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Characterization and Evaluation of the Efficiency of Organic Amendments and Native Macrophytes for the Treatment of Acid Mine Drainage in Hualgayoc—A Case Study

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Abstract: Acid mine drainage (AMD) is a major environmental issue due to its high heavy metal concentrations and low pH, posing risks to ecosystems and human health. In Hualgayoc, Cajamarca, Peru, AMD contamination from mining activities necessitates effective remediation strategies. This study evaluated the removal efficiency of organic amendments, municipal waste compost (MWC), cattle manure compost (CMC), vermicompost (HMS), corn stalk biochar (CSB), sludge biochar (SLB), pine biochar (PBC), and native macrophytes *Carex pichinchensis* (CAX), *Juncus ecuadoriensis* (JSP), and *Myriophyllum aquaticum* (MYA) in removing As, Cd, Cu, Fe, and Zn. A physicochemical characterization of AMD, amendments, and plants was conducted, and sorption capacity was determined through triplicate analyses. The results showed that SLB achieved a 100% Zn removal, while CSB removed over 90% of Cu and Fe. Among the organic amendments, CMC demonstrated the highest As and Cd removal (~100%). Regarding macrophytes, CAX achieved a ~97% Fe removal, and MYA exhibited Cd affinity. CSB had the highest sorption capacity for Cu (0.139 mg/g), Fe (1.942 mg/g), and Zn (0.149 mg/g), highlighting its potential for metal remediation. Organic amendments enhanced metal adsorption and stabilization, outperforming macrophytes in heavy metal removal. Combining organic amendments with native plants is recommended to assess their synergistic potential for AMD remediation.

Keywords: acid mine drainage; heavy metals; macrophytes; environmental remediation

1. Introduction

Acid mine drainage (AMD) is a significant environmental problem associated with the mining industry [1]. The environmental impacts of AMD can be chemical (an increase in acidity, reduction in pH); physical (modification of the substrate, sedimentation, turbidity);

biological (reproduction, osmoregulation fall in the acid-base balance of organisms, instead causing the death of species) [2]; and ecological (habitat modification, loss of niches, modification of the food chain, bioaccumulation in the food chain) [3]. AMD forms when sulfide minerals are exposed to oxidizing conditions [4], which releases extremely acidic leachates with a high mobility of metal ions [5], presenting high concentrations of sulfates and heavy metals (As, Cd, Cr, Cu, Hg, Ni, Pb, Zn) [6], which can last hundreds of years in the environment once generated [7], representing a potential risk to human health through the food chain [8].

The impacts of AMD not only persist in the environment but can also affect living beings, since these metals can be ingested by humans through drinking water supplies [9]. Causing problems such as damage to the brain, kidneys, and nervous system, lead poses serious health risks. Similarly, cadmium can lead to conditions like high blood pressure, liver damage, and even cancer. As for mercury, it can bring about a deterioration of the nervous system [10].

In Peru, there are areas that have been affected by AMD due to the mining industry, such as Quiulacocha in the province of Cerro de Pasco [11], where AMD could be originated by mining tailings, and in the province of Ancash, at the Antamina mine [12]. In Cajamarca, a province in Peru, Hualgayoc is a district with a long mining history that dates to the times of the Spanish Colony, where contamination has been reported due to the waste generated by Mining Environmental Labilities (MELs), which in turn generate AMD [13]. Despite the evident environmental and human health risks posed by AMD, remediation efforts remain limited, especially in the selection of efficient, cost-effective, and sustainable treatment approaches adapted to local conditions. This highlights the need for innovative strategies that integrate native plant species and organic amendments, to enhance AMD treatment while preserving local biodiversity.

Hence, to safeguard the ecological environment and eradicate the risk to human health, AMD should only be releasing following treatment to meet the required standards. Neutralization stands out as the prevailing method for treating AMD [14]. Some of these neutralization methods include limestone neutralization and the limestone–lime combined neutralization method [3]; however, this technique can be expensive and difficult to implement; therefore, constructed wetlands (CWs) emerge as an alternative, economic, and efficient treatment technology to minimize the negative impacts of AMD in the environment [1].

The application of CWs is a passive treatment technology [15], one that forms an ecosystem composed of substrates, microbials, and plants with a triple physical, chemical, and biological synergy [16] that allows the removal of metals and sulfates [15] through processes of absorption, hydrolysis precipitation [1], cationic exchange, complexation, biosorption, reduction, and direct assimilation by plants [17]. This system employs plants for the removal of heavy metals, which can be aquatic macrophytes, such as as those of the genus Carex [18], *Myriophyllum aquaticum* [19], or *Juncus* spp. [20]. However, studies on the effectiveness of native plant species in AMD treatment remain scarce, especially regarding their long-term metal retention capacity and adaptation to extreme pH and metal concentrations. This knowledge gap limits the development of site-specific remediation strategies that optimize metal sequestration while ensuring ecosystem resilience.

On the other hand, amendments such us biochar [21], compost [22], and humus have been widely investigated to evaluate phytoremediation in AMD. Biochar is a carbonaceous material produced under oxygen-limiting conditions, obtained from biomass [23]. Its efficiency in removing heavy metals will depend on the physicochemical properties when characterizing them; these parameters can include cation exchange capacity (CEC) and pH [24]. Furthermore, compost refers to the aerobic process produced from municipal organic waste [25]. Although different methods exist for producing compost, its quality depends on a combination of key parameters, such as the C/N ratio [26], moisture content, and microbial diversity, which in turn influence its efficiency in AMD remediation [27]. Studies have shown that compost can act as a metal-binding agent, enhancing metal immobilization [28] in contaminated environments and promoting microbial communities beneficial for bioremediation [29].

Vermicomposting, or humus, is a biological process that uses earthworms and microorganisms to convert organic waste into a stable, nutrient-rich material. This material, in addition to improving soil fertility, has shown great potential in mitigating the effects of heavy metals [30]. Recent studies have highlighted that humus can immobilize heavy metals in contaminated soils through processes such as adsorption, chelation, and complexation, significantly reducing their bioavailability [31,32].

Therefore, the aim of this research is to evaluate the efficiency of organic amendments and native macrophytes in the treatment of heavy metals (As, Cu, Cd, Fe, and Zn) from AMD pollution in Hualgayoc, Cajamarca. By evaluating native species and organic amendments, this study seeks to explore novel, site-adapted remediation strategies that maximize metal retention, improve system stability, and provide a foundation for future applications in AMD-impacted areas.

2. Materials and Methods

2.1. A Description of the Case Study

The case study is situated in the Hualgayoc district, within the province of Hualgayoc in the department of Cajamarca, Peru. This region is located at an altitude of approximately 3500 m above sea level and falls within the ecological classification of the Tropical Montane Steppe Life Zone. The climate in the region is classified as Subhumid Cold Temperate, characterized by an average annual temperature ranging between 6 °C and 12 °C. The area receives an average annual rainfall of 764.9 mm [33]. The Hualgayoc district has an extensive history of mining and is a place of some Mining Environmental Liabilities (MELs) [13], which include mineheads with emissions of AMD. This situation provides a potential risk to the ecosystems of the Hualgayoc–Arascorgue and Llaucano river basins, including their plant and animal life, as well as to human health [34].

2.2. Sampling and Characterization of AMD and Macrophytes

For a better understanding of the experimental process, a schematic diagram is presented in Figure 1. First, AMD samples were collected from the Hualgayoc district in the department of Cajamarca, as illustrated in Figure 2. An in situ assessment was promptly conducted using a portable multiparameter device (HANNA Instruments, HI98199, Woonsocket, RI, USA) [35]. The key parameters measured included pH and electrical conductivity (EC) to characterize the physicochemical properties of the AMD. This research also focused on three native macrophyte species from the region of Cajamarca, including *Carex pichinchensis* (CAX)., *Juncus ecuadoriensis* (JSP), and *Myriophyllum aquaticum* (MYA); they were identified and selected from the coordinates detailed in Figure 2. The plants were then transported to the water laboratory at the National Autonomous University of Chota in Cajamarca, where they underwent a 15-day adaptation and rooting process in their native water. This procedure was designed to ensure optimal acclimatization prior to experimental use.

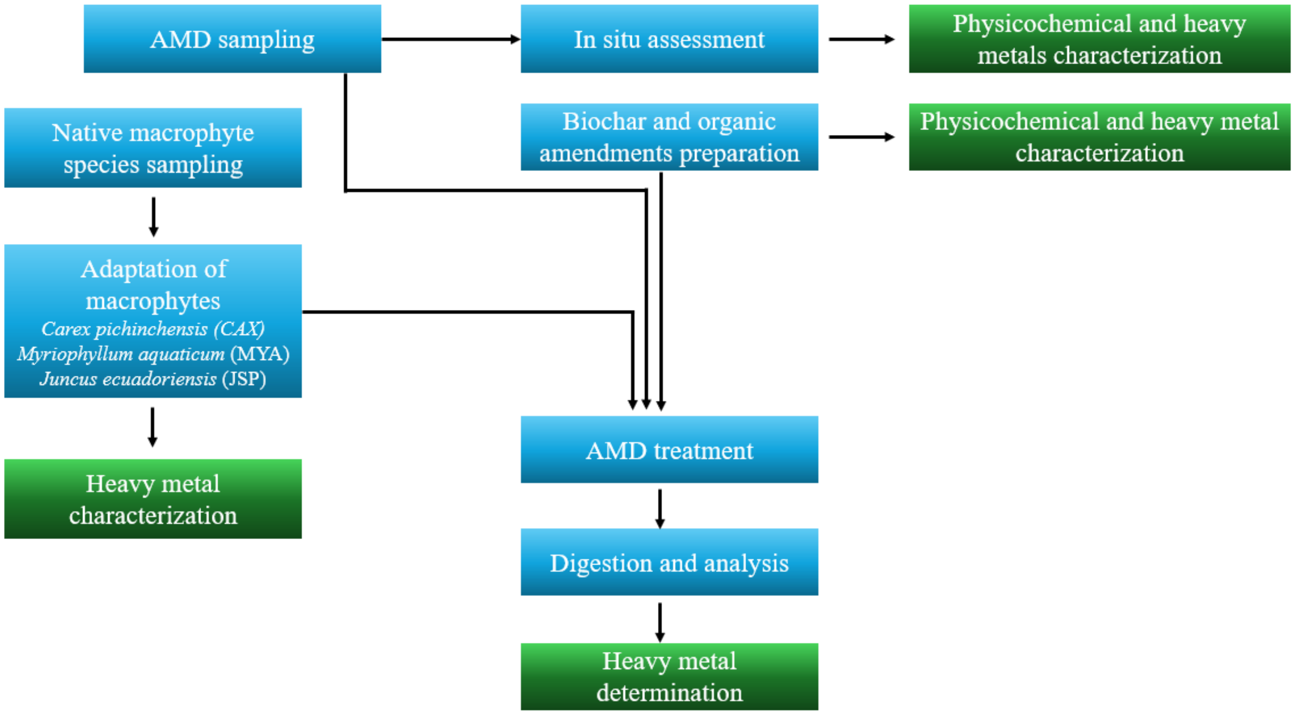

Figure 1. Schematic diagram of the experimental process.

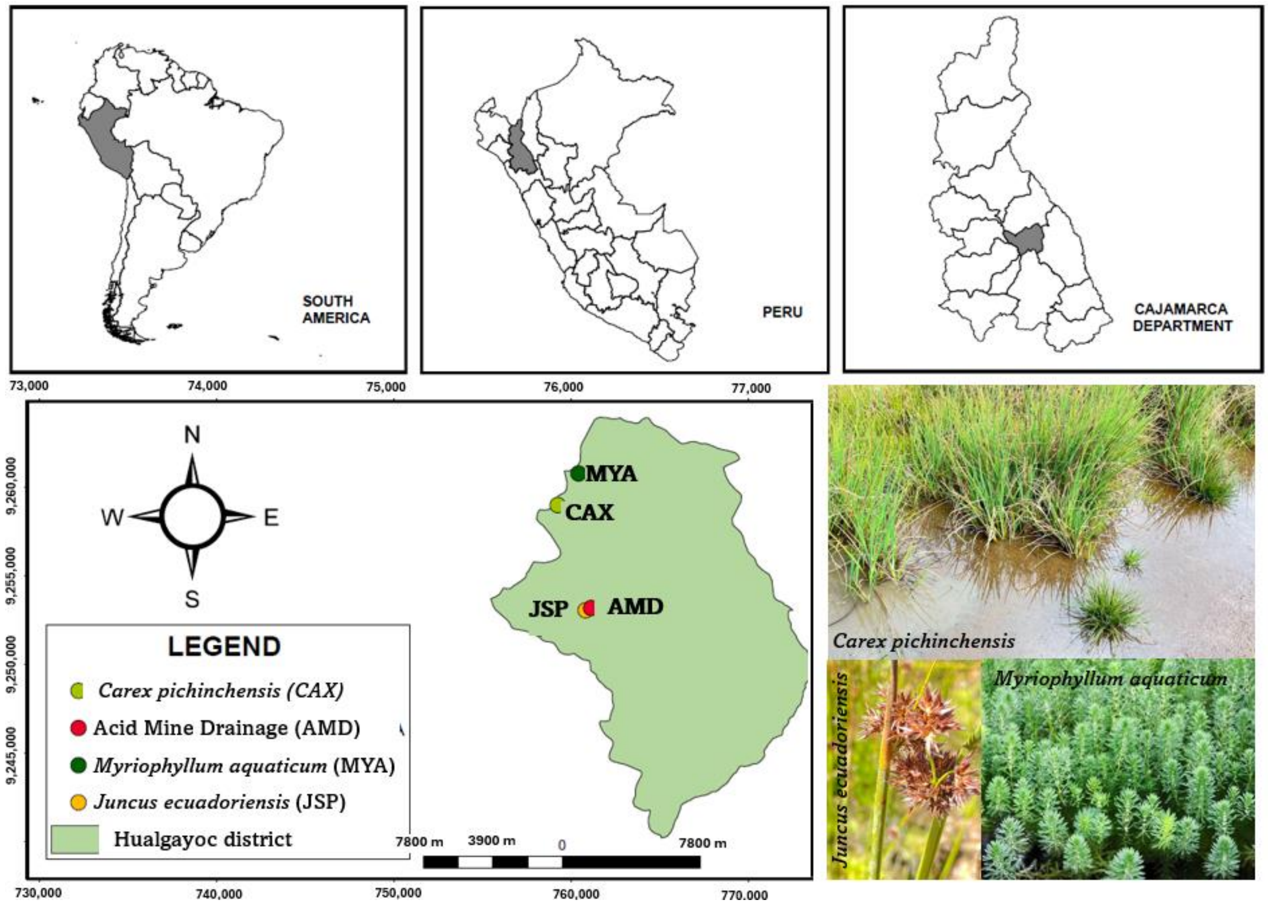

Figure 2. Sampling of AMD and macrophytes.

2.3. Production and Characterization of Amendments

Biochar was produced in the National Autonomous University of Chota from different feedstocks such as *Pinus radiata* (PBC), corn stalks (CSB), and sewage sludge (SLB). The methodology used for the construction of the pyrolytic oven was proposed by Césare et al. (2020) [36]; it is a design that integrates two cylindrical drums intended to produce small quantities of charcoal for research purposes.

The criteria considered during production were an initial material moisture of 10–12% (moisture meter—Emt01 Bside, precision of 0.5%), temperature of 500 °C (infrared thermometer type GIS 500 Bosch), and pyrolysis time of 3 h. Municipal waste compost (MWC) was produced from municipal organic waste, including food scraps (leftovers, peels, and fruit, vegetable, and herb residues), weed and pruning waste (flower, leaf, stem, and grass residues), and organic amendments (guinea pig and sheep manure) in proportions of 82% w/w, 10% w/w, and 8% w/w, respectively. The composting process lasted 90 days, with a moisture content of 40–50%, in a pile measuring 0.9 m in height, 1.50 m in width, and 2.20 m in length. Compost made from cattle manure and crop residue (CMC) was prepared with a proportion of 50% w/w each. The process lasted 90 days, with a moisture content of 40–60%. The materials were arranged in layers within a pit measuring 1.20 m in depth, 1.50 m in width, and 2.00 m in length. For vermicompost (HMS), the preparation criteria and materials used were the same as those employed for the production of municipal waste compost (MWC). Additionally, red worms (*Eisenia* spp.) were used, and the process lasted 120 days.

For biochar, compost, and humus, physicochemical parameters were characterized. In the case of pH and electrical conductivity (EC), it followed the methodology of Rajkovich et al. (2011) [37], using a HANNA HI2020-02 multiparameter. For the determination of organic matter (OM), samples were dried at 105 °C for 10 h in a desiccator, then burned at 550 °C for 6 h in a muffle furnace. The OM content was determined by the weight difference, accounting for the ash content [38].

The elemental composition of C (%) and N (%) was analyzed using a carbon analyzer (CHN628) in accordance with the ASTM D5373(40) standard method [39]. Calcium and magnesium were analyzed by shaking 1 g of compost sample with sodium acetate solution for 1 h prior to atomic absorption spectroscopy detection (Varian Spectra AA 220FS, USA). Phosphorus (P) was analyzed using colorimetry following the ascorbic acid method. Potassium (K^+) and sodium (Na^+) were determined through atomic emission spectrometry with flame photometry. Samples were extracted using a 1 M ammonium acetate solution, and emission intensities were measured at their respective wavelengths after extraction. CEC was assessed using the ammonium acetate saturation method. In this process, the samples were saturated with ammonium acetate (1 M, pH 7) to replace the existing cations with NH_4^+. Subsequently, the exchanged cations were eluted with a 1 M sodium chloride solution and quantified via spectrometry [40]. All the equipment used was calibrated with known concentration standards to ensure the results.

2.4. AMD Preparation for Heavy Metal Determination

The AMD samples were acidified with HNO_3 (1 + 1) until reaching a pH below 2 and stored under refrigeration at 4 °C for the subsequent digestion and analysis of metals and metalloids by atomic emission spectrometry [41].

2.5. Determination of Heavy Metals Form Organic Ammendments

Samples of organic amendments (CMC, HMS, and MWC) and biochar (CSB, PBC, and SLB) were dried at 50 °C for 72 h in an oven (brand: MMM Group; model: Ecocell 222). Once dried, they were ground using a mortar and sieved with a 2 mm sieve. Subsequently, they were stored in airtight containers for the further digestion and analysis of metals and metalloids by atomic emission spectrometry [42].

2.6. Preparation of Macrophytes for Heavy Metal Determination

The macrophyte samples were air-dried at room temperature for 15 days. Once dried, they were ground using a mortar and sieved with a 2 mm sieve. Subsequently, they were stored in airtight containers for the further digestion and analysis of metals and metalloids by atomic emission spectrometry [43].

2.7. Digestion and Determination of Metals and Metalloids in AMD, Organic Ammendments, and Macrophytes

The determination of metals and metalloids in CMS, HMS, MWC, CSB, PBC, SLB, and macrophytes (ex situ characterization) was carried out in the facilities of the Soil and Water Research Laboratory at the Toribio Rodriguez National University of Mendoza in Amazonas. AMD samples were digested following the APHA 3030-E method. An amount of 10 mL of each sample was taken and placed into glass tubes. Subsequently, 1 mL of HNO_3 (1 + 1) was added to the tubes, which were then sealed and placed in a heating block (Macherey Nagel, model: Vario 4). The samples were digested at 105 ± 5 °C for 2 h. The digested samples were filtered through ashless Whatman N° 42 filter paper for analysis using Microwave Plasma Atomic Emission Spectroscopy (MP-AES), following an adaptation of the APHA 3120 method [41]. Before digesting all the samples, all the containers were washed with 0.1 M HCl and rinsed with distilled water.

CMS, HMS, MWC, CSB, PBC, and SLB were digested following a modified version of EPA Method 3050B [42]. Each sample (0.5 g) was weighed and transferred into acid-resistant plastic tubes. Subsequently, 5 mL of nitric acid (HNO_3; 1:1 v/v) was added, and the tubes were covered with watch glasses. The samples were heated in a Hot Block digestion system (Environmental Express, Model SC154) for 15 min at 95 ± 5 °C. After cooling, 2.5 mL of concentrated HNO_3 was added to each tube, and the samples were returned to the Hot Block for an additional 30 min at 95 ± 5 °C. This step was repeated until no visible brown fumes were observed, indicating the complete oxidation of organic matter. The samples were then evaporated to a volume of approximately 5 mL at 95 ± 5 °C. Once cooled, 1 mL of distilled water and 1.5 mL of hydrogen peroxide (H_2O_2) was added to each sample, followed by further evaporation at 95 ± 5 °C until the volume was reduced to 5 mL. Finally, 5 mL of concentrated hydrochloric acid (HCl) was added, and the samples were heated for 15 min at 95 ± 5 °C. The digested samples were filtered using ashless Whatman No. 42 filter paper and diluted to a final volume of 50 mL with distilled water. The resulting solutions were analyzed using Microwave Plasma Atomic Emission Spectroscopy (MP-AES), following the adapted EPA Method 3050B [42].

The macrophyte samples were digested following modified methodologies described by Tokalioglu et al. (2001) [44] and Mirosławski and Paukszto (2018) [45]. A total of 1 g of each sample was weighed and placed into porcelain crucibles. The samples were then incinerated in a muffle furnace (Thermo Scientific, Waltham, MA, USA, Thermolyne model) for 6 h at 450 ± 5 °C. After cooling, the incinerated samples were moistened with distilled water, and 1 mL of hydrochloric acid (HCl) was added. The samples were left to rest for 1 h. Subsequently, 1 mL of distilled water and 1 mL of HCl was added again, and the samples were allowed to rest for an additional hour. The digested samples were filtered using ashless Whatman No. 42 filter paper and diluted to a final volume of 25 mL with distilled water. The solutions were then analyzed using Microwave Plasma Atomic Emission Spectroscopy (MP-AES), following the adapted methodologies described in [44,45].

Following digestion, the drainage, organic substrate, biochar, and macrophyte samples were analyzed using an Agilent Microwave Plasma Atomic Emission Spectrometer (Agilent

Technologies, Santa Clara, CA, USA, Model 4100 MP-AES). The instrument was equipped with a standard torch, an inert OneNeb nebulizer, and a double-pass cyclonic glass spray chamber (Agilent Technologies). Nitrogen gas, required to operate the instrument, was supplied by a nitrogen generator (Agilent Technologies, Model 4107). The pump speed was set to 15 rpm. Prior to sample analysis, the following operational parameters were configured: 12 s for sample uptake, 12 s for torch stabilization, 30 s for rinsing, and 5 s for reading time. The spectral intensity for each sample was determined as the average of three repeated measurements. The following detection wavelengths were selected for elemental quantification: 193.695 nm (As), 213.857 nm (Zn), 228.802 nm (Cd), 324.754 nm (Cu), and 371.993 nm (Fe). The instrument was calibrated using standard solutions of each element at different concentrations, prepared from 1000 ppm stock solutions traceable to Standard Reference Materials (SRMs) from the National Institute of Standards and Technology (NIST) (Agilent). After each reading, the instrument automatically retrieved the concentration and intensity values for each sample without requiring re-enrichment.

2.8. Installation and Operating Conditions of the System and Sorption Capacity

For the installation of each experimental unit, a polyethylene container measuring 0.45 m in length, 0.30 m in width, and 0.35 m in height was used, allowing for the placement of the AMD. Inside these containers, three 1-L polyethylene vessels (experimental units with two replicates) were placed, each containing biochar and/or organic substrates occupying 70% of the vessel's volume.

The AMD was supplied daily to the containers with a 4 cm water layer for 4 days, gradually reaching a 2 cm water layer above the substrates inside the 1-L vessels. This condition was maintained for 5 days. Macrophyte plants, following the adaptation process and verification of rooting, were placed in 1-L plastic vessels containing 700 mL of AMD.

The treated DAM was extracted from the biochar and organic substrates by gravity drainage, and the vessel was removed from the containers 9 days after installation. For metal determination, samples were collected in 100 mL polyethylene bottles, which had been cleaned with a 10% nitric acid solution and rinsed with distilled water. A Schott Lab 850 benchtop digital potentiometer was used for the direct measurement of pH and electrical conductivity. The water samples were transported to the Soil and Water Research Laboratory (LABISAG) at the National University Toribio Rodríguez de Mendoza de Amazonas, located in Chachapoyas. Samples were stored at −20 °C until processing [46]. In the laboratory, the water samples were filtered using cellulose filter paper (qualitative grade F1002 CHMLab, 190 µm thickness). The filtered product was acidified with nitric acid (1:1) until the pH was <2. The biochar, organic substrates, and macrophyte samples were dried at 50 °C, then ground using an agate mortar and sieved through a 200 mm sieve.

Sorption capacity is a measure that indicates how much of a specific substance can be absorbed by a material. This parameter is important to analyze for several reasons. When determining this sorption in organic materials in relation to the removed heavy metals, it can be expressed in mg/g. The concentration of heavy metals in biochar and organic amendments was calculated using the following equation [47]:

$$C_s = \frac{(C_i - C_{aq}\ ) \ \times \ V}{Sm} \tag{1}$$

where Cs = concentration of biochar (mg g^{-1}), S_m = mass of the biochar (g), Ci = heavy metals concentration measured in the blank tubes (mg^{-1}), C_{aq} = heavy metals concentration measured in the solution after sorption (mg L^{-1}), and V = solution volume (L).

2.9. Experimental Design and Data Analysis

The data analysis was conducted using Microsoft Excel 2019 (Microsoft Corp., Redmond, WA, USA) for data organization and processing. Given that the dataset for each treatment included fewer than 30 data points, the normality of the data was assessed using the Shapiro–Wilk test, with a significance level set at 0.05. For parametric comparisons between two specific treatments, a *t*-Student test was applied to evaluate significant differences in heavy metal removal percentages.

For comparisons involving multiple treatments, a one-way analysis of variance (ANOVA) was performed using JMP 16.2.0. The factors considered were the treatments (CAX, JSP, MYA, CSB, PBC, SLB, CMC, HMS, and MWC) and the percentage removal of heavy metals (As, Cu, Cd, Fe, Zn), using the differences in concentration [48] when the ANOVA indicated significant differences. Graphical representations of the data, including heavy metal removal efficiency for each treatment, were created using JMP 16.2.0.

3. Results and Discussion

3.1. Heavy Metals and Physicochemical Parameters of AMD

The physicochemical characterization of AMD in Hualgayoc, Cajamarca, revealed an acidic pH of 2.515, indicating highly acidic conditions, as shown in Table 1. The electrical conductivity (EC) measured 3.78 mS/m, suggesting a significant ionic content in the solution. The concentrations of heavy metals were quantified as follows: arsenic (As) at 0.61 mg/L, cadmium (Cd) at 0.45 mg/L, copper (Cu) at 27.09 mg/L, iron (Fe) at 374.96 mg/L, and zinc (Zn) at 32.31 mg/L. These results indicate the presence of elevated levels of heavy metals, particularly Fe and Zn, which are characteristic of AMD and represent environmental risks due to their potential toxicity and mobility under acidic conditions. This characterization serves as a baseline for assessing the effectiveness of the subsequent treatments applied to mitigate heavy metal contamination.

Table 1. Physicochemical parameters and heavy metals of treatments.

	EC (mS/m)	pH	Heavy Metals (mg/kg)				
			As	Cd	Cu	Fe	Zn
AMD	3.78	2.515	0.61	0.45	27.09	374.96	32.31

3.2. Physicochemical Properties and Heavy Metals of Amendments, Biochar, and Macrophytes

The physicochemical properties and heavy metal content of different amendments, biochars, and macrophytes were analyzed as shown in Table 2.

Table 2. Physicochemical parameters and heavy metals of biochar, amendments, and macrophytes.

Treatment	Physicochemical Parameters												Heavy Metals (mg/kg)				
	pH	EC (mS/m)	P (mg/kg)	K (mg/kg)	C (%)	OM (%)	N (meq/100 g)	CEC (meq/100 g)	Ca (meq/100 g)	Mg^{2+} (meq/100 g)	K^+ (meq/100 g)	Na^+ (meq/100 g)	As	Cu	Cd	Fe	Zn
MWC	10.03	12.78	125	28,656	22.52	38.83	1.74	154.3	33.55	19.32	72.2	29.2	20.19	0.74	0	12.6	0.46
CMC	6.33	1.8	77	1669	21.09	36.36	1.63	48	23.98	6.88	3.39	0.65	19.7	0.14	0	1.09	1.89
HMS	7.5	1.37	65	2261	14.22	24.52	1.1	37.81	26.2	5.99	4.74	0.88	18.87	0.16	0	0.4	0.07
PBC	9.81	0.63	18	1236	51.73	89.91	4.01	9.96	6.63	0.69	2.52	0.12	2.83	0.11	0	10.18	44.26
CSB	10.58	2.69	54	9328	49.77	85.82	3.96	28.9	3.59	2.03	23	0.28	11.63	0.4	0	4.79	4.72
SLB	8.59	2.52	49	233.2	7.85	13.54	0.6	21.49	19.9	0.78	0.55	0.25	63.5	0.07	0.9	0.08	0
CAX	-	-	-	-	-	-	-	-	-	-	-	-	10.93	14.16	0.62	1664.6	63.2
JSP	-	-	-	-	-	-	-	-	-	-	-	-	17.17	52.2	0.96	3809.7	99.55
MYA	-	-	-	-	-	-	-	-	-	-	-	-	7.17	3.72	0.005	1146.9	18.64

MWC and CSB showed a high pH value of 10.03 and 10.58, respectively. CMC and HMS had pH values closer to neutrality (6.33 and 7.5, respectively). PBC was also alkaline (9.81), which could help stabilize the pH of the soil. EC was highest in MWC (12.78 mS/m), with a high concentration of soluble salts, potentially limiting its use in already saline soils. In contrast, PBC and HMS showed lower EC values (0.63 and 1.37 mS/m, respectively), making them safer options in terms of salinity. MWC presented the highest concentration of P (125 mg/kg), followed by CMC and HMS (77 and 65 mg/kg, respectively). This suggests that MWC could be an excellent source of phosphorus, essential for root development in plants. The potassium (K) content was also significantly higher in MWC (28,656 mg/kg) compared to other treatments.

The carbon (C) and organic matter (OM) content was notably high in PBC and CSB, with PBC reaching 51.73% C and 89.91% OM, which can improve the soil structure and its capacity to retain water and nutrients. Nitrogen (N) was highest in PBC (4.01%), indicating a high potential to supply this essential nutrient, in contrast to SLB, which presented the lowest value (0.6%). MWC and CMC showed a high cation exchange capacity (154.3 and 48 meq/100 g, respectively), which is beneficial for nutrient retention in the soil. On the other hand, PBC and SLB showed lower CEC values (9.96 and 21.49 meq/100 g, respectively), which could limit their long-term nutrient supply capacity.

On the other hand, the heavy metal content in the treatments was analyzed, and it is shown in Table 2. The table uses a color gradient to represent the concentration levels of heavy metals across treatments, with red indicating the highest concentrations, yellow representing moderate levels, and green indicating the lowest concentrations; the heavy metal content in the biochar, amendments, and macrophytes followed distinct concentration patterns.

For arsenic (As), the order of concentration was SLB > MWC > CMC > HMS > JSP > CSB > CAX > MYA > PBC. For copper (Cu), the ranking was JSP > CAX > MYA > MWC > CSB > HMS > CMC > PBC > SLB. Regarding cadmium (Cd), the order was JSP > SLB > CAX > MYA. For iron (Fe), the order was JSP > CAX > MYA > MWC > PBC > CSB > CMC > HMS > SLB. Lastly, for zinc (Zn), the concentrations were as follows: JSP > CAX > PBC > MYA > CSB > CMC > MWC > HMS > SLB. Among all the treatments, MWC had the highest overall heavy metal content, as it exhibited elevated levels of multiple key metals. This can be attributed to its composition derived from municipal solid waste, which inherently contains diverse metal contaminants [49].

In the case of macrophytes, elevated concentrations of heavy metals, including arsenic (As), copper (Cu), cadmium (Cd), iron (Fe), and zinc (Zn), were detected in the leaves of CAX, JSP, and MYA near AMD-impacted areas in Hualgayoc, Cajamarca, highlighting the severity of environmental contamination in the region. This pollution is a characteristic outcome of acid mine drainage (AMD), which facilitates the mobilization of heavy metals into surrounding ecosystems. Despite these adverse conditions, CAX, JSP, and MYA exhibited remarkable resilience and a substantial metal accumulation, suggesting their potential for phytoremediation applications. These findings were consistent with previous studies investigating the phytoremediation potential of native plant species in mining-impacted regions. For instance, research in China demonstrated that certain plant species with a high tolerance and metal accumulation capacities contributed to the stabilization and remediation of contaminated soils [50]. Similarly, studies in Sardinia, Italy, highlighted the uptake of Zn, Pb, and Cd by native plants thriving in a highly contaminated mining site, reinforcing their role in ecological restoration [51]. Additionally, research in Jeddah, Saudi Arabia, emphasized the effectiveness of native flora in remediating anthropogenic pollutants, further supporting their deployment in highly polluted environments [52]. These collective insights underscore the ecological significance of native macrophytes such as CAX, JSP, and MYA, not only for their adaptability to metal-rich environments but also for their potential role in mitigating heavy metal contamination.

3.3. Sorption Capacity and Removal of Heavy Metals

The sorption capacity of different organic amendments for heavy metals from acid mine drainage was evaluated, as shown in Table 3, including municipal waste compost (MWC), cattle manure compost (CMC), humus (HMS), pine biochar (PBC), corn stalk biochar (CSB), and sludge biochar (SLB). The results indicate that CSB exhibited the highest sorption capacity, particularly for Cu (0.139 mg/g), Fe (1.942 mg/g), and Zn (0.149 mg/g), suggesting its high potential for metal remediation. PBC also showed a notable Fe sorption (0.879 mg/g), while CMC and HMS had similar Cu adsorption capacities (0.049 mg/g). Conversely, MWC displayed a negative As value (−0.004 mg/g), possibly indicating desorption or measurement variability. Overall, biochar-based treatments (CSB, PBC, and SLB) demonstrated greater effectiveness in metal retention, particularly for Fe and Zn, compared to compost-based amendments. These findings highlight the potential of biochar, especially CSB, as a promising material for mitigating heavy metal contamination in acid mine drainage.

Table 3. Physicochemical parameters and heavy metals of treatments and macrophytes.

	Capacidad de Sorción (mg/g)				
Treatment	**As**	**Cd**	**Cu**	**Fe**	**Zn**
MWC	−0.004	0.001	0.039	0.546	0.044
CMC	0.001	0.001	0.049	0.672	0.044
HMS	0.000	0.001	0.049	0.636	0.057
PBC	0.001	0.000	0.044	0.879	0.030
CSB	0.001	0.002	0.139	1.942	0.149
SLB	0.000	0.001	0.030	0.419	0.036

Figure 3 shows the average percentage removal efficiency (%) of As, Cu, Fe, and Zn in AMD using different treatments categorized into three groups, amendments (MWC, CMC, and HMS), biochar (PBC, CSB, and SLB), and macrophytes (CAX, JSP, and MYA). The letters indicate statistically significant differences between treatments. Treatments with the same letter do not differ significantly from one another.

For As, regarding the amendments, the highest average removal percentage was achieved by CMC (58.5%), while no removal was observed for MWC. This may be because CMC (6.33) and HMS (7.5) have pH values close to neutral, which can favor the precipitation and adsorption of heavy metals, as many metals tend to form insoluble hydroxides at these pH levels.

MWC (10.03) had an alkaline pH, which could limit the precipitation of certain metals or alter their chemical speciation, thereby reducing the effective removal of As. For biochar treatments, PBC (62.29%) and SLB (63.39%) achieved higher removal rates compared to CSB (40.98%). At an alkaline pH, heavy metals tend to precipitate into insoluble forms like metal hydroxides. As may transform into less mobile forms, such as As(III) or As(V).

At a moderately alkaline pH, the competition with alkaline cations (e.g., Na^+ and K^+) is reduced. The pH of SLB (8.59) was more stable for the formation of chemical bonds with functional groups in biochar, such as carboxyls (-COOH) and phenols (-OH), which often play a crucial role in the chemisorption of heavy metals. The biochars used in this research were unmodified, although there are some ways of doing so that guarantee much greater efficiency, such as in the study of He et al. (2018) [53] (Table 4), which used iron-impregnated biochar to remove up to 87.53% of As in AMD. The application of modified biochar showed various advantages, such as a higher adsorption capacity for As(V) [53], a greater specific surface area and porosity, and the possibility of magnetic separation; however, the additional cost associated with the iron impregnation process could be considered a disadvantage. On the other hand, Zama et al. (2022) [54] used biochar that was not modified, yet it acted as a complement to calcium in the precipitation of As, achieving a 58.1% and 54.0% removal efficiency for wood biochar and rice husk biochar, respectively.

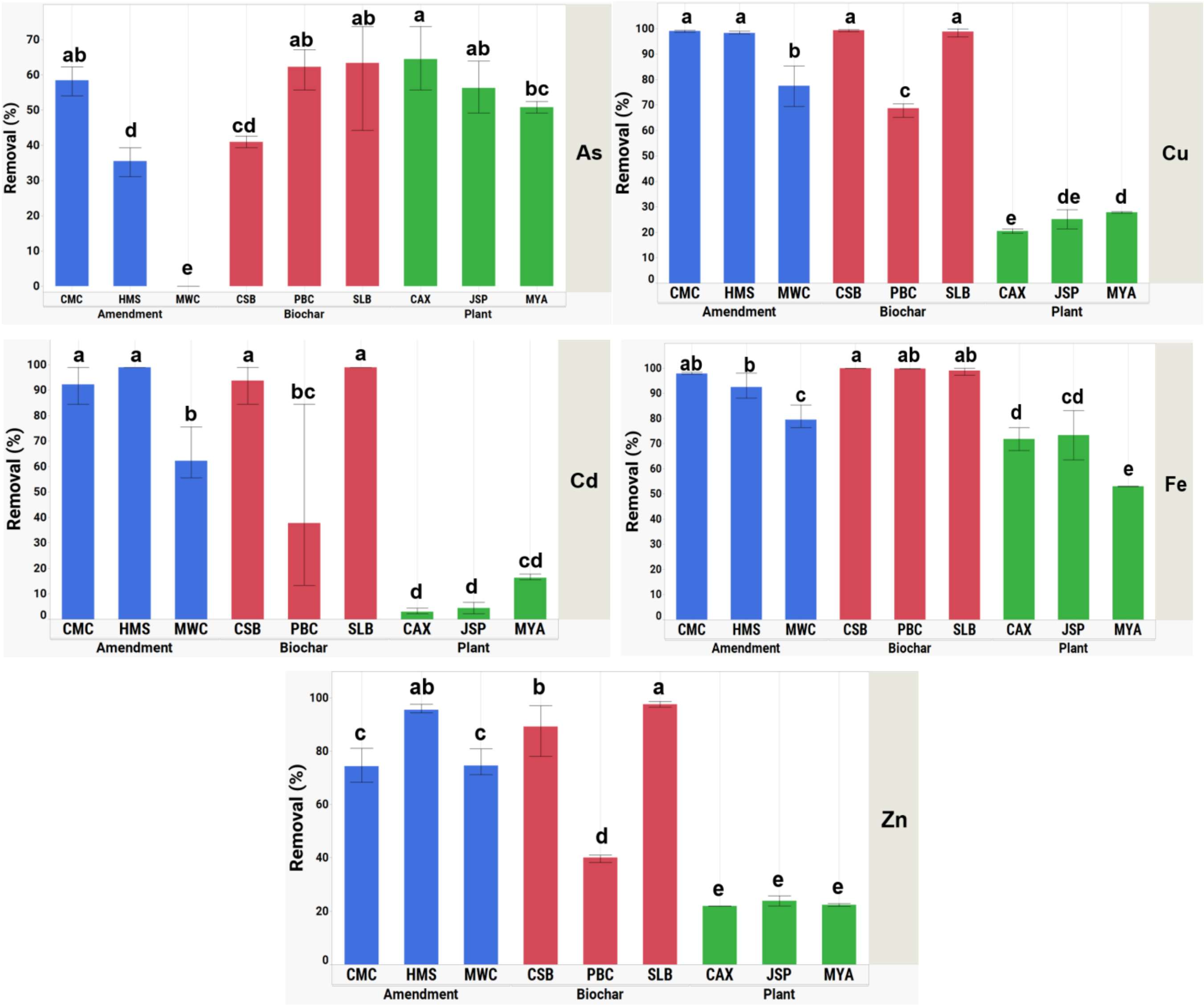

Figure 3. Efficiency of heavy metal removal from AMD by different amendments, biochars, and macrophytes.

Macrophytes demonstrated higher removal rates compared to amendments, with CAX (64.5%) showing a greater removal percentage than JSP (56.3%) and MYA (50.8%). In the case of the plants, it is evident that they have a high removal potential, similar to that of amendments (compost, humus, and biochar). A similar study demonstrated that *Carex riparia* tends to be an accumulator plant, as it achieved a BCF of 74.24 [55].

For copper (Cu), regarding the amendments, CMC, HMS, CSB, and SLB exhibited the highest efficiency in Cu removal, with percentages close to 100%. In contrast, for plants, CAX showed the lowest removal efficiency, similar to Velasco-Arroyo et al. (2024) [55], with a BCF value of 4.42 for Cu using *Carex riparia*, a relatively low value compared to other metals.

CSB and SLB stand out, achieving the highest Cu removal percentages, both exceeding 90%. This can be attributed to their physicochemical properties, where CSB presents a remarkably high content of OM (85.82%) and carbon (C) (49.77%), which enhances its capacity to adsorb Cu. The high organic matter content creates functional surfaces rich in carboxyl and phenolic groups, facilitating the immobilization of metal cations. Previous studies have demonstrated that biochars with a high carbon and organic matter content are effective adsorbents of heavy metals due to their ionic interaction capacity with contaminant metals [56,57]. CMC and HMS exhibited a Cu removal percentage close to 100%, making them the most effective in this category, while MWC showed a lower performance, with a removal rate of approximately 75%. This difference can be primarily attributed to pH values. CMC and HMS have pH levels of 6.33 and 7.5, respectively, which fall within the optimal range for heavy metal adsorption, whereas MWC has a higher pH (10.03), affecting the solubility and availability of Cu for adsorption. Additionally, the electrical conductivity (EC) of MWC is considerably higher, which could negatively impact metal retention in the soil. This finding aligns with studies indicating that amendments with a near-neutral pH optimize the immobilization of heavy metals in contaminated soils [58].

CAX (20.5%), JSP (25.2%), and MYA (27.9%), showed the lowest Cu removal percentages compared to amendments and biochars. CEC is a key factor in heavy metal removal, as negatively charged sites in the soil facilitate metal immobilization through electrostatic bonds [59]. For example, Nagy et al. (2020) [8], employed Condensed Molasses Soluble (CMS) for copper (Cu) remediation using *Carex flacca*. CMS functioned as a complexing agent, and it was observed that as the CMS concentration increased, the concentration of metals also increased, including Cu. Research has shown that the Cu accumulation in *M. aquaticum* occurs primarily in the roots, accounting for 45–64% of its total metal uptake, suggesting a limited translocation to aerial tissues [19]. This indicates that although *M. aquaticum* possesses potential for Cu remediation, its efficiency is influenced by Cu concentrations in the medium and may be less effective than other amendments or biochars, which rely on adsorption rather than biological uptake.

For Cd removal in biochar treatments, SLB and CSB achieved the highest removal percentages, both close to 100%, whereas PBC demonstrated a lower performance. As shown in Figure 3, these treatments were characterized by a high carbon (C) content and cation exchange capacity (CEC), particularly CSB, which had a CEC of 28.9 meq/100 g. The high CEC of these biochars facilitated an improved cation exchange, including Cd, enhancing its adsorption onto the biochar matrix. This finding was consistent with previous studies [57] indicating that organic matter content and CEC are key factors in the retention of heavy metals, including Cd, in contaminated soils. In the category of amendments, the CMC and HMS treatments exhibited the highest Cd removal percentages, with values close to 100%, whereas MWC showed a lower performance.

CMC and HMS had low EC values (1.8 mS/m and 1.37 mS/m, respectively), which favored the removal of heavy metals by reducing competition from other cations present in the soil. In contrast, the MWC treatment had a high EC (12.78 mS/m,), which may have limited Cd removal due to the saturation of adsorption sites with secondary cations such as sodium and potassium. In the macrophyte treatments, MYA (16.3%) exhibited the highest Cd removal percentage, although it remained lower compared to the biochar and amendment treatments.

For Fe removal, CMC (97.8%) exhibited the highest removal efficiency. HMS (92.5%) showed a slightly lower removal compared to CMC, while MWC's was 79.5%. For biochar treatments, CSB, PBC, and SLB displayed a very similar performance. Regarding macrophyte treatments, CAX achieved the highest Fe removal.

CSB, PBC, and SLB achieved removal percentages above 90%, making them the most effective in this category. These biochars had high levels of organic matter (OM) and carbon (C), which facilitated the formation of stable complexes between the functional groups of the biochar and Fe. Additionally, these treatments exhibited moderate electrical conductivity (EC) values (2.69 mS/m for CSB, 0.63 mS/m for PBC, and 2.52 mS/m for SLB), allowing for a balanced availability of cations in solution and reducing competition for adsorption sites. The presence of carboxyl and phenolic groups in high-carbon biochars also contributed to enhanced Fe adsorption, as demonstrated in previous studies on the capacity of biochars to retain heavy metals through electrostatic interactions and chelation [60]. CMC and HMS had low EC values (1.8 mS/m and 1.37 mS/m, respectively), which promoted greater Fe retention by reducing cation competition in the soil. In contrast, MWC had a much higher EC (12.78 mS/m), which could explain its lower removal capacity, as high concentrations of dissolved salts compete for adsorption sites, limiting the interaction between Fe and the adsorptive surfaces. Additionally, MWC had an alkaline pH (10.03), which could reduce Fe solubility in the soil and, consequently, decrease its availability for adsorption. Some studies indicate that soils with a moderate pH and low EC are more efficient at immobilizing Fe and other heavy metals due to improved metal complex formation. Similarly, Liu et al. (2024) [61], utilizing food waste compost for Fe removal from acid mine drainage (AMD), achieved a >90% Fe removal at a pH $\geq$ 3.00, primarily through precipitation and adsorption mechanisms.

For Zn, among the amendments, HMS (95.48%) exhibited the highest removal percentage, followed by MWC (74.58%) and CMC (74.3%). The highest removal percentage was achieved by SLB, close to 100%, while PBC (97.56%) showed the lowest removal efficiency. Regarding plant-based treatments, all three displayed similar behavior, with lower removal percentages compared to all the other treatments. In Zn removal, the SLB biochar treatment achieved the highest removal percentage, close to 100%, while CSB and PBC exhibited significantly lower removal efficiencies. As shown in Table 2, SLB had a relatively low OM content (13.54%) but presented a moderate EC of 2.52 mS/m and a CEC of 21.49 cmol/kg, which facilitated the adsorption of metal cations such as Zn. In contrast, PBC, which showed a much lower removal percentage, had an EC of 0.63 mS/m, which may have been insufficient to promote a proper balance in the soil solution and enhance Zn retention. Previous studies have demonstrated that biochars with a moderate EC and high CEC have a greater capacity to immobilize heavy metals, as they provide sufficient active sites for the ionic adsorption of metal cations such as Zn. In the category of amendments, the CMC and HMS treatments achieved a Zn removal percentages close to 90%, while MWC exhibited a slightly lower performance. CMC and HMS had relatively low EC values (1.8 mS/m and 1.37 mS/m, respectively), which helped reduce competition for adsorption sites within the soil matrix, allowing for greater Zn retention. In contrast, MWC had a high EC value (12.78 mS/m), which could lead to the saturation of secondary cations, thereby reducing the Zn adsorption capacity. Additionally, the elevated pH of MWC (10.03) may have negatively affected Zn solubility, decreasing its availability for adsorption at active soil sites. According to the literature, soils with a low to moderate EC and a near-neutral pH are more effective in immobilizing metals such as Zn. CAX, JSP, and MYA exhibited similar Zn removal percentages, though significantly lower than those achieved by biochar and amendment treatments. According to Table 4, Velasco-Arroyo et al. (2024) [55] demonstrated a Bioconcentration Factor (BCF) of 3.95 for Zn using *Carex riparia.* This aligns with the demonstrated capacity of the plant for metal adsorption and the establishment of stable root–metal interactions. In the study conducted by Romero-Hernández et al. (2017) [62], the application of *Myriophyllum aquaticum* achieved a zinc (Zn) removal efficiency of 91.91% at a concentration of 0.25 mg/L. In general, it was observed that biochar and amendments showed good removal percentages compared to native macrophytes.

Furthermore, Table 4 shows various studies examining heavy metal removal treatments with significantly varied timeframes, ranging from just 7 days to extensive 90-day periods. These temporal differences are crucial for properly understanding a treatment's effectiveness. The longer-duration studies, such as the 90-day experiments using *Carex riparia* and *Cyperus longus* species [55], provide substantially more comprehensive assessments of metal removal capacity for elements like As, Cd, Cu, Fe, and Zn. These extended periods allow researchers to observe effectiveness variations over time, evaluate treatments' sustainability, and permit biological systems to reach a natural equilibrium, revealing their true removal capabilities. Conversely, shorter studies like the 7-day *Myriophyllum aquaticum* experiment offer immediate results but may inadequately represent long-term efficacy. The intermediate 6-week study strikes a valuable balance, allowing sufficient time to observe natural system fluctuations, establish chemical equilibrium processes, and assess treatment stability under varying environmental conditions. The wide measurement ranges shown in BCF (Bioconcentration Factor) values and removal percentages provide essential information about treatments' consistency and reliability across different conditions. These ranges become particularly valuable when combined with longer study periods, capturing both temporal variability and treatment condition variations.

Table 5 shows the results of a one-way ANOVA conducted to evaluate the removal percentages of heavy metals (As, Cd, Cu, Fe, Zn) across various treatments. All p-values are <0.0001, indicating statistically significant differences among treatments for all the metals analyzed. The F Ratios highlight the strength of these differences, with Cu showing the highest variability (F Ratio: 353.221), suggesting that its removal efficiency varies significantly across treatments. In contrast, Fe has the lowest F Ratio (43.949), reflecting a more consistent removal across treatments. The Sum of Squares (SS) further emphasizes this trend, as Cd exhibits the highest SS (41,448.574), indicating greater variability in its removal percentages, while Fe has the lowest SS (6662.845), supporting its relatively uniform performance. These findings suggest that metals like Cu and Cd are more treatment-sensitive, while Fe removal is more stable across the tested methods.

The methodology ensured data normality using the Shapiro–Wilk test before applying parametric tests like ANOVA, validating the statistical reliability of these results. Post hoc analyses, such as Tukey's HSD, should be conducted to identify specific treatments responsible for the significant differences. Metals like Cu and Zn, with high F Ratios and variability, likely require more tailored treatments, whereas the consistent performance of Fe suggests that general approaches are effective. This could be due to the precipitation of Fe and adsorption mechanisms, which are less influenced by variations in treatment conditions. These results underline the importance of understanding metal-specific behavior to optimize remediation strategies, with targeted approaches for metals showing a high variability and general methods for those with stable removal efficiencies, such as Fe.

On the other hand, although different investigations highlight the potential of removing heavy metals with the application of macrophytes and organic amendments in AMD, a critical environmental concern is the fate of the contaminated biomass, as the metals removed are transferred to these materials. If not properly managed, this could result in secondary pollution through leaching or degradation processes. Alternatively, thermochemical conversion methods such as pyrolysis can transform contaminated plant material into biochar, which has shown promise in stabilizing metals and reducing environmental risks [65]. Additionally, phytomining has been proposed as a recovery strategy, where accumulated metals in hyperaccumulators are extracted for industrial reuse [65]. Given the environmental risks, future studies should evaluate the long-term stability of metal-laden macrophytes under field conditions, exploring best practices for their disposal or valorization. These findings emphasize the need for an integrated AMD remediation strategy that not only focuses on water purification but also ensures the safe and sustainable management of contaminated residual biomass.

Table 4. Physicochemical parameters and heavy metals of treatments and macrophytes.

Description of Application	Concentration of Application	Concentration of Pollutant	Time	Pollutant	% Removal (%)	BCF	Reference
Carex riparia ^{1} *Cyperus longus* ^{2} *Cyperus rotundu* ^{3}	-	As: 0.450–0.500 mg/L	90 days	As	-	74.24 ^{1}, 40.29 ^{2}, 29.37 ^{3}	[55]
		Cd: 2.000–2.500 mg/L		Cd	-	12.64 ^{1}, 15.63 ^{2}, 24.45 ^{3}	
		Cu: 160–1000 mg/L		Cu	-	4.42 ^{1}, 39.80 ^{2}, 34.94 ^{3}	
		Fe: 250–400 mg/L		Fe	-	108.58 ^{1}, 399.79 ^{2}, 413.11 ^{3}	
		Zn: 70–320 mg/L		Zn	-	3.95 ^{1}, 14.24 ^{2}, 18.91 ^{3}	
Myriophyllum aquaticum	-	Cu: 1000 ug/L TC: 3000 ug/L	12 weeks	Cu + tetracycline (TC)	>80	-	[19]
Myriophyllum aquaticum	-	Cu: 0.5 mg/L	7 days	Cu	95.20	200–300	[62]
Myriophyllum aquaticum	-	Zn: 0.25 mg/L		Zn	91.91	180–200	
Juncus effusus	-	Sulfate (SO_4^{2-}): 4000 mg/L, Mn: 18 mg/L, Zn: 10 mg/L, Cd: 0.5 mg/L	60 days	Sulfate (SO_4^{2-})	<50	-	[1]
Juncus effusus	-	Sulfate (SO_4^{2-}): 4000 mg/L, Mn: 18 mg/L, Zn: 10 mg/L, Cd: 0.5 mg/L		Mn	>99	-	
Carex flacca + Condensed Molasses Soluble (CMS) ^{1} *Phragmites australis* ^{2}	1% CSM	206 ± 45	6 weeks	As	-	2227 ^{1}, 295 ^{2}	[8]
		265 ± 100		Cu	-	371 ^{1}, 502 ^{2}	
		885 ± 50		Pb	-	1685 ^{1}, 1046 ^{2}	
		2780 ± 110		Zn	-	329 ^{1}, 388^{2}	
		-		Cd	-	401 ^{1}, 463 ^{2}	
Fe-impregnated biochar (FBC)	20% FBC	10 mg/L As(V)	48 h	As	87.53	-	[53]
Wood biochar (WB4, WB7)	-	10 mg/L As (III)	24 h	As	58.1(WB7)	-	[54]
Rice husk biochar (RB4, RB7)	-	10 mg/L As (III)		As	54.0 (RB7)	-	

Table 4. *Cont.*

Description of Application	Concentration of Application	Concentration of Pollutant	Time	Pollutant	% Removal (%)	BCF	Reference
Pine cone biochar (PBC) [1] Zn-loaded PCB [2]	-	100 µg/L As (III)	-	As	66.08 ± 3.94 [1] 87.62 ± 3.88 [2]	-	[63]
Biochar inoculated bacteria (BIC)	BIC: 0.6 g/L	5 mg/L (Cd^{2+})	Up to 120 h	Cd	67.90	-	[64]
Food waste compost		11,787.6 ppm	2 h	Fe	≈100	-	[61]
	20 g/L compost	4.5 ppm		Cu	>90	-	
	20 g/L compost	0.2 ppm		Pb	≈100	-	
	40 g/L compost	61.2 ppm		Zn	≈90	-	
	40 g/L compost	5.9 ppm		Ni	<90	-	

Note: The plant species listed in the “Description of Application” column are associated with the metals indicated in the “Concentration of Pollutant” column. The values in the “BCF” column correspond sequentially to those metals: [1] = first metal, [2] = second metal, [3] = third metal, and so on for each treatment evaluated.

Table 5. *p*-value of the heavy metals.

Heavy Metal	SS	F Ratio	*p*-Value
As	10,223.762	23.692	<0.0001
Cd	41,448.574	24.647	<0.0001
Cu	28,804.231	353.221	<0.0001
Fe	6662.845	43.949	<0.0001
Zn	25,515.522	158.865	<0.0001

4. Conclusions

The results indicate that the most effective treatments for removing heavy metals from acid mine drainage (AMD) include organic amendments such as biochar and compost, with efficiencies exceeding 90% in most cases. Specifically, sewage sludge biochar (SLB) achieved a 100% Zn removal, while cattle manure compost (CMC) reached up to a 97.8% Fe removal. Regarding metals with greater sensitivity to treatments, Cu and Zn exhibited the highest variability values (F Ratio: 353.22 and 158.86, respectively), suggesting that their removal requires more targeted strategies. In contrast, Fe showed a more stable removal across the tested treatments, indicating that general stabilization methods can be effective for this metal. Native plants such as *Carex pichinchensis*, *Juncus ecuadoriensis*, and *Myriophyllum aquaticum* demonstrated a remarkable capacity for removing heavy metals in constructed wetland systems. *Carex* sp. achieved the highest Fe removal (~97%), while *Myriophyllum aquaticum* showed a higher affinity for Cd. However, although these species have great potential for phytoremediation, their efficiency in metal removal remains lower compared to amendments such as biochar or compost. Additionally, their metal absorption capacity may be limited by the low translocation of contaminants from roots to leaves. This highlights the need to develop strategies that enhance their effectiveness by leveraging their resistance to extreme pH conditions and high metal loads. This study suggests that combining native plants with organic amendments such as biochar and compost significantly improves the efficiency of heavy metal removal from AMD. The organic matter in the amendments enhances their metal adsorption and stabilization capacity, facilitating their retention in the substrate and subsequent absorption by plants. For example, *Myriophyllum aquaticum* increased its Cd accumulation when applied in combination with compost, demonstrating that the interaction between organic matter and roots can optimize phytoremediation.

The evaluation of organic amendments for heavy metal removal from acid mine drainage revealed that biochar-based materials, particularly corn stalk biochar (CSB), exhibited the highest sorption capacity, especially for Cu, Fe, and Zn. Pine biochar (PBC) and sludge biochar (SLB) also showed notable metal retention, outperforming compost-based amendments. The superior performance of biochars suggests their potential as effective remediation materials for metal-contaminated environments. Future studies should explore their long-term stability and possible synergies with other treatment methods to optimize their application in AMD mitigation. Furthermore, future research should evaluate the effects of weather and climate on AMD variation, establishing long-term control groups. In addition, future research should focus on longer removal times; surface analysis (morphological surface area (SEM), specific surface area—BET) and molecular analysis (XRD and XPS, X-ray analysis; aromaticity and aromatic condensation (NMR); volatile organic compounds) for AMD treatment.

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