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Assessment of mercury load in river Ravi, urban sewage streams of Lahore Pakistan and its impact on the oxidative stress of exposed fish

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Abstract

The purpose of the present work was Mercury determination in river Ravi, different nalas from Lahore, Head Balloki water and their sediments located in Punjab Pakistan in the summer and winter seasons. Tekran-2500 cold vapor atomic fluorescence spectrometer employed for determination of Hg in water samples and Zeeman mercury analyzer for sediments. The results show highest Hg concentration in Hudaira drain at all three sampling areas in the summer season. Mehmood Booti nala showed high concentration at terminal point before entering into river water with 84.6 ± 28.11 ng/l concentration. Water at Head Balloki showed 67.3 ± 14.5 ng/L concentration in the side running water. The river water sampling before the studied point also showed 1.2 ± 0.4 ng/l concentration. Bhek Nala and Farrukhabad Nala showed relatively less concentration of mercury compared to other nalas. The mercury level was found highest in the sediments of Hudaira drain in both seasons. The high concentration in water tends to accumulate in the different organs of the aquatic organisms. In the current study of Tilapia and *Labeo rohita* shows the order of the concentration from higher to lower was kidney, liver, gills and muscle where in case of tilapia the accumulation order is slightly different starting from gills, kidney, liver and muscle respectively. The level of CAT was decreased in response to high concentration. Mercury treatment stimulated the liver and gill tissue to significantly increase the level of SOD which might be due to synthesis of SOD and addition to the pre-existing SOD level. The MDA level of gills and liver tissue was increased with the increase in the concentration.

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Introduction

Heavy metals are among the most common environmental pollutants, and their occurrence in water and biota indicate the presence of natural sources, associated with chemical weathering of minerals and soil leaching, or anthropogenic sources that are mainly concerned with industrial and domestic effluents, urban storm, water runoff, landfill, mining of coal and ore, atmospheric sources and inputs rural areas. Water pollution by trace metals is an important factor in both geochemical cycling of metals and in environmental health. The existence of heavy metals in aquatic environments has led to serious concerns about their influence on plant and animal life (Luoma *et al.*, 2008; Förstner and Wittmann, 2012; Ghaleno *et al.*, 2015). Rivers are one of the important fresh water reservoirs and are threatened with the pollution resulting from urban and industrial waste water which is disposed off into rivers without any treatment (Yasar *et al.*, 2010; Khan *et al.*, 2015; Nzeve *et al.*, 2015).

Pakistan is blessed with rivers and all of them flow through Punjab province Pakistan. River Ravi arises in Bara Bangal, a branch of the Dhauladhar range of Himalayas in Chamba district of Himachal Pradesh, a state of India and finally enters into Punjab Province of Pakistan after cutting Dhauladhar (Förstner and Wittmann, 2012).

Intense pollution contributed by both India and Pakistan is threatening irrigation and ground waters and also terrestrial and aquatic life in River Ravi. About 1307.08 tons of hazardous and untreated waste is going into Ravi on daily basis. The load from district Lahore is 728.75 tons per day. Food chain contamination by heavy metals has become a burning issue in recent years because of their potential accumulation in bio stems through contaminated water, soil and air. Therefore, the study of river ecotoxicity has gained immense importance because of multiple use of river water for human consumption, agriculture and industry (Javed and Hayat, 1999; Rood River, 2012; Damodharan, 2013;

Khan *et al.*, 2016).

Mercury is among the most toxic elements present in industrial and urban waste water. The major sources of mercury in the environment are increasing due to discharge from hydroelectric, mining, pulp, dental preparation, and paper and cement industries (Luoma *et al.*, 2008; Förstner and Wittmann, 2012). Although most mercury compounds and uses are now banned or about to be banned, however, mercury is still used in thermometers and dental amalgams. Also, mercury can still be found as an additive in old paints for water proofing and marine antifouling (mercuric arsenate), in old pesticides (mercuric chloride in fungicides, insecticides), in wood preservatives (mercuric chloride), cosmetic products, in embalming fluids (mercuric chloride), in germicidal soaps and antibacterial products (mercuric chloride and mercuric cyanide), as mercury-silver-tin alloys and for "silver mirrors" (Clifton, 2007).

The estimated emissions of mercury worldwide are reported to be about 2,200 metric tons per annum. Interestingly, one-third of this quantity arises from natural sources and two-third from human activities such as fossil fuels burning and medical wastes. Mercury may exist as inorganic mercury in the form of HgO, Hg⁺², or as organic mercury in the form of methyl mercury (MeHg), ethylmercury, and dimethylmercury. All forms of mercury pose risk of toxicity to humans. Analytical speciation is therefore essential to determine the various forms of Hg in order to predict their effects on biological systems including human beings (Boszke *et al.*, 2002; Baeyens *et al.*, 2007; Houston, 2011; Karagas *et al.*, 2012).

The accurate determination of Hg and other elements in environmental, biological or medical samples is a very challenging task. Extremely low levels of this metal combined with numerous interferences in the most sensitive analytical techniques are considered to be the major difficulties. The most appropriate techniques for the determination of Hg include inductively coupled plasma mass spectrometry (ICP-

MS), especially in combination with isotope dilution mass spectrometry (IDMS), inductively coupled plasma atomic emission spectrometry (ICP-OES), and electro-thermal atomic absorption spectrometry (ET-AAS). However, all of the mentioned techniques suffer from severe limitations which restrict their application to selected samples only - in particular some methods work only for a specific type of sample and are inadequate for the analysis of other sample types. For example ICP-OES and ET-AAS can be applied, but imply prior pre-concentration and/or matrix-separation steps to assess Hg and As concentrations at the required trace levels. ICP-MS suffers from matrix induced non-spectral interferences, as well as spectral interferences caused by monoatomic or polyatomic ions produced in the plasma from matrix components (Mandal & Suzuki, 2002; Mukhtar & Limbeck, 2010; Sanchez-Rodas *et al.*, 2010; Azizullah *et al.*, 2011; Yin *et al.*, 2013). The AFS could be of great interest for the determination of Hg in water samples without requiring the use of expensive ultrasensitive multi elemental instrumentation, like ICP-MS (Ammann, 2011).

A lot of work has done on the toxicity of heavy metals in aquatic environment nationally and internationally. The heavy metals enter into the aquatic organisms through gills and food web and accumulated in the different organs. After accumulation the metals causes toxicity to oxidative stress leading to the genotoxicity (Khan *et al.*, 2015a; Khan *et al.*, 2015b). In an experiment on *Oreochromis mossambicus* in Indus River in Mianwali (Pakistan) studies revealed pathological changes in liver and gill tissues, this shows these biomarkers can be employed to evaluate the toxicity of heavy metals in aquatic life (Jabeen and Chaudhry, 2013).

In current study, the Hg concentrations has been determined in River Ravi and various sewage and industrial effluents falling into the river, metal bioaccumulation and its effects on antioxidant enzymes was studied.

Materials and methods

The different waste water falls into River Ravi named Mahmood Booti, Bhek Nala, Farrukh Abad drain, Bakar Mandi Nala and Hudiara drain were selected as sampling points at three locality 1, 2 and 3 for start of nala, at the start of mixing nala waste with river water and at terminal of nala before it drops its wastes in the river respectively where in river water and samples were sampled at three localities including locality-1 middle of river, locality-2 side of river running water and locality-3 standing waters before the entrance of these nalas in the River. Similar sampling was done at head Balloki region.

The sampling of water and sediments was done during time period of August, and January, at above mentioned points. The two seasons (summer and winter) were representative of seasonal variation of heavy metal pollution of River Ravi. Additionally, sampling of fresh Ravi water and sediments was also done before the beginning of waste water tributaries in order to check the quality of non-contaminated water. The sampling of water and sediments was done in pre-cleaned 50 ml polypropylene tubes. For cleaning the tubes were soaked well before use in 2% (v/v) HNO₃ solution for 24 hours followed by drying in an oven at 70 °C till dryness. The tubes were filled with surface water from river and above mentioned points and preserved by adding HNO₃ (1% v/v). Similarly, sediments of river and waste drains were collected at the same points where from water samples were collected. All precautionary measurements were taken during sampling in order to avoid contamination of water and sediment samples from external sources.

The samples were transported to lab after sampling. For measurement of as in water samples, about 2 ml Conc. HNO₃ was added to 20 ml of water samples taken into Teflon digestion cups followed by heating at 90°C for 1 hour with cover in order to avoid sample losses. This led to the digestion of organic constituents of water samples in order to get clear solution. After digestion of unwanted material, the

water samples were allowed to cool down to room temperature and transferred to centrifugal tubes. The samples were stored in refrigerator at 4 °C until analysis.

For digestion of sediment samples, approximately 0.05-0.1 g of samples was weighed into Teflon digestion cup. To these samples, 3 ml of conc. HNO₃ were added and allowed to stand till no bubbles in the solution. The Teflon cups were placed into steel sleeve which was transferred to an oven and the samples were treated at 150-155 °C for 6 hour. Afterwards the samples were allowed to cool down to room temperature followed by addition of 1-2 ml of H₂O₂ and subsequent heating at 90 °C for 1 hour while keeping the Teflon cups covered with a lid. After complete digestion of organic matter in sediment samples, the samples were heated nearly to dry. The supernatant liquid was separated from the rest of undigested material and diluted to desired volume using double distilled water. About 5 ml of conc. HCl was added to 25 ml of supernatant solution followed by addition of 10 ml sulfocarbamide solution (10 %) and the sample solution was diluted to final volume of 50 ml. The samples were kept stored in refrigerated at 4 °C until analysis.

Total Hg in water samples was measured in accordance to United States Environment Protection Agency (USEPA 1631E) (Mukhtar & Limbeck, 2011). Before Hg analysis, the water samples were treated as described below. Approximately, 0.5% (v/v) bromine monochloride (BrCl, 25%) added into 25ml water samples with 0.4 % HCl (v/v) at least for 24 hours. The BrCl was used to convert all Hg into Hg (II) form. After oxidation, the sample is sequentially reduced with NH₂OH.HCl to destroy the free halogens, then reduced with stannous chloride (SnCl₂) to convert Hg (II) to volatile Hg(o). The Hg (o) is separated from solution by purging with nitrogen and collected on the Gold-trap. The Hg is thermally desorbed from the gold trap into an inert gas stream that carries the released Hg (o) to a second gold (analytical) trap. The Hg is desorbed from the analytical trap into a gas

stream that carries the Hg into the cell of a cold-vapor atomic fluorescence spectrometer (CVAFS) for detection.

Gold trap apparatus for capturing Hg (o)

For determination of total Hg in sediment samples, the samples were frozen dried in order to avoid Hg losses due to heating. After drying, a weight quantity (50-100 g) of sediment samples was placed in to the sample injector of Lumex RA 915M. The instrument burnt the sediment samples to determine total Hg.

Metal content in the fish tissues

30 fishes of both *L. rohita* and *talipia* were maintained in the separately in the test aquaria with capacity of 40 liters. The fishes were accumulated for 2 weeks and then test concentration of mercury (100, 200, 300, 400, 500 ng/l) was applied for a period of 28 days in both summer and winter season. The fishes were selected randomly and dissected and desired organs including liver, gills, kidney and muscles. These organs were carefully removed using clear and sterilized instruments. The samples were washed with double distil water and allowed to dried in the oven at 120C. One gram of each sample was digested in di-acid solution consisting of HNO₃ and HClO₄ in 2:1 ratio on a hot plate at 130 °C till dissolving all the materials. Each sample was then diluted with double distil water approximately to equal concentration of standards. The standards were prepared from the metal solution (Merck Germany). The metal concentration in each sample was measured through atomic absorption spectrophotometer and the results were expressed in the terms of ng/g dry weight.

Enzyme Analysis of Oxidative Stress

The activities of CAT, SOD level of MDA was recorded from the gill and liver tissue extract. Superoxide dismutase (SOD) was recoded according to the protocol of Payá *et al.*, (1992) with modifications made by Peixoto and Pereira-Moura (2008). The reaction mixture was consisted of 100 mM buffer of phosphate having pH 7.0, 10 mM NBT and 10 mM

hypoxanthine. The reaction was started by mixing 0.023 U/mol of xanthine oxidase with extracts of enzymes maintaining the temperature at 20 °C. The activity of enzyme was measured by 50 % inhibition of NBT. The results were expressed as U/mg protein.

Aebi (1984) was followed for estimating the activity of CAT. About 50 mM solution of H₂O₂ was used as substrate. The solution was prepared in 50 mM of buffer of potassium phosphate. The CAT decomposed the H₂O₂, which was measured through spectrophotometer at 240 nm wave length maintaining the pH at 7.0.

According to the Buege and Aust (1978) lipid peroxidation is the measure of MDA content (malondialdehyde). In the methodology, 1 ml sample

from homogenates was mixed with 2 ml of TBA. TCA-HCl. The mixture was then heated in a medium of water bath and then cooled, centrifuged at 4000 rpm (15 minutes). The absorbance was recorded at 535 nm using blank as standard. The MDA content was expressed as μmol/ mg protein.

Results and discussion

The Hg concentrations have been found to be varied between as low as the detection limit (less than 1 picogram/L) in the river water to one hundreds of ng/L in the water samples. During summer, the highest concentration of Hg 124.3±22.65 was observed in the Hudiarra drain at locality 1 which was the most polluted site among the selected ones. The obtained results have been shown in Table-1.

Table 1. Hg concentrations (ng/L) in the water samples during summer and winter season.

Hg concentrations (ng/L) in the water samples during summer season							
Locality	River water	Mehmood Booti	Bhek Nala	Farrukhabad Nala	Bakar Mandi Nala	Hudiarra drain	Head Balloki
Loc-1	1.2±0.4	19.4±3.54	3.6±0.87	3.8±0.87	6.3±2.34	124.3±22.65	67.3±14.51
Loc-2	LOD	33.5±8.12	8.4±2.34	5.6±1.87	18.3±7.26	89.7±26.72	56.3±18.23
Loc-3	0.87±0.05	84.6±28.11	12.4±3.77	20.5±6.91	14.4±4.87	123.6±33.65	29.6±9.31
Hg concentrations (ng/L) in the water samples during winter season							
Loc-1	LOD	36.7±12.32	34.6±15.55	19.4±7.23	13.5±5.87	167.7±51.17	64.7±12.24
Loc-2	0.3±0.02	35.6±11.45	16.8±7.33	8.6±5.37	9.3±3.45	120.4±35.72	95.7±25.71
Loc-3	LOD	53.2±19.62	16.4±6.98	22.1±6.39	20.4±9.21	95.3±33.23	102.3±31.12

The lowest Hg concentration was observed in the fresh river water with ranging from LOD to 1.2±0.4 ng/L indicating that fresh Ravi water is not contaminated with Hg before spilling these contaminated water of these nalas. Similar findings were observed in the water samples collected during winter season where concentration found slightly higher in the winter season in all the sites (Table-1).

The results of Hg reported in current study have been find far above than those reported by Hines *et al.*, (2000) in Idrija river showing total Hg concentrations of 60 ng/L. The presence of total Hg in such concentrations is hazardous for the living

organisms. The most contaminated drain was found to be the Hudiarra which deteriorates the quality of river water to a greater extent. There are several industrial units along this drain which throw their waste waters without any treatment into Hudiarra drain. The elevated Hg levels can be reduced by proper treatment of waste water effluent plants and by use of less toxic industrial chemicals in contrast to Hg containing chemicals.

While interpreting the results for sediment samples, the Hg concentration has been observed between 1.2±0.76 3.7±1.23 ng/g in summer and 1.1±0.12 ng/g 2.1±0.45 in winter season in river sediment before

accompanying any of the waste water effluent. These results were not threatening and were within the safe limits. The Hg concentrations in various selected drains during summer were found to be drastically higher than the fresh water sediments as shown in Table-2. The results indicated the highest concentration of Hg in Hudiarra drain ranging from 845.3±356.29 ng/g to 1530.4±671.01 ng/g in the

summer and 980.6±355.91 ng/g to 1284.0±321.89 ng/g in the winter season respectively.

At head Balloki point the concentration of Hg range 446.5±256.09 to 670.4±213.71 in the summer and 630.5±257.50 ng/g to 1195.3±472.81 ng/g in the winter season respectively (Table-2).

Table 2. Hg concentrations (ng/g) in the sediment samples during summer and winter season.

Hg concentrations (ng/g) in the sediment samples during summer season							
Locality	River sediment	Mehmood Booti	Bhek Nala	Farrukhabad Nala	Bakar Mandi Nala	Hudiarra drain	Head Balloki
Loc-1	1.2±0.76	997.4±338.11	24.3±8.82	12.6±4.28	13.4±5.24	1110.2±498.05	670.4±213.71
Loc-2	2.1±0.97	1213.5±472.54	29.6±7.38	11.5±4.81	8.1±4.22	1530.4±671.01	446.5±256.09
Loc-3	3.7±1.23	1256±378.23	35.9±9.55	7.9±2.10	8.9±4.67	845.3±356.29	565.4±241.83
Hg concentrations (ng/g) in the sediment samples during winter season							
Loc-1	1.1±0.12	1135.7±432.64	23.4±6.82	15.4±4.91	6.5±2.52	1284.0±321.89	788.1±341.04
Loc-2	1.4±0.23	1133.5±387.36	32.5±7.61	8.5±3.83	12.5±5.47	1135.4±387.68	1195.3±472.81
Loc-3	2.1±0.45	820.5±327.75	33.5±6.33	4.6±2.30	7.23±3.21	980.6±355.91	630.5±257.50

Such higher concentrations have been resulted in the deterioration of river water quality due to elevated Hg levels. Hg concentrations in Head Balloki sediments were increased by almost 2 folds during winter season in contrast to the summer season. This outcome can be justified by the seasonal deficiency of fresh water

in the river Ravi and by the stoppage of Qadra Abad link canal thereby increasing the Hg concentration. It has been well known that a huge quantity of soil and sediment come down into the rivers due to water erosion which is deposited on the river beds.

Table 3. Bioaccumulation of Hg concentrations (ng/g dry wight) in the different tissues of test fishes

Fish model	Liver	Gills	Kidney	Muscle
Summer session				
<i>L.rohita</i>	0.31 ± 0.22 ^A	0.23 ± 0.10 ^A	0.38 ± 0.09 ^A	0.24 ± 0.140 ^A
Tillapia	0.26±0.13 ^B	0.33±0.07 ^B	0.28±0.06 ^C	0.22±0.10 ^{AB}
Winter session				
<i>L.rohita</i>	0.29 ± 0.20 ^{AB}	0.31 ± 0.06 ^C	0.33 ± 0.08 ^B	0.21 ± 0.11 ^B
Tillapia	0.22±0.11 ^C	0.11±0.06 ^D	0.24±0.06 ^D	0.20±0.07 ^{AB}

Values are mean ±SD of five replicates.

Values sharing the same letter in the same column are not significantly different at 5% level.

Additionally, the presence of contaminated sediment has been found to be the secondary source of pollution where from the contaminants are transported to the irrigation channels and finally the agricultural fields (Boszke *et al.*, 2002; Luoma *et al.*, 2008). The reported results for Hg concentrations in

present study have been noted to be quite high than those reported by in Yu *et al.*, (2012) sediments from the Pearl River Estuary, Southern China showing Hg concentrations of 453 ng/g. In comparison to this finding, the results observed in selected drain sediments are considerably higher indicating high

level of Hg pollution. Therefore, this problem of Hg pollution should be addressed properly to control Hg pollution.

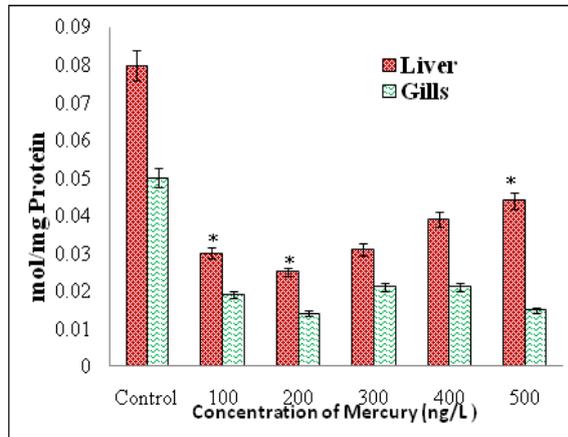


Fig. 1. Effect of low and high dose of Mercury on the activity of CAT in the laboratory condition (Asterisk represents significantly different activities at different concentration in liver and gills tissue).

Hg has found to be a lethal element which can lead to very severe diseases and thus may lead to death. Since Hg has been found to be transformed into more toxic forms like methyl mercury which can be bio-accumulated into the tissues of living organisms especially fish. The organic forms of mercury i.e., methyl and dimethyl Hg were found to be fat soluble and hence could be bio-accumulated in the tissues of living organisms and thus become source of Hg contamination in the food web. To find out such toxic effects on the antioxidant system of exposed organisms and Hg bioaccumulation in the different organs, two fishes Tilapia and *L.rohita* was exposed to different concentration under the laboratory conditions.

The fish *L. rohita* showed high tendency for accumulation of mercury in all the tissues. These values were found significantly different from the other fish Tilapia (Table-3). Kidney tissue shows high tendency for the accumulation of the metal 0.376 ± 0.09 for Rohu and 0.276 ± 0.056 tilapia respectively. Moreover, significantly higher accumulation was occurred in the summer season than winter in all tissues of both species. The order of accumulation of

mercury was kidney > liver > muscle > gills tissues (Table-3). Similar results were found in the studies of Wei *et al.*, (2014) where recorded the highest concentration of the Hg in the muscles of the studied fish.

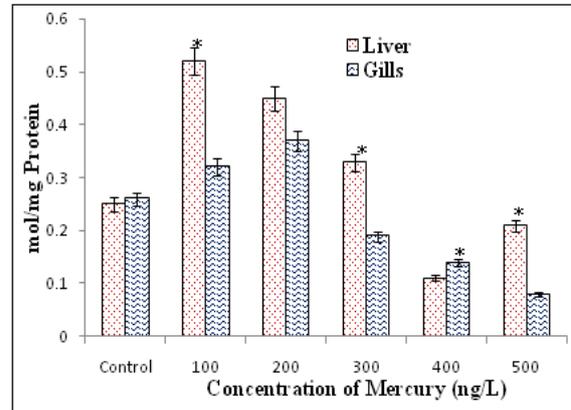


Fig. 2. Effect of low and high dose of Mercury on the activity of SOD in the laboratory condition (Asterisk represents significantly different activities at different concentration in liver and gills tissue).

The oxidative stress may be the disturbance between ROS and ability of antioxidant system to remove them in the biological systems. Jones (2006) defined it as disorder of redox signalling and control. According to Sies (1991) the term oxidative stress means serious imbalance between antioxidant and production of ROS (Datta *et al.*, 2015). ROS increase dramatically during the oxidative stress and causes lipid peroxidation, intact with nucleic acid, lipid and protein and causes loss of membrane integrity, functional changes and mutation. All these factors contribute to health disorder (Kataria *et al.*, 2012).

Metals are the main causes of oxidative stress in the contaminated environment and the main mechanism of metals toxicity is oxidative stress. Mercury triggered oxidative stress in both experimental fish. Due to oxidative stress there are typical changes in the activity of SOD, CAT and in the levels of MDA. The activities of enzyme SOD increases dose dependently and their level increases significantly in comparison to the control ($p \text{ value} \leq 0.05$), while the activity of CAT decreases dose dependently (Fig.1 &2). Wiggers *et al.* (2008) found that low concentration of

Hg causes the oxidative stress and increase the cardiovascular risk in the exposed wistar rat. They concluded from their studies that Hg at low concentration in the environmental risk of producing the cardiovascular diseases.

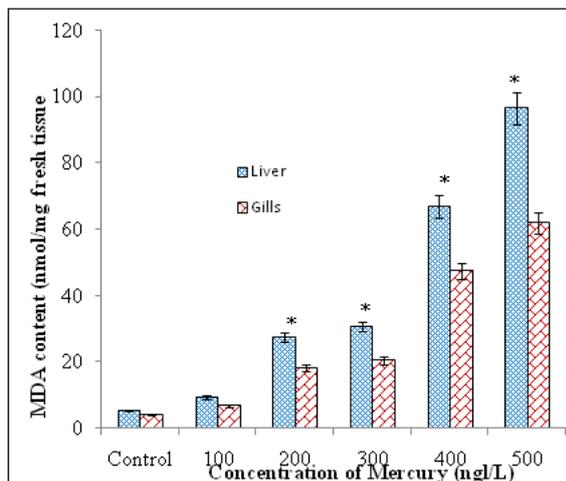


Fig. 3. Effect of low and high dose of Mercury on Lipid peroxidation of test fish in the laboratory condition (Asterisk represents significantly different activities at different concentration in liver and gills tissue).

The oxidative corrosion of lipid in cell membrane is called LPO are used as biomarker of oxidative stress and can be assessed by calculating the level of MDA. This above accretion of MDA can damage cells and activates apoptosis. The present study also presented a noteworthy rise in the activity of MDA at the higher concentration of the mercury (Fig.3). The higher oxidative stress leads to the pathological conditions including cardiovascular diseases, cancer (Sosa *et al.*, 2013), inflammation and neurodegenerative diseases and ageing (Oyinloye *et al.*, 2015).

Conclusions

It is concluded that river Ravi is continually polluted due to Mehmood Booti Nala, Bhek nala, Farrukhabad nala, Bakar Mandi nala, Hudiara drain, and Head Balloki water. Mean heavy metal Mercury concentration is increasing day by day. Among these polluting drains, the maximum concentration of Mercury is found in Hudiara drain which is the most populated site among the selected ones and the lowest

concentration of Mercury is found in fresh river water before mixing of this contaminated water. It is also found that higher concentration of mercury accumulates in the different tissues of the aquatic organisms and become major cause of oxidative stress which ultimately leads to pathological conditions. Therefore, the metals contamination in the river Ravi is the main cause of valuable species extinction. If this contamination was not minimized in the future, Our Rivers will be deprived of biodiversity.

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