Effects of road deicing salts on soil microorganisms

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ABSTRACT

In this study, the effects of road salting on the quality of forest soils near the road were monitored in the Krkonoše Mountains (Czech Republic). Physical, chemical properties and microbial parameters of soils were determined and the toxic potentials of soil water extracts were evaluated using the bacterial tests (Microtox and *Pseudomonas putida* growth inhibition test). Increased concentrations of Na^+ ions (up to 100 mg/kg) and pH values up to 8 were found closer to the road. Microbial biomass and respiration activity were significantly reduced at the roadside (ANOVA; $P < 0.05$), and the metabolic quotients showed that the microbial community was apparently under stress. Large stimulation of *Pseudomonas putida* growth was determined, especially for salinized samples closest to the road. Oppositely, results showed the unsuitability of bacteria toxicity tests in such cases of pollution. Assessment of intrinsic soil microbial communities is more ecologically relevant and shows the effects that cannot be detected by bacterial toxicity tests.

Keywords: deicing salts; salinization; soil microorganisms; bacterial tests

In many countries, large quantities of deicing agents have been used for decades to melt snow and ice from roads during the winter season; it is a serious environmental problem also in the Czech Republic. Sodium chloride (NaCl) is the most common and preferred deicing agent due to low price, easy availability, storage and handling. In the United States, an amount of eight to twelve million tons of road-salt (NaCl) are applied per year (Wegner and Yaggi 2001). Calcium, potassium, and magnesium chlorides or calcium magnesium acetate are also frequently used. In the Czech Republic, about 300 000 metric tonnes of chemical deicers are used per winter season, of which sodium chloride represents 98%. Despite all of the advantages for traffic safety improvement, there are also many adverse effects following the use of deicer chemicals, namely increased levels of chloride and sodium ions in the environment.

Sodium chloride from road runoff is responsible for increased salinity or osmolality of surface and ground waters still several months after the last road treatment (Thunqvist 2004). Ramakrishna and Viraraghavatan (2005) reported that this could reduce water circulation, reaeration in lower depth that results in changes in population or community structure of aquatic biota. Such effects of deicer salts on freshwater organisms and aquatic ecosystems have been frequently reported (Sanzo and Hecnar 2006). Acute toxicity effects of chloride on aquatic invertebrates are observed at relatively elevated concentration (thousands of mg/l), however such concentrations can occur in some ponds and wetlands (Benbow and Merritt 2004).

In soil environment, salt transport, infiltration and effects depend on a variety of factors and local conditions, such as the slope of the roadside, soil type, proportion of silt and clay, and vegetation cover (Ramakrishna and Viraraghavatan 2005). Increasing amount of Na^+ and Cl^- affects soil structure, dispersion, permeability and osmotic potential and leads to loss of soil stability and also to osmotic stress for vegetation and for soil macro- and microorganisms. It has also been found

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that deicing agents (NaCl and calcium magnesium acetate) may facilitate mobility of several heavy metals found in roadside soils (Backstroma et al. 2004). The Na^+ can also affect soil fertility by exchanging with available nutrients in the soil complex that could eventually lead to cation leaching and subsequently to nutrient deficiencies (Bouzille et al. 2001).

A number of studies reported damages to soil structure and negative effects on vegetation near roads (Butler and Addison 2000, Czerniawska-Kusza et al. 2004) or negative impacts of deicing salt on terrestrial organisms in laboratory tests (Sanzo and Hecnar 2006). On the other hand, relatively few studies have dealt with the impact of deicing salts on soil microbial community so far. The inhibition of sensitive soil microorganisms was observed at NaCl concentration of 90 mg/l and soil nitrification was significantly reduced at the concentration of about 100 mg/kg sodium and 150 mg/kg chlorides (Butler and Addison 2000). In this study we focused on evaluating effects of road salting on soil microorganisms in real field situation.

Soil microorganisms are crucial in the maintenance of structure, quality and fertility of soils. Microbial biomass, its activity and other parameters of soil microbial community are generally accepted as important indicators of soil quality and health. They can serve as early warning of any stress situation in soil environment (Schloter et al. 2003). Hence, it is necessary to investigate how microbial communities are affected by salinization related to using road deicing salts.

In our study, the effects of road salting on soil quality near a road in the Krkonoše Mountains (Czech Republic) were monitored. The state of the soil microbial community was assessed by measuring microbial biomass and respiration activity. The toxicity of soil extracts was evaluated using standard bacterial tests. Results from these two different approaches were compared and also related to the changes of soil physical chemical properties at different distances from the road.

MATERIAL AND METHODS

Soil sampling

The study was performed in the Krkonoše Mountains (located in the northern part of the Czech Republic) in August 2004. Sampling sites were chosen in a forest area along the main road, which follows a contour line in the middle of the hillside. This road is periodically treated with deicing salts (particularly by sodium chloride) during each winter season. In the season 2003/2004, more than 40 tonnes of deicing salts were used per kilometre of road in this region. Three transect lines (A, B and C) perpendicularly to the road were selected with approximately 10 m spacing. The transect lines A and B were characterized by mixed forest (about 60–80 years old) and slope of terrain approximately 30°. The transect line C was characterized by 20-years old spruce forest and slope 5–7°.

Four points were sampled at different distance from the road at each transect line. Control points free from salts (A control, B control and C control) were taken at the uphill, 50 m above the road. Points, where the effects of salting were expected, were sampled at the downslope, below the road, along the possible runoff: roadside points 1 m from the road (A 1 m, B 1 m, C 1 m), points 10 m from the road (A 10 m, B 10 m, C 10 m) and points 30 m from the road (A 30 m, B 30 m, C 30 m). Representative samples of the surface humus layer (0–10 cm) were taken at each sampling point.

Soil was sampled, manipulated and stored in accordance with ISO 10381-6 (1993). For the microbiological analysis and toxicity tests, fresh soils were sieved (< 2 mm) and stored at 4°C in darkness until analysis. For physical-chemical analyses soil was air-dried at laboratory temperature. Maximal water holding capacity (WHC) and dry matter of the soils were measured according to Forster (1995).

The following physical-chemical characteristics of samples were measured by standard methods described in Forster (1995): soil pH value, cation exchange capacity (CEC), conductivity, base saturation (BS), exchangeable acidity (portion of the CEC occupied by H^+ and Al^{3+} ions), organic carbon content (C_{org}), ion concentrations in water and $BaCl_2$ extracts and total Zn and Cd concentrations.

Microbial parameters

A set of microbiological parameters of soils was measured, including microbial biomass content, basal and substrate-induced respiration. All measurements were carried out in triplicates using 10 g portions of soil. Microbial biomass was measured as microbial carbon content (C_{bio}) by the chloroform fumigation extraction method according to ISO 14240-2 (1997). Organic carbon was extracted with 0.5M K_2SO_4 from non-fumigated and chloroform fumigated soil samples and then determined by dichromate digestion. The soil microbial biomass (C_{bio}) was calculated as differences between organic carbon in fumigated and non-fumigated variants.

Basal and substrate induced respiration were analyzed after 4 days of soil preincubation at 40% of WHC and 20 ± 2°C. Basal respiration (BR) was quantified according to ISO/DIS 16072 (2002) after 24 h at 20 ± 2°C in closed glass jars with soil adjusted to 60% of WHC and without any addition of substrate. Substrate induced respiration (SIR) was measured as the response to glucose addition in the first 6 h of incubation at 20 ± 2°C (ISO 14240- 1, 1997). Glucose (5 mg/g dry soil) was added to samples as a water solution, used at the same time for the WHC adjustment to 80%. Respiration was determined as CO_2 production using GC with H_2 mobile phase, Porapack Q stationary phase and thermal conductivity detector.

The physiological condition of the soil microbial community was described using eco-physiological quotients – qCO_2 and ratio C_{bio}/C_{org} (Anderson 1994). The metabolic quotient qCO_2 was calculated as the ratio of SIR to C_{bio}. The microbial quotient C_{bio}/C_{org} was calculated as the ratio of soil microbial biomass carbon to soil organic carbon.

Bacterial toxicity tests

Two bacterial toxicity tests were used to determine acute toxicity of soil water extract – *Vibrio fischeri* bioluminescence inhibition test – Microtox (ISO 11348-3, 1998) and *Pseudomonas putida* growth inhibition test (ISO 10712, 1995). Soil was extracted by deionised water (for *P. putida* growth inhibition test) and a solution of 2% NaCl (for the Microtox test). Soil extracts were prepared in a ratio of 1:2 (soil to water or 2% NaCl) but for two samples (A 30 m and B 30 m) the extraction ratio was 1:4 (soil to water or 2% NaCl) because of the high sorption capacity of the samples. In all cases 5 g of soil samples were used for extraction. If the pH of the extract was not in the optimum range 6–8 it was adjusted to pH 7. The *P. putida* test was performed as a miniaturized microplate growth inhibition assay according to Schmitz et al. (1998) in growth medium according to ISO 10712 (1995). The optical density of samples was measured at 436 nm after 16 h incubation at 23°C using a microplate spectrophotometer. The results were related to the response measured in the growth medium and expressed in percents. The luminescence inhibition of *V. fischeri* was detected by a luminometer after 15 and 30 min exposition at 15°C. The results were related to the luminescence in pure medium (2% NaCl) and expressed in percentual values.

Data analysis

Significant differences among control and affected points were determined by analysis of variance (ANOVA) at $P < 0.05$ using the Newman-Keuls test. The relationship between the microbiological and physical-chemical parameters was assessed by non-parametric Spearman correlation analysis because of low amounts of samples (n = 12) and non-normal distribution of the values.

RESULTS AND DISCUSSION

The results showed, that the transect line C was slightly different from transect lines A and B. The reason of this may be found mainly in different character of terrain, slope and vegetation cover. At the transect line C there was young spruce forest and relatively plain terrain (slope 5–7°) and thus soil properties and runoff conditions were different. General effects of salting were, however, obvious at all three transect lines.

The physical-chemical characteristics of the sampled soils are summarized in Table 1. All soil samples were classified as cambisols. As expected, soil properties significantly differed between the controls and runoff transect points. The most apparent effects were detected at the distance of 1 m from the road. Substantially increased levels of dissolved elements (Na^+, Ca, Mg), originating from road salts, caused an increase of pH value, base saturation and cation exchange capacity. Oppositely, exchangeable acidity, amount of organic carbon and concentrations of chloride ions increased with increasing distance from the road. Low pH levels of forest soils (3.8–4.0 at control points) were increased up to neutral or alkaline values. Several studies also reported that saline soils could have higher pH values (about 7.5–8) mainly due to sodium cation accumulation and consequently higher base saturation (Bouzille et al. 2001, Czerniawska-Kusza et al. 2004, Ramakrishna and Viraraghavatan 2005).

Table 1. Physical-chemical characteristics of the studied soils

Transect points	pH (H_2O)	C_{org} (%)	BS (%)	CEC (meq/100 g)	EC (µS/cm)	EA (meq/100 g)	Total extraction (mg/kg)		Water extraction (mg/kg dw)				0.1M $BaCl_2$ extraction (mg/kg dw)			
							Cd	Zn	Ca^{2+}	Na^+	Cl^-	SO_4^{2-}	Ca	K	Mg	Na
A control	3.78	12.00	21.4	10.5	71	8.24	0.35	49.9	31	11.9	10.1	41	331	53.1	46	17.2
A 1 m	7.63*	4.52*	99.3*	19.0*	64	0.14*	0.35	201.0*	18*	74.3*	4.7*	15*	2910*	76.8	379*	239.0*
A 10 m	5.00	11.90	94.5*	20.8*	62	1.14*	0.82*	108.0*	32	20.3	18.3*	35	3160*	180.0*	400*	40.0
A 30 m	4.10	22.50*	61.4*	21.4*	119*	8.25	1.08*	72.2*	32	20.2	–	74*	2000*	190.0*	303*	44.0
B control	3.89	5.57	13.6	9.4	55	8.15	0.35	65.7	22	5.3	4.2	43	152	65.8	40	6.8
B 1 m	7.70*	7.35	99.4*	18.9*	96*	0.11*	0.35	160.0*	12*	104.0*	4.2	27*	2380*	139.0*	297*	951.0*
B 10 m	5.50*	10.60*	92.5*	17.3*	104*	1.29*	0.35	90.2*	20	97.0*	20.0*	58*	2210*	125.0*	262*	577.0*
B 30 m	3.97	20.80*	63.2*	15.0*	68	5.52*	0.79*	42.6*	23	7.8	16.7*	28*	1560*	62.7	180*	17.3
C control	3.94	9.83	10.8	9.5	49	8.51	0.35	14.8	17	8.0	14.5	50	138	33.4	25	11.4
C 1 m	8.13*	4.43*	99.2*	12.6*	85*	0.10*	0.35	59.7*	8*	102.0*	3.5*	15*	1220*	25.2	152*	1180.0*
C 10 m	4.33	4.64*	17.6	5.6*	43	4.58*	0.35	10.5	12*	23.3*	20.0	15*	94	61.6*	24	35.8
C 30 m	4.72	5.64*	42.6*	6.2*	93*	3.54*	0.35	19.4	14	59.0*	73.0*	39*	339	22.8	50	107.0

C_{org} – organic carbon content; BS – base saturation; CEC – cation exchange capacity; EC – electric conductivity at 25°C; EA – exchangeable acidity
*statistically significant difference ($P < 0.05$) from the control point of each transect

Soil microbial properties were influenced by different physical-chemical properties but it is not easy to distinguish the effects of parameters changed by salting and the effects of natural soil properties. Microbial parameters changed at transect points showing significantly lower values at 1 m distance than at control points (Figure 1). At the transect lines A and B, increase of microbial biomass and respiration activities followed at 10 m and 30 m distance from the road. Microbial biomass and respiration were correlated significantly with organic carbon content (Spearman *R* coefficient: 0.87 and 0.81, respectively; $P < 0.05$). Therefore, the microbial quotient C_{bio}/C_{org} was calculated to eliminate the impact of C_{org} to microbial biomass values (Figure 2). High values of this quotient show potential for microbial growth, whereas low values indicate a stress situation (Anderson 1994). In our study, lower values at runoff points showed adverse conditions for the microorganisms. This negative impact was apparent up to 10 m from the road.

To avoid the influence of C_{bio} content on respiration activity, the metabolic quotient qCO_2 was calculated. Higher values indicate increased energy demands of soil microorganisms and higher maintenance energy induced by stress conditions (Anderson 1994). Increased values of qCO_2 in our study signalized a stress situation up to 10 m distance apparently caused by salinization. This is also confirmed by high correlation between qCO_2 and majority of physical chemical properties

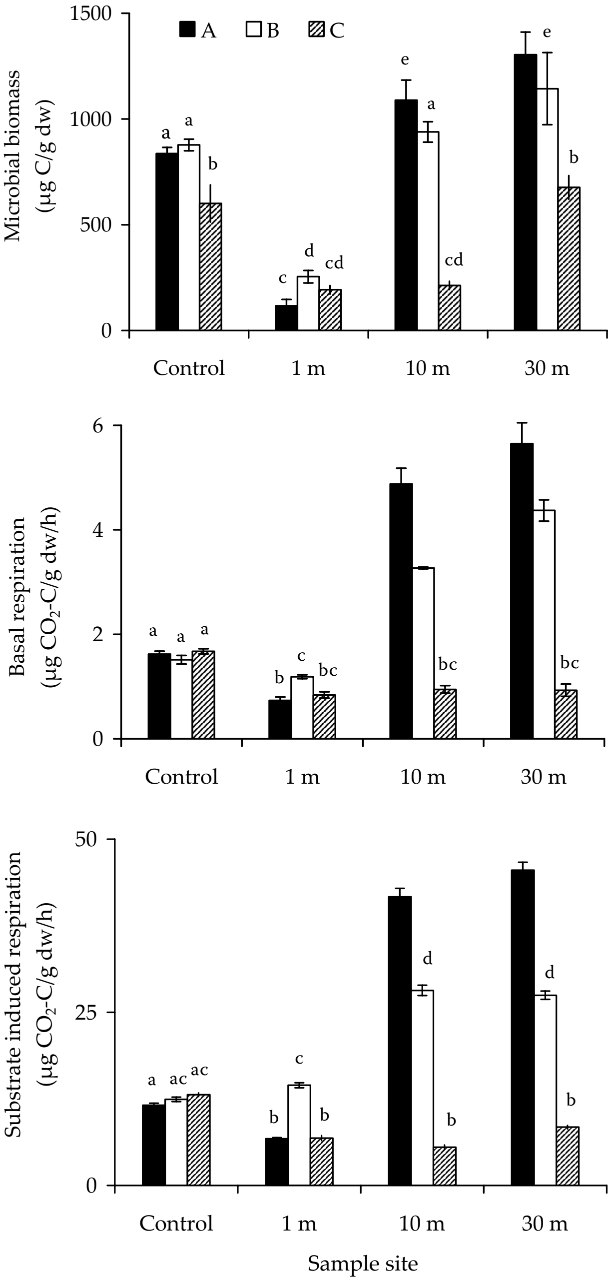

Figure 1. Microbial biomass, basal and substrate-induced respiration activity of soils. A, B, and C are transect lines. The results are expressed as mean ± standard deviation. The same lower case letters above bars mark statistically not significant differences ($P < 0.05$) among samples

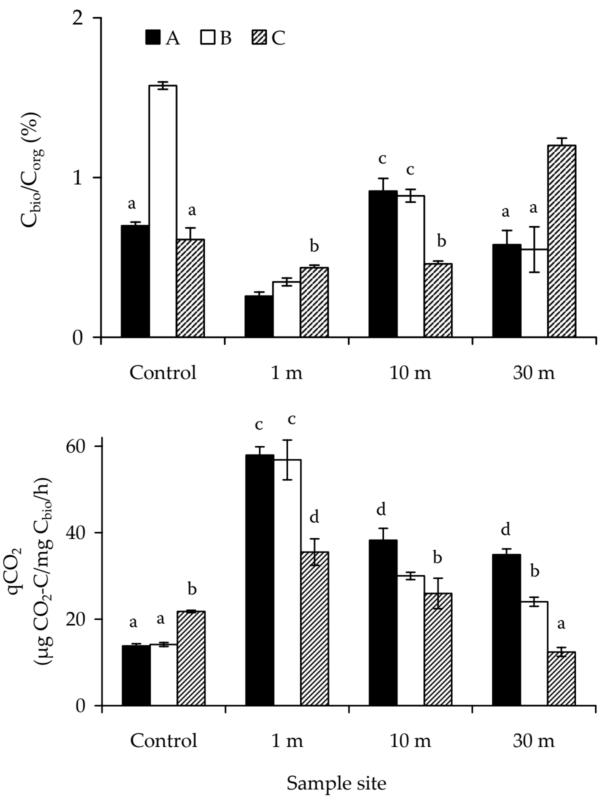

Figure 2. Microbial physiological state of soil: relative microbial biomass (C_{bio}/C_{org}) and metabolic coefficient qCO_2-SIR (SIR/C_{bio}). A, B, and C are transect lines. The results are expressed as mean ± standard deviation. The same lower case letters above bars mark statistically not significant difference ($P < 0.05$) among samples

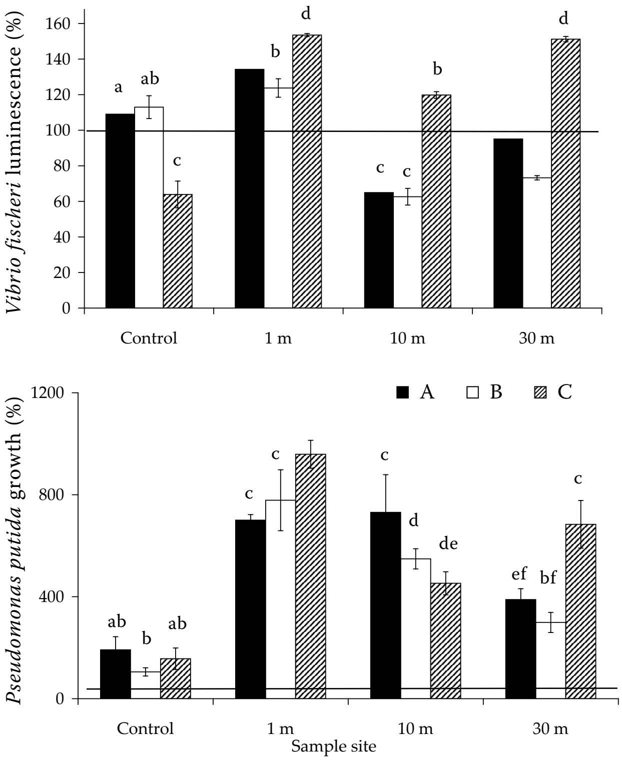

Figure 3. Bacterial toxicity tests of soil water extracts: *Vibrio fischeri* bioluminescence inhibition test – Microtox and *Pseudomonas putida* growth inhibition test. A, B, and C are transect lines. The results (mean ± standard deviation; n = 3) are expressed as percentage of the luminescence or growth in pure test medium without soil extracts (this is equal to 100%, which is shown by horizontal line in the charts). The same lower case letters above bars mark statistically not significant difference (P < 0.05) among samples

like Na^+ concentration, pH, CEC, base saturation (Spearman R coefficient: 0.59, 0.76, 0.74, 0.82; P < 0.05). Negative impact of soil salinization on soil microbial characteristic (biomass, qCO_2) was observed also in other studies. Yuan et al. (2007) reported that higher salinity in arid soil in China resulted in a smaller, more stressed, microbial community, which was less metabolically efficient. Restriction of microbial metabolism and C mineralization in salt-water wetland were detected also in Mamilov et al. (2004).

Opposite results than for indigenous microbial biomass were observed for bacterial toxicity tests (Figure 3). For *Vibrio fischeri* test, no clear relation between the luminescence and distance from the road was found. Results from the growth inhibition test with *Pseudomonas putida* indicated a strong stimulation (P < 0.05) at all runoff points with the highest effect at 1 m distance from the road. Strong stimulation was apparent also at 30 m distance from the road when compared with very low stimulation at control points. Joutti et al. (2003) found out that NaCl and other chloride deicers ($CaCl_2$ and $MgCl_2$) were not toxic for *V. fischeri* in luminescence inhibition assay in the laboratory test. The comparison with salt effects on soil natural microbial community is, however, not much relevant because these tests use laboratory bacterial strains, which prefer salt-enriched media. On the other hand, bacterial tests with soil extracts could provide additional information about toxicity and contamination of soil samples. In this case, growth stimulation could be used as an indication of salts dissolved in the soil extracts. The significant negative correlation with the amount of sodium ions and pH (Spearman R coefficient: –0.88 and –0.94, respectively; P < 0.05) also confirmed that these species were strongly stimulated by salinization.

Simple bacterial tests were proved as a very useful tool for ecotoxicity screening i.e. during bioremediation processes (Bundy et al. 2004). On the other hand *P. putida* was considered to be not sensitive enough to differentiate between contaminated and clean soil (Ahtiainen et al. 2003). Another drawback is that only soil extracts are used in these tests. It is known that soil microorganisms in natural ecosystems are, however, affected not only by dissolved but also by solid phase bound contaminants (Ronnpagel et al. 1998). From this point of view parameters of indigenous microbial community are more ecologically relevant because they indicate a long-term impact on microbial decomposition abilities and on terrestrial nutrient cycles.

In conclusion, a negative impact of the road deicing chemicals on soils was evident. Soil microorganisms showed to be stressed by environmental conditions and indicated higher energy requirement. On the other hand, increased soil salinity caused rather stimulation effect in single species bacterial tests with water extracts of soils. From this point of view, assessment of intrinsic soil microbial communities is more relevant to risk characterization in the real situation and shows the effects, which cannot be detected by bacterial toxicity tests.

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