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***Salicornia europea*, a Bioaccumulator
in Maharloo Salt Lake Region**

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Abstract: Osmotic potential, percent of ash, mineral salt content and heavy metals of the halophyte *Salicornia europea*, collected from Maharloo region, Shiraz, were determined. Sodium content in ash was found to be up to 30%, equivalent to 31 500 $\mu\text{g g}^{-1}$ dry matter. The high sodium content of the plant especially in the shoot as compared with normal content of 10 $\mu\text{g g}^{-1}$ in non-halophytes suggests that the plant can be considered as a sodium hyperaccumulator and it can be used as a means of phytoremediation for saline media, including soil and water. The content of mineral nutrients K, Mg, Ca, Zn and Cu were also determined and were compared with the normal amounts needed for plant growth. The results indicate that mobile elements such as Na, K and Mg are mostly transported to the shoot, where, they may be compartmentalized in the leaf cell vacuoles; whereas the immobile elements such as Ca, Cu, Zn and Fe are mainly retained in the root. The amount of Mg in the shoot and also the amounts of copper and iron in the root are much higher than the normal amount found in other plants; indicating that the root tissues of the plant can be further studied to be used as biosorbent for heavy metal contaminants in wastewater and soil. The amounts of heavy metals such as Ni, Cr, Cd, Pb and Hg were also determined. The results indicate that the amounts of these heavy metals are relatively high in this plant.

Key words: *Salicornia europea*, halophytes, phytoremediation, heavy metals, bioaccumulator

Introduction

With respect to salt tolerance, there is a wide spectrum of plants ranging from very sensitive glycophytes showing the effects of salt injury at a concentration of less than 50 mM; to halophytes that complete their life cycles at a salt concentration of as high as 500 mM (Clipson *et al.*, 1985; Ungar, 1999; Volkmar *et al.*, 1997). Unfortunately, the major economical plants are almost universally non-halophytic. For example, bean yield is inhibited almost completely at 50 mM NaCl (Mass and Grieve, 1987). Usually there is a threshold concentration of salt above which glycophytes begin to show signs of growth inhibition, leaf discoloration and loss of dry weight. Among crops, maize, onion, citrus, pecan, lettuce and bean are highly sensitive to salt; cotton and barley are moderately tolerant; and sugar beet and date palms are highly tolerant (Greenway and Munns, 1980).

Plant mineral nutrition, a much older field of study than phytoremediation, has recently focused on enhancing the ability of plants to accumulate mineral nutrients from the environment more efficiently. This effort has been directed at both reducing the amount of mineral fertilizer input into agricultural system and at alleviating human diseases caused by deficiencies in plant-derived from deficiency of essential and beneficial micro nutrients, such as Fe, Zn and Se. The interesting

convergence of these two fields has led to realization that phytoremediation and enhanced mineral nutrition are two sides of the same coin (Salt *et al.*, 1988). Plants possess different potential mechanisms that may be involved in the detoxification of heavy metals and thus tolerance to metal stress. Some of these mechanisms are controlled at the cellular level (Hall, 2002; Volkmar *et al.*, 1997; Hasegawa *et al.*, 2000) and some, at whole plant (Cobbet *et al.*, 2000). *Salicornia europaea* is a halophyte living around salt marshes. In the present study, the salt content of this plant ash has been determined both in the root and shoot. The aim of the research is to study the potential of this plant to accumulate salts and its capacity to be used as a means of phytoremediation in saline water and soil. Being consumed as a source of food by both human and animals, it is also a valuable source of essential micronutrients such as, Fe and Zn, protein, determining the levels of heavy metals in this plant is of interest to animal nutritionists.

Materials and Methods

This study was conducted in 2002 in Biology Department, College of Sciences, Shiraz University, Iran.

Salicornia plants were collected from the vicinity of Maharloo salt lake near Shiraz, in Fars Province. After washing the wet soil particles from the Plant surface, leaf cell sap was extracted and its osmotic potential was determined by cryoscopic method. Both shoot and root fresh and dry weights were determined. For drying, plant materials were kept in an oven set at 75°C for 24 h. To determine the mineral content,

The dry tissues were ashed in a furnace set at 600 °C for 8 h. The percentage of dry matter and ash were determined for both shoot and root. Mineral content in shoot and root ash was determined by both XRF and atomic absorption spectrophotometer.

Results and Discussion

The osmotic potential of leaves cell sap as determined by cryoscopic method, was about -8.5 MPa, which is much lower than the amount reported for glycophytes (-1 to -3 Mpa) indicating the presence of a very high soluble organic and inorganic materials. The shoot and root dry weight and the percentage of ash are shown in Table 1 and some of the elements present in both shoot and root ashes are shown in Table 2. There was a considerable amount of sodium both in shoot and root ashes, amounting to 30 and 24% of ashes in these tissues respectively, corresponding to 31500 and 11040 $\mu\text{g g}^{-1}$ dry weight. For C_4 and CAM plants, Na^+ is a required macronutrient (10 $\mu\text{g g}^{-1}$ dry weight) needed for regeneration of phosphoenolpyruvate in C_4 and CAM Pathway (Johnstone *et al.*, 1988). In some halophytes sodium can replace potassium but not completely in its physiological functions. Since NaCl is the most abundant salt encountered by plants under salinity stress, transport systems that facilitate compartmentation of Na^+ I vacuole are of critical importance (Binzel, 1988). In this study the sodium content in dry matter of the shoot is much higher than the root (Table 2). Na^+ is mainly transported to the shoots to be accumulated in the leaf vacuoles. The succulent feature of this plant leaves is apparently due to this high accumulation of Na^+ . Vacuolar compartmentation of Na^+ probably results in part from the activity of a family of Na^+ - H^+ antiporters (Aspe, 2002). According to Table 2 and 3, only a small portion of absorbed K^+ is transported to the shoot. The results also show much higher Na^+ content than K^+ both in root and shoot. Na^+ has been shown to compete with K^+ in inward rectifying channels (Anderson *et al.*, 1999). Other reports

Table 1: Content of dry matter and ash in root and shoot of *Salicornia europaea* %

	Fresh weight	Dry weight	Ash in dry matter
Root	100	20.4	4.5
Shoot	100	22.7	10.5

Table 2: Mineral content in dry matter of *Salicornia europaea* ($\mu\text{g g}^{-1}$) compared with normal content in plant dry matter

Element	Root	Shoot	Normal
Na	11040	31500	10
K	200	2800	2000
Mg	1800	2400	2000
Ca	6400	2400	5000
Cu	48	405	6
ZN	21.56	8	20
Fe	528.50	92	100

Table 3: Heavy metal content in cell sap of root and shoot of *Salicornia europaea* ($\mu\text{g g}^{-1}$)

Heavy metals	Root	Shoot	Maximum safe level in drinking water
Ni	0.8	0.8	0.10
Cr	1.7	7.2	0.10
Cd	0.0	0.0	0.005
Pb	0.43	1.1	0.0015
Hg	0.0	0.0	0.002

have suggested K^+ outward rectifying channels might facilitate Na^+ influx as well. The high affinity K^+ transporter (HKT) from wheat (Schatman *et al.*, 1991) and low affinity cation transporter (LCT1) from wheat (Schatman *et al.*, 1997) facilitate Na^+ transport with isolated membranes, providing evidence to support their possible function in Na^+ uptake since high affinity K^+ transporter (HKT1) transcripts are expressed predominately in the roots, it most probably functions in the control of Na^+ flux into the xylem for export to the shoot. Both Ca^{2+} and K^+ affect intracellular Na^+ concentration (Zhong and Lauchli, 1994).

The elements Ni, Cr, Cd, Pb and Hg were not detectable in ash, so they were checked in cell sap by means of atomic absorption spectrophotometer (Table 3).

As shown in Table 3, Cd and Hg were not detected in cell sap. The amount of Ni was shown to be $0.8 \mu\text{g g}^{-1}$ both in the root and shoot cell sap, which is 8 times the permissible amount in drinking water. The amount of Cr was shown to be up to $7.2 \mu\text{g g}^{-1}$ (72 times) and Pb $1.1 \mu\text{g g}^{-1}$ (1000 times) as compared with the permissible level in drinking water. So, the amounts of these heavy metals in the cell sap may be toxic for animal consumption. Many soil trace minerals such as Cu, Fe, Mn, Ni and Zn are absorbed by plant roots. Although these elements are essential (Epstein, 1972, 1999) they can also be potentially toxic. Plants possess complex mechanisms to control them. For reasons not yet clear, plants also have the ability to acquire and detoxify non-essential elements such as Cd, Cr and Pb (Van Hoof *et al.*, 2001).

The majority of plants grown in metal enriched soils have mechanisms that exclude these potentially toxic metals from their shoots using their roots to either pump metal ions back into the soil (Van Hoof *et al.*, 2001), or store them in their root vacuolar compartment. There are also much evidence showing that salinity increases intracellular Ca^{2+} concentration. Increased intracellular Ca^{2+} concentration in corn root protoplast (Lynch *et al.*, 1989) in response to salinity, has been attributed to the role of calcium in signal transduction (Reid *et al.*, 1993). In unicellular alga *Dunaliella salina*, Ca^{2+} absorption from external medium increases and the magnitude of this increase depends on the degree of salt

stress (Ko and Lee, 1995). Other reports indicate increased cytosolic Ca^{2+} concentration in barley root protoplast and also in cells of wheat aleurone layer in response to salinity (Bittisnich *et al.*, 1989; Bush, 1996). H^+ pumps in the plasma membrane and tonoplast provide the driving force for secondary transport of ions. A vacuolar H^+ -ATP ase generates a Δ pH and membrane potential across the tonoplast. Activity of these pumps is required for secondary transport of ions associated with plant responses to salinity stress; since the activity of these H^+ - pumps is increased by salinity (Hasegawa *et al.*, 2000). Vacuolar compartmentation of Na^+ results in part from the activity of a family of Na^+/H^+ antiporters (Aspe, 2002). Little is known about the mechanism of metal translocation to the shoot, though it has been suggested that coordination of the metal ion with free histidine may play a role (Kramer *et al.*, 1996). Within these cells metal ions are detoxified by compartmentalization in the vacuoles (Kupper *et al.*, 1999; Kramer *et al.*, 2000), where they are most likely coordinated by organic acids (Salt *et al.*, 1998; Taiz and Zeiger, 2002) the succulent feature of the plant might be an evidence for this. Recently researchers have focused their attention on the various metal hyperaccumulating species, which are close relatives of *Arabidopsis thaliana* (Macnair *et al.*, 1999B). Hyperaccumulators are species capable of accumulating metals at levels 100-fold greater than those typically measured in shoots of the common non-accumulator plants (Lasat, 2002; Baker *et al.*, 2000). Emerging knowledge will hopefully lead to identification of new plants useful for remediation of metal polluted soils as well as plants with enhanced mineral composition for improved human nutrition and health (Taiz and Zeiger, 2002). This plant is used as a salad vegetable and sometimes as main dish together with mushroom, fish and spices. Having the potential to accumulate heavy metals from the surrounding, one should make sure of its suitability as a food source when grown in polluted habitats like Maharloo Salt Lake region. Our unpublished data has shown that it can also be considered as a source of protein.

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