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Heavy metal effects on earthworms in different ecosystems

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Abstract Elevated heavy metals in soil are a serious environmental problem that threatens human health and other organisms. Earthworms are widely used as pollution bioindicators of soil ecosystems. The influence of heavy metal content on species composition and population density of earthworms in soils of urban and natural ecosystems has been previously studied. The accumulation of heavy metals in earthworm samples was measured using atomic absorption spectrometry. This study found representatives of 11 species of earthworms in biogeocenoses of the Zailiisky Alatau foothills. The low occurrence of earthworms in ecosystems with a maximum content of cadmium (0.25±0.0024 mg/kg), lead (16±0.70 mg/kg) and arsenic (2.84±0.05 mg/kg) was marked. The number and variety of species of lumbricidofauna differed in urban and natural habitats, indicating that increased heavy metal content substantially impacts earthworms. Earthworms can absorb heavy metals from contaminated soils, which simulates the actions of key elements in the body and causes diseases. Thus, one of the primary determining factors of a positive physical and chemical state of the soil is the number and species composition of earthworms. These data can be used to monitor soil contamination near industrial facilities.

Keywords: biogeocenosis, earthworms, heavy metals, species composition

1. Introduction

Earthworms (Lumbricina) are key soil architects shaping soil structure, microstructure, and fertility. Earthworms are involved in converting complex organic compounds into simple forms that can be used by plants by using nutrients and influencing microbial populations, soil moisture, and aeration through grinding and transporting plant material. In addition, they create a developed drilosphere (a zone saturated with microorganisms around the earthworm course), which contributes to the development of self-managed processes and symbiotic relationships of microflora and mesofauna (Gilyarov and Sivakumar 2015). In addition, as the prey of terrestrial vertebrates and birds, earthworms constitute an important link in the food chain that channels waste energy from dead and decaying organic matter to higher trophic levels (Zvyagintsev et al 2005). Earthworms are numerous and represented in all soil-dwelling and animal bioindicator ecosystem groups. By consuming the mycelium of pathogenic fungi, earthworms improve the environment, contribute to the binding of heavy metals and radionuclides into forms inaccessible to plants, and significantly increase nitrogen fixation by stimulating the activity of ammonificators, denitrificators, and nitrificators (Langdon et al 2003; Borges et al 2021). Soil is a complex heterogeneous system dominated by a solid phase consisting of soil organic matter, minerals, plants, microbes, and fauna. The activity of earthworms improves the soil's physical characteristics by burying and abandoning the formation of stabilized aggregates that allow easy water and air penetration (Garg et al 2009; Cheng and Wong 2002). Heavy metals are naturally present in low concentrations in soils. In high concentrations and due to their toxicity, they are major environmental pollutants that can pose a major problem.

In some cases, industrial, mining, and agricultural activities significantly contaminate soils with heavy metals. This is increasingly becoming a major environmental problem and is damaging ecosystems near emission sources. Most heavy metals eventually enter surface soil layers, where they bind to organic matter and reduce the mobility and bioavailability of useful trace elements in those parts of an ecosystem where essential biological processes, such as nutrient decomposition and mineralization, occur (Ruiz et al 2009; Lukkari et al 2006, 2004). Soils contaminated with heavy metals are an environmental problem that is considered a serious threat to human health and other organisms. Treating contaminated sites and reducing exposure to heavy metals through conventional procedures is costly and time-consuming (Narayanan et al 2016; Kapil et al 2019). It has been experimentally proven that excess heavy metals slow the rate of organic matter decomposition in soils. Nearly all heavy metals entering the atmosphere (except zinc

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and cadmium) accumulate in the litter, disrupting the life activity of organisms that perform decomposition (Rapport et al 1985; Sizmur and Hodson 2009). Earthworms constitute a significant proportion and important part of soil mesofauna (Bezkorovainaya 2001; Liu et al 2005). As ecological participants in the circulation and release of detritus-associated nutrients, they are reliable indicators for the ecological monitoring of soil quality and assessing soil contamination. This is confirmed by their wide use in soil ecotoxicological studies (Gilyarov and Krivolutskiy 1971; Straalen et al 2001; Shefali and Yadav 2018; Wen et al 2004).

Heavy metals from the soil enter earthworms through the skin and during ingestion and digestion of contaminated soil. Heavy contamination, especially combined with soil acidification, is detrimental to the earthworm population and causes a decrease in the population, followed by complete disappearance. These reactions depend on the pollutant dose and the study site's contamination duration. These factors support the relevance of studying earthworms as bioindicators of contaminated areas (Babenko 2013; Sokolova 2010). To date, the composition and structure of soil components of the mesofauna soils of Almaty City and the Almaty region remain virtually unstudied. There is minimal research on the impact of soil contamination on the state of ecosystems (Novak 2015; Isayeva 2018; Mustafayev 2015; Karimov et al 2021), which was a reason for choosing this topic and study object. The main purpose of this study was to investigate the effect of heavy metals on the abundance and species diversity of soil mesofauna in urban and background (natural) biocenoses of Almaty City and the Almaty region.

2. Materials and Methods

This research was conducted at the Department of Biology, at Abai Kazakh National pedagogical university. Soil ecological surveys were conducted between May and September in 2018-2021, during the active plant growing season, as this is the period of greatest pedobiont activity. Data comparison of the distribution of soil mesofauna, for which the lumbricide group is the most represented, in different seasons allows us to study seasonal fluctuations in numbers of this group. Suburban and urban ecosystems were selected to compare lumbricides abundance and species diversity. The areas of research included forest biotopes and agro-ecosystems. Soil samples from eight sites of Almaty City and the Almaty region were obtained and studied as follows: three background (suburban) sites on the northern slopes of Iliyskiy Alatau (spruce forest, mixed forest, and alpine meadows) and five experimental (urban) sites near oil depots, gas stations, thermal power plants, and along busy urban highways and national highways located in different parts of the city. Lumbricides were counted using a method commonly accepted in soil zoological studies by Gilyarov. Soil invertebrates were counted by sampling a 0.25 m^2 area to the depth of soil invertebrates (up to 1 m).

Twelve samples were collected at each test plot. Mesofauna in the field was counted by manual disassembly of samples taken in layers (litter, 0-5 cm, 5-10 cm, 10-20 cm, 20-30 cm, and 30-40 cm). The earthworms were fixed with a weak (0.5%) formalin solution. All materials were labeled, which indicated the date of sampling, site name, site characteristics, and sample number in the numerator and layer in the denominator, followed by cameral determination of group identity. The identification of lumbricides was performed following the identification tables by Vsevolodova-Perel and Vsevolodova-Perel (1997) and Matveeva (1982). During the study at the population level, indicators, such as species composition, number of species, occurrence density (number of individuals per unit area), the biomass of lumbricides of the studied biogeocenoses, and occurrence and number of different groups of paedobionts, were considered. Species occurrence was determined as the ratio of the percentage of samples in which lumbricides were detected compared to the total number of samples collected (Striganova 1987). Abundance was determined as the total number of individuals found in a given area (the total number of soil mesofauna). Lumbricide biomass was determined by directly weighing organisms. The heavy metal content in the soil of each study area was determined by atomic absorption spectrometry at the laboratory of the non-governmental "Kazecology" consulting organization (Almaty, Republic of Kazakhstan). The mathematical reliability of the content of toxic elements was determined using standard methods (Ivanter and Korosov 2010).

3. Results

In the study ecosystems of Almaty City and foothills of Zailiisky Alatau, we found representatives of 11 species of the *Lumbricidae* family and class Oligochaeta. Four of these species, *Lumbricus rubellus* (Hoffmeister 1843), *Lumbricus castaneus* (Savigni 1826), *Octolasion lacteum* (Orley 1885), and *Dendrobaena octaedra* (Savigni 1826), are widely distributed and environmentally plastic species typical of forest biocenoses of the mixed forest subzone of Zailiisky Alatau. The greatest number of family Lumbricidae species found in the Almaty region belong to the genus *Lumbricus*. Species of this genus make up almost 30% of lumbricidofauna of biogeocenoses as follows: *Lumbricus rubellus, Lumbricus castaneus,* and *Lumbricus terrestris*. The most widely distributed small red worm species is *Lumbricus rubellus* (Hoffmeister 1843). The body length of this species can reach 50-150 mm and widths up to 4-6 mm. The morphology peculiarities of this species are a pronounced purple pigmentation of the dorsal side of the body, especially at the anterior end of the body, and a flattened tail end (Vsevolodova-Perel 1997; Ivanter and Korosov 2010).

Lumbricus rubellus belongs to bedding species that prefer humus-rich and moist soil. Representatives of this species were found in all study biogeocenoses of the Almaty region and the Zailiisky Alatau. Such a wide distribution of this species is indicated by deciduous tree species forming rich forest litter predominating in these territories. Thus, in birch and mixed forests, the proportion of *Lumbricus rubellus* ranged from 7.4-29%. However, the small red worm was also found in suburban and urban agrocenoses, where this species accounts for 21 to 24.8% of the total lumbricid fauna. This may be due to the relatively high humus content in soils of these biogeocenoses. This was confirmed by our soil chemical analysis of these biocenoses. The mass fraction of organic matter in these samples ranged from 3.01 to 6.07%. The largest of the discovered *lumbricidae* is the *Lumbricus terrestris* (Linnaeus 1758), with a body length of 90-300 mm and a width of 6-9 mm. A purple body pigmentation characterizes this species up to the girdle lighter than *L. rubellus* and a darker median stripe behind. *Lumbricus terrestris*, a large red worm, is a mink that lives in deeper soil layers (Athmann et al 2017). This species also prefers humus-rich soils. *Lumbricus terrestris* was not found in the birch forest and agrocenosis of the Karasai District, which may be due to the relatively low content of humus in the soils of these biocenoses. A soil chemical analysis confirmed this assumption and showed that the humus content in these soils was 3.47 - 3.51%, and the moisture content was up to 12.08%.

A rather low occurrence is marked for representatives of the species *Octolasion lacteum* (Orley) genus Octolasion. This species has practically no pigment, although specimens with a light gray and bluish tint are often found with a body length of 30-180 mm and a thickness of 2-8 mm. By feeding only on soil humus, the upper tiered cosmopolitan *Octolasion lacteum* is capable of inhabiting overwatered soils and easily tolerates prolonged periods of oxygen deficiency. In Trans-Ili Alatau, this species was found in the mixed forest, where the soil humidity during the study period was 24.68%. *Octolasion lacteum* accounted for 16.9% of all lumbricides found in this biocenosis. During our research, we found a significant difference in the species composition of soil mesofaunas in urban and background biocenoses (Table 1). This may be due to the great anthropogenic impact on urban biogeocenoses and, consequently, the heavy pollution of soils with heavy metals.

The chemical composition of soil samples obtained from study sites was analyzed. In determining the toxic element content in urban and background (natural) biocenoses, four main elements affecting species and the numerical composition of mesofauna were identified: lead, cadmium, arsenic, and mercury (Table 2).

Table 1 Species composition and average abundance of soil mesofauna in suburban and urban ecosystems of the Zailiisky Alatau foothills (ex/m²).

Types of lumbricides	Suburban biocenoses			Urban biocenoses				
	1	2	3	4	5	6	7	8
Lumbriscus gubellus (Hoffinister 1843)	1.31	1.24	3.54	0.57	0.68	0.47	0.78	+
Lumbriscus castaneus (Savigni 1826)	0.89	0.84	1.7	0.36	0.57	0.26	0.62	+
Lumbricus terrestris (Linnaeus 1758)	-	0.72	3.12	0.66	+	0.32	1.5	-
Eisenia fetida (Savigni 1826)	0.62	-	1.32	0.45	0.92	-	0.72	-
Eisenia nordenskioldi (Eisen 1879)	0.24	0.11	-	-	-	+	-	-
Aroghedea goosea (Savigni 1826)	1.28	1.07	1.58	-	-	-	0.42	+
Arogena caliginosa (Savigni 1826)	0.98	1.63	+	-	-	-	0.12	0.31
Ostolanium lacaeum (Orley 1885)	-	0.84	+	-	-	-	1.1	0.7
Nisodrilus caliginosus (Eisen 1874)	1.56	1.2	+	0.13	1.02	+	+	+
Niñodrilus longus (Ude 1885)	0.93	0.25	+	-	-	+	-	-
Dendrobane remains (Savigni 1826)	1.32	-	2.02	0.51	0.14	0.7	0.5	-

Note: Suburban (background) biocenoses and northern slopes of Ili Alatau: 1 - spruce forest; 2 - mixed forest; 3 - alpine meadows. Urban biocenoses are soils near petroleum storage depots, petrol stations, and thermal power plants: 4 - soils near oil depots; 5 - soils near petrol stations located in different parts of the city; 6 - soils near thermal power plants; 7 - soils along city busy highways; 8 - soils along national highways.

Table 2 The content of heavy metals (mg/kg) in soils of study sites.

Sample site	Cadmium (Cd)	Lead (PB)	Arsenic (As)	Mercury (Hg)
1	0.05±0.0013	0.9±0.1001	0.24±0.0150	0.010±00060**
2	0.04±0.0017	0.6±0.1502	0.14±0.0140	0.009±0.0300**
3	0.03±0.0008	0.5±0.1501	0.06±0.0020	0.001±0.0120**
4	0.25±0.0024	11.2±0.1703	2.84±0.0501	0.03±0.0462
5	0.20±0.0017	9.3±0.2201	1.7±0.0802	0.016±0.0031
6	0.23±0.0027	16±0.7002	2.8±0.0701	0.048±0.0120
7	0.11±0.0010	6.7±0.1202	2.7±0.1103	0.02±0.0042
8	0.17±0.0018	10.7±0.1101	1.53±0.0201	0.018±0.0130**

Standard error of the mean value P < 0.01, except **- P > 0.99

4. Discussion

In industrial pollution, large amounts of heavy metals enter the surface soil layers and, when bound to the organic component, reduce the bioavailability of trace elements necessary for ecosystems' most important biological processes. Due to their anatomical and physiological characteristics, earthworms are among the first to react to this type of pollution (Ruiz et al 2009; Lukkari et al 2006; Lukkari et al 2004; Kapil et al 2019; Rapport et al 1985; Sizmur and Hodson 2009).

Results of our research showed direct dependence of lumbricid fauna diversity and density of urban biocenoses on the proximity of industrial objects. Thus, soil samples collected within 1 km of thermal power plants were completely devoid of earthworms, and in soils near oil depots, their numbers were also insignificant. We found that the total density of earthworms is inversely proportional to the concentration of heavy metals in soils. Some earthworm species were more sensitive to soil contamination by heavy metals and were absent in areas with more resistant species. For example, heavy metal insensitive species such as *Lumbricus rubellus, Lumbricus castaneus,* and *Lumbricus terrestris* were present in soils near petrol stations. At the same time, representatives of *Aporrectodea rosea* and *Aporrectodea caliginosa* were absent. The most widespread pollution of soils by heavy metals is related to the type of fuel used by motor vehicles. Vehicles using gas as fuel account for only 15% of all motor vehicles in Kazakhstan. The share of trucks with diesel engines in Kazakhstan does not exceed 30% on average, whereas the remaining 55% of vehicles use gasoline as fuel. Thus, most motor transport pollutes the environment and emits exhaust gases containing toxic elements. In addition, emissions from thermal power plants contain toxic substances that negatively affect all living organisms. In soil-zoological studies, it is important to determine heavy metals in the soil as many of them accumulate in the litter and negatively affect the vital activity of soil organisms.

Studies have shown that the content of such toxic elements, such as lead, cadmium, arsenic, and mercury, is within the sensitivity of the control method. However, maximum contents of cadmium and arsenic are noted in soils near oil depots. The cadmium content presented in this study was 0.25±0.0024 mg/kg, and the arsenic content was 2.84±0.05 mg/kg, which may be a consequence of burying various household wastes in this area due to a lack of a system/infrastructure for their disposal. In soils near the thermal power plant of the Algabas settlement of the Karasai District, a maximum amount of lead and mercury was detected and reached 16±0.70 mg/kg and 0.048±0.012mg/kg, respectively. Thus, we can conclude that in urban biocenoses, litter is most exposed to anthropogenic impact and accumulation of pollutants (in particular, heavy metals), so species inhabiting these areas are the most sensitive.

5. Conclusions

Differences in the abundance and species diversity of lumbricidofauna of urban and natural ecosystems revealed during this study indicate a significant impact of increased content of heavy metals in soil on the life activity of earthworms. In natural biogeocenoses, almost all species of earthworms were found in maximum quantity. In contrast, their number and species composition significantly decreased in urban ecosystems, and species most sensitive to soil pollution, such as *Aporrectodea rosea* and *Aporrectodea caliginosa*, were completely absent. Na increased anthropogenic load explains this on urban ecosystems due to many motor vehicles, oil depots, factories, and heating plants polluting the environment with heavy metals. Earthworms can absorb heavy metals from contaminated soils, mimicking the action of major elements in the body and interfering with the metabolic process, leading to diseases. Thus, the number and species composition of the earthworm population can be used as one of the main criteria for determining the soil's favorable physical and chemical states.

Conflict of Interest

The authors declare that they have no conflict of interest.

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