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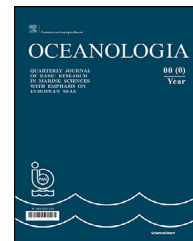
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ORIGINAL RESEARCH ARTICLE

First survey of metallic distribution in zooplankton from a south Moroccan area

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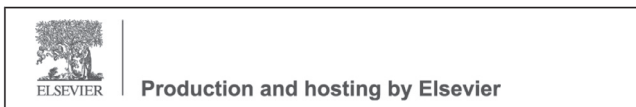
Zooplankton;
Metals;
Southern Atlantic
coast of Morocco;
Upwelling;
EPURE cruises

Abstract The Moroccan Southern Atlantic coast is mainly influenced by upwelling, Saharan dust and anthropogenic micropollutant input. These factors contribute to increasing the availability of metal elements in waters. To differentiate human impact from natural variability, knowledge of background concentrations of metals and their fluctuations in bioindicator organisms such as zooplankton is important. This work aims to determine the levels of metals elements (Zn, Mn, Pb, Cu, Cd, Cr, Co, Ni, Li, As, Sr, U, Fe and Ba) in zooplankton along the southern area of the Atlantic coast of Morocco. Zooplankton samples were collected in the summer (July 2013) and autumn (December 2013) at 27 stations from Sidi Ifni to the south of Dakhla. All stations were located on transects perpendicular to the coast. The analysis of metal elements in zooplankton was determined by ICP–MS. The results revealed that in all transects, metal concentrations were below the regulatory limits. Metal enrichments were observed in the south and decreased gradually to the north. This study can be used as baseline data for the metal contents of zooplankton in Moroccan South Atlantic coastal water. A comparison to

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worldwide reported data on zooplankton did not reveal any suggestions on increased metal presence in the area investigated.

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1. Introduction

In marine environments, metals have received considerable attention due to their toxicity and accumulation in biota. Metallic elements exist naturally in the marine environment, but they are further amplified by anthropogenic and industrial inputs, including planting N-fixing crops, fertilizer production, and wastewater disposal. The irreversibility of this pollution is of particular concern, because it is impossible to recover these metals once dissipated in nature. Despite the fact that metals are natural elements in the environment, above a certain threshold of bioavailability, they become toxic (Kucuksezgin et al., 2006). Unlike organic pollutants, metals are not metabolized; they can be transferred through the food chain and then accumulate in living matter. Metallic element contents in seawater vary over time, depending on the variability of the pollution flow and physical parameters such as the temperature, ocean roughness, flow rates and biological effects (Gaudry et al., 2007).

Metallic accumulation by zooplankton is especially achieved either by direct adsorption from water or by absorption from food and detritus. Zooplankton are a very important compartment in the processes of metal bioavailability in coastal waters. In particular, they are a major source of food for marine species, confirming their role in the transfer of metals to higher trophic levels (Robin et al., 2012). Plankton play an important role in the vertical transport of elements in pelagic surface waters; the flux of organic compounds forms a large part of the vertical flux and then influences the residence periods of metal elements adsorbed to particles in the sea (Fisher et al., 1991). Zooplankton are vital to the functioning of ocean food webs due to their sheer abundance and important ecosystem roles (Battuello et al., 2016).

Zooplankton's position as an "intermediary" in the trophic chain of aquatic ecosystems, with a major role in the transfer of material between primary producers and other higher levels, means that these organisms can be good indicators of the general conditions under which aquatic ecosystems function. Accordingly, several studies have successfully used macro- and mesozooplankton organisms as bioindicators. They are applied to assess the bioavailability of metal elements in marine ecosystems at different spatial and temporal scales (Achary et al., 2020; Battuello et al., 2016; Fang et al., 2006; Hsiao et al., 2011; Kahle and Zauke, 2003; Nair et al., 1999; Pempkowiak et al., 2006). Their utility is due to their presence on a global scale, their high contribution to the total biomass of marine systems and their major role in food chains.

The assessment of anthropogenic disturbance by zooplankton has been studied by researchers worldwide; however, information on the quality of zooplankton in aquatic

environments along African coasts is limited, including the Moroccan Atlantic coast, which is one of the richest coasts in terms of exploitable biological resources. Indeed, the south Moroccan coastline is characterized by the upwelling of deep waters, which ensures a supply of nutrients contributing to the production of the entire trophic food chain (Makaoui et al., 2020), as well as local contamination by metal elements, especially Cd in bivalve mollusks and in the liver tissue of pelagic fish. Afandi et al. (2018) and Benbrahim et al. (2006) attributed this finding to anthropogenic sources (phosphate industry) and to natural sources (upwelling activities, showing cadmium contamination). To date, no previous studies assessing the concentration of metals in zooplankton carried out in the southern zone of the Atlantic coast of Morocco are available. To fill this gap in this region of the world, we studied the levels of fourteen metallic elements in zooplankton in the lower and upper mixed layers. This study was conducted along nine transects perpendicular to the coast.

The objectives of this study are as follows: (1) Determine the quality of the coastal marine environment below the baseline metal concentrations for the first time in zooplankton samples from the southern zone of the Atlantic coast of Morocco; (2) Differentiating input from human influence versus natural variance; (3) Determine whether the concentrations of metals differ significantly during the season of high or low upwelling; (4) Determine vertical and diurnal variation in metal accumulation in zooplankton; and (5) Development of a new database of metal levels.

2. Material and methods

2.1. Study area

The study area is located in the eastern limit of the North Atlantic subtropical gyre (Figure 1). The southern Moroccan zone between 20° and 25°N is characterized by variable permanent upwelling with intense activity in summer. This zone is limited in the north and south by seasonal upwelling systems in summer and winter, respectively, with an inter-annual change (Larissi et al., 2013). It is generally in the 0–100 km coastal band that vertical upwelling-induced movements occur, as estimated from the Rossby deformation radius in northwest Africa (Chelton et al., 1998) cited by Auger et al. (2015); therefore, the coastal jet is limited near the coast (Waeles et al., 2016). In addition, the inter-annual variability in the upwelling index between 2002 and 2010 deviated from the infrared thermal imaging of the Modis-Aqua sensor along the Moroccan upwelling zone (21–33°N), indicating an evident oscillation over the years (Larissi et al., 2013). This study is particularly valuable be-

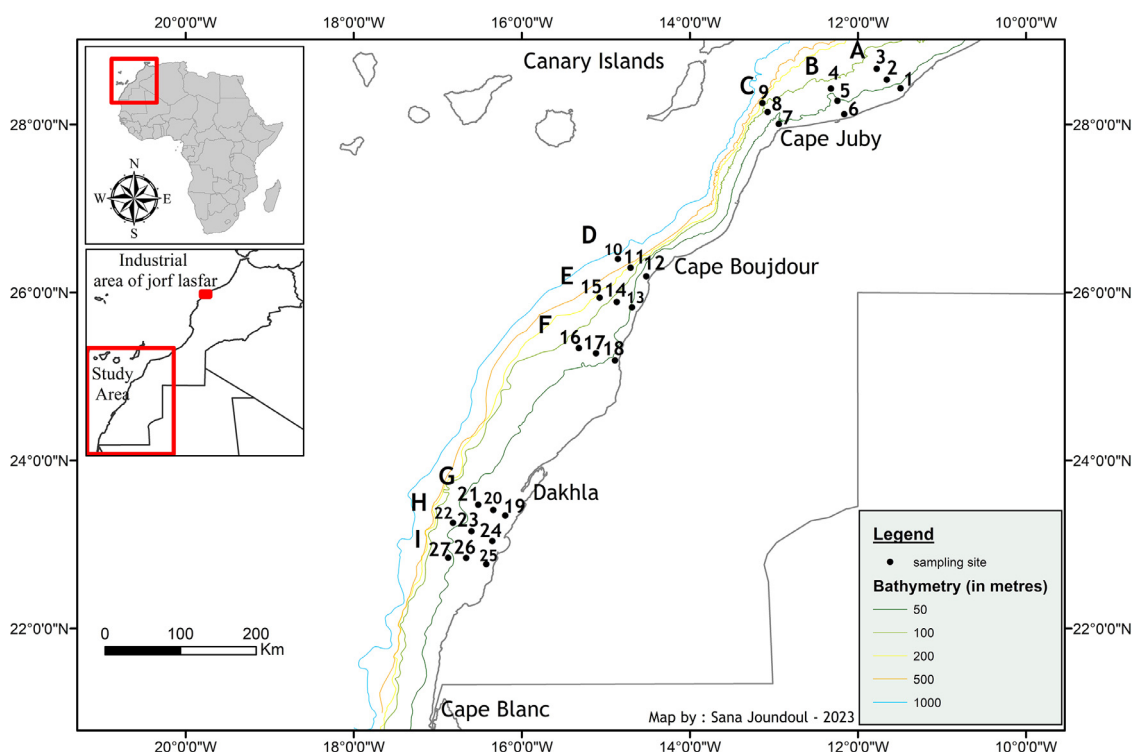


Figure 1 Locations of sampling sites (black dots) and transect definitions (capital letters) along the south Atlantic Moroccan coast, where zooplankton samples were collected during the cruise 2 and 4 aboard the *r/v Antea*.

cause this area of the Canary Current ecosystem represents a considerable fishery potential (Tito de Morais, 2013).

2.2. Sampling and sample preparation

During the EPURE cruises (cruise 2: from 11 to 31 July 2013 and cruise 4: from 21 November to 09 December 2013, *r/v Antea*), 27 hydro-biological stations were sampled. Zooplankton samples were collected using nets with a mesh size of 150 microns. The sampling strategy closely followed the Moroccan coast, integrating different areas of Moroccan upwelling. Specifically, three zones known for their coastal upwelling strength, due to their hydrobiological and bathymetric characteristics, were selected: north of Cape Juby, south of Cape Boujdour and south of Dakhla. In each zone, three sections were conducted (Figure 1), with a total of 111 zooplankton samples, of which 57 were collected in cruise 2 and 54 samples in cruise 4. At each station, samples were collected during the day and night, further 2 levels were typically sampled for 5 min., usually one near the bottom and one in the mixed layer.

The zooplankton samples were passed through a small nylon sieve, briefly rinsed after catching with ultrapure water to remove salts and frozen at -20°C . Subsequently, in the laboratory, samples were freeze-dried for 72 hours at -55°C , homogenized by mixing in rotating glass and stored in glass bottles in vacuum desiccators.

2.3. Analytical procedures

Prior to the use for digestion, all glassware and vessels were cleaned with special detergent (Micro 90), kept cov-

ered with HNO_3 (10%) for at least 24 h and rinsed 4 to 5 times with deionized water. To avoid any contamination, vessels were tested individually for blanks and screened before use. Mineralization was carried out in Teflon digestion vessels according to the Association of Official Analytical Chemists (AOAC Official Method 999.10, AOAC, 2002). Zooplankton samples were first mixed, and approximately 0.25 g of zooplankton samples were digested for 30 min. in a microwave oven at 190°C with 5 ml of HNO_3 (65% Merck-Suprapur Grade) and 2 ml of H_2O_2 (30% Fluka). The digested solutions were successively diluted to 50 ml with deionized water.

All elements analyses (Zn, Mn, Pb, Cu, Cd, Cr, Co, Ni, Li, As, Sr, U, Fe and Ba) were performed in an Ocean Spectrometry Pole at LEMAR by means of a magnetic sector inductively coupled plasma mass spectrometer (ELEMENT 2, Thermo) with low- and medium-resolution settings as described in Afandi et al. (2018).

The stock standard solution was prepared gravimetrically from primary standards. An external 6-point standard curve was prepared by serial dilution and analyzed at the beginning, middle and end of each run. We used indium (In) as an internal standard ($\sim 1 \mu\text{g L}^{-1}$, as run in the final solution) for all samples, standards and blank solutions. Final concentrations of samples and procedural blanks were calculated from In-normalized data. All results were expressed in $\mu\text{g g}^{-1}$ dry weight (dw). Precision was estimated through replicated samples (every 10th sample was a replica) and was better than 10% in all cases. A quality control program was performed, including treatment and analysis of Certified Reference Material (the CRM was fish protein DORM-4 from the National Research Council, Canada) (Table 1) and blanks with the samples.

Table 1 Limit of quantification and accuracy of standard (DORM-4) analyzed according to the procedure used for zooplankton metal analysis ($\mu\text{g g}^{-1}\text{dw}$).

CRM Dorm-4	Li	Cd	Cu	Mn	Cr	Co	Fe	Pb	Sr	As	Ni	Zn	U
LQ	5.40 10^{-04}	1.20 10^{-04}	3.03 10^{-03}	9.30 10^{-04}	3.80 10^{-04}	8.10 10^{-05}	0.173	2.99 10^{-03}	4.80 10^{-03}	1.50 10^{-04}	6.54 10^{-03}	4.20 10^{-03}	2.22 10^{-04}
Assigned value	–	0.306 ± 0.015	15.9 ± 0.9	–	1.87 ± 0.16	–	341 ± 27	0.416 ± 0.053	–	6.80 ± 0.64	1.36 ± 0.22	52.2 ± 3.2	–
Measured value	1.09	0.32 ± 0.013	15.93 ± 0.25	2.88	1.86 ± 0.026	0.263	357.58 ± 15.10	0.37 ± 0.017	9.275	6.86 ± 0.06	1.25 ± 0.046	49.61 ± 2.48	0.034

2.4. Statistical procedures

All data were subjected to statistical analysis. One-way analysis of variance (ANOVA) was used to determine the significant differences in metal levels during the two cruises in the three areas, by radials, by day/night and by in-shore/offshore. All statistical calculations were performed with XLSTAT for Windows (Version 2015.1.03.15945). Statistical significance was defined at 95% ($p < 0.05$). A Principle Component Analysis (PCA) is performed on standardized data, which is intended to classify the different groups of metals with the same origin.

3. Results

The vertical distribution of physico-chemical parameters along the nine inshore-offshore sections was explained by Waeles et al. (2016), with strong upwelling signatures observed in the northern and southern areas; in the central area, biological activity was less intense, as reflected in the fluorescence concentrations.

Zooplankton collections were examined to determine the biological composition. Copepods were the most dominant (60–98%), and their composition was dominated by *Acartia*, *Centropages*, *Euterpina*, *Oncaea* and *Paracalamus* species in summer and autumn seasons from all nine transect stations. Total density was considerably higher in the south than in the north, with minor variations between day and night. The highest total density was at Station 27 level 5 (night), with 18,158 ind m^{-3} in contrast with total biomass with the highest value at Station 22 level 5 (day), while the lowest density was recorded at Station 15 level 5 (night) with a total density of 10.66 ind m^{-3} and lower total biomass in the same station 15 level 2 (day).

3.1. Metal distribution and concentrations in zooplankton

In the present study, the mean metal concentrations in total zooplankton are given in Tables 2 and 3. The average metal concentrations in the analyzed zooplankton samples were arranged in the following order: Sr > Fe > Zn > Ba > Li > Mn > Cr > As > Cu > Cd > Ni > Pb > Co > U. However, it should be noted that the metal ranges were extremely large (Table 2 and 3). According to the metal concentration in zooplankton, the metals were divided into four classes: (Sr, Fe); (Zn, Li, Ba, Cr and Mn); (As, Cu, Cd, Ni and Pb), and the fourth subgroup included redox-sensitive elements (Co and U). Strontium showed the highest concentration, ranging from 84 to 63887 $\mu\text{g g}^{-1}\text{dw}$. The concentrations of metals in the second and third classes of elements ranged from 0.36–566 $\mu\text{g g}^{-1}\text{dw}$ and 0.17–45 $\mu\text{g g}^{-1}\text{dw}$, respectively. The values of the fourth-class elements ranged from 0.08–1.84 $\mu\text{g g}^{-1}\text{dw}$.

By separating the data into various categories, such as diurnal vs. nocturnal samples, by cruise, or by sample location, some noticeable trends were observable (Table 2 and 3). The metal spatial distribution in zooplankton samples showed some particular patterns. ANOVA indicated a significant variation in the metal concentrations in zooplankton between the northern, central and southern zones

Table 2 Concentrations of Li, Cr, Mn, Fe, Co and Ni (in $\mu\text{g g}^{-1}$ dry weight) determined in zooplankton, reported per season (summer vs. autumn), per sampling zone (north, center or south) and per time (day vs. night). Values are mean \pm standard deviation (SD). N=number of samples.

Samples	Li	Cr	Mn	Fe	Co	Ni
Mean \pm SD	15.66 \pm 43.58	10.25 \pm 18.31	12.31 \pm 17.64	1126 \pm 1169	0.60 \pm 0.30	5.22 \pm 8.21
(Min-Max) N=113	0.50–353.09	0.36–101.15	1.64–99.15	84.23–5095	0.12–1.84	0.46–45.02
Summer N=57	15.70 ^a \pm 47.73	6.04 ^b \pm 7.08	6.80 ^b \pm 4.31	895 ^b \pm 847	0.57 ^a \pm 0.31	3.00 ^b \pm 3.90
Autumn N=54	15.61 ^a \pm 39.00	14.86 ^a \pm 24.74	18.22 ^a \pm 23.85	1379 ^a \pm 1406	0.63 ^a \pm 0.28	7.64 ^a \pm 10.69
North N=38	12.95 ^{ab} \pm 27.99	3.72 ^a \pm 5.30	6.88 ^b \pm 3.28	611.43 ^b \pm 434.35	0.70 ^a \pm 0.33	3.05 ^b \pm 3.98
Center N=30	32.37 ^a \pm 74.48	8.86 ^b \pm 7.85	10.39 ^b \pm 6.91	1328.18 ^b \pm 1156.44	0.58 ^{ab} \pm 0.23	4.16 ^{ab} \pm 2.95
South N=44	62.30 ^b \pm 253.46	19.08 ^b \pm 28.28	20.25 ^a \pm 28.07	1575.47 ^a \pm 1516.23	0.49 ^b \pm 0.26	8.62 ^a \pm 12.64
Day N=76	45.96 ^a \pm 185.22	11.04 ^a \pm 18.11	12.75 ^a \pm 17.07	1154.72 ^a \pm 1146.27	0.6 ^a \pm 0.28	5.58 ^a \pm 8.3
Night N=37	11.59 ^a \pm 23.73	8.63 ^a \pm 18.85	11.41 ^a \pm 18.96	1066.72 ^a \pm 1227.31	0.59 ^a \pm 0.32	4.48 ^a \pm 8.07

The mean metal concentrations of different species from all sites sharing a common letter (a, b or ab) for a particular metal are not significantly different, $p < 0.05$.

(ANOVA, $p > 0.05$) (Tables 2 and 3), except for Zn, Sr and Ba. Therefore, we found increases from north to south in the concentrations of Cr, Mn, Fe, Ni, Cu and Pb and a decrease in Co. However, the concentration of Cd was lower in the central zone, whereas the concentrations of Li, As and U were higher.

The results showed that during both the summer and autumn seasons, no significant differences were observed for Li, Co, Cu, Cd, Pb and U concentrations; however, during autumn, we detected relatively high concentrations of Cr, Mn, Fe, Ni and As, whereas the lowest concentrations were recorded for Zn, Sr and Ba (Table 2 and 3).

Statistically significant spatial variations (by transect) were observed among all metal variables, except for Zn, Cd and Li. Transects I and H formed a group with generally noticeable concentrations of Cr, Mn, Fe, Ni, Cu, Sr, Ba and Pb (Figure 2).

However, no significant differences were found between diurnal and nocturnal zooplankton samples ($p > 0.05$). Furthermore, metal concentrations were not significantly different near the coast in comparison with offshore and were not different between surface and deep areas, except for Sr and U.

3.2. Statistical analysis

The results of metals in zooplankton also indicated the same relative origins between some metals. This was confirmed by statistical analysis (PCA) (Figure 3), which showed a very good correlation between Sr and Ba in Group I and a low correlation between Zn and Pb in group II, while Cu, Fe, Cr, Mn, and Ni formed a clustered group III on the right, and Li, Cd, U, As and Co formed a loose group IV on the left, with negative correlations between Cd and other metals, possibly indicating a different source.

4. Discussion

4.1. Essential metal elements in zooplankton

The assessment of metal concentrations in zooplankton is very important, because they are the main diet for many

predators and may contribute to transferring a variety of metals to higher trophic levels. As mentioned, this is the first study on the levels of metals in zooplankton in the south Atlantic region of Morocco (North Atlantic). Also, in this area, metal concentrations in sediments are comparable with uncontaminated sediments (Maanan et al., 2015). The absence of previous studies on the composition of metallic elements in zooplankton in the study region complicates the interpretation of our results. Indeed, published zooplankton data on some elements, such as Sr, Li, As, Mn, Fe, U, Co and Ba, are very scarce.

According to results revealed by the principal component analysis in Figure 3, the first group (G-I) contained Sr at a higher concentration, it correlates with Ba. Sr is the fifth most abundant cation after Na^+ , Mg^{2+} , Ca^{2+} and K^+ . Strontium is especially used in the reconstruction of environmental history (Fang et al., 2014), it is used as a chemical tracer for oceanic circulation. Related results were reported by Rentería-Cano et al. (2011), with high concentrations of Sr ($105\text{--}15,450 \mu\text{g g}^{-1}$ dw) on zooplankton sampled from the northern Gulf of California, while Fang et al. (2014) in northeastern Taiwan reported an average of $370 \mu\text{g g}^{-1}$ dw, with a range of $121\text{--}777 \mu\text{g g}^{-1}$ dw. This element was followed by iron (Fe), which is an essential micronutrient for the biological requirements of all marine organisms. The high levels of Fe can be attributed to its direct adsorption by zooplankton in seawater or indirect absorption by a digestion of phytoplankton, which metabolize it by photosynthesis (Ho et al., 2003; Rentería-Cano et al., 2011). The Fe concentrations were comparable to those reported by Pempkowiak et al. (2006) from the southern Baltic and by Fang et al. (2014) but higher than those reported by Ben Salem and Ayadi (2017) and Battuello et al. (2016), respectively, on the southern and northern Mediterranean coasts.

Zinc is one of the most important micronutrients, with higher levels in zooplankton than in pelagic fish (Afandi et al., 2018), reflecting the presence of Zn in the marine environment. Furthermore, according to Eisler (1993), nutrition is the main source of Zn for aquatic organisms, and it is significantly more essential than Zn uptake from seawater. Additionally, depending on Paimpillil et al. (2010), this higher concentration may be

Table 3 Concentrations of Cu, Zn, As, Sr, Cd, Sr, Cd, Ba, Pb and U (in $\mu\text{g g}^{-1}$ dry weight) determined in zooplankton, reported per season (summer vs. autumn), per sampling zone (north, center or south) and per time (day vs. night). Values are mean \pm standard deviation (SD). N =number of samples. The mean metal concentrations of different species from all sites sharing a common letter (a, b or ab) for a particular metal are not significantly different, $p < 0.05$.

Samples	Cu	Zn	As	Sr	Cd	Ba	Pb	U
Mean \pm SD	7.32 \pm 3.35	106.05 \pm 85.57	7.47 \pm 3.91	6034 \pm 10451	6.97 \pm 2.72	17.37 \pm 20.96	1.31 \pm 1.58	0.26 \pm 0.11
(Min-Max) N=113	1.62–21.75	23.17–566.09	2.05–25.21	147.93–63887	0.86–12.91	0.91–111.05	0.17–9.86	0.08–0.69
Summer N=57	7.58 ^a \pm 4.15	126.68 ^a \pm 89.79	6.10 ^b \pm 2.12	8107 ^a \pm 13129	7.34 ^a \pm 3.01	21.50 ^a \pm 25.50	1.55 ^a \pm 2.02	0.25 ^a \pm 0.12
Autumn N=54	7.03 ^a \pm 2.15	83.90 ^b \pm 75.48	8.97 ^a \pm 4.79	3806 ^b \pm 3795	6.56 ^a \pm 2.33	12.94 ^b \pm 13.49	1.05 ^a \pm 0.83	0.27 ^a \pm 0.11
North N=38	6.41 ^b \pm 3.05	102.74 ^a \pm 100.24	6.90 ^b \pm 2.10	7081.99 ^a \pm 19096.94	7.39 ^a \pm 2.38	21.11 ^a \pm 43.62	0.81 ^b \pm 0.97	0.22 ^b \pm 0.10
Center N=30	7.50 ^{ab} \pm 4.04	255.40 ^a \pm 841.89	10.79 ^a \pm 5.71	5008.39 ^a \pm 10777.58	5.93 ^b \pm 3.00	14.85 ^a \pm 21.01	1.51 ^{ab} \pm 1.98	0.35 ^a \pm 0.13
South N=44	8.26 ^a \pm 2.85	112.92 ^a \pm 78.72	5.54 ^b \pm 1.50	8818.87 ^a \pm 14029.17	7.28 ^a \pm 2.74	22.20 ^a \pm 26.79	1.75 ^a \pm 1.68	0.24 ^b \pm 0.07
Day N=76	7.5 ^a \pm 3.46	173.72 ^a \pm 535.34	7.49 ^a \pm 3.56	8017.57 ^a \pm 17971.79	6.83 ^a \pm 2.56	21.61 ^a \pm 38.35	1.45 ^a \pm 1.82	0.26 ^a \pm 0.1
Night N=37	6.96 ^a \pm 3.1	91.16 ^a \pm 36.14	7.43 ^a \pm 4.59	5262.78 ^a \pm 8379.98	7.24 ^a \pm 3.05	16.12 ^a \pm 19.06	1.02 ^a \pm 0.86	0.26 ^a \pm 0.14

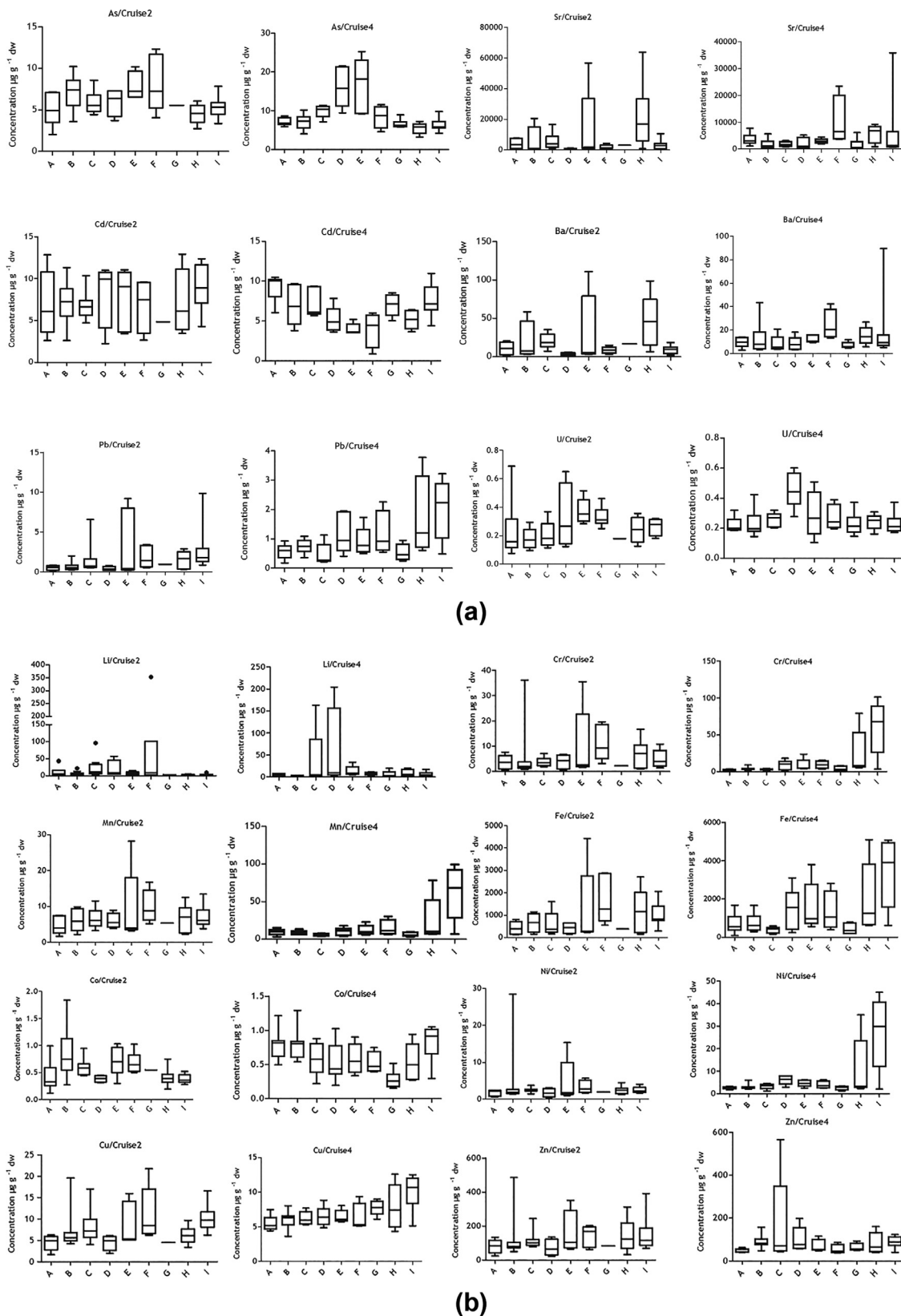


Figure 2 Metals variations in zooplankton along the nine transects of the study area (in $\mu\text{g g}^{-1} \text{ dw}$).

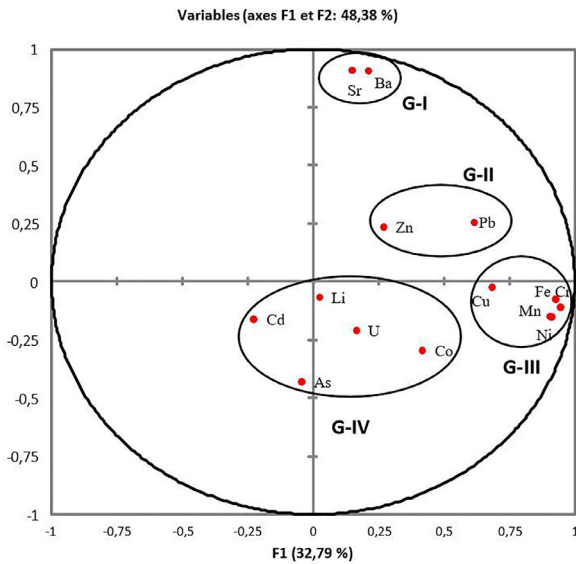


Figure 3 Principal component analysis (PCA) with different groups of metals.

due to its co-precipitation with calcium carbonate. Nevertheless, higher levels have been reported in the literature than those noted in our study (Nair et al., 1999; Pempkowiak et al., 2006; Srichandan et al., 2016).

Manganese is a naturally arising metal in seawater and can be considerably bioconcentrated by aquatic biota at lower trophic levels. In our study, the concentration of this element ranged from 1.64 to 99.15 $\mu\text{g g}^{-1}$ dw. These levels were higher than those of pelagic species analyzed in the same area (Afandi et al., 2018). Similar levels were detected in previous studies, such as Fang et al. (2006) and Kahle and Zauke (2003), but they are still lower than those recorded in more contaminated marine regions, e.g., in the Bay of Bengal (Srichandan et al., 2016) and at the northern coast of Taiwan (Hsiao et al., 2011). At the same order of metal concentrations, the average level of Cr was 10.25 $\mu\text{g g}^{-1}$ dw, which varied from 0.36 to 101.15 $\mu\text{g g}^{-1}$ dw. Seasonally, its accumulation in zooplankton was found to be higher during autumn compared to the summer season (Table 2), this difference may be due to the development cycle of zooplankton, with a short period of reproduction at the beginning of summer and a maximum biomass in autumn (Shi et al., 2020), the same observation was recorded by Battuello et al. (2016) with lower values. However, our values of Cr are noticeably low compared to the reported values, such as Hsiao et al. (2011) and Fang et al. (2014) from Taiwan, Leonova et al. (2013) in the White Sea or Achary et al. (2020) in coastal India (Table 4).

Copper is important in the zooplankton life cycle process, including egg production and growth, and is used as a component of enzymatic activity (Paimpillil et al., 2010). Their levels in zooplankton ranged from 1.62 to 21.75 $\mu\text{g g}^{-1}$ dw (Table 3) and were in accordance with those registered in other marine environments of the world in areas of low anthropogenic impact (Battuello et al., 2016; Kahle and Zauke, 2003). Ni is one of the biologically essential trace elements and has a wide distribution in terrestrial and aquatic environments. The concentration of Ni

Table 4 Metal concentrations ($\mu\text{g g}^{-1}$ dry weight) in zooplankton samples from different areas.

Location	Cu	Zn	Cr	Cd	Pb	Ni	Source
Atlantic coast off Morocco	7.32 ± 3.35	106.05 ± 85.57	10.25 ± 18.31	6.97 ± 2.72	1.31 ± 2.72	5.22 ± 8.21	Present study
Mediterranean area	1.62–21.75	24–566	0.36–101.15	0.86–12.91	0.17–9.86	0.46–45	Battuello et al. (2016)
Banc d'Arguin Mauritania	1.90–39.70	14.47–132.33	–	0.05–0.41	1.01–12.38	0.85–5.48	Everaarts et al. (1993)
Northeastern Taiwan (Temora turbine)	17–92	70–580	–	4–11	12–54	–	Hsiao et al. (2011)
White Sea	8.3–180.0	5.6–825.1	7.1–188.3	1.4–141.8	0.06–85.9	2.7–954.9	Leonova et al. (2013)
North Sea (Atlantic ocean)	33–142	284–643	59–620	0.4–3.2	9.1–36.4	3.2–13.4	Haarich et al. (1993)
Weddel Sea- Antarctic (Australia)	4.3–13.4	55–171	–	2.7–13.1	0.4–2.3	0.9–6.4	Kahle and Zauke (2003)
Southern Baltic sea	6–52	131–682	–	2.1–14.4	0.09–1.67	2.9–18	Pempkowiak et al. (2006)
Kalpakkam coast (India)	2.33–25.42	84–1600	0.00–16.22	0.07–3.23	0.71–13.68	1.42–11.58	Achary et al. (2020)
Bombay (India)	37.6 ± 24.3	356.8 ± 216.6	60.0 ± 43.4	2.3 ± 2.3	38.6 ± 21.8	43.4 ± 49.8	Nair et al. (1999)
Bay of Bengal (Indian ocean)	5.6–105.0	13.1–711.8	4.7–145.3	0.4–8.4	4.4–84.8	1.94–180.3	Srichandan et al. (2016)
Arctic	73.8–151.8	709–1809	–	1.1–2.5	3.6–5.4	4.4–8.6	Mohan et al. (2019)
Arabian Sea	2.64–14.38	16.96–1408	–	0.76–1.50	0.22–4.16	1.50–3.10	Robin et al. (2012)
	4.69–36.93	84.14–366.81	1.67–13.29	0.18–4.96	1.43–15.32	2.77–16.07	
	1.31 ± 1.13	2.77 ± 2.25	–	0.04 ± 0.03	2.57 ± 2.66	–	

in the present study ranged between 0.46 and 45.02 $\mu\text{g g}^{-1}$ dw, and is higher compared to the reported values, such as Battuello et al. (2016) and Ben Salem and Ayadi (2017). The highest Ni concentration is usually entering into the individual cells through the metabolic pathways, it replaces the metals of the metalloenzymes and disrupts metabolism in certain cases (Achary et al., 2020). Thus with the exception of Fe, the concentrations of metals in the group (G-III) (Figure 3) have the same range of magnitude, which could indicate the same metabolic needs for metals or the same pathways of metal ingestion and accumulation, while a high correlation could indicate a common source.

Cobalt is an important metallic element due to its association with vitamin B12. In the present study, the Co concentration in zooplankton varied from 0.12 to 1.84 $\mu\text{g g}^{-1}$ dw. Our findings have shown that Co levels were similar to pelagic species analyzed in the same (Afandi et al., 2018), but lower than those detected in contaminated coastal areas (Fang et al., 2014; Rejomon et al., 2008; Rentería-Cano et al., 2011; Srichandan et al., 2016). These low values indicate that the study area is not contaminated with this metal. This element is part of the redox-sensitive element in the same subgroup (G-IV) (Figure 3) such as uranium.

Published data on minor elements such as Li and Ba, in zooplankton are very limited, the lack of previous information on these elements complicates the interpretation of our results. The concentration ranges of Ba found in the present study are in the same order of magnitude as the reported data by Fang et al. (2014).

4.2. Nonessential metal elements in zooplankton

Cadmium (Cd) is a nonessential metal with no known beneficial effects. Once it is present in the environment, it can remain for a long time and does not break down into less toxic substances. It is well known that the concentrations of Cd in seawater depend on several factors, including upwelling and biogeochemical processes such as direct uptake by living species and decomposition of organic matter. Since Cd has no significant physiological role, it is probably adsorbed on the surface of zooplanktonic debris or fecal pellets (Battuello et al., 2016; Kahle and Zauke, 2003; Paimpillil et al., 2010). In this study, Cd levels ranged from 0.86 to 12.91 $\mu\text{g g}^{-1}$ dw (Table 3). These results are comparable with those reported in other marine organisms from the same area (Afandi et al., 2018; Benbrahim et al., 2006), in the upwelled water zone in Mauritania (Everaarts et al., 1993).

Lead is recognized as a nonessential metal for marine organisms, and the Pb content in the analyzed zooplankton in this study ranged from 0.17–9.86 $\mu\text{g g}^{-1}$ dw. These results are higher than those reported in bivalve mollusks and pelagic fish from the same area (Afandi et al., 2018; Benbrahim et al., 2006), but they are comparable with the data reported in zooplankton (Fang et al., 2014; Pempkowiak et al., 2006). The observed high Pb levels in zooplankton in the south and center could be attributed to high influxes from mineral dust in these regions and the formation of colloids with Pb in seawater adsorbing onto planktonic debris, which can increase the concentration of this element in this species (Robin et al., 2012). Pb and Zn

showed a positive correlation (Figure 3) which could be influenced by various processes such as recycling within surface waters from rapid remineralization of biological material.

The uranium contents in the present study were lower, ranging between 0.08 and 0.69 $\mu\text{g g}^{-1}$ dw, while these concentrations ranged from 0.5 to 6.1 $\mu\text{g g}^{-1}$ dw in the Bay of Bengal (Achary et al., 2020) and between 0.24 to 11.3 $\mu\text{g g}^{-1}$ dw in the Northern Gulf of California (Rentería-Cano et al., 2011). This element causes significant toxic effects in animals and humans. According to Skwarzec et al. (2012), the contribution of marine organisms to the geochemical migration of U between seawater and bottom sediments is insignificant, but it was shown that an increase in U accumulation in reducing conditions by comparison with oxidizing conditions (Khaustova et al., 2021).

Arsenic is present in different environmental compartments at very variable concentrations. The highest levels are found in marine organisms that are capable of both accumulating and transforming this element. In zooplankton from the study area, the levels ranged from 2.05 to 25.21 $\mu\text{g g}^{-1}$. Published data noted large variations in the concentrations registered from other marine environments of the world, e.g., in the Bay of Bengal, values ranged from 2.73 to 5.73 $\mu\text{g g}^{-1}$ dw (Srichandan et al., 2016), and in the Sea of Japan, values varied between 0.21 and 17 $\mu\text{g g}^{-1}$ dw (Shibata et al., 1996). Furthermore, Neff (1997) reported concentrations of total arsenic in zooplankton from all over the world of 0.2–24.4 $\mu\text{g g}^{-1}$ dw; these levels were in accordance with our findings. This element correlates with Cd and Co, it may indicate the same source such as upwelling and removal of surface waters by the biological pump (Figure 3) but generally, Cd may correlate with Cu and Zn in marine organisms. In our study, As and Cd doesn't correlate with other metals, while Mn, Fe, Ni, Cu, Pb, Cr and Zn discerned positive affinity and good relationship, suggesting their strong affinity and coexistence; the same observation was recorded by Srichandan et al. (2016) in contrast with Robin et al. (2012) who recorded inter-metal relationships for essential and non-essential metals.

4.3. Diverse variations in metal accumulation

Geographical variations in the concentrations of the majority of the metals studied follow the biological activity explained above, with increases in the concentrations in the north followed by the south and then the central zone. Furthermore, local upwelling may be related to increased Cd and other metal bioavailabilities in seawater, hence increasing uptake into zooplankton, as hypothesized for these regions. Additionally, despite the presence of phosphate mining activities in the south of Laâyoune, more precisely in the central zone, we did not record enrichment with Cd in this area, in contrast to the other two zones (north and south), which show evidence of permanent upwelling. Additionally, Shelley et al. (2016), in the same project EPURE, studied the metallic element compositions of aerosols at three stations (Agadir, Laâyoune, Dakhla) and quantified atmospheric deposition fluxes in the Canary Current Upwelling System; they did not observe significantly different ratios of Cd/Al between stations. Otherwise, these results correlated with those found for total dissolved Cd in the same

cruises by Waeles et al. (2016), with concentrations ranging from 40 to 370 pmol l⁻¹ in the northern area and from 40 to 420 pmol l⁻¹ in the southern area, and lower values in the central zone varying from 10 to 210 pmol l⁻¹. Furthermore, our results are generally in agreement with those proposed by these two previous studies in terms of the origin of contamination by certain metals such as Cd, suggesting that the importance of atmospheric deposition and anthropogenic inputs of Cd has not been proven in the study area, while it is the upwelling of the North Central Atlantic Waters that mainly influences the distribution of Cd. This diversity in the southern region can be explained by the quality of the environment in this zone. Indeed, according to Robin et al. (2012), the high concentrations of metals in zooplankton at both coastal and offshore stations often coincide with high concentrations of dissolved metals; thus, elevated concentrations may be attributed to their higher availability in seawater. It can be noted, as Erasmus et al. (2021) have indicated, that factors driving the accumulation of metal elements are oceanographic process and diffuse sources, (e.g., geogenic weathering, ocean currents, upwelling, etc.) and not direct point sources of pollution.

It is well documented that the metals contained in zooplankton, especially in copepods, could vary significantly inter- and intraspecies (Hsiao et al., 2011). Hamanaka and Tsujita (1981) reported large variations in Cd concentrations in copepods, amphipods and euphosids, with a lower Cd concentration in euphosids (less than 2.2 µg g⁻¹ dw) but a wide Cd range in copepods (1.66–14.55 µg g⁻¹ dw), with large similarity to our results. In a more precise way, Achary et al. (2020) reported that zooplankton taxa dominated by copepods are active in accumulating metals from the background. Other reports on metal content from the marine environment determined that zooplankton are more efficient in the accumulation of metals from the environment (Rejomon et al., 2008).

The concentrations of metals in the diurnal samples were slightly higher than those in the nocturnal samples for the main metals (Li, Cr, Mn, Fe, Zn, Sr), with no significant differences. However, for Co, Ni, Cu, As, Cd, Ba, Pb and U, the concentrations were in the same order for both the diurnal and nocturnal zooplankton. These variations between groups of metals were observed by Horowitz and Presley (1977), who recorded biological variations between day and night. In fact, zooplankton are mainly transported by ambient water currents, used to avoid predators as in diel vertical migration or to increase prey encounter rate.

The vertical variation in metal accumulation in zooplankton did not represent any significant difference, except for Sr, because of its higher concentration in surface water; and U because of its lower concentration in surface water. However, some metals have profiles that are nutrient-like, such as Cd, Cu and Zn, which is indicative of their involvement in biological cycles (Waeles et al., 2016) and their strong correlation with zooplankton biomass. Fisher et al. (1991) also indicated that the storage efficiencies of Cd, Zn and Hg indicate their need to be recycled by copepods in surface waters through the organic cycle in the sea, with a direct correlation between the retention efficiency of ingested elements in copepods and the presence of the elements in the cytoplasm of the copepods' diatom food. In contrast, other

metals seem to have a scavenged-type behavior, such as Pb, or behave in a conservative way, such as U; In our study, the concentrations of Pb tend to be maximal near mineral dust in the south and center area, it confirms this scavenged-type and conservative character. Then, some other metals, such as Mn, tend to be at maximum concentration level in the south, without any vertical variation, such elements can be considered hybrid-type metals, and their distribution is controlled by both biological uptake and scavenging processes (Bruland and Lohan, 2003). In general, metals with no apparent biological function would be expected to show some assimilation and therefore greater recycling, accounting for their longer residence times. Furthermore, the oceanic behavior of other metals, such as Sr and Ba, is still not well assessed, with limited data on Sr and Ba circulations in the global ocean, especially in the Southern Hemisphere (Fang et al., 2014).

The average metal concentrations in zooplankton during summer and autumn presented significant variations, with higher levels of Cr, Mn, Fe, Ni and As in autumn and higher levels of Zn, Ba and Sr during summer. These increases in metals during both seasons coincide with the presence of a strong upwelling activity, allowing for metal resuspension and making them bioavailable to biota (Larissi et al., 2013). In fact, this zone is limited in the north and south by seasonal upwelling systems with an inter-annual change. It is described as an ecological barrier for planktonic populations and frontal zone where two different water masses NACW (North Atlantic Central Waters) and SACW (South Atlantic Central Water) meet, hence its important richness (Tito de Morais, 2013). The mean concentrations of metals reported in this study (Table 2 and 3) were much higher than those previously reported in water by Waeles et al. (2016) and Afandi (2018), reflecting an efficient bioaccumulation of metals, such as demonstrated by Chevrollier et al. (2022). These differences may be associated with the large surface area of zooplankton relative to their unit mass and with their active metabolism, resulting in rapid adsorption of various metals (Battuello et al., 2016; Ravera, 2001). Moreover, it has been reported that the concentration levels of metals in plankton depend upon different biotic and abiotic factors, such as the physicochemical properties of the water, the productivity of the water body, quantitative and qualitative aspects of the plankton, the bioavailability of metals, and spatiotemporal variations (Dobaradaran et al., 2018; Elmaci et al., 2007).

A general comparison of the present data on the metal concentrations with those from various sea areas is compiled in Table 4. All the metal levels in the zooplankton samples appear to fall within a range of values that have been reported for similar organisms obtained in other areas (Everaarts et al., 1993; Rainbow, 1996; Srichandan et al., 2016). The reported patterns are also in good agreement with the data in the literature obtained from a great number of open ocean areas. The moderately increased Zn concentrations found in this study (Table 2 and 3) followed by Cu, Ni, Pb and Cd are generally in agreement with data from nearer regions, such as Banc Arguin in Mauritania (Everaarts et al., 1993), and indeed with other marine areas, such as the Antarctic, Arctic and the North Sea (Haarich et al., 1993; Kahle and Zauke, 2003; Mohan et al., 2019).

5. Conclusion

This study highlights, for the first time in the Moroccan marine environment, metal accumulation in zooplankton, and it is confirmed that this species plays a significant role in the transfer of metals to higher trophic levels due to its high bioaccumulation of most metals compared to bivalve mollusks and pelagic fish species previously studied in the same area. In the present study, the increases in metal concentrations in zooplankton from the northern zone to the southern zone, which has a very low anthropogenic influence, can be explained by the existence of cold-water upwelling, bringing nutrients and metal elements to the surface and making these elements bioavailable to marine organisms. Metals in zooplankton samples followed the order of $Sr > Fe > Zn > Ba > Li > Mn > Cr > As > Cu > Cd > Ni > Pb > Co > U$; there were also rather large variations in concentrations between metals and by area, the meaning of which is still poorly understood. Unfortunately, some metal concentrations found in our study were in good agreement with concentrations found in other regions that were not contaminated. The results of this study can be used as baseline data for the metal contents of zooplankton in Moroccan South Atlantic coastal water. Additionally, the results of the present study will be useful for historical evaluations by annual monitoring in the area. Finally, it is highly recommended that further studies be undertaken in the region of the southern Moroccan Atlantic coast to determine the relationship between the metal contents of zooplankton and seawater, the bioaccumulation factor (BAF) of metals in zooplankton, as well as the metal content in different species of zooplankton, to determine which method yields the most sensitive bioindicator.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

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