International Scholars Journals

African Journal of Agriculture ISSN 2375-1134 Vol. 6 (1), pp. 001-006, January, 2019. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Seasonal variations of Cd, Cu, Pb and Zn in the edible mollusc *Donax trunculus* (Mollusca, Bivalvia) from the gulf of Annaba, Algeria

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Accepted 10 November, 2018

Seasonal variations in the concentrations of four trace heavy metals (cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn)) were determined in *Donax trunculus* (Mollusca, Bivalvia) at two contaminated sites in the gulf of Annaba (East of Algeria): El Battah and Sidi Salem . The average concentrations of the metals exhibited the following order: Zn>Cu> Pb>Cd for the two sites. The statistical analysis revealed a significant effect of seasons for all metals measured, the highest values being recorded in winter for Zn and in summer for the other metals. Contrary to expectation, both sites were contaminated with trace heavy metals analysed. Although the highest levels were detected in Sidi Salem beach, there was no significant difference on trace heavy metal concentrations between sites, except for Cd. The implication of this metal contamination for local population was discussed.

Keywords: Bivalve Molluscs; *Donax trunculus*; Environmental contamination; Trace heavy metals, Biomonitoring.

INTRODUCTION

The coastal zone receives a large amount of metal pollution from agricultural and industrial activity (Usero et al., 2005) . Pollution by heavy metals is a serious problem due to their toxicity and ability to accumulate in the biota (Islam and Tanaka, 2004). There is still a general concern about the impact of metals in the aquatic environment (Grosell and Brix, 2005). Bivalves are filter- feeders and thus uptake heavy elements not only from food and water but also from ingestion of inorganic particulate materials (El-Sikaily et al., 2004). Moreover, they have been well established as bioindicators for monitoring the concentration of heavy trace metals in many areas in the world (Neuberger-Cywiak et al., 2003).

Due to their economic and ecological importance, as well as their sedentary life, molluscs have assumed a major role in monitoring contaminants worldwide (Boening, 1999). The bivalve *Donax trunculus* L. (Bivalvia: Donacidae) is a widespread clam that lives in sandy beaches of the word (McLachlan et al., 1996). This species has also been used as a monitor of baseline environmental metal concentration (Romeo and Gnassia-Barelli, 1988). This species was found in higher densities in the sand beaches of the Annaba gulf in Algeria (Vaissière and Fredj, 1963) and is widely consumed by the local population. Their habitats are exposed to several pollutants from different sources (Abdennour et al., 2004). Recently, acetylcholinesterase and glutathion S-transferase activities in *D. trunculus* were used as biomarkers of contamination in the gulf of Annaba (Abbes et al., 2003).

The present experiment completes previous reports made on the studied area concerning the trace heavy metals (Abdenour et al., 2000, 2004) or on *D. trunculus* (Abbes et al., 2003; Bouzeraa et al., 2004). Cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn) are the most present trace heavy metals in the wastewater of the gulf (Semadi

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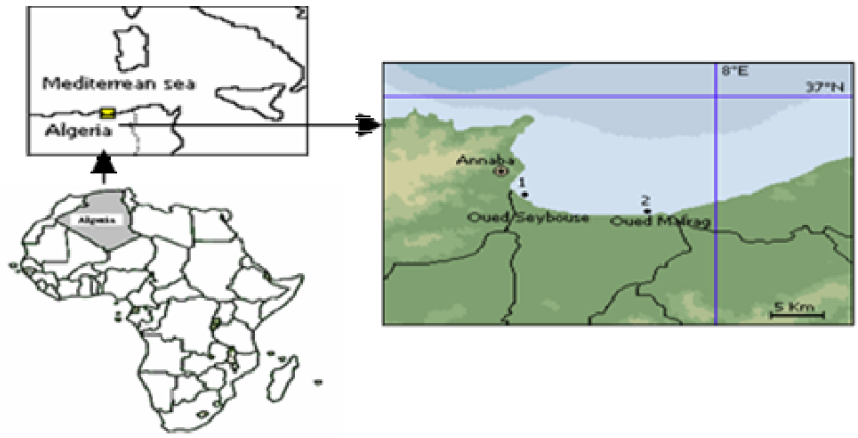

Figure 1. Location of studied area in Algeria and the two sampling sites in the Annaba gulf: Sidi Salem (1) and El Battah (2) beaches.

and Deruelle, 1993; Abdennour et al., 2000, 2004). Zn and Cu are used in small amounts as fertilizers in some soils deficient in these elements, while Zn, Cu and Cd are constituents of some fungicides. In addition, Cd and Zn occur as contaminants of phosphoric fertilizers (Migon et al., 1991). Biomonitoring programs based on measures of contaminants in marine organisms are also interesting from a human health point of view. Many factors such as season and physiological state or size of animals could affect the bioaccumulation of heavy metals in molluscs (Romeo and Gnassia-Barelli, 1988). Therefore, this study examined the seasonal variations of heavy trace metal concentrations in *D. trunculus* from coastal waters from northeast Algeria.

MATERIALS AND METHODS

Animals and collection sites

The Northeast of Algeria is characterised by its Mediterranean climate: hot and dry in summer (June-September), cold and wet in winter (December-February) and moderate in autumn and spring, with an average annual precipitation of 800 mm. Samples of standardized shell size (30 ± 5 mm) of *D. trunculus* were collected during four seasons (spring, summer, autumn and winter). Individuals were collected from two selected sites in the Annaba gulf (Figure) 1. El-Battah beach (8° 15' LE, 36° 38'lN), located about 30 km to the East of Annaba, far from any source of pollution and expected as a relatively clean site, and Sidi Salem beach (7°16'LE, 36° 68'lN) situated about 1 km to the East of Annaba and considered affected by the emission of considerable domestic, agricultural and industrial waste and locally untreated sewage (Semadi and Deruelle, 1993; Abdennour et al., 2000). Animals were collected from the two sites in May, July, September and December 2005. Analyses were made in six pooled samples per site and per season, each containing 20 individuals.

Analysis of trace metals

After collection, the clams were allowed to flush out undigested matter in filtered seawater from the sampling sites for 24 h (Sokolowski et al., 2002). The whole soft tissues of 20 individuals from each location and season were carefully removed by shelling the bivalves with a plastic knife (Chiu et al., 2000). Samples were dried at 60°C to a constant weight. All reagents were of analytical grade and obtained from Carlo Erba (Val de Rueil, France). All samples were digested in 4 ml of HNO_3 (50%) in closed polystyrene crystal tubes in a drying oven at 60°C for 48 h. Following acid digestion, all samples were analysed for Cd, Cu, Pb and Zn by atomic absorption spectrophotometry (Varian 220 FS and 220Z with Zeeman background correction). Validity of analytical methods was checked using standard biological reference materials (TORT-2, lobster hepatopancreas) provided by the National Research Council of Canada (Institute for National Measurement Standard, Ottawa, ON, Canada).

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 Student's t -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 MINITAB 12.21 software (PA State College, USA). The significance level was $p< 0.05$.

RESULTS AND DISCUSSION

From an environmental point of view, coastal zones can be considered as the geographic space of interaction between terrestrial and marine ecosystems that is of great importance for the survival of a large variety of plants, animals and marine species (Castro et al., 1999). Adverse anthropogenic effects on the coastal environment include eutrophication, heavy metals, organic and microbial pollution and oils spills (Boudouresque and Verlaque, 2002).

The concentrations of metals found in *D. trunculus* were presented in Figure 2. Generally, a similar seasonal profile was observed for the different metals in the two studied sites. The mean values recorded exhibited the following decreasing order: Zn, Cu, Pb and Cd for the two studied sites. An important concentration of Zn was also observed in the muscle of *Perna perna* (Moukrim et al., 2000). The concentrations measured in the soft tissues of *D. trunculus*

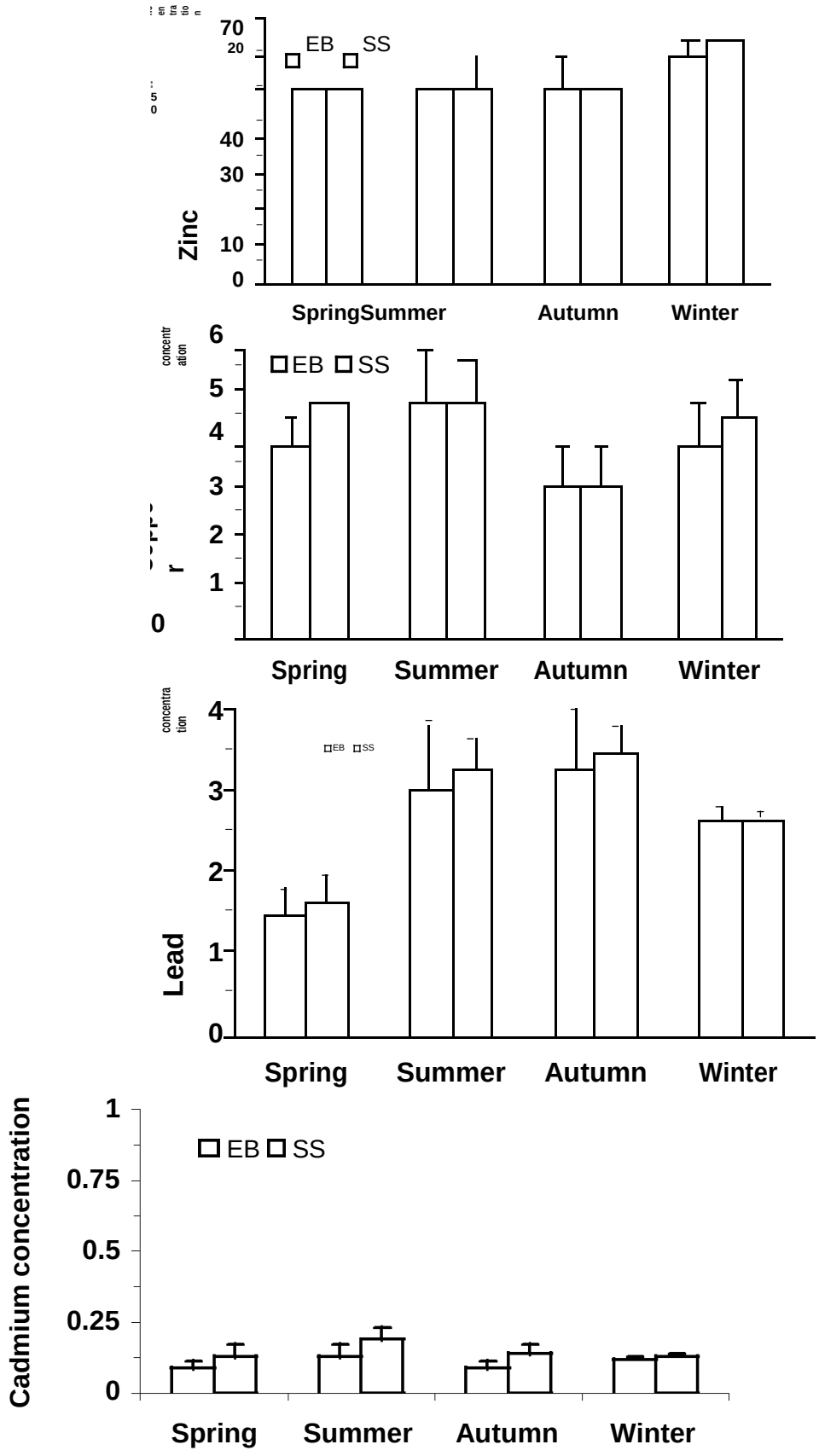

Figure 2. Seasonal variation of trace heavy metal concentrations (µg/g dry weight) in *D. trunculus* sampled at two sites (EB: El Battah; SS: Sidi Salem) in the gulf of Annaba (m±SD)

Table 1. ANOVA *p* values and significance levels for analyses of tissue trace heavy metal concentrations in *D. trunculus*.

	Cd	Cu	Pb	Zn
Season	0.000***	0.000***	0.000***	0.000***
Site	0.000***	0.954	0.320	0.228
Season x Site	0.341	0.419	0.978	0.942

*** $p < 0.001$

Table 2. Comparative trace heavy metal levels (µg/g dry weight) in some species of *Donax*.

Species	Site	Season	Cd	Cu	Pb	Zn	Reference
Donax deltoides	Contaminated	Spring Autumn		7-11 7-16	85-125 145- 155		Haynes et al., 1997
Donax trunculus	Control Contaminated	?	6-11 6-9	10-13 6-8	2-11		Fishelson et al., 1999
Donax trunculus	Control Contaminated	Autumn	0.19 0.20	60 383	1.1 3.6	63 107	Usero et al., 2005
Donax rugosus	Contaminated	Autumn Winter	0.63 1	13.3 6		53-70 64-80	Sidoumou et al.,2006

Table 3. Maximum acceptable limits (µg/g) of some trace heavy metals

Metal	µg/g	Reference
Cd	5	CEFAS, 1997
	10	NHMRC, 1987
Cu	100	BOE, 1991
	350	NHMRC, 1987
Pb	25	BOE, 1991
	50	Great Britain-Parliament, 1979
Zn	250	Ministry of Food, 1953
	750	NHMRC, 1987

revealed a significant ($p < 0.001$) effect of season for all metals measured. The temporal accumulation in *D. trunculus* varied according to the metals. Thus, the highest values were recorded in winter for Zn and in summer for the other metals.

It is generally agreed that heavy metal uptake occurs mainly from water, food and sediment. However, effectiveness of metal uptake from these sources may differ in relation to ecological needs and metabolism of animals and concentrations of the heavy metals in water, food and sediment as well as some other factors such as salinity, temperature, interacting agents (Roesijiadi and Robinson, 1994). The seasonal variation in metal concentrations observed in the gulf of Annaba could be related to the marine currents and the reproductive cycle of this species in the gulf of Annaba. The high accumulation of metals coincides with the low currents and the reproduction in the gulf of Annaba. The concentrations of trace metals were slightly higher in Sidi Salem in comparison with El Battah. However, there was no significant ($p > 0.228$) differences in Zn, Cu and Pb concentrations (Zn, $p = 0.228$; Cu, $p = 0.954$; Pb, $p = 0.320$) between the two sites, except for Cd ($p < 0.001$) (Table 1). The significant high concentrations of Cd in *D. trunculus* from site 1 (Sidi Salem) observed in summer, autumn and winter, indicate a local source of contamination. The site of Sidi Salem is located near several sources of pollution: harbour and many factories such those producing phosphoric fertilizers and pesticides. As shown in (Table 2), trace metal concentrations were also found to vary as function seasons and contamination levels of sites examined in several species of *Donax*: *D. deltoides* (Haynes et al., 1997*), D. trunculus* (Fishelson et al., 1999; Usero et al., 2005) and *D. rugosus* (Sidoumou et al., 2006). Concentrations of the trace metals Zn, Cu, Fe, Cd and Pb in four caridean decapods from northeast Algeria, varied according to locations and seasons (Abdennour et al., 2000), while in sediments they were low in clean sites, and slightly higher in the other locations in relation to anthropogenic sources (Abdenour et al., 2004). The high concentrations of Zn and Cu observed in *D. trunculus* were correlated with the metal contamination of sediments. Based on a previous report (Abdenour et al., 2004), the highest metal concentrations in sediments recorded for Annaba gulf are represented by Fe, followed by Zn and Cu. Similarly, a fair correlation between concentration of pollutants in animal tissue and in sediments was observed (Baumard et al., 1998).

Heavy metals pollutants are conservative and often highly toxic to biota (Clark, 1992; Richardson, 1995). They have been shown to be an important group of toxic contaminants because of their high toxicity and persistence in all aquatic system (Amin, 1996) . As, Cd, Cu, Hg, and Zn are the five metals with most potential impact that enter the environment in elevated concentrations through storm water and wastewater discharges as a consequence of agricultural and industrial activity. Accumulation of such contaminable amount of heavy metals can cause toxic reactions along the food chain. The values of trace heavy metals detected in this edible species were low as compared to the maximum acceptable limits (Table 3).

CONCLUSION

The determination of metal concentrations in organisms is part of an assessment and monitoring program in the coastal zone of Annaba that will bring results useful for health and environmental risk assessment. *D. trunculus* is suitable as bioindicators for monitoring the quality of water from the Annaba gulf. Concentrations of metals showed seasonal variations. The two monitored sites were contaminated by metals. The difference observed between these two sites was related to their pollution level. However, the metal contamination in the gulf of Annaba is relatively low as compared with values reported in some mollusc species from other countries. Thus, the contamination risks of local population via this edible species were low.

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