Removal of heavy metals using a wetland batch system with Carrizo (Phragmites Australis)

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Research

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Abstract

One of the greatest environmental issues related to the development of the mining activity is the generation of Acid Mine Drainage (AMD). This effluent generated by active and abandoned mines in Peru produces multiple discussions about the role of the industry for a sustainable development. Many methods have been developed for AMD treatment, being wetlands a good option for heavy metal removal. In this sense, removal of heavy metal by using laboratory constructed wetlands with Phragmites Australis was studied. During a 9-day period the variation of different physicochemical parameters and heavy metals concentrations was recorded. The pH decreased to a constant value of 8.24 after the sixth day. The electrical conductivity presented a slight variation from 1 to 1.33 µS cm⁻¹ on the ninth day. Moreover, fluctuating values of Dissolved Oxygen and Turbidity were observed. A reduction between 69% and 93% of ammonia nitrogen (NH₃-N) was registered. Cu concentration was reduced in 68% and 87%; Zn concentrations dropped in 53% and 95%; and Pb was reduced in 20% and 55%. It was concluded that Phragmites Australis can be an alternative in the treatment of the contaminants produced by AMD.

Highlights

- Physico-chemical parameters mostly were reduced due to Carrizo action.
- Heavy metal concentrations (Cu and Zn) were removed between 68%-95%.
- Lead concentration was reduced in 20% and 55%.
- Iron concentrations need more time to analyze its removal due to its variation values
- Plant saturation, and the fluctuating parameters, generated a nine days cycle

Introduction

Several challenges are produced during the development of mining activities, where the most relevant are related to environmental issues. The effect on the ecosystem is one important aspect taken into account in the elaboration of the plans in order to prevent the generation of liabilities. One of the most important issues is the contamination of water resources, usually produced by the generation Acid Mine Drainage (AMD). In addition, as well as in many countries, the relationship between lands, water and communities in Peru go beyond than economic factors, but a historical bond based on religion and other cultural traditions. In several occasions the negative effects of AMD can reduce the quality of water resources of rivers and ditches used in communities [1] and their presence in the food chain [2]. Due to this fact, social problems, usually occasioned by events as the ones mentioned before, can be prevented with solutions connected to correct policies and technologies applied by mining companies for a sustainable development.

As was explained before, AMD became an important factor considered by mines during the development of their activities. Due to its compounds, the generation of AMD can be easily produced unintentionally in the environments where these industries are located. The presence of Acidithiobacillus ferrooxidans - a
bacteria that influences the oxidation of pyrite and sulphides, generally copper, lead and zinc, the presence of oxygen and Fe⁺ in water are the common factors involved [3]. Therefore, this pollutant can occur in situations such as mineral processing, or cracks in tailing facilities, because seepage of material, with highly concentration of heavy metals, can reach nearby water sources [4].

The proposals that a mining company can consider as solutions for the AMD generation can be catalogued in Active and Passive methods. Both of them have their own characteristics, which included different implications during their application in a real situation. Active methods, the most common used in the mining industry, are based on the precipitation of heavy metals as hydroxides by the incorporation of alkaline chemicals during the process [5]. In that way, this procedure, usually developed in treatment plants, can increase the pH of the effluent.

However, the high operating costs of active methods - including the reagents used in this process, such as Sodium Hydroxide – in some occasions reduce the feasibility of its application for the treatment of AMD [6]. Thus, the use of Passive Methods has become a more accessible alternative. This alternative, which uses the constant interaction of alkaline elements such as wetlands with AMD, has increased its popularity for their economic advantages. In fact, although is used by several mining projects, the scope can include other industries for the treatment of urban waste, remained materials from cattle raising, among others [7–9]. In general, in relation to active methods, the maintenance costs can be one twentieth of the one from the previous alternative [10].

The versatility of passive methods with wetlands has produced a big number of investigations and variation of the plants used for the process. Based on the basic criteria of the procedure, local species have been adapted for the treatment of contaminated water in different regions around the world - *Eichhornia crassipes* in coal mines of Singrauli [11], *Typha domingensis*, which grows in locations close to Australian mines [12], *Schoenoplectus Californicus*, intensively used in constructed wetlands of United States [13], etc.

As well as the previous plants mentioned, the use of *Phragmites Australis* represents an alternative for the remediation of local liabilities close to Peruvian active and abandoned mines. This species can be found specially in Asia and Europe [14], but its extension reaches Australia, New Zealand, North America, and South America, in cold and tropical regions [15]. *Phragmites Australis* is part of a genus that is composed by six types of plants whose grasses vary from 2 to 6 meter [16], and it has the tendency to live in zones close to rivers, lakes and wetlands [17]. In addition, different conditions of pH and salinity do not imply a problem for the reproduction and adaptability of the plant. In fact, this species can be catalogued as a hyperaccumulator, which means that it has an optimal tolerance to high concentrations of heavy metals in their roots and leaves [18, 19].

Due to the optimal characteristics of *Phragmites Australis*, different investigations have been developed with this species as part of constructed wetlands [20–22]. Therefore, based on its proper features proved in previous studies and the availability of the plant in local mines, the main objective of this investigation is the study of the phytoremediation efficiency produced by *Phragmites Australis*, as part of the recovery
of the most common metals found in liabilities – Copper, Iron, Zinc and Lead. In addition to the recoveries obtained, the time of absorption is required as a part of the feasibility analysis of AMD treatment.

**Materials And Methods**

**Plant samples**

The samples of Phragmites Australis were obtained from the south of Lima, Peru. As was explained before, this specie can be found in different regions, and close to Peruvian mines. For the experiment gravels with diameters of 30 mm and 40 mm were use as substrate of the laboratory wetlands.

**Chemicals**

Copper sulfate (CuSO$_4$), zinc sulfate (ZnSO$_4$), lead sulfate (PbSO$_4$), iron sulfate (FeSO$_4$), sodium nitrate (NaNO$_3$) and ammonia (NH$_3$) were used. All chemicals were used as received used as received without further purification. MiliQ water was used for the preparation of the samples. Finally, 1000 ppm Standard solutions of Copper, Zinc, Iron and Lead solutions from Merck were used for the procedure in the Atomic Absorption Spectroscopy.

**Effluent Preparation**

The features of the effluents used in each cell varied according to the metals added in each one. Initially all of them included 85 mmol of sodium nitrate and 17 mmol of ammonium. After that, the four cells received different metals, but all of them contained 0.072 mM of iron sulfate. The copper-iron solution, zinc-iron solution and lead-iron solution contain 0.06 mM of copper sulfate, 0.062 mM of zinc sulfate and 0.02 mM of lead sulfate, respectively.

**Cells Construction**

The cells design was done by following the parameters given by EPA [23], where the relation of width, height and length was recommended to be 1:2:4. Eight cells of 20 cm, 40 cm and 80 cm were constructed. Once the cells were built the gravel was added immediately. Then, four plants, spaced every 20 cm, were placed in the cells. After that, 15 L of the artificial effluent were added in an approximate flow of 3 ml/min.

**Experiment Conditions**

The experiments were carried out at temperature range of 16 °C and 18 °C and 65% of humidity. Characteristic environmental conditions of the spring of the city of Lima.

**Equipment**

The analysis of the parameters was done by different instruments. For the measure of pH, Dissolved Oxygen, Conductivity, and Turbidity, two pH-meters (HI 3220 and PH211), an oxygen dissolved meter (HI 98186), an electro conductivity meter (HI 8733) and a turbidity meter (C114) were used, respectively.
addition, Multiparameter probes (HI 83200) were applied for ammonia, nitrate and sulphate analysis. For the analysis of the concentration of heavy metals, the Atomic Absorption Spectrophotometer (Varian AA240) was part of the experiment.

**Water sampling**

Water samples from the eight constructed wetlands cells were collected in a 100 ml plastic bottles during a period of 10 days. All samples were collected from the central part of each cell that is considered as the monitoring point. 80 ml of each sample was filtered using a 0.45 µm cellulose acetate filters and the other 20 ml was kept as unfiltered sample to measure the turbidity. The concentrations of parameters such as ammonia, nitrate and sulphates were determined by colorimetric methods. The concentrations of copper, zinc, lead and iron were obtained by flame Atomic Absorption Spectroscopy (AAS).

**Physicochemical characterization**

The procedures of the water quality analysis [24, 25] were used to measure the physicochemical parameters such as pH, turbidity, dissolved oxygen (DO), and electric conductivity (EC) of the samples. The calibration procedure was performed by using buffer solutions according to the reference method for pH, DO, EC and Turbidity test, respectively.

**Ammonia, nitrate and sulphate concentration**

In order to measure the concentration of ammonia nitrogen (NH₃-N), nitrate nitrogen (NO₃-N) and sulfur sulphate (SO₄-S) a multiparameter was used. For ammonia and sulphate concentration, the modification of the Nessler’s method [26] and the barium precipitation method were applied, respectively. Finally, in the case of nitrates, ferrous sulfate and cadmium reduction methods were used.

**Heavy metal concentration**

Flame Atomic Absorption Spectrometry (AAS) was used to determine the concentration of heavy metal. During the analysis, a blank solution with 2 ml of the 0.50% aqueous HNO₃ was prepared. Furthermore, with the dilution of 1000 ppm of the stock with 0.50% aqueous Nitric Acid (HNO₃), three standards solutions (100 mL) for each metal were prepared (1, 2 and 5 ppm). The blank, standard (from lowest to highest concentration) and sample solutions were placed in that order on the autosampler [27–29]. At the end all absorbance results were registered.

**Results And Discussion**

As part of the analysis, four of the most common metals in the AMD [6, 30–33] were considered in this research: Cu. Zn, Pb and Fe. In order to understand the effect of specific metals in the absorption performance of *Phragmites Australis* different solutions were prepared. Based on that, three binary solutions (Cu-Fe, Zn-Fe and Pb-Fe) and a quaternary solution (Cu-Zn-Pb-Fe) were prepared as artificial effluents (Table 1).
Table 1
Distribution of heavy metals in each wetland.

| Compound   | W1 | W2 | W3 | W4 |
|------------|----|----|----|----|
| CuSO$_4$   | x  | -  | -  | x  |
| ZnSO$_4$   | -  | x  | -  | x  |
| PbSO$_4$   | -  | -  | x  | x  |
| FeSO$_4$   | x  | x  | x  | x  |

$[\text{NO}_3^-] = [\text{NH}_4^+] = 10$ ppm; $[\text{Cu}^{2+}] = [\text{Zn}^{2+}] = [\text{Fe}^{2+}] = [\text{Pb}^{2+}] = 4$ ppm

At the beginning of the experiment, the wetlands were placed in a space covered from the sun, to avoid the deterioration of plant and the heating of water (Fig. 1). After the conditioning of artificial wetlands was finished, the data collection started. Initials water samples were collected in order to determine the amount of metal absorbed by the substrate or plants at its first contact in the wetland (day 0). These samples reported different metal concentration than solutions prepared in the laboratory.

**Physicochemical parameters**

For the measurements of pH, turbidity, dissolved oxygen and electrical conductivity a period of 10-days was used. However, this period was not considered as the effective cycle of removal generated by the plant. An effective cycle of 9-days was determined. A summary of the physicochemical parameters obtained in the initial (day 0) and nine (day 9) day are showed in Table 2.

**pH variation**

The values of pH obtained in the experiment are showed in the Fig. 2a. As can be observed, the pH variation of all solutions presents a similar behavior. According the Fig. 2A, the pH in all solutions started at 10.56 (day 0) in average and it decreased until 8.24 approximately (day 6). This decrease may be due to different chemical reactions generated in water, the oxidation process of metals and the generation of acidity [5, 6, 34, 35]. In addition, the pH remained almost constant from sixth to tenth day.

**Dissolved Oxygen**

The values of Dissolved Oxygen (DO) obtained are showed in the Fig. 2b. In the case of the binary cooper-iron solution, the concentration of DO was reduced to a minimum value on the fifth day. However, in the other solutions this reduction was observed on the seventh day. Furthermore, the lowest concentration of DO (0.08 mg/l) was obtained in the binary zinc-iron solution on the seventh day. It is known that oxygen produced by photosynthesis can be released from plant roots and leaves. Besides, Radial Oxygen Loss (ROL) is a complex process where oxygen goes through the aerenchyma from plant leaves and stems to plant roots, and is then emanated into the surrounding environment from the roots. However, plants root,
commonly, tend to reduce oxygen release during a strong oxygen consumption, which directly limits the ROL process. As a consequence, it is not possible a contribution of ROL to the DO [36]. This event can explain the minimums values of DO obtained on the sixth and seventh day.

**Turbidity**

The values of turbidity obtained are showed in Fig. 3a. As is known, turbidity is a reduction in water clarity because of the presence of suspended matter absorbing or scattering down welling light [37]. Initial values of 0 NTU in average were obtained. But in the following days these values considerably increased; this behavior can be explained by the solids formed during the experiment period. Other factor could be the presence of insoluble material, or microorganisms in the wetland cell. The binary cooper-iron solution presented mostly high turbidity values; its maximum value was 67.6 NTU on the first day. The solution that presented the minimum turbidity values in comparison to the others was the binary zinc-iron solution, where the highest value of 15.2 NTU was registered on the first day. In the case of the binary lead-iron solution, the maximum value of 14.1 NTU was obtained in the third day. Finally, for the quaternary solution that contain all metals, the maximum value of 26.5 NTU was observed on the second day.

**Electrical conductivity**

The values of conductivity obtained are showed in Fig. 3b. It can be observed that at the beginning (day 0) the average conductivity was 1.02 µS/cm and in the last day (tenth day) the average value was 1.43 µS/cm. The minimum values of conductivity (1.18 µS/cm, 1.23 µS/cm, 1.16 µS/cm) were obtained on the third day for the cooper-iron, lead-iron and quaternary solutions respectively. In the case of the binary zinc-iron solution the minimum conductivity was 1.2 µS/cm on the ninth day. Finally, the conductivity increased until 1.43 µS/cm, in average, on the tenth day. However, in this case it is not possible to conclude that the metals in the solutions have been removed because the electrical conductivity not only depends on the content of metal ions in solution [38, 39].

**Table 2** Summary of Average Performance of Treatment Wetlands: variation of pH, Conductivity, DO and Turbidity in *Phragmites Australis* wetlands.

| Solution | pH (day 0) | DO (mg/L) (day 0) | DO (mg/L) (day 9) | Conductivity (µS/cm) (day 0) | Conductivity (µS/cm) (day 9) | Turbidity (NTU) (day 0) | Turbidity (NTU) (day 9) |
|----------|------------|------------------|------------------|-------------------------------|-------------------------------|-------------------------|-------------------------|
| Cu-Fe    | 10.71      | 8.28             | 6.41             | 6.12                          | 1.03                          | 1.34                    | 0                       | 1.29                    |
| Zn-Fe    | 10.46      | 8.25             | 6.37             | 6.40                          | 1.00                          | 1.20                    | 1.84                    | 3.42                    |
| Pb-Fe    | 10.59      | 8.30             | 6.26             | 5.44                          | 1.06                          | 1.42                    | 0.83                    | 2.27                    |
| Cu-Zn-Pb-Fe | 10.54 | 8.21             | 6.50             | 5.87                          | 1.01                          | 1.35                    | 1.00                    | 2.53                    |

**Ammonia (NH₃-N) concentration**

The values of ammonia (NH₃-N) concentrations are indicated in Fig. 4a. In general, the *Phragmites australis* wetlands behaved similar for all samples, contributing with the ammonia (NH₃-N) reduction,
reaching close values on the tenth day. The zinc-iron, lead-iron binary and quaternary solutions show high concentrations of this anion (NH$_3$-N) at the outset (day 0). For the quaternary solution, an increment of the value was noticed on the fifth day and a slight increase of NH$_3$-N concentration was observed on the tenth day. Besides, in the binary cooper-iron solution there was an increase of the concentration value on the fourth and eight day. Furthermore, in the binary lead-iron solution a slight increase was registered on the second day. The removal of NH$_3$-N depends on the wetland configuration and the dissolved oxygen (DO) vacancy for nitrification, since it is the best process for the ammonia reduction [40].

**Nitrate (NO$_3$-N) concentration**

The values of nitrate (NO$_3$-N) concentrations are presented in Fig. 4b. There are three main process that took place in this anion removal from the effluent: denitrification, plant uptake and microbial assimilation/immobilization. For the quaternary solution a variation of nitrate (NO$_3$-N) is noticed through the time, reaching the lowest value on the fifth day (1.8 mg/l). The value obtained on the tenth day was the same as its initial condition (52.2 mg/l). For the others binary solutions, the values tended to increase. The maximum concentration for the binary copper-iron was on the seventh day (150.6 mg/l). For the zinc-iron solution its maximum value was reached on the eighth day (196.2 mg/l). In the case of lead-iron solution the highest value was on the ninth day (128.4 mg/l). Nonetheless, for all the binary solutions, the values obtained on the last day (day 10) were upper than the initial. Based on these results, it can be concluded that *Phragmites australis* in the outset started to increase the values of ammonia (NO$_3$-N) in the binary solutions. However, for all the solutions, on the fifth day, the values were the lowest, except for the binary zinc-iron solution. For the removal of this anion, denitrification, defined as the depletion of two gases: nitrogen gas (N$_2$) and nitrous oxide (N$_2$O) takes place under anoxic conditions [40]. It is a key factor since it is delimited by the lack of organic carbon [41, 42]. It seems that the nitrification process took place instead the denitrification one.

**Sulphates (SO$_4$-S) concentration**

In Fig. 4c, the behavior of sulfate (SO$_4$-S) concentration did not have a significant variation. For the binary copper-iron solution a maximum value (250 mg/l) on the eighth day was reported. However, since fifth to tenth day, in the other solutions, including the quaternary, the values have a constant concentration (200 mg/l).

Table 3 showed the summarized concentration of NH$_3$-N, NO$_3$-N and SO$_4$-S for the initial to the final day. The removal percentage through the experiment was 69% for the binary copper-iron solution, meanwhile the other solutions has a removal between 91%-93%. In that sense, it can be concluded that the *Phragmites australis* can be applied for the ammonia (NH$_3$-N) reduction. For nitrate (NO$_3$-N) concentrations, the removal of 47% in the quaternary solution was determined. However, due to a nitrification process in the binary solutions, the oxidation of ammonium to nitrate occurs in a biologically mediated aerobic reaction [40]. Therefore, an extra study is required. For sulfate (SO$_4$-S) concentrations,
the variations were not significant. It implies that there was a low removal despite of the presence of roots discharge and the organic matter, which help reduce sulfate ions [43, 44].

Table 3 Summary of Average Performance of Treatment Wetlands: variation of ammonia (NH$_3$-N), nitrate (NO$_3$-N) and sulphate (SO$_4$-S)

| Solution         | NH$_3$-N (mg/L) | NO$_3$-N (mg/L) | SO$_4$-S (mg/L) |
|------------------|-----------------|-----------------|-----------------|
|                  | day (0)         | day (9)         | day (0)         | day (9)   |
| Cu-Fe            | 420             | 132             | 69              |           |
| Zn-Fe            | 1276            | 121             | 91              |           |
| Pb-Fe            | 1874            | 142             | 92              |           |
| Cu-Zn-Pb-Fe     | 1554            | 113.8           | 93              |           |
| PWQS*            | 1.5             | 50              | 250             |

*Peruvian Water Quality Standards (PWQS)

Copper removal

The copper concentrations in binary and quaternary solutions are indicated in Fig. 5a. For the binary copper-iron solution, the concentration increases on the fourth day (1.49 mg/l); however, on the tenth day the concentration decreased, becoming the lowest (0.11 mg/l) compared to the initial (0.50 mg/l) and the other measurements. With regard to the quaternary solution, the concentrations decreased through the days until reached a value of 0.10 mg/l.

A comparison between copper and iron behavior in the binary copper-iron solution is presented in Fig. 5b. Based on it, the copper presented a greatest removal compared to the iron, which concentration varied through the time. The highest copper and iron concentration were 1.49 mg/l and 3.52 mg/l respectively. Nonetheless, copper had on the tenth day a lower value than its initial concentration, meanwhile iron has a slightly increment on the same day regard to the initial concentration.

Figure 5h shows the behavior of each metal in the quaternary solution. It can be noticed that copper concentrations were higher than zinc, although since seventh to tenth day, the concentrations were very close. Furthermore, copper concentrations were below that lead and iron. It can be concluded that Phragmites australis is a good alternative for copper removal despite of the coexistence of other metals.

The different recoveries for the copper concentration found during the experiment (Table 3), were similar to the results observed in other studies. For the binary and quaternary solutions, the recoveries until the ninth day were 68% and 87%, respectively. The same tendency can be found in a pilot-scale constructed wetland [45] where the removal of copper was 83%. On the other hand, Lee et al. and Marchan et al. reported copper removal up to 90% in the treatment of urban runoffs and freshwater using constructed wetlands [21, 46].

Zinc removal
The zinc concentrations in binary and quaternary solutions are presented in Fig. 5c. For the binary zinc-iron solution, the concentrations behaved fluctuant through the time. The maximum concentration was on the fifth day (0.573 mg/l), and one of the lowest one was detected on the ninth day (0.214 mg/l). Nonetheless, the concentration obtained on the tenth day was very similar to the initial one. For the quaternary solution the values decreased drastically at the beginning and the variation from first to tenth was insignificant, having 0.106 mg/l at the end of the experiment. However, on the ninth day, the value was the second lowest (0.214 mg/l). In Fig. 5d it can be observed the interaction of zinc and iron in the same effluent. The concentration of zinc presents a slightly variation compared to the iron; however, its final value was slightly upper than its initial concentration. For iron, its concentration increases through time, reaching its maximum on the eight day (4.26 mg/l). Finally, it can be concluded that zinc showed a better performance to be removed than iron.

Figure 5h presented the behavior of each metal in quaternary solution. It can be observed that zinc starts with the highest concentration (day 0), then it decreases and maintained its concentrations below than the other metals, until the seventh day, when it had slightly upper than copper, but remained less than the other metals. It can be concluded that *Phragmites australis* is a good plant for zinc removal.

The results obtained for zinc removal from different effluent show a high difference (Table 3). In the case of the binary solution, a recovery of 53% was reported. This result is similar with the results of 59% previously reported by Ha et al. [47]. In contrast, a higher absorption was observed in the quaternary solution, where approximately 95% of the metal was absorbed. In this case, a similar recovery of 92% and a recovery range between 83% and 90% were found in a pilot-scale constructed and laboratory scale project wetland respectively [45, 48].

**Lead removal**

The lead concentrations in binary and quaternary solutions are presented in Fig. 5e. For the binary lead-iron solution, the concentration of lead decreases until the fifth day, when the value was the lowest (0.06 mg/l). The following days the concentrations increased until the ninth day. Finally, in the last day (10-d), the concentration decreased to 0.21 mg/l, approximately, the half of the initial value. In the case of the quaternary solution the amounts fluctuated through time, reaching their maximum (0.53 mg/l) on the fifth day, meanwhile the lowest value was on the ninth day (0.14 mg/l). In addition, on the tenth day, it increased having the same value than its initial condition (0.31 mg/l).

The comparison between lead and iron removal in the binary lead-iron solution is presented in Fig. 5f. It can be noticed, despite of both metals started with similar initial conditions (0.46 mg/l), lead concentrations are below the iron results. However, similar values on the second, eight and tenth day were observed. The maximum values that lead (0.37 mg/l) and iron (0.68 mg/l) were observed on the ninth day and first day, respectively. Finally, both metals reached concentrations below their initial conditions.

For quaternary solution, Fig. 5h shows the interaction of the four metals. It can be noticed that lead concentration was above those of copper and zinc. In addition, they show similar values on the ninth day.
However, lead presents the maximum value on the fifth day, which corresponds to the highest value compared to the other three metals for this day. Table 3 shows lead removal about 20% and 55% for binary and quaternary solution, respectively. These results were similar to previously reported by Ha et al. in a constructed wetland project that presented 30 days of evaluation [47]. In addition, a higher range of absorption (76–95%) was reported previously by Mungur et al. in a laboratory wetland with a larger time of analysis [48]. In this case, it could be possible that the duration of the project plays an important role in the lead removal.

Iron removal

The iron concentrations and the variation during the experiment in the different cells are shown in the Figs. 5b, 5d, 5f and 5h, for the copper-iron, zinc-iron, lead-iron, and the quaternary solution, respectively.

The behavior presented in the Fig. 5b correspond to the binary copper-iron solution. In this graphic, it can be appreciated that Fe had fluctuating values during the development of the experiment. The maximum values were registered on the third and sixth day, and the values were 2.99 mg/l and 3.52 mg/l, respectively. Nonetheless, it can be appreciated that the general trend in concentration is increasing. A similar tendency is founded in the binary zinc-iron solution (Fig. 5d). From the beginning of the evaluation to the day seven, the values varied between 0.35 and 1.99 mg/l. After that, a high increase happened, and the maximum value was registered on the eighth day (4.23 mg/l).

On the other hand, erratic values of iron were found on the binary lead-iron solution (Fig. 5f). During the first days, it increased and decreased to a minimum concentration of 0.18 mg/l on the second day. After that, the values rose again until the seventh day (0.54 mg/l). The values behaved erratic again after the moment commented before. In contrast to the case of the zinc-iron solution, there was a smaller gap between the values of lead and iron during the experimental procedure.

The quaternary solution shows the variation of Fe with the influence of the other metals in the same effluent (Fig. 5h). On the ninth day the concentration of iron was 0.27 mg/l, nearly to the day zero (0.28 mg/l). Nevertheless, on the tenth day the value increased suddenly to 3.62 mg/l. If we compare the iron concentration in binary and quaternary solution, it can notice that only in the quaternary solution, a slight decrease was observed (4%). Contrary to these values, other studies have demonstrated the efficiency of *Phragmites Australis* for absorbing iron, with optimal recoveries that varied between 70% and 90% [19, 22].

The results obtained on the initial (day 0) and ninth day for copper, zinc, lead and iron removal were summarized in Table 4.

Reductions of copper in the binary copper-iron solution and the quaternary solution, were 68% and 87%, respectively. In the case of zinc, the absorption by *Phragmites Australis* were 53% (binary solution) and 95% (quaternary solution). In addition, lower recoveries of lead were recorded in the cells that contained lead (20% and 55%). Then, it can be appreciated that *Phragmites Australis* has a better performance in the absorption of copper, zinc, and lead when they are together in the quaternary solution. However, the
iron concentration increased in the majority of the effluents, and only in the quaternary solution a slight reduction of 4% was registered.

The recoveries obtained shows a different tendency in the absorption of heavy metals by *Phragmites Australis*. In this situation, the highest reduction was detected for copper, followed by zinc, lead, and finally iron. A similar trend is found by Southichak *et al.* in a comparable project about constructed wetlands [22]. However, other studies show greatest recoveries of zinc [20, 48].

Finally, we can observed, that the final concentrations of copper, zinc, and iron on the ninth day were below the Peruvian maximum permissible water limits (PMPWL) [49]. In the same way, they were lower than the Peruvian water quality standards (PWQS) [50]. In the case of lead removal, it is observed that on the ninth day, the concentrations comply only with the PMPWL. Therefore, a more detailed study should be carried out.

**Table 4** Summary of Average Performance of Treatment Wetlands: variation of copper, zinc, lead and iron concentration in *Phragmites Australis* wetlands.

| Solution       | Cu (mg/l) | Zn (mg/l) | Pb (mg/l) | Fe (mg/l) |
|----------------|-----------|-----------|-----------|-----------|
|                | day (0)   | day (9)   | day (0)   | day (9)   | day (0)   | day (9)   |
| Cu-Fe          | 0.50      | 0.16      | -         | -         | -         | 0.26      | 0.64      |
| Zn-Fe          | -         | -         | 0.45      | 0.21      | 53        | -         | 0.35      | 1.33      |
| Pb-Fe          | -         | -         | -         | -         | 0.46      | 0.37      | 0.47      | 0.54      |
| Cu-Zn-Pb-Fe   | 0.97      | 0.13      | 1.49      | 0.08      | 95        | 0.31      | 0.14      | 55        |
| PMPWL*         | 0.5       | 1.5       | 0.2       | 2         |
| PWQS**         | 2         | 3         | 0.01      | 0.3       |

*Peruvian Maximum Permissible Water Limits (PMPWL)
**Peruvian Water Quality Standards (PWQS)

**Determination of the optimal removal cycle**

Based on the heavy metal removal results obtained through the different solutions and the variation of the physicochemical parameters, an effective cycle of absorption was determined. The optimal range was 9 days, and this is due to the metal removal performance of the studied species. It was observed that, in general, after the ninth day, the heavy metal concentration started to increase again. It can be explained by the beginning of the desorption cycle of the *Phragmites Australis*. Due to this fact, it can be determined that the plant under the study condition has a short-term effect, and the removal process will require additional study.

**Conclusion**

A range from 69–93% of ammonia (NH$_3$-N) can be removed by *Phragmites Australis*. However, nitrate (NO$_3$-N) can be removed only in a 47%. An optimal removal heavy metals cycle of 9 days was established. The effect on the variation of metal concentrations was tangible. In the case of Cu, the
reduction in the binary and the quaternary solution were 68% and 87%, respectively. For Zn, *Phragmites Australis* generated the absorption of 53% of the metal in the binary solution, and 95% in the quaternary solution, which is the highest recovery produced by the species. Lower recoveries were observed in the case of Pb, with only 20% and 55%. In the quaternary solution a small reduction of Iron (4%) was obtained. *Phragmites Australis* seems to be an attractive species to remove heavy metals as Copper, Zinc and Lead. However, an extra analysis must be done.

In summary, the effect of *Phragmites Australis* in the reduction of the physicochemical parameters and metals analyzed during this investigation can be relevant during a short period of time, as was demonstrated throughout the empirical results obtained. Its behavior makes it an ideal candidate for the treatment of the local liabilities close to Peruvian mines. Nonetheless, the effect of the species in Fe concentration requires additional study due to the reverse effect that has been observed.

**Declarations**

**Acknowledgments**

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**Author's contributions**

MR and MF carried out the experiments, collected the samples, analyzed the heavy metals content in the collected water samples and analyzed the data. SB helped acquire field and laboratory data and carried out the statistical analysis. Dr. MG supervised the project, wrote, revised and approved the manuscript to be published. All authors proposed the study and participated in writing the manuscript. MR, MF and SB prepared the manuscript, Dr. MG edited the manuscript.

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**Competing interest**

The authors declare that they have no conflict of interest in the publication.

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Figures

![Figure 1](image)

**Figure 1**

Construction of the laboratory wetland, a plants conditioning in water previous to the experiment, b dimensions and general view of the constructed wetland, c details of plants distribution in the cell.
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Construction of the laboratory wetland, a plants conditioning in water previous to the experiment, b dimensions and general view of the constructed wetland, c details of plants distribution in the cell.
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Figure 2

Variation of, a pH, b dissolved oxygen in Phragmites Australis wetlands

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Figure 3
Variation of, a turbidity, b electrical conductivity in Phragmites Australis wetlands.
Figure 3

Variation of, a turbidity, b electrical conductivity in Phragmites Australis wetlands.
Figure 4

Variation of, a ammonia (NH3-N), b nitrate (NO3-N), c sulfate (SO4-S) in Phragmites australis wetlands.
Figure 5

Variation of, a copper concentration in the binary Cu-Fe and in the quaternary Cu-Zn-Pb-Fe solution, b copper and iron concentration in the binary Cu-Fe solution, c zinc concentration in the binary Zn-Fe and in the quaternary Cu-Zn-Pb-Fe solution, d zinc and iron concentration in the binary Zn-Fe solution, e lead concentration in the binary Pb-Fe and quaternary Cu-Zn-Pb-Fe solution, f lead and iron concentration in
the binary Pb-Fe solution, g iron concentration in the binary and quaternary solutions, h metal concentration in the quaternary solution.

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Variation of, a copper concentration in the binary Cu-Fe and in the quaternary Cu-Zn-Pb-Fe solution, b copper and iron concentration in the binary Cu-Fe solution, c zinc concentration in the binary Zn-Fe and in the quaternary Cu-Zn-Pb-Fe solution, d zinc and iron concentration in the binary Zn-Fe solution, e lead
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