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The Effect of Heavy Metals on the Soil–Earthworm–European Mole Food Chain under the Conditions of Environmental Pollution Caused by the Emissions of a Copper Smelting Plant

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Abstract—The paper examines the effect of heavy metals on the soil–earthworm–European mole food chain in the area affected by the operation of the Middle Urals Copper Smelting Plant (MUCSP), located in Revda (Sverdlovsk region). Compared to the surrounding territory, in the polluted zone, the increase in the content level of physiologically essential elements (Cu and Zn) is less pronounced in the tissues of earthworms than in the soil, while the content level of nonessential elements (Pb and Cd) is higher in the tissues of earthworms than in the soil. The only biomagnification that has been revealed is that of Cd: the Cd content is 8–10 times higher in the tissues of earthworms than in the soil (3.9–4.5 times higher as compared to the litter) and 4–6 times more considerable in the liver of the mole than in the tissues of earthworms. Zn, Cu and Pb do not accumulate in the liver of moles, since their levels grow in the gastric contents, while a higher Cd level in food leads to the disproportionate accumulation of this substance in the liver. Even though there is an excessively high Cd content in the organism of the mole, this animal is the ultimate "depot" of this element in terrestrial ecosystems.

Keywords: mole, *Talpa europaea*, earthworm, soil, forest litter, heavy metals, Cu, Pb, Cd, Zn, copper smelting plant, industrial pollution, Middle Urals

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INTRODUCTION

The attention of ecologists has been drawn to toxic agent accumulation in trophic chains for more than 50 years, since the first descriptions of excessive accumulation of ^{137}Cs and DDT in the bodies of fish-eating birds in comparison to the content of these pollutants in water. However, subsequent research has revealed that not all toxic agents (in particular, heavy metals) have the same accumulation characteristics. Three types of pollutant action in food chains are distinguished: accumulation (a higher content of a substance at a higher trophic level); indication (the same content of a substance at any trophic level); and elimination (a lower content of a substance). Rare cases of excessive accumulation of heavy metals, Cd in particular, were described regarding earthworms (bioaccumulation factor (BAF) of 158) and mollusks (BAF of 206) under conditions of insignificant soil pollution (Hsu et al., 2006). Most studies show that there exist moderate accumulation (BAF = 2–20) (Ma, 1987; Hendriks et al., 1995; Nahmani et al., 2007), indication (Laskowski, 1991), or elimination (Hendriks et al., 1995; Nahmani et al., 2007) of heavy metals in animals.

The accumulation of heavy metals in terrestrial animals is determined by many factors. First, great importance is attributed to the characteristics of a certain heavy metal, its compounds, its interaction with other substances, and its content rate. For instance, the content of physiologically essential metals is efficiently regulated by the gastrointestinal barrier, protein binding, and excretion (Vijver et al., 2004). Second, heavy metal intake involves edaphic factors, such as soil acidity, organic content, clay content, and cation-exchange capacity (Ma et al., 1983; Ladonin, 2003). Third, the characteristics of particular species, their trophic specialization, and their age have some significance. In particular, the comparison of herbivorous and carnivorous small mammals has shown high Cd, Pb, and Cr contents in the organisms of carnivores (Hunter et al., 1987; Hamers et al., 2006; Veltman et al., 2007a; Bezel et al., 2007).

The emissions of nonferrous metal processing plants cause considerable geochemical anomalies. This is why information on the transfer of heavy metals in food chains is important. It is reasonable to choose the soil–earthworm–European mole food chain as a model, since it is not extensively branched and all its elements are closely interrelated, in contrast to most terrestrial trophic chains. The European mole (*Talpa europaea L.*) is mostly stenophagous: the share of earthworms in its diet is 90–96% (Gogfrey and Crowcroft, 1960; Funmilayo, 1979). In this way, it is fair to expect that relatively undisturbed transfer of metals takes place in this food chain. At the same time, data on the heavy metal accumulation in the tissues of moles dwelling in territories affected by industrial plants is insufficient (Ma, 1987). The research devoted to heavy metal accumulation in the organisms of moles caused by other types of pollution is also scarce (Pankakoski et al., 1993; Komarnicki, 2000).

The objective of the work is to close the gap in the analysis of heavy metal content in European moles and in the regularities of heavy metal transfer in the soil–earthworm–European mole food chain under the conditions of the environmental pollution by the emissions of a copper smelting plant.

MATERIALS AND METHODS

Study Area

The study has been conducted in the territory affected by the operation of the Middle Urals Copper Smelting Plant (MUCSP) situated near Revda in the Sverdlovsk region. The main compounds found in the emissions are SO_2 and polymetallic dust with prevailing Cu, Pb, Zn, Cd, and As particles. By the end of the 1980s, the total volume of emissions had exceeded 140000 t/year. In particular, these emissions included 134089 t/year of SO_2, 1016 t/year of HF, 2610 t/year of Cu, 1754 t/year of Zn, 639 t/year of As, and 564 t/year of Pb. Beginning in the mid-1990s, emissions were gradually reduced and their volume amounted to less than 30000 t/year in the mid-2000s.

According to physiographic zoning, the territory belongs to the taiga area of the low-mountain belt of the Middle Urals (true altitude of 150–450 m asl). Dark coniferous forests and their related coniferous–deciduous, birch, and aspen forests prevail. The main types of soil are brown mountain-forest soil, dark gray soil, podzolized soil, and gleyed soils.

According to a snow and soil analysis, there are three zones to the west of the MUCSP characterized by different degrees of pollution: impact (up to 3 km from the facility), buffer (3–7 km from the facility), and background (more than 7 km from the facility) (Vorobeichik et al., 1994). However, the zoning varies with the state of the biota. In relation to earthworms, the impact zone extends up to 4 km from the plant, since earthworms do not dwell closer to the facility (Vorobeichik et al., 1998). The absence of earthworms determines the absence of moles. The first traces of digging activity by moles were found at a distance of 5 km to the west of the plant; regular dwelling was located at a distance of 6–7 km from the facility. At a distance of 10 km from the facility, the mole population is 1.5–1.8 times smaller than at 20–30 km (Nesterkova, 2014). Since this research deals with the European mole, it covered only the buffer (5–10 km from the plant) and background (more than 10 km from the plant) zones.

The study area was well assessed for regular reactions of the biota to industrial pollution. Detailed studies were devoted to soil invertebrates (Vorobeichik, 1998; Vorobeichik et al., 1994, 2012) and heavy metal accumulation in small mammals (Mukhacheva, Bezel, 1995; Bezel et al., 2007). Mole species, however, were not included in the research.

Soil and Forest Litter

The characteristics of the heavy metal content in the soil of the study area were elaborated using data obtained over 1995–1998 during the mapping of a 40 × 50 km territory surrounding the MUCSP (208 sample plots with an area of 25 × 25 km) (Vorobeichik, 2003). The longest distance between the closest plots did not exceed 3 km in most cases. The sample plots differed not only in pollution levels, but also in landscapes (eluvial, transient, and accumulative), soil and plant cover, and remoteness from residential areas. The territory contains birch, pine, birch–pine, and birch–spruce–fir forests of diverse plant associations. The main soil types found in the area are brown mountain-forest, gray forest, and sod-podzolic. The present study deals with 67 sample plots situated to the west of the plant: 23 of these plots belong to the impact zone (including technogenic barren), and 30 and 14 plots are located in the buffer and regular zones, respectively.

There were three combined samples of litter and upper (0–5 cm) humus accumulative soil horizon collected from each sample plot. Each sample was composed of five individual components (taken from a 1 × 1 m area according to the "envelope" scheme). The points of sample collection inside the sample plot were random.

Earthworms

Earthworms were sampled in July 2008. The sampling was conducted in four plots situated at a distance of 30, 20, 7, and 4–5 km to the west of the MUCSP (the samples of earthworms were collected in the spruce-fir and birch-aspen forests in each plot). In each plot, 5–10 small trenches randomly situated at a distance of 10–100 m from each other were dug for subsequent manual sampling. The trenches extended to both forest litter and upper (0–10 cm) humus horizon. Relatively large earthworms (4–6 cm long) of the same species—*Perelia diplotetratheca* (Perel, 1976)—prevailing in the study area were selected for the sampling. The selection included sexually mature earthworms and individuals without a clitellum. A total of 197 earthworms was involved in the study (38–57 individuals per plot).

The method based on the replacement of gut contents with agar proposed by A.D. Pokarzhevskii and his co-authors (2000) was used to evacuate soil from the intestine of earthworms. This method is more advantageous than the other methods such as the filter paper method. The substitute substance was made of microbiological agar, according to a ratio of 4.0–4.5 g of the dry matter to 1 L of distilled water (the ratio was deduced empirically during previous experiments; the highest survival rate of earthworms was observed at this ratio). Dry agar was dissolved in hot water. The solution was then brought to the boiling point and distributed into 500 mL transparent plastic containers. Afterwards, 14–15 earthworms were put into each container and the containers were covered with cotton fabric firmly fixed with a rubber ring. The containers were stored in a dark place at a temperature of 20–22°C for four days. Every day the containers were examined and dead earthworms were removed. After four days of storage, each earthworm was examined with the help of a binocular loupe for any soil left in the intestine (if there was any soil found, the specimen was not used for the further analysis). Then the earthworms were put into a freezing chamber (at a temperature of −18°C) for some time to be killed. They were then washed in distilled water once, placed into Petri dishes, and dried in a drying oven at a temperature of 60°C for 24 hours. This method ensures rapid immobilization of the earthworms and prevents them from secreting large amounts of mucus (in contrast to traditional immobilization with formalin or alcohol).

Moles

The moles were captured at a distance of 30, 20, 10, and 7 km to the west of the MUCSP in May, July, and October from 2007 to 2009. The animals were caught with live traps and regular traps set in permanent tunnels (four traps per tunnel). Since the permanent tunnels were sometimes quite far from each other, the capture areas were extended to 1–2 km in each plot. Out of a total of 153 moles captured, 111 were trapped with the help of live traps. The animals captured with regular traps were frozen and delivered to the laboratory. The living moles were put into individual plastic containers (7 L capacity) half-filled with soil. The animals were given an unlimited amount of water and were fed earthworms and fish fillet (Alaska pollock) every 4 h. After being kept in the laboratory (up to five days), the moles underwent ether anesthesia with subsequent decapitation. The weight of the body (0.001 g accuracy), the liver, and the stomach with the contents (0.002 g accuracy) were measured for each mole.

Since most toxic agents deposit in the liver ($n = 153$), it was the organ selected for chemical analysis for moles trapped with live traps. As for the moles trapped with regular traps, the analysis involved the gastric contents ($n = 42$). The samples were dried at a temperature of 60°C for two days. The age of each mole was identified according to the development of the reproductive system, weight, and color of fur. For adult moles, the age was determined more precisely by observing the growth rings in the histological section of the second and the third lower molars. The moles were divided into three age groups differing in body size and morphophysiological characteristics: 1–1.5 months old, 4–5 months old, and 11–73 months old.

Chemical Analysis

Actual acidity was determined for each forest litter sample and soil sample with the use of the ionometric technique (aqueous extract; a ratio of forest litter to water 1 : 25). A 5% extract of NHO_3 was used to measure the content of the mobile forms of Cu, Cd, Pb, and Zn (with a ratio of the sample substance to the acid of 1 : 10 and 24-hour extraction after a single shaking). Like all the other strong acids, the extractant used helps analyze not only heavy metals present in the biota but also potentially mobilized forms of heavy metal (Ladonin, 2003). Thus, this extractant makes it possible to get the data on heavy metal pollution that are more precise (compared to the use of ammonium acetate buffer and complexons). The heavy metal content in the forest litter and soil samples was measured with the help of an AAS-3 atomic absorption spectrometer (Carl Zeiss, Germany).

Samples of 100 g (0.0001 g accuracy) were taken from the animal material to measure heavy metal content in the tissues of earthworms and moles. An amount of 7 mL of concentrated HNO_3 and 1 mL of deionized water were added to the samples. Then the samples were incinerated in Teflon containers in a MWS-2 microwave oven (Berghof, Germany) at a maximum pressure of 900 kPa and maximum temperature of 155°C for 80 min. The heavy metal content (Cu, Pb, Cd, Zn) in the tissues was measured with the help of an AAS Vario 6 atomic absorption spectrometer (Analytic Jena, Germany) in the flame atomization mode. The chemical analyses were conducted in the population and community ecotoxicology laboratory based in the Plant and Animal Ecology Institute of the Ural Branch of the Russian Academy of Sciences. The laboratory is certified in conformity with the technical standards of and included in the state register of the Russian Federation (certificate ROSS.RU0001.515630).

Statistical Analysis

With the logarithm of the values preliminarily found, the significance levels of the content of elements in the samples were evaluated through the analysis of variance. Sheffe's method was used to deal with multiple comparisons. The relationship between heavy metal content in the liver (C_l) and the food (C_S) was approximated with the help of the equation $\mathrm{Log} C_l = a \log C_S + b$, where a and b are coefficients.

Table 1. Acidity (pH units) and heavy metal content (μg/g) in the forest litter and the soil in the zones of different toxic load surrounding the MUCSP

Element	Zone of toxic load		
	background ($n = 42$)	buffer ($n = 89$)	impact ($n = 69$)
		Forest litter	
pH	5.7 ± 0.1 (5.2–6.5)	5.3 ± 0.1 (4.6–6.8)	4.7 ± 0.1 (2.9–6.2)
Cu	67.6 ± 2.9 (39.3–123.3)	1556.8 ± 60.7 (661.3–3206.4)	4398.9 ± 247.1 (1889.6–12121.7)
Pb	56.4 ± 2.2 (33.5–95.0)	547.3 ± 19.1 (206.7–1122.3)	1082.5 ± 56.3 (317.5–2347.8)
Cd	3.7 ± 0.1 (2.3–5.5)	17.4 ± 0.4 (5.4–26.4)	25.4 ± 1.9 (6.2–80.3)
Zn	481.2 ± 18.4 (251.4–727.8)	1158.9 ± 37.8 (453.6–1950.3)	1443.6 ± 95.6 (297.1–4194.1)
		Soil	
pH	5.7 ± 0.1 (4.8–6.6)	5.2 ± 0.1 (4.3–6.0)	4.7 ± 0.1 (3.4–5.8)
Cu	85.6 ± 5.9 (34.9–179.6)	567 ± 27.8 (101.1–1508.6)	1374.5 ± 149.3 (183.7–6420.7)
Pb	55.3 ± 4.1 (26.4–128.8)	113.8 ± 7.0 (12.2–379.8)	177.1 ± 22.6 (13.8–849.9)
Cd	2.1 ± 0.2 (0.7–6.3)	6.7 ± 0.4 (1.4–24.8)	5.1 ± 0.4 (1.0–16.9)
Zn	121.5 ± 11.5 (31.8–313.4)	286.5 ± 17.9 (52.0–948.2)	231.1 ± 16.5 (65.9–752.2)

Average ± error; minimum and maximum values are inside the parentheses; a substrate sample is considered as a statistical unit; n represents the number of samples.

RESULTS AND DISCUSSION

Metal Content in Deposit Environments

Over the 70 years the MUCSP existed, it caused an extensive technogenic geochemical anomaly: the Cu content in the forest litter near the plant is 65 times higher compared to the regular zone. As for the other heavy metals, the content of Pb, Cd, and Zn is 19, 7, and 3 times more significant, respectively. The contents of Cu, Pb, Cd, and Zn in the humus accumulative horizon exceed 16, 3, 2.4, and 2 times, respectively, their contents in the regular zone. The metal content of the buffer zone is slightly less significant (Table1). The acidification of the soil solution resulted in the reduction in pH by one unit. This should be considered as the factor increasing the availability of the metals to the biota. The metal content of the soils situated at a distance of 30 km from the plant is in line with the regional content of the Urals, where it is higher than in other Russian regions with non-contaminated territories.

Metal Content in the Food of the Moles

Toxic agents penetrate into the organisms of mammals mainly through the digestive system (Hunter et al., 1987). There are two methods to estimate the quantity of toxic agents penetrating into the organism. The first evaluates the amount of the toxic agent in the gastric contents (Mukhacheva and Bezel, 1995). The second is based on the data about the composition of the food and the content of the toxic agent in it (Hunter et al., 1987). The toxic load is also determined by the amount of the food consumed in terms of both methods. As for the study, the weight of raw food consumed by the mole was equivalent to 5.1 ± 0.3 (average weight ± error, $n = 42$) and was not different depending on the age ($F(2, 37) = 2.15$, $p = 0.13$) or sex ($F(1, 38) = 2.47$, $p = 0.12$) of the animal or the pollution zone ($F(1, 38) = 0.02$, $p = 0.89$). In this way, the differences in the toxic load between the samples can be determined only by the differences in the heavy metal content.

The Cd content in the samples collected at a distance of 7 km from the plant was 4.4 times higher than

Table 2. Amount of heavy metals (μg/g) in the gastric contents of the moles sampled at a different distances from the MUCSP

Element	Distance from the pollution source, km			
	30 ($n = 10$)	20 ($n = 8$)	10 ($n = 14$)	7 ($n = 10$)
Cu	42.8 ± 10.0 {40.4} (11.9–95.9)	35.6 ± 8.3 {48.5} (14.2–83.1)	50.7 ± 6.3 {65.9} (17.1–92.5)	77.2 ± 9.9 {73.7}* (27.4–139.7)
Pb	8.4 ± 1.7 {20.6} (1.9–20.2)	12.4 ± 4.1 {42.9} (1.8–34.4)	32.4 ± 3.9 {93.2}*** (3.1–58.6)	62.3 ± 4.1 {64.7}*** (42.7–79.1)
Cd	18.4 ± 2.3 {58.0} (6.2–27.1)	20.9 ± 2.3 {35.0} (10.1–27.1)	47.0 ± 5.2 {31.4}** (12.2–75.1)	80.3 ± 14.7 {39.7}*** (18.4–159.0)
Zn	235.5 ± 52.6 {75.4} (58.4–553.7)	227.1 ± 43.5 {59.3} (85.5–396.6)	234.9 ± 39.5 {54.2} (75.6–634.1)	197.2 ± 47.0 {70.6} (73.0–492.7)

Here and in Table 3, average ± error; minimum and maximum values are inside the parentheses; the coefficient of variation is inside the curly brackets; an individual is considered a statistical unit. Significance levels for a 30 km distance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (Scheffe's method).

Table 3. Heavy metal content (μg/g) in the tissues of the moles sampled at a different distance from the MUCSP

Element	Distance from the pollution source, km			
	30 ($n = 55$)	20 ($n = 47$)	7 ($n = 38$)	4–5 ($n = 57$)
Cu	7.5 ± 0.1 {9.9} (5.6–10.3)	7.9 ± 0.2 {17.4} (5.3–11.1)	23.7 ± 1.7 {44.2}*** (11.9–71.7)	28.5 ± 1.9 {50.3}*** (10.3–75.4)
Pb	9.2 ± 0.7 {56.4} (1.0–26.7)	14.1 ± 1.5 {72.9} (3.1–62.6)	112.5 ± 13.6 {74.5}*** (18.3–359.5)	228.3 ± 25.1 {83.0}*** (8.6–1010.3)
Cd	16.8 ± 0.7 {30.9} (6.6–28.8)	21.3 ± 0.9 {29.0}** (9.7–35.8)	67.7 ± 3.8 {34.6}*** (24.8–131.9)	91.9 ± 4.8 {39.4}*** (28.3–193.3)
Zn	299.6 ± 10.1 {25.0} (143.6–439.6)	356.8 ± 14.8 {28.4} (193.6–705.9)	617.8 ± 36.3 {36.2}*** (230.2–1365.3)	536.6 ± 30.7 {43.2}*** (185.6–1152.4)

that of the samples collected at a distance of 30 km. As for Pb and Cu, their contents were 7.4 and 1.8 times higher. The Zn content did not vary (Table 2). The amount of Cd in the gastric content differed among moles of different ages ($F(2, 39) = 4.81, p = 0.014$). Cd was 2.5 times more prevalent in the adult moles dwelling in the polluted zones compared to pups. This can be determined by the differences in diet. On average, rodents and shrews inhabiting the same territory consume 0.6–19.8 μg of Cd per one gram of dry food. With a Cd content of 6.2–159 μg/g, the diet of the mole is more toxic.

It is a well-known fact that the basic food of the mole is earthworms and insect larvae. Depending on the source, however, the share of earthworms in the diet of the mole varies from 90–95% (Gogfrey and Crowcroft, 1960) to 38–96% (Funmilayo, 1979). The diet structure depends on the soil fauna composition of a certain habitat. With the lack of earthworms, the share of insects in the diet can become more significant, which is the case in sandy soils (Folitarek, 1932). Despite insufficient data on the food spectrum of the mole in the study area, the local abundance of earthworms (Vorobeichik, 1998) suggests that the metal content in the tissues of the earthworms is representative of the metal content in the diet of the mole.

The study area is inhabited by eight species of earthworms, with the *P. diplotetratheca*, epigeal species endemic to the Ural region prevailing (Vorobeichik, 1998). While the content of Cu, Zn, and Cd in the individuals of this species (Table 3) is in line with the existing data on other earthworm species inhabiting polluted areas, the Pb content is higher than expected for the given pollution level (Ma, 1987; Dai et al., 2004; Veltman et al., 2007b). Compared to other invertebrates dwelling in the territory affected by the operation of the MUCSP, the organisms of earthworms accumulate essentially larger amounts of Pb and Cd even at the average pollution level. Accumulation rates comparable to those of earthworms are typical only of mollusks (Bezel et al., 2009). The consumption of earthworms, therefore, leads to penetration of large quantities of heavy metals into the subsequent trophic levels. This is determined by high heavy metal content in the tissues of earthworms and the abundance of this species in forest ecosystems.

The comparison of the heavy metal content levels in the organisms of the earthworms and the gastric contents of the moles sampled in the same plots does not reveal differences in the quantities of the most toxic metals such as Cd ($p = 0.23–0.87$) and Pb ($p = 0.07–0.68$). However, there are higher Cu levels ($p < 0.001$) and lower Zn levels ($p = 0.001–0.05$) in the gastric contents than in earthworm tissues. The variation coefficients of heavy metal contents were higher in the gastric contents of the moles than in the tissues of the earthworms (F(1; 24) = 4.8; $p = 0.038$). This is likely to be explained by the fact that the moles searched for food in an area larger than that in which the earthworms were sampled.

We should expand on probable reasons of differences in the amounts of Cu and Zn in the tissues of earthworms and the gastric contents of the moles. First, while the mole consumes earthworms with partially full digestive tracts containing the remaining soil and vegetable tissues, the contents of the digestive tract of the earthworms used for the chemical analysis were removed. The studies of 28 species of mammals and birds suggest that soil particles accidentally penetrating into the organism can constitute 3–30% of the weight of food (Beyer et al., 1994). However, if it were the only reason, the quantity of heavy metals in the gastric contents of the moles would be an average between the quantities of heavy metals in the tissues of the earthworms and the soil. Therefore, the amount of Zn found would be greater. Second, besides *P. diplotetratheca*, the mole can consume other species of earthworms and insect larvae. Unfortunately, we do not have data on heavy metal content neither in other species of earthworms nor in other groups of soil mesofauna typical of the study area. According to V. Bezel and his co-authors (2009), the Cu content in the tissues of imagoes of Coleoptera, Diptera, Homoptera, and Hemiptera is 15–30 μg/g in the regular territories. It considerably exceeds the Cu content in the tissues of earthworms. However, the Zn content in the tissues of the same animal species is equivalent to 42–304 μg/g and 93–583 μg/g in the regular area and the impact area, respectively; i.e., it is lower than in the tissues of earthworms. Thus, the fact that the mole consumes insects, even if the quantity is not significant, may explain the difference in the content of heavy metals found in the gastric contents and the tissues of the earthworms. Third, the gastric contents of the mole constitute a relatively homogeneous semiliquid substance with some parts of the bodies of earthworms and insect larvae. It is a well-known fact that the mole digests food quite rapidly. The food passes through the stomach within 1 h, and it takes 4 h for food to be completely evacuated from the digestive system (Spiridonova, 1949). It is reasonable to suppose that the easily digestible soft tissues of the victims pass through the stomach more rapidly than their integuments. This affects the evaluation of the quantity of metals in the gastric contents.

Content of Heavy Metals in Tissues of Moles

The content of the metals in the tissues of the moles involved in the study did not differ for males and females (F(1;151) = 0.06–0.66). As for the age groups, differences in the content of Cu (F(2;150) = 15.2, $p < 0.001$), Pb (F(2;150) = 10.1, $p < 0.001$), and Cd (F(2;150) = 156.5, $p < 0.001$) were identified (Table 4). There is no specific tendency typical of the change in the content of Cu and Pb with age. In contrast to this phenomenon, the Cd content was 11 times higher in the adult moles studied than it was in the one-month old moles studied. The accumulation of Cd (y, μg/g) is determined by age (x, months) and can be precisely defined with the use of the logarithmic function ($y = 14.8 + 67.5\log x$ for the background area; $y = 26.5 + 208.5\log x$ for the buffer area; and the coefficient of determination is equivalent to 0.67 ($p < 0.0001$) and 0.64 ($p < 0.0001$), respectively). The age-determined accumulation of Cd is typical of many vertebrates. Some authors describe threefold age-associated increases in the Cd content in the liver of the mole (Pankakoski et al., 1993); other researchers (Komarnicki, 2000) suggest a five μg/g increase per year over the lifetime of the mole. In our case, the accumulation is considerably more intense than the rates mentioned above. During the first year of life, 94 μg/g and 256 μg/g of Cd are accumulated in the organisms of the moles dwelling in the background area and the buffer area, respectively. The respective annual increase in the Cd content is 2 μg/g and 22 μg/g. Even in the background area, the Cd content in the liver of adult moles is 2–110 times higher than in the liver of other mammals inhabiting the same territory (Mukhacheva, 2007).

The nearer the moles dwell to the pollution source, the higher the content of Cd and Zn in their liver (Table 4). Cd, however, should be considered as the element responsible for toxic impact; its contents reach 2.9, 5.4, and 3.2 times higher in age group I, II, and III, respectively.

The comparison of the results of the study with the data available in literature reveals that the content of Cd, Cu, and Zn in the liver of the moles inhabiting the background territory involved in the research is considerably higher than in the liver of the moles dwelling in non-polluted and moderately polluted areas in Finland (Pankakoski et al., 1993) and Austria (Komarnicki, 2000). The content of Cu and Zn is 1.3–1.7 times higher; the Cd content (in adult animals) is 6–8 times higher. Even the amount of Cd found in the tissues of the moles inhabiting the territory near Helsinki, a rather large city, is 1.9 times lower than the levels revealed as a result of our research. The considerable Cd content in the background territories revealed in our study is likely to be associated with the 300-year history of industrial development of the Ural region. The extensive atmospheric dispersion of cadmium

Table 4. Heavy metal content (μg/g) in the liver of the moles sampled at a different distance from the MUCSP

Element	Age group	Distance from the pollution source, km			
		30 (n = 6, 7, 9)	20 (n = 17, 13, 16)	10 (n = 20, 17, 15)	7 (n = 17, 8, 8)
Cu	I	34.8 ± 3.9	38.8 ± 5.3	30.9 ± 3.0	33.9 ± 3.5
		(24.3–49.8)	(14.2–86.3)	(11.5–65.0)	(16.4–76.6)
	II	13.9 ± 1.0	25.4 ± 3.2**	21.8 ± 1.4*	18.8 ± 1.8
		(10.9–18.5)	(14.4–57.2)	(11.1–31.6)	(12.0–25.2)
	III	29.9 ± 2.5	26.3 ± 2.7	35.8 ± 4.1	36.7 ± 8.4
		(21.1–42.4)	(9.9–44.0)	(12.7–71.2)	(14.9–84.7)
Pb	I	2.6 ± 0.6	5.0 ± 0.6	6.1 ± 0.6	5.9 ± 1.0
		(1.4–4.7)	(0.5–9.5)	(1.5–11.0)	(0.8–16.1)
	II	3.4 ± 0.7	3.0 ± 0.6	2.4 ± 0.4	3.7 ± 1.1
		(1.0–5.9)	(0.9–9.6)	(0.7–6.4)	(0.1–9.1)
	III	3.9 ± 0.8	2.9 ± 0.5	3.7 ± 0.5	4.8 ± 0.9
		(1.4–7.6)	(0.2–7.1)	(0.7–8.5)	(1.0–7.9)
Cd	I	9.0 ± 0.7	11.5 ± 1.2	20.8 ± 1.7**	26.5 ± 3.5***
		(6.6–10.9)	(4.3–21.3)	(5.9–34.1)	(7.8–61.7)
	II	34.5 ± 5.2	42.5 ± 4.0	169.7 ± 16.5***	186.8 ± 34.6***
		(22.6–63.5)	(22.8–70.4)	(62.9–264.0)	(76.3–345.0)
	III	103.0 ± 8.4	89.3 ± 8.0	309.3 ± 33.9***	325.0 ± 40.5***
		(75.7–136.3)	(39.5–136.9)	(155.0–547.4)	(159.0–534.6)
Zn	I	129.4 ± 25.8	154.1 ± 17.7	163.3 ± 14.9	163.6 ± 17.3
		(91.5–251.7)	(68.2–273.9)	(67.8–310.4)	(83.3–376.3)
	II	101.0 ± 3.2	158.6 ± 18.6*	137.4 ± 6.6	163.5 ± 16.6*
		(89.6–113.2)	(100.7–352.1)	(91.2–187.7)	(122.7–262.4)
	III	130.7 ± 12.8	119.1 ± 9.1	157.9 ± 11.8	155.7 ± 10.5
		(103.0–226.9)	(66.0–204.3)	(97.2–229.4)	(119.9–218.7)

Average ± error; minimum and maximum values are inside the parentheses; n represents the number of individuals in age groups I, II, and III, respectively. Significance levels for a 30 km distance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (Scheffe's method).

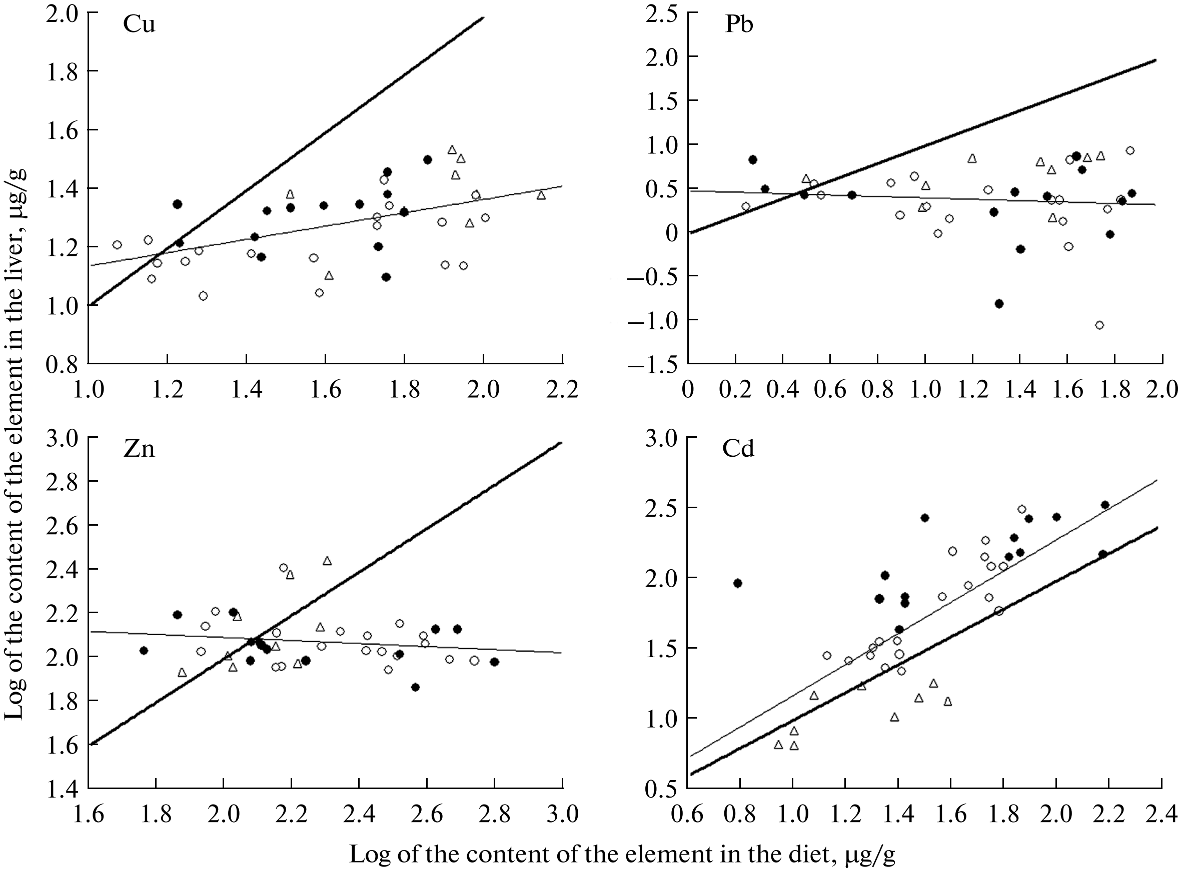
resulting from the metal processing industry caused a considerable technogenic geochemical cadmium anomaly in the Middle Urals. At the same time, our results can be correlated with the data on the metal content in the liver of the moles inhabiting the area near a zinc processing plant in the Netherlands and the control territory involved (Ma, 1987). However, the difference lies in the fact that the research conducted in the Netherlands revealed a 2–6 times higher Pb content and lower maximum Cd content typical of all the study areas.

Compared to those of five species of rodents and the common shrew dwelling in the area surrounding the MUCSP (Mukhacheva, 2007), the content levels of Zn and Cu are higher in the liver of the moles in the background territory and the polluted territory. The Pb content correlates with the rates found in the rodents and is 3.5 times lower than it is in the common shrew. While the Cd content is also considerably (six times) higher in the organisms of adult animals, one-month old moles are characterized by two times lower Cd levels than is the common shrew.

The highest Cd contents in the liver of the mole identified in the course of the study (547 μg/g) are close to the upper limit of the content of this element in all vertebrates. Among mammals, the highest Cd content was identified in the liver of *Sorex araneus*; it was equivalent to 800–1120 μg/g under natural conditions (Hunter et al., 1989) and 2059 μg/g as a result of experimental feeding (Dodds-Smith et al., 1992).

Distribution of Heavy Metals in the Food Chain

Moving from background area to the buffer area, the increase in the content of the physiologically essential metals is lower in the earthworms than in the soil: the Cu content is 3.2 and 6.6 times lower and the Zn content is 2.1 and 2.4 times lower for earthworms



The relationship between the amount of heavy metals in the liver and the gastric contents.

Regression equations: Cu—$y = 0.91 + 0.23x$, $r^2 = 0.26$; $p < 0.001$; Zn—$r^2 = 0.02$; $p = 0.36$; Pb—$r^2 = 0.009$; $p = 0.55$; Cd—$y = 0.06 + 1.12x$, $r^2 = 0.54$; $p < 0.001$. The triangles, unfilled circles, and filled circles indicate age groups I, II, and III, respectively. The bold diagonal line corresponds to BAF = 1.

and the soil, respectively. In contrast to this, the contents of the non-essential metals showed a greater increase in the organisms of the earthworms than in the soil, when comparing the background area to the buffer area: the Cd content is 4 and 3.2 times higher and the Pb content is 12.2 and 2.1 times higher for earthworms and the soil, respectively. Only Cd accumulation was observed: the content of this element is 8–10 times more significant in the tissues of the earthworms than in the soil and 3.9–4.5 times more important than in the forest litter.

The biological accumulation coefficient—or bioaccumulation factor (BAF)—which is generally used to characterize the rate of the penetration of a certain chemical element from the environment (food) into the organism, usually decreases if the content of the element in the food grows. However, the analysis of the relationship between the BAF and the content of the element in the diet can involve an artifact associated exclusively with the mathematical properties of this index (Berges, 1997). This is the reason that the regularities of bioaccumulation could be more precisely evaluated with the use of regression models (figure).

The analysis of the relationship between the content of an element in the liver and in the gastric contents of the mole shows the principal differences typical of accumulation of the studied elements. Even if the content of Zn, Cu, and Pb grows in food, the accumulation of these metals in the organism does not take place. Moreover, it is not related to the age of the animals. In its turn, a small positive angle measure of the regression line characterizing the Cu content suggests that the efficiency of the gastrointestinal barrier ensuring the internal homeostasis of the physiologically essential elements is relative, especially at a high rate of the element. The scatter in the values of the BAF deduced for Pb was the most considerable, particularly at a high rate of toxic load. Most species are not considered to be able to efficiently regulate the amount of Pb entering their organism (Mukhacheva, Bezel, 1995; Rogival et al., 2007). In addition, this metal is principally deposited in the osseous tissue with lower content in the liver. The data on the Pb content in soil and the tissues of earthworms similar to the results obtained in the course of our study suggests that the Pb accumulation in the liver of the mole be up to 40 μg/g (Ma, 1987). However, our research revealed that the sevenfold increase in the Pb content in the polluted area did not cause Pb accumulation in the liver of the mole.

The diagonal line in the figure (BAF = 1) shows the equivalent content of the metal in the liver and diet. Therefore, the points above and below this line indicate the accumulation and elimination of the element, respectively. The accumulation of Zn, Cu, and Pb takes place only if their respective amounts in the gastric contents are not significant. The reverse is true for Cd: higher Cd content in food leads to its accumulation in the liver. All the points above the diagonal line characterize individuals of 1–1.5 months old at the intensive growth phase determining the reduction in the values of the Cd content associated with the increasing weight of the liver.

In general, the Cd content of the different subjects of the study is more significant at a distance of 7 km from the MUCSP than at a distance of 30 km; it is 4.7 times higher in the litter, 3.2 times higher in the soil, 4 times higher in the tissues of the earthworms, 4.4 times higher in the food of the mole, and 3.8 times higher in the liver of the mole.

Since the mole principally consumes earthworms, a species accumulating large amounts of Cd, it can be considered evolutionarily adapted to high Cd contents. One of the mechanisms preventing the toxic effect is based on the efficient Cd binding to metallothioneins neutralizing toxicity of the metal (Vijver et al., 2004). In this manner, the species that feature this detoxification mechanism can accumulate Cd because it is not efficiently eliminated from the organism (Vijver et al., 2004; Veltman et al., 2007a).

Since the mole accumulates extremely high amounts of Cd that exceed those of all the other vertebrates, it could be dangerous for other links of in its food chain. However, the mole population is rather small and it does not play an important role in the diet of predators, compared to rodents. Thus, the mole species comprise only 0.05–4.5% of the food consumed by owls, kestrels, and buzzards (Haeck, 1969). The mole's sharp musky smell is considered the reason that other mammals do not include the mole in their diet. However, the mole can be found in the gastric contents of mustelids in 77% of cases during the years when the rodent population is scarce (Koryakov, 1962). Therefore, the mole can be considered the last Cd accumulating food chain link in terrestrial ecosystems.

CONCLUSIONS

The study deals with the soil–earthworm–European mole detritus food chain that is characterized by insignificant branching making it a convenient basis for analyzing the transfer of heavy metals. Intensive Cd accumulation in the last two links of this chain can be considered as one of its most important features. The Cd content is 8–10 times higher in the tissues of earthworms than in the soil and 4–6 times more significant in the liver of moles than in the tissues of earthworms. The study has revealed that the increase in the Cd content in the food leads to the disproportionate accumulation of this metal in the liver of the mole. The limited penetration of other metals (Zn, Cu, and Zn) into each of the subsequent trophic levels has been observed. However, it is necessary to take into consideration that the obtained rates of the accumulation of the metals in the organism of the mole are slightly overstated as the content of the elements has been evaluated only in the liver, the organ accumulating the largest amount of toxic agents, and not in other tissues. Since mole species are minimally consumed by predators, they can be considered as the last link of their food chain and therefore do not convey the threat to other vertebrates.

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