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Microbial activity and biomass in four Finnish coniferous forest soils — spatial variability and effect of heavy metals

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In the long-term monitoring of forested areas, there is a need for reference values for soil microbiological variables in different types of forest ecosystem under different pollution loads. Owing to the high spatial variation in microbial flora in the soil and to the uncertainty in the measurements, several replicate samples are needed to give a representative estimate of a study site. Four different soil microbiological variables, soil respiration rate, ATP content, ergosterol content and acid phosphatase activity, were measured in four monitoring areas belonging to the Finnish Integrated Monitoring programme. The inhibitory effect of Cd, Cu, and Ni on the soil respiration rate was investigated in a laboratory experiment. The biomass-dependent variables correlated very well with each other, but differed from the variables representing microbial activities. The number of replicate humus samples needed to accurately describe the microbiological status of a study area is clearly site dependent. In general, less than 15 replicates were sufficient to give results with 20% precision at the 5% significance level. The soil respiration rate clearly decreased in response to the experimental heavy metal addition at all four sites. The EC_{20} values of soil respiration for Cd, Cu, and Ni were 100–1 070, 100–700 and 20–510 mg kg^{-1} , respectively.

Introduction

All organic material deposited on or in the soil is decomposed and mineralised mainly through the activities of fungi, bacteria and soil animals. Soil microorganisms are therefore of prime importance in maintaining the fertility of terrestrial habitats, and factors which alter the rates of microbial processes in the soil are consequently of importance for the functioning of forest ecosystems. Moni-

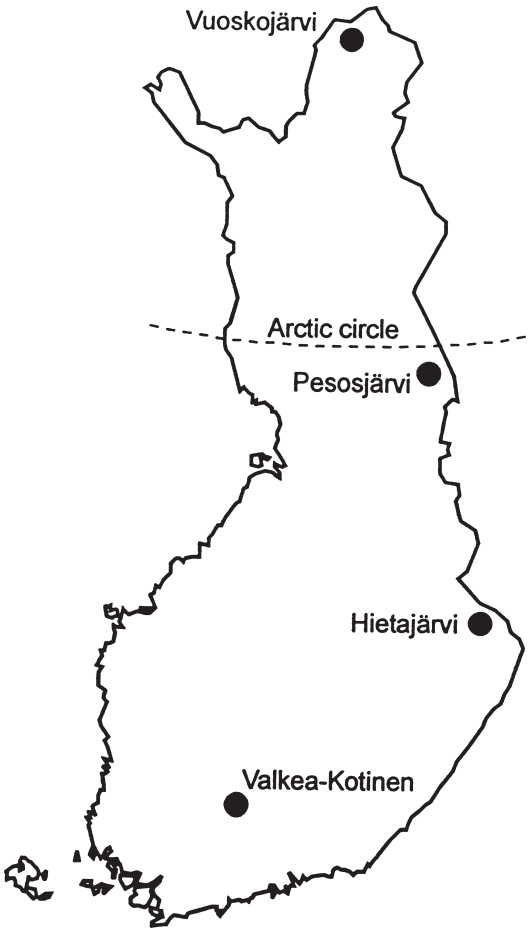

Fig. 1. Location of the study areas in Finland.

toring nutrient cycling via specific components of decomposition processes, such as the microbiological activity and microbial biomass in the soil, can serve as indicator of how ecosystems are affected by pollution stress.

In his literature survey, Ratsep (1991) listed biological variables which seem to be the most promising for monitoring the quality of soil and litter. He emphasised the value of characterising total microbial activity through the measurement of soil respiration and the determination of soil microbial biomass. Kandeler *et al*. (1993) came to the same conclusion and gave methodological recommendations on microbiological measurements for monitoring soil quality, the measurements being the soil respiration rate, microbial biomass, and soil enzymatic activity.

Soil respiration is considered to represent the overall microbial activity reflecting mineralisation of organic matter in the soil, and it is the most commonly used variable in soil studies concerning the effects of pollution such as heavy metals (Bååth 1989). The microbial biomass can be measured in several ways. The soil ATP content, for instance, is considered to be an estimate of the soil microbial biomass in arable soils (Jenkinson *et al*. 1979, West *et al*. 1986) and in forest humus (Vance *et al*. 1987, Arnebrant and Bååth 1991). Ergosterol, an indicator of fungal biomass, is a sterol found only in fungal cell walls (West *et al*. 1987, Frankland *et al*. 1990). One example of soil enzymatic activity are the soil phosphatases which catalyse the mineralisation of compounds containing organically-bound phosphorus into phosphate ions, making it available to plants. Bringmark (1989) recommended soil acid phosphatase activity for monitoring purposes.

In most cases, heavy metal pollution has little effect on the soil respiration rate at low levels of contamination, but with higher doses the soil respiration rate decreases. In laboratory experiments the addition of heavy metals such as Pb (Doelman and Haanstra 1979), or the combination of Cr, Cd, Cu, Zn, and Mn (Chang and Broadbent 1981), or of Cd, Cr, Cu, Ni, Pb, and Zn (Doelman and Haanstra 1984) to soil samples caused a decrease in the soil respiration rates. The results of field studies at forest sites surrounding heavy metal sources, such as smelters, agree with those obtained in laboratory studies (Nordgren *et al*. 1983, Nordgren *et al*. 1986, Fritze *et al*. 1989, Vanhala and Ahtiainen 1994, Fritze *et al*. 1996).

Based on literature data, it is difficult to estimate which heavy metal concentrations in the soil can alter microbial processes. Bååth (1989) reported in his review that the lowest metal concentration yielding an effect on microbial processes in forest humus varied from 1.5 to 25 000 for Cd and from 25 to 5 000 g kg^{-1} soil for Cu. For soil monitoring purposes more exact toxicity levels need to be determined for the study sites in question.

In the long-term monitoring of forested areas, there is a need for reference values for microbiological monitoring variables in different types of forest ecosystem under different pollution loads. Brookes (1995) concluded in his review that there

are problems in the interpretation of environmental measurements owing to the lack of control and background measurements. The detection levels and reliability of the monitoring methods in different types of pollution pattern should also be estimated.

The aims of this study were (i) to determine reference values for microbial activity, microbial biomass and enzymatic activity in four permanent sampling plots situated in Finnish Integrated Monitoring (IM) areas, (ii) to estimate the number of replicate humus samples needed for accurate description of soil microbiological variables in an experimental area, and (iii) to study whether the microbial activity of soils from the four monitoring areas differ with respect to sensitivity to short-term heavy metal exposure.

Material and methods

Study areas

Soil samples were collected from four coniferous forest plots located in the Finnish Integrated Monitoring programme areas (Starr *et al*. 1995; Fig. 1). The areas are situated along a north-south climate gradient running through Finland. In the areas there is no local anthropogenic impact. In Vuoskojärvi the *Uliginosum-Empetrum Myrtillus* (UEMT) site type and in Hietajärvi the *Empetrum-Vaccinium* (EVT) forest site type were both dominated by Scots pine (*Pinus sylvestris*). In Pesosjärvi the *Hylocomium-Myrtillus* (HMT) and in Valkea-Kotinen the *Oxalis-Myrtillus* (OMT) site types were dominated by Norway spruce (*Picea abies*). For further description of IM areas *see* Ukonmaanaho *et al*. (1998).

Soil sampling

The organic top soil was used because it is biologically the most active part of the soil system and it is also the most vulnerable to the effects of deposition. Soil samples were collected from the four plots in autumn 1992, proceeding from north to south in order to obtain samples representing approximately the same season. The 40×40 m study areas were divided into 1×1 m plots, and 25 randomly selected plots were sampled. From each of the sampled 25 plots, a composite sample consisting of 10 humus cores, (diameter 72 mm) was taken from the organic layer down to a maximum depth of 5 cm. The samples were taken to the laboratory, sieved to pass through 4 mm mesh, and stored at 4 °C. A part of the samples was stored for six months at –18 °C for the ergosterol determinations.

Chemical and microbiological analyses

All 25 samples from each of the four areas were handled as replicates in the chemical and microbiological analyses. However, only 10 replicates were used in the ergosterol determinations. Soil samples were dried overnight at 105 °C to determine soil dry weight (dw). The organic matter content was determined as loss in weight on ignition (LOI) for 4 h at 550 °C. All the results have been converted to an oven-dry weight basis. The chemical characteristics of the soil are presented in Table 1.

Soil respiration rate was used as a measure of total microbial activity, and the ATP content as an estimate of microbial biomass. The ergosterol content of the samples was determined in order to

Table 1. Characteristics of the organic layer samples. Loss in weight on ignition (LOI), pH, cation exchange capacity (CEC), base saturation (BS), total nitrogen (N). Concentrations are expressed on the basis of dw.

Study site	LOI %	pH_{H2O} [a)]	CEC [a)]	BS % [a)]	N % [a)]	Cd [a)] (mg kg^{-1})	Cu [a)] (mg kg^{-1})
Valkea-Kotinen	62.6	3.9	228	80	0.97	0.9	7.3
Hietajärvi	90.5	3.6	226	74	1.01	< 0.5	7.1
Pesosjärvi	93.1	3.8	250	76	0.98	0.7	6.3
Vuoskojärvi	95.6	3.7	258	76	1.07	< 0.5	5.1

[a)] Values from M. Starr and L. Ukonmaanaho (unpublished data). Methods are described in Starr *et al.* (1995) and in Manual for Integrated Monitoring (1993).

estimate the amount of fungal biomass. The acid phosphatase activity was measured as an example of soil enzymatic activity.

The soil respiration rate was measured both at *in situ* moisture content and at the water-holding capacity (WHC) of 60%. After incubating the samples (10 g fresh weight) at 20 °C for seven days to stabilise the respiration rate which had been affected by the sieving and water addition, CO_2 evolution was followed for two weeks in an automated respirometer at 20 °C (Nordgren 1988). Soil respiration rate was measured every hour. The mean value of these measurements was used as a basis for further calculations. The soil ATP content was determined from the samples (4 g dw equivalent) as described by Vanhala and Ahtiainen (1994). ATP was extracted from the samples by means of trichloroacetic acid and EDTA and measured on a BioOrbit 1253 luminometer. The fungal biomass, determined as ergosterol contents, was measured using a modified method of Grant and West (1986) according to Fritze *et al.* (1994). Ergosterol was extracted from 1.5 g of fresh soil with methanol and hexane and determined by high performance liquid chromatography.

For the measurement of acid phosphatase activity, the samples were stored at 4 °C for two months in order to stabilise the enzyme activity, which had been affected by the sieving. The samples were kept moist during storage. After the stabilising period the measurement was performed according to the instructions in the Manual for Integrated Monitoring (1993). The measurement was based on the method of Tabatai and Bremner (1969), which involves the determination of p-nitrophenole released by incubation of 1 g of soil dw with p-nitrophenyl phosphate and acetate buffer at pH 5 and at 25 °C for two hours. The reaction end-product was measured on a spectrophotometer at 400 nm.

Heavy metal experiment

The inhibitory effect of Cd, Cu, and Ni on the soil respiration rate was investigated in a laboratory experiment. Four separate composite samples were prepared from the bulk samples taken from each site. Therefore, each composite sample consisted of 25 replicate soil samples from each site. Four replicates of each composite sample were treated with an aqueous solution of $CdCl_2$, $CuCl_2$ or $NiCl_2$ to achieve final Cd, Cu and Ni concentrations of 200, 400, 1 000, 2 000, 4 000, 8 000 and 16 000 mg g^{-1} dw of soil. Metal solutions contained a sufficient amount of water to bring the soils to 60% of WHC. The moisture content was maintained throughout the experiment. Untreated samples served as controls. After 4 weeks incubation at 20 °C, the generated carbon dioxide was measured with an automated respirometer as described earlier for seven days. An incubation time of four weeks was considered to be appropriate because the effects of heavy metals will already be developed but no substrate addition is needed to maintain the microbial activity (Bringmark *et al.* 1998).

Statistical analysis

Variance analysis followed by Tukey's test was performed on the variables measured in the IM areas. As soil respiration and microbial biomass are known to be dependent on the soil organic matter content (Nohrstedt 1985, Vanhala *et al.* 1996), LOI was used as a co-variant in the analysis. We also computed the Pearson's correlation coefficients between the measured variables. In order to estimate the amount of replicate humus samples needed to obtain a measure of a 40×40 m sampling area with the microbiological methods used here, with 20% precision and 5% significance level in the four coniferous forest sites, the following formula was used (Cochran (1977):

$$N = t_v^2 \, (\mathrm{CV}/\mathrm{AE})^2,$$

where N = number of replicate samples needed, t_v = value of student t-distribution at the 5% significance level, CV = coefficient of variation (in %), and AE = allowed error (20%).

In order to compare the effects of heavy metals on soil respiration in soils with indigenous differences in respiration rates, the respiration rate was expressed as a percentage of the rate in the untreated soil. The heavy metal contents of the soil were logarithmically transformed and linear regression models were used to describe the relationship between soil Cd, Cu and Ni content and the soil respiration rate. The data for these soils

had a good fit to the linear models used. The r^2-coefficient varied from 0.81 to 0.93 and the correlation was always significant (t-test $p < 0.01$). In order to determine whether the toxicity of heavy metals varies in different soils, the F-test was used to determine whether the regression coefficients of the models used were statistically different. The regression models were also used to calculate the heavy metal concentration causing a 20% decrease in soil respiration rate compared to the unamended soils (effective concentration, EC_{20}).

Results

Microbial biomass and activity

The results of the soil respiration, ATP and ergosterol content and the acid phosphatase activity measurements are presented in Fig. 2. The soil respiration rates did not differ significantly between the soil samples with an *in situ* moisture content and those adjusted to 60% WHC. The highest respiration rate was found in Pesosjärvi, where the respiration was ca 50% higher than that in the rest of the areas, which were all at the same level (Fig. 2a). The amount of biomass measured as the ATP content and the fungal biomass measured as the ergosterol content increased towards the north (Fig. 2b and c). The ATP contents were higher in Vuoskonjärvi and Pesosjärvi than in Hietajärvi and Valkea-Kotinen. The amount of ergosterol followed the same trend (Fig 2c). Acid phosphatase activities were higher in the spruce-dominated study areas (Pesosjärvi and Valkea-Kotinen) than in the pine-dominated ones (Vuoskojärvi and Hietajärvi) (Fig.2d).

Soil respiration rate measured from *in situ* moisture content and WHC 60% samples correlated significantly ($p < 0.05$) in all four areas as well as the soil respiration rate of WHC 60% and acid phosphatase activity (Table 2). The biomass-dependent variables, i.e. ATP and ergosterol, correlated only in Valkea-Kotinen.

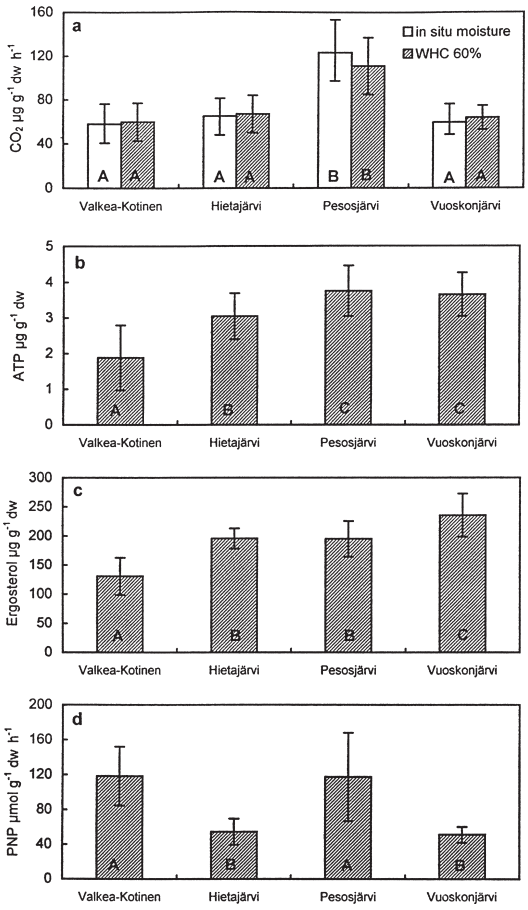

Fig. 2. Soil respiration (a), ATP content (b), ergosterol content (c), and acid phosphatase activity (d) of the soil samples (dw). Standard deviations marked on bars. Bars indexed with different letters (A, B, or C) were significantly different between study areas ($p < 0.001$).

Number of replicate samples needed to obtain estimates of different variables

The number of replicate samples needed for a desired precision in the soil respiration rate measurement was always somewhat smaller for the 60% WHC adjusted samples than for the *in situ* moisture content samples (Table 3). The number of replicate samples for *in situ* moisture content samples varied from 9 to 14, and for the 60% WHC adjusted samples from 6 to 12. Of all the methods tested, ergosterol measurements produced reliable results with the smallest number of replicates. Nine replicates were always enough to yield the desired precision. The Valkea-Kotinen study site was the most diverse of the areas tested, and the number of replicates needed was the highest for most of the variables.

Exposure to heavy metals

The effects of Cd, Cu, and Ni on soil respiration are presented in Fig. 3. Regression models for the effect of Cd, Cu and Ni on the soil respiration rate after four weeks incubation are presented in Table 4. The soil respiration rate clearly decreased due to heavy metal addition at all four sites. The regression coefficients show the effect on soil respiration of increasing heavy metal doses. Significant differences were observed between the regression coefficients in the different areas, and it thus appears that the toxicity of Cd, Cu, and Ni, is dependent on the characteristics of the individual study site.

EC_{20} values for Cd, Cu and Ni calculated from the regression models for the study sites are given in Table 5. The EC_{20} values of soil respiration for Cd, Cu, and Ni were 100–1 070, 100–700 and 20–510 mg kg^{-1} , respectively. The EC_{20} value for Cd, and Ni were lower in the spruce-dominated areas (Pesosjärvi and Valkea-Kotinen) than in the pine-dominated ones (Vuoskonjärvi and Hietajärvi).

Discussion

Nordgren *et al*. (1988) showed that an elevated water content increases the respiration rate of soil

Table 2. Correlation coefficients between the measured microbiological variables.

Area	Soil respiration (*in situ* water content)	Soil respiration (60% WHC)	ATP	Ergosterol
Valkea-Kotinen				
Soil respiration (60% WHC)	0.941*			
ATP	0.234	0.288		
Ergosterol	0.640	0.614	0.845*	
Acid phosphatase	0.548	0.645*	0.598*	0.246
Hietajärvi				
Soil respiration (60% WHC)	0.933*			
ATP	0.638*	0.543*		
Ergosterol	0.593	0.593	0.179	
Acid phosphatase	0.660*	0.668*	0.569*	0.443
Pesosjärvi				
Soil respiration 60% WHC	0.825*			
ATP	0.130	0.192		
Ergosterol	0.286	0.324	0.384	
Acid phosphatase	0.334	0.460*	– 0.201	– 0.114
Vuoskojärvi				
Soil respiration 60% WHC	0.624*			
ATP	0.592*	0.429*		
Ergosterol	0.140	0.278	0.250	
Acid phosphatase	0.485*	0.666*	0.324	0.246

* $p < 0.05$

Table 3. Number of replicate samples needed to obtain estimates of different variables with a 20% precision at the 5% significance level in the different study sites

Study site	Soil respiration (*in situ* water content)	Soil respiration (60% WHC)	Acid phosphatase	ATP	Ergosterol
Valkea-Kotinen	14	12	11	26	9
Hietajärvi	9	8	10	8	4
Pesosjärvi	9	8	21	6	5
Vuoskojärvi	11	6	6	6	5

samples. In this study, however, the soil respiration rate did not differ between samples with an *in situ* moisture content and those adjusted to 60% WHC. These conflicting results may be due to the fact that the *in situ* samples in this study had already a high water content (60%–80%).

The acid phosphatase activity correlated with the soil respiration rate but not with biomass related variables. Thus it seems that the acid phosphatase activity gives an estimate of overall microbial activity. This observation is in agreement with the results of Cochran *et al*. (1989). They reported that the phosphatase activity does not correlate with biomass estimates.

In this study we followed the instructions in the Manual for Integrated Monitoring (1993) concerning storage of the samples before measurement. The aim of storage is to allow the samples to equilibrate after the disturbance caused by sampling and homogenising the samples. Storage may have a different effect on biomass and microbial activity measurements. Further study should be carried out on the influence of storage, especially on the activity of extracellular acid phosphatase and on ergosterol, because the reported impacts are somewhat contradictory (Zelles *et al*. 1991, Davis and Lamar 1992).

Owing to the high spatial variation in microbial flora in the soil and the uncertainty in the measurements, several replicate samples are needed to give a representative estimate of a study site. In this study, 25 replicates from each site were used. In general, less than 15 replicates were sufficient to give results with 20% precision at the 5% significance level. Exceptions to this were the ATP determination at Valkea-Kotinen and the acid phosphatase measurement at Pesosjärvi. At all the sites, 9 or less replicates were needed to accurately measure the amount of ergosterol, while

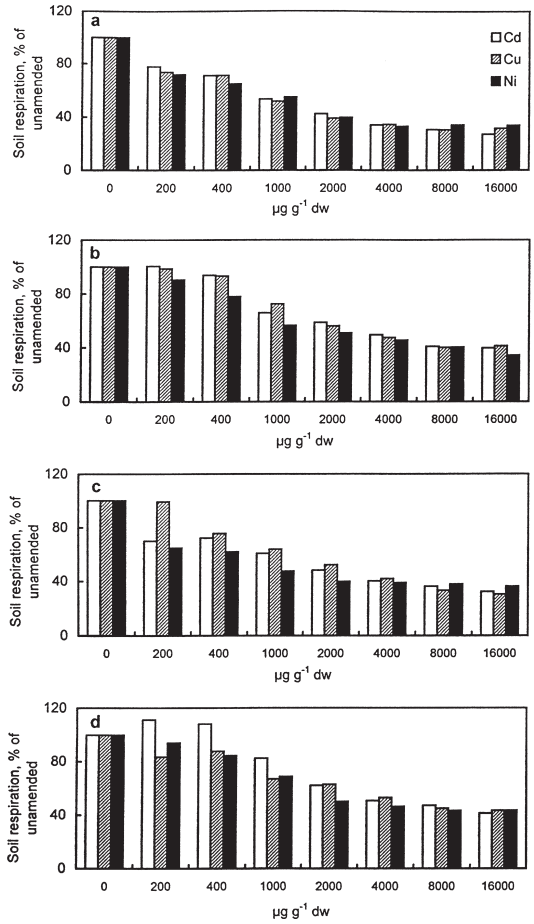

Fig. 3. The soil respiration rate (expressed as a percentage of the rate in unamended soil) of the Cd, Cu and Ni soil samples from Valkea-Kotinen (a), Hietajärvi (b), Pesosjärvi (c), and Vuoskojärvi (d).

Table 4. Linear regression models for the effect of log transformed Cd, Cu, and Ni contents on soil respiration rate in the four study sites after four weeks exposure. Regression coefficients indexed with same letter were not significantly different between metals and study areas. (*F*-test $p < 0.01$, $n = 28$).

Metal	Valkea-Kotinen	Hietajärvi	Pesosjärvi	Vuoskojärvi
Cd	–12.3[1) e,c] + 141[2)]	–15.0[a,b]+177	–10.0[d,f]+127	–16.8[a]+197
	– 0.966[3)]	– 0.945	– 0.956	– 0.930
Cu	–11.1[c,f]+131	–15.7[a]+183	–15.3[a]+172	–10.7[d,e,f]+144
	– 0.932	– 0.956	– 0.955	– 0.938
Ni	– 9.7[d,f]+121	–12.5[b,c,d]+150	– 7.0[g]+ 99	–13.5[a,c]+164
	– 0.926	– 0.909	– 0.899	– 0.939

[1)] Regression coefficient; [2)] Regression constant; [3)] Correlation coefficient.

the other variables required more replicates. The results of the soil respiration measurements indicate that the heterogeneity of the samples is reduced when the water content of the samples is adjusted to 60% WHC. The number of replicate humus samples needed to accurately describe the microbiological status of a study area is clearly site dependent. This should be taken into account when sampling strategies are being drawn up for monitoring purposes.

The toxicity of heavy metals in soils is strongly dependent on the soil texture and its physical and chemical properties, e.g. pH, inorganic cation concentrations and organic matter (Collins and Stolzky 1989). In this study the organic matter content of the samples was high, varying from 63 to 96%. In his review, Bååth (1989) reported decreasing toxicity of heavy metals in soils with high organic matter contents. Humus can serve as a buffer against heavy metals in soils with a high organic matter content, and is even more efficient than the clay in mineral soils (Dumonted and Mathur 1989). Doelman and Haanstra (1984) emphasise the importance of CEC as a factor that affects metal toxicity to microorganisms in soil. However, the soils used in this study had all almost the same CEC. Because the heavy metals were added to soil as chloride salts, the inhibitory effect on soil respiration might partly be caused by chloride. Saviozzi *et al.* (1997) found that heavy metals added as chlorides or sulphates appeared to depress soil respiration more than nitrates.

The calculations show that the lowest EC_{20} values could have been reached even by adding lower concentrations than were used in this study. The EC_{20} values determined for the monitoring areas in question were always much higher than the soil heavy metal concentrations in these areas (Table 1). Such low metal concentrations do not have any measurable influence on soil microbial activity.

Table 5. EC_{20} values of soil respiration for Cd, Cu and Ni (mg kg^{-1} dw) in the study sites.

Study site	Cd	Cu	Ni
Valkea-Kotinen	140	100	60
Hietajärvi	640	700	270
Pesosjärvi	100	380	20
Vuoskojärvi	1070	350	510

In general, the most toxic heavy metal in soil seems to be Cd, followed by Cu, Zn and Pb (Bååth 1989). However, controversial results also exist: Saviozzi *et al.* (1987) found that Cu and Ni reduce soil respiration more than Cd. Also in this study, Cd was not more toxic than the other metals. In addition, the responses to Cd and Ni seemed to be site dependent. The spruce-dominated sites seemed to be more sensitive to Cd and Ni than the pine-dominated areas.

In this study the heavy metals were added as a single dose and, furthermore, the samples were exposed to one metal at a time. In the field, pollution usually proceeds over extended periods and the soil is exposed to mixed pollution comprising a range of elements accompanied, e.g., by a change in pH. Due to long periods of exposure in the field, metals are bound to the organic material harder than in this experiment. Therefore, laboratory experiments are not directly applicable to field conditions, and it will be necessary to carry out complementary field studies. Nevertheless, the laboratory experiments showed potential effects in undisturbed environments where heavy metal pollution had not caused any changes in the composition of the microbial flora.

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