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IN SITU AND EXPERIMENTAL STUDIES ON THE INDUSTRIAL POLLUTION INDUCED ALTERATIONS IN THE ORGANS OF FISHES

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Abstract: ***Studies were carried out to compare in situ and experimental exposure to industrial pollutants on the status of oxidative stress generation and structure of liver, muscle and gills of fish. The fishes, Tilapia (Oreochromis mossambicus Peters 1852), length 15-18cm (weight 26-32g), collected from non polluted pond site, Dumad, were exposed to 10 and 20 % of industrial effluent, collected from the discharge point of CETP, Undera of Vadodara industrial area, in standard lab conditions. For insitu studies Tilapia of similar weight group were collected from polluted site, Koyali pond, located within the industrial area and brought to laboratory for studies without any further experimental exposure. Histological findings indicated prominent damage at higher exposure doses in all the tissues studied. The fishes of Koyali pond also showed structural damage comparable to those noted following high dose experimental exposure. The metabolic and oxidative stress indicator parameters were least affected by day 7 but by day 30 in the high dose exposure group and in the in situ exposed fishes the changes were significant. Among the heavy metals, cadmium, lead and copper accumulated in the fish tissues both in the 30 day high dose experimental and in situ exposure groups. Present studies suggested that at the polluted site, the continuous long term exposure may lead to patho-physiological alterations in fishes or other edible organisms which may be of people's health concern.***

Key words: Industrial pollution, Fish toxicology, Liver, Gill, Muscle

INTRODUCTION

The pollution impacts on environment, in experimental animals and in humans, are reported the world over during past few decades [1-4]. Environmentally persistent chemicals are consistently found as contaminant in air, water and vegetative as well as animal food sources and consequently in human blood, milk, urine and hair samples. The existing toxicological data mostly describe toxicity of single compound or a mixture of few chemicals of the same classes, which does not assess the toxic potentials of chemical mixtures, particularly heterogeneous industrial effluent [5,6]. The list of potential hazardous chemicals and their permissible

concentrations for release into the environment are well documented [7-9]. Nonetheless, the studies on ecotoxicology of heterogeneous chemical mixtures are sparse [10-13]. The environmental exposure levels of chemicals are usually low while in the experimental studies generally concentrations higher than these are tested; which fail to reflect the actual ecotoxicological evaluation [14,15]. Although the responsiveness of the animals may be correctly understood in experimental set up of defined environmental conditions, however, such experiments provide true picture for single toxicant or group of toxicants with similar pharmacological properties or toxicological profile. Since the industrial pollution of aquatic bodies is heterogeneous with compounds exhibiting widely variable pharmacological and toxicological profile, it is more difficult to delineate toxic manifestation and organism responses [16-18]. Therefore, in present investigation an attempt was made to compare the toxic responses of fishes to industrial effluent exposure under in situ and experimental conditions.

MATERIALS AND METHODS

***In situ* exposure studies:** Two perennial ponds were selected for in situ studies which included Koyali pond (Lat 22º36.67"N and Long 73º11.67" E) situated within the industrial area around Vadodara and Dumad pond (Lat 22º34.34" N and Long 73º11.21" E) situated in the out skirts of Vadodara (Fig. 1). Since Koyali pond is located within the industrial area it receives surface and sub surface runoff. The groundwater in the industrial area has been reported to contain heavy metals, which may lead to pollution of Koyali pond owing to subsurface drainage and recharge. Therefore, it was considered as polluted site while, Dumad pond which is away from the influences of industrial area was considered as a control site and as source of normal fishes for experimentation. Fishing was done during early morning hours by local fisherman and Tilapia (*Oreochromis mossambicus* Peters 1852), length 15-18cm (weight 26-32g), were collected and brought live to the laboratory. The fishes from Koyali pond were immediately dissected and the liver, kidney, muscle and gills were stored at -80ºC till further biochemical analysis. A portion of tissues was fixed in 10% neutral formalin, processed and stained with hematoxylin and eosin for histological studies or the tissues were digested and processed for the heavy metal analysis using AAS as per standard protocols.

Experimental exposure studies: The fish Tilapia (*Oreochromis mossambicus* Peters 1852),

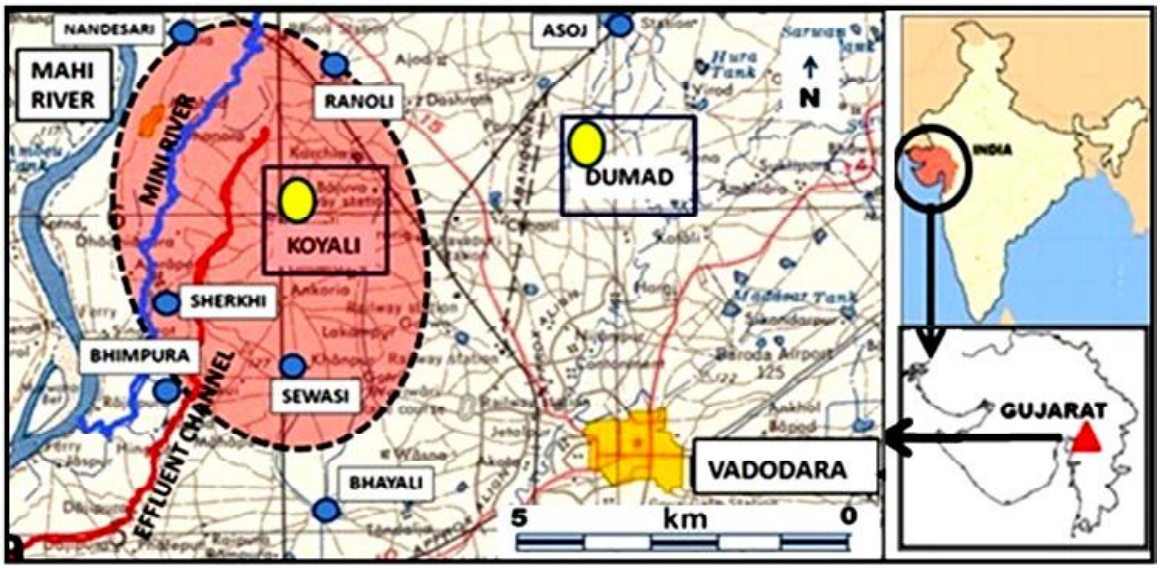

Fig. 1: Showing the map of study area and location of study ponds which were sources of fishes. The area encircled with dotted lines indicates industrial area. (based on Google map)

collected from Dumad pond (15-18 cm length, 26-30 g weight), non polluted site, were brought to the lab and transferred to bath tub of 50 l water capacity in group of 10 per tub for acclimatization. The fishes were fed standard feed for exotic fishes consisting of rice bran and oil cakes. After 1 week, the fishes were divided into several groups of four each for toxicity assay. To determine the experimental doses, the fishes were treated with the freshly collected heterogeneous industrial effluent at different concentration for durations ranging from 1 to 30 days. The effluent discharge of CETP, Undera of Vadodara industrial area was collected weekly from the J point of effluent channel near Sarod village, Bharuch district, Gujarat where it opens into Mahi estuary. The water and toxicants were changed daily to avoid any residue formation or any effect that cannot be correlated with known experimental conditions. The doses and fish mortality rate is presented in Table 1. Based on these findings, the doses were determined and the fishes were divided into three groups. Non treated fishes collected from Dumad pond served as control (Group I). Other fishes were exposed to 10% (Group II) and 20% (Group III) of industrial effluent for 7, 15 and 30 days in experimental set up. On the next day of these exposure durations, the fishes were dissected out to collect liver, kidney, muscle and gills and stored at -80°C till further biochemical analysis. A portion of tissues was fixed in 10% neutral formalin, processed and stained with hematoxylin and eosin for histological studies or the tissues were digested and processed for the heavy metal analysis using AAS as per standard protocols.

Biochemical studies: The tissues were homogenate as required for estimation of total protein [19], ascorbic acid [20], glutathione [21], acid and alkaline phosphatases [22], super oxide dismutase [23] and glutathione peroxidase [24] in liver, muscle and gills.

RESULTS

Biochemical studies: The findings of biochemical studies are presented in Tables 2-4. The

Table 1: Fish toxicity assay to determine the experimental doses

Concentration of effluent	Percent mortality					
	1 day	2 days	3 days	4 days	15 days	30 days
5%	0	0	0	0	0	0
10%	0	0	0	0	0	0
15%	0	0	0	0	0	0
20%	0	0	0	0	0	0
22%	0	0	0	6	18	30
24%	0	0	6	12	18	36
26%	0	0	12	18	24	36
28%	0	0	18	24	30	42
30%	2	8	24	30	36	60
35%	6	17	30	36	42	72
40%	12	23	36	42	60	80
45%	19	30	42	42	66	86
50%	45	60	72	100	100	100

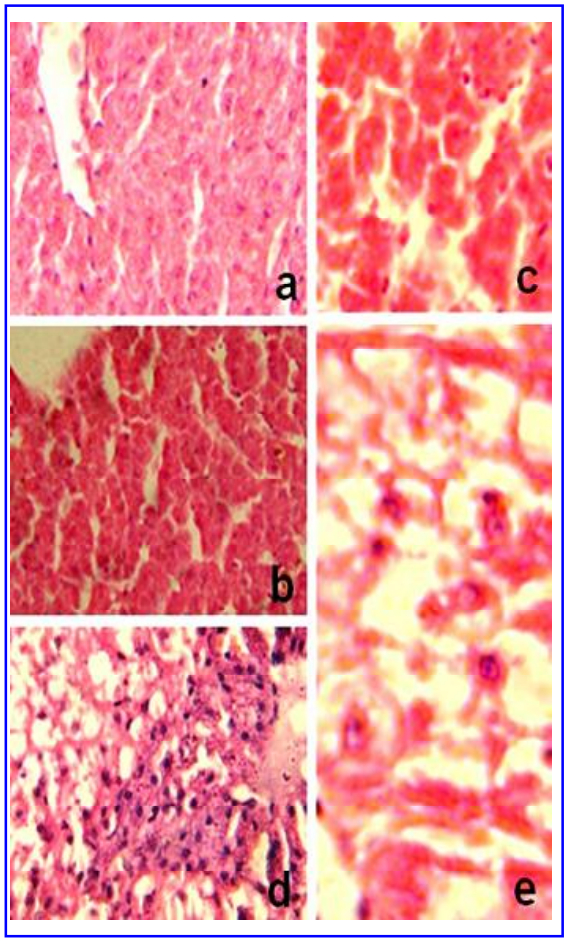

Fig. 2: Liver. a: Showing normal histoarchitecture with proper nuclear staining and arrangement of hepatocytes, b, c: Cell enlargement and sinusoidal disarrangement seen following 15 and 30 day high dose exposure, respecetively, d, e: extensive cytoplasmic vacuolation and hepatocyte cell damage is seen in liver of fish collected from Koyali. H and E. a,b X100, c,d X250, e X400.

Table 2: Effect of effluent water on fish liver biochemical parameters. The values are Mean + SE of 10 observations. In experimental studies, the treated groups were compared with respective control. For In situ studies, the omparisions were made with 30 day comtrol values. Significance: a = p ≥ 0.01, b = ≥ 0.001

		7 Days			15 Days			30 Days			Koyali
Parameter	UNIT	Control	10% Dose	20% Dose	Control	10% Dose	20% Dose	Control	10% Dose	20% Dose	In situ
Protein	mg/ 100mg tissue	4.119 ±0.025	4.097 ±0.048	4.000 ±0.03	4.210 ±.037	4.032 ±0.0380	3.281 ±0.088b	4.011 ±0.060	4.000 ±0.082	3.092 ±0.076	3.237 ± 0.075
Acid Phosphatase	µ mole of PNPP released/mg protein/h	1.896 ±0.015	1.852 ±0.018	1.802 ±0.024	1.806 ±0.024	1.796 ±0.017	1.780 ±0.050	1.900 ±0.03	1.904 ±0.09	1.994 ±0.07	1.802 ± 0.014
Alkaline Phosphatase	µ mole of PNPP released/mg protein/h	6.633 ±0.05	6.530 ±0.08	6.348 ±0.036	6.200 ±0.04	6.486 ±0.027	6.203 ±0.070	6.730 ±0.06	6.737 ±0.054	6.884 ±0.062	6.522 ± 0.028
Superoxide Dismutase	Unit/ mg protein	1.896 ±0.05	1.852 ±0.07	1.802 ±0.04	1.806 ±0.04	1.796 ±0.02	1.780 ±0.05	1.900 ±0.03	1.904 ±0.09	1.994 ±0.07	1.882 ± 0.08
Glutathione Peroxidase	µ mole GSH oxidized/min/ mg protein	1.619 ±0.06	1.639 ±0.04	1.704 ±0.03	1.713 ±0.045	1.702 ±0.017	1.694 ±0.050	1.789 ±0.030	1.836 ±0.09	1.894 ±0.07	0.0108 ± 0.014
Glutathione	µ mole GSH/ protein	0.0128 ±0.04	0.0125 ±0.08	0.0115 ±0.038	0.0126 ±0.05	0.0110 ±0.07	0.0105 ±0.07	0.0127 ±0.03	0.0129 ±0.08	0.0132 ±0.006	0.0130 ± 0.08
Ascorbic Acid	µg/100mg	0.01467 ±0.08	0.01403 ±0.05	0.01326 ±0.04	0.01310 ±0.03	0.01314 ±0.03	0.01319 ±0.05	0.01480 ±0.03	0.0129 ±0.06	0.0132 ±0.05	0.162 ± 0.07

Table 3. Effect of effluent water on fish gill biochemical parameters.The values are Mean + SE of 10 observations. In experimental studies, the treated groups were compared with respective control. For In situ studies, the comparisions were made with 30 day comtrol values. Significance: a = p ≥ 0.01, b = p ≥ 0.001

		7 Days			15 Days			30 Days			Koyali
Parameter	UNIT	Control	10% Dose	20% Dose	Control	10% Dose	20% Dose	Control	10% Dose	20% Dose	In situ
Protein	mg/ 100mg tissue	2.89 ± 0.010	2.669 ± 0.06	2.532 ± 0.92	2.592 ± 0.9	2.589 ± 0.76	2.409 ± 0.02b	2.489 ± 0.046	2.478 ± 0.04a	2.037 ± 0.08b	2.233 ± 0.061b
Acid Phosphatase	µ mole of PNPP released/mg protein/h	2.218 ± 0.07	2.027 ± 0.050	1.929 ± 0.80a	1.881 ± 0.04	1.908 ± 0.017a	1.898 ± 0.05a	1.800 ± 0.03	1.876 ± 0.09b	1.804 ± 0.07b	1.837 ± 0.08b
Alkaline Phosphatase	µ mole of PNPP released/mg protein/h	3.721 ± 0.04	3.789 ± 0.079	3.386 ± 0.04a	3.721 ± 0.06	3.672 ± 0.037a	3.285 ± 0.07a	4.292 ± 0.03	4.189 ± 0.069b	4.290 ± 0.047a	3.381 ± 0.94b
Superoxide Dismutase	Unit/ mg protein	2.758 ± 0.05	2.639 ± 0.09	2.623 ± 0.06	2.804 ± 0.035	2.842 ± 0.068	2.898 ± 0.025	2.821 ± 0.04	2.849 ± 0.9	2.899 ± 0.077	2.838 ± 0.065
Glutathione Peroxidase	µ mole GSH oxidized/min /mg protein	0.0208 ± 0.034	0.0196 ± 0.079	0.0200 ± 0.04	0.0198 ± 0.045	0.0182 ± 0.017	0.0176 ± 0.05	0.0210 ± 0.03	0.0212 ± 0.09	0.0219 ± 0.083	0.00179 ± 0.064
Glutathione	µ mole GSH/ mg protein	0.0266 ±0.050	0.0241 ± 0.09	0.0244 ± 0.084	0.0223 ± 0.075	0.0226 ± 0.072	0.0228 ± 0.05	0.0294 ± 0.06	0.0297 ± 0.084	0.0328 ± 0.074	0.0308 ± 0.083
Ascorbic Acid	µg/100mg	0.313 ± 0.050	0.310 ± 0.058	0.298 ± 0.054	0.303 ± 0.047	0.299 ± 0.082	0.242 ± 0.05b	0.292 ± 0.083	0.286 ± 0.085a	0.239 ± 0.07a	0.260 ± 0.08b

protein contents in liver and muscle did not exhibit any noticeable alterations on day 7 but in gills, 20% dose exposure resulted into significant reduction. On days 15 and 30, liver and gill protein exhibited significant reduction both in high dose experimental and Koyali pond fishes. Koyali pond fish did not exhibit any significant alteration in the protein contents of muscles. The liver did not exhibit any significant change in alkaline phosphatase content by 7 days, but after 15 days some noticeable changes were seen. In gills, after 7 and 15 days exposure both at low and high doses, there was significant reduction in the enzyme activity. The muscles exhibited no change during the study. Koyali fish tissues showed significant changes in gills only. Acid phosphatase content in liver of experimental fishes did not show any changes but in gills significant changes were seen as dose responses on 7, 15 and 30 days. Muscles showed

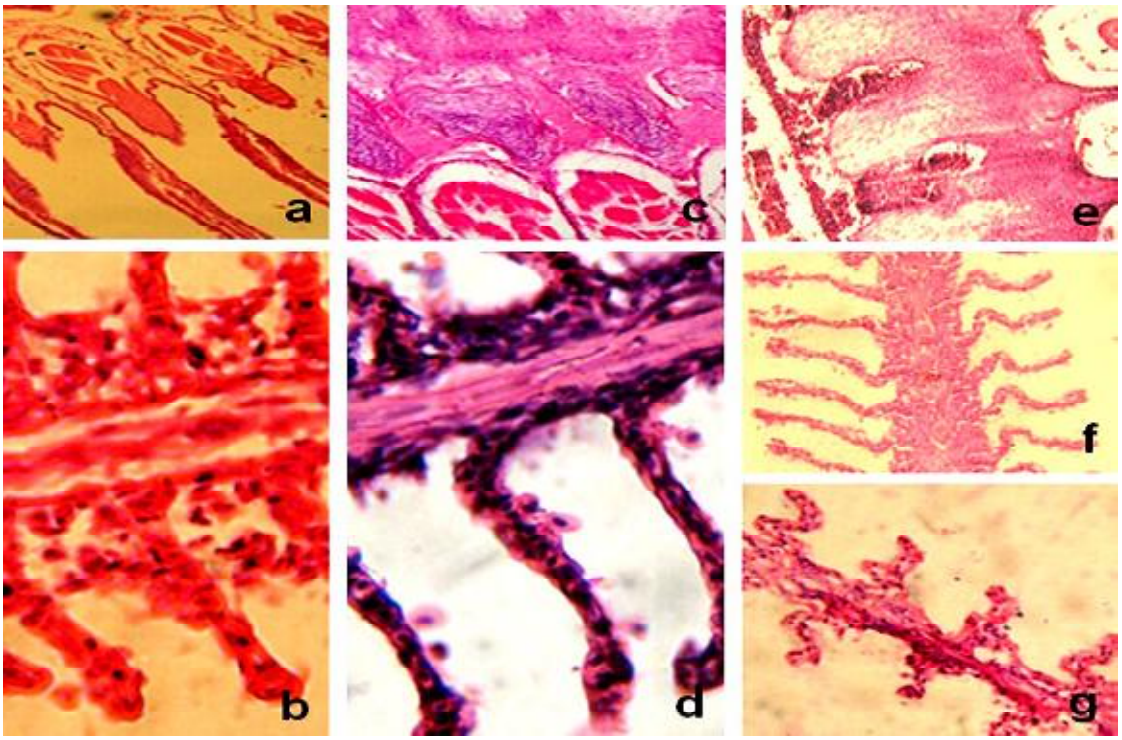

Fig. 3: Gill. a,b: Normal structure of gills showing gill arches and filaments. Normal primary and second lamellae are seen, c,d: On 30 days, the mucosal epithelium and sub mucosa of the gill racker exhibited severe damaged, the secondary lamellae exhibited clubbing at tip, e,f,g: In fishes exposed insitu at the Koyali pond the gills exhibited damage to secondary lamellae with oedema and erythrocyte infiltration. Hand E. a,c,e,f X100, g X250, b,d X400.

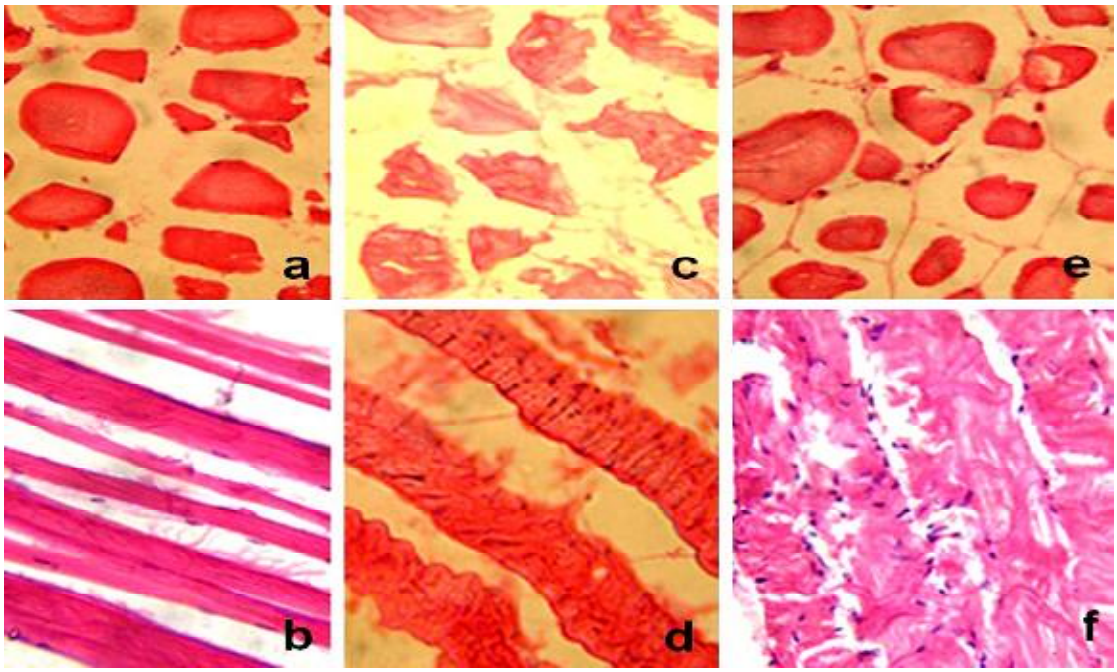

Fig. 4: Muscle. a,b: The muscle bundles packed in epimycium were appropriately arranged with prominent peripheral nuclei in control fishes, c.d: focal necrosis, aggregation of inflammatory cells, vacuolar degeneration, atrophy of muscle cells and oedema were noted on day 30 in high dose exposure group, e,f: The fish collected from Koyali showed prominent loss of connective tissues and muscle dystrophy. H and E. a,b,c,d X100, e,f X250.

Table 4: Effect of effluent water on fish muscle biochemical parameters.The values are Mean + SE of 10 observations. In experimental studies, the treated groups were compared with respective control. For In situ studies, the comparisions were made with 30 day comtrol values. Significance: a = p $\geq$ 0.01, b = p $\geq$ 0.001

		7 Days			15 Days			30 Days			Koyali
Parameter	UNIT	Control	10% Dose	20% Dose	Control	10% Dose	20% Dose	Control	10% Dose	20% Dose	In situ
Protein	mg/ 100mg tissue	2.410 ± 0.080	2.390 ± 0.060	2.331 ± 0.50	2.352 ± 0.08	2.341 ± 0.45	2.220 ± 0.17	2.341 ± 0.07	2.329 ± 0.06	2.086 ± 0.05	2.189 ± 0.09
Acid Phosphatase	µ mole of PNPP eleased /mg protein/h	3.052 ± 0.023	2.980 ± 0.012	2.180 ± 0.010	2.880 ± 0.013	2.670 ± 0.017	2.320 ± 0.015b	2.322 ±07	2.290 ± 0.05b	2.100 ± 0.041b	2.180 ± 0.04b
Alkaline Phosphatase	µ mole of PNPP leased/ mg protein/h	4.292 ± 0.03	3.800 ± 0.079	3.769 ± 0.044	3.825 ± 0.04	3.787 ± 0.043	3.646 ± 0.050 b	4.001 ± 0.03	3.946 ± 0.09b	3.999 ± 0.07b	3.609 ± 0.054a
Superoxide Dismutase	Unit/ mg protein	3.598 ± 0.050	3.477 ± 0.09	3.398 ± 0.047	3.608 ± 0.05	3.628 ± 0.017	3.881 ± 0.06	3.666 ± 0.043	3.796 ± 0.076	3.892 ± 0.068	3.786 ± 0.084
Glutathione Peroxidase	µ mole GSH oxidized/min/ mg protein	0.0208 ± 0.065	0.0310 ± 0.045	0.0306 ± 0.044	0.0304 ± 0.045	0.0302 ± 0.058	0.0281 ± 0.046	0.0320 ± 0.066	0.0330 ± 0.09	0.0332 ± 0.07	0.0299 ± 0.046
Glutathione	µ mole GSH/ mg protein	0.0250 ± 0.05	0.0248 ± 0.07	0.0237 ± 0.062	0.0304 ± 0.055	0.0231 ± 0.036	0.0229 ± 0.035	0.0268 ± 0.048	0.0248 ± 0.082	0.0237 ± 0.043	0.0210 ± 0.062
Ascorbic Acid	µg/100mg	0.378 ± 0.05	0.369 ± 0.09	0.287 ± 0.04b	0.357 ± 0.05	0.354 ± 0.07	0.276 ± 0.05 b	0.348 ± 0.03	0.343 ± 0.08b	0.264 ± 0.07b	0.300 ± 0.08b

Table 5: Heavy metals in various tissues of fish from Koyali Pond. All heavy metal levels in ug / g dry wt.

Metal	Kidney	Muscle	Liver	Gill
Cadmium	1.4 ± 0.02	0.03 ± 0.002	0.06 ± 0.01	0.01 ± 0.003
Chromium	B.D.L.	B.D.L.	B.D.L.	B.D.L.
Nickel	B.D.L.	B.D.L.	0.7± 0.03	0.7 ± 0.004
Lead	B.D.L.	0.04± 0.005	0.1 ± 0.01	0.7 ± 0.002
Copper	4.5 ± 0.003	0.01 ± 0.001	1.7 ± 0.02	0.65 ± 0.01
Cobalt	B.D.L.	B.D.L.	B.D.L.	B.D.L.

some increase in activity by day 15 followed by decrease in enzymes activity at day 30. Koyali fish tissues exhibited significant alterations in the enzyme activity. The superoxide dismutase activity was non-significantly altered in the fish tissues. As compared to 7 days, the enzyme activity was little more on 15 and 30 days in both the treatment and experimental groups. Similarly the tissues of Koyali fish also showed no change in the enzyme activity. Glutathione peroxidase activity significantly reduced in the liver and gill tissues on 15 days post exposure, however the activity in the treated groups then increased to be at par with the control values. Glutathione content in experimental were almost equal to the control tissue levels on all the experimental durations and even in the tissues of Koyali fish. The trend indicated an increment in the GSH content on 30 days as compared to that noted on 7 and 15 days. Ascorbic acid content showed increase in activity in liver on high dose exposure at 15 and 30 days while in gills and muscles the contents significantly reduced on these experimental durations. In the Koyali pond fish also similar type of changes were noted in different tissues.

Fish tissues were also analyzed for heavy metals like cadmium (Cd), nickel (Ni), lead (Pb), chromium (Cr), cobalt (Co) and copper (Cu). All these metals except chromium and cobalt were detected in fish tissues of Koyali pond while all the metals were below detection limits in the experimental fish tissues (Table 5). Cadmium and copper were present in all the analyzed tissues while lead was deposited in liver, muscle and gills and nickel was accumulated in liver and gills only. Chromium and cobalt were found to be below detection limits in all the tissues. The order of bioaccumulation of different metals in different tissues of Koyali pond fish was:
Copper:kidn-ey $\geq$ liver $\geq$ gills $\geq$ muscle, Nickel: liver $\geq$ gill $\geq$ muscle $\geq$ kidney, Lead: gill $\geq$ liver $\geq$ muscle $\geq$ kidney and Cadmium: kidney $\geq$ liver $\geq$ muscle $\geq$ gills.

Histological studies: The liver of fish has typical paranchymatous organisation, primarily of polyhedral hepatocytes with large central nuclei and prominently stained chromatin, central vein, sinusoid and portal areas with the bile ducts are appropriately organised. The blood sinus spaces are lined by endothelial cells. The reticuloendothelial cells are located at the margin of sinusoids, between the sinusoids and hepatocytes. In the portal area few lymphocytes are also seen (Fig. 2a). Following the exposure to toxicant at 10% dose level, the changes were non significant by 7 days. A few pericentral hepatocytes were relatively swollen. By 15 days in the higher dose group cytoplasmic changes were prominent. The dissolution of cytoplasm was seen, however nuclear changes were not prominent. The endothelial lining of the sinusoids and the central vein were highly damaged (Fig. 2b). By 30 days, much alteration in the typical parenchymatous appearance was seen where the cord like arrangements of hepatocytes were almost lost. The nuclei were highly disintegrated. The cytoplasm dissolution and small vacuole degeneration was seen (Fig. 2c). In one of the fish liver severe cytoplasmic changes were seen as extensive eosinophilic staining. The fish collected from Koyali exhibited many of these histological abnormalities. The cell damage and presence of large vacuoles was prominent. At several places lymphocytes infiltration was seen in the peripheral region (Fig. 2d,e).

In tilapia, four gill arches extend on either side in the buccal cavity. The anterior edges have gill arches that protect the fragile gill filament. The arches are supported by bone and cartilage with associated striated abductor and adductor muscle facilitating movements of gills (Fig. 3a). The gill filaments have central cartilaginous support, afferent and efferent arterioles and thin epithelial covering. On the superior and inferior surfaces of primary lamellae the secondary lamellae originate (Fig. 3b). The thin epithelial covering of secondary lamella rests on basement membrane supported by pillar cells. Other cell types found in primary and secondary lamellae include melanocytes, lymphocytes, macrophages, mucous and chloride cells. The mucous cells are located at the base of secondary lamellae; chloride cells are located at the base of secondary lamellae and gill filaments. Following exposure to the heterogeneous effluent hypertrophy and hyperplasia of cells was prominently seen. Fusion of secondary lamellae was also seen on day 15. On 30 days, the mucosal epithelium and sub mucosa of the gill racker exhibited severe damage. In high dose group on day 30, the damage was more prominent, the secondary lamellae significantly exhibited clubbing at tip (Fig. 3c,d). The primary lamellae showed irregular thickening in the tissue collected from Koyali fish. The conditions were little more severe, with damage to gill filaments. Due to damage to epithelial covering cells and supportive pillar cells and the architecture of secondary lamellae collapsed. The secondary lamellae were oedematous and Infiltration of erythrocytes was also seen (Fig. 3e-g).

The muscle fibres forming muscle bundle were packed by dense connective tissues, epimycium. The multiple nuclei were located at the periphery of muscle fibre (Fig. 4a,b). Exposure to the toxicant for 7 days had no prominent effect on the histoarchitecture of muscle. The major pathological changes at high dose exposure for 30 days included focal necrosis, aggregation of inflammatory cells, vacuolar degeneration, atrophy of muscle cells and oedema (Fig. 4c,d). The damage to connective tissue components actively influences histoarchitecture. The fish collected from Koyali showed prominent loss of connective tissues and some amount of muscle dystrophy (Fig. 4e,f) indicative of disintegration and dissolution of perimycium.

DISCUSSION

The extent of pollution of the Mahi River and related fish loss was first reported in October 1968 and was correlated with contamination of well and pond waters of the villages especially around the confluence of Mini and Mahi rivers. This followed with the construction of a 56 km long effluent channel to divert the industrial wastewaters from the industrial area around Vadodara for discharge into the lower estuarine

region of Mahi River at the Gulf of Khambhat. Analysis of groundwater from wells located 50–200 meters from the effluent channel showed high levels pollutants including metals [25]. There has also been rapid erosion in the quality of estuarine flora and fauna at the Gulf of Khambhat [26,27].

Heavy metals have very long biological half-life and possess greater potential to get absorbed through tissues easily owing to their lipophilic nature. Their accumulation in animal tissues is investigated the most [28-30]. In present studies, kidney and liver potentially accumulated metals compared to gills and muscles. Since the experimental model is located at the higher trophic level, presence of these metals in pond water and accumulation in the tissues of fish establish a direct link and evidence to suggest that the pollutants bioaccumulated through the food chain [31,32]. It further implies that much of the observed changes in the physiology of fishes may be due to the accumulated toxicants/ metals. Considering biomagnification through the food chain, the entero-hepatic circulation exposes primarily the liver tissue to higher toxicant concentration, thus increase probability of hepatotoxicity. The gill membrane forms a significant barrier for transportation and disposition of toxicants. The hyperplasia and hypertrophy of various cell types of gill filament, thickening of basement membrane and fusion of the tips of the secondary lamellae are regarded as adaptive responses of cellular defense. In the experimentally exposed fishes gradual and moderate modifications in histoarchitectural integrity of gills were noted in different dose groups over a period of 30 days. These pathological alterations are expected to compromise with respiratory mechanism of gills. Muscles are physiologically important to animals but more so economically with reference to the fish culture practice. Although, present findings demonstrated that metal accumulate in muscles to much lesser content as compared to liver and gills, the observed pathological changes like connective tissue dissolution, fibril disorientation and degeneration and edema are indicative of severe damage. Several researchers have demonstrated similar findings following toxicant exposure to fishes. Exposure to water born copper under experimental conditions for 21 days induced severe cellular changes in both liver and gills [33].

The metals and organicals are shown to induce oxidative stress in different experimental animals and these have been used as biochemical markers of toxicity. Livingston [34] reviewed the status of oxidative stress in fishes with reference to aquatic pollution and suggested that although pollutant induced ROS increase and depletion in glutathione has been studied by many workers, the findings do not establish direct quantitative or dose response relationships in several cases. Glutathione protects against oxidative damage due to lipid peroxidation. In present findings, the enzymes of antioxidant mechanism were not altered till 15 days in all the organs studied. However, the alterations were noted, though not significant always, on 30 days in 20% dose group and in the Koyali pond fish. The antioxidant enzyme activity slightly increased with corresponding increase in glutathione level. The phosphatases are also indicator of cellular functions which were altered to various levels of significance in the experimental and in the Koyali pond fishes. Heavy metal exposure induces oxidative stress and alters their biomarkers [35-37]. It is suggested from these studies that metal accumulation in tissues of fish could generate free radicals and superoxide anions to induce the activity of enzymes like SOD, GP_X, and Catalase. Since GSH is the substrate for GST activity, it also increases correspondingly [38].

Experimental exposure of fishes to wide variety of toxicants at different concentrations over acute to chronic durations has exhibited much similar type of lesions [39,40]. These studies are direct in-situ evidences to correlate environmental pollution and toxic manifestations in an aquatic system. In view of the fact that any aquatic body located in the vicinity of industrial region receive variety of industrial pollutants, domestic discharges and agricultural runoff result into distinctly heterogeneous environmental

conditions. The findings reported here is the outcome of the heterogeneous toxicant exposure (industrial effluent) at low doses. The correlation of toxic manifestations in experimental and in situ exposure suggest that pathological changes induced in organs of Koyali pond fish are comparable to or little more severe than those noted at low dose toxicant exposure for 30 days in experimental groups. In the experimental set up, the environmental conditions are much less diverse since they are well controlled/ regulated, while in-situ exposure involve diverse and wide array of environmental conditions. The routine physicochemical parameters, nutrient distribution and availability, natural food availability and quantity altogether influence the toxicant uptake and effects. Under such circumstances, also the toxicants accumulate within the tissues of fishes indicating the significance of regular environmental monitoring and importance of pollution control measures [41-42].

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