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Toxicity of Nickel to a Soil-Dwelling Springtail, *Folsomia fimetaria* (Collembola: Isotomidae)

Janeck J. Scott-Fordsmand,*[,1] Paul Henning Krogh,* and Stephen P. Hopkin†

*Department of Terrestrial Ecology, National Environmental Research Institute, P.O. Box 314, Vejlsøvej 25, DK-8600 Silkeborg, Denmark; and †Division of Zoology, School of Animal and Microbial Sciences, University of Reading, P.O. Box 228, Reading RG6 6AJ, United Kingdom

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Exposure of the collembolan *Folsomia fimetaria* L. to nickel via soil caused significant mortality and reduced growth and reproductive output. Nickel may be present in elevated concentrations due to anthropogenic discharge. Although collembolans are very numerous and important organisms in the soil ecosystem, the effect of nickel has not previously been studied on these organisms. The aim of this study was to investigate the toxic effects of high soil nickel concentrations on the collembolan *F. fimetaria* following a 3-week exposure in a loamy sand spiked with nickel up to 1000 mg Ni/kg. A 10% decrease in adult female numbers at 427 mg Ni/kg and at 645 mg Ni/kg for adult male numbers was observed for nickel-spiked soil. Juvenile numbers were reduced at 701 mg Ni/kg following a 3-week exposure. The corresponding EC_{50} values were 786 mg Ni/kg for females, 922 mg Ni/kg for males, and 859 mg Ni/kg for juveniles. The reproductive output seems to be the most sensitive parameter being reduced at soil nickel concentrations above 173 mg Ni/kg (EC_{10}). Adult growth was not affected by soil nickel concentrations up to 1000 mg Ni/kg, but juvenile growth was reduced at concentrations above 480 mg Ni/kg (EC_{10}). © 1999 Academic Press

Key Words: **Collembola; nickel; loamy sand soil; toxicity; *Folsomia fimetaria*.**

INTRODUCTION

In recent years, a large number of studies have found that trace metals may be toxic to soil-living species, such as microorganisms, plants, and invertebrates (Hopkin, 1989; Scott-Fordsmand and Bruus Pedersen, 1995). Although toxic effects of the trace metal nickel have been studied for plants and microorganisms, this compound has been little studied in terrestrial invertebrates (Scott-Fordsmand, 1997). Nickel is a naturally occurring element present in soil, water, and air and is believed to be essential in many organisms. Apart from being a naturally occurring element, elevated concentrations found in the environment may also be caused, for example, by deposition from the burning of fossil fuels and spreading of waste, such as sewage sludge and manure, especially pig manure (Bak *et al.,* 1997). Discharges of nickel, and in certain places natural occurring concentrations, may lead to high concentrations in soil and other ecosystems.

Some of the most abundant and widespread groups of organisms in soil are Collembola (springtails) occurring in densities of up to 10^4–10^5 m^{-2} in soil and litter (Hopkin, 1997). They are present in most soil from very cold (arctic) habitats to very hot and dry habitats and may play a vital role in the decomposition of organic material (Petersen and Luxton, 1982; Hopkin, 1997). One representative of this species is the sexually reproducing *Folsomia fimetaria* which is widely spread, mainly living in decaying material. Heavy metals, other than nickel, have been found to be toxic to collembolans, but no studies (to the authors' knowledge) have reported the toxicity of nickel toward collembolans.

The aim of this study is to evaluate the toxic effects of nickel on the sexually reproducing collembolan species *F. fimetaria*. The lethal and sublethal effects on adult and juvenile collembolans have been studied, including effects on mortality, growth, and reproductive output.

MATERIALS AND METHODS

Animal Culture

F. fimetaria was taken from a laboratory culture established from field-collected animals and maintained in petri dishes on a substrate of moistened Plaster-of-Paris and charcoal. They were fed dried yeast *ad libitum* and every 2 to 4 weeks the adult animals were transferred to new substrates with fresh food. Prior to the experiment a

[1]To whom correspondence should be addressed. Fax: +45 89 20 14 13. E-mail: jsf@dmu.dk.

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synchronized culture was produced by collecting eggs (ca. 7 days old) from these stock cultures, which were allowed to hatch over a 3-day period. Animals hatched during this time were subsequently used for the following experiments.

Preparation and Contamination of Soil

In all experiments the soil used was a LUFA-Speyer soil (LUFA-Speyer 2.2, Sp 2121, LUFA Speyer, Speyer, Germany) with a pH of 5.5, total organic carbon 2.3%, clay 5%, silt 13%, and sand 82%. Prior to the experiments the soil was dried in an oven (Memmert, Type UL40) at 80°C overnight, to eliminate undesired soil fauna and to obtain soil nickel concentrations on a dry weight basis. Nickel was added as the chloride salt ($NiCl_2 6H_2O$, Merck Pro Analysis, Germany) from a stock solution (9.953 g Ni/liter).

Experimental Design

The experiments were conducted in microcosms containing 30 g moist soil (25.5 g dry soil and 4.5 ml demineralized water). To induce microbial activity, half the water (2.25 ml) was added 1 week prior to the experiment and the remainder, in relevant cases also containing the final nickel content, was added 1 day prior to the start of the experiment. The Collembola were exposed in these microcosms to six soil nickel concentrations ranging between 0 and 1000 mg Ni/kg dry wt, viz. 0, 100, 300, 500, 700, and 1000 mg Ni/kg, with four replicates per concentration. The pH was adjusted to pH 5.5 to 6.0, by the addition of powdered $CaCO_3$ (Merck, Pro Analysis), for all but the control soils which already had a pH of 5.5 to 6.0. The experiments were run at constant temperature of 20°C, with a 12/12-h light/dark regime. The animals were fed dried baker's yeast (15 mg dry wt) on day 0 and on day 14 (for those animals exposed for 21 days). The soil was remoistened after 14 days and soil pH measured at the end of the experiment.

Effect of Nickel on Survival, Final Body Size, and Reproduction

To measure adult survival, final adult body size, and reproduction, 20 *F. fimetaria*, 10 adult females and 10 adult males (aged 19 to 23 days), were added to each replicate microcosm on day 0 and incubated for 21 days [in a synchronous culture of *F. fimetaria*, it is possible to discern the differences in sex by size (at this age) as females are much larger than males].

Juvenile survival and final body size were measured by exposing 20 juveniles (0 to 3 days old) to soils with elevated nickel concentrations in microcosms and incubating for 21 days, as described above.

Counting and Measuring the Animals

At the end of both experiments, all animals were extracted in a high-gradient Tullgren funnel of the MacFayden type and collected in a cooled (4°C) collecting dish. Surviving adults and juveniles were counted by an automated process and the following measurements determined: individual body area, length, width, sliminess, and optical gray intensity, by use of a digital image processing (DIP) system. The digital image processing system is an automated counting and measuring technique based on a video camera connected to a frame grabber and a computerized treatment of data. From each microcosm collembolans were transferred to a homogenous black surface (plaster/charcoal mixture, 1:1 by weight) and immobilized by carbon dioxide anesthetization. By scanning the surface holding the collembolans with a digital image processing system, the number and size of the individuals were quantified (for further details, see Scott-Fordsmand *et al.,* 1997; Krogh *et al.,* 1998). Using the measurements obtained from the DIP it was possible to distinguish between females, males, and juveniles by use of an ordination technique using principal component analysis (PCA) (SAS, 1989). The overall reproduction, growth (measured as final body surface area), and survival of the animals were then calculated.

Statistics

The data were checked for normality using a χ^2 test and for homogeneity of variance by Barlett's test. The NOEC (no-observable-effect concentration) and LOEC (lowest-observable-effect concentration) were estimated by Tukey's Studentized range (HSD) test (SAS, 1989). Effect concentrations, EC_{10} and EC_{50}, and confidence intervals were estimated by fitting a logistic model to the data (Lacey and Mallett, 1991). The formulae were reparameterized by incorporation of EC_{10} and EC_{50} into the equation (Krogh, 1995). The estimates with a 95% confidence interval were performed with SAS procedure PROC NLIN (SAS, 1989).

RESULTS

Significant mortality was observed for both adults and juveniles exposed to 1000 mg/kg (LOEC) soil nickel concentrations, but not at concentrations below this (NOEC = 700 mg Ni/kg) (Fig. 1). A 10% decrease in adult female number occurred at 427 mg Ni/kg, at 645 mg Ni/kg for adult males, and at 701 mg Ni/kg for juveniles. The LC_{50} values were 786, 922, and 859 mg Ni/kg for females, males, and juveniles, respectively (Table 1).

Defining differences in growth as the difference between final body surface areas of the Collembola, adult growth was not significantly affected following 21 days of exposure to nickel (Fig. 2). Juvenile final body size was more

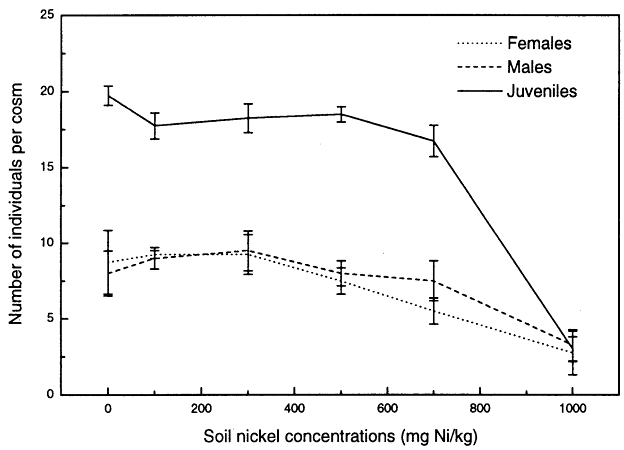

FIG. 1. Number of female, male, and juvenile (mean ± SEM) Collembola, *Folsomia fimetaria*, per microcosm following exposure of adult Collembola to increasing soil nickel concentrations (mg Ni/kg) after 21 days. Each point is based on four replicates.

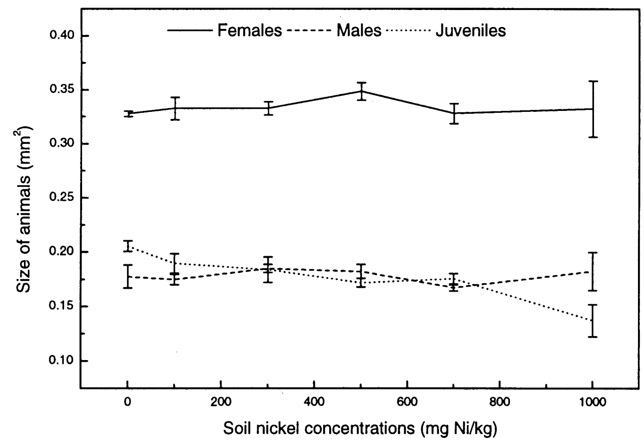

FIG. 2. Mean size (surface area) in mm^2 (± SEM) of female, male, and juvenile Collembola, *Folsomia fimetaria*, following exposure to increasing soil nickel concentrations (mg Ni/kg) after 21 days of exposure. Each point is based on four replicates.

sensitive than that for adults, being affected at a soil nickel concentration of 1000 mg Ni/kg (NOEC), with a resulting 10% decrease in growth at 480 mg Ni/kg (Fig. 2).

Springtail reproduction was clearly affected following exposure to nickel with a significant reduction ($P < 0.05\%$) at 500 mg Ni/kg, an estimated 10% reduction at only 173 mg Ni/kg, and a 50% reduction at 450 mg Ni/kg (Fig. 3, Table 1). Reproduction of springtails includes the production of two clutches during the 21-day exposure period which can be discriminated by PCA on the measured parameter (Scott-Fordsmand *et al.,* 1997; Krogh *et al.,* 1998). In the present experiment this was also the case for most concentrations. Thus, an analysis of the effect on clutch size was not performed.

Soil pH did not change during the period of the experiment.

TABLE 1

No-Observable-Effect Concentrations (NOEC), Lowest-Observable-Effect Concentration (LOEC), and Concentrations with a 10% Lethality or Effect (LC_{10}/EC_{10}) and a 50% Lethality or Effect (LC_{50}/EC_{50}) (mg Ni/kg Dry wt) for Adult and Juvenile Collembola *F. fimetaria* Following Exposure to Nickel-Contaminated LUFA-Speyer Soil

	NOEC	LOEC	LC_{10}/EC_{10}	LC_{50}/EC_{50}
Number				
Females	800	1000	427 [151–702]	786 [589–983]
Males	800	1000	645 [360–930]	922 [764–1080]
Reproduction	300	500	173 [16–332]	450 [293–607]
Juveniles	700	1000	701 [627–775]	859 [808–909]
Area				
Females	⩾1000	⩾1000	⩾1000	⩾1000
Males	⩾1000	⩾1000	⩾1000	⩾1000
Juvenile	700	1000	480	⩾1000

Note. Values enclosed in brackets are confidence intervals (5%) on the EC_{10}/EC_{50} estimates.

DISCUSSION

This study has demonstrated that *F. fimetaria* is affected by nickel-enriched soils in the laboratory at concentrations above 173 mg Ni/kg. The most sensitive toxicological parameter was reproduction, with survival being reduced only at higher soil nickel concentrations (e.g., 427 mg Ni/kg for females). Growth of adult springtails (measured as final size) was not affected at concentrations up to 1000 mg Ni/kg, while juvenile growth was reduced above 480 mg Ni/kg.

Although the toxicological effect of various metals has been reported for Collembola, to the authors' knowledge the present results are the first reports on the effects of nickel on springtails. The toxicological levels found in the present experiment are somewhat higher than for

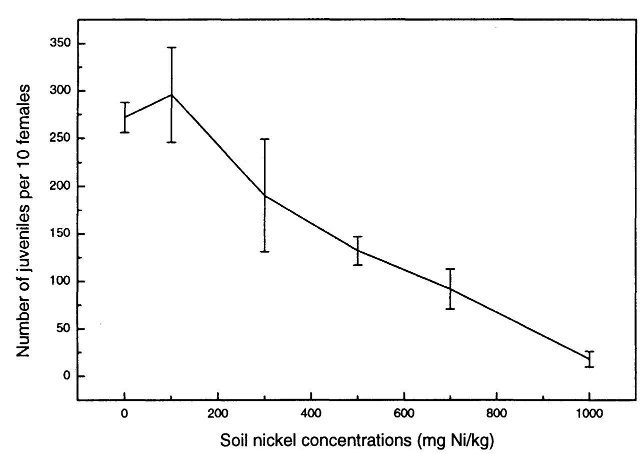

FIG. 3. Number of juveniles (mean ± SEM) per 10 female Collembola, *Folsomia fimetaria*, following exposure of 20 adult Collembola (10 females and 10 males) to increasing soil nickel concentrations (mg Ni/kg) for 21 days. Each point is based on four replicates.

earthworms exposed under similar conditions. For example, exposing the earthworm *Eisenia veneta* to nickel under similar conditions, Scott-Fordsmand *et al.* (1998) measured a 10% reduction of reproduction at 85 mg Ni/kg, somewhat lower than the lowest effect level found in the present experiment. The levels of effect measured for the collembolan *F. fimetaria* when exposed to soil nickel are in general higher than effects caused by copper, using the same type of salt (chloride) and the same experimental design (Scott-Fordsmand *et al.,* 1997), indicating that nickel is less toxic to springtails than copper. Similar differences were observed for earthworms (Neuhauser *et al.,* 1985).

Differences in the overall sensitivity between various measured end points in studies conducted with soil organisms are important when ecotoxicological risk assessment is required for a particular soil media, as discussed by, e.g., Scott-Fordsmand *et al.* (1997). If the most sensitive end point (which should be relevant at a population level) is not included, the risk assessment will not provide a reliable assessment of the harm to the animals. In accordance with observed differences between toxicological end points for other metals on springtails (Crommentuijn *et al.,* 1993, 1995b; Scott-Fordsmand and Bruus Pedersen, 1995), the most sensitive toxicological end point for the effects of nickel on springtails was reproduction. The difference in sensitivity between reproduction and mortality of a factor of two to three was similar to that observed for the earthworm *Eisenia fetida* exposed to nickel under similar conditions (Scott-Fordsmand *et al.,* 1998). Similar, or slightly higher, differences in sensitivity between these two end points have been observed for the effect of other metals on springtails (Crommentuijn *et al.,* 1993, 1995a; Jepson *et al.,* 1996). Larger differences, up to a factor of 20, between mortality and reproduction have been recorded for copper (Scott-Fordsmand *et al.,* 1997) and cadmium (van Gestel and van Diepen, 1997). Van Straalen *et al.* (1989) found female growth to be more sensitive than both reproduction and mortality when feeding *Orchesella cincta* cadmium-contaminated food. In agreement with previous results obtained for the effect of copper on springtails, the final size of juveniles was more sensitive than adult size, which may reflect the higher growth rate of juveniles (Folker-Hansen *et al.,* 1996). A higher growth rate will inevitably result in a higher metabolic turnover and as a consequence a larger risk of incorporating the nickel into enzymes and nucleic acid, which normally contain other metal ions. On the other hand, females which produce eggs may also have a high metabolic rate but their growth was not affected. One further factor involved in this could be that nickel was added as a chloride salt, which at high nickel concentrations could result in an enhanced toxic effect of chloride. If juveniles are more sensitive to chloride than adults, for example due to a relatively larger surface area of the former, this may also explain some of the differences observed between juveniles and adults.

The effect of nickel on reproduction may in the present experimental design be due to a number of factors, i.e., reduced egg-laying of females, reduced hatchability of eggs, or reduced survival of juveniles hatched. The fact that the effect level for the reproductive output is much lower than the effect of juvenile survival indicates that the effect of nickel is acting via either reduced egg-laying or reduced hatchability, but not via decreased juvenile survivability. Although the mode of action may vary between metals and species, observations on *Onychiurus armatus* have demonstrated that when fed copper-, copper/lead-, or copper/zinc-contaminated fungi the reduced reproduction is due to a reduced number of eggs rather than the ability of the eggs to hatch. A similar mode of action may also be the case for the effect of nickel on reproduction in *F. fimetaria* (Bengtsson *et al.,* 1985; Tranvik *et al.,* 1993).

In a field population, the number of springtails may be affected not only by a reduction in the production of juveniles and the number of adults within the population, but also by a reduced growth of juveniles. Reduced growth of juveniles, as observed in the present experiment, may delay maturity of the springtails which may have consequences for the lifetime reproductive output (Bengtsson *et al.,* 1985; Crommentuijn *et al.,* 1993).

CONCLUSIONS

Exposure of the collembolan *F. fimetaria* L. to nickel via soil caused significant toxicological effects at concentrations above 173 mg Ni/kg. The reproductive output was the most sensitive parameter being reduced at soil nickel concentrations above 173 mg Ni/kg (EC_{10}). A 10% decrease in adult female numbers occurred at 427 mg Ni/kg and at 645 mg Ni/kg for adult male numbers, while a 10% reduction in juvenile numbers was detected at 701 mg Ni/kg. The corresponding EC_{50} values were 786 mg Ni/kg for females, 922 mg Ni/kg for males, and 859 mg Ni/kg for juveniles. Adult growth was not affected by soil nickel concentrations up to 1000 mg Ni/kg, but juvenile growth was reduced at concentrations above 480 mg Ni/kg (EC_{10}).

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