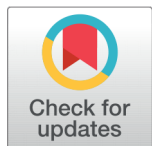


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Harpic Induced Biochemical and Histological Changes in Fresh Water Fish Common Carp (*Cyprinus Carpio* L.)

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Abstract

Objectives: To investigate the xenobiotic action of toilet cleaner 'Harpic' on gills and muscles of fresh water teleost common carp (*Cyprinus carpio* L.). **Methods:** The test organism, fingerlings of Common carp (weight: 4.5 ± 1.3 g), (length: 5.3 ± 1.5 cm) were collected from Bhadra Reservoir Project, India. Static bioassay tests were conducted in order to evaluate the acute toxicity of Harpic. In all treatments, ten fully acclimatized test organisms were held and the same was observed for experiment and placed in different concentrations of Harpic to determine LC₅₀ values after 24 hours. Behavioral responses and mortality of the fishes were recorded at the interval of 2 hours and the alternation of behavioral characteristics was recorded. Glucose was estimated by Anthrone method. Glycogen was estimated by Kemp's method and Protein content was estimated by Lowery's method. **Findings:** The glycogen muscle (0.015mg/g) and gills (0.06mg/g), and protein in muscles (0.482mg/g) and gills (0.749mg/g) levels were found to be depleted respectively in the tissues exposure to sub lethal concentration over the control. Whereas, the glucose level in the fish tissue showed an increase in muscle (0.168 mg/g) and in gills (0.108 mg/g) on pesticide exposure in comparison with the control. The depletion of glycogen level of gills (0.06mg/g) on pesticide exposure may be due to stress condition and increased metabolism. Further depletion of protein may also attribute to spontaneous utilization of amino acids in various catabolic reactions inside the organisms in order to combat the stress condition. Gill exhibited alteration such as showed desquamation of the epithelial lining, necrosis (Telangiectasia) of the secondary lamellae, shrinkage of secondary lamellae and also showed hypertrophy and hyperplasia at the base of the secondary lamellae. In case of muscle the nuclear proliferation was observed and also seen different size of the muscle fibres, disintegration of muscle bundles, atrophy of muscle bundles, marked thickening and separation of muscle bundles. **Novelty:** No such works carried out on the effect on younger developmental stages of fishes which are considered to be more susceptible and vulnerable to toxicants than those of adult stages.

Keywords: *Cyprinus carpio*; Harpic; Desquamation; Hyperglycaemia; Necrosis

1 Introduction

Detergents are the parts of a large group of chemical compounds, collectively referred as surface-active agents or surfactants because they act upon surfaces⁽¹⁾. Surfactants are the components mainly responsible for the cleaning action of detergents. In commercial detergents, the surfactant component is between 10 and 20%. The other components include bleach, filler, foam stabilizer, builders, perfume, soil-suspending agents, enzymes, dyes, optical brighteners and other materials designed to enhance the cleaning action of the surfactant. Detergents are cleaning products derived from synthetic organic chemicals. The cheapness of detergent production from petrochemical sources with its ability to foam when used in acid or hard water gives it an advantage over soaps⁽²⁾. These pollutants build up in the food chain and are responsible for the adverse effects and death in aquatic organisms⁽³⁾. Fishes are widely used to evaluate the health of aquatic ecosystem and physiological changes serves as biomarkers of environmental pollution⁽⁴⁾.

Xenobiotics compounds concentrate in the tissues of aquatic biotas and are known to produce cumulative deleterious effects^(5–7). Therefore, the application of environmental toxicology studies on non-mammalian vertebrates is rapidly expanding for the evaluation of the effects of noxious compounds⁽⁸⁾. Indiscriminate discharge of such compounds into natural waterways have harmful effects on the fish population and other forms of aquatic life and may contribute long term effects in the environment^(9,10).

Fishes are among the group of non-target aquatic organisms, which represent the largest and most diverse group of vertebrates. A number of characteristics make them excellent experimental models for toxicological research, especially for the contaminants, which are likely to exert their impact on aquatic systems⁽¹¹⁾.

Hence an attempt has been made to determine the short term (24hours) toxic effect of toilet cleaners to the fingerlings of economically important fresh water fishes *Cyprinus carpio*. As the chosen brand is easily available majority of people predominately use for cleaning the toilets. Hence, important consideration for studying the toxicity of toilet cleaners in the fingerlings of *Cyprinus carpio* species was the paucity of information on the younger developmental stages which are considered to be more susceptible and vulnerable to toxicants and than those of adult stages.

2 Material and Methods

Selection of test Organism:

The test organism, fingerlings of Common carp (weight: $4.5 \pm 1.3\text{g}$), (length: $5.3 \pm 1.5\text{cm}$) were collected from Bhadra Reservoir Project, and were transported to the laboratory in well ventilated polythene bags to avoid any injury. The test organism were kept in large plastic containers and thereafter acclimatized for laboratory conditions.

Bioassay Protocol:

Static bioassay tests were conducted in order to evaluate the acute toxicity of Harpic. Dechlorinated tap water was used for acclimation of fish as well as experiment and control. In all treatments, ten fully acclimatized test organisms were held and the same was observed by^(12,13). Fishes were fed twice daily with groundnut oil cake and rice bran with respect to the 10% body weight of fish. The moderate size fishes were selected for experiment and placed in different concentrations of Harpic to determine LC_{50} values after 24 hours.

LC_{50} -24 Determination

Commercially available Harpic brought from the market taken in term of concentration ml/l. In each four trough, each containing 10 liters of water stocked with 10 fish, containing different concentration (0.01, 0.02, 0.03, 0.04, 0.05 and 0.06 ml/l) of Harpic for Common carp. A control set was run with the same number of fish and the same volume of water but without toilet cleaners. The experiment was run in duplicates. The water was aerated and the feeding was completely stopped during experiment period. Dead fishes were removed immediately and their number was recorded at 24 hrs. LC_{50} were calculated by graphical method.

Study of Behavioral Response:

The behavioral changes in the fishes were noted right after the application of testing dose till the end of the experiment. The negative control group was also monitored in the same while for mortalities and changes in behavior including loss of balance, moving in spiral fashion with jerks, lying laterally and opened mouth with rapid opercular movement. Behavioral responses and mortality of the fishes were recorded at the interval of 2 hours and the alternation of behavioral characteristics was recorded.

Biochemical Analysis:

At the end of each exposure period fishes were sacrificed and tissues such as gills and muscle were dissected and removed for biochemical profile analysis. Glucose was estimated by Anthrone method⁽¹⁴⁾. Glycogen was estimated by Kemp's method⁽¹⁵⁾ and Protein content was estimated by Lowery's method⁽¹⁶⁾.

Histology analysis

For histological examination fish were sacrificed and tissue (gills and muscle) were removed immediately to overcome autolysis; then they were fixed in Bouin's solution dehydrated through graded series of ethanol, cleared in xylene and embedded in 56-58 °C. After taking the 4-6 micrometer they were haematoxyline and eosin under a microscope for histological analysis.

3 RESULTS

Toxicity studies:

LC₅₀ is a concentration in which 50% of the experimental animals survive. Estimation of LC₅₀ by interpolation involving plotting of data in a graph with concentration on X-axis, while percentage on Y-axis. A straight line is drawn between maximum points representing survival at maximum successive concentrations that were lethal to more and less than of the total number of test animals exposed to the toxicant. The concentration at which this crosses the 50% survival line is the LC₅₀ value (17). The LC₅₀ value was determined for the Harpic to the late fingerlings of Common carp (*Cyprinus carpio*).

Table 1. LC₅₀ values of Harpic exposure to fingerlings of *Cyprinus carpio*.

Cleaners	Fish species	Exposure period	Method	LC ₅₀
Harpic	<i>Cyprinus carpio</i>	24	Graphical	0.06ml/l

There was no mortality in the control at the end of experiment (24hrs). During the same time span, the mortalities were 100% at the highest concentration (0.1ml/l and 0.06ml/l) of floor cleaner used. The estimated 24hrs LC₅₀ value of Harpic using a static bioassay system for Common carp (*Cyprinus carpio*) was 0.06ml/l.

Behavioral Studies

Different behavioral responses of common carp shows at different concentration of Harpic were observed throughout the experimental period. The control group showed the normal behavior during the whole experimental period. Application of the lowest concentration (0.01ml/l) of floor cleaner to the fish was observed normal response. The behavioral responses of the test organism during the acute toxicity test were noticed by the sudden change in the organism response to the environment such as erratic swimming, occasional grasping for breath, loss of balance, swimming in a spiral path with jerks and revolving in water. At the highest concentration (0.06ml/l) the more severity of all these responses was observed including the loss of balance, lying laterally at the bottom, swimming down in spiral fashion with jerks rapid, opercular movements with opened mouth (Table 1).

Table 2. Behavioral response of Common carp (*Cyprinus carpio*) fingerling during the exposure to lethal concentration of Harpic

Behavior	Concentration in ml/l					
	0.01	0.02	0.03	0.04	0.05	0.06
Erratic swimming	-	-	+	+	+	+
Gasping for breathing	-	-	-	+	+	+
Loss of balance	-	+	+	+	+	+
Swimming in spiral fashion with jerks	-	-	+	+	+	+
Lying laterally at bottom	-	+	+	+	+	+
Motionlessness	-	+	+	+	+	+

(+) = Present, (-) = Absent

Biochemical Changes in Tissues

Glucose: The variation of glucose content in fish, control and expose trails represented in (Table 2, Figure 1). Glucose content (mg/g) in the gills and muscle tissue was estimated after exposing the fingerlings for 24 hours (LC₅₀ of Harpic for 0.06 ml/l). In that the exposed muscle found to have (0.168mg/g) and gills (0.108mg/g), tissues shows increased glucose consumption level as contrast to the controlled fingerlings muscle (0.131mg/g) and gills (0.085mg/g).

Glycogen: Glycogen content mg/g in the gills and muscle tissue was estimated after exposing the late fingerlings for 24 hours (LC₅₀ of Harpic for 0.06ml/l) (Table 2, Figure 1) in that exposed muscle (0.015mg/g) and gills(0.06mg/g), tissue shows decrease in glycogen consumption level as contrast to the controlled fingerling muscle (0.05mg/g) and gills (0.09mg/g).

Protein: The alteration of protein content in fish in control and exposed trials presented in (Table 2, Figure 1) protein content (mg/g) in the muscle and gills tissue was estimated after exposing the late fingerlings for 24hours(LC₅₀ of Harpic for 0.06ml/l) showed 0.433mg/g and 0.589mg/g respectively. In control fingerlings protein content found in muscles (0.482mg/g) and gills (0.749mg/g).

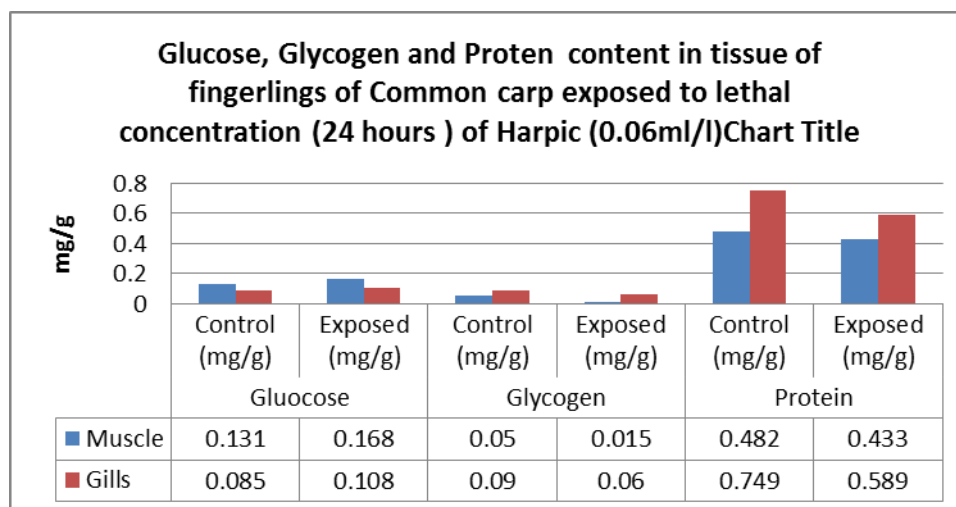


Fig 1. Biochemical Changes in Tissues

Histological Analysis

In the present study the muscle tissue exposed to 24 hr were damaged. In treated muscle the nuclear proliferation was observed and also seen different size of the muscle fibers and disintegration of muscle bundles, atrophy of muscle bundles, marked thickening and separation of muscle bundles. Where as in case of control there was no alteration were observed in the histology of muscle (Figure 2).

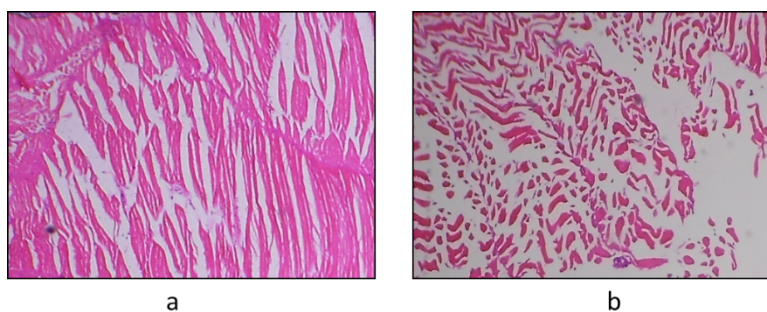


Fig 2. a. Muscle of *Cyprinus carpio* shows no changes (Control), b. Muscle of *Cyprinus carpio* exposed shows Muscle bundle degeneration Analysis of changes in gills

Histological examination gave significant indication of toxicity of Harpic. The effects include gill alteration such as desquamation of the epithelial lining, hemorrhagic and hyperplasia and fusion of the secondary lamellae. While there was no lesion in the control experiment (Figure 3).

4 Discussion

Toxicity Studies

The present studies are in agreement with the following earliest studies reveals that LC_{50} values of the toilet and bathroom cleaner were found that 0.06ml/l of Harpic is toxic to the fresh water fishes *Cyprinus carpio*. It is found that to Harpic the mortality rate increased as the concentration was increased indicating that the effect of Harpic was dose dependent.

The 96 hrs LC_{50} values of Surf Excel detergent to *Catla* and *Rohu* fingerlings were 14.2 and 11.06 ppm, respectively. In another study⁽¹⁸⁾, reported the 96hrs LC_{50} values for three different detergents namely Surf, Besto and Key as 12.7, 77.6 and 32.9 ppm, respectively to *Rasbora elonga*. According to⁽¹⁹⁾ the 24hrs LC_{50} value of a synthetic detergent, Linear Alkylbenzene Sulfonate was 0.5 ppm to *Macrobrachium lamarrei*⁽²⁰⁾ recorded a 96hr LC_{50} value of 400 ppm for wheel detergent to *Lamellidans marginalis* (Lamarck). However,⁽²¹⁾ determined the 96hrs LC_{50} values of the household detergents Det-I and Det-II as 20 and 23.5 ppm, respectively to *Mystus montanus*. One of the important advantages of using histopathological biomarkers in environmental monitoring is that this category of biomarkers allows examining specific

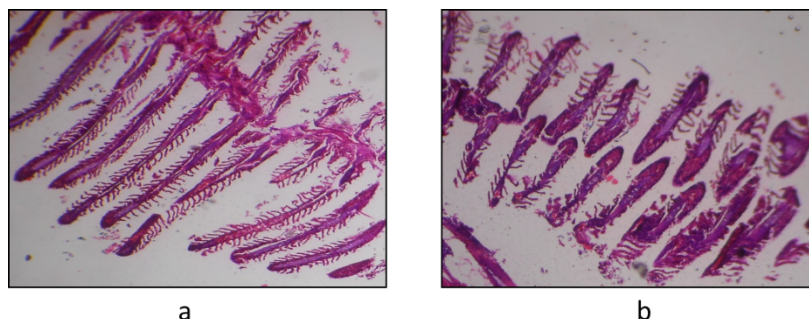


Fig 3. a. Gills of *Cyprinus carpio* Shows nochanges (Control), b. Gills of *Cyprinus carpio* exposed shows desquamationof epithelial lining

target organs, including gills. They are responsible for vital functions such as respiration and regulation of osmotic and ionic balance^(22,23).

Behavioral Studies

The result of the present study clearly reveal that the concentration (0.06ml/l /l) and shorter exposure period (24hours) of Harpic is highly toxic to the fish. The effect of other concentration cannot be ignored. Prolonged exposure to even lower concentration includes behavioral and morphological changes in fishes. These changes and responses indicated stress in fishes which can further lead to death and reduction in fish fauna.

In the present investigation, *Cyprinus carpio* exhibited a variety of behavioral responses like opercular movement was 20-25 times more faster than control, loss of nervous control, try to jump out of media. In dead fishes opercula region becomes blackish, hemorrhaging occurs of gill filaments amongst, along the belly, at the base of pectoral, anal and pelvic fins. Body was slimy due to mucus secretion from epithelium of gills. The fishes were surfacing frequently. Affected fishes were swimming on lateral side of the body; nervous control and equilibrium were lost. The body color of dead fishes turn to yellow. In higher concentrations of cleaner swimming movements of fish immediately slow down with the addition of toxicants. During exposure period, the test fish exhibited several behavioral changes before death such as restlessness, rapid swimming and respiratory distress. Opercula ventilation rate as well as visual examination of dead fish indicates lethal effects of the toilet cleaners on the fish.

The studies of^(24–27) corroborated the present study. These observations however conformed to the submissions of^(21,27–31) that increase in swimming activities with increased breathing rate, lethargic conditions and loss of equilibrium were as a result of disturbances in metabolic reaction resulting in the depletion of energy and higher respiration.

Biochemical Analysis

Glucose

In the present investigation, the exposer of fresh water fishes *Cyprinus carpio* fingerlings to lethal concentration (0.06mg/g) of Harpic have marked metabolic alteration. The total glucose content was increased significantly in gills (0.108mg/g) and muscle (0.168mg/g) tissue sample of exposed fingerlings compare to controlled tissue gills (0.085mg/g) and muscle (0.131mg/g) *Cyprinus carpio* respectively. Increase in the glucose levels during the initial period of exposure indicate the Harpic induced stress and the animal needs more sugar to meet the increased energy demand to mitigate the toxic effect of the Harpic.

Glycogen

In the present observation compare to control fish *Cyprinus* gills (0.09 mg/g) and muscle (0.05mg/g), there was decreased level of glycogen in the tissue of gills (0.06mg/g) and muscle (0.015mg/g) in the fingerlings of *Cyprinus carpio* exposed to lethal concentration of Harpic.

The decreased in the glycogen content in the exposed might have been due to the utilization of reserved glycogen to meet the extra energy demand due to the pollutant stress and also suggest the possible onset of glycogenolysis forming free glucose in the exposed tissue⁽³²⁾. Decreased glycogen synthesis is also attributed to the inhibition of the enzymes glycogen synthetase which mediates glycogen synthesis⁽³³⁾.

Protein

In the present experimental observation, fishes exposed to Harpic has showed decrease tendency in the protein content level in gills (0.589mg/g and) and in muscle (0.433mg/g) when compared to controlled gills (0.749mg/g) and muscle (0.482mg/g) of fishes *Cyprinus carpio* respectively. This indicates that the toxicant inhibits the protein synthesis during the period, the availability of amino acid have become less, that result in the depletion of protein content. The protein and amino acids were decreased gradually compared to control, when the period of exposure increased. The depletion of protein may also attributed to spontaneous utilization of amino acids in various catabolic reactions inside the organisms in order to combat the stress condition. According to Das and Mukherjee, exposure of fishes for long time to most

toxicants interferes with protein metabolism. Decrease in total protein in fish exposed to toxic levels of toxicant could be attributed to either a state of hydration and change in water equilibrium in the fish or a disturbance in liver. Similar findings have been reported in the fish. *Heteropneustes fossilis* exposed to rogor, ⁽³⁴⁾, ⁽³⁵⁾.

Histological Analysis

The treated gills showed damage at the exposure of 24hr and exhibit more destruction of the gill filaments. The gills showed desquamation of the epithelial lining, necrosis (Telangiectasia) of the secondary lamellae, shrinkage of secondary lamellae and also showed hypertrophy and hyperplasia at the base of the secondary lamellae. The histological alterations observed in the present study was in agreement with observation made by ^(36–38).

Under light microscopy, the fishes (normal) not treated with toilet cleaners showed normal anatomical and surface features of gills arches, filaments and lamellae. The gills arch had two rows of gills filaments and row of gill rakers; the former directed towards the opercular opening and the latter towards buccopharyngeal chamber. Two rows of gill filaments were directed posterior-ventrally from each arch arranged in a manner that resemble like a comb. The tips of gill filament formed a gill certain forming sieves for the passage of water passing from buccal chamber to opercular chamber. After exposure of toilet cleaners, sever histopathological changes were observed in the epithelial lining of gill arch, gill rakers and gill filaments of fishes. Fusion of gills lamellae was observed at the tip of gill filament with accumulation of blood cells in capillary and infiltration of blood cell in sub-epithelial space. The secondary lamellae of the basal region of filament were also found fused with each other leaving part free, indicating proliferation (epithelial lifting) of inter-lamellar epithelia within the lamellae. The gill rakers, large empty lymphatic spaces were formed around the central row of pillar cells. The histopathological finding of the present study may be correlated with the observation of many researchers. Similar histopathological changes, including lifting of lamellar epithelium and formation of empty space in gill have been seen earlier by some investigators ⁽³⁹⁾ in fish exposed to some insecticide and chemical substances.

In control muscle has no nuclear proliferation and there is no alternation in muscle. But in treated muscle showed damage at the 24 hr and exhibited more destruction of the muscle fibers. The muscle showed different size of the muscle bundles, marked thickening and separation of muscle bundles, edema between muscle bundles and splitting of muscle fibers. Muscle fiber, disintegration of muscle bundles, atrophy so that alteration of histopathology of muscle at the concentration of 0.06 and 0.03ml/l. The histological alterations observed in the present study was in agreement with observation made by ^(35,37,38). In ⁽³⁹⁾ concluded that all the used concentrations of thiamethoxam negatively affected the histological structure of common carp gills and also activated compensatory-adaptive mechanisms, resulting in pathological changes. These histological lesions affected the gills by disrupting their functions. There was a tendency towards enhancing the morphological alterations and their degree of expression was proportional to the increasing concentration of thiamethoxam.

5 Conclusion

Overall, the study has shown that the Harpic is toxic to *Cyprinus carpio*. Fishes exposed to acute lethal concentrations of the toilet cleaners resulted in significant behavioral, biochemical and histopathological alterations. The glycogen and protein in muscle and gills found minimum compared to control. Where as glucose has shown increasing trend compared to control. These changes suggest that the treated fish are faced serious metabolic crisis. The random damage and structural alterations in gills and muscle in the exposed fishes are indicative of stress mediated production. As The present investigations carried out only for short term exposure (Acute toxicity test) of fish that to confine to the fingerling stage. Further toxicity studies on various life stages (mainly the reproductive stages) have to be studied while exposing the fish to the chronic bioassay. For the comprehensive toxicity evaluation the studies needs to be done on enzyme assay, histochemical changes and gene toxicity. The results clearly indicate that the addition of toilet cleaners to the water body may be threat to aquatic fauna and flora as well as humans. There is a need for preventive measures to be taken in order to avoid the indiscriminate direct discharge of these toilet cleaners into nearby streams and ponds and there is need of development of eco-friendly toilet cleaners so that aquatic fauna of various water bodies will be preserved

References

- 1) Ruiswell RE, Brimblecombe P, Deut DL, Liss PS. Environmental chemistry, the earth-air-water factory. 1992.
- 2) Okpokwasili GO, Nwabuzor CN. Primary biodegradation of anionic surfactants in laundry detergents. *Chemosphere*. 1988;17:2175–2182. Available from: <https://link.springer.com/article/10.1023/A:1005709709031>.
- 3) Farkas A, Salanki J, Specziar A. Relation between growth and the heavy metals concentrations in organs of bream, *Abramis brama* L. populating lake Blaton. *Arch of Environ Contam Toxicol*. 2002;43(2):236–243. Available from: [10.1007/s00244-002-1123-5](https://doi.org/10.1007/s00244-002-1123-5).
- 4) Köck G, Triendl M, Hofer R. Seasonal patterns of metal accumulation in Arctic char (*Salvelinus alpinus*) from an oligotrophic Alpine lake related to temperature. *Canadian Journal of Fisheries and Aquatic Sciences*. 1996;53(4):780–786. Available from: <https://dx.doi.org/10.1139/f95-243>.
- 5) Cavalli LM, Cassani G, Pravettoni S, Nucci O, Larrizaria M. Surfactants in sediments. *Clear Review*. 2000;6:32–43.
- 6) Eniola K, Olayemi AB. Some Aspects of Bacterial-Detergents interaction in fresh water environment. *Bioscience Research communication*. 2002;14(6). Available from: <https://scialert.net/eboardlivedna.php?issn=1994-5426&id=234.3348>.
- 7) Ogundiran M, Fawole O, Adewoye S, Ayandiran T. Toxicological impact of detergent effluent on juvenile of African Catfish (*Clarias gariepinus*) (Buchell 1822). *Agriculture and Biology Journal of North America*. 2010;1(3):330–342. Available from: <https://dx.doi.org/10.5251/abjna.2010.1.3.330.342>.
- 8) O WH. . International programme on chemical safety environment health criterial 169 Linear Alkyl benzene Sulfonates and related compounds. 1996. Available from: https://www.who.int/ipcs/publications/ehc/ehc_numerical/en/.
- 9) Olojo EAA, Olurin KB, Mbaka G, D O. Histopathology of gills and liver tissues of the African catfish *Clarias gariepinus* exposed to lead. *African Journal of Biotechnology*. 2005;4(1):117–122. Available from: <https://tspace.library.utoronto.ca/bitstream/1807/6599/1/jb05022.pdf>.

- 10) Omoniye I, Agbon A, Sa S. Effects of lethal and sub-lethal concentrations of tobacco (Nicotianatobaccum), leaf dustextracutum on weight and haematological changes in Clariassariiepinus. *J Appl Environ Man*. 2002;6(2):37–41. Available from: <https://www.ajol.info/index.php/jasem/article/view/17174>.
- 11) Raisuddin S, Lee JS. Fish Models in Impact Assessment of Carcinogenic Potential of Environmental Chemical Pollutants: An Appraisal of Hermaphroditic Mangrove Killifish *Kryptolebias marmoratus*. Interdisciplinary Studies on Environmental Chemistry-Biological Responses to Chemical Pollutants. Murakami Y, Nakayama K, Kitamura SI, Iwata H, Tanabe S, editors; TERRAPUB. 2008.
- 12) Solbe JF. Freshwater. In: Handbook of Ecotoxicology. Peter Collins. 1995.
- 13) Rahman MZ, Hossain ZM, Ellah MFR, G A. Effect of Diazinon 60Ec on Anabastestudineus, Channapunctatus and Barbadegomonous. *Naga The ICLARM quarterly*. 2002;25:8–112. Available from: <http://aquaticcommons.org/9461/>.
- 14) Roe JH, Carroll N. V The determination of sugar in blood and spinal fluid with anthrone reagent. *J Biol Chemistry*. 1955;212:335–343. Available from: <https://pubmed.ncbi.nlm.nih.gov/13233235/>.
- 15) Carroll NV, Longley R, Roe J. Glycogen determination in liver and muscle by use of anthrone reagent. *Journal of Biol Chemistry*. 1956;22:583–593. Available from: <https://pubmed.ncbi.nlm.nih.gov/13331917/>.
- 16) Lowry O, Rosebrough N, Farr AL, Randall R. Protein measurement with the Follin-phenol reagent. *Journal of Biological Chemistry*. 1951;193(1):265–275. Available from: [https://dx.doi.org/10.1016/s0021-9258\(19\)52451-6](https://dx.doi.org/10.1016/s0021-9258(19)52451-6).
- 17) Litchfield JT, Wilcoxon F. A simple method of evaluating dose effects experiments. *Pharmacol Exp Ther*. 1949;96:99–113. Available from: <https://pubmed.ncbi.nlm.nih.gov/18152921/>.
- 18) Palanichamy R, Murugan V. Acute toxicity of household detergents to a weed fish *Rasbora elongata*. *J Environ Biology*. 1991;12(2):143–148. Available from: <http://www.isca.in/AVFS/Archive/v1/i9/3.ISCA-RJAVFS-2013-052.php>.
- 19) Maruthanayagam C, Ravi N, Arun S, Subramaniam P. Impact of detergent on biochemistry of freshwater prawn *Macrobrachium lamarrei*. *Environment and ecology*. 1997;(3):97–102.
- 20) Shingadia HU, Sakthivel V. Estimation of LC50 values for *Lamellidens marginalis* (Lamarck) with detergent 'Wheel', a component of domestic sewage. *J Aqua Biol*. 2003;18(2):187–189.
- 21) Eknath C. Studies on Toxicity of Detergents to *Mystus montanus* and Change in behaviour of Fish. *Research Journal of Animal, Veterinary and Fishery Sciences*. 2013;1(9):14–19. Available from: <http://www.isca.in/AVFS/Archive/v1/i9/3.ISCA-RJAVFS-2013-052.pdf>.
- 22) Gernhöfer M, Pawet M, Schramm M, Müller E, Triebkorn R. Ultrastructural biomarkers as tools to characterize the health status of fish in contaminated streams. *J Aquat Ecosyst Stress Recovery*. 2001;8:241–260.
- 23) Cengiz EI. Gill and kidney histopathology in the freshwater fish *Cyprinus carpio* after acute exposure to deltamethrin. *Environ Toxicol Pharmacol*. 2006;22:200–204.
- 24) Omeregbe E, Ufodiye EB, Keke IR. Tissue chemistry of *Oreochromis niloticus* exposed to sublethal concentrations of gammaun 20 and actellic 25EC. *Journal of Aquatic Sciences*. 1990;5:33–36.
- 25) Okwuosa V, Omeregbe E. Acute toxicity of alkylbenzenesulphonate (ABS) detergent to the toothed carp, *Aphyosemion gairdneri* (L.). *Aquaculture Research*. 1995;26:755–758. Available from: <https://doi.org/10.1111/j.1365-2109.1995.tb00868.x>.
- 26) Avoajah D, Oti E. Effect of sublethal concentrations of some pesticides on the growth and survival of the fingerlings of the African catfish - *Heteroclinas* (hybrid). *Nigeria Journal of Biotechnology*. 1997;8(1):40–45.
- 27) Adewoye. Haematological and biochemical changes in *Clariassariiepinus* exposed to *Trephosia vogelii* extract. *Applied Science Research*. 2010;1(1):74–79.
- 28) Oti EE. Acute toxicity of gammalin 20 to *Chrysichthys nigrodigitatus* (Lacepede). In: Proceeding of the 16th annual conference of the fisheries society of Nigeria (FISON) Maiduguri. 2005;p. 175–179. Available from: <http://aquaticcommons.org/3615/>.
- 29) Donaldson EM, M DH. Plasma corticosteroid and chloride dynamics in rainbow trout, Atlantic salmon, and lake trout during and after stress. *J. Fish Res Board can*;32:533–539. Available from: <https://scialert.net/fulltext/?doi=jfas.2010.113.119>.
- 30) Shah SL. Behavioural abnormalities of *Cyprinus watsoni* on exposure to copper and zinc. *Turkish Journal of Zoology*. 1975;26.
- 31) Vasantharaja C, Pugazhendy K, Venkatesan S, Meenambal M, Prabhakaran S, Jayachandran K. Acute Toxicity of Cypermethrin and its Impact on Biochemical Alteration in the Fresh Water Fish *Cirrhinus mrigala* (Hamilton) and Protective Effect of *Cardiospermum heliacabum* (Linn). *International Journal of Pharmaceutical & Biological Archives*. 2012;3(1):146–152.
- 32) Veeraiah K, Srinivas, Rao P, Symyuktha R, Dhilleswarao A, H. Changes in biochemical parameters of freshwater fish *Labeo rohita* exposed to lethal and sub-lethal concentrations of *Indoxacarb*. *International Journal of Bioassays*. 2013;(2):1382–1387.
- 33) Borah S, Yadav RNS. Effects of rogor (30% w/v dimethoate) on the activity of lactate dehydrogenase, acid and alkaline phosphatase in the muscle and gill of fresh water fish *Heteropneustes fossilis*. *J Environ Biol*. 1996;17(4):279–283.
- 34) Pechiammalk, Vasanthij E, Joice P. 2015. Available from: <https://ijpr.humanjournals.com/20-4/>.
- 35) Polcksic V, Karan V. Effects of Trifluralin on carp; Biochemical and Histological Evaluation. *Ecotoxicology and environmental safety*. 1999. Available from: [http://203.250.122.194/lecture/p_lecture/specialanat/science-23\(g\).pdf](http://203.250.122.194/lecture/p_lecture/specialanat/science-23(g).pdf).
- 36) Sridhar K, Joice E, P. 2012. Available from: https://www.researchgate.net/profile/Sridhar-Krishnan-4/publication/337908163_MS07-IJALS-.
- 37) Vijayalakshmi M, Sumathy R, Sharmila J. Electrophoretic pattern and Histopathological Study of Muscle Tissue *Tilapia*, *Oreochromis mossambicus*. Exposed to textile dyes. *International Journal of Science and Research*. 2015;p. 2319–2399. Available from: <https://www.ijsr.net/archive/v5i10/ART20162253.pdf>.
- 38) Konar SK. Histopathological Effects of the Insecticides, Heptachlor and Nicotine, on the Gills of the catfish, *Heteropneustes fossilis*. *Japanese Journal of Ichthyology*. 1969;15(4):156–159. Available from: <https://doi.org/10.11369/jji1950.15.156>.
- 39) Georgieva E, Stoyanova S, Velcheva I. Vesela Yancheva Histopathological Alterations in Common Carp (*Cyprinus carpio* L.) Gills Caused by Thiamethoxam 2014. *Braz Arch Biol Technol*. 2014;57(6).