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THE TOXIC EFFECT OF HEAVY METALS (NICKEL, COPPER AND MIXTURE) ON HISTOLOGICAL PARAMETERS OF THE COMMON CARP FINGERLINGS) *CYPRINUS CARPIO*

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Abstract

The fingerlings of *Cyprinus carpio* exposed to heavy metals Nickel, Copper and their mixture with reference to histopathological changes were observed. Histopathological observation were made in liver, stomach and gonad (ovary). The tissues of the fingerlings treated with the mixture showed remarkable changes in the structure when compared with the individual metal Nickel and Copper treatments. In all the above mentioned parameters, mixture treatment was more effective that too in the 30 days exposure than individual metal treatments. So the following sequence of toxicity is inferred from the present study in all the periods of exposure. Mixture> Copper> Nickel and the joint action of the metals is synergistic. This indicates Nickel and copper acted synergistically at sublethal levels in combination.

Key words: Nickel, Copper, Mixture, *Cyprinus carpio,* Liver, Stomach and Gonad.

Introduction

Heavy metals are considered as most dangerous pollutants because of their unbiodegradability and persistent nature (Akpor *et al*., 2014; Singh *et al*., 2011). Contamination with metals from mining and smelting operations poses serious threats to aquatic environments because of its toxicity, persistence, bioaccumulation and biomagnification in the foodchain (Mohamaddi *et al*. 2011). Copper is a significant trace element necessary for the normal growth and metabolism of living organisms. However, this element may become very dangerous if used beyond its limit, turning into continuous metal compounds with the ability to accumulate in water and cause imbalance to the biological system. Aquaculture activities can also be affected due to the increase in environmental pollution. Copper is observed with the ability to cause some deleterious effects on fish by its toxicity, which can be evaluated from the molecular and structural level of the organism. This is because fish is one of the aquatic organisms that are able to accumulate heavy metals in their tissue (Padrilah *et al*., 2018). Copper in a toxic form might serve as a stressor agent for fish that can inhibit several biological functions and cause some histopathological alterations (Sabullah *et al.,* 2014). These histopathological alterations are then used to indicate the condition of the environment and represent time-integrated endogenous and exogenous impact on the organism stemming from alterations at the lower level of biological organisation (Paulo *et al*., 2012). Histopathological changes in animal tissue especially fish are powerful indicators for prior exposure of aquatic environmental stressors. Various studies have been conducted using the histopathology of fish organs as a biomarker to indicate water quality. A study on histopathological effects of the acute toxicity level of copper sulphate on *Puntius conchonius* revealed that metallic salts are capable of producing severe damage in the gills and necrotic changes in the liver and kidney (Pant *et al*., 1980). The histopathology study involves cellular biomarkers, which can provide a better indication of the health of an organism and has also been proven to be a cost-effective tool for determining the health of fish population and reflecting the health of the entire aquatic ecosystem (Devi and Mishra, 2013). *Oreochromis mossambicus* exposed to sublethal Cr showed histological alterations in the liver (congestion of blood vessels; fat accumulation; increase in melano-macrophage centre's and necrosis), gills (hyperplasia of primary lamellar epithelium), ovaries (deposits in interstitial tissue) and testes (hypertrophy and vacuolation of spermatocytes)

(Ackermann C, 2008). Histological abnormalities in ovaries may be caused by several factors such as parasitic infections, mechanical injuries, toxic effluents and aquatic pollutants. Effect of all these factors on fish health leads to liquification of perinucleolar cytoplasm and condensation of nucleus, disappearance of nuclear membrane, cytoplasmic clumping and Atretic oocytes (Sakthival and Gaikwad, 2001; Abou-Seedo *et al*., 2003; Deshmukh and Kulkarni, 2005; Olfat and El-Greisy, 2007). Nickel poisoning in fishes include decreased concentrations of glycogenin muscle and liver with simultaneous increases inlevels of lactic acid and glucose in blood (Ghazaly, 1992), depressed hydrogen peroxide production in tissues and a reduction in Superoxide dismutase (Bowser *et al*., 1994) and contractions of vascular smooth muscle-signs similar to those associated with hypertension in mammals (Evans *et al*., 1990). Elenka Georgieva *et al*., (2014) evaluate the current contamination status of Topolnitsa Reservoir by measuring As, Cd, Cu, Ni, Pb and Zn concentrations in surface water samples and in the liver of *S. erythrophthalmus* in three different seasons: spring, summer and autumn. The effluents are often complex mixtures of poisons. If two (or) more poisons are present in an effluent they may exert a combined effects on an organism which is additive. Copper is more than additive with Chlorine, Zinc, Cadmium and Mercury, it decreases the toxicity of Cyanide (Mason, 1987). The main objective of the present histopathological study is to observe cellular changes that occur in the target organs such as liver, stomach and gonad of the heavy metals (Nickel, Copper and their mixture) treated fingerlings *Cyprinus carpio*.

Materials and Methods

Collection and maintenance of the experimental animals

The Commercially important exotic fish *Cyprinus carpio* was used for present studies. Fingerlings were collected from the fishery ponds in and around Chidambaram. The fingerlings were acclimated to the laboratory conditions keeping them in aquaria at room temperature (30°C±1°C). The acclimatization period lasted for 15 days. The fish were fed with feed at 5% of body weight, daily in two split doses, in the morning and evening. Uneaten food was removed from the tank after 24 hours. The feeding was started three days after the fish were stocked and stopped 24 hours prior to experimentation.

Mode of Dilution of heavy metals

Nickel, Copper and mixture were used in this experiments. Desired concentration of this heavy metals were prepared by dilution technique standard method (Apha *et al*., 1975). The sublethal concentration selected were 0.004%, 0.0015% and 0.0005%+0.0017% of Nickel, Copper and mixture on different days of exposure *i.e*, 24hrs, 15 days and 30 days.

Histopathological studies

For normal liver, stomach and gonad were fixed in Bouins fluid. The treated fingerlings to sublethal concentration of Nickel, Copper and mixture after 30 days were sacrified for histopathological studies. The materials were processed and embedded in paraffin wax. The serial sections were 6 to 7µ thickness were taken. Staining were carried out with Harry's alum haematoxylin and aqueous eosin as counter stain. DPX was used as mount, changes in various tissues of *Cyprinus carpio* were observed and compared with the control fish for the determination of histological alterations.

Results

Histopathological studies

• Normal Liver

The outer protective membranous covering is seen intact. The nucleus is found at the center of hepatocytes. The cells are more (or) less spherical in shape and cytoplasm seems to be granular. The nucleus is larger in size when compared with cytoplasmic ratio. (Fig. 1).

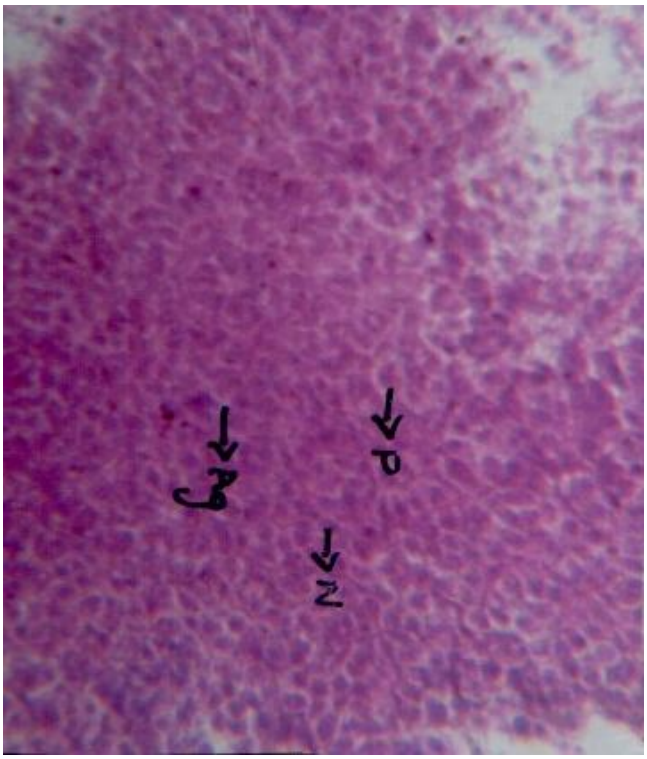

Fig. 1: Normal Liver Structure of *Cyprinus carpio*.
P - Polygonal Hepatocytes, N - Nucleus,
Ag - Agranular Cytoplasm, B - Blood cells

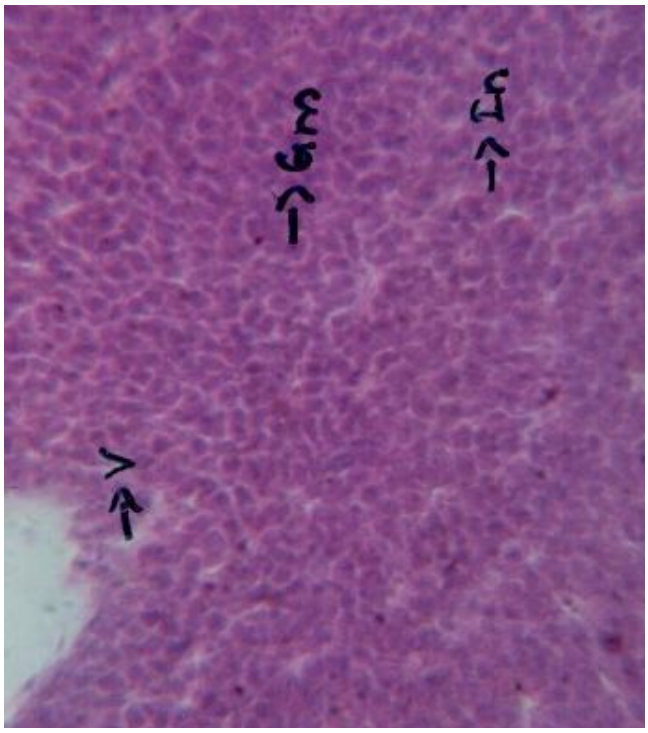

Fig. 2: Changes in liver structure of *Cyprinus carpio* after 30 Day exposure to Nickel Treatment.
V -Vacuoles, In - Irregular nucleus, Gm - Granular material

Histopathological changes in the liver of fingerlings *Cyprinus carpio* treated with sublethal concentration of Nickel.

30-days exposure:

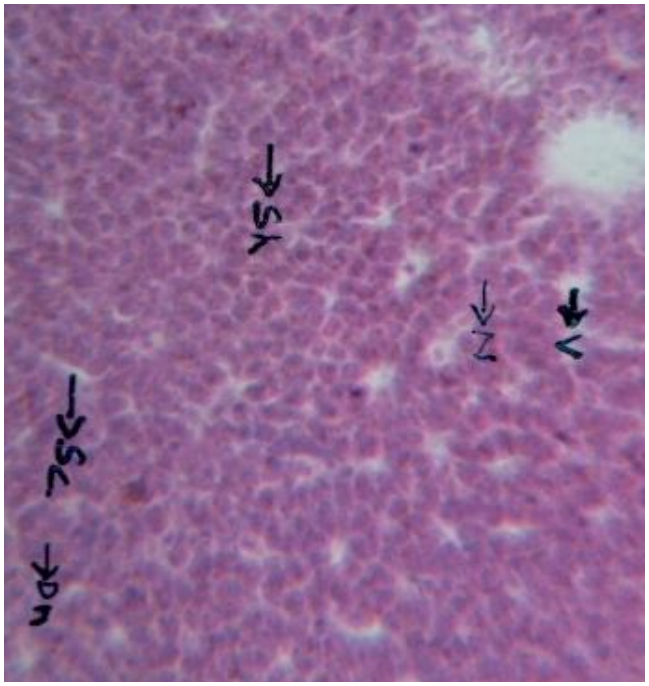

Fig. 3: Changes in liver structure of *Cyprinus carpio* after 30 Day exposure to Copper Treatment.
Sh - Shrinkage hepatic, N - Necrosis, V - Vacuoles, Sc - Splitting cells, Dn - Damaged nuclei, Sn - Swelling of Nucleus.

The liver of fingerlings exposed to sublethal concentration of Nickel revealed comparatively mild histopathological changes, vacuolization and accumulation of granular material. (Fig. 2).

Histopathological changes in the liver of fingerlings *Cyprinus carpio* treated with sublethal concentration of Copper.

30-days exposure:

Fingerlings exposed to sublethal concentration of Copper showed following histopathological changes in the liver. The polygonal shape of the hepatic cells were found to be lost. At certain places necrosis was occurred. Vacuolization and splitting also appeared at some places. Nuclei of the damaged cells got displaced and become irregular in shape. Pyconotic and swelling of nuclei were also observed. (Fig. 3).

Histopathological changes in the liver of fingerlings *Cyprinus carpio* treated with sublethal concentration of bimetal mixture.

30-days exposure:

Mixture of Nickle and Copper causes severe histopathological changes in the liver of *Cyprinus carpio*.

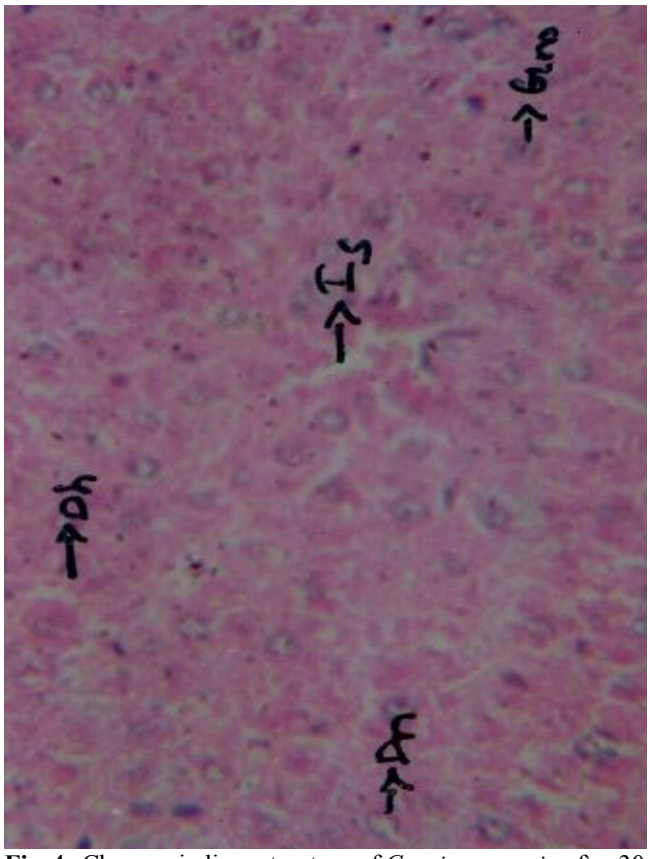

Fig. 4: Changes in liver structure of *Cyprinus carpio* after 30 Day exposure to Mixture Treatment
Dh - Damaged Hepatic cells, Dn - Damaged nuclei, Gm - Granular material, Is - Inter Cellular Space

A remarkable changes were the loss of polygonal shape of hepatic cells and the whole structure formed a compact mass of cells was studied. Vacuolization and splitting appeared at various places was noticed. Nuclei of damaged cells and irregular in shape was observed. Further accumulation of granular material was observed. (Fig. 4).

Normal stomach of *Cyprinus carpio*

The lumen of the stomach was lined by thick protective squamous epithelium. There was a number of division of layers in the stomach such as mucosa, submucosa and muscularis. The mucosa is divided histologically into 3 layers. Epithelial lining, a supporting connective tissue lamina propria and a thin smooth muscle layer the muscularis mucosa which produces local movements and folding of the mucosa. Submucosa layer was compared of loose, connective tissue supported the mucosa and contained the layer blood vessels lymphatics and nerves. It contained small mucous glands. The muscularis layer consisted of smooth muscle which was usually into two histological layers, an inner circular layer and an outer longitudinal layer. (Fig. 5).

Histopathological changes in the stomach of fingerlings *Cyprinus carpio* treated with sublethal concentration of Nickel.

30-days exposure:

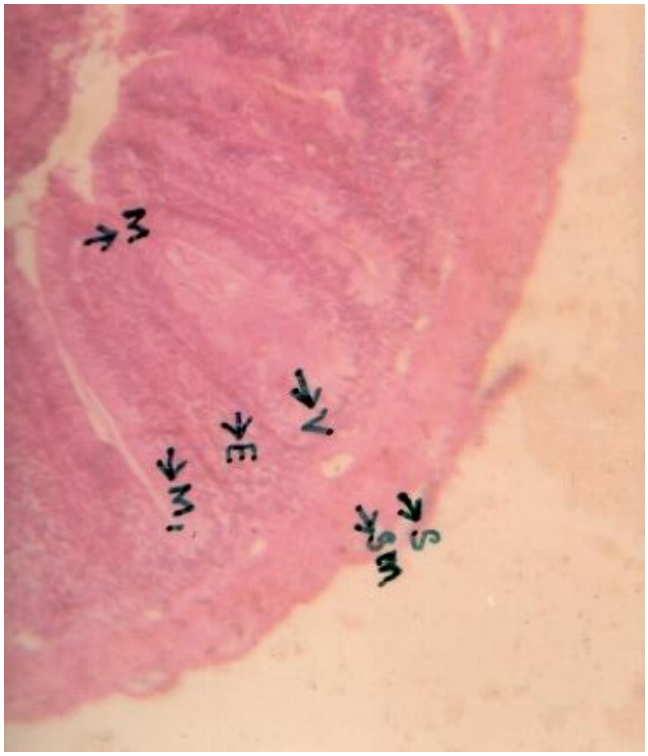

Fig. 5: Normal Stomach Section of *Cyprinus carpio*.
E - Epithelial cell, V - Vili, S - Serosa,
M - Mucosa, Sm - submucosa, M_1 - Muscularis

Nickel treated fingerlings showed a comparatively lesser pathological changes than the other treatments. Fragmentation of epithelial layer and disruption of mucosal layer was observed. Circular muscles were deformed and gave a appearance of group of nuclei was noticed. Vacuoles are seen in muscularis region of the stomach. (Fig. 6).

Histopathological changes in the stomach of fingerlings *Cyprinus carpio* treated with sublethal concentration of Copper.

30-days exposure:

Fingerlings exposed to sublethal concentration of Copper showed following histopathological changes in the stomach. Disruption of serosal layer was observed. The nuclear pycnosis was seen in the epithelial cells. Lamina propria was damaged and broadened in the disrupted epithelial lining was noticed. Reduced and damaged muscularis mucosa was observed. Damage in the tips of the folds of the villi was seen in the gut. (Fig. 7).

Histopathological changes in the stomach of fingerlings *Cyprinus carpio* treated with sublethal concentration of bimetal mixture.

30-day exposure:

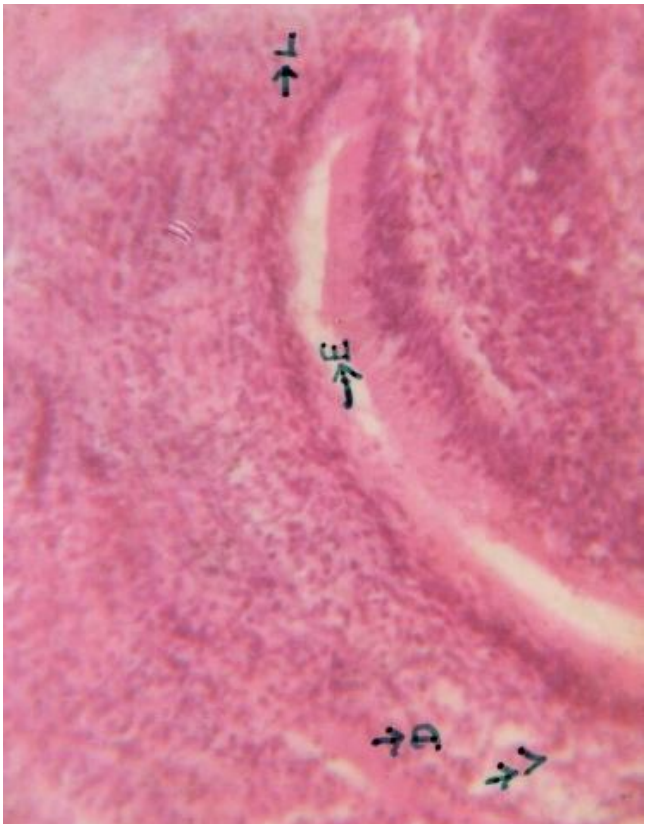

Fig. 6: Changes in Stomach of *Cyprinus carpio* after 30 - Day exposure to Nickel Treatment.
D - Destruction of Submucosa, V - Vacuoles,
L - Loss of nuclei, E - Eroded villi epithelium

Nickel and Copper mixture caused severe pathological changes on the stomach of fingerlings as indicated by disappearance of epithelial cells in the villi and formation of vacuoles in mucosa and muscularis. Lamina propria was destroyed. Mucosa and submucosa were also damaged. Longitudinal and circular muscles were severely damaged (Fig. 8).

Normal Gonad (Ovary) of *Cyprinus carpio*

Histology of normal ovary in fish shows outer wall made of 3 layers (a) an outermost their peritoneum, (b) a thicker tunica albeginea made up of connective tissue, muscle fibers and blood capillaries, (c) the inner most layer in the germinal epithelium. The germ cells (or) Oogonia are found in clusters in the lamellae consist of a lightly stained clear cytoplasm and single large nucleus with a centrally placed nucleolus. The immature Oocytes are small in size and are characterized by a large nucleus containing several nucleoli arranged along the periphery. The maturing Oocytes are yolk vesicles along the periphery. Histopathological changes in the ovary of fingerlings of *Cyprinus carpio* treated with sublethal concentration of Nickel after 30-days exposure. (Fig. 9).

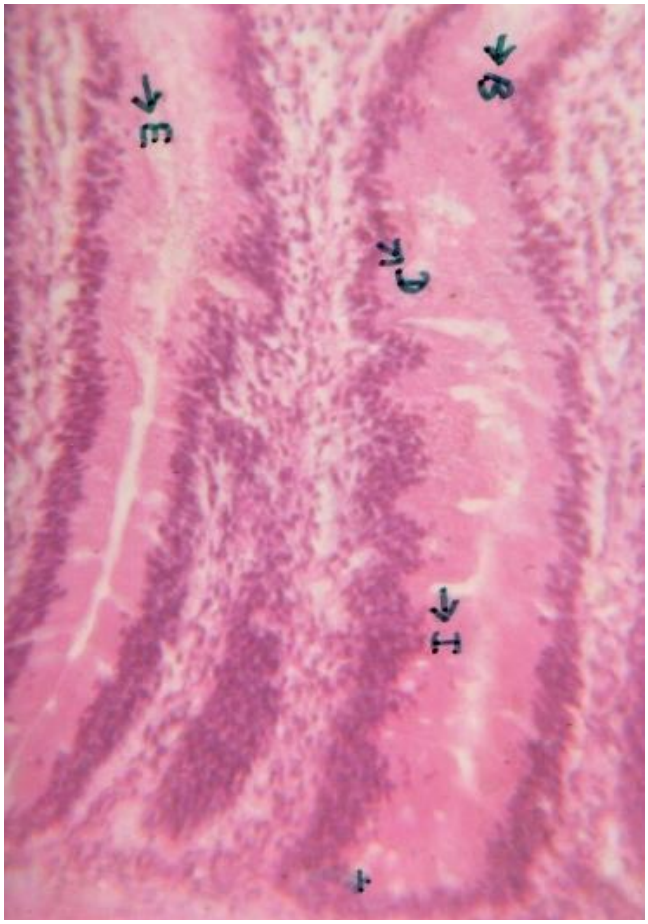

Fig. 7: Changes in Stomach of *Cyprinus carpio* after 30 - Day exposure to Copper Treatment.
E - Eroded villi epithelium, B - Boradened basal ends of Villi, I - Intercellular gaps between villi, D - Destruction of Submucosa

Histopathological changes in the gonad (ovary) of fingerlings of *Cyprinus carpio* treated with sublethal concentration of Nickel.

30-days exposure

Nickel treated fingerlings showed a comparatively lesser pathological changes than the other treatments. Disappearance of the nucleus and appearance of vacuolization is seen. In some cells the fibrous content of the nucleus was increased and less dense granules was seen clumping of cytoplasm is also noticed. (Fig. 10).

Histopathological changes in the gonad (ovary) of fingerlings of *Cyprinus carpio* treated with sublethal concentration of Copper.

30-days exposure:

Fingerlings exposed to sublethal concentration of Copper showed the following histopathological changes in the ovary. Nucleus become disappeared and

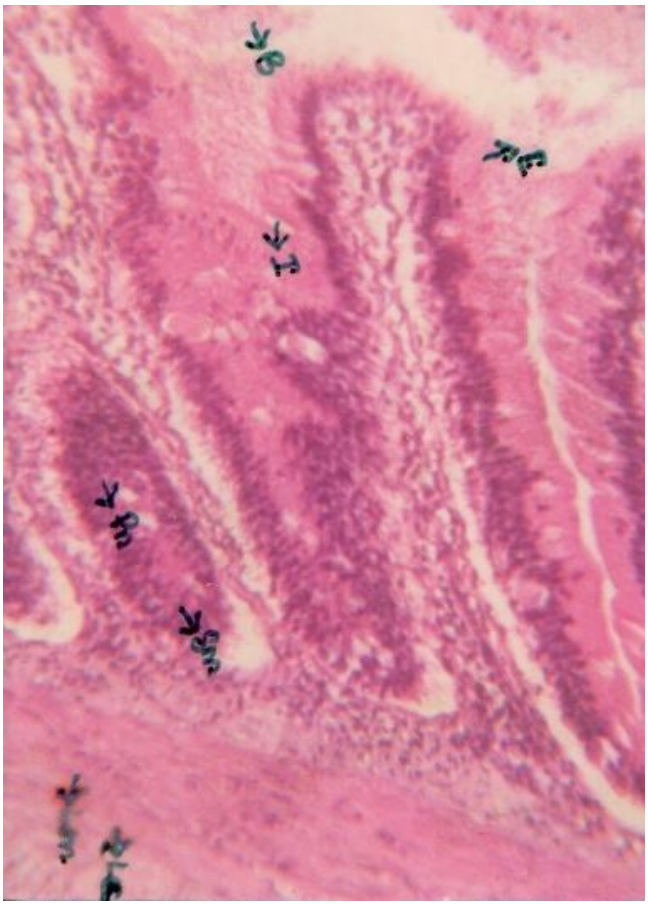

Fig. 8: Changes in Stomach of *Cyprinus carpio* after 30 - Day exposure to Mixture Treatment.
B - Broadened basal ends of Villi, I - Intercellular gaps between villi, D - Destruction of Submucosa, E - Eroded villi epithelium, Mr - Ruptured mucosa membrane, Pn - Pycnotic nucleus, Sm - Swelling of mucosa, Lm - Loss of longitudinal muscle, Lc - Loss of circular muscle

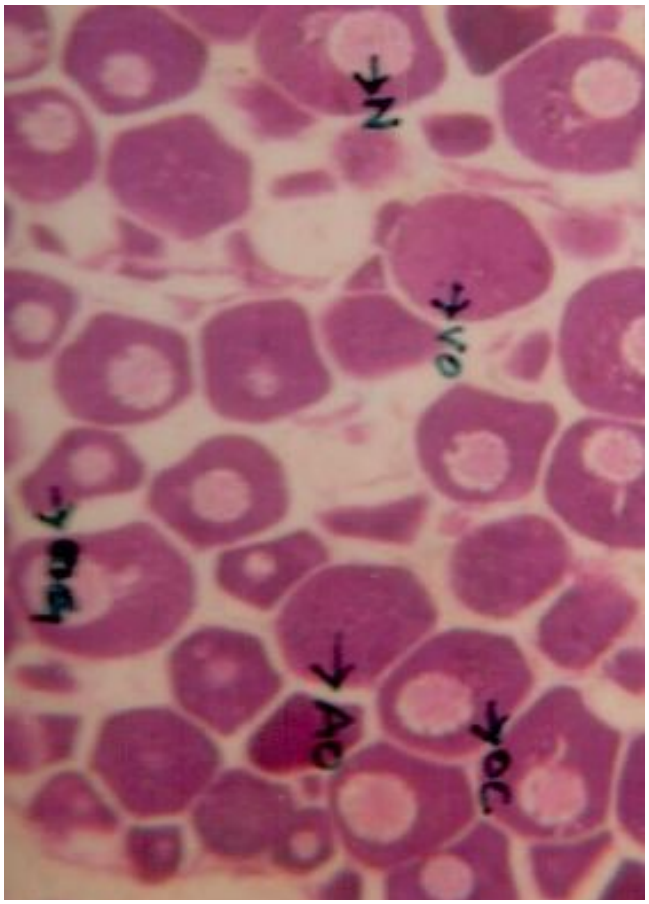

Fig. 9: Normal Gonad (Ovary) section of *Cyprinus carpio*.
N - Nucleus, Oc - Oocortex, OG - Oogonia,
Vo - Vitellogenic Oocyte, Ao - Artretic oocyte

vacuolization is seen. Clumping of cytoplasm is noticed. Less dense yolk granules are observed. Ruptured cells was observed. (Fig. 11).

Histopathological changes in the gonad (ovary) of fingerlings of *Cyprinus carpio* treated with sublethal concentration of bimetal mixture.

30-days exposure:

Mixture treatment caused severe histopathological changes in the ovary of *Cyprinus carpio*. Nucleus become greatly disintegrated. The cell membrane getting ruptured and shape of the cells also vary. Clumping of cytoplasm is noticed. (Fig. 12).

Discussion

Liver

The liver is a very important organ performing vital function like detoxification, synthesis of several components of blood plasma, storage of glucose in the form of glycogen and release of glucose. In the present observation, the histopathological studies revealed, that the liver of fingerlings were more damaged in the sublethal concentration of Nickel, Copper and mixture treatments than control. In the individual and mixture treatments there were remarkable histopathological changes including the loss of polygonal shape of hepatic cells, vacuolization in the Cytoplasm and necrosis of hepatic cells, which were more severe in mixture treatment. The more pronounced effect due to bimetal mixture may be due to the joint effect of both the metals (Nickel and Copper). The liver is the vital organ of detoxification. The alterations in liver due to toxicity impact are often associated with a degenerative necrotic condition. The changes induced by chromium in the liver hepatocytes such as vacuolization, necrosis and nuclear condensation were also reported for copper exposure (Arellano *et al*., 1999; Olojo *et al*., 2005; Figueiredo-Fernandes *et al*., 2007). Liver alteration of *Anabas testudineus* has displayed the results of necrosis, vascular haemorrhage, dilated sinusoids and vacuolar degeneration after being treated with copper (Nandan and Kumar, 2014). A similar observation of copper-induced histopathological changes in the liver of Nile tilapia *(Oreochromis niloticus*) was also reported by Abdel-Tawwab, (2016). In addition, a study carried out by Udotong and John, (2015) also showed similar alterations in the liver by displaying diffuse hepatocyte necrosis. Saravpreet Kaur *et al*., 2018 showed on histopathology changes of liver of freshwater

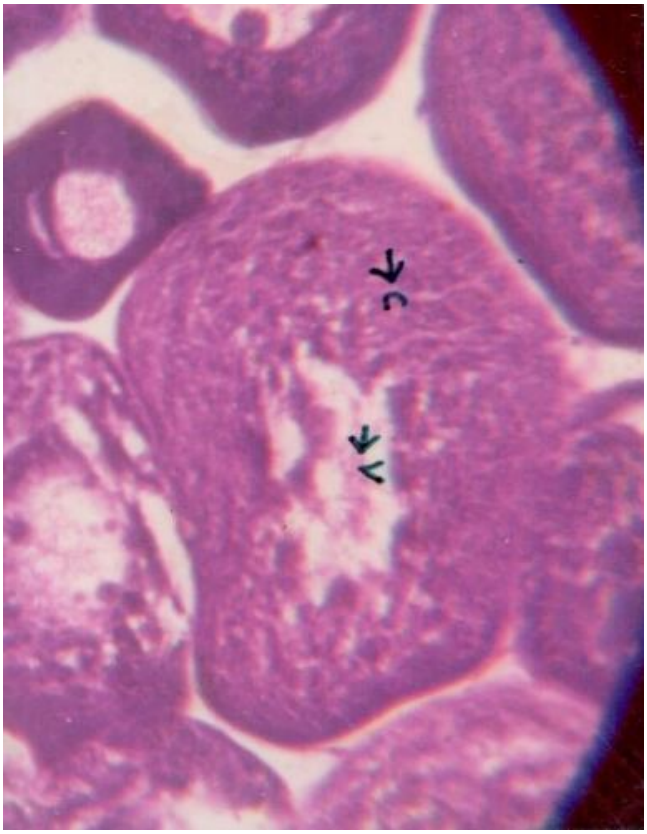

Fig. 10: Changes in Gonad (Ovary) of *Cyprinus carpio* after 30 - Day exposure to Nickel Treatment.
V - Vacuolization, C - Clumping of Cytoplasm

cyprinid, *Labeo rohita* after being treated with toxic heavy metals (arsenic, chromium, cadmium, manganese and lead).

Stomach

In the present study, the stomach of treated fingerlings of *Cyprinus carpio* was very much affected in sublethal concentration of Nickel, Copper and mixture treatment than control, which were lesser in Nickel and slightly more in Copper and more in mixture treatments. The histopathological changes including fragmentation of serosal layer, erosin of epithelial cells and broadening of lamina propria were observed. The serosal fragmentation is more in mixture treatment than the other two treatments and the order of toxicity was as follows, Mixture > Copper > Nickel. Similar observations were made by Juliet Premalatha, (1990) using Malathion and Sevin in *Cyprinus carpio*. Severe damages in the internal structure of stomach were evident following exposure to a sublethal dose of Lead nitrate for 45days. The stomach looked completely de-shaped, crumbled and shrunken, damaged epithelium of the mucosal fold, its shortened length, disintegration of the muscle layers, vacuolization's and edema in the epithelial and mucuous goblet cell. (Jha and Pandey, 1989). Present findings were further supported by the study of Sastry and Malik, (1979) in *Channa punctatus* when exposed to Dimecron. Vacuoles are seen in different layers of stomach. Amminikutty and Rege, (1977) reported the same observation in *Gymnocorymbus ternatzi* exposed to Thiodon. In the present study, the glandular cells were destroyed and the lymphatic cells were destroyed (or) disappeared. This is supported by Neelam arrora and Kulrestha, (1984) in *Channa striatus* when exposed to mixture of Carbaryl and Endosulfan and Surinder virk *et al*., (1987) in *Mystus tengara* when exposed to mixture of Endrin and Carbaryl. Saxena and Agarwal. (1971a) also reported similar histopathological changes in the stomach of *Heteropneustes fossilis* exposed to metal of Carbaryl and Endrin.

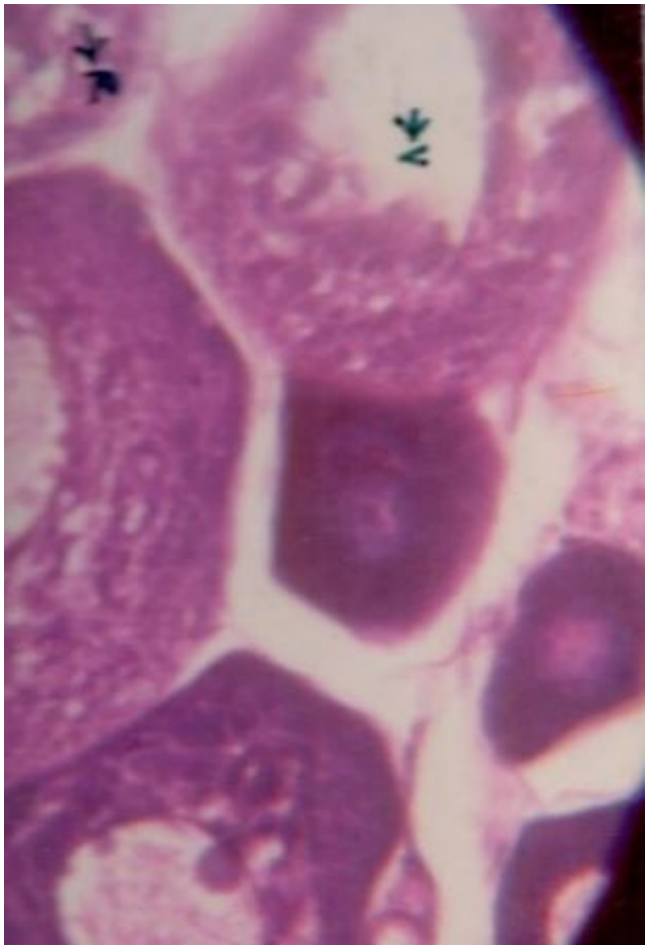

Fig. 11: Changes in Gonad (Ovary) of *Cyprinus carpio* after 30 - Day exposure to Copper Treatment
N - Nucleus disappeared, V - Vacuolization, R - Ruptured cell

Gonad (Female)

In the present study, the gonad (ovary) of treated fingerlings of *Cyprinus carpio* was very much affected in sublethal concentration of Nickel, Copper and mixture treatment than control. There was decreased in the gonadosomatic index in all the three treatments. The order of sequence of toxicity of the treatments on gonads is as follows Mixture > Copper > Nickel. The significantly

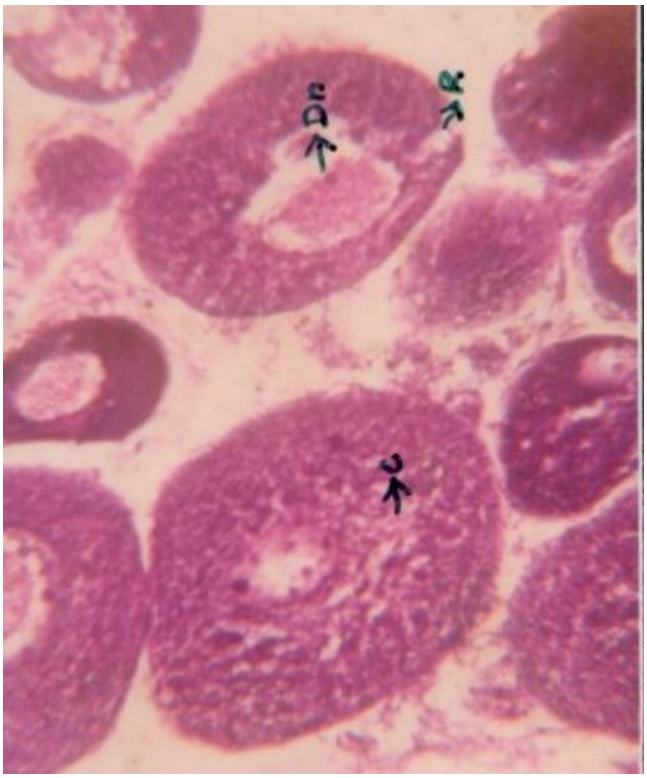

Fig. 12: Changes in Gonad (Ovary) of *Cyprinus carpio* after 30 - Day exposure to Mixture Treatment
Dn - Disintegration of nucleus, Rc - Ruptured Cell wall, C - Clumping of Cytoplasm

lower values of Gonadosomatic index indicated the inhibitory action of the toxicants on the ovarian activity which decreased the gonadotropin hormone release. Similar decrease in gonadotropin has been reported by Mckim and Benoit, (1971) in *Salvelinus fontinalis* when exposed to Copper. Contradictorily Copper acetate treatment has been observed to hasten enlargement of Oocyte thereby acting as chemical inductors for Oocyte development in *Clarias batrachus* and *Ophiocephalus punctatus* (Khosa and Chandrasekhar, 1972). Shukla and Pandey, (1984) have observed increased atresia of Oocyte due to exposure to sublethal Arsenic concentration to *Colisa fasciatus*. Ram and Sathynesan, (1983) and Saxena and Bhatia, (1983) have also reported arrested ovarian growth because of partial ganodotropin release when the fish is exposed to Mercuric Chloride. In the present study, it was noted that the ovarian weight is decreased and atretic follicle increased due to the prolonged metals treatments. These above mentioned changes were more pronounced in bimetal mixture treatment than individual metals and control. This may be due to joint action of the metals which might have acted synergistically on the ovarian structure and growth. The histological study of ovaries of *Channa punctatus* in comparison with controlled fish organs showed Atretic oocytes with broken membrane, decreased nucleoliin perinucleolar stage. Artesia was seen in maturing follicles, ovarian follicles separated due to loss of inter follicular connective tissue, inter follicular space were larger and vacuolation in developing oocytes were also observed. Dissolution of yolk globles and vacuolation in vitellogenic stage observed. Damage to yolk visicle and clumping of cytoplasm in mature oocytes was also observed (Khillare *et al*., 2017). The different pollutants such as industrial and agricultural wastes, pesticides and heavy metals have histopathological effects on the reproductive tissues of fish gonads (Pedlar *et al*., 2002; Hanna *et al*., 2005), these effects may disturb the development of germ cells and may reduce the ability of the fish to reproduce (Mehanna, 2005). In the present investigation it can be concluded, that the toxic effect is more pronounced in bimetal compared to individual metals. This indicates Nickel and Copper acted synergistically at sublethal levels in combination.

Reference

Abdel-Tawwab, M. (2016). Effect of feed availability on susceptibility of Nile tilapia, *Oreochromis niloticus* (L.) to environmental zinc toxicity: Growth performance, biochemical response and zinc bioaccumulation. *Aquaculture.*, **464:** 309-315.

Abou-Seedo, F., S. Dadzie, K.A. Al-Kanan (2003). Histology of ovarian development and maturity stages in the yellowfin seabream, *Acanthopagrus latus* (Teleostei:Sparidae) (Hottuyn, 1782) reared in cages. *Kuwait J. Sci. Eng*., **30(1):** 121-136.

Ackermann, C. (2008). A quantitative and qualitative histological assessment of selected organs of *Oreochromis mossambicus* after acute exposure to cadmium, chromium and nickel. M.Sc. dissertation, University of Johannesburg, South Africa.

Akpor, O.B., G.O. Ohiobor and T.D. Olaolu (2014). Heavy metal pollutants in wastewater effluents: sources, effects and remediation. *Adv. Biosci. Bioeng*., **2:** 37e43.

Amminikutty, K.C. and M.S. Rege (1977). The destruction of hepatocytes and pycnosis in fish on exposure to malathion. *Ind. J. Exp. Biol.*, **15:** 197.

Arellano, J.M., V. Storch and C. Sarasquete (1999). Histological changes and copper accumulation in liver and gills of the Senegales sole, *Solea senegalensis*. *Ecotoxicol. Environ. Saf*., **44:** 62-72.

Apha, Awwa and Wplf (1975). Standard methods for the examination of water and waste water. 14th Edition, APHA, INC, New York. Dc, 1.120.

Bowser, D.H., K. Frenkel and J.T. Zelikoff (1994). Effects of *in vitro* nickel exposure on the macrophage mediated immune functions of rainbow trout (*Oncorhynchus mykiss*). *Bulletin of Environmental Contamination and Toxicology.,* **52:** 367-373.

Devi, Y. and A. Mishra (2013). Histopathological alterations in gill and liver anotomy of fresh water, air breathing fish *Channa punctatus* after Pesticide Hilban® (Chlorpyrifos) treatment. *Advanced Biomedical Research.*, **4(2):** 57-62.

Deshmukh, S. and K.M. Kulkarni (2005). Effect of cadmium chloride on gonads of the fish *Channa orientalis* (Sch.). *Indian J. Environ. Ecoplan*., **10:** 239-245.

Elenka Georgieva, Stela Stoyanova, Iliana Velcheva, Tonka Vasileva, Veselin Bivolarski, IliaIliev and Vesela Yancheva (2014). Metal effects on histological and biochemical parameters of common rudd *(Scardiniuserythrophthalmus* L.). *Arch. Pol. Fish*., **22:** 197-206.

Evans, D.H., K.E. Weingarten and J.S. Walton (1990). The effect of atropine on cadmium- and nickel-induced constriction of vascular smooth muscle of the dogfish shark ventral aorta. *Toxicology.,* **62:**89-94.

Figueiredo-Fernandes, A., J.V. Ferreira-Cardoso, S. Garcia-Santos, S.M. Monteiro, J. Carrola, P. Matos and A. Fontaínhas-Fernandes (2007). Histopathological changes in liver and gill epithelium of Nile tilapia, *Oreochromis niloticus*, exposed to waterborne copper. *Pesquisa Veterinária Brasileira.*, **27(3):** 103-109.

Ghazaly, K.S. (1992). Sublethal effects of nickel on carbohydrate metabolism, blood and mineral contents of *Tilapia nilotica. Water, Air and Soil Pollution.,* **64:** 525-532.

Hanna, M.I., I.B. Shaheed and N.S. Elias (2005). A contribution on chromium and lead toxicity in cultured *Oreochromis*

niloticus. *Egypt. J. Aquatic Biol. Fisher.,* **9:** 177-209.

Jha, B.S. and S. Pandey (1989). Histopathological lesions induced by Lead nitrate in the stomach of the air breathing teleosts *Channa punctatus*. *Environmental and Ecology.*, **7 (3):** 721-723.

Juliet Premalatha, R. (1990). The toxic effect of pesticides (Malathion and Sevin) on the fish *Cyprinus carpio*, M.Phil thesis.

Khosa, D. and K. Chandrasekhar (1972). Effect of Copper acetate and Asphat on gonadal activities and the correlated changes in the pre-optic nucleus of two genera of teleosts fishes, *Clarias batrachus* (Linn) and *Ophiocephalus punctatus* (Bloch). Proc. *Indian Acad. Sci. Sect.,* **76:** 229-239.

Kiran Khillare*, Y.K. Khillare and Umesh Wagh (2017). Histological changes in gonads of fresh water fishes due to heavy metal pollution. *World Journal of Pharmacy and Pharmaceutical Science*., **6(7):** 601-609.

Mason, C.F. (1987). Biology of freshwater pollution. ISBN-0-582-45506-0. Newyork, 250.

Mckim, J.M and D.A.Benoit (1971). Effects of long term exposure to copper on survival, growth and reproduction of brook trout *(Salvelinus fontinalis*). *J. Fish. Res. Bd. Can*., **28:** 655-662.

Mehanna, S.F. (2005). Population dynamics and management of the Nile Tilapia (*O. niloticus*) in Wadi El-Raiyan lakes, Egypt. *Afr. J. Biol. Sci*., **1:** 79-88.

Mohammadi, M., A.A. Sary and M. Khodadadi (2011). Determinationof heavy metals in two barbs, *Barbus grypus* and *Barbus xanthopterus* in Karoon and Dez Rivers, Khoozestan, Iran-Bull. *Environ. Contam. Toxicol*., **87:** 158-162.

Nandan, S. and B.G. Kumar (2014). Copper toxicity: Haematological and histopathological changes and prophylactic role of vitamin C in the fish, *Anabas testudineus* (Bloch, 1792). *Journal of Zoology Studies*., **1(3):** 4-13.

Arora, Neelam and A.K. Kulshrestha (1984). Histopathological effects induced by sublethal doses of carbaryl and endosulfan on the stomach of *Channa striatus* (Bloch). *India. J. Comp. Anim. Physio.*, **2:** 50-53.

Olfat, M.N. and Z.A. El-Greisy (2007). Comparative impact of different waste sources on the reproductive parameters and histology of gonads, liver and pituitary gland of Signusrivulatus. *J. Appl. Sci. Res*., **3:** 236-244.

Olojo, E.A.A., K.B. Olurin, G. Mbaka and A.D. Oluwemimo (2005). Histopathology of the gill and liver tissues of the African catfish, *Clarias gariepinus* exposed to lead. *Afr. J. Biotechnol.*, **4(1):** 117-122.

Padrilah1, S.N. Mohd Khalizan Sabullah2, MohdYunus Abd Shukor1, Nur Adeela Yasid1, Nor Aripin Shamaan3 and Siti Aqlima Ahmad1 (2018). Toxicity Effects of Fish Histopathology on Copper Accumulation. *Pertanika J. Trop. Agric. Sci*., **41(2):** 519-540.

Paulo, D.V., F.M. Fontes and F. Flores-Lopes (2012). Histopathological alterations observed in the liver of *Poecilia vivipara* (Cyprinodontiformes: Poeciliidae) as a tool for the environmental quality assessment of the Cachoeira River, BA. *Brazilian Journal of Biology.*, ***72*(1):** 131-140.

Pant, S.C., S. Kumar and S.S. Khanna (1980). Toxicity of copper sulphate and zinc sulphate to fresh water teleost *Puntius conchonius* (Ham.) in hard water. *Comparative Physiology and Ecology.*, **5(3):** 146-14.

Pedlar, R.M., M.D. Ptashynski, R. Evans and J.F. Klaverkamp (2002). Toxicological effects of dietary arsenic exposure in Lake Whitefish *(Coregeonus clupeaformis*). *Aquat. Toxicol*., **57:** 167-189.

Ram, R.N. and A.G. Sathyanesan (1983). Mercuric chloride induced changes in the protein,lipid and cholesterol levels of liver and ovary of the fish, *Channa punctatus. Environ and Ecol*., **2:**113-117.

Sabullah, M.K., M.Y. Shukor, M.R. Sulaiman, N.A. Shamaan, M.A. Syed, A. Khalid and S.A. Ahmad (2014). The effect of copper on the ultrastructure of *Puntius javanicus hepatocyte*. *Australian Journal of Basic and Applied Science.*, **8(15):** 245-51.

Sakthival, V. and S.A. Gaikwad (2001). Histopathological changes in testis and ovary of *Gambusia affinis* (Baird and Girard) due to dimecron stress. *J. Aquat. Biol*., **16(2):** 63-65.

Kaur Saravpreet, Kuldeep Singh Khera and Jasjit Kaur Kondal (2018). Heavy metal induced histopathological alterations in liver, muscle and kidney of freshwater cyprinid, *Labeo rohita* (Hamilton). *Journal of Entomology and Zoology Studies.,* **6(2):** 2137-2144.

Sastry, K.V and P.V. Malik (1979). Studies on the effect of Dimecron on the digestive system of a fresh water fish, *Channa punctatus*. *Arch. Environm. Contam. Toxicol.*, **8:** 397-407.

Saxena, P.K and R. Bhatia (1983). *Water, Air and Soil Pollution*., **20:** 55.

Saxena, P.K and S. Agarwal (1971a). Carbaryl induced histopathological changes in the stomach, intestine and liver of *Heteropneustes fossilis*. Proc. 58th. *Ind. Sci. Con*., 569.

Shukla, J.P and K. Pandey (1984). Arsenic induced structural changes during the ovarian cycle of a fresh water Perch, *Colisa fasciatus* (Bloch and Schneider). *Bul. Inst. Zool. Academic Sinica.*, **23(1):** 69-74.

Singh, R., N. Gautam, A. Mishra and R. Gupta (2011). Heavy metals and living systems: an overview. *Indian J. Pharmacol*., **43:** 246-253.

Virk Surinder, Kamal deep Kaur and Satinderpal Kaur (1987). Histopathological and biochemical changes induced by Endrin and Carboryl in the stomach, intestine and liver of *Mystus tengara* Indian. *J. Ecol*., **14(1):** 14-20.

Udotong, J. I. and O.U. John (2015). Histopathological changes in liver and muscle of tilapia fish from QIRE exposed to concentrations of heavy metals. *World Academy of Science, Engineering and Technology.*, **9(6):** 659-662.