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Effects of Fluoride on Soil Fauna Mediated Litter Decomposition

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ABSTRACT

To evaluate the effect of potentially toxic compounds on soil fauna mediated carbon and other mineral fluxes in poplar litter, a terrestrial micro-ecosystem (MES) was developed, with the isopod Porcellio scaber *as a representative soil fauna species. An experiment was conducted, in which MES with isopods were compared to MES without isopods. Potassium fluoride was used as a model compound.*

The isopods influenced the microbial respiration negatively. No effect was found for the isopods on the nitrogen and phosphorus mineralization.

After the first four weeks of the experiment the total respiration of the MES was increased and the phosphate concentration decreased at the highest fluoride concentration. After nine weeks, fluoride decreased ammonium, nitrate and phosphate concentrations in the litter.

It is concluded that nitrogen and phosphorus mineralization in the MES are more sensitive to effects of fluoride than microbial respiration. The no observed effect concentrations for net mineralization of ammonium, nitrate and phosphorus were, respectively, 17, 5·3 and 53 μmol fluoride per gram dry weight of litter. Fluoride seems to be toxic for microbial processes at concentrations found in moderately polluted areas.

INTRODUCTION

Fluoride is widely distributed in nature and a common element in rocks and soils. Normal total concentrations are 10–15 $\mu mol\, g^{-1}$ dry mineral soil

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(Elrashidi & Lindsay, 1987), although wider ranges have been reported. Considerable amounts are added to the soil as a result of atmospheric contamination by industries. Among the emission sources are aluminium smelting works, phosphate processing operations, combustion of coal, and the manufacturing of steel, brick, tile, clay and glass products (Groth, 1975). Awareness of the environmental problems of atmospheric fluoride has been increased by the fact that fluoride causes economic damage to agricultural crops and forests (Bunce, 1984; Van der Eerden *et al.*, 1988).

The organic horizon of soils is the main site for biological activity, but also a sink for many pollutants. Accumulation of fluoride in litter layers up to 36·6 μmol g^{-1} (acid-extractable) has been found in the vicinity of an aluminium reduction plant (Tennessee, United States, Beyer *et al.*, 1987). Total F-concentrations in humus decreased from 142 to 32 μmol g^{-1} over a distance of 8·8 km from an aluminium smelter in Switzerland (Polomski *et al.*, 1982). In the organic layer of the soil, decomposition and mineralization of nutrients takes place. The decomposer community, composed of microflora and detritivorous invertebrates, may be exposed to relatively high levels of fluoride. Accumulation of fluoride in invertebrates near an aluminium reduction plant (Wales, Great Britain) has been found (Buse, 1986). The overall sequence of increasing fluoride concentrations tended to be: herbivores–omnivores–predators–detritivores. Andrews *et al.* (1989) found also highest fluoride concentrations in invertebrate detritivores, compared to invertebrate herbivores and carnivores. Detritivores play a functional role in decomposition and mineralization processes (Anderson, 1988), partly by their own contribution to these processes, but also as stimulators of microbial activity.

Accumulation of fluoride may have negative effects on the invertebrates and their ability to function. (Sub)lethal and beneficial effects for insects, exposed to fluoride, have been found. In general, toxicity was dependent on the fluoride compound being tested (Alstad *et al.*, 1982). For vertebrates, low concentrations of fluoride are indispensable for the growth of bones and teeth, but there is a narrow concentration range between beneficial and harmful effects. Toxicity for plants and vertebrates has been described (Ernst & Joosse-Van Damme, 1983, p. 112–117). For microbial processes in soils the toxicity of fluoride is poorly documented.

In order to evaluate the effects of chemical compounds on the ability to function of two different groups of decomposers, we developed a standardized experimental design with micro-ecosystems, so that the effects on microbial and invertebrate functions could be separated. The two groups are represented by the soil arthropod *Porcellio scaber* Latr. (Isopoda, Crustacea) and the undefined microbial community in poplar litter (*Populus canadensis*). A description of the system and its behaviour in an

uncontaminated situation has been given by Van Wensem (1989). In the present paper the results of an experiment, using potassium fluoride, are reported.

MATERIALS AND METHODS

Poplar litter was collected in the Roggebotzand forestry, near Dronten, The Netherlands, approximately two months after litter fall (January 1989). The leaves could be clearly distinguished from leaves of previous years; all leaves collected were approximately the same age. The leaves were air dried and cut into pieces of approximately 4 cm^2. This material was stored for two months at room temperature (20°C). No attempt was made to defaunate the litter completely. Methods used for this (e.g. freezing) have an undesired increasing effect on nutrient availability. The presence of some microfauna cannot therefore be excluded, but is assumed to be the same in all treatments.

Contamination and rewetting of litter

Prior to the experiment the litter was rewetted and contaminated with potassium fluoride (KF), by putting bulk portions of 60 g (two for uncontaminated litter and one for each F-concentration) in plastic 3-litre bowls. To each bowl, 300 ml of demineralized water was added and mixed thoroughly with the litter. After this, water was allowed to evaporate at 20°C with forced ventilation, until the litter contained about 230% water on a dry weight basis (after 36 h). In this period the litter–water mixture was regularly stirred. Contamination was achieved by adding an amount of KF to the 300 ml water, such that after evaporation the litter contained the intended concentration of F on a dry weight basis. In earlier experiments the same procedure was used for contamination with cadmium. Analyses of 5 mg milled litter samples had shown that the contamination procedure resulted in highly reproducible cadmium concentrations.

The fluoride concentrations were chosen in a geometrical series: 1·7, 5·3, 17, 53 and 170 $\mu mol\ g^{-1}$ F. Litter portions of 13·2 g, an equivalent of 4 g dry litter, were transferred to micro-ecosystems (MES). Control MES received uncontaminated litter.

Experimental design

A micro-ecosystem consists of a perspex cylinder, 12 cm high and 6 cm diameter, and is designed to allow measurement of carbon dioxide production from litter using an infra red gas analyzing system, equipped

with high precision flowmeters. For details of MES and carbon dioxide measurements see Van Wensem (1989). Each MES was filled with a litter portion which was placed on a layer of sand (approximately 4 cm). The sand was wetted to holding capacity with demineralized water. The litter and sand were separated by a nylon gauze (mesh size 500 μm) to prevent contamination. The MES were placed in containers (40 × 60 × 20 cm), which were almost closed to prevent desiccation, and kept at 15°C, 16 h dark and 8 h low light intensity. Each week, after the carbon dioxide measurements, water loss due to evaporation (0·5–2·5 ml) was determined by weighing the MES. The loss was compensated by adding an equal amount of water to the sand beneath the litter.

The experiment lasted for nine weeks. At the start, 24 uncontaminated litter portions were analyzed (see below). A total of 72 MES was used, 12 MES per concentration. In the first four weeks the MES remained undisturbed. After four weeks, four MES from each group of 12 were taken out of the experiment. The litter was removed from the MES and analyzed. From each eight remaining MES per concentration, four were supplied with eight isopods each. Isopods (*Porcellio scaber* Latr.) were obtained from a laboratory culture which was kept at 15°C on poplar litter and small amounts of pet food to provide proteins. After nine weeks, litter or litter and isopods were removed from all remaining MES. The litter was analyzed as below. Fresh weight and survival of the isopods were determined instantly. Dry weight of the isopods was determined after drying for 72 h at 60°C.

The rate of carbon dioxide production for each MES was measured eight times in nine weeks. These data were integrated to calculate the cumulated carbon dioxide production of the MES.

Analysis of the litter

Directly after removal from the MES, each litter portion was weighed and divided into two parts. One part was used for determination of dry weight and moisture content, after drying for 72 h at 60°C. The extractable concentrations of ammonium, nitrate, phosphate and dissolved organic carbon (DOC) in the litter were determined as follows. After adding 40 ml 1 M KCl to the second part of the litter and shaking for 1 h, the pH_{KCl} was measured. Thirty millilitre of fluid was then removed from the litter suspension, centrifuged and the supernatant was pressed through a 0·2 μm millipore filter. NH_4, NO_3, PO_4 and DOC were determined in the filtered supernatant. Ammonium, nitrate and phosphate concentrations were determined using auto analyzer methods (Skalar 5100). The DOC concentrations in the samples were oxidized with potassium persulphate ($K_2S_2O_5$) in ampoules at 130°C. The resulting carbon dioxide was measured

with an infra red gas analyzer (Oceanography International Corporation, model 524 B, C).

The analyses of fluoride were done by MT-TNO, Delft (extractable F) and Zeist (total F), The Netherlands. Total fluoride concentrations were determined in litter removed from the MES at the end of the experiment, with an ion selective electrode, after pyrohydrolysis of small amounts of milled litter samples (Van Leuven *et al.*, 1979). Extractable fluoride concentrations were determined after the experiment in the remainder of the bulk litter portions, which had been stored at 4°C. Fluoride was extracted by 15 mM NaOH with ultrasonic resonance for 10 min. The extract was separated in a Seppak C-18 column to remove apolar components. After this the extract was analyzed for F with ion chromatography and conductivity detection.

All NH_4, NO_3, PO_4, DOC and F concentrations were expressed on litter dry weight basis.

Statistical analysis

The data were analyzed using (two-way) analysis of variance (ANOVA). Effects of time, fluoride or isopods were considered significant as $p < 0{\cdot}05$. No Observed Effect Concentrations (NOECs) for fluoride at each time were determined by Dunnett's method for comparing all treatments with the control (Steel & Torrie 1960). The NOEC was estimated as the highest concentration below the one differing significantly from the control (at the 5% level), on condition that responses at all concentrations above the NOEC were significantly different from the control in the same direction. Dunnett's method was used when the underlying one-way ANOVA was significant. As ANOVA assumes homogeneity of variances this was also tested. The data for NH_4, NO_3, PO_4 were transformed logarithmically to satisfy this condition.

RESULTS

The total carbon and nitrogen content of the poplar litter were, respectively, 38·7% and 2·56%. The C/N ratio was 15·1, which is relatively low according to Swift *et al.* (1979, p. 142); poplar litter can be considered as a high quality substrate for microbial degradation.

The moisture content increased in the experimental period from 226 ± 18 ($n = 24$) to 260% ± 45 ($n = 48$) and became more variable. This was caused by redistribution of water in the MES. Total water content in sand and litter remained constant, but the litter absorbed water from the sand beneath the litter.

TABLE 1
Nominal and Actual F-Concentrations in Dry Poplar Litter. The Actual Concentrations were Determined at the End of the Experiment

Nominal	*Actual*	
F ($\mu mol\,g^{-1}$)	*Total F* ($\mu mol\,g^{-1}$)	*Extractable F* ($\mu mol\,g^{-1}$)
0	1·37	0·15
1·7	1·90	1·11
5·3	7·53	2·79
17	17·79	8·42
53	59·58	10·79
170	190·00	25·00

Analysis of total fluoride in the litter at the end of the experiment demonstrated that control litter contained a low concentration of fluoride; as a whole, added F slightly exceeded the nominal concentrations (Table 1). Extraction of F with an alkaline solution resulted in considerably lower measurable F concentrations in the litter. The relative amount of extractable F decreased with increasing total concentrations of F. This contradicts the generally observed pattern that the extractability of compounds increases with increasing additions due to saturation of binding capacity of the substrate.

Effects of time

Changes in decomposition and mineralization processes in the experimental period are illustrated with the results from control MES without isopods (Table 2). Extractable ammonium, nitrate and phosphate concentrations,

TABLE 2
Extractable Concentrations of Ammonium, Nitrate, Phosphate; DOC, Carbon Dioxide Production Rate and pH in the Control MES (no F Added) in Dry Poplar Litter as Determined after 0, 4 and 9 Weeks of Incubation (Mean and Standard Deviation)

Weeks	NH_4 ($\mu mol\,g^{-1}$)	NO_3 ($\mu mol\,g^{-1}$)	PO_4 ($\mu mol\,g^{-1}$)	*DOC* ($\mu mol\,g^{-1}$)	CO_2/*MES* ($\mu l\,h^{-1}$)	*pH*
$t=0$	12·14 ± 2·41	0·46 ± 0·45	4·01 ± 0·79	303 ± 11	802 ± 16	6·66 ± 0·09
$t=4$	21·57 ± 1·31	8·50 ± 3·63	9·85 ± 1·56	490 ± 70	569 ± 52	6·93 ± 0·17
$t=9$	52·9 ± 10·9	152·5 ± 31·5	16·5 ± 2·97	339 ± 32	251 ± 43	6·11 ± 0·33

the rate of carbon dioxide production and pH showed a statistical significant effect for time ($p < 0{\cdot}05$). The changes in the second period of the experiment (4th–9th week) were larger than in the first period. The rate of carbon dioxide production decreased sharply after the start and continued to decrease throughout the experiment. The concentrations of ammonium, nitrate and, to a lesser extent, phosphate, increased; this demonstrates that the nitrification rate (and ammonification rate) became very high, especially in the second period of the experiment. Probably as a result of these processes the pH was lowered by 0·5 units in nine weeks.

Effects of fluoride in weeks 1–4

In this period isopods were not present in the MES; thus only effects on microbial processes could be determined. From week 1 until week 4 the respiration rate and the total carbon dioxide production were significantly increased at the highest concentration of fluoride (Fig. 1). Values for the accumulated amounts of ammonium, DOC, and the pH in relation to F-concentration at week 4 are not shown, for they did not differ significantly from the values for the controls given in Table 2. This means that ammonium, DOC. and the pH were not affected by fluoride at week 4 ($p = 0{\cdot}255$, $p = 0{\cdot}794$ and $p = 0{\cdot}609$, respectively). The accumulated nitrate and phosphate concentrations showed a significant effect for fluoride ($p = 0{\cdot}0006$ and $p < 0{\cdot}0001$). The nitrate concentrations, however, did not show a monotonic relation with the fluoride concentration (Table 3). From the concentrations of nitrate in the controls (Table 2) it can be deduced that the nitrification rate after four weeks was low compared to the rate after nine weeks. The nitrification process in the MES sped up in the second part of the experiment. The total variance in nitrate concentrations was high after

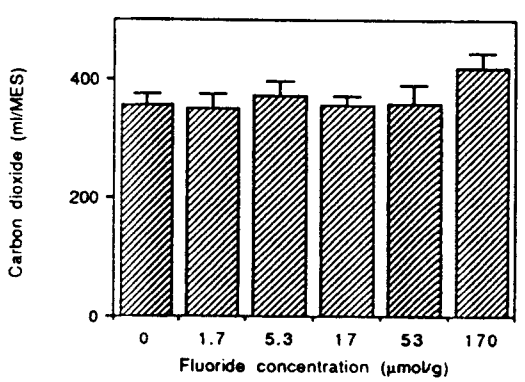

Fig. 1. Cumulated microbial carbon dioxide production (ml/MES) (mean and standard deviation) related to the fluoride concentration (μmol g^{-1}) in the first 28 days for microecosystems without isopods, containing 4 g poplar litter.

TABLE 3
Extractable Nitrate and Phosphate Concentrations at $t = 4$ Weeks, in Relation to the Fluoride Concentration in the Litter (Means and Standard Deviation)

F ($\mu mol g^{-1}$)	NO_3 ($\mu mol g^{-1}$)	PO_4 ($\mu mol g^{-1}$)
0	8·50 ± 3·63	9·85 ± 1·56
1·7	1·75 ± 0·70	10·33 ± 1·85
5·3	4·38 ± 5·01	10·17 ± 1·76
17	1·06 ± 0·77	9·41 ± 1·09
53	0·81 ± 0·43	9·72 ± 0·63
170	5·53 ± 1·53	4·89 ± 0·65

four weeks: 3·67 ± 3·62 (mean and standard deviation, $n = 24$). The significant effect of fluoride after four weeks on the nitrate concentrations is probably more a result of differences in the 'speeding up' in nitrification than an effect of fluoride. The phosphate concentration was diminished at the highest concentration compared to the control (Dunnett's test, see Table 3).

Effect of fluoride and isopods in weeks 5–9

After nine weeks MES with and without isopods were compared. Survival of the isopods was not affected by fluoride; five of a total of 192 animals died during the experiment, and these were independent of the fluoride concentration. Effects of fluoride on growth of isopods were not observed.

The cumulated MES respiration in this period, was, respectively, 222 ± 31 and 220 ± 27 ml/MES CO_2 in MES without and with isopods ($n = 24$). With an estimated isopod respiration of 0·12 $\mu l\, mg^{-1}\, h^{-1}$ at 15°C (Van Wensem, 1989) it was calculated that the isopod's contribution to the total MES respiration was 19% (42 ml per MES). Microbial respiration in the MES with isopods was thus negatively influenced by the isopods. The isopods did not have detectable effects on the other mineralization processes (Fig. 2).

Fluoride had a significant effect on the extractable ammonium, nitrate and phosphate concentrations ($p = 0·001$, Fig. 2). A slight increase of the ammonium, nitrate and phosphate concentrations in the litter could be observed at the lower fluoride concentrations, in MES with isopods. This was, however, not significant. The extractable concentrations of ammonium, nitrate and phosphate in the MES are a measure of the mineralization rate of nitrogen and phosphorus. Estimations of No Observed Effect Concentrations (NOECs) of fluoride, for mineralization, applying Dunnett's method, are shown in Table 4. Because there are two types of control MES, with and without isopods, each series of treatments was compared to its own

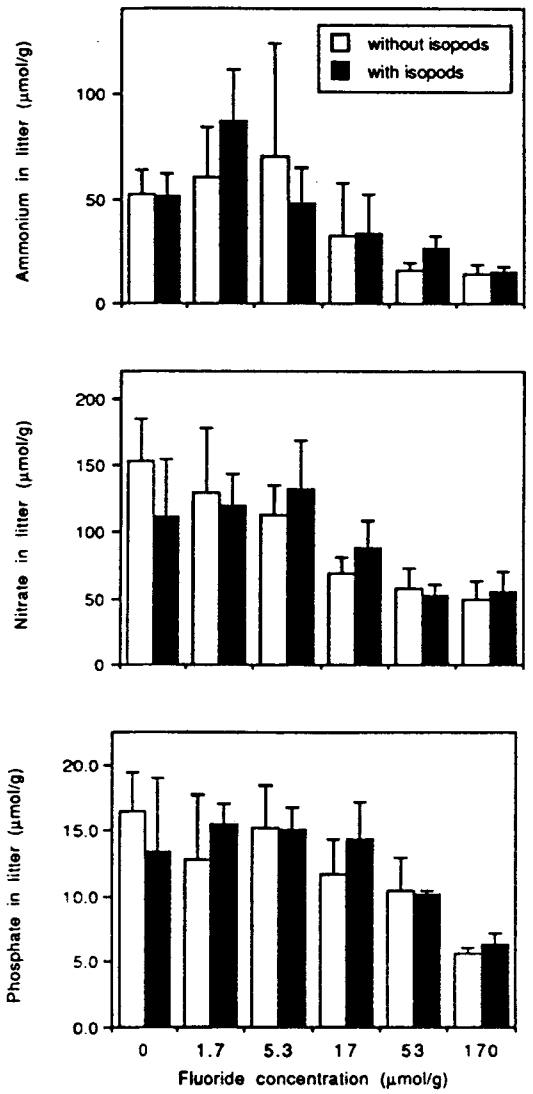

Fig. 2. KCl-extractable ammonium, nitrate and phosphate concentrations in μmol g^{-1} poplar litter after nine weeks (mean and standard deviation), plotted against the fluoride concentrations (μmol g^{-1}) in the litter. In the last five weeks eight isopods were present in part of the micro-ecosystems (black columns).

control. Since the two controls behaved differently, the NOECs for nitrate and phosphate were higher in MES with isopods than in MES without isopods. Considering the processes entirely as microbial processes and pooling the data at nine weeks for MES without and with isopods, the results demonstrate that the sequence in sensitivity of mineralization processes to fluoride is phosphate < ammonium < nitrate (Table 4).

TABLE 4
No Observed Effect Concentrations (μmol g^{-1}) for the Effects of Fluoride on Extractable Ammonium, Nitrate and Phosphate Concentrations in Dry Poplar Litter at the End of the Experiment, as Determined by Dunnett's Test

Series	*NOEC(NH_4)*	*NOEC(NO_3)*	*NOEC(PO_4)*
No isopods ($n = 4$)	17	5·3	17
Isopods ($n = 4$)	17	17	53
Pooled ($n = 8$)	17	5·3	53

Figure 3 shows the pH of the litter in the MES after nine weeks in relation to the fluoride concentration. The pH increased with higher fluoride concentrations ($p = 0{\cdot}001$). From Table 2 it was concluded that in the control MES the pH decreased in nine weeks by 0·5 unit, probably as a result of the mineralization of ammonium, followed by nitrification. The increase of pH with higher fluoride concentrations should therefore be related to these processes. It is concluded that the pH is not increased by higher concentrations of fluoride, but is prevented from decreasing because nitrification is inhibited (Fig. 2).

DISCUSSION

In this experiment the effects of fluoride on isopods and the ability to function for both isopods and microflora in the decomposition of carbon and mineralization of nitrogen, phosphorus and DOC were examined.

Survival of isopods was not affected after four weeks of exposure to

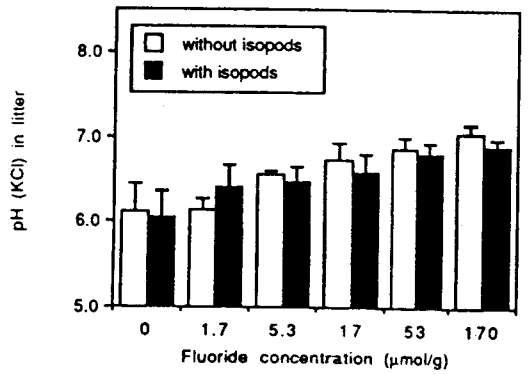

Fig. 3. Effect of the fluoride concentration (μmol g^{-1}) on the pH of poplar litter after nine weeks (mean and standard deviation).

fluoride levels up to 170 μmol g^{-1}. At this concentration Beyer *et al.* (1987) reported decrease of survival of *Porcellio scaber*; however, the acid-extractable concentration of fluoride was considerably higher in Beyer's study (258 μmol g^{-1}) than the added concentration, which points to contamination of the litter before adding the nominal dose. It is therefore doubtful that fluoride is lethal for isopods at 170 μmol g^{-1}. Silkworms appear to be relatively sensitive to fluoride derived from atmospheric pollution. A mortality of 35% was found at 1·6–2·6 μmol g^{-1} (Wang & Bian, 1988).

The toxicity of fluoride for cabbage loopers depended on the compound added to the food, fluoride salts being more toxic than HF. In contrast with HF, fluoride derived from salts accumulated in the larvae. KF and NaF at 10·5 μmol g^{-1} reduced feeding, growth and rate of development of the larvae. Addition of plant material to the food reduced the toxicity (Hughes *et al.*, 1985). Compared to the effect concentrations mentioned in these studies the litter in the MES contained high concentrations of fluoride. High accumulation of fluoride in isopods from contaminated habitats has been reported (Walton, 1987). Therefore, the present results suggest that isopods are relatively insensitive to fluoride. The occurrence of high accumulation and low toxicity is well known for heavy metals in isopods (Hopkin, 1989, pp. 121–40).

Isopods did not affect mineralization of nitrogen in this experiment, although there was a tendency for nitrate and phosphate to be lower in the controls with isopods. A slight increase of ammonium, nitrate and phosphate concentrations at intermediate fluoride concentrations was observed in MES with isopods, and therefore the NOECs for phosphate and nitrate were higher in MES with isopods than in MES without isopods. This suggests that the isopods had a positive influence on the system's ability as a whole to cope with the effects of fluoride. However, this suggestion is entirely based on non-significant differences.

The lack of influence of isopods on mineralization of nitrogen cannot be explained by effects of fluoride on isopods since the control MES showed the same results. An increase of ammonium in MES with isopods, as observed in other experiments (unpublished) and by Anderson & Ineson (1983), is thought to be a result of excretion of ammonia/ammonium as end product of protein metabolism. The data on survival and weight of the isopods do not support the hypothesis that, in the present experiment, the isopods were for some unknown reason severely stressed and did not produce ammonium in four weeks. A more likely hypothesis is that ammonium produced by the isopods was immobilized by micro-organisms. The balance between immobilization and mineralization of nutrients in litter depends very much on substrate quality, according to the concept of Swift *et al.* (1979); net mineralization would be expected, because the C/N ratio in the poplar litter

is relatively low. In MES, however, volume, time-scale and the controlled experimental conditions may have influenced this balance.

In conclusion, direct effects of fluoride on the functioning of isopods could not be proven. Consequently, the results allow evaluation of effects of fluoride on microbial processes only. Nitrification seemed to be most sensitive to fluoride in the MES. Ammonification was also decreased by fluoride, so part of the inhibition of nitrification may be caused by a lower availability of ammonium. Nitrification has also been found to be inhibited by fluoride in synthetic waste water; the oxidation of ammonium was not affected at 10·5 mM fluoride; at 26·3 and more than 42·1 mM nitrification decreased to 78% and 60–30%, respectively (Clarkson *et al.*, 1989).

In 1981, Murray hypothesized, based on observations of high fluoride concentrations in isopods (*Armadillidium*) and soils, that inhibition of some elements in the detritus cycle might occur. It is not possible to relate the NOECs found in this study directly to total F-concentrations found at polluted field sites. The lower NaOH-extractable fluoride concentrations (Table 1) indicate a low availability for fluoride in the poplar litter, which may be caused partly by high calcium concentrations in the litter and subsequent binding as insoluble CaF_2. Taking into account that the available concentrations were probably considerably lower than the nominal concentrations in this experiment, the conclusion that fluoride is toxic for microbial processes at concentrations found at moderately polluted areas, is justified.

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