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Effect of pyrotechnic chemicals in digestive tract microflora and proximate composition in fish *Oreochromis mossambicus*

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ABSTRACT

In the present study, the fish Oreochromis mossambicus were obtained from the Thiruthangal pond for the toxicological study treated with pyrotechnic chemicals, cryolite and copper acetoarsenite, which they are used for the production of sparklers as a coloring agent. After exposure with these pyrotechnic chemicals the fishes were sacrificed and the guts were taken for the study. Foregut, midgut and hindgut were separated and serially diluted then plated. Meanwhile, the tissue homogenate was used for the estimation of the proteins, carbohydrates and lipids, simultaneously a set of control was analyzed. The results indicates that decreased amount of proteins, carbohydrates and lipids also, these chemicals affect the composition of the gut micro flora thereby bring about changes in the metabolic activities in the exposed fish. In terms of extreme toxicity, it is absolutely necessary to monitor the residual deposition in the soil also in freshwater ecosystem at low concentrations. Hence, this study strongly recommends the usage of pyrotechnic chemicals should be handled carefully and discharged in highly proper way without affecting whole ecosystem.

Key words: *Oreochromis mossambicus*, copper acetoarsenite, cryolite, pollution, sivakasi.

INTRODUCTION

Aquatic pollution result in physical, chemical and biological deterioration of the water bodies causing destruction of fish and other biota. In addition the economy of a country depends on its freshwater resources by recycling the waste water for its use in agriculture and industry. At present, the rate of pollution is very fast due to the industrialization and urbanization [1]. All types of aquatic pollutants can destroy the normal environment of fish and other organisms [2-5]. The effects of pollution may be long-term or short duration. The exposure of short duration may result in changes that are not lethal but affect the physiological aspects of fish related to the feeding, growth and reproduction while the long-term exposure may leads to the death of fishes [6]. Tolerance to environmental disturbances may vary from animal to animal and species to species [7]. Fishes are now considered as the best indicators of pollution. The environmental disturbances may affect the fish production either directly or indirectly. In evaluating the effects of pollution to fish, some growth variables as length, weight and condition factors are generally used [8].

Measurement of body length gives direct evidence for growth or lack of growth [9]. Change of weight is probably the most common procedure for the assessment of the whole growth of the fish [10-12]. In addition to these two variables of growth, condition factor is also used to describe either the condition or fatness or well being of a fish as discussed by Begenal [13] and Lloyd [14].

Sivakasi town consists of fireworks, match works, offset, litho printing, dye printing, ink printing, card board printing industries and so this town is in the industrial map in India. All these industries are responsible for environmental pollution. Keeping in view the importance of this area of fishery science, the present study was conducted to see the extent of toxicity of pyrotechnic chemicals in Sivakasi by the studies on *Oreochromis mossambicus* (Cichilidae), the most common freshwater fish in this area. On this aspect, it will be helpful in the identification of various pollutants and their levels, affecting the fish growth. Generally, the physical parameters do not directly influence the fish growth but may cause indirect effects by temperature and transparency. In the case of chemical parameters indicated chemical pollution. Jobling [6] has observed that a fish, when living in polluted water, suffers by pollutants in different ways. They affect the structure and physiology of fish and thus fishes try to avoid polluted waters [15]. Chemical pollution in an aquatic environment can disturb both somatic as well as reproductive growth of fish [16]. The effluents discharges from the industries have caused ground water pollution; also it affects the living environment including human beings and pyrotechnic chemicals (Cryolite and Copper Acetoarsenite) widely used for the production of sparklers. Many chemicals used which are highly toxic to the person working and also which affects the environment and leads to bioaccumulation. In this present study, the toxicity of pyrotechnic chemicals were to be analysed morphologically, and to know the impact of symbiotic microbes inhabitating the gut of the fish also to find the proximate composition (level of proteins, carbohydrates and lipids) of the chemical exposed fish.

MATERIALS AND METHODS

Chemicals

Pyrotechnic chemicals, Copper acetoarsenite and Cryolite were purchased from Jeganathan Chemicals Pvt. Ltd., Sivakasi. These two chemicals were used to expose on fishes for morphological and proximate composition analysis.

Fish Collection

Oreochromis mossambicus is a freshwater fish collected from Thiruthangal pond near Sivakasi, Virdhunagar District, Tamil Nadu. India. Collected fishes were maintained in fish water tank.

Experimental Setup

A total number of eighteen fishes were separated into three groups. Each group comprised of Six Fishes. GROUP I serves as control, which was provided with normal feed. GROUP II was exposed to copper acetoarsenite and GROUP III was exposed to cryolite for consecutive twenty days along with feed. After treatment, the fishes of all groups were sacrificed for gut microbial study and proximate composition analysis.

Gut Microbial Study

The foregut, midgut and hindgut were separated and smashed with normal saline. It was then serially diluted. One mL of the sample (liquid suspension which contains a microbial mixture) was added it to 9mL of sterile water to give 1:10 or 10^{-1}dilution of the original sample, (i.e.) the original sample has been diluted to 1/10, similarly, 1:100(10^{-2}), 1:1000(10^{-3}), 1:10,000(10^{-4}) and up to 10^{-9} dilution of the original sample. This method is, therefore, also used for quantitative estimation of microbial cells in a known volume of original sample. Serial dilution can also be used for a pure culture and is thus called serial dilution method. The spread plate technique is used for enumerating microorganisms, dropped out 0.1 mL aliquots from serial dilutions onto the surface of an agar plate. Aseptically inoculums were spreaded across the surface using a bent glass rod. By the suspension over the plate, a dilution gradient was established to provide isolated colonies. Incubated agar plates inverted in appropriate conditions at 37^0C. Colonies were counted and calculated.

Proximate composition analysis

Estimation of Total Proteins

The total protein concentration was determined by the method of Lowry *et al.*, [17]. The BSA solution was taken from 50 to 200 µL in different tubes and it was made up with distilled water to 200 µL and similarly, in other

separate tubes, the gastric juices of about 200 µL of all groups were taken. 1 ml of alkaline copper sulphate reagent (analytical reagent) was added to all the tubes and mixed well. These solutions were incubated at room temperature for 10 minutes. Then 200 µL of folin ciocalteau reagent was added to each tube and incubate for 30minutes. Zero the colorimeter with blank and the optical density (measure the absorbance) was noted at 660 nm.

Estimation of Total carbohydrates

Hedge and Hofreiter [18] method can be used for the estimation of reducing sugars as well as non-reducing sugars (total sugars) in the sample. Pipette out into a series of test tubes with increasing volume of Sucrose solution from 0.2 mL to 1mL and make up the volume with Distilled Water. 5mL of anthrone was added to each tube and mixed well. The tubes were boiled well for 10 min and later cooled to room temperature. The absorbance was read at 625nm.

Estimation of Total Lipids

In centrifuge tubes, 0.2 mL of sample was taken. In another set of tubes, 0.2mL to 1mL of standard solution was taken and make up to1mL with distilled water. Ferric Chloride- Acetic Acid Reagent (9.8 mL) was added to each tube and mixed well. The tubes were kept at 70^0C for ten minutes. The supernatant was collected and concentrated sulphuric acid was added. The absorbance was read at 625nm [19].

RESULTS

Based on the morphological, impact of symbiotic inhabitating micro flora and proximate composition the toxicity of pyrotechnic chemicals were analyzed. The present study aimed to find the extent of toxicity of pyrotechnic chemicals by the analysis the normal gut micro flora and analyzing the levels of proteins, carbohydrates and lipids in the fish *Oreochromis mossambicus*. The pyrotechnic chemicals and their toxicity in the freshwater has the potential to change the aquatic medium, affecting the tolerance limit of aquatic fauna and flora, as well as creating danger to the ecosystem.

Morphological Studies

It has been observed that morphology of fish has changed. The color of the fish in the gill cover and roughness has been differed (Fig.1a and b). It reveals that pyrotechnic chemicals are highly affected the fishes and not suitable for edible purposes.

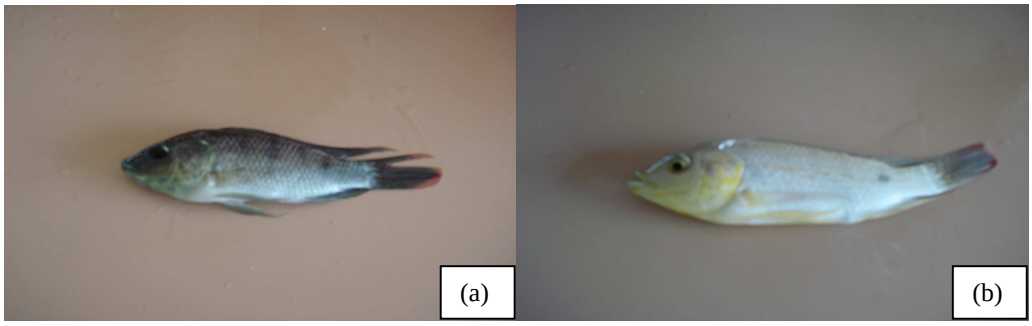

Fig.1.Morphological analysis, Normal fish (a) and Pyrotechnic chemical exposed fish (b).

Gut Microbial Studies

In the pyrotechnic chemical exposed fish, the populations of bacteria were more affected and more sensitive to the chemicals. Such differential sensitivity in bacterial genera to pyrotechnic chemical was due to their reduced metabolizing capacity and plasmid activity to degrade the molecule. The inhibitory activity of the pyrotechnic chemical on different bacterial genera further restricted their role in digestive process. The failure of microbial role in digestion leads to the suppression of immunity (Table.1 and 2).

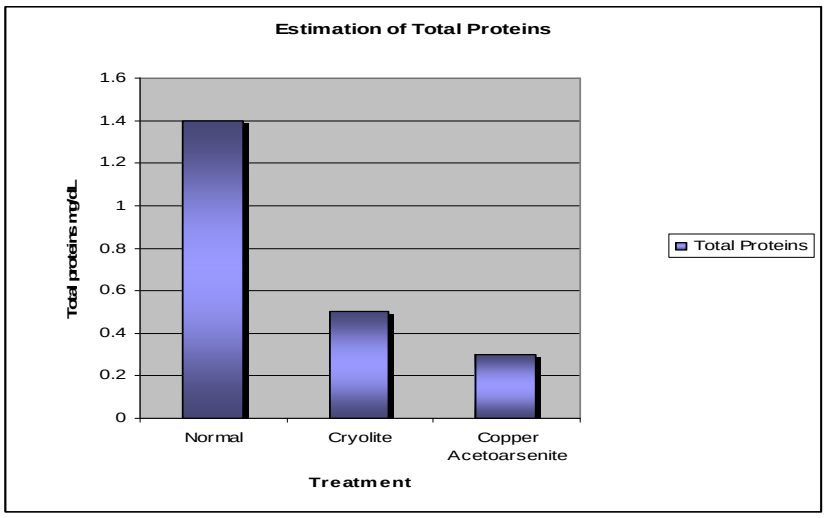

Fig.2.Estimation of total proteins.

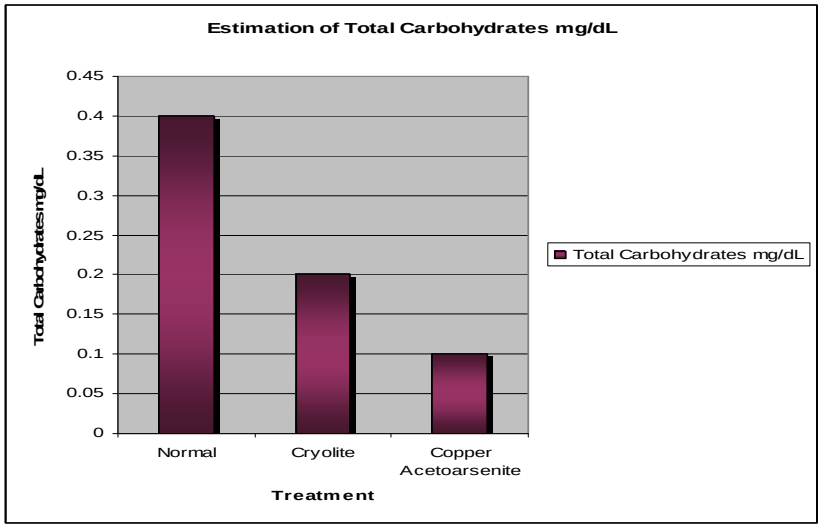

Fig.3.Estimation of total carbohydrates.

Proximate composition analysis

Total Proteins

Proteins have been estimated that pyrotechnic chemical treated fish contains low amount of protein when compared to the normal fish. The level of protein has been expressed in the form of bar diagram in Fig.2 shows the decreased level of protein. When compared to cryolite exposed fish the level of protein is much decreased in copper acetorsenite exposed fish. Breakage of peptide bonds may occur which may due to the toxicity of pyrotechnic chemicals.

Total Carbohydrates

Fish contains very little amount of carbohydrate normally. It has also been estimated and the level of carbohydrate was expressed in terms of bar diagram in Fig.3. It reveals that due to the toxicity, low level of carbohydrate was produced which shows significant decrease in Copper acetoarsenite exposed fish when compared to that of the cryolite exposed fish.

Total Lipids

It has been estimated that total lipid content was significantly reduced in copper aceoarsenite when compared to that of the cryolite exposed fish, which was expressed (Fig.4).

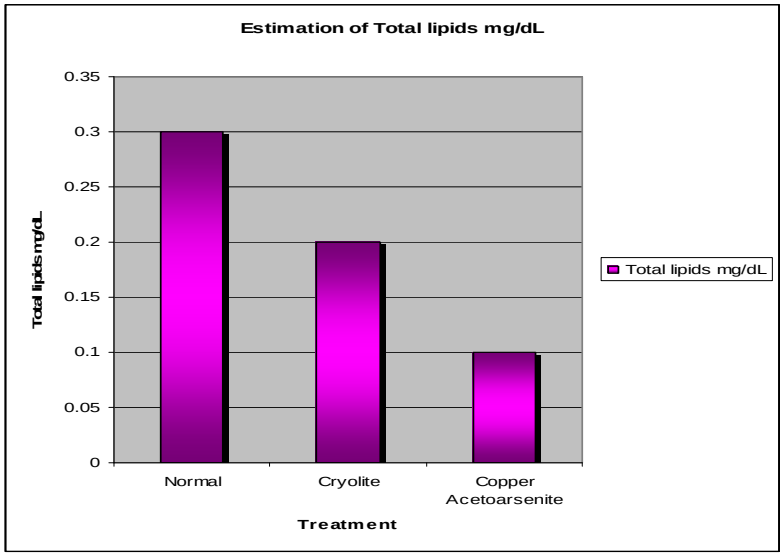

Fig.4.Estimation of total lipids.

Table.1.Morphology of strains isolated from normal and pyrotechnic chemical exposed fish digestive tract sample.

Strain's name	Gram's reaction	Cell morphology	Colony morphology
A	Negative	Coccoid, rod	White ,translucent, circular,
B	Negative	Short rod	Orange , smooth, translucent, circular
C	Positive	Short rod	Translucent, round, flat, smooth.
D	Negative	Rod	White , flat
E	Negative	Rod	Pink, smooth, round.

Table.2.Total heterotrophic bacterial population of digestive tract samples of normal and pyrotechnic chemical exposed fish.

S. No	FISH	Dilutions			
		10^4	10^5	10^6	10^7
1	**Normal**				
	Foregut	440	64	17	5
	Midgut	83	27	15	7
	Hindgut	97	32	13	4
2	**Cryolite**				
	Foregut	98	67	5	-
	Midgut	41	24	8	-
	Hindgut	46	28	11	-
3	**COP AA**				
	Foregut	63	15	-	-
	Midgut	37	12	-	-
	Hindgut	39	9	-	-

COP AA- Copper Acetoarsenite., Nil-No colonies.

DISCUSSION

The present study aimed to see the extent of toxicity of pyrotechnic chemicals by the analysis the normal gut micro flora and analyzing the levels of proteins, carbohydrates and lipids in the fish *Oreochromis mossambicus*. The pyrotechnic chemicals and their toxicity in the freshwater has the potential to change the aquatic medium, affecting the tolerance limit of aquatic fauna and flora, as well as creating danger to the ecosystem.

The intestinal lumen contains numerous and many species of bacteria. In order to preserve the integrity of the body from the continuous threat of infection by the abundant and various intestinal bacteria, the non-specific protection strategies of the gut are augmented by the presence of an immune system. The prominent feature is that mucosal immune system, i.e. the mucous membranes lining the digestive, respiratory, and urogenital systems have a combined surface area and are the major sites of entry for most pathogens. This mucosal immune system is formed by Ig A secreting plasma cells located in the connective tissue (Lamina Propira) beneath the epithelium [20]. These IgA secreting cells in the gut wall are clearly the result of B cell stimulation induced by microbial and food antigens in the gut lumen since such antibody producing cells are virtually absent in gut tissue. There are several reports on pesticides to inflict histopathological changes in the wall of gut. In this regard, more commensal bacteria live in the gut and which enhances the digestive ability, these commensal bacteria differ in their ability both to promote development of the gut-associated lymphoid tissues and to maintain its function. Total heterotrophic population count in the different regions of the gut included symbiotic as well as pathogenic microbes. Symbiotic microbes have probiotic role and promote digestive ability by producing microbial enzymes [21]. The decrease in total heterotrophic bacterial population in different regions of gut due to the pyrotechnic chemical treatment indicated the decrease in beneficial probiotic microbes. The reduction of beneficial gut micro flora had interfered with the digestive ability, food consumption energetic and overall health of the fish.

Current knowledge of the mucosa associated bacterial communities in the intestine and colon is limited owing to the greater focus on characterization of fecal diversity. Recent studies, however, indicate that the predominant mucosa associated community is host- specific and significantly different from luminal and fecal bacteria communities [22]. In the present study, the reduction in the total heterotrophic population in the gut due to the pyrotechnic chemical exposure indicates the sensitivity of the microbes to the pyrotechnic chemical as well as the loss of gut-associated epithelium, where lymphoidal tissues are present. It was found that decrease in bacterial population in the regions occurs when compared to that of the control samples. The xenobiotic stress on the inhibition of digestive enzymes, changes in gut epithelial cells and alterations in the food and feeding reduce the bacterial load. Further, the poor presence of digested food in stomach, intestine and rectum failed to provide suitable medium for the growth of micro flora in the alimentary tract. This had lead to the reduction in the total heterotrophic bacterial population [10].

The reductions in the gut of the host indirectly affect the feeding and energy budget in the host animal [10]. Targeting the immune system of the human gut with live bacterial probiotics, bacteria with health promoting properties, could provide benefit for the treatment of both acute and chronic intestinal diseases. In the present study, the total heterotrophic bacterial population in gut of the fish *Oreochromis mossambicus* affected to due to the toxicity of the pyrotechnic chemical and most of the beneficial microbes cannot survive. When the lamina propia layer was corroded in the associated lymphoidal tissues (Payer's patches) was damaged and pathogenic microbe's easily infected IgA secreting cells were damaged. Drasar and Barrow [10] and Vander Waaij [21] the bacterial micro flora of the gut is an extremely stable ecosystem, which prevents colonization of newly ingested bacteria, in conjugation with other non-specific defense mechanism to prevent infection by intestinal bacteria [23]. Hence the changes in intestinal flora were also found to be another factor for the decline of immunity.

Any damage to the physiological mechanism and to cells of the body could reduce cytokines production. Cytokines are soluble glycoprotein that is released by living cells. Most cytokines are produced by a variety of cell types. This growth factor cytokines play an important role in activating B cells, T cells, Macrophages and various other cells that participate in the immune response. Pyrotechnic chemicals treated fish were found to affect the different species of white blood cells and peripheral lymphocytes. Hence the cytological change in these cells leads to the low production of cytokines, thereby affecting the immune regulation.

In the pyrotechnic chemical exposed fish, the populations of bacteria were more affected and more sensitive to the chemicals. Such differential sensitivity in bacterial genera to pyrotechnic chemical was due to their reduced metabolizing capacity and plasmid activity to degrade the molecule. The inhibitory activity of the pyrotechnic chemical on different bacterial genera further restricted their role in digestive process. The failure of microbial role in digestion leads to the suppression of immunity.

Proximate Composition Analysis

Oreochromis mossambicus exposed to the Pyrotechnic chemicals showed decreased levels of proteins, carbohydrates and lipids in their tissues. Proteins and fats are the major nutrient groups supplied by fish. Fish generally contain very little carbohydrate. From hair to fingernails, protein is a major functional and structural

component of all our cells. Protein provides the body with roughly 10 to 15 per cent of its dietary energy, and is needed for growth and repair. Proteins are large molecules made up of long chains of amino acid subunits. Some of these amino acids are nutritionally essential as they cannot be made or stored within the body and so must come from foods in our daily diet. The protein in fish contains sufficient amounts of all the essential amino acids required by the body for growth and maintenance of lean muscle tissue. The protein in fish makes up complete protein source. The protein source can be important to lose weight because high quality proteins such as the protein in fish can be used to maintain an active metabolism. Low quality protein does not contain all essential amino acids required for use in protein synthesis and means the protein must either be used for energy or converted to fat. The protein found in fish is of high biological value, which means that fish can be used as the sole source of protein in the diet.

Lipid is the term used to describe both fats and oils. Fats are lipids that are solid at room temperature and oils are lipids that are liquid at room temperature. The lipid content of fish varies depending on the type of fish, the time of year and what the fish feeds on. White fish, such as cod, generally have between 0 and 2% lipid whereas oil-rich fish, such as mackerel, can have in excess of 16% lipid. The lipid content of farmed fish can vary widely depending on the feed used. The lipid found in fish is mostly polyunsaturated lipids, also known as PUFA, with small amounts of saturated and monounsaturated lipids.

It can be revealed that due to the toxicity pyrotechnic chemicals, decreased level of protein, carbohydrate and lipids were observed. So, if people consume this fish it systematically affects the human system thereby causing adverse effects as well as to the ecosystem.

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