Research Article

Metallic Contamination of the Muscles of Three Fish Species from the Moulouya River (Lower Moulouya, Eastern Morocco)

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1.Introduction

The Moulouya River, which extends over a 520 km length, is considered to be the blue artery of the eastern region of Morocco. It has a major socioeconomic importance; it is used for irrigation. It is also a source of drinking water and has a big wealth of flora and fauna aquatic [1]. Fishery resources are among the most present animals in the river. The study of Melhaoui and Boudo in 2009 [2] revealed the presence of 29 species. The 29 species are distributed in the upper, middle, and lower regions of Moulouya. The abundance of fishery resources is more in the lower part, with an important activity of fishing along the river. On the contrary, at the higher and medium level, fish is not much varying, is very disseminated, and has weak density. This shows us that the fishing is mainly focused at the level of lower Moulouya.

The local industrial (oil mills, the Sucrafor sugar factory in Zaio), agricultural (the plain of Triffa and of Sebra), and urban activities (the cities: Taourirt, Zaio, Aklim, Berkane) can affect the quality of waters in this river by the discharge of many contaminants [3–5], making them inappropriate to use. This contamination will be able to impact negatively on biodiversity of fauna and flora of this river, which leads to intoxication and mortality of fauna, as in the case of July 15th, 2011, when thousands of fish were found dead and floating at the level of lower Moulouya [6].

Among the possible contaminants that can constitute a danger to aquatic life in general and the fish fauna especially are trace metals. They are very toxic even in weak concentrations and are characterized by their resistance to deterioration by organisms (not biodegradable), which causes their accumulation in the living organisms (the bioaccumulation) [7, 8]. Therefore, the consumption of fishes contaminated with greater levels of trace metals can pose risks to health of humans. Among the wide range of trace metals contaminating the aquatic ecosystem, a major concern has been focused on cadmium, lead, and mercury.
They are nonessential elements occupying top positions in all lists of toxicants [9, 10].

To the best of our knowledge, there is only one single study on the contamination of the fish of the Moulouya River by trace metals [11]; this study is limited to single species (Anguilla anguilla) at the level of single site, that is, the mouth of the Moulouya.

From this official report, we have gotten the idea of doing this study again, but on different types of fish species of the Moulouya River and in different stations. We led a new study on three different fish species and in a larger zone. This study introduces three main objectives: the first is to use the fish as indicator of the metallic pollution of Moulouya River, the second is to assess the largeness of the consumers’ health risks, and the last is to know which fish species are most contaminated with trace metals.

This study is interested in lower Moulouya, from the confluence of the Moulouya and the Sebra River up to the mouth of Moulouya. This part of the river is chosen because it is the richest in fish in comparison with other zones, and it is known for fishing activities. This study also introduces a spatiotemporal evaluation of the metallic pollution of the fish fauna of lower Moulouya, by determining the most alarming and dangerous concentrations of trace metals (cadmium, lead, and mercury (for the first time)) in the muscles of three fish species.

2. Materials and Methods

2.1. Study Area and Sampling Stations. According to the different sources of water contaminations of the Moulouya River in the zone of lower Moulouya (domestic, industrial, agricultural contamination), the fish samples in this zone were collected from three stations (Figure 1):

(i) Station 3 (35°03′05.1″ N 2°25′43.1″ W): It is at the level of the ancient bridge of Ras El Ma-Moulouya, which is located downstream from confluence of the Charaâ River and Moulouya River (Figure 2). It is chosen to estimate the impact of the contributions of the Charaâ River, which drains wastewaters of the city of Berkane on the fish of Moulouya River.

(ii) Station 2 (34°57′01.2″ N 2°29′49.4″ W): This zone is at the level of the area of the farms of Aklim region and downstream from confluence of the Lakhmis River and Moulouya River. It is characterized by a good extension of agricultural fields (Figure 2). It was chosen for search due to the possible pollution of fish caused by agricultural activities and for the purpose of assessing the impact of the contributions of the Lakhmis River, which drains wastewaters of the city of Aklim on the fish of Moulouya River.

(iii) Station 1 (34°53′11.2″ N 2°39′38.9″ W): It is located at the level of confluence of the Moulouya River and the Sebra River; the latter drains the effluents from the sugar factory (Sucrafor, Zaio) and domestic wastewater from the city of Zaio and mixes them with the waters of the Moulouya River (Figure 2).

2.2. Sampling. Seasonal sampling missions were spread over the four seasons from summer of 2017 until spring of 2018. The fish samples were captured using a combination of fishing rods and gill nets of various mesh sizes; we determined the species to which every fish belonged using keys by Azeroual (2003) [12]. A total of 121 individuals representing 3 species (Lepomis macrochirius, Barbus callensis, and Barbus nasus) were collected. The standard length (cm) and weight (g) of each fish were determined using a vernier caliper and an analytical pocket balance, respectively. The individuals belonging to the same species were conditioned in the same polyethylene bags, which contained a chip of identification assuring their marking, then stocked in a cool box where the temperature is about 4°C; after that, they were routed in the laboratory, where they were frozen at −25°C until the instant of the analysis.

2.3. Metal Analysis. The fish samples were taken to the Regional Laboratory of Analysis and Research of National Office of Food Safety (RLAR, ONSSA) in Tangier, where they were dissected using stainless steel scalpels. We were interested in the edible parts of the fish that were ground and homogenized by a domestic food blender. At least three fish individuals were used as samples for trace metal analysis at each station, and each sample was analyzed three times to get the average value of metal content in fish. Mineralization was performed according to the technique described by AOAC Official Method [13], where a quantity varying between 0.5 g and 0.6 g of the homogenate mixture was treated, in a closed Teflon vessel, with 5 ml of Suprapur (69%) nitric acid (HNO₃) for mercury, and 5 ml of Suprapur (69%) HNO₃ and 2 ml of Suprapur (30%) hydrogen peroxide (H₂O₂) for
lead and cadmium. The digestion flasks were then put on a microwave oven (Berghof speedwave MWS-2) and gradually heated (for 45 min up to 185°C) until all the materials were dissolved. After digestion and cooling to room temperature, the samples were diluted by 50 ml with ultrapure water in polyethylene tubes.

The contents of trace metals were determined for lead and cadmium by a Graphite Furnace Atomic Absorption Spectrometry (GF-AAS) facility (Varian Perkin Elmer, ACE 800), equipped with a fully automated autosampler system. 2.5% NH₄H₂PO₄ and 1% Mg(NO₃)₂ were the applied matrix modifiers. Mercury was quantified by Cold Vapour Atomic Absorption Spectrometry (CV-AAS) (VARIAN FIMS 100). The reducing agent used is stannous chloride (SnCl₂) at 2.5%, and the carrier solution is hydrochloric acid (HCl) at 3%. For the two spectrometry techniques, using high purity argon as the carrier gas, the flow rate was 50 ml/min.

2.4. Quality Assurance and Quality Control. All the tools used have been cleaned by soaking overnight in HNO₃ (10%), rinsed with ultrapure water, and dried before each use. In addition to HNO₃, the Teflon vessels have been cleaned with acetone. All of the reagents employed in this study are of analytical grade. The calibration curve demonstrates good linearity for the three trace metals, with correlation coefficients (r) greater than 0.999 (Table 1). The limit of quantification (LQ), the wavelength, and the standard calibration concentration of the present study are presented in Table 1.

According to ISO 17025, the accreditation laboratories that perform analytical service must have quality control procedure for monitoring the validity of tests undertaken [14]. The methods of GF-AAS and CV-AAS were accredited in laboratory (RLAR, ONSSA), and the accuracy of the analytical methods was evaluated by participation to proficiency test schemes. The test materials distributed were canned fish at different concentrations of cadmium, lead, and mercury, obtained from the Food Analysis Performance Assessment Scheme (FAPAS). Replicate analysis of these proficiency tests showed good accuracy, with recovery rates for trace metals between 97.67% and 100.46% (Table 2).

3. Results and Discussion

The number of fish individuals caught for each station and the biometric parameters (weight and length) of the fish species are summarized in Table 3.

The results of the spatiotemporal measure of metallic contents in the muscles of the different captured fish species are presented in Table 4 and Figure 3.

At all the stations, the results cadmium concentration in the analyzed fish species are low. They vary from 0.001 mg/kg of wet weight in *Barbus callensis* and *Lepomis macrochirus* of station 2 to 0.006 mg/kg of wet weight in *Barbus callensis* captured in station 1 (Table 4).

Similar results were found in the muscles of *Barbus meridionalis* of the Ripoll River in Catalonia [15], of *Luciobarbus graellisi*, *Rutilus rutilus*, and *Lepomis gibbosus* in station 1 from the Llobregat River to Spain [16], and in *Lepomis gibbosus* from the Salek Lakes in Slovenia [17]. A weak concentration of cadmium was revealed in the muscles of *Barbus callensis* and *Liza ramada* by El Morhit et al. [18] and in the muscles of *Barbus barbus* and *Anguilla anguilla* by Boscher et al. [19]. On the contrary, high concentrations of cadmium were obtained by Rajotte and Couture [20], Yi and Zhang [21], and Arantes et al. [22] (Table 5).

No significant correlation (P < 0.05) was found between cadmium and other trace metals in different species of fish in all stations (Table 6).

The order of the bioaccumulation of cadmium in the muscles of the studied fish is slightly high in *Barbus nasus* (Table 7).

Note that the concentration of cadmium in the muscles of studied fish species is much less than the regulation limit of cadmium in the flesh of fish (0.05 mg/kg of wet weight) according to Commission Regulation (EC) No. 1881/2006 [28] (the same standard is adopted by Morocco).

The concentration of lead (Table 4) revealed in the muscles of the fish studied varies from 0.016 mg/kg of wet weight in *Barbus callensis* gotten in station 2 to 0.200 mg/kg of wet weight in *Lepomis macrochirus* of station 3. Similar levels were found by El Bouhaliet al. [26], Merciai et al. [16], El Morhit et al. [18], and Djedjibegovic et al. [23]. However, low concentrations of lead were found by Maceda-Veiga et al. [15], Boscher et al. [19], Wariaghli et al. [27], and
Table 1: The wavelength, limit of quantification (LQ), standard calibration concentration, and correlation coefficient (r) for trace metals determination.

| Trace metals | Wavelength (nm) | LQ (µg/kg) | Standard calibration concentration (µg/L) | Correlation coefficient (r) |
|--------------|-----------------|------------|------------------------------------------|----------------------------|
| Cd           | 228.8           | 0.01       | 0, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2           | 0.999830                   |
| Pb           | 283.3           | 2.5        | 0, 1, 2, 4, 8, 16, 32                     | 0.999791                   |
| Hg           | 253.7           | 0.4        | 0, 1, 2.5, 5, 10, 20                      | 0.999902                   |

SD: standard deviation.

Table 2: Trace metals determination in proficiency tests (FAPAS canned fish samples).

| Trace metals | Reference value (mg/kg) ± SD | Observed value (mg/kg) ± SD | Recovery (%) |
|--------------|------------------------------|----------------------------|--------------|
| Cd           | 6.2100 ± 1.5100              | 6.0655 ± 0.2636            | 97.67        |
| Pb           | 0.0526 ± 0.0232              | 0.0515 ± 0.0083            | 97.91        |
| Hg           | 0.1080 ± 0.0455              | 0.1085 ± 0.0129            | 100.46       |

SD: standard deviation.

Table 3: Number of fish individuals, weight, and length by sampling sites.

| Fish species  | Number of fish | Mean weight (g) ± SD | Mean length (cm) ± SD |
|---------------|----------------|----------------------|-----------------------|
| *Barbus callensis* |                |                      |                       |
| S1            | 35             | 44.23 ± 17.11        | 13.46 ± 2.64          |
| S2            | 25             | 43.56 ± 13.72        | 13.41 ± 2.17          |
| S3            | 24             | 44.12 ± 14.92        | 13.77 ± 2.01          |
| *Barbus nasus* |                |                      |                       |
| S1            | 26             | 50.84 ± 18.94        | 14.51 ± 2.54          |
| *Lepomis macrochirus* |      |                      |                       |
| S2            | 11             | 82.72 ± 22.72        | 14.54 ± 2.43          |

SD: standard deviation, S1: station 1, S2: station 2, S3: station 3.

Table 4: Contents of trace metals dosed in the muscles of studied fish species (mg/kg of wet weight).

| Species                | Cd (X ± SD) | Pb (X ± SD) | Hg (X ± SD) |
|------------------------|-------------|-------------|-------------|
| *Lepomis macrochirus*  | 0.00175 ± 0.0005 | 0.001–0.002 | 0.1085 ± 0.0691 | 0.033–0.200 | 0.1083 ± 0.0549 | 0.062–0.187 |
| *Barbus callensis*     | 0.0028 ± 0.001  | 0.001–0.006 | 0.0622 ± 0.0182 | 0.016–0.158 | 0.0549 ± 0.0237 | 0.009–0.161 |
| *Barbus nasus*         | 0.0045 ± 0.0006 | 0.004–0.005 | 0.101 ± 0.0252 | 0.065–0.119 | 0.0985 ± 0.0393 | 0.067–0.154 |

X: mean, SD: standard deviation, m: minimum, M: maximum

Figure 3: Seasonal variation in mean trace metals concentrations in muscles of the three fish species (mg/kg of wet weight).
Petkovšek et al. [17] compared to our results. On the other hand, some studies showed high contents of lead in the muscles of fish, such as those of Yi and Zhang. [21] and Arantes et al. [22] (Table 5).

Very high positive correlations were recorded between the bioaccumulation of lead and mercury at a level of significance (P < 0.05) for the fish in these stations, whose correlation coefficient is 0.9997 (Table 6).

The order of the bioaccumulation of lead in the muscles of the studied fish species is relatively high in Lepomis macrochirus (Table 7).

This signals also that lead concentration in the muscles of studied fish species is much less than the regulation limit of lead in the flesh of fish (0.3 mg/kg of wet weight) according to Commission Regulation (EC) No. 1881/2006 [28]. The results of mercury measured in the fish muscles show that the lowest value is 0.009 mg/kg of wet weight in Barbus callensis captured in station 2, and the highest value is 0.187 mg/kg of wet weight in Lepomis macrochirus captured in station 2 (Table 4).

Close values were found by Boscher et al. [19] in Barbus barbus from the Sûre River in Luxembourg, Petkovšek et al. [17] on Lepomis gibbosus from the Šalek Lakes in Slovenia, and Djedjibegovic et al. [23] in the muscles of different fish species from the Neretva River in Bosna and Herzegovina. However, low concentrations of mercury were found in the muscles of different fish species from the Yangtze River in China [21]. On the contrary, high concentrations of mercury were found in the muscles of Barbus meridionalis from the Ripoll River in Spain [15], in the muscles of Micropterus salmoides from the Sipsey River in USA [24], and in the muscles of different fish species from the Cecina River in Italy [25]. In addition, high concentrations were found in the muscles of Pseudoplatystoma corrugans from the Paraopeba River in Brazil [22] (Table 5).

Mercury and lead are strongly correlated at a level of significance (P < 0.05) for the fish in these stations, whose correlation coefficient is 0.9997 (Table 6).

The order of the bioaccumulation of mercury in the muscles of the studied fish species is relatively high in Lepomis macrochirus (Table 7).

Table 5: Comparisons of recorded trace metal levels in the muscles of the fish species during the present study with literature reported from different areas (mg/kg of wet weight).

| Fish species          | Region                                                                 | Cd     | Pb          | Hg     | References |
|-----------------------|-------------------------------------------------------------------------|--------|-------------|--------|------------|
| Barbus meridionalis   | Ripoll River in Catalonia (Spain)                                       | 0.0004 | 0.0092      | 0.2242 | [15]       |
| Barbus barbus and Anguilla anguilla | Sûre River (Luxembourg)                                               | 0.028, 0.021, resp. | 0.034, 0.034, resp. | 0.096, 0.317, resp. | [19]       |
| Hypopthalmichthys molitrix, Carassius auratus, Coreius heterodon, and Silurus asotus | Yangtze River (China)                                                | 0.062, 0.132, 0.085, 0.115, resp. | 0.529, 0.811, 0.53, 0.55, resp. | 0.006, 0.0079, 0.005, 0.0304, resp. | [21]       |
| Lepomis gibbosus      | Šalek Lake (Slovenia)                                                  | <0.01  | 0.02        | 0.08   | [17]       |
| Cyprinus carpio, Carassius auratus gibelio, and Scardinius erythrophthalmus | Neretva river (Bosna and Herzegovina)                              | 0.013, 0.045, 0.023, resp. | 0.073, 0.055, 0.066, resp. | 0.083, 0.050, 0.080, resp. | [23]       |
| Pseudoplatystoma corrugans | Paraopeba River (Brazil)                                              | 0.07–0.19 | 0.94–3.31   | 0.35–0.41 | [22]       |
| Luciobarbus graellsii, and Lepomis gibbosus | Station 1 of Llobregat River (Spain)                            | 0.007, 0.009, resp. | 0.095, 0.078, resp. | —      | [16]       |
| Barbus callensis and Liza ramada | Loukkos river estuary (Morocco)                                     | 0.02, 0.02, resp. | 0.06, 0.13, resp. | —      | [18]       |
| Perca flavescens      | A range of lakes (Canada)                                              | 0.338–2.598 | —          | —      | [20]       |
| Micropterus salmoides | Sipsey River (USA)                                                   | —      | —          | 0.87   | [24]       |
| Anguilla anguilla, Leuciscus cephalus cabeda, and Chondrostoma toxostoma | Cecina River (Italy)                                               | —      | —          | 0.82, 0.558, 0.65, resp. | [25]       |
| Gambusia holbrooki    | Sebou estuary (Morocco)                                               | —      | 0.0002–0.1967 | —      | [26]       |
| Anguilla anguilla     | Sebou estuary (Morocco)                                               | —      | 0.00036     | —      | [27]       |

Table 6: Matrix of correlation between the different trace metals in different fish species.

|        | Cd           | Pb           | Hg           |
|--------|--------------|--------------|--------------|
| Cd     | 1            | -0.02978876  | 1            |
| Pb     | -0.05063245  | 0.99978242   | 1            |

Table 7: The order of the bioaccumulation of trace metals in the muscles of the studied fish species.

| Trace metals | Order of metal bioaccumulation |
|--------------|-------------------------------|
| Cd           | Barbus nasus > Barbus callensis > Lepomis macrochirus |
| Pb           | Lepomis macrochirus > Barbus nasus > Barbus callensis |
| Hg           | Lepomis macrochirus > Barbus nasus > Barbus callensis |
**4. Conclusions**

According to the present results, we have noted that mercury and lead are both trace metals most concentrated in the muscles of the studied fish (it attained 0.187 mg/kg of wet weight) set by Commission Regulation (EC) No. 1881/2006 [28].

The seasonal variation of trace metals in the muscles of the studied fish species has slightly high concentrations during summer season (Figure 3), in view of the fact that the weak flow of Moulouya River during this season does not seem to dilute the industrial and domestic discharges of tributaries. However, the winter is the season when contents of trace metals are very low, so strong flow of currents leads to the dispersion of trace metals. Moreover, the very high temperatures in the summer could also lead to higher metabolic rates, which could induce an increase in fish food activity, and this in turn increases the concentration of metals in fish [42, 43]. Thus, the concentration of trace metals in a fish is the product of a balance between the concentration of the metal in its environment and its rate of ingestion and excretion by fish [44, 45]. This result is in agreement with the studies of Mazini [46], Orban et al. [47], Obasohan [44], and Kassegne et al. [43].

Although concentration of trace metals in the muscles of the studied species does not exceed the proposed limit values, it is correlated with other contaminants (pesticides, PCB) likely to increase toxic effects on fish-farming fauna [48]. Therefore, further studies are needed to identify all pollutants that may threaten the health of fish and fish consumers in this area and to determine the relationships between them.
weight for mercury and 0.200 mg/kg of wet weight for lead), while cadmium is the metal with the lowest concentration (does not exceed 0.006 mg/kg of wet weight). Station 1 (confluence of Moulouya River with Sebra River) includes fish most contaminated with trace metals, because of their exposure to the domestic discharges of the city Zaio and industrial discharges from the Sucrafor sugar factory in Zaio. *Lepomis macrochirus* is the species with the highest values of trace metals, while the season that records the highest concentrations of trace metals is the summer. All concentrations of three traces metals (lead, cadmium, and mercury) found in the muscles of the different studied fish species have values not exceeding the regulation limit of the European Community (EC), so the consumption of the fish in lower Moulouya of Morocco is not dangerous for human health.

**Data Availability**

The data used to support the findings of this study are available from the corresponding author upon request.

**Conflicts of Interest**

The authors declare that there are no conflicts of interest regarding the publication of this work.

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