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Cadmium bioaccumulation in three benthic fish species, *Salaria basilisca*, *Zosterisessor ophiocephalus* and *Solea vulgaris* collected from the Gulf of Gabes in Tunisia

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Abstract

To select a marine teleost fish which can be used as a bioindicator of cadmium (Cd) pollution in the Gulf of Gabes in Tunisia, Cd concentrations in liver and gill were compared in three benthic fish species including *Salaria basilisca*, *Zosterisessor ophiocephalus* and *Solea vulgaris*. Fish samples were collected from three selected sites in the Gulf of Gabes, with different degrees of Cd contamination: the industrialized coast of Sfax (S1), the coast of Douar Chatt (S2) and the coast of Luza (S3). The results shows that Cd concentrations in both sediment and water collected from S1 were significantly higher ($p < 0.0001$) than those from S2 and S3. For each species, Cd concentrations, in both liver and gill, showed the decreasing order: S1 > S2 > S3. The highest concentration of Cd was detected in the liver of *S. basilisca*, and only *S. basilisca* showed bioaccumulation factors (BAF) greater than 1 in all studied sites. In S1 and S2, BAF values respect the following order: *S. basilisca* > *Z. ophiocephalus* > *S. vulgaris*. These results of significant bioaccumulation of Cd, in terms of hepatic concentrations and bioaccumulation factors, indicated that *S. basilisca* can be used as bioindicator to evaluate the evolution of Cd pollution in the Gulf of Gabes.

Key words: bioaccumulation; cadmium; Gulf of Gabes; *Salaria basilisca*; *Solea vulgaris*; *Zosterisessor ophiocephalus*; Tunisia
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Introduction

Cadmium (Cd) is considered to be one of most toxic heavy metals. It enters the environment from natural and, essentially, anthropogenic sources (Burger, 2008). Cd dissolved in water or deposit in sediment constitutes a contamination source for the various aquatic food chain links (Romeo and Gnassia-Barelli, 1995).

The study of organisms as pollutant monitors has several advantages over the chemical of abiotic compartments. Organisms only accumulate the biologically available forms of the pollutant and are always present in the environment, thus enabling the continuous monitoring of pollutants. Most of the articles published on organisms as pollution bioindicators have concentrated on invertebrates, mainly molluscs and crustaceans. Marine clams have been proposed as suitable indicators for monitoring trace metal pollution in Tunisian coasts (Dellali *et al*., 2001; Smaoui-Damek *et al*., 2003; Banni *et al*., 2005). Several studies performed under laboratory conditions (Bebianno *et al*., 1993) or in the field (Hamza-Chaffai *et al*., 2000; Smaoui-Damak *et al*., 2003) have indicated that marine clams can accumulate a high amount of Cd in their soft tissues, but without exhibiting marked visible physiological effects. Fish are often at the top of aquatic food chain and may concentrate large amounts of Cd from water, sediment and diet (Mansour and Sidky, 2002). Therefore, the use of fish as indicators for marine pollution monitoring is widely recognized at present (Reddy *et al*., 2001; Marcovecchio, 2004).

The industrialized coast of Sfax in the Gulf of Gabes (Tunisia) concentrates a great number of industrial activities, mainly related to the industry of phosphates as well as others heavy metal transmitters such as salt works, tanneries, lead foundry, textiles, ceramics industry, soap factories, building materials. Several studies conducted in this region showed that the industrial activities are associated to Cd pollution that touched terrestrial (Messaoudi and Ben Chaouacha-Chekir, 2002) and aquatic (Hamza-Chaffai *et al*., 1995; Smaoui-Damek *et al*., 2003; Banni *et al*., 2007; Messaoudi *et al*., 2008) fauna and flora. Therefore, the aim of this work was to select a marine teleost fish which can be used as a bioindicator of Cd pollution in the Gulf of Gabes. For this purpose, the ability to accumulate Cd, in terms of amounts and bioaccumulation factors, was compared in three benthic species *Salaria basilisca*, *Zosterisessor ophiocephalus* and

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Solea vulgaris, collected from three selected sites in the Gulf of Gabes, with different degrees of Cd contamination.

1 Materials and methods

1.1 Study area and sampling

Sampling sites were selected in the Gulf of Gabes, in southeastern coast of Tunisia (Fig. 1). The industrialized coast of Sfax S1, surrounded by important industrial activities, mainly crude phosphate treatments and chemical industries, was chosen as a polluted site. This site is contaminated with heavy metals, essentially Cd (Smaoui-Damak *et al*., 2003). The coast of Douar Chatt (S2), touched only by domestic sewage inputs, was taken as the moderate polluted site. It is 30 km far from (S1) to the north. The coast of Luza (S3), located about 50 km north of S1. This site is apparently unaffected by human activities and used as reference sample site.

Fish samples were collected by professional fishermen with a 4 m beam trawl during September 2005 from the three sites. Water samples were collected at a depth of 0.5 m in clean bottles from the same area. The upper 5 cm of sediments were also collected. Sampling bottles were previously cleaned by soaking with 10% HNO_3 and rinsed with ultra pure water. Fish, water and sediment samples were transported to the laboratory in a thermos flask with ice on the same day.

1.2 Analytical procedures

Cd analysis was carried out only in males to eliminate any possible interference of sex with metal bioaccumulation. As far as possible, fish of similar size and weight were selected for Cd determination. The size and weight of fish samples destined for Cd analysis were measured and given in Table 1. The entire liver and two gill racers from each sample were removed, washed with redistilled water and dried in filter paper. Liver and gill samples were dried at 60°C for 48 h to constant weight in Pyrex test tubes. Dried tissues were weighed and digested with concentrated HNO_3 at 120°C. When fumes were white and the solution was completely clear, the samples were cooled to room temperature and the tubes were filled to 5 mL with ultra pure water (Warchałowska-Śliwa *et al*., 2005). Sea water samples were stabilized at pH 2 with 1 mol/L HNO_3 prior to direct determination of total metal concentrations (Bervoets and Blust, 2003). Sediment samples were oven-dried for 48 h at 100°C and 100 mg of each sample was mineralized at 250°C with a set of acids, composed of 1 mL of nitric acid, 2 mL of fluorhydric acid and 0.5 mL of perchloric acid, and then adjusted to 10 mL with ultra pure water (Annabi *et al*., 2008).

Cd concentrations were determined by atomic absorption spectrometry (AAS) (ZEEnit 700-Analytik-Jena, Germany). Concentrations were measured by flameless AAS with electrothermal atomization in a graphite furnace.

Table 1 Fish samples destined for cadmium analysis

Species	Site	Total length (cm)	Weight (g)
Salaria basilisca	S1	15.38 ± 2.34	28.45 ± 3.65
	S2	16.04 ± 1.58	32.41 ± 4.75
	S3	14.31 ± 3.21	30.17 ± 2.47
Zosterisessor ophiocephalus	S1	14.58 ± 2.47	29.78 ± 4.12
	S2	16.24 ± 3.84	36.12 ± 5.78
	S3	14.00 ± 2.86	33.12 ± 5.02
Solea vulgaris	S1	15.32 ± 3.22	28.17 ± 4.88
	S2	15.41 ± 2.00	34.12 ± 2.75
	S3	13.86 ± 3.65	29.84 ± 4.37

Data are expressed as mean ± SE from 7 males fish in each case.

Table 2 Cd concentrations in water and sediment from S1, S2, and S3

Site	Cd in water (μg/L)	Cd in sediment (μg/g dry weight)
S1	$0.61 \pm 0.02^{a3,b3}$	$1.78 \pm 0.15^{a3,b3}$
S2	0.26 ± 0.03^{a3}	0.22 ± 0.02^{a3}
S3	0.03 ± 0.01	0.05 ± 0.01

Data are expressed as mean ± SE from 7 samples in each case. Significance from S3: a3 $p < 0.0001$; significance from S2: b3 $p < 0.0001$.

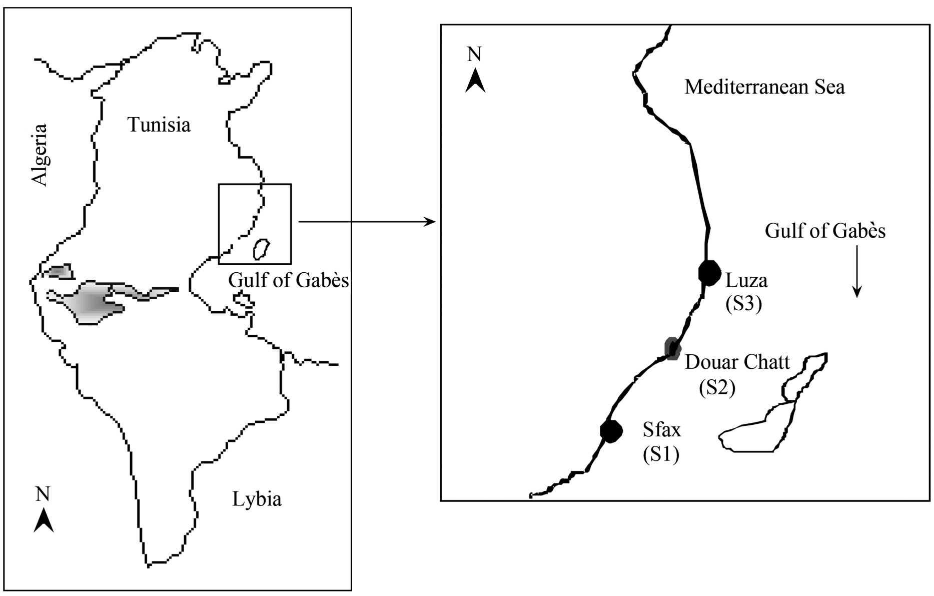

Fig. 1 Localization of the sites sampling in the Gulf of Gabes, Tunisia.

Samples were analyzed in triplicates. The variation coefficient was usually less than 10%. Cd concentrations are reported as μg/g dry weight for liver and sediment samples and as μg/L for water samples.

Bioaccumulation factors of Cd in fish from sediment (BAF) were calculated as the ratio of metal concentration in liver/metal concentration in sediment (Chen and Chen, 1999).

1.3 Statistics

All the data were expressed as mean ± SE (standard error). Differences were assessed by one-way ANOVA followed by PLSD Fisher's test. Values were considered statistically significant when $p < 0.05$.

2 Results

Cd concentrations in water and sediment from the three studied sites are summarized in Table 2. The results show that Cd concentrations in both sediment and water collected from S1 were significantly higher ($p < 0.0001$) than those from S2 and S3. In S2, water and sediment Cd concentrations were also higher ($p < 0.0001$) than those in S3.

As shown in Table 3, Cd concentrations in the three fish species varied according to the sampling site. Fish from S3 always had Cd concentrations significantly lower ($p < 0.0001$) compared to those measured in fish from other two sites. For each species, Cd concentrations, in both liver and gill, showed the decreasing order as S1 > S2 > S3.

Hepatic Cd concentrations measured in the three fish species showed a marked interspecific variation. In the three studied sites, the highest concentrations of Cd were found in the liver of *S. basilisca*. Hepatic tissues of *Z. ophiocephalus* exhibited higher Cd concentrations than those of *S. vulgaris* only in S1 and S2 (Table 3).

In all studied sites, liver tissues of *S. basilisca* have clearly shown a higher capacity to accumulate Cd compared to gill tissues (Table 3). The same pattern is noted in *Z. ophiocephalus* only in S1. In contrast, for *S. vulgaris* collected from S1, gill tissues exhibited the highest Cd concentrations when compared to liver tissues.

Table 3 Cd concentrations in liver and gill of the fish collected from S1, S2, and S3

Sites	Fish	Cd concentrations (μg/g dry weight)	
		Liver	Gill
S1	*S. basilisca*	$4.81 \pm 0.13^{c3,d3}$	$2.79 \pm 0.10^{c3,d3,h3}$
	Z. ophiocephalus	$2.59 \pm 0.16^{c3,d3,e3,f3}$	$0.80 \pm 0.06^{c3,d3,e3,f3,h3}$
	S. vulgaris	$1.35 \pm 0.12^{c3,d3,e3}$	$1.99 \pm 0.15^{c3,d3,e3,h1}$
S2	*S. basilisca*	0.24 ± 0.02^{c3}	$0.12 \pm 0.01^{c3,h3}$
	Z. ophiocephalus	$0.18 \pm 0.02^{c3,e,f1}$	$0.16 \pm 0.02^{c3,e,f}$
	S. vulgaris	$0.10 \pm 0.01^{c3,e3}$	0.12 ± 0.02^{c3}
S3	*S. basilisca*	0.06 ± 0.01	0.03 ± 0.01^{h2}
	Z. ophiocephalus	0.04 ± 0.01^{e2}	0.04 ± 0.01
	S. vulgaris	0.04 ± 0.01^{e2}	0.05 ± 0.01^{e}

Data are expressed as mean ± SE from 7 samples in each case. Significance from S3 for the same species: ^{c3} $p < 0.0001$; significance from S2 for the same species: ^{d3} $p < 0.001$; significance from *S. basilisca* for the same site: ^{e} $p < 0.05$; ^{e2} $p < 0.001$; ^{e3} $p < 0.0001$; significance from *S. vulgaris* for the same site: ^{f} $p < 0.05$; ^{f1} $p < 0.01$; ^{f3} $p < 0.0001$; significance from the Cd concentrations in the liver for the same specie and the same site: ^{h1} $p < 0.01$; ^{h2} $p < 0.001$; ^{h3} $p < 0.0001$.

Figure 2 reports BAF of Cd in the liver of fish from the sediment. *S. basilisca* has remarkably the highest BAF than other two species. Among these three fish species, only *S. basilisca* showed BAF greater than 1 in all studied sites. In S1 and S2, BAF values respect the following order: *S. basilisca* > *Z. ophiocephalus* > *S. vulgaris*.

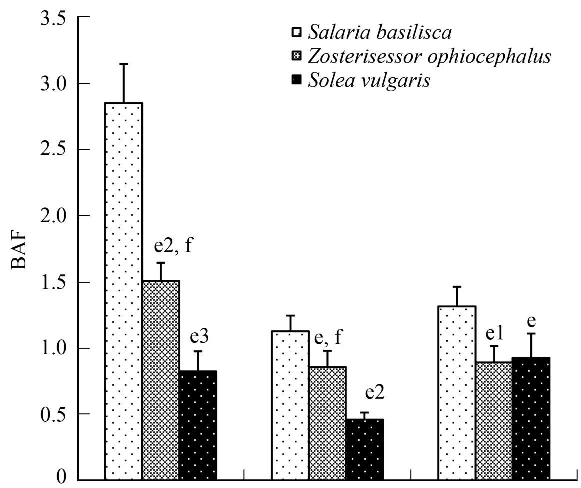

Fig. 2 Bioaccumulation factors (BAF) in liver of fish from the sediment. Data are expressed as means ± SE from 7 samples in each case. Significance from *S. basilisca* in the same site: $^{e}p < 0.05$; ^{e1} $p < 0.01$; ^{e2} $p < 0.001$; ^{e3} $p < 0.0001$. Significance from *S. vulgaris* in the same site: ^{f} $p < 0.05$.

3 Discussion

Benthic animals, which are in contact with sediment, accumulate relatively higher concentration of heavy metals and reflect well the contamination state of the aquatic environment (Aimards *et al.*, 1986; Romeo and Gnassia-Barelli, 1995). Thus, we have studied the bioaccumulation of Cd on fish having a benthic way of life. Among the benthic fish communities inhabiting the Gulf of Gabes, we selected three species: *S. basilisca*, *Z. ophiocephalus* and *S. vulgaris* based on their abundance, facility of sampling all year round as well as their easy identification (Gharred, 1999; Menif, 2000). The three species, in this study, were approximately the same size which would avoid any confounding interpretation of the amounts of Cd bioaccumulated. While age influence on mercury concentrations has been unambiguously shown for several fish species, it is still difficult to distinguish a clear tendency for Cd (Berniger and Pennanen, 1995; Andres *et al.*, 2000). Depending on previous studies, the concentrations of Cd are either positively (Kock *et al.*, 1996) negatively (Pip and Stepaniuk, 1997) or even not correlated (Andres *et al.*, 2000). In the same way, it has been reported that the bioaccumulation with age of fish appeared to be important for Hg (Sadiq, 1992) and is less pronounced for Cd (Sadiq, 1992; Sorensen; 1991; Meador *et al.*, 2005). The influence of age of the studied fish species on the Cd concentrations was determined in previous study (unpublished data). It was applied for each species and the results obtained were mostly non significant and did not enable us to discern clear pattern among the studied variable. Thus, we presented in this work the two main variables acting on the bioaccumulation of Cd: the site of capture and the species considered.

We have found that Cd concentrations in the water and the sediment from the industrialized coast of Sfax were significantly higher than those from the coast of Douar Chatt and the coast of Luza. The coast of Sfax was reported to be contaminated with heavy metals, especially Cd (Hamza-Chaffai and Pellerin, 2003; Smaoui-Damak *et al*., 2003; Banni *et al*., 2005, 2007; Messaoudi *et al*., 2008). In fact, the pollution in this coast is due essentially to the presence of continuous discharge of heavy metals from local industrial activities and from the phosphogypsum stock (Hamza-Chaffai *et al*., 1997). Our results showed that Cd concentrations in fish organs follow a similar trend with the concentrations measured in the sediment and the water. The highest Cd concentrations were found in fish collected from the industrial coast of Sfax. Consistent with our findings, several reports have also indicated high metal concentrations in soft tissues of *Ruditapes decussatus* (Smaoui-Damak *et al*., 2003; Hamza-Chaffai and Pellerin, 2003) and in the fish *Scorpaena porcus* (Hamza-Chaffai *et al*., 1995) from the industrialized coast of Sfax.

The concentrations of metals in the liver represent the storage of metals from the water and the sediment where the fish species live (Romeo *et al*., 1999; Karadede *et al*., 2004). Thus, the liver is more often recommended as environmental indicator organ of external medium pollution than other fish organs. For the three studied sites, *S. basilisca* has hepatic Cd concentrations higher than *Z. ophiocephalus* and *S. vulgaris*. Many factors can be at the origin of these differences between fish species taken in the same sites, essentially diet and physiological characteristics.

Very little is known about the trophic modes, in the Gulf of Gabes, for these species. Because of their close association with the benthic environment, these species feed generally on benthic and epibenthic organisms, including polychaetes, crustaceans, mollusks and ophiouroids (Gharred, 1999; Menif, 2000). As discussed in the available literature there is a lack of data on the trophic mode for each species. It is impossible, in this study, to establish a relationships between Cd accumulation and the consumed diet.

The gills are the main targets of a direct contamination, as they play a significant role in metal uptake, storage and eventually transfer to the internal compartments via blood transport. The gills are the predominant barriers that control the mechanisms of absorption of bioavailable metals from the external medium (direct route of exposure). It is possible that the ability of *S. basilisca* to accumulate larger amounts of Cd in liver than in gills is related to the low excreted rate of this metal or to the deficiency of efficient detoxification mechanisms when compared to *S. vulgaris* which showed the inverse pattern.

BAF indicates whether heavy metal bioaccumulation take place. A BAF greater than 1 indicates bioaccumulation (Rashed, 2001). Among the three fish species, only *S. basilisca* showed BAF greater than 1 in all studied sites, and this means that the fish undergo bioaccumulation of Cd from the sediment. Considering Cd concentrations in the liver and BAF values, *S. basilisca* exhibited a higher capability to accumulate Cd compared to *Z. ophiocephalus* and *S. vulgaris*.

The use of biological indicators of pollution is efficient when basic information on biological and ecological aspects of the assessed environments is available (Olsson and Jensen, 1975). Some requirements are necessary to select suitable bioindicators, particularly abundance and facility of sampling, the non migratory nature and easy identification of the selected organisms and their capability to accumulate the pollutant of interest (Phillips and Segar, 1986; Marcovecchio, 2004).

4 Conclusions

Considering the results of this study and the above mentioned requirement, *S. basilisca* is designated as the best potential bioindicator of Cd pollution among the three proposed species. This is supported by the fact that this species showed the greater ability to accumulate the Cd in terms of amounts and bioaccumulation factors. However, before *S. basilisca* can be used as bioindicator, further studies on its ecology and biology are critical. In addition, the investigation of other factors, such as dietary composition, reproductive cycle and the environmental differences influencing bioaccumulation are required. Since induction of metallothioneins in liver is the main form of storage and detoxication of metals in fish (Hamilton and Mehrle, 1986), it is very important to study the relationship between Cd contamination and metallothioneins induction under both field conditions and laboratory exposure in *S. basilisca*. It would also be of interest to study, in laboratory, the physiological effects of Cd in this species.

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