ISSN 2229-5518

Effects of Heavy Metals and Pathogenic Bacteria on the Antioxidant Enzyme Activities and Immune Response of *Clarias gariepinus* in Egypt

Mohamed H. Bahnasawy[1], Entsar A. Saad[2], Hasan A. Maroof[3], Samah A. Abou Shehatta[2,3,*]
[1] Zoology Department, Faculty of Science, Damietta University, Egypt
[2] Chemistry Department, Faculty of Science, Damietta University, Egypt
[3] Food Inspection Laboratory, Damietta Port, Animal Health Research Institute, Giza, Egypt

Abstract—The aim of this study was to measure chemical, bacteriological, and biochemical changes of fish to determine the pollutants level and their effects on *Clarias gariepinus* (catfish) quality. The study was applied on samples at Shatta (site1) and Lake El-Burullus (site 2). The obtained results revealed that (site1) had greater concentrations of heavy metals. Moreover, the antioxidant parameters revealed noticed increase in MDA in fish samples from both sites, reduced levels of SOD, catalase, glutathione reductase and TAO in fish samples from (site1). These results showed that antioxidant enzymes can be used as biomarkers of heavy metals pollution.

Index Terms— Antioxidant parameters, biochemical changes, *Clarias gariepinus*, heavy metals, pollution, pathogenic bacteria

1 Introduction

World consumption of fish has increased simultaneously with the growing concern of their nutritional and therapeutic benefits. In addition to its important source of protein, fish have rich contents of essential minerals, unsaturated fatty acids and vitamins [1]. Water contamination has been long reported as the major threat to the aquatic environment [2].When fish are exposed to these metals in an aquatic ecosystem, they tend to take these metals up which may accumulate in various tissues in significant amounts and are eliciting toxicological effects at critical targets [3]. Increase in agricultural pollution and industrial growth, with consequent impacts on aquatic ecosystems are a major focus of research worldwide [4].

Many pollutants mediate their toxicity through oxidative stress, resulting in changes in antioxidant defenses as well as damage to proteins, membrane lipids and DNA molecules, the result of such exposure leading to oxidative stress can impair cellular or biological function which lead to disease [5]. Fish are suitable candidates for the study of oxidative stress induced by pollutants [6]. Oxidative stress can lead to oxidative damage in cellular molecules [7].

Heavy metal pollution increase the susceptibility of aquatic animals to various diseases by interfering with the normal functioning of their immune, reproductive, and developmental processes [3]. Pollution with heavy metals influence the immune system of fish, leading to impairment of their health or even increase their mortality [8].

Total volatile base, taken as an indication for bacterial growth, while the ammonia comes from decomposition of amino acids, thus reducing the quality of the available protein [9]. *Salmonella* is a common food borne pathogen, causing major health problems [10]. Municipal sewage, agriculture pollution, and storm water runoff are the main sources of these pathogens in waters [11]. *Escherichia coli* is the best thermo-tolerant pollution indicator [13]. It is estimated that 2–7% of *E. coli* infections result in acute renal failure [11].

L. monocytogenes is a concern to public health because of its ability to survive under harsh conditions. Therefore, it is considered a serious public health risk. It spreads through the consumption of food products and its related-disease can be fatal to humans and animals [12]. The discharge of untreated sewage into seawater was involved in contamination of fish with *L. monocytogenes* [13].

Heavy metals are considered major pollutants of aquatic environments due to the difficulty of metabolization and the bioaccumulative potential in tissues of aquatic organisms [14]. Heavy metals including mercury, lead, and cadmium alter host's immune system and lead to increased susceptibility to infections and autoimmune diseases [15]. Cancer caused by Pb is a potential issue [16]. Cadmium is an extremely toxic heavy metal, result in alterations of the physiological processes in the blood and tissue of fish [17].

Hg is extremely toxic to animals and human health through various absorption pathways [18], it induced oxidative stress on exposed fish, since inhibition of antioxidant enzymes activity, increases in lipid peroxidation, DNA damage, and micronuclei frequency occurred [19]. Recently, it has the ability to bind to a variety of biomolecules, which can compromise its structure and functionality and thus promote its toxic effects [20].

2 MATERIALS AND METHODS

2.1 Study Area

The study was conducted on two areas, the first one is Shatta village, located in Damietta Governorate, is characterized by many aquaculture fish farms. The main growing water supply is an extension of the water of Lake Manzala, which lies between some Governorates in Eastern Delta, Egypt. It is exposed to constant pollution from different sources, notably the most dangerous one is the untreated domestic human sewage waste, in addition to agricultural and industrial pollution including the presence of garbage recycling factory neighboring this area leading to smoke spreads as a result of garbage burning.

The second area is Lake El-Burullus. It is an important lake, large, shallow, and fresh to brackish salt-water lagoon, located on the coastal bulge of the North Central Delta region between the

Rosetta and Damietta branches of the Nile. The Lake is located within five districts of Kafr El Sheikh Governorate. The main activities of the population in and around the lake are fishing, reed cutting, grazing, and agriculture.

2.2 Sampling

Clarias gariepinus samples with approximately the same weight were collected separately from different aquaculture farms in two locations (Damietta and Lake El-Burullus) during summer to avoid seasonal variation. Apparently-health fish samples obtained from clean aquaculture farms in the northern of the first location were distributed into rearing aquaria for 8 weeks (used as control), and fed twice daily at amounts of 2% of total biomass containing 25% protein. Air pumps were used for aeration of the aquaria. Blood samples were collected then fish samples were packed immediately in labeled and tight sterile polyethylene bags and kept in insulated icebox directly to Damietta Sea Port- food inspection laboratory, Animal Health Research Institute for analysis.

2.3 Methods

Total bacterial count was done according to [21], total coliforms count and *E. coli* were determined according to method described by [22], isolation, identification and enumeration of *Staphylococcus auerus* were determined by standard methods set by [23]. Isolation of *Listeria monocytogenes* was performed according to [24], and isolation of *Salmonella* species was performed according to [25].

Blood samples were collected according to [26]. RBCs and WBCs were counted using methods of [27]. Leukocyte were differentiated based on methods of [28]. Hematocrit was estimated using micro-hematocrit technique according to [29]. Hb-content in blood was estimated according to method of [30].

Gutathione reductase activity was determined by the method of [31]. Superoxide dismutase (SOD) activity was assayed according to [32]. Catalase activity was determined by the method of [33].The amount of malondialdehyde (MDA) was measured by the thiobarbituric acid assay according to the method of [34]. Measurement of total antioxidant (TAO) activity was done according to the method of [35].

Determination of total volatile base nitrogen was done by Conway's micro diffusion technique recommended by [36]. Moisture was determined according to method of [37]. Total lipids were determined according to [38]. Serum protein was analyzed according to [39]. Heavy metals were determined by Atomic Absorption Spectrophotometery [40].

The statistical analyses of the results were performed using MedCalc software computer program, version 11.3.3.0, Copyright ©1993-2010 (*MedCalc software, Broekstreat 52, 9030 Mariake, Belgium*). Results were expressed as mean±SD.

3 RESULTS

The results were illustrated in Tables (1 - 7). In Table 1, contamination rates with total viable aerobic count were highly significant increased in all examined samples from both sites except in catfish muscles of site 2 were not significant. Contamination rates with coliform bacteria were highly significant increased in all examined samples from both sites when compared to that of control .

In Table 2, contamination rates with *Listeria monocytogenes* bacteria were highly significant increased in all examined samples of site 1. Contamination rates with *S. auerus* bacteria were highly significant increased in muscle and liver of catfish at site 2 when compared to that of control.

Table 3 showed that heamatocrite, RBCs and WBCs of catfish in site1 were highly significant decreased. Also, Hb was highly significant decreased in the two sites when compared to that of control.

As can be seen from Table 4, lymphocyte cells levels of catfish in site 1 and site 2 were highly significant decreased. On contrary, there was significant increased count of monocyte cells and neutrophil cells in catfish of site 1and highly significant increased count of monocyte cells in site 2. Also, acidophil cells levels of catfish were highly significant increased in site 1 and site 2 when compared to that of control.

In antioxidant parameters; there was increased levels of MDA and nitric oxide, and reduced levels of SOD, catalase, glutathione reductase and TAO in fish samples of site 1. However, increased levels of SOD, catalase, glutathione reductase, MDA and TAO in fish samples of site 2 were noticed (Table 5).

In the present work, there were highly significant increase in lipid content of catfish muscles in site 1 and site 2 and in the levels of total volatile nitrogen in site 1. On the other hand, there were highly significant decrease in serum protein and moisture of catfish of site 1 (Table 6).

There were high significant increase in Pb, Cd, and Hg levels in catfish muscles, liver and gills of site 1. Also, high significant increase in Pb and Cd in catfish muscles and liver of site 2 were recorded, when compared to that of control (Table 7).

TABLE 1
TOTAL VIABLE AEROBIC COUNT (CFU/G) AND TOTAL COLIFORM COUNT (LOG_{10}CFU/G) FROM SITE 1 AND SITE 2.

Sites	Total viable aerobic count CFU/g			Total coliform count (log_{10}CFU/g)		
	Muscle	Liver	Gills	Muscle	Liver	Gills
	x10^4	x10^5	x10^5	x10^2	x10^2	x10^2
Control	0.12±	0.16±	0.19±	0	5.95±	13.32±
	0.041	0.022	0.057		1.90	3.574
Site 1	24.25±	29.87±	31.62±	0	212.30±	962.85±
	7.64**	5.98**	5.423**		93.61**	272.52**
Site 2	0.136±	7.875±	8.62±	0	49.27±	94.90±
	0.03	.23**	2.06**		14.42**	32.48**

(P≤0.05)* = significant, (P≤0.01)** = highly significant, Site 1 = Damietta Governorate, Site 2 = Lake El Burullus.

TABLE 2
L. MONOCYTOGENES (CFU/G) AND *S. AUERUS* (CFU/G) FROM SITE 1 AND SITE 2.

Sites	*L. monocytogenes* (CFU/g)			*S. auerus* (CFU/g)		
	Muscle	Liver	Gills	Muscle	Liver	Gills
	x10^3	x10^4	x10^4	x10^2	x10^2	x10^2
Control	0	0	0	0.220±	0.460±	0.425±
				0.03	0.045	0.068
Site 1	0.107±	0.174±	0.28±	2.25±	0.375±	0.044±
	0.05**	0.09**	0.10**	1.5	0.206	0.005**
Site 2	0	0	0	4.366±	4.80±	5.733±
				0.58**	0.49**	0.49**

(P≤0.01)** = highly significant, Site 1 = Damietta Governorate, Site 2 = Lake El Burullus.

TABLE 3
HB (G/DL), HEAMATOCRITE (%), WBCS×10^3 (CELL/ML) AND RBCS×10^6 (CELL/ML) OF *CLARIAS GARIEPINUS* FROM SITE 1 AND SITE 2.

Sites	Hb (g/dl)	Heamatocrite (%)	WBCs×10^3 (cell/ml)	RBCs×10^6 (cell/ml)
Control	8.13± 0.62	15.86±0.87	23.40±4.50	2.08±0.41
Site 1	5.06±0.88**	14.29±0.36**	19.84±2.89**	1.43±0.28**
Site 2	5.90±0.8**	15.60±0.376	23.08±3.27	1.86±0.57

(P≤0.01)** = highly significant, Site 1 = Damietta Governorate, Site 2 = Lake El Burullus.

TABLE 4
DIFFERENTIAL LEUCOCYTE COUNT (%) OF *CLARIAS GARIEPINUS* FROM SITE 1 AND SITE 2.

Sites	Lymphocyte cells (%)	Neutrophil cells (%)	Monocyte cells (%)	Acidophil cells (%)	Basophil cells (%)
Control	73.14±	19.785±	3.21±	1.428	2.42±
	3.13	3.51	0.69	016	1.08
Site 1	48.21±	24.78±	21.14±	2.57±	3.285±
	4.54**	4.08*	27.48*	1.22**	1.815
Site 2	62.57±	21.28±	10.64±	1.94±	2.96±
	5.35**	3.85	2.53**	0.571*	0.90

(P≤0.05)* = significant, (P≤0.01)** = highly significant, Site 1 = Damietta Governorate, Site 2 = Lake El Burullus.

TABLE 5
MEAN CONCENTRATIONS OF ACTIVITIES OF SUPEROXIDE DISMUTASE (SOD) AND THE MEAN RED BLOOD CELLS LEVELS OF MALONDIALDEHYDE (MDA), CATALASE, GLUTATHIONE REDUCTASE (GR) AND TOTAL ANTIOXIDANT (TAO) OF *CLARIAS GARIEPINUS* FROM SITE 1 AND SITE 2.

Sites	SOD % inhibition	MDA Moles/ml Packed cells	Catalase Unit/mg Protein	GR U/l	TAO mmole/l
Control	0.70±	0.0004±	12.26±	19.75±	1.95±
	0.1430	0.00008	0.87	1.06	0.67
Site 1	0.61±	0.007±	9.40±	17.70±	0.072±
	0.216	0.001**	1.00**	0.42**	0.024**
Site 2	0.82±	0.004±	12.72±	22.43±	1.97±
	0.33	0.001**	0.58**	0.26**	0.43

(P≤0.01)** = highly significant, Site 1 = Damietta Governorate, Site 2 = Lake El Burullus.

TABLE 6
CONCENTRATIONS OF SERUM PROTEIN (G%), LIPID CONTENT (G%), TOTAL VOLATILE NITROGEN (MG%) AND MOISTURE (%) IN MUSCLES OF *CLARIAS GARIEPINUS* FROM SITE 1 AND SITE 2.

Sites	Protein (g %)	Lipid (g %)	Total volatile nitrogen (mg%)	Moisture (%)
Control	6.20±	2.20 ±	18.05±	77.14±
	0.70	0.67	0.78	6.14
Site 1	3.97±	5.06±	21.26±	65.00±
	0.68**	0.54**	1.42**	6.70**
Site 2	6.12±	4.50±	18.13±	73.58±
	0.53	0.81**	0.92	5.30

(P≤0.01)** = highly significant, Site 1 = Damietta Governorate, Site 2 = Lake El Burullus.

TABLE 7
CONCENTRATIONS OF HEAVY METALS (MERCURY, CADMIUM AND LEAD) IN WET WEIGHT MUSCLES, LIVER AND GILLS OF *CLARIAS GARIEPINUS* FROM SITE 1 AND SITE 2.

	Sites	Hg (ppm)	Cd (ppm)	Pb (ppm)
In muscles	Control	0	0.014±0.003	0.049±0.073
	Site 1	0.029±0.01762**	0.40±0.057**	6.56±0.37**
	Site 2	0	0.034±0.006**	0.19±0.041**
In liver	Control	0	0.43±0.05	0.276±0.036
	Site 1	0.0345±0.017**	11.75±0.86**	55.23±5.13**
	Site 2	0	0.557±0.087**	0.31±0.085**
In gills	Control	0	0.13±0.025	3.197±0.633
	Site 1	0.03±0.02**	10.76±0.51**	42.310±3.587**
	Site 2	0	0.048±0.13	2.919±0.44

(P≤0.01)** = highly significant, Site 1 = Damietta Governorate, Site 2 = Lake El Burullus.

4 DISCUSSION

E. coli and *Salmonella* pathogens were absent in all the analyzed samples. These results are agreeable with those obtained by [41]. Fish must be free from *E. coli* and *Salmonella* according to the [42]. Aquaculture can become contaminated with *Salmonella* through the use of contaminated water [43]

Our results showed that contamination rates with total viable aerobic count were within the permissible limits according to the recommended in [42] for fish. The difference in contamination rates of total viable aerobic count could be due to the bacteriological quality of the water [44]. Nearly similar finding was declared by [45], who mentioned that total bacterial count were 1.7×10^5 CFU/g for fresh catfish.

In the present work, the coliforms bacteria was highly significant increased ($p<0.0001$) in all examined samples from both sites. Similar to that of [44], who mentioned that bacteriological results of the fish harvested from ponds showed significant differences ($p<0.0001$) in coliform counts.

The present study showed that, the highest count of *Listeria monocytogenes* was found in examined gills of catfish samples collected from site 1 (0.28±0.1 x 10^4 CFU/g). Therefore, the presence of *L. monocytogenes* pathogenic bacteria in catfish gills was due to direct contact with contaminated sediments in which catfish live in muddy habitat. Lower prevalence of *L. monocytogenes* (3.3%) was obtained from fish farm in Beheira Governorate by [46]. But, higher prevalence of *L. monocytogenes* (40%) was obtained in tilapia muscles from fish farm in Damiatta Governorate by [41]. The microbiological limit of *L. monocytogenes* at <100 CFU/g [47].

The highest count of *S. auerus* was found in examined muscle, liver and gills samples collected from site 2. *S. aureus* is uniquely resistant to adverse conditions such as low and high salt content and osmotic stress [48]. The obtained results were higher than those reported by [49] who mentioned that *S. auerus* results of catfish in flesh and gills were 0.02±0.0 and 0.02±0.0 x10^3 CFU/g.

In the present work the mean levels of Hb at site 1 and site 2 in blood of catfish were highly significant decreased when compared to that of control. These results are nearly in agreement with those reported by [50] who found that the average values for Hb concentrations ranged from 5.27 to 9.7 g%.

RBCs levels in blood of catfish of site 1 were highly significant decreased when compared to that of control. These results were similar to those reported by [51]; they found that the total RBCs count and Hb concentration decreased. The reduction of Hb and erythrocytes may be due to suppressive effect of heavy metals on Hb formation and hemolysing action through destruction of protein and mucopolysaccharide structure of walls of blood vessels.

Moreover, the mean WBCs levels in blood of catfish of site 1 were highly significant decreased when compared to that of control. These results were in agreement with those obtained by [52] where the WBCs count was $22.8\pm0.9\times10^3$ cell/ml in blood of catfish samples. Reduction in lymphocytes indicate a stress condition of the environment as shown by [53] who found that lymphocytes of *S. melanotheron* showed mean values of 70.78±6.53%. This depression in immune system in site 1 may be due to the high levels of Pb and Hg which have inhibitory effect on phagocyte activity of fish macrophages and so having an inhibitory effect on cell mediated immune response [54].

Ayoola et al. [53] showed that, neutrophils mean values (28.83±6.46%), monocyte (0.39±0.69%) and eosinophil and basophil (0%) were recorded in tilapia (*S. melanotheron*). There was an alteration in hematological profile. Hematological parameters are closely related to the response of the animal and to the environment. Therefore, there is an indication that environment where the fish lives exert some influence on the hematological characteristics.

Regarding the antioxidant parameters, there was no significant difference in serum activities of SOD in catfish of site 1 and site 2. Moreover, catalase activity showed highly significant decrease in catfish of site 1 but showed highly significant increase in catfish of site 2. The decrease in catalase activity in site 1 may be due to the flux of superoxide radicals which have been shown to inhibit catalase activity.

The effects of heavy metal contamination on the common carp (*Cyprinus carpio*) was investigated by [55], fish was systematically exposed to heavy metal such as cadmium and lead at a sub-lethal level for a period of 32 days and the analytical results indicated that heavy metal toxicity in fish organs gradually increased during the exposure period and slightly decreased at the 32nd day. The activity of the antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT), in the fish were significant increased at $P < 0.001$. This observation clearly indicated the defensive nature and the adaptive mechanism of cells against free radical induced toxicity. We obtained in the current study nearly similar results to these. Also, results obtained by [56] showed decreases in catalase and glutathione reduced form in gills and muscles due to the efficiency of these antioxidants in scavenging heavy metals which lead to decreasing of free catalase and glutathione reduced form.

The mean levels of MDA of catfish of site 1 and site 2, were highly significantly increased. Site 1 was higher in MDA than site 2, our results were in agreement with [57] who investigated the effects of chronic exposures to binary mixtures of industrial effluents, they found that MDA concentration were significantly elevated $p<0.05$ in the kidney of exposed *Clarias gariepinus* fish.

In this study, glutathione reductase and total anti-oxidant levels of catfish of site 1 were highly significantly decreased when compared to control; this may be due to the presence of Hg and *lesteria* in this site. There were highly significant increase in lipid content and decrease in serum protein. Similar results were obtained by [58]. They found that the reduction of serum protein concentration ranged from 2.05 to 3.05 g/dl. They suggested that it could be due to protein catabolism. Catfish have 81.3, 86 and 4.9% (dry weight) of moisture, protein (dry weight) and fat, respectively [59]. The high-accumulated heavy metals in fish collected from site 1 due to that the main source of water in this site is Manzalah lake which receive heavy load of inorganic and organic pollutants via several agricultural drains, waste municipal and domestic water, in addition to the industrial effluents [60].

The mean metals concentration in fish muscles decreased in the order; Pb > Cd > Hg. These results agreed with those found by [61]. It is clear that, mercury concentrations in catfish collected from site 1 were in the following manner: gills > liver > muscles. The high heavy metal content in gills can be related to accumulation of such heavy metals from the water primarily through fish gills where metallothionine enhances that bioaccumulation in gills and its uptake could be controlled by the amount of water passing through the gills [62]. Concerning mercury residues in muscles, it could be observed that, mercury levels ranged from 0.0 to 0.029 ppm (wet weight). These results were lower than the permissible limits recommended by [63], mercury as methyl was 0.3 ppm.

Concerning lead residues in muscles, it could be observed that, lead levels ranged from 0.049 to 6.56 ppm (wet weight). The results of lead in site 1 were higher than the permissible limit recommended by [63], 0.3 ppm. This could be attributed to the boats near this site with gasoline motors which lead to more pollution of water, plankton, algae and the increased nutrition rate of fish on this contaminated food [60].

Concerning cadmium residues in muscles, it could be observed that, cadmium levels ranged from 0.014 to 0.4 ppm (wet weight). Cadmium level was lower than the permissible limit 0.5 ppm according to [64]. These results were nearly similar to those reported by [41], who said that cadmium was ranged from 0.02 to 0.05 mg/kg. Current results of Cd collected from site 1 and site 2 were in the following manner: liver > gills > muscles. These obtained results agreed with those reported by [65]. In Egypt, most of fish farms are depending on agriculture drainage water mixed with industrial, herbicides and the phosphate fertilizers which are considered the main source of Cd in the environment [66].

5 Conclusion

Our results showed that increases in heavy metals and pathogenic bacteria affect the biological activity of catfish, as well as antioxidant enzymes. Therefore, fish in site 1 are not safe for human consumption since the heavy metals and bacteriological analysis revealed a public health hazard.

RECOMMENDATION

Further studies are needed to determine the environmental consequences and human health impacts associated with mercury contamination. High concentrations of heavy metals implicate fish

tissues affecting its quality and become a threat to human. So, treatment of these effluents should be carried out before their discharge to the natural water resources.

ACKNOWLEDGMENT

The authors would like to express their sincere thanks to Prof. Salem A. Habib, professor of Biochemistry, Faculty of Science, Damietta University, Dr. Randa G. Abo-samra, Dr. Medhat I. Abdallah, Dr. Mohamed S. Bazalo, Dr. Rania L. Salem, Research members of Food Inspection Laboratory, Damietta Port, AHRI, ARC, Egypt, for supports and great help.

REFERENCES

[1] R.J. Medeiros, L.M. Santos, A.S Freire, R.E. Santali and T.M. Krauss, "determination of inorganic trace elements in edible marine fish from rio de janeiro state," Brazil, 2012.

[2] C. Ogueri, E.T. Adebayo and V.C.C.A. Nwaiwu, "ssessment of heavy metals in fish species and some physico-chemical parameters of Oguta lake, Oguta, Imo state, Nigeria," International Journal of Hydrology, 2, 2018.

[3] I.A. Adebayo, "Determination of Heavy Metals in Water, Fish and Sediment from Ureje Water Reservoir Fish & Ocean," Opj 4(1): OFOAJ.MS.ID.555628, 2017.

[4] L.R. Souza-Bastos, L.P. Bastos, P.C.F. Carneiro, I.C. Guiloski, H.C. Silva de Assis, A.A. Padial and C.A. Freire, "Evaluation of the water quality of the upper reaches of the main Southern Brazil river (Iguaçu river) through in situ exposure of the native siluriform Rhamdia quelen in cages," Environ Pollut. 22. pii: S0269-7491(17)31822-5,2017.

[5] E.A. Saad, S.A. Habib, M. Eltabeey, ''Diagnostic performance of AFP, autotoxin and collagen IV and their combinations for non-invasive assessment of hepatic fibrosis staging in liver fibrosis patients associated with chronic HCV,'' *IJPQA*, 8, 165-173, 2017.

[6] V.F. Doherty, O.O. Ogunkuade and U.C. Kanife, "Biomarkers of oxidative stress as indicators of Environmental pollution in some selected fishes in Lagos, Nigeria," American-Eurasian J. Agric. Environ. Sci., 7(3): 359-365,2010.

[7] E.A. Saad, E.A. Toson, G.M. Ahmed, ''Clove or green tea administration antagonizes khat hepatotoxicity in rats,'' *Int J Pharm Pharm Sci*, 7, 72-76, 2015.

[8] M. Jordanova, K. Rebok, Z. KDragun, S. Ramani, L. Ivanova, V. Kostov,D. Valić, N. Krasnići, V.F. Marijić, D. Kapetanović, "Effects of heavy metal pollution on pigmented macrophages in kidney of Vardar chub (Squalius vardarensis Karaman)," Microsc Res Tech. 2017 Aug;80(8):930-935.

[9] B.K.K.K Jinadasa, "Determination of Quality of Marine Fishes Based on Total Volatile Base Nitrogen test (TVB-N)," Nature and Science 12(5),2014.

[10] M.A. El-Tayeb, A.S.S. Ibrahim, A.A. Al-Salamah, K.S. Almaary, Y.B. Elbadawi, " Prevalence, serotyping and antimicrobials resistance mechanism of *Salmonella enterica* isolated from clinical and environmental samples in Saudi Arabia," Braz J Microbiol. 2017 Jul - Sep;48(3):499-508.

[11] P.S.C. João, "Water Microbiology. Bacterial Pathogens and Water," Int J Environ Res Public Health. 7(10): 3657–3703,2010.

[12] S.S.T. Ahmed, A.T. Bizhar, M.A. Alind, M.M. Sowaila and H.M.S. Yousif, "Isolation and Molecular Detection of Listeria monocytogenes in Minced Meat, Frozen Chicken and Cheese in Duhok Province," Kurdistan Region of Iraq. Food Microbiol Saf Hyg 2017, 2:1.

[13] D.J. Thomas, N. Strachan, K. Goodburn, O. Rotariu, M.L. Hutchison, "A review of the published literature and current production and processing practices in smoked fish processing plants with emphasis on contamination by Listeria monocytogenes," Final FSA report, 2012.

[14] A.L. Paschoalini, L.A. Savassi , F.P. Arantes, E. Rizzo and N. Bazzoli, "Heavy metals accumulation and endocrine disruption in Prochilodus argenteus from a polluted neotropical river," Ecotoxicol Environ Saf. 169:539-550,2019..

[15] J. Bernier, P. Brousseau, K. Krzystyniak, H. Tryphonas and M. Fournier, "Immunotoxicity of Heavy Metals in Relation to Great Lakes," Environmental Health Perspectives - Vol 103(Suppl 9):23-34,1995.

[16] Q. Gao, L.Y.Q. Cheng, M. Yu, B. Hu, Z. Wang, Z. Yu, "Analysis and assessment of the nutrients, biochemical indexes and heavy metals in the Three Gorges Reservoir, China, from 2008 to 2013," Water Res. 2016 Apr 1;92:262-74.

[17] S.Z. Mona, Z. Alaa, A.E. Ismael, Abd El-Moneen Eiss and I.N. Ahmed Isamel, "Effect of cadmium toxicity on Vertebrates," Electronic Physician Volume: 8, Issue: 2, Pages: 1964-1965,2016.

[18] W. Shanqian, Z. Taiyang, C. Dongmei and Z. Xiuying, "Spatial Distribution of Mercury (Hg) Concentration in Agricultural Soil and Its Risk Assessment on Food Safety in China," Sustainability 8, 795,2016.

[19] M.S. García, M.M. Galar, O. L.M. Gómez, L.K. Ruiz, F.H. Islas ,P.E. Gasca, "Relationship between genotoxicity and oxidative stress induced by mercury on common carp (Cyprinus carpio) tissues," Aquat Toxicol. 2017 Sep 21;192:207-215.

[20] A.C. Bittarello, J.C.S. Vieira, C.P. Braga, A.W.L. de Paula, B.I. da Cunha, S.J.M. da Silva,M.A.R. Buzalaf, L.F. Fleuri, P.P. de Magalhães,"Characterization of molecular biomarkers of mercury exposure to muscle tissue of Plagioscion squamosissimus and Colossoma macropomum from the Amazon region," Food Chem. 15;276:247-254,2019.

[21] APHA, "American Public Health Association," Compendium of Methods for the Microbiological Examination of Foods. 2nd ed. APHA, Washington, DC, 1984.

[22] FDA, "Food and Drug Administration," U.S. Department of health and human services, Public Health Service, Food and Drug Administration.2002, College Pardk, MD 20740.

[23] FDA, "Food and Drug Administration," Bacteriological Analytical Manual, Staphylococcus aureus, Bennett, R.W. and G.A. Lancette, Ch 12, Rev. Jan. 2001, 8th Ed., Rev. A. AOAC, International, Gaithersburg, MD.

[24] FDA, "Food and Drug Aministration," Bacteriological Analytical Manual. Chapter 10: Detection and enumeration of Listeria monocytogenes in food, 2011.

[25] ISO, "International Organization for Standardization," Microbiology of food and animal feeding stuffs, Horizontal method for the detection of *Salmonella* (EN ISO 6579: 2002), International Organization for Standardization, Geneva, Switzerland.

[26] Z. Svobodová and B. Vykusová, "Diagnostics, prevention and therapy of fish diseases and intoxications," Research Institute of Fish Culture and Hydrobiology Vodňany, Czechoslovakia. 13(4):120-144, 1991.

[27] C. A. Natt and Herrick, "A new blood diluents for blood cells of counting the red and white chickens," Poult. Sci M. P. and. 1952,13: 335.

[28] T.W. Campbell and C.K. Ellis, "Hematology of fish,"Avian and Exotic Animal Hematology and Cytology. Carton: Blackwell publishing Asia. P 93-111,2007.

[29] International Committee for Standardization in Hematology, "Recommendation for reference method for haemoglobinometry in human blood and specifications for international hemoglobin cyanide reference preparation," J.Clinical pathology, 31:139, 1978.

[30] ICSH, "International Committe for Standardization in Hematology," Brit. J. of Hematology 13 Suppl., 71. 1967.

[31] D.M. Goldberg and R.J. Spooner, "Glutathione reductase, In: methods in enzymatic analysis," H.U. Bergmeyer (Ed.)(3rd ed.), Vol. 3, pp258-265, 1985.

[32] L.D. Dechatelet, C.E. Mc. Call, L.C. Mc phial and R.B. Johnston, "Superoxide dismutase activity in leukocytes," J. Clin. Invest., 53: 1197-1201, 1974.

[33] H. Aebi, "Catalase. In: methods in enzemology," L. Packer (Ed). Academic Pres, Orlando, 105: 121-126, 1984.

[34] H. Ohkawa, N. Ohishi and K. Yagi, "Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction," Annals of Biochemistry. 1979;95:351–358.

[35] D. Koracevic, G. Koracevic, V. Djordjevic, S. Anderjevic and V, Cosic "Methods for megerment of antioxidant activity in human fluids," J, Clin. Pathol.,54:336-361, 2001.

IJSER

[36] FAO, "Food and Agriculture Organization," Manuals control. Quality, adulteration and tests of identity. Food and agriculture Organization of the united nation, 1980.

[37] V.D. Sidwell, B.R. Stillings and G.M. Knobel, "The fish protein concentration," 10-Nutritional quality and use in foods. J. Food Techol. 1970, 14 (8): 40- 46.

[38] AOAC "Association of Official Analytical Chemists," (1984): Official Methods of Analysis of the Association of Official Analytical, 13 th edditon,1984. Whelms S. (Editor). Association of Official Analytical Chemists. Inc.

[39] R. Henery, "Clincal and chemical principles and techniques," Harper Row, New York, 1964, p.182

[40] F. Rosner and P.C. Gorfien "Erythrocytes and plasma Zinc and magnesium levels in health and disease," J. Lab. And Clin. Med., 1968, 72:213-219.

[41] T.R. MONA, G.A. NASER, L.F. AMANY and M.I.M. ABDALLAH, "Risk evaluation of some fish aquaculture". Journal of Egyptian veterinary medical association. Vol.77,No. 2. 313-336,2017.

[42] EGASQC, "Egyptian General Authority of Standardization and Quality control," 2000, Cario, Egypt. (344- 2000).

[43] FAO "Food and Agriculture Organization" Expert workshop on the application of biosecurity measures to control *Salmonella* contamination in sustainable aquaculture FAO Fisheries and Aquaculture Report No. 937 ISSN 2070-6987, Mangalore, India, 19-21 January 2010.

[44] V. Razavilar, M.R. Khani and A.A. Motallebi, "Bacteriological study of cultured silver car(Hypophthalmichthys molitrix) in Gilan province," Iran Iranian Journal of Fisheries Sciences 12(3) 689- 701, 2013.

[45] M.A.M. Ibrahim and H.A.H. Samia, "Effect of chilling on microbial load of two fish species (Oreochromis niloticus and Clarias lazera)," Am. J. Food. Nutr, 2011, 1(3): 109-113.

[46] A.M. Edris, M.S. Amany and A.F. Michael, "Incidence of listeria monocytogenes in fresh tilapia nilotica fish," Benha Veterinary Medical Journal, 26 (1): 120-126,2014.

[47] L. Mitt, "How to meet an FSO – Control of Listeria monocytogenes in the smoked fish industry," Hyg. 95, 59–67,2004.

[48] T.J. Montville and K.R. Matthews, "Food microbiology: An introduction," 2nd ed,2008, ASM Press,Washington D.C.

[49] J.A. Olugbojo and S.O. Ayoola, "Comparative studies of bacteria load in fish species of commercial importance at the Aquaculture Unit and Lagoon Front of the University of Lagos," Int. J. Fish. Aquac., ISSN: 2006-9839, 7 (4): 37-46,2015.

[50] A.H.I. Mohammed, "Biochemical studies on the Effect of Pollution on the Fish Production in Dakhlia and Damietta. Ma., Thesis, Fac., Agric. Man., University, Egypt,1999.

[51] W. EL- Eraky, G. Saleh and N. Habashy,"Effect of acute and sub acute toxicity of nitrite on health condition and growth performance of common carp fry," J. Egypt vet. Med. Ass. 62, 4: 157-174,2002..

[52] S.A. Essam, A.Y. Abbas and N.M. Mohammed, "Effect of water temperature upon the response of cultured Clarias Lazera to saprolegnia infection and the consequent haematological changes," Egypt. J. Comp. and Clinic. Path. October; 15(2): 108 – 125,2002.

[53] S. O. Ayoola, K.O. Adejumobi and O.H. Adamson, "HAEMATOLOGICAL INDICES AND ENZYMATIC BIOMAKER OF BLACK JAW TILAPIA (SAROTHERODON MELANOTHERON) FROM LAGOS LAGOON," Agrosearch (2014) Volume 14(1):62-75 http://dx.doi.org/10.4314/agrosh.v14i1.7.

[54] A.S.A.A. Omima, N.P.A. SARC and O. Obire, "Impact of pollution with lead, mercury and cadmium on the immune response of Oreochromis niloticus," New York Science Journal. 2010;3(9).

[55] V. Rajamanickam and N. Muthuswamy, "Biochemical changes of antioxidant enzymes in common carp (Cyprinus carpio L.) ater heavy metal exposure. Turk. J. Vet. Anim. Sci.; 33(4): 273-278, 2009.

[56] F.I.A. Diaa, "Effect of industrial waste discharges including heavy metals in Burullus lake on some physiological parameters and antioxidants in Tillapia niloticus and Siluriformes fish," New York Science Journal 2013;6(4).

[57] O.A. Aina, M.O. Ilelabayo, R.I. Oju, V.C. Azubuike,"Long-Term Exposure to Industrial Effluent Induces Oxidative Stress and Affects Growth in Clarias gariepinus," Research Journal of Environmental and Earth Sciences 4(7): 738-746, 2012.

[58] p.m. Marilena, c. Faggio, Caterina."Haematological and serum protein profiles of Mugil cephalus: effect of two different habitats," Ichthyological Research. Jan2013, Vol. 60 Issue 1, p36-42, 2013.

[59] A.M. Abdellhamid and A.A.M. EL-Zareef,"Further studies of the pollution status on the southern region of EL- Manzalah Lake," Proc. Conf. Food Borne Contamination and Egyptian's Health Conference. Mansoura, Nov. 26-27. pp: 141-150, 1996.

[60] A.B. Hussein, H.Z. Khalid and A.A.M. Mahmoud, "EFFECT OF SOME WATER POLLUTANTS ON THE NILE TILAPIA, OREOCHROMISNILOTICUS COLLECTED FROM THE RIVER NILE AND SOME EGYPTIAN LAKES Egypt," J. AquaL BioL A Fish., Vol 5, Aft 4: 251 - 219 (2001) 1SSS I UP- 613!

[61] J. Andreji, I. Stranai, P. Massanyi and M. Valent, "Concentration of selected metals in muscle of various fish species," J Environ Sci Health. 40A:899–912,2005.

[62] S.M. Saeed, " study on factors affecting fish production from certain fish farms in the Delta," M. Sc. Thesis. Faculty of Science, Ain Shams University, Egypt,2000.

[63] CAC "Codex Alimentarius Commission," General standard for contaminants and toxins in food and feeds, 2012.

[64] FAO (Food and Agriculture Organization), "Compilation of legal limits for hazardous substances in fish and fishery products," FAO,1983, Fishery Circular No. 464, 5 - 100.

[65] K.H. Zaghloul, "Studies on the effect of water pollution along different sites of the River Nile on the survival and production of some freshwater fishes". M.Sc. Thesis, Fac. Of Science, Cairo Univ., Egypt,1997.

[66] M.K.A. Iman, M.N.A. Mohammad and T.A. Wafaa T. Abbas, "Heavy Metal Pollution and Metallothionein Expression: A Survey on Egyptian Tilapia Farms," Journal of Applied Sciences Research, 9(1): 612-619, 2013.

IJSER