Comparison of Heavy Metal Content in *Artemisia austriaca* in Various Impact Zones

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**ABSTRACT:** A study on *Artemisia austriaca* of two anthropogenically heavy metal-polluted impact zones of the Rostov region, namely Lake Atamanskoye and Novocherkasskaya Power Station, was conducted. The influence of soil pollution on the Pb, Zn, and Cu accumulation in various organs of *A. austriaca*, which is widespread in the studied territories, was established. An extremely high level of Zn content (3051 mg/kg) was observed in the soils of the impact zone of Lake Atamanskoe, as well as an excess over the maximum permissible level for Pb and Cu (32 and 132 mg/kg accordingly). The distribution coefficient (DC) of heavy metal translocation showed the highest mobility of Zn (DC \( \geq 1 \) in 9 out of 11 sites) and the smallest of Pb (DC \( \geq 1 \) in 4 out of 11 sites) in plants of the Novocherkasskaya Power Station impact zone. The zone of increased pollution around Lake Atamanskoye was 1.5 km, which was much smaller than the Novocherkasskaya Power Station zone of high pollution (5 km). However, vehicle emissions accumulated in the soil over the past decades had a greater effect on the Pb translocation than atmospheric emissions of the enterprise.

1. INTRODUCTION

Nowadays, industrial enterprises are the main source of the anthropogenic pollution of the natural environment. One of the most common pollutants contained in industrial emissions is heavy metals (HMs). Atmospheric emissions from metallurgical plants and power stations, due to the distribution of high concentrations of HMs over considerable distances, are responsible for subjecting zones to anthropogenic stress. The foliar uptake of HM by plants can play a significant role in anthropogenic atmospheric pollution, including contamination with fine soil particles enriched with chemical elements.

Industrial enterprises closely adjacent to an agricultural land pose risks to the health of the people living in the region. Extensive industrial use of natural reservoirs such as sedimentation tanks and sludge collectors, in turn, leads to more localized zones with an extremely high level of hydrogenic pollution. In the industrial impact zones of the Rostov region of Russia, one of the most commonly grown medicinal plants belonging to the family Asteraceae around Lake Atamanskoye and Novocherkasskaya Power Station is Austrian wormwood (*Artemisia austriaca* Pall. ex. Wild). It is widely used in medicine to produce diaphoretic, choleretic, diuretic, anticonvulsant, antipyretic, and antiemetic drugs. *A. austriaca* plants in disturbed ecosystems can be contaminated with various toxicants, including HMs.

The study on HM accumulation by *A. austriaca* plants is one of the most important, and at the same time, the most challenging aspects of environmental decontamination of affected areas. *A. austriaca* is the indicator plant species of pollution with chemical elements. Various plant species, in addition to the selective absorption of certain elements from the soil and HM localization in various organs, are also characterized by specific mechanisms of resistance to anthropogenic pollution. *Artemisia* species and especially *Abronia fragrans* growing on contaminated soils are able to accumulate Zn, Cd, and Cu in their aboveground parts without displaying any toxicity symptoms. The HM accumulation in plants depends on their biological (species) characteristics and on external natural and anthropogenic factors. Thus, the qualitative characterization of HM-induced inhibition of plants

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based on external signs seems insufficient, due to the presence of evolutionarily developed mechanisms of adaptation to adverse environmental conditions.

The interest in herbal medicine has sharply increased all over the world in recent decades due to its complex of relatively simple technologies to prevent and treat diseases using medicinal plants.\textsuperscript{15,16} At present, at least 80\% of the world population use herbal remedies.\textsuperscript{16}−\textsuperscript{18} Therefore, the studies on the elemental chemical composition of medicinal plants (herbal medicines, i.e., herbal remedies) are currently of particular relevance due to the continuously detected side effects of modern synthetic medicines and the lack of drugs for treating chronic and long-term diseases. However, reduced efficacy, as well as the side effects (deleterious) of herbal remedies, is possible, which is associated with the poor quality of medicinal raw materials and final products.\textsuperscript{16}

The problem of medicinal raw material contamination with inorganic contaminants is known to be of an apparent regional character and is especially relevant for the regions of medicinal raw material procurement.\textsuperscript{19−21} Large industrial enterprises in the region increase the chances of collecting raw materials contaminated with HMs. The presence of HMs may compromise the medicinal value and induce toxicity in humans after consumption during disease treatment. To ensure the safety of medicinal raw materials and medicinal products, it is necessary to determine the content of HMs in them, along with traditional pharmacopoeial indicators. Hence, laboratory
studies are necessary to determine HM content in herbal raw materials.

Therefore, the objective of this work was to study the Zn, Cu, and Pb content in both soils and tissues of *A. austriaca* of the impact zones of the Lake Atamanskoye and Novocherkasskaya Power Station.

### 2. RESULTS AND DISCUSSION

#### 2.1. Mobile Forms of HMs in Soil.

It was determined that the soils adjacent to Lake Atamanskoye were highly polluted with Zn. The Zn total content level varied from 2.8 to 13.9 times the maximum permissible level (MPL). Moreover, the mobile forms of Zn ranged from 1.7 to 25.7 MPL (Figure 1). According to Russian state standard, the MPL for total concentration in the soil is 220 mg/kg for Zn, 32 mg/kg for Pb, and 132 mg/kg for Cu (HS 2.1.7.2041-06; HS 2.1.7.2511-09). For the concentration of the mobile form in the soil, the MPL value is 23 mg/kg for Zn, 6.0 mg/kg for Pb, and 3.0 mg/kg for Cu.

Soil pollution by the mobile forms of Cu and Pb was also recorded. The highest HM pollution level was determined in the 1.5 km zone around the discharge point of wastewater from the chemical industry, regardless of the direction to the source.
The MPL for the total Pb content as well as for the mobile forms of Pb, Zn, and Cu exceeded in the soils within the 5 km zone around Novocherkasskaya Power Station (Figure 2). The maximum total content and content of mobile forms of HMs reached their maximum value in the soils located in plots within the northwestern direction and decreased with the distance from the station, which is typical for soils of different impact zones according to the previous study.22 The concentration of the studied HM exceeded the MPL for soils. Statistically significant differences between HM content in soil were found at the monitoring plots depending on the source of pollution (Figures 1 and 2).

2.2. HM Content in Plant Tissues. Under the hydrogenous influx of pollutants into the soil—plant system of the territory of Lake Atamanskoye, mono element contamination of A. austriaca by Zn was observed. The Zn content in the aerial part of the A. austriaca plants in all plots exceeded the MPL in forage for agricultural animals23 (50 mg/kg). At the monitoring plot no. 5, the Zn content in the plant reached 3.1 MPL (Figure 3). The excess of the Pb MPL (5.0 mg/kg) was observed only at plot no. 1.

Assessment of HM content in plants by MPL for food raw materials showed a high level of contamination.24 The MPL was exceeded for Pb (0.5 mg/kg) at most of the monitoring plots on the territory of Lake Atamanskoye to 2.5–18.0 times. Cu and Pb accumulated mainly in the root system of plants, which is due to the hydrogenic type of soil pollution.25,26 However, it is noted that Zn accumulation occurred mainly in the aerial parts of A. austriaca, despite the absence of aerogenic pollution.27,28

The highest level of HM accumulation in plants was observed in the 1.5 km zone around Lake Atamanskoye (Figure 3). This is caused by the barrier-free absorption of these pollutants by plants under a high level of hydrogenic pollution of the soil. It resulted in the accumulation of active HM in the aboveground parts (stem and leaves) of plants.29 A high level of HM accumulation by plants was also observed under the anthropogenic load.30

Multi-metal pollution of the aerial parts of the A. austriaca plants with Pb (up to 4.3 MPL in forage for agricultural animals) and Zn (1.1 MPL in forage for agricultural animals) was observed in the impact zone of Novocherkasskaya Power Station (Figure 4). The MPL of Pb for food raw materials exceeded in all monitoring plots and reached 44 times at plot no. 5, the closest to the Power Station. As in the case of HM content in the soil, the highest concentration of elements was observed in plants growing within the 5 km zone around the Power Station, with a maximum in the northwest direction.

At plot nos. 1 and 10, located at a distance of 1 and 20 km from the Power Station, respectively, a high Pb content of up to 40 MPL was observed for food raw materials. This is associated with Pb accumulation in the soil resulting from vehicle emissions in the previous years.31 Zn accumulated mainly in the aerial part of the plants, while Cu and Pb were characterized by a slightly higher content in the roots (Figure 4). The obtained results are statistically significant.

It is established that the studied plants growing in the impact zone of Lake Atamanskoe are 15–30% smaller than plants in the Novocherkasskaya Power Station zone, which is caused by a difference in soil pollution levels. In the impact zone of Lake Atamanskoe, there is a tendency to decrease the size of A. austriaca plants as they approach the source of pollution. In the Novocherkasskaya Power Station zone, there are no signs of the impact of the pollution level on the growth of A. austriaca plants.

2.3. Distribution Coefficient (DC) of Translocation of HM. The highest DC values for Zn and Pb (3.5 and 4.6, respectively) are observed at site no. 1, and for Cu (1.8), at site nos. 9 and no. 14 (Table 1).

| no. of plots | direction and distance from the source, km | Zn | Cu | Pb |
|-------------|-----------------------------------------|----|----|----|
| 1           | 1.5 NNE                                 | 3.5| 1.5| 4.6|
| 2           | 1.1 NNW                                 | 1.5| 1.1| 0.6|
| 3           | 0.9 NW                                  | 0.7| 0.6| 1.3|
| 4           | 0.7 NE                                  | 2.0| 0.9| 1.1|
| 5           | 0.9 NE                                  | 1.6| 1.8| 0.6|
| 6           | 0.8 SE                                  | 0.9| 0.7| 0.8|
| 7           | 1.0 SE                                  | 1.1| 0.6| 0.6|
| 8           | 0.8 N                                   | 0.7| 0.7| 0.5|
| 9           | 1.7 S                                   | 1.9| 1.8| 1.0|
| 10          | 6.7 SSE                                 | 1.0| 0.8| 0.3|
| 11          | 6.1 N                                   | 1.4| 1.0| 1.0|
| 12          | 4.6 E                                   | 1.0| 0.6| 1.5|
| 13          | 8.4 W                                   | 1.0| 1.0| 0.6|
| 14          | 2.2 NW                                  | 1.6| 1.8| 0.5|

The most intense translocation to the aerial part of A. austriaca is observed for Zn (DC ≥ 1 in 12 out of 14 sites), the least characterized by Pb (DC ≥ 1 in 6 out of 14 sites). For DC values, no dependence was found on either the distance from the source of pollution or the content of HM in plants.

The study of HM translocation in plants of the impact zone of Novocherkasskaya Power Station also showed the highest mobility of Zn (DC ≥ 1 in 9 out of 11 sites) and the smallest mobility of Pb (DC ≥ 1 in 4 out of 11 sites) (Table 2). The maximum DC values for Zn were established at site no. 8 (2.8), and for Cu and Pb at site no. 10 (2.0 and 1.3, respectively). For Zn, intense translocation from the roots to the aerial part is observed in the 5 km zone around the enterprise, with the highest values at the sites of the prevailing wind direction. The Pb is characterized by comparatively lower DC values at sites experiencing anthropogenic load than those of the other monitoring sites at the same distance from the Novocherkasskaya Power Station.

Table 2. Values of DC, Zn, Cu, and Pb in A. austriaca Plants in the Impact Zone of Novocherkasskaya Power Station

| no. of plots | direction and distance from the source, km | Zn | Cu | Pb |
|-------------|-----------------------------------------|----|----|----|
| 1           | 1.0 NE                                  | 1.2| 1.0| 0.9|
| 2           | 3.0 SW                                  | 1.0| 0.9| 1.1|
| 3           | 2.7 SW                                  | 1.6| 0.6| 1.0|
| 4           | 1.6 NW                                  | 1.9| 1.7| 0.8|
| 5           | 1.2 NW                                  | 1.4| 1.0| 0.9|
| 6           | 2.0 NW                                  | 1.5| 0.5| 0.9|
| 7           | 1.5 N                                   | 0.9| 1.0| 0.9|
| 8           | 5.0 NW                                  | 2.8| 0.3| 1.0|
| 9           | 15.0 NW                                 | 1.4| 0.8| 0.3|
| 10          | 20.0 NW                                 | 0.8| 2.0| 1.3|
| 11          | 1.0 SE                                  | 1.7| 1.0| 0.6|

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On the basis of the revealed patterns, it can be concluded that aerogenic pollution has a significant effect on the entry of Zn into the aerial part of A. austriaca plants in the zone of influence of Novocherkasskaya Power Station. At the same time, Pb translocation is affected mainly by contaminated soil. This is confirmed by the high DC value at site no. 10, the farthest from the enterprise, however, the soil of which is an additional source of plant pollution by these metals due to its location near the highway.

3. CONCLUSIONS

The high level of Zn content was observed in the soil of Lake Atamanskoe, and relatively low levels of HMs contamination were observed in the soil of Novocherkasskaya Power Station impact zone. The exceeded limits of MPL for Zn and Pb in A. austriaca grown in both the studied impact zones were observed. The exceeded limit of MPL for Pb in food raw materials was only found in the Novocherkasskaya Power Station impact zone. The highest Zn content in plants is observed in the impact zone of Lake Atamanskoe, and Pb in the impact zone of Novocherkasskaya Power Station. Cu is characterized by the lowest content of both impact zones in plants. The predominant Zn accumulation was found in the aerial part while Pb and Cu in the root system of A. austriaca. It has been established that the main factor in the entry of elements into plants during hydrogenic pollution is the high content of pollutants in the soil, while in atmospheric conditions, the main role is played by the direction of the wind. According to the DC values, the largest Zn translocation from the roots to the aerial part of the A. austriaca in the Novocherkasskaya Power Station impact zone occurs on the prevailing wind direction sites. The significant distribution area of anthropogenic load (up to 5 km) is due to both the distance from the source of atmospheric emissions and the monitoring plot direction to the prevailing direction of the windrose. The territory of Lake Atamanskoe has a smaller pollution area (1.5 km) than that of the Novocherkasskaya Power Station, and the direction of the location of the plots did not affect the level of elements in the soil. Vehicle emissions accumulated in the soil over the past decades on plot nos. 4, 8, 9, 10 have a greater effect on the Pb translocation than those of the atmospheric emissions of the enterprise. There is no direct effect of the anthropogenic pollution level on the HM translocation in the Lake Atamanskoe impact zone. The study indicated several differences in the accumulation of elements by soils and plants under hydrogenic and aerogenic pollution of the impact zones. To minimize the negative consequences of anthropogenic pollution of soils and plants in these impact zones, it is necessary to develop remedial measures taking into account the specifics of pollution in each zone.

4. OBJECTS AND METHODS

4.1. Study Area and Sampling. Lake Atamanskoe is a horseshoe-shaped depression with a hummocky surface and is a dried lagoon (Figure 5). The lake is located in the floodplain of the Seversky Donets River, the main tributary of the Don. From the early 1950s to the mid-90s, it was used as a reservoir for dumping industrial wastewater at the Khimvolokno plant. As a result, the natural ecosystem was destroyed, turning the lake into a sludge sink. Lake Atamanskoe is no longer being used as a reservoir for dumping industrial waste, and it remains as a hydrogenic source of environmental pollution. In 2015, 14 monitoring plots were laid on the shores of Lake Atamanskoe at a distance of 0.7–8.4 km in various directions from the industrial wastewater landfill site. The soil of the plots is represented by Haplic Chernozem and Calcaric Fluvisols Loamic.

Novocherkasskaya Power Station is well-known as the main contributor to HM pollution in the Rostov region. This enterprise accounts for 1% of all pollutant emissions into the atmosphere in the Russian Federation. In detail, over 50% in the Rostov region and about 90% in Novocherkassk, pollution has been released by this power station for almost 40 years. The average emissions are near 60,000 tons per year. The negative consequences of its emissions have led to the HM accumulation in soil and consequently have been routed to plants with time.

The 11 monitoring plots around Novocherkasskaya Power Station were established in 2007 in different directions at a distance of 1–20 km from the enterprise. Following the windrose, the prevailing northwest direction was identified, along which soil and plants were sampled at monitoring plots no. 4, 8, 9, 10, and adjacent to this direction plot no. 5 (Figure 5). Plot no. 10 is located at a distance of 400 m from the
motorway. Over the decades of vehicle emissions, significant amounts of HMs have entered the soil of plot no. 10, and currently continue to move from soil to plants and are an additional source of anthropogenic load. Plot no. 1 is located in a windbreak 25 m from the road. This plot is also affected by the additional release of pollutants. The soils of the territory adjacent to Novocherkasskaya Power Station are represented by Haplic Chernozem, Calcaric Fluvisols Loamic, and Calcaric Fluvic Arenosols of Tuzlov River floodplain.

The assessment of the Cu, Zn, and Pb content in soil and plants was studied because these metals are priority pollutants in the Rostov region.\textsuperscript{3}

\textit{Artemisia austriaca} was chosen as the study object since this wild medicinal plant is widely distributed at all monitoring plots in both impact zones under study. A sampling of \textit{A. austriaca} plants in both impact zones was carried out by cuttings of herbaceous plants growing in the area in the second decade of June during the mass flowering stage. Plants were selected together with the root part, being dug out of the ground at various points of monitoring plots (point samples).\textsuperscript{35} The plant root was sampled from the soil together with the monolith of the soil to avoid the loss of the root system. The plant sample from each plot weighing 1.5 kg consisted of 8 to 10-point samples. After sampling, the plants were dried to an air-dry state and crushed. The root part was pre-cleaned of soil particles before grinding, to avoid getting them into the sample.

4.2. Determination of HMs in Soil. The total content of HMs in soils was determined by the X-ray fluorescence method. The mobile compounds of the element were transferred into the solution from the soil by parallel extractions using the reagents following the method suggested by Minkina et al.:\textsuperscript{36} (1) 1 N ammonium acetate buffer (NH\textsubscript{4}Ac) pH 4.8 (soil/solution ratio = 1:5, extraction time: 18 h), which transfers exchangeable forms of metals characterizing their actual mobility into the solution; and (2) a 1% solution of ethylenediaminetetraacetic acid (EDTA) in NH\textsubscript{4}Ac pH 4.8 (soil/solution ratio = 1:5, extraction time: 18 h), which presumably transfers relatively weak metal complexes of metals into the solution along with their exchangeable forms.

The plant sample mineralization was carried out by the dry ashing method according to GOST 26929-94. The HMs were extracted from ash by being dissolved in a 20% HCl solution and determined by atomic absorption spectrometry.\textsuperscript{37}

4.3. Assessment of HMs in Plant Tissues. Assessment of the HMs contamination level in plants was carried out by comparing the HM content in plants with the Russian state standard MPL of chemical elements for food raw materials and comparing the HM content in plants with the Russian state standard MPL of chemical elements for food raw materials and the metal content in the soil, parametric and non-parametric correlation analyses were used. For variables with a different type of distribution, the Spearman correlation coefficient was used.

4.4. Estimation of Distribution Coefficient (DC) of HM Accumulation. To assess the selectivity of HM accumulation by \textit{A. austriaca} plants under polluted conditions, distribution coefficient (DC) was calculated. To study the migration of HM to different parts of plants, element uptake barrier was considered as the ratio of the metal content in the aboveground biomass to its concentration in the roots. The DC value makes it possible to estimate the barrier function of plants under contamination.\textsuperscript{38} The value DC > 1 indicates an active translocation of metals from the roots to the stem and leaves or from the stalks to the grain; the plant is a metal accumulator;\textsuperscript{1} DC = 1 indicates the neutral behavior of the plant in relation to metals (indicator), and DC < 1 indicates that the plant excludes metals from absorption, being an excluder.\textsuperscript{3,41} The DC values are often the same for the plants of the same species; a change in the value indicates that the plant experiences environmental stress.\textsuperscript{2}

4.5. Statistical Analysis. The statistical analyses were performed using Microsoft Excel 2016 software. To analyze the dependence between the metal content in the aboveground (stem and leaves) and underground (roots) organs of plants and the metal content in the soil, parametric and non-parametric correlation analyses were used. For variables with a different type of distribution, the Spearman correlation coefficient was used.

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