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Risk elements in soils affected by long-term gold and mercury (Hg) mining related to soil microbial activity

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ABSTRACT

Representative soil samples were collected from three areas in the Czech Republic surrounding two former cinnabar mines and a former gold mine, where mercury (Hg) was historically used in the amalgamation process for gold extraction. This study aimed to assess the potential impact of long-term soil contamination by risk elements on microbial activity. The soil risk element content was determined based on location, with Hg identified as the predominant pollutant at all three sites, where the individual pollution index (I_{Hg}) ranged from 0.7 to 450. To evaluate the impact of Hg pollution on soil biological properties, a suite of soil enzyme activities was analyzed. The results indicated no significant reduction in enzymatic activities, suggesting a negligible harmful effect of long-term Hg pollution on microbial activity. However, significant ($p < 0.05$) positive correlations between soil Hg content and chitinase and cellobiohydrolase activities at one location suggested that other factors, beyond Hg pollution levels, influenced microbial activity. These findings point to the low bioaccessibility of Hg in the studied areas and/or potential microbial adaptation to elevated Hg levels in soils.

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Mercury; risk elements; soil; enzymatic activity; gold mine; cinnabar mine

Introduction

Risk elements are released from various natural and anthropogenic sources, and their accumulation in the environment can lead to contamination of water, air, soil, and food, posing significant health risks [1]. Anthropogenic activities significantly alter the global mercury (Hg) cycle, contributing to the overall Hg burden [2]. The largest source of Hg pollution is artisanal and small-scale gold mining (37.7%), followed by coal combustion (21%), non-ferrous metal production (15%), and cement and lime production (11%) [3].

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Gold mining degrades the environment and alters soil structure, affecting its physicochemical and biological properties, including microbial diversity. The amalgamation process, commonly used for gold extraction, introduces Hg into soils, leading to long-term contamination [4,5]. While active artisanal mining continues in many developing countries, historical gold mining in Europe has left legacy Hg contamination. Yevugah et al. [6] reported elevated Hg levels in Ghanaian gold mining areas due to long-term atmospheric deposition, with associated human health risks [7].

Similarly, cinnabar mining areas contribute to Hg pollution. Li et al. [8] highlighted the potential mobility of Hg in mine waste near Chinese cinnabar mines, with concentrations ranging from 0.369 to 2.62 mg/kg, posing risks to aquatic and atmospheric systems. Comparable contamination has been reported in the Idrija Hg mine in Slovenia [9]. Additionally, cinnabar mining areas often exhibit elevated levels of other metals, such as lead, zinc, and copper [10]. Hg bioavailability in soil depends on its chemical partitioning, including water-soluble, exchangeable, oxidisable, and residual fractions [11]. In well-aerated soils, Hg primarily exists as Hg(II), with smaller amounts of elemental Hg (Hg^0) and methylmercury [12].

The vertical distribution of Hg in soil varies by landscape type. Higher Hg levels are typically found in surface layers, particularly in forested areas where leaf litter decomposition enhances Hg accumulation in organic horizons [13, 14]. Zhou et al. [15] reported annual total Hg deposition fluxes of 40.5 μg/m^2 in coniferous forests and 90.9 μg/m^2 in broad-leaved forests, with similar soil Hg levels despite differences in litter decomposition rates.

Elevated risk element concentrations influence soil microbial communities by disrupting biochemical processes and altering genetic structures [16]. However, some microorganisms exhibit remarkable tolerance to Hg due to biosorption and bioaccumulation abilities [17]. Microbial transformation of Hg is facilitated by specific detoxification genes, such as *mer* and *hgc*, which are overexpressed under high Hg concentrations [18]. Enhanced microbial activity can also aid in Hg phytoremediation, where enzymatic amendments improve plant uptake of Hg [19].

Soil enzyme activities serve as bioindicators of soil quality and pollution impact, particularly in mining-impacted environments [20]. Enzymes such as dehydrogenase, β-glucosidase, urease, alkaline phosphatase, and acid phosphatase are useful for assessing soil regeneration [21]. Dehydrogenase, a key indicator of microbial metabolic activity, has shown variable responses to Hg contamination [22]. While Mahbub et al. [23] observed significant inhibition of dehydrogenase activity even at low Hg concentrations, Campos et al. [24] reported no significant effects in long-term Hg mining areas. The activities of arylsulfatase and acid phosphatase have been found to correlate with soil Hg levels [25].

To further explore these interactions, this study analyzed soil microbial activity in three Hg-contaminated areas in the Czech Republic: two former cinnabar mines (Horní Luby and Jedová Hora) and a former gold mine (Libčice). These sites differ significantly in Hg pollution origins - geogenic from cinnabar deposits [26, 27] and anthropogenic from historical gold amalgamation at Libčice in the early twentieth century [28]. Previous studies have investigated Hg speciation and soil–plant transport at Libčice and Jedová Hora, including the presence of elemental Hg (Hg^0), methylmercury ($MeHg^+$), phenylmercury ($PhHg^+$), and gaseous elemental mercury (GEM) [29–31]. However, the Horní Luby site has not been studied in detail. The objectives of this study were: (i) to assess and compare soil risk element content across these locations and (ii) to evaluate the response of soil microbial activity to Hg pollution, considering differences in contamination sources. It was hypothesised

that long-term Hg exposure had led to microbial community stabilisation, minimising enzymatic activity variations. To test this, soil samples were collected from all three sites, and the activity of key soil enzymes was analyzed based on location and depth.

Comparative studies on Hg-polluted soils and their effects on microbial communities are common due to widespread Hg contamination worldwide. However, variations in pollution levels, soil properties, climatic conditions, mining histories, and sampling strategies complicate direct comparisons. The novelty of this study lies in its direct comparison of Hg pollution from two distinct sources – lithogenic from cinnabar mining and anthropogenic from gold mining.

Material and methods

Sampling and sample processing

Two abandoned cinnabar mines, the Hg mine in Horní Luby (North Bohemia) and Jedová Hora ('Poison Mountain') in Central Bohemia, were selected for this study. In Horní Luby, the ore consists of pure cinnabar [26]. The primary mining phase occurred between 1520 and 1540, followed by a second peak from 1560 to 1570, with Hg production ranging from 6 to 15 tonnes per annum. The mine was ultimately abandoned in 1597, with several unsuccessful attempts at reactivation in the 17th to 19th centuries. In contrast, Jedová Hora's cinnabar deposits, mined as a by-product of Ordovician iron ore (mainly haematite and siderite), were exploited from the eighteenth century until the late nineteenth century, producing several tonnes of Hg per year [32]. At the former gold mine in Libčice, 8661 tonnes of ore were extracted between 1913 and 1923, yielding approximately 25 kg of gold. After 1923, mining operations were minimal, and the site is now characterised by disintegrating building ruins [28]. Previous studies by Umlaufová et al. [31] confirmed heterogeneous Hg contamination across the area.

A consistent soil sampling strategy was applied at all three locations - Jedová Hora, Horní Luby, and Libčice. Soil samples were collected near key mining-related features, such as abandoned drift outfalls, mine waste heaps, and ore-processing facility ruins. Each site included seven sampling points, with soil samples taken from a depth of 0–20 cm. At each sampling point, five subsamples were collected within an area of approximately 5 m^2. Due to variations in mining residue distribution, the sampling grid varied slightly between locations.

Soil profiles were excavated to collect distinct horizons: O horizon (organic layer, 1–5 cm deep, with litter removed prior to sampling), A horizon (mineral and humic layer, 6–13 cm deep), and B horizon (subsoil mineral layer, up to 20 cm deep). At Libčice, the shallow soil and stony subsoil limited sampling to only the O and A horizons. The precise locations of sampling points, including GPS coordinates, are provided in Supplementary Table S1. Samples were freeze-dried, ground using a mortar, and sieved through a 2-mm plastic mesh prior to analysis.

Analytical methods

Soil risk element content

Soil pH values were determined in a 0.01 mol/L $CaCl_2$ extract (1:10 w/v). The pseudototal element contents in the soils were measured following a decomposition procedure.

Approximately 0.5 g of air-dried soil was digested with 10 mL of aqua regia (a 1:3 mixture of nitric and hydrochloric acids) in an Ethos 1 microwave-assisted wet digestion system (MLS GmbH, Germany) at 210°C for 33 min. After cooling, the digests were transferred to a 25-mL glass tube, diluted with deionised water, and maintained at laboratory temperature until analysis. Elemental concentrations (As, Be, Cd, Co, Cr, Cu, Ni, Pb, V, Zn) in the soil extracts were determined using inductively coupled plasma-optical emission spectrometry (ICP-OES, Agilent 720, Agilent Technologies Inc., USA).

Total Hg content in soils was determined using an AMA-254 single-purpose atomic absorption spectrometer (LECO model, Altec, Czech Republic). The samples were first dried, then combusted in an oxygen stream. The resulting Hg vapour was trapped on a gold amalgamator and subsequently released as elemental Hg (Hg^0) through heating. The released vapour was measured using atomic absorption spectrometry at a wavelength of 253.65 nm, with a low-pressure Hg discharge lamp as the radiation source [33].

Soil enzymatic activity

Soil enzymatic activity was assessed using the spectrofluorometric method described by Baldrian [34] with labelled fluorescent substrates. The activities of β-glucosidase, phosphatase, chitinase, cellobiohydrolase, arylsulfatase, and lipase were determined using the fluorogenic substrate 4-methylumbelliferyl (MUF), while alanine aminopeptidase and leucine aminopeptidase were analyzed using the fluorogenic substrate 7-amino-4-methylcoumarin (AMC).

For the analysis, 0.25 g of freeze-dried soil sample was suspended in 50 mL of 50 mM acetate buffer (pH 5). A 200 µL aliquot of this suspension was pipetted into microplates containing 40 µL of the appropriate MUF or AMC substrate for each enzyme. Enzymatic activity was measured using a microplate reader (Infinite F200, TECAN, Switzerland) at an excitation wavelength of 355 nm and an emission wavelength of 460 nm.

Following initial measurement, samples were incubated for 2 h at 40°C, and the measurement was repeated. Each determination included three replicates. To correct for fluorescence quenching, a calibration curve was established for each soil sample and substrate (MUF and AMC). Enzymatic activity was calculated as the difference between initial and terminal values and expressed as µM of the reaction product formed per minute per gram of soil (µM/min/g).

Environmental and health risk assessment

1) The individual pollution index (I_i) was calculated as follows:

$$I_i = \frac{C_i}{S_i}$$

where C_i is the measured element concentration, and S_i is the permissible value of the element under evaluation. In this case, the preventive values of these elements in soil, as given by Czech Public Notice No. 153/2016 [35], were chosen as S_i. When the individual pollution index is $Ii \leq 0.7$, the pollution level is safe; when $0.7 < Ii \leq 1.0$, it is a clean environment (limit); $1.0 < Ii \leq 2.0$ means there is slight pollution; $2.0 < Ii \leq 3.0$ means moderate pollution; and $Ii \geq 3.0$ means serious pollution.

2) Subsequently, to evaluate the overall environmental quality, the Nemerow index (P_N) reflects both the maximum ($MaxI_i$) and average ($AvgI_i$) values of pollutant concentrations:

$$P_N = \sqrt{\frac{MaxI_i^2 + AvgI_i^2}{2}}$$

where the average is calculated as an average of all individual pollution indices regardless of the element, and the maximum means the highest individual pollution index regardless of the element. For the Nemerow index, the same scale was used to assess pollution levels as in the case of individual pollution indices [36, 37].

Statistics

All statistical analyses were performed using Statistica 12.0 software (www.statsoft.cz). The individual datasets did not match the parameters of a normal data distribution. Thus, the non-parametric Kruskal–Wallis test was used to assess statistically significant differences in the measured parameters among the locations. Dixon's test was applied to identify outliers. Spearman's rank correlation coefficients (ρ) were applied to determine the potential interrelationships between Hg levels in soils and the activity of individual enzymes. A value of $p < 0.05$ was considered significant.

Results and discussion

Soil pollution level

The pseudototal element contents (total in the case of Hg) in the soils are summarised in Table 1. Element concentrations were compared against preventive and indicative soil element levels specified by Czech Public Notice No. 153/2016 [35], despite the studied soils not being agricultural. Maximum concentrations of As, Be, Cd, Co, Cu, Hg, Ni, Pb, V, and Zn exceeded preventive limits at least at one study site. Indicative values for crop contamination risk (40 mg/kg As, 1.5 mg/kg Cd, 1.5 mg/kg Hg) were surpassed for Cd and As at some sites and for Hg at all three locations. Zn levels exceeded indicative values for plant growth and soil biological function (400 mg/kg Zn). Additionally, As and Hg values posed direct threats to human and animal health (40 mg/kg As, 20 mg/kg Hg). Except for Libčice, Hg concentrations exceeded indicative values for human and animal health risks.

No significant differences in Pb and Zn levels were observed among locations, while Cd, Co, Cr, Ni, and V concentrations were significantly ($p < 0.05$) higher at Jedová Hora. As and Cu concentrations were highest ($p < 0.05$) at Libčice, whereas Be concentrations were lowest ($p < 0.05$) at this site. Hg concentrations were lower in the gold mining area at Libčice compared to the cinnabar mining sites, but due to high variability, statistically significant differences ($p < 0.05$) were only found between Libčice and Horní Luby. The variability in Hg concentrations is attributed to the heterogeneous distribution of Hg-rich mine tailings at Horní Luby and Jedová Hora and the variable distance of sampling points from gold-bearing ore processing ruins at Libčice.

Table 2 presents individual pollution indices (I_i) and the Nemerow index for each location, showing a wide range of pollution levels from unpolluted to moderate for

Table 1. Descriptive statistics of the pseudototal (real total in the case of Hg) element contents in the soils.

Sampling point	As mg/kg	Be mg/kg	Cd mg/kg	Co mg/kg	Cr mg/kg	Cu mg/kg	Hg[a] mg/kg	Ni mg/kg	Pb mg/kg	V mg/kg	Zn mg/kg
Jedová Hora ($n = 7$)											
Minimum	25.9	1.0	0.2	5.1	39.3	12.1	0.2	11.5	38.9	73.0	78.7
Maximum	53.1	2.3	1.8	59.7	107	47.0	135	91.7	133	150	315
Average	36.7	1.7	1.0	30.6	65.7	35.4	26.5	51.2	70.2	110	178
Standard deviation	8.6	0.5	0.6	18.8	21.1	11.7	48.3	31.4	38.9	27.0	84.8
Median	37.1	1.7	0.9	36.1	62.3	37.0	8.7	53.3	49.8	107	163
MAD[b]	4.1	0.4	0.6	36.1	8.3	7.0	4.0	29.6	10.9	24.7	57.5
Libčice ($n = 7$)											
Minimum	17.9	0.4	0.2	9.5	16.9	19.4	0.7	7.9	11.8	56.4	63.4
Maximum	122	1.2	0.5	24.5	73.8	121	15.5	43.6	162	85	185
Average	58.1	0.7	0.4	13.1	28.4	46.5	5.6	15.2	38.9	66.1	97.4
Standard deviation	34.9	0.2	0.1	5.1	20.4	34.2	4.8	12.7	54.7	9.5	40.2
Median	43.9	0.6	0.4	11.6	20.3	33.1	4.4	10.3	16.3	64.1	89.8
MAD[b]	25.9	0.1	0.1	0.8	2.5	9.2	1.6	1.0	3.6	4.7	12.6
Horní Luby ($n = 7$)											
Minimum	13.7	1.4	0.1	8.4	32.1	12.8	3.3	20.4	38.6	40.4	78.5
Maximum	27.0	3.4	0.3	18.9	47.6	40.2	117	29.8	103	65.5	617
Average	17.5	2.0	0.2	12.7	41.3	26.6	40.9	25.6	61.7	57.8	193
Standard deviation	4.4	0.7	0.1	3.5	6.5	11.1	39.2	3.6	20.4	10.5	192
Median	16.8	1.8	0.1	12.6	44.0	20.8	34.2	25.2	55.0	62.0	132
MAD[b]	0.7	0.2	0.0	1.6	2.4	8.0	21.9	3.6	9.1	3.5	48

[a]total content; [b]median of absolute deviations

most elements. Serious pollution was recorded in maximum I_i values for Cd at Jedová Hora, As at Libčice, and Zn at Horní Luby. No significant ($p < 0.05$) differences were found for I_{Cu}, I_{Pb}, or I_{Zn}, while I_{Cd}, I_{Co}, I_{Cr}, I_{Ni}, and I_V were significantly ($p < 0.05$) higher at Jedová Hora. I_{As} values were highest at Libčice, and IBe was significantly ($p < 0.05$) elevated at Horní Luby. Extremely high maximum I_{Hg} values at all sites confirmed Hg as the primary pollutant. The dominance of Hg was evident from Nemerow index values, which closely followed I_{Hg} levels. Neither I_{Hg} nor the Nemerow index significantly differed across locations, likely due to the substantial variability in Hg concentrations. Spearman's correlation analysis (Supplementary Table S3) revealed various significant ($p < 0.05$) correlations among individual pollution indices, with the strongest correlation ($\rho = 1$) between I_{Hg} and the Nemerow index, highlighting Hg as the predominant environmental pollutant.

Elevated soil element concentrations in mining areas, including cinnabar mines, are well documented (e.g. Gosar et al. [9]). As expected, Hg was the dominant soil pollutant. Similarly, historical gold mining using amalgamation has led to Hg pollution [38]. Other risk elements, such as As from arsenopyrite-bearing gold ores, have also been reported [27, 31]. Abandoned mine areas without remediation measures pose risks of elemental transport into surrounding environments [39]. Mine tailings are often acidic, increasing the mobility of most risk elements However, Hg exhibits higher affinity for organic matter at lower pH levels [40]. In this study, acidic pH values (4.3–4.7) were recorded at Horní Luby and Jedová Hora, which could be attributed to natural forest soil acidity [41] rather than mining residues. At Libčice, pH values ranged from 6.2 to 6.4. Differences

Table 2. Descriptive statistics of the individual pollution indices (I_i) and Nemerow index (P_N) of the soils.

Sampling point	I_{As}	I_{Be}	I_{Cd}	I_{Co}	I_{Cr}	I_{Cu}	I_{Hg}	I_{Ni}	I_{Pb}	I_V	I_{Zn}	P_N
Jedová Hora ($n = 7$)												
Minimum	1.29	0.52	0.45	0.17	0.44	0.20	0.74	0.23	0.65	0.56	0.66	1.2
Maximum	2.65	1.05	3.51	1.99	0.80	0.75	451	1.66	2.22	1.15	2.63	320
Average	1.83	0.81	2.07	0.99	0.65	0.56	101	0.89	1.23	0.82	1.50	71.6
Standard deviation	0.47	0.21	1.33	0.68	0.13	0.19	173	0.57	0.69	0.21	0.77	123
Median	1.78	0.84	2.29	0.98	0.68	0.62	29.4	0.84	0.95	0.82	1.39	21.0
MAD[b]	0.26	0.21	1.22	0.63	0.08	0.11	28.7	0.53	0.31	0.20	0.66	19.7
Libčice ($n = 7$)												
Minimum	0.90	0.21	0.39	0.32	0.19	0.32	2.21	0.16	0.20	0.43	0.53	1.61
Maximum	6.12	0.59	1.08	0.82	0.82	2.02	51.8	0.87	2.69	0.65	1.54	36.8
Average	2.91	0.33	0.78	0.44	0.32	0.78	18.6	0.30	0.65	0.51	0.81	13.3
Standard deviation	1.74	0.12	0.25	0.17	0.23	0.57	15.9	0.25	0.91	0.07	0.33	11.3
Median	2.19	0.31	0.84	0.39	0.23	0.55	14.8	0.21	0.27	0.49	0.75	10.6
MAD[b]	1.30	0.03	0.19	0.03	0.03	0.15	5.19	0.02	0.06	0.04	0.10	3.67
Horní Luby ($n = 7$)												
Minimum	0.68	0.69	0.18	0.28	0.36	0.21	11.1	0.41	0.64	0.31	0.65	7.9
Maximum	1.35	1.71	0.59	0.63	0.53	0.64	390	0.60	1.71	0.50	5.14	277
Average	0.89	0.98	0.31	0.42	0.46	0.41	140	0.50	1.02	0.44	1.60	99.6
Standard deviation	0.24	0.37	0.14	0.13	0.08	0.17	143	0.08	0.37	0.09	1.75	101
Median	0.85	0.85	0.26	0.41	0.49	0.34	110	0.50	0.91	0.49	0.91	78.0
MAD	0.15	0.15	0.05	0.10	0.04	0.13	77.3	0.08	0.21	0.02	0.26	54.8

[b]median of absolute deviations

in soil pH can influence microbial activity, as different microbial communities thrive at varying pH levels [42].

Gold mining areas often contain As-bearing minerals like arsenopyrite, making elevated As levels expected [31]. However, other elements commonly associated with gold mining, such as Cd and Pb [43], were not elevated in this study. Supplementary Table S2 provides Spearman's correlation coefficients (ρ), showing strong interrelationships among most elements, suggesting similar mineral compositions across study sites. The pseudototal content results indicate that As followed an inverse trend compared to other elements, with the highest levels at Libčice, while the remaining elements had lower concentrations. Hg exhibited relatively weak correlations with other elements, likely due to its high variability in soil samples.

Soil enzymatic activity

The activities of soil enzymes - β-glucosidase, phosphatase, chitinase, cellobiohydrolase, alanine aminopeptidase, leucine aminopeptidase, arylsulfatase, and lipase - were determined in the soil samples (Figures 1–8). The results indicate that microbial activity varied by location. Enzyme activities, including β-glucosidase, phosphatase, chitinase, arylsulfatase, and lipase, were significantly higher ($p < 0.05$) at Jedová Hora compared to the other sites. Soil properties, particularly organic matter content and composition, strongly influence enzymatic responses to elevated risk element concentrations [44]. However, no significant ($p < 0.05$) relationships were found between soil Hg content and total or organic carbon content, despite previous studies showing higher Hg accumulation in the upper organic layer [14]. Microbial activity generally declined with soil depth, though the differences were not statistically significant. The presence of organic carbon plays a key role in supporting microbial activity [45]. Among individual enzymatic

activities, acid phosphatase and lipase exhibited the highest values (Figures 2 and 4). Acid phosphatase facilitates phosphorus release from organic matter, while lipase hydrolyzes lipids into fatty acids and glycerol [46], making them crucial components of microbial metabolism. Conversely, alanine aminopeptidase and leucine aminopeptidase, which contribute to nitrogen cycling, displayed the lowest activity [47]. Enzymatic variability appeared more dependent on site-specific soil characteristics than Hg contamination.

No significant correlations were observed between soil Hg concentrations and enzymatic activities, except for chitinase ($\rho = 0.55$) and cellobiohydrolase ($\rho = 0.57$) at Libčice ($p < 0.05$). These enzymes exhibited increased activity with higher Hg content, though Hg levels at this site were considerably lower than at the other locations. This aligns with findings by Frey et al. [18], which demonstrated that carbon and nitrogen cycling remained largely unaffected by high Hg concentrations (up to 36 mg/kg), suggesting limited microbial damage.

Previous studies have indicated that soil enzymatic activity serves as a sensitive bioindicator of soil health, often showing decreased activity in response to increased risk element concentrations [11, 48, 49]. Casucci et al. [44] noted that Hg strongly binds to thiol groups in proteins, potentially inhibiting enzymatic function. Zheng et al. [50] reported a decline in enzymatic activity in Hg-contaminated coal-mining soils, affecting urease, acid phosphatase, and catalase, with inhibition rates varying by pollutant concentration. Hg pollution can directly impact hydrolytic enzymes by either suppressing gene expression or deactivating active enzyme sites. Oliveira and Pampulha [22] documented shifts in microbial communities due to prolonged Hg contamination and verified dehydrogenase activity as a reliable bioindicator of Hg toxicity. However, dehydrogenase activity has shown no significant changes in long-term Hg-polluted areas [24], leading to its exclusion from this study's analytical protocol.

Contrastingly, Piani et al. [25] studied Hg contamination from mine spoils at the Idrija Hg mining site (Slovenia) and found no significant long-term negative trends in soil

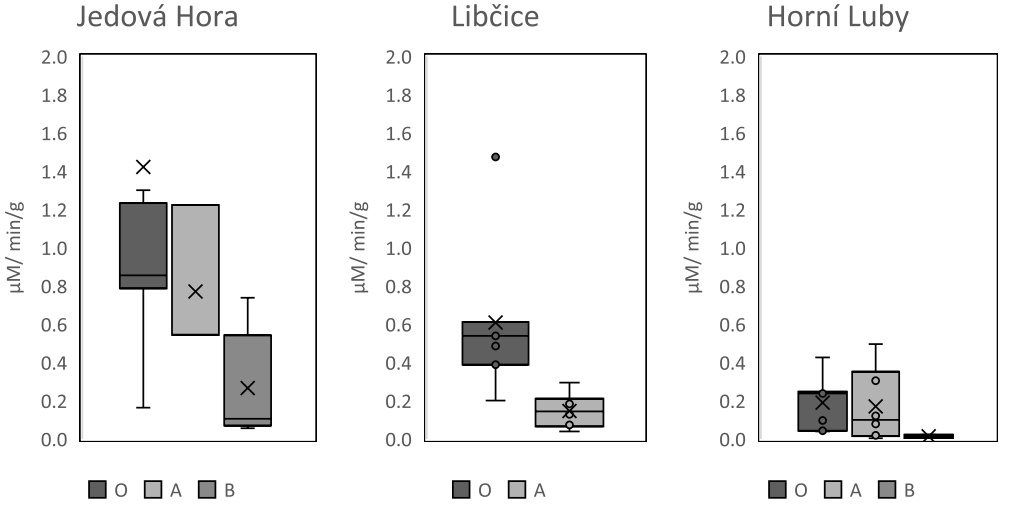

Figure 1. b-D-glukosidase activity in the soils according to the location and soil layer (n = 7 for each soil layer).

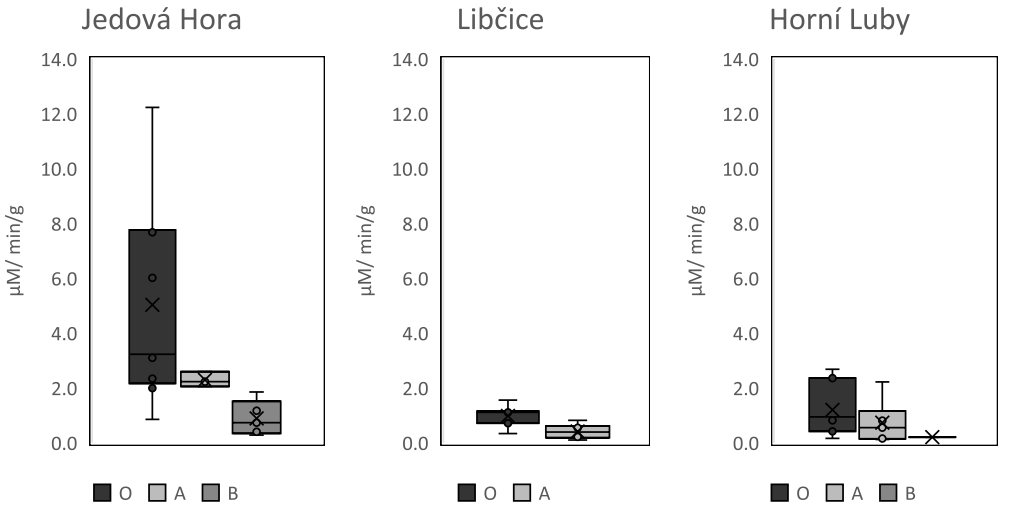

Figure 2. Acid phosphatase activity in the soils according to the location and soil layer (n = 7 for each soil layer).

biological activity, despite Hg levels reaching 80 mg/kg. Only arylsulfatase and acid phosphatase showed a tendency for negative correlations with Hg concentrations. Similarly, Ruggiero et al. [51] observed no effects of long-term Hg pollution on microbial biomass or enzyme activity, supporting the hypothesis that microbial communities may adapt to elevated Hg concentrations. Microorganisms have evolved diverse resistance mechanisms to risk elements, as summarised by Bruins et al. [16].

Another possible explanation for the minimal impact of high Hg levels on microbial activity is the low bioavailability of Hg in the studied soils. The mobility and bioaccessibility of Hg depend on soil type and regional conditions. Garcia-Ordiales et al. [52] reported

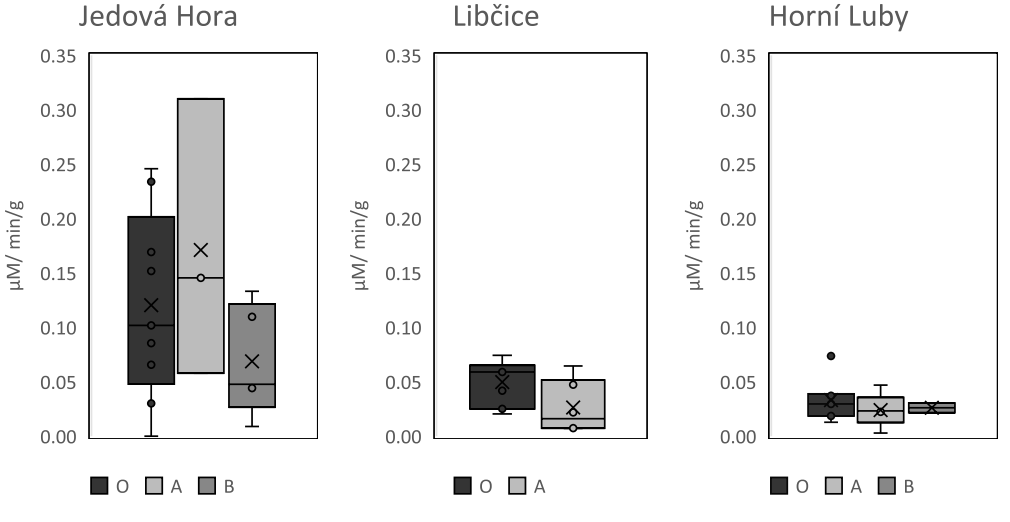

Figure 3. Arylsulfatase activity in the soils according to the location and soil layer (n = 7 for each soil layer).

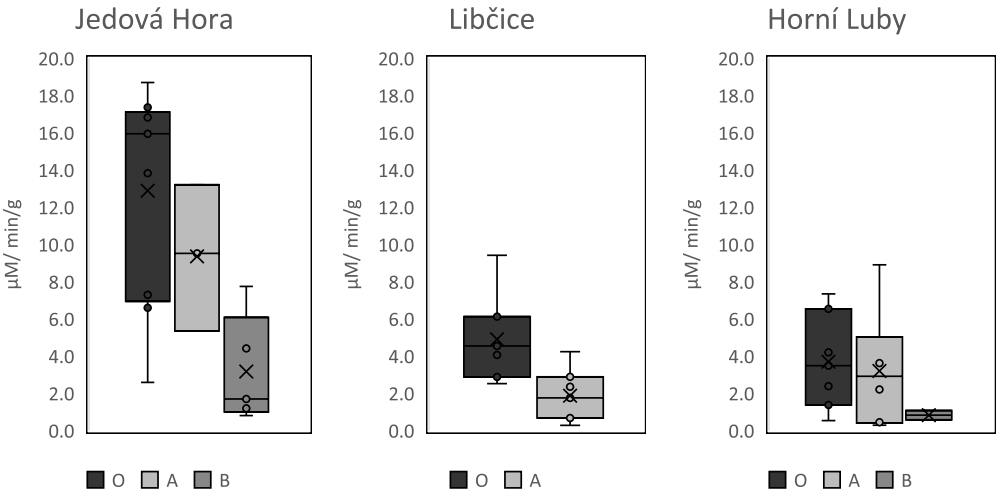

Figure 4. Lipase activity in the soils according to the location and soil layer (n = 7 for each soil layer).

that Hg in mining areas is predominantly present as HgS, with smaller proportions as Hg^{2+} and methylmercury. Although speciation analysis was not conducted in this study, prior research offers relevant insights. Hojdová et al. [39] used thermal desorption to assess Hg speciation at Jedová Hora and found that 50–80% of total Hg existed as relatively insoluble HgS, consistent with Garcia-Ordiales et al. [52]. Additionally, a significant portion of total Hg was associated with adsorption onto mineral surfaces, Fe-oxyhydroxides, or clay minerals [39].

Sysalová et al. [29] identified minor proportions of Hg°, $MeHg^{+}$, $PhHg^{+}$, and GEM in Jedová Hora soils, indicating some Hg transformation via microbial activity. Similarly, Sysalová et al. [30] found Hg^{0}, GEM, and $MeHg^{+}$ at Libčice, though $MeHg^{+}$ levels were

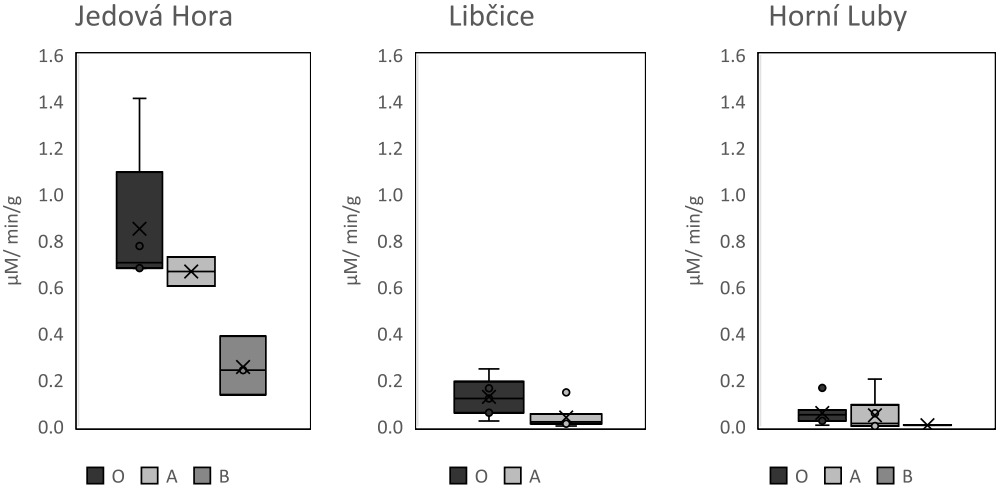

Figure 5. Chitinase activity in the soils according to the location and soil layer (n = 7 for each soil layer).

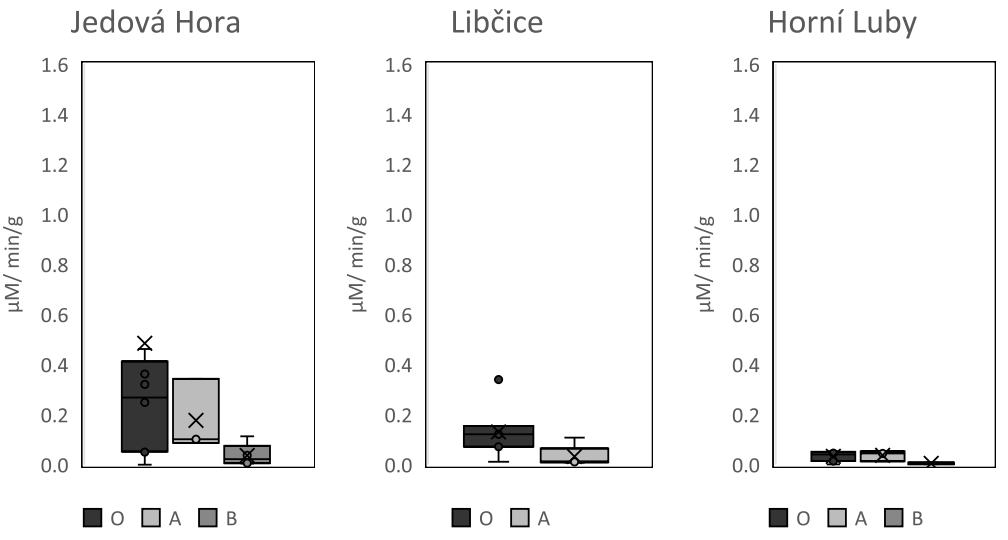

Figure 6. Cellobiohydrolase activity in the soils according to the location and soil layer (n = 7 for each soil layer).

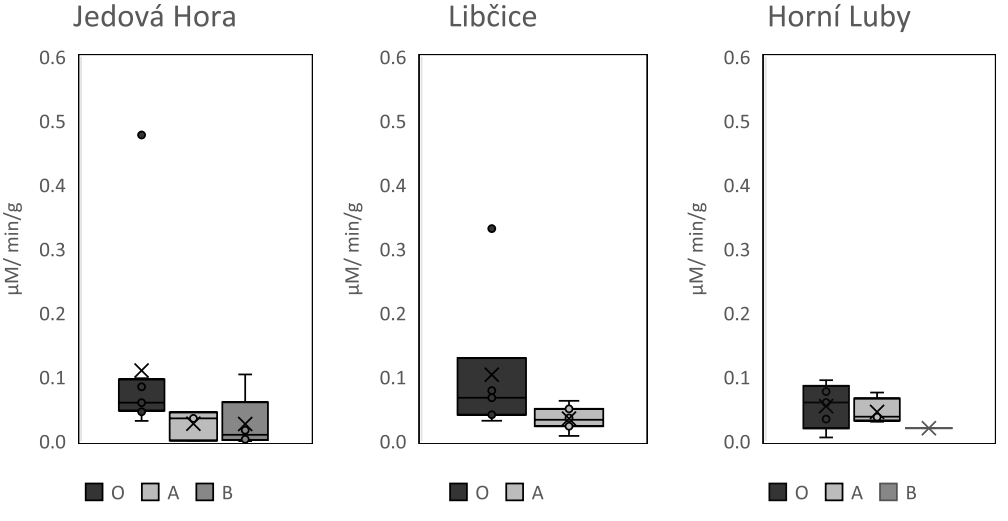

Figure 7. Alanin aminopeptidase activity in the soils according to the location and soil layer (n = 7 for each soil layer)

relatively low, as Hg-metal amalgams (Hg^{+}-Au) are less mobile than soluble inorganic Hg species and less available for methylation. Consequently, both cinnabar mining and gold amalgamation processes resulted in low microbial bioaccessibility of Hg, minimising its impact on microbial communities.

The enzymatic activity analysis of soil nutrient cycles did not indicate direct harmful effects of Hg on microbial activity. Soil nutrient status significantly influences microbial community dynamics, with organic carbon and nitrogen availability serving as key environmental factors [53]. These factors likely drive variations in enzymatic activity

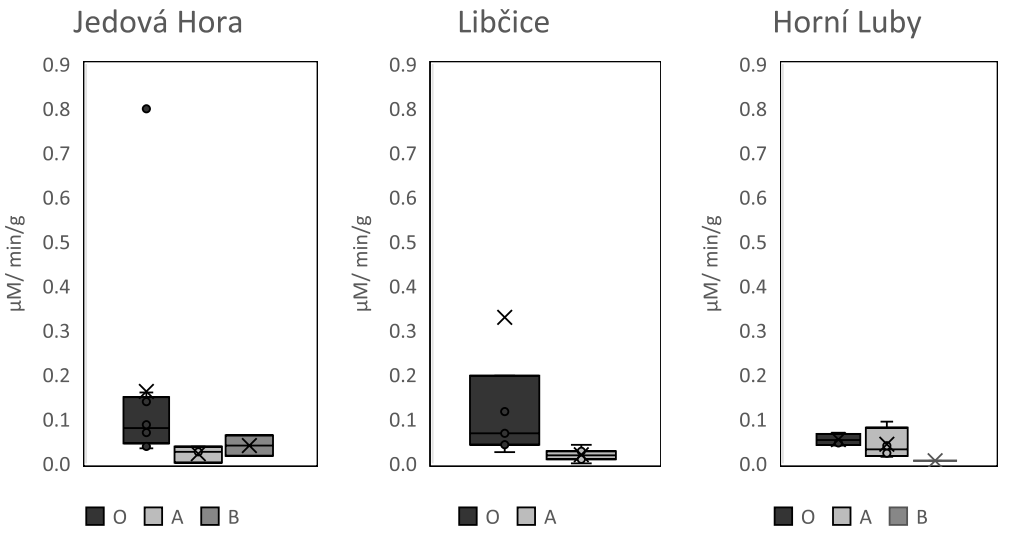

Figure 8. Leucin aminopeptidase activity in the soils according to the location and soil layer (n = 7 for each soil layer).

more than Hg contamination alone. A more detailed characterisation of the soil microbiome is necessary to fully understand microbial responses to long-term Hg exposure.

Hg fate in forest soils depends on environmental factors such as temperature, precipitation, pH, and organic matter content [54]. In this study, previous research suggested low bioaccessibility of soil Hg. While the environmental risk of Hg contamination at the study sites does not appear acute, potential Hg transfer into other ecosystem compartments cannot be ruled out. Piani et al. [25] suggested that, despite no direct threat to soil microbiomes, extreme Hg pollution may facilitate soil–plant–animal transfer. This is consistent with findings from Libčice, where Umlaufová et al. [31] reported occasional exceedances of Hg threshold limits in plant tissues (particularly Fabaceae species). Furthermore, Sysalová et al. [30] documented measurable methylation rates and GEM proportions, indicating possible Hg diffusion into adjacent environmental compartments. Further research is needed to assess these potential risks comprehensively.

Conclusions

This study did not confirm a significant adverse effect of soil Hg pollution on microbial activity, suggesting that either Hg concentrations remained below toxicity thresholds or favourable soil conditions and nutrient availability supported microbial resilience. However, soil microbial activity proved to be a weak indicator of long-term Hg contamination, making it difficult to conclusively assess its impact on the microbiome. The limited response of soil microbial activity in these areas may be attributed to the low bioaccessibility of Hg at the studied sites. This response could differ significantly in areas with ongoing mining activities, where Hg remains more bioavailable. Therefore, further detailed research is necessary to comprehensively evaluate the potential risks of Hg pollution in these locations, particularly regarding its bioavailability and transport within the environment.

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Author contribution

Luka Stefanović, Václav Tejnecký and Artem Khaustov collected the soil samples and characterised them; Tatiana Robledo Mahón, Artem Khaustov and Anna Cieslarová determined the element contents and enzymatic activities, Jiřina Száková evaluated the data and wrote the main text, Pavel Tlustoš and Jiřina Száková reviewed the final version of the text. All authors read and approved the final manuscript.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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