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RESEARCH PAPER

CRABS AS BIOINDICATORS OF TRACE ELEMENT ACCUMULATION IN MEDITERRANEAN LAGOON (BIZERTE LAGOON, TUNISIA)

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Abstract

Nine crab species samples, males and females, were collected after homogeneously prospection of sediment surface of Bizerte Lagoon. Crabs were caught by dip net from Bizerte Lagoon during spring 2012. Concentrations of metals (Zn, As, Cd, Cr, Cu, Fe, Ni, Pb and Zn) were evaluated in the carapace and muscle tissue of crabs and in surface sediment samples. Concentrations of metals accumulated in the benthic crabs tissues were compared to the reactive metals content that constitute the bioavailable fraction of the sediments. Total organic carbon and carbonate contents were also determined, since they are principal requirements associated with crab development.

Results of this work indicate that, in the study area, the metals that reach the highest concentrations in the sediments-water interface are by decreasing order Zn, Cr and Pb. The reactive concentrations of these metals are also the highest. However, the trace elements that are being accumulated in the carapace and muscle of crabs are mainly As and Cu.

Continental waters flowing into the Bizerte Lagoon are the main source and the principal cause of the enrichment of trace elements in sediment. Results highlight that the reactive concentrations of metals in sediments were the principal cause of their bioaccumulation in the crabs tissues.

The important results of this work highlight that cabs can be very useful on studies of monitoring and evaluation of environmental quality in addition to data obtained from the sediment as they also give information about the bioaccumulation of metals through the oceanic food webs.

Keywords: Sediment. Pollution. Availability of trace elements. Bioindicators. Bioaccumulation. Crabs.

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1 Introduction

Mediterranean lagoons are characterized by important human activities that favor the accumulation of metals in sediments formed by anthropogenic inputs. The accumulation of contaminants affects biota in different levels of food chains both in the water column and in the sediment compartments.

Recent studies provided evidences that the accumulation of toxic trace elements, such as Cd, Pb and Zn affect the living organisms (EEA, 1999; Wright and Mason, 1999; Reimann and De Caritat, 2005; Buccolieri et al., 2006; Zonta et al., 2007; Cukrov et al., 2011).

Crabs can be considered as important components of biota in the sediment-water interface. They interact with the sediment and sedimentary pore-water by producing organic matter and with other living organisms through the food webs.

The crab species present in the Mediterranean basin have a diverse diet which permits the absorption of different chemical elements, at various concentrations, depending on the food source. Availability of trace elements in sediments is dependent and controlled by metal interaction with pH variation, cationic exchange capacity, nutrient status, carbonates and organic matter contents, redox potential and sediments texture, as well as the contamination nature in terms of origin and characteristics of deposition and composition (Rubio et al., 2000, 2010; Liu et al., 2011; Warren et al., 2012; Kalantzi et al., 2013).

The metal industry and aquaculture have significantly increased during the last decades in Bizerte Lagoon, situated in the North of Tunisia. These anthropogenic factors may have induced together a harmful impact on marine environment, including mass mortality of several species of fishes (Sakka Hlaili et al., 2008; Ben Garali et al., 2011).

Previous woks in Bizerte Lagoon about crabs and metals in sediment water interface was discussed by Zaaboub et al. (2015). Concerning sediment toxicity and influence of metal bioavailability on biota, sediments were evaluated by Martins et al. (2015, 2016) and Oueslati et al. (2017).

The purpose of this study was to evaluate the metal accumulation in crabs living in Bizerte Lagoon and to elucidate the possibility of using crabs as bioindicators of trace elements bioaccumulation in food webs.

1.2 Study Area

Bizerte Lagoon, located in northern Tunisia (latitude: 37.1333°-37.2333° N, longitude: 9.7666°-9.9333° E), is connected to the Mediterranean Sea, from its eastern side, through an artificial inlet, a narrow channel 300 m wide (Zaaboub et al., 2016 a). It links with Ichkeul Lake, from its western side, by a channel, approximately 5 km long (Fig. 1; Zaaboub et al., 2016 a).

The lagoon has a surface area of 121.6 km^2 and maximum and mean depths of 12 m and 8 m respectively. Salinity in the lagoon ranges from 33 to 35 p.s.u, whereas in the inlet it is similar to that of sea (36 p.s.u). The total amount of fresh water discharged into the lagoon is about 125 Mm^3 y^{-1}.

The lagoon environment is threatened by anthropogenic interferences (Zaaboub et al., 2016 b). It is being affected by urban and industrial effluents and harboring activities (Martins et al., 2015, 2016). In addition, a large mussel farm operates there, mainly in the eastern zone (Fig. 1; Martins et al., 2015; Zaaboub et al., 2016 a).

2. Materials and methods

2.1. Sampling and processing

2.1.1 Crab sampling and samples preparation

Sixty-nine crab samples, including male and female, belonging to nine crab species, were collected after homogeneous inspection of surface sediment of Bizerte Lagoon. Crabs were caught by dip net, with mesh size of 32 mm, from Bizerte Lagoon, in 2012 spring season. Male and female individuals were caught and kept in polystyrene boxes with ice. When they were brought to the laboratory, they were still alive. Abundance of crabs was assessed during all the sampling period.

2.1.2. Sediment sampling

To access sediment quality in which crabs were living, 10 surface sediment samples were sampled using a Van Veen bottom grab (2000 cm^2) (Fig. 1). Sediment samples were kept in polyethylene flasks at -4°C for immediate or short-time analysis.

2.2. Trace element analysis

2.2.1. Trace element in crabs

The metal analysis was made according to Canli and Atli (2003). The carapace and muscle samples (0.1 g dry weight each) from 69 crab samples, used for metal analysis, were dried at 150 °C until reaching constant weight. Then concentrated nitric acid (4 mL, Merck, Darmstadt, Germany) and perchloric acid (2 mL, Merck) were added to the samples, which were put on a hot plate set to 150 °C until all tissues were dissolved. Inductively coupled plasma mass spectrometer (ICP-MS, Agilent, 7500ce Model) was used to determine chemical elements concentrations. The metal concentrations (Pb, Cu, Fe, Zn, Ni, Cr, As and Cd) were detected as µg metal g^{-1} dry weight, in samples of carapace and muscle separately. The mean value of the measured concentrations in the crab muscles and carapaces are used in this work. High purity multi standard (Charleston, SC, USA) was used for determination of the metals.

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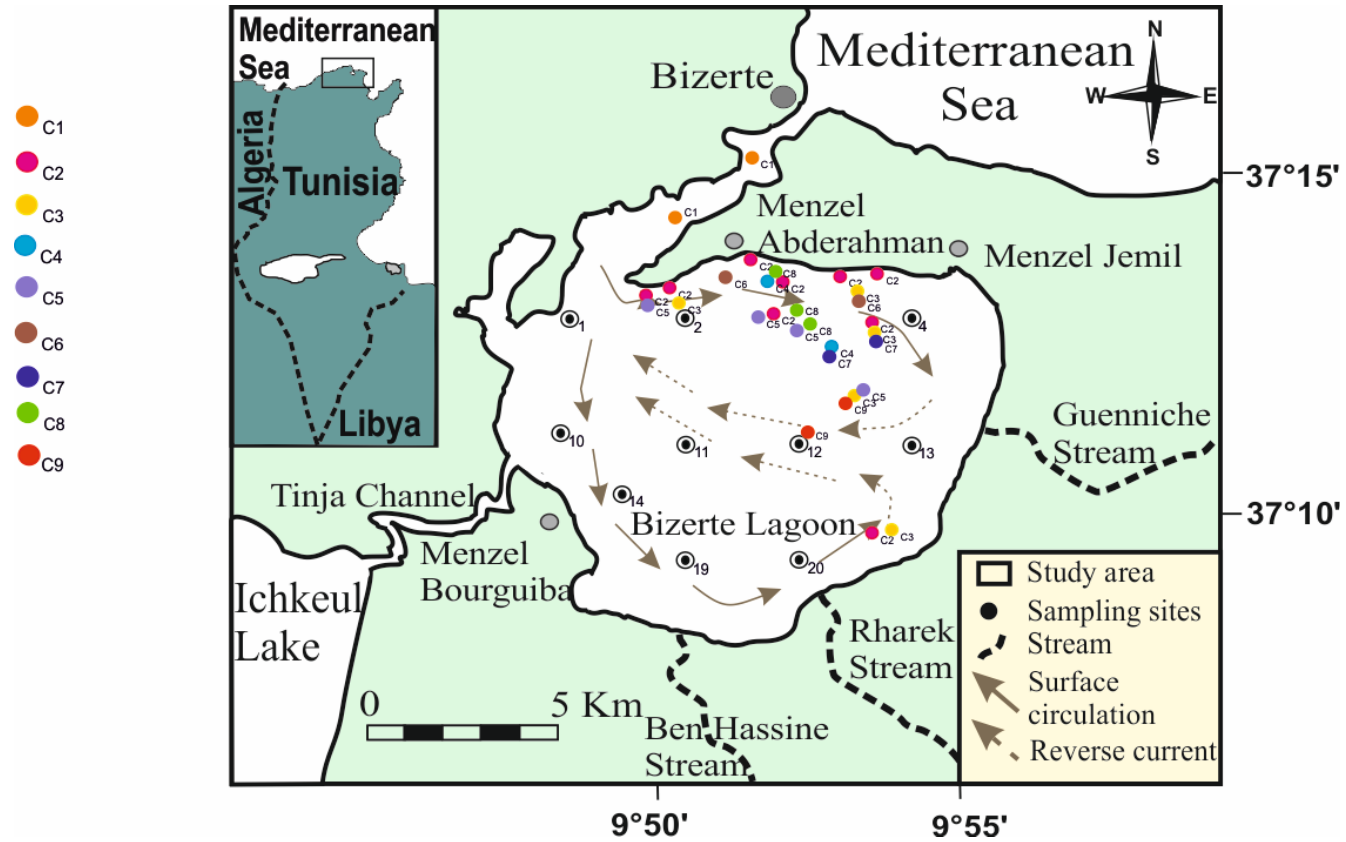

Fig. 1. The study area, sediment and crabs sampling sites. Cn: crabs; C1: *Pachygrapsus marmoratus*; C2: *Carcinus aestuari*; C3: *Pilumnus hirtellus*; C4: *Xantho poressa*; C5: *Ilia nucleus*; C6: *Eucrate crenata*; C7: *Maja squinado*; C8: *Parthenope angulifrons*; C9: *Inachus communissimus*.

In order to assess the accumulation of trace element in crab carapaces and muscle tissues, the bioaccumulation ratio (BAR) was calculated (Ferguson and Chandle, 1998; Mackay and Fraser, 2000; Ruus et al., 2005; Somerset et al., 2015): $BAR_Z = [C_{organism,\ polluted}] / [C_{organism,\ controlled}]$, where *Z* is the studied species, "$C_{organism,\ polluted}$" is the concentration of selected accumulated metal in the lagoon and "$C_{organism,\ controlled}$" is the concentration of metal in crab used as a control species.

The control species, *Maja squinado* was selected from Bizerte Lagoon based on the almost total absence of this species as burying state in the sediment. This species has also been selected in other works (Somerset et al., 2015). This species was considered adequate for this purpose since it presented the lowest metallic contamination in the study area. The same was observed in other studies based on lagoonal crab communities, such as that of Somerset et al. (2015). Indeed, it is a species that essentially lives attached to the hard and rocky substrata (Le Foll, 1993; Palomares and Pauly, 2015).

A second ratio was selected to determine the interaction between crab and sediment-water interface, the biota-sediment accumulation factor (BSAF): $BSAF = C_M(Tissue)/C_M(sediment)$, where C_M is the concentration of metal in tissue and C_M concentration of metal in sediment. The BSAF values which are greater than 1 (>1) indicate the occurrence of accumulation and biomagnification of that pollutant. The BSAF values less than 1 (<1) suggests the occurrence of trophic dilution (Burkhard, 2009).

2.2.2 Metals and Reactive metals in sediment

Metals were analyzed in fine fraction (<63 µm) by ICP-MS, after total digestion of sediment (TD) with four acids ($HClO_4$-HNO_3-HCl-HF) followed by ICP-MS analysis, at ACME Analytical Laboratories, Canada. For reactive metals, 20 ml of 6 N hydrochloric acid was introduced into the flask of sediment sample. The use of strong acid increases the remobilization of metals. The filtered solution was used in the ICP-MS analysis to determine the metals concentrations.

2.3. Total organic carbon

Sediment subsamples for total organic carbon (TOC) analysis were de-carbonated, using 1 M HCl and dried at 60 °C, and then analyzed by means of Perkin Elmer PE 2400CHN. Data are given in percentage of TOC.

2.4. Carbonates

Carbonate minerals analysis is based on the volumetric analysis of the carbon dioxide (CO_2), which is liberated

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during the application of hydrochloric acid solution HCl 4N. Each sample, for the same solution was analyzed three times (i.e., in triplicate). Results are given in percentage of the mean value of the triplicate analyses.

2.5. Statistical analysis

Statistical analysis was performed with XLSTAT (version 2014.5.03) software (Copyright Addinsoft 1995-2014). On the one hand, Principal Component Analysis (PCA, Pearson matrix correlation) was applied to find the relationship between crabs species and trace elements accumulation in its muscle and carapace. On the other hand, PCA was performed to define crab groups relative to the trace element bioavailability in the lagoon. Both PCAs were based on the total number of each species sampled in Bizerte Lagoon. The last type of PCA was applied to set stations according to bioavailable trace element as well as TOC and $CaCO_3$.

3. Results

3.1 Sedimentological results

Metals concentrations (µg/g), $CaCO_3$ (%) and TOC (%) contents in the surface sediments from regularly distributed sites (Fig. 1) in Bizerte Lagoon are presented in Table 1. In the studied sites metals concentrations ranged, by decreasing order for: Zn between 258-219 µg/g (mean 238 µg/g); Cr between 101-120 µg/g (mean 110 µg/g); Pb between 59-78 µg/g (mean 66 µg/g); Ni between 29-39 µg/g (mean 34 µg/g); Cu between 25-38 µg/g (mean 31 µg/g); As between14-24 µg/g (mean 238 µg/g) and; Cd between 02-0.5 µg/g (mean 0.3 µg/g).

Surface sediment in Bizerte Lagoon displays relatively high carbonates content (12-18 %; mean 19.6 %). TOC contents, ranging from 1.1-3.4 % (mean 1.8 %), reach the highest concentrations in the northern part of the lagoon.

Results of concentrations of reactive metals evaluated in selected sites (1 and 4), from the area where the highest abundance of crabs was found, as described in the following item, are presented in Table 2. The most reactive metals in that area are Zn, Pb and Cr (reaching concentrations of 51.4 µg/g, 12 µg/g and 7.4 µg/g, respectively). Reactive concentrations of As, Cu and Ni are relatively low (≈3 µg/g).

3.2 Identified species and their abundance in the study area

Carcinological macrozoobenthos was represented by 9 species: *Carcinus aestuari* Nardo, 1847; *Eucrate crenata* (De Haan, 1835); *Ilia nucleus* (Linnaeus, 1758); *Inachus communissimus* (Rizza, 1839); *Maja squinado* (Herbst, 1788); *Pachygrapsus marmoratus* (Fabricius, 1787); *Derilambrus angulifrons* (Latreille, 1825); *Pilumnus hirtellus* (Linnaeus, 1761) and; *Xantho poressa* (Olivi, 1792).

The abundance of these species in the study area is presented in Table 3. According to this table the most abundant species collected in Bizerte Lagoon were by decreasing order *I. nucleus* (C5: 18 specimens), *C. aestuari* (C2: 12 specimens), *I. communissimus* (C9: 9 specimens), *P. hirtellus* (C3: 8 specimens), *Eucrate crenata* (C6: 8 specimens) and *Derilambrus angulifrons* (C8: 6 specimens). The number of specimens of the other species was lower (2 or 3 specimens). Crabs were mostly found in the northeast and internal central area of Bizerte Lagoon (Fig. 1).

3.3 Concentrations of metals in crabs tissues

Mean values of trace elements concentrations (µg g^{-1}) and respective standard deviation (±SD) estimated in crabs carapace and muscle are presented in Table 4. The mean concentrations of metals measured in crabs tissues reached the highest values for Fe (between 101-2451 µg g^{-1}; mean 510 µg g^{-1}). They reached relatively high concentrations for: As (35- 269 µg g^{-1}; mean 83 µg g^{-1}), Cr (59-190 µg g^{-1}; mean 104 µg g^{-1}), Zn (37-101 µg g^{-1}; mean 77 µg g^{-1}) and Cu (8-39 µg g^{-1}; mean 21 µg g^{-1}). Concentrations of Pb, Ni and Cd in tissues of crabs of Bizerte Lagoon are much lower. The highest standard deviations were observed for Fe, As and Zn.

All the studied carb species display highest concentrations of Fe than the other metals. However, concentrations of metals varied according to the species. For instance, the species with tissues with highest concentrations of: Fe are *I. nucleus*, *I. communissimus* and *P. hirtellus*; As is *I. communissimus*; Cr is *P. hirtellus* and *I. nucleus* and; Zn and Cu is *P. hirtellus*.

Species with highest total concentrations of all these metals are: *I. nucleus* (Σmetals: 2812 µg g^{-1}); *I. communissimus* (976 µg g^{-1}); and *P. hirtellus* (890 µg g^{-1}). The species *D. angulifrons*, *C. aestuarii*, *E. crenata* presented intermediate concentrations for the study area (ranging from 435-496 µg g^{-1}) and *P. marmoratus*, *X. poressa*, M. *squinado* the lowest values (ranging from 329-397 µg g^{-1}).

3.4 Bioaccumulation ratio and biota-sediment accumulation factor

Results of bioaccumulation ratio (BAR) for metals in each species of crabs found in Bizerte Lagoon are presented in Table 5. The highest values of Bar are recorded for Fe (1.7-24.2; mean 5.5), Pb (1.2-6.1; mean 2.5), Cd (0.9-4.6; mean 2.1) and for As (0.6-4.2; 1.3). The Bar values for the other metals are relatively low (maximum values ranging from 1.3 and 2.9 and minimum from 0.5-0.9).

All the analyzed species display BAR values >1 for the analyzed metals. The species that reached highest BAR values are *I. nucleus* (24.2 for Fe), *I. communissimus* (6.1 for Pb) and *P. hirtellus* (4.9 for Fe).

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Tab. 1. Occurrences of crab species in Bizerte Lagoon.

Crab Reference	Crab species	Number of Crabs
C1	*Pachygrapsus marmoratus* (Fabricus, 1787)	3
C2	*Carcinus aestuari* (Nardo, 1847)	12
C3	*Pilumnus hirtellus* (Linnaeus, 1761)	8
C4	*Xantho poressa* (Olivi, 1792)	2
C5	*Ilia nucleus* (Linnaeus, 1758)	18
C6	*Eucrate crenata* (De Haan, 1835)	8
C7	*Maja squinado* (Herbst, 1788)	3
C8	*Parthenope angulifrons* (Latreille, 1825)	6
C9	*Inachus communissimus* (Rizza, 1839)	9

Biota-sediment accumulation factor (BSAF) values evaluated in each studied species of Bizerte Lagoon are presented in Table 6. All the analyzed species displayed highest BSAF values for As (10-79; mean 24), Cr (8-24; mean 13) and Cu (2-6; mean 5). The highest BSAF values are reached for As (79), Cr (24) and Cu (6). The species with highest BSAF values are *I. communissimus, I. nucleus* and *P. hirtellus.*

3.5 Results of Statistical Analysis

Results of PCA aiming to assess the relationship between crab species and trace element accumulation in their muscle and carapace is presented in Fig. 2A. The relationship between crab and trace element bioavailability in the lagoon was also analyzed by PCA (Fig. 2B). In the PCA analysis presented in Figure 2 (A, B), the Bartlett test shows a P-value 0.0022 and an alpha 0.05.

The results of the PCA of Fig. 2A allowed the identification of three main groups composed by the following species: Group I including *I. communissimus*; Group II composed by *I. nucleus* and *P. hirtellus* and Group III containing the other species (*C. aestuaris, M. squinado, E. crenata, P. anguliforns, X. poressa* and *P. marmoratus*).

The results of the PCA of Fig. 2B associated the refereed groups of species I and II to reactive metals. Thus, in Group I, *I. communissimus* is associated to As and Pb reactive metals concentrations and in Group II, *I. nucleus* and *P. hirtellus* are linked to Cr, Zn, Ni and Cu reactive metals concentrations.

4. Discussion

4.1 Biology of the sampled species

Ilia nucleus, the most abundant species in the study area, remains all day buried in the sediment, usually sand, silty or muddy sediment (Debelius, 2001; Çelik et al., 2007; Froglia, 2010). *Carcinus aestuari* is also one of the main species in Bizerte Lagoon, inhabiting the covered areas with seagrass and sandy or muddy areas (Çelik et al., 2007; Froglia, 2010).

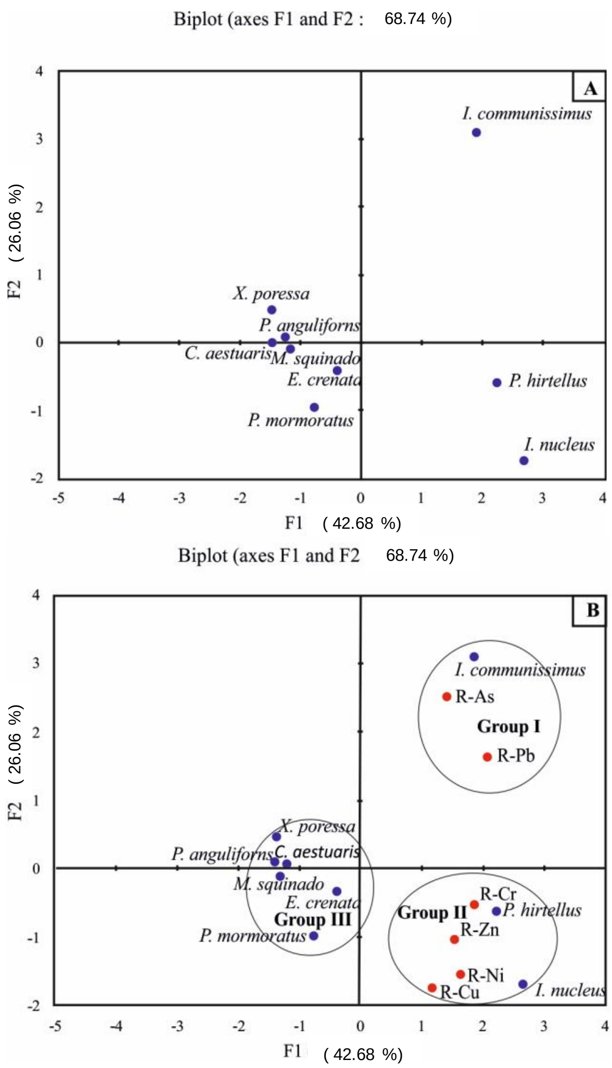

Fig. 2. PCA results. A) Relation between crab species and trace element accumulation in its muscle and carapace. B) Crab groups relative to the trace element bioavailability in the lagoon.

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Tab. 2. Levels values of trace element (Mean±SD) in crabs (μg g^{-1}) concentrations are in the average value between muscle and carapace.

Species	Cd		Pb		As		Cr		Cu		Zn		Fe		Ni	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
C. aestuarii	0.20	0.02	1.24	0.20	69.48	6.51	59.45	3.77	28.84	5.20	81.51	5.59	228.26	32.56	1.90	0.20
I. nucleus	1.07	0.11	3.00	0.31	85.81	6.18	131.49	15.86	39.22	4.41	96.78	6.09	2450.75	153.79	4.11	0.45
P. anguliforns	0.53	0.12	1.02	0.18	50.52	2.79	79.63	2.80	18.00	3.22	67.88	3.37	276.27	37.06	2.49	0.24
E. crenata	0.26	0.02	1.02	0.18	55.65	5.81	100.82	7.52	11.34	1.85	93.74	5.60	169.37	19.44	2.90	0.17
P. marmoratus	0.29	0.02	1.07	0.13	35.42	3.43	90.31	7.14	27.93	3.83	63.42	5.66	175.22	16.81	3.05	0.27
P. hirtellus	0.57	0.07	3.07	0.63	73.26	7.17	189.82	18.89	21.32	3.56	100.61	9.25	498.26	36.89	3.13	0.22
M. squinado	0.23	0.03	0.83	0.13	64.65	8.58	66.34	3.99	17.18	3.20	75.54	5.08	101.38	17.02	2.67	0.31
X. poressa	0.43	0.04	1.35	0.29	41.61	2.28	111.00	24.00	7.99	3.08	36.73	3.73	191.42	17.72	3.02	0.24
I. communissimus	0.56	0.04	5.01	1.41	268.73	24.24	107.89	13.13	16.11	2.20	74.22	8.16	500.54	32.69	2.69	0.22

Tab. 3. Bioaccumulation ratio (Bar) for trace element in each species of crabs found in Bizerte Lagoon.

BAR	Pb	Zn	Cu	Ni	Cr	Cd	Fe	As
C. aestuaris	1.50	1.08	1.68	0.71	0.90	0.88	2.25	1.07
I. nucleus	3.64	1.28	2.28	1.54	1.98	4.63	24.17	1.33
P. anguliforns	1.23	0.90	1.05	0.93	1.20	2.28	2.73	0.78
E. crenata	1.23	1.24	0.66	1.09	1.52	1.12	1.67	0.86
P. mormoratus	1.29	0.84	1.63	1.14	1.36	1.25	1.73	0.55
P. hirtellus	3.72	1.33	1.24	1.17	2.86	2.49	4.91	1.13
X. poressa	1.64	0.49	0.46	1.13	1.67	1.85	1.89	0.64
I. communissimus	6.07	0.98	0.94	1.01	1.63	2.45	4.94	4.16
Average BAR Value	2.54	1.02	1.24	1.09	1.64	2.12	5.54	1.32

Tab. 4. Trace element (μg/g), $CaCO_3$ (%) and TOC (%) content in Bizerte Lagoon surface sediment.

Studied Sites	Pb (μg/g)	Zn (μg/g)	Cu (μg/g)	Cd (μg/g)	Ni (μg/g)	Cr (μg/g)	As (μg/g)	$CaCO_3$ (%)	TOC (%)
1	73.1	249	36.4	0.4	30.9	114	24	21	3.4
2	71.1	243	28.3	0.5	33.6	120	16	16	2.1
4	60.0	237	34.1	0.5	29.4	107	19	28	2.5
13	59.0	231	33.3	0.2	32.4	108	14	23	2.1
20	59.5	225	27.1	0.3	35.5	110	14	20	1.5
19	62.5	236	27.5	<0.1	33.6	106	16	18	1.4
14	77.7	258	38.3	0.4	36.2	119	20	12	1.1
10	74.4	252	25.2	0.3	38.7	109	18	18	1.1
12	61.2	219	25.9	0.2	32.5	101	17	21	1.5
11	64.7	233	31.3	0.3	33.3	109	14	19	1.5
Average	66.3	238.3	30.7	0.3	33.6	110.3	17.2	19.6	1.8

Inachus communissimus inhabits different habitat types, which includes soft sandy - muddy substrates and detritus to bedrock (Martinelli et al., 2011). This species is not limited to the hard substrate.

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Pilumnus hirtellus has a clear preference for hard materials, such as limestone and reef (Debelius, 2001; Froglia, 2010). *Eucrate crenata* is a native of Red Sea but it has been present in Tunisian waters for nearly fifteen years and in Bizerte Lagoon from about 2005 (Shaiek et al., 2010). This crab has an affinity for sandy – clay substrates.

According to Çelik et al. (2007), *Derilambrus angulifrons* (as *Parthenope angulifrons*) is presented in all types of biotopes, including sandy areas, as well as muddy and rocky substrates.

Maja squinado is also a characteristic species of Bizerte Lagoon. It lives preferentially at surface sediment and stands of subtidal algae (Le Foll, 1993; Shaiek et al., 2010). *Pachygrapsus marmoratus* is known for its biotic affinity for hard and rocky bottoms of the intertidal area (Çelik et al., 2007; Debelius, 2001; Froglia, 2010). *Xantho poressa* has a preference for hard substrates and is commonly found on rocky and stony enclaves (Çelik et al., 2007; Palomares and Pauly, 2015).

Tab. 5. Biota-sediment accumulation factor (BSAF) in Bizerte Lagoon.

BSAF	Pb	Zn	Cu	Ni	Cr	As
C. aestuaris	0.09	1.27	6.39	0.45	7.46	20.41
I. nucleus	0.22	1.50	8.70	0.98	16.49	25.21
P. anguliforns	0.07	1.05	3.99	0.59	9.99	14.84
E. crenata	0.07	1.46	2.51	0.69	12.64	16.35
P. marmoratus	0.08	0.98	6.19	0.73	11.33	10.41
P. hirtellus	0.22	1.56	4.73	0.75	23.80	21.52
X. poressa	0.10	0.57	1.77	0.72	13.92	12.22
I. communissimus	0.37	1.15	3.57	0.64	13.53	78.95
M. squinado	0.06	1.17	3.81	0.64	8.32	18.99
Moy BSAF	**0.14**	**1.19**	**4.63**	**0.69**	**13.05**	**24.32**

The crabs' occurrence is influenced by the characteristics of the substrates. According to Zaaboub et al. (2015), the sediment grain size in Bizerte Lagoon is dominated by sandy grain particles from 0-4 m depth and by clay and silt grain particles from 4 to 12 m depth. Sediments grain size and composition are related to rivers runoff and currents activity which influence its composition in terms of mineralogical and geochemical composition, the deposition of organic matter and the biogeochemical processes that occur in it (Martins et al., 2015, 2016).

The crabs' occurrence was highly important where TOC was highest in Bizerte Lagoon, this is in the northern region. This pattern of TOC accumulation seems to affect the crab repartition zone where most samples are found in the northern part of the lagoon. Indeed, and according to Taylor and Allanson (1993), the largest populations of crabs are strongly related to zones impacted by organic matter across the surface of aquatic ecosystems, such as salt marshes and lagoons.

Tab. 6. Reactive metals (R-metal) in Bizerte Lagoon surface sediment (near crab's sites).

Studied Sites	R-Pb (µg/g)	R-Zn (µg/g)	R-Cu (µg/g)	R-Ni (µg/g)	R-Cr (µg/g)	R-As (µg/g)
2	13.4	53	3.6	3.4	7.5	3.0
4	10.6	50	3.9	3.3	7.3	3.4
Average	12.0	51.4	3.8	3.4	7.4	3.2

4.2 Bioaccumulation of metals in crabs tissues

Results displayed in Table 4 evidence that all the species accumulate relatively high concentrations of Fe, mostly *I. nucleus, I. communissimus* and *P. hirtellus.* All the analyzed crab species also accumulate relatively high concentrations of Cr > As >Zn > Cu and very low concentrations of Pb, Cd and Ni in muscles and tissues. This difference could be linked to the direct contact of each species with the sediment and the duration of the interaction with the substrate along the life cycle of each crab.

This information can be completed with the BAR values (Table 5). A BAR value >1 for Fe in all the species means that an accumulation and biomagnification of that pollutant have occurred. The BAR values are <1, for instance for Ni, Cr and Cd in *C. aestuaris* and for Zn, Cu and As in *X. poressa* and for Zn and As for *D. angulifrons* and P. *mormoratus*, meaning that trophic dilution might have taken place in that species (Newman, 2010).

According to the results of this work, Fe is the most accumulated element in crab and Pb is the second most accumulated element (Table 5). A similar result was found in crabs from the southeast coast of India (Batvari et al., 2016). This accumulation was attributed to metabolic rate and exposure route (Ariza et al., 1999; Offem and Ayotunde, 2008).

As noticed by Zaaboub et al. (2015), Zn is the most bioavailable metal in surface sediments of Bizerte Lagoon. This chemical element is mostly bioavailable in the southern part of the lagoon in the opposite sites of crab's localization.

Some authors, such as Somerset et al. (2015), Batvari et al. (2016), and Álvaro et al. (2016) have shown that metals accumulation vary strongly depending on the concerned trace element, as well as the biology and physiology of crab species. On the other hand, most of the analyzed metals are accumulated in small amounts in crabs: Cd>Cr>As>Cu>Ni>Zn.

The BAR index is considered an indicator of accumulation for crabs, since it is evaluated having as a reference a crab species (Somerset et al., 2015). However, for better understanding of the process by which metals are

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accumulated, it is important to analyze the crabs relationship with metals concentrations in the sediment.

4.3 Relationship between metals in surface sediment and crabs

Surface sediments of the western part of the lagoon, near the industrial zone of Menzel Bourguiba and Tinja Channel (Sites 1, 14 and 10) have relatively high concentrations of metals, namely Zn, As and Pb (Table 1). This should be explained by the large concentration of industrial and urban wastes, located close to the western catchment area of Bizerte Lagoon. Else, the enrichment in Pb and Zn in western area of Bizerte Lagoon should be also related to the supply of contaminated sediments by metals derived from mining activities (Decrée et al., 2010). Other reasons for such high metals content should be related to the possible occurrence of biogeochemical processes in the lagoon sediments which contribute to the metals bioavailability (Zaaboub et al., 2015).

On the other hand, residence time of water in the lagoon is high. The water mass needs several months to totally leave the lagoon for the Mediterranean Sea (Harzallah, 2003, Béjaoui, 2008, 2010). Thus, the hydrodynamic pattern of Bizerte Lagoon also contributes to the accumulation of Zn, As and Pb and other metals (Table 1). This time for water renovation is long enough to allow the exchange of metals from sediment-water interface to biota, in our case with crab. It is emphasized that the state of sediment has a strong influence on the geochemistry in Bizerte Lagoon (Daesslé et al., 2009).

According to Zaaboub et al. (2015), the most bioavailable elements in Bizerte Lagoon are Zn and Cr. However, the results of the biota-sediment accumulation factor (BSAF) evidenced that As and Cr are much more highly accumulated elements, than Zn (Table 6). As previously suggested, these results should be dependent on biological factors of crab and exposure time in sediment-water interface (Ariza et al., 1999; Offem and Ayotunde, 2008). Thus, bioavailability of metals in sediment did not always result in accumulation in muscle tissue or carapace of crabs, at least in the same proportion, because metals are accumulated indirectly from food consumption. As known, crabs have an often diversified diet and a varied alimentary behavior (Baeta et al., 2006).

4.4 Crabs as bioindicators of metals accumulation

The metal fractions introduced by anthropogenic activity include the adsorptive and exchangeable and acid soluble phases which are considered to be weakly bound and may equilibrate with aqueous phase. So, in these kind of phases, metals become more rapidly bioavailable for biota mainly when associated with organic matter since the principal source of metals for crabs is diet.

The important role of metal introduced by anthropogenic activity is added to another factor that can mobilize some reactive metals from sediments. Indeed, the Tinja Channel, during the summer season and Rharek Stream, all year (Fig. 1), present high contents of dissolved salts (Ben Garali et al., 2011). The most reactive metals concentrations are Zn and Pb (Table 2), located not far from the output point of the sewage rejects in the area where crabs were collected (Figs. 1, 3). In some specific conditions, reactive metals can be mobilized from the interstitial water and integrate the food webs.

The relationship between crab species and trace element accumulation in its tissues and reactive metals in the lagoon assessed by PCA (Fig. 2, b) show that *I. communissimus* is associated with As and Pb. According to the BSAF factor, the accumulation of As by this species is high (Table 6). Most marine animals have a limit potential to accumulate metals and mainly As from the environment (Sanders et al., 1994). Dissolved forms of As are accumulated in food webs before they reach crab organism (Neff, 2002).

The PCA results of Figure 2 (A, B) also highlights that *P. hirtellus* and *I. nucleus* are linked to relatively high reactive concentrations of Cr, Zn, Ni and Cu. According to BSAF factor results, of this group of metals, Cr is considered the most accumulated trace element of the sediments of Bizerte Lagoon (Table 6). The great affinity of crabs to assimilate Cr to muscle and carapace was also observed by Chiarelli and Roccheri (2014).

The bioaccumulation of reactive metals in *P. marmoratus*, *C. aestuari*, *X. poressa*, *E. crenata*, *M. squinado* and *D. angulifrons* was relatively low in the study area (as suggested by BSAF factor; Table 6). However, results also demonstrated that these species also accumulated reactive metals from the sediment.

The bioavailable fraction found in this work is composed mostly of Zn and Pb (Table 2), and the most accumulated elements in crabs are As and Cr (according to BSAF factor values presented in Table 6). According to Fratini et al. (2008), crabs can accumulate toxic chemical elements, such as As, Cd, Pb and Cu. Concentrations of these elements in their tissues reflects with accuracy the levels of contamination in their proximal environment.

As and Cr are reactive metals present in the studied area as well as Pb, Zn, Cu, Cd, and Ni. The increase of the reactive fraction of those metals in the sediment pore waters seems to have a high influence in concentrations of metals in crabs' tissues. Nevertheless, this affinity seems to vary according to the species, depending on the animal habitat biochemistry, the biophysiology of each species and mainly on its diet. Therefore, our results indicate that the concentration of metals in crabs depends mainly on their concentration in the food web, reflecting the degree of bioaccumulation of metals by the biota of this ecosystem. So, crabs can be seen as environmental indicators of contamination caused by metals enrichment (Somerset et al., 2015; Álvaro et al., 2016).

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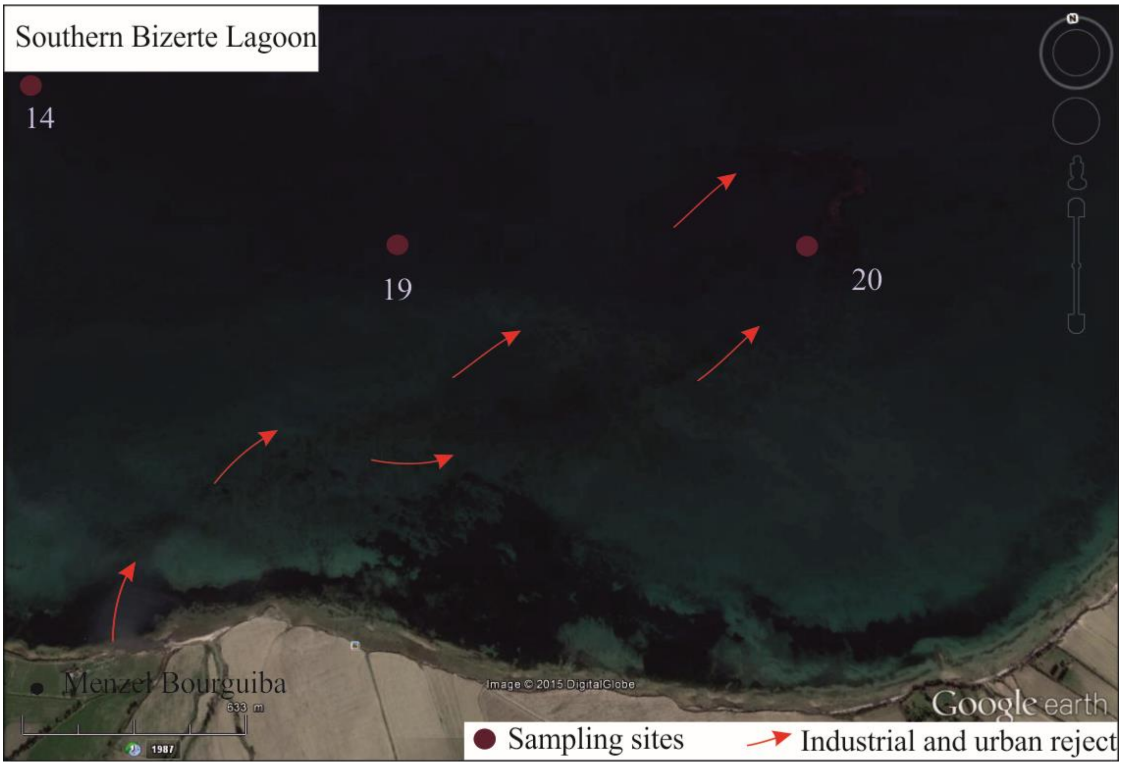

Fig. 3. Satellite photo showing continental sewage plant rejects affecting the southern area of the lagoon.

5. Conclusion

Results of this work show that the nine species of crabs collected in Bizerte Lagoon accumulate relatively high concentrations of Fe > Cr > As >Zn > Cu in muscles and tissues. The animal habitat biochemistry, the biophysiology of each species and its diet influences the accumulation of metals of each species differentially.

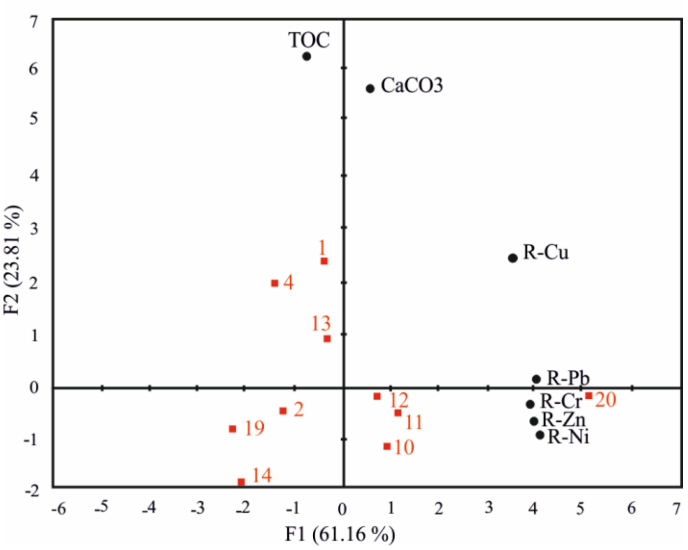

Fig. 4. ACP results, sites distribution and reactive metals, TOC and $CaCO_3$ variation.

In the study area, Zn is the most bioavailable metal in the sediment. It was expected that this metal was transported from the sedimentary interstitial water and integrated into the crabs carapace during its formation process in highest concentrations than other metals. However, the results of the biota-sediment accumulation factor (BSAF) suggested that the nine studied species of crabs accumulated mostly As and Cr from surface sediment.

These results indicated that the metals source accumulated in muscle and carapace of crabs is obtained mainly from food. These important results evidenced that crabs can be considered as potential indicators of metals bioacummulation in food webs. Thus, the evaluation of metals bioaccumulation in crabs can be very useful in studies of environmental quality assessment and monitoring of marine ecosystems. It makes it possible to obtain information about metals bioaccumulation and biomagnification in food webs and to understand how much an ecosystem is impacted.

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