HEAVY METALS ASSESSMENT IN THREE FISH SPECIES: BOOPS BOOPS, SARDINA PILCHARDUS AND TRACHURUS TRACHURUS FROM NORTH-EAST OF MOROCCO.

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Abstract

This study aims to provide information on some heavy metal concentrations in the muscles of three fish species: Boops boops, Sardina pilchardus, Trachurus trachurus, in North-East of Morocco Market and to evaluate the possible risk associated with their consumption. The concentration of Cd, As, Zn, Cr, Cu, Ni, Pb, Mn and Fe were evaluated in fish tissues by means of the total reflection X-Ray Fluorescence technique (TXRF) [Wavelength Dispersive X-ray Fluorescence (WD-XRF)]. The concentration of heavy metal measured in the fish species were expressed as µg/g dry weight. In the present study, the heavy metals concentration in the muscles of fish species were less than the permissible levels specified for human consumption by the EU, FAO/WHO guidelines. For As it was at the specified limit set by USEPA2000. However, Ni and Mn had the highest level but there is no official limit for these two metals.

Introduction:

Morocco, with more than 3500 km of coastline, finds in the marine environment one of the main basis of its economy, with fishing and tourism being the most prominent activities undertaken. For this reason, an effort is being made to establish and preserve the environmental quality of littoral zones, including the levels of different pollutants in Moroccan coast (Banaoui et al., 2004; Blinda et al., 2005; González et al., 2007).

Several pollutants are discharged daily and end up rapidly in the marine environment, which represent the ultimate repository of all anthropogenic emissions. Among these pollutants, the heavy metals are considered the most harmful for the aquatic environment due to their capacity to accumulate in marine organisms (Harte et al., 1991; Schüürmann and Markert 1998)The accumulation of heavy metals in these living matters may subsequently affect the quality of the environment and deteriorate the ecological imbalance.

Fishes are widely used as sentinel species of contamination in the aquatic environment. They are in top of the aquatic food web (Yilmaz et al., 2007; Zhao et al., 2012) and represent an important part of the human diet, consuming fish provides an important source of protein, polyunsaturated fatty acids (PUFA), liposoluble vitamins and essential minerals, which are associated with health benefits and normal growth (Daviglus et al., 2002; Verbeke...
et al., 2007). According to FAO statistics, fish accounted for about 16% of the global population’s intake of animal protein and 6% of all protein consumed (FAO, 2010b). Thus, it is not surprising that many studies have been conducted to establish the risk of contamination by metals in different species of edible fish (Kucuksezgin et al., 2001; Lewis et al., 2002; Prudente et al., 1997).

In the present work, levels of several metals (Cd, As, Zn, Cr, Cu, Ni, Pb, Mn and Fe) in the muscle tissue of some commercial fish from the Moroccan Mediterranean coast were determined. Three fish species Boops boops, Sardina pilchardus, Trachurus trachurus have been analyzed for heavy metals contents. These metals were selected based on their importance in the field of monitoring of the quality of marine environment. The aim of this work is to evaluate the current environmental status of the coast, and to compare the metals content in muscles against the recommended maximum permissible limit to assess the quality of fish and the health risk for human.

**Material and Methods:-**

**Sample collection:-**

Three fish species were collected four times during the year 2016 from local fishermen at disembarkation point in M’diq one of main port in North-East of morocco (Fig1). They were placed immediately in poly-ethylene bags, put into isolated container of polystyrene icebox and, then, brought to laboratory.

![Figure 1](image_url)
Measurements of the basic biological parameters:-
Fishes were first identified, and then the total lengths (cm) and the body-wet weights (g) of each fish specimen were measured. The detailed information is listed in Table 1.

Table 1: Total Length (cm) and Body-wet weight (g) of Fish Species used in this study with standard deviation (SD)

| Scientific name     | Local name | Common name | No. | TL (cm) mean± S.D. | BW (g) mean± S.D. |
|---------------------|------------|-------------|-----|-------------------|------------------|
| Boops boops         | Taghzalt   | Bogue       | 77  | 16,66±1,46        | 41,67±11,06      |
| Sardina pilchardus, | Sardine    | Sardine     | 84  | 16,37±2,16        | 36,13±16,93      |
| Trachurus trachurus | Chrel      | Chinchar    | 68  | 17,40±3,00        | 43,22±30,09      |

Analytical procedure:--
Fishes are dissected, the muscles tissues were removed and dried in an oven at 60°C during 24h. Then each dried fish tissue was grounded to obtain a very fine powder. Heavy metal analysis was carried out by wavelength dispersive x-ray Fluorescence (WD-XRF) at the technical unit support to scientific research (UATRS) of the National Centre of Scientific Researches and Techniques (CNRST) in Rabat, Morocco.

Data analysis:--
The data of this study were statically analyzed. A One-way analysis of variance (ANOVA) was used to determine the significant differences in metal levels among species. Statistical significance was defined as p≤0.05.

Results and Discussion:--
The port of M'diq is considered one of the important ports located in the Mediterranean cost and it is one of the main source of fishes in this area. The Figure 2 shows the importance annual production of the selected species in this harbor. Data provided by national fisheries office (ONP).

Figure 2: Annual production of *Boops boops*, *Sardina pilchardus* and *Trachurus trachurus* during 2002-2015 in kg/year.

The average mean of the heavy metals Cd, As, Zn, Cr, Cu, Ni, Pb, Mn and Fe in the muscles of three fish species are represented in Tables 2 given in µg g⁻¹ dry weight.
Table 2: Maximum metal concentrations for four collected sample (µg/g dry weight)

| Species              | Cd   | As  | Zn  | Cr  | Ni  | Pb  | Mn  | Cu  | Fe  |
|----------------------|------|-----|-----|-----|-----|-----|-----|-----|-----|
| *Trachurus trachurus*| BLD  | 1.31| 4.63| BLD | 3.48| BLD | 17.7| BLD | 49.1|
| *Boops boops*        | BLD  | BLD | 16.4| BLD | 2.62| BLD | 22.2| BLD | 67.1|
| *Sardina pilchardus* | BLD  | BLD | BLD | BLD | BLD | BLD | 52.1| BLD | BLD |

BLD: Below limit of detection which equal to 1 ppm
Mean metal concentrations of different species are not significantly different, p > 0.05

Table 3: Guidelines for maximum metal concentrations in fish tissues (ppm).

|        | Cd | As | Zn | Cr | Ni | Pb | Mn | Cu | Fe |
|--------|----|----|----|----|----|----|----|----|----|
| USEPA2000 | 4  | 1.2| -  | -  | -  | -  | -  | -  | -  |
| FAO/WHO 2004 | 1  | -  | 50 | -  | -  | 1  | -  | 10 | -  |
| Turkish Guideline | 0.1 | 1  | 50 | -  | -  | 0.1| 20 | 20 | 50 |
| FAO/WHO 1983 | 0.5| -  | -  | 0.15| 0.4| 0.5| 2.5| 30 | -  |

(-) no values were presented in the standards.

This study was held to investigate heavy metal concentrations in muscles of three commercially important fish species in North-East of Morocco because the concentration of heavy metals in commercial fish available in this region was rarely investigated. Although it is well known that fish muscle is not an active tissue in accumulating heavy metals (Bahnasawy et al., 2009) the present study concerned with the heavy metal concentrations in the fish muscles because it is the most consumed portion by the Moroccan people. Furthermore, it was documented that some fish in polluted regions may accumulate substantial amounts of metals in their tissues, which sometimes exceeded the maximum acceptable levels (Kalay et al., 1999).

There are many sources of water pollution and they are an ongoing problem in Morocco, which manifests particularly in irrigated perimeters and areas of economic activity. Among the causes of water pollution, are included industrial units, mainly concentrated in and around cities, and among these industries, some are recognized pollutants, table 1 sums up some examples of industrial and agricultural sources which may introduce metals in the environment (Othmer, 1995).

Table 4: Industrial and agricultural sources of metals in the environment. (Bouzid et al., 2011)

| Uses                        | Metals                      |
|-----------------------------|-----------------------------|
| Batteries and other electrical appliances | Cd, Hg, Pb, Zn, Mn, Ni |
| Pigments and paints          | Ti, Cd, Hg, Pb, Zn, Mn, Sn, Cr, Al, As, Cu, Fe |
| Alloys and solders           | Cd, As, Pb, Zn, Mn, Sn, Ni, Cu |
| Biocides (pesticides, herbicides, curators) | As, Hg, Pb, Cu, Sn, Zn, Mn |
| Catalyst agents              | Ni, Hg, Pb, Cu, Sn |
| Glass                       | As, Sn, Mn |
| Fertilizers                  | Cd, Hg, Pb, Al, As, Cr, Cu, Mn, Ni, Zn |
| Plastics                     | Cd, Sn, Pb |
| Dental and cosmetic products | Sn, Hg |
| Textiles                     | Cr, Fe, Al |
| Refineries                   | Ni, V, Pb, Fe, Mn, Zn |
| Fuels                        | Ni, Hg, Cu, Fe, Mn, Pb, Cd |

This investigation showed that the different fish species contained different mean concentrations of heavy metals in their muscles (Table 2).

The mean of Cd in the present study was below limit detection as well as Cr, Cu and Pb. This is in agreement with the Environmental Health Criteria of Cd WHO (WHO, 1992) and USEPA2000 (Us-Epa, 2000) which reported that the maximum permissible limit for Cd is 3.33 µg/g, 100µg/g for copper, Lead 1 µg/g according to FAO (FAO/WHO, 2004).
The Maximum permissible limit of Fe in fish muscle is 333.33 μg/g (Mokhtar et al., 2009), our results indicate much lower values for Fe 67.1 μg/g

The Maximum permissible limit of Zn in fish muscle set by FAO is 50 μg/g (FAO/WHO, 2004) our results found lower values for 16.4 μg/g.

According to USEPA2000 the Recommended mean of As is 1.2 μg/g, our result was slightly higher than the concentration allowed by USEP2000 and Turkish guideline

The higher mean was found for Magnesium and Nickel; in our study was 52.1μg/g in Sardina pilchardus and Ni 2.62 μg/g Boobps Boops after rainy season. There is no information about maximum permissible limits for Magnesium and Nickel in fish tissues except for Turkish Food Codex (2004) they set 20μg/g as maximum limit for magnesium in fish tissues and 0.4 by FAO (FAO/WHO, 1983)

Our main interest was investigate the heavy metals Cd, As, Zn, Cr, Cu, Ni, Pb, Mn and Fe but after rainy season samples showed other different metals trace as Ti, Zr, Bp, Y, Sr et Ac. These trace metal showed so low concentration, which are represented in table 5; this could be attributed to the heavy rainfall during these seasons, which increases the metal content of water, by washing down the wastes.

Table 5:- Mean concentration for Trace metal after rainy season (μg.g⁻¹ dry weight)

|            | Ti   | Zr   | Rb   | Y    | Sr   | Ac   |
|------------|------|------|------|------|------|------|
| Trachurus  | 3.87 | 3.3  | 1.93 | 7.74 | 4.71 | 4.56 |
| trachurus  |      |      |      |      |      |      |
| Boops      | 5.94 | 4.55 | 2.4  | 0.595| 2.5  | 0    |
| boops      |      |      |      |      |      |      |

Conclusion:-
The pollution of the marine environment has become a global problem and the need to investigate the safety of marine products become a necessity in any country.

Metal concentrations in the three studied fish species were within the same range or below the limits proposed for fish by various international standards and guidelines such as EU , FAO/WHO, and Turkish guidelines except for magnesium found in muscles of sardina pilchardus was high that limit set by Turkish Food Codex.

The examined fish were safe for human consumption at least with regard to residual levels of cadmium, copper, manganese, nickel, lead and zinc but a continuous monitoring of heavy metals in commercial fish in North-East of Morocco as well as other Moroccan regions is necessary to insure the prescribed worldwide limit.

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