

Ecotoxicological assessment of arsenic contamination: Biological responses and bioaccumulation capacity in *Leptodictyum riparium* and *Lymnaea stagnalis*, individually and in a microcosm

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ABSTRACT

Arsenic (As) is a widespread environmental pollutant recognised by the World Health Organization (WHO) and the International Agency for Research on Cancer (IARC) as posing a serious threat to human health due to its toxic, teratogenic and carcinogenic properties. As is highly mobile and persistent in aquatic environments, where it can bioaccumulate in organisms and enter the food chain. Although regulatory agencies have set the maximum limit for As in drinking water at 10 µg/L, concentrations often exceed this threshold, exposing millions of people worldwide to significant health risks. Phytoremediation has emerged as a promising, cost-effective strategy for mitigating metal contamination, particularly using hyperaccumulating plant species. In this study, we investigated the phytoremediation potential of the aquatic moss *Leptodictyum riparium*, examining its capacity to reduce the bioavailability and toxicity of As individually and in a freshwater microcosm. We also evaluated whether the presence of the moss could mitigate As bioaccumulation and related toxic effects in the freshwater gastropod *Lymnaea stagnalis*, a well-established biomonitor of aquatic contamination. To this aim, As bioaccumulation and bioaccumulation factor; the levels of reactive oxygen species (ROS); the antioxidant enzyme activities, and the expression of proteins related to cell death and stress were evaluated. Our results show that *L. riparium* removed efficiently As from the water, without, however, mitigating As effects in *L. stagnalis*. Moreover, our results showed that the co-presence of moss and snails impaired the accumulation capacity of both by inducing oxidative stress reactions, through mechanisms that have yet to be elucidated.

1. Introduction

Arsenic (As) is one of the most important global environmental pollutants, considered by the World Health Organization (WHO, 2019) and the International Agency for Research on Cancer (IARC) a significant risk to global health due to its toxic, teratogenic, and carcinogenic properties. As is a metalloid with toxicity to both plants and animals (Bhat et al., 2024; Tang et al., 2025), which exists in several forms, generally classified as organic, identified mainly in fish (Tang et al.,

2025), and inorganic, present mainly in aquifers used in many countries for water supply (Fatoki and Badmus, 2022; Mandal, 2017; Ng, 2005; Rahman and Singh, 2019).

The different forms of As are each endowed with different degrees of toxicity; the most common in freshwater environments are arsenite [As (III)], arsenate [As (V)], along with their methylated metabolites, monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA), which can arise from biotic and abiotic transformations (Gaur and Yadav, 2023), while organic arsenic species such as arsenobetaine

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(AsB) are commonly found in marine organisms, they are less relevant to freshwater ecosystems and dietary exposure pathways (Caumette et al., 2012). The presence of As in the environment has three main natural origins: aquifer sediments, mineral deposits and hydrothermal activity (Rehman et al., 2021), but is mainly related to human activities such as metallurgical ones, mining, pesticide use in agriculture and industrial processing (Chung et al., 2014; Fatoki and Badmus, 2022; Sevak and Pushkar, 2024). The distribution of As in the environment is vast, as it is widespread in different environmental matrices such as water, air, rocks and soil; in the environment, both As and its compounds are mobile and persistent, unable to be destroyed (Chung et al., 2014; De and Roy, 2023; Panagiotaras and Nikolopoulos, 2015; Patel et al., 2023; Stuyfzand and Bonte, 2023).

As is bioaccumulated by aquatic organisms, primarily through dietary uptake, although direct absorption from the surrounding water through gills and other permeable tissues may also contribute, depending on the species and environmental conditions. Benthic organisms, algae, mollusks, crustaceans, and fish can accumulate arsenic from contaminated sediments and dissolved arsenic in the water column. However, unlike highly lipophilic contaminants such as methylmercury or persistent organic pollutants, arsenic does not generally biomagnify through aquatic food webs. This is largely because many aquatic organisms are able to metabolize inorganic arsenic into less toxic organic forms and efficiently eliminate it, preventing a progressive increase in concentration at higher trophic levels (Hepp et al., 2017).

With regard to human exposure, although several intake routes exist, including inhalation and dermal contact, the oral route represents the primary pathway. Human exposure occurs mainly through the consumption of arsenic-contaminated drinking water, agricultural products irrigated with contaminated water, and seafood. Among these sources, drinking water is generally considered the most important contributor to inorganic arsenic exposure in affected regions, whereas seafood often contains predominantly organic arsenic species, which are substantially less toxic than inorganic forms (Chung et al., 2014; Sevak and Pushkar, 2024). Exposure to As induces in humans various pathologies such as cancer, neurodegenerative diseases, diabetes, skin and kidney diseases or developmental alterations, etc. (Chen and Costa, 2021; Chung et al., 2014; Fatoki and Badmus, 2022). Currently, the limits set by the United States Environmental Protection Agency (EPA), the WHO and the European Union for the presence of As in drinking water are 10 µg/L; however, it is estimated that the concentration of As in many regions can be significantly higher, up to thousands of µg/L, exposing >500 million people worldwide to the risk of contamination (Bhat et al., 2024; Chen and Costa 2021; Chung et al., 2014; Fatoki and Badmus 2022; Shaji et al., 2021).

One possible solution to the problem of As contamination in aquatic environments is phytoremediation, an efficient, simple and economical strategy that uses plants to remove or immobilize contaminants such as pesticides, hydrocarbons, and metals from water (Susarla et al., 2002). Phytoremediation has shown promise in the removal of heavy metals from contaminated areas thanks to the use of hyper-accumulating plant species, which are extremely tolerant to environmental metals and capable of taking up metals from water and sediments through their root systems or, in the case of aquatic plants, directly from the surrounding water (Bhat et al., 2022; Huang et al., 2006; Lwanga et al., 2025; Rai 2009). Bryophytes are particularly efficient in removing metals from water due to their large surface area, high cation exchange capacity, and ability to absorb dissolved metals directly from the water column (Barukial and Hazarika, 2023 Gezahegn et al., 2025; Papadia et al., 2020). Among them, the aquatic moss *Leptodictyum riparium*, a well-known freshwater contamination biomonitor, has been shown to be extremely effective in accumulating various heavy metals, including Cd, Cu, Pb, and Zn, both in laboratory and field studies (Basile et al., 2011; 2012; Esposito et al., 2018; Maresca et al., 2022). The aim of this study is to investigate whether *L. riparium* can remove As from aquatic environments and whether phytoremediation can reduce As

bioaccumulation in other animal species, thereby limiting the damage caused by this metalloid. To this end, we set up a microcosm with the freshwater gastropod *Lymnaea stagnalis*, which is a well-known biomonitor of aquatic environmental contamination (Kuroda and Abe, 2020; Fodor et al., 2020; Gnatyshyna et al., 2023; Otludil and Karakaş, 2024). *L. stagnalis* is widely distributed in freshwaters in Europe, North America and throughout much of Asia; it has lungs allowing adaptation to oxygen-poor environments and prefers cross-fertilization, even though it is hermaphroditic (Kuroda and Abe, 2020). *L. stagnalis* is highly sensitive to heavy metals and accumulates them with great efficiency (Crémazy et al., 2018; Karakaş and Otludil, 2020): moreover, previous studies showed that As exposure induced in the digestive gland of this species structural and functional alterations, indicative of cellular stress.

Our basic hypotheses are as follows: 1) *L. riparium* effectively accumulates As from the water, thereby proving its suitability for phytoremediation of contaminated aquatic environments; and 2) the presence of the moss reduces As accumulation in *L. stagnalis* and mitigates the stress responses typically induced by As exposure. To test these hypotheses, we analysed the following parameters in both organisms after 7 days of exposure to 1360 µg/L As in a microcosm setup: the levels of As in the water and in both organisms, the levels of reactive oxygen species (ROS), the activities of catalase (CAT) and superoxide dismutase (SOD), which are indicators of oxidative stress, and the levels of the proteins HSP70 (which is involved in protein folding); in the snails, the levels of p53 protein, a tumour suppressor and transcription factor, absent in plant and vegetables Table 1.

2. Materials and methods

2.1. Experimental organisms

Samples of *L. riparium* were collected from a small reservoir located within the Botanical Garden of the University of Naples Federico II. This site was selected because it is characterized by controlled environmental conditions and is not exposed to direct sources of anthropogenic contamination, thereby ensuring that the plant material presents a low background level of arsenic (Supplementary material). The collected samples were thoroughly rinsed with distilled water to remove any residual surface particles and subsequently used for the experiments. Adult *L. stagnalis* specimens, obtained from an authorized supplier, were acclimated for 20 days in 20-L glass aquaria, and a diet of lettuce leaves

Table 1
Bioaccumulation factor (BAF) values calculated for each organ of the snail *L. stagnalis* (S) and the moss *L. riparium* (M). BAF was calculated as the ratio between the arsenic (As) concentration measured in the tissues of each organ and the As concentration in the water microcosm (1360 µg L⁻¹).

<i>L. stagnalis</i>		
Organ	Treatment	BAF
Shell	S	0,00,094 ± 0,00,012
	S + M	0,00,027 ± 0,00,008
	S + As	0,00,300 ± 0,00,010
	S + M + As	0,00,109 ± 0,00,010
Digestive gland	S	0,00,022 ± 0,00,006
	S + M	0,00,014 ± 0,00,005
	S + As	0,00,352 ± 0,00,014
	S + M + As	0,00,281 ± 0,00,021
Body	S	0,00,011 ± 0,00,002
	S + M	0,00,014 ± 0,00,002
	S + As	0,00,500 ± 0,00,037
	S + M + As	0,00,192 ± 0,00,013
<i>L. riparium</i>		
Organ	Treatment	BAF
Gametophyte	M	0,28,229 ± 0,00,021
	M + S	0,16,347 ± 0,00,287
	M + As	11,05,814 ± 0,05,226
	M + S + As	2,27,661 ± 0,01,943

was given to them ad libitum. The water was changed twice a week and the faeces as well as uneaten food were removed daily. The animals were inspected daily to monitor mortality, as well as any signs of stress or abnormal behaviour. Throughout the acclimatisation and exposure periods, both organisms were subjected to natural temperature and photoperiod conditions. The physicochemical parameters of the water used for acclimation and exposure were as follows: Ca^{2+} : 74.5 mg L⁻¹; Cl^- : 6.75 mg L⁻¹; free CO_2 : 44.6 mg L⁻¹; HCO_3^- : 220 mg L⁻¹; K^+ : 0.57 mg L⁻¹; Mg^{2+} : 1.09 mg L⁻¹; Na^+ : 3.23 mg L⁻¹; NO_3^- : 3 mg L⁻¹; SO_4^{2-} : 2.89 mg L⁻¹; fixed residue at 180 °C: 255 mg L⁻¹; pH 7.35.

2.2. Experimental design

Prior to the start of the As exposure, the gametophytes of *L. riparium* were carefully washed with distilled water and subjected to surface sterilization using 70% ethanol for a few seconds. *L. stagnalis* specimens (shell length 3.04 ± 0.08 cm) were inspected for signs of disease. Both organisms were subjected to the same experimental conditions described above; As exposure took place in 20-L glass aquaria, each containing 12 L of water. The As concentration used for the exposure ($1360 \mu\text{g L}^{-1}$) was selected based on the environmental values reported by Bove et al. (2011) for the Regi Lagni area, a network of artificial canals in the Campania region known for heavy metal contamination. This concentration is considered environmentally realistic and has been shown to be non-lethal for these species. To achieve the required level of exposure, a stock solution of 0.1 g/L of As (Arsenic(V) oxide, CAS: 1303–28–2, Thermo Fisher Scientific Inc., MA, U.S.A.) dissolved in water was prepared. As exposure was performed both on individual organisms and within a microcosm; therefore, the following six experimental groups, each containing 24 g of moss and/or 20 snails, were established: 1) three control groups, only exposed to water (control moss, control snails and control microcosm, containing moss and snails; 2) three As exposed groups (exposed moss, exposed snails and exposed microcosm, containing moss and snails). All the experimental conditions were set up in triplicate. During the seven-day exposure period, the pH level, nitrate levels and temperature were measured daily; the water was only replenished to compensate for evaporation, not renewed. The snails were monitored daily for signs of mortality or stress and fed lettuce leaves ad libitum. According to current legislation in Italy, the scientific use of gastropods does not require authorization from the Ministry of Health, the Competent Authority for animal testing. However, throughout the experiment, institutional guidelines for the care and use of the *L. stagnalis* species were respected. At the end of the exposure period, water, moss, and snail samples (divided in shell, digestive gland and body) were collected for subsequent analysis.

2.3. As bioaccumulation: inductively coupled plasma mass spectrometry (ICP-MS) analysis

For each experimental group, approximately 0.4 g of moss and about 10 mg of digestive gland, 100 mg of shell, 150 mg of body of snails were taken up. The water samples (5 mL) were simply acidified with 2% nitric acid and analysed by ICP-MS. Concentrated nitric (UpA) and hydrochloric (UpA) acids, and certified stock standard solutions were purchased from Romil (ROMIL Ltd, The Source, Cambridge, UK). Standard solutions of As were prepared in 2% nitric acid at five different concentrations (0, 1, 10, 50, and $100 \mu\text{g L}^{-1}$). Subsequently, all samples from moss and snails were subjected to wet digestion using nitric acid and hydrochloric acid in a ratio of 1:3 v/v. The reaction was conducted for 16 h at 90 °C, after which the solution was diluted with milli-Q water and prepared for analysis. As concentrations were measured with three replicates. Measurement was performed with an Agilent 7700 ICP-MS instrument (Agilent Technologies, Santa Clara, CA, U.S.A.) equipped with a frequency-matching radio frequency (RF) generator and 3rd generation Octapole Reaction System (ORS3) operating with helium gas. The following parameters were used: RF power: 1550 W, plasma gas

flow: 14 L min⁻¹; carrier gas flow: 0.99 L min⁻¹; He gas flow: 4.5 mL min⁻¹. Agilent mix of 6-Li, Sc, Ge, Rh, In, Tb, Lu, Bi was used as internal standard.

2.4. Bioaccumulation factor

To assess the accumulation efficiency of arsenic (As) in *L. stagnalis* and *L. riparium*, the Bioaccumulation Factor (BAF) was determined for each experimental group. The BAF was calculated as the ratio between the As concentration measured in the biological samples at the end of the 7-day exposure and the initial As concentration in the corresponding water samples period. Specifically, for moss, the BAF was expressed as g L⁻¹ dry weight, while for snail tissues (whole body, digestive gland, and shell) it was expressed as g L⁻¹ wet weight. The BAF values were calculated individually for each biological replicate and subsequently averaged for each experimental condition.

2.5. Protein extraction for antioxidant activity

One gram of *L. riparium* fresh tissue was ground in liquid nitrogen and suspended in 1.5 mL of phosphate-buffered saline (PBS; 50 mM, pH 7.8, containing 0.1 mM EDTA), supplemented with PMSF and Na_2VO_4 (Elabscience, USA), and a protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA). The samples were homogenized using a Tissue Lyser (10 min, 40 Hz) and centrifuged at $12,000 \times g$ for 30 min at 4 °C. The resulting clear supernatant was collected and stored at -20 °C until further analysis. Protein concentrations were determined using the Bradford assay (Bio-Rad Protein Assay, Hercules, CA, USA; 1:5 dilution in distilled H₂O) with absorbance measured at 595 nm and results expressed as $\mu\text{g}/\mu\text{L}$. Whole-body samples of *L. stagnalis* were homogenized at 4 °C using a Potter homogenizer in 5 mL of PBS buffer supplemented with a protease inhibitor cocktail (3 mM aprotinin, 1 mM leupeptin, 1 mM E-64, 130 μM bestatin, 1 mM EDTA and 2 mM AEBSF) (Thermo Scientific, USA). Total protein concentration in the supernatant was determined using the Pierce BCA Protein Assay (Thermo Scientific, USA). Specifically, aliquots of the sample were mixed with the BCA working reagent, incubated at 37 °C for 30 min, and the absorbance was measured at 562 nm using a spectrophotometer.

2.6. Determination of ROS levels

ROS levels were quantified using a fluorometric assay based on the 2',7'-dichlorofluorescein diacetate (DCFDA) probe (Sigma-Aldrich, St. Louis, MO, USA). In a 96-well plate, 10 μL of supernatant was incubated with 90 μL of PBS and 1 μL of 1 mM DCFDA (final concentration 10 μM) for 10 min in the dark. Fluorescence was measured using a fluorimeter ($\lambda_{\text{ex/em}}$ = 485/528 nm). Control samples lacking DCFDA were used to subtract background fluorescence. Results were expressed as relative fluorescence units (RFU) normalized to protein content (RFU/ μg protein).

2.7. CAT and SOD activities

CAT activity (EC 1.11.1.6) was assessed using a colorimetric assay provided in the CAT Activity Assay Kit (Sigma-Aldrich, St. Louis, MO, USA; code MAK531), following the manufacturer's instructions. The assay quantifies the enzyme's ability to catalyse the decomposition of hydrogen peroxide (H_2O_2) into water and molecular oxygen. Enzymatic activity was calculated using an H_2O_2 standard curve (0–400 μM) and expressed as catalase units (U/L), subsequently normalized to the protein concentration of each sample. SOD activity (EC 1.15.1.1) was determined colorimetrically using the SOD Determination Kit (Sigma-Aldrich, St. Louis, MO, USA; code 19,160), according to the manufacturer's protocol. The method is based on the enzyme's ability to catalyze the dismutation of the superoxide anion radical (O_2^-) into hydrogen peroxide and molecular oxygen. Enzyme activity was expressed in

inhibition units (%) and subsequently normalized to protein content, expressed in U per μg of protein.

2.8. Western blot

The aim of the analysis was to evaluate the expression levels of proteins involved in cell death and cell stress processes (HSP70 and P53). *L. riparium* samples were accurately weighed and homogenized in ice-cold RIPA buffer (Elabscience, USA) at a ratio of 1:5 (w/v), using 5 mL of buffer per gram of tissue. Immediately before use, the RIPA buffer was supplemented with sodium orthovanadate (Na_3VO_4) (Elabscience, USA), phenylmethylsulfonyl fluoride (PMSF) (Elabscience, USA) and protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer's instructions. Samples were mechanically homogenized and incubated on ice for 60 min with intermittent mixing to ensure complete cell lysis. The lysates were then centrifuged at $14,000 \times g$ for 30 min at 4°C and the protein concentration was determined using the Bradford method (Bio-Rad Laboratories, CA, USA). *L. stagnalis* samples were homogenized at 4°C using a Potter homogenizer in 5 mL of RIPA buffer containing 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 10% NP-40, and a protease inhibitor cocktail (3 mM aprotinin, 1 mM leupeptin, 1 mM E-64, 130 μM bestatin, 1 mM EDTA, and 2 mM AEBSEF). The homogenate was centrifuged at 7000 g for 10 min at 4°C , and the protein concentration was determined using the PIERCE method. For each sample, 40 μg of protein was denatured by boiling (5 min) in SDS buffer (50 mM Tris-HCl (pH 6.8), 2 g/100 mL SDS, 10% (v/v) glycerol, 0.1 g/100 mL bromophenol blue), separated by electrophoresis on 12.5% SDS-polyacrylamide gel and transferred to nitrocellulose membranes using the Mini Trans-Blot system (Bio-Rad Laboratories, USA). The membranes were kept at room temperature and subjected to a double blocking step: initially with 7% skim milk in $1 \times$ TBS containing 0.025% Tween-20 for 45 min, followed by 7% BSA in $1 \times$ TBS-Tween-20 for a further 45 min. Incubation with primary anti-HSP70, anti-p53 and anti-GAPDH antibodies (Sigma-Aldrich, rabbit) was performed overnight at 4°C , using a 1:500 dilution in TBS-T containing 2.5% BSA. After three washes with TBS-T, the membranes were incubated for 1 h at room temperature with the secondary anti-rabbit IgG antibody (Santa Cruz, cat. no. sc-2005) diluted 1:2000 in TBS-T with 2.5% BSA. Signal detection was performed using the ChemiDoc system (Bio-Rad). The intensity of the bands was quantified and normalized with respect to GAPDH levels using dedicated image analysis software.

2.9. Statistical analysis

All biochemical analyses (ROS, CAT, SOD, HSP70, and p53) were performed on three independent biological replicates per experimental group ($n = 3$). The values were presented in terms of mean $\pm$ standard deviation of mean (SD). To meet statistical assumptions, all the data were checked for normality and homogeneity of variance. The Bartlett test was used to assess the homogeneity of variance. The data were compared by one-way analysis of variance (ANOVA), followed by Tukey's post hoc multiple-comparison test. Commercial software (SigmaStat version 2.0; SPSS) was used to perform all statistical analyses and significant differences were considered when $P < 0.05$.

3. Results

3.1. As bioaccumulation and bioaccumulation factor (BAF)

The As bioaccumulation was expressed as fold change values, calculated as the ratio between As-exposed samples and the corresponding unexposed controls, which were normalized to 1. In all the experimental groups, As accumulation was evaluated in *L. stagnalis* (shell, whole body without the digestive gland, and digestive gland), in *L. riparium*, and in the water (Fig. 1A, B). Moss showed high levels of As accumulation, which however became significantly lower in the microcosm, in the presence of snails. Snail's whole body exhibited the highest level of As bioaccumulation, followed by the digestive gland and the shell. However, in the microcosm, the As accumulation of the whole body became significantly lower, whereas that of the digestive gland and the shell increased (Fig. 1A). As residual concentration in the water was the lowest in the presence of the moss, higher in the presence of the snails and highest in the microcosm (Fig. 1B).

BAF values increased markedly in the As-exposed treatments for both organisms. In *L. stagnalis*, the highest BAF was observed in the body ($0.00,500 \pm 0.00,037$), followed by the digestive gland ($0.00,352 \pm 0.00,014$) and the shell ($0.00,300 \pm 0.00,010$). Co-exposure with *L. riparium* reduced BAF values in all snail organs. In the moss, the gametophyte showed a substantially higher BAF than the snail tissues, reaching $11,05,814 \pm 0.05,226$ in the As treatment and decreasing to 2.27661 ± 0.01943 when in co-presence with snails.

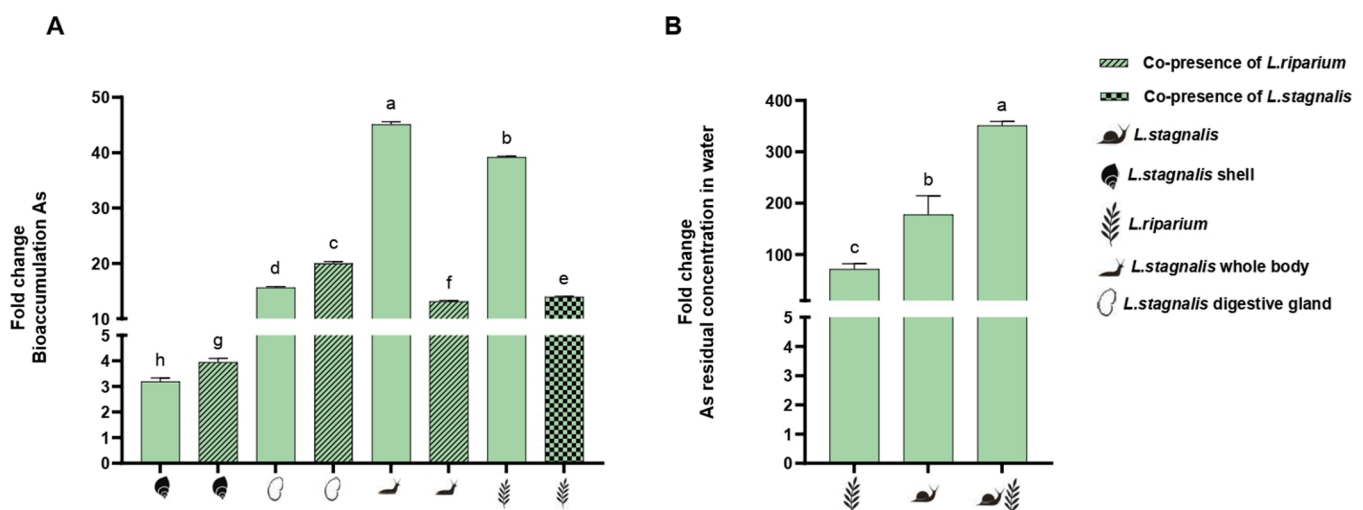


Fig. 1. As bioaccumulation in *L. stagnalis* and *L. riparium* under combined exposure. A) fold change of As bioaccumulation in *L. stagnalis* (shell, whole body and digestive gland) and in *L. riparium*; B) fold change of As residual concentration in water under combined exposure. Symbols denote the biological compartments analyzed. Different letters above bars indicate statistically significant ($p < 0.05$) differences among the experimental groups.

3.2. ROS levels and antioxidant activity

Compared with the control, in the moss ROS levels decreased after As exposure, increased following exposure to snails and even more in the microcosm, following simultaneous exposure to As and snails. Concomitantly, CAT and SOD activities increased after As exposure, and decreased following exposure to snails, and in the microcosm, following simultaneous exposure to As and snails (Fig. 2A). Compared with the control, in the snails ROS levels increased after As exposure and decreased following exposure to moss and even more in the microcosm, following simultaneous exposure to As and moss. CAT and SOD activities increased after As exposure, and even more following exposure to moss, whereas decreased in the microcosm, following simultaneous exposure to As and moss (Fig. 2B).

3.3. HSP70 and p53 protein levels in *L. stagnalis*

Compared with the control, HSP70 levels increased reaching similar values after moss and after As exposure, and decreased in the microcosm, following simultaneous exposure to As and moss, remaining in any case higher than control values. Compared with the control, p53 levels increased after moss exposure and even more after As exposure, and dramatically decreased in the microcosm, following simultaneous exposure to As and moss (Fig. 3).

3.4. HSP70 in *L. riparium*

Compared with the control, HSP70 levels significantly increased after exposure to snails and after As exposure, and even more in the microcosm, following simultaneous exposure to As and moss (Fig. 4).

4. Discussion

To the best of our knowledge, there is no data on the ability of *L. riparium* and *L. stagnalis* to accumulate As from water, individually or together, in a microcosm. The main results of our study were the following: 1) both moss and snails efficiently bioaccumulated As from the water; however, in the microcosm, when the moss and the snails were simultaneously present, their ability to accumulate As decreased significantly; 2) As induced oxidative stress, altering ROS levels and CAT and SOD activities in both organisms, both individually and in the microcosm; however, unlike what was hypothesized, the co-presence of moss and snails altered all these parameters in both organisms; 3) As increased HSP70 levels in both organisms, both individually and in the microcosm; and unlike what was hypothesized, the co-presence of moss and snails altered the levels of HSP70 in both organisms; 4) in the snails the levels of p53 were increased by both exposure to As and moss, but decreased in the microcosm, when the moss and the snails were simultaneously present. The As concentration used in this study (1360 µg/L) reflects a worst-case scenario of acute contamination, as documented for heavily polluted freshwater sites such as the Regi Lagni area (Bove et al., 2011). Although this level is higher than regulatory limits (10 µg/L), it is environmentally realistic for areas affected by industrial discharge or natural As enrichment (Shaji et al., 2021; Patel et al., 2023). This allows us to assess the full potential of *L. riparium* for phytoremediation and the physiological responses of *L. stagnalis* under extreme conditions. However, extrapolation to lower, chronic exposure levels should be made with caution, as the magnitude of stress responses may differ.

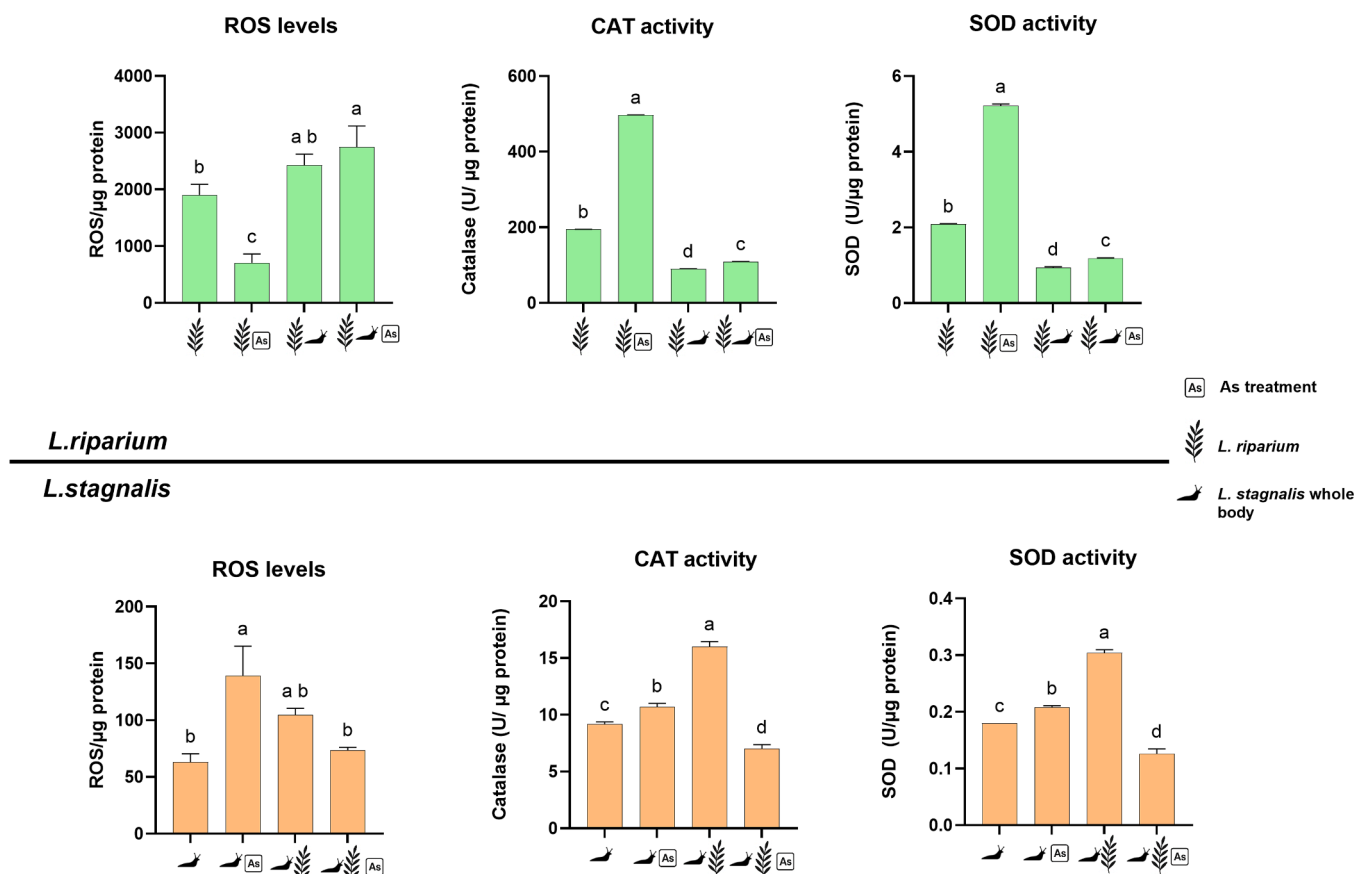


Fig. 2. Changes in ROS levels and CAT and SOD activity in upper figure) *L. riparium*; lower figure) *L. stagnalis*. Values are expressed as mean $\pm$ SD. Symbols denote the biological compartments analyzed. Different letters above bars indicate statistically significant ($p < 0.05$) differences among the experimental groups.

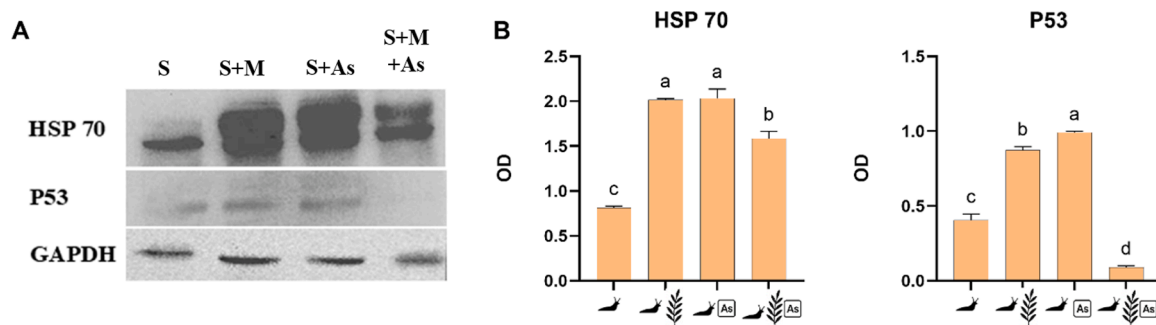


Fig. 3. Expression of HSP70 and p53 protein levels in *L. stagnalis*. A) Representative Western blot of protein extracts, stained with anti-HSP70, anti-p53 and anti-GAPDH. B) Quantitative densitometric analysis of HSP 70, P53 and GAPDH by calculation of the band intensities. HSP 70 and p53 concentration is shown relative to the GAPDH level. S=Snail, S+M= Snail and moss; S+As Snail and As; S+M+As= Microcosm.

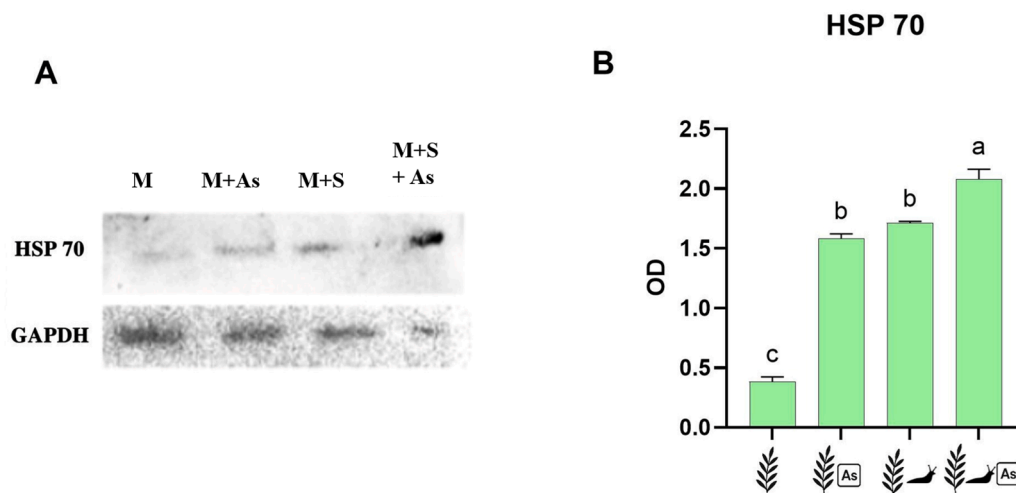


Fig. 4. Expression of HSP70 protein levels in *L. riparium*. A) Representative western blot of protein extracts, stained with anti-HSP70 and anti-GAPDH. B) Quantitative densitometric analysis of HSP 70 and GAPDH by calculation of the band intensities. HSP 70 concentration is shown relative to the GAPDH level. M=Moss; M+As= Moss and As; M+S= Moss and Snail; M+S+As= Microcosm.

4.1. As bioaccumulation

Individual responses. The ability of both organisms to accumulate As from water is consistent with previous literature. Bryophytes are well-known accumulators of metals from water and sediments (Culioli et al., 2009; McIntyre, 2003), and *L. riparium* in particular has been shown to be extremely effective in heavy metal accumulation both in vitro and in vivo (Basile et al., 2011, 2012; Esposito et al., 2018; Maresca et al., 2022). Similarly, freshwater gastropods are recognized for their capacity to accumulate metals (Krupnova et al., 2018), and *L. stagnalis* readily takes up heavy metals from the water (Crémazy et al., 2018; Amorim et al., 2019; Karakaş and Otludil, 2020).

Notably, the different body compartments of *L. stagnalis* accumulated As to different extents, with the highest levels detected in the whole body, followed by the digestive gland and finally the shell. This pattern was somewhat unexpected, as the digestive gland is generally considered the primary site of metal accumulation and detoxification in gastropods (Marigómez et al., 2002). The higher As burden in the whole body may be explained by several factors. First, As differs from cationic metals such as Cd, Cu, or Pb, as it exists predominantly as an oxyanion in aquatic environments, leading to different uptake pathways and reduced affinity for the detoxification mechanisms of the digestive gland. Second, the whole body includes multiple metabolically active tissues (foot, mantle, gonads) that collectively contribute to As accumulation. Third, As is metabolized and excreted more rapidly than other metals,

potentially reducing its accumulation in the digestive gland over time (Dallinger et al., 2020). Fourth, the relatively short exposure period (7 days) may have been insufficient for preferential sequestration in the digestive gland (Contreras et al., 2012). These findings highlight the importance of analyzing multiple tissue compartments when assessing metalloid bioaccumulation in gastropods.

When exposed individually to As, the snail's whole body exhibited a greater accumulation capacity than the moss. However, analysis of the residual As concentration in the microcosm water revealed that, in the presence of snails alone, the As content was higher than in the presence of moss alone. This apparent discrepancy reflects fundamental differences in the mechanisms governing As uptake and retention between these organisms. Aquatic mosses act as efficient passive sinks for dissolved metals through adsorption onto cell walls and ion exchange processes (Papadia et al., 2020), removing As from the water without necessarily accumulating high internal concentrations. In contrast, gastropods accumulate As primarily through dietary uptake and internal sequestration, while also releasing As back into the water via excretion, mucus production, or fecal deposition (Jugdaohsingh et al., 1998; Zhu et al., 2024). Together, these processes explain why higher tissue bioaccumulation in snails does not necessarily correspond to lower As concentrations in the surrounding water.

Microcosm. In the microcosm, the accumulation ability of the two organisms drastically decreased. As accumulation of the moss and that of snails' whole bodies decreased significantly, whereas that of the

digestive gland and the shell increased. Although further studies are needed, it is possible that the reduced accumulation capacity of the two organisms was due to the oxidative stress conditions of the two organism, which we will discuss later. A limitation of this study is that arsenic speciation was not monitored; therefore, biotransformation processes (e. g., reduction to As(III) or methylation) occurring in the organisms cannot be excluded and may have influenced the observed toxic responses.

4.2. Oxidative stress

Individual responses. Concerning the oxidative stress result, the changes induced by As exposure in ROS levels and CAT and SOD activities in both organisms, agree with the known ability of As to induce oxidative stress in plant and animals (Flora, 2011; Chi et al., 2019; Nahar et al., 2022). Although there is a lack of data on the effects of As on the moss *L. riparium* and the snail *L. stagnalis*, the observed change in the different parameters is consistent with that induced in *L. riparium* (Esposito et al., 2012) and *L. stagnalis* (Gnatyshina et al., 2020) by other metals as Zn, Cd, Cu and Pb. Our results, however, reveal that *L. riparium* and *L. stagnalis* exhibited different responses to oxidative stress; indeed, As exposure caused in moss a sharp increase in SOD and CAT activities and, consistently, decreased ROS levels, suggesting a strong activation of the enzymatic antioxidant machinery. In contrast, As exposure caused in snails a slight, though significant, increase in SOD and CAT activities and a sharp increase in ROS levels, suggesting a condition of redox imbalance, in which the production of oxidizing species exceeded the basal capacity for cellular detoxification. As is known to induce oxidative stress (Flora, 2011; Chi et al., 2019; Fatoki and Badmus, 2022) indirectly by disrupting cellular redox homeostasis, enhancing the generation of superoxide radicals and hydrogen peroxide, through interference with electron transport chains and metabolic pathways. The increase in the SOD activity likely reflects the enhanced dismutation of superoxide radicals into hydrogen peroxide, which is subsequently detoxified by CAT. The concomitant reduction in ROS levels, observed in *L. riparium*, indicates that these antioxidant mechanisms were sufficiently upregulated to maintain the cellular redox balance and limit the oxidative damage. The increase in CAT and SOD activities observed in *L. stagnalis* are consistent with the activation of detoxification mechanisms commonly reported in molluscs under metal stress (Gnatyshina et al., 2020), but the analysis of the observed parameters indicates that this species is unable to effectively counteract the effects of As.

Microcosm. In the microcosm, when the moss and the snails were simultaneously present, we observed similar behaviour in both organisms after As exposure: a decrease in CAT and SOD activities and an increase in ROS levels, that in *L. stagnalis* was not significant. These findings indicate that the coexistence of the two organisms impaired their ability to combat the oxidative stress induced by As. This hypothesis is confirmed by the results obtained in the microcosm in the absence of As, where an increase in ROS levels, accompanied by a decrease in SOD and CAT activities in the moss, and an increase in SOD and CAT activities in the snails, were observed. The results suggest that *L. riparium* and *L. stagnalis* had difficulty coexisting, and that each of the two organisms was a source of stress for the other.

4.3. HSP70 and p53 proteins

Individual responses. Regarding the Western blot results, the increase in HSP70 levels induced by As in both organisms, and the increase in p53 levels induced by As in the snails, they agree with the rise in HSP70 levels observed in MDA231 cells (Kim et al., 2005) and in cock livers (Zhang et al., 2016), and the increase in p53 levels found in immortalized prostate epithelial cells (Pacheco-Castillo et al., 2025), after As exposure. The increase in these two proteins is typical of cells exposed to unfavourable environmental conditions or molecular damage. HSP70, known for its molecular chaperone activity, plays a fundamental role in

ensuring the correct folding of proteins and preventing the accumulation of denatured proteins, thus acting as a key player in the response to oxidative stress. Increased expression of HSP70 suggests that cells were attempting to maintain protein homeostasis in the face of a toxic insult. Similarly, p53 is one of the main regulators of the cellular response to genotoxic and oxidative stress. p53 is a tumour suppressor and transcription factor found also in freshwater molluscs, and considered a biomarker of environmental pollution, infection, and genotoxic damage (Mohanty, 2006; Banni et al., 2009). In the presence of DNA damage or an increase in ROS, p53 can induce cell cycle arrest, activate repair mechanisms or, if the damage is irreversible, promote apoptosis. The increase in p53 levels observed in the exposed snails, therefore suggests that the cells were facing a significant insult, seeking to preserve their integrity through stringent control pathways.

Microcosm. In the microcosm, As increased the levels of HSP70 both in the moss and in the snails but decreased p53 levels in the snails. The increase in HSP70 indicates a cellular protective response to stress, already observed with exposure to As alone; more difficult is to explain the decrease in p53 levels. It is possible that, under conditions of high stress, represented by the simultaneous presence of As and moss, HSP70 may promote the degradation of p53, preventing it from triggering apoptosis and promoting cell survival. In fact, it has been demonstrated that HSP70 can sequester p53 in the cytoplasm and negatively regulate its stability (Boysen et al., 2019) and that the ubiquitination of p53 by the ubiquitin ligase CHIP is increased in the presence of HSP70 and its co-chaperone Hdj1 (Stankiewicz et al., 2010). Once again, the fact that moss and snails are a source of stress for one another is demonstrated by the fact that the coexistence of moss and snails, in absence of As, caused in both organisms an increase in HSP70 levels and in the snails an increase in p53 levels, suggesting the activation of defence mechanisms both in the moss and the snails.

Finally, a possible explanation for the damage caused to moss and snails by their coexistence may lie in the likely release of substances into the water by the two organisms. Gastropods secrete mucus into the water, a mixture of various molecules such as glycosaminoglycans, proteins, lectins, and antimicrobial peptides (Jugdaohsingh et al., 1998; Zhu et al., 2024), while mosses are capable of emitting biogenic volatile organic compounds (BVOCs) involved, among other things, in communication between plants and/or between plants and the ecosystem, for example, as a defence against predation (Yáñez-Serrano et al., 2024). It is possible that the substances contained in snail mucus triggered both an oxidative stress response and the release of defense molecules in the moss. These, in turn, may have also triggered oxidative stress responses in the snails. Of course, further studies are needed to clarify these mechanisms.

5. Conclusion

In conclusion, our findings demonstrate that the moss *L. riparium* accumulated As by removing it from the water, maintaining the cellular redox balance and limiting the oxidative damage. This makes it a species that is potentially suitable for phytoremediation of As-contaminated water. The snail *L. stagnalis* can accumulate As, but less efficiently than moss does, and without being able to effectively counteract the effects of As. Finally, our results show that the co-presence of moss and snails impaired the accumulation capacity of both by inducing oxidative stress reactions, through mechanisms that have yet to be elucidated.

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CRediT authorship contribution statement

Alessia Postiglione: Writing – original draft, Methodology, Investigation, Conceptualization. **Martina Dentato:** Writing – original draft, Methodology, Investigation, Data curation. **Teresa Chianese:** Software, Methodology, Data curation. **Rosaria Scudiero:** Writing – review & editing, Supervision. **Adriana Basile:** Writing – review & editing, Resources, Funding acquisition. **Karen Power:** Methodology, Investigation, Data curation. **Michele Spinelli:** Software, Methodology. **Angela Amoresano:** Visualization, Supervision, Formal analysis. **Viviana Maresca:** Writing – review & editing, Writing – original draft, Investigation, Conceptualization. **Anna Capaldo:** Writing – review & editing, Writing – original draft, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.aquatox.2026.107931](https://doi.org/10.1016/j.aquatox.2026.107931).

Data availability

Data will be made available on request.

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