Integrated environmental evaluation of heavy metals bioaccumulation in invertebrates and seaweeds from different marine coastal areas of Sardinia, Mediterranean Sea.

Francesco Corrias[a], Alessandro Atzei[a], Piero Addis[b], Marco Secci[b], Mariateresa Russo[c], Alberto Angioni[a]*

[a]Department of Life and Environmental Science, Food Toxicology Unit, University of Cagliari, University Campus of Monserrato, SS 554, 09042 Cagliari, Italy,

Francesco Corrias (francesco.corrias@unica.it), Alessandro Atzei (alessandro.atzei@unica.it),

[b]Department of Life and Environmental Science, Biology Section, University of Cagliari, via Tommaso Fiorelli 2, 09126 Cagliari, Italy, Marco Secci (msecci@unica.it), Piero Addis (addisp@unica.it).

[c] Department of Agricultural Science, Mediterranean University of Reggio Calabria, Località Feo di Vito - 89122 Reggio Calabria (RC), Mariateresa Russo (mariateresa.russo@unirc.it)

Running title: Heavy metals accumulation studies in marine coastal areas of Sardinia, Mediterranean Sea.

* Corresponding author:

Alberto Angioni

Food Toxicology Unit, University of Cagliari, University campus of Monserrato, SS 554, 09042 Cagliari, Italy; Phone: +390706758615, Fax: +390706758612; e-mail: aangioni@unica.it

Abstract

In this work, three gastropods *Patella vulgata*, *Osilinus turbinata,* and *Tahis clavigera*, one echinoderm *Parancetrotus lividus*, one coelenterate *Anemonia sulcata*, and two seaweed *Padina pavonica*, and *Cystoseira mediterranea* were collected from three different marine areas of Sardinia in the Mediterranean sea and studied for heavy metals and metalloid content and accumulation trends.

Inductively coupled plasma optical emission spectrometry (ICP-OES) was used for the determination of Al, AS, B, Ba, Be, Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Sb, Se, Sn, Sr, Te, Ti, V and Zn in the selected samples. The results showed that *gastropods* were capable of accumulating Al, Ba, Cu, Fe, Sr, and Zn; *seaweeds* can better concentrate Al, Fe, and Zn than all other species. At the same time, *echinoderms and coelenterate* had limited ability to store specific metals, showing a much more homogeneous distribution. PCA analysis allowed us to discriminate among the sites and the species. Cala Zafferano was the area with the higher values of accumulation of all metals in all species as expected, considering its proximity to industrial sites. The results of the analysis showed clearly that heavy metal and metalloid accumulation was different for each species studied. Therefore, for a correct environmental assessment of a given area, a comprehensive approach is strongly recommended by exploiting the different properties of both accumulation and concentration of the metals by different aquatic species.

Summary

Different species of aquatic living organisms have different heavy metals and metalloid accumulation trends, even if they belong to the same family and the same water column or have similar living habits.

Keywords: Heavy metals; accumulation; gastropods; *Paracentrotus, Anemonia*, seaweed, PCA

Introduction

Metals are usually found in the environment worldwide, their presence caused by natural events or because of anthropogenic activities, such as industrial sites and agricultural practice (Kamaruzzaman et al., 2011). The increase in environmental metal concentration in these areas can reach 100-1000 times the normal levels of Earth's crust (Carral et al., 1995). The hydrological cycle of water between the land, open water surface and sea can bring to significant pollution, exposing the aquatic ecosystems directly to a great variety of these metals causing severe contamination of marine organisms (Farombi et al., 2007).

Metals are mutually essential elements necessary to support biological activities and unneeded chemicals with an unidentified role (Roesijadi and Robinson, 1994). Heavy metals are problematic pollutants because, unlike most organic contaminants, they are non-biodegradable and can accumulate in living tissues and food, causing a significant threat to both human health and environmental safety. The most hazardous heavy metals and metalloids are Be, Cr, Hg, Cd, Pb, and As. These metal ions are low soluble in water, can cause toxicity, and have serious side effects on human health. After discharged in the water, they are usually accumulated by sediments and transferred to invertebrates and biomagnified in fish populations accumulating in the lipid tissue (Fergusson, 1990; Yi et al., 2011; Jakimska, 2011; Jaishankar et al., 2014; Jitar et al. 2015). An accurate assessment of the degree of metal contamination at a specific location requires an adequate estimation of these elements' natural levels in the biological communities and the natural environment. Mussels, as interdital filter feeders, accumulate pollutants coming from local contamination and distant areas influenced by sea current and thus can lead to inaccurate information on the real environmental situation of restricted territories. Therefore, environmental pollution monitoring studies relying on their ability to concentrate high levels of metal in the soft tissue used mussels, invertebrates, and marine vegetables widely (Philips, 1977; Ingston, 1982; Goldberg et al., 1983; Cossa, 1989; Bryan and Langston, 1992; Abisil et al., 1997; Hummel et al., 1997).

Commentato [IC1]: Ho messo la bibliografia dopo widely.

The use of sedentary herbivorous such as gastropods, and echinoderms, seaweeds holdfast, and anemonia has several advantages. These living organisms are available all year, simple to collect, resistant to transportation and conservation, and have enough tissues for the analysis.

In the last decades, metals bioaccumulation has been investigated in different biological communities such as invertebrate gastropods (Vinogrjidov et al., 1953; Blackmore, 2000; Perez-Lopez et al., 2003; Amin et al., 2006; Collado et al., 2006; Yüzereroğlu et al., 2009; Ramirez, 2013; Kelepertzis, 2013; Boucetta et al., 2016; Duysak and Azdural, 2017; Aydin-Onen and Ozturk, 2017; Gawad, 2018), anemone (Mitchelmore et al., 2003; Horwitz et al., 2014), or echinoderms (Salvo et al., 2014, Chiarelli et al., 2019). Moreover, some authors investigated seaweed such as *Padina* spp and *Cystoseira* spp (Aydin-Onen and Ozturk, 2017; Rodríguez-Figueroa et al., 2008; Benfares et al., 2015; Ryabushko et al., 2017). However, most articles reported pollution from 4 to 15 metals in one or two species.

The study aimed to compare metals accumulation performance of 7 living organisms (invertebrates and vegetables) in three marine areas of Sardinia characterized by the different anthropic impact. Cala Zafferano (CZ) affected by heavy metal contamination, Capo Frasca (CF) and Capo Spartivento (CS), representing potentially clean environments, exposed to NW, SW and SE currents. Three gastropods *Patella vulgata*, *Osilinus turbinata*, and *Tahis clavigera*, one echinoderm *Parancetrotus lividus*, one coelenterate *Anemonia sulcata*, and two seaweed *Padina pavonica*, and *Cystoseira mediterranea,* were collected. All samples were subjected to mineralization and analysis by ICP-OES to determine an exhaustive pattern of 23 metals and metalloids and accumulation studies.

1. **Material and methods**

2.1 Sample collection and processing

Samples were handpicked from three sites of the Sardinia coast, Capo Spartivento (38° 52'41.90 "N; 8° 50'28.77 "E), Cala Zafferano Teulada (38° 53'43.26 "N; 8° 39'42.19 "E), and Capo Frasca (39° 42'54.98 "N; 8° 26'44.14 "E). Twenty samples of the gastropods *Patella vulgata*, *Osilinus turbinata*, and *Thais haemastoma*, the echinoderm *Parancetritus lividus,* the coelenterate *Anemonia sulcate,* and the seaweeds *Padina pavonica,* and *Cystoseira mediterranea*, were collected at a maximum depth of 10 meters in August (Table 1). All species were present at all stations and individually processed.

After collection, samples were immediately stored in ice cooler "drain plug open" to let the water run out. This operation avoids deterioration during transport to the lab. Samples brought to the laboratory were weighed, and gastropods soft tissue was separated from the shell. Sea urchin's gonads were collected with a stainless-steel spatula from the shell opened with a stainless-steel cutter for sea urchins. Anemone tentacles and seaweed aerial parts were selected using a stainless-steel scissor. All samples, except gonads, were rinsed with deionized water to remove accidental impurities, and finally stored at -20°C before analysis.

Chemicals

HNO_3 67-69%, H_2O_2 solution 30%, and standards of Al, AS, B, Ba, Be, Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Sb, Se, Sn, Sr, Te, Ti, V, Zn were of ICP grade (Carlo Erba Reagents Milan, Italy), HCl 34-37% super pure quality (Romil Spa Cambridge, England). Double-deionized water with a conductivity less than 18.2 MΩ was obtained with a Milli-Q system (Millipore, Bedford, MA, USA). Stock standard solutions were prepared at 1000 mg/L in HNO_3 10%. Working standard solutions were prepared daily by diluting the stock solution with MilliQ water.

2.2 Moisture and ash

Ten grams of homogenized samples were weighed and dried at 105°C in a thermostatic heater until a constant weight was achieved (~ 24 h). After that, samples were stored in a desiccator to reach ambient temperature and weighted for dried mater assessment. Dehydrated samples were then carbonized at 450 C° in a porcelain crucible for five hours for total ash analysis. Moisture and ash determinations were calculated as a percentage of the fresh product.

Commentato [IC2]: Weighed??

2.3 Heavy metals and metalloids

About 0.2 g of ashes obtained by carbonization were mineralized in a microwave CEM Mars6 system (CEM Corporation, Milano, Italy), by adding 10 mL of a mixture of HNO3 and HCl (1:3), and 1 mL of H2O2. After the mineralization program, the solution was transferred into a 10 mL flask and brought to volume with MilliQ water with a conductivity less than 18.2 MΩ, filtered through 0.45 µm nitrocellulose membrane filter (Whatman, Milan, Italy) in another flask and finally subjected to analysis. Control solvent samples were simultaneously prepared to avoid false positives and contamination during the investigation.

Commentato [IC3]: Mettere 3 al pedice?

Commentato [IC4]: Mettere i 2 al pedice?

Commentato [IC5]: Lo ripetiamo di nuovo dopo i materiali?

Hg, As, and Se sample preparation were carried out using Agilent VGA-77 (Agilent, Milan, Italy) according to Agilent application notes (Agilent AN, 2014; Agilent AN, 2012). Analysis were carried out using a Varian 710ES ICP Optical Emission Spectrometer for the simultaneous analysis of 23 metals and metalloids. Operating conditions were as follows: radiofrequency (RF) generator power 1.2, frequency 40 MHz; argon (99.996% purity) was used both for plasma (15.5 L/min), nebulizer (200 Kpa) and optic supply (1.5 L/min). The sample uptake delay was 30 seconds, and the instrument stabilization delay 15 seconds. The spray chamber was a double-pass, glass cyclonic. Each measurement was made in triplicate. The power and pressure applied were 600 W and 100 PSI for 13 minutes. The limit of quantitation (LOQ) was calculated as ten times the standard deviation reading of the blank sample signal (Wenzl et al. 2016). Calibration curves were calculated on five points starting from the LOQ value and were considered acceptable for $R^2 \geq 0.999$.

Commentato [IC6]: Ho aggiunto questo.

2.4 Risk assessment

In this study, we used the target hazard quotient (THQ) for risk assessment. THQ represents the ratio of the estimated daily intake (EDI) / reference dose (RfD) for noncarcinogenic risk assessment methods indicating the risk level associated with xenobiotic exposure (Chien et al., 2002; Zhang et al., 2017). RfDs for oral intake were obtained from the Integrated Risk Information System (IRIS-EPA). When THQ is below 1 no adverse effect during a person's lifetime is expected. EDI was calculated as the concentration of heavy metals (mg/kg wet weight) × average daily consumption of invertebrates in the local area (g/day bw) / body weight (Bw). To evaluate the risk of heavy metals and metalloids from invertebrate consumption, we consider an average weight of 60 Kg. Total THQ for each species was calculated as the sum of single metals THQ, while total THQ of each site was calculated assuming the intake from all the selected species in the same day.

2.5 Statistical Analysis

Analysis of variance (ANOVA) was carried out with the software XLSTAT (Addinsolf LTD, Version 19.4), Mean comparisons of the effects of treatments were calculated by the Fisher's least significant difference test at $p \leq 0.05$.

Commentato [IC7]: Perché maiuscolo?

Analysis data sets were imported into SIMCA 14 (Umetrics AB, Umea, Sweden) for processing using principal component analysis (PCA). Additionally, R2 (the coefficient of determination) and Q2 (the cross-validated correlation coefficient) were used as measures for the robustness of a pattern recognition model (Atherton et al., 2006).

3. Results

The selected sites had different anthropic conditions. Cala Zafferano (CZ) is in a military area, downstream of an industrialized coastal area, and subjected to main sea NW currents. Capo Spartivento (CS), located in the Gulf of Cagliari, is protected from the main current NW and exposed to SW and SE, in contrast, Capo Frasca (CF) is located in the south of the Gulf of Oristano but totally exposed to NW current. Both sites are far from industrial and agriculture areas (Figure 1). All organisms tested were present in each site and were selected with similar size and weight among the species (Table 1). The analysis of water content and ashes showed similar values in the three sites for gastropods, ranging in average from 74.44 ± 2.43% (*Osilinus*, CZ), to 79.30 ± 1.05% (*Patella*, CZ). Ash content ranged on average from 3.75 ± 2.41% (Thais, CF), to 8.52 ± 25.75% (*Osilinus*, CZ). However, the higher water levels were detected for *Anemonia sulcata*, *Paracentrotus lividus* and *Padina pavonica* accounting in average for 90.85 ± 1.72%, 81.72

Commentato [IC8]: Non deve andare in corsivo?

± 2.77%, and 82.45 ± 1.82%, respectively, while the lower levels were for *Cystoseira mediterranea* with an average value of 72.52 ± 7.12% (Table 2).

Seaweeds showed the higher mineral content with ash average values accounting for 15.35 ± 17.98% (g/100g ± RSD, FW) and 12.86 ± 19.98% (g/100g ± RSD, FW) in *Padina* CZ and *Cystoseira* CS, respectively, almost three times higher than the analyzed invertebrates. Instead, the lowest ash levels were detected in *Anemonia sulcate* CZ and *Paracentrotus lividus* CZ with average values of 3.50 ± 7.89% and 2.10 ± 12.60% (g/100g ± RSD, FW), respectively.

Gastropods soft tissue, echinoderms gonads, and coelenterate anemone tentacles were used for the analysis, while the juvenile areal parts were selected for seaweeds.

The ICP-OES analytical method allowed the analysis of 23 heavy metals and metalloids. Calibration curves showed R^2 values ranging from 0.9967 (Pb) to 0.9997 (Co), and the limits of quantification of the method were suitable to evaluate overall pollution and risk assessment (Table 3) (Wenzl et al., 2016).

Among the heavy metals and metalloids searched within the methods, eight were missing (Be, Co, Hg, Mo, Sb, Se, Sn, Te), and only 6 (Al, Cu, Fe, Sr, V, Zn) were found in all samples from the three sites. Gastropods and seaweeds showed in all sites the presence of 12 heavy metals and metalloids, *Paracentrotus* 10, and *Anemonia* 9 (Table 4-6).

3.1 Gastropods

Patella vulgata showed higher values for seven heavy metals and metalloids (Al, Ba, Fe, Mn, Pb, Sr, Ti) in the samples from CZ, while CS and CF showed even values. B, Cd, Cr, Cu, V, and Zn had similar values in all three sites. Fe, Sr, and Al from CZ had the higher mean values accounting for 34.28 μg/g, 7.06 μg/g, and 6.44 μg/g, respectively, about four times higher than those from CF and CS (Table 3). *Osilinus turbinatus* had the higher mean values in the samples from CZ for Al, Cr, Cu, Mn, Fe, Ni, Pb, Sr, Ti, and Zn, among these, the highest concentrations were detected for Fe 40.22 μg/g, Sr 19.08 μg/g, Al 8.77 μg/g, and Ni 7.93 μg/g. B, Ba, Cd, and V had similar values in the three sites. Also, *Thais* showed higher values in the samples of CZ. In particular, Al, Ba, Cu, Fe, Pb, and V were the most concentrated, with Fe 36.89 μg/g, Cu 29.68 μg/g, Zn 21.91 μg/g, and Ba 10.04 μg/g with the higher values (Table 4).

Comparing the three gastropods in the three sites, Al, B, Cu, Fe, and Ti showed similar values in the samples from CZ for *Patella* and *Osilinus*, Mn and Sr for *Patella* and *Thais*, while Cr had similar values in all sites. In CZ, *Thais* showed higher mean values of Ba, Cd, Cu, and Zn, and lower mean values for Al, B, and V.

In CS, B, Fe, and Ti had overlapping values in *Patella* and *Osilinus*, while Mn and Sr were similar in *Osilinus* and *Thais*. Al, Ba, Cd, Pb, and Zn had different values in all sites.

CF showed more considerable variability among all metal's accumulations in the three species. Considering all sites for the single species, the samples of CZ showed higher levels of all metals, except for Zn, which showed overlapping values in the three sites for the three species.

3.2 Seaweeds

Heavy metal and metalloids distribution followed an entirely different and uneven distribution among the sites and between the two seaweed species (Table 5). *Padina* and *Cystoseira* showed both the higher values in the samples of CZ, Fe 225.46 μg/g, Al and Zn both at 104.4 μg/g, and Sr 77.46 μg/g in *Padina*, and Fe 184.03 μg/g, Al 41.85 μg/g, Sr 55.46 μg/g, Zn 41.84 μg/g, Ba 32.08 μg/g, B 12.60 μg/g, and Mn 6.58 μg/g in *Cystoseira*.

Al, Fe, and Zn in *Padina* showed higher concentration in CZ compared to CS and CF, and to *Cystoseira* samples in all sites. *Cystoseira* levels of Al, B, Ba, Cu, Fe, Mn, Sr and Zn were higher in CZ samples, while Cr, Pb, Ti and V were higher in CF.

3.3 Echinoderms and coelenterate

Parancetrotus lividus accumulated only a few metals at moderate concentrations, Al, Sr, and Zn were highly concentrate in CZ. At the same time, the other trace elements showed values in an even range in the three sites. The higher levels were detected for Zn 9.24 mg/g, Al and Sr 5.64 μg/g, and Fe 4.25 μg/g in CZ. The levels of Fe were similar in CZ and CF, and lower in CS (Table 6).

Anemonia sulcata showed the presence in the tentacles of some minerals at low levels, among the heavy metals detected, Fe 4.07 μg/g, and Al 2.21 mg/g were the most abundant in CZ. Also, Sr and Zn showed values slightly higher in CZ than in CS and CF (Table 5).

Commentato [IC9]: Tabella 6

Accumulation results from all samples showed an overall content of metals higher in Cala Zafferano, especially for Fe, Al, and Zn in seaweeds. These findings confirmed an increase in the environmental pollution in this area which suffered the anthropic impact of the industrial area due to the exposition to NW current (Figure 1).

Among the heavy metals and metalloids considered most dangerous, Hg was never detected at a value above the LOQ of the method (0.05 μg/g), which was ten times below the MRL set for fish (CR-EC No 1881/2006). Arsenic was below the LOQ in all samples except in seaweed, and *Cystoseira* showed higher values in all sites. Pb was not present in *Paracentrotus* and *Anemonia*, and the samples of *Osilinus* from CS and CF. In the other samples, its values were all below the MRL (0.3 μg/g), except in the samples of CZ accounting for 0.50 μg/g in *Patella* and 0.46 μg/g in *Thais*. Cd was not detected in *Anemonia*, while showed values below the MRL (0.05 mg/g) in *Paracentrotus* and *Padina* from CS and CF. All other samples showed values above the MRL, with *Patella* showing a value four times higher of the MRL, and *Thais* 20 times higher. No differences were detected among each species between CS and CF sites.

Commentato [IC10]: Ho aggiunto metalloids visto che poi parli di mercurio, ecc…

3.4 Multivariate analysis

Multivariate analysis statistics could help in better discriminate among the different species, due to the high number of samples and variables from the analysis. In this paper, we have used the principal component analysis (PCA) approach do describe if the various species studied had different heavy metals accumulation capacity and which were the most discerning heavy metals. PCA biplot of score and loadings for CZ and CS showed a separate group of datasets for each species, allowing to identify 12 clustering groups and to discriminate metals along PC1 and PC2 axis (Figure 3).

PCA emphasize the differences in accumulation of the seaweed *Cystoseira* and *Padina* in CZ samples to the other species studied in the same area and to all the other samples collected in CS. Among the gastropod, *Thais* was the most discriminating species. CZ and CS samples were separated from the other gastropod's samples, invertebrate and seaweed, moreover, the samples from CZ were split from CS along the PCA1 axis stressing a more influence of the residues of the most discriminating metals for this axis. The samples of *Patella*, *Osilinus* and *Paracentrotus* fall in a restricted zone of the PCA plot, despite this they were well separated between CZ and CS along the PC1 axis. The metals Pb, Ba, Fe, Mn, Al, Cr, and B were the most discriminating along the PC1 axis, while Cu, Zn, Mo, and Cd were the most discriminating in PC2 axis (Figure 3).

Risk assessment showed total THQ for each species below 1, indicating no adverse effects from the consumption of the selected organisms. In all sites, *Thais* showed higher values, while *Anemonia* showed lower values in all sites (Table 7). Moreover, total THQ for the site obtained from the sum of total THQ of the various species were below 1, confirming that no adverse effects were reached also consuming the different species in the same day.

Commentato [IC11]: Ripetizione. Lo hai già messo a inizio frase.

The values of total THQ for the site confirmed that CZ had higher harmful metals concentration than CS and CF, and the amounts consumed should be taken into consideration (Table 7).

4. Discussion

Biomonitoring studies have the scope to assess the environmental occurrence of pollutants potentially toxic for human and other living organisms. Different approaches can be used, such as bioconcentration, that studies the direct partition form the environment to target organisms, and biomagnification that is strictly related to the diet leading to increase xenobiotic concentration in the feeder than in the diet. In contrast, bioaccumulation describes the accumulation and enrichment of contaminants in a selected living organism. This process is species-dependent since different organisms have different feeding habits, mechanisms of detoxification and metabolism (Jasminska et al. 2011). Metals and metalloids are commonly present in the environment and can be essential elements supporting biological activities or have adverse effects decreasing aquatic species or being dangerous for human being trough the food chain.

Gastropods, echinoderm, and anemonia species are usually eaten in Sardinia. THQ showed that the intake of single metals from the selected areas trough the diet did not create an alarm for the population if food quantities remains in the local typical FDI (food daily intake).

Commentato [IC12]: Through?

Metals do not degrade and along the trophic pyramid tend to increase, and anthropic activity or geochemical conditions can influence their concentration, the natural levels can grow significantly, becoming harmful to human safety. Literature data reported xenobiotic accumulation on suitable species used as indicators. Although most studies have addressed local issues, their impact is important to assess the different bioaccumulation trends of the organisms used.

Perez-Lopez et al. (2003) studied the contamination of *Patella vulgata* from Zn, Cd, Pb and Cu with levels ranging in the soft tissue from 91 to 429 µg/g for Zn, 5.6 µg/g for Cd, 1-3 µg/g for Pb, and 2-10 µg/g for Cu.

Kelepertzis (2013), studying the heavy metal concentration in *Patella*, postulated that many mineralized areas give rise to higher levels of Pb (96 vs 8 µg/g), Zn (196 vs 48 µg/g), Mn (36 vs 10 µg/g), and Cu (28 vs 6 µg/g) bioconcentration. In contrast, Ni (9 µg/g), and Cr (8 µg/g) were even, the levels of metals decreased in the line Zn > Pb > Mn> Cu.

Duysak and Azdural (2017) studied the accumulation from 8 sites in the different tissues of *Patella* of Fe, Zn, Cd, Cu, Co, Ni, Mn, Pb, Cr and Al, with the higher concentration in all sites for Fe, Zn, Cd, Co, Pb, and Al.

Collado et al. (2006) found that mean total concentrations of Cd, Cu, Pb, and Zn in *P. rustica* were 0.37 ± 0.05, 1.77 ± 0.09, 1.27 ± 0.07 and 8.84 ± 0.71 µg/g DW (mean ± S.E.) respectively; whereas in *P. candei crenata* were 0.71 ± 0.10, 2.94 ± 0.11, 0.09 ± 0.01 and 33.74 ± 1.15 µg/g DW (mean ± S.E.).

Gadaw (2018) studied the accumulation in the gastropod *Lanistes carinatus* of Mn, Fe, Co, Ni, Cu, Zn, Cd, and Pb. With values ranging from 240 to 567 µg/g dw for Fe, 5.12 – 32.8 µg/g dw for Mn, Zn 5.35-28.15 µg/g dw, 0.69-3.25 µg/g dw for Cu, 4.42-13.87 µg/g dw for Ni, 0-9.24 µg/g dw for Co, 0.03-0.04 µg/g dw for Cd, and 0.24-0.40 µg/g dw for Pb.

Jakimska et al. (2011) reported the levels of Zn and Fe in gastropods. In these molluscs these two metals have essential biochemical functions, Zn is a constituent of haemocyanin, Fe is a constituent of goethite (α-FeOOH) responsible for the proper functioning of the radula. This fact can explain the high levels of Zn and Fe in gastropods. Moreover, the levels of Zn are related to Cu levels through the metabolic processes and homeostasis, metallothionein mediated.

Amin et al. (2006) studied the accumulation of Cu, Zn, Fe, Pb, Cd in the soft tissues of *Thais aculeata* with values of 150.3 ± 58.0 µg/g DW, 158.4 ± 27.9 µg/g DW, 316.7 ± 125.3 µg/g DW, 11.3 ± 5.9 µg/g DW and 0.6 ± 0.3 µg/g DW, respectively.

Ramirez et al. (2013), studied the levels of Cd (0.69-15.81 µg/g), Cu (10.82-55.68 µg/g), Pb (0.21-1.36 µg/g), and Zn (15.78-32.09 µg/g) in *Osilinus*. Later Boucetta et al. (2016) reported the determination in *Osilinus* of Cd, Cr, Cu, Ni, Pb, Zn in two stations, recording different values in the two stations, accounting for 318.10±38.20 µg/g and 6.51±1.47 µg/g for Zn and Pb in the first station. And 3.63±1.14 µg/g for Cd, 32.70±2.90 µg/g for Cu, and 24.80±20 µg/g for Ni in the station 2.

Salvo et al. (2014) studied the accumulation of Hg, As, Cr, Ni, Cu, V, Cd and Pb in P*aracentrotus lividus*, their findings suggested the use of *Paracentrotus* as a bioindicator for pollution in defined areas. Similar findings were presented by Angioni et al. (2012, 2014) analyzing polycyclic aromatic hydrocarbon (PAH) pollution in different Sardinian coastal areas.

Horovits et al. (2014) analyzed symbiotic algae and the tentacles of *Anemonia* for the bioaccumulation of 12 trace elements. *Anemonia viridis* regulates its internal trace element concentrations by compartmentalization and excretion owing a high resilience to environmental contamination from high levels of metals. Pb, Fe, and Cu were mostly associated with symbiotic algae, while Cd, As, and Zn were associated with the *Anemonia*. 85% of Fe was in the alga fraction, Pb and Cu were evenly distributed, while Zn, As, and Cd were mostly distributed in the *Anemonia*. Mitchelmore et al. (2003) studying the accumulation of heavy metals in anemone pointed out that symbiotic anemones accumulate Cd, Ni e Zn higher than aposymbiotic anemones, and the levels were depleted when recovered in seawater.

Data on the contamination of *Cystoseria* have been reported by Ryabushko et al. (2017) on Pb (35-50 µg/g), Cd (2-3.5 µg/g), Zn (40-65 µg/g), and Cu (13-24 µg/g), and by Benfares et al. (2015) on Cd, Pb (0-5.1 µg/g), Cr (0.1-1.9 µg/g), and Hg.

The same specimen with different size can give rise to different levels of xenobiotics; therefore, to avoid individual variation, we selected specimens from the same species with similar size in each site and among sites.

Literature most studied metals were Cd, Cr, Cu, Pb, and Zn, by using one or two organisms, mainly comparing polluted vs clean areas. The merged data from Sardinia obtained in the present study showed values for all living organisms in the lower range compare to literature data for Cd, Cr and Cu, while Pb and Zinc levels in gastropods were higher than the levels reported in the Canary Islands (Table 8).

Mercury detected in mussels collected along the coastline of Sardinia from two sampling campaigns showed values ranging from 0.035 to 0.11 µg/g and 0.048 to 0.83 µg/g (Ipolyi et al. 2004). Moreover, data reported on carnivorous and herbivorous or omnivorous fish showed that the seconds accumulate till 10 times less the concentration of the former (Levitan et al., 1974; Liu et al., 2014). Therefore, values of Hg below the LOQ in the samples of the present study were not surprising since most of the living organisms studied are mostly herbivorous or seaweed (Table 1).

Commentato [IC13]: Forse bisogna mettere Hg?

The results from the studies examined showed that local pollution cannot be used for global discussions but take pictures of a scenario in restricted areas. The Mediterranean basin, which is an enclosed sea, has different contamination characteristics related to the anthropic effects on the coasts and on the sea of the single surrounding countries.

Therefore, the real value of these studies is related to the global information of the accumulation trends of the different living organisms.

The present study elucidated that the species of gastropods, *Paracentrotus*, *Anemonia* and seaweed living in the same water column (upper littoral zone) had different uptake capability on metals.

These data confirm that accumulation is species and site-dependent, being related to pollution magnitude, feeding habitude and geochemical morphology of the site.

MVA analysis emphasizes the different accumulation capacity of the seven species collected at the different upper littoral areas of Sardinia, allowing to distinguish areas with different pollution contaminations in a similar contest.

5. Conclusions

The data reported showed different capabilities of the species involved in accumulating different metals. Gastropods are more capable of accumulating Al, Ba, Cu, Fe, Sr, and Zn, but in different amount. *Thais* levels of Cu and Zn are much higher than *Patella* and *Osilinus*, seaweeds can concentrate Al, Fe, and Zn (*Padina* more than *Cystoseira*) in large extent in respect to all other species. At the same time, *Paracentrotus* and *Anemonia* have limited ability to concentrate on specific metals but have a much more homogeneous distribution. THQ showed that Thais contributed for almost 50% to total THQ of the site and that among the heavy metals Cd was the most impacting on the overall health concerns for invertebrate eating.

All these species have been referred to be model organisms for biomonitoring environmental quality and pollution.

Therefore, for a correct environmental assessment of a given area, a diversified approach is strongly recommended by exploiting the different properties of both accumulation and concentration of the metals by different aquatic species.

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Table 1. Humidity and ash content of the edible part of gastropods, Paracentrotus, anemonia, and of the seaweed sampled.

	Humidity g/100g ± RSD FW			Ash g/100g ± RSD FW		
	C. Zafferano (CZ)	C. Spartivento (CS)	C. Frasca (CF)	C. Zafferano (CZ)	C. Spartivento (CS)	C. Frasca (CF)
Patella vulgata	79.46 a(a)	78.91 a(a)	78.01 a(a)	4.61 a(a)	4.29 a(a)	5.04 ab(a)
Osilinus turbinatus	74.44 a(a)	77.54 a(a)	75.62 a(a)	8.91 b(a)	5.10 a(b)	6.59 a(ab)
Thais haemastoma	76.53 a(a)	74.90 a(a)	76.20 a(a)	5.18 a(a)	3.75 ab(b)	4.24 b(ab)
Paracentrotus lividus	84.06 b(a)	79.54 a(a)	81.56 b(a)	2.10 c(a)	2.75 b(a)	3.30 b(a)
Anemonia sulcata	89.06 b(a)	91.44 b(a)	91.96 c(a)	3.50 c(a)	3.85 ab(a)	3.71 b(a)
Padina pavonica	81.67 b(a)	81.50 c(a)	84.18 b(a)	15.35 d(a)	13.13 c(a)	6.30 a(b)
Cystoseira mediterranea	70.21 c(a)	68.91 d(a)	78.44 b(b)	10.85 e(a)	12.86 c(a)	10.48 c(a)

[a] Values among species (without brackets) or among sites (in brackets) followed by unlike letters differ significantly by Fisher's least significant difference (LSD) procedure, $P \leq 0.05$. Letters without brackets relate to comparisons of the influence of species within each site. Letters in brackets relate to comparisons of the influence of the sites among each species for each parameter.

Table 2. Heavy metals method parameters.

Element	λ	MRL µg/g	LOQ	Linear regression equation	R^2
Al	237.3		0.10	y=670.89x-45.48	0.9978
AS	188.98		0.10	y=79.38x-10.14	0.9991
B	249.77		0.50	y=5151.33x+789.8	0.9995
Ba	493.40		0.05	y=8035x-289.9	0.9993
Be	313.04		0.005	y=259128x-6848	0.9985
Cd	226.50	0.05	0.005	y=4804x-3.55	0.9995
Co	228.61		0.025	y=6622.7x+58.6	0.9997
Cr	267.71		0.01	y=9908.7x+97.7	0.9994
Cu	324.75		0.01	y=17117.2x-95.93	0.9996
Fe	259.94		0.10	y=3059.9x+80.8	0.9992
Hg	194.16	0.5	0.05	y=392.2x+11	0.9994
Mn	257.61		0.01	y=70780.6x-8029	0.9990
Mo	204.59		0.05	y=604.4x-5.5	0.9988
Ni	216.55		0.05	y=1642.2x-22.3	0.9986
Pb	220.35	0.3	0.05	y=297.3x-19.4	0.9967
Sb	217.58		0.10	y=49.7x-5.8	0.9981
Se	196.02		0.025	y=157.7x-11.5	0.9992
Sn	189.92		0.05	y=74.3x-4.9	0.9986
Sr	407.77		0.10	y=829945.3x+17943	0.9984
Te	214.28		0.10	y=106.6x-10.1	0.9972

Ti	336.12		0.01	y=14275x+521.8	0.9976
V	292.40		0.005	y=13753.2x+231.2	0.9996
Zn	213.85		0.05	y=4785.9x-316	0.9986

Table 3. Heavy metals (μg/g ± RSD%) in gastropods from the three sites investigated.

metal	*Patella vulgata*			*Osilinus turbinatus*			*Thais*		
	C. Zafferano (CZ)	C. Spartivento (CS)	C. Frasca (CF)	C. Zafferano (CZ)	C. Spartivento (CS)	C. Frasca (CF)	C. Zafferano (CZ)	C. Spartivento (CS)	C. Frasca (CF)
Al	6.44a(a)	1,41b(b)	1.35b(b)	8,77 a(a)	2,94 b(c)	2.80 b(c)	0,37B a(d)	0,18 b(e)	0.15 b(e)
AS	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
B	1,63a(a)	1,44a(a)	1.37a(a)	1,54 a(a)	1,35 a(a)	1.40 a(a)	1,14 a(b)	0,90 a(b)	0.92 a(b)
Ba	1,21a(a)	0,52b(b)	0.48b(b)	1,98 a(c)	1,63 a(c)	1.57 a(c)	10,04 a(d)	7,07 b(e)	6.58 b(e)
Be	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Cd	0,19a(a)	0,23a(a)	0.20a(a)	0,08 a(b)	0,10 a(b)	0.09 a(b)	0,90 a(c)	1,14 a(c)	1.01 a(c)
Co	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Cr	0,03a(a)	0,03a(a)	0.03a(a)	0,11 a(a)	0,03 b(a)	0.03 b(a)	0,04 a(a)	0,03 a(a)	0.03 a(a)
Cu	0,28a(a)	0,26a(a)	0.27a(a)	2,29 a(a)	1,64 b(a)	1.58 b(a)	29,68 a(a)	8,71 b(a)	7.58 b(a)
Fe	34,28a(a)	7,37b(b)	6.58b(b)	40,22 a(a)	15,36 b(c)	14.91 b(c)	36,89 a(a)	10,73 b(d)	11.51 b(d)
Hg	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Mn	0,33a(a)	0,07b(b)	0.05b(b)	0,69 a(c)	0,23 b(a)	0.21 b(a)	0,30 a(a)	0,36 a(a)	0.32 a(a)

Mo	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Ni	< LOQ	0,13a(a)	0.11a(a)	7,93 a(b)	0,26 b(c)	0.25 b(c)	< LOQ	0,08 a(a)	0.05 a(a)
Pb	0,50a(a)	0,07b(b)	0.08b(b)	0,18 a(c)	< LOQ	< LOQ	0,46 a(a)	0,16 b(c)	0.14 b(c)
Sb	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Se	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Sn	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Sr	7,06a(a)	1,57b(b)	1.65b(b)	19,08 a(c)	8,04 b(a)	7.58 b(a)	6,01 a(a)	6,10 a(a)	6.05 a(a)
Te	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Ti	0,13a(a)	0,07b(b)	0.06b(b)	0,18 a(a)	0,09 b(b)	0.10 b(b)	< LOQ	< LOQ	< LOQ
V	0,28a(a)	0,30a(a)	0.31a(a)	0,60 a(b)	0,59 a(b)	0.54 a(b)	0,06 a(c)	0,51 b(b)	0.48 b(b)
Zn	1,69a(a)	1,88a(a)	1.75a(a)	0,62 a(b)	2,16 b(c)	1.98 b(c)	21,91 a(d)	20,31 a(d)	21.03 a(d)

[a] Values among species (without brackets) or among sites (in brackets) followed by unlike letters differ significantly by Fisher's least significant difference (LSD) procedure, $P \leq 0.05$. Letters without brackets relate to comparisons of the influence of species within each site. Letters in brackets relate to comparisons of the influence of the sites among each metal.

Table 4. Heavy metals seaweeds.

Element	*Padina pavonica*			*Cystoseira mediterranea*		
	C. Zafferano (CZ)	C. Spartivento (CS)	C. Frasca (CF)	C. Zafferano (CZ)	C. Spartivento (CS)	C. Frasca (CF)
Al	104,40a(a)	9,13b(b)	7,30b(b)	41,85a(c)	3,85b(d)	15,09c(e)
AS	0,73a(a)	0,25b(b)	0,26b(b)	1,06a(c)	2,56a(c)	1,05a(c)
B	< LOQ	0,74a(a)	< LOQ	12,60a(b)	5,10b(c)	< LOQ
Ba	9,96a(a)	3,52b(b)	0,68c(c)	32,08a(d)	17,77b(e)	4,46c(b)
Be	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Cd	0,07a(a)	0,01b(b)	0,02b(b)	0,08a(a)	0,02b(b)	0,08a(a)
Co	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Cr	0,18a(a)	0,02b(b)	0,06b(b)	0,15a(a)	0,03b(b)	0,24c(ac)
Cu	0,42a(a)	0,10b(b)	0,15b(b)	0,79a(c)	0,18b(b)	0,15b(b)
Fe	225,46a(a)	11,41b(b)	15,79b(b)	184,03a(a)	3,94b(c)	3,56b(c)

Hg	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Mn	2,88a(a)	0,84b(b)	< LOQ	6,58a(c)	1,40b(a)	0,73c(b)
Mo	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Ni	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Pb	0,71a(a)	0,11b(b)	0,25c(c)	0,58a(a)	0,13b(b)	0,92c(d)
Sb	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Se	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Sn	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Sr	77,46a(a)	5,39b(a)	5.65b (b)	55,46a(a)	1,53b(c)	1,15b(c)
Te	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Ti	2,60a(a)	0,20b(b)	0,92c(c)	1,18a(c)	0,03b(d)	4,41c(e)
V	0.72a(a)	0,21b(b)	0,25b(b)	1.10a(a)	0,52b(c)	1,50c(d)
Zn	104,40a(a)	0,23b(b)	11,42c(c)	41,85a(d)	1,50b(e)	2,97c(f)

[a] Values among species (without brackets) or among sites (in brackets) followed by unlike letters differ significantly by Fisher's least significant difference (LSD) procedure, P ≤ 0.05. Letters without brackets relate to comparisons of the influence of species within each site. Letters in brackets relate to comparisons of the influence of the sites among each metal.

Table 5. Heavy metals in echinoderms, and coelenterate.

Element	*Paracentrotus lividus*			*Anemonia sulcata*		
	C. Zafferano (CZ)	C. Spartivento (CS)	C. Frasca (CF)	C. Zafferano (CZ)	C. Spartivento (CS)	C. Frasca (CF)
Al	5.64a(a)	0,27a(a)	1,74a(a)	2,21a(a)	1.95a(a)	2.05a(a)
AS	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
B	1.25a(a)	0,53a(a)	1,27a(a)	0,63a(a)	0.59a(a)	0.60a(a)
Ba	0.98a(a)	0.25a(a)	0,40a(a)	0,13a(a)	0.12a(a)	0.10a(a)
Be	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Cd	0.06a(a)	0,04a(a)	0,03a(a)	< LOQ	< LOQ	< LOQ
Co	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Cr	0.02a(a)	0,04a(a)	0,02a(a)	< LOQ	< LOQ	< LOQ
Cu	0.27a(a)	0,14a(a)	0,17a(a)	0,20a(a)	0.12a(a)	0.16a(a)
Fe	4.25a(a)	1,85a(a)	4,38a(a)	4,07a(a)	3.81a(a)	2.95a(a)
Hg	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Mn	< LOQ	< LOQ	< LOQ	0,22a(a)	0.35a(a)	0.27a(a)
Mo	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Ni	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Pb	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Sb	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Se	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Sn	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Sr	5.64a(a)	0,79a(a)	2,89a(a)	0,64a(a)	0.29a(a)	0.31a(a)
Te	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Ti	0.09a(a)	0,02a(a)	0,08a(a)	< LOQ	< LOQ	< LOQ
V	0.12a(a)	0,08a(a)	0,10a(a)	0,02a(a)	0.03a(a)	0.02a(a)
Zn	9.24a(a)	4,87a(a)	2,47a(a)	0,62a(a)	0.22a(a)	0.13a(a)

[a] Values among species (without brackets) or among sites (in brackets) followed by unlike letters differ significantly by Fisher's least significant difference (LSD) procedure, $P \leq 0.05$. Letters without brackets relate to comparisons of the influence of species within each site. Letters in brackets relate to comparisons of the influence of the sites among each metal.

Figure 1. Localization of the three sites in Sardinia, main current ↑NW, and ● industrial area.

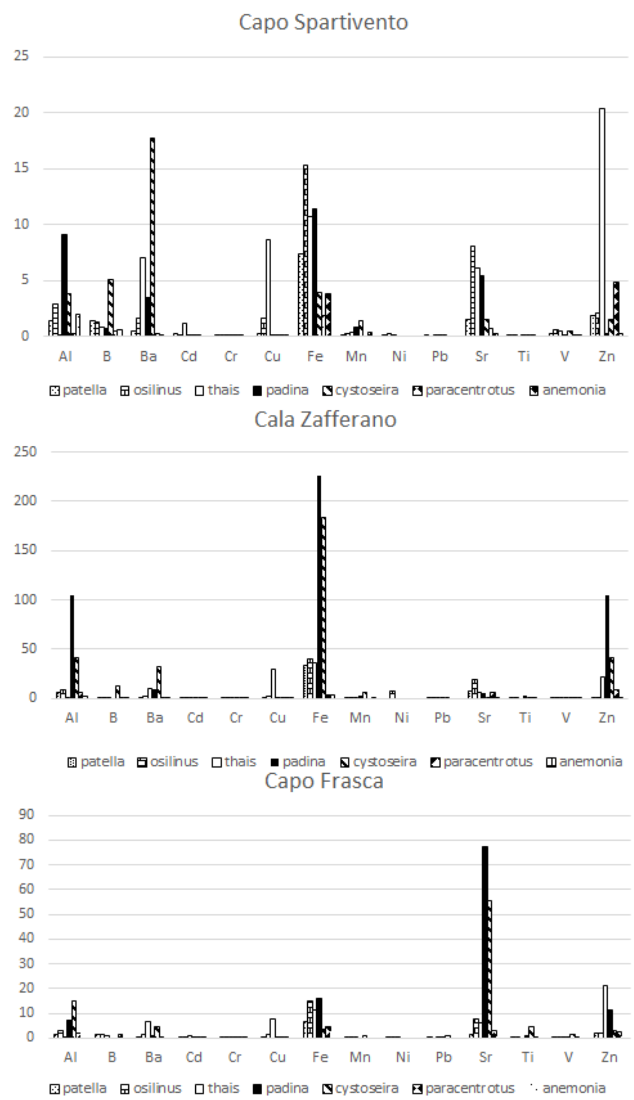

Figure 2. Histograms of the distribution of the metals among the species in the three locations.

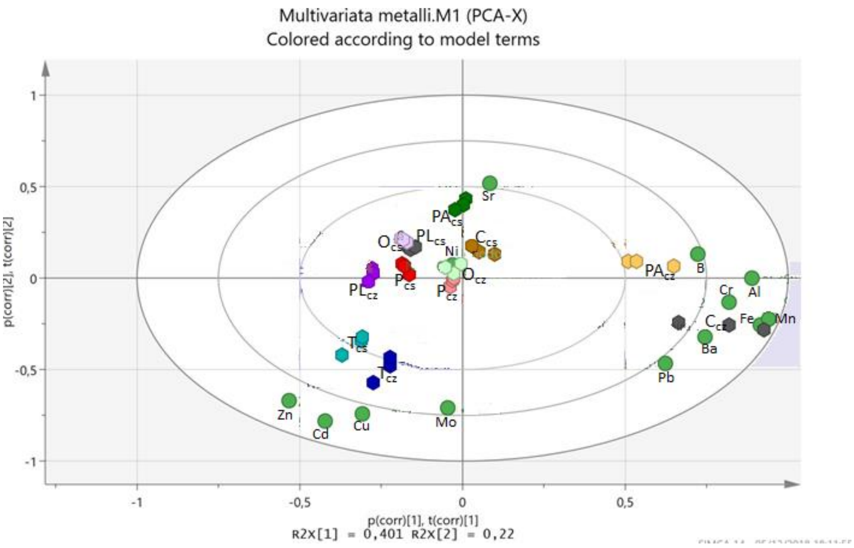

Figure 3. PCA biplot of the score and loading in CZ and CS sites.