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Article

Toxicity of Organic Waste-Contaminated Soil on Earthworm (*Eisenia fetida*)

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The toxicities of contaminated soils with 8 consecutive year applications of three levels (12.5, 25.0, and 50.0 t dry matter ha^{-1} yr-) of four organic sludge [municipal sewage sludge (MSS), industrial sewage sludge (ISS), alcohol fermentation processing sludge (AFPS) and leather processing sludge (LPS)] on earthworm (*Eisenia fetida*) were examined by using microcosm container in the laboratory. Results were compared with those of pig manure compost (PMC) treated soil. In tests with three treatment levels (12.5, 25.0, and 50.0 t per plot), ISS treated soil showed higher contents of Cu (18.9~26.2 fold), Cr (7.7~34.7 fold), and Ni (14.8~18.8 fold) at 8 years post treatment, than PMC treated soil. LPS treated soil showed higher contents of Cr (35.7~268.0 fold) and Ni (4.5~7.6 fold) than PMC treated soil. There were no great differences in heavy metal contents among MSS, AFPS, and PMC treated soils. In these contaminated soils, earthworm mortalities of MSS and AFPS treated soils at 8 weeks post-exposure were similar to those of PMC treated soil regardless of each treatment level. Toxic effect (26.7~96.7 mortality) on the ISS and LPS treated soils was significantly higher than one of PMC treated soil, with an exception of LPS soil treated with 25.0 t per plot. At 16 weeks post-exposure, earthworm mortalities of AFPS' 12.5 and 25.0 t treated soils were similar to those of PMC treated soil. Toxic effect (53.3~100 mortality) on the 12.5, 25.0, and 50.0 t treated soils of MSS, ISS and LPS, and AFPS' 50.0 t treated soils was significantly higher than those of PMC treated soil. The data suggested that the 12.5, 25.0, and 50.0 t of MSS, ISS and LPS, and AFPS' 50.0 t treated soils were evaluated to have toxicity on earthworm.

Key words : Earthworm, Organic waste, Heavy metals, Contaminants

Introduction

Soil contamination can occur from the spreading on agricultural land of sewage sludge, slurries of animal dung containing copper, and landfill disposal of industrial and municipal wastes containing heavy metals such as copper (Cu), chromium (Cr), cadmium (Cd), lead (Pb), nickel (Ni), and many other metals. Soils are the ultimate sink of many toxic materials. Ecologically earthworms are near the bottom of the terrestrial tropic levels. Concerns about contamination of soil and detrimental effects of contaminants on the living environment have resulted in a strong and growing interest in soil organisms among environmental scientists and legislators. A legislation in many countries has recently focused on the need of sensitive organisms from the soil environment for environmental monitoring.

The effects of contaminants on earthworms have been studied at the laboratory (Edwards and Thompson, 1973). These tests tended to produce consistent and reproducible results because 10 individuals of *E. fetida* were used and these worms were an intimate contact with pollutants. Van Hook (1974) demonstrated that earthworms could serve as useful biological indicators of contamination because of the fairly consistent relationships between the concentrations of various contaminants and mortality of earthworm. Burrows and Edwards (2002) have tried to use integrated soil microcosm to predict effects of contaminants on soil ecosystems. Soil microcosms have the advantage of being easy to handle and inexpensive, and can be kept under controlled condition with more replication and provide more reproducible results than field tests. In Korea, assessment system in soil pollution

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varies depending on the concentration of heavy metals. Very little work has been done on the development of biological assessment.

We conducted to assess potential toxicity of the soil treated with four organic sludges and pig manure on earthworm in the laboratory.

Materials and Methods

Waste materials and pig manure Four waste materials used in this study were as follows: municipal sewage sludge (MSS) collected from sewage treatment plants in 1994 on Gwacheon city, Gyeonggi Province, Korea; industrial sewage sludge (ISS) and alcohol fermentation processing sludge (AFPS) from Ansan industrial complex, Gyeonggi Province, in 1994; leather processing sludge (LPS) from sewage treatment plant on Cheongju, Chungnam Province, Korea, in 1994. Pig manure compost (PMC) was purchased from Anjung Nong-hyup located in Anjung, Gyeonggi Province, in 1994. These materials were kept in deep-freezer to be applied annually from 1994 to 2001.

Chemical properties of these materials were described by Na (2004). MSS had characteristics that all components of heavy metal were low level in comparison with legally allowed limit values (Cu, 300; Zn, 900; Cr, 300; Cd, 5; Pb, 150; Ni, 50 mg kg^{-1}), although the content of Zn was relatively higher as 797 mg kg^{-1}. ISS was characterized by the excess level in four components that the contents of Cu, Zn, Cr, and Ni appeared 19.3, 3.1, 3.7, and 22.8 times as high as their limits, respectively. LPS exceeded limit value in two heavy metals that the contents of Cr and Cd were 34.3 and 1.5 times as high as their limits, respectively. AFPS contained 56 mg kg^{-1} of Ni and this value was 1.5 times higher than the limit value. PMC had characteristics that one component, Cu was 1.9 times higher in comparison with limit level and the other components were low levels, with an exception of Zn.

Contents of heavy metals in soils from field lysimeter Lysimeter was installed in the upland field of Suwon, Gyeonggi Province, Korea, in 1993. Lysimeter was composed of 45 plots of 1.0 m length, 1.0 m width, and 1.1 m depth. Each plot was filled with the sandy loam soil and mixed into the soil of 15 cm in depth. Three levels (12.5, 25.0, and 50.0 t dry matter ha^{-1} yr^{-1}) of the organic materials were applied to each plot with two times split application yearly for eight consecutive years (1994-2001) and mixed into the soil of 15 cm in depth. PMC served as a standard for comparison in tests. A randomized complete block design with three replicates was used. Two radish varieties, *Raphanus sativus* var. jinmialtari and *R. sativus* var. backkyoung, were cultivated in spring and autumn, respectively.

Soil samples were taken from the 45 lysimeter plots in 2001. The samples were collected from the top 10 cm of the soil, air-dried, and crushed to pass through a 2 mm sieve. A 10-g dried soil sample from each plot was homogenized in 50 ml of 0.1 N HCl solution by shaking the preparation on a rotary shaker at 30℃ for 1 hr and filtered through Whatman No. 6 filter paper. Heavy metals were analyzed through atomic absorption spectrometer AA6701F (Shimadzu, Kyoto, Japan) according to the method of Whitney (1988). Pollution index (PI) was calculated by the formula of Kloke (1979), PI = [∑(heavy metal concentration in soil/tolerable level)/number of heavy metal].

Test organism *E. fetida* was maintained in the laboratory in an artificial soil after catching them from the heap of compost made from rice straw. They were kept in a darkened incubator at 21±1℃. The earthworms used in this assay were adults with well-developed clitellum.

Toxicity test of organic waste-contaminated soil on earthworm Microcosm container consisted of a 14 cm length, 14 m width, and 7 m depth square of commercially available high-density stable polyethylene box with 36 pores (1 mm diameter) of lid. Soil samples from plots treated with organic wastes for eight consecutive years were sieved gently through a 2 mm mesh sieve and 300 g fresh weight of soil was hydrated to 40% of water holding capacity. In microcosm preliminary test, 40% of water holding capacity was optimal for microcosm test. Hydration water required to achieve the desired hydration was calculated as follows:

Hydration water to be added (ml 100 g^{-1})
$$= \text{THWts} - \text{EHWts} \tag{1}$$

THWts (total test soil hydration water desired, ml 100 g^{-1})
$$= \text{PHYD} \times [(\text{PAS} \times \text{WHCas}) + (\text{PWS} \times \text{WHCws})] \times 100 \tag{2}$$

EHWts (existing test soil hydration water, ml 100 g^{-1})
$$= [(\text{PAS} \times \text{WCas}) + (\text{PWS} \times \text{WCws})] \times 100 \tag{3}$$

Where PHYD = proportion of hydation required (e.g. 0.4);

PAS = proportion of artificial soil in test soil;
WHCas = water holding capacity of the artificial soil;
PWS = proportion of waste sample in the test soil;
WHCws = the water holding capacity of the waste sample;
WCas = water content of the artificial soil;
WCws = water content of the waste sample.

Ten earthworms were placed into each microcosm as described above. The microcosms were kept in a controlled chamber at 20℃ and a photoperiod of 12:12 (L:D). Mortality of individual earthworm was assessed by emptying the test soil onto a tray and sorting the worms from the soil. Earthworms were considered dead when they do not respond to a gentle touch on their anterior with a pin. Live worms were placed back into their test microcosms and placed on the surface of the soil. The numbers of live and dead worms in each test microcosms were recorded every 2 weeks and the dead worms were discarded. The microcosm boxes were randomly designed with three replicates.

Results and Discussion

Contents of heavy metals in soils treated with test materials The amounts of residual heavy metals (Cu, Zn, Cr, Cd, Pb, and Ni) of the lysimeter soils sampled at 8 years post-treatment are given in Table 1. The contents of heavy metals varied according to treatment level and waste material tested. In tests with three treatment levels (12.5, 25.0, and 50.0 t per plot), ISS treated soil showed higher contents of Cu (18.9~26.2 fold), Cr (7.7~34.7 fold), and Ni (14.8~18.8 fold), than PMC treated soil. LPS treated soil showed higher contents of Cr (35.7~268.0 fold) and Ni (4.5~7.6 fold) than PMC treated soil. There were no significant differences in heavy metal contents among MSS, AFPS, and PMC treated soils.

The pollution indices (PI) are given in Table 1. The PI values of PMC treated soil (0.03~0.06) were similar to those of MSS and AFPS treated soils. Very high PI values were observed in ISS treated soil (0.31~0.77) and LPS treated soil (0.22~1.38).

Toxicity of organic waste-contaminated soil on *E. fetida* in the laboratory Survival rate of MSS, ISS, LPS, AFPS, and PMC-contaminated soils to *E. fetida* was investigated every 2 weeks (Fig. 1). The PMC soil did not affect survival rate of the earthworm, whereas MSS and AFPS began to resulted in death after 8 weeks. ISS and LPS caused death of earthworms after 4 weeks. Earthworms exposed to the soil of the four organic sludge died after 16 weeks, except for PMC' soil.

Earthworm-exposed duration was established as 8 and 16 weeks based on the result of survival data in Figure 1. Each mortality of *E. fetida* in MSS, ISS, LPS, AFPS, and PMC-contaminated soils is shown in Table 2. Mortality of Earthworm varied according to waste material,

Table 1. Contents of heavy metals and pollution index (PI) in lysimeter soils treated with three levels of test materials at 8 years post-treatment.

Material†	Rate (t dr. M ha^{-1} y^{-1})‡	Content (mg kg^{-1})						PI
		Cu	Zn	Cr	Cd	Pb	Ni	
MSS	12.5	5.5	2.7	0.1	0.09	5.0	0.2	0.02
	25.0	9.5	26.4	0.2	0.11	6.2	0.2	0.03
	50.0	14.7	29.5	0.5	0.08	6.6	0.4	0.04
ISS	12.5	186.8	27.1	2.3	0.02	9.9	4.5	0.31
	25.0	246.0	41.4	4.9	0.15	12.5	5.9	0.44
	50.0	419.6	36.0	10.4	0.19	14.2	9.4	0.77
LPS	12.5	5.2	54.2	10.7	0.23	8.0	2.0	0.22
	25.0	3.5	12.8	23.4	0.25	10.2	1.8	0.42
	50.0	3.9	25.1	80.4	0.44	12.3	3.8	1.38
AFPS	12.5	5.7	3.9	0.6	0.15	3.8	0.4	0.03
	25.0	7.8	2.4	0.1	0.11	2.9	0.2	0.02
	50.0	13.8	5.0	n.d.	0.14	1.2	0.6	0.03
PMC	12.5	9.4	21.0	0.3	0.10	3.6	0.3	0.03
	25.0	13.0	47.7	0.2	0.09	2.6	0.4	0.04
	50.0	16.0	85.5	0.3	0.19	1.5	0.5	0.06

† ‡ For explanation, see Materials and Methods.

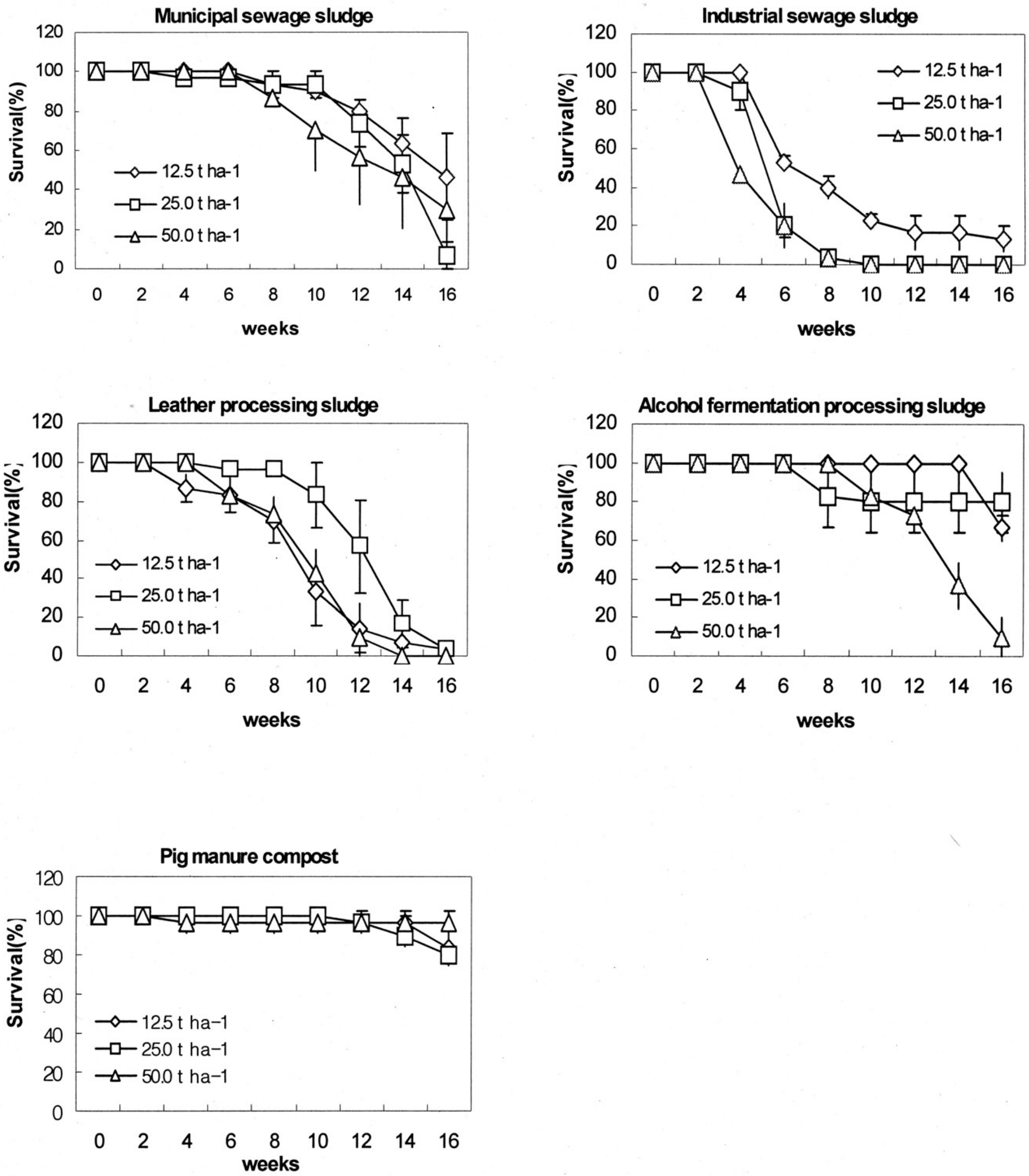

Fig. 1. Changes of the rate of survival earthworm, *E. fetida* in oils treated with four organic wastes and pig manure compost for eight years using microcosm soil test.

treatment level, and exposure duration. At 8 weeks post-exposure, earthworm mortalities of MSS and AFPS treated soils were similar to those of PMC treated soil regardless of each treatment level. Toxic effect (26.7~96.7 mortality) in the ISS and LPS treated soils were significantly observed very higher than those of PMC treated soil, with an exception of LPS soil treated with 25.0 t per plot. At 16 weeks post-exposure, earthworm mortalities of AFPS' 12.5 and 25.0 t treated soils were similar to those of PMC treated soil. Toxic effect (53.3~100 mortality) in the 12.5, 25.0, and 50.0 t treated soils of MSS, ISS and LPS, and AFPS' 50.0 t treated soils appeared significantly very higher than those of PMC treated soil.

From these results, the copper contents of ISS' soils were 3.7, 4.9, and 8.4 times as high as the threshold level (50 mg kg^{-1}) suggested by SECA (Soil Environmental Conservation Act) in Korea. Additionally, the contents of residual chromium were 1.2 to 2.6 times the higher than threshold level, 4 mg kg^{-1}. For LPS, 10.7, 23.4, and 80.4 mg kg^{-1} of chromium were accumulated in the soils, respectively, which were estimated 2.7, 5.9, and 20.1 times higher than threshold level. These high values of Cu and Cr of ISS and LPS treated soils might cause the most strong toxicity against *E. fetida.* Van Rhee (1975) studied toxic effects of copper in soil. He reported that 110 mg kg^{-1}

Table 2. Mortality of earthworm, *E. fetida* in organic sludge-contaminated soils and PMC-treated soil.

Contaminated soil samples	Mortality, % (mean±SE) Earthworm-exposed duration	
	8 weeks	16 weeks
MSS, 12.5[†]	6.7± 5.8 def	53.3±37.9 bcd
MSS, 25.0	6.7±11.5 ef	93.7±11.5 ab
MSS, 50.0	13.5± 5.8 cde	70.0±30.0 abc
ISS, 12.5	60.0±10.0 ab	86.7±11.5 ab
ISS, 25.0	96.7± 5.8 a	100± 0.0 a
ISS, 50.0	96.7± 5.8 a	100± 0.0 a
LPS, 12.5	30.0±20.0 bc	96.7± 5.8 ab
LPS, 25.0	3.3± 5.8 ef	96.7± 5.8 ab
LPS, 50.0	26.7±15.3 bcd	100± 0.0 a
AFPS, 12.5	0± 0.0 f	33.3±11.5 cde
AFPS, 25.0	16.7±28.9 cdef	20.0±26.5 efg
AFPS, 50.0	0± 0.0 f	90.0±17.3 ab
PMC, 12.5	0± 0.0 f	16.7±15.3 efg
PMC, 25.0	0± 0.0 f	0± 0.0 efg
PMC, 50.0	3.3± 5.8 ef	5.3± 5.8 g

[†] For explanation, see Materials and Methods.
Numbers followed by the same letter within a column are not significantly different(Scheffe's test, $p<0.05$).

of copper in soil were toxic to earthworm. Neuhauser *et al.* (1985) determined a 14-day LC_{50} for copper of 643 mg kg^{-1}, while NOEC (no observed effect concentration) reproduction values of between 30~120 have been calculated in natural and artificial soils (Ma, 1988). Spurgeon *et al.* (1994) determined NOECs for *E. fetida* exposed to heavy metals. The estimated NOEC values were 32 mg Cu kg^{-1}. Cr(Ⅵ) Ⅵ is even more toxic to earthworms than Cu. A concentration of only 10 mg kg^{-1} in soil contaminated by effluent containing Cr(Ⅵ) was fatal to *Pheretima posthuma* and other species (Abbasi and Soni, 1983). The critical concentration value for Cr(Ⅲ) of 32 mg kg^{-1} reported by Van Gestal *et al.* (1993) for *E. andrei* in an artificial soil spiked with metal salts.

Earthworm mortalities in the MSS' 12.5, 25.0 and 50.0 t, and AFPS' 50.0 t-treated soils were significantly different to those of PMC treated soil at 16 weeks post-exposure, although those contents of accumulated heavy metals were similar. These results may be due to the accumulation of organic compounds for long-time besides heavy metals known to exist in sewage sludge. Hence, we need to analyze organic compounds in soils in the future.

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유기성 폐기물에 의해 오염된 토양이 지렁이에게 미치는 독성

나영은* · 방혜선 · 김명현 · 이정택 · 안용준[1] · 윤성탁[2]

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유기성폐기물을 농경지에 투여하기 위하여 작물에 미치는 영향, 토양에 축적되는 중금속 농도 분석이 이루어지고 있으나, 토양에 축적된 총 오염물질이 지렁이에게 얼마나 독성을 갖고 있는지가 구명되어 있지 못한 실정이다. 본 연구는 4종류 유기성 슬러지(생활하수슬러지, 공단하수슬러지, 피혁슬러지, 주정슬러지)와 돈분퇴비를 8년 동안 매년 ha당 12.5톤, 25.0톤, 50.0톤으로 처리한 토양에 대한 지렁이의 독성을 측정하였다. 공단하수슬러지를 처리한 토양를 돈분퇴비와 비교해 보면, 축적된 구리는 18.9～26.2배, 크롬은 7.7～34.7배, 니켈은 14.8～18.8배로 나타났고, 피혁슬러지의 크롬은 35.7～268.0배, 니켈은 14.8～18.8배가 축적되었다. 이렇게 오염된 토양에 지렁이를 8주와 16주 동안 각각 노출시켰다. 8주 노출에서 생활하수슬러지와 주정슬러지의 모든 처리토양과 피혁슬러지 25.0톤 처리 토양은 돈분퇴비와 비슷한 낮은 독성을 나타냈으나, 나머지 공단슬러지와 피혁슬러지의 지렁이 치사율은 26.7～96.7%으로 높게 나타났다. 16주 노출에서 주정슬러지 12.5톤, 25.0톤의 토양만 돈분처리 토양과 비슷한 낮은 지렁이 독성을 보였고, 나머지 처리토양들은 모든 53.3～100%의 지렁이 치사율를 나타냈다. 이러한 결과들은 주정슬러지 50.0톤 처리 토양을 제외한 다른 슬러지 처리 토양은 지렁이에게 독성을 가지는 것으로 나타났다.