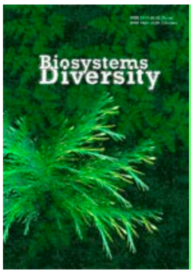

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Impact of cadmium and lead on *Megaphyllum kievense* (Diplopoda, Julidae) in a laboratory experiment

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After accumulating in plants, lead and cadmium of technogenic origin concentrate in forest litter, thus inhibiting the speed of its microbiological decomposition and causing chronic intoxication of saprophage millipedes which feed on it. The 20-day laboratory experiment described in this article determined the toxic impact of lead and cadmium (at concentrations of 0.3, 3, 30, 300, 3,000 and 30,000 mg/kg of litter) on the body weight of *Megaphyllum kievense* (Lohmander, 1928) and microbiological decomposition of forest litter. The death rate of *M. kievense* increased from 10–20% to 40% at 3 and 30 g/kg of lead and to 100% at 30 g of cadmium per 1 kg of litter. Gain in body weight of *M. kievense* reliably decreased only at 3 g/kg of cadmium (0.71 to 0.20 mg/24-hours). Under the influence of lead, gain in body weight reliably decreased compared to the control already at 100 times smaller concentration (30 mg/kg of substrate – 0.74 to 0.10 mg/24-hours). Under the influence of both cadmium and lead, the decomposition of litter by microorganisms is slowed down only under the highest of the studied concentrations (30 g/kg). The results of the laboratory experiment showed high resistance of *M. kievense* to lead and cadmium, at levels significantly higher than natural concentrations of heavy metals in technogenically transformed ecosystems.

Keywords: heavy metals; technogenic pollution; litter decomposition; growth rate; body weight; litter microbiota

Introduction

Lead and cadmium are among the most hazardous environmental pollutants, which affect the death rate of living organisms, their body weight and physiological activity of different systems of organs (Spehar et al., 1978; Dallinger, 1993; Santana et al., 2005; Valko et al., 2005; Zygmunt et al., 2006).

The average content of cadmium in the earth's crust is 130 mg/t, and about thousand times lower in sea water (0.11 mg/t). Rocks contain cadmium mostly in the form of CdS, $CdCO_3$, CdO, CdSe and $CdS(H_2O)_n$. A relatively high content of cadmium occurs in lead-zinc and copper-pyrite deposits. Global deposits of cadmium are estimated at around 560 thousand tons (according to US Geological Survey). Currently, the global production of cadmium equals around 21–24 thousand tons (the data of World Bureau of Metal Statistics). Mostly, cadmium is used as a component of soldering (for reducing the temperature of melting). Around 10% of cadmium production is processed in jewelry and low-melting alloys. Around 40% of cadmium is processed in undersealing metal products, and around 20% in making of cadmium electrodes and backup batteries (lead-cadmium and mercurial-cadmium elements). Nickel-cadmium batteries are widely used in the production of electronic devices, especially in China and countries of Southeast Asia. Most of these products become inoperable after a few years and accumulate in municipal solid waste storage sites. From there, cadmium penetrates the neighboring natural ecosystems, mostly via ground water and dust.

The average content of lead in the earth's crust is 16 g/t. In rocks, it occurs mostly in compound with silver, zinc and other metals, and is common in different types of rocks (from sedimentary to magmatic intrusive rocks). The world reserve of lead is 89 million tons (the data of US Geological Survey). World production of lead is around 3.8–5.3 mil t (the data of World Bureau of Metal Statistics). In the USA and countries of the European Union, 60–80% of lead is obtained through recycling. Around 80% of lead production is processed in the production of electric batteries. Earlier, lead was mostly used for making gasoline additives and solders. Also, lead is widely used in production of paint and pigments. After decomposition in municipal solid waste storage sites, many of these products become sources of lead contamination in ground water and surface soil.

Concentrating in different populations of animals, these metals can have a toxic effect on them. The concentration of cadmium in predatory invertebrates should increase significantly faster than in saprophages or herbivorous animals (Lindqvist et al., 1995; Maryanski et al., 2002; Lagisz, 2008). Addition of these metals to the diet of invertebrates decreases the activity of superoxide dismutase and glutathione peroxidase (Wang et al., 2015), and changes the ratio of isoforms of nonspecific esterases (Vlahovic et al., 2012), activity of alkaline phosphatase (Vlahovic et al., 2008) and other enzymes (Pedersen et al., 2007, 2008). This leads to change in the body weight (its increase or decrease), though there have been few laboratory experiments on change in the body weight of different groups of saprophages (Young & Harvey, 1988; Valko et al., 2005).

The objective of this research was to determine the toxic dosages of lead and cadmium for *Megaphyllum kievense* (Lohmander, 1928), one of the most common species of millipedes in the steppe zone of Ukraine, in laboratory conditions, and also to determine the impact of leaf litter pollution with these elements on microbiological and zoogenic decomposition of leaf litter.

Materials and methods

Adult individuals of *Megaphyllum kievense* (Lohmander, 1928) were collected manually in a ravine forest in the north part of the steppe zone (48.3564º N, 35.0651º E). The fodder substrate (leaf litter of half-decomposed leaves of *Robinia pseudoacacia* Linnaeus, 1753) was dried to air-dried condition, sieved (calibrated for placing 10–20 mm long fragments in the containers). Dry litter to the amount of 990–1010 mg was put in every container (200 ml plastic cups), uniformly moistened with 1 g of solution of metal in a certain concentration or distilled water in the control. The experiments used chemically pure $CdCl_2$ and $Pb(NO_3)_2$. The data on concentration of pollutants in the graphs and text are given in mg of metal (Cd or Pb) per 1 kg of dry litter. The data on fodder consumption and decrease in the mass of litter are calculated for a 24-hour period.

One *M. kievense* individual (an equal number of males and females was used for every variant of the experiment) was put in each container; average mass of individuals at the beginning of the experiment equaled 36.4 ± 9.3 mg (min – 19.1, max – 70.0 mg), and at the end it was 43.5 ± 1.3 mg (min – 21.9, max – 76.4 mg). The total number of *M. kievense* individuals used in the experiment was 130: 6 concentrations of each of the metals (n = 10) and one control (n = 10). The litter in 130 containers with the same concentration of metals (n = 10) and without it (n = 10) was at the same time weighed for control of microbiological decomposition of the substrate. For maintaining an optimum uniform moisture of the litter, a small amount of distilled water was added to the control and experimental containers once every two days. The experiment lasted 20 days.

The data was compared using Tukey's test. Differences between the selections were considered statistically significant at $P < 0.05$. The data was analyzed in Statistica 13 (Dell Inc., USA, 2015), the small squares in the diagrams show the median, the large rectangles show the 25% and 75% quartiles, the vertical lines show 95% of the variation, the stars and circles show the outliers.

Results

The death rate of *M. kievense* increased from 10–20% to 40% at 3 and 30 g/kg concentration of lead and to 100% at 30 g/kg concentration of cadmium (Table 1). Gain in the body weight of *M. kievense* over the 20-day laboratory experiment reliably decreased only at 3 g/kg cadmium (0.71 to 0.20 mg/24 hours, Fig. 1*a*). The impact of lead caused weight gain to reliably decrease already at the concentration of 30 mg/kg of substrate (0.74 to 0.10 mg/24 hours, Fig. 1*b*) compared to the control.

The impact of cadmium and lead caused the litter to decompose at a significantly slower rate only at the concentration of 30 g of metal per 1 kg of litter (Fig. 2). The differences in the rates of decrease in the litter mass in the presence of *M. kievense* and during the absence of the millipedes are not reliable.

Discussion

The data obtained in a previous study (Brygadyrenko & Ivanyshin, 2014) of the change in the body weight of *M. kievense* in relation to iron concentration (10^{-1} to 10^{-8} mg/g of fodder) showed similar results: the millipedes on average gained 2.5 mg/24 hours, the differences between high, average and low concentrations of Fe in the substrate were also insignificant. Copper in the diet of *M. kievense* caused a significant impact on the body weight of the millipedes (Brygadyrenko & Ivanyshin, 2015): a low dose of Cu (10^{-8} mg/g of litter) doubled the increase in weight gain, and a high concentration (10^{-1} mg/g of litter) decreased the weight gain threefold, i.e. toxically affected the invertebrates. Copper occurs in the compound of the enzymes, perhaps, that is why its low concentration causes anabolic changes in *M. kievense*. Iron caused no gain in body weight similarly to lead and cadmium in this recent study, where the metals did not affect the millipedes' body weight. Similar results (Shulman et al., 2017) were obtained after analyzing the trophic activity of *Calliphora vicina* Robineau-Desvoidy, 1830 larvae: no reliable changes in the pupa weight were found at minimum to 10^{-3} mg/g concentrations. The studies by Schmid et al. (1991, 1992) showed a decrease in the body weight of *Aiolopus thalassinus* (F.) imagoes caused by the effect of cadmium. Hatching of eggs in soil saturated with lead and cadmium significantly decreased.

Table 1
Mortality of *M. kievense* fed on litter with high concentrations of heavy metals during a 20 day experiment

Chemical element	Concentration of the metal, mg/kg of substrate						
	0	0.3	3	30	300	3000	30000
Cadmium	20	10	20	10	10	20	100
Lead	20	10	20	10	20	40	40

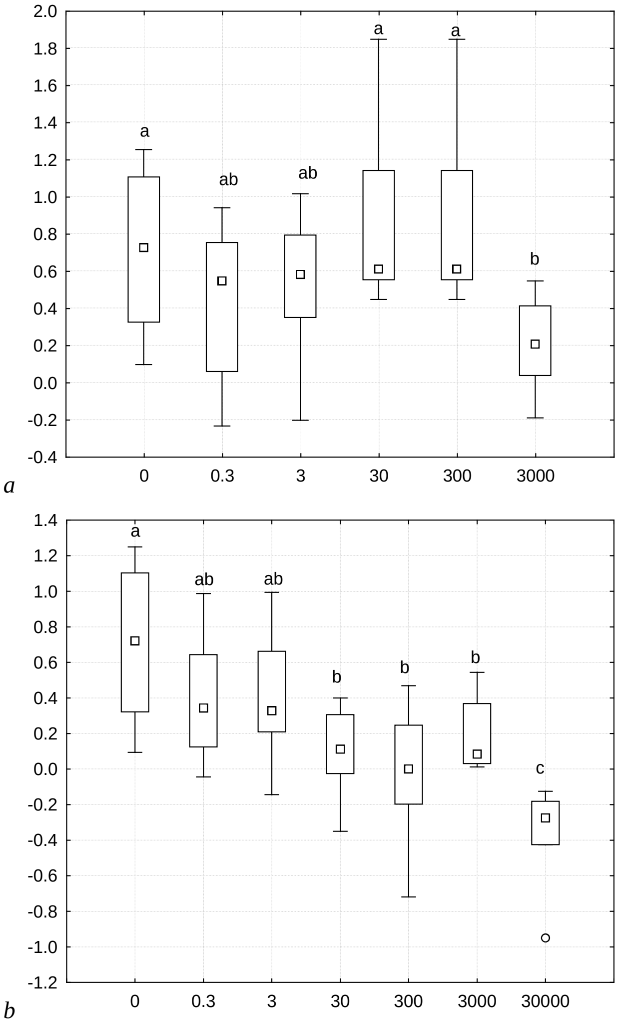

Fig. 1. Changes in the body weight of *M. kievense* during the 20-day laboratory experiment with substrate including cadmium (*a*) and lead (*b*): on the abscissa – concentration of metal in the food substrate (mg/kg of fodder substrate), on the ordinate – change in the body mass of *M. kievense* (mg/24 hours); n = 6–9 depending on death rate, indicated in Table 1; different letters correspond to the selections which had significant differences between one another according to the results of Tukey's test ($P < 0.05$)

Cadmium at 10 mg/kg concentration has reliably decreased the formation of cocoons of *Eisenia andrei* Bouche earthworms (Van Gestel et al., 1993). Change in the body weight occurred among the following saprophage insects and phytophages: *Orchesella cincta* (L.) (Posthuma et al., 1992; Hensbergen et al., 2000), *Proisotoma minuta* Tullberg (Nursita et al., 2005), *Chironomus riparius* (Meigen) (McChaton & Pascoe, 1991), *Chironomus tentans* F. (Martinez et al., 2001, 2004), *Ch. thummi* (Meigen) (Bisthoven et al., 1992), *Tanytarsus dissimilis* Johannsen (Anderson et al., 1980), *Locusta migratoria* (L.) (Martoja et al., 1983), *Oncopeltus fasciatus* (Dallas) (Cervera et al., 2004), *Lymantria dispar* L. (Vlahovic et al., 2008; Mircic et al., 2010, 2013), *Aglais urticae* (L.) (Lindqvist, 1994), *Drosophila melanogaster* Meigen (Maroni & Watson, 1985; Lauverjat et al., 1989), *Musca domestica* L. (Niu et al., 2002).

Change in the litter mass under the impact of copper and iron (Brygadyrenko & Ivanyshin, 2014, 2015) in the absence of the millipedes was insignificant compared to the control. Lead and cadmium in this study at concentrations up to 3 g/kg of litter caused no significant inhibition in the activity of the microflora which decomposes the leaf litter. Higher concentration of cadmium and lead significantly inhibited both microbiological and zoogenic decomposition of plant remains.

Conclusions

Cadmium and lead penetrates the environment over a period of decades, accumulating in the soils around large cities, thus affecting the activity of growth of saprophage millipedes. Only high concentrations (about 30 g/kg of litter) increase the death rate and inhibit the growth of millipedes. Inhibition of litter decomposition by 3–8 times occurs also at a cadmium and lead concentration of 30 g/kg of litter.

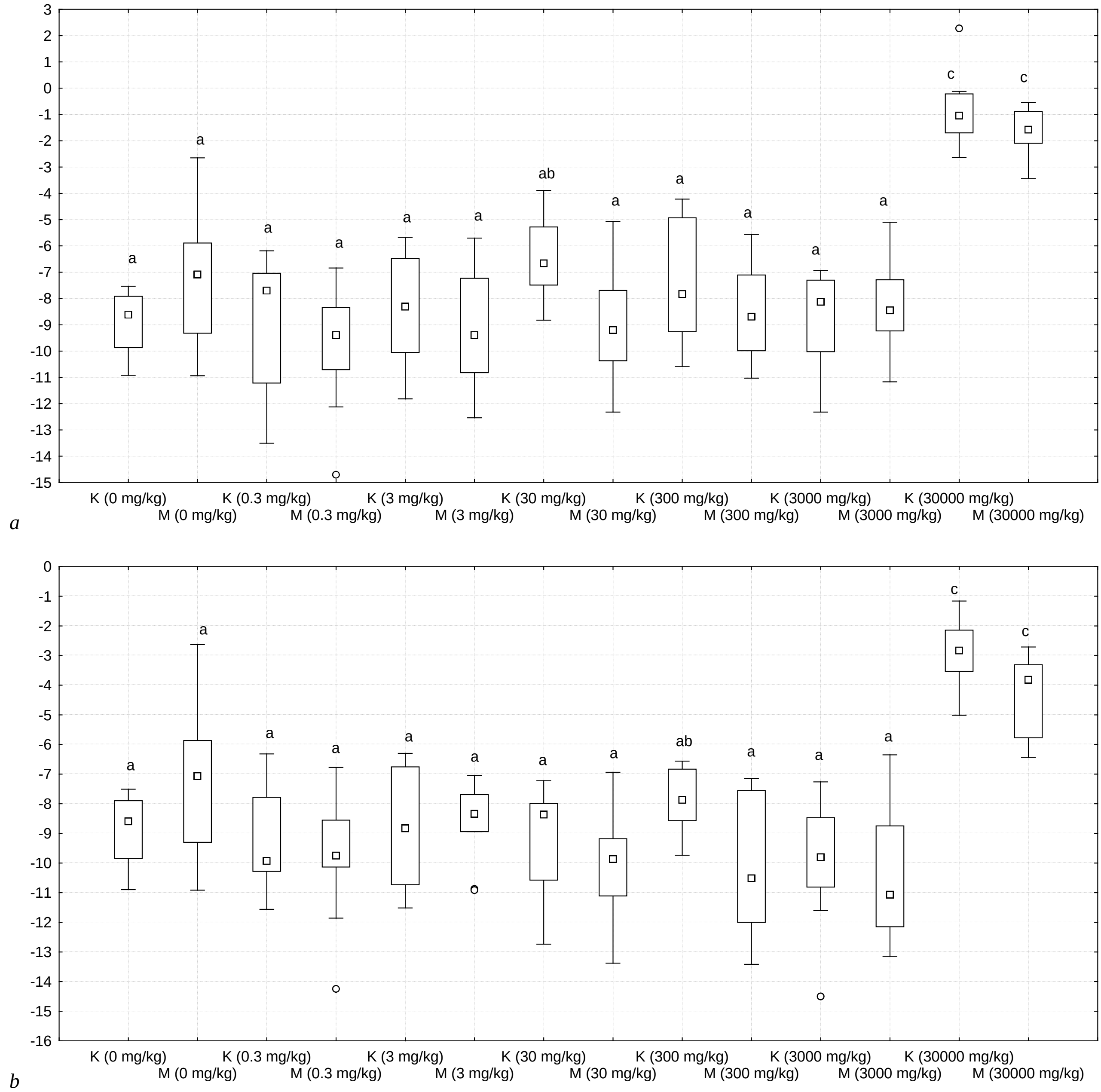

Fig. 2. Changes in the weight of food substrate including cadmium (*a*) and lead (*b*) over a 20-day laboratory experiment: on the abscissa – concentration of the metal in the food substrate (mg/kg food substrate), on the ordinate – change in weight of litter (mg/24 hours); K – variant of experiment without *M. kievense* (n = 10), M – variant of experiment with *M. kievense* (n = 6–9 in relation to death rate presented in Table 1); different letters correspond to the selections which had significant differences between one another according to the results of Tukey's test (P < 0.05)

Change in the biological activity of the microflora and of the studied species of litter invertebrate occurs at practically the same concentrations of cadmium and lead. Further research requires study of the change in the enzymic activity, the cellular composition of hemolymph and parasitic fauna of the intestine of *M. kievense* in the conditions of heightened concentrations of heavy metals.

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