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ORIGINAL RESEARCH ARTICLE

Assay and Comparison of Heavy Metals: Pb, Cu, Cd in Liver and Muscle of Tiger Cuttlefish-Sepia pharaonis- in Bahrakan Bay-Persian Gulf

A. Didaran[1]; G. Vosoughi[2]; A. Mashinchian Moradi[3]

[1] Science and research branch, Islamic Azad University, Tehran, Iran
[2] Professor of Marine Biology Group, Science and research branch, Islamic Azad University, Tehran, Iran
[3] Professor of Marine Chemistry Group, Science and research branch, Islamic Azad University, Tehran, Iran

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ABSTRACT

Tiger Cuttlefish-*Sepia pharaonis* – Herenberg 1831-is a is a seafood species that belongs to five fishing stations in the Bahrakan Bay (the waters of the Persian Gulf). 3specimens of Cuttlefish-*Sepia pharaonis* –, regardless of being male or female specimens, have been caught. Since muscle is the edible part of Tiger Cuttlefish -*Sepia pharaonis*- and the liver has the highest concentration of heavy metals, the study was conducted in the context of these two tissues to define essential heavy metal (Cu) and nonessential ones (Pb, Cd). The essential heavy metal (Cu) and nonessential (Pb, Cd) in the liver and muscle of Cuttlefish -*Sepia pharaonis*-, define whether dietary consumption of Cuttlefish -*Sepia pharaonis*-, are a danger to humans?

The amount of these heavy metals by using atomic absorption unit and spectrometer (AAS) was determined. The Range of concentrations of the heavy metals on the basis of dry weight, respectively is: The Concentrations of Cd, Cu, Pb in the samples of Cuttlefish -*Sepia pharaonis*- 0.54 to 0.25,3.98 to 2.12and from 0.09-0.03 (mg / kg) were measured. The level of contamination by heavy metals in Cuttlefish -*Sepia pharaonis*- of the Bahrakan Bay-Persian Gulf Shows that the amount of heavy metals in the species of Cuttlefish -*Sepia pharaonis*- that is studied on, according to the standard of US FDA, is within the limits permitted for human consumption and The results reveal that the concentration of the metals can be compared in this way: Cu>Pb> Cd. As a result of this study, acorrelation between climatic parameters (5 different locations of Cuttlefish -*Sepia pharaonis*-live in the Bahrakan Bay) and accumulation of heavy metals were found in the Cuttlefish -Sepia pharaonis-.

To select the location of Cuttlefish-*Sepia pharaonis* – fishing as you go from the province of Khuzestan to Bushehr, the contamination by heavy metals: lead, copper, cadmium, in this fish will behigher.

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*Corresponding Author:
Email: *aida.didar@gmail.com*

INTRODUCTION

Heavy metals have long biological half-life and therefore are considered a serious threat to aquatic. Various aquatic organism's bodies are different in their ability to regulate different metals. Most of them are able to operate only within certain range and not excrete metals remainsIn the body and the amount of these metals will Continuously increase during the period of life

that is named bioaccumulation. Risks resulting from bioaccumulation of these metals in higher levels of food chain are one of the major concerns in the issue of human health.

Among the three metals, lead, cadmium and copper, cadmium is the second toxic pollutants in the aquatic ecosystems.

Therefore, the study of cadmium accumulation in organisms and in environment is quite remarkable. The use of cadmium increased in

the twentieth century and has reached its peak in the past two decades. About 60% of the element entered into the ocean through human activity.

The metal due to high toxicity to organisms and widely distributed in the aquatic environment is considered as an important pollutant. Although researchers have not considered a biological role for heavy metals such as lead and cadmium, in fact, these metals don't have known metabolic functions. However, some metals such as copper and zinc - essential elements - have important biological functions. During recent decades, extensive research on the accumulation of heavy metals such as cadmium, lead, mercury, copper, and especially in aquatic Cephalopoda (cephalopods), is conducted. During recent decades, extensive researches on the accumulation of heavy metals such as cadmium, lead, mercury, copper in aquatic especially Cephalopoda(cephalopods) have been done. Among marine organisms, Cephalopoda is the best choice to study the bioaccumulation of cadmium, because Cephalopoda is able to excret cadmium and prevent further risk of cadmium accumulation. (Craig, 2008; Miramand, 2012; Bustament, 2008).

The Expansion of the petrochemical industry in the provinces of Khuzestan and Bushehr, and also oil docks at the south coasts of Iran over the past 40 years have created a lot of industrial pollution, particularly during the last decade. (Paphan Shushtari, 2008).

One of the most important spaces that Cuttlefish-*Sepia pharaonis* – occupiesin the ecosystem of Iran is Bahrakan Bay, that is located in the north west of Persian Gulf (Pophan Shooshtari, 2008).

Until now no one has done similar research like the research of this paper's author except Esmaili Sari in Iranin 2001 that measured the heavy metals copper, lead and zinc in the liver and muscles of the Cuttlefish-*Sepia pharaonis* – paid. Therefore, there were not enough Persian sources for the present research.

Esmaili Sari has compared the concentrations of copper(Cu), cadmium(Cd) and zinc(Zn)in the tissues of muscles and hepatopancreas of Cuttlefish-*Sepia pharaonis* – and Indian Squid -*Loligo dauvauceli*- in the North Persian Gulf in 2008.

MATERIALS AND METHODS

The Sampling in the five stations that had specified latitude and longitude in the Bahrakan Bay of the Persian Gulf was identified as follows in February 2013:

30 ° 5'29 .75 "N 49 ° 48'12 .72 " E Hendijan 30 ° 7'33 .83 "N 49 ° 53'45 .54" E 30 ° 9'30 .99 "N 49 ° 58'2 .61" E Port Boutaheri 30 ° 8'40 .55 "N 50 ° 3'6 .78" E 30 ° 2'29 .04 "N 50 ° 5'42 .20" E Deylam Port

Three examples of large-sized -*Sepia pharaonis* – were purchased from fishermen of each fishing station, and at each station, samples were put into the freezer. Also, the used freezers were encoded. The Samples were transferred to the laboratory and were kept in the freezer at a temperature of- 19° C. Each of the15 samples were weighed by sensitive digital scale and the weights between 3.82 kg to 4.13 kg were recorded.

In continuation of biometry, arm's length from the arm's top to the bottom of the mantle, minimum 38 to maximum 43 cm were recorded.

Fig. 1. The location of Bahrakan Bay in the north-west of Persian Gulf

In order to describe the sample, the Cuttlefish-*Sepia pharaonis* – was cut from the mouth and liver of each sample was isolated. The samples in plastic containers at a temperature of - 19 ° C were maintained and coded. The muscles of the back of each of the samples that is the best muscle as it is reported in Cuttlefish-*Sepia pharaonis* – to measure heavy metals were isolated in plastic containers at temperatures- 19 ° C were maintained and coded. After drying, the samples were removed from the oven and the samples were powdered by Chinese mortar. To prevent moisture, enter into the dried samples, the cans were put into the desiccator.

The preparation of standard stock solution

Preparation of standard stock solution for the metals cadmium, lead and copper, according to instructions of manufacturers that make atomic absorption were carried out (Genesis Model Made in Germany). Powder samples were removed from the desiccator and the amount of material required for the preparation of standard stock solution of proper salt as sodium chloride (Merck - Germany) was weighed by digital scale with an accuracy of 0.0001g. In this study distilled water was used with a conductivity of 1.2 micro. mouse/cm in the clean balloon that has a volume of 1000 ml. It should be noted that for every 1 g of dried sample 1 ml of nitric acid 0.65 is added to digest the heavy metals lead (Pb), copper(Cu), cadmium (Cd) and the 10, 50, 100 ml of solution will be prepared.

To unify heavy metals, lead, copper and cadmium in nitric acid, the solution was placed in the microwave. Calculating the amount of radiation absorbed by the detector device of AAS by calibration curves, unknown element concentration in solution can be calculated.

Data Analysis

For statistical calculations and plotting their corresponding graphs, to check that the average of samples taken from a random variable extent is close to the actual amount of the heavy metals lead (Pb), copper (Cu) and cadmium (Cd) in the liver and muscles of the Cuttlefish-*Sepia pharaonis* –, T-test analysis method using Excel software is used. This is used as in the case of normal data, the ANOVA test show that the heavy metals lead (Pb), copper (Cu) and cadmium (Cd) in the liver and muscles of the Cuttlefish-*Sepia pharaonis* – have significant difference or not? ($P<0.0.5$)

Results

The population of the study is the amount of the heavy metals lead (Pb), copper (Cu) and cadmium (Cd) in the liver and muscles of the Cuttlefish-*Sepia pharaonis* – and fishing

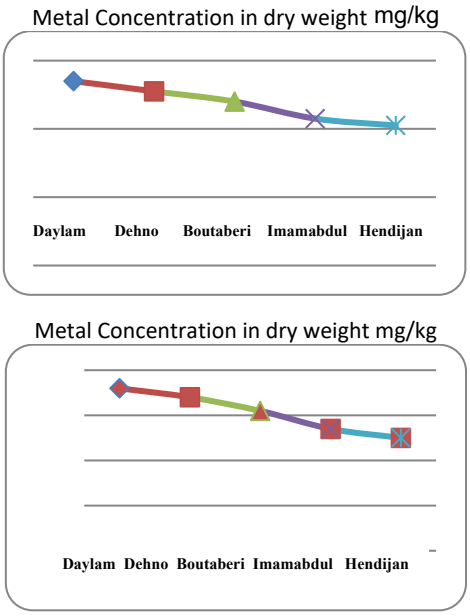

Fig.2. Comparison of lead concentration in the muscle of Cuttlefish-Sepia pharaonis –at five stations of the East (Deylam Port) to the West (Hendijan)

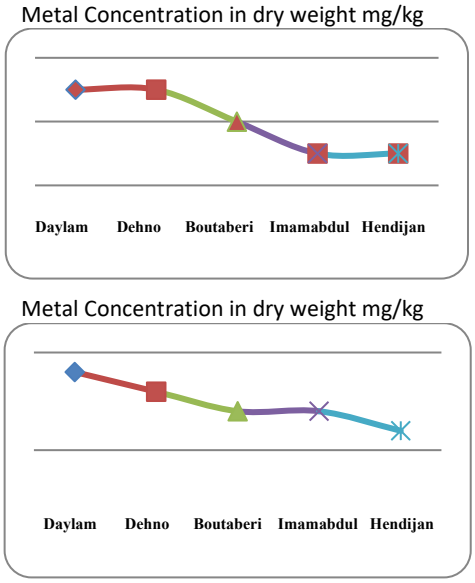

Fig.3. The Comparison of Cadmium concentration in the muscle of Cuttlefish-Sepia pharaonis –at five stations of the East (Deylam Port) to the West (Hendijan) of Baharkan Bay-Persian Gulf (top). The comparison of the amount of Cadmium in the liver of Cuttlefish-Sepia pharaonis –at five stations of the East (Port Deylam) to the West (Hendijan) of Baharkan Bay-Persian Gulf(bottom).

locations are in five different stations from the West to the East of the Bahrakan Bay. In the case of normal data, a significant difference between the first and last stations, is possible through the statistical tests such as ANOVA.

Correlation tests, parametric tests (Anova) and nonparametric (Mann-Whitney) based on comparing the mean and median are performed by software SPSS and Excel.

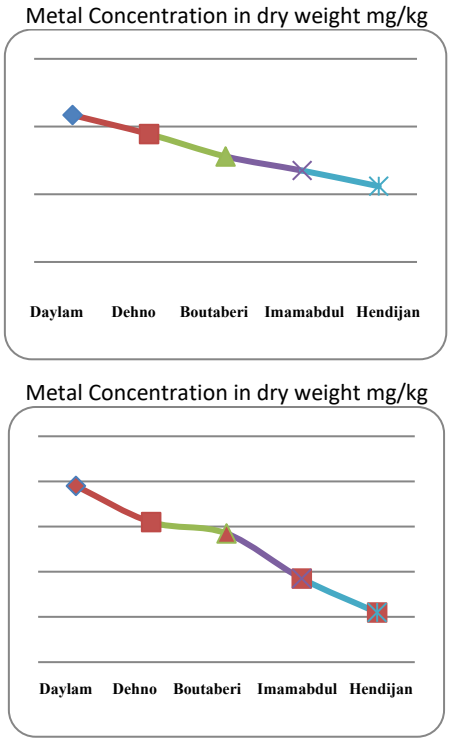

Fig.4. The Comparison of copper concentration in the muscle of Cuttlefish-*Sepia pharaonis* –at five stations of the East (Deylam Port) to the West (Hendijan) of Baharkan Bay-Persian Gulf (top). The comparison of the amount of copper in the liver of Cuttlefish-*Sepia pharaonis* –at five stations of the East (Deylam Port) to the West (Hendijan) of Baharkan Bay-Persian Gulf (bottom).

In the Baharkan Bay - the Persian Gulf (Right) --- The comparison of the amount of Lead in Cuttlefish-Sepia pharaonis –`s liver in five stations of the East (Daylam port) to the West (Hendijan) of the Bahrakan Bay – Persian Gulf. (Left)

Data Interpretation

Using measurement results obtained from Pb, Cu, Cd, by AAS (Atomic Absorption Spectrometry) in the liver and muscles of the 15 Cuttlefish - Sepia pharaonis - listed on the device AAS (Atomic Absorption Spectrometry) that is registered in the following table. The data indicates that in fishing from the Hendijan to the Deylam port, pollution of the heavy metals in the Cuttlefish`s liver and muscles will be higher.

Statistical Data Analysis

Tiger Cuttlefish - Sepia pharaonis, Hernborg 1831, is an edible marine species and the testing sample, is owned by 5 Fishing Station of Bahrakan Bay, the waters of the Persian Gulf. Essential heavy metals (Cu) and nonessential ones (Pb, Cd), in the liver and muscle of the Cuttlefish was determined by atomic absorption spectrophotometer (AAS).

The Range of Pb concentration in the samples of Cuttlefish - Sepia pharaonis - 0.54 (mg / kg) -0.25, was based on dry weight. Cd concentration in the samples of Cuttlefish - Sepia pharaonis - was measured according to dry weight 0.09-0.03 (mg / kg). Cu concentration in the samples of Cuttlefish - Sepia pharaonis - was measured according to dry weight 2.12-3.98 (mg / kg).

Through one-way ANOVA test, it can be concluded that there are significant differences between the concentration of heavy metal, non-lead (Pb) in liver and muscles of the Cuttlefish - Sepia pharaonis - caught from Hendijan (Khuzestan province), and the concentration of this metal in the liver and

muscles of the Cuttlefish - Sepia pharaonis - from Deylam Port (Boshehr province).

Through One-way ANOVA test, it can be concluded that there are significant differences between the concentration of heavy metal, non-lead cadmium cd in liver and muscles of the Cuttlefish - Sepia pharaonis - caught from Hendijan (Khuzestan province), and the concentration of this metal in the liver and muscles of the Cuttlefish - Sepia pharaonis - from Deylam Port (Boshehr province).

Through One-way ANOVA test, it can be concluded that there are significant differences between the concentration of heavy metal, non-lead copper cu in liver and muscles of the Cuttlefish - Sepia pharaonis - caught from Hendijan (Khuzestan province), and the concentration of this metal in the liver and muscles of the Cuttlefish - Sepia pharaonis - from Deylam Port (Boshehr province).

DISCUSSION AND CONCLUSIONS

The author of this study has recorded the highest concentration of Cd in the Cuttlefish - Sepia pharaonis - muscle (mg/kg0.04) that shows the lowest value in comparison with other marine shows (Lorenko and others, 2009).

The author of this study indicate that copper in edible parts (muscle) of the Cuttlefish - Sepia pharaonis - is like this: between the two blood-filled organs, liver and muscle, muscles had the lowest rate of copper. The author reaches the conclusion that copper in Cuttlefish - Sepia pharaonis - is as follows: (percentage as a percentage of total tissue):

20% in muscle, and 35%.in liver. Hence, the author's opinion, it seems that the tissues of Cuttlefish - Sepia pharaonis - muscle is not the target organ for Cu. According to this research, studying on the Cuttlefish - Sepia pharaonis - in the Bahrakan Bay- Persian Gulf indicated that copper is the essential element (average concentration mg/kg2.4) in the muscle tissue of the Cuttlefish - Sepia pharaonis.

SUGGESTION

To complete this study and the research of Ismaili Surrey, the only registered research in the field of heavy metal concentration in the liver and muscle tissues of the Cuttlefish - *Sepia pharaonis* -, it is suggested that in the coming years, the concentration of other heavy metals such as mercury, selenium, iron, silver, cobalt, vanadium, chromium,and nickel in liver and muscle tissues be measure. Consequently, other heavy metals in the tissues of the body of Cuttlefish - *Sepia pharaonis* - will be measured.

The Cuttlefish - *Sepia pharaonis* - fishing season is from late winter to early spring and the amount of waste water discharges into the Persian Gulf in winter may be more than the amount in summer. Consequently, it is recommended that researchers review polluting metals in the tissues of the liver and muscle of Cuttlefish - *Sepia pharaonis* - at the Seasons that Cuttlefish - *Sepia pharaonis* - fishing is less.

To complete this study and find that the concentration of these metals in the body of the Cuttlefish - *Sepia pharaonis* - has changed or not in different seasons of the year. Finally, it will be shown that the concentration of heavy metals in different seasons of the year in the body of Cuttlefish - *Sepia pharaonis* - is changed or remained constant.

It is recommended that in addition of Cuttlefish - *Sepia pharaonis* - liver and muscle, the concentrations of heavy metals, lead, copper, cadmium, in other tissues including kidney, gills, heart, central nervous system, blood, ear, gastrointestinal tract be measured. Consequently, these three heavy metals in the all tissues of Cuttlefish - *Sepia pharaonis* - of Persian Gulf, is investigated.

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