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COMPARATIVE STUDY ON PHYSIOLOGICAL, HEMATOLOGICAL, BIOCHEMICAL EFFECTS OF FISH BEHAVIOR ON RAW EFFLUENT AND BIOREMEDIAL TREATED WATER

Zoology

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ABSTRACT

Aim: In the present study was attempt to the effluent water were treated with microorganism and fish experiment were conducted and observed for the fish behavior, biochemical and hematological characteristics of fish were analyzed.

Methodology: The effluent water was analyzed for physicochemical parameters. After treatment with *Pseudomonas sp.* the physicochemical parameters reveals the quality of water content. The experiment was carried out by three groups. First group of fish were grown in normal water, second group of fishes were grown in direct effluent water and third group of fishes were grown in effluent treated water for 21 days exhibited the normal condition with swimming behavior. Additionally, estimated hematological, biomolecules and enzymatic parameters observed in first and third group of fishes.

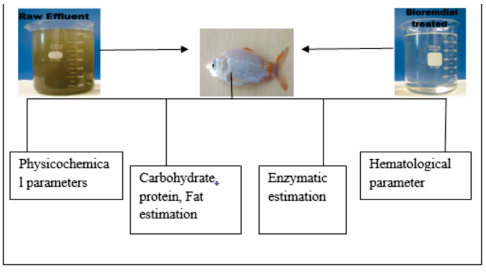
Result &Interpretation: Direct effluent water fish were not able to survive for 2 hrs and the significant difference was found in all the parameters when compared to control. No significant difference was observed between control and effluent treated water. Therefore the study was concluded *Pseudomonas sp.* can able to remove the crude toxic of effluent water and therefore the aquatic organism can able to lead a healthy survival.

KEYWORDS

Bioremediation, Biomolecules, Effluent water, Hematology, *Pseudomonas sp.*

INTRODUCTION

Fish culture is very important protein source globally and aquaculture sector is expected to play a significantly greater role in contributing to food security (FAO, 2017). The large volume of polluted water discharged in the river kills the aquatic organism whereas lower levels of discharge accumulated in the marine organism eventually cause the morbidities those aquatic population (Austin., 1998). Discharge of polluted water in the aquatic environment alters the chemical composition of water thereby modifies the physiological and biochemical alterations in the aquatic population. Among the aquatic population, fishes are very sensitive to a wide range of pollutant present in the effluents (Saroja *et al.,* 2013). Biochemical parameter in fish gives knowledge to evaluate the adverse effect of pollutant present in the water. Assessing the sensitive biochemical markers namely lactate dehydrogenase, alanine and aspartate aminotransferase in fish before and after the exposure of aquatic pollutant help to determine the pollution levels during chronic exposure (Nasser *et al* .,2015).Bioremediation is the process to remove the hazardous waste in soil or water using wide range of microorganisms like yeast fungi or bacteria (Divya *et al.,* 2015).Among the various ecosystems, aquatic ecosystem is widely affected by pollutants due to rapid growth of industrialization with urbanization, exhaustive agriculture and other human activities .The effluent water industry and human activities were termed as sewage and classified as domestic or public sewage, industrial effluents and atmospheric run off. Based on the extent of pollution these effluents should be treated before it is discharged in to the river stream (Purnima Dhall, 2012). In Bioremediation, the effluent water was treated with various types of microorganisms to break down the organic material in to a simpler form (Akpor, 2006). Bioremediation by microorganism is very useful because they can able to adapt in extreme conditions and they can able to clean even the diluted amount of pollutant present in the sewage water. This process can be augmented by using specialized microorganism which can able to break down the complex substances (Hesnawi *et al.,* 2014). Among the various organism, *Pseudomonas sp.* effectively remove the phenol from industrial wastes. Similarly *Bacillus sp.* can able to remove the quinol from the industrial waste. Standardizing the microorganism for the effective removal of waste from the effluents is vital before discharged in to the river stream. The present study aims to compare the effect of fish growth in fresh water and effluent water treated with the *Pseudomonas sp.*



MATERIALS AND METHODS

The fresh water fish were collected from Poondy reservoir at Thiruvallur district. In the laboratory 8gm weight, 12 cm sfishes were maintained in rectangular fiber glass tanks in chlorine free aerated well water. The fishes were kept for five days to acclimatize in laboratory conditions and the animals were fed with commercially available pellets. The animals were maintained at 30 ±2°C with water exchange.

Collection of Effluent water and Experimental design

The raw alcoholic distillery effluent water was collected from Caddalore, TamilNadu, India. 20 liters of water samples were collected from mouth of the outlet of the industry in polypropylene container. The physicochemical parameter of the sample was estimated according to the standard protocol of APHA (2009). Fishes used for experimental purpose were conducted in three groups (n=10). In first experimental group the fishes were maintained in normal chlorine free water with aeration. In second experimental group the fishes were grown in effluent water without any treatment with proper aeration. In third experimental group the fishes were grown in effluent water treated with *Pseudomonas sp.* In all the groups fishes were grown for 21 days with proper aeration and fed with commercially available pellet.

Maintenance of fish in effluent treated water

The 20 litter of effluent water was treated with *Pseudomonas sp.* at the concentration of 10^{-3}per ml. The water sample was incubated at 30±2°C for 3-5 days with proper aeration. After five days of water treatment samples were used for the fish experiment.20 liter of water sample in 50 L glass tank were used 5 animals per tank and at approximately 7 to 10g as of fish samples. Once the turbidity and colour of the water changes the healthy fishes were cultured in the treated water. To analyze the biochemical changes in the various organs of the fish brain, gills, liver, kidney, intestine and muscles. Before and after the treatment period the fishes were dissected to collect the various organs including brain, gills, heart, liver, intestine, muscle and kidney. The dissected organs were rinsed with saline, 100mg weighed and homogenated using PBS buffer pH 7.4. The homogenate and centrifuged sample was further used to analyze various parameters (Fathima 2012). Experiments were repeated for thrice.

Estimation of carbohydrate, protein and lipid:

0.5 ml of the supernatant 4.0 ml of anthrone reagent was added, boiled for 10 minutes in a water bath and cooled. The absorbance was measured at 630 nm.

To 500 µl of the extract 5 ml of alkaline copper sulphate solution was added and incubated for 5 minutes at room temperature. Then 0.5 ml of 1:2 folin's reagent was added and again incubated for 30 minutes and

the absorbance at 660 nm. The standard graph was plotted using BSA (100-500 µg/ml) from which the protein conc. was calculated.

100 µl of the sample was mixed with 4 ml of working $FeCl_3$-CH_3 COOH reagent and incubated at room temperature for 10 minutes. Then 3 ml of 85% H_2SO_4 was added and kept in ice for 2 minutes. The same protocol was done for the standard at the conc. of 20-100 µg/ml. Pink colour formed was measured at 540 nm.

All the experiments were repeated triplicate. The statistical analysis was performed using one way analysis of variance (ANOVA) technique. $p<0.5$ was considered as statistically significant.

Estimation of Enzymes test

Biochemical test such as lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase was estimated colorimetrically according to the standard protocol (Reitman *et al.,* 1957). The enzyme activity was expressed in terms of unit enzyme ml^{-1} of serum.

Hematological parameters analyse

Blood samples were withdrawn from caudal vein using sterile syringe and collected in tube containing heparin as an anti-coagulant. To separate the plasma samples were centrifuged at 3000 rpm for 15 min and it was stored at 4^0C. The blood samples were used to measure the RBC, WBC, Haemoglobin (Hb) content, packed cell volume (PVC), urea and calcium (Nassar *et al*.,2015)

RESULTS & DISCUSSION

Aquaculture is fast growing sectors in world wide. Detriations of quality of water is due to the discharge of waste water from domestic, agricultural and industrial wastes plagued the aquaculture sector near the coastal regions (Chua ThiaEng *et al*., 1989). Contaminated water affects the reproductive ability of the aquatic ecosystem. Bioremediation is the friendly tool to evacuate the pollution present in the coastal areas (Divya, *et al*., 2015).

Pseudomonas sp. is widely used in various industries to clean the effluent and to degrade the phenol, pesticides and heavy metals (Tabrez & Ahmad, 2014). It was widely used to treat the leather effluents and rubber processing industries. It was also examined for the bioremediation of phosphate from synthetic waste water and dairy effluent (Porwal., 2015).

The Effluent water was collected from Parry's Limit., Nellikuppam Caddalore district in Tamil Nadu. Effluent were quantitatively analyzed for their physico-chemical parameters such as water colour, pH, total dissolved solids; alkalinity were observed and tabulated (Table 1). In the effluent sample the colour, turbidity of the water changes to clear and also fishes were good in condition for a 30 days without any significant. The similar studies were reported by using *Pseudomonas sp.* which can able to change the color and turbidity of the sewage treatment plant (Dhall *et al*., 2012).

In the present study the fishes were divided in to three groups. In group I the fishes were adapted in normal water. In group II the fishes were introduced effluent water and in group III the fishes were adapted in *Pseudomonas sp.* treated effluent water. In first and third group the fishes appeared healthy, food habit and swimming behavior of the animal in the third group equal to that of the control fishes. The second group of fishes showed rapid swimming because of excitation when introduced in to the effluent water but after some period of time it showed the altered swimming behavior and died within 2 hours. Similar result was observations were noticed in *Daniorerio* after introduced into the raw effluent water (Sivakumar *et al*., 2014).

Glucose is the major carbohydrate source and acts as immediate metabolic fuel. Rapid increase in glucose concentration was observed during environmental stress through handling, thermal shock or effluent treatment and decline slowly (Javed and Usmani, 2015).In the present study the glucose concentration did not show any significantly variation in group I and II. In case of group II significantly decreased in the glucose level was observed (Table 2). Similar result was observed in the case of protein content and lipids .

Lipids usually elevated in stress condition due to the impaired clearance of liver from the serum which favours liver dysfunction (Javed, 2008).

Various important enzymes like LDH, ALT, AST, ALP were analyzed and in different organs. All the enzymes did not show significant difference found in the fish sample adapted in treated water when compared to the control experiment.(Table 3) ALT and AST are the enzymes play an important role in the conversion of amino acids and α-ketoacids. ALP is also an enzyme involved in the dephosphorylation of molecules such as nucleotides and proteins (Kroon *et al.,* 2017).

Hematological parameters are usually a less chemical specific parameter but give general information regarding general health and physiology of the organism .Total WBC, RBC and hemoglobin levels were significantly changed between group I and II. Exposure of fish in effluent water significantly reduces the RBC, WBC and hemoglobin level in the present study (Table 4). Same observation is reported the significant difference in fish exposed to dye stuff and chemical effluent water (Abdel-Moneim *et al.,* 2008*).* Hematological examination in group III does not showed any significant changes when compared to control .

The study showed *Pseudomonas sp.* were able to treat the effluent water effectively which helps in healthy adaptation of fish because fishes were more sensitive to the chemicals.

CONCLUSION

The present study showed *Pseudomonas sp.* able to treat the effluent water effectively. The treated water used for the fish cultivation were showed the normal behaviour with swimming pattern and feed. Classification of biochemical and enzymes were not significantly different between the control and treated group. The hematological examination was also normal when compared with the control. Hence the *Pseudomonas sp.* was effective tool to treat the effluent water.

Table 1: Physicochemical parameters of effluent water collected from Caddalur and treated water using *Pseudomonas spp*.

S.No	Analysis	Units mg/l	Effluent water	Treated water
1	Colour	Hazen	85	Disagreeable
2	pH @ 25°C	-	7.21	6.78
3	Conductivity	micromhs/cm	5096	1320
4	Turbidity	NTU	198	102
5	Total Dissolved Salts	mg/l	3160	788
6	Total Hardness	CaCO3	420	168
7	Calcium	Ca	152	28
8	Magnesium	Mg	10	38
9	Chloride	Cl	1757	292
10	Iron	Fe	1.89	0.1
11	Sulphate	SO4	17	62
12	Total alkalinity	CaCO3	780	142
13	P- Alkalinity	CaCO3	Nil	Nil
14	Silica	SiO2	31	14
15	BOD, 4 days incubation @ 20°C	as O2	175	257
16	COD	O2	498	38

Table 2: Estimation of carbohydrates, Proteins andLipids in all three groups.* denotes $p<0.005$ and ** denotes $p<0.05$.**

Organ	Carbohydrates (mg %)			Proteins (mg %)			Lipids (mg %)		
	1	1I	III	1	1I	III	1	1I	III
Brain	13.05	6.55***	12.03	6.54	3.08***	6.02	55.2	81.25***	54.32
Gills	32.58	25.35***	30.87	5.82	3.05**	5.74	45.15	50.25**	46.8
Heart	6.45	5.1**	5.12	3.7	2.37**	3.57	19.35	25.7**	19.65
Liver	70.26	60.05***	69.32	15.5	12.1**	14.87	64. 55	69.05**	64.12
Intestine	37.25	30.01***	35.87	13.85	9.22***	12.86	26.08	31.35**	25.15
Muscles	45	38.96***	43.12	20.37	16.49***	20.61	40.38	50.38**	41.38
Kidney	5.99	3.25***	5.9	3.33	1.07**	4.51	8.19	11.55**	9.08

Table. 3: Estimation of hematological parameters in the three groups.

Parameter	Group I	Group II	Group III
Total WBC/cmm	2301	1987***	2200
Total RBC/cmm	2.7	2.4**	2.67
Hbgrm%	14.56	11.97***	14.65
PCV %	49.02	44**	48.04
ESR mm/hr	4.98	3.2*	4.9

Table 4: Estimation of enzymes

Organ	LDH (U/L)			ALT (U/L)			AST (U/L)			ALP (U/L)		
	A	B	C	A	B	C	A	B	C	A	B	C
Brain	22.5	22.18	21.62	8.02	8.25	7.52	5.8	5.34	5.5	12	12.25	12.26
Gills	12.38	12.5	12.48	5.05	5.15	5.06	3.16	3.2	3.2	8.35	8.4	8.36
Heart	65.31	65.32	64.83	24.09	24.08	24.14	19.06	19.05	19.06	24.18	24.26	24.3
Liver	54.22	54.24	54.15	15.03	15.05	15.1	22.21	22.24	22.23	20.64	20.59	20.56
Intestine	33.04	33.05	33.08	10.8	10.72	10.65	12.17	12.24	13.14	13.09	13.08	13.14
Muscles	50.01	50.1	50.03	12.5	12.5	12.45	18.3	18.32	18.29	8.55	8.62	8.58
Kidney	28.08	28.13	28	8.62	8.5	8.45	7	7.02	7.05	5.45	5.52	5.6
Ovary	37.22	37.3	37	9.13	9.14	9.2	6.55	6.55	6.6	11.68	11.65	12.05

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