

Relationship between Extraction Methods of Copper in Soil and the Bioaccumulated Copper in Earthworm

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This study compared the correlation between the accumulated copper content in earthworms and the copper concentration rate of soil measured using several methods to extract heavy metals from soil. For the experiment, a microcosm soil test was carried out using copper contaminated soil from the vicinity of copper-roofed buildings and earthworms (*Eisenia fetida*). Soils from the study area were used to produce 6 treatments; control, 1C (contamination level with the lowest treated copper concentration rate), 2C, 4C, 8C, and 16C (contamination level with the highest treated copper concentration rate). Microcosm soil test using the 6 treatments proved that as the copper content in soil and the experiment time increased, the growth rate of and the accumulated copper concentration rate in earthworms increased as well. The degree of the increase corresponded to the order of the treated copper concentration levels in microcosm soils. Standard method of the ministry of environment and EPA method 3051 were used to obtain the copper concentration in soil and the total copper content in soil, respectively. The correlation coefficient (r) of 0.9875–0.9993 between the copper content extracted by the standard method and the total copper content shows high positive correlation. The correlation coefficient of the copper content in soil extracted by the standard method and the accumulated copper content in earthworms, and the correlation coefficient of the total copper content in soil and the accumulated copper content in earthworms were ranged from 0.9193 to 0.9728 and from 0.9282 to 0.9844, respectively, showing highly significant positive correlation. Due to the high correlation between the copper concentration in soil and the accumulated copper content in earthworms, it is concluded that earthworms are suitable to be used as biological indicator species or for bio-monitoring against copper contamination of soil.

Key words: Copper, Earthworm, Microcosm soil test, Bioaccumulation

Introduction

Among soil organisms, earthworms are abundant in their species and found worldwide. They tend to prefer soil which are affected by human (Lavelle and Spain, 2001). Earthworms increase soil fertility by digesting the organic matters of the surface soil and transporting them to subsurface layers. They also play a significant role in the translocation and mixing of soil constituents (Langdon *et al.*, 2003). Through this process, earthworms also are impacted by soil pollutants. Most of the pollutants accumulate throughout the food chain and eventually are transferred to the higher level of the food pyramid (Langdon *et al.*, 2003). Due to these characteristics and their role as a stimulant in the humification of soil organic matters, earthworms are

widely used as an important indicator in monitoring the effects of pollutants in soil and the soil quality throughout the world (Dai *et al.*, 2004; Langdon *et al.*, 2003). As a result of their contact with soil, through ingestion and dermal contact, in both the solid and aqueous phases, earthworms have a high capacity for accumulating toxic elements. However, the extent of accumulation is dependant upon the type of element and on the soil properties (Langdon *et al.*, 2003). The relationship between soil pollution and the accumulation of toxic elements in earthworms can be used to measure the level of environmental pollution (Paoletti, 1999).

There are two methods with which to extract contaminants using earthworms; carrying out the experiment directly at the contaminated sites or using collected soil from the site in the laboratory. The risk assessments of soil pollutants and toxic chemical matters using earthworms have been performed by Lofs (1992) and Kula (1992) as field experiments. In field

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experiments studying the environmental impact of various complex factors, more toxin-focused researches have been performed than the chemical-focused. Heimbach's test (1992) comparing the LC₅₀ (median lethal concentration) value between the artificial soil test and the standardized field test showed the significant result of high correlation ($r=0.86$). However, the artificial soil test had the drawback of having to employ a single species and a single experimental process.

As an alternative one, Morgan and Knacker (1994) introduced a microcosm, an ecological model built with similar conditions as in the field, which reduces the discrepancies between the laboratory setting and the field. Microcosm is a model ecosystem in which population or community which is cultivated in specific containers within controlled environment. It allows examine the interaction among organisms. In order to utilize microcosm, the species must be known, and the measuring the number of each species must be possible. The experiment reproducibility must also be superior. It enables the forecast and evaluation of the external disturbance on natural ecosystem by evaluating the impact of various contaminants (Wui *et al.*, 2002).

Microcosm plays a significant role in developing and verifying ecological theories and in connecting theory and reality (Fraser, 1999). Recently, various scales of microcosm techniques were developed and utilized for the examination of ecotoxicology (Martikainen *et al.*, 1998). Burrow and Edwards (2002) have implemented the more developed type of soil microcosm in their evaluation of the contaminants' effect on soil ecology.

Soil microcosm has the advantage of higher reproducibility when compared to field research. This is due to the controlled environment, which enables larger number of replicates to be produced economically (Na, 2004; Tarradellas *et al.*, 1996). Microcosms are now actively used in studying the effects of heavy metals - especially when researching impact of copper on soil organisms and invertebrates. They are useful in comparing various experiment results (Bogomolov *et al.*, 1996).

For this study microcosms were constructed with copper-contaminated soil, which were collected from sites near buildings with copper plate roof in order to access the indicator for soil contamination. The objective of this study was to investigate the relationship between copper concentration in earthworms and copper concentration level of soils, which were obtained using various heavy metal extraction methods.

Materials and Methods

Soil sampling Soil samples were collected from locations near by the Grand Hall and the 21st Century Building of The University of Seoul in Seoul, Korea. Both buildings have copper plate roof and were built in 1981 and 2001, respectively. In August 2005, samples were collected from locations directly under the Grand Hall's copper roof and from locations with some distance from under the roof. Three sites directly under the roof, one site which was 5 meters away from under the roof and as the control, a nearby site whose soil was not affected by the copper roof were chosen. From each site, the surface soil of 2 cm depth was collected for obtaining over 20 kg after drying. The soil samples were passed through a 4mm sieve and kept at a cool and dark place until the microcosm test began.

Physico-chemical analysis of the soil samples The soil samples were air dried and then passed through a 2mm sieve. The pH and EC of the samples (soil:deionized water = 1:5) were first shaken and then left for one hour. Soil pH (MP230, Mettler toledo, UK) (Thomas, 1996) and EC were measured with a electrical conductivity meter (MC226, Mettler toledo, UK). The organic matter content was measured by Walkley-Black method (Nelson and Sommers, 1996), while the content of the available phosphorus was examined by Bray No. 1 method (Kuo, 1996). Cation exchange capacity (CEC) and the exchangeable-Ca, -Mg, and K concentration were measured with an atomic absorption spectrophotometer (AA-6800, Shimadzu, Japan) after being extracted by 1 N ammonium acetate with pH 7.0 (Sumner and Miller, 1996). Micro-pipette method (Miller and Miller, 1987) was used for the particle size analysis. The heavy metal contents (Cd, Cu, Pb, Zn) of the soil were extracted with 0.1 N HCl, and their concentrations were analyzed with an atomic absorption spectrophotometer (AA-6800, Shimadzu, Japan) (Ministry of environment, 1996).

Cultivation of earthworm The earthworms used in the study, *Eisenia fetida* (Oligochaeta, Lumbricidae) are found worldwide. They are currently bred in farms in Korea as fishing baits and sources of health food. These farms are under the guidance of Yeosu Agricultural Technology and Extension Center. The experimental conditions for the growth of earthworm were in accordance with the manual by the Method Development

and Applications Section Environmental Technology Centre (2004).

The earthworms from the breeding farms were first kept in an incubator at $20 \pm 2^\circ\text{C}$ and relative humidity was adjusted about 50% for the laboratory setting. The substrate was kept without water overflowing on its floor, and the earthworms were exposed for 16 hours of light period and 8 hours of dark period daily for two weeks.

The density of the earthworms were maintained at below 0.03 g/cm^3 during the cultivation. In order to produce 10 L of soil material for the cultivation, 3 L of growing media and 4 L of dried moss peat were mixed. 1 L of distilled water was added to it, and then was well mixed until the water content, color and the soil texture of the mixture became consistent. Afterwards, 1.5 L of artificial soil and 1 L of distilled water were added and again mixed well. Maximum 30 g of CaCO_3 were added to the mixture and mixed well until the CaCO_3 powder was invisible, and the pH reached 6.0–7.5.

The artificial soil used as substrate was consisted of 10% of *Sphagnum* sp. peat which was passed through 2mm sieve after being air dried and 20% of kaolinite with particle size under $40 \mu\text{m}$, and 40% of grade 70 diatomite. The Quaker brand oatmeal was mixed with distilled water in the ratio of 1:2. The fully soaked mixture was cooled to room temperature and then fed to the earthworms. Five mL of the feed were given to each microcosm once a week. The survival rate of the earthworms during cultivation was maintained at above 90%.

Microcosm soil test HDPE (high-density polyethylene) pipes were used to make microcosms. Copper-contaminated soil sample collected from directly under the Grand Hall's roof was of highest copper concentration. This was mixed 1:1 ratio with the uncontaminated soil from the control site to obtain a soil sample. This was subsequently mixed again 1:1 ratio with the control soil to produce another soil sample. This mixing process was repeated to produce the soils of decreasing concentrations, which were labeled as control, 1C (contamination level 1, collected away from the 5 m site, lowest treated copper concentration level), 2C, 4C, 8C, and 16C (collected from directly under the Grand Hall roof, highest treated copper concentration rate).

The pipes were filled with soil samples 2 cm below pipe tip. The experiment was consisted with 6 treatments with 3 replications. Five earthworms, which were at least eight weeks old with fresh weight between 150–800 mg,

were put into each microcosm. After the earthworms were added, the top and the bottom of the pipes were covered with gauze to facilitate the air flow and to minimize a vaporization. It also protected the microcosm from other pollutants and prevented soil loss and the escape of the earthworms. The experiment was conducted at incubators within the greenhouse of The University of Seoul. The incubators during the cultivation were set as follows; temperature; $20 \pm 2^\circ\text{C}$, relative humidity; about 50% with 16 hours of light period and 8 hours of dark period. The experiment lasted for 12 weeks and no extra feed were given throughout the duration. 18 microcosms were collected at the end of 1, 2, 3, 4, 8 and 12 weeks. Then the earthworms of each microcosm and the soil samples were analyzed for copper concentration with an atomic absorption spectrophotometer (AA-6800, Shimadzu, Japan).

Earthworm growth Prior to the microcosm soil test, earthworms were placed on wet filter paper in petri dishes to clear their gut contents. The petri dishes were sealed to prevent the earthworms from escaping. Earthworms were placed in an incubator at $20 \pm 2^\circ\text{C}$ for 16 hours of light and 8 hours of dark periods. After 24 hours, the earthworms were removed from the petri dishes, put on dry filter paper, and were measured the fresh weight on a balance (Mettler GB204, UK). At the completion of each microcosm soil test, the earthworms were weighed again in the same procedure.

Analysis of the bioaccumulated copper concentration in earthworms After the earthworms' fresh weights were measured at the completion of a microcosm soil test, the earthworms were again put in the petri dish to be oven-dried at 65°C for 24 hours. Afterwards, the dried earthworms were ground using a mortar and pestle. The powder was then digested with concentrated nitric acid and then filtered with filter paper (Whatman No. 42). The filtrate was poured to fill up a 50 mL volumetric flask. The copper concentration of the filtrate was analyzed with an atomic absorption spectrophotometer (AA-6800, Shimadzu, Japan), and then used to calculate the copper content of the earthworms in dry weight basis.

Analysis of the copper concentration in soil by the standard method The soil copper concentration was analyzed using the standard method of the ministry of environment (1996). Ten g of air-dried soil was put into a

250 mL Erlenmyer flask. Fifty mL of 0.1 N HCl solution was added, and the mixture was shaken with a shaking incubator at 30°C and 100 rpm for one hour. The resulting suspension was filtered through a filter paper (Whatman No. 42). The copper concentration of this filtrate was analyzed with an atomic absorption spectrophotometer (AA-6800, Shimadzu, Japan).

Analysis of the total copper concentration in the soil by the EPA method 3051 The EPA Method 3051 (USEPA, 1994) was used for the analysis of the total copper content in soil. 0.5 g of dried soil was placed in a microwavable vessel (XP-1500 plus, CEM corporation, U.S.A) and was added 10 mL of concentrated nitric acid. The vessel was tightly sealed before being digested in a microwave digester (MARS5X, CEM corporation, U.S.A) at 1200 W, 70 psi, and $175 \pm 5^\circ\text{C}$ for approximately 20 minutes. When the digested solution was cooled to room temperature, it was filtered through a filter paper (Whatman No. 41) and the 100 mL of the filtrate were poured to fill up to the 100 mL volumetric flask. Copper concentration rate of the filtrate was analyzed with ICP-OES (Integra XL, GBC Scientific Equipment, Australia)

Results and Discussion

Analysis of the soils used for microcosm soil test

The physico-chemical properties of the soil sample are shown in Table 1. Soils from three different sites were used for the experiment. The copper concentration rate of the soil collected from under the copper plate roof was 312.1 mg kg^{-1} , while those of soil from the site 5 m away from the roof and the control site were 25.4 mg kg^{-1} and 3.4 mg kg^{-1} , respectively.

Growth rate of the earthworm The growth rate of earthworms was evaluated by measuring their fresh weight at the completion of the experiment in order to determine the effects of the soil copper content and their growth rate. The equation for evaluating the growth rate was as follows:

$$\text{Growth rate} = \frac{\text{fresh weight of earthworm after the test}}{\text{fresh weight of earthworm before the test}}$$

The experiment showed that the growth rate of earthworms decreased as the exposure time and copper concentration in soil increased (Fig. 1). However, for the control the growth rate increased temporarily at 4 weeks and then decreased afterwards. The earthworms in the control grew initially with its optimal conditions. Then as the organic matter in the soil was being depleted with no addition of feed, their growth began to decrease.

Analysis of the accumulated copper concentration in earthworm The effects of the copper concentration in soil and the exposure time on the changes of accumulated copper in earthworms were shown in Fig. 2. It indicated that accumulated copper concentration in earthworms changed depending on the copper content in soil and the duration of experiment.

During the cultivation of earthworm before the experiment began, their accumulated copper concentration was 21.94 mg kg^{-1} . One week after the initiation of the experiment, that of the earthworms in the control increased to 31.92 mg kg^{-1} , whereas its concentration increased to 46.82 mg kg^{-1} , 52.11 mg kg^{-1} , 61.31 mg kg^{-1} after 4, 8, and 12 weeks, respectively.

Especially, the accumulated copper concentration in the control increased rapidly after week 3. As shown in Fig. 1 which analyzed the earthworms' growth rate, the growth rate also increased rapidly at weeks 3 and 4. Their

Table 1. The physico-chemical properties of soils used for microcosm soil tests.

Site	pH (1:5)	EC (1:5)	O.M	Av. P ₂ O ₅	CEC	Exch. cation			Heavy metal				Soil texture
						Ca	Mg	K	Cd	Cu	Zn	Pb	
		dS m ⁻¹	g kg ⁻¹	mg kg ⁻¹	----- cmolc kg ⁻¹ -----			----- mg kg ⁻¹ -----					
1 [†]	8.03	0.06	1.8	26.8	9.7	9.61	0.55	1.14	0.22	312.1	14.59	19.6	SL [†]
2 [†]	8.06	0.07	2.0	33.4	9.6	12.66	0.68	1.32	0.24	25.4	13.80	10.60	SL
3 [†]	5.94	0.03	0.8	20.9	8.4	4.18	0.84	0.95	0.22	3.4	3.20	6.38	SL

[†] SL : Sandy Loam

[†] Site 1: site near the copper contamination source,

[†] Site 2: site 5 meters away from the copper contamination source,

[†] Site 3: non-contaminated site

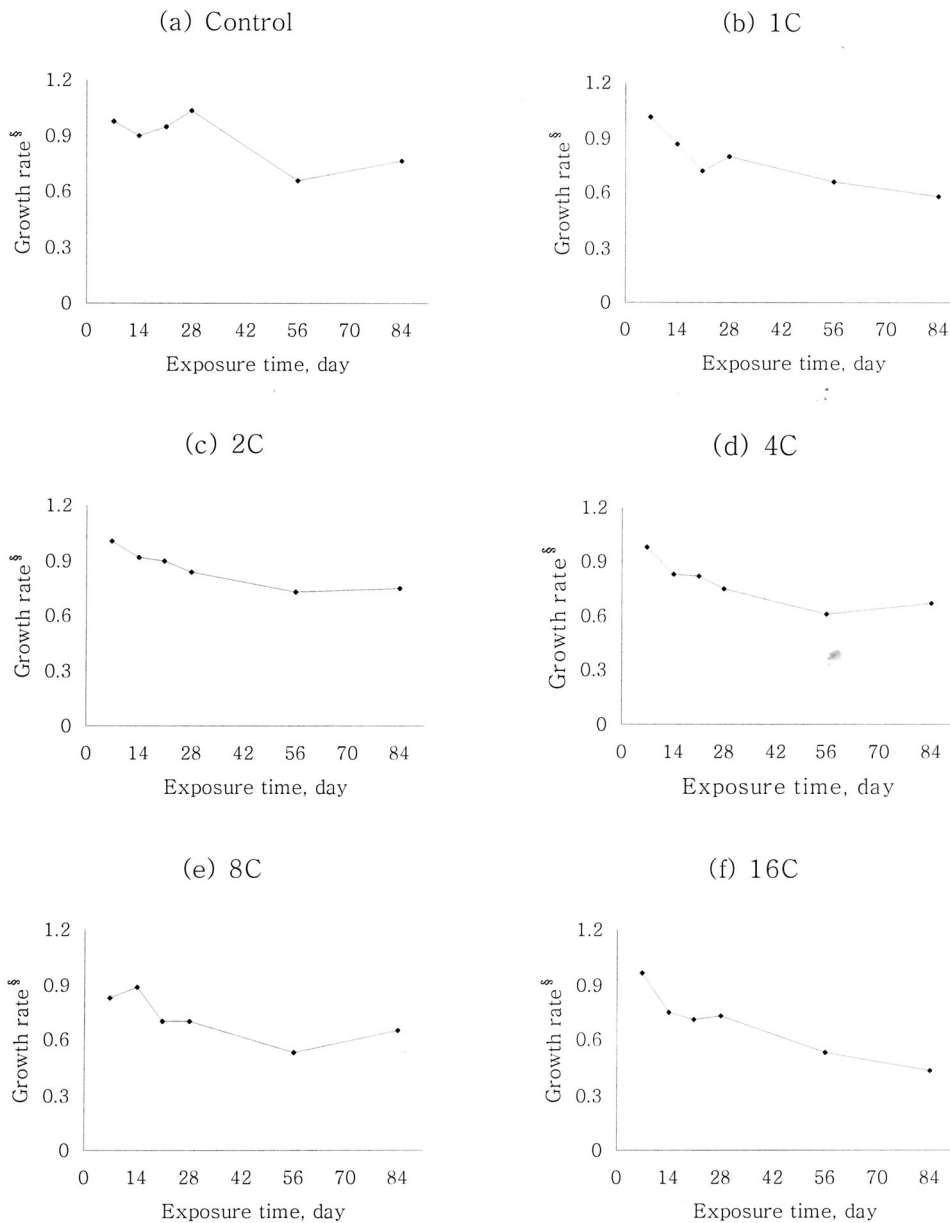


Fig. 1. Changes of growth rate of earthworm over time in soils treated with different Cu level for the control and treatments of 1C (contamination level 1, lowest treated copper concentration level), 2C (1C*2), 4C (1C*4), 8C (1C*8) and 16C (1C*16). Growth rate[§] = fresh weight of earthworm after the test/ fresh weight of earthworm before the test.

rapid growth during a relatively short period might be due to the optimal environment for the growth in the control with comparatively absent effects of copper.

In the rest of the treatments, the accumulated copper concentration in earthworms showed similar tendency to the copper concentration rate of microcosms. This was more evident after the third week into the experiment, especially for 16C with the highest copper treatment level. The accumulated copper in earthworms was similar to that of 8C during week 1 and 2, but it rapidly increased from week 3 onwards resulting in 91.93 mg kg^{-1} at week 8 and $157.42 \text{ mg kg}^{-1}$ by week 12.

From weeks 1 to 3, the order of the rate of accumulated copper concentration in earthworms were as follows, which is identical to the order of levels of copper in the 6 treatments:

$$16C > 8C > 4C > 2C > 1C > \text{Control}$$

Treatment 1C showed the accumulated concentration increasing until week 3 to 40.68 mg kg^{-1} and then decreasing to 36.4 mg kg^{-1} at week 4 and to 35.76 mg kg^{-1} at week 8. It increased to 64.47 mg kg^{-1} by week 12. The rate of increase in treatments 2C, 4C and 8C also declined from week 3 or 4 with the concentration stopping to

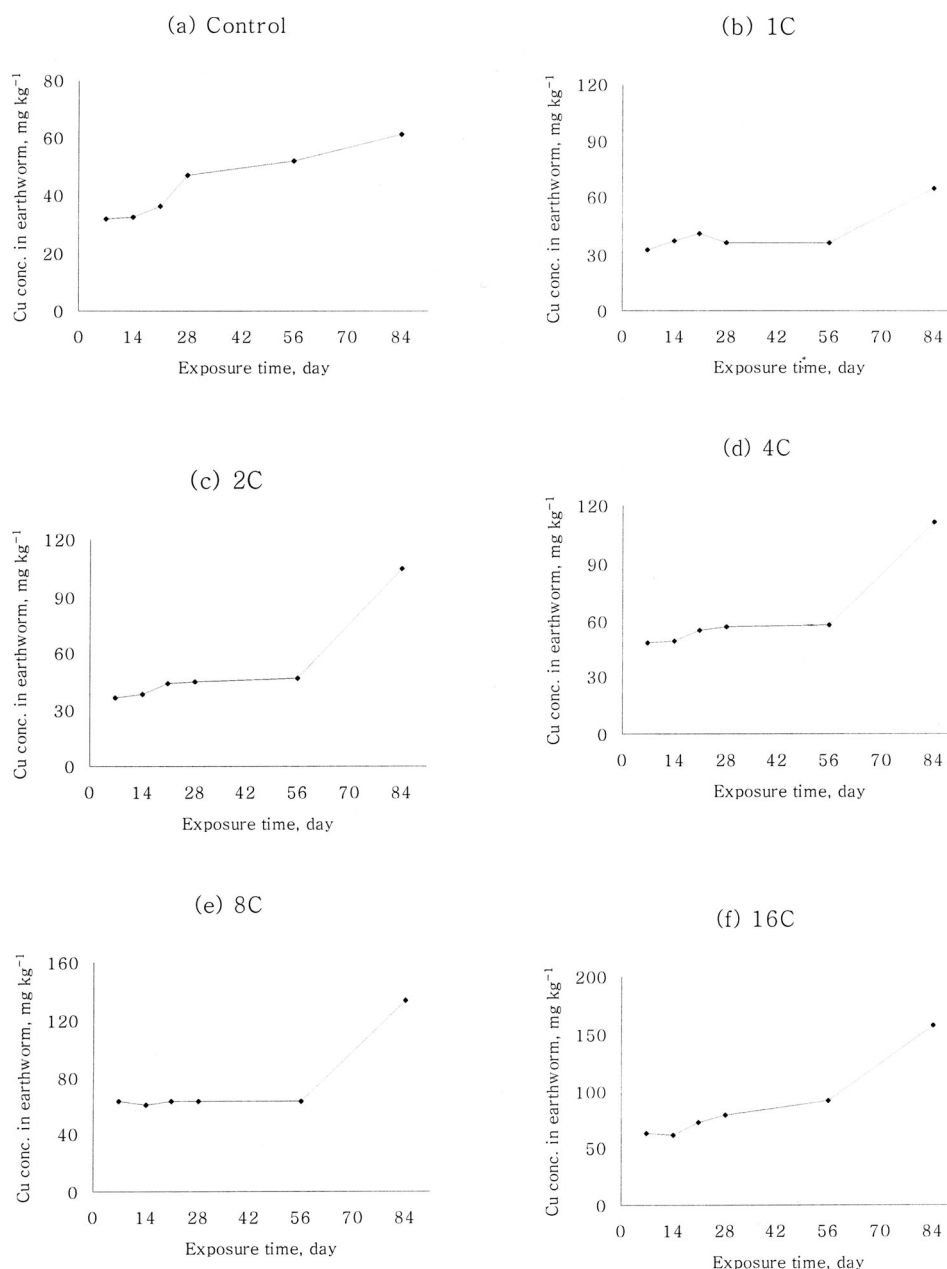


Fig. 2. Accumulation of Cu in earthworms in soils for the control and different treatments (See Fig. 1).

increase at a certain level and stabilizing. However, in both the control and all of the treatments, the accumulated copper concentration rate increased rapidly by week 12.

Among various heavy metals, copper has high affinity to organic matters in soil. This property resulted in the rate decline of copper accumulation in earthworms through their ingesting and digesting it from the limited amount of soil organic matter. As their exposure to copper-contaminated soil increased over time however, the accumulation increased again by week 12.

In treatment 16C, the accumulated copper content increased steadily from the beginning. This seems to

show that the accumulation of copper is dependent on its concentration level in soil. In addition, as shown in the significant decline of the growth rate in 16C, the accumulation of copper in earthworms is thought to have increased due to the reduction of their fresh weight.

Copper is one of the essential elements for earthworms. It is absorbed into their bodies to reach a certain level of concentration, and its excess is discharged to maintain their homeostasis. The homeostatic mechanism of earthworms are thought to maintain the copper concentration within them at a certain level without having it increase rapidly from the presence of excess copper content of soil (Neuhauser, 1995).

Analysis of copper in the soil by the standard method Copper contents of soil used in the microcosm soil test, which were extracted with 0.1 N HCl according to the standard method of the ministry of environment are shown in Fig. 3. Copper concentration rate in the beginning for the treatments of control, 1C, 2C, 4C, 8C, and 16C were 3.4 mg kg⁻¹, 25.4 mg kg⁻¹, 48.8 mg kg⁻¹, 103.8 mg kg⁻¹, 199.6 mg kg⁻¹ and 312.1 mg kg⁻¹, respectively. As the experiment progressed, the concentration rates decreased with the results of 2.9 mg kg⁻¹, 19.3 mg kg⁻¹, 41.3 mg kg⁻¹, 75.3 mg kg⁻¹, 123.8 mg kg⁻¹ and 255.9 mg kg⁻¹, respectively by week 4.

By week 12, the copper concentration rates in soil were

2.9 mg kg⁻¹, 16.29 mg kg⁻¹, 38.5 mg kg⁻¹, 71.1 mg kg⁻¹, 113.6 mg kg⁻¹, and 238.2 mg kg⁻¹, respectively. This shows the reduction of soil copper content from week 1 to week 8, in comparison to the start of the experiment, and the decrease of or similar level of copper content at week 12, in comparison to week 8.

Earthworms feed on the organic matters in soil. Copper in soil is fed together with these matters, which once ingested, are dissolved with digestive fluids, and then absorbed. The affinity of copper for organic matter in soils thought to have resulted in the copper content reduction in the microcosm soils.

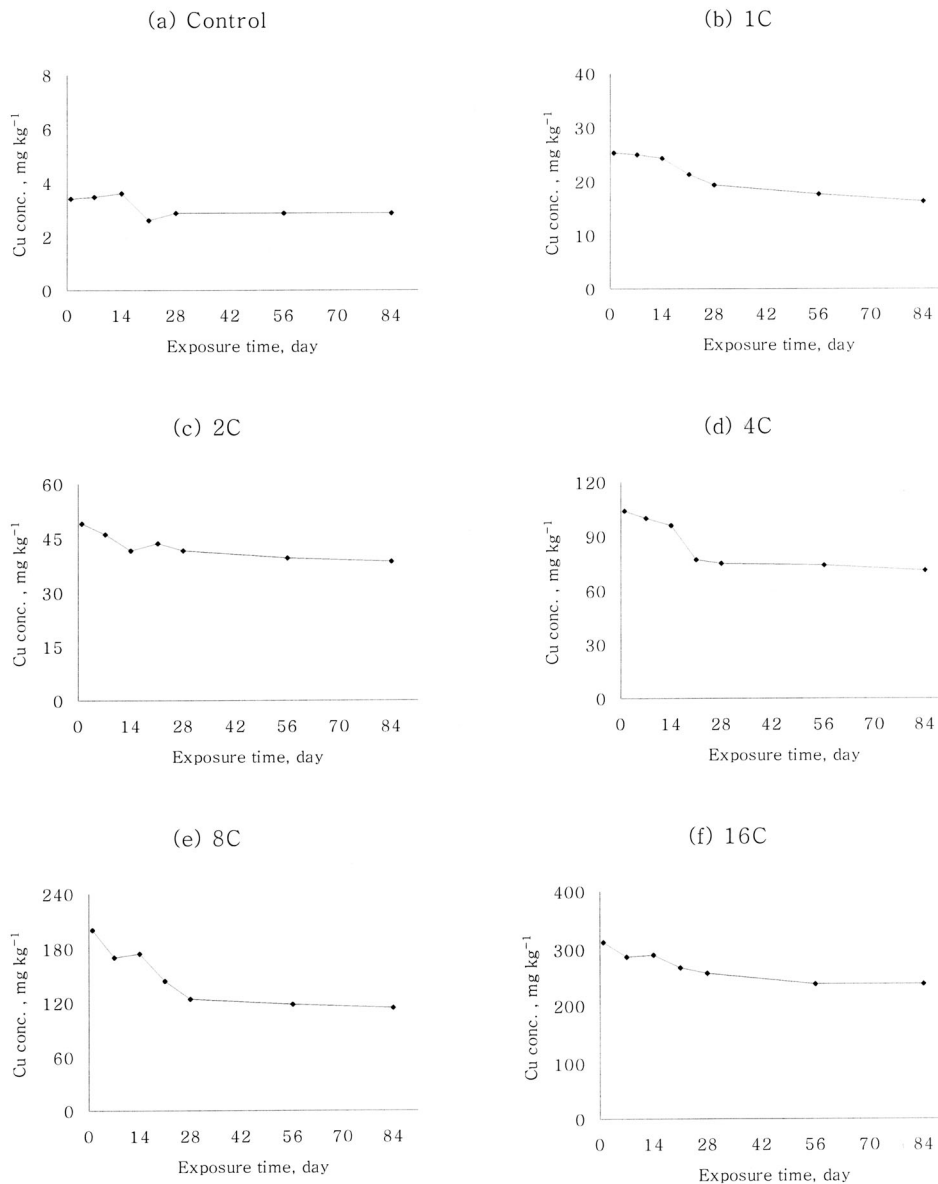


Fig. 3. Effect of exposure time on Cu concentration in soils extracted with 0.1 N HCl for the control and different treatments (See Fig. 1).

Analysis of the total copper content in the soil by EPA method 3051 Total copper content in the soils used for microcosm study using concentrated nitric acid and microwave digestion according to the EPA method 3051 is shown in Fig. 4. The initial copper content of the control, 1C, 2C, 4C, 8C, and 16C were 5.9 mg kg^{-1} , 34.0 mg kg^{-1} , 87.6 mg kg^{-1} , 148.3 mg kg^{-1} , 233.8 mg kg^{-1} , and 445.6 mg kg^{-1} , respectively. All values from the start of the experiment to week 12 obtained using nitric acid and

microwave showed higher values of copper content than those obtained using 0.1 N HCl .

At week 4, total copper content in soil for control, treatments 1C, 2C, 4C, 8C, 16C were 5.82 mg kg^{-1} , 39.2 mg kg^{-1} , 83.7 mg kg^{-1} , 135.1 mg kg^{-1} , 265.2 mg kg^{-1} , and 438.0 mg kg^{-1} , respectively, showing slight total copper content decrease in control, 2C, 4C, and 16C. By week 8, total copper contents were 6.6 mg kg^{-1} , 36.3 mg kg^{-1} , 71.3 mg kg^{-1} , 126.4 mg kg^{-1} , 245.2 mg kg^{-1} , and 417.0 mg kg^{-1} ,

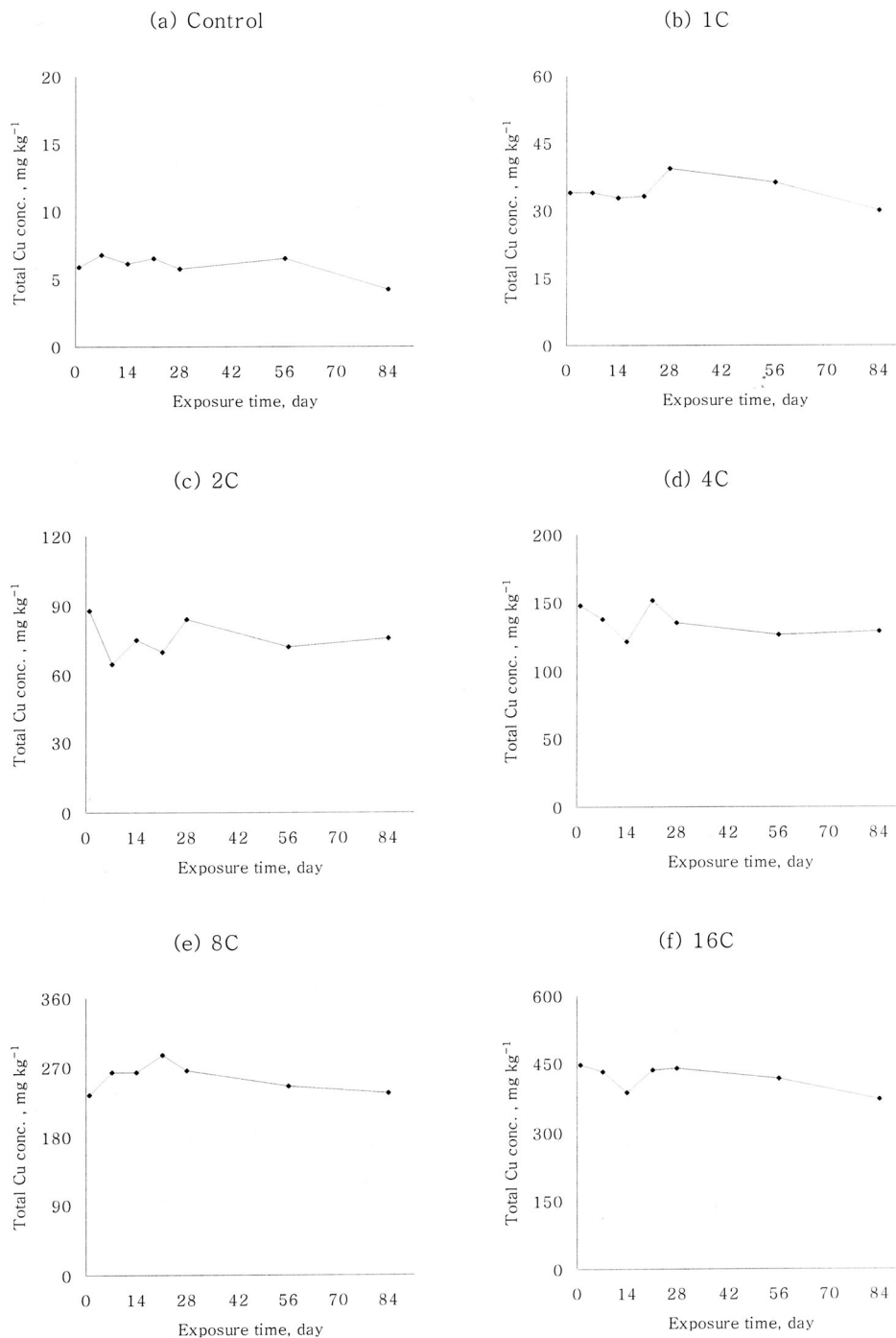


Fig. 4. Effect of exposure time on total Cu concentration in soils for the control and different treatments (See Fig. 1).

respectively with the content slightly higher in control and the rest of the treatments showing small decrease. At week 12, the control, treatments 1C, 2C, 4C, 8C, and 16C showed concentrations of 4.3 mg kg^{-1} , 30.2 mg kg^{-1} , 75.4 mg kg^{-1} , 128.2 mg kg^{-1} , 234.5 mg kg^{-1} , and 370.0 mg kg^{-1} respectively, where both the control and all of the treatments resulted in the decrease of total copper content compared to week 8.

The change of total copper content between week 2 and 4 was somewhat irregular. However, the results obtained through this method showed that the total copper content

in soil generally decreased slightly with time although not as rapidly as from the results obtained from copper content extracted using standard method.

Copper which is ingested by earthworms, and then dissolved by digestive fluids and absorbed, has high affinity for organic matters. Due to this affinity, the concentration rate changes in soil are detected more precisely when copper content extracted with 0.1 N HCl is used than with this method. This resulted in smaller total content change using this method compared to the change of copper concentration using 0.1 N HCl.

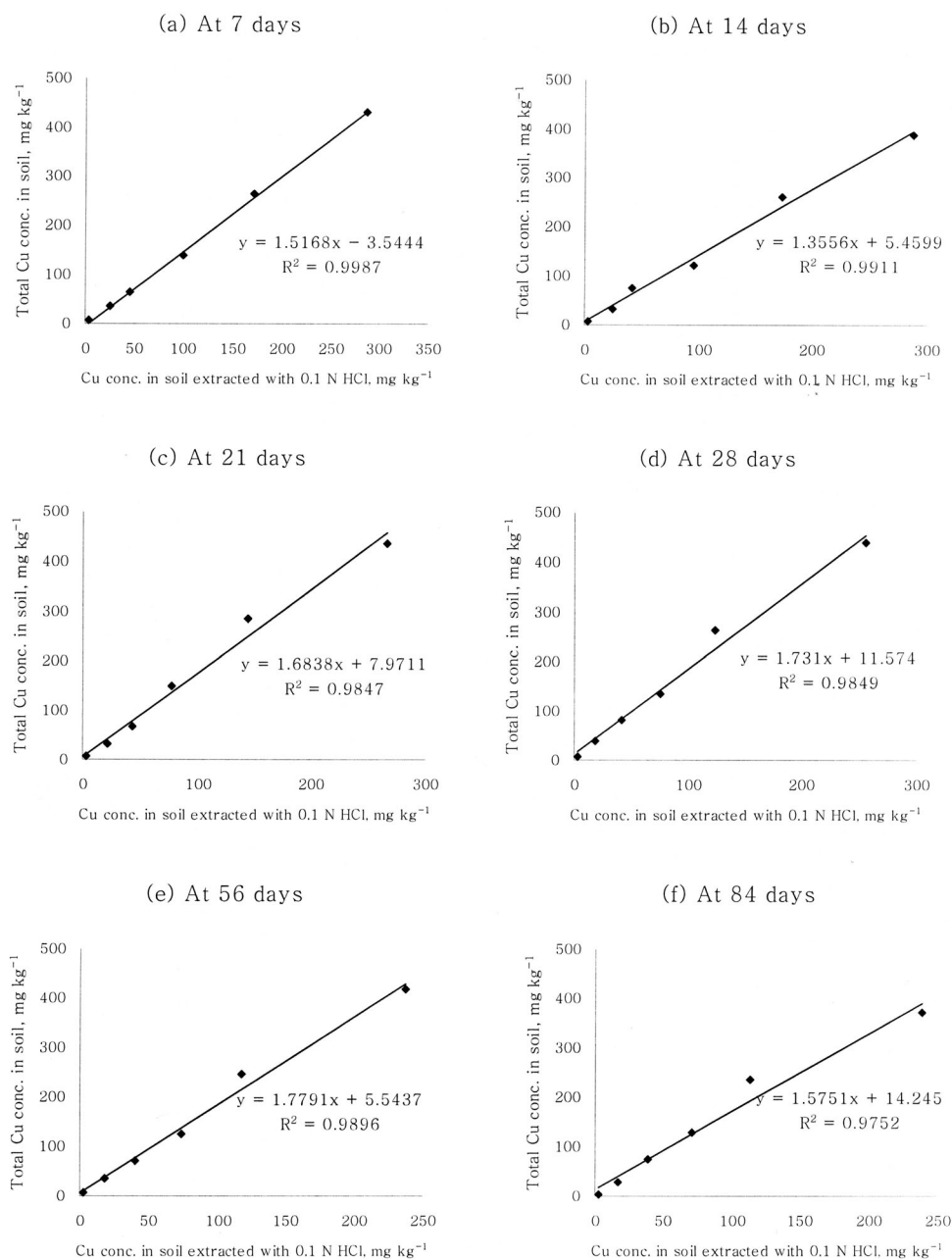


Fig. 5. Relationship between 0.1 N HCl extractable and total Cu concentration in soils sampled at 7, 14, 21, 28, 56 and 84 days from initiation of microcosm study.

As the content of organic matter in the soil used for the experiment was very low, the changes in the total copper content of the soil were smaller than the copper concentration rate of soil extracted with 0.1 N HCl.

Relations between copper contents in the soil by standard method and EPA method 3051 The relations between copper concentrations in soil extracted with 0.1 N HCl according to the standard method and the total copper content in soil obtained with method 3051 are shown in Fig. 5. The R^2 of the copper content of soils extracted with the two methods at the start of the

experiment, week 1 and week 2 were 0.9823, 0.9987 and 0.9911, respectively. The R^2 of the two methods at weeks 4, 8, and 12 were 0.9847, 0.9849, 0.9896 and 0.9752, respectively.

The analysis showed that during all treatment periods, the copper concentration rate from using standard method (Ministry of environment, 1996) and the total copper content in soil using EPA method 3051 have significant correlation. According to Jung *et al.* (2000), the relations of positive correlation of copper shows the correlation coefficient of 0.94, indicating high positive correlation with all extracting solutions. Jung *et al.* (2000) showed

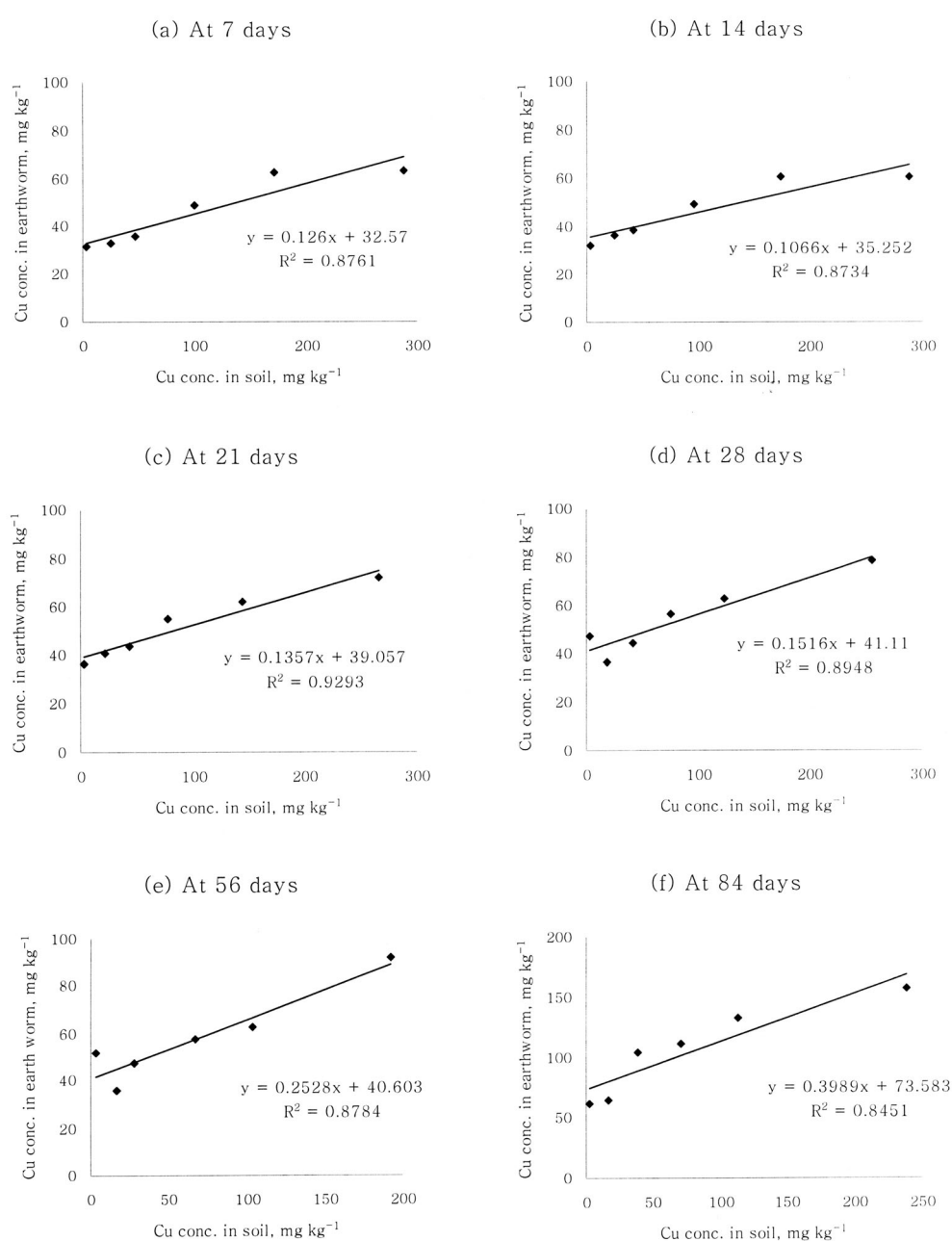


Fig. 6. Relationship between 0.1 N HCl extractable Cu in soil and Cu concentration in earthworm sampled at 7, 14, 21, 28, 56 and 84 days from initiation of microcosm study.

that the correlation coefficient of 0.971 between copper content in soil obtained using aqua regia and copper concentration from soil extracted with 0.1 N HCl. Comparing this r of 0.971 to the current analysis indicates that a high correlation exists between the two copper content analysis methods.

Relations between the copper content in the soil by the standard method and the accumulated copper contents in earthworm Relations between the analysis results of the copper content extracted with 0.1 N HCl using the standard method of the ministry of environment

and the accumulated copper content in earthworms is shown in Fig. 6. The determination coefficients (R^2) of the two results at 1, 2, 3, 4, and 8 weeks after the start of the microcosm soil test were 0.8761, 0.8734, 0.9293, 0.8948, 0.8784, and 0.8451, respectively.

There was a significant correlation between copper concentration extracted with 0.1 N HCl using the standard method of the ministry of environment and the accumulated copper content in earthworms throughout the experiment duration. This showed a much higher correlation coefficient than the $r=0.4459$ obtained at Taegu city by Lee *et al.* (1993). In their research, Lee *et*

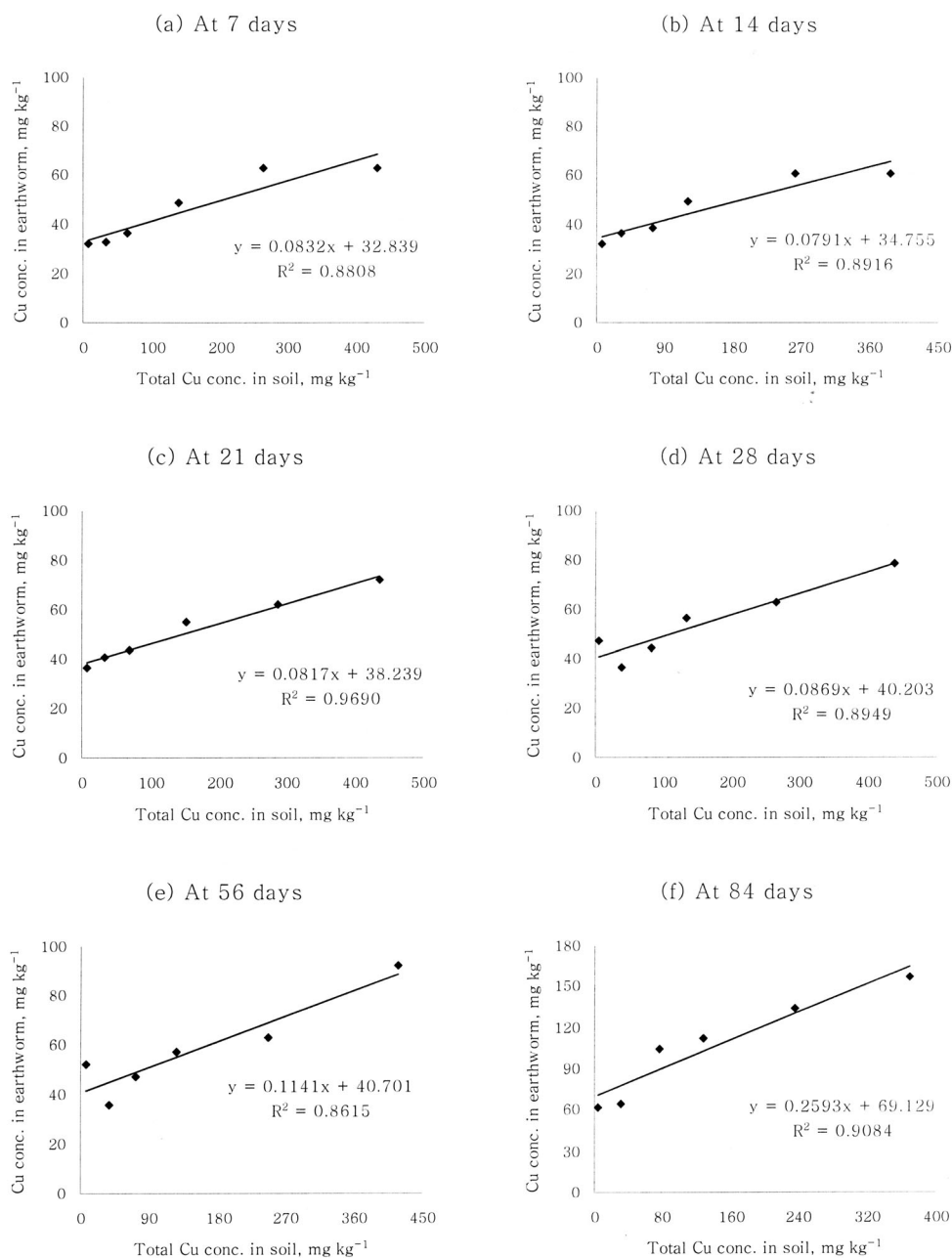


Fig. 7. Relationship between total Cu concentration in soils and Cu concentration in earthworm sampled at 7, 14, 21, 28, 54 and 84 days from initiation of microcosm study.

al. (1993) did not classify earthworm into their own species, they just evaluated copper contents in earthworm as a whole. That is why correlation coefficient they obtained, $r=0.4459$, is much lower than that obtained in our research. In addition, it was similar or slightly lower than the $R^2=0.92$ of Bogomolov *et al.* (1996) from the analysis between the copper concentration of soil extracted with 0.05 M CaCl_2 and accumulated copper content in earthworms.

Relations between total copper content in the soil by EPA method 3051 and the accumulated copper content in earthworm Relations between the analysis results of the total copper content by EPA method 3051 and the accumulated copper content in earthworms is shown in Fig. 7. The determination coefficient (R^2) of 1, 2, 3, 4, 8 and 12 weeks after the initiation of the experiment were 0.8808, 0.8916, 0.9690, 0.8949, 0.8615 and 0.9084, respectively. This R^2 is similar to the R^2 between copper concentration of soil using the standard method and the accumulated content in earthworms throughout the duration of the experiment, which were also highly significant.

Throughout the treatment period, the relation between total copper content of soil obtained using EPA method 3051 and the accumulated copper content in earthworms were highly significant. The copper concentration in soil, which was extracted with 0.1 N HCl using the standard method of the ministry of environment (1996) and the accumulated copper content in earthworms were highly significant as well. According to van Hook (1974), earthworms can be a useful biological indicator due to the consistent relations between the concentration of various pollutants and the fatality rate of earthworms. Na (2004) considered earthworms, including *E. fetida*, to be suitable as a biological indicator species or as species for biomonitoring.

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Microcosm soil test를 이용한 지렁이 체내 축적 구리 농도와 구리 침출법 간의 상관관계 비교

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본 연구는 동판 건물 주변의 구리 오염 토양과 지렁이를 이용해 microcosm soil test를 실시함으로써 지렁이 체내에 농축된 구리 농도와 몇 가지의 토양 내 중금속 침출 방법으로 측정된 토양 내 구리 농도간의 상관관계를 비교하기 위하여 수행하였다. 조사 지역의 토양 시료를 이용해 control, 1C(Contamination level, 최저 처리 농도), 2C, 4C, 8C, 16C(최고 처리 농도)의 여섯 가지 처리구를 통해 microcosm soil test를 실시한 결과, 토양 내 구리의 농도와 실험 기간이 경과함에 따라 지렁이의 성장량과 지렁이 체내 농축 구리 농도가 함께 증가하는 것으로 조사되었으며, 그 정도는 microcosm 내 토양의 구리 처리 농도와 같은 순서였다. 토양 내 구리 농도를 조사하기 위해 공정시험법을 사용하였고 토양 내 구리 총합량을 조사하기 위해 EPA 3051 방법을 사용하였다. 공정시험법으로 침출한 토양 내 구리 농도와 토양 내 구리 총합량간의 상관관계수(r)는 0.9875~0.9993로 고도의 정의 상관관계가 존재하였다. 위의 두 결과와 지렁이 체내에 농축된 구리 농도와의 상관관계를 비교해 본 결과, 공정시험법으로 침출한 토양 내 구리 농도와 지렁이 체내 농축 구리 농도간의 상관관계수와 토양 내 구리 총합량과 지렁이 체내 농축 구리 농도간의 상관관계수는 각각 0.9193~0.9728과 0.9282~0.9844로 고도의 정의 상관관계를 나타내었다. 토양 내 구리 농도와 지렁이 체내 농축 구리 농도 간에 상당히 높은 수준의 상관관계를 보임에 따라 지렁이는 토양 내 구리 오염에 대한 유용한 생물지표종(biological indicator species) 또는 생물모니터링(biomonitoring)에 적합한 종의 역할을 할 수 있을 것이라 판단하였다.