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The effects of cadmium, copper, lead, and zinc on the growth and reproduction of *Proisotoma minuta* Tullberg (Collembola)

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Abstract

Laboratory studies were conducted to evaluate *Proisotoma minuta* sensitivity toward selected heavy metals (Cd, Cu, Pb, and Zn). The experimental results showed a reduction in adult survival and no reproduction at the highest concentrations of Cd and Zn. Application of Pb at all levels resulted in large numbers of progeny and no significant mortality compared to controls. $EC_{50\,\text{reproduction}}$ values for Cd, Cu, and Zn were 125, 696, and 283 $\mu g\,g^{-1}$, respectively. No significant difference in time from the introduction of adults into the test soils to the appearance of the first-instar animals was observed between different metal treatments. The growth rate of adults decreased for all metal treatments compared to the controls. It is suggested that the accumulation of metals in *P. minuta* affects metabolism and results in a slower growth rate. The absence of any statistically significant effect on mortality at all concentrations of Pb may be due to greater tolerance of *P. minuta* to Pb than to other metals.

Keywords: *Proisotoma minuta*; Collembola; Cadmium; Lead; Zinc; Copper

1. Introduction

Soil contamination in both rural and urban environments in Australia has mainly resulted from anthropogenic activities. Agrochemicals contribute largely to soil contamination in rural areas, while municipal and industrial wastes have been the major sources of urban soil contamination (Barzi et al., 1996). Heavy metals appear to be the most common soil contaminants and have contaminated at least two-thirds of urban sites examined in a survey including sites in South Australia, Victoria, and Queensland (Tiller, 1992).

In agricultural areas, most metal contamination in soil has occurred as a result of the application of inorganic fertilizers and sewage sludge. Australian soils are strongly weathered and leached, so phosphatic fertilizers have been used to sustain agricultural production (Wild, 1958). In Australia the input of Cd from fertilizer usage has been estimated to be approximately 1.6 $g\,ha^{-1}\,yr^{-1}$ (Sumner and McLaughlin, 1996). Traditionally, sewage sludge was disposed of in the ocean. However, this practice is largely prohibited nowadays. The application of sewage sludge on agricultural land has been reported as the most cost-effective method in European countries. The adaptation of this method may improve the physical properties of low-fertility Australian soils (Whatmuff, 1996). Depending on its source, sewage sludge may contain large quantities of heavy metals that may enter into the food chain and be potentially harmful to plants, livestock, and humans. The Cd, Pb, Zn, and Cu concentrations in Sydney sewage sludge used as fertilizer have been reported as 3.9, 140, 839, and 1427 $mg\,kg^{-1}$, respectively (Ross et al., 1991). High uptake of Cd, Zn, and Cu was reported in vegetables grown on sewage-sludge amended soils at pH 4.2–4.8 in New South Wales, Australia, in the ranges 0.05–22 mg Cd kg^{-1}, 1.82–4627 mg Zn kg^{-1}, and 7.9–202 mg Cu kg^{-1}, respectively (Whatmuff, 1996). In another New South Wales study Cresswell et al. (1996) found that the uptake of metals by vegetables was 2.39 $mg\,kg^{-1}$ Cd in outer leaves of lettuce, 3.71 $mg\,kg^{-1}$ Pb in eggplant leaves, and 308 $mg\,kg^{-1}$ Zn in inner leaves of lettuce grown on soil amended with composted

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sludge containing Cd, Pb, and Zn (0.12, 49, and 35 mg kg^{-1}, respectively).

In some urban and rural areas metal contamination has resulted from mining activities dating from the first extraction of coal at Newcastle in 1796. Australia has many existing (e.g., Woodlawn in the Hunter Valley, NSW) and abandoned (e.g., Captains Flat, Sunny Corner, Broken Hill in New South Wales, Brukunga in South Australia, Mount Morgan in Queensland, Rum Jungle in the Northern Territory, and Mount Lyell in Tasmania) mine sites that have caused contamination problems. The composition of waste rock dump leachate from the abandoned Mount Lyell gold mine contains 130 mg L^{-1} Cu (Wood, 1991). Elevated concentrations of Cu and Pb (312 and 1140 mg kg^{-1}) were found in tailings at the abandoned Rum Jungle mine, Northern Territory, Australia, and at Woodlawn the waste rock dump leachate contained Cd, Zn, and Cu (24, 4980, and 88 mg L^{-1}) (Taylor, 1996). It is important to understand the impact of heavy metals from various sources on soil health in relation to agricultural production. Tests for soil contamination have been developed, which include the impact of heavy metals on soil invertebrates, including Collembola (Van Straalen and Krivolutsky, 1996).

Collembola and other soil fauna play an important role as decomposers of soil and leaf litter. They feed directly on decaying materials and soil fungi and may give an earlier indication of ecosystem disturbance than predators (Cole et al., 2001). In addition, the collembolan fecal pellets are broken down by microbes and slowly release essential nutrients to plant roots (Hasegawa and Takeda, 1995). In a study in south west Finland, it was found that the accumulation of heavy metals impacted on the soil microbiota and resulted in a decrease in the rate of decomposition and litter quality of Scots pine fine root and needle litter (McEnroe and Helmisaari, 2001). There are also cases where Collembola appeared to be tolerant and resistant to pollutants even at high levels of contamination. Dunger (1989) showed that some species of Collembola can be primary colonizers in disturbed sites, as shown in rehabilitated coal-mined areas in Germany, where Collembola were dominant from the pioneer period up to the 10th year of rehabilitation. In Australia, *Proisotoma minuta,* a cosmopolitan species, is also found abundantly in some rehabilitated mine sites in southern Western Australia, as well as in cotton-growing soils in New South Wales where crops have been treated with a range of insecticides (Greenslade and Vaughan, 2003; Park and Lees, 2004).

Many studies have been conducted in relation to Collembola sensitivity and resistance to pollutants in populations exposed to long-term contamination of soils, in particular to heavy metals (Posthuma, 1990; Posthuma et al., 1992; Tranvik et al., 1993). The study of bioaccumulation of chemicals by Collembola is important for forecasting food-chain transfer of pollutants at different trophic levels of organisms. Collembola are also known as an ideal test animal for ecotoxicological research. They have been used in different kinds of experiments in particular on the effects of environmental contaminants such as pesticides, soil fumigants, acid rain, heavy metals, fertilizers, radiation, PAH, and PCBs on animals (Wiles and Krogh, 1998). However, most studies that have been published on the effects of heavy metals on Collembola in soils are from European countries, and to our knowledge there are scarce reports on the effects of heavy metals on *P. minuta* in Australian soils.

The risk assessment of chemicals for ecosystems is generally based on the results of laboratory toxicity tests. Extrapolation methods have been developed from the results of these tests to derive safe levels of chemicals in soil ecosystems (Van Gestel, 1997). In metal risk-assessment procedures, most toxicity and uptake tests with soil organisms have been conducted with soft-bodied oligochaete species, which are known to accumulate metals through the soil solution (Vijver et al., 2001). In contrast with earthworms' soft permeable epidermis, Collembola use their cuticle and ventral tube to maintain their water balance actively (Hopkin, 1997). However, uptake kinetics and accumulation for heavy metals vary among species and with metal bioavailability in soils. *Folsomia candida* has also been selected as a standard animal for toxicity tests conducted under laboratory conditions (ISO, 1999) and most European metal accumulation studies of Collembola have used *F. candida*. In Australia, the occurrence of *F. candida* is very rare. Two Australian species that are widely distributed in nature are *Sinella communis* and *P. minuta* (Greenslade and Vaughan, 2003).

The objectives of the current research were to study the effects of four heavy metals commonly occurring in Australian sewage sludges (Cd, Cu, Pb, and Zn; Singh, 2001) in contaminated soils on the growth and reproduction of *P. minuta* in an acid soil from New South Wales, Australia.

2. Materials and methods

2.1. Test organism

A layer of 2–3 cm of a plaster of Paris and charcoal mixture (5:1, v:v) mixed with nanopure water (Singh and Moore, 1985) was placed in 200-mL glass jars (6 cm diameter, 9.5 cm height) with plastic screw-on lids. The mixture was allowed to stand for a few days at room temperature until it was solid and well dried. As the mixture solidified the surface was smoothed to prevent the appearance of fissures and holes.