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# Trace metals in the clam *Donax trunculus* L. from the Bouadisse sandy beach, discharge zone of a plant sewage outfall in Agadir Bay (Morocco)

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**Abstract.** *Donax trunculus* were sampled seasonally during 2004-2005, from six stations along the coastline of the Bouadisse sandy beach. This site is located at 10 km south of Agadir (Morocco) and subjected to the pollution generated by a wastewater treatment plant outfall. The concentrations of heavy metals accumulated in the clams' soft tissues were measured. The concentrations of Cd, Ag, Pb, Cu, Zn and Ni were determined by ICP-MS methods, whilst Cr was quantified by AAS. The results show that *D. Truncului* appear to accumulate more Pb, Cr, Zn and Ni along the north-south sites especially in the stations closest and situated in the south of the sewage outfall indicating an influence of the sewage outfall toward the South. This gradient suggests that the distribution of these heavy metals is probably affected by a coastal current flowing in this area from the North to the South. However, the concentrations of Cd and Ag were lower in the same sites. It can be related to high salinity in wastewater discharged on the beach.

**Key words:** *Donax trunculus*, wastewater treatment outfall, heavy metals, Bouadisse sandy beach, Morocco.

**Résumé.** ***Métaux traces dans le mollusque* Donax trunculus *de la plage sableuse de Bouadisse, zone de déversement de l'émissaire de la station d'épuration des eaux usées dans la baie d'Agadir (Maroc).*** Un échantillonnage saisonnier de *Donax trunculus* en 2004 et 2005 a été effectué sur six sites le long de la plage sableuse de Bouadisse, située à 10 km au Sud d'Agadir (Maroc). Cette plage est soumise à la pollution générée par l'émissaire de la station d'épuration des eaux usées du grand Agadir. Les concentrations de Cd, Ag, Pb, Cu, Zn et Ni ont été évaluées dans les parties molles de l'animal, par la technique ICP-MS, tandis que celles de Cr ont été déterminées par la spectrométrie d'absorption atomique (AAS). Les résultats montrent un gradient spatial des teneurs des métaux lourds du nord vers le sud pour Pb, Cr, Zn et Ni. Une accumulation importante de ces métaux est particulièrement notée dans les sites situés au sud et proches de l'émissaire. Ceci suggère l'influence des courants côtiers qui portent du nord vers le sud, dans la dispersion de la pollution engendrée par la station d'épuration vers les sites du sud. Cependant, les concentrations de Cd et Ag ont été plus faibles dans les mêmes sites. Ceci est probablement lié à la salinité élevée dans les eaux usées déversées sur la plage.

**Mots clés :** *Donax trunculus*, eaux usées traitées, métaux lourds, plage sableuse de Bouadisse, Maroc.

## INTRODUCTION

The Agadir bay is of great socioeconomic importance to the southern Moroccan regions. It creates high-level jobs thanks to its industrial, fishing and tourism vocation. Until November 2002, this bay was threatened by the pollution generated from the increasing urbanization and rapid industrial development (more than 40000 $m^3$/day of wastewater expected to reach 92000 $m^3$/day by 2015) (Idhalla *et al*. 1997, Najimi 1997). With the need to protect and preserve the environment of this ecosystem, a wastewater treatment plant (WWTP) has been installed at M'zar site located at 10 km south of Agadir, and has been selected taking into account the general circulation of marine current in this area, indicating a main direction towards the south (Banaoui *et al*., 2004, Chafik *et al*. 2001).

At the time of this study, the M'zar sewage treatment plant was employing a primary treatment consisted in anaerobic decanting. So, the effluents discharged from the plant are not cleared away from the contaminants and the xenobiotics residues and then can compromise the organisms and human beings by their potential hazards. The main objective of our investigations is to contribute in the assessment of the environmental impact of these effluents after been released into the receiving beach.

The extent of contamination can be assessed by measuring pollutants concentrations in the water, sediment and organic tissue samples. Due to the fact that water transports pollutants from one place to another and diluting them and that sediment integrates the contaminants via the precipitation over the time and over the water column. Among the common approaches used to survey environmental contamination, the use of bioindicators species has proven to be a valuable and informative tool. Molluscs are largely used as bioindicators and they were chosen in the monitoring programs for their economic and ecological importance, sedentary life and ability to

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concentrate contaminants in their tissues (Feldstein *et al*. 2003).

In this research, to rank the stations according to their contamination level, we have chosen the bivalve mollusc, *Donax trunculus*, as a sentinel species. Because, in addition to share the characteristics with other molluscs used in this goal, it is abundant along this sandy beach and numerous studies have shown that it could be used in monitoring of this type of ecosystems (El Hamidi 2003, Moukrim *et al.* 2004). It is suspension feeders as well as potential bioaccumulators (Mauri & Orlando 1982, Ansell 1983, Roméo & Gnassia-Barelli 1988, Moukrim *et al.* 2004, Feldstein *et al.* 2003, Sidoumou *et al*. 2006).

The purpose of this study is to obtain quantitative information on the concentrations of trace metals in the soft tissue of *D. trunculus* collected along the Bouadisse beach and to study the metal pollution distribution according to the wastewater discharge.

## MATERIAL AND METHODS

### Animals and collection sites

*D. trunculus* clams were collected seasonally between December 2004 and January 2005 from 6 sites (Fig. 1) along the Bouadisse beach at low tide from the intertidal flats. Four sites, distance by 1 km from each other, are situated in the north of plant sewage outfall (S1, S2, S3 and S4). In other side of the wastewater outfall, two sites are chosen (S5 and S6). The site S5 is located at 4 km from the source of pollution and marked the first presence of *D. trunculus* in south of plant sewage outfall.

To compare the total metal content at the different sampling sites, the metal pollution index (MPI) was used, obtained with this equation (Usero *et al*. 1997):

$$MPI= (Cf_1 \text{ x } Cf_2 \text{ x } Cf_3 \ldots Cf_n)^{1/n}$$

Where $Cf_n$ is the concentration of the metal n in the sample.

### Analysis of trace metals

After the collection of clams from the beach, they were allowed to flush out undigested matter in filtered seawater from the sampling sites for 24 h. The bivalves were stored in plastic bags and frozen in a deep freezer at -30°C until their treatment in the laboratory. The whole soft tissues of 50 individuals from each location and season were carefully removed by shelling the bivalves with a plastic knife. Samples were dried at 60°C to a constant weight, mortared, and analyzed for heavy metals. All reagents were of analytical grade.

The aliquots of dried samples (200 mg) were put into polyethylene tubes. Each sample was added with 4 ml of pure nitric acid with analytic quality and left to pre-digested overnight at ambient temperature. Then placed in the heat plate digested 3 h at 90°C. After cooling, the solution was diluted to 50 ml in fresh deionised water (MilliQ water). Ni, Cu, Zn, Ag, Cd and Pb were determined with ICP-MS. Cr was carried out by atomic absorption spectrometry (AAS).

The analytical procedure was checked using a standard reference material obtained from the community office of references, Brussels, Belgium (BCR, ref. CRM 278R mussels) and national institute of standards and technology, Gaithersbourg, USA (NITS, ref. SRM 1566b oyster). The results are shown in Table I and they are in agreement with the certified values.

### Statistical analysis

Data were expressed as mean ± standard error (SD). Comparison of mean values between sites (for the same season) and between seasons (for the same site) were estimated by LSD test. The effects of both seasons and sites of sampling were tested by a two way analysis of variance (ANOVA). Statistical analyses were performed using Statistica version 6. The significance level was $p < 0.05$.

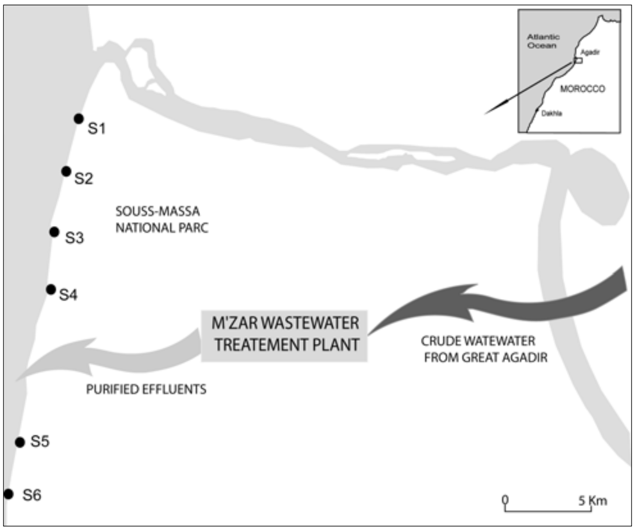


Figure 1: Location of sampling sites along the Bouadisse beach

Table I: Performances of the quality control with the CRM 278R from the BCR and the SRM 1566b from NITS

| | BCR 278 | | | SRM 1566b | | |
|---|---|---|---|---|---|---|
| | Analyzed | Certified | Recovery (%) | Analyzed | Certified | Recovery (%) |
| Cr | 0.6 | 0.78 ± 0.06 | 12.9 | 0.2 | 0.78 ± 0.06 | 426.2 |
| Ni | 1.0 | - | | 1.0 | 1.04±0.09 | 105.5 |
| Cu | 9.1 | 9.45 ± 0.13 | 103.4 | 67.7 | 71.6±1.6 | 105.7 |
| Zn | 86.1 | 83.1 ± 1.7 | 96.5 | 1595.9 | 1424±46 | 89.2 |
| Ag | 0.2 | - | | 0.6 | 0.666±0.009 | 111.0 |
| Cd | 0.3 | 0.348 ± 0.007 | 99.6 | 2.6 | 2.48±0.08 | 96.5 |
| Pb | 2.0 | 2 ± 0.04 | 100.4 | 0.3 | 0.308±0.009 | 97.9 |

## RESULTS AND DISCUSSION

### Seasonal variation in heavy metal content

As it is depicted in Figure 2, the soft tissue concentrations of heavy metals showed significant temporal variations registered in all sampling sites, within minima in winter and autumn. Depending on the station and the metal, these cycles were more or less developed and regular. The levels of Zn were relatively stable except their autumnal increase in S5 and S6. Although the changes observed in the tissue concentration of Cd, Pb, Ni, Cu, Ag and Cr, may be due to variations in the wastewater quantities reaching the beach or caused by the existence of occasional sewage inputs, it can not be exclude that these changes could be due to biological variables peculiar to the species, particularly those governing reproductive activity. It is in agreement with the results of Mubiana *et al*. (2005) where a marked spring peak was observed for several metals in soft tissues. Bayed (1990) observed that the gametogenic cycle of *D. trunculus* from the Mehdia sandy beach (Moroccan Atlantic coast) began at the end of autumn and the spawning occurred in spring. A drop in metals levels coincide with the spawning period while during gametogenesis more elevated rates of metal integration are observed. Well developed gonads have been shown to coincide with metal retention in a number of the studies (Cheggour *et al.* 1990). Moukrim *et al*. (2000) noted seasonal variations in the concentrations of Zn, Cd and Cu in *Mytilus galloprovincialis* and *Perna perna* from Anza and Cap Ghir (Agadir Bay, Morocco). These variations were explained by the mussels' reproductive cycle. Etim *et al*. (1991) reported also that the fluctuation in the dry tissue weight of the clam *Egeria radiate* exerted an influence on the temporal variations of the metal: while the peak concentration of Zn coincided with the peak of the mean dry tissue weight, the reverse was true in the case of Pb and Cd. Bryan (1976) observed that Cd concentrations in the bivalve *Scrobicularia plana* from the East Looe estuary (England) remained constant with increasing body weight while those from the highly polluted Gannel and Plym estuaries (England) increased with increasing body weight. Most authors, however, reported a reciprocal relationship between metal concentrations and body weight of mussels (Phillips 1976). Boyden (1974) reported that concentration of Pb, Cu, Zn and Fe in *Mytilus edulis* decreased with the increasing body weight whereas Ni and Cd remained constant. It is possible that gonadal development in the species leads to dilution of some of the body metal concentrations. A similar conclusion was drawn by Bryan *et al*. (1980) for the species *Scrobicularia plana* and by Amiard *et al*. (1986) for *Mytilus edulis*. Galtsoff (1964) observed that the ripe oyster gonad may comprise 31 to 41% of the total body weight. On the basis of this, Cunningham & Tripp (1975) argued that if metals were accumulated in the gonad tissues, an appreciable loss might, occur during spawning.

### Comparison of metal bioaccumulation by *D. trunculus* in the Bouadisse Beach and in others areas

Average metal concentrations (Cd, Cu, Ag, Cr, Ni, Pb and Zn) in the clams *D. trunculus* collected seasonally from Bouadisse beach can be found in the table II. These values ranged within the values reported from the Mediterranean and the Red Sea in the same species, whereas those of Zn were much higher in the Bouadisse Beach (El Skaily *et al*. 2005).

The concentrations of Zn recorded in this study were higher than those reported by Usero *et al*. (2005) in the same bivalve from the Spanish Atlantic coast. But the concentrations of Cu were higher in this latter (Table II). Ni, Cd and Pb concentrations are similar to those reported from the same species collected at several Spanish and Mauritanian sites. For Cr, the concentration was below than 2µg/g with lower values recorded in summer. This value is similar to the one reported by Usero *et al*. (2005).

### Comparison of metal bioaccumulation pattern by *D. trunculus* with others bivalves

The Zn and Cu concentrations in the clams from the Bouadisse Beach are lower than those reported in other species of molluscs from other Moroccan Atlantic sites (e.g. El Jadida and Jorf Lasfar) (Maanan 2007).

Table II: Comparison of heavy metal levels found in mussel tissues in several field studies.

| Sample area/ species | Heavy metals concentration (µg/g dry weight) | | | | | | | Reference |
|---|---|---|---|---|---|---|---|---|
| | Cr | Ni | Cu | Cd | Pb | Zn | Ag | |
| Bouadisse plage (Morocco)/ *D. trunculus* | 1.42 | 1.60 | 10.52 | 0.29 | 0.90 | 134.40 | 1.38 | Current study |
| Southern Spanish Atlantic coast / *D. Trunculus* | 1.2 | 1.2 | 175 | 0.19 | 3.6 | 107 | - | Usero *et al.* 2005 |
| Mediterranean Sea / *D. Trunculus* | - | 2.85 | 8.83 | 0.34 | 0.90 | 14.14 | - | El Sikaily *et al.* 2004 |
| Red Sea / *D. Trunculus* | - | 3.33 | 5.95 | 0.70 | 1.46 | 17.39 | - | El Sikaily *et al.* 2004 |
| Mauritania / *D. trunculus* | - | - | 8.88 | 0.74 | - | 66.98 | - | Sidoumou *et al.* 2006 |
| Jorf lasfar (Morocco)/ *Perna perna* | - | - | 72 | 29 | 5 | 150 | - | Banaoui *et al.* 2004 |
| El Jadida (Morocco)/ *M. galloprovincialis* | 8.8 | 32.8 | 26.8 | 7.2 | 9.6 | 292 | - | Maanan 2007 |
| Sidi Moussa lagoon (Morocco)/ *T. decussata* | 9.6 | 22.4 | 11.1 | 2.2 | 4.1 | 103.1 | - | Maanan 2007 |

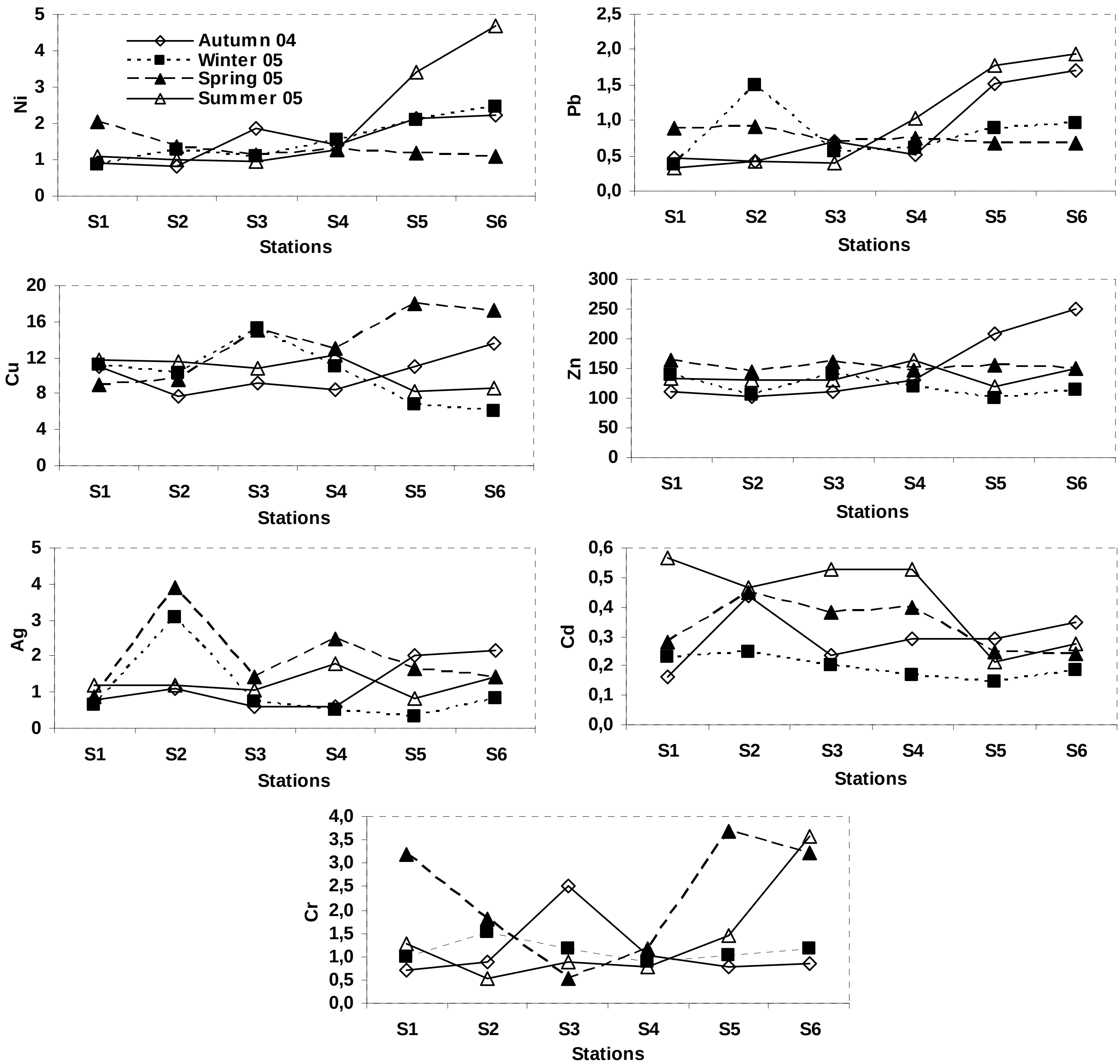


Figure 2: Seasonal variations in trace metal concentrations (µg/g) in *Donax trunculus* along the Bouadisse beach (stations) from July 2004 to July 2005.

The levels of Ni, Cd and Pb in the soft tissues of *D. trunculus* were lower than the concentrations amounts determined in *Mytilus galloprovincialis, Perna perna* and *Tapes decussata* sampled from other Moroccan sites (Maanan 2007, Zourarah *et al.* 2007, Banaoui *et al.* 2004). The Cr concentrations are very weak in comparison to those found in the Moroccan sites receiving wastewater discharge (Jorf Lasfar).

**Spatial variation in heavy metal content in Bouadisse Beach**

The spatial variation in the bioaccumulation of all seven heavy metals analysed was significantly different ($p<0.001$) between the six sampling sites (Fig. 2). The highest concentrations of Cr, Ni and Pb were consistently found at the site S6 located at 5 km in the south of the plant sewage outfall.

The concentrations of Ag in *D. trunculus* varied among the sampling sites with the ratios of the highest to the lowest values being 0.31–3.9 µg/g d. w. the highest concentration of Ag was mainly recorded in S2. The significantly lowest value was obtained from S5. This metal is considered to be a good tracer of wastewater inputs. Ag enters the animal body through inhalation, ingestion and movement across mucous membranes and broken skin. There are marked differences in the ability of animals to accumulate, retain and eliminate Ag in different species. Almost the entire Ag intake is usually excreted rapidly in faeces; less than 1% of the total Ag intake is absorbed and retained in tissues (Ribeiro Guevara *et al*. 2004, 2005).

The Cu concentration of soft tissues ranged between 9.55–11.87 µg/g. The Cu concentrations were highest in S3 and lowest in S2. The decrease of Cu was often occurred when the Ag in soft tissues was increasing. The potential interaction between Ag and Cu has been previously investigated (Ettajani *et al*. 1992, Shi & Wang 2004). The interaction between the two metals has explained by the involvement of a (Ag, Cu)-binding Protein. It has been suggested that Cu may occupy the majority of available binding sites common to both Ag and Cu, thus only a few sites may be available for additional binding with Ag. Consequently, a decrease in Ag influx by Cu pre-exposed mussels may result from the saturation of binding sites by Cu.

The spatial distribution of Cd showed higher concentrations in clams from the sites located in the vicinity of Oued Souss estuary. The lowest values were observed in organisms from S5 and S6 during summer 2005. Phillips (1976) reported that low salinities increased the net uptake of Cd and Cu, but decreased that of Pb in the bivalve *Mytilus edulis*. Bryan & Hummerstone (1973) pointed out that salinity variation will interact with sediment carried by the river, affecting the ratio of soluble to particulate metal in the water column. This is important, as the accumulation of metals depends upon their speciation and thus in their availability (Valenta *et al*. 1983). Metal ions are generally much more available for uptake by organisms that are elements bound to inert complexing ligands or particulate matter; although metal ions are never the most abundant chemical species, they are generally the most biologically important (Bryan *et al*. 1985).

Cd and Ag exhibited low values in the southern sites. This finding can be dealing with the fact that wastewater discharge from the sewage plant is known to indicate very important salinity values. Riedel *et al*. (1998) found that the uptake of Cd, Ag and Cu by oysters from solution is less important at higher salinities. This can be explained simply by the increase of the thermodynamic activity of the free ion due to the increase of ionic strength and complexation by sea water ions. Several authors pointed out that the spatial distribution of heavy metals in bivalve organisms should be explained by the variation of extrinsic interacting factors (currents pattern, mixing processes of waters of different origin, upwelling, productivity, runoff) which can generate seasonal differences in the level of metal concentration in the water column, and/or by organisms metal accumulation, storage and excretion mechanisms (Phillips 1977, Mason & Simkiss 1983). According to Frias-Espericueta *et al*. (1991), the main factors that regulate the concentrations of heavy metals in bivalve tissues are the quantity of metals in the water column (dissolved and /or particulate) and some biological parameters.

**The MPI along the Bouadisse Beach**

The calculation of the MPI (Table III) showed with the exception of high value recorded in S2, a clear north-south gradient of environmental perturbation from the sites near the sewage outfall to the further away sites. The same results were found in *Mytilus californianus* (Gutiérrez-Galindo & Munoz-Barbosa 2003) from Baja California and in *Macoma balthica* from San Francisco bay. The former was subjected to the sewage treatment plant impact. As pointed out by Pearson & Rosenberg (1978), the effects of a sewage effluent are most pronounced in the vicinity of the outlets and decrease progressively with increasing distance from the discharge points. Raffaelli & Hawkins (1996) pointed out that most of the small volumes of effluent discharge have ecological effects in the first 10 m away from the outfall. Terlizzi *et al.* (2002) provided evidence that sites located about 100–300 m apart from an outfall located in Apulian coast (Italy) were similar to each other and they differed from the site located in close proximity to the sewage. Popham *et al*. (1980) found major changes in Cu, Zn and Pb concentrations in the mussel *Mytilus edulis* within 1 km of a source. McGreer (1982) reported a similar sharp gradient in metal concentrations in *M. balthica* near a small sewage outfall, Near Palo Alto, Cu and Ag concentrations in *M. balthica* declined more than six-fold within several hundred meters north of the sewage outfall, but extended for several kilometres south of the outfall. The distinct, consistent pattern of metal distribution in our case suggests an important influence of hydrodynamic, with an indication of a consistent net southward transport. This observation reflected the general movement of coastal waters related to coastal current flowing north to south a long the Moroccan Atlantic coasts (Johnson & Stevens 2000, Chafik *et al.* 2001). It is noteworthy that the important MPI exhibited in S2 taken a short distance south of Oued Souss estuary could be related to the influence of the former as it has been subjected, for a long time, to the discharge of large amounts of sewage and industrial effluents (Moukrim *et al*. 2000, Ait alla *et al*. 2006), until November 2002 when all waste outlets were connected to the M'Zar wastewater purification plant.

The sewage input can contribute to an increase in the organic carbon content characteristics of an area and suspended solid that may deposit on the bottom (Gray 1992, Heip 1995) and it can contribute to an increase in the proportion of silts, changing the sediment type in the sites close to the sewage outfall in the study area. Herein, it is important to highlight the traditional concept of the relationship between metal content and grain size assumes that the fine fraction carries most of the metals in natural sediments (Moore *et al.* 1989). This concept is supported in many cases by strong, significant linear relationships between total-sediment metal concentrations and percentages of various fine-size fractions.

Table III: Concentrations of heavy metals and the metal pollution index values in *D .trunculus* along the Bouadisse beach.

| Location | | Heavy metals concentration (µg/g dry weight) | | | | | | | MPI |
|---|---|---|---|---|---|---|---|---|---|
| | | Cr | Ni | Cu | Zn | Ag | Cd | Pb | |
| S1 | Mean | 1,48 | 1,23 | 10,36 | 130,35 | 1,01 | 0,30 | 0,56 | 2.37 |
| | ± SD | 0,99 | 0,48 | 1,38 | 24,69 | 0,38 | 0,16 | 0,24 | |
| S2 | Mean | 1,22 | 1,14 | 9,55 | 116,70 | 2,20 | 0,37 | 0,83 | 2.70 |
| | ± SD | 0,50 | 0,22 | 1,46 | 19,02 | 1,24 | 0,12 | 0,45 | |
| S3 | Mean | 1,27 | 1,23 | 11,87 | 130,06 | 1,18 | 0,31 | 0,68 | 2.50 |
| | ± SD | 0,74 | 0,36 | 3,06 | 22,45 | 0,62 | 0,14 | 0,25 | |
| S4 | Mean | 1,13 | 1,38 | 10,80 | 134,71 | 1,36 | 0,33 | 0,85 | 2.63 |
| | ± SD | 0,40 | 0,12 | 1,95 | 21,24 | 0,83 | 0,14 | 0,34 | |
| S5 | Mean | 1,59 | 2,23 | 10,00 | 138,02 | 1,08 | 0,22 | 1,21 | 2.81 |
| | ± SD | 1,19 | 0,80 | 4,82 | 45,04 | 0,73 | 0,06 | 0,44 | |
| S6 | Mean | 1,85 | 2,42 | 10,56 | 156,54 | 1,46 | 0,25 | 1,26 | 3.19 |
| | ± SD | 1,43 | 1,36 | 4,73 | 54,95 | 0,47 | 0,07 | 0,53 | |

## CONCLUSION

The metals levels in the soft tissue of the clam *D. trunculus* indicated an evident influence of crude or partially treated sewage released by the M'zar plant and the Souss estuary. Significant variations were observed in the distribution of trace metal concentrations in the clams along the Bouadisse beach. The distribution of Pb, Cr, Zn and Ni concentrations followed distance gradient from the mouth of Oued Souss estuary and the M'zar sewage treatment plant outfall. This distribution of these tracers toward the South was apparently due to the coastal current prevailing along the Agadir Bay. For the rest of the metals, distribution did not follow predictable spatial patterns. High salinity in the wastewater coming from the plant and the interaction between metallic elements may be a more important factor explaining this distribution. Finally, the levels of metals in the clam associated with sewage outfall are clearly lower than those reported in the Moroccan sites which receive industrial or urban sewage.

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