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Effects of fluorine on crops, soil exoenzyme activities, and earthworms in terrestrial ecosystems

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ABSTRACT

Fluorine can flow into the environment after leakage or spill accidents and these excessive amounts can cause adverse effects on terrestrial ecosystems. Using three media (filter paper, soil, and filter-paper-on-soil), we investigated the toxic effects of fluorine on the germination and growth of crops (barley, mung bean, sorghum, and wheat), on the activities of soil exoenzymes (acid phosphatase, arylsulfatase, fluorescein diacetate hydrolase, and urease) and on the survival, abnormality, and cytotoxicity of *Eisenia andrei* earthworms. The germination and growth of crops were affected by fluorine as exposure concentration increased. The activities of the four enzymes after 0-, 3-, 10-, and 20-day periods varied as exposure concentration increased. According to *in vivo* and *in vitro* earthworm assays, *E. andrei* mortality, abnormality, and cytotoxicity increased with increasing fluorine concentration. Overall, fluorine significantly affected each tested species in the concentration ranges used in this study. The activities of soil exoenzymes were also affected by soil fluorine concentration, although in an inconsistent manner. Albeit the abnormally high concentrations of fluorine in soil compared to that observed under natural conditions, its toxicity was much restrained possibly due to the adsorption of fluorine on soil particles and its combination with soil cations.

1. Introduction

In recent years (2012–2014), various hydrofluoric acid leakage accidents occurred in South Korea, especially in chemical industrial complexes, and several people were killed or injured by explosions or exposure to hydrofluoric acid (An et al., 2015; Cho et al., 2013; Jung and Park, 2016; Lee et al., 2016; Shin, 2013). In 2012, the hydrofluoric acid leaked from a chemical plant in Gumi City (North Gyeongsang Province) resulted in the death of five workers (Cho et al., 2013; Jung and Park, 2016). This accident became a serious issue and highlighted the need for chemical safety, which ultimately led to the revision of the Toxic Chemicals Control Act (TCCA) in Korea—Law of the Ministry of Environment (An et al., 2015; Kim et al., 2015). In fact, the after-effects of this accident have been discussed in Korea until present. Immediately after the accident, the maximum concentration of fluorine in the soil near the chemical plant was 510 mg/kg and the damages to livestock and vegetation were very serious (Cho et al., 2013; Jung and Park, 2016). In 2014, the Korean Ministry of Environment announced that the trees and shrubs were affected immediately after the accident by hydrofluoric acid, and therefore chlorosis and leaf blight were observed. They also reported that atmosphere, water quality, groundwater, and soil showed a tendency for recovery, but that natural vegetation needed restoration and additional investigation.

Fluorine (atomic number = 9) is the lightest halogen and the most electronegative and reactive element (Fordyce et al., 2007). It is formed in the Earth crust as fluoride compounds and minerals like apatite ($Ca_5(PO_4)_3F$), cryolite (Na_3AlF_6), fluorite (CaF_2), and topaz ($Al_2(SiO_4)F_2$), expected to contain high fluorine levels, and their content depends on other soil materials (Cronin et al., 2000). In soil, fluorine usually combines with other native elements or materials, such as aluminum (Al), calcium (Ca), iron (Fe), or clay minerals producing stable fluorinated compounds (Bower and Hatcher, 1967; Omueti and Jones, 1977; Hani, 1978). There are also several anthropogenic sources of fluorine including drugs, cosmetics (toothpastes and mouth rinses), and organofluoride compounds (Ghosh et al., 2013). Fluorine is an essential nutritional element for humans and animals, but it is harmful to humans if exposure is excessive. Several toxic anions like arsenate/arsenite, bromide, chromate, cyanide, and iodide can adversely affect soil organisms (Crompton, 2006). Due to its low bioavailability by combination with other cations, the fluorine naturally present in soil is less toxic than other anions like metallic salts and other halogens (Cronin et al., 2000). Several previous studies confirmed the toxicity of chronic fluorine exposure on animals and plants. It can cause adverse effects on bones, skeletal systems, teeth and organs (kidney, gastrointestine, and thyroid

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gland) of human and animals (Ghosh et al., 2013). Chronic exposure to fluorine can also cause plant necrosis and interveinal chlorosis (Thomas, 1951; Brewer, 1966), decrease the efficiency of plant photosynthesis (Brewer et al., 1967; Robinson, 1977), and reduce flower production (Bruyn and Hulsman, 1972).

Generally, high concentrations of fluorine that can cause severe impacts on environments and organisms enter the ecosystems *via* leakage accidents or smelter emissions. According to the World Health Organization (WHO), fluorine in drinking water should be less than 1.5 mg/L (Fawell et al., 2006), and the United States Environmental Protection Agency (USEPA) suggested an oral reference dose for fluorine of 0.06 mg/kg/day. In Korea, fluoride compounds are treated as soil contaminating substances and controlled under 400 (agricultural and residential sites) and 800 (industrial and military sites) mg F^-/kg. The Occupational Safety and Health Administration (OSHA) suggested that workers should not be exposed to more than 0.1 ppm (F_2, gas) or 2.5 mg/m^3 (Na_3AlF_6, cryolite) (NIOSH-OSHA, 1981) of fluorine.

In the present study, we carried out several experiments to identify the toxicity of fluorine in soil ecosystems and its effects in soil ecospecies. Ecosystems nearby leakage accident areas were exposed to fluorine and are, therefore, seriously damaged pointing out the need to identify acute or chronic toxicities of fluorine on various organisms within soil ecosystems. To assess the toxicity of fluorine in terrestrial ecosystems, this was determined for plants, earthworms, and for the activity of soil exoenzymes, and its effects were compared regarding plant species, kind of exoenzyme, and endpoint of earthworm.

2. Materials and methods

2.1. Test soil

Natural soil was harvested at Konkuk University, from a gentle slope site nearby a greenhouse and away from the streets. Surface soil (0–5 cm) was collected, sieved (< 2 mm), and air-dried for a week in the greenhouse. Soil pH, water holding capacity, texture, organic matter, organic carbon, and concentration of soluble fluorine were characterized before the assay and are shown in Table 1.

2.2. Plant assay

Four crops, barley (*Hordeum vulgare*), sorghum (*Sorghum bicolor*), wheat (*Triticum aestivum*), and mung bean (*Phaseolus radiatus*) were used as test species. Plant assays were carried out using three test designs. First, a screening test using filter paper was conducted in Petri dishes. Seeds were sterilized with 5% sodium hypochlorite solution (NaClO, CASN 7681-52-9; Daejung, Seoul, Korea) for 10 min, rinsed three times with distilled water, and then soaked on wet cotton for 3 h in the dark. Filter papers (Φ = 95 mm, pore size 5 μm, thickness 0.26 mm; Advantec, Tokyo, Japan) were placed in Petri dishes (100 × 15 mm, sterilized, SP10100; SPL, Pochon, Korea) and saturated with NaF solution at each target concentration (0–22.62 μg F^-/cm^2 for barley and wheat and 0–11.31 μg F^-/cm^2 for sorghum and mung bean). Ten seeds were then placed in a circle on the filter paper, and the Petri dishes were sealed with plastic paraffin film and incubated in the dark at 25 °C. After 72 h, germination rate and seedling growth were measured. In a second test design, the modified Organization for Economic Co-operation and Development (OECD) method (OECD, 2006) was applied. Seeds were prepared as in the screening test, and distilled water and NaF solutions were injected into tested soil samples (25 g) and mixed with soil homogeneously (0–1357 mg F^-/kg for sorghum and wheat, 0–905 mg F^-/kg for barley, and 0–679 mg F^-/kg for mung bean). Five seeds of each crop were then planted in a circle in each soil sample, within 85-mL flat-bottomed glass vials [internal diameter (ID), 40 mm; length, 80 mm]. Vials were wrapped in plastic paraffin film and incubated at 25 °C under a 16:8 light:dark photoperiod. No further moisture was added, and germination rate and seedling growth were measured after 96 h. Lastly, in a filter-paper-on-soil assay, soil samples with the same characteristics and same weight as those used in the soil assay were prepared in Petri dishes (100 × 15 mm) and the concentration of fluorine was set as in the soil test. Filter papers with same type and size used in the screening test were placed on soil samples and pressed with a spatula to saturate filter papers with the NaF solution present in the soil. Ten sterilized seeds were then placed on the filter paper, as in the screening test, and germination rate and seedling growth were measured after 72 h of exposure to NaF.

2.3. Soil exoenzyme activity assay

The exoenzyme activities tested were acid phosphatase activity (APA) (EC 3.1.3.2), arylsulfatase activity (ASA) (EC 3.1.6.1), fluorescein diacetate hydrolase activity (FDA), and urease activity (UA) (EC 3.5.1.5). Before the test, blank soils were sterilized at 120 °C for 15 min. Spiked moisture content was 60% of the water holding capacity of tested soil samples, and distilled water and 10% formaldehyde (for sterilization) were spiked in control and blank soil samples, respectively. After spiked with NaF solution of each concentration (0–905 mg F^-/kg), soils were mixed homogeneously with a spatula. These assays were conducted in triplicate (samples from the same spiked soils) per concentration, and exoenzyme activities were measured at 0, 3, 10, and 20 d after exposure. Exposed soil was incubated at 25 °C and under a 16:8 light: dark photoperiod.

The APA and ASA assays were adapted from Tabatabai (1994) and An and Kim (2009), the FDA assay followed the methods of Adam and Duncan (2001) and An and Kim (2009), and the UA assay followed the methods of Kandeler and Gerber (1988) and An and Kim (2009).

2.4. Earthworm assay

Eisenia andrei were collected at the Nanji Water Recycling Center (Goyang, Gyeonggi, Korea) and maintained in an incubator at 20 °C under dark conditions for 7 d. Healthy adult earthworms (300–600 mg) selected for the toxicity test were rinsed with distilled water, separated from the cultures, and kept in the dark for 3 h before the test to allow them to void their gut contents. Both *in vivo* and *in vitro* assays were carried out.

In the *in vivo* assay, a modified OECD method (OECD, 1984) was applied. Ten gram of dry soil was placed in a 20-mL flat-bottomed glass vial (ID 25 mm; length 50 mm) and saturated with 3.5 mL NaF solution at each target concentration (0–452 mg F^-/kg). One earthworm was added to each glass vial and this was covered with a silicone stopper to provide aeration and to prevent the avoidance behavior of the earthworm. The test was conducted using 20 replicates for the control conditions and 10 replicates for each NaF concentration. Vials were incubated at 20 °C in dark conditions. At 3, 5, and 7 d after exposure, earthworm mortality and burrowing behavior (an indicator of the health of earthworms) were recorded. After 7 d, abnormalities (mucous secretion, bleeding) and morphological changes (swelling, thinning, and fragmentation) were investigated.

Table 1
The pH, water holding capacity, soil texture, organic matter, organic carbon, and concentration of soluble fluorine in test soil, prior to assays.

Characteristic	pH	Water holding capacity (mL/g)	Soil texture	Organic matter (%)	Organic carbon (%)	Concentration of soluble F (μg/L)
Values	4.43 (± 0.75)	65.7 (± 0.04)	Loamy sand	11.2 (± 0.34)	6.49 (± 0.19)	0.04 (± 0.00)