ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

PHENOTYPIC AND HISTOLOGICAL PLASTICITY OF *EICHHORNIA CRASSIPES* IN TWO POLLUTED AQUATIC HABITATS - AN INDICATOR OF INVASIVE ADAPTATION

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ABSTRACT

Present study reports the results of a comparative evaluation of the morphological and anatomical variations exhibited by the alien invasive-*Eichhornia crassipes* (Mart.) Solms in two polluted water bodies in Trivandrum city in relation to its normal growth responses in a non-polluted fresh water pond. The water samples from the two experimental sites-Parvathy Puthanar and Veli Lake were analysed for their physicochemical parameters. The morpho-anatomical variability in response to physico-chemical differences has been interpreted to elucidate the invasive adaptations of the species. Water samples collected were compared for BOD, COD, pH, alkalinity, carbonates and bicarbonates. Morphological variations between plant samples were analysed for parameters such as plant size, shoot and root length, root hair proliferation, colouration of roots, bulb girth, petiole length, discolouration of bulb and leaves. Transverse sections of lamina, petioles and roots were compared for variability in anatomical parameters. Water samples from Parvathy Puthanar exhibited higher BOD and COD. The pH of water samples were detected as slightly acidic in Parvathy Puthanar and above neutral in Veli Lake. The adaptive features of the plant included decline in root/shoot ratio, reduced size of petiolar bulb and petiole elongation, reduction in number of root length and lateral branches, development of chlorotic and necrotic patches during salinity stress in Veli Lake. Histological evaluation of plant parts concluded increase in stomatal indices (8.69-29.1%), variations in stomatal aperture size, enhanced epidermal and mesophyll thickness, increment of leaf vascular bundles, reduced air spaces in aerenchyma of petiole and roots.

Keywords: *Eichhornia crassipes*, Invasive adaptation, Morpho-anatomical, Invasion, Pollution, Aquatic ecosystem.

1. INTRODUCTION

In India, it has been estimated that more than two lakh hectares of water bodies are infested with invasive aquatic weeds, especially water hyacinths [1]. An outstanding feature of the coastal zone of Kerala extending north-south is the presence of discontinuous chain of temporary or perennial water bodies and estuaries, popularly known as backwaters [2]. The main problem of Kerala water bodies is the infestation of aquatic weeds, the major ones being *Eichhornia crassipes, Salvinia molesta* and *Pistia stratiotes.* A subtropical country like India pampers the arrival of such aquatic invasive weeds and Kerala with abundant sunshine and rains pave a way for their abundant growth and establishment [3]. The invasiveness of these alien species is not only confined to its high reproductive rates but also includes its ability to thrive and adapt in different types of ecosystems, whether it be a nutrient poor, a nutrient rich, a partial saline medium or a plain fresh water ecosystem.

The present study is an attempt to analyse the invasive adaptability of the alien species-*Eichhornia crassipes* (Mart.) Solms in two study sites in Trivandrum - The Parvathy Puthanar- a historically important artificial canal and Veli Lake- a well-known tourist destination in Trivandrum city. Both study sites have been drastically affected with the menace of thick mats of water hyacinths. As per the reports of the GoI-UNDP project (2013-17), the surface water quality of Trivandrum city is comparatively poor for potable use as per accepted standards in the country. In a study report released by Kerala State Council for Science, Technology and Environment in 2010, analysis of water samples collected from 20 locations along the river Parvathy Puthanar showed contamination from different sources resulting in poor water quality, especially in the downstream areas of Karamana River. About 75% of the water samples were acidic and 53% contaminated with bacteria. The report observes that the Parvathy Puthanar Canal is a major source of pollution of these

river waters. A study conducted by Rohini *et al* (2018) concluded that heavy metal concentration in water samples of Parvathy Puthanar was very high with concentrations of Cadmium, Lead and Nickel showing seasonal fluctuations along with high levels of fluoride [4].

Veli Lake, a famous tourist destination in Trivandrum city, is the smallest lakes confined to the southern part of Kerala. The Lake has no permanent connection with the sea but being separated from the sea by a narrow strip of sand bar which breaks open for few days during monsoon flooding [5]. As reported by the Deccan Chronicle (2018), Veli Lake is being continuously fed with the sewage from Parvathy Puthanar [6]. This causes a severe depletion in oxygen levels and the BOD is quite low in the lake water.

The major objectives include the screening of water samples from Parvathy Puthanar and Veli Lake to assess their physico-chemical status, evaluation of morpho-logical variability in response to physicochemical variations of water in ecosystems under comparison and the assessment of anatomical variations in plants adapted in the habitats selected.

2. MATERIAL AND METHODS

2.1. Site Description and Sample Collection

The study sites selected for the analysis of invasive adaptability of *Eichhornia crassipes* (Mart.) Solms were the Parvathy Puthanar and the Veli Lake. As a control site, a non-polluted fresh water pond (260cm x 202 cm) with the presence of fishes, micro algae, snails and water hyacinths was selected. For the sampling of water and collection of *Eichhornia crassipes,* (Mart.) Solms, three sampling sites (stations) were fixed for Parvathy Puthanar and Veli Lake.

The three sampling points of Parvathy Puthanar were- North end near Kadinamkulam (sample no. PP1), Middle zone near Chackai (sample no. PP2) and South end towards Thiruvallam (sample no. PP3). The three sampling stations of the Veli Lake were the station 1 which was on the bend of the Lake across which the floating bridge of the boat club (sample no. VL1) has been placed, station 2 was the 'pozhikkara' which is close to the sea with shallow water (sample no. VL2) with sandy bottom. The third station selected was the south-western side of the Veli Lake (sample no. VL3), under the over-bridge.

For the physico-chemical analysis, water samples were collected in 5 L bottles from three stations each of Parvathy Puthanar, Veli Lake and the control fresh water pond. *E.crassipes* were collected from the sites and washed with running water and taken directly for testing various study parameters.

2.2. Water analysis

Water samples collected from the study sites of Parvathy Puthanar and Veli and the sample from the pond (control) were analysed for parameters like BOD, COD, pH, alkalinity, carbonates and bicarbonates were estimated by the standard methods of APHA [7].

2.3. Estimation of Biological Oxygen Demand (BOD)

To the water samples, 1 N acid or 1 N alkali was added to adjust the pH to 7. It was transferred into BOD bottles without any air bubbles getting entrapped inside. One ml of allylthiourea was added to each bottle to avoid nitrification. separately 2 ml of manganous sulphate and 2 ml of alkaline iodine azide solution was added and the bottles were stoppered. The bottles were shaken gently upside down for about 6-8 times, so as to develop a brown precipitate. After a few minutes till the precipitate settled down, 2 ml of concentrated sulphuric acid was added into the bottle and mixed properly to ensure complete dissolution of brown precipitate. 50 ml of this water sample was titrated against 0.025 N sodium thiosulfate solution until a pale straw colour was developed. 2 drops of starch solution was added to flask. Colour of the content changed from pale to blue. It was titrated against sodium thiosulfate solution until the blue colour disappeared. The volume of sodium thiosulfate solution used for titration was noted. Incubated the other BOD bottle at 27°C for 5 days in a BOD incubator and measured the amount of Oxygen.

BOD (mg/ml) = [(B.R for sample at D0 - D5) - Blank correction] x Dilution factor,

Where, B.R = burette reading; D0 = initial day; D5 = Day five after incubation

2.4. Analysis of Chemical Oxygen Demand (COD)

50 ml of water sample from each experimental site and fresh water pond as control was collected in separate conical flasks. 5 ml of Potassium dichromate (0.1 N) was poured in all the flasks and incubated the flasks at 100°C for 1 h in a water bath. 5 ml of potassium iodide solution (10% w/v) and 10 ml of sulphuric acid solution (2 M) were mixed in each flask. The sample and control were titrated against 0.1 M Sodium thiosulfate solution

(0.1M) till pale yellow colour appeared. 1 ml of starch solution (1% w/v) was added to both the flasks to impart blue color and again titrated with sodium thiosulfate till complete disappearance of blue colour. Noted the volume of sodium thiosulfate used for all water samples.

COD = (mg/ml) = [8xCx (V_B-V_A)] / V_S

where, C is concentration of titrant; V_A is the volume of titrant used for blank (distilled water); V_B is the volume of titrant used for water sample; V_S is the volume of water taken.

2.5. pH, Alkalinity, Carbonates and Bicarbonates

2.5.1. pH

The pH of the control and experimental samples of water was measured using pH meter (Eutech pH meter).

2.5.2. Alkalinity

To 50 ml of water sample, 3 drops of phenolphthalein indicator was added and titrated against 0.02N sulfuric acid and estimated the phenolphthalein alkalinity.

The phenolphthalein indicator changed indication, from pink to clear, at pH 8.3. The phenolphthalein alkalinity was calculated using the formula:

Phenolphthalein Alkalinity (in mg/l as $CaCO_3$) = (A1×N ×50,000)/V

where: A1 = volume of sulphuric acid used in ml; N = normality of acid used for titration; V= volume of sample used in ml

Using the above sample, 3 drops of bromocresol green indicator was added and titrated the samples with 0.02N sulphuric acid to pH 4.5 and estimated total alkalinity (bromcresol green indicator will change colour, from blue to yellow, at pH 4.5). Amount of acid used at this moment starting from step1 (*i.e.,* A2) was used to react with the hydroxide, carbonate, and bicarbonate and it constitutes of total alkalinity and is calculated by the formula.

Total Alkalinity (in mg/l as $CaCO_3$)=(A2×N×50,000) /V (2b)

Where, A2 = volume of acid used in ml starting from step 1 (i.e., A2>A1)

Hydroxide alkalinity, carbonate alkalinity and bicarbonate alkalinity were calculated using alkalinity and pH.

Hydroxide alkalinity, (mg/l as $CaCO_3$)=$50,000\times10^{[pH-pKw]}$; pKw= 15 at 24°C

Carbonate alkalinity, (mg/l as $CaCO_3$) = 2 × (Phenolphthalein alkalinity - hydroxide alkalinity)

Bicarbonate alkalinity (mg/l as $CaCO_3$) = Total alkalinity - (Carbonate alkalinity + hydroxide alkalinity)

2.6. Collection of Plant samples-*Eichhornia crassipes* (Mart.) Solms

Eichhornia crassipes, (Mart.) Solms were collected from the selected study sites-Parvathy Puthanar and Veli Lake. The control plants were procured from a fresh water pond (control). The samples were thoroughly washed in tap water and processed for experimental analysis.

2.7. Morphological Studies

The growth of *E. crassipes* in polluted habitats and control site were monitored and real time changes in various observable parameters were recorded. The plants were compared in terms of size, shoot and root length, root hair proliferation, colouration of roots, bulb girth, petiole length, discolouration of bulb and leaves. The leaf length and breadth were calculated and the root area was estimated and compared for the plants from each site. Stress symptoms like leaf rolling, chlorosis and necrosis were also recorded. Triplicates for each parameter was taken and average mean value was compared for the morphological changes of stressed and control plants.

To calculate the root-shoot ratio, the plants from each study site and the control were dissected by cutting at the level of leaf crown bases to separate the root and the shoot. The shoots containing leaves, petiole and the stolon were cut into pieces and fresh weights were determined. Similarly, the roots of the plants were weighed for recording the fresh weights. The plant parts were allowed to oven dry (60°C) till constant weights were attained. The dried parts were separately weighed for recording the root and shoot dry weights. The root-shoot ratio was calculated using the formula indicated and comparisons were made.

Root-shoot ratio = Dry weight of roots/Dry weight of shoots

2.8. Determination of Dry matter and Moisture contents

The dry matter content of plants adapted in different sampling sites was assessed as the difference in wet and dry weight expressed as percentage of dry matter (DM) using the formula [8]:

$$DM = (y - x)/(z - x) \times 100$$

Where, 'y' and 'z' correspond to 'wet' and 'dry' weights and 'x' depicts the container weight.

The moisture contents were determined by subtracting the DM from 100.

2.9. Anatomical Studies

The plant samples were washed thoroughly in running water. The plant parts such as leaves, petioles and roots were separated and blotted to remove the water content. For stomatal estimates epidermal peelings from adaxial and abaxial surface of lamina were dissected out with a sharp razor blade. Transverse sections of lamina, petioles and roots from experimental and control groups were compared for variability in anatomical parameters. The peelings/sections were stained with safranin and mounted in glycerine on glass slides and examined under a microscope with ocular 10x and objective 40x. For micrometry, the data obtained from ocular micrometre were converted into microns (μ) with the use of stage micrometre after calibration.

The stomatal index was calculated using the formula [9]:

$$\text{Stomatal Index} = S/S+E \times 100$$

Where S = number of stomata and E = number of epidermal cells.

Stomatal frequency was measured as number of stomata per field. Three fields were taken for the measurements and average was taken. The number of epidermal cells, diameter and length of stomata and pore size of stomata were measured using micrometres and recorded.

The cross sections of leaves from experimental and control samples were taken for the comparison of adaxial and abaxial epidermal thickness, the width of palisade and spongy parenchyma and number of vascular bundles. Cross sections of petioles were taken to compare the sizes of aerenchyma and the cross section of roots to assess the variability in size of air spaces in aerenchyma.

3. RESULTS AND DISCUSSION

3.1. Physico-chemical parameters of water

The data on physico-chemical attributes of water samples from two experimental sites and control are tabulated in Table 1.

Table 1: Physico-chemical parameters of water from the experimental sites-Parvathy Puthanar and Veli Lake. (C- control; PP- Parvathy Puthanar; VL -Veli Lake)

Sample	BOD (mg/ml)	COD (mg/ml)	pH	Total Alkalinity (mg/ml)	Carbonates (mg/ml)	Bicarbonates (mg/ml)
C	1.5	0.046	7.66	0.04	1.31×10^{-6}	0.04
PP1	1.7	0.032	6.12	0.104	4.50×10^{-6}	0.103
PP2	4.1	0.602	5.63	0.064	4.20×10^{-6}	0.063
PP3	2.4	0.288	6.03	0.054	4.07×10^{-6}	0.053
VL1	1.3	0.088	7.48	0.044	3.00×10^{-6}	0.044
VL2	1.2	0.048	7.50	0.038	3.10×10^{-6}	0.038
VL3	1.3	0.088	7.55	0.039	3.00×10^{-6}	0.039

3.2. Biochemical Oxygen Demand (BOD)

The BOD was comparatively very high in water samples collected from the three sites of Parvathy Puthanar, among which the sample-PP2 from Site 2 had the highest level of BOD, *i.e.,* 4.1 mg/ml. The water samples from Veli showed relatively same BOD levels as that of the control water sample taken from the very less polluted fresh water pond. This clearly indicated that the study site Parvathy Puthanar had the highest levels of organic pollutants. BOD values have been widely adopted as a measure of pollution effect. It indicates the amount of organic matter present in the water. Low BOD content is an indicator of good quality of water, while high BOD indicates polluted water.

3.3. The Chemical Oxygen Demand (COD)

From the estimates of COD depicted in Table 1, it is inferred that the site 2 of Parvathy Puthanar had the highest levels of COD. In general, two other sites of Parvathy Puthanar, site 2 and site 3 also indicated relatively higher COD levels in comparison to the samples from the Veli sampling sites and control water. The water samples from site 1 of Parvathy Puthanar and the three sites of Veli reflected almost the same COD values representing a less polluted state. The BOD and COD measures also concluded that the Chackai area of Parvathy Puthanar *i.e.* site 2 is harbouring the highest level of particulate and soluble organic matter pollutants. The higher the BOD and COD, the more polluted the water and the water carries organic and inorganic matter as pollutants.

The COD determination is a measure of the oxygen equivalent of that portion of the organic matter in a

sample that is susceptible to oxidation by a strong chemical oxidant. During COD determination, oxygen demand value is useful in specifying toxic condition and presence of biologically resistant substances. It is important and rapidly measured parameter for industrial waste water studies and control of waste treatments. COD is mostly used to measure the load of organic pollutants in the industrial waste water [10].

3.4. Water pH

The samples collected from the sites of Parvathy Puthanar showed slightly acidic pH, ranging from 5.63 to 6.13 (Table 1). The site 2 of Parvathy Puthanar suggested a measure of 5.63, slightly higher acidic pH when compared to the other two sites. The water samples collected from Veli displayed almost same pH as the control water i.e. > 7.00. The samples from Veli indicated almost a near neutral pH, revealing absence of acidic or alkaline pollutants. pH is a measure of the acidity or alkalinity of water and is one of the stable measurements. pH is a simple parameter but is extremely important, since most of the chemical reactions in aquatic environment are controlled by any change in its value. The toxicity of heavy metals also gets enhanced at particular pH. Thus, pH is having primary importance in deciding the quality of water. The slightly acidic pH of Parvathy Puthanar could be attributed to the presence of acidic pollutants in the canal.

3.5. Components of water Alkalinity

Analysis of alkalinity level, bicarbonates and carbonates pointed out that the water from site 1 of Parvathy Puthanar was relatively higher indicator of these parameters than PP2 and PP3 samples. However, the samples from Veli exhibited a uniformly higher range of total carbonate level (Table 1).

3.6. Morphological Studies

The water hyacinths collected from the three study sites-Pond (control), Parvathy Puthanar and Veli Lake differed significantly in morphological features.

3.6.1. Shoot Morphology

The control plants were of relatively smaller in size compared to the plants from Parvathy Puthanar and Veli Lake. Though, the plants (fully developed with ten foliage) from Parvathy Puthanar and control plants had almost same sizes with an average fresh weight of 61.175 g and 52.792 g respectively those from Veli exhibited appreciably larger size (Table 3). As depicted in Table 2 they had long (an average length of 20.3 cm) and slender petioles compared to that of the control plants and the plants from Parvathy Puthanar, both of which had short and bulbous petioles, with an average length of about 12.11 cm and 10.40 cm respectively. Swollen petioles with distended bulbous part was characteristic of plants from fresh water pond (control) and Parvathy Puthanar and the growth profile in these two water bodies was monitored as non-entangled with narrow spaces in between for penetrance of sunlight. These features are adaptations that aid in buoyancy [1]. These petioles also provide a stable platform for vertical growth under non-crowded conditions. Slender and elongated petiole with reduction in girth of petiolar bulb and substantial increase in plant size in general were monitored as the general adaptive features associated with flora from Veli Lake.

3.6.2. Foliar Morphology

The control plants had healthy glabrous leaves, but small sized with an average laminar area of 44.46 cm^2 (Table 2). The leaves of plants from Parvathy Puthanar were distorted with symptoms of wilting and pathogenesis. They had an average leaf area of 31.18 $cm.^2$ The leaves from the plants of Veli Lake were characterized by severe chlorotic and necrotic lesions on lamina. This could be due to their periodic exposure to salinity. The Veli Lake is separated from the Arabian Sea by a short stretch of water body known as 'pozhi'. After heavy rain, the 'pozhi' is allowed to merge and the sea joins the lake. This causes the mixing of the saline water from the sea to the Lake. According to Sooknah and Wilkie, (2004) high levels of salinity in waste water is the only factor that can limit the growth of water hyacinth and other aquatic macrophytes [11]. The large leaf size and associated necrotic and chlorotic patches may be an adaptation for tolerating high salt levels in the Veli Lake during this time. *E. crassipes* (Mart.) Solms cannot tolerate salinity above a particular level [12]. According to Olivares and Colonnello (2000), salinity is the main obstacle for the growth of water hyacinth in coastal areas [13]. De Casabianca and Laugier (1995) suggested that in high intensity of salinity, the production of water hyacinth was reducing and necroses on leaves and petioles were predominant [14]. In the present study, massive wilting of *Eichhorniacrassipes* (Mart.) Solmswas monitored during heavy monsoon in July in the region near the 'pozhi' where mixing of sea water persisted continuously for more than seven days. This ultimately led to the wilting and senescence of plants in Veli Lake.

Table 2: Morphological parameters of *E.crassipes*(Mart.) Solms., collected from the experimental sites.

Parameters	C	PP	VL
Nature of Leaves	Small sized, healthy and glabrous	Moderate sized with chlorotic patches	Large with chlorotic and necrotic patches
Average Lamina Length (cm)	6.5	5.5	7.5
Average Lamina breadth (cm)	7.6	6.3	9.1
Total Leaf Area (cm^2)	44.46	31.18	61.42
Nature of Petiole	Short, Bulbous	Short, Bulbous	Long, Slender
Petiole Length (cm)	10.40	12.11	20.31
Nature of Roots	Long, purple coloured, bulky, numerous root hairs	Short, Whitish, less number of root hairs	Short, brownish, less number of root hairs
Average Length of Roots (cm)	22	6.75	8.52
Average number of root hairs per cm	96	42	85

(C- control; PP- Parvathy Puthanar; VL – Veli Lake)

Table 3: Root-Shoot ratio of *E. crassipes* (Mart.) Solms collected from the study sites.

Sample	Fresh Weight (g)	Dry Weight (g)	Root-Shoot Ratio	Dry Matter (%)
CR	52.792	3.034	0.884	5.806
CS	58.560	3.432		
PR	16.573	0.888	0.345	4.443
PS	61.175	2.567		
VR	21.127	1.829	0.236	6.567
VS	124.620	7.743		

(CR-Conirol root; CS-Control Shoot; PR- Parvathy Puthanar Root; PS- Parvathy Puthanar Shoot; VR- Veli Root; VS- Veli Shoot)

3.6.3. Root Morphology

The root system of water hyacinth is adventitious and fibrous. Unlike the shoot weights of the plants from the three sites, the root weights exhibited a negative trend in fresh weights. The control plants with the least shoot weight had the most extensive fibrous root system, with a mean length of 22cm (Table 2) and average fresh weight of 52.79g (Table 3). The length of roots in *Eichhornia crassipes* (Mart.) Solms. can reach up to 300 cm as dense masses covering the water surface[1]. In control plants there were as many as 96 lateral roots (root hairs) per cm length gave the root a hairy appearance. The roots of plants from Veli Lake indicated a substantial decrement with a fresh weight of 21.127g over the control plants (Table 3). They had an average length of 8.5cm with about 42 short root hairs per cm. The root system of samples from Parvathy Puthanar has the lowest average fresh weight, of 16.57g and also with the minimum root length of 6.7 cm. They had the shortest root hairs numbered an average of 42 per cm. The variations in root masses clearly indicate the nutrient availability in the water body. In Parvathy Puthanar, since there is a nutrient enrichment (high levels of organic pollutants as mentioned in Table 1), roots need not get flourished to obtain nutrients, while in a pond with comparatively lesser nutrient availability, the roots have to be elaborate enough to absorb nutrients available in the water body. Veli Lake, even though not so polluted as Parvathy Puthanar, has some levels of organic pollution consequently reduced the root length of plants. Under normal growth conditions, roots absorb water and nutrients from the soil and supply them throughout the plant body and maintaining homeostasis. This balance is altered during a stress period when roots are forced to adopt several structural and functional modifications including molecular, cellular and phenotypic changes such as hardening of cell wall and reduction in root length [15]. Analyses concluded that the root length and number of lateral roots of plants from Parvathy Puthanar and Veli were significantly reduced and this might be due to the enhanced levels of nutrients, organic matter and prevalence of stress conditions in comparison to a fresh water pond selected as control.

The roots from the three sites varied greatly in colour. The colour of roots of control plants were purplish pink indicating the presence of anthocyanin. The roots of plants from Veli Lake were brownish with apparently lesser intensity of color and that from Parvathy Puthanar were nearly whitish *which* is general for most of the

plant roots. This gradation in root pigmentation could be due to variation in anthocyanin accumulation in the roots. In a pond (control), the plants floating on clear water without forming dense mats allow effective penetration of sunlight which could favour anthocyanin biosynthesis. In experimental sites, the invasion was so intense that the plants exhibited entangled and interlocked dense mats so that the submerged roots were deprived of direct sunlight. This could be the reason for the observed differences in root pigmentation. Sunlight, especially the red and far red wavelengths is reported as a vital environmental factor governing the biosynthesis of anthocyanin in plant parts [16]. The water in Parvathy Puthanar has high organic matter content (Table 1) and thereby the dissolved oxygen content is too low. This low light penetrant water body with poor dissolved oxygen reduced the anthocyanin content of roots of *Eichhornia crassipes* (Mart.) Solms and the plants become adapted with lesser pigmented roots during invasion.

3.7. *Relative Root/Shoot ratio and Dry matter content*

The root-shoot ratio shown in Table 3 indicates that the control plants had the highest root-shoot ratio of 0.884 while the plants from Veli Lake and Parvathy Puthanar had a lower ratio of 0.236 and 0.345 respectively. Dry root and shoot weights are balanced within a certain limit which is a characteristic of that species. A relatively low root-shoot ratio indicates an increase in nutrient availability in the water column and similarly a high ratio indicated low nutrient availability in the ecosystem, so that root mass should be increased [17]. This clearly indicated the presence of nutrient enrichment in the Veli Lake and Parvathy Puthanar.

The root-shoot ratio helps to assess the overall health of a plant. According to Bray (1963), root-shoot ratio decreases with increase in size, which is true for the plants from Veli [18]. These plants had a large size and a decreased root-shoot ratio. The dry matter content (%) of the different samples were in the order VL (6.567) > control (5.806) >PP (4.443), respectively. The observations suggested that the species *Eichhornia crassipes* (Mart.) Solms exhibited reduction in root/shoot ratio and an increase in dry matter content as an invasive adaptability in habitats with high levels of salinity whereas under conditions of high BOD and COD, the dry matter was detected as lesser in relation to the control.

3.8. *Leaf Anatomical studies*

3.8.1. Epidermal and Stomatal features

The leaves displayed uniseriate epidermis with reduced cuticle on both adaxial and abaxial sides. The histological studies on epidermal peels from adaxial and abaxial sides revealed that number of stomata per field was more in number in *E.crassipes* (Mart.) Solms collected from Veli Lake compared to that of control plants and that collected from Parvathy Puthanar. This contradicts with the number of epidermal cells, which showed an increase in the plants from Parvathy Puthanar while the plants from Veli Lake had the least number of epidermal cells. Stomatal indices tabulated in Table 4 suggested an increase of 8.69% in plants from Parvathy Puthanar and increase of 29.1% in plants from Veli Lake over the control plants. The pore size of stomata showed a range from 8 to12µm, and reduction in pore size was recorded for plants from Veli indicating an adaptive measure to defend the stress of periodic fluctuations in salinity level in correspondence with mixing of sea water.

Table 4: Characteristics of epidermal peels of *E. crassipes* (Mart.) Solms, collected from the study sites.

Stomatal Parameter	Control	PP	VL
NS	16 ± 1.52	18 ±1.00	22.30 ± 1.57
NEC	147 ± 3.05	149 ± 2.51	140 ± 1.52
SD (µm)	32 ± 2.30	27 ± 2.30	39 ± 2.31
SL (µm)	40 ± 2.39	47 ± 2.31	47 ± 2.39
PS (µm)	12 ± 2.39	9.00 ± 2.3	8.00 ± 0
SI (%)	9.81 ± 0.82	10.66 ± 0.37	13.78 ± 0.83

Data shown as value ± standard deviation. NS = number of stomata per field; NEC= number of epidermal cells per field; SD= diameter of stomata; SL= length of stomata; SI= stomatal index; PS= pore size of stomata

The metrics from analysis of leaf histology are indicated in Table 5. The thickness of adaxial epidermis in leaves was found to be varying in relation to control and suggested an increase of about 50% and 60% in plants from Parvathy Puthanar and Veli Lake respectively. The abaxial epidermal thickness is significantly different in

plants collected from both the study sites, with an increase of 150% in plants from Parvathy Puthanar and only about 50% increase in those from Veli Lake.

Table 5: Anatomical characteristics of cross-section of leaves of *E. crassipes* (Mart). Solms. collected from the study sites.

Sample	AD W(µm)	ABW (µm)	PPW (µm)	SPW (µm)	V.B.W(µm)	MSW(µm)
C	25.63 ± 4.43	15.38 ± 0	153.81 ± 5.38	158.92± 2.01	133.29 ± 3.49	58.95 ± 11.74
PP	38.45 ± 7.69	38.45 ± .69	261.46 ± 0.76	379.37 ± 8.70	276.84 ± 5.38	189.68 ±23.49
VL	41.01 ± 4.43	23.07 ± 0	425.51 ± 3.49	235.82 ± 3.49	158.92 ± 3.49	82.02 ± 8.87

(C- control; PP- Parvathy Puthanar; VL – Veli Lake)

(ADW= adaxial epidermis width; ABW= abaxial epidermis width; PPW= palisade parenchyma width; SPW= spongy parenchyma width; V.B.W= Stelar (Vascular bundle) width; M SW = Marginal stelar width)

3.8.2. Assimilatory tissues

The mesophyll thickness of leaves of plants from Veli Lake has a 133.33% increase while that of the leaves of plants from Parvathy Puthanar has only 20.28% increase over that of the control leaves. As shown in Table 5, there was a considerable variation in the thickness of the spongy and palisade parenchyma of the leaves of the plants collected from the study sites from that of the control plants. There is an increase of about 70% in width of palisade parenchyma of plants from Parvathy Puthanar and about 176.66% increase in plants from Veli Lake, with respect to the control plants. The sizes of spongy parenchyma has an increase of about 138.71% in plants from Parvathy Puthanar and about 48.38% increase in plants from Veli Lake. An increase in size of spongy mesophyll may aid the leaves to float and to avoid submergence.

3.8.3. Aerenchyma and Vasculature

Large air spaces were present in the cross sections of leaves from Parvathy Puthanar while the air spaces were highly reduced in the leaves of plants from Veli Lake due to the presence of two rows of vascular bundles below the palisade and above the spongy parenchyma. The two rows of vascular bundles were of the same size. The cross sections of leaves of plants from Parvathy Puthanar had only a single well differentiated row of vascular bundles arranged below the palisade parenchyma whereas the control plants had two distinct rows of vascular bundles in which the ones on the adaxial side were smaller in size than that on the abaxial side.

The petiole anatomy of *E.crassipes* (Mart.) Solms also indicated a reduction in air space showed a steep decrease from that of the control plants. The petioles of plants from Parvathy Puthanar had a decrease in diameter of aerenchyma, which was a 15% from the central region (Table 6). Similarly, the plants from Veli Lake had a 35% decrease in aerenchyma size in the centre and a 36.11% decrease in periphery. Prominent variations in leaf anatomical attributes in response to pollutants can be interpreted as an adaptive measure for better assimilation of atmospheric carbon dioxide and similar adaptations in plants along road side areas is reported [19, 20].

Table 6: Anatomical characteristics of cross-section of roots of *E.crassipes*(Mart). Solms.,collected from the study sites

Sample	Air Space		Middle Cortex	Inner Cortex		Pith
	Diameter (µm)	Length (µm)	Width of Aerenchyma (µm)	Number of Layers	Width (µm)	Diameter (µm)
C	61.52 ± 8.87	184.56 ± 15.38	199.94 ± 23.49	76.91±8.87	76.91 ± 8.87	153.82± 5.38
PP	51.26 ± 23.49	220.44 ± 38.70	194.81 ± 32.01	61.52±0	76.91 ± 0	174.31± 8.87
VL	Homogenous outer and middle cortex			76.901 ± 0	71.77 ± 8.87	210.19±23.49

(C- control; PP- Parvathy Puthanar; VL – Veli Lake)

3.8.4. Root histology

The anatomical structure of roots of *E.crassipes*(Mart.) Solms collected from all sites showed uniseriate epidermis with thick-walled cells. While the cortex of the root section in control plants and plants from Parvathy Puthanar was divided into three regions- outer

cortex, middle cortex and inner cortex, the cortex of root cross sections in plants from Veli Lake was homogenous i.e., without differentiating into an outer, middle and inner cortical regions and the cortical aerenchyma zone was not demarcated. In the root sections of plants from Parvathy Puthanar, the outer cortex possessed prominent air spaces than in the middle and inner cortex but the diameter of the air spaces had a 16% decrease while the width of the airspaces had a 19.44 % decrease in comparison to the control plants (Table 6). The width of aerenchyma of middle cortex of the roots of plants from Parvathy Puthanar displayed an average size of 194µm and with a decrease of about 2.56% from that of the control plants. The inner cortex of the plants from the study sites showed not many differences from that of the control plants. Number of layers of the inner cortex of roots had an average width of 61 µm in the plants from Parvathy Puthanar which was a 20% increase from that of the control plants while it was about 80 µm in average number of layers in that of the plants from Veli Lake, which was the same as that of the control plants. The inner cortex aerenchyma width had not shown many differences in the plants from study site while the diameter of aerenchyma in roots had shown about 13% increase in plants from Parvathy Puthanar and about 36.66% increase in plants form Veli Lake. Adecline in cortical layers under heavy metal stress in a terrestrial species- *Trigonella* reported by Ahmad *et al* (2005) agrees with the present observations and indicated stress induced anatomical variations in terrestrial and aquatic plants [21].

4. CONCLUSION

A comparative analysis of the alien macrophytic invasive-*Eichhornia crassipes* (Mart.) Solms for its high degree of invasive adaptability concluded that the species exhibit multiple mechanisms of morphological anatomical plasticity and stress tolerance for its survival in polluted and non-polluted habitats.

5. ACKNOWLEDGEMENTS

The authors are thankful to the Principal, University College, Trivandrum for providing all laboratory facilities.

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