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The Role of Antioxidant Metabolism in Phytoremediation of Shrimp Farm Effluent by *Acrostichum aureum Linn*

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Abstract The study evaluates the phytoremediation capabilities of *Acrostichum aureum* in the treatment of shrimp farm effluent. The effluent parameters like Biochemical Oxygen Demand (BOD) (73%), Chemical Oxygen Demand (COD) (39%) and Nitrate (55%) were found reduced considerably during the treatment period of thirty days. The antioxidant concentrations of the plant were increased to cope up with the stress environment. Superoxide dismutase (SOD) in plants was increased to 70% during the treatment period. Peroxidase activity was also found to be increased from 0.02 to 0.26 mg/gFW. The increase in non-enzymatic antioxidants like chlorophyll indicated the health status of the plant during the treatment period.

Keywords: *phytoremediation, shrimp farming, antioxidant, Acrostichum aureum*

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1. Introduction

Limited natural sources for fish production had resulted in the significant booming of aquaculture and contributed the world food supply. Although this industry have given a big contribution towards economic growth of the country, however, the large volume of water consumption and the wastewater discharged into the water source caused a significant environmental problem that must be controlled properly. Pollution and environmental degradation in coastal areas due to aquaculture farms is a serious problem in many developing countries. Most of the aquaculture farms do not have treatment facilities for treating aqua farm effluents. The common way to reduce water pollution is to treat the waste water by an effective treatment method at the source itself. Another way is to recycle the wastewater for secondary aquaculture [1].

Aquaculture wastewater from shrimp cultivation activities cause increased levels of ammonia, nitrate, phosphorus and organic matter [2]. A large portion of input nitrogen and phosphorus into shrimp ponds as feed is not converted to shrimp biomass, but is released into the environment. Ammonia in water is the principal nitrogenous waste product excreted by aquatic animals and released from decomposing nitrogen containing organic matters by microbial activities. Rapid development of shrimp farming brings a series of environmental, human health and safety problems, as well as considerable profit and interests, thus causing concern about its sustainability [3,4]. Therefore; intensive culture of shrimps should properly managed to keep the shrimp cultivation sustainable. In other words, shrimp farm effluent before discharged into the environment should undergo some treatment beforehand. An inexpensive, easy, and effective technology in controlling aquaculture's wastewater is phytoremediation technology. In the 1980s, the concept of phytoremediation was born when the ability of plants species to accumulate high amount of toxic metals in their tissue and organs was proved [5,6]. Phytotechnologies, which are the application of plants and associated microorganisms-based technologies, are beginning to be accepted to examine the problems and provide sustainable solutions for effluent pollution [7].

Constructed Wetlands (CWs) have been exploited for rearing crayfish, shrimp, and commercial fish species in shallow ponds and used mainly for treatment of freshwater aquaculture effluent [8]. If relatively inexpensive land is available, CW for aquaculture can be cost effective since they require only moderate capital investment, with low energy consumption and low maintenance expenses [9]. Higher plants can be yet another solution for the assimilation (removal) of dissolved nutrients, suspended solids, and reduction of biological oxygen demand (BOD) and pathogens from land-based aquaculture systems [8]. Halophytes are salt tolerant plants and therefore have found application in agro-engineering projects such as recycling of agricultural and industrial effluents [10], bioremediation of aquaculture effluent [11], and phyto-remediation of contaminated soils [12]. Reference [13] investigated the potential of native wetland plants (*Acrostichum aureum* L. and *Rhizophora apiculata*) for phytoremediation of antibiotics used in sediment of shrimp farms. The main objective s of the study is to determine the role of antioxidants in phytoremediation of shrimp farm effluents and the characterization of shrimp farm effluent. The performance evaluation of *Acrostichum aureum* in bioremediation of the shrimp farm effluent was also studied in detail.

2. Materials and Methods

2.1. Study Area and Need of the Study

Munroe Island is a part of Ashtamudi Lake which is one of the Ramsar sites of Kerala where commercial shrimp farming is practiced. Farming systems have gradually shifted from extensive traditional systems to semi-intensive and intensive that multiplied the environmental stress to the ecosystem. The untreated shrimp farm effluents which contain high concentration of organic load, fertilizers, chemicals and antibiotics deteriorate the quality of water in the area. The effluents from the culture farms cause serious threat to the water resources and Lake Ecosystem. High nutrient content in the effluent leads to Eutrophication of the lake. The chemicals and antibiotics used in aquaculture had adverse effect on the native aquatic animals. Prevention of shrimp farm effluent pollution is important for the conservation of the Ramsar site.

2.2. Collection of *Acrostichum aureum*

The golden leather fern is found in tropical and sub-tropical areas around the world. It grows in swamps and mangrove forests, salt marshes and on river banks and is tolerant of raised salinity levels. For the present study, *Acrostichum aureum* were collected from river banks of Munroe Island in Kollam. *Acrostichum aureum* were abundant in the area and it is used for protecting the embankment of aquaculture pond ridges from erosion. Collected Plants were washed several times using tap water and distilled water to remove particles adhering to them and are kept in crates containing tap water, mud, and pebbles with a total thickness of 2 inch. Plants are allowed to grow for two weeks for acclimating to the new environment. Plants of similar size and shape were selected with an average initial weight of about 20g wet mass for the experiment.

2.3. Design of the Containers

The sizes of the containers were 18 X 18 X 24 cm. The base of the tank was filled with gravel (2 cm) and wetland soil (2 cm) up to 4cm in height. Water/Effluent level in the tank was up to 6 cm height.

2.4. Experimental Media

Effluents were collected from one selected shrimp farm were transferred to the laboratory. The experiment was conducted in plastic crates of 10L capacity and were labeled as $E50_1$, $E50_2$ (both containing 50% of farm effluent and 50% distilled water), $E100_1$, $E100_2$ (both containing 100% of farm effluent) and E50C, E100C (both as control).

2.5. Experimental Design

For treatments, the plants which maintained in the stock tanks were collected, cleaned and introduced in the experimental tanks. Approximately 250 g (wet weight) of each experimental plant is used for the study; each occupying half of crates. An experimental set up for 30 days were planned and conducted. The experimental work was carried out in a Constructed Wetland system in a greenhouse outside the lab. Plants were exposed to sunlight and the level of effluent was kept constant by adding distilled water, to compensate the water loss through transpiration, sampling and evaporation, as and when required. Samples were collected at an interval of 10 days for analysis.

2.6. Physicochemical Parameters

Physico-chemical characteristics of treated water are analyzed. Standard methods have been adopted for pH, conductivity, salinity, sulphate, nitrate, phosphate, biological oxygen demand and dissolved oxygen [14].

2.7. Estimation of Antioxidant Enzymes

The activity of Superoxide Dismutase (SOD) was assayed spectrophotometrically by measuring its ability to inhibit the photochemical reduction of Nitro blue Tetrazoilum [15]. One unit of SOD is the amount of extracts that gives 50% inhibition in the rate of NBT reduction. Catalase activity (CAT) was determined by consumption of H_2O_2 and was monitored spectrophotometrically at 240 nm for 3 min. For Peroxidase assay (POX), in the presence of the hydrogen donor pyrogallol, peroxidise converts H_2O_2 to H_2O and O_2. The oxidation of pyrogallol or dianisidine to a coloured product called purpurogalli can be followed spectrophotometrically at 430nm [16]. Glutathione s-transferase (GST) was assayed by its ability to conjugate GSH and CDNB, the extent of conjugation causing a proportionate change in the absorbance at 340nm [17].

2.8. Non-enzymatic Antioxidants

The chlorophyll content in the plant *Acrostichum aureum* was estimated by the procedure reported by Reference [18].

3. Results

3.1. Physico Chemical Characteristics of the Effluent

The shrimp farm effluent had higher level values of BOD and COD, like level of Total Dissolved Solids (TDS) of the effluent was 6350 mg/l. the water quality parameters of effluent before treatment has been measured which is given in the Table 1.

Table 1. Physico chemical characteristics of Shrimp farm effluent

Parameters	Effluent characteristics
pH	6.59
Total Dissolved Solids (mg/l)	6370.0
Salinity (ppt)	5.38
Sulphates (mg/l)	606.40
Nitrate (mg/l)	11.32
Phosphate (mg/l)	0.12
Ammonium (mg/l)	0.56
DO (mg/l)	5.87
BOD (mg/l)	17.5
COD (mg/l)	46.4

The tolerance limit of surface water for different classes were listed in IS: 2296. Tolerance limit for surface waters for aqua culture and wild life propagation were given in the Table 2.

Table 2. Tolerance limit for surface waters for aqua culture and wild life propagation

	Characteristic	Tolerance Limit
1	pH value	6.5 to 8.5
2	Dissolved Oxygen, mg/l, Min.	4.0
3	Free Ammonia (as N), mg/l, Max.	1.2
4	Electrical Conductance at 25 °C, µS, Max	1000
5	Free Carbon Dioxide (as C02),mg/1, Max	6.0
6	Oils and Grease, mg/l, Max	0.1

pH of effluent with 50% concentration decreases from an initial pH of 6.27 to 5.60 (day 30). Similar trend was observed in the case of 100% effluent (ie 6.96 to 4.78).In control, both the concentration of effluent showed decrease of pH from the initial values. Increasing the number of days of the experiment resulted in gradual decrease in pH, over a period of 30 days. The values of Total dissolved solids (TDS) in both control and experimental media was found to be decreased at the end of the experiment. The effluent with 50% concentration showed an increase in TDS value (4125 mg/l) from first day to the 20th day (2124 mg/l) and then decreased considerably in 30th day (826 mg/l).

As the biomass and treatment period increased, the salinity of the treated water increased slightly from first day to 30th day in all experimental media. This might have happened due to the water evaporation. The value of salinity for 50% and 100% concentrations varied between 2.34 to 2.42 ppt and 5.38 ppt to 5.46 ppt respectively. The nitrate values for all media with plant. The experiments using *A. aureum* indicated a reduction in nitrate concentration. For effluent with 100%and 50% concentrations the value of nitrate varied between 11.32 to 5.2mg/l and 9.3 to 2.1mg/l respectively. The values of phosphate for the effluent media found to be decreased during the experimental period. The media with 50% and 100% concentrations reported the value of phosphate between 0 .09 to 0.06 mg/l and 0.12 to 0.08 mg/l respectively.

Dissolved Oxygen (DO) has gradually increased in each effluent media as a result of growth and phytoremediation has occurred in each plant. For effluent with 100% concentrations the value of DO varied between 5.87 to 6.21 mg/l. In the case of 50% concentration both control and media showed no significant changes in DO values. Media with 50% effluent showed a minute decrease in DO value during 30th day of the experiment. Biological oxygen demand has gradually decreased in each effluent media as a result of phytoremediation. For effluent with 100% and 50% concentrations the value of BOD varied from 17.50 to 4.6 mg/l and 9.54 to 3.2 mg/l respectively. But in control, the value varied from 17.5 to 15.6 mg/l.

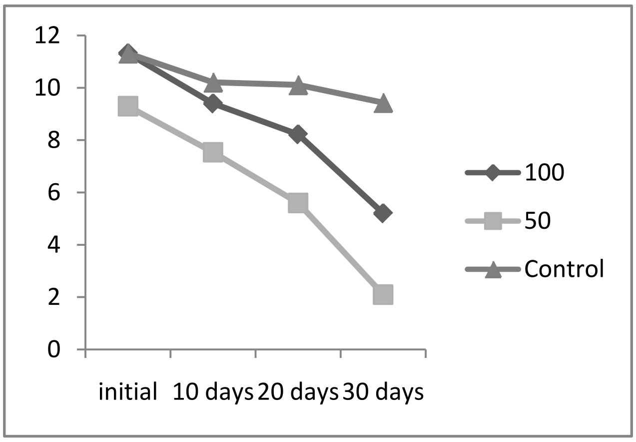

Figure 1. Variation in nitrate concentration of Effluent

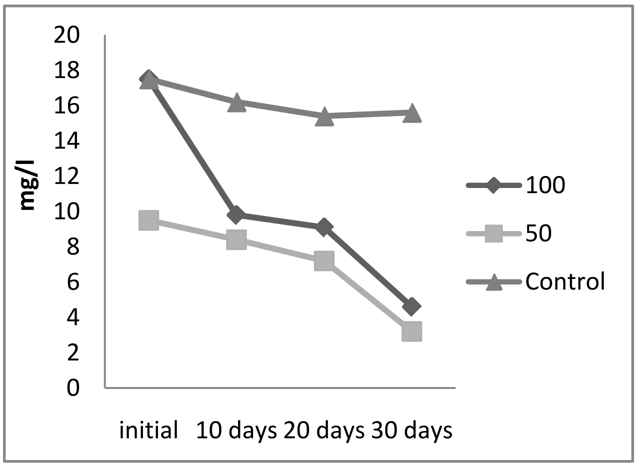

Figure 2. Variation in BOD of Effluent

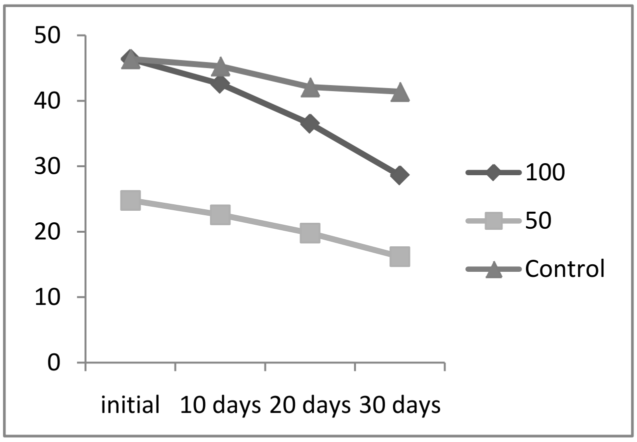

Figure 3. Variation in COD of Effluent

Chemical Oxygen Demand has gradually decreased in each effluent media but in slow pace.. For effluent with 100% and 50% concentrations the value of COD varied between 46.40 to 28.6 mg/l and 24.8 to 16.2 mg/l respectively. But in control, the value varied from 46.4 to 41.4 mg/l.

3.2. Biochemical Characterization of Plant

The activity of some enzymatic antioxidants such as superoxide dismutase, catalase, guaiacol peroxidase, glutathione s-transferase and non-enzymatic antioxidants like chlorophyll content was measured. The value of catalase for both concentration of effluent was gradually increases from first day of experiment to the 30th day. In the case of 50% effluent a sudden decrease was observed from the initial to the 20th day of sampling then it gradually increases during the 30th day. Catalase activity was found to be increased in stressed plant. The content of superoxide dismutase (SOD) showed a gradual increase throughout the experiment. The SOD activity in both 50% and 100% stressed plants varied from 10.09 to 13.34 and 10.29 to 17.03 EU/gFw throughout the experiment respectively. The Peroxidase content in plants found to be increased under effluent stress with the increase of days. POD activity for plants growing in 100% media was varied from 0.02 to 0.26 EU/gFW/minute. For the plant growing in 50% media was changed from 0.01 to 0 .19 EU/gFW/minute. The SOD activity of plants in under 50% and 100% stresses varied from 0.13 to 0.20 and 0.14 to 0.21EU/gFW throughout the experiment respectively.

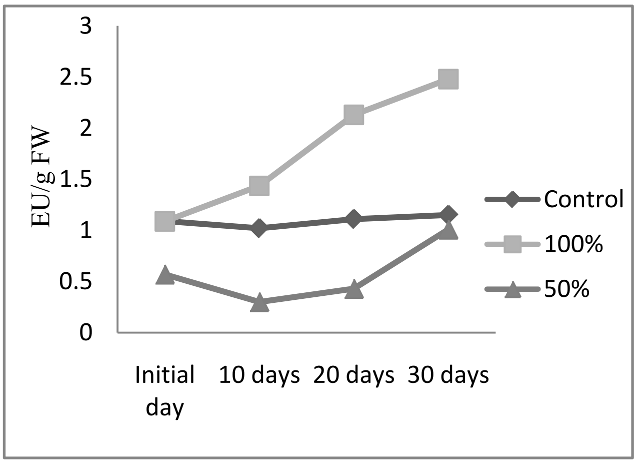

Figure 4. Variation in catalase

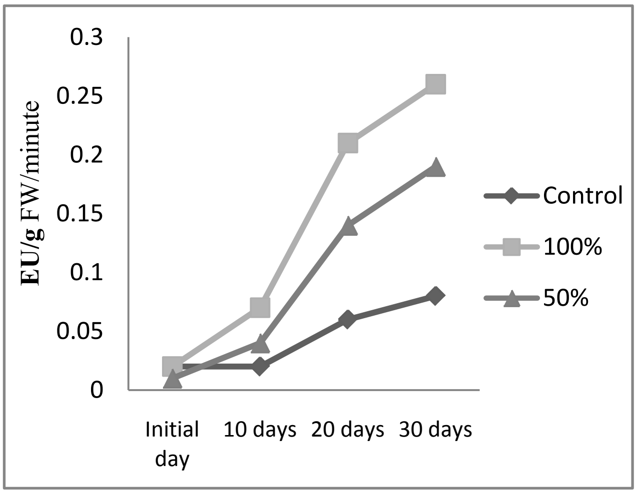

Figure 5. Variation in Peroxidase

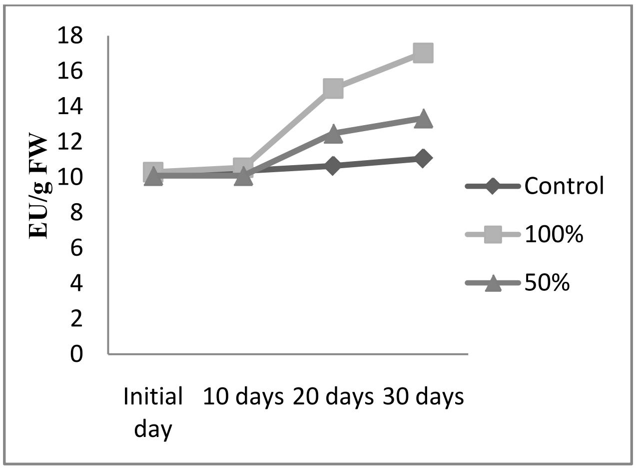

Figure 6. Variation in SOD

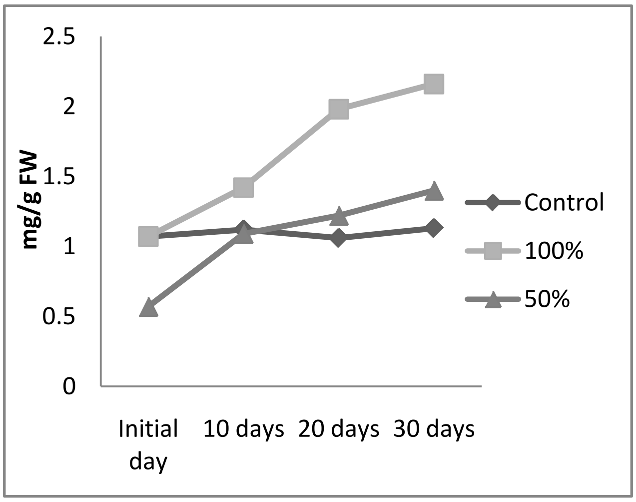

Figure 7. Variation in Chlorophyll

The calculated value of chlorophyll for effluent with 100% and 50% concentration at 663 and 645 nm were increased from 1.07 to 2.11 and 0.57 to 0.14 mg/l respectively. The content of chlorophyll showed a gradual increase during the process of phytoremediation. Maximum value of chlorophyll was observed during the last days of experiment.

4. Discussion

The decreases in pH values in the CW was likely large due to the nitrification process, as nitrification produces hydrogen ions that can lower the pH [19]. Many constructed wetlands exhibit a strong "buffer" capacity with respect to temperature, DO and pH [20]. The reduction in *pH* favored microbial action to degrade BOD and COD in the *wastewaters* [21]. In aquatic macrophyte based treatment systems, fluctuations in the pH of the growth medium are caused by the uptake of cations and anions by the root systems of the growing plants. When cations are taken up more rapidly than anions, the roots will release hydrogen ions into solution and the pH of the medium falls. Another explanation for the fluctuation in pH is the consumption of carbon dioxide (CO_2) during algal photosynthesis and the production of CO_2during organic matter decomposition [22].

Acrostichum aureum is a salt tolerant plant and can accumulate salts in the leaves. These plants contain a vacuolar sap of similarly high osmotic pressures and salt composition as mangroves. Reference [23] reported that the increase in plant density affects the decrease in the concentration of nitrogen. NO_3-N is the preferred form of inorganic nitrogen taken up by the roots of higher plants. Most of the nitrogen removal in the CW is the result of nitrification and denitrification process [20]. In some cases, CWs were reported to be poor in nitrate removal or even increased nitrate levels in the water [24].

Fish meal contains a large amount of phosphate that is in excess of the fish's dietary requirements. As a result, most dietary phosphorus is released in the effluent, excreted mainly in the faecal material. Reference [25] estimated that 47–84% of total phosphate in aquaculture effluents is particularly bound to the TSS. The key mechanism of phosphorus removal in pond systems was uptake by plants [26]. *Acrostechum aureum* took up phosphorus for their growth. This explains the decrease of phosphorus in treatments. However, the bacteria attached to the extensive root system of *Acrostechum aureum* is likely responsible for the higher reduction in treatments with plants [27]. The decrease of DO in the treatments with plant was also observed in the constructed wetland system planted with common reed in treating shrimp wastewater [24].

Reference [28] reported that, lead treated *Jatropha curcas* showed increasing catalase activity, which may be due to the scavenging role of CAT to H_2O_2. The increase of SOD activity can be considered as an indirect evidence for enhanced production of free radicals. Reference [29] states that the superoxide radicals are scavenged in stressed plants by superoxide dismutase which converts O_2 to H_2O_2. SOD activity has been reported to increase in plants exposed to various environmental stresses, including drought and metal toxicity [30]. Increase in SOD activity in response to stress appears to be probably due to de-novo synthesis of the enzymic protein. Transgenic plants over-expressing SOD, show increased tolerance towards oxidative damage caused due to harsh environmental conditions and among antioxidant enzymes the activity levels of SOD are of more relevance in maintenance of the overall defense system of plants subjected to oxidative stress [31-32].

Guaiacol peroxidase are widely accepted as stress “enzyme.” GPX can function as effective quencher of reactive intermediary forms of O_2 and peroxy radicals under stressed conditions. Reference [33] correlated increased activity of GPX to oxidative reactions under metal toxicity conditions and suggested its potential as biomarker for sublethal metal toxicity in plants [31]. The compounds, ascorbate and glutathione, are key non-enzymic antioxidants and play an essential role in protecting plants against oxidative damage. Several authors have reported increased activity of GPX under environmental stresses [32]. Chlorophyll content is often measured in plants in order to assess the impact of environmental stress, as changes in pigment content are linked to visual symptoms of plant illness and photosynthetic productivity.

5. Conclusion

The Shrimp farm effluent pollutants were found to be reduced during phytoremediation. The pollutants like BOD, COD and nitrates were also reduced considerably. The effluent stress caused a number of changes in the physico-chemical and biological characteristics of plants. The study also showed that the growth of *Acrostechum aureum* in shrimp farm effluent affects the physico-chemical characteristics of water and thereby enhancing degradation of pollutants. Plants show increased activity of catalase, super oxide dismutase, peroxidase and glutathione s-transferase activity. The chlorophyll content of the plants was increased throughout the experiment. In conclusion *Acrostechum aureum* was found to be suitable for the remediation of shrimp farm effluent.

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