ISSN: 2642-9020

Research Article

Journal of Marine Science Research and Oceanography

Evaluation of Heavy Metals and Oxidative Stress with Biochemical Parameters as Bioindicators of Water Pollution and Fish in Lake Burullus, Egypt

Mohamed YM Aly*, Dalia MK El-Gaar, Sally M Salaah and Mohammed H Abdo

National Institute of Oceanography and Fisheries (NIOF), Cairo, Egypt

***Corresponding author**
Mohamed YM Aly, National Institute of Oceanography and Fisheries (NIOF), Cairo, Egypt

Submitted: 10 Mar 2020; **Accepted**: 16 Mar 2020; **Published**: 28 Mar 2020

Abstract
Lake Burullus represents an economically important brackishwater coastal lagoon in Egypt, due to its rich fishery resources. In addition to the remains of agricultural wastes, the lake is exposed to many kinds of chemical and biological pollutants that are disposed into it. So, the study was carried out on three sectors in the lake. Western sector of the lake where agricultural drainage was discharged, eastern sector where agricultural and industrial effluents was entered, and middle sector where the least pollution was occurred among them. The present study was controlled to assess the harmful effects of heavy metals on the biochemical and antioxidant defense system in Nile tilapia collected from three sectors. The studied heavy metals in gills, liver, and muscles of the Nile tilapia (Oreochromis niloticus) collected from the eastern and western sectors of the lake was much higher than that of fish collected from middle sector. Biochemical profile and antioxidant defense system of Nile tilapia were significantly ($P<0.05$) higher in fish collected from western and eastern sectors during summer and winter seasons compared to the middle sector. The results obtained from the present study also revealed that the meat quality of the collected fish from middle sector was more superior to those of fish collected from the eastern and western sectors of the lake. The study also indicates that the alterations in the biochemistry profile and antioxidant defense system of Nile tilapia can be used as biomarkers of metal pollution for monitoring aquatic life.

Keywords: *Oreochromis niloticus*, Pollution, Industrial and Agricultural Effluents, Heavy Metals, Bioindicator, Antioxidant Enzymes

Introduction
The aquatic environment with its water quality is considered the main factor controlling the state of health and disease in both cultured and wild fishes. Pollution of the aquatic environment by inorganic and organic chemicals is a major factor posing serious threat to the survival of aquatic organisms including fish.

The northern Delta of Egypt includes Lake Edku, Borollus, Manzala, and Mariut. These Lakes are situated on the Mediterranean Coast of the Delta and cover about 6% of the non-desert surface area of Egypt. The Lakes are important natural resources for fish production in Egypt. Until 1991, these Lakes have always contributed more than 40% of the country's total fish production, but at present this has been decreased to less than 12.22% [1].

Lake Burullus represents an economically important lagoon in Egypt, due to its rich fishery resources. Unfortunately, the lake is exposed to many kinds of chemical and biological pollutants in addition to the remains of agricultural and wastes that are disposed into it. Lake Burullus is also the central part of five principal coastal lagoons of northern Egypt. To the east are Lake Manzala and Bardawil, to the west are lake Idku and lake Maryut. It is a part of the governorate of Kafr El-Sheikh in the northern part of the delta that lies between the two branches of the Nile. Lake Burullus is separated from the Mediterranean Sea by a 65 km long sand bar; the middle section of the bar is narrow and is cut by an inlet (Boughaz) that connects the sea and the lake. The lake comprises a body of water (460 km^2). The area has been decreased during the last 50 years due to land reclamation of its southern stretches. Moreover, annual fish production of lake Burullus is around 55.000 tons; which is about 40% from the northern lakes and 13.5% from the national fish production [2].

Recently, the aquatic organisms are used as indicators of trace metals pollution. Heavy metal concentrations are extremely variable in various marine and fresh water organisms depending on the geochemical background, the level of pollution in a given area and fish activity [3]. Bioaccumulation of heavy metals in fish may critically influence the growth rate, physiological and biochemical status, and consequently the meat quality of fish. Moreover, it has been observed that through biological amplification, some aquatic organisms may concentrate metals present with low amounts in the environment to levels that exceed standards, which are harmful to organisms. Fish likewise other aquatic organisms, are greatly affected with chemical pollutants present in the ecosystem. It is recommended that the developed histopathological changes in fish can be used as biondicators for environmental pollution [4].

Several studies have reported that exposure of fish to pollutants (such as agricultural wastes, industrial effluents and sewage discharges) affects the antioxidative defense enzymes such as: superoxide dismutase (SOD), catalase (CAT), glutathione-s-transferase (GST), glutathione peroxidase (GTx) and glutathione reductase (GR) [5]. It is suggested that some of these enzymes can constitute good molecular bioindicators for oxidative stress and can indicate the magnitude of response in vertebrate population chronically exposed to contaminants, such as metals and other xenobiotic [6]. Many circumstances promote the antioxidant defense response in fish, and factors intrinsic to the fish itself such as: age, reproductive, metabolic status of fish and environmental conditions include food availability, oxygen level, temperature of water, salinity, photoperiod, toxins present in the water or pathologies, can either fortify or weaken antioxidant defenses [7]. The accumulation of heavy metals can produce increasing amount of Reactive oxygen species (ROS) in fish by generating free radicals such as the hydroxyl radical (OH), proxy radical (RO_2•) and superoxide (O_2•) and some non-radical species such as hydrogen peroxide (H_2O_2), which lead to the induction of enzymatic antioxidants (SOD, CAT, GST, GR and GPx) and non-enzymatic antioxidant glutathione (GSH). These antioxidants scavenge free radicals and provide protection against this aspect of oxygen toxicity (Kadar et al., 2005). Oxidative stress occurs when the equilibrium between ROS production and the antioxidant defenses is lost. ROS can be important mediators of damage to cell structures, including lipids and membranes, proteins and nucleic acids, that can seriously alter the cell membranes and other structures such as proteins, lipids, lipoproteins, and deoxyribonucleic acid (DNA), which lead to different pathologic processes, and fish diseases [8]. The first line of defense against oxidative stress is SOD, CAT and SOD catalyzes the superoxide anion radical (O_2) dismutation into hydrogen peroxide (H_2O_2) by reduction. The oxidant formed H_2O_2, is transformed into water and oxygen (O_2) by catalase (CAT) or glutathione peroxidase (GPx) [9]. GPx enzyme removes H_2O_2 by using it to oxidize reduced glutathione (GSH) into oxidized glutathione (GSSG). Glutathione reductase GR is a flavoprotein enzyme, regenerates GSH from GSSG, using NADPH as a source of reducing power. Besides hydrogen peroxide, GPx also reduces lipid or non-lipid hydroperoxides, while oxidizing glutathione (GSH) [10].

Materials and methods

Collection of samples

Three sampling sites were chosen at the eastern, middle and western sectors of the lake Burullus:

Site (1): The eastern sector near the agricultural effluents of a drain in a region called El-Belak.

Site (2): The middle sector near the industrial and agricultural effluents of a drain in a region called Demro.

Site (3): The western sector near the agricultural effluents and fish hatcheries, where a drainage canal called El- Hoxa.

Determination of heavy metals in fish organs

Heavy metals (Cu, Fe, Pb, Mn and Zn) were detected in liver, gills and muscle according to Apha then measured using atomic absorption spectrophotometry (Perkin Elmer, 2280) [11].

Sample Preparation

Blood samples (approximately 3 mL) were collected immediately from alive *Oreochromis niloticus*; blood was withdrawn from the caudal vein using a syringe. Soon after collection, blood was left to clot and then centrifuged at 3000 rpm for 10 min. Supernatant serum was obtained using micropipette and stored at 4˚C till the determination of biochemical parameters.

Biochemical parameters

Serum aspartate amino transferase (AST) and alanine amino transferase (ALT) activities were determined colorimetrically, using transferases kits according to method described by Reitmans and Frankel [12]. Serum glucose was measured by using the GOD-PAP method (enzymatic colorimetric method) according to Trinder, using Boehringr Mannheim kits [13]. Serum creatinine was measured, using colorimetric method described by Henry [14]. Serum uric acid was determined, using enzymatic reaction according to Barham and Trinder [15].

Oxidative stress parameters

The antioxidant enzymes in blood serum were investigated using commercial test Kits (Bio-diagnostic, Egypt). Superoxide dismutase (SOD) activity in tissues was measured using the enzyme ability to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye. Catalase (CAT) activity, defined as U/g was determined by Colorimetric method. GST activity was determined by the increase in absorbance at 340 nm due to the formation of the conjugate 1-chloro-2,4- dinitrobenzene (CDNB) using as substrate at the presence of reduced glutathione (GSH).

Statistical analysis

The results were expressed as mean ± SE using a Microsoft Excel sheet on Windows 2010. The differences among treatments were analyzed using a One-way Analysis of Variance (ANOVA) followed by Duncan Multiple Range Test for multiple comparisons of the means. The statistical significance was set at $P < 0.05$, using SPSS for Windows version 23.0 (SPSS, Michigan Avenue, Chicago, IL, USA).

Results

Heavy metals concentration levels in fish organs

The heavy metal levels of Cu, Fe, Pb, Mn and Zn in fish organs (Liver, gills and muscles) collected from the different locations of the lake Burullus during winter and summer 2018 are presented in Tables 1, 2 and 3. The order of heavy metals levels in the muscles was Zn >Mn>Fe >Cu>Pb, and in the liver and gills were Zn>Mn>Fe>Pb>Cu. The highest concentration of Zn in fish liver was 29.44 μg/g at western sector during summer. Whereas the lowest concentration of Zn in fish gills was 8.72 μg/g at eastern sector during winter. The highest value of Mn in fish muscles was found to be 18.1μg/g at western sector during summer, whereas the lowest concentration of Mn was 1.04 μg/g at eastern sector during winter. The highest value of Fe in fish gills was 12.36 μg/g during winter at western sector, whereas the lowest value of Fe was 0.5μg/g during summer at eastern sector. The highest value of Cu in fish liver was 2.41μg/g at western sector during summer. The lowest concentration of Cu in fish muscles was 0.49 μg/g at eastern sector in summer. The highest value of Pb in fish gills was obtained to be 3.54 μg/g during winter at western sector, whereas the lowest concentration of Pb (0.15 μg/g) was found in fish muscles at eastern sector during winter season.

Table 1: Heavy metal concentrations (ug/g) of the Nile tilapia *O. niloticus* caught from eastern sector of the Lake Burullus during winter and summer seasons (2018)

heavy metals	winter			summer		
	liver	gills	muscles	liver	gills	muscles
Cu	1.31	0.88	0.41	1.52	0.85	0.49
Fe	2.61	3.44	1.32	4.55	3.52	0.50
Pb	2.40	3.10	0.15	2.50	2.90	0.20
Mn	9.14	1.04	2.03	13.58	1.49	2.27
Zn	18.22	8.72	10.39	21.26	10.35	15.28

Table 2: Heavy metal concentrations (ug/g) of the Nile tilapia *O. niloticus* caught from middle sector of the Lake Burullus during winter and summer seasons (2018)

heavy metals	winter			summer		
	liver	gills	muscles	liver	gills	muscles
Cu	1.14	0.72	0.36	1.32	0.93	0.55
Fe	2.99	3.32	1.038	3.78	3.9	2.2
Pb	2.9	3.3	0.22	2.7	3.1	0.24
Mn	10.04	1.47	2.34	15.06	2.22	2.34
Zn	20.29	11.1	10.24	23.31	12.72	18.26

Table 3: Heavy metal concentrations (ug/g) of the Nile tilapia *O. niloticus* caught from western sector in Lake Burullus during winter and summer seasons (2018)

heavy metals	winter			summer		
	liver	gills	muscles	liver	gills	muscles
Cu	1.55	0.93	0.53	2.41	1.41	0.61
Fe	3.6	12.36	1.42	4.1	3.8	3.6
Pb	3.2	3.54	0.29	2.9	3.3	0.32
Mn	11.2	1.71	2.56	18.1	2.57	2.77
Zn	21.41	16.15	11.12	29.44	15.24	22.3

Biochemical parameters

The mean concentration values of aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, glucose and creatinine in blood serum of Nile tilapia are shown in Tables (4 and 5). The values of AST content in fish samples from both sites are represented the highest value of 70.5 g/dl, which was estimated at western sector during summer, while the lowest value (28.3 g/dl) was obtained at eastern sector during winter. The highest value of ALT was 45.6 g/dl during summer at western sector, whereas the lowest concentration was 15.3 g/dl during winter at eastern sector. The highest value of urea was 35.4 g/dl during summer at western sector, while the lowest content was 17.7 g/dl. Also, the highest content of glucose was 136.4 g/dl at western sector during summer, whereas the lowest concentration was 80 g/dl during winter at eastern sector. The highest content of creatinine was 1.7 g/dl at western sector during winter and the lowest content was 0.63 g/dl at the same sector.

Table 4: The biochemical parameters of Nile tilapia *O. niloticus* collected from Lake Burullus during winter season (2018) (Means ±SD)

Biochemical Parameters	East	Middle	West	f-value	Sig.
AST	28.33±2.2^{b}	41.81± 1.9^{a}	53.53±2.4^{a}	18.98	<0.01
ALT	15.30±1.3^{b}	21.02±2.1^{b}	29.61±2.1^{a}	16.51	<0.01
Glucose	80.31±1.3^{c}	101.84±1.2^{b}	123.12±1.3^{a}	200.04	<0.01
Urea	17.71±0.44^{c}	27.91±0.41^{b}	33.70±0.52^{a}	522.54	<0.01
Creatinine	0.63±0.04^{b}	0.83±0.05^{b}	1.74±0.11^{a}	56.58	<0.01

Data were presented as mean ± SE (n=6 fish). Values within a row with different superscripts differ significantly (Duncan Multiple Range Test, P<0.0)

Table 5: The biochemical parameters of Nile tilapia *O. niloticus* collected from Lake Burullus during summer season (2018) (Means ±SD).

Biochemical Parameters	East	Middle	West	f-value	Sig.
AST	42.34±2.2^{c}	59.81±1.9^{b}	70.51±2.1^{a}	71.48	<0.01
ALT	23.3±2.3^{b}	35.02±2.1^{a}	45.62± 2.1^{a}	28.27	<0.01
Glucose	114.45±2.1c	127.40±1.6^{b}	136.44±1.4^{a}	35.32	<0.01
Urea	21.11±0.49c	30.85±0.35^{b}	35.40±0.44^{a}	222.17	<0.01
Creatinine	0.98±0.25^{a}	1.06±0.10^{a}	1.17±0.13^{a}	0.59	0.556

Data were presented as mean ± SE (n=6 fish). Values within a row with different superscripts differ significantly (Duncan Multiple Range Test, P<0.05).

Oxidative stress parameters

Tables 6 and 7 shows the values of SOD enzyme in the blood collected from Nile tilapia inhabiting Burullus Lake. It is obvious that the highest SOD value (118.3 u/ml) was estimated at western sector during summer, while the lowest value (58.8u/ml) was recorded from eastern sector during winter. The highest CAT value (82.3 u/l) was estimated at western sector during summer, while the lowest value (29.1 u/l) was recorded at eastern sector during winter season. The highest GST value (0.97 u/l) was estimated at western sector during summer, while the lowest value U/l was recorded at eastern sector during winter.

Table 6: The antioxidant enzymes of Nile tilapia *O. niloticus* collected from Lake Burullus during winter season (2018) (Means ±SD)

Antioxidant Enzymes	East	Middle	West	f-value	Sig.
SOD	58.8±4.4 b	73.4±5.2 a	90.4±5.6 a	10.31	<0.01
CAT	29.1±4.1 b	40.2±4.2 b	55.6±4.5 a	7.97	<0.01
GST	0.19±0.06 a	0.27±0.04 a	0.43±0.16 a	0.69	0.515

Data were presented as mean ± SE (n=6 fish). Values within a row with different superscripts differ significantly (Duncan Multiple Range Test, P<0.05).

Table 7: The antioxidant enzymes of Nile tilapia *O. niloticus* collected from Lake Burullus during summer season (2018) (Means ±SD)

Antioxidant Enzymes	East	Middle	West	f-value	Sig.
SOD	78.21±4.2[c]	91.42±4.1[b]	118.32±3.7[a]	17.87	<0.01
CAT	38.13±3.1[c]	54.44±3.4[b]	82.33±3.3[a]	70.51	<0.01
GST	0.46±0.07[c]	0.72±0.04[b]	0.97±0.16[a]	20.69	0.515

Data were presented as mean ± SE (n=6 fish). Values within a row with different superscripts differ significantly (Duncan Multiple Range Test, P<0.05).

Discussion

Heavy metals in organs fish

Heavy metals are taken up through different organs of the fish according to the affinity between these organs. So, many of these heavy metals are concentrated at different levels in different organs of the fish body [16]. Generally, the concentrations of the studied heavy metals in some selected vital organs (liver, gills and muscle) of the Nile tilapia; *Oreochromis niloticus* caught from the different sites along the El-Burullus lake are higher in the eastern and western sectors of the lake. This may be due to the large amount of agricultural and industrial effluents discharged directly into the lake. On the other side, the obtained results declared that the lowest concentrations of the heavy metals were recorded in tissues of fish collected from the middle sectors. The present data showed the order of heavy metals concentration levels in the muscles was Zn>Mn>Fe>Cu>Pb and in liver and gills were Zn>Mn>Fe>Pb>Cu. This agree with the findings of Yacoub and Gad [17], which reported that the levels of elements (Cu, Mn, Pb, and Zn) were in high degrees in the intestine of *Oreochromis niloticus* caught from river Nile at various studied areas at Upper Egypt, and the lowest values were reported in the edible parts of fish, which were less than the allowable level, so the negative effect of these elements was lowered in the muscles compared to the other organs in the investigated fish. The gill, liver is the preferred organs for heavy metals accumulation as could be deduced from the present study. This research showed that the gill and liver have almost higher metal concentration than the muscles. Such pattern has been observed in a number of other studies, covering a wide spectrum of fish species [18, 19].

Generally, the heavy metal concentration levels were higher in the gill than the muscle tissue of fish. This may be associated with the gill which may be due to mucus which is impossible to completely remove from the platelets.The adsorption of metals onto the gill surface, as the first target for pollutants in water, could also be an important influence in the total metal levels of the gill [20].

Biochemical parameters

Biochemical parameters helped to identify the target organs of toxicity and the general health status of animals. It also provided an early warning signal in stressed organism [21].

Transaminases are important enzymes which are playing a key role in mobilizing L-amino acids for gluconeogenesis and they function as links between carbohydrate and protein metabolism under altered physiological and pathological conditions [22]. In the present study, serum AST and ALT were significantly (P<0.05) elevated during summer season compared with winter. Elevations in activities of serum AST and ALT reflect hepatic impairment, leading to extensive liberation of these enzymes into the blood circulation. The elevation of the hepatic enzymes may be due to liver dysfunction as a result of the hepatocellular damage or cellular degradation [23].

Glucose level was significantly (P<0.05) higher at the different sectors during summer season compared to winter season. Elevation in glucose level may be resulted from the increase in glucogenesis and glycogenolysis as well as inhibition of glucogenolysis and glycogenesis to produce the energy used in combating the stress induced on the fish by the environmental pollution [24].

Kidneys are playing an essential role in the water and electrolyte balance and in the maintenance of a stable internal body environment [25]. Serum renal products, including creatinine and urea were significantly (P<0.05) higher at the different sectors during summer season compared with the winter. The increase in the creatinine and urea levels in the blood is an indicative of impaired kidney function, which is attributed to the high production of ROS and kidney injury [26, 27]. The kidney dysfunction may be as a result of heavy metal -induced nephrotoxicity, and glomerular insufficiency [28].

Oxidative stress parameters

Oxidative stress is another mechanism for toxicity, which leading to cell death and disturbance of the physiological processes in fish [29]. Oxidative stress results from the imbalance between antioxidant enzyme activities and ROS production, also when the antioxidant system becomes unable to eliminate or neutralize the excess of ROS [30]. The SOD and CAT system, the first line of defense system against oxidants varied according to the response of fish antioxidant system to counteract with the toxicity of metal exposure [31]. The increase in SOD and CAT enzymes may be due to the elimination of ROS from the cell induced by exposure to pollutants which convert superoxide anions (O_2-) into H_2O_2 and then into H_2O and O_2 [32]. GST plays an important role in homeostasis, detoxification and clearance of many xenobiotic compounds. Whereas, GST can stimulate pollutants of GSH, and facilitate chemical secretion [33-37].

Conclusion

The results in this study showed that the eastern and western sectors Tilapia samples were collected from them. The samples were more polluted from the middle sector. As for the heavy metals, biochemical and oxidative stress parameters, they were higher in the western sector, followed by the eastern and then the middle, and in the summer they were higher than in the winter in the three sectors. The liver was the most polluted organs than the gills and muscles and Zinc is higher in fish organs than other heavy metals.

References

1. GAFRD (2006) General Authority for Fishery Resources Development. Year-Book of fishery statistics in Egypt (1990-2005) Cairo.
2. National Water Resources Plan for Egypt (2000) Ministry of water Resources and Irrigation, Egypt Planning sector (NWRP). Fisheries and Water Resources.
3. Yilmaz F, Ozdemir N, Demirak A, Tuna AL (2007) Heavy metal levels in two fish species; Leucisws cephalus and lepomis gibbosus . Food Chem 100: 830-835.
4. Gaber HS (2003) Histopathological changes in liver of Tilapia zillii induced by aromatic hydrocarbons. J Egypt Ger Soc Zool 42: 145-154.

5. Khalil MT, Gad NS, Ahmed NA, SM Salaah (2017) Antioxidant Defense System Alternations in Fish as a Bio-Indicator of Environmental Pollution. Egyptian Journal of Aquatic Biology and Fisheries 21: 11-28.
6. Gad NS (2009) Determination of glutathione related enzymes and cholinesterase activities in Oreochromis niloticus And Clarias gariepinus as bioindicator for pollution in Lake Manzala. Global Vet 3: 37-44.
7. Melegaria SP, Perreaulut F, Costa RH, Popovic R, Matiasa WG, etal., (2013) Evaluation of toxicity and oxidative stress induced by copper oxide nanoparticles in the green alga; Chlamydomonas reinhardtii . Aquat Toxicol 142: 431-440.
8. Pereira CDS, Martins-Dias ML, Zanette J, Cesar A, Choueri R J, etal., (2011): Integrated biomarker responses as environmental status descriptors of a coastal zone (Sao Paulo, Brazil). Ecotoxicol. & Environ Saf 74: 1257-1264.
9. Stara A, Machova J, Velisek J (2012) Effect of chronic exposure to simazine on oxidative stress and antioxidant response in common carp (Cyprinus carpio L.). Environ. Toxicol & pharmacol 33: 334-343.
10. Genestra M (2007) Oxyl radicals, redox-sensitive signalling cascades and antioxidants. Review Cell Signal 19: 1807-1819.
11. APHA, American Public Health Association (1995) American Water Works Association and Water Pollution Control Federation Standard Methods for the Examination of Water and Wastewater. 19th edition.
12. Reitman S, Frankel S (1957) Glutamic – pyruvate transaminase assay by colorimetric method. Am J Clin Path 28: 56.
13. Trinder P (1969) Enzymatic colorimetric method of glucose. Ann Clin Biochem. 6: 24.
14. Henry TJ 2nd ed. Harper and Row Publishers (1974) Clinical Chemistry Principles and Techniques.
15. Barham D, Trinder P (1972) An improved colour reagent for the determination of blood glucose by the oxidase system. Analyst 97: 142-145
16. Gad N Sh (2005) Impact of environmental pollution in the southern region of lake Manzalah Egypt on some biochemical parameters of Tilapia zillii. J Egypt Ger Soc Zool 48: 279-298.
17. Yacoub AM, NS Gad (2012) Accumulation of some heavy metals and biochemical alterations in muscles of Oreochromis niloticus from the River Nile in Upper Egypt. International Journal of Environmental Science and Engineering 3: 1-10.
18. Ploetz DM, Fitts BE, Rice TM (2007) Differential accumulation of heavy metals in muscles and liver of a marine fish (King Mackerel, Scomberomorus cavalla, Cuvier) from the Northern Gulf of Mexico, USA. Bull. Environ Contam Toxicol, 78: 134-137.
19. Agah H, Leermakers M, Elskens M, Fatemi SMR, Baeyens W, etal., (2009) Accumulation of trace metals in the muscles and liver tissues of five fish species from the Persian Gulf. Environ Monit Assess 157: 499-514.
20. Heath AG (1995) Water Pollution and Fish Physiology. CRC Press Inc Boca Raton Florida 359pp.
21. David M, Ramesh H, Patil V, Marigoudar S, Chebbi S, etal., (2010) Sodium cyanide- induced modulations in the activities of some oxidative enzymes and metabolites in the fingerlings of Cyprinus carpio (L). Toxicological and Environmental Chemistry 92: 1841-1849.
22. Manjunatha B, Tirado J, Selvanayagam M (2015) Sub lethal toxicity of potassium cyanide on Nile tilapia (Oreochromis niloticus): biochemical response. International Journal of Pharmacy and Pharmaceutical Sciences 7: 379-382.
23. Mohamed FAS, Gad NS (2009) Bioaccumulation, some blood biochemical changes and histological alterations in selected tissues of Oreochromis niloticus exposed to zinc and/ or cadmium. American European Journal of Agricultural and Environmental Sciences 5: 441-455.
24. Yekeen TA, Fawole O (2011) Toxic effects of endosufan on haematological and biochemical indices of Clarias gariepinus. African Journal of Biotechnology 10: 14090-14096.
25. Palaniappan PLRM, Krishnakumar N, Vadivelu M (2009) Bioaccumulation of lead and the influence of chelating agents in Catla catla fingerlings. Environmental Chemistry Letters 7: 51-54.
26. Patel M, Rogers JT, Pane EF, Wood CM (2006) Renal responses to acute lead waterborne exposure in the freshwater rainbow trout (Oncorhynchus myskis). Aquatic Toxicology 80: 362-371.
27. Upasani CD, Balaraman R (2003) Protective effect of spirulina on lead induced deleterious changes in the lipid peroxidation and endogenous antioxidants in rats. Phototherapy Research 17: 330-334.
28. Yu CC, Lin JL, Lin Tan DT (2004) Environmental exposure to lead and progression of chronic renal diseases a four – year prospective longitudinal study. Journal of the American Society of Nephrology 15: 1016-1022.
29. Banaee M (2013) Physiological dysfunction in fish after insecticides exposure edited by Stanislav Trdan P (Ed). Insecticides-Development of Safer and More Effective Technologies.
30. Hamed HS (2015) Impact of a short-term malathion exposure of Nile Tilapia, (Oreochromis niloticus): The protective role of selenium. International Journal of Environmental Monitoring and Analysis, 3: 30-37.
31. Atli G, Canli M (2010) Response of antioxidant system of freshwater fish Oreochromis niloticus to acute and chronic metal (Cd, Cu, Cr, Zn, Fe) exposures. Ecotoxicology and Environmental Safety 73: 1884-1889.
32. Hamed HS (2016) Ameliorative effects of Spirulina platensis on deltamethrin-induced biochemical alterations and oxidative stress in the African catfish; Clarias gariepinus. Open Journal of Marine Scicence 6: 1-10.
33. Luo Y, Su Y, Lin RZ, Shi HH, Wang XR, etal., (2006) 2-Chlorophenol induced ROS generation in fish Carassius auratus based on the EPR method. Chemosphere 65: 1064-1073.
34. Habig WH, Pabst MJ, Jacoby WB (1974) Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. J. Biol. Chem 249: 7130-7139.
35. Kádár E, Costa V, Santos RS (2006) Distribution of micro-essential (Fe, Cu, Zn) and toxic (Hg) metals in tissues of two nutritionally distinct hydrothermal shrimps. Science of the total environment, 358(1-3), 143-150.
36. KORI-SIAKPERE, O AKE JEG, IDOGE E (2005) Hematological characteristics of the African Snakehead, Parachanna obscura. African Journal of Biotechnology 4: 527-530.
37. Lartillot S, Kadziora P, Athios A (1988) Purification and characterization of new fungal catalase. Prep Biochem. 18: 241-246.