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Role of Synthetic Detergents in Damaging the Tissues of *Labeo rohita*

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Abstract

The biochemical components like protein, carbohydrate and lipid were estimated quantitatively in the tissue of gills, liver, kidney and muscle of control and detergent treated fishes. The fishes were treated with sub lethal concentrations of 1.5% for 15 days exposure and another 15 days for recovery period. The protein content of the gill of the control fish was 85.18 ± 0.68 mg/g, while in treated fish it was reduced to 43.30 ± 0.04 mg/g after 15 days exposure to detergent. The protein content of the kidney, liver and muscle of the control fish were 53.44 ± 0.03, 68.28 ± 0.05 and 94.22 ± 0.05 mg/g while in treated fish it were reduced to 60.16 ± 0.04, 60.54 ± 0.04 and 78.14 ± 0.05 mg/g after 15 days of exposure respectively. The carbohydrate and lipid content of the gills, liver and muscle also showed similar declining trend at during study period. During recovery period, the restoration of protein, carbohydrate and lipid level were found be slow and gradual.

Keywords: Organ; Fish retailors; Hygienic handling; Fish consumers; Lake hawassa

Introduction

Detergents were developed due to the problems that occurred when organic soap was used in areas of hard water. Hard water contains the ions of calcium and magnesium in high amounts and these are substituted on to the soap molecule in place of sodium. The salts of calcium and magnesium are insoluble and form a precipitate; this is what leaves a 'ring' around the bath [1].

Other problems were encountered in the textile industry where acid solutions are used in the dying process. The free H+ ions replace the sodium ions reforming the fatty acid which affects the application of dyes and leaves spots on fabrics [2].

The first synthetic detergents were developed following the First World War by the Germans so that fat could be used for other purposes. The first synthetic detergents were short-chain Alkyl Naphthalene Sulphonates which were later discovered to be only moderately good detergents and so were improved but are still used today as wetting agents.

In the 1920's and 30's straight chain alcohols were sulphonated to give straight chain detergents. In the 30's long chain alkyl and aryl sulphonates with benzene as the aromatic nucleus were developed. The alkyl portion was derived from kerosene by the end of the World War II alkyl aryl sulphonates swamped the detergent market over alcohol sulphates which later became useful in the shampoo industry [3].

It was a popular complaint at this time that whites dulled after washing, they weren't as white as white should be and this was linked to the use of synthetic detergents. Even though they were just as good as soaps at removing dirt, they were poor at holding it in suspension and the particles redeposited on to the clothing. The problem was overcome by the addition of CMC (Carboxy Methyl Cellulose). More recently the limiting factor that affected the production of specific detergents was availability of raw materials [4].

This lead to the development of Igepon compounds in Germany and the USA (for example Igepon-T, the sodium salt of oleyl tauride), mesolates in Germany (alkane sulphates) and Teepol in England (a secondary Olefine Sulphate form petrochemical sources). Alkyl benzene sulphonate was top of the market due to its ease of manufacture and versatility.

Materials and Methods

Between 1950 and 1965 more than half of the detergents were based on a propylene tetramera coupled to benzene (PT benzene), but they were later blamed for a rise in eutrophication in lakes and streams.

This problem has not been fully resolved in some cases, in some countries there has been a 'gentlemans agreement' to reduce the use of phosphates but in countries **(Table 1)**.

Table 1: Effect on pollutants in different species of fishes.

Sl. No.	Species of fish used	Pesticide/pollutant employed
1	*Labeo rohita*	Quinalphos
2	*Channa punctatus*	Carbamate and synthetic pyrethroid
3	*Oncorynchus mykiss*	Cypermethrin
4	*Beleopthalmus dussumieri*	Chlorpyrifos
5	*Oreochromix mossambicus*	Chlorpyrifos
6	*Channa punctatus*	Dimethoate
7	*Cyprinus carpio*	Endosulfon
8	*Labeo rohita*	Carbofuran
9	*Catla catla*	Chlorpyrifos
10	*Clarias gariepinus*	Dimethoate
11	*Channa punctatus*	λ-Cyhalothrin
12	*Oncorhyncus mykiss*	Malachite green
13	*Oreochromis mossambicus*	Chlorpyrifos
14	*Cirrhina mrigala*	Chlorocid
15	*Cyprinus carpio*	Chlorpyrifos

Study area

The study was carried out in Alagppa University campus in fishery pond area of Sivagangai district, Tamilnadu, India (N11° 03.845'; E078° 41.007') from March 2018 to February 2019 (**Table 2 and Figure 1**).

Table 2: Scientific classification of experimental fish.

Kindgom	Animalia
Phylum	*Chordata*
Class	*Actinopterygii*
Order	*Cypriniformes*
Family	*Cyprinidae*
Genus	*Labeo*
Species	*rohita*

Figure 1: Binomial name *Labeo rohita*.

The only water source is rainfall and non-perennial for supporting pump out water through [5]. The temperature ranged from 30.0°C to 39.0°C during summer and 20°C to 26°C during the monsoon and post monsoon periods. The study area receives northeast (October-December) monsoon rains. Failure of monsoon occurs rarely and results in drought

Distribution

Rohu (*Labeo rohita*) is a fish of the carp family Cyprinidae, found commonly in rivers and freshwater lakes in and around the South Asia and South-East Asia. It is an herbivore; it is treated as a delicacy on Orissa, Bihar and Uttar Pradesh. In fact, the Kayastha community of Uttar Pradesh treats it as one of their most sacred foods: to be eaten on all auspicious occasions [6-9].

In Hindi it is called Rehu (Rawas is the Indian Salmon, which is quite different). It is called rohu in Tamil, rohi is Oriya, rui in Bengali, rou in Assamese and is popular in Orissa, Thailand, Pakistan, Bangladesh, Bihar, Uttar Pradesh, West Bengal, Assam and The Konkan region of India. It is a non-oily white fish.

The roe of rohu is also considered as a delicacy by Oriyas and Bengalis. It is deep fried and served hot as an appetizer as part of a Oriya and Bengali meal [10-12].

It is also stuffed inside pointed gourd to make potoler dolma which is a delicacy often prepared to study the palate of the

discerning guess. Rohu is also served deep fried in mustard oil, as Kalia onions and tok, where the fish is cooked in a flavourful and tangy sauce made of tamarind and mustard. Rohu is also very popular in Northern India such as in the province of Punjab. It is a specialty of lahori cuisine as in lahori fried fish, prepared with batter and species [13].

Identification

Upper body with dark scale, lower body and belly golden brown, dark brown dorsal fin and tail, pelvic, pectoral and anal fins with red tint [14,15].

Other common name

Ruee, Rui, Tapra.

Typical location

In creedy and slow flowing or standing water of lakes, ponds, pools and rivers.

Biology

During the early stages of its life cycle, it eats mainly zooplankton, but as it grows, it eats more and more phytoplankton and as a juvenile or adult is a herbivorous column feeder, eating mainly phytoplankton and submerged vegetation. It has modified thin hair-like gill rakers suggesting that it feeds by sieving the water. It is diurnal and generally solitary. It reaches maturity between two and five years. In nature, it spawns in the marginal areas of flooded rivers. When cultured, it does not breed in lentic environments, so induced spawning becomes necessary [16].

History of use

Distributed across the Indo-Gangetic flood plains of Bangladesh, India and Pakistan and also in the Irrawady and its tributaries in Myanmar. *Labeo rohita* is the most highly valued of all carp species farmed using traditional or newly-developed aquaculture systems in the Indain Subcontinent [17,18].

It has been introduced in other areas of India beyond its natural range for aquaculture in ponds and reservoirs and also to the Godavari and Krishna rivers. Because of its fast growth and high quality flesh, it has also been introduced to other countries including the former USSR, Japan and The Philippines [19].

Breeding time

Rui attains maturity towards the end of the second year in ponds. The spawning season of Rui generally coincides with the southwest monsoon. Spawning takes place in flooded rivers [20].

Economic importance

Rui is regarded an excellent game fish and seems to put up a better fight in a river than in a tank. A number of inter specific and inter generic hybrids have been produced. It is very delicious food and supplies a huge amount of protein for the people [21,22].

Ecological role

Middle dweller fish species. Sometimes come to the surface and bottom layer for finding food [23].

Marketing status

Market price is varied from 120 to 140 Tk/kg [24,25].

General description of detergents

A soap is a salt of a compound known as a fatty acid. A soap molecule consists of a long hydrocarbon chain (composed of carbons and hydrogens) with a carboxylic acid group on one end which is ionic bonded to a metal ion, usually a sodium or potassium. The hydrocarbon end is non-polar and is soluble in nonpolar substances (such as fats and oils) and the ionic end (the salt of a carboxylic acid) is soluble in water **(Figure 2)**.

Figure 2: Picture of detergent packet.

Detergents are structurally similar to soaps, but differ in the water-soluble portion. Three examples of detergent shown below **(Figures 3-5)**.

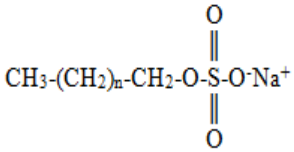

Figure 3: A sodium alkyl sulfate.

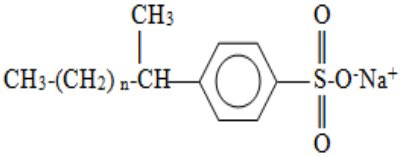

Figure 4: A sodium alkly benzene sulfonate.

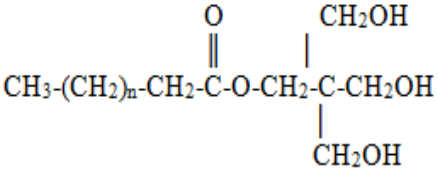

Figure 5: A glycerol (a non-ionic detergent).

When a soap or detergent is added to water, a polar solvent, the molecules form clusters, known as micelles, in which the polar ends of the molecules are on the outside of the cluster and the non-polar ends are in the middle [26-28].

The cleaning action of both soaps and detergents results from their ability to emulsify or disperse water-insoluble materials (dirt, oil, grease, etc.) and hold them in suspension in water. This ability comes from the molecular structure of soaps and detergents. When a soap or detergent is added to water that contains oil or other water-soluble materials, the soap or detergent molecules surround the oil droplets (**Table 3**).

Table 3: Physico chemical parameters of normal water and sub lethal concentration of Ariel detergent dissolved water.

Parameters	Normal water (Control)	Sub lethal concentration (Ariel detergent dissolved water)
Colour	Colourless	Light milky
Odour	Odourless	Unpleasant
Temperature °C	22 ± 0.5	22.5 ± 0.5
pH	7.5 ± 0.05	8.1 ± 0.03
DO mg/l	7.7 ± .01	5.5 ± 0.01
Free CO2	1.5 ± 0.01	2.3 ± 0.01
Total alkalinity mg/l	40.7 ± 0.5	57.1 ± 0.14
Salinity (ppt)	27.93 ± 0.3	0.63 ± 1.3

The oil or grease is "dissolved" in the alkyl groups of the soap molecules while the ionic end allows the micelle to dissolve in water. As a result, the oil droplets are dispersed throughout the water (this is referred to as emulsification) and can be rinsed away [29].

Water criteria

The chlorine free water was used as test water in all the experiments [30]. The condition of test water maintained at constant characterization as recommended by Committee on water quality criteria throughout the test were depicted.

System of treatment

In the present investigation, the commercial grade of Ariel detergent powder was selected and used by diluting it in required volume of water or any other organic solvents. This is in accordance with the method employed by the farmers who simply dilute the grade with water and apply in paddy fields [31].

Acute toxicity studies

Acute toxicity test were conducted to determine the impact of toxicant on aquatic animals within a short period of 24 hours. Simultaneously the toxicity of Ariel detergent on the test fish Labeo rohita for 3, 6, 12, 24, 48, 72, 96 and 120 hours were determined. The LC50 is a statistical estimate of the concentration of the toxic materials in water that kills 50% of the test animals under experimental conditions at specific time intervals.

This value is ideally suited for toxicity studies as it gives a more acceptable and reproducible concentration required to affect 50% of the organisms than any other value [32].

Calculation of harmless concentration (c)

A presumable harmless concentration (c) of the toxicants is calculated by using the safe factor or application factor employing the formula:

$$c = \frac{48hrsLC_{50} \times A}{S}$$

Where,

$$S = \frac{24hrsLC_{50}}{48hrsLC_{50}}$$

$$A = 0.3(cons\tan t)$$

The safe concentration is a useful unit of measurement of acceptable amount of the toxicant, which has no lethality and stress to the animal exposed.

Biochemical analysis

The fishes were collected from the control and experimental 15 days exposure and recovery period (15 days) and were subjected to the biochemical analysis individually as mentioned below.

Estimation of protein

The protein content of tissues of the normal and pesticide treated fishes were estimated quantitavely. The principle of the method lies in the reaction of carbonyl group a complex which with phosphomol acid of Folin's reagent gets reduced to tyrosine and tryptophan [33]. 1 g of tissue sample was taken, homogenized well then transferred in a centrifuge tube and to

this 1 ml of 80% ethanol was added. The mixture was centrifuged at a velocity of 3,000 rpm for 10 minutes. After discarding the supernatant, 1 N sodium hydroxide was added to dissolve the precipitate in it. From this 1 ml was taken and mixed with 5 ml of alkaline copper solution and kept for 10 minutes. To this mixture, 0.5 ml of Folinphenolciocaltue reagent was added. The blue colour of the solution was measured at 720 nm by using a spectronic. The O. D value of each sample was recorded. A blank solution of pure 1 N sodium hydroxide was treated like wise. The amount of protein present in the sample was read from the graph drawn for standard solution.

Estimation of carbohydrate

The carbohydrate content of the above said tissues of the normal and treated fishes was estimated quantitatively. The sulphuric acid present in the anthrone reagent hydrolyses di and oligo-saccharides to furfural or its derivatives. The furfural reacted with anthrone to produce a complex colour proportional to the amount of saccharides present in the sample [34].

1 gm of tissue sample was homogenized, then the supernatant taken in a centrifuge tube and to this 1 ml of 80%ethanol was added. The mixture was centrifuged at a velocity of 3,000 rpm for 10 mintues. From this 0.5 ml of the supernatant was taken and mixed with 5 ml of anthrone reagent was added to it. The test tube was kept in boiling water bath for 15 minutes. The intensity of green colour developed was measured at 620 nm by using a spectronic 21 and the O.D value of each sample was recorded. A blank solution of pure 80% ethanol was treated likewise. The amount of carbohydrate present in the sample was read from the graph drawn for standard solution.

Estimation of lipid

The lipid content of tissue of the normal and pesticide treated fishes were estimated quantitatively. This method is based on the sulphophosphovanillin reaction of Charrol and Charronat. Lipids react with sulphuric acid and phosphoric acid vanillin to give a red coloured complex.

1 gm of tissue sample was taken and homogenized, then transferred into a centrifuged tube and 2 ml of chloroform: methanol mixture (2:1) was added to it. The mixture was centrifuged at a velocity of 3,000 rpm for 10 minutes. From this 0.5 ml of lipid extract was taken into a test tube and to it 0.5 ml of concentrated sulphuric acid was added. The test tube was placed in boiling water bath for 2 to 5 minutes and cooled at room temperature. From this, 0.2 ml was taken in a separate test tube and to this 5 ml phosphovanillin reagent was added. The test tube was allowed to stand for 30 minutes. The pink colour developed was measured at 520 nm using spectronic. The OD value of each simple was recorded.

A blank sample of pure chloroform: methanol mixture (2:1) was treated likewise. The amount of lipid present in the sample was read from the graph constructed for standard solution.

Results and Discussion

Level of protein (mg/g) in different tissue of the fish *Labeo rohita* exposed to sub lethal concentration of Ariel detergent for 15 days exposure and another 15 days for recovery period. The protein content of the gill of the control fish was 85.18 ± 0.68 mg/g, while in treated fish it was reduced to 43.30 ± 0.04 mg/g after 15 days exposure to detergent. The protein content of the kidney, liver and muscle of the control fish were 53.44 ± 0.03, 68.28 ± 0.05 and 94.22 ± 0.05 mg/g while in treated fish it were reduced to 60.16 ± 0.04, 60.54 ± 0.04 and 78.14 ± 0.05 mg/g after 15 days of exposure respectively.

The level of carbohydrate (mg/g) in different tissue of the fish *Labeo rohita* exposed to sublethal concentration of Ariel detergent for 15 days exposure and another 15 days recovery period.

The carbohydrates content of the gill of the control fish was 15.84 ± 0.73 while in treated fish it was reduced to 12.41 ± 0.44 mg/g after 15 days exposure to the detergent. The carbohydrate content of the kidney, liver and muscle of control fish were 21.34 ± 0.35, 35.41 ± 0.71 and 23.21 ± 0.73 mg/g respectively while in treated fish it were reduced to 19.33 ± 0.5, 24.31 ± 0.92 and 19.14 ± 0.83 mg/g after 15 days exposure respectively. The lipid content of the control fish in gill, kidney, liver and muscle were 18.2 ± 0.43, 16.4 ± 0.45, 18.3 ± 0.04 and 14.3 ± 0.05 mg/g respectively, while in treated fish it were reduced to 13.5 ± 0.05, 14.14 ± 0.4, 13.1 ± 0.03 and 13.4 ± 0.04 respectively after 15 days exposure to the Ariel detergent. Similar trend was observed in remaining tissues [35]. The lipid content during the exposure period of the gill, kidney, liver and muscle of the control fish was 20.34 ± 0.05, 18.41 ± 0.55, 23.43 ± 0.55 and 16.4 ± 0.08 respectively, while in treated fish it were reduced to 18.35 ± 0.03, 17.34 ± 0.15, 20.41 ± 0.05 and 16.4 ± 0.05 respectively [35].

During the recovery period (15 days), it was found that after short term exposure of 15 days, restoration of protein, carbohydrate and lipid content level to their normal level were found to be slow and gradual which might be due to slow elimination of the toxicant form the tissues and reduced proteolysis and also physiological activity **(Tables 4-7)**.

Table 4: Mortality of *Labeo rohita* exposed to different concentration of Ariel detergents at different hours of exposure.

Concentration (ppt)	No. of fishes exposed	3 hrs	6 hrs	12 hrs	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs
0.10*	30	-	-	-	-	-	-	-	-
0.15*	30	-	-	-	-	-	-	-	-
0.2	30	-	-	-	3	4	6	7	9

0.25	30	-	-	3	6	7	9	10	11
0.3	30	-	-	5	9	10	13	15	19
0.35**	30	-	5	7	12	15	17	19	24
0.4	30	3	7	11	14	20	22	24	27
0.45***	30	5	10	15	19	22	26	28	30
0.5	30	10	12	19	22	25	25	30	-
0.55	30	12	17	21	25	28	30	-	-
0.6	30	15	20	24	28	30	-	-	-

Note": * LC50 Sublethal concentration, ** LC50 50% of mortality, *** LC100 100% of mortality (Lethal concentrations)

Table 5: Sublethal effect of Ariel detergent on protein content (mg/g) in different tissues of *Lebeo rohita* during exposure and recovery periods (Each value is mean ± SD (n=5)).

Period of study	Concentrations	Gill	Kidney	Liver	Muscle
Exposure period	Control	85.18 ± 0.68	53.44 ± 0.03	68.28 ± 0.05	94.22 ± 0.05
15 days	Experiment	43.30 ± 0.04	60.16 ± 0.04	60.54 ± 0.04	78.14 ± 0.05
Recovery period	Control	50.43 ± 0.04	65.43 ± 0.04	68.13 ± 0.05	83.11 ± 0.04
15 days	Experiment	45.30 ± .04	68.14 ± 0.05	65.13 ± 0.05	80.14 ± 0.05

Table 6: Sublethal effect of Ariel detergent on carbohydrate content (mg/g) in different tissues of *Lebeo rohita* during exposure and recovery periods (Each value is mean ± SD (n=5)).

Period of study	Concentration	Gill	Kidney	Liver	Muscle
Exposure period	Control	15.84 ± 0.73	21.34 ± 0.35	35.41 ± 0.71	23.21 ± 0.73
15 days	Experiment	12.41 ± 0.44	19.33 ± .05	24.31 ± 0.82	19.14 ± 0.83
Recovery period	Control	14.41 ± 0.54	20.44 ± 0.05	34.41 ± 0.04	25.41 ± 0.05
15 days	Experiment	14.81 ± 0.33	18.54 ± 0.05	30.43 ± 0.05	21.03 ± 0.04

Table 7: Sublethal effect of Ariel detergent on Lipid content (mg/g) in different tissues of *Lebeo rohita* during exposure and recovery periods (Each value is mean ± SD (n=5)).

Period of study	Concentration	Gill	Kidney	Liver	Muscle
Exposure period	Control	18.2 ± 0.43	16.43 ± 0.45	18.3 ± 0.04	14.3 ± 0.05
15 days	Experiment	13.5 ± 0.05	14.14 ± 0.43	13.1 ± 0.03	13.4 ± 0.04
Recovery period	Control	20.34 ± 0.05	18.41 ± 0.55	23.43 ± 0.55	16.4 ± 0.08
15 days	Experiment	18.35 ± 0.03	17.34 ± 0.15	20.41 ± 0.05	16.4 ± 0.05

Conclusion

The pesticides in the aquatic environment cause several effects on the physiological and biochemical aspects of fish. Biochemical changes in response to pesticides usually lead to irreversible and detrimental disturbances of integrated functions such as behavior, digestion, growth, reproduction and survival itself, which in turn may cause many changes in the population level. The results of the study showed significant decrease in protein content in all the tissues studied. Depletion in tissue protein in fishes exposed to pollutants has also been reported by earlier workers.

A number of workers have reported decline in protein level of various organs and tissues under toxic stress of various chemicals. There is depression in *de novo* protein synthesis in liver methyl mercury intoxicated *Cyprinus carpio*. It is observed considerable loss of protein content in liver and ovary of *Channa punctatus* exposed chronically to mercuric chloride. It is reported similar decreasing trend of protein in the liver due to increased utilization to overcome stress created by heavy metals. It is reported decline in liver protein in *Cyprinus carpio* exposed to nickel and chromium separately. All these observations confirm the findings of the present study.

The present investigation indicates a fall not only protein but also in the carbohydrate level in all tissues during exposure period. But the decrease was more significant in liver followed by muscle and intestine.

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