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

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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.

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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.

Acknowledgements

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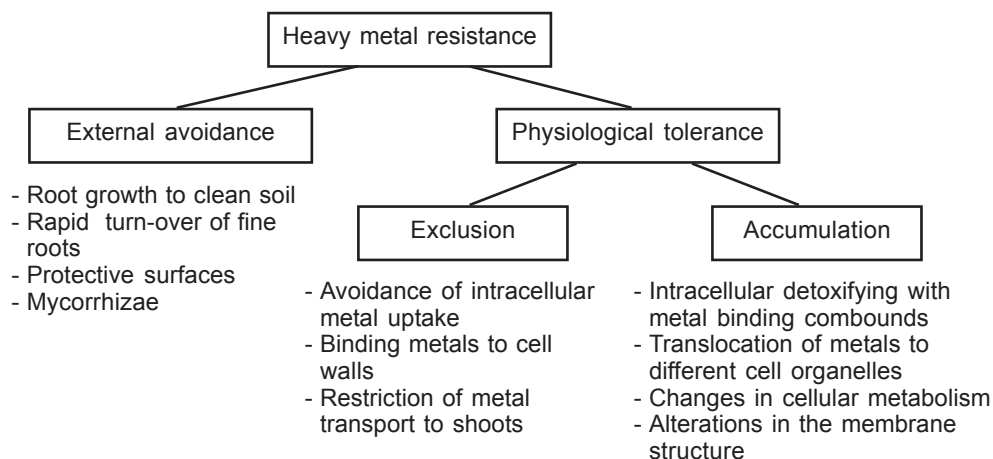
Fig. 1. Summary of the possible mechanisms involved in the resistance to elevated heavy metal concentrations according to Baker (1987), Tyler *et al*. (1989) and Hall (2002).

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$_2$O$_2$) 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 0.5, 1, 2, 3, 4 and 8 km	6 × 3	August 1993	Bryophytes Lichens Vascular plants	Species abundances (point frequency, %) Chemical composition of organic layer
	Reference: National forest inventory	12	1995	”	Species abundances (visual cover, %)
II	Harjavalta transect 0.5, 2, 4 and 8 km	4	August 1992 (1993, 1994)	Bryophytes Lichens Vascular plants	Element concentrations in species, soil and precipitation Species abundances (point frequency, %)
	Reference: Hämeenkangas and Mekrijärvi	2	Aug. – Sept. 1992	”	”
III	Harjavalta transect 0.5, 1, 2, 3, 4 and 8 km Fertilised: 0.5, 4 and 8 km	6 × 3 3 × 3	May 1994	Vascular plants	Germinated seeds and seedling survival in soil samples (greenhouse experiment)
IV	Field manipulation: 2 and 8 km forest 0.5 and 4 km forest, 5 km bog		1994 – 1995 ” (1994, 1997)	Arctostaphylos uva-ursi Vaccinium uliginosum	Compensatory growth after shoot clipping ” (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 References: Monni et al. (2000a) Monni et al. (2000b) Salemaa et al. (2003)		1999 1996 1996 2000	Arctostaphylos uva-ursi Calluna vulgaris Empetrum nigrum Vaccinium uliginosum	Survival, growth and Cu accumulation

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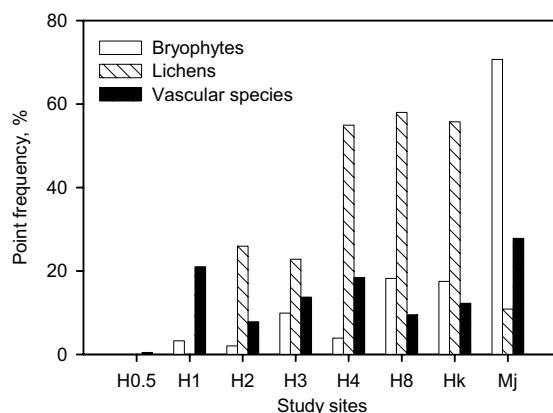
Fig. 3. The total abundances of the bryophytes, lichens and vascular plant species along the Harjavalta transect (H0.5 – H8: 0.5 – 8 km from the smelter, mean abundance of three plots per each distance in 1993), Hk = Hämeenkangas (1992) and Mj = Mekrijärvi (1992) (I, II).

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
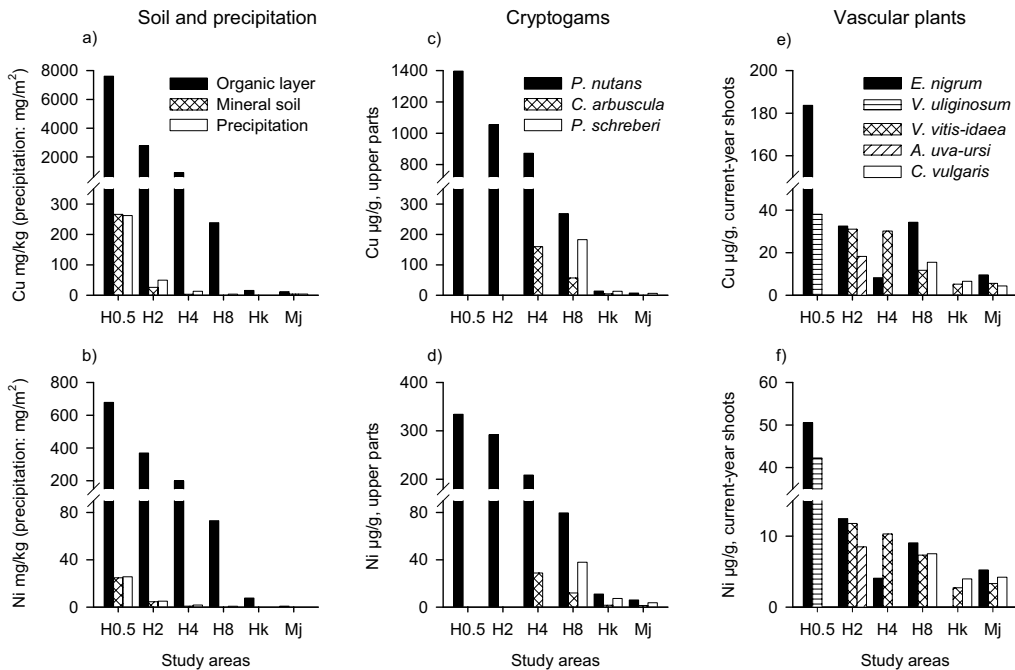
Fig 4. Cu and Ni concentrations in (a, b) the organic and mineral (0 – 5 cm) soil layers (mg/kg) and bulk precipitation (mg/m^2), (c, d) selected cryptogam species (µg/g) and (e, f) the current-year shoots of dwarf shrubs (µg/g) at different distances (km) from the Harjavalta Cu-Ni smelter (H0.5, H2, 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		F2	F8	Genotype		F0.5	F4	P5
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
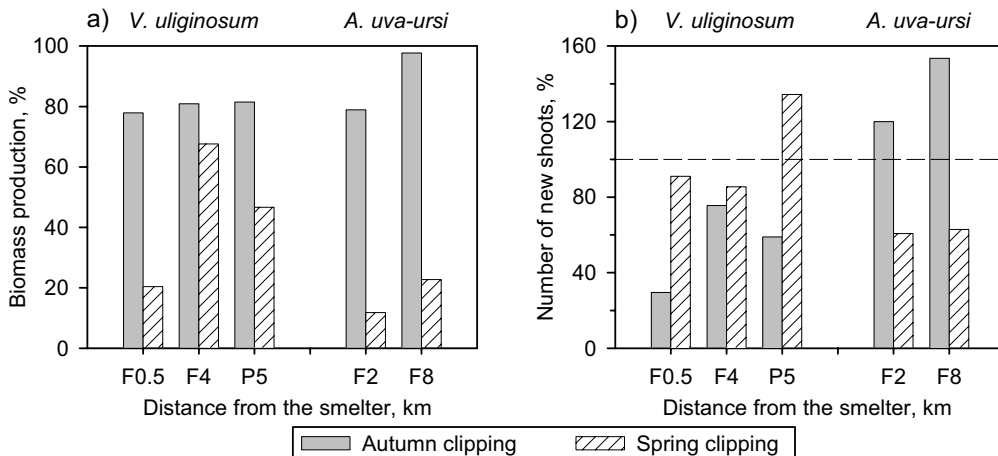
Fig. 5. The average compensatory growth of branches of *V. uliginosum* and *A. uva-ursi* after shoot clipping performed at different distances from the smelter (F = forest, P = peatland site) in autumn 1993 and spring 1994. The compensatory growth is presented as percentages of a) the biomass production, and b) the number of new shoots compared to the control branches (**IV**). The dashed line indicates the 100 % level.

a)

b)

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).

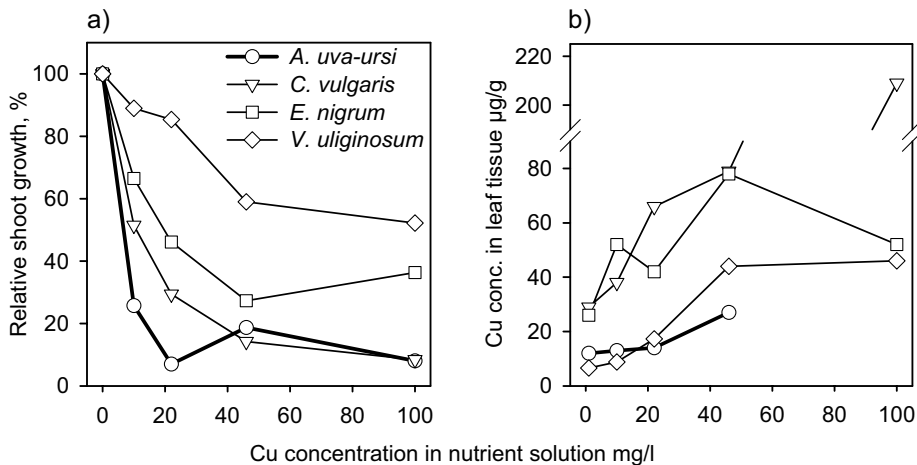Fig. 7. a) The decrease in the relative shoot growth (%) (standardized to the Cu level of 1 mg/l) of *E. nigrum*, *C. vulgaris*, *A. uva-ursi* and *V. uliginosum*, and b) the Cu concentrations of the new leaves (µg/g) at different Cu levels applied. The shoot growth was based on the biomass production (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.

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