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Effects of total cadmium and lead concentrations in soil on the growth, reproduction and survival of earthworm *Eisenia fetida*

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The effects of lead and cadmium on earthworm *Eisenia fetida* growth, reproduction and survival were evaluated following the OECD earthworm acute and reproduction toxicity protocols. The range of the test concentrations of Pb (20–2500 μgPb g^{-1} of soil) and Cd (1–100 μgCd g^{-1} of soil) was chosen to simulate the levels that are likely to occur in the environment under different scenarios (typical soil levels, levels in industrial or urbanized contaminated soil). Worms were exposed for 28 days to a single metal in standard OECD artificial soil, and the LC_{50} values for lead and cadmium could not be determined at the concentration ranges used. The risk of death of *E. fetida* increased with the concentration of lead ($\chi^2 = 28.64$, $p = 0.00001$), though there was no significant mortality at all Cd concentrations. The weight of earthworms declined in all treatments during the experiment. Earthworms' weight negatively correlated with lead concentrations in soil (14 days: $R^2 = 0.59$; 21 days: $R^2 = 0.58$; 28 days: $R^2 = 0.62$; $p < 0.05$). The weight of the earthworms exposed to cadmium tended to show a dose-related decrease, although a statistically significant decline was detected only in case of the highest concentration (100 μgCd g^{-1} soil). Cocoon production was more sensitive than mortality and growth. A regression analysis showed that cocoon production rate significantly decreased with increasing lead concentration in soil ($R^2 = 0.65$, $p = 0.0001$) and the EC_{50} value of 349 μgPb g^{-1} was estimated. Cd inhibited the cocoon production rate by 18–65%.

Key words: cadmium, earthworms, growth, lead, mortality, reproduction

INTRODUCTION

Metal pollution may disturb soil ecosystems by affecting the structure of soil invertebrate communities. Generally, the potential hazards of various environmental toxicants to soil invertebrates are assessed by bioassays with the keystone species – earthworms. Earthworms are one of the first receptors affected by soil contamination. They are more susceptible to metal pollution than many other groups of soil invertebrates, and toxicity data on earthworms are important in determining "safe levels" for metals and other contaminants in soil.

Heavy metals have been shown to cause lysosomal membrane instability, changes in gene expression, oxidative stress (Spurgeon et al., 2004a; Berthelot et al., 2008; van Gestel et al., 2009), to reduce growth (Spurgeon et al., 1994), to slow sexual development (Spurgeon, Hopkin, 1996; Spurgeon et al., 2004b), to reduce cocoon production and hatchability (Reinecke et al., 2001; Davies et al., 2003; Spurgeon et al., 2004a), juvenile viability (Bengtsson et al., 1986; van Gestel et al., 1992), to cause mortality (Neuhauser et al., 1985; Spurgeon et al., 1994; Davies et al., 2003) and affect the population size, abundance and species diversity of earthworms (Spurgeon et al., 2005). The effects are dependent on metal and soil parameters and on species. Earthworms, to a certain degree, can regulate the uptake of essential metals (such as Cu, Zn) from soil, although the regulation of non-essential metals (e. g., Cd, Pb) is probably less, if any (Morgan, Morgan, 1988; van Gestel et al., 1993; Nahmani et al., 2009).

Generally, mortality is not a very sensitive endpoint for predicting the effects on field populations. It is assumed that measures like LC_{50} (lethal concentration) are crude indices of biological effects. Sublethal effects, such as disturbances of

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growth and reproduction, are more suitable for evaluating the possible effects on populations and communities (Moriarty, 1999). Suborganism level endpoints usually respond at lower levels of contaminants than higher organization endpoints, but the endpoints in the upper level of biological organization (population, community and ecosystem) may be in the same order of sensitivity (Spurgeon et al., 2005).

Growth is an integral parameter easy to measure, and it integrates a suite of biochemical and physiological effects. It represents changes in individual energy budgets as the exposed organisms have to expand their energy to metabolism, detoxification or sequestration, and excretion of the contaminants. This additional energy requirement results in a decrease of growth. The growth and reproduction of earthworms exposed to metals were negatively correlated, i. e. if the earthworms did not grow, they produced cocoons, and *vice versa*, if they did not produce cocoons, they continued growing (Burgos et al., 2005).

The earthworm reproduction test is generally considered to be more sensitive than the acute toxicity test because it can evaluate the effects of sublethal concentrations of chemicals far below a given LC_{50} concentration for earthworms. Furthermore, the earthworm reproduction test is widely regarded to be of greater significance for predicting the impacts on soil ecosystems. Reproduction is very important in ecotoxicological assessment as it influences the population dynamics. Reproduction rate was shown to be a sensitive endpoint in toxicity tests of various metals and organic compounds (such as hydrocarbons, PCB, pesticides) (Van Gestel et al., 1989; Hubálek et al., 2007).

Earthworm acute and chronic toxicity tests are used to assess the potential hazards of various environmental pollutants to soil ecosystems, as well as in risk assessment procedures. Earthworms, especially the compost worm *Eisenia fetida*, are model organisms for assessing the effects of various chemicals on terrestrial invertebrates (Spurgeon et al., 2003; Nahmani et al., 2007). Earthworm survival and reproductions tests are relatively easy to conduct, require little maintenance and are low-cost. Acute and chronic earthworm toxicity tests were successfully applied to assess the toxicity of contaminated soils in urban areas (Pižl, Josens, 1995; Jager et al., 2005), in mining and smelting areas (Tervihuo et al., 1994; Hønsi et al., 2003; Alvarenga et al., 2008), in industrial (Saterbak et al., 1999) and other areas (Berthelot et al., 2008; van Gestel et al., 2009). Earthworms are also frequently used in various mesocosms and test batteries (Gunderson et al., 1997; Römbke et al., 2006). Screening of contaminated soils is conducted using and combining field and laboratory tests. In Lithuania, previously the earthworm test had been applied to assess the toxicity of sewage sludge compost (Eitminavičiūtė et al., 2001). The main aim of the present study was to determine the effects of different total cadmium and lead concentrations on earthworm *Eisenia fetida* growth, reproduction and survival.

MATERIALS AND METHODS

The toxicity test was carried out according to the OECD guidelines for the testing of chemicals (OECD, 1984; 2004) with earthworm *Eisenia fetida*. The effects on their mortality, growth and reproduction rate were determined.

The artificial soil of the following composition (by dry weight) was prepared: 70% of quartz sand (more than 50% of the 0.05–0.2 mm particles), 20% of clay and 10% of *Sphagnum* peat. The soil pH_{KCl} was adjusted to 6.0 ± 0.5 with powdered calcium carbonate ($CaCO_3$). The constituents of the artificial soil were air-dried, mixed thoroughly and weighed (500 g) into plastic boxes. The dry soil was moistened with distilled water to obtain approximately half of the final required water content. Solutions of lead nitrate ($Pb(NO_3)_2$) and cadmium chloride ($CdCl_2 \times 2.5\ H_2O$) were mixed with the soil to obtain the final required water content (45–50%) and metal concentrations in soil. The same volume of distilled water was added to the control. The concentrations of metals in soil (in $\mu g\ g^{-1}$ of soil): lead – 20, 250, 500, 1000, 2500; cadmium – 1, 2, 5, 10, 100. Three replicates were used for each test and control.

The range of Pb and Cd concentrations was chosen to mimic the levels of these metals in typical non-contaminated and in industrial or urbanized contaminated soil (Morgan, Morgan, 1988; Pižl, Josens, 1995; Adomaitis, Mažvila, 2001; Eitminavičius et al., 2001; Grigalavičienė, Rutkovienė, 2006; Ernst et al., 2008; van Gestel et al., 2009). The emphasis was on the situation in Lithuania as here the concentrations of heavy metals in soil are lower than in other more industrialized European countries.

The test worms were taken from a breeding culture kept at the laboratory of the Vytautas Magnus University. All selected worms were adult and fully scitellate. The selected worms were acclimated for 7 days. The mass of an individual worm varied between 500 and 660 mg. Ten washed and weighed worms were added to each container, and after 3 hours they were checked to ensure that all the worms had burrowed into the soil. All containers were covered to prevent water loss.

The experiment was run for 28 days at 20 °C under continuous illumination (600 lux). Mortality and growth were measured on a weekly basis (every 7 days) by counting and weighing the surviving worms in each container. The worms were considered alive if they were able to respond to a mechanical stimulus. Cocoons were collected by sorting through the soil after 28 days. The number of cocoons was compared to the survival data to get a cocoon production rate, i. e. cocoons per worm per week (cocoons / worm / week).

A comparison of weight and cocoon production between treatments was made using the Mann–Whitney U test and Student's t test. For the reproduction, EC_{50} value was determined by regression analysis. Cocoon production rate averages were converted to percentages (reduction in cocoon production rate) and then plotted against concentration.

RESULTS AND DISCUSSION

Data on the mortality of earthworm exposed to different concentrations of lead and cadmium in soil are presented in Fig. 1. Earthworms survived in the control artificial soil during the whole test period. *E. fetida* response was more pronounced to lead than to cadmium.

There was no significant mortality of earthworms at all the Cd concentrations tested. The good survival of earthworms exposed to cadmium may be explained by detoxification of cadmium by cadmium-binding metallothioneins (Morgan et al., 1989; Stürzenbaum et al., 2004). No significant decrease in the survival of other earthworms representative of *Lumbricus rubellus* after exposure to cadmium (5–200 μgCd g^{-1} soil) was observed by Burgos et al. (2005). Spurgeon et al. (1994) determined a 56-day NOEC for mortality for cadmium of more than 300 μgCd g^{-1} soil, while Neuhauser et al. (1985) recorded a 14-day LC_{50} of 1843 μgCd g^{-1} soil. LC_{50} for *Lumbricus rubellus* was estimated to be approximately 463 μgCd g^{-1} soil, but the authors indicate that due to the threshold nature of the mortality after exposure to cadmium, the reliability of calculated LC_{50} was very limited (Spurgeon et al., 2004a). As *Eisenia fetida* is reported to be less sensitive than *Lumbricus rubellus* (Spurgeon et al., 2003), we can also presume that LC_{50} for cadmium may be somewhat higher in case of *Eisenia fetida*.

Although *E. fetida* response to lead was more pronounced, the mortality was not very high. During the first week, the highest mortality (20%) was observed in the case of the highest Pb concentration (2500 μgPb g^{-1}); at the end of the test it reached 55%. It is noteworthy that the survival of earthworms exposed to 250–500 μgPb g^{-1} was slightly higher than of those exposed to 20 μgPb g^{-1}. A similar pattern was observed by D. J. Spurgeon and co-workers (1994). They found that the mortality of *Eisenia fetida* at a lower Pb concentration in soil was higher than a higher concentration. The results of logistic regression showed that the risk of death of *E. fetida* increased significantly with the concentration of lead ($\chi^2 = 28.64$, p = 0.00001). As earthworms store lead in a non-toxic form and die when the stored Pb reaches a critical concentrations (Morgan, Morgan, 1998), it can be presumed that the duration of the test (28 days) may be insufficient.

No effect on earthworm survival exposed to heavy-metal (Cu, Zn, Cd and Pb) contaminated soils was observed in Norway despite the fact that the concentrations of metals were high (max Pb – 8750, Cd – 110 μg g^{-1} soil) (Hønsi et al., 2003), although others reported the 14-day LC_{50} for Pb to range from 4480 to 5941 μgPb g^{-1} soil (Neuhauser et al., 1985; Spurgeon et al., 1994).

Mortality is generally accepted to be a rather insensitive parameter; therefore, other sublethal effects – changes in weight and reproduction – were assessed. The growth of earthworms was evaluated after 14, 21 and 28 days (Fig. 2). Weight loss was recorded in all treatments. The possible reasons for weight loss in the control could be insufficient food resources (the medium contained 10% of Sphagnum peat), although the mortality of worms during the test (28 days) did not exceed the limit of test validity (20%).

Earthworms exposed to different lead concentrations in soil tended to lose more weight than those in control. At the highest concentrations (1000 and 2500 μg Pb g^{-1} soil) the body weight (after 14, 21 and 28 days) of earthworms was significantly ($p < 0.05$) reduced in comparison with the control group. The linear regression analysis of a correlation between lead concentrations in soil and earthworms' weight showed a close negative relationship after 14, 21 and 28 days of exposure (14 days: $R^2 = 0.59$; 21 days: $R^2 = 0.58$; 28 days: $R^2 = 0.62$; $p < 0.05$) (Fig. 3). The weight of earthworms exposed to cadmium tended to show a dose-related decrease (Fig. 2), although a statistically significant decline

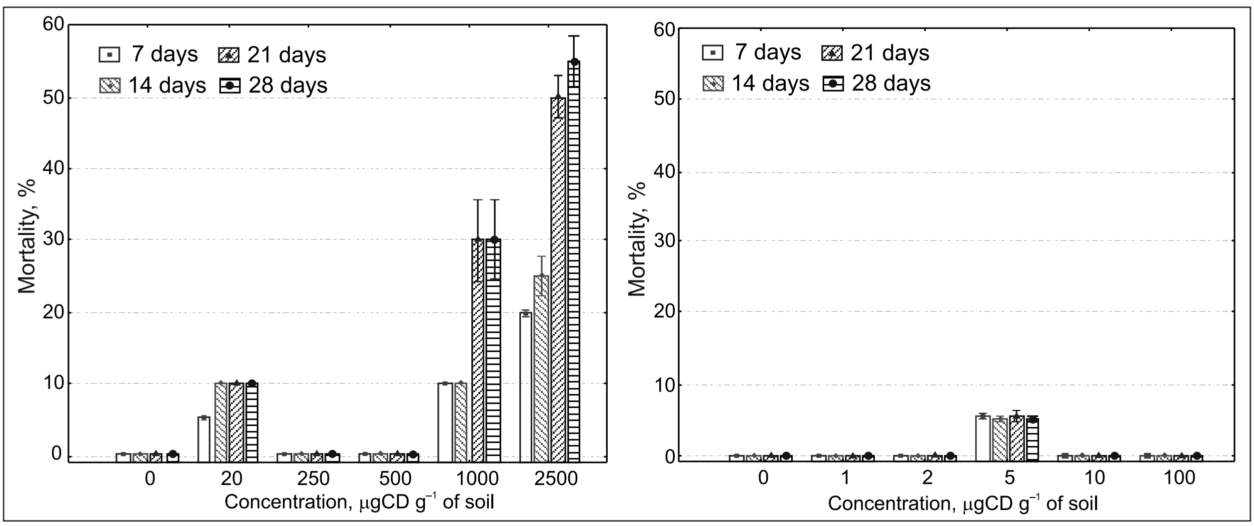

Fig. 1. Mortality of *Eisenia fetida* in OECD artificial soil contaminated with various concentrations of lead and cadmium. Error bars indicate SE of the mean value

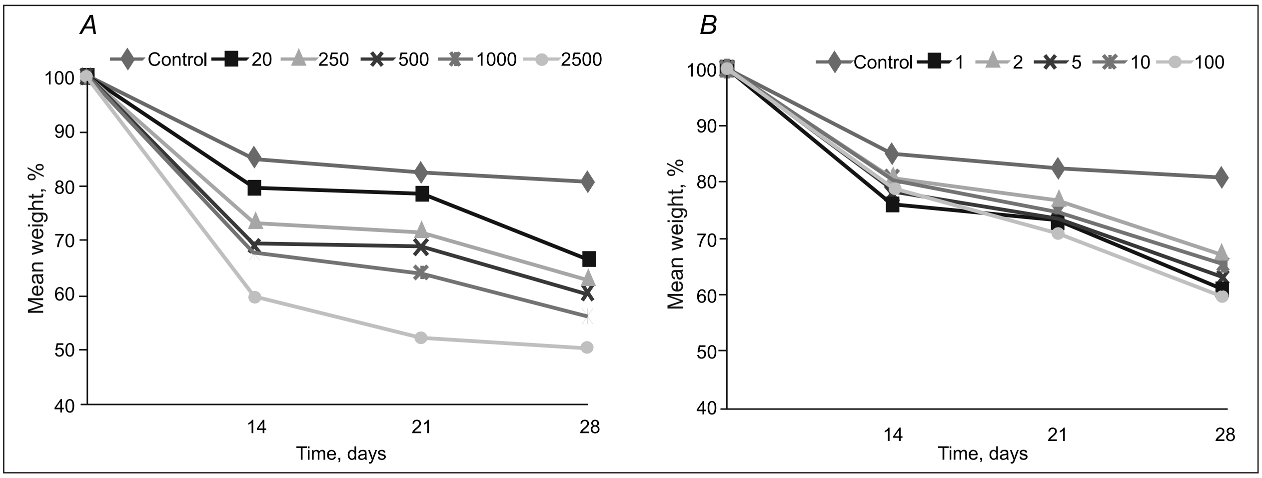

Fig. 2. Changes in the fresh weight of *Eisenia fetida* in OECD artificial soil contaminated with various lead (*A*) and cadmium (*B*) (µg g^{-1}) concentrations

was detected only in the case of the highest concentration (100 µgCd g^{-1} soil) after 21 days of exposure and in cases of 1, 2, 10 and 100 µgCd g^{-1} soil after 28 days of exposure. The relationship between cadmium concentrations and body weigh was negative, though very weak and statistically insignificant. Body weight reduction due to metal exposure was observed in other studies (Berthelot et al., 2008; van Gestel et al., 2009), but no significant impact on body weight, or even an increase in weight, were also reported (van Gestel et al., 1993; Spurgeon et al., 1994). The worms living in metal-contaminated soils reach the lower weight or need more time to reach the maximum weight than in non-polluted sites (Spurgeon, Hopkin, 1996).

As various sublethal effects can be observed at concentrations far below a given LC_{50}, acute toxicity is not the most sensitive parameter of the potential ecological impact of pollutants. Reproduction rate is of high ecological relevance. Cocoon production rate in the control was in the reported range (0.2–0.5) of production rates in artificial soil without food added (van Gestel et al., 1989). In contrast to the acute toxicity test, metals did affect earthworm reproduction at environmentally relevant soil concentrations. The lowest lead concentration had no significant adverse effect on worms' reproduction (Fig. 4). The other concentrations significantly ($p < 0.05$) inhibited the cocoon production rate (range 29–65%), and no production was observed in soil containing the

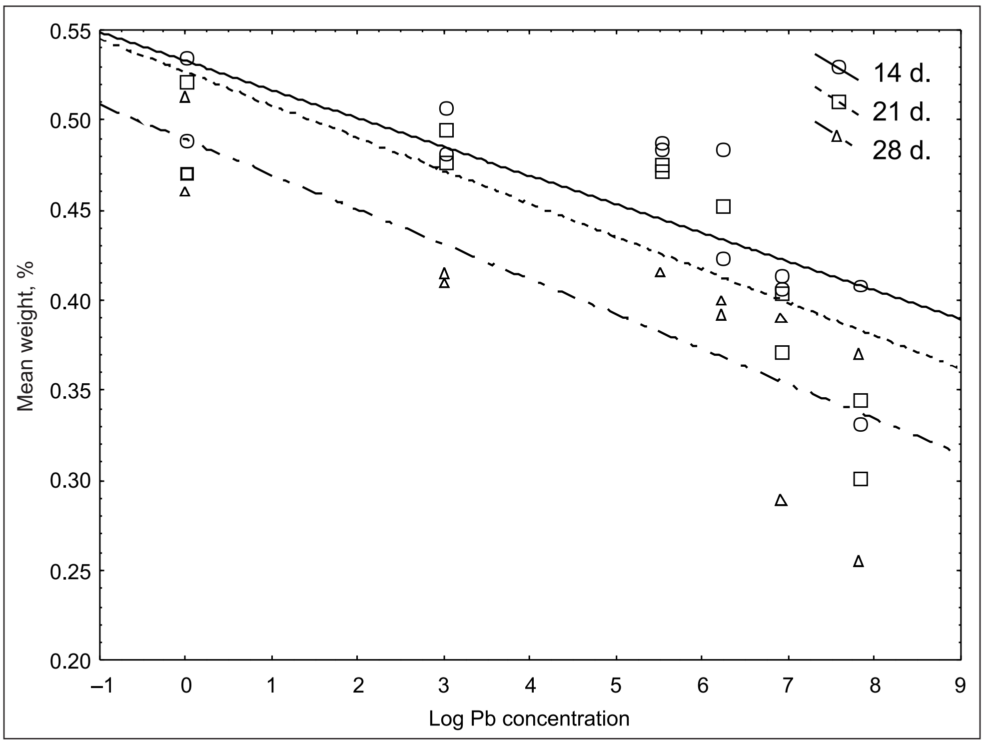

Fig. 3. Relationship between the mean weight of *Eisenia fetida* and lead concentrations (in logarithmic (ln) scale) in OECD artificial soil to which worms were exposed for 14, 21 and 28 days

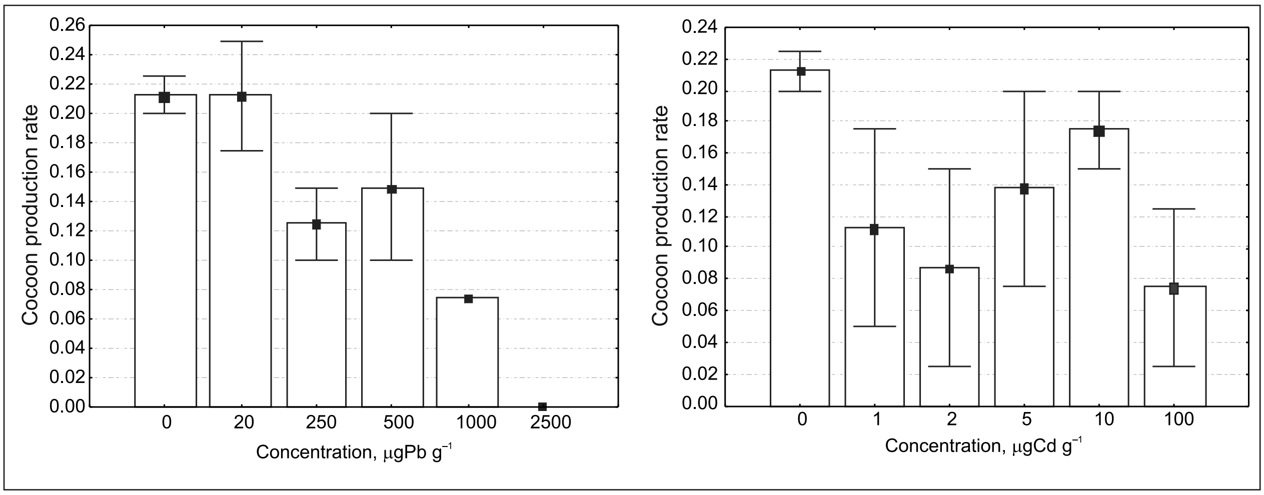

Fig. 4. Rate of cocoon production (cocoon / worm / week) by *Eisenia fetida* exposed to different concentrations of lead and cadmium. Error bars indicate SE of mean values

highest level of lead (2500 μgPb g^{-1}). A regression analysis showed that the cocoon production rate significantly decreased with increasing lead concentration in soil ($R^2 = 0.65$, $p = 0.0001$), and the EC_{50} value of 349 μgPb g^{-1} was estimated. In our study, the EC_{50} value was significantly lower than that detected in other studies (Spurgeon et al., 1994). The possible reasons for a relatively high lead toxicity to earthworms' reproduction may be that lead was not in equilibrium with the soil, and its bioavailability was relatively high. According to OECD eartworms' reproduction test, soil does not need to be equilibrated with the chemicals prior to the addition of worms. Other researchers indicate that it may increase the toxicity because when a contaminant is not in equilibrium with the soil, it becomes more bioavailable to living organisms (Davies et al., 2003). They suggest that the total Pb concentration in soil is of little relevance to its toxic effects on earthworms, and metal toxicity is related to its content in soil solution, because the most important route of uptake of dissolved metals is dermal from the soil pore water.

Cadmium also inhibited the reproductive rate of worms. The cocoon production rate under exposure to cadmium was inhibited by 18–65% in comparison with the control group, although the variability of cocoon production rate was rather high and no clear relationship had been found between cadmium concentrations in soil and cocoon production rate. Similar results, i. e. no clear relationship between cadmium concentrations in soil and cocoon production rate, were obtained by Burgos et al. (2005). The reported NOEC and EC_{50} for reproduction vary greatly in different studies. Spurgeon et al. (1994) determined a 56-day NOEC for reproduction under cadmium exposure to be of more than 300 μgCd g^{-1} soil, while others reported the range from 10 to 50 μgCd g^{-1} soil (van Gestel et al., 1992). Reduction in cocoon production due to lead and cadmium exposure was reported in several studies (van Gestel et al., 1992; 1993; Spurgeon et al., 1994; Savard et al., 2007).

Our findings indicate that mortality is the least sensitive endpoint than growth weight and cocoon production being more sensitive. Reproduction was more sensitive to metal exposure. Van Gestel et al. (1992) reported that growth was less sensitive than reproduction to most chemicals (metals, pesticides). Our study confirmed a higher sensitivity of the earthworms' reproduction test in comparison with the acute earthworm mortality test.

CONCLUSIONS

The results of this study have indicated that lead and cadmium levels in Lithuanian background and industrial areas are relatively "safe" for earthworms as they cause no significant acute toxicity, although the impact on reproduction and growth was rather significant and suggested that earthworms under such heavy metal pollution level are at risk enough to cause reproduction disturbances leading to changes in population growth rate and size. However, since contaminated field soils usually contain more than one metal or other chemicals, this can affect the toxicity and should be taken into consideration when applying the results of laboratory tests. The study confirmed a higher sensitivity of the earthworm reproduction test in comparison with the acute earthworm mortality test.

The results imply that testing should only be done when the contaminants reach an equilibrium with soil and that the duration of the test should be longer than four weeks. Also, the results indicate that future experiments should include a food source (e. g., animal manure) in the soil to prevent weight loss. Further investigations into soil properties may help to elucidate the mechanisms of toxicity to earthworms.

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KADMIO IR ŠVINO KONCENTRACIJOS DIRVOŽEMYJE POVEIKIS SLIEKO *EISENIA FETIDA* AUGIMUI, REPRODUKCIJAI IR IŠGYVENAMUMUI

Santrauka

Švino ir kadmio poveikis slieko *Eisenia fetida* augimui, reprodukcijai ir išgyvenamumui buvo tirtas remiantis OECD ūmaus ir lėtinio (reprodukcijai) toksiškumo sliekams protokolais. Tirtos Pb (20–2500 µgPb g^{-1} dirvožemio) ir Cd (1–100 µgCd g^{-1} dirvožemio) koncentracijos buvo parinktos siekiant imituoti metalų lygius skirtingomis aplinkos taršos sąlygomis (tarša foninėmis sąlygomis, pramoninėse ar užterštose teritorijose). Sliekai metalais veikti 28 dienas dirbtinio dirvožemio substrate. Tyrimo metu tirtų koncentracijų intervale LC_{50} nebuvo nustatyta. *E. fetida* rizika žūti didėjo didėjant švino koncentracijai dirvožemyje (χ^2 = 28,64, p = 0,00001), tačiau patikimo sliekų mirtingumo padidėjimo veikiant juos kadmiu nebuvo nustatyta. Sliekų svorio mažėjimas nustatytas visų metalų koncentracijų atvejais. Tarp sliekų svorio ir švino koncentracijos dirvožemyje nustatyta patikima neigiama koreliacija (14 d.: R^2 = 0,59; 21 d.: R^2 = 0,58; 28 d.: R^2 = 0,62; p < 0,05). Sliekų, paveiktų kadmiu, svoris mažėjo, tačiau statistiškai patikimas svorio sumažėjimas nustatytas tik veikiant 100 µgCd g^{-1} (didžiausia koncentracija). Kokonų skaičiaus rodiklis buvo jautresnis nei mirtingumas ar augimas. Regresinė analizė parodė, kad reprodukcija (kokonų skaičius) patikimai mažėjo didėjant švino koncentracijai dirvožemyje (R^2 = 0,65, p = 0,0001), nustatyta EC_{50} buvo lygi 349 µgPb g^{-1}. Dėl Cd poveikio reprodukcija (kokonų skaičius) sumažėjo 18–65 %.

Raktažodžiai: kadmis, sliekai, augimas, švinas, mirtingumas, reprodukcija