

## GROWTH, OSMOREGULATION AND ION ACCUMULATION IN THE COASTAL HALOPHYTE *ARTHROCNEMUM INDICUM* UNDER FIELD CONDITIONS

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**ABSTRACT:** Life cycle and population biology of a perennial halophyte *Arthrocnemum indicum* Willd. was studied from February 1992 to January 1993. During the 12 months, the population was exposed to great variations in soil salinity from 35 to 58 ms cm<sup>-1</sup> and soil moisture ranging from flood to drought levels. Seasonal changes in dry weight is directly related to soil salinity stress. When salinity levels become low, the dry matter production increases. A little increase in dry weight from April to July indicates that more negative soil water potentials were limiting plant growth. Proline content increased considerably during the dry season with a corresponding increase in salinity. Water soluble oxalate did not vary much with changes in salinity.

**KEY WORDS:** *Arthrocnemum indicum* - halophytes - osmoregulation - Karachi coast.

### INTRODUCTION

The *Arthrocnemum indicum* Willd. (Chenopodiaceae) is a perennial halophytic shrub, commonly found in tropical salt marshes which are frequently inundated with seawater. Karim and Qadir (1979) described that *A. indicum* with its bushy appearance occurs in almost pure patches rarely with other species like *Limonium stocksii*, *Cressa cretica*, *Aeluropus insignis* and *Suaeda monoica*. Williams (1960) showed that *A. indicum* requires low concentration of sodium chloride for its growth, however, frequent inundation of tidal water adds lot of sodium salts to the soil which increases its osmotic concentration. The physiological, morphological and life history characteristics of salt marsh plants are the result of severe selection for tolerance of high ionic concentrations, low water potentials, hypoxic soil conditions, periodic suspension of gas exchange with the atmosphere, and scouring currents of water to varying degrees (Davy and Costa, 1992).

Seasonal variation of soil salinity in saline habitats is well documented and is directly influenced by the fluctuations in soil moisture levels (Ungar, 1973; Waisel, 1972). Pennings and Callaway (1992) showed that growth of *Arthrocnemum subterminalis* is strongly reduced by high salinity but unaffected by intraspecific competition in high stress environment. He also showed that the K/Na and Cl/Na ratios were higher in shoots than in roots and chloride uptake rates were twice as high as sodium uptake rates in Chenopodiaceae.

Proline along with other organic solutes accumulates in the cytoplasm and lowers the solute potential, balancing a low solute potential in the vacuole due to the accumulation of salt and other solutes (Stewart and Lee, 1974, Wyn Jones *et al.*, 1977). Voetberg and Stewart (1984) showed a linear relationship between increase in proline and Na<sup>+</sup> concentrations in barley leaves. If proline does accumulate in cytoplasm to balance the accumulation of salts outside of the cytoplasm, proline concentration is expected to be directly proportional to salt concentrations in the tissue. In mature leaves of 2 halophytes, proline concentrations reached a constant proportion of Na<sup>+</sup> and Cl<sup>-</sup> concentrations (Treichel, 1975). Proline was shown to be directly proportional to osmotic

potentials, which were mostly accounted for by  $\text{Na}^+$  and  $\text{K}^+$  salts in two other halophytes (Neals and Sharkey, 1981, Storey *et al.*, 1977). The accumulation of oxalate in halophytic Chenopodiaceae affects their tissue ion relations (Albert and Popp, 1977; Austenfeld, 1974; Davis, 1981).

The purpose of this investigation is to determine the effect of seawater inundation on the growth, ionic adjustment, and osmoregulation of *A. indicum* under natural conditions.

## MATERIALS AND METHODS

### STUDY SITE

Karachi city lies between the latitude  $24^{\circ} 51'$  North and longitude  $65^{\circ} 55'$  East. The study area is located in Manora channel near Sandspit. This salt marsh is regularly inundated with seawater. The area close to sea is dominated by *Avicennia marina* followed by an almost pure population of *Arthrocnemum indicum*.

Ten soil samples were collected monthly from *A. indicum* community and were dried and sieved with a 2 mm sieve before analysis. Soil moisture was measured by weighing known quantities of samples, oven-drying them for 24 h and reweighing them to determine the water loss. Percent soil moisture was calculated as percentage weight of water in dry soil. Ten gram of soil mixed in 50 ml of distilled water were shaken and filtered using Whatman # 1 filter paper. pH and inorganic elements;  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{NO}_3^-$  and  $\text{Cl}^-$  (Radiometer Ion 85, Ion Analyzer) and soil conductivity (Radiometer CD- 83, conductivity meter) were measured.

Five plants were collected randomly from the population of *A. indicum* monthly for a year. Plants were separated into root, stem and leaves. *Arthrocnemum indicum* do not have true leaves but they form jointed, seemingly leafless stem; internodal-tube usually club shaped, shriveling and usually falling away from the stem ultimately (hence interpreted as bladeless connate bases of opposite leaves), each with a pan of vague opposite, terminal cups embracing the base of the next segment. The green leaf portion is considered here as leaf while woody portion as stem. Fresh weight was recorded before and after drying the material in an oven at  $80^{\circ}\text{C}$  for 48 h.

Five oven dried samples sorted into root, stem and leaf were ashed in a muffle furnace (National China Export Machinery Corporation) for 24 h at  $450^{\circ}\text{C}$ . The ash was dissolved in 5 ml HCl (w/v) and warmed to dissolve the residue. Half ml of concentrated  $\text{HNO}_3$  was added and then evaporated to dryness. Two ml of HCl was added and warmed slightly to dissolve the residue, filtered through a Whatman # 1 filter paper and the volume was made up to 10 ml. The  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{NO}_3^-$  and  $\text{Cl}^-$  ions were determined using ion-85 (Radiometer ion analyzer). Oxalate was determined using the Moir (1953) method and proline according to Bates *et al.* (1973) as modified by Karimi and Ungar (1986).

## RESULTS

### CHARACTERISTICS OF VEGETATION

Phytosociological survey showed (data not shown) that the frequently inundated salt marsh community has an almost pure population of *A. indicum*. Few individuals

of *Atriplex stocksii*, *Suaeda fruticosa*, *Aeluropus insignis* and *Cressa cretica* were present.

### PHYSICAL AND CHEMICAL PROPERTIES OF SOIL

Soil pH was neutral from February to May, while it was basic for the rest of the year (Table I). Soil conductivity remained high throughout the year except for the period when it received winter or summer rains (Table I). Moisture content of the soil varied from 18 to 33% (Table I). This variation corroborates with the fluctuation in the conductivity.

**Table I: Seasonal variation in the pH, conductivity (ms/cm<sup>2</sup>) and maximum water holding capacity (M.W.H.C.) (%) of the soil from *Arthrocnemum indicum* community.**

Month	pH	Conductivity (ms/cm <sup>2</sup> )	M.W.H.C. (%)
February	7.73±0.15	21.70±3.64	30.50±4.28
March	6.80±0.43	26.40±3.73	29.06±6.43
April	7.77±0.21	33.70±8.01	20.00±2.83
May	7.78±0.31	58.30±7.02	18.47±7.04
June	8.12±0.07	48.80±9.85	23.70±4.43
July	8.62±0.07	54.50±8.22	26.76±5.52
August	8.13±0.06	34.90±5.84	33.07±3.49
September	8.27±0.08	54.70±10.2	29.37±11.56
October	8.56±0.10	56.16±6.07	29.84±9.13
November	8.21±0.06	51.00±8.45	32.80±9.28
December	8.24±0.11	56.86±5.03	27.78±2.35
January	8.39±0.14	52.61±7.13	25.79±3.38

Sodium (1060 ppm), nitrate (800 ppm) and chloride (800 ppm) concentrations remained high throughout the growing season while potassium concentration remained low (100 ppm) in the soil. A one way analysis of variance shows a significant difference between months for all the ions studied (Table II).

**Table II: Results of one way analysis of variance of soil characteristics by month.**

Source of variation	df	F	P
Chloride	11	23.7	0.001
Nitrate	11	11.0	0.001
Potassium	11	22.2	0.001
Sodium	11	14.5	0.001

### TISSUE ION CONTENT

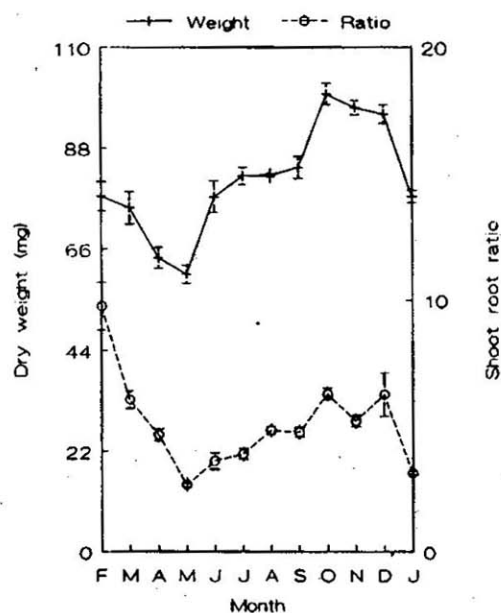
Leaf shows higher concentrations of  $\text{Na}^+$  as compared to stem and root, while nitrate concentrations were high in root, stem and low in leaf. Potassium concentration remained the same in all parts of plants studied. A two way analysis of variance of ion concentration by month and plant parts shows a significant difference among all months and plant parts for all the ions studied (Table III). Periods after summer and winter rains show a significant drop in  $\text{Na}^+$  and  $\text{Cl}^-$  concentrations.

**Table III: Results of two way analysis of variance of plant characteristics by month and plant part.**

Dependent variable	Independent variable		
	Month (M)	Plant parts (PP)	MxPP
Chloride	111.2***	714.9***	302.1***
Nitrate	5.4***	376.8***	1.8***
Potassium	19.6***	16.8***	5.3***
Sodium	9.7***	3050.9***	1.8***

### BIOMASS ALLOCATION

Highest percentage of biomass was allocated to leaf, whereas, root and stem show similar allocations (Fig. 1). Leaves show tremendous growth from September to December. Dry weight of the whole seedling shows a similar pattern (Fig. 2). Lowest weight of seedling was recorded in May and highest in October with gradual increase towards October and decrease thereafter. Shoot root ratio was highest in February, declined till March and thereafter increased gradually.



**Fig. 1. Seasonal patterns of dry weight and shoot : root ratio of plants collected from *Arthrocnemum indicum* community.**

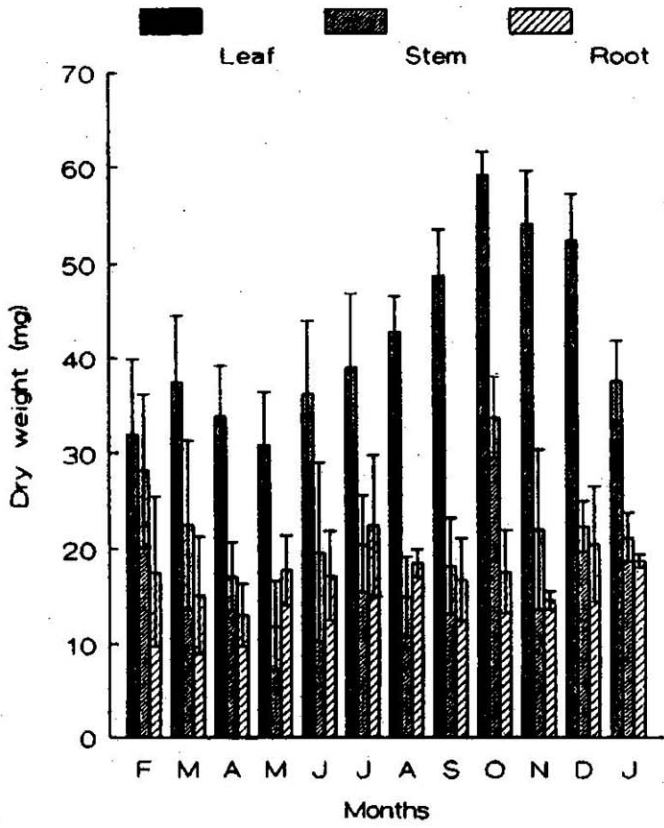


Fig. 2. Seasonal patterns of dry weight of leaf, shoot and root of plants collected from *Arthrocnemum indicum* community.

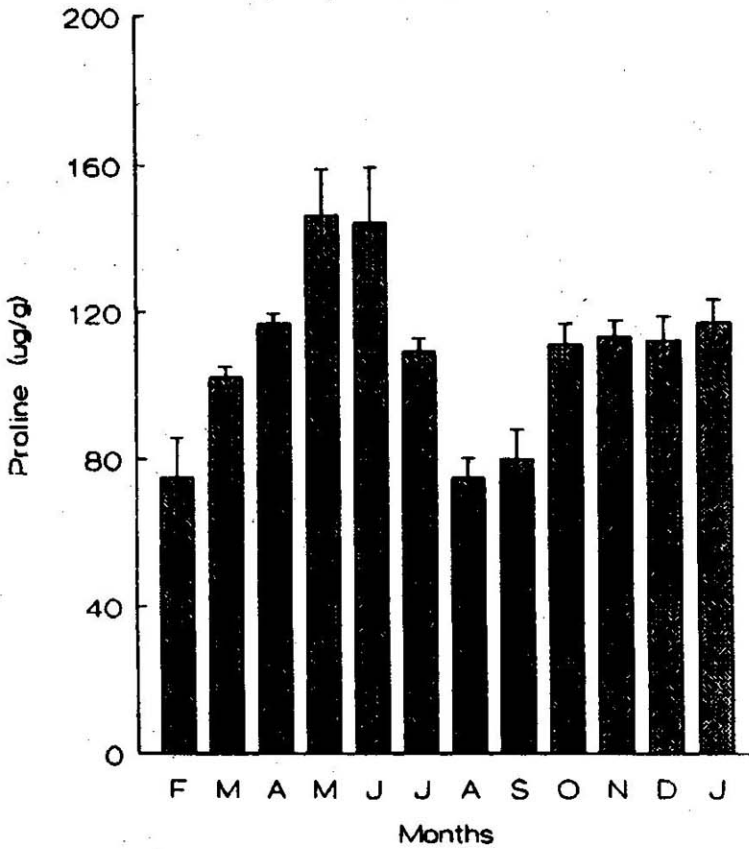


Fig. 3. Monthly variation in leaf proline content of *Arthrocnemum indicum*.

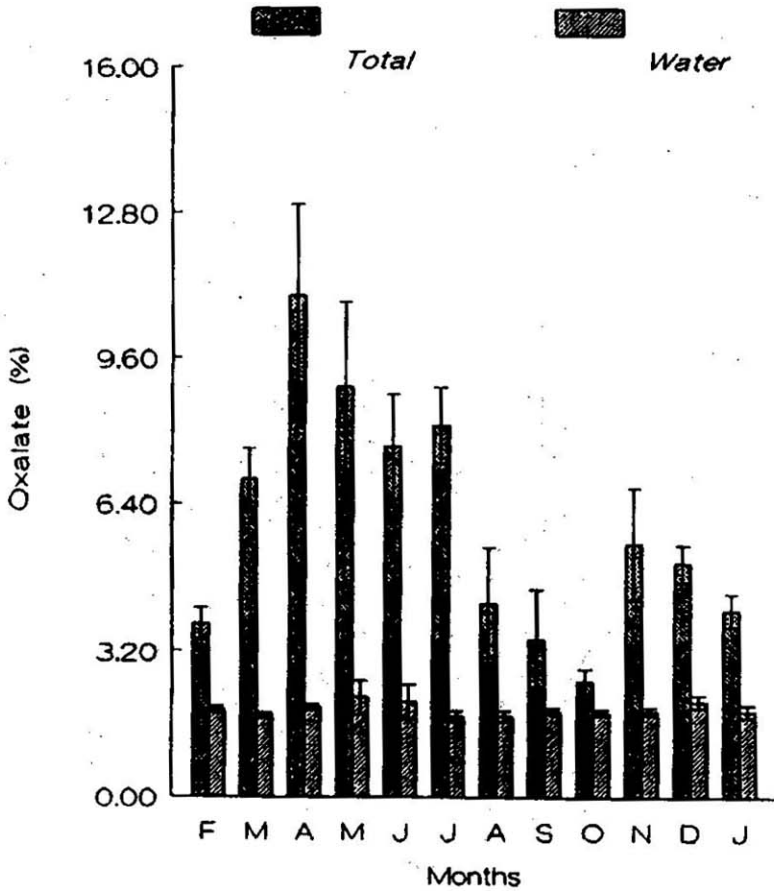


Fig. 4. Monthly variation in leaf oxalate content of *Arthrocnemum indicum*.

## OSMOTICA

The proline content of *A. indicum* showed an increase with increase in salinity (Fig. 3). After summer and winter rains; which coincided with decrease in salinity; proline content decreased. It increased again with increase in salinity.

Seasonal variation in total and water soluble oxalate content was studied in *A. indicum* (Fig. 4). The results showed that total oxalate does increase with the increase in stress while water soluble oxalate remained unchanged.

## DISCUSSION

The life history variations of *A. indicum* were studied for 12 months from February 1992 to January 1993 during which populations were exposed to great variations in edaphic conditions. This exposure to conditions such as soil salinity varying from 35 - 58  $\text{ms cm}^{-1}$  and soil moisture ranging from flooding to drought levels affected the survival and growth of *A. indicum*.

Dry matter production under saline conditions increases with decrease in salinity (McGraw and Ungar, 1981). Seasonal changes in dry weight accumulation observed in this investigation appear to be directly related to soil salinity stress. The small increase in dry weight between April and July indicated that the low soil water potentials at this time were limiting the plant growth.

The brackish salt marsh community of *A. indicum* faces changing environmental conditions throughout the year. There is an adaptive capability of *A. indicum* to grow better in high salinity areas. *Arthrocnemum subterminalis* is reported to tolerate high salinities (Pennings and Callaway, 1992). Dry mass production of plants is affected by environmental factors such as light and salinity. The soil of *A. indicum* community was very clayey and sticky. During drought conditions the saline soils become dry and are more stressful to the plants. During such conditions occurring from April to June, the FW:DW ratios of shoots and roots decrease, pH and conductivity increases which shows that dry season promotes salinity increases and affects plant growth.

*Arthrocnemum indicum* shows highest dry mass in leaves and shoots but in very high salinity the dry mass production declines. Cooper (1982) reported that the tolerance of plants to salinity and waterlogging was correlated to their position along a salt marsh gradient. *Salicornia europaea* had higher dry mass production in both drained and waterlogged saline treatments than in non saline treatments (McGraw and Ungar, 1981).

Various species of halophytes accumulate high concentrations of organic solutes in response to salinity (Stewart and Lee, 1974; Flowers *et al.*, 1977; Storey *et al.*, 1977; Wyn Jones *et al.*, 1977; Jefferies and Rudmik, 1979; Gorham *et al.*, 1980; Cavalieri and Huang, 1981). Proline accumulates in leaves of many plants in response to environmental stress (Stewart *et al.*, 1966; Aspinall and Paleg, 1981). Free proline is known to accumulate in plant tissues in response to environmental stress. One possible role of proline, along with some other organic solutes, is that it accumulates in the cytoplasm and lowers the solute potential there, balancing a low solute potential in the vacuole due to the accumulation of salts (Voetberg and Stewart, 1984).

The role of proline is described as an osmoprotectant (Chrominski *et al.*, 1989). In *A. indicum* proline content increased considerably during dry conditions when insufficient rainfall occurred but decreased with the onset of rainfall. Treichel *et al.* (1984) described the proline content in plants in response to the decreasing and increasing annual rainfall. They also reported that increasing water shortage in the soil led to a decreasing water content and osmotic potential in these succulent species, accompanied by an increasing proline content. These processes were fully reversible with the occurrence of rain.

Oxalic acid and its salts have been found to accumulate in halophytic Chenopodiaceae and affect tissue ion relations (Brown and Wadleigh, 1955; Austenfeld, 1974; Albert and Popp, 1977; Davis, 1981). The form of oxalate present in tissues was closely linked to the presence of NaCl in the media. Over 50% of oxalate in plants grown in non-saline medium, where water potential is negligible, was in the osmotically inactive acid soluble form. Water soluble oxalate generally made up less than half of the total oxalate and when salinity increases the water soluble oxalate also increase (Osmond, 1963; Austenfeld, 1974; Ewing, 1981). Water soluble oxalate which is believed to be acting as osmotica remained unchanged throughout the season in *A. indicum*. This indicates that oxalate has only a minor role to play in osmoregulation of this species. Popp (per. comm.) has opined that there is no convincing evidence that water soluble oxalate is acting as osmoticum.

The marsh community of *A. indicum* varied greatly in soil electrical conductivity from a low of 34.9 to a high of 58.3 ms cm<sup>-1</sup>. Na<sup>+</sup> concentration in this species was

highest in roots. This is due to ion immobility as has been demonstrated in other halophytes (Greenway *et al.*, 1966, Albert, 1975, Yeo, 1981). In *A. indicum*  $\text{Na}^+$  and  $\text{Cl}^-$  made up the bulk of accumulated ions, and showed a significant correlation with external soil solution conductivity. All of the species maintain a relatively high  $\text{K}^+$  concentration when compared with the external solution indicating that these halophytes can actively absorb  $\text{K}^+$  from saline soils.

Concentration of  $\text{Na}^+$  was lower in *A. indicum* root and shoot cells in comparison with the external solution. It is possible that the permeability of *A. indicum* root and shoot to  $\text{Na}^+$  is low and that the  $\text{Na}^+$  entering the root by passive diffusion is probably removed by active efflux. This further supports the idea of root control over the inflow of  $\text{Na}^+$  in higher salinity modalities.

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