Oceanological and Hydrobiological Studies

International Journal of Oceanography and Hydrobiology

Volume 52, No. 4 December 2023
pages (484-492)

sciendo

ISSN 1730-413X
eISSN 1897-3191

A biomonitoring study of *Diadema setosum*: metal bioaccumulation and current status in Iskenderun Bay, eastern Mediterranean

by

Erkan Uğurlu*

DOI: **https://doi.org/10.26881/oahs-2023.4.09**
Category: **Original research paper**
Received: **February 28, 2023**
Accepted: **July 28, 2023**

Marine Science and Technology Faculty, Iskenderun Technical University, Iskenderun, Hatay, Türkiye

Abstract

Concentrations of aluminum, chromium, cobalt, nickel, cadmium, lead, manganese, iron and zinc were determined in sea urchin species, *Diadema setosum* from Iskenderun Bay in December 2022. The level of metals was determined by Inductively Coupled Plasma-Mass Spectrometer (ICP-MS). Concentrations of the heavy metals in the examined sea urchins ranged as follows: Al 0.07-7.17 μg g^{-1}; Cr 0.07–0.8 μg g^{-1}; Co 0.01–0.2 μg g^{-1}; Ni 0.2–2.9 μg g^{-1}; Cd 0.0–0.02 μg g^{-1}; Pb 0.4-3.5 μg g^{-1}; Mn 0.03–0.4 μg g^{-1}; Fe 1.2-57.2 μg g^{-1}; Zn 0.28–2.7 μg g^{-1}, respectively. The highest accumulation of Fe and Pb metals was determined in the tissues of the sea urchin. The present study suggests that D. *setosum* could be a potential biological indicator of metal pollution in the İskenderun Bay. Furthermore, soft tissues generally had lower levels overall. For this reason, the data show that *D. setosum* is a very good heavy metal collector and therefore may be used successfully to monitor heavy metal levels in Iskenderun Bay.

Key words: Diadema, gonad, ICP-MS, metals, sea urchin, shell

* *erkn.ugurlu@yahoo.com*

online at www.oandhs.ug.edu.pl

1. Introduction

The *Diadema* sp. sea urchin is a widely distributed, abundant and ecologically important tropical genus (Muthiga et al. 2020). *Diadema setosum* (Leske, 1778) (Echinodermata: Echinoidea: Diadematidae) is one of the echinoderm species distributed in the western Indo-Pacific Ocean. This sea urchin species is dispersed in the Red Sea (Gulf of Suez, Gulf of Aqaba, Northern and Southern Red Sea) and from the coast of Africa to Japan and Australia (Lessios et al. 2001). *D. setosum* was first reported in 2006 in the Mediterranean Sea, along the southwest coast of Turkey (Yokes & Galil 2006). It has also been reported along Turkey's Mediterranean coast, Hatay (Turan et al. 2011), Aegean Sea (Yapıcı et al. 2014), and Marmara Sea (Artüz & Artüz 2019).

D. setosum is defined by its long dark spines, five white spots around the shell, and an orange hue around the anal cone (Lessios et al. 2001; Clark 1925). The *D. setosum* sea urchin is an omnivorous digger that eats free substrate and feeds on detritus. The invasive sea urchin lives in the shallow sublittoral region (0–20 m) and is mainly found at depths of 1-6 m. *D. setosum*, which is found in stony habitats and biogenic reefs where it hides in fractures and under ledges due to dense lighting, has also been reported to be distributed in sediment and seagrass meadows (Muthiga et al. 2020). The species exhibits mutable reproductive shapes in changed geographic areas that are influenced by ecological influences such as temperature, moon times, and congener and adult densities. Due to their elevated densities, species can turn stony shores into barren areas (Muthiga et al. 2020) and coral reefs, in particular, can erode biogenic substrates (Bronstein & Loya 2014).

Various studies on echinoids have shown that these species can be used as bioindicator species in metal pollution studies (Çagiltay et al. 2022; Öndes et al. 2022; Uğurlu & Duysak 2023, 2022; Al Najjar et al. 2018; Campbell, 1967; Flammang et al. 1997; Warnau et al. 1995a, b; Ablanedo et al. 1990). There are bioindicator studies of marine invertebrates in Iskenderun Bay (Duysak et al. 2021; Duysak & Uğurlu 2020; Duysak & Dural 2015; Duysak & Ersoy, 2014), but no study has been made on sea urchin species. In addition, sea urchin populations are constantly increasing in Iskenderun Bay. Therefore, the use of *D. setosum* as a bioindicator species was investigated.

The aim of the study was to determine the metal concentrations of Al, Cd, Co, Ni, Pb, Cr, Fe, Mn and Zn in the testa (shell), gonads, and soft tissues of *D. setosum* taken from the Iskenderun and Arsuz sites in the northeastern Mediterranean, Iskenderun Bay.

2. Materials and methods

D. setosum was collected in December 2022 from the İskenderun (n=50) and Arsuz (n=50) sites located on the northeastern coast of Iskenderun Bay (Figure 1). The sea urchin samples collected from the sites were transported to the laboratory in a bucket. At the laboratory, each individual's total body weight and average test diameter were measured vertically, without spines, with the aid of a calliper. The sea urchin species were dissected using laboratory scissors to remove gonads, testa (shell) and soft organs (Figure 2A-D). The dissected organs were washed with double distilled water to clean their shells and gonads from foreign matter. All samples were stored in a freezer (-20°C) until analysis.

To determine the accumulation levels of Al, Cd, Co, Ni, Pb, Cr, Fe, Mn, and Zn in the shell, soft tissue and gonad tissues of sea urchins, approximately 1 g was taken from each tissue. For the analysis of the shells, a mixture was prepared by taking the chemicals HNO_3, $HClO_4$ and H_2SO_4 at a ratio of 5:2:1, respectively. Approximately 1 g of shells was added to 10 ml of the prepared mixture, and the dissolution process was carried out at 50 ± 5°C for 50 min. Next, 10 ml of 2 N HCl was added. The resulting mixture was filtered with Whatman Quantitative filter paper (No: 42, 110 mm £). The filtered mixtures were put into polyethylene tubes and made up to 20 ml with double distilled water and stored at room temperature until analysis (FAO 1983). The soft tissue and gonads of the sea urchin samples were mixed separately and put into approximately 1 g

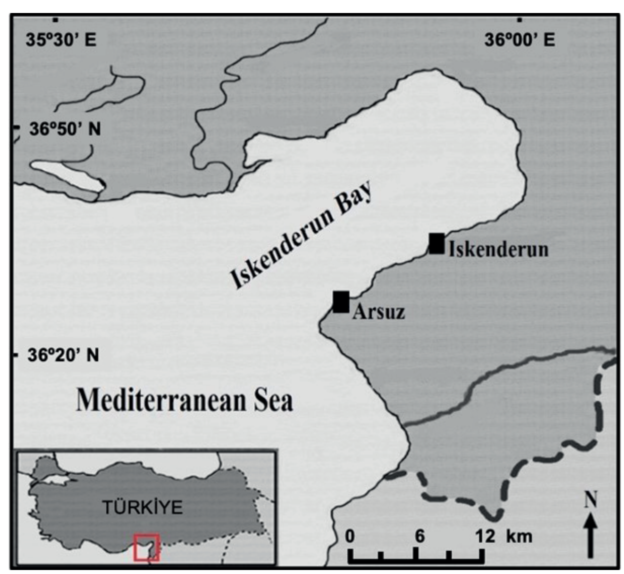

Figure 1

Study area (Modified from Koçak et al. 2005)

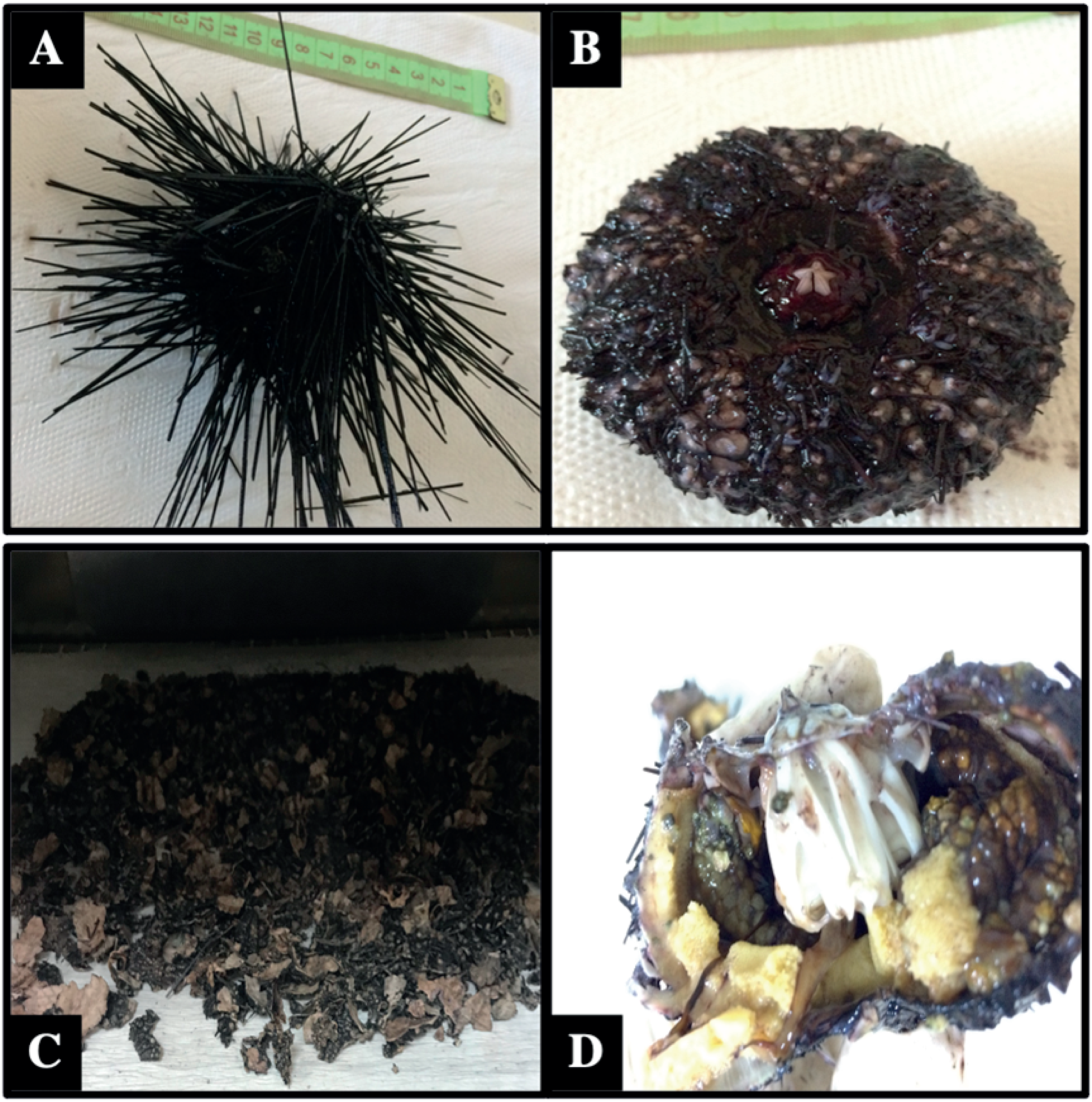

Figure 2

A-B) *Diadema setosum*, **C)** *D. setosum* shell, **D)** *D. setosum* gonad

polyethylene tubes, and 5 ml of nitric acid was added to it and allowed to dissolve at 60°C. The resulting mixture was filtered with Whatman Quantitative filter paper (No: 42, 110 mm £). The mixture was taken into polyethylene tubes and made up to 20 ml with double distilled water and stored at room temperature until analysis. All samples were made in 3 replications (Tüzen 2003).

Metal analyses of the prepared samples were measured using an Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) device (Plasma Quant MS Series, Jena, Germany) at Hatay Mustafa Kemal University. The operating conditions of the device are given in Table 1. In addition, in order to determine the accuracy of the device in heavy metal measurement, metal analysis was carried out by preparing solutions from DORM-3 (Dogfish Protein Certified Reference Material For Trace Metals) certified reference material in the same way (Table 2). Metal levels were calculated in micrograms per gram wet weight (μg g^{-1} ww).

Table 1

ICP-MS (Plasma Quant MS Series) operating conditions

RF power	1.20 kW
Plasma gas flow rate	9 l min^{-1}
Auxiliary gas flow rate	1.65 l min^{-1}
Carrier gas flow rate	1.1 l min^{-1}
Helium collision gas flow rate	ml min^{-1} (Not used)
Spray chamber T	2°C
Sample depth	6 mm
Sample introduction flow rate	1 ml min^{-1}
Nebulizer pump	0.1 rps
Extract lens	1.5 V

Table 2

Comparison of our analysed values with the standard reference material, DORM-3 (fish protein)

Element	DORM-3 reference material		
	Certified value (µg g^{-1})	Measured value (µg g^{-1})	Recovery (%)
Al	-	-	-
Cd	0.291 ± 0.03	0.281 ± 0.04	97
Co	-	-	-
Ni	1.24 ± 0.31	1.35 ± 0.23	108
Pb	0.391 ± 0.09	0.412 ± 0.08	101
Cr	1.87 ± 0.19	1.93 ± 0.14	103
Fe	348 ± 19	326 ± 15.7	94
Mn	-	-	-
Zn	51.9 ± 2.7	52.8 ± 3.9	101

The arithmetic means and standard deviations of the results were calculated. One-way Anova, Duncan and Pearson tests were performed to define the relationships of metals in sea urchin tissues between sites and between tissues (Duncan 1955; Muller & Fetterman 2002; Gravetter & Wallnau 2007). All statistical calculations of the sample results were performed with the SPSS 17.0 program.

3. Results and discussion

The average concentrations of metals determined in the gonad, shell and soft tissues of *D. setosum* taken from Iskenderun and Arsuz sites in December 2022 are presented in Table 3. As a result of the metal analysis, the highest accumulation was in the shells of the sea urchin species and Al, Ni, Pb, Zn and Fe were detected. The concentrations of Al, Ni, Pb and Fe were determined as 5.23 ± 1.04 µg g^{-1}, 2.39 ± 0.85 µg g^{-1}, 2.37 ± 0.58 µg g^{-1} and 36.57 ± 5.59 µg g^{-1}, respectively at the Iskenderun site, and 7.17 ± 1.45 µg g^{-1}, 2.91 ± 0.52 µg g^{-1}, 3.45 ± 0.46 µg g^{-1} and 57.17 ± 5.02 µg g^{-1}, respectively at the Arsuz site (Table 3).

Table 3

Metal accumulation levels (ORT ± SD) (µg g^{-1} wet weight) in *D. setosum* sea urchins

Metals	Tissues	Sites	
		İskenderun	Arsuz
Al	Soft tissues	0.071 ± 0.001^{a,x}	0.081 ± 0.001^{a,x}
	Gonad	0.711 ± 0.165^{a,x}	0.816 ± 0.152^{a,x}
	Testa (Shell)	5.231 ± 1.039^{b,x}	7.170 ± 1.449^{b,y}
Cr	Soft tissues	0.069 ± 0.011^{a,x}	0.075 ± 0.007^{a,x}
	Gonad	0.227 ± 0.122^{a,x}	0.340 ± 0.118^{b,x}
	Testa (Shell)	0.664 ± 0.111^{b,x}	0.803 ± 0.028^{c,x}
Co	Soft tissues	0.014 ± 0.002^{a,x}	0.025 ± 0.002^{a,x}
	Gonad	0.034 ± 0.012^{a,x}	0.041 ± 0.003^{a,x}
	Testa (Shell)	0.246 ± 0.082^{b,x}	0.249 ± 0.034^{b,x}
Ni	Soft tissues	0.150 ± 0.047^{a,x}	0.187 ± 0.003^{a,x}
	Gonad	0.495 ± 0.281^{a,x}	0.468 ± 0.173^{a,x}
	Testa (Shell)	2.390 ± 0.848^{b,x}	2.914 ± 0.515^{b,x}
Cd	Soft tissues	0.012 ± 0.001^{a,x}	0.011 ± 0.001^{a,x}
	Gonad	0.010 ± 0.001^{a,x}	0.010 ± 0.001^{a,x}
	Testa (Shell)	0.012 ± 0.004^{a,x}	0.016 ± 0.002^{b,x}
Pb	Soft tissues	0.374 ± 0.092^{a,x}	0.402 ± 0.092^{a,x}
	Gonad	1.058 ± 0.032^{a,x}	2.774 ± 0.178^{b,y}
	Testa (Shell)	2.371 ± 0.577^{b,x}	3.451 ± 0.463^{b,y}
Mn	Soft tissues	0.028 ± 0.007^{a,x}	0.173 ± 0.023^{a,x}
	Gonad	0.089 ± 0.059^{b,x}	0.102 ± 0.014^{a,x}
	Testa (Shell)	0.406 ± 0.242^{b,x}	0.370 ± 0.119^{b,x}
Fe	Soft tissues	1.202 ± 0.129^{a,x}	2.556 ± 0.394^{a,x}
	Gonad	7.526 ± 0.658^{a,x}	8.819 ± 0.591^{a,x}
	Testa (Shell)	36.570 ± 5.587^{b,x}	57.166 ± 5.018^{b,y}
Zn	Soft tissues	1.532 ± 0.329^{a,x}	2.650 ± 0.301^{a,x}
	Gonad	0.276 ± 0.051^{b,x}	0.593 ± 0.045^{b,x}
	Testa (Shell)	0.356 ± 0.124^{b,x}	0.596 ± 0.032^{b,x}

Vertically, a, b, c show the differences between tissues ($p < 0.05$), while the differences between x, y sites ($p < 0.05$) are given horizontally. Data shown with different letters are statistically significant at the differences $p < 0.05$ level.

It was determined that the highest Al concentration in the *D. setosum* sea urchin shell taken from two different points of the Iskenderun Bay (7.170 ± 1.449 µg g^{-1}) was in the individuals taken from the Arsuz site. The lowest concentration of Al was detected in the soft tissue of individuals taken from the Iskenderun site (0.071 ± 0.001 µg g^{-1}) (Table 3). It was established that the difference in Al concentration levels between sea urchin shell and other tissues was statistically significant ($p > 0.05$). Al accumulation levels in tissues were determined as Testa > Gonad > Soft tissue at Iskenderun and Arsuz sites. Choi et al. (2014) reported that the Al concentration level in the edible muscle tissue of *Anthocidaris crass*ispina sea urchins from South Korea was 26.9 ± 30.1 mg kg^{-1}.

In the study, chromium (Cr) levels were determined to be between 0.069 ± 0.011 – 0.803 ± 0.028 µg g^{-1}. The highest Cr concentration was detected in the shells of *D. setosum* samples taken from the Arsuz site (0.803 ± 0.028 µg g^{-1}), while the lowest was found in the soft tissue (0.069 ± 0.011 µg g^{-1}) of samples taken from the Iskenderun site (Table 3). The difference in Cr concentration level between tissues was determined to be significant ($p > 0.05$). Cr accumulation levels in tissues were determined as Testa > Gonad > Soft tissue at the Iskenderun and Arsuz sites. Cr is recognised as an occupational and environmental pollutant; studies continue on the exact mechanisms of chromium that cause carcinogenic toxicity (Hu et al. 2017). It is reported to be associated with the formation of free radicals and ROS. Cr types that can be found in different valences are different in terms of essential and toxicity. Studies have reported that the Cr (VI) type is more toxic than Cr (III). Cr (III) plays an active role in lipid metabolism and insulin function. Warnau et al. (1998) reported that Cr concentration levels in *Paracentrotus lividus* gonad tissues collected from the Calvi, Ischia and Marseille sites were 0.67 ± 0.2 – 1.04 ± 0.39 µg g^{-1}, 0.96 ± 0.19 – 2.01 ± 1.44 µg g^{-1} and 0.95 ± 0.48 – 2.16 ± 1.13 µg g^{-1}, respectively. *P. lividus* reported that Cr concentration levels in sea urchin shells were in the range of 0.14 ± 0.08 – 1.14 ± 0.19 µg g^{-1}, 0.1 ± 0.05 – 1.32 ± 0.2 µg g^{-1} and 0.03 ± 0.04 – 1.35 ± 0.19 µg g^{-1}, respectively. Bonsignore et al. (2018) reported that the Cr concentration level in the gonad tissue of the *P. lividus* sea urchins collected from the Rosignano region of the Tuscany coast was 1.53 ± 0.74 µg g^{-1}. Anagha et al. (2022) reported that in the tissue of *Salmacis virgulata* sea urchins collected from the Kerala coast in South India, the Cr concentration level was 1.71 ± 0.05 µg g^{-1}.

Cobalt (Co) is one of the most common elements on Earth. Co metal is used extensively in industry, especially in the paint and glass industries. Co poisoning occurs as a result of inhalation of Co metal in the air and skin contact with cobalt salts, and although no research has yet been conducted on the effects of cobalt on cancer, there is a risk that it may be a carcinogenic substance (Kahvecioğlu et al. 2003). In the current study, Co levels were determined to be between 0.014 ± 0.002 – 0.249 ± 0.034 µg g^{-1} (Table 3). The highest Co concentration was detected in the shells of *D. setosum* samples taken from Arsuz site (0.249 ± 0.034 µg g^{-1}), while the lowest Co concentration was determined in the soft tissue (0.014 ± 0.002 µg g^{-1}) of the samples taken from the Iskenderun site. There were determined to be no statistical differences in Co concentrations between sites and between tissues ($p > 0.05$). Co concentrations in tissues were listed as shell > gonad > soft tissue at the Iskenderun and Arsuz sites.

Nickel (Ni) levels in *D. setosum* sea urchins taken from two different sites were determined to be between 0.150 ± 0.047 – 2.914 ± 0.51 µg g^{-1}. The highest Ni concentration was determined in the shells of the samples taken from the Arsuz site (2.914 ± 0.515 µg g^{-1}), and the lowest in the soft tissues of the samples taken from the Iskenderun site (0.150 ± 0.047 µg g^{-1}). The difference between the gonad tissue and other tissues was determined to be significant at both sites ($p < 0.05$) (Table 3). Ni levels in tissues were determined to be in the form of shell > gonad > soft tissue in both sites. Ni is generally absorbed by the body as a divalent Ni ion and is spread to several organs, causing neurotoxicity. Bonsignore et al. (2018) reported that the Ni concentration level in the gonad tissue of *P. lividus* sea urchins collected from the Rosignano region of the Tuscany coast was 0.74 ± 0.45 µg g^{-1}.

Cadmium (Cd) intake may cause disorders in calcium metabolism, formation of kidney stones, kidney diseases, lung diseases and atherosclerosis (Faroon et al. 2012). Cd levels in tissues of *D. setosum* were found to be between 0.010 ± 0.001 – 0.016 ± 0.002 µg g^{-1} (Table 3). The highest concentration of Cd was found in the *D. setosum* shells taken from the Arsuz site and the lowest concentration was detected in gonad tissue (0.010 ± 0.001 µg g^{-1}) at both sites. Cd concentrations are in the form of shell > gonad > soft tissue at the Iskenderun site, and in the order of shell > soft tissue > gonad tissue at the Arsuz site (Table 3). Warnau et al. (1998) reported that the Cd concentration levels in the gonad tissues of *P. lividus* sea urchins collected from the Calvi, Ischia and Marseille sites were 0.15 ± 0.08 – 0.32 ± 0.12 µg g^{-1}, 0.26 ± 0.09 – 0.93 ± 0.42 µg g^{-1} and 0.19 ± 0.09 – 0.83 ± 0.48 µg g^{-1}, respectively. Cd concentration in the shell was reported to be between 0.1 ± 0.01 – 0.34 ± 0.06 µg g^{-1}, 0.08 ± 0.03 – 0.32 ± 0.06 µg g^{-1} and 0.04 ± 0.04 – 0.37 ± 0.07 µg g^{-1}, respectively. Soualili et al. (2008) reported that the Cd concentration levels in tissues of *P. lividus* gonad collected from the Algiers Beach, Tamentfoust and Sidi-Fredj sites in Algiers bay were 0.08 ± 0.04 – 9.14 ± 0.08 µg g^{-1}, 0.05 ± 0.01 – 0.12 ± 0.08 µg g^{-1} and 0.05 ± 0.03 – 0.614 ± 0.09 µg g^{-1} in male-female individuals, respectively. Hernández et al. (2010) reported that Cd concentration levels in internal tissue and the shell of *Diadema* aff. *antillarum*, which they collected from 12 different sites on the island of Tenerife in Spain, were between 25.95 ± 22.78 – 816.27 ± 559.24 µg g^{-1} and 72.50 ± 39.21 – 309.89 ± 242.36 µg g^{-1}, respectively. Rouane-Hacene et al. (2018) reported the levels of Cd concentrations in whole soft body tissue of *P. lividus* sea urchins taken from the Oran Harbor, Ain Defla and Hadjaj sites in Algeria as 0.86 ± 0.02 µg g^{-1}, 0.68 ± 0.02 µg g^{-1} and 0.60 ± 0.02 µg g^{-1}, respectively. Choi et al. (2014) reported that the

Cd concentration level in the edible muscle tissue of *A. crassispina* sea urchins from South Korea was 0.04 ± 0.02 mg kg^{-1}. Bonsignore et al. (2018) reported that the Cd concentration level in the gonad tissue of *P. lividus* sea urchins collected from the Rosignano region along the Tuscany coast was 0.17 ± 0.11 µg g^{-1}. A similar kind of Cd accumulation ranging from 0.01 to 0.6 µg g^{-1} was observed when previous studies were examined, whereas a study in the Spanish islands by Hernández et al. (2010) showed a higher concentration (25 – 816 µg g^{-1}) than this study.

In this study, the highest toxic metal lead (Pb) content was found in the shell (testa) of the sea urchin samples taken from the Arsuz site (3.451 ± 0.463 µg g^{-1}), and the lowest in the soft tissue of sea urchins (0.374 ± 0.092 µg g^{-1}) from the Iskenderun site (Table 3). The difference in Pb accumulation between the tissues of the individuals taken from the İskenderun and Arsuz sites was determined to be not significant ($p > 0.05$). Pb accumulation in tissues was ranked as shell > gonad > soft tissue at the Iskenderun and Arsuz sites. The higher Pb levels are found in aquatic organisms, algae, and benthic organisms such as bivalves or crabs (Lee et al. 2019). Exposure to Pb has been reported to cause neurotoxic effects, joint weakness, anemia, deterioration of kidney function, and changes in hormone levels (EPA 2014). Pb is included in the list of toxic and harmful substances (WHO 2011). High levels of Pb are believed to be caused by industrial wastewater. Warnau et al. (1998) reported that the Pb levels in the gonad tissues of *P. lividus* collected from the Calvi, Ischia and Marseille sites were 0.74 ± 0.17 – 2.25 ± 0.63 µg g^{-1}, 1.15 ± 0.5 – 3.02 ± 1.31 µg g^{-1} and 1 ± 0.6 – 2.68 ± 0.7 µg g^{-1}, respectively. Pb accumulation levels in the sea urchin shell were reported to be between 1.52 ± 0.82 – 4.64 ± 1.50 µg g^{-1}, 1.03 ± 0.49 – 5.49 ± 1.65 µg g^{-1} and 1.17 ± 0.38 – 5.36 ± 1.1 µg g^{-1}, respectively. Soualili et al. (2008) reported that Pb concentration levels in *P. lividus* gonad tissues collected from the Algiers Beach, Tamentfoust and Sidi-Fredj sites in Algiers Bay were 7.78 ± 8.77 – 6.14 ± 3.46 µg g^{-1}, 0.88 ± 0.44 – 1.5 ± 1.72 µg g^{-1} and 0.9 ± 0.41 – 0.68 ± 0.12 µg g^{-1} in male-female individuals, respectively. Hernández et al. (2010) reported that Pb concentration levels in the internal tissue and shell of *Diadema* aff. *antillarum* collected from 12 different sites on Tenerife were between 17.08 ± 6.76 – 1718.47 ± 1669.14 µg g^{-1} and 101.61 ± 62.26 – 365.99 ± 296.02 µg g^{-1}, respectively. Rouane-Hacene et al. (2018) reported the levels of Pb concentrations in whole soft body tissue of *P. lividus* sea urchins taken from the Oran Harbour, Ain Defla and Hadjaj sites in Algeria as 6.08 ± 0.30 µg g^{-1}, 4.85 ± 0.30 µg g^{-1} and 3.59 ± 0.20 µg g^{-1}, respectively. Choi et al. (2014) reported that the level of Pb concentration in edible muscle tissue of *A. crassispina* sea urchins from South Korea was 0.10 ± 0.05 mg kg^{-1}. Bonsignore et al. (2018) reported that the level of Pb concentration in the gonad tissue of *P. lividus* sea urchins collected from the Rosignano region of the Tuscany coast was 0.85 ± 0.80 µg g^{-1}. Anagha et al. (2022) reported that in the tissue of *S. virgulata* sea urchins collected from the Kerala coast in South India, the Pb concentration level was 15.21 ± 0.91 µg g^{-1}.

Manganese (Mn) has a very important place in human physiology, is a cofactor of various enzymes and when it is lacking in biological systems, it causes serious skeletal and reproductive anomalies. In the current study, manganese levels were determined to be between 0.028 ± 0.007 – 0.406 ± 0.242 µg g^{-1}. The highest levels of manganese (0.406 ± 0.242 µg g^{-1}) were determined in the crusts of the samples from the Iskenderun site (Table 3). The lowest Mn value was also detected in the soft tissue (0.028 ± 0.007 µg g^{-1}) of the samples from the Iskenderun site. The difference in Mn concentrations between tissues and sites was found to be statistically insignificant ($p > 0.05$). The levels of Mn accumulation in tissues were listed as shell > gonad > soft tissue and shell > soft tissue > gonad, respectively, at the Iskenderun and Arsuz sites.

Iron (Fe), which is the main source of animal foods, is an essential metal for human health and its deficiency causes anemia. In our study, iron (Fe) levels varied between 1.202 ± 0.129 – 57.166 ± 5.018 µg g^{-1}. The highest Fe concentration was found in the shells of the sea urchin samples from the Arsuz site (57.166 ± 5,018 µg g^{-1}), and the lowest in the soft tissue of the samples from the Iskenderun site (1.202 ± 0.129 µg g^{-1}) (Table 3). The difference between the Fe concentration levels in the shell tissue of individuals from the İskenderun and Arsuz sites was determined to be significant ($p < 0.05$). Fe accumulation levels in the tissues were ranked as shell > gonad > soft tissue for both sites. Warnau et al. (1998) reported that Fe concentration levels in *P. lividus* gonad tissues collected from the Calvi, Ischia and Marseille sites were 26 ± 12 – 60 ± 14 µg g^{-1}, 65 ± 20 – 119 ± 66 µg g^{-1} and 23 ± 9 – 45 ± 22 µg g^{-1}, respectively. Fe concentrations in the shell were reported to be between 1.71 ± 0.74 – 5.66 ± 3.57 µg g^{-1}, 4 ± 1.99 – 13.9 ± 11.2 µg g^{-1} and 2.77 ± 1.83 – 7.82 ± 2.06 µg g^{-1}, respectively. Soualili et al. (2008) reported that Fe concentration levels *in P. lividus* gonad collected from the Algiers Beach, Tamentfoust and Sidi-Fredj sites in Algiers bay were 19.3 ± 19.7 – 73.8 ± 35.5 µg g^{-1}, 112.6 ± 66 – 113 ± 37.6 µg g^{-1} and 92.7 ± 78.8 – 71.1 ± 54.8 µg g^{-1} in male-female individuals, respectively.

One of the important metals for human health is zinc (Zn). Zn is an essential trace element for both animals and humans. It is necessary for the transport of carbon dioxide and the use of vitamin A in the body (EPA 2014). Zn concentrations in sea urchin tissues were found to vary between 0.276 ± 0.051 – 2.650 ± 0.301 μg g^{-1}. The highest Zn level was detected in the soft tissue (2,650 ± 0.301 μg g^{-1}) of sea urchin samples from the Arsuz site. The lowest Zn level was determined in the gonad tissue (0.276 ± 0.051 μg g^{-1}) of the samples from the Iskenderun site (Table 3). The difference in Zn metal accumulations between sites was determined not to be significant ($p > 0.05$), while the difference between mantle tissue and other tissues was significant ($p < 0.05$). The Zn concentrations calculated in the tissues were the same at Iskenderun and Arsuz sites and were ranked as soft tissue > shell > gonad. However, Zn is not a good indicator of pollution, as it is an important element for animal metabolism and may be present in tissues in transiently high concentrations (Hambridge et al. 1986). Warnau et al. (1998) investigated the metal accumulation levels of Zn, Cd, Pb, Fe, Cr, Cu and Ti in different tissues of *P. lividus* sea urchins collected from 3 different points (Calvi, Ischia and Marseille) in the Mediterranean region. The researchers reported that the Zn levels in the gonad tissues of sea urchins collected from the Calvi, Ischia and Marseille sites were between 67 ± 72 – 383 ± 253 μg g^{-1}, 53 ± 51 – 271 ± 186 μg g^{-1} and 55 ± 38 – 168 ± 134 μg g^{-1}, respectively. Zn accumulation levels in the shell were reported to be between 1.82 ± 0.64 – 3.86 ± 0.41 μg g^{-1}, 1.37 ± 0.24 – 3.92 ± 0.22 μg g^{-1}, and 1.69 ± 0.34 – 4.09 ± 0.56 μg g^{-1}, respectively. Soualili et al. (2008) reported that Zn concentration levels in *P. lividus* gonad tissues collected from the Algiers Beach, Tamentfoust, and Sidi-Fredj sites in Algiers Bay were 32.9 ± 13.5 – 385.5 ± 344.1 μg g^{-1}, 76.1 ± 172.2 – 538.2 ± 324.3 μg g^{-1} and 52.9 ± 73.2 – 366.9 ± 178.3 μg g^{-1} in male-female individuals, respectively. Rouane-Hacene et al. (2018) reported the levels of Zn concentrations in whole soft body tissue of *P. lividus* sea urchins taken from the Oran Harbour, Ain Defla and Hadjaj sites in Algeria as 114.93 ± 3.59 μg g^{-1}, 66.23 ± 2.11 μg g^{-1} and 56.22 ± 1.33 μg g^{-1}, respectively. Bonsignore et al. (2018) reported that the level of Zn concentration in the gonad tissue of *P. lividus* sea urchins collected from the Rosignano region of the Tuscany coast was 20.4 ± 9.25 μg g^{-1}. Anagha et al. (2022) reported that in the tissue of *S. virgulata* sea urchins collected from the Kerala coast in South India, the Zn concentration level was 15.45 ± 0.61 μg g^{-1}.

The Pearson correlation coefficient analysis to find a significant relationship between the metals in the tissues of the *D. setosum* sea urchins collected from the İskenderun and Arsuz sites of the İskenderun Bay is given in Table 4. Significant positive and negative correlations were observed between metals. It has been determined that there are important positive and negative correlations among metals and significant correlations at the 0.01 level (2-tailed) between Cr, Co, Ni, Pb, Mn and Fe. It was determined that there were significant linear and positive correlations between metals.

Positive correlations were observed for Al-Cr (r = 0.922), Al-Co (r = 0.945), Al-Ni (r = 0.978), Al-Pb (r = 0.752), Al-Mn (r = 0.824), Al-Fe (r = 0.988), Cr-Co (r = 0.873), Cr-Ni (r = 0.929), Cr-Pb (r = 0.856), Cr- Fe (r = 0.925), Co-Ni (r = 0.951), Co-Mn (r = 0.916), Co-Fe (r = 0.940) Ni-Pb (r = 0,753) Ni-Mn (r = 0.855), Ni-Fe (r = 0.959), Pb-Fe (r = 0.787) and Mn-Fe (r = 0.799) metals (Table 4). In general, this study showed different correlations between different metals for many reasons. This suggested that the reason for this would depend on various factors such as body metabolism, exposure time, trophic level or dietary habits.

Table 4

Pearson correlation coefficient matrix of metals detected in *D. setosum* species collected from the İskenderun and Arsuz sites

	Al	Cr	Co	Ni	Cd	Pb	Mn	Fe	Zn
Al	1								
Cr	.922**	1							
Co	.945**	.873**	1						
Ni	.978**	.929**	.951**	1					
Cd	.599**	.501*	.619**	.637**	1				
Pb	.752**	.856**	.713**	.753**	.468	1			
Mn	.824**	.702**	.916**	.855**	.577*	.567*	1		
Fe	.988**	.925**	.940**	.959**	.627**	.787**	.799**	1	
Zn	-.464	-.602**	-.442	-.466	-.038	-.625**	-.231	-.468	1

* Correlation is significant at the 0.05 level (2-tailed); ** Correlation is significant at the 0.01 level (2-tailed)

4. Conclusion

The concentrations of Al, Cd, Co, Ni, Pb, Cr, Fe, Mn, and Zn in the shell, gonad, and soft tissues of the long-spined black sea urchin (*D. setosum*) shell, gonad, and soft tissues of the exposed sites were within the limits of international standards collected by the FAO. The results of the present study provide valuable information on the toxic metal concentrations of *D. setosum* sea urchins obtained from İskenderun Bay. Therefore, the tissue of sea urchins from the İskenderun bay does not create any health risks for human consumers with respect to these elements.

The results confirmed that the gonad and shell appeared to be good model tissues for the identification of the regions of the İskenderun bay exposed to metallic pollutants. On the other hand, soft tissues usually manifested lower metal concentrations overall. For this reason, care should be taken to continuously conduct biomonitoring studies to monitor heavy metal levels.

Acknowledgments

The author thanks Dr. Önder DUYSAK for kindly help with statistical analysis of the study. The author also would like to express his deep sorrow for Serbay BUCAK helped with the study, the esteemed staff of Hatay Mustafa Kemal University Technology and Research & Development Center, who lost his life in the earthquake on 6th February 2023. The author offers his deepest condolences to Serbay's family.

References

Anagha, B., Athira, P. S., Anisha, P., Charles, P. E., Anandkumar, A., & Rajaram, R. (2022). Biomonitoring of heavy metals accumulation in molluscs and echinoderms collected from southern coastal India. *Marine Pollution Bulletin, 184*, 114169. https://doi.org/10.1016/j.marpolbul.2022.114169 PMID:36201985

Ablanedo, N., Gonzalez, H., Ramirez, M., & Torres, I. (1990). Evaluation del erizo de mar *Echinometra lucunter* coma indicador de contaminacion por metales pesados, Cuba. *Aquatic Living Resources, 3*, 113–120. https://doi.org/10.1051/alr:1990011

Al Najjar, T., Al Tawaha, M., Wahsha, M., & Hilal, A. A. (2018). Heavy metals in the sea urchin *Diadema setosum* from the Gulf of Aqaba. *Fresenius Environmental Bulletin, 27*, 4149–4155.

Buarung, J., Tongumpon, S., Pairagsa, S., Milinthalek, J., & Yeemin, T. (1998). Heavy metal concentrations in a sea urchin, *Diadema setosum* from Khang khao island, Chonburi province. 24. Congress on Science and Technology of Thailand, Bangkok (Thailand) 1998 (p. 434-435), AGRIS - International System for Agricultural Science and Technology. https://agris.fao.org/search/en/providers/122623/records/64723a69e17b74d2224ef4a1

Bonsignore, M., Salvagio Manta, D., Mirto, S., Quinci, E. M., Ape, F., Montalto, V., Gristina, M., Traina, A., & Sprovieri, M. (2018). Bioaccumulation of heavy metals in fish, crustaceans, molluscs and echinoderms from the Tuscany coast. *Ecotoxicology and Environmental Safety, 162*, 554–562. https://doi.org/10.1016/j.ecoenv.2018.07.044 PMID:30029101

Campbell J. W. (1967) Echinoderms: Physiology of Echinodermata. Richard A. Boolootian, Ed. Interscence (Wiley), New York, 1966. pp 840. Science156,54-55 https://doi.org/10.1126/science.156.3771.54

Choi, J. Y., Habte, G., Khan, N., Nho, E. Y., Hong, J. H., Choi, H., Park, K. S., & Kim, K. S. (2014). Determination of toxic heavy metals in Echinodermata and Chordata species from South Korea. *Food Additives & Contaminants. Part B, Surveillance, 7*(4), 295–301. https://doi.org/10.1080/19393210.2014.932311 PMID:24916139

Çağiltay, F., Gökoğlu, M., Yılmaz, R., & Yıldız, A. (2022). Some Fish Species Showing Commensalism Traits with Long-Spined Sea Urchin (*Diadema setosum* Leske, 1778) in Gulf of Antalya. *Indonesian Fisheries Research Journal, 28*(1), 33–39.

Downs, R. T., Bartelmehs, K. L., Gibbs, G. V., & Boisen, M. B. (1993). Interactive software for calculating and displaying X-ray or neutron powder diffractometer patterns of crystalline materials. *The American Mineralogist, 78*(9-10), 1104–1107.

Duysak, Ö., Mazlum, Y., & Uğurlu, E. (2021). Heavy metal and Al bioaccumulation in the anemone *Actinia equina* Linnaeus, 1758 (Cnidaria: Actiniidae) from Iskenderun Bay, North-Eastern Mediterranean, Turkey. *Su Ürünleri Dergisi, 38*(2), 161–166. https://doi.org/10.12714/egejfas.38.2.04

Duysak, Ö., & Uğurlu, E. (2020). Heavy metal accumulation in different tissues of cuttlefish (*Sepia officinalis* L., 1758) in İskenderun Bay. *Journal of Anatolian Environmental and Animal Sciences, 5*(4), 556–562. https://doi.org/10.35229/jaes.750466

Duysak, Ö., & Dural, M. (2015). Heavy metal concentrations in tissues of short-finned squid *Illex coindetii* (Cephalopoda: Ommastrephidae) (Vérany, 1839) in Iskenderun Bay, North-Eastern Mediterranean. *Pakistan Journal of Zoology, 47*(2), 447–453.

Duysak, O., & Ersoy, B. (2014). A Biomonitoring Study: Heavy Metals in *Monodonta turbinata* (Mollusca: Gastropoda) From Iskenderun Bay North-Eastern Mediterranean. *Pakistan Journal of Zoology, 46*(5), 1317–1322.

EPA. (2014). EPA, U.S. Environmental Protection Agency. Manganese compounds. www.epa.gov/ttn/atw/hlthef/

manganes.html Accessed 27.12. 2022.

FAO/WHO. (2011). World Health Organization, Technical Report Series 966, Geneva: 2011.Seventy-fourth Report of the Joint Expert Committee on Food Additives. Evaluation of certain food additives and contaminants. http://www.fao.org/3/a-at873e.pdf Accessed 20.12. 2022.

Faroon, O., Ashizawa, A., Wright, S., Tucker, P., Jenkins, K., Ingerman, L., & Rudisill, C. (2012). Toxicological profile for cadmium. Atlanta: Agency for Toxic Substances and Disease Registry (ATSDR), 246-258. PMID: 24049863 https://www.ncbi.nlm.nih.gov/books/NBK158838/

Flammang, P., Warnau, M., Temara, A., Lane, D. J., & Jangoux, M. (1997). Heavy metals in *Diadema setosum* (Echinodermata, Echinoidea) from Singapore coral reefs. *Journal of Sea Research, 38*(1-2), 35–45. https://doi.org/10.1016/S1385-1101(97)00033-6

Hambridge, K. M., Casey, C. E., & Krebs, N. F. (1986). Zinc. In Trace Elements in Human and Animal Nutrition (5th ed., Vol. 2, pp. 1–137). Academic Press. https://doi.org/10.1016/B978-0-08-092469-4.50005-4

Hernández, O. D., Gutiérrez, Á. J., González-Weller, D., Lozano, G., Melón, E. G., Rubio, C., & Hardisson, A. (2010). Accumulation of toxic metals (Pb and Cd) in the sea urchin *Diadema aff. antillarum* Philippi, 1845, in an oceanic island (Tenerife, Canary Islands). *Environmental Toxicology, 25*(3), 227–233. https://doi.org/10.1002/tox.20487 PMID:19399849

Kahvecioğlu, Ö., Kartal, G., Güven, A., & Timur, S. (2003). Metallerin çevresel etkileri-I. *Metalurji Dergisi, 136*, 47–53.

Koçak, C., Katagan, T., & Ozcan, T. (2005). Anomura of the Iskenderun Bay region (southeastern Turkey). *Crustaceana, 78*(2), 247–252. https://doi.org/10.1163/1568540054020578

Lee, J. W., Choi, H., Hwang, U. K., Kang, J. C., Kang, Y. J., Kim, K. I., & Kim, J. H. (2019). Toxic effects of lead exposure on bioaccumulation, oxidative stress, neurotoxicity, and immune responses in fish: A review. *Environmental Toxicology and Pharmacology, 68*, 101–108. https://doi.org/10.1016/j.etap.2019.03.010 PMID:30884452

Ma, Y., Cohen, S. R., Addadi, L., & Weiner, S. (2008). Sea urchin tooth design: An "all-calcite" polycrystalline reinforced fiber composite for grinding rocks. *Advanced Materials, 20*(8), 1555–1559. https://doi.org/10.1002/adma.200702842

Ma, Y. R., Weiner, S., & Addadi, L. (2007). Mineral deposition and crystal growth in the continuously forming teeth of sea urchins. *Advanced Functional Materials, 17*(15), 2693–2700. https://doi.org/10.1002/adfm.200700234

Nelson, B. V., & Vance, R. R. (1979). Diel foraging patterns of the sea urchin *Centrostephanus coronatus* as a predator avoidance strategy. *Marine Biology, 51*(3), 251–258. https://doi.org/10.1007/BF00386805

Otter, G. W. (1932). Rock-burrowing echinoids. *Biological Reviews of the Cambridge Philosophical Society, 7*(2), 89–107. https://doi.org/10.1111/j.1469-185X.1962.tb01037.x

Öndes, F., Alan, V., Kaiser, M. J., & Güçlüsoy, H. (2022). Spatial distribution and density of the invasive sea urchin *Diadema setosum* in Turkey (eastern Mediterranean). *Marine Ecology (Berlin), 43*(6), 12724. https://doi.org/10.1111/maec.12724

Rouane-Hacene, O., Boutiba, Z., Benaissa, M., Belhaouari, B., Francour, P., Guibbolini-Sabatier, M. E., & Faverney, C. R. D. (2018). Seasonal assessment of biological indices, bioaccumulation, and bioavailability of heavy metals in sea urchins *Paracentrotus lividus* from Algerian west coast, applied to environmental monitoring. *Environmental Science and Pollution Research, 25*, 11238–11251. https://doi.org/10.1007/s11356-017-8946-0 PMID:28397119

Soualili, D., Dubois, P., Gosselin, P., Pernet, P., & Guillou, M. (2008). Assessment of seawater pollution by heavy metals in the neighbourhood of Algiers: Use of the sea urchin, *Paracentrotus lividus*, as a bioindicator. *ICES Journal of Marine Science, 65*(2), 132–139. https://doi.org/10.1093/icesjms/fsm183

Uğurlu, E., & Duysak, Ö. (2023). A study on the extraction of chitin and chitosan from the invasive sea urchin *Diadema setosum* from Iskenderun Bay in the Northeastern Mediterranean. *Environmental Science and Pollution Research, 30*, 21416–21424. https://doi.org/10.1007/s11356-022-23728-9 PMID:36271066

Uğurlu, E., & Duysak, Ö. (2022). Length-Weight Relationships and Gonadosomatic Index of Invasive Sea Urchin *Diadema setosum* (Leske, 1778) from Iskenderun Bay, North-Eastern Mediterranean, Turkey. *Osmaniye Korkut Ata Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, 5(3), 1579-1591. https://doi.org/10.47495/okufbed.1078408

Warnau, M., Ledent, G., Temara, A., Bouquegneau, J.-M., Jangoux, M., & Dubois, I. (1995a). Heavy metals in *Posidonia oceanica* and *Paracentrotus lividus* from seagrass beds of north-western Mediterranean. *The Science of the Total Environment, 171*, 95–99. https://doi.org/10.1016/0048-9697(95)04721-8

Warnau, M., Ledent, G., Temara, A., Jangoux, M., & Dubois, I. (1995b). Experimental contamination of the echinoid *Paracentrotus lividus* by cadmium: Influence of exposure mode and distribution of the metal in the organism. *Marine Ecology Progress Series, 116*, 117–124. https://doi.org/10.3354/meps116117

Warnau, M., Biondo, R., Temara, A., Bouquegneau, J. M., Jangoux, M., & Dubois, P. (1998). Distribution of heavy metals in the echinoid *Paracentrotus lividus* from the Mediterranean *Posidonia oceanica* ecosystem: Seasonal and geographical variations. *Journal of Sea Research, 39*(3-4), 267–280. https://doi.org/10.1016/S1385-1101(97)00064-6