Translocation and experimental adaptation of *Distichia muscoides* cushions in a wetland impacted by acid rock drainage, Ancash, Peru

Translocación y adaptación experimental de cojines de *Distichia muscoides* en un bofedal impactado con drenaje ácido de roca, Áncash, Perú

Maria Cristina Otoya Fernández [1*], Yeidy Montano [2], Marilín Sánchez-Purihuamán [3], Pedro M. Tapia [4], Junior Caro-Castro [5], Carmen Carreño-Farfán [6]

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Abstract

The deglaciation of the Andean mountain range negatively impacts ecosystems and water bodies, primarily increasing the concentration of heavy metals. However, their concentration can be reduced by applying bioremediation techniques. The objective of this study was to evaluate the effect of the translocation and adaptation of *Distichia muscoides* cushions in a wetland impacted by acid rock drainage in a high Andean region. For this purpose, the characteristics of water, peat, and *D. muscoides* tissue were compared in two wetlands, and the behavior of translocated *D. muscoides* was evaluated based on the bioaccumulation and translocation factors of metals. The quantification of Al, Fe, and Mn in peat, root, and aerial tissue of *D. muscoides* showed higher concentration values after the translocation of the cushions. Additionally, the bioaccumulation factor classified the transplanted cushions as accumulators of Al, Cu, As, Fe, Mn, and Zn, while the translocation factor classified the cushions as phytoextractors of Al, As, Cr, Fe, Mn, and Zn, and phytostabilizers of Pb and Cu. It is concluded that translocated and adapted *D. muscoides* cushions have potential for the bioremediation of wetlands contaminated with acid rock drainage.

Keywords
Bioremediation • wetland • acid rock drainage • *Distichia muscoides* • heavy metals

1 Universidad Nacional Pedro Ruiz Gallo. Facultad de Ciencias Biológicas. Laboratorio de Investigación Biotecnología Microbiana. Lambayeque-Perú. *crisotofer13@gmail.com
2 Instituto Nacional de Investigación en Glaciares y Ecosistemas de Montaña. Huaraz. Ancash-Perú.
3 Universidad Nacional Pedro Ruiz Gallo. BlyME: BS-CA. Grupo de Investigación Biodiversidad y Manejo Ecológico del Bosque Seco y Cultivos Tropicales. Lambayeque-Perú.
4 Universidad Peruana Cayetano Heredia. Facultad de Ciencias e Ingeniería. Lima-Perú.
5 Universidad Nacional Mayor de San Marcos. Facultad de Ciencias Biológicas. Laboratorio de Ecología Microbiana. Lima-Perú.
6 Universidad Nacional Pedro Ruiz Gallo. BlyME: BS-CA. Facultad de Ciencias Biológicas. Laboratorio de Investigación Biotecnología Microbiana. Lambayeque-Perú.

Resumen

La desglaciación de la cordillera andina impacta de manera negativa los ecosistemas y cuerpos de agua, incrementando la concentración de metales pesados como principal consecuencia; sin embargo, su concentración puede ser disminuida aplicando técnicas de biorremediación. El objetivo de este trabajo fue evaluar el efecto de la translocación y adaptación de cojines de *Distichia muscoides* en un bofedal impactado con drenaje ácido de roca en una región altoandina. Para ello, se compararon las características del agua, turba y tejido de *D. muscoides* en dos bofedales, evaluando el comportamiento de *D. muscoides* traslocados basados en los factores de bioacumulación y traslocación de metales. La cuantificación de Al, Fe y Mn en turba, tejido radicular y aéreo de *D. muscoides* demostró mayores valores de concentración después de la traslocación de los cojines. Por otro lado, el factor de bioacumulación calificó los cojines trasplantados como acumuladores de Al, Cu, As, Fe, Mn, Zn, mientras que el factor de traslocación calificó a los cojines como fitoextractores de Al, As, Cr, Fe, Mn, Zn, y fitoestabilizadores de Pb y Cu. Se concluye que los cojines de *D. muscoides* traslocados y adaptados tienen potencial para la biorremediación de bofedales contaminados con drenaje ácido de roca.

Palabras clave

Biorremediación • bofedales • drenaje ácido de roca • *Distichia muscoides* • metales pesados

Introduction

The Huascaran Biosphere Reserve is a natural heritage site located in Ancash, Peru, encompassing an area of 1,155,800 hectares. This includes the core area, Huascaran National Park (HNP), which contains 95% of the Cordillera Blanca (11). Between 1962 and 1970, the Cordillera Blanca covered 723 km^2 and 658 km^2, respectively (6). However, by 2016, the glacial area had decreased to 448.81 km^2, representing a 38.2% reduction and a loss of 277.45 km^2 (38). Additionally, in the Santa River, which is fed by western glaciers, heavy metals have been detected at levels exceeding the Maximum Permissible Limits (MPL) of the Environmental Quality Standards (EQS) for Water. This contamination results from glacial erosion, rock weathering, and anthropogenic activity (18, 39).

The retreat of Andean glaciers exposes rock material that typically contains sulfide minerals (pyrite), which, when oxidized and leached, generates acid rock drainage (ARD) with an acidic pH and high metal concentrations, impacting water bodies and ecosystems (17). The ARD formation process begins when sulfide minerals, exposed to atmospheric oxygen, become unstable and oxidize (34). The oxidation of pyrite, the main mineral responsible for ARD generation, requires oxygen and water and can be accelerated by microbial action. Metals in ARD originate from the oxidation of sulfides and the dissolution of acid-consuming minerals. ARD has an acidic pH, a high concentration of sulfates, and primarily dissolved metals such as iron (Fe) and aluminum (Al). However, trace metals like lead (Pb), zinc (Zn), copper (Cu), cadmium (Cd), manganese (Mn), cobalt (Co), and nickel (Ni) can reach high concentrations on certain occasions (38). Heavy metals are persistent pollutants because they bioaccumulate, are not biodegradable, and are highly toxic even at low concentrations, affecting plants, animals, and humans (27).

Wetlands are characteristic of the tropical and subtropical Andes (5, 36), where cushion plants such as *Distichia muscoides* and *Oxychloe andina* are found. These are primary species of the Andean Altiplano with the capacity to store water and elevate it above the groundwater level (31). These cushion plants regulate water release, act as sinks for organic carbon, and promote wildlife (7, 20). However, they are threatened by glacial retreat (20), overgrazing, anthropogenic extraction, and heavy metal contamination from ARD (10, 31, 36). Previous studies conducted in the Cordillera Blanca, Ancash, Peru, have demonstrated the effectiveness of transplanting species like *D. muscoides* to accelerate the bioremediation of environments impacted by ARD (17, 38).

Based on previous evidence, bioremediation is proposed as a solution, involving the application of microorganisms, plants, or derived enzymes for environmental restoration. This approach relies on the biological entities' ability to reduce or eliminate contaminants (12).

Phytoremediation, the use of plants for bioremediation, allows them to absorb, mobilize, and accumulate heavy metals and other contaminants through strategies like phytoextraction, phytostabilization, and phytovolatilization (16, 28). In phytoextraction, metals are absorbed by roots, transported, and accumulated in stems and leaves. Phytostabilization reduces contaminant mobility and prevents migration to groundwater (24, 30). Phytovolatilization involves the absorption of metals by roots, transport via the xylem, and release from the aerial parts of plants (23).

The response of plants to heavy metals classifies them as excluders, indicators or accumulators. Excluders have lower metal accumulation in their above-ground parts compared to the soil concentration. Indicators maintain a direct relationship between metal concentration in their above-ground parts and the soil. Accumulators have higher metal concentrations in their above-ground parts compared to the soil. To implement phytoremediation through phytostabilization, excluder plants are used, which accumulate metals in their roots, while for phytoextraction, accumulator plants are necessary to transport metals to their above-ground parts (1, 16, 24).

Plants like *D. muscoides* are efficient for the stabilization and extraction of heavy metals (17); however, it is necessary to analyze the technical, ecological, and economic feasibility before their use, considering the conditions of the damaged ecosystem and the possibilities of application (12). Therefore, the objective of this study was to evaluate the effect of translocating and establishing *D. muscoides* cushions in a wetland impacted by ARD located in the Ancash region, Peru, aiming to remediate ARD-affected wetlands. The specific objectives were: a) to compare the characteristics of wetlands unaffected and affected by ARD, b) to monitor the adaptation of translocated *D. muscoides* cushions by comparing the concentration of heavy metals, and c) to determine the behavior of translocated cushions in an ARD-impacted wetland.

Materials and methods

Comparison of wetlands affected and unaffected by ARD

Study area

The study was conducted within HNP, in the district of Catac, province of Recuay, Ancash region. Two wetlands were selected, designated as wetland 1 and 2, both predominantly populated with *D. muscoides* cushions. Three zones (A, B, and C) were established within each wetland (figure 1, page XXX), based on pH conditions (3.95-7.27), temperature (8.7-14.7 °C), altitude (4506-4818 meters above sea level), and the condition of the *D. muscoides* cushions. For sampling in each zone of both wetlands, nine sampling points were considered (A1, A2, A3, B1, B2, B3, C1, C2, and C3) with a 50 cm separation between them. Each wetland involved nine samples of root tissue, nine of aerial tissue, three water samples, and three peat samples.

Sampling of water from the wetlands

Water quality was assessed according to the National Protocol for the Quality of Natural Bodies of Surface Water (3). In each wetland, three water samples were collected in October 2021. Five-hundred mL of water were collected using sterile polyethylene bottles placed at the imaginary triangle center formed by the three sampling points. The samples were taken against the water flow direction at a depth of 20-30 cm, with the addition of 1 mL of nitric acid as a chemical preservative. Physicochemical parameters such as pH and temperature were measured in each wetland water sample using a handheld meter (HANNA HI8424 model).

Sampling of peat, root and foliage of D. muscoides

Sampling of organic peat was conducted following the established technique in the Peruvian Soil Sampling Guide (21). Nine cushions of *D. muscoides* were selected in each wetland, one per sampling point, at a depth of 30 cm. From each cushion, a rectangle of 20x15 cm consisting of peat, roots, and aerial tissue was extracted. In the laboratory, peat samples from each zone (three sampling points) were mixed, and a 300 g sample of the most decomposed peat was selected, totaling three samples per wetland. Root tissue was separated from aerial tissue, and 250 g from each rectangle was weighed, resulting in nine samples per wetland.

The concentration of heavy metals in all samples was determined using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) for water according to method EMW 200.7 (19). For peat, root tissue, and aerial tissue, method 3050-B (2) was employed. Metal concentration values in water were compared with EQS fow water, Category 4, Subcategory 1 (22).

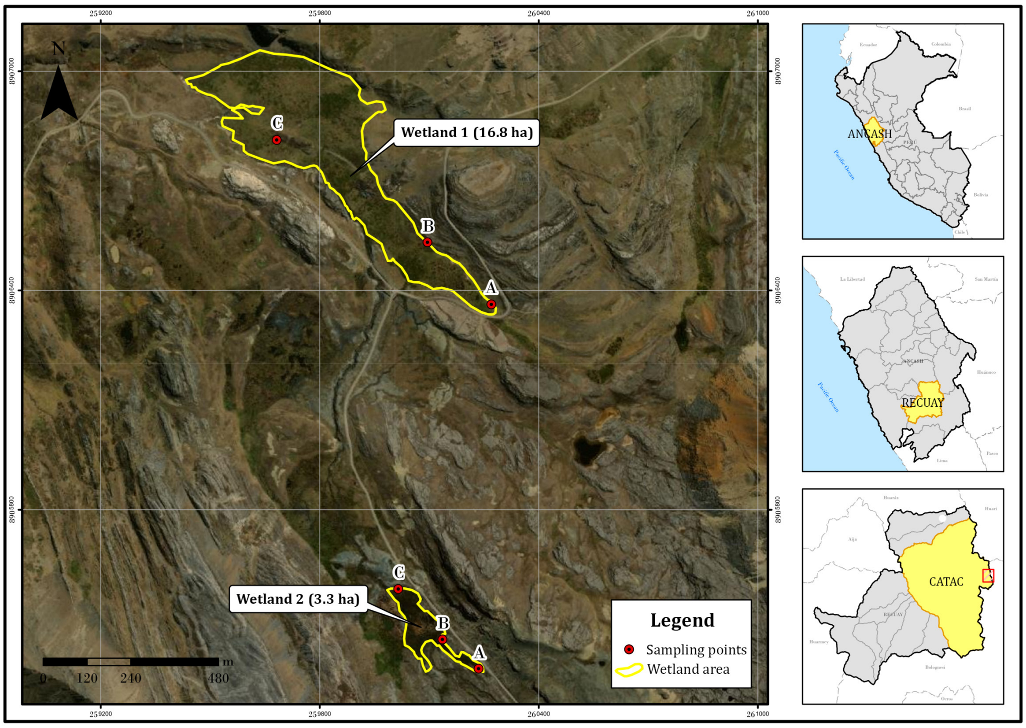

Figure 1. Location of the sampling zones in wetlands 1 and 2.

Figura 1. Ubicación de las zonas de muestreo en los bofedales 1 y 2.

Presence of sulfate-reducing bacteria in the sediment of water bodies

For sampling, three zones were considered in each wetland, with two sampling points in each zone (A1, A2, B1, B2, C1, and C2). Samples of 1000 g of sediment from the water bodies were collected using an auger that extracts perforated solid material (37) at a depth of 70 cm. The samples were immediately deposited into transparent glass jars and transported in a thermal box (10±1°C) to the laboratory, where enrichment of sulfate-reducing bacteria (SRB) was conducted in twelve Winogradsky columns (25).

Translocation and adaptation of *D. muscoides* and comparison of heavy metal concentrations

Translocation and adaptation of D. muscoides

The phase of translocation or transplantation and adaptation of *D. muscoides* took place from December 2021 to May 2022 in zone B, the experimental area of wetland 2, previously delineated (total area: 7.7 ha). This area is traversed by water from Pastoruri Creek, covering an area of 5x5 m^2 (georeferenced points E: 260088 and N: 8905452, at an altitude of 4804 meters above sea level). Eight cushions were selected from zones A and B of wetland 1, located at the edge in contact with the water, from which a rectangle (30 x 15 cm) consisting of roots, aerial tissue, and root peat was extracted to a depth of 30 cm. Among the eight rectangles extracted from the cushions, five were designated for translocation and three for the initial analysis of heavy metal concentration in peat, root tissue, and aerial tissue before transplantation. Monitoring of the adaptation of transplanted cushion rectangles was conducted over 5 months, during what is known as the rainy season due to the significant influence of wetland water levels (7). Monitoring occurred every 10 days during the first two months and subsequently every 15 days from the third to the fifth month. Vigor and persistence of green color were evaluated in each cushion rectangle.

After 5 months of transplantation, a random sampling of three cushions was conducted from among the five that were transplanted. The presence of few, moderate, or abundant roots allowed for assessing the adaptation of the cushions to the transplantation. In contrast, the absence of roots indicated inadequate adaptation of the cushions to the transplantation.

Comparison of heavy metal concentrations

The concentration of heavy metals in the peat, root tissue, and aerial tissue of three *D. muscoides* cushions before translocation was compared with the concentration determined in three cushions 5 months after translocation. Additionally, the concentration of heavy metals in the peat, root tissue, and foliage of three translocated cushions was compared with that in three native cushions (not transplanted) from the wetland impacted by ARD where the translocation occurred.

Behavior of translocated cushions in the wetland impacted by ARD

The behavior of transplanted *D. muscoides* was evaluated using the bioaccumulation factor (BAF) and translocation factor (TF) (30) of the root tissue and foliage of both translocated and non-translocated *D. muscoides*.

$$\mathrm{BAF} = \frac{\text{Concentration of root or foliage}}{\text{Concentration of peat}}$$

$$\mathrm{TF} = \frac{\text{Concentration of foliage}}{\text{Concentration of root tissue}}$$

Statistical analysis

The concentrations of heavy metals (mg/kg) in peat, root tissue, and foliage before and after the translocation of *D. muscoides* were analyzed using the paired Student's t-test for normally distributed samples and the Wilcoxon test for samples with a non-normal distribution. Additionally, the concentrations of heavy metals (mg/kg) in translocated and non-translocated cushions were analyzed using the one-sample Student's t-test. A significance level of 0.05 was applied to all statistical tests. The analyses were conducted using RStudio 2021.09.

Results and discussion

Characteristics of the studied wetlands

Wetland 2 was classified as impacted by ARD based on the acidic pH of the water, higher concentrations of metals in both the water and tissues of *D. muscoides*, as well as the presence of sulfate-reducing bacteria (table 1, page XXX).

The pH range of water in wetland 2, impacted by ARD (3.95 - 6.35), was more acidic compared to wetland 1, which showed no apparent impact from ARD (water: 7.13 - 7.30). In ARD-impacted environments, a permanent acidity (3.54-4.47) has been reported (39), or significantly lower pH (3.60-4.42) compared to non-ARD water (6.95-7.13) (15). The acidic pH is a consequence of ARD, resulting from glacier retreat and subsequent oxidation of exposed mineralized rocks. The acidic pH increases the availability of dissolved metal ions in the water, thereby increasing toxicity to living organisms (38).

Neutralization reactions also influence ARD. While most carbonate minerals dissolve rapidly, hydrolysis of Fe or Mn, following the dissolution of their respective carbonates and subsequent precipitation, can generate acidity (34). The concentration of metals in the water of wetland 2 (Fe, Mn, Zn) was higher than in wetland 1. 0.4844 mg/L of Mn was quantified in wetland 2, contrasting with wetland 1 (0.0002 mg/L), a value that exceeds the EQS for water, Category 4 (22).

Regarding water temperature, the ranges were similar in wetlands 1 and 2 (10.5 - 12.3 °C and 8.7 - 14.7 °C, respectively). Similarly, in the Quillcay basin, Ancash region, it was determined that water temperature remained constant throughout the year at different sampling points, thus not contributing to differences in water quality found in potentially ARD-affected and unaffected areas (15).

Table 1. Concentration of heavy metals (mg/kg) in *D. muscoides* from the evaluated wetlands.

Tabla 1. Concentración de metales pesados (mg/kg) en *D. muscoides* procedentes de los bofedales evaluados.

*Peruvian EQS: Category 4, subcategory 1: 0.2 mg/L.

*ECA peruano: Categoría 4, subcategoría 1: 0,2 mg/L.

Heavy metal	Water (n=3) (mg/L)	Peat (n=3) (mg/kg)	Root tissue (n=9) (mg/kg)	Foliage (n=9) (mg/kg)
Wetland 1				
Aluminum (Al)	0.0200±0.01	2.0000±0.09	2.000±0.05	2.00±0.00
Cadmium (Cd)	0.0029±0.00	4.0500±3.35	4.1844±1.67	4.00±2.25
Copper (Cu)	0.0010±0.00	1.4500±0.77	2.636±3.56	2.04±1.08
Iron (Fe)	0.6520±0.55	1545.80±191.53	1020.81±83.07	774.56±237.44
Manganese (Mn)	0.0002±0.00	119.86±10.40	118.218±12.02	74.91±27.23
Lead (Pb)	0.0060±0.00	9.720±1.98	22.112±17.15	5.99±2.04
Zinc (Zn)	0.0267±0.00	7.020±4.75	72.125±10.27	35.60±9.29
Wetland 2				
Aluminum (Al)	0.0200±0.00	2.000±0.05	2.000±0.00	2.000±0.00
Cadmium (Cd)	0.0009±0.00	11.520±1.75	14.230±7.20	5.110±3.94
Copper (Cu)	0.0010±0.05	13.170±6.25	4.190±1.35	3.130±1.09
Iron (Fe)	2.8807±0.59	16980.97±148.55	6583.57±90.60	7830.47±864.16
Manganese (Mn)*	0.4844±0.43	106.630±8.19	136.50±14.34	183.11±48.30
Lead (Pb)	0.0060±0.04	51.9000±7.90	19.790±5.86	15.64±3.11
Zinc (Zn)	0.0643±0.03	161.7400±16.8	80.34±7.67	93.29±7.23

The concentration range of metals in the peat (Cd, Cu, Fe, Pb, Zn), root tissue (Cu, Fe, Zn, Cd, Mn), and foliage (Cd, Fe, Mn, Zn) in wetland 2 was higher than that in wetland 1 (table 1). Fe and Al are the main metals dissolved in ARD, as previously reported by Zimmer *et al.* (2018). However, the high concentration of heavy metals in the peat and tissues of *D. muscoides* suggests that these plants are adapted to decontamination efforts (10). Al, Fe, and Mn are the heavy metals quantified in higher concentrations in *D. muscoides* exposed to ARD in an artificial wetland, with other metals such as Cu, Cd, Fe, Ni, and Zn also present (17).

Regarding the Winogradsky columns from sediment in water bodies, several characteristics were observed related to coloration, gas production, and turbidity, verifying the presence of sulfate-reducing bacteria (BSR), similar to Winogradsky columns processed with residual sludge from wastewater treatment (25). The black coloration of the sulfate-reducing zone results from sulfide precipitation with reduced metals such as iron, which deposits at the bottom of the columns (29). BSR produce approximately 2 moles of alkalinity per mole of reduced sulfate, thereby neutralizing the pH of acidic waters. Additionally, the generated bicarbonate ions consume protons and raise the pH of acidic water (8, 33). In Wetland 2, the presence of BSR was confirmed in 66.7% of the Winogradsky columns, compared to 16.67% in columns prepared from wetland 1 samples. This difference may be attributed to the greater impact of ARD in wetland 2. As a consequence of increased oxidation of sulfide minerals, sulfate concentrations rise, which are used as electron acceptors by BSR, while organic matter in lower layers of the peat serves as a carbon source (38).

Translocation and adaptation of *D. muscoides* and comparison of heavy metal concentrations

From days 20 to 80, the vigor and green color persisted in all cushions. By day 100, there was no discernible difference in vigor and color due to flooding. From days 120 to 160, a blackish coloration appeared on the edges and middle tissue of all translocated rectangles. At day 160, it was determined that the green coverage ranged from 34.3% to 97.9%, and the black coverage from 2.1% to 65.7% in the translocated cushions, compared to 100% green coverage in the controls. The major radius of the translocated cushions ranged from 26 to 38 cm, and the minor radius from 21 to 44 cm, compared to 30-32 cm and 20-21 cm in the non-translocated controls. Additionally, a regular to abundant number of roots was observed in the translocated cushions compared to the abundant root presence in the controls.

The growth of *D. muscoides* is very slow, requiring more than 5 months for translocated cushions to develop morphology similar to non-translocated ones. A growth rate of 1-2 cm per year has been reported, with an increase in height of more than 1 cm in summer and less than 1 cm in winter over 6 months (7). In contrast, the root production of *D. muscoides* was 2000-2800 g m-2 $year^{-1}$, a range that exceeds other cushion species such as *Plantago rigida*, which produces 1000-1080 g m-2 $year^{-1}$ (32). In areas with shallow and stable water tables, *D. muscoides* is dominant and exhibits a high capacity for peat accumulation, owing to its abundant underground biomass that can facilitate the adaptation of translocated plants (26). The establishment of translocated *D. muscoides* cushions was evidenced by both growth and the accumulation of metals in their tissues. Thus, it was demonstrated that translocation is a technique with potential for the recovery of impacted wetlands, as also corroborated by Luna (2018), who collected and transplanted *D. muscoides* cushions to artificial wetlands and determined a growth of 3.3 cm in roots and 2.8 cm in foliage after 9 months. Additionally, the pH of ARD at the inlet of the wetland was 2.9-3.6 and at the outlet was 3.87-5.30, indicating a decrease in water acidity, although the level achieved was lower than the EQS for water (6.5-8.5).

Cushions before and after translocation

The t-Student analysis for related samples revealed statistically significant differences ($p<0.05$) in the concentrations of Al, Fe, and Mn in peat, root tissue, and foliage before and after transplantation. The concentrations of these metals were higher after transplantation (figure 2).

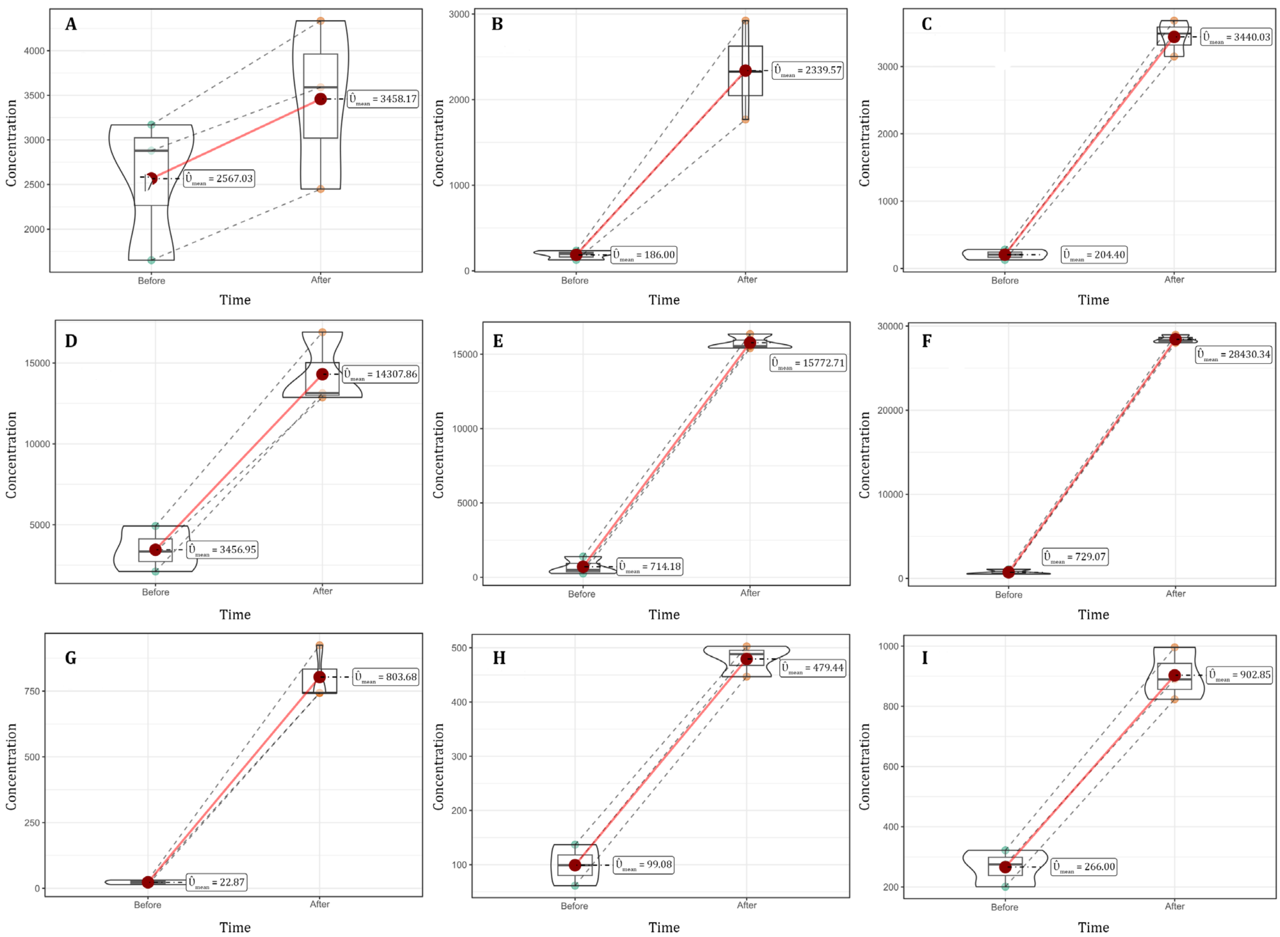

Figure 2. Concentration of heavy metals (mg/kg) in *D. muscoides* before and after transplantation (five months). A. Al (peat). B. Al (root). C. Al (foliage). D. Fe (peat). E. Fe (root). F. Fe (foliage). G. Mn (peat). H. Mn (root). I. Mn (foliage).

Figura 2. Concentración de metales pesados (mg/kg) en *D. muscoides* antes y después del trasplante (cinco meses). A. Al (turba). B. Al (raíz). C. Al (follaje). D. Fe (turba). E. Fe (raíz). F. Fe (follaje). G. Mn (turba). H. Mn (raíz). I. Mn (follaje).

In contrast, the concentrations of Cr and Ar in peat, root tissue, and foliage before and after transplantation were statistically equal (table 2, $p > 0.05$). On the other hand, concentrations of Pb were statistically different in peat; those of Zn were statistically different in root and aerial tissues; and those of Cu differed in peat and foliage. However, the Wilcoxon analysis showed that Cu concentrations in root tissue before and after transplantation were statistically equal ($p > 0.05$).

Table 2. Concentrations of heavy metals (mg/kg) before and after transplantation of *D. muscoides*.

Tabla 2. Concentraciones de metales pesados (mg/kg) antes y después del trasplante de *D. muscoides.*

Heavy metal	Cushions before transplantation			Cushions after transplantation		
	Peat (n=3)	Root tissue (n=3)	Foliage (n=3)	Peat (n=3)	Root tissue (n=3)	Foliage (n=3)
Arsenic (As)	13.84±7.22	3.09±0.16	3.00±0.00	9.32±0.99	5.95±1.71	16.65±2.17
Copper (Cu)	22.4±1.21	4.59±0.85	2.75±0.50	5.40±0.82	6.09±0.388	3.42±0.10
Chromium (Cr)	3.52±0.13	1.00±0.00	1.00±0.00	1.90±0.12	0.66±0.01	1.72±0.05
Lead (Pb)	74.84±30.32	6.47±3.08	8.42±1.94	5.74±0.83	4.36±0.38	4.22±0.09
Zinc (Zn)	376.43±59.4	71.56±16.01	58.63±6.60	21.32±0.24	23.95±3.09	27.49±1.78

The presence of metals in *D. muscoides* tissues before and 5 months after translocation demonstrated that macrophytes can easily absorb bioavailable metals (As, Cd, Cu, Se, Ni, Zn), moderately bioavailable metals (Co, Fe, Mn, Hg), and poorly bioavailable metals (Cr, Pb) from water sediments, accumulating, translocating, and eventually storing them (4, 35). This capacity persisted in the translocated *D. muscoides* cushions. The ability to accumulate metals in translocated *D. muscoides* cushions corresponds with Luna (2018), who quantified Al, Cd, Cu, Fe, Mn, Ni, and Zn in plants of this species 9 months after transplantation to artificial wetlands.

Translocated and non-translocated cushions in the ARD-impacted wetland

The t-Student analysis of a single sample demonstrated that the concentration of heavy metals was statistically different ($p<0.05$) in the peat, root tissue, and foliage of *D. muscoides* in the ARD-impacted wetland (figure 3, page XXX). The concentrations of Al, Fe, Cr, As, Zn, Cu, and Mn were higher in the foliage of translocated *D. muscoides* cushions compared to the peat and root tissue. In contrast, the concentrations of Al, Fe, Pb, Cr, and As in the peat and root tissue of non-translocated cushions were higher than in the foliage. These results indicate the absorption and movement of metals within the plant tissues of translocated *D. muscoides* cushions, where the concentrations of Cu in peat, root tissue, and foliage; Zn in peat and root tissue; and Al, Fe, Pb, Cu, and As in foliage were higher than in non-translocated cushions. The accumulation and translocation of Al, Cd, Cu, Fe, Mn, Ni, and Zn have previously been demonstrated in translocated *D. muscoides* to an artificial wetland (17). In contrast, the concentration of Mn was lower in the peat, root tissue, and foliage of translocated cushions compared to non-translocated cushions, a result that may be related to the duration of non-translocated cushions in the ARD-impacted wetland 2, where the concentration of Mn in water, root tissue, and foliage was higher than in wetland 1, which was not impacted.

Behavior of translocated cushions in the ARD-impacted wetland

The bioconcentration and translocation factors used to evaluate bioremediation capacity (1, 9, 10, 14) demonstrated the absorption of heavy metals, as well as their translocation to the foliage in transplanted *D. muscoides* cushions (17), highlighting their potential for phytoremediation of these contaminants (14). The BAF indicates the phenotypic trait of heavy metal accumulation in plant tissues and their potential for phytoextraction (14), while the TF provides information on contaminant mobility within plants (10). The BAF of heavy metals in foliage classified both translocated and non-translocated cushions as excluders (BAF < 1) of Al, Cu, Cr, Pb, and accumulators (BAF 1-10) of Mn and Zn (figure 4, page XXX).

A previous study identified *D. muscoides* as an accumulator of Zn and a hyperaccumulator of Al and Mn (17). The difference between that study and the present one may be attributed to the use of an artificial wetland in the former, where plants were exposed to a constant flow of ARD for 9 months, unlike the natural wetland used in the present study over 5 months. Additionally, manure was applied during the implementation of the artificial wetland, providing microorganisms that may promote plant growth and activity (13).

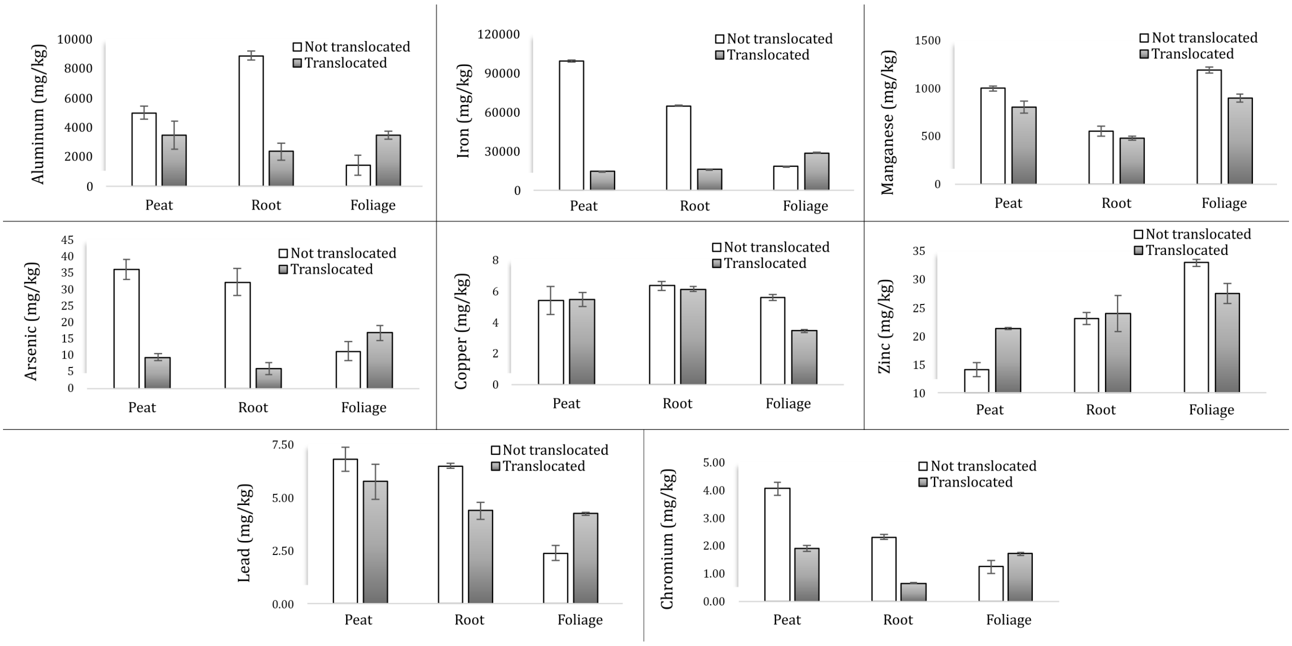

Figure 3. Concentration of heavy metals (mg/kg) in non-translocated and translocated *D. muscoides* cushions.

Figura 3. Concentración de metales pesados (mg/kg) en cojines de *D. muscoides* no translocados y translocados.

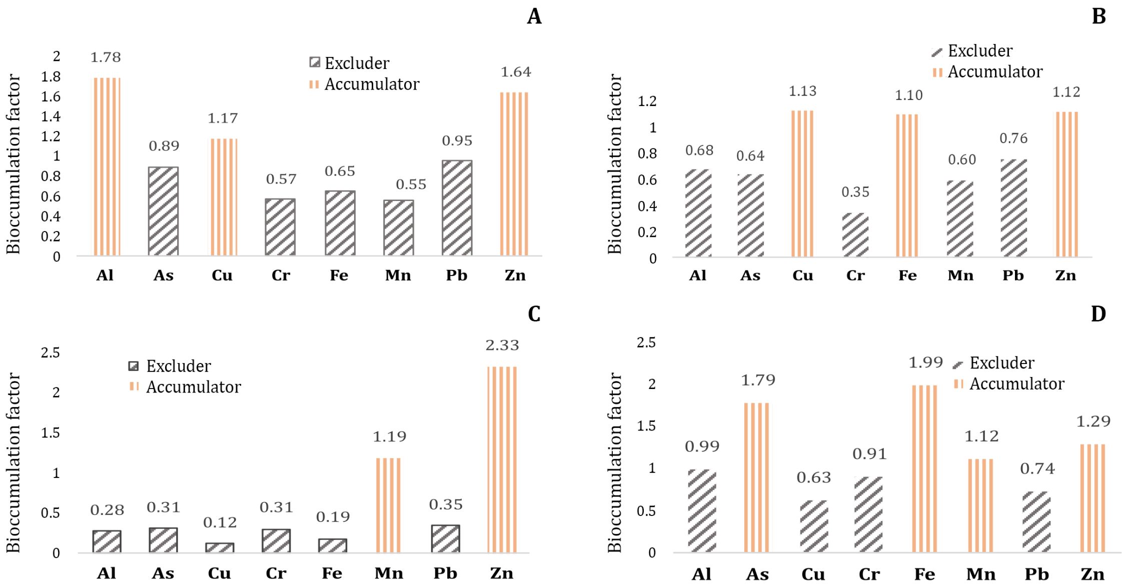

Figure 4. Bioaccumulation factor in root tissue in non-translocated cushions (A), translocated cushions (B), and in foliage in non-translocated cushions (C) and translocated cushions (D) of *D. muscoides.*

Figura 4. Factor de bioacumulación en tejido radicular en cojines no translocados (A), translocados (B), y en follaje en cojines no translocados (C) y translocados (D) de *D. muscoides.*

The BAF of metals in root tissue classified both translocated and non-translocated cushions as excluders of As, Cr, Mn, and Pb, and accumulators of Cu and Zn. The highest BAF values in roots corresponded to Fe (1.10), Cu (1.13), and Zn (1.12), which were higher than for other heavy metals (0.34-0.75). This contrasts with the values observed in *T. latifolia*, where BAF values for Ni and Zn drastically decreased, metals that reached higher concentrations (42.2 and 107 mg/kg) in wetland sediments compared to Cr (30.1 mg/kg), Cu (33.2 mg/kg), and Pb (39.3 mg/kg). This decrease is related to negative regulation or the plant's capacity to reduce or suppress a response to stimuli. At low environmental concentrations of metals, the plant can retain them; however, when concentrations increase chronically, tissues may not effectively control bioaccumulation (9). While an inverse relationship between BAF and the concentration of major metals was observed in *T. latifolia*, this relationship was not observed in *D. muscoides* regarding Fe, Cu, and Zn, metals that were found in higher concentrations in wetland 2 impacted by ARD.

The translocation factor of metals equally classified transplanted and non-transplanted cushions as phytoextractors (TF > 1) of Mn and Zn, and phytostabilizers (TF < 1) of Pb and Cu (figure 5). This aligns with the classifications attributed to *T. latifolio* as a phytoextractor of Zn, but contrasts with these plants as phytostabilizers of As, Cr, and Ni (9). Regarding Al, As, Fe, and Cr, the translocation factor classified transplanted cushions as phytoextractors, whereas non-transplanted cushions were classified as phytostabilizers. In phytoremediation, metals absorbed by the roots are transported and accumulated in the foliage, thereby permanently reducing these contaminants in peat or soil. In phytostabilization, metal mobilization is reduced, preventing migration into groundwater; however, contaminants remain in the peat or soil (24, 30).

This study had several limitations, including aspects of experimental design (such as the study area and sample size), challenges during experiment execution (such as inaccessibility to the experimental zone due to flooding and the slow growth of *D. muscoides*), and logistical issues (such as entry and handling of plants in the protected area and transportation of samples to the laboratory). Despite these challenges, the data obtained and analyzed were sufficient to determine the experimental positive effect of translocation and adaptation of *D. muscoides* cushions in a ARD-impacted wetland.

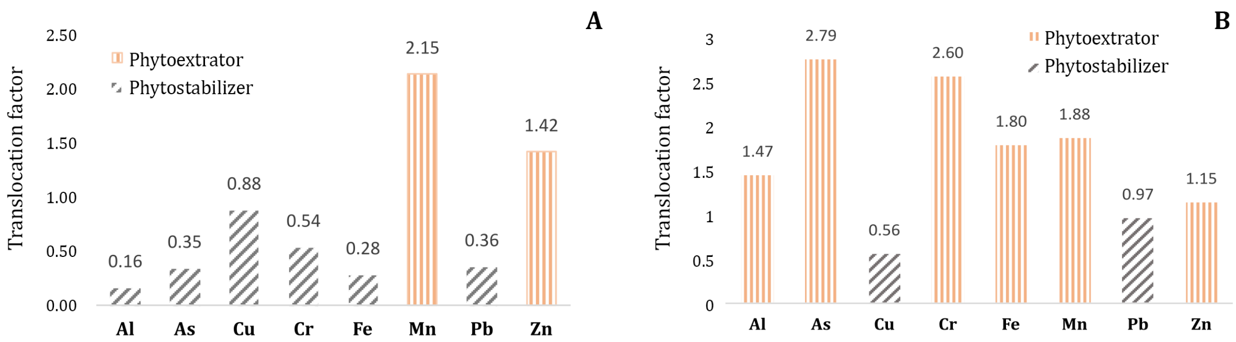

Figure 5. Translocation factor of non-translocated (A) and translocated (B) cushions of *D. muscoides*.

Figura 5. Factor de traslocación de cojines no traslocados (A) y traslocados (B) de *D. muscoides*.

Conclusion

The translocated cushions of *D. muscoides* adapted to the ARD-impacted wetland, promoting the phytoextraction and phytostabilization of heavy metals over the five months of evaluation. Particularly notable is the accumulation of Mn and Zn in the aerial plant tissue, alongside the accumulation and stabilization of Pb and Cu in the root tissue. The concentrations of some metals at certain points showed a higher standard deviation from the mean; however, these outliers had little effect on the overall results. This confirms *D. muscoides'* phytoremediation activity in extracting heavy metals from the environment, thereby preventing their deposition in the soil and surrounding water. They are proposed as suitable candidates for bioremediating ARD-impacted wetlands. However, larger-scale studies are needed that encompass a broader sample size, evaluate the concentration of heavy metals in water throughout the study period, and cover both wet and dry periods of the wetland to obtain more definitive results. Finally, special attention is emphasized on the concentration of Mn, which was the metal with the highest concentration in the evaluated wetlands.

References

1. Alcalá Jáuregui, J. A.; Rodríguez Ortiz, J. C.; Filippini, M. F.; Martínez Carretero, E.; Hernández Montoya, A.; Rojas Velázquez, A. N.; Méndez Cortés, H.; Beltrán Morales, F. A. 2022. Metallic elements in foliar material and fruits of three tree species as bioindicators. Revista de la Facultad de Ciencias Agrarias. Universidad Nacional de Cuyo. Mendoza. Argentina. 54(2): 61-72. DOI: https://doi.org/10.48162/rev.39.083
2. Arsenic, A. M.; Beryllium, A. M.; Chromium, B. N.; Cobalt, C. P.; Iron, C. S.; Lead, C. S.; Thallium, I. Z. 1996. Method 3050B acid digestion of sediments, sludges, and soils 1.0 scope and application. United States Environmental Protection Agency (EPA). Washington. DC. USA. 1-12.
3. Autoridad Nacional del Agua (ANA). 2011. Protocolo Nacional de Monitoreo de la calidad en cuerpos naturales de agua superficial. Ministerio de Agricultura. Perú. https://hdl. handle.net/20.500.12543/215 (Accessed May, 2023).
4. Awa, S.; Hadibarata, T. 2020. Removal of heavy metals in contaminated soil by phytoremediation mechanism: A Review. Water, air, Soil Pollut. 231-47. https://doi.org/10.1007/s11270-020-4426-0
5. Benfield, A.; Yu, Z.; Benavides, J. 2021. Environmental controls over Holocene carbon accumulation in *Distichia muscoides*-dominated peatlands in the eastern Andes of Colombia. Quaternary Science Reviews. 251: 106687. https://doi.org/10.1016/j.quascirev.2020.106687
6. Chávez, J.; Gonzales, J.; Iparraguirre, J. 2023. El impacto del cambio climático en la evolución glaciar desde la pequeña edad de hielo, en la vertiente occidental de la subcuenca Ranrahirca (Cordillera Blanca, Ancash). Tesis de grado en Ingeniería Ambiental y Recursos Naturales. Facultad de Ingeniería Ambiental y Recursos Naturales. Universidad Nacional del Callao. Perú. 156 p.
7. Cooper, D.; Kaczynski, K.; Slayback, D.; Yager, K. 2015. Growth and organic carbon production in peatlands dominated by *Distichia muscoides*, Bolivia, South America. Artic, Antarctic, and Alpine Research. 47(3): 505-510. https://doi.org/10.1657/AAAR0014-060
8. Ding, X.; Qian, L.; Juang, W.; Liu, H.; Yiming, A.; Zha, M.; Qu, J.; Jiang, Z. 2024. Review of bacterial sulfate reduction in lacustrine deposition and its identification in the Jimsar Sag, Junggar Basin. Marine and Petroleum Geology. 163: 106801. https://doi.org/10.1016/j.marpetgeo.2024.106801
9. Haghnazar, H.; Sabbagh, K.; Johannesson, K.; Pourakbar, M.; Aghayani, E. 2023. Phytoremediation capability of *Typha latifolia* L. to uptake sediment toxic elements in the largest coastal wetland of the Persian Gulf. Marine Pollution Bulletin. 188: 114699. https://doi.org/10.1016/j.marpolbul.2023.114699
10. Heisi, H.; Awosusi, A.; Nkuna, R.; Matambo, T. 2023. Phytoextraction of anthropogenic heavy metal contamination of the Blesbokspruit wetland: Potential of wetland macrophytes. Journal of Contaminant Hydrology. 253: 104101. https://doi.org/10.1016/j.jconhyd.2022.104101
11. Hirsh, M. 2016. Compilación sistemática de la situación actual en bofedales, bosques, paisajes, calidad de agua y praderas en la reserva de biósfera Huascarán, región Áncash. USAID, Instituto de montaña conservación, cultura, comunidad AID-527-A-14-00001. 39 p.
12. Hussein, S.; Qurbani, K.; Ahmed, S.; Tawfeeq, W.; Hassan, M. 2024. Bioremediation of heavy metals in contaminated environments using *Comamonas* species: A narrative review. Bioresource Technology Reports. 25: 101711. https://doi.org/10.1016/j.biteb.2023.101711
13. Janaki, M.; Kirupanantha, P.; Senthil, S.; Stanley, V.; Al Farraj, D.; Aljeidi, R.; Arokiyaraj. S. 2024. Beneficial role of *Burkholderia cepacia* in heavy metal bioremediation in metal-polluted soils and enhance the tomato plant growth. Biocatalysis and Agricultural Biotechnology. 57: 103032. https://doi.org/10.1016/j.bcab.2024.103032

14. Jara-Peña, E.; Gómez, J.; Montoya, H.; Sánchez, T.; Tapia, L.; Cano, N.; Dextre, A. 2017. Acumulación de metales pesados en *Calamagrostis rigida* (Kunth) Trin ex Steud. (Poaceae) y *Myriophyllum quitense* Kunth (Haloragaceae) evaluadas en cuatro humedales altoandinos del Perú. Arnaldoa. 24(2): 583-598. http://dx.doi.org/10.22497/arnaldoa.242.24210
15. La Matta, F. 2020. Influencia del drenaje ácido de roca en la comunidad de macroinvertebrados bentónicos, índices bióticos de calidad de agua y grupos funcionales alimenticios en ríos y cabeceras de La Cordillera Blanca (Subcuenca de Quillcay, Áncash). Tesis de grado en Biología. Facultad de Ciencias y Filosofía. Universidad Peruana Cayetano Heredia. Lima. Perú. 118 p.
16. López, M.; Morales, O. 2022. Fitorremediación de suelos contaminados por metales pesados: una revisión. Revista de Ciencia y Tecnología El Higo. 12(2): 15-28. https://doi.org/10.5377/elhigo.v12i2.15197
17 Luna, E. 2018. Biorremediación utilizando *Distichia muscoides* y *Calamagrostis glacialis* del drenaje ácido de roca proveniente del nevado de Pastoruri-2015. Tesis de grado en Ingeniería Ambiental. Facultad de Ciencias del Ambiente. Universidad Nacional Santiago Antúnez de Mayolo. Ancash. Perú. 103 p.
18. Magnússon, R.; Cammeraat, E.; Lücke, A.; Jansen, B.; Zimmer, A.; Recharte, J. 2020. Influence of glacial sediments on the chemical quality of surface water in the Ulta valley, Cordillera Blanca, Perú. Journal of Hydrology. 587: 125027. https://doi.org/10.1016/j.jhydrol.2020.125027
19. Martín, T. D.; Brockhoff, C. A.; Creed, J. T.; EMMC Methods Work Group. 1994. Methods EMW 200.7: Methods for the determination of metals in environmental samples. U.S. Environmental Protection Agency Cincinnati, Ohio. Methods Work Group. Revision 4.4. 1-58.
20. Martínez, V.; Benavides, J. 2023. Carbon balance shift in mountain peatlands along a gradient of grazing disturbance in the tropical Andes (Colombia). Plant Ecology. 224: 1049-1058. https://doi.org/10.1007/s11258-023-01356-8
21. Ministerio del Ambiente (MINAM). 2016. El Perú y el cambio climático, tercera comunicación nacional del Perú a la Convención Marco de las Naciones Unidas sobre cambio climático. https://www.gob.pe/institucion/minam/informes-publicaciones/2653-el-peru-y-el-cambio-climatico-tercera-comunicacion-nacional-del-peru (Accessed March, 2023).
22. Ministerio del Ambiente (MINAM). 2017. Decreto Supremo N° 004-2017. Estándares de Calidad Ambiental (ECA) para Agua y establecen Disposiciones Complementarias. https://www.gob.pe/institucion/minam/normas-legales/3671-004-2017- minam (Accessed March, 2023)
23. Narváez, C.; Kalinhoff, C.; Sánchez, A. 2024. Potencial del arbusto *Lantana camara* L. (Verbenaceae) para la fitoestabilización y volatilización de mercurio. Revista Internacional de Contaminación Ambiental. 40: 95-103. https://doi.org/10.20937/RICA.54790
24. Oggero, A.; Nakayama, H.; Ávalos, C.; Garcia, I.; Benítez, J.; Ayala, J.; Elkhalili, R., Peralta, I. 2021. Eficiencia de la absorción de cobre (Cu) y cromo (Cr), una propuesta de fitorremediación de efluentes mediada por *Typha domingensis*. Revista de la Sociedad Científica del Paraguay. 26(2): 100-113. https://doi.org/10.32480/rscp.2021.26.2.100
25. Orrego, S. 2019. Eficiencia de consorcios de bacterias sulfato reductoras en la degradación anaerobia de hidrocarburos aromáticos del petróleo en microcosmos, junio-octubre de 2018. Tesis de maestría en Ciencias en Ingeniería Ambiental. Universidad Nacional Pedro Ruiz Gallo. Lambayeque. Perú. 83 p.
26. Oyague, E.; Cooper, D.; Ingol, E. 2022. Effects of land use on the hydrologic regime, vegetation, and hydraulic conductivity of peatlands in the central Peruvian Andes. Journal of Hydrology. 609: 127687. https://doi.org/10.1016/j.jhydrol.2022.127687
27. Pabón, S.; Benítez, R.; Sarria, R.; Gallo, J. 2020. Contaminación del agua por metales pesados, métodos de análisis y tecnologías de remoción. Una revisión. Entre Ciencia e Ingeniería. 14(27): 9-18. https://doi.org/10.31908/19098367.1734
28. Pedraza, M. 2021. Fitorremediación en cuerpos de agua contaminados por metales pesados. Revista Científica de Biología y Conservación. 1(1): 61-78. https://doi.org/10.58720/ibs. v1i1.6
29. Rascón, J.; Corroto, F. 2020. Evolución fisicoquímica y de las bacterias de azufre en microcosmos de diferentes sistemas acuáticos de la Región Amazonas. Revista de Investigación Científica Tayacaja. 3(1). https://doi.org/10.46908/rict.v3i1.68
30. Reyna, D.; Cortés, L.; Sandino, G.; Martínez, M.; Rodríguez, L. 2022. Estudio del comportamiento de especies vegetales en un proceso de fitoestabilización para remediación de suelo contaminado por jal minero abandonado. Ciencia, Tecnología y Salud. 16(32): 52-57. https://doi.org/10.36790/epistemus.v16i32.197
31. Ruthsatz, B. 2022. Vegetación y ecología de los bofedales tropicales altoandinos en el norte de Chile. Chloris Chilensis. 25(1): 165-214.
32. Suárez, E.; Chimbolema, S.; Chimner, R.; Lilleskov, E. 2021. Root biomass and production by two cushion plant species of tropical high-elevation peatlands in the andean paramo. Mires and Peat. 27. https://doi.org/10.19189/MaP.2020.OMB.StA.2131
33. Wang, H.; Wang, X.; Zhang, Y.; Wang, D.; Long, X.; Chai, G.; Wang, Z.; Meng, H.; Jiang, C.; Dong, W.; Guo, Y.; Li, J.; Xu, Z.; Lin, Y. 2023. Sulfate-reducing bacteria-based bioelectrochemical system for heavy metal wastewater treatment: Mechanisms, operating factors, and future challenges. Journal of Electroanalytical Chemistry. 915 (15): 117945. https://doi.org/10.1016/j.jelechem.2023.117945

34. Wanner, C.; Moradi, H.; Ingold, P.; Cardenas, M.; Mercurio, R.; Furrer, G. 2023. Rock glaciers in the Central Eastern Alps-How permafrost degradation can cause acid rock drainage, mobilization of toxic elements and formation of basaluminite. Global and Planetary Change. 227: 104180. https://doi.org/10.1016/j.gloplacha.2023.104180
35. Yan, A.; Wang, Y.; Tan, S.; Yusof, M.; Ghosh, S.; Chen, Z. 2020. Phytoremediation: a promising approach for revegetation of heavy metal-polluted land. Frontiers in Plant Science. 30(11): 359. https://doi.org/10.3389/fpls.2020.00359
36. Yaranga, R. 2020. Humedal Altoandino del Perú: Diversidad florística, productividad primaria neta aérea, condición ecológica y capacidad de carga. Scientia Agropecuaria. 11(2): 213-221. https://doi.org/10.17268/sci.agropecu.2020.02.08
37. Zheng, H.; Bingjie, L.; Guocheng, L.; Zhaohui, C.; Chenchen, Z. 2019. Potential toxic compounds in biochar: Knowledge gaps between biochar research and safety, biochar from biomass and waste. Biochar from Biomass and Waste. 349-384. https://doi.org/10.1016/B978-0- 12-811729-3.00019-4
38. Zimmer, A.; Brito, M.; Alegre, C.; Sánchez, J.; Recharte, J. 2018. Implementación de dos sistemas de biorremediación como estrategia para la prevención y mitigación de los efectos del drenaje ácido de roca en la Cordillera Blanca, Perú. Revista de Glaciares y Ecosistemas de Montaña. 4(3): 57-76.
39. Zimmer, A.; Beach, T.; Luzzadder, S.; Rabatel, A.; Lopez, J.; Cruz, R.; Temme, A. 2024. Physico-chemical properties and toxicity of young proglacial soils in the Tropical Andes and Alps. Catena. 237: 107748. https://doi.org/10.1016/j.catena.2023.107748

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