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Histological alterations induced by 4-Nonylphenol in different organs of fish, *Channa punctatus* after acute and sub chronic exposure

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Abstract
The present study was designed to evaluate histopathological effects of 4-nonylphenol (NP), in fish, *Channa punctatus*. ½ of LC_{50} value was selected as a sublethal concentration for acute exposure (96 hours), while for sub chronic exposure safe application rate was calculated and $1/10^{th}$ of it was used for the sub chronic exposure (30, 60 and 90 days). Liver, gill and kidney tissues were analyzed for estimating histological studies. Different histopathological changes after acute exposure like necrosis, vacuolar degeneration, fatty changes and congestion were observed in the liver, while hyperplasia, congestion, degeneration was found in the gill. In the kidney focal area of necrosis, vacuolar degeneration and congestion were seen mainly. On the other hand, during sub chronic exposure gill showed fused secondary lamellae, atrophy in gill filament and telangectasia, while fatty cyst formation and 99% of vacuolar degeneration were seen in hepatocytes. Kidney after sub chronic exposure resulted in pyknosis, atrophy, hypertrophy, hyperplasia, hyperchromic haemopoietic cells, fibrosis and glomular nephritis.

Keywords: 4-nonylphenol, histopathology, acute exposure, sub chronic exposure

1. Introduction
Increased industrialization results into increased environmental pollution and has become a major problem worldwide. The list of endocrine disruptors is extensive with over 800 compounds [1] covering herbicides, plastic contaminants, biocides, personal care products and pharmaceuticals. These come under the priority list of contaminants for which concern have been raised. Among them one EDC is nonylphenol ethoxylate (NPE) and has been found in aquatic environment. NPEs are utilized as raw materials for cleaners, emulsifiers and wetting agents [2]. NPE can enter in to environment through variety of sources such as industrial waste, drain from an urbanized area. Most worrying consequences are its wide use increase possible exposure pathway for humans. It has been shown toxic to fish, amphibian, mollusks [3]. NPE break down to NP and short nonylphenol ethoxylates in the environment after microbial degradation. NP and short chain nonylphenol ethoxylate possess strong lipophilicity, toxicity, cumulative property and estrogenic effect [4].
Biomarkers are interesting tools to study the mechanism of action of toxins and to detect early responses of organism. Histopathological studies give useful data of concerning tissue changes prior to external manifestation and have recently achieved an important place in morpho-toxicology [5]. During contamination process in fish, pollutants cross biological barriers of the gill epithelium and skin for direct route and the wall of the digestive tract for indirect route [6]. They accumulate mainly in metabolically active tissues such as kidney, liver and gill.
One of the great advantages of using histopathological biomarkers in environmental monitoring is that this category of biomarkers allows examining specific target organs, including the gills, kidney and liver, that are responsible for vital functions, such as respiration, excretion and the accumulation and biotransformation of xenobiotics in the fish [7, 8]. Furthermore, the alterations can considered as warning signs of damage to animal health [9] as these easier to identify than functional ones [10].
Aquatic ecosystem is often impacted by chemical pollution originated from consumer products; therefore fish provide an excellent model for monitoring the toxicity in aquatic system because they are extremely sensitive to pollutants [11]. Fish is a suitable indicator for monitoring environmental pollution because they concentrate pollutants in their tissues directly from water and likewise through their diet,

therefore enabling the assessment of transfer of pollutants through the trophic web [12]. *Channa punctatus* is an established vertebrate model in toxicity testing and becoming more popular due to its hardy nature[13]. So the present study is conducted to see the effect of 4-NP on histopathology of fish *C. punctatus*.

2. Material and methods

2.1 Experimental fish specimen and chemicals

Freshwater air-breathing fish *C. punctatus* was procured from local fish market of Amritsar. Study was conducted in 2015. The specimens had an average weight and length of 16.50± 2.14g and 11.40 ± 2.01cm, respectively. Fish specimens were subjected to a prophylactic treatment by bathing twice in 0.05% potassium permanganate (KMnO4) for 2 minutes to avoid any dermal infections. The specimens were then acclimatized for two weeks under laboratory conditions in semi-static systems. They were fed with boiled eggs. The fecal matter and other waste materials were siphoned off daily to reduce the ammonia content in water. 4-nonylphenol used in the present study was obtained from Himedia (India). A stock solution was prepared by dissolving NP in ethanol as a carrier solvent.

2.2 Determination of sub-lethal concentrations

To determine the 96 h LC_{50} value of 4-Nonylphenol, acute toxicity bioassay was conducted in the semi-static system in the laboratory with the change of test solution on every day to maintain the similar concentration of the chemical. This study was conducted under the OECD guideline No. 203 in the semi-static test conditions. In order to eliminate the leaching potential of nonylphenol, plastic material was avoided and glass aquaria of 200 liter capacity were used for the experiment. The 96 h LC_{50} value of 4-nonylphenol was determined as 1.27 mg/l for *C. punctatus* [14], following the probit analysis method as described by [15]. Based on the 96 h LC_{50} value, the two test concentrations of NP viz; sub-lethal concentration I (SL-I; 1/2nd of LC_{50} = 0. 635mg/l) for acute exposure and concentration II (SL-II $1/10^{th}$ of LC_{50}= mg/l) for sub chronic exposure were estimated and used for the *in vivo* experiment.

2.3 *In vivo* exposure experiment

The fish specimens were exposed to the three aforementioned test concentrations of NP in a semi-static system with the change of test water on every day to maintain the concentration of the NP. The exposure was continued up to 96 h (four days) in acute exposure while for 30, 60, and 90 days in sub chronic exposure and tissue sampling was done at the rate of five fish per time interval. The specimens maintained in tap water were considered as negative and in ethanol as positive control. On each sampling day, the liver, kidney and gill cells were collected and immediately processed for MN assay. The physicochemical properties of test water, namely temperature -23.9±0.19, pH -7.4±0.20, dissolved oxygen-3.2±0.30mg/l, total alkalinity-16.65±0.30, free CO_{2-} 8±0. 23mg/l, TDS - 0.4±0.01g/l, TSS - 0.5±0.01, TS- 0.9±0.02g/l were analyzed by standard methods [16].

2.4 Histopathological test-

At the end of exposure for 96 hours for acute and 30, 60 and 90 days for sub chronic, the fish were taken from each replicate. The gill arches of the fish were excised from both sides. Fish were dissected, the abdominal cavity was operated and the liver and kidney were excised quickly and were fixed in 10% formalin buffer as a histological fixative. According to[17], the specimens were processed as usual in the recognized method of dehydration, cleared in xylene and finally embedded in paraffin wax before being sectioned at 5 µm using a rotary microtome. The specimens were stained with hematoxylin and eosin. Finally, the prepared sections were examined and photographed under 10 x and 40x magnifications using a light microscope Olympus CX21.

3. Result and Discussion

Environmental monitoring by using histopathological techniques is having the advantage that it allows us to examine specific organs like liver, gill and kidney which are the vital organs. So in the present study, we studied the histological changes in the liver, gill and kidney. Different kinds of histopathological changes are also observed in liver, gill and kidney cells. Earlier histopathological studies of NP exposed fish were mainly related to alterations in the gonads [18, 19] due to its potent estrogenic properties. Histopathological investigations involving gill, liver and kidney of fish after NP exposure specially sub chronic exposures are very scarce [20, 21, 22]. Histopathological changes has been reported in fish as a result of different kind of exposure [20, 21, 23].

In the present investigation gill showed congestion, cougulative necrosis, hyaline degeneration of muscles in muscularis mucosa and hyperplasia after acute exposure (Plate-2), while sub chronic exposure for 90 days lead to fusion of secondary lamellae, atrophy, hyperplasia, underdeveloped filament, typical congestion and telangectasia (Plate-5). Lamellar fusion is one of protective mechanism which decreases the area of the gill. Secondary lamellae fusion and hyperplasia are the defensive mechanisms as both these changes lead to formation of barrier between blood and the contaminants, as well as diminish the amount of vulnerable gill surface, but this may result in respiratory failure and problem with ionic and acid base balance [24]. pointed out that change in the gill epithelium cell such as cell hypertrophy, cell proliferation and epithelial lifting may represent a defense response. These changes increase the distance across which water borne irritants must diffuse to reach the blood stream. Hyperplasia of gill epithelial cells was also noticed in the gill of fish exposed to the Porland cement powder in solution after 96 h of exposure [22, 23]**.** reported necrosis and hyperplasia of epithelial cells in the gills of *G. affinis* after exposure to Deltamathrin [26]. observed hyperplasia in gill epithelial cells of fish *O. niloticus* after exposure to lead acetate. Similarly [27] reported hyperplasia in gill epithelial cells in rainbow trout after exposure to nanoparticles. After long term exposure telangiectasia was observed in gill tissue [28]. Similar to this [29] visualized hyperplasia, vacuolization and telangiectasia in *C. mrigala* after exposure to pesticide lambda-cyhalothrin. Further [30] observed congestion, telangiectasia and hyperplasia in gill of fish *Hydrocynus vittatus* after treatment with DDT [31]. found necrosis, vacuolar degeneration, fusion and atrophy of primary and secondary gill lamellae.

In case of liver both acute and sub chronic exposure lead to variable size fat vacuoles, necrosis of hepatocytes, vacuolar degeneration and fatty changes (Plate-1 & 4). Mild congestion is also seen in sinocytes. The vacuolization of hepatocytes might indicate an imbalance between the rate of synthesis of substances in the parenchymal cells and the rate of their release into the systemic circulation [32]. Moderate fatty vacuolation of the hepatocytes in monosex Nile tilapia exposed to deltamethrin was observed by [33]. Vacuolization of

the hepatocytes is described as a signal of the degenerative process by[34]. [35]described it as a cellular defense mechanism against chemical which are injurious to hepatocytes and may result into collecting the injurious element and preventing them from interfering with the biological activities. Vacuolization is the result of inhibition of protein synthesis, microtublues disintegration and energy depletion [36 37]. have found cytoplasmic vacuoles in hepatocytes of *Trichogaster trichopterus* after exposure to paraquat. The large vacuole forces the nuclei to the periphery. A similar change was observed by [38] in fish *O. niloticus* when exposed to ^{60}Co gamma radiation.

Depletion of the glycogen in the hepatocytes is usually found in stressed animals [39], because the glycogen acts as a reserve of glucose to supply the higher energy demand occurring in stressed situations [40]. The fatty degenerative changes seen in liver cells may be due to a decrease in the rate of utilization of energy reserve or pathological enhanced synthesis [41 42]. observed inflammation, central necrosis and cell degeneration of liver tissue of *O. aureus* juveniles after treating them to phenol [43]. reported that the hepatic parenchyma of fish exposed to copper showed cytoplasmic vacuolation and hepatocellular necrosis. Degeneration in liver tissue and necrosis of central vein, necrosis in kidney tubules in Goldfish (*C. auratus*) due to chromium exposure was found by [44]. Fatty changes degeneration and necrosis were seen in the liver of *C. carpio* in response to chromium. Swelling and vacuolation of hepatocytes and shifting of nucleus toward one side along with mild inflammation is reported in fish *Esomus danricus* exposed to malathion.

After acute exposure of fish to 4-NP, kidney cells exhibited focal area of necrosis, vacuolar degenerations and congestion (Plate-3). But sub chronic exposure (Plate-6) leads to great renal toxicity and show hypertrophy of haemopoietic tissue (a), necrosis, nephritis (e), hemorrhage (c), hyperplasia, inflammation, pyknosis, atrophy, vacuolar degeneration (f) congestion and fibrosis (e). With increase in concentration of NP, congestion, vacuolar degeneration in tubular epithelium and focal area of necrosis are also observed. Necrosis implies premature cell death due to the exposure of pollutants and leads to degeneration. Necrosis in kidney leads to affect the haemopoietic machinery [45]. observed tubular necrosis in the kidney cells exposed to herbicide and emphasized it to be an indicator of renal toxicity. Hypertrophy reduces the interlamellar space and leads to complete lamellar fusion which reduces the surface area, but if the pollutant exposure last for longer time it can lead to hemorrhagic foci. Tubular nephritis and hyperplasia in epithelial cells of the kidney were observed by [46] in fish *C. gariepinus* exposed to sniper 1000EC [46]. observed tubular necrosis, pyknotic nuclei, coaugulative necrosis, and renal tubular degeneration in kidney of rainbow trout after exposure to maneb and carbaryl [48]. observed hypertrophy of haemopoietic tissue, necrosis, pyknotic nuclei and tubular necrosis in the kidney cells of fish *Rasbora daniconius* when exposed to paper mill effluent. Chromium is reported to induce necrosis, atrophy and pyknotic nuclei in fish *C. carpio* [29]. Nephrotoxicity observed in the present study after subchronic exposure is much more extensive then the hepatotoxicity, indicating that 4-NP have more severe effect on kidney cells in long term exposure and it may impose a negative impact on the ability of kidney to perform osmoregulation and excretion. Similar results were obtained by [49] in Zebra fish after 4-NP exposure.

During the present study different kinds of histopathological changes are observed in the gill, liver and kidney of *C. punctatus* after exposure to nonionic surfactant 4-nonylphenol. The surfactants are known to exert antimicrobial activity by binding to various proteins and phospholipids of membranes. Binding leads to increase in the permeability of membranes. And the formation of vesicles, causing leakage of compounds with low molecular mass. This results in cell death due to the damage through loss of ions or amino acids [50]. Increased ion permeability and sodium efflux of gill has been reported for rainbow trout exposed to 4-nonylphenol [51]. NP found to adversely affect the active transport of calcium to sarcopalsmic reticulum and cause cell death [52, 21]. have also demonstrated that observed necrosis and a decrease in cell membrane permeability along with vocalization in liver of rainbow trout exposed to NP is mediated by inhibition of calcium pump. Similar results were found by Bhattacharya *et al*. (2008) on fish, rosy barb after the exposure to NP. Further, [53] revealed that hepatocytes of fish *O. mossambicus* after exposure to 4-NP show vacuolization, necrosis and decrease in the number of nuclei.

Table 1: Histopathological changes in gill, liver and kidney tissues after acute and sub chronic exposure of 4-NP

Tissue	96 hours (Acute exposure)	30 Days	60 Days	90 Days
Gill	• Hyperplasia • Cogulative necrosis • Congestion • Vacuolar degeneration • Necrosis in gill arches • Necrosis in secondary lamellae	• Congestion of blood vessels • Focal area of fused secondary lamellae • Cogulative necrosis • Atrophy of gill filaments • Hyperplasia of chloride cells • Underdeveloped filaments	• Fusion of secondary lamellae • Congestion of blood vessels • Cogulative necrosis at the tip of primary lamellae	• All the secondary lamellae got fused • Typical congestion (90%) of gill • Telangectasia
Liver	Severe hydrophic degeneration (99%cells effected Focal area of cogulative necrosis Fatty changes Severe congestion Fatty liver leads to the formation of fatty cyst	Mild to moderate fatty changes Focal area of cogulative necrosis	Mild to severe fatty changes (almost all cells affected) Focal area of cogulative necrosis	Severe fatty changes Variable size of vacuole in hepatocytes (pull the nucleus towards side) Fatty cyst formation Vacuolar degeneration (99% part affected)
Kidney	Deformation of kidney	Messenglio proliferative	Hypertrophy of glomerular	Severe hemorrhage

	tubules Cellular swelling Vacuolar degeneration necrosis	glomerulo nephritis Mild inflammation Hyperplasia and hypertrophy of haemopoiteic tissue Degeneration and necrosis in renal tubules Pyknosis Atrophy Hypertrophy of messenglial cells	messenglial cells Focal area of tubular necrosis Hypertophy and hyperplasia in haemopoitic cells Vacuolar degeneration Messenglio proliferative glomerulo nephritis Atrophy of haemopoitic cells Haemopoitic cells become hyperchromic, large nucleus size and irregular cell wall	Focal area of necrosis Degeneration Congestion Tubular necrosis Glomerular nephritis Focal area of interstitial nephritis Proliferation of messenglial cells Fibrosis

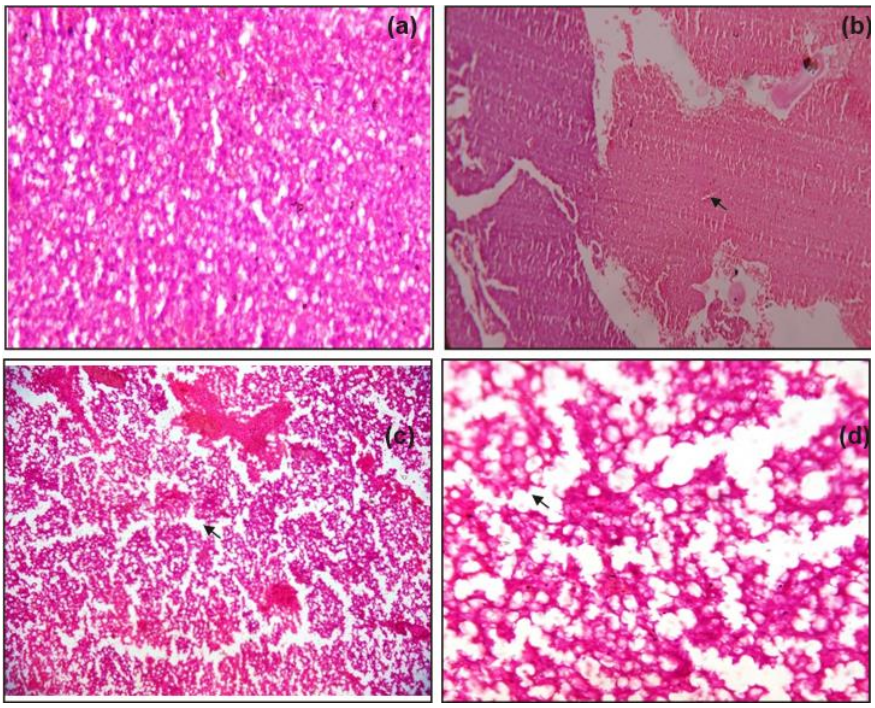

Plate 1: Photomicrographs (a-d) showing sections of liver (a) hepatocytes in control (b) necrosis (c) vacuolar degeneration (d) formation of fatty cyst

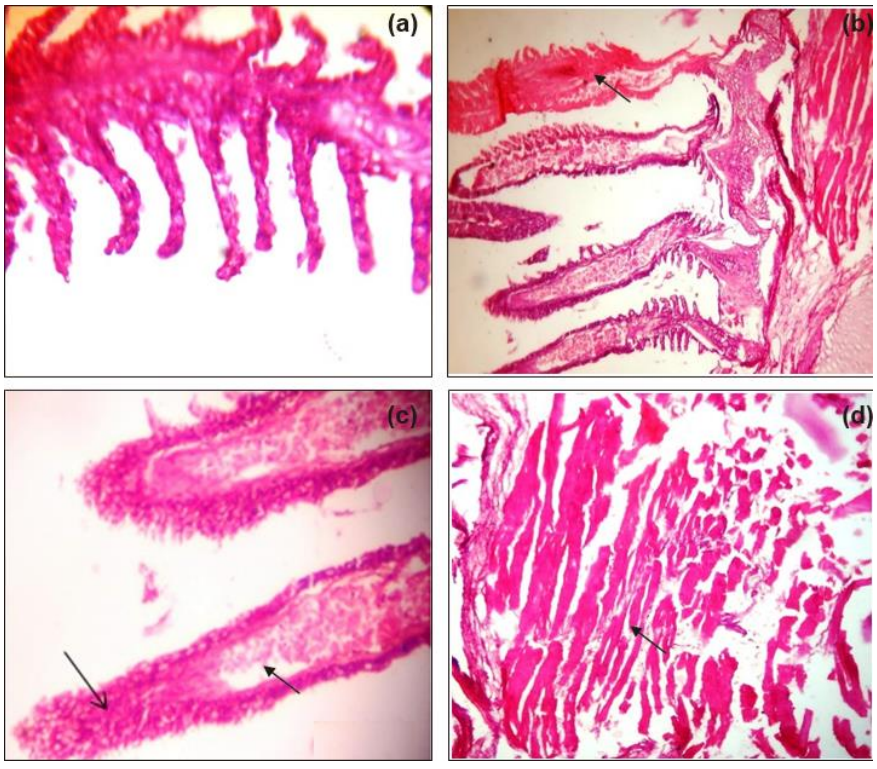

Plate 2: Photomicrographs (a-d) showing sections of gill (a) gill filament of control (b) necrosis (c) hyperplasia and congestion (d) degeneration of muscles

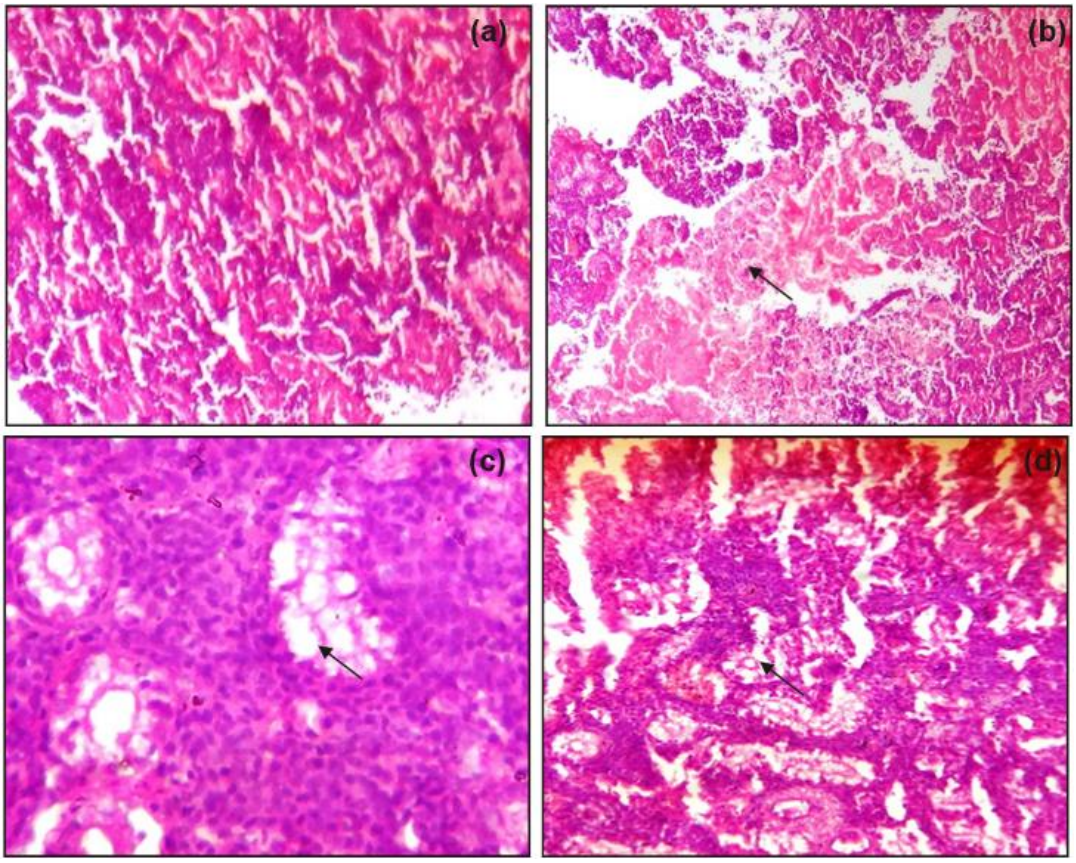

Plate 3: Photomicrographs (a-d) showing sections of kidney (a) control kidney (b) necrosis (c and d) tubular degeneration

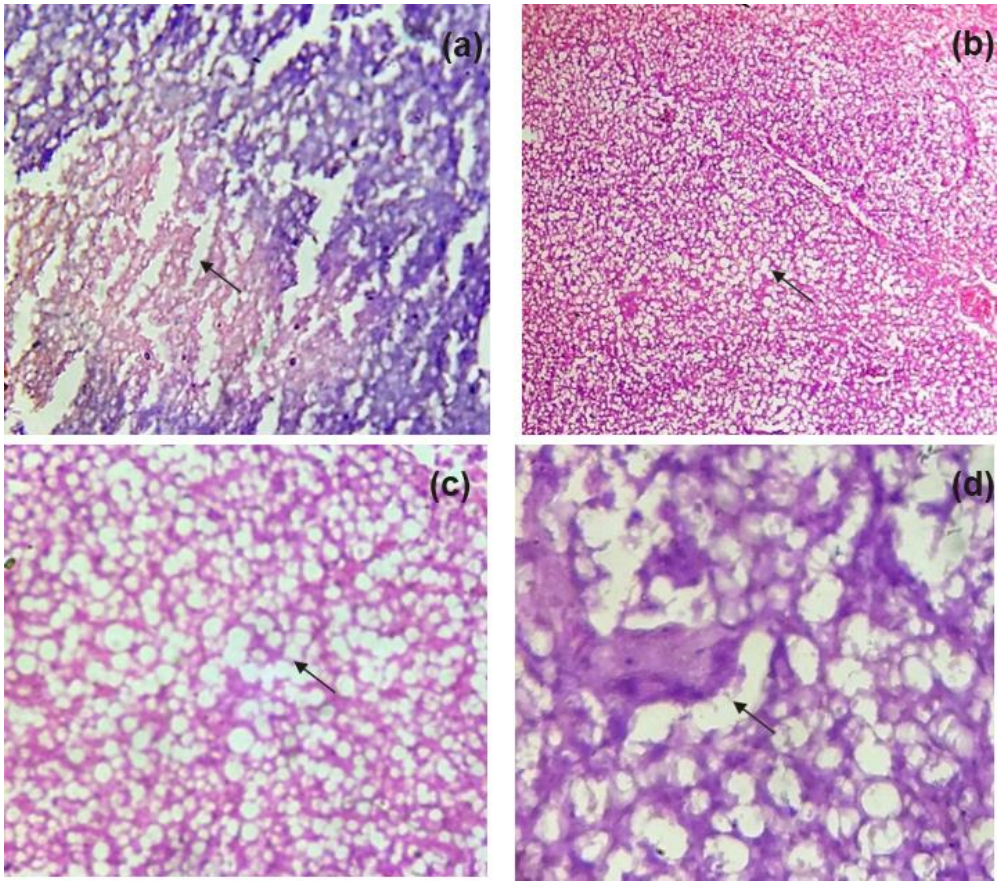

Plate 4: Photomicrographs (a-d) showing sections of liver of *C. punctatus* after sub chronic exposure (a) coagulative necrosis (b) mild fatty changes (c) severe fatty changes (d) fatty cyst formation

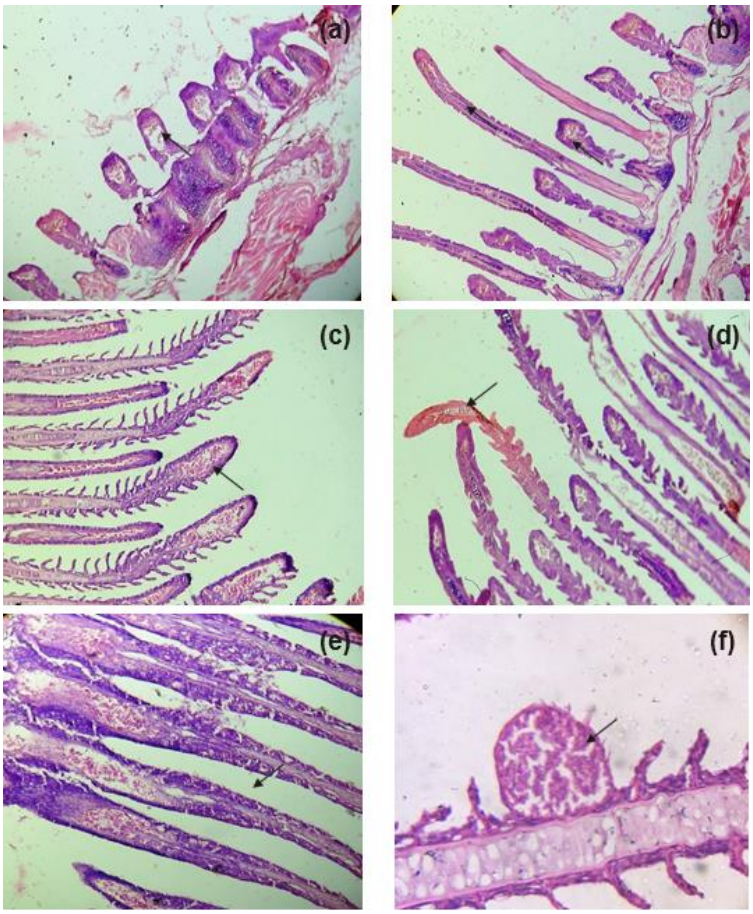

Plate 5: Photomicrographs (a-f) showing sections of gill of *C. punctatus* after subchronic exposure. (a and b) atrophy in gill filaments and underdeveloped gill filaments (c) typical congestion of blood vessels in gill (d) coagulative necrosis at the tip of primary lamalle (e) fused secondary lamellae (f) telangectasia

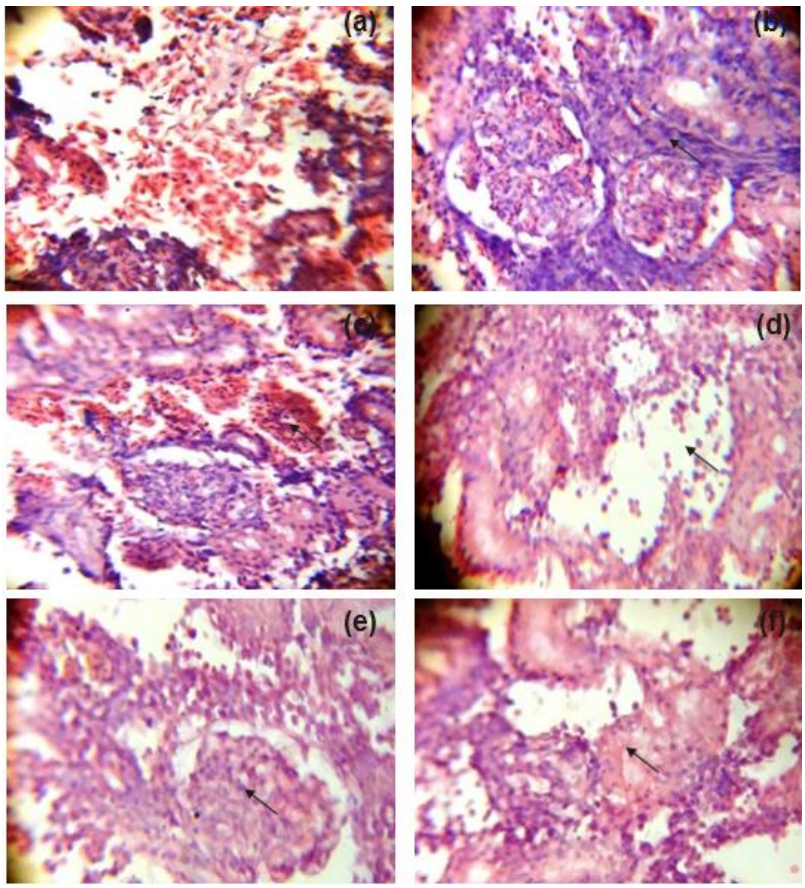

Plate 6: Photomicrographs (a-f) showing sections of kidney of *C. punctatus* after subchronic exposure. (a) hypertrophy of haemopoiteic tissue (b) fibrosis (c) severe hemorrhage (d) degeneration of haemopoietic tissue (e) nephritis (f) tubular degeneration

4. Conclusion

Conclusively our study indicated that structural modifications found in this study are a result of acute damage associated with short term exposure and chronic response due to long term exposure to 4-NP. Liver, gill and kidney were found to be sensitive tissues. Nephrotoxicity was found to be more comparative to other tissues.

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6. References

1. Bergman A, Rydén A, Law RJ, de Boer J, Covaci A, Alaee M, et al. A novel abbreviation standard for organobromine, organochlorine and organophosphorus flame retardants and some characteristics of the chemicals. Environment International. 2012; 49:57-82.
2. Sharma M, Chadha P. Toxicity of non-ionic surfactant 4-nonylphenol an endocrine disruptor: A review. International Journal of Fisheries and Aquatic Studies. 2018; 6(2):190-197.
3. Sharma M, Chadha P. Study on DNA damaging effects of 4-nonylphenol using erythrocytes from peripheral circulation, gill and kidney of fish *Channa punctatus*. Journal of Environmental Biology. 2016; 37(2):313-318.
4. Sharma M, Chadha P. Widely used non-inonic surfactant 4-nonylphenol: showing genotoxic effects in various tissues of *Channa punctatus.* Environmental Science and Pollution Research, 2017. DOI 10.1007/s11356-017-8759
5. Lamastra L, Suciu NA, Trevisan M. Sewage sludge for sustainable agriculture: contaminants' contents and potential use as fertilizer**.** Chemical and Biological Technologies in Agriculture. 2018; 5:10
6. Lloyd R. Pollution and freshwater fish. The Buckland foundation. Fishing News Books, Oxford, Luna, C. G. 1968. Manual of Histologic Staining. 1992; 35-40, 77-81, 107-110, 122-124.
7. Sachar A, Raina S, Gupta K. Stress of inorganic pollutant (Nitrate) induced histopathological alterations in haemeopoetic tissues of *Aspidoparia morar*. International Journal of Fisheries and Aquatic Studies. 2015; 2(4):07-11.
8. Ubong G, Ini-Ibehe N, Etim MP, Akpan E, Monica K. Acute Toxic Effects of *Hevea brasiliensis* on the Gills of Hatchery Reared *Oreochromis niloticus* Fingerlings. Journal of Academia and Industrial Research. 2015; 3(11):562-566.
9. Fanta E, Rios FS, Romao S, Vianna ACC, Freiberger S. Histopathology of the fish paleatus contaminated with sublethal levels of organophosphorus in water and food. Ecotoxicology and Environmental Safely. 2003; 54:119-130.
10. Hinton DE, Lauren DJ. Liver structural alterations accompanying chronic toxicity in fishes: potential biomarkers of exposure. 1990; 17-57.
11. Sharma M, Chadha P. Acute toxicity of 4-nonylphenol on haematological profile of fresh water fish *Channa punctatus*. Research Journal of Recent Sciences. 2015; 4:25-31
12. Fisk AT, Holst M, Hobson KA, Duffe J, Moisey J, Norstrom RJ. Persistent organochlorine contaminants and enantiomeric signatures of chiral pollutants in ringed seal (*Phaco hipida*) collected on the east and west side of Northwater Polynya, Canadian Arctic. Archive of Environmental Contamination and Toxicology. 2002; 42:118-126.
13. Sharma M, Chadha P. 4-Nonylphenol induced DNA damage and repair in fish, *Channa punctatus* after sub chronic exposure. Drug and Chemical Toxicology. 2016. DOI: 10.1080/01480545.2016.1223096
14. Sharma M, Chadha P, Sharma S. Acute and subchronic exposure of 4-nonylphenol to freshwater fish *Channa punctatus* to evaluate its cytotoxicity. Biochemical and Cellular Archives. 2014; 14(2):363-367.
15. Finney DJ. Probit Analysis. Cambridge University Press, Cambridge. 1971, 333.
16. AWWA, APHA, WPCF. Standard methods for the examination of water and wastewater, 21st ed. Washington, (DC): American Publication of Health Association, 2005.
17. Humason GL. Animal Tissue Techniques. 2nd Edn., W.H. Freeman and Co., San Francisco, 1967, 569.
18. Jobling S, Sheahan D, Osborne JA, Matthiessen P, Sumpter JP. Inhibition of testicular growth in rainbow trout (*Oncorhynchus mykiss*) exposed to estrogenic alkylphenolic chemicals. Environmental Toxicology Chem. 1996; 15(2):194-202.
19. Miles-Richardson SR, Pierens SL, Nichol KM, Kramer VJ, Snyder EM, Snyder SA, et al. Effects of waterborne exposure to 4-nonylphenol and nonylphenol ethoxylates on secondary sex characteristics and gonads of fathered minnows (*Pimephales promelas*). Environmental Research**.** 1999; 80(2):S122-S137.
20. Bhattacharya H, Xiao Q, Lun L. Toxicity studies of nonylphenol on rosy barb (*Punitius conchronious*): a biochemical and histopathological evaluation. Tissue and Cell**.** 2008; 40:243-249.
21. Sayed AEH, Hakeem SSA, Mahmoud UM, Mekkawy IA. 4-Nonylphenol induced morphological and histopathological malformations in *Bufo regularis* tadpoles. Global Advanced Research Journal of Environmental Science and Toxicology**.** 2012; 1(6):143-151.
22. Uguz C, Iscan M, Togan I. Developmental genetics and physiology of sex differentiation in vertabrates. Envir0onmental Toxicology and Pharmacology. 2003; 14(1-2):9-16.
23. Cengiz EI. Gill and kidney histopathology in the fresh water fish *Cyprinus carpio* after acute exposure to delmethrin. Environmental toxicology and pharmacology**.** 2006; 22:200-204.
24. Mallatt J. Fish gill structural changes induced by toxicants and other irritants. A statistical review. Canadian Journal of Fisheries and Aquatic Sciences**.** 1985, 42**:**630-648.
25. Adamu KM, Audu BS, Audu LE. Toxicity and histopathological effects of Portland cement power in solution on the structure of the gill and liver tissues of the Nile tilapia, *Oreochromis niloticus;* A microscopic study. Tropical Freshwater Bio**.** 2008, 17(1):00-00.
26. Doaa M, Hanan H, Abd-Elhafeez. Histological changes in selected organs of *Oreochromis niloticus* exposed to doses of lead acetate. Journal of Life Science Biomed**.** 2013; 3(3):256-263.
27. Monfared AL, Soltani S. Histological, Histometric and biochemical alterations of the gill and kidney of rainbow trout (*Oncorhynchus mykiss*) exposed to silver nanoparticles. European Journal of Experimental Biology**.** 2013; 3(2):391-395.
28. Van Dyk JC, Marchand MJ, Smit NJ, Pieterse GM. A

histology based fish health assessment of four commercially and ecologically important species from Okavango Delta panhandle, Botswana. African Journal of Aquatic Sciences. 2009; 34(3):273-282.
29. Velmurugan B, Selvanayaga M, Cengiz E, Unlu E. Histopathological changes in the gill and liver tissues of freshwater fish, *Cirrhinus mrigala* exposed to dichlorvos. Brazil Archives of Biology and Technology. 2009; 52(5):1291-1296.
30. McHugh KJ, Smit NJ, Van Vuren JHJ, Van Dyk JC, Bervoets L, Covaci A, et al. A histology-based fish health assessment of the tigerfish, *Hydrocynus vittatus* from a DDT-affected area. Physics and Chemistry of the Earth. 2011; 36(14):895-904.
31. Butchiram MS, Tilak KS, Raju PW. Studies on histopathological changes in the gill, liver and kidney of *Channa punctatus* (Bloch) exposed to Alachlor. Journal of Environmental Biology. 2009; 30**(**2):303-306.
32. Gingerich WH. Hepatic toxicology of fishes. In: Aquatic toxicology (LJ Weber, Ed), 55-105. Raven Press, New York, 1982.
33. El-Sayed YS, Saad TT. Sub acute Intoxication of Deltamethrin based prepration (Botox 5% EC) in Monosex Nile Tilapia, *Oreochromis niloticus* L. Basic and clinical Pharmacology and Toxicology. 2008; 102(3):293-299.
34. Pacheco M, Santos MA. Biotransformation, genotoxic and histopathological effects of environmental contaminants in European eel, *Anguilla anguilla*. Ecotoxicology and Environmental Safety. 2002; 53:331-347.
35. Mollendroff. Cytology and cell physiology. 3rd ed. Academic press. New York. 1973, 50-55.
36. Hinton DE, Lauren DJ. Integrative histopathological approaches to detecting effects of environmental stressors on fish. American Fisheries Society Symposium Series**.** 1990; 8:51-66.
37. Banaee M, Davoodi MH, Zoheiri F. Histopathological changes induced by paraquat on some tissues of gourami fish (*Trichogaster trichopterus*). Open Veterinary Journal. 2013; 3(1): 36-42.
38. Bukhari S, Mohameda AS, Broos HE, Stalin A, Singhal RK, Venubabu P. Histological variations in liver of freshwater fish *Oreochromis mossambicus* exposed to 60Co gamma irradiation. Journal of Environmental Radioactivity**.** 2012; 113:57-62.
39. Wilhelm Filho D, Torres MA, Tribess,TB, Pedrosa RC, Soares CHL. Influence of season and pollution on the antioxidant defenses of the cichlid fish acará (*Geophagus brasiliensis*). Brazilian Journal of Medical and Biological Research. 2010; 34:719-726.
40. Panepucci RA, Panepucci L, Fernandes MN, Sanchez JR, Rantin FT. The effect of hypoxia and recuperation on carbohydrate metabolism in pacu (*Piaractus mesopotamicus*). Brazilian Journal of Biological Sciences. 2001; 61:547-554.
41. Desai ID. Vitamin E analysis method for animal tissues. In: Methods of enzymology. Ed.: L. Packer. 1984; 105:138.
42. Abdel-Hameid NH. Physiological and Histopathological Alterations Induced by Phenol Exposure in *Oreochromis aureus* Juveniles. Turkish Journal of Fisheries and Aquatic Sciences. 2007; 7: 131-138.
43. Figueiredo-Fernandes A, Ferreira-Cardoso JV, Garcia-Santos S, Monteiro SM, Carrola J, Matos P, Fontaínhas-Fernandes A. Histopathological changes in liver and gill epithelium of Nile tilapia, *Oreochromis niloticus* exposed to waterborne copper. Pesq. Vet. Bras. 2007**;** 27(3):103-109.
44. Velma V, Tchounwou PB. Chromium induced biochemical, genotoxic and histopathologic effects in liver and kidney of Goldfish Carassius auratus. Mutation Research**,** 2010; 30:43-51.
45. Jiraungkoorskul W, Upatham E, Kruatrachue M, Sahaphong S, Vichasri-Grams S, Pokethitiyook P. Biochemical and histopathological effects of glyphosate herbicide on Nile tilapia (*Oreochromis niloticus*). Environmental Toxicology**.** 2003; 18(4):260-267.
46. Abubakar, Idi-ogede M. Toxicity of sniper 1000ec on respiratory dynamics of *Oreochromis niloticus* (Trewavas, 1983) under laboratory conditions. Journal Science Multidisciplinary Research. 2014; 6(1):58-63.
47. Boran H, Altinok I, Capkin E. Histopathological changes induced by maneb and carbaryl on some tissues of rainbow trout, *Oncorhynchus mykiss*. Tissue and Cell. 2010; 42(3):158-164.
48. Pathan TS, Thete PB, Shinde SE, Sonawane DL, Khillare YK. Histopathological changes in the gill of freshwater fish, *Rasbora daniconius* exposed to paper mill effluent. Iranian Journal of energy and Environment. 2010; 1(3):170-175.
49. Weber DN, Spieler RE. Behavioral mechanisms of metal toxicity in fishes. In: Malins, D.C., Ostrander, G.K. (Eds.), Aquatic Toxicology: Molecular, Biochemical and Cellular Perspectives. CRC Press, London, UK. 1994, 421-467.
50. Cserhati T. Alkyl ethoxylated and alkylphenol ethoxylated nonionic surfactants: Interaction with bioactive compounds and biological effects. Environmental Health Perspectives**.** 1995; 103: 358- 64.
51. Part P, Svanberg O, Begstrom E. The influence of surfactants on gill physiology and cadmium uptake in prefused rainbow trout gill. Ecotoxicology and Environmental Safety**.** 1985; 9:135-144.
52. Kirk CJ, Bottomely L, Minican N, Carpenter H, Shaw S, Kohli N *et al*. Environmental endocrine disrupters dysregulate estrogen metabolism and Ca2+ homeostasis in fish and mammals via receptor-independent mechanism. Comparitive biochemistry and physiology Part A-Molecular and Intergrative Physiology**.** 2003; 135:1-8.
53. Midhila EM, Chitra KC. Nonylphenol-induced hepatotoxicity in the freshwater fish, *Oreochromis mossambicus*. International Journal of Science and Research Publication. 2015; 5(3):1-5.