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Toxicokinetics and toxicodynamics of lead in the soil invertebrate *Enchytraeus crypticus*☆

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ABSTRACT

The aim of the present study was to link Pb toxicokinetics to toxicodynamics in *Enchytraeus crypticus*. The enchytraeids were exposed for 14 d to different Pb concentrations (uptake phase) in natural Lufa 2.2 soil, followed by a 14-d elimination phase in clean soil. Pb accumulation and enchytraeid mortality were determined at different time intervals. At each exposure concentration, internal Pb concentration increased with exposure time and achieved equilibrium in approximately 7 d. Median lethal concentration (LC50) based on total Pb concentration in soil decreased with exposure time, but did not reach a steady-state level. Pb toxicity, therefore, showed a delay compared to accumulation in *E. crypticus*. LC50s based on internal Pb concentrations in the surviving animals did reach steady state in approx.14 d, suggesting that linking toxicokinetics to toxicodynamics may reduce the effects of time. This study highlighted that exposure time, as an important factor in metal uptake and toxicity, should be taken into account in ecotoxicological tests for risk assessment.

1. Introduction

With intense industrialization and urbanization, metal pollution has become a significant environmental problem worldwide. In particular, heavy metals in soil have attracted great attention due to their non-biodegradability, posing a potential risk to terrestrial ecosystems. Among heavy metals, lead (Pb) has become widespread in the environment as a result of human activities. Anthropogenic lead primarily originates from industrial processes (i.e. mining, smelting), use (i.e. batteries, pigments, bullets, mineral fertilizer), combustion of fossil fuels, waste incineration, and sewage sludge application. Lead is a nonessential element, having no known physiological functions for animals, plants and microorganisms, and is acknowledged for its high toxicity (Fisher et al., 2006). High Pb concentrations may affect the survival and reproduction of soil invertebrates like earthworms, springtails and enchytraeids (Langdon et al., 2005).

It is commonly acknowledged that the dose determines the toxicity of a chemical. Usually, to assess the environmental risk of a metal in soil, toxicity tests with microorganisms, plants and soil invertebrates are performed with a fixed exposure time to develop dose-effect relationships for selected endpoints. Such traditional ecotoxicological tests relate the resulting toxicity to an external concentration. However, the potential risk of metals in soils depends on their bioavailability rather than on total concentrations. Bioavailability is defined as the fraction of metal in the environment that is available for uptake, leading to adverse effects on organisms (Peijnenburg, 2004). Therefore, to better understand the risk of metals in soil, the relationship between metal toxicity to organisms and its bioavailability in soil should be investigated. Accumulation of metals in organisms could be a good predictor of metal bioavailability in soil, as internal concentrations reflect the actual exposure in the environment. Internal concentrations however, do not only depend on exposure concentration but also on exposure time. Spurgeon and Hopkin (1999) found that internal metal concentrations increased with exposure time until reaching a steady state, when earthworms were exposed to contaminated soils. Mortality of organisms is observed when the internal concentration exceeds a certain threshold (lethal body concentration), which represents a physiological limit (Jager et al., 2011). When the metal uptake rate exceeds the elimination rate, the organism will slowly accumulate the metal, potentially leading to toxic effects in the long run. In such case, no or little toxicity could be observed in the traditional ecotoxicological tests when exposure time is too short to reach equilibrium. He and Van Gestel (2013) found that

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metal toxicity was time-dependent, with median lethal concentration (LC50) for the toxicity of nickel to the enchytraeid *Enchytraeus crypticus* decreasing with time. Time, therefore, should be a vital factor taken into account in toxicity tests.

Van Straalen et al. (2005) also highlighted that instead of body concentration in an organism, metal uptake rate was the superior predictor for bioavailability. Thus, to simulate the time course processes from metal accumulation to causing toxic effects on organisms, simultaneous assessment of toxicokinetics-toxicodynamics might be a better approach to quantify metal toxicity (Ashauer and Escher, 2010). Toxicokinetics translates the external concentration of a metal into an internal metal concentration in an organism over time, including uptake, body distribution, transformation or sequestration and elimination. Toxicodynamics describes the development with time of the toxic action at target sites or active sites, or the resulting subsequent adverse effects at the level of the organism (e.g. effects on survival, reproduction or growth), quantitatively linking the internal metal concentration to toxic effects on organisms. Toxicokinetics-toxicodynamics therefore can provide a mechanistic understanding of the processes of exposure, accumulation, depuration and toxic effects, linking mortality or sublethal endpoints to internal concentration, taking into account the factor of time.

Widely distributed in different soils, Enchytraeids (class Oligochaeta, family Enchytraeidae) play a key role in the functioning of terrestrial ecosystems. As soft-bodied organisms, enchytraeids could be exposed to different stress factors in soil, both via the soil solution and the solid phase. They therefore are recommended as suitable test species in soil ecotoxicology (Didden and Römbke, 2001). Among Enchytraeids, *Enchytraeus crypticus* has been demonstrated to be a useful model species for soil toxicity tests, because of its short generation time, good control performance and wide tolerance to distinct soil properties (e.g. pH, texture, organic matter content) (Castro-Ferreira et al., 2012).

This study investigated the development of Pb bioaccumulation and toxicity with exposure time in *E. crypticus* in a Pb-amended natural soil. Our aims were: (1) determining the kinetics of Pb uptake and elimination in *E. crypticus* at different soil Pb concentrations (toxicokinetics), (2) investigating the development of Pb toxicity with exposure time (toxicodynamics), and (3) linking toxicokinetics (Pb bioaccumulation in time) to toxicodynamics (survival in time).

2. Materials and methods

2.1. Test organism

Enchytraeus crypticus (Enchytraeidae; Oligochaeta; Annelida) has been cultured for several years at the Vrije Universiteit, Amsterdam. The worms were kept on agar prepared with an aqueous soil extract, in a climate room at 16 °C, with 75% relative humidity, and in complete darkness. The animals were fed twice a week with a mixture of oat meal, dried yeast, yolk powder, and fish oil (Castro-Ferreira et al., 2012). Adult *E. crypticus* of approximately 1 cm with white spots in the clitellum region were selected for the tests.

2.2. Test substrates

As a natural standard soil, Lufa 2.2 was chosen to be the test soil, obtained from the LUFA Institute (Landwirtschaftliche Untersuchungs-und Forschungsanstalt) at Speyer, Germany. The soil had a nominal pH-0.01 M $CaCl_2$ of 5.49, 3.5% organic matter, 12% clay and a Cation Exchange Capacity (CEC) of 9.10 cmolc/kg. To obtain nominal concentrations of 0, 100, 200, 400, 800, 1600 and 3200 mg Pb/kg dry soil, soil was spiked by adding aqueous solutions of $Pb(NO_3)_2$ (purity >99.99%; Sigma-Aldrich; USA). For each treatment, the soil was moistened to reach 50% of the maximum water-holding capacity, equaling a soil moisture content of 24% (w/w). The spiked soils were equilibrated for 14 d in a climate room at 20 °C before use in the tests.

2.3. Toxicokinetics and toxicodynamics tests

Pb uptake and elimination kinetics in *E. crypticus* exposed to the different test concentrations were assessed following OECD guideline 317 (OECD, 2010). After 14 d exposure in spiked soils (uptake phase), surviving adults were transferred to clean Lufa 2.2 soil for the 14-d elimination phase. The uptake phase was combined with the assessment of Pb toxicodynamics, and also included observations after 21 days of exposure. So, Pb uptake and toxicity were determined at seven time intervals (1, 2, 4, 7, 10, 14, 21 d), while six sampling times (15, 16, 18, 21, 24, 28 d) were used in the elimination phase for determining internal Pb concentrations. For each treatment and sampling time, ten worms were introduced into a 100 mL glass jar filled with 30 g moist test soil, and 2 mg oatmeal was added for food. The jars were covered with perforated aluminum foils and incubated at 20 °C, 75% relative humidity and 16-h light/8-h dark photoperiod cycle in a climate room. Food and soil moisture content were checked once a week and the water loss was replenished by adding deionized water. At each sampling time, three replicate jars were sampled for each test concentration, survival was determined and surviving adults were collected and transferred to petri dishes (100 mm × 15 mm) with 20 mL ISO solution (ISO, 2004a) for 24 h for gut cleaning. Subsequently, three animals from each replicate were frozen at −20 °C for Pb analysis.

2.4. Chemical analysis

Soil samples were dried at 40 °C for 48 h. To measure total Pb concentrations, soils were digested in a mixture of HNO_3 (65%, Sigma-Aldrich, USA) and HCl (37%, Sigma-Aldrich, USA) (4:1 v/v). Around 130 mg dry soil was mixed with 2 mL of the acid mixture in a tightly closed Teflon container and heated for 7 h in an oven at 140 °C. Total soil concentrations were measured by atomic absorption spectrometry (AAS; AAnalyst 100, Perkin Elmer, Germany). Quality of the analysis was checked by using the certified reference material ISE sample 989 (International Soil-Analytical Exchange), and the measured lead concentrations in the reference material were always within 10% of the certified concentration. The frozen worms were freeze-dried for at least 24 h, weighted individually and digested with 300 μL mixture of HNO_3 (65%; Mallbaker Ultrex Ultra-Pure) and $HClO_4$ (70%; Mallbaker Ultrex Ultra-Pure) (7:1 v/v) in a block heater (TCS Metallblock Thermostat) using a heating ramp ranging from 85 to 180 °C for 2 h. The lead concentrations in worms were measured by graphite furnace AAS (PinAAcle 900Z, Perkin Elmer, Germany). The certified reference material DOLT 4 (Dogfish liver, LGC Standards) was included for quality control and the Pb recoveries were 93.7%–102.2%.

2.5. Data analysis

Assuming that exposure concentration (mg Pb/kg dry soil) is constant, the development of internal concentration with time can be described by a one-compartment model (Crommentuijn et al., 1997):