World Journal of Engineering Research and Technology
WJERT
www.wjert.org
SJIF Impact Factor: 4.326

SEARCH FOR HEAVY TRACE METALS IN SPECIES SARDINA PILCHARDUS AT THE BAY OF ALGIERS

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Article Received on 24/02/2017 **Article Revised on 17/03/2017** **Article Accepted on 06/04/2017**

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ABSTRACT

Our study involves the determination of heavy metals in a fishery product from the Bay of Algiers. This study involved a representative sampling of Sardina pilchardus of homogeneous size. The study was conducted over a three-month period during which we sampled a group of fish randomly from several crates by selecting ten homogeneous size individuals in each batch up to three Lots by sampling. The latter were carried out at a fortnight interval. We then prepared the samples and assayed the following trace metals: Cd, Pb, Ni, Cu and Zn. The analysis is carried out on three distinct matrices: viscera, flesh and head, skeleton. Our results show the presence of trace metals with the exception of cadmium; Their distribution is much greater in the viscera. The head and the skeleton are the second most contaminated matrix followed by the flesh. The concentration of lead is much higher than that set by the WHO,[21] suggesting that there is potential lead pollution in the Algiers Bay. The mean concentration of Zn exceeds its Acceptable Daily Dose set at 7-15 mg / day by the WHO. The average concentration of Ni (0.22 mg / kg ± 0.07) and Cu (0.14 mg / kg ± 0.08) did not exceed the upper limit set by the AFSSA in 2001.[1] This study should extend to fish species other than sediments and waters.

KEYWORDS: Determination, Heavy metals, Sardine, contamination, Bay of Algiers.

INTRODUCTION

Our work aims at the determination of the heavy metals present possibly at the scale of traces in the species Sardina pilchardus of the Bay of Algiers, which allows us to evaluate the level of marine pollution of the studied region, Than the likely risk incurred by the Algerian Consumer. The objective of this study was to determine the following heavy metals: Lead (Pb), Cadmium (Cd), Copper (Cu), Zinc (Zn) and Nickel (Ni).

MATERIEL AND METHODES

Location of experimentation and sampling conditions

This study involved a representative sampling (n=180) of the species Sardina pilchardus (Figure N°1) of homogeneous size sins at the Bay of Algiers. Fish species were measured by standard methods used in ichthyology (length, weight and age determination were measured by scales). The study was carried out in three months, during which we sampled a group of fish by randomly selecting a group of fish by selecting ten homogeneous size individuals in each batch (Figure 2) within the limit of three lots per sampling.

The latter were carried out at an interval of fifteen days (15 days). The fish were immediately put under ice in a hermetically sealed refrigerated enclosure and transported to the laboratory.

Methodes

In this study, particular attention has been paid to samples and methods of metals. In this study we have investigated a few heavy metals, namely lead (Pb), cadmium (Cd), copper Cu), Zinc (Zn) and Nickel (Ni). The assay is performed by Atomic Absorption Spectrometry (SAA) in three replicates for each compartment (Filet / Viscere / Head and skeleton). The protocol followed is that adopted by I.A.E.A.[3]

Selection of samples

The first step consists in the selection of five individuals of homogeneous size classes in order to neutralize the age factor.[19],[20] Each lot is weighed at the tenth meadows (1/10). The second step is to open the abdominal cavities of each individual, remove the viscera, the pulpit, and then detach the skeleton and head. The sampling of the sample to be assayed is always done on the same side and corresponds to the position: head to left, tail to right, belly to the bottom and back to the top.[19] The weight of the upper pulpit, the viscera and the head and the skeleton are also weighed thereafter to the nearest one-tenth.

Conservation

Preservation after treatment is carried out in a freezer at a temperature of -24 ° C, using petri dishes, freezing sachets and pill bottles, depending on the size of each batches, which are labeled with the date of collection, the nature (Pulpit, viscera, head and skeleton) and lot number Freezing makes it possible to preserve the chemical properties of the samples and to avoid contamination with the ambient environment and loss of volatile elements such as mercury.[10]

Freeze drying

The freeze-drying is carried out for 48 hours under vacuum at (10^{11} mbar) and at very low temperature (-50 °C to -60 °C). This allows a transition from the solid phase to the vapor phase without passing through the liquid phase. This means of dehydration is preferable to that of evaporation in the oven because in the latter case the rise in temperature can be a source of losses of the most volatile metals.[10],[9]

Grinding

The lyophilized samples are ground using an automatic stainless steel grinder and porcelain mortar until a fine powder is obtained. The latter is recovered in small plastic pots and stored in a dry place (oven at 27 ° C.). The weight of each sample is weighed using a precision balance.

Homogenization

This fact for a uniform distribution of the metallic elements in the dry matter and thus to take out a representative quantity of the sample, the homogenization took place before mineralization by electric stirring for a few minutes.

Extraction of heavy metals

Clear mineralization

Prior to using the buckets, a blank mineralization is carried out. 4 ml of 69% nitric acid are placed in each Teflon cup. The cups are sealed. It is left to digest overnight. It is then heated on a hot plate at 120 °C. for 2 h 30 min. The samples are allowed to cool before opening the cups gently, then rinsed with ultra-pure water and dried in the oven at 70 °C.

Mineralization of the biological sample

According to the protocol described by A.I.E.A.,[1] the mineralization takes place under a suction hood in a Teflon cup. About 0.5 g of dry matter of samples is weighed. 5 ml of nitric acid (HNO_3) concentrated to 69% are added and then the cups are sealed. The samples are left at room temperature overnight and then placed on a hot plate at 120 °C. for 2 h 30 min. The samples are allowed to cool before the buckets are opened gently, and the mineralized material is then transferred to 50 ml polypropylene tubes. The wells are rinsed with ultra-pure water at least three times by collecting the rinses in the same polypropylene tubes. Adjust to the 50 ml mark.

The White

A blank is inserted with each set of biological samples. 5 ml of concentrated nitric acid (HNO_3) are placed at 69%. It will undergo the same treatment undergone by the biological samples. The series are stored in a refrigerator at 4°C until assayed to avoid loss and contamination.[1]

Analysis of heavy metals

The assay method used is flame atomic absorption spectrophotometry of the research center in physicochemical analysis (CRAPC).

Detection limit (LD)

The detection limit is defined by the smallest measurable concentration value giving an absorbance equal to twice the variation of the background noise of the analytical solution.[5],[6],[7],[8],[9],[18] In our case the experimental limits are cited in the annexes (Table 1).

Table 1 : Experimental conditions.

Metal	Wavelength	Detection limits (ppm)
Plomb	217.0	0 à 30
Cadmium	228.8	0 à 03
Nickel	232.0	0 à 20
Zinc	213.9	0 à 1.5
Cuivre	324.8	0 à 10

Calibration

The calibration method used is that of the standards alone. Calibration of the spectrophotometer is carried out using five standard solutions (S_1, S_2, S_3, S_4 and S_5) of increasing concentrations. For each metal to be analyzed, the standard samples were prepared

from the stock solutions. The absorbances of these five solutions are measured, which makes it possible to plot the calibration curve (Absorbance = f (concentrations) For low concentrations, that is to say for low absorbances, the curve is quasi-linear (Figure 2, 3, 4, 5), hence of the form: Y = a X + b Where: Y: Optical density (OD), X: Concentration of the metal to be analyzed, a: Slope of the curve, b: Ordinal at the origin. The unknown metal concentrations of the samples to be analyzed can be determined using the calibration line.

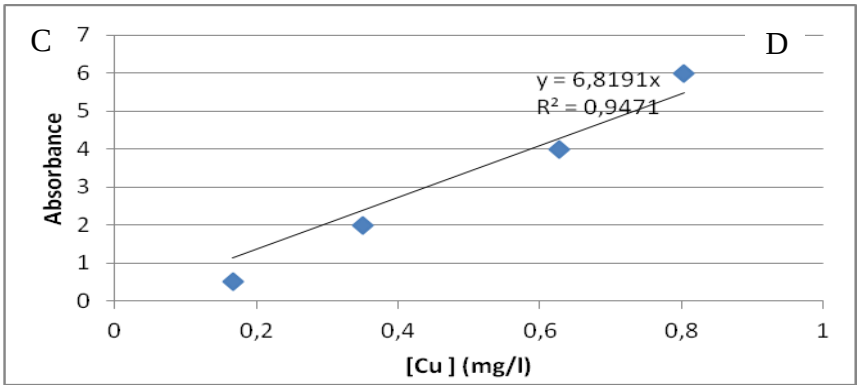

Figure N°2: Copper Calibration Copper A = f (Cu).

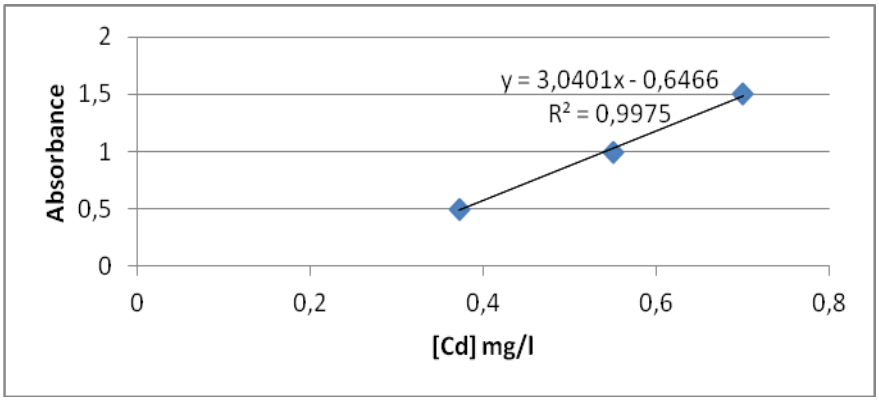

Figure N°3 : ***Cadmium calibration curve A = f (Cd).***

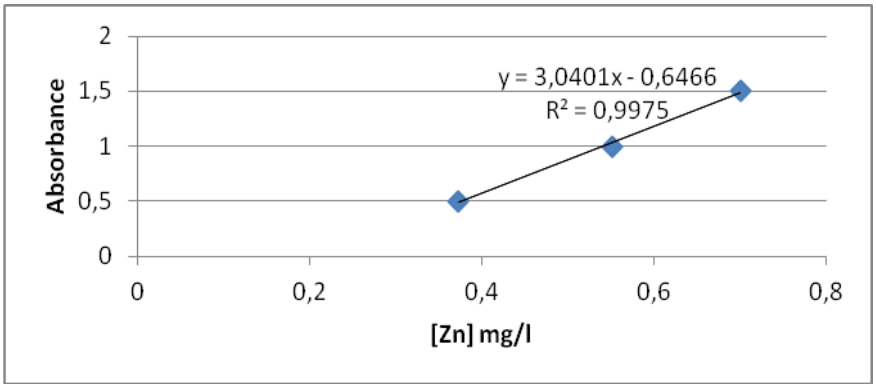

Figure N°4 : ***courbe d'étalonnage du Zinc A=f(Zn).***

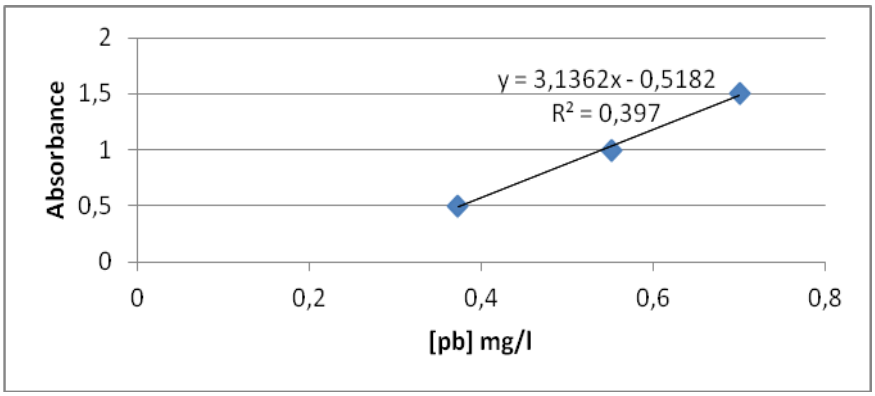

Figure N°5 : ***courbe d'étalonnage du lead A=f(pb).***

Calculation of ETM concentration

The calculation is done by the apparatus, which gives us the rate of the metal to be assayed in our samples. If the concentration is too high, which means that it exceeds the detection limits for each metal, dilution is necessary. The concentration obtained will then be multiplied by the dilution factor.[1]

Calculation of the corrected weight of the biological sample

Our samples after their lyophilization trap a certain number of particles of water due to the period that separates the freeze-drying and the analysis. Consequently, the weighed weights are not the real dry weights, for this we go through the calculation of moisture content in order to be able to correct these weights and have the real dry weights.[1]

Data processing

The variability of the metal contents is studied by an analysis of variance (ANOVA test). The multiple comparison of the averages of each metallic element and their classification is carried out by the Friedman test, whenever the analysis of variance reveals significant differences.

RESULT

Table 2: Results of average heavy metal concentrations (ppm).

Matrix	Concentration	Cd	Pb	Ni	Cu	Zn
Flesh	Average ± ET	00	0.99±0.11	0.22±0.07	0.14±0.08	27.03±23.19
Viscera	Average ± ET	00	3.11±1.68	0.92±0.92	0.52±0.52	91.88±60.89
Head and skeleton	Average ± ET	00	0.30±0.27	0.25±0.25	0.130±0.12	42.70±29.54

N°: sampling number, ET: Deviation Type

Our results are presented as concentrations of the five trace metals sought at the three matrices. The results are summarized in Table II. A comparison with other values reported in the literature is carried out when the matrix corresponds. The results of the five trace metals in three Sardina pilchardus matrices are represented by averages (with standard deviation).

- The results show a complete absence of cadmium in the three matrices analyzed (flesh, viscera, head / skeleton). They show that the average concentration of lead in the viscera (3.11 ± 1.68) is clearly higher than that found in the flesh

(0.99 ± 0.112). The concentration at the skeleton head (0.30 ± 0.28) is the lowest. The results also show an average concentration of nickel, higher in the viscera (0.92 ± 0.92), followed by that of the head / skeleton (0.25 ± 0.25). In the last place, we find the average concentration of flesh (0.22 ± 0.08). They show that the average concentration of copper is higher in the viscera (0.52 ± 0.52), than in the flesh (0.14 ± 0.09) and head + skeleton (0.13 ± 0.13). The mean concentration of Zink is much higher than the level of the viscera with a rate of 91.88 ± 60.90, followed by the head / skeleton (42.70 ± 29.54) and flesh (27.03 ± 23.198).

INTERPRETATION AND DISCUSSION

It appears that the concentrations of Pb, Ni, Cu, and Zn are higher in the viscera (which contain the liver, gonads and stomach contents). These results are in agreement with the work of EL MORHIT et al.[12] on Sardina pilchardus and on cod.[14] Then comes the flesh for the Pb and Cu, and finally the head / skeleton (which contain the gills) for Ni and Zn. For the head / skeleton, the most important concentration is Zn (42.70 ± 29.54), followed by Pb, Ni and Cu. Our results are consistent with the literature[12] on gill concentration (66 ± 22.51).

WU et al.[22] suggest that metals are first accumulated in temporary target organs such as gills and then transferred to the digestive organs such as the liver, kidneys and intestines. For flesh, the concentration of Zn is the most important (27.03 ± 23.19), which is in agreement with the results found in the scientific literature[12] and does not exceed the threshold set by WHO (1000 µg / g Of fresh weight)[21] and that described by BENEY et al.,[4] followed by Pb (0.99 ± 0.11), which far exceeds the threshold set by Algerian regulations (0.3 mg / kg fresh weight) (Table III).[15] Ni is present at an average concentration of 0.22 ± 0.07, lower than that observed by MERBOUH (1.37 ± 0.67) in Sardina pilchardus in the Golf of Oran.[16] Cu is present at a lower concentration than the other metals (0.14 ± 0.08), the observed concentration (0.31 ± 0.07) is lower than that of EL MORHIT et al.[12]

For cadmium, the results obtained could be explained either by the absence of pollution of the species studied by this metal in the fishing zones, or by the difficulty of metering the metal because of its infinitesimal presence in water (Its concentration is much greater in the sediment than the water column ,[9] it is stored in the sediments in the form of a Cd-sediment complex.[9],[13] Cd in Sardina pilchardus, in flesh (0.03 ± 0.02), gills (0.08 ± 0.01) and liver (0.05 ± 0.02).[12] However, our results do not exceed the limits established by the Algerian regulations / Kg fresh weight) (table III) and those of the WHO (2.0 µg / g fresh weight), as described by BENEY et al.

Mean lead concentrations in flesh are above the limit set by Algerian regulations (0.3mg / kg fresh weight), and this is also true for viscera, which could suggest that the lead level is high in the Bay of Algiers. On the other hand the head and the skeleton have a dose of 0.3 ± 0.27 which is equal to the regulatory threshold (Table III).

Table 03 : The limit values for lead, cadmium and mercury in Algeria.[1]

Products	Maximum levels (mg / kg fresh weight)
1 - Lead (Pb)	
1.1 - Muscle meat of fish (a) (b).	0.3
2 - Cadmium (Cd):	
2.1 - Muscle flesh of fish (a) (b).	0.05
2.2 - Muscle flesh of the following fish (a) (b):	
- Sardine (Sardina pilchardus)	0.10
3 - Mercury:	
3.1 - Muscle flesh of fish (a) (b).	0.5

(A) Muscle flesh of live fish, fresh fish, frozen and fish fillets and other fish flesh (whether or not minced), fresh or frozen.

(B) When the fish is consumed whole, the maximum level applies to whole fish.

[1]Inter-ministerial decree of 30 Moharram 1432 corresponding to 5 January 2011 fixing the thresholds for the presence of chemical, microbiological and toxicological contaminants in fishery and aquaculture products.

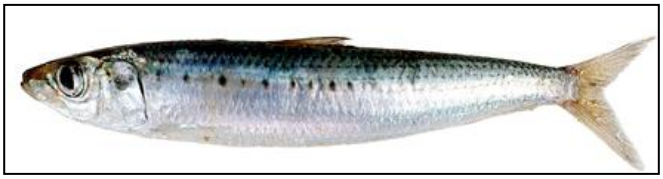

Photo taken in the laboratory of the species studied:

Figure N°1 : *Sardina pilchardus.*

The current position of Sardina pilchardus in the phylogenetic classification of osteichthyes is:

- Phylum: Vertebrates
- Sub-branch: Gnathostomes
- Superclass: Fish
- Class: Osteichthyes
- Subclass: Actinopterygii
- Superorder: Teleosteens
- Order: Clupéiformes
- Order: Clupéoidés
- Family: Clupeidae
- Genre: Sardina
- Species: S.pilchardus

In Algiers Bay, the origin of the micropollutants present is due, on the one hand, to the multiple industrial and urban discharges into the basin and to maritime traffic (presence of the port). Without forgetting the contribution by the repression of sediment, currents and swells.[11] They seem to us to be the reason why the highest concentrations are detected after the viscera.

CONCLUSION

In the light of these results, the establishment of a regular, if not systematic, systematic monitoring system for the chemicals in the fisheries becomes necessary for this sector. It is of great interest that this study be extended to different periods of the year and to several bio-accumulating marine species of pollution as well as to the sediments and waters of the same sites.

ACKNOWLEDGMENTS

Our sincere thanks go to the veterinary inspectors of the fishery of Algiers for their accompaniment in the sampling operations as well as Personnel of the laboratory of the Higher National School of the Sciences of the Sea and the Development of the Littoral of Algiers. Not to forget Mrs HEMIDOUCHE Sabra, researcher at the Center for Research in Physico-Chemical Analysis (CRAPC).

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