

## *Bioaccumulation of some heavy metals in plants and insects*

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**Abstract.** The study in this scientific paper aimed to analyze the content of potentially toxic elements – heavy metals lead, cadmium, and zinc in soil, plant samples, and insect samples from three different areas: an uninhabited area, an industrial zone, and an urbanized area. Using inductively coupled plasma mass spectrometry (ICP-MS), it was found that the levels of heavy metals in soil, plants, and insects from the three studied areas were higher for lead, cadmium, and zinc in the industrial zone – soil: Pb - 850, Cd - 21.3, Zn - 996 (mg/kg); plants: Pb - 191, Cd - 7.79, Zn - 298 (mg/kg); insects: Pb - 24.5, Cd - 1.32, Zn - 160 (mg/kg). Based on the obtained data, the degree of bioaccumulation of lead, cadmium, and zinc was traced using the bioaccumulation factor (BAF) through the pathway: soil, plants, and insects. A BAF < 1 was found for plants, and a bioaccumulation process for cadmium and zinc was observed in two insect species (BAF > 2).

**Key words:** soil, plants, insects, heavy metals.

### **Introduction**

As a dominant group of modern fauna on Earth, insects are one of the most important species in global biodiversity. They play an essential ecological role in maintaining the ecosystem. Increasing attention is being paid to the potential effects of heavy metal contamination on insects. Heavy metals can affect insects in different ways.

It has been proven that insects can be contaminated through respiration, absorption of ions through body coverings, and food intake (Hongxia et al., 2007). Host plants contaminated with copper and lead have a negative impact on the fertility of *Brevicoryne brassicae* L. (Görür, 2006).

Some chemical elements become toxicologically significant when they are accumulated in plants and taken up by animals. Such elements are copper, lead, fluorine, molybdenum and selenium. Large amounts of them enter plants when there is a high percentage in the soil (Simeonov, 1987).

Plants can accumulate and retain heavy metals in their roots and thus reduce the harmful effects on other organs as long as possible. One of the most harmful to humans and animals, cadmium ion, moves easily from the roots to the stems and leaves, posing a risk of toxicosis. The ions of heavy metals Zn, Mn, and Cu accumulate mainly in cell walls as silicates (Bringerzu, 1999).

At normal background soil content, these metals do not affect plants, but in high concentrations they become phytotoxic. These include lead, cadmium, mercury, and cesium (NBU, 2012).

When consumed by insects, these metals are likely to enter insects' bodies through the food chain. Insects differ in their ability to accumulate heavy metals and demonstrate significant differences in their tolerance to them. In their studies, Lagisz & Laskowski (2008) found that the carabid beetles *Pterostichus oblongopunctatus* (Coleoptera, Carabidae) that inhabited metal-polluted environments altered their vital signs compared to those of the populations in the reference area.

The excessive metal accumulation in insects can cause not only variations in cellular ultrastructure and alteration of genetic homeostasis, but it can also induce cell apoptosis and affect cell viability and proliferation. (Hongxia et al., 2007). Heavy metals and insecticides have a clear effect on the hemocyte profile of insects (Borowska & Pyza, 2011, Kurt & Kayış, 2015).

Moroń et al. (2014) found a clear relation between increasing metal concentrations in the soil layer and increased negative impacts on life cycle determinants of *Osmia rufa*. In some scientific studies, insects are considered as bioindicators. In monitoring the accumulation of heavy metals (Cd, Cr, Cu, Ni, and Zn) in dragonflies (*Crocothemis servilia*), grasshoppers (*Oxya hyla hyla*), and butterflies (*Danaus chrysippus*) in industrial areas, high values above the permissible levels have been reported, with relatively higher concentrations of metals found in Orthoptera than in Odonata and Lepidoptera (Azam et al., 2015).

Of all insects, grasshoppers and crickets from the order Orthoptera form a significant biomass and they are an important food source for other organisms. In 2009, the number of Orthoptera

species in Bulgaria was 213 (Chobanov, 2009). Both adults and larvae from the order Coleoptera are consumed, while species from the orders Orthoptera, Homoptera, Isoptera, and Hemiptera are primarily consumed as adults (Cerritos, 2009).

About 80 species of grasshoppers are consumed worldwide (van Huis et al., 2013). Some authors think that the only risk associated with the consumption of grasshoppers is poisoning, as insects may have been treated with pesticides (Saeed et al., 1993).

Although insects account for the largest proportion of biodiversity in all ecosystems, they remain the least studied organisms (Johnson, 2010).

The present study was prompted by several questions, primarily concerning the degree of accumulation of heavy metals and metalloids in insects, specifically grasshoppers and plants, along the trophic transfer pathway. In this sense, the research aims to analyze the content of three potentially toxic metals and to trace their bioaccumulation in soils, plants, and orthopterous insects from three different sites. Since the adults of most species of the order Orthoptera are herbivores (Bechev, 2021), and the phenology of individual insect species corresponds to the plant rhythmicity (Peshev, 1975), the plant species on which the insects fed are included in the study.

### Materials and methods

Three different sampling sites were selected for the study, including the following areas: an uninhabited site (next to the village of Gluhar, Kardzhali district, GPS: 41°34'19.9"N 25°23'29.9"E - Fig. 1); an environmentally polluted site (an industrial zone near the city of Plovdiv, GPS: 42°03'33.1"N 24°49'17.5"E - Fig. 2); an urban site (the city of Plovdiv, GPS: 42°09'14.0"N 24°43'33.9"E - Fig. 3).



Fig. 1. Village of Gluhar



Fig. 2. Industrial zone



Fig. 3. City of Plovdiv

Plant samples include leaf and stem mass of the following species: *Persicaria hydropiper* (L.) Delarbre [*Polygonum hydropiper* L.] – Gluhar village – Fig. 4; *Convolvulus arvensis* L. – southern industrial zone, Plovdiv – Fig. 5; *Rubus caesius* L. – urban region, Plovdiv – Fig. 6. The insect samples

include three species of grasshoppers from the order Orthoptera, family Acrididae: *Sphingonotus caeruleus* (Linnaeus, 1767) – Gluhar village – Fig. 7; *Chorthippus biguttulus* (Linnaeus, 1758) – southern industrial zone, Plovdiv – Fig. 8; *Aiolopus strepens* (Latreille, 1804) – urban region, Plovdiv – Fig. 9.



Fig. 4. *Persicaria hydropiper*



Fig. 5. *Convolvulus arvensis*



Fig. 6. *Rubus caesius*



Fig. 7. *Sphingonotus caeruleus*



Fig. 8. *Chorthippus biguttulus*



Fig. 9. *Aiolopus strepens*

The material was collected in the natural habitats of the insects during the autumn period of 2022, by catching them in the plant areas, and collections were made with an entomological bag by “mowing”.

The analyses of soil, plant and insect samples were carried out in the laboratories of the Faculty of Chemistry at the Department of Analytical and Computational Chemistry, at the University of Plovdiv. The samples are in accordance with the requirements and regulations of the EU for competence, testing and calibration. The active soil reaction in the respective area was measured on site. Indicators for assessing the current state of the soils for field studies were used. The soil samples were taken at a depth of 0-20 cm, with dimensions of 50x50 cm, after removal of the surface layer (Naskova et al., 2017; Malcheva, 2020). The spatial variability of the soils was achieved by 5 layer-by-layer sampling in one site and then forming an average sample for analysis (Petrova et al., 2022).

The quantification of the heavy metal contents – Pb, Cd, and Zn in the samples was performed by inductively coupled plasma mass spec-

trometry (ICP-MS), using Agilent 7700 ICP-MS (2009). The data for each individual sample are the arithmetic mean of three analytical measurements. Results are presented in mg/kg, as the mean of three parallel measurements with the corresponding relative standard deviation in percentage (RSD%). Values below the limit of quantification are given as < the corresponding limit of quantification. (LOQ).

The bioaccumulation factor (BAF), generally used in practice, was applied to study the extent of bioaccumulation (Nikanorov et al., 1985; Walker, 1990; Sijm & Hermens, 2000). Depending on the BAF value, organisms can be defined as follows: macroconcentrators /BAF>2/, microconcentrators /BAF=1 to 2/, and deconcentrators /BAF<1/ (Nikanorov et al., 1985; Dallinger, 1993; Sijm & Hermens, 2000).

### Results and Discussion

After the analyses on the active reaction of the soils for pH, the following values were recorded: pH=6.8 /Gluhar village/, pH=8 Plovdiv /industrial zone/, and pH=7.7 /Plovdiv city/. The active

reaction ranges from slightly acidic to neutral /Gluhar village/, and slightly to moderately alkaline /Plovdiv - industrial zone and the city of Plovdiv/. Similar studies have been done by Nikolov et al. (2019), in which the soil profile in the city of Plovdiv ranges from very slightly alkaline to moderately alkaline.

Analyzing the data on the content of lead, cadmium, and zinc in the three regions, in accordance with the MPC under Regulation 3, the values for lead, cadmium, and zinc increased only in the Plovdiv region - industrial zone (Table 1).

**Table 1.** Lead, cadmium and zinc content in soil samples.

Research area	Heavy metals (mg/kg)		
	Lead (RSD%)	Cadmium (RSD%)	Zinc (RSD%)
Gluhar Vill.	29.9 (5.2%)	0.57 (4.5%)	154 (4.5%)
Plovdiv /IZ/	850 (5.1%)	21.3 (5.5%)	996 (4.6%)
Plovdiv City	34.5 (4.8%)	0.31 (9%)	72.8 (5.5%)

The concentrations of lead, cadmium, and zinc measured in Plovdiv - industrial zone - were higher than the regulated maximum permissible concentrations (MPC) in soils of arable land and permanent grasslands according to Regulation No. 3 of August 1, 2008, on the standards for permissible content of harmful substances in soils. A slight excess for lead has also been found by other authors at a site in the KCM area (KCM - Bulgarian abbreviation for Non-Ferrous Metals Plant), as the site is located at the exit of Plovdiv on the road to the town of Asenovgrad, so the presence of lead, under the influence of emissions from "KCM 2000" JSC with an appropriate wind direction, can be assumed (Nikolov et al., 2019).

The concentrations of lead, cadmium, and zinc measured in the area of Gluhar village and

the city of Plovdiv were significantly lower than the regulated maximum permissible concentrations and they do not pose a danger to the environment and human health. In similar analyses of soils from the Kardzhali region, a geoaccumulation index was calculated for all transition metals, which shows that the most significant pollutant is Cd, and all the soils being "extremely contaminated" by this metal, followed by Pb and Zn (DN 14-7/2017/). In cities and their surrounding areas, as being subjects to urbanization pressure, there were reported increased concentrations of heavy metals in the surface layers of soils (Bloemen et al., 1995; Sutherland et al., 2000).

In plant samples, the highest values for lead, cadmium, and zinc were reported in the Plovdiv region - industrial zone (Table 2).

**Table 2.** Lead, cadmium and zinc content in plant samples.

Plants and Research area	Heavy metals (mg/kg)		
	Lead (RSD%)	Cadmium (RSD%)	Zinc (RSD%)
<i>Persicaria hydropiper</i> Gluhar Vill.	2.36 (6.7%)	0.25 (8.7%)	39.9 (3.4%)
<i>Convolvulus arvensis</i> Plovdiv /IZ/	191 (3.5%)	7.79 (2.4%)	298 (2.1%)
<i>Rubus caesius</i> Plovdiv City	3.01 (5.4%)	<0.1 (-)	22.9 (3.9%)

The heavy metals in the plant species were compared to Regulation (EO) No 1881/2006, and according to this regulation, only the values for cadmium (MPC - 0.2 mg/kg) can be compared. Since the three plant species are defined as herbs

(Medicinal Plants Act, 2023), according to Regulation (EO) No 1881/2006, Cd is above the permissible values for *Persicaria hydropiper* in Gluhar village (0.25 mg/kg) and for *Convolvulus arvensis* in Plovdiv - industrial zone (7.79 mg/kg).

Plants show anatomical selectivity in their uptake of heavy metals. For example, aerosol-deposited lead does not pass through the cuticle of higher plants, but adheres to the surface of the leaves (Zimdahl & Koeppel, 1977).

Lead, cadmium, and zinc content in the insect samples are shown in Table 3. In insect samples, the highest values for lead, cadmium, and zinc were reported in the Plovdiv industrial zone re-

gion. Since our country does not have regulated values for MPC of heavy metals in insects, the results for lead and cadmium content could be compared with the MPC for food additives, according to Regulation (EU) No. 2023/915, given that insects can be included in foods as additives – powder, suspension, etc. and Regulation No. 31 on maximum permissible amounts of contaminants in foods for zinc.

**Table 3.** Lead, cadmium and zinc content in insect samples.

Insects And Research area	Heavy metals (mg/kg)		
	Lead (RSD%)	Cadmium (RSD%)	Zinc (RSD%)
<i>Sphingonotus caeruleans</i> Gluhar Vill.	1.67 (7.2%)	0.90 (6%)	106 (4.8%)
<i>Chorthippus biguttulus</i> Plovdiv /IZ/	24.5 (4.4%)	1.32 (3.3%)	160 (6.0%)
<i>Aiolopus strepens</i> Plovdiv City	0.71 (8.4%)	0.15 (8.8%)	142 (3.3%)

According to Regulation (EU) No. 2023/915, the following values are taken into account for maximum permissible amounts of contaminants in food: for lead and cadmium, compared with food additives (MPC, Pb – 3.0 mg/kg; MPC, Cd – 1.0 mg/kg) in *Chorthippus biguttulus* – Plovdiv, industrial zone. According to Regulation No. 31 for zinc, the maximum permissible amounts were reported, compared to “other foods” (MPC, Zn – 50.0 mg/kg) in the three insect species from the three regions.

It is worth noting that, despite their nutritional value, insects may contain heavy metals. For example, recent studies have shown that grasshoppers can contain high and sometimes dangerous amounts of lead. (Cohen et al., 2009). The accumulation of lead in soil and plants causes

numerous negative effects as a result of its toxicity. (Pourrut et al., 2011; Petrova et al., 2024; Zou et al., 2018). Therefore, it is necessary to study methods that can prevent or reduce the concentrations of metals and metalloids in certain sources from which they can enter organisms along the food chain.

The bioaccumulation factor was calculated based on the average content of heavy metals in plants, relative to their content in the soil, as well as the average content of heavy metals in insects, relative to their content in plants (Table 4).

The analyzes obtained for the bioaccumulation factor (BAF) show selective bioaccumulation for lead, cadmium, and zinc in plants and insects from the three regions.

**Table 4.** Bioaccumulation factor (BAF), expressed as the ratio of heavy metal content between plants/soil (p/s) and insects/plants (i/p).

Research area	Heavy metals (mg/kg)					
	Pb		Cd		Zn	
	p/s	i/p	p/s	i/p	p/s	i/p
Gluhar Vill.	0.0789	0.7076	0.4385	3.6	0.2590	2.6566
Plovdiv/IZ/	0.2247	0.1282	0.3657	0.1694	0.2991	0.5369
Plovdiv City	0.0872	0.2358	0.3225	1.5	0.3145	6.2008

The plants in the region Gluhar village had a low bioaccumulation process and they are defined as deconcentrators (BAF<1).

In the same region, it was observed a bioaccumulation process for insects, in which they are defined as macroconcentrators for cadmium /3.6/ and zinc /2.6/ (BAF>2). Although there was a bioaccumulation process for lead, the bioaccumulation factor values identify insects as deconcentrators (BAF<1).

For the region Plovdiv - industrial zone, there was a bioaccumulation process for zinc along the trophic chain plants → insects, but overall it was observed a low degree of accumulation along the path soil → plants → insects in this region (BAF<1). The high content of lead, cadmium, and zinc in plants and insects in the region Plovdiv-industrial zone is probably due to other sources, besides the amounts taken up by the soil. For example, it is believed that 90-99% of Pb in the leaf mass is due to aerosol deposition and a very small part of Pb in soils is available for uptake through the root system via the xylem (Doychinova & Atanasova, 2013).

In the urban region Plovdiv, the plants are deconcentrators for the three metals, while the insects are zinc macroconcentrators /6.2/ (BAF>2), cadmium microconcentrators /1.5/ (BAF=1 to 2), and lead deconcentrators /0.08/ (BAF<1).

According to the analyses, BAF reached the highest concentrations in insects for cadmium (Gluhar village), zinc (Gluhar village and Plovdiv city), compared to its levels in plants.

It was assumed that the plants that contain more than 0.1% Ni, Co, Cu, Cr, and Pb, or 1% Zn in dry leaf substance, can be considered hyperaccumulators, regardless of the metal content in the soil (Baker & Walker, 1990).

### Conclusions

In conclusion, it can be summarized that even low concentrations of heavy metals in the soil can lead to their accumulation in higher trophic levels.

In the plant samples, the three metals exhibited selective distribution, with propensity for accumulation of lead, cadmium, and zinc in the species *Convolvulus arvensis*. In the insect samples, the highest concentrations of lead, cadmium, and zinc were found in the species *Chorthippus biguttulus*. For the soil → plant transfer pathway, a bioaccumulation factor (BAF)<1 was established for

lead, cadmium, and zinc in plants from all three investigated sites. A BAF>2 was established in *Sphingonotus caeruleus* /Gluhar village/ for cadmium and zinc, while in *Aiolopus strepens* /Plovdiv/, a BAF>2 was established for zinc, and a BAF=1 to 2 in *Aiolopus strepens* /Plovdiv/ for cadmium.

Grasshoppers are classified as agricultural pests, and therefore, the probability of being treated with insecticides is high. In this context, the potential for bioaccumulation of various pollutants, including heavy metals and metalloids in these insects, is significant. The ability of heavy metals to transfer from the abiotic environment to living organisms and accumulate within the biota at various trophic levels can lead to contamination of food chains, posing an ecological risk with serious implications for wildlife.

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