EFFECTS OF TOXIC ELEMENTS ON LEAF MORPHOLOGY OF *HALOPHILA STIPULACEA* GROWINMINE DUMP SEDIMENTS IN SOUTHEAST JORDAN

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Abstract

Plants, in particular seagrasses, that grow in contaminated areas can uptake and accumulate Potential Toxic Elements (PTEs) in their tissues. This accumulation in turn can produce several effects on plant morphology and health status. This study, carried out on seagrass (*Halophila stipulacea*) growing on the phosphate mine dump sediments in southeast Jordan, highlights the possible leaf damages at anatomical and cytological levels. The micro-morphological observations demonstrated that the damage caused by PTEs was easily found throughout the leaf, showing massive change in the overall organization of cells, both in the epidermis and the mesophyll and vascular bundles. This probably caused the blockage of liquids and nutrients, leading to necrosis of the leaf in comparison to the control. Moreover, seagrass samples collected from phosphate port area showed a swelling in the outer epidermal wall, and in some cases a collapse in parenchyma was observed. In addition, collapse in the epidermis, chloroplasts degradation, and necrosis of some cells was detected. The observed morphological change seems to be correlated to contamination levels in the sediments.

Keywords: *Halophila stipulacea, potentially toxic elements, leaf morphology*

Introduction

The evaluation of soil contamination by potential toxic elements (PTEs) has been extensively carried out through plant analysis (Wahsha et al., 2016; Fontana et al., 2010); both wild and cultivated plant species have been commonly used as passive accumulative bioindicators for large scale and local soil contamination (Bini et al., 2012).Heavy metal accumulation is well known to produce significant biological and physiological responses in vascular plants (Maleci et al., 2014a). A number of authors demonstrated that there is a strong relationship between chemical degree of contamination in the environment and the morphological and biochemical responses in plants (Bini et al., 2012, Preeti and Tripathi, 2011). Plants growing in contaminated mine sites are of particular interest in this perspective, since they are

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genetically tolerant to high toxic elements levels, as previously reported at various sites in the world (Bini, 2005; Brooks, 1998; Giuliani et al., 2008; Maleci et al., 1999). Amongst wild vascular plants, the seagrasses are the only group of higher plants adapted to life submerged under the sea. They inhabit shallow soft bottomwater areas of temperate, subtropical and tropical seas where they may form large meadows (Al-Rousan et al., 2011). Seagrass habitats are key component of the estuarine landscape supporting high productivity, abundant marine life, and provide nursery habitat for many estuarine species (Williams et al., 2016). Seagrasses are particularly sensitive to environmental stressors; they are often heralded as sentinel or indicator species because they are long lived and integrate biological, physical and chemical parameters (Bonanno and Martino, 2016). Assessing the change of environmental quality has become increasingly important worldwide. As a result, there has been a recent burgeoning of monitoring programs based either directly or indirectly on seagrass responses to environmental changes (Roca et al., 2016; Martínez-Crego et al., 2008).

Previous studies of our research group (Al-Absi et al., 2016; Al-Absi et al., 2014) investigated the toxic elements (Ac, As, Bi, Cd, Pb, Ra, Sr, Th, Tl, U) concentration of sediments derived from mine waste soil material, and the seagrass growing on those contaminated sediments, in order to determine the extent of potentially toxic elements dispersion, and the uptake by both known and unreported toxic element tolerant plant species. The results showed that seagrass (*H. stipulacea*) is a species tolerant to high toxic elements levels, and suggested to use it as a bioindicator plant. Toxic elements accumulated mainly in roots, but also in leaves as proved accumulating organs, being able to store significant quantities of uranium, for instance, with only little visible damages. In this work we report the outcomes of a study carried out on *H. stipulacea* growing on mine dump sediments in southeast Jordan to highlight possible damages at anatomical and cytological levels in the leaf part of the plant.

Materials and Methods

Study area

The Gulf of Aqaba is a unique semi-enclosed water body located at the northern end of the Red Sea. It is located at the east fork of the Red Sea (Figure 1). Its coasts are shared by Jordan, Palestine, Egypt and Saudi Arabia. The Gulf contains the only port of Jordan, Aqaba port. The maximum depth of the Gulf is about 1800 m; its length is 180 km and its width ranges between 14 and 26 km. The study area lies within the Jordanian coast of the Gulf that is 26.5 km long. Sediment and seagrass samples were collected from 2 sites: Marine Science Station (MSS) and Phosphate Port Area (PH).

Field sampling and laboratory analysis

Twenty samples of soil sediments and seagrass were collected from two selected sites (the Marine Science Station "MSS" as a protected area and the Phosphate Port

Area "PH" as contaminated area) from the Gulf of Aqaba by scuba diving using at a depth ranged between 5 and 7 m below sea surface.

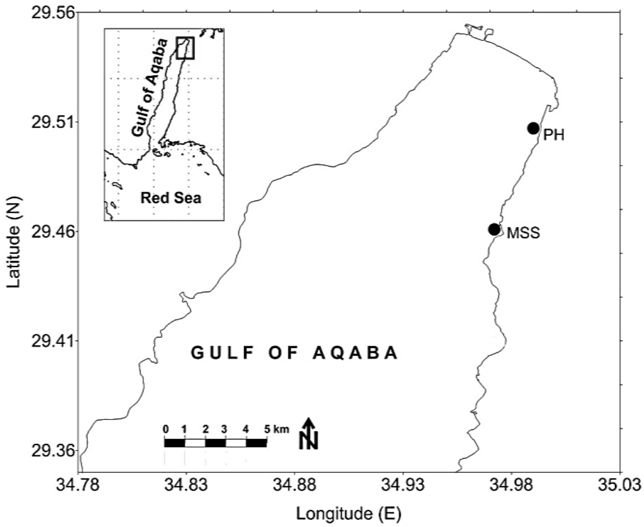

Figure 1
Location of the studied area and sampling sites.

According to the procedures described by Benton Jones (2001) and Margesin and Schinner (2005), sediment samples were collected from the upper horizon at depth of approximately 25 cm. Each sediment sample was a composite of 5 subsamples collected in a given sector (4 m^2). Samples were taken at the site, mixed, packed in containers, and then transported to the laboratory for further routine chemical and physical analysis. Seagrass samples were collected according to Benton Jones (2001) with minor modifications. At least six specimens of selected plant species (at the early vegetative phase and normal morphological appearance) were sampled at each site. Samples were carefully packed in plastic bags and transported to the laboratory. Seagrass species were classified according to Edmund et al. (2003) as "*Halophila stipulacea*". Sediment samples were dried in an oven at 105 °C overnight. They were crushed, homogenized and sieved through 63-100 µm mesh sieve. Then they became ash gradually at about 550 °C.

Small leaf portions of fresh *H. Stipulacea* specimens, were taken from the middle part of the leaf length (three replicates for each sample) then pre-fixed in 10% formaldehyde, dehydrated and then embedded in paraffin. Seven µm sections were stained with Hematoxylin and Eosin and observed with a light microscope for a general overview of leaf morphology.

Results and discussion

Sediment analysis

The characteristics of all sediment samples (total organic matter, sediment type and color) are listed in Table 1. Waste soils are clay in texture and typically unsaturated with respect to water; they have low cation exchange capacity and relatively high

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hydraulic conductivity that favours oxidation and alteration processes. The total organic matter ranged between 0.89% at the MSS and 1.725% at the PH. Moreover, sediment type and color varied from location to another due the differences in the physical, chemical, and geological properties of the marine sediments.

Site	Total organic matter (%)	Particle size	Colour
MSS	0.89	Sandy	Brown to gray
PH	1.725	Clay	Gray to black

Table 1
Selected chemo-physical properties of the studied sediments.

According to Jordan national reports 4.0 to 7.0 million tons of raw phosphates are exported through this port every year. During shipment, loading and transportation a small fraction of phosphate particles enters the water of the Gulf.
Table 2 summarizes the results of the average concentrations of selected potentially toxic elements (Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn) in the tested sediments. The area is almost not contaminated by Cd. The total concentrations of most of the investigated elements in the sediment samples (PH) were significantly higher (ANOVA $p<0.05$) than those of control (MSS) except Iron. Currently, identifying a specific benchmark for iron in soils and sediments is difficult since iron's bioavailability to plants and resulting toxicity are dependent upon site-specific soil conditions (pH and soil-water conditions) (Wahsha et al., 2012a).

Table 2. *Concentration of potentially toxic elements in soil sediments collected in the proximity of the phosphate port area. All toxic elements are expressed as mg kg^{-1}.*

	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
PH	<DL	67.4	25.6	2888	378	11.8	23.1	593
MSS	<DL	66.6	2.50	26000	522	54.6	8.9	500
* International average	0.3	200	20	-	850	40	10	50
* Excessive values	5	100	100	-	1500	100	100	250
* Residential Limits	-	150	120	-	-	120	100	150
* Industrial Limits	-	800	600	-	-	500	1000	1500

*Adopted from Wahsha et al., 2012a. DL = Detection limits
Bold value indicates a statistically significant difference with a P-value less than 0.05

The linear correlation between Pb, Cu, Zn (Cu/Pb 0.641; Pb/Zn 0.562; Cu/Zn 0.573) significant at ($P < 0.05$) is consistent with their calcophilous behavior, since these metals tend to form compounds with sulfur, as chalcopyrite ($CuFeS_2$), sphalerite (ZnS) and galena (PbS). Cr is negatively correlated with all elements. Ni, Fe and Mn are not significantly correlated with any other element, although they share the same geochemical behavior, as a result of anthropogenic activities in the area (Wahsha et al., 2014). It is important to point out, however, that the PTEs levels of contamination in the investigated area vary proportionally with the anthropogenic activities at the mining site.

Mine wastes are current sources of environmental contamination, mostly in the form of acid mine drainage. Acid mine waters have caused severe degree of pollution in the fluvial systems, with transference of large amounts of acidity and dissolved metal(loids) (Al, As, Cd, Cu, Fe, Mn, Pb, Zn), and SO_4 (see for example Van Geen et al., 1997; Frau et al., 2009). Upon further anthropogenic conditions, such PTEs can be released in the environment and constitute a potential risk to vegetation, living communities and ultimately to the human health (Bini and Wahsha, 2014). Mining affects relatively small areas, thus could not pose serious environmental problems. The environmental impact appears when ores are mined, milled, roasted, smelted, transferred, and large amounts of waste material are generated (Wahsha et al., 2012b). Based on the nature of the waste material, a wide dispersion of the potentially harmful substances in soils, surface and groundwater and sediments, in both solution and particulate forms, is expected to occur (Wahsha and Al-Rshaidat, 2014).

Microscope observations

The leaf morphology of seagrass plants grown on potentially toxic contaminated substrate (PH site) in comparison to plants grown on uncontaminated substrate (MSS site) was examined to ascertain possible toxic signals.
In this study we observed a little difference in leaves colour (a less intense green colour in specimens of the contaminated site in comparison to control). Light microscopy observations carried out on the leaf lamina (Fig. 2) demonstrated that the damage caused by toxic elements was easily found throughout the leaf, showing massive changes in overall organization of cells, both in the epidermis and the mesophyll and vascular bundles, which probably caused the blockage of liquids and nutrients, leading to leaf necrosis. Moreover, PH samples showed swelling of the outer epidermal wall, and in some cases a collapse of parenchyma was observed in comparison to the control. Furthermore, collapse of the epidermis, degradation of chloroplasts, necrosis of some cells was detected. These statements are in agreement with findings by Kupper et al. (1998), who noted that in water plants transition metals such as Cd, Cu, Ni, Pb, Zn may substitute for Mg in the chlorophyll molecule, thus reducing the photosynthetic function, which results in colour change (i.e. chloroplasts number decrease, and consequently the chlorophyll production). It is possible that an analogous process would occur in our studied plants. Moreover, some of our findings are in agreement with those of Maleci et al. (2014b) and Bini et al. (2012) in *Taraxacum officinale* plants infected by toxic heavy metals.

Conclusions

The anthropogenic influence related to the port activity on sediments of the studied area is obvious. Sediments in the studied area site are highly contaminated by PTEs, mainly U, and As. The study shows that there is a relationship between high PTEs contents in the plant ecosystem and their modified morphology, even if non-toxic visible symptoms were observed.

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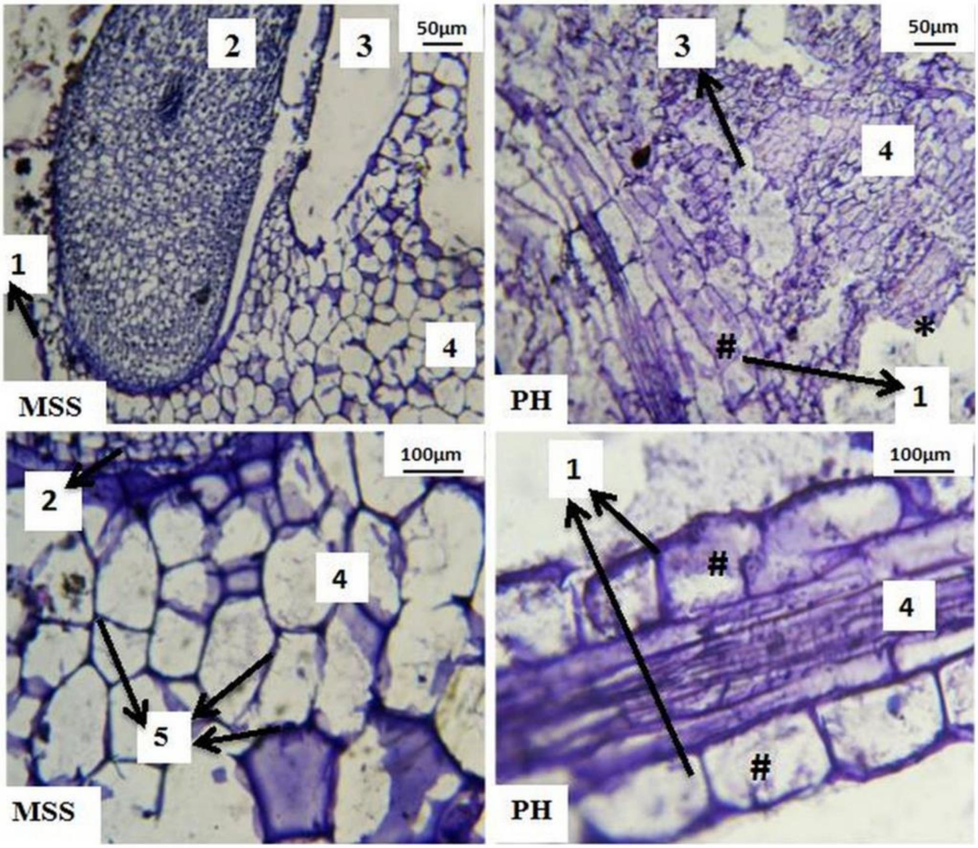

Figure 2. *Histopathological structure of seagrass leaves (H&E) at Phosphate(PH, longitudinal section) compared to Marine Science Station (MSS, cross section). 1: Epidermis, 2: Apical meristematic tissue 3: Air lacunae, 4: Mesophyllcell, 5: Chloroplasts. Phosphate seagrass showing cell necrosis (*) and swelling of Epidermis (#), loss of cell organization and parenchyma structure.*

In conclusion, the screening of new species among toxicity tolerant aquatic organisms will bring large advances in phytoremediation strategies of contaminated aquatic sites. Further investigations may help to understand if seagrass might be a toxicity tolerant model to be grown in slightly toxic contaminated sediments for phytoremediation and restoration purposes.

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