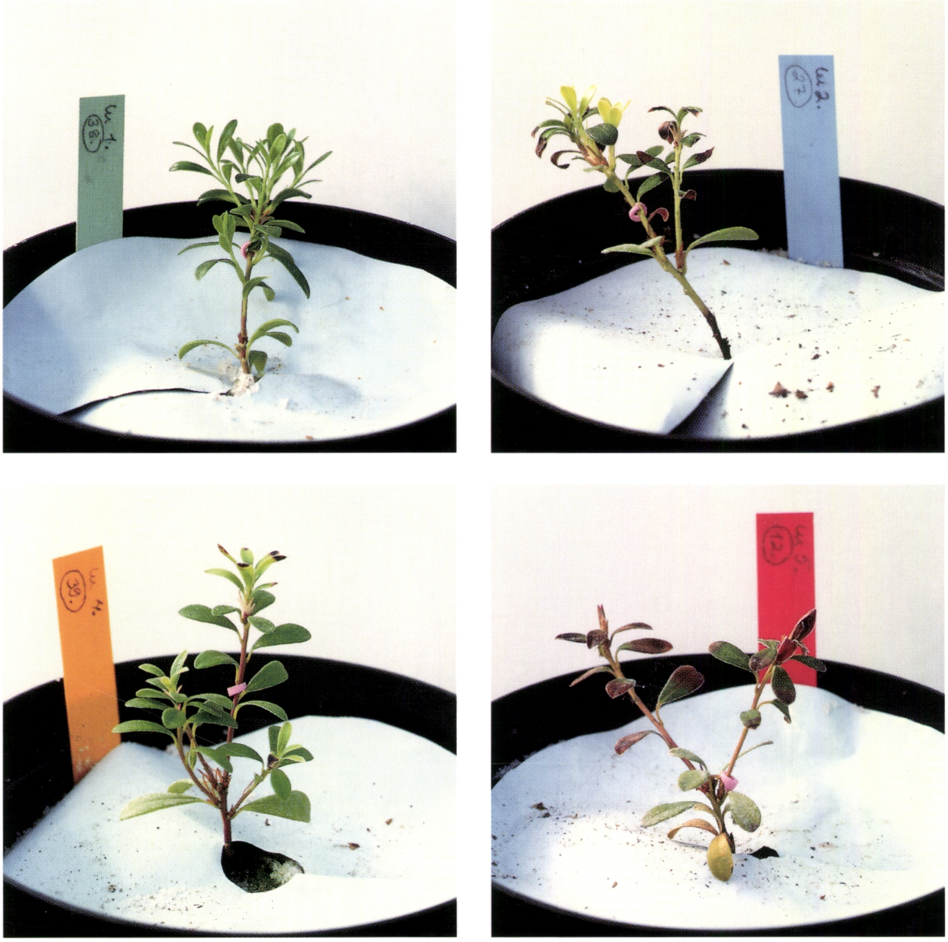
METSÄNTUTKIMUSLAITOKSEN TIEDONANTOJA 905, 2003
FINNISH FOREST RESEARCH INSTITUTE, RESEARCH PAPERS 905, 2003



Response of the understorey vegetation of boreal forests to heavy metal loading

Maija Salemaa

VANTAAN TUTKIMUSKESKUS – VANTAA RESEARCH CENTRE

METLA

METSÄNTUTKIMUSLAITOKSEN TIEDONANTOJA 905, 2003
THE FINNISH FOREST RESEARCH INSTITUTE, RESEARCH PAPERS 905, 2003

Response of the understorey vegetation of boreal forests to heavy metal loading

Maija Salemaa

Vantaa Research Centre,
Finnish Forest Research Institute

Academic dissertation in Terrestrial Plant Ecology
Faculty of Biosciences
University of Helsinki

To be presented, with the permission of the Faculty of Science of the University of Helsinki, for public criticism in the auditorium of Arppeanum (Helsinki University Museum, Snellmaninkatu 3) on February 20th, 2004, at 12 o'clock noon.

Helsinki, 2003

Supervisors:	Docent Heljä-Sisko Helmisaari Vantaa Research Center Finnish Forest Research Institute
	Docent John Derome Rovaniemi Research Station Finnish Forest Research Institute
Reviewers:	Docent Pasi Rautio Department of Biology University of Oulu
	Docent Timo Vuorisalo Department of Biology University of Turku
Opponent	Docent Kari Laine Department of Biology University of Oulu

Publisher: Finnish Forest Research Institute,
Vantaa Research Center, P.O. Box 18, FIN-01301 Vantaa, Finland

Accepted by Jari Hynynen, Research Director, 26 November 2003

Front cover: Cuttings of bearberry (*Arctostaphylos uva-ursi*) exposed to different amounts of Cu (paper VI). Photos: Erkki Oksanen /METLA

ISBN 951-40-1899-0 (printed version)
ISSN 0358-4283
Helsinki 2003
Vammalan Kirjapaino Oy 2003

ISBN 952-10-1611-6 (PDF version)
http://ethesis.helsinki.fi/
Helsinki 2003
Helsingin yliopiston verkkojulkaisut

Contents

List of original publications

The thesis is based on the following publications, which are referred to in the text by Roman numerals. All the publications are reproduced with the publishers' permission.

I Salemaa, M., Vanha-Majamaa, I. & Derome, J. 2001. Understorey vegetation along a heavy-metal pollution gradient in SW Finland. Environmental Pollution 112: 339-350.

II Salemaa, M., Derome, J., Helmisaari, H.-S., Nieminen, T. & Vanha-Majamaa, I. 2004. Element accumulation in boreal bryophytes, lichens and vascular plants exposed to heavy metal and sulfur deposition in Finland. The Science of the Total Environment (in press).

III Salemaa, M. & Uotila, T. 2001. Seed bank composition and seedling survival in forest soil polluted with heavy metals. Basic and Applied Ecology 2: 251-263.

IV Salemaa, M., Vanha-Majamaa, I. & Gardner, P. 1999. Compensatory growth of two clonal dwarf shrubs, *Arctostaphylos uva-ursi* and *Vaccinium uliginosum* in a heavy metal polluted environment. Plant Ecology 141: 79-91.

V Salemaa, M. & Sievänen, R. 2002. The effect of apical dominance on the branching architecture of *Arctostaphylos uva-ursi* in four contrasting environments. Flora 197: 429-442.

VI Salemaa, M. & Monni, S. 2003. Copper resistance of the evergreen dwarf shrub *Arctostaphylos uva-ursi*: an experimental exposure. Environmental Pollution 126: 435-443.

Studies **I** and **II** are based on initial suggestions provided by prof. Eino Mälkönen and Dr. Heljä-Sisko Helmisaari. Dr. John Derome is responsible for the soil and deposition data in all the papers. Field work and data processing were planned together with all co-authors. Maija Salemaa is responsible for data handling and writing of the first draft of papers **I** and **II**, and for the idea, data handling and writing the first draft of papers **III** – **VI**. Dr. Risto Sievänen developed the simulation model for the branching architecture of *A. uva-ursi* (**V**). Anu Blom, M. Sc., performed the electrophoresis analyses of *A. uva-ursi* and *V. uliginosum* and interpreted the zymograms.

Abstract

The detrimental effects of heavy metals on boreal forest vegetation are most evident in the surroundings of metal smelters and mines. The structure of the understorey vegetation and soil seed banks, chemical composition of plant species and branching architecture of two dwarf shrub species (*Arctostaphylos uva-ursi* and *Vaccinium uliginosum*) were studied in Scots pine forests along an 8 km transect running SE from the Cu-Ni smelter at Harjavalta, SW Finland, during 1992 – 2000. The general aim of the study was to compare the responses of bryophytes, lichens and vascular plants to pollution, and to evaluate the possibility of re-establishing the native understorey vegetation of a damaged forest. Clonal diversity and isoenzyme variation of the two dwarf shrub species were analysed in order to investigate whether the populations in the polluted sites near the smelter were genetically differentiated from those growing at further distances away. In addition to the field studies, the effect of Cu on *A. uva-ursi* was investigated in an experimental exposure and the results were compared to earlier experiments carried out using other local dwarf shrub species.

Over 50 years' accumulation of heavy metals (mainly Fe, Cu, Ni, Zn and Pb) and sulphur in the forest ecosystem near the smelter has drastically changed the plant communities. Vegetation was almost absent up to a distance of 0.5 km from the smelter. The total coverage and the number of plant species increased with increasing distance from the smelter. Vascular plants, being capable of restricting the uptake of toxic elements, grew closer to the smelter than lichens and bryophytes, which accumulated larger amounts of heavy metals. In general, vascular plants were more pollution-resistant than lichens, whereas bryophytes were the most sensitive group. A pioneer moss species (*Pohlia nutans*) was an exception to this general pattern, because it accumulated considerably higher amounts of heavy metals than the other species and still survived close to the smelter.

Viable seeds were found in the soil at all the studied distances from the smelter. Although the vegetation was very scanty at 0.5 km, the soil contained germinable seeds of local plant species. At the present time, young seedlings rapidly die in the contaminated soil, which restricts the natural recolonization of the sites.

The clonal diversity of *A. uva-ursi* and *V. uliginosum* was relatively high in both the polluted and clean sites. Further, the genotype frequencies of either species did not differ significantly between the sites, indicating that the populations have not differentiated genetically. It is suggested that the failure of seedling establishment partially prevents the evolution of heavy metal-tolerant ecotypes in the most polluted areas. The surviving clones of dwarf shrubs were tens of years old and may represent the most resistant genotypes of the populations derived from the time before the smelter started operating in the 1940's.

A. uva-ursi and *V. uliginosum* showed high regrowth after autumn clipping of current-year shoots (imitating pollution-induced shoot damage) in a field experiment carried out in polluted and clean sites. In contrast, spring clipping of new shoots was extremely detrimental, especially for the evergreen *A. uva-ursi*, which had smaller carbohydrate and nutrient reserves than the deciduous *V. uliginosum*. In an experimental exposure to Cu, *A. uva-ursi* proved to be more sensitive than the other dwarf shrubs (*Calluna vulgaris*, *Empetrum nigrum*, *V. uliginosum*). However, *A. uva-ursi* showed high plasticity in branching when studied in four habitats with varying pollution, nutrient, light and competition levels. Further, the adult clones avoided heavy metals by extending their roots into the less toxic, deeper soil layers. It is concluded that phenotypic plasticity increases the survival of this species in contaminated sites and enables it to respond to changed resource levels according to the "reserve meristem hypothesis".

The results of this thesis show that heavy metal and sulphur deposition have subjected the understorey vegetation growing in the vicinity of the Harjavalta smelter to a strong selection pressure, which is reflected as a changed species composition and disappearance of sensitive species.

In addition to heavy metals, nutrient imbalances, reduced water-holding capacity of the surface soil and the accumulation of large amounts of undecomposed, dry needle litter also restrict plant recolonisation on the degraded sites. However, the considerable reduction of emissions during the last decade has opened up new possibilities for the restoration of the forest ecosystems. The recovery of the existing vegetation and realisation of the revegetation potential of local seed banks can be promoted by soil mitigation, facilitating the formation of a new organic soil layer and the planting of tolerant plant species.

Keywords: bryophytes, copper, dwarf shrubs, ecotypes, heavy metals, lichens, nickel, resistance, phenotypic plasticity, tolerance

Acknowledgements

This thesis was carried out at the Vantaa Research Centre of the Finnish Forest Research Institute. I express my warmest thanks to my supervisors Dr. Heljä-Sisko Helmisaari (leader of the project “Recovery of boreal forest ecosystem from long-term heavy metal pollution”) and Dr. John Derome for their scientific guidance, support and help throughout the study. Heljä-Sisko introduced me to the field of nutrient fluxes of forest ecosystems, and John to chemistry and processes in forest soil. I am also deeply indebted to the other co-authors Peter Gardner, Satu Lyyra (née Monni), Tiina Nieminen, Risto Sievänen, Taru Uotila and Ilkka Vanha-Majamaa for the innovative and enjoyable cooperation. The other members of the “Harjavalta Group”, Hannu Fritze, Oili Kiikkilä, Hannu Nousiainen, Antti Reinikainen and Christian Uhlig, have contributed to this thesis by helping in different phases of the work. I am grateful to Anu Blom for performing the isoenzyme analyses, Leila Korpela for participating in the vegetation inventories, Jari Perttunen for making the branching simulations, and Arja Ojala and Marja Vieno for advice and practical help in sampling soil seed banks. I would like to thank all of you for the stimulating discussions, valuable comments, encouragement and friendly atmosphere. The opportunity to share your expertise in different fields of ecology has enormously widened my understanding about the functioning of Nature.

I owe my sincere thanks to Prof. emer. Eino Mälkönen for the possibility to start my plant ecological research in the forest fertilisation experiments at Harjavalta. I am very grateful to Prof. Erkki Tomppo and my colleagues in the Tempos Project for the possibility to use the vegetation data of the national forest inventory as reference material in my thesis. I would like to thank Maija Jarva, the former director of the Central Laboratory of Metla, and Kerttu Nyberg, Pirkko Ronkainen, Maija Ruokolainen and Arja Tervahauta for performing the chemical analyses of the plant samples. I also thank Prof. Katri Kärkkäinen, Helena Korpelainen and Tiina Nieminen for reading and commenting on parts of the summary.

I warmly thank the reviewers of the thesis, Dr. Pasi Rautio and Dr. Timo Vuorisalo, for their useful comments and constructive criticism. The encouragement of Rauno Ruuhijärvi, professor emeritus in plant ecology at the University of Helsinki, has been very important when I took my first steps along the path of science. The same positive attitude towards my studies has continued during the period of Professor Heikki Hänninen and Professor Sirkku Manninen, to whom I would like to express my sincere thanks for commenting on the manuscripts and for their speedy handling of the official papers concerning my dissertation.

Many persons have provided practical assistance in the field, laboratory and office. My special thanks go to Sinikka Levula, Teuvo Levula and Pekka Suolahti for helping in the field work. Pia Reponen prepared the plant samples for chemical analyses, Esa Ek, Kaarina Pynnönen and Satu Smolander took care of the plants in the greenhouse, and Sampsa Lommi and Mikko Piirainen identified the lichen and plant samples. Hilkka Granlund and Airi Piira recorded the data, Hannu Nousiainen made the drawings, Markku Tamminen the maps, and Sari Elomaa the layouts of many of the figures. In addition to the persons mentioned above, I want to thank all the staff of the Vantaa Research Center,

especially the library and computer services, for their highly qualified work. John Derome has made linguistic corrections to the summary and all the papers.

I wish to thank the Niemi Foundation for additional financial support. Outokumpu Harjavalta Metals Oy is acknowledged for the possibility to establish the sample plots and to collect samples in the smelter area.

Finally, thanks to my family and friends for their support and care. The "Lunch Society" and the "Friday Swimming Club" have brought me considerable enjoyment over the years when I was in danger of being buried in heavy metal dust.

1. Introduction

1.1. What are heavy metals?

The term "heavy metals" has received widespread usage for metals that are potentially toxic in high doses. The elements having a specific gravity > 4.5 g/cm^3 (sometimes defined as 5.0 or 6.0) are called heavy metals (Streit & Stumm 1993). Except for their specific gravity, heavy metals have no common chemical property or behaviour in biological or ecological systems. Therefore some authors prefer to classify the metallic group of elements into "A (oxygen-seeking), B (nitrogen/sulphur-seeking) and borderline metals", according to their ability to become a part of a chemical complex (Nieboer & Richardson 1980). Many authors use the term "trace metals" as a synonym for heavy metals. However, this term should perhaps be restricted to its original meaning, i.e. for metals required by plants in extremely small amounts, "trace" amounts. In this thesis I use the term "heavy metals" when referring to copper (Cu), nickel (Ni), iron (Fe), manganese (Mn), zinc (Zn), cadmium (Cd) and lead (Pb). Aluminium (Al), which has a specific gravity of 2.7 g/cm^3, is a metal but, according to the definition applied here, is not a heavy metal. Of the heavy metals investigated in this thesis, Mn, Zn, Cu, Fe and Ni are essential micronutrients for plants, but are toxic at higher concentrations and disturb most of the primary physiological processes of plants (Marschner 1995). Cd and Pb are non-essential elements for plants, and may be toxic or lethal even when absorbed in small amounts.

1.2. Sources and forms of heavy metals in soil-plant systems in boreal forests

Natural weathering of metalliferous rocks and anthropogenic sources provide the two major inputs of metals into ecosystems (Ross 1994a). Natural sources of these elements in the atmosphere are volcanic activity, terrestrial dust, vegetation fires, salt spray from the oceans and biogenic sources (Nriagu 1989). Anthropogenic sources in the soil-plant systems include atmospheric deposition originating from a range of industrial activities (metalliferous mining and smelting, alloying plants, petrochemical industry, fertiliser plants, coal power plants, industrial and home furnaces), agricultural amendments and motor traffic (Alloway 1995a). The amount of heavy metals emitted by natural processes into the atmosphere is small compared with the anthropogenic input of these elements (Ross 1994a).

Heavy metals are emitted from traffic and industrial sources into the atmosphere and spread over wide regions of the boreal forest zone. Atmospheric deposition of heavy metals has decreased considerably during the last 20 years in northern Europe (Ukonmaanaho *et al.* 1998), which is reflected as low concentrations in bioindicator bryophytes in background areas (Mäkinen 1994, Rühling & Steinnes 1998, Buse *et al.* 2003, Poikolainen *et al.* 2004). However, there are many industrial areas where the long-term accumulation of heavy metals, often associated with exposure to sulphur dioxide, has damaged northern forests ecosystems. For instance, there are extensive damage areas surrounding the metallurgical industry complexes on the Kola Peninsula, NW Russia (Tikkanen & Niemelä 1995, Chernenkova & Kuperman 1999, Rigina & Kozlov 2000). Heavy metal polluted areas are also to be found in the Nordic countries, primarily close to smelters, mines or steel mills (Folkeson & Andersson-Bringmark 1988, Kubin *et al.* 2000, Tammiranta 2000, Buse *et al.* 2003). In Finland, for instance, it has been estimated that there are about 800 contaminated industrial areas (also including non-metalliferous) and 35 mine tailing areas requiring restoration in the near future (Puolanne *et al.* 1994).

The atmospheric deposition of heavy metals on ecosystems occurs mainly in particulate and aerosol forms (Ross 1994a, Luttermann & Freedman 2000). The area affected by the deposition of heavy metal containing particles is usually much more local than that of gaseous pollutants. Rates of deposition, whether dry or wet, tend to be greatest near the pollution source. However, small particles are transported over longer distances, and have longer atmospheric residence times than larger ones (Hutchinson & Whitby 1977). The following factors may affect the fate of atmospheric metal deposition in the receiving plant - soil systems: 1) particle size, 2) solubility, 3) distance of the receiving system from the metal source, and 4) acidity of rainfall (Ross 1994a).

Heavy metal deposition shows a high affinity for adsorption to organic surfaces. In boreal forests, the canopies of coniferous trees effectively filter pollutant particles from the air (Tyler 1984, Zöttl 1985, Fowler *et al.* 1989). Stand throughfall and plant litter increase the load of heavy metals and sulphur on the forest floor (Heinrichs & Mayer 1980, Derome & Nieminen 1998, Nieminen *et al.* 1999). The humus layer in coniferous forests effectively retains heavy metals through adsorption and complexation with organic matter (Derome 2000b). The characteristics of the receiving soil (especially pH, oxidation-reduction potential, the amount of particulate and soluble organic matter, clay minerals and concentrations of mineral nutrients) influence the bioavailability and toxicity of most metals (Luttermann & Freedman 2000).

The phytotoxicity of heavy metals depends on their bioavailability, which is related to their occurrence in different chemical forms (Alva *et al.* 2000). Heavy metals in soils can exist in water soluble and exchangeable form, associated with insoluble organic matter, and as carbonates, oxides of Fe, Al and Mn, and layer silicates (Alloway 1995b). Generally, increasing acidity tends to increase the mobility and toxicity of heavy metals (Alloway 1995b). Organic and exchangeable forms of heavy metals are the major forms taken up by plant species (Alva *et al.* 2000).

Although the above-ground biomass of the understorey vegetation of boreal forests is small in relation to that of the trees, it plays an important role in regulating the nutrient fluxes (Mälkönen 1974), hydrology and micro climate (Sirén 1955, Päivänen 1966, Tolvanen & Kubin 1990). In contaminated environments, certain plant species can accumulate considerable amounts of heavy metals and protect the soil from erosion and the leaching of heavy metals into the groundwater (Vangronsveld *et al.* 1996). Because many heavy metals are bound on plant surfaces and tissues (Rautio & Huttunen 2003), and form stable complexes with organic matter in the soil (Alloway 1995b), they may still have a long-lasting effect on forest ecosystems after the emissions have ceased (Ross 1994a). Before we can gain a better understanding of heavy metal fluxes in boreal forests, more information is needed about the importance of the understorey vegetation in ecosystem processes.

1.3. Mechanisms of heavy metal uptake in cryptogams and vascular plants

Vascular plants mainly take up elements via their roots from the soil, although the foliar uptake of gases and soluble elements may also be substantial (Marschner 1995). The uptake of heavy metals via aerial plant parts has been demonstrated in fir (Lin *et al.* 1995) and in many crop plants including wheat (Haslett *et al.* 2001). Generally, the thick epidermis and waxy cuticle of the leaves provide external protection against toxic elements in evergreen species. For instance, Monni *et al.* (2001b) did not find ecophysiological responses when heavy metal solutions were applied to the aboveground parts of *Empetrum nigrum*. In industrial areas with high airborne deposition, large amounts of metal-containing dust become attached to the surface structures of the aerial parts, and particles may also become embedded in the cuticular waxes (Rautio *et al.* 1998, Kozlov *et al.* 2000, Rautio & Huttunen 2003).

Heavy metals dissolved in soil water enter vascular plants via mass flow or diffusion into the free space of the root cortex (Marschner 1995). Only a small proportion of the heavy metals accumulated in roots passes through the endodermis and is subsequently distributed as organic complexes into the different plant organs via the xylem and phloem (Clemens *et al.* 2002).

In contrary to vascular plants, cryptogams (bryophytes and lichens) have no real roots, epidermis or cuticle layer, and they absorb water and dissolved elements directly across their surface. Most of the bryophyte and lichen species obtain the majority of their water and nutrients from atmospheric deposition; some species also obtain nutrients from water that has been in contact with the substrate (Bates 1992, Økland *et al.* 1999, Garty 2001). Lichens, which are symbiotic organisms comprising mycobiont and phytobiont partners, have many similarities with bryophytes in their element uptake. The following element fractions occur in both taxa: 1) trapped particulate matter, 2) intercellular soluble elements, 3) extracellular elements bound to the cell wall on charged exchange sites, and 4) intracellular elements (Tyler 1990, Nash 1996, Zechmeister *et al.* 2003). Both bryophytes and lichens (especially the mycobiont partner) have a high ion exchange capacity on their cell walls, and the dead tissues also have an ability to bind ions (Tyler 1989, 1990, Chettri *et al.* 1997).

The toxic effects of heavy metals are manifested in a wide range of plant cellular activities including photosynthesis, respiration, mineral nutrition and membrane structure in all the plant groups (Tyler 1990, Marschner 1995, Garty 2001). Toxic concentrations have been found to cause membrane damage, ion leakage and decreased chlorophyll concentrations in vascular plants (Mocquot *et al.* 1996, Monni *et al.* 2001a, Pätsikkä *et al.* 2001), as well as in bryophytes (Brown & Wells 1990, Guschina & Harwood 2002) and lichens (Chettri *et al.* 1998, Tarhanen *et al.* 1999, Hyvärinen *et al.* 2000). General responses of dwarf shrubs to elevated concentrations of heavy metals are leaf discoloration and decreased growth of the shoots and roots (Monni *et al.* 2000a,b). Differences in root elongation rates in toxic solutions compared to control solutions are commonly used to determine the metal tolerance index of higher plants (Utriainen *et al.* 1997).

1.4. Plant strategies for resisting and tolerating heavy metals

Resistance is a quantitative trait that enables a plant to survive, grow and reproduce in the presence of a particular pollutant (Baker & Walker 1989). Plant populations can become resistant to heavy metals through heritable adaptation (ecotypes), or individual plants can gradually acclimatise to an increasing heavy metal load (phenotypic plasticity) (Antonovics *et al.* 1971, Baker *et al.* 1986, Dickinson *et al.* 1991, Punshon & Dickinson 1997). The broadness of phenotypic plasticity is also genetically controlled (Bradshaw & Hardwick 1989, Thompson 1991).

In some species, all the individuals show some degree of innative (constitutive) tolerance even though they are not exposed to heavy metals (Baker 1987). For instance, cuttings of *Empetrum nigrum* originating from an unpolluted area showed high survival when exposed to Cu and Ni, indicating consitutive tolerance (Monni *et al.* 2000a). However, normally less than 0.1 % of the individuals in natural populations of plant species are resistant (MacNair 1987). If heavy metal concentrations in the soil increase, resistant individuals are favoured as a result of natural selection and their abundance increases. In short-lived plant species such as grasses and herbaceous species, the whole population can change to a resistant one within a few years (MacNair 1987). It has been demonstrated that pioneer bryophytes are also able to undergo rapid evolution in response to a heavy metal load in soil (Jules & Shaw 1994). The evolution of heavy metal resistant ecotypes is often considered to be the best example of evolutionary changes in plant populations (Bradshaw *et al.* 1990).

Heritable changes take place at a slower rate in trees and dwarf shrubs owing to their longer generation times. Ecotypes are much rarer in these plants than among grasses and herbaceous species. The few known examples of metal-tolerant populations of trees occur among pioneer species, e.g. in the *Betula* and *Salix* species (Eltrop *et al.* 1991, Kahle 1993, Kopponen *et al.* 2001). High plasticity in growth and physiological characteristics, which moderates the impact of local stress, is common in clonal dwarf shrubs, e.g. in the family *Ericaceae* (Gimingham 1972, Shevtsova 1998). Phenotypic plasticity may also provide a mechanism that improves the survival of long-lived species in metal-contaminated environments (Dickinson *et al.* 1991, 1992, Turner & Dickinson 1993).

The mechanisms involved in heavy metal resistance are species-specific and are usually divided into avoidance and tolerance mechanisms (Fig. 1) (Baker 1987, Verkleij & Schat 1989). Avoidance is expressed as external protection against toxic elements or as active orientation of the roots to less toxic soil (Tyler *et al.* 1989). The avoidance of heavy metals can also be facilitated by mycorrhizal fungi. Ericoid mycorrhizas of dwarf shrubs have the ability to accumulate large amounts of heavy metals, thereby restricting metal transport to the shoots (Bradley *et al.* 1981, Meharg & Cairney 2000). Heavy metal tolerant strains of mycorrhizal fungi have been found on host plants growing in heavy metal polluted sites (reviewed by Hartley *et al.* 1997).

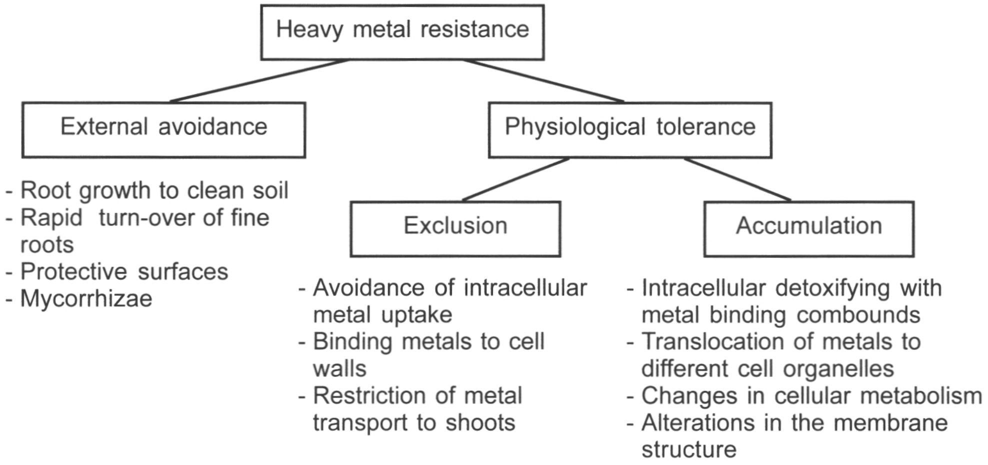


Fig. 1. Summary of the possible mechanisms involved in the resistance to elevated heavy metal concentrations according to Baker (1987), Tyler *et al.* (1989) and Hall (2002).

Although vascular plants have some degree of control over which elements are taken up by their roots, total avoidance of heavy metal uptake is not possible (Kahle 1993). Real tolerance is based on physiological mechanisms that result in the exclusion of heavy metal ions from important metabolic processes or which accumulate metals in detoxified forms (Baker 1987). Vascular plants have many species-specific mechanisms to restrict the cellular uptake of heavy metals and to detoxify them internally (Fig. 1). For instance, living plant cells can detoxify heavy metals by binding them in cell walls, chelating and storing them in vacuoles, or binding them with phytochelatines in the cytoplasm (reviewed by Hall 2002).

Mosses and lichens, which absorb nutrients directly through their surfaces, cannot prevent ions penetrating into their tissues. A number of functional groups in bryophyte and lichen structures are capable of binding metal ions on the cell walls (Tyler 1990, Nash 1996, Onianwa 2001). Intracellular complexing of metals has been found to be based on e.g. organic acids (Sarret *et al.* 1998) or phytochelatins (Pawlik-Skowronska *et al.* 2002) in lichens, and on glutathione (GSH) synthesis (Bruns *et al.* 2001) in bryophytes. Bryophytes have the ability to translocate heavy metals e.g. to vacuoles (Bruns *et al.* 2001).

1.5. Aims of the study

The general aim of this thesis is to compare the responses of bryophytes, lichens and vascular plants to heavy metal loading, and to evaluate the possibility of re-establishing the native understorey vegetation of a damaged forest in the vicinity of the Harjavalta Cu-Ni smelter. A significant decrease in emissions from the smelter, which has been operating since 1945, was achieved in the beginning of the 1990's (Section 2.2). Thus the year 1992, when I started these investigations, provides an interesting reference point to studies on the recovery of the vegetation.

The thesis is based on the field studies and manipulations carried out at different distances from the smelter (**I** - **V**) and on a controlled greenhouse experiment (**VI**). In addition, some unpublished results on the clonal diversity and isoenzyme variation in the populations of two dwarf shrub species in relation to the pollution level, are presented.

This study is based on the hypothesis that the airborne deposition of heavy metals and sulphur have subjected the vegetation near the smelter to a strong selection pressure. The fundamental evolutionary question to which this thesis seeks an answer is: What is the importance of phenotypic plasticity compared to ecotypic differentiation of long-lived dwarf shrubs in survival under a heavy metal load? One practical aim is to evaluate the applicability of local plant species in the phytostabilisation of polluted soil and as bioindicators in biomonitoring studies.

The specific aims of the thesis are

- to evaluate the sensitivity of bryophyte, lichen and vascular plant species to heavy metals according to their occurrence at different distances from the smelter and accumulation pattern of toxic elements (**I**, **II**)
- to study how the species composition of the understorey vegetation and soil seed banks change along a heavy metal and sulphur gradient near the Harjavalta smelter (**I**, **III**)
- to study seedling recruitment from the forest soil in order to determine the revegetation potential of the seed bank (**III**)
- to study the clonal diversity and isoenzyme variation of *A. uva-ursi* and *V. uliginosum* at different distances from the smelter
- to study the importance of compensatory growth (**IV**) and activation of the bud reserve (**V**) of clonal dwarf shrubs as a resistance mechanism to heavy metals
- to determine the sensitivity of *A. uva-ursi* to Cu in relation to that of the other dwarf shrub species in greenhouse conditions (**VI**)

2. Study areas and pollutant emissions

2.1. Experimental sites along the Harjavalta pollution gradient

The study area is situated near the Cu – Ni smelter at Harjavalta (61°19' N, 22°09'E), SW Finland. The Finnish Forest Research Institute established a number of experimental plots in Scots pine stands for liming and fertilization (Mälkönen *et al.* 1999, Derome 2000a), nutrient flux (Helmisaari *et al.* 1995) and restoration (Kiikkilä 2002) studies along a 8 km transect running SE from the smelter (Fig. 2). A large part of the field data of this thesis has been collected from these plots or from their immediate vicinity. The experimental stands along the transect (0.5, 2, 4 and 8 km) were 40 – 55 years old (**I** – **V**), whereas the two extra study stands (1 and 3 km) were 51 – 67 years old (**I**, **III**). All the stands, except one peatland site (**IV**), were growing on dry, nutrient-poor sandy soils of the *Calluna* site type (Cajander 1909). A detailed description of the stand characteristics is given in **I** and **II**.

2.2. Emissions from the Harjavalta smelter

The Harjavalta Metals smelter complex is one of the largest point sources of heavy metal emissions in Finland (Melanen *et al.* 1999, Tammiranta 2000, Jussila 2003). The copper smelter has been operating since 1945, and the nickel smelter since 1960. The concentrated ores contain sulphur, heavy metals and arsenic. Before the sulphuric acid plant was built in 1947, all the SO_2 produced during the smelting process (annually about 30 000 t) was emitted into the atmosphere, causing severe damage to the surrounding coniferous forests (Helmisaari 2000). Since the beginning of the 1990's, the emissions have been considerably reduced by the installation of new filters in 1990, 1991 and 1994. The temporal change in the emissions during 1985 – 1995 is presented in **II** (**II**: Fig. 2).

2.3. Ecological research in the Harjavalta area

Many investigations focusing on different aspects of forest ecosystem processes have been carried out along the Harjavalta transect during the last decade. In addition to the fertilisation experiments (Derome & Saarsalmi 1999, Mälkönen *et al.* 1999, Derome 2000a), studies on nutrient fluxes (Nieminen & Helmisaari 1996, Derome & Nieminen 1998), distribution of radiocaesium in soil and vegetation (Outola *et al.* 2003), soil microbiology (Fritze *et al.* 1989, Fritze *et al.* 1996, Kiikkilä *et al.* 2000) and ecophysiology of dwarf shrubs (Monni *et al.* 2000b, 2001a,b, Uhlig *et al.* 2001) have generated a considerable amount of information about the factors affecting the understorey vegetation.

The accumulation of Cu and Ni and other heavy metals in the soil has resulted in a severe deficit of plant-available Ca, Mg and K in the organic layer caused by the inhibition of mineralisation and the displacement of these base cations from exchange sites (Derome & Lindroos 1998). In addition to toxic element concentrations in the soil, nutrient

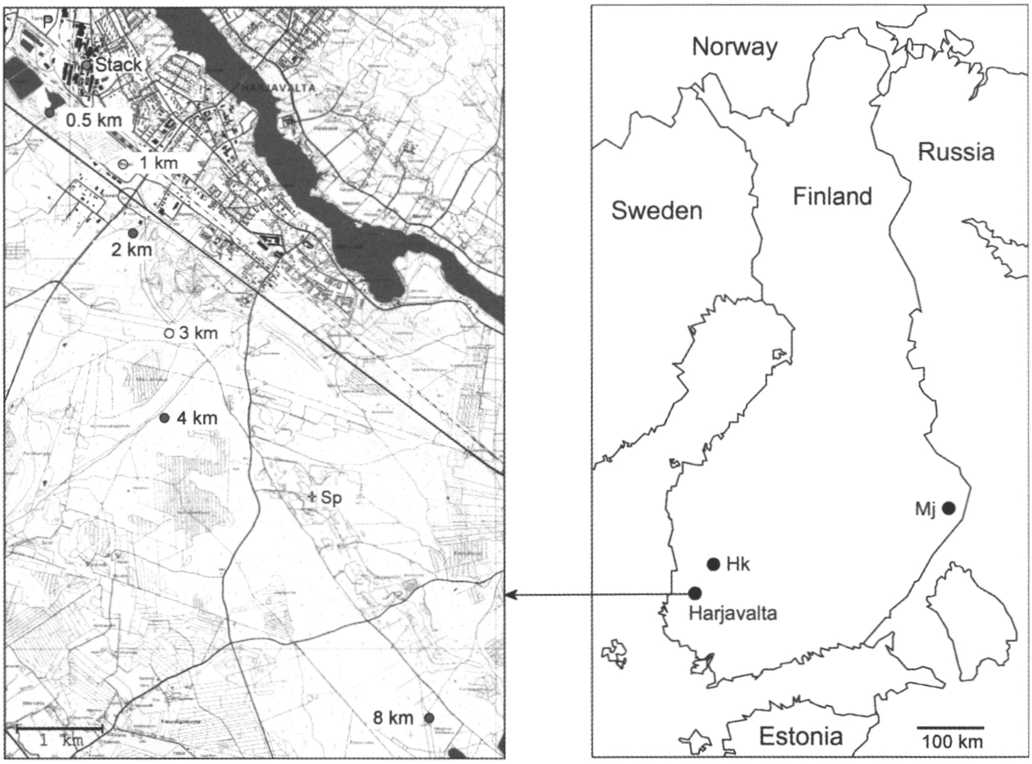


Fig. 2. Location of the three study areas. The town of Harjavalta is situated about 30 km from the coast of the Gulf of Bothnia in western Finland. The study sites at distances of 0.5, 1, 2, 3, 4 and 8 km from the Harjavalta Cu-Ni smelter (see stack on the map) have been marked on the detailed map (grey points: forest health fertilisation experiments of the Finnish Forest Research Institute). The sandpit and polluted habitats (**V**) have been marked by Sp and P, respectively. Hämeenkangas (Hk) and Mekrijärvi (Mj) represent background areas (**II**). Pohjakartta © Maanmittauslaitos lupanumero 6/MYY/03

imbalances and a decreased water-holding capacity (Derome & Nieminen 1998) have increased the stress encountered by the plants growing near the smelter.

Bioindicator studies were started in the middle of the 1970's (Laaksovirta & Silvola 1975, Hynninen 1986) and they have continued up until today (Jussila 2003). They show that, despite the reductions in emissions, the effects of the Harjavalta smelters extend to a distance of at least 10 km from the emission source. Kiikkilä (2003) has given a recent overview of all the ecological studies dealing with a range of organisms (e.g. birds, insects and endophytic fungi) carried out in the Harjavalta area.

2.4. Background areas

One study site situated in Hämeenkangas (61°45N, 22°40'E) and another in Mekrijärvi (62°47'N, 30°58'E) (Fig. 2) were selected as reference areas for the plant chemical composition (**II**). The stand in Hämeenkangas (age 44 years) was an untreated control of a fertilization experiment and represented the same forest site type (*Calluna* type) as the study stands along the Harjavalta transect. The Mekrijärvi stand (age 45 years), in contrast, represented a slightly more fertile site type (*Vaccinium* type) than the other stands. Its selection was justified because the low N, S and heavy metal deposition in the area in question made it a suitable reference level especially for bryophytes and lichens. Furthermore, it offered a wide range of data dealing with nutrient fluxes in forest ecosystems (Helmisaari 1995). The background areas had no local emission sources.

3. Materials and methods

An overview of the study sites, number of sample plots, time of sampling and the studied species and variables are given in Table 1. Detailed descriptions of the methods are given in the original articles.

3.1. Vegetation surveys (I, II) and seed bank composition (III)

The abundance of the understorey vegetation was studied using the point quadrat method at six locations (0.5, 1, 2, 3, 4 and 8 km) along the study transect in August 1993. The vegetation analysis was carried out on three sample plots (30 × 30 m) at each distance, and 16 vegetation quadrats (1 m^2) were studied on each plot. The total data consists of 288 1 m^2 vegetation quadrats. The vegetation survey of the National Forest Inventory, carried out in 1995 (Reinikainen *et al.* 2000), was used as a reference. The same method was applied in 1992, when the vegetation survey was carried out on the smaller number of sample plots selected for the studies on the chemical composition of plants (**II**).

In addition to the sites mentioned above (0.5, 1, 2, 3, 4 and 8 km), the soil samples for the seed bank analyses were collected from the fertilised sites located at 0.5, 4 and 8 km from the smelter in May 1994 (**III**). Five soil samples were taken from the buffer zones surrounding each of the three replicate plots. The soil samples were taken from the organic layer (including the litter layer) and the upper part of the mineral soil layer at a depth of 5 – 10 cm using a 9.5 × 9.5 cm metal sampler. The germination experiment with a total of 135 soil samples, spread out on trays (18.5 × 21.5 cm), was carried out in a greenhouse. The growth substrate consisted of mixed quartz sand and peat. The emerged seedlings were counted once a week.

3.2. Chemical analyses of plants (II, IV, VI), soil (I – V) and precipitation (II, IV)

Species-specific composite samples were taken in 1992 (additional samples in 1993 and 1994) for the chemical analysis of understorey bryophytes, lichens and vascular plant species growing at four distances from the smelter (0.5, 2, 4 and 8 km) and at two background sites (**II**). The plant material was not washed before chemical analysis and thus included the surface accumulation of elements. Total element concentrations (P, K, Mg, Ca, Fe, Zn, Mn, Cu, Ni, Cd, Pb and Al) were determined by dry digestion (HNO_3/ H_2O_2) and analysed by inductively coupled plasma atomic emission spectrometer (ICP-AES). Total sulphur and nitrogen concentrations were determined on the homogenized samples on LECO S-132 and LECO CHN-600 analysers. These standard methods were also applied when determining the Cu (**IV**, **VI**) and Ni (**IV**) concentrations of the shoots and roots of the dwarf shrub species in the field and greenhouse material. All the analyses were performed in the Central Laboratory of the Finnish Forest Research Institute.

Samples were taken from the organic (**I** – **IV**) and mineral soil (**II**: 0 – 5 cm, **V**: 0 – 10 cm) layers along the Harjavalta transect and in the background areas in 1992 – 1993

Table 1. General description of the study sites, number of sample plots, time of the surveys and sample collection, studied species and measured variables.

Study	Sites (distance from the smelter)	Number of plots	Time	Species	Variables
I	Harjavalta transect	6 × 3	August	Bryophytes	Species abundances (point frequency, %)
	0.5, 1, 2, 3, 4 and 8 km		1993	Lichens	Chemical composition of organic layer
				Vascular plants	
	Reference:				
	National forest inventory	12	1995	”	Species abundances (visual cover, %)
II	Harjavalta transect	4	August 1992	Bryophytes	Element concentrations in species,
	0.5, 2, 4 and 8 km		(1993, 1994)	Lichens	soil and precipitation
				Vascular plants	Species abundances (point frequency, %)
	Reference:				
	Hämeenkangas and Mekrijärvi	2	Aug. – Sept. 1992	”	”
III	Harjavalta transect	6 × 3	May 1994	Vascular plants	Germinated seeds and seedling survival in
	0.5, 1, 2, 3, 4 and 8 km				soil samples (greenhouse experiment)
	Fertilised: 0.5, 4 and 8 km	3 × 3			
IV	Field manipulation:				
	2 and 8 km forest		1994 – 1995	*Arctostaphylos uva-ursi*	Compensatory growth after shoot clipping
	0.5 and 4 km forest, 5 km bog		”	*Vaccinium uliginosum*	”
			(1994, 1997)		(Clonal diversity and isoenzyme variation)
V	Field study in four habitas:				
	Polluted and restauration 0.5 km, sand pit 6 km and forest 8 km		2000	*Arctostaphylos uva-ursi*	Branching architecture
VI	Greenhouse experiment		1999	*Arctostaphylos uva-ursi*	Survival, growth and Cu accumulation
	References:				
	Monni *et al.* (2000a)		1996	*Calluna vulgaris*	
	Monni *et al.* (2000b)		1996	*Empetrum nigrum*	
	Salemaa *et al.* (2003)		2000	*Vaccinium uliginosum*	

(Hämeenkangas in 1990). Some extra soil samples were taken at a later date (**V**). Total N was determined on a CHN analyser. Exchangeable Ca, Mg, K, Cu, Ni, Zn, Fe, Mn, Cd, Pb and extractable P and S at the Harjavalta plots were determined by extraction with 1 M ammonium acetate (pH 4.65) + 1% EDTA, followed by analysis by ICP-AES (**I**, **II**, **IV**). Ammonium acetate extraction has been extensively used for determining the plant-available fraction of elements in soils, and EDTA increases the efficiency of heavy metal (especially Cu and Fe) extraction (Lakanen & Erviö 1971). The extractant used for the samples from Hämeenkangas and Mekrijärvi did not include EDTA. The extractant used in **III** and **V** was barium chloride (0.1 M) + EDTA (Derome 2000b). The element concentrations in the organic layer were expressed on an organic matter basis in order to reduce the variation arising from the inclusion of varying amounts of mineral soil in the organic layer samples. Soil analyses were performed in the Central Laboratory of the Finnish Forest Research Institute and in the laboratory of the Joensuu Research Centre.

Bulk deposition was collected in open areas close to the study stands using five (Mekrijärvi: 20) rainfall collectors (d = 20 cm) during the snowfree period or two snow collectors (d = 36 cm) during the winter. Stand throughfall was collected using 20 rainfall collectors located systematically inside the plots during the snowfree period and six (Mekrijärvi: 10) snow collectors (**II**, **IV**). See Derome and Nieminen (1998) for details of the chemical analysis of precipitation.

3.3. Compensatory growth (IV) and branching pattern (V) of dwarf shrubs in the field

Shoot clipping manipulation was performed on *A. uva-ursi* in two Scots pine stands at 2 km and 8 km and on *V. uliginosum* at 0.5 km and 4 km distances from the smelter, as well as in a drained peatland stand at 5 km (**IV**). The two species did not occur in sufficient numbers at the same distances. A total of 30 clones per species were randomly selected at each site. Clonal diversity and isoenzyme variation of these clones were also studied (Section 3.4). The clones were divided into three groups, ten replicates in each: unclipped controls, clones clipped in autumn (1994), and clones clipped soon after bud break in spring (1995). Shoot clipping was restricted to three randomly selected main branches on each experimental clone. All the current-year shoots of the three main branches were removed and stored for further measurements. The branches were harvested for biomass measurements at the end of July 1995.

Horizontal spreading and axillary bud activation of *A. uva-ursi* was studied in four habitats in the vicinity of the smelter in September 2000: 1) restoration experiment (0.5 km to the S of the smelter), 2) treeless polluted area (0.5 km W, Torttila), 3) sand pit (6 km SE) and 4) pine forest (8 km SE) (**V**) (Fig. 2). In the restoration experiment, ten six-year-old plants were removed together with the roots. Five separate established clones were randomly selected in the other habitats. Altogether 1 – 3 branches with the six youngest annually grown shoots (formed during 1995 – 2000) were taken from the periphery of each clone. Thus all the sample branches had one parent shoot, formed in 1995, from which all the daughter shoots had developed. The following variables, used later in a simulation model, were recorded: the length, location and branching angle of the shoots; the number of activated and inactive buds; the age, hierarchy and terminal types of the shoots.

3.4. Electrophoretic analysis of isoenzyme variation in the dwarf shrub populations

Isoenzyme variation in the populations of *V. uliginosum* (Scots pine stands at 0.5 km and 4 km, peatland site at 5 km) and *A. uva-ursi* (Scots pine stands at 2 km and 8 km) were studied by means of protein electrophoresis. Leaf samples of *V. uliginosum* (40 samples per site) were collected in June 1994. Current-year leaves of *A. uva-ursi* were collected from 20 plants at 2 km and from 25 plants at 8 km in June 1997. The material consisted of samples from all the experimental clones in the shoot clipping experiment (**IV**) and some additional clones. Selection of the plants was carried out at a 5 m minimum distance between adjacent plants. The area of the studied populations ranged from 2 500 to 3 000 m^2.

A total of 10 enzyme loci for *A. uva-ursi* and 12 partly different enzyme loci for *V. uliginosum* (p. 27) were assayed by the standard starch gel electrophoresis technique as described by Mattila *et al.* (1994). The analyses were carried out at the Foundation for Forest Tree Breeding. Interpretation of the tetraploid zymograms (electrophoretic banding patterns) was performed according to Krebs & Hancock (1989). In addition to the determination of genotypes, the following genetic parameters were calculated at the population level: mean number of alleles per locus, proportion of polymorphic loci and observed mean heterozygosity (H_o) over all and over polymorphic loci. Genotype frequencies between the sites were compared using the Chi-square test.

3.5. Experimental exposure of *Arctostaphylos uva-ursi* and other dwarf shrubs to Cu (VI)

Rooted cuttings of *A. uva-ursi* originating from a distance of 2 km from the smelter were grown in quartz sand in pots and exposed to five levels of Cu (1, 10, 22, 46 and 100 mg/l as $CuCl_2$) in a greenhouse (**VI**). The Cu was added to a nutrient solution (Stribley & Read 1976) given to the plants once or twice a week. The total amount of nutrient solution given was 12 × 50 ml/pot. The duration of the experiments was 8 weeks. The Cu resistance was quantified by means of the following variables: 1) plant survival, 2) biomass production, and 3) Cu accumulation in shoots and roots.

The Cu resistance of *A. uva-ursi* was compared to that of the other dwarf shrub species grown under similar experimental conditions. Earlier experiments using cuttings of *E. nigrum* (Monni *et al.* 2000a) and seedlings of *C. vulgaris* (Monni *et al.* 2000b) differed from the present experiment in that they lasted for only 6 weeks and Cu was given in the form of $CuSO_4$. On the other hand, the experiment using cuttings of *V. uliginosum* lasted for 8 weeks, but the amount of the solution (with $CuCl_2$) applied was lower, 9 × 50 ml (Salemaa *et al.* 2003).

3.6. Statistical analysis

The vegetation survey data were ordinated using global non-metric multi-dimensional scaling (DECODA 2.04 software) (**I**). Kruskal-Wallis non-parametric analysis of variance was used in comparing the species abundances in the Harjavalta data to the national forest inventory reference (**I**), and the number of germinated seeds and mortality rate of seedlings in the soil samples from fertilised and untreated plots (**III**). Differences in the element concentrations between the life forms and species were tested by Mann-Whitney's U tests (**II**). Non-parametric statistics were used because the sample number was low, and it was not possible to test the normality of the distributions. Actuarial time tables and Wilcoxon statistic were used for analysing the survival probability of the *Calluna vulgaris* seedlings over time (**III**).

Linear and non-linear regressions were used when studying the abundance of the plants vs. chemical composition of their tissues (**II**), and the number of germinated seeds and mortality rate of seedlings (**III**) as a function of the distance from the smelter. Regression models were also applied when studying the effect of soil chemistry (fertilisation) on seedling mortality (**III**), the age dependence of the bud activation in branches of *A. uva-ursi* (**V**), and the response of *A. uva-ursi* cuttings to the applied Cu levels (**VI**).

Nested ANOVA, in which the sample was nested under the plot and the plot under the distance, was used when analysing the numbers of germinated seeds and mortality rate along the Harjavalta transect (**III**). The effects of shoot-clipping and habitat and their interaction on the growth responses of *V. uliginosum* and *A. uva-ursi* were studied using two-factor ANOVA, followed by pairwise contrasts (only the results of contrasts are given in **IV**: Figs. 2 – 3). Three-factor ANOVA was used when studying the effects of habitat, terminal type and age of the shoots on the number/proportion of activated buds of *A. uva-ursi* (**V**). Tukey's tests (**V**) and t-tests (**IV**, **V**) were used in testing pairwise differences between different factors in ANOVAs.

Two-by-two contingency tables and Chi-square tests were used in testing frequency based data in the branching morphology of *A. uva-ursi* (**V**). The branching response to different environmental conditions was simulated by means of an L-system architectural model (Prusinkiewicz & Lindenmayer 1990) based on the use of an annual time step and the demographic and morphological parameters measured in each habitat (**V**).

4. Results

4.1. Heavy metal accumulation and the distribution of species (I, II)

Heavy metal and sulphur deposition during the last 50 years has drastically affected the occurrence of plant species, their relative abundances (**I**) and chemical composition (**II**) along the Harjavalta transect. The total number of plant species decreased from 30 at 8 km to 8 at 0.5 km from the smelter (**I**). The overall coverage of the vegetation also decreased towards the smelter (Fig. 3). Elevated N, S and heavy metal concentrations (Cu and Ni distributions in Fig. 4) were found in all life forms near the pollution source. Four damage zones were distinguished along the pollution transect on the basis of the vegetation composition and the element concentrations of the organic soil layer (**I**). An overview of the species occurrence (**I**) and the highest Cu and Ni concentrations in their tissues (**II**) in these four areas is given below:

Area of severe damage (0.5 – 1 km):

The understorey vegetation was almost totally absent up to a distance of 0.5 km from the smelter. Except for pioneer species, the bryophytes and lichens typical of mature forests were missing. Only a few patches of *E. nigrum, V. uliginosum* and *Carex globularis* were present. A few seedlings of *Pinus sylvestris* and *Betula pubescens* were growing in the most polluted area. Some shoots of *Vaccinium myrtillus*, *V. vitis-idaea* and *Ledum palustre* were found at 1 km from the smelter. *E. nigrum* and *C. globularis* accumulated higher concentrations of Cu (184 – 254 µg/g) and Ni (51 – 17 µg/g) in the current-year growth than *V. uliginosum* (Cu 38 µg/g, Ni 42 µg/g) (Fig. 4e,f). However, a pioneer moss *Pohlia nutans* accumulated considerably higher amounts of Cu (1 397 µg/g) and Ni (334 µg/g) than the vascular species (Fig. 4c,d).

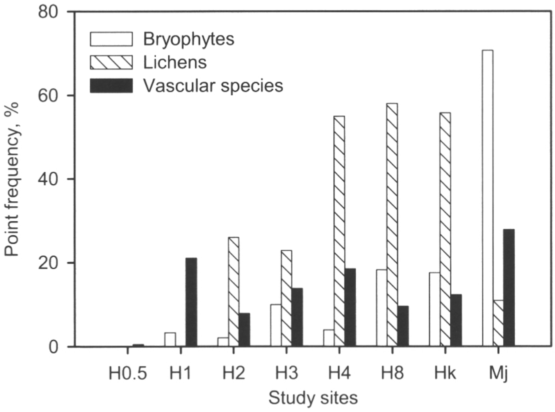


Fig. 3. The total abundances of the bryophytes, lichens and vascular plant species along the Harjavalta transect (H0.5 – H8: 0.5 – 8 km from the smelter, mean abundance of three plots per each distance in 1993), Hk = Hämeenkangas (1992) and Mj = Mekrijärvi (1992) (I, II).

Area of moderate damage (2 – 3 km):

A. uva-ursi and *E. nigrum* began to increase, but *C. vulgaris*, which is the characteristic species of *Calluna* type forests, was still very scarce. The first specimens of reindeer lichens (*Cladina* spp.*)* were recorded, but *Cetraria islandica* was more abundant than the reindeer lichens. Cup lichens (*Cladonia* spp.) and *P. nutans* reached their highest abundances halfway (3 km) along the transect (**I**). The Cu (18 – 32 µg/g) and Ni (8 – 12 µg/g) concentrations of dwarf shrubs (current-year shoots) were clearly lower than those of *C. islandica* (Cu 108 µg/g, Ni 26 µg/g) and *P. nutans* (Cu 872 µg/g, Ni 209 µg/g) (Fig 4).

Area of slight damage (4 km):

The floristic composition at 4 km resembled that of normal *Calluna* type forests. The bryophyte layer was still poorly developed (Fig. 3). Reindeer lichens occurred in normal abundances (**I**), but their heavy metal concentrations were considerably higher (Cu 160 – 260 µg/g, Ni 30 – 40 µg/g) than the background values in Mekrijärvi and Hämeenkangas (Cu and Ni 2 – 6 µg/g) (**II**).

Area of minimum disturbance (8 km):

The total coverage of the vegetation approached almost 90 % and all the species groups typical to mature forests were present at a distance of 8 km (Fig. 3). However, the

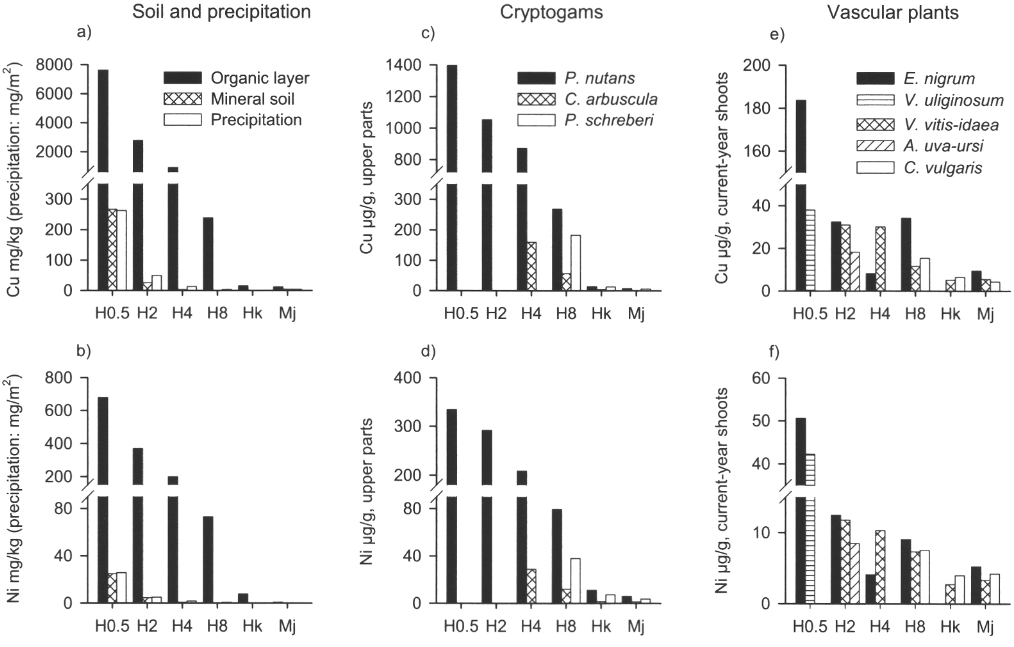


Fig 4. Cu and Ni concentrations in (a, b) the organic and mineral (0 – 5 cm) soil layers (mg/kg) and bulk precipitation (mg/m²), (c, d) selected cryptogam species (µg/g) and (e, f) the current-year shoots of dwarf shrubs (µg/g) at different distances (km) from the Harjavalta Cu-Ni smelter (H0.5, H2, H4 and H8) and in two background areas Hämeenkangas (Hk) and Mekrijärvi (Mj).

abundance of *C. vulgaris* was still lower than in the background areas (**I**). The abundance of *Pleurozium schreberi* was also lower than normal, and the Cu (180 µg/g) and Ni (38 µg/g) concentrations in its younger parts were considerably higher than those of the background areas (Cu 6 – 13 µg/g, Ni 4 – 7 µg/g). In general, when all the species grew on the same plot, heavy metal concentrations (except Mn) tended to increase in the order: vascular plants < *C. islandica* < *Cladina* lichens < bryophytes of mature forests < *P. nutans* (**II**: Fig. 3). The accumulation of Cu in the different species (excluding *P. nutans*) correlated positively with the closest distance to the smelter at which the species occurred (**II**: Fig. 4).

4.2. Seed bank composition in relation to the existing vegetation (I, III)

Viable seeds were found at all the studied distances from the smelter (**III**: Table 3). Altogether 1 300 seedlings germinated in the total of 135 seed bank samples. The emerged seedlings represented 15 taxa, of which 6 species were grasses and sedges, 4 dwarf shrubs, 3 trees and 2 herbs (Table 2). The most numerous species were *B. pubescens* and *C. vulgaris*. The average seedling density varied from 250 to 4 750 per m^2 at the different distances.

Table 2. The frequency (%) of the sample plots in which the species emerged from the seed bank (**III**: Table 3) versus aboveground occurrence of vascular plants (**I**: Table 2). Data from the untreated plots (n = 18) along the 8 km transect.

Species	Seed bank	Aboveground
Andromeda polifolia	-	5.6
Agrostis capillaris	5.6	-
Arctostaphylos uva-ursi	-	33.3
Betula pendula	-	5.6
Betula pubescens	77.8	27.8
Calluna vulgaris	72.2	44.4
Carex canescens	5.6	-
Carex ericetorum	5.6	-
Carex globularis	16.7	44.4
Deschampsia flexuosa	5.6	16.7
Empetrum nigrum	5.6	88.9
Epilobium sp.	-	38.9
Festuca ovina	5.6	-
Ledum palustre	-	22.2
Picea abies	-	33.3
Pinus sylvestris	27.8	88.9
Populus tremula	16.7	5.6
Rumex acetosella	11.1	-
Salix aurita	-	5.6
Vaccinium myrtillus	-	27.8
Vaccinium uliginosum	22.2	38.9
Vaccinium vitis-idaea	27.8	83.3

The seed bank species were rather well represented in the existing vegetation. Of the 17 vascular plant species growing on the unfertilised plots, 10 were found in the seed banks (Table 2). The percentage similarity between the species in the existing vegetation and the seed banks varied from 18 % to 67 % at different distances from the smelter. The total similarity in the data for the whole transect was about 70 % in the unfertilised, and 60 % in the fertilised plots. The number of *C. vulgaris* seedlings increased with increasing distance from the smelter, but no such trend was found for the other species (**III**: Fig. 1b).

4.3. Seedling survival in forest soil polluted with heavy metals (III)

Although germinable seeds were found even in the most polluted study area, the majority of the seedlings died at an age of a few weeks. In the life table analysis, the survival probability of *C. vulgaris* seedlings was the higher, the further away from the smelter the soil was collected (**III**: Fig. 2). The survival probability was the lowest in the soil from distances of 0.5 – 2 km (0 – 30 %), increased to over 60 % at distances of 3 – 4 km, and to 80 % at 8 km. Nutrient addition and liming increased the survival probability of the seedling slightly at 0.5 km and 4 km, but the effect was not statistically significant.

4.4. Genetic structure of the dwarf shrub populations

Isoenzyme analysis verified that the studied populations of *A. uva-ursi* and *V. uliginosum* were autotetraploid and multiclonal. An autotetraploid individual has four different alleles per locus, and its genotype can be marked e.g. as 1112 according to the electrophoretic banding pattern. The majority of the sampled plants represented different genotypes (Table 3a). *A. uva-ursi* had 4 polymorphic loci out of 10 studied, and *V. uliginosum* 5 out of 12 (Table 3a). The average number of alleles per locus was 2 and 1.4, respectively. The percentage of observed heterozygous individuals over all loci was about 21 % in the populations of *A. uva-ursi*, and 30 % in the populations of *V. uliginosum*. The corresponding values over polymorphic loci were 51 % and 73 %, respectively.

The clonal diversity of A. *uva-ursi* was the highest at the distance of 8 km and that of *V. uliginosum* at 0.5 km (Table 3a). Both species had some samples with the same isoenzyme pattern but, owing to the long distance between the samples (> 20 m), it is more realistic to assume that they represent different genotypes. Only in the peatland population (5 km) of *V. uliginosum* were there some (n = 5) large clones from which branches may have been selected twice.

The genotype frequencies of *A. uva-ursi* did not differ between the sites (Table 3b). In *V. uliginosum* there were differences only in two loci. The peatland population differed from both forest populations (0.5 km and 4 km) for diaphorase (DIA) ($\chi^2 = 12.84$, $P = 0.05$, df = 6), and the forest population at 4 km differed from those at forest 0.5 km and peatland 5 km for phosphoglucose isomerase (PGI2) ($\chi^2 = 14.72$, $P = 0.06$, df = 8).

Table 3. a) Number of genotypes, percentage of loci polymorphic (0.95 criterion) and mean observed heterozygosity over all (H_o1) and over polymorphic (H_o2) loci in the populations of *A. uva-ursi* and V. *uliginosum*.
b) Site-specific genotype frequencies of *A. uva-ursi* for 10 enzyme loci and of *V. uliginosum* for 12 enzyme loci. Both species are autotetraploid (4X). Zymogram pattern is presented.

a)

	A. uva-ursi		V. *uliginosum*		
Site	F2	F8	F0.5	F4	P5
No of samples	20	25	40	40	40
No of genot.	16	24	39	36	31
Polym. loci %	40	40	42	42	42
H_o1	0.21	0.21	0.32	0.31	0.29
H_o2	0.50	0.53	0.76	0.73	0.71

b)

Genotype				Genotype				
6PG1	1111	0.05	0.00	6PG1	1111	1.00	1.00	1.00
	2222	0.95	1.00	6PG2	1111	0.10	0.16	0.15
6PG2	2222	1.00	1.00		1112	0.55	0.37	0.27
FE1	1111	0.95	1.00		1122	0.15	0.29	0.30
	2222	0.05	0.00		1222	0.15	0.16	0.23
MDH	1111	0.05	0.00		2222	0.05	0.02	0.05
	2222	0.90	0.64	MDH1	1111	1.00	1.00	1.00
	2223	0.05	0.24	MDH2	1111	1.00	1.00	1.00
	2233	0.00	0.08	MDH3	1111	0.08	0.00	0.00
ADH	1111	0.05	0.00		1112	0.33	0.22	0.35
	1112	0.00	0.04		1122	0.47	0.70	0.55
	1122	0.05	0.04		1222	0.12	0.08	0.10
	1222	0.25	0.31	ADH	1111	1.00	1.00	1.00
	2222	0.65	0.61	DIA	1112	0.00	0.03	0.00
MNR	1111	0.05	0.00		1122	0.08	0.13	0.13
	2222	0.95	1.00		1222	0.50	0.42	0.17
PGM	1111	0.00	0.20		2222	0.42	0.42	0.70
	1112	0.20	0.16	GOT1	1111	1.00	1.00	1.00
	1122	0.35	0.12	GOT2	1111	0.05	0.10	0.03
	1123	0.10	0.00		1112	0.22	0.25	0.27
	1133	0.00	0.04		1122	0.30	0.21	0.13
	1222	0.20	0.28		1222	0.20	0.31	0.30
	1223	0.05	0.04		2222	0.23	0.13	0.27
	2222	0.10	0.16	PGI2	1111	0.18	0.48	0.22
PGI1	1111	0.95	1.00		1112	0.23	0.10	0.18
	2222	0.05	0.00		1122	0.43	0.40	0.42
PGI2	1112	0.20	0.17		1222	0.08	0.00	0.13
	1122	0.20	0.21		2222	0.08	0.02	0.05
	1222	0.40	0.33	IDH1	1111	1.00	1.00	1.00
	2222	0.20	0.29	GDH	1111	1.00	1.00	1.00
IDH	2222	1.00	1.00					

4.5. Compensatory growth and branching pattern of the dwarf shrubs (IV,V)

Both the evergreen *A. uva-ursi* and deciduous *V. uliginosum* displayed a considerable ability to activate dormant meristems (axillary buds) and regrow after shoot clipping (**IV**). The biomass of the current-year shoots during the next growing season was at least 80 % compared to the within-clone control in both species after autumn clipping (Fig. 5a). Shoot clipping in early summer was more detrimental for both species, and *A. uva-ursi* suffered more than *V. uliginosum* (Fig. 5a). *A. uva-ursi* showed overcompensation (over 100 % growth compared to the control) in the number of new shoots after autumn clipping (Fig. 5b). A similar trend was found in *V. uliginosum* at the peatland site after spring clipping (Fig. 5b). No berries developed on either species in the year following the autumn treatment because clipping removed all the flower buds. Spring clipping had no effect on the sexual reproduction of *A. uva-ursi*, but decreased the berry production of *V. uliginosum.* The degree of compensatory growth of both species was only slightly affected by the distance from the smelter.

The state of the apical buds (living or dead) in the branches of *A. uva-ursi* was extremely important in regulating lateral branching in resource-poor habitats (**V**). Apical dominance of lateral branching was strongest in the intact shoots in the polluted (nutrient limited) and forest (light limited) habitats. However, when the apical bud of the parent shoot was dead, the disruption of apical dominance caused intensive branching in the poor habitats (**V**: Fig. 3). This response was demonstrated both in the shoot clipping

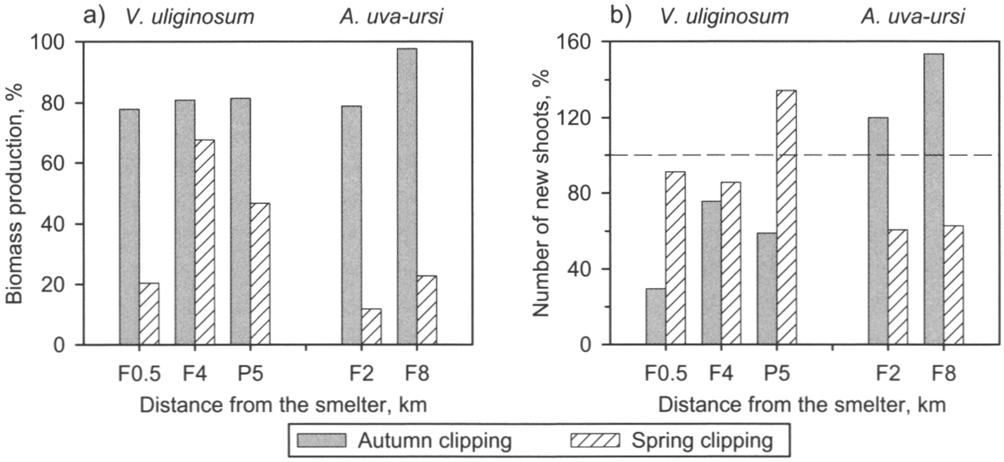


Fig. 5. The average compensatory growth of branches of *V. uliginosum* and *A. uva-ursi* after shoot clipping performed at different distances from the smelter (F = forest, P = peatland site) in autumn 1993 and spring 1994. The compensatory growth is presented as percentages of a) the biomass production, and b) the number of new shoots compared to the control branches (**IV**). The dashed line indicates the 100 % level.

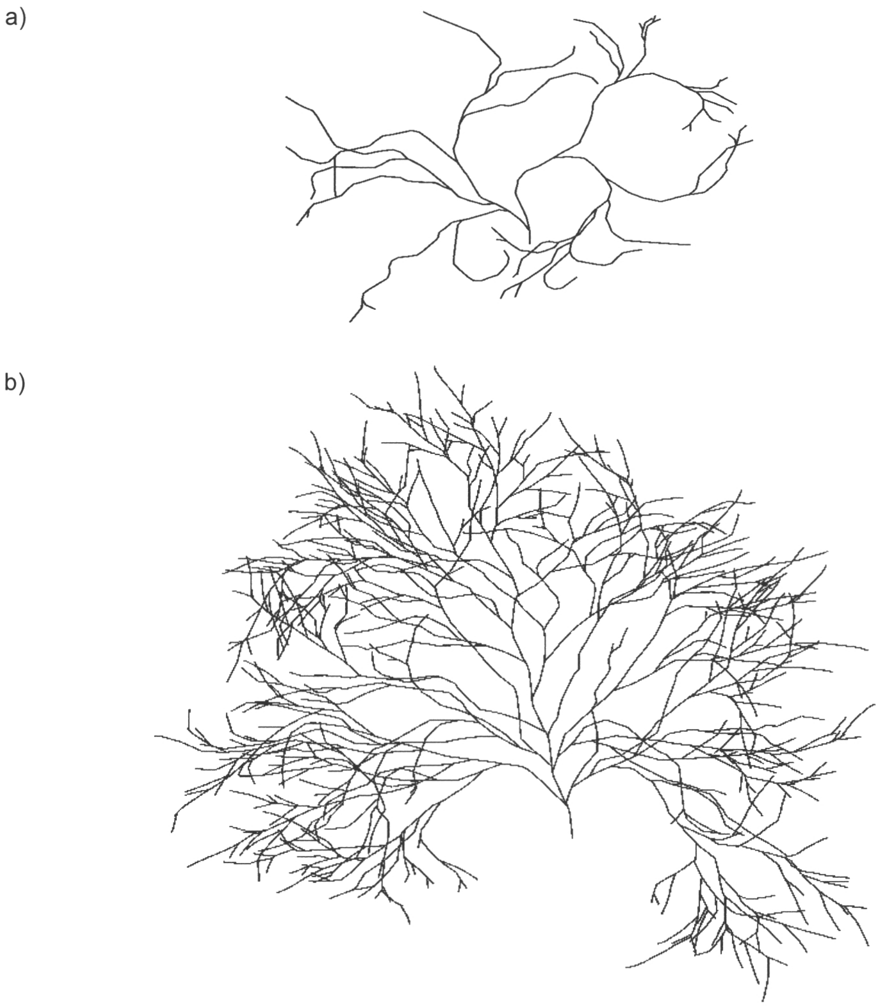


Fig. 6. Examples of the simulated branching patterns of *A. uva-ursi* in a) the polluted and b) the sand pit habitats (LIGNUM model). The growth cycle is 30 years in a) and 20 years in b). Annual shoot mortality increased from the 3rd year by 15 %, 20 % and 30 % years in dominant, subdominant and nondominant shoots, respectively in a). Corresponding parameters from the 4th year are 7 %, 10 % and 15 % for b). Dead branches have dropped off. The collision detection algorithm determines free growing space for active buds within an angle of 30 degrees and a distance of 20 cm in a) and 30 cm in b).

experiment (**IV**) (Fig. 5b) and in the analysis of the branching pattern of *A. uva-ursi* growing in forest or heavily disturbed polluted habitats (**V**). Apical dominance was much weaker in the sandpit and restoration habitats, where the nutrient availability and light level were relatively high. In these habitats branching frequency was high in both intact and terminated parent shoots (**V**: Fig. 3).

Simulations produced a variety of branching patterns for *A. uva-ursi* depending on the pollution and resource levels of the habitat (**V:** Fig. 6). A more realistic, star-like shape of the clones was produced by adding a collision detection algorithm to the model (Fig. 6).

4.6. Cu resistance of *Arctostaphylos uva-ursi* compared to the other dwarf shrubs (VI)

Growth inhibition and reduction of biomass production were the general responses of *A. uva-ursi* cuttings to exposure to Cu in a nutrient solution (**VI**). Compared to the other dwarf shrub species (*C. vulgaris*, *E. nigrum* and *V. uliginosum*) grown under similar experimental conditions, *A. uva-ursi* proved to be the most sensitive. The biomass production of the four species decreased in the order: *A. uva-ursi* > *C. vulgaris* > *E. nigrum* > *V. uliginosum* (Fig. 7a). The lowest external Cu level that reduced the growth of *C. vulgaris* and *A. uva-ursi* by 50 % was 10 mg/l. The corresponding critical concentration for *E. nigrum* was 22 mg/l. *V. uliginosum* did not reach a 50 % decrease in growth even at the highest Cu level of 100 mg/l. Also, the accumulation pattern of Cu in the new leaves indicated high sensitivity of *A. uva-ursi* to absorbed Cu. The Cu concentrations were lowest in the leaves of *A. uva-ursi*, followed by *V. uliginosum* < *E. nigrum* < *C. vulgaris* (Fig. 7b).

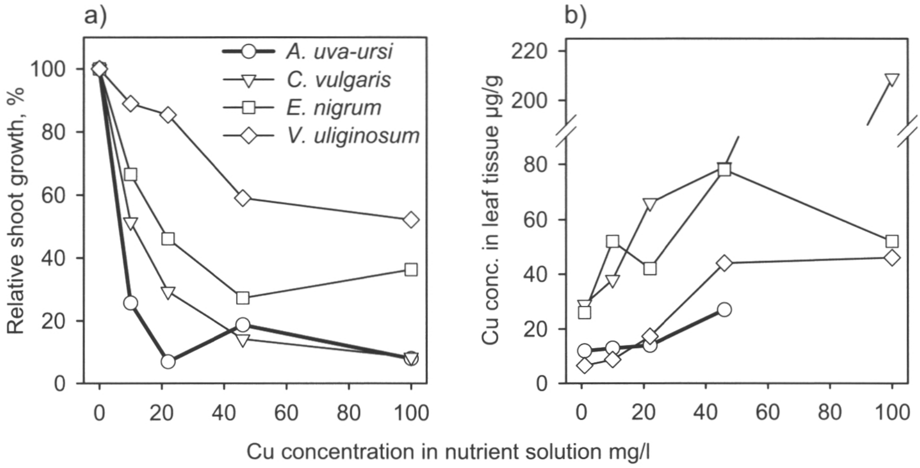


Fig. 7. a) The decrease in the relative shoot growth (%) (standardized to the Cu level of 1 mg/l) of *E. nigrum*, *C. vulgaris*, *A. uva-ursi* and *V. uliginosum*, and b) the Cu concentrations of the new leaves (µg/g) at different Cu levels applied. The shoot growth was based on the biomass production (dry weights) in the other species, except for *C. vulgaris* in which the length growth was used (Salemaa *et al.* 2003) (**VI**)

5. Discussion

5.1. Heavy metal accumulation and the relative resistance of the species

In general, vascular plants, being capable of restricting the uptake of toxic elements, grew closer to the smelter than lichens, while sensitive bryophytes began to increase at further distances from the smelter (**I**, **II**). The occurrence of the life forms which were still surviving followed, in relation to the closest distance to the smelter, the order: bryophytes of mature forests (*Dicranum* spp. and *P. schreberi*) (4 – 8 km) > reindeer lichens (2 – 4 km) > other lichens (2 km) > vascular plants (0.5 – 2 km). Pioneer mosses (dominated by *Pohlia nutans*) were exceptions to this general pattern. Despite the accumulation of large amounts of Cu and Ni in the living tissues, these species had surviving populations in the immediate vicinity of the emission source.

The order of the species along a pollution gradient may give some indication about the general resistance level of the species against pollution (e.g. heavy metals and SO_2) and other stress factors. In this respect, vascular plants were more resistant than lichens, whereas bryophytes were the most sensitive plant group. However, dwarf shrubs also apparently suffered from phytotoxic effects, which was expressed as their decreased abundance towards the smelter. Other environmental factors such as nutrient deficiencies in the soil, drought and increased illumination, frequently strengthen the selection pressure on plants in severely affected stands. It should be noted that unwashed samples have considerable amounts of dust attached to them, and actual tissue concentrations that have harmful metabolic effects are much lower than those reported in field conditions (Brown & Brumelis 1996, Bennett 1999). Therefore it is very difficult to identify the contribution of individual factors and present any maximum (toxic) limits for the survival of plant species based on field data.

Similar trends in the sequence of plant species around emission sources in coniferous forests have been reported e.g. near the Cu-Zn smelter at Gusum, SE Sweden (Folkeson 1984, Folkeson & Andersson-Bringmark 1988, Tyler *et al.* 1989), fertiliser factories in W Finland (Huttunen 1975, Väisänen 1986), a smelter complex in Sudbury, Ontario (Amiro & Courtin 1981), and the Cu-Ni smelters in Monchegorsk, Kola Peninsula, NW Russia (Rigina & Kozlov 2000).

The heavy metal concentrations were elevated especially in the older parts of the species (**II**: Appendices). For instance, the Cu and Ni concentrations of the dwarf shrubs were the highest in dead parts, and decreased from older to current-year shoots and berries. The Cu (13.78 µg/g) and Ni (8.64 µg/g) concentrations in the berries of *Vaccinium vitis-idaea* at 2 km distance from the Harjavalta smelter were considerably higher than those measured in Lapland in 1990 (Cu: 6.23 µg/g, Ni: 0.55 µg/g) but lower than the highest concentrations found near the Monchegorsk smelter on the Kola Peninsula (Cu: 33.8 µg/g, Ni: 25.2 µg/g) (Laine *et al.* 1993). Although the heavy metal concentrations of berries are usually lower than those in the other plant parts, they may be an important link in the transfer of heavy metals into food-chains via berry-eating animals and humans.

5.2. Revegetation potential of the seed bank

Although the understorey vegetation was almost totally absent at the distance of 0.5 km from the smelter, viable seeds of native plant species were present in the soil. However, seedling establishment in a greenhouse experiment presumably failed as a result of the phytotoxicity of heavy metals (**III**). One reason for the high mortality of the seedlings might be the absence of mycorrhizal infections (c.f. Bradley *et al.* 1981). In actual field conditions the thick layer of undecomposed needle litter and drought also hinder the germination of seeds near the smelter. Thus, the realisation of the revegetation potential of the local seed banks presupposes soil mitigation to immobilise heavy metals and facilitating the generation of a new organic soil layer.

5.3. Sensitivity of *Arctostaphylos uva-ursi* to Cu compared to the other dwarf shrubs

Compared to the earlier greenhouse experiments, *A. uva-ursi* (**VI**) seemed to be more sensitive to Cu than *C. vulgaris* (Monni *et al.* 2000b), *E. nigrum* (Monni *et al.* 2000a) and *V. uliginosum* (Salemaa *et al.* 2003). The ranking of the four dwarf shrub species based on survival and growth in the Cu exposure experiments was: *E. nigrum* (most resistant) > *V. uliginosum* > *C. vulgaris* > *A. uva-ursi* (most sensitive). This order was the same as that found for the closest distance to the Harjavalta smelter at which the species occurred. A few patches of *E. nigrum* and *V. uliginosum* were present at a distance of 0.5 km, *C. vulgaris* appeared for the first time at 1 km, and *A. uva-ursi* at 2 km from the smelter (**I**). The overall accumulation of Cu in *A. uva-ursi* was similar to that in the other dwarf shrubs: the concentrations were the highest in roots and stems and the lowest in green leaves (Monni *et al.* 2000a, b). A corresponding accumulation pattern of Cu has also been demonstrated in other vascular species (reviewed by Balsberg-Påhlsson 1989).

Short-term experiments using high exposure levels of heavy metals (**VI**) do not give a complete picture of the heavy metal resistance of long-lived dwarf shrubs, which may form extensive clones with modular subunits. In the real ecological conditions, the adult clones have to cope with low, but chronic exposure to heavy metals that have accumulated in the soil or entered the ecosystem in wet and dry deposition. The two possible ways in which plant populations can become resistant to heavy metals, 1) ecotypic differentiation or 2) phenotypic plasticity of individual plants (Section 1.3.), are discussed below.

5.4. Clonal diversity and genetic variation of the dwarf shrubs

In general, outcrossed plant species have a significantly higher genetic diversity than selfed or mixed-mating species (Hamrick & Godt 1990). *A. uva-ursi* and *V. uliginosum* are predominately outcrossed insect pollinated species. Both species produced flowers and berries in the study areas (**IV**). Isoenzyme analysis revealed that almost all the samples were different genotypes, indicating high clonal diversity in both species. This result is consistent with the overview of Ellstrand & Roose (1987), who showed that genetic variation in clonal plants is not rare. The percentage of polymorphic loci at the population level in both species (40 – 42 %) was slightly higher than the average value (34 %) reported for a large number of plant species (Hamrick & Godt 1990).

The distribution of the genotype frequencies in both species was rather similar at the different sites. On the basis of the restricted number of studied enzyme loci, there was no evidence of differentiation into heavy metal-specific ecotypes. However, it is also possible that isoenzyme variation is selectively neutral (e.g. Nei *et al.* 1976), and there is therefore no connection between the isoenzyme pattern and the pollution level.

Although vegetative production often predominates in the life of clonal plants, the existence of genetic variation indicates that the populations were originally established by sexual propagules (Ellstrand & Roose 1987). This was also true for the studied populations of *A. uva-ursi* and *V. uliginosum*. Nowadays, however, the birth of new clones in the most polluted areas is prevented, because the young seedlings die in the toxic surface soil (**III**). It is also possible that the failure of seedling establishment results from the absence of metal-tolerant genotypes in the study populations or that soil toxicity is too high for even the existing metal-tolerant genotypes. However, as seed banks maintain genetic diversity of plant populations (Mahy *et al.* 1999), the evolution of tolerant ecotypes may be possible in the future if the pollution level decreases.

The studied clones of both species were tens of years old. Because vegetative spreading is characteristic of clonal dwarf shrubs, some "mother clones" may date back to the time when the smelter first started operating in the 1940's. The abundance of dead clones near the smelter indicates that the heavy metal concentrations have been too high for the majority of the individual plants. The surviving clones most likely represent the most resistant genotypes of the earlier populations, or have rooted in clean "islands" in the polluted soil, having e.g. wood debris as substrate.

5.5. Phenotypic plasticity as a resistance strategy in clonal dwarf shrubs

Phenotypic plasticity is the ability of an individual organism to alter its physiology or morphology in response to changes in environmental conditions (Schlichting 1989). Morphological plasticity via growth is possible because plant development is modular in form. Vuorisalo & Tuomi (1986) define modules as partially self-maintaining, repetitive and multicellular parts of structural individuals. Integration between modules (e.g. annually grown shoots in dwarf shrubs) moderates the impact of local, adverse selection pressures (Slade & Hutchings 1987). Several studies (reviewed by Hutchings & de Kroon 1994) have described changes in the internodal length of the stems or rhizomes, lateral branching intensity and branching angle of clonal plants in response to environmental conditions (e.g. nutrients and light). Phenotypic plasticity allows both clonal and non-clonal plants to use avoidance strategies in relation to the heterogeneous distribution of a pollutant (Dickinson *et al.* 1991).

A. uva-ursi and *V. uliginosum* showed high regrowth after autumn clipping of current-year shoots (imitating pollution-induced shoot damage) in a field experiment carried out in polluted and clean sites (**IV**). In contrast, spring clipping of new shoots was more detrimental to the evergreen *A. uva-ursi* than to the deciduous *V. uliginosum*. Differences in the storage reserves and sink-source mechanisms of carbon allocation between evergreen and deciduous species probably explain their distinct response, as demonstrated e.g. by Tolvanen & Laine (1997) using *Vaccinium myrtillus* and *V. vitis-idaea* as experimenal species. Contrary to the predictions, the relative amount of activated meristems was higher in *A. uva-ursi* than in *V. uliginosum*. Architectural constraints (e.g. number of axillary buds) or differences in the rooting pattern might explain this. The creeping branches of *A. uva-ursi* could have fine adventitious roots, which made the shoots more independent of intraclonal transport of water and nutrients than shoots of *V. uliginosum*. It should be noted, however, that mechanical cutting of shoots may not have the same physiological effect on plants as pollution induced mortality.

A. uva-ursi showed high plasticity in the lateral branching, which varied according to the pollution, light and resource levels of the habitat (**V**). The plasticity in the clonal morphology was an expression of foraging behaviour that enables the clones to colonize favourable microhabitats and spread the risk of shoot mortality. Strong apical dominance, observed in resource-poor habitats, maintains a reserve of axillary buds that can be used to continue growth after damage ("reserve meristem hypothesis", Tuomi *et al.* 1994, Aarssen 1995) or after periods with low resource levels (Jonsdottir & Callaghan 1988, Hutchings & de Kroon 1994). The reserve meristem strategy partly explains why *A. uva-ursi* generally survives in severely disturbed sites such as the polluted one in this study.

In addition to dormant bud activation, rapid regrowth and plastic branching, adult dwarf shrub clones can avoid heavy metals by extending their roots into the less toxic, deeper soil layers. For instance, the deepest roots extended down to a depth of 50 cm in the clones of *A. uva-ursi* (**V**) and *E. nigrum* (Uhlig *et al.* 2001) growing at 2 km and 0.5 km distances from the smelter, respectively. Despite external avoidance of heavy metals, clonal dwarf shrubs express different degrees of real physiological tolerance (Fig. 1). For instance, *E. nigrum* has an ability to accumulate Cu in cell walls, vacuoles and cytoplasm (Monni *et al.* 2002).

5.6. Applicability of the results in biomonitoring and phytoremediation

The deposition gradient near the Harjavalta Cu-Ni smelter was very steep, resulting in strong inter-correlations between the elemental load in bulk deposition, and the concentrations in the understorey vegetation and organic layer (**II**). This makes it extremely difficult to distinguish between the role of airborne deposition, wind-blown dust and elements taken up by the substrate in the chemical composition of the plants. The relationship between deposition and plant uptake seem to be strongly dependent on the local conditions and the element ranges in deposition in polluted areas, as emphasized also by Halleraker *et al.* (1998) and Reimann *et al.* (2001). Bryophytes, lichens and vascular plants showed considerable differences in their capacity to accumulate pollutants and to grow in contaminated soil at Harjavalta (**I**, **II**). Therefore information about all the life forms in the understorey is needed when evaluating the state and recovery of forest ecosystems in heavily polluted areas.

In contaminated environments the vegetation cover protects the soil from erosion and the leaching of heavy metals into the groundwater (Vangronsveld *et al.* 1996). Through the litterfall, the understorey vegetation affects the composition of the organic layer which, in turn, is an important medium for the root growth of forest trees and also the understorey itself.

An ability to enrich heavy metals or to grow in contaminated soil makes some dwarf shrub species suitable for the revegetation of damaged forest areas. Although the growth rate of boreal dwarf shrubs is too low for the phytoextraction of heavy metals, they can be used for phytostabilisation of contaminated soil. *E. nigrum* and *A. uva-ursi* are, in fact, the two dwarf shrub species planted in a revegetation experiment in the vicinity of the Harjavalta smelter (Kiikkilä 2002). Both species have survived well after the high mortality during the first few years, and their clonal growth habit facilitates rapid expansion and coverage of the forest floor.

6. Conclusions

I found the following answers to the questions and hypotheses presented in the aims:

1) Heavy metal and sulphur deposition have subjected the vegetation growing near the smelter to a strong selection pressure. The species composition has changed, sensitive species have disappeared and competitive interactions between species may also have been altered. According to the species occurrence along the pollution gradient, vascular plants were more resistant than lichens, whereas the bryophytes of mature forests were the most sensitive taxon. The capacity of bryophytes and lichens to accumulate large amounts of heavy metals made them more sensitive than vascular plants.

2) The accumulation of heavy metals has caused chronic disturbances in the ecosystem, preventing the normal succession of plant communities. The size of the soil seed bank has decreased and young seedlings rapidly die in the contaminated soil. Natural recolonization of the vegetation in heavily polluted areas is a slow process, even though emissions have decreased. Recovery of the vegetation presupposes immobilisation of the heavy metals and the generation of a functioning organic soil layer.

3) Although the dwarf shrubs had the ability to produce berries, and there was genetic variation in the populations, the failure of seedling establishment is a factor preventing the evolution of metal-tolerant ecotypes. There was no evidence that the populations of *A. uva-ursi* or *V. uliginosum* had differentiated into heavy metal-specific ecotypes near the smelter.

4) Plasticity in dormant bud activation, rapid turnover of shoots after damage and root growth into deeper soil layers may help the long-lived dwarf shrubs in avoiding heavy metals.

5) The Cu resistance level of different dwarf shrub species varied in the greenhouse experiments as follows: *E. nigrum* (most resistant) > *V. uliginosum* > *C. vulgaris* > *A. uva-ursi* (most sensitive). This order was the same as that found for the occurrence pattern of the species along the pollution gradient near the Harjavalta smelter.

6) The results of this thesis reveal that understorey vegetation has great indicative value when studying the effects of environmental changes in forest ecosystems. However, there were considerable differences between the bryophytes, lichens and vascular plants in their capacity to accumulate pollutants and to grow in contaminated soil. Therefore, information about all plant groups in the understorey vegetation is needed when evaluating the state and recovery of forest ecosystems in heavily polluted areas.

References

Aarssen, L.W. 1995. Hypotheses for the evolution of apical dominance in plants: implications for the interpretation of overcompensation. Oikos 74(1): 149-156.

Alloway, B.J. 1995a. The origins of heavy metals in soils. In: Alloway, B.J. (ed.). Heavy metals in soil. Blackie Academic & Professional, London. pp. 38-57.

Alloway, B.J. 1995b. Soil processes and the behaviour of metals. In: Alloway, B.J. (ed.). Heavy metals in soils. Blackie Academic and Professional, London. pp. 11-37.

Alva, A.K., Huang, B. & Paramasivam, S. 2000. Soil pH affects copper fractionation and phytotoxicity. Soil Science Society of American Journal 64: 955-962.

Amiro, B.D. & Courtin, G.M. 1981. Patterns of vegetation in the vicinity of an industrially disturbed ecosystem, Sudbury, Ontario. Canadian Journal of Botany 59: 1623-1639.

Antonovics, J., Bradshaw, A.D. & Turner, R.G. 1971. Heavy metal tolerance in plants. Advances in Ecological Tolerance 7: 1-85.

Baker, A.J.M. 1987. Metal tolerance. New Phytologist 106: 93-111.

Baker, A.J.M., Grant, C.J., Martin, M.H., Shaw, S.C. & Whitebrook, J. 1986. Induction and loss of cadmium tolerance in *Holcus lanatus* L. and other grasses. New Phytologist 102: 575-585.

Baker, A.J.M. & Walker, P.L. 1989. Physiological responses of plants to heavy metals and the quantification of tolerance and toxicity. Chemical Speciation and Bioavailability 1: 7-17.

Balsberg-Påhlsson, A.M. 1989. Toxicity of heavy metals (Zn, Cu, Cd, Pb) to vascular plants. A literature review. Water, Air and Soil Pollution 47(3-4): 287-319.

Bates, J.W. 1992. Mineral nutrient acquisition and retention by bryophytes. Journal of Bryology 17: 223-240.

Bennett, J. 1999. Statistical baseline values for chemical elements in the lichen *Hypogymnia physodes*. In: Agrawal, S.B. & Agrawal, M. (eds.). Environmental Pollution and Plant Responses. Lewis Publishers, Boca Raton. pp. 343-352.

Bradley, R., Burt, A.J. & Read, D.J. 1981. Mycorrhizal infection to heavy metal toxicity in *Calluna vulgaris*. Nature 292: 335-337.

Bradshaw, A.D. & Hardwick, K. 1989. Evolution and stress-genotypic and phenotypic components. Biological Journal of the Linnean Society 37: 137-155.

Bradshaw, A.D., McNeilly, T. & Putwain, P.D. 1990. The essential qualites. In: Shaw, J.A. (ed.). Heavy metal tolerance in plants: evolutionary aspects. CRC Press, Boca Raton. pp. 323-334.

Brown, D.H. & Brumelis, G. 1996. A biomonitoring method using the cellular distribution of metals in moss. The Science of the Total Environment 187: 153-161.

Brown, D.H. & Wells, J.M. 1990. Physiological effects of heavy metals on the moss *Rhytidiadelphus squarrosus*. Annals Botany 66: 641-647.

Bruns, I., Sutter, K., Menge, S., Neumann, D. & Krauss, G.J. 2001. Cadmium lets increase the glutathione pool in bryophytes. Journal of Plant Physiology: 79-89.

Buse, A., Norris, D., Harmens, H., Buker, P., Ashenden, T. & Mills, G. 2003. Heavy metals in European mosses: 2000/2001 survey. UNECE ICP Vegetation. Centre for Ecology & Hydrology, Bangor. 45 p.

Cajander, A.K. 1909. Ueber Waldtypen. Acta Forestalia Fennica 1(1): 1-176.

Chernenkova, T. & Kuperman, G. 1999. Changes in the spruce forest communities along a heavy metals deposition gradient on Kola peninsula. Water, Air and Soil Pollution: 187-200.

Chettri, M.K., Cook, C.M., Vardaka, E., Sawidis, T. & Lanaras, T. 1998. The effect of Cu, Zn and Pb on the chlorophyll content of the lichens *Cladonia convoluta* and *Cladonia rangiformis*. Environmental and Experimental Botany 39: 1-10.

Chettri, M.K., Sawidis, T., Zachariadis, G.A. & Stratis, J.A. 1997. Uptake of heavy metals by living and dead *Cladonia* thalli. Environmental and Experimental Botany 37: 39-52.

Clemens, S., Palmgren, M. & Krämer, U. 2002. A long way ahead: understanding and engineering plant metal accumulation. Trends in Plant Science 7: 309-315.

Derome, J. 2000a. Detoxification and amelioration of heavy-metal contaminated forest soils by means of liming and fertilisation. Enviromental Pollution 107: 79-88.

Derome, J. 2000b. Effects of heavy-metal and sulphur deposition on the chemical properties of forest soil in the vicinity of a Cu-Ni smelter, and means of reducing the detrimental effects of heavy metals. Finnish Forest Research Institute, Research Papers 769. 78 p.

Derome, J. & Lindroos, A.-J. 1998. Effects of heavy metal contamination on macronutrient availability and acidification parameters in forest soil in the vicinity of the Harjavalta Cu-Ni, SW Finland. Environmental Pollution 99: 225-232.

Derome, J. & Nieminen, T. 1998. Metal and macronutrient fluxes in heavy-metal polluted Scots pine ecosystems in SW Finland. Environmental Pollution 103: 219-228.

Derome, J. & Saarsalmi, A. 1999. The effect of liming and correction fertilisation on heavy metal and macronutrient concentrations in soil solution in heavy-metal polluted Scots pine stands. Environmental Pollution 104: 249-259.

Dickinson, N.M., Turner, A.P. & Lepp, N.W. 1991. How do trees and other long-lived plants survive in polluted environments? Functional Ecology 5: 5-11.

Dickinson, N.M., Turner, A.P. & Watmough, S.A. 1992. Acclimation of trees to pollution stress: cellular metal tolerance traits. Ann. Bot. 70: 569-572.

Ellstrand, N.C. & Roose, M.L. 1987. Patterns of genotypic diversity in clonal plant species. American Journal of Botany 74: 123-131.

Eltrop, L., Brown, G., Joachim, O. & Brinkmann, K. 1991. Lead tolerance of *Betula* and *Salix* in the mining area of Mechernich/Germany. Plant and soil 131: 275-285.

Folkeson, L. 1984. Deterioration of the moss and lichen vegetation in a forest polluted by heavy metals. Ambio 13(1): 37-39.

Folkeson, L. & Andersson-Bringmark, E. 1988. Impoverishment of vegetation in a coniferous forest polluted by copper and zinc. Canadian Journal of Botany 66: 417-428.

Fowler, D., Cape, J. & Unsworth, M. 1989. Deposition of atmospheric pollutants on forests. Philosophical Transactions of the Royal Society of London: 247-265.

Fritze, H., Niini, S., Mikkola, K. & Mäkinen, A. 1989. Soil microbiological effects of a Cu-Ni smelter in southwestern Finland. Biological Fertility of Soils 8: 87-94.

Fritze, H., Vanhala, P., Pietikäinen, J. & Mälkönen, E. 1996. Vitality fertilization of Scots pine stands growing along a gradient of heavy metal pollution: Short-term effects on microbial biomass and respiration rate of the humus layer. Fresenius Journal of Analytical Chemistry 5219: 1-6.

Garty, J. 2001. Biomonitoring atmospheric heavy metals with lichens: theory and application. Critical Reviews in Plant Sciences 20(4): 309-371.

Gimingham, C.H. 1972. Ecology of heathlands. Chapman and Hall. 266 p.

Guschina, I.A. & Harwood, J.L. 2002. Lipid metabolism in the moss *Rhytidiadelphus squarrosus* (Hedw.) Warnst. from lead-contaminated and non-contaminated populations. Journal of Experimental Botany 53: 455-463.

Hall, J.L. 2002. Cellular mechanisms for heavy metal detoxification and tolerance. Journal of Experimental Botany 53: 1-11.

Halleraker, J.H., Reimann, C., Caritat de, P., T.E., F., Kashulina, G., Niskavaara, H. & Bogatyrev, I. 1998. Reliability of moss (*Hylocomium splendens* and *Pleurozium schreberi*) as a bioindicator of atmospheric chemistry in the Barents region: Interspecies and field duplicate variability. The Science of the Total Environment 218: 123-139.

Hamrick, J.L. & Godt, M.J.W. 1990. Allozyme diversity in plant species. In: Brown, A.H.D., Clegg, M.T., Kahler, A.L. & Weir, B.S. (eds.). Plant population genetics, breeding and genetic resources. Sinauer, Sunderland, USA, 1990, Sunderland, USA. pp. 43-63.

Hartley, J., Cairney, J.W.G. & Meharg, A.A. 1997. Do ectomycorrhizal fungi exhibit adaptive tolerance to potentially toxic metals in the environment? Plant and Soil 189: 303-319.

Haslett, B.S., Reid, R.J. & Rengel, Z. 2001. Zinc mobility in wheat: Uptake and distribution of zinc applied to leaves ar roots. Annals of Botany 87: 379-386.

Heinrichs, H. & Mayer, R. 1980. The role of forest vegetation in the biogeochemical cycle of heavy metals. Journal of Environmetal Quality 9: 111-118.

Helmisaari, H.-S. 1995. Nutrient cycling in *Pinus sylvestris* stands in eastern Finland. Plant and Soil 168-169: 327-336.

Helmisaari, H.-S. 2000. Function of forest ecosystems under heavy metal loading. In: Mälkönen, E. (ed.). Forest Condition in a Changing Environment - The Finnish Case. Kluwer Academic Publishers, Dordrecht. pp. 248-251.

Helmisaari, H.-S., Derome, J., Fritze, H., Nieminen, T., Palmgren, K., Salemaa, M. & Vanha-Majamaa, I. 1995. Copper in Scots pine forests around a heavy-metal smelter in south-western Finland. Water, Air and Soil Pollution 85: 1727-1732.

Hutchings, M. & de Kroon, H. 1994. Foraging in plants: the role of morphological plasticity in resource acquisition. Advances in Ecological Research 25: 159-238.

Hutchinson, T.C. & Whitby, L.M. 1977. The effects of acid rainfall and heavy metal particulates on a boreal forest ecosystem near the Sudbury smelting region of Canada. Water, Air, Soil Pollut. 7: 421-438.

Huttunen, S. 1975. Studies on forest vegetation in air pollution damage area. In: Acta Universitatis Oulensis. Acta Universitatis Oulensis, Series A, Biology 2. University of Oulu, Oulu. pp. 1-37.

Hynninen, V. 1986. Monitoring of airborne metal pollution with moss bags near an industrial source at Harjavalta. Annales Botanici Fennici 23: 83-90.

Hyvärinen, M., Roitto, M., Ohtonen, R. & Markkola, A. 2000. Impact of wet deposited nickel on the cation content of a mat-forming lichen *Cladina stellaris*. Environmental and Experimental Botany 43: 211-218.

Jonsdottir, I.S. & Callaghan, T.V. 1988. Interrelationships between different generations of interconnected tillers of *Carex bigelowii*. Oikos 52: 120-128.

Jules, E.S. & Shaw, A.J. 1994. Adaptation to metal-contaminated soils in populations of the moss, *Ceratodon purpureus*: vegative growth and reproductive expression. American Journal of Botany 81: 791-797.

Jussila, I. 2003. A bioindicator study on the effects of air pollution on forest ecosystem at Pori-Harjavalta district in SW-Finland (In Finnish, English summary). Sykesarja B 15. Turun yliopisto. Satakunnan ympäristöntutkimuslaitos, Pori. 112 p.

Kahle, H. 1993. Response of roots trees to heavy metals. Environmental and Experimental Botany 33(1): 99-119.

Kiikkilä, O. 2002. Remediation through mulching with organic matter of soil polluted by a copper-nickel smelter. Finnish Forest Research Institute, research papers. 57 p.

Kiikkilä, O. 2003. Heavy-metal pollution and remediation of forest soil around the Harjavalta Cu-Ni smelter, in SW Finland. Silva Fennica 37(3): 399-415.

Kiikkilä, O., Pennanen, T., Pietikäinen, J., Hurme, K.-J. & Fritze, H. 2000. Some observations on the copper tolerance of bacterial communities determined by (3H)-thymide incorporation method in heavy metal polluted humus. Soil Biology & Biochemistry 32: 883-885.

Kopponen, P., Utriainen, M., Lukkari, K., Suntioinen, S., Kärenlampi, L. & Kärenlampi, S. 2001. Clonal differences in copper and zink tolerance of birch in metal-supplemented soils. Enviromental Pollution 112: 89-97.

Kozlov, M.V., Haukioja, E., Bakhtiarov, A.V., Stroganov, D.N. & Zimina, S.N. 2000. Root versus canopy uptake of heavy metals by birch in an industrially polluted area: contrasting behaviour of nickel and copper. Environmental Pollution 107: 413-420.

Krebs, S.L. & Hancock, J.F. 1989. Tetrasomic inheritance of isoenzyme markers in the highbush blueberry, *Vaccinium corymbosum* L. Heredity 63: 11-18.

Kubin, E., Lippo, H. & Poikolainen, J. 2000. Heavy metal loading. In: Mälkönen, E. (ed.). Forest condition in a changing environment - the Finnish case. Kluwer Academic, Dordrecht. pp. 60-71.

Laaksovirta, K. & Silvola, J. 1975. Effect of air pollution by copper, sulphuric acid and fertilizer factories on plants at Harjavalta, W. Finland. Annales Botanici Fennici 12: 81-88.

Laine, K., Saari, E., Kemppainen, K., Pakonen, T., Havas, P., Lajunen, L., Perämäki, P., Paama, L. 1993. Lapin metsämarjojen raskasmetallipitoisuudet. Ympäristö ja Terveys 24(7-8): 443-449.

Lakanen, E. & Erviö, R. 1971. A comparison of eight extractants for the determination of plant available micronutrients in soils. Acta Agralia Fennica 123: 223-232.

Lin, Z.Q., Schuepp, P.H., Schemenauer, R.S. & Kennedy, G.G. 1995. Trace metal contamination and on balsam fir (*Abies balsamea* (L.) Mill.) foliage in Southern Quebec, Canada. Water, Air, Soil Pollut. 81: 175-191.

Luttermann, A. & Freedman, B. 2000. Risks to forests in heavily polluted regions. In: Innes, J.L. & Oleksyn, J. (eds.). Forest dynamics in heavily polluted regions. CABI Publishing, Wallingford. pp. 9-26.

MacNair, M., R. 1987. Heavy metal tolerance in plants: a model evolutionary system. Trends in Ecology & Evolution 2(12): 354-358.

Mahy, G., Vekemans, X. & Jacquemart, A.-L. 1999. Patterns of allozymic variation whithin *Calluna vulgaris* populations at seed bank and adult stages. Heredity 82: 432-440.

Mäkinen, A. 1994. Biomonitoring of atmospheric deposition in Finland, Estonia and Kola Peninsula, based on the chemical analysis of mosses. Publications from the Department of Botany, University of Helsinki 19. University of Helsinki, 1994, Helsinki. 28 p.

Mälkönen, E. 1974. Annual primary production and nutrient cycle in some Scots pine stands. Communicationes Instituti Forestalis Fenniae 84.5: 1-87.

Mälkönen, E., Derome, J., Fritze, H., Helmisaari, H.-S., Kukkola, M., Kytö, M., Saarsalmi, A. & Salemaa, M. 1999. Compensatory fertilization of Scots pine stands polluted by heavy metals. Nutrient Cycling in Agroecosystems 55: 239-268.

Marschner, H. 1995. Mineral nutrition of higher plants. Academic Press, London. 889 p.

Mattila, A., Pakkanen, A., Vakkari, P. & Raisio, J. 1994. Genetic variation in English oak (*Quercus robur*) in Finland. Silva Fennica 28(4): 251-256.

Meharg, A.A. & Cairney, J.W.G. 2000. Co-evolution of mycorrhizal symbionts and their hosts to metal-contaminated environments. Advances in Ecological Research 30: 70-112.

Melanen, M., Ekqvist, M., Mukherjee, A.B., Aunela-Tapola, L., Verta, M. & Salmikangas, T. 1999. Atmospheric emissions of heavy metals in Finland in 1990s. The Finnish Environment 329. Finnish Environment Institute, Helsinki. 92 p.

Mocquot, B., Vangronsved, J., Clijsters, H. & Mench, M. 1996. Copper toxicity in young maize (*Zea mays* L.) plants: effects on growth, mineral and chlorophyll contents, and enzyme activities. Plant and Soil: 287-300.

Monni, S., Bucking, H. & Kottke, I. 2002. Ultrastructural element localization by EDXS in *Empetrum nigrum*. Micron 33: 339-351.

Monni, S., Salemaa, M. & Millar, N. 2000a. The tolerance of *Empetrum nigrum* to copper and nickel. Environmental Pollution 109: 221-229.

Monni, S., Salemaa, M., White, C., Tuittila, E. & Huopalainen, M. 2000b. Copper resistance of *Calluna vulgaris* originating from the pollution gradient of a Cu-Ni smelter, in southwest Finland. Enviromental Pollution 109: 211-219.

Monni, S., Uhlig, C., Hansen, E. & Magel, E. 2001a. Ecophysiological responses of *Empetrum nigrum* to heavy metal pollution. Enviromental Pollution 112: 121-129.

Monni, S., Uhlig, C., Junttila, O., Hansen, E. & Hynynen, J. 2001b. Chemical composition and ecophysiological responses of *Empetrum nigrum* to aboveground element application. Enviromental Pollution 112: 417-426.

Nash, T.H. 1996. Nutrients, element accumulation and mineral cycling. In: Nash, T.H. (ed.). Lichen biology. Cambridge Univerisity Press, Cambridge, UK. pp. 136-153.

Nei, M., Furerstt, P.A. & Chakraborty, R. 1976. Testing the neutral mutation hypothesis by distribution of single locus heterozygosity. Nature 262: 491-493.

Nieboer, E. & Richardson, D.H.S. 1980. The replacement of the nondescript term 'heavy metals' by a biologically and chemically significant classification of metal ions. Enviromental Pollution 1: 3-26.

Nieminen, T. & 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.

Nieminen, T.M., Derome, J. & Helmisaari, H.-S. 1999. Interactions between precipitation and Scots pine canopies along a heavy-metal pollution gradient. Environmental Pollution 106: 129-137.

Nriagu, J.O. 1989. A global assessment of natural sources of atmospheric trace metals. Nature 338(2): 47-49.

Økland, T., Økland, R.H. & Steinnes, E. 1999. Element concentrations in the boreal forest moss *Hylocomium splendens*: variation related to gradient in vegetation and local environmental factors. Plant and Soil 209: 71-83.

Onianwa, P.C. 2001. Monitoring atmospheric metal pollution: a review of the use of mosses as indicators. Environmental Monitoring and Assessment 71: 13-50.

Outola, I., Pehrman, R. and Jaakkola, T. 2003. Effect of industrial pollution on the distribution of ^{137}Cs in soil and the soil-to-plant transfer in a pine forest in SW Finland. The Science of the Total Environment 303: 221-230.

Päivänen, J. 1966. Sateen jakautumisesta erilaisissa metsiköissä. Summary: The distribution of rainfall in different types of forest stands. Silva Fennica 119(3): 1-37.

Pätsikkä, E., Aro, E.-M. & Tyystjärvi, E. 2001. Mechanism of copper-enhanched photoinhibition in thylakoid membranes. Physiologia Plantarum 113(1): 142-150.

Pawlik-Skowronska, B., di Toppi, L.S., Favali, M.A., Fossati, F., Pirszel, J. & Skowronski, T. 2002. Lichens respond to heavy metals by phytochelatin synthesis. New Phytologist 156: 95-102.

Poikolainen, J., Kubin, E., Piispanen, J. & Karhu, J. 2004. Atmospheric heavy metal deposition in Finland during 1985-2000. The Science of the Total Environment 318: 171-185.

Prusinkiewicz, P. & Lindenmayer, A. 1990. The algorithmic beauty of plants. Springer, New York. 228 p.

Punshon, T. & Dickinson, N.M. 1997. Acclimation of *Salix* to metal stress. New Phytologist 137: 303-314.

Puolanne, J., Pyy, O. & Jeltsch, U. 1994. Saastuneet maa-alueet ja niiden käsittely Suomessa: saastuneiden maa-alueiden selvitys- ja kunnostusprojekti (In Finnish). Ministry of Environment. 218 p.

Rautio, P. & Huttunen, S. 2003. Total vs. internal element concentrations in Scots pine needles along a sulphur and metal pollution gradient. Environmental Pollution 122: 273-289.

Rautio, P., Huttunen, S., Kukkola, E., Peura, R. & Lamppu, J. 1998. Deposited particles, element concentrations and needle injuries on Scots pine along an industrial pollution transect in northern Europe. Environmental Pollution 103: 81-89.

Reimann, C., Koller, F., Frengstad, B., Kashulina, G., Niskavaara, H. & Englmaier, P. 2001. Comparison of the element composition in several plant species and their subsrate trom a 1 500 000-km2 area in Northern Europe. The Science of the Total Environment 278: 87-112.

Reinikainen, A., Mäkipää, R., Vanha-Majamaa, I. & Hotanen, J.-P. 2000. Kasvit muuttuvassa metsäluonnossa. (English summary: Changes in the frequency and abundance of forest and mire plants in Finland since 1950). Tammi, Helsinki. 384 p.

Rigina, O. & Kozlov, M. 2000. The impacts of air pollution on the Northern taiga forests of the Kola Peninsula, Russian federation. In: Innes, J.L. & Oleksyn, J. (eds.). Forest dynamics in heavily polluted regions. CABI Publishing, Wallingford. pp. 37-65.

Ross, S.M. 1994a. Retention, transformation and mobility of toxic metals in soils. In: Ross, S.M. (ed.). Toxic metals in soil - plant systems. John Wiley, Chichester. pp. 63-152.

Ross, S.M. 1994b. Sources and forms of potentially toxic metals in soil-plant systems. In: Ross, S.M. (ed.). Toxic metals in soil-plant systems. John Wiley & Sons, Chichester. pp. 3-25.

Rühling, Å. & Steinnes, E. 1998. Atmospheric heavy metal deposition in Europe 1995-1996. NORD 1998. Nordic Council of Ministers, Copenhagen. 15 p.

Salemaa, M., Monni, S. & Nieminen, T. 2003. Dwarf shrubs (*Arctostaphylos uva-ursi, Calluna vulgaris, Empetrum nigrum* and *Vaccinium uliginosum*) in the revegetation of a Cu polluted forest area. In: Gobran, G.R. & Lepp, N.W. (eds.). 7th International Conference on the Biogeochemistry of Trace Elements, vol II, Uppsala, Sweden. pp. 196-197.

Sarret, G., Manceau, A., Cuny, D., C., V.H., Deruelle, S., Hazemann, J.-L., Soldo, Y., Eybert-Berard, L. & Menthonnex, J.-J. 1998. Mechanisms of lichen resistance to metallic pollution. Environmental Science & Technology 32: 3325-3330.

Schlichting, C.D. 1989. Phenotypic integration and environmental change. What are the consequences of differential phenotypic plasticity of traits? Bioscience 39(7): 460-464.

Shevtsova, A. 1998. Responses of subarctic dwarf shrubs to climate change and air pollution. Annales Universitatis Turkuensis. Ser AII. 113. University of Turku, Turku. 22 p.

Sirén, G. 1955. The development of spruce forest on raw humus sites in northern Finland. Acta Forestalia Fennica 62.4: 1-408.

Slade, A.J. & Hutchings, M.J. 1987. Clonal integration and plasticity in foraging behaviour in *Glechoma hederacea*. Journal of Ecology 75: 1023-1036.

Streit, B. & Stumm, W. 1993. Chemical properties of metals and the process of bioaccumulation in terrestrial plants. In: Markert, B. (ed.). Plants as biomonitors. Indicators for heavy metals in the terrestrial environment. VCH, Weinheim. pp. 31-62.

Stribley, D.P. & Read, D.J. 1976. The biology of mycorrhiza in the Ericaceae. VI. The effects of mycorrhizal infection and concentration of ammonium nitrogen on groth of carnberry (*Vaccinium microcarpon* Ait.) in sand culture. New Phytologist 77: 63-72.

Tammiranta, A. 2000. Study of soil contamination in Harjavalta area and assessment of need for measures (In Finnish, English summary). The Finnish Environment 418. Finnish Environmental Institute, Helsinki. 87 p.

Tarhanen, S., Metsärinne, S., Holopainen, T. & Oksanen, J. 1999. Membrane permeability response of lichen *Bryoria fuscescens* to wet deposited heavy metals and acid rain. Environmental Pollution 104: 121-129.

Thompson, J.D. 1991. Phenotypic plasticity as a component of evolutionary change. Trends in Ecology and Evolution 6: 246-249.

Tikkanen, E. & Niemelä, I. 1995. Kola Peninsula pollutants and forest ecosystems in Lapland. Final Report of the Lapland Forest Damage Project. Gummerus, Jyväskylä. 82 p.

Tolvanen, A. & Kubin, E. 1990. The effect of clear felling and site preparation on microclimate, soil frost and forest regeneration at elevated sites in Kuusamo. Aquilo Series Botanica 29: 77-86.

Tolvanen, A. & Laine, K. 1997. Effects of reproduction and artificial herbivory on vegetative growth and resource levels in deciduous and evergreen dwarf shrubs. Canadian Journal of Botany 75: 656-666.

Tuomi, J., Nilsson, P. & Åström, M. 1994. Plant compensatory responses: bud dormancy as an adaptation to herbivory. Ecology 75(5): 1429-1436.

Turner, A.P. & Dickinson, N.M. 1993. Copper tolerance of *Acer pseudoplatanus* L. (sycamore) in tissue culture. New Phytologist 123: 523-530.

Tyler, G. 1984. The impact of heavy metal pollution on forest: a case study of Gusum, Sweden. Ambio 13(1): 18-25.

Tyler, G. 1989. Uptake, retention and toxicity of heavy metals in lichens. Water, Air and Soil Pollution 47: 321-333.

Tyler, G. 1990. Bryophytes and heavy metals: a literature review. Botanical Journal of Linnean Society 104: 231-253.

Tyler, G., Balsberg-Påhlsson, A.-M., Bengtsson, G., Bååth, E. & Tranvik, L. 1989. Heavy-metal ecology of terrestrial plants, micro-organisms and invertebrates. Water, Air and Soil Pollution 47: 189-215.

Uhlig, C., Salemaa, M., Vanha-Majamaa, I. & Derome, J. 2001. Element distribution in *Empetrum nigrum* microsites at heavy metal contaminated sites in Harjavalta, Western Finland. Environmental Pollution 112: 435-442.

Ukonmaanaho, L., Starr, M., Hirvi, J.-P., Kokko, A., Lahermo, P., mannio, J., Paukola, T., Ruoho-Airola, T. & Tanskanen, H. 1998. Heavy metal concentrations in various aqueous and biotic media in Finnish integrated monitoring catchments. Boreal Environment Research 3: 235-249.

Utriainen, M., Kärenlampi, L., Kärenlampi, S. & Schat, H. 1997. Differential tolerance to copper and zinc of micropropagated birches tested in hydroponics. New Phytologist 137: 543-549.

Väisänen, S. 1986. Effects of air pollution by metal, chemical and fertilizer plants on forest vegetation at Kokkola, W Finland. Annales Botanici Fennici 23: 303-315.

Vangronsveld, J., Colpaert, J.V. & Tichelen Van, K.K. 1996. Reclamation of a bare industrial area contaminated by non-ferrous metals: physicochemical and biological evaluation of the durability of soil treatment and revegetation. Environmental Pollution 94: 131-140.

Verkleij, J.A.C. & Schat, H. 1989. Mechanisms of metal tolerance in higher plants. In: Shaw, J.A. (ed.). Heavy metal tolerance in plants: evolutionary aspects. CRC Press, Boca Raton. pp. 179-193.

Vuorisalo, T. & Tuomi, J. 1986. Unitary and modular organisms: criteria for ecological division. Oikos 47(3): 382-385.

Zechmeister, H.G., Grodzinska, K. & Szarek-Lukaszewska, G. 2003. Bryophytes. In: Markert, B.A., Breure, A.M. & Zechmeister, H.G. (eds.). Bioindicators and biomonitors. Elsevier, Amsterdam. pp. 329-375.

Zöttl, H.W. 1985. Heavy metal levels and cycling in forest ecosystems. Experientia 41: 1104-1113.

Paper I

I

Salemaa, M., Vanha-Majamaa, I. & Derome, J. 2001. Understorey vegetation along a heavy-metal pollution gradient in SW Finland. Environmental Pollution 112: 339-350.

ELSEVIER

Environmental Pollution 112 (2001) 339–350

ENVIRONMENTAL
POLLUTION

www.elsevier.com/locate/envpol

Understorey vegetation along a heavy-metal pollution gradient in SW Finland☆

M. Salemaa [a,*], I. Vanha-Majamaa [a], J. Derome [b]

[a] Vantaa Research Centre, Finnish Forest Research Institute, PO Box 18, FIN-01301 Vantaa, Finland
[b] Rovaniemi Research Station, Finnish Forest Research Institute, PO Box 16, FIN-96301 Rovaniemi, Finland

Received 8 February 2000; accepted 30 May 2000

"Capsule": *Heavy metals and sulphur in soils decreased and floristic diversity increased with distance from a Cu–Ni smelter.*

Abstract

Understorey vegetation of Scots pine forests was studied along a 8-km transect running SE from a Cu–Ni smelter at Harjavalta, SW Finland. Long-term accumulation of heavy metals and sulphur in the forest ecosystem has drastically changed plant communities. Vegetation was almost absent up to a distance of 0.5 km from the smelter. The total coverage and the number of plant species increased with increasing distance from the smelter. Ordination by global non-metric multidimensional scaling (GNMDS) indicated that the floristic composition was differentiated in response to the pollution level. The main compositional gradient of GNMDS was correlated with the heavy metal concentrations in the organic soil layer and with the size of the overstorey trees. Vascular plants were more pollution-resistant than ground lichens, whereas mosses were the most sensitive plant group. In addition to heavy metals, nutrient imbalances and the considerably reduced water-holding capacity of the surface soil also restrict plant recolonisation on the degraded sites. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Boreal forest vegetation; Heavy metals; Sulphur; Non-metric multidimensional scaling; Point quadrat method

1. Introduction

Heavy metals are emitted from traffic and industrial sources into the atmosphere and spread over wide regions of the boreal forest zone. Although heavy metal deposition has decreased considerably during the last 20 years in northern Europe (Rühling et al., 1992; Mäkinen, 1994; Berthelsen et al., 1995; Ukonmaanaho et al., 1998; Kubin et al., 2000), there are many areas where the long-term accumulation of heavy metals, often associated with exposure to sulphur dioxide, has damaged forests ecosystems. For instance, there are extensive damage areas surrounding the metallurgical industry complexes in the Kola Peninsula, NW Russia (Kozlov et al., 1993; Tikkanen and Niemelä, 1995; Chernenkova and Kuperman, 1999; Rigina and Kozlov, 2000). Localised damage areas are also to be found in the Nordic countries, primarily close to smelters, mines or steel mills (Väisänen, 1986; Folkeson and Andersson-Bringmark, 1988; Rühling et al., 1992).

Coniferous trees efficiently filter pollutant particles from the air (Tyler, 1984; Zöttl, 1985; Fowler et al., 1989). Stand throughfall and plant litter increase the load of heavy metals and sulphur on the forest floor (Heinrichs and Mayer, 1980; Derome and Nieminen, 1998; Nieminen et al., 1999). Heavy metals depress soil microbial activity (Bååth, 1989; Pennanen et al., 1996), which is seen as an increase in undecomposed needle litter on the soil surface and retarded nutrient cycling of the whole ecosystem (Fritze et al., 1997). The accumulation of Cu and Ni in forest soil can also cause a deficiency of base cations as a result of their displacement by heavy metals (Løbersli and Steinnes, 1988; Derome and Lindroos, 1998). The toxic effects of heavy metals and changes in the nutrient status of the soil subject the vegetation to a strong selective pressure.

The above-ground biomass of the understorey vegetation is small in relation to that of the trees, but it plays

---

☆ Nomenclature as per Hämet-Ahti et al. (1998) (vascular species), Koponen et al. (1977) (bryophytes) and Vitikainen et al. (1997) (lichens).

* Corresponding author. Tel.: +358-9-8570-5565.
E-mail address: maija.salemaa@metla.fi (M. Salemaa).

0269-7491/01/$ - see front matter
PII: S0269-7491(00)00150-0

an important role in regulating the nutrient fluxes (Mälkönen, 1974), hydrology and micro climate (Axelsson and Bråkenhielm, 1980; Tolvanen and Kubin, 1990) of boreal forests. In contaminated environments, certain plant species can accumulate considerable amounts of heavy metals and protect the soil from erosion and the leaching of heavy metals into the groundwater (Vangronsveld et al., 1996). Because many heavy metals are bound on plant surfaces and tissues (Wittig, 1993), and form stable complexes with organic matter in the soil (Alloway, 1995), they may still have a long-lasting effect on forest ecosystems after the emissions have ceased (Ross, 1994). Before we can gain a better understanding of heavy metal fluxes in boreal forests, more information is needed about the importance of the understorey vegetation in ecosystem processes.

In this study, we describe how the structure of understorey vegetation changes along a heavy metal and sulphur gradient near a Cu–Ni smelter in SW Finland. Our aims were (1) to relate the changes in the vegetation to the element concentrations in the organic soil layer and stand structure, (2) to evaluate the sensitivity of plant species to heavy metals according to their occurrence in polluted soil, and (3) to compare the abundances of plant species in the contaminated stands with data from background areas.

2. Materials and methods

2.1. Study area

The study area is situated near the Cu–Ni smelter at Harjavalta (61°19′ N, 22°09′E), SW Finland. The sample plots were established in Scots pine stands along a 8-km transect running to the SE of the smelter. This was the only direction with an unbroken corridor of coniferous forest near the smelter. Apart from the pollution level, the tree stand and site type at the individual plots were originally relatively similar. The study transect has probably not been exposed to the heaviest deposition levels because southerly winds are somewhat dominant (18% of the time) in the area (Derome, 2000a). All the stands were 40–50 years old and growing on dry and relatively infertile sites of the *Calluna* site type (Cajander, 1949) in the southern boreal coniferous zone (Ahti et al., 1968) (Table 1). The soil was fine sand and was classified as orthic podsol. A number of research projects, e.g. fertilisation experiments (Derome and Saarsalmi, 1999; Mälkönen et al., 1999; Derome, 2000b), and studies on soil microbiology (Fritze et al., 1989, 1997) and nutrient fluxes (Helmisaari et al., 1995; Nieminen and Helmisaari, 1996), have already generated a considerable amount of ecological information about the transect.

The Outokumpu Harjavalta Metals smelter complex is one of the largest point sources of heavy metal emissions in Finland. Its main products are copper, nickel and sulphuric acid. The copper smelter has been operating since 1945, and the nickel smelter since 1960. Before the sulphuric acid plant was built in 1947, all the SO_2 produced during the smelting process (annually about 30 000 t) was emitted into the atmosphere, causing severe damage to the surrounding coniferous forests. During 1985–1990, the average annual emissions of Cu from the smelter were 104 t, Ni 50 t, Zn 177 t, SO_2 8100 t and dust 1200 t. During the past few years, however, the emissions have been considerably reduced: in 1993, Cu 50 t, Ni 7 t, SO_2 4700 t and dust 250 t (Helmisaari et al., 1995). The Kemira fertiliser factory produced superphosphate and PK fertilisers at Harjavalta from 1948 to 1989.

Most of the heavy metals have been deposited rather close to the smelter owing to the fact that the smelter stacks were relatively low (70 m) up until 1994, when a 140-m high stack was built. Copper deposition in stand throughfall during 1993–1996 was 369 mg/m^2 at 0.5 km, 12 mg/m^2 at 4 km and 3 mg/m^2 at 8 km. The corresponding values for Ni were 138, 2 and 1 mg/m^2 and for S 1816, 464 and 376 mg/m^2 (Derome and Nieminen, 1998). The 24-h mean SO_2 concentrations in the air have decreased from the level of 38–56 μ g/m^3 in 1987 (1 January–30 June) to 17–18 μ g/m^3 in 1992 (21 January–15 May) within a 1 km radius of the smelter. However, the peak hourly concentrations in 1992 still occasionally reached 500–1000 μ g/m^3 (Saari et al., 1993).

2.2. Sampling design and vegetation analysis

The understorey vegetation was studied at six locations (0.5, 1, 2, 3, 4 and 8 km) along the study transect in August 1993. The vegetation analysis was carried out on three sample plots (30 × 30 m) at each distance (Table 1). Each sample plot was divided into four subplots, and stratified random sampling performed on 16 vegetation quadrats (1 m^2).

Three persons assessed the coverage of plant species using a modification of the point quadrat method (Goodall, 1952). The frame (1 m^2) was divided into 10 × 10 cm squares by means of strings stretched to form grids at two levels. The intersections of the strings formed 81 points; the centre point was excluded because it was placed against the marking tube fixed in the ground. A metal pin (diameter 2 mm) was inserted vertically into the ground at each of the 80 intersection points. Touches between the pin and the same plant species were counted. The point frequency% of each species was used in the statistical analyses. One point was equivalent to a coverage of 1.25% (1/80 × 100). Species occurring in the quadrat but not touched by the pin were recorded as 0.5%, and species occurring on the sample plot but absent in the quadrat as 0.01%. The point frequency method slightly overestimates the spe-

Table 1
General characteristics of the study sites[a]

	Distance from the smelter (km)					
	0.5	1	2	3	4	8
Stand						
Forest site type	CT+	CT+	CT+	CT	CT	CT
Stand age, years	49	67	54	51	48	40
Mean pine height (m)	6.1	10.1	10.9	9.5	9.2	10.6
Mean pine diameter (cm)	10.9	13.6	13.7	12.3	10.3	13.1
Stem volume (m^3/ha)	23.2	98.0	85.3	69.3	67.8	94.5
Number of trees/ha	1008	1048	1230	1436	1517	1552
Volume increment (1981–1990) $m^3\ ha^{-1}\ year^{-1}$	0.31	–	3.78	–	2.78	6.27
Ground layer						
Thickness of organic layer (cm)	2.5	7.2	2.3	3.3	2.2	0.8
Coverage of needle litter (%)	83.3	90.3	74.3	85.0	33.0	31.7
Coverage of decaying wood (%)	8.3	7.6	15.7	5.7	9.7	5.3
Trampled area (%)	14.7	8.7	26.7	9.3	14.3	14.0

[a] The stand data are from the year 1992 and the other measurements from years 1992 and 1993. CT, *Calluna* type, + indicates slightly more fertile type. Ground layer variables are averages of the three sample plots. Coverages of needle litter, decaying wood and trampled area were visually estimated over the whole plot. Stand data according to Mälkönen et al. 1999.

cies coverages as compared to e.g. visual estimates (Vanha-Majamaa et al., 2000).

Vegetation data collected from the permanent sample plots of national forest inventory (NFI) in Finland (1995) were used as the reference level. The reference data comprised 12 sample plots representing 40- to 65-year-old Calluna type pine forests in the southern and middle boreal zones of Finland. Species coverages were visually estimated on 3–4 quadrats (2 m^2 each) on circular sample plots (300 m^2).

2.3. Chemical composition of the organic soil layer

Samples were taken from the organic layer at 25 systematically selected points on each of the same three plots as the vegetation analysis at distances of 0.5, 2, 4 and 8 km in May 1992. Corresponding soil samples were taken from one plot at distances of 1 and 3 km in September 1993. The samples were dried and milled to pass through a 1-mm sieve. pH was measured in water. Organic matter (om) content was determined by loss in weight on ignition by ashing the samples in a muffle furnace at 550°C for 3 h. Exchangeable Cu, Ni, Zn, Fe, Mn, Cd, Pb, Ca, Mg, K and extractable P and S were determined by extraction with 1 M ammonium acetate (pH 4.65) + 1% EDTA, followed by analysis by inductively coupled plasma atomic emission spectrometry. Total N was determined on a CHN analyser. EDTA was used in the extractant because a chelating agent is known to be necessary when extracting high concentrations of Cu and Fe from organic material. All element concentrations are expressed with respect to the organic matter content, in order to reduce the variation arising from the inclusion of mineral soil in the organic layer samples. Removal of the organic layer was problematic at some of the sampling sites.

2.4. Data analysis

The vegetation data were ordered by GNMDS (DECODA 2.04 software of Minchin, 1991) to relate the vegetation composition to the chemical soil properties and stand structure [see Minchin (1987) and Kent and Coker (1992) for the method]. Species occurring only once were excluded from the analysis. The Bray–Curtis (Czekanowski) coefficient was used as a measure of dissimilarity in floristic composition between the sample plots. The ordination distances were scaled to half-change units. Two- and three-dimensional GNMDS solutions were carried out with 10 randomly generated starting configurations. All 10 iterations gave effectively identical minimum stresses (two-dimensional: 0.058, three-dimensional: 0.028) within both solutions. One iteration was arbitrarily selected for the further analysis. Maximum (canonical) correlations between the environmental variables and the ordination configuration were calculated using the vector fitting procedure of DECODA. The significance of the correlations were assessed with Monte Carlo tests. Because the third dimension did not provide new information, the two-dimensional solution was selected. The weighted averages of the species were calculated in the ordination space.

The relationships between the distance from the smelter and the plotwise means of environmental variables, species richness (S), indices of Shannon diversity ($H' = -\sum_{i=1}^{s}(p_i \times \ln p_i)$, s = number of species, p_i proportion of the ith species of the total abundance) and species evenness ($E = H'/\ln S$), as well as the total abundance of the understorey, were calculated by Kendall non-parametric correlations (SAS, 1994). Dominance-diversity curves were drawn to display the log-scaled abundance of the fifteen most abundant species on the sample plots against their ranking from most to least abundant. Kruskal–Wallis non-parametric analysis of variance (SAS, 1994) was used in comparing species abundances at a distance of 8 km and in the NFI reference data.

3. Results

Clear differences in the distribution pattern of the plant species and in the chemical composition of the organic layer were observed on moving towards the smelter (Fig. 1). The total number of plant species increased from 8 at 0.5 km to 30 at 8 km (Table 2). The total coverage of the vegetation also increased with

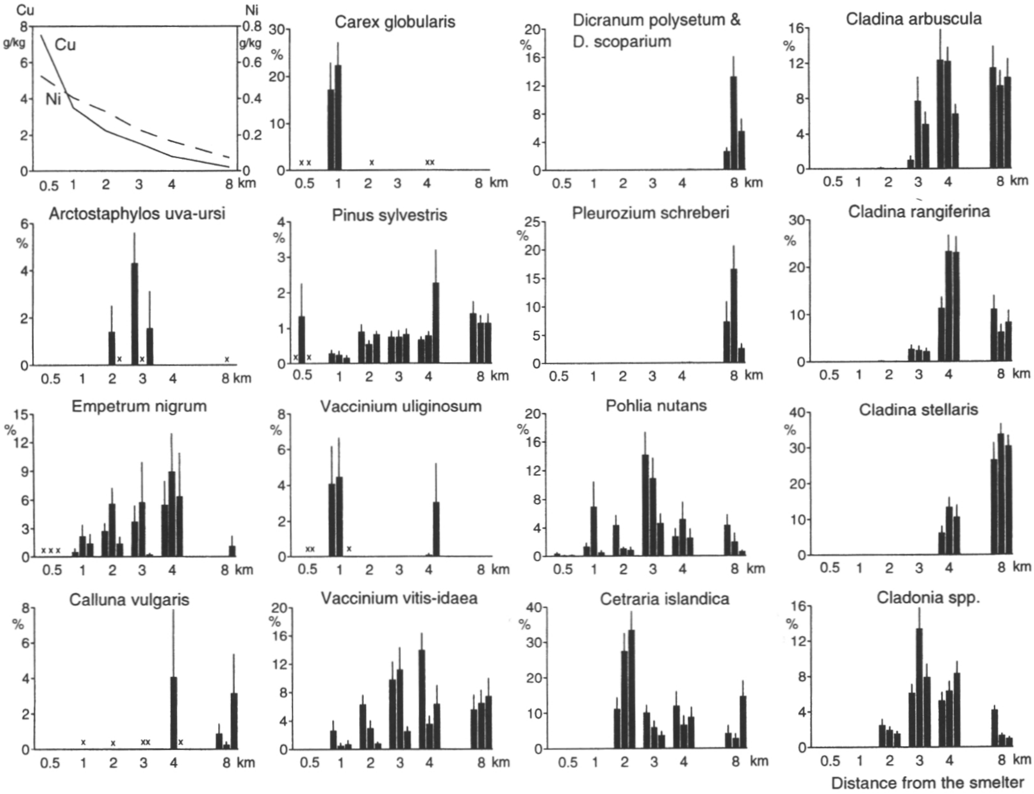


Fig. 1. The mean point frequencies (%) of the plant species on the sample plots (columns) at different distances from the smelter (km). Species observed on the sample plots but not on the sample quadrats are marked with x. The line indicates SEM. The distributions of exchangeable Cu and Ni in the organic layer are given in the upper left panel. Note the different scales on the *y* axes.

increasing distance from the smelter (Tables 2 and 4). A few species accounted for most of the abundance of the communities near the smelter (0.5–2 km), but at greater distances (3–8 km) the species distributions were more even (Fig. 2).

At 0.5 km, the exchangeable Cu concentration of the organic layer was 36 times higher (7540 mg/kg om) than that at 8 km (200 mg/kg om). The gradients were not as steep for the other heavy metals, e.g. the exchangeable Ni concentrations were 527 mg/kg om at 0.5 km and 72 mg/kg om at 8 km (Fig. 1, Table 3).

3.1. Vegetation pattern related to environmental gradients

In general, the ordination configuration was highly related to the heavy metal concentrations in the organic layer and to the stand structure in GNMDS (Fig. 3). The sample plots were positioned according to the distance from the smelter along the main compositional gradient, which runs parallel to the first dimension. The species scores were arranged in three groups: (1) sensitive species (present only at greater distances from the smelter e.g. *Pleurozium schreberi*), (2) resistant species which grew on both polluted and less-polluted sample plots (e.g. *Pohlia nutans*), and (3) resistant species, the occurrence of which near the smelter was restricted to paludified microhabitats (e.g. *Vaccinium uliginosum*).

The correlations of the exchangeable heavy metal (Cu, Ni, Fe, Pb) and sulphur concentrations in the organic soil layer and the ordination space were highly significant ($P < 0.001$), and showed an increasing trend with decreasing distance from the smelter (Fig. 3a, Table 4). An opposite trend was found for some of the macro-nutrients (e.g. Ca, K and Mg), as well as for Mn. The mean height and the number of the overstorey trees increased with increasing distance from the smelter (Fig. 3a, Table 4). The diversity and the total abundance of the plant species also increased as a function of increasing distance (Table 4). The initial heterogeneity among the sample plots was reflected in the second dimension. Mire species (e.g. *Ledum palustris* and *Carex*

Table 2
Plotwise means for point frequency% of the plant species at different distances from the smelter[a,b]

	Distance from the smelter (km)						Reference
Species	0.5	1	2	3	4	8	NFI 95
Vascular plants							
Andromeda polifolia L.	0.00	0.00	0.00	0.00	0.01	0.00	0.11±0.11
Arctostaphylos uva-ursi (L.) Sprengel	0.00	0.00	0.47	1.95	0.00	0.01	0.04±0.04
Betula pendula Roth.	0.00	0.00	0.01	0.00	0.00	0.00	0.00±0.00
Betula pubescens Ehrh.	0.01	0.01	0.01	0.00	0.00	0.01	0.15±0.11
Calluna vulgaris (L.) Hull	0.00	0.01	0.01	0.01	1.35	1.41	12.74±3.09 *
Carex globularis L.	0.01	13.13	0.01	0.00	0.01	0.00	0.09±0.05
Deschampsia flexuosa (L.) Trin.	0.00	0.01	0.00	0.00	0.00	0.01	1.18±0.83
Empetrum nigrum L.	0.01	1.34	3.20	3.20	6.89	0.36	1.53±0.81 ns
Epilobium angustifolium L.	0.00	0.01	0.01	0.01	0.00	0.01	0.04±0.04
Ledum palustre L.	0.00	1.72	0.00	0.00	0.01	0.00	0.03±0.03 ns
Picea abies (L.) H. Karst.	0.00	0.01	0.01	0.01	0.00	0.01	0.04±0.04
Pinus sylvestris L.	0.44	0.22	0.76	0.77	1.24	1.22	0.06±0.03 **
Populus tremula L.	0.00	0.00	0.00	0.01	0.00	0.00	0.06±0.04
Salix aurita L.	0.01	0.00	0.00	0.00	0.00	0.00	0.00±0.00
Vaccinium myrtillus L.	0.00	0.52	0.07	0.00	0.00	0.03	0.79±0.47 ns
Vaccinium uliginosum L.	0.01	2.84	0.00	0.00	1.04	0.00	2.52±1.61 ns
Vaccinium vitis-idaea L.	0.00	1.21	3.29	7.79	7.90	6.46	7.94±1.34 ns
Other species							0.09±0.06
Total abundance per plot	0.48	21.01	7.83	13.73	18.44	9.52	27.37±4.22 ns
Number of species per plot	4.00	9.00	8.00	5.67	5.67	5.67	
Total number of species per distance	6	12	11	8	8	10	
Bryophyta							
Ceratodon purpureus (Hedw.) Brid.	0.01	0.05	0.01	0.00	0.00	0.00	0.00±0.00
Dicranum polysetum Sw.	0.00	0.00	0.00	0.00	0.00	6.65	3.67±0.93 ns
Dicranum scoparium Hedw.	0.00	0.00	0.00	0.00	0.03	0.39	0.68±0.36 ns
Ortodicranum montanum (Hedw.) Loeske	0.00	0.00	0.01	0.00	0.00	0.00	0.00±0.00
Pleurozium schreberi (Brid.) Mitt.	0.00	0.00	0.01	0.00	0.07	8.72	30.39±6.06 ns
Pohlia nutans (Hedw.) Lindb.	0.17	2.89	2.02	9.83	3.42	2.25	0.17±0.10**
Polytrichum juniperinum Hedw.	0.00	0.31	0.01	0.00	0.01	0.12	0.92±0.66
Ptilidium ciliare (L.) Hampe	0.00	0.00	0.01	0.08	0.38	0.07	0.02±0.02
Other species							2.53±1.07
Total abundance per plot	0.17	3.26	2.06	9.91	3.90	18.21	38.46±6.44 ns
Number of species per plot	1.33	2.33	3.00	1.67	3.67	5.67	
Total number of species per distance	2	3	6	2	5	6	
Ground lichens							
Cetraria islandica (L.) Ach.	0.00	0.00	23.91	6.41	9.01	7.04	0.48±0.32 **
Cladina arbuscula (Wallr.) Hale&W.L.Club.	0.00	0.00	0.04	4.57	10.22	10.36	9.52±2.73 ns
Cladina rangiferina (L.) Nyl.	0.00	0.00	0.08	2.37	19.14	8.45	10.21±3.37 ns
Cladina stellaris (Opiz) Brodo	0.00	0.00	0.00	0.03	9.99	30.10	5.40±4.42 *
Cladonia bellidiflora (Ach.) Schaer.	0.00	0.00	0.00	0.00	0.01	0.00	0.00±0.00
Cladonia botrytes (K.G. Hagen) Willd.	0.00	0.00	0.01	0.00	0.01	0.04	0.00±0.00
Cladonia cenotea (Ach.) Schaer.	0.00	0.00	0.00	0.00	0.04	0.00	0.01±0.01
Cladonia chlorophaea coll. (Flörke ex Sommerf.) Spreng.	0.00	0.00	0.30	3.39	2.25	0.59	0.04±0.02
Cladonia coccifera (L.) Willd.	0.00	0.00	0.00	0.00	0.01	0.00	0.00±0.00
Cladonia coniocraea (Flörke) Spreng.	0.00	0.00	0.12	0.07	0.21	0.08	0.00±0.00
Cladonia cornuta (L.) Hoffm.	0.00	0.00	1.03	1.99	1.60	0.47	0.04±0.02
Cladonia crispata (Ach.) Flot.	0.00	0.00	0.00	0.35	0.29	0.21	0.01±0.01
Cladonia deformis L. (Hoffm.) + *Cladonia sulphurina* (Michx.) Fr.	0.00	0.00	0.08	0.55	0.89	0.27	0.07±0.06
Cladonia digitata (L.) Hoffm.	0.00	0.00	0.04	0.30	0.13	0.05	0.00±0.00
Cladonia fimbriata (L.) Fr.	0.00	0.00	0.00	0.03	0.05	0.05	0.00±0.00
Cladonia furcata (Huds.) Schrad. + *Cladonia turgida* Hoffm.	0.00	0.00	0.03	0.14	0.03	0.01	0.00±0.00
Cladonia gracilis (L.) Willd. ssp. *gracilis* (Flörke) + ssp. *turbinata* (Ach.) Ahti	0.00	0.00	0.31	2.24	1.02	0.29	0.03±0.02

(continued on next page)

Table 2 (*continued*)

	Distance from the smelter (km)						Reference
Species	0.5	1	2	3	4	8	NFI 95
Cladonia phyllophora Hoffm.	0.00	0.00	0.00	0.03	0.00	0.00	0.00±0.00
Cladonia uncialis (L.) F.H.Wigg.	0.00	0.00	0.00	0.20	0.01	0.00	0.01±0.01
Cladonia squamosa (Scop.) Hoffm.	0.00	0.00	0.00	0.01	0.05	0.00	0.00±0.00
Stereocaulon paschale (L.) Hoffm.	0.00	0.02	0.01	0.16	0.00	0.00	0.01±0.01
Total abundance per plot	0.00	0.02	25.94	22.81	54.96	58.02	26.04±5.93 °
Number of species per plot	0.00	1.00	8.33	13.00	14.67	12.67	
Total number of species per distance	0	1	12	17	19	14	
All species							
Total abundance per plot	0.65	24.29	35.97	46.50	77.38	85.77	91.86±8.20 **
Number of species per plot	5.33	12.00	19.33	20.33	24.00	24.00	
Total number of species per distance	8	16	29	27	32	30	

[a] $n=3$, 16×1 m^2 quadrats per plot.

[b] Reference data are based on the mean% coverages (± SEM) of the species at 12 sample plots (3–4 × 2 m^2 quadrats). Mean total abundances and number of species per plot are given. Abundances of the most abundant species are compared between 8 km and the reference data using Kruskal–Wallis tests (** = $P < 0.01$, * = $P < 0.05$, ° = $P < 0.10$, ns = not significant).

globularis) and a thick organic layer with low total N and pH were typical of the sample plots at 1 km. High exchangeable Zn, Cd and S concentrations were also found at 1 km (Fig. 3, Table 3).

3.2. The damage areas

Four damage areas were distinguished along the pollution gradient on the basis of the understorey vegetation, the condition of the overstorey trees and the element concentrations of the organic soil layer.

3.2.1. Area of severe damage (0.5 and 1 km from the smelter)

The sample plots at 0.5 and 1 km distances from the smelter deviated the most from the other plots (Fig. 3). The understorey vegetation was almost totally dead up to a distance of 0.5 km. Most of the overstorey was alive, but the pines were stunted (Table 1), damaged and suffering from needle discolouration. The forest floor was covered with relatively undecomposed needle litter and the organic layer was thin (Table 1). Lichens and mosses were absent, except for the resistant pioneer mosses *Pohlia nutans* and *Ceratodon purpureus*. Only a few patches of *Empetrum nigrum* ssp. *nigrum*, *Carex globularis*, and *Vaccinium uliginosum* were present (Fig. 1 and Table 2). The two latter species were frequently growing in paludified depressions. Some saplings of *Pinus sylvestris* and *Betula pubescens* were also found. The surviving vascular plants were suffering from a wide range of injury and damage, e.g. dead branches and leaf discolouration.

The sample plots at 1 km differed to some extent from the other plots in having a thick organic layer and older tree stand (Fig. 3a, Table 1). One plot had almost no vegetation cover, and resembled the plots at 0.5 km. However, the total number of vascular plant species was the highest ($n=12$) at 1 km distance, *Carex globularis* and *Vaccinium uliginosum* being the most abundant species (Fig. 1, Table 2). These species, as well as *Ledum palustre*, indicated paludification of the site.

Both 0.5 km and 1 km sites were classified in the severe damage class owing to the low number of moss and lichen species (< 5), low total abundance of vege-

Table 3
pH, total N (%) and exchangeable concentrations of macro-elements and heavy metals in the organic soil layer[a]

	Distance from the smelter (km)					
	0.5	1	2	3	4	8
pH (H_2O)	3.84	3.34	3.66	3.62	3.56	3.62
Macro nutrients						
tot N% om	1.92	1.31	1.74	1.58	1.65	1.68
P mg/kg om	845	387	274	370	196	213
K mg/kg om	168	485	445	586	623	607
Mg mg/kg om	51	215	157	180	188	163
S mg/kg om	387	402	198	176	170	176
Ca mg/kg om	594	1563	1926	1868	2000	1722
Heavy metals						
Fe mg/kg om	10899	5792	3800	3648	1902	1659
Cu mg/kg om	7540	3501	2238	1532	786	209
Ni mg/kg om	527	406	329	230	164	72
Zn mg/kg om	167	308	144	139	153	82
Pb mg/kg om	468	177	230	196	136	122
Mn mg/kg om	12.5	59.0	135.5	148.7	75.1	91.0
Cd mg/kg om	2.83	4.74	2.59	2.97	2.04	1.09

[a] Results are expressed on the basis of the organic matter content (om). Averages from three plots are presented for distances of 0.5, 2, 4, and 8 km, and from one plot at 1 and 4 km.

Table 4
Plotwise non-parametric Kendall correlations (tau) between the distance from the smelter and the environmental and plant community variables[a]

	Distance from the smelter	
	tau	*P*
Organic soil layer		
Thickness	−0.469	0.010**
pH	0.185	0.314
Element concentrations		
N	−0.021	0.908
P	−0.703	0.000***
K	0.532	0.004**
Mg	0.135	0.462
S	−0.547	0.003**
Ca	0.433	0.018*
Fe	−0.845	0.000***
Cu	−0.958	0.000***
Ni	−0.916	0.000***
Zn	−0.490	0.008**
Pb	−0.674	0.001***
Mn	0.419	0.022*
Cd	−0.504	0.006**
Overstorey trees		
Stand age	−0.333	0.079
Mean diameter	0.174	0.336
Mean height	0.327	0.070
Number of trees	0.475	0.009**
Stem volume	0.216	0.233
Ground layer		
Trampled area (%)	−0.099	0.589
Needle litter (%)	−0.596	0.001***
Decaying wood (%)	−0.159	0.393
Plant community		
Total abundance	0.566	0.002**
Species richness, *S*	0.733	0.000***
Evenness, *E*	0.188	0.299
Diversity, *H′*	0.508	0.005**

[a] Number of sample plots, $n=18$. P=statistical significance (***=$P < 0.001$, **=$P < 0.01$ and *=$P < 0.05$).

tation (< 25%) (Table 2) and high exchangeable heavy metal and extractable sulphur concentrations in the organic layer (Table 3). The Cu, Fe and Pb concentrations at 0.5 km were about double those at 1 km. In contrast, the Zn and Cd concentrations at 1 km were strongly elevated compared to the other distances. The concentrations of exchangeable Ca and K, and especially Mg, at 0.5 km were much lower than those at the other sites.

3.2.2. *Area of moderate damage (2 and 3 km)*

The growth of the tree stand was considerably better and the total coverage of the understorey vegetation was higher (Table 2) at distances of 2 and 3 km than those at 0.5 and 1 km. Many species typical of dry heath forests (e.g. *Calluna vulgaris*, *Empetrum nigrum* and *Vaccinium vitis-idaea*) were present, but their abundances were much lower than those in the NFI reference data (Table 2). The understorey vegetation was not closed (Table 2) and the coverage of needle litter was still 70–80% (Table 1). The abundance of *Cetraria islandica* was highest (24%) at 2 km, and *Cladonia* spp. (cup lichens) at 3 km (Fig. 1, Table 2). The first specimens of *Cladina arbuscula* and *C. rangiferina* were recorded at 2 km, and of *C. stellaris* and *Cladonia uncialis* at 3 km. *Ptilidium ciliare* and *Ortodicranum montanum* were two moss species recorded for the first time. The abundance of *Pohlia nutans* was exceptionally high. *Arctostaphylos uva-ursi* appeared for the first time at 2 km.

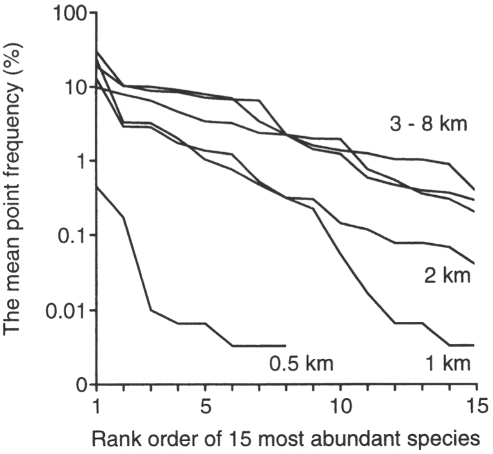


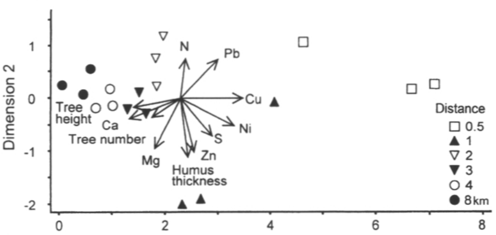
Fig. 2. Dominance–diversity curves of the log-transformed abundances (point frequency%) of the 15 most common species on sample plots at different distances from the smelter.

The exchangeable Cu and Fe concentrations were considerably lower than those at the closest sites, but still much higher than those at greater distances from the smelter (Table 3). The exchangeable Mn concentrations at these two sites were at their highest values along the transect, but the macronutrient concentrations at a relatively normal level.

3.2.3. *Area of slight damage (4 km)*

The floristic composition at 4 km resembled that of normal dry heath forests. However, typical mosses were still missing or were, as in the case of *Pleurozium schreberi* and *Dicranum* spp., very scarce (Table 2). There were no large gaps in the ground vegetation, and the coverage of needle litter was only 30% (Table 1). The highest number of cup lichen species ($n=13$) was recorded at this distance, the most abundant being *Cladonia chlorophaea* coll., *C. cornuta*, *C. deformis* and *C. sulphurina* group and *C. gracilis* ssp. *turbinata* (Table 2). Heavy metal concentrations in the organic layer, apart from Zn, were much closer to those at 8 km (Table 3).

a) Sample plots



b) Species

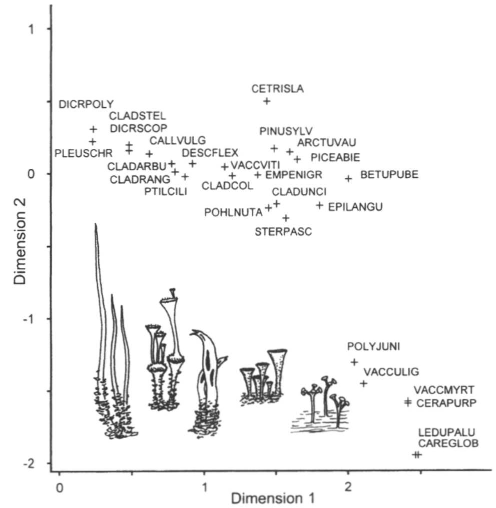


Fig. 3. Two-dimensional solution of GNMDS: (a) Ordination of the sample plots and the fitted vectors of selected environmental variables; (b) weighted averages of the species. The arrows visualize the correlations between the environmental variables and the sample plot ordination. The length of the arrow indicates the magnitude of the correlation, and the direction of the arrow the polarity. Abbreviation of species names = first four letters from generic and species names (see Table 2). km = distance from the smelter. Cup lichens (*Cladcol*) were combined. The most abundant cup lichens (*Cladonia cornuta, C. gracilis* ssp. *turbinata, C. deformis (sulphurina), C. chlorophaea and C. botrytes* are depicted in the lower left corner of the species ordination figure.

3.2.4. Area of minimum disturbance (8 km)

The abundance of some species such as *Vaccinium vitis-idaea* approached the typical coverage for dry heath forests, but that of *Calluna vulgaris*, which is one of the characteristic species of dry boreal heaths, was still low (< 2%). In the NFI reference material its coverage was significantly higher (Kruskall–Wallis 1-way ANOVA: $F = 5.35$, $P < 0.05$, df = 1), being over 10% (Table 2). The abundances of *Dicranum* spp. mosses and *Cladina arbuscula* and *C. rangiferina* were rather typical, but that of *Pleurozium schreberi* to some extent lower ($P < 0.11$). In contrast, the abundance of *C. stellaris* was higher ($P < 0.05$) than that in the NFI material. The total abundance of all species was 86% at 8 km, which was lower ($P < 0.01$) than that in the reference (92%). The heavy metal concentrations were much lower than at sites closer to the smelter (Table 3), but higher than those for background areas (e.g. 7.4 mg/kg Cu and 5.1 mg/kg Ni in the organic soil layer; Tamminen and Starr, 1990).

4. Discussion

The understorey vegetation near the smelter was more damaged than the overstorey trees. Compared to damage areas around much larger industrial complexes in the boreal forest zone, e.g. in Sudbury, Ontario (Freedman and Hutchinson, 1980; Winterhalder, 2000) or in Monchegorsk, the Kola Peninsula (Rigina and Kozlov, 2000), where the tree stands are dead over wide areas, there was no "industrial desert area" at Harjavalta. The majority of pines growing in the immediate surroundings of the smelter died during the 1940s, as a result of the high SO_2 emissions from the new smelter. The present stand was planted about 50 years ago. Today the only treeless area, restricted in size, is to be found on the opposite side of the study transect.

It is clear that, in addition to the deposition of heavy metals, high SO_2 emissions have also earlier had a marked effect on the vegetation. In the 1990s, however, the ambient SO_2 concentrations have decreased, and the heavy metals that have accumulated in the soil most likely represent the greatest threat to plant vigour. However, occasional peak SO_2 concentrations may damage the most sensitive plant species, e.g. mosses and lichens (Bates, 1992). Nutrient imbalances (Derome and Lindroos, 1998) and a decreased water-holding capacity of the soil (Derome and Nieminen, 1998) have strengthened the selection pressure on plants growing in the most polluted area. The increased illumination in sparse, defoliated stands has also had a strong effect on plant establishment.

Owing to strong abiotic control, a few tolerant species were dominant in the ground vegetation on the most polluted sites. It is probable that between-species competition was insignificant in these harsh conditions, where the total coverage of the vegetation was low. The increased heterogeneity of the environment offered more niches to a greater number of species further away from the smelter. Competition had a more profound effect when the vegetation was closed. As a consequence, the total coverage was divided more evenly between the species (Fig. 2).

Mosses and lichens cannot prevent the passage of heavy metals or other toxic ions into their shoots or thalli, because they absorb nutrients directly from rainwater or the air, and have no protective cuticle (Nash, 1989; Tyler, 1990; Bates, 1992). In addition to elements derived from the atmosphere, mosses also take up ions from water that has been in contact with the organic layer of the soil (Økland et al., 1999). Vascular plants, in contrast, can to some extent select which elements are taken up by their roots. In many cases, however, the element concentrations inside roots closely follow those in the substrate, especially when the exclusion mechanisms break down in high concentrations (Kahle, 1993; Punz and Sieghardt, 1993; Monni et al., 2000a,b).

The rooting depth of the dwarf shrubs growing in boreal forests is rather shallow, the majority of the roots growing in the organic and uppermost (0–10 cm) mineral soil layers (Makkonen and Helmisaari, 1998). *Empetrum nigrum* clones surving at a distance of 0.5 km from the Harjavalta smelter were an exception to this: their roots extended down to a depth of 50 cm into cleaner soil horizons (Uhlig et al., 2001). Compared to coniferous trees, dwarf shrubs generally have a more superficial root system, which may increase their exposure to the high concentrations of heavy metals in the topsoil.

Without experimental exposures, it is difficult to identify the contribution of individual pollutants to the injuries of plant species. The biological availability of heavy metals to plants depends on the soil characteristics (e.g. the amount of organic matter, pH and the chemical composition) (Balsberg Påhlsson, 1989; Kahle, 1993; Punz and Sieghardt, 1993). Because different metal ions have antagonistic, additive or synergistic interactions, their actual absorption rate and toxicity may be modified according to the overall element composition in the soil (Kahle, 1993).

The general responses of the lichen and moss communities along the Harjavalta transect were very similar to those described in the coniferous forests near the Cu–Zn smelter in Gusum, SE Sweden, although no SO_2 was emitted from the latter point source (Folkeson, 1984; Tyler, 1984; Folkeson and Andersson-Bringmark, 1988). Our results confirm the observation that common mosses are the most sensitive plant group in boreal forests to an increased heavy metal load (Freedman and Hutchinson, 1980; Väisänen, 1986; Mäkinen, 1994). Although the frequency of *Pleurozium schreberi* and *Dicranum* spp. began to increase at a distance of 8 km, the Cu concentrations in their tissues were considerably higher (160–180 mg/kg) than those in background areas (6–8 mg/kg) (Helmisaari et al., 1995).

The reindeer lichens (*Cladina* spp.) were more tolerant than forest mosses, but they did not increase until a distance of 4 km from the smelter. *C. stellaris* appeared to suffer more from the pollutants than *C. arbuscula* or *C. rangiferina*. *Cladina* species did not accumulate Cu as effectively as forest mosses at the same distances (Helmisaari et al. 1995), which partly explains their better resistance. The abundance distributions of *Pohlia nutans* and cup lichens (*Cladonia* spp.) along the transect were approximately bell-shaped. Their increase in abundance indicates the lack of stronger competitors halfway (3–4 km) along the transect. A similar response of *Cladonia* spp., but not of *P. nutans*, was observed near the Gusum smelter (Folkeson and Andersson-Bringmark, 1988). These species are pioneers that typically rapidly colonise vacated or disturbed habitats. Later succession stages of ground lichen communities have been found to be more sensitive to air pollutants than younger ones also on the Kola Peninsula (Gorshkov, 1993).

Stereocaulon paschale and *Cetraria islandica* proved to be rather tolerant lichen species, the former species being found at 1 km and the latter at 2 km from the smelter. *P. nutans* and *C. purpureus* were the only moss species surviving in small patches on the most contaminated site closest to the smelter. *P. nutans, S. paschale* and *Cladonia* spp. have been reported to withstand high ambient SO_2 levels and elevated Cu and Ni concentrations in the soil at distances of 3–8 km from the smelter complex in Sudbury, Ontario (Freedman and Hutchinson, 1980). *C. islandica* has been found to grow at distances of 4–30 km from the Severonikel smelter in Monchegorsk, the Kola Peninsula (Gorshkov, 1993; Chernenkova and Kuperman, 1999).

Arctostaphylos uva-ursi, Empetrum nigrum ssp. *nigrum* and *Vaccinium uliginosum* are clonal dwarf shrubs, which have survived on the most polluted sites. According to the annual rings on the oldest part of the stem, the age of the clones were at least 30–40 years (Salemaa et al., 2000). Because dwarf shrubs normally regenerate vegetatively, it is probable that some "mother clones" date back to the time when the smelter first started operating in the 1940s, thus representing the most resistant genotypes of the earlier populations.

Although *A. uva-ursi* was not present until 2 km along the study transect, it was found growing at a distance of 0.5 km on the opposite side of the smelter (unpublished observation). *A. uva-ursi* and *E. nigrum* ssp. *hermafroditum* have also been found very close to the Monchegorsk smelter on the Kola Peninsula (Lukina et al., 1993). Compared to the vegetation inventory made at Harjavalta in the 1970s, *A. uva-ursi* and *Calluna vulgaris* seem to have declined, while the abundances of *E. nigrum* and *Vaccinium vitis-idaea* have remained unchanged during the last 20 years (Laaksovirta and Silvola, 1975). Experimental Cu exposure in a greenhouse has shown that *C. vulgaris* is clearly more sensitive than *E. nigrum* (Monni et al., 2000a), which explains its absence from the most polluted site. In contrast, *E. nigrum*, has proved to be a very resistant species, and can accumulate considerably high concentrations of Cu and Ni especially in its stems and restrict their transport to the leaves (Monni et al., 2000b; Uhlig et al., 2000). However, the many signs of dead *E. nigrum* clones near the smelter indicate that the levels of heavy metals and sulphur have been too high for the majority of the *E. nigrum* population.

The tolerance mechanisms of vascular species to heavy metals have been studied to a considerable extent (e.g. Verkleij and Schatt, 1989; Turner, 1994). Resistance is achieved either by restricting metal uptake by the plant (avoidance) or by binding heavy metals in cellular compartments so that sensitive metabolic processes are not affected (tolerance) (Levitt, 1980; Baker, 1987; Tyler et al., 1989). Ericoid mycorrhizas have the ability to restrict the transport of heavy metals to shoots (Bradley et al., 1981). Clonal dwarf shrubs can live for decades, and they express high phenotypic plasticity in all their characteristics. *V. uliginosum* and *A. uva-ursi* showed strong regrowth after artificial shoot clipping in a field experiment carried out at Harjavalta (Salemaa et al., 1999). It was suggested that their high ability to compensate for the lost biomass is one factor contributing to their resistance to heavy metals. Plastic growth responses have been found to be typical also of *Empetrum nigrum* and deciduous *Vaccinium* species growing along a pollution gradient on the Kola Peninsula (Shevtsova, 1998). In general, ecotypic differentiation into metal-tolerant races is rare or very slow in long-lived plants (Dickinson et al., 1991; Turner, 1994).

Increased coverages of grasses are often found in moderately polluted zones in the vicinity of point sources (Huttunen, 1975; Väisänen, 1986; Rigina and Kozlov, 1999). It is known that many grass species are able to evolve rapidly into tolerant ecotypes (Baker, 1987). Along the Harjavalta transect, only *Deschampsia flexuosa* grew at a low frequency. On the other hand, a sedge species, *Carex globularis,* grew successfully at paludified points on the contaminated sites at 0.5–1 km. Nitrogen application seems to have slightly increased the abundance of *D. flexuosa* and *Epilobium angustifolium* on fertilised sample plots (unpublished data), but the general response to fertilisation was very low.

In contrast to the situation in many other smelter areas, soil acidification did not increase on moving towards the smelter at Harjavalta (Derome and Lindroos, 1998). Apart from the site at 1 km, the pH of the organic layer was higher than 3.5 at all sites along the transect. Soil paludification at 1 km was reflected in the increased acidity (pH 3.3). The high concentrations of exchangeable Zn and Cd, which are more mobile than the other heavy metals, may originate from the slag heaps located close to the site at 1 km. These elements may have been leached from the surrounding soil or been deposited as particles and accumulated in the paludified depressions.

The accumulated heavy metals have brought about a chronic disturbance in the ecosystem, preventing the normal succession of the plant communities. Colonisation of mosses and lichens is also prevented because the large amounts of undecomposed litter suppress their growth. Although viable seeds of many dwarf shrubs and tree species have been found in the soil seed banks in the polluted areas, seedling establishment is unsuccessful (Salemaa and Uotila, 1996). It would appear, that the closest site will continue to deteriorate, but the decreased emissions will enable the vegetation to recover gradually at further distances.

5. Conclusions

The species composition of the plant communities has changed, sensitive plant species have disappeared and competitive interactions between the species have been altered as a result of 50 years' activity of the Cu–Ni smelter at Harjavalta. According to their occurrence along the pollution gradient, forest mosses were the most sensitive plant group, followed by lichens. The vascular plants included both sensitive and resistant species. In general, the understorey vegetation seemed to respond more sensitively to the pollution load than the overstorey trees, which confirms the importance of including understorey vegetation in national and international monitoring programmes of forest health.

Acknowledgements

We are grateful to Leila Korpela, Sinikka Levula, Teuvo Levula and Pekka Suolahti for helping in the field work. Markku Tamminen and Tiina Tonteri advised in the data analysis. Krister Karttunen and Sampsa Lommi checked the identification of some of the species, and Hannu Nousiainen drew the cup lichens presented in Fig. 3. Heljä-Sisko Helmisaari, Leila Korpela and Satu Monni commented on the manuscript. We would also like to thank Professor Eino Mälkönen for the possibility to use the data collected from the forest health fertilisation sample plots and Professor Erkki Tomppo for the vegetation data from national forest inventory.

References

Ahti, T., Hämet-Ahti, L., Jalas, J., 1968. Vegetation zones and their sections in northwestern Europe. Annales Botanici Fennici 5, 9–211.

Alloway, B.J., 1995. Soil processes and the behaviour of metals. In: Alloway, B.J. (Ed.), Heavy Metals in Soils. Blackie Academic & Professional, London, pp. 11–37.

Axelsson, B., Bråkenhielm, S., 1980. Investigation sites of the Swedish coniferous forest project — biological and physiographical features. In: Persson, T. (Ed.), Structure and Function of Northern Coniferous Forests — An Ecosystem Study. Ecological Bulletins (Stockholm) 32, 25-64.

Bååth, E., 1989. Effects of heavy metals in soil on microbial processes and populations (a review). Water, Air and Soil Pollution 47, 335–379.

Baker, A.J.M., 1987. Metal tolerance. New Phytologist 106, 93–111.

Balsberg Påhlsson, A.-M., 1989. Toxicity of heavy metals (Zn, Cu, Cd, Pb) to vascular plants. A literature review. Water, Air and Soil Pollution 47, 287–319.

Bates, J.W., 1992. Bryophytes and lichens as target organisms. In: Ashmore, M.R., Wilson, R.B. (Eds.), Critical Levels of Air Pollutants for Europe. Background papers prepared for the United Nations Economic Commission for Europe Workshop on Critical Levels. Egham, UK, 23–26 March 1992, pp. 113-124.

Berthelsen, B.O., Steinnes, E., Solberg, W., Jingsen, L., 1995. Heavy metal concentrations in plants in relation to atmospheric heavy metal deposition. Journal of Environmental Quality 24, 1018–1026.

Bradley, R., Burt, A.J., Read, D.J., 1981. Mycorrhizal infection and resistance of heavy metal toxicity in *Calluna vulgaris*. Nature 292, 335–337.

Cajander, A.K., 1949. Forest types and their significance. Acta Forestalia Fennica 56, 1–71.

Chernenkova, T.V., Kuperman, R.G., 1999. Changes in the forest communities along a heavy metal deposition gradient on Kola Peninsula. Water, Air and Soil Pollution 111, 187–200.

Derome, J., 2000a. Effects of heavy-metal and sulphur deposition on the chemical properties of forest soil in the vicinity of a Cu–Ni smelter, and means of reducing the detrimental effects of heavy metals. Research paper 769, Finnish Forest Research Institute.

Derome, J., 2000b. Detoxification and amelioration of heavy-metal contaminated forest soils by means of liming and fertilisation. Environmental Pollution 107, 79–88.

Derome, J., Lindroos, A.-J., 1998. Effects of heavy metal contamination on macronutrient availability and acidification parameters in forest soil in the vicinity of Harjavalta Cu–Ni smelter, SW Finland. Environmental Pollution 99, 225–232.

Derome, J., Nieminen, T., 1998. Metal and macronutrient fluxes in heavy-metal polluted Scots pine ecosystems in SW Finland. Environmental Pollution 103, 219–228.

Derome, J., Saarsalmi, A., 1999. The effect of liming and correction fertilisation on heavy metal and macronutrient concentrations in soil solution in heavy-metal polluted Scots pine stands. Environmental Pollution 104, 249–259.

Dickinson, N.M., Turner, A.P., Lepp, N.W., 1991. How do tree and other long-lived plants survive in polluted environments? Functional Ecology 5, 5–11.

Folkeson, L., 1984. Deterioration of the moss and lichen vegetation in a forest polluted by heavy metals. Ambio 13, 37–39.

Folkeson, L., Andersson-Bringmark, E., 1988. Impoverishment of vegetation in a coniferous forest polluted by copper and zinc. Canadian Journal of Botany 66, 417–428.

Fowler, D.P., Cape, J.N., Unsworth, M.H., 1989. Deposition of atmospheric pollutants on forests. Philosophical Transactions of the Royal Society of London, Series B 324, 247–265.

Freedman, B., Hutchinson, T.C., 1980. Long-term effects of smelter pollution at Sudbury, Ontario, on forest community composition. Canadian Journal of Botany 58, 2123–2140.

Fritze, H., Pennanen, T., Vanhala, P., 1997. Impact of fertilizers on the humus layer microbial community of Scots pine stands growing along a gradient of heavy metal pollution. In: Insam, H., Rangger, A. (Eds.), Microbial Communities. Functional versus Structural Approaches. Springer-Verlag, pp. 68–83.

Fritze, H., Niini, S., Mikkola, K., Mäkinen, A., 1989. Soil microbial effects of a Cu–Ni smelter in southwestern Finland. Biological Fertility of Soils 8, 87–94.

Goodall, D.W., 1952. Some considerations in the use of point quadrats for the analysisi of vegetation. Australian Journal of Scientific Research Ser. B 5, 1–41.

Gorshkov, V., 1993. The state of moss-lichen cover in polluted and unpolluted pine forests of the Kola Peninsula. In: Kozlov, M.V., Haukioja, E., Yarmishko, V.T., (Eds.), Aerial Pollution in Kola Peninsula. Proceedings of the International Workshop, 14–16 April 1992, St. Petersburg. Kola Scientific Centre, Apatity, Russia, pp. 290–298.

Hämet-Ahti, L., Suominen, J., Ulvinen, T., Uotila, P., Vuokko, S., 1998. Retkeilykasvio (Field Flora of Finland), 4th Ed. Finnish Museum of Natural History, Botanical Museum, Helsinki.

Heinrichs, H., Mayer, R., 1980. The role of forest vegetation in the biochemical cycle of heavy metals. Journal of Environmental Quality 6, 402–407.

Helmisaari, H.-S., Derome, J., Fritze, H., Nieminen, T., Palmgren, K., Salemaa, M., Vanha-Majamaa, I., 1995. Copper in Scots pine forests around a heavy-metal smelter in south-western Finland. Water, Air and Soil Pollution 85, 1727–1732.

Huttunen, S., 1975. Studies on forest vegetation in air pollution damage area. Acta Universitatis Ouluensis Series A 33, Biologica 2, 1–37.

Kahle, H., 1993. Response of roots of trees to heavy metals. Environmental and Experimental Botany 33, 99–119.

Kent, M., Coker, P., 1992. Vegetation Description and Analysis. A Practical Approach. John Wiley & Sons, Chichester.

Koponen, T., Isoviita, P., Lammes, T., 1977. The bryophytes of Finland: an annotated checklist. Flora Fennica 6, 1–77.

Kozlov, M.V., Haukioja, E., Yarmishko, V.T. (Eds.), 1993. Aerial pollution in Kola Peninsula. In: Proceedings of the International Workshop, 14–16 April 1992, St. Petersburg, Kola Scientific Centre, Apatity, Russia.

Kubin, E., Lippo, H., Poikolainen, J., 2000. Heavy metal loading. In: Mälkönen, E. (Ed.), Forest condition in a changing environment — the Finnish case. Kluwer Academic, pp. 60–71.

Laaksovirta, K., Silvola, J., 1975. Effect of air pollution by copper, sulphuric acid and fertilizer factories on plants at Harjavalta, W. Finland. Annales Botanici Fennici 12, 81–88.

Levitt, J., 1980. Responses of Plants to Environmental Stresses. Academic Press, New York.

Løbersli, E.M., Steinnes, E., 1988. Metal uptake in plants from a birch forest area near a copper smelter in Norway. Water, Air and Soil Pollution 37, 25–39.

Lukina, N.V., Liseenko, L.A., Belova, E.A., 1993. Pollution-induced changes in the vegetation cover of spruce and pine ecosystems in the Kola Peninsula. In: Kozlov, M.V., Haukioja, E., Yarmishko, V.T., (Eds.), Aerial Pollution in Kola Peninsula. Proceedings of the International Workshop, 14–16 April 1992, St. Petersburg, Kola Scientific Centre, Apatity, Russia, pp. 312–321.

Mäkinen, A., 1994. Biomonitoring of atmospheric deposition in Finland, Estonia and the Kola Peninsula, based on the chemical analysis of mosses. Publications from the Department of Botany, University of Helsinki No. 19.

Makkonen, K., Helmisaari, H.-S., 1998. Seasonal and yearly variations of fine-root biomass and necromass in a Scots pine (*Pinus sylvestris* L.) stand. Forest Ecology and Management 102, 283–290.

Mälkönen, E., 1974. Annual primary production and nutrient cycle in some Scots pine stands. Communicationes Instituti Forestalis Fenniae 84 (5), 1–87.

Mälkönen, E., Derome, J., Fritze, H., Helmisaari, H.-S., Kukkola, M., Kytö, M., Saarsalmi, A., Salemaa, M., 1999. Compensatory fertilization of Scots pine stands polluted by heavy metals. Nutrient Cycling in Agroecosystems 55, 239–268.

Minchin, P.R., 1987. An evaluation of the relative robustness of techniques for ecological ordination. Vegetatio 69, 89–107.

Minchin, P.R., 1991. DECODA. Database for ecological community data, Version 2.04. Australian National University.

Monni, S., Salemaa, M., White, C., Tuittila, E., Huopalainen, M., 2000a. Copper resistance of *Calluna vulgaris* originating from the pollution gradient of a Cu–Ni smelter, in southwest Finland. Environmental Pollution 109, 211–219.

Monni, S., Salemaa, M., Millar, N., 2000b. The tolerance of *Empetrum nigrum* to copper and nickel. Environmental Pollution 109, 221–229.

Nash, T.H.III, 1989. Metal tolerance in lichens. In: Shaw, J.A. (Ed.), Heavy Metal Tolerance in Plants: Evolutionary Aspects. CRC Press, FL, pp. 119–131.

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

Nieminen, T., Derome, J., Helmisaari, H.-S., 1999. Interactions between precipitation and Scots pine canopies along a heavy-metal pollution gradient. Environmental Pollution 106, 129–137.

Økland, T., Økland, R.H., Steinnes, E., 1999. Element concentrations in the boreal forest moss *Hylocomium splendens*: variation related to gradients in vegetation and local environmental factors. Plant and Soil 209, 71–83.

Pennanen, T., Frostegård, Å, Fritze, H., Bååth, E., 1996. Phospholipid fatty acid composition and heavy metal tolerance of soil microbial communities along two heavy metal-polluted gradients in coniferous forests. Applied and Environmental Microbiology 62, 420–428.

Punz, W.F., Sieghardt, H., 1993. The response of roots of herbaceous plant species to heavy metals. Environmental and Experimental Botany 33, 85–98.

Rigina, O., Kozlov, M.V., 2000. Pollution impact on sub-arctic forests in the Kola Peninsula, Russia. In: Innes, J.L., Oleksyn, J. (Eds.), Forest Dynamics in Heavily Polluted Regions. CAB International, Wallingford, pp. 37–65.

Ross, S.M., 1994. Retention, transformation and mobility of toxic metals in soils. In: Ross, S.M. (Ed.), Toxic Metals in Soil — Plant Systems. John Wiley, Chichester, pp. 63–152.

Rühling, Å, Brumelis, G., Goltsova, N., Kvietkus, K., Kubin, E., Liiv, S. et al., 1992. Atmospheric heavy metal deposition in northern Europe 1990. Nord 12, 1–41.

Saari, H., Kartastenpää, R., Pohjola, V., Voltti, U., 1993. Hiukkas- ja rikkidioksiditutkimus Harjavallassa keväällä 1992. (Particle and Sulphur Dioxide Research at Harjavalta in the Spring 1992). Ilmatieteen laitos, Ilmanlaatuosasto, Helsinki (in Finnish).

Salemaa, M., Uotila, T., 1996. Kanervan taimien hengissäsäilymisen todennäköisyys raskasmetallien saastuttamassa maassa. (The survival probability of the *Calluna vulgaris* seedlings in heavy-metal polluted soil). In: Rekilä, R., Kärenlampi, L., (Eds.), 5th Meeting of Finnish Plant Scientists, University of Kuopio, Kuopio, Finland, p. 118 (in Finnish).

Salemaa, M., Vanha-Majamaa, I., Gardner, P.J., 1999. Compensatory growth of two clonal dwarf shrubs, *Arctostaphylos uva-ursi* and *Vaccinium uliginosum* in a heavy metal polluted environment. Plant Ecology 141, 79–91.

Salemaa, M., Vanha-Majamaa, I., Reinikainen, A., Nousiainen, H., 2000. Response of understorey vegetation to heavy metal loading. In: Mälkönen, E. (Ed.), Forest Condition in a Changing Environment — the Finnish Case. Kluwer Academic Publishers, pp. 266–275.

SAS, 1994. SAS/STAT User's Guide Version 6, 4th Edition. SAS Institute Inc, Cary, NC, USA.

Shevtsova, A., 1998. Responses of Subarctic Dwarf Shrubs to Climate Change and Air Pollution. Annales Universitatis Turkuensis, Ser AII, Vol. 113. Turku, Finland.

Tamminen, P., Starr, M.R., 1990. A survey of forest soil properties related to soil acidification in southern Finland. In: Kauppi, P., Anttila, P., Kenttämies, K. (Eds.), Acidification in Finland. Springer Verlag, Berlin, pp. 235–251.

Tikkanen, E., Niemelä, I., 1995. Kola Peninsula pollutants and forest ecosystems in Lapland. Final report of the Lapland Forest Damage Project. Gummerus. Jyväskylä.

Tolvanen, A., Kubin, E., 1990. The effect of clear felling and site preparation on microclimate, soil frost and forest regeneration at elevated sites in Kuusamo. Aquilo Series Botanica 29, 77–86.

Turner, A.P., 1994. The response of plants to heavy metals. In: Ross, S.M. (Ed.), Toxic Metals in Soil–Plant Systems. John Wiley, Chichester, pp. 151–187.

Tyler, G., 1984. The impact of heavy metal pollution on forests: a case study on Gusum, Sweden. Ambio 13, 18–24.

Tyler, G., 1990. Bryophytes and heavy metals: a literature review. Botanical Journal of the Linnean Society 104, 231–253.

Tyler, G., Balsberg Påhlsson, A.-M., Bengtsson, G., Bååth, E., Tranvik, L., 1989. Heavy metal ecology of terrestrial plants, micro organisms and invertebrates. Water, Air and Soil Pollution 47, 189–215.

Uhlig, C., Salemaa, M., Vanha-Majamaa, I., Derome, J., 2001. Element distribution in *Empetrum nigrum* microsites at heavy metal contaminated sites in Harjavalta, western Finland. Environmental Pollution 112, 431–438.

Ukonmaanaho, L., Starr, M., Hirvi, J.-P., Kokko, A., Lahermo, P., Mannio, J., Paukola, T., Ruoho-Airola, T., Tanskanen, H., 1998. Heavy metal concentrations in various aqueous and biotic media in Finnish Integrated Monitoring catchments. Boreal Environment Research 3, 235–249.

Väisänen, S., 1986. Effects of air pollution by metal, chemical and fertilizer plants on forest vegetation at Kokkola, W Finland. Annales Botanici Fennici 23, 305–315.

Vangronsveld, J., Colpaert, J.V., Van Tichelen, K.K., 1996. Reclamation of bare industrial area contaminated by non-ferrous metals: physiochemical and biological evaluation of the durability of soil treatment and revegetation. Environmental Pollution 94, 131–140.

Vanha-Majamaa, I., Salemaa, M., Tuominen, S., Mikkola, K., 2000. Digitized photographs in vegetation analysis — a comparison of cover estimates. Journal of Applied Vegetation Science 31, 89–99.

Verkleij, J.A.C., Schat, H., 1989. Mechanisms of metal tolerance in higher plants. In: Shaw, J.A. (Ed.), Heavy Metal Tolerance in Plants: Evolutionary Aspects. CRC Press, FL, pp. 179–193.

Vitikainen, O., Ahti, T., Kuusinen, M., Lommi, S., Ulvinen, T., 1997. Checklist of lichens and allied fungi of Finland. Norrlinia 6.

Winterhalder, K., 2000. Landscape degradation by smelter emissions near Sudbury, Canada, and subsequent amelioration and restoration. In: Innes, J.L., Oleksyn, J. (Eds.), Forest Dynamics in Heavily Polluted Regions. CAB International, Wallingford, pp. 87–119.

Wittig, R., 1993. General aspects of biomonitoring heavy metals by plants. In: Markert, B. (Ed.), Plants as Biomonitors. Indicators for Heavy Metals in the Terrestrial Environment. VCH, Weinheim, pp. 3–27.

Zöttl, H.W., 1985. Heavy metal levels and cycling in forest ecosystems. Experimentia 41, 1104–1113.

Paper II

Salemaa, M., Derome, J., Helmisaari, H.-S., Nieminen, T. & Vanha-Majamaa, I. 2004. Element accumulation in boreal bryophytes, lichens and vascular plants exposed to heavy metal and sulfur deposition in Finland. The Science of the Total Environment (in press).

II

ARTICLE IN PRESS

ELSEVIER

Available online at www.sciencedirect.com
SCIENCE DIRECT®

Science of the Total Environment xx (2003) xxx–xxx

Science of the Total Environment
An International Journal for Scientific Research into the Environment and its Relationship with Man

www.elsevier.com/locate/scitotenv

Element accumulation in boreal bryophytes, lichens and vascular plants exposed to heavy metal and sulfur deposition in Finland

Maija Salemaa[a,]*, John Derome[b], Heljä-Sisko Helmisaari[a], Tiina Nieminen[a], Ilkka Vanha-Majamaa[a]

[a]*Vantaa Research Centre, Finnish Forest Research Institute, P.O. Box 18, FIN-01301 Vantaa, Finland*
[b]*Rovaniemi Research Station, Finnish Forest Research Institute, P.O. Box 16, FIN-96301 Rovaniemi, Finland*

Received 15 June 2003; received in revised form 28 October 2003; accepted 29 October 2003

Abstract

Macronutrient (N, P, K, Mg, S, Ca), heavy metal (Fe, Zn, Mn, Cu, Ni, Cd, Pb) and Al concentrations in understorey bryophytes, lichens and vascular plant species growing in Scots pine forests at four distances from the Harjavalta Cu–Ni smelter (0.5, 2, 4 and 8 km) were compared to those at two background sites in Finland. The aim was to study the relationship between element accumulation and the distribution of the species along a pollution gradient. Elevated sulfur, nitrogen and heavy metal concentrations were found in all species groups near the pollution source. Macronutrient concentrations tended to decrease in the order: vascular plants > bryophytes > lichens, when all the species groups grew on the same plot. Heavy metal concentrations (except Mn) were the highest in bryophytes, followed by lichens, and were the lowest in vascular plants. In general, vascular plants, being capable of restricting the uptake of toxic elements, grew closer to the smelter than lichens, while bryophytes began to increase in the understorey vegetation at further distances from the smelter. A pioneer moss (*Pohlia nutans*) was an exception, because it accumulated considerably higher amounts of Cu and Ni than the other species and still survived close to the smelter. The abundance of most of the species decreased with increasing Cu and Ni concentrations in their tissues. *Cetraria islandica*, instead, showed a positive relationship between the abundance and Cu, Ni and S concentrations of the thallus. It is probable that, in addition to heavy metals, sporadically high SO_2 emissions have also affected the distribution of the plant species.

Keywords: Biomonitoring; *Cladina*; *Cladonia*; *Dicranum*; *Pleurozium*; *Empetrum*; *Vaccinium*

1. Introduction

The understorey vegetation of coniferous forests undergoes strong changes around smelter complexes emitting heavy metals, sulfur or other pollutants in the northern hemisphere (Amiro and Courtin, 1981; Folkeson and Andersson-Bringmark, 1988; Rigina and Kozlov, 2000; Salemaa et al., 2001). Different plant species show varying resistance to airborne and soil-accumulated toxic elements, which is reflected in their growth, survival and occurrence along pollution gradients. However, the actual degree of exposure to toxic elements is not

---

*Corresponding author. Tel.: +358-10-211-2539; fax: +358-10-211-2202.
E-mail address: maija.salemaa@metla.fi (M. Salemaa).

0048-9697/03/$ - see front matter © 2003 Published by Elsevier B.V.
doi:10.1016/S0048-9697(03)00629-6

the same for all the plant species growing at the same distance from an emission source because of differences in element uptake mechanisms (Tyler et al., 1989; Garty, 2001; Zechmeister et al., 2003). A range of morphological, anatomical and physiological properties affect the capacity of individual species to filter, bind and accumulate elements on their surfaces, and to take them up intracellularly.

The greatest difference in the uptake mechanisms of elements is that between vascular plants and cryptogams (bryophytes and lichens). Vascular plants mainly take up elements via their roots from the soil, although the foliar uptake of gases (e.g. NO_2, NH_3 and SO_2) and soluble elements may also be substantial (Marschner, 1995). The foliar uptake of heavy metals has been demonstrated in many crop plants (e.g. Haslett et al., 2001) but, in evergreen species, the thick epidermis and waxy cuticle of the leaves provide external protection against toxic elements. Large amounts of metal-containing dust become attached to the leaf surfaces of trees growing near the emission sources (Kozlov et al., 2000), and particles may also become embedded in the cuticular waxes (Rautio and Huttunen, 2003). Acidic deposition causes erosion of the cuticle (Manninen and Huttunen, 1995), which may increase the leaching of elements and the penetration of heavy metals into the foliar tissues.

The bioavailability of heavy metals in the soil is regulated by many physical, chemical and biological properties and processes (Ernst, 1996). The mobility and toxicity of heavy metals are strongly related to the acidity and organic matter content of the soil (Alloway, 1995). Heavy metal ions accumulate first in the cortex of the roots, from where a small proportion passes through the endodermis and is subsequently distributed into the different plant organs via the xylem and phloem. Vascular plants have many species-specific mechanisms to restrict the cellular uptake of heavy metals and to detoxify them internally (recently reviewed by Hall, 2002). The role of mycorrhizal fungi in retaining heavy metals in the root system is important in providing resistance to the host plants (Meharg and Cairney, 2000).

Cryptogams, however, have no real roots, epidermis or continuous cuticle layer, and they absorb water and dissolved elements directly across their surface. Most of the bryophyte and lichen species obtain the majority of their water and nutrients from atmospheric deposition; some species also obtain nutrients from water that has been in contact with the substrate (Bates, 1992; Garty, 2001). The following element compartments occur in both taxons: (1) trapped particulate matter; (2) intercellular soluble elements; (3) extracellular elements, bound to the cell wall on charged exchange sites; and (4) intracellular elements (Tyler, 1990; Garty, 2001; Zechmeister et al., 2003). Both bryophytes and lichens (especially the mycobiont partner) have a high ion exchange capacity on their cell walls, and the dead tissues also have an ability to bind ions (Tyler, 1989, 1990; Chettri et al., 1997).

The pollution gradients extending from emission sources can be used as ‘field tests’ for studying the relative resistance of different plant species in real ecological conditions. Non-resistant species or populations show a sudden or a gradual decrease in abundance as the pollution level increases (Tyler et al., 1989; Lepp and Salmon, 1999). However, without the use of ultrastructural analysis to identify the intracellular accumulation of toxic elements and experimental exposures, it is almost impossible to identify the contribution of individual pollutants to the occurrence of a species.

In this study we compare the element concentrations of unwashed samples of the understorey bryophyte, lichen and vascular plant species (later refered as life forms) growing in coniferous forests at four distances from the Harjavalta Cu–Ni smelter, and in two background areas in other parts of Finland. Our aim is to answer the following questions:

1. Does the accumulation of elements differ between the different life forms, species and plant parts in polluted and background areas?
2. How is the accumulation pattern of heavy metals and S related to the occurrence and abundance of the plant species along the pollution gradient?
3. What is the potential of different plant species in biomonitoring of heavy metals?

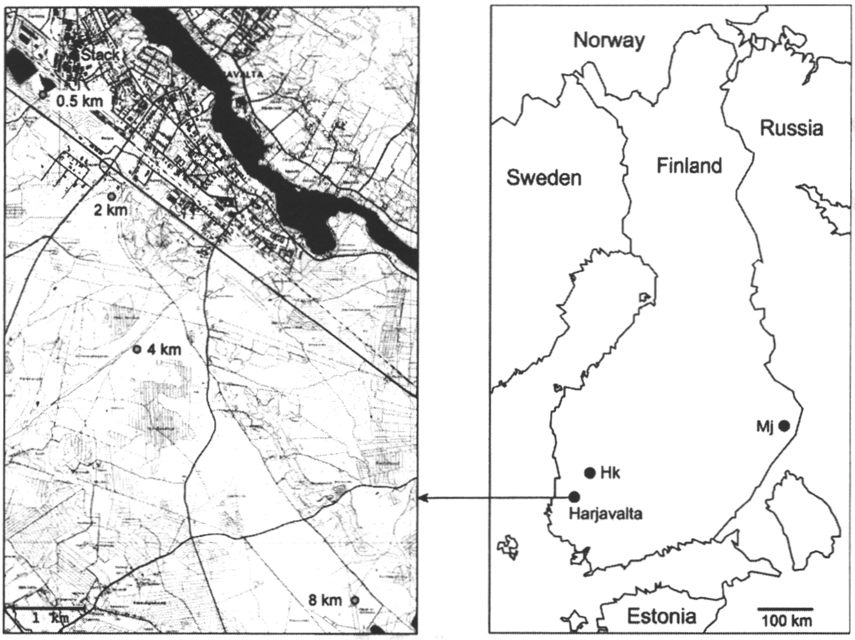


Fig. 1. Location of the three study locations. Mekrijärvi (Mj) and Hämeenkangas (Hk) represent background areas. The town of Harjavalta is situated approximately 30 km from the coast (Gulf of Bothnia) in Western Finland. The study plots at distances of 0.5, 2, 4 and 8 km from the Harjavalta Cu–Ni smelter (see stack on the map) have been marked on the detailed map. © National Land Survey of Finland 554/myy/03.

2. Material and methods

2.1. Study areas

The study plots are located in Scots pine (*Pinus sylvestris* L.) stands at four distances (0.5, 2, 4 and 8 km) (later called H0.5, H2, H4 and H8) to the south and south-west of the Harjavalta copper–nickel smelter (61°19′ N, 22°9′ E). The two background areas, Hämeenkangas (Hk) (61°45′ N, 22°40′ E) and Mekrijärvi (Mj) (62°47′ N, 30°58′ E), are located at a distance of 60 and 420 km from Harjavalta, respectively (Fig. 1). The size of the sample plots was 30×30 m (H0.5: 26×26 m). The plots were established for nutrient flux studies (Helmisaari, 1995) and as control plots for liming and fertilization experiments (Mälkönen et al., 1999). The plots at Harjavalta and Hämeenkangas were xeric and Mekrijärvi sub-xeric heath forests (Table 1). Although Mekrijärvi represented slightly more fertile forest than the other plots, it was selected for a clean reference area especially for bryophytes and lichens. The soil on all the plots is sorted fine sand and the soil type ferric podzol. The age of the stands ranged from 40 to 50 years. Table 1 summarizes the general stand characteristics.

Carpet-forming bryophytes, bilberry (*Vaccinium myrtillus*) and lingonberry (*V. vitis-idaea*) dominated in the understorey at Mj, whereas reindeer lichens (*Cladina* spp.) and evergreen dwarf shrubs were typical species at Hk and H8 (Table 2). The cover of the understorey vegetation and the species richness decreased with decreasing distance to the Cu–Ni smelter at Harjavalta (Table 2). The nearest plot to the smelter (H0.5) was almost devoid of vegetation, and the forest floor was covered with a thick layer of undecomposed needle litter.

Table 1
General stand characteristics

	Harjavalta gradient				Background areas	
	H0.5	H2	H4	H8	Hk	Mj
Forest site type	CT+	CT+	CT	CT	CT	VT
Stand age, years	49	54	48	40	45	44
Number of trees/ha	1008	1230	1517	1552	2017	2660
Mean pine height, m	6.1	10.9	9.2	10.6	9.4	7.4
Stem volume, m^3/ha	23.2	85.3	67.8	94.5	49.8	63.1
H ✓ Volume increment, m^3/ha⊢/year⊢	0.31	3.78	2.78	6.27	3.29	5.2
Distance to the Harjavalta Cu–Ni smelter, km	0.5	2	4	8	60	420

CT = Calluna type (xeric heath type), VT = Vaccinium type (sub-xeric heath type), + indicates slightly more fertile type. Results from Harjavalta published by Mälkönen et al. (1999), Hämeenkangas (Hk) by Mälkönen et al. (2000) and Mekrijärvi (Mj) by Helmisaari and Mälkönen (1989).

2.2. Air pollutant emissions

The Harjavalta Metals smelter complex is one of the largest point sources of heavy metal emissions in Finland. The copper smelter has been operating since 1945, and the nickel smelter since 1960. The concentrated ores contain sulfur, heavy metals and arsenic. Before the sulfuric acid plant was built in 1947, all the SO$_2$ produced during the smelting process (annually approx. 30 000 t) was emitted into the atmosphere, causing severe damage to the surrounding coniferous forests. At the end of the 1980s the average annual emissions of SO$_2$ were 8050 t and total dust 1200 t, including Cu 110 t, Ni 53 t and Zn 180 t. Since the beginning of the 1990s, however, the emissions have been considerably reduced: in 1992, Cu 60 t, Ni 10 t, Zn 12 t, SO$_2$ 4800 t and total dust 280 t (Fig. 2). The 24-h mean SO$_2$ concentrations in the air have decreased from the level of 38–56 μg/m^3 in 1987 (1 January–30 June) to 17–18 μg/m^3 in 1992 (21 January–15 May) within a 1 km radius of the smelter. However, the peak hourly concentrations in 1992 still occasionally reached 500–1000 μg/
/3 m^3 (Saari et al., 199~~2~~). The Kemira fertilizer factory produced superphosphate and PK fertilizers at Harjavalta from 1948 to 1989. The study plots have probably not been exposed to the heaviest deposition levels in the vicinity of the smelter, because they were not located under the predominant southern winds (Derome, 2000).

The background areas (Hk, Mj) had no local emission sources. The mean annual bulk deposition of sulfate in open areas varied between 300 and 500 mg/m^2 during 1988–1996 in South Finland (Kulmala et al., 1998). Atmospheric emissions of heavy metals have decreased substantially in Finland during the 1990s, which are clearly reflected as low heavy metal concentrations in background bryophytes in the national surveys (Poikolainen et al., ~~1985~~). ⊢ 2004

2.3. Plant sampling and chemical analysis

The plant samples for elemental analysis were collected from the surrounding buffer zones of the study plots in August–September 1992. Additional samples from the Harjavalta gradient (H0.5 and H2) were taken in September 1993 and 1994. Random sampling was performed using 30 small quadrats (using a 20×20 cm frame) stratified on different sides of the plots (H4, H8, Hk and Mj). The total sampled area was 1.2 m^2 per plot. Subjective selection of the plant material had to be applied on the highly polluted plots (H0.5 and H2, see species in Appendices A and B) owing to the very low abundance of understorey vegetation (Table 2). The plant samples were cut off level with the surface of the organic layer, and thus excluded rhizomes and roots. The samples were placed in plastic bags, and stored frozen up until chemical analysis.

Table 2
The average abundance (point frequency %) of the bryophyte, lichen and vascular plant species at the six study sites in 1992

	Harjavalta gradient				Background	
	H0.5	H2	H4	H8	Hk	Mj
Bryophytes:						
Ceratodon purpureus (Hedw.) Brid	0.0	0.1	0.0	0.0	0.0	0.0
D. polysetum Sw.	0.0	0.0	0.0	1.2	9.9	11.2
D. scoparium Hedw.	0.0	0.0	0.0	0.5	0.5	0.2
P. schreberi (Brid.) Mitt.	0.0	0.0	0.1	3.6	9.8	77.0
P. nutans (Hedw.) Lindb.	0.1	2.4	2.2	5.2	0.8	0.0
P. juniperinum Hedw.	0.0	0.0	0.0	0.0	0.9	0.1
Ptilidium ciliare (L.) Hampe	0.0	0.0	0.2	0.3	0.0	0.0
Lichens:						
C. islandica (L.) Ach.	0.0	26.5	9.5	6.0	2.3	5.2
C. arbuscula (Wallr.) Hale and Culb.	0.0	0.0	9.2	19.1	31.7	3.4
C. rangiferina (L.) Nyl.	0.0	0.2	13.9	10.4	12.2	3.9
C. stellaris (Opiz) Brodo	0.0	0.0	5.2	32.0	18.6	+
C. uncialis (L.) F.H.Wigg.	0.0	0.0	0.0	0.0	0.1	0.0
Cladonia spp.	0.0	3.7	5.7	6.6	4.6	1.1
Vascular plants:						
A. uva-ursi (L.) Sprengel	0.0	1.8	0.0	0.0	0.3	0.0
Betula pubescens Ehrh.	+	+	0.0	0.0	0.0	0.0
C. vulgaris (L.) Hull	0.0	+	+	0.9	11.7	8.5
C. globularis L.	+	0.0	0.0	0.0	0.0	0.0
D. flexuosa (L.) Trin.	0.0	0.0	0.0	+	0.2	0.0
Epilobium angustifolium L.	0.0	0.1	0.0	+	0.0	0.0
E. nigrum L.	0.1	10.3	6.8	0.1	+	0.1
Juniperus communis L.	0.0	0.0	0.0	0.0	+	0.0
Ledum palustre L.	+	0.0	0.0	0.0	0.0	0.0
P. sylvestris L.	+	0.6	1.3	1.6	0.5	0.0
Sorbus aucuparia L.	0.0	0.0	0.0	0.0	0.0	0.1
V. myrtillus L.	0.0	0.1	0.0	0.0	+	16.4
V. uliginosum L.	0.1	0.0	0.0	0.1	0.0	0.0
V. vitis-idaea L.	0.0	2.5	11.8	6.3	3.3	9.7

Averages from 16 sample quadrats (each 1 m^2) per sample plot. + = species present on the sample plot but not on the sample quadrat. Nomenclature as per Hämet-Ahti et al. (1998): vascular plants, Koponen et al. (1977): bryophytes and Vitikainen et al. (1997): lichens.

The plant biomass was divided according to species and age class. Current-year shoots, older living parts and dead biomass were separated on vascular plants. The upper living part of the thalli of bryophytes and lichens was separated from the lower decomposing (darker) parts on the basis of their color difference. In the case of bryophytes, the upper part consisted of 2–3 year's growth. The plant material was not washed before chemical analysis and thus included the surface accumulation of elements. All the plant samples were handled using cotton gloves.

The species-specific plant samples were combined into composite samples per side or whole plot in order to obtain sufficient material for elemental analysis. The dry weight of the composite samples ranged from 0.5 to 1.0 g for bryophytes, and from 1.0 to 3.0 g for other plants. The samples were oven-dried (+60 °C), weighed, homogenized and dry digested (+550 °C). The ash was extracted with 2–3 ml of 6 M HCl (p.a.) in a water bath (approx. +80 °C). The dry residue was diluted with 10 ml 1 M HCl for 20 min and filtered, and the filter paper rinsed with 0.1 M

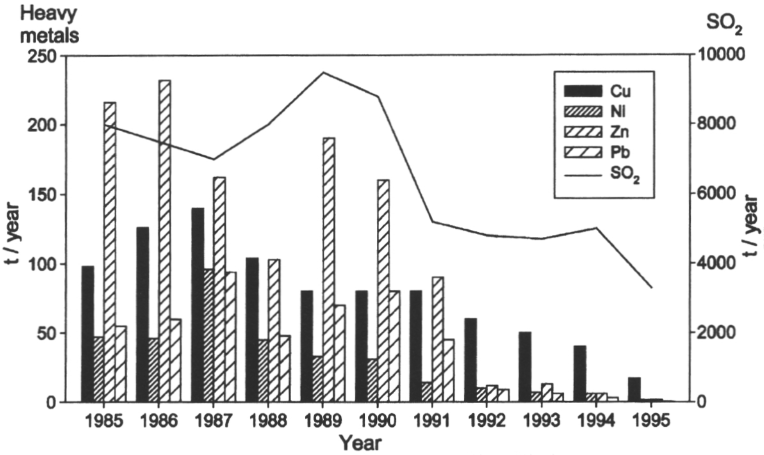


Fig. 2. Cu, Ni, Zn and Pb emissions (bars) and SO_2 emissions (t/year) from the Harjavalta smelter during 1985–1995. *Source*: Outokumpu Harjavalta Metals Oy.

HCl. The final volume of the solution was 25–100 ml depending on the sample weight. Total element concentrations (P, K, Mg, Ca, Fe, Zn, Mn, Cu, Ni, Cd, Pb and Al) were determined by inductively coupled plasma atomic emission spectrometer (ICP-AES) (Dahlquist and Knoll, 1978). Total sulfur and nitrogen concentrations were determined from the homogenized samples on a LECO S-132 and LECO CHN-600 analyzers. All the analyses were performed in the central laboratory of the Finnish Forest Research Institute. The laboratory included its own blank and standard samples in all the batches, and the laboratory participated, with satisfactory results, in international inter-calibration exercises (e.g. during the analysis period in question, IUFRO Inter-laboratory Sample Exchange 1993).

The abundances of the plant species growing on the study plots (30×30 m) were measured in 1992 using the point quadrat method (Salemaa et al., 2001). Each plot was divided into four subplots, and stratified random sampling performed on 16 vegetation quadrats (1 m^2).

2.4. Soil and precipitation sampling, and chemical analyses

Samples were taken from the organic and mineral soil layers (0–5 cm) at 25 systematically selected points in 1992 (Hk in 1990). The organic layer samples were dried and milled to pass through a 1 mm sieve. The mineral soil samples were passed through a 2 mm sieve to remove stones and large roots. pH was measured in water. Organic matter content was determined as loss in weights on ignition by ashing the samples (550 °C, 3 h). Total N was determined on a CHN analyzer. Exchangeable Ca, Mg, K, Cu, Ni, Zn, Fe, Mn, Cd, Pb and extractable P and S at the Harjavalta plots were determined by extraction with 1 M ammonium acetate (pH 4.65)+1% EDTA, followed by analysis by ICP-AES. EDTA was used in the extractant for the samples from the polluted sites because it was known that they contained high concentrations of Cu and Fe (Derome and Lindroos, 1998). The extractant used for the samples from Hämeenkangas and Mekrijärvi did not include EDTA due to the low levels of Cu and Fe. The element concentrations in the organic layer were expressed on an organic matter basis in order to reduce the variation arising from the inclusion of varying amounts of mineral soil in the organic layer samples. The chemical properties of the soil along the Harjavalta gradient are described in more detail in Derome and Lindroos (1998) and Derome (2000).

A description of the sampling of precipitation and its chemical analysis is given in Derome and Nieminen (1998).

2.5. Statistical analysis

Plotwise means of the element concentrations of the species were calculated in the cases where there were 2–4 composite samples per plot. Differences in the element concentrations between the life forms and species were tested by Mann–Whitney's U tests. Non-parametric statistics were used because the sample number was low, and it was not possible to test the normality of the distributions. The relationship between the element accumulation (Cu, Ni and S) in different species at H8 (where all life forms were present) and the closest distance to the smelter at which the species occurred, was analyzed by Pearson's correlation coefficients. The abundances of the species were plotted against the Cu concentrations of their tissues using the data of the Harjavalta gradient and the nearest background area (Hk).

3. Results

3.1. Precipitation and soil chemistry

The effect of emissions from the Harjavalta smelter was clearly reflected in the composition of bulk precipitation and stand throughfall (Table 3). The deposition of heavy metals (except Mn) was tens or hundreds of times higher at H0.5 than in the background areas. The bulk deposition of Cu and Ni increased exponentially towards the smelter (Fig. 3a,b). Furthermore, elevated N, SO_4 and Mg deposition was recorded near the smelter. Of the two background areas, Mj had lower N and SO_4 deposition than Hk (Table 3). The throughfall values, which also include elements leached from the canopy, were higher than those of the bulk precipitation except for nitrogen (H8, Hk) on all the study plots.

The heavy metal concentrations in the organic layer increased strongly towards the smelter (distributions for Cu and Ni given in Fig. 3a,b), whereas those of the macronutrients (K, Mg and Ca) decreased (Table 4). The effect of emissions was also seen as elevated concentrations of N, S and P at H0.5 (Table 4). Both the macronutrient and heavy metal concentrations were higher in the organic layer than in the mineral soil (0–5 cm). The enrichment factor (H0.5 vs. Mj) for the Cu concentration was 645 in the organic layer and 66 in the mineral soil. The corresponding value for Ni in the organic layer was 848. Mj differed from the other plots by having higher macronutrient, Fe and Mn concentrations in the mineral soil (Table 4).

3.2. Element accumulation in ~~the plant species~~ vegetation

3.2.1. Variation between the life forms (bryophytes, lichens and vascular plants)

When the life forms growing on the same plots were compared, the macronutrient concentrations tended to be the highest in vascular plants and decreased from bryophytes to lichens (Appendices A and B). There were some exceptions to this pattern, which depended on the element, area and age of the plant part. For instance, higher concentrations of N, P, Mg and Ca (H0.5) and S (H4) were found in *Pohlia nutans* compared to the other species. The average N and S concentrations of *Pleurozium schreberi* and *Dicranum* spp. (upper parts, N: 1.11%, S: 0.98 mg/g, $n=7$) and vascular plants (current-year shoots, N: 1.10%, S: 1.25 mg/g, $n=6$) were relatively similar (N: $U=19.0$, $P=0.836$; S: $U=12.0$, $P=0.234$) in the combined data of the background areas, whereas those of *Cladina* and *Cetraria* lichens (upper parts, N: 0.62%, S: 0.54 mg/g, $n=14$) were significantly lower (N: $U=0.0$, $P=0.001$; S: $U=1.0$, $P=0.001$).

The heavy metal concentrations were the highest in bryophytes, followed by lichens, and the lowest in vascular plants in the Harjavalta data (distributions for Cu and Ni given in Fig. 3c,d). The only exception was Mn, which followed the order: vascular plants > bryophytes > lichens. *Vaccinium* species, especially *V. myrtillus*, had high Mn concentrations in Mj. The differences in the heavy metal concentrations between the life forms were greater in the polluted sites at Harjavalta than in the background areas. This was especially the case

Table 3
Annual precipitation (mm) and deposition of elements (mg/m^2) in bulk precipitation (BP) and stand throughfall (TF)

Parameter	Type of precip.	Harjavalta gradient				Background areas	
		H0.5	H2	H4	H8	Hk	Mj
Amount, mm	BP	493	556	638	505	734	478
	TF	445	m.d.	395	409	639	430
PH	BP	4.6	4.7	4.6	4.5	4.5	4.5
	TF	4.3	m.d.	4.2	4.2	4.3	4.3
N tot	BP	673	389	471	522	650	338
	TF	703	m.d.	497	418	510	402
P	BP	10	10	15	14	18	20
	TF	16	m.d.	10	6	10	18
K	BP	40	74	56	15	40	77
	TF	198	m.d.	139	137	204	149
Mg	BP	86	11	23	20	29	32
	TF	71	m.d.	55	43	46	48
SO_4–S	BP	762	384	424	467	382	325
	TF	1162	m.d.	551	567	540	306
Ca	BP	136	39	74	56	75	113
	TF	283	m.d.	188	136	145	144
Fe	BP	94.0	25.0	15.0	5.7	<LOQ	8.5
	TF	186.4	m.d.	11.9	6.7	<LOQ	9.5
Zn	BP	24.7	7.7	3.4	2.6	<LOQ	3.7
	TF	47.6	m.d.	5.6	3.4	2.2	5.7
Mn	BP	3.5	1.6	1.7	1.0	<LOQ	6.1
	TF	11.1	m.d.	11.3	8.6	15.3	11.2
Cu	BP	262.3	49.6	13.2	3.7	<LOQ	4.0
	TF	471.1	m.d.	16.5	4.2	0.5	2.9
Ni	BP	25.6	5.0	1.7	0.7	<LOQ	<LOQ
	TF	58.5	m.d.	2.5	0.6	<LOQ	<LOQ
Cd	BP	0.7	0.3	0.1	0.1	<LOQ	<LOQ
	TF	0.9	m.d.	0.0	0.1	<LOQ	<LOQ
Pb	BP	4.6	4.6	3.3	2.8	<LOQ	2.5
	TF	5.9	m.d.	0.5	2.3	<LOQ	<LOQ

Sampling period: 6 June 1992–21 June 1993 (H0.5, H4, H8, Hk), 12 January 1992–15 December 1992 (H2), and 19 October 1992–27 October 1993 (Mj). m.d. = missing data, throughfall was not collected at H2. <LOQ = below the limit of quantification for the analysis. For description of sampling and analysis methods, see Derome and Nieminen (1998).

for Cu and Ni; the bryophytes (average of upper parts, Cu: 173.5 μg/g, Ni: 36.8 μg/g, $n=5$) had 5–12 times higher concentrations than vascular plants (current-year shoots, Cu: 14.8 μg/g, Ni: 8.0 μg/g, $n=3$) at H8 (Cu and Ni: $U=0.0$, $P=0.036$), whereas the differences were much smaller in the background areas (Cu: bryophytes 10.0 μg/g, $n=7$, and vascular plants 6.4 μg/g, $n=6$, $U=8.0$, $P=0.073$; Ni: 5.4 μg/g and 4.2 μg/g, respectively, $U=14$, $P=0.317$).

3.2.2. Variation between the species within the life forms

The pollution level in the growing area affected

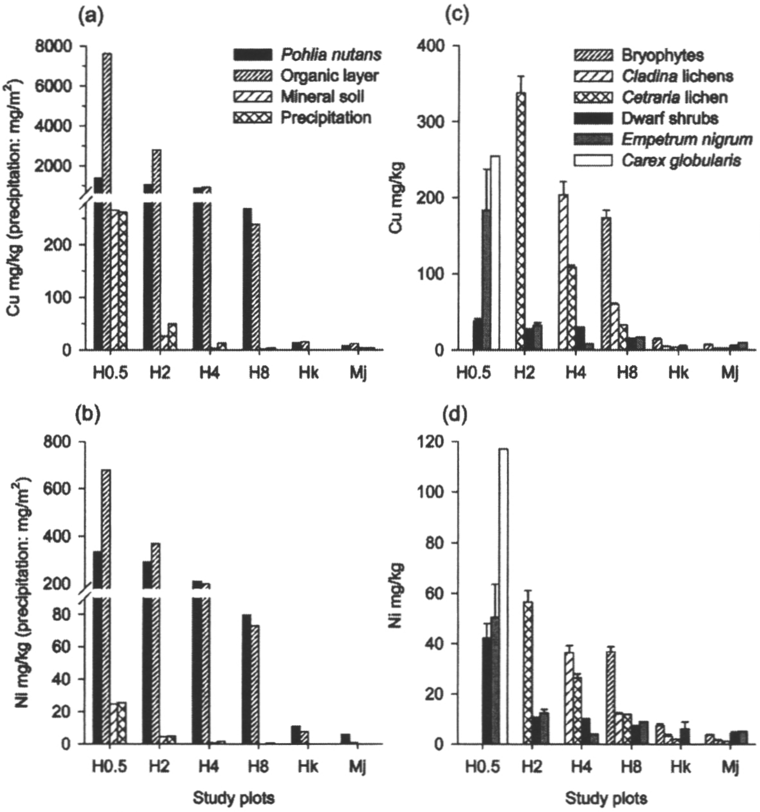


Fig. 3. Cu and Ni concentrations (mg/kg) in (a, b) a pioneer moss *P. nutans*, the organic and mineral (0–5 cm) soil layers and bulk precipitation (mg/m²) and (c, d) the upper parts of bryophytes (*Dicranum* spp. and *P. schreberi*) and lichens, and the current-year shoots of vascular plants at different distances (km) from the Harjavalta Cu–Ni smelter (H0.5, H2, H4 and H8) and in two background areas Hämeenkangas (Hk) and Mekrijärvi (Mj). Vertical line = S.E. of mean. See Tables 3 and 4 and Appendices A and B for individual values. Observe different scaling on the *y* axis.

the interspecies differences in macronutrient and heavy metal concentrations within the life forms. In general, the interspecies variation was relatively low in the background areas, but increased in the polluted areas (Appendices A and B). *Dicranum* spp. accumulated slightly higher amounts of macronutrients and heavy metals than *P. schreberi* in the background areas but, at H8, the Cu, Ni and Pb concentrations were slightly higher in the latter species. *P. nutans*, in contrast, had higher N, S and heavy metal concentrations than the other bryophytes at H8. Within the bryophyte group, however, the Mn concentration was always the highest in *Dicranum* spp. *C. stellaris* accumulated more S (1.05 mg/g) and Cu (258.7 μg/g) than *C. arbuscula* (S: 0.82 mg/g, Cu: 159.9 μg/g) or *C. rangiferina* (S: 0.85 mg/g, Cu: 214.3 μg/g), and all the *Cladina* species (average of upper parts, S: 0.90 mg/g, Cu: 203.7 μg/g, $n=7$) more than *Cetraria islandica* (upper parts, S: 0.58 mg/g, Cu: 108.5 μg/g, $n=3$) (S and Cu: $U=0.00$, $P=0.02$) at H4. Cup lichens (mainly *Cladonia chlorophaea*, *C. cornuta*, *C. sulfurina* and *C. gracilis*), in which the whole thallus was sampled, had relatively

Table 4
pH, total N (%) and exchangeable concentrations (mg/kg) of macronutrients and heavy metals in organic soil layer (Org) (organic matter basis) and 0 to 5-cm thick mineral soil layer (Min)

Parameter	Soil layer	Harjavalta gradient				Background areas	
		H0.5	H2	H4	H8	Hk	Mj
PH	Org	3.6	3.8	3.5	3.4	3.5	3.7
	Min	3.9	3.8	3.7	3.9	4.6	m.d.
N tot	Org	2.06	1.92	1.49	1.58	1.84	1.75
	Min	m.d	m.d.	m.d.	m.d.	m.d.	m.d.
P	Org	801	326	232	193	375	364
	Min	27	11	7	15	18	47
K	Org	215	422	753	577	581	717
	Min	14	16	13	9	26	31
Mg	Org	65	171	189	167	142	225
	Min	5	5	3	4	4	7
S	Org	349	223	184	185	232	176
	Min	13	5	4	9	27	41
Ca	Org	953	1953	2008	1753	1369	1407
	Min	41	32	14	11	18	21
Fe	Org	9763	4686	2257	1460	2089	2268
	Min	260	119	63	203	350	787
Zn	Org	205.3	167.3	149.0	93.6	66.6	37.0
	Min	10.3	3.4	1.9	1.1	1.6	3.4
Mn	Org	21.9	112.2	91.9	72.5	67.0	138.3
	Min	1.1	2.1	0.6	0.6	1.5	13.2
Cu	Org	7616.9	2786.3	915.8	238.7	15.8	11.8
	Min	265.8	25.7	3.3	1.5	0.4	4.0
Ni	Org	678.7	369.4	199.1	73.1	7.7	0.8
	Min	24.8	4.5	0.8	0.0	<LOQ	<LOQ
Cd	Org	3.6	2.9	2.2	1.2	1.3	0.4
	Min	0.2	0.0	0.00	0.0	<LOQ	0.1
Pb	Org	418.0	252.5	166.6	106.3	141.0	44.8
	Min	8.7	2.8	1.9	3.0	3.3	3.9
Al	Org	447	1039	963	1252	2963	2037
	Min	93	118	110	263	721	967

m.d. = missing data. <LOQ = below the limit of quantification for the analysis.

similar heavy metal concentrations to the average value of the upper and lower parts of the *Cladina* species growing on the same plots.

Interspecies differences in the macronutrient concentrations were relatively small in vascular plants, the highest variation occurring in the K and Ca concentrations of the current-year shoots. There was more variation in the heavy metal concentrations. *Vaccinium* species, especially *V. myrtillus*, accumulated more Mn and Al than the other species (Mj). In general, *Empetrum nigrum* had higher Cu and Ni concentrations than the other dwarf shrubs on the polluted plots (H2 and H0.5), especially in the older plant parts. However, a sedge, *Carex globularis*, had the highest heavy metal concentrations in the current-year biomass (green leaves: Cu 254 μg/g and Ni 117 μg/g).

3.2.3. Variation between the study plots

The N and S concentrations of the cryptogams increased from the background areas to Harjavalta, as well as towards the smelter (Appendix A). In the vascular plants (current-year shoots), N and S concentrations were elevated only on the stand closest to the smelter (H0.5) (Appendix B). In contrast, the Mg and Ca concentrations of the vascular plants decreased towards the smelter. There were some differences in the macronutrient concentrations between the two background areas. Cryptogams had higher N and S concentrations, but lower concentrations of the other macronutrients in Hk than in Mj. This pattern was related to the input of elements to the ecosystems: Mj received less N and S deposition (Table 3), but had higher nutrient concentrations in the soil (Table 4).

All the heavy metal concentrations (except Mn) in the cryptogams, and the Cu, Ni, Fe and Al concentrations in the vascular plants (*V. vitis-idaea* and *E. nigrum*), increased on moving from the background areas to Harjavalta, as well as towards the smelter. The Zn and Pb concentrations in *V. vitis-idaea* were relatively similar between the plots, but increased with pollution level in *E. nigrum*. The Cu and Ni concentrations increased linearly in *P. nutans* and exponentially in *E. nigrum* towards the smelter (Fig. 3). The enrichment factors (H2 or H0.5 vs. Mj) of the heavy metals were much higher for the cryptogams (*C. islandica* and *P. nutans* Cu: 150-185, Ni: 45-55) than for the current-year shoots of vascular plants (*V. vitis-idaea* and *E. nigrum* Cu: 6–19, Ni: 4–10).

3.2.4. Variation between the plant parts within the species

In general, the younger (upper) plant parts had higher macronutrient concentrations and lower heavy metal concentrations compared to the older (lower) parts in the all life forms (Appendices A and B). In some cases, however, also macronutrient concentrations of lichens (except K) were higher in the lower, than in the upper parts (Appendix A). The S and heavy metal concentrations increased especially in the older parts towards the smelter in all the species. Berry samples were obtained only from *Arctostaphylos uva-ursi* and *V. vitis-idaea* (H2) and *V. uliginosum* (H0.5). The Cu concentrations of the berries were slightly lower than those of the current-year shoots at H2, but an opposite trend was found in *V. uliginosum* growing at H0.5 (Appendix B).

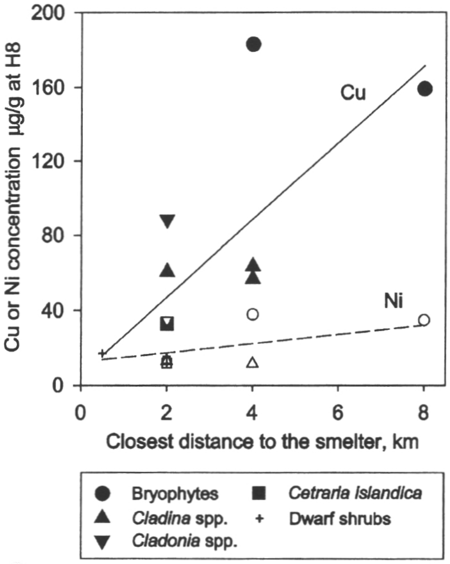


Fig. 4. The relationships (linear regressions) between the closest distance (km) to the smelter at which the species occurred (*x* axis) and Cu (black symbols, solid line) and Ni (open symbols, dashed line) accumulation (μg/g) in the upper parts/current-year shoots of different species at H8 (*y* axis). *P. nutans* excluded from bryophytes.

3.3. The maximum Cu, Ni, Zn and S concentrations of tissues

The maximum concentrations of Cu (160–180 μg/g), Ni (35–38 μg/g) and Zn (60–62 μg/g) in the upper parts of *Dicranum* spp. and *P. schreberi* occurred at H8 (Fig. 4). The corresponding values for the reindeer lichens (*Cladina* spp.) were relatively similar (Cu: 160–260 μg/g, Ni: 30–40 μg/g, Zn: 47–61 μg/g), but these concentrations were reached at H4, at which distance the bryophytes were absent. *C. islandica* showed the highest resistance within the lichen group, and even increased in abundance at 2 km distance (Table 2). The Cu (338 μg/g), Ni (57 μg/g) and Zn (115 μg/g) concentrations (whole thallus) of this species were higher than those of the other lichens. Some patches of *C. rangiferina* and cup lichens (*Cladonia* spp.) were also found at H2. *P. nutans* had almost 100 times higher concentrations of Cu (1397 μg/g) and Ni (334 μg/g) than the maximum of the other bryophytes, and was still alive at H0.5.

A few patches of *E. nigrum*, *C. globularis* and *V. uliginosum* were present close to the smelter (H0.5). *E. nigrum* and C. *globularis* accumulated higher concentrations of Cu (184–254 μg/g), Ni (51–117 μg/g) and Zn (175–243 μg/g) in the current-year biomass than *V. uliginosum* (Cu 38 μg/g, Ni 42 μg/g, Zn 68 μg/g) at H0.5 (Appendix B). The other dwarf shrubs (*A. uva-ursi*, *Calluna vulgaris* and *V. vitis-idaea*) appeared in the understorey vegetation at 2 km distance. Their maximum Cu (18–31 μg/g) and Ni (8–12 μg/g) concentrations in the current-year shoots were considerably lower than those of the cryptogams growing on the same plots.

The maximum S concentrations in the upper parts of all the cryptogams were below 1200 μg/g, except in *P. nutans*, which had an S concentration of over 1700 μg/g at H0.5 (Appendix A). In vascular plants, the highest S concentration of the current-year shoots was found in *V. uliginosum* (2500 μg/g) growing close to the smelter at H0.5, but in the other species the S level was clearly below 2000 μg/g (Appendix B).

3.4. The occurrence and abundance of the species in relation to Cu, Ni and S concentrations

The only distance where most of the species were present at Harjavalta, was 8 km from the smelter (Table 2). The Cu and Ni concentrations of the species at H8 (Appendices A and B) were plotted against the closest distance to the smelter at which they occurred (Table 2) in order to determine whether the accumulation pattern of these elements correlated with the sequence of species along the pollution gradient (Fig. 4). *P. nutans* was excluded from the comparison because it differed from all the other species. The accumulation of Cu in the different species at H8 correlated positively with the closest distance to the smelter at which the species occurred ($r=0.727$, $P=0.017$, $n=10$). A positive, but weaker trend was found for Ni ($r=0.460$, $P=0.180$, $n=10$). No correlation was found with respect to S ($r=-0.212$, $P=0.555$, $n=10$) although, within the cryptogams, lichens had lower S concentrations and they grew closer to the smelter than bryophytes.

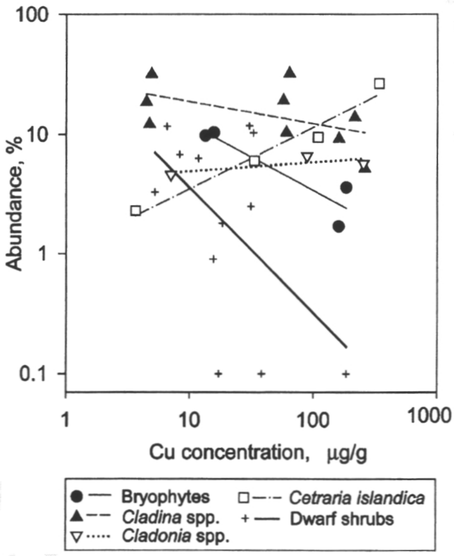


Fig. 5. The relationships (linear regressions) between the Cu concentration (μg/g) of the upper parts/current-year shoots (*x* axis) and the abundance (point frequency, %) of the species (*y* axis). Both axes in log scales. *P. nutans* excluded from bryophytes. Data from Harjavalta and Hämeenkangas.

The abundance (Table 2) of most of the species (dwarf shrubs, bryophytes and reindeer lichens) decreased on moving from the background area at 60 km distance (Hk) towards the Harjavalta smelter; their heavy metal, especially Cu (Fig. 5) and Ni, concentrations correspondingly increased. The abundance of dwarf shrubs was also inversely related to the S concentration of the current-year shoots. In contrast, *C. islandica*, which slightly increased towards the smelter, showed a positive trend between the abundance and Cu (Fig. 5), Ni and S concentrations of the thallus.

4. Discussion

4.1. Factors influencing pollutant accumulation in the understorey vegetation

The dust emitted from the Harjavalta smelter mainly consists of heavy metal sulfates and oxides fused with silica (Derome, 2000). The pattern of heavy metal deposition was relatively local in the surroundings of the smelter up until 1994 when the old 70-m high stacks were replaced by a 140-m high one. The most polluted area was restricted to a radius of 4 km.

In addition to the amount, quality and temporal development of the emissions, many edaphic, weather and biological factors also regulate pollutant accumulation in vegetation (Reimann et al., 1999; Kozlov et al., 2000). Pollutant-containing particles and aerosols are efficiently filtered by the canopies of conifers, and stand throughfall and litterfall increase the total deposition of heavy metals on the understorey and forest floor (Derome and Nieminen, 1998; Nieminen et al., 1999). The amount and acidity of the precipitation, altitude, aspect and wind direction, for instance, affect the accumulation of heavy metals in the cryptogams especially (reviewed by Conti and Cecchetti, 2001; Zechmeister et al., 2003). Furthermore, the uptake of heavy metals by the roots of vascular plants from the soil, and subsequent leaching from living or dead plant material, transfer, e.g. Mn to bryophytes (Berg et al., 1995). The effects of SO_2 fumigation on plants depend, e.g. on the concentration, time of exposure, air humidity and the transformation of SO_2 to other compounds (Wellburn, 1988).

The chemical form and stability of heavy metals emitted by industrial activities determine their toxicity to living organisms (Ross, 1994). The surface and intercellular accumulation of inert heavy metal-containing particles may account for a significant proportion of the total element concentrations of the unwashed plant samples in the Harjavalta material. In addition to atmospheric deposition, wind-blown dust originating from the forest floor or smelter area, may also be trapped by understorey vegetation and affect the chemical composition of the plants (Nieminen et al., 1999). However, only the phytoavailable fraction of heavy metals (mainly dissolved and exchangeable ions) has intracellular access and may be toxic at high concentrations (Ross, 1994). The accumulation of heavy metals in forest soil also indirectly affects the chemical composition of vascular plants by depressing microbial activity in the soil (Tyler et al., 1989), and by displacing base cations from the exchange sites in the organic layer (Derome and Lindroos, 1998).

4.2. Element accumulation compared to the relative resistance of plant species

In general, the species accumulating the largest amounts of Cu and Ni appeared in the understorey vegetation at further distances than those species capable of restricting the uptake of toxic elements. The occurrence of the life forms followed, in relation to the closest distance to the smelter at which they were present, the sequence: vascular plants (0.5–2 km) < *C. islandica* and some other lichens (2 km) < reindeer lichens (2–4 km) < bryophytes (*Dicranum* spp. and *P. schreberi*) (4–8 km). One pioneer moss (*P. nutans*) was an exception to this general pattern.

The sequence of the species along a pollution gradient may give some indication about the general resistance level of the species against pollution (e.g. heavy metals and SO_2) and other stress factors. In this respect, vascular plants were more resistant than lichens, whereas bryophytes were the most sensitive plant group. However, dwarf shrubs also apparently suffered from phytotoxic effects, which was expressed as their decreased abundance towards the smelter. Other environmental factors such as nutrient deficiencies in the soil, drought, the presence of needle litter and increased illumination, frequently increase the stress on plants in severely affected stands.

It is probable that the earlier relatively high SO_2 emissions have also affected the distribution of the plant species at Harjavalta. Although SO_2

emissions have decreased considerably since the end of the 1980s (Fig. 2), the mean concentration of SO_2 was still at the level of 17–18 μg/m^3 near the smelter in the beginning of the 1990s (Saari /3 et al., 199~~2~~). The critical level of SO_2 for sensitive lichen and bryophyte species is 5–10 μg/m^3 (Kashulina et al., 2003). Thus, in addition to current deposition and soil-accumulated heavy metals, the ambient SO_2 level may also represent an obstacle to recolonization by sensitive plant species.

Similar trends in the sequence of plant species around emission sources in coniferous forests have been reported, e.g. near the Cu–Zn smelter at Gusum, SE Sweden (Folkeson and Andersson-Bringmark, 1988; Tyler et al., 1989), a fertilizer factory in W Finland (Väisänen, 1986), a smelter complex in Sudbury, Ontario (Amiro and Courtin, 1981) and the Cu–Ni smelters in Monchegorsk, Kola Peninsula, NW Russia (Rigina and Kozlov, 2000).

4.2.1. *Maximum concentrations of Cu, Ni, Zn and S in cryptogams in the field studies*

The maximum concentrations of Cu in the upper parts of bryophytes (*P. schreberi* and *Dicranum* spp.) were 160–180 μg/g, and those of Ni 30–40 μg/g and S 1100–1200 μg/g in the Harjavalta data. Reimann et al. (1999) reported approximately similar median Cu (120–136 μg/g) and S (1190–1470 μg/g) concentrations, but considerable higher Ni (>200 μg/g) concentrations in bryophytes (composite samples of *P. schreberi* and *Hylocomium splendens*) growing near the smelter complexes on the Kola Peninsula (10 km from Zapoljarnij and 25 km from Monchegorsk). The same pattern was found in the reindeer lichens: the maximum Cu and S concentrations were at the same level in both areas, but the Ni concentrations were higher on the Kola Peninsula (Reimann et al., 1999). The highest Cu concentrations of *P. schreberi* and *D. polysetum* were much lower (70–80 μg/g) near (6–7 km) the Gusum smelter (Folkeson and Andersson-Bringmark, 1988). However, the same species accumulated larger amounts of Zn at Gusum (300–330 μg/g) than at Harjavalta (60 μg/g). The upper parts of *C. rangiferina* accumulated more Cu at Gusum (350 μg/g) than at Harjavalta (214 μg/g).

The variation in the maximum concentrations of elements in different geographical regions indicates that it is very difficult to present any common, maximum limits for the survival of cryptogams based on field data. According to our results and values from the literature, it is amazing how high metal concentrations cryptogams can withstand in field conditions compared, e.g. to the experimentally determined toxic limits of vascular plants (see below). However, it should be noted that unwashed samples have considerable amounts of dust attached to them, and the actual tissue concentrations that have harmful metabolic effects are much lower than those reported in field conditions (Brown and Brumelis, 1996; Bennett, 1999).

P. nutans, which accumulated exceptionally high concentrations of heavy metals along the Harjavalta gradient, has been considered to be a pollutant-resistant species also in other investigations (Amiro and Courtin, 1981; Tyler, 1990). Lepp and Salmon (1999) suggested that pleurocarpous bryophytes (those with a horizontal growth form) are more sensitive to toxic elements than acrocarpous ones (upright growth form) like *P. nutans*. This sensitivity may be related to differences in the water-conducting systems and uptake of soluble metals between the growth forms.

As Pakarinen (1981) has presented earlier, there are between-species differences within the *Cladina* genus in the capacity to accumulate toxic elements (*C. stellaris* > *C. arbuscula*). This was especially evident under an increasing pollution load. These results agree well with the observations of Garty (2001 ~~1999~~), who gave examples of how lichen species with finely divided thalli had a greater affinity to collect particulate matter than undivided ones. Accordingly, *C. islandica*, which is characterized by a flat, coarsely divided thallus, contained smaller amounts of pollutants than *Cladina* spp. growing at the same location. *C. islandica* proved to be the most resistant lichen species at Harjavalta, and it has also been found to grow near the smelter complexes on the Kola Peninsula (Chernenkova and Kuperman, 1999). However, *C. islandica*, as well as cup lichens (*Cladonia* spp.), may benefit from the disappearance of reindeer lichens in moderately polluted areas (Folkeson and Andersson-Bringmark, 1988).

4.2.2. Maximum Cu, Ni, Zn and S concentrations in vascular plants

Vascular plants were growing close to the pollution source. However, the resistance level varied between the individual vascular plant species. For instance, *C. vulgaris* and *A. uva-ursi* were absent from the most polluted area, where a few populations of *C. globularis*, *E. nigrum* and *V. uliginosum* had survived. The ranking of dwarf shrubs in experimental Cu exposures was the same as the order of the species occurrence along the pollution gradient: *E. nigrum* (most resistant) > *C. vulgaris* > *A. uva-ursi* (most sensitive) (Monni et al., 2000a,b; Salemaa and Monni, 2003). When we exclude the study plot closest to the smelter (H0.5), the maximum heavy metal concentrations measured in the current-year shoots of the dwarf shrubs (Cu 8–30 μg/g, Ni 4–12 μg/g, Zn 9–35 μg/g) were generally lower than the critical tissue values given in the literature. Marschner (1995) reported that toxic foliar concentrations of most crop species are above 20–30 μg/g for Cu, 10–50 μg/g for Ni and 100–300 μg/g for Zn. However, there are considerable differences between the vascular plant species and ecotypes in their toxic limits (Balsberg Påhlsson, 1989). The concentrations in the dwarf shrubs at Harjavalta increased with shoot age, and surface accumulation seemed to be especially high on the dead parts of vascular plants (e.g. *E. nigrum* had Cu > 4000 μg/g at H0.5).

The maximum Cu, Ni and S concentrations in the current-year shoots of *E. nigrum* growing closest to the Harjavalta smelter were lower than the values reported by Reimann et al. (1999) in *E. nigrum* growing at 5 km distance from Monchegorsk, the Kola Peninsula: median (max) Cu = 408 (626) μg/g, Ni = 329 (626) μg/g and S = 1390 (2020) μg/g. The Zn concentrations, however, were higher at Harjavalta. *E. nigrum* is able to accumulate very high concentrations of Cu and Ni, especially in its older stems, but also in older leaves (Monni et al., 2000a; Uhlig et al., 2001). In contrast, *V. uliginosum* seems to base its resistance on the restriction of metal transport to leaves. Uhlig and Junttila (2001) found a similar relationship between *E. nigrum* spp. *hermaphroditum* and *V. myrtillus* in Northern Norway, near to the adjacent Nikel and Zapoljarnij smelters on the Russian side of the border. Relatively low Cu and Ni concentrations were also found in the leaves and stems of lowbush blueberry *V. angustifolium*, growing near the smelter complexes at Sudbury, Canada (Bagatto and Shorthouse, 1991).

4.3. Applicability of the results in biomonitoring

Emissions from the Cu–Ni smelter and fertilizer factor at Harjavalta were clearly reflected in the elemental load in bulk deposition, and the concentrations in the understorey vegetation and organic layer. The deposition gradient was very steep, resulting in strong inter-correlations between all three compartments. This makes it extremely difficult to distinguish between the role of airborne deposition, wind-blown dust and elements taken up by the substrate in the chemical composition of the plants. As emphasized by other authors (e.g. Halleraker et al., 1998; Reimann et al., 2001), we conclude that the local conditions and the element ranges in deposition strongly influence the relationship between deposition and plant uptake in polluted areas.

The heavy metal and S concentrations were elevated especially in the older plant parts, which have been exposed to deposition for a longer time. The age (Pakarinen, 1981) and the growth rate (Zechmeister, 1995) affect the element accumulation and should be taken into account when using plants as accumulation indicators. Our results reveal that there are considerable differences between the bryophytes, lichens and vascular plants in their capacity to accumulate pollutants and to grow in contaminated soil. We conclude that information about all the life forms in the understorey is needed when evaluating the state and recovery of forest ecosystems in heavily polluted areas.

Acknowledgments

We thank the staff of the Mekrijärvi Research Station, University of Joensuu, and Pia Reponen,

M. Sci., for helping in the data handling phase of the study. The comments of Dr Pasi Rautio and Dr Timo Vuorisalo on the manuscript are greatly acknowledged. The final version of the manuscript was greatly improved by the suggestions and comments of Dr James Bennett and an anonymous referee, to whom we express our sincere thanks.

Appendix A:

The mean element concentrations in lichens and bryophytes. Parts: 1 = living upper part, 2 = decomposing lower part, 3 = the whole thallus. *n* = number of composite samples.

			Macronutrients mg/g						Heavy metals and Al μg/g							
Area and year	Part	*n*	N%	P	K	Mg	S	Ca	Fe	Zn	Mn	Cu	Ni	Cd	Pb	Al
Mekrijärvi 1992																
C. islandica	1	1	0.49	0.60	3.00	0.33	0.48	0.86	111.7	33.0	169.3	2.27	1.26	0.16	3.06	101.9
	2	1	0.37	0.70	1.89	0.36	0.51	1.34	331.0	41.4	224.7	3.70	2.66	0.17	5.56	271.7
C. arbuscula	1	1	0.51	0.55	1.92	0.33	0.44	0.76	139.4	19.9	181.0	2.13	1.30	0.13	2.79	131.6
	2	1	0.48	0.48	1.00	0.22	0.46	0.73	342.1	17.7	194.9	2.82	2.03	0.08	3.49	281.3
C. rangiferina	3	1	0.52	0.43	1.41	0.28	0.37	0.68	211.4	16.8	147.8	2.29	1.78	0.14	4.79	191.4
C. stellaris	3	1	0.35	0.34	1.12	0.18	0.28	0.47	134.7	16.6	80.5	1.69	2.01	0.13	3.52	117.3
Dicranum spp.	1	1	1.10	1.35	5.74	1.17	0.72	2.37	343.0	44.4	878.5	8.45	4.25	0.25	8.70	380.8
	2	1	0.94	1.11	4.45	0.91	1.03	2.43	649.6	59.0	916.1	7.34	4.55	0.27	13.91	724.0
P. schreberi	1	3	0.96	1.22	5.23	1.08	0.89	3.20	282.6	41.0	705.3	6.48	3.65	0.20	9.44	303.6
	2	3	0.80	0.84	2.96	0.57	0.85	2.96	624.3	47.8	583.1	5.70	5.05	0.30	15.02	632.0
P. nutans	3	1	1.18	1.44	4.17	0.82	–	3.69	480.0	56.5	731.6	7.55	6.06	0.41	22.25	564.9
Hämeenkangas 1992																
C. islandica	1	1	0.51	0.31	1.86	0.17	0.39	0.34	113.9	24.0	40.1	3.66	2.13	0.17	6.74	100.8
	2	1	0.50	0.35	0.97	0.17	0.35	0.67	505.7	34.1	61.8	7.01	5.22	0.20	12.03	373.8
C. arbuscula	1	4	0.67	0.42	1.55	0.21	0.56	0.44	196.8	28.1	53.0	4.91	1.59	0.16	4.90	165.2
	2	4	0.70	0.44	0.73	0.17	0.56	0.55	474.4	22.2	55.3	7.25	2.95	0.15	8.75	346.6
C. rangiferina	1	3	0.67	0.42	1.67	0.21	0.49	0.41	283.4	27.2	48.1	4.73	5.70	0.20	6.00	158.9
	2	1	0.68	0.40	0.65	0.17	0.51	0.57	637.4	27.4	48.5	8.84	3.85	0.19	18.90	446.5
C. stellaris	1	4	0.61	0.36	1.30	0.18	0.65	0.36	248.1	26.8	37.3	4.47	3.09	0.12	5.26	182.4
	2	4	0.64	0.42	0.59	0.16	0.51	0.51	576.8	22.6	48.9	8.12	3.88	0.14	9.96	409.5
Cladonia spp.	3	1	0.85	0.64	1.80	0.23	0.76	0.61	454.6	46.6	63.9	7.05	2.81	0.22	9.17	426.9
C. uncialis	3	1	0.57	0.34	1.26	0.18	0.45	0.30	229.9	32.2	33.6	4.97	1.92	0.15	5.49	180.7
Dicranum spp.	1	1	1.31	0.98	3.85	0.86	1.28	3.49	681.7	58.2	370.3	15.63	7.85	0.48	20.63	548.8
	2	1	1.20	0.97	2.59	0.64	1.48	3.46	1013.4	77.1	350.3	16.85	10.17	0.61	24.73	926.3
P. schreberi	1	2	1.24	0.86	3.20	0.74	1.12	2.81	547.4	55.6	342.1	13.35	7.34	0.39	20.09	523.3
	2	1	0.90	0.77	2.02	0.47	1.14	2.80	825.8	55.4	237.5	14.40	8.65	0.36	25.30	804.9
P. nutans	3	1	1.36	0.96	1.91	0.61	1.04	2.76	848.4	82.7	209.1	13.82	10.99	0.70	47.74	922.5
Polytrichum juniperinum	3	1	1.10	0.81	2.66	0.58	0.85	2.23	436.7	54.3	222.3	16.26	6.04	0.44	22.97	797.0
Harjavalta 8 km 1992																
C. islandica	1	1	0.48	0.42	2.30	0.18	0.52	0.36	220.1	39.7	27.5	33.03	12.02	0.20	4.44	162.6
	2	1	–	0.42	1.41	0.25	–	1.00	869.4	52.4	74.9	109.87	25.13	0.34	8.44	499.2
C. arbuscula	1	4	0.78	0.51	1.78	0.28	0.76	0.50	394.4	37.6	35.0	56.94	11.92	0.27	7.69	253.7
	2	4	0.77	0.61	0.92	0.61	0.90	1.28	1179.3	42.6	59.0	162.05	29.95	0.31	14.31	609.6
C. rangiferina	1	3	0.78	0.51	1.71	0.24	0.75	0.35	448.7	37.0	39.6	60.26	13.10	0.33	10.83	284.3
	2	2	0.83	0.54	0.88	0.26	0.91	0.58	1191.1	40.2	54.3	148.78	29.58	0.33	18.39	603.8
C. stellaris	1	4	0.75	0.53	1.67	0.25	0.82	0.39	426.4	40.9	29.2	63.60	11.74	0.26	9.62	264.6
	2	4	0.80	0.63	0.78	0.35	0.93	0.97	1291.1	40.6	52.9	175.24	30.60	0.32	19.75	612.7
Cladonia spp.	3	1	0.85	0.64	2.07	0.27	1.00	0.61	569.0	54.4	56.6	88.41	19.36	0.33	12.01	389.3
Dicranum spp.	1	2	1.14	1.26	5.14	0.78	1.11	2.82	962.3	61.5	272.6	159.14	34.97	0.83	24.81	772.6
	2	2	1.12	1.11	3.36	0.67	1.38	3.35	1684.2	87.1	296.9	231.67	65.01	0.84	41.99	1264.9
P. schreberi	1	3	1.19	1.15	4.25	0.66	1.19	2.25	1054.8	59.7	206.8	183.10	37.97	0.64	30.48	801.7
	2	2	0.98	0.98	2.05	0.51	1.27	2.29	1884.9	73.5	195.8	276.49	66.41	0.86	55.24	1237.8
P. nutans	3	1	1.48	1.36	2.14	0.58	1.56	2.57	1518.9	94.1	189.2	268.72	79.64	1.20	61.35	1110.6
Harjavalta 4 km 1992																
C. islandica	1	3	0.61	0.37	1.66	0.17	0.58	0.32	365.0	54.9	22.3	108.47	26.40	0.34	14.48	190.3

	2	1	1.06	0.68	1.03	0.25	1.05	0.79	1254.2	70.4	52.0	365.43	76.24	0.66	29.44	483.5
C. arbuscula	1	3	0.77	0.51	1.52	0.22	0.82	0.30	631.5	46.5	24.0	159.90	28.87	0.50	13.38	246.0
	2	1	0.93	0.71	0.96	0.28	1.35	0.65	1959.4	72.8	41.3	447.93	70.15	0.71	25.93	552.6
C. rangiferina	1	2	0.81	0.54	1.60	0.24	0.85	0.37	875.7	54.7	30.2	214.29	42.36	0.53	19.68	309.2
	2	1	0.97	0.69	0.89	0.32	1.44	0.64	2638.2	82.1	47.4	586.27	96.55	0.70	37.84	773.3
C. stellaris	1	2	0.85	0.62	1.55	0.26	1.05	0.40	1032.8	61.1	24.0	258.70	41.96	0.53	22.40	342.0
	2	1	0.88	0.58	1.42	0.24	1.16	0.30	1215.6	62.7	22.9	324.14	48.37	0.51	26.27	383.6
Cladonia spp.	3	4	0.93	0.64	2.02	0.28	0.99	0.69	886.2	74.0	45.1	252.31	51.54	0.58	23.06	395.4
P. nutans	3	1	1.49	1.27	1.60	0.54	1.64	2.93	2576.1	141.1	165.6	872.03	208.66	1.87	82.08	1198.6
Harjavalta 2 km 1992–1994																
C. islandica (-92)	3	4	0.69	0.52	2.10	0.24	0.69	0.52	890.7	114.6	31.2	338.25	56.63	0.54	21.81	239.0
P. nutans (-94)	3	1	1.57	2.19	5.68	0.92	1.62	3.55	2332.6	202.4	348.0	1054.71	291.83	2.68	57.85	708.3
Harjavalta 0.5 km 1994																
P. nutans	3	1	1.46	2.61	4.22	1.65	1.72	4.24	4022.1	298.2	39.7	1397.19	334.22	3.04	71.39	271.8

Appendix B:

The mean element concentrations in vascular plant species. Parts: 1 = current living shoots, 2 = older living parts, 3 = dead parts, 4 = berries. *n* = number of composite samples.

			Macronutrients mg/g						Heavy metals and Al µg/g							
Area and year	Part	*n*	N%	P	K	Mg	S	Ca	Fe	Zn	Mn	Cu	Ni	Cd	Pb	Al
Mekrijärvi 1992																
C. vulgaris	1	1	1.25	1.16	5.97	1.87	1.63	4.24	65.4	17.0	469.9	4.38	4.21	0.06	2.65	61.5
	2	1	0.72	0.74	2.65	0.97	1.10	2.81	97.2	18.1	879.8	5.87	4.79	0.11	3.38	97.0
E. nigrum	1,2	1	0.83	0.99	3.26	1.03	0.78	3.32	61.7	17.5	1023.3	9.59	5.24	0.13	4.07	68.4
V. myrtillus	1	1	1.22	1.32	5.07	1.78	1.02	2.97	49.1	18.4	2580.9	6.71	5.68	0.06	2.93	123.0
	2	1	0.64	0.83	3.02	0.92	0.73	2.20	34.5	32.0	2358.9	7.07	4.82	0.14	2.25	141.9
V. vitis-idaea	1	1	0.93	1.14	4.71	1.28	1.07	2.66	44.4	22.3	1054.0	5.62	3.32	0.04	1.27	115.8
	2	1	0.73	0.84	3.29	0.91	0.89	2.56	58.0	22.1	1158.4	6.22	3.46	0.08	2.42	136.8
Hämeenkangas 1992																
C. vulgaris	1	1	1.29	1.02	5.15	1.17	1.55	3.94	73.3	27.4	351.2	6.57	3.98	0.02	3.81	79.0
	2	1	0.81	0.69	2.72	0.69	1.04	2.69	138.8	22.3	556.6	7.05	4.45	0.09	5.14	122.3
	3	1	0.80	0.62	2.54	0.64	1.22	3.03	130.3	23.1	707.0	7.07	3.36	0.18	7.02	146.6
V. vitis-idaea	1	1	1.11	0.96	4.11	0.98	1.45	3.93	60.2	33.8	529.0	5.24	2.72	0.01	2.90	120.0
	2	1	0.92	0.88	3.41	0.81	1.42	3.79	97.5	47.2	684.6	7.76	3.40	0.07	7.47	169.5
Deschampsia flexuosa	1	1	0.78	1.10	4.61	0.30	2.01	0.70	295.6	40.80	218.4	4.11	11.8	0.17	6.68	186.4
Harjavalta 8 km 1992																
C. vulgaris	1	1	1.30	1.13	6.32	1.11	1.80	3.44	112.6	15.8	157.5	15.52	7.52	0.02	1.28	82.3
	2	2	0.61	0.57	3.39	0.68	1.14	2.20	159.7	13.4	537.9	34.29	11.27	0.12	4.82	119.1
E. nigrum	1	1	1.25	1.63	10.49	1.16	1.55	3.52	71.7	20.8	107.8	17.16	9.06	–	1.02	37.8
	2	1	0.75	0.78	3.90	0.69	0.79	3.33	128.7	21.5	177.5	30.33	12.41	0.07	4.75	94.8
V. vitis-idaea	1	1	1.08	1.16	5.20	1.31	1.45	4.26	57.2	35.3	579.8	11.75	7.32	–	0.78	118.3
	2	1	0.91	0.79	3.32	0.86	1.17	2.59	150.1	43.8	668.5	27.95	12.70	0.20	6.22	197.6
Harjavalta 4 km 1992																
E. nigrum	1	1	1.15	0.60	3.18	0.52	1.16	1.83	31.0	8.8	27.5	8.30	4.08	0.05	0.91	13.8
	2	1	0.59	0.56	2.53	0.53	0.73	2.61	372.5	31.4	174.4	149.32	36.42	0.38	13.97	174.4
V. vitis-idaea	1	1	0.88	0.89	4.49	0.91	0.88	4.78	91.3	30.7	396.2	30.18	10.31	0.38	2.19	105.2
	2	2	0.74	0.67	3.06	0.68	1.36	3.25	192.4	47.1	477.0	79.76	21.68	0.28	10.54	189.9
Harjavalta 2 km 1993																
E. nigrum	1	4	1.16	1.50	8.00	0.99	1.30	3.86	189.6	24.9	43.6	32.54	12.48	0.02	2.40	27.0
	2	4	0.61	0.84	3.78	0.70	0.97	2.96	1000.0	64.7	108.6	403.40	53.57	0.55	16.69	206.4
	3	1	0.56	0.55	1.22	0.53	1.06	3.16	1995.0	114.9	90.5	873.82	103.74	1.10	31.04	363.9
V. vitis-idaea	1	4	0.85	0.99	5.24	0.99	1.88	2.80	142.5	31.3	313.5	31.08	11.78	0.04	1.77	143.3
	2	3–4	0.74	0.74	4.48	0.75	2.08	2.46	368.2	46.0	366.6	97.94	22.13	0.15	6.44	228.2
	4	1	–	1.34	7.73	0.66	–	2.52	59.5	15.8	102.7	13.78	8.64	–	6.54	53.9
A. uva-ursi	1	4	0.79	1.35	6.87	0.95	0.72	2.95	102.5	30.3	11.0	18.28	8.49	0.03	1.16	33.4
	2	4	0.55	1.02	4.88	0.86	0.53	2.10	522.4	95.1	153.8	202.77	35.49	0.35	9.50	163.7

	3	2–3	0.68	0.80	2.08	0.66	0.72	4.86	933.2	120.6	106.7	413.29	78.59	1.02	22.09	282.2
	4	1	0.61	1.22	7.39	0.62	0.43	1.67	44.1	16.5	8.6	12.41	11.99	–	6.11	4.1
Harjavalta 0.5 km 1993																
E. nigrum	1	3	1.38	1.72	7.00	1.33	1.72	3.40	2981.0	174.6	215.7	183.69	50.55	0.37	22.40	119.2
	2	4	0.72	0.90	2.88	0.94	1.87	2.60	8632.4	446.6	276.8	1453.66	166.96	1.79	99.54	411.2
	3	1–4	0.70	1.40	2.38	1.54	2.14	4.82	20570.8	1041.2	280.9	4134.83	473.12	4.12	229.31	967.4
V. uliginosum	1	4	1.43	2.05	10.89	0.82	2.51	2.67	451.7	67.8	138.9	38.07	42.25	0.62	5.68	57.2
	2	4	0.66	0.71	2.07	0.38	0.88	1.83	2157.0	162.5	137.1	329.94	61.76	0.99	36.64	131.7
	3	1	–	0.84	4.52	0.99	–	3.66	4037.2	235.7	60.1	561.06	86.06	1.17	43.04	157.5
	4	1	–	1.57	6.86	0.41	2.44	1.12	590.4	47.6	21.6	55.49	15.56	0.20	9.87	93.8
C. globularis	1	1	–	1.41	21.29	0.56	–	2.11	1625.1	242.7	233.5	254.65	117.02	0.74	13.64	72.4
	3	1	1.53	1.02	1.92	0.66	–	1.95	16984.0	844.3	140.0	3978.90	488.11	4.64	209.60	888.2

References

Alloway BJ. Soil processes and the behaviour of metals. In: Alloway BJ, editor. Heavy metals in soils. London: Blackie Academic and Professional, 1995. p. 11–37.

Amiro BD, Courtin GM. Patterns of vegetation in the vicinity of an industrially disturbed ecosystem, Sudbury, Ontario. Can J Bot 1981;59:1623–1639.

Bagatto G, Shorthouse JD. Accumulation of copper and nickel in plant tissues and an insect gall of lowbush blueberry, *Vaccinium uliginosum*, near an ore smelter at Sudbury, Ontario, Canada. Can J Bot 1991;69:1483–1490.

Balsberg Påhlsson AM. Toxicity of heavy metals (Zn, Cu, Cd, Pb) to vascular plants. A literature review. Water Air Soil Pollut 1989;47:287–319.

Bates JW. Mineral nutrient acquisition and retention by bryophytes. J Bryol 1992;17:223–240.

Bennett JP. Statistical baseline values for chemical elements in the lichen *Hypogymnia physodes*. In: Agrawal SB, Agrawal M, editors. Environmental pollution and plant responses. Boca Raton: Lewis Publishers, 1999. p. 343–353.

Berg T, Røyset O, Steinnes E. Moss (*Hylocomium splendens*) used as biomonitor of atmospheric trace element deposition: estimation of uptake efficiencies. Atmos Environ 1995;29:353–360.

Brown DH, Brumelis G. A biomonitoring method using the cellular distribution of metals in moss. Sci Total Environ 1996;187:153–161.

Chernenkova TV, Kuperman RG. Changes in the forest communities along a heavy metal deposition gradient on Kola Peninsula. Water Air Soil Pollut 1999;111:187–200.

Chettri MK, Sawidis T, Zachariadis GA, Stratis JA. Uptake of heavy metals by living and dead *Cladonia* thalli. Environ Exp Bot 1997;37:39–52.

Conti ME, Cecchetti G. Biological monitoring: lichens as bioindicators of air pollution assessment—a review. Environ Pollut 2001;114:471–492.

Dahlquist RL, Knoll JW. Inductively coupled plasma-atomic emission spectrometry: analysis of biological materials and soils for major, trace and ultra-trace elements. Appl Spectrosc 1978;32:1–30.

Derome J. Effects of heavy-metal and sulfur deposition on the chemical properties of forest soil in the vicinity of a Cu–Ni smelter, and means of reducing the detrimental effects of heavy metals. Finn For Res Inst, Research papers 2000;769:1–77.

Derome J, Lindroos AJ. Effects of heavy metal contamination on macronutrient availability and acidification parameters in forest soil in the vicinity of Harjavalta Cu–Ni smelter, SW Finland. Environ Pollut 1998;99:225–232.

Derome J, Nieminen T. Metal and macronutrient fluxes in heavy-metal polluted Scots pine ecosystems in SW Finland. Environ Pollut 1998;103:219–228.

Ernst WHO. Bioavailability of heavy metals and decontamination of soils by plants. Appl Geochem 1996;11:163–167.

Folkeson L, Andersson-Bringmark E. Impoverishment of vegetation in a coniferous forest polluted by copper and zinc. Can J Bot 1988;66:417–428.

Garty J. Biomonitoring atmospheric heavy metals with lichens: theory and application. Crit Rev Plant Sci 2001;20:309–371.

Hall JL. Cellular mechanisms for heavy metal detoxification and tolerance. J Exp Bot 2002;53:1–11.

Halleraker JH, Reimann C, de Caritat P, Finne TE, Kashulina G, Niskavaara H, Bogatyrev I. Reliablity of moss (*Hylocomium splendens* and *Pleurozium schreberi*) as a bioindicator of atmospheric chemistry in the Barents region: interspecies and field dublicate variability. Sci Total Environ 1998;218:123–139.

Hämet-Ahti L, Suominen J, Ulvinen T, Uotila P, editors. Retkeilykasvio (Field Flora of Finland), Edn. 4. Finnish Museum of Natural History, Botanical Museum, Helsinki, 1998, p. 656.

Haslett BS, Reid RJ, Rengel Z. Zinc mobility in wheat: uptake and distribution of zinc applied to leaves and roots. Ann Bot 2001;87:379–386.

Helmisaari HS. Nutrient cycling in *Pinus sylvestris* stands in Eastern Finland. Plant Soil 1995;168–169:327–336.

Helmisaari HS, Mälkönen E. Acidity and nutrient content of throughfall and soil leachate in three *Pinus sylvestris* stands. Scand J For Res 1989;4:13–28.

Kashulina G, Reimann C, Banks D. Sulfur in the Arctic environment (3): environmental impact. Environ Pollut 2003;124:151–171.

Koponen T, Isoviita P, Lammes T. The bryophytes of Finland: an annotated checklist. Flora Fennica 1977;6:1–77.

Kozlov MV, Haukioja E, Bakhtiarov AV, Stroganov DN, Zimina SN. Root vs. canopy uptake of heavy metals by birch in an industrially polluted area: contrasting behaviour of nickel and copper. Environ Pollut 2000;107:413–420.

Kulmala A, Leinonen L, Ruoho-Airola T, Salmi T, Walden J. Air quality trends in Finland. Air quality measurements. Helsinki: Finnish Meteorological Institute, 1998. p. 91

Lepp NW, Salmon D. A field study of the ecotoxicology of copper to bryophytes. Environ Pollut 1999;106:153–156.

Mälkönen E, Derome J, Kukkola M, Saarsalmi A. Improving forest condition by means of slow-release fertilisers. In: Mälkönen E, editor. Forest condition in a changing environment—the Finnish case. Dordrecht: Kluwer Academic Publishers, 2000. p. 301–323.

Mälkönen E, Derome J, Fritze H, Helmisaari HS, Kukkola M, Kytö M, Saarsalmi A, Salemaa M. Compensatory fertilization of Scots pine stands polluted by heavy metals. Nutr Cycl Agroecosys 1999;55:239–268.

Manninen S, Huttunen S. Scots pine needles as bioindicators of sulfur deposition. Can J For Res 1995;25:1559–1569.

Marschner H. Mineral nutrition of higher plants, 2nd ed. London: Academic Press, 1995. p. 889

Meharg AA, Cairney JWG. Co-evolution of mycorrhizal symbiosis and their hosts to metal-contaminated environments. Adv Ecol Res 2000;30:69–112.

Monni S, Salemaa M, Millar N. The tolerance of *Empetrum nigrum* to copper and nickel. Environ Pollut 2000a;109:221–229.

Monni S, Salemaa M, White C, Tuittila E, Huopalainen M. Copper resistance of *Calluna vulgaris* originating from the pollution gradient of a Cu–Ni smelter, in Southwest Finland. Environ Pollut 2000b;109:211–219.

Nieminen T, Derome J, Helmisaari HS. Interactions between precipitation and Scots pine canopies along a heavy-metal pollution gradient. Environ Pollut 1999;106:129–137.

Pakarinen P. Nutrient and trace metal content and retention in reindeer lichen carpets of Finnish ombrotrophic bogs. Ann Bot Fenn 1981;18:265–274.

14 Poikolainen J, Kubin E, Piispanen J, Karhu J. Atmospheric heavy metal deposition in Finland during 1985–2000 using mosses as bioindicators. Sci Total Environ 2003; ~~in press.~~ 318: 171–185

Rautio P, Huttunen S. Total vs. internal element concentrations in Scots pine needles along a sulfur and metal pollution gradient. Environ Pollut 2003;122:273–289.

Reimann C, Halleraker JH, Kashulina G, Bogatyrev I. Comparison of plant and precipitation chemistry in catchments with different levels of pollution on the Kola Peninsula, Russia. Sci Total Environ 1999;243–244:169–191.

Reimann C, Niskavaara H, Kashulina G, Filzmoser P, Boyd R, Volden T, Tomilina O, Bogatyrev I. Critical remarks on the use of terrestrial moss (*Hylocomium splendens* and *Pleurozium schreberi*) for monitoring airborne pollution. Environ Pollut 2001;113:41–57.

Rigina O, Kozlov MV. Pollution impact on sub-arctic forests in the Kola Peninsula, Russia. In: Innes JL, Oleksyn J, editors. Forest dynamics in heavily polluted regions. Wallingford: CAB International, 2000. p. 37–65.

Ross SM. Retention, transformation and mobility of toxic metals in soils. In: Ross SM, editor. Toxic metals in soil–plant systems. Chichester: John Wiley, 1994. p. 63–152.

Saari H, Kartastenpää R, Pohjola V, Voltti U. Particle and sulfur dioxide research at Harjavalta in the spring 1992. Helsinki: Finnish Meteorological Institute, 1993. p. 66 (In Finnish. Hiukkas- ja rikkidioksiditutkimus Harjavallassa keväällä 1992).

Salemaa M, Monni S. Copper resistance of the evergreen dwarf shrub *Arctostaphylos uva-ursi*: an experimental exposure. Environ Pollut 2003;126:435–443.

Salemaa M, Vanha-Majamaa I, Derome J. Understorey vegetation along a heavy-metal pollution gradient in SW Finland. Environ Pollut 2001;112:339–350.

Tyler G. Uptake, retention and toxicity of heavy metals in lichens. A brief review. Water Air Soil Pollut 1989;47:321–333.

Tyler G. Bryophytes and heavy metals: a literature review. Bot J Linn Soc 1990;104:231–253.

Tyler G, Balsberg Påhlsson AM, Bengtsson G, Bååth E, Tranvik L. Heavy metal ecology of terrestrial plants, micro organisms and invertebrates. Water Air Soil Pollut 1989;47:189–215.

Uhlig C, Junttila O. Airborne heavy metal pollution and its effects on foliar elemental composition of *Empetrum hermaphroditum* and *Vaccinium myrtillus* in Sør-Varanger, Northern Norway. Environ Pollut 2001;114:461–469.

Uhlig C, Salemaa M, Vanha-Majamaa I, Derome J. Element distribution in *Empetrum nigrum* microsites at heavy metal contaminated sites in Harjavalta, Western Finland. Environ Pollut 2001;112:435–442.

Väisänen S. Effects of air pollution by metal, chemical and fertilizer plants on forest vegetation at Kokkola, W Finland. Ann Bot Fenn 1986;23:305–315.

Vitikainen O, Ahti T, Kuusinen M, Lommi S, Ulvinen T. Checklist of lichens and allied fungi of Finland. Norrlinia 1997;6:1–123.

Wellburn A. Air pollution and acid rain. The biological impact. London: Longman group, 1988. p. 274

Zechmeister HG. Growth rates of five pleurocarpous moss species under various climatic conditions. J Bryol 1995;18:455–468.

Zechmeister HG, Grodzinska K, Szarek-Lukaszewska G. Bryophytes. In: Markerts BA, Breure AM, Zechmeister HG, editors. Bioindicators and biomonitors. Amsterdam: Elsevier, 2003. p. 329–375.

Paper III

Salemaa, M. & Uotila, T. 2001. Seed bank composition and seedling survival in forest soil polluted with heavy metals. Basic and Applied Ecology 2: 251-263.

III

Basic Appl. Ecol. **2**, 251–263 (2001)
© Urban & Fischer Verlag
http://www.urbanfischer.de/journals/baecol

Basic and Applied Ecology

Seed bank composition and seedling survival in forest soil polluted with heavy metals

Maija Salemaa[1]*, Taru Uotila[2]

[1]Vantaa Research Centre, The Finnish Forest Research Institute, P.O. Box 18, FIN-01301 Vantaa, Finland
[2]Department of Limnology and Environmental Protection, University of Helsinki, PB 62 Info Centre, Viikinkaari 11 A, FIN-00014 University of Helsinki, Finland

Received January 2, 2001 · Accepted March 26, 2001

Abstract

Seedling recruitment from forest soil polluted with heavy metals was studied in order to determine the revegetation potential of the seed bank. The soil samples were collected from untreated and fertilised Scots pine stands along a 8 km transect running SE from a copper-nickel smelter in SW Finland. The composition and size of the active seed bank and the survival of the germinated seedlings were studied in greenhouse conditions. The average densities of germinated seeds of 15 species ranged from 250 to 4750 plants per m^2 at the six sites. Although vegetation was almost totally absent near the smelter, germinable seeds of *Betula pubescens*, *Calluna vulgaris*, *Pinus sylvestris* and *Vaccinium uliginosum* were found in the most contaminated soil. The number of *Calluna vulgaris* seedlings increased with increasing distance from the smelter, but no such trend was found for the other species.

The mortality rate of the seedlings was highest in the soil samples collected near the smelter. Nutrient addition (stand-specific fertilisation with N, Ca, P and Mg) did not affect the number of germinated seeds, but liming slightly reduced heavy metal induced death of the seedlings. The survival probability of *Calluna vulgaris* seedlings decreased with proximity to the smelter.

The results suggest that the recovery of *Calluna vulgaris* near the smelter was prevented by a low availability of seeds in the soil, unfavourable germination conditions and unsuccessful seedling establishment. We conclude that, despite the presence of viable seeds in polluted soil, revegetation from seed banks is not successful without soil mitigation to immobilise heavy metals.

Das Revegetationspotential einer Samenbank in einem mit Schwermetall belasteten Waldboden wurde in einem Treibhausexperiment untersucht. Die Bodenproben stammten von insgesamt 6 Kontroll- und Düngungsflächen, die auf einem 8 km langen Transekt angelegt worden waren. Der Ausgangspunkt des Transekts war eine Kupfer-Nickel-Gießerei im Südwesten Finnlands. Die Samenbanken brachten 15 Planzenarten hervor, deren Mittelwerte von 250 bis 4750 Exemplaren pro m^2 variierten. Trotz einer fast nicht existierenden Vegetation neben der Gießerei wies die Samenbank des belasteten Standorts Sämlinge der Arten *Betula pubescens*, *Calluna vulgaris*, *Pinus sylvestris* and *Vaccinium uliginosum* auf. Nur die Anzahl der *Calluna vulgaris* Sämlinge nahm zu mit wachsender Distanz zur Gießerei.

Die Mortalität der Sämlinge war am höchsten in den belasteten Bodenproben neben der Gießerei. Eine standortspezifische Düngung mit N, Ca, P und Mg erzielte das gleiche Ergebnis, aber durch Kalkung konnte die Mortalität der Sämlinge geringfügig vermindert werden. Die Überlebensrate der *Calluna vulgaris* Triebe verminderte sich mit abnehmender Distanz zur Gießerei.

***Corresponding author:** Maija Salemaa, Vantaa Research Centre, The Finnish Forest Research Institute, P.O. Box 18, FIN-01301 Vantaa, Finland, Phone: ++358-9-8570 5565, Fax: ++358-9-8570 5569, E-mail: maija.salemaa@metla.fi

1439-1791/01/02/03-251 $ 15.00/0

Unsere Ergebnisse zeigen, dass die Ansiedlung der Art *Calluna vulgaris* in der Nähe der Gießerei durch die niedrige Anzahl von Samen im Boden, schlechte Keimungsbedingungen und erhöhte Mortalität kontrolliert wird. Eine Revegetation kann trotz keimungsfähiger Samen in mit Schwermetall belasteten Böden nicht erfolgen, solange die Schwermetalle nicht immobilisiert werden.

Key words: actuarial life tables – *Calluna vulgaris* – fertilisation – liming – revegetation

Introduction

Many studies on post-fire and clear-cut succession (Moore & Wein 1977, Archibold 1979, Johnson & Bradshaw 1979) and gap dynamics of forests (Mladenoff 1990, McGee & Feller 1993) emphasise the importance of soil seed banks in the regeneration of forest vegetation after a disturbance. The potential role of seed banks in restoring ecologically valuable communities, e.g. in heathlands (Pywell et al. 1997, Mitchell et al. 1998) and forests (Halpern et al. 1999, Onaindia & Amezaga 2000), has also been studied. However, only a few studies have focused on seed bank dynamics after a disturbance caused by airborne pollution of forest soil (Vieno et al. 1993, Komulainen et al. 1994, Huopalainen et al. 2000, Winterhalder 2000).

The zonal degradation of forest vegetation has been widely documented around smelter complexes emitting heavy metals, sulphur and other pollutants in the boreal coniferous zone (Freedman & Hutchinson 1980, Folkeson & Andersson-Bringmark 1988, Aamlid et al. 2000, Rigina & Kozlov 2000, Salemaa et al. 2001). The absence of a vegetation cover enhances the wind erosion of metal-contaminated particles, decreases the water-holding capacity of the soil and facilitates the leaching of heavy metals into the ground water (Vangronsveld et al. 1996, Derome & Nieminen 1998). Revegetation of degraded sites is therefore essential to stabilise the soil and to reduce the adverse environmental impacts (Johnson & Bradshaw 1979, Kiikkilä et al. 2001). Recolonization of the vegetation in disturbed areas can be realised via (1) propagules (e.g. seeds, spores, buds, rhizomes or roots) stored in the soil, (2) propagules dispersed from the neighbouring areas, or (3) vegetative or sexual reproduction of the existing remnants of the plant populations (Moore & Wein 1977, Komulainen et al. 1994).

Although heavy metal deposition has considerably decreased during the last decades in northern Europe (Kubin et al. 2000), natural recolonization of the vegetation in heavily polluted areas still faces many problems. In areas where the vegetation has been absent for decades, the shortage of propagules may be a factor limiting revegetation. Furthermore, the initial development of the seedlings is sensitive to metals and acidity (Bradley et al. 1981, Patterson & Olson 1983, Bell & Teramura 1991). Natural recovery of the vegetation in industrial barrens, e.g. in the Sudbury area, Canada, has proved to be a very slow process without soil mitigation and the sowing or replanting of tolerant plant species (Winterhalder 2000).

Information about the germination capacity of the soil seed banks and the critical early phases of seedling establishment helps us to choose the most viable method for restoring forest areas suffering from the impacts of severe pollution. In this paper we evaluate the possibility of re-establishing the native understorey vegetation of a Scots pine forest damaged by the deposition of heavy metals and sulphur. Our aims were to determine:

(1) the size and species composition of the active seed bank in Scots pine stands at different distances from a Cu-Ni smelter,

(2) the effect of soil fertilisation and liming on the number of germinable seeds and the mortality rate of seedlings growing in soil samples representing different pollution levels, and

(3) the survival of *Calluna vulgaris* seedlings germinated from the seed bank samples collected along the pollution gradient.

Materials and methods

Study area

The seed banks in forest soil were studied along a 8 km transect running SE from the Harjavalta Cu-Ni smelter (61°19'N, 22°09'E) in SW Finland. This was the only direction with suitable coniferous stands near the smelter. The study area is dry and infertile Scots pine (*Pinus sylvestris* L.) forest. The soil is sorted fine sand and the soil type ferric podsol (Mälkönen et al. 1999).

The Cu smelter was founded in 1945 and Ni smelting started in 1960. More than 50 years' accumulation of heavy metals and sulphur has drastically changed the forest ecosystem (Helmisaari et al. 1995, Fritze et al. 1997, Derome & Lindroos 1998, Derome & Nieminen 1998). The understorey vegetation, which was originally dominated by *Calluna vulgaris* (L.) Hull and a well-developed moss and lichen layer, was almost totally absent up to a distance of 0.5 km from the smelter in the beginning of the 1990's. Only some

Table 1. Stand characteristics, element concentrations in the organic soil layer and the coverage percentages of needle litter (visual estimation) and plant species (point frequencies). Plant-available concentrations of Cu and Ni in the organic layer were determined by extraction with NH_4 acetate + EDTA and expressed on the basis of the organic matter (om) content (for details, see Salemaa et al. 2001).

	Distance from the smelter, km					
	0.5	1	2	3	4	8
Pine stand:						
Stand age, years	49	67	52	51	56	40
Mean height, m	6.1	10.1	10.9	9.5	9.2	10.6
Number of trees ha^{-1}	1008	1048	1230	1436	1517	1552
Element concentrations in the organic soil layer before fertilisation (1992):						
Thickness of organic layer, cm	2.5	7.2	2.3	3.3	2.2	0.8
N tot % om	1.92	1.31	1.74	1.58	1.65	1.68
S, tot mg/kg om	387	402	198	176	170	176
Cu, exc. mg/kg om	7540	3501	2238	1532	786	209
Ni, exc. mg/kg om	527	406	329	230	164	72
Coverage (%):						
Needle litter	83.3	90.3	74.3	85.0	33.0	31.7
Mosses and lichens	0.17	3.28	28.14	32.77	58.94	76.25
Calluna vulgaris	0.00	0.01	0.01	0.01	1.35	1.41
Vascular plant species	0.48	21.01	7.83	13.73	18.44	9.52
All plant species	0.65	24.29	35.97	46.50	77.38	85.77

Table 2. The effect of stand-specific fertilisation (SSF) on the element concentrations (mg/kg) and pH of the organic soil layer at three distances from the Harjavalta smelter in 1996 (data from Derome 2000). Plant available concentrations of Cu, Ni, Ca, Mg and P were determined by extraction with $BaCl_2$ + EDTA. All concentrations have been recalculated per dry mass of organic matter. The statistical differences between the averages of the control and SSF plots (n = 3) are given by * ($P < 0.05$) and ° ($P < 0.10$) (t-tests).

		Distance from the smelter, km		
		0.5	4	8
Cu	control	4517.8	210.1	39.1
	SSF	2520.9*	91.5°	37.4
Ni	control	697.1	143.5	61.5
	SSF	495.7	96.9	53.1
Ca	control	904.3	2295.8	2357.7
	SSF	5550.9*	5891.7*	1915.0
Mg	control	105.8	256.1	316.6
	SSF	557.1*	693.1*	218.0
P	control	40.4	127.9	151.1
	SSF	40.6	187.6	113.8
N tot	control	2.55	1.67	1.89
	SSF	2.01	1.54	1.31
pH	control	3.57	3.11	3.20
	SSF	4.25	3.80	3.23

Nutrient applications (kg ha^{-1}) in 1992: 0.5 km: 150 N, 50 Mg, 1500 Ca
4 km: 150 N, 30 P, 30 Mg, 1000 Ca, 8 km: 120 N

patches of the most resistant vascular plant species e.g. *Empetrum nigrum* ssp. *nigrum* L. (Uhlig et al. 2001), *Carex globularis* L. and *Vaccinium uliginosum* L. were present. The overstorey pines were alive, but they were stunted and seriously defoliated. The coverage and floristic diversity of the understorey vegetation increased with increasing distance from the smelter (Salemaa et al. 2001). The characteristics of the tree stands, the existing vegetation and the concentrations of exchangeable Cu, Ni and total S and N in the organic soil layer are given in Tab. 1.

Emissions from the smelter were considerably reduced in the 1990's. During 1985–1990 the average annual emissions of Cu were 104 t, Ni 50 t and SO_2 8100 t. In 1993, the year before the seed bank samples were collected, the corresponding values were Cu 50 t, Ni 7 t and SO_2 4700 t (Helmisaari et al. 1995).

Sampling and fertiliser treatments

The soil samples for the seed bank analyses were collected from untreated sites at six distances (0.5, 1, 2, 3, 4 and 8 km), and from fertilised sites located at 0.5, 4 and 8 km from the Harjavalta smelter on 5 May 1994. The sites at distances of 0.5, 2, 4 and 8 km represented experimental plots in a forest correction fertilisation project (1992–1996) carried out to alleviate detrimental changes in the forest soil caused by heavy metal and SO_2 deposition (Mälkönen et al. 1999). The samples were collected from the plots in one (stand-specific fertilisation) of the four fertilisation treatments. There were three replicates of each untreated or fertilised plot (30 x 30m). Five soil samples were taken from the buffer zone of each plot. The soil samples were taken from the organic layer (including the litter and the upper part of the mineral soil layer) to a depth of 5–10 cm using a 9.5 x 9.5 cm metal frame.

The stand-specific fertiliser treatment was applied as a single broadcast dose in spring 1992. The composition of the fertilisers was different at the individual distances (Tab. 2) and was based on soil and needle analyses in each Scots pine stand (Mälkönen et al. 1999, Derome 2000). Nitrogen was given in the form of slow-release methylene urea and fast-release ammonium nitrate, and Ca and Mg as Mg-rich granulated limestone. The aim of liming and fertilisation was to reduce heavy metal toxicity and to correct nutrient imbalances in order to improve the vitality of the tree stand.

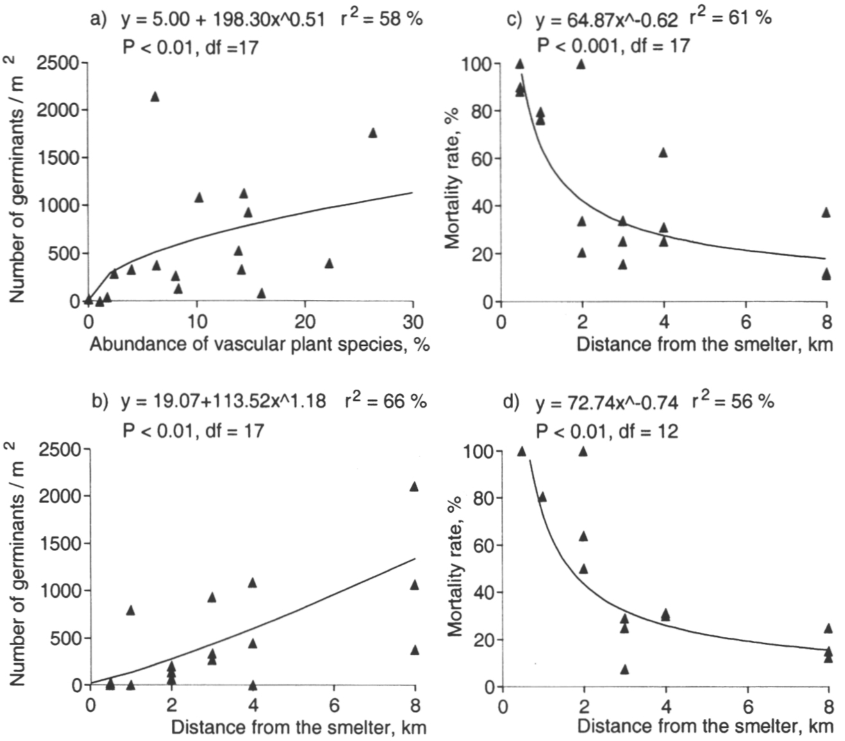


Fig. 1. The relationships between a) the total number of germinated seedlings (excluding *Betula pubescens*) (y) and the abundance of vascular plant species in the existing vegetation measured as point frequencies, % (x), b) the number of *Calluna vulgaris* seedlings (y), c) the mortality rate of seedlings of all species (including *Betula pubescens*) (y), and d) the mortality rate of the *Calluna vulgaris* seedlings (y) and the distance from the smelter, km (x).

The plant available concentrations of heavy metals and macronutrients in the organic soil layer four years after the treatments are given in Tab. 2. The limestone application at distances of 0.5 and 4 km considerably reduced the Cu and to some extent also the Ni concentrations in the organic layer. The pH of the organic layer increased slightly at the corresponding distances. Nitrogen application at 8 km had no significant effects on the heavy metal concentrations of the organic soil layer (Derome 2000). Ca and Mg concentrations were higher in the fertilised than in the untreated plots at 0.5 and 4 km. At 8 km, however, the concentrations were lower on the fertilised plots, presumably due to natural inter-plot variation. (Tab. 2).

Only very slight changes occurred between 1992 and 1994 in the understorey vegetation after fertilisation. The coverage of *Calluna vulgaris* increased by a few percentage units. Some new patches of *Epilobium angustifolium* L. and *Deschampsia flexuosa* (L.) Trin. also appeared (unpublished results).

Germination

The germination experiment with a total of 135 soil samples (total surface area 1.2 m^2) was carried out in the greenhouse of the Finnish Forest Research Institute at Tuusula (60°21'N, 25°00'E). Because the samples were taken immediately after the end of winter, stratification was not considered necessary. Rhizomes and large roots were removed and the samples spread out on trays (18.5 x 21.5 cm). The trays contained a 2–3 cm layer of mixed quartz sand and peat as growth substrate. Six control trays containing only substrate were used to check whether any seeds originated from the greenhouse environment. The trays were arranged randomly on greenhouse tables. The light conditions were natural. The temperature was maintained at +20°C during the day and +15°C at night. However, the temperature occasionally reached 30°C on sunny days. The relative humidity in the greenhouse was 60–70%. The samples were watered several times a week.

The emerged seedlings were counted once a week, and the species were identified as soon as possible. The seedlings were left to grow on the trays, the location of each seedling having been marked on a map. The seedlings of *Calluna vulgaris* were monitored individually up until 21 weeks (from 10 May to 19 October 1994), and the other species up until 16 weeks. Some gramineous seedlings were replanted when the trial was finished and identified at a later stage.

Statistical analysis

The five seed bank samples from each plot were germinated individually, thus making it possible to study both within- and between-plot variation in the response variables. The statistical analyses were performed on all species combined, and separately on *Betula pubescens* Ehrh. and *Calluna vulgaris* when there was sufficient data. The ln(x+1) transformed numbers of emerged seedlings were compared by nested ANOVA, in which the sample was nested under the plot and the plot under the distance from the smelter (GLM proc., SAS Institute Inc. 1994). The plot means of fertilised and untreated samples were compared pairwise within each distance using Kruskal-Wallis tests based on chi-square values (NPAR1WAY proc., SAS Institute Inc. 1994). The effect of the fertilisation could not be compared across the distances, because of the varying treatments. The relationships between the number of germinants and the abundance of the existing vegetation, the distance from the smelter and the heavy metal concentrations in the organic layer were analysed by regression models (REG and NLIN proc., SAS Institute Inc. 1994). The corresponding tests were also carried out on the mortality rates of the seedlings.

The correspondence between the species composition of the existing vegetation and the seed bank at each distance was estimated by Sørensen similarity indices (Sørensen 1948) using the presence-absence data. Similarity = 2W/A+B, where A and B are the numbers of species occurring separately in the aboveground vegetation (A) and in the soil seed bank (B), and W the number of species common to A and B.

Actuarial life tables were used for analysing the survival probability of the *Calluna vulgaris* seedlings over time (SPSS 9.0.1 software, SPSS Inc. 1999). If a seedling did not die during the experiment, the case was handled as censored. The survivor function gives the probability that a seedling will survive for a specified time at least, or longer, without a response (death) (McCullagh & Nelder 1989). The equality of the survival functions of the seedlings originating from different distances from the smelter, and between the fertilised and untreated samples, were compared using the Wilcoxon (Gehan) statistic.

Results

The size and composition of the seed banks

Viable seeds were found at all distances from the smelter. Altogether 1300 seedlings germinated in the total of 135 seed bank samples. The emerged seedlings represented 15 taxa, of which 6 species were grasses and sedges, 4 dwarf shrubs, 3 trees and 2 herbs (Tab. 3). The most numerous species were *Betula pubescens* (696 seedlings) and *Calluna vulgaris* (490), both of which were found at all distances from the smelter. The average seedling density varied from 250 to 4750 per m^2 at the different distances (Tab. 3). Excluding the seedlings of *Betula pubescens*, which were most probably derived from the previous-year seed crop, the average size of the persistent seed banks ranged from 15 to 1200 seeds per m^2. There was very high variation in seedling numbers between the plots at the same distance, but no differences within each plot (Tab. 4, Fig. 1). No seedlings emerged from the control trays.

Although aboveground vegetation was very scanty at 0.5 km, the samples contained germinable seeds of *Betula pubescens*, *Calluna vulgaris*, *Vaccinium uliginosum* and *Pinus sylvestris* (the last species only on the fertilised plots). *Betula pubescens* was the most numerous species at 0.5 and 1 km, and *Calluna vulgaris* dominated in the seed bank at distances of 2 km or more. In addition to birches, a large number of *Carex globularis* seedlings emerged from the samples taken at 1 km. *Vaccinium vitis-idaea* L. appeared for the first time in the seed bank at 1 km. Only one *Empetrum nigrum* seedling was found at a distance of 4 km. Seedlings of the other species, especially grasses and herbs, also appeared in low numbers and sporadically along the study transect (Tab. 3).

Germination in relation to the existing vegetation, the distance from the smelter and the fertilisation treatments

The seed bank species were rather well represented in the existing vegetation. Of the 17 vascular plant species growing on the untreated plots, 11 were found in the seed banks (Tab. 5). The percentage similarity between the species in the existing vegetation and the seed banks varied from 18% to 67% at different distances from the smelter. The total similarity in the data for the whole transect was about 70% in the untreated, and 60% in the fertilised plots (Tab. 5).

The four species that were absent from the existing vegetation but present in the seed bank were *Festuca ovina* L., *Agrostis capillaris* L., *Carex ericetorum* Pollich and *Rumex acetosella* L. In contrast, no seedlings of *Arctostaphylos uva-ursi* (L.) Sprengel, *Ledum palustre* L. and *Vaccinium myrtillus* L. emerged from

Table 3. a) The average number of germinated seeds per m² ± se in the soil samples collected from the untreated and fertilised plots at different distances from the Harjavalta smelter. The averages have been calculated from the plot-specific data (n = 3 plots), each including 5 soil samples. b) The average mortality rate (%) of the seedlings during the 16 week germination trial. The plots where no seedlings emerged were excluded from the mortality rate data (plot number lower than 3 is given in parantheses). One seedling is equivalent to a density of 7.4 per m².

Distance from the smelter, km	Untreated stands						Fertilised stands		
	0.5	1	2	3	4	8	0.5	4	8
a) Germinated seeds									
Agrostis capillaris	–	–	–	–	–	7.4 ± 7.4	–	–	–
Betula pubescens	517.1 ± 429.2	4011.0 ± 2537.1	22.2 ± 12.8	59.1 ± 7.4	36.9 ± 26.6	7.4 ± 7.4	435.8 ± 140.4	7.4 ± 7.4	44.3 ± 33.9
Carex canescens	–	–	–	–	–	7.4 ± 7.4	–	–	–
Carex ericetorum	–	–	44.3 ± 44.3	–	–	–	–	–	–
Carex globularis	–	376.7 ± 280.6	–	–	–	–	–	–	–
Calluna vulgaris	7.4 ± 7.4	265.9 ± 265.9	133.0 ± 38.4	509.7 ± 211.4	509.7 ± 315.2	1181.9 ± 502.5	103.4 ± 57.7	524.5 ± 372.9	384.1 ± 240.2
Deschampsia flexuosa	–	–	–	–	7.4 ± 7.4	–	–	–	–
Empetrum nigrum	–	–	–	–	7.4 ± 7.4	–	–	–	–
Epilobium spp.	–	–	–	–	–	–	–	14.8 ± 14.8	–
Festuca ovina	–	–	7.4 ± 7.4	–	–	–	–	–	–
Pinus sylvestris	–	36.9 ± 19.5	22.2 ± 12.8	–	7.4 ± 7.4	–	7.4 ± 7.4	–	14.8 ± 14.8
Populus tremula	–	–	7.4 ± 7.4	–	7.4 ± 7.4	7.4 ± 7.4	–	7.4 ± 7.4	–
Rumex acetosella	–	–	–	–	14.8 ± 7.4	–	–	7.4 ± 7.4	7.4 ± 7.4
Vaccinium uliginosum	7.4 ± 7.4	22.2 ± 12.8	–	22.2 ± 22.2	–	–	22.2 ± 12.8	29.5 ± 19.5	–
Vaccinium vitis-idaea	–	36.9 ± 36.9	14.8 ± 7.4	–	22.2 ± 12.8	–	–	7.4 ± 7.4	29.5 ± 14.8
All excluding *Betula*	14.8 ± 7.4	738.7 ± 527.1	229.0 ± 48.4	531.8 ± 199.4	583.6 ± 301.8	1204.0 ± 515.2	133.0 ± 46.1	590.9 ± 394.8	435.8 ± 270.3
All species	531.8 ± 433.3	4749.6 ± 3062.6	251.2 ± 48.4	590.9 ± 192.1	620.5 ± 322.9	1211.4 ± 509.3	568.8 ± 186.4	598.3 ± 390.4	480.1 ± 303.1
b) Mortality rate, %									
Betula pubescens	93 ± 3	82 ± 2	50 ± 50(2)	33 ± 19	25 ± 25(2)	100(1)	90 ± 6	0(1)	8 ± 8(2)
Calluna vulgaris	100(1)	80(1)	71 ± 15	21 ± 7	31 ± 1(2)	17 ± 4	56 ± 22(2)	10 ± 7	30 ± 11
Other species	0(1)	60 ± 5	5 ± 5(2)	100(1)	33 ± 22	0(2)	33 ± 33(2)	23 ± 15	35 ± 15(2)
All species	93 ± 4	77 ± 1	51 ± 2	25 ± 5	40 ± 1	20 ± 8	83 ± 5	17 ± 9	24 ± 5

Table 4. The effect of the distance from the smelter on a) the number of germinated seedlings, ln(x + 1) transformation, and b) the mortality rate (%) of the seedlings. F and P values for nested ANOVA. df = degree of freedom. Data from untreated plots.

Source of variation	*Betula pubescens*			*Calluna vulgaris*			All species		
	df	F	P	df	F	P	df	F	P
a)									
Distance	5	25.70	0.000	5	5.92	0.000	5	6.55	0.000
Plot (distance)	10	3.65	0.001	10	3.19	0.002	10	2.67	0.009
Sample (plot)	12	0.71	0.736	12	0.67	0.769	12	0.73	0.716
b)									
Distance	5	4.90	0.006	5	4.89	0.005	5	12.12	0.000
Plot (distance)	6	10.96	0.090	5	0.67	0.653	10	2.00	0.067
Sample (plot)	12	7.05	0.021	12	0.94	0.535	12	1.22	0.308

Table 5. The number of vascular plant species in the aboveground vegetation (AV) and in the soil seed bank (SB) in the a) untreated and b) fertilised plots. Common species (C) were found both in the aboveground vegetation and in the seed banks. Percentage similarity (Sim%) was measured by Sørensen index (100% = max similarity). Data for three plots combined at each distance.

	Distance, km	AV	SB	C	Sim%
a)	0.5	6	3	2	44.4
	1	12	6	6	66.7
	2	11	7	4	44.4
	3	8	3	1	18.2
	4	8	8	4	50.0
	8	10	5	2	26.7
	total	17	15	11	68.8
b)	0.5	5	4	3	66.7
	4	7	7	3	42.9
	8	10	5	4	53.3
	total	12	8	6	60.0

the soil samples, although these species were present in the existing vegetation.

The total density of the germinated seeds (*Betula pubescens* excluded) was positively related ($r^2 = 58\%$, $P < 0.01$) to the total cover of the vascular species in the existing vegetation on the untreated plots (Fig. 1a), but there were no similar species-specific trends. For instance, *Calluna vulgaris* was absent or very scarce in the existing vegetation up to a distance of 4 km, although the density of viable seeds varied between 7–100 per m^2 even at 0.5 km (Tab. 3).

The number of *Calluna vulgaris* and *Betula pubescens,* as well as the total number of germinated seedlings, varied between different distances (Tab. 4). *Calluna vulgaris* was the only species in which the germinant number increased according to increasing distance from the smelter ($r^2 = 66\%$, $P < 0.01$) (Fig. 1b). A similar trend was also found in the combined data of all species (excluding *Betula pubescens*) ($r^2 = 59\%$, $P < 0.01$). The fertilisation treatments had no effect on the number of germinated seeds (Tab. 6).

Table 6. The Kruskal-Wallis statistics (χ^2) for the effect of the stand-specific fertilisation on the number of germinated seeds and the mortality rate (%) of the seedlings. The means of the untreated and fertilised plots are compared separately at each distance, because the fertilisation treatment was not comparable over the distances. n = number of untreated/fertilised plots. – = insufficient data.

	Germinated seeds			Mortality rate, %		
	χ^2	n	P	χ^2	n	P
0.5 km						
Betula pubescens	0.43	3/3	0.513	0.00	3/3	1.000
Calluna vulgaris[1]	1.34	3/3	0.246	2.40	2/2	0.121
All species	0.43	3/3	0.513	1.19	3/3	0.275
4 km						
Betula pubescens	0.89	3/3	0.346	–		
Calluna vulgaris	0.04	3/3	0.827	3.00	2/3	0.083
All species	0.04	3/3	0.827	2.33	3/3	0.127
8 km						
Betula pubescens	0.89	3/3	0.346	–		
Calluna vulgaris	2.33	3/3	0.127	1.19	3/3	0.275
All species	1.76	3/3	0.184	0.43	3/3	0.513

[1] Mortality rate data from untreated plots in 0.5 and 1 km because small n

Mortality rate

The mortality rate of the seedlings of all species was the highest in the untreated soil samples collected near the smelter (Tab. 3, Tab. 4). The mortality rate decreased with increasing distance from the smelter in the data of all species ($r^2 = 61\%$, $P < 0.001$) (Fig. 1c) and in *Calluna vulgaris* ($r^2 = 56\%$, $P < 0.01$) (Fig. 1d).

The mortality rate of *Calluna vulgaris* was slightly lower ($P = 0.121$) on the fertilised and limed plots at 0.5 km (56%) compared to that on the untreated plots at distances of 0.5–1 km (80%). A slightly decreasing trend in the mortality rate of the *Calluna vulgaris* ($P = 0.083$) and in the data for all species ($P = 0.127$) was also observed in the fertilised and limed plots at 4 km (Tab. 6). On the other hand, nitrogen fertilisation alone had no effect on heavy metal and macronutrient concentrations in the organic soil layer at 8 km

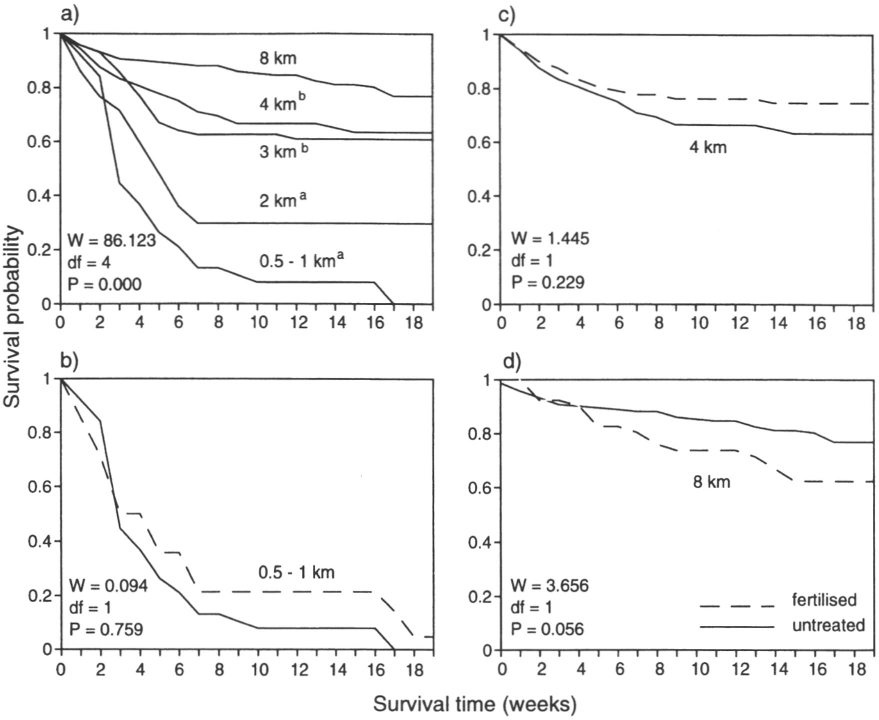


Fig. 2. The survival functions of the seedlings of *Calluna vulgaris* grown a) in the substrate collected from the untreated stands at different distances (km) from the smelter, and b) – d) in the untreated and fertilised substrate representing varying heavy metal and nutrient concentrations (Tab. 2) at different distances from the smelter. The functions have been compared using the Wilcoxon (Gehan) tests. The same letter indicates non-significant differences between the functions in the panel a), otherwise the pairwise differences are significant at the $P < 0.03$ level.

(Tab. 2), and had no significant effect on the mortality rates of the seedlings (Tab. 6).

When explaining the mortality rate of *Calluna vulgaris* in the combined data of the untreated and fertilised plots (distances 0.5, 4 and 8 km, $n = 14$) on the basis of the 1996 soil data (Tab. 2), Cu ($P < 0.001$) and Ca ($P < 0.04$) were significant explanatory factors, but the other elements (Ni, Mg, N, P) were non-significant. The mortality rate of the seedlings increased with increasing Cu concentrations, but decreased with increasing Ca concentrations:

$y = 33.032 + 0.018x_1 - 0.004x_2$, $r^2 = 81\%$, $P < 0.001$, df = 13.

y = mortality rate (%) of the *Calluna vulgaris* seedlings,
x_1 = Cu concentration in the organic layer,
x_2 = Ca concentration in the organic layer.

Life tables of the *Calluna vulgaris* seedlings

The first seedlings of *Calluna vulgaris* emerged during the second week in the greenhouse trial, and the germination rate was highest by the fourth week. A few seedlings emerged at the end of the experiment in the 21th week. The survival probability of the seedlings was the higher, the further away from the smelter the soil was collected (Fig. 2). The death risk increased the most in young, under five-week-old seedlings and was relatively stable after this period. The survival probability was the lowest in the soil from distances of 0.5–2 km, and varied between 0–30% at the end of the trial. The survival probability was over 60% at distances of 3–4 km, and 80% at 8 km. The survival distributions differed significantly between the three distances of 0.5–2 km, 3–4 km and 8 km (Fig. 2a).

Although nutrient addition and liming increased the survival probability of the seedling slightly at 0.5 km and 4 km, the effect was not statistically significant (Fig. 2b–c). The survival probability was lower in the soil samples from plots given nitrogen than in the untreated soil samples at 8 km (Fig. 2d). However, the overall nutrient level was higher in the untreated than in the fertilised plots at this distance (Tab. 2).

Discussion

The size and composition of the seed banks

The importance of soil seed banks in regenerating forest vegetation varies according to plant species and the disturbance history of the stands (Archibold 1989, Mladenoff 1990, McGee & Feller 1993). The persistent seed banks in boreal coniferous forests are considered to be rather small (Archibold 1989) compared to those in e.g. temperate deciduous forests (Pickett & McDonnell 1989, Smallidge & Leopold 1995). The densities of germinable seeds found in this study (15–1200 per m^2 excluding seedlings of *Betula pubescens*) were lower than the range of 900–3700 per m^2 presented by Huopalainen et al. (2000) for undrained pine bogs near the Harjavalta smelter, or the range of 90–5000 per m^2 in *Calluna* type pine forests in Sweden (Granström 1986). Some of the seedlings may have died before cotyledons emerged on the soil surface in our experiment. In any case, the seedling emergence method used here gives an underestimate of the actual size of the seed banks (Brown 1992).

In general, the seed bank density of the present study well reflected the abundance of the existing vegetation, the pollution level in the organic soil layer and the distance from the smelter. The fertilisation and liming treatments did not appear to have any effect on the number of germinated seedlings. Compared to the seed bank data collected from Scots pine forests of heavily polluted areas in the Kola Peninsula, NW Russia (Komulainen et al. 1994), more viable seeds were found in our samples. Seedling density varied between 430 and 1200 per m^2 in the soil taken at a distance of 8 km from the Harjavalta smelter, while it was approximately 280 per m^2 at the corresponding distance from the Monchegorsk smelter (Komulainen et al. 1994). The number of dormant or dead seeds was not known in our study, and it is therefore impossible to determine whether heavy metals had any detrimental effects on the viability of the seeds stored in the soil. In the data from the Kola Peninsula, for instance, only 3% of the total seed pool was germinable at a distance of 15 km from the smelter (Komulainen et al. 1994).

Many studies have shown that the seed germination capacity of tree and dwarf shrub species is not reduced by acidity or metals (Patterson & Olson 1983, Percy 1986, Scherbatskoy et al. 1987, Legg et al. 1992), even though Helsper & Klerken (1984) reported that the germination of Dutch *Calluna vulgaris* seeds decreased significantly when the pH was lower than 3.2. In our study, the pH values of the untreated plots were close to this critical value, ranging from 3.1 to 3.6 in the organic layer (Tab. 2). Vieno et al. (1993) observed that six years of acidic irrigation did not affect the viability of forest seed banks at a site in Finnish Lapland.

A lowered seed input owing to the paucity of vegetation is a more probable reason for the reduced seed bank close to the Harjavalta smelter than soil toxicity. The total coverage of the understorey vegetation was under 1% (Tab. 1). *Calluna vulgaris*, which was still growing close to the smelter in the 1970's (Laaksovirta & Silvola 1975), had completely disappeared. Experimental exposures have shown that *Calluna vulgaris* is more sensitive to Cu than *Empetrum nigrum* (Monni et al. 2000a, b), the latter species growing in small patches in severely heavy metal contaminated sites.

Seed production may also be hindered by disturbances in different stages of sexual reproduction. For instance, the clones of wind-pollinated dioecious *Empetrum nigrum* produced a large number of flower buds, but practically no berries in the most polluted study area in 1994 (unpublished results). Whether this was caused by poor pollen quality (Wolters & Martens 1987) or insufficient pollen for successful dispersion from male to female clones, is unknown. According to Bell & Tallis (1973), the seeds of this species need at least four years' dormancy in the soil before germination.

The frequency of insect pollinators may also be lower in heavily polluted ecosystems, thus affecting the seed production of insect-pollinated species. However, this did not seem to be the case with *Vaccinium uliginosum*, the berry production of which was enhanced near to the smelter, probably as a response to the increased illumination in the damaged stand (Salemaa et al. 1999).

Clonal dwarf shrubs, which are the most dominant group of vascular plants in boreal forest understorey, normally regenerate vegetatively in mature stands. For instance, the seedling recruitment of *Vaccinium* species is possible only after a forest fire or in disturbed microsites or gaps, which have a suitable substrate with high moisture and organic matter contents (Erikson & Fröborg 1996). Although *Vaccinium* species invest highly in seed production, their seed banks are considered to be small and short-lived (Granström 1982, Vander Kloet & Hill 1994). However, *Vaccinium uliginosum* and *V. vitis-idaea* were well represented in the seed banks of our study, reflecting the composition of the existing vegetation. In contrast, *V. myrtillus* was absent from the seed bank, but it was also rare in the aboveground vegetation.

The absence of *Arctostaphylos uva-ursi* from our samples, despite berry-producing clones at distances of 2–3 km from the smelter, may be due to deep dormancy of the seeds. The berries of this species are also relatively heavy and probably fall close to the mother clones. The number of soil samples in our study might be too low to reflect the patchy spatial pattern of the *Arctostaphylos* seeds in the soil. Also birds can eat berries and spread them over relatively long distances.

Almost all the soil samples from the study transect had viable seeds of *Calluna vulgaris*. *Calluna* seed banks have been widely studied in heathlands (e.g. Hester et al. 1991, Legg et al. 1992, Pywell et al. 1997, Mahy et al. 1999, Valbuena et al. 2000) and forest plantations (Hill & Stevens 1981, Granström 1988). Compared to the other dwarf shrub species growing in boreal forests, it has large and persistent seed banks that enable it to colonise exposed soil soon after clear cutting or fire (Granström 1988). *Calluna* seeds have been shown to remain viable for 30–40 years under a heathland canopy (Gimingham 1972), and at least 90 years in forest soil (Granström 1988). Thus, it is possible that the seeds originating from the most polluted area date back decades in the past. However, our samples represented only the surface layers of the soil, from which the oldest part of the *Calluna* seed population has probably drifted down to the deeper soil layers (cf. Bekker et al. 1998).

Both *Calluna vulgaris* and *Betula pubescens* belong to the early successional species, which produce large numbers of seed with good dispersion ability. The seed crop of the *Betula* species was exceptional high in the year before the sampling, 1993. Because the majority of *Betula* seeds lose their viability within one year (Granström & Fries 1985), it is probable that most of the *Betula* seedling in our samples were derived from the previous-year seed crop. The huge number of seedlings in the samples taken 1 km from the smelter especially can be explained by the presence of a birch stand growing in the vicinity of the study plots.

Seedling establishment

Seedling survival was strongly affected by the heavy metal concentrations in the soil. Most of the germinated seedlings originating from a distance of 0.5–2 km from the smelter died at an age of a few weeks, probably after the depletion of the seed's nutrient reserves. Cu and Ni are known to reduce especially the root growth of many tree (Patterson & Olson 1983, Kahle 1993) and dwarf shrub species (Monni et al. 2000b). Seedlings that managed to grow their roots into the clean substrate in our experiment could have escaped the toxicity of the polluted soil. The mycorrhizas of ericaceous plants, in addition to facilitating the uptake of nutrients and water, also provide resistance to heavy metals (Bradley et al. 1981, Leake et al. 1990). One reason for the high mortality of the *Calluna vulgaris* seedlings growing in the most polluted soil might be the absence of mycorrhizal infections.

The actual germination and growth conditions close to the Harjavalta smelter are much more severe than those in the greenhouse. Drought and the thick layer of undecomposed needle litter hinder seed germination. If a seed successfully germinates, the roots of the young seedling still have to grow through the heavy metal enriched surface layer down into the less-polluted, moister soil horizons. In addition to the toxic effects of heavy metals, a deficiency of macronutrients such as Ca and Mg (Derome & Lindroos 1998) and the decreased water-holding capacity of the soil (Derome & Nieminen 1998) increase the stress encountered by the plant individuals. The seedlings of *Calluna vulgaris*, particularly, are sensitive to summer desiccation and winter browning (Legg et al. 1992). In general, protection from the wind and withering seem to increase the survival rate of seedlings growing in polluted soil (Kozlov & Haukioja 1999).

The fertiliser treatments that included limestone decreased the Cu and Ni concentrations in the organic soil layer (Mälkönen et al. 1999, Derome 2000). The Ca and Mg concentrations and pH correspondingly increased, as well as the microbial activity of the soil (Fritze et al. 1996). These changes presumably contributed to the increase in seedling survival in our samples. Although *Calluna vulgaris* is, according to its habitat preference, a calcifuge species (Gimingham 1960), its young seedlings seemed to benefit from liming when growing in heavy metal polluted soil. Legg et al. (1992) found that liming had no negative effect on the survival of *Calluna* seedlings in a transplant experiment, but liming decreased the shoot mass by 13%.

The nitrogen application without limestone had no significant effects on the nutrient status or pH of the organic layer compared to the untreated plots at a distance of 8 km. In contrast, the N, P, K, Ca and Mg concentrations were slightly higher in the untreated than in the fertilised plots (Tab. 2). The better overall nutrient status of the substrate seemed to explain the better survival probability of the *Calluna* seedlings growing in the untreated soil (Fig. 2d). The nitrogen input, although no longer reflected in the nutrient concentrations of the organic layer in 1996 (Tab. 2), might also have had a detrimental effect on the survival of the *Calluna* seedlings in 1994. For instance, Helsper & Klerken (1984) found that repeated applications of nitrogen inhibited the growth of *Calluna*, while Ca had little apparent effect.

Revegetation efforts in heavy metal polluted industrial areas have produced promising results when combined with soil amelioration e.g. in Sudbury, Canada (Winterhalder 2000). The reduction in emissions has opened up new possibilities for the recovery of forest ecosystems close to the Harjavalta smelter. Although the understorey vegetation was almost totally absent at 0.5 km from the smelter, viable seeds of native plant species were stored in the soil. Seed banks maintain genetic diversity of plant populations (Mahy et al. 1999) which may enable evolution of tolerant ecotypes. However, seedling establishment fails as a result of the phytotoxicity of heavy metals, a thick layer of undecomposed needle litter, drought and nutrient deficiencies. Our results stress that the realisation of the revegetation potential of the local seed banks presupposes soil amelioration directed at immobilising the heavy metals and facilitating the generation of a new organic soil layer.

Acknowledgements. We extend our sincerest thanks to Marja Vieno and Arja Ojala for advising on the sampling and germination procedures, to Mikko Piirainen for identifying some difficult species, and to Heljä-Sisko Helmisaari and Satu Monni for commenting on the earlier version of the manuscript. John Derome kindly checked the English language and provided the soil data.

References

Aamlid D, Vassilieva N, Aarrestad PA, Gytarsky ML, Lindmo S, Karaban R, Korotkov V, Rindal T, Kuzmicheva V, Venn K (2000) The ecological state of the ecosystems in the border areas between Norway and Russia. Boreal Environment Research 5: 257–278.

Archibold OW (1979) Buried viable propagules as a factor in postfire regeneration in northern Saskatchewan. Canadian Journal of Botany 57: 54–58.

Archibold OW (1989) Seed banks and vegetation processes in coniferous forests. In: Leck MA, Parker VT, Simpson RL (eds) Ecology of soil seed banks. Academic Press Inc, San Diego, pp 107–122.

Bekker RM, Bakker JP, Grandin U, Kalamees R, Milberg P, Poschlod P, Thompson K, Willems JH (1998) Seed size, shape and vertical distribution in the soil: indicators of seed longevity. Functional Ecology 12: 834–842.

Bell JN, Tallis JH (1973) *Empetrum nigrum* L. Journal of Ecology 61: 289–305.

Bell R, Teramura AH (1991) Soil metal effects on the germination and survival of *Quercus alba* L., and *Q. prinus* L. Environmental and Experimental Botany 31: 145–152.

Bradley R, Burt AJ, Read DJ (1981) Mycorrhizal infection and resistance of heavy metal toxicity in *Calluna vulgaris*. Nature 292: 335–337.

Brown D (1992) Estimating the composition of a forest seed bank: a comparison of the seed extraction and seedling emergence methods. Canadian Journal of Botany 70: 1603–1612.

Derome J (2000) Detoxification and amelioration of heavy-metal contaminated forest soils by means of liming and fertilisation. Environmental Pollution 107: 79–88.

Derome J, Lindroos AJ (1998) Effects of heavy metal contamination on macronutrient availability and acidification parameters in forest soil in the vicinity of Harjavalta Cu-Ni smelter, SW Finland. Environmental Pollution 99: 225–232.

Derome J, Nieminen T (1998) Metal and macronutrient fluxes in heavy-metal polluted Scots pine ecosystems in SW Finland. Environmental Pollution 103: 219–228.

Eriksson O, Fröborg H (1996) "Windows of opportunity" for recruitment in long-lived clonal plants: experimental studies of seedling establishment in *Vaccinium* shrubs. Canadian Journal Botany 74: 1369–1374.

Folkeson L, Andersson-Bringmark E (1988) Impoverishment of vegetation in a coniferous forest polluted by copper and zinc. Canadian Journal of Botany 66: 417–428.

Freedman B, Hutchinson TC (1980) Long-term effects of smelter pollution at Sudbury, Ontario, on forest community composition. Canadian Journal of Botany 58: 2123–2140.

Fritze H, Vanhala P, Pietikäinen J, Mälkönen E (1996) Vitality fertilisation of Scots pine stands growing along a gradient of heavy metal pollution: short-term effects on microbial biomass and respiration rate of the humus layer. Fresenius Journal of Analytical Chemistry 354: 750–755.

Fritze H, Pennanen T, Vanhala P (1997) Impact of fertilisers on the humus layer microbial community of Scots pine stands growing along a gradient of heavy metal pollution. In: Insam H, Rangger A (eds) Microbial communities. Functional versus structural approaches. Springer-Verlag, Berlin, pp 68–83.

Gimingham CH (1960) *Calluna vulgaris* (L.) Hull. Biological Flora of the British Isles. Journal of Ecology 48: 455–483.

Gimingham CH (1972) Ecology of heathlands. Chapman and Hall, London.

Granström A (1982) Seed banks in five boreal forest stands originating between 1810 and 1863. Canadian Journal of Botany 60: 1815–1821.

Granström A (1986) Seed banks in forest soils and their role in vegetation succession after disturbance. Swedish University of Agricultural Sciences, Department of Forest Site Research, Stencil no. 6.

Granström A (1988) Seed banks at six open and afforested heathland sites in southern Sweden. Journal of Applied Ecology 25: 297–306.

Granström A, Fries C (1985) Depletion of viable seeds of *Betula pubescens* and *Betula verrucosa* sown onto some north Swedish forest soils. Canadian Journal of Forest Research 15: 1176–1180.

Halpern CB, Evans SA, Nielson S (1999) Soil seed banks in young, closed-canopy forests of the Olympic Peninsula, Washington: potential contributions to understory reinitiation. Canadian Journal of Botany 77: 922–935.

Helmisaari HS, Derome J, Fritze H, Nieminen T, Palmgren K, Salemaa M, Vanha-Majamaa I (1995) Copper in Scots pine forests around a heavy-metal smelter in south-western Finland. Water, Air and Soil Pollution 85: 1727–1732.

Helsper HPG, Klerken GAM (1984) Germination of *Calluna vulgaris* (L.) Hull in vitro under different pH-conditions. Acta Botanica Neerlandica 33: 347–353.

Hester AJ, Gimingham CH, Miles J (1991) Succession from heather moorland to birch woodland. III. Seed availability, germination and early growth. Journal of Ecology 79: 329–344.

Hill MO, Stevens PA (1981) The density of viable seed in soil of forest plantations in upland Britain. Journal of Ecology 69: 693–709.

Huopalainen M, Tuittila ES, Vanha-Majamaa I, Nousiainen H, Laine J, Vasander H (2000) The potential of soil seed banks for revegetation of bogs in SW Finland after long-term aerial pollution. Annales Botanici Fennici 37: 1–9.

Johnson MS, Bradshaw AD (1979) Ecological principles for restoration of disturbed and degraded land. Applied Biology 4: 141–200.

Kahle H (1993) Response of roots of trees to heavy metals. Environmental and Experimental Botany 3: 99–119.

Kiikkilä O, Perkiömäki J, Barnette M, Derome J, Pennanen T, Tulisalo E, Fritze H (2001) In situ bioremediation through mulching of soil polluted by a Cu-Ni smelter. Journal of Environment Quality (in press).

Komulainen M, Vieno M, Yarmishko VT, Daletskaja TD, Maznaja EA (1994) Seedling establishment from seeds and seed banks in forests under long-term pollution stress: a potential for vegetation recovery. Canadian Journal of Botany 72: 143–149.

Kozlov M, Haukioja E (1999) Performance of birch seedlings replanted in heavily polluted industrial barrens of the Kola peninsula, Northwest Russia. Restoration Ecology 7: 145–154.

Kubin E, Lippo H, Poikolainen J (2000) Heavy metal loading. In: Mälkönen E (ed) Forest condition in a changing environment – the Finnish case. Kluwer Academic Publishers, Dordrecht, pp. 60–71.

Laaksovirta K, Silvola J (1975) Effect of air pollution by copper, sulphuric acid and fertilizer factories on plants at Harjavalta, W. Finland. Annales Botanici Fennici 12: 81–88.

Leake JR, Shaw G, Read DJ (1990) The role of ericoid mycorrhizas in the ecology of ericaceous plant. Agriculture, Ecosystems and Environment 29: 237–250.

Legg CJ, Maltby E, Proctor MCF (1992) The ecology of severe moorland fire on the North York Moors: seed distribution and seedling establishment of *Calluna vulgaris*. Journal of Ecology 80: 737–752.

Mahy G, Vekemans X, Jacquemart AL (1999) Patterns of allozymic variation within *Calluna vulgaris* populations at seed bank and adult stages. Heredity 82: 432–440.

Mälkönen E, Derome J, Fritze H, Helmisaari HS, Kukkola M, Kytö M, Saarsalmi A, Salemaa M (1999) Compensatory fertilisation of Scots pine stands polluted by heavy metals. Nutrient Cycling in Agroecosystems 55: 239–268.

McCullagh P, Nelder JA (1989) Generalized linear models. 2nd edition. Monographs on statistics and applied probability 37. Chapman and Hall, London.

McGee A, Feller MC (1993) Seed banks of forested and disturbed soils in southwestern British Columbia. Canadian Journal of Botany 71: 1574–1583.

Mitchell RJ, Marrs RH, Auld MHD (1998) A comparative study of the seedbanks of heathland and successional habitats in Dorset, Southern England. Journal of Ecology 86: 588–596.

Mladenoff DJ (1990) The relationship of the soil seed bank and understorey vegetation in old-growth northern hardwood-hemlock treefall gaps. Canadian Journal of Botany 68: 2714–2721.

Monni S, Salemaa M, White C, Tuittila E, Huopalainen M (2000a) Copper resistance of *Calluna vulgaris* originating from the pollution gradient of a Cu-Ni smelter, in southwestern Finland. Environmental Pollution 109: 211–219.

Monni S, Salemaa M, Millar N (2000b) The tolerance of *Empetrum nigrum* to copper and nickel. Environmental Pollution 109: 221–229.

Moore JM, Wein RW (1977) Viable seed populations by soil depth and potential site recolonization after disturbance. Canadian Journal of Botany 55: 2408–2412.

Onaindia M, Amezaga I (2000) Seasonal variation in the seed banks of native woodland and coniferous plantations in Northern Spain. Forest Ecology and Management 126: 163–172.

Patterson W, Olson J (1983) Effects of heavy metals on radicle growth of selected woody species germinated on filter paper, mineral and organic soil substrates. Canadian Journal of Forest Research 13: 233–238.

Percy K (1986) The effect of simulated acid rain on germinative capacity, growth and morphology of forest tree seedlings. New Phytologist 104: 473–484.

Pickett STA, McDonnell MJ (1989) Seed bank dynamics in temperate deciduous forest. In: Leck MA, Parker VT, Simpson RL (eds) Ecology of soil seed banks. Academic Press Inc., San Diego, pp 123–147.

Pywell RF, Putwain PD, Webb NR (1997) The decline of heathland seed populations following the conversion to agriculture. Journal of Applied Ecology 34: 949–960.

Rigina O, Kozlov MV (2000) Pollution impact on sub-arctic forests in the Kola Peninsula, Russia. In: Innes JL, Oleksyn J (eds) Forest dynamics in heavily polluted regions. CAB International, Wallingford, pp 37–65.

Salemaa M, Vanha-Majamaa I, Gardner PJ (1999) Compensatory growth of two clonal dwarf shrubs, *Arctostaphylos uva-ursi* and *Vaccinium uliginosum* in a heavy metal polluted environment. Plant Ecology 141: 79–91.

Salemaa M, Vanha-Majamaa I, Derome J (2001) Understorey vegetation along a heavy-metal pollution gradient in SW Finland. Environmental Pollution 112: 339–350.

SAS Institute Inc. (1994) SAS/STAT User's Guide Version 6. 4th Edition. SAS Institute Inc., Cary, North Carolina, USA.

Scherbatskoy T, Klein RM, Badger GJ (1987) Germination responses of forest tree seed to acidity and metal ions. Environmental and Experimental Botany 27: 157–164.

Smallidge PJ, Leopold DJ (1995) Watershed liming and pit and mound topography effects on seed banks in the Adirondacks, New York, USA. Forest Ecology and Management 72: 273–285.

SPSS Inc. (1999) SPSS 9.0.1. software. SPSS Inc., Chicago, USA.

Sørensen T (1948) A method of establishing groups of equal amplitude in plant sociology based on similarity of species content. Det Kongelige Danske Videnskabernes Selskab, Biologiske Skrifter, Bind V, Nr 4, Copenhagen.

Uhlig C, Salemaa M, Vanha-Majamaa I, Derome, J 2001. Element distribution in *Empetrum nigrum* microsites at heavy metal contaminated sites in Harjavalta, western Finland. Environmental Pollution 112: 435–442.

Valbuena L, Tárrega R, Luis-Calabuig E (2000) Seed banks of *Erica australis* and *Calluna vulgaris* in a heathland subjected to experimental fire. Journal of Vegetation Science 11: 161–166.

Vander Kloet SP, Hill NM (1994) The paradox of berry production in temperate species of *Vaccinium*. Canadian Journal of Botany 72: 52–58.

Vangronsveld J, Colpaert JV, Van Tichelen KK (1996) Reclamation of a bare industrial area contaminated by non-ferrous metals: physico-chemical and biological evaluation of the durability of soil treatment and revegetation. Environmental Pollution 94: 131–140.

Vieno M, Komulainen M, Neuvonen S (1993) Seed bank composition in a subarctic pine – birch forest in Finnish Lapland: natural variation and the effect of simulated acid rain. Canadian Journal of Botany 71: 379–384.

Winterhalder K (2000) Landscape degradation by smelter emissions near Sudbury, Canada, and subsequent amelioration and restoration. In: Innes JL, Oleksyn J (eds) Forest dynamics in heavily polluted regions, CABI Publishing, Wallingford, pp 87–119.

Wolters JHB, Martens MJM (1987) Effects of air pollutants on pollen. The Botanical Review 53: 372–414.

Paper IV

Salemaa, M., Vanha-Majamaa, I. & Gardner, P. 1999. Compensatory growth of two clonal dwarf shrubs, *Arctostaphylos uva-ursi* and *Vaccinium uliginosum* in a heavy metal polluted environment. Plant Ecology 141: 79-91.

Plant Ecology **141:** 79–91, 1999.

Compensatory growth of two clonal dwarf shrubs, *Arctostaphylos uva-ursi* and *Vaccinium uliginosum* in a heavy metal polluted environment

M. Salemaa[1], I. Vanha-Majamaa[1] & P. J. Gardner[2]
[1]*Vantaa Research Centre, The Finnish Forest Research Institute, FIN-01301 Vantaa, Finland (e-mail: maija.salemaa@metla.fi);* [2]*Environment Agency, Ridgeworth House, Liverpool Gardens, Worthing, BN11 1YH, UK*

Key words: Bearberry, Bog bilberry, Branching architecture, Metal resistance, Overcompensation, Phenotypic plasticity

Abstract

The effect of artificial shoot clipping on the vegetative growth and sexual reproduction of the evergreen bearberry, *Arctostaphylos uva-ursi*, and the deciduous bog bilberry, *Vaccinium uliginosum*, was studied in the vicinity of a copper-nickel smelter in SW Finland. According to the research hypothesis, heavy metal induced shoot death breaks the apical dominance in the clones growing in a polluted environment. This causes activation of dormant axillary and adventitious buds and an increase in branching on the older parts of the stem. Regrowth after shoot death was studied by clipping off all the current-year shoots from experimental branches in autumn (1994) and spring (1995). Within-clone and between-clone control branches were used to test the data.

Both species displayed a considerable ability to activate dormant meristems after the damage. Regrowth of the current shoots during the next growing season (1995) was about 80% compared to the within-clone control in both species after autumn clipping. Shoot clipping in early summer was more detrimental for both species, and the regrowth of *A. uva-ursi* was less than that of *V. uliginosum*. Differences in the storage reserves and source-sink mechanisms of carbon allocation between evergreen and deciduous species probably explain their distinct response. When the removed biomass was added to the living biomass of the branches, there was overcompensation in the total dry weight of *A. uva-ursi* after autumn clipping, and the weight was almost 90% of the control after spring clipping. The total dry weight of *V. uliginosum* also equalled that of the control when the removed biomass was added. No berries developed on either species in the year following the autumn treatment, because clipping removed all the flower buds. Spring clipping had no effect on the sexual reproduction of *A. uva-ursi*, but decreased the berry production of *V. uliginosum.* The degree of compensatory growth of both species was only slightly affected by the distance from the smelter. It is suggested that dormant bud activation, rapid regrowth and plastic branching contribute to the resistance mechanism to heavy metals.

Introduction

The ability of plants to compensate for lost biomass is an important means of recovering from different kinds of biotic and abiotic damage (Belsky et al. 1993). Compensatory growth has most often been described following herbivory (Marquis 1996), but it can also be considered as a generalised response of plants to all kinds of damage. Heavy metal induced changes in vegetation are common in the surroundings of metal processing factories, smelters and on metalliferous waste. Although elevated heavy metal levels generally injure plants, resistant populations are known, especially among vascular plants (Ernst 1974). Resistance to heavy metals can be achieved through either avoidance or tolerance strategies (Levitt 1980; Baker 1987; Verkleij & Schat 1989). Avoidance is expressed as an external protection against toxic elements or as active orientation of the roots to a less toxic substrate (Balsberg 1982; Tyler et al. 1989). Tolerance is usually understood as a physiological mechanism, which results in the exclusion or accumulation of heavy metals and their conversion into a detoxified form (Baker 1981).

Rapid turnover of the roots or above-ground parts of plants may also serve as a resistance strategy, which makes it possible to exclude heavy metals from living tissues (Tyler et al. 1989) or to recover from biomass loss (Belsky et al. 1993). Release of apical dominance after damage makes regrowth possible from activated dormant buds in metameric plant species (Aarssen 1995; Marquis 1996). In long-lived trees and clonal dwarf shrubs, ecotypic differentiation into metal-tolerant races is rare or very slow (Dickinson et al. 1991). In these species, phenotypic plasticity in morphological and physiological characteristics is a more probable mechanism for surviving in a polluted environment.

In this paper we study two clonal dwarf shrubs, the evergreen *Arctostaphylos uva-ursi* L. (bearberry) and the deciduous *Vaccinium uliginosum* L. (bog bilberry) in a heavy metal polluted environment. Both of the studied species express high resistance to heavy metals (Laaksovirta & Silvola 1975; Väisänen 1986; Salemaa & Vanha-Majamaa 1993) and have a good regrowth ability after damage. High plasticity in growth response is common in the *Ericaceae* family (Gimmingham 1972; Chester & McGraw 1983; Shevtsova et al. 1995), but different species display different degrees of compensation, depending on the life form and the type of damage (Tolvanen & Laine 1997). It is proposed that deciduous *Vaccinium* species are more resistant to herbivory and other tissue loss than evergreen *Vaccinium* species. This is due to their larger carbohydrate and nutrient reserves, more rapid movement of nutrients to the leaves, and weaker architectural constraints on shoot growth (Tolvanen et al. 1993, 1995). Also, the time of damage and environmental conditions strongly affect the regrowth ability of plants (Chapin 1980a; Maschinski & Whitham 1989; Hjälten et al. 1993). If damage occurs late in the growing season in *Vaccinium* species, the regrowth response is absent or lower than that after damage taking place early in the growing season (Tolvanen & Laine 1997). Vigorous regrowth is suggested to occur in resource-rich environments where plants are not subjected to stress or competition (Belsky et al. 1993).

The object of this study is to compare the short-term ability of *V. uliginosum* and *A. uva-ursi* to recover after artificial shoot clipping along a heavy metal gradient. Special attention is paid to the significance of compensatory growth as a tolerance mechanism to heavy metals. Three hypotheses were tested as follows:

- Deciduous *V. uliginosum* is more resistant to shoot clipping than evergreen *A. uva-ursi.*
- Autumn shoot clipping at the time when growth has already ceased has a less deleterious effect on regrowth than the clipping of new growth in the spring.
- Shoot clipping is more deleterious in a highly polluted than in a less polluted environment.

Materials and methods

Life histories of V. uliginosum *and* A. uva-ursi

Both deciduous *V. uliginosum* and evergreen *A. uva-ursi* are clonal, widespread circumpolar members of the *Ericaceae.* Their global range extends from the Arctic Circle down to the northern temperate zone, and their main distribution area is in the boreal vegetation zone (Tutin et al. 1972; Packer & Denford 1974; Jacquemart 1996).

V. uliginosum grows in southern Finland mainly on peatlands and, further to the north, also on mineral soils. It has horizontal subterranean rhizomes and grows laterally by producing erect 30–100 cm long shoots and may form large, pure stands. It generally grows on nutrient-poor acid soils and, as a heliophilious species, avoids substantially shaded habitats (Jaquemart 1996). It is regarded as cold-tolerant, and the limiting factor in its appearance in southern regions seems to be relatively high temperatures (Stewart & Bannister 1973). *V. uliginosum* has a sympodial branching habit with the terminal bud producing flowers and the uppermost lateral buds producing shoots (Figure 1a). The leaves are annual but the stem and buds overwinter. Fertility varies according to the site, and the berries are produced exclusively after cross-pollination (Fröborg 1996). However, vegetative reproduction appears to be much more important than that via seeds (Eriksson & Fröborg 1996).

Prostrate *A. uva-ursi* grows throughout Finland. It colonises open sandy heathlands and moraine slopes, eroded banks, and a variety of habitats in the boreal forest; however it does not grow on peat bogs, as opposed to the situation in North America (Remphrey et al. 1983). It has a deep tap and numerous adventitious roots which make it a good coloniser of disturbed sites. The branching habit of *A. uva-ursi* is predominantly sympodial, but some horizontally

Figure 1. Branching habits of the studied dwarf shrubs. (a) *Vaccinium uliginosum*: (1) sympodial vegetative shoots with annual leaves, (2) sympodial vegetative shoot developed from the basal bud on the old stem (elongated and thick rejuvenation shoot), (3) terminal short-shoots with flowers, and (4) old dead shoots. (b) *Arctostaphylos uva-ursi*: (1) monopodially produced leading vegetative shoot with an active terminal bud, (2) sympodial lateral vegetative shoot with an active terminal bud, (3) sympodial lateral generative shoot with a terminal inflorescence, (4) sympodial generative shoot with berries, and (5) sympodial lateral shoots with a dormant terminal bud. Stages 1–3 represent current-year, and 4–5 previous-year shoots.

elongated shoots are produced monopodially from the terminal bud of the parent shoot (Figure 1b). The occurrence of neoformed growth (produced by the apex of the current-year shoots) is common and suggests an opportunistic growth strategy (Remphrey & Steeves 1984a). Terminal inflorescences overwinter and berries develop in the next year. The shoots do not grow during the year that they produce berries. *A. uva-ursi* regenerates rather poorly from seeds and spreads mainly vegetatively (Remphrey et al. 1983). Activation of lateral buds on creeping branches (Remphrey et al. 1983; Remphrey & Steeves 1984b; Bowles 1983), or latent buds on roots (Tiffney et al. 1978), produces new shoots and aids re-establishment after disturbance.

Study sites

The study area is situated near the Cu-Ni smelter at Harjavalta (61°19′N, 22°9′E), SW Finland, in the southern boreal vegetation zone. *A. uva-ursi* was studied in two heath forest (*Pinus sylvestris* L.) stands at distances of 2 (F2) and 8 km (F8) from the smelter. *V. uliginosum* was studied in two heath forest stands at 0.5 km (F0.5) and 4 km (F4) and also in a drained peatland stand at 5 km (P5). The two species did not occur in sufficient numbers at all the distances. The 0.5–2 km zone represents highly polluted sites and the 4–8 km zone less polluted ones. General characteristics of the stands and data on deposition and element concentrations in the organic layer and vegetation are presented in Table 1. The mean annual temperature was 5 °C in 1995 and the annual precipitation 698 mm at the nearby weather station of the Finnish Meteorological Institute.

Copper production at the smelter started in 1945 and that of nickel in 1960. Emissions from the smelter have considerably decreased since the end of the 1980s (Cu 110, Ni 53 and SO_2 8000 t/yr) to the middle of 1990s (1994: Cu 40, Ni 6 and SO_2 5000 t/yr), when the study was started. However, accumulation of heavy metals in the soil during the last 50 years has changed the element fluxes of the ecosystem and damaged the vegetation (Helmisaari et al. 1995; Salemaa & Vanha-Majamaa 1993).

Experimental design and measured variables

A total of 30 clones were randomly selected at all sites. The distance between each clone was at least 5 m, and only visually separate patches were sampled. Unpublished data on isoenzyme variation revealed that almost all the studied clones represented different autotetraploid genotypes. The age of a few typical clones of both species growing at 0.5–4 km distance from the smelter was determined by counting the number of annual rings on the oldest part of the stem. The age of the clones ranged from 30 to 40 years, and it is probable that some 'mother clones' date back to the time when the smelter was founded in the 1940s.

The clones were divided into three groups, ten replicates in each: unclipped controls, clones clipped in autumn (14–16 September 1994) and clones clipped in spring, soon after bud break (*V. uliginosum* 6–7 June, *A. uva-ursi* 14 June 1995). Clipped clones are later called experimental clones. Shoot clipping was restricted to three randomly selected main branches on each experimental clone. All the current-year shoots were removed and stored for further measurements. In addition, three branches were randomly selected for a within-clone control and three for an unclipped control. This enables compensatory growth of the clipped branches to be compared with the unclipped branches of the same clone and with the unclipped control

Table 1. Site characteristics, element concentrations in the organic layer and in bulk precipitation, and Cu and Ni concentrations in *A. uva-ursi* (*A. u-u.*) and *V. uliginosum* (*V. u.*) tissues. Exchangeable Cu, Ni and Fe concentrations in the organic layer were determined with NH_4acetate + EDTA. All organic layer concentrations have been calculated per dry mass of organic matter. Data from Finnish Forest Research Institute: peatland (Veijalainen, unpublished), stand data on heath forest (Kukkola, unpublished), concentrations in forest humus and deposition (Derome, unpublished), concentrations in plant tissues (Salemaa & Vanha-Majamaa, unpublished).

Site	Forest (Scots pine)				Peatland (Scots pine)
	F0.5	F2	F4	F8	P5
Distance from smelter, km	0.5	2	4	8	5
Direction from smelter	S	SE	SE	SE	NW
Forest site type	*Calluna*	*Calluna*	*Calluna*	*Calluna*	Dwarf shrub pine bog
Stand age, yrs	49	52	56	90	30
Basal area, m^2/ha	9.0	14.8	15.9	–	9.8
Thickness of humus, cm	2.5	2.3	2.2	–	3.0
Vegetation coverage, %	1	24	77	100	100
Element concentrations in organic layer(1991):					
N, tot %	1.92	1.74	1.65	1.68	1.49
S, tot mg/kg	387	198	170	176	219
Exc. Cu, mg/kg	7540	2238	786	209	376
Exc. Ni, mg/kg	528	329	164	72	122
Exc. Fe, mg/kg	10899	3800	1902	1659	1240
Deposition in bulk precipitation (1994):					
N, tot mg/m^2	449	447	490	396	–
S04-S, mg/m^2	628	427	399	311	–
Cu, mg/m^2	119	30	9	3	–
Ni, mg/m^2	26	5	2	0.8	–
Fe, mg/m^2	38	17	10	7	–
Element concentrations in current leaves (1994):	*V.u.*	*A.u-u.*	*V.u.*	*A.u-u.*	*V.u.*
Cu, mg/kg	42.4	13.5	22.3	4.4	14.0
Ni, mg/kg	40.5	10.7	19.3	3.5	12.8
in fine roots (1994):					
Cu, mg/kg	718.7	313.0	102.3	39.7	102.8
Ni, mg/kg	163.3	119.4	49.3	29.7	48.4

clones. All the branches were harvested for biomass measurements on 25–27 July 1995.

A section was removed from the lowest part of the stem of each branch for age determination by dendrochronology in the laboratory. The length of the branch was measured and all living current shoots, dead shoots and berries were removed. The numbers of shoots and berries were counted and the dry weight of each separated biomass category and the remaining branch was determined. The number of clipped shoots was counted and their dry weight measured. Six branch-specific response variables to shoot clipping were recorded: (1) the total living biomass of the branch including the removed biomass, (2) the total biomass of the current shoots in 1995, (3) the number of current shoots, (4) the average weight of the current shoots, (5) the total biomass of dead shoots, and (6) the total biomass of berries.

In bush-like *V. uliginosum* the studied branches represented the oldest branches and were directly connected to the common stem or rhizome. In *A. uva-ursi* the studied branches were only distal parts of the creeping branches which grew from the common rhizome. Thus the size and age of the branches represented the real population in *V. uliginosum* but the sampled branches of *A. uva-ursi* were arbitrarily clipped to a length of about 24 cm.

The definition of compensation is according to Belsky (1986). Overcompensation occurs when the cumulative total dry weight of clipped plants (including removed tissue) is higher, exact compensation when it is equal to, and undercompensation when it is less than the total dry weight of the control plants.

Data analysis

Branchwise data were used in analysing the responses to shoot clipping, and in comparing the between-site differences in the unclipped control clones. The effect of site and treatment and their interaction was tested by two-factor ANOVA (SAS Institute Inc. 1994). The response to clipping depended on branch size, which varied between the sites (Table 2). Therefore covariates which eliminated the effect of branch size were added to the variance model. The relationship between the response variables and branch size of unclipped control clones was studied by means of regression analysis. Depending on the variable or the species, either branch weight or length gave the best coefficient of determination. Both branch weight and length were therefore selected as covariates in the model.

The model for the response is:

$$y_{ij} = \mu + \alpha_i + \beta_j + \gamma_{ij} + ax_{ij} + bz_{ij} + e_{ij},$$

where μ = the overall grand mean, α_i = site effect, $i = 1, 2$ (*A.u-u.*) or $i = 1, 2, 3$ (*V.u.*), β_j = treatment effect, $j = 0, \ldots, 4$, γ_{ij} = site by treatment interaction, a = the coefficient of the covariate x (weight), b = the coefficient of the covariate z (length), e_{ij} = random error.

Comparison between the treatment means within the sites was carried out using pairwise contrasts by ANOVA (F-values). No statistics for the clipping response of berries is presented because of the negative values given by the model. Unclipped controls were compared between the sites by 1-way ANOVA and pairwise contrasts.

The degree of compensation was studied by calculating the difference between the means of the control and of the clipped branches within each experimental clone. Between-site comparison of the degree of compensation was made by 1-way ANOVA and pairwise contrast analysis, the differences in within-clone branch weight and length being used as covariates.

Results

Unclipped controls at different sites compared to within-clone controls

Despite the similar lengths, the branches of *A. uva-ursi* were heavier and one year older at site F2 than at site F8 (Table 2a). The average length of the branches of *V. uliginosum* was highest at site P5, followed by F0.5 with F4 having the shortest branches (pairwise contrast, $p < 0.01$). The weight and age of the branches were highest at site F0.5 ($p < 0.05$) (Table 2b).

The means of the response variables predicted by the model of the unclipped and within-clone controls are presented in Figures 2 and 3, and the original data in Table 2. The total biomass of current-year shoots of *A. uva-ursi* was similar at both sites (pairwise contrasts, $p = 0.752$), but the average shoot weight was significantly higher ($p = 0.004$) at site F8 than at site F2 (Figures 2b and 2d). In contrast, the total weight of berries was higher at site F2 than at site F8 ($p = 0.002$, Table 2a). The number of current shoots or the biomass of dead shoots did not differ between the sites ($p = 0.965$ and $p = 0.574$ respectively) (Figure 2c,e). The total biomass of current-year shoots of *V. uliginosum* was lower ($p < 0.05$) at site F4 than at the other sites (Figure 3b). The number of current-year shoots was similar at all sites (Figure 3c). The average weight of the current shoots, the biomass of dead shoots and that of berries were highest at site F0.5 ($p < 0.09$, Figures 3d, e, Table 2b).

Although the model predicted number of current shoots did not differ between the sites in either species, there were differences when the shoot number was calculated per cm of stem length. In *A. uva-ursi* the shoot number was slightly higher at site F2 (0.3 shoots/cm) than at F8 (0.2 shoots/cm) ($p = 0.122$). In *V. uliginosum* the number was highest (0.5 shoots/cm) at site F0.5, followed by site P5 (0.4 shoots/cm) and F4 (0.3 shoots/cm) ($p < 0.01$) (Table 2a, b).

In general, the growth of the unclipped controls was slightly better or similar to that of the within-clone controls in *V. uliginosum*, but the differences were statistically significant only in a few cases (Figure 3b, c). The autumn clipping of *A. uva-ursi* at site F2 was exceptional, because both the control and

Table 2. Means ± standard errors of five treatments for branch size, removed biomass and response variables of a) *A. uva-ursi* and b) *V. uliginosum.* n = number of branches. Total biomass = branch weight + removed biomass + current shoots. Average shoot weight = current shoots / number of shoots. Shoot weight for removed biomass of *V. uliginosum* includes only stems in treatment 2. Treatment codes as in Figure 2.

a) *Arctostaphylos uva-ursi*

		Branch size				Removed biomass		Response variables					
Site	Treat-ment	n	Age, yrs	Length, mm	Weight, g	Shoot weight, g	Number of shoots	Total biomass, g	Current shoots, g	Number of shoots	Av. shoot weight, mg	Dead branches, g	Berries, mg
Forest 2 km	0	30	6.1±0.3	247±10	2.2±0.2			2.90±0.22	0.64±0.10	8.4±1.0	81±7	0.03±0.01	204±46
	1	28	6.5±0.4	251±20	2.6±0.4			3.79±0.54	1.15±0.22	13.1±1.8	77±8	0.05±0.02	177±44
	2	30	7.2±0.3	286±18	2.4±0.3	1.18±0.13	11.7±1.4	4.57±0.46	0.98±0.15	15.4±1.4	61±8	0.19±0.07	0
	3	29	7.0±0.2	224±14	2.6±0.3			3.09±0.34	0.54±0.09	9.1±1.5	62±5	0.05±0.02	207±57
	4	28	7.0±0.3	234±13	4.1±0.5	0.17±0.03	13.1±1.9	4.51±0.53	0.24±0.05	9.6±1.7	27±3	0.09±0.04	260±57
Forest 8 km	0	30	5.3±0.2	230±14	1.0±0.1			1.53±0.12	0.51±0.04	5.3±0.6	115±10	0.01±0.01	21±6
	1	30	5.8±0.2	256±15	1.5±0.2			2.04±0.28	0.51±0.08	6.9±1.2	97±10	0.04±0.01	12±6
	2	30	5.4±0.3	225±14	1.1±0.1	0.84±0.10	7.4±0.9	2.28±0.30	0.37±0.06	9.8±1.5	37±3	0.09±0.03	0
	3	29	5.5±0.3	289±18	1.9±0.2			2.48±0.27	0.58±0.09	7.7±1.2	86±12	0.05±0.02	27±13
	4	30	6.0±0.3	277±15	2.3±0.2	0.18±0.03	10.0±1.1	2.73±0.21	0.20±0.04	5.7±0.6	36±5	0.02±0.01	25±11

Table 2. Continued

b) *Vaccinium uliginosum*

		Branch size				Removed biomass		Response variables					
Site	Treatment	n	Age, yrs	Length, mm	Weight, g	Shoot weight, g	Number of shoots	Total biomass, g	Current shoots, g	Number of shoots	Av. shoot weight, mg	Dead branches, g	Berries, mg
Forest 0.5 km	0	30	7.6±0.3	423±17	3.8±0.4			5.54±0.50	1.70±0.16	22.4±2.5	88±9	1.05±0.21	455±115
	1	28	9.5±0.4	457±15	5.4±0.5			7.26±0.53	1.86±0.17	28.0±3.0	75±8	1.17±0.20	97±31
	2	30	10.2±0.6	448±17	6.3±0.6	0.59±0.09	28.7±2.3	8.57±0.71	1.68±0.19	16.1±1.3	102±8	1.55±0.41	0
	3	30	9.0±0.4	545±27	5.9±0.6			7.50±0.75	1.55±0.22	23.2±2.6	71±7	1.26±0.22	531±178
	4	30	8.9±0.4	552±29	7.1±0.8	0.76±0.08	24.5±1.9	8.63±0.90	0.79±0.12	26.0±3.8	37±7	1.29±0.17	26±19
Forest 4 km	0	30	6.1±0.3	363±10	1.3±0.1			2.09±0.17	0.78±0.06	12.4±1.2	70±5	0.11±0.04	5±3
	1	29	5.7±0.3	348±20	1.4±0.2			2.06±0.25	0.66±0.08	14.1±1.6	51±4	0.12±0.03	3±3
	2	29	5.8±0.3	326±26	1.6±0.3	0.16±0.03	15.9±2.4	2.30±0.33	0.52±0.07	10.1±1.4	57±5	0.12±0.03	0
	3	30	5.5±0.3	393±16	1.8±0.3			2.48±0.32	0.65±0.07	11.7±1.1	65±9	0.23±0.08	7±4
	4	30	6.4±0.3	378±20	1.8±0.2	0.16±0.02	11.9±1.6	2.28±0.30	0.33±0.04	9.1±1.1	40±4	0.21±0.05	2±2
Peatland 5 km	0	30	6.7±0.3	503±23	3.4±0.6			4.82±0.73	1.46±0.17	22.0±2.7	75±6	0.25±0.10	3±3
	1	27	7.3±0.4	541±25	3.3±0.3			4.53±0.37	1.26±0.10	20.4±1.8	66±4	0.36±0.05	7±5
	2	27	8.0±0.4	571±28	5.2±0.5	0.46±0.05	25.7±2.6	7.05±0.59	1.34±0.15	17.5±1.8	79±6	0.30±0.05	0
	3	30	6.9±0.3	497±19	3.8±0.4			5.64±0.61	1.81±0.20	23.5±2.6	86±6	0.26±0.04	34±12
	4	30	7.6±0.3	501±19	5.5±0.6	0.94±0.11	32.8±3.9	7.60±0.83	1.14±0.15	38.3±4.5	32±3	0.34±0.08	3±3

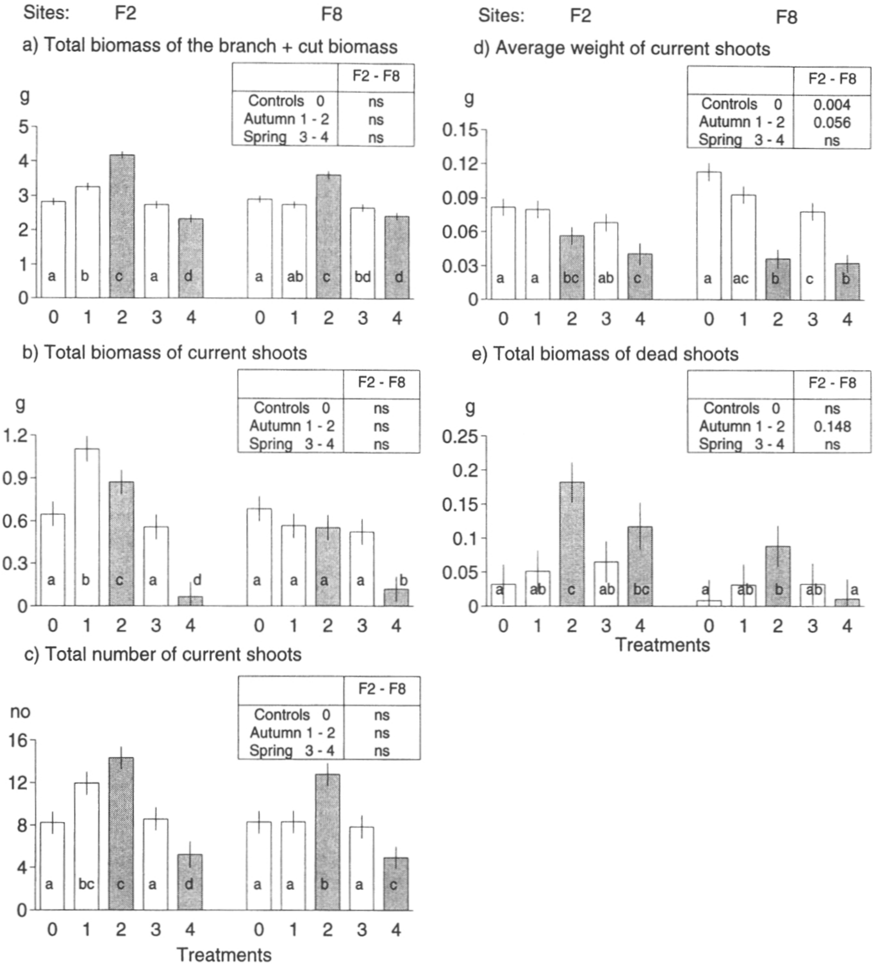
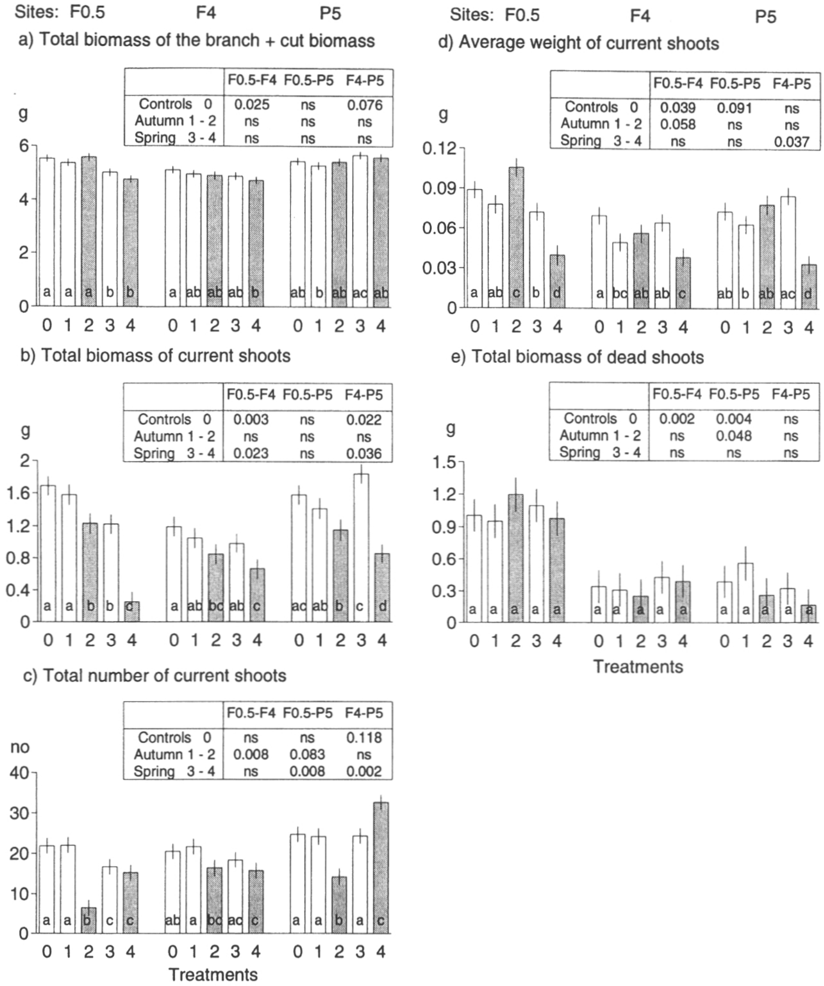


Figure 2. Model predicted means ± standard errors of five treatments of *A. uva-ursi* at two sites (F2 and F8) on a) total biomass of the branch including the removed biomass, b) total biomass of current shoots, c) total number of current shoots, d) average weight of current shoots and e) total biomass of dead shoots. Treatments: 0 = unclipped control, 1 = within-clone control for autumn clipping, 2 = autumn clipping (shaded), 3 = within-clone control for spring clipping and 4 = spring clipping (shaded). Pairwise contrast analyses are presented with the letters inside the bars, the same letter indicating non-significant differences ($p > 0.05$) between the means. Three between-site comparisons are presented in the boxes (contrast analysis, $p < 0.150$ given): (1) unclipped controls, (2) the degree of compensatory growth (within-clone difference between the means of the control and tratment branches) in autumn and (3) in spring. ns = non-significant ($p > 0.05$).

treatment branches grew better than unclipped control ($p < 0.05$). No differences in growth between the unclipped and within-clone controls of *A. uva-ursi* were found at site F8 (Figure 2b, c).

Vegetative growth after shoot clipping compared to within-clone control

Both species displayed a good ability to compensate for lost biomass during the growing season following the autumn treatment. Overcompensation occurred in the total biomass of the branches of *A. uva-ursi* when removed biomass was added to the living biomass of the branches. The total biomass of the clipped branches, including removed biomass, was about 30% higher than that of the control (pairwise contrast analysis, $p < 0.001$) at both sites (Figure 2a). In *V. uliginosum* the total biomass of the branches, including removed biomass, was similar to that of the control at all sites after autumn clipping (Figure 3a). The total



a) Total biomass of the branch + cut biomass

	F0.5-F4	F0.5-P5	F4-P5
Controls 0	0.025	ns	0.076
Autumn 1 - 2	ns	ns	ns
Spring 3 - 4	ns	ns	ns

b) Total biomass of current shoots

	F0.5-F4	F0.5-P5	F4-P5
Controls 0	0.003	ns	0.022
Autumn 1 - 2	ns	ns	ns
Spring 3 - 4	0.023	ns	0.036

c) Total number of current shoots

	F0.5-F4	F0.5-P5	F4-P5
Controls 0	ns	ns	0.118
Autumn 1 - 2	0.008	0.083	ns
Spring 3 - 4	ns	0.008	0.002

d) Average weight of current shoots

	F0.5-F4	F0.5-P5	F4-P5
Controls 0	0.039	0.091	ns
Autumn 1 - 2	0.058	ns	ns
Spring 3 - 4	ns	ns	0.037

e) Total biomass of dead shoots

	F0.5-F4	F0.5-P5	F4-P5
Controls 0	0.002	0.004	ns
Autumn 1 - 2	ns	0.048	ns
Spring 3 - 4	ns	ns	ns

Figure 3. Model predicted means ± standard errors of different treatments of *V. uliginosum* at three sites (F0.5, F4 and P5). Explanations as in Figure 2.

biomass of the branches equalled or was very close to that of the control after the spring clipping for both species (Figures 2a, 3a).

The total biomass of current-year shoots of *A. uva-ursi* was 21% lower at site F2 ($p = 0.061$) and equalled to that of the control at site F8 ($p = 0.912$) after the autumn treatment (Figure 2b). The corresponding biomass of current-year shoots of *V. uliginosum* was about 20% lower than that of the controls at all sites (significant difference only at site F0.5, $p = 0.039$) (Figure 3b). Shoot clipping in the spring had more detrimental effects on the regrowth ability. In *A. uva-ursi* the total biomass of the current-year shoots was only 12% of that of the control at site F2 ($p = 0.001$) and 25% at site F8 ($p = 0.001$) (Figure 2b). Regrowth of *V. uliginosum* was slightly better than that of *A. uva-ursi* after the spring treatment, being 20% compared to the control at site F0.5 ($p = 0.001$), 68% at site F4 ($p = 0.054$) and 47% at site P5 ($p = 0.001$) (Figure 3b).

The number of current-year shoots on the clipped branches increased in *A. uva-ursi* (F2: $p = 0.114$, F4: $p = 0.003$) but decreased in *V. uliginosum* (F0.5 and P5: $p = 0.001$, F4: $p < 0.05$) after autumn clipping (Figures 2c and 3c). The shoot number of the clipped branches did not approach the control after spring clipping in *A. uva-ursi* ($p < 0.05$). Compensation in the shoot numbers of *V. uliginosum* was almost exactly the same after spring clipping at sites F0.5 and F4, and slight overcompensation occurred at site P5 ($p = 0.002$).

The average weight of the current-year shoots of *A. uva-ursi* was lower in the clipped branches than in the control after both autumn and spring clipping ($p < 0.03$) (Figure 2d). Accordingly, when the number of shoots increased, the average weight of the shoots decreased after autumn clipping. Both the shoot number and the average shoot weight decreased ($p < 0.02$) after spring clipping in *A. uva-ursi.* The opposite relationship was found in *V. uliginosum* after autumn clipping. The number of shoots was lower but the average weight of the shoots higher than those in the control (significant difference only at F0.5: $p < 0.03$), (Figure 3c, d). After the spring clipping the average shoot weight in *V. uliginosum* was also lower than that in the control (all sites: $p < 0.004$).

The total biomass of dead shoots increased at site F2 after autumn clipping in *A. uva-ursi* ($p = 0.002$) (Figure 2d). There were no statistically significant differences in the biomass of dead branches between the treatment and the control in *V. uliginosum* within the sites (Figure 3d).

Sexual reproduction after shoot clipping

All the flower buds were lost when current-year shoots were clipped in the autumn in both species. Consequently, no berries developed after the autumn treatment (Tables 2a, b). Spring clipping did not have any effect on the berry production of *A. uva-ursi*, and only a few flowers of *V. uliginosum* developed into berries following spring clipping (Table 2a, b).

Effect of the distance from the smelter on the degree of compensation

In general, the distance from the smelter did not significantly affect the degree of compensatory growth of either species. Although the regrowth level of the current shoots of *A. uva-ursi* was slightly lower at site F2 than at site F8, this difference was not statistically significant ($p = 0.335$) (Figure 2b). Only the average weight of shoots decreased more at site F8 than at site F2 ($p = 0.056$) after autumn clipping (Figure 2d). In *V. uliginosum* the degree of regrowth of the current-year shoots was slightly higher at site F4 than at the two other sites ($p < 0.04$) after spring clipping (Figure 3b). In addition, the decrease in the number of current-year shoots was highest at site F0.5 ($p < 0.100$) after autumn clipping (Figure 3c).

Discussion

The effect of life form and the time of damage on the response

Mechanical cutting of shoots may not have the same effect on plants as heavy metal induced shoot mortality. Heavy metal stress damages all the primary metabolic processes of plants (Balsberg-Påhlsson 1989), but the actual damage mechanism is unknown at the cellular level in the studied species. *V. uliginosum* had a high abundance of dead branches in the most polluted environment. Both species also had a higher number of current shoots per cm of stem than clones growing further away from the smelter. This suggests that the breakage of apical dominance is somehow connected to shoot death caused by heavy metal toxicity. Many of the current shoots of *V. uliginosum* grew from old wooden stems and were elongated and thicker than the shoots in the apical parts of the branches (rejuvenation shoots, Figure 1a). Besides direct toxic effects on the living tissues of plants, heavy metals may also accentuate natural stress factors such as frost (Sutinen et al. 1996) or desiccation in the spring when the snow cover no longer protects the understorey vegetation.

Autumn clipping did not appear to damage either *A. uva-ursi* or *V. uliginosum* seriously. Although only the short-term response was studied, the current-year growth of the clipped branches was about 80% of that of the within-clone control. It is probable that the resource loss caused by autumn clipping was rather small at the time when the growing season was already over. Thus clipping merely activated the dormant meristems and resulted in vigorous lateral branching the following summer. Contrary to the predictions, the relative number of activated meristems was higher in *A. uva-ursi* than in *V. uliginosum.* This kind of flexible branching seems to be very typical of *A. uva-ursi* after damage (Remphrey et al. 1983, Bowles 1983). However, the slight overcompensation observed in the total dry weight of *A. uva-ursi* was not exceptional, but probably represented only the upper range of normal growth (cf., Belsky et al. 1993). Aarssen (1995) suggests that overcompensation in terms of increased fitness can be expected when species that usually benefit from apical dominance are found in conditions where apical dominance affords little or no selective advantage. In the case of *A. uva-ursi*, it seems that the strength of apical dominance is rather plastic and that the 'reserve of dormant meristems' (Tuomi et al. 1994; Aarssen 1995) enables the species to recover after damage. While autumn-clipped branches of *V. uliginosum* produced a few heavy shoots, *A. uva-ursi* produced many lighter ones. Chester & McGraw (1983) have also observed the tendency of *V. uliginosum* to increase shoot size as a response to nitrogen fertilization, in contrast to *V. vitis-idaea* L. in which many lateral meristems are activated after fertilization. The weight of dead shoots after autumn clipping increased in *A. uva-ursi*, suggesting that the reserves in the older plant parts were depleted as a result of nutrient translocation to the new shoots.

Both species showed a marked reduction in shoot growth after spring clipping. The effect was more detrimental to *A. uva-ursi* than to *V. uliginosum.* This result is in accordance with many earlier studies, in which deciduous species are reported to recover faster from early summer damage than evergreen species (Chapin 1980a; Archer & Tieszen 1980; Tolvanen & Laine 1997). For instance, deciduous *V. myrtillus* L. produced many shoots with a low carbohydrate content, while evergreen *V. vitis-idaea* produced less shoots with an increased carbohydrate content after artificial herbivory (Tolvanen & Laine 1997). It has been emphasised that the distinct responses after damage observed between evergreen and deciduous species are a consequence of differences in their storage reserves and source-sink aspects of carbon and nutrient allocation (Chapin 1980a; Archer & Tieszen 1980; Tolvanen & Laine 1997). In addition to reallocation of resources after damage, plant architecture and sectoriality and the number and distribution of meristems determines the general range of tolerance (Marquis 1996).

A. uva-ursi and *V. uliginosum* differ from each other in three essential factors affecting their regrowth ability. Firstly, the belowground storage of carbohydrates and nutrients is generally greater in deciduous than in evergreen species (Chapin 1980a). For instance, the proportion of the belowground biomass of *V. uliginosum* was about 79% whereas that of *V. vitis-idaea* was about 51% in a subarctic area (Karlsson 1987). The proportion of the latter species is of course not comparable with that of *A. uva-ursi* which has a tap and many adventitious roots. While the carbohydrate and mineral nutrient stores of deciduous shrubs are located in stems or roots, the leaves are important as storage sites in evergreen species (Chapin 1980b, 1983; Karlsson 1985). Secondly, the photosynthesis and growth rates of deciduous species are higher than those of evergreen species (Johnson & Tieszen 1976; Chapin 1980a; Karlsson 1989). Although the photosynthesis of evergreen species starts earlier (Karlsson 1989), their growth begins later than that of deciduous species. The shoot growth of *V. uliginosum* is based on both current-year photosynthesis and the stored resources, whereas, e.g., *V. vitis-idaea* mostly uses early-summer assimilates produced by old leaves (Karlsson 1985). It is probable that *A. uva-ursi* resembles *V. vitis-idaea* in this respect. Finally, the absorption and translocation of nutrients is faster in deciduous than in evergreen species (Chapin 1980a). The spring decrease in the nitrogen content of the belowground parts of deciduous shrubs suggests that much of the nitrogen in their leaves is translocated from belowground stores (Chapin 1980b). Current growth in *V. uliginosum* is observed to be a strong sink for nitrogen (Chester & Oechel 1986).

The branches of *A. uva-ursi* and *V. uliginosum* appeared to be rather autonomous in carbon allocation because there was no marked decrease in the growth of within-clone control branches compared to the unclipped control. This observation is in agreement with results reported for other woody plants, e.g., for *Pinus sylvestris* (Honkanen & Haukioja 1995). It is known that the sectoriality of plants causes resources, in particular carbon, to flow more freely within morphological units (e.g., branches) than between such units (Watson & Casper 1984; Marquis 1996; Marshall 1996). The development of fine roots on the creeping branches of *A. uva-ursi* made the annual growth segments (modules) more independent of intraclonal transport of water and nutrients than modules of *V. uliginosum.*

The developing fruits are known to be a strong sink for resources (Watson & Casper 1984; Marshall 1996). *V. uliginosum* had started blooming when the young current shoots were clipped in the spring. Because berries were obviously supported by the assimilates of the neighbouring leaves, the removal of all the shoots dramatically decreased the number of berries. In contrast, overwintered flower buds in the previous-year shoots of *A. uva-ursi* (Figure 1b) were not affected by the removal of new current shoots. The berries of this species were evidently supported by the older leaves of the same unclipped shoots.

The effect of the distance from the smelter

The degree of compensatory growth was not clearly affected by the distance from the smelter in either species, although the biomass of dead branches was highest at the most polluted sites. The prediction of a higher regrowth level in a low-stress environment (Belsky et al. 1993) was only partially fulfilled. *A. uva-ursi* showed some signs of decreased growth after autumn clipping near the smelter, but this reduction was not statistically significant. The degree of regrowth of *V. uliginosum* was also slightly lower near the smelter compared to the other sites after spring clipping. Both species accumulate heavy metals in roots and this may restrict their translocation to leaves (Table 1). In addition to the ability to prevent heavy metals passing into assimilating tissues, the low competition for resources resulting from the low number of other plant species growing near the smelter, may partly explain the unexpectedly good compensation.

Conclusions

Contrary to predictions, *A. uva-ursi* showed as vigorous regrowth as *V. uliginosum* after shoot clipping in autumn. Both species suffered more from spring than from autumn clipping, and the compensatory growth of *A. uva-ursi* was lower than that of *V. uliginosum* after spring clipping. The degree of compensatory growth was only slightly lower in the highly polluted environment near the smelter than at greater distances. Heavy metals and sulphur have subjected the vegetation growing nearest to the smelter to a strong selection pressure. The surviving clones probably represent the most resistant genotypes of the populations established at least 30–40 years ago. It is suggested that the vigorous regrowth resulting from activation of the dormant buds and the rapid shoot turnover have significantly improved the survival of the studied clones in this polluted environment. In long-lived dwarf shrubs, phenotypic plasticity in morphological and physiological responses probably provides resistance to heavy metals when the evolution of tolerant ecotypes is restricted by the failure of seedling establishment.

Acknowledgements

Päivi Lindholm, Pekka Suolahti, Taru Uotila and Outi Välilehto helped in the field and laboratory work. Yrjö Sulkala determined the age of the branches and Airi Piira assisted with the data and figures. Erkki Tomppo advised in statistical analyses. John Derome and Satu Monni commented on the manuscript. Hannu Nousiainen made the drawings of the species. We wish to thank all the above-mentioned persons and the Forest Health Fertilization Project of the Finnish Forest Research Institute for the opportunity to use the data collected on the sample plots of the project.

References

Aarssen, L. W. 1995. Hypothesis for the evolution of apical dominance in plants: implications for the interpretation of overcompensation. Oikos 74: 149–156.

Archer, S. & Tieszen, L. L. 1980. Growth and physiological responses of tundra plants to defoliation. Arct. Alp. Res. 12: 531–552.

Baker, A. J. M. 1981. Accumulators and excluders – strategies in the response of plants to heavy metals. J. Plant Nutr. 3: 643–654.

Baker, A. J. M. 1987. Metal tolerance. New Phytol. 106: 93–111.

Balsberg, A.-M. 1982. Plant biomass, primary production and litter disappearance in a *Filipendula ulmaria* meadow ecosystem, and the effects of cadmium. Oikos: 72–90.

Balsberg-Påhlsson, A.-M. 1989. Toxicity of heavy metals (Zn, Cu, Cd, Pb) to vascular plants. Water, Air, Soil Pollut. 47: 287–319.

Belsky, A. J. 1986. Does herbivory benefit plants? A review of the evidence. Am. Nat. 127: 870–892.

Belsky, A. J., Carson, W. P., Jensen, C. L. & Fox, G. 1993. Overcompensation by plants: herbivore optimization or red herring? Evol. Ecol. 7: 109–121.

Bowles, J. M. 1983. Burl sprouting in *Arctostaphylos uva-ursi* as a response to trampling damage. Can. J. Bot. 61: 3543–3545.

Chapin, F. S. III 1980a. Nutrient allocation and responses to defoliation in tundra plants. Arct. Alp. Res. 12: 553–563.

Chapin, F. S. III 1980b. The mineral nutrition of wild plants. Ann. Rev. Ecol. Syst. 11: 233–260.

Chapin, F. S. III 1983. Nitrogen and phosphorus nutrition and nutrition cycling by evergreen and deciduous understorey shrubs in an Alaskan black spruce forest. Can. J. For. Res. 13: 773–781.

Chester, A. L. & McGraw, J. B. 1983. Effects of nitrogen addition on the growth of *Vaccinium uliginosum* and *Vaccinium vitis-idaea.* Can. J. Bot. 61: 2316–2322.

Chester, A. L. & Oechel, W. C. 1986. Effects of leaf nitrogen availability and leaf position on nitrogen allocation patterns in *Vaccinium vitis-idaea* and *Vaccinium uliginosum.* Oecol. (Berl.) 69: 121–125.

Dickinson, N. M., Turner, A. P. & Lepp, N. W. 1991. How do tree and other long-lived plants survive in polluted environments? Funct. Ecol. 5: 5–11.

Eriksson, O. & Fröborg, H. 1996. 'Windows of opportunity' for recruitment in long-lived clonal plants: experimental studies of seedling establishment in *Vaccinium* shrubs. Can. J. Bot. 74: 1369–1374.

Ernst, W. H. O. 1974. Schwermetallvegetation der Erde. Gustav Fischer Verlag, Stuttgart.

Fröborg, H. 1996. Pollination and seed production in five boreal species of *Vaccinium* and *Andromeda* (Ericaceae). Can. J. Bot. 74: 1363–1368.

Gimmingham, C. H. 1972. Ecology of heathlands. Chapman and Hall, London.

Helmisaari, H.-S., Derome, J., Fritze, H., Nieminen, T., Palmgren, K., Salemaa, M. & Vanha-Majamaa, I. 1995. Copper in Scots pine forests around a heavy-metal smelter in south-western Finland. Water, Air Soil Pollut. 85: 1727–1732.

Hjälten, J., Danell, K. & Ericson, L. 1993. Effects of simulated herbivory and intraspecific competition on the compensatory ability of birches. Ecology 74: 1136–1142.

Honkanen, T. & Haukioja, E. 1995. Why does a branch suffer more after branch-wide than after tree-wide defoliation. Oikos 71: 441–450.

Jacquemart, A.-L. 1996. *Vaccinium uliginosum*, L. Biological flora of the British Isles. J. Ecol. 84: 771–785.

Johnson, D. A. & Tieszen, L. L. 1976. Aboveground biomass allocation, leaf growth, and photosynthesis patterns in tundra plant forms in Arctic Alaska. Oecol. (Berl.) 24: 159–173.

Karlsson, P. S. 1985. Patterns of carbon allocation above ground in a deciduous (*Vaccinium uliginosum*) and an evergreen (*Vaccinium vitis-idaea*) dwarf shrub. Physiol. Plant. 63: 1–7.

Karlsson, P. S. 1987. Micro-site performance of evergreen and deciduous dwarf shrubs in a subarctic heath in relation to nitrogen status. Holarctic Ecol. 10: 114–119.

Karlsson, P. S. 1989. In situ photosynthetic performance of four coexisting dwarf shrubs in relation to light in a subarctic woodland. Funct. Ecol. 3: 481–488.

Laaksovirta, K. & Silvola, J. 1975. Effect of air pollution by copper, sulphuric acid and fertilizer factories on plants at Harjavalta, W. Finland. Ann. Bot. Fenn. 12: 81–88.

Levitt, J. 1980. Responses of plants to environmental stresses. Academic Press, New York.

Marshall, C. 1996. Sectoriality and physiological organisation in herbaceous plants: an overview. Vegetatio 127: 9–16.

Marquis, R. J. 1996. Plant architecture, sectoriality and plant tolerance to herbivores. Vegetatio 127: 85–97.

Maschinsky, J. & Whitham, T. G. 1989. The continuum of plant responses to herbivory: the influence of plant association, nutrient availabilty, and timing. Am. Nat. 134: 1–19.

Packer, J. G. & Denford, K. E. 1974. A contribution to the taxonomy of *Arctostaphylos uva-ursi*. Can. J. Bot. 52: 743–753.

Remphrey, W. R., & Steeves, T. A. 1984a. Shoot ontogeny in *Arctostaphylos uva-ursi* (bearberry): the annual cycle of apical activity. Can. J. Bot. 62: 1925–1932.

Remphrey, W. R., & Steeves, T. A. 1984b. Shoot ontogeny in *Arctostaphylos uva-ursi* (bearberry): origin and early development of lateral vegetative and floral buds. Can. J. Bot. 62: 1933–1939.

Remphrey, W. R., Steeves, T. A. & Neal, B. R. 1983. The morphology and growth of *Arctostaphylos uva-ursi* (bearberry): an architectural analysis. Can. J. Bot. 61: 2430–2450.

Salemaa, M. & Vanha-Majamaa, I. 1993. Forest vegetation change along a pollution gradient in SW Finland. Proc. First Finnish Conference of Environmental Sciences. Kuopio Univ. Pub. C. Nat. Env. Sci. 14: 101-104.

SAS Institute, Inc. 1994. SAS/STAT User's Guide Version 6. 4^{th} Edition. SAS Institute Inc., Cary, North Carolina, USA.

Shevtsova, A., Ojala, A., Neuvonen, S., Vieno, M. & Haukioja, E. 1995. Growth and reproduction of dwarf shrubs in a subarctic plant community: annual variation and above-ground interactions with neighbours. J. Ecol. 83: 263–275.

Stewart, W. S. & Bannister, P. 1973. Seasonal changes in carbohydrate content of three *Vaccinium* spp with particular reference to *V. uliginosum* L. and its distribution in the British Isles. Flora Bd. 162: 134–155.

Sutinen, M.-L., Raitio, H., Nivala, V., Ollikainen, R. & Ritari, A. 1996. Effects of emissions from copper-nickel smelters on the frost hardiness of *Pinus sylvestris* needles in the subarctic region. New Phytol. 132(3): 503–512.

Tiffney, W. N., Benson, D. R. & Eveleigh, D. E. 1978. Does *Arctostaphylos uva-ursi* (Bearberry) have nitrogen-fixing nodules? Amer. J. Bot. 65: 625–628.

Tolvanen, A. & Laine, K. 1997. Effect of reproduction and artificial herbivory on vegetative growth and resource levels in deciduous and evergreen dwarf shrubs. Can. J. Bot. 75: 656–666.

Tolvanen, A., Laine, K., Pakonen, T. & Havas, P. 1995. Recovery of evergreen clonal dwarf shrub *Vaccinium vitis-idaea* after simulated microtine herbivory in a boreal forest. Vegetatio 116: 1–5.

Tolvanen, A., Laine, K., Pakonen, T., Saari, E. & Havas, P. 1993. Effect of habitat and time of clipping on the recovery of the bilberry (*Vaccinium myrtillus*). Ann. Bot. Fenn. 30: 15–20.

Tuomi, J., Nilsson, P. & Åström, M. 1994. Plant compensatory responses: bud dormancy as an adaptation to herbivory. Ecology 75: 1429–1436.

Tutin, T. G., Heywood, V. H., Burges, N. A., Moore, D. M., Valentine, D. H., Walters, S. M. & Wegg, D. A. 1972. Flora Europaea. Vol. 3. Cambridge University Press, Cambridge.

Tyler, G., Balsberg Påhlsson, A.-M., Bengtson, G., Bååth, E. & Tranvik, L. 1989. Heavy metal ecology of terrestrial plants, micro organisms and invertebrates. Water, Air, and Soil Poll. 47: 189–215.

Verkleij, J. A. C. & Schat, H. 1989. Mechanisms of metal tolerance in higher plants. Pp. 179–193. In: Shaw, J. A. (ed.), Heavy metal tolerance in plants: evolutionary aspects. CRC Press, Florida.

Väisänen, S. 1986. Effects of air pollution by metal, chemical and fertilizer plants on forest vegetation at Kokkola, W Finland. Ann. Bot. Fenn. 23: 305–315.

Watson, M. A. & Casper, B. B. 1984. Morphogenetic constraints on patterns of carbon distribution in plants. Ann. Rev. Ecol. Syst. 15: 233–258.

Paper V

Salemaa, M. & Sievänen, R. 2002. The effect of apical dominance on the branching architecture of *Arctostaphylos uva-ursi* in four contrasting environments. Flora 197: 429-442.

V

Flora (2002) **197**, 429–442
http://www.urbanfischer.de/journals/flora

FLORA

The effect of apical dominance on the branching architecture of *Arctostaphylos uva-ursi* in four contrasting environments

MAIJA SALEMAA* & RISTO SIEVÄNEN

Vantaa Research Centre, The Finnish Forest Research Institute, P.O. Box 18, FIN-01301 Vantaa, Finland
* e-mail corresponding author: maija.salemaa@metla.fi

Submitted: Mar 28, 2002 · Accepted: Jul 19, 2002

Summary

We studied horizontal spreading and axillary bud activation of colonizing branches of a clonal dwarf shrub, *Arctostaphylos uva-ursi*, in SW Finland in four habitats with varying pollution, nutrient, light and competition levels. *A. uva-ursi* showed high plasticity in the proportion of released buds, which changed according to the stress and resource level of the habitats. Apical dominance of lateral branching was the strongest in the resource-poor habitats (low soil nutrient or low light levels). However, when the apex of the parent shoot was terminated (due to mortality of an apical bud or the formation of an inflorescence) the disruption of apical dominance caused intensive branching in the poor habitats. Apical dominance of the dominant shoots was much weaker in the resource-rich habitats. When nutrient availability and light level were relatively high, branching frequency was high in both intact (expanded) and terminated parent shoots. Shoot mortality and the proportion of terminated shoot apices was the highest in the polluted habitat, as a result of soil toxicity. This caused intensive branching in all shoot hierarchies, which may enhance the plant's survival in heavy metal polluted soil. We conclude that plastic branching enables this species to recover after damage, or to respond to changed resource levels according to the 'reserve meristem hypothesis'. The branching response to different environmental conditions was simulated by means of an L-system architectural model based on the demographic and morphological parameters measured in each habitat.

Key words: bearberry, clonal growth, heavy metals, L-system architectural model, meristem activation, revegetation

Introduction

Modular growth enables clonal plants to respond to environmental variation by modifying their shape (e.g. HUTCHINGS & DE KROON 1994; DE KROON & HUTCHINGS 1995). Several studies have described changes in the internodal length of the stems, lateral branching intensity and branching angle of clonal plants in response to environmental conditions (SLADE & HUTCHINGS 1987; CALLAGHAN et al. 1990; DE KROON & KNOPS 1990; DONG et al. 1997; MOEN et al. 1999). It has been demonstrated that simple computer algorithms can generate large differences in clonal morphology (BELL et al. 1979; BELL & TOMLINSON 1980; SUTHERLAND & STILLMAN 1990).

The role of apical dominance is crucial in determining the form of many plant species (HUTCHINGS & MOGIE 1990). The control exerted by the shoot apex over the outgrowth of lateral buds causes directional growth of elongated primary shoots. Death or removal of the shoot apices as a result of damage disrupts apical dominance and enables regrowth through lateral branching (CLINE 1991, 1997). A number of hypotheses on the selective advantages of apical dominance have been proposed, e.g. competition for light, reduction in within-plant competition (AARSSEN 1995; IRWIN & AARSSEN 1996), keeping dormant meristems in reserve as a strategy against possible damage (TUOMI et al. 1994; LORTIE & AARSSEN 2000), and the function of dominant apices as a metabolic sink under nutrient and water stress (LORTIE & AARSSEN 1997). The strength of apical dominance varies between species and between different environmental conditions (HUTCHINGS & DE KROON 1994; BONSER & AARSSEN 1996). The growth of the plant may be limited by the number and activity of meristems, which affect its ability to respond to damage or changes in resource availability (HARPER 1977; SALOMONSON et al. 1994; TOLVANEN & LAINE 1997).

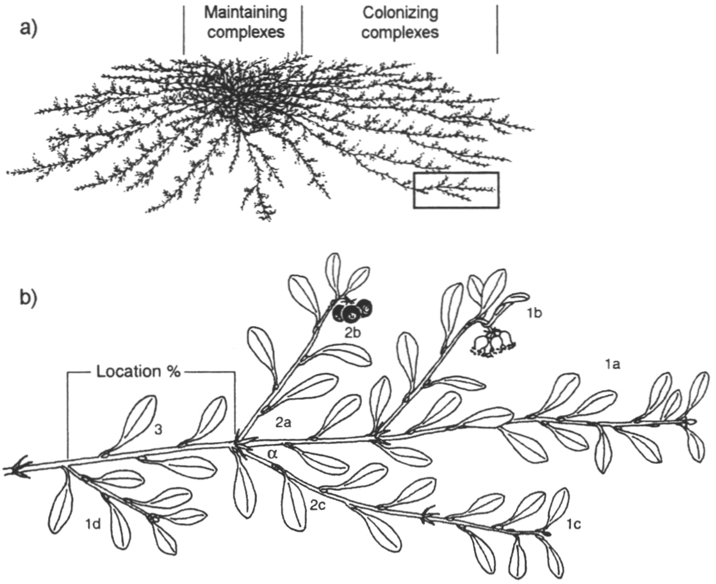


Fig. 1. Morphological structure of a) a compact clone, and b) a colonizing branch of *A. uva-ursi*, with three age classes of shoots (yearly grown segments). Current-year shoots: 1 a) monopodial dominant (D) shoot with an intact apical bud, 1 b) sympodial subdominant (SD) shoot with an inflorescence, 1 c) monopodial SD shoot with a dead apical bud, 1 d) sympodial nondominant (ND) shoot with an intact apical bud. Previous-year shoots: 2 a) monopodial expanded D shoot, 2 b) sympodial SD shoot with berries, 2 c) sympodial expanded SD shoot. 3rd year shoots: 3 monopodial expanded D shoot. Shoot hierarchy levels: D dominant, SD subdominant and ND nondominant. Location %: the distance between the attachment point of the lateral shoot from the apex of the parent shoot / the total length of the parent shoot. Branching angle (α) between the lateral and parent shoots is marked.

Bonser & Aarssen (1996) presented general predictions of the optimal pattern of axillary meristem allocation of herbaceous plants (here only polycarpic perennials) along light (L) and nutrient (N) gradients. They propose that

1) Where soil nutrients are abundant, but light is limiting due to crowding from neighbours (N+L-), keeping axillary meristems inactive (strong apical dominance) is favoured in order to enable vertical growth/extended horizontal axes.
2) Where both nutrients and light are abundant (N+L+), releasing axillary meristems (weak apical dominance) is favoured in order to maximize resource utilization through intensive branching.
3) Where nutrients are limiting, but light is abundant (N-L+), keeping axillary meristems in an inactive state (strong apical dominance) is favoured in order to extend access to richer nutrient patches in the soil or to hold inactive meristems in reserve, from which growth can be increased if a nutrient rich patch is encountered or if apical meristems are destroyed (Aarssen 1995).

In this paper we test the above predictions using a clonal evergreen dwarf shrub *Arctostaphylos uva-ursi* L. (bear berry) as the study plant (Fig. 1). This species is suitable for studying the state of meristems (i.e. keeping them inactive, or activating them to form vegetative or reproductive shoots) because of existing detailed analyses of its architecture (Remphrey et al. 1983a, b; Remphrey & Steeves 1984 a, b), and because it is relatively easy to determine the terminal status of the shoots and to identify the axillary buds on leaf axils.

We address the hypotheses by considering the prostrate growth habit of *A. uva-ursi* in four habitats with varying pollution, nutrient, light and competition levels. We designed a model for the branching architecture using the results of measurements. The purpose of the model is to summarize the empirical results and to study qualitative features of the branching patterns by simulations.

Material and methods

The study plant

The xeromorphic evergreen dwarf shrub, *Arctostaphylos uva-ursi* (L.) Spreng., grows circumpolarly in boreal and temperate forests and in dry subalpine sites above the forest limit (Schütt & Lang 2000). It colonises open sandy heathlands and moraine slopes, eroded banks, sand dunes and a variety of other habitats in the boreal forest (Remphrey et al. 1983a; Salemaa et al. 1999). It is a pioneer of light, dry and nutrient-poor habitats, but occurs also in the understorey of dry Scots pine forests in its Nordic range. It is one of the few understorey species which survives in polluted soil close to non-ferrous smelters (Väisänen 1986; Lukina et al. 1993; Salemaa et al. 2001).

The clones growing in open sites form compact patches, with a deep tap root (50–100 cm) in the centre and prostrate branches (stems) spreading radially around the plant (Fig. 1a). The stems may produce superficial adventitious roots as they get older. *A. uva-ursi* regenerates rather poorly from seed and spreads mainly vegetatively.

In this study we follow the terminology presented by Remphrey et al. (1983a, b) on the morphological units of *A. uva-ursi*. The outer zone of the patch consists of branches with extended shoots and a linear growth pattern (colonizing complexes), and the inner zone shorter branches with more upright orientation (maintaining complexes) (Fig. 1a). The growth segments formed during one year (later on called shoots) are divided into three hierarchy types: dominant (D) shoots are long and low, whereas subdominant (SD) and non-dominant (ND) shoots are shorter and grow with higher elevation angles (Fig. 1b). The differences between the lengths of D, SD and ND shoots were statistically significant ($p < 0.05$) in most cases in the study material (Fig. 2a).

Each leaf axil has a single meristem (axillary bud), which is initially dormant but may be released in subsequent years. Shoot apices are terminated either by vegetative buds or by inflorescences. Intact apical buds of parent shoots expand monopodially into daughter shoots in the next growing season or remain dormant. If apical buds die or produce inflorescences, branching continues sympodially from axillary buds during the next year (Fig. 1b). Thus, in contrast to the meristem allocation model of Bonser & Aarssen (1996), reproductive shoots of *A. uva-ursi* have the capacity to continue lateral growth after flowering. Terminal inflorescences overwinter and berries develop during the following year. The shoots do not grow during the year that they produce berries.

Study sites

The study area is a dry, infertile Scots pine (*Pinus sylvetris* L.) forest of the southern boreal coniferous zone, located close to the Harjavalta Cu-Ni smelter (61°19′N, 22°09′E) in SW Finland. The soil is sorted fine sand and the soil type ferric podzol (Mälkönen et al. 1999). The forest ecosystem near the smelter has been drastically changed by heavy-metal and

Table 1. Site characteristics and element concentrations in the growing substrate in four habitats of *A. uva-ursi*. Plant available (exchangeable) element concentrations in the mineral soil layer (at 0–10 cm depth) for the polluted (data 0.5 km S from the smelter in the year 1999), sand pit and forest habitats (year 2001) and in the mulch (year 1998) added to the restoration habitat. All element concentration in sand were determined with $BaCl_2$ + EDTA. Concentrations in the mulch were determined with $BaCl_2$ and calculated per mass organic matter. The relative competition level for light and belowground resources was subjectively estimated according to the overall density of the ground vegetation.

Habitat	Polluted	Restoration	Sand pit	Forest
Distance from smelter, km	0.5	0.5	6	8
Direction from smelter	W	S	SE	SE
Shading of overstorey	none	open canopy	none	dense canopy
Vegetation coverage, %	<1	<10	<10	100
Substrate	sand	mulch	sand	sand
Element conc.				
K, mg/kg	12.6	375.6	25.5	29.5
Mg, mg/kg	2.8	649.0	4.5	10.6
Ca, mg/kg	20.8	7888.0	62.8	73.0
P, mg/kg	3.8	87.6	4.4	7.4
Cu, mg/kg	84.2	190.7	17.7	8.3
Ni, mg/kg	11.3	11.8	2.6	2.3
Pollution level	+	+	–	–
Nutrient level	–	++	+	+
Light level	+	±	+	–
Competition level	–	–	–	+

sulphur emissions during the past 50 years (Helmisaari et al. 1995; Fritze et al. 1997; Derome & Lindroos 1998; Derome & Nieminen 1998; Salemaa et al. 2001). During 1985–1990, the average annual emissions of Cu were 104 t, Ni 50 t and SO_2 8100 t. By 1999, the corresponding values had decreased to Cu 6 t, Ni 1 t and SO_2 3400 t (data from Outokumpu Harjavalta Metals smelter). Since 1995 the smelter has been emitting considerable amounts of gaseous NH_3, which has been reflected as elevated pH values in precipitation and increased nitrogen deposition (Derome 2000). The long-term accumulation of Cu and Ni in the soil close to the smelter has resulted in a severe deficit of plant available Ca, Mg and K in the organic layer (Derome 2000).

The understorey vegetation is almost totally absent up to a distance of 0.5 km. Visible toxic effects of heavy metals on the vegetation extend up to a distance of 3–4 km (Salemaa et al. 2001). The distances of 6 and 8 km represent "clean" sites in this study, although even at these distances the heavy metal concentrations in the organic layer exceed background levels (Derome 2000). The general characteristics, element concentrations in the upper soil layers and the relative light (L), nutrient (N), pollution and competition levels of the four habitats are given in Table 1.

The polluted habitat (N–L+) is a severely disturbed Scots pine stand at a distance of 0.5 km to the W of the smelter. The forest floor is almost completely devoid of vegetation and covered with undecomposed needle litter. Only a few patches of *A. uva-ursi*, *Empetrum nigrum* L. and resistant pioneer mosses survive there. The clones of *A. uva-ursi* may be relicts and date back to the time before the smelter was founded in the 1940's. The soil is very dry and the nutrient status poor, and the toxic effects of heavy metals restrict nutrient uptake by the roots. Light availability is high in this open, damaged stand. Interspecific competition is insignificant because the site is almost empty of species.

The restoration habitat (N+L±) is a revegetation experiment in an open Scots pine stand 0.5 km to the S of the smelter. The experiment was established in spring 1996 by spreading a mulch consisting of compost and wood chips onto the polluted forest floor (Kiikkilä et al. 2001). *A. uva-ursi* was one of the species that were planted on one-species, 5 × 5 m experimental quadrats in attempts to revegetate and stabilise the polluted soil. The rooted cuttings of *A. uva-ursi* were of local Harjavalta origin (taken in April 1996), and had one shoot (formed in 1995) when planted. The light level is relatively high because the stand is seriously defoliated. The nutrient-rich mulch pockets (see Table 1 for chemical data from 1998) ensure high nutrient availability to the roots and protect them against heavy metals in the soil. The understorey is not closed, indicating a low competition level between the plants.

The sand pit habitat (N+L+) 6 km to the SE of the smelter represents one of the most favourable sites for *A. uva-ursi*. At this site the light, nutrient and moisture conditions are all sufficiently high to allow maximum biomass production. The clones have regenerated naturally decades ago along the open edges of the abandoned sand pit. The pioneer mosses and lichens and Scots pine seedlings have low competitive effects on *A. uva-ursi*.

The forest habitat (N+L–) 8 km to the SE of the smelter is relatively fertile and half-shaded by the overstorey Scots pines. The naturally regenerated *A. uva-ursi* clones may be decades old. The *A. uva-ursi* clones are intermixed within the closed understorey (mosses, *Cladina* lichens and other dwarf shrubs e.g. *Vaccinium vitis-idaea* L., *Calluna vulgaris* (L.) Hull). Between-species competition for light and space is probably high.

Branch sampling

We randomly selected five separate clones of *A. uva-ursi* in the polluted, sand pit and forest habitats in September, 2000. Altogether 1–3 branches with the six youngest shoots (formed

Table 2. Number of studied clones, branches and shoots in different habitats. n_1 = number of all shoots (age classes 1996–2000) (Fig. 2a, b), n_2 = number of lateral shoots (1996–2000) (Fig. 2c, d), n_3 = number of shoots with intact apex (1996–1999) (Fig. 3) and n_4 = number of shoots with terminated apex (1996–1999) (Fig. 3). Shoot hierarchy types: D = dominant, SD = subdominant and ND = nondominant.

Habitat	Clones	Branches	Type	n_1	n_2	n_3	n_4
Polluted	5	13	D	101	37	55	28
			SD	104	52	45	21
			ND	125	68	7	14
Restoration	10	10	D	120	34	77	14
			SD	338	151	166	30
			ND	483	126	135	24
Sand pit	5	5	D	42	11	27	6
			SD	100	52	48	14
			ND	232	140	89	25
Forest	5	9	D	78	27	41	17
			SD	108	49	53	15
			ND	62	35	20	12

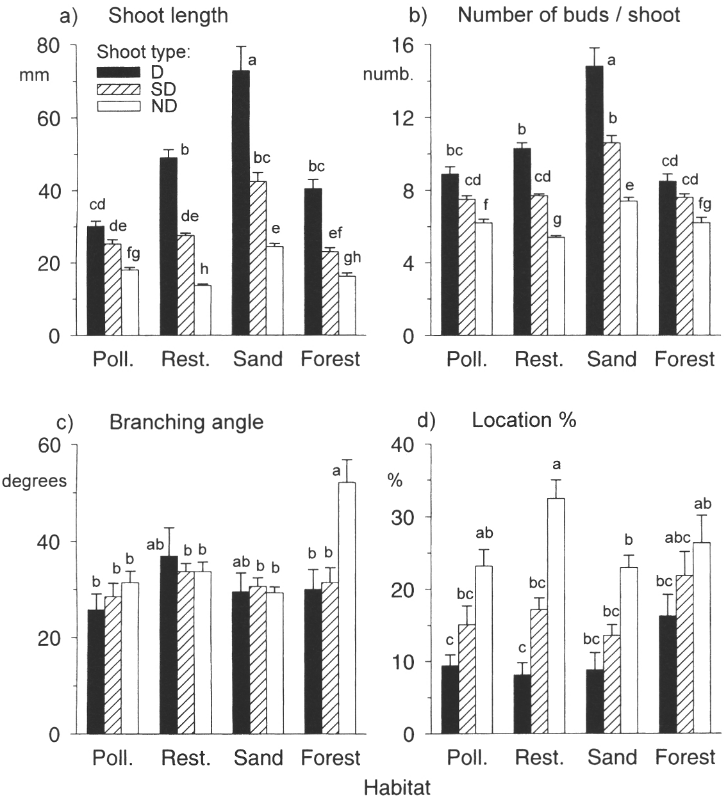


Fig. 2. Shoot-specific means (± se) of morphological variables measured on living shoots in the 1996–2000 age classes in four habitats (Poll. = Polluted, Rest. = Restoration, Sand = Sand pit and Forest = Forest habitats). Pairwise comparisons between the means were performed by Tukey's tests. The same letter indicates that there are no significant differences ($p > 0.05$) between the means. Shoot hierarchy types: D = dominant, SD = subdominant and ND = nondominant. The number of clones, branches and shoots given in Table 2 (n_1 = number of all shoots and n_2 = number of lateral shoots).

during 1995–2000) were taken from the colonizing zone of each clone. In the restoration habitat we removed ten plants, each comprising six shoots (1995–2000) together with the roots. Thus all the sample branches had one parent shoot, formed in 1995, from which all the daughter shoots had developed. In the sand pit habitat, two branches had the parent shoot from 1996. Detailed morphological analysis of the branches was carried out in the laboratory. This included measuring the length, location of attachment and branching angle of the shoots, counting the number of activated and inactive buds, and determining the age, hierarchy and terminal types of the shoots. The total number of branches and shoots analysed is given in Table 2.

Statistical analyses

We calculated shoot-specific mean values for the morpholocial variables from the data representing the age classes 1996–2000 (1996–1999 for lateral branching because current-year shoots do not branch). The pairwise differences in the mean values over the habitats and shoot hierarchy types were tested by Tukey's tests. The effect of the habitat, terminal type (intact or terminated apex) and age of the shoots, and their interaction in the number and proportion of released buds on D shoots, were tested using three-factor ANOVA. The differences between the intact and terminated parent shoots were compared by two-sample t tests and between the habitats within the same shoot hierarchy type by Tukey's tests. The time delay in the production of released buds was studied by means of linear regression models. All the tests were carried out using SAS software (SAS Inc. 2000).

The frequency of dead and living shoots and terminal types were calculated separately for the current-year shoots (2000) and for the combined data of the older (overwintered) shoots (1996–1999). The mortality rates and frequencies of the terminal types were tested pairwise between the habitats using two-by-two contingency tables and between intact or terminated parent shoots using chi-square tests (STATISTIX 7, Analytical Software 2000).

Model for the morphological development of *A. uva-ursi*

We constructed a morphological model in order to summarize the experimental results and to gain an understanding of the processes affecting the branching pattern of *A. uva-ursi*. We applied the modelling approach of REMPHREY et al. (1983b). Our model is based on the use of an annual time step and a set of rules, derived from measurements, to control the production of new shoots. The plant is a collection of D, SD, and ND shoots. The shoot may be intact or terminated. In a growth cycle of one year a parent shoot produces a number of daughter shoots. We used the field measurements from the four habitats to parameterize the model. It is presented in detail in Appendix.

We evaluated the validity of the model by comparing the rate of increase (r) in the number of living shoots during the first five years, calculated from the mean of ten simulations with the parameters measured in the field. We linearized the exponential growth equation ($N_t = N_0e^{rt}$, in which N_0 = initial number of shoots, t = time in years, r = rate of increase) as $\ln N_t = \ln N_0 + rt$. Differences in the values of r parameters (regression slopes) between the observed and model-simulated data were tested by t-tests (STATISTIX 7, Analytical Software 2000).

Results

Basic morphology

The length of the D shoots of *A. uva-ursi* followed the order: sand pit > restoration > forest > polluted (Fig. 2a). The SD and ND shoots were also the longest in the sand pit, but there were no differences between the other habitats. D shoots were clearly longer than SD and ND shoots within the habitats, except in the polluted one where the length of D and SD shoots did not differ significantly (Fig. 2a). There were no statistical differences in the lengths of intact or terminated D shoots. The total number of axillary buds (Fig. 2b) correlated positively with the shoot length ($r = 0.774$, $p < 0.0001$, $n = 371$ in the combined data of D shoots for all sites).

The branching angles of D, SD and ND shoots were relatively similar, varying between 26–36 degrees in all the habitats, except for the ND shoots in the forest which had a wider angle (52°) and upright orientation (Fig. 2c). The relative location of attachment of most of the lateral shoots was near to the apex of the parent shoot (Fig. 2d). However, the lower the shoot hierarchy, the further away from the shoot apex they grew.

The number of shoots with green leaves was the highest in the restoration habitat where the four youngest year classes (1997–2000) had leaves. The other sites followed the order: polluted (3 green year classes) > sand pit (2.5) > forest (2). The length of the main roots was about 30 cm in the plants excavated from the restoration experiment, but no adventitious roots had yet developed. In the other habitats the branches had occasional adventitious roots along the branches in the 1995–1999 year classes. A number of second flushes (sensu REMPHREY & STEEVES 1984a) produced by the current-year shoots were found in the restoration habitat.

Lateral branching

The most important factors that affected lateral branching of the D shoots were the habitat, terminal type and age of the parent shoots (Table 3). The effects of the last two factors varied according to the resource level of the habitat (significant interaction terms in ANOVA). The overall pattern of lateral branching was similar, irrespective of whether it was calculated on the basis of the number or the proportion of released buds.

In general, apical dominance of the intact D shoots on lateral branching was stronger in the resource-poor (polluted and forest) than in the resource-rich (restoration and sand pit) habitats (Fig. 3a). The proportion of released buds was significantly lower ($p < 0.001$) on intact than on terminated D shoots in the polluted and forest habitats (Fig. 3a). However, when the apex of the parent shoot had been terminated, the breakage of apical dominance caused intensive branching in the poor habitats. Apical dominance of D shoots was much weaker in the resource-rich habitats. This was expressed in the similar

Table 3. The effect of the habitat, terminal type (intact or terminated) and age of the dominant shoots on the number of released buds and the proportion of released buds. F and p values for three-factor ANOVA. Only significant interactions given. df = degree of freedom. Data from years 1996–1999.

Source of variation	df	Number of released buds		Proportion of released buds	
		F	p	F	p
Habitat	3	9.68	<0.0001	4.93	0.0024
Terminal type	1	17.90	<0.0001	28.16	<0.0001
Age	3	5.89	0.0007	8.13	<0.0001
H×T	3	4.34	0.0053	11.73	<0.0001
H×A	9	3.87	0.0001	3.59	0.0003
T×A	3	–	–	2.77	0.0422
Error	234				

($p > 0.05$), high proportion of released buds in both intact and terminated D shoots in the restoration and sand pit habitats (Fig. 3a). The proportion of released buds of intact D shoots was higher ($p < 0.05$) in the resource-rich habitats than in the resource-poor habitats (Fig. 3a). In contrast, the proportion of released buds on terminated D shoots was relatively similar between the habitats.

Intact SD and ND shoots had a significantly lower ($p < 0.05$) proportion of released buds than terminated ones in all the habitats (Fig. 3b, c). There were no great between-habitat differences in lateral branching of SD and ND shoots, although the proportion of released buds in terminated ND shoots tended to be higher in the polluted (19%) than in the other habitats (9–15%). In general, the proportion of released buds tended to decrease according to shoot hierarchy type in the order D > SD > ND.

The proportion of released buds increased with the age of the intact parent D shoots (significant positive regression slopes in the restoration, sand pit and forest habitats) (Fig. 4). The age trend was not as clear in the terminated D shoots, although the proportion of released buds increased from 1- to 3-year-old shoots in the other habitats except for the polluted one. The decrease in the branching in the 4-year-old shoots may be an artefact caused by the small amount of data and the problems in identifying points at which dead branches have disappeared.

Shoot mortality and terminal types

The mortality rate of the current-year shoots (2000) was lower than that of the older over-wintered age classes (1996–1999) (Table 4a, b). Most of the dead shoots represented the ND hierarchy type. The mortality rate of the older ND shoots was higher in the resource-poor (57% in the polluted and 30% in the forest habitats) than in the resource-rich habitats (7% in both the restoration and sand pit habitats) (Pearson's $X^2 = 70.76$, $p < 0.001$, $n = 390$) (Table 4b).

The current-year shoots terminating in the inflorescences were the most abundant in the polluted and sand

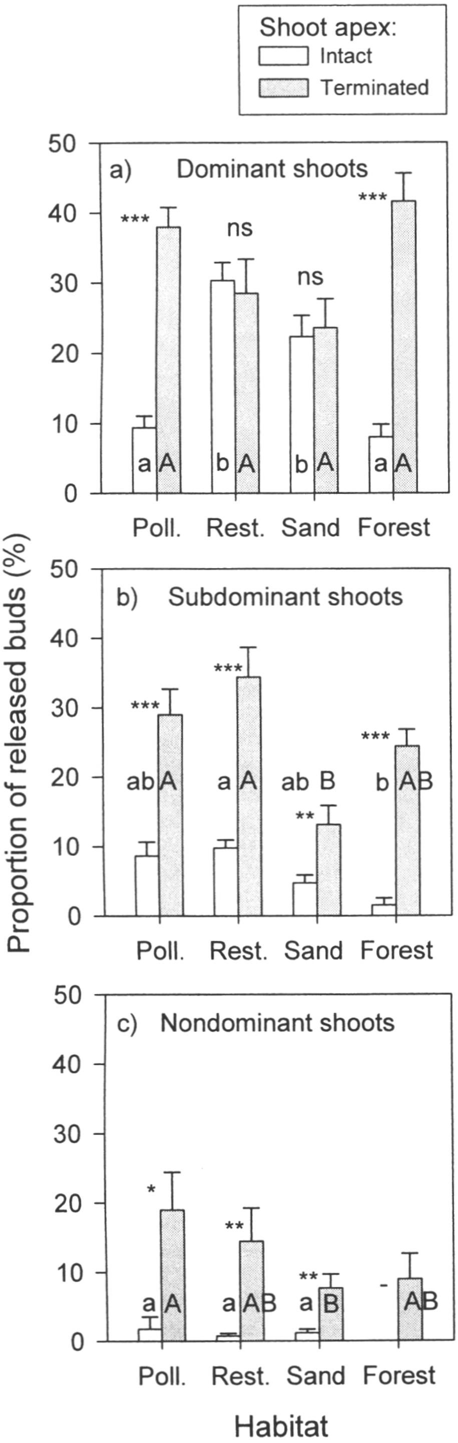


Fig. 3. The mean proportion (%) (± se) of released buds in a) dominant, b) subdominant and c) nondominant shoots. The intact (white bar) and terminated (hatched bar) shoots compared by two-sample t-tests (significancies $p < 0.001$ = ***, $p < 0.01$ = **, $p < 0.05$ = *, ns = not significant). The statistical differences (Tukey's tests) between the habitats within the same terminal type marked with letters: the same letter indicates that there are no significant differences ($p > 0.05$) between the means. The number of shoots in each shoot category in Table 2 (n_3 = number of intact shoots and n_4 = number of terminated shoots, 1996–1999). Abbreviations as in Fig. 2.

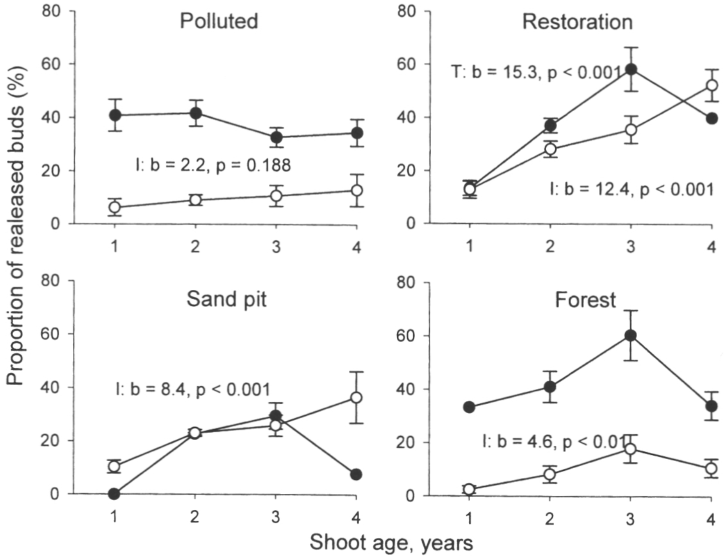


Fig. 4. The proportion (%) of released buds (x̄ ± se) on the intact (I, white circles) and terminated (T, black circles) parent D shoots as a function of the shoot age (data from the years 1996–1999). The significant slopes (b) of linear regressions are given.

pit habitats where the light level was high (Table 4a). However, the frequency of shoots bearing berries was much lower than that assumed according to the number of inflorescences. The frequency of dead apices in the older D shoots was higher in the resource-poor habitats (polluted and forest combined 32%) than in the resource-rich habitats (restoration and sand pit combined 16%) ($\chi^2 = 8.88$, $p < 0.0029$, $n = 265$) (Table 4b). The occurrence of shoot apices with dormant buds was considerable only in the polluted habitat.

Hierarchy types of the daughter shoots

The terminal daughter shoot that expanded from the apical bud mainly represented the same hierarchy type as the parent shoot. The type of lateral daughter shoot was never higher than that of the parent. Thus D parents produced all types of hierachy (D, SD and ND), but ND parents only ND daughters. When the apex of the parent shoot had been terminated, the proportion of lateral shoots with a higher hierarchy increased (Fig. 5). The increase was the most significant in the polluted (D shoots: $\chi^2 = 10.8$, $p = 0.004$, $df = 2$) and the restoration (D shoots: $\chi^2 = 13.2$, $p = 0.002$, $df = 2$) habitats.

Simulations of the branching pattern

Simulations produced a variety of branching patterns to the colonizing complexes of *A. uva-ursi* depending on the pollution and resource levels of the habitat (Fig. 6). The between-habitat differences in the size, shape and lateral spreading of the branches were clearly expressed already during the five-year growth cycle. When the frequency of terminated D shoots and shoot mortality rate were decreased in the polluted habitat in order to predict the possible effect of decreasing emissions, allocation to lateral branching also decreased (Fig. 6a, b).

Both the observed and simulated number of living shoots increased exponentially over time in all the habitats. The observed / simulated values of the rate of increase (r) were: polluted 0.62 / 0.65 ($t = 1.19$, $df = 1, 116$, $p = 0.277$), restoration 0.89 / 0.91 ($t = 0.56$, $df = 1, 96$, $p = 0.458$), sand pit 0.91 / 0.89 ($t = 0.10$, $df = 1, 71$, $p = 0.748$), and forest 0.63 / 0.63 ($t = 0.01$, $df = 1, 91$, $p = 0.927$). The close correspondence between the simulated and observed r values showed that the model is able to produce the basic features of branching dynamics during the first five years.

Discussion

The general predictions of optimal meristem allocation presented by Bonser & Aarssen (1996) were valid for the branching pattern of D shoots of *A. uva-ursi*. Relatively high allocation in lateral branching (weak apical control) in order to maximize growth was found when light and nutrients were abundant. In contrast, a relatively high proportion of meristems were kept inactive (strong apical control) under light or nutrient limitation. However, the latter pattern was expressed only in the

Table 4. The number of living and dead shoots, the frequency (%) of dead shoots and the frequency (%) of terminal types of living shoots in a) current-year shoots (2000) and b) the combined data of the over-wintered shoot age classes 1996–1999.

a)

					Apices of living shoots, %			
Habitat	Type	Living	Dead	Death %	Intact	Dead	Dormant	Flower
Polluted	D	18	0	0.0	83.3	5.6	0.0	11.1
	SD	38	0	0.0	60.5	5.3	0.0	34.2
	ND	104	5	4.6	76.9	5.8	0.0	17.3
Restoration	D	2	1	3.3	89.7	10.3	0.0	0.0
	SD	14	0	0.0	93.0	6.3	0.0	0.7
	ND	324	6	1.8	94.4	5.6	0.0	0.0
Sand pit	D	9	0	0.0	100.0	0.0	0.0	0.0
	SD	38	0	0.0	68.4	5.3	0.0	26.3
	ND	118	0	0.0	78.8	2.5	0.0	18.6
Forest	D	20	0	0.0	95.0	0.0	0.0	5.0
	SD	40	0	0.0	100.0	0.0	0.0	0.0
	ND	30	0	0.0	100.0	0.0	0.0	0.0

b)

					Apices of living shoots, %			
Habitat	Type	Living	Dead	Death%	Intact	Dead	Dormant	Berry
Polluted	D	83	3	3.5	66.3	32.5	0.0	1.2
	SD	66	5	7.0	68.2	30.3	1.5	0.0
	ND	21	28	57.1	33.3	42.9	23.8	0.0
Restoration	D	91	0	0.0	84.6	15.4	0.0	0.0
	SD	196	1	0.5	84.7	15.3	0.0	0.0
	ND	159	13	7.6	84.9	13.2	1.9	0.0
Sand pit	D	33	0	0.0	81.8	18.2	0.0	0.0
	SD	62	0	0.0	77.4	17.7	0.0	4.8
	ND	114	9	7.3	78.1	18.4	1.8	1.8
Forest	D	58	0	0.0	70.7	29.3	0.0	0.0
	SD	68	6	8.1	77.9	22.1	0.0	0.0
	ND	32	14	30.4	62.5	37.5	0.0	0.0

intact shoots of plants growing in resource-poor sites. The predictions were also confirmed by the simulations (Fig. 6).

The frequency of dead shoot apices in the polluted habitat was high owing to the direct and indirect effects of air pollution (Fig. 6a). This caused abundant lateral branching in all shoot hierarchy types. The number of lateral shoots per unit length was high because the parent shoots were short, increasing the "branchy" appearance of the clones at the polluted site. However, high annual mortality decreased the shoot density. Rapid shoot turnover may serve as a resistance mechanism, which enables the plant to exclude heavy metals from the living biomass and to recover from biomass loss (SALEMAA et al. 1999). Inactive buds were kept in reserve on the intact shoots. We predict that the frequency of terminated shoots and shoot mortality will decrease in the future as a consequence of decreasing pollution emissions. However, nutrient levels in the soil will still remain low and the light level high. Although the overall increase in the vigour of the plants is expected to weaken apical dominance, the deficiency of nutrients affects oppositely. In this situation the simulations generated a plant architecture with linearly extended branches (Fig. 6b).

The rate of increase of the living shoots was the highest in the restoration experiment. Addition of the mulch has increased plant available nutrients and decreased heavy metal concentrations (KIIKKILÄ et al. 2001). The plants responded to the nutrient addition (from the

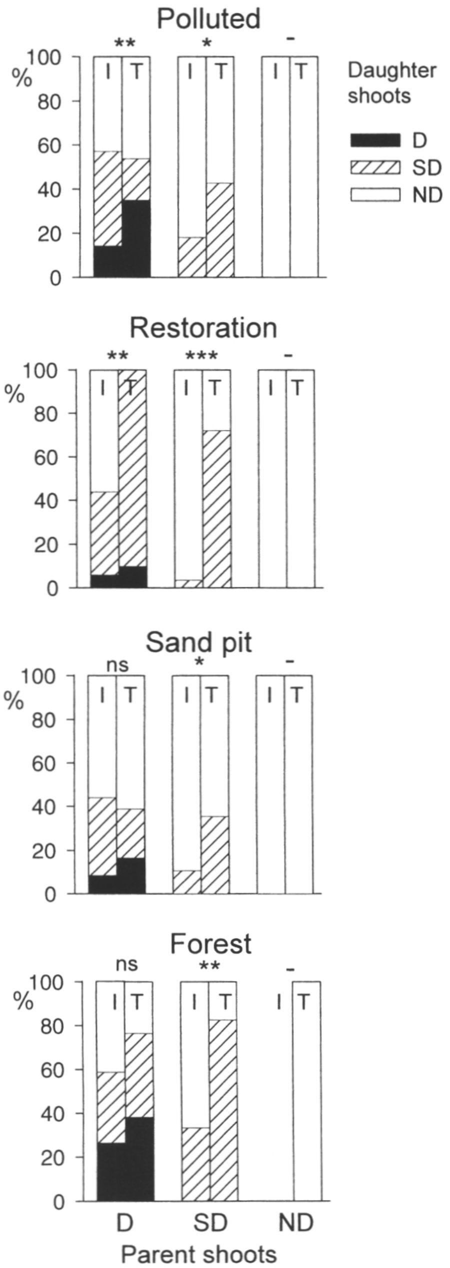


Fig. 5. The proportion (%) of different hierarchy types of the lateral daughter shoots produced by intact (I) and terminated (T) parent shoots. D = dominant, SD = subdominant and ND = nondominant shoot. Significant differencies between I and T parent shoots (χ^2 test): $p < 0.001$ = ***, $p < 0.01$ = **, $p < 0.05$ = *, ns = not significant). df = 2 for D and I for SD parent shoots. Data from the 1996–2000 age classes.

mulch) and improvement in the moisture conditions by vigorous growth and lateral branching (Fig. 6c). The number of shoot age classes with green leaves was also higher than that at the other sites. The plants had main roots and this may improve their ability to take up nutrients and water compared to the sample branches that had only adventitious roots. The abundant branching will most probably decrease in the future when the added nutrients have been consumed and the roots grow into the toxic soil. The signs of toxic effects were visible already in summer 2000 as an increase in the mortality of current-year shoots (Table 4a).

The simulations generated tall branches with long colonizing axes in the sandpit habitat (Fig. 6d). Lateral branching was almost as high as that in the restoration experiment although the nutrient level was much lower than in the mulch. The relatively high frequency of terminated shoots was caused by the production of reproductive shoots with inflorescences (Table 4). No rapid changes in the growing conditions are to be expected in the near future, but within-clone crowding will decrease the probability of branching. Shoot mortality may increase unpredictably as a result of winter damage, drought, burying by sand, insect herbivory or pathogens.

Apical dominance was the strongest in the intact shoots in the forest habitat. The simulations produced long, extended axes with low branching intensity of lateral branches (Fig. 6e). The general appearance of the branches resembled those predicted for the polluted habitat after decreasing emissions (6b). Although the pollution level was relatively low, the mortality rate of the shoots was surprisingly high (Table 4). This may be caused by shading from neighbouring plants, winter damage, drought, insects or pathogens. The carbohydrate and nutrient reserves may also be low in the shaded habitat, which would limit compensatory growth after damage (SALEMAA et al. 1999). Only two youngest age classes of shoots had green leaves in the forest habitat. The leaves of evergreen dwarf shrubs are important storage sites (CHAPIN 1983).

A. uva-ursi showed high plasticity in the apical dominance of lateral branching in different environments. This kind of opportunistic branching behaviour increases the plant's fitness in environments where unpredictable damage is frequent and the resources are heterogeneously distributed. Strong apical dominance maintains a reserve of lateral buds that can be used to continue growth after damage ('reserve meristem hypothesis', TUOMI et al. 1994; AARSSEN 1995) or after periods with low resource levels (JONSDOTTIR & CALLAGHAN 1988; HUTCHINGS & DE KROON 1994). The reserve meristem strategy partly explains why *A. uva-ursi* generally survives in severely disturbed sites such as the polluted one in this study. On the other hand, strong apical dominance leads to horizontally extended branches that can explore

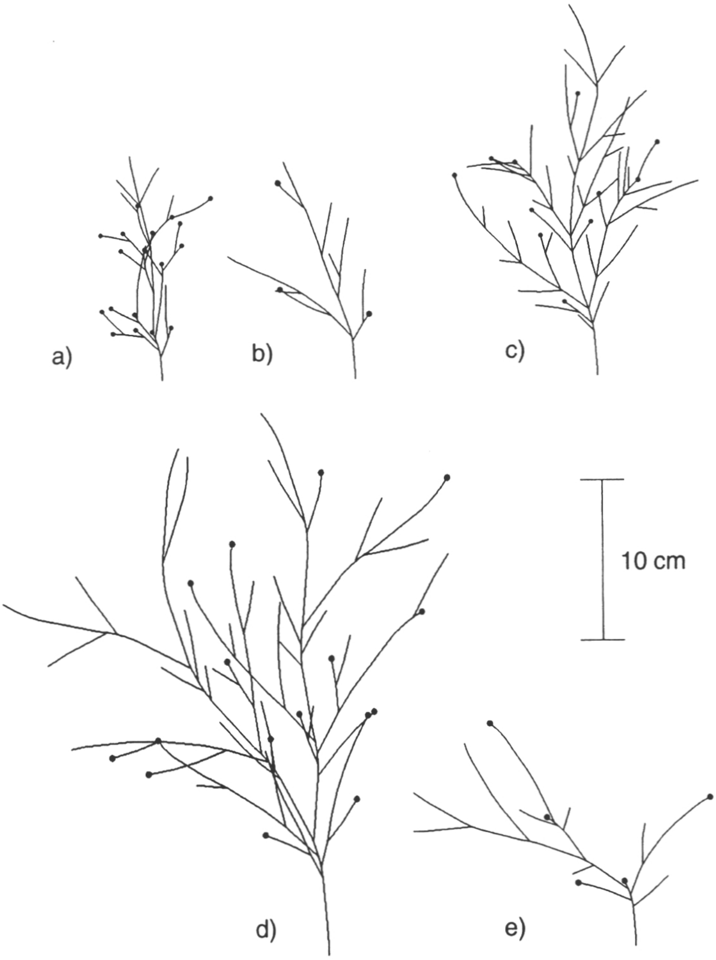


Fig. 6. Examples of the simulated branching patterns of *A. uva-ursi* during the five-year growth cycle on different habitats: a) polluted (N–L+), b) polluted when shoot mortality and the frequency of terminated D shoots were changed to the same level as in the restoration habitat, c) restoration (N++L±), d) sand pit (N+L+), and e) forest (N+L–). N = relative nutrient and L = relative light level. Terminated apices marked with black dots.

for better resource patches or escape from crowded or toxic patches. In addition, strong apical dominance regulates the within-clone density of shoot and minimizes within-clone competition (AARSSEN 1995). Weak apical dominance of the intact D shoots of *A. uva-ursi* was observed when resource availability was high. Intensive branching enables maximum growth and the exploitation of abundant resources.

The model for architectural growth was based on the earlier model by REMPHREY et al. (1983b). There were some differences in the implementation of the models. We concentrated on the type of shoots that a bud can produce, whereas they focused on the shoots that can grow out of a shoot. We also investigated the time delay in the release of lateral buds. Our model was constructed using L system language on a computer, while they performed the simulations manually.

The branching response was successfully simulated by means of the architectural model. It produced similar estimates for the living shoot number as those measured in the field. However, the validification of the model was not based on the independent measurements, and thus requires further research with a new data set. By means of the simulations, it was possible to study the effect of stochastic events on the spreading of *A. uva-ursi* under varying ecological conditions. For instance, changing the probabilities for shoot apex death demonstrated how the disruption of apical dominance increased branching intensity, even though the resource level of the habitat had an opposite effect (Figs. 6a, b).

In conclusion, we interpret the plasticity in the clonal morphology of *A. uva-ursi* as an expression of foraging behaviour that enables the clone to colonize favourable microhabitats and to spread the risk of shoot mortality.

Our results support the observations of earlier studies concerning the regeneration of *A. uva-ursi*, which have emphasised its good regrowth ability after damage (Tiffney et al. 1978; Bowles 1983; Remphrey & Steeves 1984a; Del Barrio et al. 1999; Salemaa et al. 1999). In this respect it is a very suitable species e.g. for the revegetation of disturbed areas.

Appendix

Model for architectural growth of *A. uva-ursi*

The basic structure of the model is based on the earlier morphological model by Remphrey et al. (1983b). We present the main features of the model in the following sections.

The plant grows horizontally, and consists of shoots (i.e. annual growth units) that we divided into dominant (D), subdominant (SD) and nondominant (N) hierarchy types as explained in „Material and methods". The shoots consist of axillary buds and one apical bud, and the internodes between the buds (Fig. A1a). The buds are of the D, SD or ND type, and the type of apical bud determines the type of shoot. In addition to the hierarchy type, a shoot can be either intact or terminated according to the status of the apical bud (living or dead). All axillary buds are living, i.e. we ignore dead axillary buds because they do not contribute to the growth. A living bud produces a shoot (intact or terminated) of its own hierarchy type. In each growth cycle, the buds either produce a shoot of their own type or remain inactive (Fig. A1a).

The simulation of morphological growth requires the following information for each type (D, SD, ND) of shoot produced by releasing buds:

1. Length
2. Branching angle
3. Number of axillary buds and their type (only living lateral buds are considered)
4. Locations of the axillary buds
5. Time delay in releasing each axillary bud
6. Type of apical bud (living or dead)

The measurements made in the four habitats (polluted, restored, sand pit, and forest) were utilized in determining the parameters as follows:

Rule 1. The lengths of the shoots were determined according to Fig. 2a.

Rule 2. The branching angles of the lateral shoots were determined according to Fig. 2c. Lateral shoots fork off alternately to the left or the right, the left/right orientation for the first shoot being random.

Rule 3. The number of living axillary buds depends on the type of shoot and was obtained by combining Figs 2b and 3. The releasing bud produces buds of different hierarchy type (D, SD, ND) in the proportions given in Fig. 5. The proportion of shoots with a dead apical bud (i.e. terminated shoots) were obtained from Table 4.

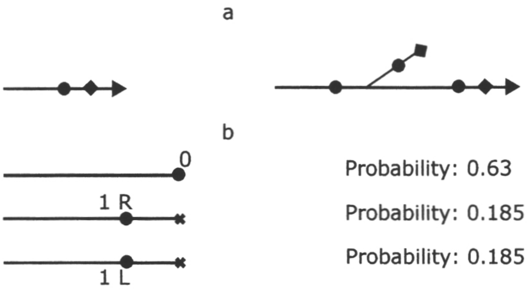


Fig. A1. a) The functioning of the model clarified with an example: Left: a D shoot consisting of an apical D bud (▸), an SD bud (◆), an ND bud (●), and internodes (line segments). Right: The apical D bud has produced an identical D shoot, and the SD bud has produced an SD shoot (which has one axillary ND bud). The ND bud has remained inactive.
(b) Three different ND shoots that can be produced by an ND bud in the forest habitat. The number next to the living bud (●) indicates the delay (years) in releasing. In the case of a lateral bud, L means forking to the left and R forking to the right, x indicates a dead apical bud. The probability of the occurrence of different shoots in the simulation is given on the right.

Rule 4. The location of attachment of axillary buds along the shoots corresponded to the mean distance from the shoot apex in accordance with Fig. 2d.

Rule 5. The data indicated that all the axillary buds did not release during the next growth cycle, but showed delays. The proportions of released lateral buds in the years 1–4 were determined on the basis of Fig. 4. The delays were implemented into the model (Fig. A1a).

Rule 6. The new shoot generated by a living bud in a growth cycle can be either intact or terminated (i.e. its apical bud is either living or dead); the proportion of intact and terminated shoots was obtained from Table 4.

We used L system language (Prusinkiewicz & Lindemayer 1990) to implement the model with L studio™ software. The generation of new shoots by living buds in accordance with the conditions described in rules 3–6 hold was accomplished as follows. In the model, a bud can produce a number of slightly different shoots. The different shoots are randomly selected with specified probabilities so that the characteristics of generated shoots correspond to measured relationships as specified in rules 3–6. This mimics the observed variability of the shoots.

As an example of this procedure, we can take the forest habitat and a living ND bud (either apical or axillary) that releases and produces an ND shoot (Fig. A1b). An ND bud can produce three different types of shoot, one intact and two terminated. The probability to produce intact shoots is 0.63 (Table 4b). The intact ND shoot does not have any releasing axillary buds (Fig. 3b). The terminated shoots are otherwise identical, only the axillary buds (and later the shoots) forking off to the left or right. Altogether the proportion of terminated shoots is 0.37 (= 0.185 + 0.185) (Table 4b). Their axillary buds release after a delay of one year.

A similar principle was applied in the generation of other kinds of shoot. The number of different kinds of shoot generated varied according to the observed proportions. The maximum number of different shoots that one bud could produce was six (D shoots for the restoration habitat). Producing the observed characteristics and variability of the growth of *A. uva-ursi* in this manner is only an approximation. However, it was deemed satisfactory for the present study.

In addition to the rules described above, collision detection and shoot bending were realized in the simulations. Collision detection prevents shoots and branches from growing over each other. Collision detection prevented a bud from releasing if other buds (on the other branches) were very close (0.5 cm). Shoot bending was realized such that, at each axillary bud, the shoot bent 5 degrees to either the left or the right. The bending direction was the same for all the axillary buds on the same shoot, and was randomly to the left or to the right.

Acknowledgements

We wish to thank the Forest Restoration Project of the Finnish Forest Research Institute for the possibility to use the data collected from its sample plots. MINNA KARPPINEN and SATU SMOLANDER helped in the field work and in measuring the plants. HEIKKI HÄNNINEN and HARRI HAUTALA kindly commented the manuscript. HANNU NOUSIAINEN made the drawings of the species, SARI ELOMAA helped in preparing the figures, and JOHN DEROME checked the English language.

References

AARSSEN, L. W. (1995): Hypothesis for the evolution of apical dominance in plants: implications for the interpretation of overcompensation. – Oikos **74:** 149–156.

BELL, A. D. & TOMLINSON, P. B. (1980): Adaptive architecture in rhizomatous plants. – Bot. J. Linn. Soc. **80:** 125–160.

BELL, A. D.; ROBERTS, D. & SMITH, A. (1979): Branching patterns: the simulation of plant architecture. – J. theor. Biol. **81:** 351–375.

BOWLES, J. M. 1983. Burl sprouting in *Arctostaphylos uva-ursi* as a response to trampling damage. – Can. J. Bot. **61:** 3543–3545.

BONSER, S. P. & AARSSEN, L. W. (1996): Meristem allocation: a new classification theory for adaptive strategies in herbaceous plants. – Oikos **77:** 347–352.

CALLAGHAN, T. V.; SVENSSON, B. M.; BOWMAN, H.; LINDLEY, D. K. & CARLSSON, B. Å. (1990): Models of clonal plant growth based on population dynamics and architecture. – Oikos **57:** 257–269.

CHAPIN, F. S. III (1983): Nitrogen and phosphorus nutrition and nutrition cycling by evergreen and deciduous understorey shrubs in Alaskan black spruce forest. – Can. J. For. Res. **13:** 773–781.

CLINE, M. G. (1991): Apical dominance. – Bot. Rev. **57:** 318–358.

CLINE, M. G. (1997): Concepts and terminology of apical dominance. – Amer. J. Bot. **84:** 1064–1069.

DEL BARRIO, J.; LUIS-CALABUIG, E. & TÁRREGA, R. (1999): Vegetative response of *Arctostaphylos uva-ursi* to experimental cutting and burning. – Plant Ecol. **145:** 191–195.

DEROME, J. (2000): Effects of heavy-metal and sulphur deposition on the chemical properties of forest soil in the vicinity of a Cu–Ni smelter, and means of reducing the detrimental effects of heavy metals. – Finnish Forest Research Institute, Research Papers 769.

DEROME, J. & LINDROOS, A.-J. (1998): Effects of heavy metal contamination on macronutrient availability and acidification parameters in forest soil in the vicinity of Harjavalta Cu–Ni smelter, SW Finland. – Environ. Pollut. **99:** 225–232.

DEROME, J. & NIEMINEN, T. (1998): Metal and macronutrient fluxes in heavy-metal polluted Scots pine ecosystems in SW Finland. – Environ. Pollut. **103:** 219–228.

DE KROON, H. & KNOPS, J. (1990): Habitat exploration through morphological plasticity in two grassland perennials. – Oikos **59:** 39–49.

DE KROON, H. & HUTCHINGS, M. J. (1995): Morphological plasticity in clonal plants: the foraging concept reconsidered. – J. Ecol. **83:** 143–152.

DONG, M.; DURING, H. J. & WERGER, M. J. A. (1997): Clonal plasticity in response to nutrient availability in the pseudoannual herb, *Trientalis europaea* L. – Plant Ecol. **131:** 233–239.

FRITZE, H.; PENNANEN, T. & VANHALA, P. (1997): Impact of fertilisers on the humus layer microbial community of Scots pine stands growing along a gradient of heavy metal pollution. In: INSAM, H. & RANGGEER, A. (eds.): Microbial communities. Functional versus structural approaches, 68–83. – Springer, Berlin.

HARPER, J. L. (1977): Population biology of plants. – Academic Press. London.

HELMISAARI, H.-S.; DEROME, J.; FRITZE, H.; NIEMINEN, T.; PALMGREN, K.; SALEMAA, M. & VANHA-MAJAMAA, I. (1995): Copper in Scots pine forests around a heavy-metal smelter in south-western Finland. – Water, Air and Soil Poll. **85:** 1727–1732.

HUTCHINGS, M. J. & MOGIE, M. (1990): The spatial structure of clonal plants: control and consequences. In: VAN GROENENDAEL, J. & DE KROON, H. (eds.): Clonal growth in plants: regulation and function, 57–76. – SPB Academic Publishing, The Hague.

HUTCHINGS, M. J. & DE KROON, H. (1994): Foraging in plants: the role of morphological plasticity in resource acquisition. – Adv. Ecol. Res. **25:** 159–237.

IRWIN, D. L. & AARSSEN, L. W. (1996): Testing for cost of apical dominance in vegetation: a field study of three species. – Ann. Bot. Fenn. **33:** 123–128.

JONSDOTTIR, I. S. & CALLAGHAN, T. V. (1988): Interrelationships between different generations of interconnected tillers of *Carex bigelowii*. – Oikos **52:** 120–128.

KIIKKILÄ, O.; PERKIÖMÄKI, J.; BARNETTE M.; DEROME, J.; PENNANEN, T.; TULISALO, E. & FRITZE, H. (2001): In situ bioremediation through mulching of soil polluted by a copper-nickel smelter. – J. Environ. Qual. **30:** 1134–1143.

LORTIE, C. J. & AARSSEN, L. W. (1997): Apical dominance as an adaption in *Verbascum thapsus*: effects of water and nutrients on branching. – Int. J. Plant Sci. **158:** 461–464.

LORTIE, C. J. & AARSSEN, L. W. (2000): A test of the reserve meristem hypothesis using *Verbascum thapsus* (*Scrophulariaceae*). – Am. J. Bot. **87:** 1789–1792.

Lukina, N. V.; Liseenko, L. A. & Belova, E. A. (1993): Pollution-induced changes in the vegetation cover of spruce and pine ecosystems in the Kola North region. In: KOZLOV, M. V.; HAUKIOJA, E. & YARMISHKO, V. T. (eds.): Aerial pollution in Kola Peninsula. – Proceedings of the International Workshop, April 14–16, 1992, St. Petersburg, Apatity, 312–321.

MOEN, J.; INGVARSSON, P. K. & WALTON, W. H. (1999): Estimates of structural complexity in clonal plant morphology: comparison of grazed and ungrazed *Acaena magellanica* rhizomes. – Can. J. Bot. **77:** 869–876.

MÄLKÖNEN, E.; DEROME, J.; FRITZE, H.; HELMISAARI, H.-S.; KUKKOLA, M.; KYTÖ, M.; SAARSALMI, A. & SALEMAA, M. (1999): Compensatory fertilization of Scots pine stands polluted by heavy metals. – Nutrient Cycling in Agroecosyst. **55:** 239–268.

PRUSINKIEWICZ, P. & LINDENMAYER, A. (1990): The algorithmic beauty of plants. – Springer, Berlin.

REMPHREY, W. R. & STEEVES, T. A. (1984a): Shoot ontogeny in *Arctostaphylos uva-ursi* (bearberry): the annual cycle of apical activity. – Can. J. Bot. **62:** 1925–1932.

REMPHREY, W. R. & STEEVES, T. A. (1984b): Shoot ontogeny in *Arctostaphylos uva-ursi* (bearberry): origin and early development of lateral vegetative and floral buds. – Can. J. Bot. **62:** 1933–1939.

REMPHREY, W. R.; STEEVES, T. A. & NEAL, B. R. (1983a): The morphology and growth of *Arctostaphylos uva-ursi* (bearberry): an architectural analysis. – Can. J. Bot. **61:** 2430–2450.

REMPHREY, W. R.; NEAL, B. R. & STEEVES, T. A. (1983b): The morphology and growth of *Arctostaphylos uva-ursi* (bearberry): an architectural model simulating colonizing growth. – Can. J. Bot. **61:** 2451–2458.

SALEMAA, M.; VANHA-MAJAMAA, I. & GARDNER, P. J. (1999): Compensatory growth of two clonal dwarf shrubs, *Arctostaphylos uva-ursi* and *Vaccinium uliginosum*, in a heavy metal polluted environment. – Plant Ecol. **141:** 79–91.

SALEMAA, M.; VANHA-MAJAMAA, I. & DEROME, J. (2001): Understorey vegetation along a heavy-metal pollution gradient in SW Finland. – Environ. Pollut. **113:** 339–350.

SALOMONSON, A.; OHLSON, M. & ERICSON, L. (1994): Meristem activity and biomass production as response mechanism in two forest herbs. – Oecologia **100:** 29–37.

SCHÜTT, P. & LANG, U. M. (2000): *Arctostaphylos uva-ursi* (Linné) Spreng., 1825. In: SCHÜTT, P., WEISGERBER, H., SCHUCK, H. J., LANG, U. M. & ROLOFF, A. (eds.): Enzyklopädie der Holzgewächse. – Handbuch und Atlas der Dendrologie, 19. Erg.Lfg. 3/00.

SLADE, A. J. & HUTCHINGS, M. J. (1987): The effects of nutrient availability on foraging in the clonal herb *Glechoma hederacea*. – J. Ecol. **75:** 95–112.

SUTHERLAND, W. J. & STILLMAN, R. A. 1990. Clonal growth: insights from models. In: VAN GROENENDAEL, J. & DE KROON, H. (eds.): Clonal growth in plants, 95–111. – SPB Academic Publishing, The Hague.

TIFFNEY, W. N.; BENSON, D. R. & EVELEIGH, D. E. (1978): Does *Arctostaphylos uva-ursi* (Bearberry) have nitrogen-fixing nodules? – Amer. J. Bot. **65:** 625–628.

TOLVANEN, A. & LAINE, K. (1997): Effect of reproduction and artificial herbivory on vegetative growth and resource levels in deciduous and evergreen dwarf shrubs. – Can. J. Bot. **75:** 656–666.

TUOMI, J.; NILSSON, P. & ÅSTRÖM, M. (1994): Plant compensatory responses: bud dormancy as an adaptation to herbivory. – Ecology **75:** 1429–1436.

VÄISÄNEN, S. (1986): Effects of air pollution by metal, chemical and fertilizer plants on forest vegetation at Kokkola, W Finland. – Ann. Bot. Fenn. **23:** 305–315.

Paper VI

Salemaa, M. & Monni, S. 2003. Copper resistance of the evergreen dwarf shrub *Arctostaphylos uva-ursi*: an experimental exposure. Environmental Pollution 126: 435-443.

Available online at www.sciencedirect.com

SCIENCE DIRECT

ELSEVIER

Environmental Pollution 126 (2003) 435–443

ENVIRONMENTAL POLLUTION

www.elsevier.com/locate/envpol

Copper resistance of the evergreen dwarf shrub *Arctostaphylos uva-ursi*: an experimental exposure

Maija Salemaa[a,*], Satu Monni[b]

[a]*Vantaa Research Centre, The Finnish Forest Research Institute, PO Box 18, FIN-01301 Vantaa, Finland*
[b]*Electrowatt-Ekono Oy, PO Box 93, FIN-02151 Espoo, Finland*

Received 20 May 2002; accepted 6 June 2003

"Capsule": *Root extension into deeper layers of soil may aid in avoidance of heavy metals.*

Abstract

The copper (Cu) resistance of *Arctostaphylos uva-ursi* was tested in a pot experiment (lasting 8 weeks) using rooted cuttings originating from an area near the Harjavalta Cu–Ni smelter, SW Finland. The fine roots were moderately infected by arbutoid mycorrhizae. The plants were exposed to five Cu levels (1, 10, 22, 46 and 100 mg l^{-1}) given repeatedly together with a nutrient solution. The critical Cu concentration in the nutrient solution inhibiting the growth of *A. uva-ursi* was below 10 mg l^{-1} Cu (EC_{50} value for biomass production 3.3 mg l^{-1} Cu). This concentration was clearly lower than the value we have found earlier for other dwarf shrubs under similar experimental conditions. Most of the Cu given accumulated in the roots and old stems. The results suggest that *A. uva-ursi* cuttings were relatively sensitive to Cu despite the ability of the adult clones to grow in Cu-contaminated soil. The adult clones extend their roots into the less toxic deeper soil layers, which may facilitate the avoidance of heavy metals.

Keywords: Arbutoid mycorrhizae; Accumulation; Bearberry; Dose–response; Heavy metals; Tolerance

1. Introduction

Many dwarf shrub species in the order Ericales are capable of surviving in heavy metal contaminated environments (Bradley et al., 1981; Chernenkova and Kuperman, 1999; Meharg and Cairney, 2000; Uhlig and Junttila, 2001). However, the resistance level of different dwarf shrub species seems to vary, which is reflected in their distribution along pollution gradients. For instance, *Arctostaphylos uva-ursi* L. (bearberry) has disappeared almost completely from the most polluted forested site close (0.5 km) to the Cu–Ni smelter at Harjavalta, SW Finland, where e.g. *Empetrum nigrum* ssp. *nigrum* L. still survives (Salemaa et al., 2001). The presence of some residual *A. uva-clones* clones and the many signs of dead clones in the vicinity of the smelter show that it has earlier been much more abundant. Both *A. uva-ursi* and *Calluna vulgaris* (L.) Hull are growing in Scots pine forests at a distance of 2–3 km from the smelter. This distance was classified as "moderately damaged" on the basis of the understorey vegetation and the element concentration of the organic soil layer (Salemaa et al., 2001). In experimental exposures to copper, *E. nigrum* appeared to be more resistant than *C. vulgaris* (Monni et al., 2000a,b). In the present study we test the copper resistance of *A. uva-ursi* orginating from a moderately polluted area near the Harjavalta smelter in controlled experiments comparable to those carried out earlier with *E. nigrum* and *C. vulgaris*.

Although Cu is an essential element in plant nutrition and acts as a cofactor for many enzymes catalysing metabolic processes, elevated levels of Cu are harmful to plants (Marschner, 1995). A high concentration of Cu disturbs the primary reactions of photosynthesis and electron transport, leading to the inhibition of shoot and root growth (Shainberg et al., 2001). An excess of Cu leads to oxidative stress, which increases lipid peroxidation and damages cell membranes (Weckx and

---

* Corresponding author. Tel.: +358-102112539; fax: +358-102112202.

E-mail addresses: maija.salemaa@metla.fi (M. Salemaa), satu.monni@poyry.fi (Satu Monni).

0269-7491/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0269-7491(03)00235-5

Clijsters, 1996; Shainberg et al., 2001). However, higher plants possess many mechanisms that enable them to defend themselves against and to resist high concentrations of heavy metals.

The evolution of heavy-metal resistant races (ecotypes) is a much slower process in long-lived trees and dwarf shrubs than in short-lived grasses and herbaceous species (Dickinson et al., 1991). There is evidence that long-lived species can survive in metal contaminated soil because they are constitutively adapted (all individuals of a species show some degree of inherent resistance) (e.g. *E. nigrum*, Monni et al., 2000a) or possess phenotypic plasticity (acclimation to increasing heavy metal load) (Bradshaw and Hardwick, 1989; Dickinson et al., 1991). The mechanisms involved in heavy metal resistance are species-specific, and are usually divided into avoidance (external protection against toxic elements) and tolerance mechanisms (physiological mechanisms to exclude or detoxify toxic elements) (Levitt, 1980; Baker, 1987; Verkleij and Schat, 1989; Hall, 2002). The role of inducible resistance to metal stress as an acclimation mechanism may be especially important for long-lived species living under fluctuating stresses (Cumming and Tomsett, 1992; Turner, 1994).

It has been suggested that part of the heavy-metal resistance of the Ericales dwarf shrubs is derived from their general ability to grow in acid, nutrient-poor soil where metal availability (e.g. aluminium, iron and manganese) is high (Bradley et al., 1981; Meharg and Cairney, 2000). One feature common to the Ericales species is that the ericoid mycorrhiza in their roots provides protection against the direct effects of heavy metals (Bradley et al., 1981; Read, 1983). *Arctostaphylos* differs from most of the genera in the family Ericaceae in having an arbutoid mycorrhiza (Read, 1983). The Ericales dwarf shrubs also have other mechanisms to avoid heavy metals. For instance, leaf exudates may play a role in heavy metal detoxification. Uhlig et al. (2001) suggested that leaf phenolics from *E. nigrum* litter immobilize metals in the organic layer and, furthermore, that they can avoid metals by extending their tap root deeper into the soil.

The clonal growth-habit enables dwarf shrubs to respond morphologically to environmental stress (Hutchings and de Kroon, 1994). For instance, *A. uva-ursi* changes its branching architecture as a response to the resource level of the habitat (Salemaa and Sievänen, 2002). Apical bud necrosis and shoot mortality of *A. uva-ursi* were higher in a polluted area close to the Harjavalta smelter than at greater distances (Salemaa et al., 1999). This breaks apical dominance and causes active branching via lateral buds. Plastic branching enables *A. uva-ursi* to recover after damage according to the 'reserve meristem hypothesis' (Aarssen, 1995).

In this paper we study the Cu resistance of *A. uva-ursi* in controlled conditions and compare its Cu resistance with that of other boreal dwarf shrub species. This knowledge is important when trying to find local plant species that are suitable for the revegetation and remediation of contaminated soil. We hypothesize that the differences in the resistance level of the dwarf shrubs can be predicted according to their occurrence pattern along a pollution gradient. The specific aims of the study are (1) to determine the level of copper toxic to growth and survival of *A. uva-ursi*, (2) to determine the accumulation pattern of copper in different plant parts, and (3) to compare the responses in a laboratory experiment to those observed in the field.

2. Materials and methods

2.1. *Arctostaphylos uva-ursi*

A. uva-ursi is a circumpolar xeromorphic evergreen dwarf shrub, which grows in boreal and temperate forests and in dry subalpine sites above the forest limit (Remphrey et al., 1983; Schütt and Lang, 2000). It is a pioneer of open and eroded soil. Its typical habitats are extremely dry and poor in nutrients, and include open sandy pine forests, moraine slopes, sand dunes and the edges of sand pits. The pH of the soil in its habitats ranges from neutral to acid.

In open sites the clones form compact patches of prostrate branches (stems), but in a closed understorey they develop long colonizing branches (Salemaa and Sievänen, 2002). The age of clones can be determined by counting the annual rings in the cross-section of the woody stem (Salemaa et al., 2000), and they have been reported to reach an age of 100 years (Schütt and Lang, 2000).

A. uva-ursi has a deep tap root (max 100 cm) and its fine roots are normally infected by arbutoid mycorrhiza (Zak, 1976; Read, 1983; Scannerini and Bonfante-Fasolo, 1983). The arbutoid mycorrhiza differs from the ericoid type in that, in addition to the intracellular infection, it also forms a sheath and Hartig net (Read, 1983) (Fig. 1). Most of the fungi involved are Basidiomycetes and are probably the same species that form the ectomycorrhizae of forest trees (Zak, 1976). To our knowledge, the heavy metal resistance of the arbutoid mycorrhizae has not been investigated.

A. uva-ursi has been reported to grow in heavy metal polluted soil near smelter complexes e.g. in the Kola Peninsula (Lukina et al., 1993), near chemical and fertilizer plants in western Finland (Väisänen, 1986), and in zinc-contaminated soil in Flin Flon, Manitoba (K. Winterhalder, pers. comm.). It has been transplanted in revegetation experiments on metal-contaminated soil near the Sudbury smelters, Ontario (Winterhalder, 1984) and near the Harjavalta smelter, SW Finland (Kiikkilä, 2002).

2.2. Sampling site and plant material

The cuttings used in the experiments were collected from eight separate *A. uva-ursi* clones growing in a moderately polluted Scots pine stand at 2 km distance from the Harjavalta copper-nickel smelter (61°19′N, 22°09′E), SW Finland, in autumn 1997. The age of the mother clones is tens of years. The smelter has been operating since 1945 and the neighbouring forest ecosystems have been drastically changed by heavy-metal and sulphur deposition (Mälkönen et al., 1999; Helmisaari, 2000; Derome, 2000a; Salemaa et al., 2001). Emissions from the smelter decreased considerably during the latter half of the 1990s. During 1985–1990 the average annual emissions of Cu were 104 t and of Ni 50 t, and during 1996–1997 Cu 60 t and Ni 2 t (Helmisaari, 2000). The soil texture of the study site is fine sand and the soil type ferric podzol (Mälkönen et al., 1999). In 1996 the exchangeable Cu concentration in the organic layer was 408.8 mg kg^{-1}, and decreased in the mineral soil layers as follows: 11.1 mg kg^{-1} (0–5 cm), 2.6 mg kg^{-1} (5–10 cm) and 6.0 mg kg^{-1} (10–20 cm) (Derome, 2000b and unpublished data). Annual Cu deposition in bulk precipitation was 32 mg m^{-2} (mean concentration 0.42 mg l^{-1}) during 1993–1998 (Derome, 2000a).

The clearest sign of the effects of deposition on the understorey vegetation in the sampling site was the total absence of forest mosses that are normally dominating in boreal *Calluna*-type stands. The understorey vegetation was not closed, but the coverage of needle litter was high (70–80%). The following dwarf shrub species were present at low abundances: *A. uva-ursi*, *C. vulgaris*, *E. nigrum*, and *Vaccinium vitis-idaea* L. (Salemaa et al., 2001).

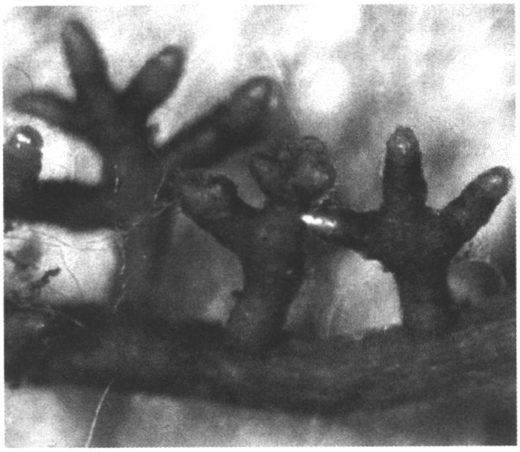


Fig. 1. Microscopic view of the arbutoid mycorrhizae of fine roots of *A. uva-ursi* cuttings before the Cu application. The roots are covered by well-developed sheaths. ×100.

The *A. uva-ursi* cuttings had two annual growth segments (1996, 1997) when taken, and the third segment (1998) developed during the rooting period in a greenhouse. The rooting substrate was an unpolluted peat-sand mixture. The fine roots were moderately colonized by arbutoid mycorrhizae at the beginning of the experiment (Fig. 1).

2.3. Experimental design

The experiment was carried out in a greenhouse of the Finnish Forest Research Institute in Tuusula (62°21′N, 25°00′E) in summer 1999. The *A. uva-ursi* cuttings were planted in pots containing 1 l of quartz sand. The roots were cut back to 10 cm to standardize the material, and the initial fresh weight and total length of the cuttings were measured before planting. The cuttings were watered once or twice a week (50 ml/pot) with a nutrient solution modified by Stribley and Read (1976). It contained the following nutrients (mg l^{-1}):

P 11.3 ($Na_2HPO_4 \times 2H_2O$), K 28.5 (KCl), Ca 29.2, S 23.4 ($CaSO_4 \times 2H_2O$), Mg 8.8 ($MgCl_2 \times 6H_2O$), Fe 3 ($FeCl_3 \times 6H_2O$), Mn 0.5 ($MnCl_2 \times 4H_2O$), B 0.5 (H_3BO_3), Zn 0.1 ($ZnCl_2$), Mo 0.1 ($Na_2MoO_4 \times 2H_2O$), N 65 (ammonium and nitrate as NH_4NO_3 and ammonium as NH_4Cl in the ratio NH_4:NO_3 70:30). Five levels of Cu (1, 10, 22, 46 and 100 mg l^{-1} as $CuCl_2$) were given in the nutrient solution. The pH of the nutrient solution was adjusted to 4.5–5.0, but the variation in pH across the five Cu levels was not determined.

The cuttings were planted in quartz sand on 21 April, and the treatments with nutrient solution lasted for 8 weeks during the active growing period in May–July 1999. The total amount of nutrient solution given was 12×50 ml = 600 ml/pot.

In order to minimize the potential differences in the response of different clones, the cuttings were randomised so that two cuttings from each of the eight clones were selected for each Cu level (total 16 cuttings/Cu level). There were no statistically significant differences in the average initial fresh weights (mean±S.D.: 1.4±0.5 g, $n=80$) ($F_{4,75}=0.04$, $P=0.996$) or lengths (10.0±2.9 cm) ($F_{4,75}=0.57$, $P=0.682$) between the plants selected for the different Cu levels (ln transformation in one-way ANOVA).

The light conditions were natural, and the mean temperature was regulated at +20 °C during the day and 15 °C at night. The relative humidity in the greenhouse was approximately 60–70%.

2.4. The response variables

The variables used to quantify the Cu resistance of *A. uva-ursi* were (1) survival of the plants, (2) growth (leaf and stem biomass), elongation and number of new shoots, (3) total aboveground and root biomass (living and dead biomass separately), and (4) accumulation of Cu in different plant parts. Biomass was determined as dry weight after drying the samples for 24 h in an oven at 60 °C.

The plant material was divided into the following 11 parts for determining the Cu concentration and uptake: current-year, previous-year and older leaves and stems, (living and dead leaves separately), detached leaves and roots. Altogether 16 replicate samples were combined to obtain enough material for the chemical analyses. The bulk samples consisted of both living and dead plants. The samples were homogenized, dried at +60 °C and weighed, and the total Cu concentrations of the plant parts were determined by dry ashing (+550 °C), extraction of the ash with 0.2 M HCl, and analysis on an inductively coupled plasma atomic emission spectrometer (ICP-AES) (Dahlquist and Knoll, 1978).

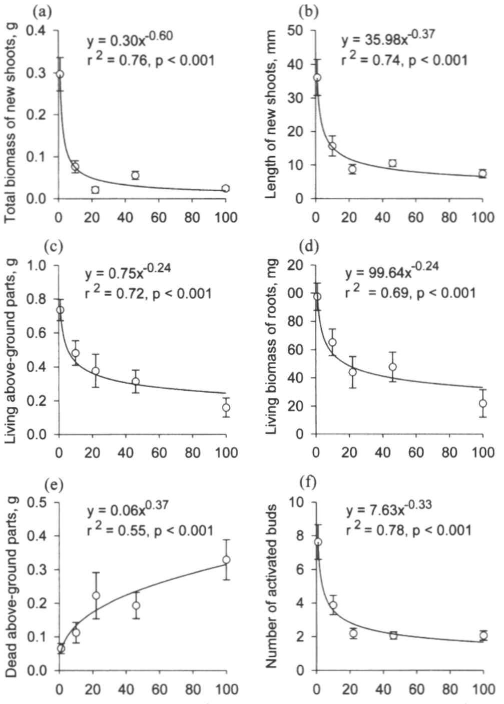


Fig. 2. Dose–response curves (non-linear regressions) for (a) biomass production and (b) length growth of the current-year shoots, living biomass of (c) the above-ground parts and (d) the roots, (e) dead biomass of above-ground parts, and (f) the number of activated buds of *A. uva-ursi* cuttings exposed to different Cu levels in a nutrient solution. Mean and standard error of mean ($n = 16$ plants) are given for each response variable. All biomass values are dry weights.

2.5. Statistical analysis

Dose–response curves were determined using non-linear regression equations (NLIN procedure in SAS 8.01 software, 2000). The effective Cu concentrations at which growth was inhibited by 50% (EC_{50}) were estimated from these curves. Regression equations were also calculated for the accumulated Cu as a function of the applied amount using the NLIN procedure. The initial fresh weights of the living and dead plants were compared by *t*-test.

3. Results

3.1. Survival at different external copper levels

All 16 replicate plants survived at the lowest Cu level (1 mg l^{-1}) during the 8-week experiment. Mortality increased in proportion to the increasing Cu concentration in the nutrient solution: 1 mg l^{-1} (0% mortality), 10 mg l^{-1} (19%), 22 mg l^{-1} (38%), 46 mg l^{-1} (25%) and 100 mg l^{-1} (69%). The first cuttings died during the fourth week of the experiment at Cu levels of 22–100 mg l^{-1}. From the sixth week onwards three plants died already at the Cu level of 10 mg l^{-1}. The initial fresh weight of the dead plants (mean ± S.D.: 1.28 ± 0.56 g, $n = 24$) was significantly lower than that of the surviving ones (1.52 ± 0.50 g, $n = 56$) ($t = 2.11$, $P < 0.04$, df = 78, ln transformation).

3.2. The effect of Cu exposure on growth, biomass and discoloration

Biomass production was strongly inhibited by increasing Cu levels in the nutrient solution (Fig. 2). The dry weight of the new shoots (leaves and stems together) decreased by over 70% and elongation by over 50% already at the Cu level of 10 mg l^{-1} compared to the lowest level (Fig. 2a,b). At the higher Cu levels the decrease in growth continued but was not as steep. The effective Cu level inhibiting growth by 50% (EC_{50}) was 3.3 mg l^{-1} for biomass production (Fig. 2a) and 6.5 mg l^{-1} for elongation (Fig. 2b). The corresponding EC_{50} value for the dry weight of the living biomass of the aboveground parts (Fig. 2c) or roots (Fig. 2d) was 20 mg l^{-1} Cu, and for the number of activated buds 8.3 mg l^{-1} (Fig. 2f). In contrast, the dry weight of the dead aboveground biomass increased in proportion to the Cu level, and was greater than the living biomass at the highest Cu level (Fig. 2e).

Growth was activated the earliest at the lowest Cu level, and the new leaves were of normal size with a healthy green colour. In the case of Cu levels of 10–22 mg l^{-1}, on the other hand, development of the new leaves was retarded and they were light green or chlorotic, and the older leaves had brown tips. A small size and necrotic tips or reddish colour were typical stress symptoms in the new leaves at the Cu level of 46 mg l^{-1}. Practically no new leaves developed at the highest Cu level and the colour of the older leaves turned dark reddish or brown.

3.3. Cu concentrations in different plant parts

The Cu concentrations increased with the age of the aboveground plant parts at all the external Cu levels (Table 1). The lowest concentrations were found in the current-year shoots. The concentrations in the new stems remained close to 10 µg g^{-1} at all Cu levels (Table 1). In contrast, the Cu concentrations in the new leaves were similar to those of the stems at the lower Cu levels, but increased strongly at the Cu level of 46 mg l^{-1}. When the dry weights and Cu concentrations in the leaves were plotted against the Cu levels in the nutrient solution (Fig. 3), a relatively small increase in the Cu concentrations of the new leaves correlated with a strong decrease in the growth of leaves. The upper tolerance limit for leaf production of *A. uva-ursi* was below 10 mg l^{-1} Cu in the nutrient solution. Growth was inhibited when the Cu concentration exceeded 13 µg g^{-1} in the tissues of the new leaves.

The dry weight of the living, previous-year leaves decreased with increasing external Cu levels as a result of Cu-induced leaf shedding (Fig. 3). Leaf and stem Cu concentrations of the previous-year shoots were relatively similar (20–30 µg g^{-1}) at the lower Cu levels, but at the higher levels the stem concentrations increased more strongly (Table 1).

The Cu concentrations of the older (over 2-year-old) shoots were considerably higher than those of the

Table 1
Copper concentrations (conc.) µg g^{-1} dry weight and amount (µg) of accumulated Cu in different parts of *A. uva-ursi*

Plant parts.[a]	Field conc.	Experiment									
		Cu concentration mg l^{-1} and corresponding total Cu amount (µg) given in the nutrient solution									
		1 conc.	(600) (µg)	10 conc.	(6000) (µg)	22 conc.	(13 200) (µg)	46 conc.	(27 600) (µg)	100 conc.	(60 000) (µg)
Current-year shoots											
Berries	9.8	–	–	–	–	–	–	–	–	–	–
Green leaves	13.5	11.9	(2.7)	13.1	(0.7)	14.0	(0.8)	27.4	(0.8)	–	–
Dead leaves		26.4	(0.1)	13.9	(0.2)	15.6	(0.4)	23.5	(0.4)	16.9	(0.3)
Stems	38.4	11.3	(0.7)	12.8	(0.2)	9.2	(0.1)	14.8	(0.1)	8.5	(0.0)
Previous-year shoots											
Green leaves	13.8	20.3	(2.8)	26.6	(3.3)	21.6	(3.0)	29.1	(3.0)	97.5	(5.4)
Dead leaves		19.8	(0.2)	12.1	(0.1)	26.3	(0.7)	24.8	(0.7)	27.0	(2.8)
Stems	59.7	21.9	(1.0)	24.5	(1.1)	23.4	(1.8)	44.3	(1.8)	461.8	(20.4)
2-year-old/older shoots											
Leaves	20.0	47.4	(5.9)	60.3	(6.6)	72.8	(6.1)	83.2	(6.1)	319.4	(7.9)
Dead leaves	135.6	52.3	(0.7)	42.4	(0.5)	167.3	(0.9)	81.9	(0.9)	93.3	(6.7)
Detached leaves	–	48.5	(3.0)	68.6	(4.6)	111.0	(23.2)	253.4	(23.2)	126.7	(5.2)
Stems[b]	200.8	307	(42.4)	1400	(207.8)	2630	(381.7)	4140	(524.4)	7810	(944.5)
Roots											
Fine roots[c]	313.0	1090	(98.0)	3141	(201.5)	6764	(424.8)	11728	(677.3)	19267	(1116.6)
Coarse roots	124.0	–	–	–	–	–	–	–	–	–	–
Tap root	107.0	–	–	–	–	–	–	–	–	–	–
Aboveground uptake[d]			(59.4)		(225.2)		(407.5)		(561.6)		(993.2)
Aboveground uptake (%)			9.9		3.7		3.1		2.0		1.6
Total uptake[e]			(157.4)		(426.7)		(832.4)		(1238.9)		(2109.9)
Total uptake (%)			26.2		7.1		6.3		4.5		3.5

Field samples (1994) were taken at a distance of 2 km from the smelter and the concentrations represent the average of the three clones (Salemaa and Vanha-Majamaa, unpublished). Field samples were washed with distilled water before elemental analysis to remove surface accumulation. In the Cu exposure experiment the bulk samples comprise 16 plants (living and dead plants combined) at each external Cu level. Amount of accumulated Cu (µg) was calculated as dry weight of the plant part (g)×Cu conc. µg g $^{-1}$ and is given in the parentheses in both experiments. Uptake % is the proportion of the amount of Cu taken up out of the amount applied. – = no biomass.

[a] Regression equations for the accumulated amount (µg) of Cu (y) as a function of the applied amount of Cu (x) in older stems, fine roots, aboveground parts and the whole plant.

[b] $y = 0.72x^{0.65}$, $r^2 = 99\%$, $P < 0.001$.

[c] $y = 0.77x^{0.66}$, $r^2 = 99\%$, $P < 0.001$.

[d] $y = 0.92x^{0.63}$, $r^2 = 99\%$, $P < 0.001$.

[e] $y = 1.67x^{0.65}$, $r^2 = 99\%$, $P < 0.001$.

younger aboveground parts (Table 1). Both the leaf and stem concentrations increased with increasing external Cu level, but the rate of increase was clearly higher in the stems (Table 1). The biomass of the older leaves decreased steeply with increasing Cu concentration in the tissues (Fig. 3).

The highest Cu concentrations of all the plant parts occurred in the roots (Table 1). At the lowest Cu level (1 mg l^{-1}), at which all the plants survived up until the end of the experiment, the Cu root concentration was already over 1000 μg g^{-1}. The Cu concentrations of the dead leaves varied inconsistently in relation to the external Cu level (Table 1).

The overall accumulation pattern of Cu in the experiment plants was similar to that observed in the adult clones growing in the field: the concentrations were the highest in the roots and old stems and decreased with shoot age (Table 1).

3.4. Copper uptake

The total amount of Cu accumulated in the new leaves and stems was very low (0.1–3 μg per plant), and it did not increase with increasing external Cu level (Table 1). This indicates that the increase in concentrations with increasing Cu levels was a consequence of decreased biomass. The largest amounts of Cu were found in the older stems (42–944 μg) and roots (1090–19 267 μg). The amount of Cu taken up from the nutrient solution increased as a function of the amount of Cu applied in both the roots and aboveground parts (equations in Table 1). Roots accumulated slightly higher amounts of Cu than the shoots. The proportion of the total amount of accumulated Cu in the whole plant (roots and shoots together) decreased from 26 to 3.5% along with an increasing amount of Cu applied (Table 1).

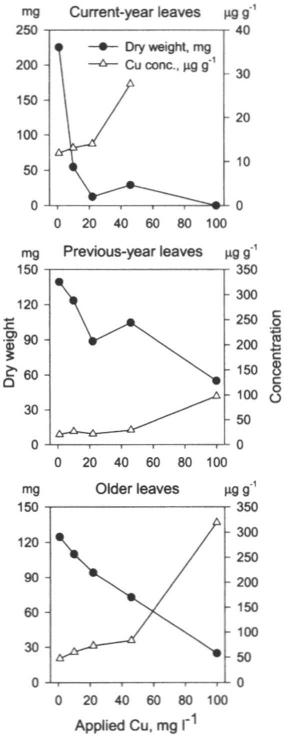


Fig. 3. The mean dry weights (mg) (left) and the Cu concentrations (μg g^{-1}) (right) of the current-year, previous-year and older living leaves of *A. uva-ursi* as a function of applied Cu level (mg l^{-1}) in the nutrient solution (*x*-axis).

4. Discussion

4.1. The external toxic concentration

Repeated application of Cu during the course of the 8-week study meant that the Cu exposure level increased steadily towards the end of the experiment. Although there are problems in relating the responses to specific concentrations, the magnitude of the responses of the various measurement endpoints increased relative to the increased Cu exposure levels. The degree of toxicity in the external media depends on the biological availability of the metals and other elements, the conditions under which uptake occurs (e.g. temperature) and the nutritional status of the plant (Balsberg Påhlsson, 1989).

The critical Cu concentration in the nutrient solution inhibiting the growth of *A. uva-ursi* was below 10 mg l^{-1} Cu (EC_{50} value for biomass production 3.3 mg l^{-1}). This concentration is clearly lower than the value we obtained earlier for other dwarf shrubs under similar experimental conditions. For instance, the biomass production of *E. nigrum* (Monni et al., 2000a) and length growth of *C. vulgaris* (Monni et al., 2000b) showed a 50% decrease at Cu levels of 22 and 10 mg l^{-1}, respectively. These species were exposed to the same amounts of Cu (given as $CuSO_4$) in a similar nutrient solution as *A. uva-ursi* in this study, but their exposure time was two weeks shorter (6 weeks) than the present one (8 weeks). The Cu levels applied proved to be too high for *A. uva-ursi*. Especially the increase in the exposure level from 1 to 10 mg l^{-1} was too large to determine the detailed shape of the growth response.

The relative sensitivity of *A. uva-ursi* compared to the other two species was also expressed in its higher mortality at lower Cu concentrations. All the *E. nigrum* cuttings had survived at the highest Cu level (100 mg l^{-1}) by the end of the experiment (Monni et al., 2000a), even though they were smaller than the *A. uva-ursi* cuttings. The seedlings of *C. vulgaris* did not survive as well as those of *E. nigrum* (Monni et al., 2000b), but their mortality was slightly lower than *A. uva-ursi* especially at the lower Cu levels. The smaller the *A. uva-ursi* cutting, the sooner it appeared to reach toxic concentrations in the tissues, leading eventually to plant death.

4.2. *The Cu concentrations in the tissues*

The toxic Cu concentrations in the leaves of *A. uva-ursi* were age dependent. In the new leaves the toxic concentration was estimated to be about 13 μg g^{-1} and in the previous-year and older leaves above 30 μg g^{-1}. For most crop species the level of Cu toxicity in the leaves is estimated to be above 20–30 μg g^{-1} (Marschner, 1995), whereas the growth of seedlings of coniferous trees is often affected at 15–20 μg g^{-1} (Bahlsberg Påhlsson, 1989). However, the great variation in the architecture across tissues (e.g. amount of cell walls, free space and vacuoles) makes it is very difficult to relate the measured tissue concentrations to the real effective dose.

New growth of evergreen dwarf shrubs (e.g. *Vaccinium vitis-idaea* L.) is initially based on the photosynthesis of the previous-year leaves (Karlsson, 1985). It takes new leaves about 2 weeks to reach full photosynthetic capacity. Older leaves show a lower photosynthetic intensity, but they also act as a storage site from which nutrients and carbohydrates are translocated to new growth. Thus the toxic Cu concentrations in both new and old leaves resulted in the strong collapse in the growth of *A. uva-ursi* at relative low Cu levels.

The growth-limiting Cu concentrations in the leaves of *A. uva-ursi* were clearly lower than those found earlier in *C. vulgaris* and *E. nigrum* in similar experimental exposures (Monni et al., 2000a,b). However, compared to the two other species, the cuttings of *A. uva-ursi* were taller and had more leaf biomass in relation to the stems, which was reflected in the relative leaf concentrations.

Most of the Cu taken up to the shoots accumulated in the old stems of *A. uva-ursi* (Table 1). The cuttings were able to regulate to some extent the access of Cu to the leaves at low Cu levels (Fig. 3). Living plant cells can detoxify heavy metals by binding them in cell walls, chelating and storing them in vacuoles or binding them with phytochelatines in the cytoplasm (e.g. Hall, 2002). The young shoots may have had lower Cu concentrations because young cells have more cytoplasm relative to vacuoles than the older cells. On the other hand, stems have relatively larger proportions of non-living tissues and the passive accumulation of Cu might increase the concentrations of older stems. Furthermore, roots contain a considerable amount of cortex tissue that passively accumulates Cu (Kahle, 1993), which may explain the relatively high concentrations of Cu in the roots. The sheath of the arbutoid mycorrhizae may also retain some Cu in the roots.

4.3. *The effect of arbutoid mycorrhizae*

Generally, ericoid mycorrhizae confer Cu resistance to the host dwarf shrubs (Meharg and Cairney, 2000; Perotto et al., 2002). The symbiont fungal endophyte accumulates heavy metals in its hyphae and restricts their transport to the tissues of the host (Galli et al., 1994). Bradley et al. (1982) found an inhibition of growth in non-mycorrhizal seedlings of *C. vulgaris*, *Vaccinium macrocarpon* and *Rhododendron ponticum* at 10 mg l^{-1} Cu. Monni et al. (2000b) did not find mycorrhizal infection in *C. vulgaris* seedlings, but the *E. nigrum* cuttings were moderately colonized by ericoid mycorrhiza (Monni et al., 2000a).

Although information about the heavy metal resistance of arbutoid mycorrhizae is lacking, the close taxonomic relationship with ectomycorrhizal fungi suggests functional similarities between the two mycorrhiza types (c.f. Read, 1983). For instance, *Laccaria laccata*, which has an ability to colonize roots of *A. uva-ursi* (Zak, 1976), has been found to reduce heavy metal transport from the roots to the shoots when forming ectomycorrhizae in coniferous trees (Galli et al., 1993). Fungal ecotypes from heavy metal contaminated sites are more tolerant to heavy metals than strains from uncontaminated sites (Galli et al., 1994). In the present experiment, the response of fungi to Cu was not studied. We can only suspect that the fungus forming the arbutoid mycorrhizae might be sensitive to Cu (the rooting substrate was from an uncontaminated site) or the colonization rate was insufficient to retain Cu in the hyphae.

4.4. *Experimental results versus field observations*

The clearest difference between tolerant and non-tolerant individuals is in their ability to establish, survive and reproduce in metal-contaminated substrates (Baker and Walker, 1989). The cuttings of *A. uva-ursi* did not show any signs of constitutive resistance to Cu in the experiment although the mother clones are growing in Cu contaminated soil at 2 km from the smelter. The mother clones may have originated before the smelter was established about 50 years ago. Thus the currently surviving clones represent the most resistant genotypes of the earlier population. However, the decreased shoot growth of the clones at 2 km compared to those growing in a less polluted area at 8 km from the smelter (Salemaa et al., 1999) indicates that the mother clones are also suffering from phytotoxic effects.

Short-term experiments using only the apical parts of long branches and high exposure concentrations do not give a complete picture of the heavy metal resistance of the long-lived, adult clones (c.f. Ernst and Nelissen, 2000). In the real ecological conditions the clones of *A. uva-ursi* have to cope with chronic low exposure to heavy metals that have accumulated in the soil or entered the ecosystem in wet and dry deposition.

The thick epidermal and cuticle layers of the leaves of *A. uva-ursi* afford them protection against foliar uptake of heavy metals. The long prostrate branches extending over the ground form adventitious roots that attach them to the surface soil. These short roots are in contact with the highly contaminated organic layer. However, the lateral roots of the deep tap root are more important for the acquisition of nutrients. When three clones were excavated for elemental analyses at the study site in 1994, the longest tap roots extended down to a depth of 50 cm. Because the concentration of exchangeable Cu decreases with soil depth (see Section 2.2), we suggest that the adult clones avoid the high concentrations in the upper soil layers by growing their roots down into deeper soil layers. The real toxicity level of the soil is modified by many factors, e.g. the amount of organic matter, pH and the overall element composition (Kahle, 1993), as well as interactions with mycorrhizae.

5. Conclusions

A. uva-ursi cuttings proved to be relatively sensitive to Cu in the experimental exposure despite the ability of the adult clones to grow in Cu-contaminated soil. The growth-limiting Cu concentrations in the nutrient solution (<10 mg l^{-1}) and in the youngest leaf tissues (about 13 $\mu g\ g^{-1}$ dw) were lower than those reported earlier for *C. vulgaris* and *E. nigrum*. Extension of the roots of the adult clones into the less toxic deeper soil layers facilitates the avoidance of heavy metals. The role of arbutoid mycorrhizae in reducing heavy metal transport to shoots requires further research.

The differences in resistance level between the species on the basis of survival, growth and accumulation of Cu in the experimental exposures, all ranked the species in the same relative order. This order was the same as that reported for the distribution of the species along the pollution gradient near the Harjavalta Cu–Ni smelter: *E. nigrum* (most resistant) > *C. vulgaris* > *A. uva-ursi* (most sensitive).

Acknowledgements

Kaarina Pynnönen and Satu Smolander helped in the greenhouse work. Hilkka Granlund and Airi Piira assisted in weighing the samples and recording the data. Heljä-Sisko Helmisaari, Tiina Nieminen and Pasi Rautio commented on the manuscript, and John Derome checked the English language. We express our sincere gratitude to all.

References

Aarssen, L.W., 1995. Hypothesis for the evolution of apical dominance in plants: implications for interpretation of overcompensation. Oikos 74, 149–156.

Baker, A.J.M., 1987. Metal tolerance. New Phytology 106, 93–111.

Baker, A.J.M., Walker, P.L., 1989. Physiological responses of plants to heavy metals and the quantification of tolerance and toxicity. Chemical Speciation and Bioavailability 1, 7–17.

Balsberg Påhlsson, A.-M., 1989. Toxicity of heavy metals (Zn, Cu, Cd, Pb) to vascular plants. Water, Air, and Soil Pollution 47, 287–319.

Bradley, R., Burt, A.J., Read, D.J., 1981. Mycorrhizal infection and resistance of heavy metal toxicity in *Calluna vulgaris*. Nature 292, 335–337.

Bradley, R., Burt, A.J., Read, D.J., 1982. The biology of mycorrhiza in the *Ericaceae*. VIII. The role of mycorrhizal infection in heavy metal resistance. New Phytologist 91, 197–209.

Bradshaw, A.D., Hardwick, K., 1989. Evolution and stress—genotypic and phenotypic components. Biological Journal of the Linnean Society 37, 137–155.

Chernenkova, T.V., Kuperman, R.G., 1999. Changes in the forest communities along a heavy metal deposition gradient on Kola Peninsula. Water, Air and Soil Pollution 111, 187–200.

Cumming, J.R., Tomsett, A.B., 1992. Metal tolerance in plants: signal transduction and acclimation mechanisms. In: Adriano, D.C. (Ed.), Biogeochemistry of Trace Metals. Boca Raton Publishers, Lewis, pp. 329–364.

Dahlquist, R.L., Knoll, J.W., 1978. Inductively coupled plasma-atomic emission spectrometry: analysis of biological materials and soils for major, trace, and ultra-trace elements. Applied Spectroscopy 32, 1–30.

Derome, J., 2000a. Effects of heavy-metal and sulphur deposition on the chemical properties of forest soil in the vicinity of a Cu-Ni smelter, and means of reducing the detrimental effects of heavy metals. Finnish Forest Research Institute, Research papers 769, 77. p.

Derome, J., 2000b. Detoxification and amelioration of heavy-metal contaminated forest soils by means of liming and fertilisation. Environmental Pollution 107, 79–88.

Dickinson, N.M., Turner, A.P., Lepp, N.W., 1991. How do tree and other long-lived plants survive in polluted environments? Functional Ecology 5, 5–11.

Ernst, W.H.O., Nelissen, H.J.M., 2000. Life-cycle phases of zinc- and cadmium-resistant ecotype of *Silene vulgaris* in risk assessment of polymetallic mine soils. Environmental Pollution 107, 329–338.

Galli, U., Meier, M., Brunold, C., 1993. Effects of cadmium on non-mycorrhizal and mycorrhizal Norway spruce seedlings [*Picea abies* (L.) Karst.] and its ectomycorrhizal fungus *Laccaria laccata* (Scop. ex Fr.) Bk. & Br.: sulphate reduction, thiols and distribution of the heavy metal. New Phytologist 125, 837–843.

Galli, U., Schüepp, H., Brunold, C., 1994. Heavy metal binding by mycorrhizal fungi. Physiologia Plantarum 92, 364–368.

Hall, J.L., 2002. Cellular mechanisms for heavy metal detoxification and tolerance. Journal of Experimental Botany 53, 1–11.

Helmisaari, H.-S., 2000. Areas polluted by heavy metals. In: Mälkönen, E. (Ed.), Forest condition in a changing environment—the Finnish case. Kluwer Academic, pp. 248–251.

Hutchings, M., de Kroon, H., 1994. Foraging in plants: the role of morphological plasticity in resource acquisition. Advances in Ecological Research 25, 159–237.

Kahle, H., 1993. Response of root of trees to heavy metals. Environmental and Experimental Botany 33, 99–119.

Karlsson, P.S., 1985. Patterns of carbon allocation above ground in a deciduous (*Vaccinium uliginosum*) and an evergreen (*Vaccinium vitis-idaea*) dwarf shrub. Physiologia Plantarum 63, 1–7.

Kiikkilä, O. 2002. Remediation through mulching with organic matter of soil polluted by a copper-nickel smelter. Finnish Forest Research Institute, Research Papers 831, 57p.

Levitt, J., 1980. Responses of plants to environmental stresses. Academic Press, New York.

Lukina, N.V., Liseenko, L.A., Belova, E.A., 1993. Pollution-induced changes in the vegetation cover of spruce and pine ecosystems in the Kola Peninsul. In: Kozlov, M.V., Haukioja, E., Yarmishko, V.T. (Eds.), Aerial Pollution in Kola Peninsula. Proceedings of the International Workshop, 14–16 April 1992. St. Petersburg, Kola Scientific Centre, Apatity, Russia, pp. 312–321.

Marschner, H., 1995. Mineral nutrition of higher plants, 2nd ed. Academic Press, London.

Meharg, A.A., Cairney, J.W.G., 2000. Co-evolution of mycorrhizal symbiosis and their hosts to metal-contaminated environments. Advances in Ecological Research 30, 69–112.

Monni, S., Salemaa, M., Millar, N., 2000a. The tolerance of *Empetrum nigrum* to copper and nickel. Environmental Pollution 109, 221–229.

Monni, S., Salemaa, M., White, C., Tuittila, E., Huopalainen, M., 2000b. Copper resistance of *Calluna vulgaris* originating from the pollution gradient of a Cu–Ni smelter, in southwest Finland. Environmental Pollution 109, 211–219.

Mälkönen, E., Derome, J., Fritze, H., Helmisaari, H.-S., Kukkola, M., Kytö, M., Saarsalmi, A., Salemaa, M., 1999. Compensatory fertilization of Scots pine stands polluted by heavy metals. Nutrient Cycling in Agroecosystems 55, 239–268.

Perotto, S., Girlanda, M., Martino, E., 2002. Ericoid mycorrhizal fungi: some new perspectives on old acquaintances. Plant and Soil 244, 41–53.

Read, D.J., 1983. The biology of mycorrhiza in the Ericales. Canadian Journal of Botany 61, 985–1004.

Remphrey, W.R., Steeves, T.A., Neal, B.R., 1983. The morphology and growth of *Arctostaphylos uva-ursi* (bearberry): an architectural analysis. Canadian Journal of Botany 61, 2430–2450.

Salemaa, M., Sievänen, R., 2002. The effect of apical dominance on branching architecture of *Arctostaphylos uva-ursi* in four contrasting environments. Flora 197, 429–442.

Salemaa, M., Vanha-Majamaa, I., Gardner, P.J., 1999. Compensatory growth of two clonal dwarf shrubs, *Arctostaphylos uva-ursi* and *Vaccinium uliginosum* in a heavy metal polluted environment. Plant Ecology 141, 79–91.

Salemaa, M., Vanha-Majamaa, I., Derome, J., 2001. Understorey vegetation along a heavy-metal pollution gradient in SW Finland. Environmental Pollution 112, 339–350.

Salemaa, M., Vanha-Majamaa, I., Reinikainen, A., Nousiainen, H., 2000. Response of understorey vegetation to heavy metal loading. In: Mälkönen, E. (Ed.), Forest Condition in a Changing Environment — the Finnish Case. Kluwer Academic, pp. 266–275.

Scannerini, S., Bonfante-Fasolo, P., 1983. Comparative ultrastructural analysis of mycorrhizal associations. Canadian Journal of Botany 61, 917–943.

Schütt, P., Lang, U.M., 2000. *Arctostaphylos uva-ursi* (Linné) Spreng., 1825. In: Schütt, P., Weisgerber, H., Schuck, H.J., Lang, U.M., Roloff, A. (Eds.), Enzyklopädie der Holzgewächse. Handbuch und Atlas der Dendrologie, 19. Erg.Lfg. 3/00.

Shainberg, O., Rubin, B., Rabinowitch, H.D., Tel-Or, E., 2001. Loading beans with sublethal levels of copper enhanches conditioning to oxidative stress. Journal of Plant Physiology 158, 1415–1421.

Stribley, D.P., Read, D.J., 1976. The biology of mycorrhiza in the Ericaceae. VI. The effects of mycorrhizal infection and concentration of ammonium nitrogen on growth of cranberry (*Vaccinium microcarpon* Ait.) in sand culture. New Phytologist 77, 63–72.

Turner, A.P., 1994. The response of plants to heavy metals. In: Ross, S.M. (Ed.), Toxic Metals in Soil–plant Systems. John Wiley, Chichester, pp. 151–187.

Uhlig, C., Junttila, O., 2001. Airborne heavy metal pollution and its effects on foliar elemental composition of *Empetrum hermaphroditum* and *Vaccinium myrtillus* in Sør-Varanger, northern Norway. Environmental Pollution 114, 461–469.

Uhlig, C., Salemaa, M., Vanha-Majamaa, I., Derome, J., 2001. Element distribution in *Empetrum nigrum* microsites at heavy metal contaminated sites in Harjavalta, western Finland. Environmental Pollution 112, 435–442.

Verkleij, J.A.C., Schat, H., 1989. Mechanisms of metal tolerance in higher plants. In: Shaw, J.A. (Ed.), Heavy Metal Tolerance in Plants: Evolutionary Aspects. CRC Press, FL, pp. 179–193.

Väisänen, S., 1986. Effects of air pollution by metal, chemical and fertilizer plants on forest vegetation at Kokkola, W Finland. Annales Botanici Fennici 23, 305–315.

Weckx, J.E.J., Clijsters, H.M.M., 1996. Oxidative damage and defence mechanisms in primary leaves of *Phaseolus vulgaris* as a result of root assimilation of toxic amounts of copper. Physiologia Plantarum 96, 506–512.

Winterhalder, K., 1984. Environmental degradation and rehabilitation in the Sudbury area. Laurentian University Review XVI (2), 15–47.

Zak, B., 1976. Pure culture synthesis of bearberry mycorrhizae. Canadian Journal of Botany 54, 1297–1305.

ISBN 951-40-1899-0
ISSN 0358-4283