

Evaluation of four metals (Cd, Pb, Zn and Cu) contamination in the superficial sediment and in salema, *Sarpa salpa* caught in the Gulf of Skikda (Mediterranean coast, East of Algeria)

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Abstract. The Gulf of Skikda is subject to a large influx of effluent discharges from the industrial zone "Sonatrach" and wastewater from the city, resulting in heavy pollution of the sea by metallic trace elements (MTE). This study aims to determine the bioaccumulation of heavy metals in the muscle of salema *Sarpa salpa* from gulf of Skikda in the eastern part of the Algerian coast. Elements such as Cd, Pb, Cu, and Zn were assayed using Atomic Absorption Spectrophotometry. The distribution of TME in sediments and fish shows a significant spatiotemporal fluctuation. In muscle, the seasonal variation is related to the physiological state of the fish. Cd and Pb concentrations were above the prescribed levels. The analyzes carried out made it possible to identify the samples of the Mollo station as the most contaminated ones. Indeed, the contamination of *S. salpa* tissues reflects the contamination of the site. Mean concentrations in sediments followed the sequence Zn > Pb > Cu > Cd and in muscles Pb > Zn > Cu > Cd, respectively and this, whatever the season. A significant difference was noticed in the level of TME in the two compartments (ANOVA, $p < 0.001$).

Key Words: heavy metals, pollution, bioaccumulation, muscle tissue, seasonal variation.

Introduction. The pollution of marine environment by heavy metals is of a great interest for scientists (Shulkin et al 2003). Indeed, the metals, existing as trace elements are the normal constituent of the environment (Brayan 1984), unlike to several pollutants (pesticides) which become toxic at certain threshold (Kucuksezgin et al 2006). Thus, the trace metal elements (TME) are either considered as essential (role in the biological processes: Cu, Fe and Zn), or non-essential due to their toxic effects (any role in the biological processes, like Pi and Cd), when their concentration goes beyond certain acceptability threshold (Türkmen et al 2005). In marine environment, the trace metal elements may stay in solution, for being absorbed on the sedimentary particles or precipitate on depth or being ingested by the organisms and accumulate in their tissue until reach the toxic concentrations (Türkmen et al 2005). Hence, these elements are considered as potentially toxic, persistent and able to accumulate in the ecosystem posing serious aquatic environmental pollution (Ikem & Egiebor 2005). The presence of these hazards in marine environment is due to industrial and agricultural wastes, as well as the geochemical structure, the exploitation manner (Yilmaz et al 2007) made directly or through the rivers, the precipitation or the atmospheric deposit (Zoller 2006). There is currently a great interest in the use of living organisms as pollution biomonitors in aquatic ecosystems (Zhou et al 2008). Since fish are at the top of the food web, they are

able to accumulate the metals present in the water column, sediment, and food. Different studies in the laboratory or in the field show that accumulation in metals in the tissues mainly depends on metal concentrations in the environment but also on the duration of exposure or other environmental factors like salinity, pH and temperature (Yilmaz & Yilmaz 2007). Most studies have focused on measuring the accumulation of metals in muscle tissue, for reasons of public health or consumption (Storelli et al 2006; Keskin et al 2007). The present study focused on the Skikda city, this jewel of the Mediterranean, known as an industrial pole, and dominated by the biggest petrochemical complex in North Africa of the international importance. Nevertheless, this industrial zone is established close to coast waters concentrated the polluting sectors. The environmental contamination, in particular, contamination of coastal waters by the industrial wastes leading to serious problems to public health, where the Skikda population is mainly exposed to pollutants due to the increased industrial activities from the beginning of 1970. Skikda has a petrochemical pole with a surface area of 1,200 ha including of 17 high-tech petrochemical industrial units. Furthermore, the studied region contains an estimated population of 982.915 habitants (2012). In absolute value, the average annual increase is 21.575 people. This has led to growing concern about the potential public health effects caused by exposure of the environment to different contaminants. This research aims to evaluate trace metals (Cd, Pb, Cu and Zn) in fish and superficial sediment of three sites of the gulf because of their geographical conditions of different backgrounds. Therefore, we thought that it is useful to compare the concentrations of ETM to both compartments (sediment and fish), in order to understand the phenomenon of transferring pollutants within an ecosystem.

Material and Method

Study and sampling zone. Skikda city is located in the north-east of Algeria, surrounded from the north by the Mediterranean Sea, between latitude of 36°75'N; 36°15'N, and les longitudes of 7°15'E; 7°30'E, covering a land area of 4,137.68 km² along with coastal fringe of 142 km of length, showing, thus 12% of Algerian coastal (Figure 1).

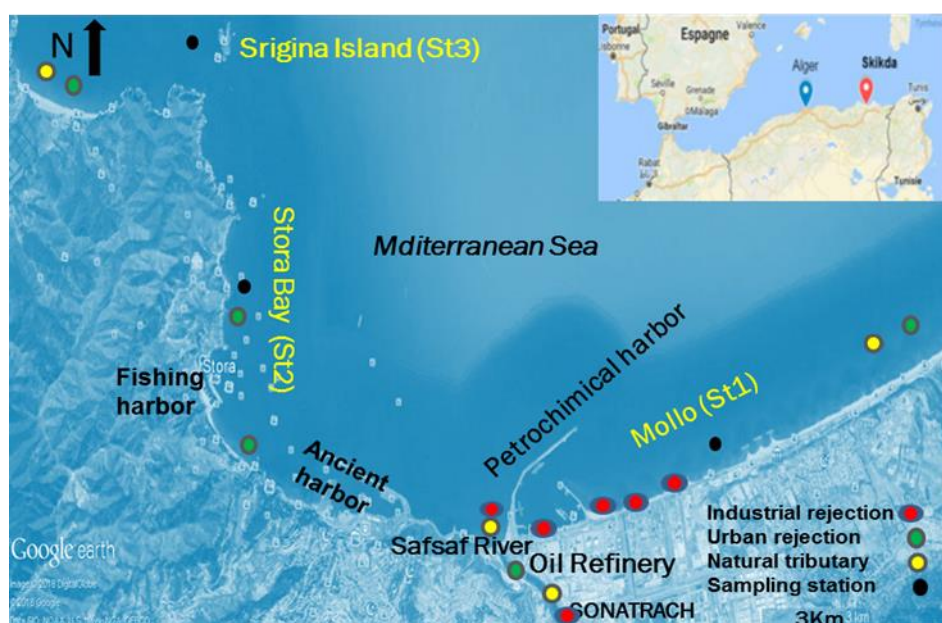


Figure 1. Satellite image of the study area and location of sampling sites and discards in the gulf of Skikda (Google Earth edited).

This study was carried out in Skikda Gulf, characterized by a coastline about 160 km of length. It is limited from the west by the cap Bougaroune, and the Iron cap from the east

between the two longitudes 06°27'10"E and 07°10'02"E (Figure 1). The coastline of Skikda is filled with important structures (fishing, tourism and oil industry) but urban planning threatens the biodiversity and leads to the gradual fade of this coastal ecosystem. We have adopted a sampling a regular quarterly period from winter 2010 to summer 2011, which makes it possible to apprehend the seasonal variations. However, the knowledge of dumping network of the urban and industrial wastewaters, accessibility to site and availability of the biological materials promote to select for the three sampling points presented in Figure 1, in which we find GPS and station characteristics as follow:

Mollo (St1) (6°52'34"E 36°54'94"N): Station located in the middle of Gulf, close to the industrial zone of Skikda (Sonatrach). It receives the tributary of this industrial complex and the hydrographic basin wastes of river Safsaf. The industrial wastewaters were collected by the urban sewage system, which goes to river Safsaf, and are discharged into the environment without any preliminary treatment.

Stora Bay (St2) (6°53'43.94"E 36°53'51.4"N): The developed footpaths containing a small fishing harbor with its fishing boats; has a naval construction activity. Of note, the rivers located at the Stora coast receive a part of untreated wastewaters coming from the habitations located on the top, surrounding the national road number 29. This phenomenon considerably affects the ecological wealth of these rivers, as well as condemns the presence of certain aquatic ecosystems.

Srigina Islands (St3) (6°53'09.93"E 36°56'16.13"N): Island located between the west of roadstead of Skikda. The land area of Srigina Island is estimated as 2.85 ha, receiving the emissary of wastewaters of environmental conurbations.

The superficial sediments (5 cm thick and about 500 g) were collected using polyethylene boxes. The samples taken were kept at low temperature where they will be stored in a freezer in preparation for their analysis. After thawing, the sediment was subjected to a pre-analytical phase, which is drying in an oven at 105°C for 24 hours. The *Sarpa salpa* fish sampling was also carried out during over the period from winter 2010 to summer 2011. A total of 36 fishes, grouped as 3 fishes per site and per season were harvested. The samples were visually inspected, in order to identify *S. salpa*.

Samples preparation and metal determination in sediments and fish. Each sample of fish (muscle) was carefully dissected. To prevent the metal contamination, special care has been taken into account, and the tissues were dissected with special ceramic knife, scissors and plastic clips. The samples were cleaned with double distilled water, and cut out into small pieces (2-3 cm) (UNEP 1982). Thereafter, the tissues were dried overnight in oven at 65°C, and were allowed to cool at ambient temperature, then were pulverized using glass mortar, sieved through 1 mm size mesh, and next they were stocked in containers with hermetic plastics in the interior of the desiccators. The dried fish muscles were digested as described elsewhere (Rahman et al 2012). A slice of *S. salpa* muscle (0.5 g) as a dried powder was added to 2.5 mL of concentrated sulfuric acid and 4 mL of concentrated nitric acid. Then the mixture was heated slowly, using a hot-plate during 20 min at 130°C, followed by cooling at ambient temperature. As a result, the double distilled water content was filtered by Whatman filter (0.45 mm), and thereafter the solution was completed to a final volume of 100 mL. The detection of heavy metals (Cd, Pb, Cu and Zn) in all samples (sediments and fish) was carried out according Flame atomic absorption spectrometric method as described by Aminot & Chaussepied (1983). All samples were collected and analyzed in three replications, and average results were used for data presentations. All elements were afterwards expressed as $\mu\text{g g}^{-1}$ of dry weight.

The amount of superficial sediment collected was cleared of boulders and debris and then a sample of 2 g of sediment was dried in a stove of 110°C, in presence of ammonium nitrate (NH_4), and then was passed through a muffle oven at 450°C (Rodier et al 1996). Concentration of metallic trace elements (Cd, Pb, Cu and Zn) contained in the samples (tissues of plant and sediments) were determined using an Atomic

Absorption Spectrophotometer (AAS) with acetylene flame (Shimadzu model AA-6200). The metal contents of the samples represent the average values obtained from replicas and were expressed in micrograms per gram dry weight. The concentration (C_{me}) of the metallic element was obtained by the formula of Joanny et al (1983).

$$C_{me} (\mu\text{g g}^{-1}) = \frac{CE \cdot V}{M} \quad (1)$$

Where, CE is the read concentration on the calibration curve ($\mu\text{g/mL}$), V is the volume of the final solution after digestion (mL) and M is the mass of mineralized sediment (g). The precision of the method was checked by several measures on the standard reference materials of the international agency of Atomic energy (IAAE): IAEA-407 (fish tissue) and IAEA-SL-1 (sediment of Custer). The coverings were superior to 90% for all the measured metals (Table 1).

Table 1
Analysis of reference materials certified by IAEA 140 (sample *Fucus*) and IAEA-SL-1 (sediment of Custer): certified and obtained values (mean \pm SD)

Metal	IAEA-407 (fish tissue) ($\mu\text{g g}^{-1}$ dry wt)		IAEA-SL-1 ($\mu\text{g g}^{-1}$ dry wt)	Sediment
	Certified	Observed	Certified	Observed
Zn	67.1 \pm 3.8	65.5 \pm 0.7	223 \pm 10	228.04 \pm 14.08
Cu	3.28 \pm 0.40	3.52 \pm 0.18	30.0 \pm 5.6	34.03 \pm 6.39
Pb	0.12 \pm 0.06	0.137 \pm 0.006	37.7 \pm 7.4	37.29 \pm 8.95
Cd	0.189 \pm 0.019	0.187 \pm 0.003	0.260 \pm 0.050	0.26 \pm 0.06

Quality standards. From a regulatory viewpoint, the metallic pollution treatment in the surface sediments and the fish tissues differs from one country to another. In Table 2, we noticed the allowable limits linked to the superficial marine sediments known by the French laws (ABRMC 1991), as well as at the fish tissue levels compared to allowable maximal limits recommended by the world health organization (WHO 2004) and the Environmental Protection Agency of USA (USEPA 2002).

Table 2
International Security standards of heavy metals in sediments and fish (ABRMC 1991; USEPA 2002; WHO 2004)

Heavy metal $\mu\text{g kg}^{-1}$ dry wt	Sediments	Fish muscles
	ABRMC	WHO/ USEPA
Cd	0.6	1
Cu	26	30
Pb	22	2
Zn	88	100

Statistical analysis. The results were expressed as mean \pm standard error (mean \pm SE). The data analyses were performed using the statistical software (Minitab 16, version 1.1.0). Comparisons of multiple parameters (stations, seasons and the rate of each metal) were tested by two-ways ANOVA.

Results

Trace metals concentrations in the sediment and fish. The mean seasonal concentrations of Cd, Pb, Cu and Zn found in superficial sediments and fish from different *S. salpa* locations and seasons are given in Figure 2 and 3. In sediments, the concentrations of Cd and Pb were higher in fall and winter and lowest in spring and

summer. Indeed, of the trace metals are accumulated in the muscle with high concentrations in the winter and summer and small amounts in the fall and spring. Mean concentrations in sediments followed the sequence Zn > Pb > Cu > Cd and in muscles Pb > Zn > Cu > Cd, respectively and this, regardless of season.

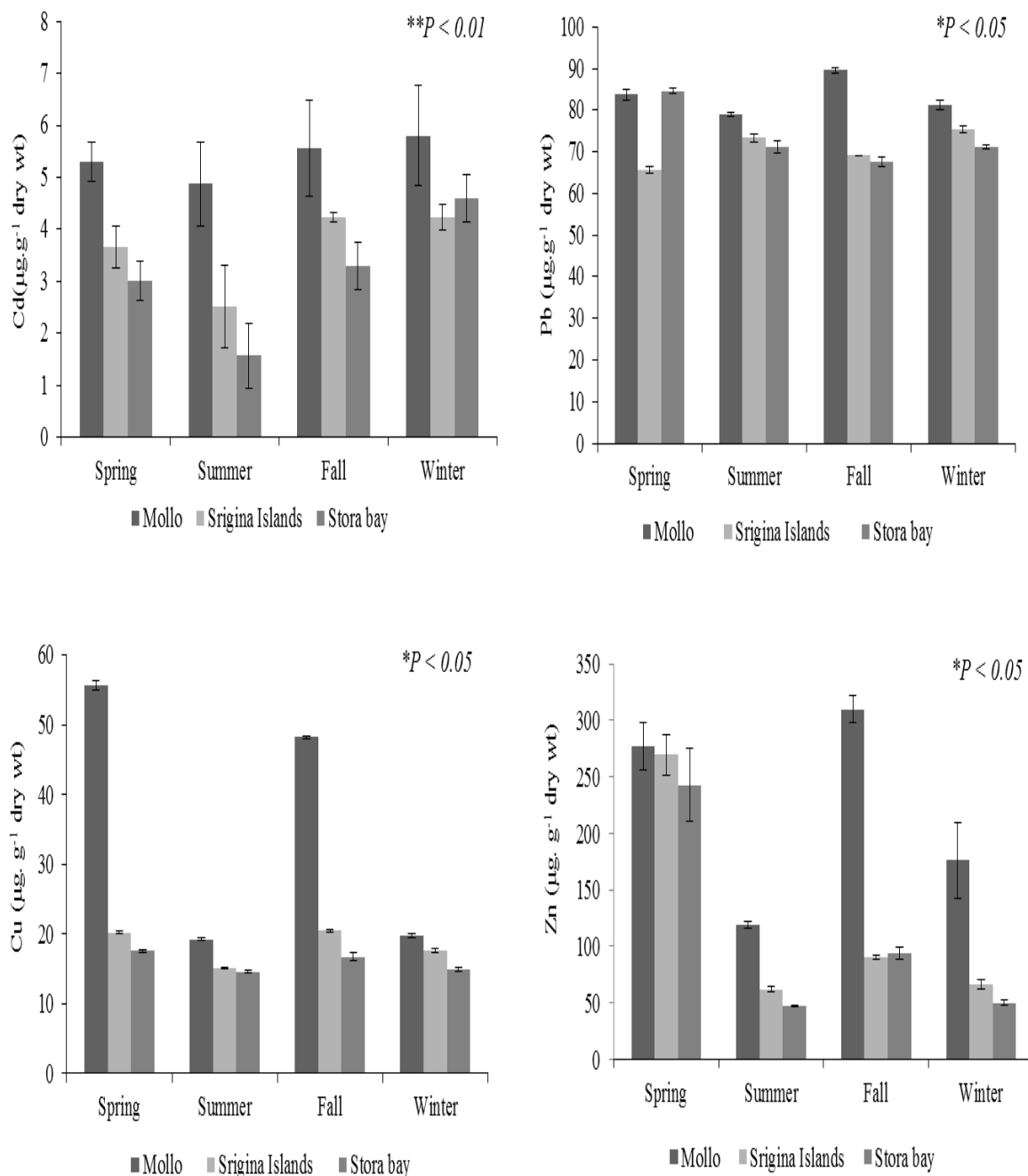


Figure 2. Spatiotemporal variations of mean metal concentration ($\mu\text{g g}^{-1}\text{dry wt}$) \pm SE in superficial sediment of gulf of Skikda.

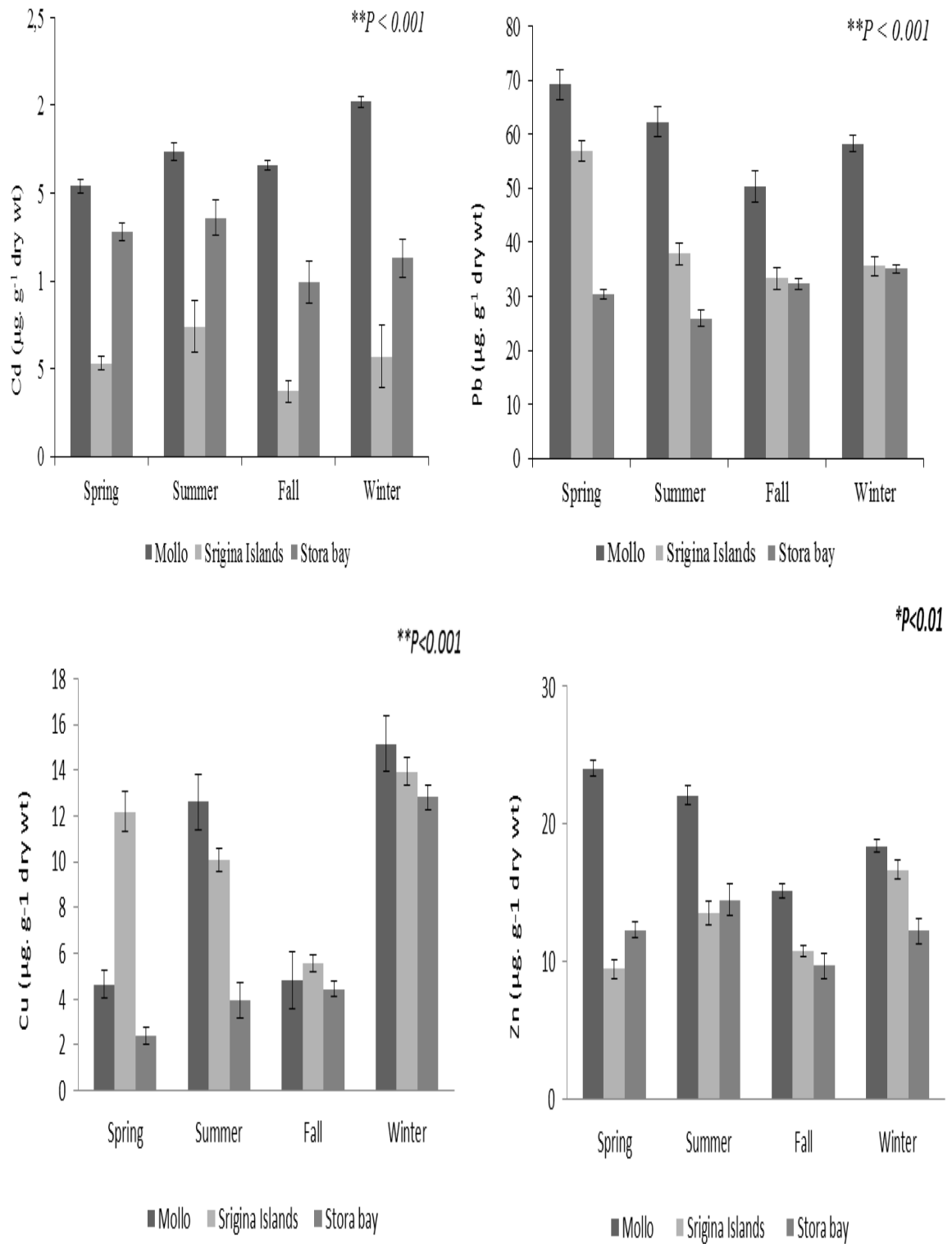


Figure 3. Spatiotemporal variations of mean metal concentration ($\mu\text{g} \cdot \text{g}^{-1}$ dry wt) \pm SE in muscle of *Sarpa salpa* of gulf of Skikda.

For all seasons and stations, the metal concentrations were higher in Mollo station (Table 3). The content of sediment and fish metals was significantly different in the three study sites ($P < 0.001$), Zn and PB rate remained the highest (Table 3).

Table 3

Order of enrichment of the studied stations for each metal in the sediment and fish

<i>ETM</i>	<i>Minimum concentration</i> ($\mu\text{g g}^{-1}$ dry wt)	<i>Maximum concentration</i> ($\mu\text{g g}^{-1}$ dry wt)	<i>Station order</i>
Sediment			
Cd	1.57	5.8	St3>St1>St2
Pb	67.69	89.62	St1>St2>St3
Cu	14.6	55.65	St1>St3>St2
Zn	47.42	309.54	St1>St3>St2
Fish			
Cd	0.53	2,02.	St1>St2>St3
Pb	25.89	69.24	St1>St3>St2
Cu	2.41	15.15	St3>St1>St2
Zn	9.41	24.03	St1>St3>St2

Discussion. The total enrichment order of the fish samples for the metallic elements obeys to their concentration order in sediments. Noteworthy, the higher levels of metals were recorded in fish samples collected from the proximity of human activities, such as Mollo.

Mollo station is the most contaminated; this could be related, on the one hand, by its particular position in the Gulf, it is located near the Safsaf River and receives the urban and industrial wastewater from the Sonatrach complex (oil refinery and liquefied natural gas complex), and others industrials activities (national ENA marble company, Hadjer Soud cement plants, large agro-food units, overall metallurgical) developed around Skikda city. These waters contaminated by copper, zinc and in particular lead and cadmium are collected and evacuated to the river to reach the sea without any prior treatment. The Pb is used as an anti-detonator in gasoline since the contamination by this element is related to anthropic activities (Li et al 2003). The agriculture activity could also promote the increase of Cu, Zn and Cd concentrations. On the other hand, the contamination of sediments of Mollo station, could be explained by the presence of a general current closer to the coast, causing the flow of contaminants to the east (LEM 1998). Currents and hydrodynamism undoubtedly influence the spatial distribution of contaminants in the marine environment. The presence of the fishing harbor subjected to various sources of pollution including two important (urban discharges in the harbor in addition to washing water trawlers and sardines), are the potential sources of pollution of the Srora Bay. The metal contamination of sediments and fish in the far western Gulf (Srigina Island), can be explained by the urban waters drained by the Guebli River. The pollutants will be accumulated in this part of the gulf, semi-closed. Our results are similar to those obtained for the same study area (LEM 1998), but above the French standards (ABRMC 1991) (Table 4).

The direct transfer of chemicals from sediments to organisms is considered to be a major route of exposure for many species (Zoumis et al 2001). In this study we focused on trace metal levels in fish because of their importance for human consumption. Also, it should be noted that pelagic fish species have less concentrated trace elements than demersal and benthopelagic species. This is because benthopelagic species are closer to sediments that are often studied as reservoirs or wells for many chemical pollutants, especially trace elements (Yao et al 2009; Chouti et al 2010). Species that are in contact with sediments will be the most contaminated in accordance with their lifestyle. In fact, the benthic species live in a more polluted environment of the aquatic system and feed on benthic organisms or even mud. It is shown that *S. salpa* is an omnivorous species with seaweed dominance according to Verlaque (1990). This confirms the results of studies conducted in France on the species *Anguilla anguilla* (burrower and feeding in sediments) was found to be the most contaminated by trace elements (Noppe 1996). The same is the situation in the case of *S. salpa*, which has higher levels of Cd and Pb than the maximum allowable limits recommended by the World Health Organization (WHO 2004) and the US Environmental Protection Agency (USEPA 2002).

Table 4

Concentrations of heavy metals found in the sediments of different marine zones in the world

<i>Area</i>	<i>Zn</i>	<i>Cu</i>	<i>Pb</i>	<i>Cd</i>	<i>References</i>
Gabes Gulf, Tunisia (mg/kg)	5.2–7165	3.8–13.9	0.11–950	-	El Zrelli et al 2016
Gulf of Guinea (mg/kg)	2.6–82.2	0.2–29.3	2.1–22.2	0.1–0.4	Mahu et al 2015
Bohai Bay, China ($\mu\text{g g}^{-1}$)	58–332	7.2–63	4.3–138	0–0.98	Zhou et al 2014
San Pietro Island, Italy ($\mu\text{g g}^{-1}$)	0.49	1.45		3.91	Di Leo et al 2013
Annaba harbor, Algeria ($\mu\text{g g}^{-1}$)	190.23–301.17	15.01–60.32	10.04–186.19	0.9–2.62	Belabed et al 2013
Andaman Islands (mg/kg)	10.4– 27.72	6.64–7.04		0.69–1.96	Nobi et al 2010
Toulon Harbor ($\mu\text{g g}^{-1}$)	15–1,880	5.8–1,080	14–710	0.004–3.4	Tessier et al 2011
Skikda, Ancient harbor (Algeria) (mg/ kg)	166.51	59.64	118.73	< 10	LEM 1998
Skikda Gulf, Algeria ($\mu\text{g g}^{-1}$)	150.38	23.34	75.99	4.05	Present study
French Standard	88	26	22	3	ABRMC 1991

In general, changes in trace element levels confirm that bioaccumulation depends on the species considered and physiological capacities for the assimilation and excretion of ingested trace elements (metal elimination results from reproduction and/or from direct excretion), and their anatomy (size, nature of the integuments, contact area with water, etc.) (Miquel 2001; Casas 2005). However, bioaccumulation also depends on the micronutrient concerned (molecular size, chemical speciation, bioavailability, etc.) (Casas 2005). In fact, pollutants whose absorption kinetics is greater than the kinetics of elimination tend to accumulate in the body. Thus, the trace elements can cross the plasma membranes either by passive diffusion or by active transport via ionic ion pumps.

The bioaccumulation of metallic trace elements in the various fish species has been the subject of several studies. Table 5 shows the levels of metallic trace elements obtained by some authors in some species, as well as those of the present work.

Table 5

Review of heavy metal concentrations in the muscle of salema (*Sarpa salpa*) and daurade (*Sparus aurata*) from different locations of the world (mg/kg⁻¹ dry weight)

Area	Species	Zn	Cu	Pb	Cd	References
Honaine Bau (Algérie)	Salema	0.856	1.723	0.005	0.019	Dali et al 2014
Bou smail Bay (Algeria)	Saupe	45	-	0.01	-	Akli 2016
Northeast Mediterranean Sea	Slema	16.48	2.34	2.98	0.45	Canli & Atli 2003
Coast of the island of Gran Canaria (Canary Islands)	Slema	10.18	0.5	0.029	0.0021	Afonso et al 2017
Arsuz (Turkey)		3.131	0.961	1.985	1.441	
Iskudruem Harbor (Turkey)		6.303	1.281	2.291	1.783	
Petrotrans (Turkey)	Daurade	5.184	1.474	2.667	1.404	Türkmen et al 2005
Iskudruem Bay (Turkey)		4.873	1.239	2.314	1.341	
Çamlık Lagoon (Turkey)	Daurade	33.4	-	-	0.13	Dural et al 2006
Black Sea (Turkey) (µg/g)	Daurade	56.3	0.86	0.62	0.5	Uluozlu et al 2007
Sea Bream (Turkey) (µg/g)	Daurade	-	-	120-185	5-18	Creti et al 2010
Iskudruem Bay (Turkey)	Daurade	14.34	6.23	3.80	1.25	Dural et al 2011
Skikda Bay (µg/g)	Salema	14.87	8.55	43.95	1.16	Present study

Conclusions. The research allowed us to determine the levels of trace metals in the superficial sediment of the Skikda Gulf and in the muscle of *S. salpa*. The range of values for these metals can provide, on the one hand, useful information on the transfer of potentially toxic elements from abiotic compartments (sediments and/or water) to higher consumers and, on the other hand, on the existence of anthropogenic contamination of the Gulf due mainly to discharges from all sources without adequate treatment: this should require the installation of treatment plants to reduce pollution. The Gulf of Skikda was subjected to intense entropic pressure. Indeed, strong urbanization and strong industrial harbor and petrochemic activities, contribute to strong coastal pollution.

Conclusively, the Stora bay and Srigina Island form a natural coherent with landscape and natural patrimony, reaching the big national Mediterranean parks, and therefore, it deserves a strong protection.

Acknowledgements. The authors are pleased to acknowledge the Professor Delimi Rachid team leader: Ionic Membrane; Industrial Effluents Treatment and teacher and researcher at Annaba University for the help to conduct the present research.

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Received: 02 May 2018. Accepted: 21 August 2018. Published online: 30 August 2018.

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How to cite this article:

Zeghdoudi F., Tandjir L., Ouali N., Rachedi M., 2018 Evaluation of four metals (Cd, Pb, Zn and Cu) contamination in the superficial sediment and in *Sarpa salpa* caught in the Gulf of Skikda (Mediterranean coast, East of Algeria). *AACL Bioflux* 11(4):1311-1322.