

## Bioaccumulation of Cadmium and Lead in the Muscle Tissue of *Mullus barbatus* in Skikda and Jijel Bays Eastern Algeria

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**Abstract.** The bays of Skikda and Jijel present an ecosystem of great biological diversity and a significant economic interest (fishing and trading ports, industrial zones and tourism). They are threatened by the inputs of industrial effluents that are loaded with different substances, especially heavy metals. These pollutants have the distinction of being toxic and non-biodegradable, they accumulate in the different levels of the food chain which represents a danger for human health. The present work aimed to evaluate the impact of metal pollution in both bays via the study of the bioaccumulation of heavy metals namely, cadmium (Cd) and lead (Pb) in red mullet *Mullus barbatus* Linnaeus, 1758. Forty two (42) fish samples were obtained from 4 sites, 2 from bays of Skikda and 2 from bays of Jijel. After preparation, lyophilisation and mineralisation, samples were analysed by Atomic Absorption Spectroscopy (AAS) for detection of Cd and Pb concentrations in  $\mu\text{g/g}$  of dry weights. Lead has reached the values of  $141.666 \pm 5.238$  and  $89 \pm 3.464$   $\mu\text{g/g}$  in Skikda sites and  $20 \pm 1.527$  and  $10 \pm 0.577$   $\mu\text{g/g}$  in Jijel sites, while Cd has reached  $0.76 \pm 0.023$  and  $0.3 \pm 0.011$   $\mu\text{g/g}$  in Skikda, with lowest values in Jijel  $0.116 \pm 0.008$  and  $0.1 \pm 0.005$   $\mu\text{g/g}$ . The highest levels were recorded in the areas that are subject to anthropogenic pollution, namely the port areas and the oil industry (Skikda bays). While the low concentrations were found in less polluted areas like Jijel bays. The results obtained in this study are alarming and reflect a significant level of pollution especially in the bays of Skikda. Preventive and remedial measures and awareness raising are needed.

### Introduction

Marine and coastal ecosystems in many parts of the world are under continuous pressure from industrial activities producing several pollutants whose marine environment is the final receptacle [1]. In aquatic environment, metals may have adverse ecotoxicological and environmental consequences. They are persistent and can be toxic at low concentrations, and can accumulate in biota [2].

The Mediterranean sea is a semi-closed basin rich in biodiversity but vulnerable in the process of degradation, because of its exposure to a set of nuisances due to populated coastline, marine traffic, complex currents, riverine inputs and tidal flow, in addition to the limited water exchange through the Strait of Gibraltar and the Suez Canal [3-5].

The bays of Skikda and Jijel present an ecosystem of great biological diversity and a significant economic interest (fishing and trading ports, industrial zones and tourism). They are threatened by the inputs of industrial effluents that are loaded with different substances, especially heavy metals. These pollutants have the distinction of being toxic and non-biodegradable, they accumulate in the different levels of the food chain which represents a danger for human health.

The use of bioindicators from marine communities, mainly affecting benthic and sessile species, is one of the means of assessing the impact of human activities on marine ecosystems. This

is the principle of quantitative bio indicators based on the fact that marine organisms concentrate contaminants, especially divalent metals, in relation to the concentrations present in the environment [6].

The present work aims to evaluate the impact of metal pollution in both bays via the study of the bioaccumulation of heavy metals namely cadmium and lead in red mullet *Mullus barbatus*.

## Material and Methods

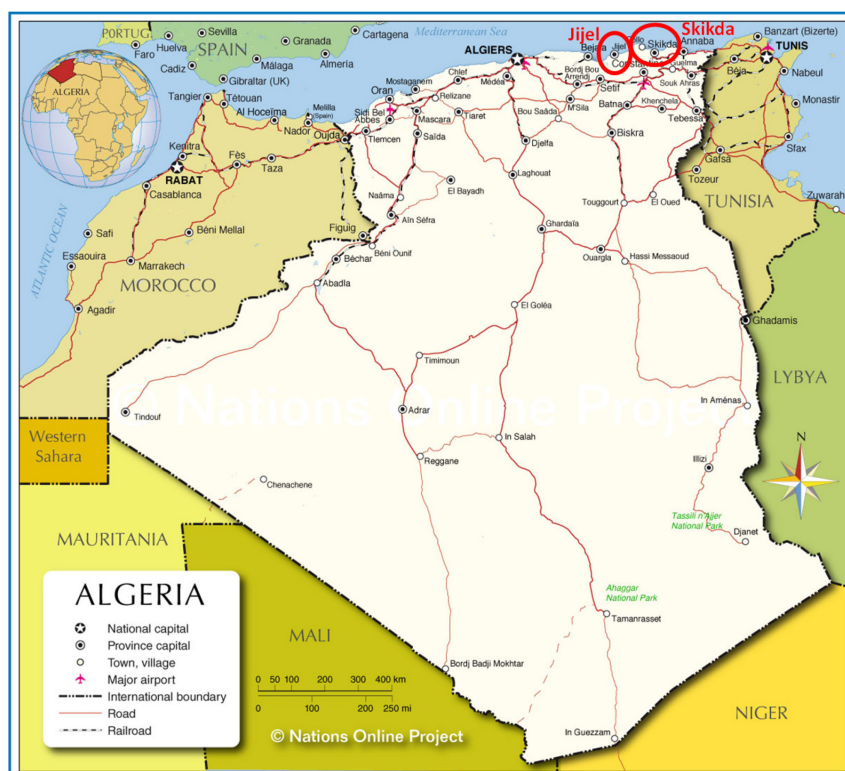
### Study area

The province of Skikda is located in the north-east of Algeria (Fig. 1), it is bounded on the north by the Mediterranean Sea, on the east by the province of Annaba, on the west by the province of Jijel, on the south by Constantine and Guelma, and by Mila to the southwest, between elevations  $36^{\circ} 5'N$  and  $36^{\circ} 15'N$  and longitudes  $7^{\circ} 15'E$  and  $7^{\circ} 30'E$ . Covering a total area of 4137.68 km<sup>2</sup> with a littoral fringe of 142 km of long, representing 12% of the Algerian coastline [7].

The province of Jijel is located in the north-east of Algeria (Figure 1) about 314 km east of Algiers, 99 km east of Bejaia and 135 km north of Setif. Spread over an area of 2,396.63 km<sup>2</sup>, with a coastline of 120 km, it lies between latitude  $36^{\circ} 30'$  and  $37^{\circ}$  North and longitude  $5^{\circ} 30'$  and  $6^{\circ} 15'E$ ; the region belongs to the North Atlas domain known locally under the name of the chain of Babors. It is backed by the mountains of small Kabylie and limited to the North by the Mediterranean Sea. The seafront of Jijel, commonly known as Boulevard Rouibah Houcine is characterized by a predominantly rocky shore. The latter is in the form of a curvilinear. It is directed East-West in the eastern end then it rushes and pivots to become almost North - South in the western part. This configuration has created a relatively sheltered area of agitations from the West sector and to a lesser degree from the favorable northern sector for sandy accumulations that have created a small beach called Rabta Beach.

From the topographical point of view, the height difference of the rocky cliff with respect to the sea level is almost constant of the order of a few meters.

The adjacent seabeds are rocky in slope and irregular underwater topography with the presence, locally, of large blocks of disintegrated rocks and rocky plateaus visible at low tide [8].



**Figure 1.** Map of Algeria highlighting sample sites in Jijel and Skikda provinces.

### *Sampling strategy*

In this work, we selected *Mullus barbatus*, for the monitoring of metallic contamination, as a bioindicator according to the recommendations of FAO-UNEP [9]. In spring 2017, we sampled at two sites: Skikda and Jijel.

**Table 1.** Sampling procedure.

Sites	Date	Male number	Female number	Total number /Site
Skikda	23/03/2017	4	12	16
	07/04/2017	2	6	8
Total	/	6	18	24
Jijel	28/03/2017	1	7	8
	11/04/2017	3	7	10
Total		4	14	18

Samples from the Skikda harbor (n = 24) were treated the same day. And those from Jijel harbor (n = 18) were kept refrigerated for the night and treated the next day.

### **Samples Analysis**

To avoid contamination, any instrument and glassware used in our work are thoroughly cleaned and packaged according to standard protocol. As soon as the samples arrive at the laboratory, the fish are placed on a glass plate cleaned with distilled water. An identification of the species is carried out to keep the species under study and eliminate other species that merge into the sample [10]. A series of biometric measurement is performed on each individual. This first makes it possible to separate the sample into two subgroups as a function of the total length (Lt); Group 1 with Lt <15cm, and Group 2 with Lt ≥15cm. Total length (Lt) is measured using a tip-end ichthyometer to the tip of the intact caudal fin. Our study is conducted on group 2 individuals (Lt ≥15cm), which are adult individuals of minimum market size [11]. The dissection is performed using a new stainless steel material and cleaned with bidistillée water. The right dorso-lateral muscle, without the skin, is taken for the analysis of the metals [10].

#### **Freeze**

Lyophilization, consists in gradually extracting the water contained in a previously frozen product (solid phase) by passing to the vapor phase, without passing through the liquid phase at low pressure; it is sublimation [12]. It was made using a CHRIST-Beta 1-8 lyophilizer, for 48 hours under vacuum (10-1 mbar) and at very low temperature (-50 ° C to -60°C). This method avoids the loss of the most volatile metals, in particular mercury [13].

#### **Grinding and homogenisation**

Freeze-dried biota samples are milled using an automatic stainless steel grinder until a fine powder is obtained. The latter is collected in plastic pillboxes and stored in a dry place. The homogenization is done before mineralization by manual stirring [14].

#### **Extraction of heavy metals**

#### **Mineralization of muscle tissue**

The mineralization was carried out at the Center for Scientific and Technical Research in Physico-Chemical Analysis (CRAPC) Bousmail - Tipaza. According to the protocol described by the IAEA (2001) [14], the mineralization takes place under hood in a Teflon cup. A mass of 0.5 g of dry flesh was weighed in teflon cups, and then a volume of 5 ml of 69% concentrated nitric acid

(HNO<sub>3</sub>) was added. The buckets are sealed with tongs and left at room temperature overnight. Subsequently the cups were placed on a hot plate at 120 °C for 2h30min. After cooling, the samples were transposed into 50 ml polyethylene conical tubes prepared beforehand (No., species, date, etc.). The cups are rinsed several times with bidistilled water, the conical tubes are completed up to 50 ml with bidistilled water. An analysis blank is inserted with each series of samples. The latter is prepared by placing 5ml of 69% concentrated nitric acid (HNO<sub>3</sub>) in a Teflon cup. Series are stored in a refrigerator at 4°C until analysis to avoid loss and contamination [14]. The series of mineralized samples were three tests for each sample (13 samples in total).

### *Analysis of metals by Atomic Absorption Spectroscopy (AAS)*

Atomic Absorption Spectroscopy (AAS) is used for sample analysis. It is a sensitive quantitative analysis technique; it is based on the phenomenon of absorption of light radiation by free atoms [15]. The apparatus used is a Perkin Elmer® Analyst 700 consisting of a photon generator intended to provide a flow of photons of constant intensity in time and with a well-defined frequency corresponding to the element to be assayed, It is equipped with a hydride generator, a graphite furnace and an automatic injector flame system (AS 800 Perkin Elmer) driven by the Winlab 32 software for atomic absorption which allows very high analysis performance. In this study, the assessment of the degree of contamination of *Mullus barbatus* in trace metals is based on a comparison with threshold values taken as reference values.

### *Statistical analysis*

Data were expressed as mean values  $\pm$ SEM from triplicates of independent experiments (n=3). Comparisons were made using one-way ANOVA followed by Turkey's Post hoc test. P<0.05 was considered as statistically significant.

## **Results and Discussion**

The analysis of metallic trace elements, namely lead and cadmium *Mullus barbatus*, used to evaluate the level of contamination at the Jijel and Skikda littoral zones. The results of analysis are summarized in Table 2.

**Table 2.** Concentrations of trace metals in the muscle tissue of red mullet *Mullus barbatus* in Skikda and Jijel bays.

Sites	Pb ( $\mu$ g/g) Mean $\pm$ SEM	Cd ( $\mu$ g/g) Mean $\pm$ SEM
Skikda 1	89 $\pm$ 3.464	0.3 $\pm$ 0.011
Skikda 2	141.666 $\pm$ 5.238	0.76 $\pm$ 0.023
Jijel 1	10 $\pm$ 0.577	0.1 $\pm$ 0.005
Jijel 2	20 $\pm$ 1.527	0.116 $\pm$ 0.008
<i>P</i> values		
Skikda 1 vs Skikda 2	0.00111	0.0000583
Jijel 1 vs Jijel 2	0.0036	0.189
Skikda1 vs Jijel 1	0.0000231	0.000101
Skikda 1 vs Jijel 2	0.0000533	0.000227
Skikda 2 vs Jijel 1	0.0000152	0.00001006
Skikda 2 vs Jijel 2	0.0000239	0.0000139

Results are expressed as mean  $\pm$ SD of dry weight, n=3.

For lead, the highest concentrations in red mullet meat were recorded at two sites, namely the Skikda 1 and Skikda 2 sites with a maximum of (141.67  $\pm$  7.41  $\mu$ g / g) in the site of Skikda 2, and a minimum of (10 $\pm$  0.82 $\mu$ g/g) at the Jijel 1 site. This may be due to the presence of boat species leaks

[16]. The largest flux from lead to ocean comes from the atmosphere [17, 18]. On the other hand, the low lead content is observed at Jijel 1, this site is located far from urbanized areas.

The results also show that the large values of cadmium concentration were observed in the Skikda 2 and Skikda 1 port areas ( $0.76 \pm 0.032 \mu\text{g/g}$  and  $0.3 \pm 0.016 \mu\text{g/g}$  respectively), with maximum at Skikda 2 site ( $0.76 \pm 0.032 \mu\text{g/g}$ ) and a minimum at Jijel 1 site ( $0.1 \pm 0.008 \mu\text{g/g}$ ). This increase may be due to the presence of the port areas activities and the oil industry.

The results of the comparison of trace metal levels in red mullet *Mullus barbatus* with those obtained in other studies at the Algerian coast are reported in Table 3.

**Table 3.** Comparison of trace metal concentrations in *Mullus barbatus* in Jijel and Skikda berries with those obtained in other studies.

Species	Zone of study	Cd $\mu\text{g/g}$	Pb $\mu\text{g/g}$	References
<i>Mullus barbatus</i>	Skikda 1	$0.3 \pm 0.011$	$89 \pm 3.464$	Current study
	Skikda 2	$0.76 \pm 0.023$	$141.666 \pm 5.238$	
	Jijel 1	$0.1 \pm 0.005$	$10 \pm 0.577$	
	Jijel 2	$0.116 \pm 0.008$	$20 \pm 1.527$	
<i>Mullus barbatus</i>	Oran bay	$0.08 \pm 0.02$	/	[19]
<i>Mullus barbatus</i>	Oran bay	$0.09 \pm 0.002$	/	[10]
<i>Mullus surmuletus</i>	Oran bay	$0.15 \pm 0.01$	$0.234 \pm 0.98$	[20]
<i>Mullus surmuletus</i>	Gulf of Arzew	/	1.32	[21]
<i>Mullus surmuletus</i>	Gulf of Arzew	$0.08 \pm 0.02$	$1.32 \pm 0.08$	[22]
<i>Mullus sephalus</i>	Oran bay	$0.3 \pm 0.02$	$0.4 \pm 0.021$	[23]

The analysis of data shows, in general, that the highest concentrations of trace metals are from Skikda bays (Skikda 1, Skikda 2). However, the concentrations of Cd in *Mullus sephalus* obtained in Oran bay are similar to those obtained in the current study in Skikda 1 site. Lead concentrations are very high in coasts of Skikda compared to all the different studies.

For the bay of Jijel, the lead levels are also very high while for cadmium, the values are close to those recorded except for Oran bay which has shown higher values of Pb in *Mullus sephalus*.

With reference to other studies, heavy metals are found in different organs and tissues of fish that are on the top of marine food chain. They accumulate in marine organisms with different ways such as respiratory, adsorption and dietary [24, 25]. The major factors of heavy metals contamination of the environment are heavy metal fabrication and use by different enterprises, fertilizer industry, incinerators, thermal power stations, vehicles, iron-steel, cement and glass production [26]. All these factors require a deep study to confirm their implication in the contamination of marine sites investigated in the present study, and to discuss the possible remedies to reduce this pollution level and to protect fish consumers from heavy metal risks.

The Provisional Tolerably Weekly Intake (PTWI) set by JECFA (Joint FAO/WHO Expert Committee) for Cd and Pb are, respectively:  $2.5 \text{ mg kg}^{-1} \text{ b.w.}$  for Cd and  $25 \text{ mg kg}^{-1} \text{ b.w.}$  for Pb [27]. In this study Cd and Pb concentrations were higher than levels reported in literature for the Mediterranean basin [28,29] and suggest higher pollution levels for fish resources, this implies that the high consumption of these contaminated resources, especially in Skikda province, could indicate a risk of public health.

## Conclusion

According to this study, it can be deduced that lead and cadmium levels in *Mullus barbatus* reflect a big level of marine pollution, particularly in Skikda bays. The highest levels were recorded in the areas that are subject to anthropogenic pollution, namely the port areas and the oil industry in Skikda bays. While the low concentrations were recorded at the lowest polluted areas of Jijel bays.

## Conflict of Interest

The authors declare that there is no conflict of interest.

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