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A Study of The Impact of Distillery Effluent on The Estuarine Catfish Mystus Gulio

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ABSTRACT

Pollution may be defined as fouling of the environment. It is an undesirable change in the physical, chemical or biological characteristics of the land, air and water that may or will harmfully affect human life or that of desirable species. Distillery effluent affects the many physico-chemical characteristic of aquatic medium. The turbidity of water is increased by the dark colour of the distillery effluent. The distillery industry is one of the most polluting of industries, not only in terms of the volume of effluent generated, but also in terms of its characteristics as well. The physiological, biochemical and histological changes in Mystus gulio after exposure to sublethal concentrations of the distillery effluent have been investigated. The results of acute toxicity test showed that the LC50 was 20 ml/liter, means that this wastewater is highly toxic. The results of physiological, biochemical and histological changes in Mystus gulio showed that the fish were under considerable stress during exposure to sublethal doses of this effluent. Physiological response of fish revealed significant disturbances in respiratory system, metabolism and ionic osmoregulation. Biochemical changes of protein, lipid and carbohydrate content were recorded. Pathological changes attributed to dairy industry effluents were observed in the gills, liver and kidney.

KEYWORDS :

INTRODUCTION

Today most environmental problems are attributed to the production and release of toxic chemical capable of interacting with the environment and disrupting the ecosystem. Water pollution has many sources. The most polluting of them are the city sewage and industrial waste discharged into the rivers. Industrial waste is defined as waste generated by manufacturing or industrial processes. The types of industrial waste generated include cafeteria garbage, dirt and gravel, masonry and concrete, scrap metals, trash, oil, solvents, chemicals, weed grass and trees, wood and scrap lumber, and similar wastes. Industrial waste - which may be solid, liquid or gases held in containers, is divided into hazardous and non-hazardous waste. Hazardous waste may result from manufacturing or other industrial processes (Ramamurthy *et al*., 2015).

In India, the indiscriminate discharge of raw sewage and industrial effluents has been the major source of pollution of rivers. Uncontrolled discharge of industrial effluent in Alexandria has led to severe impact on ecological balance and appreciable environmental deterioration. The effluents concentrations usually show a considerable rise in the waters receiving industrial wastes. Alteration in the chemical composition of a natural aquatic environment by industrial effluents, usually induce changes in the behavioural, biochemical and pathological aspects of the inhabitants, particularly fish (Ramamurthy *et al.,* 2014). The effluents released from distilleries, tanneries, pulp factories, paper mills, sugar factories etc., are having large amounts of toxic chemicals, which cause death of organisms. The level of toxicity of these chemicals may either increase or decrease, when the effluent is stored.

The human health and environmental impacts of many of these chemicals are largely unknown. High levels of toxic contaminants have been found in animals and humans, particularly those, like farm workers and oil and gas workers, who are continually exposed to such waste streams. Waste water from manufacturing or chemical processes in industries contributes to water pollution. Industrial waste water usually contains specific and readily identifiable chemical compounds. Water pollution is concentrated within a few subsectors, mainly in the form of toxic wastes and organic pollutants. Out of this a large portion can be traced to the processing of industrial chemicals and to the food products industry. Most major industries have treatment facilities for industrial effluents but this is not the case with small-scale industries, which cannot afford enormous investments in pollution control equipment as their profit margin is very slender. The effects of water pollution are not only devastating to people but also to animals, fish, and birds. Polluted water is unsuitable for drinking, recreation, agriculture, and industry. It diminishes the aesthetic quality of lakes and rivers. More seriously, contaminated water destroys aquatic life and reduces its reproductive ability. Eventually, it is a hazard to human health. Nobody can escape the effects of water pollution.

Man's endeavour to advance has placed more strain on the environment in the last fifty years than in the previous 500 years put together. Man has been the greatest single factor in the ecological imbalance of modern times. Industry, in general the distillery industry in particular has been a major source of pollution. In a day, this distillery produces 90 000 litres of alcohol and discharges about 1.2 million litres of effluents, producing 15 times more waste than the product itself. The effluents of this industry not only causes pollution, but if reused can enhance profitability as well.

Alcohol is one of the raw materials for various chemical industries. It has a tremendous potentiality to replace petrol in future. At present India have more than 350 distilleries with an annual production of 2,00,000 Lakhs litres of alcohol. Tamil Nadu has more than 20 distilleries. The main raw material of distillery industry is molasses, a by-product obtained from sugar mill industry, which contain 50% of sugar for each litre of alcohol produced 20 to 50 litres of "spent wash" is discharged. The major toxic constituents in distillery effluent are high dissolved solids, chlorides, sulphate, sulphite and very high putrecibility and low volume of highly toxic sulphide.

Effluent from distilleries affects the physico-chemical characteristic of aquatic medium. The turbidity of water is increased by the dark brown colour of the distillery effluent. High organic and inorganic constituents present in the effluent, increase the production of algal blooms and create a severe oxygen reduction in the freshwater bodies which affect the normal life activities of aquatic flora and fauna.

Distillery effluent enters into the aquatic bodies which results in rapid depletion of oxygen making the environment unfit for fish life. Water temperature is one of the most important environmental factors. It exhibits dial and seasonal fluctuations in freshwater body. It plays a vital role in determining the distribution, survival, growth, metabolism and reproduction of organisms. The environmental temperature influences the survival, growth, conversion efficiency and biochemical composition of fishes.

Although there are several works available on the effects of the effluent on the aquatic organisms (Hamza *et al.* 1985, Mourad, 1995,

Aboul-Naga and Allam 1996), yet no strong information has been published about physiological and histological changes following the exposure to the distillery waste water. The measurement of these changes may provide a sensitive method for predicting the effect of chronic exposure on survival, reproduction and growth. Therefore, the main objective of this study is to assess the physiological, biochemical and histological changes in *Mystus gulio* after exposure to sublethal concentrations of the distillery effluent.

MATERIALS AND METHODS

Effluent was collected from distillery industry waste, Trichy, Tamil Nadu, India. Samples were collected in large sterilized container and brought to the laboratory. Physico-chemical characteristics were done on the same day when the samples were brought to the laboratory. The effluent samples were filtered through cotton to remove suspended coarse particles before use. The effluent contains chlorides (350 ± 17 mg/l), Sulphate (275 ± 12 mg/l), Sulfide (57 ± 28 mg/l), Chromium (11 ± 02 mg/l), Copper (14 ± 14 mg/l), Total suspended solids (3040 ± 72 mg/l) and total dissolved solids (4630 ± 55 mg/l). About 100 litre of raw effluents from the canal was collected in clean polyethylene containers and stored at room temperatures.

The fish is abundant, inhabiting mouths and tidal parts of rivers, and adjacent coastline in and around Adirampattinam. Live specimens were caught from natural habitats of local backwaters of Agniyaru estuary, situated in the Thanjavur District, Tamil Nadu, India and reared in large aquarium tanks with continuous circulation of the estuarine water. Later the collected fish were acclimatized to the laboratory conditions. For the experiments fishes with 9 – 10 cms length were selected because the minimum length of the mature fish is 8 cm (Raveendran, 2000).

Acute toxicity test was carried out according to Standard Methods for the Examination of Water and Wastewater (1975). To study the effects of sublethal concentrations of this wastewater, ten fish were introduced to each aquarium containing 50 litres of different dilutions (20 effluent and 80 ml water). The time of experiment was 30 days. To determine the effects of sublethal concentrations on some haematological parameters of fish, the blood was collected directly from the caudal artery into heparinized capillary tubes. Plasma protein and glucose were measured using Standard kits (Modern Laboratory Chemicals). Plasma ion concentrations of sodium and potassium were measured using Gallenkamp flame analyzer. Hematocrit was determined using microhematocrit tubes. Muscle protein concentration was measured using the method of Biuret (Gornall *et al* 1949). Muscle lipid concentration was measured using the method of Knight *et al* (1972). Moisture was determined by drying at 125°C for 3 hours and ash was measured by heating at 55^{0}C for 3 hours.

Biopsy specimens were collected for microscopic investigation from the gills, liver and kidney of control and treated fish. Fixed in formalin solution, dehydrated, cleared and embedded in paraffin wax, sectioned at 5-µm-thick sections, stained using Ehrlish haematoxylin and eosin, mounted for microscopically examination.

RESULTS AND DISCUSSION

The results of acute toxicity test for *Mystus gulio* exposed to different concentrations of the distillery effluent showed that the LC_{50} was 20 ml/l, which means that this wastewater is highly toxic. The toxicity of this waste water is attributed mainly to combination of several synergistic factors e,g. high concentration of heavy metals and solids besides low pH and dissolved oxygen (Mourad, 1995).

Table 1 represents the physiological response of *Mystus gulio* after exposure to sublethal concentrations of the distillery effluent (10 and 20 ml/l) for 30 days. With respect to haematology the present study is an illustrative of the fact that there was a gradual decrease in the haematological parameters such as RBC number and Hb content and gradual increase in WBC number, such a change was actually caused by the use of the effluent. The effluent shows significant dose dependent. The minimum reduction of red blood cells was noticed at lower concentration and also at short term exposures i.e. in different percentage of distillery effluent for 30 days. The same fishes were treated at a higher a concentrations of 1.5% and 2.0% of dairy industry effluent even though there was a short term exposure results was considerable number of decreased RBC. At the same time during an exposure for a long period, the maximum reductions were observed from the tables. A significant increase in hematocrit from 23.8% to 31.8 and 31.5% was observed after exposure to this distillery effluent that may be attributed to gill damage or increased demand for oxygen by certain tissues (Andersson *et al.,* 1988). Several authors also observed hematocrit value increase after exposure to heavy metals (McKim *et al.,* 1970 and Hilmy *et al.,* 1987).

A significant hyperglycaemia was also recorded after exposure to this dairy effluent to control fish had mean plasma glucose of 65.5 mg while the effluent treated fish exhibited an increase in the levels of plasma glucose to 75.5 and 91.00 mg, respectively. This means that the fish were subjected to some sort of hypertoxic stress. It is well known that stressful stimuli elicit rapid secretion of both glucocorticoids (Wedemeyer, 1969) and catecholamines (Nakano and Tomlinson 1967) from the adrenal tissues of fish and both of these hormones produced hyperglycaemia (Oguri and Nace, 1966). The obtained results are in agreement with Dange, (1986) and Benson *et al.,* (1987) who recorded an increase in plasma glucose levels after exposure to heavy metals.

The protein content of fish was diminished with increasing the sublethal concentrations of industry effluent. The protein content of *M. gulio* when reared in distillery effluent for 30 days of the exposure periods In this study, tissue and plasma total protein were generally influenced by this effluent which may be attributed to the relative changes in the mobilization of protein, changes in the plasma protein concentrations may be a result of increased production of metallothionein which is a sequestering agent (Cousins, 1982). On the other hand, the elevation of plasma glucose that runs parallel to a decrease in muscle protein content may be on indication of a gluconeogenetic response. This additional source of glucose may support the fish with the required energy highly demanded to cope with the presence of a potentially harmful substances such as effluents.

An increase in the levels of plasma sodium and potassium concentrations was also observed after exposure to this wastewater. This may be attributed to the changes in the permeability to sodium and potassium at the branchial site. The obtained results are in accordance with Stagg and Shuttleworth (1982) who found disturbances in plasma electrolyte concentrations after exposure of the fish to effluents.

The condition of fish exhibited a significant depression after exposure to this wastewater, which might be a result of elevation of the fish metabolic rate and cessation of feeding. Buckley *et al.* (1982) showed also a decrease in the condition of fish after exposure to effluents. Changes in the muscle lipid, ash, and water content were statistically insignificant.

Histological examination of the fish after exposure to sublethal concentrations of the distillery effluent revealed serious disturbances in the gills, liver and kidney tissues. As shown in control gills had normal structure of lamellae and wide interlamellar space. The fish exposed to diluted effluent showed hyperplasia of primary lamellar epithelium leading to obstruction of the water passage and reduction of the respiratory surface area. Similar results were recorded in other teleosts exposed to copper (Gardner and Roche, 1973; Daoust 1981; Khadre, 1990).

Fish have five pairs of gill arches. The front four pairs, slender gill filaments form two lines facing towards the back and these two lines are joined to each other at the base by a gill septum. The last pair of gill arches generally transforms into the pharyngeal bone and does not play a role in respiration.

Numerous semicircular secondary gill lamellae are line up along both sides of the gill filament. The surface of the gill lamellae is covered with simple squamous epithelial cells and many capillaries separated by pillar cells run parallel along the surface. Numerous semi circular secondary gill lamellae are lined up along both sides of primary gill lamellae. The primary gill lamella consists of centrally placed rod like supporting axis (SA) with blood vessels on either side. The secondary lamellae, also termed respiratory lamellae (RL), are highly vascularised and covered with thin layer of epithelial cells (EC). Blood vessels (BV) are extended into each of the secondary gill filaments. The blood cells of the secondary gill lamellae have single nucleus which are flattened

in appearance. The region between the two adjacent secondary gill lamellae is known as inter lamellar region.

The structural organization of the liver from the control group fish and the treated livers from the experimental group fish possessed a hexagonal shaped lobule with the hepatoxytes displaying arrangements around the central vein and, the glycogen being distributed in a homogeneous way along the hepatic tissue. The liver of control fish group showed the usual polyhedral hepatocytes, homogeneous cytoplasm and rounded nuclei. The exposed fish showed fatty infiltration with nuclear pycnosis. This fatty infiltration may be due to metabolic disturbances in liver tissue that enhanced activity of biotransformation enzymes. Histological lesions involving extensive fatty infiltration due to abnormal lipid accumulation in present work is in agreement with those finding of Backer (1969) and Khadre (1991).

The posterior kidney of freshwater fishes is adaptated to produce diluted urine and it has little participation in ion or acid-base balance. It receives the vast majority of post bronchial blood and because of that we expect renal lesions when the fish are exposed to pollutants. Therefore a study of these possible kidney changes could be a good response of environmental pollution. The body fluids of freshwater fish have a higher ionic concentration compared to surrounding water, a condition referred as hyperosmotic. To maintain the concentration gradient, the removal and conservation of ions prior to the excretion of purified water is required. This aim is accomplished in the kidney by filtration of water through glomerular nephrons comprising of a renal corpuscle and renal tubule. When the magnitude of the pollutant-induced stress is enough to cause cellular lesions but not the death of the organism, changes may be noticed in light microscopy.

The kidney of control fish showed normal structure of renal tubules (RT) and haemapoietic tissue in between. The exposed fish showed tubular destruction and epithelial ceil oedema. This oedema caused detachment of the epithelial cells from the underling basement membrane, pycnosis of nuclei and swelling of others. Deposition of dark granules inside glomeruli was also observed. These darkly stained granules may be a result of alteration in urinary pH or urinary stasis as a consequence of tubular destruction. Backer (1969) and Wahbi (1998) found the same results in winter flounder and *Solea vulgaris* due to copper and industrial effluent toxicity.

Generally, distillery effluents are xenobiotic compounds which are usually washed into water bodies and are made up of several compounds of which the active components are the surface-active agents or surfactants. The major toxic constituents in distillery effluent are high dissolved solids, COD and BOD. This effluent has variable composition whose values are far exceed the permissible limits there by posing great danger to the aquatic biota. The dilution and biological treatment requirements are needed before discharge in the distillery effluent safely to the ecosystem. Therefore, it is needs that the authorities concerned to ensure that treated effluent discharge comply with acceptable standard to save our environment from destruction.

Table 1. Physiological and Biochemical response of *Mystus gulio* exposed to sublethal concentrations of distillery industry effluent for 30 days

S. No	Parameter		Concentration (effluent volume/ water volume)	
		Control	10 ml/l	20ml/l
1	Hematocrit (%)	23.5 ± 1.22	30.5 ± 1.15*	31.5 ± 1.28*
2	Plasma protein (mg/100 cm^3)	4.29 ± 0.52	4.60 ± 0.12	4.99 ± 0.24
3	Plasma glucose (mg/100 cm^3)	65.5 ± 0.25	75.5 ± 0.21*	91.0 ± 0.33*
4	Plasma sodium (mmol/dm^3)	135 ± 0.11	141 ± 0.18	145 ± 0.28*
5	Plasma potassium(mmol/dm^3)	14.5 ± 1.22	15.3 ± 1.31	16.5 ± 1.14
6	Muscle protein (mg/100 mg)	22.5 ± 1.14	29.5 ± 1.31*	32.6 ± 1.17*
7	Muscle lipid (mg/100 mg)	1.58 ± 0.27	1.73 ± 0.12	1.85 ± 0.13
8	Water content (%)	74.3 ± 0.15	74.5 ± 0.22	74.9 ± 0.37
9	Ash content (%)	6.10 ± 0.12	6.34 ± 0.33	6.76 ± 0.51
10	Fish condition (Kf)	1.97 ± 0.18	1.68 ± 0.25	1.31 ± 0.16

* Significant difference in comparison to control group.
Average of 10 fish ± standard deviation.

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