Trace elements and arsenic speciation in Paracentrotus lividus from North-West Mediterranean Sea

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Abstract

Levels of 14 trace elements (Al, Ti, V, Cr, Mn, Co, Ni, Zn, As, Se, Cd, Sn, Hg and Pb) and 5 arsenic species (arsenite/As(III), arsenate/As(V), monomethylarsonic acid/MA, dimethylarsinic acid/DMA and arsenobetaine/AsB) were assessed in the gonads of sea urchin samples (*Paracentrotus lividus)* from North-West Mediterranean Sea (French coast). The samples were collected from 13 sites characterized by different types and levels of chemical contamination. Trace elements levels were measured by inductively coupled plasma-mass spectrometry (ICP-MS) following microwave (acid) digestion in a closed system, whereas As speciation analysis was carried out by ion-exchange liquid chromatography coupled with ICP-MS after microwave assisted extraction. High levels of trace elements were found in sea urchins sampled from Corsica whereas the samples from Théoule were found to be the least contaminated. From all the analyzed urchin samples, none showed Cd, Hg or Pb concentrations above the regulatory levels set by the European Community (EC) No. 1881/2006 for seafood or bivalve mollusks. Regardin arsenic specation, AsB was confirmed to be the predominant species. Inorganic As (As(III) + As(V)) was mainly constituted by As(III), which was quantified in all samples. Methylated As forms (MA and DMA) represent 9 to 23% of the total As.

Introduction

Seafood represents a substantial part of a balanced diet for humans as they are a source of proteins and omega-3 acids with beneficial effects for the human health (Çoğun et al. 2006, Dural et al. 2006, Copat et al. 2012, FAO 2016). Seafood consumption has been steadily increasing worldwide during the last 5 decades (FAO 2016). It is worth to highlight that seafood may also be an important source of toxic chemicals (Storelli 2008) as they are known to accumulate trace metals and metalloids (Pastorelli et al. 2012, Olmedo et al. 2013). So far, at European level, only maximum levels of Pb, Cd and Hg are regulated in several types of seafood (European Commission 2006), and no regulatory limits have been set for other toxic trace elements such as total arsenic and its species.

Thus, assessing human exposure to these elements via diet is very important. Several studies focused on the health risks related to the consumption of contaminated seafood along the Western Mediterranean basin in particular Warnau et al. (1998), Salvo et al. (2014), Scanu et al. (2015), Salvo et al. (2016), Ternengo et al. (2018), Camacho et al. (2018), Corrias et al. (2020). However, none of these works have studied the As speciation in seafood.

Our previous study highlighted significant Al, Ti and V contaminations of fish species from the Cassidaigne Canyon area; regarding inorganic arsenic, the results showed that the concentrations in fish collected close to the alumina refinery pipe were higher than those from the reference area (Bouchoucha et al. 2019).

The purple sea urchin, *Paracentrotus lividus* (Lamarck, 1816), is an echinoderm, wildly distributed within the Mediterranean and North-Eastern Atlantic in rocky intertidal and shallow subtidal habitats and in seagrass meadows (Boudouresque and Verlaque 2001). This species is consumed mainly in France, Spain and Italy (Le Direach 1987, Guidetti et al. 2004, Gianguzza et al. 2006, Fernández-Boán et al. 2013) but it has been or is currently exploited, mostly for exportation, over larger areas (Barnes and Crook 2001, Bertocci et al. 2014). Furthermore, because they are frequently found in shallow waters, edible sea urchins are also subject to recreational fishing (Gianguzza et al. 2006, Pais et al. 2007).

This study aims at the assessment of the contamination features of sea urchins (*Paracentrotus lividus)* samples from various sites from the from North-West Mediterranean Sea with 14 trace elements (TE) such as aluminium (Al), titanium (Ti), vanadium (V), chromium (Cr), manganese (Mn), cobalt (Co), nickel (Ni), zinc (Zn), arsenic (As), selenium (Se), cadmium (Cd), tin (Sn), mercury (Hg) and lead (Pb)) as well as 5 arsenic species (arsenite/As(III), arsenate/As(V), monomethylarsonic acid/MA, dimethylarsinic acid/DMA and arsenobetaine/AsB). The total content of the trace elements was measured by inductively coupled plasma-mass spectrometry (ICP-MS), whereas the speciation analysis of arsenite (AsIII), arsenate (AsV), monomethylarsonic acid (MA), dimethylarsinic acid (DMA) and arsenobetaine (AsB) was carried out by ion-exchange high performance liquid chromatography (HPLC) coupled to ICP-MS after microwave assisted extraction (both methods were accredited).

Material and methods

Reagents and chemicals

Ultrapure water (18.2 MΩ/cm, Millipore Milli-Q™, Merck Millipore, Saint Quentin en Yvelines, France) was used throughout the study. HNO_3 (67% m/m, Suprapur) and methanol (HPLC gradient grade) were purchased from VWR (Fontenay sous-Bois, France).

For elemental analysis, mono-elemental commercial solutions containing 1000 mg/L of each analyte (Al, Ti, V, Cr, Mn, Co, Ni, Zn, As, Se, Cd, Hg, Pb, Sc, Y, In, Re, and Bi) purchased from LGC Standards (Molsheim, France) were used to prepare multi-elemental intermediate standard solutions for calibration and internal standardization. Calibration standard solutions were prepared daily in 6% (v/v) HNO_3.

For speciation analysis, standard solutions of individual As species (1000 mg/L, as As) were prepared from the following substances: disodium methyl arsnonate hexahydrate (MA, ≥ 99.4%) (Greyhound chromatography, Birkenhead, UK); cacodylic acid (DMA, ≥ 98.0%) and arsenate heptahydrated (As(V), ≥ 98.5%) (Fisher Scientific, Illkirch, France); arsenic trioxide (AsIII, ≥ 99.5%) and arsenobetaine (AsB, ≥ 95.0%) (Sigma Aldrich, Saint-Quentin-Fallavier, France). A multi-species intermediate solution of As(III), As(V), MMA, DMA at 1.0 mg/L and AsB at 5 mg/L was prepared and stored in the dark at 4°C (during maximum two months) to prevent decomposition or oxidation. Calibration standard solutions were prepared daily from these stock solutions by appropriate dilution in ultra-pure water.

Several certified reference materials (CRMs) purchased from LGC Standards (Molesheim, France) were used throughout the study for the internal quality control (IQC), as follows: NIST SRM 1548a (Typical diet), NRC TORT-2 (Lobster hepatopancreas) and SRM 278k (Mussel tissue) for total element determination, and BCR 627 (Tuna fish) and NIST SRM 1566b (Oyster tissue) for As speciation analysis.

Instrumentation

Both sample digestion (for total trace elements determination) and extraction (for speciation analysis of As) were carried out using a closed microwave system (Multiwave 3000, Anton Paar, Les Ulis, France).

An ICP-MS (7700x type, Agilent, Les Ulis, France) equipped with a concentric nebulizer and an impact bead spray chamber at 3°C was used for total element determination. Kinetic energy discrimination (KED) mode using helium (He) as a collision gas was employed for the detection of the interfered elements (see Table 1).

For As speciation analysis, an iCAP Q ICP-MS (ThermoFisher Scientific, Les Ulis, France) equipped with a PFA standard nebulizer and a cyclonic quartz spray chamber was coupled to an ICS-5000$^+$ HPLC system (ThermoFisher Scientific). The ICP-MS was directly connected with the outlet of the HPLC column via a 0.18 mm i.d. PEEK tubing (80 cm length). The online separation of As species was carried out on a Dionex IonPac AS7 HPLC column (250 × 4 mm; 10-µm particles) and a Dionex IonPac AG7 guard column (50 × 4 mm; 10 µm particles) (both from ThermoFisher Scientific).

ICP-MS measurement conditions were optimised daily to maximise the sensitivity and minimise the interference effects from oxides formation; short term stability tests were performed in both detection modes (standard and collision mode). Data were processed using the Scientific MassHunter (Agilent Technologies) for total element determination and Qtegra software (ThermoFisher Scientific) for As speciation analysis.

The optimum operating conditions of ICP-MS solely (total element determination) and for HPLC-ICP-MS coupling (arsenic speciation analysis) are summarized in Table 1.

Sampling and samples preparation

Commercial-sized sea urchins (*Paracentrotus lividus*, body diameters > 5 cm) were collected in April 2017 by scuba divers (5–10 meters depth) from 13 sites along the North-West Mediterranean Sea: Porquerolles (Por), Port Cros (PC), la Ciotat (Cio), Calanques (Cal), Riou (Rio), Théoule (The), Couronne (Cou), Carry (Car), Frioul (Fri), les Embiez (Emb), Cap Garonne (CG), Canari (Can) and Bastia Nord (Bas) (Fig. 1). These areas were chosen due to the diversity of anthropogenic pressures they encounter. Por and PC, both located on small islands inside marine protected areas are considered as reference sites in this study. Cal and Rio were located in the vicinity of the industrial discharge of the Alteo Gardanne alumina refinery, one of the most important industrial discharge in the west Mediterranean (Dauvin 2010). From 1966 to 2015, the Gardanne refinery discharged millions of tons of bauxite residue (called red mud) through a pipe at 320 m depth in the Cassidaigne Canyon in the northwest French Mediterranean. These red muds contained high levels of TE, in particular titanium (Ti), vanadium (V), aluminum (Al), and to a lesser extent arsenic (As) (Dauvin 2010, Fontanier et al. 2012). Can is under the influence of the former asbestos mine of Canari (Corsica). Between 1948 and 1965, this mine discharged more than 11 millions of tons of serpentinite rubble directly into the sea (Bernier et al. 1997). Contamination by cobalt (Co), chromium (Cr) and nickel (Ni) has been reported in areas close to this mine (Andral et al. 2004, Lafabrie et al. 2007). Cou is located in the industrial zone of the Gulf of Fos. This Gulf hosts the most important commercial harbor of the Mediterranean. Fri is located on the Frioul Island, in the Marseilles Bay. Marseilles is the second largest French city. Finally, Cio, The, Car, Emb, CG and Bas represent intermediate human pressure levels.

At each site three samples were collected, each one being composed of 10 sea urchins (5 females and 5 males). Therefore, taking into account the 13 sampling sites, 39 urchin samples corresponding to 390 individuals were analyzed in this study. Samples were transported alive to the laboratory where the body diameter of each individual were measured and weighted after having been drained with several incisions around its Aristotle's lantern. After dissection, each individual was sexed and the gonads were extracted for further analysis taking into account that there are the mostly consumed part of the sea urchin. Samples were homogenized using a stainless steel free mixer and then stored at -20°C before analysis.

Analytical procedures

Determination of total trace element levels by ICP-MS

The levels of Al, Ti, V, Cr, Mn, Co, Ni, Zn, As, Se, Cd, Hg and Pb were measured by ICP-MS using an accredited method (French Accreditation Comitee - COFRAC) described elsewhere (Chevallier et al. 2015). Briefly, ~ 0.6 g of sample were digested with a mixture of 3 mL of nitric acid (HNO_3) 67% (m/m) and 3 mL of ultrapure water using a closed microwave digestion system. After cooling, the sample digests were quantitatively transferred into 50 mL calibrated polypropylene tubes. A solution of a mixture of internal standards (scandium (Sc), indium (In), bismuth (Bi), rhenium (Re), and yttrium (Y)) was added to reach a final concentration of 2 µg/L and the digest was diluted to 50 mL with ultrapure water before ICP-MS analysis.

Speciation analysis of arsenic by HPLC-ICP-MS

Speciation analysis of As was carried out using an accredited method (Leufroy et al. 2011) based on HPLC-ICP-MS. Briefly, ≅ 0.15 g of frieze-dried sample were prone to extraction with 10 mL of ultrapure water at 80°C for 6 min in a closed-microwave system. The extracts were 25-fold diluted with ultrapure water and then filtrated using 0.45 µm polyvinylidene fluoride (PVDF) syringe filters. Five arsenic species, namely As(III), As(V), MA, DMA, and AsB were separated using an ion exchange HPLC column and a mobile phase consisting of a mixture of HNO_3 and methanol at 1% (v/v) using a pH gradient (variation of pH from 3.3 to 1.3) as shown in the Table 1. The limits of detection (LOD) and quantification (LOQ) were respectively 0.003 mg/kg and 0.006 mg/kg for each As species.

Internal quality control

Several internal quality controls (IQCs) were used to ensure the data reliability. Each run included a calibration curve to check the linearity ($r^2 \geq 0.995$), blanks solutions to monitor the eventual cross-contamination or memory effects (blank values < LOQ), CRMs and/or spiked samples to assess the trueness (100 ± $3 \times CV_R$ % of the theoretical value, where CV_R is the relative standard deviation/RSD reflecting the laboratory intermediate reproducibility) and duplicate samples to check the repeatability (RSD ≤ 20% when mean ≥ 5×LOQ and RSD ≤ 40% when LOQ ≤ mean value < 5×LOQ). A mix solution containing each analyte/species at the LOQ level was also analyzed daily to assess data reliability at ultra-trace levels (100 ± $3 \times CV_R$ % of the theoretical value); a mid-range standard was also analyzed every ten samples (and at the end of the sequence) to monitor the instrumental drift (100 ± 20% of the theoretical value).

Uncertainty calculation

The expanded uncertainty (U, k = 2) associated to each measurement in this study was assessed as the standard deviation characterizing the intermediate precision (CV_R) during a time span of approximately 3 months, as reported elsewhere (Chevallier et al. 2015, Ghosn et al. 2019).

Food safety thresholds

Among all trace elements measured in this study, the European Commission (EC) have fixed maximum levels (MLs) only for Hg, Pb and Cd in various types of seafood. In urchin gonads, only Hg is regulated with a ML of 0.5 mg/kg wet weight (ww) (European Commission 2006). Nevertheless, the MLs for Pb (1.50 mg/kg) and Cd (1.0 mg/kg) in bivalve mollusks were used in this study for comparison purposes.

Only two countries apply regulatory limits for the presence of inorganic As (iAs) in seafood. China established maximum levels for iAs in aquatic animals (0.5 mg/kg) and fish (0.1 mg/kg) (Ministry of Health of the People's Republic of China 2012) and Australia and New Zeeland have set MLs to fish and crustaceans (2 mg/kg) and to molluscs (1 mg/kg). In the European Union such regulation is not yet implemented.

Statistical analysis

A Kruskal Wallis test was performed on body diameter data in order to test its spatial variation and a post-hoc tests according to Dunn for pairwise multiple comparisons of the ranked data including the Bonferroni correction in which the p-values are corrected by the number of comparisons to prevent the inflation of type I error rates. Principal component analysis and a hierarchical cluster analysis were carried out on the element concentration and sample matrix (element concentration as columns and individual as rows) in order to describe the ordination of the sites. All statistical analyses were performed to the error threshold of 5% in the statistical environment R (R Development Core Team; www.r-project.org).

Pearson correlation tests to check for significant relationships between TE in the analyzed samples were performed with StatGraphics Centurion XVII (version17.1.04) (probability level of 0.05). Left censored data (< LOD or < LOQ) were excluded from statistical analysis.

Results and discussion

Distribution of the urchins' size amongst the sampling sites

The urchins with the smallest body diameter (5.0-6.4 cm) were collected from les Embiez while the largest specimens (6.4–7.2 cm) were sampled from Riou (see Fig. 2).

The Krustal Wallis test confirmed this spatial variation of body diameter (χ^2=33.37, df = 12, p-value = 0.001). The post-hoc test indicated significant smaller sea-urchins from les Embiez and Cap Garonne than sea-urchin from Riou. As contamination levels in marine organisms is driven both by extrinsic and intrinsic factors, variation in body diameter can induce variation in TE accumulation in gonads and should therefore be taken into account in the interpretations of the results.

Occurrence data of total trace elements

The individual (total) levels of As, Pb, Cd, and Hg, and Mn, Ni, Se, Ti, V, Al, Co, Cr, and Zn measured in the urchin gonad samples of this study associated with their uncertainty are shown in Table 2**and Supplementary material**, respectively. The levels of Sn are not reported here because all values were < LOD. Mean concentrations discussed in the text are expressed with standard deviation (SD, n = 3).

Arsenic

Among the 39 studied samples, As was found at a mean level of 5.37 ± 2.01 mg/kg. The lower mean level was measured in samples from Théoule (2.90 ± 0.27 mg/kg), whereas the higher mean levels were found in Canari (8.47 ± 0.49 mg/kg), followed by Port Cros and Porquerolles (8.41 ± 0.82 mg/kg and 8.22 ± 0.73 mg/kg respectively). Similar As levels were measured by Salvo et al. (2016) in urchins samples collected in France. The values obtained in our study are lower than those reported by Scanu et al. (2015) in *Paracentrotus lividus* from northern Tyrrhenian Sea. However, the geographical distribution of arsenic contamination observed here is different from that obtained for other seafood species. Indeed, As concentration in mussels from measured by Andral et al. (2004) are twice as high in Cal and Rio than in Can. This reflects different trace accumulation processes between marine organisms.

Aluminum

Aluminum was found at an average level of 10.9 ± 8.3 mg/kg (all samples). The highest mean levels were found in Bastia Nord with 29.6 ± 5.9 mg/kg followed by Les Embiez (19.4 ± 6.3 mg/kg) and Cap Garonne (18.4 ± 10.3 mg/kg). Similar levels have previously been measured in Corsica as reported by Ternengo et al. (2018). The lowest mean level was measured in Port Cros (1.26 ± 0.14 mg/kg). The measured Al values are in good accordance with the values reported by Corrias et al. (2020) in Sardinia and El Idrissi et al. (2020) in Corsica.

Cadmium

Cadmium was measured at a mean level of 0.047 ± 0.022 mg/kg in the different areas. The highest mean concentrations were found in Calanques (0.082 ± 0.020 mg/kg) and Riou (0.082 ± 0.010 mg/kg), followed by Frioul (0.065 ± 0.010 mg/kg) and Canari (0.064 ± 0.007 mg/kg). The lowest mean level was found in Théoule (0.012 ± 0.001 mg/kg). Similar levels were reported in *Paracentrotus lividus* from different areas in NW Mediterranean sea in other similar studies (Warnau et al. 1998, Ternengo et al. 2018, Corrias et al. 2020, El Idrissi et al. 2020) and in northern Tyrrhenian Sea (Scanu et al. 2015). Rouane-Hacene et al. (2018) reported higher mean level in urchins collected from the Algerian coasts, whereas Salvo et al. (2016) reported higher level in urchins from France, but similar levels for urchins from Morocco and Spain. From all the analyzed samples, Cd concentrations were well below the maximum level of 1.0 mg/kg imposed by the EC Regulation in bivalve mollusks (EU 1881/2006).

Chromium

The mean Cr concentration was 0.182 ± 0.248 mg/kg. The highest mean levels were found in Corsica (0.928 ± 0.460 mg/kg in Canari and 0.253 ± 0.032 mg/kg in Bastia Nord). These values are very close to those reported by other studies (Ternengo et al. 2018, El Idrissi et al. 2020) in several sites of Corsica and consistent with what is measured in mussels (Andral et al. 2004). The high concentrations of Cr but also Ni and Co (see here after) observed in northern Corsica are related on one hand to the presence of the Canari asbestos mine and on the other hand to the geochemical nature of the bedrock in Cap Corse. The lowest concentrations were found in the samples from Théoule with an average of 0.057 ± 0.018 mg/kg. Values measured in the other stations are in good agreement with those reported by Salvo et al. (2016) and Scanu et al. (2015) in Mediterranean Sea but lower than those measured by Warnau et al. (1998) in Marseille and higher than those reported by Corrias et al. (2020) in Sardinia.

Cobalt

Cobalt was found at a mean level of 0.066 ± 0.034 mg/kg. The highest levels were measured in Corsica with average values of 0.150 ± 0.00017 mg/kg in Canari and 0.102 ± 0.016 mg/kg in Bastia Nord.

These results are very close to those reported by El Idrissi et al. (2020) and Ternengo et al. (2018) in specimen sampled in Saint Florent station, close to the asbestos mine of Canari. In contrast, Corrias et al. (2020) did not quantified Co in *Paracentrotus lividus* sample in Sardinia, in the south of Corsica. In the other sampling station, Co mean level ranged from 0.022 ± 0.003 mg/kg in Théoule to 0.077 ± 0.008 mg/kg in Riou.

Lead

The mean level measured for Pb (0.050 ± 0.033 mg/kg) in the different sampling sites ranged from 0.017 ± 0.003 mg/kg in Port Cros and Canari to 0.107 ± 0.006 mg/kg in Couronne. This site is located in the industrial zone of the Gulf of Fos, hosting the most important commercial harbor in the Mediterranean, which is also one of the most contaminated areas of the French Mediterranean coast, in particular by Pb (Benon et al. 1978). These results are in good agreement with those published by other authors on *Paracentrotus lividus* in Mediterranean sea (Scanu et al. 2015, Salvo et al. 2016, Ternengo et al. 2018, Corrias et al. 2020, El Idrissi et al. 2020).

In the 39 analyzed samples, Pb levels were below the maximum value of 1.50 mg/kg (ww) set out in the Regulation (CE) 1881/2006 for bivalve mollusks.

Manganese

Manganese was measured at a total mean level of 0.404 ± 0.157 mg/kg. The highest individual mean levels were found in Bastia Nord (0.818 ± 0.032 mg/kg) and the lowest levels in Théoule (0.243 ± 0.017 mg/kg). Corrias et al. (2020) did not quantify Mn in urchin samples from Sardinia whereas El Idrissi et al. (2020) and Ternengo et al. (2018) reported similar levels in Corsica.

Mercury

Hg was quantified only in 26 out of the 39 samples analyzed in this study at a mean level of 0.015 ± 0.006 mg/kg. The highest mean Hg levels were found in Couronne (0.029 ± 0.004 mg/kg) followed by Riou (0.018 ± 0.002 mg/kg). The concentrations for all samples were well below the ML of 0.5 mg/kg defined by the European Commission (EC 1881/2006) relative to the average Hg content in edible parts of fishery products. Salvo et al. (2014) and Corrias et al. (2020) reported similar Hg levels in urchins collected in Sardinia and Sicilia, but Salvo et al. (2016) found higher levels (up to 3.7 mg/kg) in urchins collected in France (with no precise information about the exact sampling site).

Nickel

Nickel was quantified in most of the samples except for one sample in Théoule; the mean individual levels ranged from 0.052 ± 0.000 mg/kg in Théoule up to 0.727 ± 0.374 mg/kg in Canari. Ternengo et al. (2018) and El Idrissi et al. (2020) reported similar Ni levels in urchins collected near Canari. Our results are in good agreement with those published by Corrias et al. (2020), Salvo et al. (2016) and Scanu et al. (2015) in Mediterranean Sea.

Selenium

Se was found at a mean level of 0.394 ± 0.077 mg/kg with a minimum mean level of 0.252 ± 0.035 mg/kg measured in La Ciotat and a maximum mean level of measured in Riou (0.465 ± 0.083 mg/kg). Similar levels were reported by El Idrissi et al. (2020) and Ternengo et al. (2018) in urchins from Corsica and by Camacho et al. (2018) in urchin bought in French market, whereas Corrias et al. (2020) reported non quantified results in urchins from Sardinia.

Titanium

The mean concentration of Ti from all areas was 0.399 ± 0.438 mg/kg. The mean values ranged from 0.078 ± 0.025 mg/kg in Port Cros to 1.70 ± 0.574 mg/kg in Bastia Nord. Warnau et al. (1998) reported lower levels in gonads from specimen collected in North-West Mediterranean Sea.

Vanadium

The mean level measured for V was 0.544 ± 0.164 mg/kg with a minimum mean value of 0.237 ± 0.053 mg/kg measured in Théoule and a maximum of 0.722 ± 0.220 mg/kg measured in Port Cros. Salvo et al. (2016) found higher levels in urchins from France (with no indication about the sampling site) and similar levels in Sardinia, whereas Corrias et al. (2020) found lower levels in urchins from Sardinia.

Zinc

The mean level measured for Zn was 28.1 ± 15.1 mg/kg. The highest levels were found in Couronne (54.0 ± 24.0 mg/kg), followed by La Ciotat (48.6 ± 34.4 mg/kg) whereas the lower level was found in Les Embiez (20.9 ± 2.2 mg/kg). It is worth to emphasize that Zn levels measured in our study are significantly higher than those published by Corrias et al. (2020) in Sardinia but agree with those reported by El Idrissi et al. (2020) and Ternengo et al. (2018) in Corsica and Warnau et al. (1998) in Marseille, Corsica and Ischia. Rouane-Hacene et al. (2018) found similar levels in urchins from Algerian West cost in the South of Mediterranean Sea.

Principal component analysis of total trace elements data

The output of PCA for the trace elements in gonad tissues for *Paracentrotus lividus* samples investigated in this study is shown in Fig. 3. The first and second axes of the PCA explained 76.3% and 22.6% of the total element concentration matrix variation.

The first axis clearly separated samples with the highest Zn concentration in gonad such as one sample from Cio (88.1 ± 35.2 mg/kg) and two samples from Cou (78.0 ± 31.2 mg/kg and 54.2 ± 21.7 mg/kg). On the second axis, samples from Bas and Emb were clearly separated from the other samples because theses samples presented the highest Al concentration in gonad tissues (Al concentration range 20.76–36.41 mg/kg). The three groups were corroborated by the result of the clustering analysis (Fig. 4).

Therefore, the multivariate analysis based on element concentrations has not allowed to demonstrate the influence of the industrial effluent on element content in gonads for *Paracentrotus lividus.*

Correlations between trace elements levels

Regarding the relationship between TE contents and urchin size (diameter), a moderate correlation was observed for Al, Cd, Ni and V with correlation coefficients of r = 0.56; 0.59; 058 and 0.48 respectively, indicating accumulation of these metals in urchin.

The Pearson correlation test showed a significant positive correlation between total TE contents in the urchins gonads analyzed mainly for Co, Cd, Cr, Ni and V (Fig. 5). This indicates that the behavior of these TE in terms of bioavailability and accumulation in sea urchins were similar, and suggesting the same origins, probably from anthropogenic sources (Hossain et al. 2022).

Arsenic speciation

Arsenic speciation analysis was performed on a selection of 18 over the 39 samples of the study. The samples were chosen according to their sampling station, so as to include all types of area: 2 samples from the impacted area, 1 sample from the non-impacted area, 1 sample in a reference station, and 2 samples from a protected area. The concentrations of As(III), MA, DMA, As(V) and AsB in the urchin samples in the 6 sampling sites are provided in Table 3.

As(III) species was quantified in all the samples of our study within a concentration range of 0.217 ± 0.069–1.02 ± 0.33 mg/kg (ww), whereas As(V) was quantified in only 5 samples at ultra-trace levels (0.007 ± 0.001 to 0.020 ± 0.004 mg/kg). The highest levels of iAs (As(III) + As(V)) were measured in Port Cros and Porquerolles with a mean level of 0.707 ± 0.365 mg/kg and 0.537 ± 0.148 mg/kg respectively, whereas the lower mean level was observed in Riou (0.226 ± 0.014 mg/kg). These values are significantly higher than those reported by Camacho et al. (2018) in urchin from North-West Atlantic and very similar with those published by Sirot et al. (2009) in the French Calipso study (no indication regarding the sampling location).

AsB was the predominant species in the analyzed samples, with levels ranging between 1.28 ± 0.28 mg/kg and 4.42 ± 0.97 mg/kg, representing 17 to 51% of the total As. It must be noted that the measured fractions of AsB in the analyzed urchins are lower than those commonly found in marine (fatty) fish (Hackethal et al. 2021) and shellfish (Liu et al. 2022). However, our results are in good agreement with those reported by Nawrocka et al. (2022) in bivalve mollusks.

The methylated As species such MA and DMA represent together 9–23% of the total As, as also observed by Hackethal et al. (2021), Liu et al. (2022), Nawrocka et al. (2022) in different bivalve mollusks, with DMA in majority compared to MA (74 to 96% of the MA + DMA level). Among the different sampling sites, the lower concentrations of the different As species were measured in Riou, except for MA.

Several unknown As species were also detected in the urchin samples analyzed in this study. According to the herbivore feeding behavior of *Paracentotus lividus*, it is assumed that the unknown species detected in the samples are arsenosugars.

The ratios between the sum of the levels of identified As species and the total As level (refereed here as extraction efficiency, EE) are also reported in Table 3. Low EE (0.49–0.78) indicates that a fraction of As is not extracted. Similar results have previously been observed in Atlantic salmon, tuna, herring, pelagic and oily fishes (Lischka et al. 2013, Hackethal et al. 2021, Nawrocka et al. 2022), suggesting that the non-extractable compounds in our study are arsenolipids.

Conclusions

This study reports the levels of 14 potentially trace elements as well as As speciation (five species) in a selection of urchin samples from different sites in the North West Mediterranean Sea. The results showed that the level of Pb, Cd and Hg were lower than the maximum levels set by the EC in seafood or bivalve mollusks. Moreover, the measured levels were very similar to those reported in other studies in the Mediterranean Sea. It is also worth noting that the obtained results showed that the urchin samples collected in the impacted areas were not more contaminated than those collected in non-impacted areas. The correlations observed between the potentially trace elements indicate similar types of contamination from the same source, and same behavior in the aquatic system among the different sampling areas.

Declarations

Conflict of Interest

The authors have declared no conflict of interest.

Ethical Approval

Not applicable

Consent to Participate

All authors gave explicit consent to participate.

Consent to Publish

All authors gave explicit consent to submit.

Authors Contributions

Author	CONTRIBUTION									
	Conceptualization	Data curation	Formal analysis	Funding acquisition	Investigation	Methodology	Project administration	Resources	Supervision	V
Axelle Leufroy	X	X	X			X				X
Marc Bouchoucha	X	X		X	X	X	X	X		X
Gilles Riviere	X				X		X			X
Thierry Guérin					X		X			X
Petru Jitaru	X			X	X		X	X	X	X

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Competing Interests

The authors have no competing interests to declare that are relevant to the content of this article.

Availability of data and materials

Raw data and derived data supporting the findings of this study are available from the corresponding author on request.

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Tables

Tables 1-3 is available in the Supplementary Files section.

Figures

Figure 1

Map showing the sampling sites in French coast (North West Mediterranean Sea)

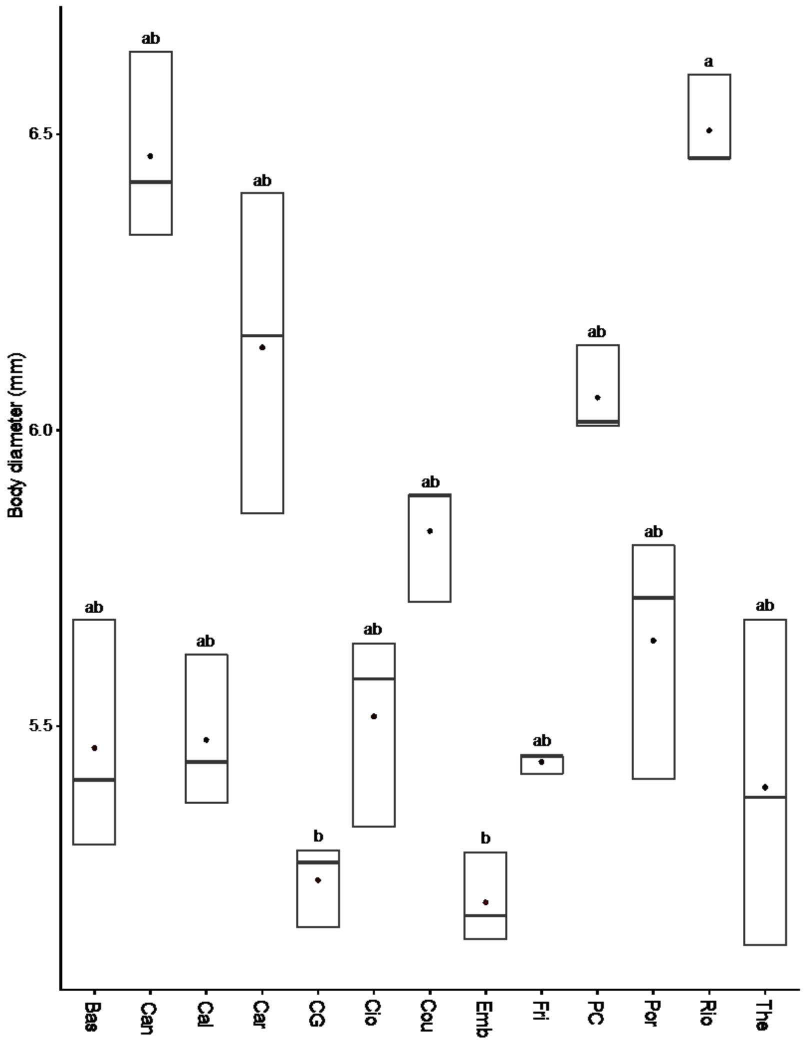

Figure 2

Spatial variation of body diameter for Paracentrotus lividus sampled from the North-West Mediterranean Sea. Boxplot: the bottom and top of the boxes are the first and the third quartiles of the data distribution, the median is drawn as a horizontal line and the mean value as a point. Letters indicate significant differences ($p < 0.05$) between sites.

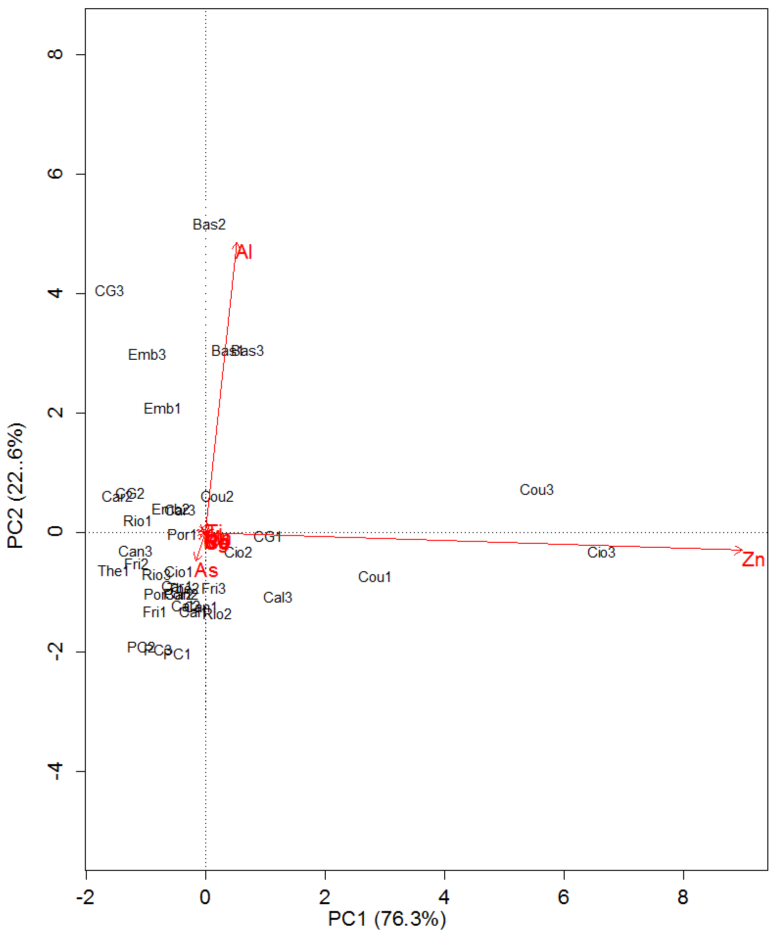

Figure 3

Plot of the first two axes of the PCA performed on element concentration in gonad tissue for Paracentrotus lividusfrom North-West Mediterranean Sea.

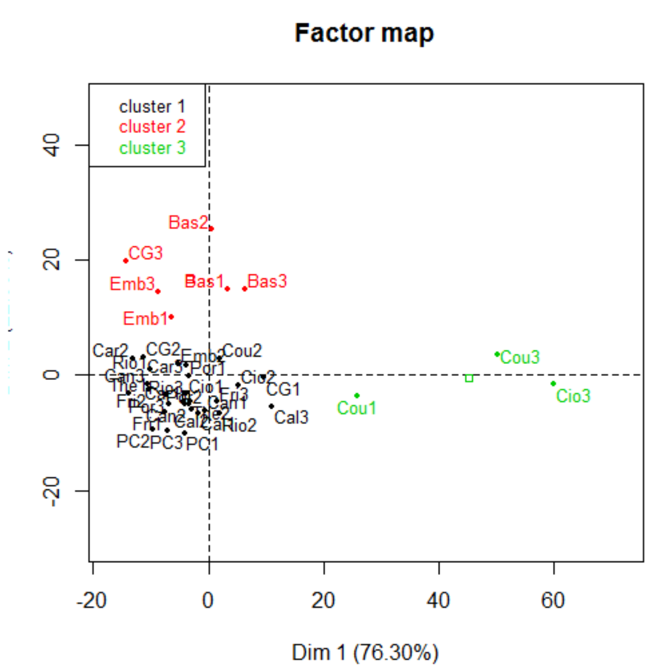

Figure 4

Sampled stations categorized in three groups resulting from the clustering analysis performed on element concentrations in gonad tissue for Paracentrotus lividus from North-West Mediterranean Sea.

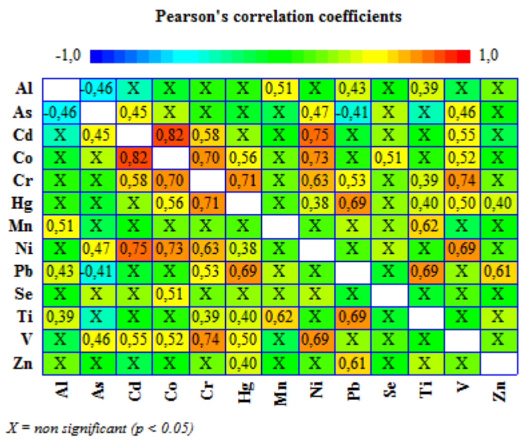

Figure 5

Pearson correlation matrix of TE contents in sea urchin gonads.

Supplementary Files

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