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Toxicological Efficiency Evaluation of the ASEC Technology for Contaminated Mining Water Using *Lemna minor*

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Abstract

The Adiabatic Sonic Evaporation and Crystallization (ASEC) technology was developed as a disruptive zero-liquid discharge system to treat contaminated mining effluents. This study evaluates its ecotoxicological efficacy using *Lemna minor*, a freshwater macrophyte, as a sensitive bioindicator. Acute growth inhibition tests were conducted using OECD Guideline 221. *Lemna minor* was exposed for 7 days to untreated and treated effluents from the Tharsis mine and the Tinto River in southern Spain. The results revealed 100% inhibition of frond growth and biomass in untreated samples (pH < 2.6), indicating acute toxicity. In contrast, effluents treated with ASEC showed growth and biomass accumulation statistically indistinguishable from the control, confirming the system's efficiency in reducing toxicity and restoring water quality. These findings support the environmental viability of ASEC technology for mine and port effluent treatment.

Keywords: *Lemna minor*; ASEC; zero liquid discharge; phytotoxicity; ecotoxicological bioassays; mine effluents

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1. Introduction

Mining activities in the Iberian Pyrite Belt (IPB) have led to one of the most persistent and extensive acid mine drainage (AMD) problems in Europe. The oxidation of pyritic materials (pyrite, arsenopyrite, galene, etc.) produces highly acidic waters enriched in dissolved metals and sulfates, which affect more than 40% of the river basin [1]. Notably, the Odiel and Tinto rivers drain large portions of the IPB and exhibit extreme chemical conditions, with pH values frequently below 3, sulfate concentrations exceeding 10,000 mg/L, and EC ranging from 2000 µS/cm to over 15 mS/cm depending on the flow conditions and proximity to mine sites [2–4]. These conditions support virtually no aquatic life and contribute significant pollutant loads to the Ría de Huelva estuary, impacting downstream ecosystems over more than 100 km of riverine and estuarine networks [4–6].

The ecological and chemical degradation resulting from these discharges presents a critical challenge regarding compliance with the European Union's Water Framework Directive (2000/60/EC), which requires member states to achieve "good ecological and chemical status" in all surface waters by 2027 [7].

In response to the chronic contamination caused by AMD, several remediation strategies are currently being investigated and applied at various scales. These include passive systems such as constructed wetlands [8], active treatments involving alkaline dosing and oxidation [9], and advanced technologies like membrane filtration [10] and zero-liquid-discharge (ZLD) processes. One such promising ZLD approach is the Adiabatic Sonic Evaporation and Crystallization (ASEC) system. This technology integrates continuous evaporation, crystallization, and distillation in a single energy-efficient unit (<20 kWh/m^3), offering a compact and modular solution for highly contaminated effluents. It allows for the complete separation of liquid–solid phases in a single step, resulting in distilled water and dried solids. This technology has already been successfully tested for the treatment of mining waters, as demonstrated in recent studies [11,12] that evaluated the performance of ASEC on AMD samples from the IPB. The treated water showed significant improvement, with pH levels rising to near-neutral (7.8–7.9), EC < 50 µS/cm, and metal concentrations falling below detection limits. Their findings confirmed that the system effectively reduced concentrations of key contaminant elements such as Fe (from >1000 mg/L to <0.01 mg/L), Cu (from 50 to 100 mg/L to undetected concentrations), Zn (from 30 to 80 mg/L to <0.02 mg/L), and sulfates (from >10,000 mg/L to <10 mg/L), achieving treated water characteristics comparable to deionized water. The study [11] also emphasized the potential for recovering valuable elements such as Mg, Fe, and rare earth elements (REEs) from the crystallized solid phase, highlighting ASEC's dual role in environmental remediation and circular economy-oriented resource recovery.

While the chemical improvements achieved by technologies like ASEC are well documented, there remains a critical gap in understanding the biological implications of such treatments. Specifically, the extent to which these chemically improved waters can support aquatic life remains poorly characterized. The European Water Framework directive requires the ecological restauration of fluvial courses. Living conditions must be achieved, and therefore, the water quality has to meet chemical and biological standards. Through toxicity tests, it is possible to check whether the living conditions are adequate for aquatic organisms. Some common target species to test freshwater quality are invertebrates (e.g., *Daphnia magna*, *Artemia* sp.), aquatic plants, (such as *Pistia stratiotes*), or microalgae species (*Selenastrum capricornutum* or *Chlorella* sp.) For example, studies have shown that treated AMD may still pose residual toxicity to aquatic organisms, even when meeting the chemical discharge criteria [13–16]. Many AMD-impacted waters undergo substantial physicochemical transformation during treatment, but without concurrent ecotoxicological assessment, it is unclear whether the detoxification is ecologically meaningful. Toxicity tests with sensitive bioindicators such as *Lemna minor* are therefore essential to determine the ecological relevance of treatment outcomes, bridge this knowledge gap, and guide informed decisions regarding water reuse or discharge.

To evaluate the ecotoxicological implications of these improvements, bioassays were performed using *Lemna minor* (L.) Griffith 1851 (duckweed), a standard aquatic macrophyte recommended in OECD Guideline 221 [17]. *L. minor* is a small, fast-growing, floating freshwater plant from the family Araceae, which is widely distributed and highly responsive to environmental contaminants. It is considered an ideal model for phytotoxicity testing due to its clonal reproduction, ease of cultivation, and well-established endpoints. Typical endpoints used in toxicological studies include the number of fronds, frond area, biomass accumulation (fresh or dry weight), and specific growth rate. These parameters provide sensitive and quantitative measures of the physiological status of the plants under stress conditions, particularly in response to high metal concentrations, low pH, and other emerging contaminants [18,19].

2. Materials and Methods

2.1. Sampling and ASEC Treatment

Mining water samples were collected from two locations representative of AMD pollution in the IPB: the Tharsis mine drainage channel (Odiel River watershed) and the Puente Gadea site on the Tinto River (Figure 1). Both sites exhibit highly acidic conditions and elevated concentrations of dissolved metals. The collected raw effluents (Type C) were stored in high-density polyethylene containers, kept at 4 °C, and shielded from light until analysis.

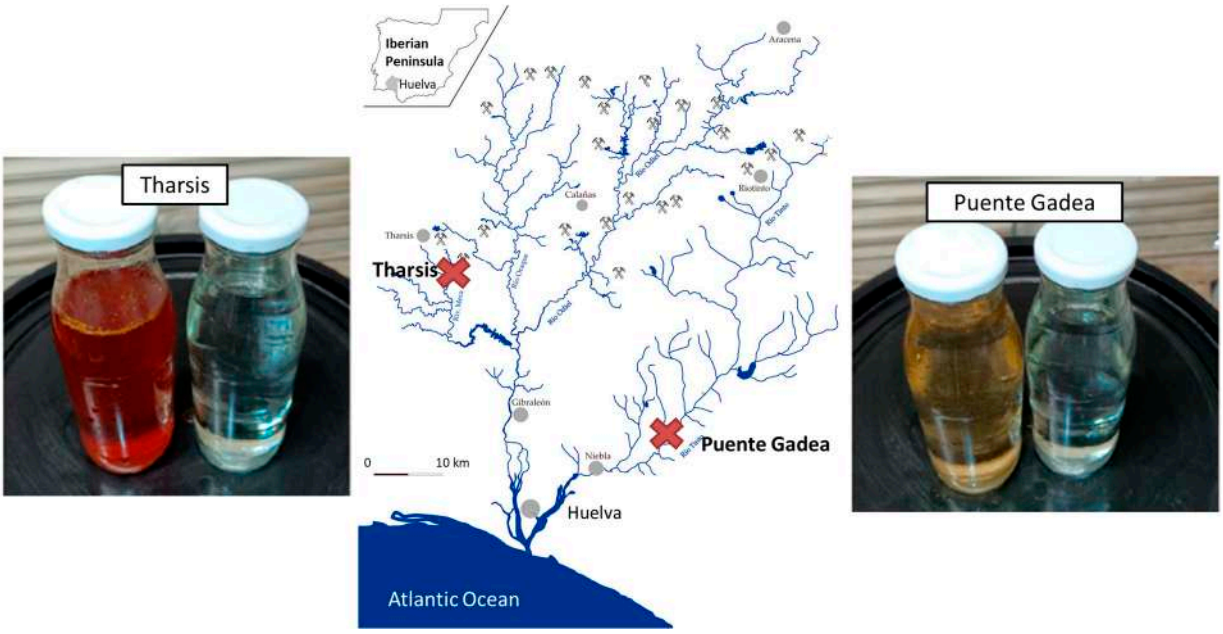

Figure 1. Location of the sampling points in the Odiel–Tinto River watershed: Tharsis (Odiel River basin), and Puente Gadea (Tinto River basin); and pictures of water samples before (**left**) and after (**right**) ASEC treatment.

A detailed hydrochemical characterization of the influent and effluent waters from the Tharsis and Puente Gadea sites was conducted as part of the study by DelValls et al. [12]. Briefly, untreated samples from Tharsis exhibited a pH of 3.15, electrical conductivity (EC) of 12,140 µS/cm, and total dissolved solids (TDS) of 7530 mg/L. Similarly, the Puente Gadea sample (Río Tinto) had a pH of 3.70, EC of 2450 µS/cm, and TDS of 1516 mg/L. Both were highly acidic and rich in metals such as Fe (2845 g/L in Tharsis) and Cu (186,798 mg/L).

The treatment was conducted using a pilot-scale Adiabatic Sonic Evaporation and Crystallization (ASEC). The ASEC system integrates evaporation, crystallization, and condensation to separate the aqueous and solid phases in a continuous-flow setup. The system operates with an energy consumption of less than 20 kWh/m^3 and produces a highly purified aqueous fraction (Type D) with conductivity values below 50 µS/cm and near-neutral pH. After ASEC treatment, the effluents showed substantial improvements: pH increased to 6.69–6.79, conductivity decreased to 35.8–55.5 µS/cm, and TDS dropped below 0.05 mg/L. All metal concentrations, including Fe, Cu, Zn, and As, were below detection limits [12]. These results confirm that ASEC technology achieves near-distilled water quality and can serve as the hydrochemical basis for subsequent ecotoxicological assays.

2.2. Toxicological Bioassays with Lemna minor

The phytotoxicity of the untreated and treated effluents was assessed using a 7-day static growth inhibition assay following OECD Guideline 221 [17]. *Lemna minor* cultures were maintained in Steinberg medium under controlled laboratory conditions (24 ± 1 °C;

16:8 h light–dark photoperiod). Before the assay, healthy colonies with three fronds were selected and pre-acclimatized for 8 weeks.

Test solutions were prepared by mixing the effluents (Type C or D) with Steinberg medium in a 1:1 volume ratio. Each treatment and control condition were tested in triplicate using glass crystallizers containing 400 mL of test solution and 12 fronds. The crystallizers were randomized and exposed under uniform light and temperature conditions. Every two days, the number and area of fronds were recorded through digital image analysis.

Biomass production was quantified using total frond area, measured via image analysis at the end of the 7-day exposure period, as recommended in OECD Guideline 221. The specific growth rate (μ) was determined for each replicate using the following formula:

$$\mu = [\ln(Nj) - ln(Ni)]/(tj - ti) \quad (1)$$

where Nj and Ni represent the number of fronds at the end (tj) and beginning (ti) of the exposure period, respectively. This metric allows for the estimation of relative growth regardless of initial frond number and is considered more robust than using biomass alone.

Growth rate inhibition ($Ir\%$) and biomass inhibition ($Ib\%$) were calculated according to the OECD guidelines:

$$Ir\% = [(\mu C - \mu T)/\mu C] \times 100 \quad (2)$$

$$Ib\% = [(bC - bT)/bC] \times 100 \quad (3)$$

where μC and μT are the average growth rates in control and treatment groups, and bC and bT are the natural logarithmic increases in biomass. This dual approach captures both the proliferation and size response of *Lemna minor* under different exposure scenarios [17,19].

2.3. Statistical Analysis

Bioassays were repeated twice under the same laboratory conditions. Prior to data pooling, the results were statistically compared and no significant differences were found, indicating that they formed homogeneous groups. To justify combining data from both assays, run tests were performed separately for biomass and growth using the *randtest* function in R. All data were found to be random ($p > 0.05$), supporting their combination. However, due to the high number of zeros and relatively low sample size, which may reduce statistical power, a mixed-effects model was applied (with bioassay as a random factor) to account for potential temporal variability.

Data normality and homoscedasticity were verified using the Kolmogorov–Smirnov and Levene's tests, respectively. Two-way ANOVA was applied to detect differences among treatments, with bioassay as random factor, followed by Tukey's HSD test for post hoc comparisons. All statistical analyses were conducted at a significance level of 0.05 using the software R (version 4.2.2). To integrate the water quality through physico-chemical characterization with the toxicity responses, a Spearman correlation between biomass and growth and physico-chemical variables was carried out. Furthermore, a distance-based redundancy analysis (dbRDA) was conducted to assess the multivariate response of *Lemna minor* to the different water treatments. These analyses were carried out using the BiodiversityR v. 2.8–4 packages in R [20].

3. Results

3.1. Physicochemical Characterization

The hydrochemical properties of the mine water samples from Tharsis and Puente Gadea (Tinto River) were analyzed before and after treatment with ASEC technology; they are detailed in [12]. Physicochemical parameters were monitored during the toxicity tests

(7 days, temperature 21 °C ± 0.40, lux 5100 ± 1205) and are summarized in Table 1. The untreated samples (Type C) showed typical characteristics of AMD, with extremely low pH (2.32–3.15), high EC (2.5–12 mS/cm), and elevated concentrations of Fe (2844 mg/L in Tharsis), Cu (186 mg/L), and sulfates (8266 mg/L).

Table 1. Physicochemical characterization of AMD waters before (Type C) and after (Type D) ASEC treatment from Tharsis and Puente Gadea.

Parameter	Control	Tharsis Type C	Tharsis Type D	Puente Gadea Type C	Puente Gadea D Type D
$pH_{bioassays}$	7.93 ± 0.17	2.32 ± 0.03	7.8 ± 0.09	2.57 ± 0.03	7.81 ± 0.09
EC (µS/cm)	269	12140	55.5	2450	35.8
TDS (mg/L)	55	7530	0.05	1516	0.05
Fe (mg/L)	<DL	2844.6	<DL	161.8	<DL
Cu (mg/L)	<DL	186.8	<DL	116.2	<DL
Zn (mg/L)	<DL	36.9	<DL	18.2	<DL
As (mg/L)	<DL	5.8	<DL	2.6	<DL
Mg (mg/L)	8.5	1.29	<DL	88.14	<DL
K (mg/L)	3	4.6	<DL	6.0	<DL
Mn (mg/L)	<DL	134	<DL	8.4	<DL
Na (mg/L)	12	67.44	<DL	41.65	<DL
Ca (mg/L)	33.3	20.2	<DL	4.3	<DL
Cd (mg/L)	<DL	0.09	<DL	0.97	<DL
B (mg/L)	0.02	0.09	<DL	0.25	<DL
Co (mg/L)	<DL	9.35	<DL	0.55	<DL
Sulfates (mg/L)	55	7300	<DL	8266.6	<DL

Note: <DL = below detection limit. Data source of physicochemical characterization [12,21].

After ASEC treatment (Type D), both samples showed marked improvements in water quality. The pH increased to near-neutral values (6.69–6.79), EC dropped below 55 µS/cm, and TDS were reduced to <0.05 mg/L. All target metals were below the detection limits. These results validate the ability of ASEC to produce high-purity water from heavily contaminated AMD sources [12].

3.2. Biological Responses of *Lemna minor*

The maximum temperature measured throughout the 7 days of exposure was 21° ± 0.40. The average light intensity was 5100 Lux ± 1205. The average pH in each treatment was 7.93 ± 0.17 (control), 7.81 ± 0.09 (Puente Gadea Type D), 7.8 ± 0.09 (Tharsis Type D), 2.57 ± 0.03 (Puente Gadea Type C) and 2.32 ± 0.03 (Tharsis Type C).

Each physiological variable measured in *Lemna minor* was analyzed separately to elucidate the differential responses to untreated (Type C) and ASEC-treated (Type D) effluents from Tharsis and Puente Gadea.

Exposure to untreated effluents (Type C) from both Tharsis and Puente Gadea resulted in the complete inhibition of *Lemna minor* growth and biomass accumulation. Visual observations revealed signs of necrosis and frond disintegration within the first 24–48 h (Figure 2). No fronds remained viable at the end of the 7-day exposure period.

Regarding the Specific Growth Rate (µ) (Figures 2 and 3a, Table 2), untreated samples caused complete growth inhibition (100%), with µ values of 0.00 d^{-1}, confirming the acute toxicity of acidic, metal-rich AMD. This is consistent with the extreme acidity (pH < 3) and high metal concentrations reported in these samples (Table 1). In contrast, the ASEC-treated samples showed growth rates similar to or even slightly higher than the control, with no signs of chlorosis or necrosis. Specifically, Puente Gadea (Type D) induced the highest specific growth rate (0.069 d^{-1}), exceeding the control (0.055 d^{-1}), while Tharsis (Type D) showed a minimal inhibition of 4.87% (Table 2), which was not biologically significant. Two-way ANOVA revealed significant treatment effects ($F = 9.11$, $p < 0.01$) but not between bioassays ($F = 4.35$, $p < 0.05$), with Tukey's post hoc test confirming that treated and control groups were not significantly different ($p > 0.05$), while both differed significantly from

untreated samples ($p < 0.001$). These results suggest that the ASEC treatment successfully neutralized phytotoxicity.

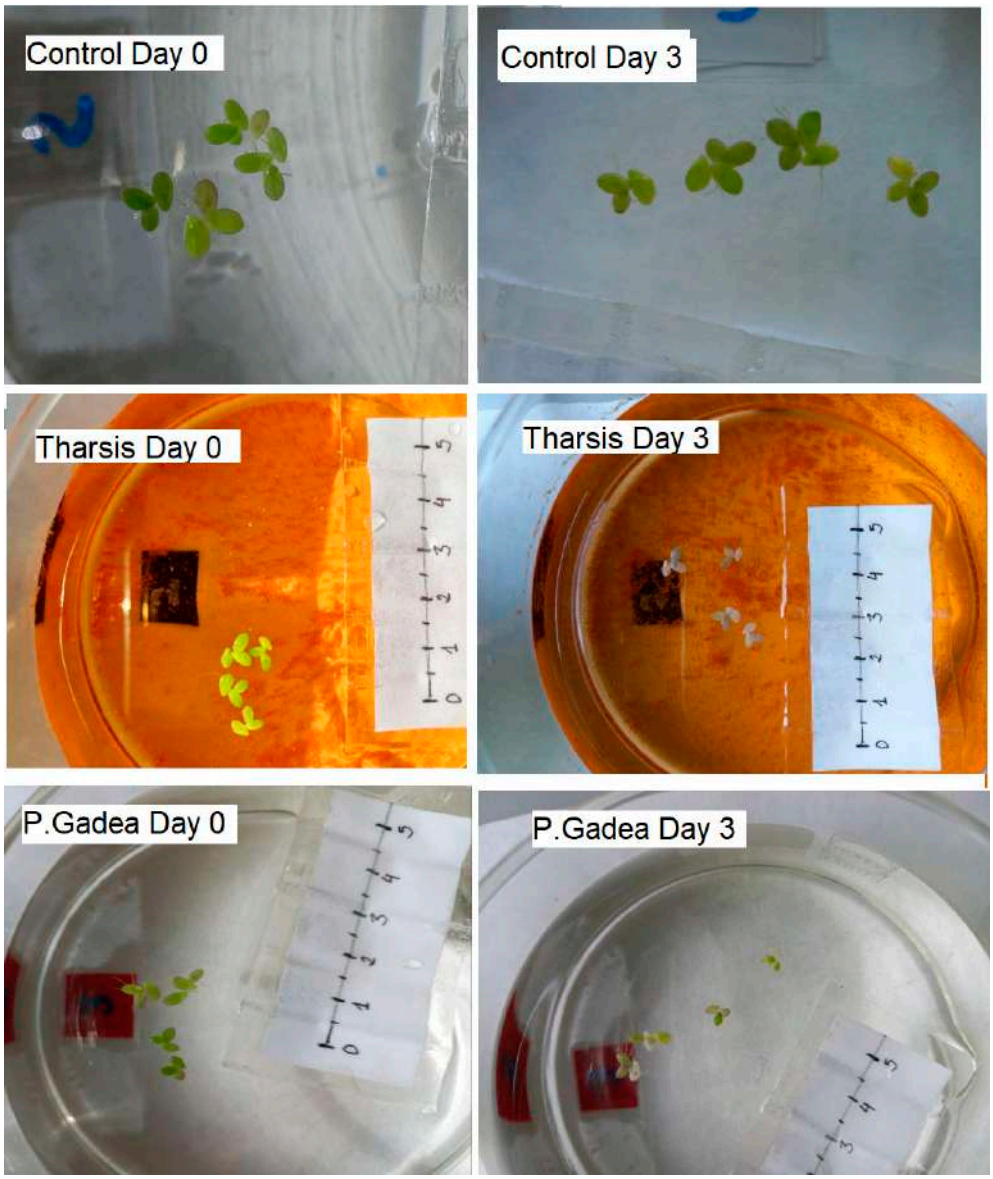

Figure 2. Changes in *Lemna* sp. organisms from day 0 to the end of exposure to the control, Tharsis, and P. Gadea samples.

The biomass results mirrored the growth rate patterns (Figure 3b, Table 2). Both untreated samples completely inhibited biomass accumulation (100% inhibition). However, treated effluents supported robust growth: 0.405 fronds for Tharsis (Type D) and 0.440 fronds for Puente Gadea (Type D) compared to 0.166 in the control (Tukey's test, $p < 0.05$). The ANOVA confirmed a significant overall treatment effect ($F = 30.51$, $p < 0.01$) that was not observed between bioassays ($F = 0$, $p > 0.05$). This increase in frond count beyond control levels suggests that ASEC-treated water not only supports plant viability but may also contain growth-promoting micronutrients within acceptable thresholds.

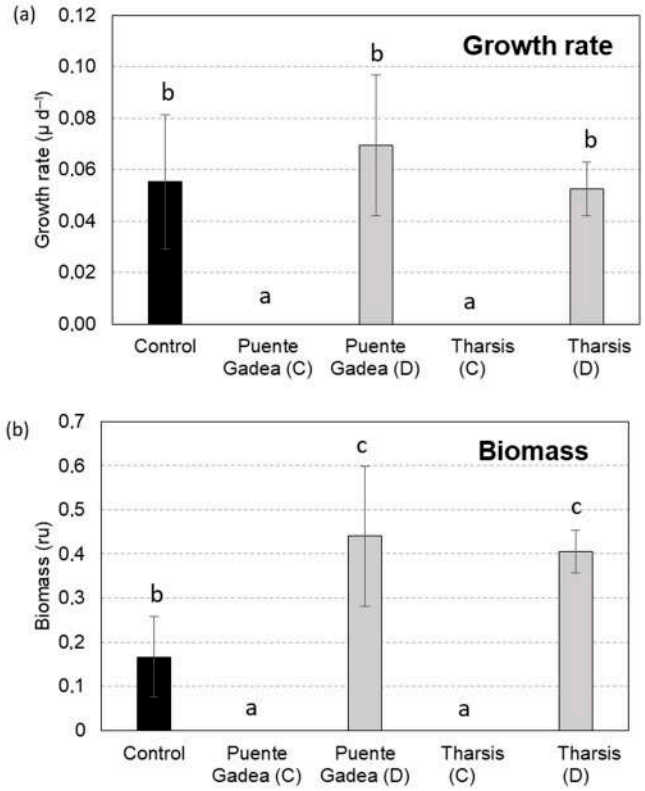

Figure 3. (**a**) Specific growth rate (μ) of *Lemna minor* exposed to untreated (Type C) and ASEC-treated (Type D) waters. (**b**) Biomass accumulation of *Lemna minor* after 7 days of exposure to each treatment. Bars show mean ± SD (n = 6). Different letters indicate statistically significant differences between treatments (Tukey HSD, $p < 0.05$). Treatments sharing a letter do not differ significantly.

Table 2. Mean specific growth rate (μ) and biomass accumulation in *Lemna minor* after 7 days of exposure to untreated (Type C) and treated (Type D) waters from Tharsis and Puente Gadea.

Treatment	Growth Rate (μ, d^{-1})	%Inhibition Growth Rate	Biomass (Fronds)	%Inhibition Biomass
Control	0.055	-	0.166	-
Tharsis (Type C)	0	100	0	100
Tharsis (Type D)	0.052	4.868	0.405	0
Puente Gadea (C)	0	100	0	100
Puente Gadea (D)	0.069	0	0.44	0

According to Spearman coefficient, growth was negatively correlated (>0.8, $p < 0.01$) with Fe, Cu, Zn, As, Mn, B, K, Na, and Co and positively correlated with pH (>0.8, $p < 0.001$). Biomass was negatively correlated with TDS, K, Na, and B (>0.9, $p < 0.01$) and with Fe, Cu, Zn, As, Mg, and Co (>0.8, $p < 0.01$). Contrarily, biomass was positively correlated with pH (>0.6, $p < 0.001$).

The dbRDA analysis showed a clear separation between Type D and the control from Type C (Figure 4). The model best explaining this distribution included the pH of Type D and the control, and the K (highly correlated with Fe, Cu, Zn, Mg, As, Na, and Mn) and sulfates (highly correlated with B and Mg) of Type D (MonteCarlo permutation test, $p < 0.001$).

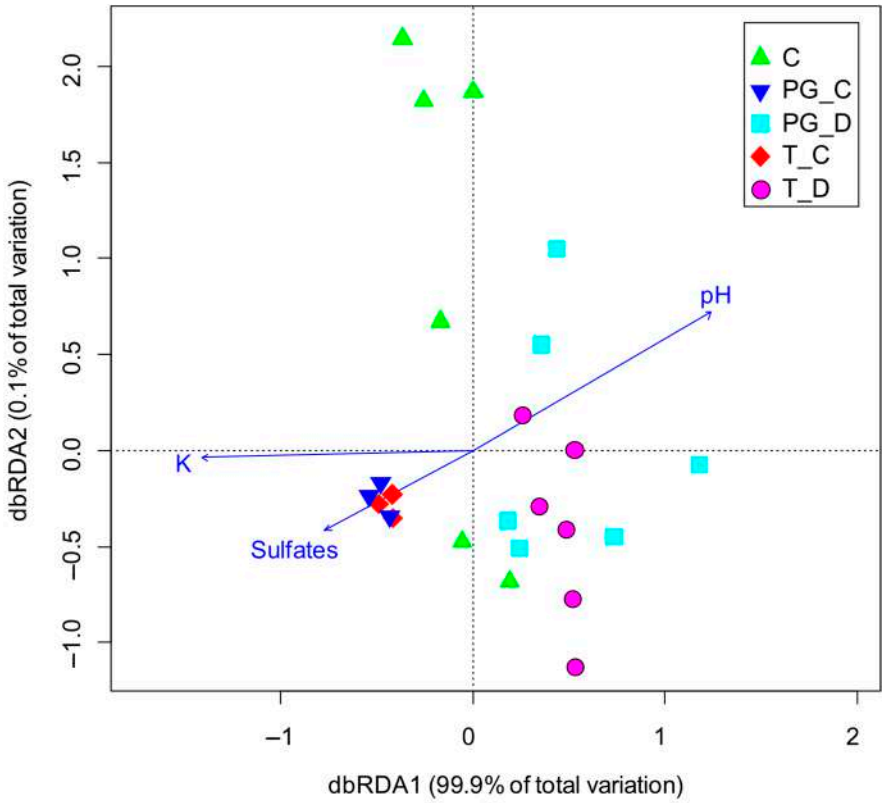

Figure 4. dbRDA analysis based on *Lemna minor* endpoints (growth and biomass) and treatments: C: control; PG_C: Puente Gadea Type C; PC_D: Puente Gadea Type D; T_C: Tharsis Type C; T_D: Tharsis Type D). The percentage of variability explained by the axis is shown.

4. Discussion

Lemna minor is the most widely distributed species within the Lemnaceae family, colonizing freshwater ecosystems globally. Morphologically, it consists of three to four dorsiventrally flattened fronds and a single adventitious root, which aids in buoyancy control and stabilization [22,23]. Under axenic and environmentally controlled laboratory conditions, *L. minor* exhibits rapid clonal propagation, with biomass doubling times of approximately 48 h under optimal nutrient and light regimes [24–26]. These attributes have established *L. minor* as a robust model system for ecophysiological studies and fundamental plant biology research [27]. Its high surface-area-to-volume ratio and simple architecture also make it particularly suitable for ecotoxicological assays [28,29].

This study demonstrates that although untreated acid mine drainage (AMD) waters are acutely toxic to aquatic plants, the ASEC system effectively neutralizes toxic elements (Table 1) and restores the chemical conditions necessary to support primary producers. This aligns with previous research emphasizing the importance of coupling chemical remediation with biological validation [30]. *L. minor* has been widely used as a sensitive indicator of industrial and AMD toxicity and metal stress [31,32], supporting its role in rapid water-quality assessments. Gerhardt et al. [33] for instance, employed *Lemna gibba* in a phytoecotoxicological study to evaluate AMD toxicity in southern Portugal using both laboratory and in situ diatom-based assessments. Their findings revealed that *L. gibba* was more sensitive to AMD than to acidified water, with growth inhibition serving as a reliable indicator of AMD-induced stress.

Similarly, refs. [34,35] demonstrated comparable results with *L. minor* cultivated in palm oil mill effluent (POME), showing greater resistance to POME toxicity compared to *Azolla pinnata*. Sasmaz Kislioglu [32] further demonstrated its efficacy in removing Ag, Au, and As from AMD waters, while ref. [26] reported substantial nutrient and chemical oxygen

demand (COD) removal from landfill leachates. Duckweed has proven more effective in removing heavy metals from industrial effluents than from municipal ones [36], likely due to the more complex composition of industrial pollutants [37]. These studies support the reliability of duckweed-based bioassays in evaluating water remediation outcomes.

Metals in ionic form can rapidly bind to the surface of *L. minor*, either integrating into the membrane or being absorbed through the roots [38]. When simultaneously exposed to multiple elements, plants experience combined metal stress and nutrient competition [39]. While essential micronutrients such as Cu and Zn are critical for enzymatic function, excessive amounts can exceed the plant's tolerance threshold and impair physiological processes [37]. In this study, *L. minor* cultivated in untreated water samples from both rivers showed growth inhibition beginning as early as day 1, with visible signs of necrosis from day 2. Similar trends were reported by [40], who observed growth inhibition in *L. minor* exposed to POME concentrations, with necrosis becoming evident at higher concentrations toward the end of the experimental period [41].

Growth inhibition may be mild or severe, with potential implications for the plant's remediation capacity. Severe inhibition often induces reactive oxygen species (ROS) production, leading to cell mortality [42]. For instance, cadmium accumulation in *L. minor* was shown to significantly inhibit plant growth [43]. Toxic levels of Zn and Al in effluents have been associated with oxidative stress and decreased enzymatic activity, prompting the activation of antioxidative mechanisms [44]. Chromium exposure also significantly inhibited growth in *L. minor* [45,46].

In our study, the growth and biomass accumulation of *L. minor* were negatively correlated with most metal concentrations in Type C waters, while a positive correlation with pH was observed. Under laboratory conditions, duckweed thrives within a pH range of 6–7.5 [37], and Type C samples are markedly more acidic. By contrast, *L. minor* exposed to treated waters showed growth and biomass accumulation that were statistically indistinguishable from the control, indicating the absence of acute phytotoxicity (Figures 3 and 4; Table 2). These biological outcomes align with the observed improvements in water quality parameters, including pH increases from <3 to ~6.7, reductions in electrical conductivity from >12,000 µS/cm to <60 µS/cm, and the removal of toxic concentrations of Fe, Cu, Zn, and As to below detection limits (Table 1).

Distance-based redundancy analysis (dbRDA) revealed a clear separation between *Lemna* cultivated in Type C waters (Figure 4), which exhibited suppressed growth, and those in Type D and control samples, which displayed enhanced growth and biomass. This confirms that the biological responses were positively influenced by the ASEC treatment. Untreated samples aligned closely with sulfate concentrations, which are known to modulate the uptake of certain pollutants in *L. minor*. This multivariate analysis reinforces the univariate findings, demonstrating that ASEC not only neutralizes toxicants but also restores biological functionality, validating the ecological relevance of the remediation process.

In this context, ASEC emerges not only as a water treatment solution, but as a holistic technology capable of restoring ecological function. It represents a replicable model for environmentally sustainable post-mining water management, aligning with circular economy principles and promoting long-term biological sustainability.

These findings highlight ASEC's potential not only as a water purification technology but also as an integrated ecological restoration solution. Its application supports the goals of the EU Water Framework Directive (2000/60/EC), which calls for good chemical and ecological status to be achieved in surface waters [7]. Additionally, ASEC contributes to several United Nations Sustainable Development Goals (SDGs), including SDG 6 (Clean Water and Sanitation), SDG 12 (Responsible Consumption and Production), and SDG 15 (Life on Land and Aquatic Ecosystems). Nevertheless, broader ecological evaluations—

including multi-species bioassays and chronic exposure studies—are recommended to fully validate the long-term sustainability of ASEC-treated discharges.

5. Conclusions

This study demonstrates the high sensitivity of *Lemna minor* to untreated acid mine drainage (AMD) and confirms its utility as a reliable bioindicator for assessing water quality and remediation success. The acute phytotoxicity observed in untreated samples, characterized by early growth inhibition and necrosis, underscores the ecological risks posed by AMD-impacted waters. However, the application of the ASEC system resulted in significant improvements in water quality parameters—such as pH neutralization, reduction in electrical conductivity, and removal of heavy metals to below detection limits—which corresponded with restored growth and biomass production in *L. minor*.

The integration of chemical and biological indicators, supported by multivariate analysis, confirms that ASEC not only removes toxic elements but also reinstates the biological functionality of aquatic systems. These findings validate the ecological relevance of the ASEC system and highlight its potential as a sustainable, circular solution for post-mining water management. Furthermore, its alignment with key environmental policy goals, including the EU Water Framework Directive and multiple United Nations Sustainable Development Goals, positions ASEC as a promising technology for advancing ecological restoration alongside water purification.

Future studies should incorporate longer-term and multi-species evaluations to fully assess the ecological resilience and long-term sustainability of treated discharges under real-world conditions.

Author Contributions: M.C. and J.E.S.-M. were involved in investigation, methodology, software, validation, formal analysis, resources, data curation, writing, reviewing and editing, visualization, supervision. E.B. was involved in formal analysis, investigation, data curation, writing, reviewing and editing, visualization. I.R. was involved in validation, formal analysis, investigation, writing, reviewing and editing. T.Á.D. was involved in conceptualization, validation, writing, reviewing and editing, visualization, supervision, project administration and funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author due to privacy or ethical restrictions.

Conflicts of Interest: Author TAD was employed by the company Water Challenge. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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