Comparative study of some heavy metal residues in Om-Elkhlol "*Donax tranculus*" retailed in markets of Alexandria and Ismailia Governorates

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Abstract

The levels of three heavy metals (cadmium, lead and copper) were estimated in 60 samples of Bivalve molluscs Om-Elkhlol"*Donax tranculus*", which were collected from both Alexandria (El-Max and Maadia Bays) and Ismailia (Timsah and Bitter Lakes) Governorates during summer 2012**.** Our results showed the hightest level of Cd in Om-Elkhlol from Bitter Lakes (0.709±0.280) followed by Om-Elkhlol Timsah Lake (0.389±0.147), El-Max Bay (0.237±0.133) and the lowest level was detected in Om-Elkhlol Maadia (0.154±0.073).Moreover the study also showed that 100, 80, 60and 33.33 % of examined Om-Elkhlol samples, respectively, containing Cd levels above the maximum permissible limit for human consumption recommended by Egyptian Standard "ES" ***(ES, 2360/1993)***. Highest Pb level was found in Om-Elkhlol Bitter Lakes (0.516±0.330) and the lowest in Om-Elkhlo Maadia(0.176±0.089). Highest Cu level was found in Om-Elkhlol Maadia (4.008±2.147) and the lowest was in Om-Elkhlol Bitter Lakes (2.225±1.338) samples. All pb levels in Timsah and Bitter Lakes Om-Elkhlol examined samples were above the MPL of ES and 80% of El-Max and Maadia samples were above the MPL of ES. On the other hand Cu levels in both Alexandria and Ismailia Om-Elkhlol samples were within the MPL. From the obtained results, it could be concluded that Om-Elkhlol tested samples contaminated with cadmium and lead in various concentrations. The obtained high levels of both cadmium and lead in Om-Elkhlol samples collected from Bitter and Timsah Lakes **(**affected mainly by agriculture effluents, industrial discharge, shipyard of the Suez Canal and sewage discharge from Ismailia City and other small villages) and El-Max Bays, industrial discharging regions, may reflect the elevated estuaries environmental pollution in those coastal regions. Bio-monitoring of environmental pollution in these molluscs harvesting coastal areas and the hygienic requirements to protect consumer against hazard of studied metals were discussed and clarified to be employed.

Key words: *Om-Elkhlol , Timsah , cadmium, lead , Cu levels .*

Introduction

Om-Elkhlol is a local name of the most famous Egyptian bivalve molluscs "Donax Trunculus" sold in regional fish markets along Alexandria and Ismailia coasts where destined for human consumption. Despite it contains high quality protein which is easier to digest than other muscle meat since it has little connective tissue, rich in vitamins, minerals and other essential nutrients **(FDA, 2009)**; it can cause serious health hazard to consumers.

This hazard return to the facts that: Om-Elkhlol is filter-feeder, they fed and respire by passing water between the shells and over gill surfaces where particulate matter is strained off for ingestion, during this process it may accumulate and concentrate pollutant pathogens and metals which may found in the water **(Messina *et al*, 2009)** ,lived near shore or at estuarine on bottom over surface of mud or sand where exposed to many types of pollutants and sediments contain high concentrations of contaminants and the whole animal is consumed (including all organs)

Om-Elkhlol is considered bio-indicator of metal contamination and used as a monitor of baseline environmental metal concentrations because its ability to absorb dissolved organic matter causes it to accumulate these metals. However, heavy metals are the most important pollutants in the coastal areas because of their toxicity and capacity to accumulate in living cells as well as in bottom sediments. So, Om-Elkhlol can concentrate more than fourfold in their tissue particles present in the surrounding water **(Butt *et al* 2004; Gutierrez *et al,* 2007; Hedge et al, 2009).**

Estuaries, especially those located in areas of intensive agricultural, industrial or sewage run-off, face the largest potential risks and suffer mostly from the toxic effects of exposure to many pollutants; the deterioration effects of those pollutant compounds is especially acute on molluscs and, eventually, on human health as a results of its consumption **(Fulton *et al*, 1999; Hyland *et al*, 2000; Yuan *et al*, 2001).**

Heavy metals are stable and persistent contaminants of aquatic environments, the toxic elements as cadmium and lead are harmful, even at low concentrations, when ingested over a long time period, and essential elements as copper may produce toxic effects when intake is excessively high **(Akin and Unlu, 2007; Storelli, 2009).**

Egyptian Organization for Standardization and Quality Control (**ES 2360/1993**) Stated that heavy metals residues in seafood should not exceed the Maximum Permissible Limits (MPL) of 0.1, 0.1 and 20 ppm for cadmium, lead and copper, respectively.

Although Cadmium (Cd) is widely distributed in the earth crust, food is considered the major source of it to the non-smoking population **(Elinder, 1985).** 2-6 % of ingested cadmium is absorbed from the gastrointestinal tract and the absorption may reach 20% of a given dose in an individual with low body stores of iron **(Nadig, 1990).** Filter- feeding Om-Elkhlol can accumulate significant concentrations of cadmium even in costal localities that were only moderately contaminated **(Byran *et al*., 1980).** It is a non essential trace element which progressively accumulates inside the body, particularly the kidney. Thus, incidence of kidney stone is increased in people affected with cadmium poisoning. Also it is a major contributor to thyroid disease **(Watanabe *et al*., 2000).**

Lead is one of the most important pollutants in our environment which accumulates in the body due to its low elimination rate. Its biological half life in bone is about 27 years **(Shibamoto and Bjeldanes, 1993)**. Lead is a special hazard for young children whom are particularly susceptible to lead exposure due to their high gastrointestinal uptake and the permeable blood–brain barrier and exposure of children to high levels may cause encephalopathy and/or irreversible mental retardation. Recent data indicated that there may be neurotoxic effects of lead at lower levels of exposure than previously anticipated **(Goyer, 1996; Lars, 2003).**Also it has hematological effects (inhibition of hemoglobin synthesis leads to anemia); renal effects which clinically, cause hyperuricaemia and gout. Gastrointestinal effects which cause constipation or diarrhea, epigastric pain, nausea, indigestion and loss of appetite **(Schardein, 1998)**

Copper is naturally occurring substances in the Earth's crust. Its release into the environment primarily through mining, sewage, treatment plant, solid waste disposal, welding and electroplating processes. In addition it is a common component of fungicides, this agricultural use of copper can result in its presence in soil, ground water and farm animals. **(ATSDR, 1990).** Copper is an essential trace element for animals and man, it is required for normal biological activity of many enzymes, hemoglobin formation and hair keratin **(Prohaska and Gybina, 2004**) .On the other hand, the excessive intake of cu may lead to toxicity which is manifested by nausea, vomiting, diarrhea and intestinal pain and the congenital inability to excrete copper and its accumulation in the body known as "Wilson S. disease". While copper deficiency results in anemia. **(Greenwood and Earnshaw, 1986).** El-Max bay receives a heavy load of waste water from industrial outfalls, agricultural drain water and sewage waste, also Boughaz El-Maadiya (in which molluscs were living) receives drainage from Lake Edko and pour it into Abu-Kir Bay **(Aboul-Dahab and Halim, 1988).** Suez Canal passes in its way through a number of Lakes, called Lake Timsah (in the north at km76 from Port Said) and the Great and Little Bitter Lakes in the southern part (between km 97.5 at Diversion and km 134.5 at Ginefa), Suez Canal environment affected mainly by agriculture effluents, shipyard of the Suez Canal and sewage discharge from Ismailia City and other small villages. In Suez Canal, the principal processes dominating the ecosystem are the mean sea level, the velocity and direction of current which are responsible for the distribution of pollutants along Suez Canal **(El Samra *et al*., 1983)**

However, in recent years, there has been a remarkable population growth, accompanied by intense urbanization, an increase of industrial activities and a higher exploitation of cultivable land. These transformations have brought about a huge increase in the quantity of discharges and a wide diversification in the types of pollutants, including heavy metals that reach sea waters and/or Suez Canal and have undesirable effects on their environment.

Aquatic systems are very sensitive to heavy metal pollutants and the gradual increase in the levels of such metals in aquatic environment, mainly due to anthropogenic sources, became a problem of primary concern. This is due to their persistence as they are not usually eliminated either by biodegradation or by chemical means, in contrast to most organic pollutants. Moreover, the decay of organic materials in aquatic systems together with detritus formed by natural weathering processes provides a rich source of nutrients in both the bottom sediments and overlying water body **(Forstner and Wittmann, 1983).**

The study of organisms as pollutant monitors has several advantages over the chemical analysis of abiotic comportments. Chemical, toxicological and ecological approaches have been studied extensively in assessing impacts of heavy metals pollution in aquatic environments. Bivalve molluscs are used as pollution bioindicators which able to bioaccumulate heavy metals in their tissues and as a result toxic effects to humans consuming these molluscs, (**Akin and Unlu, 2007).**

So, the present work was built to monitor the environmental pollution with cadmium, lead and copper in Om-Elkhlol "*Donax tranculus*" in Alexandria compared to Om-Elkhlol "*Donax tranculus*" in Ismailia Governorates, and to suggest a protocol for prevention of this pollution to protect consumers.

Materials and Methods

***Sampling sites**:

In Alexandria Governorate: El-Max Bay and Maadia

In Ismailia Governorate: Timsah Lake and Bitter Lakes

Collection of samples:

From each site, 15 samples of Om-Elkhlol "*Donax tranculus*" were collected from fishermen in the main molluscs-harvesting coasts of Alexandria and Ismailia province during summer 2012 with a total of 60 samples. Each sample composed of an average 20 organisms. All collected samples were transferred to laboratory without delay to be examined.

Preparation of samples: Om-Elkhlol of each sample were shucked with a clean knife and Om-Elkhlol tissues were removed and dissected with clean scissors and forceps, then ground and thoroughly homogenized for examination according to **Al-Baggou (2002)**.

Digestion of samples: Two grams from each prepared sample were digested by adding 10 ml of nitric/perchloric acid mixture (4:1) according to the methods described by **Salisbury and Chan (1985)**. Initial digestion was made for 4 hours at room temperature, followed by heating at 40-45ºC for an hour in water bath, and then the temperature was raised to 75ºC until the end of digestion. In case of incomplete digestion, the above experiment was repeated till the solution becomes clear. After cooling at room temperature, the digests were diluted to 20 ml. with ultrahigh purity water and filtered.

Estimation of metals: The filtrates were examined for the concentration of three heavy metals cadmium**,** lead, and copper by using Unicam 969AA spectrometer atomic absorption, Ingland according to **(Iwegbue,2008)** at Toxicology Unit; Biochemistry, Food Deficiency Diseases and Toxicology Department; Animal Health Research Institute, Dokki, Giza, Egypt.

Statistical analysis

The statistical analysis of the results was preformed using student T test and analysis of variance (ANOVA test) ***(*Snedecor and Cochran, 1980).**

Results

Table (1). Cadmium levels (ppm, wet weight of tissues) in examined Om-Elkhlol samples (n=60)

Samples sources	No. of samples	Minimum	Maximum	Mean± SE
Alexandria				
El-Max	15	0.016	0.621	0.237±0.133
Maadia	15	0.013	0.487	0.154±0.073
Ismailia				
Lake Timsah	15	0.019	0.962	0.389±0.147
Bitter Lakes	15	0.034	1.076	0.709±0.280

Table (2). Lead levels (ppm, wet weight of tissues) in examined Om-Elkhlol samples (n=60).

Samples sources	No. of samples	Minimum	Maximum	Mean± SE
Alexandria				
El-Max	15	0.093	0.597	0.196±0.087
Maadia	15	0.078	0.273	0.176±0.089
Ismailia				
Lake Timsah	15	0.014	0.986	0.432±0.316
Bitter Lakes	15	0.031	0.962	0.516±0.330

Table (3). Copper levels (ppm, wet weight of tissues) in examined Om-Elkhlol samples (n=60).

Samples sources	No. of samples	Minimum	Maximum	Mean± SE
Alexandria				
El-Max	15	0.790	6.330	3.844±1.593
Maadia	15	1.830	7.116	4.008±2.147
Ismailia				
Lake Timsah	15	1.265	6.134	2.880±1.470
Bitter Lakes	15	1.111	5.386	2.225±1.338

Table (4). Statistical comparison of lead, cadmium and copper levels in examined Om-Elkhlol samples according to sources in Alexandria

Criteria	Om-Elkhlol Alexandria					
	(El-max)			(Maadia)		
	Cd	Pb	Cu	Cd	Pb	Cu
Mean± SE	0.237±0.133	0.196±0.087	3.844±1.593	0.154±0.073	0.176±0.089	4.008±2.147
S D	A	A	A	B	B	B
MPL (ppm)	0.1	0.1	20	0.1	0.1	20
Samples above MPL%	9 60	12 80	0 0	5 33.33	12 80	0 0
No. of Samples	15			15		

-MPL=Maximum permissible limit -SD= Significant Difference ($p< 0.05$)

-The same letter means no significant differences using ANOVA test at 0. 5%

Table (5). Statistical comparison of lead, cadmium and copper levels in examined Om-Elkhlol samples according to sources in Ismailia

Criteria	Om-Elkhlol Ismailia					
	Timsah Lake			Bitter Lakes		
	Cd	Pb	Cu	Cd	Pb	Cu
Mean± SE	0.389±0.147	0.432±0.316	2.880±1.470	0.709±0.280	0.516±0.330	2.225±1.338
S D	A	A	A	B	B	A
MPL (ppm)	0.1	0.1	20	0.1	0.1	20
Samples above MPL	12	15	0	15	15	0
%	80	100	0	100	100	0
No. of Samples	15			15		

Discussion

Food is the major source of cadmium to the non-smoking population. It is a cumulative toxic metal with biological half life ranging from 20-40 years **(Shibamoto and Bjeldanes, 1993).** While the time of accumulation continues, the quantities of metal in organs also increase. These properties of cadmium make it especially dangerous. Egyptian Organization for Standardization and Quality Control **(ES, 2360/1993)** reported that the MPL of cadmium in seafood must not be more than 0.1 ppm. The present study revealed that the highest mean concentration of cadmium (ppm, wet weight of tissues) was found in Om-Elkhlol (Bitter Lakes) followed by Om-Elkhlol (Lake Timsah), Om-Elkhlol (El-Max)and the lowest in Om-Elkhlol(Maadia) samples (Table, 1).In addition 15(100%), 12(80%), 9(60%) and 5 (33.33%) of these Om-Elkhlol samples, respectively, were exceed MPL(Table 4&5), which may constitute a toxic hazard to their consumers. There is a significant difference was noticed in cadmium levels between samples from El-Max and from Maadia, also in between samples from Lake Timsah and from Bitter Lakes. Nearly similar results of cd levels in Om-Elkhlol (El-Max- Maadia) were reported by **Saleh (2004)** and in clams (Ruditapes decussatus and Venerupis pullastra) samples from lake Timsah by **Howaida *et al* (2008).**Lower results in Om-Elkhlol (El-Max-Maadia) samples were reported by **youssef and Basem(2009)** and in bivalves Paphia undulata and Gafrarium pectinatum samples from Lake Timsah were recorded by **Khalid and Mohamed (2005)**. Slight higher results of cadmium level in Om-Elkhlol (Maadia) were reported by **Abd El-Massih (1994)**; in mussels in Italy recorded by **Storelli *et al* (2000)**; in two bivalves (Mactra spp. and Mytilus spp.) at Alexandria region and in Bivalve(Paphia undulate) samples from Bitter Lakes were reported by **El-Gamal (2011,).** Much higher results were recorded by **Gutierrez *et al* (2007)** in Bivalve molluscs (Chlamys varia,) in Spain.

Regarding Lead levels in examined Om-Elkhlol samples (Table 2) the highest mean concentration of lead was found in samples from (Bitter Lakes) 0.709±0.280 followed by (Lake Timsah) 0.432±0.316, (El-Max) 0.196±0.087 and the lowest level was found in samples from (Maadia) 0.176±0.089. Data represented in tables 4&5 showed that 15-15 (100%) and12-12 (80%) of Om-Elkhlol samples in both (Bitter Lakes -Lake Timsah) and (El-Max- Maadia), respectively, were exceed MPL. Significant difference was clear between Om-Elkhlol samples from Lake Timsah and Bitter Lakes. Meanwhile there no significant difference between Om-Elkhlol samples from El-Max and Maadia. **.** Nearly similar results of pb levels in Om-Elkhlol (El-Max- Maadia) were reported by **Saleh (2004)** and in clams (Ruditapes decussatus and Venerupis pullastra) samples from lake Timsah by **Howaida *et al* (2008**) .Lower results in Om-Elkhlol (El-Max-Maadia) samples were reported by **youssef**

and Basem (2009). Higher results of lead level in bivalves (Paphia undulate) and (Gafrarium pectinatum) samples from Lake Timsah recorded by **Khalid and Mohamed (2005)** and in bivalves (Paphia undulate) samples from Bitter Lakes reported by **El-Gamal (2011).** The obtained high results of lead levels in mostly all examined Om-Elkhlol samples may reflect the elevated aquatic environmental pollution ,where El-Max bay it consider as one of the main fishing sources in Egypt, it has several industrial plants(cement, Miser chemical and petroleum refining companies) situated close to the coast and directly discharge its effluents into it. **(Abdallah and Abdallah, 2008).** While Maadia is affected by brackish agricultural runoff. **.**Regarding to Suez Canal environment comprises almost all types of land based sources of pollution and severely affected by most types of pollutants **(Abd El-Azim, 1996).** Results of Lead levels in examined Om-Elkhlol samples in Ismailia were higher than ones in Alexandria may be return to that Pb and Cd gave their highest level at Bitter Lakes and Lake Timsah respectively .due to receiving some sewage effluents from Ismailia City and other small villages as well as agriculture effluents, fresh water from Ismailia channel, land drainage and different water currents caused by the passage of ships **(El-Moselhy *et al*, 2005)**
Human absorbs lead in small amounts from food, water and / or air. About 15-18 million children in the developing countries have permanent suffering from brain damage as a result of lead poisoning **(Tong *et al*., 2000).**
The obtained results in table (3) shows the mean values of Cu in examined samples of Om -Elkhlol El-Max, Om-Elkhlol Maadia Om-Elkhlol Lake Timsah and Om-Elkhlol Bitter Lakes were 3.844±1.593, 4.008±2.147, 2.880±1.470, 2.225±1.338, respectively, the highest mean was observed in Om-Elkhlol Maadia, while the lowest in Om-Elkhlol Bitter Lakes samples. **McLaughlin *et al*., (1999)** reported that Cadmium interacts with a number of minerals mainly zinc, iron and copper due to chemical similarities and competition for binding stage**.** This fact may explain why Cu levels in Om-Elkhlol Bitter Lakes samples in our study were the lowest and their Cd levels were the highest. According to Egyptian Standard **(ES, 2360/1993)** the Maximum Permissible Limits (MPL) of copper in molluscs are 20 ppm. (Tables 4&5) revealed that all examined samples in Alexandria and Ismailia Governorates were within the MPL. There was significant difference between Om-Elkhlol El-Max and Om-Elkhlol Maadia, while it was not found between Om-Elkhlol Lake Timsah and Om-Elkhlol Bitter Lakes. Despite the existence of a number of detoxifying and storage systems for Cu, it is the most toxic metal after mercury and silver, to a wide spectrum of marine life, hence its value in antifouling preparations **(Clark, 1989).** Similar results in Om-Elkhlol Maadia and El-Max were reported by **Saleh (2004).** Dissimilar results obtained by **Howaida *et al* (2008)** where cu level exceeded MPL in clams (Ruditapes decussatus and Venerupis pullastra) samples from Lake Timsah. Higher results of cu levels in (Paphia undulate) samples from Bitter lakes were reported by **El-Gamal (2011)** and in Om-Elkhlol Maadia were reported by **Abd El-Massih (1994),** while his results of cu levels in El-Max Bay were lower than ours. Lower results of cu level in bivalves (Paphia undulata and Gafrarium pectinatum) samples from Lake Timsah recorded by **Khalid and Mohamed (2005)**. It is important to note that using filter feeding organisms, such as bivalve molluscs, will give an ‘overall' measurement of the accumulation caused by both particle ingestion and membrane transport **(Rainbow, 2006).**

Conclusion and Recommendations

The results of the present study concluded that the examined Om-Elkhlol contaminated with lead in high concentrations in both Alexandria and Ismailia Governorates and the high residual cadmium contents were recorded in Om-Elkhlol samples collected from Ismailia Governorate may reflect elevated estuaries and aquatic environmental pollution in both Governorates .Concerning copper, it detected in safety levels in all Alexandria and Ismaialia om-

Elkhlol samples may return to its wide range of the permissible limit. We **recommend** that effective ecological measures should be taken to reduce toxic metals in the environment and continuous monitoring for metals in environmental (biotic and abiotic) samples is necessary

Government should prohibit the industrial companies to discharge their effluents into molluscs-producing regions before application of effective hygienic treatment and/or safe disposal of hazard waste. Awareness of public about toxic metals which may accumulate in bivalve molluscs and causing health problems.

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دراسة مقارنة لبقايا بعض المعادن الثقيلة فى ام الخلول المباعة فى اسواق محافظة الاسكندرية والاسماعيلية

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الملخص العربى

الرخويات الصدفية (ام الخلول) لها اهمية غذائية كبيرة حيث انها تحتوى على الاملاح والبروتينات والعناصر المختلفة وايضا اهمية صحية حيث انها تتغذى عن طريق فلترة مكونات البحر وينتج عن ذلك تركيز لكل مكوناتة شاملا المعادن الثقيلة داخل انسجتها. لذا تم تجميع عدد 60 عينة من ام الخلول من كل من محافظة الاسكندرية (بوغاز المكس والمعدية) ومحافظة الاسماعيلية (البحيرات المرة و بحيرة التمساح) خلال صيف عام 2012 وتم تقدير متبقيات الكادميوم والرصاص و النحاس وقد اسفرت النتائج ان متوسط التركيز الاعلى للكادميوم فى عينات ام الخلول البحيرات المرة – ثم ام الخلول بحيرة التمساح- ثم المكس واخيرا التركيز الاقل فى المعدية .وايضا اوضحت النتائج ان 100-80-60و 33و33%من العينات بالترتيب تحتوى على نسب من الكادميوم اعلى من الحدود المسموح بها للاستهلاك الادمى فى المواصفات القياسية المصرية (2300/ 1993) . وقد وجد ان متوسط المستوى الاعلى للرصاص فى عينات ام الخلول البحيرات المرة والاقل فى عينات ام الخلول المعدية وعلى العكس فمتوسط تركيز النحاس الاعلى فى ام الخلول المعدية والاقل فى ام الخلول البحيرات المرة. وكانت 100% عبنات ام الخلول كل من البحيرات المرة و بحيرة التمساح و80% من كل من عبنات ام الخلول المكس والمعدية محتوية على تركيزات للرصاص اعلى من الحدود المسموح بها للاستهلاك الادمى بالمواصفات القياسية المصرية . كما احتوت جميع العينات على تركيزات من النحاس فى حدود المسموح بها فى المواصفات القياسية المصرية . وكذلك قد تم مناقشة الاهمية الصحية و التأثيرات السامة لهذه المعادن الثقيلة وكيفية التحكم أو منع وصول هذه العناصر الى الرخويات الصدفية وحماية المستهلكين من اثارها الضارة.