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Biodegradation of Sewage Waste Water Using Azolla Microphylla and Its Reuse for Aquaculture of Fish Tilapia Mossambica

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Abstract: *This study focused on the biomonitoring capacity of Azolla microphylla for purification of sewage waste water. 100% untreated and treated sewage samples were prepared and the Azolla microphylla were introduced into the tubs. After 96hrs, sewage waste water samples were analysed and the results showed an active reduction in physicochemical parameters and heavy metals. The biotreated (Azolla microphylla) sample was reused for Aquaculture of fish - Tilapia mossambica for a period of 60 days. At the end of 60 days, this study was extended to show the effects of 100% untreated, treated, biountreated (Azolla microphylla) and biotreated (Azolla microphylla) sewage water on fish - Tilapia mossambica by examining the potential target organs namely gills, liver and muscles for biochemical estimation - Protein, Carbohydrate and Lipid. The results revealed a decreasing trend of biochemical constituents in different organs of T.mossambica exposed to 100% untreated and treated compared to control but an increasing trend was recorded in 100% biotreated and biountreated samples compared to other samples.*

Keywords: *Sewage waste water treatment, Physico chemical parameters, Azolla microphylla, Tilapia mossambica, Biochemical parameters*

I. Introduction

Water pollution is one of the most serious problems of today's civilization. The consumption of water has been doubling on every twenty years. Fresh water disposition on Earth is only one third of its territory is well provide with water, and if drastic efforts in water protection are not made by year 2025, 2.3 billion people will live in areas with chronic water shortage (WHO, 2005). The effects of water pollution can be broadly classified into physiochemical, biological, toxic and pathogenic. The waste water resulting from various human activities, domestic, agricultural and industrial is technically referred to as sewage. Sewage contains about 99% of water and 1% of organic solids (Athalye et al., 2001).Sewage disposal in our country is done in so haphazard manner that causes serious water and land pollution (Thanunathan et al., 2000).In most cases the domestic sewage in generally discharged as such in the untreated, treated or partially treated form into the nearby water pollution problems (Sathya narayanan, 2007).

Therefore, there is a need for recycling of waste water is currently used for the production of fish both in India and in Aboard (Paulami, 2002). Hence it is very important to treat sewage waste water before it is disposed. Several treatment methods were evolved to make this pollutant water to less toxic and make use of them for aquaculture, agriculture and other purposes (Nagarajan and Sasikumar,2002). Degradation is a technique to degrade rapidly hazardous organic contaminants to environmentally safe levels in soils, water sludges and residues by using microorganism, plants and animals (kannagi, 2007).

Recently the researches has highlighted about the awareness on bioabsorption, bioaccumulation by plants and animals involving water plants like water hyacinth, Azolla, bivalves, algae and fungi. There are many reports on aquatic plants and weeds which have been used for bioaccumulation or bioremediation of the sewage waste water (Jamuna and Noorjahan etal., 2009). Today, there is an increasing interest in using the Azolla for other purposes, such as its utilization as a decontaminant plant in low-cost wastewater treatment systems, (Culley and Epps, 1973 and Forni et al., 2001). The biomass produced can be used for inoculating paddy fields or for other applications and wastewaters can be recycled for irrigation purposes (Arora and Saxena, 2005). The wastewater treatment from piggery farms by using Azolla for agriculture may be one of the alternative solutions for soil fertility, wastewater management and suitable for the developing country's wastewater treatment.

Phytoremediation is considered one such remedy to solve the problem of heavy metal pollution in water by using plants (Gardea Torresdey, 2003). The ability of aquatic plants to accumulate toxic metals from water is well documented (Rai and Chandra, 1989, 1992; Rai et al., 1995; Gupta and Chandra, 1990; Sinha et al., 1996; Rai, 2007a, 2007b, 2007c; Rai and Tripathi, 2007a). Azolla has proved to be a potent aquatic water fern for the biofiltration of various toxic metals (Noraho and Gaur, 1996; Zhao and Duncan, 1997, 1998; Antunes, Watkins, and Duncan, 2001; Cohen-Shoel et al., 2002; Rai, 2007c).

Numbers of researches are being carried out in countries like USA for stripping of nutrients from sewage waste water and natural water by cultivating aquatic vascular plants (Sinha and Sinha,1982). Azolla microphylla., the free floating aquatic fern, has distinct advantages as it has high biomass productivity coupled with high rate of N fixation, ability to grow in varied environments and multiple applications as biomonitor, biofertilizers, animal feed, biofilter, bioweedicide, ability to concentrate nutrients and heavy metals from flood waters . Azolla has proved to a potent aquatic water fern for the biofilteration of various toxic metals (Xin zhang et al., 2008).

Recently researchers has well documented on the ability of other species of Azolla plants has been tested as a biofilter to purify water, to remove nitrogen and phosphorus, elements that cause water eutrophication. Also, it can remove sulfa drugs (Forni et al., 2001) and metals like Sr, Cu, Cd, Zn, Cr, Ni, Pb, Au, Pt and even radioactive elements as U (Zhao and Duncan, 1997, 1998; Fogarty et al., 1999; Antunes et al., 2001; Cohen-Shoel et al., 2002).

Fishes are the bioindicator of metal pollution and are particularly sensitive to wide variety of toxicants. As fish fauna serves as a food source, it is essential to know the impact of water pollution on these organisms. Reports available in the recent years suggest the contamination of freshwater bodies by heavy metals coming through industrial wastes leads to biological magnification resulting into severe alteration in physiological and biochemical parameters of fish (Palanichamy and Baskaran 1995).

Shoba rani, 2001, showed the effect of arsenite on certain aspect of protein metabolism in fresh water Telost, Oreochromis mossambicus(Peters). Cruz and Miranda, 2006 have showed the sublethal effects of Titanium dioxide industrial effluents on Biochemical composition of the fish Oreochromis mossambicus.

This study is designed to understand the bioaccumulation capacity of Azolla microphylla. Hence this study was focused on the treatment of sewage waste water and reutilization of the treated sewage water for aquaculture (i.e.) fish culture by examining the potential target organs Viz., Gills, liver and muscle for biochemical estimation - Protein, Carbohydrate, Lipid by standard methods.

II. Materials And Method

2.1 Collection of Sewage Waste Water, Plants -Azolla microphylla and Fish –Tilapia mossambica

Untreated and treated sewage waste water were collected from the sewage treatment plant situated in Koyembedu, Chennai, Tamilnadu, India, in polythene containers (5 litres capacity). Azolla microphyllaplants of similar size was harvested from the Chetpet Pond, Chennai. The plants were washed thoroughly and maintained in tap water for 10 days to acclimatize to laboratory conditions. The fingerlings of fish Tilapia mossambica were brought from the hydro biological research station, Tamilnadu Fisheries Department, Chetpet. The fishes were acclimatized for a period of 10 days and were fed with pelletized feed daily and sufficient oxygen was maintained by an aerator.

2.2 Analysis of physicochemical parameters of sewage waste water:

The physico-chemical parameters of sewage waste water such as colour, odour, pH, EC, Total Suspended Solids (TSS), Total Dissolved Solids (TDS), Biological Oxygen Demand $(BOD)_5$, Chemical Oxygen Demand (COD) and heavy metals such as Chromium, Copper and Zinc present in the sewage samples were studied following the Standard methods outlined by APHA (1995) before and after introduction of the Azolla microphylla plants in the sewage waste water samples.

2.3 Experimental set up:

Batch experiments were conducted in 4 plastic tubs (15litres capacity). The outer surfaces of the tubs were covered with black polythene papers to prevent entry of excessive light from the sides. The initial level of physico-chemical parameters and heavy metals in the sewage waste water samples were estimated in the Atomic Absorption Spectrophotometer (AAS) as per the procedure of Kannagi (2007). The fishes ranging from 4-7cms in length and body weight of 2-4 gms were introduced in 10 litres of 100% untreated, treated, biountreated (after treatment of untreated sample with Eichhornia sp) and biotreated (after treatment of treated sample with Eichhornia sp) sewage samples separately for a period of 60 days . After a period of 60 days, the biochemical estimation such as Protein, Carbohydrate, Lipid of different potential organs such as gills, liver and muscle of fish T. mossambica were carried out after exposing T.mossambica to 100% untreated, treated, biotreated and biotreated sewage samples for a period of 60 days .

2.4 Biochemical Estimations:

At the end of the experimental period (60^{th} day), experimental fishes belongs to different groups were sacrificed, dissected to separate the target organs such as gills, Liver and Muscle, homogenates were prepared for the measurement of the following biochemical parameters.

2.4.1 Estimation of Proteins:

The protein content of the samples was determined by the method of Lowry et al.,1951. Standard Curves were prepared with different concentrations of BSA .Values have been expressed as mg/100mg of tissue.

2.4.2 Estimation of Carbohydrates:

The Anthrone method of Roe, 1955 was used for the estimation of Carbohydrates level in the tissue. The optical density was compared with a set of glucose standard of varying concentrations. Results have been expressed as mg/100mg of tissue.

2.4.3 Estimation of Lipids:

Lipids was extracted as per the method of Folch et al., 1957 and the estimation was done as described by Barnes and Blackstock., 1973. Standard curves using the same procedure were drawn with known amounts of Cholesterol. Results obtained were expressed as mg/100 mg of tissue.

III. Results And Discussion

Recent awareness and growing global deterioration in the quality of environment has induced great interest in the identification of pollution and their effects on plants, animals and human being .This study is an attempt to determine the physicochemical parameters and heavy metal content in the untreated and treated sewage waste water .The physicochemical parameters of sewage sample were carried out before and after the introduction of Azolla microphylla and the results of the same are shown in Table-1. Colour, odour, pH, EC, TSS, TDS, BOD, COD and heavy metals of sewage samples were found to be higher than the permissible limits of CPCB (1995) before the introduction of Azolla microphylla plants, but at the end of 96 hrs, maximum reduction of BOD (Biountreated 83.33% and Biotreated 80%), COD (Biountreated 78.93% and Biotreated 88.69%) and Heavy metals such as Chromium (Biountreated 84.74% and Biotreated 100%), Copper (Biountreated 87.21% and Biotreated 89.43%), Zinc (Biountreated 99.07% and Biotreated 100%) were recorded using Azolla microphylla plants.

The present study was further extended to reuse the biountreated and biotreated sewage water for culturing the fish Tilapia mossambica. The results of the biochemical constituents such as proteins, carbohydrates and lipids estimation present in the organs like Gills, liver and muscle of fish T.mossambica were estimated after exposing the fish to 100% untreated, biountreated (Azolla microphylla), treated and biotreated (Azolla microphylla) are presented in Table -2. The results revealed a decreasing trend of biochemical constituents in different organs of T. mossambica exposed to 100% untreated and treated compared to control but an increasing trend was recorded in 100% biotreated and biountreated samples. Since major biochemical constituents – Carbohydrates, Proteins and lipid of the body play an important role in body construction and energy metabolism. The principle and immediate energy resource is the carbohydrate, which normally comes to the rescue. The results of the study revealed that carbohydrate content is reduced in the gills, followed by liver and muscles of T. mossambica exposed 100% untreated and treated sample.

Carbohydrate represents the principal and immediate energy source for animals exposed to stress conditions and that carbohydrate reserves deplete to meet energy demand. The loss of protein in tissue (gills, liver and muscle) due to heavy metal stress as observed the present investigation after 10 days of exposure may be due to excessive proteolysis to overcome the metabolic stress. Sridevi, 2002 also reported gradual and significant reduction in the protein contents in the tissues like muscle, intestine, kidney liver and gill of the Heteropneustes fossils after chronic exposure to nickel. Mudhakkira fathima and Noorjahan et al., 2006 also showed the significant reduction in the biochemical parameters of fish T.mossambica when exposed to untreated soap effluent.

The depletion of proteins was high in the organs of fish exposed to 100% untreated and treated sewage sample compared to control and this depletion of the protein level may be due to the defective protein synthesis and alterations between the ribosomes and membranes of endoplasmic reticulum as suggested by Debrium, 1976. Though other factors such as increased mobilization of sugars and protein growth related changes could also be responsible, it is like that the presence of optimum organic matter which is naturally required for the growth of fishes might have enhanced the levels of these constituents. (Goel,1997).

Lipid forms an important fuel reserves stored in large quantities and it is an essential component of protoplasm and even during extreme starvation, considerable amount would be extracted from the tissue (Hoar, 1984).The results of the lipid content in this study also shows a declining trend in the organs of the fish exposed to 100% untreated and treated sewage waste water. Lipid is the fuel reserve of the fish and during stress there may be a change in the lipid content of the animal.

This study has demonstrated that biotreated sewage sample could be utilized for aquaculture of fishes. Biochemical parameters like carbohydrates, protein and lipid of the fish T.mossambica showed favorable increase when cultured in 100% biountreated and biotreated sewage water. Moreover among all the three different organs (gills, liver and muscle) of the fish, T.mossambica lipid was most depleted followed by carbohydrates and protein. Among the three organs as they are vulnerable to the toxic stress to 100 % untreated and treated sewage waste water followed by liver and muscle.

IV. Conclusion

In conclusion, the present investigation demonstrates the feasibility of adopting a "sustainable" and eco-friendly approach to sewage waste water treatment using aquatic plant Azolla microphylla emphasizing on degradation and recycling / reuse of sewage water for biocompost production irrigation and aquaculture (Fig.1). Since this is only a laboratory scale base **-** line study, further investigations should be carried out in future on a large scale particularly focussing on phyto-remediation and resource utilisation.

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Table 1. Physicochemical characteristics of sewage waste water before and after treatment using *Azolla microphylla*

SI.NO	Characteristics	Permissible Limits (CPCB, 1995)	Before Treatment		After Treatment using *Azolla microphylla* (at the end of 96 hrs.)		Percentage removal	
			Untreated	Treated	Biountreated (*Azolla microphylla*)	Biotreated (*Azolla microphylla*)	Biountreated	Biotreated
1.	Colour	Colourless	Grayish Black	Brown	Colourless	Colourless	--	--
2.	Odour	Odourless	Offensive	Earthy	Odourless	Odourless	--	--
3.	pH	5.5 - 9.0	6.9	6.7	6.9±0.08	7.0±1.58	--	--
4.	EC (µmhos/cm)	400	1702	1410	623±1.58	402±1.414	63.39%	71.48%
5.	TSS (mg/l)	100	360	18	9±0.015	7±1.58	97.5%	61.11%
6.	TDS (mg/l)	2100	690	810	466±1.29	362±1.06	32.46%	55.30%
7.	$(BOD)_5$ (mg/l)	30	180	55	35±1.06	9±1.29	83.63%	83.63%
8.	COD (mg/l)	250	497	65	131±5.09	26±0.01	73.64%	60%
9.	Chromium (mg/l)	2	0.00513	0.00035	0.00118±0.0518	Nil	76.99%	100%
10.	Copper (mg/l)	3	0.1547	0.00157	0.02554±0.0002	0.00092±0.00002	83.49%	98.72%
11.	Zinc (mg/l)	1.5	0,0781	0.065	0.00141±2.302	Nil	99.19%	100%

± = Standard Deviation

%=Percentage Removal

TABLE – 2. Change in Carbohydrates, Protein and lipid content of fish exposed to Untreated, Biountreated (Azolla microphylla.), Treated and Biotreated (Azolla microphylla.) of Sewage Waste Water on Biochemical Constituents of the fish, *T.mossambica*

Samples	Organs mg/100mg of tissue	Biochemical Constituents		
		Carbohydrates	Protein	Lipid
Control	Gills	1.342 ± 0.08	1.553 ± 0.92	0.202 ± 0.40
	Liver	1.456 ± 0.08	1.111 ± 0.35	0.157 ± 0.36
	Muscle	1.664 ± 0.01	1.931 ± 0.35	0.294 ± 0.45
Untreated	Gills	0.138 ± 0.03	1.166 ± 0.44	0.151 ± 0.35
	Liver	0.804 ± 0.39	0.878 ± 0.32	0.0171 ± 1.70
	Muscle	0.807 ± 0.38	1.053 ± 0.22	0.142 ± 0.34
Biountreated (*Azolla microphylla*.)	Gills	1.608 ± 0.09	1.547 ± 0.91	0.257 ± 0.43
	Liver	1.651 ± 0.02	1.441 ± 0.79	0.159 ± 0.36
	Muscle	1.368 ± 0.06	2.367 ± 1.79	0.314 ± 0.46
Treated	Gills	1.318 ± 0.07	1.175 ± 0.45	0.170 ± 0.37
	Liver	1.014 ± 0.09	0.871 ± 0.33	0.165 ± 0.37
	Muscle	1.232 ± 0.04	1.492 ± 0.85	0.288 ± 0.45
Biotreated (*Azolla microphylla.*)	Gills	2.931 ± 0.09	2.084 ± 1.50	0.291 ± 0.45
	Liver	3.590 ± 0.04	3.567 ± 3.02	0.228 ± 0.41
	Muscle	1.506 ± 1.45	2.733 ± 2.17	0.394 ± 0.48

± = Standard Deviation

FIG-1 SUGGESTED INTEGRATED APPROACH FOR SEWAGE WASTE WATER TREATMENT AND RECYCLING