

Phytochelatin Are Bioindicators of Atmospheric Metal Exposure via Direct Foliar Uptake in Trees near Sudbury, Ontario, Canada

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Plants produce phytochelatin in response to copper and nickel, the primary metal pollutants emitted by the dominant smelting operation in Sudbury. Copper and nickel concentrations in soils decline sharply with distance from this facility, primarily as a result of early smelting practices. Phytochelatin concentrations in Sudbury-area trees, however, do not correlate with metal levels in soils. Rather, phytochelatin production in tree leaves is driven by metals currently released to the atmosphere through the 381 m emissions stack. Phytochelatin concentrations in the foliage of three tree species growing in situ are highest 20–30 km from the stack, correlated with maximum acid-leachable concentrations of deposited copper and nickel. Similar results observed in potted trees placed adjacent to indigenous trees confirm that aerially deposited metals are the source of current metal stress patterns. The addition of peat moss “filters” to potted soils did not alter this response, indicating that direct foliar metal uptake is responsible.

Introduction

One of the most dramatic and familiar examples of acute metal pollution is that of the smelter operations near Sudbury in the Province of Ontario, Canada. There, extensive historical pollution with copper and nickel has resulted in almost complete elimination of plants in the vicinity of the smelters. Undoubtedly, there must also be more subtle effects farther away, but it is difficult to ascertain and quantify their extent and gravity. Metal concentrations in the soil can easily be measured, but these concentrations are not necessarily related to metal uptake by the plants as a host of other chemical factors, in the soil and the atmosphere, also influence uptake. Even metal concentrations in plant tissues are not always a good indicator of physiological effects for they include extracellular metal deposits, and contamination of samples is a common and difficult problem. Therefore, it

would be most helpful to have a specific and sensitive biological indicator of metal stress to study the sublethal effects of metal pollution in areas such as Sudbury.

Phytochelatin, a class of metal binding peptides with the general formula $(\gamma\text{Glu-Cys})_n\text{-Gly}$, where $n = 2-11$, are induced in plants subjected to intracellular metal stress. They are synthesized in the cytoplasm in response to an excess of certain metals, including Cu and Ni, the primary atmospheric metal pollutants in the Sudbury region (1). All plants surveyed produce phytochelatin (2) or related compounds (3–6) which may thus provide a useful quantitative link between metal exposure and physiological stress. The applicability of phytochelatin for quantifying metal stress in natural ecosystems has previously been reported in studies on high-altitude forests (7), phytoplankton (8), and aquatic mosses (9). Using these compounds as bioindicators, we study here the effects of soil and atmospheric metal contamination on trees growing in the vicinity of the smelting operations at Sudbury. We show that current atmospheric metal emissions from the smelters are having a measurable physiological impact on the surrounding forests up to 40 km away from the smelters and that direct foliar uptake of metals deposited to the leaves is the dominant pathway for metal exposure to trees.

Site History

Sudbury is located approximately 60 km northeast of Lake Huron on the Canadian Shield geological formation. Since 1883, following the completion of the Canadian Pacific Railroad, the ore deposits have been increasingly exploited and have yielded a large percentage of the world's copper and nickel supply as well as a significant amount of iron, cobalt, gold, silver, and selenium (10–12).

Until banned in the late 1920s, open-bed roasting of unprocessed ore created ground-level smoke containing high concentrations of sulfur dioxide and metals. Damage to the surrounding vegetation was severe, creating a barren wasteland in a 3–8 km radius around the pits (13). The present-day signature of this early smelting technique is a distinct pattern of metal concentrations. Measurements taken in the 1970s reveal that the highest levels of Cu and Ni (3700 and 3000 ppm, respectively) in forest litter and surface soils occur in the area immediately adjacent to the site of the old roaster beds. The concentrations of Cu and Ni then decrease rapidly with distance from the INCO site, dropping below 300 ppm within 35 km (14). Since the ban on open pit roasting, emission stacks of various heights have been used to disperse SO₂ and metal-containing aerosols over a larger area. However, local meteorological instabilities have been found to cause plume looping, resulting in the periodic deposition of high levels of metals and acids in close proximity to the smelters (15).

In 1972, a 381 m “superstack” was completed at International Nickel Company Limited's (INCO) Copper Cliff plant, 8 km west of Sudbury, as a means of complying with environmental regulations designed to improve ground level air quality in the surrounding area. This stack, with an effective release height of approximately 600 m, was intended to elevate emissions above the inversion layer that plagued earlier, shorter stacks. However, “looping” conditions still continue to affect the plume during the summer months, even at this height, resulting in episodes of plume impingement within 3–10 km of the stack (16). At the same time that the “superstack” was brought online, INCO closed down its nearby Coniston facility and incorporated all smelting operations at its Copper Cliff plant. Falconbridge Nickel Mines

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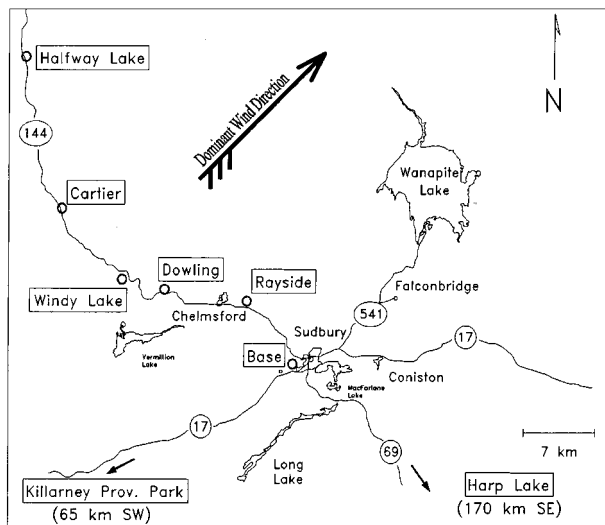


FIGURE 1. Map of Sudbury, Ontario, area showing sampling locations and distances from the INCO "superstack". Distances to the Killarney Provincial Park and Harp Lake control sites are not to scale. The dominant wind direction for May to October is indicated.

Limited operates the only other significant smelter in the area, 16 km northeast of Sudbury, but the INCO plant emits 20 times the metals that the Falconbridge facility discharges (16). Therefore, the largest current source of atmospheric metals emissions in the Sudbury region is the "superstack" at INCO's Copper Cliff facility.

Materials and Methods

Sampling stations were established along a transect beginning 1 km from the base of the "superstack" and extending almost 60 km to the northwest, following highway 144 [Figure 1]. Winds in the Sudbury area blow predominantly out of the southwest from May to October, as indicated in Figure 1. As a result, the primary direction of transport for smelter emissions is toward the northeast. Unfortunately, the Sudbury-Falconbridge urban corridor also extends away from the INCO smelter in the same northeasterly direction, potentially confounding attempts to differentiate smelter effects from urban sources. Therefore, the northwest transect orientation chosen for this study—and by other researchers (17)—maximizes smelter emission effects while minimizing potentially confounding sources of metals found in urban areas. Six sites were located along this transect—Base (1 km from the "superstack"), Rayside (15 km), Dowling (23 km), Windy Lake (32 km), Cartier (44 km), and Halfway Lake (59 km)—and control sites were established at Harp Lake (170 km southeast of Sudbury) in 1993 and then in Killarney Provincial Park (65 km southwest of Sudbury) in 1994 and 1995. Although the control site at Harp Lake, like the northwest sampling transect, is located in a direction perpendicular to the dominant wind direction, the 170 km distance from the "superstack" is assumed to negate the influence of smelter emissions. The Killarney control site was established opposite the prevailing wind direction and thus avoids this uncertainty. All sites were at least 0.5 km from the highway to minimize the impact of automobile metal emissions.

In May of 1993, foliage samples were collected from the north and south sides of three trees at each site. Leaves and petioles only (approximately 1.5 mL of packed foliage) were taken from branches approximately 5–8 ft above the ground. Repeat sampling in October of that year was carried out in the same manner but did not include the Base and Rayside locations. *Betula papyrifera* trees were sampled at all locations and times, with the exception of the control site at Harp Lake

where *Populus alba* was used in May and replaced with *B. papyrifera* in October. All samples were placed in separate cryovials, immersed immediately in a portable dewar containing liquid nitrogen, and sent to MIT where they were kept in liquid nitrogen prior to analysis.

Sampling was repeated in May, June, and August of 1994 at the same locations, with the exception of the control site, which was moved to Killarney Provincial Park for easier access. In 1994, samples were pooled from the north and south sides of each tree as the previous year's results showed no consistent relationship between exposure aspect and phytochelatin measurements. *B. papyrifera*, *Abies balsamea*, and *Populus tremuloides* (three trees for each species) were sampled at each site. *A. balsamea* was not found growing at the Base site, and thus there are no data reported for this species at that location.

Three potted 2-year-old *B. papyrifera* saplings from nursery stock were placed on the ground surface at each of the sites on June 27, 1994, thus preventing direct exposure to metals in the soils while allowing wet and dry atmospheric deposition. A ceptometer was used to measure photosynthetically active radiation (PAR) at the point of placement to ensure that exposure to atmospheric sources would be consistent. At the point of placement, 95% or greater of the incident PAR reached the ground, while 90% or greater reached the ground over a 15 ft radius from the potted sapling. Samples were collected—as described above for 1994 in situ vegetation—on the date of placement and again on August 10 of the same year. Theft of the saplings from the Rayside site prevented sampling in August at that location.

A similar experiment utilizing potted saplings was carried out in 1995. The saplings were sampled on the date of placement, June 22, and again prior to removal on August 11. A greater number of foliage samples were collected this year; two samples from each sapling were collected and analyzed separately in June, while three samples were taken from each tree in August. Before placement of these trees at the sampling sites that year, crude "filters" for atmospheric metals were added to the potted saplings. These "filters" consisted of approximately 2 in. of packed commercial peat moss placed on top of the soil in the pots and held in place by a screen. An overflow conduit was also included on each pot so that excess water could drain away.

Samples of the peat moss used in 1995 were collected in August and placed in sealed vials in liquid nitrogen for shipping and storage. These peat samples, along with "control" samples taken from the bag prior to use, were dried at 80 °C for 3 days in an oven. Acid-leachable metals were extracted from approximately 0.1 g of dry peat moss by shaking overnight in centrifuge tubes with 5 mL of 0.12 N HCl. Extracts were centrifuged at 27 000 rpm for 30 min, filtered through 0.2 μm syringe filters, and stored in the refrigerator prior to analysis. These extracts were analyzed by graphite furnace atomic absorption spectrophotometer for Cu and Ni. Method blanks showed insignificant contamination during sample preparation (~5 ppb Cu and undetectable Ni), and analyzed peat moss controls had only low levels of Cu and Ni (12.7 ± 1.8 nmol/g dry peat and 11.3 ± 0.5 nmol/g dry peat, respectively).

For phytochelatin quantification, foliage samples were pulverized in liquid nitrogen in a ceramic mortar and pestle and homogenized on ice (Brinkmann Instruments' Polytron Homogenizer) in 5 mL of 0.01 M methanesulfonic acid. Homogenates were centrifuged at 4 °C for 30 min at 16 000g, and the supernatant was stored in the refrigerator prior to analysis. Details of the HPLC separation procedure and fluorometric detection using monobromobimane have been published elsewhere (18). Concentrations of phytochelatin have been normalized either to soluble protein concentrations (19) in the foliage or to foliar wet weight. Normalization

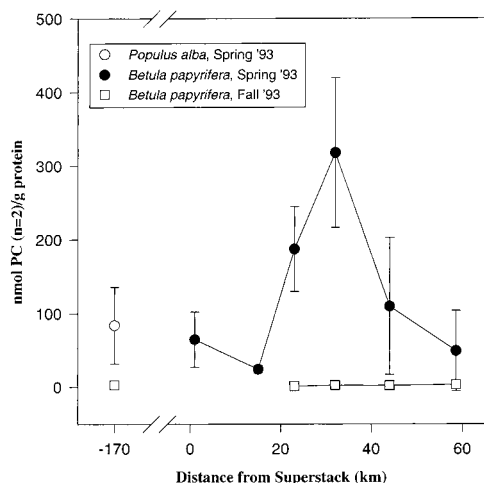


FIGURE 2. Concentrations of phytochelatin ($n = 2$ chainlength only) normalized to soluble protein in the foliage of trees growing in situ near Sudbury, Ontario, in the spring (circles) and fall (squares) of 1993. Concentrations of the longer phytochelatin chainlengths (e.g. $n = 3$ and 4) were not significant in comparison to the $n = 2$ chainlength and therefore have been excluded. Error bars are one standard deviation of the mean for each sampling location ($N = 6$) and are hidden behind symbols in the fall. The negative distance for the Harp Lake "control" site indicates that this sampling location is 170 km in the opposite direction.

to protein is thought to more meaningfully reflect the degree of physiological stress induced by metals as this directly relates phytochelatin production to the total pool of protein available to the plants and excludes morphological differences between species.

A one-way ANOVA was performed on data from each species for each sampling date to test the significance of the relationship between distance from the INCO stack and phytochelatin concentrations in foliage. Statistical significance is reported as p -values.

Results and Discussion

In the spring of 1993, phytochelatin concentrations normalized to protein in *B. papyrifera* were highest ($P < 0.05$) at the Windy Lake site, 32 km away [Figure 2]. The sites closest to the stack (Base and Rayside) had levels similar to the most distant site (59 km) and the control, although the same species was not sampled at the control site in the spring. In October, near the end of the growing season, phytochelatin concentrations had declined to a baseline level at all sites (~ 2.6 nmol phytochelatin/g protein). A similar pattern of high foliar phytochelatin concentrations in early spring, declining toward fall, has already been observed in *Picea rubens* in the Adirondack Mountains and is most likely the result of metabolic senescence (7).

Sampling the following year was designed to test whether the spatial distribution of phytochelatin concentrations observed in 1993 is unique to *B. papyrifera* or an indication of a more widespread phenomenon. In May 1994, all three species sampled produced similar results. Phytochelatin concentrations normalized to protein were highest in *B. papyrifera* at the 23 and 32 km sites ($P < 0.05$) [Figure 3a], in *A. balsamea* at the 32 km site ($P < 0.001$) [Figure 3b], and in *P. tremuloides* at the 16, 23, and 32 km sites ($P < 0.001$) [Figure 3c]. Elevated phytochelatin levels were also measured in May at the 59 km site for all species. The phytochelatin concentrations were similar in *B. papyrifera* and *P. tremuloides* but equal to only half of the concentrations measured in *B. papyrifera* in 1993. Concentrations in *A. balsamea* were much lower than in the other two. The relatively high standard

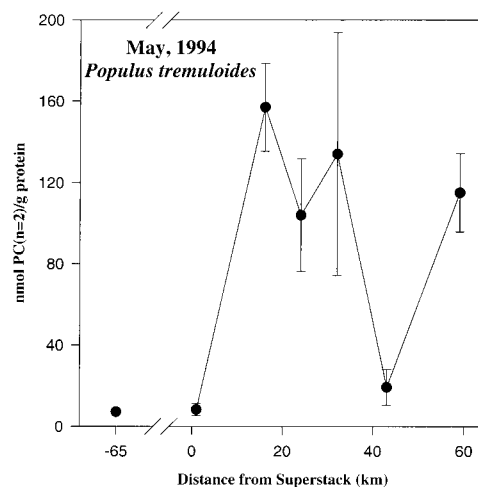
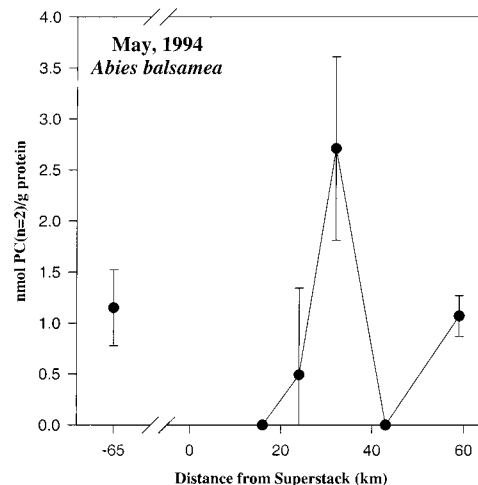
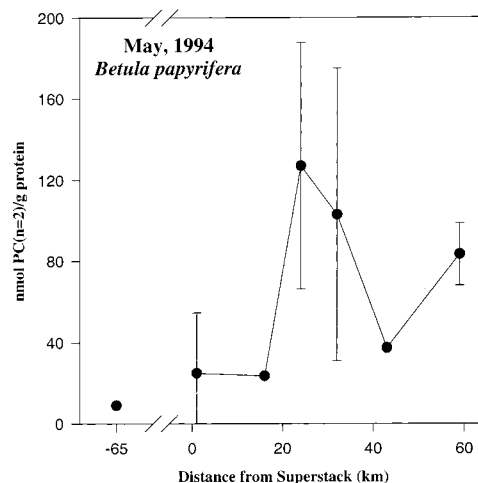


FIGURE 3. Concentrations of phytochelatin ($n = 2$ chainlength only) normalized to soluble protein in the foliage of (a) *Betula papyrifera* (paper birch), (b) *Abies balsamea* (balsam fir), and (c) *Populus tremuloides* (trembling aspen) growing in situ near Sudbury, Ontario, in the spring of 1994. Error bars are one standard deviation of the mean for each sampling location ($N = 3$).

deviations for these measurements in 1994, in comparison to those collected in 1993, reflect the smaller number of samples analyzed at each site in 1994 ($N = 3$) than in 1993 ($N = 6$). Phytochelatin concentrations remained low for all species at the control site in Killarney Provincial Park and at the Base site near the stack. By June of 1994, phytochelatin

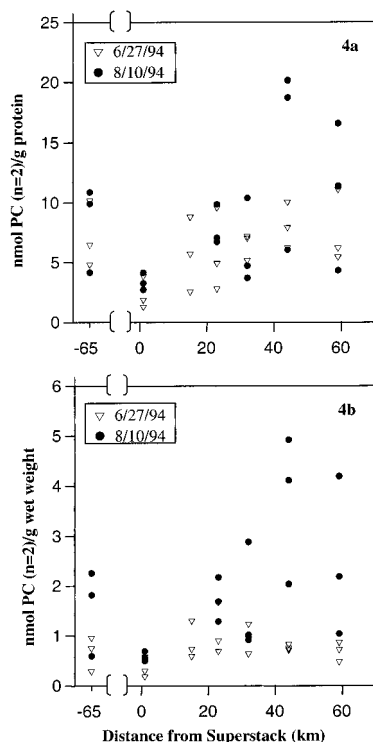


FIGURE 4. Phytochelatin concentrations ($n = 2$ chainlength only) normalized to (a) soluble protein and (b) wet weight in potted *Betula papyrifera* saplings placed at each sampling location in 1994. All samples are shown individually.

levels had decreased throughout the transect for all species, with the exception of *A. balsamea* at the 59 km site (data not shown). Normalization to wet weight of foliage produced similar results (data not shown). Samples collected in August of 1994 showed brown, dry areas on the foliage that put in doubt the validity of normalizations to both wet weight and protein. The data for August have therefore not been included. The consistent geographic pattern of phytochelatin production exhibited by all three species is evidence that the source of metal stress to these forests is not specific to a particular species, and the repetition of this pattern from year to year points to a relatively constant metal source. More importantly, it is obvious that soil metal concentrations (which are known to decrease with distance from the smelter) cannot solely account for the pattern of phytochelatin production near Sudbury.

To examine the relative importance of metal exposure from soil and atmosphere, we performed an experiment in 1994 with potted trees to eliminate exposure to contaminated soils. In late spring 1994, potted *B. papyrifera* were placed at the same sampling locations used the previous year. The phytochelatin response exhibited by these potted trees is qualitatively similar to the response of trees growing in situ [Figure 4a,b]. The potted trees were exposed for less than 2 months in 1994 (June 27 to August 10), and yet a peak in phytochelatin concentration normalized to protein ($P = 0.12$) and wet weight ($P = 0.06$) is evident at the Cartier site, 44 km from the stack. Since potted soils were chemically uniform and all saplings were from identical stock, neither differing soil chemistry nor differential metal adaptation can account for this pattern of metal stress. It appears that current deposition of atmospheric metals near Sudbury is driving the metal stress response in tree leaves. Differences in metal inputs caused by variable meteorological conditions are probably responsible for the shift in the phytochelatin peak from 30 to 40 km and perhaps for the much lower absolute

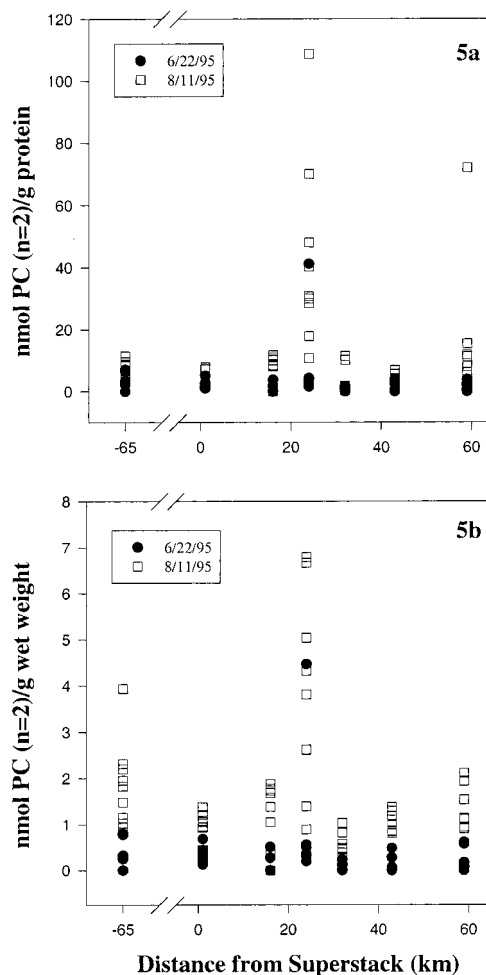


FIGURE 5. Phytochelatin concentrations ($n = 2$ chainlength only) normalized to (a) soluble protein and (b) wet weight in potted *Betula papyrifera* saplings containing an added peat moss filter, placed at each sampling location in 1995. All samples are shown individually.

phytochelatin concentrations observed in 1994. The low concentrations may also have resulted from the late date of the sampling since the maximum phytochelatin concentrations in leaves occur in early spring.

To confirm these results and determine the dominant pathway for metal uptake in trees near Sudbury, we repeated the potted tree experiment in 1995, with one important modification: a layer of peat moss was added as a simple metal filter for the potted soils. The metal binding capacity of peat moss has been documented previously (20). We also collected a larger number of foliage samples to improve the statistical significance of the data. Our results from 1995 show that phytochelatin production normalized to both protein ($P < 0.001$) and wet weight ($P < 0.001$) peaked that year at 23 km from the stack [Figure 5a,b]. In addition, maximum phytochelatin levels in the potted *B. papyrifera* in 1995 were within a factor of 2 of the levels measured in indigenous *B. papyrifera* in 1994 [Figure 3a]. These results demonstrate that direct foliar uptake by *B. papyrifera* is a dominant pathway, perhaps the dominant pathway, for intracellular metal exposure in the Sudbury area. The potential for direct foliar uptake of metals has been documented by other researchers (21, 22).

To test the efficacy of the peat moss filters and to document metal deposition at our sampling sites, we measured the acid-leachable fraction of Cu and Ni that accumulated in the peat moss over the experimental period. The results of these

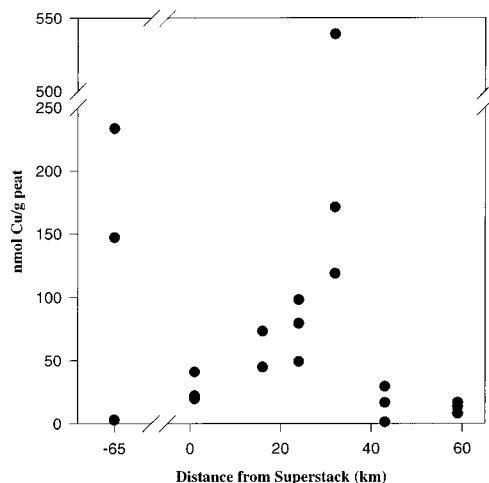


FIGURE 6. Copper concentrations extracted from peat moss filters recovered from potted *Betula papyrifera* saplings after exposure for two months in 1995. Samples are shown individually. Note the break in the vertical scale for the highest sample at the 32 km sampling site.

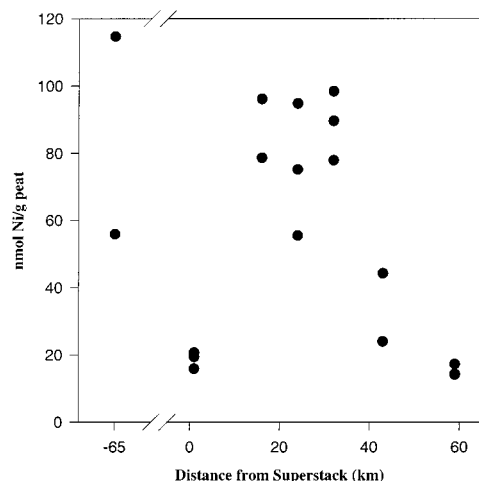


FIGURE 7. Nickel concentrations extracted from peat moss filters recovered from potted *Betula papyrifera* saplings after exposure for two months in 1995. Samples are shown individually.

analyses are shown in Figures 6 and 7 for Cu and Ni, respectively. Both metals show elevated concentrations away from the INCO stack, similar to our phytochelatin measurements. Excluding the control site, Cu was significantly higher at the 32 km site ($P < 0.05$), while Ni was higher at the 15, 23, and 32 km sites ($P < 0.001$). Unfortunately, no additional data were collected to help explain the high concentrations of Cu and Ni in peat moss at the control site in Killarney Provincial Park, where phytochelatin levels are consistently low. As discussed previously, metal concentrations alone may not be strictly correlated with physiological stress for a number of reasons. Nevertheless, the results of this experiment suggest that the (acid-leachable) concentrations of Cu and Ni deposited to Sudbury-area forests generally correlate with phytochelatin production in the trees.

Our finding that current "superstack" emissions are resulting in elevated metal concentrations 15–40 km away from the stack is supported by the data of Gundermann and Hutchinson (23). Their transect to the southeast of the closed Coniston smelter facility, 8 km east of Sudbury, is 180° from the direction of our transect, but both are perpendicular to the dominant wind direction (data from the Atmospheric Environment Service, Environment Canada). They find that

soils within 7 km of the Coniston smelter (i.e. within ~15 km of the "superstack") have much lower Cu and Ni concentrations and higher pH values now in comparison to measurements made before the "superstack" was in place. However, soils collected from sites over 30 km away from the "superstack" show lower pH values now than previously measured. Therefore, Gundermann and Hutchinson similarly conclude that current emissions from the INCO "superstack" are having a greater detrimental effect on forests more than 30 km away. In addition, our calculations using the Gaussian plume model for a continuous source (24)—with an effective stack height of 600 m, an average wind speed of 20 km/h (data from the Atmospheric Environment Service, Environment Canada), and stability class C/D—yield a distance to maximum ground-level emissions of 20–30 km from the "superstack". Although this model is very simplistic, the calculations support our findings and those of Gundermann and Hutchinson (23). Unfortunately, samples were not collected during our study for determining current metal concentrations in soils along our northwest transect, and to our knowledge no recent soil metals data have been published in the literature for this transect either. Therefore, we do not know for certain whether the spatial distribution of soil metal concentrations along our transect has changed significantly over time. However, given the findings of Gundermann and Hutchinson (23) described above, change seems likely.

Our results do not rule out the possibility that soil metal contamination may have some effect on trees in the Sudbury area. To the contrary, we have independent evidence that under different conditions metal contamination in the subsurface can be monitored using phytochelatin in foliage (25). Phytochelatin is produced in the roots as well as the foliage of plants, and laboratory and field studies have shown that metal concentrations and phytochelatin levels in the foliage of trees subjected to metal-contaminated soils are generally orders of magnitude less than in the roots themselves. It is thus conceivable that, in the Sudbury region, phytochelatin concentrations in the roots would display a signature resembling soil metal concentrations, while phytochelatin levels in foliage would reflect atmospheric sources. In other words, metal stress in roots may be greater near the Sudbury smelter, where metal concentrations in the soil are highest, while metal stress in the foliage may reach a maximum 20–30 km away as a result of current atmospheric metal deposition. Laboratory experiments carried out by Gundermann and Hutchinson (23) have shown that surface soils collected near the closed Coniston smelter have a greater negative impact on root growth of lettuce than soils farther away. Further work is necessary to investigate the combination of soil and atmospheric metal contamination on plant stress responses. Nevertheless, under present conditions, in the Sudbury area, current atmospheric metal deposition seems to have a dominant impact on tree foliage.

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