

## Article

# The Treatment of Acid Mine Drainage Using Vertically Flowing Wetland: Insights into the Fate of Chemical Species

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**Abstract:** In this study, the treatment of acid mine drainage (AMD) using vertically flowing wetland was explored. The wetland was enriched with *Vetiveria zizanioides* as a decontaminating media and soil as the substrate. Water was percolated through the substrate and the throughput samples were collected and characterized every five days for a period of 30 days. The obtained results revealed a tolerant index of 1.03 for *Vetiveria zizanioides*, and a net reduction of metals and sulfate. The removal efficacy of chemical species was observed to obey the following order: Fe (71.25%) > Zn (70.40%) > Mn (62%) > Al (56.68%) > SO<sub>4</sub><sup>2-</sup> (55.18%) > Ni (35%) > Cu (18.83%). The removal of chemical species was further aided by the used substrate, and this could be attributed to the accumulation of chemical species on the soil through precipitation, adsorption, and phyto-retention. As such, it could be deduced that the substrate plays a significant role in the removal of metals, while the grass and external factors accounted for the rest of the chemical species attenuation. The translocation assessment revealed that the distribution of chemical species was observed to be predominant in the roots, except manganese, which was transferred in the shoot (67%). The XRF, XRD, FTIR, and SEM-EDS analysis revealed the presence of AMD chemical species in the substrate and the grass components, hence confirming that the plants are playing a huge role in the removal of contaminants from AMD. The PHREDOX EQUILIBRIUM (in C language) (PHREEQC) geochemical model confirm that metals existed as di-and-trivalent complexes in AMD. Lastly, available metals were precipitated as metals hydroxides and oxy-hydrosulfates by the substrate. In light of the obtained results, vertically flowing wetland could be used for the passive treatment of AMD, and it will play a huge role in active and abandoned mines. However, prolonged assessment should be undertaken to understand its performance over a notable period of time.

**Keywords:** acid mine drainage; vertically flowing wetland; treatment; phytoremediation; *Vetiveria zizanioides*



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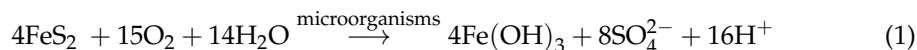


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## 1. Introduction

Over many decades, the mining of gold and coal has played an indispensable role in the economy of any given country. The latter has been widely used for its high colorific value for power generation, while gold has been used for jewelry [1]. Despite their enormous socio-economic benefits, mining has been associated with numerous environmental problems, such as land destruction and the generation of acid mine drainage (AMD), among others [2,3]. Due to the nature and magnitude of its ecological impacts, AMD has been a topical issue that has been troubling the mining industry over the years [4,5]. In particular, during the mining of coal and gold, the associated sulfide-bearing minerals such as pyrite and arsenopyrite which are embedded in the surrounding geology and stratas get

exposed to oxidizing conditions [1,6,7] During rainfall and underground leakages, sulfide minerals come into contact with water and oxygen, leading to the formation of highly acidic mine effluent known as acid mine drainage (AMD) [8]. With the use of pyrite as an example, the formation of acid mine drainage (AMD) could be explained by the following equation (Equation (1)) [1]:



This reaction is also mediated by microorganisms [9]. The acidity in AMD promotes the leaching of heavy metals and metalloids from the surrounding geology [10–12]. According to the literature, AMD comprises Al, Fe, Mn, and  $\text{SO}_4$  as major elements, and traces of Cu, Zn, Ni, Pb, Cr, As, and radionuclides, among others [1,5,13,14]. These chemical species exceed the recommended limits for discharge as stipulated by the World Health Organization (WHO) [15]. As such, the discharge of AMD may lead to the degradation of receiving ecological systems and compartments [5], hence affecting the integrity of the ecosystem and its ability to support life [3,6,8]. According to ecotoxicological and epidemiological reports, constituents embodied in AMD can pose various carcinogenic, mutagenic, and teratogenic conditions to living organisms on exposure [1,3,16,17]. As such, stringent regulatory frameworks require AMD effluent to be treated before it can be discharged into different receiving environments [18,19].

In light of the above, various technologies have been developed for the treatment of AMD, which include both active and passive treatment systems [20,21]. Specifically, active treatment technologies include ion exchange [22], adsorption [23], filtration [24,25], neutralization [15,26], and crystallization [27], whereas passive treatment methods include constructed wetlands [28], phytoremediation [29–31] bio-sorption [32], permeable reactive barriers (PRB) [33], sulfate-reducing bioreactors [34], anoxic limestone drains [35], and slag leach bed [36]. These technologies have their advantages and disadvantages; the active treatment technologies require energy and frequent chemical inputs, whereas passive treatment systems are the opposite. Furthermore, active treatment technologies are not cost effective and require constant maintenance and skilled staff among others; furthermore, they are grossly deemed not environmentally friendly since they generate a considerable volume of sludge, which in turn leads to a new environmental problem to be solved due to secondary pollution from the highly mineralized and heterogeneous sludge [24,34,37]. Therefore, they cannot be considered a long-term solution for AMD treatment. Following the persisting environmental impacts of AMD and the limitation of existing technologies, there is a need to investigate, develop, and implement an environmentally friendly treatment technology for the removal of contaminants from AMD. Phytoremediation has been reported as the most eco-friendly way of attenuating contaminants from different spheres of the environment, and it plays a crucial role in the reduction of heavy metals and sulfates from AMD [30,38]. Phytoremediation is an in situ intervention and there is no need for transportation and off-site processing cost except for floral plantations. In addition, the phytoremediation process prevents metals from leaching due to high metals being retained intensively post extraction. The metals will then be stored in the plant biomass or released in volatile form into the atmosphere through a process known as phyto-volatilization. To this end, pollutants are removed by a wetland via (i) phyto-extraction by the plants roots [39], (ii) phyto-stimulation, which is the breaking down of organics contaminants in plant root zone by microbial activity [40], (iii) phyto-degradation, which is the metabolization of contaminants in plant tissue to less toxic level [41], and lastly, (iv) the phyto-stabilization process, which basically entails the de-mobilization of heavy metals in soil by plant roots [42].

Noting that plants play an important role in the bioremediation, the selection of plant species to be used is crucial since they will improve the overall pollutants removal process considering that metals accumulated by plants are stored in their biomass or released as volatile form into the atmosphere through the phyto-volatilization process. Various studies

have revealed that *Vetiveria zizanioides* (VZ) is tolerant to very harsh environments, such as the acidic and metalliferous conditions in AMD. Furthermore, it grows well in aquatic and terrestrial environments [31,43], is tolerant to salinity conditions, grows well in all seasons, and is tolerant to heavy metals and others pollutants. However, its use with the substrate and on a vertically flowing modality has merely been considered, specifically considering AMD. The vertical flow allows for maximum reaction and interaction between the substrate and plants so as to enhance the attenuation of chemical constituents in relation to horizontal flows. To the best of our knowledge, the subsurface vertical flow constructed wetlands (SSVF-CWs) has never been explored for AMD treatment using VZ with the substrate. Engineering-wise, the SSVF-CW is the type of wetland where water is poured onto a surface and percolates through the soil vertically to the bottom of the cell, where it is drained horizontally to the outlet, where it is collected and characterized. Furthermore, SSVF-CWs are characterized by an abundance of oxygen and substrate pores which are intermittently filled and drained with water, thereby creating an aerobic environment for metals precipitation, facilitation of certain biochemical reactions, and aerobic decomposition [44,45]. In light of the above, SSVF-CWs have demonstrated their abilities to remove pollutants and other contaminants from waste water and are, therefore, considered as viable low-cost replacements of traditional methods which are expensive, require skilled personal, and generate waste that requires additional treatment [45,46]. The ultimate goal of this study is to highlight the accumulation and translocation of chemical species from contaminated water to the plants. The impact of the used substrate will also be evaluated.

## 2. Materials and Methods

### 2.1. Acquisition of Reagents, Substrate, and Plants

All reagents used in this study were of analytical grade (AG) and were obtained from Merck, South Africa. Prior to the construction of subsurface vertical flow constructed wetland (SSVF-CW), shoots of *Vetiveria zizanioides* were purchased from the NANDADRAM ECOVILLAGE farm in Kwa-Zulu Natal, South Africa and transported to the University of South Africa's sciences campus in Florida, Johannesburg. Once on campus, the shoots of *Vetiveria zizanioides* were submerged under potable fresh water for two weeks in order to allow the new seeds to germinate. After that, seedlings of *Vetiveria zizanioides* were transferred into small pots containing organic matter used as wetland substrate (soil purchased from garden world Johannesburg, RSA) as growing medium. Plants were allowed to grow naturally without the addition of nutrients and were monitored and watered daily.

### 2.2. Sampling and Characterization of AMD Water

Acid mine drainage (AMD) water used in this study was collected at the Gold mine in Krugersdorp, Gauteng, South Africa (Latitude: 26.0963 and Longitude: 27.7752). During AMD collection, some parameters of AMD water were determined on site using a multi-parameter meter (HANNA instruments, Johannesburg, RSA). These included pH, total dissolved solids (TDS), and Electrical conductivity (EC). Initial concentration of metals (Al, Cu, Fe, Mn, Ni, and Zn) in AMD was analyzed using inductively coupled plasma optical emission spectrometry (ICP-OES), 5110 ICP-OES vertical dual view, Agilent technologies Australia, made in Malaysia. The ICP-OES was coupled with Agilent SPS 4 Auto sampler, whereas the  $\text{SO}_4^{2-}$  concentration was analyzed using an Ion chromatography (IC) (850 professional IC Metrohm, Herisau, Switzerland). All the parameters were analyzed following the "Standard Methods of the Examination of Water and Wastewater" [47].

### Quality Assurance and Quality Control (QA/QC)

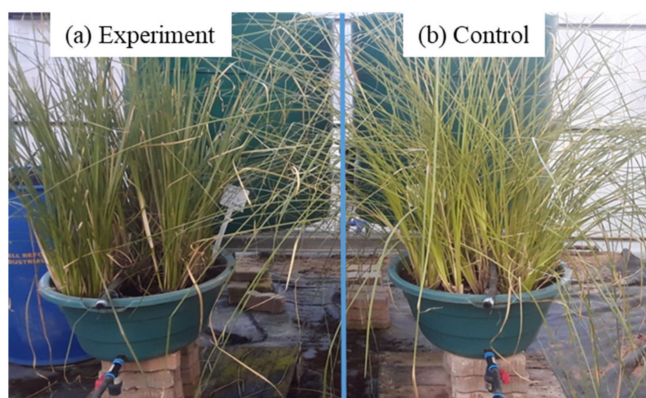
A QA/QC process was applied in this study to ensure the collection and production of trustworthy results. The QA/QC process embraced the collection and preservation of the samples. Analysis was done in triplicates, and data reported as mean values was considered

acceptable when the difference within triplicate samples was less than 10%. The value below detection limit (BDL) was in line with EPA guidelines for analyzing data [48], while the accuracy of the analysis was monitored using the National Institute of Standards and Technology (NIST) water standards. The water-Q4 database was utilized. The plant substrate was modeled using PH REdox EQUilibrium (in C language) (PHREEQC). The water potential precipitation of metals was determined using saturation indexes (SI), in which  $SI \leq 1$  denotes under-saturation,  $SI \approx 1$  denotes saturation, and  $SI \geq 1$  denotes super-saturation.

### 2.3. Experimental Setup

#### 2.3.1. Vertically Flowing Wetland Design and Description

The pilot plants consisted of the following: (a) one reservoir tank to contain AMD and (b) another reservoir tank to contain fresh water for control. Two plastic basins with a capacity of 100 L were used, each measuring 100 cm L  $\times$  60 cm W  $\times$  50 cm H, for treatment and control (Figure 1). The new germinated seedlings of Vetiver, described in Section 2.1, were transplanted into the two basins containing soil as substrate to have two wetlands (one for experiment and one for control). The wetlands were continuous flow systems, where water was flowing continuously for the duration of the experiment. The wetlands were connected to the tank via a perforated conduit pipe and contained a total of 20 plants planted at equidistance to each other. A control valve was incorporated at the baseline of each tank to regulate the flow rate of water from tank to the wetland cell. The outlet structure of each wetland cell was an orifice with a valve to collect leachate for the analysis. Preventive maintenance measures, such as trash and debris removal and the elimination of mosquito breeding habitats, was applied in a routine basis to maintain the operation and safe function of the wetland, while corrective maintenance measures were applied in a non-routine basis, i.e., when needed.



**Figure 1.** Experimental set-up of the SSVF-CW. (a) experiment and (b) control.

#### 2.3.2. Pre-Treatment of AMD Water and Chemical Composition of the Substrate

Prior to the treatment, AMD water was filtered to remove total suspended solid (TSS) susceptible to clog the pipe, while the chemical composition of the substrate before and after contact with water (AMD) was done using X-ray fluorescence (XRF) and X-ray diffraction (XRD). The analyses were performed using a Thermo Fisher ARL-9400 XP+ Sequential XRF with winXRF Software. Mineralogical composition was ascertained using X-ray diffraction (XRD). The system was calibrated using a drip plastic bottle, and fed into the system as continuous drops. The average flow per unit time was calculated using the Darcy's law Equation (2):

$$Q = \frac{Q_{in} + Q_{out}}{2} = K A S \quad (2)$$

where  $Q$  is the average flow per unit time,  $K$  is the hydraulic conductivity of a unit area in the medium perpendicular to the flow direction ( $m^3/d$ ),  $A$  is the total cross-section area

perpendicular to the flow ( $m^2$ ), and  $S$  is the hydraulic gradient of the water surface in the flow system ( $m/m$ ).

Once the average flow rate (3 L/day) was calculated ( $Q$ ), the results were used to determine the hydraulic retention time (HRT), as illustrated in Equation (3) [49], taking into account the sizes of the wetland cell and the porosity of substrate:

$$HRT = \frac{V \times d}{Q} \quad (3)$$

where  $V$  is the volume of the wetland cell ( $m^3$ ),  $d$  is the porosity of the wetland substrate (%), and  $Q$  is the average flow through the wetland ( $m^3/d$ ):

$$HRT = \frac{1 \times 0.6 \times 0.25 \times 0.6}{0.003}$$

The maximum determined HRT was 30 days, and during that period, the inlet flow rate and outlet flow rate were monitored daily to ensure a steady flow of water in and out of the wetland. The flow rate was maintained low (3 L/Day) and the HRT (30 days) long in order to allow the water to spend more time in the wetland cells, thereby improving the performance of the wetland, since the studies of Conn and Fiedler [50], Ewemoje et al. [51], and Piñeyro et al. [52] revealed that the performance of the wetland is improved by high hydraulic retention time (HRT). At the end of the experiment (just after 30 days), the plants were harvested from both wetlands (treatment cell and control) for the assessment of the uptake and translocation of chemical species.

### 2.3.3. Product Water and Soil Sampling

Water samples (experiment and control) were collected daily from each wetland from the first day (Day 0) of HRT to the last day (Day 30) of HRT. An analysis was performed and the average result of five-day period was considered. Water samples were collected into amber glass bottles of 500 mL. After collection, samples were divided into two sub-samples of 400 mL and 100 mL. The sub-samples of 400 mL were used for the analysis in situ of pH, TDS, and EC, while the sub-samples of 100 mL was used for the analysis of ex situ parameters, which included metals (Al, Cu, Fe, Mn, Ni, Zn) and  $SO_4^{2-}$ . Soil samples were air dried for one week followed by an analysis for metal concentration.

### 2.3.4. Analytical Methods

Product water from both wetlands (control and treatment cell) were analyzed in triplicate following standard methods. Electrical conductivity (EC), pH, and TDS were determined using Hanna HI 83200 multi-parameter and the manufacturer's manual was followed with reference to standard methods for the examination of water and wastewater [47]. Prior to the analysis of the metal concentration, water samples were filtered through a 0.22- $\mu m$  pore syringe filter membrane to remove particles and preserved by adding two drops of nitric acid ( $HNO_3$ ) concentrated to prevent ageing and immediate precipitation of metals. The filtrates were refrigerated at 4 °C prior to analysis by ICP-OES.  $SO_4^{2-}$  was determined using IC. Soils samples were air dried for one week, sieved, and one gram (1 g) was digested in a mixture of 5 mL of 55% ( $HNO_3$ ) and 2 mL 30% Hydrogen peroxide ( $H_2O_2$ ) using microwave digestion. Once digested, the samples were cooled to room temperature and the solutions were filtered through a 0.22- $\mu m$  pore syringe filter membrane and analyzed for metal concentrations by ICP-OES with a detection limit of 0.0001 mg/L and a margin of error of 10%.

## 2.4. Treatment Efficiency of Subsurface Vertical Flow Constructed Wetland (SSVF-CW)

The efficiency of SSVF-CW in AMD water treatment was determined gradually during the duration of the experiment. The parameters of concern were analyzed daily and the removal efficiency (RE) was calculated after every five-day period for the duration of the experiment (30 days). The RE was determined using Equation (4) [53,54]:

$$RE = \frac{C_i - C_f}{C_i} \times 100 \quad (4)$$

where  $C_i$  is the initial concentration of each parameter in the AMD water,  $C_f$  is the final concentration of each parameter after 30 days of retention time, and  $RE$  is the removal efficiency of each parameter after 30 days of retention time (in percentage).

### 2.5. Plant Harvesting

Plants from each wetland (treatment cell and control cell) were harvested without damaging the roots and were rinsed separately with distilled water to remove dust, soil, and mineral particles. Plants were air dried at 25 °C for one week and weighed to determine the tolerance index, as shown in Equation (5). The tolerance index (TI) according to Wilkins (1979) is the ratio between a variable measured in treated plants and that in control plants, and was calculated considering the dry plant weight. The tolerance index (TI) was calculated as the mean weight (biomass) of a dry plant grown in the AMD wetland divided by the mean weight of a dry plant grown in a control wetland [55]:

$$\text{Tolerance index (TI)} = \frac{\text{biomass of plant growing in AMD cell}}{\text{Biomass of plants growing in potable fresh water cell}} \quad (5)$$

#### 2.5.1. Digestion of Plants and Metal Analysis

The plants' biomasses were separated into two samples (shoots and roots). Each part of the plant was dried and grinded using a mortar and pestle, and 1 g was digested with 10 mL of 65%  $\text{HNO}_3$  (AR grade; BDH). The digestate samples were then filtered using a 0.22- $\mu\text{m}$  pore syringe filter membrane followed by a metal content analysis using ICP-OES. The concentration of metals in each portion of the plant allowed the researchers to determine the bio-concentration factor (BCF), translocation factor (TF), and the contribution of plant, substrate, and external factors in chemical species removal. The (BCF) of each metal was calculated, as illustrated in Equation (6), to determine the quantity of metals that moved from the wetland to the plant [56], while the TF indicates the ability of the plant to translocate metal from the roots to the aerial part of the plants [57]. The following equations were used:

$$\text{BCF} = \frac{\text{Metal concentration in plant tissue}}{\text{Final concentration of metals in AMD water}} \quad (6)$$

$$\text{TF}_x = \frac{\text{metal concentration in shoots}}{\text{metal concentration in roots}} \quad (7)$$

#### 2.5.2. Functional Group and Morphology of Vetiveria Zizanioides Roots

Functional groups of grinded grass roots were determined using a PerkinElmer Spectrum 100 Fourier Transform Infrared Spectrometer (FTIR) equipped with a PerkinElmer Precisely Universal Attenuated Total Reflectance (ATR) sampling accessory equipped with a diamond crystal, while morphological properties and spot analysis of grass roots and was determined using SEM-EDS. Grinded roots samples were mounted on carbon tape that was attached to Al ends. Samples were viewed in a JEOL 5400 LV SEM with an attached KEVEX electron detector (JEOL, Tokyo, Japan). They were viewed in low vacuum mode and the metals were detected with a Sigma EDS spectrometer.

### 2.6. Partitioning of Metals between Substrate, Plant, and External Factors

Prior to the start of the experiment, the initial concentration of metals in substrate was determined. After 30 days of the experimental period, the quantity of metal retained in the wetland ( $M_w$ ) was determined using Equation (8):

$$M_w = X - Y \quad (8)$$

where  $X$  is the initial concentration of metal in AMD water,  $Y$  is the final concentration of metal in AMD water, and  $M_w$  is the concentration of metal retained in the wetland.

The substrate's contribution ( $M_s$ ) to overall metal removal was also determined; it is the difference between the final metal concentration in the substrate and the initial metal concentration in the substrate Equation (9):

$$M = Z - T \quad (9)$$

where  $T$  is the initial concentration of metal in the substrate and  $Z$  is the final concentration of metal in the substrate.

To estimate the contribution of external factors (ExF) in the overall metal removal, the metal concentration in the substrate ( $M_s$ ) was summed with the metal concentration in plants ( $M_p$ ), and the total was compared to wetland metal ( $M_w$ ) content, as per Equation (10):

$$\text{ExF} = M_w - (M_s + M_p) \quad (10)$$

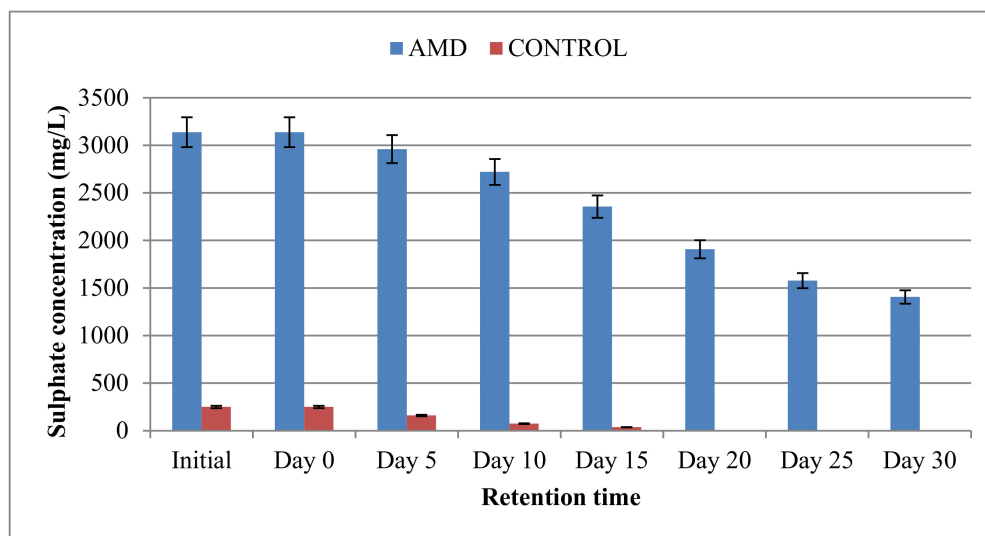
### 3. Results and Discussion

The results for this study will be categorized into the aqueous samples and solid samples. This will emphasize the findings which were acquired from different experiments and techniques.

#### 3.1. Characterization of Aqueous Samples

##### 3.1.1. Effect of SSVF-CW on the Sulfate Concentration

The effect of SSVF-CW on the removal of  $\text{SO}_4^{2-}$  from AMD is reported in Figure 2.



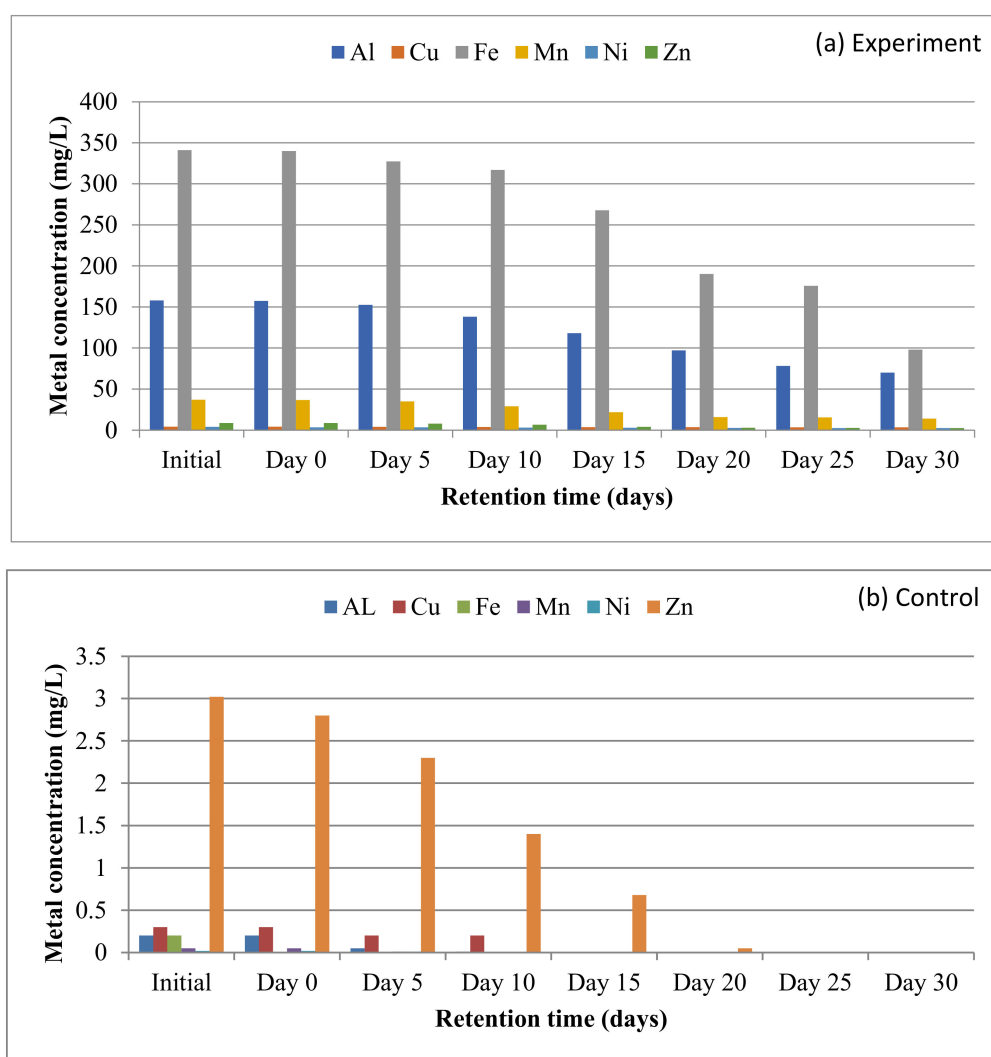
**Figure 2.** The effect of SSVF-CW on the removal of sulfate from AMD in 30 days HRT under a hydraulic loading rate of 3 L/day.

Figure 2 shows that the concentration of  $\text{SO}_4^{2-}$  decreased from 3137 mg/L on the 1st day of retention time to 1406 mg/L on the 30th day, resulting in a removal efficiency of 55.18% (Figure 2). The results, therefore, revealed that SSVF-CW with *Vetiveria zizanioides* can remove  $\text{SO}_4^{2-}$ , and thereby, confirms other studies [58]. However, the removal percentage depends on the retention time and the initial concentration of  $\text{SO}_4^{2-}$  in AMD. In the meantime, the concentration of sulfate in control wetland gradually decreased from day 1 to day 25 and remained constant until the end of experiment. The process of  $\text{SO}_4^{2-}$  removal in SSVF-CW can be attributed to chemicals reaction occurring within the wetland. According to Chen et al. [59] and Balci et al. [60], the oxidation-reduction (redox) reaction occurring within the wetland can transform  $\text{SO}_4^{2-}$  into elemental or mixed-valence-state form of sulfur, which facilitates its absorption by plants. Others compounds, such as

thiosulfate and tetrathionate, have been detected in oxidized sulfide minerals and may have a crucial role in  $\text{SO}_4^{2-}$  reduction in SSVF-CW [60]. The conversion of  $\text{SO}_4^{2-}$  to sulfide ( $\text{S}^{2-}$ ) can be seen as an initial step toward  $\text{SO}_4^{2-}$  reduction in wetland due to the fact that  $\text{S}^{2-}$  can be precipitated in the presence of metals such as Mn and especially Fe from AMD water [59]. Dissimilatory Sulfate Reduction (DSR) and sulfide oxidation have been reported with constructed wetlands containing *Phragmites australis* and *Juncus effusus*, though DSR was the main removal mechanism [61]. The removal of  $\text{SO}_4^{2-}$  can also be attributed to the DBR, since  $\text{SO}_4^{2-}$  reduction generates  $\text{H}_2\text{S}$ , which can form metals  $\text{S}^{2-}$  with divalent ions, such as  $\text{Fe}^{2+}$  or  $\text{Cu}^{2+}$  [62]. The reduction of  $\text{SO}_4^{2-}$  may also be attributed to the increase of the pH value following the metal solubility reduction in AMD water and especially iron due to sedimentation. Plants' uptake, metals sedimentation, and DSR may, thus, be considered as possible mechanisms of  $\text{SO}_4^{2-}$  removal.

### 3.1.2. Effect of SSVF-CW on Heavy Metal Concentration

The effect of SSVF-CW on the removal of metals from acid mine drainage (AMD) is reported in Figure 3a,b.



**Figure 3.** (a) Variation of the metal concentration in treatment wetland in 30 days of retention time under a hydraulic loading rate of 3 L/day. (b) Variation of metal concentration in control wetland in 30 days of retention time under a hydraulic loading rate of 3 L/day.

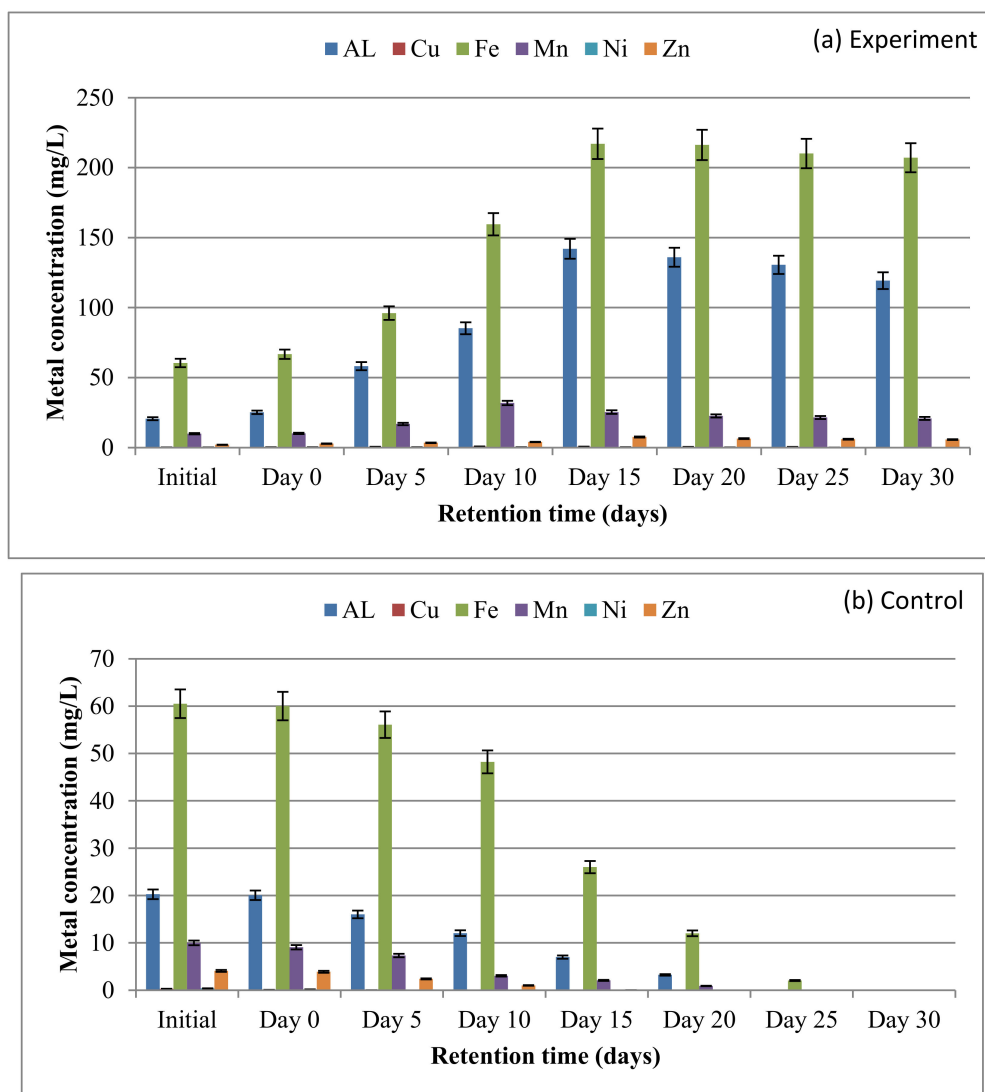


Figure 3a reveals that the metal concentration significantly decreased during the 30 days of retention time but with different removal efficiency for each metal. For instance, iron decreased slowly for the first 10 days and then drastically until the end of the experiment, whereas others metals decreased slowly from the beginning until the end (Figure 3a). The metal concentration reduction can be attributed to sedimentation and accumulation by *Vetiveria zizanioides*. In the meantime, the concentrations of metals in the control wetland decreased and were below the detection limit of 0.0001 mg/L at the end of the experiment for all metals (Figure 3b). This can be explained by the sedimentation and accumulation by *Vetiveria zizanioides*. In fact, a metal such as Fe plays a crucial role in plant metabolism processes such as DNA synthesis, respiration, and photosynthesis, and its deficiency in plants leads to common nutritional disorders [63]. Metals such as Cu, Fe, Zn, and Mn act as important co-factors for many enzymes and are essential for both mitochondrial and chloroplast functions [64]. Furthermore, zinc is one of the eight essential micronutrients. It is needed by plants in small amounts, and is crucial to plant development as it plays an important role in a wide range of processes, such as growth hormone synthesis [65]. Many processes in plant metabolism, such as chloroplast formation, photosynthesis, nitrogen uptake, and synthesis of some enzymes, are directly linked to the presence of Mn in plant [66]. In addition, the sorption of metals in oxide form is widely known, as reported by Stumm and Morgan [67]. The oxide form plays an important role in metal removal in constructed wetland if Fe/Mn oxides are present.

### 3.1.3. Metal Concentration in Substrate

The effect of SSVF-CW on the removal of metals from acid mine drainage (AMD) is reported in Figure 4a,b.

From Figure 4a, it can be seen that the metal concentration in substrate increased gradually from day 1 up to day 15. Thereafter, the removal efficacy was observed to decrease until the end of the experiment. The increase in metal concentration during the first 15 days of retention time may be attributed to the continuous load of metals in the wetland, since it was a continuous flow system. After 15 days, the concentration of metals in the substrate decreased until the end of the experiment, and this may be attributed to plant accumulation of metals. In the control wetland (Figure 4b), the concentration of metals in the substrate remained almost constant for the first five days and then decreased to below the detection limit at the end of experiment; this may be attributed to the accumulation of metals by *Vetiveria zizanioides* (Figure 4b). The finding of this study showed that sedimentation plays an important role in metal removal by sequestering metals and facilitates their accumulation by plants. Sheoran and Sheoran [68] added that others processes, such as precipitation, must precedes sedimentation, since plants only accumulate precipitated metals. The use of compost soil as substrate in SSVF-CW seems to be recommended due to sorption capacity and ability to form new storage sediment without clogging the wetland. The reduction of pollutants in wetland is accomplished by diverse mechanisms, such as sedimentation, filtration chemical precipitation, adsorption, and microbial interactions in wetland media [69]. From the results above, it follows that substrate in wetland is one of the most important components in constructed wetland, since it provides a viable condition for maximum removal of pollutant. Metal removal in wetland varies depending on the type of plant species, the microbial diversity, and the physical and hydrodynamic characteristic, such as the depth and types of wetland [70] and temperature [71]. Elements have different affinities to different plant tissues once accumulated/complexed depending on their chemistry. This determines the easy or difficulty in the de-complexation from the plant tissues. Moreover, all elements are precipitated in the substrate, but their accumulation in plants depends on the pattern of metabolism of the plants.



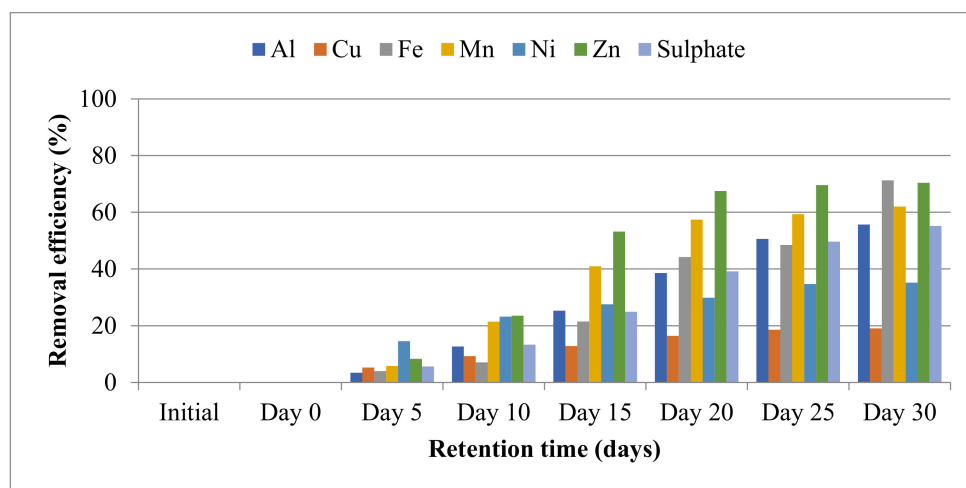
**Figure 4.** (a) Variation of the metal concentration in substrate (treatment wetland) within 30 days HRT and with a hydraulic loading rate of 3 L/day. (b) Variation of the metal concentration in substrate (control wetland) within 30 days HRT and with a hydraulic loading rate of 3 L/day.

### 3.1.4. Removal Efficiency of Metals and Sulfate

The removal efficiency of metals and sulfate by SSVF-CW is reported in Figure 5.

The removal efficiency is the percentage of a molecule of a given compound removed or destroyed during the treatment process (Figure 5). It was calculated gradually for each metal of concern and  $\text{SO}_4^{2-}$  for the duration of experiments. The results showed that the metal removal percentage is ranked in the order: Fe (71.25%) > Zn (70.40%) > Al (68.93%) > Mn (62%) >  $\text{SO}_4^{2-}$  (55.18%) > Ni (35%) > Cu (18.83%). (Figure 5). These results were in line with the studies of Roongtanakiat et al. [72] for Fe and Mn, who used vertical subsurface constructed wetland. Two processes (Sedimentation and plants uptake) contribute to heavy metal removal in SSVF-CW [58]. Plants accumulate metals for their metabolism, and this may be the reason why metals such as iron are easily accumulated by *Vetiveria zizanioides*. Fe is the third most limiting nutrient for plant growth and metabolism, primarily due to the low solubility of the oxidized ferric form in aerobic environments [73]. In that regards, Fe deficiency results in common nutritional disorder in plants, leading to poor yields and reduced nutritional quality. The  $\text{SO}_4^{2-}$  removal may also be attributed to the reduction of sulfur (S) in water, since S is an important nutrient for optimal plant growth. It is one of the key macro elements essential for plant growth and it is taken up from the soil/water by

the plant in sulfate form ( $\text{SO}_4^{2+}$ ). S is a component of methionine, cystine, and cysteine, which are three of the 21 amino acids that are the essential building block of proteins; see Brosnan and Brosnan [74] and Colovic et al. [75].



**Figure 5.** Removal efficiency of heavy metals and sulfate by SSVF in 30 days HRT under a hydraulic loading rate of 3 L/day.

### 3.1.5. Tolerance Index

For this experiment, the TI value was 1.03 (>1), which, according to Wilkins [76], reflects a net increase in plant biomass, revealing that *Vetiveria zizanioides* has developed tolerance. In both treatment and control wetland, all the plants were green and seemed healthy at the end of the experiment. The fact that *Vetiveria zizanioides* growing in AMD water remained green and seemed healthy after 30 days was not expected by the researchers, but this demonstrates that Vetiver is tolerant of a wide range of extreme conditions, such as very acidic medium (pH = 2.7) or very alkaline conditions (pH = 12) [77]. The *Vetiveria zizanioides* is a hydrophyte (wetland plant) due to its well-developed Sclerenchyma or air cell network [78], hence its high tolerance degree. Vetiver is also tolerant to high concentration of a wide range of heavy metals, whether individually or a combination of several heavy metals [77]. Following these characteristics, it can be concluded that *Vetiveria zizanioides* is an excellent candidate for a wide range of phytoremediations, as demonstrated in this study. The fact that Vetiver adapts itself to a very harsh conditions means Vetiver may have a specific cytogenetic composition allowing it to modify its metabolism according to the physico-chemical condition of the medium. However, this was not the objective of this project and may be the subject of further research.

### 3.1.6. Bio-Concentration Factor (BCF)

The bio-concentration factor (BCF) demonstrates the potential success of the plant species for phytoremediation. A BCF greater than 1 is an indicator of how probable a metal can be bio accumulated by the plant species. It shows the heavy metal movement from soil to different plant parts, including their roots and shoots [57]. The BCF was calculated only for metals in the AMD-treated wetland, since the final concentration of all metals in control wetland was below the detection limit. The results are presented in Table 1.

**Table 1.** Bio-concentration factor of the metals of concern for *Vetiveria zizanioides*.

Metals	Al	Cu	Fe	Mn	Ni	Zn
BCF	0.25	0.81	0.75	0.42	0.58	0.24

As shown in Table 1, the BCF of metals obeyed the following order: Cu (0.81) > Fe (0.75) > Ni (0.58) > Mn (0.42) > Al (0.25) > Zn (0.24). The dissimilitude of BCF results may be

attributed to the variation in the heavy metal concentration in the medium and the ability of plants (*Vetiveria zizanioides*) to accumulate a specific metal. These results demonstrate that the concentration of heavy metals in plants is related to their availability in the wetland and their importance in plant metabolism. The results showed that *Vetiveria zizanioides* has not accumulated sufficient quantity of metals to be considered as hyper accumulator, since the BCF of metals was less than 1. This can be attributed to the versatile ability of *Vetiveria zizanioides* to adapt under various harsh environmental conditions, and the ability to resist a complexity of heavy metal toxicities [79]. Furthermore, low intermediate accumulation may be explained by the flow pattern of the designed system. Although the current results show that *Vetiveria zizanioides* is not a hyper accumulator, but due to its high biomass content and tolerance index (TI), which is higher than 1, and its ability to accumulate various metals simultaneously further shows its high capability for phytoremediation in relation to others plants such as *Pistia stratiotes* and *Eleocharis dulcis*, which selectively accumulate only Mn and Fe, respectively [46,73].

### 3.1.7. Metal Distribution and Translocation

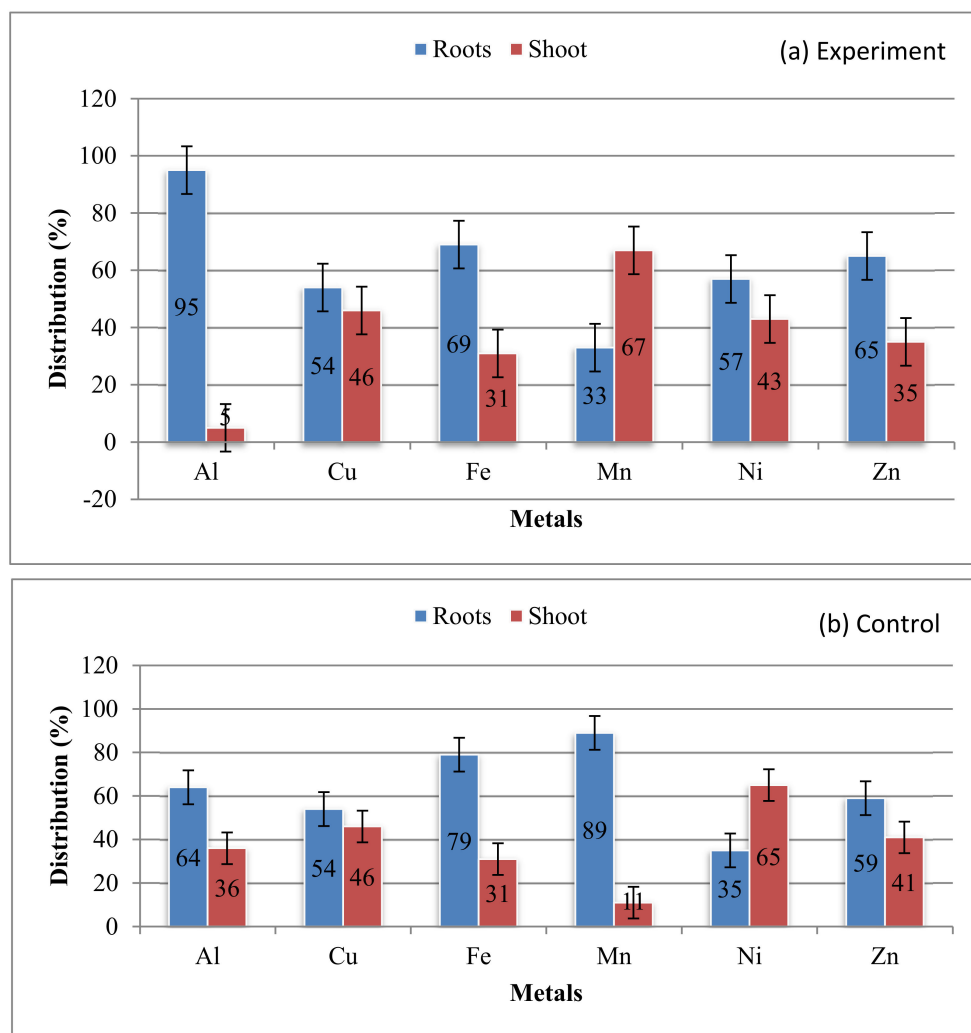
The translocation factors of metals grown in treatment and control wetland is shown in Table 2.

**Table 2.** Translocation factor (root/shoot) of AMD and control grown vetiver.

TF	Al	Cu	Fe	Mn	Ni	Zn
AMD	0.05	0.85	0.44	2.06	0.75	0.53
CONTROL	0.03	0.83	0.27	0.12	1.85	0.41

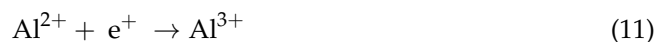
After 30 days of the experiment, *Vetiveria zizanioides* accumulated Al (95%), Fe (69%), and Zn (65%) in the roots (Figure 6a), with a TF of 0.05, 0.44, and 0.53, respectively (Table 2). Figure 6a shows that the majority of Cu (54%) and Ni (57%) were accumulated in the roots with a TF of 0.85 and 0.75, respectively, whereas there was great translocation of Mn (67%) with a TF of 2.06 (Table 2). However, in the control wetland, *Vetiveria zizanioides* had greater Mn (89%) and Fe contents (79%) in the roots (Figure 6b), with a TF of 0.12 and 0.27, respectively, while the majority of Al (64%), Cu (54%), and Zn (59%) were accumulated in the roots with a TF of 0.03, 0.83, and 0.41, respectively. A minority of Ni (35%) was accumulated in the roots with a TF of 1.85. The roots that grew in AMD-treated wetland showed amorphous and irregularly spaced build up along their surface from plaques, thereby reducing the translocation of metals from roots to shoots [80] (Figure 6a), which is not the case for roots that grew in control wetland (Figure 6b).

The difference between the TF of Mn in AMD water and control water may be attributed to the pH of the medium. The bioavailability of Mn in soil is, therefore, influenced by both pH and redox conditions [77]. Within a harsh environment such as AMD wastewater with various metals, including Mn and Fe, Mn oxides form co-precipitates with Fe, leading to the acidity of the medium with an increased redox potential of Mn oxides, which can be easily reduced in the soil exchange sites [81], thereby increasing the concentration of soluble  $Mn^{2+}$  [82], which is the predominant form of Mn in the soil solution and the most available Mn form for plants [83]. In contrast, when the pH is basic, chemical  $Mn^{2+}$  auto-oxidation is favored over  $MnO_2$ ,  $Mn_2O_3$ ,  $Mn_3O_4$ , and even  $Mn_2O_7$ , which are not normally available to plants [82]. In addition, alkaline pH promotes the chemisorption of Mn into soil particles, decreasing their availability [84]. Moderate translocation of Ni in vetiver grown in both AMD and control wetlands can be attributed to the biochemical processes of sequestration and translocation, since Ni can be transported as a nickel-histidine complex [85]. These two biochemical processes ensure high mobility of Ni within the plant, which can justify the moderate translocation of Nickel [86]. The moderate translocation of Cu by vetiver grown in both wetlands may be attributed to plaque inhibition, thereby requiring more time to accumulate copper [87].



**Figure 6.** (a) Percentages of the metal distribution partitioned into roots and shoots (treatment wetland). (b) Percentages of the metal distribution partitioned into roots and shoots (control wetland).

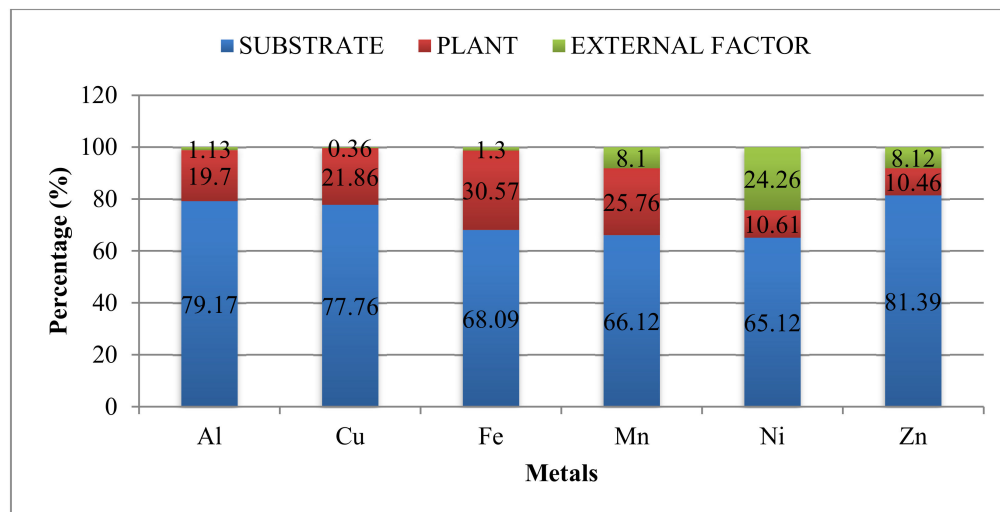
The low translocation of Al may be attributed to plant tolerance strategy, since in an acidic condition, Al becomes a major constraint for the growth of plant. At a pH value less than 6,  $\text{Al}^{2+}$  is oxidized to  $\text{Al}^{3+}$  following Equation (11):



The  $\text{Al}^{3+}$  released to the medium enters into the root tip cell and delays the growth of the plant [88]. There was a greater translocation of Fe by Vetiver grown from both wetlands: 69% and 79% for AMD and control, respectively. The higher translocation of Mn and Zn in both wetlands may be attributed to the need of the aforementioned metals for plant metabolism, since Mn and Zn are essential nutrients [87]. A number of factors, including types of metals, plant species, types of substrate, and the initial concentration of metals, anatomical, biochemical, and physiological factors influence the metals' translocation from roots to shoots [89]. In fact, metals are taken up from the soil by roots and transported via the plasma membrane driven by ATP-dependent proton pumps that catalyze  $\text{H}^+$  extrusion across the membrane. Furthermore, along with cationic nutrients, plant transporters are also involved in shuttling potentially toxic cations across plant membranes [85]. Cytogenetic make-up and others unknown factors can be attributed to different patterns of metal translocations from roots to shoots [90].

### 3.1.8. Metal Removal Partitioned between Substrate, Plant, and External Factors

The contribution of substrate, Vetiver, and external factors in the removal efficiency of metals was determined and the results are shown in Figure 7.



**Figure 7.** Removal efficiency of metals partitioned between Vetiver, substrate, and external factors.

The determination of the final concentration of metals in AMD water, substrate, and in plant tissue allowed the researchers to calculate the contribution of each wetland component (substrate and plant) and external factor in overall metal removal process. The results revealed that metals were more removed by the wetland substrate, followed by vetiver and external factors, with substrate contributing to 81.39%, 79.17%, 77.76%, 68.09%, 66.12%, and 65.12% removal of Zn, Al, Cu, Fe, Mn, and Ni, respectively (Figure 7). The substrate provides support to plant growth, serves as an energy source for biogeochemical reactions, and also plays a major role in pollutant reduction, since it absorbs pollutants such as heavy metals, thereby facilitating their accumulation by the plants roots [91].

Plant contribution in the overall metal removal process was ranged in the order: Fe (30.57%) > Mn (25.76%) > Cu (21.86%) > Al (19.70%) > Ni (10.61%) > Zn (10.46%) (Figure 7). The contribution of vetiver to the metal removal was small compared to the substrate fraction in the overall metal removal, and this may be attributed to the duration of experiment (30 days) and the need of metals for plant metabolism. For instance, Cu and Zn are essential elements for plant metabolism; however, at elevated concentrations, they become toxic [92]. Therefore, more time may be required for the plant (VZ) to accumulate considerable quantities of metals. Despite its smaller contribution in overall metal removal in SSVF-CW, Vetiver, similar to other plants used in constructed AMD-treatment wetlands, contributed to the overall efficiency of CW in many ways. It favored the settlement of total suspended solid (TSS) and provides optimum conditions for the growth of microorganisms, thereby promoting the sedimentation and accumulation of metals in CW [93]. The contribution of external factors was ranged in the order: Ni (24.26%) > Zn (8.12%) > Mn (8.10%) > Fe (1.3%) > Al (1.13%) > Cu (0.36%) (Figure 7). They include: evaporation, adsorption, biological assimilation, decomposition, chemical transformation, and volatilization; sulfides and others minerals in the sediment may also contribute significantly to metal removal in CW [94]. The finding was in line with the study by Mustapha et al. [46], who used SSVF-CW with three different plants (*Typha. latifolia*; *Cyperus alternifolius*; *Cynodon dactylon*) for heavy metal removal from refinery wastewater and found that metal accumulation by plants and externals factors accounted only for a small fraction of the overall metal removal in subsurface vertical flow wetland.

### 3.2. Characterization of the Solid Samples

#### 3.2.1. X-ray Fluorescence (XRF) Analysis

An XRF analysis was done to determine the elemental composition of initial soil, soil from control wetland, and soil from treatment wetland, and the results are shown in Table 3.

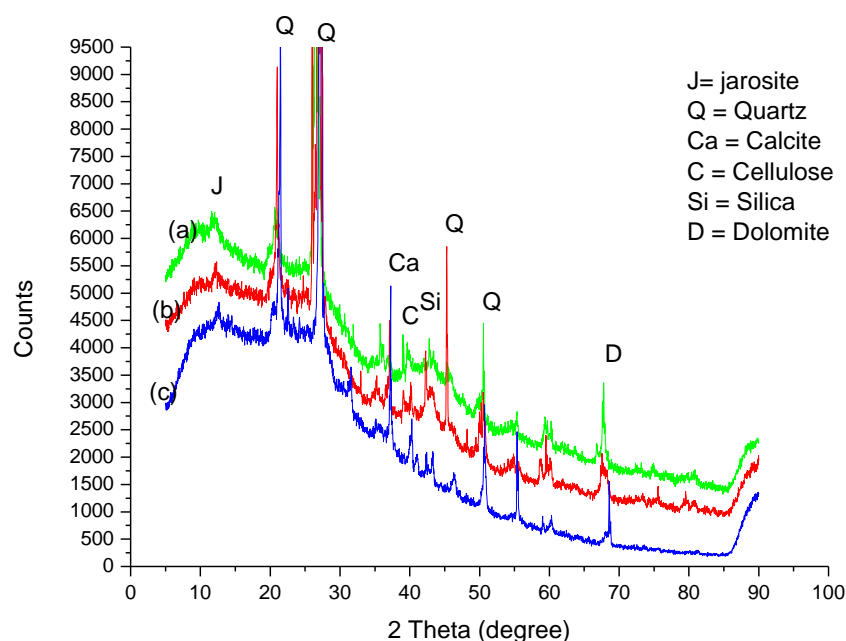
**Table 3.** The elemental composition of initial soil, soil from control wetland, and soil from treatment wetland.

Samples (wt.%)	Initial Soil	Control Soil	AMD Reacted Soil
Fe	84.9504	84.9532	88.8688
Ti	4.6093	2.6774	4.3403
Rb	3.2519	3.8190	2.3403
Sr	2.7486	3.7355	2.7808
Mn	2.3030	2.6018	3.4293
Zn	0.6970	0.7001	0.4873
Si	0.1393	0.3210	0.0291
Al	—	—	0.0393
Na	—	—	0.0324
Ni	0.4505	0.0007	0.0291

From the results in Table 3, it follows that the percentage of Fe and Mn was the same for the initial and control soil. However, the percentage of Fe and Mn were found to increase after reaction of soil with AMD, and this may be attributed to the addition of metals content and formation of new phases. This corroborates results reported in the water quality assays. The percentage of Zn, Ni, and Si decreased after contact of substrate (soil) with AMD water, indicating the possible dissolution of these metals. Al and Na were found to be present, and this may have originated from AMD water. The results also revealed that there was no noticeable difference in the elemental composition between initial soil and soil from control wetland, which may be attributed to the potable fresh water used in control wetland, which in fact does contain an insignificant quantity of chemicals.

#### 3.2.2. X-ray Diffraction (XRD) Analysis

An XRD analysis was done to determine the mineralogical composition of initial soil, soil from treatment wetland, and soil from control wetland, and the results are shown in Figure 8. In addition, Table 4 lists the mineral phases identified with their various peaks.



**Figure 8.** (A–C) XRD patterns of substrate: treatment (A), control (B), and initial substrate (C).

**Table 4.** The measured XRD and the identified mineral phase of the initial substrate, substrate from experimental wetland, and substrate from control wetland.

Substrate	2 Theta (Degree)	Mineral Phase	References
Initial soil	Quartz	21	[96]
	Quartz	28	[96]
	Calcite	37.5	[97]
	Cellulose	40.1	
	Silica	46	
	Quartz	50	[96]
Soil from control wetland	Quartz	55	[96]
	Dolomite	68	
	Quartz	21	[95]
	Quartz	27	
	Calcite	37	
	Cellulose	38	[98]
Soil from treatment wetland	Silica	44	
	Quartz	45	[96]
	Quartz	50	[96]
	Dolomite	67	[99]
	Jarosite	8	[98]
	Quartz	21	[96]
	Quartz	26	[96]
	Calcite	35	[99]
	Silica	42	[96]
	Quartz	45	[98]
	Quartz	50	
	Quartz	55	[96]
Calcite	61	[98]	
Dolomite	65	[99]	

As shown in Figure 8, the X-ray diffraction patterns of substrate from experimental wetland (a) and control wetland (b) and initial substrate showed several peaks spread over the range  $2\theta$  from  $10^\circ$  to  $68^\circ$ , although at different intensities. The peaks at  $2\theta = 20.6^\circ$ ,  $28^\circ$ ,  $45^\circ$ , and  $50^\circ$  may correspond to Quartz ( $\text{SiO}_4$ ), which is the major elemental component of compost [95]. Jarosite is present in a substrate from the experimental wetland with peak at 8, and this may be a ferric iron-sulfate mineral with the formula  $(\text{K}, \text{H}_3\text{O}) \text{Fe}_3 (\text{SO}_4)_2 (\text{OH})_6$  that originated from AMD water with acidic pH.

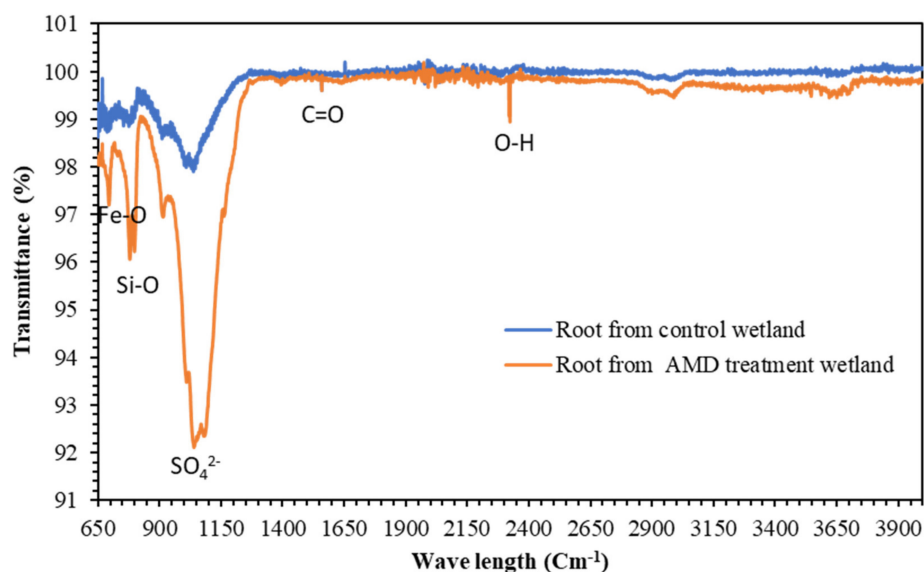
### 3.2.3. Fourier Transforms Infrared Spectroscopy Analysis

Fourier transforms infrared spectroscopy (FTIR) analysis of roots grown from both wetlands (treatment and control) was done and the results are presented in Figure 9 while Table 5 lists the identified metals' functional groups and their respective wavelengths.

**Table 5.** The metals' functional groups and their references.

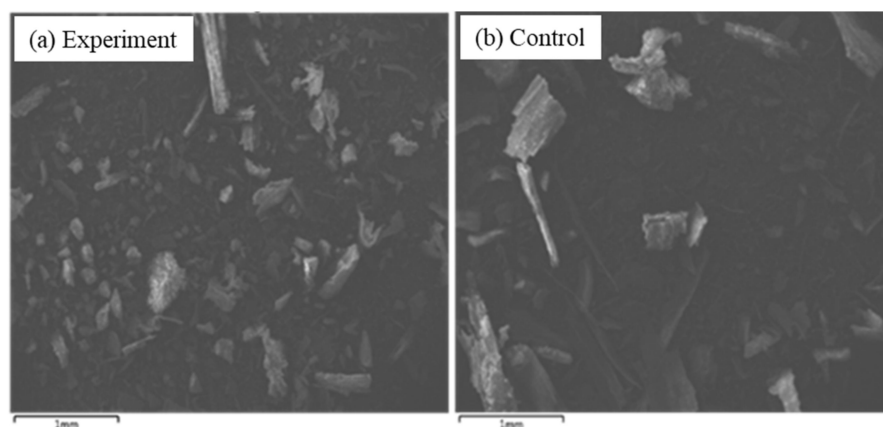
Wavenumber	Functional Group	Reference
698	Fe-O	[6]
780	Si-O	[99]
1080	$\text{SO}_4$	[12]
1559	C=O	[100]
2222	O-H	[101]
2996.5	O-H	[99]





**Figure 9.** Fourier Transfer Infrared (FTIR) spectra of root from AMD treatment wetland and root from control wetland.

FTIR spectra of *Vetiveria zizanioides* grown in AMD-treated wetland and control wetland in Figure 9 indicates the complex formations between the pollutants in AMD water and different part of *Vetiveria zizanioides* cell wall. The bands in the roots grown in control wetland are characteristic of vibration, corresponding to the band at  $3498\text{ cm}^{-1}$ . The stretching vibration of the roots from control wetland correspond to the bands at  $3032$ ,  $2814.5$ ,  $1998.5$ ,  $1489.5$ , and  $1274\text{ cm}^{-1}$ , while in the roots from AMD-treated wetland, the stretching vibration corresponds to the bands at  $3495$ ,  $2937$ ,  $2323.5$ , and  $1345.5\text{ cm}^{-1}$ . The doublet at  $1026$  and  $1105$  for roots from AMD wetland and the doublet at  $857$  and  $966.5$  for roots from control wetland may be attributed to the formation of carbonate ( $\text{CaCO}_3$ ) [102] from calcium present in roots from both wetlands, as revealed by the EDS results (Figure 10a,b). Furthermore, sulfates ( $900\text{--}1200\text{ cm}^{-1}$ ) [14] and iron oxides ( $450\text{--}500\text{ cm}^{-1}$ ) were also present [6].



**Figure 10.** (a,b). SEM of *Vetiveria zizanioides* roots from AMD-treated wetland water (a) and control wetland (b).

### 3.2.4. Scanning Electron Microscope Electron Dispersion Spectrometry Analysis

To understand the mode of interaction of AMD with *Vetiveria zizanioides* and the formation of mineral phases, a scanning electron microscope (SEM) was used to assess the change in morphology of *Vetiveria zizanioides* roots reacted with AMD water (Figure 10a) and *Vetiveria zizanioides* root reacted with potable fresh water (Figure 10a). The SEM images of roots grown in AMD water (Figure 10a) and the SEM images of *Vetiveria zizanioides* roots

grown in potable fresh water (Figure 10b) showed a noticeable difference in morphology. This result concurs with the findings obtained by Kiiskila et al. [103]. In fact, plants use their cell wall as a defense compartment to respond to toxic conditions and accumulate pollutants. Pollutants are accumulated by plants using the cell wall, which is rich in proteins, amino acids, and phenolics able to bind pollutants in order to render them less or non-toxic. When plants accumulate metals using the cell wall, it leads to the formation of crystal-like deposits, which saturates the cell wall, creating cell thickening. This demonstrates that *Vetiveria zizanioides* has accumulated and sequestered toxic pollutants, as illustrated by Figure 10a. The SEM image of *Vetiveria zizanioides* roots from control wetland showed less thickening compared to the SEM image of roots from AMD wetland, thereby demonstrating that VZ growth in control wetland accumulated fewer pollutants.

The EDS results of vetiver roots grown in AMD water revealed the presence of C, O, Mg, Au, Si, and Fe. The presence of Ca indicates the presence of Calcite. The reacted *Vetiveria zizanioides* with AMD contained O, Al, K, and Si as the major components. The presence of oxygen at high levels may be the results of precipitation of AMD elements to metal oxides. High levels of Fe and Al are possibly due to the phenomena of ion exchange on the soil surface (substrate) and precipitation following the slight increase of pH. The ion exchange process leads to the release of some metals [22] and the presence of Potassium (K), Calcium (Ca), and Magnesium (Mg) in root growth in the AMD environment may be the result of an ion exchange reaction, whereas the presence of S compounds could be attributed to the sink of heavy metals released into the environment (Figure 11a). The EDS of roots from control wetland revealed the presence of the same components in addition to Na but with a very low percentage, thereby confirming the X-ray fluorescence analysis results (Figure 11b). The components of roots from control wetland may be attributed to their presence in substrate used as wetland media.

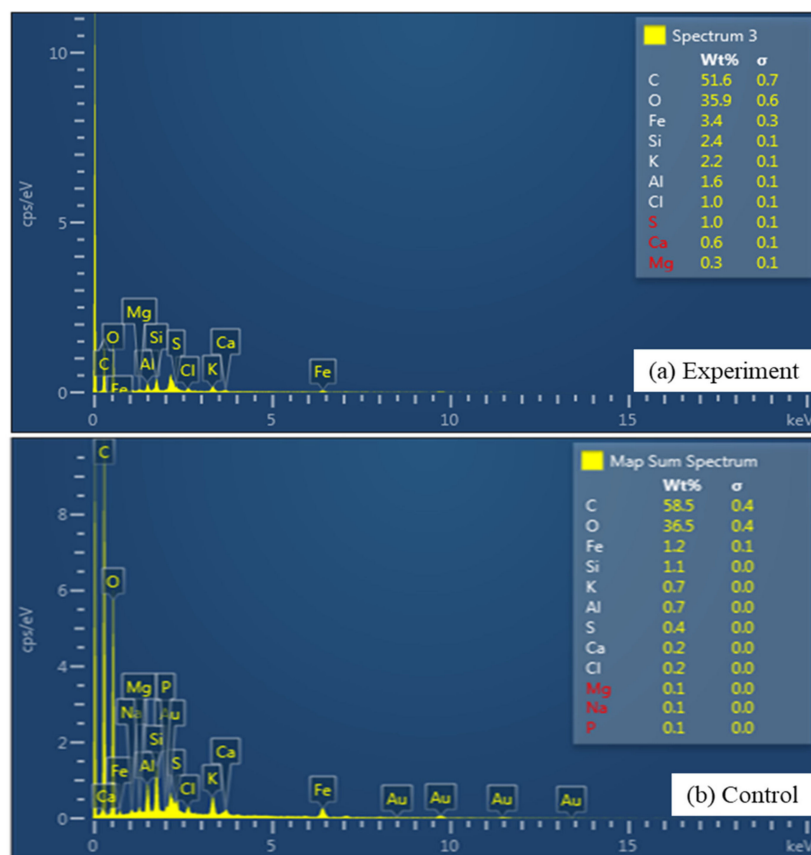


Figure 11. (a,b) EDS of *Vetiveria zizanioides* roots from AMD-treated (a) and control wetland (b).

#### 4. Chemical Species for Untreated and AMD-Treated Wetland with SSVF-CW

The results of chemical species of AMD were used to compare the guidelines standards for effluent from the department of environmental affairs (DEA) and DWAS, and the results are presented in Table 6.

**Table 6.** Concentrations of chemical species for untreated and AMD-treated wetland with SSVF-CW (all units in mg/L except pH and EC).

Parameters	Raw AMD	DEA/DWAS Guidelines for Effluent Discharge	AMD-Treated Water	Percentage of Pollutant Removed
pH	2.6	6–12	3.8	—
TDS	3380	2400 mg/L	1400	58.57
EC	5000	150 $\mu$ S/cm	3200	36.00
Al	158	20 mg/L	70.02	56.68
Fe	341	50 mg/L	98.03	71.24
Mn	37	20 mg/L	14.06	62
Cu	4.2	20 mg/L	3.40	19.04
Zn	8.55	20 mg/L	2.53	70.40
Ni	3.92	10 mg/l	2.54	35
Sulfate	3137	2400 mg/L	1406	55.18

The pH is very acidic (2.6), with high TDS and EC. Higher TDS and EC may be attributed to high levels of Al, Fe, and Mn, other macro-elements, such as Na, Ca, and Mg, and microelements, such as Cu, Zn, Pb, Co, Ni, B, Cr, Mo, Se, As, K, and Si, and a high concentration of sulfate. After treatment of AMD with SSVF-CW using *Vetiveria zizanioides*, the pH increased slightly from 2.6 to 3.01, whereas EC and TDS decreased. The concentrations of metals (Al, Cu, Fe, Mn, Ni, and Zn) and  $\text{SO}_4^{2-}$  decreased significantly after treatment with SSVF-CW, and this can be attributed to sedimentation/precipitation and accumulation by *Vetiveria zizanioides*. However, the DWAS water quality guidelines have not been met, and therefore, there is a need to associate or integrate subsurface vertical flow constructed wetland with another type of passive treatment, such as free water constructed wetland, or with active technology, such as neutralization or filtration, in order to improve the AMD water quality.

#### 5. Conclusions

*Vetiveria zizanioides* was successfully explored for the removal of contaminants from acid mine drainage (AMD). Findings from this study further revealed that *Vetiveria zizanioides* has high tolerance to AMD, and this has been confirmed by a zero to trivial sign of toxicity response after 30 days of hydraulic retention and hydro-dynamics. Interestingly, (i) a lower removal efficiency for Ni and Cu were observed, with (ii) moderate removal of  $\text{SO}_4^{2-}$  reported, while (iii) higher removal efficiency was observed for Fe, Zn, Al, and Mn. The finding further revealed that the attenuation of metals could be explained by the partitioning of extracted chemical species between wetland substrate, plant, and external factors with a substrate contribution of approximately 65.12% to 81.39% with  $\leq 31\%$  of Al, Cu, Fe, Mn, Ni, and Zn stored in the plant. The removal efficacy of chemical species was observed to obey the following order: Fe (71.25%) > Zn (70.40%) > Mn (62%) > Al (56.68%) >  $\text{SO}_4^{2-}$  (55.18%) > Ni (35%) > Cu (18.83%). Furthermore, metals were more concentrated in the roots, except Mn, which was more concentrated in the shoot. The PH REDox EQUilibrium (in C language) (PHREEQC) geochemical model confirmed that metals existed as di- and-trivalent complexes in the solution. Lastly, the available metals were precipitated as metal hydroxides and oxy-hydrosulfates. A significant quantity of pollutants of concern was removed from AMD water, but we did not reach the guideline standards for effluent discharge as established by the DEA/DWAS and World Health Organization (WHO). As such, we recommend the coupling with a secondary AMD treatment technology to ensure that the treated water is of an acceptable standard.

**Author Contributions:** Conceptualization, B.N., V.M., T.A.M.M., and M.T.; Formal analysis, B.N.; Methodology, B.N., V.M., T.A.M.M., and M.T.; Data curation, B.N., V.M., and T.A.M.M.; writing—original draft, B.N.; Supervision, V.M. and T.A.M.M.; writing—review and auditing, B.N., V.M., T.A.M.M., and M.T. All authors have read and agreed to the published version of the manuscript.

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**Informed Consent Statement:** Verbal agreement was obtained from mine where AMD water was collected.

**Data Availability Statement:** Not applicable.

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**Conflicts of Interest:** The authors declare that this research project has no conflict of interest.

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