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Phytoremediation with *Schoenoplectus Americanus* and *Eichhornia Crassipes* in Cyanide Effluents

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In Peru, the gold mining industries use cyanide in leaching processes, being the main sources of contamination of ecosystems, current techniques with living organisms are being used to absorb and inhibit the effect of contaminants, such as phytoremediation that through green plants are used to remove exposed contaminants from an area, remediating it to its natural state. The objective of the research was to evaluate the phytoremediation capacity of Schoenoplectus americanus and Eichhornia crassipes, on the cyanide concentration in effluents from the Paltarumi gold mine. A laboratory-scale phytoremediation system was implemented as an alternative treatment, it was executed in pots with different treatments, water from a wetland near the sea, plus cyanide effluent. The results showed that the color of the stems, leaves and roots changed to darker colors with necrotic points, it was evidenced that cyanide affected the growth of the plants, highlighting the Schoenoplectus americanus that achieved a greater height. In root length, Eichhornia crassipes had more extensive roots. In the remaining cyanide in the aqueous solution, the Eichhornia crassipes macrophyte had the highest cyanide retention capacity and in relation to absorption, it was the treatment with Schoenoplectus americanus + Eichhornia crassipes that absorbed over the greatest amount of cyanide with 18,673 $mg.L^{-1}$ equivalent at 84.8 %, followed by Schoenoplectus americanus and Eichhornia crassipes with 80.5 % and 78.3 % respectively. Therefore, the use of phytoremediation plant species for cyanide absorption is a good alternative as it has environmental advantages due to the non-use of chemical products, low cost and easy implementation.

1. Introduction

The amount of wastewater generated by the use of water for human activities and industry is accelerating rapidly, the problems associated with wastewater will intensify in the future due to water scarcity and population growth, so Therefore, prompt actions must be taken to create a wastewater treatment technique to produce water of a certain quality (Kanafin et al., 2022). In Latin America, extractivism is an intensive and extensive form of extraction of common goods, as evidenced in the case of mining, which for more than a decade became one of the most important productive activities, and which produced a high degree of conflict over the use of land, water and damage caused to the environment (Yacoub et al., 2015). Mining companies are one of the main sources of contamination of ecosystems, due to the development of their mining activities, transportation, construction and processing of minerals; during its beneficiation stage, cyanide effluents from these mining companies are discharged into the soil and water sources, generating contaminated areas with high concentrations of toxic metals. It is estimated that, for a long period, cyanide leaching will continue to dominate gold (Dong et al., 2021). In 1887, a team of Glasgow chemists and physicians developed what became known

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as the Macarthur-Forrest cyanidation process. In essence, it involves dissolving gold in a dilute cyanide solution, which is then brought into contact with zinc, so that the gold (Verbrugge et al., 2021). In Peru, the gold mining industries use cyanide leaching processes, which is why over the years the contamination of natural resources has been observed, putting people's health at risk. The mining industry has been an important part of the economy for many decades. Phytoremediation technology may represent a low-cost option for the remediation of industrially contaminated areas (Latan, 2021). Eichhornia crassipes is a floating macrophyte, and its availability in mining regions of South America makes it an appropriate candidate for the treatment of cyanide effluents (Ebel et al., 2007). This research proposes to evaluate the phytoremediation capacity of Schoenoplectus americanus and Eichhornia crassipes on the concentration of cyanide from the effluent of the Paltarumi mine, in order to reduce environmental impacts in areas contaminated with cyanide.

2. Methodology

There are different experimental steps in this investigation, the operating conditions for each of the steps are explained in the following subsections.

2.1 Place of development of the investigation

The analyzes were carried out in a laboratory certified by INACAL located in the province of Huaura-Peru. The research process lasted seven months, beginning with the preliminary phase, followed by the field, laboratory, and office phases. The investigation followed a non-probabilistic quantitative experimental design and a correlational scope to know the relationship between the variables, two phytoremediation plants such as Schoenoplectus americanus and Eichhornia crassipes were subjected to a single concentration of cyanide, allowing to establish comparative groups of the control sample with the treatments and repetitions, to determine results that allow validating or rejecting the proposed hypothesis (Jaramillo & Flores, 2012).

2.2 Collection of cyanide effluent and water from the Carquín-Hualmay wetland

The collection of cyanide effluent samples was carried out by the company CERTIFICAL S.A.C., strictly following the sampling protocols, according to Guide No. 4 Use and management of cyanide in small-scale mining (MINEM, 2012). The containers with the cyanide sample were labelled. A total of 2 L was taken for the execution of the test preserved in the laboratory of the company Felles E.I.R.L and 500 mL for the laboratory of the CERTIFICAL S.A.C. analysis, allowing to obtain a knowledge of the initial concentration of cyanide in the effluent. The methodology used for cyanide analysis by the CERTIFICAL S.A.C. It is the SM 4500-CN which consists of analyzing by means of a titrimetric selective ion electrode procedure using an alkaline distillate allowing the quantification of cyanide complexes in a sample. The samples of water contaminated with cyanide were made in the Carquín-Hualmay wetland, for the quantification of total cyanide, 500 mL was delivered to the CERTIFICAL S.A.C. laboratory, while 64 L were transported to the Felles E.I.R.L test laboratory for study. After the recognition of the same place, the Schoenoplectus americanus and Eichhornia crassipes plants were collected, taking into account the following criteria in the collection of macrophytes: coloration, youth, homogeneity in root and leaf size.

2.3 Obtaining aquatic macrophytes

For the extraction of aquatic macrophytes, the methodology of permanent sampling plots was used, using a grid built with 1 x 1 m polyethylene tubes. In the sampling areas, apart from Schoenoplectus americanus and Eichhornia crassipes, other species such as Torulinium odoratum, Sesuvium portulacastrum, Amaranthus quitensis, and Ludwigia peploides were found. At this level, the first thing that was done was the recognition and description of the state of the collected Schoenoplectus americanus and Eichhornia crassipes plants through metric measurement and detailed observation of the pigmentation, discarding the necrotic areas, with the purpose that the plants used in the treatment are homogeneous and thus show the differences in each evaluation phase after having been (Jaramillo & Flores, 2012)

2.4 Laboratory stage (Adaptation, nutrition and intoxication phase)

In the adaptation phase, the plants selected for the experimental evaluation followed an adaptation period of three days under laboratory conditions. Sixteen plasticized experimental containers with a height of 24 cm, a diameter of 23 cm and a capacity of 6 L were prepared, filled with 2 kg of sand that served as a support for the macrophytes plus 3,900 mL of water from the Carquín wetland and its respective number of phytoremediation plants. In the experiment, a total of 80 Eichhornia crassipes plants and 64 previously selected Schoenoplectus americanus plants were used, which were distributed within the containers according to the four treatments with four repetitions (Montesdeoca, 2019). In the nutrition phase, 11.7 mL of the hydroponic solution "A" and 7.8 mL of the concentrated solution "B" were applied to each container of 19.5 mL capacity. In the intoxication phase, the cyanide concentration of the effluent was analyzed. Using a measuring cylinder, 100 mL of previously homogenized cyanide effluent was added. For the measurement of pH and electrical conductivity, a rigorous control of each treatment was carried out, measured every 24 hours.

3. Results and Discussion

The results obtained are presented below, the color, height of the foliar and root system were evaluated, in its 3 phases, adaptation, nutrition and intoxication, as well as the physicochemical characteristics, performing a detailed interpretation. The means were compared through Duncan's significance test ($p \leq 0.05$).

3.1 Leaf system color

The evaluation of the color of the plants under study, between the nutrition and intoxication phases, showed notorious changes such as in the foliar area with a green color change with the beginnings of a necrosis on the edge of the leaves at the end of the intoxication phase, manifesting in this way the effect of the added cyanide that also affected the normal growth of the plants of both species studied except in the control treatment that remained with its color as at the beginning of the experiment. The change in color of the leaves of a plant or rather the change from bright green to dark brownish green in the case of Eichhornia crassipes (Salas Ruiz, 2019) and yellowish green in Schoenoplectus americanus are signs of necrosis because the plant has extracted toxic elements, among them cyanide, meaning that the plant has been affected by the presence of cyanide effluent and that its death is imminent.

3.2 Root system color

In the nutrition phase, color changes were observed in the root system of Eichhornia crassipes with the appearance of dark pigments, which have the function of generating protection for the roots of the aquatic plant against ultraviolet radiation and viral or microbial contamination, being This condition is a sign that the roots were in optimal conditions of development, especially taking into account that the treatments received the necessary nutrients (Salas Ruiz, 2019). On the contrary, in the intoxication phase it was observed that the roots had a much darker coloration and with the beginnings of a progressive death of the cells caused by the application of the effluent contaminated with cyanide, being a sample that the Schoenoplectus americanus and Eichhornia crassipes were absorbing the contaminant.

3.3 Evaluation of plant height and root length

The height of the plants in the adaptation phase, the treatment with Schoenoplectus americanus was 51.78 cm, followed by the Eichhornia crassipes + Schoenoplectus americanus with 31.15 cm, presented the greatest stem length and the control treatment with Eichhornia crassipes showed the lowest growth taking values of 31.15 cm and 12.75 cm, indicating that the height of the plant is different due mainly to genetic factors. As reported (Montesdeoca, 2019) who indicates that the resistance, adaptation, growth and useful life is influenced by the genetics of the plants. As reported by (Gomez & Guarín, 2020) plant growth is influenced by light, temperature, humidity, water and nutrients, which are essential components for its successful development. For the height of the plants in the nutrition phase, the treatment with Schoenoplectus americanus was 52.1 cm, followed by the Eichhornia crassipes + Schoenoplectus americanus with 32.3 cm, presented the greatest length of roots and the control treatment with Eichhornia crassipes showed the lowest growth taking values of 31.2 cm and 15.55 cm, indicating that the plant height is different due to genetic factors. As reported (Domínguez et al., 2016), They affirmed that plant nutrients are assimilated according to the genetics of each plant, influencing its development. Also, as reported by (Paredes & Ñique, 2015) indicated that plants absorb nutrients through their leaf stomata and from the soil dissolution through their roots, allowing them to develop normally and fulfill their life cycle. For the height of the plants in the intoxication phase, the treatment with Schoenoplectus americanus was 52.103 cm, followed by Eichhornia crassipes + Schoenoplectus americanus with 32.3 cm, presented the highest leaf height and the control treatment with Eichhornia crassipes showed the lowest growth taking values of 31.6 cm and 15.55 cm. (Paredes & Ñique, 2015) reported that in contaminated media the development of plants is influenced by the genetic characteristics of adaptation and resistance. Furthermore, as reported by (Ayala et al., 2018), indicated that according to the variety of plant species and the dose of contamination that is exposed, the propagation of seeds is reduced and their growth is retarded. For the length of the roots in the adaptation phase, the treatment with Eichhornia crassipes was 24.55 cm, followed by the Eichhornia crassipes + Schoenoplectus americanus with 24.3 cm, presented the greatest length of roots and the control with Schoenoplectus americanus showed the lowest growth taking values of 24.025 cm and 19.025 cm, which is related to what was reported (Paredes & Ñique, 2015), who indicate that the development of the root system is basically conditioned by factors such as temperature, aeration, humidity from where it absorbs nutrients and mineral salts for the formation of primary and secondary roots. For the root length in the nutrition phase, the treatment with Eichhornia crassipes was 26.45 cm, followed by the Eichhornia crassipes + Schoenoplectus americanus with 26.325 cm, presented the greatest length of roots and the control with Schoenoplectus americanus showed the lowest growth taking values of 24.55 cm and 19.925 cm. For the length of the roots in the intoxication phase, the treatment of Eichhornia crassipes + Schoenoplectus americanus with 26.6 cm followed by Eichhornia crassipes was 26.45 cm, they presented the greatest length of roots and the control treatments, Schoenoplectus americanus showed the lowest growth taking values of 24.85 cm and 19.925 cm. As pointed out by (Sandoval, 2019) indicated that it is attributed to the genotypic differences between species that were affected by the presence of contaminants in the water.

3.4 Assessment of plant mortality

None of the phases that were considered in the investigation died any plant. However, in the intoxication phase necrosis was observed at the edges of the leaves, both Schoenoplectus americanus and Eichhornia crassipes are species with a high degree of resistance to this type of contaminant and that probably have the capacity to remediate media contaminated with cyanide. . It is probable that, if the plants of both species remained for a longer time, the death of many of them would occur ((Gomez & Guarín, 2020)

3.5 Evaluation of the physical-chemical characteristics

For the pH in the intoxication phase, the results showed that the treatment with Schoenoplectus americanus had the highest pH with 8.08, significantly higher than the other treatments, followed by Eichhornia crassipes + Schoenoplectus americanus with 7.85, then the control treatment with Eichhornia crassipes, which showed a lower pH increase taking values of 7.837 and 7.736. For electrical conductivity in the intoxication phase, the results showed that the treatment with Schoenoplectus americanus had the highest EC with 4,757 $dS.m^{-1}$, significantly higher than the other treatments, followed by Eichhornia crassipes + Schoenoplectus americanus with 4,693 $dS.m^{-1}$ after the control treatment with Eichhornia crassipes, which showed a lower increase in EC, taking values of 4,233 and 3,757 $dS.m^{-1}$.

3.6 Analysis of cyanide effluent, wetland water, final concentration of cyanide remaining in the water and in plants

The contaminated effluent had a concentration of 880.3 mg.L-1 of cyanide, the cyanide concentration of the wetland water was in a very low range of 0.005 mg.L-1. Table 1 shows the cyanide, pH and electrical conductivity contents of both the mining effluent studied from the company Paltarumi S.A.C, as well as in the water from the Carquín - Hualmay wetland.

Table 1: pH, electrical conductivity, and initial cyanide content in effluent and wetland water l

Sample	pH	Electric conductivity ($dS.m^{-1}$)	cyanide content ($mg.L^{-1}$)
Paltarumi Mine Effluent	12.5	2.43	880.3
Water from the Carquín wetland	7.71	3.70	0.005

The analysis of variance showed a high statistical significance (p-value < 0.01) in the treatment source, indicating that the final concentration in the water is different in the treatments, due to the absorption of cyanide by the plants. The results of the Duncan test showed that the treatment with Eichhornia crassipes stands out in the final concentration in the water with 4,774 g of cyanide, significantly higher than the other treatments, followed by Schoenoplectus americanus with 4,281 g, then the Schoenoplectus americanus + Eichhornia treatment. crassipes with 3,339 g and finally the control treatment that had a concentration of 0 since it only presented wetland water and no cyanide effluent.

Table 2. final concentration of cyanide in the water

Repeticiones	Schoenoplectus americanus (g)	Eichhornia crassipes (g)	Schoenoplectus americanus + Eichhornia crassipes (g)	Control (g)
I	4.567	3.233	3.577	0.005
II	3.921	4.979	2.784	0.005
III	4.635	5.455	3.921	0.005
IV	4,002	5.429	3.074	0.005
AVERAGE	4.281	4.774	3.339	0.005

Regarding the higher concentration of cyanide in the remaining solution, it is clearly noted that Eichhornia crassipes was more efficient in cyanide removal than Schoenoplectus americanus, this coincides with what was reported by(Vera, 2016) who highlights the efficiency of the plant in the removal of contaminants. Table 2 shows the results obtained from the final concentration of cyanide in the water used in the experiment in the intoxication phase.

It is observed that the treatments have the following order of absorption by the plant: Schoenoplectus americanus + Eichhornia crassipes > Schoenoplectus americanus > Eichhornia crassipes > Control. By verifying whether the data from the population from which the sample comes has a normal distribution because the p-value is greater than 0.05 ($\alpha = 0.05$) in all treatments. The analysis of variance shows high statistical significance (p-value < 0.01) in the treatment source, indicating that the amount absorbed by the plant is different in the treatments and the results of the Duncan test showed that the treatment with Schoenoplectus americanus + Eichhornia crassipes with 18,673 stands out in the concentration assimilated by the plant followed by Schoenoplectus americanus with 17,731 then the Eichhornia crassipes treatment with 17,238 and finally the control treatment that had the lowest concentration with 0 and was significantly lower than the other treatments. Figure 1 shows amount of cyanide absorbed by plants.

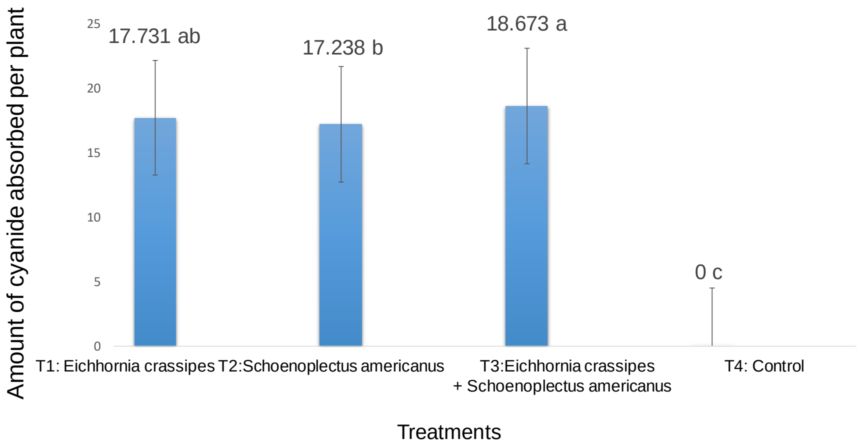

Figure 1. Duncan's test for the amount absorbed by the plant

Regarding the concentration of cyanide extracted by the plants, verifying that the Schoenoplectus americanus + Eichhornia crassipes stands out in the extraction of cyanide, as indicated by (Sandoval, 2019), by ensuring that the species Eichhornia crassipes can achieve up to 83.57% of the cyanide present in a contaminated environment, such as the cyanide effluent, the subject of this study. Which is somewhat related to what was reported by (Calle & Coello, 2015), who indicated that aquatic plants such as Eichhornia crassipes, Schoenoplectus americanus have the ability to absorb between 65 to 95% of polluting metals in their roots and leaves.

4. Conclusion

There were no significant differences in the height of the plants or the length of the roots, this was due more to the genetic factor of each of the species under study, where Schoenoplectus americanus achieved greater height, and Eichhornia crassipes achieved greater growth in root length. The species Schoenoplectus americanus is more efficient in the absorption of cyanide metal, managing to eliminate up to 80.55 %, while Eichhornia crassipes achieved 78.31 %, demonstrating that both species are phytoremedial species of said metal. A greater absorption of cyanide was obtained in the combined treatment Schoenoplectus americanus + Eichhornia crassipes plants in 84.83 %, so it is advisable to use it in this way.

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