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# Passive Treatment of Acid Mine Drainage Effluents Using Constructed Wetlands: Case of an Abandoned Iron Mine, Morocco

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**Abstract:** This study presents the effectiveness of two vertical subsurface flow (VF) constructed wetlands (CWs), one planted with *Juncus effusus* (PCW) and the other unplanted (CCW), for the remediation of acid mine drainage (AMD) from the Ouixane abandoned mine site located in Morocco. The VFs were fed with highly acidic AMD (pH < 2.5) and were evaluated over a period of 150 days. The substrate was composed of limestone, as a neutralizing agent, river gravel, and natural peat moss, with the goal of promoting the growth of sulfate-reducing bacteria (SRB) and metals precipitation. The results showed that both VFs successfully neutralized the acidity, with effluent pH values ranging from 3.57 to 8.5, indicating effective alkalinization of the AMD. Significant differences ($p < 0.05$) were observed between the metal removal rates of the CCW and the PCW, except for Mn. Both types of constructed wetlands (CWs), the planted system (PCW) and the unplanted system (CCW), exhibited similar efficiencies in metal removal from the influent. The rates of metalloid removal were as follows: 99.9% vs. 99% for Cr, 99% vs. 80% for As, 96% vs. 94 for Zn, 99.94% vs. 99% for Fe, and 90% vs. 81% for Al. Microbial sulfate reduction was increased from 43% to 50% by the presence of plants. Sediment analysis revealed that metals were primarily in stable forms: Fe and Zn were mostly associated with Fe-Mn oxides, while Mn and Ni were predominantly present as carbonates. These observations indicate a relative stability of metals in the CWs' sediment. This study highlights the effectiveness of the studied CWs, particularly those with vegetation, for AMD remediation, emphasizing the importance of neutralizing agents, plants, and organic substrates in the treatment process.

**Keywords:** vertical flow constructed wetlands; metal remediation; acid mine wastewater phytoremediation; metal recovery; *Juncus effusus*

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## 1. Introduction

The environmental threat posed by mining activities is a global challenge. To date, acid mine drainage (AMD) remains one of the primary environmental impacts associated with mining operations [1,2]. These waters are harmful to the environment due to their high acidity and concentrations of metals, metalloids, and sulfates, which negatively affect surface and groundwater, sediments, and soils [2–4]. The oxidation process of sulfide minerals (e.g., pyrite, pyrrhotite) in solid mining waste can occur under natural conditions, depending on seasonal climatic changes, generating AMD in the presence of minimal concentrations of neutralizing minerals [5–8]. During interactions between water and rock, the mobility and leaching of metals, metalloids, and sulfates can be constrained by the formation of secondary minerals (e.g., oxides, hydroxides, sulfates). However, variations in parameters such as pH, Eh, temperature, and climatic conditions may induce the dissolution of these secondary minerals, leading to the potential release of metals, metalloids, and sulfates into the natural environment [9,10]. In cases where sulfide mining wastes also show significant proportions of neutralizing minerals, the concentration of these secondary minerals can significantly increase, since pH remains close to neutral, where the precipitation of metals such as iron are favored [11,12].

Consequently, regulating the formation of acidic effluents proves to be highly complex, particularly at abandoned mining areas. To minimize the detrimental effects of contaminants on the receiving environment, it is imperative that contaminated water undergoes treatment prior to its discharge. To avoid the harmful impacts of contaminants on the receiving environment, several different approaches for managing AMD have been developed, namely incorporating both active and passive techniques to neutralize acidity and promote the precipitation of metals and metalloids [13]. **Active treatments** involve the continuous application of alkaline compounds to neutralize the acid effluents generated at mining sites and facilitate the precipitation of metallic contaminants [14]. Conversely, **passive methods** utilize cost-effective and environmentally sustainable processes to achieve similar outcomes with long-term treatment efficacy and, in some cases, with the advantage of material reuse at minimal cost [15]. Both treatment methods are frequently used together. Thus, to limit the production of AMD and extend the lifespan of passive treatments, alkaline amendments are used in situ [16,17]. Under acidic conditions, the dissolution of these amendments enhances alkalinity and restricts the mobility of contaminants by promoting the precipitation of iron oxyhydroxides, coprecipitation, adsorption, passivation of sulfide surfaces, and reduction of acidophilic bacterial activity when transitioning to neutral conditions [18]. Passive abiotic treatment systems encompass anoxic or oxic limestone drains, open limestone trenches, and limestone ponds [19]. By contrast, passive biochemical treatment methods include artificial wetlands, biochemical reactors, and permeable reactive barriers [20,21]. In passive treatment systems, AMD effluents traverse reactor filling materials, which include organic matter and neutralizing agents such as calcite [22]. The interaction between the drainage water and these substrates produces adequate alkalinity to promote pH rise and facilitate metal removal through sorption, precipitation, and coprecipitation. The use of these materials represents a cost-effective and environmentally sustainable remediation approach, as previously highlighted [23].

Passive treatment through constructed wetlands (CWs) is distinguished by its eco-sustainable approach to AMD treatment [19]. Previous studies have highlighted the significant contribution of using neutralizing materials in CWs, such as shells, in the Nador region (Morocco). This approach not only increases the pH but promotes the precipitation of sulfates, metals, and metalloids from the AMD [24]. In addition, vegetation plays a critical role in CWs by facilitating the removal of potential toxic elements through phytostabilization and/or phytoaccumulation. To date, numerous studies have demonstrated that metal accumulation occurs in both the root and shoot tissues of plants, influenced by their capacity for metal uptake and their preferential affinity for metal ions [25]. However, the scientific literature presents conflicting views on the contribution of wetland plants to AMD remediation [19,26]. The effectiveness of metal removal in CWs is contingent upon several factors, including plant species, growth conditions, the plant's ability to thrive in the substrate or with organic amendments, metal stress tolerance, and adaptability to the varying metal concentrations present in the AMD [27]. Furthermore, research has highlighted significant variability regarding the role of plants in sulfate removal in CWs [28]. In addition to dissimilatory sulfate reduction by sulfate-reducing bacteria (SRB), biological sulfur assimilation by plants also contributes to the overall sulfate removal in these systems [29]. Additional concerns have been raised about metal leaching from the CWs substrate (primary pit) following their long-term use, due to increased metal bioavailability [30].

This study seeks to evaluate the feasibility of CWs as sustainable methods for treating the AMD in the Ouixane mine (Morocco), and to focus on the mechanisms of metal and metalloid removal, as well as their operational speciation/fractionation in the substrate. To assess the importance of configuration and the presence or absence of plants in metal and metalloid removal, two types of vertical subsurface flow (VF) along with planted and unplanted wetlands, were studied. The selected plant species was *J. effusus*, which is commonly found at mining sites affected by AMD.

The presence of plants is expected to slow the flow rate and increase the hydraulic retention time (HRT), in addition to promoting the growth of bacteria that actively participate in metal transformation.

## 2. Materials and Methods

### 2.1. Site Description and AMD Composition

The iron ore Ouixane mine is located at the northern end of the Beni Bou Ifrour massif, approximately 10 km S-SW of the port of Nador, Morocco. This area is sensitive and significant due to its high capacity to regulate and maintain the quality and availability of water resources, as well as its endemic biodiversity. The primary ore is composed of iron oxides (hematite, magnetite), iron carbonates (siderite, ankerite), and sulfides (pyrite, pyrrhotite) [31]. The composition of the AMD from the Ouixane mine was collected during the dry season and evaluated for trace metal elements (Fe, Mn, Al, Cu, Cd, Zn, and Pb) and trace metalloid elements (As and Cr). Acid mine drainage is highly acidic, acting as an oxidizing agent with elevated concentrations of iron and sulfate, while exhibiting lower levels of other metals.

### 2.2. Design and Operational Conditions of the Laboratory-Scale CWs

Two small-scale CW systems (planted—PCW; and unplanted—CCW; Figure 1a) were developed using polyethylene containers with dimensions of (0.48 m × 0.38 m × 0.33 m), providing a surface area of 0.22 $m^2$ and a volume of 0.06 $m^3$. The experimental cells contained, at the base, a layer of neutralizing agent, specifically limestone (10 cm), covered with a mix of river gravel (approximately 15 mm diameter, washed and without fine particles), and a source of organic carbon (natural *Sphagnum* moss) (Figure 1b). A water-soluble plant fertilizer (Miracle-Gro N:P 24:8:16) was added to the PCW system in order to promote the initial development of plants. These organic substrates were added to promote the growth of sulfate-reducing bacteria (SRB) and the precipitation of metals and metalloids in the form of carbonates and metal sulfides. *J. effusus* has been reported to be a species that adapts well in natural wetlands receiving mining wastewater with low pH (<2) [32]. Thus, young specimens of these plants (40–50 cm) growing abundantly around the Ouixane mine were transplanted into the PCW system at a planting density of 19 stems $m^{-2}$.

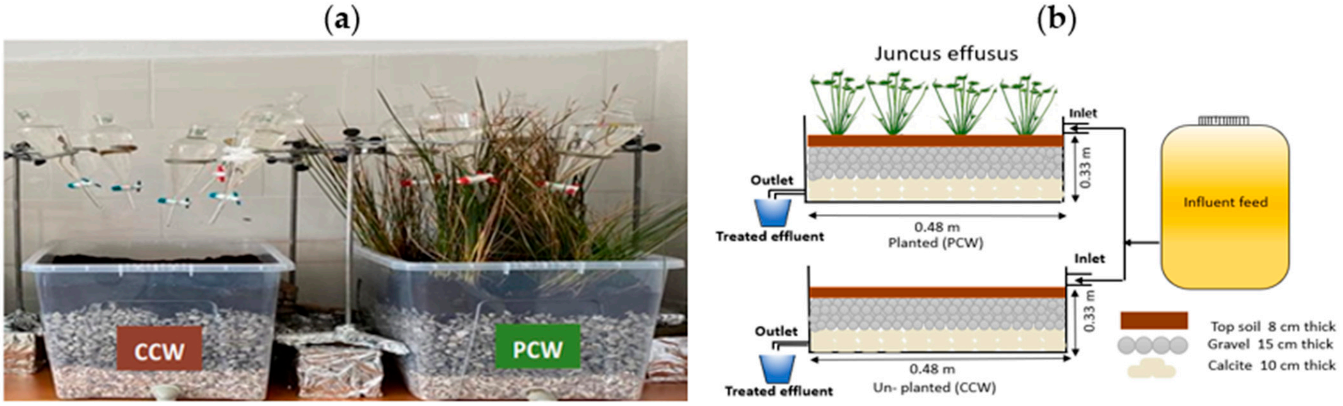

**Figure 1.** (**a**) Experimental lab constructed wetlands systems operated with plants (PCW) and without plants (CCW), and (**b**) the schematic design of the lab CW systems showing the vertical flow and the details of the substratum layers.

The wetlands were placed indoors and continuously supplied with AMD collected in the mining site from a 60-L influent reservoir (Figure 1b). The effective volumes for the CCW and PCW were approximately 27 and 26 L, respectively. The flow rate was maintained at 1.5 mL $min^{-1}$ in a continuous flow and the resulting nominal hydraulic residence time (HRT) was 10 days [33]. A water height of approximately 3–5 cm above the substrate was maintained to ensure a saturated environment. The temperature was kept at around 22.5 °C during the day for a photoperiod of 16 h. Water samples were collected, and the physicochemical parameters, as well as the chemical analyses of the treated effluent, were carried out over a period of 105 days for the CCW and PCW, respectively, excluding the two-week acclimation period.

## *2.3. Analytical Procedures*

### 2.3.1. Water (Influent and Effluent) Analysis

Water samples from both the influent (AMD) and the effluent were collected and analyzed weekly. These analyses involved measuring the oxidation-redox potential (ORP), which were then converted to Eh relative to a standard hydrogen electrode (SHE), electrical conductivity (EC), temperature, and pH. Temperature and pH were measured using a HI 8519N pH-meter (Hanna Instruments, Woonsocket, RI, USA), after a calibration with standard solutions of pH 4.01 and 7.01 (at 25 °C). Electrical conductivity was measured using a CO 411 m (FLMETRON, Witosa, Poland), a Thermo ORION 105 (USA), and a Eutech Instruments SAL6+ meter (CAKTON, Bethesda, MD, USA), respectively. Alkalinity and acidity tests were performed in the laboratory according to the Standard Method 2310 (APHA, 2005). Samples reserved for chemical analysis (i.e., sulfates, metals, and metalloids) were stored at 4 °C. The aliquots for metal and metalloid analysis were filtered (0.45 μm) and acidified to a pH below 2 with concentrated analytical-grade $HNO_3$. The chemical concentrations of metals and metalloids were determined by inductively coupled plasma mass spectrometry (ICP-MS; Agilent Technologies 7700 Series). Sulfate content was measured by ion chromatography (IC, Thermo Fisher Scientific—Dionex™ Integrion HPIC) with a hydroxide-based eluent on a gradient flow rate of 0.23 mL $min^{-1}$, while the ion separation was achieved through an IonPac™ AS11-HC-4 μm 2 × 250 mm column and a pre-column IonPac AG11-HC-4 μm 2 × 50 mm.

### 2.3.2. Substrate Analysis

Prior to their analysis, solids collected from the CWs (after their use in the experimental remediation assay) were sealed in sterile polyethylene sample bags (Whirl-Pak®, B01196WA), and stored at a temperature < 2 °C to prevent further alteration. Samples were dried for 24 h at 60 °C and sieved to a fraction <150 µm to obtain a homogeneous sample. To meet the granulometric requirements for chemical and mineralogical analyses, a portion of each material substrate was also pulverized using a planetary mill (Fritsch).

Physicochemical parameters, namely pH and EC, were determined by intermittently mixing the solids (non-milled) with deionized water in a 1:10 (*w*/*v*) ratio for one hour [34].

The chemical analysis of the limestone (before and after the remediation treatment) was carried out in the milled samples after an acidic digestion ($HNO_3/Br_2/HF/HCl$). The chemical composition was obtained by inductively coupled plasma atomic emission spectroscopy (ICP-AES, Agilent 4200) and inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7500 CE). Elemental analyses targeted 15 elements, but only those with values above the detection limits (DL) are presented here. The objective of the analysis was to evaluate the potential precipitation of metals and metalloids in the substrate during the passive treatment.

The limestone used in the substrate and the minerals forming coatings in the rocks after the treatment were analyzed by X-ray diffraction (XRD) using a Bruker AXS Advance D8 equipped with Cu radiation and a scintillation counter, scanning with a diffraction angle (2θ) ranging from 5° to 70° at a rate of 0.005°/s. Samples were prepared using a micronizing apparatus (McCrone, DL 1% *w*/*w*) and the powder method on dried samples, pulverized, and then micronized to 10 µm in isopropanol. DiffracPlus EVA version 4.2 and TOPAS version 6 software were used for mineral identification and quantification, respectively. In addition, scanning electron microscopy (SEM) equipped with an energy dispersive X-ray spectroscopy (EDS) probe was utilized for the identification of minerals and physical structures through imaging and elemental analyses. The equipment used was a Hitachi TM3030 SEM (20 kV, 140 µA, 25 kPa pressure, and a 1% weight detection limit). Images, chemical composition, and elemental maps were recorded using an INCA (Oxford Energy 450) data processor. Chemical analyses and spatial resolution were obtained by SEM in backscattered electron (BSE) detection mode.

### 2.3.3. Metal Speciation and Fractionation

The sequential chemical extraction (SCE) method, following Tessier's protocol [35], was applied to all marsh materials to identify the association of metals and metalloids within solid phases. This method involves the progressive application of chemical reagents to selectively release elements from their host minerals [28,36]. While it does not directly identify the specific chemical forms of metals and metalloids, it classifies them into several geochemical fractions: (F1) soluble and exchangeable; (F2) carbonate-bound; (F3) associated with iron and manganese oxides and hydroxides; (F4) bound to organic matter and sulfides; (F5) present in the residual mineral fraction [33].

Fractions F1 and F2 are generally considered the most mobile and bioavailable, as they can be easily released into the environment under fluctuations in pH, redox potential (Eh), and organic matter degradation. By contrast, metals bound to Fe-Mn oxides (F3), organic matter (F4), and particularly the residual fraction (F5) are less available in the short term. However, under specific environmental conditions, these fractions may undergo progressive release, contributing to potential remobilization of contaminants [37].

This method is therefore crucial for assessing the potential risk of metal and metalloid remobilization and their bioavailability, which may influence their uptake by plants and microorganisms in the context of contaminated water treatment [35,38].

#### 2.3.4. Plant Analysis

After 5 months of the experiment, plants reached maturity and were harvested for chemical analysis. Samples were separated into aerial (stems) and root parts, then dried, ground and homogenized into a fine powder. One gram of the powdered sample was digested using a triacid mixture ($HNO_3$, $HClO_4$, and $H_2SO_4$; 5:1:1) at 80 °C. Quantification of trace elements in stems and roots was performed according to the method described [35].

The translocation factor (TF), which evaluates the ability of the plant to translocate metals and metalloids from the underground parts to the aerial parts, was calculated as the ratio between the concentration of metals and metalloids in the stems ($C_S$) and in the roots ($C_R$), as given by Equation (1) [19]:

$$TF = \frac{C_S}{C_R} \tag{1}$$

A TF greater than 1 indicates the ability of a metal or metalloid to be translocated to the aerial parts, while a TF lower than 1 indicates that this is mainly retained in the roots.

#### 2.3.5. Statistics

The open-source software R version 4.3.3 was employed to perform a statistical analysis of the chemical results obtained. After the detection and removal of outliers, the efficiencies of the different CWs were compared by applying an ANOVA test. Since it is a parametric test, normality (Shapiro–Wilk test) and homoscedasticity (Bartlett test) of the data was checked. The non-parametric Kruskal–Wallis test was used when these assumptions were not met. A 95% confidence level was adopted. Thus, differences between the group means were considered significant if *p*-values were below 0.05.

Removals (%) were determined through Equation (2).

$$\text{Removal (\%)} = \left(\frac{C_i - C_e}{C_i}\right) \times 100 \tag{2}$$

where $C_i$ is the concentration of the metal or metalloid in the influent and $C_e$ is the concentration of that trace element in the effluent.

## 3. Results and Discussion

### *3.1. Time Evolution of Physicochemical Parameters*

Table 1 presents the values of the physicochemical parameters and the chemical concentrations of the main metals, metalloids, and sulfates of the AMD collected from the Ouixane mining site, which was used as the influent in the CW assays. The AMD sample is characterized by high EC values (21 mS $cm^{-1}$), low pH (3.57), an Eh of 493 mV (indicating oxidizing conditions), and high acidity levels (4875 mg $CaCO_3$ $L^{-1}$), while the alkalinity was not determined since the pH is below 4.5 and no alkalinity species of carbonate exists under this acidic condition. This sample evidenced high concentrations of metals and metalloids, namely Al, Fe, Mn, As, Cd, Cr, Cu, and Pb, as well as sulfates, which surpass the standard Moroccan reference levels for drinking waters by several orders of magnitude (Table 1).

The effluents resulted from the AMD lab CW treatment showed a significant increase of pH to 8.53 (PCW) and 8.47 (CCW) (Figure 2a), which was mainly due to the neutralizing action of calcite present in both filtration systems. In addition, several studies have also reported the positive influence of wetland plants on the increased pH of metal-rich wastewater, likely due to the release of organic acids and exudates from plant roots [19,39,40]. Neutralizing measures are not only important to reduce negative effects caused by high acidity, but because alkaline pH promotes the precipitation of divalent metals [38], and in the adsorption of metals onto solid surfaces [20]. Statistical analysis revealed no significant difference between the pH of the PCW and CCW effluents ($p > 0.05$). This indicates that, during the initial phase of the CW operation, the main process contributing to the increase in pH is probably the dissolution of carbonates. Subsequently, the precipitation of iron hydroxides on the surface of carbonates makes the role of organic matter, including the formation of organic acids (such as humic, formic, and oxalic acids) and microbial processes, more relevant. However, the production of alkalinity through sulfate reduction is the primary mechanism governing long-term operations [39]. In fact, the reduction of Fe-hydroxides or sulfate in the presence of bacteria are important in promoting pH increase [40].

**Table 1.** Physicochemical parameters and chemical composition of acid mine drainage of the Ouixane mine and the Moroccan standards.

| Parameters | Units | AMD | Moroccan Standards |
|---|---|---|---|
| pH | - | 3.57 | <8.5 |
| Electrical conductivity (EC) | mS cm$^{-1}$ | 21 | 0.00643 |
| Redox potential (Eh) | mV | 483 | - |
| Acidity | mg CaCO$_3$ L$^{-1}$ | 4875 | 200 |
| Alkalinity | mg CaCO$_3$ L$^{-1}$ | n.d | 30 |
| Al | mg L$^{-1}$ | 142 | 0.2 |
| Fe | mg L$^{-1}$ | 91 | 0.3 |
| Mn | mg L$^{-1}$ | 40 | 0.1 |
| As | mg L$^{-1}$ | 0.0439 | 0.01 |
| Cd | mg L$^{-1}$ | 11.82 | 0.003 |
| Cr | mg L$^{-1}$ | 70 | 0.05 |
| Cu | mg L$^{-1}$ | 2.64 | 0.001 |
| Pb | mg L$^{-1}$ | 4.59 | 0.01 |
| Zn | mg L$^{-1}$ | 3.41 | 3 |
| SO$_4$$^{2-}$ | mg L$^{-1}$ | 52,120 | 400 |

The EC increased during treatment of the effluents, with final values of 38.2 mS cm$^{-1}$ and reported for the CCW and PCW, respectively (Figure 2b). Higher electrical conductivity (EC) of the effluents during the first weeks of system operation can be explained by the dissolution of materials present in the substrate, especially carbonates, which release $Ca^{2+}$ and $HCO_3^-$ ions into the solution. This phenomenon is particularly marked at the beginning of the treatment, when the substrate is still in interaction with the polluted water. Although the mineralization of organic matter contained in the peat can also contribute to the release of ions, this process seems to have a less significant impact on the increase of EC compared to the dissolution of carbonates [41].

During the initial phase of the operation, the organic substrate provided a continuous carbon source, which is crucial when the appropriate conditions for sulfate-reducing bacteria (SRB) are established (i.e., pH > 5, Eh < −50 mV) [42]. In this study, the recorded redox potential values were not representative of a reducing environment, with positive measurements ranging from 36.5 to 73 mV across the wetlands (Figure 2c). The speciation of metals in acidic waters is strongly influenced by pH and redox potential [43]. Metals such as Fe, Al, and Zn precipitate as hydroxides when acidity is neutralized, which are also important for the immobilization of other metals and metalloids in the bottom materials of the constructed wetlands. The solubility of such trace elements is low at alkaline pH (pH = 8.0), higher at slightly acidic pH (pH = 5.0), and increases significantly at pH levels below 3.3 [44]. At pH < 3 the hydrolysis of ferric iron is slow, while at pH < 5 bacterial activity and plant performance are limited. This parameter also plays a crucial role in the formation of complexes of metals and metalloids with organic matter, although other factors, such as the type of organic matter, also influence this process. Additionally, a decrease in redox potential enhances the solubility of metals and metalloids [45].

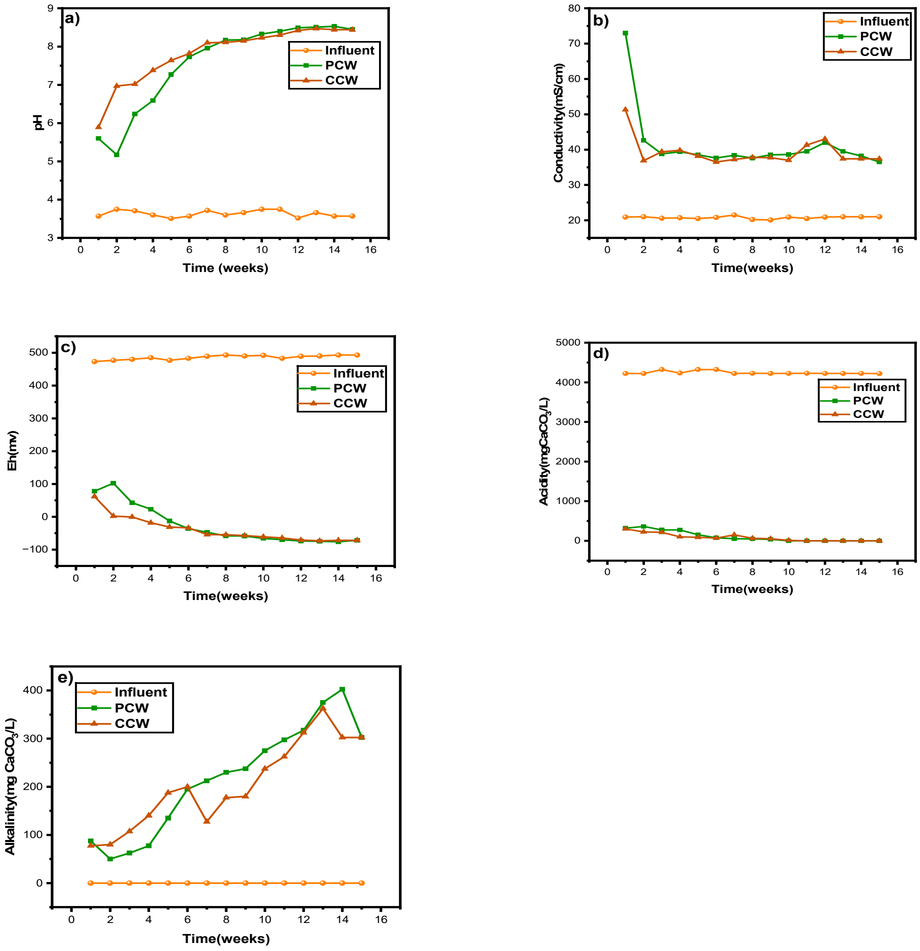

**Figure 2.** (**a**–**e**) Time evolution of pH, EC, Eh, acidity, and alkalinity in the constructed wetlands with the planted PCW and the non-planted CCW.

### *3.2. Metal, Metalloid, and Sulfate Removal*

The chemical results of the metal, metalloid, and sulfate concentrations obtained in the effluents of the PCW and CCW treatment systems are presented in Figure 3. In both the PCW and CCW systems the concentrations of Fe, Al, Cu, As, Cd, Cr, Zn, and Pb were below the detection limit of the equipment, while the other metals are in concentrations below the threshold levels set by the Moroccan government. Thus, these results demonstrate that the CCW and PCW systems were effective in the removal of metals and metalloids from the AMD, with exception of Mn, which shows increasing concentrations over time and above the AMD initial concentration (Figure 3).

The concentration of Fe changed from 97 mg $L^{-1}$ in the AMD to an average of 0.06 mg $L^{-1}$ in the PCW effluent and 0.07 mg $L^{-1}$ in the CCW effluent (Figure 3b), which represents a reduction of about 1617 and 1386 times, respectively. Similarly, the results obtained for Zn show that the CCW and PCW systems had the capacity to retain this metal. The average Zn concentration measured after treatment was 0.40 and 0.146 mg $L^{-1}$ for the experimental cells with and without *Juncus effusus*, respectively, compared to an average inflow value of 3.41 mg $L^{-1}$ (Figure 3i). Minimum concentrations of Zn 0.109 and 0.057 mg $L^{-1}$ and maximum concentrations of 0.929 and 1.28 mg $L^{-1}$ were measured at the outlet of the wetlands, with and without plants. The strong retention of Fe and Zn in the wetlands is likely favored by an increase in pH (>7), as well as by the redox potential of the surrounding environment [46]. According to [47] the redox potential varies significantly from one system to another, even within a single wetland. For example, the aerobic layer at the soil–water interface is created by a low diffusion of oxygen, which promotes the oxidation of metals, while the underlying layers, which are lower in oxygen, may allow the reduction of metals, such as $Fe^{3+}$.

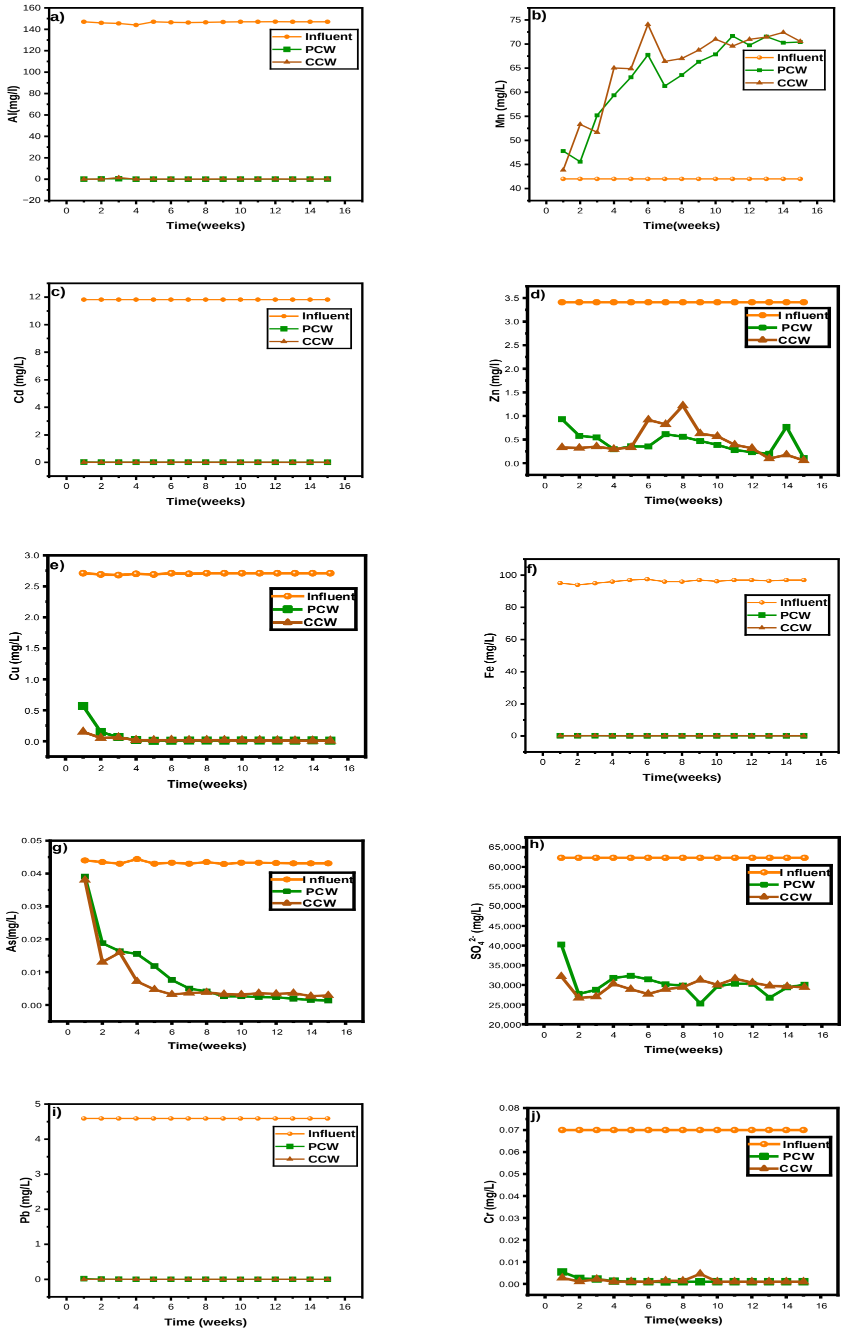

**Figure 3.** (**a**–**j**) Time evolution of the Fe, Cu, As, Cd, Cr, Zn, Pb, Mn, and sulfate concentrations in the effluents of the PCW and CCW systems.

Statistical analysis showed a significant difference ($p < 0.05$) between the overall metal removal efficiencies of the CCW and PCW systems, except for Mn, for which values vary from highly variable to negligible among the two systems (Figure 3c). It is noteworthy that Mn is among the most difficult metals to remove due to the complexity of the interactions governing its solubility [20]. With a $p$-value lower than 0.05, a confidence interval of 95%

is assumed. Thus, as previously mentioned, the average concentrations of Mn increase from the influent (42 mg $L^{-1}$) to minimum and maximum values of 44–46 mg $L^{-1}$ and 72–74 mg $L^{-1}$ in the planted and unplanted wetlands, respectively. Although, Mn concentrations were significantly increased ($p = 4.6 \times 10^{-12}$) in the CWs, there were no significant differences between both reactors. The negative removal or leaching of Mn may result from the release of weakly adsorbed $Mn^{2+}$ ions from the substrate material, as the precipitation of this metal as oxyhydroxide requires a pH > 8. Under anaerobic/anoxic conditions, $Mn^{2+}$ remains in a soluble reduced state, making its removal particularly challenging [48]. Reductive dissolution of manganese oxides has also been reported as an additional cause for the release of Mn in cases where the concentration of $Fe^{2+}$ is relatively higher than that of Mn, i.e., Fe/Mn > 4 [18,20].

The process of Al removal primarily occurs in the neutral pH range through abiotic pathways, such as hydrolysis or oxidation, to more stable forms, which would be less soluble at pH > 7.0 [49]. Copper, Cd, Pb, and Cr were present at lower concentrations and coprecipitation with Fe and Mn oxyhydroxides could be a major removal mechanism and could even form insoluble sulfides under anaerobic conditions [50].

Sulfate is one of the main pollutants in mining wastewater. Its removal primarily occurs through dissimilatory sulfate reduction and the heterotrophic oxidation of organic matter [48]. To support this mechanism, sulfate concentrations in both the influent and the effluent were analyzed using ion chromatography. The results indicate that sulfate concentration is higher in the influent than in the effluent (Figure 3j), suggesting significant sulfate reduction. In the planted subsurface wetlands, the minimum and maximum values (2528.45–40,284.45 mg $L^{-1}$) were lower than in the unplanted systems (26,711.17–32,151.5 mg $L^{-1}$), although these values remain above the threshold levels set by the Moroccan government.

In a nutshell, the removal of metals and metalloids significantly follows a general efficiency trend Fe > Cd > Al > Cu > Pb > Cr > As > Zn > $SO_4{}^{2-}$ > Mn, which depends on their ionic forms, the substrate type, and the plants colonizing the wetland. Maintaining a circumneutral pH in the remediation wetlands promotes the precipitation of metals and metalloids, reducing their bioavailability, toxicity, and transfer to other ecosystems. Furthermore, the removal of contaminants is greatly affected by seasonal (temperature and precipitation) changes [51], as well as by the fluctuating pollutant load over time, which inevitably influences the short-term performance of the wetland. However, these conditions were not assessed in this lab systems. In the long term, there may be changes in retention capacity due to substrate accumulation and the development of preferential pathways that affect the system's ability to retain contaminants [52].

### *3.3. Metal and Metalloid Accumulation in the Solid Media*

The mineralogy of limestone used as a substrate in the CWs assays show that the sample is composed mainly of calcite, while the coatings precipitated in limestone after the remediation assays are mainly composed of gypsum and Al- and Ca-phosphates (Figure 4). In fact, limestone is generally recommended as a substrate for wetlands because of its ability to neutralize acidity and improve pH levels, rather than for its heavy metal content [53].

Higher concentrations of Fe, Al, As, Mn, and P, followed by Zn, Ni, and Pb, were found in the limestone coatings after the assays (Table 2). The results revealed that more than 90% of the total mass of the metals and metalloids removed was retained in the wetland itself. This indicates that the medium is the main reservoir for the retention of metals, potentially in the form of metal sulfides or associated with carbonates.

The SEM-EDS analysis of the limestone used in the CW as a substrate showed that particles are mainly composed of calcite, as previously confirmed by XRD (Figure 5). On the other hand, after the remediation assays, it is clear that gypsum is the main mineral phase present in both the CCW and PCW limestone affected substrate (Figure 5), due to dissolution of calcite that release Ca to the sulfate rich AMD solution. The result is the precipitation of gypsum.

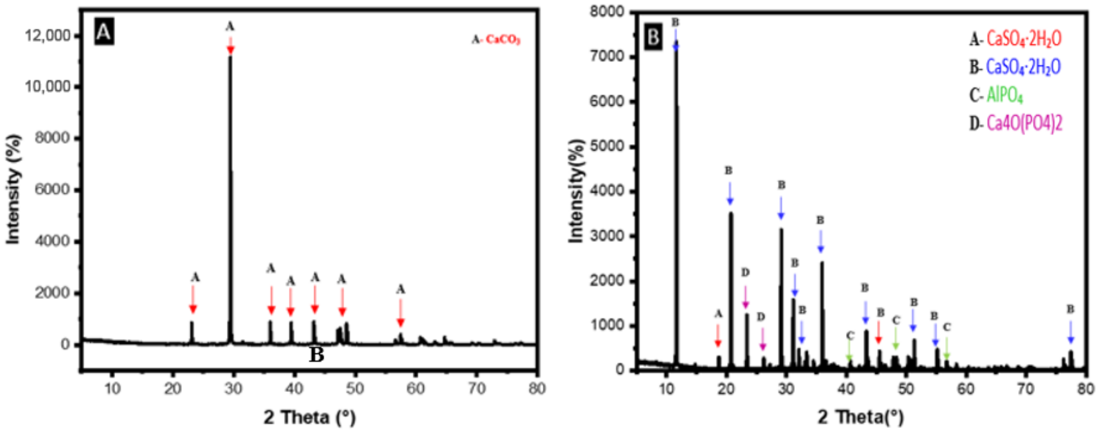

**Figure 4.** The X-ray powder diffraction pattern of (**A**) pure limestone and (**B**) limestone coatings after the CW assays.

**Table 2.** Accumulation of metals and metalloids in limestone pure vegetated end non-vegetated in the CWs.

| Sample | Al | Fe | Mn | As | Ni | Pb | P | Cd | Cr | Cu | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | mg kg$^{-1}$ | | | | | | | | | | |
| Pure limestone | 66.5 | 3.5 | 1.50 | 13 | 0.54 | 0.09 | 177.9 | 7.50 | 1.45 | 0.21 | 1.02 |
| Limestone vegetated | 90 | 13.5 | 24.87 | 19 | 2.75 | 0.10 | 4777 | 5 | 1.12 | 1.95 | 24.03 |
| Limestone non-vegetated | 110.5 | 20.0 | 11.02 | 18.5 | 1.42 | 0.13 | 1816 | 8.5 | 1.86 | 0.70 | 7.59 |

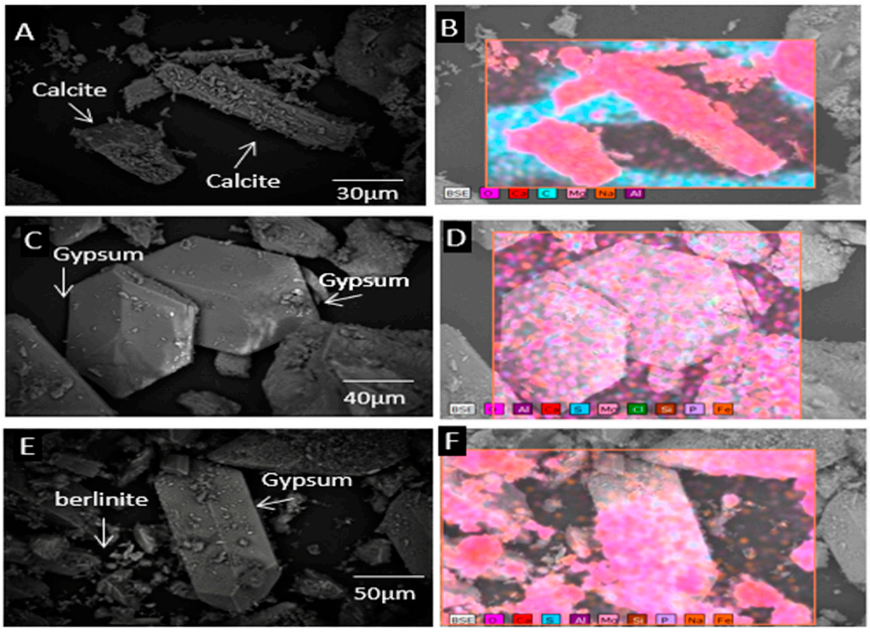

**Figure 5.** SEM images and EDS analysis of limestone substrate samples before the assays (**A**,**B**), and from the non-vegetated limestone cell (**C**,**D**) and the vegetated limestone cell (**E**,**F**) after the remediation assays.

### 3.4. Metal and Metalloid Partitioning

Sequential extraction allows for accurate estimation of the geochemical partitioning of metals and metalloids in the substrate, indicating their potential bioavailability. Therefore, it is considered to be a crucial method for assessing the environmental impact of human activities such as mining [54].

The results of the chemical selective extractions (Table 3) show that the exchangeable fraction, which can be easily released (bioavailable fraction), accounts for about 48% of the studied metals and metalloids, while the amounts released as carbonates are close to 38%. Residual fractions constituted the primary source of metals and metalloids (54%), while those associated with Fe-Mn oxides are present at concentrations (82%). Nevertheless, the main support phases of each metal or metalloid vary considerably: Cr exhibits particularly low concentrations in the carbonate-bound fraction compared to other fractions, whereas Zn is primarily associated with the residual fraction. Both the CCW and the PCW exhibit high concentrations of iron and arsenic in Fe-Mn oxides (507 mg kg$^{-1}$ and 189.50 mg kg$^{-1}$, respectively), although low amounts are associated with sulfides and organic matter. Manganese is primarily associated with carbonates but is also partially bound to Fe-Mn oxides, while Ni predominantly occurs in the form of carbonates. Data confirm the elevated concentration of iron in Fe-Mn oxides and the residual fraction, while low proportions of zinc and manganese were found in organic matter and sulfides. Nickel, Pb, Zn, Cr, and Mn showed significantly higher concentrations in the residual fraction, representing more than 50% of the total metal concentration.

**Table 3.** Concentrations of metals and metalloids obtained in the sequential chemical extraction solutions of substrate samples collected from vegetated and non-vegetated artificial wetlands.

| Sample | As | Fe | Cd | Cr | Cu | Mn | Ni | Pb | Zn |
|---|---|---|---|---|---|---|---|---|---|
| Unit | mg kg$^{-1}$ | | | | | | | | |
| PCW sample | | | | | | | | | |
| F1 | 63.00 | 9.50 | 0.03 | 0.41 | 0.06 | 3.10 | 0.10 | 0.03 | 0.27 |
| F2 | 75.50 | 18.00 | 0.03 | 0.05 | 0.08 | 3.92 | 0.31 | 0.05 | 2.08 |
| F3 | 189.50 | 507.00 | 0.03 | 0.07 | 0.09 | 1.83 | 0.36 | 0.08 | 4.00 |
| F4 | 146.00 | 266.50 | 0.03 | 0.14 | 1.46 | 0.18 | 0.37 | 0.10 | 0.82 |
| F5 | 25.50 | 55.50 | 0.16 | 2.05 | 0.88 | 16.19 | 1.39 | 8.00 | 7.48 |
| CCW sample | | | | | | | | | |
| F1 | 76.00 | 30.00 | 0.03 | 0.35 | 0.06 | 1.20 | 0.06 | 0.05 | 0.12 |
| F2 | 79.00 | 22.00 | 0.03 | 0.04 | 0.07 | 0.66 | 0.09 | 0.05 | 0.20 |
| F3 | 163.50 | 435.00 | 0.03 | 0.06 | 0.05 | 0.89 | 0.14 | 0.05 | 0.37 |
| F4 | 199.50 | 348.50 | 0.03 | 0.10 | 0.69 | 0.12 | 0.17 | 0.05 | 0.33 |
| F5 | 31.50 | 33.50 | 0.38 | 1.80 | 7.94 | 150.35 | 10.26 | 5.50 | 90.20 |

Notes: F1: Exchangeable fraction. F2: Fraction associated with carbonates. F3: Reducible fraction (fraction associated with Fe-Mn oxides). F4: Oxidizable fraction (fraction associated with organic matter). F5: Residual fraction.

### 3.5. Plant Analysis

As shown in Table 4, plants in the limestone cell exhibited higher concentrations of Al, Cr, Pb, and Cd, in both shoots and roots, while the concentration of As, Fe, Cr, P, Mn, and Zn was higher in the roots. The concentration of As, Fe, Cr, Mn, and Zn in the shoots tended to decrease from the inlet to the outlet in both types of cells.

**Table 4.** Concentration of metals and metalloids in plant roots and shoots in the PCW cells, and the respective translocation factors (TF).

| *Juncus effusus* | Al | Fe | Mn | As | Cd | Cr | Pb | Cu | Ni | P | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | mg kg$^{-1}$ | | | | | | | | | | |
| Roots | 152.5 | 85.5 | 12.695 | 16.5 | 7 | 2.34 | 0.11085 | 1.35 | 1.04 | 45.11 | 7.76 |
| Shoots | 332 | 50.5 | 8.455 | 10.5 | 9 | 7.22 | 1.2065 | 1.48 | 1.40 | 42.6 | 6.09 |
| TF | 2.17 | 0.6 | 0.66 | 0.63 | 1.28 | 3.08 | 10.8 | 1.10 | 1.35 | 1.05 | 0.78 |

The TF was used to assess the accumulation and translocation of metals and metalloids from roots to shoots. Translocation factor values greater than 1 were determined for Al, Cr, Ni, Cu, P, Pb, and Cd, suggesting that these metals are more easily accumulated by shoots and the potential of these plants for phytoremediation, namely phytoextraction. By contrast, a TF < 1 was found for As, Fe, Mn, and Zn, indicating that these metals and metalloids are mainly phytostabilized in roots.

Several studies have shown that the substrate is the primary reservoir for metals and metalloids in constructed wetlands (CWs) [55]. When effective alternative substrates are used, the role of plants becomes even more significant; conversely, when conventional substrates (e.g., gravel) are employed, plant absorption may become more critical for overall removal of metals and metalloids [56]. Limited vegetation cover can also lead to increased absorption by plants. *Juncus effusus* has been widely utilized in CWs, not only for its accumulation capabilities, but for its resistance to acidic conditions and high metal concentrations [57]. Some authors have reported that metal and metalloid immobilization is higher in vegetated systems [58], while others suggested that plants might increase their mobility, which could have a detrimental effect [59]. Anaerobic processes are believed to occur in areas far from the rhizomes, while aerobic processes are thought to take place near the rhizomes [19].

## 4. Conclusions

Constructed wetlands are a cost-effective and applicable method for treating AMD. A vertical subsurface flow constructed wetland system, using limestone as the primary substrate and *J. effusus* as vegetation (PCW), alongside a non-vegetated system (CCW), successfully demonstrated that these wetland systems were effective in raising the pH of the AMD (from <2.5 to approximately 8.42), and removing metals and metalloids, except for manganese. The negative removal of Mn indicated the inefficiency of the constructed wetlands (CW) due to the anoxic conditions present in the system, which may also be attributed to excessive leaching of adsorbed manganese from the depleted wetland bed. Microbial sulfate reduction using organic substrates as a carbon source proved to be highly effective for sulfate removal (53%).

Both CW systems showed an excellent metal and metalloid removal capacity and a moderate sulfate removal capacity in the following order: Fe > Cd > Al > Cu > Pb > Cr > As > Zn > $SO_4^{2-}$ > Mn in limestone cells. However, vegetated cells did not show significant differences compared to non-vegetated cells in terms of pH. Therefore, the impact of vegetation on treatment performance was found to be dependent on several factors, including the duration of the experiment, the type of substrate used, water quality parameters, and the targeted metals and metalloids. Notwithstanding, a greater accumulation of metals and metalloids was observed in plant tissues. The neutralizing agent used, in this case calcite, had a predominant influence on the overall neutralization capacity of the system, the latter remaining high throughout the experimental period.

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