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Physio-biochemical characteristics of *Acrostichum aureum* L. In the coir retting areas of Kadinamkulam Estuary, South India

Abstract

The Kadinamkulam lake a temporary estuary lying in the southern part of Kerala, is the largest of its kind in Thiruvananthapuram district, Kerala. Connected with the Anchuthengu kayal on the north and the Veli kayal on the south, the north and the Veli kayal on the south, the Kadinamkulam kayal remains connected with the Lakshadweep Sea for varying periods depending on rain fall and river discharge. *Acrostichum aureum* L. is only pteridophyte genus fern in the mangrove ecosystem of Indian coast. So the present study was conducted in the plant *Acrostichum aureum* L. identified in the selected stations of Kadinamkulam estuary. The major objective of the study is to assess the changes in the physiological and biochemical characteristics of the plant *Acrostichum aureum* L in coir retting areas. For this, surface water samples and plant samples were collected from the six selected stations of the coir retting areas and one station in the non-retting area of Kadinamkulam estuary. The results show reduction in leaf pigments, total proteins, carbohydrates in the *Acrostichum aureum* L. plants in polluted stations compared to that of control station/non retting area. The malondialdehyde, antioxidants and proline were increased in the *Acrostichum aureum* plants in retting stations and it vividly points out the stressful environment in the study area. It may be due to the presence of pollutants in the coir retting effluents and sewage disposed to this lake. The results of the study in lake water samples show that the values of some water quality parameters in coir retting areas were above the permissible limits of surface water quality standards.

Keywords: *acrostichum aureum* l, antioxidants, coir retting, kadinamkulam estuary, physio-biochemical parameters

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Introduction

The adaptation of plants to its environment is the most important issues in evolutionary biology. Acrostichum belonging to the family Pteridaceae, is the only fern genus of mangroves.[1] *Acrostichum aureum* L. is the only species that occurs in Indian coastline of Kerala in association with mangroves. The stem (rhizome) of this species is stout, erect, and covered with relatively large scales that are about 4 by 1.8 cm. Whole plant is used for anthelmintic and styptic purposes. Powdered rhizome paste is used to treat wounds, ulcers and boils. Fronds are used as antimicrobial agent and used to stop bleeding.[2] The present study aims to assess the changes in the biochemical and physiological characteristics of the estuarine plant *Acrostichum aureum* L. in the coir retting areas of Kadinamkulam estuary.

Materials and methods

Study area: The Kadinamkularn Kayal a temporary estuary lying in the southern part of Kerala, South India (Lattitude 8^{0}35' - 8^{0}40'N; Longitude 76°44' - 76°51'E) is the largest of its kind in Thiruvananthapuram district connected with the Anchuthengu Kayal in the north and the Veli Kayal in the south (Figure 1). This temporary estuary has no direct connection with the Arabian Sea, but seasonally it becomes connected through the opening of the sand bar at Perumathura.

For the study, surface water samples and the plant *Acrostichum aureum* L. (commom name: Golden leather fern) samples were collected in the morning from the seven selected stations (six retting and one non retting /control station) of the Kadinamkulam estuary during the summer season of 2017. Scientific classification of the plant is given in Table 1. It is a large species of fern that grows in mangrove swamps and other wet locations. All plant samples were immediately stored in an ice-chest at 4^{0}C and transported to the laboratory for analysis. The surface water samples were collected in clean one liter screw caped glass bottles from seven selected stations of the lake including six coir retting zones and one non-coir retting area. The mean depth of the Kadinamkulam estuary in the study stations was 1.328 m. The samples were carried to the laboratory on the same day after sampling.

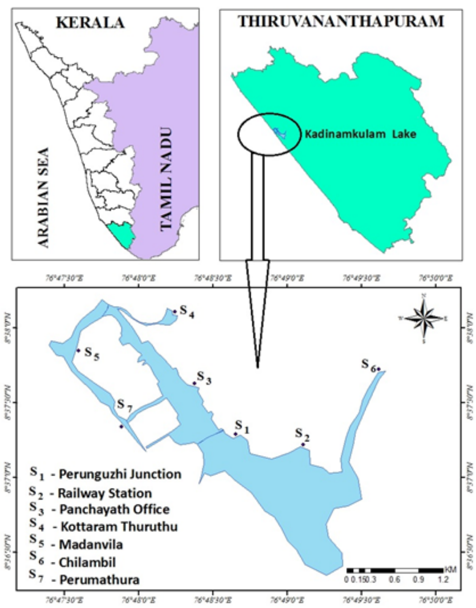

Figure 1 Location Map of the Study Area (Kadinamkulam Lake).

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Table 1 Scientific Classification of *Acrostichum aureum* L

Rank	Scientific name and common name
Kingdom	Plantae– Plants
Subkingdom	Tracheobionta– Vascular plants
Division	Pteridophyta– Ferns
Class	Filicopsida
Order	Polypodiales
Family	Pteridaceae– Maidenhair Fern family
Genus	Acrostichum L. – leather fern
Species	Acrostichum aureum L. – Golden leather fern

The different physio-biochemical parameters (pH, total chlorophyll, carotenoids, total carbohydrates, total proteins, proline, ascorbic acid, malondialdehyde, catalase, peroxidase) of the experimental plants were determined following the standard procedure by Sadasivam and Manickam [3] and the physico-chemical parameters of surface water samples were analysed following the standard procedure by Trivedy and Goel.[4]

Results and discussion

Biometric parameters of plants in retting and non retting area of Kadinamkulam estuary

The biometric parameters of *Acrostichum aureum* L. in the study stations are shown in Figure 2. The plant height recorded in the study area is in the range 62 cm- 95 cm. The highest value (95 cm) of plant height was noted in the plants in non coir retting area (control station). It is may be due to the less pollution in the control station and its effective growth. The root length is in the range of 16cm to 22 cm. The highest values are recorded in station 4. The total number of leaves in the plants in the study stations ranges from 18 to 32. The number of leaves recorded in the control plant was more compared to that of the plants in the coir retting stations. This may be due to the retting zone activity that leads to the reduction in the growth of plants in the stations.

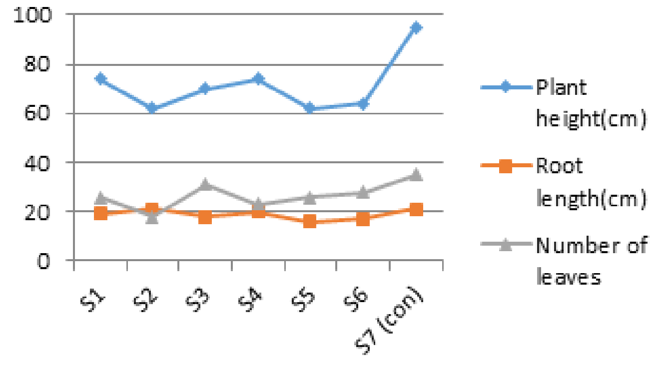

Figure 2 Biometric parameters of *Acrostichum aureum* L. in study stations.

Physio-biochemical parameters in plants in the retting and non retting areas of Kadinamkulam estuary

The results of the concentration of total chlorophyll and carotenoides are shown in Figure 3. The results of the concentration of total chlorophyll is in the range of 0.351 mg/g FW to 1.877 mg/g FW. This shows that the retting zone activities reduced the activities of some enzymes involved in photosynthesis in these plants. And in the control station, the total chlorophyll content is 1.877 mg/g FW. Caroteniods are the pigments that absorb light energy for use in photosynthesis, and they protect chlorophyll from damage. The concentration of caroteniods in the plant is in the range 0.025 mg/g FW to 0.261 mg/g FW. The control station plants showed highest carotenoid value (0.261 mg/g FW).The recorded leaf extract pH values are shown in Figure 4. The leaf extract pH of plants are in the range 4.47 to 8.1. The leaf extract pH of plants in the control station (Permathura) is alkaline. It may be due to the less amount of retting activities in that station. And in station 2, plant leaf extract pH is 4.47 and is acidic. It may be due to the retting activities in this area and production of organic acids and hydrogen sulphide.

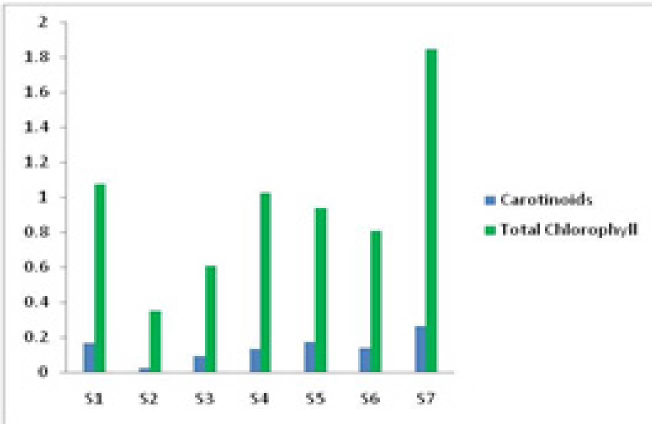

Figure 3 Foliar pigment content (mg/g FW).

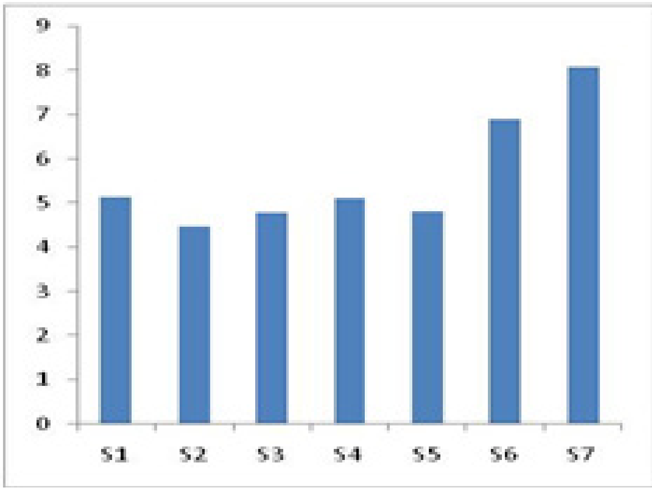

Figure 4 pH of the plant leaf extract.

The total carbohydrates, proteins, proline, MDA and ascorbic acid content are given in Table 2. The recorded protein content in the plant leaves are in the range of 220.7 mg/g FW to 369.12 mg/g FW. The control station plant has high protein content compared to other stations. The values of total carbohydrates in leaves are in the range 1.08 mg/g FW to 2.71 mg/g FW. The plants in the non coir retting area (control station) showed highest carbohydrate content.

Ascorbic acid is an abundant component of plants in relation to cell growth. The recorded values of ascorbic acid content in *Acrostichum aureum* leaves are in the range 1.993 mg/g FW to 13.37 mg/g FW. The results indicate that the plants in the stress condition regions the ascorbic acid values are increased compared to that of the control station. Ascorbic acid is found in all eukaryotes and is an antioxidant. In association with other components of the antioxidant system, protect plants from oxidative damage resulting from aerobic metabolism. In the abiotic stress condition, the ascorbic acid values are increased.[5]

Table 2 Biochemical characteristics of *Acrostichum aurium* L. In study station

Station	Parameter								
	Total Proteins (mg/g FW)		Total Carbohydrates (mg/g FW)		Proline (µ mol/g FW)		MDA (µ mol/g FW)		Ascorbic acid (mg/ g FW)
	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf
S1	221.75	181.75	1.23	1.75	0.374	0.23	8.78	3.8	12.57
S2	325.96	104.91	1.6	1.65	0.265	0.15	5.73	3.28	5.23
S3	217.54	102.87	2.54	1.7	0.438	0.23	4.21	4.86	7.9
S4	221.75	173.33	1.08	0.33	0.856	0.11	8.9	6	13.37
S5	221.75	121.75	1.53	1.17	0.401	0.23	4.05	4.82	4.29
S6	325.96	128.07	1.98	1.42	0.261	0.14	5.98	5.75	2.76
S7 (Control)	369.12	283.85	2.71	1.07	0.186	0.06	3.83	2.71	1.99

The proline content in *Acrostichum aureum* L. leaves are in the range 0.186 µmoles/g to 0.856 µmoles/g. The recorded values of proline content in plant roots are in the range of 0.064 µmoles/g to0.23 µmoles/g.The proline accumulations in the plant parts are proportional to increase in stress exposure. Proline increases the stress tolerance of the plants through such functions as osmoregulation, the protection of enzymes against denaturation and the stabilization of protein synthesis. The proline content was increased in the plants collected from the stations with retting activity. The stressful environment results in an overproduction of proline in plants which in turn imparts stress tolerance by maintaining cell turgor or osmotic balance; stabilizing membranes thereby preventing electrolyte leakage; and bringing concentrations of reactive oxygen species (ROS) within normal ranges, thus preventing oxidative burst in plants.[6] Proline treatment promotes plant growth in high-salt environments by increasing grain production, biomass, photosynthesis, gas exchange, and seed germination. Better nutrient intake, water uptake, and biological nitrogen fixation are the key causes of these beneficial impacts.[7]

The malondialdehyde content in the leaves of *Acrostichum aureum* L. plants in different stations ranged from 3.83µ mol/gm FW to 8.78 µmol/gm FW. The values of MDA content in plant roots ranged between 2.71µmol/gm FW to 6µmol/gm FW. The results show that the control station plants has less MDA content compared to that in the coir retting areas. The retting zone activity enhances the concentration of lipid peroxidation product, malondialdehyde in the plants in Kadinamkulam estuary. The malondialdehyde content was increased in the roots and leaves of *Acrostichum aureum* L. in the retting stations and indicates the stress in plants in the retting areas. Plant cells' capacity to detoxify reactive oxygen species (ROS) is hampered by osmotic stress. Cells do not produce much ROS under typical growth circumstances. But when a stressor upsets a plant's cellular equilibrium, the concentration of ROS-producing molecules increases dramatically.[8]

Peroxidase and catalase are the two important antioxidant enzymes in plants. The peroxidase activity recorded in the *Acrostichum aureum* L. plant leaves in the estuary varies from 7.14 Units/L to 12.5 Units/L extract. The highest activity of peroxidase (12.5 Units/L) was recorded in the plants in station 3. The leaves of *Acrostichum aureum* showed catalase activity in the range 121.42 Units/ml extract to 425 Units/ml extract (station 6). The increase in the activities of these antiperoxidative enzymes denotes the stressed condition of plant in the coir retting stations. Higher values of these enzymes indicate the oxidative stress in the plants of coir retting areas in Kadinamkulam estuary (Figures 5 and 6).

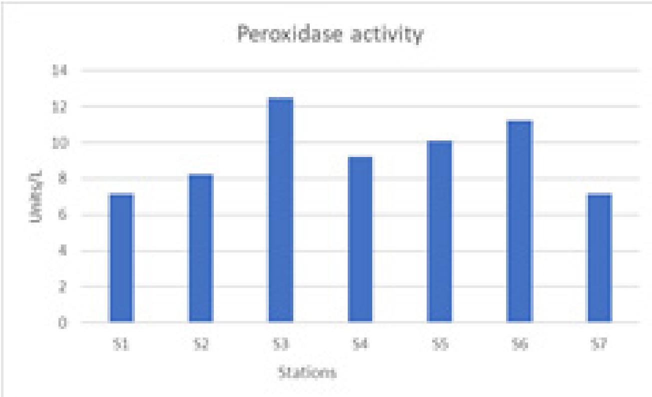

Figure 5 Peroxidase activity in plant leaves.

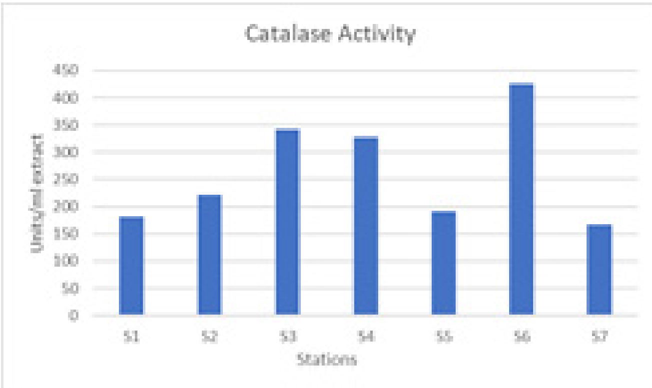

Figure 6 Catalase activity in plant leaves.

Physico-chemical characteristics of lake water in retting and non retting area

The results of the physico-chemical characteristics of surface water samples of are given in Table 3. The colour range in the different stations of coir retting area varied from 5 Hazen units to 25 Hazen Units. It may be due to the leaching of lake water containing coir retting effluent. The control station has 5 Hazen Units, it indicates control has less coir retting activity. The temperature of water in the study stations are in the range of 30^{0}C to 32^{0}C. The highest temperature (32^{0}C) was recorded in station 1. The recorded pH value in the study area is in the range of 6.72 - 8.1. These results indicate the pH of the lake and it varies in different station from slightly acidic to alkaline. The conductivity of lake water samples collected from the study area ranged between 25.4 µS/cm to 43.8µS/cm. This indicate the Kadinamkulam lake water may have more dissolved solids due to coir retting activity. And therefore, high electrical conductivity value was also recorded in retting stations. The control station showed the electrical conductivity value of 25.4mS/cm. The values of turbidity of water in the coir retting area are in the range 6.2 NTU to 35.5 NTU. The S_1 to S_6 stations have high turbidity values compared to the control station, and the highest turbidity value was recorded in station 2 (35.5 NTU). The greater the amount of total suspended solids in water, higher will be the turbidity values.

Table 3 Physico-chemical characteristics of surface water of Kadinamkulam estuary

Parameter	Station						
	S1	S2	S3	S4	S5	S6	S7 (Control)
Colour (HU)	10	15	15	25	15	25	5
Temperature (°C)	32	30	30	30	30	30	30
Turbidity (NTU)	22.6	35.5	22.1	35.3	11.9	35.3	6.2
Electrical Conductivity (mS/cm)	40.1	36.2	41.3	43.8	37.1	37.4	25.4
pH	7.6	6.95	7.4	6.72	7.63	7.29	8.1
TDS(mg/L)	22500	21700	25100	27700	26900	22400	20100
DO(mg/L)	6.9	7.31	4.06	8.53	6.5	7.94	7.72
Total Alkalinity (mg/L as $CaCO_3$)	40	40	60	60	40	20	20
Total Hardness (mg/L as $CaCO_3$)	580	536	624	360	598	606	542
Sodium (mg/L)	5992	5528	6211	4001	6939	6091	4537
Potassium (mg/L)	137.6	127.4	136.9	106.2	143.7	132.8	138.9
H_2S (mg/L)	7.48	6.8	7.14	7.82	6.46	6.8	2.04
Chloride (mg/L)	2220	1374.8	1852.4	1084.6	2820	1484.5	1039.8
Salinity (ppt)	25.8	22.9	26.5	27.4	18.2	23.4	17.3
Sulphate (mg/L)	67.97	64.44	77.76	50.81	79.48	67.37	79.48
Nitrate (mg/L)	0.337	0.932	0.25	0.565	0.473	0.515	0.742
Calcium (mg/L $CaCO_3$)	51.01	72.87	47.36	47.36	54.65	80.16	58.29
Magnesium (mg/L $CaCO_3$)	127.4	118.3	182	118.3	136.5	200.2	145.6

The dissolved oxygen (DO) of lake water ranges from 4.06 mg/L to 8.94 mg/L. The BOD values are in the range of 1.41 mg/L to 8.13 mg/ L. The highest BOD value (8.13 mg/ L) recorded in the station S4 indicates more organic pollution in this station. The lowest BOD value (1.41 mg/L) was recorded in the control station. Alkalinity is a measure of the capacity of water to neutralize acids. The alkalinity values of water in the study stations range from 20 mg/L as $CaCO_3$ to 60 mg/L as $CaCO_3$. The station 3 and station 4 showed highest alkalinity. In the study, the total hardness values recorded for water samples collected from sampling stations S_1 to S_7 are in the range 360 mg/L as $CaCO_3$ to 624 mg/L as $CaCO_3$ (Table 3). The highest value of hardness (624 mg/L as $CaCO_3$) was recorded in the station 3. It is due to the discharge of sewage and coir retting activities occurring in this area. It may also impart more calcium and magnesium ions in this lake.

In this study, the calcium and magnesium content recorded in the study stations are shown in the Table 3. The recorded values of Ca content range from 47.36 mg/L as $CaCO_3$ to 80.16 mg/L as $CaCO_3$. The station 6 has recorded the highest value of calcium i.e. 80.16 mg/L as $CaCO_3$. In this station, many local people use lake water for domestic purposes. And the lake water is getting mixed with underground drainage and sewage water from the nearby areas. Ca^{2+} and Mg^{2+} ions if present in excess may get deposited in the soft tissues of the living bodies leading to various kinds of chronic diseases. The highest value of Mg content (200.15 mg/L as $CaCO_3$) was recorded in station 6. The magnesium ions are increased in this station due to the coir retting and domestic activities.

The chloride and hydrogen sulphide content in surface water recorded in the study stations are shown in Table 3. The chloride value ranged from 1039.8 mg/L to 2820 mg/L. The station 5 recorded the highest chloride value (2820 mg/L). The hydrogen sulphide content in the surface water samples ranges from 2.04 mg/L to 7.82 mg/L. The highest concentration of hydrogen sulphide was recorded in station 4 (Kottaram Thuruthu). In this station intensive retting activities are going on compared to other stations. This leads to water pollution and cause damage to the aquatic plants and fishes. The control station water showed least hydrogen sulphide content (2.04 mg/L). Studies by Remani *et al.*[9] reported that the coconut husk adversely affects the productivity of the backwaters and is harmful to estuarine flora and fauna. Studies conducted on Vemabanad Lake have demonstrated the high level of pollution in the lake's backwaters, as well as the irreversible harm to productivity. The retting area had a higher concentration of hydrogen sulphide than the non-retting area. The discharge of hydrogen sulphide from the retting material is what causes the water pollution, which has an impact on the aquatic environment's dissolved oxygen concentration and productivity.[10]

The recorded value for salinity in the surface water of study stations varies from 17.3 mg/L to 27.4 mg/L. The highest value 27.4 mg/L was recorded in the station 4. It may be due to the leaching of salts from the domestic activities. The control station showed less salinity, 17.3 mg/L. Nielsen *et al.*[11] reported that aquatic biota will be adversely affected as salinity increases. Salinisation can lead to changes in the physical environment that will affect ecosystem processes. The surface water in the study area recorded sodium content in the range 4001 to 6939 mg/L. The station 5 showed higher value for Na content compared to other stations. The study area shows the concentration of potassium is in the range 106.2 mg/L to 143.7 mg/L. Potassium compounds are predominantly soluble and rarely precipitated.

In the present study the recorded value of sulphates in surface water ranges from 50.81 to 79.48 mg/L (Table 3). The control station showed higher sulphate value (79.48 mg/L) than the other study stations. In the coir retting areas, the sulphate content was found less and may be due to the conversion of sulphate to hydrogen sulphide. In the study area, the nitrate values ranges from 0.25 to 0.932 mg/L. The lowest value for nitrate content 0.25 mg/L was detected in the control station. This indicates that the coir retting activities altered the water quality parameters of this surface water body.

Conclusion

In this study, the water pollution due to open retting practice was revealed in the Kadinamkulam lake. The results show that H_2S content was increased and there is reduction of the DO in the water in retting area. This changes in water quality of the lake that adversely affects the aquatic organisms. Salinity stress detected reduces the water uptake and this reduces the plant growth in this area. The plant species *Acrostichum aureum* L. in coir retting /polluted areas recorded reduction in plant pigments compared to that of the control area. Also, the concentrations of biomolecules (total proteins, carbohydrates) are reduced in the plants in coir retting stations compared to that of the non-retting area. The malondialdehyde, proline and antioxidants are increased in the plants in retting stations, which vividly points out the stressful environment in the study stations due to the pollutants in coir retting effluents. Therefore the study concludes that open retting activity affects the physiological and biochemical processes of the plants in the banks of Kadinamkulam lake.

The study also recommends to implement closed retting process, the green technology for controlling coir retting pollution of backwaters. Awareness programs should be conducted among public by local authorities to avoid the disposal of domestic wastes and sewage to this water body.

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Conflicts of interest

The authors declare there is no conflicts of interest.

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