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Assessment of trace element contamination and effects on *Paracentrotus lividus* using several approaches: Pollution indices, accumulation factors and biochemical tools

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ABSTRACT

Among the most common contaminants in marine ecosystems, trace elements are recognized as serious pollutants. In Corsica (NW Mediterranean Sea), near the old asbestos mine at Canari, trace elements from the leaching of mine residues have been discharged into the sea for several decades. The aim of this study was to assess the levels of contamination in this area and the potential effects on *Paracentrotus lividus* (Lamarck, 1816) using pollution indices, accumulation factors and biochemical tools. For this purpose, the concentration of 24 trace elements was measured in sea urchins (gonads and gut content), macroalgae, seawater column and sediment collected at 12 stations nearby the old asbestos mine and at a reference site. The bioaccumulation of trace elements occurs as follows: macroalgae > gut > gonads. TEPI contribute to highlight contamination gradients which are mainly due to the dominant marine currents allowing the migration of mining waste along the coastline. This hypothesis was supported by TESVI, which identified characteristic trace elements in the southern area of the mine. High hydrogen peroxide content, associated with elevated catalase and glutathione-S-transferase enzyme activities, were also identified at these sites and at the reference site. Trace elements contamination as well as several abiotic factors could explain these results (*e.g.* microbiological contamination, hydrodynamic events, *etc.*). The results obtained in this study suggest that oxidative stress induced by contamination does not affect the health of *Paracentrotus lividus*. This work has provided a useful dataset allowing better use of sea urchins and various tools for assessing trace element contamination in coastal ecosystems.

1. Introduction

Coastal ecosystems are at the interface between the marine and terrestrial domains and provide many goods and ecosystem services of high value (Costanza et al., 2017). Coastal marine waters are particularly vulnerable to a wide range of disturbances such as climate change effects and the release into the sea of a wide variety of contaminants (Livingston et al., 1994; Mostofa et al., 2013). These habitats are subject to an increase in anthropogenic activities that can lead to high levels of contamination (Islam and Tanaka, 2004). The presence of these contaminants can have a devastating effect on organisms and their ecosystems, making marine pollution a global concern (Abdel-Shafy and Mansour, 2016; Torres et al., 2016).

Among the major pollutants, trace elements are a real ecological issues because of their toxicity, their persistence and their ability to accumulate in marine organisms and even be biomagnified through the trophic chain (Rainbow and Luoma, 2011; Bonanno and Di Martino, 2017). The inputs of trace elements in marine environment derived from natural geogenic pollution (geological phenomena) and/or anthropogenic sources (*e.g.* mining, agriculture, petrochemical industry, aquaculture, sewage waste water) result in increased concentration levels (D'Adamo et al., 2008; Barhoumi et al., 2014). The trace elements can be classified as essential or non-essential (Jiang et al., 2014). Essential elements have a biological function in organisms, such as copper, iron and zinc and non-essential trace elements are not involved in any metabolic mechanism, such as cadmium, mercury and lead (Amiard,

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2011). Above a certain threshold, all trace elements present a potential threat for organisms (Nordberg et al., 2007). As a result of the threats caused by trace elements, continuous monitoring of their presence and concentration must be undertaken (Richir and Gobert, 2014).

The Mediterranean Sea, which represents 0.82 % of the global ocean surface area, is one of the main hotspots of marine biodiversity in the world, with 4–18 % of identified marine species (Coll et al., 2010). However, the coastal areas surrounding this semi-enclosed sea are subjected to the increasing synergistic effects of global changes and intensive anthropogenic activities leading to significant alterations in the structure and functioning of trophic webs and to the degradation of coastal and marine ecosystems (Coll et al., 2012; Ramírez et al., 2018; Stock et al., 2018a). Located in the northwestern Mediterranean Sea, Corsica Island has long been considered as a pristine region with low anthropogenic disturbances resulting in low levels of contamination (Gobert et al., 2017). However, recent studies have revealed major trace elements contamination near an old asbestos mine in northern Corsica (Cary et al., 2013; Ternengo et al., 2018; El Idrissi et al., 2020). These high levels originate from untreated material directly discharged into the sea during the active period of the asbestos mine between 1948 and 1965 (BRGM, 2017; Cary et al., 2013). The continuous leaching of these mining residues, still present on the sides of the mine, contributes to the dispersal of nickel, chromium and cobalt along the coastline (Galgani et al., 2006; Kantin and Pergent-Martini, 2007). Apart from the threat associated with the old asbestos mine, this region is characterized by relatively low levels of anthropogenic pressure (*i.e.* industrial, agricultural and urban activities; ASR, 2011). Because of these specificities, this sector represents a privileged study area to assess the trace elements contamination from a specific source within the coastal ecosystems.

The bioaccumulation of trace elements by marine organisms can occur through various pathways such as absorption and/or adsorption directly from sediments and interstitial seawater but also through ingestion, which will potentially affect other species along the food chain (Adamo et al., 2005; Chen et al., 2007; Türkmen et al., 2010). In order to assess bioaccumulation and biomagnification to clarify the toxicity and fate of trace elements in organisms, it is important to determine the concentrations in the different biological and environmental matrices (water column, sediment, plants, animals; Jha, 2004). Echinoderms, and in particular sea urchins, are particularly suitable organisms for use in bioindication studies (Parra-Luna et al., 2020). Along the Mediterranean coast, *Paracentrotus lividus* (Lamarck, 1816) is frequently considered as a good bioindicator of local pollution because of its wide distribution, abundance, benthic behavior, sedentary habits, rapid response and its recognized sensitivity to a variety of pollutants (Sugni et al., 2007; Amri et al., 2017; Rouhane-Hacene et al., 2017). Described as the organs that accumulate the most trace elements, its gonads and gut are of interest in studies assessing contamination levels in coastal marine ecosystems (Augier et al., 1989; Warnau et al., 1998; Geraci et al., 2004). *P. lividus* is also a species of economic importance due to its valued consumption in several countries (Fernández-Boán et al., 2013; Powell et al., 2014; Sun and Chiang, 2015), and ecological significance, controlling the dynamics of seaweeds and seagrass through its grazing activity (Lawrence and Sammarco, 1982; Boudouresque and Verlaque, 2013).

The use of biomarkers as bioindicators is an important approach in aquatic biomonitoring to assess the relationships between exposure to environmental pollutants and increased effects on individuals and populations (Bouzahouane et al., 2018). The effects of pollutants on the marine ecosystem can be expressed in terms of biochemical endpoints (Kamel et al., 2014). The exposure of aquatic organisms to trace elements can lead to the production of reactive oxygen species (ROS; Nieto et al., 2010), causing an imbalance between the production of ROS and endogenous antioxidant activity (Beyersmann and Hartwig, 2008). Oxidative stress is involved in DNA damage, protein oxidation and lipid peroxidation (Winston and Di Giulio, 1991). In view of this, it is relevant to assess the activities of antioxidant enzymes acting against oxidative stress such as catalase (CAT), glutathione peroxidase (GPX), glutathione-s-transferase (GST) and superoxide dismutase (SOD). The content of malondialdehyde (MDA), a by-product of lipid peroxidation, and the level of hydrogen peroxide (H_2O_2) in the tissues of organisms is also a good indicator of oxidative stress intensity. Therefore, oxidative stress biomarkers are widely used in marine ecotoxicology (Kamel et al., 2014; Benedetti et al., 2015; Ghribi et al., 2020).

The aims of this study are (i) to assess the reliability of different matrices (sea urchin, macroalgae, water column and sediment) to characterize 24 trace elements contamination levels; (ii) to test the effectiveness of several indices used in ecotoxicological studies in order to confirm their use in the assessment of contamination; (iii) to determine the bioaccumulation, biomagnification and biota-sediment accumulation factor of trace elements, and (iv) to estimate the effects of trace elements contamination on the oxidative stress of *P. lividus*.

2. Material and methods

2.1. Study area

The study area is located on the northwest coast of Corsica (NW Mediterranean Sea; Fig. 1A, B). This rocky coast is characterized by the occurrence of several cliffs (40 to 50 m high) formed of ophiolitic rocks (pillow-lavas and prasinites, gabbros, serpentinites and peridotites) and of mineral characteristics of the rubble sand belongs to the serpentine, olivine, pyroxene and amphibole groups (Bernier et al., 1997; Lafabrie et al., 2008). In this area, the old asbestos mine of Canari extends along 1 km of rocky coastline (42°49′15″N, 9°19′41″E; Fig. 1C). After the discovery of the asbestos deposit in 1898, the mine was industrially exploited from 1948 to 1965 (BRGM, 1997). During this active period of exploitation, up to 28,000 tons of asbestos was produced yearly and approximately 4.5 million m^3 of solid mine waste was directly discharged into the Mediterranean Sea (BRGM, 1997; Méria, 2004). The soils surrounding the old mine are covered by dense scrubland vegetation (upstream of the mine: over 400 m) while the natural revegetation of the exploited areas (downstream of the old mine) remains very reduced (BRGM, 1999).

Although the mine has been closed for over 50 years, the leaching of mining residues, present along the coastline of the mine, still contributes to the dispersal of nickel, chromium and cobalt into the marine environment (Galgani et al., 2006; Kantin and Pergent-Martini, 2007). Indeed, in the southern part of the shoreline, there are many beaches (*e.g.* Albo, Nonza) resulting from the southward transport of discharged rubble (360,000–400,000 $m^3\ yr^{-1}$) associated with the old asbestos mine of Canari (Fig. 1C; Bernier et al., 1997; Méria, 2004). The sediment enrichment of these beaches results from two sources: a natural enrichment by coarse sediments from small coastal rivers and an artificial enrichment due to discharged rubbles (Pouquet, 1958; Bernier et al., 1997). Greater amounts of sediment are provided by artificial sources (mixture of cobbles and pebbles, sands and silts) continually eroded and winnowed by wave action (Bernier et al., 1997). Currently, the low levels of anthropogenic activity in this area and the local sources of contamination associated with the old mine provide a unique mesocosm to study the contamination in the marine environment (ASR, 2011).

Six sampling sites located along the coast were selected (Fig. 1C): one in front of the old asbestos mine (Old Mine: OM), two to the north (Punta di Canelle: PC; Canelle: CN) and three to the south (Punta Bianca: PB; Albo: AB; Nonza: NZ). Samples were also collected at a reference site (RF), recognized as having a low level of trace elements contamination (Fig. 1D; Gobert and Richir, 2019; El Idrissi et al., 2020). These sites were selected to provide a basis for assessing the differences in trace elements concentration in order to establish a potential gradi-

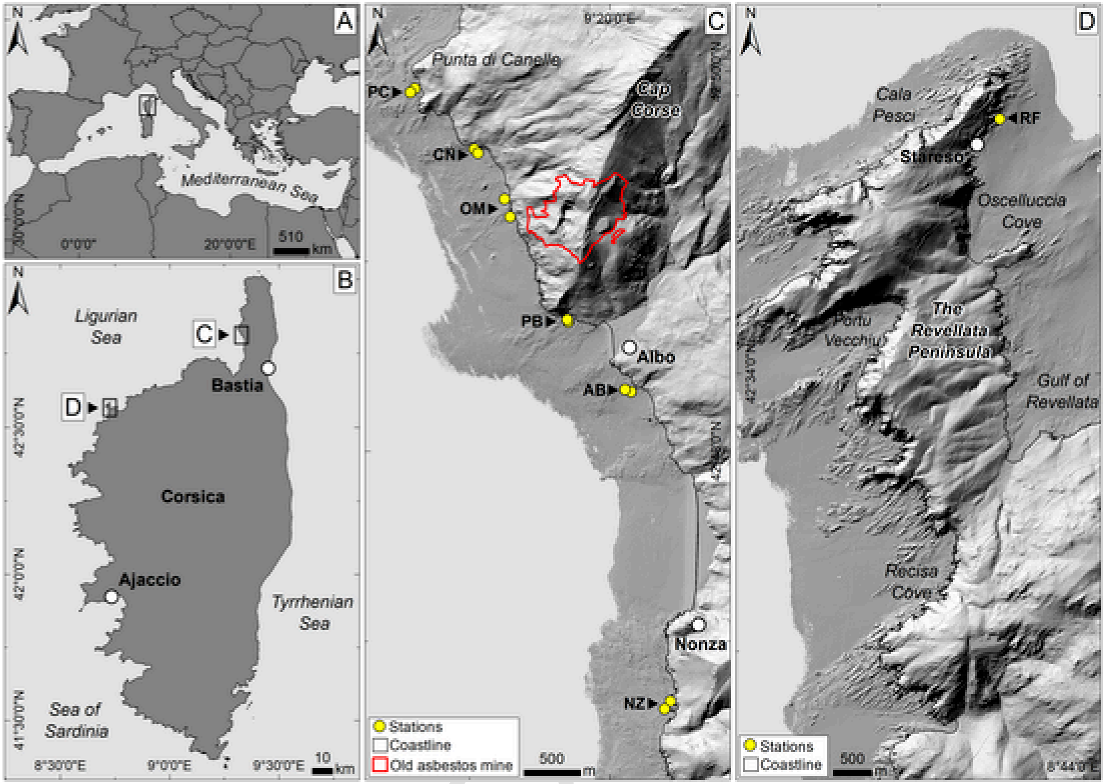

Fig. 1. Location of the study area in Corsica Island (A, B) and the sampling sites (C, D). PC: Punta di Canelle; CN: Canelle; OM: old mine; PB: Punta Bianca; AB: Albo; NZ: Nonza; RF: reference site.

ent of contamination and also to better understand the effects of these different concentrations on the organisms.

2.2. Collection and processing of biological samples

Individuals ($n = 28$) of *P. lividus* sea urchin and macroalgae species observed at each sampling site were collected at two distinct depths for each site (3 m and 6 m depth) in winter (Fig. 1C, D). As indicated by El Idrissi et al. (2020), the winter season avoids bias due to the reproductive stage of the urchins. In total, 364 sea urchins of commercial size (~50 mm) were collected and directly transported in a cooler with oxygenated seawater to the laboratory. Among the macroalgae collected at each site, the three most dominant taxa were retained and presented in this study *(Ericaria amentacea* (C.Agardh) Molinari & Guiry, 2020, *Dictyota dichotoma* (Hudson) J.V.Lamouroux, 1809 and *Ellisolandia elongata* (J.Ellis & Solander) K.R.Hind & G.W.Saunders, 2013). Three samples of each macroalgae (≈500 g wet weight) were collected at the different sites.

After sampling, macroalgae were rinsed with ultrapure MilliQ™ water and placed at −20 °C until chemical analysis. The specimens of *P. lividus* were measured, weighed and dissected. The sex ratio has been respected to avoid bias. Gonads and guts collected from sea urchins ($n = 16$) from each station were immediately weighed and stored at −20 °C. Frozen samples were lyophilized (CHRIST LCG Lyochamber Guard 121550 PMMA/Alpha 1-4 LD plus) and ground with an agate mortar. Approximately 0.2 g of each dried material was mineralized in Teflon digestion vessels, in a closed microwave digestion labstation (Ethos D, Milestone Inc. Sorisole, Italy), using nitric acid (HNO_3, 60 %) and hydrogen peroxide (H_2O_2, 30 %) as reagents (Suprapur grade, Merck, Darmstadt, Germany).

2.3. Collection and processing of sediment samples

Sediment samples were collected at each station using a 0.1 m^2 Van Veen grab from the bottom (top 20 cm). After collection, the sediments were stored at −20 °C until chemical analysis. Frozen sediments were lyophilized (BenchTop 3L, VirTis Company Inc.) and were eluted for 4 h at room temperature with 30 mL of HCl 1 N (Suprapure grade, Merck, Darmstadt, Germany), according to Townsend et al. (2007). A 4-h extraction time in HCl 1 N ensures the removal of the available precipitated trace elements while not favouring the extraction of natural geogenic metals (Snape et al., 2004). Eluates were then diluted to an appropriate volume of 50 mL, centrifuged for 10 min at 2000 rotations per minute and separated from their remaining culot prior to being analyzed.

2.4. Trace element concentrations

2.4.1. Bioavailable concentrations in the seawater column

The concentration of trace elements in the seawater column at each site was measured using diffusive gradients in thin films (DGTs) devices (DGT Research Ltd., UK). DGT units, fixed with a nylon fishing line to a plastic stage buried in the sediment, floated freely in the seawater column for one week. After recovery, DGTs were rinsed with ultrapure MilliQ™ water and stored at 4 °C until analysis. As trace metal diffusion coefficients through the diffusive layer depend on water temperature, average temperatures were recorded using HOBO TidbiT® v2 loggers (accuracy: ±0.21 °C).

2.4.2. Trace element analysis

The concentration of 24 trace elements (Ag, Al, As, Ba, Be, Bi, Cd, Co, Cr, Cu, Fe, Hg, Li, Mn, Mo, Ni, Pb, Sb, Se, Sn, Sr, U, V, Zn) were determined by Inductively Coupled Plasma Mass Spectrometry using Dynamic Reaction Cell technology (ICP-MS ELAN DRC II, Perkin Elmer), according to the method described by Richir and Gobert (2014). In order to check the purity of the chemicals used, analytical blanks were analyzed similarly to the samples and were performed every 40 samples. Analytical accuracy was checked by analyzing Certified Reference Materials (CRM): DORM-4 (fish protein), NIST1566b (oyster), NIST2976 (mussel tissue), BCR-60 (*Lagarosiphon major*), BCR-661 (*Platyhypnidium riparioides*) and GBW07603 (bush twigs and leaves). For each TE, detection limit (LD) and quantification limit (LQ) were calculated, depending on their specific blank distribution (Grinzaid et al., 1977; Currie, 1999). The total Hg (THg) content of biological samples was determined using atomic absorption spectrometry at 254 nm, in a Direct Mercury Analyser (DMA 80 153 Milestone, Minnesota, USA). Quality assessment was operated using replicates, standards (THg 100 ng g^{-1}), blanks (HCl 1 %) and CRM at the beginning and the end of each series. The results are expressed in milligrams of element per kilogram of dry weight (mg kg^{-1} DW) for tissue and sediment, and in milligrams of element per litre (mg L^{-1}). TEs with values below the detection limit were removed from the database. For the others, concentrations below the LD were replaced with a value of LD/2, as reported by Skrbić et al. (2010).

2.5. Histological study

The gonads of six sea urchins from different stations were fixed in formaldehyde in order to perform a histological study and determine the reproductive stage. This enables us to determine whether trace elements levels are influenced by the reproductive stage in this study. Gonadal tissues were dehydrated using ethanol (from 70 to 100 %) then placed in Neo-Clear™, xylene substitute, before being embedded in paraffin. After cooling the blocks, two duplicate sections (thickness: 5 μm) were taken using a microtome, mounted on slides, and air-dried for the deparaffinization of the tissue in preparation for rehydration. After that, the sections were stained with Masson's Trichrome and observed under a light microscope to determine the stage of maturity based on the stages described by Byrne (1990): stage 1, recovery; stage 2, growing; stage 3, premature; stage 4, mature; stage 5, partly spawned; stage 6, spent.

2.6. Biochemical analyses

For the oxidative stress study, the gonads of six sea urchins per station were fixed in liquid nitrogen before storage at −80 °C. Samples were homogenized using a Potter-Elvehjem homogenizer in chilled phosphate buffer (100 mM, pH 7.4; 25 mg *w*/w per mL of buffer) containing 20 % glycerol and 0.2 mM phenylmethylsulfonyl fluoride as a serine protease inhibitor. The homogenates were centrifuged at 15,000 ×*g* for 30 min at 4 °C and the supernatant was used for biochemical assays. Protein concentration was measured as described in Bradford (1976) and was used to normalize the final unit for biomarker responses. Biomarkers including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and glutathione-S-transferase (GST) and determination of malondialdehyde (MDA) content were assessed as described by Greani et al. (2017). The hydrogen peroxide (H_2O_2) concentration was determined using a PeroxiDetect Kit (Sigma-Aldrich, St. Louis, MO, United States) as described by Lourkisti et al. (2020).

2.7. Elemental and isotopic analyses

Gut content of *P. lividus* ($n = 12$ per site) and food sources (*D. dichotoma, E. elongata and E. amentacea*) were analyzed through elemental and isotopic analyses. The sea urchins and macroalgae samples were dried at 60 °C for 48–96 h and successively ground to a homogenous powder with agate mortal. As acidification is known to alter N stable isotope ratios (Mateo et al., 2008), acidified samples (*E. elongata*) were analyzed twice: once for stable C isotopic ratios, using decarbonated material, and once for N isotopic ratios, using native material. Samples of food source (1–5 mg) and gut content (2–3 mg) were subsequently loaded into tin capsules (8 × 5 mm, Elemental Microanalysis). Stable isotope ratio measurements were performed *via* continuous flow-elemental analysis-isotope-ratio mass spectrometry (*CF*-EA-IRMS) at University of Liège, using a Vario Micro Cube elemental analyser (Elementar Analysensysteme GmBH, Hanau, Germany) coupled to an Isoprime 100 mass spectrometer (Isoprime, Cheadle, UK). Isotopic ratios of C and N were expressed conventionally (Coplen, 2011), using standard delta (δ) notation relative to their respective international standards, Vienna-Pee Dee Belemnite (VPDB) and atmospheric N_2. Certified reference materials (CRM) of sucrose (IAEA-C6, $\delta^{13}C = -10.8 \pm 0.5$ ‰) and ammonium sulphate (IAEA-N2, $\delta^{15}N = 20.3 \pm 0.2$ ‰) obtained from the International Atomic Energy Agency (IAEA, Vienna, Austria), were used for the measurement of isotopic ratios. These CRM, procedural blanks and internal replicates (*i.e.*, glycine and in-house seagrass reference materials) were used to assess the analytical precision. Standard deviations on multi-batch replicate measurements were 0.1 ‰ for $\delta^{13}C$ and 0.2 ‰ for $\delta^{15}N$.

The relative contribution of macroalgae to the diet of the sea urchin *P. lividus* was estimated with a Bayesian isotopic mixing model (MixSIAR, Stock and Semmens, 2016; Stock et al., 2018b). The application of the mixing model provided accurate information concerning the contribution of macroalgae species to the sea urchin tissues and recognized the main components of the diet under different conditions (Peterson, 1999; Fry, 2006; Wing et al., 2008; Cabanillas-Terán et al., 2016). The model runs included the isotopic signatures of each individual, isotopic compositions of food sources (mean ± SD) and trophic enrichment factors (TEFs; mean ± SD) corresponding to the isotopic composition difference of consumer tissues and the potential food sources (Mascart et al., 2018). Though the use of suitable TEFs is required to run mixing model, there are no specific TEF for the taxa studied here, and we used widely applicable values (*i.e.*, 0.4 ± 1.2 ‰ for C, 2.3 ± 1.6 ‰ for N) from McCutchan et al. (2003). The model was run using the MixSIAR 3.1.12 package in R 4.1. The results of the mixing model showing the calculated sea urchin dietary proportions were represented using mean ± SD and 95 % credible intervals (CI_{95}) indicating the intervals of probability density function distributions.

2.8. Numerical procedures

In order to compare the contamination levels at the different sites, the Trace Element Pollution Index (TEPI) was calculated for each site. The TEPI allows a reliable comparison of study sites, regardless of trace elements or the biological model used (Richir and Gobert, 2014). The index was calculated with concentrations measured in gonads, digestive tract and macroalgae to confirm its robustness. The data were standardized by mean normalization as recommended for the calculation by Richir and Gobert (2014). TEPI values were calculated using the following formula:

$$TEPI = \left(Cf_1 \ast Cf_2 \ast \ldots \ast Cf_n\right)^{1/n}$$

where Cf_n is the mean normalized concentration of the trace element at each site and n is the number of trace elements examined. The higher the TEPI value, the more contaminated the site is. In order to classify and compare the trace elements according to their spatial variability on all the studied sites, the Trace Element Spatial Variation Index (TESVI) was determined for each element according to Richir and Gobert (2014). For each trace element, TESVI was calculated as follows:

$$TESVI = \left[\left(x_{\max} / x_{\min}\right) / \left(\sum \left(x_{\max} / x_i\right) / n\right)\right] \ast SD$$

where x_{max} and x_{min} are the maximum and minimum mean concentrations recorded among the n sites, x_i are the mean concentrations recorded at each of the n sites, and SD is the standard deviation of the mean ratio $\sum(x_{max}/x_i)/n$. For a given trace element, the higher the value of the TESVI, the more its environmental levels will vary overall across the study area. Therefore, the higher the TESVI, the more representative it is of a site.

Bioaccumulation is generally referred to as a process in which the chemical concentration in an organism achieves a level that exceeds that in the environment, the diet, or both (Gobas et al., 2009). The extent to which chemicals bioaccumulate is expressed by several values, including bioaccumulation factor (BAF), biomagnification factor (BMF) and biota-sediment accumulation factor (BSAF). BAF is ratio of the steady chemical concentrations in an aquatic water-respiring organism (CB, g kg^{-1} WW) and the water (CW, g L^{-1}) determined from field data in which sampled organisms are exposed to a chemical in the water and in their diet. BAF is calculated using the following formula: CB/CW. BFM is the ratio of the steady state chemical concentrations in an organism (CB, g kg^{-1} WW) and the diet of the organism (CD, g kg^{-1} WW) determined from field data. Lastly, BSAF is the ratio of the chemical concentrations in an organism (CB, g kg^{-1} DW) and the sediment (CS, g kg^{-1} DW).

Data were expressed as mean ± standard error (SE) and analyzed using XLSTAT software. The data were transformed in order to meet the conditions of application of the parametric tests and to reduce the effect of outliers skewing the data distribution. Analyses of variance (ANOVA) followed by post hoc Tukey's honestly significant difference (HSD) tests were performed. The relationship between enzymatic activities and trace elements was measured by Pearson correlation coefficient. A significant difference was considered a *p*-value <0.05.

3. Results

3.1. Distribution of trace element content

The levels in Be and Bi levels were below the detection limit in all matrices analyzed, so they were not considered in the statistical analyses of the results. In addition, Hg concentrations were also below the detection limit in the sediments and the seawater column. For the sake of clarity, only 14 representative examples chosen among the 24 studied trace elements are graphically illustrated throughout the paper, and discussion mainly revolves around these selected examples. The mean trace element concentrations measured in the different matrices (sea urchins, macroalgae, sediment and seawater column) are presented in Fig. 2.

Trace element concentrations follow the sequence: Fe > Zn > Al > Ni > Cr > Mn > Cu > V > Se > Co > Mo > Cd > Ag > Pb in sea urchin gonads; Fe > Al > Cr > Ni > Zn > Mn > Cu > V > Se > Co > Cd > Mo > Ag > Pb in sea urchin guts; Fe > Al > Ni > Cr > Mn > Cu > V > Zn > Co > Pb > Mo > Cd > Ag in macroalgae; Fe > Pb > Al > Ni > V > Zn > Se > Mn > Cr > Cu > Co > Mo > Cd > Ag in seawater column and Fe > Al > Ni > Cr > Mn > V > Co > Cu > Zn > Se > Pb > Mo > Cd > Ag in sediment (Fig. 2).

3.2. Spatial variations of trace elements

There is no significant difference in trace elements concentrations between the samples collected at 3 m depth and those collected at 6 m depth. The TEPI was calculated with concentrations in sea urchin gonads, sea urchin guts and macroalgae (Fig. 3). In gonads and guts, a gradient of contamination clearly appeared, with higher contamination to the south of the old asbestos mine, particularly at Albo (gonads: 1.367, guts: 1.172) and Nonza (gonads: 1.161, guts: 1.201; Fig. 3A, B). In contrast, the reference site is the least contaminated, with a TEPI of 0.456 for the gonads and 0.529 for guts (Fig. 3A, B).

The TEPI calculated with macroalgae showed a more contrasting gradient with the Canelle site, located north of the mine, similarly contaminated to Nonza (0.902 and 0.907, respectively; Fig. 3C). The four trace elements with the highest TESVI in gonads, guts and macroalgae are Co, Cr, Fe and Ni (Table 1). The highest concentrations were noted at Albo for the four trace elements (Table 1). Therefore, Co concentrations are 10 to 20-fold higher at Albo than at the reference site, Cr 96 to 172-fold, Fe 2 to 9-fold and Ni approximately 38-fold.

3.3. Bioaccumulation, biomagnification and biota-sediment accumulation factors

The BAF and BSAF were calculated with the concentrations in the gonads and guts of the sea urchin and with concentrations measured in macroalgae (Table 2). Most trace elements were most bioaccumulated in macroalgae then the guts and finally the gonads (Al, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, V; Table 2). Only Zn had a higher accumulation in the gonads in comparison to the guts and macroalgae (Table 2).

Among the macroalgae studied, the isotopic analysis highlighted that sea urchins collected fed mainly on *E. elongata* (96.6 ± 2.3 %; CI_{95}: 90.8–99.6 %) and very little on *D. dichotoma* (2.5 ± 2.2 %; CI_{95}: 0.1–8.4 %) and *E. amentacea* (0.9 ± 0.9 %; CI_{95}: 0–3.2 %; Fig. 4). In order to calculate the BMF, a weighted average of macroalgae was used for accuracy. Therefore, the formula used in this study was:

$$CB/\left(CD_1 * 0.966 + CD_2 * 0.025 + CD_3 * 0.009\right)$$

where, CB is the concentration measured in guts, CD_1 the concentration in *E. elongata*, CD_2 in *D. dichotoma* and CD_3 in *E. amentacea*. The highest BMFs were obtained for Ag, Cd and Mo, elements for which the guts accumulate more than the other matrices (Table 2).

3.4. Biotic factors of the sea urchin

The histological analysis highlighted that sea urchins were mostly at stage 4 (mature) and stage 5 (partly spawned; Fig. 5). However, variations were observed at Punta Bianca where ~30 % of the sea urchins had already spawned and at the reference site where 50 % of the sea urchins had reached the premature stage (Fig. 5). Significant differences were noted between the concentrations measured in the male and female gonads. Females exhibited higher concentrations of Cd, Mn, Se, and Zn while high Mo, Pb, and V levels were found in males (*p*-value $<$ 0.05). In contrast, no significant differences were found for biomarkers of stress. In the guts, the only significant difference between males and females is measured for Zn with a concentration 1.5-fold higher in females (p-value $<$ 0.001).

3.5. Biochemical analyses

There was no significant difference between sites regarding MDA content and specific activities of SOD and GPX (Fig. 6). At the same time, the specific activity of CAT and the H_2O_2 levels were higher in sea urchins collected at Albo, Nonza and at the reference site (Fig. 6). Finally, the highest specific activity of GST was measured in sea urchins at Albo and three sites (Reference site, Nonza and Old Mine; Fig. 6). Specific activities of CAT and GST as well as H_2O_2 levels in sea urchin gonads showed significant positive correlation (p-value $<$ 0.001, Table A.1). In addition, there were significant positive correlations for several trace elements with CAT and H_2O_2 (Table A.1). Accordingly, increased concentrations of Al, Cr, Cu, Fe, Mn, Pb, and Se resulted in higher specific CAT activity and H_2O_2 levels in the gonads (p-value $<$ 0.05).

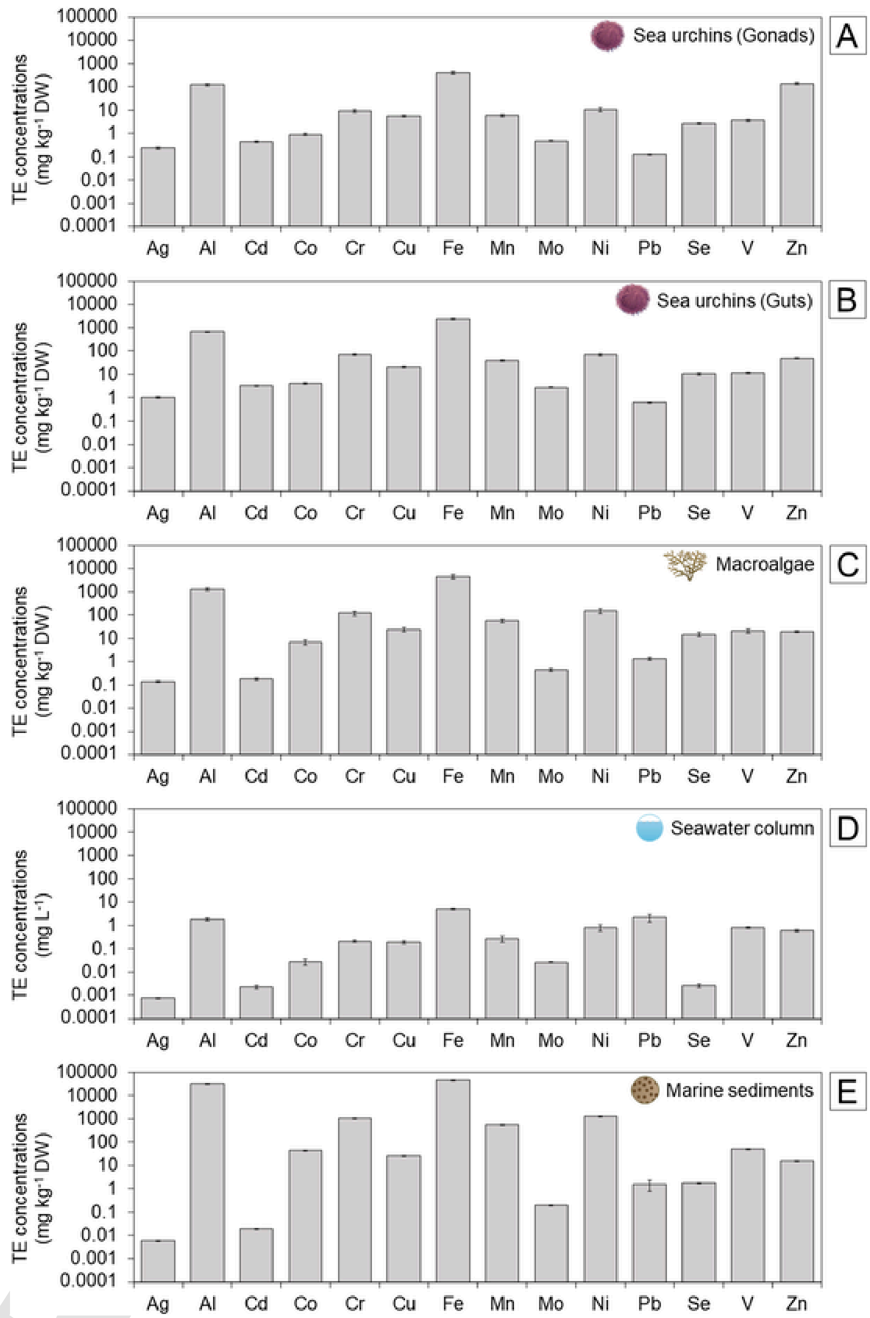

Fig. 2. Distribution of trace element (TE) concentrations in sea urchins *P. lividus* (gonads: A, guts: B), macroalgae (C), seawater column (D) and marine sediments (E).

4. Discussion

The study of environmental contamination requires a deep knowledge of the distribution of pollutants and their concentrations in biotopes and organisms (Lagadic et al., 1998; Ramade, 2007). For this purpose, trace element concentrations were analyzed in sea urchins (gonads and guts), three macroalgae species, the seawater column and sediment. The trace elements follow an almost similar sequence regardless of the environmental matrices studied, although there are some notable differences. Fe is the predominant trace element resulting from its essential character (Phillips and Rainbow, 1989; Lohan and Tagliabue, 2018) or from the contamination of the ecosystem as described in the literature (Brik et al., 2018). Fe is an essential trace element playing a major role for many species due to its role in various physiological processes (*e.g.* photosynthesis, enzymatic activity, reproduction, *etc.*) and it is also included in geochemical processes (Sunda, 2001; Thuróczy et al., 2011). However, in contrast to the literature, Fe concentrations exceeded Zn concentrations in the gonads (Strogyloudi et al., 2014; Ternengo et al., 2018; El Idrissi et al., 2020). This is due to the remarkably high concentrations of Fe, 2 to 8-fold higher than in many studies (Warnau et al., 1998; Storelli et al., 2001; Soualili et al., 2008; Strogyloudi et al., 2014). According to Blum et al. (2006), the serpentinites of Cap Corse could generate significant Fe content explaining these high contents at the studied sites. Although Fe is an essential requirement for marine organisms, an excessive concentration could be harmful to their health and must therefore be monitored (Fosmire, 1990). Zn is the second element measured in high concentration in the gonads. It is the only trace element with a higher accumulation factor in the gonads than in the guts and macroalgae. This accumulation could be due to its intervention in gametogenesis and in particular in ovogenesis explaining its high content in females compared to males (Unuma et al., 2003, 2007). In contrast to the sediment, macroalgae, gonads and

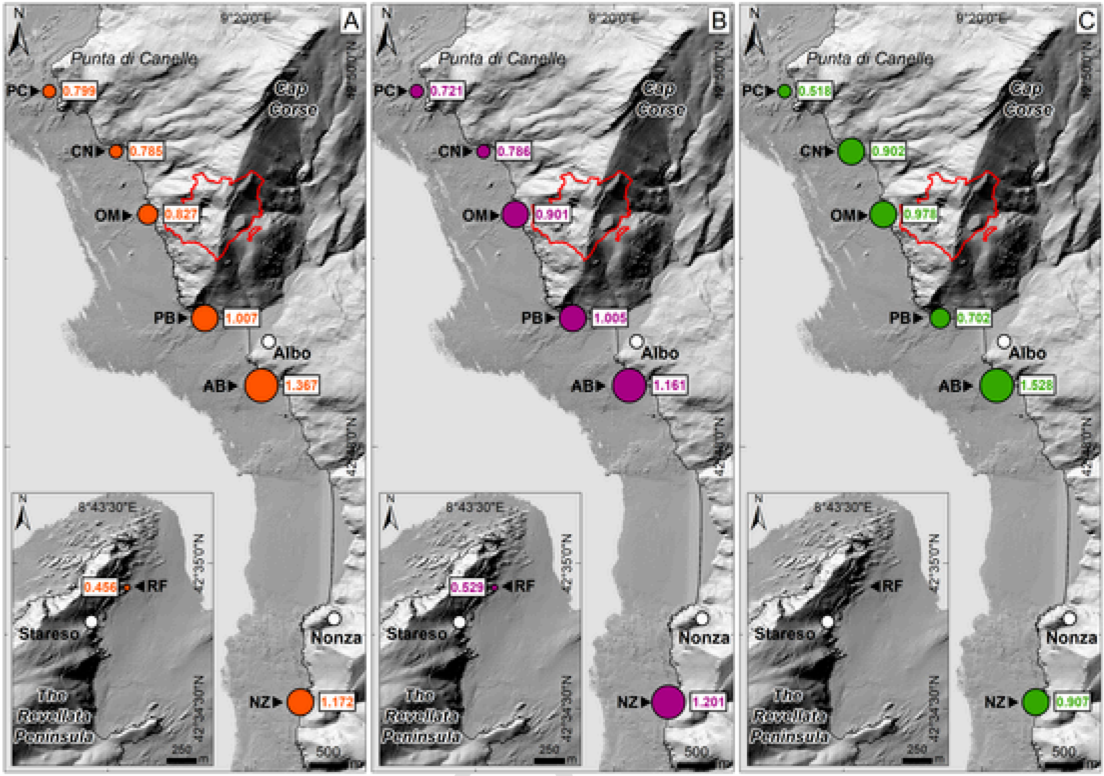

Fig. 3. Trace Element Pollution Index (TEPI) determined using sea urchins (gonads: A, orange points, guts: B, purple points) and macroalgae (C, green points), of the seven sites (PC: Punta di Canelle; CN: Canelle; OM: old mine; PB: Punta Bianca; AB: Albo; NZ: Nonza; RF: reference site). No macroalgae samples were collected at RF. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

guts of sea urchins where Pb is among those with the lowest concentrations, this trace element is the second most abundant in the distribution sequence of the seawater column. It is one of the most abundant and common non-essential trace elements in the environment (Mishra et al., 2006). Its concentration in the seawater column (2.17 ± 0.83 mg L^{-1}) is considerably higher than previously reported in the literature (Lin et al., 2000; Bruland and Lohan, 2003). This is may be due to point contamination of the water column during the period of this study or contamination of DGTs during material handling. Seawater column analysis is reported to be less relevant in the literature for trace element monitoring due to its high fluctuations caused by several factors such as hydrodynamic energy (Jahan and Strezov, 2019). Moreover, it is not always possible to analyze all the contaminants in this biotope, the concentration levels being too low and the analytical techniques not being sensitive enough in this study for Hg.

Calculation of the TEPI through measurement of trace element contents in the gonads and guts of the sea urchin enabled us to determine a gradient of contamination with a higher concentration to the south of the old asbestos mine, in particular at Albo and Nonza. The occurrence of a similar north-south gradient within this area has already been demonstrated in other matrices such as mussels (Kantin et al., 2015). In the literature, it has been established that asbestos wastes were accumulated to the south of the old asbestos mine (BRGM, 1997; Cary et al., 2013). These materials migrated along the coastline due to the effect of swell and prevailing marine currents (BRGM, 1997). As a result, the wastes expanded the coastline southward for 5 km from the old mine, progressively filling the beach at Albo and creating the beach at Nonza (about 1500 m long; BRGM, 1997). A total of 1 million tons of excavated material was transported to the artificial beaches during the 35 years of operation of the site (Boulmier et al., 1999; Hervé et al., 1997; Cary et al., 2013). This observation highlights the important role that the marine environment plays in the diffusion and distribution of contaminants in the environment. Hence, it is necessary to take care during the selection process of the sampling site for ecotoxicological studies and to consider which can contribute to the variability of contaminants at local scale. Moreover, sites at 3 and 6 m depth are similarly impacted by diffusion, indicating that the contamination affects the whole of the studied bathymetric range.

The Albo and Nonza beaches are characterized by black pebbles constituted by serpentine (BRGM, 1997). The serpentinites are characterized by high levels of Fe and Mg (Morrison et al., 2009) which may explain the high Fe concentrations found at Albo with a high TESVI whatever the matrices studied. Co, Cr, and Ni are also present in high concentrations at Albo with significant concentration differences compared to the reference site. Previous works have also highlighted a high contamination of Ni, Cr and Co in marine sediments in the area adjacent (15 km of coastline) to the old asbestos mine at Canari (Andral and Tomasino, 2007; Kantin and Pergent-Martini, 2007) and an accumulation of these elements in several marine organisms (Bouchoucha & Andral, 2010; El Idrissi et al., 2020). According to several authors, in addition to Fe, serpentinites are naturally enriched in other trace elements such as Cr, Ni and Co (Morrison et al., 2009; Siebecker, 2010; Tashakor et al., n.d.), explaining these high concentration levels in the region and particularly to the south of the old mine. The general formula of serpentine is Mg_5 {Si_2O_4} $(OH)_4$ and substitution of Mg by Fe (II), Fe (III), Cr, Al, Ni and Mn may occur (Mével, 2003). In the same way, large amounts of Mg and/or Fe are reported, disproportionate richness in Ni, Cr and Co and poorness in Ca in the serpentinite (Lee et al., 2004; Pal et al., 2006). Thus, even though mine drainage was the main cause of these contamination variations, there is a general sensitivity related to a particularly concentrated geochemical background. The assessment of ecosystem quality requires good knowledge of the natural geochemical context in order to distinguish the trace elements naturally present in the environment from those resulting from anthropogenic activities.

Serpentine soils are considered a source of geogenic pollution by many researchers because of the trace elements they contain and their

Table 1
Trace Element Spatial Variation Index (TESVI) of 22 trace elements examined in sea urchins (gonads and guts) and macroalgae from six sites near the old asbestos mine (Corsica, NW Mediterranean Sea; PC: Punta di Canelle; CN: Canelle; OM: Old Mine; PB: Punta Bianca; AB: Albo; NZ: Nonza; RF: Reference site). The higher the TESVI value, the greater the spatial variation of that element among the sampling locations. In dark grey, the higher values.

Trace elements	Sea urchins				Macroalgae	
	Gonads		Guts			
	TESVI	Site X_{max}	TESVI	Site X_{max}	TESVI	Site X_{max}
Ag	1.622	NZ	3.964	CN	0.216	PC
Al	**4.896**	**AB**	1.321	AB	0.776	AB
As	0.704	AB	0.962	OM	2.357	AB
Ba	0.710	PC	0.182	PB	n.a	n.a
Cd	1.354	PC	0.370	CN	0.639	PC
Co	**8.243**	**AB**	**31.789**	**AB**	**10.124**	**AB**
Cr	**194.926**	**AB**	**406.518**	**AB**	**13.335**	**AB**
Cu	0.589	NZ	0.750	NZ	**4.422**	**AB**
Fe	**16.854**	**AB**	**15.238**	**AB**	**10.913**	**AB**
Hg	1.703	AB	0.576	RF	n.a	n.a
Li	0.160	AB	0.247	PB	n.a	n.a
Mn	3.078	AB	**6.650**	**AB**	3.897	AB
Mo	0.454	PC	0.652	RF	1.871	AB
Ni	**56.107**	**AB**	**50.454**	**AB**	**17.229**	**AB**
Pb	0.296	RF	0.488	RF	0.422	CN
Sb	0.794	CN	0.304	RF	0.185	AB
Se	0.790	NZ	3.457	NZ	3.451	AB
Sn	1.002	AB	2.190	RF	2.893	AB
Sr	0.960	AB	0.520	PB	0.237	PC
U	3.293	CN	0.120	PB	n.a	n.a
V	3.735	AB	1.310	AB	2.000	OM
Zn	1.429	PC	0.287	PC	0.204	CN

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potential deleterious effect at high concentrations in the environment (Caillaud et al., 2009; Tashakor et al., n.d.). In this regard, recent experiments have been performed to determine the impacts of concentrations measured in this area on *Paracentrotus lividus* but no significant effect were highlighted on the larvae, even in the presence of chronic contamination (El Idrissi et al., 2022a, 2022b).

The TEPI of macroalgae also indicated strong contamination at Albo but no north-south contamination gradient has been highlighted. Indeed, the macroalgae located in the northern part of the mine exhibited contamination levels similar to those located in the south, resulting in a gradient around the mine rather than north-south. This result may be related to a reversal phenomenon of the littoral drift (from south to north) during a strong storm in 1973 causing a transport of sediment from the rubble discharge towards the north (Bernier et al., 1997).

Macroalgae may be better able to accumulate than sea urchins as indicated by the different accumulation factors calculated in this study. Thus, the macroalgae can be particularly useful to determine the presence of a contaminant at the specific location. However, it is necessary to be careful when interpreting contamination levels at the risk of overestimating them (Richir, 2013). Moreover, if the aim of the study is to identify the magnitude of well-integrated contamination, the sea urchin may be a better tool for assessing a contamination gradient.

It is commonly recognized that the accumulation of contaminants in marine organisms occurs through three compartments: the seawater column, food and sediments (Bouzahouane et al., 2018). Accumulation can lead to high internal concentrations resulting in toxicity, even when external concentrations are low (ECHA, 2017). According to some authors, sediment often plays the role of a local sink for contaminants, which can increase the concentrations of benthic organisms that indiscriminately ingest sediment particles while feeding (Li et al., 2019; Jacobs, 1998). In the present study, the BSAFs are mostly low (<1) indicating low bioaccumulation (Warnau et al., 1998). These low values are notably related to high trace element concentrations in the sediment. Only Ag, Cd, Mo, Se and Zn have BSAFs higher than 1 with concentrations in organisms equivalent to those recorded in the literature (*e.g.* Guendouzi et al., 2017; Rouhane-Hacene et al., 2017; Warnau et al., 1998). A high BSAF does not necessarily indicate a risk and rather suggests high concentrations in the organism due to its essential role in physiological processes and low concentrations in sediments, as with Zn for instance. Therefore, the efficiency of trace element absorption from different sources may vary according to ecological needs, organism metabolism and concentrations in different compartments (Bouzahouane et al., 2018). Variations in trace elements within the same species can therefore have different origins. The reproductive stage of the sea urchin is known as a parameter influencing trace element concentrations (El Idrissi et al., 2020). In the present study, the sea urchins are almost all at the same stage of reproduction except at Punta Bianca where about 30 % of the sea urchins have already spawned (stage 5) and at the reference site where 50 % of the gonads are premature (stage 3). This information probably indicates a low overestimation of gonadal contamination levels at Punta Bianca and the reference site compared to the other investigated sites where a dilution effect occurs (El Idrissi et al., 2020). In this study, BAFs indicate that the majority of trace elements are most bioaccumulated in macroalgae then the gut and later the gonads. Therefore, the use of guts might be more interesting than gonads in studies assessing local variations in trace element contamination. Indeed, this compartment of the sea urchin bioaccumulates more making concentrations higher and therefore the analytical analysis more accurate. In addition, in contrast to the gonads, the majority of the trace elements do not vary according to the sex. Investigations should be conducted to assess whether reproductive stages and the source of food have an impact on the concentrations in the guts. In the present study, we estimated BMF using the guts and macroalgae that the urchin could potentially feed on. Although in some conditions, *P. lividus* can be omnivorous (Wangensteen et al., 2011), it remains mainly herbivorous (Agnetta et al., 2013; Boudouresque and Verlaque, 2013). Several factors such as seasonality (Verlaque, 1987), abiotic parameters or resources available within the biotope (Paine and Vadas, 1969; Frantzis et al., 1988) can influence its choice. Therefore, for this work, three species of macroalgae were collected because of the presence of *P. lividus* in their beds and their high abundance in the sites. Isotopic analyses identified *E. elongata* as the main food source compared to *D. dichotoma* and *E. amentacea*. Frantzis and Grémare (n.d.) have already demonstrated a preference of *P. lividus* for this macroalgae which is consistent with our results. The most biomagnified trace elements (Ag, Cd and Mo) are those measured at low concentrations and are

Table 2
Bioaccumulation (BAF), biomagnification (BMF) and biota-sediment accumulation (BSAF) factor of 14 trace elements. TE: trace elements; SW: seawater.

TE	BAF			BMF	BSAF		
	Gonads/SW	Guts/SW	Macroalgae/SW	Guts/macroalgae	Gonads/sediment	Guts/sediment	Macroalgae/sediment
Ag	50.3526	163.6319	29.8274	5.5936	38.7261	167.7989	22.9402
Al	11.2258	46.8450	117.4314	0.1401	0.0038	0.0210	0.0395
Cd	33.4045	181.6255	13.1774	6.9715	23.6158	171.2038	9.3159
Co	5.5608	18.1816	41.7896	0.1351	0.0205	0.0894	0.1541
Cr	7.5379	45.2430	98.6556	0.1514	0.0086	0.0691	0.1131
Cu	5.0336	14.3122	21.8451	0.2092	0.2221	0.8419	0.9638
Fe	13.1917	59.3457	148.6595	0.1393	0.0086	0.0516	0.0970
Mn	3.7815	18.2950	36.8864	0.1556	0.0109	0.0702	0.1061
Mo	3.1471	13.0912	2.7865	3.2869	2.5737	14.2747	2.2788
Ni	2.2670	11.1937	32.2017	0.1214	0.0081	0.0532	0.1149
Pb	0.0097	0.0361	0.1022	0.1169	0.0794	0.3938	0.8352
Se	170.7810	502.6702	958.6628	0.1535	1.5232	5.9777	8.5503
V	0.7858	1.8492	4.5681	0.1535	0.0720	0.2259	0.4185
Zn	38.8205	10.4672	5.4270	0.8050	8.7230	3.1360	1.2195

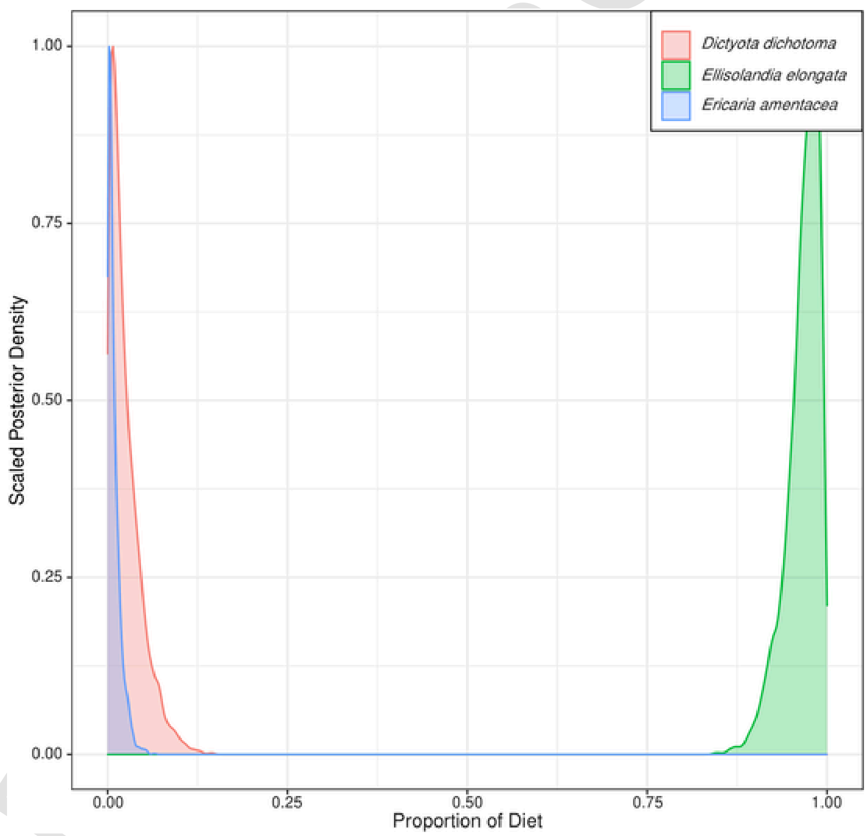

Fig. 4. Relative contributions of three food sources (*Dictyota dichotoma, Ellisolandia elongata* and *Ericaria amentacea*) to the diet of *P. lividus* individuals sampled near the old asbestos mine at Canari (Corsica, NW Mediterranean).

among the last elements in the distribution regardless of the matrices studied. Luy et al. (2012) have also studied these elements and, according to them, the levels of Ag and Mo measured in seagrass reflect the background level of agriculture in this area.

Several studies suggest that exposure to trace element contamination can induce a cascade of events such as ROS production (Farombi et al., 2007; Nieto et al., 2010). Thus, antioxidant defense mechanisms are crucial to maintain the redox balance between pro-oxidants and antioxidants in aerobic organisms (Limon-Pacheco and Gonsebatt, 2009; Chan and Wang, 2019). In order to estimate the effects of trace element contamination on the oxidative stress of *P. lividus*, analyses were performed on the gonads of sea urchins collected at different locations of the contamination gradient (north to south). The highest specific activity of CAT, GST and H_2O_2 was reported in the south of the old asbestos mine where the contamination is the highest. A positive correlation between the specific activity of CAT, H_2O_2 and some trace elements is noted confirming an effect of contaminants on the oxidative stress of sea urchin and on the reliability of biomarkers used in this study. In the mitochondrial respiratory chain, H_2O_2 can react directly with metal ions such as Fe or Cu, by the Fenton reaction, and form the hydroxyl radical which is a powerful oxidant (Regoli and Giuliani, 2014; Mejdoub et al., 2017). Consequently, the elimination of H_2O_2 is a key strategy of organisms against oxidative stress (Regoli et al., 2002a; Mejdoub et al., 2017). According to Giarratano et al. (2013), the simultaneous induction of GST and CAT activities suggests a similar pattern for hydrogen peroxide removal. Therefore, the increase in these en-

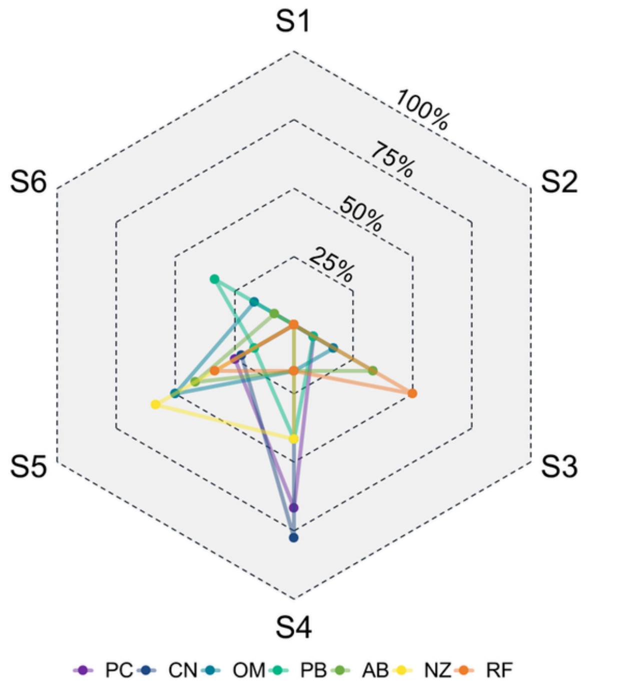

Fig. 5. Maturity stage of sea urchin gonads (%) at each site (PC: Punta di Canelle; CN: Canelle; OM: Old Mine; PB: Punta Bianca; AB: Albo; NZ: Nonza; RF: Reference site).

zymes suggests an activation of detoxification processes, probably reflecting high stress (Louiz et al., 2016). A large number of other studies have shown higher activity of CAT and GST in organisms collected from contaminated sites (Bougherira et al., 2015; Keblouti et al., 2015; Bouzenda et al., 2017). Regoli et al. (2002b) consider CAT a sensitive and important biomarker of oxidative stress superior to SOD, explaining the invariance of specific SOD activity in this study. These high specific activities of CAT and GST as well as this high H_2O_2 content were also measured at the reference site which however has a low concentration of trace elements. The main difficulty in using biomarkers in the natural environment is the interference with other environmental factors (Lagadic et al., 1998). In contrast to experiments under controlled conditions, various factors can lead to responses of biochemical parameters in the natural environment (Lagadic et al., 1998; Ramade, 2007). As a result, many factors such as meteorological conditions, interactions with other contaminants than those studied, or interspecific relationships can complexify the interpretation of oxidative stress responses (Lagadic et al., 1998). In this case, the same habitat at the reference site was chosen as for the sites near the old asbestos mine in order to minimize variation; it thus appears that other factors led to these high values. Further study would be of interest to determine the source of this significant oxidative stress response. Finally, despite the higher levels of H_2O_2 in sea urchin gonads collected to the south of the mine and at the reference site, the level of MDA, a by-product of lipid peroxidation, did not significantly vary, suggesting that the antioxidant enzyme system of *P. lividus* prevented oxidative damage from occurring (Amri et al., 2017; Ding et al., 2018).

5. Conclusion

This study assessed the reliability of sea urchins and macroalgae in the assessment of contamination in coastal ecosystems. The sea urchin gut appears to be a good bioindicator tool for assessing trace element levels. In this context, it would be interesting to perform complementary studies in order to understand the influence of several parameters such as the season and the food sources of the sea urchin. TEPI allowed identification of the different levels of contamination by highlighting the gradients of contamination in the old asbestos mine using sea urchins and macroalgae. TESVI enabled us to determine with efficiency the trace elements characteristic (Co, Cr, Fe, Ni) of the Albo site. The high levels of these trace elements in the different matrices are due to the discharges of the old mine swept along by the sea current and the geology of the region composed by serpentinites.

The contamination generated by the old asbestos mine causes oxidative stress very well regulated by the antioxidant mechanisms of the sea

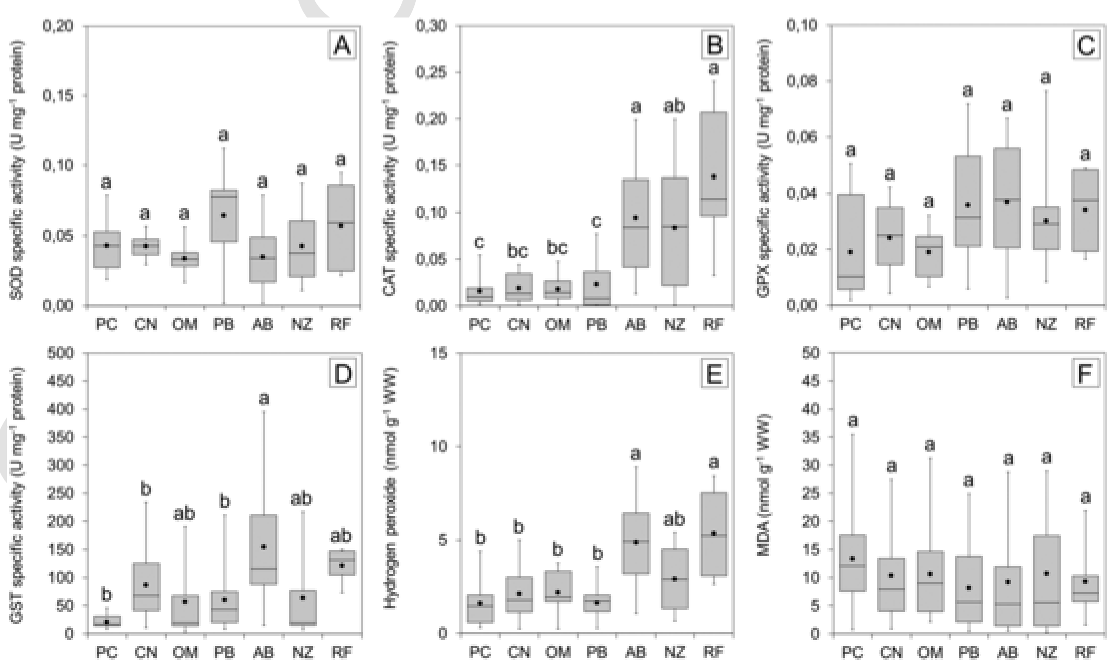

Fig. 6. Changes in specific activities of (A) superoxyde dismutase (SOD), (B) catalase (CAT), (C) glutathione peroxidase (GPX), (D) glutathione-S-transferase (GST) and changes in (E) hydrogen peroxide (H_2O_2) and (F) malondialdehyde (MDA) contents in gonads of *P. lividus* collected at seven sites (PC: Punta di Canelle; CN: Canelle; OM: old mine; PB: Punta Bianca; AB: Albo; NZ: Nonza; RF: reference site). Dissimilar letters denote significant differences between groups (p-value $<$ 0.05).

urchin. This study also highlighted the need for caution when interpreting biomarkers of stress under environmental conditions due to the different factors involved. Finally, this research shows that the effects of the old asbestos mine are still present and in order to prevent them, it is essential to stabilize the surrounding environment and soils through revegetation action and to maintain monitoring of the marine environment. Currently, a large-scale project to remediate and to rehabilitate the area is underway. A study of the effects of this project on the surrounding marine ecosystems would be relevant at short-term, because of the increased risk of contamination from this rehabilitation work, and in the long-term to monitor potential beneficial effects.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.161686.

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Bouchoucha et al., 2012
Duchaud, 2018

CRediT authorship contribution statement

O. El Idrissi : Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **S. Ternengo :** Writing – review & editing, Funding acquisition, Resources. **B. Monnier :** Formal analysis, Writing – original draft. **G. Lepoint :** Investigation. **A. Aiello :** Funding acquisition, Resources. **R. Bastien :** Investigation. **R. Lourkisti :** Investigation. **M. Bonnin :** Investigation. **J. Santini :** Writing – review & editing, Funding acquisition, Resources. **V. Pasqualini :** Writing – review & editing, Funding acquisition, Resources. **S. Gobert :** Writing – review & editing, Funding acquisition, Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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