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VANTAAN TUTKIMUSKESKUS – VANTAA RESEARCH CENTRE

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Plant biology
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Academic dissertation

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II Nieminen, T. M., Ukonmaanaho, L. and Shotyk, W. 2002. Enrichment of Cu, Ni, Zn, Pb and As in an ombrotrophic peat bog near a Cu-Ni smelter in Southwest Finland. The Science of the Total Environment 292: 81—89.

III Nieminen, T. M. 2004. Effects of soil copper and nickel on survival and growth of Scots pine. Journal of Environmental Monitoring 6: 888—896.

IV Nieminen, T. M. and Saarsalmi, A. 2002. Contents of Cu, Ni and Zn in smelter-polluted soil-plant systems. Geochemistry: Exploration, Environment, Analysis 2: 167—174.

V Nieminen, T. and Helmisaari H.-S. 1996. Nutrient retranslocation in the foliage of Pinus sylvestris L. growing along a heavy metal pollution gradient. Tree Physiology 16: 825—831.

VI Nieminen, T. M., Derome, J. and Saarsalmi, A. 2004. The applicability of needle chemistry for diagnosing heavy metal toxicity to trees. Water, Air, and Soil Pollution 157: 269—279.

Author's contribution

Tiina Nieminen was responsible for the data handling, preparation and writing of all the papers (**I–VI**), as well as for the idea of papers **I**, **III**, **IV** and **VI**. The experimental design of papers **II, III**, **IV** and **VI** was also planned by her. Heljä-Sisko Helmisaari was the supervisor of the thesis and paper **V** is based on her suggestion. The field work of paper **V** was planned by Heljä-Sisko Helmisaari. The design of the field experiments (**I**, **V**, **VI**) was provided jointly by Prof. Eino Mälkönen, Heljä-Sisko Helmisaari and John Derome. The idea for paper **II** was initiated by William Shotyk, Liisa Ukonmaanaho and Tiina Nieminen, who also jointly planned the sampling protocol. Anna Saarsalmi provided the litterfall (**IV**) and tree needle results (**VI**) and gave advice on their interpretation. John Derome provided advice in the calculations and in interpreting the results of paper **I** and **VI**.

Abstract

The aim of my thesis was to evaluate the lifelong response of Scots pine to a chronic Cu and Ni exposure in the vicinity of a smelter complex, as well as to study the fate of the pollutants in the studied forest ecosystems. Four study sites were established in pure Scots pine stands growing along an esker at distances 0.5, 2, 4 and 8 km southeast from the main stack of the smelters. In addition, the response of Scots pine to soil Cu and Ni was also studied by performing simulation experiments in a greenhouse.

The rate of Cu and Ni deposition on the forests was estimated by monitoring the current Cu and Ni concentrations in bulk precipitation and stand throughfall, and by estimating the past pollution loads on the basis of the amounts of Cu and Ni accumulated in the surface peat of an adjacent ombrotrophic bog. The measured Cu and Ni deposition at the forest site nearest to the smelters did not appear to be a reliable estimate of current metal input into the ecosystem, because of the high level of internal metal cycling *via* soil dust. The elevated Cu and Ni concentrations in the surface peat sediments of the ombrotrophic bog were interpreted as signs of a higher level of Cu and Ni deposition in the past compared to current deposition in the immediate vicinity of the smelters. In addition, the vertical distribution pattern of Cu suggests that the input of Cu to the peat *via* atmospheric deposition is retained in the top-most peat layers, whereas Ni showed a more even vertical distribution pattern reflecting downward migration. Nickel appeared to be more mobile than Cu also in the polluted forest soils, but this was not reflected as relatively higher Ni uptake by pine roots. The uptake of both Cu and Ni corresponded to their soil contents in the smelter-polluted forest soil, although the uptake rate of inorganic Ni from an artificial quartz sand substrate was, in some cases, higher than that of Cu.

The performance of experimental pine seedlings cultivated in smelter-polluted soil was similar to that of the mature pine stands growing along the study gradient. The variation in the biomass of the seedlings appeared to be related both to the toxicity of Cu and Ni and to the differences in the nutrient status of the experimental soils. However, smelter-induced pollution may affect pines also indirectly through changes in soil nutrient status, which are difficult to distinguish from the natural variation in fertility. The autumnal nutrient retranslocation from senescing needles to overwintering tree compartments was less efficient at the most polluted site compared to that at further distances from the smelters. According to the results of the artificial exposure experiment the lethal threshold for Cu concentration in pine roots would be approx. 1 000 mg kg^{-1}, while the corresponding value for Ni would be 100 mg kg^{-1}, thus indicating a higher toxicity of Ni. The corresponding thresholds for pine stem concentrations were 70 mg Cu kg^{-1} and 8 mg Ni kg^{-1}. The needle concentrations did not appear to be reliable indicators of Cu and/or Ni toxicity. This was especially true in the field, where the surface contamination of needles by metal-containing particles in the heavily polluted environment further complicated the interpretation of the measured Cu and Ni concentrations. The chloroform washing did not remove all of the metal-containing material attached to the needle surfaces.

1 Introduction

1.1 Copper and nickel pollution in terrestrial ecosystems

Although air pollutants may originate from natural sources, such as volcanoes, vegetation fires and salt spray from the oceans, human activities currently have major impacts on the global and regional cycles of most of the trace elements (Nriagu and Pacyna 1988, Nriagu 1990, Ross 1994). Anthropogenic sources of atmospheric copper and nickel are metal mining, smelting and refining, alloying, the petrochemical and fertilizer industries, the burning of fossil fuels, refuse incineration and the use of agricultural amendments (Nriagu and Pacyna 1988, Alloway 1995). The most traditional agrochemical application is the use of copper compounds as fungicides in vineyards since 1885 (Bordeaux mixture: $Ca(OH)_2$ + $CuSO_4$). This practise has resulted in substantial accumulation of Cu in the surface soils of vine growing areas (Brun *et al.* 2001, Parat *et al.* 2002, Ribolzi *et al.* 2002, Chaignon *et al.* 2003). Irrigation by metal-containing sewage water on agricultural fields dates back to the end of the 19th century around some old urban agglomerations in Central Europe (Rebele 2001, Kirpichtchikova 2003). The worldwide use of CCA (chromated copper arsenate) impregnated wood causes environmental contamination by Cu, As and Cr (Chirenje *et al.* 2003, Townsend *et al.* 2004). In Finland, the major sources of Cu are the metallurgical industry, and those of Ni energy production and the use of oil fuel in industry (Jalkanen 2000).

Copper and Ni particulates emitted from mining and smelting are primarily deposited locally, and thus the most severe environmental damage tends to be restricted to limited areas. In many areas, however, metal emissions are associated with SO_2 emissions. The effects of heavy metals on forest trees are connected to irreversible changes in soil processes, while the SO_2 emissions have a direct impact on the above-ground part of trees, but a less permanent effect on the surrounding soils (Winterhalder 1995). Two of the largest and most widely studied Cu-Ni mining and smelting areas in the northern hemisphere are the Sudbury region in Canada (Hutchinson and Whitby 1974, Lozano and Morrison 1981, Winterhalder 1996, Nriagu *et al.* 1998), and the large industrial agglomerations in the Kola Peninsula in NW Russia (Tikkanen and Niemelä 1995, Nöjd *et al.* 1996, Lindroos 1998, Rautio *et al.* 1998, Rigina and Kozlov 2000, Steinnes *et al.* 2000). In Sudbury, the barren land area has been estimated to cover a total surface of 170 km^2 and a large surrounding semi-barren area is reported to be about 720 km^2 (Winterhalder 1995, 2000). According to Oleksyn and Innes (2000), the area affected by forest death in the Kola Peninsula is 600–1 000 km^2. Extensively documented, smaller Cu/Cu-Ni smelting complexes are situated at Gusum, Sweden (Tyler 1984, Bååth 1989, Köhler 1999) and Harjavalta, Finland (Laaksovirta and Silvola 1975, Hynninen 1986, Heliövaara *et al.* 1987, Fritze 1989, 1996, Helmisaari *et al.* 1995, Kiikkilä 2003) as well as a mining and smelting complex at Sulitjelma, Norway (Lobersli and Steinnes 1988).

As a result of the long-range transport of metal-containing aerosols, heavy metals originating from anthropogenic sources have also reached remote areas. For instance, the trace metal profiles determined on Arctic ice cores are in reasonable accord with the calculated historical changes in the rates of anthropogenic emissions into the atmosphere (Nriagu and Pacyna 1988, Boutron *et al.* 1995). Peat and lake sediments can also be used as archives of the past atmospheric metal deposition rates (Gubala *et al.* 1995, Shotyk 1997, Sternbeck and Östlund 2001, Renberg *et al.* 2002). Valuable information about pre-industrial metal concentrations in forest plants can also be obtained from the analysis of old herbarium plants (Lobersli *et al.* 1990).

During the past decade, emissions of heavy metals have decreased in Northern Europe (Melanen *et al.* 1999), which has been reflected in national bioindicator surveys as lowered metal concentrations in forest mosses (Mäkinen 1994, Rühling and Tyler 2001, Steinnes 2001, Poikolainen *et al.* 2004). In Norway, it has been estimated that long-range transport is less important than local sources for Cu and Ni contamination in forest mosses (Steinnes 2001). In Finland, too, local sources have a great impact on the nation-wide distribution of Cu and Ni concentrations in forest mosses (Poikolainen *et al.* 2004).

The weathering of primary minerals is the most important source of trace elements in terrestrial ecosystems in non-polluted areas (Ross 1994, Henderson 1998). Since the amounts of trace elements in the solid soil phase are usually low and weathering processes slow, the release of elements results in very low quantities of available forms (Kabata-Pendias 2001). However, in some cases the weathering of specific metalliferous minerals can result in metal concentrations toxic to non-tolerant-plants, *e.g.* soils derived from ultramafic (serpentine) rocks have a distinctive stunted vegetation adapted to the prevailing elevated Ni concentrations (Brooks 1998).

1.2 Plant responses to excess soil Cu and Ni

Accumulation of metals in toxic amounts in plant substrates affects many metabolic processes, although the sensitivity of different plant species varies widely. According to Jackson *et al.* (1990), Cu toxicity is related to its high affinity for sulfhydrul groups, causing inactivation of sulfhydrul-containing enzymes or altering their catalytic specificity or control. High Cu concentrations stimulate peroxidative degradation of the lipids of membranes, which leads to increased membrane permeability (Marschner 1995, Adriano 2001). Although Cu is a toxic metal at high concentrations, it is also an essential micronutrient (Lipman and MacKinney 1931). Most of the functions of Cu as a plant nutrient are based on the participation of enzymatically bound Cu in redox reactions (Marschner 1995). Consequently, plants need a copper homeostasis mechanism that provides the necessary Cu ions at the required enzymatic sites, while reducing or eliminating their deleterious interactions (Jackson *et al.* 1990).

Nickel has quite recently also been found to be an essential micronutrient for plants (Brown *et al.* 1987), although the role of Ni in plant metabolism remains still largely unknown. At the present time, urease is the only known nickel-containing enzyme in higher plants (Marschner 1995). Only a few studies have been addressed at Ni toxicity (Jackson *et al.* 1990), and the mechanism of Ni toxicity to plants is not well understood (Kabata-Pendias 2001). Leaf chlorosis, stunted growth and dark colour of the roots are

described as symptoms in both Ni and Cu toxicity cases (Marschner 1995, Adriano 2001, Kabata-Pendias 2001).

Baker (1987) divides plants into two main categories with respect to their response to excess amounts of metals in their growing substrate: excluders and accumulators. Excluders survive in polluted soils through avoidance, whereas accumulators survive through a physiological tolerance mechanism. Hyperaccumulator is a term established by Brooks *et al.* (1977) for extreme accumulators that can enrich metal concentrations higher than 1 000 mg kg^{-1} in their above-ground tissues. The most famous hyperaccumulator is a small perennial shrub (*Alyssum bertolonii* Desv.) that can contain up to 7 900 mg Ni kg^{-1} in its leaves (Brooks 1998). It grows on ultramafic rocks in Tuscany, and was scientifically documented already in the 16th century by the Italian botanist Cesalpino (Brooks 1998). Even today, there are many more plant species that are known to hyperaccumulate Ni than those hyperaccumulating Cu.

Several biochemical mechanisms appear to be involved in the Cu tolerance of higher plants. Chelating molecules are apparently of crucial importance in Cu tolerance, although their exact role is not well understood (Jackson *et al.* 1990). The immobilization of Cu in cell walls, in cell vacuoles and in nondiffusible Cu-protein complexes has also been related to Cu tolerance (Jackson *et al.* 1990, Kabata-Pendias 2001). Organic acids are believed to play a central detoxifying role in Ni accumulating plants (Lee *et al.* 1978, Yang *et al.* 1997).

Some plant species are suggested to show constitutive tolerance, *i.e.* they possess a tolerance mechanism even though they are not exposed to metals (Baker 1987, Baker and Proctor 1990, Ye *et al.* 1997, Monni *et al.* 2000). Plant populations can also evolve tolerance through heritable adaptation (Baker *et al.* 1986). Although the evolution of metal-tolerant grasses in nature is a classic example of local adaptation (Antonovics *et al.* 1971, Dickinson *et al.* 1996), tolerant ecotypes of long-lived trees are much rarer due to their long generation times. In an exposure study by Kopponen *et al.* (2001), birch clones (*Betula pendula* Roth, *B. pubescens* Ehrh.) from the vicinity of the Harjavalta Cu-Ni smelters in Finland showed better Cu tolerance than birch clones from Zn polluted or non-polluted areas. However, most plant species do not evolve tolerance and are either eliminated from toxic sites (Bradshaw and McNeilly 1981) or survive through gradual acclimatisation by individual plants (Dickinson *et al.* 1991).

The metal excluders possess a restricted metal uptake and transport (Baker 1987). Besides the control of metabolic root uptake, also apoplasmic metal uptake can be restricted by plants through the establishment of a suberin-rich transport barrier in their roots (Schreiber *et al.* 1999). The binding of metals on extracellular binding sites in the root cortex can also be regarded as an exclusion strategy (Baker 1987, McLaughlin 2002). In addition, root uptake restriction can also be based on simple avoidance, expressed as the orientation of the roots into less toxic soil microsites (Turner and Dickinson 1993). Metal avoidance can be facilitated by root associated ectomycorrhizal species, since the spatially large ectomycorrhizal mycelium can efficiently immobilize metals (Galli *et al.* 1994, Colpaert and Assche 1992, Tichelen 1999) and it ameliorates the ability of roots to reach clean soil layers. Metal tolerant strains of mycorrhizal fungi have been isolated from metal polluted soils (Hartley *et al.* 1997). However, according to the review of Godbold *et al.* (1998), ectomycorrhizas do

not universally ameliorate metal toxicity, and amelioration is dependent on the species and on the strain of the ectomycorrhiza, as well as on the metal in question.

1.3 Phytoavailability of soil Cu and Ni

The rate of element uptake from a solution into plants can be described using Michaelis-Menten kinetics: $F = V_{max}\, c / (K_m + c)$ (Marschner 1995). In a fundamental work carried out by Epstein and Hagan (1952) ion transport through the membranes of plant cells was regarded as formally equivalent to the relationship between an enzyme and its substrate. Ion uptake by plants has features of saturation kinetics, which is related to the assumption of control (*e.g.* number of binding sites: carriers, permeases; capacity of H^+ efflux pumps). According to the equation, the ion transport rate is dependent on two factors: V_{max}, which is the maximal transport rate when all available binding sites are loaded, and K_m a constant, equal to the substrate ion concentration (c) giving half of the maximal transport rate. The basic scheme has stood the test of time, and this model is still commonly used as a component in plant uptake models.

However, the formal application of the Michaelis-Menten kinetics is not always in accordance with the experimental results (Marschner 1995). The relationship between the substrate metal content and its uptake is regulated by many factors, such as interactions with other ions in solution or changes in plant uptake processes. For instance, at high metal concentrations damage to cell membranes and transport proteins may also cause a reduction in metal uptake rates, so that the effects of phytotoxicity may be confused with saturation of the uptake mechanisms (McLaughlin 2002). Furthermore, the Michaelis-Menten model depicts basically the short-term element uptake pattern in controlled nutrient solution cultures, which cannot be directly generalized to *in situ* conditions. The presence of the solid matrix in soil strongly affects metal availability, thus turning the substrate metal concentration into a complex concept. As a general rule, the partitioning of metals between the solid and solution phases reduces metal availability, although the distribution between the soil phases is not constant, but varies with time (Allen 2002, Sauvé 2002). The strength of binding of a metal by the soil varies markedly across soils as a consequence of differences in soil properties (Allen 2002). According to Kabata-Pendias (2001), the association of trace elements with specific soil phases and their affinity for each soil constituent is the key factor determining their behaviour in soils (Fig. 1).

Total metal contents in the soil depict the potential availability of metals; in fact total concentrations refer to the complete dissolution of the solid matrix and quantification of its chemical constituents (Sauvé 2002). In mineral soils the real total content is of little relevance in terms of trace element availability, and strong acid digestions are more frequently used instead. The basic aim of the number of more subtle extraction schemas is to estimate the metal pool available to plants or other soil biota (McLaughlin 2002). Since plants access metals in the soil principally through the soil solution, it would be expected that the determination of metal concentrations in the soil solution would provide the best predictor of their availability. However, the prediction capacity of soil solution concentrations appears to be unclear. For example, the effect of pH is contradictory. Although a decrease in pH increases soil solution metal concentrations, it does not enhance the metal uptake by plants to the same extent

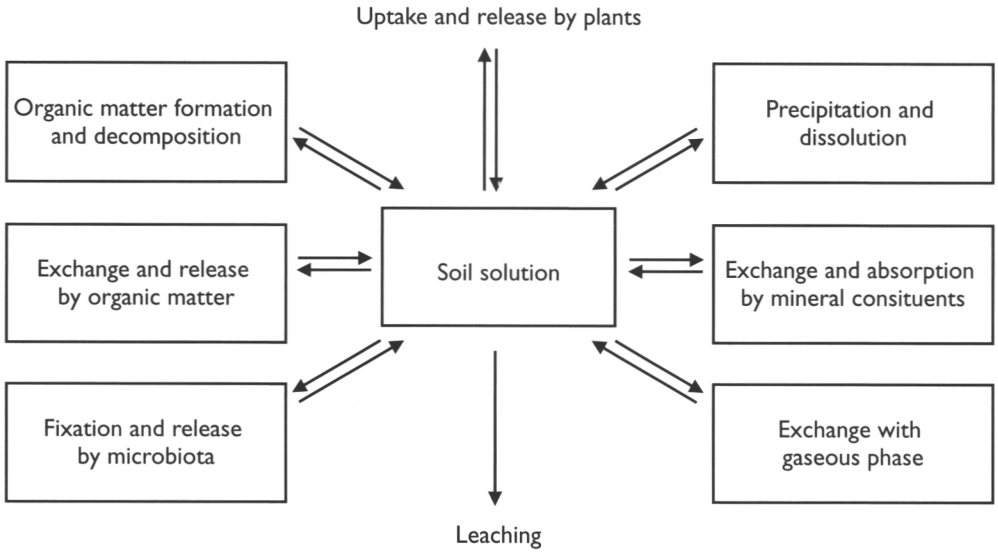


Fig. 1. Schematic diagram of the key interactive processes in the soil system (modified from Kabata-Pendias 2001).

(McLaughlin 2002). On the basis of a model developed in aquatic toxicology (Free Ion activity Model, FIAM), it has been supposed that the use of free ion activities of soil solution would give the best indication of their availability also to terrestrial plants (Sauvé 2002). Nevertheless, at the present time there are insufficient data to determine whether soil solutions or soil extracts are the best indicators to use, and the additional utility of considering free metal ion activities also remains questionable (Allen 2002, McLaughlin 2002).

Copper is known to be a rather immobile element in soils, which is related to the formation of organic complexes (Adriano 2001, Kabata-Pendias 2001). The mobility of Cu in soils is highly dependent on soil pH (Kabata-Pendias 2001). Nickel appears to be relatively mobile in soils with a high complexation ability, due to the apparent remobilization of Ni from solid phases in the presence of organic acids (Kabata-Pendias 2001). The sorption of Ni in soils is largely pH-dependent (Adriano 2001). An average value of 30 mg kg^{-1} for the total Cu concentration and 20 mg kg^{-1} for total Ni of world soils have been reported by Adriano (2001).

1.4 Ecological characteristics of Scots pine

Scots pine (*Pinus sylvestris* L.) has the widest geographical amplitude of all pine species. It forms the northern timberline in Fennoscandia, and the southernmost Scots pine populations can be found in Spain (Atlas Flora Europaeae 1973). It grows on a wide range of soils, including peat, and tolerates both acid and slightly alkaline soil conditions, although its volume production is highest on well-drained acid soils (Kujala 1958). Scots pine is not susceptible to drought but, as a pioneer tree species (Hämet-Ahti *et al.* 1989), it does not tolerate shade. Abundant seed crops are produced

irregularly, every 3 to 6 years, with light crops in intervening years (Sarvas 1962a, Koski and Tallqvist 1978). The rooting system of Scots pine is deep (Laitakari 1927), and it is associated with ectomycorrhizal fungi.

Natural fires, as well as slash-and-burn cultivation, have historically played an important role in Finnish forests (Tolonen 1983), and this has lead to a dominance of Scots pine since it is relatively resistant against fires and has an efficient colonization capacity on burned soils (Sarvas 1962b). At the present time, Scots pine is still the dominant tree species in Finnish forests. Being the most important raw material for the Finnish forest industry, it has been favoured by forest management practices (Tomppo 2000). However, in several studies Scots pine has been reported to be relatively sensitive to air-borne pollutants (Huttunen *et al.* 1983, Laine *et al.* 1994, Bäck *et al.* 1995, Innes 1995), and it appears to be especially susceptible to sulphur dioxide (Huttunen and Laine 1983, Katainen *et al.* 1984, Huttunen *et al.* 1985, Turunen *et al.* 1997). Extensive studies on the performance of Scots pine under the impact of sulphur dioxide and heavy metal emissions from the Ni-Cu smelters on the Kola Peninsula have recently been reported by Nöjd (1996) and Rautio (2000).

1.5 Element cycles at the ecosystem level

In the 1950s element cycling at the ecosystem level became a subject of extensive study. The concept of whole-ecosystem studies *in situ* was introduced by H.T. Odum's early works on strontium (Sr) cycling and energy and material flows in aquatic ecosystems (Odum 1951, 1957), in which he connected element geocycles with biotic cycles. Later, he pioneered large-scale whole-ecosystem studies in rain forests of Puerto Rico and in ecosystems under the impact of anthropogenic inputs, *i.e.* surface waters and swamps affected by waste water (referred to by Mitsch and Day 2004).

Already in the 1950s, Ovington (1957, 1959) studied biomass production and nutrient cycling in Scots pine plantations at different stages of development. However, in the 1970s the application of the whole-ecosystem concept in forests became strongly developed in research on the relationship between acidic deposition and forest functioning. Mathematical models can serve as important tools in describing and predicting the characteristics of biogeochemical cycling, *e.g.* the MAGIC model developed by Cosby *et al.* (1985). Also processes that cannot be directly measured can be described by modelling, *e.g.* mineral weathering by the PROFILE model (Sverdrup and Warfvinge 1990, Jonsson *et al.* 1995). Ulrich (1974, 1981, 1992) built up the system analysis concept of forest functioning by dividing the forest ecosystem into compartments that exchange elements with each other and across ecosystem boundaries. The aim of this kind of approach is a quantitative evaluation of the compartments and element fluxes within the forest ecosystem.

In Finland, Mälkönen (1974) published the annual nutrient budgets of Scots pine stands at different developmental stages, and in a birch stand (Mälkönen 1977) already in the 1970s. Later on, Helmisaari (1995) and Helmisaari *et al.* (2002) studied biomass production and nutrient cycling in a chronosequence of Scots pine stands in eastern Finland. Saarsalmi *et al.* (1985) and Saarsalmi and Mälkönen (1989) studied nutrient cycling in *Alnus incana* (L.) Moench stands, and Saarsalmi (1984) also in a *Salix* 'Aquatica Gigantea' plantation. Major nutrient and acidity budgets at the catchment

scale have been determined in remote areas by Forsius *et al.* (1995), and at the forest stand level by Ukonmaanaho and Starr (2002).

Paavilainen (1980, 1984) studied the relationship between fertilization and nutrient cycling in peatland forests. Furthermore, Finér (1992) published an extensive report about the nutrient dynamics of Scots pine in drained peatland ecosystems. Nieminen (2003) studied the consequences of clear-cutting on nutrient output from drained Scots pine mires, while Piirainen *et al.* (2004) reported the effects of forest clear-cutting on the nutrient fluxes in podzol soils. Although studies on nutrient cycling in Finnish forests are relatively frequent, the cycling of heavy metals has rarely been included in such studies. Ukonmaanaho *et al.* (2001) presented heavy metal budgets for two forested catchments in background areas of Finland, Bringmark and Lundin (2004) recently reported results on heavy metal stores and fluxes in several European sites including Finland, while Starr *et al.* (2003) estimated the importance of weathering for ecosystem heavy metal budget in a background forest area. However, no studies on either nutrient or heavy metal cycling in heavy metal polluted forest ecosystems have been realised in Finland prior to the present study.

1.6 Aims of the study

The aim of my thesis was to evaluate the lifelong response of Scots pine to a chronic Cu and Ni exposure in the vicinity of a smelter complex. The focus of the research articles of this thesis is on juvenile (**III**, **IV**, **VI**) and mature phases (**I**, **V**, **VI**) of the lifecycle of pine. The rate of Cu and Ni exposure was studied by **1)** monitoring the current deposition level (**I**, **VI**), **2)** estimating the past pollution load on the basis of the accumulated amounts of metals in the surface peat of an adjacent ombrotrophic bog (**II**), **3)** estimating the soil Cu and Ni content and their phytoavailability (**III**, **IV**), and **4)** performing simulation experiments (**III-IV**).

The determination of metal exposure rates and plant responses for long-lived trees is a complex task, because only the current status can be measured by direct means. I have traced the past metal deposition and plant response patterns on the basis of production and emission records provided by the smelter company, vertical metal distributions in peat sediments, and pine annual ring chronologies.

Most of the studies dealing with the effects of heavy-metals on plants have been conducted as a short-term exposure without considering the relevance of the assessment for long-term exposure. The life-cycle approach with terrestrial plants has been rare, even with annual crops and herbaceous plants. In order to cover a full lifecycle of long-lived plants, such as trees, extensive long-term investigations are needed. Our current understanding of the response of trees to high metal loads is mostly based on data from the early life phases, *i.e.* on the responses of newly germinated seedlings or on seed germination trials. Therefore, in my experimental manipulations (**III**, **IV**), I used seedlings that had already reached the age of 4 years. The duration of the experiments was extended to 17 months in order to study the responses over two successive growing seasons.

As the persistence of a tree stand in a polluted environment is largely dependent on the functioning of nutrient cycling in the ecosystem, my studies on the responses in the mature phase were concentrated on different aspects of the nutrient cycling in

the studied forests (**I**, **V**). In addition, the fluxes of Cu and Ni were studied. Therefore, the approach of this study could be included in the field of ecotoxicology which, according to Cairns and Mount (1990), is a study of the fate and effect of toxic agents in ecosystems.

The interactions between the tree canopy and metal deposition loads (**I**), as well as the internal nutrient cycling of the trees (**V**), were studied along a pollution gradient. In addition, the consistency between foliar metal concentrations over successive time intervals and measured metal deposition loads was studied, and the diagnostic value of foliar concentrations evaluated (**VI**).

The specific aims of the thesis were:

- to determine the current metal input to the ecosystems along the smelter pollution gradient
- to trace the past input loads and amounts accumulated in the soil
- to evaluate the availability of Cu and Ni to Scots pine in polluted soils
- to define the responses of Scots pine seedlings to soil Cu and Ni exposure in controlled conditions
- to determine the efficiency of internal nutrient cycling in mature pine stands growing along the smelter-pollution gradient
- to estimate the critical toxicity thresholds for Cu and Ni concentrations in plant tissues and soil
- to estimate the proportion of surface contamination out of needle total metal concentrations

2 Material and methods

2.1 Study area

Harjavalta (61°19´N 22°9´E) is a small industrial town situated in southwestern Finland, about 30 km from the coast, in the southern boreal coniferous zone (Ahti *et al.* 1968). The long-term (1960–1990) mean annual temperature at a near-by weather station of the Finnish Meteorological Institute is +4.0°C and the annual precipitation 558 mm. The area has been subjected to a heavy pollution load since 1945, due to the relocation of a large metal smelter from eastern Finland to Harjavalta during the final stages of World War II. At that time Harjavalta was a completely rural area with practically no industry (Poutanen and Kuisma 1998).

The smelter was reconstructed on a forested esker running in a NW-SE direction. The sorted sand heathland was considered as ideal for the rapid reconstruction of the plant owing to the urgent need for copper for military purposes (Poutanen and Kuisma 1998). Copper smelting started at Harjavalta in 1945 and nickel smelting in December 1959, and a nickel refinery was built in 1960. The blister copper produced by smelting has been transported to a copper refinery in Pori, located some 30 km from Harjavalta. There has never been any ore mining at Harjavalta.

The heathland Scots pine forest growing in the immediate vicinity of the smelter died during the first years of industrial activities (Poutanen and Kuisma 1998). The dead pine stands were left uncut until the mid 1950s, which aroused a lot of publicity and gave a nationwide negative image to the smelter. However, owing to the lack of any scientific documentation of the damage, the exact rate and extent of this forest damage remains unclear. Poutanen and Kuisma (1998) report that Docent Esko Kangas was asked by the smelter company to evaluate the damaged area in 1946. He concluded that the damage was limited to the immediate vicinity of the smelter. However, it is worth noting that the forested area in this part of Finland was, and still is, relatively limited due to the intensive agricultural use of land. In practice, only the relatively infertile sites not suitable for crop cultivation, like this sorted sand esker, had been left in a forested state. Poutanen and Kuisma (1998) further report that, in 1946, altogether 47 farmers reported that pollution-induced damage had taken place in their fields. The total surface area of the affected fields was reported to be almost 100 hectares.

Public concern arose again in the 1970s because of the visible damage in gardens and forest trees in the vicinity of the smelters. This initiated several scientific research works carried out in the area. A comprehensive review of the studies carried out since 1975 in and around the Harjavalta area has been published by Kiikkilä (2003). A systematic survey of the condition of forests in Harjavalta and neighboring municipalities was carried out for the first time in 1979 by the regional Forest Centre of Southwest Finland, and it has subsequently been repeated every fifth year. The purpose of the survey is to determine the reduction of growth rates in forests considered to be affected by smelters, in order to determine appropriate compensation to be paid to forest owners by the smelter company. In 1985 the surface area, estimated to be affected by smelting, was 550 hectares. Fifteen years later, in 2000, the area had

enlarged to 700 hectares, and the most distant forest site considered to be affected was located 6.6 km from the smelters. The mean reduction in volume growth of the affected stands compared to control stands was 16%.

In 1992, the Finnish Forest Research Institute established study plot clusters (each plot 625–900 m^2 in size) in pure Scots pine stands growing along the esker at distances of 0.5, 2, 4 and 8 km southeast from the main stack of the smelters (Fig. 2.)

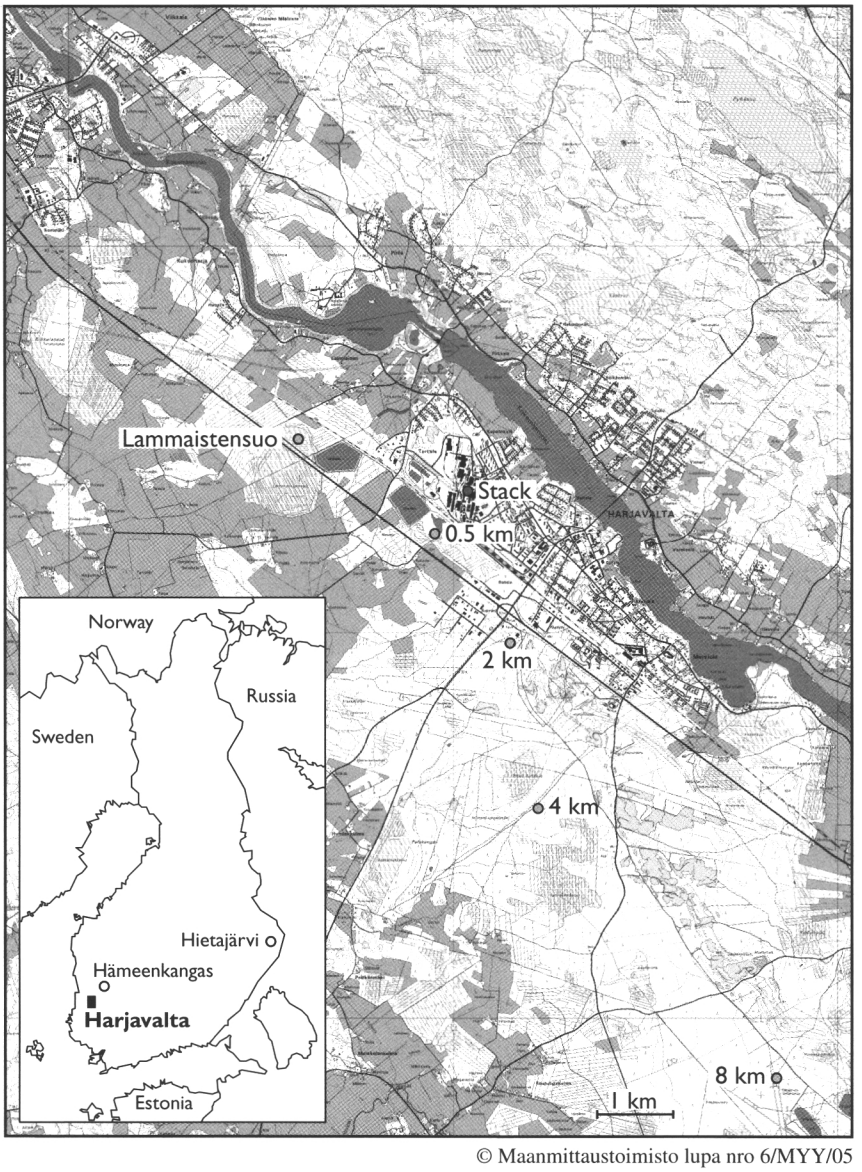


Fig. 2. Location of the Harjavalta study area, the forest background site at Hämeenkangas, and the reference peat bog at Hietajärvi in Finland. The study sites at Harjavalta are situated at distances of 0.5, 2, 4 and 8 km from the main stack of the Cu-Ni smelters, and the peat bog sample plot (Lammaistensuo) 2.4 km west from the stack.

(Mälkönen *et al.* 1999). The field data of this thesis (**I**, **III–VI**) have mostly been collected from these plots. The study plots are located on bedrock consisting of Jotnian olivine diabase. The soil comprises sorted glaciofluvial sediments, which poorly reflect the chemical properties of the underlying bedrock. One sampling site, situated at Hämeenkangas, 60 km northeast from Harjavalta (Fig. 2), in an area without any local pollution sources, was chosen as a background site. The texture of the mineral soil at all sites is fine sand, excluding the sites at 2 (fine/coarse) and 60 (coarse) km, and the soil type is orthic podzol (according to FAO-UNESCO 1998). The organic layer is mor, with a thickness ranging from 1 to 3 cm.

After almost 50 years of smelting activities the esker was still mostly covered by Scots pine forests, even though the large industrial complex and numerous sand pits caused by intensive sand extraction, have made the forests relatively fragmented (Fig. 2). The understorey vegetation of all sites is, or has originally been, typical of xerophilous forest sites: *Calluna vulgaris* (L.) Hull., *Empetrum nigrum* L., *Vaccinium vitis-idaea* L., *Pleurozium schreberi* (Brid.) Mit., *Dicranum spp.*, *Cladina spp. etc.* The understorey vegetation in the stand at 0.5 km is seriously damaged. According to a survey of understorey vegetation carried out in 1993 by Salemaa *et al.* (2001), lichens were absent up to a distance of 2 km, and mosses, excluding *Pohlia nutans* (Hedw.) Lindb., were not frequent until a distance of 8 km. In 1996, a remediation experiment (Kiikkilä 2002) was started at the same Scots pine site where the 0.5 km study plot is located. Needles of field-grown Scots pine seedlings in this experiment were used in Paper **VI**.

In addition, two ombrotophic peat bogs were chosen as sampling sites in 1999 for studying airborne metal accumulation (**II**): Lammaistensuo bog situated 2.4 km west from the main stack of the smelter and a reference site, Hietajärvi bog, situated in the Patvinsuo National Park in eastern Finland, without any local pollution sources within tens of kilometres (Fig. 2). Hietajärvi catchment has been systematically monitored since 1989 as a part of the UN-ECE International Cooperative Programme of Integrated Monitoring for the effects of long-range transboundary air pollution on forest ecosystems (Ukonmaanaho *et al.* 1998). The polluted Lammaistensuo site is located in the concentric raised bog zone, and the Hietajärvi site in a transition area between the eccentric raised bog zone and the northern aapa mire zone according to Ruuhijärvi (1983).

Both bog sites were undrained ombrotrophic bogs with a sparse Scots pine cover. The field layer of both sites was dominated by *Eriophorum vaginatum* L., but at the polluted Lammaistensuo bog only two species, *Pohlia nutans* (Hedw.) Lindb. and *Cladopodiella fluitans* (Nees) Buch, occurred in the ground layer and a large part of the bog consisted of unvegetated peat surfaces. The ground layer vegetation of the background site in Hietajärvi consisted of *Sphagnum* species typical to a pristine ombrotrophic pine bog, *e.g., S. angustifolium* C. Jens. and *S. fuscum* (Schimp.) Klinggr.

2.2 Emissions from the smelters

The chemical composition of the raw material used for smelting has naturally a great impact on the composition of the emissions. Up until the early 1970s, the ores

used for smelting were mainly domestic sulphidic minerals from the Outokumpu mine in eastern Finland. Chalcopyrite ($CuFeS_2$) was the most important copper and pentlandite ($(Ni, Fe+Co)_9S_8$) nickel mineral of the Outokumpu ore body (Mäkinen 1938, Disler 1953, Parkkinen and Reino 1985). Only ore concentrates have been used at the Harjavalta smelters, so no ore crashing or concentrating has taken place at Harjavalta. However, the transport and handling of the ore concentrates have produced dust emissions especially during the earlier periods of smelters' lifetime but, after some technical improvements realised in the 1970's, the direct ore-concentrate-dust emissions have been drastically decreased (Poutanen and Kuisma 1994). At present, the ore concentrates used for smelting are bought worldwide from different mining companies.

Metals are emitted from the smelter stacks as components of fugitive dust release. Regular monitoring of stack emissions was started in 1985 by Outokumpu Harjavalta Metals Oy (Table 1). Most of the combustion gases were emitted from a 70 m high main stack up until 1994, when it was replaced by a 140 m-high stack. At present, 80% of the Ni and Zn is emitted from the 140-m-high main stack (Saari *et al.* 1998). Copper is mainly (60%) emitted from a smaller 40-m-high stack (Saari *et al.* 1998). The Harjavalta smelter complex is one of the largest point sources in Finland (Melanen *et al.* 1999), and its metal emissions account for a large proportion of the total national emissions (Table 2). During the past decade, the emissions from Harjavalta smelters have drastically decreased owing to improvements in process technology and the installation of more efficient filter systems.

Stack emissions for the earlier operating period of the smelters (1945–1984) have been estimated according to the production rates provided by Outokumpu Harjavalta

Table 1. Annual sulphur dioxide and heavy metal emissions from the Harjavalta smelters during 1985–2003. Arsenic (As) measurements were started in 1993 (Source: Outokumpu Harjavalta Metals Oy).

Year	SO_2	Cu	Ni	Zn	Pb	As
			tonnes per year			
1985	8000	98	47	216	55	
1986	7500	126	46	232	60	
1987	7000	140	96	162	94	
1988	8000	104	45	103	48	
1989	9500	80	33	190	70	
1990	8804	80	31	160	80	
1991	5200	80	14	90	45	
1992	4800	60	10	12	9.0	
1993	4700	50	7.0	13	6.0	11
1994	5000	40	6.0	6.0	3.0	5
1995	3230	17	1.4	1.7	0.5	0.2
1996	3200	49	1.2	5.0	1.7	4.0
1997	3000	69	2.9	13.9	3.9	9.7
1998	3041	23	1.7	6.1	2.3	10
1999	3392	5.9	0.8	4.2	1.0	1.8
2000	3002	6.6	1.2	1.1	0.2	0.8
2001	3387	7.4	0.8	3.0	0.7	1.6
2002	3300	11.6	0.6	1.5	0.4	0.5
2003	3000	6.0	0.6	0.9	0.3	0.4

Metals Oy (Fig. 3). During the early 1940s, the sulphur in the combustion gases was not covered, and the SO_2 emissions were even greater than the amounts of copper produced. In the worst year, 1947, the copper production was 26 000 tonnes and the SO_2 emissions were 35 000 tonnes (Poutanen and Kuisma 1994). The construction of a sulphuric acid factory in 1947 considerably reduced the SO_2 emissions, especially after the start of full-time processing in 1949 (Poutanen and Kuisma

Table 2. Annual total sulphur dioxide and heavy metal emissions in Finland during 1990–2002 (Source: Finnish Environment Institute).

Year	SO_2	Cu	Ni	Zn	Pb	As
			tonnes per year			
1990	260000	94	67	571	326	33.2
1991	194000	91	45	381	248	22.1
1992	141000	66	37	284	175	10.0
1993	123000	54	26	260	100	14.3
1994	114000	49	34	316	60	9.3
1995	96000	27	34	322	57	3.5
1996	105000	55	25	191	35	7.2
1997	99000	72	28	70	19	12.3
1998	90000	27	21	71	20	12.4
1999	87000	-	-	-	-	-
2000	74000	19	33	71	38	4.6
2001	85000	19	33	69	38	5.2
2002	82000	28	36	88	40	3.7

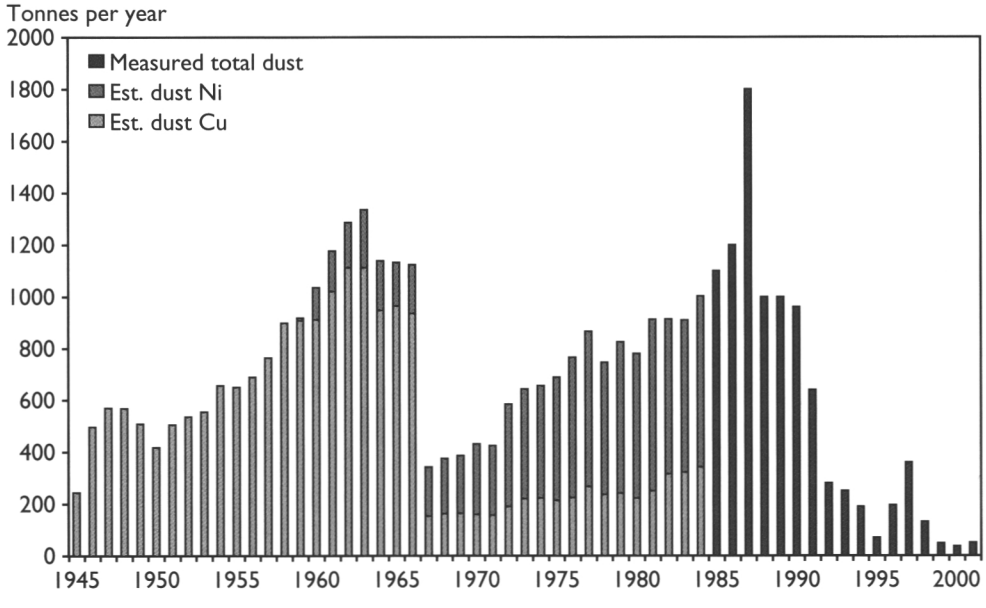


Fig 3. Production-based estimates of dust emissions from the Cu and Ni processes during 1945–1984. Regular measurement of stack-emitted dusts was started in 1985 (Source: Outokumpu Harjavalta Metals Oy).

1994). The flash-smelting process, an innovation of the Outokumpu company that was introduced in metal smelting in 1949, further improved the recovery of the SO_2 gases.

Important sources of heavy metals in the surroundings of the industrial plant are the different types of slag generated by the smelting processes, which have primarily been stored at the plant site. During the piling period, the uncovered slag heaps are a source of wind-borne dust emissions. The recycling and use of slag as land-fill and construction material further complicates the distribution pattern of slag-originating dust particles. An Environmental Impact Assessment report (EIA, Finnish law 10.6.1994/468), carried out by Geoinsinöörit Oy (2000) gives some details about the recent use of slag. Copper slag has been used in railroad ballast between Kokemäki and Pori, as well as in constructing a sound abatement barrier at the eastern end of the industrial area in the middle of the 1990s. The slag-cooling and handling area, which adjoins one of the study sites (0.5 km), is based on a Ni slag foundation, and the Ni slag has been used in the ballast of two railroad bridges inside the industrial area. In 1990, in order to reduce the overall dust emissions, the company started to transport the Cu slag in sludge form and to store it in land basins.

The composition and amounts of industrial wastes generated by smelting activities are given in the EIA report (Geoinsinöörit 2000). The main components of both Cu and Ni slag are fayalite (Fe_2SiO_4), magnetite (Fe_3O_4) and a glassy matrix. The elemental impurities are bound in fayalite and the glassy matrix (Table 3). The annual amounts produced in the end of the 1990s were *ca.* 320 000 tonnes of Cu slag and *ca.* 150 000 tonnes of Ni slag. In addition, gypsum (*ca.* 5 000 tonnes per year) and iron (*ca.* 20 000 tonnes per year) precipitates are generated in small amounts.

The EIA report also sums up the wide range of fuels used by the complex (Geoinsinöörit 2000). Most of the energy requirements are supplied by the ignition of sulphur in the ore concentrates (flash smelting), but also oil, coke and propane are used to some extent. The annual consumption of fossil fuels is *ca.* 25 000 tonnes, and

Table 3. Total mean concentrations of the potentially harmful elements in smelter slag in 1996 compared to the mean background values of mineral soils in Finland (Koljonen 1990). Concentrations in slag are determined by Outokumpu Harjavalta Metals Oy. Table modified from Geoinsinöörit (2000).

element	background concentration ppm	Cu slag		Ni slag	
		concentration ppm	enrichment	concentration ppm	enrichment
As	7.2	700	100 x	5	0.7 x
Cd	0.3	22	70 x	<1	<3.3 x
Co	10	400	40 x	2100	210 x
Cr	50	n.d.	-	1100	22 x
Cu	25	4100	160 x	1400	56 x
Ni	20	1400	70 x	2800	140 x
Pb	17	3800	220 x	99	6 x
Sb	0.7	370	530 x	0	0 x
Sn	5	2100	420 x	40	8 x
Zn	50	18100	360 x	500	10 x

the annual amount fuel needed by the smelters' transport vehicles is about 3 000 tonnes. Kerosene is used in the nickel fabrication processes, and its annual losses through ventilation of the industrial hall are *ca.* 30 tonnes according to the measurements made by the factory. The corresponding emissions of Ni as ventilation losses are about one tonne per year.

In 1995 a new reduction method for the production of nickel was adopted. Ammonia (NH_3) is used in the process, and ammonium sulphate ($(NH_4)_2SO_4$) is formed as a by-product. According to the EIA report, the ammonia emissions through process gas ventilation were *ca.* 750 tonnes in the year 2000 (Geoinsinöörit 2000). Sporadic emissions of ammonium sulphate -dust can also take place during loading operations.

2.3 Bulk deposition and stand throughfall

Bulk (free) precipitation and stand throughfall were collected at three sites along the Harjavalta study gradient, at 0.5, 4 and 8 km distances, as well as at the 60 km Hämeenkangas background site. Bulk deposition was collected in open areas close to the tree stands using 5 rainfall collectors (diameter = 20 cm) during the snow-free period, and 2 snow collectors (d = 36 cm) during the winter. Stand throughfall was collected using 20 rainfall collectors located systematically inside the stand on a 30 m × 30 m plot, and 6 systematically located snow collectors during the winter. Samples were collected at 1-month intervals during the period June 1992 to December 1998.

Water samples from the rainfall collectors on the same plot were combined to give one composite sample per plot at each sampling date. The volume and pH of the samples were measured and the remaining part of the samples filtered through a 0.45 µm membrane filter. Samples for the determination of Ca, Mg, K, Fe, Cu, Ni and Zn by inductively coupled plasma/atomic emission spectrometry (ICP-AES) were acidified with ultrapure 65% HNO_3 (0.5 ml/100 ml sample). Ammonium, NO_3^-, SO_4^{2-} and PO_4^{3-} were determined by ion chromatography. Dissolved organic carbon (DOC) was determined by digestion with sodium persulphate under UV radiation on a total organic carbon analyzer.

Bulk precipitation and stand throughfall samples at 0.5, 4 and 8 km distances during the snow-free period from June 1992 to December 1996 were used in Paper **I.** Bulk precipitation from June 1992 to December 1996 at the 0.5 km stand was used in Paper **II**. Both snow-free and winter period samples of stand throughfall during the year 1993 at 0.5, 4, 8 and 60 km were used in Paper **IV** in order to calculate the Cu and Ni fluxes *via* throughfall. Bulk precipitation and stand throughfall samples of the autumnal needle senescence period from July 1992 to September 1992 were used in Paper **V**. Both snow-free and winter period samples of the bulk precipitation and stand throughfall during the period from June 1992 to December 1998 were used in Paper **VI**.

In Papers **I**, **V** and **VI**, the net throughfall deposition was calculated as the difference between throughfall deposition (rainfall collectors inside the stand) and bulk deposition (rainfall collectors in an open area). In Paper **I** the sums of anions and cations were derived from the ionic concentrations ($mol_c\ L^{-1}$) of each sample. The Pearsson

coefficients of correlation between DOC and the anion deficit were calculated. Deposition loads (mol_c m^{-2}) were calculated by multiplying the concentrations by the amount of water (L m^{-2}) at each sampling date for both bulk precipitation and throughfall. The average deposition for each element was calculated as the arithmetic mean of the deposition values of all sampling dates from July 1992 to November 1996 (n = 34).

Canonical discriminant analysis was carried out in order to obtain the best combination of element variables in bulk precipitation and throughfall that discriminated the study stands from each other. The statistical significance of the effect of the canopy on the amounts of water and elements in precipitation was tested using the paired t-test (difference between the amount in bulk precipitation and that in throughfall).

In Paper **V**, nutrient net throughfall values for the autumnal senescence period were used as indices of needle nutrient leaching for this period at 0.5 and 8 km distances. Net throughfall from the whole canopy (mg m^{-2}) was transformed to net throughfall from the senescing needle mass by multiplying the net throughfall from the whole canopy by the ratio between the three-year-old needle mass and the total needle mass (equation (4) in **V**).

In Paper **VI**, mean annual Cu, Fe, Ni and Zn deposition in net throughfall and stand throughfall were calculated for the calendar years 1993–1998 on the basis of the amount of these metals in precipitation and the metal concentrations in the precipitation samples.

2.4 Litterfall

Tree litterfall was collected by 12 litter traps systematically located inside the pine stand at 0.5, 4 and 8 km distances along the Harjavalta study gradient and at the Hämeenkangas background study site at 60 km distance during the period July 1992 to December 1998. The litter traps were emptied every second week during March-July, and weekly during August-November up until the formation of a permanent snow cover.

Green and senescent needles, as well as pine seeds, were separated from the rest of the litterfall. The number of seeds and needles were calculated, and all litter compartments were dried at 70°C for 48 h, weighed and milled. Needle unit weight (mg $needle^{-1}$) was determined for each sample. Total P, Ca, K, Mg, Mn, Fe, Cu, Ni and Zn concentrations were determined by dry ashing, followed by extraction with a concentrated HCl. The solutions were analyzed using ICP-AES. Nitrogen and sulphur concentrations of finely ground needles were determined by Leco analyzers. Annual seed crops were calculated for the period 1993–1998.

In Paper **IV**, area-specific Cu, Ni and Zn deposition values (mg m^{-2}) were calculated from the concentrations and amounts of sample related to the surface area of the collectors for the sampling period of the year 1993.

In Paper **V**, the litter needle mass per unit area was calculated by dividing the total mass of the collected litter needles (kg) by the surface area of the litter traps (m^{-2}). The nutrient content of litter needles (mg m^{-2}) was calculated by multiplying the nutrient concentrations of litter needles (mg kg^{-1}) by the litter needle mass (kg m^{-2}) (equation (3)

in **V**). The relative element content per needle (μg needle^{-1}) was calculated by dividing the area-based content by the number of needles per unit area.

2.5 Needle sampling in the pine stands along the study gradient

Summer needles were collected in the middle of July 1992 from 8–10 trees per study site at 0.5, 4 and 8 km along the Harjavalta gradient and at the 60 km Hämeenkangas background site. The sample trees were randomly selected from five size-class groups representative of each stand. One random branch from the upper, middle and lower crown was sampled on each tree. One-hundred needle pairs were collected from each needle age-class on each branch, and the needles from the upper, middle and lower branch of the same tree were combined to form a sample of 300 needle pairs *per* sample tree.

The needle samples were dried at 70°C for 48 h, weighed and milled. Needle unit weight (mg needle $^{-1}$) was determined for each sample, and total element concentrations were determined in the same way as for the litter needles.

In Paper **I**, the needle element pools (mg m^{-2}) were calculated by multiplying the needle element concentrations (mg kg^{-1}) by the corresponding needle mass (kg ha^{-1}).

In Paper **V**, three-year old needle samples were used to calculate the senescing needle mass by multiplying the litter needle mass (kg m^{-2}) by the ratio between three-year-old green needle unit mass and litter needle unit mass (equation (1) in V). The nutrient content of the needles prior to senescence (mg m^{-2}) was calculated by multiplying the three-year-old needle mass (kg m^{-2}) by the nutrient concentration of the three-year-old green needles (equation (2) in **V**).

Winter needles for Paper **VI** were collected twice from three replicate plots in the 0.5 km stand: in January 1992 and in February 1998. The needle samples were taken from five sample trees, randomly selected from the dominant crown layer on each of the plots, *i.e.* from 15 trees in both sampling years. Only current-year needles (C) growing on the third to fifth branch of the whorl, counting from the top, on the southern side of the crown were sampled. The needle samples were dried (70°C for 48 h) and analyzed separately for each tree. The concentrations of Cu, Fe, Ni and Zn were determined from finely ground needles by wet digestion (HNO_3 + H_2O_2), followed by analysis by ICP-AES. To estimate the internal pool of metals in the needles, additional needles were collected in 1998 from five pine trees from the buffer zone around two of the replicate plots. The needles were sampled in the same way as described above. Half of the needles were washed with chloroform as recommended by Raitio (1995), and the other half were dried (70°C for 48 h) without any washing. The fresh needles were washed in chloroform for 1 min while stirring with a glass rod. The chloroform was then decanted off, the needles dried on filter paper, and then dried in the same way as the unwashed needles. The Cu, Fe, Ni and Zn concentrations of both washed and unwashed needles were determined according to the same method as for the regular needle samples. The statistical significance of the differences in needle nutrient concentrations between the washed and unwashed samples and between the two sampling years were tested using paired t-test.

2.6 Experimental approaches

2.6.1 Soil sampling along the gradient at Harjavalta

Intact volumetric soil profiles including the litter layer and ground vegetation were taken with an auger (diameter 25 cm, depth 30 cm) at the five sampling sites (0.5, 2, 4, 8, and 60 km) and placed in 10-liter pots. One 4-year-old, bare-rooted pine seedling (*Pinus sylvestris* L.) was planted on the 2nd of June 1994 in each pot. The soil profiles were taken at 25 points in 5 clusters on each site. A smaller volumetric soil sample was taken for chemical analysis next to each sampling point with a small auger (diameter 3.8 cm, depth 30 cm). The loose litter was removed from the top of the sample, and the sample was divided into the humus layer and two mineral soil layers: 0–10 and 10–20 cm. The samples from each cluster (5) were bulked to give five composite samples per layer per site. These samples were used in Papers **III** and **IV**.

2.6.2 Artificial exposure treatment

Pine seedlings of the artificial exposure experiment of Paper **III** were from the same 4-year-old seedling lot as those planted in the smelter-pollutes soil cores. They were planted on the 2nd of June 1994 in similar 10-litre pots as for the smelter-polluted-soil. Each pot contained 8 litres (11.34 kg) of quartz sand (particle size 0.5–1.5 mm). On the following day the soil-plant systems were treated with increasing doses of 1) copper sulphate ($CuSO_4 \cdot 5H_2O$), 2) nickel sulphate ($NiSO_4 \cdot 6H_2O$), or 3) a combination of both in equal doses (Table 1 in **III**). Two replicate seedlings were treated with each treatment dose in order to ensure a sufficient amount of plant material for chemical analysis. All the treatments were given as a water solution. The seedlings were fertilized twice during the experiment: 5th of July 1994, and 2nd of July 1995 using a commercial fertilizer. The total amount of nutrients added per pot in fertilization were 155 mg N, 55 mg P, 355 mg K, and 22 mg Mg. These doses were relatively low because achieving optimal nutrient conditions, which are unlikely to occur in natural conditions, was not the aim of the fertilization. At the end of the experiment soil samples were taken for chemical analysis with a small auger (diameter 3.8 cm) from the artificial quartz sand exposure pots. The samples from both replicates were combined to give one composite sample per treatment level.

2.6.3 Experimental conditions, harvesting and soil sampling at the end of the experiments

The four-year-old pine seedlings used in the experiment were bare-rooted and had been raised from selected seed in the forest nursery of the Finnish Forest Research Institute at Suonenjoki, eastern Finland. A set of 50 reference seedlings of the same 4-year-old seedling lot as those planted in the experimental pots were measured (average height 31 cm, standard deviation 5.8), weighed, and the element concentrations in the different compartments (roots, stem, needles) analysed at the start of the experiment. All the soil-plant systems (total number 185) were cultivated for 17 months (from June, 1994 until October, 1995) in controlled greenhouse conditions at the Ruotsinkylä field station

(60°21'N, 25°00'E) of the Finnish Forest Research Institute. A period consisting of two growing seasons was considered to be the optimal length for the experiment, since the primary stem growth of the woody test plant (*Pinus sylvetris* L.) starts to be predominantly prederminative already at the age of 5 years (Lanner 1976, Kanninen 1990). Hence, the primary stem growth of the experimental seedlings during the first year still largely reflected the environmental conditions of the previous year, which naturally also affects the biomass production.

Day temperatures were allowed to follow the ambient temperature during the summer months, but the night temperature was kept at 15°C. A constant temperature of +4°C was maintained throughout the winter period. The seedlings were watered by drip irrigation using ordinary tap water with a pH of 5.9, Cu concentration 0.35 mg L^{-1}, Zn concentration 0.03 mg L^{-1} and Ni concentration below the quantification limit (< 0.018 mg L^{-1}).

At the end of the experiment the pine seedlings were harvested and divided into root, stem and needle compartments. Green needles were collected by age classes: current needles (C), one-year-old (C+1) needles, and two-year-old and older needles (C+2). Senescent needles were collected throughout the course of the experiment and were stored in a dry place prior to analysis. The pine seedling data are presented in Papers **III–IV**.

At the end of the experiment soil samples for chemical analysis were taken using a small auger from each pot. The soil samples were divided into layers and those belonging to the same clusters were bulked together to give five composite samples per layer per site, as in field sampling prior to the experiment. These soil samples were compared to those taken in the field prior to the experiment in Paper **IV**.

2.6.4 Chemical analysis of the greenhouse experiments

The pine seedling samples were dried at 70°C for 48 h and weighed in order to obtain the biomass of the individual seedling compartments. The compartments of the seedlings grown in the smelter-polluted soil pots, and belonging to the same cluster, were bulked to give five composite samples per compartment per site. The replicate seedling compartments of the artificial quartz sand exposure were combined to give one composite sample per compartment per treatment level. The humus samples were dried, weighed and milled to pass through a 1-mm sieve. Total Cu, Ni, P, Ca, Mg and K concentrations were determined on the humus and pine seedling samples by dry ashing at 550°C, followed by extraction with concentrated HCl. The solutions were analysed by ICP-AES, and the total N and S concentrations were determined on the milled humus samples on LECO analysers. Exchangeable element concentrations were determined on the humus samples by extraction with 1M ammonium acetate (pH 4.65) with 2% EDTA (25 ml humus *per* 250 ml extractant, shaking for 1 h), followed by filtration and analysis by ICP-AES. The mineral forest soil samples were dried, weighed and passed through a 2-mm sieve to remove stones and large roots. The sieved portion of each sample was weighed. Exchangeable element concentrations were determined from the sieved mineral forest soil and from the quartz sand samples in the same way as for the humus samples. The pH of the quartz sand samples was determined in water (15 ml sample *per* 25 ml water).

2.6.5 Calculations and statistical treatment of the greenhouse experiments

The net uptake of Cu and Ni by the pine seedlings was determined by calculating the amount of metal per compartment sample (roots, stem, green needles and senescent needles). As these compartment samples were composite samples taken from 5 seedlings, the values were divided by five in order to obtain the value per seedling. Finally, metal uptake was obtained by summing up all the compartments per seedling, and then subtracting the average element content of the reference seedlings from the element content of each experimental seedling.

The data were analysed using SAS V8 statistical package. Simple linear regression equations were calculated for the relationships between the exchangeable Cu and Ni content of the quartz sand series at the end of the experiment and the amount of Cu and Ni originally added. The dependence of seedling biomass on the Cu and Ni content of the quartz sand media was calculated as a simple linear regression equation for all treatment series using ln-transformed treatment dose values. The independent variables were logarithmically (ln) transformed in order to linearize the asymptotic relationship with the dependent variable.

To find the variables that best explained the variation in biomass of the smelter-polluted-soil- grown seedlings, multiple regression analysis was performed using the Cu, Ni, Ca, K, Mg, and P contents of the smelter-polluted soil cores as independent variables. The calculation of the element contents of the smelter-polluted soil cores (mg *per* pot) is explained in detail in Paper **IV**. All possible regression models were fitted to the data with all possible combinations of the independent variables. The best model was found to be the model with Cu, Ni and P as independent variables. The multiple regression procedure was repeated using the total Cu, Ni, N, S, P, Ca, K, and Mg concentrations in the humus as independent variables. The humus Cu and N concentrations were found to be the best explaining variables.

Regression equations between seedling metal uptake and soil Cu and Ni content were calculated as simple linear models. Pearson correlation coefficients were calculated between soil Cu and Ni contents and the Ca, K, Mg, P, Cu and Ni concentrations of the roots, stem, senescent needles and green needles.

In Paper **IV**, the soil Cu, Ni and Zn concentrations are presented as an average value of the five replicate samples. The nonexchangeable metal concentration was obtained by subtracting the exchangeable concentration from the corresponding total concentration. The statistical significance of the differences between the concentration before and after the incubation period was tested by paired t-test.

2.6.6 Pine seedling experiment in the field

The field grown pine seedlings of Paper **VI** were planted in June, 1996, as 2-year-old containerised (peat containers) seedlings in the immediate vicinity of the study stand at 0.5 km distance from the smelters. Three experimental squares (5 m × 5 m) were established, and 49 seedlings were planted in each square. The seedlings were planted in soil pockets filled with 'clean' mulch consisting of a mixture of municipal compost

and woodchips. These seedlings were therefore grown in unpolluted soil in the field, but exposed to the heavy-metal deposition from the smelters.

Needles from the seedling experiment in the field were collected by needle-age classes in September 1998, when the seedlings had reached the age of 5 years. Needle samples were taken from three seedlings per square, and then bulked together to give one sample per square, *i.e.* a total of 3 samples of each needle-age class. The needle samples were dried (70°C, 48 h) and analysed by dry ashing and extraction with HCl, followed by analysis by ICP-AES.

2.7 Peat sampling

The peat core (15 cm depth × 5 cm × 5 cm) in Paper **II** was taken from the surface layer of the Lammaistensuo bog at Harjavalta, 2.4 km the Cu-Ni smelters. A reference core was taken from the background bog at Hietajärvi in eastern Finland. The cores were frozen and cut into 1 cm slices using a stainless steel band saw at the University of Berne. All peat samples were dried at 105°C in acid-washed Teflon bowls, and milled in a centrifugal mill equipped with a titanium rotor and 0.25 mm titanium sieve. The milling was carried out in a Class 100 laminar flow clean air cabinet to prevent possible contamination of the peat samples by laboratory dust. Selected trace elements were measured using the Energy-dispersive Miniprobe Multielement Analyzer (EMMA-XRF) (Cheburkin and Shotyk 1996) at EMMA Analytical Inc., Elmvale, Ontario, Canada. The instrument was calibrated using certified standard reference plant materials.

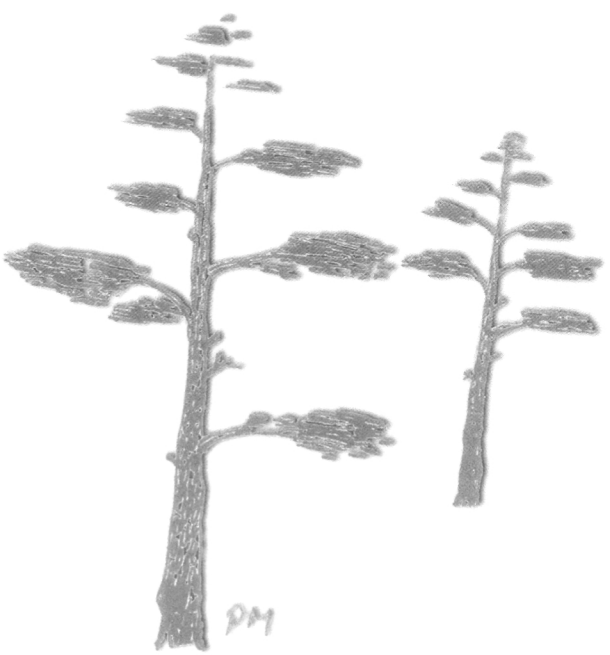
In order to separate the natural variation in element concentrations with depth from changes due to anthropogenic deposition, enrichment factors (EF) were calculated according to the following formula:

$$EF = (X/Ti)_{peat} / (X/Ti)_{crust},$$

where the data of Wedepohl (1995) was used for the values of the Earth`s crust. The calculated enrichment factors (EF) show the extent of the changes in element abundances in the profile relative to crustal values. We acknowledge that pre-anthropogenic (X/Ti) peat values may exceed those of the crust, but it is helpful to have a common reference level for comparison between the two cores, and with our published values.

Pollution factors (PF) were calculated for comparison between the polluted (Harjavalta) and the reference site (Hietajärvi). The average and median element concentrations of the Harjavalta core were divided by the corresponding concentrations of the Hietajärvi core. These PF values thus merely indicate the extent of pollution at the Harjavalta bog compared to that of the Hietajärvi bog.

The bulk density value of 87 g dm^{-3} reported by Veijalainen (1984) for the Harjavalta peat bog was used to estimate the total amounts of the studied elements in the 15-cm-surface-peat layer. It was assumed that this 15-cm surface-peat contains all the peat formed since the smelting activities started. Thus the total amount of Cu and Zn was divided by the duration of Cu smelting (55 years) in order to obtain a rough estimate of the average annual deposition during the smelter history. Nickel was correspondingly divided by 40, since Ni smelting started in 1959.



3 Results and discussion

3.1 Recent and past metal deposition at the study area

3.1.1 Bulk precipitation and stand throughfall

The deposition of metals (Ca^{2+}, Mg^{2+}, Fe^{3+}, Zn^{2+}, Cu^{2+}, Ni^{2+}), NH_4^+ and SO_4^{2-} through bulk precipitation and stand throughfall was the greatest at 0.5 km (Fig. 1 in **I**). As an average for the whole study period, Cu^{2+} and Ni^{2+} were clearly the most abundant heavy metals in both bulk and throughfall deposition. The element concentration of bulk precipitation changes during passage through the tree canopies (Helmisaari and Mälkönen 1989, Hyvärinen 1990, Bringmark *et al.* 2001, Ukonmaanaho 2001). Forest canopies are efficient at intercepting dry deposition (Hultberg 1985), which is reflected in stand throughfall as a strong enhancement of the dry deposited elements. In addition to canopy-intercepted dry deposition, throughfall also contains elements leached out from the living and dead foliar tissues (Tukey 1980, Parker 1983, Godt *et al.* 1986). Despite the sparse tree cover at 0.5 km, the amounts of metals intercepted as dry deposition by the canopy were extremely high, since a high proportion of the metals in throughfall were derived from the canopy (Fig. 1 in **I**). The results of canonical discrimination analysis demonstrated that the composition of both bulk precipitation and stand throughfall at 0.5 km was clearly different from that at 4 and 8 km (Fig. 2 in **I**).

The monthly average amounts of canopy derived Cu^{2+} (net throughfall) during 1992–1996 at 0.5, 4 and 8 km were 0.27, 0.004 and 0.0007 $mol_c\ m^{-2}$, respectively, and the corresponding amounts of Ni^{2+} 0.047, 0.0007 and 0.0007 $mol_c\ m^{-2}$ (Fig. 1 in **I**). Although foliar leaching of Cu^{2+} and Ni^{2+} cannot be completely ruled out, most of the canopy derived Cu^{2+} and Ni^{2+} at 0.5 km originates from the wash-off of previously dry-deposited material. This can be concluded from the ratios between the annual fluxes (mg m^{-2} $year^{-1}$) and needle pools (mg m^{-2}) of Cu and Ni (Table 5 in **I**). The annual Cu flux during 1992–1996 was nine times greater than the needle pool of Cu, and that of Ni almost 14 times higher. According to Pfirrmann *et al.* (1990), the leaching of most cations from spruce foliage represents only slightly more than 1% of the total content in the needles. In our experimental needle washing approach, a statistically significant proportion (40%) of Ni was removed from needles by chloroform washing (Table II in **VI**). In contrast, the difference between the Cu concentrations in the chloroform-washed and unwashed samples was not statistically significant. These results suggest that although the overall deposition of Ni is lower, the canopy deposited Ni is relatively more susceptible than Cu to wash-off from the canopy by rainfall.

The relatively high amount of NH_4^+ in both bulk precipitation and stand throughfall at 0.5 km (**I**) was caused by NH_3 emissions from the nickel-processing plant (Derome *et al.* 2004). The NH_3 emissions have had an increasing effect on S deposition by scavenging SO_2 from the air (Derome *et al.* 2004). Although SO_4^{2-} was the most abundant ion, both in bulk precipitation and in throughfall at all sites (**I**), only the amounts at 0.5 km were greater than those reported for Finnish background areas

(Helmisaari and Mälkönen 1989, Hyvärinen 1990, Ukonmaanaho 2001). Throughfall enhancement of SO_4^{2-} has also been reported in several earlier studies, and it is assumed to be mainly due to the canopy interception and subsequent wash-off of sulphur-rich, dry deposition (Mayer and Ulrich 1978, Probst *et al.* 1990, Cape *et al.* 1992, Hultberg and Grennfelt 1992, Lindberg and Lovett 1992).

The annual metal throughfall deposition values during 1993–1998 at the 0.5 km site (Fig. 2 in **VI**) do not follow the trends of the stack emissions (Table 1). Copper deposition in throughfall remained relatively constant during the study period, while there was a decrease in throughfall deposition of Zn. In the case of Ni, there was a very strong increasing peak in throughfall deposition in 1998 (Fig. 2 in **VI**), which is consistent with the observation of increased Ni concentrations in forest mosses in 2000 compared to 1995 in the surroundings of the Harjavalta smelters (Poikolainen *et al.* 2004), but inconsistent with the simultaneous decrease in stack-emissions of Ni (Table 1). Therefore, other sources of metals have to be of more importance for the measured metal deposition than the current stack-emissions. Possible sources of metals are the slag produced during smelting (*cf.* Chapter 2.2.), as well as wind-borne dust derived from the forest floor, where a considerable accumulation of metals has taken place during the more than 50 years lifetime of the smelters (**I**).

3.1.2 The extent of past metal deposition

The extent of pollution in the immediate vicinity of the Harjavalta smelters was determined by comparing metal concentrations in peat and precipitation samples to the values measured in corresponding media at the reference site Hietajärvi in eastern Finland (Fig. 2) The metal contents in the surface peat of the Lammaistensuo bog adjacent to the smelters (Fig. 2) reflect much higher Cu and Ni pollution rates than the current bulk precipitation at the 0.5 km site (**II**). This can, at least partly, be explained by the earlier considerably higher emission levels. (Table 1, Fig. 3). In addition to the stack emissions, possible sources of Cu and Ni are the slags formed during the smelting processes (Table 3). The wind-borne slag dust may have had a greater impact on metal deposition in the surrounding areas in earlier days prior to the recent technical improvements in slag handling. On the other hand, the direct effect of a recent influx of sludge runoff on the surface peat of the study bog cannot be completely ruled out. In spring 1998, the holding dam of a sludge basin adjacent to the bog burst and a considerable amount of sludge was released into the surroundings despite active clean-up efforts. Furthermore, the precipitation data are not fully comparable with the peat contents, since insoluble particles were excluded from the bulk precipitation samples by filtration, and the peat samples, parting turn, included all chemical and mineralogical forms.

Peat sediments can be used as indicators of past metal deposition only in the case of relatively immobile elements, such as Pb (Mackenzie *et al.* 1998, Shotyk *et al.* 1998, Weiss *et al.* 1999). According to our current study, Cu also appeared to be strongly retained by the peat (**II**). In contrast, Ni is known to be much more mobile than Cu in an organic matrix (Bergkvist *et al.* 1989, Kabata-Pendias 2001), and hence also more susceptible to downward migration in a peat profile.

3.2 Performance of Scots pine in smelter-polluted environment at different phases of its life-cycle

3.2.1 History of the forests under study

The deleterious effect of SO_2 gases on coniferous trees has been documented in numerous studies (*e.g.* Whitby 1939, Kikuzawa 1973, Huttunen 1975, Hutchinson and Whitby 1977, Legge *et al.* 1996), and *Pinus sylvestris* L. has proved to be among the most SO_2 sensitive species of the *Pinaceae* family (Caput *et al.* 1978, Genys and Heggestad 1978, Katainen *et al.* 1984). Therefore, it is most probable that the forest dieback, known to have occurred during the first years of smelting activities at Harjavalta, was caused by the direct toxic effects of SO_2. However, after the start of efficient sulphur recovery in 1949 and consequently improved air quality, the pine stands in the vicinity of the smelter were still able to recover. As the mean age of the 0.5 km study stand, 52 years in 1996, is approximately the same as the duration of smelting activities, it appears that the forest dieback of the early days was not totally complete. At least some of the young seedlings survived or new ones were rapidly established.

The radial growth series of the study stands indicate an abrupt decrease in growth rates of the 0.5 km stand in the mid-1970s (Fig. 4), which reflects the deterioration in growth conditions at the site. The critical threshold in soil metal accumulation may have been passed by that time. The vegetation damage area appeared to be at its largest in the 1970s (Laaksovirta and Silvola 1975), and public concern was also aroused at that time by the visible vegetation injuries in the gardens and forests surrounding smelters (Poutanen and Kuisma 1994). The decrease in radial growth of the 0.5 km stand was followed by a sudden increase (Fig. 4) due to the NPK fertilization applied

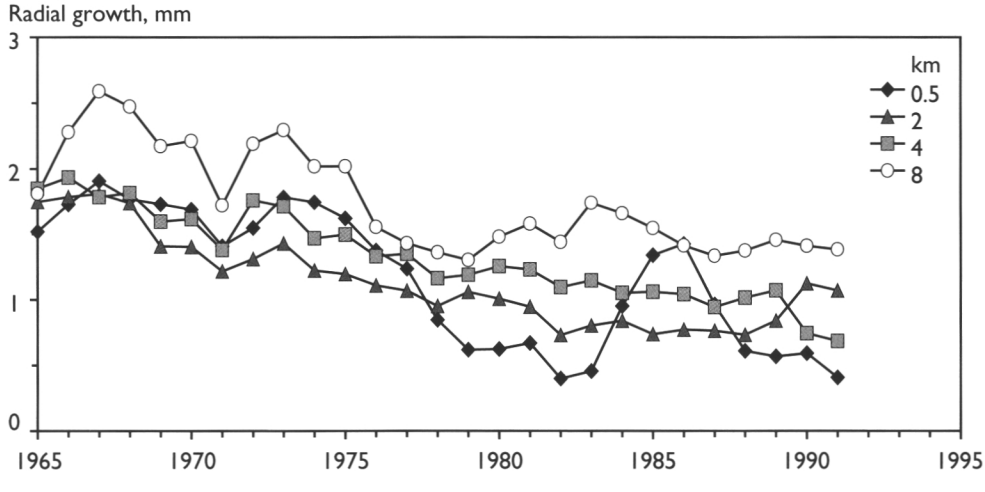


Fig. 4. Radial growth of trees in the experimental stands during 1965–1991 (modified from Mälkönen *et al.* 1999)

in 1983 (Mälkönen *et al.* 1999) containing *ca.* 120 kg N ha^{-1}. The effect of fertilization followed a normal response pattern and levelled off in the late 1980s.

3.2.2 Current growth rate of the study stands

All of the study stands are naturally regenerated middle-aged stands in which thinnings were carried out at an early stage (Mälkönen *et al.* 1999). The Harjavalta tree stands were measured in 1992 and 1996, and the Hämeenkangas background site in 1991 and 1995, in order to determine the volume increment, *i.e.* the difference in stand volume between the measurement years (Mälkönen *et al.* 1999). The overall low growth rate of the study stands (Table 4) reflects the poor fertility of the sites. The extremely low growth of the stand growing next to the smelters is related to the adverse effects of smelting activities (**I** and **III–V**), but the differences between the other stands are much more difficult to interpret. Their growth characteristics are not clearly related to distance and can simply indicate silvicultural and natural, between-site variation (Mälkönen *et al.* 1999, Nieminen *et al.* 2000). However, the role of pollution as a factor affecting growth also at greater distances cannot be completely ruled out. The indirect effects of metal pollution on tree growth, through deterioration of the nutrient status of the soil, are difficult to distinguish from the natural variation in soil fertility between sites subjected to low to moderate pollution conditions. The current growth rate of the stands, expressed as the annual volume increment during the last 5-year-period, is clearly different from the predicted increment estimate modelled by functions presented by Nyyssönen and Mielikäinen (1978), only in the case of the 0.5 km stand (Table 4).

3.2.3 Comparison with the experimental seedling cultures

The results of the greenhouse experiment, in which pine seedlings were cultivated in soil cores transported from the field plots, showed a relatively similar variation in the biomass production rates as the mature pine stands (Fig. 1 in **III**, Figs. 5 and 6). These results indicate that soil factors have a major impact on the current biomass

Table 4. Stand characteristics at different distances from the Harjavalta smelters. (Modified from Nieminen *et al.* 1998.)

Distance from the smelter, km	0.5	2	4	8	60	
Age of the study stands in 1992, years	49	54	48	40	in 1991	45
Stems, no ha^{-1} in 1992	1008	1230	1517	1552	in 1991	2063
H_{dom}, m in 1992	7.6	12.5	11.1	12.4	in 1991	9.2
Stem volume in 1992, m^3 ha^{-1}	23	85	68	95	in 1991	48
Annual volume increment, m^3 ha^{-1} $year^{-1}$ (1992–1996)	0.3	3.8	2.8	6.3	(1991–1995)	3.3
Predicted volume increment for the five year period, m^3 ha^{-1} $year^{-1}$ (according to Nyyssönen and Mielikäinen 1978)	1.6	4.4	4.2	6.2		3.7

production of Scots pine at the study plots, and that the current level of deposition is of minor importance.

However, the production rate of the 0.5 km seedlings was, in relative terms, even lower than that of the corresponding pine stand in the field. In fact the seedlings in the 0.5 km soil cores hardly grew at all, and they did not have any net biomass production during the experiment (**III**). Due to their limited photosynthetic capacity, survival has probably been strongly based on the consumption of storage carbon compounds. Furthermore, only four of the original 25 seedlings survived up until the end of the 17-month experimental period (**III**); at first site, this appears to conflict with the fact that the pine stand, with a current density of more than 1 000 trees per hectare, has been growing in the same polluted site for more than 50 years. However, in reality, the trees have not been growing for over 50 years in soil that is as polluted as it is now. Soil pollution has taken place gradually during the period of smelting activities, and metal accumulation has reached a level at which no natural seedling establishment can take place at 0.5–1 km distance from the smelters (Riissanen 1998, Salemaa and Uotila

Fig. 5. Needle mass of Scots pine seedlings grown in soil cores transported from a smelter-pollution gradient. C = current needles, C+1 = previous-year needles and C+2 = two-years-old and older needles.

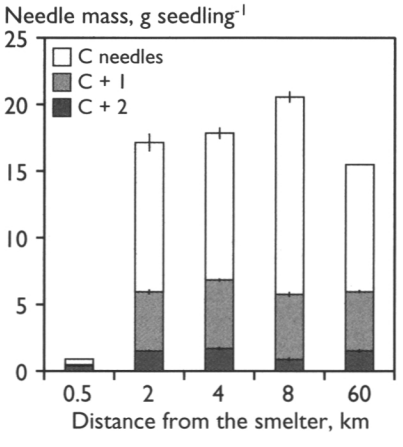
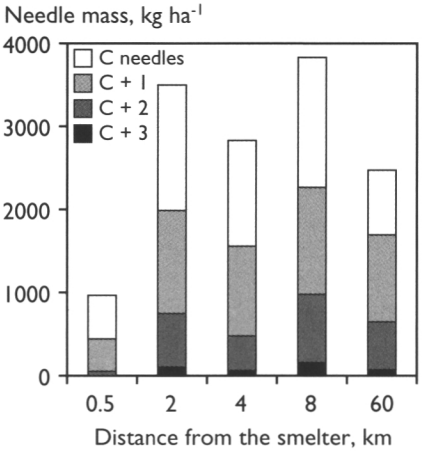


Fig. 6. Needle mass of Scots pine stands growing along a smelter pollution gradient (Nieminen *et al.*1998). C = current needles, C+1 = previous-year needles, C+2 = two-years-old needles and C+3 = three-years-old and older needles.



2001). According to Dickinson *et al.* (1996) trees can remain relatively healthy in the presence of high levels of contamination that build up slowly over time. Lepp et al. (1997) report that seedlings of broad-leaved herbs in a vicinity of a copper rod rolling factory in England were more sensitive to Cu than older herbs. Due to the cessation of reproduction close to Harjavalta smelters, the Scots pine stand at 0.5 km can no longer be considered as tolerant to the prevailing pollution level. According to the definition given by Baker and Walker (1989), tolerance enables a plant to establish, survive and reproduce in the presence of a particular pollutant.

Another possible factor explaining the better survival and growth rate of the mature trees at the 0.5 km field site compared to the greenhouse grown 0.5 km-seedlings is the impact of selection pressure. The heavy metal load, and also the SO_2 exposure in the early period of smelter activities, may have subjected the trees to a strong selection pressure with the result that only the most resistant individuals have survived at the site. During their *ca.* 50-year life-cycle, the pines have also had time to acclimatise to the gradually increasing heavy-metal concentrations in the soil. According to Baker (1987), Baker and Walker (1989), and Dickinson *et al.* (1991), the metal resistance of long-lived plants, such as trees, is primarily based on the phenotypic plasticity of individuals rather than on a constitutive tolerance, although the precise mechanisms allowing survival in metal-contaminated environments still remain unclear (Turner and Ross 1994).

Trees growing in metalliferous soils can also avoid metals by penetrating their roots in less polluted soil pockets and deep uncontaminated soil layers (Tyler *et al.* 1989, Dickinson *et al.* 1991, Turner and Dickinson 1993, Watmough and Dickinson 1995). Thus, a heterogeneous dispersal and availability of metals in soil may explain the survival of mature trees at 0.5 km.

Finally, the genetic tolerance of root-associated mycorrhizal species plays an important role in the heavy metal resistance of woody plants (*e.g.* Bradley *et al.* 1982, Dixon 1988, Wilkinson and Dickinson 1995). The spatially large ectomycorrizal mycelium may immobilize metals in their cell walls (Colpaert and Assche 1992, Tichelen 1999, Ahonen-Jonnarth 2000), and facilitate the ability of roots to reach clean soil layers, thus avoiding the metals (Turner and Dickinson 1993). The mycorrhizas may have alleviated heavy-metal toxicity in the field in the mature stands (Nieminen *et al.* 2000). In the 0.5 km plant-soil systems grown in the greenhouse, on the other hand, there was no possibility for mycorrhizal infection by new fungal species during the experiment, because a relatively high supply of carbohydrates from the host plant is needed for the initiation of a mycorrhizal infection (Harley and Smith 1983).

The major contribution of soil contamination to the high mortality rate of the greenhouse-grown-seedlings is confirmed by the low mortality of the field-grown pine seedlings (see **VI**). These field-seedlings were planted in June 1996 in soil pockets filled with "clean" mulch as a part of the remediation experiment (Kiikkilä 2002) established next to the 0.5 km study site. In September 2004, after nine growing seasons, the survival rate of the seedlings was as high as 96% (unpublished results). Hence, planting in unpolluted organic mulch proved to be an efficient way to mitigate the toxic effects of soil contamination, even under the impact of the current atmospheric smelter emissions.

3.2.4 Seed crop and seedling establishment

The annual seed crops on the three study plots showed an overall decreasing trend with decreasing distance to the smelters (Table 5); this was of course partly due to the decreasing stand density. However, even the seed crops of the most polluted 0.5 km stand are at a normal level. Lehto (1956) reported 16 seeds *per* m^2 as a mean value for CT stands, and 30 seeds *per* m^2 for VT stands. Sarvas (1949) found that the average annual crop from seed-tree stands on CT sites in southern Finland was 18.5 seeds *per* m^2. He also concluded that sites with a poor growth rate produced smaller seed crops than those with a higher growth rate. According to Koski and Tallqvist (1978), the seed crop of Scots pine varies from 18 to 216 seeds *per* m^2. Therefore, the seed production of the study stands closest to the smelter cannot be the reason for the lack of natural seedling establishment. Similar results were obtained by Kozlov and Zvereva (2004) near the Monchegorsk Ni-Cu smelters in northwest Russia. They concluded that the high level of pollution had not decreased the seed production of mountain birch (*Betula pubescens ssp. czerepanovii* (Orlova) Hämet-Ahti.

Salemaa and Uotila (2001) concluded from their seed bank studies in Harjavalta soils that viable pine seeds occurred even in the most contaminated soil, but that seedling establishment was unsuccessful. According to several studies on the lack of tree seedling establishment in metal-polluted substrate, seed germination does not seem to be affected, but the development of the radicle is disturbed (Patterson and Olson 1983, Wotton *et al.* 1986, Komulainen *et al.* 1994). Niini and Raitio (1993) found that the radicles of germinated pine seedlings were not able to penetrate into soil derived from a forest in the vicinity of the Harjavalta smelters.

Consequently, pine seedlings appear to be more susceptible than mature pines to the current elevated soil metal contents. The seed production of mature trees, which is considered to be a decisive phase in the assessment of population survival by Ernst and Nelissen (2000), was not severely affected. Nevertheless, the disturbed development of the seedling radicle is most likely the crucial phase preventing the establishment of seedlings at the most polluted study site. According to Ernst *et al.* (1992), long-term survival of plants in metal-enriched environments is possible only if they can detoxify the metals and protect their roots from an excess of metals. At the most polluted Harjavalta site, the pine seedlings do not appear to be able to protect their roots, although mature pines survive at the same site.

Table 5 . The annual pine seed crops during 1993–1998 at plots at three distances from the Cu-Ni smelters.

Distance from the smelters	1993	1994	1995	1996	1997	1998
	number of seeds per m^2					
0.5 km	45	45	28	26	105	12
4 km	26	32	64	60	111	32
8 km	188	133	57	42	98	41

3.3 Availability of soil Cu and Ni to Scots pine

3.3.1 Soil and soil solution Cu and Ni concentrations as indicators of their availability

Based on the present study, the uptake pattern of Scots pine appeared to be closer to that of an accumulator plant than of an excluder plant (Baker 1987) in both the case of Cu and Ni (Figs. 4 and 5 in **III**). The soil Cu, Ni, N and P contents were the best explainers of the biomass production of the seedlings grown in smelter-polluted soil in paper **III**. The exceptionally low production rate of the 60 km background site is undoubtedly due to the poor soil nutrient status (Table 6), while the stunted growth

Table 6. The mean total and exchangeable concentrations in the humus layer along the smelter pollution gradient, as well as the exchangeable mineral soil concentrations (n = 5, standard deviation is given beside the mean value).

Distance, km	0.5		2		4		8		60	
Humus, pH	4.1		3.8		3.5		3.9		4.1	
Mineral soil pH										
0-10 cm	4.1		4.1		3.9		4.2		4.9	
10-20 cm	4.1		4.6		4.6		4.6		4.8	
	Humus concentrations $g\ kg^{-1}$									
N_{tot}	4.68	1.0	5.58	0.8	5.32	1.4	6.10	1.2	2.58	0.4
S_{tot}	0.65	0.12	0.56	0.06	0.40	0.05	0.33	0.04	0.30	0.04
P_{tot}	0.42	0.08	0.49	0.1	0.47	0.04	0.42	0.1	0.26	0.02
P_{extr}	0.14	0.02	0.18	0.05	0.15	0.01	0.14	0.03	0.07	0.01
Ca_{tot}	0.45	0.1	1.13	0.3	1.07	0.1	0.91	0.2	0.50	0.2
Ca_{exch}	0.24	0.06	1.13	0.2	0.80	0.1	0.80	0.2	0.35	0.1
K_{tot}	0.25	0.03	0.38	0.05	0.43	0.03	0.40	0.05	0.28	0.02
K_{exch}	0.06	0.01	0.19	0.04	0.29	0.03	0.26	0.05	0.11	0.01
Mg_{tot}	0.17	0.03	0.26	0.03	0.26	0.04	0.28	0.06	0.35	0.09
Mg_{exch}	0.02	0.006	0.10	0.02	0.13	0.03	0.11	0.03	0.07	0.03
	Mineral soil exchangeable concentrations $mg\ kg^{-1}$									
P 0-10 cm	7.1	1.2	35.6	20.1	13.0	7.2	32.4	6.6	8.3	3.7
P 10-20 cm	42.6	21.2	37.3	16.1	22.9	9.0	19.7	3.2	4.2	0.8
Ca 0-10 cm	21.9	7.0	36.0	17.7	10.7	2.4	16.5	5.1	8.6	3.4
Ca 10-20 cm	11.6	3.4	13.2	6.7	6.5	1.4	5.5	1.5	4.0	0.8
K 0-10 cm	13.4	5.3	18.6	8.8	10.0	1.1	13.6	1.6	25.0	7.3
K 10-20 cm	9.0	1.4	12.8	5.1	8.7	1.6	10.3	2.1	12.4	5.5
Mg 0-10 cm	3.0	0.9	8.0	4.5	3.5	1.3	5.5	2.1	4.5	2.2
Mg 10-20 cm	1.8	0.4	1.6	0.5	1.4	0.3	1.5	0.5	1.2	0.4

of the 0.5 km site mainly reflects the deleterious effects of the high soil Cu and Ni contents (Table 7). The low soil nutrient content at Hämeenkangas has been reported also by Raitio (1990a).

Since plants access metals in the soil primarily through the soil solution, the soil solution concentration of a specific metal would appear to be a good indicator of its availability. The Cu and Ni concentrations in zero-tension soil water collected at the Harjavalta field sites followed the same decreasing pattern with increasing distance as did the soil concentrations (Table 8). However, although the soil solution Ni concentrations were almost as high as those of Cu at the 0.5 km site, this was not reflected in the Cu:Ni ratio of the pine tissues of the present study. According to the tissue concentrations of the greenhouse grown seedlings of the present study (Fig. 7 and 8), as well as to their net uptake rates (Fig. 5 in **IV**), the ratio between Cu and Ni availability at the most polluted 0.5 km site would be relatively close to 10:1, which is about the same as their ratio in the soil (Fig. 8, Table 7, **III**, **IV**). Thus, the soil concentrations of the present study appeared to be better indicators of Cu and Ni availability than the soil solution concentrations of the same site presented by Derome (2000). However, the use of root concentration as an availability indicator is problematic due to the strong affinity of Cu for the negatively charged exchange sites in the root cortex (McLaughlin 2002). This cortex-bound Cu would appear as elevated root Cu concentrations, even if no real uptake through the plasma membrane had taken place (cf. **III**).

The inconsistency between the Cu:Ni ratio in soil solution and the Cu:Ni ratio in the aboveground tissues of the experimental pine seedlings, may be partly due

Table 7. The mean Cu and Ni concentrations of the experimental soil cores collected along the pollution gradient (n = 5, standard deviation is given beside the mean value. n.d. = not detected *i.e.* below the detection limit).

Distance from the smelter, km	Humus layer concentration, total		Humus layer concentration, exchangeable		Mineral soil concentration, 0-10 cm layer,		Mineral soil concentration, 10-20 cm layer,	
	mg kg^{-1} d.w.							
	mean	sd	mean	sd	mean	sd	mean	sd
Cu								
0.5	2665	1178	2072	380	59	23	22	18
2	1456	487	1188	145	17	4	3	0.5
4	522	230	385	79	3	1	0.9	0.2
8	102	34	74	10	2	0.4	0.4	0.2
60	6	1.7	3	0.9	0.8	0.7	0.4	0.1
Ni								
0.5	216	105	138	31	10	2.6	n.d.	
2	158	39	111	12	3	0.8	n.d.	
4	100	27	70	10	0.6	0.6	n.d.	
8	32	12	21	3	n.d.		n.d.	
60	16	3	1	0.3	n.d.		n.d.	

Table 8. Mean element concentrations and pH in soil solution at different distances from the Harjavalta smelter (n.m. = not measured, s.e. = standard error of the mean). Modified from Derome (2000).

Distance	Depth	0.5 km		4 km		8 km	
		mg L^{-1}					
		mean	s.e.	mean	s.e.	mean	s.e.
Cu	5 cm	0.65	0.09	0.06	0.01	0.02	0.002
	20 cm	1.19	0.08	0.03	0.003	0.01	0.002
	40 cm	0.41	0.05	0.01	0.001	n.m.	
Ni	5 cm	0.54	0.06	0.02	0.003	0.01	0.001
	20 cm	0.95	0.05	0.02	0.003	0.01	0.001
	40 cm	0.64	0.05	0.01	0.001	n.m.	
NH_4-N	5 cm	0.61	0.16	0.42	0.09	0.44	0.10
	20 cm	0.98	0.36	0.20	0.03	0.27	0.05
	40 cm	0.24	0.06	0.12	0.12	n.m.	
NO_3-N	5 cm	0.21	0.04	0.21	0.05	0.08	0.02
	20 cm	0.21	0.10	0.03	0.01	0.06	0.02
	40 cm	0.25	0.05	0.03	0.01	n.m.	
SO_4-S	5 cm	4.43	0.29	2.28	0.18	1.98	0.18
	20 cm	6.59	0.44	1.82	0.13	1.89	0.13
	40 cm	5.29	0.50	1.49	0.11	n.m.	
PO_4-P	5 cm	0.11	0.02	0.18	0.02	0.11	0.02
	20 cm	0.07	0.01	0.09	0.01	0.08	0.02
	40 cm	0.06	0.01	0.04	0.01	n.m.	
Ca	5 cm	1.53	0.12	1.12	0.11	0.91	0.09
	20 cm	3.05	0.45	0.44	0.04	0.82	0.07
	40 cm	1.59	0.15	0.38	0.04	n.m.	
K	5 cm	1.47	0.13	2.03	0.16	1.78	0.17
	20 cm	2.76	0.28	2.38	0.20	1.97	0.21
	40 cm	2.59	0.26	1.52	0.16	n.m.	
Mg	5 cm	0.45	0.05	0.27	0.03	0.27	0.03
	20 cm	0.81	0.12	0.21	0.02	0.30	0.02
	40 cm	0.57	0.06	0.15	0.01	n.m.	
pH	5 cm	4.07	0.04	4.38	0.09	4.27	0.09
	20 cm	3.96	0.03	4.34	0.06	4.56	0.05
	40 cm	4.43	0.03	4.76	0.04	n.m.	

to the fact that the sampled soil solution was zero-tension soil solution, which does not necessarily correspond to the soil water fraction surrounding the roots of the vegetation. It is generally assumed that soil solution sampled by tension lysimeters would give a better idea of plant-available element concentrations, whereas gravimetric soil solution provides better information about the movement of elements between the soil horizons (Derome *et al.* 2002).

Furthermore, in the case of Cu, the soil solution concentrations appear to be relatively close to the chemical equilibrium status of a saturated system, where pH is a more important controlling factor than the soil Cu pool. In an experimental

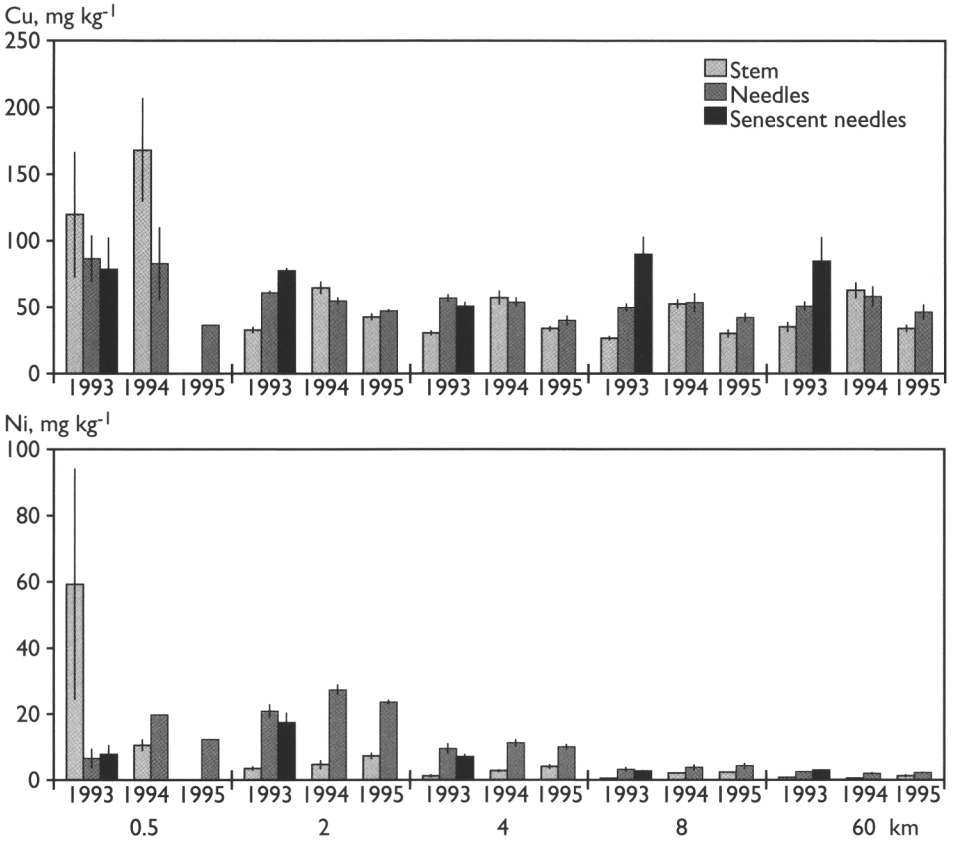


Fig. 7. The mean Cu and Ni concentrations of the above-ground compartments of the seedlings grown in smelter-polluted soil , n = 5, except for the 1994 (n = 4) and 1995 (n = 1) compartments of the 0.5 km. The bar indicates the standard error of the mean. (Note also that the 1994 and 1995 samples of 0.5 km represent only 1 seedling, while the other samples represent 5 seedlings bulked together.)

approach reported by Herms and Brümmer (1980) and Brümmer and Herms (1983), Cu concentration in an equilibrium solution of a sandy podzol with constant total Cu content varied between 0.4 and 4 as a function of pH (Fig. 9). The soil used in this experiment was sampled from the A horizon of an uncontaminated sandy gleyic podzol site, and adjusted to give an excessive total concentration of 100 mg Cu kg^{-1} (Herms 1982). The soil solution Cu concentrations of the 0.5 km study site (Table 7) follow relatively closely the pH dependence curve of the equilibrium solutions of the sandy podzol (Fig. 9), even though the experimental soil solution was obtained by vacuum filtration (Herms 1982). Consequently, the differences in the Cu concentrations of the soil solution between different depths at the 0.5 km site are presumably merely due to pH changes rather than to changes in the total soil Cu concentration. According to Herms and Brümmer (1980), the increase in Cu solubility over the pH range 6 to 8 (Fig. 9) is due to increasing solubility of Cu complexing organic substances. The increase at low pH values is supposed to be mainly due to dissociation of the metal-organo-complexes, resulting in the release of free Cu^{2+} ions (Herms and Brümmer

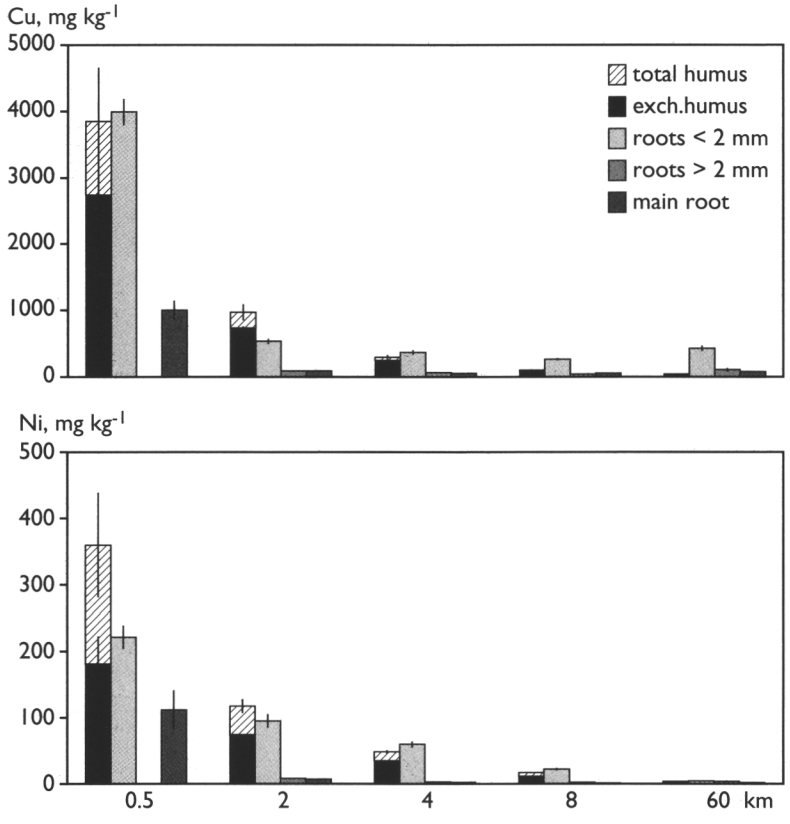


Fig. 8. The mean Cu and Ni concentrations of the humus and below-ground compartments of the pine seedlings. n = 5. The bar indicates the standard error of the mean.

1980), which are considered to be the most toxic Cu fraction (Sauvé 2002). According to McLaughlin (2002), the pH-related changes in soil solution metal concentrations are not clearly reflected in plant metal uptake.

Higher solubility of Ni in comparison to Cu in acid soils enriched with organic matter has been reported by several authors (Adriano 2001, Kabata-Pendias 2001). According to Ashworth and Alloway (2004), the role of organic matter in Cu and Ni mobilization is of dual nature. It promotes the mobility of both metals, since neither of them are very mobile if added in inorganic form to an inorganic substrate, but the mobility of Ni is enhanced more by the organic matter than that of Cu. The high immobilization rate of Cu and especially Ni in the quartz sand media of study **III** (Fig. 10) supports the statement of Ashworth and Alloway (2004) about the low mobility of inorganic Cu and Ni in an inorganic matrix.

Although the higher Ni mobility was reflected as higher leaching of Ni compared to Cu in the field at the sites adjacent to the smelters (**II**, Derome and Nieminen 1998), Ni uptake by Scots pine did not appear to be more efficient than that of Cu on the basis of the experiments of the present study (**III**, **IV**). According to Sauvé *et al.* (1996), studies on the link between metal concentrations in soil solution and metal uptake are rather rare, but there are numerous limitations to assume that the phytoavailable portion of

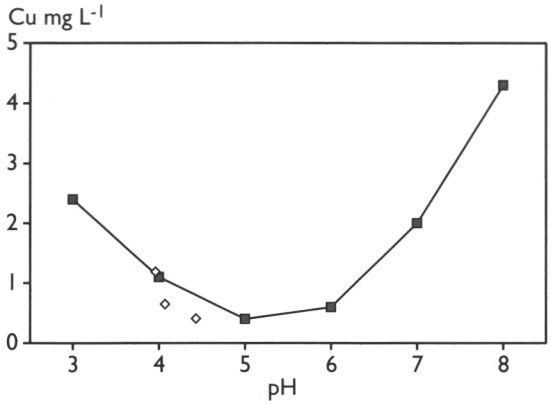


Fig. 9. Cu concentrations in relation to pH in an equilibrium solution of a sandy podzol. (Modified from Herms and Brümmer 1980). ◇ symbols refer to the Cu concentrations measured at different depths of the 0.5 km soils by Derome (2000).

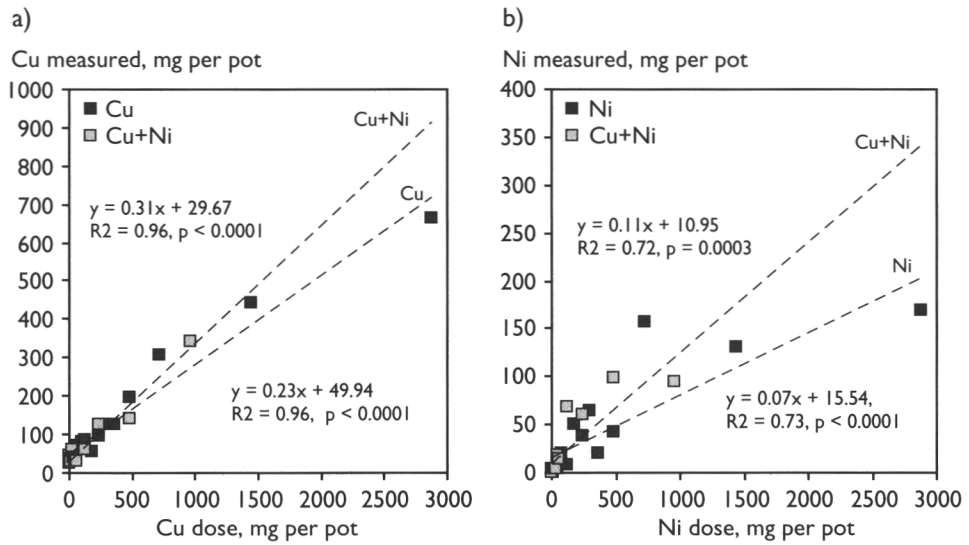


Fig. 10. The exchangeable Cu (a) and Ni (b) contents of the quartz sand substrates after the 17-month experimental period in relation to the Cu/Ni treatment doses originally added.

a metal would be that occurring in the soil solution. Furthermore, when plant uptake causes a depletion of a metal in solution, it is highly unlikely that determining the metal concentration in solution will provide a good predictor of plant metal concentrations or uptake (McLaughlin 2002). The soil's capacity to supply metal to the soil solution is of crucial importance for availability (Ernst and Nelissen 2000, McLaughlin 2002). And, *vice versa*, according to Allen (2002) it is the strength of binding of the metal by the soil that controls its phytoavailability. Assessment of the pool of solid-phase metal that buffers the solution metal concentrations is a means of accounting for this supply rate term. In fact, all the wide range of extraction techniques developed so far aim at measuring this pool (McLaughlin 2002).

3.3.2 Cu and Ni concentrations as diagnostic criteria of toxicity

The lethal substrate thresholds estimated on the basis of the artificial quartz sand treatment series (**III**) are quite different (32 mg kg^{-1} for Cu and 3.2 mg kg^{-1} for Ni) from the critical threshold values for the solution concentrations presented *e.g.* by Lozano and Morrison (1982). They report substantial growth reductions in hydroponically cultivated white pine and white spruce seedlings when the concentrations of Ni and/or Cu had reached 10 mg L^{-1}. However, solution concentrations cannot as such be directly compared with mineral soil concentrations. On the basis of Chapter 3.3.1., it is evident that soil solution concentration as high as 10 mg L^{-1} are not realistic. Neither can the mineral soil concentrations be directly compared with the concentrations of an organic matrix. Thus, the experimentally defined lethal thresholds for Cu and Ni concentrations in the substrate (**III**) cannot be generalized to cover different edaphic conditions. According to Patterson and Olson (1983), pine seedlings can support ten times higher metal concentrations when growing in a mineral soil substrate compared to seedlings growing in nutrient solution, and hundred times higher concentrations when growing in an organic substrate.

The lethal threshold values for root and stem Ni and Cu concentrations responded correspondingly to the substrate threshold: 940 mg Cu kg^{-1} and 80 mg Ni kg^{-1} in the roots, and 70 mg Cu kg^{-1} and 8 mg Ni kg^{-1} indicating a higher toxicity of Ni compared to Cu. However, the greater toxicity of Ni was evident only in the presence of Cu (**III**, Nieminen 1998). The differences in pine mortality between the Cu and Ni treatments were small when given as inorganic single metal treatment in quartz sand media. Both Cu and Ni concentrations in the roots and stems of the 0.5 km soil cores clearly exceeded the lethal threshold values of the quartz sand experiments, and the root Ni concentrations of the 2 km soil cores were close or even greater than this limit (**III**). On the basis of the present study (**III**), root and stem concentrations appeared to be relatively reliable indicators of Cu and Ni toxicity.

The results of the Cu and Ni concentrations in the needles of the experimental seedlings were rather contradictory (**III**). The mobility of Cu and Ni within a plant is most probably related to the chemical form of the metal taken up by the plant (Jackson *et al.* 1990). According to the present study (**III**), Ni would appear to be more mobile in Scots pine in an inorganic form than in the forms present in forest soils. Therefore, needles did not prove to be as advantageous as roots and stem as toxicity indicators (**III**).

Furthermore, the needles of mature pines and young seedlings sampled from the field at the 0.5 km site had much higher Cu and Ni concentrations than those of the experimental seedlings (**VI**). This was undoubtedly due to the high surface contamination of the needles as a consequence of the high dry deposition load of metal-containing particles from the current smelter emissions and from the degraded forest floor (**VI**). Surface contamination may bias also the stem concentrations in the field. Hence, the metal concentrations of above-ground plant tissues in a heavily polluted environment have little relevance in terms of a plant's physiological response, although they may be useful as indicators of metal accumulation. Conifer needles have been widely used as indicators of the sulphur deposition rate, both in nationwide studies (Raitio *et al.* 2000) and in the vicinity of industrial towns (Huttunen *et al.* 1985).

3.4 Biogeochemical cycling of elements in the studied forest ecosystems

3.4.1 Consequences of a long-term Cu an Ni exposure for element cycling

The biogeochemical cycling of elements involves the exchange of elements between the various compartments within the ecosystem (Adriano 2001). Element cycles and exchange processes of the degraded 0.5 km forest site have been greatly affected by the long-term pollution load (Fig. 11). The atmospheric input has brought large amounts of heavy metals (**II**), especially Cu and Ni, derived from the smelter emissions. These have been deposited on the tree canopy (**VI**) and washed down to the forest-floor by

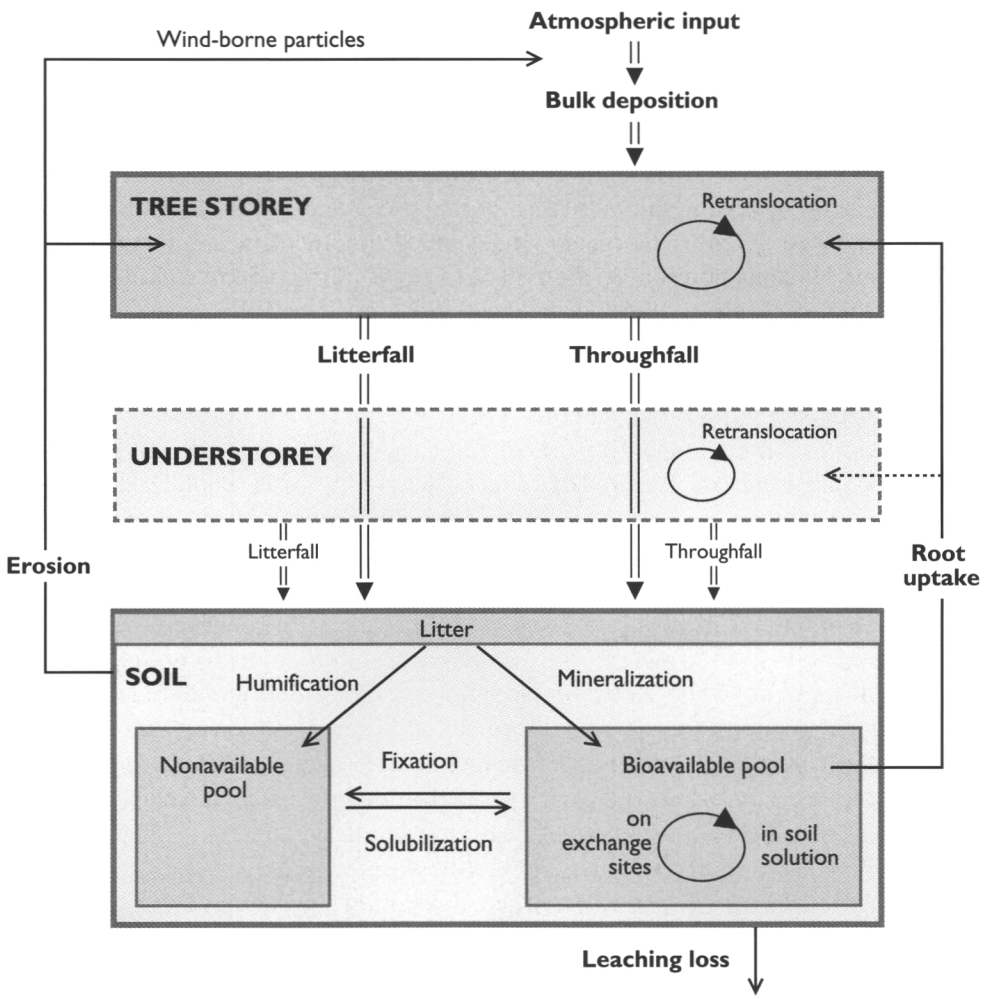


Fig 11. A generalized model depicting element cycles in a degraded forest ecosystem.

rain in the throughfall flux (**I**), as well as transported as surface contaminants of the litter compartments in the litterfall flux (**IV, V**). The wind-borne dust from the degraded forest floor has recycled these metals back into the tree canopy, from where they have been again deposited onto the forest floor in the throughfall and litterfall fluxes (**I**).

The almost completely lacking understorey vegetation does not participate in the element cycling, and the denuded soil is exposed to wind and soil erosion. Copper accumulates in the upper-most organic layers of the soil, preventing seedling establishment and the formation of a new understorey. Nickel, on the other hand, has a higher mobility and higher potential for downwards leaching (**II**), but is most probably also involved in the inhibition of seedling establishment. Both metals cause nutrient depletion from the phytoavailable pool in the soil through displacement and by retarding litter mineralisation due to their toxicity to soil microbes. The displaced nutrient cations are susceptible to leaching from the root zone. Both Cu and Ni interfere with root nutrient uptake by impairing the normal functioning of the roots (**III**). The deterioration of the roots causes severe growth retardation of the above-ground parts of the trees, which is reflected as a low internal nutrient retranslocation efficiency (**V**) and low organic matter input to the forest floor *via* the litterfall flux. However, despite the low litter input, large amounts of litter have accumulated on the forest floor as a consequence of the strongly retarded mineralisation processes.

3.4.2 Canopy filtration of dry deposition as a source of element fluxes in the ecosystems

The lack of understorey vegetation (Salemaa *et al.* 2001), as well as the retarded organic matter mineralization (Fritze *et al.* 1989, Fritze *et al.* 1996) and, consequently, low water holding capacity of the organic layer (Derome and Nieminen 1998), expose the barren soil to wind-erosion, which seldom takes place under normal conditions in boreal forests. The wind-borne soil dust contains large amounts of heavy metals that have accumulated in the soil during the *ca.* 50-year-period of smelting activities (Table 7, Derome and Lindroos 1998). This affects the composition of bulk precipitation and especially the stand throughfall fluxes, thus making it difficult to quantify the atmospheric input by means of precipitation sampling (**I**).

The current emissions from smelting activities most probably cause an increased atmospheric input of metals into the forests close to the area. However, the relatively effective methods used in our studies for deposition measurement do not correctly quantify the input flux in degraded forests, although they are supposed to give reliable estimations of atmospheric inputs in normal conditions (Helmisaari and Mälkönen 1989, Hyvärinen 1990, Ukonmaanaho and Starr 2002). The stand throughfall flux contains exudates from the canopy, elements originating from foliar leaching, and elements from the wash-off of previously dry deposited material. Distinguishing between these three sources is difficult, and the separation of dry deposition derived from the internal cycling of dust from dry deposition originating from outside the ecosystem boundaries is even more problematic. Therefore, the exact atmospheric input into the 0.5 km ecosystem remains unclear, although our results suggest that sources other than the stack emissions make a major contribution to metal enrichment in bulk precipitation and stand throughfall (**I**, **V** and **VI**).

Canopy filtration of soil-borne dust also affects the litterfall fluxes. Although the quantity of annual litterfall was at its lowest at 0.5 km, the Cu and Ni fluxes *via* litterfall were *ca.* 100 times higher at 0.5 km than at 4 km during 1993 (Nieminen *et al.*1998). An undeterminable proportion of the dry deposited metal particles attached to the surface of litter needles and other litter compartments is derived from internal dust cycling in the ecosystem. In addition, the litter traps can, to some extent, directly capture wind-born soil dust.

3.4.3 Foliar leaching of potassium

The high rate of K leaching from the needle tissues at 0.5 km stand suggested that K cycling has been altered by the heavy pollution load (**I**). As a monovalent cation, K^+ is highly susceptible to foliar leaching even in normal forest conditions (Helmisaari and Mälkönen 1989, Hyvärinen 1990, Stachurski and Zimka 2000, 2002) and the throughfall flux is usually a more important pathway for K cycling than the litterfall flux (Ranger *et al.* 1994, Helmisaari 1995). Enhanced canopy leaching of K has been observed in forest stands suffering from low tree vitality in a number of earlier studies (*e.g.* Alenäs and Skärby 1988, Gjengedal 1996).

The tree canopy was an important source of K at all the distances studied (Fig. 1 in **I**). However, the amount of K in net throughfall was greatest at 0.5 km, even though the stand K needle pool was the lowest at this site (Fig. 1 and 3 in **I**). Since net throughfall K is considered to be almost completely derived from foliar leaching (Parker 1983), and no smelter-derived deposition of K was observed, the biological cycling of K in the soil-plant system at 0.5 km appeared to be accelerated (**I**). This is supported by the fact that the needle K concentrations were at their highest at 0.5 km (Appendix in **I**).

Accelerated root K uptake would be needed to maintain the needle concentrations at the observed level under the constant loss of K through foliar leaching. However, the results of the greenhouse-grown experimental seedlings do not support the assumption of a more efficient K uptake by the roots (**III**). A decrease in root K concentrations with increasing metal exposure was found both in the smelter-polluted-soil grown and in the artificially treated seedling series (**III**). In the smelter-polluted-soil grown seedling series, this pattern was also reflected in the above ground parts of the seedlings (**III**). Excessive amounts of both Cu and Ni have been reported to cause damage to the cell membranes, which is reflected as an enhanced efflux of K from plant tissues (Baker and Walker 1989, Marschner 1995, Adriano 2001, Kabata-Pendias 2001).

One possible reason for the differences in K status between the field-grown trees and the greenhouse grown seedlings, could be that the pine roots in the field could be associated with a highly adapted microsymbiont. Wallander and Wickman (1999) reported that *Paxillus involutus,* growing in symbiosis with *Pinus sylvestris*, was able to access the K in microcline, which is a common mineral in acid bedrock. In contrast, the other ectomycorrhizal fungi used in their experiment, *Suillus variegatus*, could not mobilise K from the mineral, nor could the pine seedlings cultivated without root symbionts (Wallander and Wickman 1999).

A shortage of K in the smelter-polluted –soil cores could have developed relatively rapidly in the kind of experimental conditions in which the throughfall

and litterfall fluxes of K were completely absent. Since K does not form stable organic complexes, it is the most mobile nutrient and is rapidly cycled in soil-plant systems (Switzer and Nelson 1972, Marschner 1995). According to Ranger (1995), the same K^+ ion can be absorbed several times during one growing season by the same plant. However, this kind of K deficiency would affect all the experimental seedlings at all distances, and should not be aggravated with increasing soil Cu and Ni concentrations.

3.4.4 Nutrient retranslocation in pine foliage

The autumnal nutrient retranslocation of mobile nutrients, especially P and K, was less efficient in the 0.5 km pine stand than at the other distances (Table 3a in **V**). This was not in agreement with the supposition that internal retranslocation would be of more importance in trees growing on infertile soils (Zimka and Stachurski 1976, Miller *et al.* 1979). Conditions in polluted soils are often relatively similar to those in infertile soils, as continuous exposure to pollution leads to a continual deterioration in soil nutrient status (Freedman and Hutchinson 1980, Bååth 1989, Fritze *et al.* 1989, Derome and Lindroos 1998). However, according to Nambiar and Fife (1987), Nambiar *et al.* (1991) and Saur *et al.* (2000), the growth rate of the trees, rather than the availability of nutrients in the soil, is the main factor controlling retranslocation, which is fully consistent with our results concerning less efficient retranslocation in the pine stand with the lowest growth rate (**V**).

Furthermore, retranslocation is not necessarily related to senescence (Nambiar *et al.* 1991). Fife and Nambiar (1982, 1984) reported that significant retranslocation occurred even in young needles of *Pinus radiata* L. Retranslocation can be divided into two phenomena: autumnal nutrient withdrawal from senescing foliage, which was determined in our study (**V**), and nutrient translocation from the living foliage during aging (Meier *et al.* 1985). There is no mathematical formula that could fully take into account both of these phenomena (Ranger 1995); mathematical means can only provide an estimate of the net retranslocation amount during the time lag between measurements.

At the ecosystem scale, the retranslocation process determines the quality of the falling needle litter which, in turn, has a great effect on litter decomposition and mineralization processes (Zimka 1991). According to our results, the N, P and K status of the litter needles was highest at 0.5 km (Fig. 1 in **V**).

3.4.5 Element losses *via* percolation water

The fluxes of Ca, Mg, K and P carried down to a depth of 5 cm in the soil as soil leachates were much higher than the fluxes entering the soil as bulk precipitation, while at 40 cm depth there was a net loss of only Mg and K (Derome and Nieminen 1998). The Mg loss in the 0.5 km stand was reflected as low soil (Table 5) and needle (Appendix in **I**) Mg concentrations but, in the case of K, the needle concentrations were even higher than at further distances from the smelters (Appendix in **I**).

The satisfactory K status of the needles suggests, however, that the trees are in fact obtaining sufficient K from the soil. Since the rooting system of pine stands growing

on sandy soils is typically deep (Laitakari 1927), we would expect that the roots are able to reach soil layers deeper than 40 cm, and satisfy their K requirements from these layers. However, the root depths measured at Harjavalta (Helmisaari *et al.* 1999) and Hämeenkangas (Raitio 1990b) do not support this assumption, since most of the fine roots in both areas were found in the upper 10 cm layer. Direct access to K in minerals by ectomycorrhizal pine roots has also been reported (*cf.* Wallander and Wickman 1999 in Chapter 3.4.2).

There were clear differences in the partitioning of Cu and Ni between the solid and solution phases in the soil at 0.5 km site. Copper was retained to a greater extent than Ni in the uppermost soil layers because the flux of Cu in percolation water at 5, 20 and 40 cm depths decreased more sharply with increasing depth (Derome and Nieminen 1998). The flux of Ni at 5 cm depth was greater than that entering the stand in precipitation both at 0.5 and 4 km (Derome and Nieminen 1998). The relatively low Cu:Ni ratio in percolation water concentrations at the 0.5 km site compared to the corresponding ratio in soil concentrations (Table 6 and 7) reflects greater solubility of Ni compared to Cu.

The behaviour of Cu and Ni in percolation water fluxes is consistent with the results obtained in study **II**. The vertical gradient in Cu concentrations in the surface peat of the Harjavalta bog suggests that the Cu supplied to the peat through atmospheric deposition was strongly retained by the bog (**II**). The relatively high Ni values at deeper depths compared to Cu indicated post-depositional downward migration, since Ni smelting in the Harjavalta area started 15 years later than Cu smelting (**II**). However, the changes that occurred in the exchangeable Cu and Ni pools in different layers of the smelter-polluted soil cores during the 17-month–experimental period of study **IV** (Table 4 in **IV**) did not indicate higher downwards migration of Ni compared to Cu.

The Cu:Ni ratio of ca. 10:1 observed in the peat of the Harjavalta bog (*cf.* Chapter 3.1.2) is in good correspondence with the Cu:Ni ratio in the humus layers of the 0.5 and 2 km sites (Table 6), which suggests that the binding capacity of organic matter is the key factor determining their current distribution pattern in these soils. Copper has been reported to form much more stable complexes with natural organic ligands than does either Ni or Zn (Bergkvist *et al.* 1989, Baker and Senft 1995). According to Kabata-Pendias (2001), Ni in peaty soils is in easily soluble organic forms. Ashworth and Alloway (2004) stress the importance of dissolved organic matter in promoting the mobility of both Cu and Ni in a sandy loam soil, but point out that the soil mobility of Ni is more enhanced by the presence of organic matter.

3.4.6 Nutrient disturbances

Based on the results of the experimental approach applied in the present study, the Ca, K and Mg dynamics of pine seedlings was affected by exposure to soil Cu and Ni (**III**). The decreased concentrations of these nutrients in the roots of both the artificial treatment series and the smelter-polluted-soil grown seedlings as a function of increasing Cu and Ni concentrations, indicate adverse effects of excess Cu and Ni. According to Ross and Kaye (1994), toxic heavy metals have disruptive effects on the structure and functioning of the plasma membrane of roots, and thus the kinetics of

nutrient uptake. An efflux of K has been reported to occur as a consequence of metal-induced damage to the plasma membrane of the root cells (Baker and Walker 1989).

The nutrient disturbances in the smelter-polluted-soil grown seedlings were more severe and were also reflected in the above-ground parts of seedlings (**II**). In addition, the low needle Mg concentrations of the mature pines growing at the 0.5 km site indicated Mg deficiency (**I**). In the smelter-polluted soil-plant systems, the changes in nutrient status can be caused both by the interference by Cu and Ni in the root nutrient uptake processes and by the low soil nutrient contents caused by excess Cu and Ni. The deficiency of macronutrients in polluted forest soil at Harjavalta has been caused both by the displacement of base cations by Cu and Ni (Derome and Lindroos 1998), and by retarded mineralisation of the litter due to the toxicity of soil Cu and Ni to litter-decomposing microbes (Fritze *et al.* 1989, 1996) and soil fauna (Haimi and Siira-Pietikäinen 1996). Litter accumulation is a well documented phenomenon in heavily metal-impacted forest ecosystems (Tyler 1975, Strojan 1978, Freedman and Hutchinson 1980, Berg *et al.* 1991). According to Berg *et al.* (1991), the low mineralisation rate at heavy metal polluted site was caused by changes both in litter quality and in soil factors. At moderate and low pollution levels, metal-induced nutrient disturbances are difficult to distinguish from natural variations in soil fertility.

4 Conclusions

Accurate determination of the current Cu and Ni input to the forest ecosystem closest to the smelter (0.5 km) was not possible by means of deposition measurements owing to the high importance of internal cycling *via* soil dust. The lack of understorey vegetation and the low water-holding capacity of the soil organic layer at the 0.5 km site make the forest soil susceptible to wind erosion. Wind-borne, metal-containing dust is an additional source of Cu and Ni in bulk deposition, and especially in stand throughfall, due to the high capacity of forest canopies to intercept and filter out dry deposition. In contrast to the situation at 0.5 km, the amounts of deposition measured at greater distances along the smelter-pollution gradient can be treated as relatively reliable indicators of the atmospheric input to the ecosystems.

The surface peat sediments of an ombrotrophic bog adjacent to the smelters revealed that the estimated mean annual accumulation of Cu and Ni based on peat contents is higher than the current annual bulk deposition load at the 0.5 km site. The vertical distribution pattern of Cu suggests that Cu supplied to the peat by atmospheric deposition is strongly retained in the top-most peat layers, whereas Ni shows a more even distribution pattern reflecting downwards migration. The behaviour of Ni was different to that of Cu also in the polluted forest soil at distances of 0.5-4 km. The humus layer was the most important sink for Cu, since it was clearly enriched in the uppermost soil layer, but in the case of Ni this was less evident. The exchangeable Ni pools of the underlying *ca.* 20 cm thick mineral soil layer appeared to be equal or higher than the corresponding pools of the humus layer along the whole smelter-pollution gradient.

There were no clear differences between the availability of Cu and Ni to Scots pine from polluted forest soil, since the uptake of both elements to the above ground parts of the pine seedlings responded according to their soil contents. In this study, both total and exchangeable Cu and Ni concentrations in the forest soil horizons appeared to be related to the available pools of soil Cu and Ni. In the artificial quartz sand experiment, on the other hand, the uptake of inorganic Ni was enhanced in the presence of equal amounts of inorganic Cu, while the uptake of Cu appeared to be inhibited in the presence of Ni.

The variation in the biomass of the experimental pine seedlings cultivated in smelter-polluted-soil cores appeared to be related both to the toxicity of Cu and Ni and to differences in the nutrient status of the experimental soils. However, Cu and Ni pollution may also affect pine stands indirectly through changes in soil nutrient status, and this is difficult to distinguish from the natural fertility variation in low-to-moderate pollution conditions. The biomass production of the experimental seedlings showed a very similar pattern to those of the growth and biomass production parameters measured in the field in earlier studies along the same smelter-pollution gradient.

Autumnal nutrient retranslocation from senescing needles to overwintering tree compartments was less efficient at the most polluted 0.5 km site compared to further distances along the smelter-pollution gradient. This finding is in good agreement with the current, widely accepted concept about the growth rate of trees as a driving force behind the retranslocation intensity.

The critical Cu and Ni concentrations determined in the present study for the quartz sand substrate give only a very approximate indication of the toxicity limits for natural soils. They can, at least to some extent, be compared to metal concentrations in the mineral soil horizons, but they are not valid in organic-rich substrates. However, the threshold concentrations determined for the pine seedling compartments should also be valid in a broader sense. The lethal threshold of *ca.* 1000 mg kg^{-1} for Cu in roots and 100 mg kg^{-1} for Ni in roots indicates that Ni has a higher toxicity. The corresponding thresholds for stem concentrations were 70 mg Cu kg^{-1} and 8 mg Ni kg^{-1}. The needle concentrations were rather contradictory, and did not appear to be reliable indicators of Cu and/or Ni toxicity.

In heavily polluted environments the surface contamination of above-ground tree compartments by metal containing particles further complicates the interpretation of the measured tissue concentrations. Washing the samples prior to analysis is generally considered to remove the surface contaminants, but the results of the present study demonstrated that a routine needle washing procedure did not remove all the metal-containing material attached to the needle surfaces. In this study, more than half of the Cu in the pine needles was present as surface contaminants at the most polluted 0.5 km site. In the case of Ni, the pine needles exposed to aerial deposition contained tens of times more Ni than the needles protected from aerial deposition. Therefore, surface contamination poses a severe risk to herbivores by considerably increasing their metal intake.

The prevention of Cu and Ni dispersion through soil erosion and leaching losses is of ultimate importance in avoiding the spread of pollution to surrounding ecosystems. The downwards leaching of Ni is a potential risk for groundwater quality, while inhalation and exposure through the digestive tract can pose health risks for the wild fauna and local human population. This is especially the case because soil-derived dust appears to be of more importance from the point of view of air quality than the current stack emissions in the immediate vicinity of the smelters.

The sustainability of pine stands growing in soils as polluted as that at 0.5 km cannot be maintained without some form of soil remediation to ensure natural seedling establishment. The aim of remediation is to either remove or immobilize the toxic metals in the soil. Several techniques are available for cleaning up polluted soil, of which the phytoextraction techniques are currently among the most studied ones. In phytoextraction, metal-accumulating plants are cultivated in polluted soil and harvested at the time when their metal content is at its maximum.

The primary aim of applying soil ameliorating agents, such as lime, zeolites, apatite or Fe and Mn oxides, is to immobilize toxic metals, as well as in some cases to facilitate the recovery of soil microbial activity, *e.g.* by spreading "clean" organic matter on the polluted soils. However, the long-term effects of these treatments on metal mobility in different soil conditions are difficult to predict. Therefore, considerable care should be taken to avoid the risk of enhanced metal leaching. A deeper understanding of the effects of ameliorating agents on metal mobility would be a challenge for future research.

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Paper I

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Interactions between precipitation and Scots pine canopies along a heavy-metal pollution gradient

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Abstract

Bulk precipitation and stand throughfall were collected during 1992–96 at distances of 0.5, 4 and 8 km from the Harjavalta Cu–Ni smelter, southwestern Finland. The amounts of heavy metals (Cu, Ni, Zn, Fe) and mineral nutrients in bulk precipitation and throughfall were highest at 0.5 km. Although the canopy coverage was low at 0.5 km, the amounts of heavy metals intercepted by the canopy were extremely high. The proportion of foliar leaching relative to the wash-off of dry deposition from the needle surfaces decreased on moving towards the smelter for all elements, except for K. The high rate of K leaching from the needle tissues close to the smelter demonstrated that the K throughfall flux has been greatly altered by the heavy pollution load. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Bulk precipitation; Canopy interaction; Cu; Ni; K; Throughfall

1. Introduction

In forested areas precipitation undergoes considerable quantitative and qualitative changes as a result of interaction with the stand canopy. The flux of elements in throughfall is an important pathway in the cycling of nutrients within forest stands (Tukey, 1980; Parker, 1983). The effect of the tree canopy on element concentrations in throughfall varies between conifers and broadleaves, as well as between individual species (e.g. Tukey, 1980; Hyvärinen, 1990). Furthermore, the composition of the precipitation falling on the canopy regulates the exchange processes taking place between the canopy and precipitation (Tukey, 1980).

The quantification of throughfall is a routine method in nutrient budget studies (Parker, 1983). In coniferous forests, the annual input of, for example, K to the forest floor in throughfall is clearly greater than that via litterfall (Ranger, 1981; Ranger and Nys, 1994; Helmisaari, 1995). Throughfall measurements are also widely used to characterise and quantify the atmospheric pollution load in heavily polluted forest ecosystems (Ulrich, 1983; Hultberg and Grennfelt, 1992; Dambrine et al., 1995; Derome and Nieminen, 1998). Throughfall chemistry is generally considered to provide a relatively good estimate of the external input into the ecosystem, since it includes both wet and dry deposition intercepted by the canopy. However, the estimated deposition rates of elements that are strongly retained by the canopy (e.g. N; Lovett and Lindberg, 1993; Lovett, 1994), or easily leached from foliar tissue (e.g. K and Mg; Parker 1983), are not usually reliable. In such cases, throughfall enhancement resulting from crown leaching more strongly reflects internal nutrient cycling processes and not the real input to the ecosystem.

Tree stands subjected to high levels of heavy-metal deposition are often sparse and suffering from defoliation, and foliar leaching has lesser impact on throughfall nutrient concentrations due to the low canopy coverage. However, foliar leaching from the remaining needles may be increased as a result of their poor condition and the effect of high levels of pollutants. The role of foliar uptake and the canopy filtration of dust, aerosols and gases in modifying throughfall composition may become more important, and lead to abnormally high throughfall enhancement of certain elements. The throughfall flux in polluted forests will be strongly

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modified according to the site conditions and pollution climate.

The aims of this investigation were to study the interactions between heavy-metal and nutrient deposition and the tree canopy along a heavy-metal pollution gradient, and to determine the extent to which heavy metals have disturbed the nutrient throughfall flux in the stands.

2. Materials and methods

2.1. The study stands

Harjavalta (61° 19′ N 22° 9′ E) is situated in southwestern Finland, about 30 km from the coast, in the southern boreal coniferous zone (Ahti et al., 1968). The long-term (1960–90) mean annual temperature at a near-by weather station of the Finnish Meteorological Institute is 4.0°C and the annual precipitation 558 mm. The area has been subjected to a heavy pollution load since the 1940s, mainly from an industrial complex producing Cu and Ni. Table 1 shows the trends in SO_2 and heavy-metal emissions during 1985–96. During the past few years, S and dust emissions have been drastically decreased owing to the changes in the process technology and installation of new filters. In 1995 a new reduction method for the production of Ni was adopted. Ammonium hydroxide is used to neutralise the sulphuric acid formed during the reduction process. The produced ammonium sulfate is stored in the industrial area before transportation. The ammonia used in the neutralising process is transported and stored in liquid form and, hence, there may have been some emissions of gaseous ammonia.

Three sample plots were established in July 1992, at distances of 0.5, 4 and 8 km from the Cu–Ni smelter in pure Scots pine stands growing on dry mineral soil sites along an esker running to the southeast of the smelter.

Table 1
Emissions of SO_2 and some heavy metals (in thousand kg $year^{-1}$) from the Harjavalta smelter during 1985–96 (Outokumpu Harjavalta Metals Oy)

Year	SO_2	Dust	Cu	Ni	Zn
1985	8000	1100	98	47	216
1986	7500	1200	126	46	232
1987	7000	1800	140	96	162
1988	8000	1000	104	45	103
1989	9500	1000	80	33	190
1990	8800	960	80	31	160
1991	5200	640	80	14	90
1992	4800	280	60	10	12
1993	4700	250	50	7	13
1994	5000	190	40	6	6
1995	3300	70	17	1.4	1.7
1996	3200	195	49	1.2	5.3

The soil type is a well-developed sorted sandy podsol, relatively poor in plant-available nutrients. The pine stand at 0.5 km was suffering from retarded growth and severe needle loss (Table 2), but the stands located at 4 and 8 km appeared to be relatively unaffected. The vegetation was originally typical of xerophilous forest sites: *Calluna vulgaris* (L.) Hull., *Empetrum nigrum* L., *Vaccinium vitis-idaea* L., *Pleurozium schreberi* (Brid.) Mit., *Dicranum* spp., *Cladina* spp., etc. The understorey vegetation in the stand at 0.5 km was seriously damaged (Salemaa and Vanha-Majamaa, 1993).

2.2. Bulk precipitation and stand throughfall

Bulk deposition was collected in open areas close to the three stands using five rainfall collectors (diameter = 20 cm), and stand throughfall using 20 rainfall collectors located systematically inside the stand on a 30×30 m plot during the same period. The height of the rainfall collectors was 40 cm above ground level. Each collector was supported inside the top of a vertical black plastic pipe, and connected to a collection bottle located in the bottom of the pipe at a depth of 40 cm. The rainwater sample was thus kept in cool, dark conditions. Bulk deposition and stand throughfall samples were collected at 1-month intervals during the snow-free period (on average June–November) from July 1992 to December 1996.

Water samples from the rainfall collectors on the same plot were combined in the field to give one composite sample per plot at each sampling date. The volume and pH of the samples were measured, and the remaining part of the samples filtered through a 0.45-μm membrane filter. Ca, Mg, K, Fe, Cu, Ni and Zn were determined by inductively coupled plasma/atomic emission spectrometer (ICP/AES), and NH_4^+, NO_3^-, SO_4^{2-} and PO_4^{3-} by ion chromatography. Dissolved organic carbon (DOC) was determined by digestion with sodium persulphate under UV radiation on a total organic carbon analyser. The sums of anions and cations were derived from the ionic concentrations ($mol_c\ l^{-1}$) of each sample. The Pearson coefficients of correlation between DOC and the anion deficit were calculated.

Table 2
Characteristics of the tree stands on the study plots in 1991 (Mälkönen et al., 1999)

	Distance from the smelter (km)		
	0.5	4	8
Stand age (years)	49	48	40
Number of trees (ha^{-1})	1008	1517	1552
Mean height (m)	7.6	11.1	12.4
Stem volume ($m^3\ ha^{-1}$)	23.2	67.8	94.5
Volume increment ($m^3\ ha^{-1}\ year$)	0.31	2.78	6.27

Deposition loads ($mol_c\ m^{-2}$) were calculated by multiplying the concentrations by the amount of water ($l\ m^{-2}$) at each sampling date for both bulk precipitation and throughfall. Net throughfall deposition was calculated as the difference between throughfall deposition (rainfall collectors in a stand) and bulk deposition (rainfall collectors in an open area). The average deposition for each element was calculated as the arithmetic mean of the deposition values of all sampling dates from July 1992 to November 1996 ($n = 34$).

Canonical discriminant analysis was carried out in order to obtain the best combination of element variables in bulk precipitation and throughfall that discriminated the study stands from each other. The statistical significance of the effect of the canopy on the amounts of water and elements in precipitation was tested using the paired t-test (difference between the amount in bulk preciptation and that in throughfall).

2.3. Needle sampling

Needles were collected in the middle of July 1992 from 8–10 trees per study stand. The sample trees were selected randomly from five size-class groups representative of each stand. One random branch from the upper, middle and lower crown was sampled from each tree. One-hundred fascicles were collected from each needle age class on each branch, and the needles from the upper, middle and lower branch of the same tree were combined by needle age classes to form a sample of 300 fascicles per sample tree.

The needle samples were dried at 70°C for 48 h, weighed, and stored in paper bags. Concentrations of P, K, Ca, Mg, Mn, Zn, Fe, Cu, Ni were determined on a dry weight basis from finely ground needles by dry ashing and extraction with HCl. The filtered solutions were analysed by ICP/AES. The needle element pools ($mg\ m^{-2}$ land area) were calculated by multiplying the needle element concentrations ($mg\ kg^{-1}$) by the corresponding needle mass ($kg\ ha^{-1}$). The ratio between the annual net throughfall flux ($mg\ m^{-2}\ year^{-1}$) derived from Derome and Nieminen (1998) and the needle element pool ($mg\ m^{-2}$) was calculated for each element in order to express the relative amount leached or washed off from the canopy.

3. Results

3.1. Composition of bulk precipitation and stand throughfall

There were clear differences in the average monthly composition of bulk precipitation and throughfall at different distances from the smelter (Fig. 1a, b). The deposition of most cations (NH_4^+, Ca^{2+}, Mg^{2+}, Fe^{3+}, Zn^{2+}, Cu^{2+}, Ni^{2+}) and SO_4^{2-} was the greatest at 0.5 km in both bulk precipitation and throughfall, despite the fact that the amount of precipitation was approximately the same at all distances (Table 3). However, the average H^+ deposition was lowest at 0.5 km. The average deposition of K^+, PO_4^{3-} and NO_3^- in bulk precipitation was relatively constant at all distances, but the corresponding values in throughfall were at their highest at 0.5 km. Bulk deposition of Mn^{2+} was slightly higher at 0.5 km (0.007 $mol_c\ m^{-2}$) compared to that at 4 and 8 km (0.0004 and 0.003 $mol_c\ m^{-2}$), but in throughfall deposition there were no differences between the plots (0.02, 0.01 and 0.02 $mol_c\ m^{-2}$, respectively). The DOC concentrations and the pH values were at their highest at 0.5 km (Table 3).

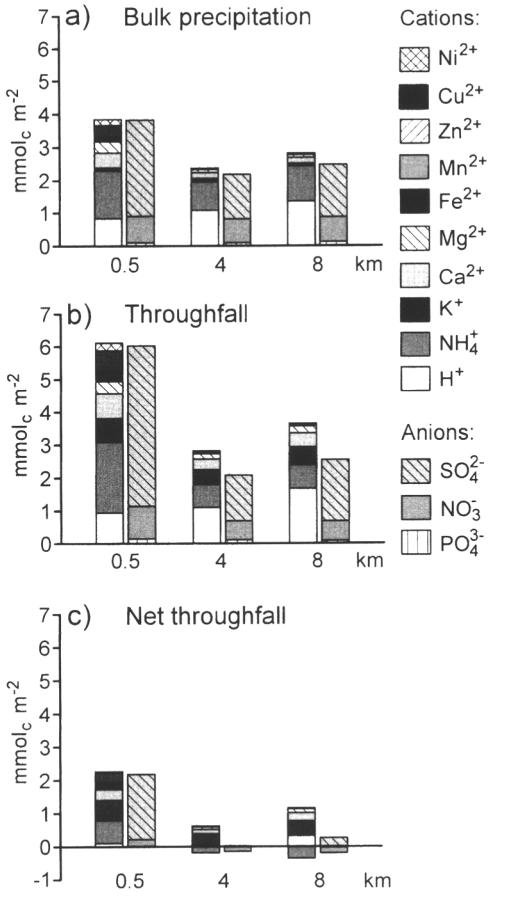

Fig. 1. The average ionic composition ($mol_c\ m^{-2}$) of (a) bulk precipitation, (b) throughfall and (c) net throughfall at different distances from the smelter.

Canonical discriminant analysis indicated that the composition of bulk precipitation and throughfall at

0.5 km was clearly different from that at 4 and 8 km (Fig. 2). The first canonical discriminant function was clearly stronger than the second. The canonical correlation coefficient of the first function was 0.985, while that of the second function was 0.496. The first discriminant function correlated strongly with the amounts of Cu^{2+}, Zn^{2+} and Ni^{2+} in throughfall and bulk precipitation, and the bulk precipitation and throughfall samples of the 0.5 km stand formed a distinct group. Correlations between the second discriminant function and the element variables were low. The strongest positive correlation was found with throughfall PO_4^{3-} and the strongest negative with throughfall Mn^{2+}.

3.2. Canopy interactions

The net throughfall amounts of cations and anions indicate the amounts derived from the canopy (Fig. 1c, Table 4). The canopy was an important source of heavy metals and K^+, NH_4^+ and SO_4^{2-} at 0.5 km. The net throughfall values of all cations and anions were positive at 0.5 km, i.e. there was no net interception of these ions at the plot nearest to the smelter. At 0.5 km the amounts of sulphate and heavy metals were clearly higher in throughfall than in bulk precipitation. However, at the other sample plots there was no statistically significant increase in heavy metals in throughfall, except for Zn^{2+} at 8 km. The increase in H^+ in throughfall was statistically significant only at 8 km distance. At 4 km the net throughfall values of NH_4^+ and NO_3^- were negative, and at 8 km those of NH_4^+, NO_3^-, PO_4^{3-} and Ni^{2+}. However, only the decrease in NH_4^+ and NO_3^- at 8 km was statistically significant. The throughfall enhancement of K^+, Ca^{2+} and Mg^{2+} was statistically significant at all the plots. The difference between the amounts of water in bulk precipitation and throughfall was not statistically significant at 0.5 km, but at 4 and 8 km a statistically significant amount of rainwater was intercepted by the canopy.

Table 3
The mean monthly amounts of precipitation, dissolved organic carbon concentration (DOC) and median pH values at different distances from the pollution source[a]

Distance (km)	Bulk precipitation		Throughfall		
	mm	pH	mm	pH	DOC (mg l^{-1})
0.5	32.1 (20.3)	4.9	29.6 (18.3)	4.7	19.3 (34.8)
4	38.2 (22.2)	4.7	25.6 (19.0)	4.4	16.0 (12.6)
8	34.3 (25.5)	4.6	28.4 (20.3)	4.4	16.5 (8.9)

[a] Standard deviations are marked in parentheses.

3.3. The importance of net throughfall flux as a pathway for element cycling

The net throughfall of K^+ was greater than the amount of K^+ in bulk deposition at all the plots (Fig. 1). Hence, the canopy was a more important source of K^+ than bulk deposition. This was also the case for Ca^{2+}, Mg^{2+} and Mn^{2+} at 8 km, and for Fe^{3+} at 0.5 km. Net throughfall of Cu^{2+} and Zn^{2+} was approximately equal to the amounts in bulk precipitation at 0.5

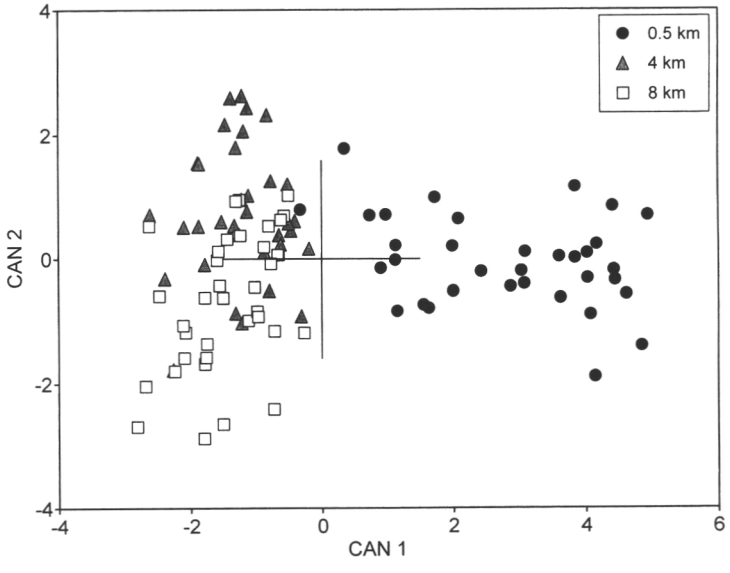

Fig. 2. Distribution of the study stands along the two discriminant function gradients obtained by canonical discriminant analysis.

Table 4
The mean difference (i.e. average net throughfall values) between the element amounts in throughfall and bulk precipitation at different distances from the smelter (paired *t*-test statistics)

	Distance (km)					
	0.5		4		8	
	Mean difference ($mol_c\ m^{-2}$)	*p*	Mean difference ($mol_c\ m^{-2}$)	*p*	Mean difference ($mol_c\ m^{-2}$)	*p*
mm	−2.44	0.0524	−12.58	0.0018*	−5.95	0.0001***
Ni^{2+}	0.10	0.0002***	0.0007	0.5097	−0.001	0.1715
Cu^{2+}	0.26	0.0001***	0.004	0.4150	0.0007	0.3330
Zn^{2+}	0.03	0.0001***	0.003	0.0562	0.003	0.0229*
Mn^{2+}	0.01	0.0001***	0.01	0.0001***	0.02	0.0001***
Fe	0.14	0.0001***	0.01	0.4728	0.008	0.0694
Mg^{2+}	0.11	0.0141*	0.07	0.0003***	0.12	0.0001***
Ca^{2+}	0.32	0.0001***	0.14	0.0012**	0.25	0.0001***
K^+	0.62	0.0001***	0.35	0.0001***	0.44	0.0001***
NH_4^+	0.64	0.1189	−0.18	0.0968	−0.36	0.0047**
H^+	0.11	0.4767	0.03	0.8552	0.33	0.0149*
SO_4^{2-}	2.01	0.0001***	0.02	0.9018	0.27	0.0290*
NO_3^-	0.17	0.1683	−0.14	0.2108	−0.17	0.0123*
PO_4^{3-}	0.03	0.4110	0.01	0.8326	−0.03	0.3192

km. Furthermore, there was an anion deficit in throughfall deposition at 4 and 8 km, but not at 0.5 km. The anion deficit values correlated with the amounts of DOC at 4 ($r=0.497$, $p=0.0028$) and 8 km ($r=0.594$, $p=0.0028$), but not at 0.5 km.

Needle element pools were estimated for each stand from the needle element concentrations and needle masses presented in the Appendix. In most cases the needle element pools were at their lowest at 0.5 km (Fig. 3), closely reflecting the trend in needle mass. However, the pools of Fe, Cu and Ni were at their highest at 0.5 km.

The relative importance of the annual net throughfall flux as a pathway for element cycling was evaluated by calculating, for each element, the ratio between the annual net throughfall flux and the needle element pool (Table 5). When the ratio is greater than 1, the annual net throughfall exceeds the needle pool of this element, i.e. the theoretical time needed for the total removal of the needle pool by canopy leaching (or wash-off) is less than 1 year. The annual net throughfall fluxes exceeded the needle pools of Mg, Cu, Ni and Zn, and the annual flux of Ca and Fe was approximately equal to the needle pool at 0.5 km. Furthermore, the ratios of all the elements were greatest at 0.5 km.

4. Discussion

Interception of rainwater by the tree canopy at 0.5 km was only 8%, compared to 33 and 18% at 4 and 8 km, respectively. Average interception values reported for Scots pine stands vary from 20 to 30% (Päivänen, 1966; Helmisaari and Mälkönen, 1989; Hyvärinen, 1990). The reduction in interception was undoubtedly due to the low needle biomass and, hence, greatly reduced leaf area at 0.5 km, resulting from higher tree mortality and retarded growth caused by emissions from the smelter.

Both bulk precipitation and throughfall at the stand nearest to the smelter were strongly contaminated with sulphate and heavy metals, and the amounts of all mineral nutrients measured were highest at 0.5 km. Possible reasons for these elevated element amounts include current emissions from the smelter, and dust from the degraded forest floor and slag heaps located near-by. Forest canopies are efficient at intercepting dry deposition (Hultberg, 1985), which is reflected as elevated deposition levels in throughfall. In addition to canopy-intercepted dry deposition, throughfall also contains elements leached out from the living and dead foliar tissues (Tukey, 1980; Parker, 1983; Godt et al., 1986).

Since emissions from the smelter have been drastically reduced during the past decade, it is highly likely that dry deposition derived from soil dust has a strong effect on throughfall quality. The earlier high emissions from the Harjavalta smelter have resulted in the accumulation of sulphate and heavy metals in the organic layer of the forest floor (Derome and Lindroos, 1998). An increasing trend was found for Cu, Ni, Fe, Zn, S and P concentrations in the organic layer at decreasing distances from the smelter. The water-holding capacity of the organic layer at 0.5 km is strongly reduced (Derome and Nieminen, 1998), and the forest floor is covered with a thick layer of dry, undecomposed litter.

NH_4^+, K^+, Ca^{2+} and H^+ are usually the most abun-

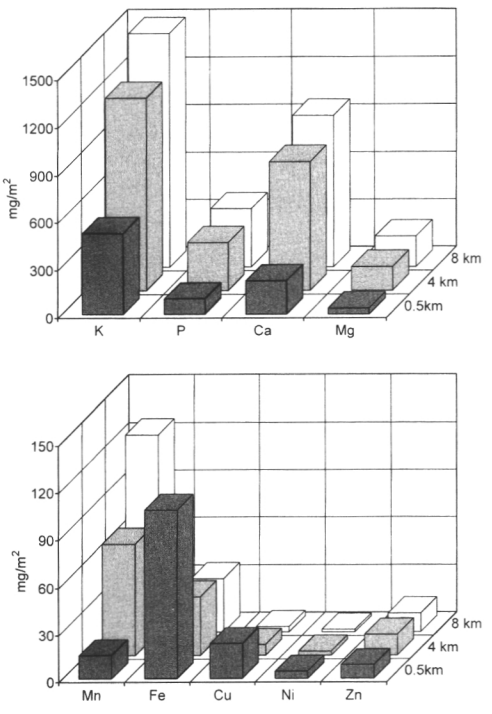

Fig. 3. Needle element pools (mg m^{-2}) at different distances from the smelter.

Table 5
The ratio between the annual net throughfall flux (mg m^{-2} $year^{-1}$) and the needle element pool (mg m^{-2}) at different distances from the smelter[a]

	Distance (km)		
	0.5	4	8
P	0.04	Intercepted	Intercepted
K	0.67	0.13	0.10
Ca	0.99	0.11	0.06
Mg	1.14	0.15	0.08
Fe	0.91	0.12	0.05
Mn	0.56	0.10	0.04
Cu	9.07	0.61	0.06
Ni	13.69	0.13	Intercepted
Zn	3.01	0.15	0.11

[a] When the net throughfall value was negative (= element was intercepted by the canopy), the ratio is not given.

dant inorganic cations in throughfall (Parker, 1983; Hyvärinen, 1990; Strand, 1997). In our study, the amount of NH_4^+ (in mol_c values) was the greatest, and that of Cu^{2+} roughly equal to that of K^+ at the plot nearest the smelter. Inorganic N is usually intercepted by coniferous canopies in background areas (Helmisaari and Mälkönen,1989; Hyvärinen, 1990). However, positive net throughfall of inorganic N has been reported in areas where N deposition is high, e.g. in the Netherlands, in France and in southern Scandinavia (Ivens, 1990; Probst et al., 1995; Strand, 1997). At 4 and 8 km NH_4^+ was intercepted by the canopies and K^+, Ca^{2+} and H^+ were the predominant cations in throughfall. The relatively high amount of NH_4^+ in bulk precipitation and throughfall at 0.5 km was most probably related to emissions of NH_3 gas from the smelter. The behaviour of NO_3^- was rather similar to that of NH_4^+, although the amount of NO_3^- was less than that of NH_4^+ and there was no trend in bulk precipitation with respect to distance to the smelter. Since there was no known point source of NO_3^-, some nitrification has undoubtedly occurred either in the rainfall collectors or on the canopy surfaces.

The amount of net throughfall K^+ was greater than bulk precipitation K^+ at all the plots. Hence the canopy was an important source of K^+ in throughfall. According to Parker (1983), net throughfall K^+ is almost completely derived from foliar leaching. The K^+ in net throughfall was greatest at 0.5 km, even though the stand K^+ needle pool was the lowest, suggesting a faster rate of K^+ cycling at 0.5 km. This is supported by the fact that the needle K^+ concentrations were also highest at 0.5 km, and also by the absence of any trend in K^+ bulk precipitation, thus ruling out any smelter-derived deposition of K^+. Furthermore, enhanced canopy leaching of K^+ has been observed in forest stands suffering from low tree vitality in a number of earlier studies (e.g. Alenäs and Skärby, 1988; Gjengedal, 1996).

The net leaching loss of K from the uppermost 40-cm-thick soil layer, observed by Derome and Nieminen (1998) at the same site at 0.5 km, does not at first sight support the supposition of more efficient K uptake. However, the fact that needle K concentrations at 0.5 km were high and the efficiency of internal K retranslocation in the foliage low (Nieminen and Helmisaari, 1996), suggests that the trees are obtaining sufficient K from the soil. Furthermore, because of the relatively deep rooting system of pine stands growing on sandy soils (Laitakari, 1927), we can assume that high losses of K from the uppermost 40-cm-thick layer do not necessarily mean that K is being lost from the soil–plant cycle.

The canopy-derived amounts of Ca^{2+} and Mg^{2+} were greater than the amounts in bulk precipitation at 8 km. At 0.5 km the situation was the opposite, and bulk deposition of Ca^{2+} and Mg^{2+} exceeded the amounts in net throughfall. A high proportion of net throughfall Ca^{2+} and Mg^{2+} is known to be derived from foliar leaching (Tukey, 1970), and the importance of the wash-off of dry-deposited Ca falls off rapidly with increasing distance from Ca sources, since Ca is primarily trans-

ported in large particles which have a high settling velocity (Parker, 1983). Furthermore, since the stand needle pool of Ca and Mg was lowest at 0.5 km, it is evident that the proportion of foliar leaching in enhancing the throughfall of Ca^{2+} and Mg^{2+} decreased on moving towards the smelter. Correspondingly, the importance of the wash-off of dry-deposited particles increased on moving towards the smelter. There was no trend in the deposition of Mn^{2+} in relation to the distance from the smelter, and the amounts were extremely low in both bulk precipitation and throughfall.

H^+ ions was the only component which showed a decreasing trend towards the smelter, and the throughfall enhancement of H^+ was statistically significant only at 8 km. In addition, there was no anion deficit in the throughfall at 0.5 km. This suggests that the influence of normal canopy exchange processes was lowest at 0.5 km, since enrichment of H^+ in throughfall, together with an anion deficit, is a common phenomenon in coniferous forests (Helmisaari and Mälkönen, 1989; Probst et al., 1995). This is caused by the increase in weak organic acids in throughfall during passage through the canopy (Hoffman et al., 1980). The increase in organic acids due to canopy interactions is usually confirmed by positive correlation between DOC and the anion deficit, as was observed in this study at 4 and 8 km, but not at 0.5 km. The relatively high DOC concentration in the stand throughfall at 0.5 km, coupled with the lack of an anion deficit, suggests that throughfall contained considerable amounts of organic matter in an undissociated form; possibly organic dust derived from the forest floor.

Both negative and positive estimates of the amounts of Cu^{2+}, Ni^{2+}, Zn^{2+} and Fe^{3+} have been reported in net throughfall of background areas, presumably due to the very low concentrations involved (Parker, 1983). This was the case at 4 and 8 km, but at 0.5 km the concentrations were considerably higher. At 0.5 km the net throughfall of Cu^{2+}, Zn^{2+} and Fe^{3+} was approximately equal to the input in bulk deposition, but for Ni^{2+} net throughfall was less than the input. Hence, the canopy was an important source of Cu^{2+}, Zn^{2+} and Fe^{3+} as bulk precipitation, but for Ni^{2+} of less importance. The fact that the needle pool of Zn was lowest and the net throughfall of Zn^{2+} was highest at 0.5 km, indicates that Zn^{2+} was much more susceptible to crown leaching or wash-off at 0.5 km than at 4 and 8 km.

SO_4^{2-} was the most abundant anion, both in bulk precipitation and in throughfall. However, only the amounts at 0.5 km were greater than those reported for Finnish background areas (Helmisaari and Mälkönen, 1989; Hyvärinen, 1990). The drastic decrease in S emissions from the smelter during the last decade has been reflected in the SO_4^{2-} deposition levels. Bulk deposition was a much more important source of SO_4^{2-} than the canopy at all the sites. Throughfall enhancement of SO_4^{2-} has been reported in several studies, and it is assumed to be mainly due to the canopy interception and wash-off of S-rich, dry deposition (Mayer and Ulrich, 1978; Probst et al., 1990; Cape et al., 1992; Hultberg and Grennfelt, 1992; Lindberg and Lovett, 1992).

The effect of the canopy on PO_4^{3-} is usually rather weak (Päivänen, 1974; Parker, 1983; Helmisaari and Mälkönen, 1989), and the amounts of PO_4^{3-} in both bulk precipitation and throughfall are extremely low (Parker, 1983), as was also the case in our study. There was an increasing trend in net throughfall PO_4^{3-} on moving towards the smelter. This may originate from the increasing role of soil dust, which is supported by the fact that the total P concentration in the organic layer is high at 0.5 km (Derome and Lindroos, 1998).

The ratios between the annual net throughfall flux and the needle pool of the individual elements provided further evidence to support the importance of sources other than foliar leaching in the throughfall enhancement of all the elements, except K^+, at 0.5 km. This ratio was greatest at 0.5 km for all the elements. The ratio for Cu and Ni was especially high at 0.5 km, even though their needle pools were also greatest at 0.5 km. Such a high cycling rate could not reflect the internal, root–plant–soil pathway; presumably, other processes are involved. According to Pfirrmann et al. (1990), leaching of most cations from spruce foliage does not represent much more than 1% of the total content in needles. Hence, the removal of particles and aerosols deposited on the needle surface was most probably the main cause of the strong throughfall enhancement of heavy metals observed in our study at 0.5 km. Leaching and wash-off processes from canopy components other than needles, e.g. bark and endophytic biota, may also contribute in heavily polluted forests, although it is supposed to be of minor importance under normal conditions (Mayer and Ulrich, 1978).

5. Conclusions

The low canopy coverage close to the smelter was reflected as low interception of precipitation and depressed chemical canopy interaction. Despite this, the canopy intercepted high amounts of heavy metals. Apart from K^+, the proportion of foliar leaching relative to dry deposition in enhancing throughfall decreased on moving towards the smelter. The high rate of K^+ leaching from the needle tissues close to the smelter demonstrated that the K^+ throughfall flux has been altered by the heavy pollution load.

Appendix

Needle masses from 1991 (Mälkönen et al., 1999) and element concentrations in the Scots pine stands at different distances from the Harjavalta smelter[a]

		Distance (km)		
		0.5	4	8
Needle mass (kg ha^{-1})	Current needles	522	1272	1564
	1-year-old needles	393	1078	1289
	2-year-old needles	52	416	822
	3-year-old needles	0.4	63	157
K (g kg^{-1})	Current needles	7.4 a	7.3 a	6.9 a
	1-year-old needles	5.3 a	4.0 b	4.4 c
	2-year-old needles	4.4 a	3.4 a	3.7 a
	3-year-old needles	4.4 a	3.2 b	3.4 c
P (g kg^{-1})	Current needles	1.5 a	1.5 a	1.6 b
	1-year-old needles	1.0 a	0.9 a	1.0 a
	2-year-old needles	0.9 a	0.9 a	1.0 a
	3-year-old needles	0.9 a	0.9 a	1.0 a
Ca (g kg^{-1})	Current needles	0.8 a	1.1 b	0.9 a
	1-year-old needles	1.9 a	2.1 a	1.9 a
	2-year-old needles	2.1 a	2.6 a	2.3 a
	3-year-old needles	2.5 a	3.0 a	2.3 a
Mg (g kg^{-1})	Current needles	0.4 a	0.7 b	0.7 b
	1-year-old needles	0.4 a	0.5 a	0.6 b
	2-year-old needles	0.3 a	0.4 a,b	0.5 b
	3-year-old needles	0.3 a	0.3 a	0.4 a
Fe (mg kg^{-1})	Current needles	329.5 a	36.2 b	37.3 b
	1-year-old needles	968.0 a	88.9 b	74.1 b
	2-year-old needles	1096.9 a	122.1 b	95.3 b
	3-year-old needles	889.6 a	150.1 b	108.7 b
Mn (mg kg^{-1})	Current needles	69.6 a	133.9 b	143.1 b
	1-year-old needles	146.2 a	259.9 b	304.4 b
	2-year-old needles	135.9 a	301.3 b	356.2 b
	3-year-old needles	151.4 a	324.2 b	342.6 b
Cu (mg kg^{-1})	Current needles	89.2 a	9.3 b	6.8 b
	1-year-old needles	210.5 a	20.0 b	8.7 b
	2-year-old needles	225.5 a	20.8 b	9.3 b
	3-year-old needles	173.3 a	23.9 b	9.9 b
Ni (mg kg^{-1})	Current needles	26.0 a	11.2 b	9.2 b
	1-year-old needles	43.5 a	7.8 b	5.1 b
	2-year-old needles	49.4 a	8.2 b	4.8 b
	3-year-old needles	43.6 a	8.6 a	3.9 b
Zn (mg kg^{-1})	Current needles	39.6 a	28.0 b	27.7 b
	1-year-old needles	82.8 a	36.9 b	33.4 b
	2-year-old needles	86.9 a	42.0 b	32.0 b
	3-year-old needles	78.8 a	46.2 a	29.9 b

[a] Values for needle age classes marked with different letters differ significantly from each other ($p < 0.05$, $n = 10$, pairwise comparisons by the Tukey test).

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Paper II

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II

Corrections:

p. 85, Chapter 3.3., line 3. The metal amounts are 250.5, 69.2, 78.3, 6.9 and 2.9 kg ha^{-1} (and not 2505, 692, 783, 69 and 29 kg ha^{-1}).

p. 87, Chapter 4.2., Table 8. The annual depositions are 455.0, 173.0 and 142.0 (and not 4550, 1730, and 1420).

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Enrichment of Cu, Ni, Zn, Pb and As in an ombrotrophic peat bog near a Cu-Ni smelter in Southwest Finland

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Abstract

The accumulation of selected trace elements (Cu, Ni, Zn, Pb, As) in the surface peat layer of an ombrotrophic bog 2.4 km from a Cu-Ni smelter at Harjavalta, Finland was studied using a peat core. A reference core was taken from an ombrotrophic bog at a background site, Hietajärvi, in eastern Finland. Element concentrations were analysed from 1-cm slices and enrichment factors (EF) were calculated. The enrichment factors of both Cu and Ni in the Harjavalta peat bog are extremely high compared to the Hietajärvi site. However, only the 6-cm surface peat Pb values are higher in Harjavalta compared to Hietajärvi. Precipitation was collected during 1992–1996, in the vicinity of the Harjavalta smelter, in order to estimate the current atmospheric deposition load. Comparison between the precipitation and peat data reveals that at Harjavalta the surface peat is relatively much more polluted than the current precipitation. The variation in EF of the Harjavalta peat core with respect to depth shows two patterns: Cu and Pb are similar, as are Ni, Zn and As. The vertical gradient in Harjavalta Cu EF suggests that Cu supplied to the peat by atmospheric deposition is very well preserved by the bog.

Keywords: Deposition; Heavy-metal pollution; Peat analysis; Precipitation

1. Introduction

In Finland, the analysis of surface peat from ombrotrophic bogs has proven to be an effective tool in estimating the regional distribution of atmospheric heavy-metal deposition (Pakarinen, 1981; Pakarinen and Tolonen 1976; Pakarinen et al., 1983). Furthermore, the recent progress in natural sciences and analytical techniques has revealed new perspectives for the use of peat sediments as historical records. Modern, sensitive and accurate analytical techniques make it possible to study vertical element distributions in peat profiles, and even to create historical chronologies of metal depositions.

Peat cores taken from ombrotrophic bogs have been successfully used to reconstruct historical records of atmospheric Pb deposition, (e.g. Shotyk, 1996; Shotyk et al., 1997, 1998). However, peat

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Table 1
Estimated dust emissions from copper smelter and nickel smelter at Harjavalta in tonnes per 5-year-period

Period	Dust from Cu production	Dust from Ni production
1945–1949	2388	0
1950–1954	2671	0
1955–1959	3908	10
1960–1964	5105	870
1965–1969	2379	780
1970–1974	951	1787
1975–1979	1182	2708
1980–1984	1452	3064

Data from Outokumpu Harjavalta Metals Oy.

cores can also serve as an excellent tool in studying the mobility of different trace elements in organic soil profiles. The distribution of Cu and Zn, being important micronutrients, has been studied rather intensively in peatland forests (Veijalainen, 1984; Kaunisto and Paavilainen, 1988) as well as in pristine raised bogs when Pb is also included (Pakarinen and Tolonen, 1977; Damman, 1978; Pakarinen, 1981). In contrast, relatively few studies have included analyses of Ni (Tanskanen, 1972; Veijalainen, 1998) or As (Shotyk, 1996) in peat bogs.

Most of the studies on the vertical distribution and mobility of trace elements in ombrotrophic bogs have been carried out at background sites, where the overall concentrations are low. In heavily polluted conditions, the distribution and mobility of elements may drastically change due to vegetation damage and the elevated burden of the accumulated elements.

In this study, trace element concentrations of a peat core taken from a polluted ombrotrophic bog near a Cu-Ni smelter were compared with smelters emission records and with element concentrations of a reference peat core taken from an ombrotrophic bog at a background site. However, this study was intended as a preliminary analysis based on a limited data set. The aim was to quantify the changes in element accumulation during the 50-year period of smelting activities, as well as to further our understanding about the mobility of the elements of concern in organic soil.

2. Material and methods

2.1. Study sites

The polluted study site is located in the concentric raised bog zone (Ruuhijärvi, 1983) in SW Finland, in the area of Harjavalta township (61°20′N, 22°10′E). A copper smelter has been operating in the vicinity of the study site since 1945 and a nickel smelter since 1959 (Outokumpu Harjavalta Metals Oy). Heavy metals and arsenic are emitted as components of fugitive dust release. Particulate emissions during 1945–1984 were estimated on the basis of dust emitted per tonne of metal produced from data supplied by the smelter company (Table 1). Monitoring of emissions was started in 1985 by the smelter company (Table 2). During the past few years, dust emissions have been drastically decreased owing to changes in

Table 2
Emissions from Harjavalta smelters in tonnes per year during 1985–1999

Year	Dust	Cu	Ni	Zn	Pb	As
t year^{-1}						
1985	1100	98	47	216	55	
1986	1200	126	46	232	60	
1987	1800	140	96	162	94	
1988	1000	104	45	103	48	
1989	1000	80	33	190	70	
1990	960	80	31	160	80	
1991	640	80	14	90	45	
1992	280	60	10	12	9	
1993	250	50	7	13	6	11.0
1994	190	40	6	6	3	5.0
1995	70	17	1.4	1.7	0.5	0.2
1996	195	49	1.2	5.3	1.9	4.2
1997	360	70	3	14	4	10.0
1998	132	23	1.7	6.1	2.4	10.1
1999	48	6	0.8	4.2	1.0	1.8

Arsenic monitoring started in 1993 (data from Outokumpu Harjavalta Metals Oy).

process technology and the installation of new filters.

Until the early 1970s, the ores used for smelting were mainly domestic sulfidic minerals from the Outokumpu mine in eastern Finland. At present, the ore concentrates used form a varied group originating from different parts of the world: the Cu concentrate coming from, e.g. Ireland and Chile and the Ni concentrate mainly from Australia. The slags generated by smelting are stored in land basins at the plant site. The Cu-slag is pumped into the storage area as sludge, and the granulated Ni-slag is piled in heaps and landscaped. During the piling period, the uncovered slag heaps are an additional source of dust emissions.

The reference site, Hietajärvi, is located in the transition area between the eccentric raised bog zone and northern aapa mire zone (Ruuhijärvi, 1983) in eastern Finland (63°10′N, 30°43′E). There is no agriculture or roads nearby and no point sources of heavy-metal air pollution within tens of kilometres. Hence, the only source of air pollution is from long-range transported emissions. This rural site is used to provide a record of the 'local background' for comparison with Harjavalta.

The study sites were undrained ombrotrophic bogs with a sparse tree (*Pinus sylvestris* L.) cover. The field layer of both sites was dominated by *Eriophorum vaginatum* L., but at the polluted Harjavalta site only two species, *Pohlia nutans* (Hedw.) Lindb. and *Cladopodiella fluitans* (Nees) Buch, occurred in the ground layer and a large part consisted of unvegetated peat surfaces. The ground layer vegetation of the background site in Hietajärvi consisted of *Sphagnum* species typical to a pristine ombrotrophic pine bog, e.g. *S. angustifolium* C. Jens. and *S. fuscum* (Schimp.) Klinggr.

2.2. Peat sampling and analysis

A peat core (15-cm depth×5×5 cm) was taken from the surface layer of the Harjavalta bog, 2.4 km SW-W from the Cu-Ni smelter. A reference core was taken from the background bog at Hietajärvi in eastern Finland. The cores were frozen and cut into 1-cm slices using a stainless steel band saw at the University of Berne. All peat samples were dried at 105 °C in acid-washed Teflon bowls and macerated in a centrifugal mill, equipped with a Ti rotor and 0.25-mm Ti sieve. The milling was carried out in a Class 100 laminar flow clean air cabinet to prevent possible contamination of the peat samples by lab dust. Selected trace elements were measured using the Energy-dispersive Miniprobe Multielement Analyzer (EMMA) at EMMA Analytical Inc., Elmvale, Ontario, Canada. The instrument was calibrated using certified standard reference plant materials.

The relationships between the element concentrations in the Harjavalta peat profile were analysed by calculating the Pearson's product moment correlation coefficients.

In order to separate the natural variations in element concentrations with depth from changes due to anthropogenic deposition, enrichment factors (EF) were calculated according to the following formula:

$$EF = (X/Ti)/(X/Ti)_{crust},$$

where the data of Wedepohl (1995) was used for the values of the Earth's crust.

The calculated enrichment factors (EF) show the extent of the changes in element abundances in the profile relative to crustal values. We acknowledge that pre-anthropogenic (X/Ti) peat values may exceed those of the crust, but it is helpful to have a common reference level for comparison between the two cores and with our published values.

Pollution factors (PF) were calculated for comparison between the polluted (Harjavalta) and the reference site (Hietajärvi). The average and median element concentrations of the Harjavalta core were divided by the corresponding concentrations of the Hietajärvi core. These PF values thus indicate simply the extent of pollution at the Harjavalta bog compared to the Hietajärvi bog.

The bulk density value 87 g dm^{-3} reported by Veijalainen (1984) for the Harjavalta peat bog was used to estimate the total amounts of the studied elements for the 15-cm surface peat layer. It was assumed that this 15-cm surface peat contains all peat formed since the smelting activities started. Thus, the total amount of Cu and Zn was divided by the duration of Cu smelting (55 years) in order to give a rough estimate of the average annual

Table 3
Metal concentrations (mg kg^{-1}) of a peat core from an ombrotrophic bog near a Cu-Ni smelter at Harjavalta, SW Finland

Depth, cm	Cu mg kg^{-1}	Ni	Zn	Pb	As	Ti
1	3528	913	500	204	58	942
2	4384	865	559	131	40	729
3	4524	652	537	82	25	239
4	4497	540	515	68	19	196
5	4659	578	594	63	17	170
6	3233	595	688	43	17	172
7	1092	567	678	21	16	135
8	349	529	677	17	15	181
9	158	459	624	15	16	190
10	136	482	707	23	17	215
11	107	365	626	18	15	119
12	100	321	575	24	15	123
13	61	286	545	19	14	160
14	46	260	574	22	15	166

deposition during the smelter history. Ni was correspondingly divided by 40, since Ni smelting started in 1959.

2.3. Precipitation sampling and analysis

Precipitation was collected monthly from June 1992 to December 1996, in an open area in the immediate vicinity of the Harjavalta Cu-Ni-smelter (0.5 km SW) using five rainfall collectors ($d=20$ cm) during the snowfree period and two snow collectors ($d=36$ cm) during the winter. Samples were filtered (0.45-μm membrane filter) and Cu, Ni, Zn and Pb concentrations were determined by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP/AES) in the Central Laboratory of the Finnish Forest Research Institute, Vantaa, Finland. The laboratory has a continuous quality control programme and it participates regularly in national and international intercalibration exercises. Medians of Cu, Ni, Zn and Pb concentrations in bulk precipitation at Hietajärvi background site (during 1990–1996) reported by Ukonmaanaho et al. (1998) were used for comparison. The precipitation samples from Hietajärvi have been analysed unfiltered by inductively coupled plasma mass spectrometry (ICP/MS) in the Finnish Meteorological Institute. Estimation of average annual Cu, Ni and Zn deposition at Harjavalta was based on element fluxes presented by Derome (2000).

3. Results

3.1. Enrichment factors and pollution factors of the peat profiles

The element concentrations in the Harjavalta peat core are much higher when compared to the corresponding values in the Hietajärvi peat core (Tables 3 and 4). The strongest correlation in the Harjavalta peat core is found between As and Pb (Table 5). However, As, Pb and Ni correlated all strongly with each other (Table 5).

The variation in EF of the Harjavalta peat core with respect to depth shows two patterns: Cu and Pb are similar, as are Ni, Zn, and As (Fig. 1). The amounts of both Cu and Ni in the Harjavalta peat bog are extremely high (Table 3; Fig. 1). The Harjavalta Cu EF values are hundreds of times greater than those of the Hietajärvi background site (Figs. 1 and 2), and Ni values correspondingly tens of times higher. Lead is exceptional in that at Hietajärvi Pb is almost as enriched as at Harjavalta. In addition, the pollution factors (PF) demonstrate high amounts of accumulated metals at Harjavalta

Table 4
Metal concentrations (mg kg^{-1}) of a peat core from an ombrotrophic bog at background site Hietajärvi, eastern Finland

Depth, cm	Cu	Ni	Zn	Pb	As	Ti
	mg kg^{-1}					
1	8	4	50	4	n.d.	93
2	6	3	53	7	n.d.	132
3	6	5	53	6	n.d.	135
4	7	6	63	8	1.4	111
5	6	5	80	13	3.5	75
6	6	6	76	16	1.9	88
7	6	n.d.	78	17	2.2	73
8	3	5	78	17	2.6	88
9	3	9	84	14	2.5	77
10	5	14	78	18	3.8	87
11	8	10	71	18	3.9	152
12	6	9	70	19	1.9	95
13	8	n.d.	69	18	4.6	129
14	5	7	60	19	3.5	91
15	7	8	68	21	3.2	110
16	4	7	61	20	5.2	86

n.d. = not detected.

Table 5
A correlation matrix of the metal concentration in the Harjavalta peat core

	Cu		Ni		Zn		Pb		As	
	r	p	r	p	r	p	r	p	r	p
Cu	1.00	0.0								
Ni	0.74	0.003	1.00	0.0						
Zn	−0.45	0.105	−0.22	0.105	1.00	0.0				
Pb	0.69	0.007	0.86	0.0001	−0.61	0.022	1.00	0.0		
As	0.51	0.060	0.84	0.0002	−0.52	0.055	0.97	0.0001	1.00	0.0

peat bog, with the exception of Pb (Table 6). The median Cu PF and Pb PF are lower than the corresponding averages reflecting the uneven vertical distribution of these elements.

3.2. Precipitation

Comparison of the monthly median concentrations of Cu, Ni and Zn in bulk precipitation at Harjavalta to the corresponding precipitation data from Hietajärvi indicates an elevated contamination rate at Harjavalta (Table 7). The Pb concentrations in most of the precipitation samples were below the detection limit of the analytical equipment of 0.025 mg l^{-1}. Arsenic was not measured in the precipitation samples.

3.3. Total element amounts in peat and annual deposition estimates

The total amounts of metal accumulated in the 15-cm surface peat for Cu, Ni, Zn, Pb and As are 2505, 692, 783, 69 and 29 kg ha^{-1}, respectively. Estimates of the average annual deposition of Cu, Ni and Zn during the period of smelting activities, derived by simply dividing the total amounts by the duration of smelting activity, are much greater than the current annual deposition values based on precipitation analyses (Table 8).

4. Discussion

4.1. Enrichment and mobility of the studied elements

The vertical gradient in Cu EF (from 4420 to 45 over a distance of only 9 cm) in the peat profile at Harjavalta suggests that Cu deposited on the peat from the atmosphere is very well preserved by the bog. The relatively high Ni EF values compared to Cu EF at depths of more than 7–8 cm, indicates post-depositional migration, since the emissions of Ni from the smelter started 15 years later than those of Cu. In addition, the fact that the peat Ni values are consistently much lower than the Cu values, although Ni production-induced dust emissions during 1970–1985 were superior to Cu dust emissions, gives further evidence of greater Ni mobility. However, the transportation distance of Ni-containing particles may be larger than that of Cu-containing particles and hence lead to a relatively lower Ni deposition in the immediate vicinity of the smelter.

Derome (2000) reported relatively similar accumulation for the organic layer of forest soil in the vicinity of the Harjavalta smelter: the Cu concentrations were 200–800 times higher than the average background Cu value for the same forest type and the Ni concentrations were correspondingly 40–90 times higher

Greater mobility of Ni compared to Cu in the organic layer of mineral forest soil has also been reported by Derome and Nieminen (1998) on the basis of soil water fluxes in the vicinity of the Harjavalta smelter. This was also supported by the Cu/Ni ratios in the organic layer and the uppermost mineral soil layer at the same site (Derome and Lindroos, 1998). Copper is known to form much more stable complexes with natural organic ligands than does either Ni or Zn (Bergkvist et al., 1989; Baker and Senft, 1995).

The clearly higher Zn and As values in Harjavalta peat compared to Hietajärvi peat indicate accumulation of smelter-derived Zn and As, even

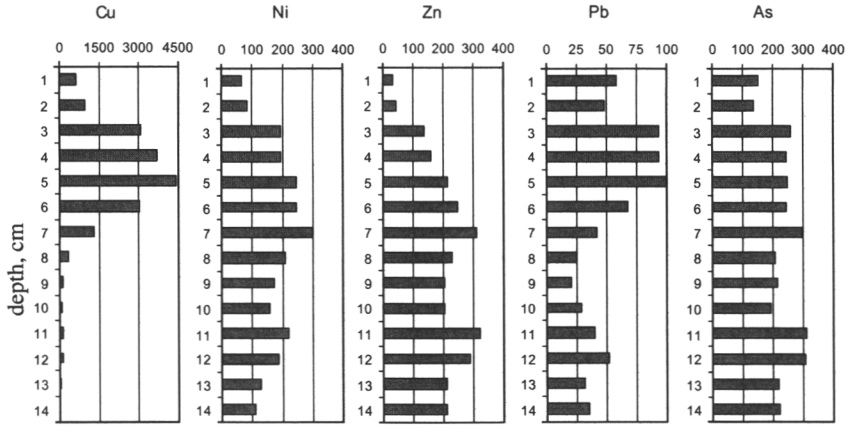

Fig. 1. The metal enrichment factors (EF) of a peat core taken from the Harjavalta bog near a Cu-Ni smelter.

though there are no clear gradients in their vertical distribution. However, the Zn values in Harjavalta peat are surprisingly low compared to the relatively high Zn emissions, which suggests considerable post-depositional Zn migration. The lack of correlation between Zn and the other elements in the Harjavalta peat core gives more evidence about the great mobility of the Zn in the peat profile. The assumption of the Zn mobility is supported by the findings of Derome and Nieminen (1998), who reported a clear net loss of Zn in percolation water from the 40-cm-thick surface layer of an upland site close to the smelter.

Veijalainen (1998) has reported results from an old ore-prospecting simulation experiment in Alkkia, SW Finland. Extremely high doses of Cu, Ni

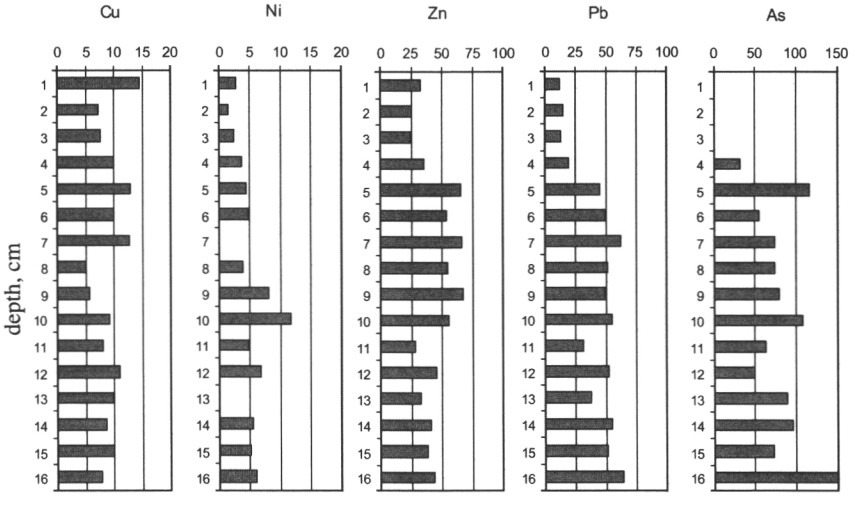

Fig. 2. The metal enrichment factors (EF) of a peat core taken from the background site at Hietajärvi.

Table 6
Median and average pollution factors (PF) of the Harjavalta peat core

	Median	Average
Cu PF	126	337
Ni PF	89	88
Zn PF	8	9
Pb PF	1	4
As PF	6	8

and Zn were applied to the surface of an ombrotrophic peat bog in July 1962. In 1990, the surface peat (0–20 cm) still contained 93% of the applied Cu (400 kg ha^{-1}), but in Ni plots, the surface peat contained only 63% of the applied Ni dose (400 kg ha^{-1}). The corresponding Zn retention was even lower, only 47%.

The Pb concentrations were surprisingly low despite Pb emissions from the smelter in both the bulk precipitation and peat, below the analytical detection limits in precipitation and in only the top 6 cm were peat Pb EF values higher than those of the background peat core from Hietajärvi. However, the vertical gradient for Pb EF is similar to the pattern for Cu EF. Several studies have shown that Pb is very well preserved in peat bogs (Shotyk, 1996 and references cited therein).

The decrease in Pb concentrations in the four uppermost centimetres at the background site Hietajärvi is in accordance with the observed decrease in Pb concentrations of forest mosses in the Hietajärvi catchment (Ukonmaanaho et al., 1998) and with a general Pb decrease in forest mosses observed in Finland (Kubin et al., 2000). Forest mosses have been analysed for the national level heavy metal survey in 5-year intervals since 1985 and the decrease in moss Pb concentrations (on average, 60% decrease) has been attributed to the introduction of Pb-free petrol (Kubin et al., 2000). Moss Pb concentrations in the Hietajärvi catchment indicate a near 50% decrease from 1991 levels to 1996 levels (Ukonmaanaho et al., 1998).

4.2. The extent of heavy-metal pollution at Harjavalta

The difference in peat PF values for Cu, Ni and Zn between the two sites is greater than the difference in Cu, Ni and Zn concentrations in precipitation, which reflects the earlier high emission levels in contrast to the current situation of strongly reduced emissions. However, caution should be paid when comparing the peat values to the precipitation data. Since precipitation samples of Harjavalta were filtered prior to analysis, they do not contain insoluble particles with a diameter greater than 0.45 μm, but peat concentrations, for their part, include all chemical and mineralogical forms. On the other hand, the element concentrations of precipitation are not only due to atmospheric inputs, since the internal cycling of dust plays a role in the area of precipitation collection in the immediate vicinity of the smelter (Nieminen et al., 1999). The earlier high emissions have resulted in the accumulation of metals in the organic layer of the soil and vegetation is almost completely lacking. In addition the water holding capacity of the organic layer is considerably reduced (Derome and Nieminen, 1998). Hence, the vegetation-free, dry soil is very susceptible to

Table 7
The median concentrations of Cu, Ni and Zn in bulk precipitation in the vicinity of the Harjavalta Cu-Ni smelter during 1992–1996 and the corresponding metal concentrations in precipitation at Hietajärvi site (1990–1996) reported by Ukonmaanaho et al. (1998)

	Cu	Ni	Zn
	μg l^{-1}		
Harjavalta	340	60	30
Hietajärvi	1.00	0.31	3.3

Table 8
Average annual deposition of Cu, Ni and Zn via bulk precipitation for the period from 1993 until 1998 (based on fluxes presented by Derome, 2000) and estimates based on peat stock of the average annual deposition for the whole period of smelting activities in Harjavalta

	Cu	Ni	Zn
Current annual deposition (1993–1996), mg m^{-2} $year^{-1}$	149	64	17
Average annual deposition for the whole period of smelting activities, mg m^{-2} $year^{-1}$	4550	1730	1420

erosion. Thus, in heavily polluted conditions of this sort, atmospheric input estimates based on precipitation samples are more likely over-estimations than under-estimations.

The estimation of the total amount (kg ha^{-1}) in the 15-cm surface peat layer gives a reasonable indication of the total deposition rate only in the case of relatively immobile Cu and Pb. Nickel, Zn and most probably As are more mobile and the amount lost through migration remains unknown. However, the annual deposition estimates based on this peat inventory are much greater than the current deposition even for Ni and Zn. This is undoubtedly due to accumulation during the past high emission level periods.

4.3. Local sources of heavy-metal deposition

The main sources of heavy-metals at Harjavalta are the particulate emissions from the stacks of the smelter complex. At present, over 90% of Pb and As are emitted from the 140 m high main stack, and the corresponding proportions for Zn and Ni are approximately 80% (Saari et al., 1998). This might, at least partly, explain the strong correlation between Pb and As in the Harjavalta peat core. Copper, for its part, is mainly (60%) emitted from a smaller, 40 m high stack (Saari et al., 1998).

Additional sources of heavy metals are the slags formed during the smelting processes. In the immediate vicinity of the slag heaps or basins, the windborne slag dust may have an important impact on the heavy-metal deposition. According to the information given by the smelter company, the slags may contain also small amounts of Ti, especially the slag from Ni-production, since an ilmenite-based concentrate from the Titania mine in southern Norway has occasionally been used as a raw material in Ni smelting. Consequently, the use of Ti-related enrichment factors may to some extent underestimate the heavy metal enrichment in the peat. However, the Ti concentrations at Harjavalta are only slightly higher than the Ti concentrations at the rural background site Hietajärvi.

In order to reduce the dust emissions, the company started to transport the Cu slag in sludge form and a basin for the sludge was constructed in the 1990s, beside the eastern margin of the peat bog under study. In spring 1998, a dam was broken and the sludge was released to the surrounding fields and forests by ditches. Despite active cleaning efforts, the dispersed slag increased the heavy metal load to the bog under study, especially since even a direct contamination of the peat surface by the sludge may have occurred. Therefore the uppermost concentrations and enrichment values of the Harjavalta peat core may be biased. The surface contamination may also be reflected in the correlations between the element concentrations, as the slag contains small amounts of all the studied elements.

Regardless of the source of the elements in the peat profile, their vertical distributions demonstrate the different behaviour of Cu and Pb vs. Ni, Zn and As. The retention mechanisms remain as a subject for future studies, as well as the elaboration of deposition chronologies by the aid of an appropriate age-dating technique.

5. Conclusions

The inventories of both Cu and Ni in polluted Harjavalta peat bog are extremely high compared to the background site at Hietajärvi. At present, the surface peat is relatively much more polluted than the current precipitation at Harjavalta. With respect to Pb, however, only the top 6 cm at Harjavalta are enriched compared to Hietajärvi. The variation in EF of the Harjavalta peat core with respect to depth shows two patterns; Cu and Pb are similar, as are Ni, Zn and As. The vertical gradient in Harjavalta Cu EF suggests that Cu supplied to the peat by atmospheric deposition is very well preserved by the bog.

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Paper III

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PAPER

Effects of soil copper and nickel on survival and growth of Scots pine

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The contribution of soil Cu and Ni pollution to the poor vitality and growth rate of Scots pine growing in the vicinity of a Cu–Ni smelter was investigated in two manipulation experiments. In the first manipulation, Cu–Ni smelter-polluted soil cores were transported from a smelter-pollution gradient to unpolluted greenhouse conditions. A 4-year-old pine seedling was planted in each core and cultivated for a 17-month period. In the second manipulation, pine seedlings from the same lot were cultivated for the same 17-month period in a quartz sand medium containing increasing doses of copper sulfate, nickel sulfate, and a combination of both. The variation in the biomass growth of the seedlings grown in the smelter-polluted soil cores was very similar to that of mature pine stands growing along the same smelter-pollution gradient in the field. In addition, the rate of Cu and Ni exposure explained a high proportion of the biomass growth variation, and had an effect on the Ca, K, and Mg status of the seedlings. According to the lethal threshold values determined on the basis of the metal sulfate exposure experiments, both the Cu and Ni content of the 0.5 km smelter-polluted soil cores were high enough to cause the death of most of the seedlings. The presence of Cu seemed to increase Ni toxicity.

1. Aim of investigation

Contamination of the environment by heavy metals has been one of the major environmental problems ever since the beginning of metallurgical industries. Although a considerable number of studies have been carried out on agronomic and herbaceous plants,[1,2] much less is known about the influence of heavy metals on forest trees. This is despite the fact that the largest mining and smelting plants in the boreal vegetation zone are situated in forested areas. The gradual accumulation of metals in forest soil continues to affect the trees even after the emissions have ceased. It is therefore extremely important to determine how chronic metal exposure affects the functioning of forest trees.

Scots pine is one of the most interesting species for studies on the effects of heavy metals on trees. It appears to be one of the most resistant plant species near the huge Monchegorsk and Severonickel smelters in north-western Russia,[3] and in the vicinity of a much smaller Cu–Ni smelter at Harjavalta, south-western Finland.[4–6] In addition, Scots pine is the most extensively distributed pine species in the boreal and temperate zone,[7] and is economically the most valuable tree species in northern Europe.

The vitality and growth rate of Scots pines growing in the immediate vicinity of the Harjavalta smelter have been greatly reduced.[5,6,8] On the Kola Peninsula, the radial growth of mature Scots pines has been affected by the long-term pollution load up to a distance of 30 km south from the Monchegorsk smelter, and radial growth has totally ceased within 15 km.[9,10]

Most of the studies dealing with the effects of metals on trees have been conducted for short periods in nutrient solutions with young, often newly germinated seedlings. However, small seedlings are physiologically very different from forest trees, and it is therefore often difficult to generalize the results to cover natural conditions. In order to avoid this problem, seedlings that had already reached an age of 4 years were used in this study. Although the use of nutrient solutions as a growing media has many advantages, one major disadvantage is the fact that the culture conditions are different from those in a natural environment in terms of metal phytoavailability. The toxic threshold values determined on the basis of nutrient solution cultures do not necessarily apply in a natural soil environment, where several factors, such as soil texture, pH, and organic matter content, have a considerable influence on metal availability. As a result, it has been claimed that toxicity tests with soil-grown plants would be ecologically much more meaningful than those carried out with nutrient solution cultures.[11]

The aim of the study was to evaluate the response of Scots pine to soil heavy metal pollution, with respect to survival, growth, nutrition, and distribution of the heavy metals within the plant. Scots pine seedlings were cultivated in soil cores obtained from pine stands located along a heavy metal pollution gradient near the Harjavalta smelter. Since an earlier study had shown that Cu and Ni are the main soil pollutants at Harjavalta,[12] special attention was paid to the effects of soil Cu and Ni. A parallel set of artificial Cu and/or Ni treatments was established for comparison purposes.

2. Description of the experimental procedures

2.1. Site description

The Cu–Ni smelter complex is situated on an esker close to the town of Harjavalta (61°20′N, 22°10′E) in the southern boreal coniferous zone.[13] The long-term (1960–1990) mean annual temperature at a nearby weather station of the Finnish Meteorological Institute is 4.0 °C, and the annual precipitation 558 mm. Four of the sampling sites were located in pure Scots pine stands on the esker at distances of 0.5, 2, 4 and 8 km southeast from the main stack of the smelter. One sampling site, situated at Hämeenkangas, 60 km northeast from Harjavalta, in an area without any local emission sources, was chosen as a background site. The texture of the mineral soil is fine or coarse sand, and the soil type is orthic podzol. The organic layer is mor, with a thickness ranging from 1 to 3 cm.

The vegetation on all the sites is, or has originally been, typical of xerophilous forest sites: *Pinus sylvestris* L. as the dominant tree species, and ground vegetation layers mainly consisting of *Calluna vulgaris* (L.) Hull., *Empetrum nigrum* L.,

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Vaccinium vitis-idaea L., *Pleurozium schreberi* (Brid.) Mit., *Dicranum spp.* and *Cladina spp.* The sites are of the *Calluna* type, according to the Finnish forest site classification of Cajander.[14] However, in the immediate vicinity of the smelter the understorey vegetation is almost completely absent,[15] and the Scots pine stand is suffering from retarded growth and severe needle loss.[5] According to a survey realised in 1993 by Salemaa *et al.*,[15] lichens were absent up to a distance of 2 km, and mosses, excluding *Pohlia nutans* (Hedw.) Lindb., were not frequent until a distance of 8 km.

The copper smelter has been operating in the vicinity of the sampling sites since 1945 and the nickel smelter since 1959 (Outokumpu Harjavalta Metals Oy). Until the early 1970s the ores used for smelting were mainly domestic sulfidic minerals from the Outokumpu mine in eastern Finland. The ore concentrates currently used at the smelters originate from different parts of the world: the Cu concentrate is imported from *e.g.* Ireland and Chile, and the Ni concentrate mainly from Australia.

Heavy metals are emitted as components of fugitive dust release. During the past few years the dust emissions have been decreased drastically owing to changes in process technology and the installation of new filters. The slag produced during smelting is stored in land basins at the plant site. The Cu slag is pumped into the storage area as sludge, and the granulated Ni slag is piled in heaps and landscaped. During the piling period the uncovered slag heaps are an additional source of dust emissions.

2.2. Soil sampling

Intact volumetric soil profiles including the litter layer and ground vegetation were taken with an auger (diameter 25 cm, depth 30 cm) at the five sampling sites (0.5, 2, 4, 8 and 60 km) and placed in 10-liter pots. One 4-year-old, bare-rooted pine seedling (*Pinus sylvestris* L.) was planted on the 2nd of June 1994 in each pot. The soil profiles were taken at 25 points in 5 clusters on each site. A smaller volumetric soil sample was taken for chemical analysis next to each sampling point with a small auger (diameter 3.8 cm, depth 30 cm). The loose litter was removed from the top of the sample, and the sample was divided into the humus layer and two mineral soil layers: 0–10 and 10–20 cm. The samples from each cluster (5) were bulked to give five composite samples per layer per site.

2.3. Artificial exposure treatment

Pine seedlings from the same 4-year-old seedling lot were planted on the 2nd of June 1994 in similar 10-litre pots as for the smelter-polluted soil. Each pot contained 8 litres (11.34 kg) of quartz sand (particle size 0.5–1.5 mm). On the following day the soil–plant systems were treated with increasing doses of (1) copper sulfate ($CuSO_4 \cdot 5H_2O$), (2) nickel sulfate ($NiSO_4 \cdot 6H_2O$) or (3) a combination of both in equal doses (Table 1). Two replicate seedlings were treated with each treatment dose in order to ensure a sufficient amount of plant material for chemical analysis. All the treatments were given as a water solution. The seedlings were fertilized twice during the experiment: on the 5th of July 1994 and the 2nd of July 1995 using a commercial fertilizer. The total amount of nutrients added per pot in fertilization were 155 mg N, 55 mg P, 355 mg K and 22 mg Mg. These doses were relatively low because achieving optimal nutrient conditions, which are unlikely to occur in natural conditions, was not the aim of the fertilization. At the end of the experiment soil samples were taken for chemical analysis with a small auger (diameter 3.8 cm) from the artificial quartz sand exposure pots. The samples from both replicates were combined to give one composite sample per treatment level.

Table 1 The Cu and Ni exposure doses of the artificial treatment series, as well as the corresponding soil concentrations and area-based doses

Treatment dose/mg per pot	Soil concentration/ mg kg^{-1} d.w.	Deposition/ mg m^{-2}
Cu or Ni treatment		
0	0	0
2.4	0.2	50
6	0.6	125
12	0.7	250
24	2.1	500
48	4.2	1000
72	6.3	1500
96	8.5	2000
120	10.6	2500
180	15.9	3700
240	21.2	5000
300	26.4	6000
360	31.7	7500
480	42.3	10000
720	63.5	15000
1440	126.9	30000
2880	253.9	60000
Cu + Ni treatment		
0 + 0	0	0
1.2 + 1.2	0.1	25
2.4 + 2.4	0.2	50
4.8 + 4.8	0.4	100
12 + 12	1.1	240
24 + 24	2.1	480
36 + 36	3.2	730
48 + 48	4.2	980
60 + 60	5.3	1220
120 + 120	10.6	2450
240 + 240	21.2	4900
480 + 480	42.3	9800
960 + 960	84.6	19600

2.4. The pine seedlings and experimental conditions

The 4-year-old pine seedlings used in the experiment were bare-rooted and had been raised from selected seed in the forest nursery of the Finnish Forest Research Institute at Suonenjoki, eastern Finland. A set of 50 reference seedlings from the same 4-year-old seedling lot as those planted in the experimental pots, were measured (average height 31 cm, standard deviation 5.8), weighed and the element concentrations in the different compartments (roots, stem, needles) analysed at the start of the experiment. All the soil–plant systems (total number 185) were cultivated for 17 months (from June 1994 until October 1995) in controlled greenhouse conditions at the Ruotsinkylä field station (60°21′N, 25°00′E) of the Finnish Forest Research Institute. A period consisting of two growing seasons was considered to be the optimal length for the experiment, since the primary stem growth of the woody test plant (*Pinus sylvetris* L.) already starts to be predominantly predermination at the age of 5 years.[16,17] Hence, the primary stem growth of the experimental seedlings during the first year still largely reflected the environmental conditions of the previous year, which naturally affects also the biomass production.

Day temperatures were allowed to follow the ambient temperature during the summer months, but the night temperature was kept at 15 °C. A constant temperature of +4 °C was maintained throughout the winter period. The seedlings were watered by drip irrigation using ordinary tap water with a pH of 5.9, Cu concentration 0.35 mg $litre^{-1}$, Zn concentration 0.03 mg $litre^{-1}$ and Ni concentration below the quantification limit (<0.018 mg $litre^{-1}$).

At the end of the experiment the pine seedlings were harvested and divided into root, stem and needle compartments. Green needles were collected by age classes: current needles (C), one-year-old needles (C + 1), and two-year-old and older needles (C + 2). Senescent needles were collected throughout the course of the experiment and were stored in a dry place prior to analysis.

2.5. Chemical analysis

The pine seedling samples were dried at 70 °C for 48 h and weighed in order to obtain the biomass of the individual seedling compartments. The compartments of the seedlings grown in the smelter-polluted soil pots, and belonging to the same cluster, were bulked to give five composite samples per compartment per site. The replicate seedling compartments of the artificial quartz sand exposure were combined to give one composite sample per compartment per treatment level. The humus samples were dried, weighed and milled to pass through a 1 mm sieve. Total Cu, Ni, P, Ca, Mg and K concentrations were determined on the humus and pine seedling samples by dry ashing at 550 °C followed by extraction with concentrated HCl. As a quality control measure, each furnace loading had one empty crucible (a blank) and a reference sample of known chemical composition which was used to ensure repeatability of the analysis.

The solutions were analysed by inductively coupled plasma atomic emission spectrometry (ICP-AES). The total N and S concentrations were determined on the milled humus samples on LECO analyzers. Exchangeable element concentrations were determined on the humus samples by extraction with 1 M ammonium acetate (pH 4.65) with 2% EDTA (25 ml humus–250 ml extractant, shaking for 1 h), followed by filtration and analysis by ICP-AES. The mineral forest soil samples were dried, weighed and passed through a 2 mm sieve to remove stones and large roots. The sieved portion of each sample was weighed. Exchangeable element concentrations were determined from sieved mineral forest soil and from quartz sand samples in the same way as for the humus samples. The pH of the quartz sand samples were determined in water (15 ml sample, 25 ml^{-1} water).

2.6. Calculations and statistical analysis

The net uptake of Cu and Ni by the pine seedlings was determined by calculating the amount of metal per compartment sample (roots, stem, green needles and senescent needles). As these compartment samples were composite samples taken from 5 seedlings, the values were divided by five in order to obtain a value per seedling. Finally, metal uptake was obtained by summing up all the compartments per seedling and then subtracting the average element content of the reference seedlings from the element content of each experimental seedling.

The data were analysed using the SAS V8 statistical package. Simple linear regression equations were calculated for the relationships between the exchangeable Cu and Ni content of the quartz sand series at the end of the experiment and the amount of Cu and Ni originally added. The dependence of seedling biomass on the Cu and Ni content of the quartz sand media was calculated as a simple linear regression equation for all treatment series using ln-transformed treatment dose values. The independent variables were logarithmically (ln) transformed in order to linearize the asymptotic relationship with the dependent variable.

To find the variables that best explained the variation in biomass of the smelter-polluted soil-grown seedlings, multiple regression analysis was performed using the Cu, Ni, Ca, K, Mg and P contents of the smelter-polluted soil cores as independent variables. The nutrient contents of the soil cores were calculated in the same way as the Cu and Ni contents described by Nieminen and Saarsalmi.[18] All possible regression models were fitted to the data with all possible combinations of independent variables. The best model was found to be the model with Cu, Ni and P as independent variables. The multiple regression procedure was repeated using the total Cu, Ni, N, S, P, Ca, K and Mg concentrations in the humus as independent variables. The humus Cu and N concentrations were found to be the best explaining variables.

Regression equations between seedling metal uptake and soil Cu and Ni content were calculated as simple linear models. Pearson correlation coefficients were calculated between soil Cu and Ni contents and the Ca, K, Mg, P, Cu and Ni concentrations of the roots, stem, senescent needles and green needles.

3. Results

3.1. Cu and Ni contents of the potting media

The Cu and Ni contents (*i.e.* exposure doses) of the smelter-polluted soil series increased with decreasing distance from the smelter (Tables 2 and 3). The mean exchangeable Cu concentrations in humus ranged from 3 mg kg^{-1} d.w. (sd 0.9) at 60 km pots to 2072 mg kg^{-1} d.w. (sd 380) at 0.5 km pots. The corresponding values for Ni were 1 mg kg^{-1} d.w. (sd 0.3) and 138 mg kg^{-1} d.w. (sd 31).

The exchangeable amount of Cu and Ni in the potting media of the artificial quartz sand treatments at the end of the experiment increased linearly with increasing treatment doses. The regression equations between the added metal doses and the measured exchangeable metal amounts for the single treatment series were: $Cu_{exch} = 0.23\ Cu_{dose} + 49.94$ ($R^2 = 0.96$, $p < 0.0001$) and $Ni_{exch} = 0.07\ Ni_{dose} + 15.54$ ($R^2 = 0.73$, $p < 0.0001$). For the combination treatment series the corresponding equations were: $Cu_{exch} = 0.31\ Cu_{dose} + 29.97$ ($R^2 = 0.96$, $p < 0.0001$) and $Ni_{exch} = 0.11\ Ni_{dose} + 10.95$ ($R^2 = 0.72$, $p = 0.0003$). Hence, the exchangeable amounts compared to the originally added doses were proportionally higher for Cu than the corresponding values for Ni. The pH values varied between 5.8 and 7.5, but showed no clear trends along the treatment series.

3.2. Survival of the seedlings and Cu and Ni concentrations in the plant compartments

All the seedlings grown in the quartz sand pots treated with doses of more than 360 mg per pot died in the Cu and Ni treatments series within a few days (Tables 4 and 5).[19] However, there were more dead seedlings below this limit in the Ni treatment series than in the Cu series. In the Cu + Ni treatment series the lethal threshold was clearly lower. None of the seedlings survived more than a few days in pots treated with a Cu + Ni dose of more than 36 mg + 36 mg per pot. In contrast to the artificial Cu and/or Ni treatment series, all the smelter-polluted soil-grown seedlings survived for the first few weeks, but by the end of the first experimental growing season, 8 of the seedlings growing in the most polluted (0.5 km) soil cores died. Finally, only four of the original 25 seedlings growing in the most polluted soil cores survived until the end of the experiment (Tables 2 and 3). In addition to these deaths in the 0.5 km soil–plant systems, one seedling growing in soil originating from the 60 km background site died by the end of the first experimental growing season.

3.3. Biomass growth and metal uptake of the seedlings

The total biomass of the seedlings decreased steeply with decreasing distance from the smelter (Fig. 1) and increasing metal exposure doses in all the manipulations (Figs. 2 and 3). Seedling biomass showed a statistically significant negative relationship with the Ni and Cu + Ni treatment doses

Table 2 The exchangeable Cu content of the smelter-polluted soil pots and Cu concentrations in different compartments of the seedlings grown in the pots. Each value represents the compartments of 5 seedlings bulked together prior to analysis

Content of exchangeable Cu/mg per pot	Roots/mg kg^{-1}	Stem/mg kg^{-1}	Senescent needles/mg kg^{-1}	Green needles/mg kg^{-1}	Number of surviving seedlings	Distance from the smelter/km
5	434	38	—	46	5	60
5	305	25	67	45	5	60
6	583	43	103	63	4	60
7	393	43	—	45	5	60
8	428	26	—	55	5	60
31	195	30	—	45	5	8
19	283	23	—	40	5	8
22	311	32	64	55	5	8
21	285	24	97	53	5	8
13	251	25	108	56	5	8
57	278	26	41	54	5	4
65	464	35	—	60	5	4
55	418	26	51	65	5	4
61	286	31	57	58	5	4
50	394	35	52	47	5	4
262	535	37	—	59	5	2
186	654	36	81	57	5	2
187	427	25	77	62	5	2
203	594	37	80	63	5	2
227	460	29	71	63	5	2
1374	3568	103	196	90	1	0.5
1332	4148	72	41	54	1	0.5
1885	4522	167	68	—	0	0.5
1349	3513	83	84	—	0	0.5
732	4205	81	56	115	2	0.5

(seedling-biomass$_{Ni}$ = $-5.68 \ln(Ni_{dose} + 1) + 48.65$; $R^2 = 0.63$, $p < 0.0001$ and seedling-biomass$_{Cu + Ni}$ = $-7.76 \ln(Cu + Ni_{dose} + 1) + 53.38$; $R^2 = 0.64$, $p < 0.0011$). When only those seedlings that were still alive at the end of the experiment were taken into account, there was a statistically significant relationship only in the Ni treatment series (seedling-biomass$_{Ni}$ = $-8.32 \ln (Ni_{dose} + 1) + 53.19$; $R^2 = 0.65$, $p < 0.0089$). In the smelter-polluted soil treatment, the seedling biomass was related to the Cu, Ni and P content of the soil pots (seedling-biomass$_{smelter\text{-}soil}$ = $-0.0123Cu_{dose} - 0.100Ni_{dose} + 0.088P_{dose} + 49.4$; $R^2 = 0.70$, $p < 0.0001$). When the total humus concentrations were used as independent variables, the

Table 3 The exchangeable Ni content of the smelter-polluted soil pots and the Ni concentrations in different compartments in the seedlings grown in the pots. Each value represents the compartments of 5 seedlings bulked together prior to analysis. <d.l. = below detection limit

Content of exchangeable Ni/mg per pot	Roots/mg kg^{-1}	Stem/mg kg^{-1}	Senescent needles/mg kg^{-1}	Green needles/mg kg^{-1}	Number of surviving seedlings	Distance from the smelter/km
0.8	7	<d.l.	—	2	5	60
0.8	5	<d.l.	—	2	5	60
0.1	5	<d.l.	3	3	4	60
0.8	2	<d.l.	3	3	5	60
0.5	2	2	—	2	5	60
5	18	<d.l.	—	2	5	8
2.3	25	<d.l.	—	2	5	8
1.0	28	<d.l.	2	3	5	8
2.7	19	<d.l.	3	2	5	8
0.9	21	<d.l.	3	6	5	8
12	57	2	5	15	5	4
19	62	2	8	6	5	4
12	77	<d.l.	—	11	5	4
9	48	1	9	7	5	4
8	52	<d.l.	6	9	5	4
41	118	2.5	—	28	5	2
19	73	2.4	16	17	5	2
27	110	4.4	26	18	5	2
27	106	5.9	14	23	5	2
26	68	2.5	13	18	5	2
164	262	36	22	2	1	0.5
196	237	24	7	12	1	0.5
101	184	21	3	—	0	0.5
148	249	94	4	—	0	0.5
81	174	9	7	5	2	0.5

Table 4 Cu concentrations in different plant compartments and the number of surviving seedlings for each treatment dose. The highest dose at which one of the replicates remained alive and the highest concentrations found in living tissues (both replicates alive) are marked in bold

Cu dose/mg per pot	Roots/mg kg^{-1}	Stem/mg kg^{-1}	Senescent needles/mg kg^{-1}	Green needles/mg kg^{-1}	Number of surviving seedlings
Cu treatment series					
0	592	58	—	45	2
2.4	433	62	—	64	2
6	385	66	—	95	2
12	551	28	—	42	2
24	672	745	224	28	1
48	677	46	50	67	2
72	665	49	186	51	2
96	783	252	64	58	1
120	3542	1432	677	—	0
180	4786	1384	713	—	0
240	**941**	**69**	121	**106**	2
300	1761	115	55	104	1
360	1183	912	210	105	1
480	8465	2557	954	—	0
720	6024	1606	341	—	0
1440	12892	4477	2318	—	0
2880	12618	6569	2674	—	0
Cu + Ni treatment series					
0	352	48	116	64	2
1.2	263	54	47	32	2
2.4	355	47	101	81	2
4.8	**330**	**56**	80	62	2
12	832	55	50	—	0
24	411	144	71	**84**	1
36	417	107	53	46	1
48	2983	367	116	—	0
60	2796	343	204	—	0
120	6119	1543	940	—	0
240	2568	115	96	—	0
480	4935	563	433	—	0
960	7214	1177	549	—	0

biomass was best explained by the Cu and N concentrations (seedling-biomass$_{smelter\text{-}soil} = -0.0236Cu_{conc} + 0.0243N_{conc}$; $R^2 = 0.57$, $p < 0.0002$).

Copper uptake by the seedlings from the artificial treatment series was strongly related to the Cu content of the quartz sand pots in the Cu treatment series ($Cu_{uptake} = 0.022Cu_{dose} + 10.37$; $R^2 = 0.92$, $p < 0.0001$), but in the Cu + Ni treatment series there was no statistically significant relationship between these variables (Fig. 4). Nickel uptake was related to the Ni content of the experimental pots both in the Ni treatment ($Ni_{uptake} = 0.0084Ni_{dose} + 6.20$; $R^2 = 0.66$, $p < 0.0001$) and in the Cu + Ni treatments series ($Ni_{uptake} = 0.022Cu + Ni_{dose} + 3.27$; $R^2 = 0.79$, $p < 0.0001$), (Fig. 5). However, when only living seedlings were taken into account, only the Ni relationship in the Cu + Ni treatment series was statistically significant ($Ni_{uptake} = 0.176\ Ni_{dose} + 0.109$; $R^2 = 0.96$, $p < 0.0007$).

There were no statistically significant relationships between the seedling Cu and Ni uptake and the corresponding contents in smelter-polluted soil pots (Figs. 4 and 5).

3.4. Nutrient, Cu and Ni concentrations of the plant compartments in relation to the treatment doses

There was a negative correlation between the root P, Ca, K and Mg concentrations and the Cu (Pearson correlation coefficients were -0.54, $p = 0.0261$; -0.78, $p = 0.0002$; -0.80, $p = 0.0001$ and -0.86, $p < 0.0001$, respectively) and Cu + Ni treatment doses (Pearson correlation coefficients were -0.56, $p = 0.047$; -0.72, $p = 0.006$; -0.70, $p = 0.007$ and -0.62, $p < 0.023$, respectively). Only the Ca and K concentrations of the roots correlated negatively with the Ni treatment doses (Pearson correlation coefficients were -0.60, $p = 0.011$ and 0.60, $p = 0.011$, respectively). There were no correlations between the nutrient concentrations of the other plant compartments and the treatment doses of the artificial manipulations. The Cu and Ni concentrations of all the plant compartments correlated with the corresponding treatment doses in the Cu and Ni single treatment series. However, in the Cu + Ni combination series treatment, the doses correlated only with the root and stem Cu and Ni concentrations.

In the smelter-polluted soil series, there were relatively similar correlations between the root Ca, K and Mg concentrations and Cu and Ni doses (Pearson correlation coefficients were -0.53, $p = 0.006$ for Cu_{dose} *vs.* Ca and -0.48, $p = 0.015$ for Ni_{dose} *vs.* Ca; -0.83, $p < 0.0001$ for Cu_{dose} *vs.* K and -0.75, $p < 0.0001$ for Ni_{dose} *vs.* K; -0.90, $p < 0.0001$ for Cu_{dose} *vs.* Mg and -0.83, $p < 0.0001$ for Ni_{dose} *vs.* Mg). In contrast to the artificial treatment series, the Ca, K and Mg concentrations of the needles of the smelter pollution series also correlated negatively with the Cu and Ni doses (Pearson correlation coefficients were -0.81, $p < 0.0001$ for Cu_{dose} *vs.* Ca and -0.74, $p < 0.0001$ for Ni_{dose} *vs.* Ca; -0.64, $p = 0.0011$ for Cu_{dose} *vs.* K and -0.59, $p = 0.003$ for Ni_{dose} *vs.* K; -0.57, $p = 0.0044$ for Cu_{dose} *vs.* Mg and -0.52, $p = 0.010$ for Ni_{dose} *vs.* Mg).

4. Discussion

4.1. Biomass growth

The trend in the biomass response of the smelter-polluted soil–plant systems was relatively similar to that obtained in the field in the Scots pine stands growing along the same smelter-pollution gradient:[8] extremely low in 0.5 km soils, but increasing with increasing distance from the smelter, apart from the 60 km background site. The Cu, Ni and P content of the soil cores, as well as the humus N and Cu concentrations, explained

Table 5 Ni concentrations in different plant compartments and the number of surviving seedlings for each treatment dose. The highest dose at which one of the replicates remained alive and the highest concentrations found in living tissues (both replicates alive) are marked in bold

Ni dose/mg per pot	Roots/mg kg^{-1}	Stem/mg kg^{-1}	Senescent needles/mg kg^{-1}	Green needles/mg kg^{-1}	Number of surviving seedlings
Ni treatment series					
0	23	1.9	6	3	2
2.4	18	2.0	15	7	2
6	18	2.2	11	10	2
12	115	22.9	13	62	1
24	**81**	**4.5**	34	27	2
48	349	361	46	94	1
72	2882	587	121	—	0
96	283	173	212	55	1
120	451	42	77	**119**	1
180	3472	622	177	—	0
240	3422	831	246	—	0
300	5238	1134	530	—	0
360	466	755	270	105	1
480	3914	1092	468	—	0
720	7767	2727	847	—	0
1440	10916	3455	3798	—	0
2880	9534	3482	2276	—	0
Cu + Ni treatment series					
0	5	4.7	5	2	2
1.2	16	5.0	2	2	2
2.4	17	4.9	15	10	2
4.8	**26**	**8.3**	24	21	2
12	583	38	23	—	0
24	107	91	20	64	1
36	332	299	82	**93**	1
48	1585	501	174	—	0
60	1279	688	938	—	0
120	1854	1156	1394	—	0
240	4393	912	92	—	0
480	5580	1357	258	—	0
960	5374	3075	1403	—	0

a large proportion of the biomass variation of the seedlings. Hence, the relatively low biomass at the 60 km background site was most probably related to the poor nutrient status of the soil, and the decreasing trend in biomass with decreasing distance to the smelter to the increase in soil Cu and Ni content. The overall growth rate of these smelter-polluted soil seedlings was higher than those of the artificial treatment series, most probably due to the better nutrient status of the forest soil compared to the weakly fertilized pure quartz sand medium.

The biomass growth of the artificial treatment series was related to the Ni and Cu + Ni treatment series, but the relationship with the Cu treatment doses was not statistically significant. The lack of a clear growth trend along the increasing Cu treatment series is partly due to the fact that the dose

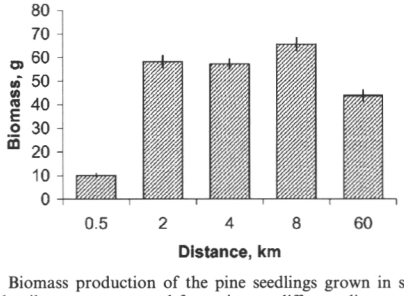

Fig. 1 Biomass production of the pine seedlings grown in smelter-polluted soil cores transported from sites at different distances along the pollution gradient. The bars indicate the standard error of the mean, $n = 25$ for each distance.

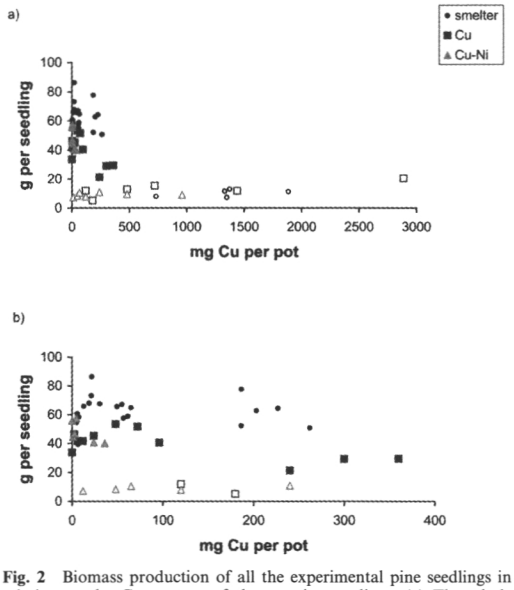

Fig. 2 Biomass production of all the experimental pine seedlings in relation to the Cu content of the growing medium. (a) The whole treatment scale. (b) The treatment doses below 400 mg Cu. Symbols of the smelter gradient refer to the average biomass of a cluster (= 5 seedlings), while those of the Cu or Ni treatments refer to the average biomass of the 2 replicates. Open symbols refer to cases where the seedlings died.

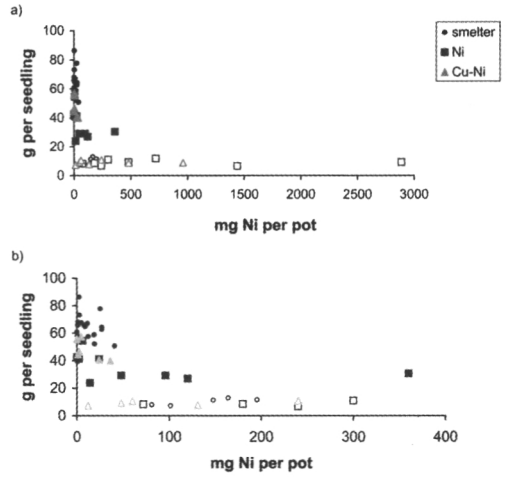

Fig. 3 Biomass production of all the experimental pine seedlings in relation to the Ni content of the growing medium. (a) The whole treatment scale. (b) The treatment doses below 400 mg Ni. For other explanations see Fig. 2.

response at low treatment levels was positive but negative at higher levels. Growth stimulation followed by inhibition is a normal response pattern for essential elements.[20,21] Nickel has also been shown to be an essential nutrient for higher plants,[20] but the requirement of Scots pine for Ni seems to be extremely low since no stimulation was detected with the Ni doses used in this experiment.

Both the survival and growth rates of the artificial treatment series reflected the duality of the dose response results: the immediate death of the seedlings with no growth at all, and the later stabilized situation of the remaining soil–plant systems with lowered metal availability and toxicity. Consequently, the available metal pools of the surviving soil–plant systems were sufficient to produce only weak growth retarda-

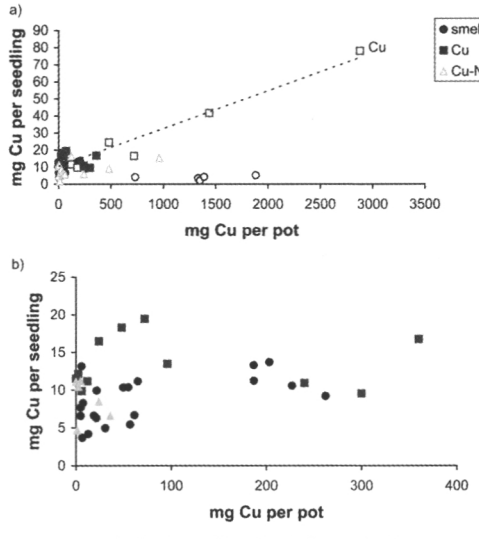

Fig. 4 Cu uptake by the seedlings in relation to the Cu content of the growing medium. a) All seedlings. b) The seedlings that survived up until the end of the experiment. Only statistically significant regression equations are presented. For other explanations see Fig. 2.

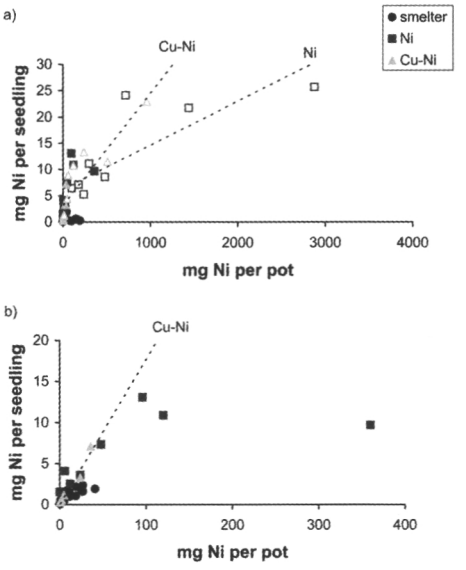

Fig. 5 Ni uptake by the seedlings in relation to the Ni content of the growing medium. a) All seedlings. b) The seedlings that survived up until the end of the experiment. Only statistically significant regression equations are presented. For other explanations see Fig. 2.

tion responses suggesting, however, greater toxicity of Ni in relation to Cu.

4.2. The available Cu and Ni pool of the potting media

The present study showed immobilization of Cu and Ni in the quartz sand media during the 17-month incubation period, since the exchangeable amounts of Cu and Ni were relatively low. The proportion of exchangeable to total Cu was higher than the corresponding ratio for Ni, indicating higher Ni sorption. This finding is in agreement with the report of slightly greater Cu mobility compared to Ni in the plough layer of metal-polluted agricultural soils near the Harjavalta smelter.[22]

As a general rule, the aging of metals in soils tends to immobilize them and render them less available than freshly added metals.[23] Consequently, metal availability in the present experiment was at its greatest immediately after metal addition. According to Sauvé,[23] secondary precipitation occurs when the solubility product of a metal compound is exceeded in the soil solution or at the soil surface. Metal hydroxide precipitates may form on the surface of minerals at a lower saturation ratio than would be required in solution because of the effect of heterogeneous nucleation.[24] Even though the coarse quartz sand is considered to be an inert soil matrix, the adsorption of soluble metals cannot be ruled out completely. In general, Si is released from minerals into the soil solution at near-equilibrium concentrations and it is usually more mobile in alkaline than in acidic soils.[20,25] The pH range of the quartz-sand series was near neutral, but clearly more alkaline than the pH range of the smelter-polluted soil cores (4.0–5.4).[18] The presence of monosilicic acid in solution increases the sorption of heavy metals.[25]

The ratio between the exchangeable and added metal amounts was slightly higher in the combination treatment than in the single treatment series, especially in the case of Ni. The metals thus seem to have inhibited the sorption mechanisms of each other.

The strong retention rate of the added metals, together with the fact that all the seedling deaths occurred during the first few

days of the experiment, suggest that death was caused by acute Cu and Ni toxicity. At the very beginning of the experiment the amounts of phytoavailable Cu and Ni were still close to the total amounts added, but presumably decreased within a few days to levels which were not sufficient to induce death or strong growth reduction of the surviving seedlings.

In contrast to the artificially treated seedlings, the seedlings growing in the smelter-polluted soil cores were subjected to chronic metal exposure. There was no decrease in the ammonium acetate exchangeable metal pool during the experiment. In contrast, in the most polluted soil cores (0.5 km) there was even an increase in the amounts of exchangeable Cu and Ni, most probably due to mobilization of the metals accumulated in the thick litter layer on top of the soil.[18] This increase in toxicity may have played a role in processes that lead to the death of most of the seedlings grown in the 0.5 km soil cores.

4.3. Uptake and mobility of Cu and Ni within the plant

In the artificial, single-metal treatment series the uptake of Cu and Ni was linearly related to the treatment dose, while in the combination treatment only Ni uptake was significantly related to the treatment doses. Therefore, Ni seemed to play a stronger role than Cu in the response of the seedlings in the combination treatment. Furthermore, Ni uptake increased more sharply in the combination treatment than in the Ni treatment, which strongly suggests that Ni uptake by the seedlings was enhanced by the presence of equal doses of Cu. Furthermore, the Cu uptake rate seemed to be inhibited by the presence of equal doses of Ni. Rautio *et al.*[26] found no clear enhancement (or decline) of pine root Ni concentrations in the presence of Cu doses higher than those of Ni, but an increase was observed in wood and needle Ni concentrations. They also reported a slight decrease in root Cu concentrations along with increasing Ni doses, even though the Ni doses were smaller than those of Cu.[26]

The total Cu and Ni uptake of the smelter-polluted soil grown seedlings was not significantly related to the metal contents of their growing substrates. However, since a strong correlation was found between the soil metal contents and the corresponding concentrations in the seedling roots and stems, the lack of a relationship between metal uptake and soil metal exposure is strongly explained by the poor biomass growth of the seedlings growing in the most polluted soil cores. A low biomass naturally also results in a low total uptake rate.

The generally much higher rate of Cu uptake by the experimental seedlings compared to that of Ni was largely explained by the high rate of Cu accumulation in the roots. Copper has a high affinity for the negatively charged carboxylic groups of the cell walls in the apoplastic free space of the roots.[20] According to McLaughlin,[27] this apoplastic extracellularly bound amount should not be taken into account when determining the plant metal uptake. In *sensu stricto*, only the symplastic intracellular proportion that has crossed the plasmamembrane into the cytoplasm of root cells is taken in by the plant. However, since there is hardly any appropriate technique for removing the extracellular cortex bound pool of elements prior to chemical analysis, this extracellular proportion has also been commonly included in plant uptake by several authors.[26,28]

Even somewhat misleading interpretations may have been made when comparing the root-to-shoot transport of Cu to that of Ni, which has no corresponding affinity for the negatively charged cation exchange sites in the root cortex.[25] Kozlov *et al.*,[28] for instance, claimed that Ni but not Cu was effectively translocated from birch seedling roots to shoots in transplanting experiments. Nevertheless, their conclusion was simply based on a higher root-to-shoot ratio of the Cu concentrations compared to that of Ni, which could simply be a consequence of the higher rate of cortex-bound Cu, rather than a reflection of a metabolically more intensive transport of Ni from root to shoot.

In the present study, the Ni concentrations in the shoots *vs.* the exposure dose were at approximately the same level as those of Cu: even lower in the single treatment series, and slightly higher in the Cu + Ni combination treatment. In the smelter-polluted soil cores, the uptake of both Cu and Ni into the shoots was at approximately the same level proportionally to the soil exchangeable content, apart from the two least polluted soil sites. However, Cu is generally considered to have a relatively low mobility within plants, whereas Ni is considered to be more mobile.[20,25]

4.4. Lethal thresholds

If we define the highest artificial single treatment dose in which one of the replicates still survived as the lethal threshold dose for Scots pine, then it appears that only the Cu contents in the 0.5 km smelter-polluted soil cores were high enough to cause the death of the pine seedlings. However, since Ni seemed to become more toxic in the presence of Cu, the highest tolerable dose of the combination treatment should be considered as a more suitable estimate for the lethal Ni threshold. In this case the Ni contents of both the 0.5 km and 2 km soil cores exceed this lethal threshold dose. The lethal Cu threshold dose corresponds to a soil Cu concentration of approximately 32 mg kg^{-1}, and that of Ni to a soil concentration of 3.2 mg kg^{-1}.

The lethal thresholds for root and stem concentrations are defined as the highest values of the artificial treatment series in which both of the replicates still survived: 940 mg Cu kg^{-1} and 80 mg Ni kg^{-1} in the roots, 70 mg Cu kg^{-1} and 8 mg Ni kg^{-1} in the stem. These values were exceeded in the 0.5 km soil–plant systems in the case of both Cu and Ni, and even in some of the 2 km soil–plant systems in the case of root Ni.

The maximum Ni concentrations of the living needles from the artificial treatment series (119 mg kg^{-1}) were extremely high compared to those found in the smelter-polluted soil–plant systems. In contrast, the needle Cu concentrations of the smelter-polluted soil–plant systems reached the same level as the maximum values of the artificial Cu treatment series (106 mg kg^{-1}). According to Kabata-Pendias,[25] the uptake and transport of Ni within plants is metabolically controlled, while Cu is taken up and translocated within a plant more passively. The extremely low growth rate of the 0.5 km soil–plant systems suggests a low metabolic activity, which could explain the relatively low needle Ni concentrations. In addition, the available Ni pool of the smelter-polluted soil–plant systems was presumably primarily organically bound, while that of the artificial treatment soil–plant systems had remained in an inorganic form. These organic Ni complexes may have a different mobility within the plant compared to that of inorganic Ni.

Furthermore, the needle Cu and Ni concentrations of the smelter-polluted 0.5 km soil–plant systems were lower than those found in the field in the needles of mature pines at the corresponding 0.5 km site: 167 and 43 mg kg^{-1}, respectively, for Cu and Ni.[8] This is most probably due to the high rate of surface contamination of the field-grown needles caused by a relatively high atmospheric load of Cu and Ni.[29] Since the needle metal concentrations seem to be strongly affected by several factors other than the soil metal exposure alone, it appears that clear toxicity thresholds for Cu and Ni in Scots pine needles cannot be defined.

4.5. Nutrient status of the experimental seedlings

The artificial treatments appear to have affected the root nutrient concentrations. The increasing Cu and Cu + Ni doses tended to decrease root P, Ca, K and Mg concentrations. The increasing Ni doses decreased the root Ca and K

concentrations. Toxic metals can have disruptive effects on the structure and functioning of the plasma membrane of roots, thus altering the kinetics of element uptake.[30] According to Baker and Walker,[31] enhanced K efflux is a consequence of metal-induced damage to the plasma membrane of root cells.

A similar nutrient decrease with increasing metal exposure was also found in the smelter-polluted soil–plant systems for root Ca, K and Mg. In contrast to the artificial treatments, this pattern was also reflected in the above ground parts of the seedlings. In addition to the metal-induced disruptions in root functioning, the decrease in soil Ca and especially Mg concentrations with increasing Cu and Ni concentrations along the smelter-pollution gradient may have affected the nutrient status of the seedlings, and further aggravated the toxic effects of these metals. Magnesium deficiency disturbs phloem loading and leads to impaired carbohydrate retranslocation.[20] A sufficient Ca supply is known to alleviate the effects of toxic metals, at least in the case of Cu, which is believed to be due to the important role of Ca in maintaining the integrity of the cell membranes.[20] The positive correlation found between the metal exposure doses and stem P concentrations is most probably due to a simple enrichment phenomenon, caused by the negligible growth of the most polluted (0.5 km) soil–plant systems.

5. Conclusions

The exchangeable Cu and Ni contents in the soil explained the survival pattern and a high proportion of the variation in biomass growth of the Scots pine seedlings cultivated in the smelter-polluted soil core series. The biomass growth pattern of this seedling series corresponded to the biomass production rates measured in the mature pine stands growing along the same smelter-pollution gradient in the field. Long-term artificial Ni exposure *via* the soil did not appear to be clearly more toxic than that of Cu as a single metal exposure, but the toxicity of Ni appeared to be increased in the presence of equal doses of Cu, presumably due to enhanced Ni uptake.

The lethal threshold values determined on the basis of the artificial exposure doses and metal concentrations of the seedling tissues corresponded approximately to the lethal values found in the smelter-polluted soil–plant system series. The root Ni uptake was generally more restricted than that of Cu, and the mobility within the plant seemed to be more dependent on the metabolic activity of the seedlings than that of Cu. The Ca, K and Mg status of the seedlings was affected by the high metal exposure.

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Paper IV

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IV

Contents of Cu, Ni and Zn in smelter-polluted soil–plant systems

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ABSTRACT: Intact soil profiles including litter layer and ground vegetation were taken at five distances (0.5, 2, 4, 8 and 60 km) from a Cu–Ni smelter in Harjavalta, SW Finland. The soil cores were placed in 10 l pots and a 4 year-old, bare-rooted pine seedling (*Pinus sylvestris* L.) was planted in each. The containers were incubated for 17 months in controlled greenhouse conditions. At the end of the experiment the seedlings were harvested and soil samples were taken from each pot and analysed for Cu, Ni and Zn. The absence of atmospheric metal deposition had no reducing effect on the exchangeable heavy-metal contents of the soil pots. Metal uptake by the plant seedlings during the experiment had a negligible effect on the exchangeable heavy-metal content of the soil cores from the most polluted sites. The increase in both the exchangeable and the total humus Cu, Ni and Zn concentrations in the 0.5 km soil cores during the experiment suggested that there were pools of accumulated metals in the thick litter layer on top of the humus.

KEYWORDS: *heavy-metal pollution, plant uptake, podzol*

INTRODUCTION

Heavy metals from anthropogenic sources have been distributed over wide areas of the boreal forest zone, and large-scale damage to the forests has been observed in areas close to industrial complexes. The effects of emissions from nickel and copper mining and smelting activities in Sudbury, Canada (e.g. Hutchinson & Whitby 1974, 1977; Freedman & Hutchinson 1980) and in the Kola Peninsula, NW Russia (e.g. Boltneva *et al.* 1983; Kalabin *et al.* 1994; Tikkanen 1994; Nöjd *et al.* 1996) are amongst the most extensively documented ones. A much smaller Cu–Ni smelter unit has been operating since 1945 in Harjavalta, SW Finland, where 50 years' accumulation of pollutants has seriously affected the adjacent Scots pine ecosystems (Laaksovirta & Silvola 1975; Fritze *et al.* 1989; Heliövaara & Väisänen 1989; Helmisaari *et al.* 1995; Mälkönen *et al.* 1999).

The Harjavalta smelter is rather unique because there are no mines or metal-rich ores in the bedrock of the area. Hence, the elevated metal concentrations in the surface layers of forest soils surrounding the smelter reflect the metal amounts deposited from the air due to the smelter emissions. A number of studies have been carried out in the area using moss, conifer needles and deposition samplers to determine heavy-metal deposition patterns. Elevated levels of Cu and Ni have been found up to 10 km from the smelter (Hynninen 1986; Jussila & Jormalainen 1991). According to a soil study carried out in 1992 by Derome & Lindroos (1998) the total Cu concentrations in the organic layer at 0.5 km distance from the Harjavalta smelter was 5800 mg kg^{-1}, and the corresponding Ni and Zn concentrations were 460 and 515 mg kg^{-1}, respectively. The total concentrations at 8 km were still elevated: 150 mg kg^{-1} for Cu, 40 mg kg^{-1} for Ni and 60 mg kg^{-1} for Zn, compared to the mean total Cu, Ni and Zn concentrations in the organic layer of Finnish forest soils: 6.6, 8.2 and 47 mg kg^{-1}, respectively (Tamminen 2000).

The recent development in the industrial process technology has lead to a drastic decrease in heavy-metal emissions in many regions of western Europe. However, the pollutants that have accumulated in forest ecosystems during the high emission periods continue to affect the soil functioning and pose a potential risk of migration. Because the mobility and the fate of heavy metals are poorly known, the time and measures needed for soil recovery cannot be accurately defined.

The aim of this study was to assess the changes in heavy-metal contents of soil cores from a pollution gradient during a controlled experiment without atmospheric metal deposition. An additional aim was to determine the effect of metal uptake by a woody plant species on the soil heavy-metal content.

MATERIALS AND METHODS

Site description

A Cu–Ni smelter complex is situated on an esker in the area of Harjavalta township (61°20′N, 22°10′E, Fig. 1) in the southern boreal coniferous zone (Ahti *et al.* 1968). The long-term (1960–1990) mean annual temperature at a nearby weather station of the Finnish Meteorological Institute is 4.0°C and the annual precipitation is 558 mm. Four sampling sites were located in pure Scots pine stands at distances of 0.5, 2, 4 and 8 km SE from the main stack of the smelter along the esker. One site, situated at Hämeenkangas, 60 km NE from Harjavalta, in an area without local emission sources, was chosen as a background site. The bedrock of the Harjavalta area consists of Jotnian olivine diabase, and the bedrock of the Hämeenkangas area consists of granites. The soils comprise sorted glaciofluvial sediments, which poorly reflect the chemical properties of the underlying bedrock. The texture of the mineral soils is fine or coarse sand, and the soil type is orthic podzol (Table 1). The organic layer is mor, with a thickness ranging from 1 to 3 cm (Table 1).

The vegetation of all the sites has originally been typical of a xerophilous forest site: *Pinus sylvestris* L. as a dominant tree species, and *Calluna vulgaris* (L.) Hull., *Empetrum nigrum* L.,

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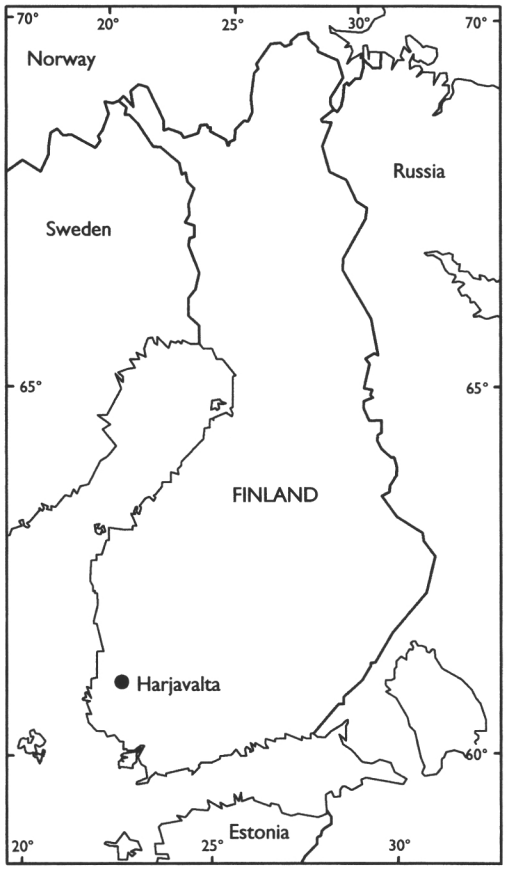

Fig. 1. Location of the Cu–Ni smelter complex at Harjavalta, SW Finland.

Vaccinium vitis-idaea L., *Pleurozium schreberi* (Brid.) Mit., *Dicranum* spp., *Cladina* spp., etc., the most common species of the ground vegetation layers. The sites are of the *Calluna* type, according to the Finnish forest site classification of Cajander (1949). However, in the immediate vicinity of the smelter the understorey vegetation is almost completely absent (Salemaa *et al.* 2001), and the Scots pine stand is suffering from retarded growth and severe needle loss (Mälkönen *et al.* 1999). According to the survey realized in 1993 by Salemaa *et al.* (2001) lichens were absent up to a distance of 2 km, and mosses, excluding *Pohlia nutans*, were not frequent until a distance of 8 km.

Table 2. *Annual emissions of Cu, Ni and Zn from the Harjavalta smelter during 1985–1993*

Year	Cu (t year^{-1})	Ni (t year^{-1})	Zn (t year^{-1})
1985	98	47	216
1986	126	46	232
1987	140	96	162
1988	104	45	103
1989	80	33	190
1990	80	31	160
1991	80	14	90
1992	60	10	12
1993	50	7	13

The copper smelter has been operating in the vicinity of the sampling sites since 1945 and a nickel smelter since 1959 (Outokumpu Harjavalta Metals Oy). Until the early 1970s the ores used for smelting were mainly domestic sulphidic minerals from the Outokumpu mine in eastern Finland. At present the ore concentrates used form a varied group originating from different parts of the world: the Cu concentrate coming from, for example, Ireland and Chile, the Ni concentrate mainly from Australia.

Heavy metals are emitted as components of fugitive dust release. Monitoring of stack emissions was started in 1985 by the smelter company (Table 2). Over the past few years, dust emissions have been drastically decreased owing to changes in process technology and the installation of new filters. The slags generated by smelting are stored in land basins at the plant site. The Cu-slag is pumped into the storage area as sludge, and the granulated Ni-slag is piled in heaps and landscaped. During the piling period the uncovered slag heaps are an additional source of dust emissions.

Stand throughfall and litterfall collection

Stand throughfall was collected using 20 rainfall collectors located systematically inside a pine stand at 0.5, 4, 8 and 60 km sites during the snow-free period and six systematically located snow collectors during the winter. The height of the rainfall collectors was 40 cm above ground level. Precipitation samples were collected at 1 month intervals during the year 1993.

Tree litterfall was collected by 12 litter traps systematically located inside a pine stand at each site. The traps consisted of funnels made of plastic-impregnated, heavy-duty cloth with a collection area of 0.5 m^2 at a height of 1.5 m above ground level. A cotton bag was attached to the bottom of the funnel

Table 1. *Physical properties of the organic layer and genetic mineral soil horizons of the sampling sites; stoniness was determined according to Viro (1952)*

	Distance from the smelter (in km)				
	0.5	2	4	8	60
Humus					
type	mor	mor	mor	mor	mor
thickness (cm)	2.7	1.7	2.0	2.0	2.0
Mineral soil					
parent material	sorted sand	sorted sand	sorted sand	sorted sand	sorted sand
texture	fine	fine/coarse	fine	fine	coarse
stoniness (vol%)	0	0	0	0	0
Horizon thickness (cm)					
E	15	6	11	8	4
Bs	31	39	26	27	31

and changed at each sampling. The litter traps were emptied every second week during March–July 1993, and weekly during August–November 1993 up until the formation of a permanent snow cover.

Soil sampling and experimental conditions

Intact volumetric soil profiles including litter layer and ground vegetation were taken using an auger (diameter 25 cm, depth 30 cm) from the five sampling sites (0.5, 2, 4, 8 and 60 km) and placed in 10 l pots. A 4 year-old, bare rooted pine seedling (*P. sylvestris* L.) was planted in each pot. The soil profiles were taken at 25 points located as five clusters on each site. A smaller volumetric soil sample for chemical analysis was taken beside each sampling point using a small auger (diameter 3.8 cm, depth 30 cm). The loose litter was removed from the top of the sample, and the sample was divided into the humus layer and two mineral soil layers: 0–10 and 10–20 cm. The samples of each cluster (five) were bulked together to give five composite samples per layer per site for chemical analysis.

The pine seedlings used in the experiment had been raised from selected seeds in the forest nursery of the Finnish Forest Research Institute at Suonenjoki, eastern Finland. A set of 50 reference seedlings, from the same 4 year-old seedling lot as those planted in the experimental pots, were measured (average height 31 cm, standard deviation 5.8), weighed and analysed for Cu, Ni and Zn content (0.30, 0.03 and 1.04 mg per seedling, respectively) at the start of the experiment. The soil–plant systems (total number 125) were incubated for 17 months (two growing seasons, from June 1994 until October 1995) in controlled greenhouse conditions at Ruotsinkylä field station of the Finnish Forest Research Institute (60°21′N, 25°00′E). Day temperatures were allowed to follow ambient temperatures during summer months, but the night temperature was kept at 15°C. The constant 4°C level was maintained throughout the winter period. Watering was realized by drip irrigation using normal tap water, of which the pH was 5.9, Cu concentration was 0.35 mg l^{-1}, Zn concentration was 0.03 mg l^{-1} and Ni concentration was below 0.018 mg l^{-1}. By the end of the experiment soil samples for chemical analysis were taken using a small auger from each pot. Soil samples were divided into layers and those belonging to the same cluster were bulked together to give five composite samples per layer per site, as in the field sampling prior to the experiment. Pine saplings were divided into root, stem, green needle and senescent needle compartments, and those grown in the pots belonging to the same cluster were bulked together to give five composite samples per component per site.

Chemical analysis

The pH of the water samples was measured, and the remaining part of the samples filtered (0.45 µm membrane filter), and Cu, Ni and Zn were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) in the Central Laboratory of the Finnish Forest Research Institute, Vantaa, Finland. The laboratory has a continuous quality control programme, and it participates regularly in national and international intercalibration exercises.

Litter, humus and pine seedling samples were dried, weighed and milled to pass through a 1-mm sieve. Humus samples were divided into two parts for the total and exchangeable analysis. Total Cu, Ni and Zn concentrations were determined from litter, humus and pine seedling samples by dry ashing at 550°C followed by extraction with concentrated HCl. As a quality control measure, each furnace loading had one empty crucible (a blank) and a reference sample of known chemical content which was used to ensure repeatability of the analysis. The solutions were analysed using ICP-AES. Exchangeable Cu, Ni and Zn concentrations were analysed from the humus samples by extraction with 1 M ammonium acetate (pH 4.65) with 2% EDTA (25 ml humus–250 ml extractant, shaking for 1 h) followed by filtration and analysis by ICP-AES. Mineral soil samples were dried, weighed and passed through a 2 mm sieve to remove stones and large roots. Subsequently, the sieved proportion of each sample was weighed. Exchangeable Cu, Ni and Zn concentrations were then analysed as from the humus samples. The pH for both humus and mineral soil was determined in water (15 ml sample per 25 ml of water).

Calculations and statistical analysis

Area-specific Cu, Ni and Zn deposition values (mg m^{-2}) for stand throughfall and litterfall were calculated from the concentrations and sample amounts related to the surface area of the collectors for each sampling period. The annual depositions for 1993 were calculated by summing up the sampling period depositions.

The soil Cu, Ni and Zn concentrations are presented as an average value of the five replicate samples. The non-exchangeable metal concentration was obtained by reducing the exchangeable concentration from the corresponding total concentration. The statistical significance of the differences between the concentration before and after the incubation period was tested by paired *t*-test.

The soil Cu, Ni and Zn content per incubation pot was achieved by calculating first the metal content separately for humus and mineral soil layers by multiplying the metal concentration by the dry mass per pot of the corresponding layer, and subsequently summing up the contents of the soil layers. The dry mass of each soil layer per pot (M_1) was calculated as

$$M_1 = (A_1 \times M_2/5)/A_2$$

where A_1 is the surface area of the incubation pot (490.87 cm^2), M_2 is the dry mass of the five small-core composite humus or sieved mineral soil sample, and A_2 is the surface area of a small-core sample (11.34 cm^2).

This data calculation provided five 'soil-metal content per pot' estimates for each distance. Results are presented as an average of these five estimates.

The area-based metal amounts (mg m^{-2}) were calculated by relating the amount per pot to the surface area of the pot (mg per 0.049087 m^2).

The Cu, Ni and Zn uptake by pine seedlings was determined by calculating first the metal amounts per compartment samples (roots, stem, green needles and senescent needles) and then summing up these amounts. As these sums were based on composite samples (consisting of five seedlings), the values were divided by 5 to give an estimate per seedling. The metal uptake per seedling was obtained by reducing the average metal contents of the reference seedlings (0.30, 0.03 and 1.04 mg per seedling for Cu, Ni and Zn, respectively) from the metal contents of the experimental seedlings. The number of replicate samples was five per distance (5 × composite sample consisting of five seedlings per distance), and hence the seedlings metal uptake per distance is presented as an average of the five replicates.

RESULTS

Stand throughfall and litterfall at Harjavalta

The pH of the stand throughfall was fairly consistent (Table 3). Copper was the most abundant element in both throughfall and

Table 3. *The median pH values and the annual deposition of Cu, Ni and Zn via stand throughfall and via litterfall in 1993 in Scots pine stands at different distances from the Cu–Ni smelter*

Distance (km)		Stand throughfall			Litterfall		
	pH	Cu (mg m^{-2})	Ni (mg m^{-2})	Zn (mg m^{-2})	Cu (mg m^{-2})	Ni (mg m^{-2})	Zn (mg m^{-2})
0.5	4.6	380	70	50	612	95	275
4	4.4	14	2	5	26	5	9
8	4.4	4	0.5	3	12	2	9
60	4.4	1	<0.018	2	2	0.4	10

litterfall deposition at the polluted stands, but at 60 km there was more Zn than Cu.

Metal concentrations in humus

In humus, Cu, Ni and Zn concentrations decreased with increasing distance from the smelter (Fig. 2). Copper concentrations were roughly tens of times higher than those of Ni and Zn. Both the total and exchangeable metal concentrations decreased in humus samples of all sites during the incubation, excluding only the most polluted site, and in the case of Cu and Zn also the less polluted site, where the concentrations increased. However, the decrease was statistically significant only in few cases.

The relation between the exchangeable and the non-exchangeable concentrations remained constant during the incubation, except for the 2 km distance, where there was a decrease solely in exchangeable concentrations, and for Zn at the 0.5 km distance, where the increase in non-exchangeable concentration was stronger. In the two most polluted soils less than 50% of the total Zn was in exchangeable form, while at further distances the proportion was clearly higher than 50%. For Cu and Ni the exchangeable proportion was clearly more than 50% of the total concentrations along the whole gradient.

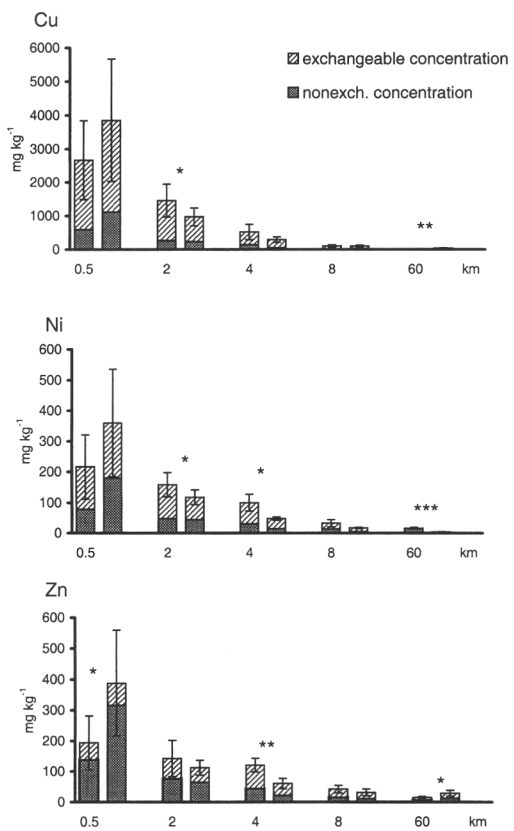

Fig. 2. The exchangeable and non-exchangeable Cu, Ni and Zn concentrations (mg kg^{-1}) in the humus samples prior to (left column) and after (right column) the incubation period. The total height of the columns indicate the total metal concentrations. The bars indicate the standard error of the mean of the total concentrations (n = 5). The difference between the concentration before and after the incubation period was tested by paired t-test. The p value is symbolized as *** for <0.001, ** for <0.01 and * for <0.05.

EXCHANGEABLE METAL CONCENTRATIONS IN MINERAL SOIL

There was a decreasing trend in mineral soil Cu, Ni and Zn concentrations with increasing distance from the smelter (Fig. 3). However, during the incubation period there was either no change or an increase in concentrations in most cases. Only the Zn concentration of the uppermost layer at 2 km decreased, but the change was not statistically significant. With depth the concentrations decreased. The exchangeable Cu concentrations were roughly 10 times higher than Ni concentrations, and Ni concentrations about twice the Zn concentrations at the 0.5 km soils.

Soil pH

Prior to incubation there was no clear difference between the pH values of the soil profiles from different sites, except the mineral soil 10–20 cm layer at the 0.5 km pots was slightly more acidic than the other corresponding layers. However, there was a slight increase in the pH values of both the humus and mineral soil layers at all distances during the incubation period (Table 4).

Exchangeable metal contents of the soil pots and the metal uptake by pine seedlings

The increase in the total amount of exchangeable Cu during the incubation period was statistically significant at the 0.5, 8 and 60 km pots (Fig. 4). There was no change at 4 km and even a decrease in exchangeable Cu at the 2 km pots. In the case of Ni there was a statistically significant increase only at the 8 and 60 km pots, and in the case of Zn at the 8 km pots. For all the other pots there was no significant change, hence most of the significant decreases in humus during the incubation have been compensated by an increase in mineral soil layers.

Most of the exchangeable Cu was in the humus layer in pots originating from the three most polluted sites, both prior to and after the incubation. About half of the exchangeable Ni and Zn was in the humus layer in the 0.5 km pots, while Ni and Zn amount was in most cases higher in mineral soil at 2, 4 and 8 km.

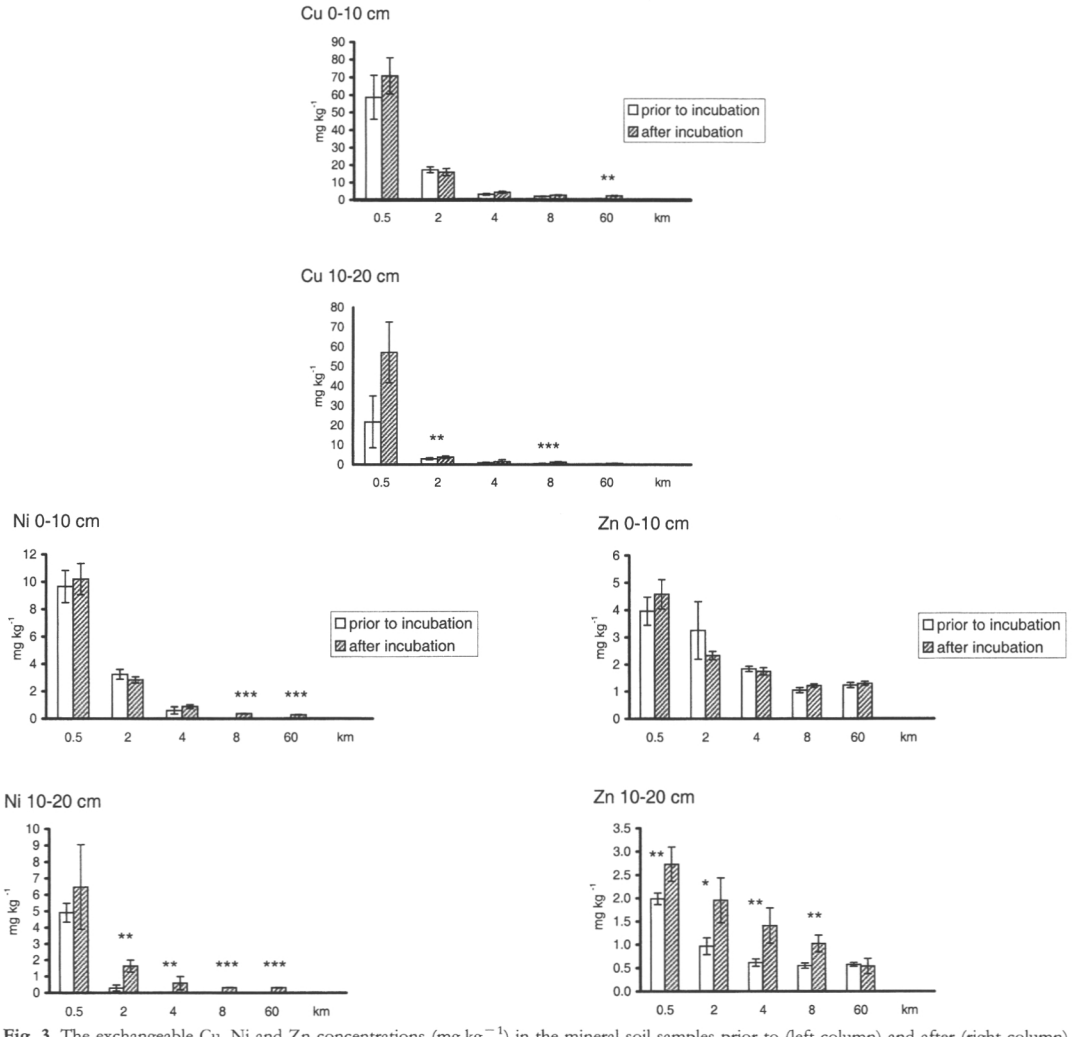

Fig. 3. The exchangeable Cu, Ni and Zn concentrations (mg kg^{-1}) in the mineral soil samples prior to (left column) and after (right column) the incubation period. The bars indicate the standard error of the mean (n = 5). For an explanation of the asterisks see caption to Figure 2.

The amounts of exchangeable Cu, Ni and Zn in the pots have also been presented as area-based values (Table 5) to make possible comparison with the annual Cu, Ni and Zn deposition via throughfall and litterfall at Harjavalta sites (Table 3).

The metal uptake by pine seedlings was at its lowest in the 0.5 km pots (Fig. 5) due to extremely poor growth of the pines. The seedlings growing in the 0.5 km pots started to die during the second growing season and only three of the original 25 seedlings were alive at the end of the experiment. The Cu and Ni uptake was at its highest in the 2 km pots, but for Zn there was not much difference between the rest of the distances. The metal amounts taken up by pine seedlings are small compared to the exchangeable soil-metal contents.

DISCUSSION

The absence of atmospheric metal deposition had no reducing effect on the exchangeable heavy-metal contents of the soil pots. There was a decrease in soil-metal content (mg per pot) during the incubation period only in the case of Cu in the 2 km soil pots. In contrast, there was an increase in exchangeable Cu content in most of the soil pots, and in the less polluted soil pots in exchangeable Ni and Zn content. Metal mobilization from solid pools of the soil during the incubation period is a probable explanation for the observed increase in exchangeable metal content.

The slight pH increase observed in the humus layers may have affected the mobility of metals, for example due to an increased dissolution of organic compounds (Brümmer *et al.* 1986). According to Temminghoff *et al.* (1998) the humic acid mobility, which enhances Cu mobility, depends strongly on pH. However, they observed only a slight pH-induced enhancement of Cu mobility between pH 4 and 6.

Leaching from a heavy-metal pool of the thick litter layer on the surface of the 0.5 km soil pots has most probably also increased the metal amounts in the underlying humus of these

Table 4. *The median pH values of the humus and mineral soil profiles from a heavy-metal pollution gradient prior to (June 1994) and after the incubation period (October 1995)*

Distance (km)	Soil profile	Depth (cm)	pH prior to incubation	pH after incubation
0.5	humus		4.1	4.2
	mineral soil	0–10	4.1	4.3
	mineral soil	10–20	4.1	4.9
2	humus		3.8	3.8
	mineral soil	0–10	4.1	4.2
	mineral soil	10–20	4.6	4.9
4	humus		3.5	3.6
	mineral soil	0–10	3.9	4.1
	mineral soil	10–20	4.6	5.1
8	humus		3.9	4.0
	mineral soil	0–10	4.2	4.4
	mineral soil	10–20	4.6	5.0
60	humus		4.1	4.4
	mineral soil	0–10	4.9	5.2
	mineral soil	10–20	4.8	5.4

pots. The continuous drip irrigation used in this experiment moistens the litter layer efficiently and may thus enhance metal leaching. In the field conditions the water-holding capacity of the litter layer at the 0.5 km site is strongly depressed due to low decomposition activity and subsequent accumulation of undecomposed litter (Fritze *et al.* 1989; Derome & Nieminen 1998).

At the less polluted soils the consequences of the constant moisture were the opposite. In the absence of a thick litter layer the enhanced leaching during the experiment resulted in decreased humus metal concentrations. Downwards movement of metals from the top layers is also suggested by the increase in metal concentrations of the underlying mineral soil observed especially in the lower mineral soil layers. There was no water outflow from the bottom of the pots, and hence the migration of metals out from the pots was not possible. The impurities of the irrigation water could partly explain the increases in the Cu and Zn concentrations observed, even in the soil pots originating from the background site, but not the increase in Ni.

The pattern of the changes in humus concentrations that had taken place during the incubation period was very similar for all of the metals under study, although in earlier studies from the same sites Cu has been considered to be more strongly retained in the humus layer than Ni or Zn (Derome & Nieminen 1998). According to Brümmer *et al.* (1986) Cu should be less mobile than Ni or Zn in the pH range of this experiment, but, on the other hand, results reported from the Sudbury Cu–Ni mining and smelting area (Adamo *et al.* 1996) reveal that Cu occurs in more mobile forms than Ni in these acid Cu–Ni polluted soils. According to Henderson *et al.* (1998) Zn in the humus is associated primarily with labile phases in background soils, but adjacent to a metal smelter a high proportion of Zn was in a non-labile phase, indicative of smelter-derived particulates. This is in good accordance with the high proportion of non-exchangeable Zn in the most polluted 0.5 km soil pots.

The humus metal concentrations were higher than the corresponding concentrations of the mineral soil layers. This has also been observed in many earlier studies in polluted forests, where humus has been considered to be the most important sink for heavy metals (e.g. Derome & Lindroos 1998; Kabata-Pendias & Pendias 1992; Räisänen *et al.* 1997). However, the amount of exchangeable metals calculated per pot is higher in the humus layer than in the 20 cm depth mineral soil layer only in the case of Cu in the polluted soil pots (0.5–4 km).

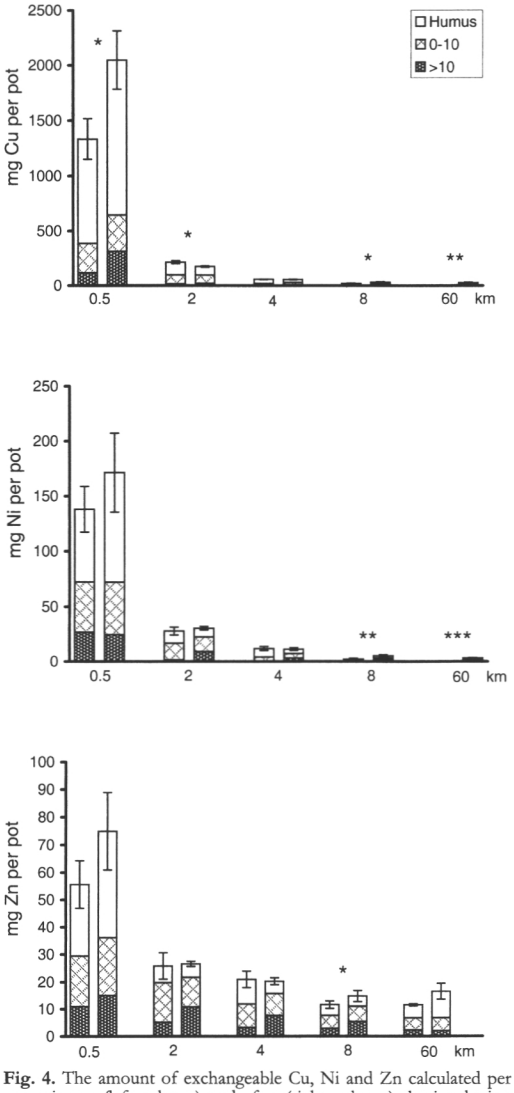

Fig. 4. The amount of exchangeable Cu, Ni and Zn calculated per pot prior to (left column) and after (right column) the incubation period. The metal content of the pot was obtained by summing up the contents of humus and the two mineral soil layers. The bars indicate the standard error of the mean (n = 5).

The Ni and Zn amount (mg per pot) in humus is about the same as the corresponding amounts in the mineral soil in the 0.5 km pots, and clearly less in the soil pots from 2, 4 and 8 km distances. Hence, in this study the humus layer was a stronger sink for Cu than for Ni and Zn.

The metal deposition in the field via throughfall and litterfall in 1993 was elevated at the 0.5–8 km sites compared to the 60 km background site, despite the recent reductions of metal emissions from the smelter. The exchangeable amounts of Ni at 0.5 km was about 20 times higher than the total annual deposition of Ni via throughfall + litterfall, but in the case of Zn the exchangeable pool is only five times higher than the

Table 5. *The amount of exchangeable Cu, Ni and Zn in the studied soil layer at different distances from the smelter calculated as mg m^{-2} prior to (June 1994) and after the incubation period (October 1995)*

Distance (km)	Cu		Ni		Zn	
	Prior to (mg m^{-2})	After (mg m^{-2})	Prior to (mg m^{-2})	After (mg m^{-2})	Prior to (mg m^{-2})	After (mg m^{-2})
0.5	27 000	42 000	2800	3500	1000	1500
2	4000	3600	600	600	525	550
4	1000	1000	250	250	425	400
8	400	650	50	100	250	300
60	100	550	10	70	235	340

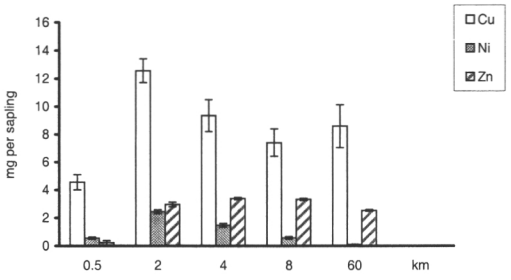

Fig. 5. The Cu, Ni and Zn uptake by pine seedling during the 17 month experimental period. The bars indicate the standard error of the mean (n = 5).

annual deposition. Most probably, an important part of metals in these polluted soils is in immobilized phase and a part has migrated out of the soil system.

The uptake rate of metals by the pine saplings in the 0.5 km soil pots was negligible due to very poor growth of the seedlings. The Cu and Ni amounts incorporated in the stem-wood production of the pines growing in the vicinity of Harjavalta smelter have also been reported to be low, due to the extremely poor growth of Scots pine in these kinds of heavily polluted soils (Nieminen *et al.* 2000). The metal uptake was low also in the less polluted and the background sites, in general lower than the changes in the metal amount during the incubation period. But, on the other hand, trees are long-living, and hence the metal uptake could have an effect on soil-metal amounts during their lifespan in the less polluted soils.

Plant roots may enhance metal mobilization from non-labile soil pools. The most cited example is the extraction of siderophores from roots of plants suffering from Fe deficiency (Zhang *et al.* 1991). These kinds of root exudates are usually non-specific and may mobilize Zn or Cu as well as Fe. According to Taylor & Foy (1985), Cu toxicity may induce Fe deficiency.

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Paper V

Nieminen, T. and Helmisaari H.-S. 1996. Nutrient retranslocation in the foliage of *Pinus sylvestris* L. growing along a heavy metal pollution gradient. Tree Physiology 16: 825—831. Reproduced by permission of Heron Publishing.

V

Correction:

p. 829, Figure 1 a). The dry weight is in mg needle^{-1} (not in µg needle^{-1}).

Tree Physiology 16, 825–831

Nutrient retranslocation in the foliage of *Pinus sylvestris* L. growing along a heavy metal pollution gradient

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Summary Retranslocation of N, P, K, Ca, Mg, Mn, Fe, Zn, Cu and Ni within the foliage of Scots pine (*Pinus sylvestris* L.) was studied during autumnal needle senescence along a heavy-metal pollution gradient in western Finland. The stands were located at distances of 0.5 (Har 0.5), 4 (Har 4) and 8 km (Har 8) to the southeast of a copper–nickel smelter at Harjavalta. A background study stand was located at Hämeenkangas (Häm), 60 km northeast of the smelter. During needle senescence, the mobile nutrients N, P and K decreased by 67–88% and needle dry mass decreased by 31–43%. Retranslocation of P and K was less efficient in the stand nearest the smelter (Har 0.5) than in the other stands. During needle senescence at stand Har 0.5, heavy metal content either decreased slightly (Zn, Ni) or increased (Fe, Cu), whereas in the background study stand, retranslocation efficiency of Cu was 75%. Net throughfall (the amount of a nutrient in throughfall minus the amount in open precipitation) was higher in stand Har 0.5 than in stand Har 8 for all of the elements measured, except N and P which were intercepted by the canopy. In stand Har 8, deposition of S, Cu and Ni was intercepted by the canopy, and net throughfall of Fe and Zn was very low. In contrast, S and heavy metal contents of net throughfall in stand Har 0.5 were greater than the decrease in these elements in the needles indicating that accumulation on needle surfaces and subsequent wash-off accounted for a major part of the fluxes. Furthermore, the Cu content in net throughfall during the senescence period was higher than the original Cu content of the needles in July, providing additional evidence that much of the needle Cu content was comprised of dry deposits of Cu on the needle surface.

Keywords: copper, heavy metal accumulation, leaching, needle litter, net throughfall, nickel, Scots pine, senescence.

Introduction

Efficient nutrient retranslocation allows trees to withstand fluctuations in soil nutrient availability. During autumnal nutrient retranslocation, nutrients from senescing needles are transported primarily to remaining needles for overwinter storage (Chapin and Kedrowski 1983). This storage is of special importance in forests exposed to a pollution load that leads to continuous deterioration of soil nutrient status.

Heavy metal accumulation disturbs decomposition and nutrient mineralization processes in forest soil (e.g., Freedman and Hutchinson 1980, Berg et al. 1991), leading to low nutrient availability. According to Berg et al. (1991), the low mineralization rate is caused by changes in both litter quality and soil factors. Studies carried out in a heavy-metal polluted area at Harjavalta, western Finland, have shown that heavy metals have direct toxic effects on soil fauna (Haimi and Siira-Pietikäinen, 1996) and soil microbes (Fritze et al. 1996). In addition, a decrease in needle litter decomposition rate has been observed in the most polluted area of Harjavalta (Helmisaari et al. 1995), and the understory vegetation has disappeared almost totally up to a distance of 0.5 km from the emission source (Salemaa and Vanha-Majamaa 1993); however, Scots pine has survived even at this distance. The ability of long-lived trees to retranslocate nutrients from older to younger tissues may play an important role in the survival of Scots pine in polluted soils with decreased nutrient availability.

The aims of our study were to estimate nutrient retranslocation efficiency in Scots pine foliage of a heavily polluted stand in Harjavalta at a distance of 0.5 km from the emission source and to compare it with retranslocation efficiencies of moderately polluted and background stands.

Material and methods

Study stands

Harjavalta (61°19′ N, 22°9′ E) is situated in southwestern Finland, about 30 km from the coast, in the southern boreal coniferous zone (Ahti et al. 1968). The long-term (1960–1990) mean annual temperature at a nearby weather station of the Finnish Meteorological Institute is 4.0 °C and the annual precipitation is 558 mm. The area has been subjected to a heavy pollution load since the 1940s, mainly from a metallurgical plant producing copper and nickel. Table 1 shows the sulfur dioxide, dust and heavy metal emissions from 1985 to 1992.

A transect to the southeast of the metallurgical plant was marked out in 1991. Three study sites were established in Scots

Table 1. Heavy metal and SO_2 emissions from the Outokumpu Harjavalta smelter from 1985 to 1992.

Year	SO_2 (Mg year^{-1})	Dust (Mg year^{-1})	Cu (Mg year^{-1})	Ni (Mg year^{-1})	Zn (Mg year^{-1})
1985	8000	1100	98	47	216
1986	7500	1200	126	46	232
1987	7000	1800	140	96	162
1988	8000	1000	104	45	103
1989	9500	1000	80	33	190
1990	8800	960	80	31	160
1991	5200	640	80	14	90
1992	4800	280	60	10	12

pine stands along this transect at distances of 0.5 (Har 0.5), 4 (Har 4) and 8 km (Har 8) from the copper and nickel smelter. One stand (Häm), situated at Hämeenkangas, 60 km northeast from Harjavalta, in an area without local emission sources, was chosen as a background site. Table 2 presents characteristics of the study stands.

At all study sites, the soil is a well developed sandy podsol that is relatively poor in available nutrients. The vegetation is typical of a xerophilous forest site: *Calluna vulgaris* (L.) Hull., *Empetrum nigrum* L., *Vaccinium vitis-idaea* L., *Pleurozium schreberi* (Brid.) Mit., *Dicranum spp.*, *Cladina spp.*, etc. The sites are of the *Calluna* type, according to the Finnish forest site classification of Cajander (1949). The effects of industrial pollution on the vegetation in stand Har 0.5 are visible, although emissions have decreased during recent years.

Sampling

Needles were collected in mid-July 1992 from eight to 10 trees per study stand. The sample trees were randomly selected from five size classes representative of each stand. One branch from the upper, middle and lower crown was randomly sampled for each tree. Because the autumnal litter in southern Finland consists mostly of three-year-old needles (Jukola-Sulonen et al. 1990), 100 three-year-old fascicles were collected from each selected branch and combined to form a sample of 300 fascicles per tree.

Litter needles were collected in 12 litter traps per stand. The traps consisted of polyethylene funnels located at 1 m above ground level, with cotton bags fixed under the funnels to collect the litter. The collection surface area of each funnel was 0.50 m^2. The litter samples were removed weekly from July 21 to September 28, 1992. Litter was dried, and needles separated from other litter components. The litter needles from all 12 traps on each plot were combined to give one composite sample from each study stand for each sampling date.

Twenty throughfall collectors were located in each study stand (4 × 5 m lines) to collect rainwater. The total surface area of the 20 rainwater collectors was 0.339 m^2. Free precipitation was collected in five collectors placed in an adjacent open area.

Laboratory analyses and data processing

The concentrations of K, Ca, Mg, Mn, Fe, Zn, Cu, Ni and S in precipitation collected both in an open area and within the stand were determined by inductively coupled plasma–atomic emission spectrometry (ICP–AES). Area-specific deposition values (mg m^{-2}) were calculated from the concentrations and sample volumes. Net throughfall was determined as the difference between deposition in throughfall and deposition in open precipitation.

Both green and litter needle samples were dried at 70 °C for 48 h, weighed, and stored in paper bags. Needle unit weight (mg needle^{-1}) was determined for each sample. Concentrations of P, K, Ca, Mg, Mn, Zn, Fe, Cu and Ni were determined on a dry weight basis from finely ground needles by dry ashing and extraction with HCl. The filtered solutions were analyzed by ICP–AES. Nitrogen and sulfur contents of finely ground needles were determined by Leco-analyzers. The litter needle mass per unit area was calculated by dividing the total mass of

Table 2. Characteristics of the study stands in 1991.

	Har 0.5	Har 4	Har 8	Häm
Distance from smelter (km)	0.5	4	8	60
Age (years)	46	42	35	25
Density (trees ha^{-1})	1280	1711	1356	1967
Basal area (m^2 ha^{-1})	9.0	15.9	16.5	10.9
Stem volume (m^3 ha^{-1})	34.9	85.7	92.3	44.9
Mean diameter (cm)	10.8	12.9	14.0	10.0
Mean height (m)	6.8	10.2	10.7	7.3
Total needle mass (kg ha^{-1})	903	2827	3614	2230
Volume increment (1981–90) (m^3 ha^{-1} year^{-1})	1.2	3.7	5.8	3.1

the collected litter needles (kg) by the surface area of the litter traps (m^2). The mass of senescing needles (M_1, kg m^{-2}) was calculated according to the equation:

$$M_1 = (W_1/W_2)(M_2), \quad (1)$$

where W_1 is three-year-old green needle unit mass (g $needle^{-1}$), W_2 is litter needle unit mass (g $needle^{-1}$) and M_2 is litter needle mass (kg m^{-2}). The nutrient content of senescing needles (NC_1, mg m^{-2}) was calculated as:

$$NC_1 = M_1C_1, \quad (2)$$

where M_1 is three-year-old green needle mass (kg m^{-2}) and C_1 is nutrient concentration of three-year-old green needles (mg kg^{-1}). The nutrient content of litter needles (NC_2, mg m^{-2}) was calculated correspondingly as:

$$NC_2 = M_2C_2, \quad (3)$$

where M_2 is litter needle mass (kg m^{-2}) and C_2 is nutrient concentration of litter needles (mg kg^{-1}). The relative element content per needle (μg $needle^{-1}$) was calculated by dividing the area-based content by the number of needles per unit area.

To estimate the importance of leaching and wash-off from the needles during the senescence period, the cumulative net throughfall for an interval corresponding to the needle senescence period was calculated for two of the stands (Har 0.5 and Har 8). Net throughfall from the whole canopy was transformed to net throughfall from the senescing needle mass (NT_1, mg m^{-2}) by the following equation:

$$NT_1 = (M_1/TM)(NT), \quad (4)$$

where TM is total needle mass (kg ha^{-1}), M_1 is three-year-old needle mass (kg ha^{-1}) and NT is net throughfall from the total needle mass (mg m^{-2}).

Results

Nutrient retranslocation and net throughfall

The decrease in nutrient content during autumnal senescence (the difference in needle nutrient content before and after senescence) is presented in Tables 3a (macronutrients) and 3b (micronutrients). The percentage decreases indicate the efficiency of retranslocation for each element. The mobile macronutrients N, P and K always decreased relatively more than the dry mass of the needles, as did Mg and S. Conversely, the relatively immobile nutrients Ca and Mn usually decreased less than the dry mass of the needles, with the exception of Ca in stand Har 0.5. The contents of Fe, Zn, Cu and Ni also decreased less than the dry mass of the needles, with the exception of Cu in stand Häm.

Retranslocation of P and K was less efficient in stand Har 0.5 than in the other stands, and retranslocation of N was less efficient in stand Häm than in the other stands (Table 3a). The decrease in heavy metal content was low in stand Har 0.5, and the amount of Fe and Cu in needles increased during senescence. There was also an increase in Zn content of stand Häm.

The net throughfall values given in Table 3 are estimates of the proportion of element content in net throughfall that originated from senescing needles. Net throughfall was relatively greater in stand Har 0.5 than in stand Har 8 for all elements studied, except N and P which were intercepted by the canopy

Table 3a. Dry mass and macronutrient content of green and litter needles. Percentage values show the proportional change in dry mass or nutrient content during senescence. Net throughfall values were determined as described in the text.

	Needle dry mass (g m^{-2})	N (mg m^{-2})	P (mg m^{-2})	K (mg m^{-2})	Ca (mg m^{-2})	Mg (mg m^{-2})	S (mg m^{-2})
Har 0.5							
Green needles	48.0	470.7	43.0	209.3	121.1	14.4	33.5
Litter needles	33.2	145.5	11.3	42.3	81.5	7.9	19.2
Percent decrease	−30.8%	−69.1%	−73.7%	−79.8%	−33.0%	−45.1%	−42.7%
Net throughfall		Intercepted	Intercepted	10.9	14.3	3.3	18.0
Har 4							
Green needles	103.7	791.6	95.1	330.2	311.0	34.9	38.6
Litter needles	59.2	221.3	16.3	46.5	214.6	17.9	17.5
Percent decrease	−42.9%	−72.0%	−82.9%	−85.9%	−31.0%	−48.7%	−54.7%
Har 8							
Green needles	134.3	1241.9	127.8	456.5	312.5	49.6	47.9
Litter needles	81.9	320.5	23.0	56.3	285.4	28.3	23.3
Percent decrease	−39.1%	−74.2%	−82.0%	−87.7%	−8.7%	−42.9%	−51.4%
Net throughfall		Intercepted	Intercepted	13.2	6.1	2.0	Intercepted
Häm							
Green needles	107.4	894.6	92.3	281.0	291.6	36.4	32.2
Litter needles	69.5	297.0	18.2	39.8	230.3	20.6	18.5
Percent decrease	−35.3%	−66.8%	−80.2%	−85.8%	−21.1%	−43.4%	−42.5%

Table 3b. Micronutrient content of green and litter needles. Percentage values show the proportional change in nutrient content during senescence. Net throughfall values were determined as described in the text.

	Mn ($mg\ m^{-2}$)	Fe ($mg\ m^{-2}$)	Zn ($mg\ m^{-2}$)	Cu ($mg\ m^{-2}$)	Ni ($mg\ m^{-2}$)
Har 0.5					
Green needles	7.3	42.7	3.8	8.3	2.1
Litter needles	5.5	52.5	3.7	11.9	2.0
Percent decrease	−24.7%	+23.0%	−2.6%	+43.4%	−4.8%
Net throughfall	0.8	7.1	1.6	14.0	2.2
Har 4					
Green needles	33.6	15.6	4.8	2.5	0.9
Litter needles	24.6	10.5	3.1	2.1	0.7
Percent decrease	−26.8%	−32.7%	−35.4%	−16.0%	−22.2%
Har 8					
Green needles	46.0	14.6	4.0	1.3	0.50
Litter needles	36.8	12.0	3.7	1.1	0.47
Percent decrease	−20.0%	−17.8%	−7.5%	−15.4%	−6.0%
Net throughfall	0.9	0.3	0.05	Intercepted	Intercepted
Häm					
Green needles	44.1	10.7	3.6	0.4	0.10
Litter needles	32.4	8.0	4.5	0.1	0.08
Percent decrease	−26.5%	−24.9%	+25.0%	−75.0%	−20.0%

in both stands. The percentage net throughfall of K from senescing needles in stand Har 0.5 was almost double that in stand Har 8, and the percentage net throughfalls of Ca, Mg, Mn and S in stand Har 0.5 were about five times greater than the corresponding values in stand Har 8. Differences in net throughfall between the two stands were even greater for the heavy metals: the percentage net throughfall of Fe and Ni in stand Har 0.5 was close to 10 times greater, Zn net throughfall 40 times greater, and Cu net throughfall 200 times greater, than the corresponding net throughfall values for stand Har 8.

The cumulative deposition of N, P and K during the study period was slightly greater near stand Har 8 than near stand Har 0.5 (Table 4). For all other elements studied, deposition was higher at stand Har 0.5 than at stand Har 8 and the differences were significant for Fe, Zn, Cu and Ni.

The amount of an element in net throughfall indicates the proportion of the total decrease in that element that is caused by foliar leaching and by washing previously deposited material from needle surfaces. We subtracted the K, Ca, Mg, and Mn contents of net throughfall from the total decrease in the content of these elements in needles in stands Har 0.5 and Har 8. We then recalculated the percentage decrease in the values to obtain a corrected estimate of retranslocation efficiency of these elements (Table 5). The corrected values indicate less efficient retranslocation of K, Mg and Mn in stand Har 0.5 compared with stand Har 8 than was indicated by the raw percentage decreases given in Table 3.

Table 4. Element deposition and free precipitation during the needle senescence period (July 16–October 6, 1992).

	Har 0.5	Har 8
Macronutrients ($mg\ m^{-2}$)		
N	220.5	226.4
P	5.3	6.3
K	0.9	3.4
Ca	28.4	26.6
Mg	7.3	6.6
S	219.2	200.8
Micronutrients ($mg\ m^{-2}$)		
Mn	0.4	0.3
Fe	4.5	0.7
Zn	2.1	0.7
Cu	20.9	0.7
Ni	1.4	0.2
Precipitation		
Amount (mm)	155	160
pH range	4.2–4.6	4.2–4.6

Relative nutrient content and relative net throughfall

Figures 1 and 2 present the contents of each element studied ($\mu g\ needle^{-1}$) in senescing needles in July and in litter needles collected from August to October. These relative values take into account the variation in total needle mass between stands, and thus also the filtering capacity of the tree canopy. A linear regression analysis on distance from the emission source was performed for green senescing needles, and *F*- and *P*-values are presented for significant cases in Figures 1 and 2. With increasing proximity to the emission source, needle K content increased slightly and the sulfur content and all heavy metal contents, with the exception of Zn, increased significantly. Needle contents of N, P, Ca, Mg and Mn showed no clear trend with distance from the emission source.

Table 5. Nutrient decrease percentages corrected for nutrient content of net throughfall. The nutrient content of the net throughfall was subtracted from the total nutrient decrease, and the percentage decrease was recalculated.

	K (%)	Ca (%)	Mg (%)	Mn (%)
Har 0.5	74.6	21.2	22.2	13.7
Har 8	84.8	6.7	38.9	18.0

Net throughfall of sulfur and heavy metals, especially Cu and Ni, in stand Har 0.5 was high compared to the needle content of these elements. Net throughfall of all measured elements was higher in stand Har 0.5 than in stand Har 8.

Discussion

Retranslocation efficiencies of 67–74% for N, 74–83% for P and 80 to 88% for K are in good agreement with the results of an earlier study on *Pinus sylvestris* stands reported by Helmisaari (1992*a*). In our study, P and K retranslocation was less efficient in stand Har 0.5 than in the other study stands, which does not support our hypothesis that there is more efficient nutrient retranslocation in heavily polluted forests.

The internal nutrient retranslocation of elements that are highly susceptible to foliar leaching—e.g., K, Ca, Mg and Mn (Helmisaari and Mälkönen 1989)—can be overestimated if leaching losses are not taken into account. Usually, leaching loss is considered to be equivalent to net throughfall (throughfall minus free precipitation), and the nutrient net throughfall

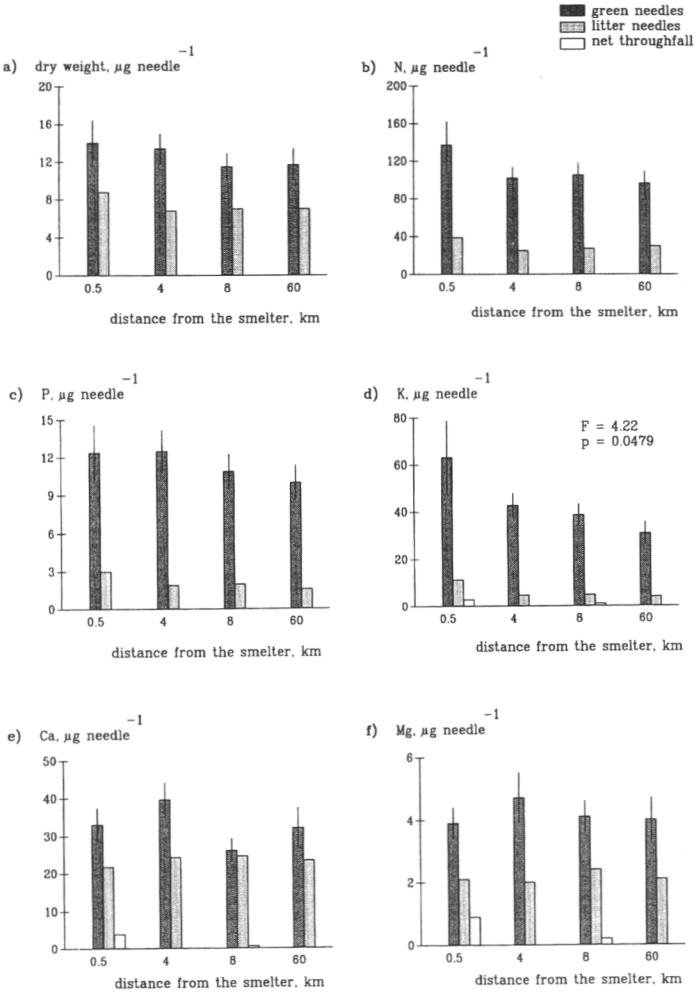

Figure 1. Dry mass and macronutrient content per needle (µg needle^{-1}) in green needles (before senescence) and litter needles. Vertical lines represent standard errors of the means. *F*- and *P*-values of linear regression analysis of content over distance from the smelter are shown for significant cases. Net throughfall was calculated for stands Har 0.5 and Har 8 only.

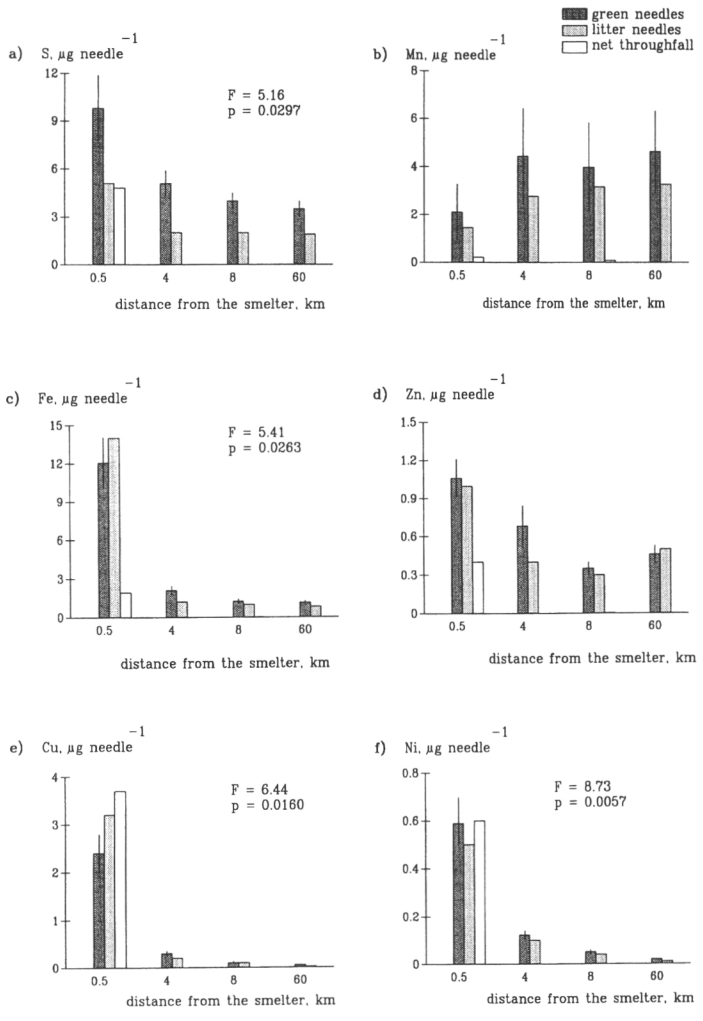

Figure 2. Micronutrient content per needle (µg needle^{-1}) in green needles (before senescence) and litter needles. Vertical lines represent standard errors of the means. *F*- and *P*-values of linear regression analysis of content over distance from the smelter are shown for significant cases. Net throughfall was calculated for stands Har 0.5 and Har 8 only.

percentage is subtracted from the percentage decrease in total nutrient content of senescing needles to provide a more accurate estimate of nutrient retranslocation (e.g., Ostman and Weaver 1982, Helmisaari 1992*b*). In addition to leached nutrients, net throughfall also contains dry deposition components washed off from needle surfaces by rain (e.g., Rosén and Lundmark-Thelin 1985, Helmisaari and Mälkönen 1989, Hyvärinen 1990). Although foliar leaching is generally the major process controlling throughfall enrichment, canopy filtration of dust, aerosols and gases is important under certain conditions (Parker 1983), for example, in the vicinity of a pollution source.

According to the net throughfall measurements made in stands Har 0.5 and Har 8, N and P were intercepted by the tree canopy during the study period, as observed in earlier studies on Scots pine stands (e.g., Helmisaari and Mälkönen 1989, Hyvärinen 1990). Because no emission-induced K deposition occurred during the study period (cf. Table 4), foliar leaching was probably the main source of K enrichment of throughfall. Potassium retranslocation in stand Har 0.5 was less efficient than in stand Har 8, and when the K net throughfall percentages were subtracted from the percentage decreases in total K for these two stands, this difference in retranslocation between the two stands increased.

The retranslocation efficiencies of Ca, Mg and Mn were within the same range as the retranslocation estimates reported previously by Helmisaari (1992*a*) for Scots pine stands. Net throughfalls of Ca, Mg and Mn were slightly higher in stand Har 0.5 than in stand Har 8, but the distribution between foliar leaching and wash-off remains uncertain.

Sulfur was retranslocated in all stands except Har 0.5, where the retranslocation estimate was not reliable because of the high net throughfall of S. The major portion of S net throughfall consists of dry deposited S, although the exact proportion cannot be determined (Lindberg and Garten 1988, Kazda 1990, Cape et al. 1992). Retranslocation did not account for the decrease in heavy metal content of senescing needles, except for Cu in the background stand (Häm), where the retranslocation efficiency of Cu was 75%. According to Loneragan et al. (1980), the mobility of Cu is related to nitrogen metabolism. The decrease in Cu content of senescing tissue reflects the hydrolysis of proteins and was concurrent with the decrease in N. The net throughfall measurements revealed a high load of dry deposition of Fe, Cu, Ni and Zn in the Scots pine stand situated closest to the smelter (Har 0.5). Thus, the calculated percentage decreases in heavy metals in stand Har 0.5 probably reflected needle surface accumulation and wash-off rather than internal nutrient retranslocation. The calculation of retranslocation efficiencies for micronutrients is sensitive to bias because the foliar heavy metal contents are usually low and the estimates are therefore subject to greater error than estimates for macronutrients.

The relative sulfur and heavy metal (except Zn) contents of the needles decreased significantly with increasing distance from the smelter. The decrease in relative Zn content was more gradual and not statistically significant and was probably related to interactions between Cu and Zn uptake by roots (Alva and Chen 1995). The decreases in S and heavy metal contents are in agreement with recent air quality measurements (Saari et al. 1993) and earlier studies carried out in the Harjavalta area (i.e., Laaksovirta and Silvola 1975, Heliövaara and Väisänen 1989).

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Paper VI

Nieminen, T. M., Derome, J. and Saarsalmi, A. 2004. The applicability of needle chemistry for diagnosing heavy metal toxicity to trees. Water, Air, and Soil Pollution 157: 269—279. Reproduced with kind permission of Springer Science and Business Media.

THE APPLICABILITY OF NEEDLE CHEMISTRY FOR DIAGNOSING HEAVY METAL TOXICITY TO TREES

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Abstract. To estimate the validity of needle chemistry as a tool for diagnosing heavy metal toxicity, we collected needle samples from mature pines and young seedlings and monitored metal deposition in the vicinity of a Cu–Ni smelter in SW Finland. Needle Cu, Ni, Zn and Fe concentrations in a pine stand growing next to the smelter were determined in 1992 and in 1998. Deposition was collected as bulk precipitation (open area) and as stand throughfall (below the canopy) during 1992–1998. Copper deposition in stand throughfall (TF) was relatively constant during 1992–1998, but for Ni in stand TF there was a strong increase from 100 mg m^{-2} in 1997 to 600 mg m^{-2} in 1998. There was a decreasing trend during the study period for both Zn and Fe deposition in TF, indicating a decrease in the emissions of these metals from the smelter. Washing the needles with chloroform removed a part of the Fe and Ni, but there was no clear difference for Cu or Zn between the washed and unwashed samples. However, needle Ni, Fe and Cu concentrations in pine seedlings grown in unpolluted soil close to the Cu–Ni smelter were considerably higher than in seedlings grown in a greenhouse in polluted soil taken from close to the smelter. Hence, our results suggest that not all the needle Ni, Fe and Cu derived from aerial deposition is washed-off from the canopy by rainfall (or by the washing of needle samples with e.g. chloroform).

Keywords: aerial deposition, Cu, Fe, Ni, Scots pine, surface contamination, wash-off, Zn

1. Introduction

Needle analysis is widely used as a diagnostic tool in studies on forest nutrient status as well as in air pollution monitoring. Stand TF measurements are also extensively used in nutrient budget studies (Parker, 1983) and to characterise and quantify the atmospheric pollution load (e.g. Hultberg and Grennfelt, 1992; Derome and Nieminen, 1998). Forest canopies, especially conifer foliage, are efficient filters of heavy-metal dry deposition (Hultberg, 1985). In polluted environments, the dry deposition accumulating on needle surfaces is an important source of metal deposition on the forest floor via stand TF, and strongly affects the needle metal concentrations (Hyvärinen, 1990; Nieminen *et al.*, 1999; Wulff and Kärenlampi, 1993). Consequently, the needle chemistry in heavily polluted environments is assumed to be strongly affected by the occurrence and intensity of rainfall events prior to needle sampling. Washing the needles prior to analysis is recommended to decrease

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the variation in the amount of dry deposition onto the needle surfaces (Raitio, 1995).

The quantitative importance of surface contamination by dry deposition on foliar chemistry and on the composition of stand TF is still relatively unknown. Dry deposited particles can be identified by scanning electron microscopy of the needle surfaces (Turunen and Huttunen, 1996; Rautio *et al.*, 1998), but their proportion out of the total metal concentration of the needles is more difficult to determine. A number of different washing techniques have been developed for removing or separating surface-deposited contaminants from the needles. The simplest method is washing with distilled water, but this removes only part of the metals bound to the needle surfaces (Raitio, 1995). More drastic extraction methods using e.g. chloroform, weak acids or a chelating agent, have also been extensively used to dissolve the epicuticular wax layer of the needles, thus removing all the elements fixed in the wax (Simmleit *et al.*, 1989; Raitio, 1995; Rautio and Huttunen, 2003).

The most important drawback to these washing techniques is the possibility of elements leaching from the tissues inside the needles during the washing procedure, i.e. that metals may be extracted from the physiologically active pools, and not only from the surface of the needles. Another disadvantage is the non-quantitative nature of the method. It is extremely difficult to define the time and intensity of the washing treatment needed for total dissolution of all the epicuticular waxes.

The goal of our study was to test the validity of needle chemistry as a tool for diagnosing heavy metal toxicity under heavily polluted conditions. We estimated the contribution of aerial contamination to the total metal concentrations in the needles by means of a routine chloroform washing procedure and by manipulation experiments using pine seedlings grown in unpolluted soil next to the Harjavalta smelter and seedlings grown in a greenhouse in heavy-metal polluted soil obtained from close to the smelter.

2. Material and Methods

2.1. Study site and experimental design

Three experimental plots (30 m × 30 m) were established in an even-aged, pure Scots pine (*Pinus sylvestris* L.) stand in July 1992 at a distance of 0.5 km from the Cu–Ni smelter at Harjavalta (61°19′N, 22°9′E), SW Finland. Emissions from the smelter primarily consist of SO_2 and of heavy metals in particulate form. SO_2 emissions until the end of the 1980s was ca. 8000 tonnes per year but, during the past decade, they have been reduced to below 4000 tonnes per year. There has also been a corresponding decrease in heavy metal emissions (Table I). The site type is a relatively infertile dry heath (*Calluna* forest site type; for Finnish site type classification see Cajander, 1949), and the soil type orthic podzol (FAO-UNESCO, 1988). The mature pine stand has been naturally regenerated, but there was neither tree understorey nor any signs of natural seedling establishment during the study

TABLE I

Annual emissions (tonnes) of Cu, Ni and Zn from the Harjavalta smelters during 1992–1998 (data from Outokumpu Harjavalta Metals Oy)

Year	Cu	Ni	Zn
1992	60	10.0	12.0
1993	50	7.0	13.0
1994	40	6.0	6.0
1995	17	1.4	1.7
1996	49	1.2	5.3
1997	70	3.0	14.0
1998	23	1.7	6.1

period. In 1998 the pine stand had reached an average age of about 50 years, but it was suffering from retarded growth and severe needle loss (Mälkönen *et al.*, 1999). The ground vegetation was almost completely lacking; only a few patches of *Empetrum nigrum* and *Vaccinium uliginosum* were present (Mälkönen *et al.*, 1999).

In May 1994, intact soil profiles including the humus and litter layers were taken with an auger (diameter 25 cm, length 30 cm) from the buffer zone of the experiment and transferred to 10 L pots. A 4-year-old, bare-rooted pine seedling was planted in each pot. The seedlings were grown in controlled greenhouse conditions in the absence of heavy-metal deposition. The total number of soil-plant systems was originally 25, but only 4 of the seedlings survived until October 1995, when the plants were harvested.

In June 1996, 2-year-old containerised (peat containers) pine seedlings were planted in the immediate vicinity of the experimental plots near to the smelter. Three experimental squares (5 m × 5 m) were established, and 49 seedlings were planted on each square. The seedlings were planted in soil pockets filled with 'clean' mulch consisting of a mixture of municipal compost and woodchips. These seedlings were therefore grown in unpolluted soil in the field, but exposed to heavy-metal deposition from the smelter.

2.2. Bulk Deposition and Throughfall Sampling and Chemical Analyses

Bulk deposition was collected in an open area close to the plots using five rainfall collectors ($d = 20$ cm) during the snow-free period, and two snow collectors ($d = 36$ cm) during the winter. Stand TF was collected on one of the three plots using 20 rainfall collectors located systematically inside the stand during the snow-free period, and 6 systematically located snow collectors during the winter (for details of the sampling design, see Mälkönen *et al.*, 1999). Bulk deposition and

stand TF were collected at ca. 1-month intervals during the period June 1992 to December 1998. The difference between TF and bulk deposition was regarded as net TF.

The pH of the bulk precipitation and stand TF was measured from a sub-sample, and the remaining part of the samples filtered (0.45 μm membrane filter). Cu, Fe, Ni and Zn were determined on the filtrate by inductively coupled plasma atomic emission spectrometry (ICP/AES) on samples acidified with ultrapure 65% HNO_3 (0.5 mL/100 mL sample).

2.3. Needle sampling and chemical analyses

Needle samples from the pine trees were collected from the three experimental plots in January 1992 and in February 1998. The needle samples were taken from five sample trees, randomly selected from the dominant crown layer on each of the plots, i.e. from 15 trees in both sampling years. The needles were collected from the current needles (C) growing on the third to fifth branch whorl, counting from the top, on the southern side of the crown. The needle samples were dried (70 °C, 48 h) and analysed separately for each tree. The concentrations of Cu, Fe, Ni and Zn were determined from finely ground needles by wet digestion (HNO_3 + H_2O_2) (Huang and Schulte, 1985), followed by analysis by ICP/AES. To estimate the internal pool of heavy metals in the needles, additional needles were collected in 1998 from five sample pine trees from the buffer zone around two of the plots. The needles were sampled in the same way as described above. Half of the needles were washed with chloroform as recommended by Raitio (1995), and the other half were dried in an oven (70 °C, 48 h) without any washing. The fresh needles were washed in chloroform for 1 min while stirring with a glass rod. The volume of the chloroform solution was 100 ml greater than the volume of the needle sample. The chloroform was then decanted off, the needles dried on filter paper, and then dried in the same way as the unwashed needles (70 °C, 48 h). The concentrations of Cu, Fe, Ni and Zn in the washed and unwashed needle samples were determined according to the same method as for the regular needle samples.

Needles from the 6-year-old seedlings in the greenhouse experiment were collected in October 1995 as three separate needle-age classes. However, only one of the seedlings formed needles during the second growing season in 1995. Thus, the number of samples is 4 for both the C + 2 and C + 1 needles, but only one for the C needles. Needles from the seedling experiment in the field were collected by needle-age classes in September 1998, and hence the C + 1 needles of the seedlings were formed in the same year as the C needles of the trees (sampled in January–February 1998). The field-grown seedlings had reached the age of 5 at the time of sampling. Needle samples were taken from three seedlings per square, and then bulked together to give one sample per square, i.e. a total of three samples of each needle-age class. The needle samples were dried (70 °C, 48 h) and analysed by dry ashing and extraction with HCl followed by analysis by ICP/AES.

2.4. Calculation of the results and statistical analyses

Annual bulk deposition and stand TF were calculated for the calendar years 1993–1998 on the basis of the amount of precipitation and the ion concentrations in the precipitation samples. Net TF deposition was calculated as the difference between TF deposition and bulk deposition. The statistical significance of the differences in needle nutrient concentrations between the washed and unwashed samples and between the two sampling years were tested using the paired t-test.

3. Results and Discussion

3.1. Heavy metal deposition and concentrations in the needles of the pine stand

The needle concentrations of Cu, and especially of Ni, were strongly elevated in 1998 compared to the corresponding concentrations in 1992 (Figure 1). The needle Ni concentration in 1998 was more than ten-fold that in 1992. In contrast, the needle Fe and Zn concentrations were lower in 1998 than in 1992.

According to the emission information provided by the company (Outokumpu Harjavalta Metals Oy), the Cu and Zn emissions in 1997 were higher than those in 1992, but decreased to a much lower level in 1998 (Table I). Nickel emissions decreased from 1992 to 1998, which is inconsistent with the much higher needle Ni concentrations found in 1998 compared to 1992 in this study. Hence, the needle metal concentrations do not appear to reflect the changes in annual Ni emissions. A similar inconsistency with the decrease in Ni emissions was reported in a national forest moss survey; the Ni concentrations in mosses in the surroundings of the Harjavalta smelters were found to have increased from 1995 to 2000 (Poikolainen *et al.*, 2004).

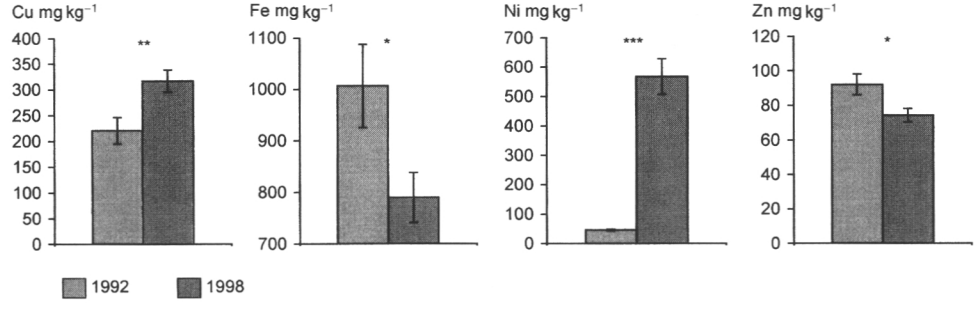

Figure 1. The C-needle Cu, Fe, Ni and Zn concentrations at the beginning of the study period in 1992 and at the end 1998. The bars indicate the standard error of the mean. Statistically significant differences (paired t-test) between the two sampling years are indicated by asterisks: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

The annual metal deposition values during 1993–1998 showed a similar trend as in the needles (Figure 1 vs. Figure 2). There was a very strong peak in Ni deposition in 1998, whereas the Cu TF deposition remained relatively stable. However, net TF Cu was higher in 1998 than at the beginning of precipitation sampling in 1993. A decrease in Fe deposition was observed during 1993–1998. For Zn there was a decrease in TF deposition during the study period, but not in Zn net TF deposition (Figure 2). Our results are consistent with the air quality measurements carried out at Harjavalta in 1992 and 1997 by the Finnish Meteorological Institute (Saari *et al.*, 1998). An increase in the mean daily Cu and Ni concentration in the air, and a decrease in the corresponding Zn concentration was recorded in 1997 compared to the situation in 1992.

The changes in deposition levels in the immediate vicinity of the smelter complex are probably not solely dependent on the variation in total emissions from the smelter. There are two main stacks at the smelter, one 140 m and the other 40 m high. The distribution of metal emissions between these two stacks greatly affects the deposition pattern of the metals. Eighty percent of the total amount of Zn and 75% of the total amount of Ni are emitted from the taller stack, whereas the corresponding value for Cu is only 40% (Saari *et al.*, 1998). In addition, the slag produced during smelting is stored in land basins at the plant site. During piling, the uncovered slagheaps represent an additional source of metal-containing dust. Furthermore, there has been considerable accumulation of heavy metals in the surface soil during the 50-year lifetime of the smelter (Derome and Lindroos, 1998) and, because the understorey vegetation has been almost completely destroyed by SO_2 and heavy metal emissions (Salemaa *et al.*, 2001), wind-borne dust derived from the forest floor also has a considerable effect on needle chemistry and on the quality of stand TF (Nieminen *et al.*, 1999).

The needle Zn concentrations in this study were higher and those of Fe and Cu many times higher than the mean element concentrations in the current-year needles of 98 Scots pine stands (years 1987–1989) growing in different parts of Finland (Raitio *et al.*, 2000). Furthermore, the mean annual TF deposition of Cu at Harjavalta during 1993–1998 was 100-fold, that of Ni almost 500-fold, and of Zn well over 7-fold compared to the mean annual (1994–1996) values at a background-monitoring ICP/Integrated Monitoring plot in eastern Finland, (Ukonmaanaho *et al.*, 2001).

A high proportion of the total heavy metal concentration in needles in areas subjected to high particle deposition, as in the Harjavalta area, is in the form of dust that accumulates on the needle surfaces and has not penetrated into the needles (Kozlov *et al.*, 2000; Nieminen *et al.*, 1999; Turunen, 1997). Thus, high concentrations of heavy metals on the needle surfaces do not necessarily pose any acute toxic hazard to plant metabolism. As a result, care should be taken in comparing the results of foliar analyses with known levels of element toxicity in foliage (Rautio and Huttunen, 2002). To avoid this problem, many researchers recommend that needle samples should be washed prior to chemical analysis in order to estimate the internal pool of heavy metals (Raitio, 1995). According to Raitio (1995), almost

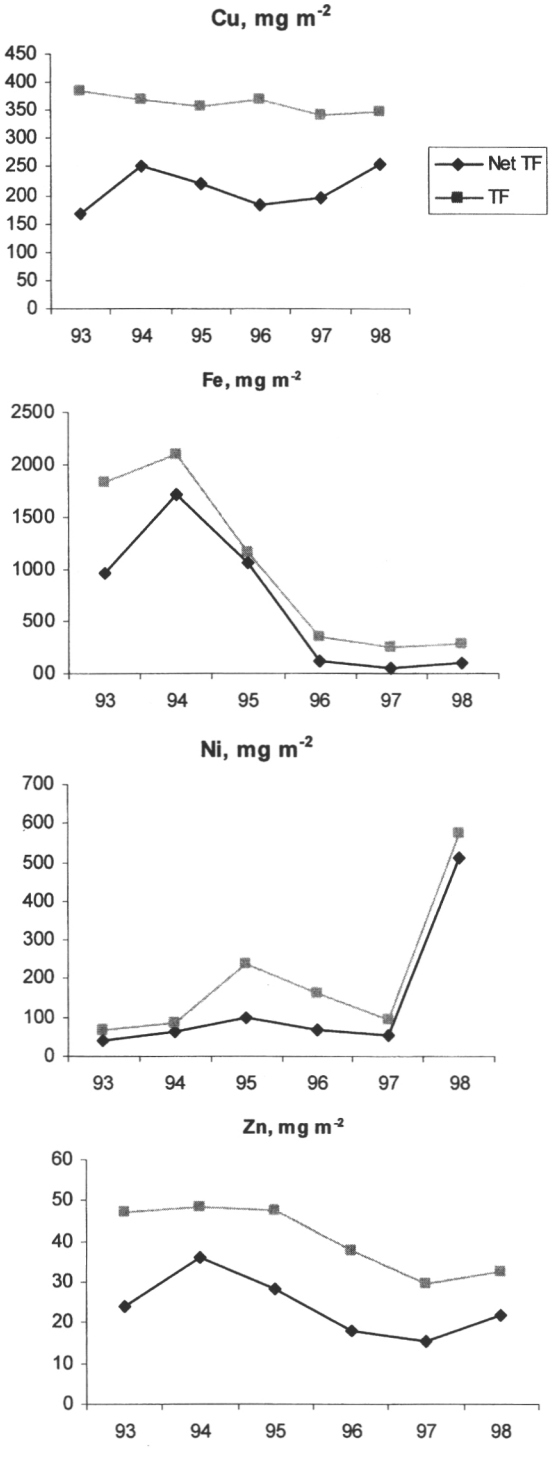

Figure 2. The mean annual throughfall (TF) and net throughfall (Net TF) deposition of Cu, Ni, Zn and Fe during 1993–1998.

TABLE II

Copper, Fe, Ni and Zn concentrations in the current needles in the Scots pine stand in 1998

Metal	Treatment	Significance	Mean mg kg^{-1}	S.E.
Cu	Unwashed		160.6	18.7
	Washed		126.2	16.0
Fe	Unwashed	**	482.5	57.5
	Washed		256.4	33.7
Ni	Unwashed	*	265.8	31.2
	Washed		161.9	19.7
Zn	Unwashed		53.8	2.7
	Washed		47.13	2.3

Statistically significant differences between unwashed and washed samples are indicated by using asterisks: $^{*}p < 0.05$, $^{**}p < 0.01$. $n = 10$. S.E. = standard error of the mean.

complete removal of surface deposition can be achieved by washing the samples with chloroform. Nevertheless, although root uptake is believed to be the main pathway for heavy-metal access in plants in polluted environments, partial penetration through the leaf cuticula has also been reported (e.g. Hagemayer *et al.*, 1986; Tyree *et al.*, 1990; Turunen *et al.*, 1997). In the case of Cu, the foliar spraying of trace amounts is a routine method for curing Cu deficiency in thin-leaved agricultural crops (Marschner, 1995). Majid and Ballard (1990) reported that foliar application of copper sulphate + a surfactant solution on lodgepole pine crowns resulted in a substantial increase in foliar Cu concentrations.

In our study a high proportion of the total concentration of Fe and of Ni were removed by chloroform washing (Table II): needle Fe decreased by 46%, and needle Ni by 34%. In contrast, the difference between the Cu concentration in the washed and unwashed samples was not statistically significant, although the Cu concentration of the washed samples was somewhat lower than that of the unwashed samples. No major differences were found in the concentrations of Zn between the washed and unwashed samples. Therefore, the needle Fe and Ni concentrations in the field are more likely to reflect temporal changes in dry deposition than those of Cu and Zn.

The results of chloroform washing, which indicated that surface contamination of the needles was highest for Fe and Ni, were relatively consistent with the results of the pine seedling experiments.

3.2. Heavy metal concentrations in the field- and greenhouse-grown pine seedlings

The needle Cu concentrations of the seedlings grown in the greenhouse in smelter-polluted soil were much lower than those of the seedlings grown in unpolluted

TABLE III

Copper, Fe, Ni and Zn concentrations in the needles of Scots pine seedlings grown in unpolluted soil close to a Cu–Ni smelter (source of metal from aerial deposit, $n = 3$) and smelter polluted soil in a greenhouse in (source of metal from soil, $n = 4$ for C + 2 and C + 1 needles, $n = 1$ for C needles)

		Needle age class (mg kg^{-1})					
Metal	Source of metal	C + 2	S.E.	C + 1	S.E.	C	S.E.
Cu	From aerial deposit	224.3	10.7	156.3	13.2	124.7	10.4
	From soil	86.4	17.5	82.6	27.4	36.3	
Fe	From aerial deposit	882.9	94.8	522.9	37.6	262.5	16.2
	From soil	69.0	4.8	56.6	13.0	25.3	
Ni	From aerial deposit	1059.8	104.0	679.7	66.9	424.3	38.5
	From soil	6.6	3.0	19.7	0.2	12.2	
Zn	From aerial deposit	106.3	5.5	78.2	3.4	59.0	5.0
	From soil	82.2	5.8	43.5	15.3	19.0	

S.E. = standard error of the mean.

soil close to the smelter (Table III). Furthermore, the difference between these two experiments was considerably greater than the difference between the washed and unwashed samples, apart from Zn. The needle Fe concentrations of the seedlings grown in unpolluted soil near the smelter were about 7-fold higher than those of the seedlings grown in the greenhouse, the Ni concentrations almost 40-fold, and the Cu-concentrations about 2-fold. This suggests that the washing treatment removed only a part of the Ni, Fe and Cu derived directly from dry deposition. Consequently, the elements derived from dry deposition may perhaps not be as effectively removed by washing procedures as has been previously suggested by Raitio (1995). In the case of Zn, needle surface contamination made only a minor contribution to the needle total concentrations of the seedlings due, in part, to the relatively small aerial input of Zn at our study site.

Furthermore, as the element concentrations of plants grown indoors are generally higher than those of the same-aged plants grown in the same soil in the field (Marschner, 1995), it is highly unlikely that our results for the polluted soil in the greenhouse would have greatly underestimated the potential uptake of metals by the roots of the pine seedlings. On the other hand, the needle element concentrations of seedlings are usually very different from those of mature trees. In the present study, however, the needle Ni concentrations in the C + 1 needles of the seedlings grown in unpolluted soil in the field (Table III) were surprisingly similar to the corresponding needles of the mature pine trees (Figure 1) growing in the immediate vicinity. This further supports the dominance of deposition over root uptake as a pathway into the foliage in heavily polluted conditions of this sort. In the case of Zn, on the other hand, root uptake appears to be more important than deposition.

4. Conclusions

Our results question the validity of needle chemistry analysis as a tool for diagnosing heavy metal toxicity under heavily polluted conditions. Although, the deposition of Cu, Fe and Ni dominated over root uptake as a pathway of metals into the foliage, only a part of the surface-deposited metals are likely to be washed-off by rainfall events (or artificial washing). Furthermore, the wash-off efficiency of individual metals varies. Consequently, care should be taken when comparing the needle metal concentrations with the toxicity limits, because surface-deposited metals do not directly affect the metabolism of the plant. In the case of Zn, on the other hand, deposition seemed to contribute only a minor part of the total needle concentrations at our study site.

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