Seasonal variations in four trace metals (Cu, Zn, Pb, Cd) in sponges *Sarcotragus spinosulus* of the Gulf of Annaba, Northeast Algeria

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Abstract. The main objective of this study was to evaluate for the first time the contamination of the Gulf of Annaba (extreme east of Algeria) by several metallic trace elements (Cu, Zn, Pb and Cd) using sponges (*Sarcotragus spinosulus*). Samples of this species were collected in the Gulf for four consecutive seasons (2015/2016) at two separate sampling stations, one located near to an industrial and agricultural zone (Alzon) and the other far from anthropic activities (Cap de Garde). The levels of the metals were determined by flame atomic absorption spectrophotometry. Analysis of the spatial distribution of the trace elements revealed the existence of a highly significant difference for all of the assayed elements, with the exception of Cu which exhibited no significant difference between the two sampling stations. The seasonal variations between the two sampling stations revealed elevated levels of metals in the tissues of *S. spinosulus* during winter and spring, with the exception of Zn for which we only noted a peak in summer that is probably correlated with the reproduction cycle of the species.
Key Words: heavy metals, pollution, Mediterranean, monitoring, bio-indicator.

Introduction. Due to the solubility of heavy metals, the analysis of sea water cannot be considered to be a reliable way to determine the degree of metal contamination in the marine environment (Philips 1977). Studying the bioaccumulation of trace metals in organisms that are exposed to them therefore constitutes a more effective means for evaluation of the metallic pollution (Lagadic et al 1997).

The qualitative and quantitative bioaccumulation of various mineral and organic contaminants along the Algerian coast has been documented in bivalve mollusks and gastropods in particular (Boucetta et al 2016; Boujema et al 2016; Merad & Soltani 2017). The majority of these authors focused on sedentary fauna that can perform limited vertical or horizontal movements, which could lead to underestimation of the bioaccumulative capacities of heavy metals, unlike fixed species such as sponges for which bioaccumulation of heavy metals are more significant. Indeed, the vast majority of these macrozoobenthic organisms are filter feeders and they consume a larger range of particles (e.g., bacteria, organic debris, and microalgae), including dissolved organic material. In light of this, they generally reflect the levels of contamination, both of the dissolved and particulate compartments. Moreover, several examples have shown the potential of these organisms as ecological indicators, bioaccumulators of pollutants, and a matrix for research of biochemical markers (Perez et al 2004; Basuyaux et al 2013; Gentric et al 2016). In light of their broad geographical and bathymetric distribution, their low level of tissue differentiation, and the capacity to assimilate both dissolved and particulate matter, these invertebrates can provide information complementary to bivalves and even compensate for some of the latter's limitations (Perez et al 2000).

The objective of this study was to evaluate for the first time the contamination of the Gulf of Annaba by heavy metals, using sponges (*Sarcotragus spinosulus*) as a biological bioindicator model of the metal pollution in this region.

Material and Method

Study area and sampling. The study was carried out in the Gulf of Annaba, a coastal area situated between ''Cape Rosa'' (8°15' E - 36°38' N) to the east and ''Cape of Garde'' to the west (7°16' E - 36°68' N). This sector is considered of economic interest (industry, recreational fishing, balneary tourism).

The sampling area was demarcated based on the following parameters: accessibility to the site, availability of the biological material (i.e., sponges), and proximity of sources of pollution (domestic and industrial). For this, we chosed two sampling stations, one situated in a periurban area, on the west coast of the port of Annaba (station I: Alzon, geographical location: 36°54'57.54''N - 07°46'13.29''E) and the other one far from domestic and industrial pollution (station II: Cape of Garde, geographical location: 38°58'3.25''N - 07°47'28.33''E) (Figure 1).

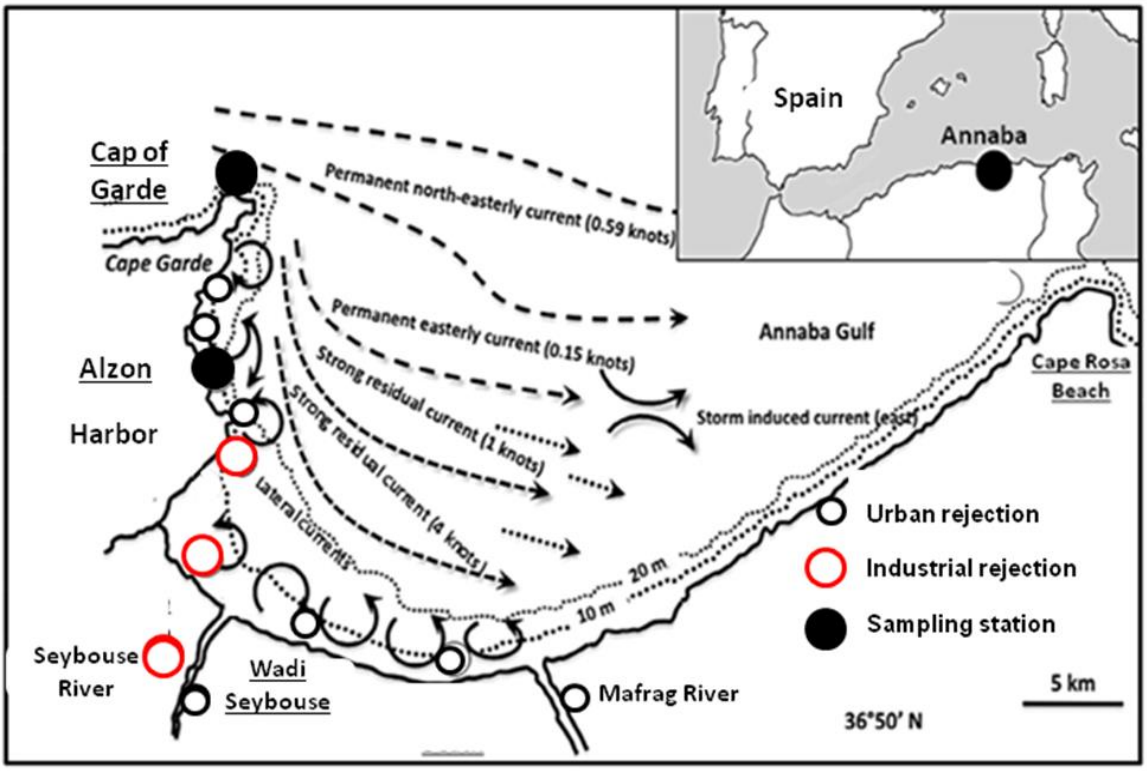

Figure 1. Study area and sampling of *Sarcotragus spinosulus* (in Belabed et al (2013), amended).

The choice of the sponge *S. spinosulus* is justified by its presence in the two sampling areas. Samples of *S. spinosulus*, with thickness of approximately 3 cm (from the surface to the base) and at a rate of 6 individuals per station, were collected by free dive between 0 and 3 meters using a thoroughly cleaned stainless steel knife, over the course of four seasons (autumn 2015/winter-spring-summer 2016). Once harvested, the samples were stored at -25°C until the time of measurements.

Method for analyzing the metals. Assessment of the level of the four metals (Pb, Cd, Zn, and Cu) in the sponges was carried out by flame atomic absorption spectrophotometry (AAS) using a Shimadzu AA6500 device connected to a microcomputer equipped with an integrated calculator. Each sample first underwent mineralization by heating. Fragments of 1 g were recovered and dried in an oven at 60°C until a constant weight was obtained. After cooling, the various samples were mineralized in the presence of 4 mL of ultra-pure NHO_3 acid (soaked for at least 48 h). The mineralized material was then filtered using a Whatman N°41 filter and adjusted to 18 mL with distilled water (Amiard et al 1987). The product obtained thereby was stored in hermetically sealed glass flasks until its analysis. The results were expressed as average values (± the standard deviation). The comparison of the average values between the two sampling stations (for the same season) and between seasons was carried out using the Student's t-test. The effects of the seasons and the sampling stations were tested by an analysis of the variance with two classification criteria (ANOVA). The statistical analyses were carried out using Minitab16 software (version 1.1.0).

Results. The various metallic pollutants (Cu, Zn, Pb, and Cd) that we investigated were present at different concentrations in the samples of *S. spinosulus* collected at the two study stations (Table 1).

At station I, the lowest average concentrations of metals were recorded in the autumn for Cu (24.82±2.37 μg/g DW), Zn (30.8±8.7 μg/g DW), lead (2.11±0.47 μg/g DW), and cadmium (0.65±0.006 μg/g DW). Whereas the highest concentrations were seen in winter for Cu (33.36±3.77 μg/g DW), in spring for Zn (51.77±6.32 μg/g DW), and in summertime for Pb and Cd, with values of 53.9±1.23 μg/g DW and of 0.74±0. 06 μg/g DW, respectively (Table 1).

At station II, the maxima for the MTE were obtained in spring for the full set of the assayed elements, with the exception of Cu for which the maximal levels were recorded in winter (31.77±2.84 μg/g DW) and in summer for Zn (152.76±35.5 μg/g DW). By contrast, the minimal concentrations were noted in summer for Cu, in winter for Zn and Pb, and in the autumn for Cd (Table 1).

Table 1

Seasonal variations in the concentrations of trace metals (Cu, Zn, Pb, Cd) in the samples of *Sarcotragus spinosulus* from the Annaba Gulf

Stations	*Seasons*	*Concentrations (μg/g DW)*			
		Cu	*Zn*	*Pb*	*Cd*
Station I (Alzon)	Autumn	24.82±2.37	30.8±8.7	2.11±0.47	0.65±0.06
	Winter	33.36±3.77	39.99±5.48	4.84±1.35	0.68±0.04
	Spring	30.57±4.76	51.77±6.32	2.34±0.61	0.74±0.10
	Summer	25.33±3.59	43.07±3.97	5.39±1.23	0.74±0.06
Station II (Cap of Garde)	Autumn	29.35±1.07	114.70±14.6	5.91±0.76	0.02±0.001
	Winter	31.77±2.84	32.27±2.98	5.17±0.87	0.48±0.08
	Spring	27.45±2.65	33.16±2.93	8.47±0.94	0.57±0.06
	Summer	21.81±2.85	152.76±35.5	6.16±0.92	0.07±0.01

The results are expressed as μg/g of dry weight (DW).

The two-way ANOVA (sampling station as fixed factors and the season as random factors) revealed highly significant differences ($p<0.001$) between the two sampling stations and the different seasons, with the exception of copper which did not exhibit any variation between the sampling stations (Table 2).

Table 2

Results for the variance analysis at two classification criteria (ANOVA) of the various MTE (i.e., Cu, Zn, Pb, Cd) analyzed in the samples of *Sarcotragus spinosulus* from the Annaba Gulf

	Metal	F_{obs}	*P*	*Signification*
Stations	Cu	1.03	0.317	n.s
	Zn	101.19	0.000	***
	Pb	102.96	0.000	***
	Cd	490.16	0.000	***
Seasons	Cu	16.91	0.000	***
	Zn	47.36	0.000	***
	Pb	7.84	0.000	***
	Cd	64.37	0.000	***

The results are expressed as μg/g of dry weight (DW). n.s.- not significant ($p>0.05$), *** - very significant ($p<0.001$).

Discussion. Sponges, like numerous invertebrate biological models (e.g., annelids, bivalves, echinoderms, etc.) are considered to be species that are indicative of pollution of coastal waters.

This study carried out in the Gulf of Annaba allowed us to find evidence for spatial variations in the concentrations of four heavy metals measured in the tissues of *S. spinosulus*. Thus, the lowest concentrations were recorded at sampling station I for Zn and Pb and at station II for Cu and Cd. Furthermore, the highest levels of Co and Cd were recorded at station I, unlike Pb and Zn for which the highest levels were found at station II.

Ambient environmental variations linked to areal (Blackmore & Wang 2003) and/or seasonal influences (Regoli 1998) may affect or promote the accumulation of heavy metals. The metallic contamination of tissues of the biological model that was used could be mainly attributed to local sources of pollution and to environmental conditions in the Gulf of Annaba. Indeed, station I (Alzon) is more exposed to metallic pollution emanating from the port of Annaba and the Seybouse Wadi, than station II (Cape of Garde) which is further away than the first station (7 km as the crow flies). With a catchment area of 6,471 km² and an average flow of 11.5 m^3/s; the Seybouse Wadi is one of the largest watersheds in Algeria that discharges it polluted waters into the Gulf of Annaba, without prior treatment (Abdennour et al 2011; Belabed et al 2013; Bouchelagem 2017). Similar findings for Pb, Cu, and Cd have been reported in other Mediterranean countries (e.g., Spain, Liban and Greece) (Abed 2011). The results obtained with the sponge *S. spinosulus* are close to those found with other macrozoobenthic species of the Gulf of Annaba, as is the case for the gastropod *Phorcus turbinatus* (Boucetta et al 2016) and the mussel *Perna perna* (Belabed et al 2013).

Cape of Garde, which is considered to be a eutrophic area that is spared from anthropic actions (Frehi et al 2007), is a site that has been proposed as a Specially Protected Area of Mediterranean Importance (SPAMI) (Derbal & Amarouayache 2015). However, this maritime section exhibits high concentrations of Zn and PB, which could be related to the mass tourism that has been developed near this area (e.g., heavy road and maritime traffic) and the presence of Zn that occurs naturally in the earth's crust, often in association with lead (Casas 2005).

The measurement of four metals in the tissues of *S. spinosulus*, collected at the two sampling stations, revealed highly significant seasonal variations. Despite the significant variations between the sampling stations, the concentrations of the trace elements that were studied were consistently higher in winter than in summer, with the exception of Zn for which the peak was recorded in summer. The level of Zn is most likely correlated with the reproduction cycle of the studied species. Indeed, we observed the start of the gametogenesis activity during this period (Bensafia et al study in progress). The high concentration of zinc could be related to the enzymatic activity in aquatic invertebrates (Boucetta et al 2010; Belhaouari et al 2011). The increase in trace metals over the course of the cold and rainy season is mainly due to the high load of metallic pollutants in the water runoff from the first floods of the season. The seasonal nature of bioaccumulation of heavy metals along the Algerian coastline has also been observed of various species of bivalve and gastropods mollusks (Belabed et al 2013; Boucetta et al 2016; Boujema et al 2016). These seasonal variations may be caused by a combination of several biotic and abiotic factors, as well as by the bioavailability of metals (Rainbow et al 2004).

Conclusions. In conclusion, this study has shown that the levels of metallic trace elements detected in the tissues of *S. spinosulus* varied from one sampling station to another and from one season to another, based on the extent of disturbance of the site. In light of this, these levels clearly reflect the degree of pollution in the Gulf of Annaba. Our results support the use of the sponge *S. spinosulus* as a sentinel species for biomonitoring of the Annaba coast.

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