IN SITU COMPARATIVE STUDY OF SEVERAL MEDITERRANEAN SPONGES AS POTENTIAL BIOMONITORS OF HEAVY METALS

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ABSTRACT

The heavy metals content of sponges is investigated in order to assess their suitability as biomonitors. The concentration of 10 elements is determined in six species of massive Demosponges well represented in a polluted and a clean site: *Cliona viridis*, *Cacospongia scalaris*, *Chondrosia reniformis*, *Spongia officinalis*, *Spongia agaricina* and *Agelas oroides*. Wide interspecific variations are observed between them, no doubt as a result of their morphological and physiological differences. *Cliona viridis* appears to be unique with regard to the higher concentrations found for several elements, especially Cd that is from 42 to 375 times more concentrated than in the other species. However, this species displays highly variable results and gives a rather poor image of the level of contamination of the sites, as only three trace elements out of ten have a significantly higher concentration in the polluted site. Three species, *S. officinalis*, *S. agaricina* and *A. oroides*, which give a consensual indication of the metals' bioavailability, with sufficiently high and homogenous concentrations, appear to be well suited for consideration in the overall assessment of the marine environment quality.

KEY WORDS

Porifera, heavy metals, biomonitor, bioavailability, Mediterranean Sea.

INTRODUCTION

An ideal biomonitor should be an opportunistic species, tolerant of physical-chemical fluctuations, sedentary and abundant at polluted sites and easy to collect and identify (RAINBOW & PHILLIPS, 1993). It should strongly accumulate and not regulate and thus reflect accurately any variations in environmental contamination.

Sponges have some of the characteristics of good bioindicators, in other words they are organisms which may offer a practical and safe way to characterize the state of an ecosystem and to highlight their natural or man-made alterations (PEREZ, 2000). They are important components of hard-bottom assemblages, and have an extensive depth and geographical range. Thus, sponge communities or indicator species (like clionid sponges) have frequently been used to indicate environmental crises in temperate as well as in tropical ecosystems (MURICY, 1991; CARBALLO *et al.,* 1996; HOLMES, 1996). Their sophisticated aquiferous system give them the ability to actively filter a water volume equivalent to their own volume in 3.7 to 20 seconds

(REISWIG, 1974; LARSEN & RIISGÅRD, 1994). They have excellent retention rates for particles from 0.2 to 50 μm, thus including colloids and small procaryotic cells that are not retained by most other filter feeders (REISWIG, 1971; THOMASSEN & RIISGÄRD, 1995; RIBES *et al.,* 1999). Most of the sponges are symbiotic with microorganisms providing a significant amount of dissolved organic matter in their diet (REISWIG, 1981). Because of all these properties, sponges can collect a very large variety of pollutants from the suspended or dissolved phases. Several studies have already demonstrated the potential of sponges for the monitoring of pollutant levels in the marine environment: hydrocarbons (FÉRAL *et al.,* 1979; ZAHN *et al.,* 1981), organochlorinated compounds (VERDENAL *et al.,* 1990, ARNOUX *et al.,* 1992), and metals (PATEL *et al.,* 1985; VERDENAL *et al.,* 1990; RICHELLE-MAURER *et al.,* 1994; HANSEN *et al.,* 1995). Experimental in vitro studies have shown that the concentration factors are very high for most elements, and accumulation is a function of the quantity present in the environment (RICHELLE-MAURER *et al.,* 1994; HANSEN *et al.,* 1995).

The ultimate goal of the study of sponges as bioindicator is to set up a monitoring strategy for Mediterranean hard substratum communities (PEREZ, 2001). In the present paper, we report metal concentrations recorded in several species of sponges well represented on hard-bottom and we check their advantages as biomonitors. According to the concentration capability, the homogeneity and representativeness of the results, we intend to identify the best Mediterranean sponge species for long-term biomonitoring of heavy metals within the infra and circalittoral zones of the Mediterranean.

MATERIALS AND METHODS

Sponge material. Six species of sponges belonging to different orders were examined: *Cacospongia scalaris* Schmidt, 1862; *Spongia officinalis* Linné, 1759; *Spongia agaricina* (Pallas, 1766), order Dictyoceratida; *Agelas oroides* (Schmidt, 1864), order Agelasida; *Chondrosia reniformis* Nardo, 1847, order Chondrosida; *Cliona viridis* (Schmidt, 1862), order Hadromerida. All these species are tolerant, abundant in both clean and polluted hard-bottom communities, and easy to identify while SCUBA diving.

Sampling sites (Fig. 1) are located within a zone under the influence of the waste water effluent emanating from the urban area of Marseilles (Mediterranean, France). A sewage plant has treated the water since 1987 and the control office of the sewage plant (Fig. 1B) now regularly analyses its composition. Effects on both the environment and benthic communities in this area have been reported in a number of studies (for a review see BELLAN *et al.,* 1999). Thus the dispersion of the pollutants and their effects on hard-bottom assemblages including sponge communities (MURICY, 1991) are well known. Site 1, located 300 m east of the discharge outlet, is in a heavily polluted area, whereas site 2, located 5 km south of site 1 and protected by an island, is in a clean area.

Treatment of the samples for metal analysis. Six specimens of each species of sponges were collected in both sites at a depth between 10 and 15 m by SCUBA diving on vertical rock-faces or on the ceiling of small caves during summer 1998. Specimens thus non-exposed to the sedimentation were chosen of the same size for each given species in order to avoid as far as possible variations of the metal levels due to different ages. Twenty-five specimens of mussels, *Mytilus galloprovincialis* Lamarck, 1819 were collected at 1 m in depth in site 1, and treated at the same time as the sponges. The samples were frozen at –25° C soon after collection, and subsequently lyophilised. One gram of each freeze-dried sample was mineralised with 10 ml of nitric acid 65 % Merck (suprapur quality) in PTFE bombs using a microwave oven (Prolabo A301 Microdigest). The same treatment was used for three replicates of the 25 mussels pooled. The digests were analysed for 10 metal elements : iron, copper and zinc by flame atomic absorption spectrometry (PYE UNICAM SP9 Atomic Absorption Spectrophotometer Philips); lead and cadmium by a graphite furnace atomic absorption spectrometry (UNICAM 939 QZ Atomic Spectrophotometer Absorption with graphite furnace "Zeeman 6790" SOLAR); chromium, arsenic, titanium and vanadium by Inductively Coupled Plasma Spectrometry (JOBIN YVON "JY24"); and mercury by X-ray Fluorescence (Merlin Mercury Fluorescent Detector). The analytical methods were validated through internal and external calibration (Analytical quality approved by the French Ministry of the Environment). The results given as means and standard deviation expressed in μg/g of dry weight were analysed statistically for differences between the sites by a Student test T.

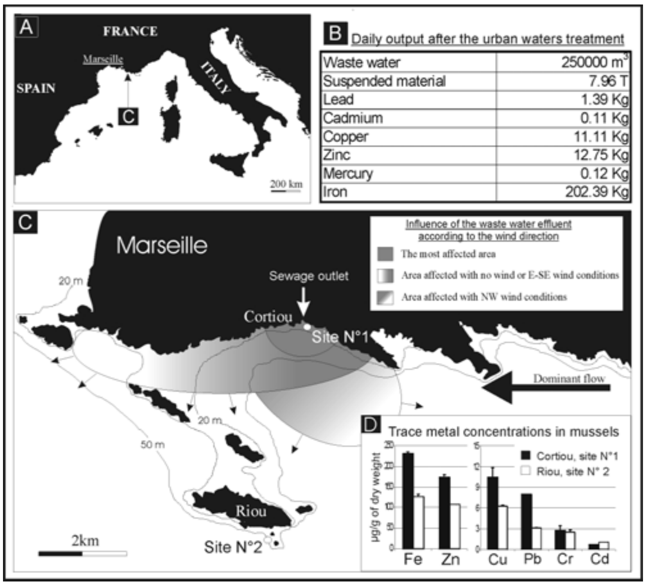

Fig. 1. A, localization of the Cortiou area. **B,** the two sampling sites are located within a zone influenced by the waste water effluent of Marseilles (annual mean results for 1995, Control Office of the sewage plant, pers. comm.). **C,** the dispersion of the waste water effluent in the area studied; site 1 is at 300 m from the outlet and site 2 is at a distance of 5 km. **D,** the contamination levels of mussels have been already studied (from ARNOUX *et al.,* 1993).

RESULTS

The comparison between sponges and mussels from site 1 shows that all sponges concentrate heavy metals more than mussels (Tab. I). According to the elements, the maximum concentrations measured in sponges are from 6 to 145 times higher than in mussels. The only exception is Zn, which is a little more concentrated by mussels than by most of the sponges tested.

Tab. I. Mean (m) and standard deviation (s) of concentration in metals (µg/g of dry weight = ppm of dry weight) in the polluted site (1, Cortiou). Ao = *Agelas oroides*; Cs = *Cacospongia scalaris*; Cr = *Chondrosia reniformis*; Cv = *Cliona viridis*; So = *Spongia officinalis*; Sa = *Spongia agaricina*; Mg = *Mytilus galloprovincialis*.

		Fe	Cu	Zn	Pb	Cd	As	Cr	V	Ti	Hg
Ao	m	2588.4	23.5	59.3	4.4	1.4	217.3	5.4	11.0	7.0	1.7
	s	*66.8*	*0.3*	*0.9*	*0.2*	*0.8*	*0.4*	*0.1*	*1.0*	*0.4*	*0.2*
Cs	m	676.9	151.6	49.7	1.1	0.3	100.4	0.6	4.7	4.2	0.8
	s	*37.9*	*2.7*	*0.8*	*0.0*	*0.1*	*1.9*	*0.1*	*0.6*	*0.1*	*0.0*
Cr	m	351.6	10.8	73.8	0.4	0.5	152.6	5.4	6.0	3.3	0.8
	s	*12.6*	*1.3*	*4.9*	*0.1*	*0.1*	*22.2*	*0.1*	*0.5*	*0.0*	*0.1*
Cv	m	1806.1	10.2	3734.7	13.7	58.1	75.5	1.8	12.7	4.2	0.3
	s	*432.2*	*1.3*	*1144.4*	*0.8*	*3.9*	*70.9*	*0.4*	*1.2*	*0.2*	*0.4*
So	m	9229.5	63.7	68.0	10.5	0.3	116.7	9.1	34.6	15.9	0.9
	s	*1111.2*	*0.6*	*0.2*	*0.6*	*0.1*	*4.1*	*0.2*	*0.6*	*0.1*	*0.1*
Sa	m	5491.8	44.6	57.5	6.9	0.2	89.7	6.8	20.7	17.8	0.3
	s	*92.1*	*0.9*	*3.3*	*0.1*	*0.0*	*4.2*	*0.1*	*0.6*	*0.3*	*0.0*
Mg	m	171.1	9.4	114.7	1.6	0.4	33.6	1.0	4.3	1.9	0.2
	s	*6.1*	*0.4*	*6.4*	*0.1*	*0.0*	*0.6*	*0.1*	*0.1*	*0.1*	*0.0*

Wide variations between sponge species were observed at a given site (Tab. I), each species presenting specificities. Except in *Cliona viridis*, Fe is the most accumulated metal, the maximum concentrations being observed in the *Spongia* spp. These species also exhibit the greatest concentrations of Cr, V and Ti. *C. viridis* shows very high concentrations of Zn and Cd, with Cd being from 42 to 375 times more concentrated than in the other species. This species also shows the maximum Pb concentration. *Cacospongia scalaris* exhibits Cu concentration 2 to 15 times higher than the other species and *Agelas oroides* shows the maximum concentrations of Hg and As.

The inter-individual variability of the results shown by the six specimens analyzed for each species differs according to the species (Tab. I). *Spongia agaricina* has the most homogenous results, the coefficient of variation being lower than 10% for all the elements concentrated by the sponge. The variability in this sponge is lower than that found in *Mytilus galloprovincialis*. *A. oroides*, *Spongia officinalis* and to a lesser extent *C. scalaris* also display homogenous results, with the exception of Cd concentrations which are very variable. The species which present the worst results in terms of inter-individual variability are *Chondrosia reniformis* and *C. viridis*, both presenting coefficients of variation higher than 10 % for 5 elements out of 10.

The comparison between site 1 and site 2 (Fig. 2) shows that some species give a better image of the level of contamination than others, with metal concentrations in more or less good agreement with the exposure to the main source of contamination. Although unique in regard to the high concentrations found for several elements, *C. viridis* gives a rather poor image of the level of contamination of the sites, as only three trace elements (Fe, Pb, V) out of 10 have a significantly higher concentration at the polluted site (Fig. 2), whereas the seven other elements do not vary significantly between the two sites. *Chondrosia reniformis* (Fig. 2) has five trace elements (Fe, As, Cu, Cr and Hg) more concentrated in the polluted site, whereas Cd concentration is higher at the clean site, and the content in four elements (Zn, Pb, V and Ti) does not vary significantly between sites. *C. scalaris* (Fig. 2) shows Fe, Cu, Pb, Ti and Hg concentrations higher at the polluted site, whereas Zn, As, V and Cd concentrations are higher at the clean site, and Cr does not vary significantly. *S. agaricina* (Fig. 2) has six metals (Fe, Zn, Pb, Cr, V, and Ti) more concentrated in the polluted site, three (Cu, As and Hg) more concentrated at the clean site, and Cd does not vary. *S. officinalis* (Fig. 2) shows seven metal concentrations (Fe, Cu, Zn, Pb, Cr, V and Ti) higher at the polluted site, and three (As, Cd and Hg) which do not vary significantly. *A. oroides* (Fig. 2) has eight metals (Fe, Cu, Zn, Pb, Cr, V, Ti and Hg) more concentrated at the polluted site and two (As and Cd) which do not vary significantly.

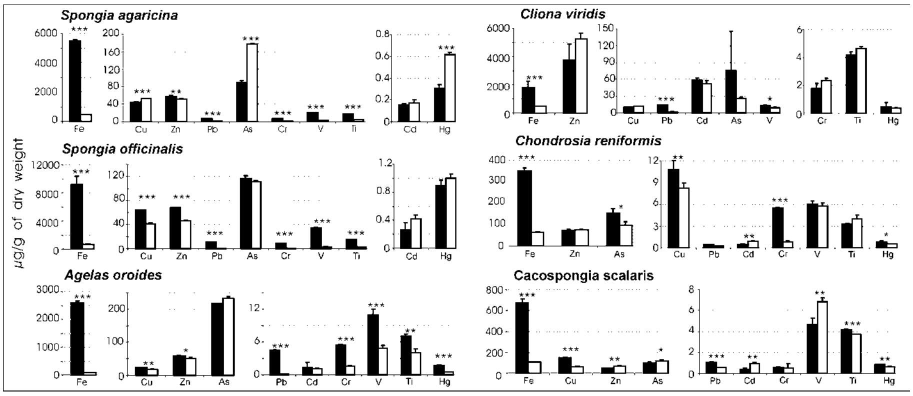

Fig. 2. Heavy metal mean (+ s, n = 6) concentrations (ppm dry weight) in samples from sites 1 (polluted in black) and 2 (clean in white). Differences between sites are statistically tested. Significant levels of the differences * = 5 %, ** = 1 %, *** = 0.1 %.

The indications also differ according to the heavy metals. Among the metals analyzed, Fe is the only element for which all sponge contents are much higher at the polluted site than at the clean site. Pb is also overall more concentrated in sponges collected at the polluted site, the only exception being *C. reniformis* for which the difference recorded is not significant. In contrast to these two elements, Cd does not vary very much between sites, the only exceptions being for *C. scalaris* and *C. reniformis* which both show higher concentrations at the clean site. But the same trend is also observed in mussels (Fig. 1D). For the other metals except As, the majority of the sponge contents are much higher at the polluted site than at the clean site, at least three or four out of the six species giving a similar indication.

DISCUSSION AND CONCLUSIONS

Heavy metal contents of sponges revealed impressive differences with mussels, the most widely used marine biomonitors. Although wide interspecific variations have been observed in the six species of sponges, the concentrations are higher by generally more than one order of magnitude in sponges than in mussels from the same site. These differences could be explained by the differences in life strategies of sponges and bivalve molluscs. Both sponges and bivalves are suspension-feeders that filter large amounts of water. However, the nature of the organic material and the size of the particles that are retained differ significantly. Sponges have a high retention rate for particles of bacteria size, and appear to be able to meet their entire carbon requirements by feeding on particles smaller than 1 µm (STUART & KLUMPP, 1984; RIBES *et al.,* 1999). Furthermore, there is reasonable evidence that "bacteriosponges", such as all the species tested in this study except *Cliona viridis*, are able to significantly use dissolved organic matter (REISWIG, 1981). In contrast, bivalves have a good retention rate for particles larger than 4 µm, with a regime including a significant amount of particles larger than 100 µm (STUART & KLUMPP, 1984; NEWELL *et al.,* 1989; RIISGARD *et al.,* 1996). The higher concentrations in sponges than in mussels may also be explained by differences in their respective life span. Mussels are short-lived, and thus are well suited for the monitoring of short and marked phenomena (AMIARD *et al.,* 1986). In contrast, sponges may live several decades and thus accumulate during a longer time. A comparison with the results obtained at site 1 on *Spongia officinalis* collected in 1983 (VERDENAL *et al.,* 1990) shows that heavy metal concentrations impressively decreased in sponges between 1983 and 1998. This is particularly clear for Pb, Cr and Cd that are respectively 9, 8 and 5 times lower in 1998. Such a decrease is most likely due to the establishment of a sewage treatment plant in 1987.

Among sponges, we observed interspecific variations of the accumulation patterns, probably as a result of their physiological and skeletal differences. This result differs from what has been observed for four species of fresh-water sponges (RICHELLE-MAURER *et al.,* 1994). These fresh-water sponges, however, belonged to the same family and had a similar morphology and physiology, while the present study includes a greater variety of sponges.

The diversity of morphology of the aquiferous system may be related to their ability to up-take a wide array of particles and to their powerful pumping activity influencing the water volumes passing through the body (REISWIG, 1975), and thus the pollutant accumulation. The three-dimensional architecture of the aquiferous system of three of the species tested in the present study, *Chondrosia reniformis*, *S. officinalis* and *C. viridis,* has been shown to be similar (BAVESTRELLO *et al.,* 1988; BURLANDO *et al.,* 1990), although the three species display different accumulation rates. For instance, *C. viridis* and S. *officinalis*, which differ widely in their metal content in the same environment, have very similar choanocyte chambers (respectively 23 ± 4 µm and 20 ± 4 µm in diameter and approximately 12000 choanocyte chambers / mm^3).

Among the six species used in this study, *C. viridis* appears unique with regard to the higher concentrations found for several elements, by the high inter-individual variability, and results that are generally not in agreement with the distance from the main source of pollutants. This sponge, which contains a large mineral fraction, made of calcareous debris and of skeletal siliceous spicules, also has a very different mode of life. All clionid sponges (family Clionaidae) excavate calcareous substrates. Under special conditions, especially eutrophication, they may develop as encrusting or massive form after more or less complete erosion of the calcareous substratum. The special behavior of *C. viridis* for accumulation of metals may be related to this ability to bio-erode calcareous substrates, which in the study area are frequently biological concretions made primarily by calcareous algae, which are themselves able to accumulate heavy metals mainly from the dissolved fraction. The high value found for several metals may thus result from the magnification by this clionid of the elements already accumulated in the biological constructions dominated by calcareous algae, whereas all the other species take metals directly from the surrounding seawater. *Cliona viridis* is also unique in its symbiotic association. In contrast to the other species that have been tested, which are "bacteriosponges" containing a large number of symbiotic bacteria, *C. viridis* is associated with a large quantity of light-dependent zooxanthellae (SARÀ & LIACI, 1964; ROSELL & URIZ, 1992), and with only very few bacteria. It is possible that this unique association influences the accumulation capacity and the peculiarities found for this species. Like other unicellular algae (RILEY & ROTH, 1971; BONIN *et al.,* 1986), zooxanthellae could be capable of accumulating metals from the dissolved phase.

The structure and biological composition of the sponge are probably the most important factors for its ability to retain or to eliminate the metals. The skeletal structure, whose size varies considerably in sponges, is able to concentrate metals differently from the living tissue (VERDENAL *et al.,* 1990). Iron is concentrated by *Spongia* spp. in lepidocrocite granules in spongin fibres (TOWE & RÜTZLER, 1968; VACELET *et al.,* 1988). The spongin fibres also contain Pb, Cr, Zn and V, possibly associated with the lepidocrocite crystals. Our results show that these metals, that are trapped in these skeletal structures, are more abundant in the *Spongia* spp. than in the species which have a less developed fibre skeleton or no spongin at all.

Because of the efficiency of modern sewage plants, the contaminants discharged in the marine environment are now primarily conveyed by very fine particles. Sponges are well suited for the monitoring of this type of chemical contamination. More studies are needed for a better correlation between the behaviour patterns with regard to heavy metals and the characteristics of the diverse species, such as the structure of the aquiferous system, the amount of symbiotic micro-organisms, the life-history, the nature of the mineral or organic skeleton.

According to the selective criteria tested above, three species appear to be good candidates for the assessment of the marine environment health. *Agelas oroides*, *Spongia officinalis* and *Spongia agaricina* show a good accumulation ability, with homogenous results in good agreement with the theoretical pollution level of the surrounding medium for a large number of elements. Fe and Pb, which are undoubtedly of urban origin, are correctly indicated by all the sponge species, with significant higher concentrations at the polluted site. In addition, the three good candidates also give a consensual indication of the bioavailability in Zn, Cr, V, Ti, and Cd, the latter being the only element, which is not more concentrated near the main source of pollution. *Cliona viridis*, although showing interestingly high accumulation rates for some metals and having a high abundance in eutrophic areas, could not be adopted until the variability of the results is better understood.

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