JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

An Overview of Heavy Metals induced Contamination and the Adverse Effects on Fish Health *Channa Punctatus* with Multiple biomarker responses.

Irfan Ahmad Parray[1], Syed Danish[2] , Pritesh Kothekar[3] , Aqib Hussain Lone[4], Jayashree Dhote[5].
Department of Zoology, Shri Shivaji Science College, Amravati (444603),
[1]Research scholar department of Zoology, Shri Shivaji Science college Amravati Maharashtra.
(Parrayirfan920@gmail.com)
Corresponding Author: - [5]Professor department of Zoology, Shri Shivaji Science college Amravati Maharashtra.

Abstract

Heavy metals are widely used in every industrial application; therefore, they form the core group of pollutants of any industrial discharge. Some of the heavy metals such as Fe, Mn, Co, Ni, Cu, Zn and Cr are essential as they form the cofactor for many of the enzymes and also needed in metabolic activities. On the contrary, their exceeding amount is also detrimental to both animals and human beings. Increased pace of the industrialization and use of advance technologies for the quest of development leads to the problem of environmental pollution. One such condition has been witnessed in the present study in MIDC Area of Amravati region of Maharashtra India. Rapid industrialization results in the production of huge amounts of solid and/or liquid wastes, which is usually discharged into the nearby water bodies, leading to the damage of the important ecosystems and seafood products. Therefore, Investigations on the accumulation of heavy metals (Cu, Ni, Fe, Co, Mn, Cr and Zn) were carried out on Channa punctatus (murrel). The accumulation was observed in tissues of muscles, liver, kidney and gills. The results revealed that the Fe and Ni concentrations were the highest in all tissues analyzed, followed by Co, Zn, Cu and Cr. The present overview aims to highlight the issue of pollution of aquatic ecosystems and fish health around MIDC Amravati region Maharashtra. Based on the current review, it has been observed that to monitor the health of indicator organism (fish), battery of bioassays or biomarkers are required. The rationale of using the few selected parameters such as, marker enzymes of tissue damage, oxidative stress, genotoxicity and histopathology in describing, the aquatic pollution has also been emphasized. All these parameters are significantly affected by heavy metals and hence proved as useful tools in biomonitoring or toxicity assessment studies. Since fishes are consumed by large mass of population due to their high protein and polyunsaturated fatty acid content, human health is also under danger.

Keywords:- Heavy metals, MIDC Amravati, Biochemical parameters, Oxidative stress, Geno toxicity, Histopathology.

Significance statement

This overview highlights the issues of river water pollution induced by the industrial effluents and fish health due to heavy metals. It includes the polluted water bodies of the Amravati region adjacent to MIDC region and their source of pollution. In addition, it also emphasizes upon the relevance of biomarkers which can suitably be used to assess the fish health.

Introduction

Aquatic pollution from sources like effluents from industries, power plants untreated domestic and sewage waste etc. have adverse effects on aquatic ecosystem. Due to which the animals thriving in these water bodies exposed to unnaturally high levels of contaminants. Since water is essential for all forms of life. Seas and oceans contribute approximately 97%, while the freshwater resources consist only 3% of the entire water reserve of the earth. Therefore, water has a key role in sustaining ecological balance. Moreover, it is not only the main component of the biosphere but also a major part of the living organisms (Gleick, 1996). The widespread scarcity, the gradual destruction and the aggravated pollution of the water resources also lead to degradation of ecosystem. Nowadays, water quality issues are gaining recognition as river waters are getting heavily polluted at many places. Due to industrialization, the number of factories and population has increased rapidly. The contamination of freshwaters with a wide range of pollutants has become a matter of concern over the last few decades (Canli et al., 1998; Dirilgen, 2001). Moreover, groundwater quality, at many places, is beginning to deteriorate to cause serious implications on the supply of water for drinking, irrigation and industrial use as all of them are important determinants of public health. The natural aquatic systems have been extensively contaminated with heavy metals released from domestic, industrial and other man- made activities (Velez and Montaro, 1998). The level of natural contaminants and chemical pollutants is high and also is increasing at several places. Environmental pollution became all the more hazardous as the urban life became more and more prevalent. Rather, it has increased parallel to the industrial development. In the second half of twentieth century, increasing environmental pollution due to rapid industrialization and population growth has caused natural resources to become more polluted so that destruction of ecosystem became an acute issue. The effluents discharged from the industries into the water bodies contain many toxic compounds like phenols, oils, pesticides, heavy metals, xenobiotics and polyaromatic hydrocarbons. The toxic effects of heavy metals have been reviewed, including their bioaccumulation by several workers (Rani, 2000; Waqar, 2006). The effluents affect the physicochemical parameters of water such as temperature, pH, dissolved oxygen, total solids, dissolved solids and suspended solids. These parameters are often employed to assess the water quality. In addition, the heavy metals form the core group of pollutants in the industrial and daily life activities. Heavy metal contaminations have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms (Ashraj, 2005; Vosyliene and Jankaite, 2006). It is known that heavy metals have toxic effects even at places away from the source of pollution as they have the ability of biological accumulation (Barlas, 1997). Fishes are the inhabitants that cannot escape from the detrimental effects of these pollutants (Olaifa et al., 2010). Fish are susceptible to acute and chronic environmental changes and they show a classical stress response. This stress influences plasma glucocorticoids and catecholamines. Environmental changes may cause hypoxia, metabolic acidosis and alkalosis, hypotension and hypoglycemia (Fabbri et al., 1998). Fish accumulate xenobiotic chemicals, especially those with poor water soluble occurs because of the very intimate contact with the medium that carries the chemicals in solution or suspension and also because fish have to extract oxygen from the medium by passing the enormous volumes of water over gills. The studies carried out on various fishes have shown that these metals alter the physiological activities and biochemical parameters both in tissues and blood (Basa and Rani, 2003). In a nut shell, this degradation of water quality has led to water scarcity for the human consumption. Toxic substances may knock down immune, reproductive, nervous and endocrine systems in animals and these effects can be at organ, tissue and cell level (Geeraerts and Belpaire, 2009).

Gill tissue is an organ having a large surface and separates blood from water in fish and is very susceptible to changes in concentrations of the variables (heavy metals, temperature, pH etc.) in the environment. These variables affect the structural integrity of the gill and cause morphological changes. For this reason, gills are good indicators of water pollution (Bhagwant and Elahee, 2002; Koca et al., 2008). Liver plays an important role in protecting inner homeostasis in vertebrates. The highly dynamic nature of liver and its regulation in many metabolic and physiological processes make this organ a valuable model for study (Segner, 1998). Muscle tissue forms a major part of the body weight of fish when compared to other vertebrates (Fabbri et al., 1998) and is also economically valuable. The heavy metals, being conservative in nature have the maximum probability of biomagnification, when they are transferred to the human beings through the various members of different trophic levels in the food chain. Human beings are affected negatively as a result of their accumulation. in order to evaluate the degree of contamination, the present study was conducted to examine the contamination of rivulet situated at MIDC (Maharashtra Industrial Development Corporation) District Amravati, Maharashtra, India (20° 56′ 14.73″ N 77° 46′ 46.38″ E). Experiments were conducted to investigate the health of fish Channa punctatus inhabiting heavy metal- loaded waste water.

A Brief Introduction of Selected Heavy Metals

Heavy metals, in general, apply to a group of metals and metalloids with atomic density greater than 4 g/cm3 or 5 times or more, greater than water (Hawkes, 1997). However, being a heavy metal has little to do with density but concerns mainly with the chemical properties. If the electronegativity is more, the toxicity will also be more (Wittmann, 1979). Heavy metals are non-biodegradable and persist in the environment for a quite long time even after the source has been removed. Therefore, heavy metals are considered most dangerous in the toxicological studies. The present review focuses on the metals most frequently found in surface water, and these include ^{24}Cr, ^{25}Mn, ^{26}Fe, ^{27}Co, ^{28}Ni, ^{29}Cu, and ^{30}Zn. These metals, belonging to first transition series of the periodic table, are known to stimulate the production of reactive oxygen species (ROS) in the living systems. This production of ROS accounts for the toxicity. All heavy metals exist in surface waters in colloidal, particulate and dissolved phases, although dissolved concentrations are generally low. The solubility of heavy metals in surface waters is predominately controlled by the water pH, the type and concentration of ligands on which the metal could adsorb, and the oxidation state of the mineral components and the redox environment of the system (Kennish,1992).

Chromium

It is one of the most common ubiquitous pollutants in the environment and does not occur naturally in the pure metallic form. This element is present in different oxidation states. However, its divalent (Cr^{2+}), trivalent (Cr^{3+}), and hexavalent (Cr^{6+}) oxidation states are stable. It is the hexavalent form of Cr, which is allowed to cross biological membranes of aquatic organisms (Oldewage, et al.,2010). In natural waters, the concentration of Cr is low and is within the range of between 1 and 2 lg/L.

Manganese

It exists in several oxidation states. The most stable oxidation states of Mn are +2, +3, +4 and +7. It is released into the environment by several industrial applications like steel industries, emission from welding rods, fuel additive, dry cell batteries, alloys, electric coils and the burning of fossil fuels.

Iron

It also exists in several oxidation states like +2, +3, +4, +5 and +6. However, the most stable states that exist in solution are the ferrous (Fe^{2+}) and ferric (Fe^{3+}); the latter is the most common form found in surface waters (Department of Water Affairs and Forestry, 1993). The main source of increased iron concentrations in the aquatic environment is the mineral, iron and steel processing industrial runoff, alloys, acid mine drainage, chemical industries, dye industries, fertilizers, organic chemicals, etc.

Cobalt

It exists in +2, +3 and +4 oxidation states. However, (Co^{2+}) and (Co^{3+}) are its most stable forms. The main sources of increased cobalt concentrations in the aquatic environment are electroplating, preparation of drying agents, production of catalysts, turbine making, alloys, high-speed tools, cobalt metal powders utilized as an indicator of humidity and also those used in painting porcelain.

Copper

Natural concentrations in water are at B5 lg/L. Cu reaches into the aquatic systems through anthropogenic sources such as industrial, coolant water discharge, corrosion of pipe lines, municipal drainage/ sewage, combustion of coal, fly ash, mining, plating operations, antifouling paints, pulp and paper board mills, fertilizers, petroleum refining, steel works, foundries, copper fungicides, use of copper salts in control- ling aquatic vegetation and influx of Cu-containing fertilizers (Nussey, 1998).

Zinc

Major sources of zinc in water are galvanized materials, and domestic products containing zinc are batteries, pigments and paints, electroplating, fly ash, combustion of coal, petrochemicals, organic chemicals, fertilizers, steel work foundries, zinc, lead and copper smelting industries, brass alloy manufacturing, galvanizing iron and steel, dry batteries, municipal waste, pesticides, automobiles, fungicides, pigments and printing.

Description of study area

The freshwater fish, *Channa punctatus* has been selected for the present work as its availability and easy to maintain in the laboratory conditions. The fishes were obtained from local fishermen District Amravati, Maharashtra, India (20° 56′ 14.73″ N 77° 46′ 46.38″ E). Locally the fish is known as *Dhoke* and it is edible fish. Channa punctatus were brought from the area of concern and transported in aerated aquaria to the Postgraduate Laboratory of Shri Shivaji Science College Amravati.

Materials and Methods

Collection and processing of fish samples

Fish *Channa punctatus* were collected from the MIDC Amravati. Procured fishes were immediately kept in pre-cleaned polythene bags, sealed and stored in an ice box for further analysis. fishes were euthanized for gill, liver and kidney removal and immediately after dissection they were carefully washed with phosphate buffer. The present study was conducted to investigate the accumulation of heavy metals (Cu, Ni, Fe, Co, Mn, Cr,and Zn) in various tissues (gills, liver, kidney, muscle and integument). Tissues were removed and oven dried at 60 °C. The dried samples were ground into fine powder using pestle and mortar, and sieved (0.5 – 1.0 mm). Tissue samples, (0.5g) were digested in 15 ml of solution containing concentrated HNO3 and HClO4 (4:1). The digested solution was filtered through Whatmann filter paper (No.42), and washed with distilled water. It was then raised to 50 ml in a volumetric flask (Javed and Usmani , 2011). These tissue samples were immediately processed for heavy metal estimation as per standard methods of APHA 49. For instrument calibration, working standards were prepared by diluting the stock standards (1000 ppm) supplied by Wako Pure Chemical Industry Ltd., Japan. Analytical blanks were also used. The applied analytical procedure accuracy was tested using the certified reference material Dorm-2 (dogfish muscle, National Research Council, Canada) for investigated metals. Replicate analyses of the reference materials showed good accuracy, with recovery rates for Cu 99%, Zn 99%, Fe 99.2%, Ni 98.5%, Cr 98%, Mn 97.7 and Co 97%. Gill, liver and kidney were also used for oxidative stress biomarkers, comet assay and histopathology.

Collection and analysis of water sample from MIDC Amravati.

Water was collected in a pre-cleaned and acidified glass bottles. The bottles were immediately brought to the laboratory and acidified with concentrated HNO3 to pH less than 2.0. Water samples were then analyzed for the presence of heavy metals (Cu, Ni, Fe, Co, Mn, Cr and Zn) according to APHA (2005). On spot fixation of water was done to measure the dissolved oxygen (D.O). Total solids (T.S), total dissolved solids (T.D.S) and suspended solids (T.S.S) were determined using standard techniques (APHA, 2005).The temperature and pH were recorded at the site using laboratory thermometer (Deluxe, 6) and pH strips (S.D Fine chemicals, 0 - 0.1).

Preparation of Blank

Blanks were prepared along with each set of the sample. Preparation of standards Standard solutions for heavy metals were prepared using standard techniques (APHA 2005).

Instrumentation

The water and fish tissue samples were analyzed for heavy metals Cu, Ni, Fe, Co, Mn, Cr and Zn by Atomic Absorption Spectrometer (Perkin Elmer, AA 800, multiple cathode lamps) with specific cathode lamps for each metal and Nitrous oxide-Acetylene was used as flame. The following analytical conditions of the instrument were used for atomic absorption of these metals.

Statistical analysis

Samples were taken in triplicates. The values are given as Mean ± S.D. The data was subjected to ANOVA. Significant differences among the means was calculated using Duncan's multiple range test (Duncan 1955).

Biochemical assays

a) Blood glucose estimation

Prior to sacrifice of fishes blood was collected through cardiac puncture and was placed into the vials. Blood samples taken were centrifuged at 3500 rpm for 10 min to obtain serum.

The glucose levels in the serum were analyzed using the diagnostic kit Eco-Pak glucose (Accurex Biomedical Pvt. Ltd., India). The glucose levels in samples were measured spectrophotometrically (UV–VIS Systronics, 118) against blank at 505 nm.

b) Liver and muscle glycogen estimation

Fishes were dissected to remove the liver and muscle tissue to estimate the glycogen level. The glycogen levels in liver and muscle were measured by Anthrone reagent according to the protocols of Carrol et al. (1956).

c) Protein profile of serum, liver and muscle

Total protein was determined according to the protocols of Bradford (1976) as modified by Spector (1978), taking BSA as a standard. Albumin was quantitated using the diagnostic kit (Siemens Ltd., Gujarat, India). The intensity of color developed was measured by a spectrophotometer (UV–VIS Systronics, 118) at 595 and 628 nm for total protein and albumin, respectively. Globulin was calculated after subtracting the albumin content from the total protein. Albumin to Globulin (A:G) ratio was also calculated.

Oxidative stress markers.

Homogenates of gill, liver and kidney were prepared in 0.1 M phosphate buffer at pH 7.4. Superoxide dismutase (SOD) activity was assayed by auto-oxidation of pyrogallol as per the standard protocols of Marklund & Marklund, (1974). The reaction mixture contains 2.85 ml of tris-succinate buffer (0.05 M, pH 8.2) and 50 µL of sample. Reaction was started by addition 100 µL of pyrogallol (8 mM) to the reaction mixture and the change in absorbance was measured at 412 nm at an interval of 30 s for 3 min. The specific activity of SOD is expressed as Units mg−1 protein. One enzyme unit of SOD is defined as the amounts which cause 50% inhibition of pyrogallolauto-oxidation in a total volume of 3 ml. Catalase (CAT) was assayed as per the protocols of Aebi56 with minor modifications. The reaction mixture containing 50 µL of sample in 1.95 mL of potassium phosphate buffer (50 mM, pH 7.0). After addition of 1 ml of H2O2 the reaction mixture was monitored at 240 nm for 3 min at an interval of 30 s. The activity of glutathione S transferase (GST) was measured by the method of Habig et al. (1974). The assay mixture contained 2.8 mL of sodium phosphate buffer (0.1 M, pH 6.5), 0.1 ml reduced glutathione and 50 µl sample. GST activity was monitored at 340 nm for 3 min at an interval of 1 min by the addition 50 µl of 1 mM 1-chloro 2, 4 dinitrobenzene (CDNB). The level of reduced glutathione (GSH) was estimated by method of Jollow et al.(1974) with minor modifications. Equal amount of homogenate sample and sulphosalicylic acid were mixed and incubated at 4 °C for 1 h and centrifugation at 12000 rpm for 15 min. The supernatant (0.2 ml) was mixed with 2.6 ml of potassium phosphate buffer (0.1 M, pH 7.4). The reaction was initiated by the addition of 0.2 ml 5, 5′-dithiobis-2-nitrobenzoic acid (DTNB) and the absorbance was monitored at 412 nm.

Results and Discussion

The aquatic environment fed with effluents, for Fish Channa punctatus is subjected to many stressful factors. Heavy metals are one of the serious pollutants that reach the aquatic habitat and also a matter of concern. For this reason, this work is projected to examine the hazardous effects of heavy metal on one of the most common fish species, Channa punctatus in the effluent fed-rivulets of Amravati region. The estimated heavy metals were recorded in the order Fe > Cu > Zn > Mn > Ni >Co > Cr. Physicochemical analysis of water has long been employed to assess its quality. Temperature, pH, DO, TSS and TDS are often used as measures of water quality because any change in them reflects the pollution status of natural waters. They were suitable for sustenance of fishes however Fe and Ni content exceeded the recommended guidelines set by the United Nations Environment Programme Global Environment Monitoring System (UNEPGEMS, 2006).

Table 1: Physicochemical parameters of Heavy metal water of MIDC Amravati.

Parameters	Water
Temperature	31.0°C
pH	7.0
Dissolved oxygen (D.O)	5.6mgL^{-1}

Table 2: Concentrations of particular heavy metal in different organs of Channa punctatus.

S.No.	Heavy metal	Concentration
1	Copper	53.33 ± 0.29
2	Iron	145.13 ± 1.5
3	Chromium	13.33 ± 0.16
4	Zinc	73.31± 0.06
5	Cobalt	83.28± 0.06
6	Nickle	96.22±0.18

Values are Mean ± S.D, (n= 5), Values are expressed in mg kg^{-1}.dry weight Means with similar letters in a row are statistically similar at $P > 0.01$

Table- 3: Accumulation of heavy metals in the organs of *Channa punctatus*

Heavy metals	Gills	Liver	Kidney	Muscle	Integument
Cu	38.83±0.82	45.83±.0.83	29.19±0.53$_c$	17.32±0.32	24.29±0.49
Fe	387.83±0.83	1659.73±0.83	401.27±0.59	177.31±0.31	179.31±0.37
Mn	17.3±0.89	ND	16.13±0.27	21.2±0.329	ND
Cr	12.5±0.00	0.033±0.002	5.06±0.00	4.33±0.30	ND
Zn	83.23±0.79	0.29±0.004	36.27±0.55	51.87±0.43	90.17±0.49
Ni	96.23±0.79	113.03±0.81	83.23±0.45	103.27±0.47	83.12±0.37

Avg length (12.20±1.5 cm); Avg weight (29 ± 0.42 g);Values are Mean ± SD, (n = 15), ND = not detected, ANOVA and DMRT was used to test the statistically significant difference. Means with similar letters in a column and row are statistically similar at $P < 0.01$.

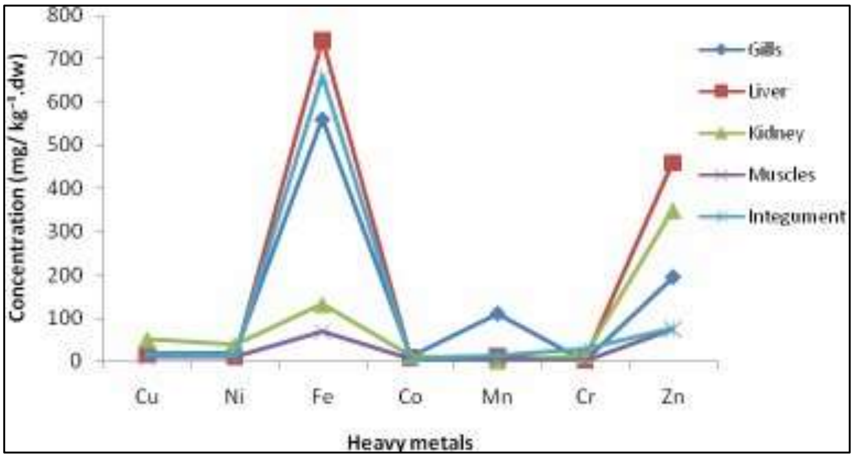

Figure 1: Shows the accumulation range of heavy metals in different organs of *Channa punctatus*.

In the present study possible reason for high levels of heavy metals could be attributed to the fact that MIDC region of Amravati may not have efficient wastewater treatment plant inside its premises due to which these metals are present beyond the maximum permissible limits. Although these metals are essential in traces but their excess becomes toxic and gets accumulated in tissues of fish Channa punctatus due to which fish comes under stress.

Biochemical Parameters

The principal components of body like carbohydrates, proteins and lipids, which play a significant role in body construction and energy production, are also affected by heavy metal pollution (Javed and Usmani 2015). The analysis of biochemical levels in serum and tissue is of considerable importance to assess the toxicity in environmental toxicology.

Protein

The heavy metal stress causing change in the glucose content indicates a change in the energy requirement and expenditure because anoxic or hypoxic condition increases carbohydrate consumption. When the glycogen reserves deplete, the tissue protein supplies keto acids by the process of deamination of amino acids. So a study of the protein content of serum is necessary to understand the changing demands and expenditure of energy during metal stress. Estimation of total protein, albumin and globulin in serum is of considerable diagnostic value in fish as it relates to general nutritional status. The albumin: globulin ratio serves as a useful index to track relative changes in the composition of protein in serum. Moreover, the necrosis of the hepatic tissue may result in decrease in protein synthesis (Jacobs, et al.,2002).

In Channa punctatus, serum total protein, albumin and globulin increased when intoxicated with Cu (0.1 g/kg). However, Labeo rohita when reared in sewage-polluted (Hebbal Lake) and highly polluted (Chowkalli Lake) lakes of Bangalore showed gradual reduction in serum total protein (Nagaraja et al., 2010). Gopal et al., 1997 exposed C. carpio to lethal and sub- lethal concentrations of Cu (8–0.8 ppm, respectively) and Ni (10–1.0 ppm, respectively) for 72 h and reported somehow same readings. In our study Serum protein and globulin level showed an initial sharp increase from 3 to 24 h and then decline by 72 h; however, serum albumin showed an initial decrease from 3 to 5 h and increased over a period of 72 h.

Impact of heavy metals has also been observed on the protein profile of different tissues of C. punctatus and is shown in Table 3. Within the body albumin and globulin makes most of the proteins and alteration in the quantities of these proteins occur due to which the A:G ratio gets disturbed when intoxicated with xenobiotics/heavy metals. In the present study significant increase was observed in the serum total protein. Albumin level fall significantly ($p < 0.01$) and globulin showed significant ($p < 0.05$) rise over reference. Thus A:G ratio decline significantly ($p < 0.01$) as compared to the reference.

Table 3: - Protein profile of reference and exposed C. punctatus.

Tissues	Total protein	Albumin	Globulin	A:G
Reference serum (mg/ml)	1.43 ± 0.04	1.11 ± 0.01	0.464 ± 0.004	2.43 ± 0.001
Exposed serum (mg/ml)	1.55 ± 0.03	0.175 ± 0.001	1.443 ± 0.03	0.121 ± 0.003
Reference liver (mg/g wet weight)	1.09 ± 0.02	0.303 ± 0.003	0.86 ± 0.033	0.36 ± 0.016
Exposed liver (mg/g wet weight)	12.04 ± 0.49	7.1 ± 0.13	6.14 ± 0.56	1.13 ± 0.085
Reference muscle (mg/g wet weight)	0.83 ± 0.06	0.162 ± 0.001	0.76 ± 0.07	0.24 ± 0.005
Exposed muscle (mg/g wet weight)	13.35 ± 0.32	3.69 ± 0.05	11.00 ± 0.30	1.69 ± 1.39

All values are expressed as mean ± SEM, (n = 7).

Gopal et al. (1997) observed increase in serum total protein and globulin but decline in albumin in Cyprinus carpio when intoxicated with Cu and Ni. Increase in total protein, albumin and globulin was observed in liver and muscle in the present study. Corroborating results of total protein in the muscle was also reported in M. cavasius (Palanisamy et al., 2011). A:G ratio is an index used to track changes in the composition of serum or plasma (Mazeoud et al., 1977) and its normal value lies between 0.8 and 2.0, reported for mammalian models.

In the present study the ratio of A:G was less than 0.8 in serum and muscle of exposed fish. However in liver of exposed fish the A:G ratio was 1.13 which was higher than 0.8 but below 2.0. Since albumin is entirely produced by the liver so an increase in albumin and total protein in the present study could be attributed toward the protein synthesis for utilization to meet high energy demand. The low albumin level than globulin in all the cases including the present case (excluding liver) could be ascribed as the utilization of albumin to meet the immediate energy demand hence rapid synthesis takes place in the liver, and higher globulin levels to meet the immunotoxic challenges. It is also said that the fishes which have low levels of globulins were less likely to survive in polluted waters because it imparts immune resistance. Moreover, the increased protein levels in the liver could also be due to the effort of liver to repair their damaged tissues as a result low levels were reported in serum despite synthesis. Therefore, estimation of total protein alone to check health status of fish would be misguiding. After all necrosis of hepatic tissue was more likely the cause of the disturbance of protein constituents in industrial heavy metal exposed fish C. punctatus

Lipid

Biomembranes consist of lipid bilayers with various types of protein embedded in or associated with them. Phospholipids are the major lipids present in all biomembranes. Maintenance of the correct composition of the lipid component is critical for the functioning of the biomembrane. During stress, the biomolecules that help the fish to cope up are stored carbohydrates, lipids and proteins. In acute stress or toxicity conditions, it is the stored glycogen which relieves the fish but on the continuous exposure to heavy metals, intensity of stress is strong then lipids and proteins start playing their role. Lipids may be mobilized to meet the energy demand either through the oxidation process or by gradual in saturation (Shelke Abhay, 2013). According to Perrier et al.,1979, HDL is the main serum lipoprotein followed by LDL and VLDL in the fish rainbow trout. Triglycerides/triacylglycerols are the prominent form of reserve lipids, which are always mobilized before phospholipids during starvation. Heavy metals are known to induce changes in the lipid levels. Hence, the study of lipid profile also serves as a biomarker of fish health. In Channa punctatus, serum total lipids increased significantly (p\0.06) from 0.89 to 1.97 g/L after Cu intoxication (0.2 g/kg). the same was observed in C. carpio when exposed to a mixture of Cr, Ni, Cd, Pb (1.25 mg/L of each metal ion) for a period of 32 days the concentrations of total cholesterol elevated by (Vinodhini and Narayanan, 2009.

When C. carpio was exposed to sublethal concentration (1/10th of LC50) for 28 days, it showed significant (p\0.05) increase in serum total cholesterol (Parvathi et al.,2011). Shaheen and Akhtar (2012), exposed C. carpio to sublethal concentrations of Cr (VI) (0, 25, 50, 75, 100, 125, and 150 mg/L) for 6 months; then, they observed that the serum cholesterol decreases significantly (p\0.05) with increasing toxicant concentration. Serum lipid profile of both the samples of C. punctatus is shown in Table 4. In the exposed fish lipid fraction occurs in the order total lipid > phospholipid > total cholesterol > HDL > LDL > triglycerides > VLDL however in reference fish the trend was total lipid > phospholipid > total cholesterol > triglyceride > LDL > HDL > VLDL. Alterations observed in profile were significant increase in the total lipid ($p < 0.01$), cholesterol ($p < 0.01$), phospholipids ($p < 0.05$), HDL and LDL ($p < 0.01$) levels when compared to reference. Other workers also recorded significant elevations in these parameters (Hanan et al., 2013).

Table 4: - Serum lipid profile of reference and exposed C. punctatus.

Profile	Reference C. punctatus	Exposed C. punctatus
Total lipid	387.23 ± 0.41	455.43 ± 0.47
Total cholesterol	139.31 ± 0.32	203.75 ± 0.41
Phospholipid	189.06 ± 0.32	227.13 ± 0.36
Triglycerides	137.60 ± 0.23	36.45 ± 0.15
HDL	35.31 ± 3.00	117.65 ± 0.07
LDL	83.18 ± 0.84	79.17 ± 0.18
VLDL	27.8 ± 0.76	9.29 ± 0.15

All values are given in mg/dl and as mean ± SEM, (n = 7).

Elevation in these parameters particularly cholesterol is ascribed due to the mobilization of lipid either through oxidation or a process of gradual instauration of lipid molecules from the synthesis site for subsequent utilization. Elevation in lipid profile is either due to disturbance in the metabolism of lipids or may be due to impaired clearance from plasma which favors liver dysfunction. As a result, hyperlipidemia, hypercholesterolemia, premature atherosclerosis and excessive deposition of fat take place Very limited work had carried out both in field and laboratory based studies in fish pertaining effect of heavy metals on lipid profile.

These biomolecules particularly proteins and polyunsaturated fats (PUFA) are also prone to oxidative damage caused due to these transition metal ions. The elevation or decline in these parameters could be due to carbonylation or peroxidation by free radicals/ROS generated as a result of metal toxicity (Tabrez et al., 2011). The free radicals/ROS either induce or suppress the antioxidant defense system leading to the imbalance between the two, and consequently cause oxidative stress (Sheriff et al., 2014).

Oxidative Stress

Lipid Peroxidation (LPO)

Biomembranes are made up of lipid bilayer. The polyunsaturated fatty acid (PUFA) present in them are highly susceptible to the oxidative deterioration. The oxidative degeneration of lipids by the ROS/[free radicals] generated as a result of exposure to toxicant is known as lipid per- oxidation. LPO is the basic deteriorative mechanism, which leads to the damage of membranes, enzymes and nucleic acids (Tadashi, 2002).

GST (glutathione S- transferase) was previously called as ligandins. It comprises a family of eukaryotic and prokaryotic phase II metabolic isozymes, which are best known for their ability to catalyze the conjugation of the reduced form of glutathione (GSH) to xenobiotics for the purpose of detoxification. GSH is synthesized from glutamate, cysteine and glycine, and the reaction is catalyzed sequentially by two cytosolic enzymes, namely c-glutamyl cysteine synthetase (GCS) and GSH synthetase. The cysteinyl part of this molecule has a sulfhydryl (SH) group, which accounts for its strong electron-donating character. Hence, GSH is readily oxidized non-enzymatically to glutathione disulfide (GSSG) by electrophilic substances (free radicals and ROS). Moreover, GSH is an extremely important cell protectant since it directly quenches reactive OH radicals, hydrogen and lipid peroxides, and radical centers on DNA and other biomolecules. Cellular GSH concentrations are reduced markedly in response to protein malnutrition, oxidative stress and many pathological conditions (Griffith, 1999).

The effects of heavy metals on LPO and antioxidant defense system (enzymatic and non-enzymatic) of fish had also been investigated by many workers. In our experiment, Zn, Cu, Cd, Fe and Mn were detected due to which CAT activity reduced in liver, kidney and gills of Channa puntatus, while SOD, GST and GSH activities in liver and kidney were increased and fall in gills. Also, LPO levels also showed elevation in all these organs (Farombi et al.,2007). Almroth et al., 2008, reported increase in antioxidant activities of liver GST and GSH, whereas CAT and SOD decrease in female Symphodus melpos, inhabiting heavy metal-polluted site Visnes, near Norway. The activity of the antioxidant enzymes SOD, CAT and GST in Cyprinus carpio was increased after exposure to heavy metal Ni, Cr, Cd and Pb solution (Vinodhini and Narayanan 2009). Velma and Tchounwou in 2010, performed antioxidant enzyme assay in liver and kidney tissues of C. auratus, exposed to different concentrations of Cr (VI) (LC12.5, LC 25 and LC50). In both tissues, CAT activity was decreased, whereas SOD activity and lipid hydroperoxide levels were increased.

Genotoxicity

DNA is another molecule that is also susceptible to oxidative damage by ROS. The polyanionic nature of DNA provides a useful substrate for adherence of metal cations, thus facilitating the formation of HO radicals adjacent to these critical biological targets (Cerutti, 1985). In addition to this, the heterogeneity of DNA allows for HO attacks including the nucleobases and sugar–phosphate backbone. When the attack of HO directed toward the sugar– phosphate backbone, cause lesions like fragmentation of deoxyribose with single-strand breaks and oxidation of sugar moiety (Breen and Murphy, 1995). Among the variety of methods developed for assessment of genotoxicity such as micronuclei test, chromosomal aberration test, DNA ladder assay and comet assay or alkaline single cell gel electrophoresis (SCGE) proves useful and is recommended by several workers. Comet assay is highly sensitive, easy and reliable technique to detect the single breaks in DNA molecule.

Moreover, in this assay the tail length (migration of DNA from the nucleus in micrometer), tail moment and tail intensity are used as an index of DNA breakage. However, most commonly used is the tail length. Furthermore, these heavy metals (24Cr, 25Mn, 26Fe, 27Co, 28Ni, 29Cu and 30Zn) are also known to cause DNA strand breaks, which may be double-strand or single-strand breaks. Ahmad et al.,2006, reported that DNA integrity lost at 1 Mm Cr concentrations, while at 100 lM no change was observed in gills of Anguilla anguilla. Mai et al. 2012, evaluated genotoxicity in larvae of oyster (Crassostrea gigas) and observed DNA strand breaks at 0.1 lg/L Cu concentrations. Barsiene et al.,2013, assessed environmental genotoxicity in blood erythrocytes of fishes, namely dab (Limanda limanda) and haddock (Melanogrammus aeglefinus) collected from North Sea and Atlantic Coastal waters. Results of our investigation reported that fishes under experiment showed significantly higher level of genotoxicity than others from normal water.

Glycogen metabolism

The industrial effluents which contain most of the heavy metals brought about a number of significant changes in the carbohydrate metabolism in Channa punctatus. There was significant elevation in blood glucose over control (+12.73%) while glycogen in liver (77.43%) and muscle (−74.35%) decline significantly. This hyperglycemic (increase in blood glucose) condition were also detected in fishes, Heteropneustes fossilis and Saccobranchus fossilis, exposed to Ni and Cr (Radhakrishaniah et al. 1992), Labeo rohita and Clarias gariepinus subjected to Cu (James et al. 1996), Oreochromis aurues exposed to mixture of Cu and Pb salts. During the present study increase in blood glucose content as a result of heavy metals present in industrial effluent could be attributed to the enhanced glycogenolysis, resulting in formation of glucose to meet the energy demand during stress. In the present study liver and muscle glycogen dropped significantly under the stress of heavy metals. Srivastava and Srivastava (2008) reported that glycogen consistently decreased from 8.18 to 5.3 mg g-^{1} in Channa punctatus when exposed to sublethal concentrations of ZnSO4. Similarly, other studies also subscribes to the above view in fishes such as Channa punctatus subjected to distillery effluent (Maruthi and Subba Rao 2000), Mystus cavasius to electroplating industrial effluent (Palanisamy et al. 2011). In this study it has been noticed that liver has highest Metal Pollution Index therefore it is assumed that high accumulation levels of heavy metals in liver impaired the activity of enzymes which contribute to glycogen synthesis, leading to decrease in glycogen content.

Glucose and glycogen levels of tissues of Channa. punctatus are shown in Table 2. In the present study non-significant fall (21.0%) was depicted in the blood glucose levels of the fish. Similarly, glycogen reserves of the liver depleted significantly ($p < 0.001$). Moreover, muscle glycogen level also declined significantly ($p < 0.01$) when compared to the reference. Other workers also observed that serum glucose first increased and then decreased upon chronic exposure until depleted (Zutshi et al., 2010). C. punctatus exposed to distillery effluent (Maruthi and Subba Rao, 2000). Srivastava and Srivastava (2008) reported that glycogen reserves consistently decreased from 8.18 to 5.3 mg g^1 in C. punctatus when exposed to sublethal concentrations of $ZnSO_4$. Since carbohydrates serve as the instant energy source during stress so during acute condition blood glucose level increases due to glycogenolysis but reduction can be correlated to utilization of stored glycogen to meet the energy demand or chronic exposure. In liver, glycogen mobilized to glucose whereas in muscle glycogen/glucose served as readily available source of energy, thus hypoglycemia was observed.

Table 2:-Impact of Heavy metal concentrations on glucose and glycogen content of Channa punctatus.

Tissues	Reference Glucose/Glycogen content	Exposed	Percent change over control (%)
Blood (glucose)	1.52 ± 0.02	1.23 ± 0.17	18.0
Liver (glycogen)	3.73 ± 0.02	1.40 ± 0.01	58.0
Muscle (glycogen)	1.13 ± 0.01	0.49 ± 0.02	45.0

Values are mean ± S.D (n = 5).

Blood glucose is given in mg %.
Glycogen values are given in mg/g.
Student's t- test was used to test the significance.

Histopathology

Histological examination of tissues is a useful method to determine the effects of environmental pollutants. Heavy metal toxicity is mediated by producing ROS and free radicals. They produce lesions and damage the tissues. Histological examination of tissues is a useful method to determine the effects of environmental pollutants. Since majority of metals accumulate in gills, major damage has been reported in other organs by most of the workers. Liver is the main metabolic organ where detoxification occurs; therefore, it would also be susceptible to damage by the toxicants. In the present study the liver of fish also exhibited the severe histopathological lesions like deshaped hepatocytes, vacuolization, necrosis of parenchyma, pyknosis and infil- tration of leucocytes. Similar results have been reported in liver of different fishes, Oreochromis mossambicus exposed to cadmium and zinc), Clarias gariepinus exposed to fuel oil for 14 days (Gabriel 2007).

In gills of fishes, the mean lengths of primary and secondary lamellae decreased, curving of primary lamellae, separation of respiratory epithelium and lamellar fusion in secondary lamellae, loss of secondary lamellae, ballooning dilation and clavate lamellae formation at the tip of secondary lamellae, cystic structures within secondary lamellae epithelium were observed in Channa punctatus.

The present study revealed that industrial effluents induced histopathological alterations in gills, liver and kidney of Channa punctatus. Under present investigation, it has been observed that gills of fish exhibited several histological alterations like complete fusion of lamellae coupled with hyperplasia, hypertrophy and epithelial lifting, necrotic and shrinked/curved lamellae. Prolonged exposure to heavy metals can lead to degeneration of the epithelium. It can therefore be argued that gill epithelium was the principal entry point of contamination which on exposure to heavy metals multiplied causing hyperplasia. The alterations observed in gill of Channa punctatus prove that it was under chronic exposure and these changes could be interpreted as defense responses of the fish, as these alterations increase the distance across which the dissolved heavy metals must diffuse to reach the blood stream. Similar results have been reported in fishes such as Labeo rohita exposed to Cr (VI) (Sesha Srinivas and Rao, 1998), Cyprinus carpio to Cr (Parvathi et al. 2011).

Similarly, kidney of Channa punctatus exhibited dilation and vacuolation of kidney tubules, hypertrophy of renal tubules, degeneration of glomeruli and necrosis of hematopoietic tissue. These degenerative changes in kidney were also reported in various fishes such as Clarias batrachus to ZnSO4 (Prasanna Subhash Joshi 2011), Cyprinus carpio to lethal concentrations of Cr (Parvathi et al. 2011). The main function of kidney is washing/filtration of body fluids and to maintain the homeostasis. Severity of lesions observed in the present study showed that uriniferous tubules and hematopoietic tissue was badly damaged which could impair the renal function and as a consequence heavy metals get accumulated in various organs or muscle of the fish.

Human Health

It is unfortunate that there is no water body, which does not have finger prints of human beings. Every water body receives the effluents containing heavy metals either from point or from nonpoint sources. Worst thing about heavy metals is their persistence in environment due to their unbiodegradable nature. It is the reason that aquatic fauna particularly fish bioaccumulate them, and thus, they remain in the tissues of the fish for long time. Fishes are the important source of protein and PUFA; therefore, American Heart Association (AHA) recommended fish twice a week to the human adults. Unfortunately, fishes are now becoming the major source of heavy metals due to the pollution caused by industries. According to United States Environmental Protection Agency (USEPA), these metals generally cause two types of health effects. One is carcinogenic and other is non-carcinogenic effects. According to New York State Department of Health (NYSDOH), the TR categories are described as: TR B 10-6 = low; 10-4 to 10-3 = moderate; 10-3 to 10-1 = high; C10-1 - = very high. Pregnant women, lactating mother and children are more prone to heavy metal health hazards. In one of the recent study on risk assessment via consumption of M. armatus, it has been reported that accumulation of Co (9.06 mg/kg dry weight) and Ni (58.98 mg/kg dry weight) pose non-carcinogenic risk to adult male and female individuals, whereas carcinogenic risk posed by this fish was in the range 3.43 9 10-3 and 3.91 9 10-3, respectively, for Ni (Javed and Usmani, 2016).

Conclusion

The effluent from the industries containing heavy metals influenced the water quality of the rivulet under study. Based on the current review reports and opinion of other investigators from the aquatic toxicology field, it is believed that for biomonitoring studies only single parameter is insufficient, rather there is a need of battery of biomarkers. Concentrations of heavy metals assessed Fe, Ni, Mn and Cr were found to exceed the permissible limits set for water quality for ecosystem and human health. These heavy metals also influence fish physiology. Hence, some scientific method of detoxification is essential to improve the health of these economic fish in the stressed environmental conditions. These not only influence quality of water, but they directly influence flora and fauna Hence, biomarkers such as bioaccumulation, lipid and protein profiles, pathological marker enzyme activities, enzymatic and non-enzymatic antioxidants, lipid peroxidation, DNA damage and tissue damage could serve as useful tools to monitor the health of aquatic fauna. These heavy metals will enter the food web through water and food, to cause the adverse health effects like that in indicator organisms. There is no denying that industries are necessary for development, but on the other hand they are also creating heavy loss to the livelihood of humans. Since virtually all metals investigated were found in higher concentration, so government should intact laws which will ensure that industries make use of standard waste treatment plants for the treatment of their wastes before they are being discharged into water bodies. Some monitoring programs should also be launched from time to time in order to prevent the misuse of valuable water resources, to check their water quality status, and to restore them for the welfare of society and to protect the natural environment. Since these fishes share the local market therefore they must be screened by food agencies before they reached the humans in order to avoid the epidemics as occurred in Japan (1956) due to consumption of heavy metal contaminated fish and fishery products. Further studies are suggested particularly on the reproductive aspects of the fish in order to check its reproductive health/potential which will help to conserve the species.

Acknowledgements The authors wish to acknowledge Supervisor, Pofessor Department of Zoology for providing necessary facilities. The abides in Compliance with Ethical Standards.

Conflict of interest The authors declare that they have no conflict of interest.

References

Ahmad I, Maria VL, Oliveira M, Pacheco M, Santos MA (2006) Oxidative stress and genotoxic effects in gill and kidney of *Anguilla anguilla L*. exposed to chromium with or without pre- exposure to b-naphthoflavone. *Mutat Res 608*:16–28.

APHA (1998). American Public Health Association: Standard Method for Examination of Water and Waste Water. 20th ED. Lenore S. C., Arnold E.G. and Andrew D. E.

Ashraj,W, (2005), Accumulation of heavy metals in kidney and heart tissues of *Epinephelus micodon* fish from the Arabian Gulf. *Environmental Monitoring Assessment, 1-3(103),* pp 311- 316.

Barlas N, (1997), A pilot study of heavy metal concentration in various environments, fishes in the upper Sakarya River Basin, Turkey. *Environmental Toxicology, 14*, pp 367- 373.

Barsiene J, Aleksandras R, Thomas L, Laura AN, Aleksandras M (2013) Environmental genotoxicity and cytotoxicity levels in fish from the North Sea offshore region and Atlantic coastal waters. *Mar Pollut Bull 68*:106–116.

Basa Siraj and Usha Rani (2003). Cadmium induced antioxidant defense mechanism in freshwater teleost *Oreochromis mossambicus* (tilapia). *Ecotoxicology and Environmental Safety, 56 (2*), pp 218-221.

Bhagwant, S. and K.B. Elahee, (2002), Pathologic gill lesions in two edible lagoon fish species, *Mulloidichtys flavolineatus* and *Mugil cephalus*, from the of poudre d' or, mauritis. *Western Indian Ocean Journal of Marine Sciences, 1*, pp 35-42.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein–dye binding. *Anal. Biochem. 2*, 248–254.

Breen AP, Murphy JA (1995). Reactions of oxyl radicals with DNA. *Rev Free Radic Biol Med 18*:1033–1077.

Canli, M. Ay, O. Kalay, M, (1998), Levels of heavy metals (Cd, Pb, Cu and Ni) in tissues of *Cyprinus carpio, Barbus capito* and *Chondrostoma regium* from the Seyhan river. *Turkish Journal of Zoology, 3(22*), pp 149- 157.

Carrol NV, Longley RW, Roe JH (1956). Glycogen determination in liver and muscle by use of anthrone reagent. *Journal of Biological Chemistry* 22:583–593.

Cerutti PA (1985) Prooxidant states and tumor promotion. *Science 227*:375–381.

Connell BS, Cox M, Singer I (1984) Nickel and Chromium. In: Brunner F, Coburn JW (eds) Disorders of minerals metabolism. *Academic Press, New York*, pp 472–532.

Crafford D, Avenant-Oldewage A (2010) Bioaccumulation of non-essential trace metals in tissues and organs of *Clarias gariepinus* (sharptooth catfish) from the Vaal River system— strontium, aluminium, lead and nickel. *Water SA 36*:621–640.

Department of Water Affairs and Forestry (1993) South African water quality guidelines. *Volume 1: Domestic use*, 1st edn. *Department of Water Affairs and Forestry*, Pretoria.

Dirilgen, N, (2001), Accumulation of heavy metals in fresh water organisms: Assessment of toxic interactions. FAO. *Fischer. Technology, 212*, pp 1- 13.

Duncan, D.B. (1955). Multiple ranges and multiple F- tests. Biometrics. Fisheries Resources. 11:1-42.

Eaton, Andrew D. "Standard methods for the examination of water and wastewater." *APHA-AWWA-WEF* (2005).

Fabbri, E., A. Capuzzo and T.W. Moon, (1998), The role of circulating catecholamines in the regulation of fish metabolism: An overview, *Comp. Biochemical Physiology C Pharmacology Toxicology Endocrinology, 120,* pp 177- 192.

Farombi EO, Adelowo OA, Ajimoko YR (2007) Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African cat fish (*Clarias gariepinus*) from Nigeria Ogun River. *International Journal of Environ Res Public Health 4*:158–165.

Gabriel UU, Ezeri GNO, Amakiri EU (2007) Liver and kidney histopathology: biomarkers of no. 1 fuel toxicosis in African catfish, *Clarias gariepinus*. *J Anim Vet Adv 6*:379–384.

Geeraerts, C. and C. Belpaire, (2009), The effects of contaminants in European eel: A review. *Ecotoxicology, 19,* pp 239- 266.

Gleick PH (1996) Water resources. In: Schneider SH (ed) Encyclopedia of climate and weather, vol 2. *Oxford University Press, New York,* pp 817–823.

Gopal, V., Parvathy, S., Balasubramanian, P.R., 1997. Effect of heavy metals on the blood protein biochemistry of the fish *Cyprinus carpio* and its use as a bioindicator of pollution stress. *Environ. Monit. Assess. 48, 117–124*.

Griffith OW (1999) Biologic and pharmacologic regulation of mammalian glutathione synthesis. *Free Radic Biol Med 27*:922–935

Habig, W. H., Pabst, M. J., & Jakoby, W. B. (1974). Glutathione- S-transferases. The first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry, 249*, 7130 –9.

Hanan SG, El-Kasheif MA, Ibrahim SA, Authman MMN (2013) Effect of water pollution in El-Rahawy drainage canal on hematology and organs of freshwater fish *Clarias gariepinus*. *World Appl Sci J 21*:329–341.

Hawkes JS (1997) Heavy metals. *J Chem Educ 74*:1374.

Jacobs MN, Covaci A, Schepens P (2002) Investigation of selected persistant organic pollutants in farmed Atlantic salmon (*Salamo salar),* salmon aquaculture feed and fish oil components of the feed. *Environ Sci Technol 36*:2797–2805.

James R, Sampath K, Alagurathinam S (1996) Effects of lead on respiratory enzyme activity, glycogen and blood sugar levels of the teleost *Oreochromis mossambicus* (Peters) during accumulation and depuration. *Asian Fishery Sci9*:87–100.

Javed M, Usmani N (2011) Accumulation of heavy metals in fishes: a human health concern. *Int J Environ Sci 2*:659–670.

Javed M, Usmani N (2015) Stress response of biomolecules (carbohydrate, protein and lipid profiles) in fish *Channa punctatus* inhabiting river polluted by Thermal Power Plant effluent. *Saudi Journal of Biological Science 22*:237–242.

Javed M, Usmani N (2016) Accumulation of heavy metals and human health risk assessment via the consumption of freshwater fish *Mastacembelus armatus* inhabiting, thermal power plant effluent loaded canal. *SpringerPlus 5:*776. doi: 10.1186/s40064-016-2471-3.

Jollow, D. J., Mitchell, J. R., Zampaglione, N. & Gillette, J. R. Bromobenzene induced liver necrosis. Protective role of glutathione and evidence for 3,4- bromobenzene oxide as the hepatotoxic metabolite. *Pharmacol. 11, 151–169,* doi:10.1159/000136485 (1974).

Kennish L (1992) Toxicity of heavy metals: effects of Cr and Se on human's health. *J Indian Public Health Educ 2*:36–64.

Koca, S., Koca, Y. B., Yildiz, S., & Gurcu, B. (2008). Genotoxic and histopathological effects of water pollution on two fish species, *Barbus capito pectoralis* and *Chondrostoma nasus* in the Buyuk Menderes River, Turkey. *Biology of Trace Elements and Research, 122(3)*, 276–291..

Mai H, Je´roˆme C, Justine B, Oliver G, Angel B, He´le`ne B, Be´ne´dicte M (2012) Embryotoxic and genotoxic effects of heavy metals and pesticides on early life stages of Pacific oyster (*Crassostrea gigas*). *Mar Pollut Bull 64*:2663–2670.

Marklund, S., & Marklund, G. (1974). Involvement of the super- oxide anion radical in the autooxidation of pyrogallol and a convenient assay for superoxide dismutase. *European Journal of Biochemistry, 47(3*), 469–474.

Maruthi, Y.A., Subba Rao, M.V., 2000. Effect of distillery effluent on biochemical parameters of fish, Channa punctatus (Bloch). J. Environ. Pollut. 7, 111–113.

Mazeoud, M.M., Mazeoud, F., Donaldson, E.M., 1977. Primary and secondary effect of stress in fish: some new data with a general review. Trans. Am. Fish. Soc. 106, 201–212.

Nussey G (1998) Metal ecotoxicology of the upper Olifants River at selected localities and the effect of copper and zinc on fish blood physiology. Ph.D. thesis, Rand Afrikans University, South Africa.

NYSDOH (New York State Department of Health) (2007) Hopewell precision area contamination: appendix C-NYS DOH. Procedure for evaluating potential health risks for contaminants of concern. http://www.health.ny.gov/environmental/investiga tions/hopewell/appendc.htm.

Olaifa, F., Olaifa, A., Adelaja, A., & Owolabi, A. (2010). Heavy metal contamination of *Clarias gariepinus* from a lake and fish farm in Ibadan, Nigeria. *African Journal of Biomedical Research*, *7*(3). https://doi.org/10.4314/ajbr.v7i3.54185.

Oner M, Atli G, Canli M (2008) Changes in serum biochemical parameters of freshwater fish Oreochromis niloticus following prolonged metal (Ag, Cd, Cr, Cu, Zn) exposures. Environ Toxicol Chem 27:360–366.

Palanisamy, P.G., Sasikala, D., Mallikaraj, N.B., Natarajan, G.M., 2011. Electroplating industrial effluent chromium induced changes in carbohydrates metabolism in air breathing cat fish *Mystus cavasius* (Ham). *Asian J. Exp. Biol. Sci. 2*, 521–524.

Parvathi K, Palanivel S, Mathan R, Sarasu (2011) Sublethal effects of chromium on some biochemical profiles of the fresh water teleost, *Cyprinus carpio*. *Int J Appl Biol Pharm Technol 2*:295–300.

Perrier H, Perrier C, Peres G, Gras J (1979) The lipoproteins of the plasma of the rainbow trout (*Salmo gairdnerii Richardson*): immunoelectrophoresis, selective precipitation and lipid composition. *Comp Biochem Physiol 62 B*:245–248.

Prasanna SJ (2011) Studies on the effects of zinc sulphate toxicity on the detoxifying organs of fresh water fish *Clarias batrachus* (Linn.). *Gold Res Thoughts 1*:1–4.

Radhakrishaniah K, Venkataramana P (1992) Effect of lethal and sublethal concentrations of copper on glycolysis in the liver of the fresh water teleost, *Labeo rohita* (Ham). *J Environ Biol 13*:63–68.

Rani Usha A, (2000), Cadmium induced bioaccumulation in tissue of freshwater teleost *Oreochromis mossambicus*. *Ann. N.Y. Academy,1(919)*, pp 318-320.

Segner, H, (1998), Isolation and primary culture of teleost hepatocytes. *Comp. Biochemical Physiology A Molecular and Integrative Physiology, 120*, pp 71-81.

Sesha Srinivas, V., & Rao, B. M. (1998). Chromium induced alterations in the gill of the freshwater teleost fish, *Labeo rohita*. *Indian Journal of Comparative Animal Physiology, 17*, 31 –33.

Shaheen T, Akhtar T (2012) Assessment of chromium toxicity in *Cyprinus carpio* through hematological and biochemical blood markers. *Turk J Zool 36*:682–690.

Shelke Abhay D (2013) Comparative study of cholesterol alterations in a freshwater teleost fish, *Ambylpharyngodon mola* exposure to heavy metals. *Bioscan 8*:1001–1004.

Sheriff, S.A., Balasubramanian, S., Baranitharan, R., Ponmurugan, P., **2014.** Synthesis and in vitro antioxidant functions of protein hydrolysate from backbones of Rastrelliger kanagurta by proteolytic enzymes. Saudi J. Biol. Sci. 21, 19–26.

Spector, T., 1978. Refinement of the Coomassie blue method of protein quantitation. A simple and linear spectrophotometric assay for less than or equal to 0.5 to 50 micrograms of protein. Ann. *Biochem. 86, 142–146*.

Srivastava, R., Srivastava, N., 2008. Changes in nutritive value of fish, Channa punctatus after chronic exposure to zinc. J. Environ. Biol. 29, 299–300.

Tabrez, S., Shakil, S., Urooj, M., Damanhouri, G.A., Abuzenadah, A.M., Ahmad, M., 2011. Genotoxicity testing and biomarker studies on surface waters: an overview of the techniques and their efficacies. J. Environ. Sci. Health, Part C 29, 250–275.

Tadashi F (2002) Formation and removal of reactive oxygen species, lipid peroxides and free radicals, and their biological effects. *Yakugaku Zasshi 122*:203–218.

UNEPGEMS (United Nations Environment Programme Global Environment Monitoring System/ Water Programme) (2006) (Water Quality for Ecosystem and Human Health). ISBN 92-95039-10-6.

USEPA (United States Environmental Protection Agency) (2011) USEPA Regional Screening Level (RSL) summary table: November 2011. http://www.epa.gov/regshwmd/risk/human/ Index.htm.

Velez, D. Montoro, R, (1998). Arsenic speciation in manufactured seafood products: a review. *Journal of food Protect, 9(61*), pp 1240-1245.

Vinodhini R, Narayanan M (2009) The impact of toxic heavy metals on the hematological parameters in common carp (*Cyprinus carpio L*.). *Iran J Environ Health Sci Eng 6*:23–28.

Vosyliene, M. Z. Jankaite, A, (2006), Effect of heavy metal model mixture on rainbow trout biological parameters *Ekologija, 4*, pp12-17.

Waqar, A, (2006), Levels of selected heavy metals in Tuna fish. *Arab Journal of Science and Engineering, 1A (31)*, pp 89– 92.

Wittmann GTW (1997) Toxic metals. In: Forstner U, Wittmann GTW (eds) Metal pollution in the aquatic environment. *Springer, Berlin*, pp 3–70.

Zutshi BSG, Prasad R, Nagaraja R (2010) Alteration in hematology of *Labeo rohita* under stress of pollution from Lakes of Bangalore, Karnataka, India. *Environ Monit Assess 168*:11–19.