The short-term effects of low-level contamination by heavy metals (As, Cd, Cu, and Pb) on the soil health were examined by analyzing soil nematode community in soils planted with tomatoes. For this, the soils were irrigated with five metal concentrations ([1, 1/4, 1/4², 1/4³, and 0] × maximum concentrations [MC] detected in irrigation waters near abandoned mine sites) for 18 weeks. Heavy metal concentrations were significantly increased in soils irrigated with MC of heavy metals, among which As and Cu exceeded the maximum heavy metal residue contents of soil approved in Korea. In no heavy metal treatments controls, nematode abundances for all trophic groups (except omnivorous-predatory nematodes [OP]) and colonizer-persister (cp) values (except cp-4–5) were significantly increased, and all maturity indices (except maturity index [MI] of plant-parasitic nematodes) and structure index (SI) were significantly decreased, suggesting the soil environments might have been disturbed during 18 weeks of tomato growth. There were no concentration-dependent significant decreases in richness, abundance, or MI for most heavy metals; however, their significant decreases occurred in abundance and richness of OP and cp-4, MI2-5 (excluding cp-1) and SI, indicating disturbed soil ecosystems, at the higher concentrations (MC and MC/4) of Pb that had the most significant negative correlation coefficients for heavy metal concentrations and nematode community among the heavy metals. Therefore, the short-term effects of low-level heavy metal contamination on soil health can be analyzed by nematode community structures before the appearance of plant damages caused by the abiotic agents, heavy metals.

**Keywords**: ecological index, heavy metal contamination, nematode community, soil health

Heavy metal pollution from abandoned mines is an important component of hazards to soil health (Järup, 2003; Li and Yang, 2008; MOE, 2005). Soil near mines is subject to contamination by heavy metals such as Cd, Cu, Cr, Pb, and Zn during mining processes and/or through mine drainage and waste (Alloway, 1990; Min et al., 2005; Park and Kim, 1998; Shao et al., 2008). Acid drainage water containing heavy metals flowing out from abandoned mines infiltrates into the soil, polluting agricultural soils (Alloway, 1990; Min et al., 2005; Park and Kim, 1998). In addition, manufacturing and the use of synthetic products such as pesticides, paints, batteries, industrial waste, and industrial and domestic sludge can result in heavy metal contamination of soils (Soil Quality Institute, 2000).

Plants growing in heavy metal-contaminated soils undergo several physiological disorders such as reduced sprouting rates, poor root growth and seed sprouting, and stunting, necrosis and chlorosis of seedlings (Foy et al., 1978; Gemmell, 1977; Wong and Bradshaw, 1982). Soil microfloral communities of fungi, algae, and photosynthetic bacteria living belowground can be disturbed.
by increased heavy metal concentrations (Mhatre and Pankhurst, 1997). In particular, nematode abundance, community structure, and a variety of community-related indices are negatively affected by concentrated heavy metals in soil, which influences on the plant growth and health (Bakonyi et al., 2003; Bongers et al., 2001; Nagy et al., 2004; Park et al., 2011a; Weiss and Larink, 1991). Soil nematodes with a variety of feeding habitats occupy several key positions, feeding on most soil organisms such as organic matter-decomposing organisms and prey and predator organisms in the soil ecosystems (Freckman, 1988; Yeates and Bongers, 1999). They provide numerous advantages for monitoring spatial and temporal disturbances in environmental conditions and soil ecosystem function (Bongers and Ferris, 1999; Wang et al., 2008). Owing to the usefulness of nematodes as ecological indicators, many studies have reported on the ecological hazards of heavy metals based on nematode assemblages and community structure (Bakonyi et al., 2003; Bongers et al., 2001; Nagy et al., 2004; Park et al., 2011a; Weiss and Larink, 1991). However, all of these studies were primarily concerned with the harmful effects of long-term exposure and high levels of heavy metal stress; few attempts have been made to examine nematode communities in soils exposed to low levels of heavy metals for a short period of time in relation to soil and plant health. This frequently occurs in agricultural soils under the initial influences of mine drainage, manufacturing, and use of the synthetic products mentioned above.

Remediation of heavy metal contaminated soils by cleaning is extremely expensive and difficult because of the immobility and water-insolubility of heavy metals, and thus prevention is the best method for the protection of soil ecosystems from heavy metal contamination (Kumar Sharma et al., 2007; Soil Quality Institute, 2000). Moreover, it is almost impossible to remediate soils after serious heavy metal contamination because of their high persistence after introduction into the soils (Scanferla et al., 2012). Thus, remediation should begin as early as possible at the initial stages of heavy metal contamination, which requires early detection of threshold heavy metal concentrations that are detrimental to soil ecosystems and plant health. Soil nematodes have been adopted as bioindicators of soil and environmental disturbance and stress, as they are sensitive to minimal alterations caused by short periods and low levels of heavy metal contamination (Bongers and Ferris, 1999; Mhatre and Pankhurst, 1997; Sánchez-Moreno and Navas, 2007; Shao et al., 2008). Therefore, this study aimed to examine alterations in nematode community structure in soils exposed to low levels of heavy metal contamination for a short period of time by simulating the infiltration of irrigation waters contaminated with heavy metals into agricultural soils. This will provide information on the early detection of heavy metal contamination for the initiation of remediation programs before the occurrence of serious heavy metal contamination detrimental to soil and plant health.

**Materials and Methods**

**Soil characteristics and analysis.** A sandy loam soil was collected from an experimental field of the National Academy of Agricultural Science, Rural Development Administration, Suwon, Korea. The field had been cropped with tomatoes and cabbages. The soil was passed through a 10-mm sieve to remove stones and roots, and then used for pot experiments. Prior to pot experiments, the soil was analyzed for texture, pH, electrical conductivity (EC), organic matter (OM), total-N, inorganic N (NH₄⁺-N and NO₃⁻-N), available P (Lancaster P), and exchangeable Ca²⁺, Mg²⁺, K⁺, and Na⁺. Soil texture was determined based on the USDA classification scheme using the pipette method (Gee and Bauder, 1986). Soil pH was measured potentiometrically in 1:1 (w:v) soil/water suspensions, and soil-EC was measured in 1:5 (w:v) aqueous extracts. Available P was determined using the Lancaster P method, and OM was determined using the Tyurin method (National Institute of Agricultural Science and Technology, 2000). Ten grams of oven-dried soil samples were extracted with 100 ml of 2 M KCl solution, and the extracts were analyzed for NH₄⁺-N and NO₃⁻-N by steam distillation using Devarda’s alloy (Keeney and Nelson, 1982). Soil samples were passed through a US standard 100-mesh sieve, digested using a block digester (B-435, 238; Büchi Labortechnik AG, Flawil, Switzerland), and the digests were analyzed for total-N by steam distillation (Bremner, 1986). Exchangeable Ca²⁺, Mg²⁺, K⁺, and Na⁺ were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES) (GBC, Braeside, Australia). Following completion of the experiment, the soil in each pot was mixed thoroughly. A sub-sample was collected to determine the heavy metal contents (As, Cd, Cu, and Pb) in the soil. For this, 0.1 M HCl-extractable heavy metals (As, Cd, Cu, and Pb) were determined in their concentrations using ICP-AES (GBC) following the procedures of the MOE (2005), and compared to the maximum heavy metal residue contents of soil approved in Korea (MHMRC) (MOE, 2005).

**Pot experiment and plant growth.** A pot experiment with tomato plants (*Lycopersicon esculentum* cv. Rutgers) using the sandy loam soil was conducted in a plastic-film greenhouse for 18 weeks from June to October 2009. Tomato seedlings raised for three weeks were transplanted
individually into 15-cm (diameter) × 18-cm (height) plastic pots filled with the soil (dry weight: ~650 g), and were raised throughout the experiment. Four levels of heavy metal (As, Cd, Cu, and Pb) solutions were prepared for treatments from fourfold serial dilutions (4⁻¹, 4⁻², 4⁻³, and 4⁻⁴) of the stock solutions containing the maximum (4⁻¹) concentrations of heavy metal species (MC) (3.2 mg l⁻¹ for As, Cu, and Pb, and 0.32 mg l⁻¹ for Cd) that were observed in irrigation waters near abandoned mine sites (Park and Kim, 1998). The minimum (4⁻³) concentration level of each heavy metal species (0.05 mg l⁻¹ for As, Pb, and Cu, and 0.005 mg l⁻¹ for Cd) corresponds to the maximum contamination level allowable in Korean agricultural irrigation water (MOE, 2007). Accordingly, four treatments were chosen for comparison and laid out in a randomized block design in five replications. During the pot experiments, a 200-ml aliquot of each heavy metal solution was irrigated daily by manual watering for 18 weeks (126 days). Water without heavy metals was irrigated for the control. Plants were pulled from the soil at the end of the experiment and visually examined for the symptoms of heavy metal treatment on plant growth (stunting and chlorosis). Contents of heavy metals (As, Cd, Cu, and Pb) in soil were determined as mentioned above. For the treatments with maximum heavy metal concentrations, their daily accumulations were calculated as \((f_c - i_c) / 126\), where \(f_c\) and \(i_c\) are the final and initial concentrations of heavy metals, respectively, and 126 was the total number of days of treatment. Accumulation rates were calculated as (amount of heavy metals accumulated in the soil) / (amounts of heavy metals irrigated into the soil (concentration × total volume of irrigated heavy metal solution)) × 100.

**Isolation and enumeration of soil nematodes.** Soil samples for the analysis of nematode populations in soil were collected twice: before planting tomato seedlings and after 18 weeks of tomato growth. The nematode population was extracted from 100-g soil samples from each pot by sieving using US standard 30-mesh, 200-mesh, and 325-mesh sieves, followed by the Baermann funnel procedure for 48 hours (Southey, 1986). The extracted nematodes were fixed in triethanolamine formalin at 80°C and mounted on glass slides (Southey, 1986). A maximum of 100 individuals from each soil sample were identified, most to the genus level and a few to family level, using a DMRD compound light microscope (Leica). The life strategy of each nematode group was determined under a DMRD compound light microscope (Leica). The life strategy of each nematode group was determined based on a continuum of colonizers-persisters (cp) following the classification of soil and freshwater nematodes described by Bongers and Bongers (1998).

**Analysis of soil nematode communities.** In this study, for each treatment, the nematode community properties were evaluated based on the values of their ecological indices. The characteristics of the nematode communities were described by several indices. (1) All of the nematode genera (or families) found in soils before and after the experiment were examined to determine nematode population changes during tomato growth. (2) A comparison of the initial and final nematode communities in the no-treatment controls was performed by measuring the initial ecological indices of the nematodes before the experiments and averaging the representative indices in the heavy metal controls after completion of the experiment. (3) The total number of taxa and their population densities in 100-g soil samples were examined as a measure of nematode community richness and abundance for total nematodes (TNe), each trophic group (Ba, Fu, PP, and OP), each cp value ranging from colonizer (cp-1) to persistor (cp-5), and each guild (the combination of trophic group and cp value such as Ba for bacterivores with cp-1; Ferris et al., 2001; Pen-Mouratov et al., 2003, 2004; Steinberger and Loboda, 1991; Steinberger and Sarig, 1993). (4) Maturity indices (MI) for nematode communities were calculated with the equation \(\text{MI} = \sum_{n}^{\text{MI}} \frac{v_i}{n} \), where \(v_i\) is the cp value of the ith taxa, \(f\) is the frequency of the ith taxa in the sample, and \(n\) is the number of TNe (excluding PP). The same method was used to calculate MI 2–5 (MI excluding cp-1), \(\Sigma\text{MI} 2–5\) (MI excluding cp-1 but including PP), and plant-parasitic nematodes (PPI) (MI for PP only), all of which were included as general maturity indices in our study (Bongers, 1990; Yeates, 1994). (5) Enrichment index (EI), structure index (SI), and channel index (CI) were included as expanded maturity indices for expanded assessment of the soil food web via analysis of the nematode communities, and were calculated as follows: \(\text{EI} = 100 \times e / (e + b)\) and \(\text{SI} = 100 \times s / (s + b)\) where \(e = \Sigma \kappa n_{k}\), \(s = \Sigma \kappa n_{k}\), and \(b = \Sigma \kappa n_{k}\), where \(k\) is the weightings assigned to respective guilds indicating basal (Ba₁, Fu₁), enrichment (Ba₂, Fu₂), and structure (Ba₃, Ba₄, Fu₃, Fu₄, omnivore (Om₁, Om₂), predatory (Pr₁, Pr₂, Pr₃) characteristics, respectively; and \(n\) is the abundances
of nematodes in those guilds; and CI = 100 × {0.8Fu2 / (3.2Ba1 + 0.8Fu2)} (Ferris et al., 2001; Wang and McSorley, 2005).

**Statistical analysis.** The data were analyzed by ANOVA. Duncan’s multiple range tests (DMRT) were carried out to identify significant differences ($P \leq 0.05$) among controls and treatments of heavy metal species at different concentrations. Correlation coefficients ($r$) were computed with the square root total variation to test for significant relationships between the heavy metal contents in soils and nematode community indices, including abundances of trophic groups, cp values, and general and expanded maturity indices at $P \leq 0.2$, $P \leq 0.1$, $P \leq 0.05$, and $P \leq 0.01$. Statistical computations were carried out using SAS version 4.2 (SAS Institute Inc., Cary, NC, USA).

**Results**

**Initial soil characteristics.** The soil used in this study was a sandy loam soil composed of 62.6% sand, 28.0% silt, and 9.3% clay, with a pH of 5.6, an EC of 0.82 dS m$^{-1}$, OM of 22.0 g kg$^{-1}$, available P (P$_2$O$_5$) of 608.0 mg kg$^{-1}$, total-N of 0.9 g kg$^{-1}$, NH$_4^+$-N of 20.0 mg kg$^{-1}$, NO$_3^-$-N of 29.0 mg kg$^{-1}$, and concentrations of exchangeable Ca$^{2+}$, Mg$^{2+}$, K$^+$, and Na$^+$ of 4.40, 0.60, 0.14, and 0.77 cmol kg$^{-1}$, respectively (data not shown).

**Soil heavy metal accumulation and plant growth.** The heavy metal concentrations in soil were highest for Cu (76.6–143.7 mg kg$^{-1}$), followed by Pb (15.2–35.0 mg kg$^{-1}$), As (4.4–25.5 mg kg$^{-1}$), and Cd (0.0–2.6 mg kg$^{-1}$), and soil contents increased in close proportion to the treatment concentration (Table 1). For all heavy metal species, the soil contents were significantly higher in MC or in MC/4 except for Pb. Heavy metal accumulation in soils exceeding MHMRC were only observed in the soils treated with MC of As and Cu, exceeding 70.0% and 15.0% of MC, respectively. The accumulation rates relative to the treatment amounts were highest for Cu (4.9%), followed by As (1.6%) among the treatments at MC. There were no significant differences in tomato plant growth among controls and treatments for all heavy metal species and no symptoms related to heavy metal toxicity appeared in any heavy metal treatment (data not shown).

**Changes in nematode community abundance and richness in soils treated with heavy metals.** A total of 12 nematode taxa (11 genera and 1 family) were identified in the soil before the experiment, including three PP genera, four Ba genera and one family, two Fu genera, and two OP genera. The number of taxa in the soils increased remarkably after 18 weeks of tomato growth to a total of 31 genera and 2 families (33 taxa): 8 PP taxa, 17 Ba, 4 Fu, and 4 OP, with relative population densities of 38.2%, 47.7%, 12.0%, and 1.5%, respectively (data not shown).

A remarkable increase in nematode community abundance was noted after the experiment, with significantly increased population densities of TNe, for which the increases of the abundance for trophic groups and cp values were the highest for PP ($\times$ 40.3) and cp-3 ($\times$ 41.8), the second highest for Ba ($\times$ 19.4) and cp-1 ($\times$ 31.0), and the lowest for OP ($\times$ 1.7) and cp-4–5 ($\times$ 2.7–1.9), respectively (Table 2). The most striking increases in the abundance of

| Treatment (concentration)* | As    | Cd     | Cu      | Pb      |
|----------------------------|-------|--------|---------|---------|
| MHMRC‡                     | 15.0  | 4.0    | 125.0   | 300.0   |
| 0.0                        | 4.36 ± 0.54 X | 0.00 ± 0.00 X | 76.64 ± 8.63 X | 16.97 ± 0.24 XY |
| 4$^{-3}$                   | 5.54 ± 0.40 X | 0.00 ± 0.00 X | 124.58 ± 25.75 Y | 15.19 ± 0.55 X  |
| 4$^{-2}$                   | 5.41 ± 0.53 X | 0.00 ± 0.00 X | 110.69 ± 13.47 XY | 15.54 ± 0.42 XY |
| 4$^{-1}$                   | 9.92 ± 0.54 X | 0.67 ± 0.17 Y | 119.06 ± 12.11 Y | 18.08 ± 0.67 Y  |
| 4$^0$                      | 25.53 ± 1.26 Z | 2.63 ± 0.12 Z | 143.74 ± 27.35 Y | 34.98 ± 2.57 Z  |
| DAA§                       | 0.16 (1.6%) | 0.02 (1.9%) | 0.53 (4.9%) | 0.14 (1.3%) |

The same letters (X, Y, Z, etc.) in a column denote no significant difference at $P \leq 0.05$ based on Duncan’s multiple range test.

* Dilutions of the maximum ($4^0$) concentrations of heavy metal species (3.2 mg l$^{-1}$ for As, Cu, and Pb, and 0.32 mg l$^{-1}$ for Cd) in irrigation waters nearby abandoned mine sites (Park and Kim, 1998).

† Averages and standard deviations of four replications.

‡ Maximum heavy metal residue contents of soil approved in Korea (MHMRC) (MOE, 2005).

§ Daily accumulation amount (DAA) of heavy metals at the maximum concentration ($4^0$). Accumulation percentages of heavy metals in soil relative to the treatment amounts.
| Heavy metal treatment (mg l⁻¹) | TNe | Trophic groups | cp values* |
|-------------------------------|-----|----------------|------------|
|                               |     | Fu            | Ba         | OP         | PP          | cp-1 | cp-2 | cp-3 | cp-4 | cp-5 | cp-6 |
| Before experiment             |     |               |            |            |             |       |      |      |      |      |      |
| As                            | 0.0 | 131.4 ± 112.0a | 449.0 ± 371.8a | 17.0 ± 15.7a | 294.0 ± 146.6a | 10.3 ± 3.8a | 14.0 ± 6.6a | 6.0 ± 4.4b | 6.0 ± 2.6a | 5.3 ± 2.5a |
| 0.05                          | 1,251.0 ± 374.0ab | 252.0 ± 156.7a | 511.0 ± 340.2a | 24.0 ± 15.2a | 464.0 ± 539.3a | 345.0 ± 363.3a | 395.0 ± 222.9a | 454.0 ± 537.6a | 35.0 ± 24.0a | 22.0 ± 12.0a |
| 0.2                           | 1,914.2 ± 1,235.6a | 212.8 ± 143.7a | 785.0 ± 702.2a | 19.6 ± 18.1a | 896.8 ± 861.2a | 623.0 ± 713.5a | 344.0 ± 208.3a | 899.6 ± 863.8a | 30.8 ± 23.5a | 16.8 ± 12.7a |
| 0.8                           | 784.0 ± 486.2b | 226.8 ± 170.1a | 355.6 ± 211.2a | 9.8 ± 15.3a | 191.8 ± 123.2a | 128.4 ± 47.6a | 441.0 ± 321.4a | 193.2 ± 125.0a | 11.2 ± 11.7a | 9.8 ± 15.3a |
| 3.2                           | 1,510.0 ± 751.3ab | 301.0 ± 221.2a | 483.0 ± 136.0a | 11.0 ± 8.9a | 715.0 ± 801.0a | 232.0 ± 103.7a | 535.0 ± 321.4a | 714.0 ± 798.5a | 19.0 ± 8.9a | 10.0 ± 10.0a |
| Cd                            | 0.0 | 1,005.6 ± 1,074.3b | 24.8 ± 14.8b | 714.4 ± 1,107.7a | 12.8 ± 9.1a | 253.6 ± 276.3b | 640.0 ± 1,116.1a | 256.8 ± 273.2b | 25.6 ± 15.2a | 14.0 ± 10.0a |
| 0.005                         | 979.2 ± 705.2b | 48.8 ± 33.6b | 260.0 ± 313.5a | 11.2 ± 10.7a | 652.9 ± 798.1b | 195.2 ± 288.8a | 97.6 ± 35.4bc | 661.6 ± 792.5b | 16.8 ± 25.0a | 8.0 ± 7.5ab |
| 0.02                          | 5,680.0 ± 4,956.0a | 137.0 ± 79.2a | 545.0 ± 478.1a | 19.0 ± 11.9a | 4,979.0 ± 5,107.9a | 423.0 ± 498.6a | 184.0 ± 70.9a | 4,984.0 ± 1,676.7a | 29.0 ± 16.7a | 14.0 ± 10.0a |
| 0.08                          | 602.0 ± 334.5b | 73.6 ± 22.9a | 380.0 ± 321.7a | 22.0 ± 24.1a | 144.0 ± 50.8a | 230.0 ± 118.3a | 230.0 ± 50.8a | 144.0 ± 50.8a | 29.0 ± 16.7a | 14.0 ± 10.0a |
| 0.32                          | 791.2 ± 386.8b | 73.6 ± 22.9a | 380.0 ± 321.7a | 22.0 ± 24.1a | 144.0 ± 50.8a | 230.0 ± 118.3a | 230.0 ± 50.8a | 144.0 ± 50.8a | 29.0 ± 16.7a | 14.0 ± 10.0a |
| Cu                            | 0.0 | 541.8 ± 431.5b | 51.8 ± 30.8a | 387.8 ± 373.9a | 9.8 ± 11.7a | 296.8 ± 346.5a | 138.6 ± 104.6a | 91.0 ± 63.2a | 7.0 ± 9.9a | 8.4 ± 9.1a |
| 0.05                          | 1,728.8 ± 1,222.1a | 106.6 ± 90.4a | 1,388.4 ± 1,390.9a | 4.2 ± 3.8a | 229.6 ± 273.2a | 1,258.0 ± 1,422.3a | 242.4 ± 157.3a | 224.0 ± 267.7a | 0.0 ± 0.0a | 4.2 ± 3.8a |
| 0.2                           | 614.6 ± 416.3b | 56.0 ± 49.7a | 317.8 ± 170.6a | 4.2 ± 9.4a | 236.6 ± 226.4a | 128.8 ± 79.0a | 238.0 ± 124.0a | 231.0 ± 221.5a | 12.6 ± 28.2a | 4.2 ± 9.4a |
| Pb                            | 0.0 | 592.2 ± 445.5a | 33.6 ± 31.5b | 175.0 ± 60.8ab | 7.0 ± 8.6ab | 376.6 ± 499.5a | 70.0 ± 39.3a | 114.8 ± 58.9a | 380.8 ± 501.1a | 22.4 ± 24.4ab | 4.2 ± 3.8a |
| 0.05                          | 1,067.0 ± 910.9a | 49.0 ± 36.1ab | 538.0 ± 594.9a | 21.0 ± 13.9a | 459.0 ± 859.2a | 411.0 ± 579.0a | 128.0 ± 51.2a | 465.0 ± 855.9a | 46.0 ± 39.3a | 17.0 ± 14.4a |
| 0.2                           | 535.0 ± 353.9a | 16.0 ± 10.8b | 156.0 ± 754.6ab | 16.0 ± 14.7ab | 347.0 ± 403.6a | 81.0 ± 29.2a | 87.0 ± 55.9a | 344.0 ± 402.3a | 12.0 ± 5.7b | 11.0 ± 11.4a |
| 0.8                           | 825.0 ± 840.3a | 12.0 ± 14.0b | 121.0 ± 19.8b | 4.0 ± 8.9b | 686.0 ± 863.1a | 50.0 ± 19.7a | 82.0 ± 29.7a | 686.0 ± 865.1a | 3.0 ± 2.7b | 4.0 ± 8.9a |
| 3.2                           | 530.0 ± 97.0a | 70.0 ± 46.5a | 211.0 ± 159.7ab | 5.0 ± 5.0b | 244.0 ± 226.0a | 119.0 ± 90.0a | 153.0 ± 91.0a | 243.0 ± 226.5a | 11.0 ± 8.2b | 4.0 ± 4.2a |

Numbers are averages and standard deviations of three replications (for Before experiment), four replications (for After experiment) and five replications (for As, Cd, Cu and Pb).

TNe, total nematodes; Fu, fungivorous; Ba, bacterivorous nematodes; OP, omnivorous and predatory nematodes; PP, plant-parasitic nematodes.

*Colonizer-persister (cp) values ranging from colonizer (cp-1) to persister (cp-5).

1No treatment controls.

The same letters (a, b, etc.) in a row denote no significant difference at $P \leq 0.05$ based on Duncan’s multiple range test.
| Heavy metal treatment (mg l⁻¹) | TNe | Trophic groups | cp values* |
|-----------------------------|-----|---------------|------------|
|                             |     | Fu            | Ba         | OP          | PP          | cp-1        | cp-2         | cp-3        | cp-4        | cp-5        |
| Before experiment           |     |               |            |             |             |             |              |              |             |             |
| As 0.0                     | 11.67 ± 0.58a | 1.33 ± 1.15a | 5.00 ± 1.00a | 2.00 ± 0.00a | 2.67 ± 0.58a | 1.00 ± 0.00a | 5.67 ± 1.15a | 2.33 ± 0.58a | 1.67±0.58a   | 1.00±0.00a  |
| After experiment†           | 12.10 ± 2.52a | 2.40 ± 0.82a | 4.80 ± 1.49a | 1.10 ± 0.53b | 3.70 ± 0.70a | 1.15 ± 0.30a | 5.20 ± 1.95a | 3.40±0.67a   | 1.50±0.50a   | 0.85±0.30a  |
| As 0.05                    | 14.80 ± 3.56a | 3.60 ± 0.55a | 6.20 ± 2.49ab | 0.60 ± 0.55a | 4.40 ± 1.52a | 1.60 ± 0.89a | 8.00 ± 2.00a | 3.00±1.22a   | 1.60±0.55a   | 0.60±0.55a  |
| Cd 0.0                     | 13.60 ± 4.04a | 2.00 ± 0.71b | 5.60 ± 2.61a | 1.80 ± 1.10a | 4.20 ± 1.30a | 1.00 ± 0.00a | 5.00 ± 2.12a | 4.40±1.52a   | 2.00±1.00a   | 1.20±0.84a  |
| Cd 0.005                   | 11.80 ± 5.54a | 2.80 ± 1.64ab | 4.60 ± 1.82a | 1.40 ± 1.67a | 3.00 ± 1.67a | 1.00 ± 0.00a | 5.40 ± 1.34a | 3.20±2.17a   | 1.40±1.95a   | 0.80±0.84ab |
| Cu 0.0                     | 9.40 ± 3.91a | 2.20 ± 1.48a | 2.80 ± 0.84a | 1.20 ± 1.30a | 3.20 ± 0.84a | 1.00 ± 0.00a | 3.60 ± 1.95a | 3.00±0.71a   | 0.80±1.30ab  | 1.00±1.00a  |
| Cu 0.05                    | 9.00 ± 2.12a | 1.80 ± 0.45a | 2.80 ± 1.30a | 0.60 ± 0.55a | 3.80 ± 1.30a | 1.20 ± 0.45a | 3.80 ± 1.79a | 3.40±1.14a   | 0.00±0.00b   | 0.60±0.55a  |
| Pb 0.0                     | 10.60 ± 4.72ab | 1.80 ± 1.30a | 4.60 ± 1.67ab | 0.80 ± 0.84ab | 3.00 ± 1.41ab | 1.00 ± 0.00a | 4.20 ± 1.79a | 3.20±1.79a   | 1.60±1.14ab  | 0.60±0.55ab |
| Pb 0.05                    | 13.60 ± 3.36a | 2.20 ± 0.84a | 6.20 ± 1.30a | 2.00 ± 1.22a | 2.60 ± 1.14ab | 1.00 ± 0.00a | 5.60 ± 1.52a | 3.20±2.28a   | 2.40±1.14a   | 1.40±0.55a  |
| Pb 0.2                     | 11.60 ± 3.36ab | 1.80 ± 1.10a | 4.00 ± 1.41b | 1.40 ± 1.52a | 4.00 ± 0.71a | 1.00 ± 0.00a | 4.60 ± 1.82a | 3.60±0.50a   | 1.40±0.55ab  | 1.00±1.00ab |
| Pb 0.8                     | 8.20 ± 1.64b | 1.00 ± 1.22a | 4.20 ± 1.30b | 0.20 ± 0.45b | 2.60 ± 1.14ab | 1.00 ± 0.00a | 4.00 ± 1.41a | 2.40±1.14a   | 0.60±0.55b   | 0.20±0.45b  |
| Pb 3.2                     | 10.80 ± 3.11ab | 2.20 ± 1.10a | 4.40 ± 1.14ab | 1.00 ± 1.00ab | 2.40 ± 0.89b | 1.00 ± 0.00a | 5.40 ± 2.07a | 2.20±0.84a   | 1.40±0.55ab  | 0.80±0.84ab |

Numbers are averages and standard deviations of three replications (for Before experiment), four replications (for After experiment), five replications (for As, Cd, Cu and Pb).

TNe, total nematodes; Fu, fungivorous; Ba, bacterivorous nematodes; OP, omnivorous and predatory nematodes; PP, plant-parasitic nematodes.

*Colonizer-persister (cp) values ranging from colonizer (cp-1) to persister (cp-5).

†No treatment controls.

The same letters (a, b, etc.) in a row denote no significant difference at $P \leq 0.05$ based on Duncan’s multiple range test.
### Table 4. Effects of heavy metal treatments on general and expanded maturity indices of nematode communities in soils treated with different concentrations of heavy metals

| Heavy metal treatment (mg l<sup>-1</sup>) | General maturity indices | Expanded maturity indices |
|-----------------------------------------|--------------------------|---------------------------|
|                                         | MI | MI<sub>25</sub> | ∑MI<sub>25</sub> | PPI | EI | SI | CI |
| Before treatment                        |    |               |                |     |    |    |    |
| As Control                              | 2.48 ± 0.29a              | 3.15 ± 0.06a            | 3.09 ± 0.02a | 2.90 ± 0.10a | 77.29 ± 10.30a | 81.76 ± 1.43a | 12.30 ± 5.84a |
| After treatment*                        | 1.72 ± 0.19b              | 2.36 ± 0.15b            | 2.70 ± 0.11b | 2.96 ± 0.04a | 78.29 ± 10.48a | 41.40 ± 14.90b | 12.94 ± 9.18a |
| As Control                              | 1.84 ± 0.42a<sup>†</sup>  | 2.31 ± 0.24ab           | 2.61 ± 0.13ab | 2.90 ± 0.11b | 67.93 ± 20.33a | 37.39 ± 21.06a | 24.27 ± 16.56a |
| 0.05                                    | 1.84 ± 0.41a              | 2.38 ± 0.21a            | 2.63 ± 0.22ab | 2.94 ± 0.10ab | 74.16 ± 16.30a | 45.86 ± 16.54a | 28.18 ± 24.89a |
| 0.2                                     | 1.68 ± 0.37a              | 2.37 ± 0.20a            | 2.66 ± 0.30a | 3.00 ± 0.00a | 79.57 ± 15.41a | 45.42 ± 18.35a | 19.76 ± 19.29a |
| 0.8                                     | 1.79 ± 0.21a              | 2.09 ± 0.10b            | 2.36 ± 0.04b | 2.99 ± 0.02ab | 66.49 ± 11.11a | 14.23 ± 14.98b | 27.81 ± 14.96a |
| 3.2                                     | 1.75 ± 0.20a              | 2.15 ± 0.08ab           | 2.56 ± 0.24ab | 2.99 ± 0.01ab | 71.11 ± 15.11a | 23.73 ± 9.81b  | 26.27 ± 20.13a |
| Cd Control                              | 1.61 ± 0.32a              | 2.57 ± 0.09a            | 2.83 ± 0.07a | 3.00 ± 0.01a | 88.57 ± 7.12a  | 61.21 ± 6.35a  | 4.27 ± 2.68a  |
| 0.005                                   | 1.74 ± 0.25a              | 2.38 ± 0.36a            | 2.78 ± 0.25ab | 2.98 ± 0.04a | 76.34 ± 17.89a | 39.75 ± 33.91a | 9.04 ± 8.81a  |
| 0.02                                    | 1.74 ± 0.42a              | 2.42 ± 0.25a            | 2.87 ± 0.22a | 3.00 ± 0.01a | 81.65 ± 12.88a | 48.73 ± 14.60a | 14.61 ± 13.71a |
| 0.08                                    | 1.72 ± 0.38a              | 2.33 ± 0.24a            | 2.54 ± 0.14b | 2.90 ± 0.14a | 81.06 ± 10.02a | 40.49 ± 26.38a | 15.13 ± 11.54a |
| 0.32                                    | 1.77 ± 0.19a              | 2.52 ± 0.39a            | 2.75 ± 0.22ab | 2.96 ± 0.06a | 81.66 ± 10.22a | 53.14 ± 23.54a | 9.54 ± 5.07a  |
| Cu Control                              | 1.52 ± 0.43a              | 2.22 ± 0.28a            | 2.59 ± 0.16a | 2.98 ± 0.05a | 85.96 ± 12.85a | 25.36 ± 28.47a | 6.82 ± 7.32a  |
| 0.05                                    | 1.33 ± 0.33a              | 2.06 ± 0.08a            | 2.47 ± 0.24a | 2.96 ± 0.05a | 87.46 ± 15.55a | 10.52 ± 12.67a | 9.61 ± 16.10a |
| 0.2                                     | 1.72 ± 0.14a              | 2.10 ± 0.22a            | 2.45 ± 0.21a | 2.98 ± 0.05a | 70.45 ± 8.34a  | 11.55 ± 25.83a | 8.73 ± 4.43a  |
| 0.8                                     | 1.67 ± 0.39a              | 2.30 ± 0.38a            | 2.64 ± 0.25a | 2.98 ± 0.02a | 81.20 ± 9.58a  | 31.85 ± 26.50a | 10.15 ± 6.92a |
| 3.2                                     | 1.61 ± 0.18a              | 2.12 ± 0.11a            | 2.49 ± 0.15a | 2.99 ± 0.03a | 73.97 ± 20.90a | 18.15 ± 16.98a | 9.39 ± 6.91a  |
| Pb Control                              | 1.91 ± 0.37a              | 2.35 ± 0.26ab           | 2.76 ± 0.16a | 2.98 ± 0.03a | 70.71 ± 15.87a | 41.67 ± 26.96ab | 16.39 ± 16.76a |
| 0.05                                    | 1.93 ± 0.53a              | 2.73 ± 0.27a            | 2.87 ± 0.13a | 2.97 ± 0.07a | 77.45 ± 20.14a | 67.22 ± 14.26a | 8.62 ± 8.16a  |
| 0.2                                     | 1.91 ± 0.29a              | 2.65 ± 0.51ab           | 2.81 ± 0.19a | 2.97 ± 0.03a | 82.47 ± 5.88a  | 56.84 ± 27.54ab | 4.45 ± 2.70a  |
| 0.8                                     | 1.76 ± 0.11a              | 2.25 ± 0.39b            | 2.81 ± 0.17a | 2.98 ± 0.05a | 71.66 ± 12.12a | 24.80 ± 30.47b | 6.65 ± 8.35a  |
| 3.2                                     | 1.73 ± 0.15a              | 2.19 ± 0.05b            | 2.64 ± 0.20a | 2.99 ± 0.01a | 75.52 ± 7.87a  | 29.20 ± 5.65b  | 16.66 ± 8.42a |

Numbers are averages and standard deviations of three replications (for Before experiment), four replications (for After experiment), five replications (for As, Cd, Cu and Pb).

MI, maturity index; PPI, plant-parasitic nematodes; EI, enrichment index; SI, structure index; CI, channel index.

*No treatment controls.

†The same letters (a, b, etc.) in a row denote no significant difference at \( P \leq 0.05 \) based on Duncan’s multiple range test.
TNe occurred concurrently with those of PP and cp-3 in As and Cd treatments and with those of Ba and cp-1 in Cu and Pb treatments, respectively (Table 2). No significant concentration-dependent effects on nematode community abundance were for most trophic groups or cp values; however, a significant decrease in nematode abundance was observed in OP and cp-4 at the higher concentrations (MC and MC/4) of the Pb treatment (Table 2). On the other hand, Fu and cp-2 populations were relatively higher in soils treated with higher concentrations (MC, MC/4 and MC/4²) than those with lower concentrations (0 and MC/4²) of Cd.

No significant differences in nematode community richness were noted for all cp values and trophic groups except for OP of which the richness was significantly reduced after 18 weeks of tomato growth with no heavy metal treatment (Table 3). For most of the heavy metal treatments, there were no concentration-dependent significant differences in the richness of TNe, trophic groups and cp values; however, the nematode richness largely decreased at higher concentrations (maximum [4⁰] and four-fold dilution [4⁻]) of the Pb treatment, especially for OP and cp-4–5 (Table 3).

Changes in general and expanded maturity indices in heavy metal-treated soils. In the untreated controls, significant reductions occurred in all general maturity indices except for PPI, and among the expanded maturity only in SI that was the most remarkably reduced (by 50.6%), compared to the initial general and expanded maturity indices (Table 4). For heavy metal treatments, there were no significant differences in most maturity indices at any heavy metal treatment concentrations except for MI 2–5 and SI that showed the lowest values at their maximum (3.2 mg l⁻¹) or four-fold diluted (0.8 mg l⁻¹) concentrations of As and Pb (Table 4).

Correlations between ecological indices of nematode communities and heavy metal concentrations in soil. Significant (P ≤ 0.1) negative correlations between ecological indices of nematode communities and heavy metal contents in soil were observed for only two pairs, Ba abundance with As, and MI with Pb (Table 5). Significant positive (P ≤ 0.1) correlations were observed for richness of cp-3 and abundance of cp-2 with As, MI with Cd, and CI with Cu. Pb had the strongest negative influences on nematode community structure, with significantly negative values for 12 (44.4%) of the 27 ecological indices examined (P ≤ 0.5, r = 0.404). Medially negative influences on nematode community structure were observed for Cu and As with significantly (P ≤ 0.5) negative values for five (18.5%) and six (22.2%) ecological indices, respectively. No significantly negative influences on the ecological indices were observed for Cd at P ≤ 0.5.

Discussion

In our study, significantly increased heavy metal accumulations were noted for all heavy metal species treatments at MC. However, accumulated heavy metals contents exceeding MHMRC were observed only for As and Cu, but

| Table 5. Pearson’s correlation coefficients† for heavy metal concentrations and ecological indices of nematode communities in soils |

| Ecological indices‡ | Concentrations of heavy metals in soil | As | Cd | Cu | Pb |
|---------------------|----------------------------------------|----|----|----|----|
| Richness TNe        | 0.342 | 0.164 | 0.181 | -0.177 |
| Fu                  | 0.761† | 0.284 | -0.050 | 0.342 |
| Ba                  | 0.258 | 0.401 | -0.372 | -0.449 |
| OP                  | 0.348 | -0.133 | -0.813† | -0.201 |
| PP                  | 0.514 | -0.322 | 0.576 | -0.498 |
| cp-1§               | 0.256 | NA | 0.255 | NA |
| cp-2                | -0.072 | 0.500 | 0.571 | 0.395 |
| cp-3                | 0.874* | -0.058 | 0.790† | -0.756† |
| cp-4                | -0.331 | -0.099 | -0.069 | -0.183 |
| cp-5                | 0.475 | 0.106 | -0.763† | -0.135 |
| Abundance TNe       | 0.185 | -0.354 | 0.202 | -0.448 |
| Fu                  | 0.747† | 0.035 | 0.093 | 0.733† |
| Ba                  | -0.877* | 0.351 | -0.563 | -0.660 |
| OP                  | -0.643 | 0.574 | -0.735† | -0.472 |
| PP                  | 0.276 | -0.351 | 0.366 | -0.516 |
| cp-1§               | -0.389 | -0.330 | 0.153 | -0.194 |
| cp-2                | 0.922* | 0.238 | 0.235 | 0.707† |
| cp-3                | 0.284 | -0.350 | 0.375 | -0.523 |
| cp-4                | -0.393 | 0.372 | -0.208 | -0.355 |
| cp-5                | -0.695† | 0.409 | -0.647 | -0.498 |
| Maturity indices MI | -0.548 | 0.816* | 0.056 | -0.921* |
| MI25                | -0.738† | 0.123 | -0.341 | -0.589 |
| ∑MI25               | 0.426 | -0.390 | NA | NA |
| PPI                 | 0.536 | -0.005 | NA | NA |
| EI                  | -0.188 | -0.050 | -0.388 | -0.120 |
| SI                  | -0.571 | 0.153 | -0.294 | -0.575 |
| CI                  | 0.274 | 0.022 | 0.835* | 0.625 |

TNe, total nematodes; Fu, fungivorous; Ba, bacterivorous nematodes; OP, omnivorous and predatory nematodes; PP, plant-parasitic nematodes; cp, colonizer-persister; NA, not available.

Critical values for Pearson’s correlation coefficient in a two-tailed test (df = 3) are 0.404 for P ≤ 0.5, 0.687 for P ≤ 0.20, 0.805 for P ≤ 0.10, and 0.934 for P ≤ 0.05.

Critical values ranging from a colonizer (cp-1) to a persister (cp-5).
not for Cd and Pb, which may be related to the different accumulation rates of heavy metal species over a short period of time (18 weeks) (Table 1). This suggests that when waters draining from abandoned mines contain low levels of heavy metals, especially Cd and Pb, the total accumulations will not exceed MHMRC in agricultural soils irrigated by these waters within several months. In our experiment, even under conditions in which heavy metal accumulation in soil exceeded MHMRC, no symptoms or damages from heavy metal toxicity were observed on the tomato plants, which is in contrast to severe damages observed in tomato plants grown in soil contaminated with high concentrations of heavy metals (Park et al., 2011b). This suggests it is difficult to detect any visible adverse short-term effects of heavy metal contamination at low levels on physiological disorders caused by heavy metals.

In our study, nematode community abundance in the untreated controls was remarkably increased for most of trophic groups, especially those of PP and cp-3 that shared common taxa such as Meloidogyne and Pratylenchus (data not shown). Also in the heavy metal treatments, the remarkable nematode population increases were attributed to the outnumbered increases of PP and cp-3 or Ba and cp-1 that shared only with Ba in our study (Table 2). These population increases in trophic groups of PP and Ba may be derived from their enhanced replication rates due to the increased nutritional sources such as plants and bacterial decomposers of plant-derived organic materials (plant debris and root exudates), respectively (Coleman et al., 1984; Freckman and Caswell, 1985). On the other hand, Fu abundance were not increased, probably owing to the slower colonizing and lower reproductive characteristics of Fu with higher cp values than those of Ba (Bongers and Bongers, 1998). Furthermore, the abundances of OP for all with cp-4 or cp-5 increased the least and even their richness was reduced significantly after tomato growth probably because they all have characteristics of persister nematode species with long life cycles and low reproduction rates, and also they are most susceptible to environmental disturbance caused by agricultural practices, i.e., soil preparation for tomato planting in our study (Bongers and Bongers, 1998; Bongers and Ferris, 1999). Considering that no new nematode species had a chance to be introduced to the soils during our study, the increased numbers of nematode genera (or families) after plant growth might have resulted from increased population densities leading to the detection of previously present but undetected taxa.

Decreases in nematode community abundance and richness resulting from heavy metal contamination have been reported in many studies (Georgieva et al., 2002; Korthals et al., 1996a, 1996b; Park et al., 2011b; Popovici, 1994; Yeates et al., 1993). However, only abundance of sensitive nematode species is reduced in the soils exposed to light to moderate heavy metal contamination, while the abundance of tolerant species is not changed or increased in these soils (Korthals et al., 1996a, 1996b). This may be one of the reasons that no significant decreases in nematode community abundance and richness were shown for most trophic groups and cp values in the soils with low-level of heavy metal contaminations in our study. Another reason may be derived from the increased nematode populations during tomato growth that have surpassed the influence of the heavy metals, which was evidenced by the remarkable abundance increases of PP and Ba in our study (Table 2). Probably because of these reasons, the trophic group of OP and cp values of 4–5, which are most susceptible to soil disturbance caused by tomato planting as well as heavy metal contamination (Bongers and Bongers, 1998), were only reduced in the higher concentrations of Pb that showed the highest negative influences on the nematode communities in our study (Table 5).

The changes in the abundance and richness of nematode trophic groups and cp values were fully reflected on the changes of general and expanded maturity indices, which are determined by the number and productivity characteristics of nematodes (Odum, 1985). The nematode community structure is shifted towards dominance by opportunistic species such as cp-1 and cp-2, decreasing the MI and especially SI (Bongers and Ferris, 1999; Korthals et al., 1996b). In the same manner as above, SI and all of general maturity indices were significantly reduced in the untreated controls after the tomato growths for 18 weeks except for PPI that surpassed the negative influences of heavy metal contamination. Also only MI 2–5 (excluding PP and cp-1 of Ba) and SI were significantly reduced in soils treated with higher concentrations of Pb and even As with less negative influences on nematode community than Pb (Table 5). In particular, the SI values of the final nematode communities were clearly reduced compared to that of the initial community, which may reflect the selective increase of nematode populations with low cp values under food-rich conditions—conditions under which nematodes with high cp values are suppressed due to their sensitivity to such disturbances (Bongers and Bongers, 1998). This is consistent with the results of our previous study on soil nematode communities exposed to high levels of heavy metal contamination for a long period of time, which shows mainly the reduced SI indicating disturbed soil ecosystems induced by the heavy metal contamination (Park et al., 2011a).

All of these results suggest Pb is the most adversely influential heavy metal species on nematode community structure at low concentrations of heavy metal accumu-
tion over a short period of time, and thus on soil health and agricultural ecosystems. The ecological indices influenced most by Pb were abundance (decreased) of OP and cp-4, and a decrease in SI that is indicative of a disturbed/degraded soil ecosystems, which implies the impacts of heavy metal contamination were most prominent on the OP and cp-4–5 nematode groups, which have long life cycles and low fecundity and possibly, low capacities for such environmental changes, providing reasonable evidence for their sensitivity to heavy metal stress. Therefore, the minimal ecological changes induced by short-term, low-level contamination of some heavy metals can be detected by the changes of soil nematode community structure before the appearance of physiological disorders of plants caused by the abiotic agents (heavy metals).

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