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Efficiency of Bioreactors in the Removal of Heavy Metals in Acidic Metallic Mining-Influenced Water in Ponce Enríquez, Ecuador

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ABSTRACT

The mining effluent, acid mine drainage (AMD), is a major global environmental concern due to its high heavy metal content and highly acidic pH, which contaminates water and compromises the well-being of ecosystems and human health. Mining activity in southern Ecuador is characterised by artisanal and small-scale mining that exploits gold, silver, and copper, registering environmental problems associated mainly with river pollution. The objective of this study was to assess river water quality at 28 points in the Camilo Ponce Enríquez canton and subsequently evaluate the efficiency of various AMD bioremediation techniques with different components, including two different types of bacteria and sugarcane bagasse, by applying statistical methods and considering regulatory criteria. The proposed methodological approach consists of i) physicochemical characterisation of AMD, ii) implementation of pilot bioreactors, and iii) statistical study of bioreactor efficiency. The results show significant water contamination in rivers by AMD, resulting in heavy metal content of at least 0.1 ppb and greater than 1000 ppb in areas close to mining activity, exceeding the Ecuadorian maximum permissible limits. Statistical analysis of the bioreactor performance indicates that bioreactors containing bagasse and sulfate-reducing bacteria (SRB) demonstrated the most efficient techniques for the removal of heavy metals, reaching an average removal range of 85.35% and 89.64%, respectively, for metals such as Al, Cd, As, Cu, Fe, and Ni. This study provides a solid basis for using agricultural waste, such as sugarcane bagasse combined with SRB, to remove heavy metals in situ on a large scale to mitigate the environmental impacts of mining activity.

1. INTRODUCTION

Mineral resources are the basis of socioeconomic development, and mining is the most important activity for extracting metals and non-metals globally [1]. However, the generation of high quantities of solid and liquid waste from mining activities represents an environmental and social threat [2] associated with air, soil, and water pollution [3-6].

Surface and groundwater resources are constantly threatened by climate change and pollution caused by anthropogenic activities such as agriculture, energy production, industry, and mining [7, 8]. Water pollution caused by the accumulation of heavy metals from the runoff of mining effluents and tailings in active and abandoned mines leads to acid mine drainage (AMD) [9, 10], also known as acid rock drainage (ARD). This drainage is characterised by low pH levels (usually less than 4) with a high content of metallic ions such as iron, zinc, aluminium, lead, arsenic, and manganese [11].

The discharge of untreated AMD into soil or water bodies contaminates resources and compromises aquatic and terrestrial flora and fauna. AMD also causes crop contamination, posing a potential risk to human health [12], [13]. In this context, there is an evident need for remediation strategies or methodologies to mitigate environmental contamination [14].

AMD-related research has focused on sustainable and cost-effective remediation techniques to reduce or eliminate AMD and avoid the generation of byproducts, which are very common in current traditional mining methods [15]. AMD remediation techniques are divided into two broad categories: active and passive systems, distinguished by their monitoring, the addition of chemicals, infrastructure, or applied maintenance [16, 17]. Passive remediation techniques use natural and biological processes that stimulate microbial sulfate reduction and contaminant absorption [18, 19], including constructed wetlands, limestone, vertical-flow wetlands, and sulfate-reducing anaerobic bioreactors [20-22].

One bioremediation method involves using bacteria under controlled environmental conditions to mitigate the contamination of degraded sites or to reduce the contamination levels to levels that are tolerable to the environment [15]. This methodology is closely related to the type of bacteria used and the control of parameters, such as pH, temperature, oxygen, and nutrients [23]. Other bioremediation methodologies include oxidation-reduction [24], biomineralisation-bioprecipitation [25], bioleaching [26], biosurfactant technology [25], biovolatilisation [27], biosorption-bioaccumulation [28], and microbe-assisted phytoremediation [29, 30]. These procedures can be applied in situ and ex situ, the latter being a process that consists of removing or excavating contaminated materials or water from the area to treat it in containers, generally in a laboratory where monitoring of the procedure is facilitated [31]. These methodologies still present limitations for their implementation, highlighting the sensitivity of biological mechanisms, operation time, large-scale applicability, and economic aspects that tend to reduce the efficiency of heavy metal elimination [32, 33].

In this context, the present study seeks to contribute to the existing research gap in the efficiency of bioremediation techniques for the removal of heavy metals from water through a statistical analysis of the performance of bioreactors operating on AMD samples taken in the Camilo Ponce Enriquez Canton in southern Ecuador for subsequent studies in the Water Quality Laboratory of the Escuela Superior Politécnica del Litoral (ESPOL). The Camilo Ponce Enriquez mining area in southern Ecuador has one of the highest gold extractions in the country, with more than 350 mining concessions, of which more than 65% are related to artisanal mining [34]. This intensive activity has affected the ecosystem, contaminated surface and underground water bodies and compromising human well-being [35]. An example is the presence of heavy metals in crops that exceed the permissible limits in national and international regulations, representing a carcinogenic risk to inhabitants of the area [36]. These effects are caused by the scarce or non-existent treatment of mining effluents discharged into nearby water bodies, resulting in contamination by heavy metals [37, 38]. Another reason for selecting this study area was the proximity to the sugar mill. Sugar bagasse is one of the key elements in the bioremediation techniques being tested. The proximity of the mill facilitates future in situ implementation of the selected method. The selection of Ponce Enriquez as the study area for this research was also based on the openness provided by one of the mines in the sector to carry out the necessary sampling in its facilities. This is often a controversial topic owing to the impact of mining activity in this area.

This study aimed to evaluate the efficiency of various bioremediation techniques using a Permutational Multivariate Analysis of Variance (PERMANOVA) applied to the removal effectiveness results of 25 heavy metals by seven different bioreactors containing AMD samples. This study identifies the most efficient technique that decision-makers can implement as a large-scale tool to reduce water pollution from mining activities.

2. MATERIALS AND METHODS

This research proposes a methodological approach to evaluate the efficiency of bioremediation techniques for removing heavy metals contained in AMD, combining statistical methods of multivariate analysis. Once the efficiency of the evaluated techniques is known, this research defines the recommended pilot bioreactor for the study area and areas with similar characteristics. The study was carried out in three phases: i) water quality study, ii) implementation of pilot bioreactors, and iii) statistical study of the efficiency of bioremediation techniques (Figure 1).

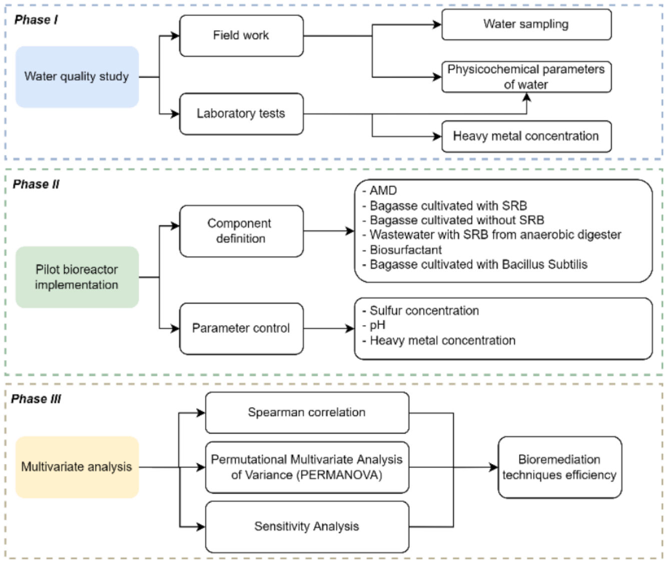

Figure 1. General methodological scheme of the study

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2.1 Phase I: Water quality study

This phase included collecting 28 water samples from the Fermín, Pagua, Siete, and Estero Guanache Rivers (Figure 2). The location of the sampling stations depended on accessibility to the study area, proximity to mines or mineral processing plants, and the location of populated areas. In situ measurements of physicochemical parameters such as temperature, oxygen, pH, electrical conductivity, and dissolved oxygen (DO) were performed at the sampled stations. On the other hand, the collected samples were transferred to the laboratory (content in 100 ml plastic containers, and preservation was carried out by adding 2% v/v HNO_3) to analyse heavy metal. The heavy metal study was conducted at the University of Colorado (Boulder), where the concentrations of 25 heavy metals were identified (Table S1). A Perkin Elmer SCIEX inductively coupled plasma mass spectrometer (Elan DRC-e) was used with indium as the standard solution.

The concentrations of heavy metals from the dry season sampling (where the highest concentrations were recorded) were used as a basis for the definition of the geographic distribution of the 13 metals relative to current national regulations in the Unified Text of Secondary Legislation of the

Ministry of the Environment (TULSMA, acronym in Spanish) [39], aimed at the preservation of aquatic and wildlife life in freshwater. The geographic distribution of heavy metals was determined by the average concentration by season and interpolation of data using ArcGIS version 5.0, allowing the visualisation of different categories of concentrations and the metals present.

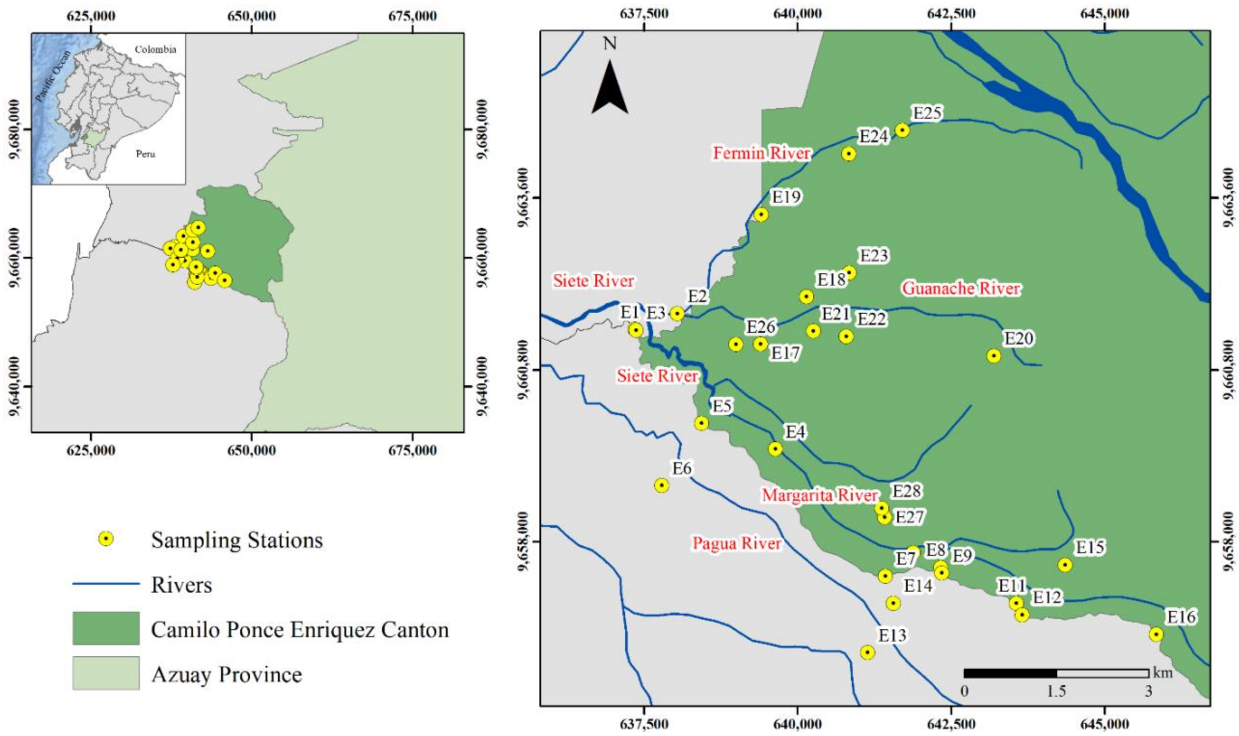

Figure 2. General location map of the studied area and water sampling points

2.2 Phase II: Pilot bioreactor implementation

The methodological process for the implementation of bioremediation techniques consists mainly of the use of sulfate-reducing bacteria (SRB) and agricultural wastes such as sugarcane bagasse [40]. Sugarcane bagasse fulfilled two objectives: i) as a food source for the bacterial colony, and ii) as a retention matrix for heavy metals. This technique was used because of satisfactory results reported by Grubb et al. [41]. Seven bioreactors (R) were installed, and their characteristics are listed in Table 1. Five bioreactors were designed using a combination of AMD, bagasse, and SRB. Two bioreactors used diverse elements, including another variety of bacteria (Bacillus subtilis) and a biosurfactant.

Table 1. Bioreactor characteristics

Bioreactor (R)	Combinations
R1	AMD + Bagasse cultivated with SRB
R2	AMD + Bagasse cultivated with SRB
R3	AMD
R4	AMD + Bagasse not cultivated with SRB
R5	AMD + domestic wastewater with SRB from anaerobic digester
R6	AMD + biosurfactant (laboratory-made)
R7	AMD + bagasse cultivated with Bacillus Subtilis type bacteria

AMD samples were taken from one of the mines in the study area, which facilitated access to and sampling of mine effluents as well as SRB from domestic wastewater produced by an anaerobic biodigester at the mine. Bioreactor operational assessment was based on weekly monitoring of the concentration of sulphide produced to verify bacterial activity and pH to verify the acidity of each bioreactor. Heavy metal concentrations were measured by taking water samples from each bioreactor every two weeks for four months (March-June).

2.3 Phase III: Multivariate analysis

The analysis phase employed a flat file (CSV) containing data from the bioreactors and the measurement of 25 heavy metals (Al, Fe, Pb, and Cu). Statistical analysis using the Shapiro-Wilk test determined that these data were not normally distributed ($W=0.92$, p-value$=0.0000023$, p-value<0.001) [42]. Therefore, correlation analysis (magnitude of relationship) was evaluated using the nonparametric Spearman correlation coefficient. The correlation ranges are: 0.2 – 0.39 (weak correlation), 0.4 – 0.59 (moderate), 0.6 – 0.79 (strong), and 0.8 to 1 (very strong) [42].

Subsequently, a Permutational Multivariate Analysis of Variance (PERMANOVA) was performed to demonstrate the significant differences in the behavior of the seven bioreactors [43]. This type of analysis facilitates the evaluation of the efficiency of the bioreactors in the removal of heavy metals in AMD. To do so, the following statistical methods were used to evaluate the similarity of the removal behavior of the bioreactors and the validation of their efficiency:

i) Non-metric MultiDimensional Scaling (NMDS) was used to determine the similarity of the behaviour of each bioreactor, that is, the concentration (significantly less different) or dispersion (significantly more different) of the heavy metal samples in each bioreactor [44].

ii) Bray-Curtis distance matrix using the vegdist function to calculate dissimilarity indices [45].

iii) Homogeneity of multivariate dispersion through betaspider function and permutational dispersion analysis (perm disp) determine whether bioreactor groups differ significantly in multivariate dispersion [46].

iv) Analysis of Variance (ANOVA), where the p-value was more significant or less than 0.05, was used to determine whether the groups differed significantly in multivariate dispersion [47].

v) PERMANOVA using the adonis2 function to establish significant differences in the bioreactor characteristics for heavy metals [48].

The statistical tests employed in this study allow for a detailed analysis of bioreactor performance. In particular, the PERMANOVA method was used to evaluate bioreactor performance. ANOVA was used to analyze the differences observed between the various bioreactor behaviors, and NMDS graphically represented the variations in microbial communities, thus facilitating the identification of behavioral patterns. This combination of statistical tools provides a comprehensive analysis of bioreactor performance because of its capacity and robustness in handling heterogeneous multivariate data, assumptions of normality, and identification of significant differences in performance [42]. Unlike other methods that may present challenges in interpretation and in obtaining direct statistical inferences owing to requirements related to data behavior, approaches such as t-distributed stochastic neighbor embedding (t-SNE) and Linear Discriminant Analysis (LDA) offer distinct alternatives for the analysis and visualization of results [49, 50]. Statistical tests were performed using the RStudio program version R-4.1.2, custom programming, and standardized libraries (vegan) [51].

3. RESULTS

3.1 Water characterization

The water in the rivers sampled in the study area reflects an evident variation by sampling season (Figure 3). This variation consists mainly of the acidification of the water resources during the dry season, in which pH values below the minimum permissible limit according to the country's current regulations (6.5) are recorded. However, from a general point of view, three pH clusters (Figure 3) were identified related to the seasons, of which it is essential to highlight that 20% of the sampled points (orange cluster), maintained a pH below the minimum permissible limits regardless of the season. On the other hand, 30% of the points (light blue cluster) indicated acidity only during the dry season, while the remaining 50% of the sampled points were within regulation for pH regardless of the season. DO measurements revealed that 94% of the stations recorded values within the country's current regulations (Figure 4).

Heavy metals in the water within the study area include concentrations of aluminum, copper, iron, and lead that exceed the maximum permissible limits according to current local regulations [39]. Figure 5 shows the spatial distribution of heavy metal concentrations within the study area. These concentrations can be classified into five categories, with the highest concentrations being located in areas close to the mining activity (Figure 5a). The minimum average heavy metal concentrations detected were 6.2 ppb for different metals, and maximum concentrations of 1928.7 ppb in metals such as aluminum, copper, iron, and manganese. Both contrasts in heavy metal concentrations indicate contamination in accordance with the maximum permissible limits according to local regulations. This level of concentration compromises water quality, and in contrast to land use, it is possible to identify potentially affected areas downstream, mainly in shrimp farms, mangroves, banana plantations, and short-cycle crops (Figure 5b).

3.2 Water characterization

Heavy metal concentration measurements from March to June at the bioreactors reflect removal percentages higher than 50% depending on the efficiency of the bioreactors. For example, metals such as Al, Ce, Co, Fe, Gd, and Ni can be removed using R1 and R2. Figure 6 and Table 2 show the metal removal percentage for each bioreactor.

Table 2. Removal percentages (%) of each heavy metal per bioreactor

	R1	**R2**	**R3**	**R4**	**R5**	**R6**	**R7**
Al	69	84	20	30	54	7	7
As	95	97	67	27	16	6	7
Ba	28	19	17	15	10	16	5
Cd	90	79	0	1	22	1	12
Ce	64	81	0	20	51	4	13
Co	87	96	0	7	10	6	17
Cr	29	57	17	14	0	8	3
Cu	6	93	3	2	72	1	7
Eu	21	72	5	2	40	4	2.6
Fe	69	88	18	17	96	34	0
Gd	51	77	0	10	31	10	0
Mn	10	29	0	1	1	4	3
Ni	64	81	0	0	15	6	5
Pb	56	59	54	58	58	14	24
Se	0	0	0	0	0	0	0
Y	11	84	1	3	17	4	4
Zn	21	88	0	1	7	3	4

3.3 Correlation analysis

Heavy metal removal values show highly significant correlations for 25 heavy metals. Figure 7 shows the potential relationship between these values (correlation >0.8) using the R1 bioreactor treatment. Additionally, a similar behavior is highlighted with the other treatments (R2 to R7). These behaviors reflect the significant groups of contaminants, such as Al, that impact Y, Zn, Fe, As, and Cs. In addition, the bioreactors showed a substantial correlation between metals, such as Zn and Y, Dy, Gd, and Mn. An incidence was also observed in metals such as Fe with Zn, Mn, and Pb with P.

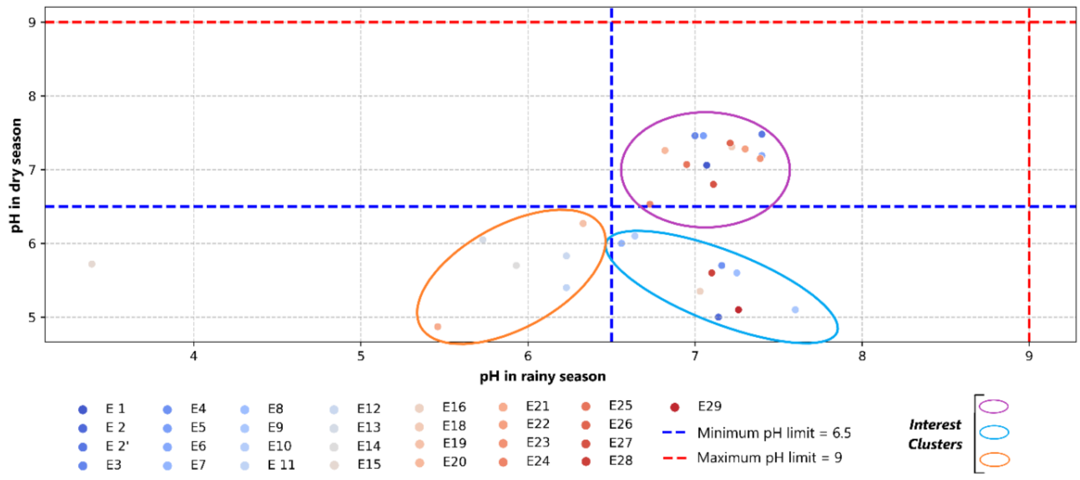

Figure 3. pH level per sampled station. pH limits are in accordance with the country's current regulations [39]

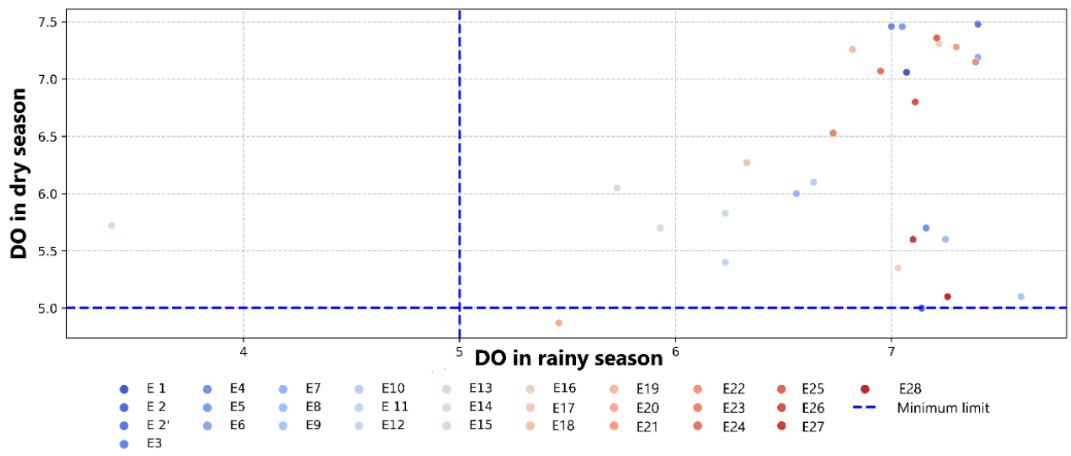

Figure 4. DO level per sampled station. DO limits are in accordance with the country's current regulations [39]

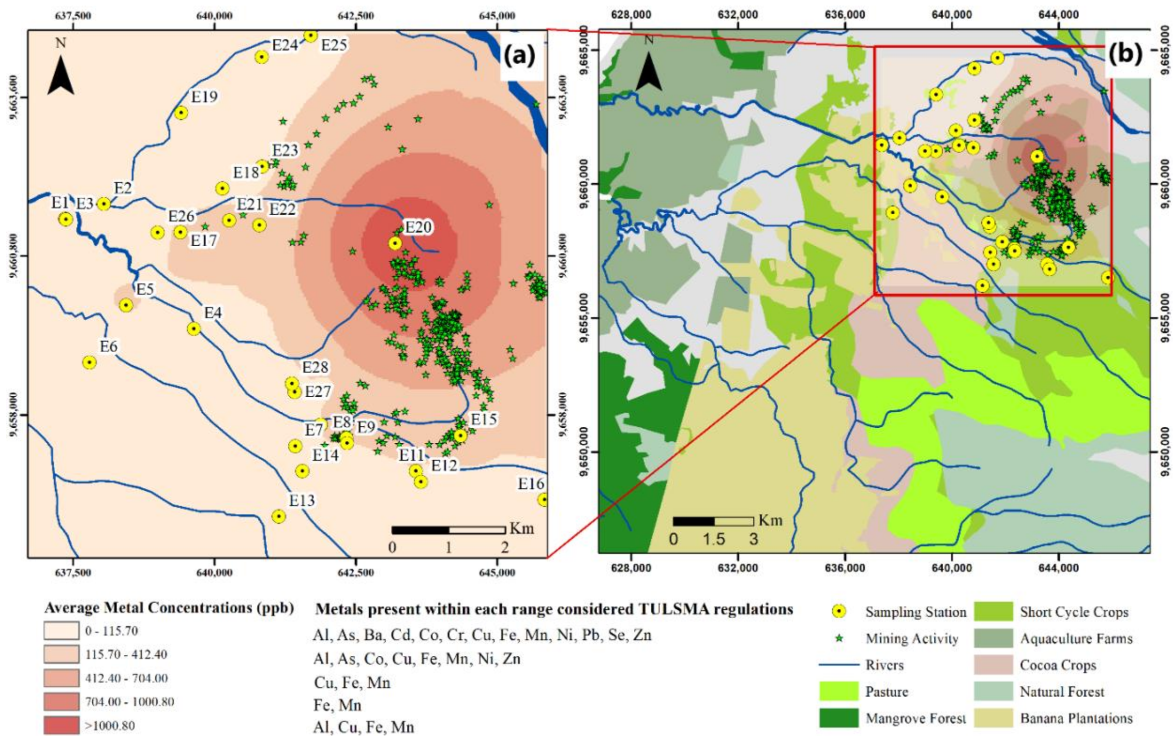

Figure 5. Spatial distribution of average heavy metal concentrations in the study area. a) Spatial distribution of the average concentration of heavy metals in the study area and b) Land use and cover for the analysis of downstream impacts in relation to heavy metal concentration.

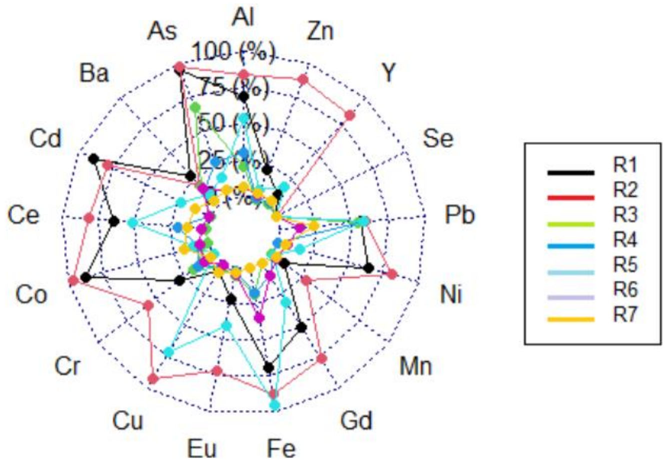

Figure 6. Remarkable removal percentages among metals per bioreactor analysed

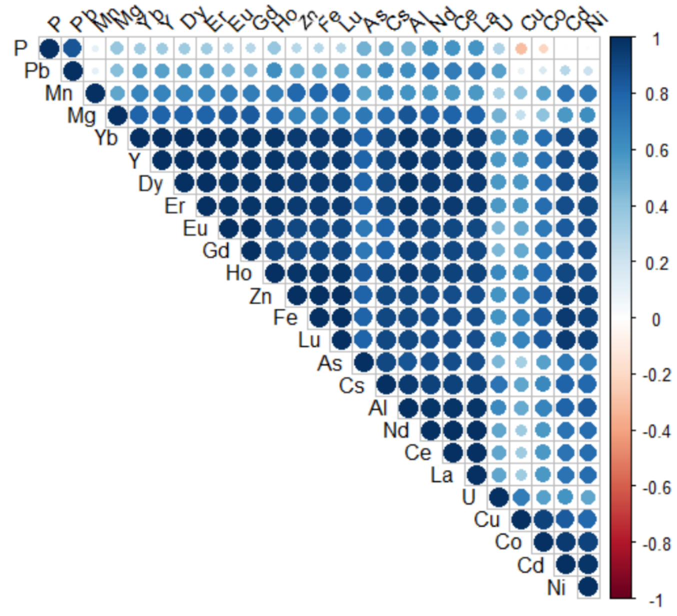

Figure 7. Correlation analysis of heavy metal removal behaviour according to the treatment of the R1 bioreactor

Therefore, this correlation analysis suggests significant relationships between the heavy metal removal values that could be related to the operation of the bioreactors and would subsequently allow their efficiency to be analysed. The coefficient of determination R2 (72% to 86%) confirms and ensures the reliability of the results.

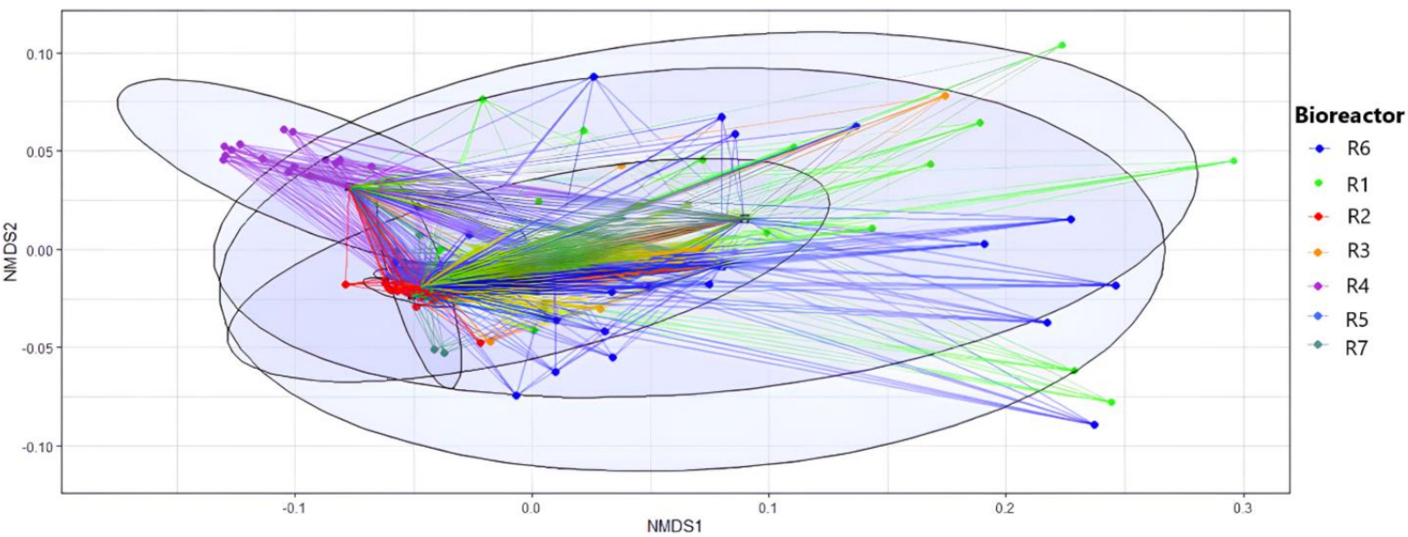

Figure 8. Non-metric multidimensional scaling (NMDS) of heavy metal patterns in the seven bioreactors

3.4 Dissimilarity analysis

Figure 8 shows the behaviour of the data dispersion patterns through NMDS (Bray Curtis distance analysis). NDMS compares heavy metal concentrations after treatment between bioreactors through its dimensions NMDS 1 and 2 (significant variability of removal values). According to the NMDS, the bioreactors with significantly different patterns were R1 (green), R2 (red), and R4 (purple). This suggests that these bioreactors show a significant medium-high performance in the treatment of AMD containing heavy metals, such as Al, Fe, Zn, and Cu.

To determine the reliability of the NDMS results, i.e., the evaluation of bioreactor performance, the stress level in multidimensional space is calculated, which is 0.08 at 20 runs. This shows that the stress test was positive (>0.20), suggesting reliability in the efficiency results of the incidence of the bioreactors on the heavy metals removal (Table 2).

Table 2. Data behavior stress level

	Run 0	**Run 1**	**Run 5**	**Run 10**	**Run 15**	**Run 20**
Stress	0.07	0.07	0.07	0.09	0.09	0.08
RMSE	0.02					

3.5 Significance and validation of the impact of bioreactors on heavy-metal removal

Table 3. Eigenvalues for axes PCA

	PCO A1	**PCO A2**	**PCO A3**	**PCO A4**	**PCO A5**	**PCO A6**	**PCO A7**
Eigenv alues	2.77	0.59	0.14	0.13	0.07	0.06	0.06

Due to the effect of the pattern behavior in Figure 9, it was demonstrated that the bioreactors differed significantly in multivariate dispersion. Figure 8 shows the principal component ordination analysis (PCOA), presenting the eigenvalues for each PCOA that coincide with the NMDS analysis. Table 3 provides the eigenvalues that demonstrate the homogeneity of the multivariate dispersion. This analysis reaffirms the significant differences between the treatments found by NMDS, indicating that bioreactors R1, R2, and R4 achieve higher efficiency in AMD treatment.

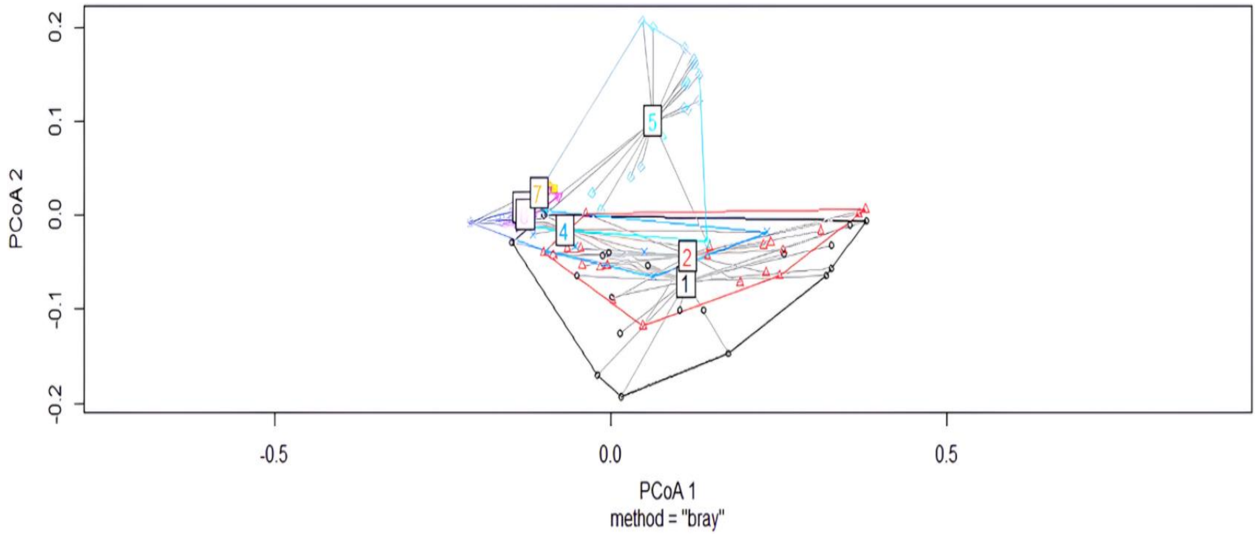

Figure 9. Principal component ordination analysis via Bray Curtis distance

Additionally, Table 4 shows the efficiency of the bioreactors in removing heavy metals (distances between removal values). The bioreactors with the highest performance in the removal of heavy metals were R2 (0.15419) and R1 (0.15113) because their mean distances between groups to each centroid were significant compared to the other bioreactors (R3, R6, and R7). This suggests the efficiency of heavy metal removal between the groups of bioreactors. Additionally, the distances between groups R4 and R5 suggest moderately significant efficiency behaviour compared to the other groups.

Table 4. Average of the distances to the centroid of each bioreactor

	R1	R2	R3	R4	R5	R6	R7
Average	0.15	0.15	0.03	0.11	0.10	0.03	0.02

Finally, the results of the ANOVA analysis consider that the statistical analysis of this study explains the efficiency of the bioreactors according to the behaviour of the heavy metal removal values. The F test statistic is superior and highly significant (p-value<0.001), indicating that the removal values acceptably explain the efficiency of the bioreactors, with a distribution error of 0.02% (Table 5).

Table 5. ANOVA analysis of multivariate dispersion

	Df	Sum Sq	Mean Sq	F value	P-value
Biorreactores	7	0.32	0.05	20.08	5.99e-16
Residuals	115	0.31	0.002		

*Df: degrees of freedom, Sum Sq: Sum of squares, Mean Sq: Mean Squares, F value: Statistician F

4. INTERPRETATION OF RESULTS AND DISCUSSION

The study evaluating the efficiency of bioremediation techniques for the removal of heavy metals from AMD presents a quantitative methodological approach through statistical analyses for the mitigation of water body contamination by direct discharge of mining effluents. According to the results obtained, the concentrations of toxic heavy metals considered by the country's current regulations exceeded the permissible limits at all sampled stations. These measurements, with minimum average concentrations of 0.1 ppb and a maximum of 1000 ppb in metals such as Al, As, Ba, Cd, Cr, Ni, and Pb, clearly indicate high contamination of water bodies by artisanal mining activity that compromises human well-being and the ecosystem in general. The spatial increase in concentrations is directly related to the proximity to mining activity in the area, which includes active and inactive mines and mineral processing plants (Figure 5) that influence downstream contamination, mainly in agricultural areas, as documented by Romero-Crespo et al. [36]. These levels of contamination have been investigated in different studies, such as that of Escobar-Segovia et al. [35], which reaffirms the high concentrations of As, Cd, and Pb in water and sediments sampled in rivers, as well as the impact of AMD classified as moderate to high on the quality of groundwater in the Ponce Enríquez area [52].

Heavy metal concentration measurements in AMD carried out in the different bioreactors revealed removal percentages higher than 50%, mainly in R1 and R2, which are those that employ sugarcane bagasse and sulphate-reducing bacteria (Figure 6), for metals such as Al, Ce, Co, Fe, Gd, and Ni, reflecting their potential in mitigating toxic metal contamination. This supports the effectiveness of using agricultural waste, specifically sugarcane bagasse, to remove heavy metals such as Pb, Hg, Ni, Cu, Cd, and Cr [53-56].

Statistical tests based on PCOA and NMDS identified the best-performing bioreactors, highlighting the effectiveness of sugarcane bagasse in combination with SRB (R1 and R2) in the removal of heavy metals, such as Al (69-84%), Ce (64-81%), Co (87-96%), Cu (6-93%), Eu (21-72%), Fe (69-88%), Gd (51-77%), Ni (64-81%), Y (11-84%), and Zn (21-88%), in approximately four months. Additionally, R5 containing domestic wastewater with SRB from anaerobic digester showed an average efficiency of 68.25% for removing metals such as Al, Ce, Cu, and Fe. In comparison, Nogueira et al. [57] removed heavy metals such as Co and Ni from AMD using sugarcane vinasse after 230 days of treatment, with removal percentages similar to those found in this study that used sugarcane bagasse (greater than 80%). However, this study found higher removal percentages for metals such as Co, Cu, Fe, and Zn (greater than 88%) than the average rate of 76% reported by Nogueira et al. [57].

In contrast, other studies have used various biological treatments, such as Song et al. [58], who used fungal compost, which efficiently reduced sulfate by removing metals such as Al (100%), Fe (68-92%), and Mn (49-61%) from AMD over a period of approximately two to five months. Wang et al. [59] applied two bioreactors based on lignocellulosic waste and river sand, achieving high efficiency in the removal of metals such as Fe (92.5%), Cd (99%), Zn and Cu. In addition, the study by Sato et al. [60] applied bioreactors based on rice bran to remove metals such as Zn, Cu, and Cd with reduction rates of 20.7-77.9% in periods of approximately 54 to 242 days.

This study demonstrates the efficiency of removing heavy metals from AMD with agricultural waste and SRB bacteria, which are easily obtained for the subsequent discharge of the effluent into nearby water bodies and comply with the country's current regulations. Despite the favorable results obtained during the sampling period, one of the limitations of this study is the time required to implement bioreactors and measure heavy metal concentrations. This could be extended from four months to one year of weekly measurements to determine the most efficient bioremediation technique more accurately.

In the future, this project will contemplate the application of the in situ technique with pilot projects in different mines and mineral processing plants for large-scale projection. Finally, it is recommended that an efficiency analysis of the bioreactors be carried out through AI methods, such as machine learning, which creates efficiency patterns that consider various pollutants and SRB.

5. CONCLUSIONS

Contamination of water bodies by directly discharging AMD from artisanal mining activities in southern Ecuador is a national and cross-border environmental problem that threatens ecosystems and human health. The results of this study show that the investigated bioremediation techniques present significant variability in their efficiency depending on the type of components used. The PERMANOVA statistical analysis identified that the bioreactors that combine sugarcane bagasse and SRB (R1 and R2) present a greater efficiency in the removal of contaminants, reaching an average removal range of 85.35% and 89.64%, respectively, which is decisive in the effectiveness of the process.

The spatial distribution of pollutants in the water bodies shows that the areas with the highest contamination levels coincide with points close to the mining activity. This research highlights some limitations, such as the type of evaluation of bioreactor efficiency (under controlled laboratory conditions) that could influence the actual in situ behavior and the need to expand to more water sampling stations in rivers for greater precision of the interpolation map.

Future lines of research may include validation in situ of the bioremediation techniques on a large scale, considering specific environmental variables, such as changes in AMD volume, temperature, and pH; as well as the exploration of alternative bacterial strains. It is also recommended that the scope of the spatial analysis of contaminants be expanded with a higher sampling density integrated into predictive models that allow the evaluation of future contamination scenarios in the investigated area. Finally, considering land use and cover, the need for future studies on the impact of heavy metal contamination on shrimp farms, one of the main economic activities in the country.

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NOMENCLATURE

pH	Hydrogen potentia
DO	Dissolved oxygen
ppb	Parts per billion

APPENDIX

Table S1

Station	Latitude	Longitude	Al	As	Ba	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Se	Zn
E1	9661467	637364	116.2	25.8	9.7	0.2	5.5	0.3	19.3	414.2	408.6	5.6	0.8	0.8	14.7
E2	9659855	636446	42.3	1.4	7.3	DL	0.1	DL	DL	105.0	19.5	DL	0.1	DL	2.5
E3	9661351	637396	145.9	25.7	7.6	0.1	4.3	0.4	22.4	468.8	434.9	DL	1.2	0.5	14.9
E4	9659509	639639	86.1	8.8	5.3	0.0	0.5	0.8	DL	157.0	16.1	DL	0.1	0.6	17.7
E5	9659933	638450	178.8	27.8	7.0	0.1	4.8	0.7	51.3	492.8	348.7	4.5	1.2	DL	10.3
E6	9658876	637783	40.6	1.3	7.1	DL	0.1	0.3	DL	67.3	8.2	DL	0.0	DL	6.2
E7	9657443	641420	102.5	35.5	4.8	0.1	6.0	0.3	118.7	709.8	328.1	6.4	1.1	0.5	7.3
E8	9657536	642336	109.7	55.1	16.3	0.3	28.0	0.8	766.2	964.3	1294.2	37.0	0.2	3.0	22.3
E9	9657520	642323	127.1	37.9	3.8	DL	0.8	0.4	24.8	314.3	61.1	DL	1.0	0.4	1.9
E10	9657825	641881	53.7	92.6	2.2	0.1	1.2	0.5	DL	297.4	40.8	DL	0.1	0.6	5.1
E11	9657095	643604	389.1	164.7	6.4	0.1	4.9	1.6	89.1	2368.5	237.8	6.0	8.6	0.8	18.1
E12	9656824	643679	82.1	2.6	4.6	DL	0.1	0.5	DL	117.5	4.7	DL	0.1	DL	2.7
E13	9656334	640959	138.1	1.0	6.5	DL	0.1	0.3	DL	203.8	3.3	DL	0.1	DL	2.9
E14	9656992	641559	256.5	0.6	10.6	DL	0.2	0.9	DL	244.5	9.6	DL	0.1	0.4	2.7
E15	9657610	644336	6831.2	736.3	16.9	1.5	30.5	16.6	177.6	28318.5	566.8	27.8	65.9	0.6	188.6
E16	9656491	645839	55.1	3.7	5.3	DL	0.0	0.3	DL	85.5	2.6	DL	0.1	DL	11.2
E17	9661190	639389	136.1	12.5	15.5	0.6	29.7	0.6	366.5	256.2	820.8	26.5	0.2	DL	29.5
E18	9661956	640140	90.9	4.9	5.8	0.0	1.4	0.5	DL	366.7	210.5	DL	0.1	0.9	2.4
E19	9663306	639401	58.2	5.6	7.2	DL	0.1	DL	DL	182.8	5.6	DL	0.1	0.5	7.4
E20	9661019	643188	1069.4	5.5	16.6	1.5	73.0	0.3	254.9	494.3	1215.9	81.5	0.3	0.9	180.3
E21	9661429	640272	226.1	15.1	15.4	0.5	35.9	0.7	648.9	360.4	761.1	32.7	0.2	1.0	15.1
E22	9661341	640801	245.7	7.0	13.5	0.6	41.2	0.6	833.1	383.3	801.5	37.6	0.1	1.5	42.2
E23	9662358	640840	132.7	8.0	6.1	0.1	2.9	DL	3.3	246.1	221.4	DL	0.1	1.1	8.0
E24	9664299	640828	86.4	8.7	6.7	0.0	0.2	0.2	DL	230.4	9.4	DL	0.1	0.7	32.7
E25	9664678	641695	264.8	5.3	5.6	DL	0.3	0.7	3.1	302.3	11.3	DL	0.1	DL	4.9
E26	9661202	638995	41.2	8.2	14.3	0.3	11.8	0.7	28.4	162.0	396.9	10.2	0.0	1.1	15.9

E27	9658353	641384	92.4	30.4	4.0	0.0	0.7	4.6	DL	240.9	71.3	6.3	0.5	DL	3.2
E28	9658547	641382	49.2	2.7	2.3	DL	2.3	1.8	DL	91.1	6.6	DL	0.1	DL	2.9