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Research Paper

Water hyacinth (*Eichhornia crassipes*) biomass characterization for a potential exploration as an agriculture soil enhancer: Linking multi-location biogeochemical profiles to ecotoxicological safety

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ABSTRACT

Water hyacinth (WH) is an invasive aquatic species for which no universal biomass management strategy exists, although many developing countries use it in agriculture with limited understanding of its potential environmental impacts. As WH is an effective bioaccumulator it is essential to assess its composition and quantify potentially harmful elements before this surplus green biomass can be effectively valorised. Determining the thresholds for their effects is crucial to define safe and sustainable uses. In this context, this study characterized WH biomass from six Portuguese locations (four northern and two southern), focusing on nutrient and potentially toxic element (PTE) profiles, sugar, protein, and structural composition. Furthermore, the ecotoxicological profile of aqueous extracts from each WH biomass was evaluated using several freshwater species (*Raphidocelis subcapitata, Brachionus calyciflorus, Daphnia magna,* and *Danio rerio*) and multiple endpoints, to benchmark safe agricultural application rates. Structural analysis revealed tissue type (leaves, floaters, roots) had greater influence than sampling location, with roots showing highest absorbance linked to lignin, proteins, and cellulose. These wall components provide metal-binding sites, explaining root PTE levels being higher than other tissues. Elemental composition showed high primary nutrients (e.g., potassium, phosphorus), meeting EU requirements for organic soil improvers (EU Regulation 2019/1009). Whole-plant WH water extracts had high conductivity (≥ 6.98 mS/cm), nutrient and PTE concentrations, and caused adverse effects on all aquatic species. No clear toxicity ranking emerged, though Bico and Pateira extracts were least toxic, and Sorraia extract most severe (algal inhibition, zooplankton mortality, zebrafish effects at 0.78 % dilution). The findings indicate that WH biomass incorporation into soils should be considered on a site-specific basis, owing to variations in PTEs accumulation across locations, requiring contaminant screening and regulatory guidance before large-scale use. The results evidenced multispecies, multi-endpoint ecotoxicity that might justify the need for dilution strategies and controlled application rates of WH biomass on soils to minimize putative downstream impacts.

1. Introduction

Water hyacinth (WH; *Eichhornia crassipes*) is one of the most prolific aquatic invasive species native to South America, but is now widespread across Asia, Africa, Europe, and the Americas [1]. It forms dense surface mats that degrade aquatic ecosystems, obstruct water transport, and foster conditions for disease vectors [2]. Although eradication is often deemed unfeasible, its rapid biomass accumulation (up to 17.5 metric tons/ha/day) [3], offers opportunities for valorisation. Recent studies have explored WH potential agricultural applications due to its favourable biochemical properties. With a high organic matter content (~70 %) and significant levels of nitrogen (N), phosphorus (P), and potassium (K), WH shows promise as a soil amendment [4–6]. Its low lignin and high cellulose and hemicellulose contents support microbial

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degradation and nutrient release [7,8]. Additionally, the high ash content, moderate protein levels, and low fat of WH biomass underscores its potential as a soil enricher [9]. However, concerns remain regarding the ecotoxicological risks associated with the use of WH biomass in agricultural soils, decurrent of contaminant leaching that could be concerning, especially for freshwater environments. Due to its documented phytoremediation capacity, WH tends to accumulate metals, metalloids, and other contaminants, particularly in its roots [10]. This makes essential to thoroughly characterize its biogeochemical profiles, including metal content, speciation, and bioavailability, before land application. Importantly, WH biomass composition may act as a fingerprint of its surrounding environment, reflecting the specific contaminants and nutrient profiles of the waters in which it grows. This implies that the use of WH biomass across different geographic regions may entail variable and location-specific risks, a factor often overlooked in existing studies. As WH matures, its biomass has the potential to induce phytotoxic effects because of the release of such contaminants [11,12].

In resource-limited agricultural contexts, WH biomass is frequently applied in raw or minimally processed form (e.g., after air or sun-drying), with limited assessment of its potential environmental impacts (e.g., [13]). Given this, an increased risk of metal leaching, for instance, is expected, which may lead to ecotoxic effects in nearby aquatic systems, particularly during the early stages of biomass degradation when the release of soluble and potentially toxics fractions is likely to be higher [14,15]. In fact, considering the high moisture content of WH biomass, leaching in terrestrial environments could theoretically be as high as that in aquatic environments [16]. Thus, the use of WH aqueous extracts in toxicity tests could serve as a proxy for worst-case exposure scenarios but could also serve a secondary purpose by simultaneously allowing to understand whether the lack of maturation of WH biomass could in fact be an interesting route for farmers compared to more laborious processes, such as composting. Previous studies have reported the antibacterial and antialgal activity effects of crude methanolic WH biomass extracts [16] and 100 % mortality rates in insects at percentages of crude WH extracts at dilutions equal to or greater than 2 % over a period of 15 days [17]. A study focusing on the impacts of WH biomass extracts on the survival, growth, and metamorphic state of the green toad *Bufotes sitibundus*, has reported that a six-day period of exposure to a 5 % dilution of WH water extract (1:5 W:V) significantly affected the organisms survival, and endangered time to metamorphosis in the surviving ones [18]. Other studies with plants have reported significant reductions in germination and growth of plants (*Mimosa pigra* and *Vigna radiata*) exposed to dilutions of 2.5 % and 5 % WH extracts [19], or reduced root and shoot length and weight [20], or reduction in the growth of *Microcystis aeruginosa* after exposure to fresh WH biomass [21]. Nevertheless, the few available studies rely on single-species assays, which narrows the capture of the range of sensitivities in an ecosystem.

This study provides an integrated assessment of WH biomass by experimentally determining ecotoxicological thresholds of aqueous extracts across several freshwater species, while also characterizing nutrient composition, structural components, and tissue-specific PTEs burdens. To gain a broader perspective on the range of sensitivities to WH extracts in an aquatic ecosystem, the ecotoxicological characterization encompassed four model organisms from different trophic and functional groups. Furthermore, this work also included the characterization of biomass collected from several geographic locations, which will allow to perceive potential generalizations between ecosystems and more comprehensively inform environmental policymakers. This second step can assist local authorities and stakeholders in decision-making on the use of WH biomass as a soil additive and potentially establish a sustainable, safe, and circular use model for WH biomass in agricultural systems.

2. Materials and methods

2.1. Biomass sampling and processing

Water hyacinth (*E. crassipes*) biomass was collected at six locations: Estação Experimental António Teixeira (EEAT) and Praia Fluvial do Rio Sorraia (Sorraia) in Coruche, Santarém; Vila Valente (VV), and São João de Loure (SJL) in Cacia, Aveiro; Pateira de Fermentelos (Pateira) and Observatório do Bico (Bico) in Pateira de Fermentelos, Águeda, Aveiro (Fig. 1). These locations were selected based on their different ecological diversity and geographic significance and because they are known for significant infestations of WH [22], thus relevant for studying biomass utilization and environmental risk. These locations are also under ongoing management interventions; therefore, the data collected in this study may be applied to real-case scenarios (Fig. 1).

At the time of biomass harvest, water parameters, such as temperature (°C), conductivity (mS/cm), and pH, were measured using appropriate probes. After arriving in the laboratory, the harvested biomass was washed with tap water to remove excess dirt and sand. A portion was immediately stored at −80 °C for biochemical (chlorophylls, sugars, protein) and elemental characterization (FTIR), while another was dried at low temperature (approximately 40 °C) for several days until weight was stabilized, and then ground into small pieces. The dried biomass was used for extract preparation.

2.2. Biomass characterization

2.2.1. Photosynthetic pigments determination

For photosynthetic pigments determination, about 10 g of 6 randomly selected plants (without signs of senescence) were harvested from biomass collected form each location. For each of the 6 locations, 3 replicates were done. Afterwards, leaves were deep-freeze, then lyophilized (− 80 °C, 1 bar), and macerated into powder. The photosynthetic pigment analysis was carried out using the whole-pigment extraction method described by Lichtenthaler [23]. Briefly, a known weight of freeze-dried plant tissue was rehydrated in 100 μL of distilled water for 10 min. Subsequently, 1 mL of 96 % ethanol was added, and the mixture vortexed. The test tubes were wrapped in aluminum foil to protect them from light and incubated overnight at room temperature. The following day, samples were centrifuged at 8000 rpm for 8 min and the supernatant absorbance was then measured at three wavelengths: 648 nm, 664 nm, and 470 nm (A_{648}, A_{664}, and A_{470}, respectively). Absorbance values were considered reliable within the range of 0.2 to 0.8 [23]. The concentrations ($mg.g^{-1}$.dw) of chlorophyll *a* (Chl_a), chlorophyll *b* (Chl_b), total chlorophyll ($Chl_a + Chl_b$), the Chl_a/Chl_b ratio, and total carotenoids (Chl_{x+c}) were calculated based on dry weight (DW) using Lichtenthaler's [19] equations as follows:

$$Chl_a = \left(13.36 \times A_{664,2} - 5.19 \times A_{648.6}\right)/DW$$

$$Chl_b = \left(27.43 \times A_{648.6} - 8.12 \times A_{664,2}\right) \times 8.1/DW$$

$$Chl_{a+b} = \left(5.24 \times A_{664,2} - 22.24 \times A_{648.6}\right) \times 8.1\right)/DW$$

$$Chl_{x+c} = \left(4.785 \times A_{470} + 3.657 \times A_{664,2} - 12.76 \times A_{648.6}\right) \times 8.1/DW$$

$$Chl_{a/b} = Chl_a/Chl_b$$

knowing that, $A_{648.6}$ is the absorbance at 648.6 nm; $A_{664.2}$ the absorbance at 664.2 nm; A_{470} the absorbance at 470 nm and DW the dry weight of plant tissue.

2.2.2. Protein and energy reserves

Protein determination followed the Biuret colorimetric method [24] using bovine serum albumin (BSA) as standard. The colorimetric reaction was carried out at room temperature for 10 min and absorbance was

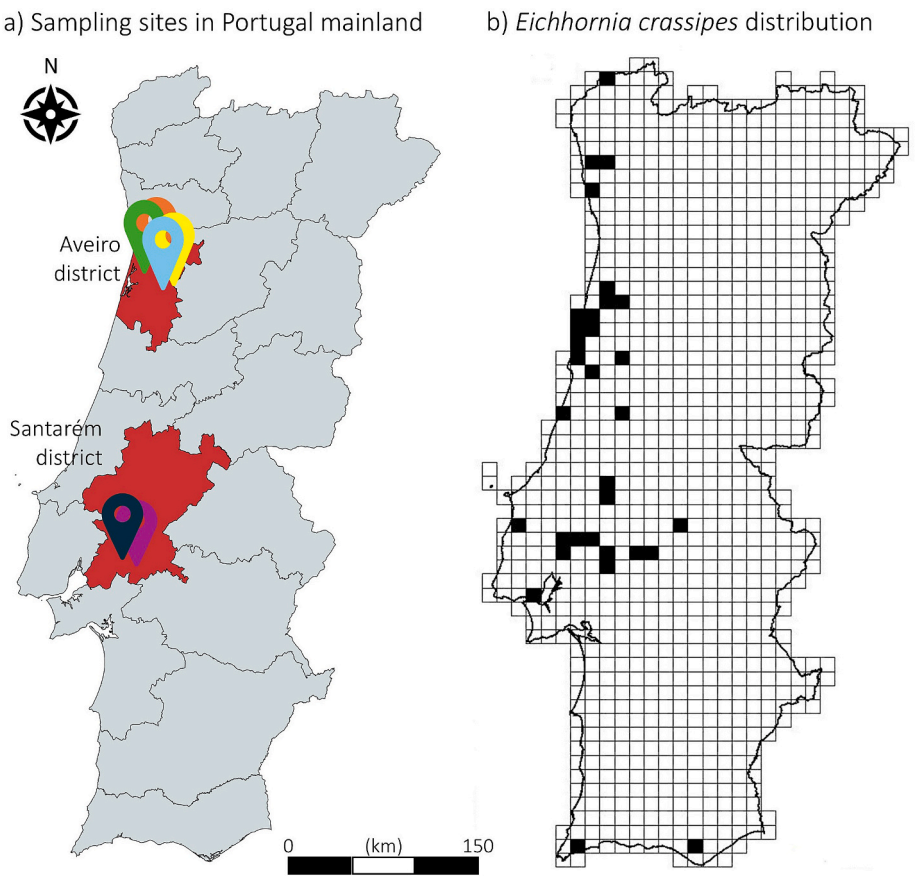


Fig. 1. (a) Map with marked locations from where water hyacinths (*Eichhornia crassipes*) were harvested. These locations are highlighted in colors: Estação Experimental António Teixeira (EEAT, dark blue) and Praia Fluvial do Rio Sorraia (Sorraia, purple), at Coruche, Santarém; Vila Valente (VV) e São João de Loure (SJL) at Cacia, Aveiro (green and orange); and Pateira de Fermentelos (Pateira) and Observátorio do Bico (Bico) in Pateira de Fermentelos (light blue and yellow), at Águeda, Aveiro. (b) Sampling sites were cross-referenced with [22], Flora-on (https://flora-on.pt/#1*Eichhornia+crassipes*; consulted on september 13th 2025). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

measured at 540 nm. Three replicates were performed for each sampling site. Results were expressed in mg per g of fresh weight (FW). Energy reserves, quantified by means of starch, were quantified in the supernatant (soluble sugars) and in the pellet (reducing sugars) according to the method described by Dubois et al. [25], after a one-hour incubation period at 95 °C in 2.5 mM sulfuric acid (1:2 *w/v*) [26]. The absorbance of the samples and standards use for calibration (0.1–3 mg of formaldehyde/mL) were measured at 492 nm, and the results were expressed in mg/g of dry weight.

2.2.3. Fourier transform infrared spectroscopy (FTIR)

FTIR characterization was also carried out for the biomass collected at each one of the six locations and allowed to further characterize its structural and chemical features that may help understand its potential toxicity but also if they may be used as a suitable "fingerprint". Spectra were obtained using a JASCO FT/IR-4200 spectrometer (Jasco Corporation, Tokyo, Japan), equipped with a universal ATR (attenuated total reflectance) accessory. Measurements were taken in the range of 500–4000 cm^{-1}, with a spectral resolution of 4 cm^{-1}, and each spectrum was averaged over 128 scans to improve signal-to-noise ratio. The FTIR analysis focused on five main absorbance regions, corresponding to specific functional groups and molecular structures: i) the 805–1200 cm^{-1} region that provides information on C–OC and C–O–P stretching vibrations, which are typical of polysaccharides; ii) the 1200–1495 cm^{-1} region which includes CH_2 and CH_3 bending vibrations, related to proteins and lipids, as well as P—O stretching, characteristic of phosphatases; iii) between 1500 and 1750 cm^{-1} corresponding to the Amide *I* and II bands, reflecting the presence of proteins; iv) between 2765 and 3000 cm^{-1} range provides indication for C—H stretching, primarily associated with lipid content; and, v) between 3000 and 3650 cm^{-1} provides indication for O—H and N—H stretching vibrations, which are indicative of carboxyl, hydroxyl, phenol, and amide groups. Peak areas were determined by integration under the curve as follows:

$$f \int f(x)dx\, ba \approx \sum [f(a) + f(b)2x\,|(\mathrm{b} - \mathrm{a})|]$$

where a and b are wavelength (cm^{-1}), with a < b, and f(x) is absorbance.

2.3. Biomass extracts

2.3.1. Extracts preparation

Extracts were prepared using whole-plant biomass from each of the locations previously described. Three replicates were made per location. Extracts were made in a proportion of 1:10 (dry weight: water volume), heated at a temperature around 60 and 80 °C for at least 2 to 4 h while stirring manually occasionally (adapted from [27], and references therein). The temperature range was selected because its represents an optimal range for polysaccharides extraction without the occurence of significant degradation [28]. After letting the mixture cool at room temperature (20–22 °C, overnight), extracts were filtered (Whatman no 1) and the pH and conductivity (mS/cm) measured using a multiparameter Lovibond® SD 335. The extracts were stored under refrigerated conditions (≤ 4 °C) until being tested in the ecotoxicological assays. Water extracts were used as they better represent the readily soluble fraction of nutrients and contaminants that could be quickly released

and cause immediate effects. Also, the use of water extracts could fit as a worst case-scenario.

2.3.2. Extracts and biomass characterization

Extracts for elemental characterization by ICP-MS were acidified with HNO_3. Biomass from each of the six locations were submitted to acid digestion to extract trace elements for analysis. Approximately 0.2 g of biomass was digested with 3 mL of concentrated nitric acid (HNO_3, 65 %) on a digestion block (DigiPrep, SCP Science, Baie-d'Urfé, QC, Canada) at 85 °C for over 120 min. After cooling, 2 mL of hydrogen peroxide (H_2O_2, 30 %) were added and the samples re-heated at 85 °C for 120 min. Acid and peroxide were evaporated at 50 °C until near dryness and the samples diluted to a final volume of 25 mL with 1 % HNO_3 solution.

The elemental composition of biomass extracts was analysed using an Agilent 7700 ICP-MS equipped with an octopole reaction system (ORS) collision/reaction cell technology to minimize spectral interferences. Elements analysed included metals/metalloids (e.g., As, Al, Pb, Cd, Cr, Ni, Cu, Zn) and essential micronutrients (e.g., Fe, Mn, Mg, Ca, K) to check for compliance with the below permissible limits according to Council Directive 89/530/EEC supplementing and amending Directive 76/116/EEC, which was herein adopted for comparison purposes as a proxy in view of the limited availability of more specific regulatory frameworks. Blanks and certified reference materials were treated using the same method. For water extracts analysis accuracy was monitored by analysis of CRM SPS-SW1 (Quality Control Material – Surface Water Level 1) with recoveries between 92 and 110 % (average = 99 %). For biomass accuracy of the method was evaluated by the analysis of certified reference material BCR-279 (Sea lettuce, *Ulva lactuca*) with recoveries between 82 % and 95 % of the certified values. Precision was estimated by the relative standard deviation (RSD) of duplicate samples and was ≤20 %.

2.4. Ecotoxicity assays with extracts

The ecotoxicity of the extracts was also evaluated. For that purpose, monospecific assays with four freshwater organisms were performed: the producer *Raphidocelis subcapitata*, the primary consumers species *Brachionus calyciflorus* and *Daphnia magna*, and the secondary consumer *Danio rerio*. These species were selected because they are recommended by several standard guidelines (e.g., OECD, ISO), for hazard assessment, and will allow to capture the range of sensitivities to WH extracts in an ecosystem because they are representative of distinct trophic and functional levels.

2.4.1. Raphidocelis subcapitata

Cultures of the microalgae *R. subcapitata* were maintained in 250 mL Erlenmeyer flasks under sterile conditions in Woods Hole Marine Biological Laboratory medium, with weekly renewal (Woods Hole MBL, [29]), and under controlled settings of light and temperature (100 μE m^2 s^{-1} and 23 °C; [30]). The microalgae *R. subcapitata* 72-h growth inhibition test followed a modified version of the OECD guideline no. 201 [30], adapted to 24-well plates [31]. The experiments began with inocula from exponentially growing cultures. The range of dilution tested spanned from 1.56 % to 100 % extract, with the control consisting solely of MBL medium [29]. Three replicates were used per treatment/control, each containing 1800 μL of the extract desired dilution or control medium (MBL) combined with 200 μL of microalgae inoculum (at an initial concentration of 10^5 cells/mL algae cells). The test conditions were controlled (23 ± 1 °C of temperature and constant illumination of 100 μE m^2 s^{-1}), and all wells were resuspended daily to prevent algal settling and shading. Additional wells of each extract dilution were included with no algae added to account for extract colour interference later in the readings. Cell density (*CD*, cells/mL) was determined by measuring absorbance (*ABS*) at 440 nm (Multiskan Spectrum, Thermo Scientific). ABS values, adjusted by subtracting the extract blank readings, were converted to CD using a pre-established calibration curve [32]. These data were then used to calculate *R. subcapitata* yield (*Y*, biomass produced during the assay, cells/mL) and growth rate (*r*, day^{-1}) for each extract dilution and control, using the equations available at the standard guideline [30].

2.4.2. Brachionus calyciflorus

For ecotoxicity tests involving the planktonic rotifer *B. calyciflorus*, neonates were produced by hatching commercially available cysts on demand (MicroBioTests; Ghent, Belgium). The cysts were incubated in the American Society for Testing and Materials (ASTM) moderately hard synthetic freshwater medium under specific conditions: 23 °C, 3000–4000 lx for 24 h. The 24-h mortality assay with the rotifer *B. calyciflorus* followed the standard operation procedures of the Rotoxkit F® (MicroBioTests Inc., Ghent, Belgium). Five newly hatched neonates were assigned per replicate, each replicate containing 1 mL of each extract dilution or control medium, with five replicates per treatment and control. No food was added nor the medium change. Dilutions tested ranged from 0.78 % to 100 %. Mortality was checked after 24 h of incubation, at 23 °C in the dark.

2.4.3. Daphnia magna

Cultures of the planktonic cladoceran *D. magna* (K6 clonal lineage) were reared in ASTM synthetic hard water [33] with an organic extract supplement [34], at 20 ± 1 °C with a 16:8 h light:dark period. Medium and feeding (using *R. subcapitata* at a concentration of 3.0×10^5 $cells^{-1}$ ml^{-1} day^{-1}) was renewed every other day. The 48-h acute exposure assay with *D. magna* followed the OECD guideline 202 [35]. Briefly, < 24-h old neonates, collected from 3rd to 5th broods, were exposed to a series of dilutions ranging from 1.56 % to 100 % extract, plus a control consisting solely of ASTM). Four replicates, each with 8 mL of test extract dilution, were assembled per treatment or control, with five randomly assigned neonates. During the 48-h exposure, no food was added nor the medium renewed. Assays run under the controlled temperature of 20 ± 1 °C and 16 hL: 8 hD photoperiod. Mortality was checked at 24 and 48 h by gentle prodding of the organisms, considering them dead if no movement was observed within a 15-s period.

2.4.4. Danio rerio

The embryos of *D. rerio* were obtained from adult zebrafish breeding pairs. Fish are kept in a continuous water-carbon filtered system (26 ± 1 °C, pH 7.5 ± 0.5, 0.75 ± 0.05 mS/cm) and are fed twice daily with ZM 400 granular fish food. The 96-h Fish Embryo Acute Toxicity (FET) test with *D. rerio* followed the OECD test guideline 236 [36], with embryos being collected within one hour after fish naturally mating. Prior to the assay, embryos were carefully screened under a stereomicroscope (Stereoscopic Zoom Microscope-SMZ 1500, Nikon, Nikon Corporation, Japan) to discard injured and coagulated eggs and washed twice with clean, sterilized maintenance water from the fish facility. The extract dilutions ranged from 0.39 % to 100 %, with control conditions consisting of zebrafish maintenance water solely. Ten embryos (~3 h post-fertilization) were assigned per dilution of each extract dilution or control. Assays were assembled in 24-well microplates, with one single egg assigned per well, filled with 2 mL of test solution or water, under controlled temperature and light conditions (26 ± 1 °C and 16 h^L: 8 h^D photoperiod). Every 24 h, mortality and the developmental state of the embryos was recorded. At 48 h, the heartbeat rate of the embryos was evaluated under a stereomicroscope with beats counted for 15 s. At the end of the assay, all alive larvae were measured using the ImageJ software.

3. Data analysis

Water hyacinth biomass was compared among locations using one-way ANOVA with Holm–Sidak post hoc testing ($p < 0.05$).

The estimation of dilutions provoking 20 % (LD_{20}) and 50 % (LD_{50}) of mortality in *B. calyciflorus*, *D.magna*, and *D. rerio* were performed

using the software Priprobit [37]. Estimation of dilutions causing 20 % (ED_{20}) and 50 % (ED_{50}) reduction of *R. subcapitata* yield and growth rate were made by fitting the logistic or sigmoid model to the data with the program Statistica for Windows 4.3 (StatSoft, Aurora, CO, USA). The identification of significant differences between control and each treatment level was done using one-way analysis of variance (ANOVA), after confirmation of the validation of the assumptions of normality and homoscedasticity, followed by Dunnett's, with a significance threshold of $p < 0.05$.

4. Results

4.1. Water hyacinth biomass collection and characterization

At time of biomass sampling, water parameters from each collection site were measured (Table S1). The pH values ranged between 7 and 8 across all sites, whereas conductivity never surpassed 525 µS/cm, with SJL and Valente locations with the lowest registered values (129 and 145 µS/cm, respectively). From the remarks column is relevant to highlight the agricultural activity near collection sites EEAT and Sorraia (Table S1).

The concentrations of the principal photosynthetic pigment (Chl_a) were found to be within the same range across all locations (between 3.34 and 4.44 mg/g DW), but the ratio of Chl a/Chl b was found to be significantly lower in plants collected in Pateira and Bico compared to the other locations (Fig. 2a). The highest Chl a + b value was found in the leaves of plants collected at Valente (Fig. 2a). This was also reflected in the other measured pigments, (Chl_b and Chl_{x+c}) which showed similar concentrations in all WH biomass samples (Fig. 2a). The $Chl_{a/c}$ ratio was slightly lower for the Pateira- and Bico-associated biomasses (2.4 and 2.8, respectively; Fig. 2a). For protein (Fig. 2b), the WH biomass collected at Sorraia, Valente, and SJL presented the highest concentrations (between 20 and 30 mg/g DW), with biomass from EEAT, Pateira, and Bico, presenting the lowest protein content and performing a homogenous group (between 15 and 17 mg/g DW; Fig. 2b). Although, EEAT, Pateira, and Bico also shared group with Sorraia and Valente (Fig. 2b). As for sugars, both reduced and soluble, biomass collected from all locations presented similar concentrations, except for Bico and SJL (highest and lowest concentrations of reducing sugars of 0.94 and 0.24 mg/g DW, respectively, performing distinct homogenous groups; Holm-Sidak, $p < 0.05$; Fig. 2c) and Sorraia and Valente (highest and lowest concentration of soluble sugars, 0.25 and 0.06 mg/ g DW, respectively, performing distinct homogenous groups; Holm-Sidak, $p < 0.05$; Fig. 2d).

The FTIR spectra analysis for the biomass of each site and by tissue type (leaves, floaters, and roots) are presented in Fig. 3, and the integration of the areas under the curves are presented in Table S2. Differences were greater between tissues than locations. Floaters showed low to mid-range FTIR spectra at all sites, with peaks of highest intensity around 3650–3000 cm^{-1} (high O—H stretching number), ranging areas under the curve of 110.01 and 268.46 cm^{-1} (Fig. 2; Table S2). Next, leaf tissues present a specific profile with absorbance peaks around 1750–1500 and 1495–1200 cm^{-1}, which correspond to C═O and C—O vibrations ranging from area values of 32.91 to 97.91 cm^{-1} and from 34.22 to 102.39 cm^{-1}, respectively (Fig. 3; Table S2). Roots consistently presented the highest absorbance values across the entire wavelength

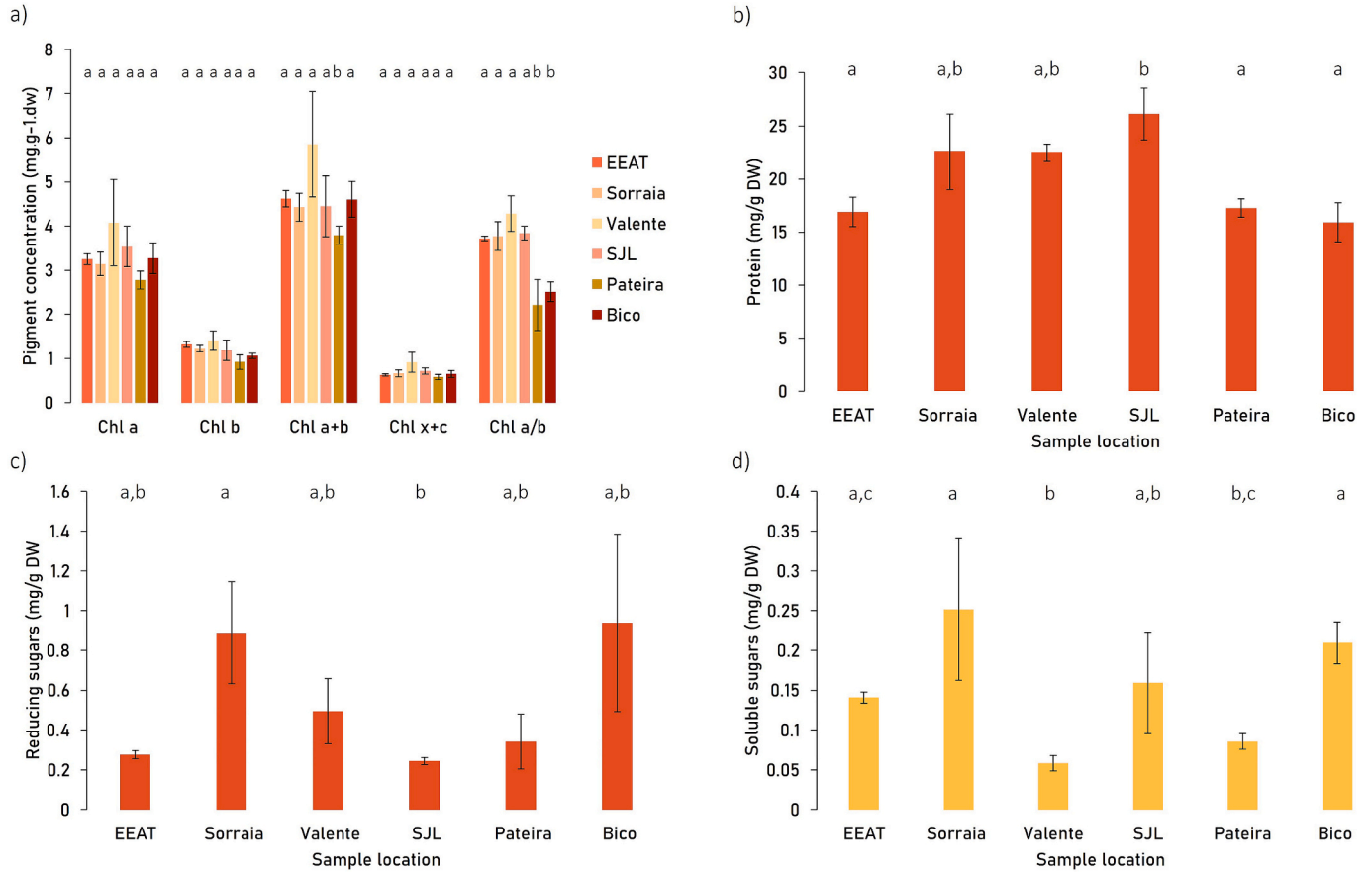


Fig. 2. Mean concentration and standard deviation of photosynthetic pigments (**a**), protein content (**b**), reduced (**c**) and soluble sugars (**d**) measured in water hyacinth (*Eichhornia crassipes*) biomass collected in different locations across Portugal. EEAT (Estação Experimental António Teixeira) and Sorraia (Praia Fluvial do Rio Sorraia) both at Santarém district. Valente (Vila Valente), SJL (São João de Loure), Bico (Observatório do Bico at Pateira de Fermentelos), and Pateira (Pateira de Fermentelos), all at Aveiro district. Letters sharing (a,b,c) indicate homogenous groups (Holm-Sidak, $p < 0.05$).

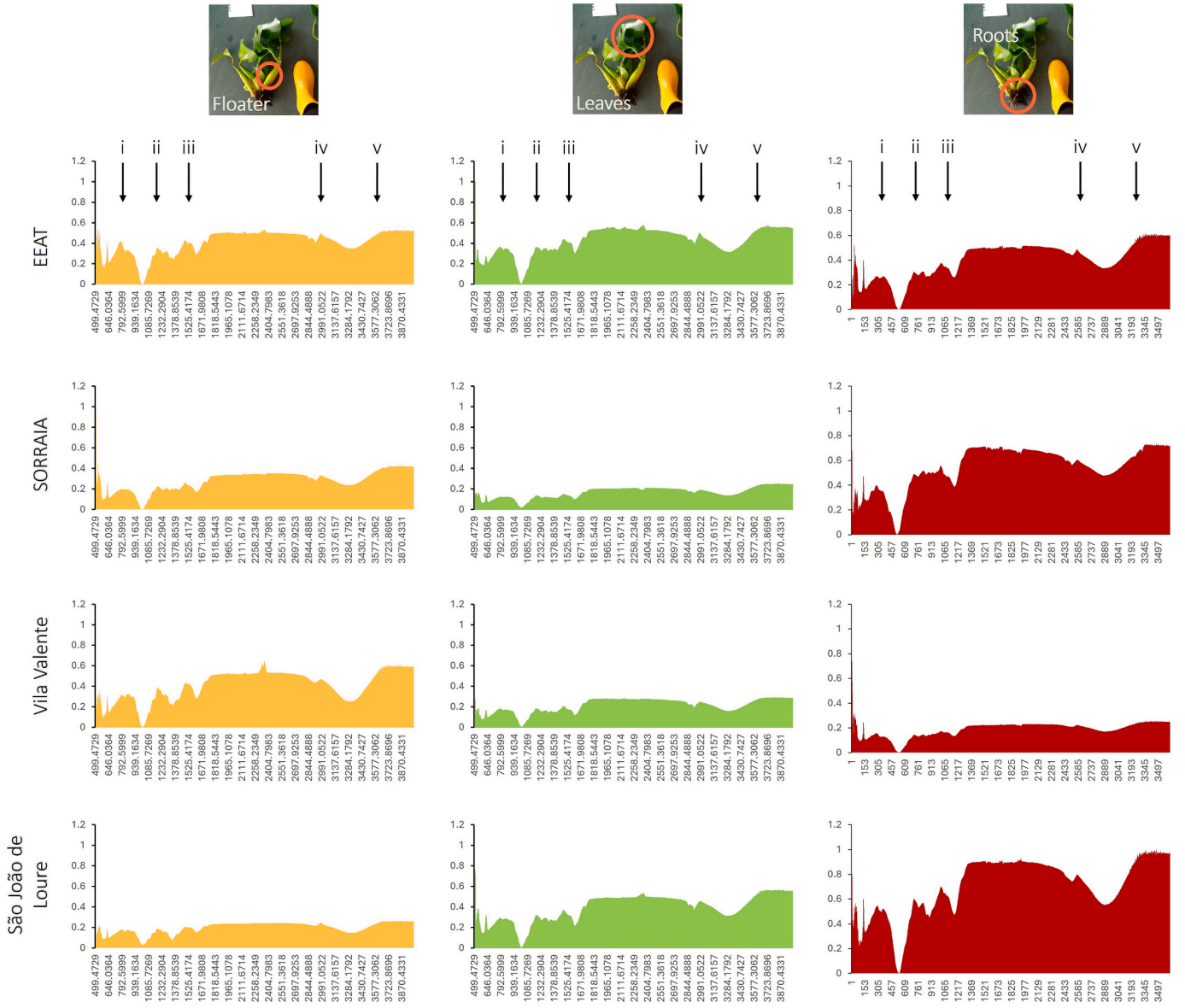


Fig. 3. FTIR-spectra of the water hyacinth (*Eichhornia crassipes*) biomass collected in six different locations across Portugal. EEAT (Estação Experimental António Teixeira) and Sorraia (Praia Fluvial do Rio Sorraia), both at Santarém district. Valente (Vila Valente), SJL (São João de Loure), Bico (Observatório do Bico at Pateira de Fermentelos), and Pateira (Pateira de Fermentelos), all at Aveiro district. Indentions on the spectra correspond to: (i) peak in 805–1200 cm^{-1} range for polysaccharides with functional groups C-O-C and C-O-P; (ii) peak in 1200–1495 cm^{-1} range for proteins and lipids with functional groups, CH_2 and CH_3 and phosphate compounds with functional group P=O; (iii) 1500–1750 cm^{-1} corresponded to the Amide *I* and II bands, reflecting the presence of proteins; (iv) peak in 2765–3000 cm^{-1} range for lipids with functional groups CH; and, (v) peak in 3000–3650 cm^{-1} was examined for O—H and N—H stretching vibrations, which are indicative of carboxyl, hydroxyl, phenol, and amide groups.

range. As for locations, the roots of plants collected in Pateira, Bico, and Valente showed strong peaks around 3650–3000 (ranging from areas of 446.92 to 454.86 cm^{-11}) and 1200–805 cm^{-1} (ranging from areas of 128.00 to 132.91 cm^{-1}). These results reflect the elevated presence of lignocellulosic material and adsorption-prone sites in roots, supporting their role in metal and contaminant accumulation—a finding corroborated by the elemental analyses.

Regarding the elemental analysis to the biomass, the most present PTEs in biomass samples were As, Pb, Cr, and Co, while for macro- and micronutrients it is to highlight the high contents of K, Mn, Fe, Ca, and Mg in the WH biomasses (Fig. 4). For instance, WH biomass collected at Valente and EEAT presented the highest values of K (8.76 and 8.00 %, respectively; Table S3), while SJL presented the highest values of Ca and Mg (2.62 and 0.78 %, respectively; Table S3). The biomass collected from Bico was the one showing the highest levels of several PTEs, namely As (12.7 mg/kg), Pb (47.4 mg/kg), Cr (6.94 mg/kg) and Co (19.0 mg/kg) (Table S3).

4.2. *Water hyacinth extracts characterization*

The pH values of WH extracts were mainly in the 7–8 units range, with the lower values reported for WH extracts made with biomass collected at Bico and Pateira (values of 5.87 and 5.30, respectively) (Table S4). The conductivity of the WH extracts followed a similar grouping, with most extracts within the range of 19.0 to 22.0 mS/cm, with WH extracts from Bico and Pateira presenting the lowest conductivity values of 11.2 and 6.98 mS/cm, respectively (Table S4).

The elemental composition of WH extracts regarding potentially toxic elements and macro- and micronutrient is presented in Fig. 5 and Table S5. The extracts from WH biomass collected at Sorraia stood out,

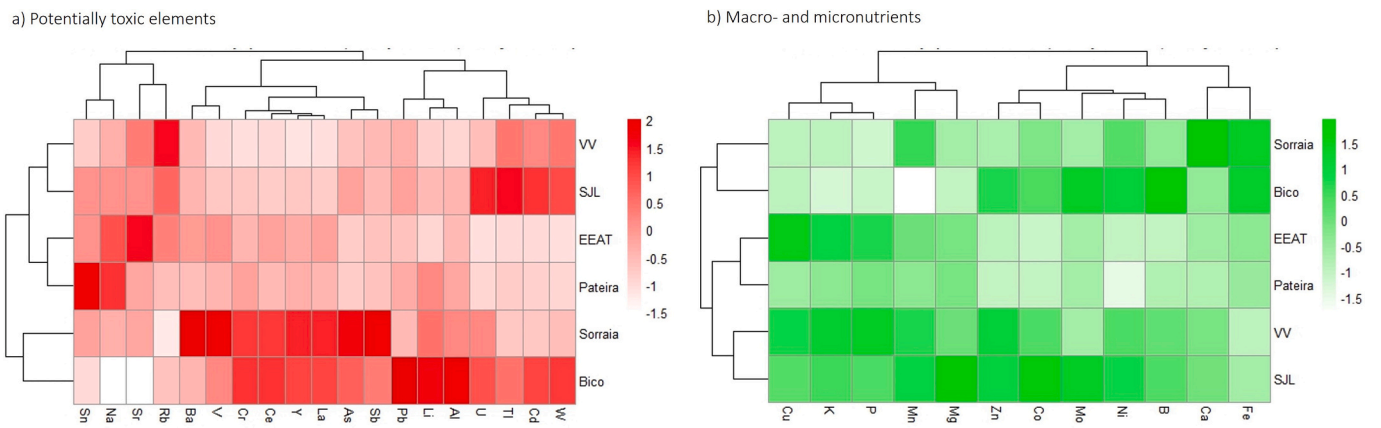


Fig. 4. Heatmap representing **a)** potentially toxic elements, and **b)** macro- and micronutrients composition in water hyacinth (*Eichhornia crassipes*) biomasses collected in six different locations across Portugal. EEAT (Estação Experimental António Teixeira) and Sorraia (Praia Fluvial do Rio Sorraia), both at Santarém district. Valente (Vila Valente), SJL (São João de Loure), Bico (Observatório do Bico at Pateira de Fermentelos), and Pateira (Pateira de Fermentelos), all at Aveiro district. Analytes were standardized. Heatmap created using Euclidean distance and complete linkage.

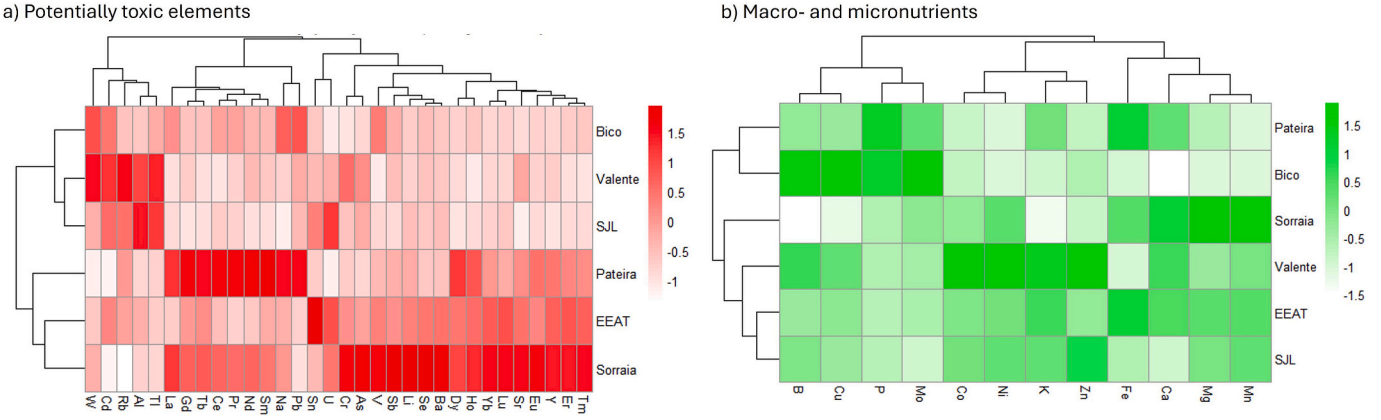


Fig. 5. Heatmap representing **a)** potentially toxic elements, and **b)** macro- and micronutrients composition in water hyacinth extracts (*Eichhornia crassipes*) with biomass collected in six different locations across Portugal. EEAT (Estação Experimental António Teixeira) and Sorraia (Praia Fluvial do Rio Sorraia), both at Santarém district. Valente (Vila Valente), SJL (São João de Loure), Bico (Observatório do Bico at Pateira de Fermentelos), and Pateira (Pateira de Fermentelos), all at Aveiro district. Analytes were standardized. Heatmap created using Euclidean distance and complete linkage.

Table 1
Toxicological endpoints measured in five freshwater organisms after exposure to water hyacinth (*Eichhornia crassipes*) extracts made with biomass collected at six different locations across Portugal. EEAT (Estação Experimental António Teixeira) and Sorraia (Praia Fluvial do Rio Sorraia), both at Santarém district. Valente (Vila Valente), SJL (São João de Loure), Bico (Observatório do Bico at Pateira de Fermentelos), and Pateira (Pateira de Fermentelos), all at Aveiro district. The LD_{20} and LD_{50} are the dilutions causing 20 % and 50 % of mortality, respectively (presented in % of dilution) and the ED_{20} and ED_{50} are the dilutions causing 20 % and 50 % of sublethal effect, respectively (presented in % of dilution). CL 95 % - confidence limits at 95 %. n.c. – not calculated.

	Raphidocelis subcapitata Yield, 72 h		*Raphidocelis subcapitata* Growth rate, 72 h		*Brachionus calyciflorus* Mortality 24 h		*Daphnia magna* Mortality, 24 h		*Danio rerio* Mortality, 96 h	
	L(*E*)D$_{20}$ CL 95 %	**L(*E*)D$_{50}$** CL 95 %	**L(*E*)D$_{20}$** CL 95 %	**L(*E*)D$_{50}$** CL 95 %	**L(*E*)D$_{20}$** CL 95 %	**L(*E*)D$_{50}$** CL 95 %	**L(*E*)D$_{20}$** CL 95 %	**L(*E*)D$_{50}$** CL 95 %	**L(*E*)D$_{20}$** CL 95 %	**L(*E*)D$_{50}$** CL 95 %
					1.37	**3.67**	**21.1**	**22.4**	**29.9**	**40.5**
EEAT	n.c.	n.c.	n.c.	n.c.	0.28–2.67	1.64–7.31	19.9–22.5	21.1–23.8	17.4–37.7	30.6–53.9
			75.2	**91.4**	**1.32**	**3.63**	**11.6**	**15.3**	**6.13**	**11.7**
Sorraia	n.c.	n.c.	n.c.	n.c.	0.26–2.58	1.65–7.38	2.58–16.8	9.09–36.1	0.87–11.6	5.12–34.3
	11.0	**12.0**	**5.77**	**28.6**	**2.25**	**4.97**	**21.2**	**22.7**	**25.0**	**26.9**
Vila Valente	n.c.	n.c.	n.c.	n.c.	1.58–2.93	3.90–6.36	20.1–22.5	21.4–24.0	23.1–27.1	24.9–29.2
	23.0	**40.0**	**48.3**	**83.2**	**1.04**	**2.29**	**8.11**	**10.5**	**5.41**	**57.3**
SJL	14.0–32.0	30.0–51.0	33.3–62.3	67.8–98.5	0.66–1.40	1.75–2.93	6.07–9.51	8.88–12.4	5.05–5.81	53.4–61.5
		6.0	**13.1**	**36.6**	**14.7**	**24.2**	**26.1**	**28.2**	**5.50**	**15.7**
Pateira	n.c.	1.0–10.0	0.24–25.9	17.5–55.7	7.04–21.3	16.0–39.3	24.3–28.0	26.3–30.4	0.24–13.6	4.90–82.6
	9.0	**17.0**	**26.1**	**29.6**	**9.91**	**14.3**	**21.1**	**22.4**	**19.4**	**27.7**
Bico	n.c.	7.10–27.0	20.8–31.3	7.63–51.6	5.16–13.5	10.1–22.2	20.0–22.5	21.1–23.8	12.1–25.0	21.0–38.1

presenting the highest concentrations of potentially toxic elements (PTEs), with very high levels of As (144 μg/L), followed by Cr (11.3 μg/L), and at a lower extent Li (12.4 μg/L), Ni and Se (Fig. 5a; Table S5). Following, EEAT extracts have also shown high levels of As (77.3 μg/L) and Cr (7.15 μg/L) (Fig. 5a, Table S5). As for the remaining extracts, they have shown moderate to very low levels of these elements.

The heat map clustering the locations by the nutrient content showed that all extracts have presented high levels of P (>6000 μg/L), with Bico water extracts exceeding 50,000 μg/L (Fig. 5b; Table S5). Pateira and Bico water extracts have also presented greatest levels of Na, and Mo and moderate to high levels of Fe and K with the remaining locations with a moderate to weak presence of nutrients in the extracts (Fig. 5b; Table S5). Comparing the results of PTEs and micro- and macronutrients present in the water extracts with those discussed above for the respective biomasses, it is to highlight that the concentrations of elements leaching to water are much lower (in the order of mg/kg in biomass, and in the order of μg/L in the water extracts).

4.3. Water hyacinth extracts ecotoxicity

Table 1 summarizes the lethal and sublethal dilutions that caused 20 % and 50 % of effect on the model organisms [L(*E*)D_{20} and L(*E*)D_{50}, respectively]. Extracts produced with WH collected in all locations (except EEAT) exerted toxicity both on *R. subcapitata* yield and growth rate or solely on growth rate (Table 1). Regarding yield, for EEAT and Sorraia, both the ED_{20} and ED_{50} values could not be estimated, although results have shown that EEAT extract percentages equal to or higher than 25 % and all Sorraia extract dilutions were able to induce significant reductions in algal yield (Fig. S1). The same was true for the EEAT extract regarding the algal growth rate: no EC_x could be estimated, although results have shown that exposure to 100 % EEAT extract resulted in almost complete suppression of algal growth (Fig. 2S). In both cases, (EEAT and Sorraia) it is to highlight that, the dose-response relationship has hindered the calculation of effective concentrations, there were still very significant declines in algal growth. Based on the ED_{50} of the algae growth rate, Valente, Pateira, and Bico were the most toxic extracts to *R. subcapitata* with ED_{50} values of 28.6 %, 36.6 %, and 29.6 %, respectively (Table 1), while for Sorraia and SJL, the ED_{50} for growth rates were above 80 % (Table 1).

Regarding the assays with both primary consumer species, *B. calyciflorus* and *D. magna*, all extracts were toxic and caused mortality of the rotifer and daphnid (Table 1). Overall, Pateira and Bico were the least toxic extracts (LD_{50}'s of 24.2 % and 28.2 %, for *B. calyciflorus* and *D. magna*, respectively for Pateira, and LD_{50}'s of 14.3 % and 22.4 %, for *B. calyciflorus* and *D. magna*, respectively for Bico), while EEAT and SJL were the most toxic (LD_{50}'s values ranging from 2.29 % to 22.4 %; Table 1). It is to highlight that values representing the onset of toxicity (LD_{20} values) were very close to the LD_{50} estimated for both species and all extracts, indicating a steep dose–response relationship and a narrow margin between sublethal and lethal effects. This suggests that once a certain threshold concentration of the extract is reached, toxicity rapidly escalates, leaving little room for gradual adaptation or tolerance.

Toxicity tests with *D. rerio* allowed the calculation of the LD_{20} and LD_{50} values for all extracts (Table 1). The LD_{20} values were separated into two groups, one for Sorraia, SJL, and Pateira (ranging between 5.41 % and 6.13 %) and the other for EEAT, Valente, and Bico (19.4 % to 29.9 % extract dilution). These same groups remained largely unchanged for LD_{50}, except for the Valente extract (26.9 %), which is now closer to the values of the Sorraia, SJL, and Pateira group (11.7 % to 27.7 % extract dilution). Additionally, it was found that extracts of EEAT, Valente, and SJL induced significant differences in survival rates at extract dilutions equal to or greater than 50 %, whereas for Sorraia and Pateira biomass extracts, significant differences were observed at dilutions equal to or greater than 25 % (Fig. S5). Regarding other parameters for which it was not possible to determine ED_{20} or ED_{50} values, heart rate varied significantly in relation to the control in embryos exposed to dilutions greater than 3.13 % of the EEAT extract. However, for other extracts such as Sorraia and Bico, these changes were observed at extract dilutions of 0.39 % and 0.78 % (Fig. S6). The total length of the larvae was significantly reduced for Sorraia and SJL extracts at dilutions of at least 1.56 % and for dilutions of 3.13 % and 6.25 % for Valente and Bico extracts, respectively (Fig. 7S). The EEAT extract caused a significant increase in the total length of the larvae for dilutions of 0.78 %, and a significant reduction in the dilution of 3.13 % (Fig. 7S). Overall, *B. calyciflorus* was the most sensitive to WH biomass extracts, followed by *D. magna* and *D. rerio*, highlighting the role of primary consumers as a quick screening tool in short-term assays. The WH biomass extracts from Sorraia and SJL were ranked as the most toxic to these aquatic species. Although EEAT and Vila Valente some L(*E*)D_x values were not estimated, and thus, they may be seen as potentially the extracts with the least toxic potential. It is to highlight that they were able to induce significant effects at high dilution levels, although the dose-response pattern impeded the determination of effective concentrations.

5. Discussion

Characterizing water hyacinth (WH) biomass and assessing the ecotoxicity of its aqueous extracts is essential for understanding its potential benefits and limitations as a sustainable agricultural input, particularly as a fertilizer, and for safeguarding against potential downstream effects. Therefore, this study aimed to comprehensively evaluate WH biomass collected from diverse geographic locations, with an emphasis on nutrient and contaminant profiles as well as the ecotoxicological effects of aqueous extracts on aquatic organisms. This would inform environmental policymakers about the feasibility of integrating WH into a safe and circular agricultural model.

Water hyacinths are known for their excellent capacity to adsorb and retain elements from the surrounding environment, including organic and inorganic compounds [5,38–40]. On the other hand, this capacity is a major concern if the aim is to monetize them in agriculture. The environmental conditions where WH grow may shape their composition: although temperature and pH were relatively consistent across all sampling sites, conductivity was relatively high at four of the six sites (except SJL and Valente), considering the reference values for non-impacted freshwater bodies (10–100 μS/cm, [41]), which could indicate some type of underlying contamination. However, in the present study, the structural differences (analysed by spectroscopy) between tissues were greater than those observed in plants collected from different geographic locations. Structural analysis showed that spectral complexity increases from floaters to leaves and roots, which is clearly related to the complexity of each tissue and its biological function [42]. Floaters showed greater variability in H-linked compounds, likely associated with water or simple C carbohydrate content, which is possibly related to the floaters' function of enabling the upright WH posture (buoyancy) and vegetative propagation. In floaters, peaks associated with proteins or amines were minimal, suggesting a limited involvement in nutrient uptake or photosynthetic activity. In leaves, the intensity of the peak areas characteristic of proteins or amines is likely related to photosynthetic activity, as this activity fuels their synthesis. Roots, in turn, showed the highest values of integration areas associated with structural components, such as lignin, proteins, and cell wall components (cellulose), confirming the results obtained elsewhere [43]. Cellulose, lignin, hemicellulose, and proteins provide active functional groups (–OH, –COOH, –NH_2) that serve as high-affinity binding sites for inorganic compounds such as metals/metalloids [38,39,42,44], and which may be related to some physicochemical parameters of the water at the sample collection points. Indeed, elemental analyses performed by ICP-MS of the different WH tissues (from all sites) revealed that PTEs concentrations were mainly centered in the root, sometimes in orders greater than 20 times when compared to leaves or stem aerenchyma-based tissue, which has been previously corroborated by others [44]. From the perspective of application and profitability in agriculture, it is

necessary to understand whether these levels can completely prevent WH incorporation into agricultural soils or whether a limit on the WH application rate can be established. Because there are no specific international regulatory guidelines for the application of WH biomass to soils, its use will have to be classified within the existing regulations for other soil amendments, such as compost and/or organic soil improver, which impose limits on PTEs such as Pb, Cd, inorganic As, Cr(VI), and Ni (Council Directive 89/530/EEC supplementing and amending Directive 76/116/EEC). Moreover, newer regulations on the safe marketing of fertilizer products also reinforce compliance across all member states (EU Regulation 2019/1009). Considering the total values (sum of PTEs in the leaves, floaters, and roots), the concentrations were within the EU permitted levels (for arsenic, total contents did not exceed inorganic As threshold). For Cr, total levels exceeded the maximum level for Cr(VI); however, because speciation was not determined compliance cannot be conclusively evaluated. Nevertheless, previous studies suggest that *E. crassipes* predominantly accumulate Cr(III) due to the reduction of Cr (VI) at the root surface as part of its detoxification mechanism [45], suggesting that most of the chromium in the biomass may be present in a less toxic form. Conversely, inorganic arsenic species tend to dominate in aquatic macrophytes [46], implying that total arsenic values close to regulatory limits could still represent a potential environmental risk. These considerations highlight the importance of future studies incorporating speciation analysis (e.g., HPLC-ICP-MS) to better assess the compliance and environmental safety of WH-based soil amendments. Furthermore, considering the EU reference values for solid organic fertilizers for declared primary nutrients, the studied biomass presented values above the quota for potassium (values above 2.5 % compared with the base value of 2 % K) and one-tenth for phosphorus (values between 0.15 % and 0.2 % compared with the base value of 2 % P [47]). Although one must assume this element richness might not directly translate into agronomic performance, some studies using transformed WH biomass reported increased in total N and P [43]. Examples are the work of Kassa et al. [48] that reported that WH biochar may double total N and total P increase beyond a factor of 4. On the same line the work, Gezahegn et al. [49] showed that WH compost incorporation resulted in an increase of 24.3 % in total N and 28.6 % in total P. These data corroborate the initial hypothesis that WH can be reused as an agricultural fertilizer, although not from the perspective of total replacement of traditional fertilizers, but partially replacing fertilizers of chemical origin and providing essential elements for soil and crop productivity [50,51].

Although WH biomass can contribute to nutrient recycling and sustainable agriculture, caution and extensive monitoring are necessary to avoid adverse downstream effects, for example, on aquatic ecosystems. This is important because, as previously discussed, WH contains not only high amounts of essential nutrients (easily leachable), but also PTEs that may percolate [5,40]. Therefore, assessing the impact of WH aqueous extracts can be a useful tool for understanding their potential effects on freshwater biota and predicting worst-case scenarios. The aqueous extracts from Sorraia, Pateira, and Valente generally contained higher concentrations of PTEs and nutrients, but all tested extracts induced measurable adverse effects on aquatic organisms. Comparing the ICP-MS analysis of WH biomass with the same analysis performed on water extracts, it is possible to confirm that PTEs and nutrients are leached from the biomass even when extracted with water. Differences varied among the different extracts, and therefore, the toxicity varied according to the species and biomass collection sites. Within the geographic group, Pateira, Bico, and São João de Loure had caused the lowest effects, whereas Sorraia was consistently among the most toxic extract, producing the strongest inhibition of *R. subcapitata* yield endpoint, was the most toxic to *B. calyciflorus*, and ranked second in lethal toxicity to *D. magna* and *D. rerio* embryos. Despite the encouraging characteristics of Bico and Pateira biomasses for potential agricultural use (based mostly on its content of essential nutrients such as potassium and phosphorus), these high nutrient loads and the relatively high concentrations of certain PTEs may, in the long-term, contribute to osmotic stress in sensitive species, and thus these biomasses application warrants caution. Therefore, any agricultural application of Bico- or Pateira-derived biomasses should be preceded by thorough elemental characterization and regular monitoring to ensure safe and sustainable use. As for the exceptional high toxicity of Sorraia water extracts, this result may be explained by the fact that this region is one of Portugal's largest irrigation areas, dominated by intensive cultivation of corn, rice, and tomato. Thus, the Sorraia basin is subject to multiple pressures, including diffuse pollution from agrochemicals, municipal discharges, and water abstraction, which may lead to elevated nutrient and contaminant loads [52].

However, no uniform toxicity classification could be defined, emphasizing the need for a complex evaluation of multiple species and endpoints as herein evaluated. For example, for producers, extract dilutions of approximately 30 % halved algal growth rates. In some cases, the effective concentrations could not be estimated; however, 50 % dilutions of the extracts completely suppressed algal growth (e.g., aqueous extract of EEAT). For representative freshwater zooplankton species, much higher extract dilutions already cause significant mortality (e.g., dilutions between 2 and 10 % of extracts could induce 50 % mortality in these organisms). Early life stages of vertebrate organisms demonstrated even greater sensitivity (extract dilutions as low as 0.78 to 1.56 %) and produced significant effects on sublethal parameters of *D. rerio*, such as heartrate and final body length, potentially impairing developmental success and future fitness. This variability may be related to the differential sensitivity of organisms to the determining elements. For instance, Cojocaru et al. [53] have reported 48 h-LC_{50} values of 0.33 mg Zn/L, 0.31 mg Cd/L, and 1.70 mg Cu/L for *D. magna*, while Venâncio et al. [54] reported a 48 h-LC_{50} of 0.26 mg Cu/L for *D. longispina*. For zebrafish, other studies reported 96 h-LC_{50} of 0.3 mg/L for Cu [55], and of 19 mg Cd/L and 15 mg Cd/L for lethal and sublethal effects [56]. Another study of young life stages of a vertebrate species (*B. sitibundus*) exposed to aqueous extracts of WH (dilutions of 1 %, 1.5 %, 2.5 %, and 5 %) demonstrated that only 6 days of exposure to the 5 % dilution were necessary for all organisms to die. In the remaining dilutions, growth inhibition, increased mortality with development, and various morphological abnormalities in the limbs were observed, with less than 10 % of individuals achieving the metamorphic state compared to the control treatment (approximately 25 %; [18]). The authors hypothesize that toxicity could be driven by metal content in the plants (for instance, zinc, aluminum; [18]). Considering the values for some elements in the WH extracts of this study (e.g., 0.026 to 1.4 mg Zn/L or 0.03 to 0.06 mg Cu/L), it is possible reasoning that WH water extracts toxicity may be primarily driven by PTEs. It is important to note that metal bioavailability can greatly influence toxicity profiles and can be a justification as to why extracts with higher concentrations of PTE did not rank among the most toxic for some of the organisms. Nevertheless, other compounds not evaluated in this study (such as oils or phytotoxins) could have contributed to the overall toxicity, a hypothesis also highlighted by Pesarakloo et al. [18]. Previous studies have shown that WH-based extracts and essential oils may cause deleterious effects on *D. magna* [57], while Lv et al. [58] and Cheng et al. [59] reported algicidal activity of WH-based extracts. Similar results were reported for zebrafish (mortality in embryos and larvae), although not specifically for WH but when exposed to *Enydra fluctuans* extracts [60] or *Antirhea borbonica* extracts [61]. Despite the variability in the ecotoxicity results, they not only reinforce the importance of multispecies testing in environmental safety assessments, as they indicate that smaller or rapidly reproducing species are often the most sensitive, making them promising early indicators of WH extract toxicity in future studies [62].

6. Conclusion

This study assessed water hyacinth (WH) biomass from several sites and examined beneficial nutrients and potential contaminants, which

are essential for the risk/benefit analysis of its reuse in agriculture. Water hyacinth biomass from Bico and Pateira showed the most promising potential for sustainable agricultural use. Particularly Bico biomass exhibited high concentrations of key nutrients, especially P and K (two of the fertilizer nutrient triad), making it a suitable candidate for partial replacement of conventional fertilizers. Nevertheless, this does not rule out a preceded basic elemental analysis to ensure its safe and consistent use. Contamination levels, especially for As and Cr, were in the intermediate range between Bico and the other four sites, although within the limits permitted by EU Regulation 2019/1009 for fertilizers. Despite the hypothesis that WH is a sustainable alternative to fertilizers due to their high nutrient load, the findings regarding PTE highlight that there may still be some environmental safety concerns in the short-term. Toxicity assessment of extracts in algae, zooplankton (*Daphnia magna* and *Brachionus calyciflorus*), and fish embryos (*Danio rerio*) highlighted species-specific responses and the complexity of ecotoxicological risk. Variation in growth and mortality data reveals the complexity of the real ecological risks posed by WH extracts, helping to identify hotspots, select sensitive bioindicators, and guide WH management actions. By demonstrating the variability of WH biomass in chemical composition and ecotoxicological responses across sites and species, this study underscores the need for thorough, site-specific assessments prior to its use in agricultural systems. Therefore, it is advisable that pre-screening of WH biomass for contaminants, along with recommended ecotoxicological screening, become standard practice to ensure safe and sustainable application, while providing essential data to support circular strategies for WH use in agroecosystems.

CRediT authorship contribution statement

Carla Patinha: Writing – review & editing, Writing – original draft, Resources, Conceptualization. **Carlos Silva:** Writing – review & editing, Validation, Investigation. **Rodrigo Neves:** Writing – original draft, Validation, Investigation. **Pedro Pato:** Writing – review & editing, Validation, Investigation. **Solange Magalhães:** Writing – review & editing, Validation, Investigation. **Isabel Lopes:** Writing – review & editing, Validation, Resources. **Cátia Venâncio:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, 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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.enceco.2025.11.005.

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