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Anguilla anguilla L.: Evaluation of the degree of heavy metal contamination in the Sebou estuary and in Moulay Bousselham lagoon reserve (Morocco)

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Abstract: This work involves an assessment of the degree of heavy metal contamination (Zn, Fe, Cu, Pb and Cr) in liver, gills, kidney and muscle of eel (*Anguilla anguilla*) inhabiting two ecosystems along the Moroccan Atlantic coast: the Sebou estuary and the Moulay Bousselham lagoon reserve in April 2006. In this study, heavy metals were determined with flame atomic absorption spectrometry. Chromium levels in liver as well as copper levels in kidneys were significantly higher ($p<0.05$) in the Sebou estuary than in the Merja Zerga reserve. Accumulation of chromium and copper in eel tissues was likely due to anthropogenic activities. The metals ratios between liver and muscle indicated that the liver accumulated higher levels of metals than the muscle tissue. All metal levels in muscle tissues are lower than the limits of European Dietary Standards and Guidelines.

Key Words: *Anguilla anguilla*, Sebou estuary, Moulay Bousselham lagoon, Morocco, heavy metals

Introduction

Aquatic ecosystems are prone to receiving and accumulating contaminants (Ahmad *et al*., 2006), in particular heavy metals which have been identified as a general cause of aquatic ecosystem deterioration over the past decades (Benasser *et al*., 1996; Tahiri *et al*., 2005; Dehn *et al*., 2006; Sivaperumal *et al*., 2007). Aquatic organisms, such as fish, inhabit these

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ecosystems and are thus constantly exposed to heavy metals (Devez, 2004).

Fish species such as the European eel (*Anguilla anguilla*) depend on freshwater and estuarine habitats for their juvenile growth phase. The major routes of exposure for this fish are water, food, sediment, and suspended particulate material (Hardersen and Wratten, 1998; Fernandes *et al*., 2007). The impact of heavy metal pollution and that of other chemical compounds due to anthropogenic activities is of considerable interest and may be one of the major causes of the species' decline (Maes, 2005).

The European eel is currently on the Helcom red list of threatened and declining species in the Baltic Sea (Helcom, 2007) and has been rated as an endangered species by the Convention on International Trade in Endangered Species (CITES) of Wild Fauna and Flora (FAO 2007; CITES 2008).

In 2003 an International Eel Symposium provided evidence, based on the four longest glass eel collection series of European eel population, that the recruitment of young eels to the continental stock had declined to as low as 1% of its former level in the late 1970s (ICES 2003).

This species has been set outside safe limits and precautionary actions must be taken immediately (Feunteun, 2002; Dekker, 2003; Maes, 2005; Boera, 2010; Geeraerts and Belpaire 2010).

The impact of pollutants (Robinet and Feunteun, 2002), including metal accumulation in eel tissues has been documented in many studies (Barak and Mason, 1990; Bruslé, 1994; Sures *et al*., 1994 ; Zimmermann *et al*., 1999, Ribeiro *et al*., 2005; Klavins 2009), making them useful indicators of heavy metal contamination in aquatic ecosystems (Gunkel, 1994).

They are also interesting for water quality monitoring because they are tolerant to a wide range of environmental conditions, such as variations in oxygen availability, waste products and in salinities (Tesch, 1977) which is of particular importance for wetland sites.

Morocco is the southern limit of eel distribution (28°N at Dr'a river (Qninba *et al*., 2011) (Fig. 1), which means that this source is extremely vulnerable and incurs dangers. In the absence of security measures for protecting eels in Morocco, this fish species could follow a probable disappearance like Moroccan Shad (*Alosa alosa*).

In Moroccan aquatic ecosystems, European eel are major importance, in particular around the Sebou River and its estuary, among the most polluted ecosystems in country (Koukal *et al*., 2004), and the Moulay Bousselham Lagoon Reserve, which is rich in avifauna diversity and well known for commercial shell and eel fishing (Labbardi *et al*., 2005; Yahyaoui *et al*., 2006).
The meat of *Anguilla anguilla* is highly valued in Europe and parts of east Asia while in Morocco

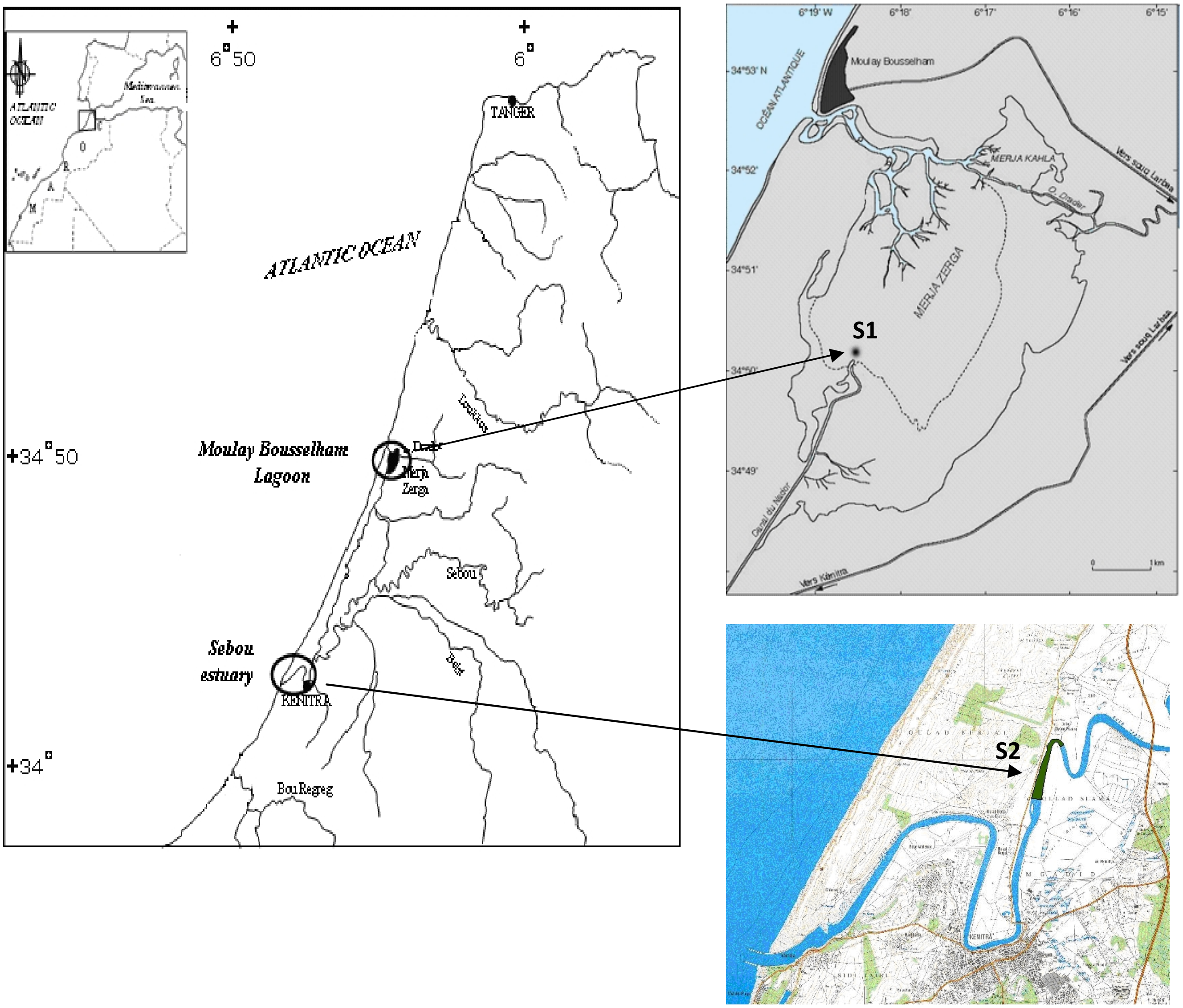

Fig. 1: Map showing sampling locations along the Moulay Bousselham lagoon and the Sebou estuary (S1, S2: study sites)

the consumption of this fish varies throughout the stage of eel. Yellow and silver eel are most consumed (2.6 €/kg). Glass eels and elvers are strictly designed to export because almost all meat production of eel is based on aquaculture of wild caught young eel stages. In Morocco glass eels are less valued than Europe (13-104 €/Kg) and trade particularly by Spain and other Europe countries market where the value of glass eels is very high (200-1,000 €/Kg).

In Moroccan aquatic ecosystems, European eel are major importance, in particular around the Sebou River and its estuary, among the most polluted ecosystems in country (Koukal *et* *al.*, 2004), and the Moulay Bousselham Lagoon Reserve, which is rich in avifauna diversity and well known for commercial shell and eel fishing (Labbardi *et al.*, 2005; Yahyaoui *et al.*,2006).

This study has been investigated for the first time heavy metal concentrations in European eel from these Moroccan ecosystems. Five heavy metals (iron, zinc, copper, chromium, and lead) were selected for examination in four eel tissues (liver, kidney, gills, and muscle). We also compared between the two study sites the metal concentrations detected in the tissues and evaluated the relationship between eel size and heavy metal accumulation in the target organs.

Materials and Methods

Study sites

European eel were collected from the Sebou estuary and the Moulay Bousselham lagoon reserve (MBLR).

Sebou river is located on the Moroccan Atlantic coast 34°27'N/6°64'W. The Lalla Ai-cha guard dam is located 40 km upstream (Figure 1). The Sebou drainage basin has a surface area of 40, 000 km^2. One of its tributaries passes through the Rharb plain, which has extensive agricultural and industrial activities. As for its harbors, Kenitra is about 10 km from the ocean, has commercial traffic, while Mehdiya is only 1 km from the river's mouth and known for its fishing activities. The average Sebou river output reaches 200 m^3. The annual input volume is about 5109 m^3 of freshwater and may double in rainy seasons. According to Combe (1966), the tidal height varies from 0.9 to 3.10m along the estuary.

The Sebou is the main purveyor of water to the Gharb plain, a major agricultural region, where fertilizers and pesticides are widely used. Along its banks, various industries (paper mills, sugar plants, tanneries, food industries, wool mills, chemical plants, etc.) have settled and contribute to the development of large towns which are not equipped with facilities for the treatment of either industrial or domestic waste.

Eel and glass eel are fished throughout the estuary, an ecosystem heavily influenced by urban, agricultural and industrial sewage.

Merja Zerga 34°86'N/06°28'W Permanent Biological Reserve, Ramsar Site (1980) is a tidal lagoon located 70 km north of Kenitra on the Atlantic coast (Fig. 1). The outlet to the ocean lies at the seaside resort and fishing village of Moulay Bousselham: hence the site's alternative name of Moulay Bousselham lagoon. In addition to its tidal inflow, the lagoon receives freshwater from the Oued Drader and the underlying water-table, which is very close to the surface here. The lagoon itself covers 4500 ha, of which 30% is open water, and has an average depth of 1.5 m.

The lagoon of Merja Zerga, presence of heavy metals carried by the Oued Drader and Nador Canal is noted especially during floods

(Mergaoui 2005).

Nador Canal and Oued Drader drained several paddy field (culture of rice) and other crops using pesticides and agricultural inputs threatening the ecological balance of the site.

Eel fisheries in Morocco occur in inland waters (rivers, estuaries and lagoons) as well as in coastal. The aim of this current study was to investigate Heavy metals to assess the degree of pollution of eels one of the most heavily exploited species in Morocco especially in Sebou estuary and Merja Zerga lagoon considered as the main important fishing sites of eels (Wariaghli *et al*. 2011 in ICES 2011).

Analytical procedure

Eels (n = 67) were caught in April 2006. Fishing for eels was undertaken by fishermen using fyke nets from the MBLR (N=33); precisely at the outlet channel of Nador (S1) and from Sebou (N=34) at S2 (Fig. 1). They were then kept at low temperature and transported (4ºC) to the laboratory, where standard length (cm) and body weight (g) were recorded. Eels were selected and sorted according to body size (cm). They were subsequently dissected by removing the gills, kidney, liver and muscle tissues in a half-frozen state to avoid any contamination between adjoining parts and the loss of metals through the movement of fluid (Stinson and Eaton, 1983; Bouachrine *et al*., 1998). The tissues were then labelled and frozen individually at -20ºC before analysis.

The material used for the dissection was previously soaked in nitric acid 10% (HNO_3) and then rinsed several times with double distilled water. For mineralization, tissue eels samples were weighed in Teflon bombs. In each vessel, 5 ml of concentrated HNO3 (65% for analysis, ISO, Merck) was added. The vessels were then capped with Teflon seals and left to heat in a sand bath for 4 hours at 80 ºC. After the digestion was completed, the vessels were cooled. The solution was then transferred into a volumetric flask and completed with double distilled water up to 50ml. The reagent blank was prepared in the same manner.

Levels of heavy metals were determined with flame atomic absorption spectrometry: Varian AA 240 FS for zinc (Zn), iron (Fe) and copper (Cu), and Varian AA 240 Z, along with a graphite furnace (AWG 120) for lead (Pb) and chromium (Cr) (Auger, 1989).

The analytical method was checked during each series of measurements against a standard biological reference material (DORM-2, dogfish muscle, NRCC-CNRC, Ottawa, Canada). Cr, Cu, Pb, Zn and Fe values were consistently within certified ranges (Table 1).

Statistical Analysis

The results were expressed as mean concentration ± standard deviation (SD). Significant differences were determined by Student’s t-test using SPSS 13.0. A p-value ≤

0.05 was considered significant.

Results

Tissue bioaccumulation

Table 2 shows the metal concentrations analysed in European eel sampled from the Sebou estuary and the Moulay Bousselham lagoon. Bioaccumulation in tissues varied depending on the metal.

Our results revealed the presence of high metal concentrations. For both sites metal concentrations in liver and kidney were considerably higher than in muscle and gills. Copper concentrations in kidney were signify-cantly higher at the Sebou estuary than the MBLR ($p \leq 0.05$). Levels detected from the Sebou estuary reached 92 µg/Kg wet weight whereas in muscle, copper concentrations were lower (0.72 µg/Kg wet weight). However, when comparing the two sites, no significant difference in copper concentrations was detected in other tissues ($p < 0.05$).

Tab. 1: Comparison between measured values and certified values of Cr, Cu, Pb, Zn and Fe concentrations using the standard biological reference material: DORM-2 (Dog fish muscle from NRCC-CNRC, Ottawa, Canada.

Element	Measured values INH Laboratory, Morocco	Certified values DORM-2 (mean±IC)
Cr (mg/Kg)	29.0±1.6	34.7±5.5
Cu (mg/Kg)	2.59±0.20	2.34±0.16
Pb (mg/Kg)	0.069±0.007	0.065±0.007
Zn (mg/Kg)	22.3±3.5	25.6±2.3
Fe (mg/Kg)	136±5	142±10

Chromium accumulation in liver was greater than in other organs. Furthermore, chromium levels in liver from the Sebou estuary were significantly higher than those from the MBLR ($p = 0.027$): 1.15 µg/Kg wet weight and 0.31 µg/Kg wet weight.

Even though no significant differences in lead concentrations were observed between the two sites, lead accumulated and was mainly detected in kidney (0.90 µg/Kg wet weight) and in gills (0.89 µg/Kg wet weight) in eels from the Sebou estuary. Those caught from the MBLR contained 1.70 µg/Kg wet weight of lead in kidney, 2.09 µg/Kg wet weight in muscle, and 0.90 µg/Kg wet weight in liver.

Of all the metals selected for this study, iron concentrations were highest in all the target organs, especially in liver and in kidney from

Tab. 2: Level of heavy metals (µg/Kg wet weight) detected in *Anguilla anguilla* tissue originating from the Sebou estuary and the Moulay Bousselham lagoon (means ± SD and ranges).

Sites	Organs	Metals				
		Fe	Zn	Cu	Pb	Cr
Moulay Bousselham Lagoon	Muscle	31.95±42.06 (5.56-132.40)	44.23±69.50 (6.00-231.20)	2.12±2.57 (0.50-8.40)	2.09±2.70 (0.01-6.79)	1.22±1.20 (0.13-3.86)
	Gills	66.60±45.91 (10.00-137.30)	46.05±35.35 (23.30-107.80)	5.41±7.64 (0.10-23.80)	0.38±0.52 (0.03-1.27)	0.57±0.44 (0.03-1.27)
	Liver	245.30±335.31 (10.01-972.20)	152.72±338.8 (0.30-989.00)	20.38±19.20 (3.80-65.30)	0.90±14.14 (0.06-41.00)	0.31±0.49 (0.01-1.50)
	Kidney	281.20±208.27 (24.30-672.30)	152.37±143.90 (30.80-478.00)	8.25±5.06 (2.40-18.60)	1.70±1.53 (0.01-3.75)	0.70±0.43 (0.01-1.57)
Sebou Estuary	Muscle	14.80±16.86 (4.00-39.60)	46.30±64.10 (5.70-27.70)	0.72±0.30 (0.60-1.20)	0.36±0.30 (0.08-0.65)	1.46±0.90 (0.49-2.57)
	Gills	47.95±48.21 (9.80-139.90)	43.15±50.69 (18.30-146.30)	2.88± 2.58 (0.20-7.20)	0.89±1.00 (0.03-2.51)	1.51±1.32 (0.11-2.9)
	Liver	71.22±70.00 (8.90-174.50)	52.32±112.86 (9.00-272.50)	5.32±2.82 (1.00-8.50)	0.23±0.36 (0.02-0.78)	1.15±0.95) (0.41-2.51)*
	Kidney	87.82±112.87 (10.90-260.00)	119.91±50.78 (22.80-167.40)	92.00±1.87* (0.20-5.50)	0.90±0.73 (0.03-2.20)	0.79±0.59 (0.16-1.46)

*Significant at an 85% confidence level (based on a two-tailed test).

both sites. Although not statistically significant, iron levels in these two organs were three times higher in eel from the MBLR (281.20 µg/Kg wet weight in kidney and 245.30 µg/Kg wet weight in liver) compared to the Sebou estuary (87.82 µg/Kg wet weight in kidney and 71.22 µg/Kg wet weight in liver).

As for zinc, an essential metal for fish, especially European eel, no significant difference between the two sites was observed. Similar concentrations were observed in liver (152.72 µg/Kg wet weight) and kidney (152.37 µg/Kg wet weight) of MBLR eel, where as levels from the Sebou estuary were 52.32 µg/Kg wet weight in liver (three times lower than the average levels found in MBLR) and 119.91 µg/Kg wet weight in kidney.

Organ specificity

The heavy metals in this study (Fe, Zn, Cu, Pb and Cr) mainly accumulated in liver and kidney (Fig.2a and Fig 2b).

Zinc concentrations were significantly higher in kidney compared to muscle ($p = 0.009$) and gills ($p = 0.007$).

Copper values were significantly higher in

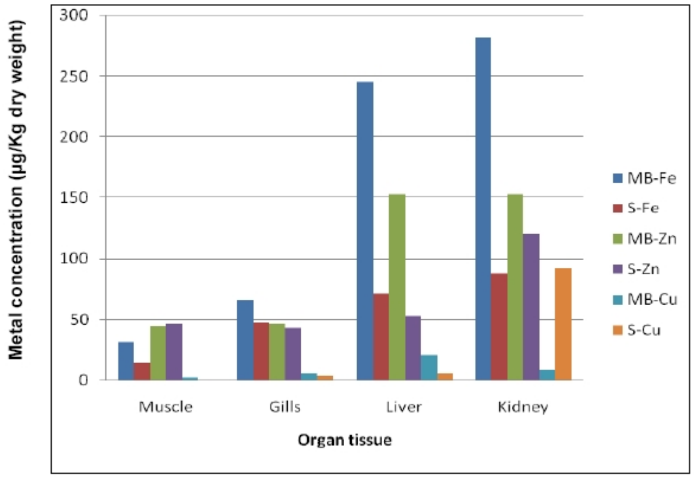

Fig. 2a: Distribution of Fe, Zn, Cu in organ tissue of *Anguilla anguilla* from the Moulay Bousselham (MBLR) and the Sebou (S)

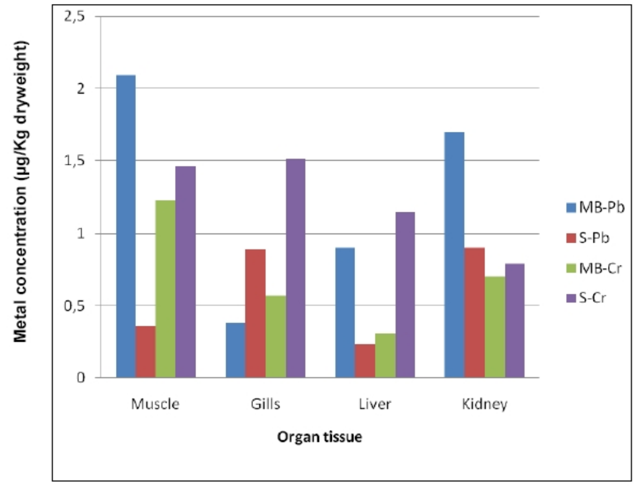

Fig. 2b: Distribution of Pb and Cr in organ tissue of *Anguilla anguilla* from the Moulay Bousselham (MB) and the Sebou (S)

kidney than in muscle ($p = 0.009$), and lead concentrations were significantly higher in kidney than in gills ($p = 0.038$). For chromium, no significant differences among tissues were detected.

Distribution of Heavy metals according eel size

The distribution of iron, zinc, copper, lead and chromium depended on the eel size. Eels caught from the MBLR were selected according to four size classes (cm): 1= 20-30, 2 = 30-40, 3 = 40-50 and 4 = 50-60 (Table 3).

In muscle, Copper concentrations were significantly higher in eels of class 3 than class 4 ($p = 0.035$). For lead eels of class 3 accumulated higher levels than those in class 2 ($p = 0.03$) and 4 ($p = 0.05$).

Tab. 3: The mean and range of total body length, total body weight according eel size in Sebou estuary and Moulay Bousselham lagoon.

Eel size class (cm)	Sebou		Moulay Bousselham lagoon	
	Length (cm) ±SE	Weight (g) ±SE	Length (cm) ±SE	Weight (g) ±SE
20-30	26.1±2.9	27.2±2.9	26.5±3.4	22.8±9.8
30-40	35.0±2.8	67.0±17.4	35.4±2.7	80.4±29.7
40-50	44.1±1.0	140±16.9	44.8±4.12	157.3±84.9
50-60	56.0±1.1	307.8±3.2	53.6±1.8	288.8±64.2

In gills, lead presented a very significant variation between different classes of eel size. The results showed higher accumulation for classes 1 and 2, compared to other classes. Differences in accumulation were found between classes 1 and 3 ($p = 0.002$) and between 1 and 4 ($p = 0.0026$). Differences were also observed between classes 2 and 3 ($p = 0.0001$) as well as 2 and 4 ($p = 0.004$), showing that lead mostly accumulated in medium-sized eel (30-40 cm). As for chromium, a significant difference was detected between classes 1 and 4 ($p = 0.028$). For Fe, Zn and Cu, no significant differences were found.

In liver, copper was the only metal for which a significant difference was observed between classes 1 and 2 ($p = 0.028$), with a higher concentration in smaller eels. No significant difference was detected for the other heavy metals examined among the other size classes.

While, metal accumulation in kidney was similar, regardless of eel size. For Fe and Pb, significant differences were detected between classes 2 and 4, both metals presenting higher concentrations in medium-sized eels ($p = 0.019$ for Fe and $p = 0.002$ for Pb). Zinc levels were higher in the class 1 ($p = 0.02$) than in the class 3. However, chromium levels were higher in class 3 than class 1 ($p = 0.034$). As for copper, no significant differences were identified between any of the size classes.

Discussion

In our study, a general contamination of four heavy metals was determined through the analysis of various organs from the European eel.

An even distribution of most of the heavy metals (Fe, Zn, Cu, Pb and Cr) was observed in the target organs kidney, liver and gills. Eel muscle, however, exhibited the lowest metal concentrations, which was also shown by Barak and Mason (1990) and Zimmermann *et al*. (2004). The accumulation of heavy metals in eel organs is most probably due to the high fat content, reflecting the actual pollution stress in the organism (Collings *et al*., 1996; Maes, 2005). This species is thus more prone to accumulate chemical compounds and may have the potential as a bioindicator.

In eel collected from the Sebou estuary, kidney and liver were the main organs in which metals accumulated. This pattern was also observed by Bouachrine *et al*. (1998) in *Barbus callensis, Cyprinus carpio and Liza ramada* collected from different sites on the Sebou River. Copper levels in liver and kidney, respectively were 201 µg/Kg wet weight and 83 µg/Kg wet weight in *L. ramada*, 156 µg/Kg wet weight and 37 µg/Kg wet weight in *C. carpio*, and 171 µg/Kg wet weight and 38 µg/Kg wet weight in *B. callensis*. Fernandes *et al*. (2006) reported higher levels than in our study. Liver of *A. anguilla* accumulated 304.89 µg/Kg wet weight of Cu, a much higher concentration than that observed in gills (10.34 µg/Kg wet weight) thus one of the main storage organs. The authors also showed that copper significantly accumulated in kidney, where it reached its highest concentration. Furthermore chromium was also detected in liver. Thus accumulation of Cu and Cr in liver and kidney of eels can undoubtedly be attributed to the presence of these metals in water and sediment in these areas which receive effluents polluted by industrial and mining activities as identified by Parvez *et al*. (2006). The literature (Bouachrine *et al*., 1998; El Kihel, 2003) also shows that the Sebou River is one of the most heavily polluted regions in Morocco. Along its banks are various industries (paper mills, textiles, tanneries…) which meet urban, polluted effluents emitted from Kenitra city. Pollution from agriculture activities also contribute. The lower part of the Sebou is more affected by the more poisonous metals: chromium followed by mercury, then lead and copper (Benasser *et al*., 2000).This can explain the contamination of eel liver by chromium.

As for the Moulay Bousselham lagoon, it is also affected by sediment pollution, especially near the Nador channel. Bayed *et al*., (1998) and Mergaoui (2005) showed increasing sediment pollution by heavy metals such as iron, zinc, copper, with lead concentrations being highest.

Concerning heavy metal accumulation in liver and kidney, natural occurring values of essential elements such as Fe, Zn and Cu should be taken into account in comparison to the high concentrations detected among the different tissues. Cu and Zn concentrations are under regulatory control by metallothioneins in liver and kidney. Though copper is essential for good health, high intakes can cause adverse health problems, such as liver and kidney damage (Tuzen, 2006). On the other hand, Pb accumulated mostly in gills, probably due to eels' constant contact with water and sediments, exposing the species to dissolved contaminants (Ahmad *et al*., 2006). Gills have often been selected as an indicator for monitoring any fish intoxication (Fekhaoui and Keck, 1986). Our results show that lead (a non-essential metal) accumulates in eel of different body sizes, mainly eels sized (20-30 cm and 30-40 cm) caught from the Moulay Bousselham lagoon. At this medium size eels are more prone to be contaminated due to their life stage (Yellow eel) where eel is sedentary and they could stay from 5 to 20 years in fresh water where they grow and feed (Belpaire and Goemans 2007); but after they become sexually mature, their eyes grow larger, their flanks become silver and belly white in color. In this stage the eels are known as "silver eels", and they begin their migration back to the Sargasso sea to spawn (40-50cm and >50 cm) at this size they don't feed anymore and they start migrating.

Barak and Mason (1990) found similar results showing highly significant correlations between body size and lead concentrations in eels caught from five rivers in the United Kingdom.

Metal concentrations in muscle from Moulay Bousselam lagoon were 0.36 µg/Kg wet weight for Pb and 1.46 µg/Kg wet weight for Cr. Compared to Canadian (Cu: 100 µg/Kg wet weight ; Zn:100 µg/Kg wet weight), Hungarian (Cu: 60 µg/Kg wet weight ; Zn: 80 µg/Kg wet weight) and Australian (Cu: 10 µg/Kg wet weight ; Zn: 150 µg/Kg wet weight) food standards (Table 4).

Tab. 4: Standards levels of some toxic metals in fish for human consumption.

Standard norms	Cu (µg/kg wet wt)	Zn (µg/kg wet wt)	Pb (µg/kg wet wt)
Spain	20	-	1
Canada	100	100	-
Hungary	60	80	-
Australia	60	80	-
OMS/FAO/EPA		70	
CE/R n°466/2001	-	-	0.4

Conclusion

This study revealed high concentrations of Zn, Fe, Cu, Pb, and Cr in *Anguilla anguilla* in the selected organs: liver, kidney, gills and muscle. Cu and Cr concentrations were higher than other metals and mainly accumulated in liver and kidney. Metal accumulation was also highest in medium sized eel (20-30 cm and 30-40 cm). The study also showed that eel from the Moulay Bousselham lagoon had higher heavy metal burdens. Identified as a Ramsar site and due to its great ecological, economical and halieutic value, an urgent biomonitoring programme program should be implemented for this lagoon. Finally results enforce the importance of eel as a bioindicator of pollution in these wetlands and should be carefully monitored in order to prevent extinction.

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