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Original article

Determination of Lead and Cadmium in Marine Water and Crabs (*Pachygrapsus marmoratus*) from Tolmeitha Coast, Libya

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Abstract
In this study, the concentrations of Lead and Cadmium metals were determined in water and Crab (Pachygrapsus marmoratus) samples collected from some locations around Tolmaitha city coast (Eastern North) of Libya. The samples were collected during two different seasons (Winter and Summer). The atomic absorption method was used to estimate the studied heavy metals. The results of this study recorded that the concentrations of Lead ranged between (0.3 – 52.15 ppm), and for the Cadmium contents ranged between (0.05-0.084 ppm). High concentrations were recorded for the lead in Crab samples (1.25 -169.7 ppm) and the cadmium concentrations (0.16 -4.32 ppm). The results of this indicated for presence of small variations of the selected metals in the organs and shields of the studied crabs. But in general, High concentrations were recorded in the carapace, more than the permissible limit of WHO values.
Keywords: Lead, Cadmium, Sea Water, Crabs, Libya.

Introduction

The Mediterranean Sea is considered one of the most important seas, with an area of about 2.5 x 610 square kilometers. It is considered one of the semi-closed seas, whose waters are renewed once every 75-80 years [1]. The Mediterranean receives pollutants from different land sources through rivers and drains, and the coastal areas in the Mediterranean are the most polluted, as the beaches in coastal cities have fallen under bad exploitation as a result of false beliefs about the endless potential of the seas for self-purification due to the vastness of the area and the huge quantities of water that It can absorb pollutants without harming them [2].

Making it the best landfill for most people, as about 80% of human and industrial wastewater is discharged into rivers and seas without decontamination [3]. The Libyan coastal cities depend on the discharge of untreated sewage directly into seawater through networks that lead to special exits, and the city of five is among the cities overly affected [4]. The Mediterranean Sea, which contains about three sewage outlets, which flows directly into untreated coastal waters, which contain pollutants through dilapidated networks [5]. These pollutants affect the chemical and physical properties of seawater, which in turn is reflected on the organisms that live in coastal areas and cause their pollution [6]. The coastal area represents about 9% of the seabed area, but it is the most vital [7].

It is home to a large number of mollusks, crustaceans, and algae [8]. Most crustaceans live in the seas, some in fresh water, and there are a few species that live on land. It includes seven types of crustaceans, and the largest is the giant spider crab in Japan, Macrocheira kaempferi, with a distance between the two dogs of 3.5 m, and the smallest of which is the daphnia Cladocera, which is 1 mm in length [9]. Marine crustaceans of all kinds are economically important, as their small individuals represent a link in the food chain in the sea, and some of them are the main food for whales [10]. Benthic animals (crustaceans and mollusks) are among the most marine organisms that meet most of the specifications that must be met in the biological evidence, as the wide range of heavy metal accumulation and the rate of absorption of these elements vary according to the different species [11,12]. Also, crustaceans and mollusks can absorb heavy metals dissolved in water through gills, and through the gut [13].

Mollusks accumulate heavy metals in higher concentrations than the aquatic environment in which they are located, where studies indicate that their accumulation is more than fish [14,15]. Many studies have shown that most heavy metals accumulate in soft tissues more than in shells and outer armor, but some studies have shown that the accumulation of heavy metals in shells can be more accurate as they provide a historical record of the organism's content of elements over its life and this record keeps preserved after death [16-18].

Because the shells and shields are sites of stored heavy elements, either by entering into their composition or the possibility of adsorbing them on them, which leads to their accumulation in high concentrations in and on the body of the organism, and for this reason, the shells and the shield are better indicators of pollution [19,20]. This study aimed to estimate the bioaccumulation of some heavy elements (lead and cadmium) in the crab (Pachygrapsus marmoratus). These elements were also estimated in the seawater samples surrounding these crab samples, which were collected from three sites in the city of Tolimitha.

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Methods

The Studied area

This study was conducted on the beach of the city of Talmitha, located in the northern part of Libya, between latitudes (16-14°) north, and longitudes (39-32°) east, and on the Mediterranean coast east of Benghazi, 120 km. Three important sites were chosen, distributed on the beach of the city of Talmitha, namely the beach"Al-Mina", "Fondo Beach, The Beach and Elaf Resort", which were described as study sites (the first, second, and third).

In this study, some chemicals were used: perchloric acid $HClO_4$ 60%, hydrogen peroxide H_2O_2 12%, nitric acid HNO_3 65%, hydrochloric acid HCl 36% and distilled water to complete the digestion and extraction processes. Several devices were used for this purpose, the most important of which are the atomic absorption spectrometer, the Hot plate, heated oven, for drying, absorption (CONTRAA700analytikjena), Blender, electric grinder, Sensitive balance, Water path, Reflux device, as well as many glasses.

Sample description (Crab Pachygrapsus marmoratus)

This type of crustacean is widespread and is found on the beach rocks and can be found up to 4 meters deep. At all times of the year, it goes out on the beach and spends a long time outside the water, as it has a wide environmental adaptation. The body of the animal is covered with a shield (shield) square in shape, the frontal edge is wide, it has weak spines, and the lateral front edge has two spines, and the eyes have a wide distance between them. (4.5 cm), (Figure 1).

The Scientific classification can be shown as follows: The Kingdom is Animalia, the Phylum is Crustacea, the Sub-phylum is Decapoda, the Class is Malacostraca, the Family is Gropsidae, and the Genus is Pachygrapsus.

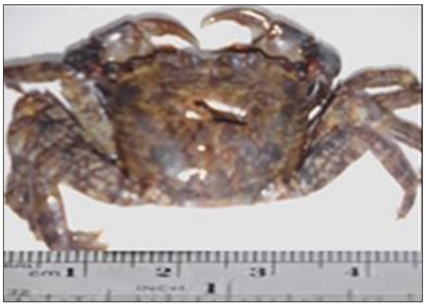

Figure 1. The studied (Crab Pachygrapsus marmoratus) samples

Samples collection

Samples were collected from the coastal area from the distances ranging between 7-10 m from the beach. Forty-four samples were collected from three sites quarterly between the fall of 2022 and winter 2023 by 3 individuals/location, where samples of beach crabs were collected from pits and under rocks, and the samples were cleaned well with sea water at the sample collection site to remove sand residues and suspended materials, then washed with tap water and kept in plastic containers and placed in frozen until transported to the laboratory. Seawater samples (10 liters) were collected at a depth of 20-30 cm from the three sites.

Samples preparation

The samples were brought to the Chemistry Department, the Inorganic Chemistry Laboratory at Faculty of Arts and Science, Almarj, Benghazi University, the (crab) samples were washed with distilled water for several times, dried then the viscera were separated from the outer shield, using laboratory dissection tools, and placed in watch bottles, the samples were placed in the oven at 105°C for 24 hours to obtain a constant weight, and then the samples were ground using the Blender and then the powder was kept in clean, tightly closed plastic containers until the chemical extraction process was carried out.

Extraction of heavy metals from seawater samples

Samples of the sweater were digested according to the method used previously. Where the weight of 50 of the sample (the carapace and the inner viscera) was taken separately, and it was placed in a conical flask with a capacity of 100 ml, and the digestion solution (5 ml of nitric acid HNO_3 and 5 ml of prochloric acid $HClO_4$) was added to it until the digestion was completed, then the samples were filtered and placed in polyethylene containers, for examination by atomic absorption spectrophotometer Absorption CONTR AA700 analytikjena at Al-Sadeem Laboratory in Tripoli, as described in many studies [21-30].

Extraction of heavy metals

Samples were digested according to the method used previously. Where the weight of 1 gm of the sample (the carapace and the inner viscera) was taken separately, and it was placed in a conical flask with a capacity of 100 ml, and the digestion solution (5 ml of nitric acid HNO_3 and 5 ml of prochloric acid $HClO_4$) was added to it until the digestion was completed, then the samples were filtered and placed in polyethylene containers,

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this method was used in many studies for estimate heavy metals in solid samples [31-40], for examination by atomic absorption spectrophotometer Absorption CONTR AA700 analytikjena at Al-Sadeem Laboratory in Tripoli.

Statistical analysis

Statistical analysis was conducted using Analysis of variance to compare the differences between the averages, and the New Multiple Range Test (Duncan) was used to verify the locations of differences at a significant level ($P<0.05$). Water and samples. To draw the figures, Microsoft Excel 2007 was used. The bioaccumulation coefficient was calculated according to the following equation: Bioconcentration factor (BCF) = the concentration of the element in the organism (µg/L) **/** its concentration in water.

Results

The results of this study showed that the highest concentration of lead in water samples (15.20 mg/l) was recorded during the summer in the second location, while the lowest concentration was (03.0 mg/l) during the autumn in the third location. (Table 1) show the analytical results of the average concentrations of heavy elements in seawater. The statistical presence of significant differences ($P<0.05$) in the values of lead concentrations according to the change of seasons, where the summer season differed significantly with each of the autumn, winter and spring seasons, and the results of the analysis of variance (MANOVA) in (Table 2) showed that there is a significant effect (for the seasons). Seasons * locations (on lead in sea water) ($P < 0.05$), while there is no significant effect (locations) on lead ($P<0.05$). While the concentrations of cadmium in the current study were similar between the three sites, the highest concentration of cadmium was (0.084) mg/l during the summer in the third site, and the lowest concentration (0.05) mg/l in the fall in the second site, as the results of the analysis showed.

Table 1. Concentration of heavy elements lead and cadmium (mg/liter) in seawater samples of the study sites during the four seasons of the year, and the permissible limit

Summer	Spring	Winter	Autumn	locations	Elements
* 1.0 ± 10.37	* 0.02 ± 0.18	0.03 ± 0.05	* 0.01 ± 0.06	First	Pb permissible limit according to WHO/FAO 2003 0.05
* 0.02 ± 15.2	* 0.01 ± 0.78	*0.02 ± 0.08	*0.03 ± 0.08	Second	
*1.0 ± 15.01	* 0.02 ± 0.18	0.03 ± 0.05	0.02 ± 0.03	Third	
* 0.03 ± 0.06	* 0.02 ± 0.07	* ±20.0 0.08	* 0.01 ± 0.068	First	Cd Permissible limit according to WHO/FAO 2003 0.05
* 0.02 ± 0.06	* 0.02 ± 0.06	* 0.02 ± 0.068	0.04 ± 0.05	Second	
* 0.03 ± 0.084	* 0.01 ± 0.06	* 0.04 ± 0.06	* 0.03 ± 0.07	Third	

As for crab samples, the results shown in (Table 2) showed that there is an accumulation of lead in the carapace and guts of crab P. marmoratu. Its highest concentration was recorded in the carapace (250.10) µg/g dry weight, in the spring season in the third location, and the lowest concentration in the shield (0.02) µg/g dry weight, in the autumn season in the third location, while the highest concentration of lead in the internal organs was (1.250) µg/g dry weight, in the spring in the third location, and the lowest concentration. The element lead in the intestines was (0.08) µg/g dry weight, in the winter season in the first location, and the results of the statistical analysis showed that there were significant differences ($P < 0.05$) between the averages of the element lead for the seasons between spring, autumn, winter and summer, and there are significant differences ($P<0.05$) between the averages of lead for the sites, between the third site and both the first site and the second site. On lead in crab ($P < 0.05$).

Table 2. The concentrations of Lead and Cadmium in the studied Crab samples.

Summer	**Spring**	**Winter**	**Autumn**	**Sample**	**Locations**	**Elements**
b c 0.1±2.09 cde 0.1±2.65	1.1±4.1 1.01±9.65	0.21±5.25 0.02±0.08	0.02 ±0.07 0.1±0.19	*P.marmoratu* shields *P.marmoratu* internal organs	First	Pb Permissible limit according to FAO/W HO1983
a b c 0.1±1.43 d	f 1.1±5.47 i 2.01±37.86	1.1±3.3 0.09±2.9	0.01±0.0380 0.1±0.746	*P.marmoratu* shield	Second	

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e 0.03±3.64				*P.marmoratu* internal organs		6-0.5
e 0.04±3.83 l 0.05±2.31	8.7±169.2 8.3±250.1	a b 0.01±0.666 a 0.03±0.431	0.01±0.02 1.1±8.11	*P.marmoratu* shields *P.marmoratu* internal organs	Third	
f g 1.05±2.09 * g 0.02±2.65	* a 0.03±0.205 a b 0.03±0.582 *c	* a 0.1±0.788 * e 0.1±1.313	a 0.01±0.182 * h 0.1±4.22	*P.marmoratu* shields *P.marmoratu* internal organs	First	Cd permissible limit according FAO/WHO1983 5.5-0.05
* c d 0.04±0.83 a 0.03±0.16	* a 0.03±0.179 * d e 0.02±1.123	a 0.01±0.3 * a b c 0.07±0.4	a 0.1±0.187 *a b 0.1±0.387	*P.marmoratu* shield *P.marmoratu* internal organs	Second	
h 0,03±3.82 *f g 0.03±2.31	a b 0.02±0.567 *c b c 0.04±0.696 *d	*a b c 0.1±0.524 *c d 0.02±0.829	*a b c 0.1±0.491 *g 0.2±2.50	*P.marmoratu* shield P.marmoratu internal organs	Third	

Discussion

(Table 3) indicates that the highest concentration of cadmium in the shield of P. marmoratu was (3.82) µg/g dry weight, in the summer in the third location, and the lowest concentration was (0.179) µg/g dry weight in the spring. In the second site, while the highest concentration of cadmium was recorded in the internal organs of P. marmoratu (4.22) µg/g dry weight, in the fall season in the first site, and the lowest concentration was (06.1) µg/g dry weight, in the summer in the second site. (Figure 3,4), as the results of the statistical analysis showed that there are statistically significant differences ($P < 0.05$) between the averages of the cadmium element for the seasons, between the summer and each of the autumn, winter and spring seasons, and it is also clear that there are statistically significant differences ($P<0.05$) between the averages of the cadmium element for the three sites, between the second site and each of the first site and the third site. Cadmium in crab. (Table 2) shows that the values of BCF for the three elements in the carapace and tissues of P. marmoratu ranged between (0.09 – 0.9) µg/L and (0.2 – 0.5) µg/L, respectively. They are considered normal because (BCF<1), and they were. The highest values for lead and cadmium in the shield were as follows: (45.5 – 9.40) µg/L, respectively, times their concentration in water. As for tissues, the highest BCF values for lead and cadmium were as follows: (62 – 270) µg/L, respectively, times as much as their concentration in water, where the BCF values were abnormal because (BCF>1).

Table 3. The ANOVA analysis of lead and Cadmium in the studied Crab samples.

Cd		**Pb**		**Heavy elements**	
P- Value Model level	F Test statistic	P- Value Model level	F Test statistic	Df Degrees of freedom	Sources variance
P<0.000	12.198	P<0.000	152.090	3	Sessions
P<0.000	12.105	P<0.000	86.225	2	Locations
P<0.000	5.653	P<0.000	83.171	6	* Sessions locations

The results of the study on P. marmoratu crab recorded low concentrations of the elements, ranging between (0.02 and 0.08) µg/g dry weight, and although they are low, they may pose a danger to the consumer because they are among the elements that have toxic effects on most living organisms. High concentrations were recorded in the carapace and internal viscera of the crab and were higher than the permissible limit and were also higher than their concentration in water, due to the processes of adsorption and absorption [11], where liquids are absorbed through the outer surface of the animal's body, which is in contact with water and sediment and Perhaps the reason for its accumulation in the carapace is due to its entry with calcium during the construction of their structures and the high concentrations in the gut may be due to nutrition. Crab. High concentrations of the elements may be through association with Metallothioneins [13]. The (Table 4) results showed that the highest concentrations of elements (Cd, Pb) in the crab shield were as follows: (41.3, 45.5) µg/g dry weight, respectively, in this study. It was also found that the concentrations of the

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elements in the internal bowels were higher, where the concentrations of the elements (Cd, Pb) were as follows:(53.6, 45.5) mcg/g, respectively.

Table 4. The concentrations of lead and Cadmium in the studied Crab samples.

Summer	Spring	Winter	Autumn	Sample	Locations	Elements
0.2 0.3	22.8 53.6	10.5 1.6	1.2 3.2	P.marmoratu shields P.marmoratu internal organs	First	Pb
0.09 0.2	7 48.5	41.3 36.3	0.5 9.3	P.marmoratu shields P.marmoratu internal organs	Second	
0.3 0.2	9.40 1.4	13.3 8.6	0.7 2.70	P.marmoratu shields P.marmoratu internal organs	Third	
34.8 44.2	2.9 8.3	9.9 16.4	2.7 62	P.marmoratu shields P.marmoratu internal organs	First	Cd
13.8 2.7	3 18.7	4.4 5.9	3.7 7.7	P.marmoratu shields P.marmoratu internal organs	Second	
45.5 27.5	9.5 11.6	8.7 13.8	7 35.7	P.marmoratu shields P.marmoratu internal organs	Third	

The study also showed that there is a difference in the concentrations of elements according to the seasons and locations. The highest concentrations of elements were in the spring and summer seasons, and the reason may be due to the high temperatures that cause evaporation and an increase in the solubility of gases and elements in water [8-10]. The results also recorded an increase in the concentrations of elements on the beach of Tumaitha city, which may be due to the increase in the liquid waste (wastewater, and in the paints used by fishing vessels. A high level of Cd was found, and the reason may be due to the presence of agricultural fields near the coast that may use chemical pesticides and fertilizers that contain Cd, which are transported with the air to the sea and then moved with the sea currents48 and the winter season at the end of the breeding season, because they need the basic elements during metabolic processes after the reproductive period. Many studies have taken place in different areas of Libya during the last years to estimate and determine different types of pollutants as heavy metals, petroleum compounds, radioactive materials, and others. These studies included different samples as drinking water, marine water, sediment, algae, soil, air, plants, vegetables, and other samples. These studies attributed the contamination were mainly due to human activities [41-70].

Conclusion

The study showed high accumulation of the two elements in marine organisms compared to their concentrations in water. It was also noted that there were changes in the concentrations of the two elements from one season to another. It was found that the highest concentration of lead was in the spring season, the highest concentration of cadmium was in the autumn season, and the highest concentration of lead was in the summer.

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Conflict of interest. Nil

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