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Arsenic accumulation in intertidal macroalgae exposed to sewage discharges

Joana Cabral-Oliveira[1] · **Helena Coelho**[2,3] · **João Pratas**[4,5] · **Susana Mendes**[1,6] · **Miguel A. Pardal**[1]

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Abstract Arsenic is a widely distributed element in marine ecosystems. The main anthropogenic sources of this contaminant are domestic and industrial wastewaters, and since it can be harmful to humans even at low concentrations, it has been ranked as one of the top hazardous substances. Therefore, the analysis of arsenic is an essential task to assess the potential environmental and human health risk associated with sewage discharges. The accumulation of total arsenic on intertidal rocky shore macroalgae exposed to contaminated sewage discharges was measured in five macroalgae species (*Asparagopsis armata*, *Codium* sp., *Plocamium cartilagineum*, *Saccorhiza polyschides* and *Ulva* sp.). Differences in the concentrations of arsenic were examined in the seawater and in the macroalgae species. The results showed significantly higher concentrations of arsenic near the sewage discharges in all the species except *S. polyschides*. Although the information obtained from total arsenic determination is not enough to assess the toxicological risk in the environment, this paper gives an important contribution on contamination risks and helps in choosing potential good biomonitors.

Keywords Arsenic · Sewage · Macroalgae · Bioindicators · Rocky shores

Introduction

Sewage pollution is normally associated to organic pollution and nutrient enrichment (Arévalo et al. 2007). However, contaminants like trace elements can also be associated with this source of pollution, especially when the sewage treatment plants receive industrial effluents (Álvarez et al. 2002). An example is arsenic (As), a widely distributed element in marine ecosystems, which can enter the environment through both natural and anthropogenic sources. The main anthropogenic sources of this contaminant are domestic and industrial wastewaters, fuel combustion, mining and agricultural pesticide production (World Health Organization 2010). In fact, the toxicity of As is highly dependent on its chemical form, but since it can be harmful to humans even at low concentrations (Mieiro et al. 2012), it has been ranked as number one of the top ten most hazardous substances of the Agency for Toxic Substances and Disease Registry (2015). Therefore, As poses serious environmental and health risks emphasizing the importance of collecting sufficient monitoring data to provide knowledge concerning the magnitude of the problem.

Intertidal rocky shore macroalgae have been considered suitable biomonitors of water quality due to their sedentarism, easy sampling and taxonomic identification, worldwide distribution and capacity to respond to several anthropogenic impacts (Rainbow 1995; Ballesteros et al. 2007). More

✉ Joana Cabral-Oliveira
joanaco@ci.uc.pt

[1] Department of Life Sciences, CFE—Centre for Functional Ecology, University of Coimbra, 3000-456 Coimbra, Portugal

[2] Bioinsight – Rua Antero de Quental, N°52 Loja B - Urbanização Colinas do Cruzeiro, 2675-690 Odivelas, Portugal

[3] Department of Biology, CESAM—Centre for Environmental and Marine Studies, University of Aveiro, Campus de Santiago, 3810-193 Aveiro, Portugal

[4] Department of Earth Sciences, MARE UC, University of Coimbra, 3030-790 Coimbra, Portugal

[5] IPG, Institute of Petroleum and Geology, Comoro-Elemloi, Díli, Timor

[6] MARE—Marine and Environmental Sciences Centre, ESTM, Polytechnic Institute of Leiria, P-2520-064 Peniche, Portugal

specifically, macroalgae have high affinity for several trace elements and revealed sensitivity to environmental changes in As concentrations in the seawater (Chaudhuri et al. 2007). Macroalgae seemed the most suitable biomonitor of trace elements because, in contrast with marine fauna, they take up the trace elements directly from the water column (Rodríguez-Figueroa et al. 2009; Benkdad et al. 2011), responding exclusively to the contaminant levels in the seawater. The concentrations of As in intertidal macroalgae have already been the focus of previous studies. Some obtained baseline data to evaluate inter-site and inter-sample differences (Chaudhuri et al. 2007; Brito et al. 2012); others examined the As concentrations in industrialized coastal areas (Benkdad et al. 2011) or assessed As speciation in natural conditions (Slejkovec et al. 2006; Llorrent-Mirandes et al. 2010). Also, Maher and Clarke (1984) have compared the total As concentrations found in macroalgae collected from clean and contaminated areas due to anthropogenic activities. However, to our best knowledge, no attempt has been made to understand the impact of As contamination due to sewage discharges and, more specifically, the effects of the As accumulation by intertidal macroalgae.

In addition to the choice of good biomonitors, the selection of an adequate sampling design is also important. The majority of previous studies compared the accumulation of trace elements between one reference area and one contaminated area, which could lead to problems of pseudoreplication. In ecology, the necessity to find methods able to detect anthropogenic impacts led to the development of before-after, control-impact (BACI) procedures. This method argues that the use of multiple sampling times, before and after the disturbance, is essential to avoid temporal confounding (Chapman et al. 1995; Glasby 1997). However, in many cases, pre-impact data are not available. In order to overcome this problem, Underwood (1991) developed a beyond-BACI design (ACI design) where the use of two or more control areas helps to distinguish between natural variability that characterizes assemblages and variability induced by a particular form of disturbance, such as sewage discharges. Accordingly, this paper aims to understand the accumulation of As (due to sewage discharges) by intertidal macroalgae. The specific objectives were (i) to collect data on As loads in different intertidal macroalgae; (ii) to compare the As accumulation by macroalgae from one impacted and two references areas; (iii) to evaluate the potential of the macroalgae species as biomonitors of As contamination.

Materials and methods

Study area and data collection

The study was conducted in the Peniche peninsula (Fig. 1), on the central western Portuguese coast. In the peninsula, a sewage treatment plant was built in 1998 releasing secondary-treated effluents. It serves a human population of 40,000 and discharges the effluent directly into the intertidal area of the rocky shore. Previous studies (Cabral-Oliveira et al. 2015) found that the sewage discharges are a source of As contamination, probably due to the industries present in the area (e.g. shipyards, metal smelting) and also nutrient enrichment. The lack of pre-impact data led to the choice of an ACI (after control/impact) experimental design (Chapman et al. 1995; Glasby 1997). Consequently, three locations were selected: one impacted, near the sewage discharges (Imp) and two references (R1 and R2) to account for the natural differences among uncontaminated sites (Fig. 1). All locations had comparable environmental conditions, with regard to slope, orientation, wave exposure and type of substrate.

Macroalgae specimens were collected in November 2010 in the three locations. Five species (*Plocamium cartilagineum*,

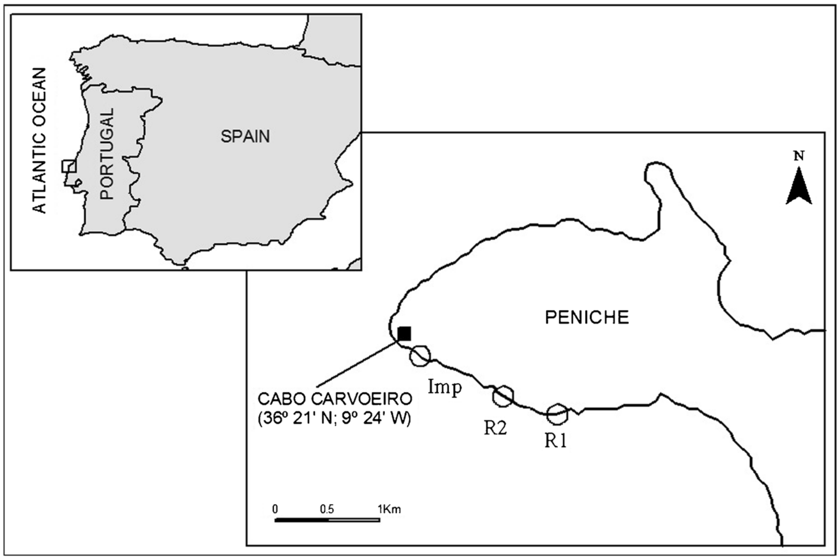

Fig. 1 Map of Peniche peninsula, western coast of Portugal, showing the three sampling locations. *Imp* impacted, *R1* and *R2* references

Asparagopsis armata, *Codium* spp., *Ulva* spp. and *Saccorhiza polyschides*) were chosen due to their different morphological and physiological characteristics and because they are common species in Atlantic shores. The specimens collected were all adults in order to avoid misinterpretations due to size-dependent accumulation trends, as previously reported (e.g. Szefer et al. 2002; Cravo and Bebianno 2005). At all locations, water samples were collected for laboratory determination of As concentrations. Water samples were filtered (Whatman GF/F glass-fibre filter, 0.45 μm) and stored frozen at −18 °C until analysis.

Laboratory procedures

At the laboratory, the macroalgae were washed with seawater (collected at the corresponding location) and epiphytes were eliminated manually. Five replicates of all the studied species were analysed. Algae were accurately weighed in dry, pre-cleaned Teflon digestion vessels. Approximately 1 g of fresh algal sample was digested using 8 mL of HNO_3 (65 %, high purity grade) and 2 mL of H_2O_2 (30 %, high purity grade), purchased from Merck (Germany) and Riedel-de-Haën (Germany). Fresh samples were use in our analysis because some arsenic compounds can be lost during the drying process by volatilization. The vessels were totally sealed and placed in the microwave chamber (Multiwave 3000—Anton Paar, Austria) for digestion. The digestion conditions were specified in the equipment, with some adjustments (digestion time: 40 min; maximum operational temperature and pressure: 180 °C and 15 bar, respectively). The digests were then diluted with nanopure-distilled water and filtered. The analytic determinations of As were performed using an Atomic absorption spectrophotometer (GFAAS Termo Unican Solaar M séries, USA), with graphite furnace and autosampler. The accuracy and precision of the analytical methodology for the trace elements determinations were assessed by replicate analysis of certified reference materials (Virginia tobacco leaves CTA-VTL-2, Institute of Nuclear Chemistry and Technology, Poland).

The recovery efficiency was 96.8 % and the detection limits was 0.01 $\mu g\ g^{-1}$ for As. Water samples were filtered (Whatman GF/F, 0.45 μm), and analyses followed the methods described in Bermejo-Barrera et al. (1998) for the determination of As. Finally, for ammonium (NH_4^+) and phosphate (PO_4^{3-}) determinations, water samples were analysed following the standard methods described in Ferskvandsbiologisk Laboratorium (1985). The concentration of ammonium was determined by using the indophenol blue method (Berthelot reaction). Water samples were treated with phenol and an alkaline hypochlorite forming an intense blue colour measured spectrophotometrically. In order to determine the concentration of phosphate, the seawater reacted with a composite reagent containing ammonium molybdate, ascorbic acid and potassium antimonyl-tartrate. The absorbance of the resulting complex (a blue-coloured solution) was measured spectrophotometrically.

Data analysis

A two-way analysis of variance (ANOVA) with replication was used to assess the differences for As concentrations between locations and species (Zar 1996). All data were checked for normality and homoscedasticity. Dunnett's multiple-comparison tests were applied whenever the respective ANOVA revealed significant differences between species among different areas (impacted and reference). For all statistical tests, the significance level was set at 0.05. All calculations were performed with IBM SPSS Statistics 19.0.

Results

The physical and chemical parameters (Table 1) presented differences when comparing locations: the reference areas (R1 and R2) having high values of salinity, dissolved oxygen and pH in contrast with the impacted area, which was characterized by high temperatures and concentrations of As and nutrients (Table 1).

Since As was found to be in higher concentrations in the seawater near the sewage discharges, total As concentrations in the intertidal macroalgae species from reference and impacted areas were analysed (Fig. 2). The As concentrations increased in the order: *A. armata* < *P. cartilagineum* < *Ulva* sp. < *S. polyschides* < *Codium* sp. and were higher in the impacted area (Fig. 2). Two-way ANOVA revealed significant differences in species among locations (Table 2). Moreover, the interaction between species and locations was also significant and therefore the comparisons were analysed. The multiple-comparison tests showed that the presence of sewage discharges changed the As concentrations ($R1 = R2 \neq Imp$) in all the studied species, with exception of the phaeophyte *S. polyschides* (Table 2).

The potential for macroalgae to be used as bioindicator for As pollution was also tested (Fig. 2). The concentrations of As in the macroalgae tissues followed the concentrations found in the seawater, in all studied species with the exception again for the results for the phaeophyte *S. polyschides*.

Discussion

In this study, several environmental parameters changed with the presence of sewage discharges, especially the salinity, oxygen, nutrients (NH_4 and PO_4) and As concentration in the seawater. Although there are no statistically significant differences, other parameters (seawater temperature, pH) revealed changes (Cabral-Oliveira et al. 2014). This occurs due to the

Table 1 Variation of environmental parameters in the three sampling areas (R1 and R2—reference areas; Imp—impacted area)

	Temp (°C)	O_2 (mg L^{-1})	Salinity	pH	Nutrients		[As] (μg L^{-1})
					NH_4	PO_4	
R1	15.4 ± 1.8	9.35 ± 0.5	35.9 ± 0.3	8.01 ± 0.2	0.07 ± 0.03	0.02 ± 0.01	0.83
R2	15.9 ± 1.8	9.38 ± 0.8	35.9 ± 0.3	8.07 ± 0.1	0.04 ± 0.03	0.02 ± 0.01	1.16
Imp	18.4 ± 1.7	6.89 ± 3.5	27.99 ± 7.1	7.87 ± 0.3	1.1 ± 1	2.5 ± 2.3	5.54

Adapted from Cabral-Oliveira et al. 2014

Temp seawater temperature, *O_2* dissolved oxygen, salinity, pH and nutrients, *NH_4* ammonium, *PO_4* phosphate; (mean ± SD)

dilution in the marine water and because the discharges are not continuous. In this situation, the values of standard deviation were higher, which attenuates the detection of statistically significant differences. In reality, with secondary treatment, some of these parameters should not be so affected by the presence of sewage discharges. However, this could be explained by the type of industrial effluents received. According to the United Nations Synthesis Report on Arsenic in Drinking Water (United Nations 2002), the average arsenic concentrations in open seawater usually show little variation and are typically around 1–2 μg L^{-1}. In this study, similar concentrations were found in the reference areas (0.83 μg L^{-1} in R1 and 1.16 μg L^{-1} in R2) but near the sewage discharges were found higher concentrations (5.54 μg L^{-1}), thus confirming the As contamination in that area.

Along with higher concentrations of As in the seawater near the sewage discharges, it was also noticed significantly higher concentrations of As in the tissues of all the macroalgae found near sewage discharges, except for *Saccorhiza polyschides*. It is in agreement with Chaudhuri et al. (2007), which have suggested that macroalgae have high affinity for several trace elements and revealed sensitivity to environmental changes in the concentrations of As in the seawater. The possibility to identify the samples affected by As contamination with respect to their geographical location (references against impacted area) attest the use of macroalgae species as suitable biomonitors. The only exception was the phaeophyte *S. polyschides*. Brown algae generally accumulate the highest concentrations of As (Slejkovec et al. 2006; Brito et al. 2012; Malea and Kevrekidis 2014) and accumulate more As due to the high phosphate concentrations found in its tissues. Phaeophyta take up and bioaccumulate arsenate from seawater as a phosphorus analogue, and then biotransform inorganic As into less toxic organical arsenical species (Malea and Kevrekidis 2014). Moreover, Maher and Clarke (1984) showed that *Cystoseira* spp. and *Sargassum* spp. accumulate more As in polluted areas (exposed to mining activities) than in clean areas. In another study, it was found that *Cystoseira* spp. had low ability to transform inorganic As absorbed from seawater into organical arsenical species. This low capacity to excrete As seems to explain the higher retentions of this element (Malea and Kevrekidis 2014). However, the phaeophyte used in this study (*S. polyschides*) showed significant differences between all the sampling areas. A similar As uptake should lead to higher values near sewage discharges (higher concentration of As in seawater reflected in higher concentration of As in *S. polyschides* tissues). Nevertheless, this has not been observed. Therefore, the

Fig. 2 Concentration of arsenic in the five macroalgae species ($n = 5$) studied (μg g^{-1} ± SD fresh weight) and in the seawater (μg L^{-1}) for all locations. *Imp* impacted; *R1* and *R2* references

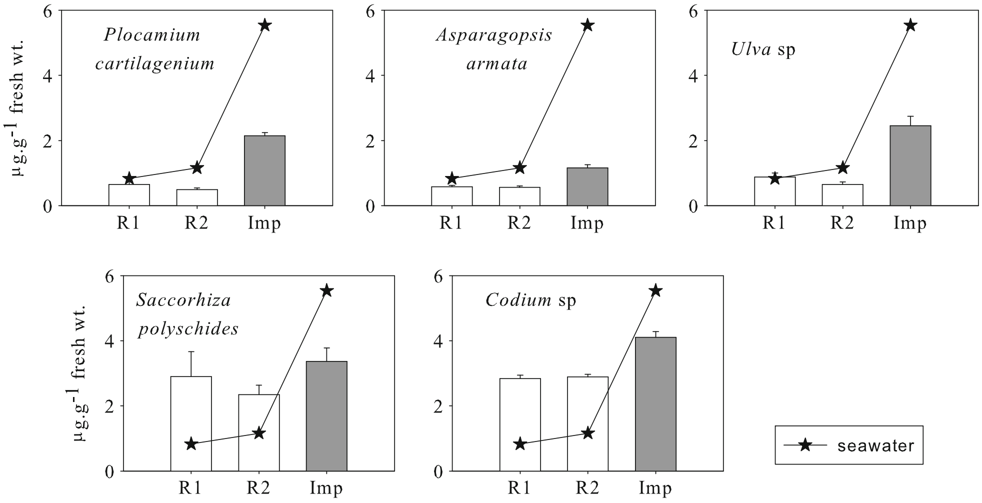

Table 2 ANOVA analysis and multiple-comparison tests for concentrations of arsenic (As) among species and for all the studied locations: R1, R2—references; Imp—impacted

Source of variation	*df*	SS	MS	*F*	*P*
Species (Sp)	4	76.454	19.113	277.805	<0.0001
Location (Lo)	2	23.195	11.597	168.561	< 0.0001
Sp x Lo	8	3.788	0.474	6.883	< 0.0001
Error	60	4.128	0.069		
Multiple comparisons	Imp vs R1		Imp vs R2		R1 vs R2
Plocamium cartilagineum	<0.0001		<0.0001		0.327
Asparagopsis armata	0.001		0.0006		0.879
Codium sp	<0.0001		<0.0001		0.741
Ulva sp	<0.0001		<0.0001		0.172
Saccorhiza polyschides	0.007		<0.0001		0.002

uptake efficiency seems to be different in each sampling area, probably related with other abiotic parameters (pH, temperature, salinity, nutrients concentration). However, to our best knowledge, there are no previous studies on the kinetics of arsenic uptake by *S. polyschides*, nor studies about differences on arsenic accumulation due to anthropogenic activities that could support our hypothesis. Consequently, *S. polyschides* does not seem a suitable biomonitor of As contamination. This supports the idea that methods based on indicator-species may be better than those based on functional-form groups (Arévalo et al. 2007), due to the variations in the rates of As accumulation of each species.

Regarding Chlorophyta, both species (*Ulva* sp. and *Codium* sp.) accumulate higher concentrations of As near the sewage discharges. Previous studies (Slejkovec et al. 2006) have found baseline values for *Ulva* sp. (1.35 ± 0.07 μg g^{-1} fresh wt) similar to those found in the reference areas of this study. However, there is no agreement if different environment As levels may affect the accumulation rates of this species. Some studies found similar As accumulations in clean and polluted areas (Maher and Clarke 1984), while others noticed the opposite (Chaudhuri et al. 2007), which raises some questions about the use of *Ulva* spp. as biomonitor of As contamination. With respect to *Codium* sp., to our best knowledge, this is the first attempt to compare the As accumulation among clean and polluted areas. Nevertheless previous studies (Llorrent-Mirandes et al. 2010) have also found higher concentrations of As in *Codium* sp. than in *Ulva* sp. (27.7 ± 2.9 μg g^{-1} and 5.3 ± 0.8 μg g^{-1}, respectively), such as in the present study. This could be related to two factors: life cycle and tidal level that the species occupy. While *Ulva* spp. are summer annuals, renewing its tissues every year, *Codium* spp. are perennial, thus absorbing and bioaccumulating more As from the seawater. Also, the species are found in different tidal levels: *Ulva* spp. in the lower eulittoral and *Codium* spp. in the upper sublittoral, with higher times of immersion which can explain the higher bioaccumulation of As present in the seawater.

Concerning Rhodophyta, both species (*Asparagopsis armata* and *P. cartilagineum*) accumulate higher concentrations of As near the sewage discharges. However, those species have different key functional traits (Table 3). Orfanidis in 2001 classified marine macroalgae in two ecological groups: ecological status group I (ESG I) and II (ESG II). The main differences between the perennial species ESG I and the opportunistic species ESG II can be found in Table 3. The fast growth and opportunist *A. armata* (ESG II) seems a more appropriate choice for As contamination biomonitor. Previous studies have showed that this information can be useful in the selection of suitable biomonitors. The use of these functional groups has already been tested to water quality (Orfanidis et al. 2001) and more recently to detect pollution (Díez et al. 2003), where ESG I prefer the clean areas and ESG II the impacted areas. Consequently, it seems more appropriate to choose an opportunist species, due to their ability to resist to anthropogenic stress. *Asparagopsis armata* is native of Southern Australia and New Zealand and has become widely distributed in Europe (Pacios et al. 2011). The ease of identification due to the characteristic harpoon-like hooks in its branches (Andreakis et al. 2007) and the lack of predators resulting from the production of defence toxic substances (Pacios et al. 2011) also support the use of *A. armata* as a bioindicator for As contamination. However, some precautions are necessary when interpreting biomonitoring data and choosing good biomonitors. First, during the collection and processing of the samples, it is important to reduce incident trace elements contamination (Gledhill et al 1998) and

Table 3 Key functional traits of the genus *Plocamium* and *Asparagopsis* and ecological status groups (ESG). Adapted from Orfanidis et al. 2001

	Plocamium ESG I	*Asparagopsis* ESG II
Thallus morphology	Thick	Fleshy
Growth	Slow	Fast
Thallus longevity	Perennial	Annual
Succession	Late-successional	Opportunistic

standardize the methods used to quantify the contaminants concentration in macroalgae tissues (Eklund and Kautsky 2003). Second, there is a need to understand that several factors (e.g. tidal level, growth rate, season) may change growth rates and consequently alter the species effectiveness as a bioindicator (Burridge and Bidwell 2002; Eklund and Kautsky 2003). It is essential to eliminate as many variables as possible: selecting locations with comparable environmental conditions and an experimental design allowing correct interpretation of the results obtained. The use of two reference areas reduces the uncertainty and increases the confidence in understanding if the concentrations of As in the macroalgae tissues follow the concentrations found in the seawater. Finally, in order to confirm the use of *A. armata* as biomonitor for As contamination it is necessary to understand better the uptake mechanism of arsenic in this species and to discriminate between organic and inorganic forms, assessing the toxicological risk and increasing the confidence in the biomonitor selection.

To sum up, this study shows that the accumulation of As in macroalgae tissues may be used as a measure of the bioavailability of As in the habitat. From all the studied species, together with the ecological characteristics of each species lead to the proposal of *A. armata* as the best biomonitor for As contamination in coastal areas. Nevertheless, more detailed research would be needed in order to confirm the potential of this species as biomonitor of As contamination.

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References

Agency for Toxic Substances and Disease Registry (2015) Detailed data table for the 2015 priority list of hazardous substances. http://www.atsdr.cdc.gov/SPL/resources/. Accessed 8 Jan 2016

Álvarez E, Callejón MM, Jiménez Sánchez JC, Ternero Rodríguez M (2002) Heavy metal extractable forms in sludge from wastewater treatment plants. Chemosphere 47:765–775

Andreakis N, Procaccini G, Kooistra W (2007) *Asparagopsis taxiformis* and *Asparagopsis armata* (Bonnemaisoniales, Rhodophyta): genetic and morphological identification of Mediterranean populations. Eur J Phycol 39:273–283

Arévalo R, Pinedo S, Ballesteros E (2007) Changes in the composition and structure of Mediterranean rocky-shore communities following a gradient of nutrient enrichment: descriptive study and test of proposed methods to assess water quality regarding macroalgae. Mar Poll Bull 55:104–111

Ballesteros E, Torras X, Pinedo S, García M, Mangialajo L, Torres M (2007) A new methodology based on littoral community cartography dominated by macroalgae for the implementation of the European Water Framework Directive. Mar Poll Bull 55:172–180

Benkdad A, Laissaoui A, Tornero MV, Benmansour M, Chakir E, Garrido IM, Moreno JB (2011) Trace metals and radionuclides in macroalgae from Moroccan coastal waters. Env Monit Assess 182: 317–324

Bermejo-Barrera P, Moreda-Pifieiro J, Moreda-Pifieiro A, Bermejo-Barrera A (1998) Direct determination of arsenic in sea water by electrothermal atomization atomic absorption spectrometry using D2 and Zeeman background correction. Microchim Acta 128:215–22l

Brito GB, Souza TL, Bressy FC, Moura CW, Korn MGA (2012) Levels and spatial distribution of trace elements in macroalgae species from the Todos os Santos Bay, Bahia, Brazil. Mar Poll Bull 64:2238–2244

Burridge TR, Bidwell JR (2002) Review of the potential use of brown algal ecotoxicological assays in monitoring effluent discharge and pollution in southern Australia. Mar Poll Bull 45:140–147

Cabral-Oliveira J, Dolbeth M, Pardal MA (2014) Is sewage pollution affecting the secondary production of rocky shore macroinvertebrates? Mar Freshwat Res 65:750–758

Cabral-Oliveira J, Pratas J, Mendes S, Pardal MA (2015) Trace elements in edible rocky shore species: effect of sewage discharges and human health risk implications. Hum Ecol Risk Assess 21:135–145

Chapman MG, Underwood AJ, Skilleter GA (1995) Variability at different spatial scales between a subtidal assemblage exposed to the discharge of sewage and two control assemblages. J Exp Mar Biol Ecol 189:103–122

Chaudhuri A, Mitra M, Havrilla C, Waguespack Y, Schwarz J (2007) Heavy metal biomonitoring by seaweeds on the Delmarva Peninsula, east coast of the USA. Bot Mar 50:151–158

Cravo A, Bebianno MJ (2005) Bioaccumulation of metals in the soft tissue of *Patella aspera*: application of metal/shell weight indices. Estuar Coast Shelf Sci 65:571–586

Díez I, Santolaria A, Gorostiaga JM (2003) The relationship of environmental factors to the structure and distribution of subtidal seaweed vegetation of the western Basque coast (N Spain). Estuar Coast Shelf Sci 56:1041–1054

Eklund BT, Kautsky L (2003) Review on toxicity testing with marine macroalgae and the need for method standardization—exemplified with copper and phenol. Mar Poll Bull 46:171–181

Ferskvandsbiologisk Laboratorium (1985) Limnologisk Metodik. Kobenhawns Universitet. Akademisk Forlag, København

Glasby TM (1997) Analysing data from post-impact studies using asymmetrical analyses of variance: a case study of epibiota on marinas. Aust J Ecol 22:448–459

Gledhill M, Brown MT, Nimmo M, Moate R, Hill SJ (1998) Comparison of techniques for the removal of particulate material from seaweed tissue. Mar Env Res 45:295–307

Llorrent-Mirandes T, Ruiz-Chancho MJ, Barbero M, Rubio R, López-Sánchez JF (2010) Measurement of arsenic compounds in littoral zone algae from the Western Mediterranean Sea. Occurrence of arsenobetaine. Chemosphere 81:867–875

Maher WA, Clarke SM (1984) The Occurrence of arsenic in selected marine macroalgae from two coastal areas of South Australia. Mar Poll Bull 25:111–112

Malea P, Kevrekidis T (2014) Trace element patterns in marine macroalgae. Sci Total Env 494–495:144–157

Mieiro CL, Coelho JP, Pacheco MP, Duarte AC, Pereira ME (2012) Trace elements in two marine fish species during estuarine residency: non-essential versus essential. Mar Poll Bull 64:2844–2848

Orfanidis S, Panayotidis P, Stamatis N (2001) Ecological evaluation of transitional and coastal waters: a marine benthic macrophytes based model. Med Mar Sci 2:45–65

Pacios I, Guerra-García JM, Baeza-Rojano E, Cabezas MP (2011) The non-native seaweed *Asparagopsis armata* supports a diverse crustacean assemblage. Mar Env Res 71:275–282

Rainbow PS (1995) Biomonitoring of heavy metal availability in the marine environment. Mar Poll Bull 31:183–192

Rodríguez-Figueroa GM, Shumilin E, Sánchez-Rodríguez I (2009) Heavy metal pollution monitoring using the brown seaweed *Padina durvillaei* in the coastal zone of the Santa Rosalía mining region, Baja California Peninsula, Mexico. J Appl Phycol 21:19–26

Slejkovec Z, Kápolna E, Ipolyi I, Elteren JT (2006) Arsenosugars and other arsenic compounds in littoral zone algae from the Adriatic Sea. Chemosphere 63:1098–1105

Szefer P, Frelek K, Szefer K, Lee CB, Kim BS, Warzocha J, Zdrojewska I, Ciesielski T (2002) Distribution and relationships of trace metals in soft tissue, byssus and shells of *Mytilus edulis* trossulus from the Southern Baltic. Env Pollut 120:423–444

Underwood AJ (1991) Beyond BACI: experimental designs for detecting human environmental impacts on temporal variations in natural populations. Aust Mar Freshwater Res 42:569–587

United Nations (2002) United Nations synthesis report on arsenic in drinking water. http://www.who.int/water_sanitation_health/dwq/arsenic3/en/. Accessed 8 Jan 2016

World Health Organization (2010) Exposure to arsenic: a major public health concern. World Health Organisation, Geneva

Zar JH (1996) Biostatistical analysis. Third editions Prentice-Hall International Editions, New Jersey