JOURNAL OF THE
ARABIAN AQUACULTURE SOCIETY

Vol. 8 No 1 **Arabian Aquaculture Conference** **June 2013**

Effect of Addition of Penicillum Spores and Cadmium on Heavy Metal Concentration in Cultured Marine Fish

Khalil, R. H.; Saad, T. T.; *Samia El-Hoshy and Abu Khashaba; A. F.
Poultry and fish Disease Department, Faculty of Vet. Med. Alex. University
*Animal research institute, Alexandria Branch

ABSTRACT

The present work aimed to, study identification of the effect of heavy metals on marine fish cultures, recording the clinical signs and PM lesions associated with such toxicity and finally monitoring of some heavy metal concentrations in both fish tissues and water that the fish live in it. In this study we used 600 fish (300 fish) O. niloticus and 300 seabream fish (Seabream *Sparus aurata L.*) that were collected and showing clinical signs from private fish farms in Alexandria and Behaira governorate, Egypt. The fish were subjected to full clinical, postmortem (PM) lesions, parasitic and mycological as well as bacteriological examinations. The fish in this study were allotted into 6 groups, Group I: of 100 O. niloticus fish that not take any treatment and considered as a control group, Group II: of 100 seabream fish and considred as a control group, Group IIII, of 100 O. niloticus fish and treated with 9 X 10^2 CFU penicillum, Group IV: of 100 O. niloticus fish treated with 0.7 mg/L cadmium, Group VI: of 100 Seabream fish treated with 9 X 10^2 CFU penicillum, Group IV: of 100 Seabream fish treated with 0.7 mg/L cadmium. The experimental period extended to 7^{th} weeks, this study coblcuded that, the toxicity of the fish with heavy metals differ according to the type of fish species and level of heavy metals that commonly polluted the water and presence of cadmium in the water can facilitate the toxicity of fish with other types of the heavy metals as copper, lead, and copper and the higher concentration of heavy metals observed in water, liver and flesh of fish, respectively.

INTRODUCTION

Fish considered as one of the main sources of national income that Egypt depends on, stimulating local market economies, and important source of foreign exchange. Moreover, marine waters are the immediate alternative sources for water needed for mariculture and fortunately, Egypt has numerous marine resources of the Mediterranean and Red Seas. *(Sadek,2000).*

The world aquaculture production in 2001 was approximately of 37.9 million tons, which represents around 41% of that obtained from extensive captures for human consumption. Also, Marine fish culture is dominated by Atlantic salmon (***Salmo salar***) led by Norway, then Chile, United Kingdom, Canada and Ireland. Other economically important marine fish are Gilthead Seabream (***Sparus aurata***), Seabass (***Dicentrarchus labrax***) and Turbot (***Scophthalmus maximus***) in countries such as Greece, Italy, France, Spain and Portugal, and Yellowtail (***Seriola quinqueradiata***), Ayu (***Plecoglossus altivelis***), Flounder (***Paralichthys olivaceus***) and Seabream (***Pagrus major***) in Japan. (FAO, 2003). In 2008, the world aquaculture production in major species groups showed that freshwater fishes continued to dominate with a production of 28.8 million tonnes (54.7 percent) valued at US$40.5 billion (41.2 percent), followed by mollusks (13.1 million tonnes), crustaceans (5 million tonnes), diadromous fishes (3.3 million tonnes), marine fishes (1.8 million tonnes) and other aquatic animals (0.62 million tonnes) (FAO, 2010).

In general, many metals occur naturally in marine environments; some of them are classified as pollutants which are only when added by man in sufficient amounts to produce deleterious effects on some features of the ecological system (Freedman, 1989). In aquatic systems, heavy metals have received considerable attention due to their toxicity and accumulation in biota (Mason, 1991). Stephen et al. (2000) mentioned that metals generally enter the aquatic environment through atmospheric deposition, erosion of the geological matrix, or due to anthropogenic activities caused by industrial effluents, domestic sewage, and mining wastes. Some of these metals, such as Cd and Pb, are toxic to living organisms even at quite low concentrations, whereas others, such as Zn and Cu, are biologically essential and natural constituents of aquatic ecosystems, and generally only become toxic at very high concentrations. The effects of heavy metals on human health and the environment are of great interest today, especially for aquatic products. (Uluozlu et al., 2007).

The important heavy metals and their recommended international permissible limits (PL) in water (mg / L.) and fish tissues (µg / g dry wt) were summarized in table the following:

Premissble limit of most important heavy metals that causes toxicity to the fish.

Metals	PL in water	Reference	PL in fishes	Reference
Lead (Pb)	0.050	***WHO (1984)***	0.1	***EOSQC (2005)***
			0.5	***FAO/WHO (1992)***
Mercury (Hg)	0.001	***WHO (1984)***	0.5	***EOSQC (2005)***
			0.5	***FAO/WHO (1992)***
Cadmium (Cd)	0.005	***WHO (1984)***	0.10	***EOSQC (2005)***
			0.05	***FAO/WHO (1992)***
Copper (Cu)	0.2	***FAO (1985)***	10	***FAO/WHO (1984)***
Zinc (Zn)	2.0	***FAO (1985)***	60	***FAO/WHO (1984)***
Iron (Fe)	< 1.0	***WHO (1984)***	30	***Food Stuff (1972)***

Also, Wong et al. (2001) evaluated the levels of six heavy metals (Cd, Cr, Cu, Ni, Pb, and Zn) in different tissues of three species of cultured marine fishes (***Epinephelus areolatus***, ***Lutjanus russelli***, and ***Sparus sarba***) collected from three fish culture sites in Hong Kong and they found that, in general, tissues of all three species contained high concentrations of Zn and Cu, but much lower concentrations of Ni, Pb, Cd, and Cr and Similar pattern of heavy metal concentrations was observed in sea water and sediment. Moreover, Gonads of all three species contained high concentrations of Zn. On the other hand, liver seemed to be the primary organ for Cu accumulation. Also, (Coetzee et al., 2002) illustrated that the fish gills have a high tendency to accumulate heavy metals. While, (Zehra et al., 2003) monitored the heavy metal distribution in the edible marine fish, 'Dandya' and they found that the concentration level of Cu, Cd, Zn, and Pb in nine different samples of fish muscles have been found to range from 0.3-0.55 (Cu), 0.04-0.15 (Cd), 3.65-4.32 (Zn) and 0.25-0.5 (Pb) µg / g. Fish muscles have a low tendency to accumulate the heavy metals to which they are exposed (Canlı and Atlı, 2003; Karadede et al., 2004 and Yılmaz, 2005). Dugo et al. (2006) investigated the bioaccumulation of trace metals (Zn, Cu, Se, Cd, and Pb) in cultured ***Dicentrarchus labrax*** tissues, liver and muscle, from the Sicilian coasts of Mediterranean Sea.

Dural et al. (2006) investigated the bioaccumulation of the heavy metals (Fe, Zn, Cd) in the liver, gill, gonad and muscle tissues of ***Dicentrarchus labrax, Mugil cephalus*** and ***Sparus aurata*** and found that the heavy metal levels were generally higher in the liver and gill than the gonad and muscle tissues in three species and the concentrations of Zn concentrations in the muscle tissues exceeded the acceptable levels for a food source for human consumption and other metals in the edible parts of the investigated fish were in the permissible safety levels for human uses.

Gill tissue is an organ having a large surface and separates blood from water in fish and is very susceptible to changes in concentrations of the variables (heavy metals, temperature, pH etc.) in the environment. These variables affect the structural integrity of the gill and cause morphological changes. For this reason gills are good indicators of water pollution (Koca et al., 2007).

Bahnasawy et al. (2011) stated that in Lake Manzala, the heavy metals in the water in water and fish tissues followed the same order: Zn > Cu > Pb > Cd. The mean concentrations of metals in the water were as follow: Cu, (0.055); Zn, (0.311); Cd, (0.020); and Pb, 0.022 mg/L. Authors mentioned that the Cd level in the water was found to be higher than the permissible limit recommended for drinking water. Moreover, Gills of the examined fish contained the highest concentration of all of the measured metals, while muscles retained the lowest. Also, ***Yabanli et al. (2012)*** studied the trace metals, Cadmium (Cd), Mercury (Hg) and Lead (Pb) concentrations of 76 pieces of frozen European Seabass (***Dicentrarchus labrax*** Linnaeus-1758) and gilthead Seabream (***Sparus aurata*** Linnaeus-1758) fillets, produced and marketed in Turkey, were determined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) after microwave damp burning process, and results obtained were assessed in terms of public health and they found that the maximum heavy metal levels for sea bass and sea bream fillets were determined as 256.50, 216.22 µg / kg for Cd; 414.79, 338.46 µg / kg for Hg; 1047.61, 147.14 µg / kg for Pb, respectively. At the end of the study, the levels of cadmium (for 3 samples) and lead (for 1 sample) were higher than the recommended legal limits of the European Union for human consumption.

It was observed that exposure to copper toxicity resulted in coagulation of the mucus layer of the gills, which inhibited oxygen transport and caused respiratory stress or reduced the number of lymphocytes and granulocytes in the blood, leading to reduced phagocytosis (Mushiake et al., 1985).

Generally, Heavy metals are chemical stressors and the development of disease will reflect interactions between the host, the disease causing situation and stressors (Austin and Austin, 1993). Also, Suppression of immune system and immune response may results from the action of several pollutants including heavy metals which provide opportunities for entering of many pathogens, but till now the effect of heavy metals on the immune system and immune response is not fully understood (Storelli et al., 2002).

Knittel (1981) demonstrated that Steelhead trout were exposed to sublethal concentrations of Copper at 7 and 10 µg / L for 96 hours and infected with ***Yersinia ruckeri*** caused more fish to die of infection than control fish (no copper). Infection susceptibility increased with time of exposure to a single dosage of copper (10 µg/1), reaching a maximum at 48 hrs. Lowering the copper concentration to 5 µg/1 caused the infection susceptibility to occur at 24 hours. The infectious dose of ***Y. ruckeri*** was lower in fish exposed to 10 µg/1 copper for 48 h than control fish. Moreover, Hetrick et al. (1982) stated that there was high susceptibility of Striped bass (***Morone saxatilis***) challenged with ***V. anguillarum*** and ***Pasteurella piscicida*** that exposed to sublethal doses of copper delivered to the aquaria for 96 hours prior to challenge.

Baker et al. (1983) tested Chinook salmon, *Oncorhynchus shawytscha* (Walbaum), and Rainbow trout, *Salmo gairdneri*, to determine if sublethal copper exposure would increase their susceptibility to *V. anguillarum* infection and found that the higher copper concentrations produce peak susceptibility to infection in shorter time periods. Rainbow trout stressed by copper required about 50% fewer pathogens to induce a fatal infection than non-copper exposed fish. Furthermore, exposure to toxic level of copper lead to increase the susceptibility to infections by *Edwardsiella tarda* (Mushiake et al., 1984).

Omima Aboud (2010*)* evaluated the effects of lead, mercury and cadmium on both humoral and cellular immune response of *Oreochromis niloticus* toward *Ps. fluorescence*. The results revealed that, lead, mercury and cadmium have inhibitory effect on phagocytic activity of fish macrophages and so having an inhibitory effect on cell mediated immune response also these metals having inhibitory effect on humoral immune functions which is manifested by low levels of antibodies and high mortality rates in fish exposed to these metals than in the control fish after experimental infection by *Ps. fluorescence*.

The prevalence of heavy metals by a high levels have been well documented in several cultured and wild freshwater fish species, however, only a few surveys on the prevalence of heavy metals toxicity responsible for outbreaks in marine fishes. Therefore, we have to explore this field to know how we can protect the Egyptian Mari culture against these toxicity.

The present work aimed to, study identification of the effect of heavy metals on marine fish cultures, recording the clinical signs and PM lesions associated with such toxicity and finally monitoring of some heavy metal concentrations in both fish tissues and water that the fish live in it.

MATERIALS AND METHODS

Fish

In this study we used 600 fish (300 fish) O. niloticus and 300 seabream fish (Seabream *Sparus aurata L.*) that were collected showing clinical signs from private fish farms in Alexandria and behaira governorate, Egypt. The fish were subjected to full clinical, postmortem (PM) lesions, parasitic and mycological as well as bacteriological examinations.

Experimental design

The fish in this study were allotted into 6 groups:

Group I: 100 *O. niloticus* fish that not take any treatment and considered as a control group.

Group II: 100 seabream fish and considred as a control group.

Group IIII: 100 *O. niloticus* fish and treated with 9 X 10^2 CFU penicillum.

Group IV: 100 *O. niloticus* fish treated with 0.7 mg/L cadmium.

Group VI: 100 Seabream fish treated with 9 X 10^2 CFU penicillum.

Group IV: 100 Seabream fish treated with 0.7 mg/L cadmium.

Gross clinical examination

Clinical examination of naturally and experimentally infected fishes was performed to investigate any clinical abnormalities according to the method described by ***Amlacher (1970***).

Postmortem (PM) examination

Necropsy was performed on variable number of freshly dead and moribund fishes for detection of PM lesions according to ***Conroy and Herman (1981***).

Parasitological and Mycological examinations

Parasitological examination methods were carried out according to ***Williams and Jones (1994)***.

Mycological examination was applied using the primary isolation medium for fungi culture on Sabouraud's dextrose agar medium (SDA) according to ***Whitman (2004).***

Bacteriological examination

a. Sampling

The fish surfaces were swapped with 70 % ethyl alcohol for surface sterilization and then bacterial inocula were taken from liver, Kidney, spleen and heart under complete aseptic condition.

Spectrophotometeric method for detection the levels of heavy metals in water and fish tissues

a. Sampling

b. Water samples

Water samples were collected from the cage water at which the fish live in it for examination the concentration of the heavy metals in water of each cage.

c. Fish sampling

At the same time, specimens of O. niloticus and Seabream were collected from the random fish samples of each group about (20 fish). After dissection of fishes, parts of dorsal musculature and liver were carefully removed and prepared for metal analysis.

Determination of heavy metals levels

The method for analysis of the heavy metals in the water was carried out according to APHA (1995) and in the fish tissues according to Clesceri (1998) that was carried out using Atomic Absorption Spectrophotometery. Atomic Absorption Spectrophotometer (Model Thermo Electron Corporation, S. Series AA Spectrometer with Gravities furnace, UK,) instrument was used to detect the heavy metals. The concentrations of heavy metals were expressed as mg/l for water and µg/g dry wt. for fish tissues. Fish specimens were digested according to AOAC (1996). All frozen fish samples were allowed to thaw at room temperature, washed with distilled water and placed on filter paper to remove the excess liquid. Their gills and musculature tissues were dissected separately and minced using a domestic blender, then approximately 1.0 g was placed in a 150 ml beaker and 10 ml concentrated nitric acid was added. After a short soaking period, 5 ml of 60% perchloric acid was added and the mixture was slowly heated on a hot plate until the conclusion of growth (approximately 2hrs). The mixture was then heated until the appearance of dense white fumes that indicate the nitric acid had evaporated and perchloric acid had reached its boiling point. The mixture was cooled; 10 ml of 25% hydrochloric acid was added then, the solution was transferred to a 100 ml volumetric flask that was subsequently brought to volume with de-ionized water. Blank solution was prepared for the background correction. Atomic absorption spectrophotometer instrument was used to determine cadmium, copper, lead and mercury concentrations which were expressed as µg / g dry weight in the Toxicology Unit of Central Laboratory, Faculty of Veterinary medicine, Alexandria University, Egypt.

The clinical signs and PM lesions were recorded daily for about one week and specimens for histopathological studies were collected. The Specificity of death was determined by re-isolation of injected bacteria from freshly dead fish during the period of observation (One week) according to (Soliman, 1988). The injected ***V. alginolyticus*** was re-isolated from freshly dead fish for verification of deaths.

Histopathological examination

Specimens for histopathological techniques were freshly taken from infected organs and tissues of the experimentally infected ***M. capito*** during both LD_{50} and chronic experiments. Samples were trimmed and fixed in 10 % phosphate buffered formalin. Then washed in running tap water for 24 hours then dehydrated in different concentration gradients of alcohol and cleared in xylol. Samples then embedded in paraffin wax and sectioned into thin sections of 5 microns thickness. Sections were stained with H & E stain and examined microscopically according to Roberts, (2001) .

Statistical analysis

The data of bacteriological examinations and heavy metal concentrations were statistically analyzed using Chi-square and ANOVA tests according to *SAS, (1987)* . After that the results presented in the form of figures according to Harvard graphics 4 computer programs.

RESULTS AND DISCUSSION

The results observed in Tables (1), (2), (3) and (4) cleared that, there is a significant ($P < 0.05$) differences of the concentration of cadmium, copper, lead and mercury among treated groups of fish with penicillium, cadmium

Table (1*): Effect of treatment with (9× 10 ²CFU) penicillium spores and (0.7 mg / L) cadmium on cadmium concentrations in O. niloticus and Seabream throughout one week.*

item	Control groups					
	O. niloticus			Seabream		
	W	F	L	W	F	L
1st day	B 2.73 ± 0.30	C 1.52 ± 0.50	C 1.37 ± 0.30	B 2.31 ± 0.30	C 1.41 ± 0.30	C 1.21 ± 0.30
2nd day	B 2.61 ± 0.60	C 1.41 ± 0.40	C 1.26 ± 0.30	2.21 ± 0.40	C 1.37 ± 0.30	C 1.08 ± 0.30
3rd day	B 2.41 ± 0.40	C 1.26 ± 0.20	C 1.11 ± 0.30	B 2.03 ± 0.40	C 1.14 ± 0.30	C 1.01 ± 0.30
4th day	B 2.17 ± 0.3	C 1.12 ± 0.20	C 1.03 ± 0.30	C 1.96 ± 0.30	C 1.17 ± 0.30	D 0.48 ± 0.10
5th day	B 2.01 ± 0.20	C 1.04 ± 0.30	D 0.95 ± 0.04	C 1.61 ± 0.30	D 0.93 ± 0.04	D 0.91 ± 0.04
6th day	C 1.95 ± 0.50	D 0.99 ± 0.04	D 0.89 ± 0.04	C 1.51 ± 0.30	D 0.88 ± 0.04	D 0.81 ± 0.04
7th day	C 1.31 ± 0.30	D 0.71 ± 0.001	D 0.53 ± 0.04	C 1.21 ± 0.30	D 0.61 ± 0.04	D 0.54 ± 0.04

W = water, F= Flesh, L= liver . Means of different letters are significantly different at (P < 0.05).

Table (2): Effect of treatment with (9× 10 ²CFU) penicillium spores and (0.7 mg / L) cadmium on Copper concentrations in *O. niloticus* and Seabream throughout one week.

item	Control groups					
	O. niloticus			Seabream		
	W	F	L	W	F	L
1st day	G 3.81± 0.80	G 3.91± 0.9	2.13± 0.40	C 7.58± 1.12	B 8.89± 1.12	E 5.34± 1.12
2nd day	G 3.42± 0.40	G 3.53± 0.50	H 2.01± 0.10	C 7.19± 2.12	B 8.13± 2.40	E 5.12± 1.12
3rd day	G 3.12± 0.20	G 3.24± 30	J 1.83± 0.30	C 7.01± 1.12	C 7.91± 0.40	E 5.01± 1.12
4th day	G 3.01± 0.3	G 3.17± 0.30	J 1.72± 0.30	D 6.71± 1.12	C 7.42± 1.12	F 4.91± 1.12
5th day	H 2.91± 0.20	G 3.08± 0.30	J 1.61± 0.30	D 6.65± 1.12	C 7.26± 1.12	F 4.79± 0.80
6th day	H 2.83± 0.30	H 2.98± 0.40	J 1.51± 0.30	D 6.31± 0.04	C 7.01± 0.04	F 4.41± 0.04
7th day	H 2.64± 0.40	H 2.74± 0.40	J 1.23± 0.30	D 6.11± 1.40	D 6.58± 1.15	F 4.13± 0.41

W = water; F= Flesh, L= liver. Means of different letters are significantly different at (P < 0.05).

Table (1)*: Effect of treatment with (9× 10 ²CFU) penicillium spores and (0.7 mg / L) cadmium on cadmium concentrations in O. niloticus and Seabream throughout one week.*(Continued).

item	*O. niloticus* group						Seabream group					
	9×10 ² CFU penicillium			0.7 mg / L cadmium			9×10 ² CFU penicillium			0.7 mg / L cadmium		
	W	F	L	W	F	L	W	F	L	W	F	L
1st day	C 1.97± 0.30	C 1.41± 0.40	C 1.21± 0.30	B 2.31± 0.40	C 1.46± 0.30	C 1.33± 0.30	C 1.37± 0.30	C 1.31± 0.30	B 2.11± 0.40	C 1.48± 0.30	C 1.39± 0.30	C 1.99± 0.30
2nd day	C 1.81± 0.30	C 1.23± 0.30	C 1.12± 0.30	B 2.81± 0.40	C 1.73± 0.30	C 1.39± 0.30	C 1.21± 0.30	C 1.11± 0.30	C 1.97± 0.30	C 1.57± 0.30	C 1.42± 0.30	C 1.51± 0.30
3rd day	C 1.32± 0.40	C 1.11± 0.30	C 1.02± 0.30	B 2.83± 0.30	C 1.77± 0.30	C 1.39± 0.30	C 1.11± 0.30	C 1.04± 0.30	C 1.61± 0.30	C 1.68± 0.30	C 1.47± 0.30	C 1.53± 0.30
4th day	C 1.11± 0.30	D 0.93± 0.40	D 0.99± 0.40	B 2.89± 0.40	C 1.94± 0.30	C 1.52± 0.30	C 1.02± 0.30	D 0.97± 0.55	C 1.24± 0.30	C 1.81± 0.30	C 1.51± 0.30	C 1.61± 0.30
5th day	D 0.91± 0.04	D 0.87± 0.04	D 0.91± 0.04	A 3.11± 0.40	B 2.07± 0.41	C 1.64± 0.30	D 0.97± 0.04	D 0.91± 0.04	C 1.13± 0.30	C 1.99± 0.30	C 1.62± 0.30	C 1.65± 0.30
6th day	D 0.78± 0.04	D 0.79± 0.04	D 0.81± 0.04	A 3.41± 0.30	B 2.18± 0.40	C 1.72± 0.30	D 0.87± 0.04	D 0.85± 0.14	C 1.02± 0.30	B 2.11± 0.30	C 1.81± 0.30	C 1.74± 0.30
7th day	D 0.51± 0.04	D 0.58± 0.04	D 0.67± 0.04	A 3.64± 0.02	B 2.41± 0.40	C 1.92± 0.40	D 0.63± 0.04	D 0.69± 0.04	D 0.93± 0.04	B 2.54± 0.40	C 1.95± 0.30	C 1.89± 0.40

W = water, F= Flesh, L= liver . Means of different letters are significantly different at (P < 0.05).

Table (2): Effect of treatment with (9× 10 ²CFU) penicillium spores and (0.7 mg / L) cadmium on Copper concentrations in *O. niloticus* and Seabream throughout one week. (Continued).

item	*O. niloticus* group						Seabream group					
	9×10 ² CFU penicillium			0.7 mg / L cadmium			9×10 ² CFU penicillium			0.7 mg / L cadmium		
	W	F	L	W	F	L	W	F	L	W	F	L
1st day	H 2.57± 0.40	B 8.99± 2.12	D 6.98± 1.12	G 3.68± 0.40	B 8.66± 2.12	C 7.11± 2.15	D 6.78± 1.12	B 8.51± 1.12	D 6.77± 1.12	G 3.88± 1.12	J 1.95± 0.30	1.21± 0.30
2nd day	H 2.21± 0.40	B 8.46± 2.40	D 6.42± 1.12	G 3.61± 0.40	B 8.57± 2.12	C 7.23± 1.12	D 6.31± 1.12	B 8.19± 2.12	D 6.42± 1.12	G 3.89± 0.40	J 1.94± 0.30	F 1.27± 0.40
3rd day	H 2.03± 0.30	B 8.17± 1.12	D 6.19± 1.12	G 3.68± 0.30	B 8.61± 1.12	C 7.33± 1.12	D 6.11± 1.12	B 8.05± 2.12	D 6.23± 1.12	G 3.91± 0.50	H 2.03± 0.40	F 1.33± 0.30
4th day	J 1.88± 0.30	C 7.19± 2.12	E 5.81± 0.40	G 3.91± 0.40	B 8.93± 1.40	C 7.52± 1.40	E 5.61± 0.40	C 7.81± 1.10	E 5.47± 1.10	F 4.11± 1.12	H 2.25± 0.30	F 1.57± 0.30
5th day	J 1.55± 0.30	C 7.05± 2.12	E 5.61± 1.12	F 4.15± 0.14	A 9.02± 1.15	C 7.61± 1.15	E 5.21± 0.40	C 7.61± 0.40	E 5.31± 0.40	F 4.41± 0.40	H 2.37± 0.41	F 1.64± 0.30
6th day	J 1.21± 0.30	D 6.81± 1.12	E 5.11± 1.12	F 4.51± 1.12	A 9.19± 2.17	C 7.74± 0.04	F 4.91± 0.44	C 7.22± 1.12	E 5.11± 1.12	F 4.61± 0.04	H 2.51± 0.40	F 1.78± 0.30
7th day	J 0.88± 0.04	E 5.13± 0.40	F 4.66± 0.40	F 4.67± 1.12	A 9.34± 1.04	C 7.86± 1.04	F 4.11± 0.40	D 6.27± 1.04	F 4.87± 0.44	F 4.72± 0.44	H 2.63± 0.40	F 1.83± 0.44

W = water; F= Flesh, L= liver. Means of different letters are significantly different at (P < 0.05).

Table (3): Effect of treatment with (9× 10^2CFU) penicillium spores and (0.7 mg / L) cadmium on lead concentrations in O. niloticus and Seabream throughout one week.

item	Control groups					
	O. niloticus			Seabream		
	W	F	L	W	F	L
1st day	C 3.82± 0.20	E 1.92± 0.3	E 1.58± 0.30	B 4.92± 1.12	E 1.91± 0.40	E 1.43± 0.30
2nd day	C 3.55± 0.50	E 1.76± 0.70	E 1.33± 0.30	B 4.62± 1.12	E 1.62± 0.30	E 1.31± 0.30
3rd day	C 3.18± 0.40	E 1.55± 0.50	E 1.18± 0.30	B 4.31± 1.12	E 1.36± 0.30	E 1.12± 0.30
4th day	C 3.01± 0.10	E 1.43± 0.40	E 1.03± 0.30	B 4.11± 1.12	E 1.18± 0.30	E 1.05± 0.30
5th day	C 3.01± 0.10	E 1.35± 0.30	E 1.03± 0.30	B 4.11± 1.12	E 1.13± 0.30	E 1.02± 0.30
6th day	D 2.89± 0.30	E 1.21± 0.30	F 0.92± 0.04	C 3.88± 0.40	E 1.01± 0.30	F 0.91± 0.04
7th day	D 2.71± 0.30	E 1.03± 0.30	F 0.81± 0.04	C 3.41± 0.40	F 0.91± 0.40	F 0.78± 0.04

W = water, F= Flesh, L= liver. Means of different letters are significantly different at ($P < 0.05$).

Table (4): Effect of treatment with (9× 10^2CFU) penicillium spores and (0.7 mg / L) cadmium on mercury concentrations in O. niloticus and Seabream throughout one week.

item	Control groups					
	O. niloticus			Seabream		
	W	F	L	W	F	L
1st day	B 0.91± 0.10	C 0.51± 0.01	C 0.17± 0.10	C 0.61± 0.40	C 0.43± 0.40	C 0.18± 0.21
2nd day	B 0.83± 0.30	C 0.47± 0.40	C 0.15 0.10	C 0.59± 0.04	C 0.41± 0.40	C 0.15 0.10
3rd day	B 0.81± 0.10	C 0.42± 0.20	C 0.14 0.10	C 0.51± 0.10	C 0.4± 0.01	C 0.13± 0.01
4th day	B 0.74± 0.3	C 0.37± 0.30	C 0.11± 0.10	C 0.43± 0.10	C 0.36± 0.10	C 0.11± 0.10
5th day	B 0.66± 0.60	C 0.3± 0.001	D 0.09± 0.001	C 0.38± 0.001	C 0.31± 0.004	D 0.09± 0.004
6th day	B 0.57± 0.50	C 0.28± 0.001	D 0.08± 0.001	C 0.31± 0.04	C 0.27± 0.05	D 0.07± 0.01
7th day	C 0.46± 0.40	C 0.19± 0.001	D 0.06± 0.001	C 0.27± 0.04	C 0.24± 0.04	D 0.04± 0.001

W = water, F= Flesh, L= liver. Means of different letters are significantly different at ($P < 0.05$).

Table (3): Effect of treatment with (9× 10 ²CFU) penicillium spores and (0.7 mg / L) cadmium on lead concentrations in O. niloticus and Seabream throughout one week. . (Continued).

item	O. niloticus group						Seabream group					
	9×10 ² CFU penicillium			0.7 mg / L cadmium			9×10 ² CFU penicillium			0.7 mg / L cadmium		
	W	F	L	W	F	L	W	F	L	W	F	L
1st day	C 3.63± 1.12	E 1.83± 0.30	E 1.52± 0.30	C 3.91± 0.40	D 2.03± 0.30	E 1.61± 0.30	B 4.81± 0.40	E 1.98± 0.30	E 1.62± 0.30	B 4.97± 0.40	E 1.94± 0.30	E 1.57± 0.30
2nd day	C 3.35± 1.11	E 1.61± 0.30	E 1.25± 0.30	C 3.47± 1.12	E 1.89± 0.30	E 1.42± 0.30	B 4.31± 0.40	E 1.79± 0.30	E 1.57± 0.30	B 4.83± 1.12	E 1.97± 0.30	E 1.59± 0.30
3rd day	C 3.14± 0.30	E 1.31± 0.30	E 1.11± 0.30	C 3.51± 0.40	E 1.94± 0.30	E 1.55± 0.30	B 4.11± 0.40	E 1.51± 0.30	E 1.41± 0.30	B 4.91± 0.40	D 2.11± 0.40	E 1.64± 0.30
4th day	D 2.69 0.30	E 1.09± 0.30	E 1.02± 0.30	C 3.71± 0.40	D 2.07± 0.40	E 1.61± 0.30	C 3.91± 0.30	E 1.33± 0.30	E 1.11± 0.30	B 4.98± 1.10	D 2.18± 0.50	E 1.71± 0.30
5th day	D 2.17± 0.40	F 0.99± 0.04	F 0.93± 0.04	C 3.71± 0.40	D 2.68± 0.40	E 1.68± 0.14	C 3.67± 0.40	E 1.27± 0.30	E 1.07± 0.30	A 5.13± 0.45	D 2.21± 0.40	E 1.79± 0.30
6th day	E 1.91± 0.30	F 0.71± 0.10	F 0.77± 0.50	C 3.99± 0.04	D 2.91± 0.04	E 1.87± 0.50	C 3.13± 0.30	E 1.01± 0.30	F 0.82± 0.04	A 5.61± 1.04	D 2.61± 0.40	E 1.91± 0.30
7th day	E 1.21± 0.30	F 0.61± 0.30	F 0.64± 0.04	B 4.11± 1.12	C 3.13± 0.17	E 1.98± 0.30	D 2.58± 0.40	F 0.93± 0.60	F 0.62± 0.04	A 5.91± 1.04	D 2.83± 0.40	D 2.11± 0.40

W = water, F= Flesh, L= liver. Means of different letters are significantly different at ($P < 0.05$).

Table (4): Effect of treatment with (9× 10 ²CFU) penicillium spores and (0.7 mg / L) cadmium on mercury concentrations in O. niloticus and Seabream throughout one week. . (Continued).

item	O. niloticus group						Seabream group					
	9×10 ² CFU penicillium			0.7 mg / L cadmium			9×10 ² CFU penicillium			0.7 mg / L cadmium		
	W	F	L	W	F	L	W	F	L	W	F	L
1st day	B 0.81± 0.40	C 0.42± 0.40	C 0.16± 0.10	B 0.82± 0.40	C 0.47± 0.40	C 0.19± 0.40	C 0.51± 0.40	C 0.46± 0.40	C 0.17± 0.10	0.59± 0.40	C 0.51± 0.40	C 0.18± 0.01
2nd day	B 0.74± 0.40	A 1.37± 0.30	C 0.14± 0.10	C 0.27± 0.40	C 0.49± 0.40	C 0.21± 0.10	C 0.47± 0.10	C 0.41± 0.10	C 0.15 0.10	B 0.61± 0.10	C 0.51± 0.10	C 0.19± 0.10
3rd day	B 0.67± 0.10	C 0.33± 0.40	C 0.12± 0.04	B 0.91± 0.04	C 0.51± 0.04	C .24± 0.04	C 0.43± 0.04	C 0.39± 0.40	C 0.13± 0.10	B 0.63± 0.40	C 0.51± 0.40	C 0.21± 0.01
4th day	B 0.59± 0.10	C 0.28± 0.10	C 0.08± 0.10	C 0.98± 0.10	B 0.63± 0.10	C 0.28± 0.10	C 0.39± 0.10	C 0.32± 0.10	C 0.11± 0.10	B 0.68± 0.10	B 0.59± 0.10	C 0.24± 0.10
5th day	C 0.51± 0.04	C 0.19± 0.10	D 0.07± 0.001	A 1.11± 0.30	B .71± 0.01	C 0.34± 0.04	C 0.31± 0.04	C 0.28± 0.04	D 0.08± 0.001	B 0.74± 0.04	B 0.62± 0.10	C 0.28± 0.10
6th day	C 0.41± 0.04	C 0.11± 0.01	D 0.05± 0.01	A 1.28± 0.30	B 0.91± 0.51	C 0.41± 0.04	C 0.18± 0.10	C 0.21± 0.04	B 0.07± 0.001	C 0.81± 0.14	B 0.77± 0.14	C 0.31± 0.04
7th day	C 0.22± 0.10	D 0.07± 0.001	D 0.01± 0.001	A 1.31± 0.30	A 1.07± 0.15	C 0.51± 0.15	D 0.48± 0.20	D 0.29± 0.40	D 0.18± 0.04	D 0.23± 0.04	D 0.29± 0.04	C 0.61± 0.04

W = water, F= Flesh, L= liver. Means of different letters are significantly different at ($P < 0.05$).

and control group of either O. niloticus or seabream. And also among water in which the fish live in it and flesh and liver of examined fish.

The results indicated that, the level of cadmium, copper, lead and mercury concentration in the groups treated with cadmium of a higher concentration for heavy metals than the groups treated with pencillum and in control.

This results attributed to the cadmium causes stress condition on the fish and causes decreaseing of the fish to resistance and immunity of the fish against toxicity with heavy metals as well as the dysfunction of the liver caused by cadmium causes increasing concentration of the heavy metals in the fish body. This results agreed with those of (Mieiro et al., 2012 and Dsikowitzky et al., 2013) where they reported that, dietary cadmium causes severe dangerous effect on the internal organs of the fish that causes increasing concentration of heavy metals to the fish especially in area contaminated with heavy metals.

The concentration of heavy metals in the seabream showed lower level than that of the *O. niloticus* group and control groups. This results attributed to the seabream fish live commonly in marine water which of lower level for heavy metal pollution than the other types of water of either brackish or fresh water and the *O. niloticus* commonly fed many type of foods of either animal or plant origin and this conditions causes the heavy metals in environment of *O. niloticus* higher than that of seabream fish. This results agreed with those of (Dsikowitzky et al., 2013) where they reported that, o. niloticus fish commonly exposed to the heavy metal intoxication than the other type of fish.

The results also indicated that the heavy metal concentration level decreased progressively from the 1st week to the 7th week of the experiment. Our results attributed to the size of the fish decreased with of the progresssion of the experiment and this causes decreasing of the accumulation of the heavy metals with advancement of the experiment due to this concentration of the heavy metals directly proprotional to the size of the fish this results agreed with those of (El-Sadaawy et al., 2013), where they reported that, by increasing the size of the fish there is an increasing of the heavy metals concentration and accunulation in the fish.

The results indicated that, the concentration of heavy metals in water higher than that of flesh and the lowest concentration observed in liver. (Table, 1). Ou results in agreement with those of (Abdolahpur Monikh et al., 2013) where they reported that the hevay metals in water that the fish live in its of a higher concentration than its level in the fish flesh or organs. But this results disagree with ou results in which they found that the liver have a higher concentration of the heavy metals than the other organs of the fish. Our results agreed with those of (Puel et al., 1987) where they reported that, heavy metals have the tendency to accumulate in various organs of marine organisms, especially fish, which in turn may enter into the human metabolism through consumption causing serious health hazards.

This study coblcuded that, the toxicity of the fish with heavy metals differ according to the type of the fish species and the level of heavy metyals that commonly polluted the water and presence of cadmium in the water can facilitated the intoxication with other types of the heavy metals as copper, lead, and copper and the higher concentration of heavy metals observed in water, liver and flesh, respectively.

CONCLUSION

This study concluded that, the toxicity of the fish with heavy metals differ according to the type of the fish species and the level of heavy metals that commonly polluted the water and presence of cadmium in the water can facilitated the intoxication with other types of the heavy metals as copper, lead, and copper and the higher concentration of heavy metals observed in water, liver and flesh, respectively.

REFERENCES

Abdolahpur Monikh F, Maryamabadi A, Savari A, Ghanemi K. (2013): Heavy metals' concentration in sediment, shrimp and two fish species from the northwest Persian Gulf. Toxicol Ind Health. 2013 Feb 13.

Amlacher, E. (1970): Textbook of fish diseases, T.E.S. Publication, NewJersey. USA, P. 117-135.

AOAC (Associatian of Official Analytical Chemists) (1996): "Official Methods for Minerals in Plants. Method 975.03. B.b." In: Official Methods of Analysis of AOAC International, 16th ed., V (1), Arlingoton (Va.): AOAC International Section 3, pp 3-4.

APHA (American Public Health Association) (1995): Standard Methods for the Examination of Water and Wastewater. 19th Edn., Washington DC.

Austin, B. and Austin, D. A. (1993): Bacterial Fish Pathogens: Disease of Famed and wild Fish, 2nd ed. Chichester: Ellis Horwood.

Bahnasawy; Liao, W. R.; Lin, J. Y.; Shieh, W. Y. and Jeng, W. L. (2011)*:* Antibiotic activity of lectins from marine algae against marine vibriosis. J. Ind. Microbial. Biotech. 30, 433 – 439.

Baker, R. J.; Knittel, M. D. and Fryer, J. L. (1983): Susceptibility of Chinook salmon, Oncorhynchus tshawytscha (Walbaum), and Rainbow trout, Salmo gairdneri Richardson, to infection with Vibrio anguillarum following sub-lethal copper exposure. Journal of Fish Diseases, (3): 267 – 275.

Canlı, M. and Atlı, G. (2003): The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, and Zn) levels and the size of six Mediterranean fish species. Environmental Pollution, (121): 129 -136.

Clesceri, L. S. (1998): Standard methods for the examination of water and waste water. In Collection and Preservation of Samples and Metals (eds Arnold, E., Greenbergy and Eaton, A. D.), APHA, AWWA, WEF, Washington, DC.

Coetzee, L.; du Preez, H. H. and van Vuren, J. H. (2002): Metal concentrations in Clarias gariepinus and Labeo umbratus from the Olifant and Klein Olifants River, Mpumalanga, South Africa: Zinc, copper, manganese, lead, chromium, nickel, aluminium and iron. Water SA, (28): 433 - 448.

Conroy, D. A. and Herman, L. R. (1981): Text book of fish diseases. T.F.H. publ., West Sylvania.

Dsikowitzky L, Mengesha M, Dadebo E, de Carvalho CE, Sindern S. (2013): Assessment of heavy metals in water samples and tissues of edible fish species from Awassa and Koka Rift Valley Lakes, Ethiopia. Environ Monit Assess. 2013 Apr;185(4):3117-31. doi: 10.1007/s10661-012-2777-8. Epub 2012 Jul 22.

Dugo, G.; La Pera, L.; Bruzzese, A.; Pellicano`, T. M. and Lo Turco, V. (2006): Concentration of Cd (II), Cu (II), Pb (II), Se (IV) and Zn (II) in cultured Seabass (Dicentrarchus labrax) tissues from Tyrrhenian Sea and Sicilian Sea by derivative stripping potentiometry. Food Control, (17): 146 – 152.

Dural, M.; Lugal, M. Z.; Ksu, G.; Akif, A. and Derici, B. (2006): Bioaccumulation of some heavy metals in different tissues of Dicentrarchus labrax L., 1758, Sparus aurata L., 1758 and Mugil cephalus L., 1758 from the Camlik lagoon of the eastern coast of the Mediterranean (Turkey). Environmental Monitoring and Assessment, (118): 65 – 74.

El-Sadaawy MM, El-Said GF, Sallam NA. (2013): Bioavailability of heavy metals in fresh water Tilapia nilotica (Oreachromis niloticus Linnaeus, 1758): potential risk to fishermen and consumers. J Environ Sci Health B. 2013;48(5):402-9. doi: 10.1080/03601234.2013.742719.

EOSQC (2005) : The role of adjuvants in the immune response of the fish. Zag. Vet. Med. J. 18: 126 - 136.

FAO (1985) : Direct immersion vaccination of Juvenile rainbow trout, Salmo gairdneri Richardison, and Juvenile coho salmon, Onchorhynchus kisutch (Walbaum), with a Yersinia ruckeri bacterin. Journal of Fish Diseases. 5, 339 - 341.

FAO (2003): Overview of Fish Production, Utilization, Consumption and Trade. FAQ, Rome, Italy.

FAO (2010): Overview of Fish Production, Utilization, Consumption and Trade. FAQ, Rome, Italy.

FAO; WHO (1984) *:* Effect of green seaweeds (Ulva Sp.) as feed supplements in Red tilapia (Oreochromis Sp) diet on growth performance, feed utilization and body composition. J. of Arab. Aqu. Cult. Soc. 5 (2): 179 - 194.

FAO; WHO (1992) : Serodiagnosis and vaccines, Lee town, W. Va, U.S.A. Develop. Biol. Standard. 49: 337 - 352. Karger, Based in International Symposium on Fish Biologics.

Food Stuff (1972) *:* Blood changes in Carp induced by ulcerative Aeromonas Salmonicida infection. Veterinary Immunology and Immunopathology. 2: 321 - 330.

Freedman, B. (1989): Environmental Ecology. The Impacts of Pollution and other Stresses on Ecosystem Structure and Function, Academic Press, London.

Hetrick, F. M.; Robertson, B. S. and Tsai, C. F. (1982): Effect of heavy metals on the susceptibility and immune response of Striped bass to bacterial pathogens. National Oceanographic Atmospheric Administration Publication 82112603: 32.

Karadede, H.; Oymak, S. A. and Ünlü, E. (2004): Heavy metals in mullet, Liza abu, and catfish, Silurus triostegus, from the Atatürk Dam Lake (Euphrates), Turkey. Environment International, (30): 183 - 188.

Knittel, M. D. (1981): Susceptibility of Steelhead trout, Salmo gairdneri Richardson to red mouth infection Yersinia ruckeri following exposure to copper. Journal of Fish Diseases, (4): 33 - 40.

Koca, S.; Koca, Y. B.; Yildiz, S. and Gurcu, B. (2007): Genotoxic and Histopathological effects of water pollution on two fish species, Barbus capito pectoralis and Chondrostoma nasus in the Buyuk Menderes River, Turkey. Biology of Trace Element Research, (122): 276 - 291.

Mason, C. (1991): Biology of freshwater pollution. Longman Scientific and Technical, Harlow, England.

Mieiro CL, Coelho JP, Pacheco M, Duarte AC, Pereira ME. (2012): Trace elements in two marine fish species during estuarine residency: non-essential versus essential. Mar Pollut Bull. 2012 Dec;64(12):2844-8. doi: 10.1016/j.marpolbul.2012.08.033. Epub 2012 Sep 25.

Mushiake, K.; Muroga, K. and Nakai, T. (1984): Increased susceptibility of Japanese eel, Anguilla japonica to Edwardsiella tarda and Pseudomonas anguilliseptica following exposure to copper. Bulletin of the Japanese Society of Scientific Fisheries, (50): 1797 - 1801.

Mushiake, K.; Nakai, T. and Muroga, K. (1985): Lowered phagocytosis in the blood of eels exposed to copper. Fish Pathology, (20): 49 - 53.

Omima, A. S. A. Aboud (2010): Impact of pollution with lead, mercury and cadmium on the immune response of Oreochromis niloticus. J. New York Science, 3(9): 12 – 16.

Puel, D.; Zsuerger, N. and Breittmayer, J. P. (1987): Statistical assessment of a sampling pattern for evaluation of changes in Hg and Zn concentration in Patella coerulea. Bull. Environ. Contam. Toxicol., (38): 700 –706.

Roberts, R. J. (2001): Fish Pathology, 3rd edn. W. B. Saunders, Philadelphia, PA.

Sadek, S. (2000): Seabream culture in Egypt; status, constraints and potential. Fish Physiol. Bioch., (22): 171 – 178.

SAS (1987): Statistical analysis system. User's Guide Statistics. SAS Institute Cary, North Carolina.

Soliman, M. K. (1988): Studies on Aeromonas hydrophila on some cultured freshwater fish “Oreochromis niloticus”. Ph. D. Thesis, Avian and Aquatic Anim., Med., Fac. of Vet. Med. Alex. Univ.

Stephen, D. E. and Jones, S. H. (2002): Microbiology of Summer flounder, Paralichthys dentatus L. fingerling production at a marine fish hatchery. Aquac., (211): 9 – 28.

Stephen, T. E.; S. D. H, Lewis and L. C. Grumbles. (2000): Comparison of selected diagostic tests for detection of motile Aeromonas septicaemia in fish. Am. J. Vol. Res., 39 (8): 1384-1386.

Storelli, M.; Giacominelli-Stuffler, R. and Marcotrigiano, G. (2002): Mercury accumulation and speciation in muscle tissue of different species of sharks from Mediterranean Sea, Italy. Bull. Environ. Contam. Toxicol. (68): 201 – 210.

Uluozlu, O. D.; Tuzen, M.; Mendil, D. and Soylak, M. (2007): Trace metal content in nine species of fish from the Black and Aegean Seas, Turkey. Food Chemistry, (104): 835 - 840.

Whitman, K. A. (2004): Finfish and Shellfish Bacteriology Manual; Techniques and Procedures. ISBN 0-8138-1952-0 – Iowa State Press.

WHO (1984) : Algae. In: Dietary supplements for the health and quality of cultured fish. Edited by Nakagawa, H., Sato, S. and Gatlin III. D. CABI North American Office Cambridge, MA 02139 USA, 133 - 168.

Williams, H. and Jones, A. (1994): Parasitic worms of fish. Taylor & Francis Ltd, London, 371 - 443.

Wong, C. K.; Wong, P. P. K. and Chu, L. M. (2001): Heavy metal concentrations in marine fishes collected from fish culture sites in Hong Kong. Arch. Environ. Contam. Toxicol., (40): 60 - 69.

Yabanli, M.; Alparslan, Y. and Baygar, T. (2012): Assessment of cadmium, mercury and lead contents of frozen Eurorean Seabass (Dicentrarchus labrax L., 1758) and gilthead Seabream (Sparus aurata L., 1758) fillets from Turkey. Agricultural Sciences, Vol.3, No.5, 669 - 673.

Yılmaz, A. B. (2005): Comparison of heavy metal levels of grey mullet (Mugil cephalus) and Seabream (Sparus aurata) caught in İskenderun Bay (Turkey). Turk. J. Vet. Anim. Sci., (29): 257 - 262.

Zehra, I.; Kauser, T.; Zahir, E. and Naqvi, I. I. (2003): Determination of Cu, Cd, Pb and Zn Concentration in Edible Marine Fish Acanthopagurus berda (DANDYA) Along Baluchistan Coast–Pakistan. International journal of agriculture & biology (1): 80 – 82.

تأثير وجود حويصلات البنسليوم فى العلف وعلاقتها بالمعادن الثقيلة فى أسماك المياه المالحة

رياض حسن خليل   طلعت طلعت سعـد   سامية الحوشى   أحمد أبو خشبة

فى هذه الدراسة تم التعرف على أهم أنواع المعادن التى تؤثر على أسماك المياه المالحة المستزرعة وتم تسجيل أهم الأعراض الداخلية والخارجية الناتجة من التسمم بالمعادن الثقيلة سواء فى الأنسجة أو المياه التى تعيش بها الأسماك .

فى هذه الدراسة تم استخدام (300) سمكة من أسماك البلطى النيلى و (300) من أسماك الدنيس تم تجميعها من مزارع خاصة بمحافظة الإسكندرية وتم تسجيل أهم الأعراض الداخلية والخارجية وكذلك العزل الفطرى والبكتريولوجى .

تم توزيع الأسماك فى 6 مجموعات كل مجموعة (100) سمكة (300) للبلطى النيلى و (300) للدنيس عدد مجموعتين تم استخدامها كمجموعة ضابطة إحداها للبلطى وإحداها للدنيس . أما المجموعتين الثالثة والرابعة فتم حقنهما بـ 9×10^2 من البنسليوم فى حالة البلطى وحالة الدنيس . أما المجموعتين الخامسة والسادسة تم حقنها بـ 0.7 مم / لتر كادميوم إحداها للبطى والأخر للدنيس .

استمرت التجربة لمدة 7 أسابيع تم تسجيل نسب المعادن الثقيلة وخصوصا الكادميوم وأيضا علاقته ببعض المعادن الأخرى مثل النحاس والرصاص وتم عمل فحص فطرى وبكتيرى من الأسماك المصابة وكذلك المياه والكبد والعضلات .