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Remediation through mulching with organic matter of soil polluted by a copper-nickel smelter

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VANTAAN TUTKIMUSKESKUS – VANTAA RESEARCH CENTRE

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Front cover: A mulched plot at Harjavalta. Photograph by M. Salemaa (2000).

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Original publications

This thesis is based on the following articles, referred to in the text by their Roman numerals:

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

II Kiikkilä, O., Pennanen, T., Perkiömäki, J., Derome, J. and Fritze, H. 2002. Organic material as a copper immobilising agent: a microcosm study on remediation. Basic and Applied Ecology (in press).

III Kiikkilä, O., Perkiömäki, J., Barnette, M., Derome, J., Pennanen, J., Tulisalo, E. and Fritze, H. 2001. In situ bioremediation through mulching of soil polluted by a copper-nickel smelter. Journal of Environmental Quality 30: 1134–1143.

IV Kiikkilä, O., Derome, J., Brügger, T., Uhlig, C. and Fritze, H. 2002. Copper mobility and toxicity of soil percolation water to bacteria in a metal polluted forest soil. Plant and Soil (in press).

Studies were carried out mainly by Oili Kiikkilä. Taina Pennanen was responsible for the phospholipid fatty acid analyses and John Derome for the fractionation of copper.

List of abbreviations

d.m.	dry matter weight
o.m.	organic matter weight
Cu_{exc}	exchangeable copper
Cu_{comp}	complexed copper
Cu_{tot}	total copper
BR	microbial respiration activity
m.l.	mass loss of litter, litter decomposition
TdR	[^{3}H]-thymidine incorporation rate describing bacterial growth rate
IC_{50}	inhibition concentration describing bacterial copper tolerance
AO	the number of bacterial cells
PLFA	phospholipid fatty acid
mol%	the relative abundance of the PLFA in question
$PLFA_{tot}$	an indicator of microbial biomass
$PLFA_{bact}$	an indicator of bacterial biomass
$PLFA_{fung}$	an indicator of fungal biomass

Abstract

Remediation through mulching with organic matter of soil polluted by a copper-nickel smelter

Remediation of soil polluted by a Cu-Ni smelter was studied by adding organic matter onto polluted soil. The study sites were at 2 km (laboratory experiment) and 0.5 km (field experiment) distance from the Outokumpu Harjavalta smelter which has had a severe effect on the forest ecosystem. The total copper concentrations in the organic soil were 1 600 mg kg^{-1} dry matter (2 km) or 5 800 mg kg^{-1} dry matter (0.5 km). A laboratory microcosm study with nine different organic mulch materials and a field study with one, a mixture of compost and woodchips, were carried out. The success of remediation treatments was assessed by measuring exchangeable copper concentration in the soil, the speciation of copper in soil water, and the toxicity of soil water to bacteria. An important objective in remediation is the recovery of microbial activities, since the nutrient cycling in heavy metal polluted soil is in general disturbed. The microbial activity, the number of bacterial cells, the bacterial growth rate, the bacterial copper tolerance and the structure of the microbial community in the organic soil were studied during a 16 months period in the laboratory and a 27 months period in the field. The toxicity of the soil water to bacteria, the bacterial growth rate and copper tolerance was measured using the [^{3}H]-thymidine incorporation method. The procedure to prepare the bacterial suspension for the [^{3}H]-thymidine incorporation into the macromolecules of bacteria was modified for the soils of low microbial activity.

During the 16 months of laboratory incubation the exchangeable copper concentration in the polluted soil remained on the same level in the control and in the treatments involving barkchips, humus, or peat. The exchangeable copper concentration in the soil decreased during the incubation when the soil was mulched with compost, mixture of compost and woodchips, mixture of compost and barkchips, sewage sludge, or garden soil. However, the Cu^{2+} concentration in soil water or the toxicity of soil water to bacteria was on the same level in all the treatments after 16 months of incubation. No microbial response direct attributable to remediation was detected in the laboratory.

In the field, mulching the polluted soil with the mixture of compost and woodchips converted copper into less toxic forms, which was detected as a lower exchangeable Cu concentration in the soil, a lower Cu^{2+} concentration in the soil water, and as decreased toxicity of soil water to bacteria after

mulching. This was mainly a consequence of increased complexation with particulate and dissolved organic matter, and increased soil pH. However, also leaching of copper down the soil profile increased slightly after mulching the forest floor. The microbial response to remediation was clear. The microbial activities increased and the tolerance of the bacteria to Cu decreased in the organic layer. Since the structure of the microbial community did not differ between the treatments it was the original microbiota that became measured.

Mulching the exposed mineral soil after the removal of the polluted organic layer had a similar but greater influence on Cu speciation and the toxicity of soil water than mulching the forest floor. However, also the leaching of Cu down the soil profile was the highest when the exposed mineral soil was mulched. Thus mulching the polluted forest floor was a more successful remediation treatment.

1. Harjavalta as a case study of environmental pollution

In Finland, the largest heavy metal polluted areas are situated around Harjavalta and Tornio (Kubin *et al*. 2000) where the area of forest death is less than 1 km^2. The largest heavy metal polluted area in the northern hemisphere is situated in NW Russia in the Kola Peninsula where the area of forest death is 600–1 000 km^2 and the area of visible damage is 39 000 km^2 (Oleksyn and Innes 2000). Another large area is found in Sudbury, Canada, where mining and smelting activities have created 170 km^2 of barren land and 700 km^2 of stunted open woodland (Winterhalder 2000).

Many studies on the effects of heavy metals on the forest ecosystem have been performed at Harjavalta, in SW Finland, which is part of the southern boreal coniferous zone. Harjavalta is situated on an esker that runs to the SE of the Cu-Ni smelter. Along the forested esker runs the pollution gradient, from 0.5 to 8 km in length, which has often been examined. The forest on the esker consists mainly of Scots pine, *Pinus sylvestris* L., and is situated on dryish, relatively nutrient-poor sites (Mälkönen *et al*. 1999). According to the Finnish forest site type classification (Cajander 1949), the forest along the esker varies from *Vaccinium* to *Calluna* type (Derome and Lindroos 1998b). According to Mälkönen *et al*. (1999) the soil comprises of sorted glaciofluvial sediments and the texture of the mineral soil is classed as sorted fine or fine/coarse sand with no stones. The soil type is ferric podzol, with organic mor layer (F+H) ranging between 1 to 3 cm, an E horizon 6 to 15 cm, and a B_s horizon 26 to 39 cm, in thickness.

This section reviews the work on heavy metal deposition and the effects of pollution on the forest ecosystem that has been published in scientific journals since 1975. The aim is to outline the extent the metal pollution affects the ecosystem.

1.1 Emissions, deposition and contamination

A copper smelter started to operate at Harjavalta in 1945 and a nickel smelter in 1960. In addition to copper and nickel, the emissions contain also zinc, lead, cadmium, arsenic, mercury, and sulphur (Table 1).

Elevated Cu concentration in forest mosses was detected as far as 30–40 km distance from the smelter between 1985 and 1995 (Kubin *et al*. 2000). With moss bags, a method where moss (*Spaghnum sp*.) bags hang in trees to collect deposition, was observed high airborne pollution of copper, nickel,

Table 1. Annual emissions from Harjavalta smelter. Emissions before 1985 are estimated. (Data from Outokumpu Harjavalta Metals Ltd)

Year	Dust	Cu	Ni	Zn	Pb	As
	t year^{-1}					
1945-84	1100	98	47	216	55	
1985	1100	98	47	216	55	
1986	1200	126	46	232	60	
1987	1800	140	96	162	94	
1988	1000	104	45	103	48	
1989	1000	80	33	190	70	
1990	960	80	31	160	80	
1991	640	80	14	90	45	
1992	280	60	10	12	9	
1993	250	50	7	13	6	11
1994	190	40	6	6	3	5
1995	70	17	1.4	1.7	0.5	0.2
1996	195	49	1.2	5.3	1.9	4.2
1997	360	70	3	14	4	10
1998	132	23	1.7	6.1	2.4	10
1999	48	6	0.8	4.2	1.0	1.8

zinc, lead, cadmium (Hynninen 1986) and mercury (Hynninen and Lodenius 1986) between 1981 and 1982 up to the distance of 9 km. Up to 4 km during 1992–1996 bulk deposition in open areas and stand throughfall, i.e. deposition inside the stand, was contaminated with sulphate and heavy metals (Derome and Nieminen 1998). The deposition of Cu was high close to the smelter the bulk deposition being 160 mg m^{-2} y^{-1}, and stand throughfall 360 mg m^{-2} y^{-1} (Derome and Nieminen 1998). At 8 km both the bulk deposition and stand throughfall were ca. 3 mg m^{-2} y^{-1}. The respective values for Ni close to the smelter were 70 and 140 mg m^{-2} y^{-1} and for Zn 20 and 40 mg m^{-2} y^{-1}, whilst at 8 km the values were ca. one tenth of these. In addition to emissions the dust from the degraded forest floor and slagheaps located near-by increased the deposition of metals (Nieminen *et al.* 1999).

A clear, logarithmically decreasing gradient, studied at the distances of 0.5, 2, 4 and 8 km, was found in the forest soil for Cu, Ni, Zn, Cd, Fe, Pb, Cr and S (Derome and Lindroos 1998b). Elevated concentrations of heavy metals were also found in peatlands near the smelter (Veijalainen 1998; Nieminen *et al.* 2001) (Table 2). The total Cu concentration in the organic layer of the forest soil was 5 800 mg kg^{-1} d.m. (Derome and Lindroos 1998b) and the exchangeable ($BaCl_2$) Cu concentration was 4 700 mg kg^{-1} d.m. at 0.5 km from the smelter (Derome and Nieminen 1998). The respective values for Ni were 460 and 420 mg kg^{-1} d.m. and for Zn 520 and 130 mg kg^{-1} d.m.

Table 2. Total concentrations of Cu, Ni, Zn and Cd in soil near the smelter (0.5–1 km). The concentration in the reference area (at 8 km or further) is in parentheses.

	Cu	Ni	Zn	Cd	Reference
	mg kg^{-1} d.m.				
Peat					Veijalainen 1998
Surface	3600 (170)	470 (50)	460 (86)	3.7 (0.9)	
0–10	1200 (75)	240 (16)	240 (16)	3.7 (0.9)	
10–20	180 (10)	5 (0)	110 (29)	1.5 (0.1)	
Peat					Nieminen *et al.*
2 cm	4400 (6)	870 (3)	560 (53)		2001
14 cm	45 (5)	260 (7)	570 (60)		
Soil					
Organic layer	5800 (150)	460 (40)	520 (60)	4.6 (0.7)	Derome and Lindroos 1998b
Organic layer[a]	4700 (120)	420 (37)	130 (47)		Derome and Lindroos 1998
Mineral Soil					Derome and
0–5 cm	270 (1.5)	25 (0)	10 (1)		Lindroos 1998
5–10 cm	27 (0.4)	5.4 (0)	2.9 (0.4)		
10–20 cm	16 (0)	3.2 (0)	1.8 (0)		
20–30 cm	12 (0.2)	2.1 (0)	1.4 (0)		
Percolation water					Derome and
5 cm	0.6 (0.02)	0.5 (0.01)			Lindroos 1998a
20 cm	1.1 (0.01)	0.9 (0)			

[a] Exchangeable ($BaCl_2$) metals

(Table 2). Total Fe concentration in organic soil was 18 600 mg kg^{-1} d.m. (Derome and Nieminen 1998). Uhlig *et al.* (2001) found extremely high total Cu concentration in organic soil under *Empetrum nigrum* patches, 49 000 mg kg^{-1} d.m. at 0.5 km and 12 000 mg kg^{-1} d.m. at 4.0 km. At the vertical scale, leaching of Cu, Ni, Zn and SO_4-S down to 40 cm depth in the soil profile was observed, Zn being the most mobile element and Cu being strongly bound to organic layer (Derome and Nieminen 1998).

Cu, Ni, Zn and Cd concentrations in different trophic levels have been reported to be high near the smelter. Cu has usually been found in higher concentrations than Ni or Zn although Zn has been emitted more than Cu until 1992 (Table 1). Elevated heavy metal concentration have been reported in understorey vegetation (Helmisaari *et al.* 1995), pine (Derome and Nieminen 1998), spruce (Heliövaara and Väisänen 1991), birch (Koricheva and Haukioja 1995), insects (Heliövaara *et al.* 1990), spiders (Koponen and Niemelä 1995), and birds (Eeva and Lehikoinen 1995).

1.2. Vegetation

The Scots pine forest stand close to the smelter has clearly suffered from pollution. The tree growth has been extremely poor (Mälkönen *et al*. 1999) and the understorey vegetation has drastically changed (Salemaa *et al*. 2001). The total coverage and the number of species decreased towards the smelter and vegetation was almost absent up to a distance of 0.5 km from the smelter (Salemaa *et al*. 2001) (Table 3). On the most polluted sites, *Empetrum nigrum*, *Arctosthaphylus uva-ursi*, and *Vaccinium uliginosum*, clonal dwarf shrubs, have survived in small patches. Vigorous regrowth and phenotypic plasticity have improved the survival of *A. uva-ursi* and *V. uliginosum* (Salemaa *et al*. 1999). *E. nigrum* possesses an internal heavy metal tolerance (Monni *et al*. 2000a). However, decreased chlorophyll and organic acid, and an increased abscisic acid concentration in stems and leaves indicated a reduction in the physiological activity of *E. nigrum* near the smelter (Monni *et al*. 2000c). The tolerance mechanisms of *E. nigrum* may include accumulation of heavy metals in older tissues, the restriction of the metal transport to the green leaves (Uhlig *et al*. 2001), localisation of metals in certain cell compartments (vacuoles, cell walls, cytoplasm), possible detoxification of metals by phenolics (Monni *et al*. 2001), and accumulation and immobilisation of metals in the litter beneath *E. nigrum* patches (Uhlig *et al*. 2001). Of the dwarf shrubs, *Calluna vulgaris*, growing first at 1.2 km to the NW of the smelter, proved to be least resistant to Cu (Monni *et al*. 2000b). Although germinable seeds of *C. vulgaris, Betula pubescens*, *Pinus sylvestris* and *V. uliginosum* were found in the most contaminated soil, seedlings of trees and dwarf shrubs were absent close to the smelter (Salemaa and Uotila 2001).

With regards to moss, *Pohlia nutans* and *Ceratodon purpureus* were the only moss species surviving in small patches on the most contaminated site (Salemaa *et al*. 2001) (Table 3). At 8 km distance the frequency of *Pleurozium schereberi* and *Dicranum spp*. began to increase (Salemaa *et al*. 2001). However, the Cu concentration in their tissues were still considerably higher at 8 km than those in background areas (Helmisaari *et al*. 1995). The reindeer lichens (*Cladina spp*.) appeared to be more tolerant than forest mosses, they increased in frequency at 4 km (Salemaa *et al*. 2001). Epiphytic lichens were absent up to 2 km, on an area of 8.8 km^2, in 1970 (Laaksovirta and Silvola 1975) and up to 4 km in 1987 (Fritze *et al*. 1989).

Table 3. The change in species abundance close to the smelter, - damaged, + benefit. The number in the parentheses refers to the distance where the point frequency % is more than 0.02. + in parentheses refers to the benefit in the moderately polluted area. The year in the parentheses refers to the study year. [a]rare, except at the distance in the parentheses

Species		Reference
Vascular plants		
Arctostaphylus uva-ursi (L.) Sprengel	- (2)	Salemaa *et al.* 2001 (1993)
Calluna vulgaris (L.) Hull	- (4)	
Carex clobularis L.	(1)[a]	
Empetrum nigrum L.	- (1)	
Pinus sylvestris	- (0.5)	
Vaccinium uliginosum L.	- (1)	
Vaccinium vitis idaea L.	- (1)	
Mosses		
Dicranum polysetum Sw.	- (8)	Salemaa *et al.* 2001 (1993)
Dicranum scoparium Hedw.	- (8)	
Cerantodon purpureus (Hedw.) Brid.	- (1)	
Polytrichum juniperum Hedw.	(1)[a]	
Pleurozium schreberi (Brid.) Mitt.	- (8)	
Pohlia nutans (Hedw.) Lindb.	- (0.5)	
Ground lichens		
Cetraria islandica (L.) Ach.	- (2)	Salemaa *et al.* 2001 (1993)
Cladina rangiferina (L.) Nyl.	- (3)	
Cladina arbuscula (Wallr.) Hale&W.L.Club	- (3)	
Cladina stellaris (Opiz) Brodo	- (4)	
Cladonia spp.	- (2-3)	
Epiphytic lichens		
Hypogymnia physodes L.	- (4)	Fritze *et al.*1989 (1987)
Pseudevernia furfuracea (L.) Zopf	- (4)	
Usnea hirta (L.) Wigg.	- (7)	
Bryoria fuscescens (Gyelnik) Brodo & Hawksw	- (7)	
Platismatia glauca (L.) Culb&Culb	- (7)	
Epiphytic algae		
Scoliciosporum chlorococcum	+	Fritze *et al.*1989 (1987)
Endophytic fungi		
Cenangium ferrucinosum Fr:Fr	-	Helander 1995 (1992)
endophtic fungi total	-	Lappalainen *et al.* 1999 (1993-94)
Hormonema sp.	+	
Fusicaldium sp.	-	
Gnomonia setacea (Pers.) Ces. and de Not	-	
Soil animals		
Enchytraeids (Oligochaeta, Enchytraeidae)	-	Haimi and Siira-Pietikäinen 1996 (1993-94)
Microarthropods	-	
Collembolans (Collembola)	-	
Nematodes (Nematoda)	-	

Table 3. (continued)

Bark bug (Heteroptera, Aradidae)		
Aradus cinnamomeus Panzer	- (+)	Heliövaara and Väisänen 1990a (1987-89)
Tortricid moths (Lepidoptera, Tortricidae)		
Retinia resinella L.	- (+)	
Rhyacionia pinicolana Doubleday	- (+)	
Blastesthia turionella L.	- (+)	
Blastesthia posticana Zetterstedt	- (+)	
Leaf- miners (Lepidoptera, Eriocraniidae)		
Eriocrania, solitary species	-	Koricheva 1994 (1992-93)
Eriocrania cicatricella Zetterstedt	+	
Geometrid moth (Lepidoptera, Geometridae)		
Epirrita autumnata Bkh.	-	Ruohomäki *et al*, 1996 (1990)
Aphids (Homoptera)		
Cinaria pini L.	+	Heliövaara and Väisänen, 1990a
Pineus pini Gmelin	+	Heliövaara and Väisänen, 1989b (1987)
Schizolachnus pineti Fabricius	+	
Diprionid (Hymenoptera, Diprionidae)		
Diprion pini L.	- (+)	Heliövaara *et al*. 1990
Ants (Hymenoptera, Formicidae)	(+)	Koponen and Niemelä 1995 (1992)
Formica fusca L. or *F. lemani* L.	+	Koricheva *et al*. 1995 (1993)
Beetles (Coleoptera, Scolytidae)		
Xylechinus pilosus Ratzb.	+	Heliövaara and Väisänen 1991
Tomicus piniperda L.	+	
Pityogenes chalcographus L.	-	
ground living beetles (Coleoptera)	-	Koponen and Niemelä 1995 (1992)
Coccinella septempunctata L.	+	
Mites (Acarina, Eriophyidae)		
Aceria leionotus Nalepa	-	Koricheva *et al*. 1996 (1993)
Aceria longisetosus Nalepa	-	
Acalitus rudis Canestrini	-	
Aceria varia Nalepa		
Eriophyes diversipunctatus Nalepa		
Phyllocoptes populi Nalepa		
Spiders (Araneae)		
Xerolycosa nemoralis Westring	+	Koponen and Niemelä 1993
Alopecosa aculeata Clerck	-	
Oedothorax apicatus Blackwall	+	
Erigone atra Blackwall	+	
Agyneta rurestris C.L. Koch	+	
Zelotes petrensis C.L. Koch	-	
Tapinocyba pallens O.P.-Cambridge	-	
Silometopus elegans O.P.-Cambridge	-	
Walckenaeria antica Wider	-	
Walckenaeria atrotibialis O.P.-Cambridge	-	
Birds		
Parus major L.	-	Eeva and Lehikoinen 1996 (1993)
Ficedula hypoleuca Pallas	-	

1.3. Nutrient cycling and soil organisms

Inhibition of nutrient cycling and the displacement of base cations from cation exchange sites by Cu and Ni cations has resulted in a decrease of base cation (Ca, Mg, K) concentrations in the organic layer (Derome and Lindroos 1998b). Trees have not been able to utilise the nutrient pools in the mineral soil presumably due to the toxic effects of Cu and Ni in the plant fine roots, including ectomycorrhizal root tips (Helmisaari *et al*. 1999) since Mg, Ca, and Mn concentrations in Scots pine needles were low (Derome and Nieminen 1998). In contrast, trees obtained sufficient K from the soil, since despite K leached from the needle tissues close to the smelter, the needle K concentrations were relatively high (Nieminen *et al*. 1999). Autumnal nutrient retranslocation, i.e. transport of nutrients from the senescing needles to the remaining organs for overwinter storage, of P and K in Scots pine was less efficient close to the smelter than at 8 km (Nieminen and Helmisaari 1996). The retranslocation of nutrients was suggested to be inhibited by non-pathogenic endophytic fungi (Wilson 1993). However, endophytes seemed not to be a reason for the decreased nutrient retranslocation since the number of endophyte infected needles was lower close to the smelter than further away (Helander *et al*. 1995).

The number of soil animals has clearly decreased (Table 3) and their community structure strongly altered close to the smelter (Haimi and Siira-Pietikäinen 1996). Since at 2 km the number of soil animals has only slightly decreased, soil animals appeared to be quite resistant to heavy metals. An indication of increased Cu resistance of the enchytraeid worm, *Cognettia sphagnetorum*, Vejdovsky, usually the only abundant enchytraeid species found in northern coniferous forest soils, has been found near the smelter (Salminen and Haimi 2001). It seems that the presence of patches of lower metal concentrations was mitigating the effects of the metals on enchytraeid populations (Salminen and Haimi 1999).

The overall microbiological activity in the soil has decreased drastically near the smelter. Microbial respiration activity, the amount of fungal hyphae, starch, pectin, xylan and cellulose hydrolysers, i.e. physiological groups of bacteria (Fritze *et al*. 1989), microbial biomass, measured as fumigation-extraction and substrate induced respiration, ATP content, and fungal biomass, measured as ergosterol concentration (Fritze *et al*. 1996) decreased towards the smelter. The toxicity of a soil extract, measured with a standard *Photobacterium phosphoreum* test, increased towards the smelter (Vanhala and Ahtiainen 1994). The structure of the microbial community, measured with phospholipid fatty acid analysis, had changed and the bacterial community was highly resistant, measured using thymidine incorporation method, to Cu but not to Cd, Ni, or Zn (Pennanen *et al.* 1996; Fritze *et al.* 1997). The fungal part of the microbial biomass was more sensitive to heavy metals than bacterial part (Pennanen *et al.* 1996). The decreased microbial activities have been reflected in a decreased rate of litter decomposition which could be seen as a changed structure of the humus layer (F+H) and as a 6–8 cm thick layer of accumulated brown needle litter on the top of the forest floor near the smelter (Fritze *et al.* 1989). The rate of litter decomposition has been influenced by the accumulation of Cu, Ni and Zn in brown needle litter and root litter, collected at the site (McEnroe and Helmisaari 2001). The accumulation of metals to unpolluted green needle litter was also observed (Ohtonen *et al.* 1990).

1.4. Herbivores and pathogens on trees

The adverse effects caused by forest pests increased with pollutant load as bark bugs, diprionids, tortricids, aphids, and bark beetles were abundant in the moderately polluted pine stands (Heliövaara and Väisänen 1990a), and near the smelter the Scots pines were heavily infested by aphids and bark beetles – *Xylechinus pilosus* being the most abundant bark beetle species in spruce and *Tomicus piniperda* in pine (Heliövaara and Väisänen 1991) (Table 3). Close to the smelter the cocoons of the defoliator species were smaller than further away (Heliövaara and Väisänen 1989a) but the smaller females produced more viable eggs (Heliövaara and Väisänen 1990a). Many insect species, however, suffered from severe pollution. *Pityogenes chalcographus*, which is one of the most common bark beetle species associated with spruce in Finland, was almost absent near the smelter (Heliövaara and Väisänen 1991). Also bark bugs, diprionids and tortricids were scarce in the immediate vicinity of the smelter (Heliövaara and Väisänen 1990a) (Table 3). Insects such as a moth *Epirrita autumnata* (Ruohomäki *et al.* 1996) and gall mite species on birch (*Betula pubescens* and *B. pendula*) (Koricheva *et al.* 1996) were also scarce near the smelter. In contrast, densities of mites on European aspen (*Populus tremula* L.) were not affected by the pollution (Koricheva *et al.* 1996) (Table 3).

Great differences in metal concentrations between the insect species feeding on Scots pine were observed near the smelter (Table 4). The highest concentration was measured in a sap-feeding aradid bug (*Aradus cinnamomeus*, the Cu concentration being 800 mg kg^{-1}. The lowest Cu concentration was measured in a gall-forming tortricid moth (*Retinia resinella*), 40 mg kg^{-1} (Heliövaara *et al.* 1987). Metal levels were higher in the needles (500 mg kg^{-1}) than in the insects *Neodiprion sertifer* (50 mg kg^{-1}), except in the case of Cd. Cd accumulated in the insects, the

Table 4. The concentrations of Cu, Ni, Zn and Cd in different plant species, cocoons of the insects, ants, spiders and faeces of birds near the smelter. The concentration in the reference area (8 km or further) is in parentheses.

Species	Cu	Ni	Zn	Cd	Reference
	mg kg^{-1} d.m.				
Pohlia nutans	1390 (270)				Helmisaari *et al.* 1995
Empetrum nigrum					
Last annual shoot	180 (20)				Helmisaari *et al.* 1995
	86 (22)[a]	30 (13)	50 (16)	0.5 (0.1)	Uhlig *et al.* 2001
Older living parts	1500 (30)				Helmisaari *et al.* 1995
	340 (90)[a]	120 (40)	220 (40)	1.1 (0.5)	Uhlig *et al.* 2001
Cladina arbuscula	160[a] (60)				Helmisaari *et al.* 1995
Picea abies (L.) Karsten					
Bark	600 (40)	100 (15)	300 (180)	1.2 (1.1)	Heliövaara and Väisänen
Phloem	75 (6)	80 (6)	340 (170)	1.1 (1.2)	1991
Wood	6 (1)	8 (1)	40 (10)	0.2 (0.1)	
Pinus sylvestris					
Bark	1500 (30)	390 (9)	190 (21)	5.6 (0.5)	Heliövaara and Väisänen
Phloem	66 (6)	35 (5)	120 (56)	5.1 (1.5)	1991
Wood	11 (3)	6.9 (1.2)	25 (7.8)	0.7 (0.2)	
Trunk wood	0.9[a] (1.1)	0.4 (0.2)	7.9 (5.2)	0.3 (0.3)	Harju *et al.* 1997
Needles	500 (10)	140 (10)		1.3 (0.2)	Heliövaara and Väisänen 1990b
Needles	210 (9)	44 (5)	83 (33)		Derome and Nieminen 1998
Stems (1-22 years)	2 (0.4)				Helmisaari *et al.* 1995
Fine roots	480 (75)				
Fine roots	590 (21)	110 (15)	70 (90)	2.1 (0.6)	Helmisaari *et al.* 1999
Betula pubescens Ehrh.		51 (10)	250 (210)		Koricheva and Haukioja
Foliage	96 (10)				1995
Betula pendula Roth.		40 (10)	220 (180)		
Foliage	64 (10)				
Aradus cinnamomeus[b]	800 (40)	110 (10)		13 (7)	Heliövaara *et al.* 1987
Retinia resinella	40 (5)	7 (2)		1.6 (0.2)	
Panolis flammea (Denis and Schiffermüller)	70 (10)	10 (1)		2 (0.1)	Heliövaara and Väisänen
Bupalus piniarius L.	90 (10)	1.6 (0)		0.6 (0.1)	1990c
Diprion pini L.	70 (10)	8 (1)			Heliövaara *et al.* 1990
Gilpinia socia Klug	60 (20)	10 (2)			
Neodiprion sertifer (Geoffroy)	80 (20)	7 (1)		2 (0.5)	Heliövaara and Väisänen 1989c
Gilpinia virens Klug	60 (10)	5 (0)		1 (0)	
G. frutetorum Fabricius	90 (20)	8 (2)		2 (0)	
Microdiprion pallipes Fallén	130 (20)	20 (2)		4 (1)	
ground living ants[b]	300 (30)			6 (4)	Koponen and Niemelä 1995
	180 (20)	30 (5)			Eeva and Lehikoinen 1996
ground living spiders	2000 (800)			20 (20)	Koponen and Niemelä 1995
Parus major	320 (50)	45 (5)	550 (350)		Eeva and Lehikoinen 1996
Ficedula hypoleuca	420 (70)	55 (5)	700 (250)		

[a] 4-6 km distance, [b] adults

concentration in the adults was 2.6 mg kg^{-1} which is higher than that in their food (1.3 mg kg^{-1}) or in their faeces (0.7 mg kg^{-1}) (Heliövaara and Väisänen 1990b). The low nutritional quality and high metal contents of pine needles increased the mortality of diprionids (Heliövaara and Väisänen 1990d) although the outbreaks of diprionids were also common (Heliövaara *et al.*

1991). The susceptibility of *N. sertifer* to virus and other diseases increased near the smelter but the mortality of *N. sertifer* caused by parasitoids decreased.

Means of defence against herbivores for trees include the production of resin and the phenolics in the bark, phloem, and foliage (Herms and Mattson 1992). Phenolics can also act as antidesiccation agents (Loponen *et al*. 1997). The resin flow decreased towards the smelter, indicating a decreased defence level, but the phenolic concentration increased, as a response to pollution, in Scots pine (Kytö *et al*. 1998) and in birch (Loponen *et al*. 1997). Compensatory growth, as a response to simulated herbivore, of two willow species, *Salix borealis* (Fries.) Nasar. and *S. caprea* L., was reduced near the smelter (Zvereva and Kozlov 2001). The endophytic fungal flora may affect their host plants positively by enhancing the resistance of the plant to pathogens (Butin 1992). Suppression of these non-pathogenic endophytes by air pollution did not promote the development of pathogenous *Gremniella abietina* (Lagerb.) Morelet, causing Scleroderris canker disease (Ranta *et al*. 1994).

Increased densities of leaf-miner species, which as pathogens are of minor importance, have been recorded around pollution source (Kozlov and Haukioja 1993). The solitary *Eriocrania* species (Koricheva and Haukioja 1992) were found to be scarce whilst the gregarious *Eriocrania cicatricella* (former *E. haworthi*) was abundant near the smelter (Koricheva and Haukioja 1994). The authors suggested that *E. cicatricella* possesses higher tolerance for pollutants than solitary species. Only host plant quality was found to be related to the population density of the solitary *Eriocrania* species (Koricheva and Haukioja 1992; 1995) when also several other aspects, such as: larval parasitism (Koricheva 1994), ant predation of miners (Koricheva *et al*. 1995), and the densities of endophytic fungi (Lappalainen *et al*. 1999) were studied.

Some changes in the ground-living arthropod fauna have also been reported. Beetles, except *Coccinella septempunctata*, were scarce near the smelter (Koponen and Niemelä 1995). Differences in diversity and species composition of spiders (Table 3), ants and bugs were observed along the pollution gradient although there were little differences in the total numbers (Koponen and Niemelä 1993 and 1995).

1.5. Birds

During 1991–1997, the survival (Eeva and Lehikoinen 1998) and behaviour (Eeva *et al*. 2000b) of two hole-nesting passerines, Pied Flycatcher (*Ficedula hypoleuca*) and Great Tit (*Parus major*) were studied around Harjavalta.

F. hypoleuca was more susceptible to pollutants than *P. major*, the response of which was weaker in many aspects. The breeding success of *P. major* was below background levels up to 3–4 km from the smelter (Eeva and Lehikoinen 1996) whilst *F. hypoleuca* was affected severely only next to the smelter (ca 1 km) (Eeva and Lehikoinen 1995; 1996). No clear differences in the female condition (Eeva *et al*. 1997a), or in the density of ectoparasites in the nestlings (Eeva *et al*. 1994) of these two bird species in relation to the pollution were found. The different responses of these two bird species were probably due to their different diet (Eeva and Lehikoinen 1996).

The poor breeding success of *P. major* was suggested to be related to habitat changes that have taken place around the smelter, e.g. a scarcity of suitable insect food for nestlings (Eeva and Lehikoinen 1996). The proportion of green larvae in the diet of the nestlings was smaller (Eeva *et al*. 1997b) and the nestlings were lighter (Eeva *et al*. 1998) in the vicinity of the smelter than further away. Air pollution was found to fade the yellow colour in plumage of the *P. major*. Pale plumage might affect mate choice, and predict reduced winter survival (Eeva *et al*. 1998). However, better wintering conditions next to human habitation may in general compensate for the possible detrimental effects of pollutants on the *P. major* population (Eeva and Lehikoinen 1998).

The low local survival rate of *F. hypoleuca* adult females near the smelter was suggested to be caused by higher emigration from the low quality habitat (Eeva and Lehikoinen 1998). However, *F. hypoleuca* nestlings were directly affected by increased amount of heavy metals and the low availability of calcium-rich food items in their diet near the smelter (Eeva *et al*. 2000a). The pollution related stress of *F. hypoleuca* was detected in biomarkers from blood and liver (Eeva and Lehikoinen 1998) and as growth abnormalities of legs and wings and changes in egg shell quality near the smelter (Eeva and Lehikoinen 1995; 1996). The authors suggested that heavy metals might accumulate more in ground living, mobile, often adult, prey items of *F. hypoleuca* than in foliage living, less mobile, often larval, prey items of *P. major*. The concentrations of Cu, Ni and Pb in ants were higher close to the smelter than further away (Table 4) and correlated positively with *F. hypoleuca* nestling faecal concentrations (Eeva and Lehikoinen 1996). Close to the smelter the heavy metal concentrations in ground-living ants and spiders (Koponen and Niemelä 1995) were higher than the concentrations of defoliator species (e.g. Heliövaara and Väisänen 1990c) (Table 4).

The breeding success of *P. major* and *F.hypoleuca* has markedly improved in the vicinity of the smelter between the years 1991 and 1997, and the lead concentrations of the nestlings have decreased by about 90% during this time (Eeva and Lehikoinen 2000). The birds have probably benefited from the decrease in emissions and the practical remediation actions, revegetation after replacing the excavated polluted soil, in parks and along roadsides at Harjavalta. The vegetation recovery has probably promoted the recovery of herbivorous insect populations.

In conclusion, the effects and mechanisms of heavy metal deposition on forest ecosystem are diverse. Cu concentration is high in the forest soil and in different trophic levels of the forest ecosystem. Clear effects on organisms were observed up to ca. 4 km distance from the smelter. However, slight changes in organisms were observed as far as 8 km and in deposition 30 km distance from the smelter.

2. Remediation through *in situ* stabilisation of heavy metal polluted soil

Remediation of heavy metal polluted soils aims to improve the soil properties by removing or immobilising the pollutants. In the immobilisation process metals are converted into forms that are less mobile and less available for plants and microbiota.

Remediation actions for the recovery of plants and animals are needed on soils polluted by atmospheric emissions from burning of fossil fuels or mining and smelting activities, industrial wastes, and on abandoned mine workings. On agricultural soils remediation may also be needed following the spreading of polluted industrial or municipal sewage sludge, fertilisers or pesticides. Vangronsveld and Cunningham (1998) and Knox *et al*. (2000a, b) have recently reviewed remediation techniques of heavy metal polluted soils. Remediation has primarily been based on techniques where the polluted soil is excavated and transported to special landfills. Less commonly soils have been remediated by soil washing e.g. by different extractants. New perspectives on remediation include phytoextraction, i.e. removing pollutants using specific metal accumulating plants, microbial-based techniques, and electroreclamation. Many techniques are expensive and environmentally invasive. Environmentally gentle and less expensive techniques in the remediation are often based on *in situ* stabilisation through revegetation.

Revegetation of industrial barren land was studied around the Severonikel smelter, NW Russia, where the seedling performance was improved by wind sheltering and watering during two growing seasons (Kozlov and Haukioja 1999). Revegetation is often combined with immobilisation of the metals by different soil additives (i.e. immobilisation agents). In this overview the emphasis is placed on copper and the remediation through *in situ* stabilisation of the natural landscape that has been degraded mainly by atmospheric metal pollution. *In situ* stabilisation has also been used to vegetate tailings and mine spoils by adding sewage sludge into soil (e.g. Sabey *et al*. 1990; Kramer *et al*. 2000) and on agricultural soils after the use of polluted sewage sludge. Remediation for agricultural use has been studied by adding gravel sludge (Krebs *et al*. 1999), zeolites (Edwards *et al*. 1999), steel shots, or beringite (Boisson *et al*. 1998; Vangronsveld *et al*. 2000a) into soil.

2.1. Immobilisation agents

In laboratory conditions immobilisation of Zn, Cd, Ni, Pb or Cu in soil has been found with several inorganic and organic agents (Table 5).

In laboratory conditions Cu_{exc} concentration in soil decreased by 28% and Cu concentration in *Phaseolus vulgaris* by 77% after the addition of beringite. The respective values after compost addition were 24% and 51% (Vangronsveld and Clijsters 1992). Synthetic zeolites have decreased Cu_{exc} in soil by 60% (Edwards *et al*. 1999) and Cu in *Lolium perenne* L. by 82% (Rebedea and Lepp 1995). The respective values after a polyacrylate polymer addition were 72% and 77% (Torres and De Varennes 1998). Hydroxyapatite has decreased Cu_{exc} concentration in soil by 97% and Cu in *Zea mays* L. by 72% (Boisson *et al*. 1999a).

Zeolites, gravel sludge, lime, beringite, compost, and sewage sludge have been used as immobilisation agents in the field. These *in situ* stabilisation experiments were often made on soils severely polluted by Zn, Cd, and Pb. Only few reports were found on soils polluted severely by Cu. The results were reported mainly 1–6 years after the start of the experiment.

Table 5. Remediation agents studied in laboratory.

Inorganic agents	
Zeolites	Gworek 1992a and b, Rebedea and Lepp 1995, Chlopecka and Adriano 1996 and1997, Garcia-Sanchez *et al.* 1999, Edwards *et al.* 999
Beringite	Vangronsveld and Cljisters 1992, Mench *et al.* 1994b, Boisson *et al.* 1999b, Oste *et al.* 2001
Fe-oxides	Chlopecka and Adriano 1996 and 1997
Mn-oxides	Mench *et al.* 1994a and b, Boularbah *et al.* 1996, Sappin-Didier *et al.* 1997, Hettiarachchi *et al.* 2000
Apatite	Ma *et al.* 1993, Chlopecka and Adriano 1996, Laperche *et al.* 1997, Chen *et al.* 1997, Boisson *et al.* 1999a and b
Phosphate rocks	Ma *et al.* 1995
Lime	Chlopecka and Adriano 1996, Shuman and Li 1997
Modified montmorillonite compounds	Lothenbach *et al.* 1997
Steel shots	Mench *et al.* 1994a and b, Sappin-Didier *et al.* 1997
Polyacrylate polymer	Torres and De Varennes 1998
Organic agents	
Biomass residues	Fisher *et al.* 1998
Sewage sludge	Sabey *et al.* 1990
Compost	Vangronsveld and Clisters 1992, Shuman and Li 1997

2.1.1. Zeolites

Zeolites are crystalline, hydrated aluminosilicate of alkali and alkaline earth cations that possess infinite three dimensional crystal structure (Mench *et al*. 1998). Nearly 50 natural chemical forms have been recognised and more than 100 forms have been synthesised. The use of zeolites for pollution control primarily depends on their ion exchange and ion-trapping capabilities (Mench *et al*. 1998).

Copper emissions from a copper rod rolling factory at Prescot, Merseyside, UK, since 1975, has resulted in significant changes in the surrounding grassland species composition the copper tolerant *Agrostis capillaris* L. being the dominant plant species (Lepp *et al*. 1997). At this site Vangronsveld *et al*. (2000a) performed a study in which large quantities of dead undecomposed plant material was removed before synthetic zeolites were added, at a rate of 1 l m^{-2}, as a slurry. The authors found that 18 months later, the water extractable Cu concentration in soil had decreased (Table 6) and the root growth of *A. capillaris* had increased. They also performed another experiment on arable soil contaminated with Cd that had been emitted by a Cd pigment manufacturer in 1970s at Staffordshire, UK. After zeolites was added a clear reduction in the Cd concentration of sown plants were recorded (Table 6) (Vangronsveld *et al*. 2000a).

2.1.2. Gravel sludge

Gravel sludge consisted of 42% clay minerals, 31% $CaCO_3$, and to a lesser extent quartz, plagioclase, dolomite and organic matter (Krebs *et al*. 1999). Gravel sludge in a powder form was tilled into 20 cm depth (8–15 g kg^{-1} d.m.) into a soil contaminated with Zn and Cd emissions from a metal-smelter at Giornico, Switzerland, and into a soil contaminated with Zn, Cu and Cd polluted sludge at Dotticon, Switzerland (Krebs *et al*. 1999). Gravel sludge application increased soil pH by 0.5 unit and decreased metal concentrations in soil and in sown plants (Table 6). The metal immobilisation effect of gravel sludge was at least partially a pH effect mostly due to calcium carbonate.

2.1.3. Lime

The first large scale attempt to remediate a heavy metal polluted landscape was in Sudbury, Ontario, Canada. More than 30 km^2 of barren land polluted by mining and smelting activities has been treated since 1978 with surface application of ground dolomite limestone (1 kg m^{-2}), with or without an accompanying fertiliser, and grass-legume seed application (Winterhalder

Table 6. Metal concentrations in soil or in plants before the remediation treatment. The percentage of decrease after the remediation treatment is in the parentheses.

Immobilisation agent	Cu	Zn	Cd	Ni
	mg kg^{-1} d.m.			
Synthetic zeolites				
Vangronsveld *et al.* 2000a				
grassland soil	15^a (74)	2.8^a (50)	0.8^a (44)	
Lactuca sativa L. (arable soil)			11 (54)	
Spinacia oleracea L. (arable soil)			8.1 (40)	
Gravel sludge				
Krebs *et al.* 1999				
arable soil	1.0^b (28)	1.9^b (99)		
Lactuca sativa L.	12 (0)	290 (24)	1.1 (36)	
Lolium perenne	60 (36)	600 (77)	0.4 (50)	
Lime				
Winterhalder 1996				
forest soil	3^a (50)			5^a (66)
Deschampsia caespitosa (L.) Trin.	35 (29)			60 (50)
Pinus banksiana Lamb.	42 (81)	65 (58)		32 (34)
Li *et al.* 2000				
town park soil		195^c (20)	2.0^c (17)	
Festuca rubra L.	12 (20)	620 (40)	3.9 (20)	
Mälkönen *et al.* 1999				
forest soil, sandy	1700^d (36)			300^d (2)
Pinus sylvestris, needles	260 (8)	160 (0)		
Helmisaari *et al.* 1999				
Pinus sylvestris, roots	590 (49)	70 (0)		110 (44)
Beringite and compost				
Vangronsveld *et al.* 1996				
sandy soil		10^a (79)		
Phaseolus vulgaris L.		900 (88)	8.2 (86)	
Sewage sludge and fly ash				
Kelly and Tate 1998				
sandy loam soil		1100^e (85)	63^e (83)	
Sewage sludge				
Li *et al.* 2000				
town park soil		195^c (98)	2.0^c (98)	
Festuca rubra	12 (22)	620 (70)	3.9 (20)	

Extracts used ^{a} H_2O, ^{b} $NaNO_3$, ^{c} $Sr(NO_3)_2$, ^{d} $BaCl_2$, ^{e} $CaCl_2$

1996). Nine years later the treatment had lead to an increase in pH by 2 units to pH 6, a decrease in soil and plant metal concentrations (Table 6) and revegetation by grasses and woody plants.

Li *et al.* (2000) reported on a remediation experiment in a town park in Palmerton, USA, where the soil, with sparse vegetation of Zn-resistant grasses, was badly disturbed by erosion. Limestone treatment, which consisted of applying 0.9 kg m^{-2} $CaCO_3$, the equivalent using dolomite agricultural limestone, increased soil pH from 6 by 0.6 unit and reduced the availability of metals to plants (Table 6).

In the forest soil polluted by the Cu-Ni smelter at Harjavalta, a soil remediation experiment was started in 1992 (Mälkönen *et al.* 1999). The treatments consisted of liming (0.2 kg m^{-2}, granulated Mg rich limestone), applying a powdered slow release mineral mixture, and stand-specific fertilisation determined on the basis of needle and soil analyses (Mälkönen *et al.* 1999). Liming had positive effects on soil chemistry during the 5 study years. It increased exchangeable Ca and Mg concentrations (Derome 2000) and reduced exchangeable Cu and Ni in the soil (Mälkönen *et al.* 1999) (Table 6) with the decreased leaching of metals down the soil profile (Derome and Saarsalmi 1999). Positive effects on tree growth and survival were also detected, liming being the most successful treatment. All the fertiliser treatments increased volume growth of Scots pine (Mälkönen *et al.* 1999) and liming increased the growth and survival of fine roots (Helmisaari *et al.* 1999) and reduced the detrimental effects of heavy metals on experimental seedling survival (Salemaa and Uotila 2001). Liming also increased the phenolic concentration of the phloem, indicating an improvement in the defence level against pathogens of Scots pine (Kytö *et al.* 1998) but did not affect soil decomposer animal community (Haimi and Mätäsniemi 2001). The microbiological response to remediation is reviewed in details in the Results and discussion section.

2.1.4. Beringite and compost mixture

Beringite, a modified aluminosilicate, also called cyclone ashes, originated from the burning of coal refuse from the former coal mine in Beringen, Belgium, (Mench *et al.* 1998). The product contains 52% SiO_2 and 30% Al_2O_3. The pH of the product was strongly alkaline (ca. 11), because of the presence of MgO and CaO. The high metal immobilising capacity of beringite was supposed to be based on the combination of chemical precipitation, ion exchange, and crystal growth (Mench *et al.* 1998).

In Maatheide, Belgium, a zinc smelter operated from 1904 until 1974 and created a desert like area of about 1.4 km^2 where the highest observed total Zn concentration in soil was 16 000 mg kg^{-1} d.m. (Vangronsveld *et al.* 1995). An experimental area of 3 ha was treated with a mixture of beringite and compost. Beringite (12 kg m^{-2}), and compost from municipal waste (10 kg m^{-2} wet weight) was mixed in the upper 35 cm topsoil layer. A seed mixture of metal- and drought-tolerant grasses was sown (Vangronsveld *et al.* 1995). After 5 years the soil pH had increased by ca. 2 units to 7.5, the organic matter content and cation exchange capacity in the soil increased and the exchangeable Zn concentration in the soil decreased (Table 6) (Vangronsveld *et al*. 1996; 2000a). The papers report successful revegetation with clearly decreased phytotoxicity symptoms. Vegetation was healthy and regenerating by vegetative means and seeds. Also the total number of nematodes in soil was increased.

2.1.5. Sewage sludge

At Palmerton, Pennsylvania, the smelting of zinc ore in a narrow valley between 1898–1980 has contaminated the naturally acid soil with Zn, Cd, Cu, and Pb giving rise to 8 km^2 of barren to sparsely vegetated land (Sopper 1989). An experimental area was successfully revegetated when a mixture of sewage sludge (0.5 kg m^{-2} d.m.), power plant fly ash (1–2.5 kg m^{-2}), and lime (0.2 kg m^{-2}) was applied on the soil surface and metal-tolerant ecotypes of grasses were sown (Oyler 1988; Sopper 1989). An increase in pH from 4.5–6.0 to 6.2–6.9 and a decrease in soluble metal concentrations in soil was detected (Table 6) (Kelly and Tate 1998). Li *et al*. (2000) mixed composted sewage sludge, 22 kg m^{-2} d.m., containing 4% Fe and 30% calcium carbonate equivalent, and NPK fertilisation, into soil 15 cm deep in a town park at Palmerton and sowed grasses. The treatment increased the soil pH from 6 by 1.3 units and reduced of the shoot metal concentrations (Table 6) – the effects remaining stable for over 6 years. The treatment resulted in successful revegetation and a disappearance of visual symptoms of metal phytotoxicity.

3. The aim of the study

The aim of the study was to investigate the remediation of forest soil polluted by a Cu-Ni smelter. The *in situ* stabilisation through mulching with organic matter was studied. Since the nutrient cycle in heavy metal polluted soils is, in general, disturbed an important objective in remediation is the recovery of microbial activities. Low microbial activities are accompanied by the increased tolerance to the heavy metals. If the metal stress is removed it is possible that microbial population might lose the tolerance previously acquired thus leaving more energy for metabolism and thereafter also the microbial activities could increase.

The addition of new organic material onto the polluted soil aims to decrease the fractions of heavy metals that are toxic to microbiota by increasing heavy metal complexation. Applying an organic mulch has also other advantages. It can increase the soil pH and thus precipitation of heavy metals, and it can prevent drying and erosion of soil thus promoting revegetation. Organic matter introduces new microbiota into the polluted soil and provides a nutrient source for them.

The Harjavalta area is suitable for studying the remediation of heavy metal polluted forest ecosystem. Knowledge about the forest ecosystem around Harjavalta provides a good possibility to follow the effect of remediation actions on the whole ecosystem. My focus in the thesis is in Cu because it is the dominant pollutant at Harjavalta, it is very toxic to microbiota (Bååth 1989), and it forms complexes with organic matter (Baker and Senft 1995) that are less toxic to bacteria (Hughes and Poole 1991).

I hypothesised that the soil copper fractions that are toxic to bacteria would decrease after mulching. I further hypothesised that if remediation occurred the bacterial tolerance to copper would decrease, the microbial activities would increase, and the microbial community would change.

4. Material and methods

4.1. The modification of the [^{3}H]-thymidine incorporation method (I)

The bacterial growth rate, the bacterial copper tolerance and the toxicity of the soil water to bacteria was studied by the [^{3}H]-thymidine incorporation method. For this the preparation of the bacterial suspension was modified. Two homogenisation techniques (crushing and rotary shaking) and three filtering techniques (glass wool, acid washed glass wool and polyester net) for preparing the bacterial suspension were tested.

The polluted soil was sampled at a distance of 2 km from the Harjavalta Cu-Ni smelter. Undecomposed litter, dwarf shrubs, and lichens were removed, and the organic layer (F+H) (1 to 3 cm) was sampled.

4.2. Experimental design of the microcosm study (II)

Remediation of heavy metal polluted forest soil was studied using nine different organic immobilisation agents in the laboratory microcosm study. The organic soil was sampled at a distance of 2 km from the Harjavalta Cu-Ni smelter. The microcosms were established in 2 l plastic pots. A 50 mm layer (0.8 l) of mulch (organic remediation agent) was spread over the surface of the polluted organic soil (1 l). The treatments are presented in Table 7. The microcosms were kept at 20–28°C in the dark, and moistened with distilled water to 50% of water holding capacity once a week. After 1, 4, 10 and 16 months of incubation three microcosms of each treatment were destructively sampled.

4.3. Experimental design in the field (III and IV)

Remediation through mulching with organic matter was studied at 0.5 km distance from the Harjavalta smelter. The mulch in the field consisted of a mixture of compost and woodchips, which was also used in the microcosm study (II). Woodchips was added to the mulch in order to increase the amount of slow-release carbon source for microbiota and especially for fungi since the biomass of fungi is decreased on heavy metal polluted soils (Pennanen *et al*. 1996). The input of C through mulching was 2 kg m^{-2}. Compost (excluding the woodchips) was added on plots at the rate of 5.4 kg m^{-2} d.m.

Table 7. The treatments, the origin, pH and C:N ratio of the mulch materials in the beginning of the microcosm experiment.

Mulch material		**pH**	**C:N**
Composted sewage sludge	Helsinki Metropolitan area sewage plant	7.7	16:1
Compost	Mature compost from Ämmässuo Waste handling Centre, Helsinki Metropolitan area	7.7	11:1
A mixture of compost and woodchips	The woodchips (diameter < 20 mm) from Scots pine and Norway Spruce stemwood (1:1, volume)	6.4	17:1
A mixture of compost and barkchips	The barkchips from Scots pine and Norway Spruce (Kekkilä Ltd) (diameter < 50 mm) (1:1, volume)	6.1	23:1
Garden soil	Commercial soil (Kekkilä Ltd)	6.7	29:1
Green leaves	Downy birch (*Betula pubescens*)	5.8	11:1
Barkchips		4.9	160:1
Humus	F+H layers, mixed Scots pine and Norway Spruce stand	4.1	33:1
Peat	Low humufied *Sphagnum* peat (Kekkilä Ltd)	4.5	46:1
Control	Polluted F+H layers	3.9	25:1

Each of the 36 sample plots was 5 × 5 m, including a 1 m-wide buffer zone. Eighteen of the plots were covered with a 5 cm-thick layer of mulch (mulch treatment), and the other 18 plots were left untreated (control). The mulch was spread directly over the layer of undecomposed plant litter on the forest floor in summer 1996.

In 24 plots seedlings of four plant species were planted in the pockets (2 l) filled with mulch. In each plot were 49 pockets. For each plant species three plots were then mulched and three was not mulched. Plots with empty mulch pockets and plots with no pockets were established, respectively. One additional treatment without mulch pockets was established in summer 1997. The polluted litter layer (from 0 to 15 cm) and organic soil layer were removed before mulching (the exposed mineral soil mulched). The treatments are shown in Fig. 1.

Soil samples were taken from the organic layer (F+H) below the polluted litter layer on each plot (III). Five samples (each area 10 cm^2) to form a composite sample was taken from each plot using a spoon after the litter layer or the mulch and the litter layer had been removed. Soil samples were collected 4, 11, 16, 23 and 27 months after mulching between October 1996 and September 1998. In 1998 the roots of the planted seedlings were still totally in the mulch pockets and thus the effects of plants on remediation were not included in the experimental layout of this thesis.

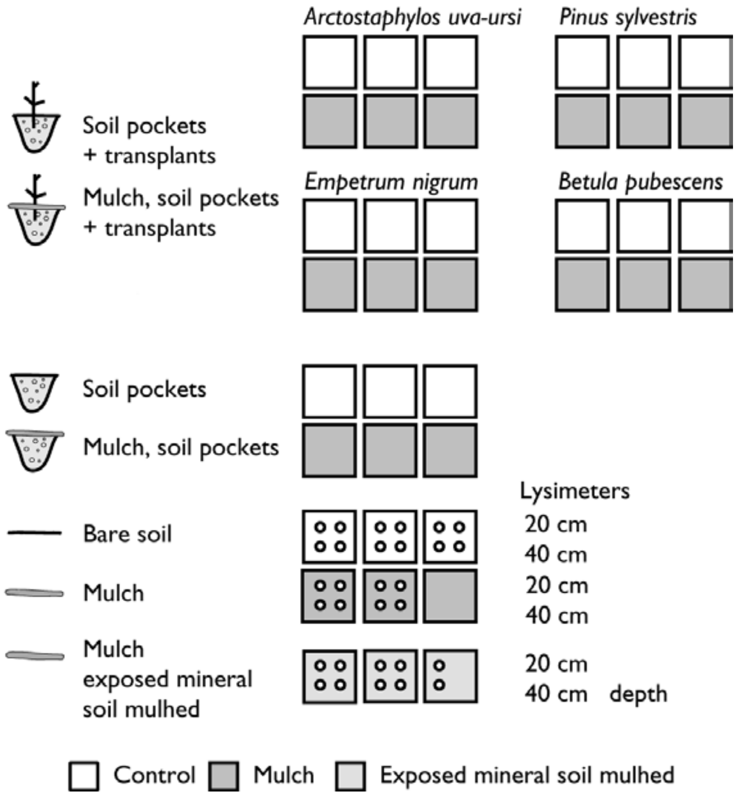

Fig. 1. The experimental design in the field.

Soil percolation water was collected by zero tension lysimeters from control, mulch treatment, and the exposed mineral soil mulched treatment (IV). The plate lysimeters (40 × 25 cm, stainless steel) were installed at the depths of 20 and 40 cm in the plots without mulch pockets. The replicate number of lysimeters at both 20 cm and 40 cm depth was 6 in the control, 4 in the mulch treatment and 5 in the exposed mineral soil mulched treatment (Fig. 1). The lysimeters were sampled once a month between April and October 1999. A total of 36 samples at 20 cm and 38 at 40 cm depth were collected.

4.4. Heavy metal pollution of the soils studied

The total heavy metal concentrations in the organic layer at 2 km distance from the smelter (I, II) were as follows: Cu 1 600 (Derome and Lindroos 1998b) or 3 000 (Pennanen *et al*. 1996), Ni 220, Fe 6 100, Zn 160, Pb 130, Cr 19, and Cd 2.1 mg kg^{-1} d.m. (Derome and Lindroos 1998b). The organic matter content, determined as loss in weight on ignition, was 33%.

The organic layer at a distance of 0.5 km from the smelter (III, IV) contained total Cu 5 800, Ni 460, Fe 20 000, Zn 520, Pb 300, Cr 31, and Cd 5 mg kg^{-1} d.m. (Derome and Lindroos 1998b) and the average organic matter content varied from 26 to 54%.

4.5. Physico-chemical and microbiological analyses

A brief summary of the methods is given here. The detailed descriptions of the methods are in the publications I–IV. The references and analyzed samples are presented in Table 8. The samples consisted of mulches, organic soil, extracts from mulches and soil (bacterial suspension), and soil water fractions. The soil water fractions are referred to as the soil solution and percolation water throughout the text. The soil solution of the organic layer was obtained from the fresh organic soil samples by centrifugation and percolation water was obtained with lysimeters.

From soil samples, dry matter weight, organic matter content, pH, and the concentrations of exchangeable Cu, Cu^{2+} and complexed Cu were measured (Table 8). The concentrations of total Cu, Cu^{2+}, complexed Cu and dissolved organic carbon were measured from soil water samples.

The bacterial growth rate and copper tolerance were determined using the $[^3H]$-thymidine incorporation method. The method was modified for soils with low microbial activity by using shaking and filtering through a polyester net to prepare the bacterial suspension (I). Then the $[^3H]$-thymidine incorporation procedure was performed as Bååth *et al*. (1992b). In the bacterial copper tolerance assay, the bacterial suspension was mixed with a range of $CuSO_4$ concentrations. The growth inhibition was calculated as the Cu concentration (M) giving a 50% reduction in $[^3H]$-thymidine incorporation. The higher the inhibition concentration, the greater is the tolerance of the bacterial community. The toxicity of the soil solution and percolation water to bacteria is expressed as the degree of inhibition of soil water on the growth rate of bacteria extracted from humus of an unpolluted forest site. After staining with acridine orange, the bacterial cells in the suspension were counted.

Microbial respiration activity was measured as the CO_2 evolved. Litter decomposition was studied with litterbags containing green Scots pine needles collected from an unpolluted area. The litterbags were inserted immediately under the polluted litter layer. The bags were removed 27 months after mulching. The litter weight lost was calculated. The growth rate of bacteria was also used as a variable describing microbial activities.

Table 8. The parameters and methods that were used in the articles. Articles are referred I, II, III, and IV, the numbers in the parentheses refer to the sampling occasions (months after mulching) when the parameter was not measured each sampling.

Abbreviation	**Parameter**	**Method**	**Publication for method reference**	**Sample**	**Used in articles**
C N	Total carbon and nitrogen	dry combustion	Nelson and Sommers 1996	mulch	II, III
d.m.	Dry matter	105°C		soil	I, II, III
o.m.	Organic matter	550°C	Howard and Howard 1990	soil	I, II, III
pH	pH	water suspension		mulch soil soil water[a]	II, III I, II, III II(16), III(27), IV
DOC	Dissolved organic carbon	TOC analyser	Greenberg *et al.* 1981	soil water	II(16), III(27), IV
Cu_{exc}	Exchangeable copper	$BaCl_2$ extraction	Tamminen and Starr 1990	soil	II, III
	Total Cu	Ion exchange column	Berggren 1989	soil extract[b]	I
Cu_{comp} Cu^{2+}	Complexed Cu Free Cu			soil water	II(16), III(27), IV
BR	Basal respiration	CO_2 evolved	Pietikäinen and Fritze 1995	soil	I, II, III
m.l.	Mass loss of litter	litter bags	Fritze 1989	soil	III(27)
TdR	[^{3}H]-Thymidine incorporation rate	[^{3}H]-thymidine incorporation	Bååth 1992a	soil	I, II(10-16), III(11-27)
IC_{50}	Inhibition concentration	[^{3}H]-thymidine incorporation	Bååth 1992b	soil	I, II(10-16), III(11-27)
DI	Degree of inhibition	[^{3}H]-thymidine incorporation	–	soil water	II(16), III(27), IV
AO	Number of bacterial cells	Acridine orange staining	Bååth and Arnebrant 1994	soil	II(10-16), III(16-27)
PLFA	Phospholipid fatty acid pattern	Phospholipid fatty acid	Frostegård *et al.* 1996; Pennanen *et al.* 1999	soil extract soil mulch extract[b]	I II, III II(1,16)
 $PLFA_{tot}$ $PLFA_{bact}$ $PLFA_{fung}$	Indicator of total microbial bacterial fungal biomass	Phospholipid fatty acid	Frostegård *et al* 1996	soil	II, III

[a] Soil water was obtained by centrifugation i.e. the soil solution, or collected by lysimeters i.e. percolation water, [b] Soil extract and mulch extract were obtained after homogenisation by shaking and centrifugation.

The structure of the microbial community was analysed by extracting the microbial-derived phospholipid fatty acids from the soil samples. Different subsets of the microbial community have different PLFA patterns in their cell membrane, and a treatment-induced change in the PLFA pattern is an indication of a changed microbial community.

The individual PLFAs were expressed as a mole percentage of the total amount of PLFAs detected in a soil sample. The total amount of PLFAs was used as an indicator of soil microbial biomass. The sum of PLFAs i15:0, a15:0, 15:0, i16:0, 16:1ω9, 16:1ω7t, i17:0, a17:0, 17:0, cy17:0, 18:1ω7, and cy19:0 was chosen as an index of the bacterial biomass, and the amount of PLFA 18:2ω6,9 was used as an indicator of the fungal biomass (Frostegård and Bååth 1996).

4.6. Data analyses

The results of the soil analysis were calculated per organic matter content. The effect of the different types of mulch on the chemical and biological variables was detected using the a priori Dunnett's test to compare the treatments to the control. Canonical correlation analysis (Gittins 1985) was used to investigate the relationships between chemical and biological variables. The PLFA pattern was explored with global non-metric multidimensional scaling (Clarke 1999). Percolation water results were subjected to analysis of variance, with depth and treatment as the main effects, and followed by Tukey's test. Linear regressions of DOC and the toxicity of the soil water to bacteria on metal fractions in soil water were performed. Prior to the analyses, the necessary logarithmic, square root, or exponential transformations were made to normalise the distribution of the variables.

5. Results and discussion

5.1. The [^{3}H]-thymidine incorporation method (I)

The [^{3}H]-thymidine incorporation method, used to measure the bacterial growth rate and Cu tolerance in soil, was modified for the purposes of this study (I). The polluted soil, which had a very low microbial respiration activity (5 µg CO_2 g^{-1} d.m. h^{-1}), gave low mean incorporation values with high standard deviations. This made it difficult to assess the change in the inhibition concentration (IC_{50}) values of Cu after remediation treatments. Therefore the preparation of the bacterial suspension was investigated in order to raise the thymidine incorporation rate (TdR) of soil.

The method is based on the incorporation of radioactive [^{3}H]-thymidine into the macromolecules of bacteria extracted from soil after homogenisation and centrifugation (Bååth 1992a). The technique was previously modified by Bååth (1992b) to determine the heavy metal tolerance of soil bacterial communities and applied to field samples of heavy metal polluted soils by Pennanen *et al*. (1996).

In the original method of Bååth (1992a), bacteria were extracted from soil by intensive crushing and the bacterial suspension was filtered with glass wool to remove humus particles. However, the glass wool increased the pH of the suspension from ca 5 to 7, and gave a lower TdR (8 pmol thymidine g^{-1} d.m.) than the unfiltered suspension (61 pmol thymidine g^{-1} d.m.). In addition, the IC_{50} value was clearly lower after glass wool filtration (0.03 mM Cu) than in the unfiltered suspension (100 mM Cu). Therefore acid washed glass wool and polyester net was tested to filtrate the suspension. When acid washed glass wool or polyester net filtration was used TdR increased compared to glass wool filtration. Therefore the former was preferred as better filtering methods. Also the alternative homogenisation method, rotary shaking, which however had only a minor effect on TdR and the IC_{50} value was tested. In the analysis of articles II, III and IV, the rotary shaking and the filtration with polyester net was used to process the bacterial suspension.

5.2. Remediation studies (II, III and IV)

To study the bioremediation through mulching of heavy metal polluted soil nine mulch materials were tested in the microcosms (II). In the field (III, IV) one of them, the mixture of compost and woodchips, was tested. I focus on this treatment when I compare the laboratory and field experiments.

5.2.1 Copper

During the 16 months of laboratory incubation (II) the Cu_{exc} concentration in the underlying polluted soil was 25–40% lower in the treatments mulched with garden soil, compost, the mixture of compost and woodchips, the mixture of compost and barkchips, and sewage sludge than in the control. The mixture of compost and woodchips, the mulch used also in the field experiment, decreased Cu_{exc} in soil by 33% from 300 mg kg^{-1} d.m. compared to the control. However, in the laboratory incubation the decrease in Cu_{exc} concentration was not reflected in the Cu^{2+} concentration of the soil solution (II).

In the field, the Cu_{exc} concentration in soil was 26% lower in the mulch treatment than in the control (2100 mg kg^{-1} d.m.) 27 months after mulching (III). Cu^{2+} in the soil solution was 81% lower (III) and in percolation water at 20 cm depth 61% (IV) lower in the mulch treatment than in the control. In percolation water at 20 cm depth the total Cu concentration was 31% lower in the mulch treatment than in the control. Liming reduced Cu_{exc} concentration in the organic soil by 36% in 4 years at Harjavalta from 1 700 mg kg^{-1} d.m. (Table 6) (Mälkönen *et al*. 1999), and by ca 50% in soil percolation water at 20 cm depth (Derome and Saarsalmi 1999). Thus the decrease in Cu concentration was on the same level after mulching and after liming. Other remediation experiments shown in Table 6 were performed on soils with a relatively low Cu concentration. Cu_{exc} concentration had decreased by 28%, from 60 mg kg^{-1} organic C, after the addition of gravel sludge (Krebs *et al*. 1999) and 74%, from 15 mg kg^{-1} d.m. in grassland, after the addition of zeolites (Vangronsveld *et al*. 2000a).

The lowest obtained Cu_{exc} concentration in organic soil in the mulch treatment was 1 200 mg kg^{-1} d.m. (III). At 2 km, the Cu_{exc} concentration has been 300 mg kg^{-1} d.m. (II) or 1 000 mg kg^{-1} d.m. (Derome and Lindroos 1998b). Thus, compared to the pollution gradient at Harjavalta, the Cu_{exc} concentration remained at higher level after mulching than it was at 2 km distance from the smelter. The total Cu concentration in the soil solution was 2.7 mg l^{-1} in the mulch treatment and 0.06 mg l^{-1} at 4 km distance (Derome and Lindroos 1998a). In the percolation water at 20 cm depth the total Cu was 1.1 mg l^{-1} in the mulch treatment (IV) and 0.03 mg l^{-1} at 4 km (Derome and Lindroos 1998b). Thus, the total Cu concentrations in soil water after mulching remained clearly higher than at 4 km.

One sink for the observed decrease in Cu_{exc} concentration after mulching was probably the precipitation of Cu due to the increased soil pH (Alloway 1995). In the field (III), the addition of the mulch resulted in ca. one pH-unit increase in the organic layer varying in the mulch treatment between 4.8

and 5.4 during the study. In the laboratory the soil pH was 4.4 in the control and 4.1 in the mixture of compost and woodchips treatment after 16 months of incubation (II). Thus, the pH of the underlying polluted soil did not markedly change in the microcosms. Another possible sink for the observed decrease in Cu_{exc} concentration was the formation of complexes with particulate organic matter (Ross 1996) carried down into the underlying polluted soil from the mulch. It was assumed that relatively large amounts of particulate material were released especially by the compost and the sewage sludge. However, no increase in the organic matter content, measured by the weight loss on ignition (a rough method), was detected in the microcosms (II) or in the field (III). An additional sink for the decreased Cu^{2+} concentration in soil water in the field was probably the formation of complexes with dissolved organic carbon after mulching. DOC concentration was 76 mg l^{-1} in the control and 130 mg l^{-1} in the mulch treatment (III). The respective values for Cu_{comp} concentration were 0.25 and 1.1 mg l^{-1}. In percolation water, the concentrations of both DOC and Cu_{comp} were also higher in the mulch treatment than in the control (IV). In the microcosms, the complexation with DOC was not detected since DOC and Cu_{comp} were not correlated, and in the compost, the mixtures of compost, and sewage sludge microcosms, the DOC concentration was lower than in the control microcosms after 16 months of incubation. DOC concentration was 130 mg l^{-1} in the compost and woodchips mixture and 220 mg l^{-1} in the control (II).

The formation of soluble organo-metal complexes can increase the mobility of heavy metals down the soil profile (Li and Shuman 1997a, b). This has been shown in soils amended with organic wastes, e.g. poultry litter (Jackson *et al.* 1999) or sewage sludge (Brown *et al.* 1997). Mulching the forest floor slightly increased the mobility of Cu compared to the control but leaching was higher after the polluted organic soil and litter was removed before mulching (exposed mineral soil mulched treatment) (IV). Increased leaching of heavy metals is obviously an undesirable phenomena in remediation, although it has been rarely studied. In a semi-field experiment with *ex situ* lysimeters, Vangronsveld *et al.* (2000a) found a strong increase in Cu percolating from polluted soils mixed with compost. When a combination of compost and beringite was mixed with polluted soil the authors reported a slight reduction in percolated Cu compared to untreated soil. There was little information available about whether organo-metal complexes are leached into groundwater. One study detected increased DOC concentration in groundwater after 15 years of heavy application of spent mushroom substrate, rich in DOC, on an agricultural field (Kaplan *et al.* 1995). Albeit, the concentration of DOC in the groundwater was only 2–7%

of that at a depth of 1 m. A laboratory study by Giusquiani *et al*. (1992) also showed that only 5–10% of the DOC extracted from composted sewage sludge leached down to a depth of 50 cm, and 70–80% retained in the upper 10 cm layer. The soil therefore has a high absorption capacity for water-soluble organic matter and mulching the forest floor with compost is not likely to markedly increase the leaching of heavy metals into the groundwater.

5.2.2. The toxicity of soil to bacteria

The change in the toxic fractions of Cu can be assessed by measuring the toxicity of soil to bacteria. Boularbah *et al.* (1996) used a ß-galactosidase-producing strain of *Escheria coli*, which responds to low levels of heavy metals (Bitton *et al*. 1996), and found that the toxicity of soil decreased following the addition of hydrous manganese oxides into polluted soil. Vangronsveld *et al*. (2000b) found the toxicity of soil to bacteria to be decreased after remediation treatment with beringite, when they applied the bioluminescence method with heavy metal-specific (Zn, Cd) biosensor strain *Alcaligenes eutrophus*. Bacteria extracted from unpolluted coniferous humus were used as biosensors in the present study. The inhibition (DI) that the soil water exhibited to bacterial growth rate was measured by TdR method.

The regression of DI on Cu^{2+} concentration in the bacterial suspension was calculated using the samples of II, III and IV articles. The regression was found to be approximately linear between 20 and 80% DI and between 0.05 and 1.5 mg l^{-1} Cu^{2+} ($DI = 77+17 \times \ln Cu^{2+}$, $p < 0.001$, $r=0.73$, $n=88$) (Fig. 2). The regression equation was used to calculate the comparable DI values for the soil solution of the field experiment (III), which was diluted 2-fold compared to the other solutions (II, IV) in the analysis procedure in order to reach the optimum Cu^{2+} concentration to measure the bacterial growth rate.

DI varied very little in the microcosms, between 69–75% (Fig. 2). DI in the control (69%) was lower than in the compost and woodchips mixture (74%). In the field, however, the toxicity of the soil solution to bacteria was clearly lower in the mulch treatment than in the control (Fig. 2). DI in the soil solution was 56% in the mulch treatment and 85% in the control. In percolation water, DI was 29 and 36% at 20 cm and 23 and 25% at 40 cm in the mulch treatment and in the control, respectively.

Cu^{2+} seemed to be the best variable to explain the toxicity of Cu in soil because the correlation between DI and Cu_{tot} was lower than between DI and Cu^{2+} calculated from the data of II, III, and IV articles. The Cu^{2+} in the soil solution also explained more of the biological variation than Cu_{exc} in soil when the canonical correlation analysis was performed (III). Cu^{2+} is generally assumed to be the most toxic form to bacteria, but according to

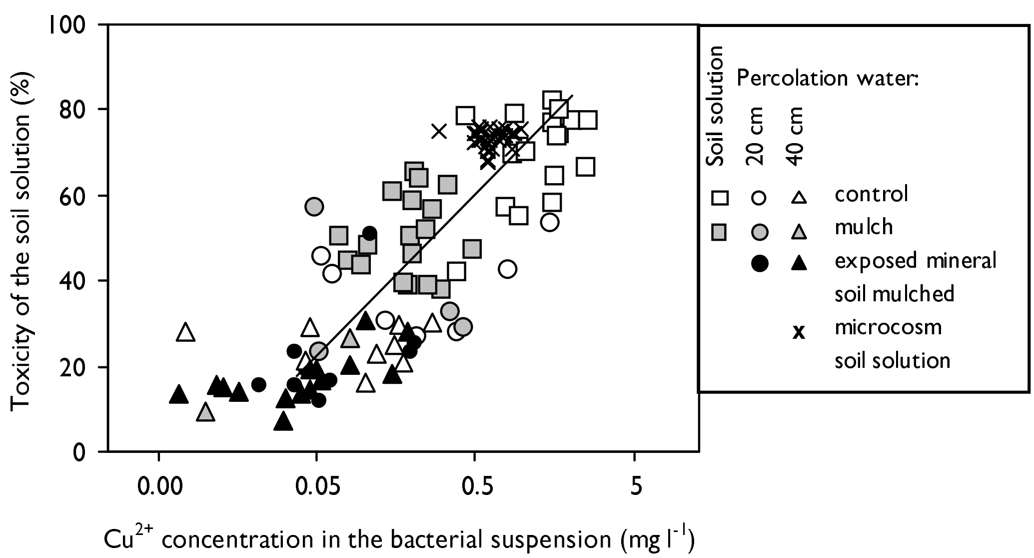

Fig 2. The relationship between the toxicity of solution (DI), measured by TdR method, and Cu^{2+} concentration in the bacterial suspension. Samples are the soil solution and the percolation water at 20 and 40 cm depth in the field, and the soil solution of the microcosm samples.

the review of Giller *et al.* (1998) this has rarely been demonstrated. The most toxic forms of Cu to microbiota were dilute HCl and $CaCl_2$ extractable Cu when the microbial response was measured as the microbial biomass (C_{mic}/ Organic C), dehydrogenase activity (Aoyoma and Nagumo 1997), or the bacterial Cu tolerance (Kunito *et al.* 1999).

5.2.3. Microbial activities and the number of bacterial cells

Microbial activities in the microcosms (II) were in general slightly lower in the compost, mixtures of compost, sewage sludge and garden soil treatments than in the peat, humus or control treatments after 16 months of incubation. Microbial respiration activity was 4.8 µg CO_2 g^{-1} h^{-1} in the control and 4.1 µg CO_2 g^{-1} h^{-1} in the compost and woodchips mixture treatment. The respective values for bacterial growth rate were 26 and 20 pmol thymidine g^{-1} h^{-1} and for the number of bacterial cells 72 and 59 × 10^9 cells g^{-1}. In the field, 27 months after mulching (III), BR was 6.7 µg CO_2 g^{-1} h^{-1} in the control and 10.5 µg CO_2 g^{-1} h^{-1} in the mulch treatment. TdR was respectively 17 and 30 pmol thymidine g^{-1} h^{-1} and AO 41 and 60 × 10^9 cells g^{-1}. BR was 7–80%, TdR 8–180% and AO 8–100% higher in the mulch treatment than in the control during the study. The litter was decomposed 9% more in the mulch treatment than in the control. Thus, the microbial activities in the field were remarkably increased after mulching with compost and woodchips mixture. After sewage sludge and fly ash addition on Zn and Cd polluted soil,

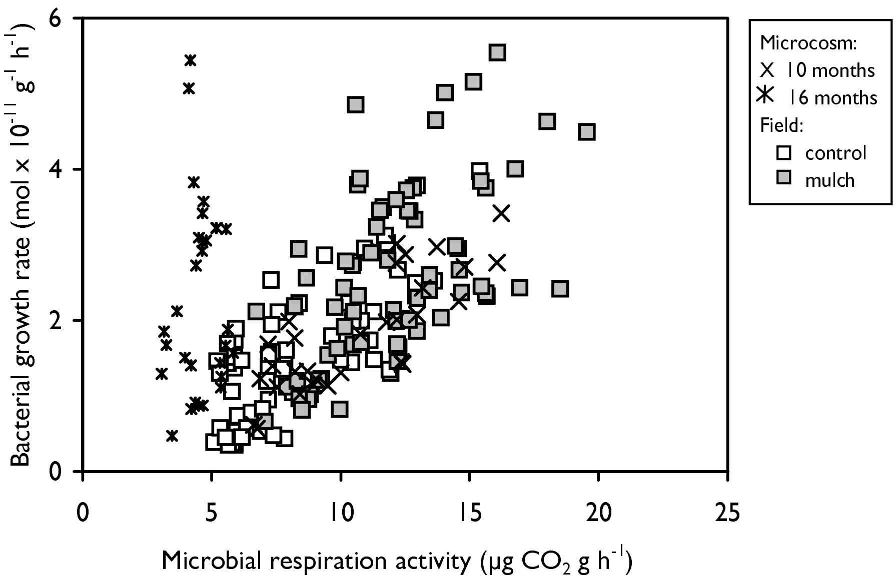

Fig. 3. Bacterial growth rate and microbial respiration activity in the organic soil from the microcosm experiment after 10 and 16 months of incubation and in the control and mulch treatment in the field.

dehydrogenase activity and total viable plate counts increased (Kelly and Tate 1998). The number of bacterial cells increased (Vangronsveld *et al.* 2000a) and mycorrhizal network established in the roots of the sown grasses (Vangronsveld *et al.* 1996) after the addition of compost and beringite. After liming microbial respiration activity increased ca 50% in the heavily Cu polluted soil at Harjavalta (Fritze *et al.* 1996).

The correlation between bacterial growth rate and microbial respiration activity was studied. In figure 3, TdR and BR of all 195 samples from microcosms (II) and field experiment (III) are plotted. When the samples taken after 16 months (II), were excluded the correlation was fairly strong ($r=0.72$, $n=165$).

5.2.4. The microbial community

The structure of the microbial community varied during the study, however no clear change attributable to remediation was detected in the laboratory (II) or in the field (III). Liming of polluted soil at Harjavalta changed the PLFA pattern (Fritze *et al.* 1997). The relative quantities of PLFAs 20:4 and 16:1ω5 increased and i15:0, 16:1ω7t, br18:0 and cy19:0 decreased on the limed plots, as was the case in general for the mulch treatment. The relative quantity of 20:4 increased and br18:0 decreased with decreasing Cu concentration in soil along the heavy-metal pollution gradient at Harjavalta

(Pennanen *et al*. 1996). After mulching most of the PLFAs did not change as could be predicted from Pennanen *et al*. (1996). Thus the microbial community seemed not to change towards the unpolluted sites but rather the slight change might be a consequence of the increase in pH or some other factors related to that. However, the differences in the relative abundance of PLFAs after mulching were very small, and therefore these signs of the changes in the microbial community are only tentative. Changes in microbial community have been found in other remediation studies, such as after liming as mentioned above. Liming changed the PLFA pattern at 4 km from the smelter where Cu_{tot} concentration in soil was 1100 mg kg^{-1} d.m. (Fritze *et al*. 1997). On these sites, also the metabolic profiles of the microbial community, measured using BIOLOG method, changed. The BIOLOG method also indicated change after the application of sewage sludge and fly ash on Zn and Cd polluted soils (Kelly and Tate 1998).

The tolerance of the bacterial community to Cu did not reflect the decreased Cu_{exc} concentration in the microcosms. However, tolerance to Cu decreased in the field after mulching. In the laboratory, 50% inhibition concentration (IC_{50}) was 8 mM Cu in both the control and the mixture of compost and woodchips treatment after 16 months of incubation (II). In the field IC_{50} in the control was 60, 25, 6, and 20 mM Cu 11, 16, 23, and 27 months after the treatment, respectively (III). In the mulch treatment the respective values were 30, 1, 3, and 3 mM Cu. Thus 16 months after mulching, the tolerance was clearly decreased in the mulch treatment to about the same level as at 2 km, where the microcosms soil samples were collected. Pennanen *et al*. (1996) detected about the same IC_{50} values, 2 mM Cu at 2 km and 20 mM Cu at 0.5 km.

When the metal stress was removed by reinoculating the metal tolerant bacteria in an unpolluted soil sample the bacterial tolerance to copper, measured by the TdR method, was shown to decrease within the first week (Díaz-Raviña and Bååth 2001). I found the tolerance to Cu to decrease 16 months after mulching in the field. Preliminary results of Vangronsveld *et al*. (2000a) suggested a loss of bacterial tolerance to several metals 5 years after the addition of compost and beringite into polluted soil. A contrary result was obtained by Kelly and Tate (1998), who found no change in bacterial tolerance to Zn, measured using the plate count method, although the soluble Zn concentration decreased and microbial activity increased 3 years after the application of sewage sludge and fly ash.

Tolerance and adaptation of microorganisms to heavy metals are common phenomenon. The increased or decreased abundance of tolerant organisms can be due to genetic changes, to physiological adaptations involving no alterations to the genotype, or to the changes in species composition (Bååth 1989). A change in species composition has been proposed as the main reason for both the increase (Frostegård *et al*. 1993; Díaz-Raviña *et al*. 1994; Pennanen *et al*. 1996) and decrease (Díaz-Raviña and Bååth 2001) in metal tolerance of microbial populations. In those studies, the change in heavy metal tolerance of the bacterial community, determined by TdR method, was accompanied by a change in the microbial community structure, determined by the PLFA technique. In the present study the microbial community structure showed no changes after 23 months, and only slight changes after 27 months. Despite this, the copper tolerance of the bacterial community decreased 16 months after the exposure to the mulch. Therefore, the results support the alternative hypotheses of genetic change or physiological adaptation of Cu tolerant bacteria to diminishing toxic concentrations of heavy metals. The microbiota that were carried from the mulch down into the underlying polluted soil were presumably not able to maintain their activity in polluted soil either in the laboratory or in the field. This was confirmed with the result that the PLFA pattern of the underlying polluted organic soil in the microcosms did not reflect the PLFA patterns of the mulch extracts (II).

One can hypothesise that the decreased IC_{50} value, measured by the TdR method, may reflect the increased bacterial growth rate instead of decreased tolerance, since rapid growing bacteria are in general more sensitive to external disturbances. Fritze *et al*. (2000) used the TdR method and found that increased microbial activity was accompanied by the increased sensitivity to Cd, after wood ash application. Therefore one possible interpretation could be that in the mulch treatment the faster growing bacteria were more sensitive to external disturbances and thus also to Cu. However, this hypothesis is not supported by the fact that TdR did not correlate with IC_{50}, when the data from articles II and III were included (n=200) (Fig. 4).

The data suggests that the bacterial community had lost its Cu tolerance as a result of decreased toxic Cu fractions in the soil. An alternative hypothesis is that instead of the decrease in the toxic Cu in soil, the main factor for decreasing IC_{50} values in the mulch treatment was the increase in soil pH by one unit (III). This hypotheses is supported by the result of the laboratory experiment where neither the soil pH nor IC_{50} value changed although the Cu_{exc} decreased. If the bacteria had not been adapted to higher pH after mulching, this might have also caused the sensitivity to Cu, since the tolerance of an organism to toxic compounds may change depending on the growth conditions e.g. pH (Bååth 1989). The effect of elevated pH on Cu tolerance was detected in article I, where bacteria growing in pH that was

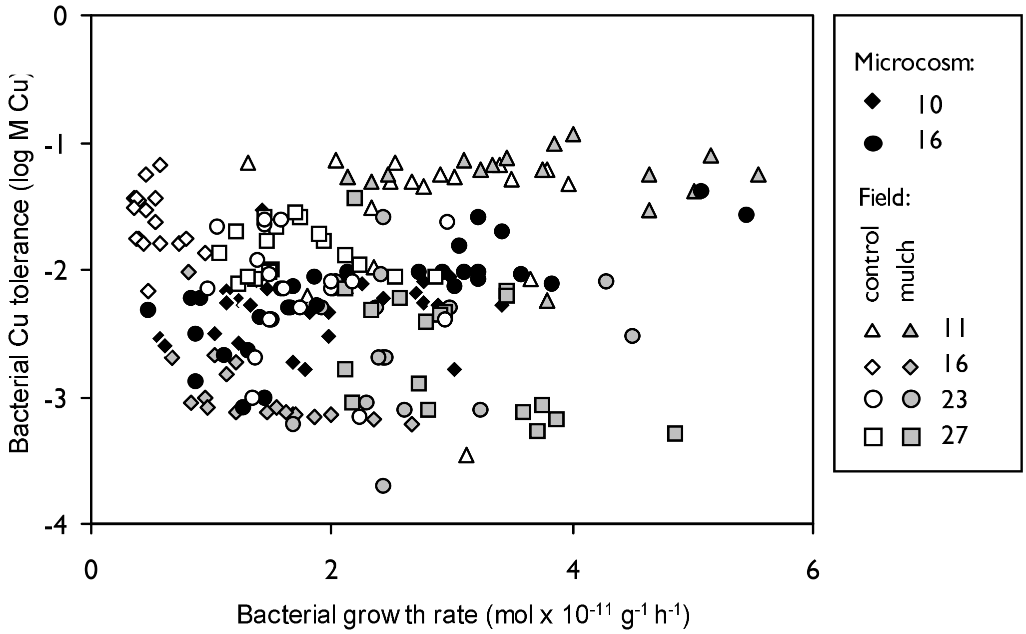

Fig. 4. Bacterial Cu tolerance and growth rate of the samples.

higher than the actual soil pH were more sensitive to Cu than the bacteria growing near the actual soil pH. However, the results from the field (III) showed that pH had increased 4 months after mulching but a clear decrease in IC_{50} was detected only 16 months after mulching. Bååth (1996) found that TdR decreased if the pH of the growth medium was changed from the actual soil pH. The same was detected in article I when the TdR decreased if the pH in the growth medium was higher than in soil. The pH adaptation developed rapidly, in one year, after wood-ash fertilisation Bååth *et al.* 1995; Bååth 1996). Thus it seems probable that following mulching, the bacteria was adapted to the increased pH and that the increased pH was not the main factor explaining the decreasing Cu tolerance in the mulch treatment. The reason was rather the decreased toxic Cu concentration in the soil. In the microcosms there were other microbial successional factors (discussed in Section 5.2.5.) which may have affected the microbial variables rather than the soil Cu or pH.

Soil pH, however, has an indirect effect on the bacterial tolerance to Cu because the availability of metals to microbiota is pH dependent. Metal tolerance increased in soil with relatively low total Cu concentration (94 mg kg^{-1} d.m.) when the soil pH had decreased from 7 to 5 after the addition of polluted sewage sludge (Witter at al. 2000). On the other hand, Pennanen *et al.* (1998) found no increased bacterial Cu tolerance after a low metal and acid load, when the soil pH had decreased from 4.1 to 3.95.

The soil pH affects also microbial activities. The higher pH itself, or some factors related to that, like the change in the organic matter quality, is beneficial to bacteria. I suppose that the quality of organic matter changed after mulching since DOC concentration increased (III, IV). It is well known that microbial activities, including bacterial growth rate, increase with increasing pH in forest soil (Bååth *et al*. 1992; Bååth and Arnebrant 1994). Also in polluted soil samples, the microbial respiration activity increased after liming at Harjavalta (Fritze *et al*. 1996; 1997). Therefore, the increased soil pH, or related factors, after mulching had probably increased microbial activities.

To summarise the changes in polluted organic soil after mulching; the microbial activities increased as a consequence of the increased pH. The increase in pH and the immobilisation effect of the mulch decreased the toxic Cu concentration. Because the metal stress decreased also the Cu tolerance decreased and that further increased the activities of the original microbiota.

5.2.5. Assessment of the microcosm experiment (II)

Several contrary results were obtained in the laboratory and in the field experiment. In the microcosms the decrease in the Cu_{exc} concentration was not reflected in the Cu^{2+} or Cu_{comp} concentrations in the soil solution, toxicity of the soil solution, or microbial activities as was hypothesised. Cu_{exc} in the soil did not correlate with Cu^{2+} but did correlate positively with Cu_{comp} in the soil solution. These results were contrary to my hypotheses and the results obtained in the field study.

One explanation could be that the $BaCl_2$ extractable Cu_{exc} in the microcosms did not reflect the available amount of Cu to microbiota. At the end of the experiments the mean and SD of Cu_{exc} concentration calculated from all microcosms (n=30) was 720±150 mg kg^{-1} o.m. (II), in the field in the mulch treatment (n=18) 2 900±500 mg kg^{-1} o.m., and in the control (n=18) 3 800±400 mg kg^{-1} o.m (III). In the microcosms, where the Cu_{exc} was lower than in the field, the Cu^{2+} concentration and DI of the soil solution remained at relatively high levels being 2.2±0.5 mg l^{-1} and 73±2%, respectively. In the field the Cu^{2+} concentration in the mulch treatment was 1.7±0.9, and DI 56±7%. In the control the respective values were 9.1±3.8 mg l^{-1} and 85±8%.

The soil pH did not increase in any of the laboratory treatments contrary to the one unit increase after mulching in the field. Thus, in the laboratory experiment the pH did not affect the chemical speciation of Cu or the microbial activities. Microbial activities were very low at the end of the incubation. Cells in the underlying polluted soil probably died gradually owing to the toxicity of the organic soil, the stressful conditions, or the loss of available carbon sources in the microcosms. As an indication of the loss of available carbon sources Frostegård *et al*. (1996) suggested the decrease in the abundance of the PLFA 16:1ω5. The amount of 16:1ω5 was higher in the extract of sewage sludge, compost, the compost mixtures or garden soil than bark, peat or humus at the end of the experiment. Therefore the carbon sources seemed not to be depleted in the microcosms mulched with sewage sludge, compost, the compost mixtures or the garden soil. This was, however, not supported by the result that DOC was lower under these mulches than under the control. Microbial cells dye throughout the course of the incubation. The differences in microbial activities may therefore also be due to the successive breakdown of dead cells, and a concomitant increase in the microbiota feeding on them. This could similarly be the reason for the succession in the PLFA patterns of the soil during the incubation (Frostegård *et al*. 1996). The microbial activities were among the highest in the control between 4 and 16 months, indicating stressful and maybe partly anaerobic conditions in the mulched microcosms. The conditions in the microcosm environment for microbiota are different from those in the field. The lack of living roots, the destroyed physical structure of the soil and the limited amount of available carbon set limits when the microbiological properties of soil are studied in a laboratory experiment.

6. Conclusion

In the microcosm study no microbial response direct attributable to remediation was detected. In the field, mulching the polluted soil with the mixture of compost and woodchips converted copper into less toxic forms. The tolerance of the bacteria to Cu decreased and the activities of the original microbiota increased in the polluted organic layer after mulching.

Mulching the polluted forest floor was a successful remediation treatment although the mulch had not yet completely decomposed, and there is a need to study remediation for a longer time period. A possible disadvantage in the studied remediation treatment is the leaching of metals into groundwater. This might be possible to avoid by adding some chemical immobilisation agent to the mulch. Organic mulch addition has advantages, which might promote the vegetation recovery, compared to the addition of solely chemical agents.

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