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# Microbial Biomass and Rhizosphere Soil Properties in Response to Heavy Metal-Contaminated Flooding

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**Abstract:** Mining and metallurgy are the main sources of soil contamination with harmful metals, posing a significant threat to human health and ecosystems. River floodplains in the vicinity of metal mines or industrial plants are often subject to flooding with sediments containing heavy metals, which can be harmful to the soil ecosystem. This study aimed to investigate the microbial properties of the soil at a metal-contaminated site and to determine the significant relationships between the biological and chemical properties of the soil. The study site was located near the village of Gyöngyösoroszi, in the Mátra mountain region of Northwest Hungary. A phytoremediation experiment was conducted in a metal-polluted floodplain using willow and corn plantations. The soil basal respiration, substrate-induced respiration, soil microbial biomass carbon (MBC), acid phosphatase activities, and soil chemical properties were measured. The soil of the contaminated sites had significantly higher levels of As, Pb, Zn, Cu, Cd, and Ca, whereas the unpolluted sites had significantly higher levels of phosphorus and potassium. The substrate-induced respiration showed a positive correlation with MBC and negative correlations with the metabolic quotient ($qCO_2$). The soil plasticity index and phosphorus showed a positive correlation with MBC, whereas salinity and the presence of Cd, Pb, Zn, As, and Cu showed a negative correlation. Acid phosphomonoesterase activity negatively correlated with the plant-available phosphorus content and MBC, but was positively correlated with the contents of toxic elements, including cadmium, lead, zinc, arsenic, and copper. This study found a significant correlation between the $qCO_2$ and the toxic element content. This suggests that an enhanced metabolic quotient ($qCO_2$), together with a decreased MBC/SOC ratio, could be used to indicate the harmful effect of soil contamination by heavy metals in floodplain soils.

**Keywords:** heavy metal; lead and zinc mine; pollution gradient; soil microbial biomass; microbial activity; phosphatase activity; soil respiration



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## 1. Introduction

The mining and metal industries are the primary sources of soil contamination by harmful metals, which pose environmental risks to both human health and ecosystems. Large quantities of spoils, tailings, and flotation slurry with high heavy metal contents are generated in mining areas. Various remediation techniques are used to address the environmental problem of soil contamination by metals. The stabilization of contaminants through the use of additives [1], followed by revegetation of the contaminated area, is likely to be the most cost-effective method. River floodplains near metal mines or industrial centers are often inundated with sediments containing heavy metals, such as the Rhine in Germany and the Netherlands, the Maas in the Netherlands, and the Tisza in Hungary [2–4]. Heavy metals can be toxic to soil biota, leading to a reduction in the number and activity of soil microorganisms and interfering with key microbial processes [5–7]. As a result, the biological properties of these soils are often severely affected. Prolonged exposure to heavy metals may enhance the tolerance of bacterial communities [8] and fungi, including arbuscular mycorrhizae (AMs), which may be beneficial for the restoration of contaminated

ecosystems [9–11]. The presence of extreme metal contamination around smelters has led to visible effects, such as the accumulation of deep layers of organic matter on the soil surface, due to inhibition of the decomposing activity of soil microorganisms and fauna, as noted in some cases [12,13]. A decrease in soil microbial biomass C has been reported following the application of metal-enriched (Cu, Ni, Zn, and Cd) sewage sludge. [14]. Fliessbach et al. [15] found that adding metal-enriched (Cr, Cu, Cd, Pb, Hg, Ni, and Zn) sewage sludge to the soil not only reduced microbial biomass, but also the microbial biomass C/soil organic C ratio. Other studies have shown that the microbial biomass C per total organic C [15–18], specific respiration activity or soil microbial metabolic quotient ($qCO_2$) [15–19], and the formation of biomass C from added C sources [20] can also serve as indicators of soil pollution. The microbial metabolic quotient—that is, the soil respiration per unit of microbial biomass—was increased; particularly the fungal respiration, which increased to a greater extent due to the heavy metal-enriched sewage sludge treatments [21]. Knight et al. [22] investigated soils with Cu, Cd, and Zn at the current UK limit values. They discovered that Cd and Cu treatments reduced the microbial biomass C, while Cu and Zn decreased the metabolic potential of the soil microbial community. The soil microbial biomass C remained significantly lower even six years after artificial contamination by Cd, Cu, or Ni salts in field samples [23]. Sediment originating from metal mines contains not only high concentrations of heavy metals but also lower volumes of nutrient elements, such as nitrogen or phosphorus. The contents and bioavailability of these nutrient elements, particularly nitrogen and phosphorus, are determinant factors for microbial processes. The effects of heavy metals on the available phosphorus content are both direct and indirect. Direct effects include the formation of insoluble inorganic compounds with metals such as Pb. It is important to note that these effects can also have an impact on the phosphorus cycle. Indirect effects include a reduction in microbial biomass in soils [24,25], and also the inhibition of the transformation of soil organic matter, leading to an increase in the ratio of labile to total fractions [26]. The enzymes associated with C decomposition are less affected by metal treatments, while arylsulfatase and phosphatase activities are among the most sensitive microbial properties. Several studies have shown that the soil phosphatase activity is sensitive to metal contamination [27,28]. Furthermore, alterations in the forms and concentrations of metals, as well as soil properties such as the pH value and texture, can affect the soil phosphatase activity [12,24]. Ore mining began in the Middle Ages, but large-scale vein mining took place between 1954 and 1986 at Gyöngyösoroszi in the Mátra Mountains, Hungary. Mining and ore processing were halted when significant contamination was revealed along the entire length of the creek Toka and its environments, which flows into a reservoir on the outskirts of a town called Gyöngyös. Mine tailings, flotation tailings, various water reservoirs, and mine water were identified as potential sources of metal contamination [29]. The most severe contamination was in the upper soil layer along the stream, which can be explained by the recurrence of flooding [30]. To the contrary, although the floods significantly contaminated the soil in many of the village gardens, the calculated human health risk—taking into account the consumption of home-grown vegetables and soil ingestion—was within acceptable limits [31]. Although many studies have investigated the effect of metal contamination on soil biological properties, very few of them have focused on flood contamination from Pb/Zn ore. Therefore, more information is needed, especially for planning and monitoring the success of the phytoremediation of contaminated floodplains. This study focused on a metal-contaminated site resulting from historical and immediate flooding contaminated by abandoned Pb and Zn mine tailings to determine: (i) if there are differences in the soil metal accumulation at the site under phytoremediation depending on the plant and its distance from the stream; (ii) if there are differences in the soil microbial properties depending on the plant and its metal accumulation, and; (iii) if there are significant relationships between soil biological properties, soil physical and chemical properties, and metal accumulation.

## 2. Materials and Methods

The study site, a 65 × 18 m fenced field, was located about 300 m south of the village of Gyöngyösoroszi, between the village and the so-called agricultural water reservoir on the bank of the Toka Creek in northeastern Hungary (47°49′ N, 19°54′ E; 211 m above sea level, Figure 1). The longer side of the field is parallel with the stream.

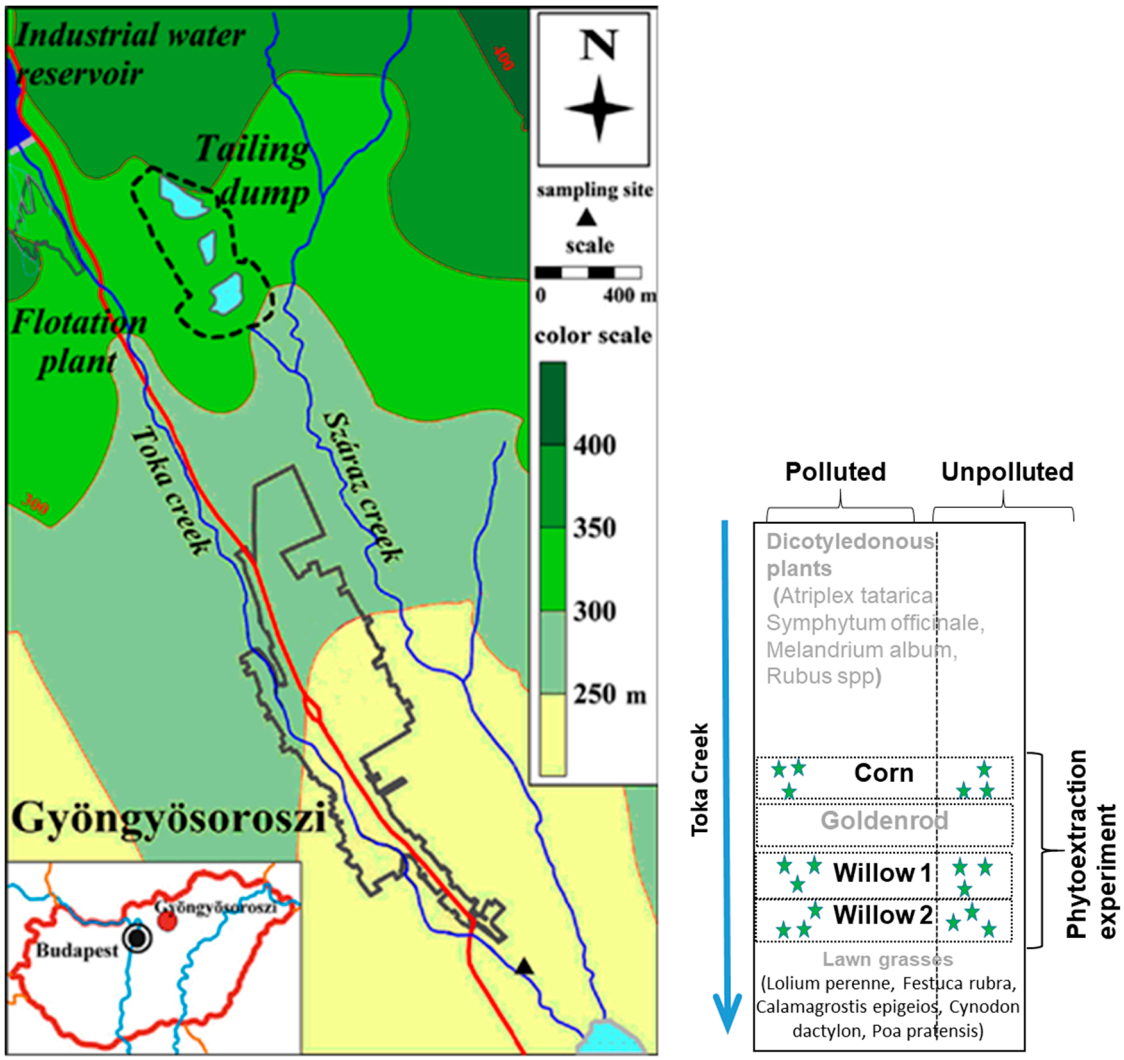


**Figure 1.** Soil sampling location of the contaminated stream in northeast Hungary, adapted from Sipter et al. (2008) [31]. The black triangle shows the sampling site, where the phytoremediation experiment was conducted, and the green stars show the sampling points within the "phytoextraction experiment" in the corn, willow 1, and willow 2 rows.

The soil type was Fluvisol, with 2.32% organic C, 0.15% total N, $pH_{CaCl2}$ 7.1, and 0.9% lime content. The soil has a particle composition of 31% sand, 49% loam, and 20% clay. Several floods have polluted the soil along the stream in the last few decades. The last major flooding was caused by heavy summer rainfall in 2004, when the river flooded the site and deposited newly transported sediment. A zone approximately 5–10 m wide along the stream was contaminated by fresh sediment, while a zone more than 15 m from the stream was considered unpolluted according to the soil analyses in that area. The sediment contained heavy metals such as zinc (Zn), lead (Pb), cadmium (Cd), copper (Cu), and mercury (Hg), and the non-metallic elements arsenic (As) and sulfur (S), in excess concentrations. The distribution of contamination was quite uneven due to the micro-topography of the site, but in general, the zone closest to the stream was the most

contaminated [30]. Dominant plant species of the site, before the establishment of the phytoremediation experiment, were *Calamagrostis epigeios* L., *Salix caprea* L., and *Populus* sp. The surrounding field was primarily dominated by orchards growing *Prunus domestica* L. and *Malus domestica* Borkh. shrubs, such as Common Blackberry (*Rubus fruticosus* L.), and vegetables such as potatoes (*Solanum tuberosum* L.) and tomatoes (*Solanum lycopersicum* L.). In 2003, a phytoremediation experiment was initiated by planting Goat willow (*Salix caprea* L.) and corn (*Zea mays* L.) in three rows perpendicular to the flow of the stream. Rapeseed (*Brassica napus subsp. napus* L.) was the previous crop before corn. European goldenrod (*Solidago virgaurea* L.) was also planted in the phytoextraction experiment, but was not used in this study. All vegetation was cleared from the site before planting. Soil samples were collected in the fall of 2004 at 18 points (Figure 1) perpendicular to the stream along the three planting rows, following a severe flood. We sampled 3–3 sampling points from the rooting zone along the two planted willow rows and one corn row in both the contaminated and uncontaminated zones. Each sample consisted of six soil cores (0–20 cm) taken from around a plant, resulting in a composite of rhizosphere soils. The soil samples were divided for soil moisture, soil chemical, and soil microbiological analysis after thorough mixing in the laboratory. The gravimetric moisture content (GWC %) of the soil samples was measured after drying at 105 °C. The soil plasticity index ($K_A$) was used as a textural characteristic of the soil samples. Soil chemical analyses were carried out on air-dried, thoroughly mixed, sieved (<2 mm) soil samples. The soil organic carbon (SOC) was measured via dichromate oxidation. A calcimeter was used to measure the lime content of the soil. The soil pH was measured in a 1:2.5 soil-to-water suspension ($pH_{H2O}$), and in a soil-to-KCl solution (1 mol) suspension ($pH_{KCl}$) with a glass electrode attached to a pH meter. The total salt content was calculated from the electrical conductivity in a water-saturated paste. The total elemental content of the soils for Cd, Hg, Pb, Zn, As, Cu, P, K, and Ca was determined according to MSZ 21470-50:1998 [32] after soil extraction with hydrochloric acid/nitric acid and microwave-assisted digestion. The potentially bioavailable fraction of the elements was measured using Lakanen–Erviö (LE) extract [33]. The extractant solution contained 0.5 M ammonium acetate, 0.02 M EDTA, and 0.5 M acetic acid, buffered at pH 4.65, and shaken with the sample for one hour. The elemental contents of both the aqua regia and LE extract were measured using inductively coupled plasma atomic emission spectrometry (ICP-AES: JY Ultima2, Jobin Yvon, Villeneuve d'Ascq, France). The soil samples for biological analyses were sieved at their original moisture contents (<2 mm) and stored at 4 °C until the analyses were performed.

Using the measurements of the soil basal respiration (BRESP) and substrate-induced respiration (SIR), the $CO_2$ evolution was determined using gas chromatography [34]. A 25 $cm^3$ vessel was filled with 2.0 g of moist soil. The evolved $CO_2$ was measured at 4 and 24 h after the closure of the vessels, and the difference between them was taken as the rate of $CO_2$ production (i.e., basal respiration). Incubation was carried out in a shaking water bath at 22 °C ± 0.1 °C. Following the basal respiration assay, the substrate-induced respiration was measured in the same samples. A 200 µL glucose solution (8 mg glucose $g^{-1}$ soil) was added to a sample, and the evolved $CO_2$ was measured after 180 min. The $CO_2$ was measured using a gas chromatograph (FISONS GC 8000) on a 250 µL gas sample. The soil microbial biomass C was measured via the chloroform fumigation extraction method from 15 g of soil [35]. From the filtered soil extract, the organic C was measured with a combustion TOC analyzer (Apollo 9000, Teledyne Tekmar, Mason, OH, USA). The equation used for the microbial biomass calculation was MBC = (Cfum − Cnfum)/$K_{EC}$, where MBC is the microbial biomass C, Cfum is the organic C from the fumigated extract, *Cnfum* is the organic C in the non-fumigated extract, and $K_{EC}$ is the conversion factor ($k_{EC}$ = 0.45; [36]). The metabolic quotient ($qCO_2$)—that is, the basal respiration per unit of microbial biomass C (BRESP/MBC)—and the ratio of microbial biomass C to soil organic C (MBC/SOC) were also calculated. Acid phosphatase activities (APAs) were determined according to Tabatabai and Bremner [37] from 1 g of moist fresh soil after adjustment to soil pH 5.5. After the addition of a buffered p-nitrophenyl phosphate solution, the soil samples were

incubated at 37 °C for 1 h. The nitrophenol released by phosphomonoesterase activity was extracted, colored with sodium hydroxide, and determined photometrically at 400 nm. All analyses were performed in triplicate. The statistical analysis was conducted using R 4.1.2. ANOVA with two factors was performed to evaluate the experimental data. Factor 1 consisted of the plantation rows (corn, willow 1, and willow 2), while factor 2 was the pollution (unpolluted and polluted). The normality (Shapiro–Wilk test) and homogeneity of variances (Levene test) were checked before the analysis. If applicable, Tukey's HSD post hoc test was used. Furthermore, Pearson's correlation was conducted to examine the relationships between the measured variables. Graphs were created using the ggplot, ggsignif, hmisc, and corrplot packages.

## 3. Results

In the floodplain soil, the organic C and $pH_{KCl}$ did not differ between the polluted and unpolluted zones, nor between the planting rows. The soil $pH_{H2O}$ was significantly lower in the polluted than the unpolluted soils in all planting rows, while there was no significant difference between the planting rows. Although the soil plasticity index ($K_A$) was lower in the polluted zones compared to the unpolluted in all planting rows, only that in the corn row had a significantly lower value. The water-soluble salt content was consequently higher in the polluted zone ($p < 0.05$) in all planting rows. Although the lime content was only detectable in trace amounts, the calcium content of the soil was significantly higher in the contaminated zone than in the uncontaminated zone, except for in the corn row. The total phosphorus and potassium contents of the soils were higher in the unpolluted than the polluted zone of all planting rows (Table 1).

**Table 1.** The main properties of soil samples collected from the polluted and unpolluted floodplain.

| Soil Code | Organic C (%) | $pH_{H2O}$ | $pH_{KCl}$ | $K_A$ | Salt (%) | Ca # | P # | K # |
|---|---|---|---|---|---|---|---|---|
| Cunp | 2.05 ± 0.05 $^{a}$ | 7.4 ± 0.0 $^{a}$ | 6.6 ± 0.0 $^{a}$ | 46 ± 0 $^{a}$ | 0.07 ± 0.01 $^{a}$ | 10,132 ± 689 $^{ab}$ | 1104 ± 46 $^{b}$ | 11,211 ± 1893 $^{b}$ |
| Cp | 1.45 ± 0.33 $^{a}$ | 6.7 ± 0.3 $^{b}$ | 6.3 ± 0.4 $^{a}$ | 33 ± 1 $^{b}$ | 0.11 ± 0.01 $^{b}$ | 17,192 ± 90 $^{b}$ | 695 ± 22 $^{a}$ | 4652 ± 538 $^{a}$ |
| W1unp | 1.92 ± 0.46 $^{a}$ | 7.3 ± 0.1 $^{a}$ | 6.6 ± 0.2 $^{a}$ | 49 ± 2 $^{a}$ | 0.08 ± 0.01 $^{a}$ | 9163 ± 956 $^{a}$ | 1119 ± 120 $^{b}$ | 11,205 ± 1970 $^{b}$ |
| W1p | 1.94 ± 0.14 $^{a}$ | 6.7 ± 0.0 $^{b}$ | 6.4 ± 0.1 $^{a}$ | 44 ± 1 $^{a}$ | 0.13 ± 0.01 $^{b}$ | 16,603 ± 5886 $^{b}$ | 833 ± 17 $^{ab}$ | 5381 ± 1307 $^{a}$ |
| W2unp | 2.05 ± 0.30 $^{a}$ | 7.3 ± 0.1 $^{a}$ | 6.6 ± 0.0 $^{a}$ | 48 ± 3 $^{a}$ | 0.08 ± 0.02 $^{a}$ | 9688 ± 117 $^{a}$ | 1105 ± 49 $^{b}$ | 11,073 ± 397 $^{b}$ |
| W2p | 1.58 ± 0.48 $^{a}$ | 6.8 ± 0.3 $^{b}$ | 6.6 ± 0.3 $^{a}$ | 45 ± 3 $^{a}$ | 0.13 ± 0.00 $^{b}$ | 21,226 ± 3374 $^{b}$ | 733 ± 258 $^{a}$ | 4557 ± 792 $^{a}$ |

# Concentration is presented as mg $kg^{-1}$; means ± SD ($n = 3$), followed by different letters indicating significant differences ($p < 0.05$) between soils. Soil codes are: corn unpolluted (Cunp), corn polluted (Cp), willow row 1 unpolluted (W1unp), willow row 1 polluted (W1p), willow row 2 unpolluted (W2unp), and willow row 2 polluted (W2p).

The soil in the polluted zone contained significantly higher levels of Cd, Hg, Pb, Zn, As, and Cu compared to the unpolluted zone. The willow 2 row contained the highest average level of these elements except for mercury, but it did not differ significantly from the willow 1 or corn rows. (Table 2). The Hg content of the soils in the unpolluted zone was always below the detection limit (<0.12 mg $kg^{-1}$). Additionally, the soil sulfur content was significantly higher in the polluted zone (16,546 ± 6125 mg S $kg^{-1}$) compared to the unpolluted zone (495 ± 92 mg S $kg^{-1}$). The bioavailable part of the toxic element content of the soils was better indicated with the Lakanen–Erviö (LE) extract compared to the "total" soil element content determined using the aqua regia extract (Table 3). The concentration of mercury was below the detection limit in all soil samples with the LE extract (Table 3). The soil Cd, Pb, Zn, and Cu contents determined with the LE extract were significantly higher in the polluted than the unpolluted zone except for As. In the polluted zone of the willow 2 row, the soils had significantly higher amounts of LE-extractable Cd and Zn than in the corn row, while the contents of these elements in the soils of the willow 1 row were between them and not significantly different from either.

**Table 2.** The contents of the aqua regia-extracted toxic elements in the soil samples collected from the polluted and unpolluted zones of the floodplain.

| Soil Code | Cd | Hg | Pb | Zn | As | Cu |
|---|---|---|---|---|---|---|
| Cunp | 0.59 ± 0.06 $^{a}$ | <0.12 ± 0.0 $^{a}$ | 52 ± 4.7 $^{a}$ | 214 ± 26 $^{a}$ | 22.5 ± 0.4 $^{a}$ | 101 ± 7.1 $^{a}$ |
| Cp | 17.97 ± 2.12 $^{b}$ | 3.33 ± 2.38 $^{b}$ | 955 ± 203 $^{b}$ | 3283 ± 315 $^{b}$ | 252 ± 56 $^{b}$ | 276 ± 44 $^{b}$ |
| W1unp | 0.62 ± 0.29 $^{a}$ | <0.12 ± 0.0 $^{a}$ | 49 ± 15 $^{a}$ | 205 ± 67 $^{a}$ | 23.1 ± 2.1 $^{a}$ | 83 ± 19 $^{a}$ |
| W1p | 16.16 ± 3.93 $^{b}$ | 1.50 ± 0.48 $^{b}$ | 1251 ± 468 $^{b}$ | 2748 ± 507 $^{b}$ | 189 ± 37 $^{b}$ | 279 ± 71 $^{b}$ |
| W2unp | 0.79 ± 0.23 $^{a}$ | <0.12 ± 0.0 $^{a}$ | 56 ± 8.7 $^{a}$ | 239 ± 40 $^{a}$ | 23.0 ± 1.4 $^{a}$ | 93 ± 10 $^{a}$ |
| W2p | 21.03 ± 6.93 $^{b}$ | 2.16 ± 0.91 $^{b}$ | 1995 ± 822 $^{b}$ | 3417 ± 906 $^{b}$ | 257 ± 77 $^{b}$ | 385 ± 99 $^{b}$ |
| **MAC** | **1** | **0.5** | **100** | **200** | **15** | **75** |

Concentration is presented as mg $kg^{-1}$; means ± SD ($n$ = 3), followed by different letters indicating significant differences ($p < 0.05$) between soils. Soil codes are: corn unpolluted (Cunp), corn polluted (Cp), willow row 1 unpolluted (W1unp), willow row 1 polluted (W1p), willow row 2 unpolluted (W2unp), and willow row 2 polluted (W2p). MAC—maximum admissible concentration for geological media established by the Decree of the Hungarian Ministry of Environment and Water, Ministry of Health, and Ministry of Agricultural and Rural Development No. 6/2009 (14.04.2009), Appendix 1; "B"-value (6/2009. (IV. 14.) KvVM-EüM-FVM).

**Table 3.** The contents of the LE-extracted toxic elements in the soil samples, collected from the polluted and unpolluted zones of the floodplain.

| Soil Code | Cd | Hg | Pb | Zn | As | Cu |
|---|---|---|---|---|---|---|
| Cunp | 0.469 ± 0.037 $^{a}$ | <dl | 23.0 ± 4.36 $^{a}$ | 67.3 ± 5.51 $^{a}$ | 0.87 ± 0.06 $^{a}$ | 46.7 ± 4.13 $^{a}$ |
| Cp | 7.083 ± 0.516 $^{b}$ | <dl | 312 ± 39.0 $^{b}$ | 1007 ± 66.3 $^{b}$ | 1.77 ± 0.15 $^{a}$ | 84.8 ± 15.7 $^{b}$ |
| W1unp | 0.505 ± 0.200 $^{a}$ | <dl | 22.0 ± 5.57 $^{a}$ | 68.3 ± 32.0 $^{a}$ | 1.33 ± 0.50 $^{a}$ | 44.0 ± 5.08 $^{a}$ |
| W1p | 9.685 ± 1.913 $^{bc}$ | <dl | 391 ± 144 $^{b}$ | 1519 ± 384 $^{bc}$ | 1.90 ± 0.30 $^{a}$ | 106 ± 27.3 $^{b}$ |
| W2unp | 0.631 ± 0.196 $^{a}$ | <dl | 25.0 ± 5.20 $^{a}$ | 85.3 ± 19.7 $^{a}$ | 1.06 ± 0.35 $^{a}$ | 45.9 ± 2.43 $^{a}$ |
| W2p | 11.78 ± 2.806 $^{c}$ | <dl | 578 ± 269 $^{b}$ | 2075 ± 742 $^{c}$ | 1.90 ± 0.92 $^{a}$ | 114 ± 12.6 $^{b}$ |

Note: dl—detection limit; all values are presented as mg $kg^{-1}$; means ± SD ($n$ = 3), followed by different letters indicate significant differences ($p < 0.05$) between soils. Soil codes are: corn unpolluted (Cunp), corn polluted (Cp), willow row 1 unpolluted (W1unp), willow row 1 polluted (W1p), willow row 2 unpolluted (W2unp), and willow row 2 polluted (W2p).

The soil basal respiration was marginally ($p$ = 0.052) higher in the polluted than the unpolluted zone while being significantly different between the planting rows ($p$ = 0.002). However, in comparing the polluted and unpolluted zones, Tukey's post hoc test indicated no significant difference in the soil basal respiration between any planting rows (Figure 2a). Substrate-induced respiration (SIR), using glucose as a substrate, showed no significant difference between the planting rows, but the SIR was significantly higher in the unpolluted soils of the corn row and willow 2 row compared to the polluted soils (Figure 2b). The soil microbial biomass C was significantly higher in the soils from the unpolluted zone than the polluted zones in all planting rows, whereas no significant difference was found between the planting rows (Figure 3a). The phosphomonoestarase activity of soil was only significantly higher in the willow 1 polluted zone compared to the willow 1 unpolluted zone, whereas no significant difference in the soil APA was detected between the polluted and unpolluted zones in the corn row and willow 2 rows (Figure 3b). According to the two calculated indices, the metabolic quotient ($qCO_2$) and the ratio of the microbial biomass C to the soil organic C (MBC/SOC) were not significantly altered between the planting rows (Figure 4).

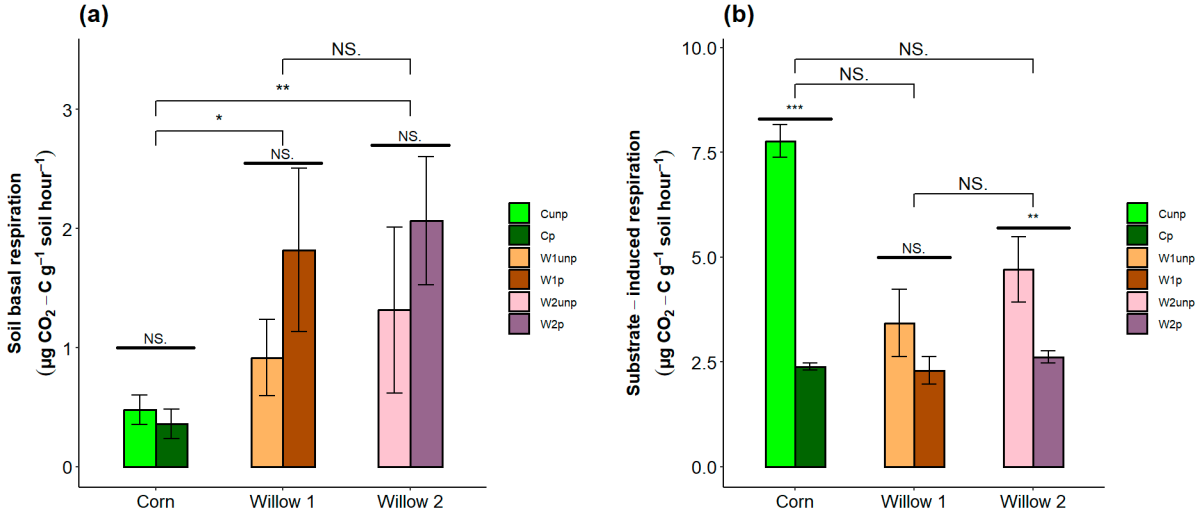


**Figure 2.** The means ± standard deviations of the soil basal respiration (**a**) and the substrate-induced respiration rates (**b**) of the samples originated from the experimental site. The samples were: corn unpolluted (Cunp) and corn polluted (Cp), willow row 1 unpolluted (W1unp), willow row 1 polluted (W1p), willow row 2 unpolluted (W2unp), and willow row 2 polluted (W2p). The significance levels between the planting rows are shown in horizontal brackets, while the unpolluted and polluted samples are in black lines above the corresponding bars; NS. $p > 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

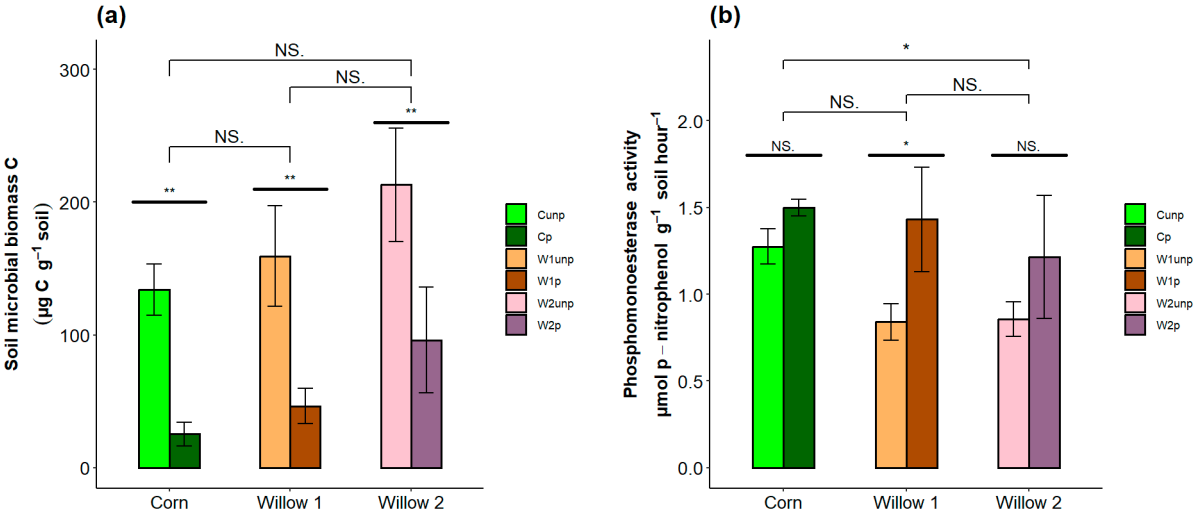


**Figure 3.** The means ± standard deviations of the soil microbial biomass C (**a**) and the acid phosphomonoesterase activity (**b**) of the samples originating from the experimental site. The samples were: corn unpolluted (Cunp) and corn polluted (Cp), willow row 1 unpolluted (W1unp), willow row 1 polluted (W1p), willow row 2 unpolluted (W2unp), and willow row 2 polluted (W2p). The significance levels between the planting rows are shown in horizontal brackets, while the unpolluted and polluted samples are in black lines above the corresponding bars; NS. $p > 0.05$; * $p < 0.05$; ** $p < 0.01$.

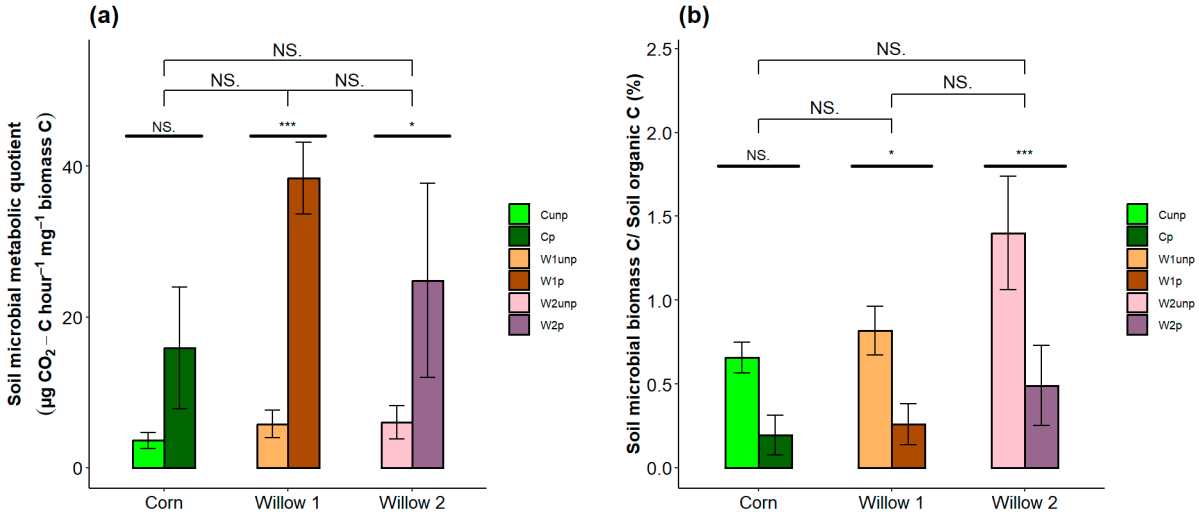


**Figure 4.** The means ± standard deviations of the metabolic quotients (**a**) and the ratio of the soil microbial biomass C to soil organic C (**b**) of the samples originating from the experimental site. The samples were: corn unpolluted (Cunp) and corn polluted (Cp), willow row 1 unpolluted (W1unp), willow row 1 polluted (W1p), willow row 2 unpolluted (W2unp), and willow row 2 polluted (W2p). The significance levels between the planting rows are shown in horizontal brackets, while the unpolluted and polluted samples are in black lines above the corresponding bars; NS. $p > 0.05$; * $p < 0.05$; *** $p < 0.001$.

The $qCO_2$ was significantly higher in the soils of the polluted zone of the willow 1 and willow 2 rows, whereas only marginally significantly higher in the soil of the polluted zone of the corn row compared to the unpolluted zone (Figure 4a). Similarly to the $qCO_2$ but in the opposite direction, the MBC-to-SOC ratio was significantly higher in the soils of the unpolluted zone of the willow 1 and willow 2 rows than that of the polluted zones, and only marginally higher in the soils of the unpolluted corn row than in the polluted one (Figure 4b).

The correlation analysis revealed several significant relationships between the physical, chemical, and biological properties of the investigated soil (Figure 5). The plasticity index ($K_A$) of the soil exhibited a negative correlation with the concentration of toxic metals such as Cd, Pb, Zn, As, and Cu, but a positive correlation with the SOC, soil phosphorus, and soil pH. A positive correlation was found between the total salt content and the concentrations of toxic metals Cd, Pb, Zn, As, and Cu, while a negative correlation was observed with the soil phosphorus content (Figure 5). Significant positive correlations were found for key pollutants, including zinc, cadmium, lead, copper, and arsenic.

The substrate-induced respiration (SIR) was positively correlated with the soil microbial biomass C (MBC), MBC/SOC, pH, and LE-P content, and negatively correlated with the $qCO_2$, total salt content, and also with Cd, Pb, Zn, As, and Cu (Figure 5). The soil microbial biomass C (MBC) also had a negative correlation with the $qCO_2$ and the acid phosphatase activity, total salt content, and Cd, Pb, Zn, As, and Cu contents, while it was positively correlated with the soil pH, soil plasticity index, and LE-P (Figure 5). The acid phosphomonoesterase activity (APA) was negatively correlated with the LE-P content, the MBC, MBC/SOC, and the soil plasticity index, while being positively correlated with the toxic elements (Cd, Pb, Zn, As, and Cu) (Figure 5).

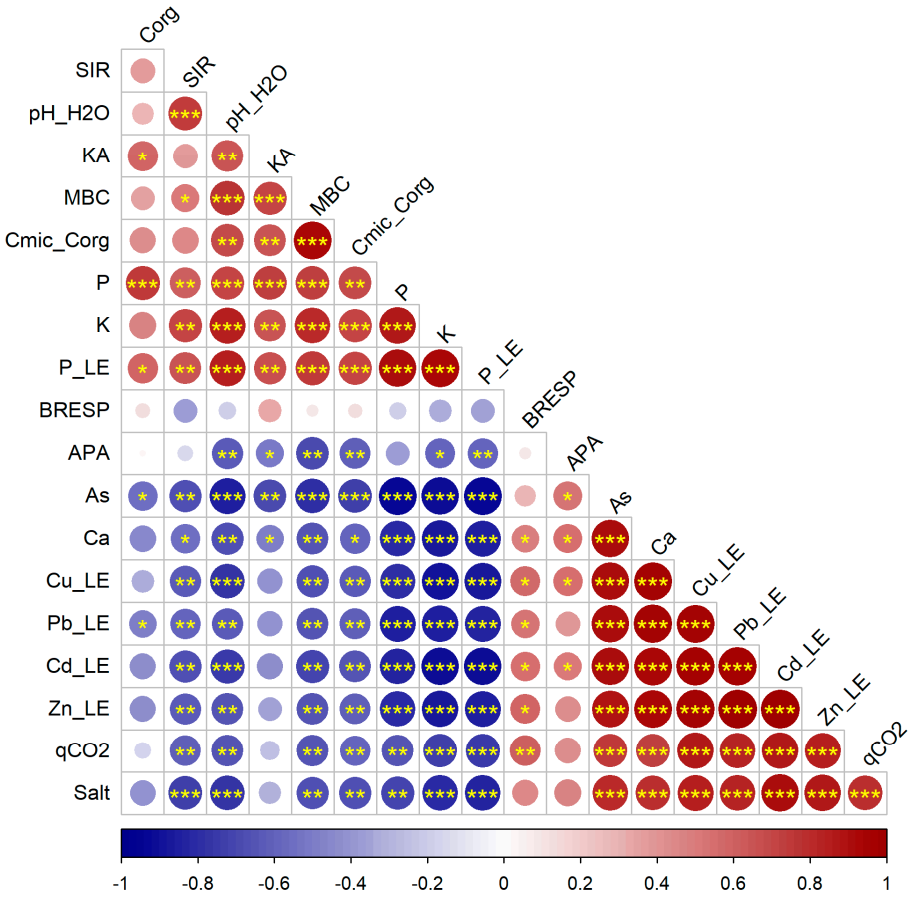


**Figure 5.** Pearson's correlations between several soil physical, chemical, and biological properties. Significant correlations are indicated by asterisks; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Abbreviations: APA = acid phosphomonoesterase activity; BRESP = soil basal respiration; $qCO_2$ = metabolic quotient; SIR = substrate-induced respiration; MBC = soil microbial biomass C; Cmic_Corg = MBC/Corg ratio; KA = soil plasticity index; Salt = total soluble salt content; Corg = soil organic C; As = arsenic; Ca = calcium Cu_LE = cupper; Pb_LE = lead; Cd_LE = cadmium; Zn_LE = zinc; P_LE = phosphorus. LE = Lakanen–Erviö-extracted. For the elements without LE indication, the analysis refers to aqua regia extract.

## 4. Discussion

The development of biological and biochemical indicators to evaluate the state of soil quality is an increasingly important field of research and also of interest to policymakers [38]. It is well known that the soil biota has a significant role in many soil processes and supports many ecosystem services, such as nutrient transformations, water and air cleaning, and carbon sequestration [39]. Therefore, soil degradation by the chemical pollution of heavy metals that are potentially toxic to living organisms can cause changes to soil processes [40]. In this study, we investigated six potentially applicable soil biological indicators if they change as a consequence of potentially toxic element accumulation in floodplain soil.

The soil samples from three planting rows (corn, willow 1, willow 2) showed elevated levels of heavy metals, arsenic, and sulfur compared to the samples taken farther away, suggesting that flooding has significantly contaminated a part of the floodplain near the stream. The concentration of inorganic contaminants in the soil was found to be more than ten times higher in the polluted zone of the floodplain compared to the unpolluted zone, exceeding the maximum permissible limit of environmental safety. This observation aligns with the findings of a previous survey that analyzed soil from multiple sites along the Toka Stream [30,41]. The sediment that was deposited had sandy characteristics, resulting in a lower plasticity index ($K_A$) and lower levels of total phosphorus and potassium in the soil

at the contaminated zone. Previous data indicated that soil pH was higher at this site than at other sites along the creek Toka, which was attributed to the use of liming technology for water cleaning, resulting in sulfur oxidation [30]. Arsenic solubility is strongly controlled by soil properties [42], and the lowest soluble As concentrations were in the clayey and iron-rich samples. The presence of iron oxides in soil increases its retention ability for As [43]. Soils with increasing levels of carbonates and organic C decrease the solubility of Pb, Zn, and Cu in metal-contaminated soils [44,45]. The oxidation of metal sulfides within the deposited sediment resulted in elevated soil acidity, as indicated by a decline in the soil pH. This, in turn, contributed to an increase in the solubility of these sulfides and, consequently, the metals they contain will be more available to plants and microbes.

Earlier reports have well documented that heavy metal contamination of mining soils, such as Cu, Zn, Pb, and Cd, results in decreased sucrase, urease, and acid phosphatase activities, MBC, MBN, MBP, and N mineralization, as well as increased basal respiration and $qCO_2$ [46–52].

Soil basal respiration is defined as the steady rate of respiration in soil, which originates from the mineralization of soil organic matter and can be detected from oxygen consumption or $CO_2$ production rates [53]. Soil basal respiration is probably the most frequently used property to quantify changes in the activity of the soil microbiota [54]. The measurement of soil basal respiration has been applied across a variety of research studies, and both soil microbial respiration and the mineralization of organic matter are commonly accepted as key indicators for measuring changes in soil quality [55]. Soil basal respiration is known to be strongly correlated with soil organic matter and is also influenced by soil pH, salinity, and texture [56,57]. However, the effects of pollutants on soil basal respiration are quite contradictory [42], leading to divergent respiration responses. Thus, some studies found no decrease in soil respiration with the increased level of pollution [58,59]. Additionally, no inhibition of soil respiration by arsenic pollution was observed [60]. In other studies, metal-contaminated soils presented higher respiratory activity than unpolluted soil [61]. Increased soil organic matter content, or the introduction of organic matter into the soil, usually means better nutrient availability for microorganisms, which can help them cope with metal contamination stress, which can also lead to increased microbial activity [62,63]. There was no significant change in the soil basal respiration rates of the metal-contaminated floodplain soils in our study compared to the unpolluted zone of the floodplain soil. Although the soil basal respiration rate was significantly higher in both the willow-planted plots than in the corn row plots, it cannot be explained by the organic C, pH, or soil texture variations between the plantations, leaving this question unanswered. The mean soil basal respiration rates in both willow rows were higher in the metal-contaminated zone compared to the uncontaminated zone, but this was not significant at $p = 0.05$, probably because of the high standard deviations among the samples due to the uneven distribution of the contamination.

The soil microbial biomass C was determined using two indirect methods: chloroform fumigation extraction (CFE) and substrate-induced respiration (SIR). Heavy metal contamination has been shown to reduce soil microbial biomass, as demonstrated in several studies [48,49,51,52]. This suggests that it would be a good indicator for soils polluted by heavy metals. We also found a decreased soil microbial biomass C in the metal-contaminated zone compared to the uncontaminated zone for all planting rows using the CFE method, but when using SIR, the contaminated zone of the corn row and the willow 2 row showed a significant decrease compared to the uncontaminated zone, but this was not the case for willow 1. Microorganisms in soils under heavy metal stress may be able to divert energy away from growth toward cell maintenance functions [64]. Moreover, in contaminated soils, microorganisms need more energy to survive in harsh conditions. Therefore, a higher percentage of energy is lost, resulting in lower amounts of C, N, and P being incorporated into organic components [65]. These findings are not in line with Wang et al.'s [66] reports, which indicated that the soil microbial biomass C did not have a correlation with heavy metals and was not suggested as a sensitive indicator for assessing the environmental

impacts of heavy metal pollution. The oligotrophic bacteria were the only group to show a significant negative correlation with the soil heavy metal content. This group is known to be the most sensitive to metal contamination. Additionally, the ratio of available to total Pb and Cu was found to have a negative correlation with the substrate-induced respiration rate [67].

The ratio of soil MBC to SOC has also been proposed to assess soil ecosystem maturity and indicate heavy metal stress. The ratio of soil MBC to SOC is an indicator of relative substrate availability for soil microorganisms [68], and the ratio of respiration to biomass carbon is an important indicator of substrate use efficiency and, thus, microbial stress [69,70]. The average MBC/SOC ratio decreased below 0.5% in the polluted zone in all rows, while this ratio averaged above 0.5% in the unpolluted zone. As with the $qCO_2$, the difference between the polluted and unpolluted rows was significant for the willow 1 and willow 2 rows, but not the corn row.

The metabolic quotient ($qCO_2$) is an index of the physiological characteristics of microorganisms found in soil and is used for the evaluation of the effects of environmental conditions on microbial biomass [35,71]. This parameter enables discrimination between the maturity levels of ecosystems, as it incorporates the assumption that mature systems respire less per biomass unit because less energy is canalized toward metabolism [17]. A high respiration rate may indicate either an ecological disorder or a high level of productivity in the ecosystem [72]. The respiration rate per unit of microbial biomass, or the metabolic quotient ($qCO_2$), is a variable that is easier to interpret. The $qCO_2$ has been used as a microbial stress indicator and interpreted as "microbial efficiency" [73]. This is because it measures the energy required to maintain the metabolic activity relative to the energy required for synthesizing new biomass [17]. Therefore, soils under stress may present higher $qCO_2$ values than non-stressed soils. Enhanced $qCO_2$ levels in plantations indicated a microbial community under stress with high-maintenance carbon demand [61]. The $qCO_2$ in our study was significantly higher in the willow 1 and willow 2 polluted zone compared to the unpolluted zone, whereas no significant difference was found in the corn row. In all plantations, the standard deviation of $qCO_2$ was higher in the polluted zones for all planting rows. This suggests that the metal contamination in polluted zones has a different effect on the $qCO_2$ change, but in all cases, an average increase in the $qCO_2$ could be observed under heavy metal stress situations.

Enzyme activities are also considered good indicators of soil quality and health because of their sensitivity to heavy metal contamination [74]. In a meta-analysis, the activities of two endoenzymes, arylsulfatase and dehydrogenase, were found to be the most responsive to heavy metals in soils [74]. Other enzymes (mainly exoenzymes, such as β-glucosidase, urease, acid phosphatase, and alkaline phosphatase) showed two times less reduction in the activities. The negative effects of heavy metal contamination on enzyme activities are weakened because these enzymes can be stabilized on the surface of clay and organic materials. The biochemical parameters of the soil samples indicate that the high heavy metal content and the low phosphorus content of the soil interacted. Ekenler and Tabatabai [75] explained the adverse effect as being caused by metal ions that may inactivate enzymes by reacting with the sulfhydryl groups of enzymes to form metal sulfides. Sulfhydryl groups in enzymes can function as essential components of the catalytic active sites or as groups involved in maintaining the correct structural relationship of the enzyme protein. Metals can also inhibit enzymes by forming a complex with the substrate or by reacting with the enzyme–substrate complex [76]. The activation of enzymes in the soil may be attributed to a shift in the microbial composition structure after prolonged exposure to heavy metals. Lower enzyme activities may also be due to energy diversion into physiological adaptations necessary to tolerate heavy metals. These adaptations include the synthesis of intra- and extracellular metal-sequestering proteins or saccharides, as well as biochemical reactions to precipitate or trap metals onto microbial surfaces [47]. The impact of heavy metals on soil enzyme activities is typically linked to key soil characteristics, such as the clay content, SOC, and pH [77]. In this study, the APA showed a weak negative correlation with

the soil plasticity index, with higher clay content leading to a higher soil plasticity index ($K_A$), no correlation with the SOC, and a weak negative correlation with the soil pH. The high metal content significantly stimulated APA, but it could be speculated that the lower availability of soil P in contaminated soils may have provoked an increase in APA. Renella et al. [46] reported that alkaline phosphatase was more susceptible in acidic soil, whereas acid phosphatase was more susceptible in alkaline soil.

It is well established that microbial biomass and activity depend mainly on the soil organic carbon, clay, and pH [70]. Accordingly, our study also shows a positive correlation between the MBC and soil plasticity index, as well as with the soil pH. However, there was little to no correlation with the SOC. In addition, possibly due to the higher salt content in the metal-contaminated soils, the MBC was found to be negatively correlated with the soil salinity. The accumulation of Pb and Zn in soils over time may result in a positive correlation between the microbial biomass and the metal content, due to a reduced rate of organic matter decomposition, leading to the accumulation of organic matter [78,79], although such a phenomenon was not observed in the current study.

## 5. Conclusions

Flooding from the metal-contaminated stream resulted in significant accumulation of Cd, Zn, Pb, Cu, Hg, and As in the soil, which decreased with increasing distance from the edge of the stream. Comparing three planted rows in the study field along the metal-contaminated floodplain revealed a significant increase in the metabolic quotient ($qCO_2$) and a significant decrease in the soil microbial biomass C and MBC/SOC in soils in the polluted zone compared to those in the unpolluted zone. No significant difference was found in the soil basal respiration rate between the soils from the polluted and unpolluted zones. Although no significant difference in metal contamination was observed between the planting rows (except between the willow 2 and corn rows), the level of pollution decreased in the order of willow 2, willow 1, and corn. The heavy metal contents of the soils in the floodplain soil were negatively correlated with the soil pH, soil plasticity index, potassium, and phosphorus content of the soil, whereas they were positively correlated with the salt content, while no correlation was found with the soil organic carbon. The soil microbial biomass was positively correlated with the soil plasticity index and phosphorus, but negatively correlated with the total salinity and Cd, Pb, Zn, As, and Cu contents in the contaminated floodplain. There was a negative correlation between the plant-available phosphorus and the acid phosphomonoesterase activity. The potentially toxic element contents had a strong positive correlation with the metabolic quotient ($qCO_2$), while a strong negative correlation with the MBC/SOC ratio, suggesting that the combination of the metabolic quotient with the MBC/SOC ratio could be a reliable indicator of metal contamination in floodplain soils.

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