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Toxicity Effect of Fish *Channa punctatus* from Vaigai River

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Research Article

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ABSTRACT

In this study aimed investigate the water quality data and heavy metal analysis results in Vaigai River by using multivariate statistical analysis methods. Samples were collected from different sites in Vaigai River and analyzed for the level of heavy metals such as Cd, Cr, Cu, Fe, Pb, Ni, and Zn. Also hematological parameters like RBC, WBC, PCV, HB, platelets, MCV, MCH, MCHC, and enzymes like ALS, ATL, CK, TP were analyzed for fresh water fish *Channa punctatus*. Histological study reveals the morphological changes in kidney, liver, heart, intestine and mussels. Influence of heavy metals, pesticide and industrial waste, changes normal to abnormal physiological characters in *Channa punctatus*. The result indicated that the concentrations of these heavy metals not fall within the respective acceptable permissible limits as recommended by the WHO/FAO/FEPA. Hence the consumption of *Channa punctatus* may pose threat to human health. However periodic monitoring of these metals in the fish and water is highly essential to public health due to bioaccumulation.

INTRODUCTION

Heavy metal pollution in many River systems of the world is a common environmental problem due to rapid population growth, industrialization and economic development [1-3]. The main natural sources of metals into the aquatic system are the weathering of soils and rocks and from anthropogenic activities, whereby industrial and urban wastes are discharged into water bodies [4-6].These metals are persistent and once released the environment for a prolonged period [7,8]. These heavy metals are well known pollutants, which are often encountered in many ponds, Lakes, Rivers and dams of India and the most important aquatic fauna being subjected to stress caused by these heavy metals [9]. The entry of municipal, industrial, and agricultural waste into the environment is another way of the environment pollution by human. Water resources are among the most critical resources. The importance of water resources, particularly surface waters (Rivers). Addition of unwanted substances into the water bodies cause changes in the physical, chemical and biological characteristics of the aquatic system which lead to ecological imbalance. The industrial effluents contribute a lot to water pollution forming a threat to aquatic plants and animals [10].

The hematological studies in fishes have assumed greater importance because these parameters were used as an efficient and sensitive index to monitor the physiological and pathological changes induced by natural or anthropogenic factors such as bacterial or fungal infection or pollution of water resources [11]. Blood parameters Therefore considered as a useful tool in diagnosing the functional status of the body in response to various stressors [12,13]. The toxicants are stressors which are accumulated in the fish through the food chain or absorption through the general body surface and severely affect the life supporting system at molecular and biochemical levels. The Pollutants generally produce relatively quick changes in hematological characteristics of fish [14]. Enzyme activity are considered as sensitive and ALP) and haematological parameters in *Chrysichthys* biochemical indicators before hazardous effects occur in *nigrodigtatus* and *Phthonichthys macrurus*. Fish and are important parameters for testing water for the presence of toxicants. Agricultural, industrial and domestic effluents generally contain a wide variety of organic and inorganic pollutants, such as solvents, oils, heavy metals, pesticides, fertilizers and suspended solids and are, invariably, discharged into small Rivers and streams, without proper treatment. Such contaminants change water quality and may cause many problems to fish, such as diseases and structural alterations [15]. Histopathological changes have been widely used as biomarkers in the evaluation of the health of fish exposed to contaminants, both in the laboratory and field studies, however to our knowledge, little or no information is available on the effect of endosulfan on the haemato-immunological and histopathological responses of *C. puncatus*. Considering the importance of this fish species both from commercial as well as conservation point of view, this study was conducted to elucidate the effect of Pollutants exposure on the alterations in haemato immunological parameters and histopathological changes in *C. punctatus*.

MATERIALS AND METHODS

Analyzing of heavy metals in vaigai River water samples

Sample collection: Water samples were collected from 4 different sites in Vaigai River Samples were collected in screw capped high density pre sterilized polypropylene bottles, each of 1 lt capacity, labeled properly and analyzed in laboratory for trace metals by Atomic Absorption Spectrometer (AAS).

Preparation of water samples: One litres of each collected water sample was first concentrated on a sandy oven at 80°C until the volume reached 50 ml. Then 4 ml concentrated sulfuric acid (Merck, 98%) was added to each sample and Digested by digs Dahl apparatus for 3 minutes. The 10 ml hydrogen peroxide (Merck, 30%) was then added and heated until oxidation was completed. After cooling, each sample filtered by filter (Whatman filter Merck, 0.45 μm).The filtrate was diluted by demonized water to a final volume of 50 ml.

Samples analysis: The prepared samples were analyzed by a Shimadzu atomic absorption spectrometry (Model analyst 7000) to determine the metals.

Analyzing of hematology and biochemistry parameter Vaigai River fish in *Channa punctatus*

Sample collection: *Channa punctatus* fishes collected (males and females) were obtained from Vaigai Rivers (to rule out environmental effect on the study) in permonson and also post monsoon studying period 4 different sites (Annai Patti, Solavanthan, Arapalayam, Anna Nagar). The fishes (*Channa punctatus*) weight of 25 g ± 500 g and mean total length of 5 ± 30 cm. The fish were transported to the laboratory in water obtained from the class dank. The fish's further analyzed of water was used as an anesthetic agent.

Blood samples (2 ml) were collected from *Channa punctatus* in EDTA coated container (BD Company). Furthermore, the blood parameters were analyzed with the help of hematology semi auto analyzer (Eraba). Some other blood samples were collected and left to coagulate for 15–20 min at 4°C prior to centrifugation for 20 min at 3,000 rpm to separate serum. The fresh serum was subjected to biochemical analysis. Total protein (g/100 ml) content and total lipids (g/l) contents were determined calorimetrically using assay kits supplied by Alere Dignostics. Activities of Aspartate Aminotransferase (AST, U/l) and Alanine Amino Transferase (ALT, U/l) were determined calorimetrically using assay kits (Agapai diagnostics,) according to Reitman and Frankel method (1957). The blood samples were determination of hematological parameters PCV, Hb, TEC, TLC and ESR in method described by Blaxhall and Daisley (1973) (Figure 1).

Figure 1. Dorsal view of *Channa punctatus*.

Histological procedures

Paraffin section method and technique: Live specimens of test organism *C. punctatus* were collected from Vaigai River Madurai. Average length and weight of the experimental fish was 20.74 cm ± 1.34 and 99.58 g ± 33.71 respectively. The experiments were conducted in the laboratory using glass aquarium (40 × 27 × 26 cm^3) they were randomly selected from each group and immediately sacrificed by pinning through the brain and tissue samples like kidney, liver and intestine were collected from the dissected fish. When the fish showed moribund condition, they were immediately removed for histological examination. The tissues were washed in physiological saline and preserved in plastic vials with Bouin's fluid for at least 18 hours. The samples (liver, kidney and intestine) were then dehydrated, embedded in paraffin wax. The embedded blocks were sectioned (6 µ) by a microtome machine. Fixed

and prepared slides were kept for one night. Then the sections were stained with hematoxylin and eosin and were then mounted for later examination under compound microscope.

Acid digestion method for AAS samples analysis

The prepared samples were analyzed by atomic absorption spectrometry (Model analyst 7000) to determine the metals.

Atomic Absorption Spectrometer (AAS): The method follow the Beer's law, the states that absorbance is straight comparative to concentration each atomic absorption spectrophotometer a light basis i.e. lamp, sample cell, monochromatic, detector and output mechanism. The graphic diagram of an atomic absorption spectrophotometer is certain as, the mainly common type of burner is a premix, which introduces the spray into a condense cavity for deletion of larger depletes.

Analyzing of hematology and Biochemistry parameter vaigai River fish in *Channa punctatus*

Sample collection: *Channa punctatus* 20 fishes were collected (male and female) Average length and weight of the experimental fish 1.34 cm ± 20.74 and 33.71 g ± 99.58 were obtained from Vaigai Rivers (to rule out environmental effect on the study) in permonson and also post monsoon studying period 4 different sites (Annai Patti, Solavanthan, Arapalayam, Anna Nagar). The fish were elated to the lab in water obtained from the class dank. The fish's further analyzed of water was use in a painkilling agent.

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Quantitative determination of Aspartate Amino-Transferase (AST) IVD

Principle of the method: Aspartate Amino-Transferase (AST) previously called Glutamate Oxaloacetate (GOT) catalyses the reversible transmit of an amino group from aspartate to α-ketoglutarate. The oxalacetate created is decrse to malate by (MDH) and NADH: Charge of reduce in concentrations of NADH, calculated photometrically, is comparative to the catalytic concentrations of AST there in the sample.

Clinical significance: The AST is a cellular enzyme, is originate in uppermost concentrations in the cells in the liver, heart muscle, in smaller amounts in other weaves and the cells in the gaunt muscle. Although an elevated level of AST in the serum is not specific of the hepatic disease, is use mostly to diagnostic and to confirm the lessons of this disease by further enzymes like ALP and ALT.

Also it is use to organize the patient following myocardial infarction, in skeletal muscle disease and other. Clinical diagnosis must not be prepared on a single test result; it should combine clinical and further laboratory data.

Quantitative determination of Alanine Amino-Transferase GPT (ALT) IVD

Principle of the method: Glutamate Pyruvate Transaminase or (GPT) Alanine Amino-Tranferase (ALT) catalyse the reverse transmit on amino group as of alanine to α-ketoglutarate form glutamate and piruvate.

Preparation: Alanine Amino Transferase (ALT) and Aspartate Aminotransferase (AST) examine in take a 20 µl serum sample adding with Regent 1000 µl in (prepare to reagent in 4:1 ratio Regent 2 in 200 µl and Regent 1 in 800 µl) imminent read the analyzer catch the result (kinetic method).

Total Protein (TP) assay in take 100 µl serum samples add with 1000 µl Regent incubated with 10 minutes after read the sample in analyzer get the result (End point Method).

Creatine Kinase (CK) assay take 100 µl serum samples add with (preparation of reagent in 1:1 ratio Regent 1 in 500 µl and Regent 2 in 500 µl) incubated with 15 minutes after read the sample in analyzer get the result (Fixed Time).According to Reitman and Frankel method the samples in blood were determined of hematological parameter Hb, PCV, ESR, TEC, and TLC in methods.

Histological procedures: Histological study has been conduct to assist set up fundamental associations among pollutant coverage and a variety of biological response Histological investigation have prove to be a perceptive tool to sense straight effect of Heavy metal and Pesticide inside target organ of fish in laboratory experiment. This experiment was expected to finded the histological effect of *Channa punctatus* liver and kidney. At Arapallayam and Anna Nagar site the fish were under with benzocaine and then sacrifice by cervical section. Pieces liver were excise, rinse in physiological saline more paraffin part methods follow.

Hematological and biochemical assay analysis: The experimentation was conduct in 4 different sites for premonsoon period, and the result the red blood characteristics were haematocrite (PCV) (26.20 ± 1.06 %), Haemoglobin (Hb) (8.57 ± 0.14 (mg/dl), White blood cell (WBCs) (15300.00 ± 355.90 (103 µl), Red Blood Cell (RBCs) (2.12 ± 0.49 (106 µl) MCV (192.20 ± 4.16 (g/dl) MCH 28.01 ± 2.36 (g/dl), MCHC (24.881 ± 2.05 (g/dl). The value of revealed that the total protein percentage of *Channa punctatus* range between (3.9 ± 0.1 mg/dl), Alkaline Phosphatase (ALP), range between 75 ± 18 U/L, Creatine Kinase (CK) 1258 ± 500 U/L. The 4 different sites the total protein of post monsoon period, and the result The red blood characteristics were haematocrite (PCV) (22.79 ± 1.04%), Hemoglobin (Hb) (7.22 ± 0.14 (mg/dl), value in the White Blood Cell (WBCs) (9387.93 ± 164.533 (103 µl), Red Blood Cell (RBCs) (2.00 ± 0.2 (106 µl) MCV (156.31 ± 4.74 (g/dl) MCH 25.00 ± 2.7 (g/dl), MCHC (27.13 ± 1.73 (g/dl). The value of revealed that the total protein percentage of *Channa punctatus* range between (3.3 ± 0.1 (g/dl), Aspartate Aminotransferase (AST) (61 ± 15 U/L) Alkaline Phosphatase (ALP) range between 75 ± 18 U/L, Creatine Kinase (CK) 1258 ± 500 U/L.

The 4 different sites the total protein of control period, and the result The red blood characteristics were packed cell volume (PCV) (28.06 ± 0.84%), Hemoglobin (Hb) (9.16 ± 0.75 (mg/dl), White Blood Cell (WBCs) (9510.410 ± 300.1453 (103 µl), Red Blood Cell (RBCs) (3.42 ± 0.47(106 µl) MCV (233.60 ± 2.20) MCH (27.13 ± 1.73(g/dl), MCHC (24.90 ± 1.42 (g/dl). the value of revealed that the total protein percentage of *Channa punctatus* range between (3.9 ± 0.1(g/dl) Aspartate Aminotransferase (AST) 75 ± 22 U/L , Alkaline Phosphatase (ALP) range between (2.1 ± 0.1 g/dl) Creatine Kinase (CK) 1258 ± 500 U/L, get in the value. Biochemical parameters in four different sites -1 and 2, (Annaipatti, Solavanthan) are same, but the sites 3 and 4 (Arapalayam, Anna Nagar) showed different variant with blood parameters. The biochemical parameters of fish vary very much from one species to anther and within individuals depending on sex, age, atmosphere and period. The difference in biochemical parameter of fish is intimately connected to feed intakes, traveling swim and sexual modify in relationship with spawning.

Histological parameters

Liver: The fish liver appear, as do the liver of explanation appendage which controls many life functions and plays a prominent role in fish physiology, both in anabolism (proteins, lipids and carbohydrates) and catabolism (nitrogen, glycogenolysis and detoxication etc). In wide-ranging, liver is aim limbs billed to its great blood provide that cause evident toxicant experience and gathering, its permission function and its marked metabolic ability the liver in fish

is a ventrally situated dense organ. Its shape and size, volume is modified to the break accessible flanked by other instinctive organs (esophagus, stomach, spleen and intestine). Fish liver is usually reddish brown because of its affluent vascularization treatment toward yellow, after fat storage is elevated. Light microscopy exposed the liver structure in *Channa punctatus* was parallel to so as to of additional teleosts. The majority of the cells in the hepatic parenchyma obtainable a polygonal form round nuclei and granular cytoplasm. In this cells are dispersed erratically among the sinusoidal vessels, canaliculi and larger vessels, as well as about the bile duct. In some individuals, the fibroblasts are also experiential among the hepatocytes. In the hepatic parenchyma, it is potential to examine exocrine pancreas, frequently close to blood vessels and duct. Exocrine pancreas complete of polygonal cells with a famous essential nucleus and with the attendance of emission granules in some of the cytoplasm.

RESULTS AND DISCUSSION

Chromium

The value of Chromium was ranging from 0.04 to 31 mg/l. The minimum amount of Chromium (0.04 mg/l) was recorded in the sampling unit Annaipatti. The value of calcium was increased in the sampling point Anna Nagar (31 mg/l) as maximum. In the sampling point Solavathan the maximum amount of chromium was recorded as (0.0397 mg/l).

Cobalt

The value of cobalt was ranging from 0.0341 to 0.0199 mg/l. The minimum amount of cobalt (0.0199 mg/l) was recorded in the sampling unit Anna Nagar. The value of cobalt was increased in the sampling point Solavathan (0.0623 mg/l) as maximum. In the sampling point Annaipatti the maximum amount of cobalt was recorded as (0.0341 mg/l).

Copper

The minimum value of copper (0.0215 mg/l) was recorded in the sampling point Annaipatti. The maximum value of copper (0.0942 mg/l) was recorded in the solavanthan. In the sampling point Aarappalayam, Anna Nagar the copper value was ranging from 0.0985 to 0.3568 mg/l.

Iron

The minimum amount of iron (0.2057 mg/l) was recorded in the Anna Nagar, Aarappalayam, the maximum value of iron was recorded as 0.4654 mg/l in the sampling point Solavanthan. In Annaipatti sampling point the value of iron was ranging from 0.2087 to 0.3592 mg/l.

Magnesium

The value of magnesium ranged between 3.0304 mg/l to 0.1238 mg/l. The Maximum value of magnesium was recorded as (1.7108 mg/l) in the sampling point solavanthan. The minimum value of magnesium was recorded as (0.3238 mg/l) in the sampling point Anna Nagar, Arapalayam. The amount of magnesium was increased (5.9649 mg/l) in the Annaipatti.

Hematological analysis

The experiment was conducted in 4 different sites for Premonsoonperiod, and the result The red blood characteristics were haematocrite (PCV) (26.20 ± 1.06 %), Haemoglobin (Hb) (8.57 ± 0.14 (mg/dl), White Blood Cell (WBCs) (15300.00 ± 355.90 (103 µl), Red Blood Cell (RBCs) (2.12 ± 0.49 (106 µl) MCV (192.20 ± 4.16 (g/dl) MCH 28.01 ± 2.36 (g/dl), MCHC (24.881 ± 2.05 (g/dl). The value of revealed that the total protein percentage of

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Histopathology of the intestine

The histological alterations found in the intestine of the fish enslaved at the 4 sites at Vaigai River are detailed in (showed fig). The commonest heavy metals found were considered to be at , the intestine is relatively short, typically around one and a half times the length of the fish's body. It commonly has a number of pyloric caeca, small pouch-like structures along its length that help to increase the overall surface area of the organ for digesting food. There is no ileocaecal valve in teleosts, with the boundary between the small intestine and the rectum being marked only by the end of the digestive epithelium its intestine affects the heavy metals length and size change in surface area of the digesting problem.

Histopathology of the kidney

The alterations found in the kidney most important change found in the glomerulus of *Channa punctatus* kidney was glomerular expansion, resulting in reduction of Bowman's space. In the tubules, the most frequent alterations were: cloudy swelling, occlusion of tubular lumen and hyaline droplet degeneration.

Histopathology of the liver

The main alterations found in the liver were irregular-shaped nuclei, nuclear hypertrophy, nuclearvacillation and the presence of eosinophilic granules in the cytoplasm. Bile stagnation was identified as brownish-yellow granules in the cytoplasm. Cytoplasm and nuclear degeneration was also very common melanomacrophages were identified as rounded aggregates of cells containing dark-yellowish granules of various sizes, normally close to the vessels. The majority of the alterations found in the liver of the caged animals belonged to the tissue was slightly too moderately damaged, and recuperation was still possible, if the water quality improved.

DISCUSSION AND CONCLUSION

Heavy metal pollution is a major problem for the Vaigai River, Madurai. In the present study concentrations of Cr, Cu, Co and Fe, Mn were higher than the safe values which indicated that the river Vaigai polluted by studied heavy

metals and might create an adverse effect on this Riverine ecosystem. Changes in the hematological and biochemical parameters of *Channa punctatus* in the alterations of these parameters may provide early warning signals for the determination of heavy metal and pesticides their effects in aquatic system. The findings of the present study also provide a better understanding of the toxicological endpoint of aquatic pollutants and to ascertain a safer level of these chemicals in the aquatic environment and protection of aquatic habitants. The analysis of the seasonal variation in the histological parameters leads to the conclusion that the changes observed in the Three organs It must be emphasized the histopathology is able to evaluate the early effects.

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