



Impact of water pollution with Heavy Metals on sea bream fishes (*Sparus Aurata*) Health in Marriott Valley

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Abstract

This research was conducted in the outskirts of Alexandria City, in Lake Marriott between December 2021 and June 2022. Samples of water and sea bream fish were brought from a fish farm. Through analysis of water and fish samples, it became clear that the water samples were poor due to the presence of high levels of ammonia and heavy metal such as cadmium and lead. And it resulted in stress for the fish and it was evident by the analysis of antioxidants that consider biological indicators of the lack of water and soil quality in which the fish live.

Keywords: Sea bream, ammonia, heavy metals and antioxidants

Introduction

After decades of rapid urbanization, population growth and industrialization, developing countries are now home to many of the world's most critical air, water and solid waste problems. Early studies have identified the rise in the pollution of particular heavy metals in freshwater systems around the world, particularly in rivers. The pollution has mainly been caused by industrial processes and industrial waste, typically from rubber and oil palm mills (1). The heavy metal contamination of aquatic ecosystems above the natural background load has drawn the attention of many researchers. Heavy metals may accumulate in aquatic species, enter the food chain and cause

serious harm to human health when the contamination content and exposure are significant (2). In Egypt, the lakes are economically important fishing source as they represent about 20% of the total areas of fish catch in Egypt (3). Lake Marriott is one of the four shallow lakes in the northern Nile Delta of Egypt. It is the smallest and most polluted of these lakes. It is situated along the Mediterranean coast of Egypt south of Alexandria city between latitude $31^{\circ} 70'$ N and longitude $29^{\circ} 50'$ E. It has a surface area of 60 km^2 and ranges in depth from 1 to 3 m. The lake has no direct connection to the sea, and its surface is maintained at 2.8 m below mean sea level by pumping water from the lake to the sea at El-Mex Bay. The lake environment was continuously subjected to quality degradation due to human pressure as well as land reclamation reducing the area of the lake over the years (4). Examples of heavy metals include mercury (Hg), cadmium (Cd), arsenic (AS), chromium (Cr), thallium (Ti) and lead (Pb). Heavy metals are natural components of the earth's crust. They can't be degraded or destroyed. Heavy metals (HM) are natural trace components of the aquatic environment, but their levels have been increased due to industrial wastes, geochemical structure, agricultural and mining activities (5). When metals accumulate in fish, they trigger reduction-oxidation reactions capable of generating reactive oxygen species (ROS) which can cause oxidative stress, morphological and biochemical changes in their tissues, Antioxidant enzymes are a vital component of the body's natural antioxidant defense system. Susceptibility of aquatic organisms to oxidative effects from pollution or pathogens in the environment is high, most importantly when they have the ability to generate or enhance the production of reactive oxygen species (ROS). Superoxide dismutase (SOD), catalase (CAT) and glutathione S-transferase (GST) are three prominent enzymes in this antioxidant defense system (6). Heavy metal concentrations in fish were fish during the dry season were due to high temperatures, which increased the activity, ventilation, metabolic rate and feeding sessions (7). Therefore, this study was aimed to study impact of water pollution with heavy metals on sea bream fishes *Sparus Aurata* health in Marriott Valley.

Materials and Methods

The tools used for determination of Physico-chemical properties of water quality

- Dissolved Oxygen meter for measuring the level of Dissolved oxygen in the water.
- Salinometer for measuring of % of water salinity.

- PH, DO and salinity were analyzed in the field using portable meters (HANNA, HACH).
- Total ammonia nitrogen (TAN); NH_3 ; nitrite nitrogen ($\text{NO}_2\text{-N}$) were analyzed by DR 4000 Spectrophotometer using powder pillows (DR - 4000, HACH).
- Thermometer for measuring of temperature.

The tools used for Determination of heavy metals

- Sterile polyethylene bottles
- Atomic absorption spectrophotometer (Thermo, Electron Corporation: 3150)
- Deep freezer to frozen at -20°C .

Chemicals used for determination of heavy metal

- Perchloric acid
- Nitric acid $\text{HNO}_3\text{-HClO}_4$,
- Sulphuric acid
- Hydrochloric acid solution

Kits for biochemicals and antioxidant parameters

The assay kits used for biochemical measurements of catalase (CAT), glutathione reductase, Superoxide Dismutase, glutathione peroxidase (GPx), lipid peroxide (malondialdehyde, MDA) were purchased from Biodiagnostic Company, ARE.

All other chemicals were of reagent grade and were commercially available from local scientific distributors in Egypt.

Apparatus and instruments:

Spectrophotometers (AQUA MATE PLUS,Thermo) ; sterile eppendorf tubes used during serum collection; Thermometer (for determination the water temperature), and Grinding Mortem. Haemocytometer used for count of Red and white blood cells.

Solutions, buffers, reagents and stains:

- Phosphate buffer saline (PBS pH 7.4) prepared according to (8).
- Normal saline (0.85% sodium chloride).
- Formalin solution 40 W/V, (Chemajit).
- Natt-Herrick's solution May-Grunewald stain and used as RBCs and WBCs diluting fluid was prepared according to (9, 10).
- Loffler's alkaline methylene blue, Giemsa and methylene blue stains were prepared according to (8).
- N/10 hydrochloric acid (Commercial) (Chemajit).

➤ Methanol (Commercial) (Chemajit).

➤ **Methods**

Water samples and analysis:

Representative water samples were taken from different areas and depths accompanied with the sea bream fish samples, were succumbed to physico-chemical analysis.

Physico-chemical analysis of water samples

Water samples were taken parallel to fish samples and subjected to complete water analysis according to (11). The tools used for determination of Physico-chemical properties of water quality were namely; Dissolved Oxygen meter for measuring the level of Dissolved oxygen in the water, Salinometer for measuring of % of water salinity. PH, DO and salinity were analyzed in the field using portable meters (HANNA, HACH). Total ammonia nitrogen (TAN); NH_3 ; nitrite nitrogen ($\text{NO}_2\text{-N}$) were analyzed by DR 4000 Spectrophotometer using powder pillows (DR - 4000, HACH).

Determination of heavy metals in water samples

Water samples were collected in sterile polyethylene bottles from the Mariout lake. Each water sample was subjected to chemical analysis for the heavy metals; lead, cadmium, copper, zinc and iron using atomic absorption spectrophotometry after (12), phosphorus and sulphates according to (13).

Determination of heavy metals in water samples:

Surface and depth water samples at two fish farms were seasonally taken by a vertical PVC water sampler at depth of a half meter from the water surface. Samples of 1 liter were placed in polyethylene bottles and transferred to the laboratory for heavy metal analysis. All water samples were collected from each fish farm in triplicate and each sample was measured also in triplicate. Heavy metals considered here were; Fe, Cu, Zn, Pb and Cd. Concentrations of heavy metals in the water were determined after the digestion by nitric acid according to (14). Heavy metals were measured by atomic absorption spectrophotometer (model; Perkin Elmer, 3150) was used for measuring the optical density for each element at its corresponding wavelength (Fe 248.3, Cu 324.8, Zn 213.9, Pb 217 nm and Cd 225 nm). Results were expressed as $\mu\text{g/L}$.

The concentrations of heavy metals were measured according to the following equation:

$$\text{Metal concentration } (\mu\text{g/L}) = A \times B/C$$

Where:

A = Conc. of metal in digested solution $\mu\text{g/L}$.

B = Final volume of digested solution, ml.

C = Sample volume, ml.

Permissible levels reported by (15) were 1, 0.05, 5, 0.3 and 0.01 (ppm) for water Cu, Pb, Zn, Fe and Cd respectively.

Fish samples:

Sea bream (*Sparus auratus*) of nearly the same size and weight 420-550 grams body weight were collected alive from the same site of water collection and at the same time from three sites in order to know the most polluted place in Marriott Valley in December 2021. Fish samples immediately were transported in ice box to the laboratory. The fish were immediately subjected to examination. Musculature specimens were taken from the fish.

*note : on the other hand fishes are brought to the laboratory as **a control group**, this fish was caught from a fish farm in the valley and transferred to the laboratory. In the laboratory for adaptation of fish with environmental conditions, fish stocked at aquarium (110) L and aeration with air pump, the fish were allowed to acclimate to laboratory condition for two weeks before measuring the concentration of antioxidants in the ideal condition, where the water was pure and the temperature was ideal, as well as aeration.

Determination of heavy metal concentrations in musculature

The collected fish species sea bream was immediately preserved on ice in an ice chest and transferred to the laboratory where they were classified, weighed, with total length recorded, and kept frozen at -20°C until further analysis.

Determination of Metal Concentrations

The fish samples were rinsed with distilled water and scales of sea bream were removed. The fishes were dissected into separate musculature using stainless steel instruments and digested by the method described by (16, 17). The lead, cadmium, copper and zinc levels in the homogenates were measured

according to (12).

In the procedure 1 g of the samples was digested with perchloric acid and nitric acid ratio (1:1) $\text{HNO}_3\text{-HClO}_4$, followed by sulphuric acid, and the mixture was heated at 200°C for 30 mins. The complete digest was then cooled down to room temperature and made up to 50 ml scale with distilled water and analysed for Cu, Zn, Pb, and Cd using Atomic Absorption Spectrophotometer (AAS model Agilent AA55) after selecting the various wavelengths at which the heavy metals were determined. An analytical blank was prepared in a similar manner. The obtained results were expressed as mg/kg wet weight.

Methods for Determination of Antioxidants Enzyme assay in serum:

Catalase: reacts with a known quantity of H_2O_2 . The reaction is stopped after exactly one minute with catalase inhibitor

GSH: The method based on the reduction of 5, 5 dithiobis (2-nitrobenzoic acid) (DTNB) with GSH to produce a yellow compound. The reduced chromogen is directly proportional to GSH concentration and its absorbance can be measured at 405 nm.

Serum Superoxide dismutase: activity was determined by the kit of Biodiagnostic and/or Biotechnology Company.

Serum Glutathione peroxidase (GPx): activities were determined by the kit of Biodiagnostic Company.

MDA: Thiobarbituric acid (TBA) reacts with malondialdehyde (MDA) in acidic medium at temperature of 95°C for 30 minutes to form thiobarbituric acid reactive product the absorbance of the resultant pink product can be measured at read at 534 nm.

Total antioxidant capacity (TAC) assay: Serum Total antioxidant capacity (TAC) levels were determined by the kit of Biodiagnostic Company

Apparatus and instruments: Spectrophotometers (Stat Lab, Germany); sterile eppendorf tubes used during serum collection; Thermometer (for determination the water temperature) and Grinding Mortem. Haemocytometer used for count of Red and white blood cells.

Solutions, buffers, reagents and stains:

➤ Phosphate buffer saline (PBS pH 7.4) prepared according to (8).

- Normal saline (0.85% sodium chloride).
- Formalin solution 40 W/V, (Chemajit).
- N/10 hydrochloric acid (Commercial) (Chemajit).
- Methanol (Commercial) (Chemajit).

Results

The result in 6-12-2021

Physico–chemical characters in water : Table 1 show high level in NH_3 and NO_3 and NO_2 and H_2S than optimum range

Table 1: Physico–chemical characters in water of private fish farms at area of Marriott Valley during 6th December 2021

Parameters	Marriott Valley	Optimum ranges
Temperature (°C)	22	-
pH	7.6	7.5-8.5
DO (mg/l) dissolved oxygen	4.5	5.1-5.9
H_2S (mg/l) hydrogen sulfide	220	70-120
Total soluble solids (mg/L)	389	300
T. hardness (mg/l)	272	>20
NO_3 (mg/l) nitrite	0.22	0.02
NO_2 (mg/l) nitrate	0.32	0.03
NH_3 (mg/l) ammonia	0.07	0.01
Sulphate (mg/L)	50.6	100
Phosphorus (mg/L)	5.45	0.3

Heavy metals (mg/l) in water

Table 2 show high level in Cd and Pd

Table 2: Heavy metals (mg/l) in water of private fish farms at area of Marriott Valley during 6th December 2021

Parameters	Mariout Valley	Reference aquaculture
Fe Iron (mg/L)	0.07	0.05
Zn Zinc (mg/L)	1.27	0.03
Cu Copper (mg/L)	0.07	0.002
Pb Lead (mg/L)	0.05	0.5
Cd Cadmium (mg/L)	0.03	0.001

Heavy metal concentrations (ppm) in sediment**Table 3:** Heavy metal concentrations (mg/kg) recorded in sediment samples collected from different private fish farms at area of Marriott Valley during 6th December 2021

Heavy metals	Marriott Valley	Optimum range
lead	421.21	100
Cadimum	74.28	1.5

Heavy metals in flesh (mg/kg, w/w) of sea bream

Samples were taken from more than one site in the lake in order to know the most polluted place

Table 4: Heavy metals in flesh (mg/kg, w/w) of sea bream (*Sparus auratus*) collected from Mariotte Valley in 6th December 2021 and Maximum Permissible Limits (MPL) international standard.

Heavy metals	Concentrations of metals (mg/kg, w/w)				Optimum range	
	Site 1	Site 2	Site 3	Site 4	FAO/WHO (1989)	WHO (1995)
Pb	1.62	2.44	2.78	2.51	0.5	2
Cd	0.54	0.63	0.53	0.45	0.5	1

Antioxidant parameters in serum of sea bream (*Sparus auratus*) collected from Mariotte Valley in December 2021

Table 5 show the level of antioxidants from fish that taken from 3 sites in order to know the most polluted place

Table 5: Antioxidant parameters in serum of sea bream (*Sparus auratus*) collected from Mariotte Valley in 6th December 2021

Parameters	Reference range (control fish) <i>in vitro</i>	Fish from site 1	Fish from site 2	Fish from site 3
GSH (nmol /ml.)	4.52	4.83	4.85	4.88
GPx (GPx, U/ml.)	15.66	27.99	28.50	29.13
CAT (μmol O ₂ /min/mg)	2.66	4.22	4.34	4.16
SOD (U/min/mg)	0.46	0.63	0.58	0.59
MDA (nmol (μM MDA/mg)	26.77	21.79	20.07	21.36
TAC (mML-1/ml.)	0.89	1.14	1.17	1.26

B- The results in 30-6-2022

Table (6): Biochemical results in water

Parameters	Marriott Valley	Optimum ranges
Temperature (°C)	22	-
pH	8	7.5-8.5
DO (mg/l)	4	5.1-5.9
H2S(mg/l)	250	70-120
Total soluble solids (mg/L)	450	300
T. hardness (mg/l)	320	>20
NO ₃ (mg/l)	0.29	0.02
NO ₂ (mg/l)	0.40	0.03
NH ₃ (mg/l)	0.23	0.01
Sulphate (mg/L)	50.6	100
Phosphorus (mg/L)	7	0.3

Table 7: Heavy metal results in water

Heavy metal in water(mg/L)	Marriout Vally	Optimum range
Zinc	1.1	0.5
Iron	0.13	1
copper	0.27	0.3
cadmium	0.006	0.001-0.003
lead	0.879	0.5

Table 8: Heavy metal results in sediment

Heavy metal	Marriout valley	Optimum range
Copper	ND	0.3
Magnesium	0.005	0.015-0.003

Cadimum	263.2	0.003-0.001
lead	1.451	0.5

Table 9: Heavy metals in flesh (mg/kg, w/w) of sea bream (*Sparus auratus*) collected from Mariotte Valley in 6th June 2022 and Maximum Permissible Limits (MPL) international standard

Heavy metals	Concentrations of metals (mg/kg, w/w)				Optimum range	
	Site 1	Site 2	Site 3	Site 4	FAO/WHO (1989)	WHO (1995)
Pb	2.62	2.70	2.78	2.90	0.5	2
Cd	1.54	1.60	1.70	1.20	0.5	1

Results of antioxidant parameters in serum analysis: -

Table 10: show the level of antioxidants from fish that taken from 3 sites

Parameters	Reference range (control fish) <i>in vitro</i>	Fish from site 1	Fish from site 2	Fish from site 3
GSH (nmol /ml.)	4.52	5.03	4.77	5.88
GPx (GPx, U/ml.)	15.66	29.99	23.50	24.13
CAT (μmol O2/min/mg)	2.66	4.22	4.34	4.16
SOD (U/min/mg)	0.46	1.63	1.58	0.99
MDA (nmol (μM MDA/mg)	26.77	22.79	20.77	21.00
TAC (mML-1/ml.)	0.89	1.14	1.17	1.26

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Discussion:

Through this study, it was found that the levels of heavy metals in the

water of lake Mariout were higher than the natural rates, and were higher in the summer than in the winter, as well as what was mentioned by (18), also the antioxidants which are considered a vital indicator of the presence of pollution or microbial infection, their rates were higher than normal, and this is consistent with what was mentioned by (19) and through laboratory tests of the blood of the fish, it was found that there was a decrease in the immunity of the fish as result of the bad aquatic environment in which the fish live.

Conclusion

The water samples are poor and are considered a very high source of stress for the fish in the fish farming process, as the non-ionized ammonia is higher than the permissible levels, the immunity of fish is very low as a result of various stresses, whether from soil, water and external parasites.

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تأثير تلوث المياه بالعناصر الثقيلة علي صحة اسماك الدنيس في بحيرة مريوط

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المستخلص العربي

تم اجراء البحث في ضواحي مدينة الاسكندرية بالتحديد في بحيرة مريوط في الفترة ما بين ديسمبر 2021 و يونيو 2022 وتم جلب عينات من المياه و أسماك الدنيس من مزرعة لتربية الاسماك واتضح من خلال التحليل لعينات المياه والاسماك ان عينات المياه كانت رديئة نظرا لوجود نسب عالية من الامونيا والعناصر الثقيلة كالكادميوم والرصاص ونتج عنه حدوث اجهاد للسماك واتضح جليا بواسطة أجراء تحليل مضادات الاكسدة التى تعتبر مؤشر بيولوجى هام لقلّة جودة المياه والتربة التي تعيش فيها الاسماك

الكلمات المفتاحية: سمك الدنيس، العناصر الثقيلة ، مضادات الاكسدة