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Toxicity of Copper to *Porcellio scaber* Latr. (Isopoda) Under Different Nutritional Status

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Isopods are members of the soil fauna important in decomposition. Their role in the decomposition of leaf litter in forest soils has been reported for many years (Gere 1956; Griffiths et al. 1989). Isopods are reported to contain high concentrations of copper in their haemolymph (70 mg/ml) and in hepatopancreas (216 mg/g d.w.) (Gunnarson and Hedlund 1987). The role that copper plays in the physiology and ecology of terrestrial isopods was first examined in detail by Wieser (1966, 1968), Dallinger (1977) and Dallinger and Wieser (1977). Furthermore, Wieser (1966, 1968) and Wieser et al. (1976) showed that copper concentrations of terrestrial isopods reflect the degree of environmental soil and litter contamination. In the last decade, pollution of the environment with heavy metals has led many environmental scientists to search for suitable methods to monitor distribution and effects of such pollution. Laboratory tests using terrestrial isopods are recommended for assessing the ecotoxicological effects of chemicals (Drobne and Hopkin 1994). Isopods are able to accumulate large amounts of several metals in their hepatopancreas (Hopkin 1989, 1990) therefore they are useful biological indicators of metal pollution (Dallinger et al. 1992). Because of its worldwide distribution and the high metal accumulation capacity, Hopkin et al. (1993) proposed that *Porcellio scaber* could be a suitable "bioindicator" of metal contaminated soils.

In spite of the fact that copper is an essential metal in isopods, it may become toxic if it is highly concentrated in the environment (Dallinger 1977; Dallinger and Wieser 1977), or released from the degraded haemocyanin (Gunnarson and Hedlund 1987) during a long period of starvation. The accumulation, metabolism, storage, detoxication and excretion of copper in isopods have been examined previously (Hopkin 1989). However, detailed information on the sublethal effects of copper in isopods and the influence of the nutritional status on the copper toxicity are sparse.

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In this paper we describe the effects of high concentrations of copper on consumption, growth rates and on reproduction correlates (gravids/females, offsping/females) applied in different foods to *Porcellio scaber* using a standard laboratory method.

MATERIALS AND METHODS

Parent specimens of *Porcellio scaber* were collected from an uncontaminated area around Balatonfenyves (Hungary) in 1993. They and their offspring were cultured under standard laboratory conditions (20° C, 16 hr light and 8 hr dark period, 100% relative humidity) in plastic boxes on artificial soil (OECD standard 1984). High temperature and long light period is used to facilitate development and maintain reproductive processes (Hornung and Warburg 1993; Hopkin and Hames 1994). The toxic endpoints were: mortality: food consumption, growth and reproduction. Food consisted of a mixture of ground maple leaf litter (*Acer platanoides*), potato powder, and commercial rabbit food (50%: 10% :40% w/w) pressed into pellets. Maple leaves were collected from an uncontaminated area in autumn. For examination of the food quality effect on the food consumption and on growth, some isopods were fed on maple leaf litter exclusively.

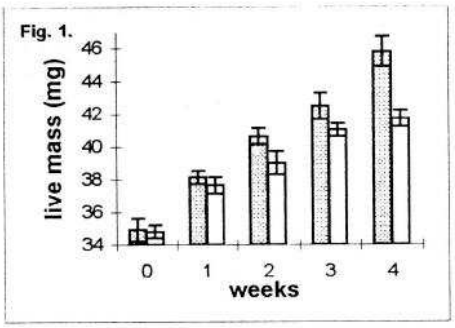
Two hundred preadults, 30-35 mg live mass, were separated into 30 replicates for feeding experiments. Half of them were fed on pellets, the other half on ground maple leaves. Cupric chloride ($CuCl_2 x\ 2\ H_2O$) used as toxicant was introduced via food in concentrations of 0 (control), 100, 500, 1000, and 5000 mg/kg d.w. Untreated maple leaves contained 26.7 mg/kg, and untreated food pellets 28.2 mg/kg copper, measured by an energy-dispersive X-ray emission spectrometer.

For reproduction studies. females were cultured with males fed on control and contaminated food pellets. After marsupial moult, gravid females (10 specimens in each treatment) were separated and kept individually until they released their young. Mass of females and number of juveniles were recorded. Both percentage of gravid females in the original cultures and rate of females successfully releasing their juveniles were recorded.

Data were statistically evaluated using ICP (Norberg and King 1993) TSK (Hamilton 1989) TOXSTAT (Gulley Boelter and Bergman 1988) programs. To make reproduction data comparable, numbers of juveniles were standardized for 100 mg female live mass.

RESULTS AND DISCUSSION

Growth rates of woodlice were much higher when fed with complex food pills than ground *Acer* leaves (Fig. 1). Similar growth rates to those we fed with food pellets were describedfor *P. spinicornis* (McQueen and Carnio 1974) fed on powdered rabbit food, goldenrod leaves and leaf litter. Stöckli (1990) found that *Acer* leaves provide ideal nourishment for isopods only if well colonized by fungi. This may be



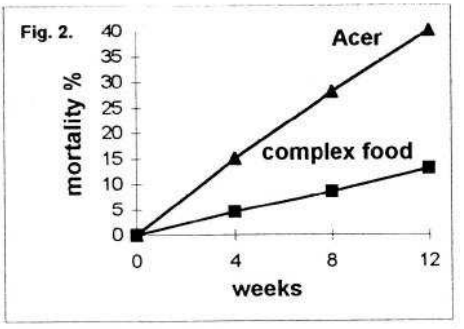


Figure 1. Effect of food quality on body mass gain of *Porcellio scaber.* Dotted columns: complex food; empty columns: fallen *Acer* leaves. There is significant difference from the 4th week. (N = 2 x 50, P = 5%. t-test) **Figure 2.** Effect of food quality on mortality of *Porcellio scaber.* Triangles: the mortality of the group fed on *Acer* leaves; squares: the mortality of the group fed on complex food. There is significant difference between the mortality of the two groups from the 4th week. (N = 5 x 10 in both groups, P = 5%, t-test)

the reason of the very slow weight increase in the case of *Acer* leaves food. It is very important for correct evaluation of toxication experiments, to establish that the spontaneous mortality of woodlice, especially of the larger ones increased when complex food was changed for a long period with ground Acer leaves (Fig. 2).Copper contamination resulted in higher mortality (see LC50 values in Table 1) when introduced via maple leaves compared to food pills. Hopkin and Hames (1994) found 100% decay of the same species during a 3 month experiment, before reaching reproductive age, when fed with *Acer* leaves containing 100 mg/kg added copper and 121 mg/kg natural zinc. They used solution of copper nitrate, applied topically to the leaves as small droplets and allowed to dry. In our copper toxicity trials 48.4% of the woodlice survived 3 months when fed with 500 mg/kg, and 30.4% survived when fed with 1000 mg/kg copper content in their *Acer* leaf food. This great difference between the data of Hopkin and Hames (1994) and our present data on the lethal level of copper may be explained either by the different treatments of the food and/or by the different origin and genetic variation of the used isopods.

Woodlice consumed more *Acer* leaves than complex food pellets (Fig. 3.). This difference could be explained by the fact, that wodlice are hyperphagous when feeding with litter not in an advanced state of decay (Dallinger and Wieser 1977) i.e. with food of low nutritive value. However, food consumption rate markedly decreased at 500 mg/kg copper content of food in both cases. Dallinger and Wieser (1977) used overwintered birch litter as food, and found a diminished ingestion rate at 340 mg/kg copper content.

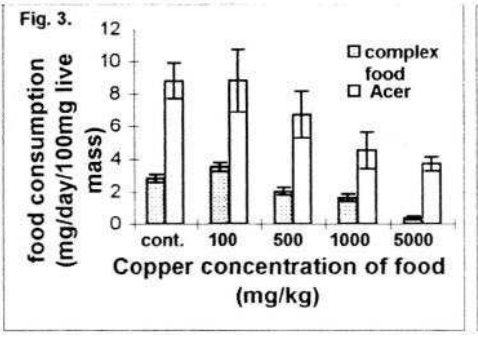
Table 1. Acute (LC) and sublethal (EC) effects of copper on *Porcellio scaber* Latr.

Output parameter	Type of the food in the experiment	Duration of the experiment (weeks)	Effective copper concentration (mg/kg)	
mortality	complex food	8	LC 50	2880
	Acer leaves	8	LC 50	1117
body mass gain	complex food	4	EC 10	349
	complex food	4	EC 50	2421
	Acer leaves	4	EC 10	45
	Acer leaves	4	EC 50	263

Hopkin and Hames (1994) described a discrepancy between the laboratory experiments and field observations, as populations were able to survive 5000 mg/kg zinc content in the field, while 1000 mg/kg was the critical concentration in the laboratory at which all isopods died before reproducing offspring. Our copper toxicity experiments showed that body mass gain could be retarded much more by increasing concentrations of copper when feeding isopods on *Acer* leaves (Fig. 4. and Table 1). The reason for such discrepancy may be the lower amount of ingested copper-enriched food pellets than the Acer leaves containing the same amount of copper and/or the low nutritional value of Acer leaves. The resistance of isopods against toxicants also can be higher in the field when they can find their preferred foods (Nair et al. 1994).

Under the described laboratory conditions, reproduction appeared at the age of 3-4 months, at a mass of about 40 mg. Donker (1992) and Donker et al. (1993) also found early reproduction in the same species collected from mine or smelter sites. In their studies, early reproduction and increased reproductive allocation could result from genetic selection. In our experiments, isopods were not previously exposed to any toxicants. Early gravidity occurred also in the control group and may have resulted in by the favorable laboratory conditions and food quality. A 25 °C temperature and 18 hr light regime accelerated the reproductive processes compared with lower temperature and shorter light period reported by Hornung and Warburg (1993).

One hundred mg/kg of additional copper in food resulted in a significant increase in percentage of gravid females (Table 2). In the present study reproduction occurredat each copper concentration. The number of offspring was the highest in the control group and differed significantly among all the treatments (t-test, Bonferroni adjustment, Log_{10} transformation). There were significant differences among treatments in the number of births. Not all of gravid females



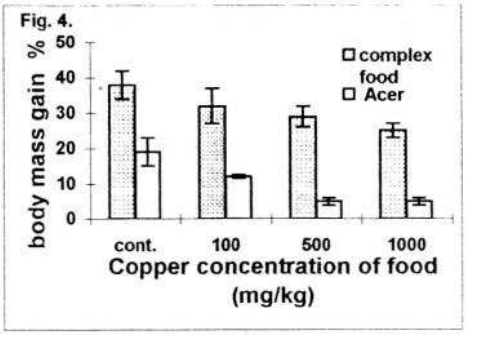


Figure 3. Effect of copper on foodconsumption of *Porcellio scaber.* Dotted columns: fed on complex food; empty columns: fed on *Acer* leaves. (N = 5 x 50, P = 5%, t-test.) There were significant differences at each concentration. **Figure 4.** Effect of copper on body mass gain of *Porcellio scaber.* Dotted columns: mass gain of the group fed on complex food; empty columns: mass gain of the group fed on *Acer sp* leaves. (Duration: 4 weeks, N = 5 x 10 in both groups, P = 5%, t-test) There were significant differences at each concentrations

Table 2. Reproductive correlates of *Porcellio scaber* at different copper concentrations in the food. (standard deviation in brackets)

Copper concentration in food (mg/kg)	control	100	500
% gravid females	33	43	31
average mass (mg) of females	63.53 (7.86)	58.29 (9.42)	50 (5.66)
average no. of juv./ female	47 (6.6)	26 (12.36)	25 (10.08)
average no. of juveniles/100mg female	74	45	49

gave birth to juveniles. Reproduction rate of succesfully breeding females was the highest at 100 mg/kg (100%), and lowest at 1000 mg/kg (10%). Isopods under stressful conditions showed increased oosorption resulting in less juveniles (Hornung and Warburg 1994). Metal pollution can be an environmental stress on soil animals.

Some laboratory test methods with isopods used only leaf litter as food (Drobne and Hopkin 1994, Hopkin and Hames 1994) but in long-term breeding methods addition of rabbit food (Donker et al. 1993) or pet food (McQueen and Carnio 1974) to leaf litter is recommended. Our results showed that woodlice tolerated higher copper concentrations in their food, expressed lower mortality and higher growth rate, when fed on complete food. One hundred mg/kg of added copper to the food resulted in higher percentage of gravid females and number of births. These observations agree with the results of Donker et al. (1993), showing that

isopods from mine and smelter sites reproduced earlier and at lower weight than controls. However, the total number of released larvae of isopods fed with 100 mg/kg of added copper in their food was significantly lower when compared to controls. This may be explained by the increased oosorption, i.e. the disruption of oocytes within the ovarium during vitellogenesis, resulted in by the gradual accumulation of copper during the breeding period. Reproductive efficiency studies showed that more than 500 mg/kg copper in the food may have fatal effect on the isopod population. Consequently, beside the determination of accumulated metals in the body of Porcellio scaber, the analysis of their local population size alternations may contribute to application of this species in pollution biomonitoring system.

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