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Biochemical and Histopathological Changes Induced by Nickel in the Striped Mullet, *Mugil cephalus* (Linnaeus 1758)

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Abstract The present study is focused on determining the acute and chronic toxicity of nickel (Ni) to fish fingerlings, *Mugil cephalus*. The 96-h median lethal concentration (LC_{50}) for Ni was found to be 42.2 ± 3.9 mg L^{-1}. Based on the chronic toxicity test for 30 days, "No Observed Effect Concentration", "Lowest Observed Effect Concentration" and "Chronic value" were found to be 2.9 ± 0.14, 4.7 ± 0.14 and 3.7 ± 0.14 mg Ni L^{-1}, respectively. The activities of biomarker enzymes including esterase, superoxide dismutase and malate dehydrogenase showed differential expression and cellular anomalies like hyperplasia and detachment of bipolar cells from photoreceptor cells in the retina of eye of mullet. Cellular anomalies in the retina of fish eye affect the primary function of retina, which is to convert light energy into nerve impulses transferred to the brain via the optic nerve, leading to loss or poor vision.

Keywords *Mugil cephalus* · NOEC · LOEC · Biomarker enzymes · Histology · Nickel

Nickel (Ni) is considered to be a nutritionally essential trace metal in animals, microorganisms, plants and has been a constituent of enzymes and proteins and therefore either toxicity symptoms or deficiency can occur when too much or too little Ni is assimilated. Creeks are the intertidal regions developed along the coasts and are sites of major port, industrial, urban and recreational activities (Ridgway and Shimmield 2002). Overall assessment of metal pollution in the coastal regions of Chennai, Tamil Nadu, India revealed that bioaccumulation of heavy metals has increased in all compartments, viz., water, sediment and biota during the past decade (Seshan et al. 2011). Overexploitation, mismanagement and untreated or not properly treated industrial effluents from more than 25 industries were discharged into North Chennai coastal region, which brings a great challenge for the ecosystem balance (Kannan et al. 2007).

The imbalance between generation and neutralization of Reactive Oxygen Species (ROS) by antioxidants in the organism is called oxidative stress (Davies 1995). ROS are induced by substances such as transitional metal ions, pesticides and petroleum pollutants (Slaninova et al. 2009). Gernhofer et al. (2001) studied ultrastructural responses in different organs of fish to be useful tools to characterize the health status and also to assess the impact of environmental contaminants on fish. Biomarker enzymes reflect the stress elicited due to exposure to environmental contaminants (Tu et al. 2010). Consequently, studies on histology of marine organisms exposed to heavy metals suggested that increased exposure concentrations produced significant effects like mortality, while lower concentrations caused physiological stress during chronic exposure (Adil et al. 2011).

The striped mullets (*Mugil cephalus*) are usually found in schools over sand or mud bottom between 0 and 10 m depths. The species is highly commercial, occurring equally in tropical, sub-tropical and temperate waters. Mainly diurnal, both juvenile and adult fish feed on zooplankton larvae, detritus, micro-algae and benthic organisms. The species were used as the test organism for toxicity studies due to being ecologically important and playing a key role in the food chain. Other considerations behind the choice of

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the test species were availability, amenability to laboratory testing, and its genetic stability. Adequate background data on the species (i.e. its physiology, genetics, taxonomy, and their place in the environment) were also considered. These criteria are closely matched with the US EPA criteria (US Environmental Protection Agency (USEPA 2002) for selection of organisms for toxicity studies.

The present study was aimed at determining the acute and chronic toxicity of Ni to *M. cephalus* and deriving respective levels of "No Observed Effect Concentration" (NOEC) and the "Lowest Observed Effective Concentration" (LOEC). The activity of enzymes like esterase, superoxide dismutase, malate dehydrogenase and histopathological changes in the gills and retina of eye of mullet were studied after exposure to Ni for 30 days under controlled laboratory conditions.

Materials and Methods

Fingerlings of *M. cephalus* (about 3.0–3.5 cm in length), a natural inhabitant of the estuaries, were collected from the mud banks of Kovalam creek located on the southern part of Chennai, Tamil Nadu, India (Lat. 12.792°N, Long. 80.252°E). Water quality parameters like salinity, pH, water temperature and dissolved oxygen (DO) were monitored by using a pre-calibrated Hydrolab water quality probe (Quanta, USA). The background concentration of Ni in 'Ennore' seawater was monitored throughout the study period. The same seawater was used for conducting toxicity experiments under flow-through conditions. The stock solution of Ni (1000 mg L^{-1}) was prepared by dissolving 4.05 g of Nickel chloride hexahydrate ($NiCl_2.6H_2O$; Merck, Germany) in 1 L of ultrapure deionized water (Millipore-Milli-Q) and the required test concentrations were prepared from the main stock solution. The bioassay for acute and chronic toxicity tests were conducted by using customized continuous flow through test method with fish fingerlings as described by Sprague (1973) and Stephan et al. (1985). For the acute toxicity tests, healthy fish fingerlings were separated from the storage tanks and divided into six groups, viz., 10 numbers each were exposed in duplicate to the definitive test concentrations—(i) control; (ii) 35.0 (iii) 42.0 (iv) 50.0 (v) 60.0 and (vi) 73.0 mg L^{-1} Ni. The acute exposure period was 96 h and totally three successive flow through tests were performed for confirmation. Similarly, for chronic toxicity tests, healthy fish fingerlings were divided into six groups, viz., 10 numbers each were exposed in duplicate to the following concentrations: (i) control; (ii) 3.0, (iii) 5.1, (iv) 8.7, (v) 14.7 and (vi) 25.1 mg L^{-1} Ni. The concentrations were selected based on 96 h LC_{50} already derived for *M. cephalus*. The chronic toxicity test was continued for 30 days of exposure and it was repeated once for confirmation. The seawater samples were collected at 24 h intervals (i.e. 24, 48, 72 and 96 h) for acute toxicity tests and between 10 days intervals (i.e. 10th, 20th and 30th day) for chronic tests. The experimental seawater samples were filtered through 0.45 µm Millipore filters and acidified with suprapure nitric acid (Fluka) to pH 2–3. The nickel was extracted from the experimental seawater and sea water samples spiked with nickel standard (Merck) and nickel chloride hexahydrate for recovery analysis by using ammonium pyrrolidinedithiocarbamate and methyl iso-butyl ketone following the method of Grasshoff et al. (1999). The total dissolved nickel concentrations in seawater samples were analysed by atomic absorption spectrometry (Varian SpectrAA Model 220FS). A standard solution of nickel (1000 mg L^{-1}, Merck, Germany) was used as the standard. To validate the results, the known standard and nickel chloride hexahydrate recovery samples were measured between the samples.

Towards the end of 30 days of chronic exposure, live test mullets were sacrificed and stored in Ultra freezer (−80°C) until further analysis. Frozen samples were thawed over ice, wiped with clean tissue paper and washed with ice cold phosphate buffer solution (0.1 M PBS pH 7.4). The samples were homogenized with liquid nitrogen followed by ice-cold extraction buffer using mortar and pestle in an ice bath. After homogenization, a clear supernatant was obtained through centrifugation repeated for 3 times, each for 10 min (12,000 rpm/10 min/4°C), which served as the enzyme source. The protein content of the extracts was estimated by the method described by Bradford (1976) using bovine serum albumin as the protein standard. Native PAGE (PolyAcrylamide Gel Electrophoresis) technique was carried out at 25 ± 1°C using Tris–glycine as running buffer (pH 8.3) under non-denaturing and non-reducing conditions (Laemmli 1970). A uniform amount of protein (100 µg) was loaded to each lane along with native sample buffer (devoid of sodium dodecyl sulphate and *b*-mercaptoethanol). Samples were then subjected to electrophoresis at 50 V through the stacking gel (4%) and at 100 V through the resolving gel (8%). Activity staining for SOD, MDH and EST on the gel was performed by following the method of Beauchamp and Fridovoch (1971) and Wendel and Weeden (1989). Activity staining for Esterase (EST): Activity staining for esterase was performed by placing the gel in a staining solution containing 100 mL of 100 mM phosphate buffer (pH 6.2), 50 mg alpha-napthyl acetate, 50 mg beta-napthyl acetate and 100 mg fast blue RR salt and incubated at room temperature (25°C) for 1 h to develop black, red or magenta colour bands. Both the substrates (alpha-napthyl acetate and beta-napthyl acetate) were dissolved quickly in 1 mL of acetone and mixed with the staining solution just before transferring of gel (Wendel and Weeden 1989). Activity staining for Superoxide

Table 1 Nominal and measured Ni concentrations (mg L^{-1}) in seawater used for acute and chronic toxicity tests and relative percentage of recovery

Acute toxicity test	Nominal concentration	Measured concentration acute test 1 (mg L^{-1})	Percent of recovery	Measured concentration acute test 2 (mg L^{-1})	Percent of recovery	Measured concentration acute test 3 (mg L^{-1})	Percent of recovery
	Control	BDL	NC	BDL	NC	BDL	NC
	35	30.9 ± 2.59	88.3	34.56 ± 0.99	98.7	29.74 ± 0.97	85
	42	39.3 ± 1.30	93.6	40.23 ± 1.74	95.8	35.47 ± 0.97	84.5
	50	43.3 ± 2.13	86.6	49.09 ± 2.67	98.2	43.09 ± 0.97	86.2
	60	51.4 ± 1.41	85.7	58.62 ± 2.39	97.7	49.97 ± 0.97	83.3
	73	64.6 ± 3.46	88.5	71.4 ± 2.48	97.8	57.62 ± 0.97	78.9
Chronic toxicity test	Nominal conc	d1 (measured conc. mg L^{-1})	d10 (measured conc. mg L^{-1})	d20 (measured conc. mg L^{-1})	d30 (measured conc. mg L^{-1})	Average	Percent of recovery
	Control	Control	BDL	BDL	BDL	NC	
Test-I							
	3	2.95	2.91	2.88	3.08	3.0 ± 0.09	98.42
	5.1	4.81	4.73	4.56	5.05	4.8 ± 0.20	93.85
	8.7	8.54	8.05	7.69	6.86	7.8 ± 0.71	89.45
	14.7	14.11	13.47	12.83	14.64	13.8 ± 0.79	93.61
	25.1	23.22	22.90	21.86	22.57	22.6 ± 0.58	90.17
Test-II							
	3	2.82	2.80	2.75	2.80	2.8 ± 0.03	93.04
	5.1	4.76	4.65	4.55	4.55	4.6 ± 0.10	90.71
	8.7	8.96	8.50	8.10	8.00	8.4 ± 0.44	96.44
	14.7	14.41	13.80	13.00	12.85	13.5 ± 0.73	91.94
	25.1	22.70	22.35	21.45	21.25	21.9 ± 0.70	87.40

BDL below detection level, *NC* not calculable; d1, d10, d20, d30 indicates number of days of exposure treatment

Table 2 Lethal concentration [LC_{50} (mg L^{-1})] of Ni for *M. cephalus* and 95 % CI estimated after exposure time (24–96 h)

Acute toxicity	24-h LC_{50} (mg L^{-1})	48-h LC_{50} (mg L^{-1})	72-h LC_{50} (mg L^{-1})	96-h LC_{50} (mg L^{-1})
Test-1	80.16 (69.67–155.42)	59.15 (55.43–64.90)	46.73 (44.65–49.27)	40.81 (38.47–43.51)
Test-2	76.62 (68.83–192.06)	65.49 (61.22–72.13)	56.02 (52.7–59.63)	46.63 (43.72–50.0)
Test-3	66.49 (60.21–142.1)	53.02 (49.89–57.69)	45.87 (42.93–49.52)	39.22 (36.60–41.78)
Mean ± SD	74.42 ± 7.09 (66.24–163.22)	59.22 ± 6.23 (55.51–64.91)	49.54 ± 5.62 (46.76–52.81)	42.22 ± 3.9 (39.6–45.10)

dismutase (SOD): Activity staining of total superoxide dismutase on the gel band was performed by following the method of Beauchamp and Fridovich (1971). The gel was soaked in the staining solution containing 50 mL of 50 mM Tris–Cl (pH 8.00), 10 mg nitroblue tetrazolium chloride (NBT), 1 mg ethylene diamine tetra acetic acid, and 2 mg riboflavin for 30 min in the dark at room temperature (25°C) and then illuminated in a light box with white fluorescent light for 30 min or until achromatic bands appeared. Activity staining for Malate dehydrogenase (MDH): Activity of malate dehydrogenase was detected by soaking the gel in 100 mL of Tris–Cl (50 mM, pH 8.5) containing 300 mg of malic acid, 20 mg of nicotinamide adenine dinucleotide (NAD), 20 mg of NBT (Hi-Media, India) and 4 mg of phenazine methosulphate (Hi Media, India). The gel was incubated at 25°C until the blue or indigo bands appeared (Wendel and Weeden 1989). Fish tissue samples such as gills and retina of eye were dissected freshly at the end of 30 days chronic exposure and fixed in Bouin's solution. After fixation, the tissues were dehydrated through a graded alcohol series, cleared in xylene and embedded in paraffin wax (58–60°C). Tissue sections of 5 µM thickness were made and stained with hematoxylin and eosin and observed under light microscope to characterize the cellular changes.

Table 3 Percentage of Survival, LOEC, NOEC, and Chronic values of Ni exposed to *M. cephalus*

Chronic toxicity	Nominal concentration	Measured concentration	Percent survival	NOEC (mg L^{-1})	LOEC (mg L^{-1})	Chronic value (mg L^{-1})
	Control	Control	100			
Test-I						
	3.0	3.0	100			
	5.1	4.8	90	3.0	4.8	3.79
	8.7	7.8	85			
	14.7	13.8	70			
	25.1	22.6	60			
Test-II						
	3.0	2.8	100			
	5.1	4.6	90			
	8.7	8.4	85	2.8	4.6	3.59
	14.7	13.5	75			
	25.1	21.9	55			
Mean ± SD				2.9 ± 0.14	4.7 ± 0.14	3.69 ± 0.14

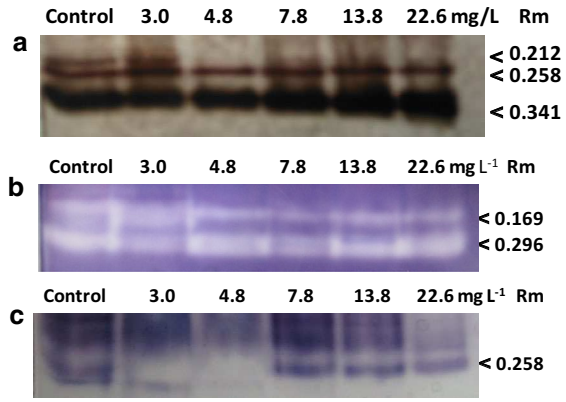

Fig. 1 Changes in isoenzyme expression profile of esterase (**a**), superoxide dismutase (**b**), and malate dehydrogenase (**c**) on different concentration of Ni (mg L^{-1}) exposed to *M. cephalus* for 30 days

Both acute and chronic toxicity values were calculated based on the measured dissolved Ni concentration in the treatment seawater. The 96 h LC_{50} and 95 % confidence limits were calculated based on the mortality against number of the test organisms exposed in each treatment concentration by using Probit Analysis software tool (Finney 1971). From the chronic test, NOEC, LOEC and chronic values were derived based on the survival of the test organisms at the end of 30-day of the experiment. One-way analysis of variance in conjunction with Dunnett's (1964) test was used to determine whether the treatments were significantly different from the control group ($p \leq 0.05$). The data were arc sine transformed. The enzyme profiles were analyzed by software provided with the gel documentation system (Biovis Gel 2012F, India).

Results and Discussion

In the present study, the temperature, DO, pH, salinity and Ni in experimental sea water were estimated to be 28 ± 1.16°C, 5.1 ± 1.46 mg L^{-1}, 7.9 ± 0.2, 32.74 ± 1.64 psu and 6.5 ± 0.76 µg L^{-1} respectively. The nominal test concentrations were measured for dissolved Ni concentration in all the test chambers at 24 h intervals for acute and 10 days intervals for chronic toxicity tests respectively. For all toxicity tests, the nominal and measured concentrations of Ni were compared to evaluate the stability of Ni concentration in the test chamber and to determine the accuracy of analytical method. The mean dissolved concentration and percent recovery of Ni between nominal and measured concentrations are summarized in Table 1 and the acute and chronic toxicity values were calculated based on the dissolved and recoverable Ni concentration in the test medium. The average 96 h LC_{50} value derived in *M. cephalus* was found to be 42.2 ± 3.9 (39.6–45.1) mg L^{-1} Ni based on the mean values of three repetitive flows through experiments (Table 2). There was no mortality initially from exposure to different concentrations of Ni, the mortality increased with longer exposure time and elevated metal levels. Previous authors reported the 96 h LC_{50} for Ni was 7.96 mg L^{-1} in *Menidia menidia* (Cardin 1985); 10.0 mg L^{-1} in *Danio rerio* (PAN database 2012); 21.0 mg L^{-1} in *Morone saxatills* (Palawski et al. 1985); 26.56 mg L^{-1} in *Atherinops affinis* (Hunt et al. 2002); 38.0 mg L^{-1} in *Menidia peninsulae* (Hansen 1983) and 70.0 mg L^{-1} in *Leiostomus xanthurus* (Hansen 1983) and such reports concluded that the survival of the species depends on their life stage and tolerance limit and factors like salinity, temperature and hardness of the test medium used. The percentage survival of exposed organisms at the

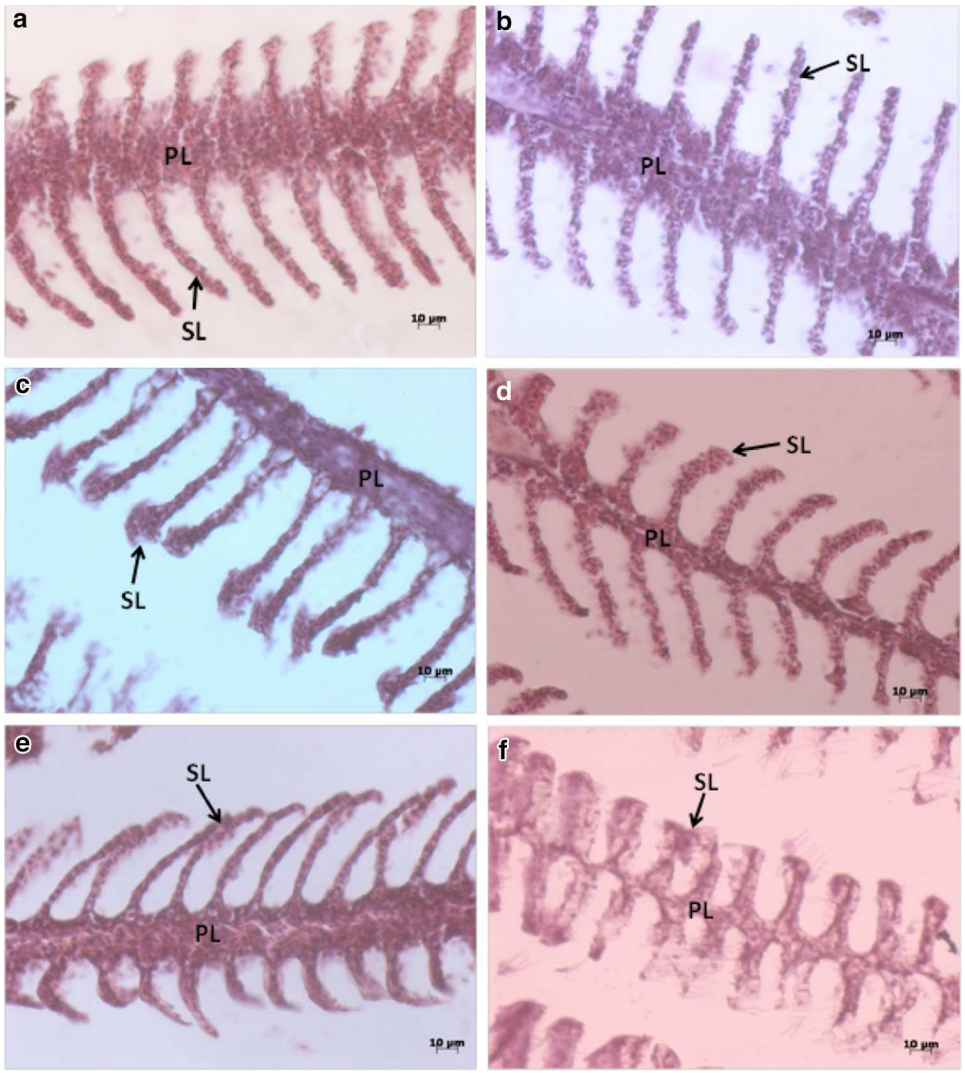

Fig. 2 Section through the gill of *M. cephalus* exposed to different concentration of Ni (mg L^{-1}) for 30 days stained with heamatoxylin and eosin (400X); **a** control fish showing normal arrangement of primary lamellae (PL) and secondary lamellae (SL); **b** 3 mg L^{-1} Ni (NOEC) exposed fish gill showing normal arrangement of gill filament (GF) and secondary lamellae (SL); **c** 4.8 mg L^{-1} Ni (LOEC) exposed fish gill showing hyperplasia at the ends of secondary lamellae (SL); **d** 7.8 mg L^{-1} Ni exposed fish gill showing hyperplasia of secondary lamellae (SL); **e** 13.8 mg L^{-1} Ni exposed fish gill showing hypertrophy and fusion at the ends of secondary lamellae (SL) and irregular interlamellar space; **f** 22.6 mg L^{-1} Ni exposed fish gill showing fusion, hypertrophy and necrosis of secondary lamellae; *scale bar* 10 µM

end of chronic exposure, the NOEC, LOEC, and chronic values are given in Table 3. From the results, it was ascertained that the survival of mullet decreased with increased exposure levels during the chronic toxicity test. Minimum percent (60 %) survival of organisms was observed at the highest concentration of Ni (22.6 mg L^{-1}) in Chronic I test and 55 % of survival at 21.9 mg L^{-1} Ni observed in Chronic II test after 30 days exposure. The average chronic value of 3.69 ± 0.14 mg L^{-1} was derived based on the geometric mean of the NOEC (2.9 ± 0.14) and LOEC (4.7 ± 0.14) respectively.

Sub lethal toxicity of Ni for 30 days in mullets caused an induction of EST, SOD and MDH activities (Fig. 1). Three isoforms of esterase activities were observed. The isoform 1 (Rm 0.212) of esterase activity was observed both at control and NOEC (3.0 mg L^{-1}) and there is no esterase activity in different concentrations as evidenced with no band formations. There is no variation in esterase activity levels of isoforms 2 and 3 (Rm 0.258; 0.341 values). SOD1 isoform activity levels (Rm 0.296) was decreased and SOD2 isoform activity levels (Rm 0.169) was increased compared to control level. MDH activity levels (Rm 0.258) was increased when compared to control level with the exception of absence of gel band formation at LOEC (4.8 mg L^{-1}) concentration. Enzymes have the ability to stabilize the effect of Ni toxicity and have their own specificity to respond and neutralize the reactive oxygen species formation. When the SOD activity was induced, it would be beneficial for the process of dismutation when there is an excess production of superoxide anion and to protect the cells from oxidative damages. Exposure to sodium hypochlorite in *M. galloprovincialis* reported inhibited acetylcholine esterase activity in gills (Lopez-Galindo et al. 2010). Padmini et al. (2009) studied low SOD activity in *M. cephalus* from Ennore estuary, in India. SOD activity increased significantly in zebrafish exposed to atrazine (Jin et al. 2010). Expression of MnSod was slightly increased when compared to control level but Cu/ZnSod expressions

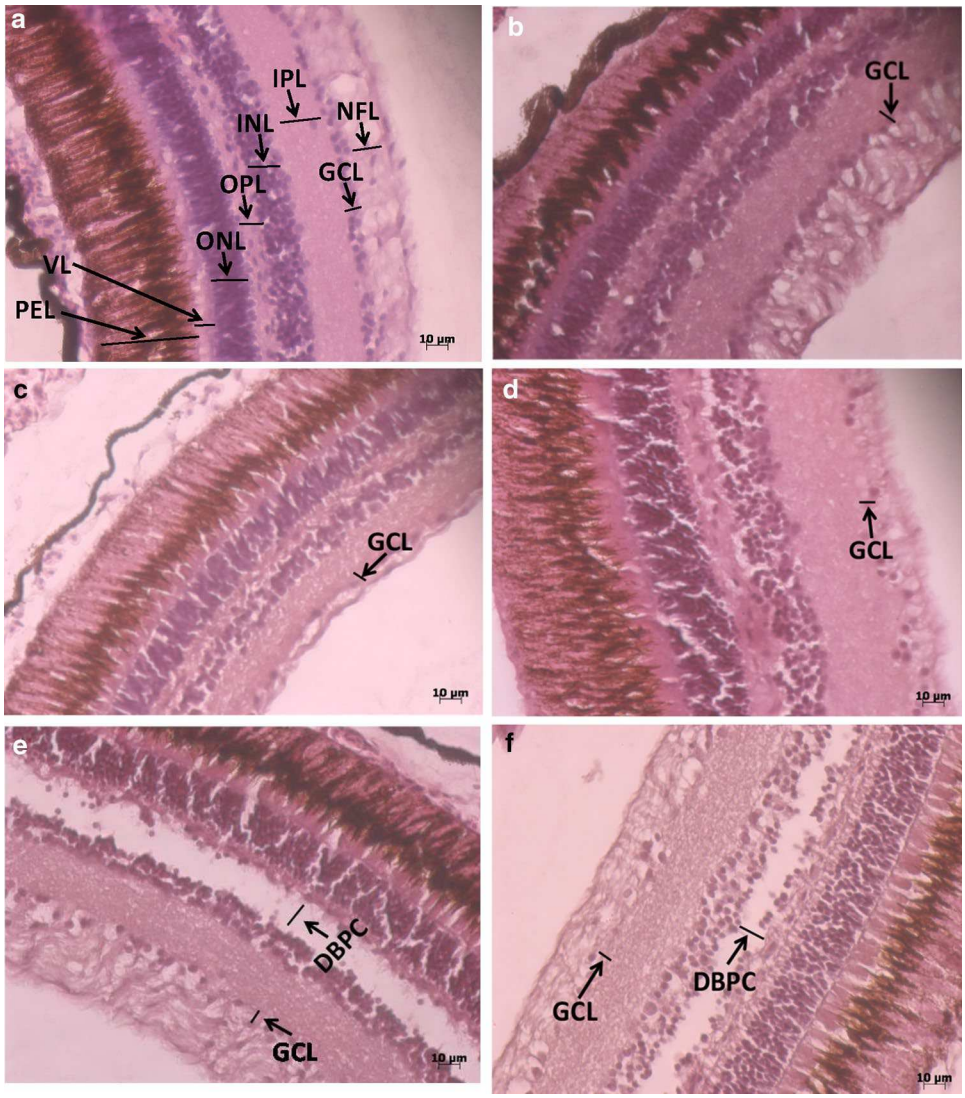

Fig. 3 Section through the retina of eye of *M. cephalus* exposed to different concentration of Ni (mg L^{-1}) for 30 days stained with heamatoxylin and eosin (400X); **a** retina of fish eye showing normal arrangement of pigment epithelium layer (PEL), visual layer (VL), outer nuclear layer (ONL), outer plexiform layer (OPL), inner nuclear layer (INL), inner plexiform layer (IPL), ganglion cell layer (GCL) and nerve fibre layer (NFL); **b** 3 mg L^{-1} Ni (NOEC) exposed fish retina of eye showing normal arrangement of eight specific layers; **c** 4.8 mg L^{-1} Ni (LOEC) exposed fish retina of eye showing reduced number of granular spherical cells in ganglion cell layer (GL); **d** 7.8 mg L^{-1} Ni exposed fish retina of eye showing reduced number of granular spherical cells in ganglion cell layer (GL); **e** 13.8 mg L^{-1} of Ni exposed fish eye retina showing reduced number of granular spherical cells in ganglion cell layer and detachment of bipolar cells from photoreceptor cells (DBPC); **f** 22.6 mg L^{-1} Ni exposed fish eye retina showing reduced number of granular spherical cells in ganglion cell layer and detachment of bipolar cells from photoreceptor cells (DBPC); *scale bar* 10 µM<\ fig>

remain unchanged in the liver of *Danio rerio* exposed to sodium fluoride (Mukhopadhyay and Chattopadhyay 2014). Low SOD activity has been reported in the gill of *M. cephalus* from Bizerte lagoon, Tuniria (Ameur et al. 2015). Livingstone (2001) reported that the responses of antioxidant enzymes to pollution vary for different species, enzymes, and single or mixed contaminants, and even greater variability is found in field situations. Elevated ROS level in tissues leads to cellular damage when the rate of its generation surpasses the rate of its decomposition by antioxidant defense systems.

Further, gills are considered a prime target to contaminants and significant route for uptake, bioconcentration and excretion of toxicants, due to the broad surface area reduce distance between internal and external medium and direct contact with the environment. Structurally the gills of fish showed the primary lamellae arranged in double rows, projecting towards the lateral side with a series of alternatively arranged secondary lamellae (respiratory lamellae). Each lamella was made up of single layer of cells (Fig. 2a). Towards the end of exposure no change was observed in the secondary lamellae of fish gill exposed to 3 mg L^{-1} Ni (NOEC) (Fig. 2b), however exposure to 4.8 mg L^{-1} (LOEC) and 7.8 mg L^{-1} Ni resulted in changes such as hyperplasia at the ends of secondary lamellae (Fig. 2c, d). Similarly, hypertrophy and fusion at the ends of secondary lamellae and irregular inter-lamellar space were observed in the gills of fish exposed to 13.8 mg L^{-1} Ni (Fig. 2e). The fusion of secondary lamellae, lamellar swelling, and reduced inter-lamellar space (Fig. 2f) were also observed in the gills of fish exposed to 22.6 mg L^{-1} Ni. Hence in the present study, the effect of Ni on the gills was particularly severe since they serve as a major organ for gaseous exchange, osmotic and ionic regulation, acid-base balance and nitrogenous waste. Epithelial hypertrophy and hyperplasia, necrosis of secondary lamellae, lamellar swelling,

fusion of adjacent lamellae and reduced inter-lamellar space were observed in the gills of mullets after long term exposure treatment to Ni under chronic stress (Fig. 2e, f). Vacuoles in branchial lamellae, necrosis and hypertrophy of epithelial cells in fish exposed to Fe(II) were reported (Zhihao et al. 2012). Ameur et al. (2015) studied thickening of primary lamellae, cellular hyperplasia, aneurism, curving, shortening and fusion of secondary lamellae in *M. cephalus* from Bizerte lagoon, Tuniria.

The retina of eye of control fish showed normal arrangement of the eight specific layers, viz., pigment epithelium layer (PEL), visual layer (cone and rod receptor cells) (VL), outer nuclear layer (ONL), outer plexiform layer (OPL), inner nuclear layer (INL), inner plexiform layer (IPL), ganglion cell layer composed of a narrow chain of granular spherical cells surrounded by a fine connective tissue network (GCL) and nerve fibre layer (NFL) (Fig. 3a). No changes were observed in the retina of fish exposed to 3.0 mg L^{-1} Ni (NOEC) (Fig. 3b), while fish exposed to 4.8 mg L^{-1} (LOEC) and 7.8 mg L^{-1} Ni showed reduced number of granular spherical cells in ganglion cell layer (Fig. 3c, d). However, reduced number of granular spherical cells in ganglion cell layer and detachment of bipolar cells from photoreceptor cells at 13.8 mg L^{-1} (Fig. 3e) and 22.6 mg L^{-1} Ni (Fig. 3f) exposed to fish were observed. The detachment of bipolar cells from photoreceptor cells in the retina of the fish eye exposed to Ni for long term exposure under chronic stress was also observed, which affects the primary function of retina by converting light energy into nerve impulses which are transferred to the brain via the optic nerve consequences in loss of vision or poor vision. Choudhury et al. (2015) reported that accumulation of selenium in the eye lens may be the cause of ocular impairments in exposed fish. Cellular anomalies like epithelial hypertrophy and hyperplasia, necrosis of secondary lamellae, lamellar swelling in gills and detachment of bipolar cells from photoreceptor cells in the retina of eye of mullet are the net result of adverse physiological and biochemical changes inside the cells of organism. These changes indicated toxic effects of nickel on cellular functioning. A practical advantage of using fish histopathology is that multiple organs can be examined; and this increases the sensitivity at which pollution impacts can be detected. The acute and chronic toxicity values are important for environmental monitoring since the presence of Ni has been identified as an indicator of pollution. Further, exposure of fish to Ni in particular induces a number of biochemical alterations reflecting the imbalance of antioxidant related parameters which lead to onset of oxidative stress.

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