

Trace metal levels in the edible tissues of sea cucumbers (*Holothuria tubulosa* and *Holothuria polii*) from Sardinia (Western Mediterranean)

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Abstract

Sea cucumbers represent an important part of the diet in Asian and Pacific regions and are also used in traditional medicine. These habits have led to the overexploitation of local sea cucumber populations in these areas, driving the pursuit of new stock regions, such as Mediterranean areas. In Italy, contrarily to that observed for other Mediterranean countries, the exploitation of sea cucumber stocks is not extensive, which opens a new market opportunity. Thus, from a food safety perspective, this work aims at reporting the first assessment of trace metal concentrations (As, Cd, Cr, Cu, Hg, Ni, Pb) in the edible tissues of *Holothuria polii* and *Holothuria tubulosa* collected in Sardinia, the second-largest island in the Mediterranean Sea. Metal concentrations found in *H. polii* were generally higher than in *H. tubulosa*. However, in both species, they were lower than those reported for other areas of the Western Mediterranean. Cd, Hg, and Pb were below the limits established for seafood in Europe. As concentrations were in the range of those measured in other commercial seafood species in the Mediterranean. Thus, these species may be harvested and traded to fulfil the demands of local and international markets.

Introduction

The aim of a healthy lifestyle has triggered the search of new marine resources to be included in the diet and for medicinal purposes (e.g., Suleria *et al.* 2015). Sea cucumbers play an important role as a food and in traditional medicine in Asian and Pacific regions (catches of 20,000-40,000 tons/year) (Toral-Granda *et al.* 2008). Sea cucumber farming and fishing is not a

common practice outside Southeast Asian and Pacific countries, but the overexploitation of local sea cucumber populations in these areas has driven the pursuit of new stock regions (Purcell *et al.* 2013; González-Wangüemert *et al.* 2018a). Thus, due to their commercial importance, currently, several Mediterranean countries (e.g., Turkey, Spain, Greece) are exploiting local sea cucumber resources for exporting (Conand 2017; González-Wangüemert *et al.* 2018a). In Italy, contrarily to that observed for other Mediterranean countries, the exploitation of sea cucumber stocks is not extensive, which opens a new market opportunity (Sicuro and Levine 2011). However, between 2015 and 2017, the illegal fishing of 53 tons of sea cucumbers was reported in Italy, specifically in Sardinia and the Puglia region (Meloni and Esposito 2018). The species displaying a greater commercial interest are *Holothuria polii*, *H. tubulosa*, *H. mammata* and *Parastichopus regalis* (González-Wangüemert *et al.* 2018a). Previous knowledge of these species refers mainly to nutritional and biometric studies (e.g., Aydin 2018), while few works have been published from a food safety perspective (e.g., Sicuro *et al.* 2012; González-Wangüemert *et al.* 2018b). Sea cucumbers belonging to the genus *Holothuria*, have been considered as useful biomonitors of environmental quality (Culha *et al.* 2016; Aydin *et al.* 2017). This is favored by their local-scale migratory movements, relatively long life-span and higher bioaccumulation capacity of pollutants than other organisms (Parra-Luna *et al.* 2020).

Seafood consumption is the major dietary source of exposure to arsenic (As), cadmium (Cd), mercury (Hg) and lead (Pb), which have non-established biological functions and are considered non-essential metals (e.g., Domingo 2016). The potential danger of chemicals to the biota present in different ecosystems and human health has been recognized by the implementation of several national and international laws. Accordingly, in Europe, maximum levels for Cd, Hg and Pb in seafood are set under Regulation No 1881/2006 (European Commission 2006) and posterior amendments. Regarding arsenic, Francesconi and Edmonds (1996) reported that its presence in seafood mainly consists of complex organic compounds that are generally non-toxic. However, the content of inorganic arsenic, a well-characterized carcinogen, should be considered in toxicity assessments (Luvonga *et al.* 2020).

Sardinia (Italy) is the second-largest island in the Mediterranean Sea. Information about the levels of trace metals in the tissues

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of autochthonous sea cucumber populations in this area of the Western Mediterranean is lacking. Thus, in the present study, *H. tubulosa* and *H. polii* sea cucumbers were collected in the Gulf of Cagliari (Sardinia) aiming to determine the levels of As, Cd, Cr, Cu, Hg, Ni, and Pb in their edible tissues, to compare them with the limits set by current seafood regulations. Moreover, comparing the obtained results with the most relevant sea cucumber data published in the Mediterranean in the last 20 years, this work provides an overall picture of trace metal concentrations in sea cucumbers in the Western Mediterranean area.

Materials and methods

Study area

Two areas, Giorgino and Foxi, were identified in the Gulf of Cagliari (Sardinia) as potential sites for the fishing of sea cucumbers based on the low levels of *Escherichia coli* in coastal waters (European

Commission 2015; ARPAS 2017) (Figure 1). The Gulf of Cagliari is in the southern coast of Sardinia. It is bordered on the north by the urban area of Cagliari (about 400,000 inhabitants) and on the west by a heavily industrialized zone, which includes the largest oil refinery in the Mediterranean. Giorgino (39.165600 N-9.080100 E) on the west side of the gulf, is located in front of a loading and unloading terminal utilized by several medium and small size chemical companies. Foxi (39.217500 N-9.233167 E) is in the north side, in front of a touristic area only crowded in the summertime and not exposed to industrial pollutants or maritime traffic. Further information, regarding the environmental assessment of the Gulf of Cagliari, based on the heavy metal accumulation in sediments, and the geomorphological characteristics of the area can be found in Schintu *et al.* (2016) and Buosi *et al.* (2019), respectively.

Sampling campaigns

Sea cucumbers were collected in June and July 2017. *Holothuria polii* (Delle Chiaje 1823) was exclusively found in Foxi, while *Holothuria tubulosa* (Gmelin 1788) was only present in Giorgino. Nineteen specimens were fished in Foxi and fifteen in Giorgino, at a depth of 7-10 m. Sea cucumbers were collected by scuba divers and delivered to the laboratory in cooled sampling bags. Samples were stored in the freezer, at -20°C, until analysis. Physico-chemical parameters (Foxi: Temperature 24.4°C, Salinity 35.8 ppt, pH 8.3, Dissolved oxygen >90%; Giorgino: Temperature 24.8°C, Salinity 35.7 ppt, pH 8.1, Dissolved oxygen >90%) were measured in situ using a YSI multiprobe (Geoves, mod. MICROHYD1, B&C electronics). The length of *H. polii* and *H. tubulosa* ranged from 9.1 to 13.0 cm (\bar{x} = 11.4±1.2 cm) and from 12.1 to 23.1 cm (\bar{x} = 18.6±2.7 cm), respectively; the weight ranged from 14.0 to 39.8 g (\bar{x} = 26.6±5.7 g) for *H. polii* specimens and from 31.9 to 58.0 g (\bar{x} = 48.4±6.5 g) for *H. tubulosa*.

Sample processing and analysis

Following routine laboratory procedures when working with trace metals, all the material was soaked overnight in a 10% HNO₃ acid bath and then rinsed with Milli-Q (Zeener Power I Water Purification System, Human Corporation, South Korea) water before use.

The preparation of samples followed the procedure of Culha *et al.* (2016). Briefly, sea cucumbers were thawed, washed with tap and Milli-Q water and drained before dissection. The organisms were dissected longitudinally, from the anus to the mouth, and left to dry on absorbent paper. Visceral organs, gonads and pulmonary apparatus were completely removed. Edible muscle tissue was lyophilized using a LIO-5PDGT freeze-dryer (5Pascal, Italy) and consecutively reduced to powder. Following USEPA (1996), each sample (0.3 g) was transferred to 100-mL Teflon digestion vessels, 7 mL of HNO₃ (Suprapur 65%, Merck Millipore, Germany) and 1 ml of H₂O₂ (Suprapur 30%, Merck Millipore,

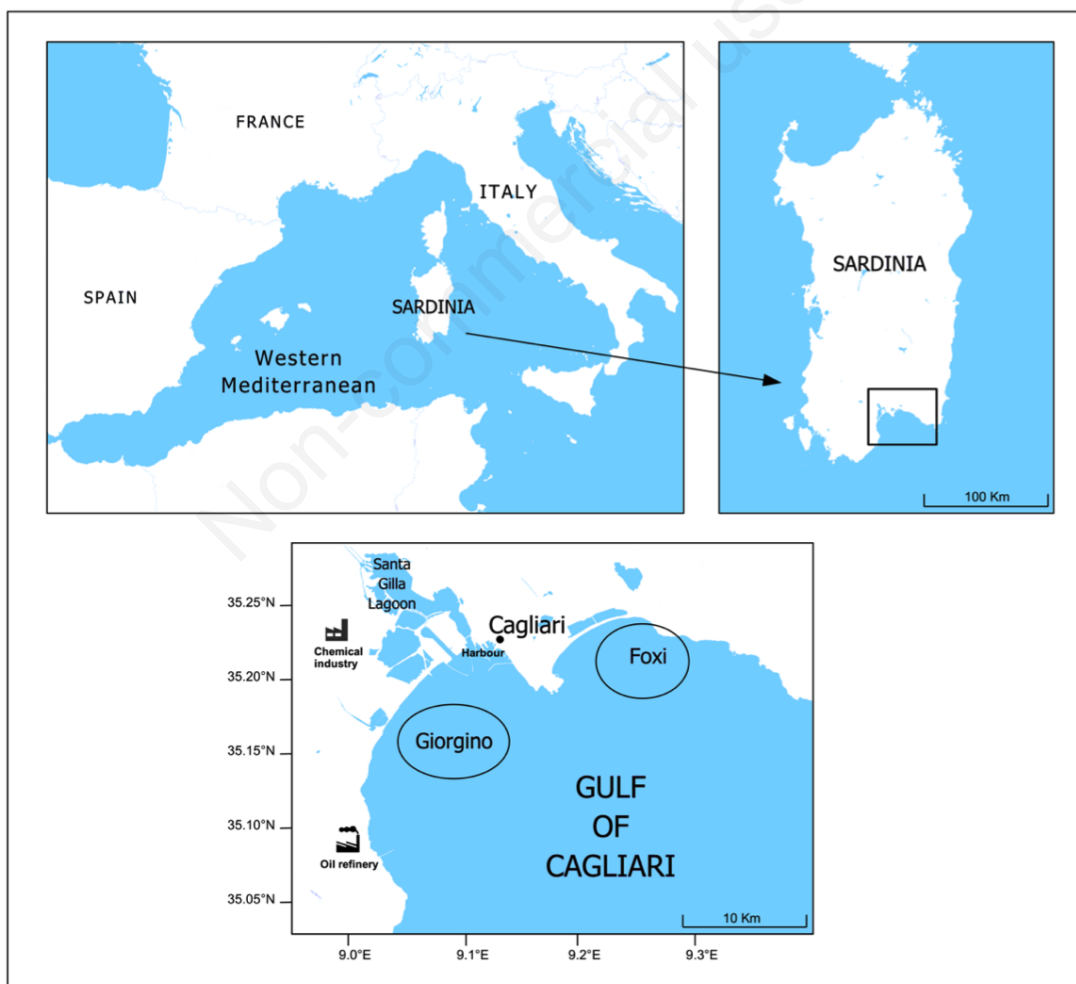


Figure 1. Sampling sites of *Holothuria polii* (Foxi) and *Holothuria tubulosa* (Giorgino) in the Gulf of Cagliari (Sardinia, Western Mediterranean).

Germany) were added and samples were left to rest for 15-20 min. Samples were microwaved (see Schintu *et al.* 2016) and then, after cooling, samples were filtered through Whatman 41 filters (Whatman International Ltd., Maidstone, UK), made up to 50 mL with Milli-Q water and analyzed. Blanks were prepared following the same procedure, but without the addition of the samples.

Metal concentrations (As, Cd, Cr, Cu, Ni and Pb) were determined, following USEPA (1994), by graphite furnace atomic absorption spectroscopy (GFAAS Varian GTA120-AA240Z) using the Zeeman background correction. Total Hg was measured using a DMA-80 Direct Mercury Analyzer (Milestone, US). The quality control (QC) of the analysis was assured utilizing multi-element solutions that were prepared analogously to the calibration samples. The instrumental detection limits were as follows: $1 \mu\text{g L}^{-1}$ for As, $0.05 \mu\text{g L}^{-1}$ for Cd, $1 \mu\text{g L}^{-1}$ for Cr, $0.2 \mu\text{g L}^{-1}$ for Cu, $0.5 \mu\text{g L}^{-1}$ for Hg, $1 \mu\text{g L}^{-1}$ for Ni and $0.5 \mu\text{g L}^{-1}$ for Pb. The accuracy and precision (QA) of the analyses was checked by analyzing seven replicates of the certified reference material (CRM) IAEA-461 ('Trace elements and methylmercury mass fractions in clam *Gafrarium tumidum* sample'). The accuracies were within 10% of the certified values for all elements. Metal concentrations were expressed on a mg kg^{-1} dry weight basis.

Statistical analysis

Metal concentrations measured in the tissues of sea cucumbers are reported as the mean \pm standard error of the mean (SEM). Significant differences between species were determined by Student's t-test using the open-source Jamovi software (www.jamovi.org). Grubb's test was used for detecting outliers and corresponding values were not used for calculating the average

concentration of metals. Shapiro-Wilk and Levene's tests were applied for the assessment of normality and homogeneity of variance, respectively. Significance level was set at $\alpha=0.05$.

Results and discussion

Results of metal concentrations measured in the edible tissues of sea cucumbers are presented in Figure 2. Considering that accumulation processes in these sea cucumber species might be different and that *H. polii* was only found in the sampling area Foxi and *H. tubulosa* in Giorgino, it is difficult to compare both species. However, in general, *H. polii* showed higher trace metal concentrations than *H. tubulosa*. The concentration of trace metals in *H. polii* decreased in the order $\text{As} > \text{Cu} > \text{Pb} > \text{Cr} > \text{Ni} > \text{Cd} > \text{Hg}$, while in *H. tubulosa* the order was $\text{As} > \text{Cu} > \text{Cr} > \text{Pb} > \text{Ni} > \text{Hg} > \text{Cd}$. Arsenic and Cu were the elements found in the highest concentrations in both species. Total As concentrations ranged from 18.3 to 30.5 mg kg^{-1} dw in *H. polii*, and from 13.2 to 24.8 mg kg^{-1} dw in *H. tubulosa*. Cu concentrations showed no statistically significant differences between the two species, while *H. polii*, collected in Foxi, presented significantly ($p < 0.001$) higher concentrations of As, Cd, Cr, Ni, and Pb than *H. tubulosa* from Giorgino. Contrarily, *H. tubulosa* presented significantly ($p < 0.001$) higher Hg concentrations than *H. polii*. Since the fishing area of Foxi, in the north side of the Gulf, is less exposed to heavy metal pollution than the west side (ARPAS 2018), the higher metal concentrations present in sea cucumbers of this area is unexpected and requires further understanding of the processes taking place in that part of the gulf. On the other hand, the higher Hg

concentrations in *H. tubulosa* can be explained by the exposition for decades to the legacy Hg pollution arising from a dismantled chlor-alkali plant located in the Santa Gilla Lagoon (Atzori *et al.* 2018; Figure 1). Among all studied metals, only As levels in the tissues of sea cucumbers were similar to those previously found in sediments of the Gulf of Cagliari (Schintu *et al.* 2016; 14-25 mg kg^{-1} dw). This might be explained by the use of sediments as food source and the lack of regulation of this metal (*e.g.*, Storelli *et al.* 2001; Culha *et al.* 2016).

There is no previous information about trace metal concentrations in sea cucumbers in this part of the Mediterranean, thus, the levels found in the present study were compared with those measured in other areas of the Mediterranean (Table 1). In general, metal concentrations found in this study were lower. However, to assess the potential health risk of consuming these species, wet weight metal concentrations (mg kg^{-1} ww) were calculated, using the measured water content corresponding to *H. tubulosa* (81-

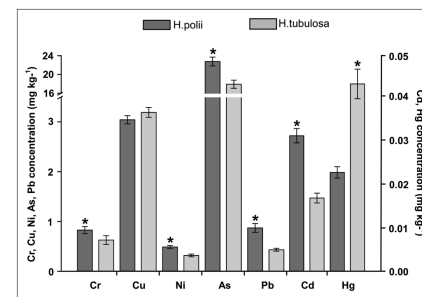


Figure 2. Trace metal concentrations (mg kg^{-1} dw) measured in the edible tissues of *H. polii* and *H. tubulosa* collected in the Gulf of Cagliari. Data are expressed as the mean \pm SEM. *Denotes a statistically significant difference between species (Student's t-test, $\alpha=0.05$).

Table 1. Trace metal concentration (mg kg^{-1} dw) in the edible tissues of *H. tubulosa* (HT) and *H. polii* (HP) from other Mediterranean areas.

Authors - Location	As	Cd	Cr	Cu	Hg	Ni	Pb
Storelli <i>et al.</i> (2001) – Southern Adriatic Sea	-	0.04 (HP)	-	71 (HP)	0.96 (HP)	-	1.3 (HP)
Warnau <i>et al.</i> (2006) – Calvi (Corse), Ischia (Italy), Marseille (France)	-	0.4-2.8 (HT)	-	0.8-5.8 (HT)	-	-	1.2-18.0 (HT)
Sicuro <i>et al.</i> (2012) – Southern Adriatic Sea	33.0 (HP) 22.0 (HT)	0.07 (HP) 0.07 (HT)	0.88 (HP) 0.86 (HT)	2.5 (HP) 2.5 (HT)	-	-	0.65 (HP) 1.2 (HT)
Culha <i>et al.</i> (2016) – Dardanelles Strait (Turkey)	-	0.09-0.63 (HT)	-	0.02-6.6 (HT)	-	8.3-24.2 (HT)	0.7-5.3 (HT)
Aydin <i>et al.</i> (2017) – Izmir coast (Turkey)	1.3-13.3	0.02-0.38	0.05-4.3	0.35-1.27	-	0.01-0.65	0.07-0.92
González-Wanguemert <i>et al.</i> (2018b) – Murcia (Spain)	-	0.09 (HP) nd (HT)	0.77 (HP) 15.2 (HT)	-	-	nd (HP) 2.5 (HT)	3.1 (HP) 6.5 (HT)
Current study – Sardinia (Italy)	22.9 (HP) 18.0 (HT)	0.03 (HP) 0.02 (HT)	0.84 (HP) 0.64 (HT)	3.1 (HP) 3.2 (HT)	0.023 (HP) 0.043 (HT)	0.50 (HP) 0.33 (HT)	0.88 (HP) 0.44 (HT)

nd, non-detected.

Table 2. Mean (\pm SD) metal concentrations (mg kg^{-1} ww) measured in *H. tubulosa* and *H. polii* collected in the Gulf of Cagliari and maximum limit concentrations set by the European Commission (2006) seafood regulation.

	As	Cd	Cr	Cu	Hg	Ni	Pb
<i>H. tubulosa</i> (mg kg^{-1} ww)	2.2 \pm 0.5	0.002 \pm 0.001	0.07 \pm 0.03	0.41 \pm 0.08	0.005 \pm 0.002	0.04 \pm 0.01	0.05 \pm 0.01
<i>H. polii</i> (mg kg^{-1} ww)	3.7 \pm 0.7	0.005 \pm 0.001	0.13 \pm 0.05	0.50 \pm 0.11	0.004 \pm 0.001	0.08 \pm 0.02	0.15 \pm 0.07
EC (mg kg^{-1} ww)	-	1.0	-	-	0.5	-	1.5

94%) and *H. polii* (79-92%) specimens, and compared with the maximum permissible limits set for human consumption (Table 2). The concentrations of Cd, Hg, and Pb were below the maximum levels set for seafood by the European Commission (2006) and posterior amendments. There are not guideline values for As in seafood in Europe, but total As concentrations found in tissues of *H. polii* (3.7 \pm 0.7 mg kg^{-1} ww) and *H. tubulosa* (2.2 \pm 0.5 mg kg^{-1} ww) were similar to those reported by Ferrante *et al.* (2019) in commonly consumed fresh seafood (mollusks 4.0 \pm 3.6 mg kg^{-1} ww; pelagic fish 6.5 \pm 7.2 mg kg^{-1} ww; demersal fish 5.1 \pm 5.4 mg kg^{-1} ww), sampled along the Mediterranean coast. Thus, *H. tubulosa* and *H. polii* sea cucumbers found in the Gulf of Cagliari may be suitable for human consumption and their fishing for trading to Asian countries or their inclusion into the local marketplace could be pursued. In Sardinia, in 2015 and 2016, two areas were identified as potential sites for the fishing of sea cucumbers, following EC Regulation 2285/2015 (European Commission 2015). However, in February 2018 their harvesting in Italy was forbidden (IMAFFP 2018), following the precautionary principle, to counteract the effect of illegal fishing (Meloni and Esposito 2018). The banning was revoked at the end of 2019, but the sustainable fishing of sea cucumbers will be only obtained combining severe laws, to combat illegal fishing, with the searching of new harvesting grounds, to reduce the pressure in single fishing areas. The results obtained in the present study open the possibility of including additional harvesting sites in the Western Mediterranean, based on a low level of contaminants, to limit the pressure over specific sea cucumber populations and ensure their long-term sustainable exploitation (*e.g.*, Anderson *et al.* 2010).

Conclusions

The present study provides a first insight into the level of metals found in the edible tissues of sea cucumbers from Sardinia (Western Mediterranean). The results were

compared with the data published in other areas of the Mediterranean in the last 20 years and evaluated for the limits set by food authorities. *H. tubulosa* and *H. polii* sea cucumbers found in the Gulf of Cagliari may be suitable for human consumption, based on the low metal concentrations present in tissues. This opens the possibility of including additional harvesting sites in the Western Mediterranean, contributing to the economic development of local fisheries.

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