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Ecological Risk Assessment of Potential Toxic Elements in Salt Marshes on the East Coast of the Red Sea: Differential Physiological Responses and Adaptation Capacities of Dominant Halophytes

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Citation: Ibraheem, F.; Al-Hazmi, N.; El-Morsy, M.; Mosa, A. Ecological Risk Assessment of Potential Toxic Elements in Salt Marshes on the East Coast of the Red Sea: Differential Physiological Responses and Adaptation Capacities of Dominant Halophytes. *Sustainability* **2021**, *13*, 11282. <https://doi.org/10.3390/su132011282>

Academic Editor: Adriana Najar-Rodriguez

Received: 31 August 2021

Accepted: 4 October 2021

Published: 13 October 2021

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Abstract: The impact of the mutual interactions between salinity and the phytoavailability of potential toxic elements (PTEs) on the adaptation of halophytes in their natural habitat is complex and far from clear. Herein, we aimed to evaluate salinity- and PTE-induced oxidative stress in selected halophytes and the antioxidant responses of these plants. For that, five salt marshes were selected, and the physiological responses of dominant halophytes (*Tamarix nilotica*, *Heliotropium crispum*, *Zygophyllum coccineum*, *Haloepplus perfoliata*, and *Avicennia marina*) were evaluated against the physicochemical features of their rhizosediments. The tested locations varied in their physicochemical properties and showed various levels of salinity stress and a low fertility status. Distinct variations in ten PTE concentrations were recognized among locations and within plants, with Cr and Co showing the highest ecological risk indices. The high levels of salinity and PTEs were associated with higher foliar levels of malondialdehyde, particularly in *A. marina* and *Z. coccineum*. The bio-concentration ratio revealed hyperaccumulating potentials of PTEs by the tested halophytes. *Z. coccineum* showed effective accumulation of Co, Fe, and Pb, while *T. nilotica* exhibited effective accumulation of Cu, Cd, and Zn. *H. perfoliate* had higher accumulation of Cr and Hg, whereas *A. marina* accumulated a significant amount of Hg, Cd, Zn, and Mn. *H. crispum* leaves accumulated the highest Ni levels among the tested halophytes. Altogether, our results highlight the potential risk of pollution of the tested areas with PTEs and the efficient physiological adaptation of each of the tested halophytes as a unique biological system. They also reflect the high capabilities of the tested halophytes as phytoextractors of their corresponding PTEs and their potential as efficient tools for phytoremediation of salt- and PTE-affected lands.

Keywords: salt marshes; salinity; halophytes; potential toxic elements; oxidative stress; phytoremediation

1. Introduction

Coastal and inland salt marshes are important ecological habitats with unique soil physicochemical properties and halophytic vegetation [1]. In such habitats, soils usually contain high levels of salinity along with other physicochemical properties that significantly impact metal phytoavailability and accessibility across biological systems. These soils also act as sinks for PTEs, which are significant environmental pollutants. These elements are usually released as a result of various anthropogenic activities such as industrial waste, domestic waste dumps, oil spilling, rapid urbanization, marine construction, and agricultural practices [2–5]. The eastern coast of the Red Sea in Saudi Arabia extends for about 1700 km

from Yemen to the border with Jordan and represents a unique arid and semiarid habitat. The severe climatic conditions (high temperature, limited precipitation, and increased evapotranspiration), the oligotrophic nature of the soil, and the high potential release of PTEs from oil trading and other anthropogenic activities exacerbate the deleterious effects of salinity and PTEs on halophytes inhabiting these areas [6–8]. Such conditions highlight the potential risk of soil contamination and potentiate the importance of continuous monitoring of soil and plant responses in the region. Therefore, monitoring the levels of PTEs has been considered of great environmental, ecological, nutritional, and toxicological concern [9,10].

In higher plants, high levels of salinity and heavy metals induce various detrimental effects on growth and physiological processes such as osmotic imbalance, ion toxicity, disruption of the carbon balance, nutrient deficiency, and oxidative stress [11–13]. Such detrimental effects restrict growth in salt marshes to halophytic plants which have natural capabilities to tolerate salinity levels from 40 to 200 mM NaCl or even more [14–16]. In addition, halophytes can take up and accumulate high levels of PTEs with various uptake/tolerance capacities [12,16]. These unique plants belong to various taxonomic plant families, and their tolerance capabilities depend on plant species, metal type/concentration, and growth stage [17]. Moreover, halophytes are successfully adapted to other abiotic stress conditions occurring simultaneously in their harsh environments [15]. To tolerate these severe conditions, halophytes employ numerous combinations of morphological and physiological adaptations [18]. At the leaf level, various halophytes have special structures such as trichomes, salt glands, or succulent tissues to sequester or alleviate toxic ions and thus balance ion concentrations in plant tissues [19,20]. They have also developed specific mechanisms for maintaining a balance between the generation of reactive oxygen species (ROS) and their detoxification mechanisms under salinity, PTEs, and their associated stresses in arid and semiarid habitats [21].

A common response among most halophytes is the generation of ROS, which is an indispensable consequence of any type of salinity- and PTE-induced stress such as water deficit, and ionic and osmotic stresses [16,22]. ROS are mainly produced as a result of a stress-induced reduction in the electron transport chain in both chloroplasts and mitochondria or as a consequence (particularly H_2O_2) of oxidation of glycolate to glyoxylic acid in peroxisomes [23,24]. In fact, H_2O_2 is the most stable among ROS with a half-life of minutes compared to $<1\ \mu s$ for other ROS such as singlet oxygen (1O_2), superoxide ($O_2^{\bullet-}$), and hydroxyl ions ($OH^{\bullet}$), and thus only H_2O_2 has the ability to diffuse among neighboring cellular components and cells [25,26]. In the presence of transition metals such as Fe, Cu, and Mn, and through the Haber–Weiss and Fenton reactions, H_2O_2 can generate highly toxic hydroxyl radicals ($OH^{\bullet}$), which activates ion transport via the plasma membrane such as Ca^{2+} influx and K^+ efflux [27,28]. ROS act as stress sensors at their optimum cellular levels; however, beyond such levels they are toxicity inducers [29,30]. Their toxic effect occurs via induction of peroxidation of lipids in cellular membranes, protein denaturation, DNA damage, pigment breakdown, carbohydrate oxidation, and disturbance of enzyme activity [27,31,32]. In general, halophytes have developed an efficient antioxidant system that maintains cellular ROS levels below the phytotoxic threshold, which varies significantly among halophytes depending on their differential cellular tolerance against ROS [22]. Under increased salinity and other associated stresses, the antioxidant protective mechanisms can become insufficient to counter the oxidative damage [18]. For instance, photosynthesis is one of the most sensitive physiological processes to salinity- and PTE-induced ROS since the photosynthetic photosystem (PSI and PSII) reaction centers are the main sites for ROS production in chloroplasts [33,34]. Under salinity and other stresses, the level of ROS increases and the overall photosynthetic activity and, consequently, carbon assimilation decreases because of the detrimental effects of ROS on thylakoid membrane organization and the modulation of photosystem II (PSII) activity in chloroplasts [32]. Therefore, monitoring photosynthetic pigments and key photosynthetic products has been

an effective biomarker in anticipating stress-induced events and their consequences on carbon assimilation at the organism level [35].

In the current investigation, the study area included five Saudi salt marshes on the east coast of the Red Sea which is a key maritime route for the world oil and goods trade. These salt marshes are directly and/or indirectly affected by pollutants derived from various anthropogenic activities developing along the Red Sea. In addition, the selected salt marshes are close to the oldest maritime port in Saudi Arabia (AlQunfodah port). These factors, along with the high salinity and hyperarid and oligotrophic environment would significantly affect the chemical features and the levels of PTEs in the soil of these salt marshes. Such effects have not been previously reported in the selected area. Interestingly, these salt marshes are inhabited by halophytes that thrive in such harsh conditions. The successful adaptation of these halophytes suggests that these plants might have a strong potential as phytoextractors of PTEs and thus provide efficient tools for phytoremediation. Little is known about both the impact of the above pollution-inducing factors on soil chemical features and their consequences on the growth and physiology of halophytic plants in the area. Therefore, these salt marshes provide a unique natural system to investigate the degree of the anthropogenic activity-induced contamination by PTEs such as Cd, Cr, Co, Cu, Fe, Hg, Mn, Ni, Pb, and Zn and the differential physiological responses, if any, of the dominant halophytic plants in the area. In addition, the current study will shed light on the potential of these halophytes as phytoextractors of contaminant elements. Moreover, this study provides necessary knowledge for making future decisions on the management and conservation of the area. Therefore, the objectives of the current study were to: (i) study the physicochemical properties of five selected salt marsh soils located at various proximities to the eastern Red Sea coast (south Saudi Arabia), (ii) assess the potential contamination hazard of PTEs in soils of the studied locations and their accumulation in the dominant halophytes, and (iii) assess the impact of the mutual interaction between current rhizospheric soil conditions and possible PTE contamination on physiological responses of the dominant halophytes.

2. Materials and Methods

2.1. Study Area and Description of Selected Plant Species

Five salt marsh locations representative of the common environment of the Red Sea coast were selected and designated as L1, L2, L3, L4, and L5 according to their proximity to the coast. L1 and L5 were the farthest and the closest to the seacoast, respectively. From each location, the most dominant plant species was identified according to [36,37] and used for downstream analysis. These plants belong to different taxonomic families and included *Tamarix nilotica* (Tamaricaceae), *Heliotropium crispum* (Boraginaceae), *Zygophyllum coccineum* (Zygophyllaceae), *Halopeplus perfoliate* (Amaranthaceae), and *Avicennia marina* (Acanthaceae). All these plants are perennials. Additionally, all these plants are chamaephytes, except for *Tamarix nilotica*, which has a phanerophyte life form.

2.2. Collection of Plant and Soil Samples

Five individual plants from each dominant species of interest in each location were selected, labeled, and guarded. Three replications of leaf samples were collected from each plant, washed thoroughly using sterilized water, and divided into two sets. Depending on the downstream analysis, samples were either dropped immediately in liquid N or brought on ice to the laboratory for oven drying (70 °C until constant weight) for elemental measurements. Corresponding rhizospheric soil samples were collected by uprooting plants, removal of coarse soil particles, and collection of soil particles that were tightly adhered to the root. The collected soil samples were stored in polyethylene bags, transferred to the laboratory, air dried at room temperature, sieved at 2 mm, and stored for soil physical and chemical analysis.

2.3. Soil and Plant Elemental Analyses

2.3.1. Soil Analysis

Soil physical and chemical analyses were carried out using standardized methods [38]: particle size distribution by sieve analysis [39], soil porosity calculation from values of bulk and solid phase densities, water holding capacity by the standardized gravimetric determination of soil moisture content, hydraulic conductivity by the Darcy equation, total concentration of calcium carbonate using the gasometric determination method after adding HCl solution (6M), soil pH value of 1:2.5 distilled water suspension using a Jenway 3505 pH/mV/Temperature Meter, and electrical conductivity of 1:2.5 soil/water extract by a HANNA (HI9835) EC meter. Water-soluble ions were determined in a soil/water extract: Ca^{2+} , Mg^{2+} , and K^{+} using inductively coupled plasma (iCAP™ 7000 Plus Series ICP-OES, Thermo Scientific™, Waltham, MA, USA), Na^{+} using a Jenway PFP7 flame photometer, CO_3^{2-} and HCO_3^{-} by titration with standardized H_2SO_4 solution, and Cl^{-} by titration with standardized AgNO_3 solution. Concentrations of total organic elements (C, H, N, and S) were determined by a CHNS analyzer (Thermo Scientific Flash 2000). The available concentration of PTEs was determined by ICP in the extraction of diethylene tri-amine penta acetic acid (DTPA).

2.3.2. Plant Elemental Analysis

Dried leaf samples were ground to a homogenous fine powder and used for determination of organic and inorganic elements. Organic element (C, N, H, and S) concentrations in dry biomass samples were determined following the dry combustion technique using a CHNS analyzer (Thermo Scientific Flash 2000). For determination of inorganic elements, oven-dried samples were digested using aqua regia solution (HCl/ HNO_3 mixture, 3:1 v/v) in a microwave digester (Milestone MLS 1200 Mega), and PTE concentrations were determined using ICP-OES.

2.4. Ecological Risk Assessment

2.4.1. Geo-accumulation Index (I_{geo})

The geo-accumulation index (I_{geo}) is widely used to evaluate the contamination degree of PTEs either in A or O horizons as referenced to their geochemical background values in carbonate rocks [40].

$$I_{\text{geo}} = \log_2 \left[\frac{C_n}{1.5 \cdot B_n} \right] \quad (1)$$

where C_n is the concentration of PTEs in the rhizospheric soil layer, and B_n is the geochemical background concentration of PTEs in the Earth's crust. The geochemical background values were multiplied by 1.5 in order to minimize the natural fluctuations in the lithologic variations [41]. The I_{geo} index is categorized into six classes to interpret the corresponding contamination intensities of PTEs (Supplementary materials Table S1).

2.4.2. Enrichment Factor (E_f)

To quantify the potential anthropogenic impact on soil contamination, the enrichment factor (E_f) of PTEs was calculated in relation to elements with a low variability of occurrence (LV) as a reference in both the tested and geochemical background samples [42].

$$E_f = \frac{\left(\frac{C_n}{LV} \right)_{\text{Sample}}}{\left(\frac{C_n}{LV} \right)_{\text{Background}}} \quad (2)$$

where C_n is the PTE concentration in the soil, and LV is the reference element concentration in the examined environment (Al).

Typical reference elements are Al, Fe, Mn, and Rb according to the literature [43]. This investigation used Al as a reference lithogenic element based on its low vertical mobility and high stability in the rhizosphere.

2.4.3. The Contamination Factor (C_F)

The contamination factor (C_F) was quantified by dividing the PTE concentrations in the studied locations (C_x) by the background values (C_b) according to the equation adopted by [44].

$$C_F = \frac{C_x}{C_b} \quad (3)$$

The contamination factor (C_F) consists of four classes as illustrated in Supplementary materials Table S1.

2.4.4. Potential Ecological Risk Index E_r^i

This criterion was introduced to evaluate the contamination load according to the toxicity coefficient values of PTEs [44].

$$E_r^i = T_r^i \times \frac{C_i}{C_0} \quad (4)$$

where T_r^i is the toxicity coefficient of PTEs (Cd = 30, Cr = 2, Co = 5, Cu = 5, Fe = 1, Hg = 40, Mn = 1, Ni = 5, Pb = 5, and Zn = 1), C_i is the concentration of PTEs in the investigated soil, and C_0 is the background value of the PTEs.

2.4.5. The Pollution Load Index (PLI)

The integrated pollution load index (PLI), which has four classes (Supplementary materials Table S1), was introduced by [45] as the root of the contamination factor of PTEs to provide an understanding of their potential deteriorative impact.

$$PLI = \sqrt[n]{C_{F1} \times C_{F2} \times \dots \times C_{Fn}} \quad (5)$$

2.4.6. The Contamination Degree (CD) and the Modified Degree of Contamination (mCD)

These integrated indices were introduced by [44] to simplify the load of contamination according to the following equations:

$$CD = \sum_{i=1}^n C_F^i \quad (6)$$

$$mCD = \frac{\sum_{i=1}^n C_F^i}{n} \quad (7)$$

where C_F is the calculated contamination factor. The contamination levels of these indices are illustrated in Supplementary materials Table S1.

2.5. Plant Physiological Analysis

2.5.1. Determination of Oxidative Damage in Leaves

Oxidative damage in leaves was assessed via monitoring the levels of both hydrogen peroxide (H_2O_2) and malondialdehyde (MDA). For H_2O_2 , 500 mg of frozen leaf tissues was crushed into a fine powder in liquid nitrogen, homogenized in 5 mL cold potassium phosphate buffer (50 mM potassium phosphate, 1 mM EDTA, pH 7.5), and centrifuged at 4000 rpm for 15 min at 4 °C. The supernatant was then collected and used for the determination of H_2O_2 spectrophotometrically using a hydrogen peroxide assay kit (Bio-diagnostic, HP 25, Giza, Egypt) following the manufacturer's instructions. Leaf MDA concentration was determined spectrophotometrically as described previously [46] with slight modification. Quantities of 500 mg of frozen leaf tissues were homogenized in 5 mL of 10% trichloroacetic acid (w/v) and centrifuged at 4000 rpm for 10 min at 4 °C. Aliquots of 500 μ L of the supernatant were mixed with 500 μ L of 0.6% (w/v) thiobarbituric acid and the mixture was incubated at 95 °C for 15 min, cooled in an ice bath, and centrifuged at 4000 rpm for 10 min at 4 °C. The absorbance of the resultant pink color was measured at

450, 532, and 600 nm. The MDA content was estimated using the extinction coefficient of 155 (nmol/L/cm) and expressed as nmol g⁻¹ DW.

2.5.2. Estimation of Antioxidant Substances

Flavonoids and phenolic compounds in 100 mg of powdered leaves were extracted three times in acetone to remove chlorophyll, dried, and resuspended in distilled water. Total flavonoids were determined spectrophotometrically at 410 nm using AlCl₃ and quercetin as a standard [47]. Total phenolics were determined spectrophotometrically at a wavelength of 760 nm using the Folin–Ciocalteu method and gallic acid as a standard [48]. The concentrations of total flavonoids and total phenolics were expressed as mg/g DWT.

2.5.3. Determination of Photosynthetic Pigments

Photosynthetic pigments were extracted by grinding 50 mg of frozen leaves in cold 80% aqueous acetone, and the concentrations of chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoids (Cars) were determined spectrophotometrically at 663.2, 646.8, and 470.0 nm according to [49]. Pigment concentrations were expressed as mg/g FWT.

2.5.4. Determination of Carbohydrate Fractions

Quantities of 100 mg powdered dry leaves were extracted with 80% ethanol, and the ethanolic extracts were completed to specific volumes and used for the spectrophotometric determination of total soluble sugars (TSS) and sucrose at 620 nm using anthrone reagent [50,51]. Carbohydrate fractions were calculated using standard curves of pure glucose and sucrose and expressed as mg/g DWT.

2.6. Quality Control and Statistical Analysis

Chemical reagents of analytical grade (Merck-Darmstadt, Germany) were used without extra purification. Preparation of chemical solutions was carried out using deionized water (18.2 MΩ) (Nanopure water, Barnstead). Soil and plant analyses were carried out by an ISO/IEC 17025 accredited laboratory to ensure data verification. All soil and plant analyses were carried out under constant temperature (25 ± 0.5 °C) with appropriate replications, controls, and blanks. Certified soil reference materials (BIPEA, France) were used to optimize the quality control of soil analysis. The accuracy of organic element measurements was verified using the BBOT standard (C₂₆ H₂₆ N₂ SO₂; C = 72.52%, H = 6.09%, N = 6.51%, and S = 7.44%). In addition, the accuracy of inorganic element determination was verified using standard calibration solutions (R² ≥ 0.99). The recovery values of elemental determination oscillated in the range between 93.25 and 103.35%, and the precision of data was optimized through adjusting the maximum relative standard deviation (RSD) at ≤5%. Limits of detection (LODs) for inorganic elements (μg L⁻¹) were: As (33.12), B (25.32), Ba (26.52), Ca (95.2), Cd (53.43), Cr (31.75), Cu (20.76), Fe (46.82), Hg (23.54), K (26.7), Li (34.63), Mg (128.9), Mn (29.73), Ni (40.17), Pb (54.93), Ti (55.41), V (26.18), and Zn (40.95).

Standard deviations were calculated using Microsoft Excel software (version 2010, Microsoft Corporation, Redmond, Washington, DC, USA). Correlation analysis was performed using CoStat (Version 6.303, CoHort, Berkeley, CA, USA, 1998–2004) at significance levels of 0.05 and 0.01 to test the relationship among selected soil physicochemical characters and the extractability potentials of PTEs.

3. Results and Discussion

Despite the harsh climatic conditions and the unique location of the selected salt marshes in the current study (proximity to the oldest port in Saudi Arabia, location on the world's key maritime route of the oil and goods trade, intensive construction, and industrial projects), very little information about the extent of possible soil pollution induced by the above factors and its consequences on the physiology of the dominant plants is available. Therefore, the impact of the interplay among the above factors on

chemical properties and the levels of PTEs in soils of these salt marshes was investigated. In addition, the physiological responses of the dominant plants were tested and compared. Further, the phytoremediation potential of the dominant halophytes was monitored to extend the ecological significance of the study.

3.1. Soil Physicochemical Analysis

All soils in the investigated locations were sandy in texture (*Typic Torripsamment*) according to the USDA soil taxonomy [52]. Particle size distribution comprised 60–66% coarse sand fraction in L₁–L₄ (Table 1). The coarse sand fraction was lower in L₅ (38.4%). Contrarily, its silt and clay fraction was the highest among the studied locations (10.7%). The high coarse sand fraction increased soil porosity (~45.1%) and the macro-pore volume of the soil matrix. Therefore, the studied soils may not impose significant restrictions on the root growth of the tested halophytes. The soil organic matter content in most locations was very low and was below the detection limit in L₁ and L₂. This low organic matter content is mainly attributed to the fast decomposition of soil organic matter due to the high temperature, low precipitation, and high aeration in the soil matrix [53]. Unlike other studied locations, the coastal location (L₅) recorded the highest organic matter content (2.69%), which is mainly attributed to the belowground accumulation of plant litter derived from mangrove forests under waterlogged conditions. In addition, this coastal location might accrete plant litter and solutes as the sea level rises, which provides a continuous accumulation and burial of soil organic matter [54]. The water holding capacity of soils in most locations was very low (32–35%) due to the low colloidal content (clay and organic matter, in particular) in the soil matrix [55,56]. However, the higher content of soil organic matter in L₅ improved its water supply potential and maximized its water holding capacity (40.12%). This finding is further supported by the data of soil hydraulic conductivity, which show high values in the L₁–L₄ locations (13.39–16.76 cm ha^{−1}). Meanwhile, L₄ and L₅ showed lower conductivity values (2.99 and 0.79 cm ha^{−1}, respectively). The total concentration of CaCO₃ varied among locations (Table 1; 0.55, 1.25, and 2.44% for minimum, median, and maximum concentrations, respectively). The low CaCO₃ concentrations in L₄ and L₅ (0.79 and 0.55%, respectively) could be attributed to the continuous dissolution by the tidal currents. The studied soils were all alkaline in nature with pH values ranging from 7.76 to 8.44 (Table 1). Salinity values varied greatly among locations (2.48–12.14 dSm^{−1}) with noticeable higher values in the coastal locations (L₃, L₄, and L₅). The high EC values in the tested arid saline habitats are shaped by interplay among metrological data in such arid conditions (high temperature, low precipitation, and high rate of evaporation, Supplementary Materials Table S2) along with the high salt contents derived from the Red Sea. The increased rate of evaporation that exceeds freshwater inputs can lead to hypersaline conditions due to the high accumulation of salts in the soil matrix [54]. Soil salinity in low-lying coastal locations was significantly higher than other locations due to the periodic inundation by the tidal water. However, other non-coastal locations might receive freshwater inputs from precipitation that can leach the accumulated salts. Water-soluble CO₃^{2−} was not detected since calcium carbonate was the predominant carbonate form in the studied locations. However, Cl[−] and HCO₃[−] were the major anions in soils at different locations (Table 1). The HCO₃[−] concentration was higher than that of Cl[−] in L₁ and L₂. The concentration of Cl[−] anions, however, was higher in the other locations. These results are in harmony with the data of soil salinity, which showed higher EC values in L₃, L₄, and L₅ since the chlorinated soil solutions often induce a higher ionic strength than bicarbonated solutions [57]. The median concentration of dominant cations was ranked as Ca²⁺ > Na⁺ > Mg²⁺ > K⁺.

Table 1. Some Physical and Chemical Analyses of the Experimental Soils.

Locations	Soil Physical Properties										Soil Chemical Properties								
	Particle Size Distribution (%)						Porosity	WHC	HC	EC	pH	CaCO ₃	Water Soluble Cations and Anions Concentration (cmol kg ⁻¹)						
	2–0–1.85	1.85–0.425	0.425–0.25	0.25–0.125	0.125–0.075	<0.075	Texture (%)	(%)	K, cm h ⁻¹	ds m ⁻¹		(%)	HCO ₃ ⁻	Cl ⁻	Ca ²⁺	Mg ²⁺	K ⁺	Na ⁺	
L ₁	2.59	2.21	6.09	55.01	26.73	7.37	Sandy	40.05	32.08	13.39	3.74	8.14	2.44	6.25	4.46	2.91	0.72	0.32	0.89
								± 3.66	± 2.85	± 1.29	± 0.34	± 0.4	± 0.28	± 0.51	± 0.41	± 0.33	± 0.17	± 0.07	± 0.13
L ₂	3.04	5.21	6.88	49.65	30.66	4.57	Sandy	48.15	30.12	13.65	2.46	8.04	1.87	6.17	2.90	3.13	0.74	1.45	0.16
								± 4.51	± 2.56	± 1.30	± 0.25	± 0.32	± 0.25	± 0.50	± 0.29	± 0.39	± 0.14	± 0.22	± 0.02
L ₃	2.04	2.37	4.14	57.23	29.70	4.53	Sandy	39.29	31.05	16.76	9.94	7.71	1.25	9.75	22.42	5.10	2.10	0.87	2.50
								± 3.73	± 2.76	± 2.15	± 1.04	± 0.33	± 0.09	± 0.86	± 2.15	± 0.52	± 0.34	± 0.15	± 0.33
L ₄	8.35	9.69	6.04	40.23	31.47	4.21	Sandy	43.74	35.19	2.99	12.10	7.96	0.79	4.20	53.38	4.35	4.68	1.30	7.58
								± 4.13	± 3.35	± 0.15	± 1.22	± 0.33	± 0.11	± 0.41	± 4.28	± 0.40	± 0.61	± 0.24	± 0.52
L ₅	3.94	11.01	8.42	14.89	41.16	20.58	Sandy	49.89	40.12	0.79	9.92	8.27	0.55	6.07	22.04	1.58	3.04	1.19	2.81
								± 4.55	± 3.70	± 0.08	± 1.02	± 0.41	± 0.05	± 0.62	± 2.30	± 0.21	± 0.49	± 0.19	± 0.29

Values are the average values of three replications $\pm$ standard deviations. L₁ (soil colonized with *T. nilotica*), L₂ (soil colonized with *H. crispum*), L₃ (soil colonized with *Z. coccineum*), L₄ (soil colonized with *H. perfoliata*), and L₅ (soil colonized with *A. marina*): WHC (water holding capacity), HC (hydraulic conductivity), and EC (electrical conductivity). The organic carbon concentration in the soil was the highest in L₅ (1.56%) taking into consideration its relatively higher organic matter content (Table 2). This carbon content was very low in L₃ and L₄ (0.022 and 0.086%, respectively), and it was below the detection limit in L₁ and L₂. The nitrogen concentration in L₅ was very low (0.081%); however, it was not detected in the other locations, which may be ascribed to the general deficiency of N in seawater [58]. Likewise, the sulfur concentration was below the detection limit in all studied locations. On the contrary, the hydrogen concentration was the highest among the other organic elements (0.211–0.515%). The relatively high hydrogen content might be derived from the soil-bound water. This finding is supported by the data of the water holding capacity of the soils (Table 1). The concentrations of most plant nutrients reflected the low fertility status of the soil, and the urgent need to add fertilizer application for improving the growth and phytoremediation potential of the tested halophytes or for the potential cultivation by economic crops [59]. This low nutrient content is mainly attributed to the low colloidal content, which minimized the nutrient supply potentials of the soil [60,61]. The available phosphorus concentration in all locations was very low (6.91–9.20 mg kg⁻¹) and insufficient to meet the adequate phosphorus supply in light soils (50–85 mg kg⁻¹) [62] which could be attributed to the high soil pH that reduces phosphorus availability [63]. Likewise, the available calcium concentration was below the adequate concentration in light soils (3000–4000 mg kg⁻¹) [62]. In this regard, the Ca²⁺ concentration in L₅ was the lowest (630.93 mg kg⁻¹), presumably due to the continuous dissolution of calcium salts by the inflow of seawater during tidal entry. Concentrations of K⁺ and Mg²⁺ were relatively high taking into consideration marine inputs by different mechanisms (e.g., seawater spray from winds, tidal surges, and seawater intrusion into shallow groundwater). The potassium concentration in L₁ was 124.67 mg kg⁻¹, and this level is inadequate for sufficient plant nutrition (200–400 mg kg⁻¹) [62]. This concentration, however, varied among other locations between 351.27 and 593.81 mg kg⁻¹. On the other hand, the Mg²⁺ concentration in L₁, L₂, and L₃ was inadequate for plant nutrition (<600–800 mg kg⁻¹). Meanwhile, the L₄ and L₅ locations showed adequate concentrations (1119.6 and 737.76, respectively).

Table 2. Total Organic and Available Nutrient Concentrations in the Experimental Soils.

Locations	Total Organic Elements Concentration (%)					Available Nutrients Concentration (mg kg ⁻¹)				
	C	N	H	S	OM (%)	P	K	Ca	Mg	Na
L ₁	N.D	N.D	0.211 ± 0.0200	N.D	N.D	3.21 ± 0.12	124.67 ± 11.1	1162.19 ± 108	169.35 ± 3.3	204.60 ± 24.9
L ₂	N.D	N.D	0.260 ± 0.0245	N.D	N.D	6.89 ± 0.41	593.81 ± 50.8	1250.90 ± 116	178.61 ± 0.3	35.81 ± 3.5
L ₃	0.0215 ± 0.0016	N.D	0.277 ± 0.0221	N.D	0.037 ± 0.0028	7.07 ± 0.74	351.27 ± 30.1	2039.95 ± 197	500.53 ± 44.4	575.85 ± 70.2
L ₄	0.0858 ± 0.0076	N.D	0.551 ± 0.0434	N.D	0.148 ± 0.0131	8.90 ± 0.98	541.44 ± 48.5	1740.83 ± 173	1119.6 ± 96.4	1743.97 ± 167.4
L ₅	1.5600 ± 0.0818	0.081 ± 0.008	0.427 ± 0.0365	N.D	2.690 ± 0.1411	9.13 ± 0.81	496.00 ± 43.9	630.93 ± 59	737.76 ± 70.0	646.46 ± 59.9

3.2. Soil Available Concentrations of PTEs and Their Correlation Matrix (*r*) with Soil Physicochemical Properties

The median, maximum, and minimum values of soil available PTE concentrations are illustrated in box-and-whisker diagrams (Figure 1). The median values of PTE concentrations (mg kg⁻¹) were ordered as follows: Mn (19.83), Fe (11.21), Pb (3.48), Cu (2.10), Zn (1.62), Hg (1.09), Cr (0.60), Co (0.45), Ni (0.36), and Cd (0.04). Distinct variations in PTE concentrations were recognized between the studied locations (Figure. 1). Among locations, L₄ showed the highest concentrations of Cd (0.069 mg kg⁻¹), Co (0.590 mg kg⁻¹), Cu (3.764 mg kg⁻¹), and Pb (4.654 mg kg⁻¹). Meanwhile, L₃ exhibited the highest concentrations of Cr (0.783 mg kg⁻¹), Hg (1.257 mg kg⁻¹), Mn (40.697 mg kg⁻¹), and Zn (8.475 mg kg⁻¹). The high levels of PTEs in these two locations may be attributed to their proximity to a dumping area.

Figure 1. Available concentrations of PTEs (mg kg⁻¹) in soil at different locations. The box chart is symbolized by the median (center line), mean (dot), lower and upper quartiles (the lower and upper borders of the box, respectively), and whiskers-error bars (the minimum and maximum values): (a) cadmium, (b) chromium, cobalt, and copper, (c) iron, manganese, lead, and zinc, and (d) mercury and nickel.

Pearson's correlation was applied to illustrate correlations between the available concentrations of PTEs and some specific soil physicochemical properties (Table 3). The correlation between PTEs and soil cations (Ca²⁺, Mg²⁺, K⁺, and Na⁺) was calculated based

on the available forms of these cations (not the water-soluble form) to be consistent with the DTPA-extractable PTEs.

Table 3. Correlation Matrix (r) Between Soil Physicochemical Properties and Available Concentrations of PTEs, n = 15.

PTEs	Silt and Clay Fraction (%)	Organic Matter (%)	EC (dS m ⁻¹)	pH	CaCO ₃ (%)	Ca ²⁺ (cmol kg ⁻¹)	Mg ²⁺ (cmol kg ⁻¹)	K ⁺ (cmol kg ⁻¹)	Na ⁺ (cmol kg ⁻¹)	HCO ₃ ⁻ (cmol kg ⁻¹)	Cl ⁻ (cmol kg ⁻¹)
Cd	−0.21	−0.62 *	0.02	−0.14	0.23	0.39	0.28	−0.01	0.53 *	−0.63 *	0.44
Cr	−0.79 **	−0.30	−0.64 *	0.08	0.84 **	−0.10	−0.79 **	−0.90 **	−0.71 *	0.39	−0.73 **
Co	−0.60 *	−0.87 **	0.17	−0.83 **	0.22	0.94 **	0.12	−0.17	0.37	0.17	0.37
Cu	0.28	−0.14	0.83 **	−0.58 *	−0.66 *	0.67 *	0.84 **	0.43	0.88 **	−0.10	0.92 **
Fe	−0.27	0.15	−0.32	0.60 *	0.47	−0.51	−0.31	−0.78 **	−0.26	−0.20	−0.34
Hg	−0.84 **	−0.94 **	−0.14	−0.81 **	0.51 *	0.86 **	−0.24	−0.36	0.01	0.37	0.01
Mn	0.56 *	−0.60 *	0.01	−0.93 **	0.14	0.82 **	−0.23	−0.02	−0.15	0.77 **	−0.07
Ni	−0.63 *	−0.29	−0.43	0.26	0.73 **	−0.15	−0.44	−0.89 **	−0.28	−0.07	−0.37
Pb	0.73 **	0.17	0.35	0.05	−0.62 *	0.01	0.62 *	0.95 **	0.54 *	−0.56 *	0.56 *
Zn	−0.38	−0.38	0.43	−0.94 **	−0.18	0.86 **	0.14	−0.07	0.19	0.75 **	0.27

The symbols (* and **) refer to the degree of significant correlation at confident levels of 0.05 and 0.01, respectively.

Cd: A significant correlation ($r = -0.62$, $p < 0.05$) was recorded between the available Cd²⁺ concentration and soil organic matter content given the high tendency of labile Cd²⁺ toward bonding with organic matter in immobile forms [64]. Additionally, a positive significant correlation ($r = 0.53$) was noticed with HCO₃⁻ ions. According to [65], Cd predominantly exists in the form of CdHCO₃ in alkaline soils, and higher concentrations than the background values are signals for anthropogenic inputs.

Cr: A negative significant correlation ($r = -0.79$, $p < 0.01$) was noticed between Cr ions and the silt and clay fraction. According to [66], the particle size distribution of soil significantly affects the extractability/leachability, and thereby the bioaccessibility, of Cr(VI) ions in soil based on the variation of the distribution factor among different soil size fractions (0.7, 0.79, 1.35, and 1.6 for coarse sand, medium sand, fine sand, and silt–clay, respectively). Negative correlations were also recorded with Ca²⁺ (-0.79 , $p < 0.01$), K⁺ (-0.90 , $p < 0.01$), and Na⁺ (-0.69 , $p < 0.05$) ions. The high concentration of these cations might reduce the extractability of Cr(VI) ions through increasing their binding capacity by the colloidal phase via cation bridging formations with active sorption sites [67]. These colloidal–chromate complexes were stable and showed low extractability compared to most of the other PTEs [68]. In addition, negative correlations were recorded with EC (-0.64 , $p < 0.05$) and Cl⁻ ions (-0.73 , $p < 0.01$). High values of ionic strength might lead to an increase in the Debye–Huckel screening influence, thereby reducing the electrostatic interaction between active sorption sites and Cr(VI) ions [69]. Reducing the affinity between the sorbent and sorbate might increase the extractability of Cr(VI) ions. Furthermore, the competition between Cr (VI) and Cl⁻ ions onto sorption sites might reduce the binding capacity of Cr(VI) ions by the soil colloidal phase.

Co: In general, the Co²⁺ concentration shows higher values in arid and semiarid regions, whereas its concentrations in soils of glaciated regions are often low [65]. Cobalt concentrations showed negative significant correlations with both silt and clay and organic matter fractions ($r = -0.60$ and -0.87 , respectively), which indicates the importance of these fractions in the stabilization of Co²⁺ ions [70]. Similarly, the soil pH value significantly correlated with Co²⁺ extractability due to the activation of chemisorption forces at high pH values of the soil [71]. Conversely, Co²⁺ extractability showed a strong positive correlation ($r = 0.94$, $p < 0.01$) with available Ca²⁺ due to the competition between Ca²⁺ and Co²⁺ ions onto the active sorption sites. Consequently, soluble calcium salts are often used as efficient Co²⁺ extractants in calcareous soils [72].

Cu: A notable highly significant correlation ($r = 0.83$, $p < 0.01$) was observed between extractable Co²⁺ and the EC value of the soil since the increment in the ionic strength of the soil solution might condensate the diffused double layer of the colloidal phase, which constrains the approaching Cu²⁺ ions to the active sorption sites [73]. In contrast, pH and CaCO₃ showed negative significant correlations with Cu²⁺ ions ($r = -0.58$ and -0.66 , respectively). The binding capacity of Cu²⁺ ions onto the colloidal soil components might increase significantly with the increasing pH value of the soil due to hydroxylation of

pH-dependent charges and the increasing electronegativity of the soil [73]. Furthermore, the formation of hydroxyl and carbonate complexes at high pH and CaCO_3 values might inhibit the extractability of Cu^{2+} ions [65]. Meanwhile, the positive significant correlations with Ca^{2+} , Mg^{2+} , and Na^+ ions might be attributed to the competition between Cu^{2+} and those metal ions onto the available sorption sites.

Fe: Unlike most reported studies, a positive significant correlation was obtained between soil pH and extractable Fe^{2+} ($r = 0.60$, $p < 0.05$). In our study, the coastal locations were marked with higher pH values. The higher Fe^{2+} extractability in these locations could be attributed to the reduction of Fe^{3+} to Fe^{2+} and the dissolution of iron carbonate complexes upon waterlogging by tidal entry. The negative significant correlation ($r = -0.78$, $p < 0.01$) between Fe^{2+} extractability and available K^+ ions is linked to their competition onto sorption sites.

Hg: The negative significant correlations between extractable Hg^{2+} ions and silt and clay (-0.84 , $p < 0.01$) and organic matter (-0.94 , $p < 0.01$) fractions are interlinked with the tendency of these components to form insoluble fractions with Hg^{2+} ions [65]. The soil reaction (pH) also showed a negative significant correlation with extractable Hg^{2+} ions ($r = -0.81$, $p < 0.01$) as the Hg^{2+} flux from the soil matrix at alkaline soil conditions is often low [72]. Calcium carbonate and DTPA-extractable Ca^{2+} , however, showed positive correlations with Hg^{2+} ions (0.51 and 0.86, respectively). It is widely accepted that CaCO_3 and active Ca^{2+} ions can increase the aggregation/stability of soil organic matter, thereby reducing its capacity toward the chemisorption of Hg^{2+} ions [74].

Mn: The extractable Mn^{2+} ions were positively correlated with the silt and clay fraction (0.56, $p < 0.05$), extractable Ca^{2+} (0.82, $p < 0.01$), and soluble HCO_3^- ions (0.77, $p < 0.01$). Negative correlations, however, were observed with organic matter (-0.60 , $p < 0.05$) and pH (-0.93 , $p < 0.01$). Manganese oxides (e.g., vernadite and birnessite) are typical constitutions of clay minerals [75]. Mn^{2+} ions also showed a high affinity toward precipitation as bicarbonate and organic manganese complexes. Those are governed by multiple soil reactions, of which soil pH and redox reactions are the most important [65]. The positive correlation between Mn^{2+} and Ca^{2+} ions is mainly related to the ion exchange mechanism, which increased the desorbability of Mn^{2+} ions.

Ni: A negative significant correlation ($r = 0.63$, $p < 0.05$) was observed between Mn^{2+} ions and the silt and clay fraction given their high binding forces by clay minerals (montmorillonite, in particular) [76]. Extractable Ni^{2+} ions also showed a positive correlation with CaCO_3 (0.73, $p < 0.01$); however, it showed a negative correlation with K^+ ions (-0.89 , $p < 0.01$). Nickel is slightly mobile in soil and exists mainly in immobile forms [65]. Meanwhile, the sorbed Ni^{2+} ions onto the nanoscale calcium carbonate fraction might exchange with K^+ ions onto the active sorption sites.

Pb: The positive significant correlation between Pb^{2+} ions and the silt and clay fraction agrees with that obtained by [65], who reported that Pb mineral distribution in soil shows a correlated trend of values with the fine granulometric fraction. In arid and semiarid conditions, Pb sulfides are oxidized slowly and show a high tendency toward forming immobile carbonate fractions with less extractability potentials [77]. This might be the reason for the negative significant correlations with CaCO_3 and HCO_3^- ions (-0.62 and -0.56 , respectively, $p < 0.05$). Positive significant correlations were further noticed with Mg^{2+} (0.62, $p < 0.05$), K^+ (0.95, $p < 0.01$), and Na^+ (0.54, $p < 0.05$), probably due to their easy exchange with Pb^{2+} ions onto the active sorption sites of the colloidal phase [78]. The positive significant correlation between available Pb^{2+} and Cl^- ions (0.56, $p < 0.05$) is mainly related to the positive effect of Cl^- ions on increasing the extractability potentials of Pb^{2+} ions from active soil fractions.

Zn: Unlike most other PTEs, Zn appeared to exist in more soluble forms with high leachability potentials; however, high HCO_3^- values precipitated Zn species into $\text{Zn}(\text{HCO}_3)_2$ with a lower degree of leachability [79]. This could be the reason for the positive significant correlation with HCO_3^- (0.75, $p < 0.01$). A negative significant correlation was observed between Zn^{2+} ions and the pH value of the soil (-0.94 , $p < 0.01$). The

desorbability of Zn^{2+} ions decreased continuously with the increasing pH value of the soil, and the proportion of sorbed Zn^{2+} that could be extracted declined dramatically as the pH value increased above 6.5 [80]. On the contrary, the positive significant correlation with Ca^{2+} ions ($0.86, p < 0.01$) might be due to maximizing the desorbability of Zn^{2+} ions by the ion exchange mechanism.

3.3. Ecological Risk Assessment of PTE Concentrations in Soil

The calculated I_{geo} values showed an uncontaminated effect ($0 < I_{\text{geo}} < 1$) of most PTEs (Cd, Cu, Fe, Ni, Pb, and Zn). Other elements, however, varied in their contamination level among rhizospheric soils (Figure 2). The contamination level of Cr and Co was the highest among other PTEs and reached a highly contaminated degree in some rhizospheric soils ($3 < I_{\text{geo}} < 4$). The contamination level of Hg and Mn was slight in most cases, except for L_3 , which showed a moderate Mn contamination level ($2 < I_{\text{geo}} < 3$).

Figure 2. Ecological risk assessment of PTEs in soil: (a) geo-accumulation index (I_{geo}), (b) enrichment factor (E_f), (c) contamination factor (C_f), (d) potential ecological risk index (E_r^i), (e) pollution load index (P_{LI}), and (f) contamination degree (C_D) and modified degree of contamination (mCD).

The data of the enrichment factor (E_f) show no enrichment of the Cu, Fe, Pb, and Zn elements ($0 < E_f < 1$), minor enrichment of Ni in most rhizospheric soils, except for L_1 (moderate enrichment), moderate to severe enrichment of Hg and Mn in most samples, except for L_2 and L_3 (severe enrichment), and very severe enrichment of Co ($25 < E_f < 50$).

The data of the single contamination factor show no enrichment of most TEs; however, Cr and Mn reached the limit of moderate to severe enrichment, and Co reached the limit of severe enrichment. Likewise, the potential ecological risk index indicated a low risk of all TEs except Co, which showed a considerable risk. The pollution load index values ranged between unpolluted to moderately polluted. The contamination degree pointed

to a very high degree of contamination, although the modified degree of contamination showed only moderate contamination.

3.4. PTE Concentrations in Plants

The data presented in the box-and-whisker diagrams (Figure 3) show the median, maximum, and minimum concentrations of PTEs in the tested halophytes. The median concentrations of PTEs in plants (mg kg^{-1}) were ranked as follows: Fe (1112), Zn (114.4), Cu (81.3), Mn (76.3), Cr (30.0), Pb (13.9), Ni (3.6), Co (3.2), Cd (2.8), and Hg (1.95). *Z. coccineum* leaves contained the highest levels (mg kg^{-1}) of Co (26.4), Cu (121), Fe (2808), Pb (84.1), and Zn (138.2). *H. perfoliata*, however, showed the highest concentrations (mg kg^{-1}) of Cd (6.4) and Hg (8.1). Meanwhile, *T. nilotica* recorded the highest concentration (mg kg^{-1}) of Cr (38.06) and Mn (92.9). On the other hand, *H. crispum* showed relatively lower PTE concentrations in general and recorded the minimum values (mg kg^{-1}) of Cr (7.7), Co (N.D.), Hg (ND), and Zn (98.6). Meanwhile, *A. marina* attained high values of Hg (2.7) and Mn (84.5). The recorded concentrations of most PTEs exceed the permissible limits ($\mu\text{g g}^{-1}$) that have been justified by standard regulatory bodies (e.g., the WHO, FAO): Cd (0.1), Cu (73), Fe (425), Mn (500), Ni (67), and Zn (100) [81], suggesting their potential use for phytoremediation purposes and medical applications [82,83] rather than other uses involving human consumption.

Figure 3. Total concentrations of PTEs (mg kg^{-1}) in halophytes. The box chart is symbolized by the median (center line), mean (dot), lower and upper quartiles (the lower and upper borders of the box, respectively), and whiskers-error bars (the minimum and maximum values): (a) cadmium, cobalt, mercury and nickel (b) copper, manganese, and zinc, (c) chromium and lead, (d) iron.

The soil-to-plant transfer factors including the bio-concentration ratio ($\text{BCR} = \text{PTE concentrations in plants} / \text{available PTE concentrations in soils}$) [84] can interpret the hyperaccumulating potentials of PTEs by wild plants, thereby indicating the usability of these plants for phytoremediation techniques (plants having $\text{BCR} > 1$ are considered as hyperaccumulators). The tested halophytes showed hyperaccumulating potentials for all studied PTEs with BCR values > 1 (Table 4).

Table 4. Bioaccumulation Factor (BAF) of PTEs in Endemic Wild Plants.

PTEs	TN	HC	ZC	HP	AM
Cd	120.31 ± 1.95	80.15 ± 0.28	128.10 ± 1.79	92.72 ± 0.82	189.47 ± 4.88
Cr	26.80 ± 0.24	12.69 ± 0.10	38.33 ± 0.24	219.02 ± 6.07	57.27 ± 0.45
Co	1.12 ± 0.01	N.D.	45.35 ± 0.08	5.45 ± 0.08	22.63 ± 0.22
Cu	64.97 ± 13.26	43.41 ± 6.50	40.85 ± 21.44	2.22 ± 0.57	11.91 ± 3.74
Fe	29.15 ± 2.84	198.28 ± 20.15	443.85 ± 42.55	82.83 ± 7.33	22.95 ± 1.88
Hg	N.D.	N.D.	1.74 ± 0.15	7.33 ± 1.64	2.42 ± 0.59
Mn	5.33 ± 0.47	2.35 ± 0.23	1.87 ± 0.17	2.66 ± 0.23	7.42 ± 0.63
Ni	3.57 ± 1.02	184.88 ± 42.40	72.53 ± 34.55	9.84 ± 0.96	8.20 ± 0.87
Pb	44.41 ± 4.37	3.27 ± 0.76	37.50 ± 3.56	0.50 ± 0.08	4.19 ± 1.91
Zn	116.53 ± 11.68	61.04 ± 6.46	16.30 ± 1.45	35.30 ± 3.56	137.31 ± 13.13

3.5. Halophyte Physiological Analysis

Our chemical analysis of rhizospheric soils in the tested salt marshes revealed that their inhabiting species suffer from varying levels of salinity (Table 1), nutrient deficiency (N, P, several micronutrients; Table 2), and elevated levels of PTEs (Figure 1). These results agree with the general belief that halophytes in their natural habitats suffer from various types of stress [15]. Such multiple stresses induce both secondary water stress and oxidative damage to cellular structures. However, the tested halophytes did not show any obvious symptoms of toxicity, suggesting that such multiple stress-induced signals might trigger cross-talk among various physiological pathways that end with activation of “cross-tolerance” allowing the tested species to respond effectively to single or multiple stresses [15]. Consistent with that, salinity improved the growth of halophytes such as *Sesuvium portulacastrum*, *Spartina densiflora*, and *Mesembryanthemum crystallinum* under PTE stress such as Cd and Zn. Such an enhancing effect is attributed to the possible role of Na⁺ in osmotic adjustment and induction of synthesis of proline, polyamines, and glutathione as well as a reduction in ethylene and abscisic acid [85,86]. The output of such a cross-talk, the possible species-specific anatomical structures, and the extent of the internal cellular tolerance to each of such multiple stresses stand behind the differential physiological responses and adaptability of the tested halophytes and make each halophyte behave as a unique biological system.

The consequences of salinity, nutrient, and PTE stresses in the soils of the current study areas on the oxidant and antioxidant systems in the dominating halophytes were monitored (Figure 4). Our measurements of oxidative stress biomarkers revealed much lower levels of MDA in the leaves of *T. nilotica*, *H. crispum*, and *Haloepplus perfoliata* (Figure 4a), and such a response was associated with relatively low levels of H₂O₂ (Figure 4b). These results indicate a reduced extent of oxidative stress in these species. In *T. nilotica* and *H. crispum*, the reduced levels of MDA can be ascribed mainly to (i) the low salinity in their rhizospheric soils (3.74 dS m^{−1}, 2.46 dS m^{−1}, Table 1), and (ii) their relatively higher antioxidant compounds such as total flavonoids (Figure 4c), phenolics (Figure 4d), and carotenoids (Figure 5d). These compounds can contribute significantly to the overall antioxidant potential and thus reduce ROS and, consequently, MDA. Consistent with this, significant content of phenolics and high antioxidant activity have also been confirmed in *Tamarix gallica* [87]. (iii) The salt glands and trichomes in *T. nilotica* as well as the succulence in *H. crispum* may reduce the PTE-induced ROS formation. The above reasons thus synergistically reduce ROS and, consequently, MDA. The reduced oxidative stress in *H. perfoliata*, regardless of the high EC values and PTE content in its rhizospheric soil, suggests that this halophyte has optimized its antioxidant defense to minimize the oxidative damage it encounters in its natural environment. In support of this hypothesis, [18] reported high catalase activity in *H. perfoliata* at 150 mM NaCl; this approximately matches the level of total soluble salts in the rhizospheric soil of *H. perfoliata* in the current study [18]. They also reported a salinity-induced reduction in the activities of both SOD and APX with minimal effect on GR, suggesting that active superoxide (O₂^{•−}) radicals are not synthesized

in response to salinity stress in *H. perfoliata* [18]. Further, its succulence may also minimize the detrimental effects of both salinity and PTEs.

Figure 4. Oxidative stress and antioxidant indicators: (a) lipid peroxidation (malondialdehyde; MDA), (b) leaf hydrogen peroxide (H₂O₂), (c) total flavonoids, and (d) total phenolics. Shown are the mean values of three biological replicates $\pm$ standard deviations. On the abscissa axis, the initials of the tested species are reported.

Unlike the above-mentioned species (*T. nilotica*, *H. crispum*, and *H. perfoliata*), both *A. marina* and *Z. coccineum* had much higher MDA levels in their leaves (Figure 4a), and such a response was associated with the higher H₂O₂ level in *A. marina* only (Figure 4b). These results indicate a disturbance of the equilibrium between the oxidative and antioxidative capacities at the cellular level in *A. marina* and *Z. coccineum* [21,88]. High MDA levels in *A. marina* and *Z. coccineum* leaves may be ascribed, in part, to the high salinity in their rhizospheric soils (9.92 dS m⁻¹ and 9.94 dS m⁻¹, Table 1), and the simultaneous toxicity of PTEs in their leaves, particularly Hg, Co, and Mn in *A. marina*, and Co, Cu, Fe, Zn, and Ni in *Z. coccineum* compared to *T. nilotica* and *H. crispum*. High salinity levels impair electron transport in chloroplasts and mitochondria and lead to the formation of ROS which, in turn, leads to MDA accumulation [89,90]. On the other hand, the higher level of H₂O₂ in *A. marina* in the presence of transient PTEs can be a major source of highly reactive hydroxyl radicals, of which cells do not have an enzymatic system to detoxify, and thus they induce high MDA levels in *A. marina* leaves [27]. Further, the level of Hg in *A. marina* leaves exceeded the toxic threshold of Hg in plants [91], thereby interfering with mitochondrial activity and triggering ROS generation and MDA accumulation [92]. Mercury (Hg) can also bind to water channel proteins, inducing stomatal closure and thus hindering water flow in *A. marina* [93]. Interestingly, despite the high foliar levels of H₂O₂ and MDA in *A. marina* leaves, they exhibited growth vigor and efficient C assimilation as indicated by their high TSS and sucrose levels (see below). These results suggest that chloroplasts in *A. marina* seem to be protected against ROS damage, which indicates that *A. marina* has developed adaptive mechanisms that could facilitate the persistence of elevated cellular H₂O₂ as a stress signal [27], as it has been described in the *Eutrema salsugineum* halophyte [94–96]. Interestingly, in *Z. coccineum* leaves, high MDA levels (Figure 4a) were associated with the highest levels of total PTEs among the tested halophytes. Yet, they contained relatively lower levels of H₂O₂, suggesting the involvement of ROS other than

H₂O₂ in lipid peroxidation. Toxic levels of PTEs can indirectly deplete low-molecular weight antioxidants [9]. Consistent with that, *Z. coccineum* leaves had the lowest level of total flavonoids (Figure 5a) and total phenolics (Figure 5b) among the tested species. Toxic levels of the tested PTEs might also have suppressive effects on the activities of some antioxidant enzymes [21,97]. Altogether, these heavy metal-induced responses trigger ROS generation [9], which, in turn, induces high MDA accumulation and lipid peroxidation in *Z. coccineum*.

Figure 5. Leaf photosynthetic pigments and C assimilation: (a) chlorophyll a (Chl a), (b) chlorophyll b (Chl b), (c) total chlorophyll (Tot. Chl), (d) carotenoids (Cars), (e) total soluble sugars (TSS), and sucrose (f). Shown are the mean values of three biological replicates ± standard deviations. On the abscissa axis, the initials of the tested species are reported.

The impact of the above differential oxidative stress on C metabolism in the tested species was also monitored. *H. crispum*, *T. nilotica*, and *A. marina* had significantly higher chl a, chl b, and total chl than *Z. coccineum* and *H. perfoliata* (Figure 5). Such a high chlorophyll content was associated with both the high carotenoid (Figure 5d) and total leaf N (Figure 6) levels, which are crucial for both chlorophyll synthesis and activity. The relatively high carotenoid levels protect photosynthetic pigments via quenching ¹O₂ and peroxy radicals

that are generated during excess excitation of chlorophyll as a result of intensive light and high temperatures [34,98]. In addition, the importance of adequate leaf N for chlorophyll synthesis is well documented. Altogether, the two factors improve C assimilation and thus explain the higher foliar TSS and sucrose in *H. crispum* and *A. marina* (Figure 5e,f). In *T. nilotica*, the higher chlorophyll and carotenoid levels were not translated into high TSS and sucrose, and such a response can be ascribed to its high foliar levels of PTEs, particularly Cd, which inhibits the leaf photosynthetic rate and intracellular CO₂ concentration of plants and consequently reduces carbohydrate synthesis [92,99]. In *H. perfoliata* and *Z. coccineum*, the high salinity in their rhizospheric soil (Table 1) reduced photosynthesis and overall CO₂ assimilation via a reduction in the chlorophyll content (Figure 6), decreasing mesophyll CO₂ availability as a result of stomatal closure to minimize water loss [100–102] and inhibiting Rubisco activity [103]. In addition, high salinity often leads to an over-reduction in the photosynthetic electron transport chain, resulting in a higher demand for alternative energy sinks to prevent photoinhibition of photosystem II (PSII) [104].

Figure 6. Leaf N content. Shown are the mean values of three biological replicates $\pm$ standard deviations. On the abscissa axis, the initials of the tested species are reported.

4. Conclusions

The soils of the tested regions are under high risk of contamination with various PTEs as a result of active oil trading and modernization activities. In addition, the successful growth and adaptation of the tested halophytes in the tested salt marshes regardless of their higher salinity and PTE contents indicates that these plants successfully maintain a viable physiological status of their cells under multiple stresses in such harsh environments. In addition, the leaves of the tested halophytes showed differential intercellular tolerance against high levels of most of the tested PTEs which were considerably above their toxic levels. *A. marina* accumulated exceptionally high Cd, Zn, and Mn along with higher levels of Cr, Co, and Hg, whereas *H. perfoliata* accumulated the highest levels of Cr and Hg. On the other hand, the leaves of *Z. coccineum* accumulated high levels of Co, Fe, and Pb, while *T. nilotica* had higher levels of Cu, Pb, Cd, and Zn. *H. crispum* leaves accumulated the highest Ni levels among the tested halophytes. These results suggest that the tested halophytes may have high PTE accumulation potentials at the whole plant level since the bio-concentration factors of metals such as Cu, Zn, Cd, Cr, and Hg are usually higher in roots than in leaves [105]. In addition, these results, along with the high biomass of the tested halophytes, highlight their capabilities as phytoextractors of their corresponding PTEs and their high potential as efficient, cost-effective tools for phytoremediation of salt- and PTE-affected lands. However, the increased levels of PTEs in the leaves of the tested halophytes, compared to their permissible levels in vegetables, might limit their uses related to direct human consumption.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/su132011282/s1>, Table S1: Ecological Risk Assessment Parameters and Their Contamination

Levels, Table S2: Median Values of PTE Concentrations (mg kg^{-1}) in Soil (S) and Halophyte (h) Samples in the Current Study Compared to Former Reported Result.

Author Contributions: Conceptualization, F.I. and A.M.; software, N.A.-H.; validation, F.I. and A.M.; formal analysis, F.I. and A.M.; resources, F.I.; data curation, F.I. and M.E.-M.; writing—original draft preparation, N.A.-H. and M.E.-M.; writing—review and editing, F.I. and A.M.; visualization, N.A.-H. and M.E.-M.; supervision, F.I.; project administration, F.I. and N.A.-H.; funding acquisition, F.I. and N.A.-H. All authors have read and agreed to the published version of the manuscript.

Funding: The authors would like to thank the Deanship of Scientific Research at Umm Al-Qura University for supporting this work by Grant Code: 17-SCI-1-03-0005.

Acknowledgments: The research team would like to thank Mohamed Al-Helasi for his help during samples collection and processing.

Conflicts of Interest: The authors declare no conflict of interest.

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