Ecological Risk Assessment of Soil Pollution Using Soil Animals.

Development of the toxicity scaling method for evaluation of specific risk in mixed contaminated soils.

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Abstract

Soils has been polluted by mixture of various artificial chemical compounds even in distant area from industrial activity. Ecological risk of soil pollution to soil nematode and collembola have been estimated by the toxicity scaling method. Maturity Index of nematode reflected artificial Cu contamination in a short period (2 weeks). Dose-response curve obtained from acidified (contaminated) and non-acidified (low contaminated) soils were analyzed. These two soils were originated from same soil and there was no difference in *Folosomia candida* Willem. (Collembola) growth at field condition, whereas EC_{50} to Cu contamination was 700 and 235 mg kg^{-1} in dry soil at acidified and non-acidified soil, respectively. Reduction in EC_{50} in the acidified soil seemed to be caused by mixed toxicity effects of metals and probably unknown low-level anthropogenic contaminants. Detailed analysis of composing metals and chemicals will not explain the observed toxicity to animals before the interaction of toxicity will be revealed in every combination of substances. The difference in EC_{50} corresponded to total toxic effect of every toxicant if there were no interaction in toxicity (e.g. additive toxicity) between the scaling substance (Cu) and the substances in target soil. It is concluded that the toxicant-scaling method for soil ecological risk assessment may be useful for estimation of low-level and mixed substance pollution.

1. Introduction

Soil is fundamental to all terrestrial life. However, soils in industrial area seem to be polluted by various toxic compounds, such as heavy metals (Cd, Cu, Zn etc.), pesticides, herbicides, dioxins and polycyclic aromatic compounds (PAHs) from diverse sources (Weltje

1998). It may be impossible to find any soils that contain completely no artificial chemical compounds. Soils are not polluted by single toxicant, rather pollution by mixed substances will be common in modern times. The question "are there any soils that show some symptom of degradation by mixed artificial toxicants?" will be hardly answered, because the concentrations of each toxicant are mostly future below NOECs except in heavily contaminated sites, for example near point source. Then, do these low levels but mixed pollution of soils have no potential ecological risk? Diverse microorganisms and animals are found in natural soils and there are rather many studies on ecotoxicology of single toxicant (Lokke and van Gestel, 1998). However, there is no study on evaluation of ecological risk of low level but mixed toxicant soil pollution. Ecological risk management depends on accounting both adverse effects of pollution and cost of restoration. Single toxicant test is basic approach to estimate ecological risks, however if there are interactions among composing toxic substances, total risk of polluted soils could not be estimated by adding specific risks of each substance.

Soil animals have important controlling role on soil biological process, such as organic matter decomposition and maintenance of soil physical structure via interactions with soil microorganisms (Lavelle *et al.*, 1998). Nematodes are plant and insect parasites as well as free living in soil, aquatic and marine sediments. Free living nematodes are bacterivores, fungivores, predators and detritus feeders, and feeding habits and ecological characteristics are common among taxonomic family members (Bongers and Bongers, 1999). More than 60 species of free living nematodes were found in temperate forest soils (Boag and Yeates 1998). Nematode feeding habits are well reflected in their mouth shape, therefore feeding guild analysis of nematode community is rather easy (Bongers, 1990). Because of their short generation time and rapid dispersal to new habitat by insect phoresy, nematode communities clearly reflect environmental condition of soils (Bongers, 1990; Bongers and Bongers, 1998). Collembola is a wingless insect inhabiting soils and often a numerically dominant arthropod in natural soils (Petersen Luxton 1982). Foods of Collembola are soil microorganisms, higher plants, algae, detritus and mineral soil particles. Both nematodes and Collembola have significant effects on soil microbial community by their feeding activity (Ingham *et al.*, 1985; Anderson 1988).

In this study, I developed a new method to estimate specific ecological risk by getting dose-effect curve with copper addition to test soils. This method、a toxicant scaling method, will describe an actual but hidden ecological risk of soil pollution irrespective of composition and concentration of mixed toxicants.

2. Study sites and methods

2-1. Nematode community response to Cu pollution

Bongers (1990) developed the Maturity Index of nematodes. He classified soil nematode families into five classes according to c-p index; 1 for colonizers (MI1) and 5 for persisters (MI5). The Maturity Index is calculated by weighted mean of c-p value of each community. A studied soil was collected from a larch stand at Yatsugatake Experimental Forest of Tsukuba University. Soil type is Wet Kuroboku soils (Gleyic Andosols (FAO/UNESCO)). A surface to 5 cm in depth was collected on 9 October 1999. Sample soil was sieved through 2mm-mesh sieve and 22.1 g each (10 g dry, water content = 54.8%) was put into 50ml polystyrene cup with airtight cover. Soil samples were pre-incubated at 22.5 °C in incubator in dark condition for 7 days. Soils were treated with solutions of copper chloride ($CuCl_2(2H_2O)$, Wako, purity 99.5%) in deionized water to obtain nominal copper concentrations of 0, 100, 1000 and 10000 mg kg^{-1} dry soil. Treatments were replicated five times and incubated for 2 weeks in 22.5 °C in incubator in dark condition.

Nematode in the sample soils were extracted by modified Cobb's sieving method (Kammenga *et al.*, 1996). Extracted nematodes were identified into family according to Shishida (1999). Maturity Index was calculated by the method of Bongers (1990).

2-2. Collembola toxicity test

Surface soils (0-5 cm) were collected at a Japanese Cedar (*Cryptomeria japonica*) plantation forest in Sanbe Experimental Forest of Shimane University. Soil type is Typical Kuroboku (Typic Andosols). On 31 October 1999, two contrasting soils were collected; the stemflow soil from near around a tree trunk (within 20 cm from the trunk) and the throughfall soil from distant at least 60cm to tree trunks. The stemflow soil has been acidified by acidic stemflow of *C.japonica* trees, whereas the throughfall soil has relatively not influenced by the stemflow. There were clear difference in soil acidity, soil microbial biomass, exchangeable Ca and Mg, and soil microarthropod abundance (Table 1.).

Table 1. Characteristics of studied soils (0-5cm) at Sanbe Experimental Forest of Shimane University, Shimane, Japan (after Kaneko & Kofuji (in prep.), and Kofuji (1997)).

	Stemflow soil	Throughfall soil
Soil $pH(H_2O)^a$	4.22 ~ 4.85	5.25 ~ 5.28
Microbial biomass carbon (mg C g-1 dry soil)[a]	4.7	7.6 ~ 11.2
Total carbon (%)[b]	19.4 ~ 21.2	17.1 ~ 17.3
Total nitrogen (%)[b]	1.27 ~ 1.30	1.03 ~ 1.06
Exchangeable calcium (meq $100g^{-1}$)[b]	7.42 ~ 8.66	10.86 ~ 10.90
Exchangeable magnesium (meq $100g^{-1}$)[b]	0.10 ~ 0.16	0.51 ~ 0.54
Density of soil microarthropods (m^{-2})[a]	102775	49550

a: Kaneko & Kofuji (in prep.), and b: Kofuji (1997).

Also the community structure of oribatid mites reflected soil acidification (Kaneko & Kofuji, submitted). Acidification of soils lead changes in cation composition; reduction of essential ions to plants (Ca, Mg) and increase in toxic ions (*Al*-ions and Mn^{2+}) (Ulrich 1989). Toxicity of Al-ions and Mn^{2+} to soil animals is not known.

Soils were sieved with 2 mm-mesh sieve and freezed-sowed for twice to eliminate soil microarthropods. Soils were air-dried and treated with solutions of copper chloride ($CuCl_2(2H_2O)$, Wako, purity 99.5%) in deionized water to obtain nominal copper concentrations of 0, 31.6, 100, and 316.2 mg kg^{-1} dry soil. Water content of each soil was adjusted to 60%. One day after Cu addition, each 5g (dry) soils was separated into 50 ml glass tube and five collembola (*Folsomia candida Willem.*) were introduced. Three replications were prepared. Toxicity test protocol has been done according to Wiles & Klogh (1998). *F.candida* were collected at the University Nursery of Shimane University in 1992, and then the culture have been maintained in incubator with dry yeast (Nisshin, Japan) as a food. Synchronized population was used in this experiment. Individuals at 19 days from egg were collected and the body length of each individuals was measured by video microscope (VH-6300, KEYENCE, Japan). Five collembolans were introduced to each tube, and the tubes were covered by airtight polyethylene caps. The tubes were incubated for two weeks under dark condition at 22.5 °C. Three granules of dry yeast were put on soil surface of each tube at the begging and 1-week after the start of the experiment. Body length and mortality of collembola were recorded at the end of the incubation.

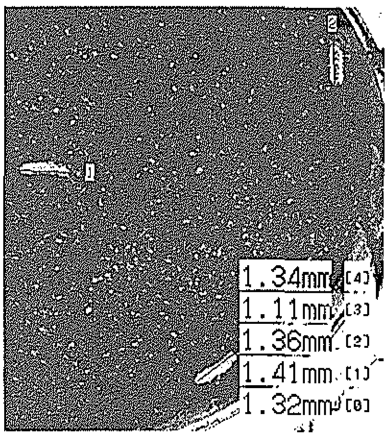

Fig. 1. Body length measurement of *Folosomia candida* by video-microscope (VH-6300, KEYENCE, Japan).

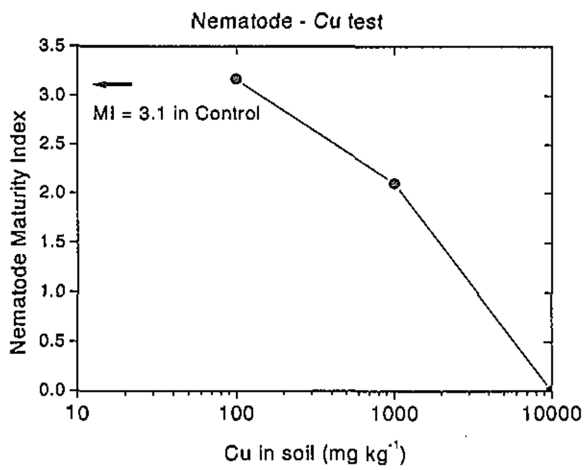

Fig.2. Maturity Index of artificial Cu contaminated soil in a larch plantation at Yatsugatake Exp. Forest, Tsukuba University.

2. Results

2-1. Nematode community response to Cu pollution

A change in Maturity Index with increase dose of Cu is shown in Figure 2. MI at the control was 3.1, therefore there was no effect in nematode community until 100mg kg^{-1} and in 1000 and 10000 mg kg^{-1}, clear responses were observed.

2-2. Collembola toxicity test

Cu toxicity effect on collembola growth is shown in Figure 3. Growth at control was 0.45 mm and 0.41 mm in the stemflow soil and in the throughfall soil, respectively. In the stemflow soil, growth was reduced in the 100 mg kg^{-1} contamination. In the 316.2 mg kg-1 contamination, reduction in growth was observed in the both treatments.

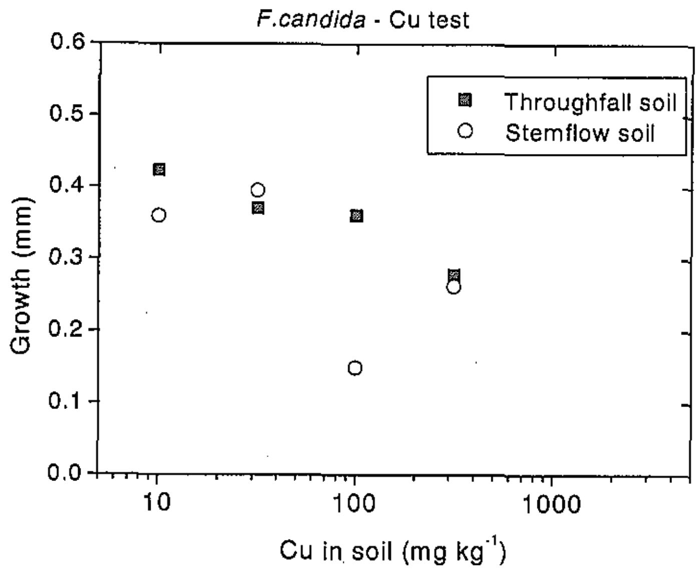

Fig.3 Effect of Cu contamination on the two-weeks growth of *Folsomina candida* in the stemflow and the throughfall soil of a Japanese Cedar plantation in Sanbe Exp. Forest of Shimane Univ.

A power function was fitted to the observed response,

$$Growth(x)=\gamma_{max}(1-(x/\alpha)^{\beta})$$

where x is an exposure concentration, γ_{max} is maximum growth rate without exposure (control) and α and β are parameters to be estimated (Tanaka & Nakanishi, 1998). Parameters were estimated by maximum likelihood method and the results are shown in Table 2.

Table 2. Parameters for dose-response curve of Copper-*Folomia candida* test.

parameters	Stemflow soil	Throughfall soil
α	4.426	3.489
β	1.112	3.395
γ_{max}	0.453	0.413
EC_{50} (Cu mg kg^{-1})	235	700

EC_{50} was 235 mg kg^{-1} and 700 mg kg^{-1} in the stemflow and the throughfall soil, respectively.

3. Discussion

Community structure of free living soil nematode has been successfully used as a bioindicator of soil pollutions (Bongers, 1990: Bongers and Bongers, 1998). This method has an advantage as follows; 1) rapid response to a treatment, 2) applicable to rice paddy soils. Community level assessment of soil pollution has a further advantage because it will reflect the actual sensitivity distribution of organisms, however theoretical background of quantitative evaluation of the community is still unclear (Bongers and Bongers, 1998).

In mixed contaminated soils, the dose-effect test for each organisms with each contaminants is impossible and also the interaction of substances may influence the shape of dose-response curve. Wiltje (1998) reviewed the heavy metal toxicity on earthworms, and showed that toxicity unit among Cd, Cu and Zn was almost unity. In this case, one can measure each heavy metal concentration and estimate total toxicity by adding each toxic unit. Soils are not only polluted by heavy metals but pesticides, herbicides and PAHs. In the stemflow soil around a Cedar trunk has been acidified by acidic stemflow water, and soil chemical properties have been changed. Retardation of biological activity also has been observed in the stemflow soil; reduction in soil microbial biomass and litter decomposition rate and increase in soil microarthropod density (Kaneko and Kofuji, *submitted*). Decrease in soil pH leads the following change; exchangeable Ca, Mg and K are subject to leaching because of ion exchange with proton, and with the further acidification Al-ions and other heavy metal ions will be increased in soil solution. This process explains forest degradation by acid rain (Ulrich 1989), because Al-ions and Mn^{2+} have plant root toxicity. In this study, these chemical changes in soil was not fully analyzed, however reduction in exchangeable Ca and Ma together with pH reduction in the stemflow soil than in the throughfall soil suggests that increase in heavy metal toxicity in the stemflow soil. The stemflow soil also receives dry deposition which once adsorbed by tree leaves and twigs, therefore contamination by air pollutant in the stemflow soil seems to be more intense than in the throughfall soil.

The growth of *F.candida* was similar when no copper has been added. Addition of copper to the two soils revealed that EC_{50} was observed in the lower concentration of Cu in the acidified soil compared to the less acidified soil. Soil acidification (c.a. 1 unit in pH) corresponded to 465 mg kg^{-1} reduction of EC_{50} for *F.candida*. Acidity itself may not be responsible to collembola growth, since Sandifer and Hopkin (1996) could not show pH effects on the reproduction of *F.candida* using artificial soil system having no other contaminants. Factors affected on the collembolan growth in this study is difficult to analyze, since pH may be responsible to ion composition in the test soils. The test soils are safe for *F.candida* in the present condition, however EC_{50} was smaller in the acidified soil. Therefore, tolerance to copper contamination has been already reduced in the acidified soil. This index by copper addition; the toxicity-scaling is shown in Figure 4.

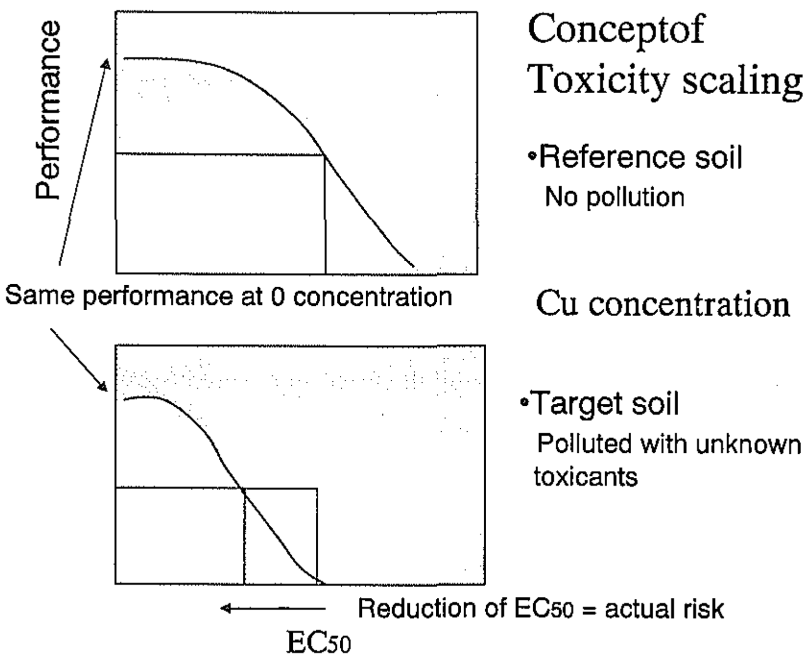

Fig.4. Hypothetical model of the toxicity scaling.

Detailed analysis of composing metals and chemicals will not explain the observed toxicity to animals before the interaction of toxicity will be revealed in every combination of substances. The difference in EC_{50} corresponded to total toxic effect of every toxicant if there were no interaction in toxicity (e.g. additive toxicity) between the scaling substance (Cu) and the substances in target soil. It is concluded that the toxicant-scaling method for soil ecological risk assessment may be useful for estimation of low-level and mixed substance pollution. .

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