Larhyss Journal, ISSN 1112-3680, n°15, Septembre 2013, pp. 21-34

METALLIC CONTAMINATION IN MUSCLE OF THREE FISH SPECIES IN THE SOUTHERN ATLANTIC COAST THE LAÂYOUNE (MOROCCO)

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ABSTRACT

The purpose of this study was to assess concentration of selected metals (Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn) in the muscle of three common southern Atlantic coast fish species (*Pagellus acarne*, *Sardina pilchardus* and *Diplodus vulgaris*). Furthermore, correlations among the selected metals and order of metal accumulation in the fish muscle were determined. Large scale fishing was used for collecting the fish from the southern Atlantic coast (Morocco) in January, March and June (2005). Concentrations of selected metals were measured using an atomic absorption spectrophotometer. Significant correlations ($P < 0.05$) between Cr–Cu, Fe–Zn and Mn–Pb were observed. In our study, the heavy metals content in fish muscle of the three species did not exceed the upper limit allowed by European communities. On average, the order of metal concentrations in the fish muscle was: Zn>Fe>Ni>Cu>Mn>Cd>Cr>Pb.

Keywords: Metals, Contamination, Fish, Atlantic Coast, Morocco.

INTRODUCTION

Fish are a major part of the human diet and it is therefore not surprising that numerous studies have been carried out on metal pollution in different species of edible fish (Prudente et al., 1997; Kucuksezgin et al., 2001; Lewis et al., 2002; El Morhit et al., 2009).

Aquatic environmental quality, and how humans may be affecting this, is an area which as received increasing attention in recent years. Marine organisms, among them fish, accumulate contaminants from the environment and therefore have been extensively used in marine pollution monitoring programs (e.g., ICES: OECD, 1991; UNEP, 1993). Two main objectives are being pursued in these programs, (i) to determine contaminant concentration in fish muscles in ordr to asses the health risk for humans, and (ii) to use fish as environmental indicators of aquatic ecosystems quality (Adams, 2002; El Morhit et al., 2013).

Fish muscle provides low metal content because of its low metabolic activity. However, it is very important to study the metal levels in this tissue in order to estimate metal quantities that enter to human by the consumption of fish (Philips, 1995; El Morhit et al., 2012).

Fe is essential for cellular respiration in animals and photosynthesis in plants, and is an integral cofactor of ribonucleotide reductase. However, in excess Fe is toxic, acting as a catalyst in the Fenton reaction generating free radicals (Crichton et al., 2002). Fe is a vital micronutrient for the teleost fish, being an integral component involved in cellular respiration and oxygen transfer (Bury et al., 2003).

Mn is an essential micronutrient that does not occur naturally in aquatic ecosystems (Dallas and Day, 1993). Although Mn is not a significant pollutant (Hellawel, 1986), it is one of the first metals that increase in concentration in acidified waters (Bendell-Young and Harvey, 1986).

Zn is an essential nutrient for aquatic organisms but in excess may become an environmental contaminant. The most important route of Zn absorption is the intestine (Glover and Hogstrand, 2003). Water hardness decreases accumulation of Zn in both gills and whole fish body and thus reduces the acute toxicity of Zn (Everall et al., 1989).

Cu is essential for fish metabolism (Canli et al., 2002). Cu in fish from unpolluted water is absorbed mainly from food (Ogino and Yang, 1980). The uptake of Cu through the gills is a minor part of the whole body Cu content. However, in Cu polluted water the gills show a 10-fold increase in Cu accumulation within a few hours of exposure, which occurs simultaneously with the appearance of Cu in the blood (Grosell et al., 1997).

Ni is a naturally occurring element in water (Snodgrass, 1980). Ni is soluble at a pH gradient lower than 6.5. A pH higher than 6.7 forms an insoluble Ni hydroxides (Dallas and Day, 1993). The toxicity of Ni to aquatic life is generally low and varies significantly with species, pH, and water hardness

(Birge and Black, 1980). Increased concentrations of Ni may cause sublethal effects (Khangarot and Ray, 1990).

Pb has no known role in biological systems (Canli et al., 2002). Pb, in the bivalent form, is a stable element that is mainly bioaccumulated by aquatic organisms. The effect of toxicity of Pb depends on the life stage of the fish, pH, water hardness, and the presence of organic materials (Hellawel, 1986).

Cd is a nonessential element that causes severe toxic effects in aquatic organisms in very low concentrations (Wong and Rainbow, 1986; Sorensen, 1991). Cd can damage the gills (Voyer et al., 1975), which represents the key mechanism of acute toxicity (Verbost et al., 1989).

Cr is a chemical element of relatively elevated mass that is toxic even to weak dose, especially if him there a cumulative effect by ingestion repeated of the food or continuous elevation of the general level of pollution in a food (Laurent, 1981).

The purpose of this study was to determine concentrations of selected metals in the muscle of three common fish species in the southern Atlantic coast the Laâyoune, which may pose risks to human health. Furthermore, correlations among the concentration of metals and order of their accumulation in the fish muscle were determined. This three common fish species collected from the Atlantic coast the Laâyoune, which is situated on the southern Atlantic coast (Morocco). This area the Laâyoune, in which there are large quantities of untreated industrial and domestic sewage, has one the most polluted coastal waters of Laâyoune.

MATERIALS AND METHODS

In this study three fish species (*Pagellus acarne, Sardina pilchardus* and *Diplodus vulgaris*) were collected in January, March and June 2005 from the southern Atlantic coast (Morocco) (Figure 1).

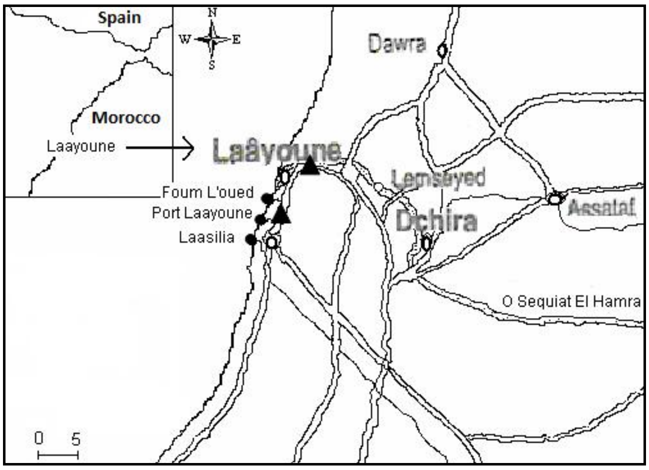

Figure 1: The site of sample collection (Atlantic coast Laâyoune).

Caught fishes (n=91) were evaluated by standard methods used in ichthyology (standard length and weight measurements, age determination by scales). After the biometric data were recorded, the samples of fish muscles were obtained from the left side of fish body in dorsal part, without skin and bones (Figure 2).

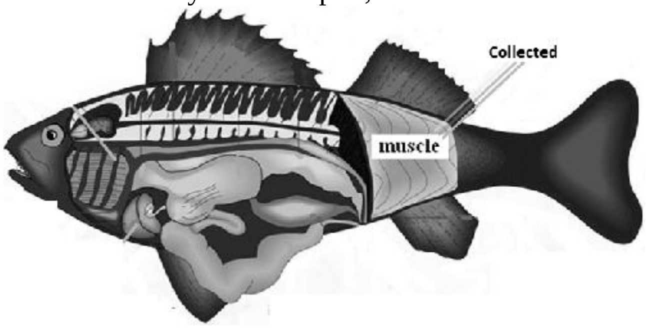

Figure 2: The muscle of sample collection (Atlantic coast Laâyoune).

After collection the tissue samples were kept at −18°C. For analysis, two grams of each muscle sample were dissolved in a solution of nitric acid P.A. (HNO_3: H_2O = 2:1) at 130°C for 2 h. Undissolved particles were filtered off and the solution diluted to 25 ml. The digested samples were analyzed by atomic absorption spectrophotometer (VARIAN AA 240 FS) by flame for Fe, Mn, Zn and (VARIAN AA 240 Z) provided with graphite furnace (AWG 120) for Cd, Cr, Cu, Ni, and Pb (Auger, 1989). Values of monitored heavy metals are presented on a wet-weight basis in µg/g and compared with European regulations. Maximal acceptable values in human food are as follows (in mg/kg wet weight): Pb—0.3 and Cd—0.1 (C.E., 2006).

Statistical analysis: For statistical analysis, the Anova One Way test, Kruskal-Wallis test, and Kolmogorov-Smirnov two-sample test were used with computer program Statgraphics Plus.

RESULTS AND DISCUSSIONS

The biometric characteristics (age, standard length, and weight) of the analyzed fish are listed in Table 1.

Table 1: Characteristics of analyzed fishes

			SL (mm)		BW (g)	
Species	n	Age	Mean±SD	Range	Mean±SD	Range
Pagellus acarne	30	3-6	132.9±29.81	100-250	116.06±37.10	68-185
Sardina pilchardus	35	2-9	150.97±34.15	150-200	126.42±19.87	94-150
Diplodus vulgaris	26	3-7	220.96±23.77	180-250	392.34±152.91	150-640

SL = standard length, BW = body weight, SD = standard deviation, n = number of individuals.

Concentrations of Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn in the muscle of the three fish species are listed in tables 2 and 3 and Figures 3, 4 and 5.

Table 2: Contents of selected metals (Cu, Fe, Mn and Zn) in muscle of analyzed fish (in mg/kg wet weight).

	Cu		Fe		Mn		Zn	
Species	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range
Pagellus acarne	0.51±0.50	0.10-1.46	5.34± 1.29	3.14-6.99	0.14±0.05	0.07-0.22	14.00±4.10	9.00-21.00
Sardina pilchardus	0.40±0.47	0.01-1.10	13.42±1.40	11.67-15.90	0.08±0.04	0.02-0.18	6.40±2.68	3.54-12.70
Diplodus vulgaris	0.37±0.29	0.08-0.87	3.34±0.81	1.97-4.67	0.21± 0.32	0.01-1.05	48.00±21.34	18.00-75.00
Total	0.42±0.42	0.01-1.46	7.36±4.60	1.97-15.90	0.15±0.19	0.01-1.05	24.03±22.47	3.54-75.00

Table 3: Contents of selected metals (Cd, Cr, Ni and Pb) in muscle of analyzed fish (in mg/kg wet weight).

	Cd		Cr		Ni		Pb	
Species	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range
Pagellus acarne	0.11±0.02	0.07-0.16	0.02±0.02	0.001-0.070	1.80±0.66	0.90-3.10	0.003±0.002	0.001-0.008
Sardina pilchardus	0.09±0.06	0.01-0.23	0.01±0.01	0.001-0.020	1.70±0.54	1.10-2.80	0.002±0.001	0.001-0.008
Diplodus vulgaris	0.09±0.05	0.01-0.19	0.02±0.01	0.004-0.039	1.60±0.53	1.10-2.70	0.01±0.02	0.001-0.080
Total	0.10±0.05	0.01-0.23	0.01±0.01	0.001-0.070	1.72±0.56	0.90-3.10	0.009±0.015	0.001-0.080

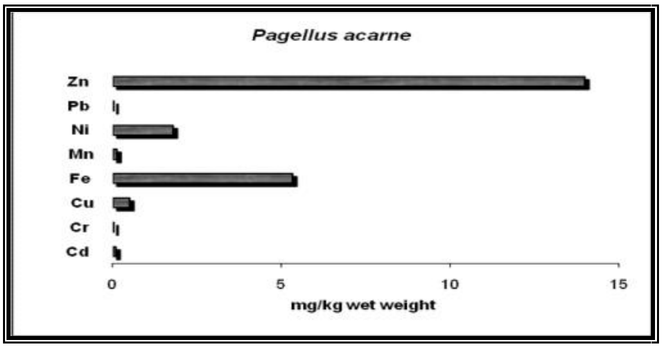

Figure 3: Concentration of selected metals in *Pagellus acarne.*

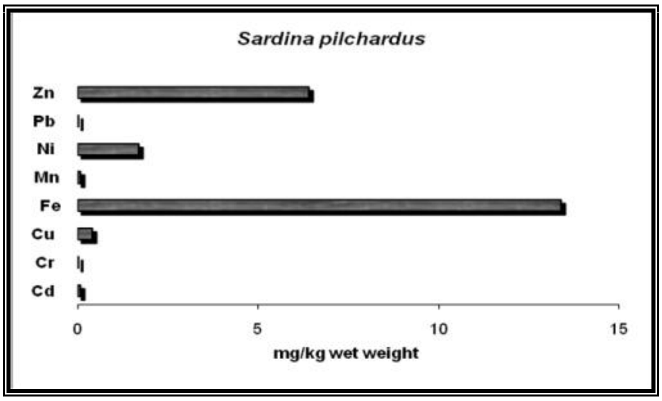

Figure 4: Concentration of selected metals in *Sardina pilchardus.*

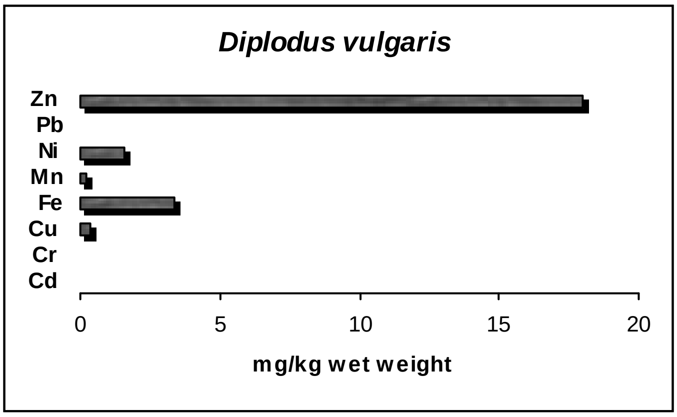

Figure 5: Concentration of selected metals in *Diplodus vulgaris*.

The Fe concentration in the muscle of the three analyzed fish species showed wide variation (Table 2). Differences among species were significant ($P < 0.05$)

for Fe/Mn and Fe/Pb. Fe concentrations fluctuated between 1.97 mg/kg wet weight (*Diplodus vulgaris*) and 15.90 mg/kg wet weight (*Sardina pilchardus*). Similar results are found in the muscle of *Lisa abu* and *Silurus triostegus* (6.88 and 6.38 µg/g wet weight, respectively) from the Atatürk Dam Lake (Karadede and Ünlü, 2000; Karadede et al., 2004) and muscle of *Leuciscus cephalus, Rutilus rutilus* and *Perca fluviatilis* (7.67, 5.90 and 5.05 mg/kg wet weight, respectively) from the Nitra River in Slovakia (Andreji et al., 2005). The lower results are found in the muscle of *Lisa aurata*, *Anguilla anguilla* and *Solea vulgaris* (4.11-7.13, 4.11-5.89 and 2.13-5.01 µg/g wet mass, respectively) from the southern Atlantic coast of Spain (Usero et al., 2003). Higher concentrations of Fe in fish muscle were reported in the Olifants River (Nussey et al., 1999). The general order of muscular Fe bioaccumulation was *Sardina pilchardus>Pagellus acarne>Diplodus vulgaris*. A significant (P<0.05) negative correlation was confirmed between bioaccumulation of Fe–Zn (Table 4).

The bioaccumulation of Mn between different fish species was significantly different (P<0.05) for Mn/Ni, Mn/Pb and Mn/Zn. Concentrations of Mn fluctuated between 0.02 mg/kg wet weight in *Sardina pilchardus* and 1 mg/kg wet weight in *Diplodus vulgaris*. Similar Mn concentrations were found in the muscle of *Lisa abu* and *Silurus triostegus* from the Atatürk Dam Lake (0.40 and 0.35 µg/g wet weight, respectively) (Karadede et al., 2004) and muscle of *Leuciscus cephalus, Rutilus rutilus* and *Perca fluviatilis* (0.35, 0.37 and 0.24 mg/kg wet weight, respectively) from river Nitra in Slovak republic (Andreji et al., 2005). In another study from the Atatürk Dam Lake, comparable results were found in three fish species (*Acanthobrama marmid*, *Capoetta trutta*, and *Cyprinus carpio*), but in another three fish species (*Chondrostoma regium*, *Chalcalburnus mossulensis*, and *Carasobarbus luteus*) the mean manganese concentration was higher (Karadede and Ünlü, 2000). In the muscle of *Lisa abu*, levels almost 40 times higher (6.65 µg/g) in comparison with our results were detected (Ünlü et al., 1996; Nussey et al., 2000). The highest results are found in the muscle of *Lisa aurata*, *Anguilla anguilla* and *Solea vulgaris* (2.25-2.50, 4.71-14.1 and 1.27-7.06 µg/g wet mass, respectively) from the southern Atlantic coast of Spain (Usero et al., 2003). The bioaccumulation order of manganese was *Diplodus vulgaris>Pagellus acarne>Sardina pilchardus*. A significant positive correlation between Mn–Pb (P<0.05) bioaccumulation was detected (Table 4). The limit for manganese in CE is not defined.

The concentrations of Zn varied greatly from 3.54 mg/kg wet weight (*Sardina pilchardus*) to 75 mg/kg wet weight (*Diplodus vulgaris*). Comparable levels of Zn concentrations in the muscle of *Pagellus erythrinus* (3.8 µg/g wet weight in May 1996) from the Eastern Aegean Sea, Turkey (Uluturhan et al., 2007). In the muscle of *Prochilodus lineatus* and *Mugil cephalus* from the Rio de la Plata River were observed and muscle of *Cyprinus carpio* also similar levels were found (12.5 µg/g wet weight) (Colombo et al., 2000). However, in the muscle of

Labeo umbratus from the upper Olifants River, levels of Zn concentrations reaching 13.83–187.63 µg/g dry mass were found (Nussey et al., 1999). Fish muscle from the Bavarian part of the Danube River was reported to be 7.2 mg/kg wet weight of Zn (Wachs, 1982). Similar mean Zn concentrations in *Chondrostoma regium* and *Capoetta trutta* (7.93 and 5.32 ppm wet weight, respectively) from the Atatürk Dam Lake were detected (Karadede and Ünlü, 2000). Higher amounts of Zn were reported in other fish species from the Atatürk Dam Lake (Karadede et al., 2004), from the Tigris River (Ünlü and Gumgum, 1993; Gumgum et al., 1994; Ünlü et al., 1996), and from the upper Negro River (Arribere et al., 2003). Slightly lower amounts of Zn were found in fish muscle from Taimyr Peninsula Lakes (Allen-Gil et al., 2003). The lower results are found in the muscle of *Lisa aurata*, *Anguilla anguilla* and *Solea vulgaris* (3.10-8.41, 10.1-13 and 4.17-8.52 µg/g wet mass, respectively) from the southern Atlantic coast of Spain (Usero et al., 2003). The bioaccumulation of Zn between different fish species was not significantly different ($P < 0.05$) for Zn/Cd, Zn/Cr, Zn/Cu, Zn/Mn and Zn/Pb. The general order of Zn bioaccumulation was *Diplodus vulgaris>Pagellus acarne>Sardina pilchardus*. The relationship between bioaccumulation of Zn and monitored metals showed negative correlations (Zn-Fe), (Table 4). The level for Zn in C.E. is not defined.
Our results showed relatively low Cu concentrations ranging from 0.08 µg/g wet weight (*Diplodus vulgaris*) to 1.46 µg/g wet weight (*Pagellus acarne*). Similar results are found in the muscle of *Lisa aurata, Anguilla anguilla* and *Solea vulgaris* (0.2-0.6, 0.5-1.5 and 0.4-0.5 µg/g wet mass, respectively) from the southern Atlantic coast of Spain (Usero et al., 2003) and *Pagellus erythrinus* (0.15-0.23 µg/g wet weight; Uluturhan et al., 2007). Higher Cu concentrations were reported in fish from the Atatürk Dam Lake (Karadede and Ünlü, 2000; Karadede et al., 2004). In fish (*Prochilodus lineatus, Cyprinus carpio,* and *Mugil cephalus*) from the Rio de la Plata River, Cu concentration 0.44–0.47 µg/g wet weight were detected (Colombo et al., 2000). In *Labeo umbratus*, Cu concentrations in muscle 1.53–13.08 µg/g dry mass were found (Nussey et al., 2000). Mean Cu concentrations reaching 0.73–1.24 µg/g dry mass in three fish species from four Taimyr Peninsula Lakes were recorded (Allen-Gil et al., 2003). Very high Cu concentrations in the fish muscles from the Tigris River were observed (Ünlü and Gumgum, 1993; Gumgum et al., 1994; Ünlü et al., 1996; Gumgum et al., 2001). The mean Cu concentration in the muscle of fishes from the Bavarian part of Danube River is 0.56 mg/kg (Wachs, 1982). Similar results are found in the muscle of *Leuciscus cephalus, Rutilus rutilus* and *Perca fluviatilis* (0.43, 0.39 and 0.56 mg/kg wet weight, respectively) from the Nitra river in Slovakia (Andreji et al., 2005). We found significant differences in Cu accumulation among the three species ($P<0.05$) for Cu/Fe, Cu/Pb and Cu/Zn. The order of Cu bioaccumulation was *Pagellus acarne>Sardina pilchardus>Diplodus vulgaris*. The relationship between bioaccumulation of Cu and monitored metals showed negative correlations, but significant differences

were not detected (table 4). Significant differences were not detected, correlation ($P > 0.05$). The level for Cu in C.E. is not defined.

Ni concentrations in the fish muscle are listed in Table 2. The bioaccumulation of Ni between different fish species was significantly different ($P<0.05$) for Ni/Pb. Ni concentrations fluctuated between 1.1 mg/kg wet weight (*Diplodus vulgaris*) and 3.1 mg/kg wet weight (*Pagellus acarne*). Much higher levels of Ni concentrations were found in the fish muscles from the Tigris River (Gumgum et al., 1994; Ünlü et al., 1996). Comparable results to ours were published from the upper Olifants River (Nussey et al., 1999) and from the Taimyr Peninsula Lakes (Allen-Gil et al., 2003). The most similar mean values (0.56 mg/kg wet weight) of Ni concentrations were reported from the Bavarian part of the Danube River (Wachs, 1982). But the lower results are found in the muscle of *Lisa aurata*, *Anguilla anguilla* and *Solea vulgaris* (0.021-0.070, 0.015-0.020 and 0.022-0.067 µg/g wet mass, respectively) from the southern Atlantic coast of Spain (Usero et al., 2003). The general order of Ni bioaccumulation was *Pagellus acarne> Sardina pilchardus>Diplodus vulgaris*. The bioaccumulation of Ni between different fish species was not significantly different ($P>0.05$, Table 4). A positive, but not significantly different, correlation was found between Ni and Zn (Ünlü et al., 1996). The level for Ni in C.E. is not defined.

Cr concentrations (Table 3) varied from 0.001 mg/kg wet weight (*Sardina pilchardus*) to 0.07 mg/kg wet weight (*Diplodus vulgaris*). The mean value was 0.01±0.01 mg/kg wet weight. Similar results were published for the fish muscles from the Rio de la Plata (Colombo et al., 2000), from the Nitra River in Slovakia (Andreji et al. 2005) and muscle of *Lisa aurata* and *Solea vulgaris* (0.029-0.038 and 0.033-0.045 µg/g wet mass, respectively) from the southern Atlantic coast of Spain (Usero et al., 2003). In fish muscles from the Tigris River Cr concentrations ranged from 18.89 to 25.96 µg/g wet weight (Ünlü et al., 1996). High variations of Cr concentrations (5.57–197.12 µg/g dry mass) were found in the muscle of *Labeo umbratus* from the upper Olifants River (Nussey et al., 2000) and muscle *Anguilla anguilla* (0.143-0.368 µg/g wet mass from the southern Atlantic coast of Spain (Usero et al., 2003). The bioaccumulation of Cr between different fish species was significantly different ($P<0.05$) for Cr/Cu, Cr/Fe, Cr/Ni and Cr/Zn. The general order of Cr bioaccumulation was *Pagellus acarne>Diplodus vulgaris>Sardina pilchardus*. The relationship between bioaccumulation of Cr and monitored metals showed negative correlations (Cr-Fe; table 4). The level for Cr in C.E. is not defined.

Pb concentrations were between 0.001 mg/kg (*Sardina pilchardus*) and 0.08 mg/kg wet weight (*Diplodus vulgaris*) comparable levels of Pb concentrations in the muscle of *Pagellus erythrinus* (0.07 mg/kg wet weight in May 1996 from the Eastern Aegean Sea, Turkey (Uluturhan et al., 2007) and muscle of *Lisa aurata, Anguilla anguilla* and *Solea vulgaris* (0.03-0.05, 0.03-0.09 and 0.03-0.05 mg/kg wet mass, respectively) from the southern Atlantic coast of Spain

(Usero et al., 2003). Also comparable Pb levels (0.025–0.896 mg/kg) were found in fish muscle from the lower Mississippi River (Watanable et al., 2003). However, in the muscle of *Leuciscus cephalus* (0.88), *Barbus barbus* (0.43), *Rutilus rutilus* (0.31) and *Perca fluviatilis* (1.80) mg/kg wet weight from the Nitra River in Slovakia higher levels were found (Andreji et al., 2005). Very low Pb concentrations (0–0.11 µg/g dry mass) for three fish species from four Taimyr Peninsula Lakes were found (Allen-Gil et al., 2003). The bioaccumulation of Pb between different fish species was significantly different (P<0.05) for Pb/Zn. The bioaccumulation order of Pb was *Diplodus vulgaris>Pagellus acarne>Sardina pilchardus*. The relationship between bioaccumulation of Pb and monitored metals showed positive correlations (Pb-Mn) (table 4). The European limits for Pb content in fish muscle is 0.3 mg/kg wet weight.

The mean value of Cd bioaccumulation in fish muscle is 0.10 ± 0.05 mg/kg wet weight. The concentration of Cd varied from 0.01 mg/kg wet weight (*Diplodus vulgaris*) to 0.16 mg/kg wet weight (*Pagellus acarne*). Lower levels of Cd concentrations were found in the fish muscles (*Leuciscus cephalus* (0.06), *Barbus barbus* (0.06) and *Perca fluviatilis* (0.09) mg/kg wet weight from the Nitra River in Slovakia (Andreji et al., 2005). Also lower values of Cd content for the fish muscle from the Bavarian part of the Danube River was recorded (0.04 mg/kg wet weight; Wachs, 1982) and muscle of *Lisa aurata*, *Anguilla anguilla* and *Solea vulgaris* (0.013-0.030, 0.015-0.050 and 0.010-0.028 µg/g wet mass, respectively) from the southern Atlantic coast of Spain (Usero et al., 2003). The mean Cd concentrations in fishes from four Taimyr Peninsula Lakes varied from 0–0.02 µg/g dry mass (Allen-Gil et al., 2003). Similar values of Cd concentrations were found in fish muscle from the lower Mississippi River (Watanable et al., 2003). High values of Cd content for the fish muscle in the *Pagellus erythrinus* (1.1-2.6 mg/kg wet weight) from the Eastern Aegean Sea, Turkey (Uluturhan et al., 2007). The bioaccumulation of Cd between different fish species was significantly different (P<0.05) Cd/Fe, Cd/Ni, Cd/Pb and Cd/Zn. The general order of Cd bioaccumulation was *Pagellus acarne>Sardina pilchardus>Diplodus vulgaris.* A significant relationship between the bioaccumulation of Cd and monitored metals was not recorded (Table 4). The European (C.E., 1990) limit for Cd content in fish muscle is 0.1 mg/kg wet weight.

CONCLUSION

In summary, Zn was found to have the highest concentration of all the monitored metals in fish muscle. Pb had the lowest concentrations. The general order of monitored metal bioaccumulation was: Zn>Fe>Ni>Cu>Mn>Cd>Cr>Pb. This order might be attributed to the different uptake, metabolism, and detoxication of the metals in the fish. In the individual fish species the order of the studied elements was as follows:

Pagellus acarne : Zn>Fe>Ni>Cu>Mn>Cd>Cr>Pb
Sardina pilchardus : Fe>Zn>Ni>Cu>Cd>Mn>Cr>Pb
Diplodus vulgaris : Zn>Fe>Ni>Cu>Mn>Cd>Cr>Pb

Our results show that consumption of fish of Atlantic of coast (Morocco) cannot have a risk for health to those that consume fish. We recommend the consumption of fish because of weak content of these metals.

ACKNOWLEDGEMENTS

Much of this work was performed under the thematic program of support for Scientific Research within the Faculty: sewage and health, Faculty of Sciences Kenitra in collaboration with the Scientific Institute and the National Institute of Hygiene (Rabat).

The authors thank the anonymous reviewers for comments and remarks that helped improve the manuscript.

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