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Effects of cadmium, mercury and lead on the survival and instantaneous rate of increase of *Paronychiurus kimi* (Lee) (Collembola)

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Abstract

The invertebrate springtail species *Paronychiurus kimi* (Lee) was selected for use in toxicity testing because it is more ecologically relevant to Korean soils than *Folsomia candida* Willem, which is the standard animal for toxicity tests. Responses of *P. kimi* to cadmium, mercury and lead were evaluated in artificial soils following the standardized ISO protocol. Although, reproduction of *P. kimi* was not as high as that of *F. candida*, 30 adults produced at least 200 juveniles over 28 days. For each of the three heavy metals, LC_{50} and EC_{50} for reproduction and NOEC and LOEC for the effect on reproduction and instantaneous rate of population increase (r_i) were also estimated. The 7 days LC_{50} was 532, 3.9 and 1322 mg/kg dry soil for cadmium, mercury and lead, respectively. As exposure time increased from 7 to 28 days, the LC_{50} values decreased for cadmium but not or only slightly for mercury and lead. The 28 days EC_{50} was 60.0 for cadmium, 0.23 for mercury and 428 mg/kg for lead. Significant changes in r_i of *P. kimi* were closely followed by the changes in the sublethal endpoint measured (reproduction) and populations were heading toward extinction ($r_i = 0$) at concentration of 129, 2.0 and 1312 mg/kg dry soil for cadmium, mercury and lead, respectively. *P. kimi* was found to be more sensitive to all heavy metals tested than *F. candida*, confirming its suitability as a bioindicator species for soil toxicological testing in Korea.

Keywords: Soil health; Heavy metals; Indigenous species; Reproduction; Population effect assessment

1. Introduction

Soil invertebrates, which respond to a wide range of chemicals at various concentrations in soils, have been used as reliable bioindicators of soil health in combination with chemical and physical analyses (Fountain and Hopkin, 2001; Greenslade and Vaughan, 2003). Among the soil invertebrates, Collembola are useful as indicator organisms because most species have short life cycles and several are present in high densities in terrestrial ecosystems (Greenslade and Vaughan, 2003). Most importantly it is known that Collembola are very sensitive to various soil contaminants (Crouau et al., 1999).

An internationally standardized, single-species Collembola reproduction bioassay using *Folsomia candida* Willem has been developed (ISO, 1999) mostly for European soils. *F. candida* was chosen because of its widespread distribution in Europe, because of the extensive knowledge of its cultivation and because acute and reproductive toxicity tests using *F. candida* had been established (Greenslade and Vaughan, 2003).

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The bioassay developed in Europe using *F. candida* is sensitive and useful for ecological risk assessments of contaminated soils (ISO, 1999). However, this species has little ecological relevance to Korean soil ecosystems because *F. candida* is rarely found in Korean soils (Anonymous, 1994) and Korean soil characteristics are different from those of European soils. Korean soils tend to have lower pH and organic matter content than European soils (Jung and Thornton, 1997).

Using indigenous species for ecotoxicological assessments greatly increases the ecological relevance and reliability of bioindicator testing. Recently, *Paronychiurus kimi* (Lee) has been suggested as a useful alternative to *F. candida* in Korea (Kang et al., 2001). *P. kimi* is found commonly and abundantly in Korean paddy soils and plays an important role in the soil ecosystem as a decomposer. This white springtail species reaches maturity at 6 weeks at 20 °C and is 2–3 mm long, with reduced furcula and short antennae. This species is easy to culture in the laboratory using the same method developed for *F. candida* (Snider et al., 1969).

Robertson and Worner (1990) recommended that the population response, rather than the response of individuals selected for their uniform characteristics, must be emphasized in laboratory bioassays to predict the responses likely to occur in a field population. One approach for evaluating the total effect of heavy metals at population levels is the use of demographic toxicological endpoints. Population growth, and in particular the intrinsic rate of increase (r_m) or instantaneous rate of increase (r_i), has been recommended as a superior laboratory bioassay endpoint to that of the LC_{50} because it combines the lethal and sublethal effects into one meaningful measure (Stark et al., 1997; Kammenga and Laskowski, 2000). Clearly, studies that address population growth versus individual survival after exposure to environmental pollutants are needed so that relationship among different levels of biological organization can be elucidated.

In Korea very few studies have been conducted in relation to Collembola sensitivity to pollutants in populations exposed to soil contaminants (Kang et al., 2001), in particular to heavy metals. It is difficult to make a direct comparison of the European reports on heavy metal toxicity with those for Korean soils because of differences of ecological relevance and soil characteristics. Therefore, the development of a soil testing system using indigenous species is the crucial step for ecological risk assessment of Korean soils.

The objectives of the current research were to study the effects of selected heavy metals on the survival and reproduction of *P. kimi* estimate consequences for the population level in order to assess its usefulness as a test species. Two endpoints of toxicological effects were compared: the first, survival of *P. kimi* were determined 7 and 28 days after exposure of cadmium, mercury and lead; the second endpoint involved determining population growth 28 days after exposure with the instantaneous rate of increase (r_i) as measure of interest. The response of *P. kimi* to cadmium, mercury and lead was compared with literature data on the standard species, *F. candida*.

2. Materials and methods

2.1. Collembola culture and test chemicals

P. kimi individuals were collected from paddy fields using an extraction from soils by floating on water (Choi et al., 2002). *P. kimi* cultures were reared on a mixture of plaster of Paris and powdered activated charcoal mixed in a ratio of 4:1:4 by volume. Plastic Petri dishes (95 mm diameter, 15 mm high) were filled to a depth of about 1 cm with the breeding substrate. Water was added to the substrate almost to saturation point prior to the introduction of *P. kimi*. The breeding containers were tightly closed and the high moisture content of the substrate was maintained by periodically adding a few drops of water.

The *P. kimi* were kept in an incubator at 20 ± 1°C under continuous darkness. Cultures were fed with diluted brewers yeast once a week in small amounts to avoid spoilage by fungi. To obtain a cohort of adult Collembola (6 weeks from egg stage), 100 adults were introduced into a breeding container, allowed to lay eggs and then removed after 3 days. The container with *P. kimi* egg clusters was incubated under the same condition described above. Several sets were prepared to ensure a large number of *P. kimi* eggs. After 12 days, the eggs were hatched and the instars were fed. Adults were collected after a further 4 weeks of incubation. Adult cohorts (6-week old) produced from this procedure were used throughout this study. All toxicity tests were performed at 20 ± 1°C without additional light source.

Cadmium chloride ($CdCl_2 \cdot 2.5H_2O$, Sigma, St. Louis, USA), mercury chloride ($HgCl_2$, Merck, Schuchardt, Germany) and lead chloride ($PbCl_2$, Merck, Darmstadt, Germany) were used as heavy metal sources. Stock solutions of each chemical were prepared in deionized water.

2.2. Preparation and contamination of the artificial soil

The artificial soil used in the test was prepared according to ISO (1999). The medium consisted of 10% finely ground *Sphagnum* peat, 20% kaolinite clay and 70% sand, with the pH adjusted to 6.0 ± 0.5 by addition of calcium carbonate. Water holding capacity (WHC) of the artificial soil was determined according to the method proposed by OECD (2000). Solutions of the metals were mixed thoroughly with the artificial soils to give the required moisture content of 65% WHC and metal concentrations in the soils. The mixed soil was introduced into the test containers, which were glass jars (90 mm diameter, 90 mm high) with plastic screw-on lids. Each test container was filled with 140 g wet mass of the artificial soil.

2.3. Toxicity bioassay

Toxicity tests with *P. kimi* were carried out according to ISO (1999). For the tests on survival, 30 adults were introduced to each test container and the containers were placed within the incubator. The nominal concentrations used were 100, 300, 500 and 700 mg/kg for cadmium, 1, 2, 5 and 6 mg/kg for mercury and 100, 500, 1000 and 2000 mg/kg for lead. Individual mortality was recorded after 7 and 28 days of exposure. For each concentration and control, four replicates were prepared. No food was added for the 7 days survival test, but 30 mg of brewers yeast was added biweekly for the 28 days survival test.

The reproduction test determined the effect of different concentrations of heavy metals on the reproductive ability of *P. kimi* adults in the artificial soil. The tests were started with 6-week-old adults to allow rapid population growth within a short time frame, because first reproduction of *P. kimi* was observed at the age of 6 weeks (Kang et al., 2001). Thirty adults were introduced to each container and 30 mg brewers yeast was added biweekly. The containers were aerated and their moisture contents checked weekly and adjusted as necessary by adding the appropriate amount of distilled water to replenish the weight loss. The number of *P. kimi* present was determined after 28 days. The nominal concentrations used for the reproduction tests were 0, 25, 50, 100, 200, 400, 800 and 1600 mg/kg for cadmium, 0, 0.25, 0.5, 1, 2 and 4 mg/kg for mercury and 0, 250, 500, 1000, 2000 and 3000 mg/kg for lead. For each concentration and a control, four replicates were prepared.

At the end of the survival (7 and 28 days) and reproduction (28 days) bioassays, the test containers were flooded with deionized water and gently stirred. The number of Collembola that came up to the surface of the water was counted under a stereo microscope. The surviving adults and resulting juveniles were counted and recorded separately. The adults and juveniles were separated based on a headcapsule width of 0.28 mm for *P. kimi* (Son, 2004). If the headcapsule width was >0.28 mm, the Collembola were considered to be adult.

2.4. Data analysis

Median lethal concentration (LC_{50}) of heavy metals for *P. kimi* was estimated using a POLO-PC computer program (Russel et al., 1977). Differences in toxicity were considered significant when 95% confidence intervals did not overlap. Median effective concentrations (EC_{50}) (Suter, 1993) that reduced the reproductive rate by 50% were estimated by fitting the data to a logistic model Eq. (1) presented by Haanstra et al. (1985):

$$y = \frac{c}{[1 + \exp(b(x - a))]}, \quad (1)$$

where y is the number of progeny, x the natural logarithm of the test concentrations, a the natural logarithm of EC_{50}, b the slope parameter and c is the number of progeny per adult collembolan of the control.

The instantaneous rate of population increase (r_i) was calculated using Eq. (2) (Stark et al., 1997):

$$r_i = \frac{\ln((N_f + 1)/(N_0 + 1))}{\Delta T}, \quad (2)$$

where N_f is the final number of animals, N_0 the initial number of animals and ΔT is the change in time (number of days the experiment was run). The r_i values were calculated for individual replicates and trends plotted by fitting of a four-parameter logistic regression of r_i values against soil chemical concentrations. From this fitted equation, a soil chemical concentration at which $r_i = 0$ was estimated. Positive values of r_i, indicate a growing population and $r_i = 0$ indicates a stable population, while negative r_i values indicate a population in decline and heading toward extinction.

Dunnett's test was performed to determine NOEC (No Observed Effect Concentration) and LOEC (Lowest Observed Effect Concentration) values for the effect of each test chemical on reproduction and population increase rate. Differences of number of progeny and $r_i = 0$ between the controls and treatments were

analyzed using ANOVA followed by Dunnett's test (at the 5% level of significance) (SAS Institute, 1990).

3. Results

3.1. Acute toxicity test

The survival of 6-week-old adults declined as the concentration of test chemicals increased ($P < 0.01$), regardless of exposure times (Table 1). At 7 days exposures, the most toxic chemical was mercury (3.9 mg/kg), followed by cadmium (532 mg/kg) and lead (1322 mg/kg). The same order resulted with the 28 days exposure. A comparison of LC_{50}s between 7 and 28 days exposures indicated that effects of mercury and lead on the survival of *P. kimi* were unaffected by exposure times, but that cadmium toxicity increased with increasing exposure time. The ratio of 7 days LC_{50} to 28 days LC_{50} was 5.9, 1.5 and 1.0 for cadmium, mercury and lead, respectively.

3.2. Reproduction toxicity test

The mean number of offspring produced per adult *P. kimi* by the end of the 28 days population growth study was fitted to equation 1 (Fig. 1). Mean reproduction in the control was 6.54 offspring per adult. Offspring production decreased in a concentration dependent manner for all heavy metals tested (ANOVA, $P < 0.01$). No offspring were produced at concentrations (mg/kg) of 800 for cadmium, 4 for mercury and 3000 for lead. Least square fitting of reproduction with equation 1 gave a good agreement ($R^2 > 0.88$) between the model and the observed values. The 28 days EC_{50} was 60.0, 0.23 and 428 mg/kg for cadmium, mercury and lead, respectively (Table 2).

For cadmium, NOEC and LOEC values for the effect on reproduction were 25 and 50 mg/kg, respectively (Dunnett's, $P < 0.01$) (Fig. 1). No NOEC value for mercury was determined because all the concentrations tested in this study significantly reduced reproduction

Table 1
LC_{50} estimates (mg/kg) with 95% confidence limits for the effect on survival of 6-week-old *Paronychiurus kimi* after 7 and 28 days exposure to different concentrations of cadmium, mercury and lead in artificial soil

Heavy metal	7 days LC_{50}	28 days LC_{50}	Ratio[a]
Cadmium	532 (429–738)	90.1 (28.3–139)	5.9
Mercury	3.9 (3.4–4.5)	2.6 (2.1–3.0)	1.5
Lead	1322 (927–2074)	1299 (520–2256)	1.0

[a] Ratio = 7 days LC_{50} /28 days LC_{50}.

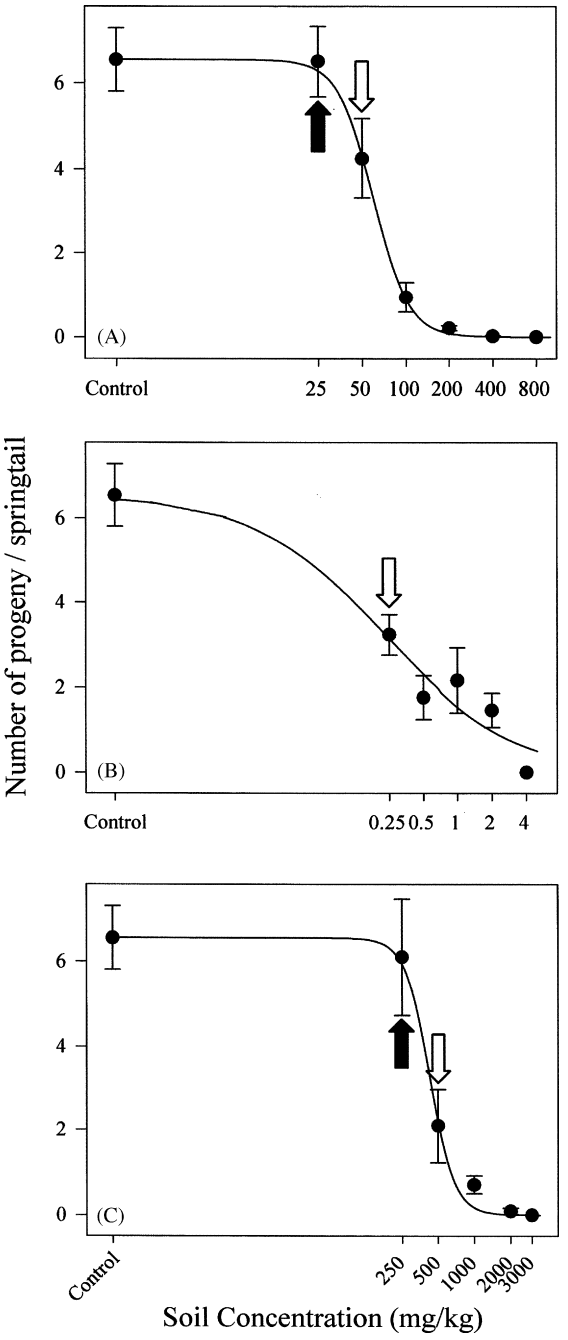

Fig. 1. Mean number of offspring produced by 6-week-old *Paronychiurus kimi* after 28 days exposure to cadmium (A), mercury (B) and lead (C) in artificial soil. The relationship between reproduction rate and heavy metal concentration was fitted using Eq. (1). Solid line indicates the fitted regression based on observed values. Black and white arrows indicate no observed effective concentration (NOEC) and lowest observed effect concentration (LOEC), respectively. No NOEC was determined for mercury because all the concentrations tested in this study significantly reduced the reproduction compared with the control (Dunnett's test, 5% level).

Table 2
Twenty-eight days EC_{50} with 95% confidence limits for the effect on reproduction of 6-week-old *P. kimi* exposed to several different concentrations of cadmium, mercury and lead in artificial soil

Heavy metal	28 days EC_{50} (mg/kg)	28 days $r_i = 0$ (mg/kg)
Cadmium	60.0 (50.7–71.3)	129
Mercury	0.23 (0.10–0.52)	2.0
Lead	428 (345–531)	1312

Also given is the concentration that reduced population growth rate (r_i) to a value of 0.

compared with the control. The LOEC for the effect on reproduction was 0.25 mg Hg/kg. For lead, NOEC and LOEC for the effect on reproduction were 250 and 500 mg/kg, respectively.

3.3. Instantaneous rate of increase (r_i)

The mean control r_i was calculated to be 0.07. Changes of r_i were similar to those of reproduction; the r_i values declined as the concentration of heavy metal increased (Fig. 2). However, r_i value declined in a more gradual manner with increasing heavy metal concentrations, while reproduction declined rapidly within a narrow range of concentrations. Least square fitting of r_i with four-parameter logistic model gave a good agreement ($R^2 > 0.96$) between the model and the observed values. From this regression the concentration at which $r_i = 0$ was calculated to be 129, 2.0 and 1312 mg/kg for cadmium, mercury and lead, respectively.

For cadmium, NOEC and LOEC values for the effect on r_i were 50 and 100 mg/kg, respectively (Dunnett's, $P < 0.01$) (Fig. 2), which were higher than those observed in the reproduction test. The NOEC values for the effect of lead on r_i and reproduction were similar. For mercury, reproduction was more sensitive than r_i.

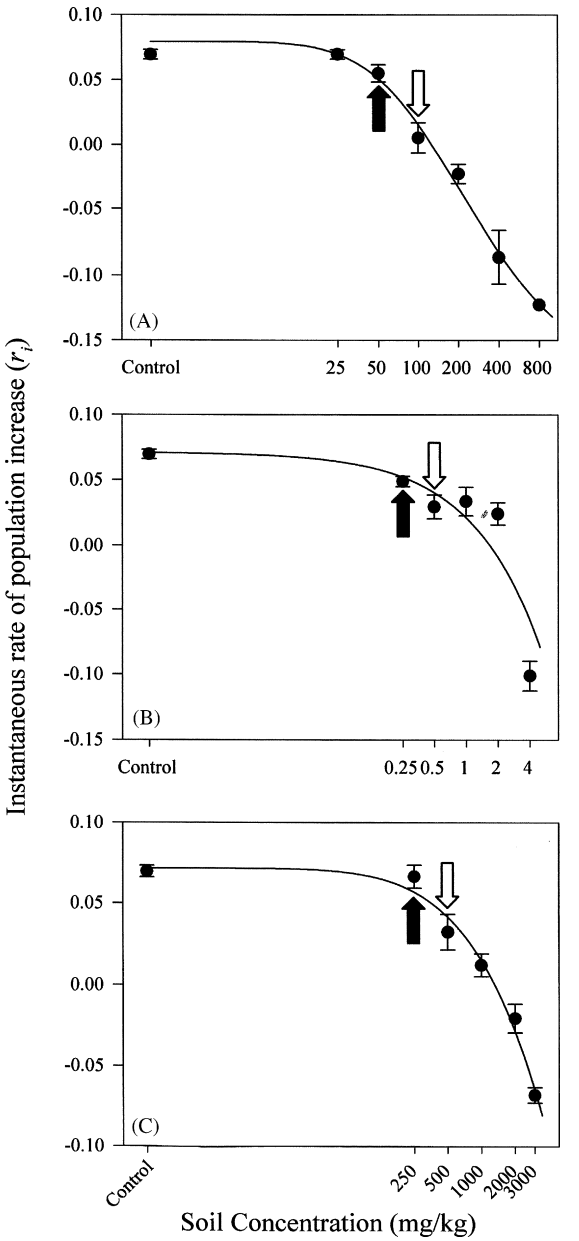

Fig. 2. Changes of instantaneous rate of population increase (r_i) for 6-week-old *P. kimi* exposed to cadmium (A), mercury (B) and lead (C) for 28 days in artificial soil. The relationship between r_i and heavy metal concentration was determined using four-parameter logistic model. Solid line indicates the fitted regression based on observed values. Black and white arrows indicate no observed effective concentration (NOEC) and lowest observed effect concentration (LOEC), respectively (Dunnett's test, 5% level).

4. Discussion

The suitability of *P. kimi* as a test species was compared with the Collembola reproduction test developed for *F. candida* (ISO, 1999). The performance of *P. kimi* on the control was similar to that of *F. candida*, a standardized test species. Adult mortality in the control was very low (<10%). The reproduction rate was not as high as that of *F. candida* but 30 *P. kimi* adults produced at least 200 offspring over 28 days. Fountain and Hopkin (2005) suggested that the mortality of adult *F. candida* in the control should not exceed 20%, and there should be at least 100 juveniles in each control vessel for the test to be valid. All tested heavy metals significantly affected *P. kimi* both at the individual and population level.

Comparisons of effect concentrations determined in this study with literature values indicated that *P. kimi*

was more sensitive to heavy metals than *F. candida*. For cadmium, the recorded 7 days LC_{50} of 532 mg/kg was lower than the value of 900 mg/kg (14 days exposure) reported by Herbert et al. (2004). When the 28 days LC_{50} value was compared, the difference was even larger. The 28 days LC_{50} value for the effect of cadmium observed in this study was 90.1 mg/kg, while van Gestel and van Diepen (1997) found 28 days LC_{50} values for *F. candida* ranging from 617 to 1275 mg/kg. The EC_{50} and $r_i = 0$ values for effects on reproduction were also lower than those for *F. candida* reported by Herbert et al. (2004). The recorded values of EC_{50} and $r_i = 0$ for the effect of cadmium in this study were 60.0 and 129 mg/kg, respectively, compared to 112 and 398 mg/kg reported by Herbert et al. (2004). Similar responses (EC_{50}) of *F. candida* to cadmium were reported by many researchers (van Gestel and Hensbergen, 1997; Crouau et al., 1999; Greenslade and Vaughan, 2003).

Relatively few data are available concerning the effects of mercury and lead on *F. candida*. For mercury, the recorded 7 and 28 days LC_{50} for the effects on *P. kimi* were 3.9 and 2.6 mg/kg, respectively. No comparable LC_{50} data for mercury were available for *F. candida*. Lock and Janssen (2001) reported that all *F. candida* died at 10 mg/kg, whereas the mortality at 5.6 mg/kg mercury was less than 10% (21 days exposure). Estimated EC_{50} value (0.23 mg/kg) for *P. kimi* was also lower than that of *F. candida*. Lock and Janssen (2001) reported that the EC_{50} of mercury for *F. candida* was 3.26 mg/kg, which suggests that *P. kimi* is over ten times more sensitive to mercury than *F. candida*.

Lead was the least toxic of the metals tested, having the highest LC_{50} and EC_{50} values. Collembola may be less sensitive to lead than other metals, as observed by Fountain and Hopkin (2001) who found that *F. candida* feeding on yeast contaminated with lead up to 49,200 mg/kg exhibited no significant changes in survival at all concentrations. The 28 days LC_{50} value for the effect of lead on the survival of *P. kimi* was 2.2 times lower than that for *F. candida* (2900 mg/kg) reported by Bongers et al. (2004), who determined the lead toxicity on LUFA soils. Sandifer and Hopkin (1996) reported that EC_{50} was 2970 mg/kg for the effect of lead at pH 6.0, which is seven times higher than that of *P. kimi*.

The present study showed that differences in cadmium exposure time between 7 and 28 days was important for the determination of effects level during short-term survival tests with *P. kimi*, indicated by the high ratio of 7 days LC_{50} to 28 days LC_{50} (Table 1). The reason why cadmium toxicity is greatly affected by exposure time can be partially explained by a distribution coefficient (K_d), the ratio of the sorbed phase concentration to the solution phase concentration at equilibrium. Cadmium has a tendency to have smaller K_d value than mercury and lead for most soils (USEPA, 1999), so that mercury and lead are strongly adsorbed, and become less bioavailable to *P. kimi*. It is usually assumed that metal uptake by soil-inhabiting organisms is governed mainly by the concentration in pore water (van Leeuwen et al., 1992; van Gestel, 1997).

All tested heavy metals exhibited sublethal effects in the form of significant reductions in juvenile production at nominal soil concentrations lower than the LC_{50} (Fig. 1). When the 28 days LC_{50} was compared with the EC_{50} for cadmium, mercury and lead, the reduction ratio was 1.5, 11.3 and 3.0, respectively. The highest value of 11.3 found for mercury suggests that sublethal effects of mercury may be more sensitive than those of cadmium and lead in *P. kimi*. Although the effect of exposure time on the survival in the cadmium treatment was very high (Table 1), the ratio of 28 days LC_{50} to EC_{50} was relatively small, indicating that both survival and reproduction of *P. kimi* were affected by cadmium with similar intensity compared to mercury and lead.

In this study, the demographic endpoint r_i was used to integrate several life cycle traits to estimate effects of heavy metal contamination on population growth rate. The results presented here showed that r_i had sensitivity similar to the sublethal endpoint for reproduction. However, the EC_{50} values were always two- to nine-fold lower than the concentration at which $r_i = 0$ (Table 2). These differences were due, in part, to the fact that r_i incorporated both the numbers of offspring produced and the number of surviving adults, whereas EC_{50} was just based on the number of juveniles produced. This study also showed that significant reductions in r_i were closely followed by significant reductions in juvenile numbers. This suggests that when sublethal effects occur, they can be displayed and therefore measured through the use of an integrating endpoint such as r_i, (Walthall and Stark, 1997; Herbert et al., 2004). However, several authors have concluded that population growth is not as sensitive a measure as other endpoints of toxicological effect such as reproduction (Stark et al., 1997; Kang et al., 2001; Herbert et al., 2004). If reproduction was the only endpoint evaluated, the effects of heavy metals on population growth would be overestimated. Moreover, traditional endpoints such as adult survival and reproduction provide little information concerning the actual fates of exposed populations. In this respect, the most significant change

for a population will occur when r_i changes from a positive to a negative value. However, to integrate fully the effects of heavy metals on all life cycle parameters, exposures should be long enough to encompass the full life cycle. In this respect, the standard protocol used herein (ISO, 1999) may not fully assess a population responses (Herbert et al., 2004).

P. kimi is a suitable test species to assess heavy metal toxicities in artificial soils as proposed by ISO (1999). The most sensitive toxicological endpoint was 28 days EC_{50} for reproduction, followed by 28 days $r_i = 0$, 28 days LC_{50} and 7 days LC_{50}. To assess the toxicity of Korean soils contaminated either naturally or artificially with metals, *P. kimi* is ecologically relevant species, being sensitive to a range of toxicants and having an acceptable rate of reproduction in artificial soil. Because Collembola have an important role in the transfer of pollutants to different trophic levels, *P. kimi* is an ideal test animal for monitoring and forecasting the effect of pollutants in Korean soil ecosystems. To effectively use this species as a bioindicator of soil contaminations in Korea, further research should be conducted on various soil properties and contaminants which are related to the Korean soils.

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