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PHYTOREMEDIATION POTENTIAL EVALUATION OF FOUR SELECTED AQUATIC MACROPHYTES

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Abstract *Heavy metal pollution in the water bodies is a serious threat to the world. Phytoremediation is the better solution of this serious threat, since most of the conventional methods are less effective, time consuming and expensive when compare to phytoremediation technology. In the present research work the phytoremediation potential of four aquatic macrophytes (Azolla pinnata R.Br., Lemna minor L., Pistia stratiotes L., and Salvinia molesta D. Mitch.) were compared by studying their stress related metabolism, photosynthetic pigment content and by estimating heavy metals using atomic absorption spectrophotometry. The various physiological and biochemical parameters as well as anatomical attributes were analyzed in the selected four aquatic macrophytes growing in less polluted and highly polluted aquatic habitats. From the results it was found that, all the selected four aquatic macrophytes growing in less polluted aquatic habitat showed more dry weight percentage and less moisture percentage. The photosynthetic pigments like chlorophyll-a, chlorophyll-b and carotenoids decreased under stressed conditions. The most common stress indicating compounds like proline and MDA was also higher in the samples collected from highly polluted aquatic habitats, except in few cases. Quantification of heavy metal showed high Fe content in all samples than Mg, Mn, Zn and Cu. Fe and Mg was the heavy metals which present in all the four aquatic macrophytes under investigation. While analyzing the anatomical attributes, the major difference was seen in the measurements of air cavities and body hairs which provide them buoyancy. Even though the stress related metabolism showed certain differences among the four aquatic macrophytes, they all showed their potentiality to be used as a phytoremediators.*

Keywords: Heavy metal pollution, phytoremediation, abiotic stress, metabolism, macrophytes

Introduction

The industrial revolution has gifted with a number of environmental problems, such as extensive devastation of forests and grasslands, large-scale desertification of arable and habitable regions severe environmental pollution and unfavourable climatic changes. Of all the current environmental issues heavy metal contamination of aquatic habitats is of major concern, because of their persistent and bio accumulative nature (Chang et al., 2009; Yadav et al., 2009). They are added to the aquatic systems either naturally by slow leaching from soil rocks to water or through anthropogenic sources. In recent times, anthropogenic inputs, such as discharge of untreated effluents (waste water), have contributed to the predominant causation. The chemical methods to effectively decrease heavy metals to acceptable levels require a large excess of chemicals, which increase the costs because of generating the voluminous sludge. The conventional methods applied to clean heavy meal pollution have benefits and limitations but in general none of them is cost effective (Volesky, 2001; Rai, 2009).

Nowadays most of the water bodies are invariably polluted with heavy metals. Most of the pollution is due to human activities. Thus it is our responsibility to make our water bodies free from these pollutants. If we could identify the aquatic plants which can effectively remove these heavy metals from the water, it could be a great achievement in mitigating the adverse effects of heavy metal pollution in water bodies. Several research works were conducted to analyze the phytoremediation potential of different aquatic plants, but a comparative study of the different aquatic macrophytes in terms of heavy metal stress is lacking. By taking account these factors, our work is intended to identify the most suitable aquatic plant which can effectively carry out phytoremediation.

In the present research work, the phytoremediation potentials of four selected aquatic macrophytes were compared. The plants selected for the study include *Azolla pinnata* R.Br., *Lemna minor* L., *Pistia stratiotes* L., *and Salvinia molesta* D. Mitch. These macrophytes were shown high biomass production. These were commonly used as fodder and organic fertilizer. The phytoremediation potentials of these plants were assessed through heavy metal quantification (Cu, Zn, Mn, Fe, Mg) and by analyzing anatomical and major biochemical parameters like proline content, Malone dialdehyde (MDA) content, photosynthetic pigment content etc.

Materials and Methods

The research was carried out with four aquatic macrophytes. They were *Azolla pinnata* R.Br (Salviniaceae), *Lemna minor* L. (Lemnaceae), *Pistia stratiotes* L. (Araceae), and *Salvinia molesta* D. Mitch (Salviniaceae). All the plants were collected from two aquatic habitats ie, from a highly polluted and less polluted aquatic habitats. The area which taken as the polluted habitat was a canal at P. Vemballur, and a paddy field at Chalakudy was considered as the less polluted aquatic habitat. In both habitats luxurious growth of aquatic macrophytes were seen. For further reference, the plants growing in the highly polluted aquatic habitat are referred as “plants (HP)”, plants growing in less polluted aquatic habitats are referred as “plants (LP)”.

Physiological studies

For dry weight percentage and moisture content percentage measurements, the samples were weighed using electronic balance. For fresh weight and dry weight measurements, the samples were blotted and wrapped separately in paper boats. Fresh weight of the samples was determined by weighing them immediately after wrapping. For dry weight measurements, the samples were kept in hot air oven at 100˚C. After 48 hours the samples were allowed to cooled and then weighed. Then dry weight percentage was calculated by using the following formula:

$$Dry\ weight\ prcentage = \frac{Dry\ weight}{Fresh\ weight} * 100$$

Moisture content percentage was calculated by using the following formula

$$\text{Moisture content percentage} = \frac{\text{Fresh weight - Dry weight}}{\text{Fresh weight}} \text{ x } 100$$

Anatomical studies

The anatomical characters were studied to find out the differences in the anatomical characters between the plants growing in less and highly polluted aquatic habitats by taking hand sections of the fronds of the four macrophytes. Fresh specimens directly from their natural habitats were used for the study. Thin sections were made and were temporarily mounted on a clean slide using glycerine and a cover-glass. Digital photographs of the plant sections were taken by using digital camera attached to microscope.

Biochemical parameters

Estimation of chlorophyll and carotenoids were done according to the method of Arnon (1949). Proline content was estimated according to the method of Bates *et al* (1973) and the MDA content estimation was done according to Health and Packer (1968).

Quantitative estimation of heavy metals

For quantitative estimation of heavy metals, all the selected plant samples were dried at 60^oC in a hot air oven. Known weight of the dried sample were digested by refluxing in 10:4 ratio of Nitric acid and perchloric acid until the solution become colourless using Kjeldahl's flask heated in a sand bath. Then the digest was transferred to a standard flask and volume was made up to 50 ml and kept in screw capped containers. Same method was adopted for the water samples also. Atomic absorption spectrophotometer (ICPOES Optima 8000) available at Agriculture university, Kasargode was used for the estimation of heavy metals present in the digested samples.

Statistical analysis

Data from observations were recorded and analyzed in the Microsoft office excel sheet. Standard deviation and standard error were determined in the MS Excel programme.

Results

Dry weight percentage and moisture content percentage

In the present study, dry weight percentage and moisture content percentage were varied in high polluted and less polluted samples. Dry weight percentage was found to decreases with increase in the rate of pollution. Moisture percentage was increased with increase in the pollution level. Result showed in table 1.

***Table 1**:* Dry weight percentage and moisture percentage of four aquatic macrophytes collected from high polluted and less polluted aquatic habitats.

PLANTS	DW % (HP)	DW % (LP)	MC % (HP)	MC % (LP)
Azolla pinnata	11.02	3.5	88.97	96.5
Lemna minor	51.23	47.62	48.77	52.38
Pistia stratiotes	9.213	5.54	90.79	94.46
Salvinia molesta	11.07	6.06	88.92	93.93

Anatomical studies

The anatomical studies of samples collected from less and highly polluted aquatic habitats showed some clear cut differences, including the length and width of air cavities, the number of chloroplasts presents, width and size of rhizoids etc.

In *Azolla pinnata,* difference in the air cavity measurements was noticed between the samples collected from less and highly polluted aquatic habitats. The sample collected from the less polluted habitat had a cavity measurement (Length-364.09 µm, Width-199.05 µm) (Fig. 1A) higher than that collected from highly polluted habitats (Length-241.03 µm, Width-103.91 µm) (Fig. 1B). The samples collected from the highly polluted habitats has a hair larger (Length-419.21 µm) (Fig. 1B) than that collected from less polluted habitats (Length-241.03 µm, Width-103.91 µm) (Fig. 1A). The samples collected from the highly polluted habitat have been found to have a symbiotic association with *Anabaena* (Fig. 1C & 1D). *Lemna minor* shows large sized air cavities in the samples that collected from highly polluted aquatic habitats (Length-164.50 µm, Width-199.10) (Fig. 2A) than those collected from less polluted aquatic habitats (Length-101.38 µm, Width-41.41 µm) (Fig. 2B). No characteristic hairs are seen on both sections. *Pistia stratiotes* have larger cavities in the samples from highly polluted aquatic habitats (Length-527.99 µm, Width-297.36 µm) (Fig. 3A) than those collected from less polluted aquatic habitats (Length- 96.01 µm, Width-67.03 µm) (Fig. 3B). The hair length of *Pistia stratiotes* varies among the less polluted and highly polluted aquatic habitats. The samples from highly polluted aquatic habitat have larger hairs (Length-358.32 µm, Width-60.65 µm) (Fig. 3C) than those collected from less polluted aquatic habitat (Length-138.26 µm, Width-17.42 µm) (Fig. 3D).

Large sized cavities are present in *Salvinia molesta* collected from highly polluted aquatic habitats (Length-397.96 µm, Width-122.21 µm) (Fig. 4A) than those collected from less polluted aquatic habitats (Length-24.74 µm, Width-14.75 µm) (Fig. 4B). The amount of chloroplast is also decreases as the degree of pollution increases (Fig. 4C) & (Fig. 4D).

Photosynthetic pigment content

Samples collected from highly polluted aquatic habitat (HP) showed a decreased amount of total chlorophyll content those collected from comparatively less polluted aquatic habitats (LP). The amount of total chlorophyll varies as *Salvinia molesta* > *Azolla pinnata* > *Pistia stratiotes* > *Lemna minor* (Fig. 5A). The aquatic macrophytes collected from highly polluted aquatic habitats (HP) have a decreased amount of carotenoid content than those collected from less polluted aquatic habitats (LP). The amount of carotenoid varies as *Salvinia molesta* > *Lemna minor* > *Pistia stratiotes* > *Azolla pinnata* (Fig. 5B).

Proline and MDA content

Under polluted condition the amount of proline is significantly increased. The amount of proline is higher in the samples collected from highly polluted habitats than those collected from less polluted habitats, except in *Azolla pinnata.* The proline amount varies as *Pistia stratiotes* > *Salvinia molesta* > *Azolla pinnata* > *Lemna minor* (Fig. 6A). The plants under the two habitats showed variation in the MDA content. The samples from less polluted habitat showed comparatively lower amount of MDA compared to samples from highly polluted habitat, except in *Pistia stratiotes.* The MDA amount varies as *Salvinia molesta* > *Pistia stratiotes* > *Azolla pinnata* > *Lemna minor* (Fig. 6B).

Heavy metal quantification

Quantification of heavy metal revealed that among the four metals quantified, amount of Fe was higher in all the samples when compared to other metals. It was found to be highest in the *Lemna minor* sample collected from highly polluted aquatic habitats. Among the tested metals, Mg is the metal which showed presence in all samples other than Fe. Its amount was found to be higher in the *Pistia stratiotes* sample collected from highly polluted aquatic habitats. Mn was found to absent in *Pistia stratiotes* collected from less polluted aquatic habitats, whereas, Zn was absent in *Salvinia molesta* and *Pistia stratiotes* collected from less polluted aquatic habitats. Cu is the metal which was absent in three samples namely *Salvinia molesta* and *Pistia stratiotes* collected from less polluted and *Lemna minor* collected from highly polluted aquatic habitats. Among the selected and tested macrophytic samples *Azolla pinnata* is the only one aquatic macrophyte which showed the presence of all the five heavy metals tested. Fe is the heavy metal which present abundantly in it (Table 2).

***Table 2*:** Heavy metal quantification in four aquatic macrophytes under less polluted and highly polluted habitats.

PLANT	LP/HP	Cu (µg/g fw)	Zn (µg/g fw)	Mn (µg/g fw)	Fe (µg/g fw)	Mg (µg/g fw)
Azolla pinnata	LP	0.08	0.33	1.61	4.71	4.59
	HP	0.24	0.4	6.78	21.5	5.07
Lemna minor	LP	4.43	0.17	5.82	9.14	4.89
	HP	_	0.37	14.8	36.6	4.61
Pistia stratiotes	LP	_	_	_	20.8	4.64
	HP	0.03	1.53	3.93	6.17	6.38
Salvinia molesta	LP	_	_	2.04	16.2	5.03
	HP	0.29	0.18	8.78	27.1	4.69

Discussion

Phytoremediation include green and environment-friendly methods which employ plants to remove pollutants from the surrounding environment. It has attracted increasing attention in ecological studies because of its safety, high efficiency, low cost, and recyclability of plant harvests. But this method is often limited by its time consuming features because the life cycle of most plants used for phytoremediation is excessively long (Agunbiade *et al.*, 2009). However, this disadvantage is not significant to aquatic macrophytes because of their enormous biomass production.

In the present study various physiological and biochemical parameters as well as anatomical attributes were analyzed in the selected four aquatic macrophytes namely, *Azolla pinnata* R.Br, *Lemna minor* L., *Pistia stratiotes* L., *and Salvinia molesta* D.Mitch growing in less polluted and highly polluted aquatic habitats. In the case of dry weight and fresh weight, there is a considerable difference seen between the macrophytes selected from the less polluted and highly polluted aquatic habitats. Under polluted conditions, it was already reported that growth attributes like fresh weight and dry weight decreases in aquatic macrophytes when compared with the plants which are growing under unpolluted or less polluted aquatic habitats. It may be due to increased utilization of metabolites for competing with stress rather than for growth and reproduction of plants. Treatment of *Azolla pinnata* with As, Pb, Cu, Cd and Cr (2 and 5 mg 1^{-1} each), decreased dry weight percentage in with respect to control (Sarkar and Jana, 1986). The chlorophyll content under stressed condition is highly reduced when compared with that of macrophytes under unstressed conditions, because the chlorophyll is most sensitive to environmental stress. Chlorophylls are important to maintain photosynthesis activity in plants (Nageswara et al., 2001). Thus changes in environmental conditions certainly caused decline in the quantity of chlorophylls.

The macrophytes collected from the highly polluted aquatic habitats showed high accumulation of proline when compared to the samples collected from less polluted aquatic habitas, with the exception of *Azolla pinnata*. *Azolla pinnata* showed a higher amount of proline in the samples collected from less polluted aquatic habitats than in the samples collected from highly polluted aquatic habitats. Proline, an imino acid, is well known to get accumulated in wide variety of organisms ranging from bacteria to higher plants on exposure to abiotic stress (Saradhi *et al*., 1993; Ahmad *et al*., 2006). Quantification of MDA content in the samples revealed slight increase in highly polluted aquatic habitats than in the less polluted aquatic habitats, except in *Pistia stratiotes.* In *Pistia stratiotes* the amount of MDA is higher in the less polluted aquatic habitats in comparison with the highly polluted aquatic habitats. MDA is the decomposition product of fatty acids of membranes and its increase shows plants are under high-level oxidative stredd. MDA is already known as a stress indicator. So in the present study also, increase in MDA denotes the increased stress for plants. Under stress condition plant cell undergoes membrane damage and leakage due to the action of free radicals. According to Anjum et al. (2012), water stress increases the MDA content in *Zea mays*.

Fe and Mg accumulation was found to present in all samples. From the data it was clear that accumulation of heavy metals was higher in those samples which were collected from highly polluted aquatic habitats than those collected from less polluted aquatic habitats. The accumulation of Cu, Zn and Fe was higher in *Lemna minor*, and that of Mn was higher in *Salvinia molesta*, and more Mg content was present in *Pistia stratiotes*. *Azolla pinnata* was the only one aquatic macrophyte which showed the presence all the studied heavy metals inboth less and highly polluted aquatic habitats. The order of heavy metal accumulation was Fe > Mn > Mg > Zn > Cu in *Azolla pinnata*, and Fe > Mn > Mg > Cu > Zn *in Lemna minor*, and Fe > Mg > Mn > Zn > Cu in *Pistia stratiotes*, and Fe > Mn > Mg > Cu > Zn in *Salvinia molesta.* There were several noticeable differences in the anatomical features also. The samples collected from highly polluted aquatic habitats have larger air chambers and trichomes than when compared to the samples collected from less polluted aquatic habitats. The number of layers in the palisade parenchyma is higher in the samples collected from highly polluted habitats than that collected from less polluted habitats.

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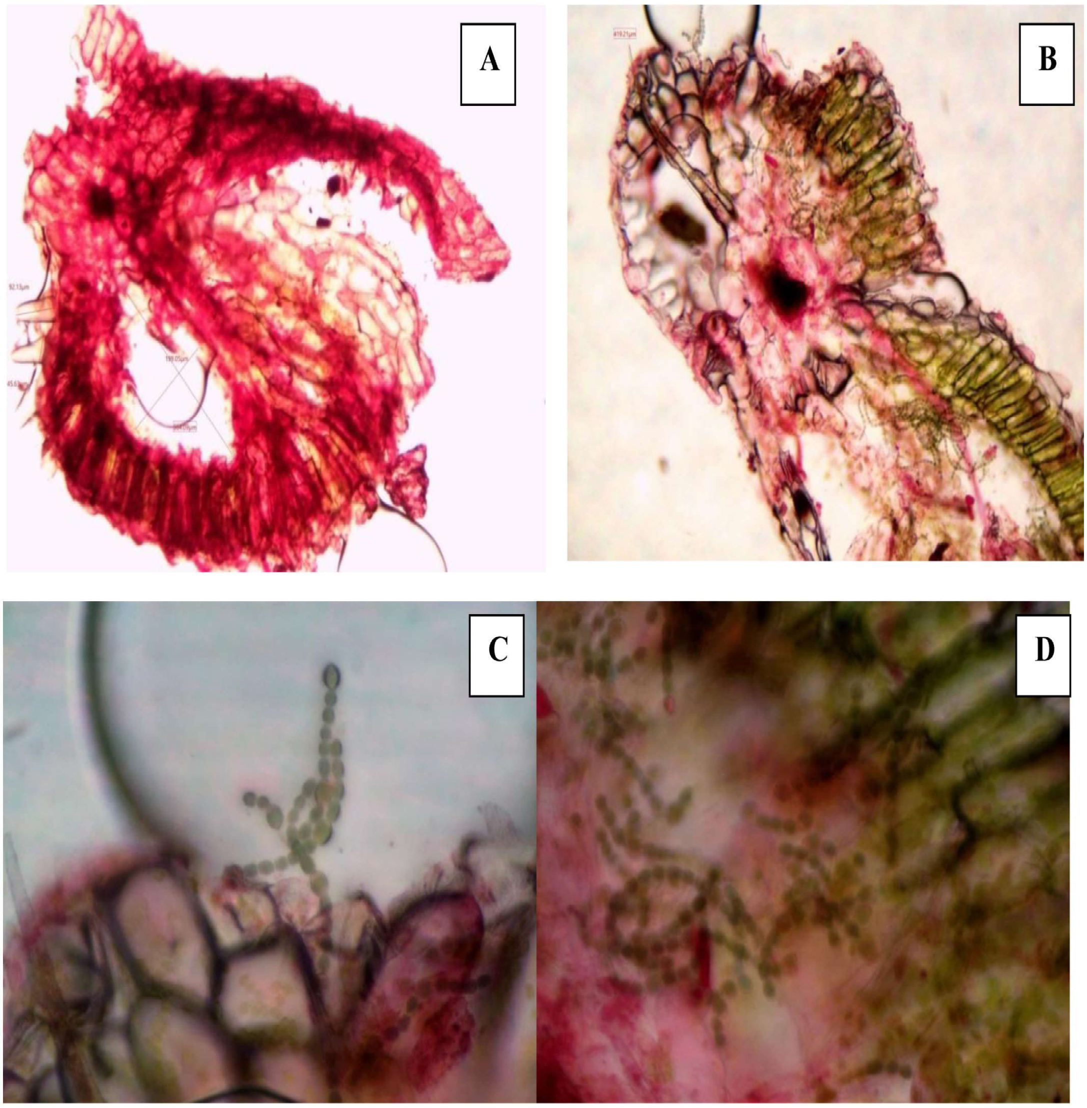

Figure 1: T.S. of *Azolla pinnata* frond. **1A**: Plant LP (Air cavity and hair measurements); **1B**: Plant HP (Air cavity and hair measurements); **1C and 1D**: Symbiotic association with *Anabaena*.

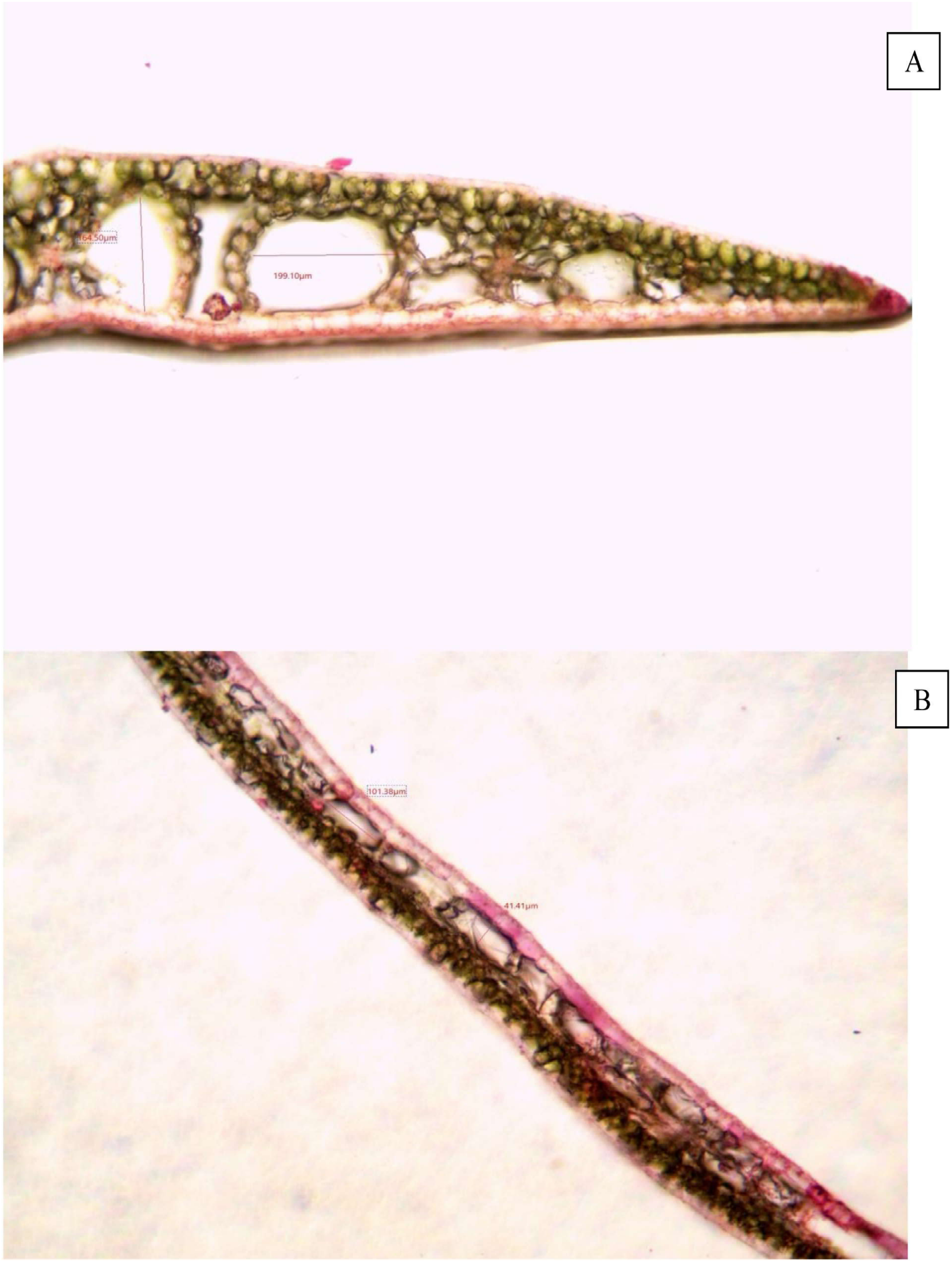

Figure 2: T.S. of *Lemna minor* frond. **2A**: Plant LP (Air cavity and hair measurements); **2B**: Plant HP (Air cavity and hair measurements).

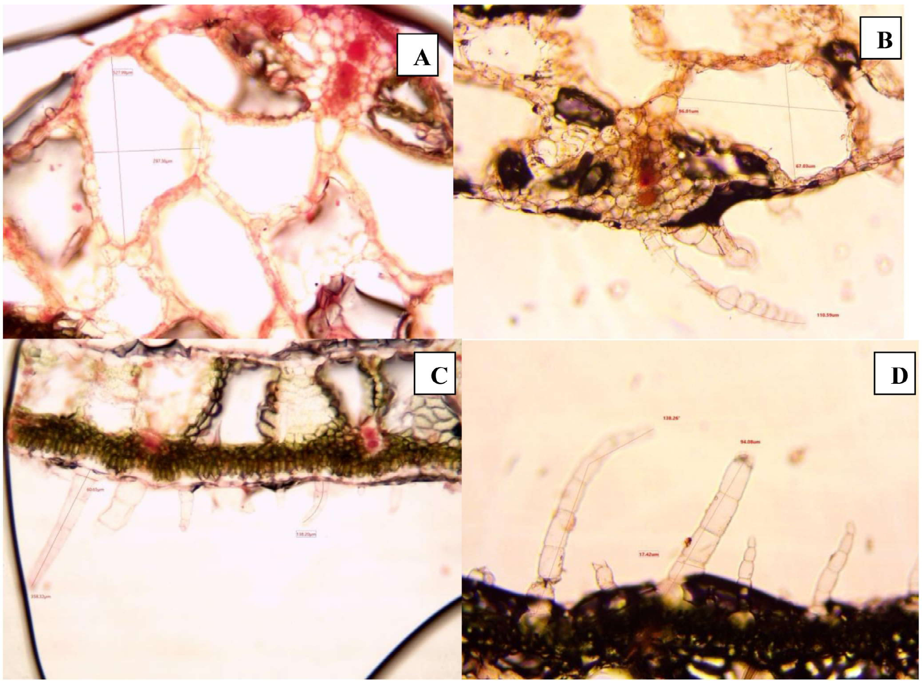

Figure 3: T.S. of *Pistia stratiotes* frond. **3A**: Plant HP (Air cavity); **3B** Plant LP (Air cavity); **3C**: Plant HP (hair measurements); **3D**: Plant LP (hair measurements).

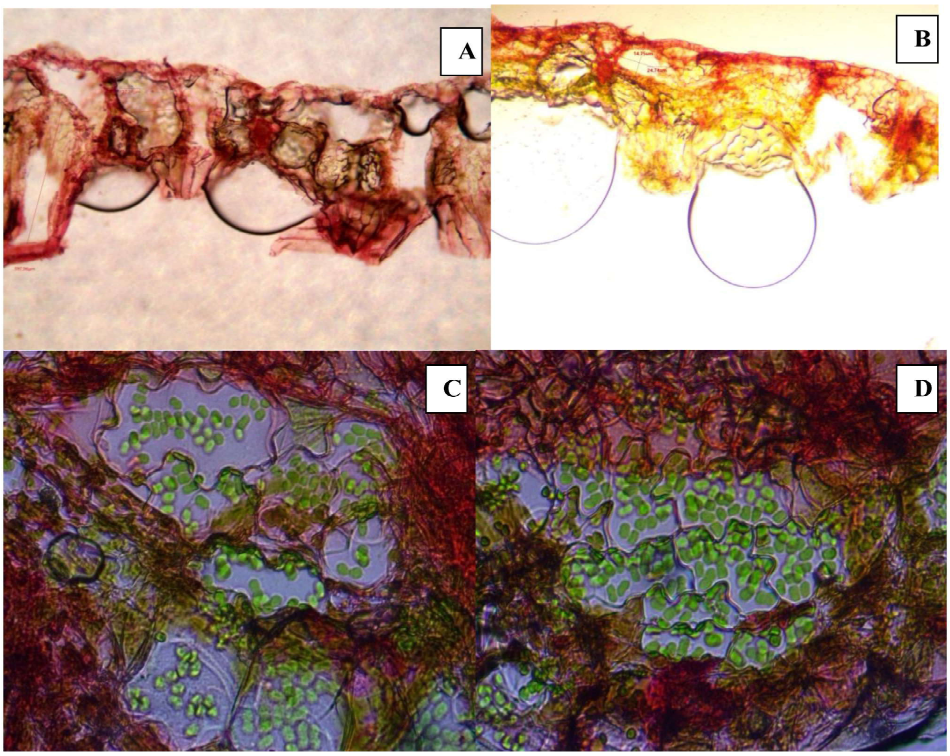

Figure 4: T.S. of *Salvinia molesta* frond. **3A**: Plant HP (Air cavity); **3B** Plant LP (Air cavity); **3C**: Plant HP (chloroplast); **3D**: Plant LP (chloroplast).

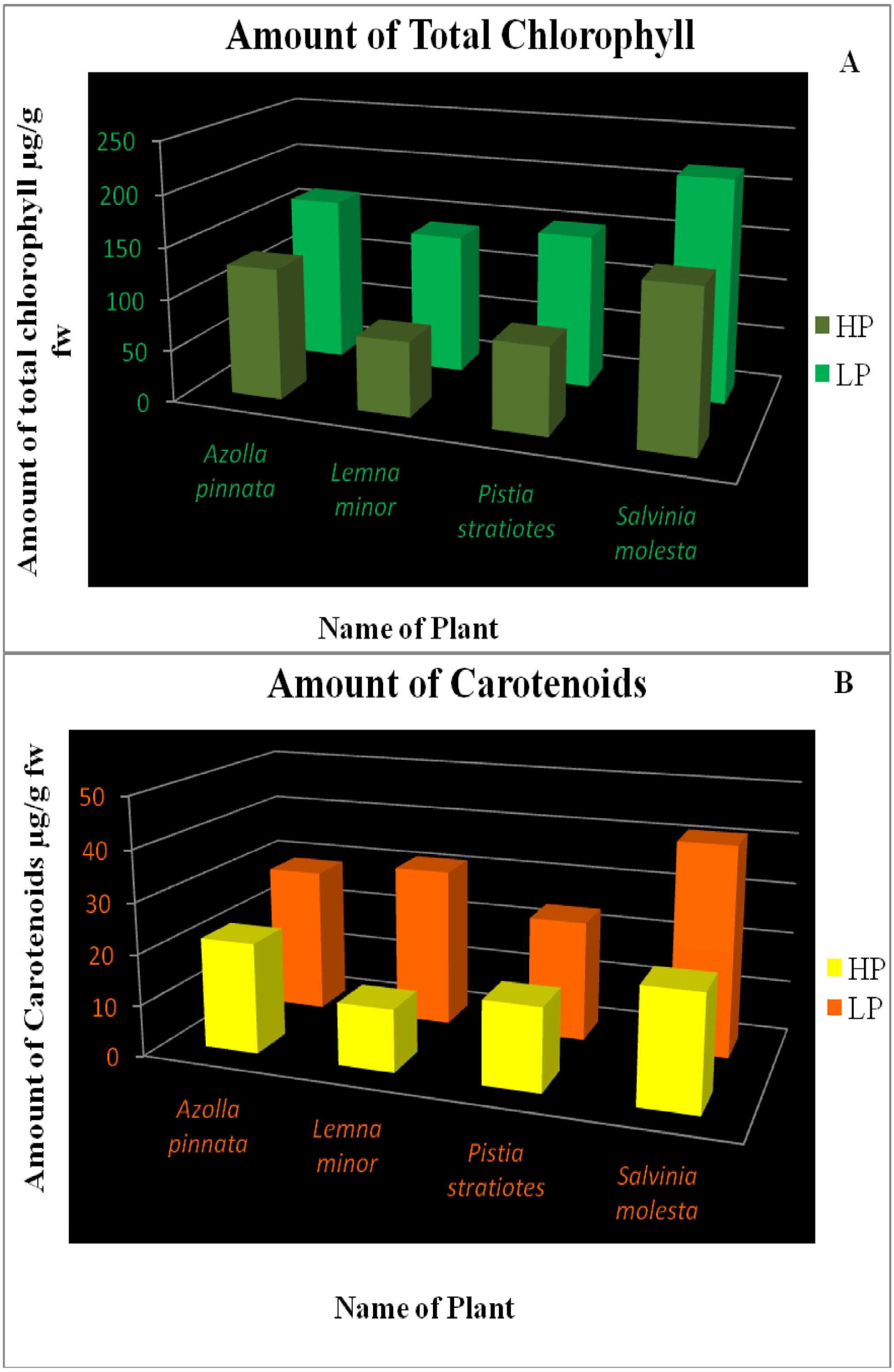

Fig. 5: Total chlorophyll content (Fig. 5A) and carotenoid content (Fig. 5B) of four selected aquatic macrophytes under high polluted and low polluted habitats.

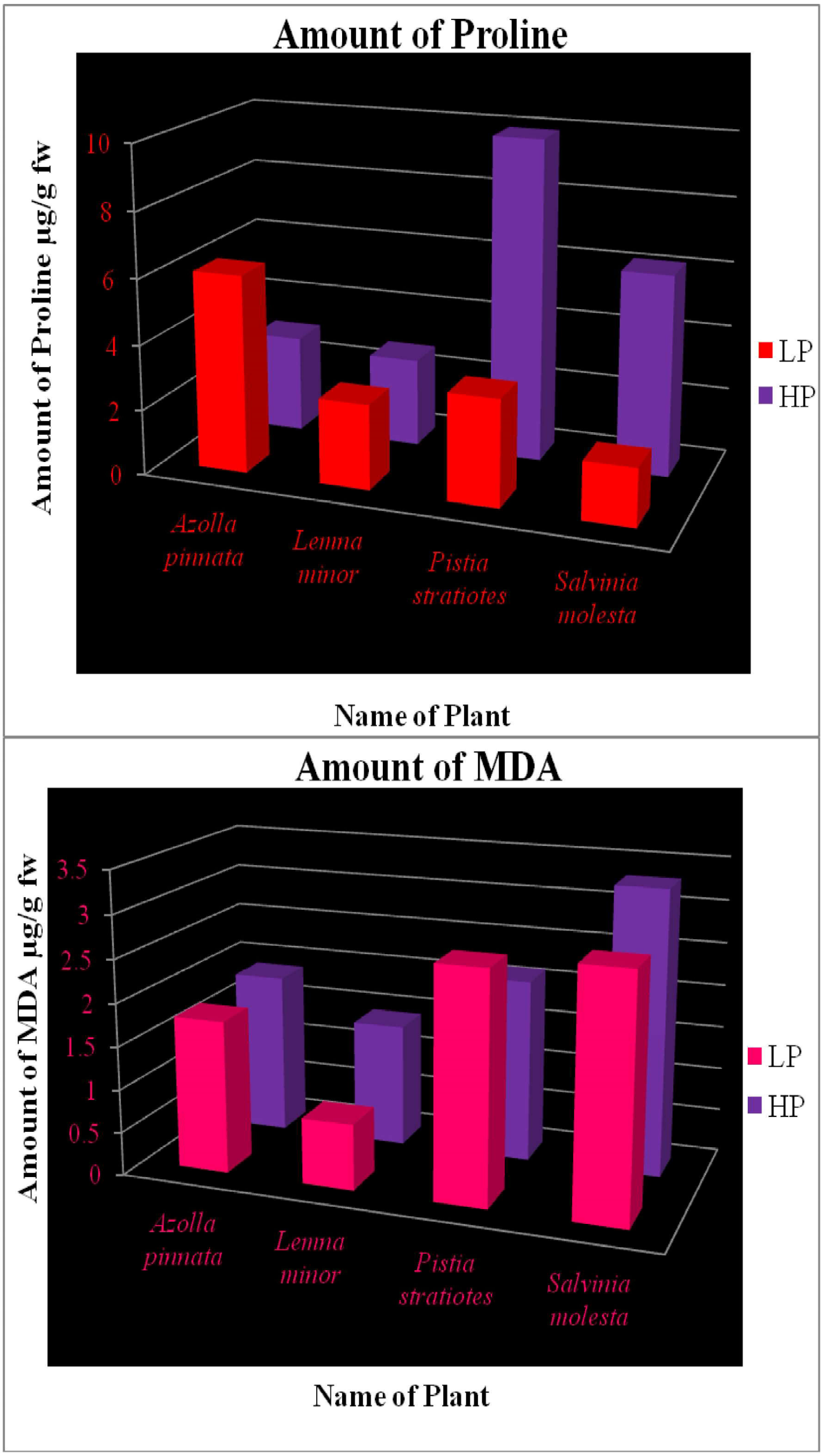

Fig. 6: Proline (**A**) and MDA (**B**) content of four selected aquatic macrophytes under high polluted and low polluted habitats.