applied sciences

MDPI

Article

Ecological Characterization and Bio-Mitigation Potential of Heavy Metal Contamination in Metallurgically Affected Soil

Varuzhan Hovhannisyan [1,2], Ashok Vaseashta [3,4,*], Lyudmila Avanesyan [2], Ruzanna Sadoyan [2], Armen Gasparyan [2], Sirine Shogheryan [2], Laura Harutyunova [1], Lyudmila Mirumyan [1] and Gor Gevorgyan [1]

1 Scientific Center of Zoology and Hydroecology, National Academy of Sciences of Republic of Armenia, Yerevan 0014, Armenia; varugh_zool52@mail.ru (V.H.); zool_laura@yahoo.com (L.H.); lmirumyan@mail.ru (L.M.); gev_gor@mail.ru (G.G.)
2 Chair of Ecology and Sustainable Development, Armenian State Pedagogical University after Khachatur Abovyan, Yerevan 0010, Armenia; avanesyanlyudmila11@aspu.am (L.A.); ruzannasad@mail.ru (R.S.); armengasparyan1969@mail.ru (A.G.); shogheryansirine11@aspu.am (S.S.)
3 Office of Applied Research, International Clean Water Institute, Manassas, VA 20112, USA
4 Faculty of Civil Engineering, Transylvania University of Brasov, 500152 Brasov, Romania
* Correspondence: prof.vaseashta@ieee.org

Abstract: Heavy metals (HMs) remain persistent in soil for a long time and thus present a long-term threat of environmental pollution. In this study, the concentrations of some HMs (As, Cu, Zn, Pb, and Mo) in soil, potentially affected by the ex-operation of a metallurgical plant—specifically, a copper smelter in Alaverdi Town, northeastern Armenia—were measured, based on which, the HM contamination and its ecological, biological, and health effects were assessed. Concentrations of HMs (Cu, Zn, and Pb) were also measured in different plant (leaves) and invertebrate species that occur in the soil over the metallurgical factory site in order to assess the potential for the bioremoval of HMs from the soil. The results showed that the ex-operation of the metallurgical facility created such contamination in the soil that it caused a noticeable loss of invertebrate biomass in the soil and posed ecological, non-carcinogenic (for children), and carcinogenic health hazards. The investigated plant and invertebrate species were characterized by different capacities for the accumulation of HMs from the soil, based on which the plant species *Fraxinus excelsior*, *Acer platanoides*, *Robinia pseudoacacia*, and *Aesculus hippocastanum* and the invertebrate species *Deroceras caucasicum*, *Limax flavus*, and *Eisenia rosea* are recommended to be used for the selective removal of HMs (Cu, Zn, and Pb) from the soil.

Keywords: heavy metal; contamination; soil; ecological hazard; health effect; bio-mitigation

Citation: Hovhannisyan, V.; Vaseashta, A.; Avanesyan, L.; Sadoyan, R.; Gasparyan, A.; Shogheryan, S.; Harutyunova, L.; Mirumyan, L.; Gevorgyan, G. Ecological Characterization and Bio-Mitigation Potential of Heavy Metal Contamination in Metallurgically Affected Soil. *Appl. Sci.* **2022**, *12*, 6312. https://doi.org/10.3390/app12136312

Academic Editor: Raffaele Marotta

Received: 1 June 2022
Accepted: 13 June 2022
Published: 21 June 2022

1. Introduction

Soil pollution is a pressing environmental issue around the world. In this regard, heavy metals (HMs) present special concerns. Globally, more than five million soil sites are contaminated with HMs [1]. Soil serves as a major sink of HMs released into the environment. Unlike organic contaminants, HMs are biologically or chemically non-degradable. However, their chemical forms and bioavailability are changeable [2]. Therefore, they can accumulate in soil and remain persistent for long periods of time, posing serious environmental threats [2,3]. Rapid industrialization and urbanization have caused the discharge of wastewater containing high concentrations of HMs, which has contributed to the elevated levels of HMs in urban environments, especially in developing countries [4]. Residents in industrial areas are most vulnerable to exposure to HMs, since many of these elements are closely related to industry [5]. Growing evidence demonstrates that HM pollution in mining areas has caused serious health issues in local residents, as well as damage to soil health [3]. Therefore, the prevention of toxic effects from these contaminants has become an existential challenge [6].

Many studies have been conducted on the reduction in or removal of HMs from polluted soil, including in situ and ex situ remediation techniques [7]. Physical and chemical remediation techniques do not completely solve the environmental problems associated with HM contamination, whereas biological techniques (e.g., use of plants) have been proven to be the most environmentally and economically effective approaches [6].

The mining industry makes significant contributions to the Armenian economy. It is highly concentrated in Lori Province, northern Armenia. Generally, improper management of industrial discharges is one of the most concerning environmental issues facing this area [8]. Earlier hydro-ecological investigations suggested that the rivers in the mining areas of Lori Province were negatively influenced by HM contamination, which was caused by discharges from the Alaverdi copper smelter and Akhtala mountain enrichment combine, as well as effluents from the non-operational tailing dump of the former Alaverdi mining and metallurgical combine and the landfills of the non-operational Alaverdi underground copper mine [8–10], while other studies have shown that the As and Pb contamination in soil in Alaverdi Town was significantly associated with the Alaverdi copper smelter [11].

The present study aims to characterize HM contamination in metallurgically affected soil, its environmental effects, and its bio-mitigation potential for the soil. To achieve the goal of this study, the authors investigated HMs in the soil, plants, and animals near the copper smelting plant in Alaverdi Town, Lori Province.

2. Materials and Methods

2.1. Study Site

The mineral industry is one of the main sectors of the Armenian economy, accounting for 30.1% of its exports in 2017. According to the Armenian Development Agency, Armenia has more than 670 mines of construction and aggregate minerals, including 30 base metal and precious metal mines. The study site for this investigation is Alaverdi Town, which is located in the northeastern part of Armenia, close to the border with Georgia (41°05′28.3″ N, 44°39′32.4″ E) at an altitude of 1000 m a.s.l. The area is surrounded by high mountains and forests. The largest Armenian polymetallic smelter is located in the middle of Alaverdi Town. The Alaverdi copper smelter has been operating since its privatization in 1997 [12]. With the advent of Armenia's independence in 1991, the operation of the copper metallurgical factory of Alaverdi was temporarily stopped due to financial issues caused by the economic crisis and the absence of a market, and the factory resumed its operations in 1997 at full capacity [13]. The smelter, once again, suspended its operations in October 2018 because of financial problems of the operating company.

2.2. Soil and Biological Sampling

Soil and biological sampling were performed at different distances from the Alaverdi copper smelter, as outlined in Figure 1 and Table 1. Surface soil (0–20 cm upper layer) and invertebrate samples for HM and biological analyses, respectively, were collected from 5 locations and a reference site located about 50–700 m and 25 km north of the copper smelter in June 2020. Plant leaf and invertebrate samples for HM measurements were gathered from one site, located about 800 m south-west of the copper smelter, and the reference site in June 2021. The surface soil, plant leaf, and invertebrate samples were collected from 5 different points for each location and mixed to make an integral sample for each site. The soil samples were stored in polyethylene bags. Leaves were collected from all plant species identified at the investigation site and stored in polyethylene containers according to species. The invertebrate species occurring at the investigation sites were gathered using a frame for determining the surface area of sample collection and stored in polyethylene containers. The animals were kept alive in cool boxes under low-temperature conditions.

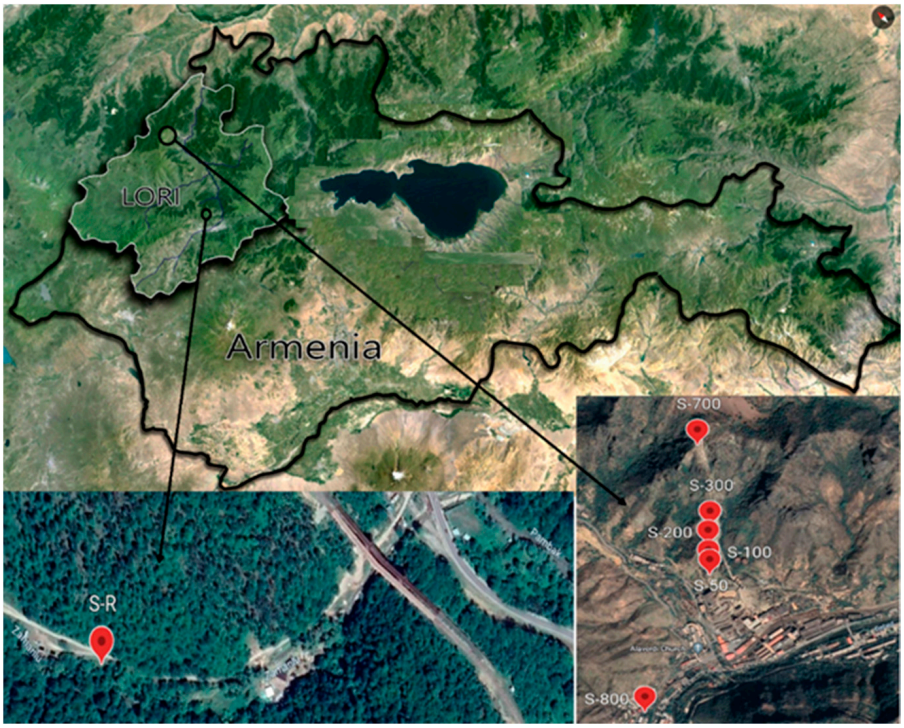

Figure 1. Location of the study area in Armenia and investigation sites as coded in Table 1.

Table 1. Coordinates of sampling sites located at different distances from the Alaverdi copper smelter.

Sampling Site Code	N/Lat	E/Long	Sampling Site Location
S-50	41°06′13.7″	44°39′35.4″	Investigation site located about 50 m north of the Alaverdi copper smelter
S-100	41°06′16.5″	44°39′36.6″	Investigation site located about 100 m north of the Alaverdi copper smelter
S-200	41°06′18.4″	44°39′38.2″	Investigation site located about 200 m north of the Alaverdi copper smelter
S-300	41°06′21.2″	44°39′41.0″	Investigation site located about 300 m north of the Alaverdi copper smelter
S-700	41°06′32.8″	44°39′47.2″	Investigation site located about 700 m north of the Alaverdi copper smelter
S-800	41°05′57.7″	44°39′07.9″	Investigation site located about 800 m southwest of the Alaverdi copper smelter
S-R	40°52′53.2″	44°34′54.6″	Reference site located about 25 km southwest of the Alaverdi copper smelter

2.3. Sample Preparation and Analyses for Biomass and HMs

In the laboratory, the invertebrate samples for biological analysis were dried in an incubator at 40 °C and then weighed to obtain the total dry mass. The animals for HM analysis were identified to the lowest taxonomic level and stored according to species. The soil and biological (invertebrates and plant leaves) samples for HM analysis were dried in an incubator at 40 °C. The samples were ground into powder by a mortar and pestle and digested on a hot plate using aqua regia solution (conc. HCl and conc. HNO_3 in ratio of 3:1) for the soil samples and conc. HNO_3 and H_2O_2 at a ratio of 3:2 for the biological samples. The digested samples were analyzed for some HMs (As, Cu, Zn, Pb, and Mo for the soil samples and Cu, Zn, and Pb for the biological samples) using an atomic absorption spectrometer (AAS) according to the guidelines of the Federal State Institution

Federal Center for Analysis and Assessment of Technogenic Impact (FSI FCAATI-2011) [14]. All chemicals used were of certified analytical grade. Deionized water was used for the preparation of calibration standards and in the analyses. All glassware used was pre-washed with 10% HNO_3, followed by rinsing with distilled water prior to use. To ensure that the AAS remained calibrated during the experiments, certified reference materials were analyzed for soil and biological samples [14].

2.4. Assessment of HM Contamination, Ecological and Health Hazards

HM contamination in soil was assessed with a geo-accumulation index, I_{geo} [15], developed based on a reference value.

$$I_{geo} = \log_2\left(\frac{C_m}{1.5C_r}\right) \quad (1)$$

where C_m is the measured concentration of the element in soil samples and C_r is the reference value of the element. Contamination degree based on I_{geo} values was classified into the following categories: uncontaminated ($I_{geo} < 0$), uncontaminated to moderately contaminated ($0 \leq I_{geo} < 1$), moderately contaminated ($1 \leq I_{geo} < 2$), moderately to heavily contaminated ($2 \leq I_{geo} < 3$), heavily contaminated ($3 \leq I_{geo} < 4$), heavily to extremely contaminated ($4 \leq I_{geo} < 5$), and extremely contaminated ($I_{geo} \geq 5$; [15]). The ecological risks of the investigated HMs in the surface soil were assessed with the potential ecological risk index (PERI; [16]).

$$C_r^i = \frac{C_s^i}{C_n^i}, \quad (2)$$

$$E_r^i = C_r^i.T_r^i, \quad (3)$$

$$PERI = \sum E_r^i, \quad (4)$$

where C_r^i is the pollution factor of a single element in soil, C_s^i is the measured concentration of a single element in soil, C_n^i is the background concentration of a single element in soil, E_r^i is the potential ecological risk of a single element, and T_r^i is the toxic response factor for a single element. The reference value of each element was considered the background. The T_r^i values for Cu, As, Pb, and Zn were derived from [17] and for Mo from [18]. The potential ecological risk of a single element based on the E_r^i values was classified into the following categories: low risk ($E_r^i < 40$), moderate risk ($40 \leq E_r^i < 80$), considerable risk ($80 \leq E_r^i < 160$), high risk ($160 \leq E_r^i < 320$), and very high risk ($E_r^i \geq 320$; [16]). The overall potential ecological risk based on the PERI values was classified according to [16] as follows: low risk (PERI < 150), moderate risk ($150 \leq$ PERI < 300), considerable risk ($300 \leq$ PERI < 600), and very high risk (PERI $\geq$ 600).

Human health risks of HMs in surface soil were examined with the risk assessment methodology adopted from the USDOE (2011) [19] and USEPA (2011) [20]. The non-carcinogenic exposure doses through ingestion, dermal absorption, and inhalation were calculated using Equations (5)–(7):

$$ED_{ing-nc} = \frac{C \times IngR \times ED \times EF \times CF}{BW \times AT_{nc}}, \quad (5)$$

$$ED_{derm-nc} = \frac{C \times ABS \times AF \times ED \times EF \times SA \times CF}{BW \times AT_{nc}}, \quad (6)$$

$$ED_{inh-nc} = \frac{C \times ET \times ED \times EF}{PEF \times 24 \times AT_{nc}}, \quad (7)$$

where C is measured HM concentration (mg kg^{-1}), IngR is the soil ingestion rate for the receptor (mg day^{-1}), CF is the unit conversion factor (kg mg^{-1}), ED is the exposure duration (year), EF is the exposure frequency (day $year^{-1}$), ABS is the dermal absorption factor (unit less), AF is the soil to skin adherence factor (mg cm^{-2}), SA is the skin surface

area available for exposure (cm^2), ET is the exposure time (h day^{-1}), BW is the average body weight (kg), AT_{nc} is the averaging time for non-carcinogens (day), and PEF is the soil-to-air particulate emission factor (m^3 kg^{-1}). The non-carcinogenic hazard quotient of a single HM was calculated using Equation (8):

$$HQ_{ing/derm/inh} = \frac{ED_{ing/derm/inh-nc}}{RfD_{ing/derm/inh}}, \quad (8)$$

where $RfD_{ing/derm/inh}$ is the non-carcinogenic reference dose (mg kg^{-1} day^{-1}) through oral ingestion, dermal absorption, and inhalation. RfD_{ing} values for As, Cu, Mo, and Zn were derived from the USEPA (2003) [21] and the RfD_{derm} values for these elements were obtained from the RfD_{ing} values by their conversion based on oral absorption efficiency for dermal absorption derived from the USEPA (2004) [22]. RfD_{ing} and RfD_{derm} values for Pb were derived from [23]. HQ_{inh} was calculated for As only, the RfD_{inh} value for which was derived from the Agency for Toxic Substances and Disease Registry (ATSDR-2007) [24]. The single metal non-carcinogenic hazard index via ingestion, dermal, and inhalation contact with soil was calculated using Equation (9):

$$HI_{nc} = \sum HQ = HQ_{ing} + HQ_{derm} + HQ_{inh}. \quad (9)$$

Non-carcinogenic health risks posed by all metals, expressed as the overall hazard index (OHI), were assessed by the following equation:

$$OHI_{nc} = \sum_{i=0}^{n} HI_{nc}. \quad (10)$$

The carcinogenic exposure doses for only As and Pb were used to assess carcinogenic health risks. The exposure doses through ingestion, dermal absorption, and inhalation were calculated using Equations (11)–(15):

$$ED_{ing-ca} = \frac{C \times IR \times EF \times CF}{AT_{ca}}, \quad (11)$$

$$IR = \frac{ED_{child} \times IngR_{child}}{BW_{child}} + \frac{(ED_{adult} - ED_{child}) \times IngR_{adult}}{BW_{adult}}, \quad (12)$$

$$ED_{derm-ca} = \frac{C \times ABS \times EF \times DFS \times CF}{AT_{ca}}, \quad (13)$$

$$DFS = \frac{ED_{child} \times SA_{child} \times AF_{child}}{BW_{child}} + \frac{(ED_{adult} - ED_{child}) \times SA_{adult} \times AF_{child}}{BW_{adult}}, \quad (14)$$

$$ED_{inh-ca} = \frac{C \times ET \times ED \times EF}{PEF \times 24 \times AT_{ca}} \times 10^3, \quad (15)$$

where IR is soil ingestion rate-age adjusted (mg year kg^{-1} day^{-1}), DFS is soil dermal contact factor-age adjusted (mg year kg^{-1} day^{-1}), and ATca is averaging time for carcinogens (day). The single metal non-carcinogenic hazard index via ingestion, dermal, and inhalation contact with soil was calculated by Equations (16) and (17):

$$HI_{ing/derm/inh-ca} = ED_{ing/derm/inh-ca} x\, CSF_{ing/derm/inh}, \quad (16)$$

$$HI_{ca} = HI_{ing-ca} + HI_{derm-ca} + HI_{inh-ca}, \quad (17)$$

where $CSF_{ing/derm/inh}$ is the cancer slope factor (mg kg^{-1} $day^{-1})^{-1}$ through oral ingestion, dermal absorption, and inhalation. The $CSF_{ing/derm/inh}$ value for As was derived from [25]. CSF through only oral ingestion and inhalation was used for Pb, and the values were

derived from the Office of Environmental Health Hazard Assessment (OEHHA-2009) [26]. The overall carcinogenic hazard index was assessed using the following Equation:

$$OHI_{ca} = \sum_{i=0}^{n} HI_{ca}. \quad (18)$$

2.5. Statistical Analyses

Since Geary's test of normality showed a nearly normal distribution of HMs and biomass [27], Student's *t*-test was used to determine the statistical significance of differences between HM/biomass value averaged for the sites located at 50–700 m distance from the Alaverdi copper smelter and HM/biomass value measured at the reference site [28]. The relationships between HMs and between HMs and biomass were determined by Pearson's correlation coefficient [29]. Statistical analyses were performed using Statistica, ver. 8 (StatSoft, Hamburg, Germany) and Excel, ver. 2019 (Microsoft, Redmond, Washington, USA) software programs.

3. Results and Discussion

3.1. HM Contamination in Soil

The average concentrations of the investigated HMs (except Pb) in the soil located within 50–700 m of the Alaverdi copper smelting plant (sites S-50–S-700) were significantly higher ($p < 0.05$) than those at the reference site (S-R). The contents of As, Cu, Zn, Pb, and Mo at sites S-50–S-700 exceeded the reference levels by 3.6–23.7, 6.2–10.0, 2.0–3.6, 0–1.6, and 3.6–6.8 times, respectively (Figure 2). The concentrations of As, Cu, Zn, and Mo mostly decreased with an increase in distance from the smelter, while the levels of Pb between sites S-50 and S-200 increased slightly, then decreased at the further sites (Figure 2). Pearson's correlation analysis showed a strong positive relationship between the concentrations of the investigated HMs (except Pb; Table 2), which indicates that they were likely induced by the same source.

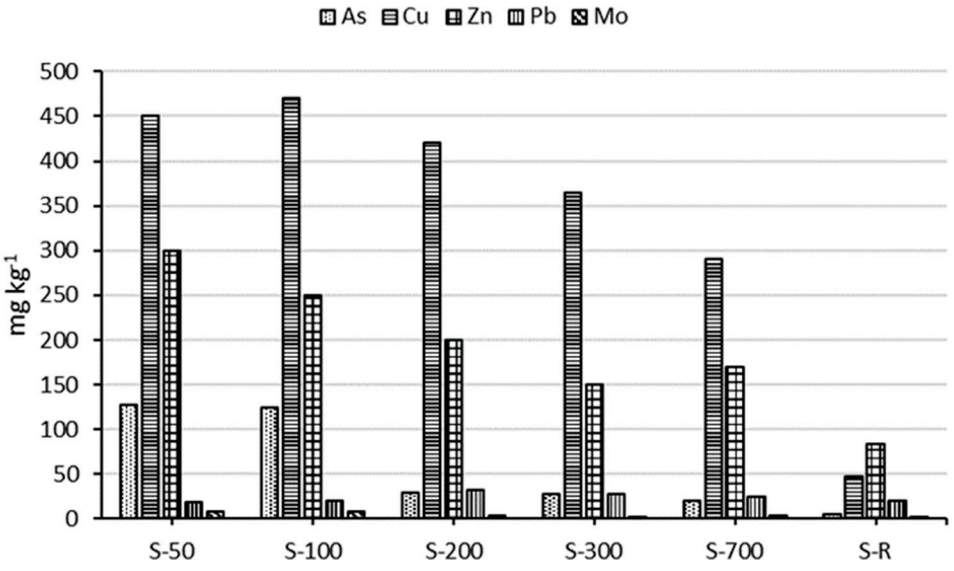

Figure 2. Concentrations of some HMs in the soil located at different distances from the Alaverdi copper smelter.

Table 2. Pearson's coefficients of correlations between HMs and between HMs and invertebrate biomass.

	As	Cu	Zn	Pb	Mo	Biomass
As	1					
Cu	0.703	1				
Zn	0.901	0.863	1			
Pb	−0.574	0.159	−0.273	1		
Mo	0.970	0.796	0.944	−0.428	1	
Biomass	−0.504	−0.948	−0.741	−0.326	−0.621	1

According to the I_{geo} values (Table 3), the soil located nearest the copper smelter (sites S-50 and S-100) was heavily and moderately contaminated with As and Zn, respectively, while the contamination decreased with an increase in distance from the smelter, and the soil at sites S-200–S-700 showed moderate contamination and non-contamination to moderate contamination with As and Zn, respectively. Cu showed moderate to heavy contamination at all investigated sites (S-50–S-700). Mo was also an ecologically risky element in soil, particularly at sites S-50–S-200 where the soil was moderately to heavily (S-50–S-100) and moderately (S-200) contaminated with Mo. The ex-operation of the Alaverdi copper smelter did not cause ecologically risky contamination with Pb in the investigation area where the soil was either uncontaminated or uncontaminated to moderately contaminated with this metal.

Table 3. I_{geo} of the investigated HMs in the soil located at different distances from the Alaverdi copper smelter.

	As	Cu	Zn	Pb	Mo
S-50	3.98	2.67	1.27	−0.74	2.19
S-100	3.94	2.74	1.01	−0.58	2.29
S-200	1.86	2.57	0.68	0.09	1.26
S-300	1.79	2.37	0.27	−0.10	0.58
S-700	1.27	2.04	0.45	−0.26	0.96

3.2. Adverse Ecological and Biological Effects of HM Contamination in Soil

The assessment of the potential ecological risks of soil contamination with single HMs showed that E_r^i values for Zn and Pb at all the investigated sites, as well as As and Cu at sites S-700 and S-300–S-700, respectively, were lower than 40, indicating a low risk (Figure 3). As, Cu, and Mo contamination at sites S-200–S-300, S-50–S-200, and S-200–S-700, respectively, showed a moderate risk, while As and Mo contamination at sites S-50–S-100 indicating high and considerable risks, respectively (Figure 3), was of particularly high concern. The investigated HMs according to E_r^i values can be ranked as As > Mo > Cu > Pb > Zn at sites S-50–S-100 and S-300, and Mo > As > Cu > Pb > Zn at sites S-200 and S-700 (Figure 3). As and Mo were the ecologically riskiest elements in the investigated soils; therefore, more attention should be paid to these metals to prevent potential ecotoxicity from these contaminants. Soil contamination with all the investigated HMs showed a low, moderate, and considerable risk at sites S-300–S-700, S-200, and S-50–S-100, respectively, according to the PERI values (Figure 3). The investigated sites can be ranked according to the overall potential ecological risk (PERI) in decreasing order as follows: S-100>S-50>S-200>S-300>S-700 (Figure 3). The highest overall potential ecological risk was posed by the sites closest to the smelter (S-50 and S-100). The risk noticeably decreased at site S-200 and continued to decrease with an increase in distance from the smelter (Figure 3).

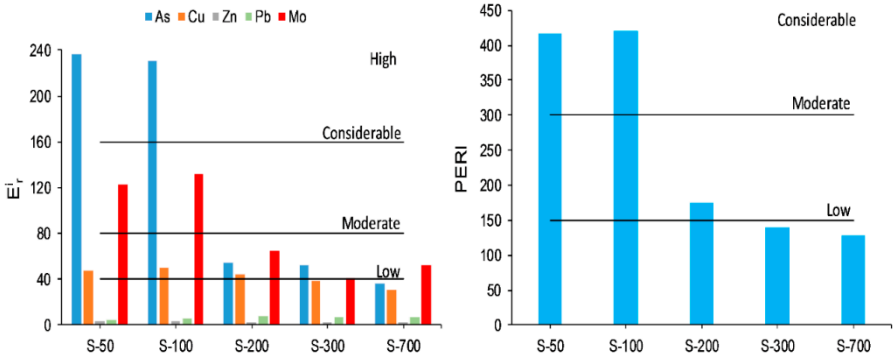

Figure 3. Potential ecological risk index of single (E_r^i) and total (PERI) HMs in the soil located at different distances from the Alaverdi copper smelter.

The biological analysis showed that the biomass of invertebrates occurring in the investigated soils noticeably increased with an increase in distance from the smelter (Figure 4). The biomass at the reference site was 11,886.5 mg m^{-2}, so no organisms were observed at the site closest to the smelter (S-50; Figure 4). The loss of biomass was 100, 99.83, 99.44, 97.33, and 88.20% at sites S-50, S-100, S-200, S-300, and S-700, respectively, compared to the reference site (Figure 4). The mean biomass value in the soil potentially affected by the ex-operation of the smelter (sites S-50–S700) was significantly lower ($p < 0.001$) than the biomass registered at the reference site.

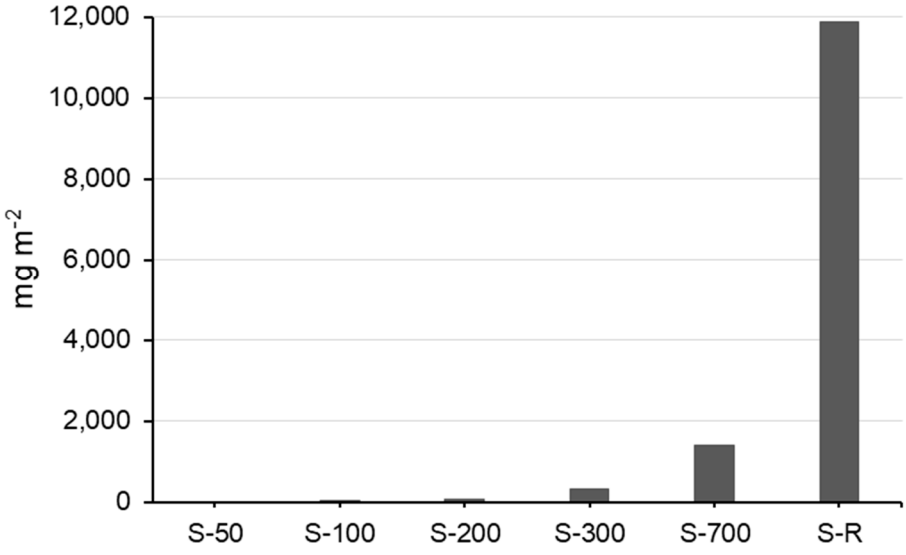

Figure 4. Biomass of invertebrates occurring in the soil located at different distances from the Alaverdi copper smelter.

Statistical analysis revealed a strong negative correlation between the biomass value and the concentration of single elements such as As, Cu, Zn, and Mo and a moderate negative correlation between the biomass value and the Pb concentration (Table 2). However, a statistically significant correlation was only observed between the biomass value and the Cu concentration ($p < 0.005$; Table 2). All of this indicates that As, Zn, Mo, and Pb were potential factors causing biomass changes, while Cu can be considered a driver of biomass loss.

3.3. Adverse Health Effects of HM Contamination in Soil

HM contamination in soil may cause not only ecological risks but also human health hazards through different pathways [30]. The calculation of OHInc through the ingestion, dermal absorption, and inhalation of HMs from soil showed that the adverse non-

carcinogenic health effects of the investigated HMs at sites S-50–S-700, as well as the reference site, were negligible for adults, while the OHInc for children was noticeably higher at sites S-50–S-700 than the threshold value of 1 (Figure 5), which indicates that HMs in this area had a probability of non-carcinogenic health effects on children. Children are more susceptible to HM effects than adults, which is explained by the difference in their physiological properties [17]. The OHIca of HMs (As and Pb) at sites S-50–S-700 also noticeably exceeded the threshold value of 10^{-4} (Figure 5), which indicates that this contamination of the soil had a probability of carcinogenic health effects.

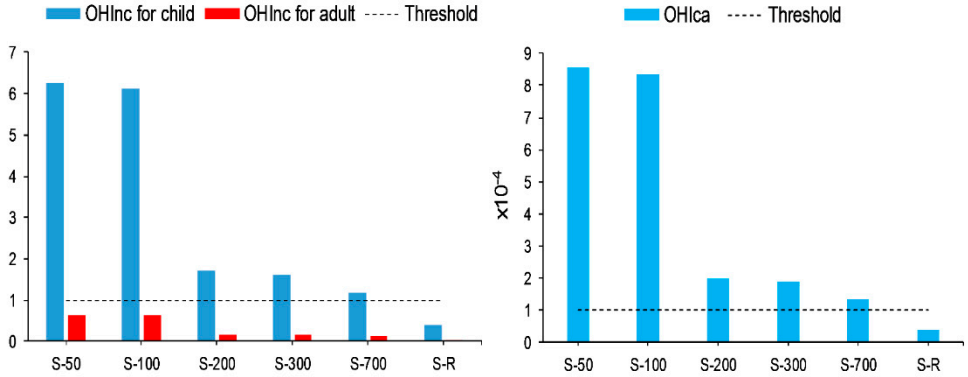

Figure 5. Overall hazard index (OHI) of HMs in the soil located at different distances from the Alaverdi copper smelter.

The investigated HMs can be ranked according to health hazard quotient (HQ) in decreasing order as follows: As > Cu > Pb > Mo > Zn and As > Cu > Pb > Zn > Mo at sites S-50–S-700 and the reference site, respectively. It should be noted that the concentration of even a single element such as As also had probability of non-carcinogenic (for children) and carcinogenic health effects, particularly at sites S-50–S-300 and S-50–S-700, respectively. The non-carcinogenic and carcinogenic health risks of single and total HMs at almost all the investigated sites that were potentially affected by the ex-operation of the Alaverdi copper smelter were even posed through a single pathway, such as the ingestion or inhalation of metals.

3.4. HMs in Plants and Invertebrates

The characteristics of the accumulation of some HMs (Cu, Pb, and Zn) by plant (leaves) and invertebrate species occurring in soil potentially affected by the ex-operation of the copper smelter in Alaverdi Town were also investigated. The accumulation level was considered the difference between the total and reference concentrations of an element. The highest concentrations of anthropogenic Cu, Zn, and Pb were accumulated in the leaves of the species *Fraxinus excelsior*, *Robinia pseudoacacia*, and *Aesculus hippocastanum*, respectively, with the lowest levels in the leaves of the species *Salix acutifolia*, *Populus canadensis*/*Ulmus minor,* and *Populus canadensis*, respectively (Figure 6). The plant species can be ranked according to decreasing HM accumulation level as follows: *Fraxinus excelsior–Acer platanoides–Robinia pseudoacacia–Aesculus hippocastanum–Populus canadensis–Ulmus minor–Salix acutifolia* in the case of Cu accumulation, *Robinia pseudoacaci–Fraxinus excelsior–Acer platanoides–Aesculus hippocastanum–Salix acutifolia–Ulmus minor–Populus canadensis* in the case of Zn accumulation, and *Aesculus hippocastanum–Robinia pseudoacacia–Fraxinus excelsior–Acer platanoides–Salix acutifolia–Ulmus minor*/*Populus canadensis* in the case of Pb accumulation (Figure 6).

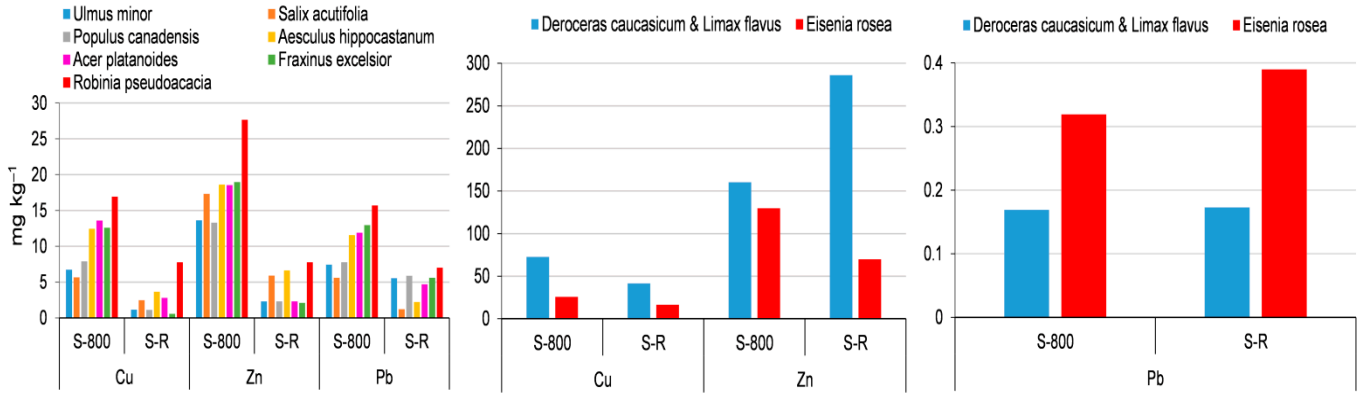

Figure 6. Concentrations of some HMs in the plant leaves and invertebrates from the areas located at different distances from the Alaverdi copper smelter.

Higher concentrations of anthropogenic Cu were accumulated in the tissues of the slug species *Deroceras caucasicum* and *Limax flavus*, while anthropogenic Zn accumulated more in the earthworm species *Eisenia rosea* (Figure 6). The investigated invertebrate species did not accumulate anthropogenic Pb (Figure 6).

It can be stated that the investigated plant and invertebrate species showed different capacities for the accumulation of HMs from the soil, and the plant species *Fraxinus excelsior*, *Acer platanoides*, *Robinia pseudoacacia*, and *Aesculus hippocastanum*, as well as the invertebrate species *Deroceras caucasicum*, *Limax flavus*, and *Eisenia rosea* can be used for the selective removal of HMs from the soil over the copper smelting facility in Alaverdi Town.

4. Conclusions

This study showed that the ex-operation of the copper smelting plant in Alaverdi Town negatively affected the environment, which was expressed by the noticeable HM contamination in the soil even 700 m from the smelter. Although the contamination decreased with an increase in distance from the smelter, the average concentrations of almost all the investigated HMs in the soil affected by the ex-operation of the smelter significantly exceeded the reference levels. Such contamination caused a substantial loss of invertebrate biomass in the investigated soil and posed serious ecological risks, as well as demonstrating an alarming probability of non-carcinogenic (for children) and carcinogenic health effects. The investigated plant and invertebrate species inhabiting the land near the smelter showed different levels of HM accumulation, indicating opportunities to use the plant and invertebrate species with high capacities for HM accumulation for the selective bioremoval of HMs from the soil. The results presented may be very useful for the environmental monitoring and management of soil contamination in mining and metallurgical areas.

5. Recommendations and Future Directions

Based on the capacities for HM accumulation by the investigated plant and invertebrate species, the plant species *Fraxinus excelsior*, *Acer platanoides*, *Robinia pseudoacacia*, and *Aesculus hippocastanum* should be grown in the soil around the Alaverdi copper smelter to mitigate environmental impacts in the ecologically vulnerable areas of Alaverdi Town, while the slug species *Deroceras caucasicum* and *Limax flavus* can be used to remove Cu from the soil, and the earthworm species *Eisenia rosea* used to remove Zn from the soil. For implementing such mitigation actions, it is necessary to place large- and medium-sized stones or make small piles in the area around the smelter, which will create ecological conditions attractive to the invertebrates. Plants and animals should be regularly isolated from the area and replaced with new ones for observation.

Author Contributions: Conceptualization, V.H. and G.G.; methodology, V.H., G.G., L.A. and S.S.; software, G.G. and V.H.; validation, V.H., G.G. and A.V.; formal analysis, G.G. and V.H.; investigation, V.H., L.H., L.M., L.A., R.S., A.G. and S.S.; resources, V.H., L.H., L.M., R.S. and A.G.; data curation, V.H.; writing—original draft preparation, G.G. and V.H.; writing—review and editing, A.V.; visualization, A.V.; supervision, V.H. and G.G.; project administration, V.H.; funding acquisition, V.H., A.V. and L.A. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Science Committee of MESCS RA in the frame of the research project no. 10-5/I-4 and under the theme of the "Investigation of biodiversity of fauna and hydrobionts in the specially protected areas of the northern regions of Armenia and Lake Sevan basin".

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All related data are contained in this manuscript. Additional explanation may be available upon request.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Nuralykyzy, B.; Wang, P.; Deng, X.; An, S.; Huang, Y. Heavy Metal Contents and Assessment of Soil Contamination in Different Land-Use Types in the Qaidam Basin. *Sustainability* **2021**, *13*, 12020. [CrossRef]
2. Wuana, R.A.; Okieimen, F.E. Heavy Metals in Contaminated Soils: A Review of Sources, Chemistry, Risks and Best Available Strategies for Remediation. *Int. Sch. Res. Not.* **2011**, *2011*, 402647. [CrossRef]
3. Štofejová, L.; Fazekaš, J.; Fazekašová, D. Analysis of Heavy Metal Content in Soil and Plants in the Dumping Ground of Magnesite Mining Factory Jelšava-Lubeník (Slovakia). *Sustainability* **2021**, *13*, 4508. [CrossRef]
4. Gebeyehu, H.R.; Bayissa, L.D. Levels of Heavy Metals in Soil and Vegetables and Associated Health Risks in Mojo Area, Ethiopia. *PLoS ONE* **2020**, *15*, e0227883. [CrossRef] [PubMed]
5. Kinuthia, G.K.; Ngure, V.; Beti, D.; Lugalia, R.; Wangila, A.; Kamau, L. Levels of Heavy Metals in Wastewater and Soil Samples from Open Drainage Channels in Nairobi, Kenya: Community Health Implication. *Sci. Rep.* **2020**, *10*, 8434. [CrossRef] [PubMed]
6. Gaur, V.K.; Sharma, P.; Gaur, P.; Varjani, S.; Ngo, H.H.; Guo, W.; Chaturvedi, P.; Singhania, R.R. Sustainable Mitigation of Heavy Metals from Effluents: Toxicity and Fate with Recent Technological Advancements. *Bioengineered* **2021**, *12*, 7297–7313. [CrossRef]
7. Zhao, H.; Wu, Y.; Lan, X.; Yang, Y.; Wu, X.; Du, L. Comprehensive Assessment of Harmful Heavy Metals in Contaminated Soil in Order to Score Pollution Level. *Sci. Rep.* **2022**, *12*, 3552. [CrossRef]
8. Gevorgyan, G.; Mamyan, A.; Boshyan, T.; Vardanyan, T.; Vaseashta, A. Heavy Metal Contamination in an Industrially Affected River Catchment Basin: Assessment, Effects, and Mitigation. *Int. J. Environ. Res. Public Health* **2021**, *18*, 2881. [CrossRef]
9. Mamyan, A.S.; Gevorgyan, G.A. Comparative Investigation of the River Phytoplankton of the Debed River Catchment Basin's Mining and Non-mining Areas. *Biol. J. Armen.* **2017**, *69*, 50–55. Available online: https://arar.sci.am/dlibra/publication/260457 (accessed on 24 May 2022).
10. Gevorgyan, G.A.; Mamyan, A.S.; Hambaryan, L.R.; Khudaverdyan, S.K.; Vaseashta, A. Environmental Risk Assessment of Heavy Metal Pollution in Armenian River Ecosystems: Case Study of Lake Sevan and Debed River Catchment Basins. *Pol. J. Environ. Stud.* **2016**, *25*, 2387–2399. [CrossRef]
11. Akopyan, K.; Petrosyan, V.; Grigoryan, R.; Melkomian, D.M. Assessment of Residential Soil Contamination with Arsenic and Lead in Mining and Smelting Towns of Northern Armenia. *J. Geochem. Explor.* **2018**, *184*, 97–109. [CrossRef]
12. Petrosyan, V.; Orlova, A.; Dunlap, C.E.; Babayan, E.; Farfel, M.; von Braunf, M. Lead in Residential Soil and Dust in a Mining and Smelting District in Northern Armenia: A Pilot Study. *Environ. Res.* **2004**, *94*, 297–308. [CrossRef]
13. Varagyan, V.M.; Varagyan, A.L.; Gevorgyan, G.A. Investigation of Heavy Metal Pollution of Soil-Crop Agroecosystems in Tumanyan Region of Lori Province of RA. *Educ. Sci. Artsakh* **2013**, *3–4*, 76–81. (In Armenian)
14. FSI FCAATI. Methods for Measuring the Mass Concentration of Iron, Cadmium, Cobalt, Manganese, Nickel, Copper, Zinc, Chromium and Lead in Samples of Natural and Waste Waters Using Plasma Atomic Absorption Spectrometry. In *Quantitative Chemical Analysis of Water*; PND F 14.1:2:4.214-06; Federal State Institution Federal Center for Analysis and Assessment of Technogenic Impact (FSI FCAATI): Moscow, Russia, 2011. (In Russian)
15. Müller, G. Heavy Metals in the Sediments of the Rhine—Changes since 1971. *Umschau* **1979**, *79*, 778–783. (In German)
16. Hakanson, L. An Ecological Risk Index for Aquatic Pollution Control. A Sedimentological Approach. *Water Res.* **1980**, *14*, 975–1001. [CrossRef]
17. Weissmannová, H.D.; Mihočová, S.; Chovanec, P.; Pavlovský, J. Potential Ecological Risk and Human Health Risk Assessment of Heavy Metal Pollution in Industrial Affected Soils by Coal Mining and Metallurgy in Ostrava, Czech Republic. *Int. J. Environ. Res. Public Health* **2019**, *16*, 4495. [CrossRef]

18. Li, H.; Yang, J.; Ye, B.; Jiang, D. Pollution Characteristics and Ecological Risk Assessment of 11 Unheeded Metals in Sediments of the Chinese Xiangjiang River. *Environ. Geochem. Health* **2019**, *41*, 1459–1472. [CrossRef]
19. U.S. Department of Energy (USDOE). *The Risk Assessment Information System (RAIS)*; U.S. Department of Energy (USDOE), Office of Environmental Management, Oak Ridge Operations Office: Oak Ridge, TN, USA, 2011.
20. USEPA. *Regional Screening Level (RSL) Table for Chemical Contaminants at Superfund Sites*; U.S. Environmental Protection Agency (USEPA): Washington, DC, USA, 2011.
21. USEPA. *Human Health Toxicity Values in Superfund Risk Assessments*; OSWER Directive 9285.7-53; U.S. Environmental Protection Agency (USEPA): Washington, DC, USA, 2003.
22. USEPA. *Risk Assessment Guidance for Superfund; Volume I: Human Health Evaluation Manual (Part E, Supplemental Guidance for Dermal Risk Assessment)*; Final; OSWER Directive 9285.7-02EP; U.S. Environmental Protection Agency (USEPA): Washington, DC, USA, 2004.
23. Liang, Y.; Yi, X.; Dang, Z.; Wang, Q.; Luo, H.; Tang, J. Heavy Metal Contamination and Health Risk Assessment in the Vicinity of a Tailing Pond in Guangdong, China. *Int. J. Environ. Res. Public Health* **2017**, *14*, 1557. [CrossRef]
24. ATSDR. *Toxicological Profile for Arsenic*; U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry (ATSDR): Atlanta, GA, USA, 2007. Available online: https://wwwn.cdc.gov/TSP/ToxProfiles/ToxProfiles.aspx?id=22&tid=3 (accessed on 24 May 2022).
25. Kamunda, C.; Mathuthu, M.; Madhuku, M. Health Risk Assessment of Heavy Metals in Soils from Witwatersrand Gold Mining Basin, South Africa. *Int. J. Environ. Res. Public Health* **2016**, *13*, 663. [CrossRef]
26. OEHHA. *Technical Support Document for Cancer Potency Factors*; Appendix B: Chemical-Specific Summaries of the Information Used to Derive Unit Risk and Cancer Potency Values; California Environmental Protection Agency, California Office of Environmental Health Hazard Assessment (OEHHA): Sacramento, CA, USA, 2009. Available online: https://oehha.ca.gov/air/crnr/technical-support-document-cancer-potency-factors-2009 (accessed on 24 May 2022).
27. Geary, R.C. The Ratio of the Mean Deviation to the Standard Deviation as a Test of Normality. *Biometrika* **1935**, *27*, 310–332. [CrossRef]
28. Student. The Probable Error of a Mean. *Biometrika* **1908**, *6*, 1–25. [CrossRef]
29. Benesty, J.; Chen, J.; Huang, Y.; Cohen, I. Pearson Correlation Coefficient. In *Noise Reduction in Speech Processing*; Springer: Berlin/Heidelberg, Germany, 2009; pp. 1–4. [CrossRef]
30. Mohammadi, A.; Mansour, S.N.; Najafi, M.L.; Toolabi, A.; Abdolahnejad, A.; Faraji, M.; Miri, M. Probabilistic Risk Assessment of Soil Contamination Related to Agricultural and Industrial Activities. *Environ. Res.* **2022**, *203*, 111837. [CrossRef] [PubMed]