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POTENTIALLY TOXIC ELEMENTS IN SALTMARSHES: A REVIEW ON METAL(LOID)S TOLERANCE AND ACCUMULATION, BIOMARKERS AND POTENTIAL BIOINDICATORS

(Elementos potencialmente tóxicos em marismas: uma revisão sobre tolerância e acúmulo de metais(loides), biomarcadores e potenciais bioindicadores)

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ABSTRACT

Saltmarshes are important coastal ecosystems that provide numerous ecosystem services. Despite their ecological importance, marshes are exposed to potential toxic elements (PTE) from different natural and anthropogenic sources. Metal(loid)s are one of the most dangerous environmental pollutants due to their toxicity and persistence in the environment, bioaccumulation along the food chain, disruption of the ecosystem, and being a risk for the biodiversity of these areas and to human well-being. This review provides an overview of the most used species of halophytic plants and invertebrates as potential bioindicators of metal exposure and the biomarkers used in saltmarshes based on field monitoring studies published in the last four years. The most studied PTE in plants were Zn, Pb, Cu, Cd, Cr and Mn, being the pattern of accumulation higher in roots/rhizomes than in aerial tissues (leaves, stems). In invertebrates, Zn, Pb, Cu, Cd, Cr, Ni and Fe were the most analyzed elements. This review highlights and remarks on the importance of studies on PTE accumulation and tolerance of halophytes. It is essential to assess and monitor polluted saltmarshes using organisms that not only have the potential to act as sentinel species, but also could be used for bioremediation. It is necessary a deeper understanding of the metal accumulation, transformation, and tolerance by dominant taxa that inhabit saltmarshes, as well as PTE effects to define appropriate ecosystem management and restoration measures.

Keywords*: Heavy metals, Bioindicators, Biomarkers, Invertebrates, Plants.*

RESUMO

As marismas são importantes ecossistemas costeiros que fornecem numerosos serviços ecossistêmicos. Apesar de sua importância ecológica, essas áreas estão expostas a elementos tóxicos potenciais (ETP) provenientes de diversas fontes naturais e antropogênicas. Os metais e metalóides estão entre os poluentes ambientais mais perigosos devido à sua toxicidade, persistência no ambiente, bioacumulação ao longo da cadeia alimentar e por causarem a interrupção do ecossistema, representando um risco para a biodiversidade dessas áreas e para o bem-estar humano. Apresenta-se aqui uma visão geral das plantas halófitas e invertebrados mais utilizados como potenciais bioindicadores de exposição a metais e dos biomarcadores utilizados em marismas, com base em estudos de monitoramento de campo. Os ETP mais estudados em plantas foram Zn, Pb, Cu, Cd, Cr e Mn, com o padrão de acumulação maior nas raízes/rizomas do que nos tecidos aéreos (folhas, caules). Em invertebrados, Zn, Pb, Cu, Cd, Cr, Ni e Fe foram os elementos mais analisados. Esta revisão destaca a importância dos estudos sobre a acumulação e tolerância de ETP em halófitas. É essencial avaliar e monitorar marismas poluídas usando organismos que não apenas têm o potencial de atuar como espécies sentinelas, mas que também possam ser utilizados para a biorremediação. Se faz necessário um entendimento mais profundo sobre a acumulação, transformação e tolerância aos metais por parte dos táxons dominantes que habitam as marismas, bem como os efeitos dos ETP, para definir medidas adequadas de manejo e restauração dos ecossistemas.

Palavras-chave: Metais pesados, Bioindicadores, Biomarcadores, Invertebrados, Plantas.

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INTRODUCTION

Saltmarshes are ecosystems found globally at the land-sea interface. These coastal wetlands are regularly flooded and drained by seawater following the tidal cycle, further connecting saline and freshwater ecosystems. Marshes hold significant ecological value, contributing for the ecosystem in primary production, nutrient cycling, denitrification, and source and sink of carbon dioxide and pollutants (HE *et al.*, 2016; GIULIANI and BELLUCI, 2019; ERMGASSEN *et al.*, 2021). Moreover, these environments provide habitat, nurseries and reproductive support for a wide number of animals species, as well as foraging, working as important link in the food chain of coastal animals (ANJUM *et al.*, 2014; GIULIANI and BELLUCCI, 2019).

Despite their importance, saltmarshes have been modified, reclaimed, and destroyed by humans. Among several anthropogenic and natural factors contributing to their decline, pollution remains a substantial threat to these ecosystems (ROE *et al.*, 2021). Pollutants are transported by tidal and retained in saltmarsh as a result of the slow water exchange rate and the characteristics of sediments. These ecosystems are typically characterized by high soil salinity and other physicochemical properties, which influence the bioavailability of metals and their interaction with biological systems (IBRAHEEM et al., 2021).

In this way, marshes constitute a sink of pollutants that not only disrupt ecosystem components but also could threaten the health of humans (HORWITZ *et al.*, 2012; RENDÓN *et al.*, 2019). Species inhabiting these ecosystems are not only facing high salt concentrations but also multiple anthropogenic pressures including metal(loid)s pollution (ANJUN *et al.*, 2014). These elements are one of the most toxic contaminants present worldwide due to their harmful effects on organisms and their environmental persistence. Metals can be taken up by plants and animals, biomagnified through the food chain, and in the last instance could affect human health (LI *et al.*, 2022).

Heavy metals, also named potentially toxic elements (PTE), are a natural component of the Earth’s crust, but there are also several anthropogenic sources introducing these elements into the environment such as urban and industrial sewages, mining deposit drainage, pesticides, fungicides, fertilizers, agriculture, aquaculture, among others (SOUZA *et al.*, 2018; LI *et al.*, 2022). Essential PTE such as copper (Cu), zinc (Zn), manganese (Mn), selenium (Se) and iron (Fe) have a biological role for organisms. However, essential elements become toxic when concentrations exceed a specific umbral. Non-essentials PTE such as cadmium (Cd), lead (Pb), arsenic (As), and mercury (Hg) do not have an identified role and are toxic even in very low concentrations (PANDEY and MADHURI, 2014).

Metals can produce sublethal effects on organisms at different levels: morphological, physiological, biochemical, and genomic. PTE are known to induce reactive oxygen/nitrogen species (RONS), which induce stress in cells. Upon exposure to metal ions different cellular signaling machinery activates cellular response (GARCÍA CAPARRÓS *et al.*, 2022). In order to counteract that effect, organisms are able to protect cellular components with biomolecules involved in metal transport and detoxification and with antioxidant enzymes (ROE *et al.*, 2021). In this sense, changes in biological markers (biomarkers) are considered useful and cost-effective

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tools to predict effects on population parameters, and community structure and for assessing ecological risks (ALJAHDALI and ALHASSAN, 2020; ROE *et al.*, 2021).

Given these considerations, it is crucial to include saltmarshes in environmental monitoring programs to assess their condition and ensure the continuation of the vital ecosystem services they provide. This study aims to review the state of knowledge on potentially toxic elements and their effects on relevant biological endpoints in saltmarshes globally, based on field monitoring studies published between 2018 and 2022. The primary goal is to compile and analyze the most studied PTE and the key species used as bioindicators.

MATERIAL AND METHODS

Data collection

This study employs an integrative review with a comprehensive approach, aimed at compiling and synthesizing relevant information from existing scientific research. A bibliographic search was conducted covering the period from 2018 to 2022, utilizing original scientific articles available on platforms such as Google Scholar, ScienceDirect, Scielo, PubMed and Frontiers. The goal was to gather and discuss literature published in the mentioned period on the accumulation of essential and non-essential metals in saltmarsh flora and fauna and their potential biomarkers under field conditions. The following descriptors were used: saltmarsh, metal, heavy metal, trace metal, potentially toxic element, bioindicator, biomarker, oxidative stress, biomonitoring.

Using the previously established inclusion criteria, 29 articles were identified, of which 6 were reviews. Following the screening, 23 articles were screened by abstract, 2 of which were excluded for being laboratory studies. Twenty-one (21) records were classified for full text analysis; due to eligibility, 5 articles were excluded because they were out of the scope and for not present concentration of metals in numbers, totaling 16 articles for inclusion in bibliometric analysis (Fig. 01).

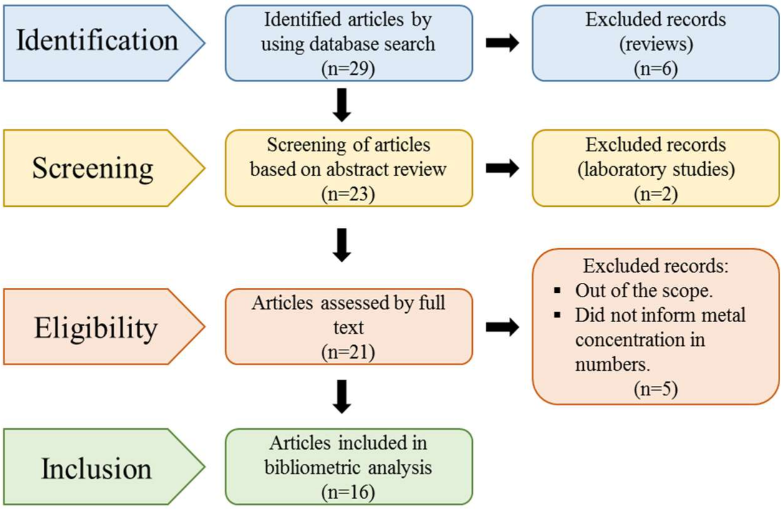

Figure 01: Flowchart for identification, screening, eligibility, and inclusion of articles.

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Statistical Analysis

Data was compiled into a table with Microsoft Word®, including authors, PTE analyzed, species and key findings. To identify trends, the number of articles published per year and by country was quantified without distinguishing between plants and invertebrates, and the absolute frequency was represented in a bar graph. The proportion of studies using a single organism type or both sentinel types was also calculated. The relative frequency of metals studied by organism type was assessed, and pie charts were used to display the results. Target tissues for metal quantification and studies using biomarkers were also identified. All graphs were made and analyzed using Microsoft Excel®.

RESULTS AND DISCUSSION

The literature review covering the period from 2018 to 2022 indicates that 16 studies were published on PTE in plants and invertebrates in saltmarshes under field conditions, with publication numbers ranging from 1 to 5 per year (Fig. 02a). Most studies are from Argentina (6) and Australia (5) (Fig. 02b). In Argentina, the most frequently studied elements were Cu, Cd, Cr, Zn, and Pb, while in Australia, the focus was on Cu, Cd, Zn, Pb and As.

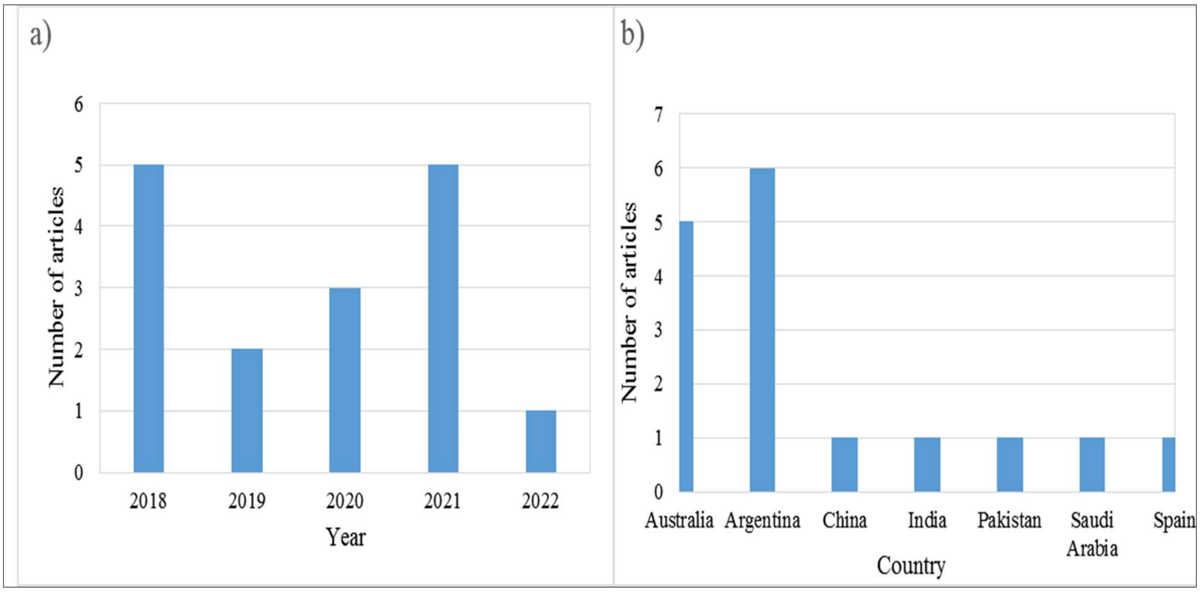

Figure 02: Number of publications in the period 2018-2022, per year (a) and per country (b).

Notably, the analysis of geographical distribution reveals distinct regional contamination patterns, with Cu, Cd, Zn, Pb and Cr emerging as the most evaluated PTE in saltmarshes. Four of the articles of Argentina studied the Bahía Blanca estuary in Buenos Aires province (BUZZI and MARCOVECCHIO, 2018; NEGRIN *et al*., 2019; SIMONETTI *et al*., 2018; TRUCHET *et al*., 2021). This area includes various harbors, cities and industrial complexes (such as oil, chemical, and plastic factories), that discharge poorly treated sewages into the estuarine waters, contributing

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to high levels of trace metals in sediments and local organisms. Additionally, port activities, including periodic dredging which mobilizes contaminated sediments, increase exposure risk. In the inner part of the estuary, rural areas and an artisanal fishing/recreational port also face these contamination pressures.

The two other studies from Argentina were focused on San Antonio Bay in Río Negro province (MARINHO *et al*., 2018; POLLICELLI *et al*., 2018), where passive mining wastes generated between 1960 and 1980 from Pb, Zn, Ag and V electrolysis has led to a persistent dispersion of metals (Pb, Cu, Zn and Cd). These residues continue to leach into the environment, contributing to metal contamination in the saltmarshes and highlighting the lasting ecological impacts of historical mining activities (GIARRATANO *et al*., 2016).

The five studies on PTE in Australian saltmarshes reveal distinct patterns of contamination that vary according to industrial activities and geographic locations. Lake Maquarie (LM), the most extensively studied and polluted estuary, is heavily impacted by historical Pb and Zn smelting that took place from 1897 to 2003 (VÅRHAMMAR *et al*., 2019; TRAN *et al*., 2020; STEIN *et al*., 2021; ALAM *et al*., 2022). Pollution in LM persists due to ongoing emissions near coal-fired power stations, which introduce Cd, Pb, Zn and other metals into the ecosystem. Steel smelting and industrial wastewater further contribute to contamination, with locations such as Cockle Creek and Fibe Island Wetlands showing particularly high levels of these metals in sediments. The most released metals are Cd, Cu, Pb and Zn, exceeding quality guidelines (ANZECC/ARMCANZ, 2000).

On the other hand, the Hunter Estuary, one of the world´s largest coal export port has created additional environmental pressures. The estuary has experienced contamination from a now-decommissioned steel plant, an aluminum smelter and various petrochemical industries. Historical drainage of wetlands for agricultural and industrial development has compounded the exposure of toxic metals, further degrading the area. Similarly, the Georges River estuary is affected by both urban and industrial pollution, containing elevated levels of As, Cu, Zn and Ni, primarily due to underground coal mining activities upstream (TRAN *et al*., 2020; ALAM *et al*., 2022).

Among different types of organisms, plants were the most widely studied. Twelve articles used halophytes as bioindicators, 3 used invertebrates, and only 1 research employed one species of both groups, halophyte and invertebrates (Fig. 03). Regarding metal studies using halophytes as bioindicators, most of them evaluated one species, while 3 articles compared the response of 3, 5, and 6 species of dominant halophytes. Among the former, there is *Spartina alterniflora* (3), different species of Juncus (3), *Sarcocornia quinqueflora*, shrub *Suaeda australis, Cressa truxillensis* and grass *Sporobolus virginicus*. The results showed a higher number of publications about seagrass plants in contrast with shrubs that were less representative.

In studies with plants, the most analyzed metals were Zn, Pb, and Cu, followed by Cd, then Cr and Mn, and, to a lesser extent As, Fe and Se, being Ni and Hg the least studied elements (Fig. 04). Most of the articles evaluated the accumulation of metal in above and ground tissues, some of them (6) distinguishing between leaves and stems. A notable pattern emerged, where halophytes typically exhibited species-specific metal accumulation, with higher concentrations

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generally found in below-ground tissues. Some studies considered seasonal variations; however, no consistent trend was observed.

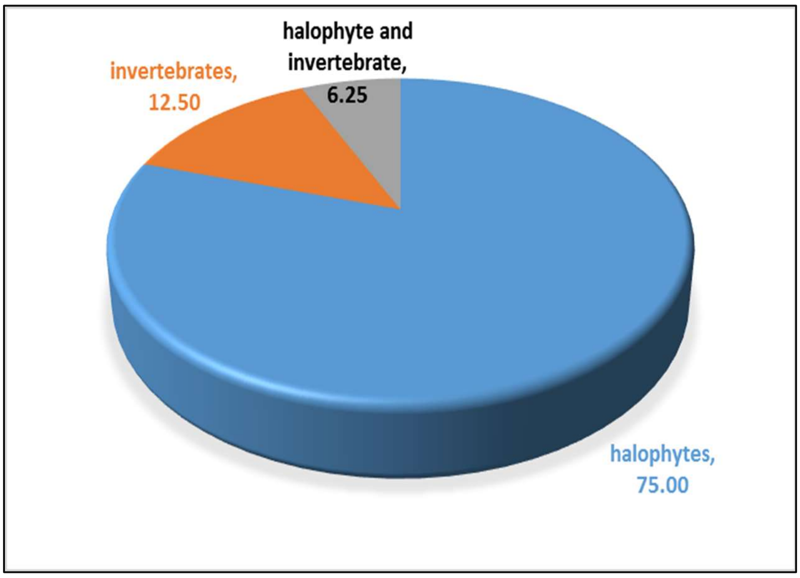

Figure 03: Percentages of articles that used halophytic plants, invertebrates and both types of organisms.

This review reveals that all of the PTE studies made on invertebrates were done in Argentina. Three of them used as bioindicators the crab *Neohelice granulata* and one used the mussel *Brachidontes rodriguezii*. Three out of four researchers measured the accumulation of metals in whole soft tissues and one in the hepatopancreas of crabs. The four reports evaluated the accumulation of Cd, Cu, Pb and Zn, four included Cr, Fe and Ni, meanwhile only two considered Mn (Fig. 04b). Physicochemical factors such as organic matter and pH modulate metal bioaccumulation, highlighting the importance of spatial analyses in identifying pollution sources and enhancing remediation strategies.

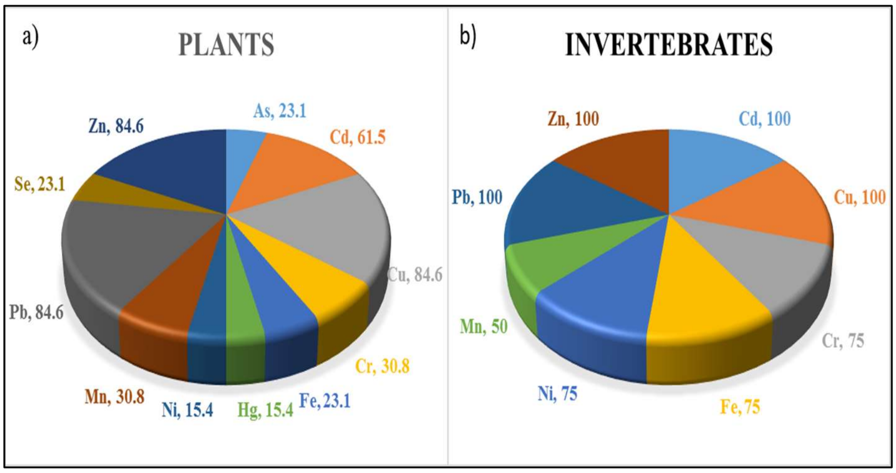

Figure 04: Relative frequency of each element analyzed in plants (a) and invertebrates (b).

Finally, concerning biomarkers as early warning signals to detect metal pollution only 2 studies employed them. One using the crab *N. granulata* as sentinel species evaluated the biochemical biomarkers metallothioneins (MT), catalase (CAT), glutathione-s-transferase (GST), hydrogen peroxide (H_2O_2) and proteins content (TRUCHET *et al.*, 2020). None of the biomarkers showed spatial differences. However, all of them showed higher values in winter, evidencing that biochemical responses were influenced by seasonality rather than by anthropogenic impacts. The other work considered changes in leaf shape of *Cressa truxillensis* as a morphometric tool to monitor soil pollution (POLLICELLI *et al.*, 2018). They did not find differences in sizes, but they did in shape being leaves more lanceolate close to the pollutants source and more oval on the farthest point. Furthermore, each search was examined for significant information, which is presented in Table 01.

Table 01: Articles included in the literature review (continued).

Authors	Potential toxic elements and species	Main findings
Alam *et al.* (2021a)	PTE: As species (inorganic As: III and V and organic As: MMA DMA), Se, Cu, Zn, Cd, Pb. Species: *Juncus acutus*	Metal accumulation root>aerial tissues. Cu>Zn>Pb>As>Se and Cd. AsIII and AsV were higher in roots, with limited translocation to culm tissues. Root metal(loid) concentration significantly predicted the sediment loading.
Alam *et al.* (2021b)	PTE: Zn, Cu, Cd, Pb, As, Se. Species: *Suaeda australis*	Root BCFs >1: Cu>Cd>Zn/Se; root BCFs <1: As/Pb; Stem TFs; 0.07–0.68: Zn>Cd>Se>Cu/Pb/As; Leaf TFs ≥ 1: Se>As>Pb/Cd>Zn/Cu. Roots could be good bioindicators of Zn and Cu and linear relationships between sediment and root for Zn and Pb were observed. *S. australis* has a good phytostabilization efficiency as most metal(loid)s, and a high accumulation of Se in stems and leaves.
Buzzi and Marcovecchio (2018)	PTE: Cd, Cu, Pb, Zn, Ni, Cr. Species: *Brachidontes rodriguezii*	Two coastal areas were studied. Higher concentrations of some heavy metals (e.g., Cd, Cr, Ni) were found in mussels collected at Pehuen-Có, while no spatial differences were observed in sediments. The two sites were classified between the class unpolluted and moderately polluted and between low and medium pollution because of the high Cd, Cr, and Cu concentrations detected.
Chen *et al.* (2018)	PTE: Cr, Pb, Cu, Zn, Mn. Species: *Spartina alterniflora*	Pattern of accumulation: root>leaf>stem, Mn>Zn>Pb>Cu>Cr. Seasonal trend in roots and stem: high in summer and fall, low in winter and spring. Dead roots, stems, and leaves release a proportion of heavy metals into the surrounding environment every year. *Spartina alterniflora* can be cropped at an appropriate time to remediate contaminated soil.
Garcia-Ordiales *et al.* (2020)	PTE: Hg. Species: *Juncus maritimus*	Most of the absorbed Hg (76.2 to 97.3%) was retained in the root system, phytostabilising the element. *J. maritimus* accumulates a high concentration of Hg in its organs, mainly in the buried parts, with enrichments from 3 to 24 times the bioavailable Hg concentration detected in the sediments. This species could be used to stabilize Hg holding it in the root system which could be useful in phytoremediation and phytostabilisation of saltmarsh areas contaminated by Hg.

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Table 01: Articles included in the literature review (continued).

Authors	Potential toxic elements and species	Main findings
Ibraheem *et al.* (2021)	PTE: Cd, Cr, Co, Cu, Fe, Hg, Mn, Ni, Pb, Zn. Species: *Tamarix nilotica, Heliotropium crispum, Zygophyllum coccineum, Halopeplus perfoliata* and *Avicennia marina*	High levels of salinity and PTE were associated with higher foliar levels of malondialdehyde, particularly in *A. marina* and *Z. coccineum*. The bio-concentration ratio revealed hyperaccumulating potentials of PTE by the tested halophytes. *Zygophyllum. coccineum* showed effective accumulation of Co, Fe, Pb and *T. nilotica* of Cu, Cd, and Zn. *Heliotropium perfoliata* had a higher accumulation of Cr and Hg. *Avicennia marina* accumulated significant amount of Hg, Cd, Zn and *H. crispum* leaves accumulated the highest Ni levels.
Joshi *et al.* (2020)	PTE: Zn, Fe, Mn, Cu, Cr, Cd. Species: *Suaeda monoica, Tamarix indica* and *Cressa critica*	Pattern of accumulation among species: *S. monoica* > *T. indica* > *C. critica*. For all species the general pattern of storage in leaves>stems following Fe>Mn>Zn>Cu>Cd>Cr. BCF: *S. monoica* > *T. indica* > *C. critica* both leaves and steam Cd>Cr>Zn>Cu>Mn>Fe. Biomarkers: osmoprotective compounds (protein content, proline, soluble sugar), antioxidant activity (SOD, FRAP and DPPH) and total phenolic content (TPC).
Marinho *et al.* (2018)	PTE: Cu, Cd, Fe, Pb, Zn. Species: halophyte *Spartina* spp. and the crab N*eohelice granulata*	In *Spartina*: Cd<Cu and Pb<Zn<Fe belowground tissues > aboveground tissues (this could be related to a compartmentation mechanism). *Neohelice granulata* in soft tissues: Cd<Pb<Zn<Cu<Fe. This crab was a useful biomonitor for Pb, but not for the other metals.
Mujeeb *et al.* (2021)	PTE: Mn, Zn, Pb, Cr. Species: *Aeluropus lagopoides, Arthrocnemum macrostachyum, Atriplex stocksii, Avicennia marina, Cressa cretic*a and *Suaeda fruticosa*	Halophytes showed spatial and seasonal variations in metal accumulation. *Aeluropus lagopoides* may be used for phytoremediation of Mn, Zn, Pb, and Cr of highly polluted marshes. *Atriplex stocksii, C. cretica, A. macrostachyum* and *S. fruticosa* potential bioindicators of Pb and Zn for phytoremediation of mud flats and wetland species *A. marina* for cleaning Pb polluted mangrove swamps.
Negrin *et al.* (2019)	PTE: Pb, Ni, Cu, Zn. Species: *Spartina alterniflora*	Zn> Cu > Pb ≥ Ni. All metals were higher in the belowground tissues. Regarding the above ground tissues, the levels of metals were usually higher in dead tissues than in live ones. *Spartina alterniflora* was efficient in metal accumulating, which implies that it could be used for phytoremediation purposes.
Pollicelli *et al.* (2018)	PTE: Zn, Pb, Cu. Species: *Cressa truxillensis*	Biomarker: leaf shape as biomarker of stress by contamination of metals in plants associated with marshes. The leaves of the polluted site (high Zn>Pb>Cu) were associated with lanceolate leaves shapes, while in cleaner sites showed oval shapes.

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Table 01: Articles included in the literature review (continued).

Authors	Potential toxic elements and species	Main findings
Simonetti *et al.* (2018)	PTE: Cd, Cu, Pb, Ni, Zn, Mn, Cr, Fe. Species: *Neohelice granulata*	Concentrations above the detection limit in soft tissues of male and female crabs for all metals except Pb and Cr. BCF > 1 for Cd, Cu, and Zn, indicating that these metals were accumulated and biomagnified. BCF values < 1 were found for the rest of the metals (Mn, Ni, and Fe). *Neohelice granulata* would play a major role in the transference of pollutants to higher trophic levels.
Stein *et al.* (2021)	PTE: Cu, Zn, As, Se, Cd, Pb. Species: *Juncus kraussii*	Zn, As, Se and Pb accumulated from sediment to root linearly. As and Se were the only metal(loid)s to show linear uptake into the culm. Cu and As showed active uptake into the culms. *Juncus kraussii* may be a candidate for hyperaccumulator of Zn and Pb.
Tran *et al.* (2020)	PTE: Zn, Cu, Pb, Cd. Species: *Sporobolus virginicus*	Of the evaluated estuaries, Lake Macquarie was the most contaminated, with high levels of contamination by sediment metals, Hunter had the lowest wet load, essential metals such as Cu and Zn were more mobile accumulated in the roots in higher concentration. In the translocation of the roots to the culm and culm of the leaves, these tissues would be unsuitable for bioindication, *S. virginicus* sequesters most of the metals in the roots.
Truchet *et al.* (2021)	PTE: Cd, Cu, Pb, Cr, Zn, Mn, Ni, Fe. Species: *Neohelice granulata*	Fe ≥ Cu > Zn > Mn > Cd; meanwhile Cr, Ni and Pb were below detection limit. Biomarkers: MT, CAT, GST and H_2O_2 were higher in winter. The biomarkers only suggest seasonal variations and not heavy metal pollution. *Neohelice granulata* was ruled out by seasonality rather than by anthropogenic impacts.
Vårhammar *et al.* (2019)	PTE: Zn, Cu, Pb, Cd. Species: *Sarcocornia quinqueflora*	BCF ≥ 2 for Zn and Cd; BCF ≤ 1 for Pb; root:non-photosynthetic stem (PS) TF < 1 for Zn, Cu, Pb and Cd; non-PS:PS TF > 1 for Zn and Cu. Appropriate bioindicator tissues to assess relative sediment metal load and bioavailability. Roots for all metals and the non-PS stem for Pb and Cd exhibited significant linear relationships.

CONCLUSIONS

This review highlights the wide use of halophytic plants as a good sentinel organism for monitoring saltmarsh metal contamination. However, there is limited literature exploring the oxidative stress caused by PTE on plants and even more on invertebrates. Thus, targeted studies on a species-by-species basis that allow correlating early biochemical changes to later effects for early detection utility are still missing. Specific biomarkers that can be mechanistically linked to the adverse effects of PTE have not yet been confirmed and thus more efforts are needed to identify the most appropriate biomarkers. Moreover, it is important to determine the levels of trace metals in different taxa of saltmarsh able to have sublethal effects for inclusion in regulatory guidelines. Future studies in field conditions, where complex interactions exist among the different taxa of organisms, and between organisms and the environment are still needed to clarify unexplored facts in the current context. We highlight the need of this type of research to advance in the comprehension of the impact of PTE on coastal saltmarsh to produce guides for potential interventions and to protect biodiversity. Such an integrated and systematic approach will allow to predict ecological consequences and enable the design of accurate strategies for monitoring saltmarsh. Finally, obtained data will be useful to develop improved environmental and health policy.

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