. The soil to water ratio for mineral soils was 2:1 while that for organic soils was 1:4. For example, to prepare a mineral soil sample with an amendment of 50 ppm of copper, 20 grams of dry soil, 9 grams of water, and 1 gram of 1,000 ppm copper solution (equivalent to 1 mg Cu) were mixed. This adds 1 mg of copper to 20 grams of soil which increases the copper content of the soil by approximately 50 ppm. The saturated pastes were stirred thoroughly and allowed to stand at least 16 hours before being used in the germination and growth tests.

Analysis of stock solutions and soil extracts

Analyses of metal solutions and soil water extracts were performed to check the concentration of the stock solutions and to determine the degree of binding of each metal on the soils and filter paper. Atomic adsorption analyses of the samples were performed by the Minnesota Department of Public Health.

The five types of samples listed below were collected for anlysis:

1. Cu, Ni, and Co stock solutions.
2. Extracts from filter paper saturated with Cu, Ni, or Co solutions.
3. Saturation extracts from mineral and organic soils with added Cu, Ni, or Co at levels of 0 to 10,000 ppm.
4. Saturation extracts from mineral and organic soils to which all three metals had been added.
5. Saturation extracts from mineral and organic soils which were allowed to air dry between repeated additions of metals.

The soil extracts were collected by vacuum filtration from saturated soil pastes. The filter paper extracts were also collected by vacuum filtration. Information on the samples and the results of the analyses are given in appendix table A-2.

Germination and radicle elongation experiments

All the germination and radicle growth experiments used seedlings germinated in petri dishes. The substrate (i.e., metal solution, saturated soil paste or saturated tailings) was placed in a 100 mm petri dish and covered with a sheet of filter paper. Twenty-five to 50 seeds (depending on size and expected germination) were placed on the filter paper. The dishes were covered and placed in the greenhouse for the duration of the experiment.

There were 48 treatment groups - different combinations of species, metal, and substrate. Each treatment group, with the exception of the tailings experiments, consisted of a series of treatments covering a range of metal concentrations. The tailings experiments consisted of a series of control treatments on each tailings sample.

The species, substrate, metal, concentration, duration, and number of petri dishes (replicates) for each treatment are listed in appendix table A-3.

Each treatment group was designed to provide specific information on the effects of heavy metals on the germination, morphology, and radicle growth of the seedlings. The metal solution-filter paper treatments permitted the observation of morphological changes induced by each metal. These treatments also established the range of concentrations over which a particular metal had an effect on radicle growth. The treatments using mineral and organic soils compared the changes in radicle length associated with increasing heavy metal contamination of the soil. The treatment where both copper and nickel were added was designed to examine possible synergistic effects of the two metals. Since the metal salts used in the experiments were either nitrates or sulphates, treatments with sodium nitrate and sodium sulphate were conducted to determine if the effects on radicle growth were attributable to the heavy metals or to the associated anions. The tailings treatments were designed to detect differences in the phytotoxicity of the tailings samples.

The change in mean radicle length of the within group treatments was the primary criterion for assessing the impacts of heavy metals on radicle growth. The procedures for comparing the mean radicle length and the results of the various treatments will be presented later.

Data collection and statistical analysis

The data collected from the germination and growth experiments consisted of germination counts, radicle measurements, and notes on the physical appearance of the seedlings. The germination and radicle length data were used to compute statistics that allowed comparisons between the treatments.

Percent germination was determined by dividing the number of germinated seedlings in a treatment by the number of filled seeds used in the treatment. A seed was considered germinated if the radicle was at least 2 mm long. Germination data were collected only for those treatments where most of the seed coats were still attached to the seedlings at the end of the growth period. In treatments where the seed coats were not attached, it was often impossible to distinguish between shed seed coats and unfilled seeds.

The radicle length of each seedling was measured to the nearest 1 mm at the end of the treatment period. The morphology of the coniferous seedlings was such that it was difficult to determine the border between hypocotyl (stem) and radicle (root) tissue. However, the radicles of the deciduous species were clearly demarcated by a distinct angle in the plant axis and the presence of root hairs. Thus the radicle measurements for the coniferous species include all tissue below the base of the needles while the measurements for the deciduous species are for the radicle only.

At the end of the growth period, notes were taken on the morphology, color, and development of the seedlings.

The mean, variance, and variance of the mean radicle length were calculated for each treatment. The mean radicle lengths within each treatment group were compared using a relative scale where the mean of the control treatment equaled 100. The mean radicle lengths within treatment groups were tested for significant differences using the analysis of variance and modified least significant difference capabilities of the Statistical Package for the Social Sciences (SPSS) (Nie, et al. 1975).

Results

The large number of treatments makes it impossible to discuss the germination and radicle growth results for all combinations of species, metals, and substrates tested. Thus only the results of the red pine treatments will be discussed in detail. Results of treatments using other species will be mentioned when they contrast with the red pine results.

Germination

The percent germination for the 98 red pine treatments ranged from 63 to 100 percent. The lowest germintion rates occurred in five treatments that had considerable fungal growth in the petri dishes. Excluding those treatments, the percent germination ranged from 77 to 100 percent with a large majority of the treatments having greater than 85 percent germination.

The variation in percent germination within the treatment groups seemed to be completely random. There were no consistent patterns of increasing or decreasing germintion in response to the concentration of heavy metals. There were no detectable effects of heavy metals on germination over the range of concentrations tested.

The percent germination for various treatments are listed in appendix table A-4.

Radicle growth

As explained above, the two major objectives of the radicle growth experiments were (1) to observe the response of the various species to increasing concentrations of each of the three metals and (2) to use the seedlings in a bio-assay procedure to determine the level at which heavy metal contamination of the soil has a significant impact on radicle growth.

The results of the radicle growth experiments are given in appendix tables A-5 and A-6. The analysis of variance calculations led to rejection of the null hypothesis (i.e. that all means within a treatment group were equal at the 95 percent confidence level) for all groups where heavy metals were added to the substrate. Appendix table A-6 also indicates which means in a group were shown to be significantly different by the modified least significant difference comparison at the 90 percent protection level. It should be noted that the within treatment standard deviation was often quite large in comparison to the mean. This may be due to factors such as seed size and time of germination which also influence radicle length. The data in table A-6 were used to construct the graphs in Figures 1, 2, and 3.

The inverse relationship between concentration and radicle length did not hold for the treatment groups using sodium salts. The F-ratios for these treatments were much lower than those for the treatments with heavy metals. Each of these two treatment groups had only one pair of significantly different means (see appendix table A-6, treatments 020917 and 020918). Thus the inhibitory effects on radicle growth observed in the heavy metal treatments cannot be ascribed to the presence of nitrate or sulphate ions.

Radicle growth - filter paper treatments

The treatments using metal solutions on filter paper substrates were designed to show the effects of each metal on the development of the seedlings and to determine the range of concentrations over which the metals have effects on radicle growth. It was assumed that the effects of the metal solutions on radicle growth would be similar to the effects of soil water containing the same concentration of heavy metals.

Copper and cobalt had similar effects on the development of all species at low concentrations. At concentrations of less than 5 ppm, both metals either stimulated radicle growth or had no effect on growth. Nickel either had no effect or inhibited radicle growth slightly at low concentrations. At concentrations above 5 ppm, all three metals caused increasing inhibition of radicle growth with increasing concentration. All three metals completely inhibited radicle growth of black spruce, paper birch, and honeysuckle at concentrations over 50 ppm. All three metals caused death of the radicle tips on all species at 100 ppm.

The impacts on the morphology of the seedlings were very similar for all three metals. The control seedlings had long, tapered, white radicles. Root hairs were present on paper birch and honeysuckle. The hypocotyls of all species were well developed and green in color. As the metal concentration increased, the radicles of all species were shorter, blunt-tipped, dark brown or black in color (necrotic), and predisposed to fungal attack. The root hairs of paper birch and honeysuckle failed to develop at concentrations above 10 ppm. At the highest concentration in each treatment group, the radicles were reduced to small necrotic tips at the base of the hypocotyl. The effects on hypocotyl growth were much less severe. Only at the higher concentrations were the color or development of the hypocotyl affected. Plate 1 shows the effects of the three metals on red pine, white spruce, and black spruce.

Figure 1 depicts the effects of increasing concentrations of heavy metals on the radicle length of red pine seedlings germinated on filter paper. The inverse relationship between metal concentration and radicle length is evident. No one metal was clearly most toxic to all species at a given concentration.

The other species showed similar patterns of decreasing radicle length with increasing metal concentration. Paper birch and honeysuckle were the least tolerant of heavy metals.

The treatment group testing a 1:1 mixture of copper and nickel did not indicate significant synergistic effects (see Figure 1). This result does not preclude the possibility of synergistic effects of copper and nickel on other substrates or in the environment.

In general, the treatment groups using filter paper as a substrate were less consistent and provided fewer significantly different means than the treatments with soil substrates. The differences between the filter paper and soil results may have been caused by the smaller range of concentrations tested and the smaller number of seedlings used in the filter paper treatments (see appendix table A-5).

Radicle growth - mineral soil treatments

The purpose of the mineral soil treatments was to determine the level at which heavy metal contamination of the soil results in a significant reduction of radicle growth for each species. Mineral soil samples with amendments of 0, 50, 100, 150, 250, and 500 ppm of copper, nickel, or cobalt were used as substrates for the germination of seedlings.

The effects of these treatments on the appearance of the seedlings were similar to those described for the filter paper treatments.

Figure 2 gives the relative radicle lengths of red pine seedlings grown on mineral soil amended with various concentrations of heavy metals. Note that the range of radicle lengths is nearly the same as in Figure 1. Thus soil metal concentrations of 50 to 500 ppm produced the same reduction in radicle growth as metal solutions of 0.5 to 100 ppm on filter paper.

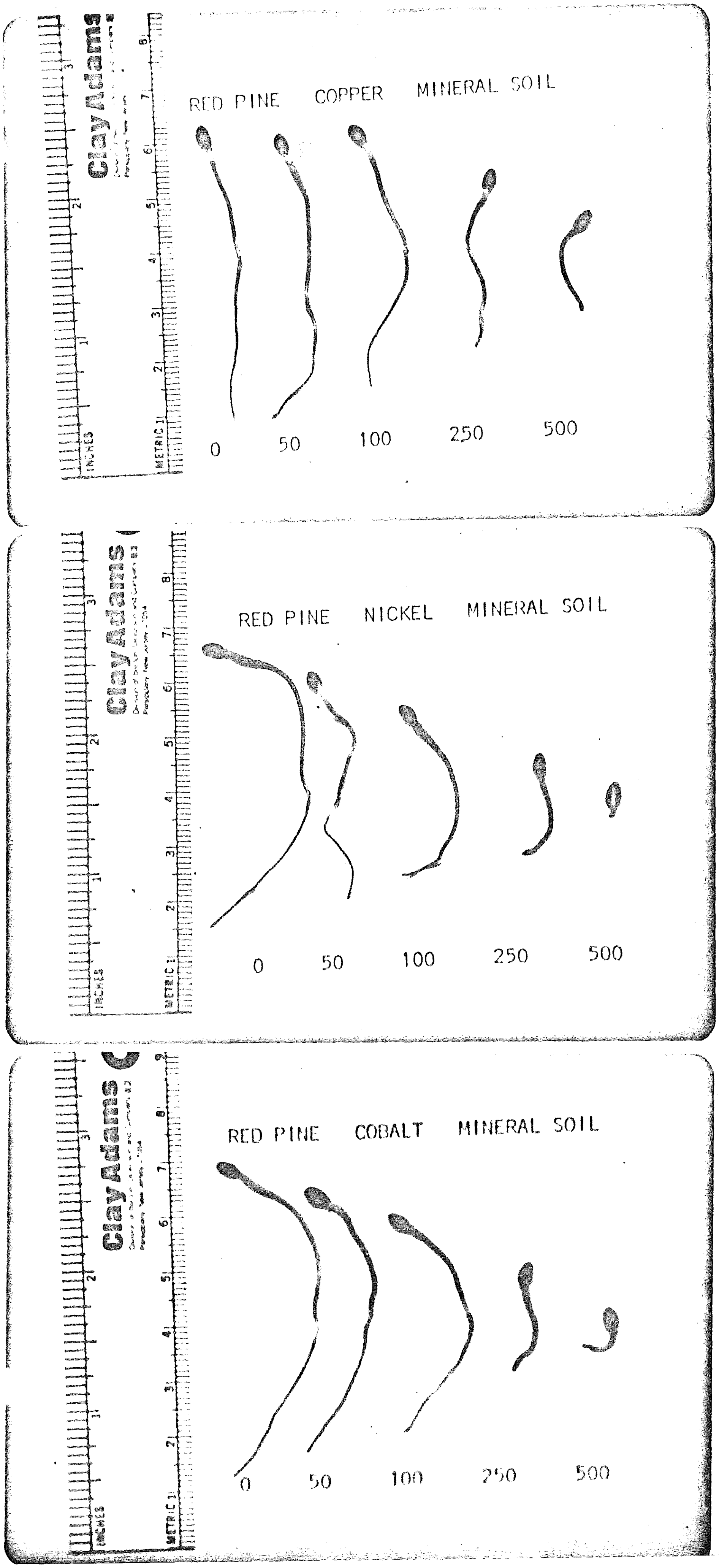
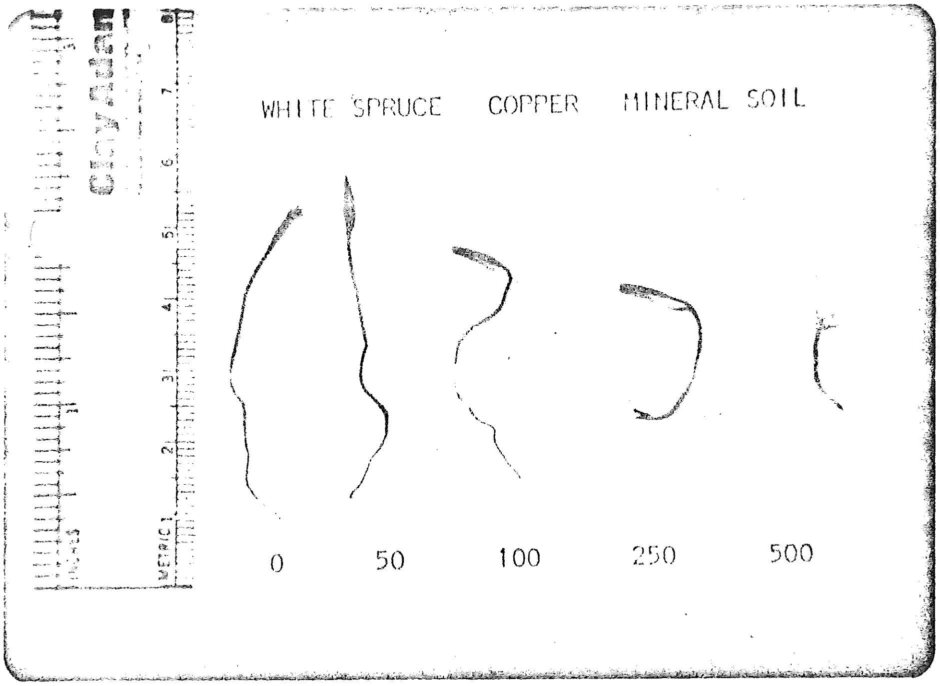
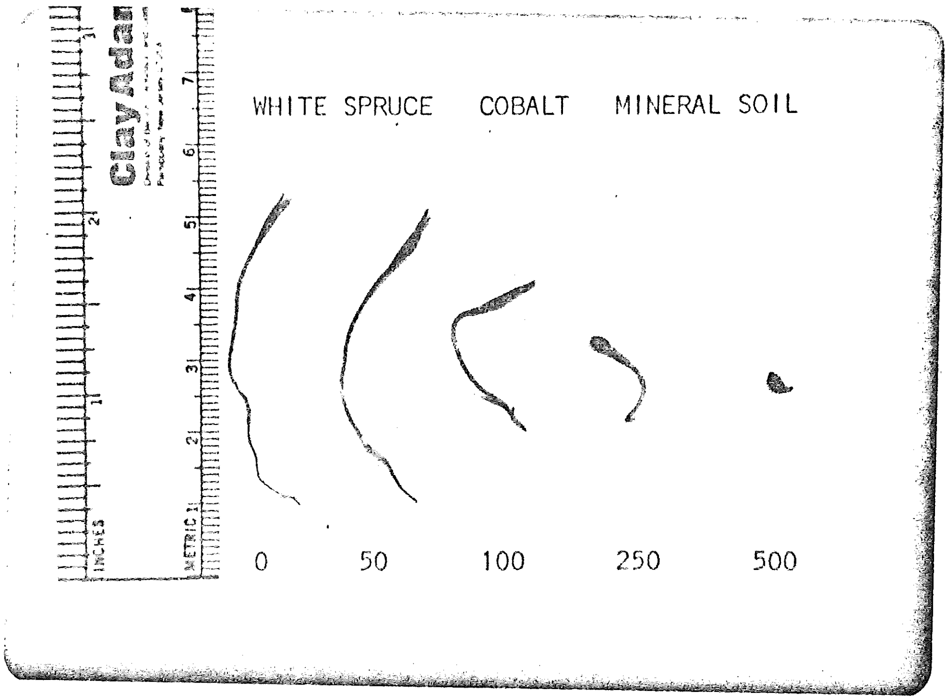


Plate 1. The morphological effects of heavy metals on seedlings.





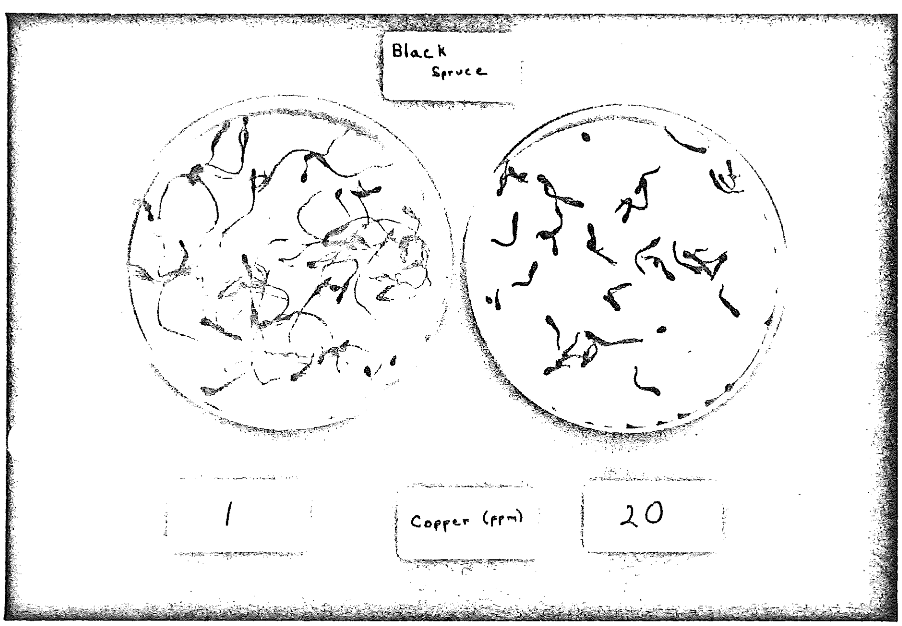


Plate 1. (continued)
The morphological effects of heavy metals on seedlings.

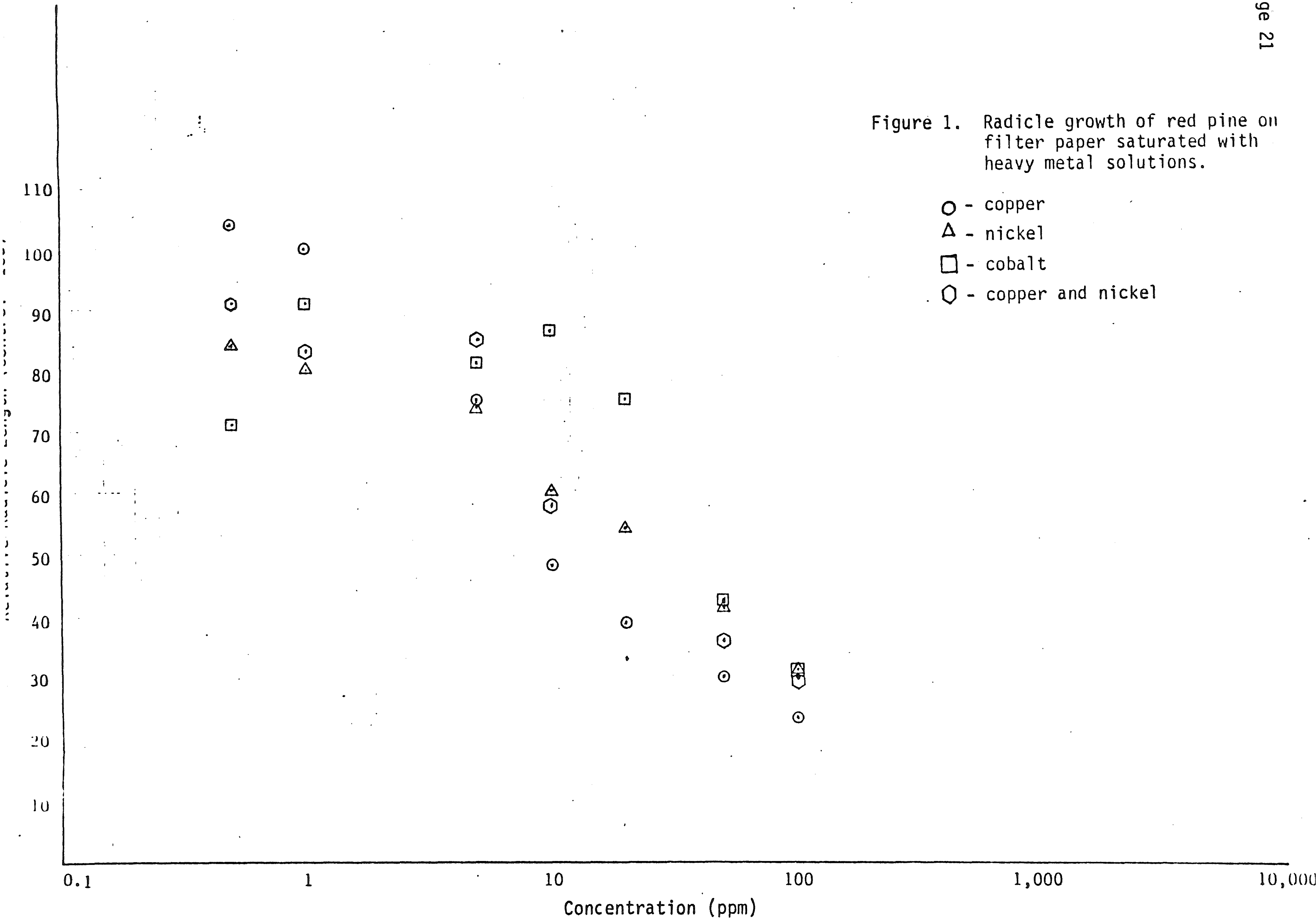


Figure 1. Radicle growth of red pine on filter paper saturated with heavy metal solutions.

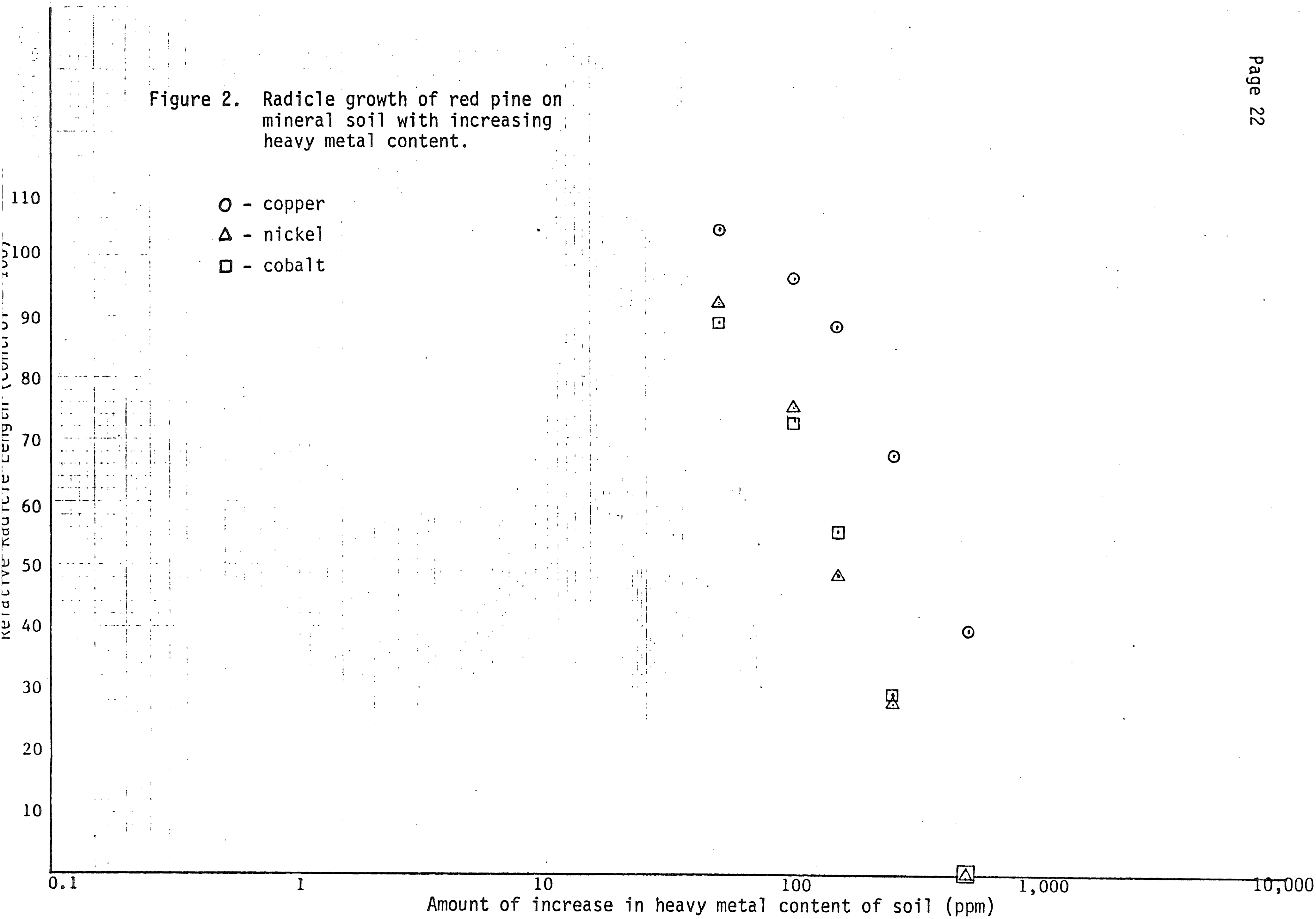


Figure 2. Radicle growth of red pine on mineral soil with increasing heavy metal content.

Nickel was most toxic to all species at concentrations $\geq$ 150 ppm. This contrasts with the filter paper treatments where no metal was most toxic to all species at the higher concentrations.

Table 3 lists the levels at which radicle lengths of the test species were reduced to approximately 75 percent of the control treatment length. This 25 percent reduction in length correlates with the point at which most treatment groups began to have seedlings with necrotic radicle tips.

Table 3. Soil metal concentrations (in ppm) that reduced mean radicle length to approximately 75 percent of control.

	Cu	Ni	Co
Jack pine	100	< 100	< 100
Red pine	200	100	100
White pine	150	50	75
White spruce	150	< 50	< 50
Paper birch	75	< 50	< 50

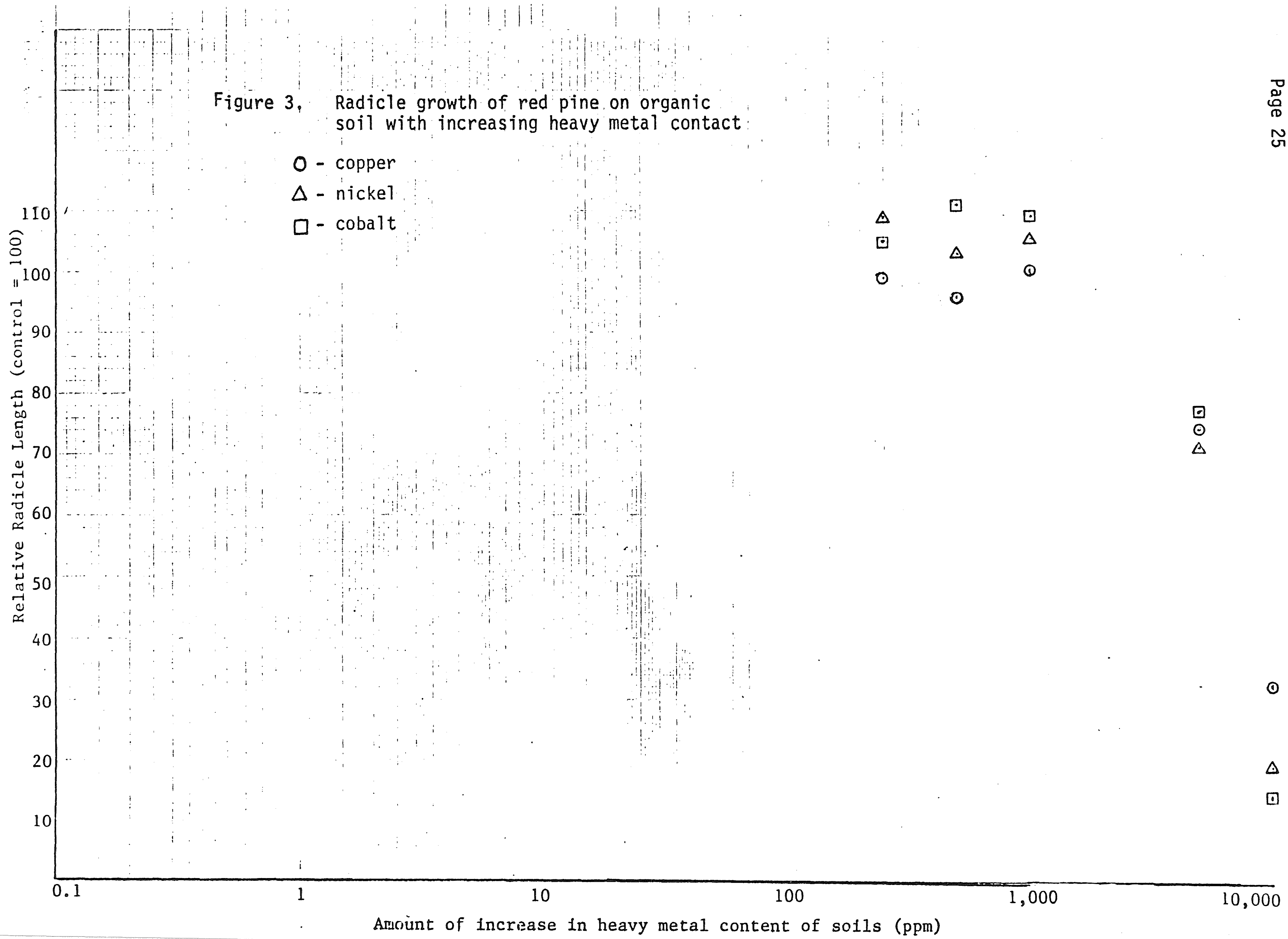


Figure 3, Radicle growth of red pine on organic soil with increasing heavy metal contact

Paper birch was the most sensitive to increasing concentrations of heavy metals. Nickel and cobalt reduced mean radicle length to 75 percent of controls at concentrations of less than 100 ppm. Copper generally did not reduce growth to 75 percent of controls until the soil metal concentration reached 150 ppm. The metals, ranked in terms of the level at which they reduce radicle growth to 75 percent of controls, are $Ni \leq Co < Cu$.

Radicle growth - organic soil treatments

Red pine and black spruce were germinated on organic soil substrates amended with 0, 250, 500, 1,000. 5,000, and 10,000 ppm of copper, nickel, or cobalt.

Figure 3 presents the results of the red pine treatments. There were no significant differences among the mean radicle lengths of the 0, 250, 500, and 1,000 ppm treatments for each metal. The seedlings grown on organic soil amended with 1,000 to 10,000 ppm of heavy metals showed the same range of morphological effects and growth reduction as seedlings grown on filter paper at concentrations of 0.5 to 100 ppm.

All three metals reduced mean radicle lengths to approximately 75 percent of controls at 5,000 ppm. Nickel and cobalt were more toxic than copper at concentrations above 1,000 ppm.

Radicle growth - tailings treatments

The tailings treatments were designed to identify tailings samples that exhibit phytotoxic properties. Red pine, black spruce, and paper birch were grown on tailings saturated with deionized distilled water. The control treatments consisted of seedlings grown on mineral soil saturated with water.

The following treatments produced seedlings that had a mean radicle length significantly different from the control group at the 90 percent confidence level:

1. Red pine on samples AX9001-200T, AX9005-65T, AX9003-200T, AX9004-200T DP9002-65T, DP9002-200T, and US9001-200T all had means less than that of the control group.
2. Black spruce on sample DP9002-65T had a mean greater than the control, while US9001-65T seedlings had a mean less than the controls.
3. Paper birch on sample AX9004-200T had a mean greater than the control and sample US9001-65T produced a mean less than the control.

The results of all the tailings treatments are given in appendix tables A-5 and A-6.

No single tailings sample reduced the growth of all three species significantly. However, US9001-65T produced significant reductions in black spruce and paper birch and a nearly significant reduction in red pine.

Only four treatments reduced growth to less than 75 percent of controls. They were red pine/AX9004-200T (57 percent), red pine/DP9002-200T (67 percent), black spruce/US9001-65T (53 percent), and paper birch/US9001-65T (40 percent). None of the treatments produced the blunt-tipped, necrotic radicles characteristic of seedlings grown in the presence of high heavy metal concentrations. Thus the observed reductions in radicle length may be caused by either moderate levels of heavy metals or other phytotoxic materials used in processing the samples.

Chemical analysis of solutions and extracts

A description of each sample and the results of the analyses for heavy metals are presented in appendix table A-2.

Copper was more readily bound than either nickel or cobalt on all substrates. Organic soil samples bound higher levels of heavy metals than did mineral soils.

It should be noted that the concentration of heavy metals in the extracts were always considerably less than the concentration of heavy metals added to the substrate. Thus, care must be exercised to distinguish between total concentrations and water soluble or available concentrations.

The analyses indicate that some of the heavy metals were bound on the glassware, filter paper, and/or other laboratory apparatus. Thus, the effects observed in these experiments might occur at lower concentrations in the environment.

Discussion

Copper, nickel, and cobalt did not affect the percent germination of any of the species used in this study. Whitby and Hutchinson (1974) germinated lettuce, tomato, cabbage, and radish seeds in water extracts from heavy metal contaminated soils. They observed that the heavy metals had no impact on the germination of those species. Mishra and Kar (1974) reviewed several studies on the effects of nickel on germination. The germination of some agricultural species increased after treatment with nickel. Other species showed reduced germination. They conclude that the effects of nickel on germination are species specific. Since heavy metals affect radicle growth, the major impacts of heavy metals on forest tree reproduction will probably result from decreased seedling survival rather than decreased germination.

Whitby and Hutchinson (1974) found that heavy metals caused an inhibition of seedling root growth while the cotyledons of the same seedlings remained green and nearly normal in appearance. Similar effects of heavy metals on seedling growth and morphology were observed in the present study. Radicle growth is often of critical importance to newly emerged seedlings. The root must reach a moist substrate if the seedling is to survive. Any reduction in radicle growth reduces the seedling's chance of obtaining an adequate supply of water and minerals, especially if shoot growth continues at the normal rate.

The fact that copper and cobalt are micronutrients may explain the increased radicle growth observed in treatments with low concentrations of these metals. Since nickel is not an essential element, it would not be expected to increase growth, except as it may affect the availability of other elements. Some soils (especially organic soils) are deficient in copper and cobalt (Bowen 1966, Brady 1974). Thus, slight additions of these elements to the environment may result in increased plant growth.

Whitby and Hutchinson (1974) noted in their study of the effects of heavy metals on seedlings that the within treatment variability of radicle length was often quite large. Some treatments in the present study also displayed large variations in radicle lengths, although variability decreased as the metal concentration increased. Thus, at higher concentrations, the natural variation of radicle growth rates due to genetic variation, seed size, and time of germination was eliminated and all seedlings were similar in appearance.

The fact that fungal growth was more prevalent on plates with higher metal concentrations is interesting. Apparently some species of fungi are quite tolerant of heavy metals. The increase in available nutrients resulting from the death and decay (caused by heavy metals) of the radicles may also have influenced the occurrence of fungal growth. In some cases, it was not obvious whether the heavy metals or the fungi were the ultimate cause of radicle death.

The results of this study point to the importance of distinguishing between total and available heavy metal concentrations.

For example, total concentrations of 50 to 500 ppm in mineral soil and 1,000 to 10,000 ppm in organic soil had similar effects on seedling growth. Analysis of the water extracts showed similar levels of available heavy metals for the two soils. The chemistry and physics of heavy metal binding are not completely understood, but the following have been shown to be among the factors which influence the relationship between total and available concentrations:

1. Acidity. The available metal concentration tends to increase at lower pH's (Brady 1974).

2. Clay content. The quantity and structure of the clay particles in a

soil will affect the availability of heavy metals (Brady 1974).

2. Clay content. The quantity and structure of the clay particles in a soil will affect the availability of heavy metals (Brady 1974). Generally, soils with a higher clay content will have a higher cation exchange capacity and will be able to, at least temporarily, make the metal ions less available. The 2:1 clays are able to incorporate some metal ions (especially cobalt) in their structure making them unavailable.

3. Organic matter content. The quantity and type of organic matter in the soil is very important in determining the availability of heavy metals. On a weight basis, organic matter has a higher cation exchange capacity than clay (Brady 1974). The heavy metals may combine with organic groups in organic complexes and be rendered unavailable (Brady 1974).

The distribution of heavy metals within the soil profile is also important in determining the effects on seed germination and growth. Hodgson (1963) states that metallic trace elements are rather uniformly distributed throughout the profile, except in podzols. Hutchinson and Whitby (1974) cite studies that indicate copper, nickel, and cobalt concentrations normally increase with depth. Thomas (1965), Jordan (1975), and Whitby and Hutchinson (1974) found that the normal pattern of uniform or increasing concentration of heavy metals with depth did not occur in soils near metal smelting operations. Jordan (1975) and Whitby and Hutchinson (1974) found increases in both total and water soluble heavy metal concentrations in surface soils near smelters in Pennsylvania and Ontario, Canada. Jordan (1975) concludes that the zinc smelter emissions have been the critical factor preventing the revegetation near the smelter in Palmerton, Pennsylvania.

Summary

The following statements are based on the results of this study.

1. Copper, nickel, and cobalt have no effect on the germination of any of the species tested.

2. Copper, nickel, and cobalt inhibit radicle growth much more than they inhibit hypocotyl growth of seedlings.

3. At low concentrations (less than 5 ppm on filter paper, less than 50 ppm on mineral soil, and less than 1,000 ppm on organic soil), copper and cobalt either have no effect on radicle growth or cause slight increases in radicle length while nickel has no effect or decreases growth slightly.

4. At higher concentrations all three metals inhibit radicle growth of all species tested. There is an inverse relationship between radicle length and heavy metal concentration.

5. Nickel and cobalt inhibit radicle growth at lower concentrations than does copper on mineral soils.

6. Small seeded species were more severely inhibited than were larger seeded species.

7. Paper birch and honeysuckle (the hardwood species tested) were the species most sensitive to heavy metals. Factors such as seed size, acidity

and nutrient supply may be involved.

8. At higher heavy metal concentrations, the seedlings were more susceptible to fungal attack.

9. The within treatment variability of radicle length decreased as heavy metal concentrations increased.

10. This study did not provide sufficient information to rank the species in terms of their sensitivities to each metal.

11. Seedlings germinated on the tailings samples did not develop signs of heavy metal toxicity. Nonetheless, some tailings samples did reduce radicle growth.

12. Given equal rates of addition to the environment, nickel would probably be the first of the three metals to become a serious environmental pollutant.

13. Given equal rates of addition to the soil, heavy metal contamination would affect forest reproduction on mineral soils before it would affect forest reproduction on organic soils.

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APPENDIX

Table A-1. Tailings characteristics.

Sample designation[1]	Water Soluble Heavy Metal Content[2] (ppm) Cu	Ni	Co	Total Heavy Metal Content[3] (ppm) Cu	Ni	Co
AX9001-65T				275	223	65
AX9002-200T				188	221	62
AX9002-65T	0.0082	0.06	N.D.[4]	362	334	97
AX9002-200T	0.0095	N.D.	N.D.	161	287	78
AX9003-65T	0.010	N.D.	N.D.	360	270	65
AX9003-200T	0.022	N.D.	N.D.	195	285	62
AX9004-200T				217	214	44
DP9002-65T	0.009	N.D.	N.D.	419	182	63
DP9002-200T	0.007	N.D.	N.D.	135	219	61
IP9002-65T	0.0032	N.D.	N.D.	297	302	75
IP9002-200T	N.D.	N.D.	N.D.	122	273	70
US9001-65T	0.010	0.23	N.D.	320	260	62
US9001-200T	0.005	N.D.	N.D.	124	266	62

[1]Codes used in sample designation are as follows:
- -location; AX = AMAX, DP = Dunka pit, IP = INCO pit, US = US Steel pit.
- -mesh size; 65 and 200 are the mesh sizes
- -T; indicates the sample consists of flotation tailings

[2]Souce: Cu-Ni Project data. (Liquors or Waters)
Matrix: aqueous - 1 day readings

[3]Source: Cu-Ni Project data. (Ground Ores, etc.)
Matrix: Aqueous 1

[4]N.D. = Not detectable

Table A-2. Chemical analyses of solutions and extracts.

Sample Description		Analysis (ppm) Cu	Ni	Co
1.	Deionized distilled water	0.0026	0.029	0.002
2.	1,000 ppm Cu stock solution			
3.	1,000 ppm Ni stock solution			
4.	1,000 ppm Co stock solution			
5.	Water extract of filter paper	0.019	0.049	<0.0002
6.	Extract from filter paper saturated with solution of 1.0 ppm Cu	0.028		
7.	10 ppm Cu	0.71		
8.	50 ppm Cu	31		
9.	100 ppm Cu	59		
10.	1.0 ppm Ni		0.049	0.0034
11.	10 ppm Ni		2	
12.	50 ppm Ni		33	
13.	100 ppm Ni		60	
14.	1.0 ppm Co			0.039
15.	10 ppm Co			2
16.	50 ppm Co			35
17.	100 ppm Co			70
18.	Water extract of mineral soil	0.012	0.021	<0.0006
19.	Extract of mineral soil amended with 50 ppm Cu	0.056		
20.	100 ppm Cu	0.079		
21.	150 ppm Cu	0.15		
22.	250 ppm Cu	3.4		
23.	500 ppm Cu	22		
24.	50 ppm Ni		3.8	
25.	100 ppm Ni		15	
26.	150 ppm Ni		36	
27.	250 ppm Ni		130	
28.	500 ppm Ni		430	
29.	50 ppm Co			6.9
30.	100 ppm Co			28
31.	150 ppm Co			63
32.	250 ppm Co			130
33.	500 ppm Co			420

a. The stock solutions were diluted before analysis so that the sample concentration would fall within the sensitivity range of the analyzer.

Table A-2. (continued)

Sample Description	Analysis (ppm) Cu	Ni	Co
34. Water extract of organic soil	0.015	0.067	0.0029
35. Extract of organic soil amended with 250 ppm Cu	0.23		
36. 500 ppm Cu	0.60		
37. 1,000 ppm Cu	1.7		
38. 5,000 ppm Cu	3.7		
39. 10,000 ppm Cu	42		
40. 250 ppm Ni		0.47	
41. 500 ppm Ni		0.49	
42. 1,000 ppm Ni		1.8	
43. 5,000 ppm Ni		55	
44. 10,000 ppm Ni		290	
45. 250 ppm Co			0.33
46. 500 ppm Co			1.2
47. 1,000 ppm Co			4
48. 5,000 ppm Co			100
49. 10,000 ppm Co			360
50. Extract of mineral soil amended with 250 ppm Cu + 250 ppm Ni + 250 ppm Co	18	190	210
51. Extract of mimeral soil amended with 500 ppm Cu + 500 ppm Ni + 500 ppm Co	100	520	610
52. Extract of organic soil amended with 500 ppm each Cu, Ni, and Co	0.57	1.5	3.8
53. Extract of organic soil amended with 1,000 ppm each Cu, Ni, and Co	0.59	4.4	12
54. Extract of organic soil amended with 5,000 ppm each Cu, Ni, and Co	19	250	360
55. Extract of mineral soil amended with 50 ppm Cu	0.12		
56. Extract of mineral soil amended twice with 50 ppm Cu	0.34		
57. Extract of mineral soil amended thrice with 50 ppm Cu	0.22		
58. once with 250 ppm Cu	2		
59. twice with 250 ppm Cu	6		
60. thrice with 250 ppm Cu	9.2		
61. once with 500 ppm Cu	34		
62. twice with 500 ppm Cu	54		
63. thrice with 500 ppm Cu	140		
64. once with 50 ppm Ni		6	
65. twice with 50 ppm Ni		13	
66. thrice with 50 ppm Ni		20	

Table A-2. (continued)

	Sample Description		Analysis (ppm) Cu	Ni	Co
67.	Extract of mineral soil amended	once with 250 ppm Ni		150	
68.	↓	twice with 250 ppm Ni		250	
69.	↓	thrice with 250 ppm Ni		470	
70.	↓	once with 500 ppm Ni		270	
71.	↓	twice with 500 ppm Ni		840	
72.	↓	thrice with 500 ppm Ni		1,700	
73.	Extract of organic soil amended	once with 500 ppm Cu	0.32		
74.	↓	twice with 500 ppm Cu	1.1		
75.	↓	thrice with 500 ppm Cu	0.93		
76.	↓	once with 5,000 ppm Cu	4.5		
77.	↓	twice with 5,000 ppm Cu	18		
78.	↓	thrice with 5,000 ppm Cu	49		
79.	↓	once with 10,000 ppm Cu	34		
80.	↓	twice with 10,000 ppm Cu	400		
81.	↓	thrice with 10,000 ppm Cu	1,900		
82.	↓	once with 500 ppm Ni		0.83	
83.	↓	twice with 500 ppm Ni		0.79	
84.	↓	thrice with 500 ppm Ni		1.4	
85.	↓	once with 5,000 ppm Ni		47	
86.	↓	twice with 5,000 ppm Ni		200	
78.	↓	thrice with 5,000 ppm Ni		500	
88.	↓	once with 10,000 ppm Ni		170	
89.	↓	twice with 10,000 ppm Ni		1,000	
90.	↓	thrice with 10,000 ppm Ni		2,100	

Table A-3. Germination and Growth Treatments.

Treatment Number	Substrate	Species	Metal	Length of treatment (days)	Number of petri dishes (repititions) at each concentration (ppm)															
					0.0 Control	0.1	0.5	1	5	10	20	50	100	150	250	500	750	1,000	5,000	10,000
020811	filter paper	Jack pine	Cu	5	2	1	1	1	1	1	1	1								
020812	"	"	Ni	5	2	1	1	1	1	1	1	1								
020813	"	"	Co	5	1		1	1	1	1	1	1	1							
020911	"	Red pine	Cu	10	3		1	1	1	1	1	1	1							
020912	"	"	Ni	10	3		1	1	1	1	1	1	1							
020913	"	"	Co	10	3		1	1	1	1	1	1	1							
021011	"	White pine	Cu	14	3		1	1	1	1	1	1	1							
021012	"	"	Ni	14	3		1	1	1	1	1	1	1							
021013	"	"	Co	14	3		1	1	1	1	1	1	1							
020711	"	Black spruce	Cu	11	3		1	1	1	1	1	1	1							
020712	"	"	Ni	11	3		1	1	1	1	1	1	1							
020713	"	"	Co	11	3		1	1	1	1	1	1	1							
020611	"	White spruce	Cu	11	3		1	1	1	1	1	1	1							
020612	"	"	Ni	11	3		1	1	1	1	1	1	1							
020613	"	"	Co	11	3		1	1	1	1	1	1	1							
126911	"	Paper birch	Cu	9	3		1	1	1	1	1	1	1							
126912	"	"	Ni	9	3		1	1	1	1	1	1	1							
126913	"	"	Co	9	3		1	1	1	1	1	1	1							
457711	"	Honeysuckle	Cu	24	3		1	1	1	1	1	1	1							
457712	"	"	Ni	24	3		1	1	1	1	1	1	1							
457713	"	"	Co	24	3		1	1	1	1	1	1	1							
020914	"	Red pine	Cu&Ni	15	1		1	1	1	1	1	1	1							
020917	"	"	$NO_3^=$	15	1		1	1	1	1	1	1	1							
020918	"	"	$SO_4^=$	15	1		1	1	1	1	1	1	1							
020821	Mineral soil	Jack pine	Cu	7	16								4		4	4	4	4		
020822	"	"	Ni	7	16								4		4	4	4	4		
020823	"	"	Co	7	16								4		4	4	4	4		
020921	"	Red pine	Cu	12	16							4	4	4	4	4				
020922	"	"	Ni	12	16							4	4	4	4	4				
020923	"	"	Co	12	16							4	4	4	4	4				

Table A-3. Germination and Growth Treatments. (continued)

Treatment Number	Substrate	Species	Metal	Length of treatment (days)	Number of petri dishes (repititions) at each concentration (ppm): 0.0 Control	0.1	0.5	1	5	10	20	50	100	150	250	500	750	1,000	5,000	10,000
021021	Mineral soil	White pine	Cu	14	16							4	4	4	4	4				
021022	"	"	Ni	14	16							4	4	4	4	4				
021023	"	"	Co	14	16							4	4	4	4	4				
020621	"	White spruce	Cu	13	16							4	4	4	4	4				
020622	"	"	Ni	13	16							4	4	4	4	4				
020623	"	"	Co	13	16							4	4	4	4	4				
126921	"	Paper birch	Cu	18	16							4	4	4	4	4				
126922	"	"	Ni	18	16							4	4	4	4	4				
126923	"	"	Co	18	16							4	4	4	4	4				
020931	Organic soil	Red pine	Cu	12	16										4	4		4	4	4
020932	"	"	Ni	12	16										4	4		4	4	4
020933	"	"	Co	12	16										4	4		4	4	4
020731	"	Black spruce	Cu	14	16										4	4		4	4	4
020732	"	"	Ni	14	16										4	4		4	4	4
020733	"	"	Co	14	16										4	4		4	4	4

Treatment Number	Substrate	Species	Metal	Length of treatment (days)	0.0 Control	Number of petri dishes (repititions) for each sample: AX 9001 65T	AX 9001 200T	AX 9002 65T	AX 9002 200T	AX 9003 65T	AX 9003 200T	AX 9004 200T	IP 9002 65T	IP 9002 200T	DP 9002 65T	DP 9002 200T	US 9001 65T	US 9001 200T
02092	Mineral soil	Red pine	None	14	4													
02094	Tailings		"	14		4	4	4	4	4	4	4	4	4	4	4	4	4
02072	Mineral soil	Black spruce	"	14	4													
02074	Tailings		"	14		4	4	4	4	4	4	4	4	4	4	4	4	4
12692	Mineral soil	Paper birch	"	14	4													
12694	Tailings		"	14		4	4	4	4	4	4	4	4	4	4	4	4	4

Table A-4. Germination results.

Treatment Code	Species	Substrate	Metal	Code 01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16
				Percent Germination at Various Concentrations															
				Concentration Control 0.0	0.1	0.5	1	5	10	20	50	100	150	250	500	750	1,000	5,000	10,000
020811	Jack pine	Filter paper	Cu	94	96	96	88	84	100	92	81	NT[a]							
020812	"	"	Ni	94	80	96	92	88	92	96	93	NT							
020813	"	"	Co	87	NT	93	100	87	93	82	97	93							
020911	Red pine	"	Cu	92	NT	97	87	85	89	93	90	97							
020912	"	"	Ni	92	NT	100	93	87	83	87	91	87							
020913	"	"	Co	92	NT	83	100	86	97	90	100	100							
021011	White pine	"	Cu	68	NT	64	56	72	68	64	75	75							
021012	"	"	Ni	68	NT	72	76	68	92	80	84	68							
021013	"	"	Co	68	NT	68	92	64	96	64	76	88							
020711	Black spruce	"	Cu	89	NT	95	95	93	93	87	88	90							
020712	"	"	Ni	89	NT	90	90	76	80	83	90	88							
020713	"	"	Co	89	NT	91	81	95	95	90	91	88							
020611	White spruce	"	Cu	73	NT	72	75	75	75	83	73	73							
020612	"	"	Ni	73	NT	71	88	70	75	83	73	80							
020613	"	"	Co	74	NT	55	93	75	85	85	78	83							
020914	Red pine	"	Cu&Ni	93	NT[a]	93	97	98	63[b]	73[b]	85	89							
010917	"	"	NO_3	77	NT	86	80	89	87	86	73[b]	96							
020918	"	"	SO_4	89	NT	84	81	74	81	82	86	87							
020821	Jack pine	Mineral soil	Cu	92							NT[a]	91	NT	87	93	NE[c]	NE		
020822	"	"	Ni	92							NT	89	NT	86	NE	NE	NE		
020823	"	"	Co	92							NT	90	NT	90	NE	NE	NE		
020921	Red pine	"	Cu	99							99	100	100	98	97	NT	NT		
020922	"	"	Ni	99							98	92	98	90	NE	NT	NT		
020923	"	"	Co	99							100	100	100	99	NE	NT	NT		
021021	White pine	"	Cu	89							86	82	84	87	83	NT	NT		
021022	"	"	Ni	89							85	84	80	15[b]	NE	NT	NT		
021023	"	"	Co	89							88	87	82	85	53[b]	NT	NT		
020621	White spruce	"	Cu	86							89	83	82	86	85	NT	NT		
020622	"	"	Ni	86							86	86	84	NE	NE	NT	NT		
020623	"	"	Co	86							82	86	87	80	NT	NT	NT		
020931	Red pine	Organic soil	Cu	99										97	100	NT[a]	98	99	98
020932	"	"	Ni	99										100	98	NT	98	96	74[b]
020933	"	"	Co	99										96	99	NT	100	98	88[b]

Table A-4. Germination results. (continued)

Treatment Code	Species	Substrate	Metal	Code 01 Concentration Control 0.0	02 0.1	03 0.5	04 1	05 5	06 10	07 20	08 50	09 100	10 150	11 250	12 500	13 750	14 1,000	15 5,000	16 10,000
02092	Red pine	Mineral soil	—	95															
			Tailing sample #		US9001 200T	US9001 65T	DP9002 65T	DP9002 200T	AX9003 200T	AX9001 200T	AX9001 65T	AX9003 65T	AX9004 200T	AX9002 65T	AX9002 200T	IP9002 65T	IP9002 200T		
					97	100	95	88	99	100	95	99	93	97	97	100	100		

a. NT = No treatment at this concentration. Blank spaces also indicate no treatment.

b. Fungal growth on seedlings. Seed coat splits but radicle doesn't elongate on affected seedlings.

c. NE = No elongation of radicle. Some seeds have split seed coat but the seedling is killed by the metal.

Table A-5. Radicle growth results.

				Number of seedlings, mean radicle length, std. deviation at each concentration (ppm)[a]															
Treatment Number	Substrate	Species	Metal	0.0 Control	0.1	0.5	1	5	10	20	50	100	150	250	500	750	1,000	5,000	10,000
020811	Filter paper	Jack pine	Cu	27.92 50/7.6	31.00 24/7.3	28.79 24/9.3	27.00 22/7.6	24.86 21/8.2	15.46 26/5.3	13.78 23/4.1	8.66 25/?	NT[b]							
020812	"	"	Ni	27.92 50/7.6	28.45 20/8.9	28.33 24/8.1	28.78 23/7.5	21.96 23/8.0	20.35 23/6.4	14.77 22/5.6	10.05 28/?	NT							
020813	"	"	Co	17.56 27/7.4	NT	20.22 27/6.2	18.93 30/6.8	21.34 26/5.6	15.21 28/5.9	12.78 23/4.9	8.21 28/2.6	6.00 28/2.2							
020911	"	Red pine	Cu	34.89 80/7.9	NT	36.41 29/7.5	34.92 26/11.5	26.32 28/8.0	16.88 24/7.3	13.54 28/4.6	10.48 27/2.7	7.89 28/2.2							
020912	"	"	Ni	34.89 80/7.9	NT	29.20 30/8.3	28.07 28/8.4	25.77 26/13.5	20.77 22/7.8	18.84 26/7.8	14.38 29/4.3	10.88 26/3.1							
020913	"	"	Co	34.89 80/7.9	NT	24.76 25/12.7	31.83 29/10.9	28.32 25/11.8	29.86 29/8.9	26.23 26/7.2	14.50 30/4.9	10.90 30/3.3							
021011	"	White pine	Cu	47.86 51/16.3	NT	53.06 16/16.4	55.00 15/7.4	43.44 18/12.9	48.12 17/19.2	42.19 16/13.2	38.22 18/12.7	27.78 18/12.9							
021012	"	"	Ni	47.86 51/16.3	NT	41.33 18/20.9	31.79 19/11.7	45.12 17/13.4	57.17 23/13.7	42.35 20/13.9	36.48 21/11.7	13.71 17/10.8							
021013	"	"	Co	47.86 51/16.3	NT	51.39 18/23.5	51.65 23/14.0	42.13 16/14.1	49.58 24/16.5	40.81 16/17.4	45.53 19/8.6	22.50 22/12.0							
020711	"	Black spruce	Cu	28.26 88/5.0	NT	28.78 41/5.0	27.45 40/3.8	20.59 39/5.1	13.22 37/3.3	8.91 34/2.6	NE[c]	NE							
020712	"	"	Ni	29.26 88/5.0	NT	28.78 38/4.3	26.80 35/6.7	18.87 31/5.1	12.41 35/5.1	5.55 33/2.3	NE	NE							
020713	"	"	Co	28.26 88/5.0	NT	29.44 39/5.8	28.49 35/4.0	23.95 38/5.7	21.82 38/5.2	12.44 36/5.7	6.95 40/2.0	NE							
020611	"	White spruce	Cu	22.56 89/11.4	NT	23.69 26/11.0	26.52 27/11.1	20.50 30/9.2	19.13 30/8.8	9.97 33/4.5	6.97 29/3.2	6.57 30/3.4							
020612	"	"	Ni	22.56 89/11.4	NT	23.70 27/13.2	27.46 35/10.2	17.84 31/11.9	18.27 30/6.6	11.97 33/6.4	NE	NE							
020613	"	"	Co	22.56 89/11.4	NT	22.64 22/10.2	28.03 37/9.8	25.57 30/9.7	23.70 33/9.1	20.76 34/9.2	10.51 31/4.8	5.32 34/1.8							

Table A-5. Radicle growth results. (continued)

Treatment Number	Substrate	Species	Metal	Number of seedlings, mean radicle length, std. deviation at each concentration (ppm)[a] 0.0 Control	0.1	0.5	1	5	10	20	50	100	150	250	500	750	1,000	5,000	10,000
126911	Filter paper	Paper birch	Cu	8.26 46/2.9	NT	8.15 13/2.9	5.00 10/1.7	2.29 14/0.6	NE	NE	NE	NE							
126912	"	"	Ni	8.26 46/2.9	NT	7.36 14/2.7	6.50 14/2.8	4.15 13/1.3	2.93 15/1.1	NE	NE	NE							
126913	"	"	Co	8.26 46/2.9	NT	10.08 13/2.9	8.36 14/2.4	4.39 23/1.7	2.95 20/0.9	NE	NE	NE							
457711	"	Honeysuckle	Cu	17.26 34/6.5	NT	19.50 10/11.8	14.93 15/4.9	21.13 16/10.9	13.79 14/4.4	14.11 18/6.4	NE	NE							
457712	"	"	Ni	17.26 34/6.5	NT	19.55 11/9.2	15.85 13/9.2	10.50 14/4.5	12.08 13/6.3	7.30 10/5.3	NE	NE							
457713	"	"	Co	17.26 34/6.5	NT	12.2 5/1.8	19.00 19/7.6	11.25 8/8.2	12.56 16/7.1	11.30 10/5.3	5.35 17/3.7	NE							
020914	"	Red pine	Cu/Ni	49.76 37/19.7	NT	45.12 25/17.5	41.45 31/18.3	42.16 49/17.2	28.89 19/4.8	16.26 19/8.8	18.00 22/7.4	14.42 33/5.1							
020917	"	"	NO_3	38.53 34/21.5	NT	41.84 25/22.9	34.00 28/23.9	50.00 32/16.9	45.21 33/14.4	35.17 30/21.5	33.81 16/11.8	30.78 18/15.7							
020918	"	"	SO_4	37.15 41/14.3	NT	29.86 21/18.2	40.44 25/19.1	33.09 33/21.1	44.14 29/16.3	46.59 27/17.0	45.07 30/20.6	40.64 33/22.5							
020821	Mineral soil	Jack pine	Cu	23.50 365/7.5							NT	17.90 91/7.1	NT	12.41 79/5.6	7.75 92/2.3	NE			
020822	"	"	Ni	23.50 365/7.5							NT	13.07 89/5.4	NT	6.34 86/2.2	NE	NE			
020823	"	"	Co	23.50 365/7.5							NT	15.52 90/4.7	NT	8.60 90/3.3	NE	NE			
020921	"	Red pine	Cu	53.42 395/9.8							55.37 109/7.9	51.25 99/9.4	47.14 100/12.3	35.69 98/12.9	21.04 97/7.8	NT			
020922	"	"	Ni	53.42 395.9.8							49.10 61.12.3	40.05 86/11.5	25.81 90/7.7	14.18 83/5.7	NE	NT			
020923	"	"	Co	53.42 395/9.8							47.80 35/8.5	39.22 93/9.0	29.44 97/7.7	14.94 99/4.9	-NE	NT			

Table A-5. Radicle growth results. (continued)

Treatment Number	Substrate	Species	Metal	0.0 Control	0.1	0.5	1	5	10	20	50	100	150	250	500	750	1,000	5,000	10,000
				Number of seedlings, mean radicle length, std. deviation at each concentration (ppm)[a]															
021021	Mineral soil	White pine	Cu	61.88 355/24.6							56.43 77/24.7	52.47 81/20.4	47.93 84/20.0	37.11 87/17.0	30.54 83/11.5	NT			
021022	"	"		61.88 355/24.6							43.70 76/18.5	29.37 83/11.4	20.81 80/9.9	8.06 17/6.8	NE	NT			
021023	"	"		61.88 355/24.6							52.11 79/20.9	39.09 86/14.0	28.10 81/13.2	17.63 82/7.9	8.76 51/3.5	NT			
020621	"	White spruce		39.59 324.13.1							37.38 88/11.3	33.40 82/11.3	29.41 80/10.3	27.95 74/8.9	18.35 83/8.4	NT			
020622	"	"		39.59 324/13.1							26.30 40/9.6	20.19 26/8.7	12.53 34/7.5	NE	NE	NT			
020623	"	"		39.59 324/13.1							26.95 82/9.3	20.42 83/8.2	14.40 84/7.2	7.53 68/3.2	NE	NT			
126921	"	Paper birch		10.64 180/3.7							8.67 76/2.8	7.04 76/3.3	5.24 55/2.8	~NE	NE	NT			
126922	"	"		10.64 180/3.7							4.24 43/1.3	~NE	~NE	NE	NE	NT			
126923	"	"		10.64 180/3.7							6.18 50/2.5	~NE	~NE	NE	NE	NT			
020931	Organic soil	Red pine		49.08 391/9.5										48.59 97/9.2	47.06 100/9.3	NT	49.07 97/9.9	36.43 99/10.2	15.56 98/5.7
020932	"	"		49.08 391/9.5										53.59 97/7.8	50.79 97/8.6	NT	51.62 97/10.3	34.66 96/10.9	9.36 74/7.2
020933	"	"		49.08 391/9.5										51.50 90/10.5	54.50 98/9.9	NT	53.46 100/8.5	37.85 97/10.9	6.85 89/3.7
020731	"	Black spruce		36.04 166/5.8										37.96 50/5.7	36.89 46/36.89	NT	37.10 30/5.7	26.74 50/8.3	17.86 64/4.6
020732	"	"		36.04 166/5.8										37.69 32/5.0	35.13 39/5.6	NT	34.69 51/6.0	24.51 41/6.5	NE
020733	"	"		36.04 166/5.8										36.36 56/5.9	37.12 25/5.5	NT	34.32 22/3.6	26.50 48/6.7	~NE

Table A-5. Radicle growth results. (continued)

Treatment Number	Substrate	Species	Metal	Control	AX 9001 65T	AX 9001 200T	AX 9002 65T	AX 9002 200T	AX 9003 65T	AX 9003 200T	AX 9004 200T	IP 9002 65T	IP 9002 200T	DP 9002 65T	DP 9001 200T	US 9001 65T	US 9001 200T
02092	Mineral soil	Red pine	None	43.66 58/14.6													
02094	Tailings	"	"		40.00 57/12.1	34.08 63/8.8	35.27 66/12.6	40.58 69/15.6	36.32 76/11.9	35.58 72/12.5	24.70 67/11.8	36.05 44/11.3	38.54 50/10.4	35.02 57/11.1	29.20 49/16.5	36.27 64/2.1	34.97 67/17.1
02072	Mineral soil	Black spruce	"	35.30 76/6.1													
02074	Tailings	"	"		38.43 70/7.8	34.27 60/8.2	36.92 74/7.4	38.74 61/6.4	32.80 59/6.7	33.31 77.78	34.96 76/6.4	36.36 55/8.5	37.29 63/7.1	39.65 54/4.9	37.68 73/7.5	18.88 66/5.2	35.47 79/8.4
12692	Mineral soil	Paper birch	"	14.61 46/5.1													
12694	Tailings	"	"		14.88 60/4.8	13.6 62/3.7	13.25 59/3.8	15.20 60/4.8	12.76 70/3.8	14.61 61/4.2	18.05 64/6.3	13.30 60/4.1	13.17 128/4.9	16.78 64/5.2	17.00 57.5.9	5.83 66/3.3	12.92 60/5.4

[a] Mean / N/std. dev.

[b] NT = No treatment at this concentration. Blank spaces also indicate no treatment.

[c] NE = No elongation - total inhibition of radicle growth. ~NE = Nearly total inhibition. At least 50 percent of radicles were less than 1mm in length.

Table A-6. Relative radicle lengths.

TREATMENT NUMBER	SUBSTRATE	SPECIES	METAL	CONTROL 0.0	0.1	0.5	1	5	10	20	50	100	150	250	500	750	1,000	5,000	10,000
				RELATIVE RADICLE LENGTHS AT VARIOUS CONCENTRATIONS (ppm)(Control=100)															
020811	Filter Paper	Jack Pine	Cu	a[3]	a	a	a	a	b	b	c								
				100	111	103	97	89	55	49	31	NT[1]							
020812	"	"	Ni	ab	ab	ab	a	bc	cd	d	e								
				100	102	101	103	79	73	53	36	NT							
020813	"	"	Co	ab		a	ab	a	bc	c	d	d							
				100	NT	115	108	122	87	73	47	34							
020911	"	Red Pine	Cu	a		a	a	b	c	cd	d	d							
				100	NT	104	100	75	48	39	30	23							
020912	"	"	Ni	a		b	b	bc	cd	cd	de	e							
				100	NT	84	80	74	60	54	41	31							
020913	"	"	Co	a		c	ab	bc	abc	bc	d	d							
				100	NT	71	91	81	86	75	42	31							
021011	"	White Pine	Cu	ab		ab	a	ab	ab	abc	bc	c							
				100	NR	111	115	91	101	88	80	58							
021012	"	"	Ni	ab		b	c	abc	a	bc	c	d							
				100	NT	86	66	94	119	88	76	29							
021013	"	"	Co	a			a	a	a	a	a	b							
				100	NT	107	108	88	104	85	95	47							
020711	"	Black Spruce	Cu	a		a	a	b	c	d									
				100	NT	102	97	73	47	32	NE[2]	NE							
020712	"	"	Ni	a		a	a	b	c	d									
				100	NT	102	95	67	44	20	NE	NE							
020713	"	"	Co	a		a	a	b	b	c	d								
				100	NT	104	101	85	77	44	25	NE							
020611	"	White Spruce	Cu	ab		ab	a	ab	b	c	c	c							
				100	NT	105	118	91	85	44	31	29							
020612	"	"	Ni	ab		ab	a	bc	bc	c									
				100	NT	105	122	79	81	53	NE	NE							
020613	"	"	Co	ab		ab	a	ab	ab	b	c	c							
				100	NT	100	124	113	105	92	47	24							

Table A-6. Relative radicle lengths (continued)

TREATMENT NUMBER	SUBSTRATE	SPECIES	METAL	CONTROL 0.0	0.1	0.5	1	5	10	20	50	100	150	250	500	750	1,000	5,000	10,000
				RELATIVE RADICLE LENGTHS AT VARIOUS CONCENTRATIONS (ppm)(Control=100)															
126911	Filter Paper	Paper Birch	Cu	a		a	b	c											
				100	NT	99	61	28	NE	NE	NE	NE							
126912	"	"	Ni	a		a	ab	abc	c										
				100	NT	89	79	50	35	NE	NE	NE							
126913	"	"	Co	a			a	a	b	b									
				100	NT	122	101	53	36	NE	NE	NE							
457711	"	Honeysuckle	Cu	a		a	a	a	a	a									
				100	NT	113	86	122	80	82	NE	NE							
457712	"	"	Ni	a		a	ab	bcf	bc	c									
				100	NT	113	92	61	70	42	NE	NE							
467713	"	"	Co	ab		abc	a	bc	ab	bc	c								
				100	NT	71	110	65	73	65	31	NE							
020914	"	Red Pine	Cu&Ni	a		a	a	a	b	bc	bc	c							
				100	NT	91	83	85	58	33	36	30							
020917	"	"	NO_3	ab		ab	ab	a	ab	ab	ab	b							
				100	NT	109	88	130	117	91	88	80							
020918	"	"	SO_4	ab		b	ab	ab	ab	a	ab	ab							
				100	NT	80	109	89	119	125	121	109							
020821	Mineral Soil	Jack Pine	Cu	a								b		c	d				
				100							NT	76	NT	53	33	NE			
020822	"	"	Ni	a								b		c					
				100							NT	56	NT	27	NE	NE			
020823	"	"	Co	a								b		c					
				100							NT	66	NT	37	NE	NE			
020921	"	Red Pine	Cu	ab							a	b	c	d	e				
				100							104	96	88	67	39	NT			
020922	"	"	Ni	a							b	c	d	e					
				100							92	75	48	27	NE	NT			
020923	"	"	Co	a							b	c	d	e					
				100							89	73	55	28	NE	NT			
021021	"	White Pine	Cu	a							ab	b	b	c	c				
				100							91	85	77	60	49	NT			

Table A-6. Relative radicle lengths (continued).

TREATMENT NUMBER	SUBSTRATE	SPECIES	METAL	CONTROL 0.0	0.1	0.5	1	5	10	20	50	100	150	250	500	750	1,000	5,000	10,000
					RELATIVE RADICLE LENGTHS AT VARIOUS CONCENTRATIONS (ppm)(Control=100)														
021022	Mineral Soil	White Pine	Ni	a 100							ab 71	b 47	b 34	c 13	c NE	NT			
021023	"	"	Co	a 100							b 84	c 63	d 45	e 28	e 14	NT			
020621	"	White Spruce	Cu	a 100							ab 94	bc 84	cd 74	d 71	e 46	NT			
020622	"	"	Ni	a 100							b 66	b 51	c 31	NE	NE	NT			
020623	"	"	Co	a 100							b 68	c 52	d 36	e 19	NE	NT			
126921	"	Paper Birch	Cu	a 100							b 82	c 66	d 49	∿ NE	NE	NT			
126922	"	"	Ni	a 100							b 40	∿ NE	∿ NE	NE	NE	NT			
126923	"	"	Co	a 100							b 58	NE	NE	NE	NT				
020931	Organic Soil	Red Pine	Cu	a 100										a 99	a 96	NT	a 100	b 74	c 32
020932	"	"	Ni	b 100										a 109	ab 103	NT	ab 105	c 71	d 19
020933	"	"	Co	b 100										ab 105	a 111	NT	a 109	c 77	d 14
020731	"	Black Spruce	Cu	a 100										a 105	a 102	NT	a 103	b 74	c 50
020732	"	"	Ni	a 100										a 105	a 97	NT	a 96	b 68	NE
020733	"	"	Co	a 100										a 101	a 103	NT	a 95	b 74	∿ NE

Table A-6. Relative radicle lengths (continued).

TREATMENT NUMBER	SUBSTRATE	SPECIES	METAL	CONTROL	AX 9001 65T	AX 9001 200T	AX 9002 65T	AX 9002 200T	AX 9003 65T	AX 9003 200T	AX 9004 200T	IP 9002 65T	IP 9002 200T	DP 9002 65T	DP 9002 200T	US 9001 65T	US 9001 200T
02092	Mineral Soil	Red Pine	None	a 100													
02094	Tailings	Red Pine	"		ab 92	bc 78	bc 81	ab 93	abc 83	bc 82	d 57	abc 83	ab 88	bc 80	cd 67	abc 83	bc 80
02072	Mineral Soil	Black Spruce	"	bcde 100													
02074	Tailings	Black Spruce	"		ab 109	cde 97	abcd 105	ab 110	e 93	de 94	bcde 99	abcde 103	abc 106	a 112	abc 107	f 53	bcde 100
12692	Mineral Soil	Paper Birch	"	bc 100													
12694	Tailings	Paper Birch	"		bc 102	c 90	c 91	bc 104	c 87	bc 100	a 123	c 91	c 90	ab 115	ab 116	d 40	c 88

[1]NT means no test at that concentration.

[2]NE means a test was performed but that there was no radicle elongation. NE means almost total inhibition- more than half of radicles were less than 1mm in length.

[3]a,b,c,d,e. Any values within a treatment group (horizontal row) that have the same letter are not significantly different at the 90% confidence level.