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# Effects of heavy metal contamination from an abandoned mine on nematode community structure as an indicator of soil ecosystem health

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ABSTRACT

Soil nematode community structure reflects soil ecosystem health and is influenced by the soil environment directly and/or indirectly by affecting the soil micro-flora and fauna that they graze. In this study, ecological indices for soil nematode community structure and microbial populations in soils contaminated with mine drainage (CS) from an abandoned mine and of a nearby non-contaminated area (NC) were examined during the seven seasons from July 2007 through December 2008 to reveal influences of mine drainage (especially heavy metals) on the soil nematode community structure. Of the soil physicochemical characteristics measured, nutritional properties such as organic matter content, nitrogen content, and soluble cations were not significantly different between CS and NC; however, significant differences were detected in pH, electrical conductivity (EC), available phosphorus (av.$P_2O_5$), and most strikingly, the concentrations of heavy metals such as Cd, Pb, Zn, and Ni. Nematodes were less abundant in CS than in NC, especially for long-living persistor-type nematodes. Comparison of ecological indices between CS and NC indicates that abundance, maturity, richness, and diversity of the soil nematode community were decreased in CS soil, indicating that soil health and function were adversely affected. Of the weighted-soil food-web indices, the structural index (SI) of the soil nematode community was significantly lower in CS than in NC, while no significant difference in the enrichment index (EI) was observed between CS and NC, suggesting that the heavy metal contamination may have disturbed the soil ecosystem by suppressing biological activity. Seasonal changes in the ecological indices during the study period showed that the discrepancies between CS and NC persisted throughout most of the seasons, suggesting that the effect of mine drainage (heavy metal) contamination on nematode community structure may be little influenced by seasonal changes in environmental conditions.



## 1. Introduction

Soil health is defined as the continued capacity of soil to sustain its biological productivity, maintain the quality of the surrounding air and water environments, and promote plant, animal, and human health (Doran et al., 1996). Soil health is threatened by various materials derived from human activities, which include industrial pollutants, pesticides, livestock wastewater, mine drainage, and petroleum contamination (Thornton, 1983; Alloway, 1990; Yeo and Kim, 1997; Kim et al., 2002). Among these, contamination of soil, especially by heavy metals from mines, is one of the most important causes of soil health decrease, as excess amounts of heavy metals are detrimental to both human health and plants (Järup, 2003; Li and Yang, 2008).

A variety of heavy metals such as Pb, Cd, Cr, Cu, and Zn accumulate in soils near mines via mining processes and/or mine drainage and waste (Alloway, 1990; Park and Kim, 1998; Min et al., 2005; Shao et al., 2008). Pine, bicolored lespedeza and Japanese alder seeds planted in soils contaminated with heavy metals were found to have reduced sprouting rates (Seo et al., 2006). The symptoms of reduced root growth, reduced seed sprouting and seedling stunting, necrosis, and chlorosis appear in susceptible plants grown in soils contaminated with heavy metals (Gemmell, 1977; Foy et al., 1978; Wong and Bradshaw, 1982). Some crop plants growing in highly contaminated soil contain heavy metals at concentration levels hazardous to human health (Kim et al., 1998, 2002). With increasing heavy metal concentrations, the activities of microbes, soil enzymes, and nitrogen fixation are inhibited, and growth of microfloral communities such as fungi, algae, and photosynthetic bacteria is reduced (Mhatre and Pankhurst, 1997). The activities and species richness of a faunal community containing nematodes, protozoa, and earthworms were also reduced in soils contaminated with heavy metals (Mhatre and Pankhurst, 1997). Long-term effects

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of heavy metals on nematodes recorded in a number of studies show different degrees of influence on nematode assemblage and community structure depending on the specific heavy metals and their concentrations (Weiss and Larink, 1991; Bongers et al., 2001; Bakonyi et al., 2003; Nagy et al., 2004).

Various abiotic and biotic soil characteristics can be used as indicators for evaluating soil health. Plants and soil-inhabiting organisms such as soil microflora, fungi, earthworms, nematodes, mites, protozoa, and insects have been used as biotic indicators of soil conditions (Doran and Parkin, 1994; Cortet et al., 1999; Ferris and Bongers, 2006). Among these, the soil nematode community has received much attention as a valuable biotic indicator because soil nematodes are simple metazoans ubiquitously inhabiting diverse soil environments; they are in intimate contact with their surroundings by living in soil water and occupying several positions at several trophic levels in the soil food-web (Ferris et al., 2001). Also, their identity, including trophic type, can be visually determined under a light microscope without dissection. Because of their influence in soil food-webs and plant–soil interactions, the composition of the soil nematode community has been suggested to be a useful indicator of the status of soils exposed to disturbances and management by humans (Bongers, 1990; Bongers and Ferris, 1999; Ferris et al., 2001). Nematode abundance and community structure analyses have proven to be sensitive indicators of stress caused by soil pollutants and ecological disturbance (Sochova et al., 2006). Therefore, the purpose of this study was to assess the changes in a soil ecosystem due to heavy metal contamination from an abandoned mine by analyzing the soil nematode community structure, especially taxal composition and aspects of trophic structure affecting soil food-webs.

## 2. Materials and methods

### 2.1. Study sites

The study was conducted around an abandoned mine located in Gijang county, Busan, South Korea (35°31′N, 129°22′E). The mine was developed in 1930; copper and silver mining activities continued until 1979 but were discontinued from 1980 onward. The land adjacent to the abandoned mine has been contaminated with heavy metals by mine drainage for the past 30 years and has become representative of heavy metal-polluted areas in South Korea. Six plots of about 10–15 $m^2$ in size were selected for the study; three for the contaminated site (CS) along the mine drainage area (CS1; 10 m away from the mine entrance, CS2; 50 m downslope from CS1, and CS3; 150 m downslope from CS2) were presumed to be contaminated with heavy metals and the other three for the non-contaminated site (NC) were located away from the mine drainage area (NC1; 70 m away from CS1, NC2; 40 m downslope from NC1, and NC3; 50 m away from CS3) at the same altitudes as the corresponding CS plots. During the study period, CS1 was densely covered with eulalia grass (*Miscanthus sinensis*), with little litter. The vegetation of CS2 and CS3 consisted of red-leaved hornbeam (*Carpinus laxiflora*) and patchy zoysiagrass (*Zoysia japonica*) on ground covered with hornbeam leaves, with about 1 and 2-cm deep litter layers in CS2 and CS3, respectively. NC1 was located in a pine tree (*Pinus densiflora*) stand, with a carpet of pine needles and a *c.* 5-cm-deep litter layer, while NC2 and NC3 were in red-leaved hornbeam tree stands with a dense ground cover of hornbeam leaves and a two-cm deep litter layer. The growth of all plant species was largely retarded in CS compared to NC plots.

### 2.2. Soil sampling

For analysis of the physicochemical characteristics of the soil (including heavy metal concentrations), soil samples of approximately 1 kg were taken in July 2007 from 3 places in each plot and mixed together. Soil samples were dried under shade conditions for 2 weeks and then sieved through a 2.0-mm-aperture sieve prior to use for analyzing physicochemical characteristics of the soil. For the isolation of soil nematodes and microorganisms such as fungi (including pseudofungi) and bacteria, soil samples were taken seven times, once in each season from July 2007 until December 2008. Six core samples randomly taken within a 1 $m^2$ central area of each plot with a 2-cm-diameter core sampler to a depth of about 30 cm after removal of fallen leaves and litter were combined to form a composite sample for each plot. Soil samples were stored at room temperature before use.

### 2.3. Analysis of soil characteristics

Ten grams of soil samples from each plot was subjected independently to the analysis of physicochemical soil characteristics as follows. Soil texture was determined by measuring the amount of clay, silt, and sand in a 10 g soil sample suspended with a soil dispersant (using a pipette) after removal of organic matter by boiling in $H_2O_2$ for 1 week and of Ca by HCl (Klute, 1986). According to the methods described in NIAST (2000), the following analyses were conducted: soil pH and electrical conductivity (EC) using pH and EC meters, organic matter by the Tyurin method, total nitrogen (T-N) by the Kjeldahl method, available phosphates (av.$P_2O_5$) by the Lancaster method using a spectrophotometer, and $NO_3$–N and $NH_4$–N extracted in 2 M KCl using an automatic ion analyzer. Soluble cations ($Ca^{2+}$, $Na^+$, $K^+$, and $Mg^{2+}$) were determined by a flame photometer. Heavy metal concentrations were determined with an ICP atomic emission spectrophotometer (ICP-AES) using 10% soil solutions with the addition of 0.1 N HCl in a glass flask while shaking at 30 °C for 1 h and then filtered through Whatman No. 2 filter paper (MOE, 2005).

### 2.4. Isolation and enumeration of soil nematodes and microorganisms

The nematode population was extracted from a 100 g sample of soil from each plot, by sieving through 30-mesh, 200-mesh, and 325-mesh sieves, followed by the Baermann funnel procedure for 48 h (Southey, 1986). Recovered organisms including nematodes were preserved in triethanolamine-formalin (TAF) fixative at 80 °C and mounted on glass slides (Southey, 1986). A maximum of 100 individuals from each soil sample was identified according to order, family, and genus (if possible) using a compound light microscope (magnifications of 400× and 1000×), referring to Choi (2001) for identification of Tylenchida and Aphelenchida, Jairajpuri and Ahmad (1992) for Dorylaimida, and Bongers (1987) for the other saprophytic nematode orders. Trophic types were determined based on known feeding habitats or stoma and esophageal morphology, and nematodes were assigned to four trophic groups: bacterivores (BF), fungivores (FF), plant parasites (PP), and omnivores–predators (OP) (Yeates et al., 1993). The nematode families found were also classified on the basis of a colonizer–persistor (*c–p*) continuum of 1–5 (Bongers, 1990). The number of nematodes belonging to each taxon, trophic type, and *c–p* group was determined under a compound light microscope.

For the isolation of soil microorganisms, 1 g of soil was diluted serially in sterile distilled water (SDW) and pour-plated onto potato-dextrose agar (PDA) acidified with 0.1% lactic acid for fungal (pseudo-fungal) isolation, and nutrient agar (NA) for bacterial isolation, each with three replicates, which were incubated at 25 °C and 28 °C in incubation chambers, respectively (Dhingra and Sinclair, 1985). Numbers of fungal and bacterial colonies formed on PDA and NA, respectively, were counted visually and multiplied by the dilution factors for their enumeration as colony-forming units (CFU)