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Biomonitoring of trace metal pollution using the Mollusc Bivalve *Donax Trunculus* and the surface sediment from the Mediterranean coast of Northern Tunisia

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Abstract

In recent years, as many other Mediterranean coasts, the Gulf of Tunis, situated in the northern eastern coast of Tunisia has been subjected to significant urban, agricultural and industrial extension that might have affected the exploitation of its maritime resources and contaminated its coastal environments. Therefore, a seasonal assessment of contamination by trace metals was made using the surface sediment and the most abundant species of bivalve living along this coast (*Donax trunculus*). Zinc, copper, lead, manganese, nickel, chromium and cadmium were analysed by atomic absorption spectrophotometry (Air acetylene) in three sites from the Gulf of Tunis (BorjCedria, Rades and Kalâat El Andalous). Results have shown that sediments from these coasts were considered to be polluted by lead and chromium with the highest metal concentrations found in the surface sediments' samples taken from Kalâat El Andalous. This finding is probably attributed to the fact that Kalâat El Andalous is subject to the impact of the most regular wadi in the Gulf of Tunis, Wadi Medjerda, formerly known by intense mining activities in its catchment area. The clams, *D.trunculus*, commonly consumed in the region, had different amounts of metals in their tissues. Zinc was the most abundant element, while cadmium was recorded at its lowest concentrations and nickel was not at all detected. Seasonal patterns of trace metals accumulation seem to be affected not only by metal availability but also by the reproductive cycle of the species. The evaluation of the risks to human health suggested that *D.trunculus* from these coasts pose no health risk for moderate shellfish consumption. Furthermore, data show that measuring metals in *D.trunculus* themselves does not accurately reflect true contamination levels in the surface sediments from the Gulf of Tunis.

Keywords: Biomonitoring; Bivalve; Donax trunculus; Gulf of Tunis; Sediment pollution; trace elements.

Introduction

With the rapid development of human activities, our environment continually undergoes disturbances mainly due to pollution. The highest levels of contaminants are especially detected in coastal areas and estuaries (Nie et al., 2005, Buccolieri et al., 2006). Indeed, around 80% of the marine environment pollution comes from land-based sources, mainly urban, industrial and agricultural wastes and run-off, (Hildering et al., 2009) often loaded in trace metals, pesticides, hydrocarbons and detergents.

As the Mediterranean is almost entirely landlocked, its waters have a very low renewal rate of 80 to 90 years, making them excessively sensitive to pollution and to the build-up of pollutants. A large number of industries located along the coast regularly pump thousands of tons of toxic waste directly into the sea. As a result, the Mediterranean basin has now become one of the most polluted semi-enclosed basins in the world. Pollution reaches the Mediterranean through its minor and major river systems which carry substantial amounts of urban, agricultural and industrial wastes. These pollution sources are considered to be a major environmental problem in a large majority of countries in the region (EEA, 2005).

Given its interesting geographic position in the Mediterranean sea along the northeastern coastal zone of Tunisia, the study area, the Gulf of Tunis, (160 Km of coast) is one of the most productive areas in Tunisia, as it supports abundant mollusc (416 species), crustacean (120 species) and fish (106 species) populations (Afli et al., 2005) and many of them are harvested for human consumption. For few years, the Gulf of Tunis has been subjected to significant urban, agricultural and industrial extension that might have affected the exploitation of its maritime resources and contaminated its coastal environments. This gulf is particularly sensitive to the accumulation effect due to the geomorphology of the bay and the winds and currents' conditions.

Wastes from these human activities reached the marine environment either from direct discharges into coastal waters or from the two principal Wadis: Medjerda following Kalâat El Andalous plain and Meliane following Rades beach. However, several studies have focused on the assessment of the degree of pollution in the sediment of the Gulf of Tunis by toxic trace metals (Rais, 1999; Added et al., 2003; Ennouri et al., 2010), by polycyclic aromatic hydrocarbons (PAHs) (Mzoughi et al., 2010) and by some residues of polychlorinated biphenyls (PCBs) (Masmoudi et al., 2007).

Heavy metals are considered to be serious pollutants of aquatic ecosystems because of their environmental persistence, toxicity and ability to be accumulated into food chains (Forstner and Wittman, 1983; Demirbas et al., 2005). All metals are toxic above some threshold bioavailable level. In fact, prolonged exposure to toxic metals such as cadmium, copper, lead, nickel, and zinc can cause detrimental health effects in humans such as the development of retardation or malformation, kidney damage, cancer, abortion and even death in some cases where exposure to very high concentrations were noted (ATSDR, 2005, 2008).

Water and sediment have been the matrices mostly used to monitor the trace metal pollution in aquatic ecosystems (Betoux et al., 1990; Chouba and Mzoughi, 2006; El Ati-Hellal et al., 2007; Hoda and Khaled, 2009). Nevertheless, the results defined by theses matrices have not yet been satisfactory and have not yet provided information about the toxic effects upon living organisms. Only toxic trace metals with high bio-availability could be absorbed by organisms and then did great harm to organisms themselves and human beings.

Bivalves especially mussels and oysters have been used extensively as sentinel organisms in the monitoring programs around the world under the design of "Mussel Watch" (Lauenstein et al., 1990) because they are known to be sedentary, suspension feeders, ubiquists, bioaccumulators, and reflect better the magnitude of environmental contamination (Phillips, 1977, 1980, 1990). However, these bivalves are present exclusively in the rocky coasts and they cannot be used as sentinel species in the monitoring programs of sandy beaches' ecosystems. *Donax trunculus* (Linnaeus 1758) (Mollusca, Bivalvia), living in the sandy beaches and largely spread on West African and West European Atlantic and on Mediterranean coasts, exhibits the same characteristics as other mussels in that they are suspension feeders as well as potential bio-accumulators (Moukrim et al., 2004; Tlili et al., 2010; Beldi et al., 2006; Neuberger-Cywiak et al., 2007). In the gulf of Tunis, this species shows a large distribution with high density along the sandy beaches especially in the relatively narrow zone in depths of 30 cm to 50 cm (2- 250 individuals/m^2) (unpublished work).

An awareness of the dangers of pollution to public health has led specialized agencies to reflect on the problem of urban, industrial and agricultural wastes and in particular the future of wastewater. In Tunisia, these waters are treated in sewage treatment stations. They are, then, used for irrigation or discharged directly into the sea. This treatment is far from complete (Masmoudi, 2010), and we would question the impact of these discharges on the aquatic environment.

Therefore, the main objective of this study was to evaluate the concentrations of copper (Cu), zinc (Zn), lead (Pb), cadmium (Cd), chromium (Cr), nickel (Ni) and manganese (Mn) in the sediment and the bivalve, *D. trunculus*, from the Gulf of Tunis. Samples were collected from two polluted sites: Radès(R) and Kalâat El Andalous (KA) and from a comparative reference one: BorjCedria (BC), in an attempt to assess the potentiality of using this bivalve as trace metal biomonitor. Given that, *D. trunculus* are commonly consumed, information on the trace metal concentrations in their tissues is potentially useful when we consider human health implication of the Gulf of Tunis contamination.

Materials and methods

Study sites

The present work was carried out in three coastal sites in the Gulf of Tunis : ‘‘Kalâat El Andalous", close to the Wadi Medjerda, ‘‘Rades" in the proximity of ‘‘Meliane" wadi's mouth and "Borj Cedria" chosen as a comparative reference site which was far from urban and industrial areas (Fig. 1). The Medjerda wadi is the longest and the unique perennial river in Tunisia with a catchment area of about 23,500 km2 and a length of 430km. It carries about 1 078.53 million m^3 of water and 2.2 million tons of sediment annually (MEDD-DGEQV, 2008).

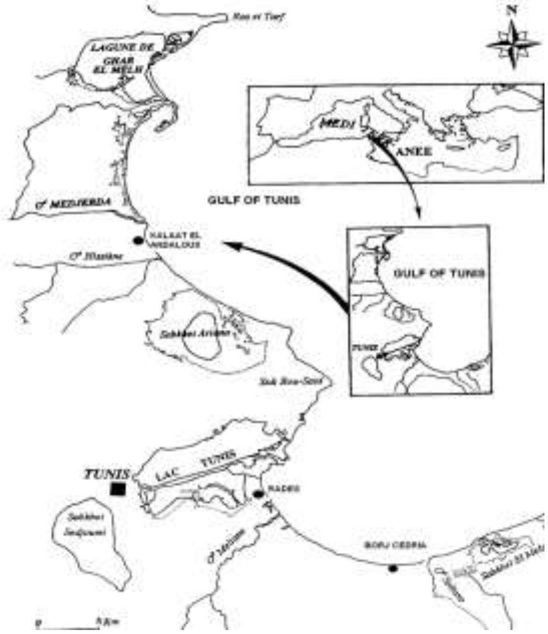

Figure 1: Location of Borj Cedria (BC), Rades (R) and Kalâat El andalous (KA) in the Gulf of Tunis (North Tunisia).

It is, therefore, a crucial waterway for irrigation and plays an important role in the national economy of the country. Sources of water pollution identified in the Medjerda valley consist mainly of the discharge of industrial waste water not connected to the network of the national Tunisian office for sanitation (ONAS); the discharge of treated wastewater from sewage treatment plants (STEP); the discharge of untreated wastewater and the drainage of irrigation water containing pesticides and nitrates (ANPE, 2008). Meliane wadi is the second most important one in Tunisia; with a catchment area of 2280 km2 and 160 km of length. It drains about 26.47 million m^3 of water and 96266 tons of sediment per year (MEDD-DGEQV, 2008) and its waters are highly polluted by 50% of wastewater, in its raw state, into the downstream part of the wadi (Ben Charrada, 1997). The discharge of the two pumping stations of sabkhet Sijoumi waters and the telluric and industrial wastes from three canneries and from a pharmaceutical unit in the area has exacerbated the situation.

Sampling procedure

Specimen of *Donax trunculus* was collected using a specially-designed hand-dredge during four seasons (summer 2004, autumn 2004, winter 2005 and spring 2005). At each station and season, 30 samples of similar size have been selected (21- 30 mm antero-posterior shell length) to prepare a pooled sample to reduce individual variations in heavy metal concentrations (Daskalakis, 1996; Szefer et al., 1997). Collected samples were immediately transported to the laboratory and placed in running filtered sea water (24 hours at least) so that their guts could be emptied before dissection. This way the food contents are expelled avoiding the presence of metals in the mid-gut (NAS, 1980). Stainless steel scalpel blades were used to cut open the clams and remove the soft tissues, which were, then, thoroughly rinsed with Milli-Q water to remove extraneous material. Samples were, then, stored in glass bottles at -30°C ready for analysis. For water content (WC) determination, soft tissues of 30 individuals were dried in an oven in a constant weight at 60°C. [WC% = ((WW-DW)/WW) ×100] where WW: wet weight and DW: dry weight.

Samples of the 15 cm polyvinyl chloride (PVC) corers of sediment were taken by hand at a depth of 30 cm of water column from the same stations where the clams were harvested. Six replicates were collected and mixed at each station and season. The sediment was transferred to polyethylene bag and stored at -30°C until analysis. Following freezing, samples were air dried (Thomas at al., 1994), and finely grounded with an agate mortar and then sieved using vibrating stainless sieves with 63µm mesh size. The fine fraction (<63 µm) was chosen for chemical analysis (Friedler et al., 1994).

Toxic element analysis

Each pooled sample of 30 individuals was weighed, lyophilized (Chiu et al., 2000) and grounded to a fine homogeneous powder with an agate mortar (Ruelas-Inzunza and Paèz-Osuna, 2000) before analysis. Triplicate tissue aliquots of 0.5 g dry weight (DW) were digested in 10 ml of concentrated nitric acid overnight. Following acid digestion, the container was placed upon a hotplate at 100 °C. The temperature was then increased to 250 °C until a transparent solution by total evaporation of nitric smoke has been obtained. After cooling, the solution was transferred into a 50 ml PET bottle and diluted with Milli-Q water. Reagent blanks were processed simultaneously. Samples were analysed for Mn, Cu, Zn, Ni, Cr, Cd and Pb by atomic absorption spectrophotometry (AAS) (Air acetylene) (Thermo elemental (Solaar AA Series) spectrophotometer) (detection limit for all metals = 0.1ppm). For sediment, the total element content was determined by digesting the samples (0.5g) with a mixture of concentrated perchloric acid $HClO_4$ (5ml) and hydrofluoric acid HF (10 ml) overnight. After digestion, the mixture was placed upon a hotplate at 100 °C. The temperature was then increased to 250-300°C until total acid evaporation. The residue, dissolved in 5ml nitric acid HNO_3 and few millilitres of Milli-Q water (perchloric, hydrofluoric and nitric acids, ratio (1:2:1)) was put back upon the hotplate at 100°C to be boiled. After cooling for at least 1 hour, the digested sample was transferred into a 100 ml PET bottle and diluted with Milli-Q water. The

metal content of each sample was analysed in the same way as the organisms were analysed. All analyses were carried out three times, using the external calibration method.

To compare the total metal content at different sampling clams, the metal pollution index (MPI) was used, as stated by Usero et al. (1997).

$MPI = (CF_1 \times CF_2 \times \ldots\ldots CF_n)^{1/n}$,

Where CF_n = concentration of the metal n in the sample, n= the number of analysed minerals.

Reagents and quality assurance

High-purity, deionized water purified with a Milli-Q water purification system was used in preparation of reagents and standards. The digestion and analytical procedures were checked by analysis of blanks and a standard reference materials in every batch digestion (sediment: BCR32; bivalve: GBW-08571 mussel tissus). Triplicate analysis of these reference materials showed a fair level of consistency with the certified values given for the reference material (table 1). Precision was verified by analysing a triplicate sample in every batch digestion. Metal concentrations were calculated in mg/kg dry weight (DW).

Statistics and correlations between parameters:

The results of the seasonal variations of the toxic metal concentrations in sediment of the three sites of collection and in *Donax trunculus* tissues are reported as means ± standard deviations. One-way analysis of variance was performed using the Statistica 6.0. The Tukey Honest Significant Differences (HSD) multiple comparisons test was conducted to determine differences at a significance level of 5 % ($p<0.05$). The Pearson's correlation coefficient was used to study relationships between the metal concentrations in the mollusc bivalve and in the sediments.

Principal components analysis (PCA) reducing the multi-dimensionality of the data and creating new variables from correlations among metals were conducted using STATISTICA ver. 10.0. The number of significant principal components was selected on the basis of the Kaiser criterion with Eigenvalue higher than 1 (Kaiser, 1960).

Table 1. Means and standard deviations of data for the references materials GBW-08571 (Bivalve) and BCR32 (Sediment) (mg/kg dry weight).

	GBW-08571(Bivalve)		BCR32(Sediment)	
Element	Certified values	Found values	Certified values	Found values
Cd	4.5±0.5	4.1 ± 0.4	20.8±1.8	22±2.1
Cr	0.57 ± 0.08	0.52 ± 0.05	257± 12	251± 13.5
Cu	7.7 ± 0.9	7.4 ± 0.04	33.7± 2.5	33.9±1.9
Mn	10.2 ± 1.8	11.1± 1	18.8±0.89	20.1±0.75
Pb	1.96 ±0.09	2 ± 0.03	No certified	No certified
Zn	138±9	140 ± 5	253± 22	250± 23.5
Ni	1.03 ± 0.07	1.12 ± 0.08	34.6± 1.45	31.2± 1.09

Results

Metal concentrations in sediments

The seasonal metal concentrations in the coastal sediments of the Gulf of Tunis are presented in table 2. Metal concentrations ranged from 28.53 to 177.8 for Mn, from 36.48 to 55.89 for Pb, from 3 to 38.5 for Zn, from 1.55 to 8.09 for Cu, from 90.32 to 118.57 for Cr, from 0.1 to 6.43 mg/kg DW for Ni and no detected concentrations of Cd were found in the sediment of the three sites. The highest concentrations of Zn, Ni, Cu and Mn were registered in KA sediment all the year round. However, Cr levels in R sediments (115.2 mg/kg DW) were higher than those recorded in KA (111.7 mg/kg DW) and BC (101.3 mg/kg DW) ($p< 0.05$). So, lead concentrations showed no significant ($p\leq 0.05$) spatial variation. As far as seasonal variation of trace metal concentrations in sediments from each station, we reported no effect of season on their distributions.

Correlation between elements in the sediment

The principal component analysis, applied to our elemental analytical results, showed a differentiation between the samples according to their sampling sites. The elements were correlated with two principal components (PCs) in which

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Table 2. Seasonal metal concentrations (mg/kg dry weight, n=6, means of three replicates ± standard deviation), in the sediment of Borj Cedria (BC), Rades (R) and Kalâat El Andalous (KA). (n: number of cores, 15 cm length, from each station and season).

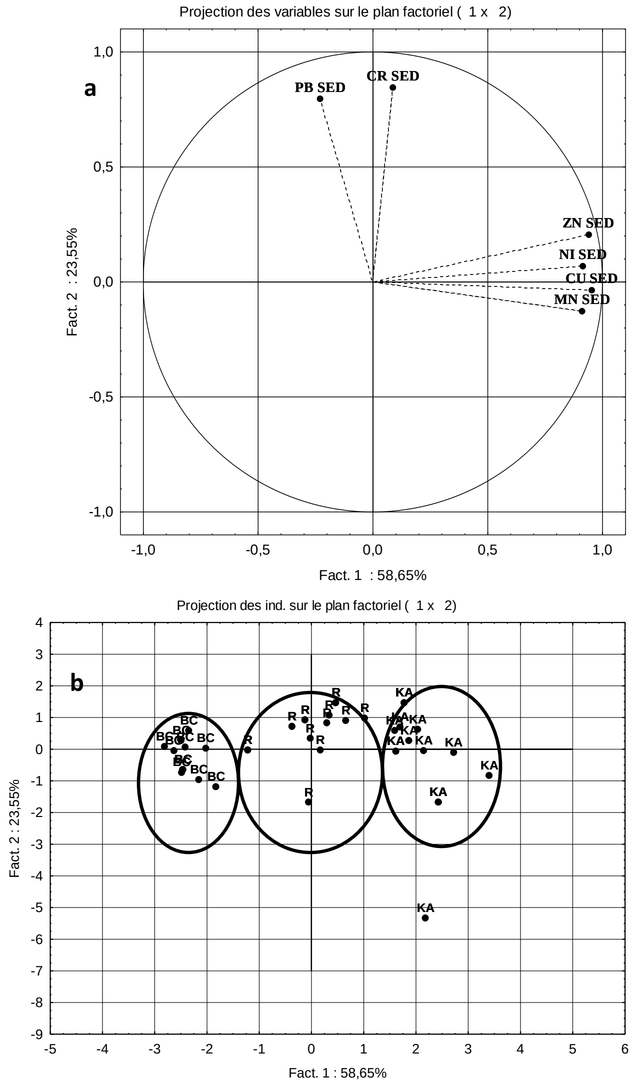
Sampling site	Element						
	Mn	Pb	Zn	Cu	Cr	Ni	Cd
Summer R	99.72±7.8 ^{a}	55.89±3.3^{b}	22.37±0.8 ^{a}	4.3±0.8 ^{a,c}	114.1±9.3 ^{a,b}	3.46±0.4^{a,b}	<0.1
Autumn R	101.67±16.7 ^{a}	49.25±4.9^{a,b}	24.05±0.7 ^{a}	4.49±0.9 ^{a,b,c}	115.29±9.3 ^{a,b}	3.08±0.5^{a,b}	<0.1
Winter R	68.32±16.7 ^{a,b}	54.17±1.8^{b}	34.51±7.4 ^{a}	5.35±1.3 ^{a,b}	112.84±3.1 ^{a,b}	5.07±3.1^{a,b}	<0.1
Spring R	48.54±9.3 ^{a,b}	51.79±4.2^{a,b}	27.70±6.9 ^{a}	4.74±1.3 ^{a,b,c}	118.57±11.2 ^{a}	2.53±2.9^{a,b}	<0.1
Means	79.5	52.77	27.16	4.7	115.20	3.54	<0.1
Summer BC	33.35±1.4 ^{b}	45.89±2.1^{a,b}	8.12±2.7 ^{b}	1.92±0.5^{c}	98.69±2.8 ^{a,b}	0.77±0.9^{a,b}	<0.1
Autumn BC	32.57±2.6 ^{b}	51.3±2.5^{a,b}	6.65±0.6 ^{b}	1.74±1.2^{c}	114.91±4.0 ^{a,b}	0.1±0.05^{b}	<0.1
Winter BC	33.41±2.1 ^{b}	53.5±1.7^{a,b}	4.86±3.7 ^{b}	1.55±0.2^{c}	101.55±2.1 ^{a,b}	1.17±1.5^{a,b}	<0.1
Spring BC	28.53±1.7 ^{b}	51.99±2.5^{a,b}	3.00±0.9 ^{b}	3.25±0.3 ^{c}	90.32±2.3 ^{b}	0.1±0.02^{b}	<0.1
Means	31.9	50.6	5.6	2.1	101.3	0.52	<0.1
Summer KA	162.78±16.8 ^{c}	36.48±2.5^{a}	34.31±0.9 ^{a}	7.64±0.3 ^{b}	107.45±7.2 ^{a,b}	5.27±0.2^{a,b}	<0.1
Autumn KA	175.38±28.6 ^{c}	48.66±3.8^{a,b}	36.81±0.6 ^{a}	8.09±1.7 ^{b}	108.81±13.6 ^{a,b}	6.43±1.3^{a}	<0.1
Winter KA	177.83±35.3 ^{c}	49.95±5.7^{a,b}	38.13±0.3 ^{a}	6.98±0.2 ^{b}	116.07±3.6 ^{a,b}	3.40±2.3^{a,b}	<0.1
Spring KA	129.62±13.0 ^{a,c}	52.62±11.4^{a,b}	38.50±4.3 ^{a}	6.51±0.7 ^{b,c}	114.64±8.7 ^{a,b}	5.30±0.3^{a,b}	<0.1
Means	161.40	46.9	36.9	7.31	111.7	5.10	<0.1

Values in the same column with different superscript letters (a, b, c) are significantly different ($p<0.05$).

82.14% of the total variance in the data were found. Hence, reduced dimensionality of the descriptor space is two. Figure 2 (a) shows the projection of the variables (metals) in the factorial plan (1:2). The first component (PC1), which accounts for 58.6% of the variance among the metals, is characterized by high positive contributions of Mn, Ni, Zn and Cu, which were positively correlated between each other's (Mn/Zn: r= 0.78 ; Mn/Cu: r= 0.88 ; Mn/Ni: r= 0.72 ; Zn/Cu: r=0.80 ; Zn/Ni: r= 0.86 ; Cu/Ni: r= 0.819). However, the percentage of the variance of the second component (23.55 %) represents high positive loads of Pb and Cr (Pb/Cr: r= 0,365, p=0.016).

According to metal concentrations in the fine grain size fraction from our sites, the projection of individuals (each samples from each station) on the same factorial plan (1: 2) (fig. 2b) shows that the KA station contributes positively to PC1. In this station the sediments contain the highest concentrations of Zn, Ni, Cu and Mn. Instead the level of contamination by these metals will be lower in the sediments of station BC which contributes negatively to the axis 1. On the PC 2, the positive contribution of Rades station coincides with a relatively higher contamination of sediment from this station by Cr compared to BC and KA sediments.

Figure 2: Projection of variables (a: correlation circle) and individuals (b) on the first two components (Fact. 1 and Fact. 2) resulting from the principal component analysis (PCA) based on trace metal levels of sediments sampled from three sites in the Gulf of Tunis (BC, R and KA).



Metal concentrations in organisms

Donax trunculus from the Gulf of Tunis has different amounts of metals in their tissues (table 3). In fact, Zinc concentrations were the highest amongst analysed metals (81.05, 82 and 77.39 mg/kg DW in R, BC and KA specimen respectively) and followed in decreasing order by Mn, Cu, Pb, Cr and Cd in R and BC specimens and by Cu, Mn, Pb, Cr and Cd in KA clams. Nickel was not detected in the tissues of *D .trunculus* harvested from all the sampling sites.

No significant differences ($p<0.05$) in the levels of Cr, Zn, Pb and Cd were observed between stations. In contrast, the concentrations of Cu and Mn varied notably ($p<0.01$) depending on the location of the sampling sites: Cu levels detected in KA clams (12 mg/kg DW) were significantly higher than those registered in BC (9.82 mg/kg DW, $p<0.05$) and R specimen (6.28 mg/kg DW, $p<0.001$). However, the highest concentrations of Mn have been reported in *D.trunculus* from R (48.58 mg/kg DW).

Statistical analysis showed no seasonal effect ($p< 0.05$) in the distribution of Pb in the clams from different sites. Amongst all analysed metals, only Cr concentrations varied notably between seasons in the tissues of *D.truncculus* from BC. Indeed, summer Cr levels were significantly lower than autumnal ($p< 0.05$) and Springer ones ($p< 0.05$).

In R clams, the Zn, Cr and Cd varied seasonally with lowest levels recorded either in autumn or in summer. Likewise, the distribution of Mn, Zn, Cu, Cr and Cd in KA clams revealed a significant effect ($p< 0.05$) of season with generally spring maximum levels of Mn, Zn and Cu and autumnal maximum values of Cr and Cd.

Table 3. Seasonal variation in trace metal concentrations (mgkg^{-1}dry weight, n=30, means of three replicates ± standard deviation), metal pollution index (MPI), percentage of moisture and mean bio-sediment accumulation factor values (BSAF) in Donax trunculus from Borj Cedria (BC), Rades (R) and Kalâat El Andalous (KA). (n: number of individuals per season and per station).

Sampling site	Element							MPI	Moisture (%)
	Mn	Pb	Zn	Cu	Cr	Cd	Ni		
Summer R	$41.11\pm 6.3^{c,b}$	3.36 ± 3^{a}	81.15 ± 3.6^{a}	6.32 ± 0.2^{c}	1.22 ± 0.6^{a}	<0.1	<0.1	4.53	82.24
Autumn R	70.46 ± 10^{c}	4.61 ± 0.9^{a}	74.16 ± 5.3^{b}	5.76 ± 0.3^{c}	6.04 ± 0.6^{b}	<0.1	<0.1	6.62	73.25
Winter R	$29.16\pm1.9^{c,b}$	4.84 ± 1.7^{a}	86.98 ± 3.5^{a}	6.34 ± 1^{c}	4.93 ± 0.2^{b}	0.1 ± 0.01^{b}	<0.1	5.81	76.02
Spring R	53.62 ± 39.6^{c}	3.97 ± 1.9^{a}	81.93 ± 3.1^{a}	6.73 ± 1.2^{c}	4.17 ± 1.8^{b}	0.66 ± 0.28^{a}	<0.1	8.38	73.68
Mean	48.58	4.19	81.05	6.28	4.09	0.38		6.30	
Summer BC	10.22 ± 3.3^{b}	3.59 ± 0.5^{a}	80.83 ± 2.3^{a}	$8.41\pm2.4^{b,c}$	1.8 ± 0.9^{a}	<0.1	<0.1	4.06	79.49
Autumn BC	17.03 ± 6.6^{b}	5.36 ± 2.3^{a}	$78.57\pm3.2^{a,b}$	$8.69\pm1.4^{b,c}$	5.38 ± 0.5^{b}	0.1 ± 0.02^{b}	<0.1	5.67	78.84
Winter BC	13.37 ± 3.8^{b}	3.36 ± 2.6^{a}	85.12 ± 2.5^{a}	$10.3\pm2.7^{b,c}$	2.2 ± 2.5^{b}	<0.1	<0.1	4.53	78.28
Spring BC	13.49 ± 3.7^{b}	5.51 ± 3.3^{a}	83.34 ± 2.4^{a}	11.3 ± 1.6^{b}	4.79 ± 1.6^{b}	0.1 ± 0.01^{b}	<0.1	5.68	75.22
Mean	13.52	4.45	81.96	9.68	3.54	0.1		4.98	
Summer KA	11.98 ± 1.6^{b}	4.03 ± 1.1^{a}	76.17 ± 5^{b}	10.6 ± 0.7^{b}	3.34 ± 1.3^{b}	<0.1	<0.1	4.85	87.71
Autumn KA	$10.09\pm1.9^{a, b}$	3.51 ± 3.2^{a}	68.27 ± 3.2^{b}	$9.64\pm0.1^{b,c}$	4.29 ± 0.3^{b}	0.64 ± 0.1^{a}	<0.1	6.32	78.88
Winter KA	7 ± 1.4^{a}	5.11 ± 1.8^{a}	77.67 ± 6.7^{b}	$12.3\pm1.9^{a, b}$	1.45 ± 0.1^{a}	<0.1	<0.1	4.13	81.34
Spring KA	12.43 ± 1.9^{b}	3.48 ± 1.1^{a}	87.46 ± 1.9^{a}	15.3 ± 2.4^{a}	3.72 ± 1.9^{b}	0.21 ± 0.17^{b}	<0.1	5.98	78.71
Mean	10.37	4.03	77.39	11.98	3.20	0.42		5.32	
Total metal Means	24.12	4.13	80.16	9.36	3.54	0.31	-		

Values in the same column with different superscript letters (a, b, c) are significantly different ($p<0.05$).

Metal pollution index (MPI) increased considerably in R (6.30) clams compared to what was found in BC (4.98, $p<0.05$) and KA specimen (5.32). This increase was especially marked by very high Mn concentrations detected in R specimen (Table 3).

Correlation between elements in organisms:

The principal component analysis of the trace metal variations in the soft mass of *D. truncullus* depending on the stations BC, KA and R is represented by the figure 3(a and b).

The first two factorial axes explain 51.67% of the total variance with 31.5% for axis 1 and 20.15% for the second axis. The first PC opposes the copper which, negatively, correlated with this axis to Mn. However, the Cd is the only metal that boasts the strongest inertia on axis 2.

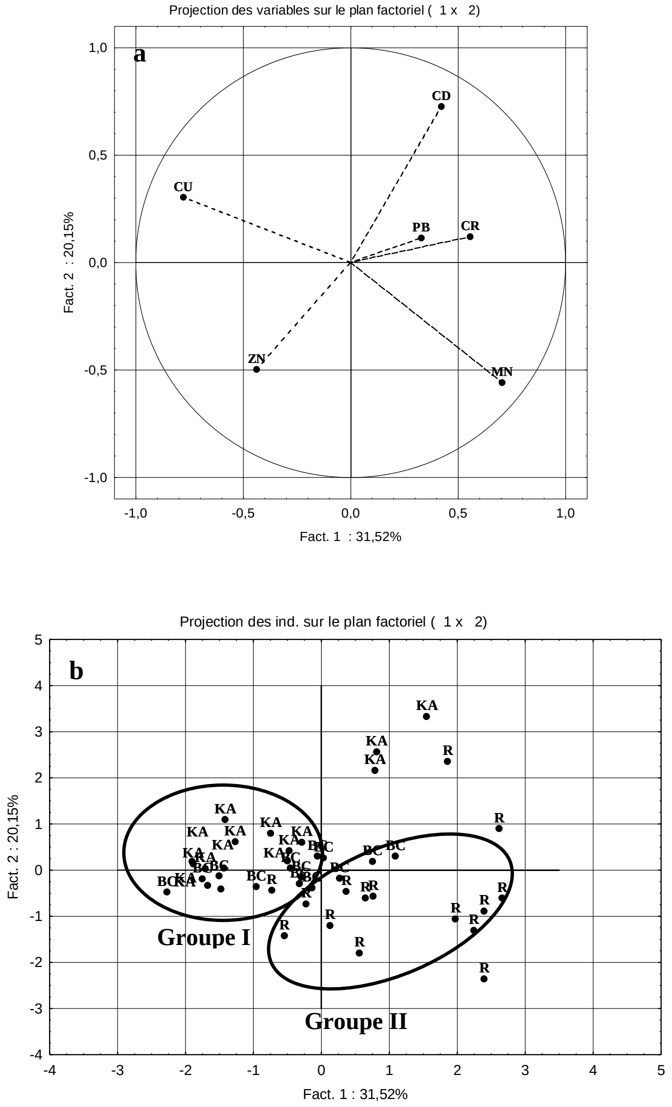
According to mineral element concentrations in the soft mass of *D.trunculus*, the specimens from the three stations in the gulf of Tunis were clustered into two groups (I and II). The group I, which is composed of the two stations KA and BC, shows a negative contribution on the first component (PC1). This correlation coincides with high Cu concentrations and Low Mn levels. On the contrary, in the group II composed by R specimen and contributing positively on the PC1, Mn concentrations increase when those of Cu decrease. A significant relation ($r= 0.31$, $p< 0.05$) for the accumulation of Cu in *D.tunculus* tissues relative to its concentrations in the surface sediments was found. The correlation coefficient obtained for Pb was positive but with a low-level of confidence ($p>0.05$). Coefficients close to zero or negative were found for Cr, Mn and Zn which indicate that the amount of these trace elements is not directly shown in the tissues of *D. trunculus*.

Discussion

Sediment:

The determination of toxic trace metal concentrations in the surface of coastal marine sediments is of great importance to the fact that this abiotic compartment has the ability to sequester these elements and interact with other biotic and abiotic compartments of the ecosystem by processes of sedimentation, flocculation, etc... In fact, the concentrations of these metals in marine sediments may vary over time for various reasons namely the direct anthropogenic inputs, continental transfer by fluvial and wind sources, ocean currents and atmospheric deposition.

Figure 3: Projection of variables (a: correlation circle) and individuals (b) on the first two components (Fact. 1 and Fact. 2) resulting from the principal component analysis based on trace metal levels accumulated in the clams Donax trunculus sampled from three sites in the Gulf of Tunis (BC, R and KA).



In the present study, concentrations of seven trace metals were monitored seasonally in the surface sediment of the Gulf of Tunis and the results showed that no seasonal variation in concentrations of these metals ($p \leq 0.05$) has been reported in sediments from BC, R and KA. However, in comparison with what was found in BC and R sediment, KA presented the highest concentrations of Mn (161.4 mg/kg DW, $p\leq 0.01$), Ni (5.09 mg/kg DW, $p\leq 0.05$), Zn (36.93 mg/kg DW, $p\leq 0.01$) and Cu (7.3 mg/kg DW, $p\leq 0.001$). In fact, sediment from KA station was taken near the old mouth of most regular wadi in the Gulf of Tunis: Medjerda wadi formerly known by intense mining activity in the catchment area (Added et al., 2003). Furthermore, sewage from the city surrounding this station, waste from industrial estates and agriculture area (Ben Hamza, 1994), are discharged into this wadi.

The concentration's of Cu, Zn, and Ni detected in the present study is to be considered lower than levels mentioned in other studies carried out in the sediment of the Gulf of Tunis by Rais (1999) (8-79, 70-226, 30-72 ppm for Cu, Zn and Ni respectively), Added et al. (2003) (9-20, 45-150, 39-67 ppm for Cu, Zn, Ni respectively), Ouelhazi et al. (2009) (0.9-43 et 30-75 ppm for Cu and Ni respectively) and Ennouri et al. (2010) (7.28-89.3 and 75-249 mg/kg DW for Cu and Zn respectively) (table 4). This could be explained by the unequal distribution of trace elements which depends on the sampling points, current patterns and the type of sediment characterizing these areas. However, the present study was conducted on the settlement area of *D. truncululs* which was collected from a relatively narrow zone in the shallow sub-littoral in depths of 30 cm to 50 cm of water column. In this coastal area, hydro-dynamism is quite pronounced allowing rapid turnover of the water masses and prevents the sedimentation of fine particles associated with minerals being transported off the Gulf.

In fact, according to these authors, accumulation zones for metals in the Gulf of Tunis were also located near the mouth of Medjerda wadi and the centre sector of the Gulf of Tunis. This implies that the inputs from the Mejerda wadi are the main source of pollution along the coast with regard to the previously mentioned metals (Ennouri et al., 2010), whereas, the accumulation of metals in the central area was due to the gyratory direction of the current crossing the Gulf of Tunis (Added et al., 2003; Ennouri et al., 2010).

The Cu, Zn, Ni and Cd levels were also lower than levels reported in other coastal marine areas affected by anthropic pressure (Gargouri et al., 2011; Abdallah and Abdallah, 2008; Cheggour et al., 2005; Usero et al., 2005; Bellucci et al., 2002). These levels, lower than the proposed limits set by the sediment quality guideline (SQG) (Long et al., 1995) (150 ppm DW for Zn, 34 ppm DW for Cu, 20.9 ppm DW for Ni and 1.2 ppm DW for Cd) indicated that there are no harmful effects from these metals. However, Mn, Pb and Cr levels were higher than those stated by Usero et al. (2005) in the Atlantic coast of Southern Spain and by Cheggour et al. (2005) in the sediments of Moroccan estuaries and the concentrations of Pb and Cr exceeded the SQG limits (for Pb: 46 ppm DW and for Cr: 81 ppm DW) in all samples (BC, R and KA). These fundings indicate that these stations are at potential risk. According to Morillo et al. (2004), the most mobile and bioavailable metal, present in high percentage in the acid-soluble fraction, was Zn followed in a decreasing order by, Mn> Cd> Cu> Ni> Pb> Cr. Thus Pb and Cr are strongly bound to the sediments which explain their high concentrations in the sediment of the Gulf.

The PCA analysis show a significant positive correlation between Mn, Zn, Cu and Ni ($p<0.001$) and between Pb and Cr ($p<0.05$) in sediments from the three sampling sites. This result was similar to what was found by Daka et al. (2003) and Ennouri et al. (2010) on the analysis of Zn, Pb, Cu and Fe in the marine sediments. According to Calace et al. (2005), this finding could mean that these metals have a common anthropogenic source and have similar properties.

Organisms:

It has been generally agreed that heavy metals uptake occurs mainly in water, food and sediment. However, effectiveness of metal uptake from these sources may differ in relation to the ecological needs, metabolism of animals (physiological state, fat content, age, size, reproduction (Phillips, 1980) and concentrations of trace metals in water, food and sediment as well as some other factors such as salinity, temperature, interacting agents (Winner, 1985; Roesijiadi and Robinson, 1994). As a result of the influence of these factors, toxic trace metal concentrations can vary considerably among season and stations within the same species.

The present study carried out on the Mollusc bivalve *Donax trunculs*, showed the accumulation of Zn, Cu, Cr, Pb, Cd and Mn in their tissues, for nickel, it has not been detected in the tissues of *D. trunculus* from all sites. In fact, this metal (Ni) is found more in plants than in animals and its toxicity in humans is not very common, as its absorption by the body is very low (Onianwa et al., 2000). Unlike, Zn is the most present trace metals in the soft mass of this organism. A significant high level of Zn was also observed in *D.trunculus* collected from various other marine coasts (Romeo and Gnassia Barelli, 1988; Sidoumou et al., 1992; Beldi et al., 2006; Abdallah and Abdallah, 2008; Drif and Cherif, 2010; Idardare and al., 2011). This is also true for some other species of bivalve as *Modiolus auriculatus* (El Sikaly et al., 2004), *Ruditapes decussatus* and *Ruditapes philippinarum* (Castro et al., 1996; Usero et al., 1997).

Although, Zinc is an essential element needed by human body in small amounts, excess may be harmful for their health (ATSDR, 2005) because zinc

Table 4. Trace metal concentrations (mg/kg DW) of marine surface sediments from various sources:

Location	Cd	Pb	Cu	Zn	Cr	Ni	Mn
Present study (Gulf of Tunis)	<0.1	36.48-55.89	1.56-8.09	3-38.13	90.32-118.57	0.05-5.29	28.53-177.83
Rais, (1999) (Gulf of Tunis)	1-7	12-112	8-79	70-226	_	30-72	230-550
Added et al. (2003) (Gulf of Tunis)	_	50-142	9-20	45-150	_	39-67	_
Ouelhazi et al. (2009) (SW de petit Golfe de Tunis (R)	0.2-2.2	_	0.9-43	_	_	30-75	246-454
Ennouri et al. (2010) (Gulf of Tunis)	0.07-0.67	18.7-98.8	7.28-89.3	75-249	_	_	_
Gargouri et al. (2011) (Southern Tunisian coast)	5.5-7	18–88	13–29	39–117	41–82	7–55	_
Bellucci et al. (2002) (Venice Lagoon)	0.2-70	21-929	_	101-8295			
De Mora et al. (2004) (Gulf and Gulf of Oman)	0.02-0.21	0.25-99	0.6-48.3	1.61-52.2	6.46-303	0.74-1010	13.2-360
Usero et al. (2005) (Spain, Atlantic coast)	0.26-0.72	2-46	6-92	18-460	10-33	3-13	_
Cheggour et al. (2005) (Morocco, Atlantic coast)	1.1-2.8	_	27-57	125-192	_	50-103	38-172
Abdallah & Abdallah, (2008) (Egyptian coast)	3.8	_	14.1	64.5	31.0	_	95.1

has now been identified as a slow and powerful carcinogen (Augier, 2008). Nevertheless, *D.trunculus* from all studied areas in the Gulf of Tunis did not exceed the maximum acceptable limits recommended by FAO (1983) (30 mg/kg WW) and by MAFF (1995) (50 mg/kg WW) for this metal (table 5).

Table 5. Maximum acceptable limits (mg/kg) of some trace metals.

Metal	Concentrations	Reference
Cd (WW)^a	0.02 – 0.17 1 1.5	Present study European Communities, 2001 European Communities, 1997
Cr (DW)^b	1.22 - 6.04 8	Present study EEC, 1979
Cu (WW)	1.12 – 3.26 20 30	Present study MAFF, 1956 FAO, 1983
Zn (WW)	9.36 – 21.56 30 50	Present study FAO, 1983 MAFF, 1995
Pb (WW)	0.5 – 1.24 0.5 1 2 10	Present study FAO/WHO, 2004 European Communities, 2001 European Communities, 1997 Great- Britain-Parliament, 1979

a: (WW): wet weight, b: (DW): dry weight.

Spatial distribution of trace metals

Concerning contamination levels by Zn, Cr and Pb, no significant difference ($p < 0.05$) was reported among specimens from the three stations. As against, those of Cu, Mn and Cd varied notably ($p < 0.01$) depending on the locations. The PCA analysis showed that R specimen had the lower contents of Cu and the higher levels of Mn compared with clams of BC ($p \leq 0.001$) and KA stations ($p \leq 0.05$).

High concentrations of Mn in R clams might indicate the fact that the station is subject to the impact of inflows of Meliane wadi, which opens out in the littoral coast of R. This wadi receives water from sewage treatment plants and from the treatment of industrial wastewater of the manufactures of textile and ceramic (Masmoudi, 2010). These industries represent the main sources of marine environment contamination by Mn. Since the supply of small amounts of Mn is necessary to human health, exposure to high levels of this metal is toxic. Symptoms of manganese toxicity can cause permanent neurological disorders known as manganism Symptoms that include tremors, difficulty walking, and facial muscle spasms (ATSDR, 2008). In the present study, the concentrations of Mn in the flesh of R clams (29.16 to 70.46 mg / kg DM) were generally higher than the values recorded in *D. trunculus* at various other marine coasts (Romeo and Gnassia Barelli, 1988; Abdallah and Abdallah, 2008; Özden et al., 2009) and in *Ruditapes decussatus* from Tunisian coasts (Trigui El Menif et al., 1999) (Table 6). However, Mn contamination levels of these bivalve species from different localities are very close and sometimes superior to those found in BC and KA clams.

In the soft mass of *D.trunculus* from KA, levels of Cu (12 mg / kg DW) were significantly higher than those reported in specimens from BC (9.82 mg / kg DW) and R (6.28 mg / kg DW). This could be explained by the excessive use of chemical fertilizers in intensive agricultural activity that characterizes the surrounding cities of the station and its proximity to the fishing port. Copper compounds are used in agriculture, especially as fungicides and insecticides and copper-containing antifouling paints are a further source of Cu inputs in the marine environment, particularly in areas of high boasting/shipping activity such as harbours and marinas (CEFAS Lowestoft, 2000). Although it is a trace element that is essential to the proper functioning of the body and plays an essential role in the transport of oxygen in molluscs (Förestner and Witmann, 1981), copper could, however, inhibit acetylcholinesterase activity (AChE) in *Donax trunculus* (Moukrim et al., 2004) and in several other species of mollusk. Moukrim et al. (2004) reported that lipid peroxidation as well as the content of malondialdehyde in *D.truncul*us increase when the copper concentration is 10^{-3} M in the water. In fact, this transition metal is capable of generating reactive fatty acids particularly arachidonic acid, which is the primary substrate for the formation of malondialdehyde (Moukrim et al., 2004).

These relatively high concentrations of Cu in KA specimens excess those in *D.trunculus* from Algerian coast (Beldi et al., 2006; Drif and Cherif, 2010) and remain lower than those reported in *D.trunculus* from the Atlantic (Spain) (Usero et al.,

Table 6. Comparison of mean metal concentrations (mg/kg) in whole soft tissue of Donax trunculus from the Gulf of Tunis with metals levels in some species of Donax from different locations and other clams from different Tunisian coasts. (NT: Northern Tunisia. ST: Southern Tunisia).

Species	locations	Mn	Pb	Zn	Cu	Cr	Cd	References
D. tunculus (DW)	Gulf of Tunis (Mediterranean)	7-70	3.3-5.3	68.3-87.5	5.8-15.3	1.2-6	0.1-0.66	Present study
D. tunculus (DW)	Mauritania (Atlantic)	10.4-14.1	_	90-120	10.3-15.3	_	0.5-0.7	Roméo and Gnassia Barelli, (1988)
D.rugosus (DW)	Mauritania (Atlantic)	7.9-21.1	_	103-127	14.9-27.8	_	0.5-1.2	Sidoumou et al. (1992)
D.deltoides (DW)	Australia (Pacific)	_	_	620-795	47.1-50	_	2.67-3	Haynes et al. (1995)
D. tunculus (DW)	Spain (Atlantic)	_	2.35	85	49.15	1.2	0.195	Uséro et al. (2005)
D. rugosus (DW)	Mauritania (Atlantic)	_	---	68.5	9.65	_	0.85	Sidoumou et al. (2006)
D. trunculus (DW)	Algeria (Mediterranean)	_	1.75-4	50-63	3.5-5.8	_	0.12-0.25	Beldi et al. (2006)
D. trunculus (DW)	Egypt (Mediterranean)	21.21	_	73.99	12.57	_	3.93	Abdallah and Abdallah, (2008)
D. trunculus (WW)	Turkey (Marmara sea)	9.76	0.57	13.19	1.93	_	0.053	Özden et al. (2009)
D. trunculus (DW)	Algeria (Mediterranean)	_	0.5-3	20-160	3.5-13	_	_	Drif and Cherif, (2010)
R.decussatus(DW)	Menzel Jmil (NT)	4.28	_	19.6	8.04	0.42	0.19	Trigui El Menif et al. (1999)
R.decussatus(DW)	Gulf of Gabes (ST)	2.94	_	31.26	15.33	1.26	0.96	Trigui El Menif et al. (1999)
R.decussatus(DW)	Ajim (Djerba, ST)	2.84	_	36.39	13.2	0.67	0.42	Trigui El Menif et al. (1999)
R.decussatus(WW)	Bizerte lagoon (Northern Tunisia)	_	0.17	_	_	_	0.20	Chouba et al. (2006)
R.decussatus(WW)	Lac of Tunis (NT)	_	0.14	_	_	_	0.10	Chouba et al. (2006)
Clams (WW)	Boughrara (ST)	_	0.05	_	11.54	0.19	1.09	Mensi et al. (2008)

2005) and in *Donax deltoïdes* from the Pacific (Australia) (Haynes et al., 1995). Furthermore, these levels are below the maximum allowed limits set by MAFF (1956) (20 mg/kg WW) and by the FAO (1983) (30 mg/kg WW) for this metal.

Cadmium is of no biological function in human system and is potentially toxic even at trace concentrations (Misra et al., 1997). Accumulated in human body, Cd may induce kidney mal-function, skeletal damage, and reproductive deficiencies (European Communities, 2001). Spatial distribution showed higher concentrations in the soft mass of *D. trunculus* from KA (during autumn) and from R (during spring). These punctual accumulations of Cd are higher than those in *D.trunculus* from other marine coasts (Usero et al., 2005; Beldi et al., 2006; Özden et al., 2009) and in *Ruditapes decussatus* from northern Tunisian coasts (Trigui El Menif et al., 1999; Chouba et al., 2006). However, they are still below the maximum permissible values set by the European Communities (1997, 2001). Phillips, (1976) reported that low salinity increased the net uptake of Cd in bivalve *Mytilus edulis*. In fact, R and KA are subject to the impact of Meliane and Medjerda wadis that are loaded by large amounts of fresh water especially during the rainiest seasons (autumn and spring) allowing the dilution of marine water masses encountered in their mouths and therefore decreasing salinity in these stations. Thus, very low salinities have been reported in February (05) (23 ‰) and May (05) (28 ‰) in R station (Boussoufa, 2013). Bryan and Hummerstone, (1973) pointed out that the change in salinity would interact with the sediment transported by the river, affecting the ratio of soluble metal / particulate metal in the water column. This is important since the accumulation of metals depends on their speciation and hence on their availability (Valenta et al., 1983). Likewise, Riedel et al. (1998) found that the uptake of Cd by oysters is less important at high salinities. This is simply due to the increase in the thermodynamic activity of the free ion due to the increase in ionic strength and the complex ions in seawater.

Seasonal distribution of trace metals:

No seasonal variations have been encountered in the concentrations of the analyzed trace metals in the BC clams except for Cr. However, the concentrations of Cu, Mn, Zn, Cr and Cd reported in the soft mass of KA's population and only the contents of Cr, Zn and Cd in Rades specimens revealed a significant effect ($p < 0, 05$) of season. Lead concentrations (3.36 to 5.51 mg / kg DW) remain stable over time but they are considered higher than those obtained in *D. trunculus* from other localities (Usero et al., 2005; Idardare et al., 2011; Beldi et al., 2006; Drif and Cherif, 2010; Özden et al., 2009). In some bivalves, Pb inhibits their burrowing activities and therefore let the mollusc in precarious conditions of protection overlooked predators (Mac Greer, 1979). Likewise, Brenko et al. (1977) have demonstarted that Pb inhibits embryonic development of mussel and increases the percentage of abnormal larvae. In humans, lead can damage the brain and kidneys in adults and children and ultimately cause the death. Due to its high toxicity, the FAO (FAO/WHO, 2004) and the European Communities (1997 and 2001) fixed the maximum limit of accumulation of Pb in marine products to 0.5 mg/kg, 1 mg/kg and 2 mg/kg WW respectively. However, the Great Britain Parliament (1979) suggested that lead levels in shellfish should not exceed 10 mg/kg WW. In the present study, Pb would always remain below the maximum permissible limits laid down by the Great Britain Parliament (1979) and by the European Community (1997) but above those of FAO and the European Community (2001) for this metal. These relatively high concentrations in *D.trunculus* from the three sites are probably due to the high sediment pollution by this metal.

The accumulation of Cr in the soft mass of *D. trunculus* occurs essentially during autumn (04) in R, BC and KA stations. These autumnal levels are significantly higher than those reported in summer (04) for R and BC specimens ($p < 0.01$) and in winter (05) for those of KA ($p < 0.05$). The values reported in this study were higher than those recorded in *D.trunculus* from the Atlantic coasts (Usero et al., 2005; Idardare et al., 2011). At low concentrations (few mg/kg), this metal is necessary to many biochemical reactions. But present in too high quantities in the body, Cr may be toxic and could cause inflammatory reactions of the skin and mucous membranes. However, Martin et al. (1976) have shown that Cr does not appear to be accumulated significantly in the human body which reduces the risks resulting from the ingestion of contaminated fish and shellfish. In our study, the contamination levels by Cr (3.54 mg / kg DW) could be considered high if we compared them to those usually found for this element in other bivalve species like *Laternula elliptica* from the King George Island (Antarctica) (2.1 µg/g DW) (In-Young Ahn et al., 1996), *Ruditapes decussatus* from different Tunisian coasts (0.42-2.53 µg/g DW) (Trigui El Menif et al., 1999), *Chamelea gallina* (0.70 µg/g DW) from the Atlantic coast of southern Spain (Usero et al., 2005) and various clams from Boughrara lagoon (South Tunisian coast) (0.19 mg/kg WW) (Mensi et al., 2008). Though, these levels are below maximum allowable limits (8 mg/kg DW) reported by the EEC (1979) for this metal.

Generally the highest levels of Donax contamination by trace metals have been reported mainly in autumn and / or in spring. Indeed, the period from November (04) to April (05) is the period during which rainfall is the largest, with a monthly average of between 40.5 and 177 mm per month (Boussoufa, 2013). This intense precipitation results in the leaching of agricultural land loaded with chemical fertilizers and the rise in water levels of the surrounding wadis (Medjerda and Meliane), which receive wastewater from industrial areas and wastewater treatment plants. Therefore, bivalves which have long been known for their ability to accumulate essential and non-essential trace elements in aquatic ecosystems (Dallinger and Rainbow, 1993) represent then a very important target for this pollution. Since 1977, Phillips had pointed out that the spatial and seasonal distribution of trace metals in the water column and / or bivalve molluscs must be explained by the variation of extrinsic interrelated factors such as currents, brewing process, upwelling and runoff.

Although the observed changes in tissue concentration of Cd, Cu, Mn, Zn and Cr, may be due to variations in the amount of wastewater reaching the beach or caused by the existence of occasional wastewater, it cannot be excluded that these changes could be also attributed to species-specific biological variables, in particular those governing the reproductive activity. Generally gametogenesis in *D.trunculus* occurs in winter and spring and the emission of gametes begins in late spring and continues until early autumn (Moueza and Frenkiel-Renault, 1973; Manca Zeichen et al., 2002; Dhaoui Ben Kheder, 2003 and Boussoufa, 2014). However, the high accumulation of Pb, Zn and Cu in this study coincides with the period of gametogenesis.

In a previous study, Boussoufa et al. (2011) have been reported that dry weight, which closely follows that of the condition index (CI), decreased during summer and late autumn and increased during the spring in BC, R and KA specimens. Indeed, we reported a positive correlation between the CI and the concentrations of Cu in Donax from KA ($r = 0.74$, $p = 0.0016$), Pb from R Clams ($r = 0.72$; $p = 0.0024$) and Zn from those of BC ($r = 0.6$, $p = 0.03$) and KA ($r = 0.80$, $p = 0.0003$). Other studies have also shown that the development of the gonads during gametogenesis of bivalves coincides with the retention of metals (Cheggour et al., 1990; Mubiana et al., 2005; Beldi et al., 2006; Idardare et al., 2011). Moukrim et al. (2000) noted seasonal variations in the concentrations of Zn, Cd and Cu in *Mytilus galloprovincialis* and *Perna perna* in the bay of Agadir (Morocco). These changes were explained by the reproductive cycle of these mussels. Similarly, Mubiana et al. (2005) found a very strong springer peak for concentrations of several metals in tissues of mussel *Mytilus edulis*. These authors showed that seasonal variations in metal concentrations appear to be largely controlled by biological processes.

The metal pollution index (MPI) mentioned in R clams was significantly higher (6.30) than in Donax from BC (4.98) and from KA (5.32). This difference was especially marked by very high concentrations of Mn detected in samples from R. Likewise, an earlier study on the monitoring of the acetylcholinesterase activity (AChE) (biomarker) in *D.trunculus* from R, BC and KA showed a significant inhibition of the AChE activity at the mantle of R specimens compared with the other two stations (Boussoufa et al., 2012). Moreover, Masmoudi et al. (2007) demonstrated that muscle tissue of Muge (*Liza aurata*) from Rades station contains amounts of PCBs residue (45-194 ng / g WW) higher than those accumulated in Raoued fishes (43-65 ng / g WW) which is close to the KA station. Springer and autumnal values of this index in the three study sites are higher than those of summer and winter. Springer values can be connected to both the gametogenesis stage of the species, previously explained, and also to intense rainfall, while those of the autumn could be attributed to periods of intense precipitations (Boussoufa, 2013).

Relationships between metals in organisms and sediments:

Several authors (Amiard et al., 1987; Castro et al., 1996) argued that the concentrations of non-essential metals (Cd, Pb) in organisms are heavily dependent on concentration levels in the environment. Regarding essential metals (Zn, Cu), studies on marine organisms (Amiard-Triquet et al., 1986; Sidoumou et al., 1992) indicate that bivalves have a marked ability to regulate internal metal concentrations. In the present study, only one significant correlation ($r = 0.31$, $p < 0.05$) was reported between Cu in the soft mass of *Donax tunculus* and those in surface sediments. The correlation coefficient obtained for Pb was positive but insignificant while coefficients near zero or negative were found for Cr, Mn and Zn showing that the amount of these trace elements in the sediment is not directly reflected in the tissue of *D. trunculus*. Usero et al. (2005) explained this behavior by the fact that concentrations of Cr, Mn and Zn are low in sediments, probably lower, than the threshold below which these organisms are able to regulate the accumulation of metals in their bodies. Abdallah and Abdallah (2008) have found that Zn and Fe concentrations in the tissues of *Paphia textile* are positively correlated ($p < 0.05$) to their concentrations in the sediment and that only concentrations of Co in the soft mass of *D. trunculus* evolving simultaneously with those in surface sediments ($p < 0.05$). Similarly, Usero et al. (2005) have reported the existence of a significant correlation between the concentrations of trace metals (Cu, Pb, Zn and Hg) in *D. trunculus* and *Chamelea gallina* from the Atlantic coast (Southern Spain) and those in surface sediments.

Although sediments from KA station have the highest concentrations of Mn, Ni, Cu and Zn, specimens from R station appear to be most affected by this metal pollution. This suggests the fact that most adsorbed pollutants on the sediments are not readily available for aquatic organisms; the variation of some physical and chemical parameters like pH, salinity, redox potential and the content of organic chelators of the overlying water may provoke the release of the metals back to the aqueous phase, thus under changing environmental conditions sediments may become important pollution sources themselves (Soares et al., 1999). In the water and sediment metal pollution, the interactions sediment-metal cation plays a key role which regulates the behaviour of these metals, their distribution in the solid phase and their transfer into the water. The exchanges of metals process sediment-water interfaces, in particular, in relation with the balance of adsorption and desorption are a function of various parameters of water (temperature, pH and ionic strength). According to Serpaud et al. (1994), if the water temperature is high, the decrease of the adsorption is especially important in the case of Cu, Zn and Cd the contrary, if the pH increases (basic), it promotes the adsorption of metals on inorganic and organic compounds. In an earlier study that was conducted in BC, R and KA stations during the same study period, Boussoufa (2013) has shown that R station is characterised by the highest temperature (due to the presence of the electric central) and the lowest pH compared to the other two stations. These conditions reduce the adsorption of metals that find themselves in dissolved form in solution and could probably explain the high levels of trace metals accumulated in the soft mass of *D.trunculus* from R station.

Conclusion

In the present study, trace metal levels were evaluated in the sediment and in *Donax trunculus* harvested in three locations in the Gulf of Tunis (KA, R and BC). Results have shown that KA sediment presented the highest concentrations of Mn, Ni, Zn and Cu. These levels, however, don't exceed the limit values set by the sediment quality guideline. Whereas, Pb and Cr levels are above their limit values in the three stations. Nevertheless, the contents of Mn, Pb, Cd, Cu, Zn and Cr accumulated in the soft mass of *D.trunculus* from the Gulf of Tunis are below acceptable limits for the shellfish consumption. If these metals were considered individually, then *D.trunculus* should be safe for human consumption. Although sediments from KA station have the highest concentrations of metals, specimens from R station appear to be most affected by this metal pollution. These results would suggest that, the trace metal accumulation in the soft mass of these species didn't reflect the real pollution state in the sediment where they live. This could be explained by the fact that most adsorbed pollutants on the sediments are not readily available for aquatic organisms.

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