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Assessment of coastal pollution in Abu-Qir bay by determination of minerals and antioxidant enzymes in different tissues for *Mullus barbatus*

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Abstract
This study was aimed to determine the levels of minerals and antioxidant enzymes in the muscles, liver and gills of *Mullus barbatus* collected from Abu-Qir Bay during December (2015). These minerals were chosen because at higher concentrations there might be toxic to the fish. The mean contents of minerals were carried out using Flame Atomic Absorption Spectrophotometer (Perkin Elmer) 2280 and expressed in mg/100gdry weight. Results showed that the highest concentrations were in liver and gills while the lowest concentrations were in muscles. The mean concentrations of Zn varied from (2.15 to 3.71),Cu (2.48 to 4.76), Cd (1.34 to 2.96), Na (119.16 to 148.12), K (182.64 to 205.96), P (2.35 to 4.09), Ca (2.09 to 5.52) and Mg (2.42 to 3.43).The concentrations of minerals were identified to have the following decreasing sequence: K > Na > Ca > Cu > Zn > Mg >P>Cd. Significant differences are observed in the activity of oxidative stress at investigated organs. There is an increased in GSH content and CAT activity in liver and gills than in muscles. Mineral concentrations differences among the investigated organs were statistically significant ($p<0.05$) and various degrees of correlations coefficient. It can be concluded that it is necessary to make a regular analysis to observe and assess the high levels of minerals and the possible effects on the ecosystem in the Abu-Qir Bay, and using the antioxidant enzymes as environmental monitoring.

Keywords: Minerals, glutathione, catalase, *Mullus barbatus*

1. Introduction

Monitoring of the environmental quality is a great importance to determine if more steps are required to improve the quality of the environment. Due to their toxicity and accumulation in biota, the level determinations of heavy metals in commercial fish species have received considerable attention in different countries [1]. There has been an increasing interest in the utilization of fishes as bio-indicators of the integrity of aquatic environmental systems in recent years [2]. Marine coastal areas are extremely impacted by human activities. The industrial and agricultural activities which are performed by human beings from past to present had caused to increase the metal contamination levels in marine ecosystems. The contamination of water by metal compounds is a worldwide environmental problem. The negative effects of heavy metal accumulations on ecosystems are increasing day by day, these effects compared with other contaminants cannot be observed in a short period, but it will be observed more in a long period [3]. The metals must be taken from water, food, or sediment for the normal metabolism of fish. However, similar to the route of essential metals, nonessential ones are also taken by fish and accumulate in their tissues [4]. Knowledge of mineral levels of muscle is important for human health, so muscle tissues of marine species are consumed more by humans compared to other parts. Levels of minerals in muscle tissues of marine species have been determined by many researchers [5-9]. Minerals discharged into the aquatic environment can damage both of aquatic organisms and species diversity, due to their easy uptake into the food chain, toxicity and accumulative behavior and bio-magnification in the food chain [10-12]. Minerals have an important role in human metabolism. Although these metals are necessary in normal concentrations, they may show toxic effects in higher concentrations [13]. Lack of essential minerals (e.g., Na, Mg, Cl, P, S, K, Ca, Mn, Fe, Cu, Zn, Se) leads to improve enzyme-mediated metabolic functions and results in organ malfunctions, chronic diseases, and ultimately death.

Trace minerals are a threat to public health, even at low concentrations, since long-term chronic exposure may result in the accumulation of toxic levels [14].

The Eastern Harbor, Western Harbor, El-Mex, El-Dekhila and Abu-Qir, which are located along the coast of the Alexandria city, are vital for fisheries and marine activities. However, these coasts are under constant threats of petroleum pollution in the form of heavy tar loads. This may result from untreated domestic sewage, oil transportation, oil spillage and ballast water from tankers, as well as industrial waste waters [15, 16]. Abu-Qir Bay is a shallow semi-circular basin lying35 Km east of Alexandria City. The bay is considered as a fertile marine habitat when compared with the other Egyptian Mediterranean coastal waters. The bay is used for commercial fishing, shipping, recreational boating, swimming and as a repository for sewage effluents. It is exposed to industrial and agricultural waste, discharged through El-Tabia outfall, Maadiya outlet and the Rosetta branch of the Nile River. The importance of Abu-Qir site and its suffering from different sources of pollution has made many researchers study the area in biological, ecological [17, 18]. The estimated amount of untreated sewage and industrial wastes from 22 different factories pumped to Abu-Qir Bay through TPS is of about 2 millions m^3/day [19]. The main enzymes that reactive oxygen species (ROS) detoxify in all organisms are functionally divided into antioxidant defense enzyme activities (catalase-CAT, reduced glutathione-GSH and biotransformation phase II components for instance, glutathione-S-transferase-GST) [20]. Catalase is an enzyme belonging to the cellular antioxidant system that counteracts the toxicity of reactive oxygen species. Several classes of pollutants, including trace metals or organic compounds, are known to enhance the formation of reactive oxygen species. Variations in the activities of antioxidant enzymes have been demonstrated in several studies and proposed as biomarkers of pollutant mediated oxidative stress [21-23]. Red mullet are among the most valuable and highly priced fish species in Egypt, though widely distributed along the entire coast of Mediterranean, their major fisheries are located in the area from Alexandria to Port Said [24]. In this study, the benthic fish red mullet (*Mullus barbatus* L.) was chosen as the bioindicator species because it is a territorial fish of commercial interest that has been used in several studies of coastal pollution monitoring [25]. The red mullet can be considered a key indicator species for the Adriatic Sea [26, 27].

The aim of the present study was to determine the levels of minerals in the muscles, liver, and gills of *M.barbatus* and assess the use of antioxidant enzymes for monitoring the marine environment at Abu-Qir bay particularly exposed to different pollutants.

2. Materials and Methods

2.1 Sample collection and preparation for Mineral determination

Twenty five samples of *Mullus barbatus* were collected directly from local fishermen from Abu-Qir bay during December (2015). This region receives diverse pollutants contributing to various wastes. Fish samples were kept immediately in ice on polyethylene bags and transported to laboratory for biochemical analysis. The average length and weight were measured (17.32cm, 85.12g) respectively. Muscles, liver, and gills of the fishes were separated and kept frozen in cold storage at -20ºc until analysis. The samples were dried in an oven at 80 °C even get a constant weight. One gram of each dry investigated tissue was weighed, transferred into polyethylene bottle and 10 ml of concentrated nitric acid was added to the sample and digested with boiling until all the contents dissolved. After cooling, the solution was filtered and diluted the sample with deionized water. The concentrations of minerals (Zn, Cu, Cd, Na, K, P, Ca, and Mg) were determined by using Atomic Absorption Spectrophotometer (Perkin Elmer) 2280. Minerals concentrations were expressed in terms of mg/100g dry weight.

2.2 Biochemical Analysis

2.2.1 Protein and lipid determination

Total protein concentration in the supernatant was determined according to the method of [28] and total lipid concentration was determined according to the method of [29] and expressed in mg/g.

2.2.2 Antioxidants biomarkers

Samples from each organ were homogenized in Tris-buffer 0.1M, pH 7.5, therefore; centrifugation was carried out at 3000*xg* for 20 min. The supernatant was collected and stored at -20ºc for enzymatic determination.

2.2.3 Determination of Reduced glutathione (GSH) content

GSH content was determined by using the method described by (Beutler *et al.,* 1963) [30]. The method based on the reduction of 5, 5` dithiobis (2-nitrobenzoic acid) (DTNB) with glutathione (GSH) to produce a yellow compound. The reduced chromogen directly proportional to GSH concentration. The intensity of the color was measured at 412 nm. Glutathione content was expressed as mg/g tissue wet weight.

2.2.4 Determination of Catalase Activity (CAT)

The activity of CAT was measured according to the method described by Aebi (1984) [31]. 0.2 gm of fresh tissue was homogenized in 2 ml of extraction buffer under cold conditions. The homogenate was centrifuged at 10,000*xg* for 20 min at 4ºc. The CAT reacts with a known quantity of H_2O_2, and the reaction is stopped after 1 min with a CAT inhibitor. In the presence of peroxidase, the remaining H_2O_2 reacts with 3, 5-dichloro-2-hydroxybenzene sulfuric acid and 4-aminophenazone to form a chromophore, with a color intensity inversely proportional to the amount of CAT in the sample. The supernatant was used for quick assay was determined by observing the disappearance of H_2O_2 by measuring the spectrophotometer at 510 nm absorbance.

2.3 Statistical analyses

Statistical analysis of data was carried out using SPSS in version (16) statistical package program. One-way analysis of variance (ANOVA) was used to assess whether metal concentrations varied as significantly among tissues. The correlation between the element pairs in tissues was determined by using the Pearson's correlation coefficient matrix.

3. Results

Protein and lipid concentrations in the muscles, liver, and gills of the *M.barbatus* are shown in Table1.The obtained results demonstrate significantly higher protein and lipid concentrations in the muscles than liver and gills ($P<0.01$).

Table 1: Proximate compositions of muscles, liver and gills of *M.barbatus*

Organ / Parameters	Muscles	Liver	Gills
Protein (mg/g)	17.82±2.84	15.96±2.53	14.39±2.45
Lipid (mg/g)	1.28±0.29	1.41±0.38	2.13±0.09

*Means and standard deviation are significantly different (P < 0.01)

Minerals concentrations of the muscles, liver, and gills of *M. barbatus* with the corresponding mean standard deviations (expressed as mg/g dry weight) are summarized in table (2). The results showed that the concentrations of Zn and Cu were higher in liver and gills than muscles while Cd (2.96±0.89) mg/100 g was higher in gills than muscles and liver. The connection of Na (148.12±9.31) mg/100 g in muscles was more than in liver and gills while K, P and Ca concentrations (205.96±33.12, 4.09±0.52, 5.52±3.27)mg/100 g respectively were higher in gills than in liver and muscles. The concentration of Mg (3.43±0.67) mg/100g was more in muscles than liver and gills. Significantly difference (P<0.01) was showed between the investigated tissues and protein, lipid, Zn, Cu, Cd Na, P, Ca and Mg,while there was no significant difference with K (P > 0.01).

Table 2: Minerals compositions of *M.barbatus* (mg/100 g).

Organ / Parameters	Muscles	Liver	Gills
Zn	2.15±0.3	3.71±0.46	2.56±0.39
Cu	2.48±0.39	3.63±0.69	4.76±1.13
Cd	1.34±0.19	2.53±0.53	2.96±0.89
Na	148.12±9.31	119.16±20.29	126.52±7.12
K	182.64±22.4	199.32±52.76	205.96±33.12
P	2.35±0.44	3.02±0.69	4.09±0.52
Ca	2.55±0.96	2.09±0.52	5.52±3.27
Mg	3.43±0.67	2.45±0.04	2.42±0.52

*Means and standard deviation are significantly different (P < 0.01)

Differences in the concentration of glutathione (GSH) and catalase enzyme activity (CAT) in muscles, liver and gills showed in Tables 3.The higher concentration of GSH and CAT enzyme activity value recorded (39.8±5.05) mg/g and (37.08±8.54) U/g tissue in liver while the lower value was (29.2±11.65) mg/ g and (31.28±9.04) U/g tissue in muscles. The Significant difference (P <0.01) was observed between the investigated tissues and GSH while no Significant difference (P >0.01) with CAT.

Table 3: GSH level (mg/g wet tissue) and Catalase (CAT, U/g tissue) activity in the muscle, liver and gills of *M.barbatus*.

Organ / Enzyme	Muscles	Liver	Gills
GSH (mg/g)	29.2±11.65	39.8±5.05	32.2±5.19
CAT (U/min/mg/tissue)	31.28±9.04	37.08±8.54	34.36±9.09

All values are expressed as means ± SD (P < 0.01)

The correlation coefficients between the elements in investigated tissues of *M. barbatus* were represented in table (4). From the table, the relations are highly significant with a p<0.01 (marked with"**") while others are less significant with a p<0.05 (marked with"*"). The results indicated that there was a positive significant relation between protein (lipid - Na) (P<0.01), lipid (Na-Mg), Sodium with Mg (P<0.01), potassium with P (P<0.01) and (Zn - Cu and CAT) (P<0.05), phosphorus (Ca - Cd- Cu and CAT) (P<0.05), (P<0.01) and Calcium with Cd (P<0.01).Zn (Cd,Cu and GSH*)* (P<0.01), Cadmium had positive correlation with Cu (P<0.01). Copper had positive significant correlation with GSH and CAT (P<0.05).While a negative significant relation were detected between protein and K, P, Zn, Cd and Cu. (P<0.01). Lipid (P, Zn, Cd, Cu and CAT). Sodium (K, P, Zn, Cd) (P<0.01) and Cu (P<0.05). Phosphorous (Mg) (P<0.01), Magnesium was a negative significant relation with (Ca-Zn-Cd and CAT (P<0.01).calcium with Zn (P<0.01).Cd with CAT (P<0.05) and CAT had negative significant with GSH (P<0.01).

Table 4: Correlation matrix between protein, lipid, minerals and antioxidant enzymes in investigated tissues of *M. barbatus*

	protein	lipid	Na	K	P	Mg	Ca	Zn	Cd	Cu	CAT	GSH
protein	1	0.299**	0.517**	-0.358**	-0.319**	0.088	0.124	-0.413**	-0.621**	-0.569**	0.223	-0.214
lipid		1	0.414**	-0.085	-0.495**	0.730**	-0.083	-0.297**	-0.387**	-0.540**	-0.256*	-0.170
Na			1	-0.626**	-0.595**	0.350**	0.182	-0.749**	-0.668**	-0.626*	0.012	-0.111
K				1	0.510**	-0.013	-0.025	0.231*	0.178	0.236*	0.238*	-0.041
P					1	-0.609**	0.287*	0.139	0.540**	0.710**	0.316**	-0.187
Mg						1	-0.314**	-0.368**	-0.530**	-0.474**	-0.379**	-0.157
Ca							1	-0.305**	0.336**	0.002	0.092	-0.158
Zn								1	0.601**	0.342**	-0.051	0.457**
Cd									1	0.700**	-0.232*	0.190
Cu										1	-0.264*	0.274*
CAT											1	-0.324**
GSH												1

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

There was no significant difference in the protein values between investigated organs in (Mg-Ca-CAT-GSH) (P>0.01), in lipid (K-Ca and GSH) and between sodium (Ca-CAT and GSH).

4. Discussion

Fish lie at the top of the aquatic food chain and may concentrate large amounts of some metals from the water. Among the compounds that can lead to pollution of water ecosystems, special focus should be placed on elements, particularly minerals. They originate primarily from anthropogenic sources (the production of iron and steel, mining wastewaters, municipal and industrial sewage, the production and application of artificial fertilizers and pesticides and water and overland transportation [32]. Heavy metal deposition occurs not only in the internal organs such as liver, kidney and spleen but also in skeletal muscles. These results observed that the concentration of Zn and Cu was higher in liver and gills than muscles while the concentration of Na and Mg was higher in muscles than both liver and gills also each of K, P, Ca and Cd were higher in gills than in liver and muscles. The present results are agreement with [33, 34], they found that trace metals such as Copper (Cu), Zinc (Zn), Cadmium (Cd) and Iron (Fe) were bioaccumulated in liver followed by gills and muscles in fish. The concentrations of metals were found to be higher in the liver, kidneys and gills than in the gonad and muscle tissues in some fish species [35, 36]; their studies are coincided with the present result. The gills are considered the main site of entry for the dissolved minerals. They are sensitive to any change of water components and serve as a good indicator of water quality. Therefore, the concentration of metals in gills reflects their concentration in water where the fish live [37-39]. These results are in a similar study, Abdallah (2008) [40] who reported that Cu and Zn levels of some commercially valuable fish species were 0.01-9.69 mg/kg, and 3.90-57.20 mg/kg, respectively, in El-Mex Bay and Eastern harbour, in Egypt. Cu and Zn levels were higher because of the availability of more industrial facilitates in El Mex Bay and Eastern harbour. Also Tepe *et al*., [41] determined Zn and Cu levels in *Mullus barbatus* tissues captured in Mersin Bay and he observed that Zn, Cu levels were higher than the findings of this study. Copper mean concentration in this study was almost similar to the results of Canli and Atli [4], but higher than Sina *et al*., [42]. Higher Cu contents in muscle and skin of fish samples in compares with other HMs are related to the importance of Cu in biota metabolisms [43]. The present result is agreement with Ali *et al*., [44] who observed the levels of Cu in all fish samples were between 6.68 μg/g for common sole and 0.12 μg/g for tub gurnard. Cadmium like some other HMs such as Pb and Hg has no biological function in human system [45]. Kidney damage, testicular tissue destruction, high blood pressure and red blood cells destruction are some adverse effects of acute cadmium toxicity [46]. Minerals play a key role in the maintenance of osmotic pressure and thus regulate the exchange of water and solutes within the animal body. It serves as essential componenents of many enzymes, vitamins, hormones and respiratory pigments or as cofactors in metabolism, catalysts and enzyme activators. The lowest levels of minerals were found in the muscle compared with other tissues and organs. Similar results have been reported in other fish species [47-49]. Muscle is the major tissue of interest under routine monitoring of metal contamination because it is consumed by people. The order of metal accumulation in liver were k> Na> Zn> Cu> P> Cd> Mg> Ca. The liver and gills in the present investigation accumulated the highest metal load when compared to muscles. Similar results have been reported in other fish species. Begum *et al*., [50] investigated the elements take part in some metabolic processes and are known to be indispensable to all living things. The accumulation of K, P and Ca increased in gills and Mg increased in muscles. The most important mineral salts are that of calcium, sodium, potassium, phosphorus, iron, and chlorine while many others are also needed in trace amounts. Calcium plays a key role in the regulation of the permeability of cell membranes and consequently over the uptake of nutrients by the cell [51]. Searches in literature on the accumulation of minerals showed that the accumulation level may change according to the length, weight, sex and age [52, 49].

Oxidative stress is a general response to toxicity induced by many contaminants and is often used as a biomarker of the effects of exposure to environmental pollution in aquatic environments. Antioxidative status in species of marine fish seems to be related to tissue oxygen consumption or to organism activity level [53]. Catalase (CAT) catalyzes the dismutation of hydrogen peroxide in water and oxygen [54]. CAT activity was spectrophotometrically determined, by following the absorbance decrease correspondent to the decomposition of hydrogen peroxide to oxygen and water. The differences between CAT activity in liver and white muscle probably represent different tissue reactions to seasonal changes, rather than a reaction to pollution in the environment (Pavlović *et al*., 2008) [55].

Fish tissues, specifically the liver and kidney are endowed with antioxidant defense systems to protect the oxidative stress caused by metals [56-58]. Elevated levels of minerals can induce oxidative stress by generating highly reactive oxygen species (ROS), such as hydrogen peroxide, superoxide radical and hydroxyl radical via Haber–Weiss and Fenton reactions that can oxidize proteins, lipids and nucleic acids, often leading to damage in cell structure or even cell death[59]. Organisms have developed several protective mechanisms to remove ROS before the detrimental effects occur in cell. Catalase activity was significantly elevated in red mullets this result is agreement with [60] who observed that CAT enzyme catalyses the production of oxygen and water from H_2O_2. Thus, catalase normally plays a relatively minor role in the catabolism of H_2O_2 at low rates of H_2O_2 generation [61], but becomes indispensable when the rate of H_2O_2 production is enhanced, as during oxidative stress. This would be in agreement with our findings and those reported by other authors [62, 63]. Furthermore, factors other than pollutants such as food availability, reproductive stage, salinity, dissolved oxygen might be sources of additional stress, and could also influence biochemical markers. The set of biomarkers used indicated different levels of stress in red mullets paralleling a coastal pollution, this may be adaptation with a polluted environments.

5. Conclusion

There was a clear difference between the concentrations of minerals within liver, gills and muscle tissues of *Mullus barbatus*. The mean concentrations of minerals analyzed in the muscles were lower than the maximum permitted concentrations recommended by FAO/WHO [64]. The highest levels of all minerals were observed in liver and gills, while muscle showed the lowest value. The results are in accordance with similar monitoring studies and represent a further support in the assessing the health of coastal areas. The analysis of GSH and CAT activity in fish is important for the evaluation of fish abilities to protect against pollutants and keep their life and biodiversity environments.

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