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GLUTATHIONE S-TRANSFERASE AS INDICATOR OF POLLUTION OF *MUGIL CEPHALUS* IN THE MEDITERANEAN

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ABSTRACT

A total approximate number of 50 individuals of adult *Mugil cephalus* (L.) were caught at random from the selected locations. All fish used were of uniform size ((14-15.50cm) and weight 85-100gm. levels of cadmium (Cd), Cromium (Cr), manganese (Mn), nickel (Ni) and lead (Pb) increased significantly in loc#1 (polluted location) compared toloc#2 (less polluted). Also concentrations of these metals in location #1 exceeded the USEPA permissible levels. Levels of total protein and albumin were decreased significantly. Levels of AST and ALT were increased significantly compared to loc#2. GSH and GST were depleted in blood of fish of loc #1 indicating the role of GSH as scavenger of reactive oxygen species.

KEYWORDS

Mediteranean, *Mugil cephalus* and S-Transferase.

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INTRODUCTON

Fishes not only meet the food requirements of man within a country but also earn valuable foreign exchange and so fishing industries do help to provide employment to millions of people all over the world. Besides anthropogenic ally induced changes, natural changes in the aquatic environments also have an appreciable impact on the fish populations and ultimately on fisheries. Usually these effects are not possible to quantify because natural changes or fluctuations in the environment may occur simultaneously with the anthropogenically induced changes (Richardson, 2001)[1].

The pollution of aquatic environment with wide array of xenobiotics has become a menace to the aquatic flora and fauna and is a problem of immediate concern. Aquatic pollutants undoubtedly have direct effect on fish health and survival. All these stresses may result in significant alteration in such important functions as feeding, reproduction, growth, haematology etc. Such studies have generally been used as an effective and sensitive index to monitor physiological and pathological changes in fishes (Kayode and Shamusideen, 2010)[2].

Area of study

This Study was taken in Mediteranean sea in the harbor of Tubruk (32°05' N 23°55' E): (location Loc #1) and fish farm (Loc #2) as control. The gray mullet, *Mugil cephalus*, belonging to Mugilidae, is an important commercial species, which has been cultured in several countries in extensive and semi-intensive ponds for many years (Akbary and Jahanbakhshi, 2016)[3]. This study here was undertaken to determine the potential effects of heavy metals pollution on aquatic animals.

Water sampling

Water was sampled for heavy metals determination. Each water sample (2-3 L) represented the mean of surface and bottom water. Surface water samples were collected about 20cm below surface to avoid floating matter. Stoppered, acid-washed, polyethylene bottles were used for sampling. Water samples were filtered in the field using a polypropylene syringe fitted with a 0.45µm Millipore cellulose acetate filter and acidified for preservation (Abdulnabi *et al*, 2013)[4].

Fish sampling

A total approximate number of 50 individuals of adult *Mugil cephalus* (L.) were caught at random from the selected locations. All fish used were of uniform size ((14-15.50cm) and weight 85-100gm. Fish were collected in closed meshed nets before being transferred into large vessels filled up with aerated lake water. In laboratory, fish were allocated into groups (7 individuals per aquarium) and placed in two thirds-filled glass aquaria (80cm × 40cm × 40cm), according to the specific sampling location. Aquaria were supplied by a freshwater system equipped with physical and biological filters and aeration was monitored continuously. To avoid handling stress reactions, fish were slightly anesthetized (Abdulnabi *et al*, 2013)[4].

Water analysis

The determination of heavy metal concentrations in filtered water samples was carried out according to Riley and Taylor (1986)[5] using Graphite Furnace Atomic Absorption spectroscopy (Perkin- Elmer model 2380) under the recommended conditions and the detection limits (DL) in the manual for each metal.

Sampling of Blood for Biochemical Analysis

In order avoid stress effects of handling, fish were slightly anaesthetized. Blood was rapidly drawn from the caudal vessel using untreated sterile plastic syringes fitted with 21-gauge needles (Hrubec *et al*, 1997)[6]. For serum preparation, blood was allowed to clot on ice for 1 h. Serum was separated from whole blood by centrifugation at 14 000g for 5 min. Blood samples from 3 fish were pooled to give one composite specimen. 5 composite specimens for each type of measurement were analyzed for statistical analysis.

Blood biochemical parameters and enzyme activities

Stored serum samples were analyzed for total protein (TP) by the Biuret method according to Armstrong and Carr (1964)[7]. Albumin (A) concentration was determined by the method of Doumas *et al*, (1977)[8]. The activities of plasma aspartate transaminase (AST; EC 2.6.1.1) and alanine transaminase (ALT; EC 2.6.1.2) were assayed by the method of Reitman and Frankel (1957)[9]. Glutathione was determined according to Beutler *et al*, (1963)[10]. Glutathione S-transferase (GST; EC 2.5.1.18) activity was determined according to Habig *et al*, (1974)[11].

Statistical analysis

Statistical analysis was carried out by Minitab software statistics. Significance was assessed using two samples T-test analysis. $P \leq 0.05$ is considered significant (Paulson, 2008)[12].

RESULTS AND DISCUSSION

Analysis of heavy metals

Table No.1 shows that levels of cadmium (Cd), Cromium (Cr), manganese (Mn), nickel (Ni) and lead (Pb) increased significantly compared to

control also heavy metal concentrations exceeded the permissible limit of USEPA.

Data presented are means ± SE (standard error). Asterisks denotes significant difference from the control value.*P≤ 0.05.

Table No.2 show levels of biochemical parameters in blood of fish of Loc #1 and Loc #2 (control). Levels of total protein and albumin decreased significantly in the blood of the fish caught in the Loc #1 (harbor) compared to control. Levels of ALT and AST increased significantly in the blood of fish caught in the Loc #1 compared to control. Levels of GSH and glutathione -s transferase increased significantly in the blood of fish caught in the Loc #1compared to control.

Discussion

Results of this study showed that serum total protein and albumin decreased significantly in fish of location #1 (Tubruk harbor) compared to control (fish farm). Also concentrations of Cd, Pb, Cu, Ni and Zn in location #1 exceeded the USEPA permissible levels. This result suggested that the elevated level of these heavy metals were inhibitory to serum total protein. These results were confirmed by earlier reports. Abdulnabi *et al*, (2002)[4] found that serum total protein was depressed in *oreochromis niloticus* after exposure to heavy metals from Lake Mayut. The hexavalent chromium induced depletion in the profiles of total protein has been reported (Saxena and Tripathi, 2007)[13].

The present data showed that there was a significant depression in albumin level in fish of location #1 compared to location #2. Several earlier reports linked fluctuations in both albumin and serum total protein with environmental stressors and pollutants. Gopal *et al*, (1997)[14] reported a decrease in blood serum total protein and albumin in *Cyprinus caprio* after exposure to two non-essential (mercury and lead) and two essential (copper and nickel) heavy metal salts at lethal and sublethal concentrations. Several serum proteins can bind normal constituents in the body as well as foreign compounds (such as heavy metals) leading to albumin and total protein reduction (Mohiseni *et al*, (2016)[15].

Plasma protein mainly contains albumin and globulin. Research on the function and uses of plasma albumin continue to be a great interest not only because of its dominant position in the plasma protein, but also its dramatic alterations in the level of this plasma protein in many diseases and other stress situations. The complex interactions of immunocompetent cells, required to produce an immunological response of fish, are susceptible to the many biochemical and physiological disturbances, which can be induced by heavy metals (Gopal *et al*, 1997)[14]. Such adverse effects may suppress the immune response of fish and make them more vulnerable to the environmental pathogens (Banaee *et al*, 2014)[16].

The present data showed that there was a significant increase in alanine aminotransferase (ALT), Aspartate transaminase (AST) in serum of fish of location #1 compared to location #2. These results were in agreement with the results obtained by Akbary *et al*, (2017)[17], who found an increase. In ALT, AST, and LDH activities of *M. cephalus* in response to copper oxide exposure when compared to the control group during 21 days. Zaki *et al*, (2011)[18], observed a significant increase in alanine transaminase(ALT), aspartate transaminase (AST) in serum and body weight of *Clarias gariepinus in* fish fed with 15mg/kg diet mercuric oxide for 4 weeks.

The results of our study showed that GSH and GST activity decreased significantly in location #1 by the effect of heavy metals. These results were in agreement with results obtained by Akbary *et al*, (2017)[17], who observed decreased level of GSH in *Mugil cephalus* in response to copper oxide exposure when compared to the control group during 21 days. Aquatic organisms have some enzymatic antioxidants such as GSH and GST to protect against ROS-induced damages (Sevcikova *et al*, 2016)[19]. GSH scavenges hydoxyl radical and singlet oxygen directly, detoxifying hydrogen peroxide and lipid peroxides by the catalytic action of glutathione peroxidase (Masella *et al*, 2005)[20]. Soluble glutathione-S-transferase (GST) enzymes belong to the group of phase II metabolizing enzymes that facilitate the biotransformation of a large range of exogenous toxic xenobiotics and endogenous compounds (Glatt, 2000)[21].

Table No.1: Concentration of heavy metals in water of the harbor and fish farm expressed as ppm

S.No	Element	Loc #1	Loc #2	USEPA permissible limit (mg/L)
1	Cd	0.30* ± 0.13	0.001	0.01
2	Cr	0.37*± 0.12	0.027	0.01
3	Mn	0.14* ± 0.03	0.03	0.05
4	Ni	0.87*± 0.14	0.08	0.05
5	Pb	3.3*± 0.17	0.001	0.05

Table No.2: Concentration of some biochemical parameters in blood of fish caught in the harbor (Loc #1) and control (Loc #2)

S.No	parameter	Loc #1	Loc #2
1	T.protein	4.5* ± 0.23	6 ± 0.29
2	albumin	0.5*± 0.012	0.7 ± .05
3	ALT (U/L)	35*± 2.3	25 ± 1.5
4	AST (U/L)	99*± 6.5	75 ± 4.6
5	GSH (U/ml)	4.5*± 1.3	2.4 ± .92
6	GST (µmol/hr/ml	2.5*± 0.19	1.1 ± .11

Data presented are means ± SE (standard error). Asterisks denotes significant difference from the control value. *P≤ 0.05.

CONCLUSION

Concentrations of Cd, Pb, Cu, Ni and Zn in location #1 (the harbor of Tubruk (32°05' N 23°55' E) in the Mediteranean sea (exceeded the USEPA permissible levels. This may pay attention to increased pollution in these areas of the Mediteranean, which reflects on health of fish and community in these areas.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Richardson K. Anthropogenically induced changes in the environment: Effect on fisheries, *Reykjavik Conference on Responsible Fisheries in the Marine Ecosystem Reykjavik, Iceland,* 2001, 1-4.
2. Kayode S J and Shamusideen S A. Haematological studies of *Oreochromis niloticus* exposed to diesel and drilling fluid in Lagos, Nigeria, *Inter Jou of Biodi and Cons,* 2(5), 2010, 130-133.
3. Akbary P, Pirbeigi A, Jahanbakhshi A. Analysis of primary and secondary stress responses in bighead carp (*Hypophthalmichthys nobilis*) by anesthetization with 2-phenoxyethanol, *Int J Environ Sci Technol,* 13(4), 2016, 1009-1016.
4. Abdulnabi B M. Xenobiotics and the susceptibility of *oreochromis niloticus* (Lineaus, 1757) from Lake Maryut to environmental interactions and hepatic biotransformations, 2002.
5. Riley J P and Taylor D. Cleating resin for concentration of the trace elements from sea water and their analytical use in conjunction with atomic absorption spectrometry, *Anal. Chim. Acta,* 40, 1968, 479-485.
6. Hrubec T C, Robertson J L and Smith S A. Effects of temperature on hematologic and serum biochemical profiles of hybrid striped bass (Moronechryscope ×Morone Saxatitlis), *J .J. Vet. Res,* 58(2), 1997, 126-130.
7. Armstrong W D and Carr C W. Physiological Chemistry Laboratory Directions, *Burges Publishing Co, Minneapolis, Minnesota,* 3rd Edition, 1964.
8. Doumas B T, Watson W A and Biggs H G. Albumin standards and the measurement of

serum albumin with bromocresol green, *Clinic. Chem. Acta,* 31(1), 1977, 87-96.
9. Reitman S and Frankel S. A colorimetric method for the determination of serum glutamic oxalocetic and glutamic pyruvic transaminases, *An. J. Clin. Path,* 28(1), 1957, 56-63.
10. Beutler E, Duron O and Kelly B M. An improved method for the detection of blood glutathione, *J. Lab. Clin. Med,* 61, 1963, 882-888.
11. Habig W H, Pabst M J and Jakoby W B. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation, *J. Biol. Chem,* 249(22), 1974, 7130-7139.
12. Paulson D S. Biostatistics and microbiology, A survival Pack, USA, Rahman, NA (1968), A course in theoretical statistics, *Springer, New York,* 1st Edition, 2008, 216.
13. Saxena D and Tripathi M. Hexavalent chromium induces biochemical alterations in air-breathing fish, *Channapunctatus*, *J. Ecophysiol. Occup. Health,* 7(3), 2007, 171-175.
14. Gopal V, Parvathy S, Balasubramanian P. Effect of heavy metals on the blood protein biochemistry of the fish *Cyprinus carpio* and its use as a bioindicator of pollution stress, *Environ Monit Assess,* 48(2), 1997, 117-124.
15. Mohiseni M, Asayesh S, Shafiee Bazarnoie S, Mohseni F, Moradi N, Matouri M, Mirzaee N. Biochemical alteration induced by cadmium and lead in common carp via an experimental food chain, *Iranian Journal of Toxicology,* 10(4), 2016, 25-32.
16. Banaee M, Haghi B N, Ibrahim A T A. Sub-lethal toxicity of chlorpyrifos on Common carp, Cyprinuscarpio (Linnaeus, 1758): Biochemical response, *Int J Aqu Biol,* 1(6), 2014, 281-288.
17. Akbary P, Yarahmadi S S, Jahanbakhshi A. Jahanbakhshi. Hematological, hepatic enzymes' activity and oxidative stress responses of gray mullet (Mugilcephalus) after sub-acute exposure to copper oxide, *Environ Sci Pollut Res,* 25(2), 2017, 1800-1808.
18. Zaki M S, Osman A H, Fawzi O M, Mostafa S O, Ata N S, Khafagy M. Clinic pathological and biochemical studies on tilipia zilli exposed to climate change and cadmium chloride (0.25p.p.m.), *Report and Opinion,* 1(2), 2009, 80-89.
19. Sevcikova M, Modra H, Blahova J, Dobsikova R, Plhalova L, Zitka O, Hynek D, Kizek R, Skoric M, Svobodova Z. Biochemical, haematological and oxidative stress responses of common carp (Cyprinuscarpio L.) after sub-chronic exposure to copper, *Vet Med,* 61(1), 2016, 35-50.
20. Masella R. Di, Benedetto R, Vari R, Felisi C and Giovannini C. Novel mechanisms of natural antioxidant compounds in biological systems: Involvement of glutathione and glutathione-related enzymes, *J. Nutr. Biochem,* 16(10), 2005, 577-586.
21. Glatt H. Sulfotransferases in the bioactivation of xenobiotics, *Chem. Biol. Interact,* 129(1-2), 2000, 141-170.

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