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Excess zinc uptake in *Paronychiurus kimi* (Collembola) induces toxic effects at the individual and population levels

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Abstract: The purpose of this study was to investigate the toxic effects of zinc in collembolan *Paronychiurus kimi* at the individual (survival and juvenile production) and population (population growth and age structure) levels after 28 days of exposure in artificially spiked soil. These toxic effects were interpreted in conjunction with the internal zinc concentrations in *P. kimi*. The EC_{50} value for juvenile production based on the total zinc concentration was 457 mg Zn kg^{-1} dry soil, while the LC_{50} value for adult survival and $r_i = 0$ value for population growth were within the same order of magnitude (2,623 and 1,637 mg Zn kg^{-1} dry soil, respectively). Significant differences in adult survival, juvenile production, and population growth compared with the control group were found at concentrations of 1,500, 375, and 375 mg Zn kg^{-1} dry or higher, respectively, whereas significant differences in the age structure, determined by the proportion of each age group in the population, were observed in all treatment groups. It appeared that the internal zinc level in *P. kimi* was regulated to some extent at soil zinc concentrations of $\leq$375 mg Zn kg^{-1} dry soil, but not at high soil zinc concentrations. These results indicate that, despite zinc being regulated by *P. kimi*, excess zinc exceeding the regulatory capacity of *P. kimi* can trigger changes in the responses at the individual and population levels. Given that population dynamics are affected not only by individual level but also by population level endpoints, it is concluded that the toxic effects of pollutants should be assessed at various levels.

Keywords: internal metal concentration, population growth rate, age structure

INTRODUCTION

Conventional ecotoxicity experiments that explicitly measure endpoints related to survival, growth, and reproduction have been widely used for assessing the effects of pollutants as an alternative to conventional chemical analyses. It is true that these various endpoints can provide relevant information on adverse effects caused by pollutants at an individual level. Such ecotoxicity data derived from laboratory tests on standard test species have been fundamental to deriving regulatory screening levels using an extrapolation approach based on species sensitivity distributions (SSD) and widely used for ecological risk assessment. In such a case where SSD approach is not available, a deterministic approach using assessment factors applied to single-species toxicity data may be used. However, it should be noted that the population is the smallest level of ecological organization that must be protected (Suter 1993). Thus, such individual level endpoints may suffer from criticisms, not the least of which is whether they can provide enough

conservative information to link the likelihood and extent of adverse effects of pollutants at the population level.

Another issue to be addressed is that total metal concentrations have traditionally been used as a basis for assessing metal toxicity. However, there is also criticism of using total metal concentrations as the dose metric to assess ecological risk. Such criticism may be attributed to the fact that not all the total metal concentrations in the soil are available to soil organisms, but only bioavailable metal fractions that are taken by them (*i.e.* internal body concentration) are responsible for the toxicity, which should be interpreted in conjunction with the impact at the individual and population levels. Therefore, a better understanding of the link between soil metal concentration and the actual uptake in and toxicity to the organism is needed.

Selecting ecologically relevant endpoints is one of the key aspects that need to be considered for assessing the potential effects caused by pollutants. Among the population level endpoints, the population growth rate is one of the most widely used endpoints for assessing ecological risk at the population level (Walthall and Stark 1997; Forbes and Calow 2002; Lin *et al.* 2005; Hanson and Stark 2011). This endpoint can provide the cumulative effects of chemical stressors on individuals (Sibly 1996; Calow *et al.* 1997) and unravel important relationships that cannot be detected using biological endpoints at the individual level, such as mortality, reproduction, or physiological responses (Stark and Wennergren 1995; Spromberg and Meador 2005; Landis and Kaminski 2007). Another endpoint available at the population level is age structure. The age structure, defined as the proportion of individuals of each age group in the population, is also an important feature that can be used to project population growth. The increasing population is typically dominated by young organisms, while the decreasing population is not (Krebs 1994). Thus, change in the age structure of population followed by exposure to a pollutant can be used to judge the status of a population. Even more, change in the age structure of a population may also be an important indicator of stability, age/stage-specific vulnerability, and demographic transition of a population to chemical exposure. Yet its use in ecotoxicological research has been rarely investigated.

In this study, a collembolan species *Paronychiurus kimi* was chosen as the test species because arthropods belonging to this order generally play an important role in litter decomposition and energy cycling in soil ecosystems (Fountain and Hopkin 2005). Zinc was chosen as a test chemical because it is an essential metal but excessive zinc uptake by an organism can have adverse effects on the organismal performance (*e.g.* growth, fecundity, survival, etc.). Furthermore, zinc is very mobile and bioavailable (Kabata-Pendias and Pendias 1993), thus elevated zinc levels in soil is likely to pose a potential risk to soil organisms, especially those organisms living in the pore space of the soil. The present study aimed to determine the effects of zinc on responses at the individual (survival and juvenile production) and population (population growth and age structure) levels in *P. kimi* and to assess whether the effects at the individual level could provide a sufficiently conservative estimate of the impact at the population level. Also, this study investigated how toxic effects at the individual and population levels were related to the internal zinc concentration. To end this, 28-d chronic toxicity test of zinc in *P. kimi* was conducted and the difference in the age structure determined by the relative proportion of each age group (grouped based on head capsule width reported by Son *et al.* (2009)) was compared between the control and zinc treatment groups.

MATERIALS AND METHODS

1. Test species

P. kimi extracted from paddy soils in Korea were kept at 20 ± 1°C on a moist substrate of the mixture of plaster of Paris and charcoal in continuous darkness, and fed with an aqueous suspension of yeast as a food source weekly. Age-synchronized cohorts of 42−44 days old adults were obtained by transferring adult collembolans from a breeding culture onto a new moistened plaster substrate (plaster of Paris charcoal plate) and allowing them to lay eggs for 2 days. Seven days after the egg clutches appeared, egg clutches were transferred onto a new moistened plaster substrate. Once the eggs hatched (approximately 14 days after oviposition), they were maintained as described above.

2. Test soil and spiking

Artificial soil consisted of 70% quartz sand, 20% kaolin clay, and 10% finely ground sphagnum peat (OECD 2009) was prepared. Soil pH was adjusted to 6.0 ± 0.5 by adding $CaCO_3$ (99% purity; Sigma-Aldrich, USA). An aqueous stock solution of zinc chloride ($ZnCl_2$, $\geq$98% purity; Sigma-Aldrich, USA) was prepared in deionized water. Six nominal zinc concentrations (93.75, 187.5, 375, 750, 1,500, and 3,000 mg Zn kg^{-1} dry soil) were prepared by diluting a stock solution with deionized water. The pre-moistened

artificial soil was mixed with each of the diluted solutions, and, if necessary, deionized water was added to achieve 50% of the maximum water holding capacity (WHC) of the soils. Control soil without zinc application was moistened to 50% WHC by adding deionized water.

3. Toxicity test

Toxicity test with *P. kimi* was carried out according to the OECD guideline 232 (OCED 2009). For each control and test soil, four replicates were conducted. Ten age-synchronized *P. kimi* (42−44 d old) were transferred into each polystyrene container (120 mL), containing 30 g of test soil as prepared above. The test ran for 28 days and all test containers were kept at 20 ± 1°C in a temperature-controlled incubator under dark conditions. Granulated dried brewer's yeast was added to each test container biweekly, and the containers were opened weekly to aerate and replenish water loss. At the end of the toxicity test, soil samples were collected from three containers, randomly selected, of each replicate for chemical analysis. All surviving adults and juveniles were collected and counted using water floatation and then transferred on a moistened plaster substrate for head capsule width measurement.

4. Head capsule width measurement

All surviving adult and juvenile collembolans collected from each control and treatment group were subjected to head capsule width measurement. Digital images of the collembolans were produced by taking them using a DIMIS M 50× LED-illuminated digital magnifier, which was calibrated with a micrometer scale (Siwon Optical Technology, Korea). The head capsule width, measured at the widest point of the head (in mm) of each individual, was analyzed using ImageJ software (National Institutes of Health, USA), and then classified into one of the five age groups based on head capsule width reported by Son *et al.* 2009.

5. Internal zinc concentration in *Paronychiurus kimi*

After image acquisition, all surviving adult collembolans were split randomly into two replicates for each concentration, rinsed with distilled water, and placed on a glass dish lined with a moistened filter paper for 24 h to allow them to empty gut contents. After 24 h, collembolans were rinsed again with distilled water, transferred to a pre-weighed vial, oven-dried at 70°C for 24 h, and weighed. Each dried sample was digested in 1 mL of a concentrated acid mixture of HNO_3 with $HClO_4$ (7:1, v/v) at 100°C until completely evaporated. After digestion, the residues were resuspended and dissolved in 2% HNO_3 and the zinc concentration in the solution was quantified using an inductively coupled plasma-mass spectrometry (Varian 820-MS, Varian Inc., Australia). Quality of the analysis was checked with certified reference material (ERM-CE278k mussel tissue) and measured zinc concentration did not deviate more than 20% from the certified reference value.

6. Chemical analysis

Soil samples were dried at room temperature. For total zinc analysis, 0.5 g of air-dried soil was digested in a concentrated acid mixture of HCl and HNO_3 (1:3, v/v) at 120°C for 2 h, diluted with distilled water to 50 mL, and then filtered through a filter paper (No.5B, Advantec, Japan). For water- and 0.01 M $CaCl_2$-extractable zinc concentrations, 5 g of air-dried soil was shaken with either 25 mL of deionized water or 0.01 M $CaCl_2$ solution, respectively, for 2 h at 200 rpm, and then filtered through 0.45 μm cellulose nitrate membrane filter (Sartorius, Germany). Zinc concentrations in the filtrates were determined using an inductively coupled plasma-optic emission spectrometry (Vista Pro, Varian Inc., Australia). The quality of analysis was ensured by analyzing the recovery using reagent blank solution fortified with zinc from an independent standard.

7. Data analysis

All statistical analyses were performed using R Studio version 1.2.1335 (RStudio Team 2016). To determine the desorption of zinc from the test soil, the amounts of zinc adsorbed on the soil were related to either the water- or 0.01 M $CaCl_2$-extractable zinc concentrations using a Freundlich isotherm: $\log(C_s) = \log(K_f) + 1/n \times \log(C_w)$, where C_s is the zinc concentration adsorbed by the soil (mg kg^{-1} dry weight), C_w is the metal concentration in the water or 0.01 M $CaCl_2$ filtrate at equilibrium (mg L^{-1}), K_f is the Freundlich adsorption constant (L kg^{-1}), and $1/n$ is a shape parameter of the Freundlich isotherm (dimensionless). The amount of zinc adsorbed on the soil was determined by calculating the difference between the initial concentration and the concentration of the leachates extracted with either water or 0.01 M $CaCl_2$ using a mass-balance relationship.

Survival and juvenile production data were fit using nonlinear least-squares regression using R package 'drc'. A 2-parameter log-logistic function (LL.2) with fixed upper limit

of 1 was used to fit the binomial data of survival, while a 3-parameter log-logistic function (LL2.3) with fixed upper limit of the mean number of juveniles in the control was used to fit the continuous data of juvenile production. The total, water-extractable, and 0.01 M $CaCl_2$-extractable zinc concentrations in the soils and internal zinc concentrations in the animals were used as a dose-metric to calculate the effect concentration LC_{50} and EC_{50} values for adult survival and juvenile production. The instantaneous rate of population increase (r_i) was calculated using the following equation (Stark *et al.* 1997): $r_i = [\ln((N_f+1)/(N_0+1))/\Delta T]$, where N_f is the final number of collembolans, N_0 is the initial number of collembolans, and ΔT is the exposure duration (28 days). Positive values of r_i indicate a growing population, and $r_i = 0$ indicates a stable population, while a negative r_i value indicates population decline and heading towards extinction. The r_i values were fit against the total, water-extractable, and 0.01 M $CaCl_2$-extractable zinc concentrations in the soils and internal zinc concentrations in the animals using a four-parameter log-logistic model, where the upper limit was fixed as the mean value of r_i in the control group. From this fitted equation, the concentration at which $r_i = 0$, a stable population, and corresponding 95% confidence intervals were obtained using R package 'drc'.

Prior to executing one-way analysis of variance (ANOVA), all variables were checked for homogeneity of variance (Levene's test) and normality (Shapiro-Wilk's test) and, if violated, appropriate data-transformations were made. When significant differences in the survival, juvenile production, r_i, and internal zinc concentration between the control and the treatment groups were found, a post-hoc Dunnett's multiple comparison test was used to further analyze differences between groups ($p < 0.05$). The bioaccumulation factor (BAF) was calculated as the ratio of the measured Zn concentrations in the surviving adult collembolans to the total Zn concentrations in the soils. A chi-square goodness of fit test was performed to determine whether the proportion of each age group in the population of the treatment group is different from that of the control group.

RESULTS AND DISCUSSION

1. Zinc concentrations and its adsorption to artificial soil

In general, the measured total zinc concentrations in the test soils were in agreement with nominal ones (usually < 12% deviation, except for 750 mg kg^{-1} where 22% deviation was observed). Thus, the nominal zinc concentrations in the soil were used as the total zinc concentrations in the soil throughout this study. For both extractants (water and 0.01 M $CaCl_2$), the extractable zinc concentrations increased with increasing total zinc concentrations due to the saturation of adsorption sites at high concentration. Moreover, the relatively small $1/n$ values (0.55–0.60, Table 1), regardless of the extracts, also indicate a strong concentration-dependent effect on the adsorption of zinc to the soil. This may be due to the fact that an excessive amount of zinc, which exceed the adsorption capacity of the soil itself, is applied to the soil (van Gestel and Hensbergen 1997). As reflected by the smaller Freundlich adsorption constant for 0.01 M $CaCl_2$ given in Table 1, a larger amount of zinc was extracted by 0.01 M $CaCl_2$ than water.

Table 1. Freundlich adsorption constants for the sorption of zinc to artificial soil

Metal	Extraction	Freundlich constants		
		K_f	$1/n$	r^2
Zinc	Water	268.6	0.553	0.994
	0.01 M-$CaCl_2$	84.8	0.596	0.997

Although a direct comparison between the results found in this study and those reported in the literature may be hampered due to the heterogeneity of soil properties affecting equilibrium partitioning of zinc in the soil, the values obtained in this study were within the same order of magnitude as reported in the literature. Smit and van Gestel (1996) reported K_f values of 220–238 mL g^{-1} with corresponding $1/n$ values of 0.42–0.47 in natural soils. The K_f value of 446 mL g^{-1} with a corresponding $1/n$ value of 0.47 in artificial soil was reported by van Gestel and Hensbergen (1997) on the basis of water-extractable zinc concentration. Considering the generally small $1/n$ values below 1 reported in the literature as well as in this study, it was clear that a considerable amount of added zinc could readily be desorbed from the soil at high concentrations because of saturation of adsorption sites available to zinc. This may be responsible for the increased zinc toxicity at high zinc concentrations as bioavailable zinc concentrations to organisms increased.

2. Internal zinc concentration and bioaccumulation factor (BAF)

Unexpectedly, a high zinc concentration of 69.0 mg Zn kg^{-1} dry body weight in *P. kimi* was measured in the control group (Fig. 1), even though the measured total zinc

concentration in the control soil was low at 2.11 mg Zn kg^{-1} dry soil. This might be due to the zinc in the brewer's yeast that was used as a food source (Bekatorou *et al.* 2006; USDA 2014). Similar magnitude but slightly lower levels of internal zinc concentration in the control group found in this study were also reported by van Gestel and Hensbergen (1997) (20–50 mg Zn kg^{-1} dry body weight) and Smit and van Gestel (1996) (44.5 mg Zn kg^{-1} dry body weight) in *F. candida*.

The internal zinc levels in *P. kimi* seemed to be regulated at concentrations of 87–114 mg Zn kg^{-1} dry body weight at soil zinc concentrations of 93.75–375 mg Zn kg^{-1} dry soil; but those at soil zinc concentrations of 750 mg Zn kg^{-1} dry soil or higher were 2.4 to 5.1 times higher than that of the control group (Fig. 1). Similar increasing trend in the internal zinc level at higher soil zinc concentrations was reported for other collembolan *F. candida* (Smit and van Gestel 1997; van Gestel and Hensbergen 1997), whereas a constant internal zinc concentration in *F. candida* (2,986 μmol Zn kg^{-1} dry body weight, which is corresponding to 195 mg Zn kg^{-1} dry body weight) was also reported by Vijver *et al.* (2001) regardless of external zinc concentrations in 10 out of 16 field soils surveyed. In general, internal metal concentration can be affected by a range of factors, including metal/soil properties, exposure concentrations, and physiology of the species (Spurgeon and Hopkin 1999; Ardestani *et al.* 2014). However, considering the comparable internal zinc concentrations in *P. kimi* (87–114 mg Zn kg^{-1} dry body weight) to those reported for other collembolan *F. candida* (70–270 mg Zn kg^{-1} dry body weight) (Smit and van Gestel 1997), *P. kimi* appear to be able to regulate their internal zinc concentrations to some extent at a certain range of soil zinc concentrations, but not at high soil zinc concentrations (Smit and van Gestel 1995; van Gestel and Hensbergen 1997; Sterenborg *et al.* 2003).

When the internal zinc concentrations were related to the total, water- and 0.01 M $CaCl_2$-extractable zinc concentrations, strong positive correlation coefficients ($r > 0.96$) were observed, indicating that these chemically available fractions can be used as chemical measures of bioavailability. The bioaccumulation factor (BAF), which is often used to describe metal accumulation through trophic transfer (Smith *et al.* 2010), ranged from 0.30 to 0.93 at soil zinc concentrations of 93.75–375 mg Zn kg^{-1} dry soil but ranged from 0.12 to 0.22 mg Zn kg^{-1} dry soil at concentration of 750 mg Zn kg^{-1} dry soil or higher. When BAF was related to the external zinc concentration, a hyperbolic decrease in BAF was observed with increasing external exposure concentration, revealing that internal zinc accumulation does not rise as quickly as exposure levels. This indicates that the internal zinc level in *P. kimi* can be, but not always, regulated at a stationary level at a certain range of external zinc concentrations, implying that the use of BAF as an indicator of potential risk of zinc through trophic transfer is questionable and BAF should be evaluated on a case-by-case basis (Ardestani *et al.* 2014).

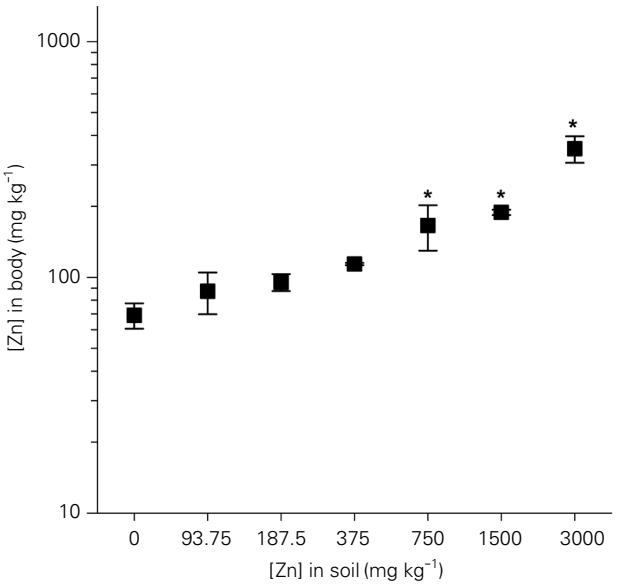

Fig. 1. Internal zinc concentrations (mg kg^{-1} of dry body weight; mean ± S.E., $n = 2$) in *Paronychiurus kimi* related to the zinc concentration in the soil. Asterisk (*) indicates significant differences between the control and the treatment groups after one-way analysis of variance followed by Dunnett's multiple comparison test.

3. Toxic effects of zinc on *Paronychiurus kimi* at individual and population levels

The LC_{50}, EC_{50}, and $r_i = 0$ values for the effects of zinc on the survival, reproduction, and population growth rate of *P. kimi* are presented in Table 2 and Fig. 2. When individual level responses in the treatment groups were compared with the control group, a significant decrease in the adult survival was observed at soil zinc concentrations above 1500 mg kg^{-1} dry soil compared with the control group, whereas a significant reduction in juvenile production was observed at concentrations of 375 mg kg^{-1} dry soil or higher. The EC_{50} values based on total, water-extractable, and internal zinc concentrations were within the same order of magnitude to those reported by van Gestel and Hensbergen

(1997), who reported that EC_{50} values for reproduction in *F. candida* were 626, 8, and 97 mg kg^{-1}, respectively.

When population level response in the treatment groups were compared with the control group, a significant decrease in population growth (r_i) compared with the control group was observed at soil zinc concentrations of 375 mg kg^{-1} dry soil or higher. The concentration at which population growth is zero ($r_i = 0$) was between EC_{50} and LC_{50} values, indicating that reduced juvenile production can trigger initial population decline and further increased adult mortality can lead to population extinction. These results indicate that zinc could affect not only individual level response (*i.e.* juvenile production) but also population level response (*i.e.* population growth), even at concentrations where zinc is regulated by *P. kimi*. The age structure of all treatment groups showed statistically significant differences from the control, as assessed by chi-square tests, even at concentrations much lower than those at which population growth was affected (Table 3). When comparing the proportion of juveniles (age groups of 1–2) and adults (age groups of 3–5) in the total population of the treatment groups with that of the control group, a significant difference was found at soil zinc concentrations of 750 mg kg^{-1} or higher. These results clearly showed that a significant reduction in the juvenile production could trigger the change in the age composition of *P. kimi*, which in turn subsequently could result in the population extinction at higher zinc concentrations. However, given that treatment groups treated at 375 mg kg^{-1} or less had a triangular-shaped age structure with a high proportion of juvenile groups, it is expected that these groups would be stable and growing populations. Although there have been few studies on the effect of chemicals on age structure, our previous study have shown that the proportion of juvenile and adult stage in the total population was affected when exposed to cadmium at or below EC_{50} (Son *et al.* 2009), whereas in this study a significant effect on the proportion of juvenile and adult stage was observed at concentrations above the EC_{50} of zinc. This difference might be because nonessential metals, such as cadmium, may not be as efficiently regulated and detoxified as essen-

Table 2. LC_{50}, EC_{50}, and $r_i = 0$ values (95% confidence intervals) for the effects of zinc on the adult survival, reproduction, and instantaneous rate of population increase of *Paronychiurus kimi* after 28 days of exposure to zinc. These values are expressed based on total, water-extractable, 0.01 M $CaCl_2$-extractable concentrations in the soil and on internal zinc concentrations in *P. kimi*

	LC_{50}	EC_{50}	$r_i = 0$
Total concentration (mg kg^{-1})	2202 (1710–2694)	464 (373–578)	1783 (1624–1942)
Water-extractable concentration (mg kg^{-1})	241 (103–378)	11.5 (7.88–16.7)	138 (117–159)
0.01 M $CaCl_2$-extractable concentration (mg kg^{-1})	886 (384–1388)	62.3 (45.2–85.7)	485 (421–550)
Internal concentration (mg kg^{-1})	281 (240–322)	131 (120–143)	245 (220–269)

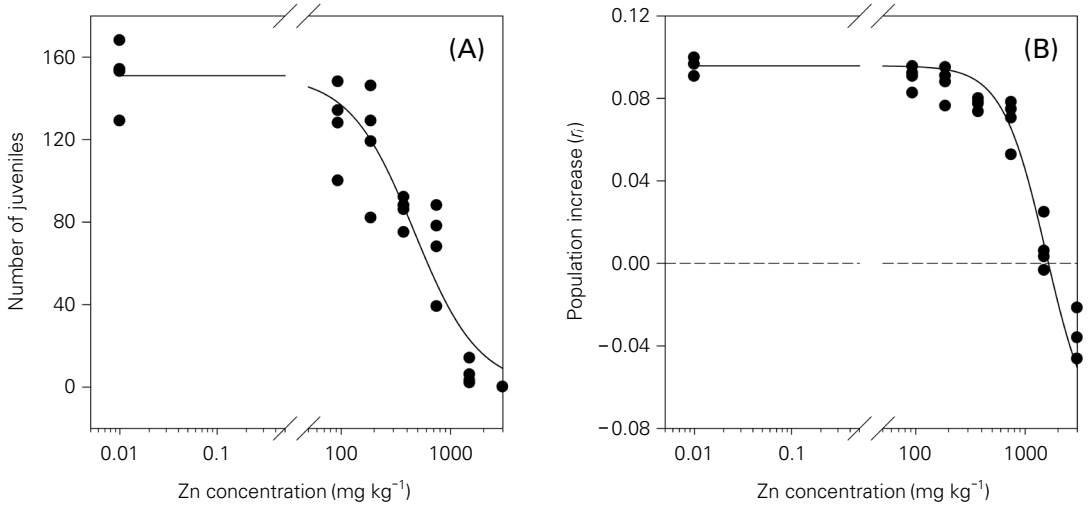

Fig. 2. Effects of zinc on juvenile production (A) and instantaneous rate of population increase (r_i) (B) in *Paronychiurus kimi* after 28 days of exposure to zinc in artificial soil. The solid line indicates the fitted regression (based on observed values). The dotted line in panel (B) represents $r_i = 0$ (stable population).

Table 3. The number of *Paronychiurus kimi* in each age group and the relative percentage (in parenthesis) of each age group, in the total population after 28 days of exposure to various concentrations of zinc

Developmental stage	Age group	Zinc concentration (mg kg^{-1})						
		0	93.75	187.5	375	750	1500	3000
Juvenile	1	311 (48.4)	210 (38.2)	221 (42.9)	128 (33.7)	134 (43.2)	12 (22.6)	0 (0.0)
	2	278 (43.3)	290 (52.7)	252 (48.9)	213 (56.1)	139 (44.8)	13 (24.5)	0 (0.0)
Adult	3	15 (2.3)	10 (1.8)	3 (0.6)	0 (0.0)	0 (0.0)	8 (15.1)	11 (84.6)
	4	11 (1.7)	3 (0.5)	9 (1.7)	10 (2.6)	14 (4.5)	15 (28.3)	2 (15.4)
	5	27 (4.2)	37 (6.7)	30 (5.8)	29 (7.6)	23 (7.4)	5 (9.4)	0 (0.0)
χ^2-value[a]		–	36.6	17.0	52.7	31.0	271	403
p-value[a]		–	< .0001	< .005	< .0001	< .0001	< .0001	< .0001

[a]A chi-square goodness of fit test was performed to determine whether the proportion of each age group in the treatment group differed from that observed in the control group.

tial metals, such as zinc, and thus may produce a cumulative toxic effect on the population, even at concentrations below those causing adverse effects at the individual level.

An understanding of not only the changes of age structure but also population growth rate is important because they can provide information on how demographic transitions and dynamics of a population would be in the future, which cannot be detected at the individual level. It is, of course, true that populations with different developmental stages and age structures may respond differently to toxic compounds (Pieters and Liess 2006). Although this study started with populations of the synchronized adult cohorts, the results of this study clearly showed that unacceptable effects at the individual level can trigger the change in the age structure of *P. kimi* populations and lead to the extinction of populations, which may ultimately affect the future population dynamics. A possible explanation for this change in age structure might be due to the trade-off between energy allocation for maintenance of homeostasis (*e.g.* regulation of internal zinc level) and less essential activities (*e.g.* reproduction and somatic growth). In spite of zinc being regulated by *P. kimi*, the influx of excess zinc into the body can increase the energy consumption to eliminate zinc from the body, which can offset the energy allocation for growth and reproduction, producing cumulative toxic effects at the individual and population levels.

CONCLUSIONS

From the results of this study, it can be concluded that considerable zinc applied to the soil can be easily desorbed from the soil, which becomes bioavailable for *P. kimi*. The internal zinc level in *P. kimi* appeared to be regulated to some extent (87–114 mg Zn kg^{-1} dry body weight) at soil zinc concentrations of $\leq$375 mg Zn kg^{-1} dry soil, but not at high soil zinc concentrations. A significant decrease in juvenile production and population growth (r_i) compared with the control group was observed at soil zinc concentrations of 375 mg Zn kg^{-1} dry soil or higher, whereas a significant difference in the age structure was observed at all treatment groups. These results indicate that excessive amounts of zinc, which are greater than the physiological needs of the individual, can adversely affect the response at the individual level, subsequently leading to adverse effect at the population level. In conclusion, a thorough understanding of the effects of chemicals on age structure together with other effects at the individual level may provide a better insight into the current and future demographic transition of population.

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