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Research Article

Elemental analysis of *Potamogeton pusillus* leaves from shatt al-arab river and al-hawizeh marshes in southern Iraq

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Abstract: *Potamogeton pusillus* is reported from Iraqi wetlands in two sites of Shatt al-Arab River and Al-Hawizeh marshes after its disappearance in 2003. This research also aimed to study the habitat, growth, distribution, and ecological condition in the collected area as well as providing SEM/EDX to determine the elemental composition of the plant leaf. The samples collected in 2019 from Shatt al-Arab and Al-Hawizeh, southern Iraq. Physical and chemical parameters of the water were measured as well. Concentrations of Na, K, Ca, Mg and total hardness were measured using Ethylenediaminetetraacetic acid (EDTA) method. Flame photometry method was used to determine Na+ and K+ ions. PO^4, Cl^- and $NO3^-$ were determined by spectrophotometry method. Trace elements were measured in the leaves of *P. pusillus* by SEM-EDX. The results showed that the soil was loam clay with neutral to slightly alkaline conditions and low conductivity values were typical for these moderately closed systems. The concentrations of Ca and Mg in the water were high (538 and 212 mg/l, respectively). Salinity and dissolved oxygen were 3.5 ppt and 7.48 mg/l, respectively. $PO^4$was 0.64 mg/l, and average turbidity 16.9 NTU. Those of the Shatt al-Arab River have more carbon (58.10%) and oxygen (40.14%), but no F, Mg, Zn, and Sm; The samples of Al-Hawizeh had lower oxygen (40.12%) and carbon (35.79%). The results showed moderate amounts of Na in Al-Hawizeh and Shatt al-Arab (5.97and 0.61%, respectively). Mg (2.37%), Cl (1.12%), K (0.88%) and carbon were found at trace levels. *Potamogeton pusillus* can accumulate pollutions found in its habitat even containing levels of salinity. It can be concluded that the pollution by oil pipelines beneath the water in the Shatt al-Arab region have stunted growth, *P. pusillus* plant, the leaves was fewer in number and smaller in size. The concentrations of F, Na, and Mg were higher in the Al-Hawizeh group.

Keywords: Al-Hawizeh, Elements analysis, SEM-EDX, Shatt al-Arab.

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Introduction

Among aquatic macrophytes, the pondweeds of the genus *Potamogeton* L. (Potamogetonaceae) are common wetland plants growing in freshwater lakes, rivers, ponds, and streams. *Potamogeton* species can be found even in brackish waters due to its highly plastic morphological features (Catling & Dobson 1985; Kaplan & Štěpánek 2003). Pondweeds can tolerate a wide variety of environmental conditions and they are found most commonly in water depths between 1-3m, but can survive 7m deep as well. *Potamogeton* plants are best grown in soft organic sediments occurring in sandy and vigorous sediments (Tobiessen & Snow 1984; Nichols 1992; Bolduan et al. 1994). The genus *Potamogeton* is cosmopolitan (Wieglet & Kaplan 1998) with about 72 species and 99 hybrids. Also, chromosomes for *Potamogeton* species are small that do not make them reliable for cytology studied, including chromosome numbers and hybridization (Les & Philbrick 1993; Iida &

Kadono 2002; Wang et al. 2007).

In Iraq, six species of the genus *Potamogeton* including, *P. pectinatus*, *P. pusillus*, *P. crispus*, *P. perfoliatus*, *P. nodosus,* and *P. lucens* are found (Alsaadi & Almousawi 1984; Al-Saadi & Al-Mousawi 1988; Abdulhasan 2009; Fadhel 2013; Hassan et al. 2016). These plants are sometimes annuals but are often perennial and typically produce rhizomes; all leaves are submerged (Townsted & Guest 1985; Monferrán et al. 2009). In the last decade, several exotic and rare plant communities were discovered in aquatic and marsh vegetation in Iraq (Al-Mayah & Al-Saadi 2013). After 2003 some species of *Potamogeton* have disappeared from Iraq. *Potamogeton pusillus* had disappeared from the Iraqi wetlands for 50 years. However, we report it in two sites in Iraq viz. Shatt al-Arab and Al-Hawizeh.

Potamogeton pusillus is an annual aquatic plant with 25-40cm long, slender, or terete, thin and branched stems. The leaves can be submerged, sessile, and narrowly linear with acute or mucronate features at the apex. It is found in freshwater, pools, canals, and ditches from April to October (Townsend & Guest 1985). Many studies have reported that submerged aquatic plants are essential to aquatic ecosystems. They have a crucial role in water quality, water purification, and environmental restoration (Jones 2002; Díaz & Massol-Deyá 2003; Demırezen & Aksoy 2004; Chen et al. 2009; Takeda et al. 2014). Many researchers have suggested that aquatic macrophytes also can be used for biomonitoring because they can accumulate heavy metals without apparent noxious effects. Biomonitoring has determined sub-lethal levels of bioaccumulation and phytoremediation within the tissues of plants and indicate the quantity of accumulated within the period (Shine et al. 1998; Mishra et al. 2006; Srivastava et al. 2006; Monferrán et al. 2009). *Potamogeton pusillus* can accumulate a substantial amount of Cu and Cr (Monferrán et al. 2012). Many researchers have studied the metal accumulation properties of *Potamogeton* species (Fritioff & Greger 2006; Duman et al. 2006; Peng et al. 2008). Harguinteguy et al. (2016) reported that *P. pusillus* is a good bioindicator of heavy metal pollution in wetlands. It can be used for bioremediation of aquifers polluted with heavy metals. It also affects water quality and plankton (Abbasi et al., 1992; Monferrán et al. 2009; Takeda et al. 2014).

Scanning Electron Microscopy with Energy Dispersive Spectroscopy (SEM/EDS) are used to study contamination in industrial places (Miler & Gosar 2009). SEM can also evaluate morphological changes in cell composition after metal binding. When combined with EDX, it can determine the distribution of different heavy metals in polluted places (Raize et al. 2004). Hence, this research aimed to study the habitat, growth, distribution, and ecology condition of *P. pusillus* in Iraq and SEM/EDX analysis to determine the elemental composition of the plant leaf.

Material and Methods

Study area*:* *Potamogeton pusillus* were found in the Basrah city between 47°45.202N, 30°39.844E and Al-Hawizeh 48°49.891N-29°39.209E. Several trips to the marshlands of southern Iraq were made in the autumn-winter of 2019. The specimens were found in Shatt al-Arab River (Hartha region 25km north of Basrah) and the Al-Hawizeh area in the southern Iraqi. Fresh *P. pusillus* were collected in January and February of 2019 at two different locations and photographed (Fig. 1). The samples were taken to the Department of Biology, College of Science, University of Basrah.

Measured parameters*:* Physical and chemical parameters of the water, including air temperature, turbidity, pH, and electrical conductivity (EC) were measured in the sampling stations. In addition, Ca, Mg, Na, No_3, and Po_4 were measured based on APHA (1995) and Motsara & Roy (2008). Water samples in 1-liter polythene bottles were collected and transferred to the lab at 4°C. The bottles had been soaked in 10% nitric acid (HNO_3) and rinsed twice with the distilled water before sampling. Water temperature, pH, and electrical conductivity (EC)

Fig.1. The collected *Potamogeton pusillus*.

were determined onsite using pre-calibrated electrodes. Triplicate samples for their chemicals were analyzed according to APHA (1985).

Concentrations of Na, K, Ca, Mg, and total hardness were measured using the Ethylenediamine-tetraacetic acid (EDTA) method. The flame photometry method was used to determine Na^+ and K^+ ions. PO_4 was determined via the ascorbic acid method using a spectrophotometer. Major anions Cl^-, NO_3^- was determined by the ultraviolet spectro-photometry method.

SEM analysis of leaves*:* EDX spectroscopy was performed at the Physics Department, College of Science, Basrah University. The elemental composition of the plants and the potential for monitoring heavy metals was evaluated by studying plant uptake. Three specimens were collected from the collected *P. pusillus* species from the Shatt al-Arab Rive and Al-Hawizeh area

Na, Mg, Cl, K, Ca, and P were measured in the water and leaves of the plants (Table 1). SEM/EDS data were collected for all samples, and one typical microphotograph and spectrum are shown in Figures 2 and 3. The percentages of the mass and atom elements of samples from studied sites are reported in Table 2. Measurements and diagnostic features of the species were noted. Herbarium specimens were prepared and deposited in Basrah University Herbarium. Plant associations and those species accompanying *P. pusillus* were identified by a light microscope and photographed.

Table 1. Physical and chemical analysis of water.

Factors	Value	Factors	Value
pH	8.5-8.8	Salinity	3.5ppt
Water temperature	14°C	No_3	2.92mg/L
Air temperature	21°C	Po_4	0.64mg/L
Turbidity	16.9NTU	Ca	538mg/L
Dissolve oxygen	7.48mg/L	Mg	212mg/L
Electric conductivity	6.44mS/cm	Na	732.4mg/L

Results and Discussion

The chemical characteristics and metals concentrations of the water are presented in Table 1. Average water turbidity was 16.9 NTU, and phenolphthalein assays indicated pH between 8.5 and 8.8. The metals with the greatest concentrations in wetland water were Ca and Mg. Some samples had high Ca, Na, and Mg (538, 732.4, and 212mg/l, respectively). The lowest concentration of phosphate was 0.64mg/l, and dissolved oxygen was moderate.

The elemental analyses and areas where EDX was performed have been presented in Figures 2 and 3; the data are presented in Table 2. The *P. pusillus*

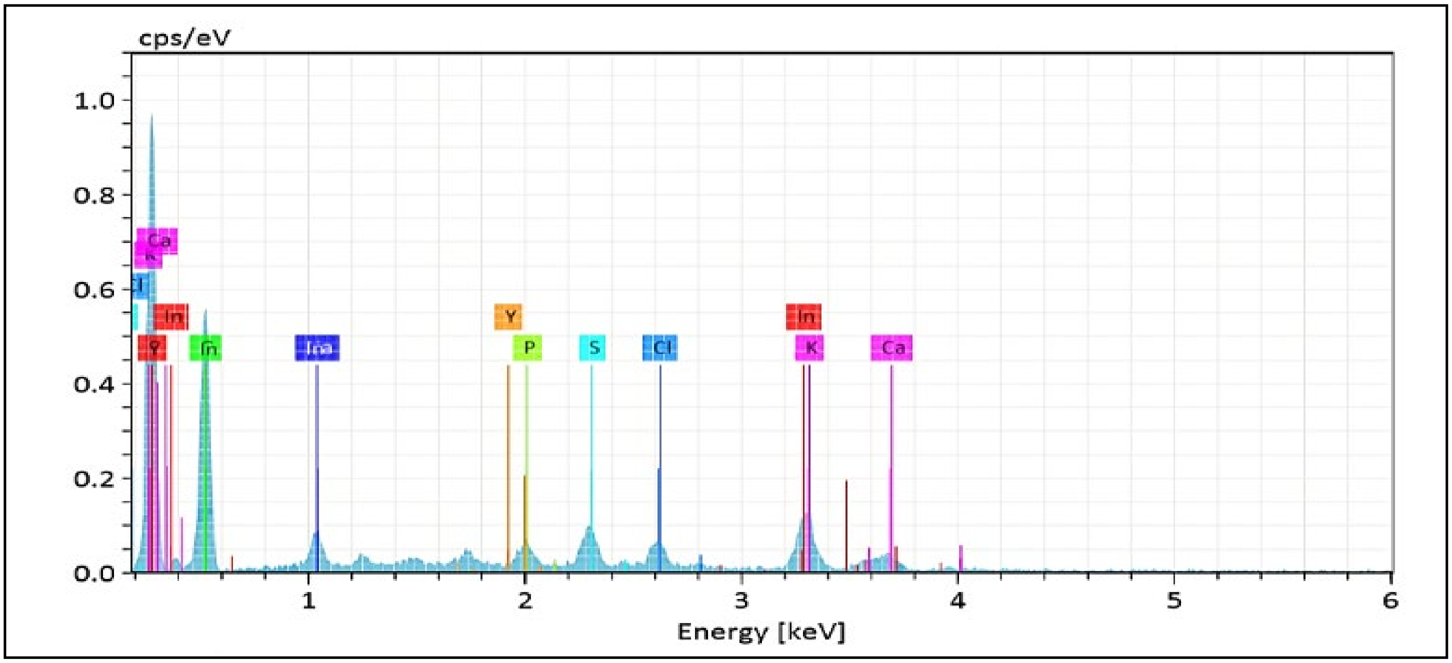

Fig.2. SEM/ EDX of *Potamogeton pusillus* in leaves of Shatt al-Arab site.

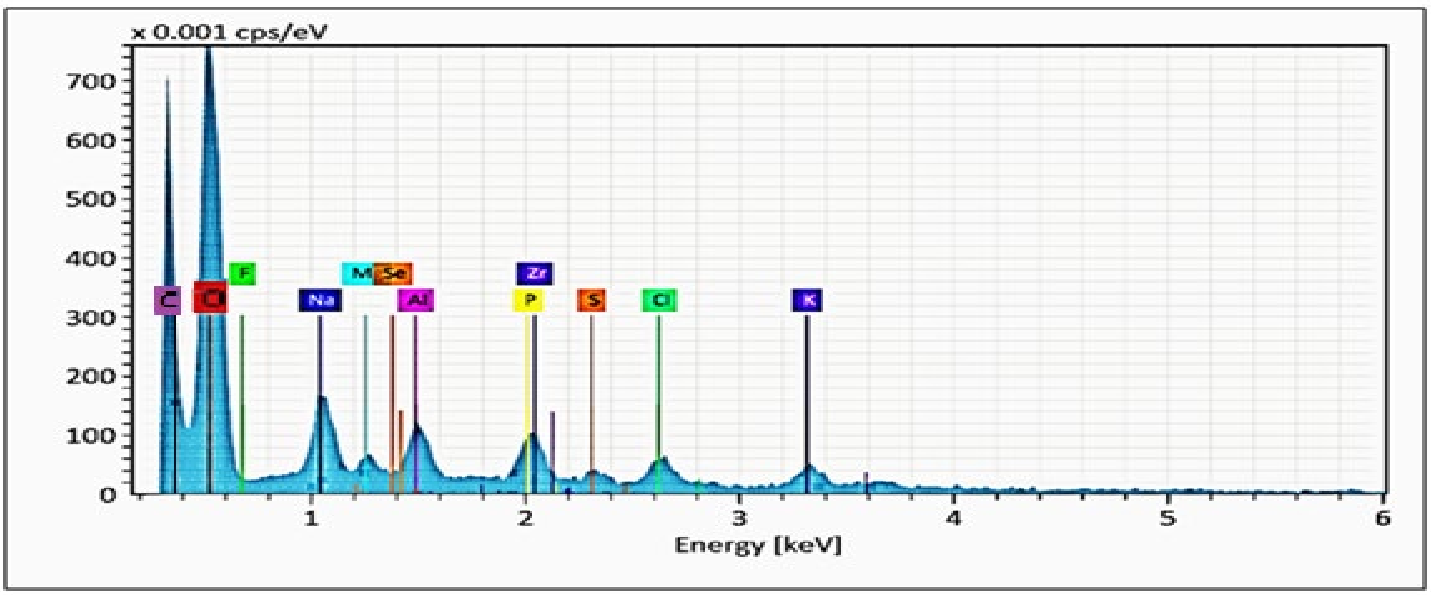

Fig3. SEM/ EDX of *Potamogeton pusillus* in leaves of Al-Hawizeh site

leaves at the Al-Hawizeh and Shatt al-Arab sites contained 12 and 11 elements, respectively, but their qualitative and quantitative analyses were different. Those of the Al-Hawizeh contain oxygen (40.12%), carbon (35.79%), F (11.18%), Na (5.97%), Mg (2.37%), and Po_4 (1.32%). Low phosphorous (0.06%) at the Shatt al-Arab site (Table 2) is a significant factor in productivity of the plants in many agriculture ecosystems; it can play essential roles in enzymatic phosphorylation reactions (Zhong et al. 2018). The quantities of sulfur, chlorine, potassium, zirconium, and samarium were less than 1% each, but sodium was found in moderate level (0.61% at the Shatt al-Arab site). In addition to Mg (2.37%), the results showed trace amounts of Cl (1.12%) and S (0.71%) at the Al-Hawizeh site.

Table 1. Elements components of *Potamogeton pusillus*.

Element	Al-Hawizeh Site		Shatt al-Arab Site	
	Mass %	Atom %	Mass %	Atom %
Oxygen	54.12	40.12	45.44	40.14
Carbon	43.27	35.79	49.38	58.10
Fluorine	17.03	11.18	-	-
Sodium	11.01	5.97	1	0.61
Magnesium	4.62	2.37	-	-
Phosphorus	3.28	1.32	0.14	0.06
Sulfur	1.83	0.71	0.40	0.18
Chlorine	3.18	1.12	0.31	0.12
Potassium	2.75	0.88	1.17	0.42
Zirconium	3.56	0.49	-	-
Selenium	0.22	0.03	-	-
Aluminum	0.12	0.02	-	-
calcium	-	-	0.56	0.20
Yttrium	-	-	0.38	0.06
Indium	-	-	0.83	0.10
Percentage of Mass and Atom in areas study	144.99	100	99.61	100

Plant samples from the Shatt al-Arab were rich in carbon (58.10%) and oxygen (40.14%) and containing calcium (0.20%), yttrium (0.06%), and indium (0.10%), but no-fluorine, magnesium, zirconium, or samarium. The leaves in the Al-Hawizeh had lower carbon and oxygen. The phreatic aquifer has been shown to interact with wetland water closely and could have contributed to its chloride content (Azeez et al. 2000). Morgan (1985) reported that heavy elements gave greater detection limits because the background was higher because the salinity was high. The trace elements of Fe, Mg, Na, K, and Ca were low in the plants. Potassium, necessary for synthesizing some proteins and enzymatic cofactors, has also been essential for transporting nutrients into cells. Without the trace elements, nutrients would not be able to enter cells. Minerals have been described as essential parts of nucleoproteins, metalloproteins, chromoproteins, and lipoproteins (Krupnova et al. 2018).

The results showed difference in the accumulation of elements between in the *P. pusillus* in two sites. The results showed the concentration of Na, K, and Mg was higher in the Al-Hawizeh, this may be due to the large size of leaves and the surface area, which agree with (Warwick & Bailey 1997) suggesting that plants capable of absorbing Na^+ into leaf vacuoles, and the concentration of Na^+ and K^+ ratio can be reached to 35 in the leaves.

In submerged macrophytes, elements absorbed from the water column directly into the leaves, and transfer to the upper plant tissues by absorption across the roots; this transfer from the roots to leaves is important in the cycling of chemicals in aquatic ecosystems (Warwick & Bailey 1997; Jackson 1998). Some researchers expected that increased temperatures, pH, the shape of leaves, and salinities could alter the nutritive content, morphometrics, and chemical traits of some Potamogetonaceae family species (*Stuckonis pectinata*) (Wittyngham et al. 2019) i.e. aquatic macrophytes with a well-developed root-rhizome system (Demırezen & Aksoy 2004).

Elements uptake by leaves is important when the metal concentrations in the environment are high or metals in the sediment are bound in not readily available compounds. Demırezen and Aksoy (2004) reported that the accumulation of elements depends on biotic and abiotic factors such as temperature and pH. Different elements accumulations may also be due to different proportions of stems to leaves. Some elements have toxic effects at high concentrations, and other non-essential nutrients are toxic to organisms even at low concentrations. Many metals cause inhibition of some enzyme associated with chlorophyll, changes the lipid composition of the plasma membrane, protein synthesis, carotenoid content decreased, limited plant growth, changes in leaf color, reduced both intracellular and extracellular, inhibited seed germination, high capacity to accumulate heavy metals in its tissues (Abbasi et al. 1992; Upadhyay et al. 2014; Harguinteguy et al. 2016).

The alkaline earth elements of Na, Mg, and K were higher in the Al-Hawizeh site. Both sulfur and chlorine were slightly higher in the Al-Hawizeh site. These results were in agreement with report of Takeda et al. (2014). Because the *P. pusillus* leaves had no exclusion mechanism, as its roots did; they seem to absorb some metals from the atmosphere during photosynthesis (Ramamurthy & Kannan 2009; Krupnova et al. 2018).

Because *P. pusillus* accumulated pollution and was in a saline environment, its growth in the leaves, stems, and roots was reduced at the Shatt al-Arab site. Pollution was discovered to affect the size and texture of the leaves, the number of leaves, their thickness, branching of the veins, and loop formation (Jang et al. 2005; Yeh & Wu 2009). The collected samples in this study from the Shatt al-Arab had smaller leaves, fewer leaves, and less growth than those of the Al-Hawizeh, where the plants were comparatively healthier, larger, and thriving.

Finally, we found no harmful heavy metals in any samples collected from either location. Not only has their presence shown depend on plant location, but accurate quantitative analyses have also been proven fundamental to both basic and applied plant studies, given their impact on human health. Thus, the morphology and chemical properties of plant extracts are useful for herbal medicines.

Conclusions

This study concluded due to pollution, plants in the Shatt al-Arab region with stunted growth by fewer and smaller leaves. The concentrations of fluoride, sodium, and magnesium are higher at the Al-Hawizeh site, possibly because of the greater salinity. The use of EDX facilitates sample analyses of this kind.

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