



# Heavy metal contamination of the sea cucumber *Holothuria poli* cultured in integrated multi-trophic aquaculture in a multi-use coastal area

Karl Cutajar<sup>\*</sup>, Lynne Falconer, Trevor C. Telfer

Institute of Aquaculture, University of Stirling, Stirling, Scotland, UK

## ARTICLE INFO

### Keywords:

Sea cucumber  
IMTA  
Heavy metals, contaminant transfer  
Bioaccumulation

## ABSTRACT

The accumulation of heavy metals in the edible tissue of the white spot sea cucumber, *Holothuria poli*, revealed the transfer of metal contaminants to sea cucumbers when produced below fish cages in a Mediterranean port area. Sea cucumbers were cultured on the seafloor directly below a fish cage at 0 m, then at 10 m and at 25 m away from the cage, as part of an open-water integrated multi-trophic aquaculture (IMTA) system, and then at a reference site over 1 km from the fish farm, over a one-year period. At the end of the study, sea cucumbers and seafloor sediments were sampled from the IMTA sites near the fish cages, except at 0 m due to mass sea cucumber mortalities within the first month of the study, and again at the reference site. The concentrations of cadmium (Cd), copper (Cu), chromium (Cr), nickel (Ni), and zinc (Zn) were significantly higher in sediments near fish cages than the reference site. Localised enrichment from marine aquaculture could explain the significant concentration of metals in sediments below fish cages that are typically ascribed to their use in aquaculture. Arsenic (As), lead (Pb) and mercury (Hg), which are not associated with commercial fish diets, did not vary between sites. Concentrations of iron (Fe), which is available in commercial diets, were similar near fish cages and at the reference site. The body wall/muscle tissue of the sea cucumber *H. poli* revealed high concentrations of the essential metals Fe and Zn near fish cages and in natural sediments at the reference site. *H. poli* can regulate these essential metals that characterised the edible tissue of the sea cucumbers. Non-essential metals like Hg and Cd had the lowest concentrations of all analysed metals in the sea cucumber tissue. However, the bioaccumulation of toxic metals, Hg and As, reveal the bioavailability of these contaminants in sediments and the propensity of bottom-dwelling sea cucumbers to bioconcentrate these metals, when cultured under a commercial fish cage in IMTA and elsewhere in natural sediments in this industrial environment. *Holothuria poli* did not exhibit bioaccumulation of Cu, Cr, Fe, Ni, Pb and Zn in its body wall/muscle tissue. The bioaccumulation of Hg and As reveal the need to account for the potential effects of farm-level variability throughout longer production cycles and bay-wide dynamics on sediment contamination and bioaccumulation in sea cucumbers until harvest. Site-specific dynamics in ports, whether natural or anthropogenic, can be expected to influence bioaccumulation of metal contaminants and therefore require long-term and fine resolution monitoring for better representation in open-water IMTA production.

## 1. Introduction

The potential of the Mediterranean white spot sea cucumber, *Holothuria poli*, to uptake organic wastes in integrated multi-trophic aquaculture (IMTA) has been validated through growth (Cutajar et al., 2022a) and dietary assimilation of fish farm organic wastes from inshore cage aquaculture (Cutajar et al., 2022b). However, few studies have been published on sea cucumber uptake of contaminants (but see Sicuro et al., 2012; González-Wangüemert, 2018b; Montero et al., 2021;

Marrugo-Negrete et al., 2021). *Holothuria poli* has yet to be commercially cultured; however, as Mediterranean sea cucumber species (e.g. *H. poli* and *Holothuria tubulosa*) become increasingly popular in IMTA research (Tolon et al., 2017; Neofitou et al., 2019; Grosso et al., 2021; Sadoul et al., 2022; Cutajar et al., 2022a, b), knowledge about the bioaccumulation of contaminants in sea cucumbers under fish cages becomes more relevant. Consequently, contamination from complementary integrated aquaculture systems and wider sources need to be addressed (Rosa et al., 2020). This is particularly crucial where

<sup>\*</sup> Correspondence to: Institute of Aquaculture, University of Stirling, Stirling, Scotland FK9 4LA, UK.  
E-mail address: [kac4@stir.ac.uk](mailto:kac4@stir.ac.uk) (K. Cutajar).

<https://doi.org/10.1016/j.aqrep.2024.102102>

Received 20 November 2023; Received in revised form 13 April 2024; Accepted 13 April 2024

Available online 21 April 2024

2352-5134/© 2024 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

aquaculture co-exists with other marine and maritime activities in port areas and industrial bays and considers IMTA as potential means for expansion in these multiple-use coastal spaces found throughout the Mediterranean region, as exemplified in studies by Yucel-Gier et al. (2013) and Israel et al. (2019).

Among the various contaminants, metals are important elements that can change chemical form yet persist in the environment without degradation and be transferred and bioaccumulated along the food chain. Heavy metal exposure has been linked with fish deformities (Sfakianakis et al., 2015) and known to influence the metabolic and physiological behaviour of crustaceans (Barbieri and Paes, 2011). Much less is understood about the acute and chronic effects of metal exposure on deposit-feeding sea cucumbers that spend a lifetime reworking and feeding in seafloor sediments. This is despite sea cucumbers having greater bioaccumulation capacity for metals than most marine organisms (Parra-Luna et al., 2020; Marrugo-Negrete et al., 2021; Montero et al., 2021) and being considered efficient bioindicators of these contaminants in sediments (Aydın et al., 2017).

*Holothuria tubulosa*, another Mediterranean sea cucumber species, tends to accumulate different metals in separate tissues with muscle having a high affinity for iron (Fe) and nickel (Ni), whereas cadmium (Cd), copper (Cu), zinc (Zn) and lead (Pb) tend to accumulate in the mucopolysaccharide-rich body wall tissue of this sea cucumber (Warnau et al., 2006). The toxic nature of metals can have chronic effects on growth and activity of sea cucumbers like *Apostichopus japonicus* (Li et al., 2016) and this can affect their potential production (economic potential) and bioremediation or bioturbation of excess sediment nutrients (ecosystem services), two of the key potential benefits of IMTA. Studies have revealed different physiological responses to metals with Wang et al. (2015) substantiating an elevated burden of Pb bioaccumulation when the sea cucumber *A. japonicus* was fed Pb-supplemented diets under controlled conditions. Pb bioaccumulation in the body wall did not influence growth but the antioxidant capacities decreased after metal exposure (Wang et al., 2015). In other studies, the metals mercury (Hg) (Rabeh et al., 2019; Telahigue et al., 2020) and Zn, Cu, and Cd (Li et al., 2016) induced genotoxicity, oxidative damage and histopathological injuries in various tissues (respiratory tree, intestine, and muscle) of the sea cucumber *Holothuria forskali*.

Most of these metals are commonly found in aquatic environments and have been associated with coastal and maritime activities, including shipping and cage aquaculture (Sutherland et al., 2007; Basaran et al., 2010). In the Mediterranean, surficial sediments directly below and close to fish cages are enriched by metals and trace elements (e.g. Cd and Zn) found as constituents in fish feed and faeces that settle to the seafloor (Kalantzi et al., 2013). Sediment enrichment near fish cages has been attributed to anthropogenic sources of Cd, Pb, Fe, and Zn (Kalantzi et al., 2013). These metals are often linked to the use of antifouling paints not only on fish farms (Basaran et al., 2010) but also from shipping activities and industrial effluent discharges across a broader geographical area (Sutherland et al., 2007). In urban port areas where aquaculture is prevalent, the socio-ecosystems are well-known for their high levels of pollutants from terrestrial effluents and other human sources (Andral et al., 2004; Benali et al., 2015; Lafabrie et al., 2008). Despite this, sea cucumbers placed under fish cages in these industrialized Mediterranean areas did not exhibit a higher concentration of metal contaminants in Pillet et al. (2023). Nevertheless, the presence of contaminants in these holothuroids still warrants further assessment. This study compares the total concentration of a range of heavy metals in seafloor sediments and in sea cucumber tissues near and away from commercial fish cages in a busy Mediterranean port area. We discuss potentially important implications concerning the production of sea cucumbers in open-water IMTA in heavily industrialised coastal spaces, in terms of contaminant transfer and uptake.

## 2. Materials and methods

### 2.1. Study site

In October 2019, after 12 months of being cultured on the seafloor as part of an IMTA system set up in Marsaxlokk Bay, Malta (35°49'39.90" N, 14°32'30.73" E) (Fig. 1A) (further information in Cutajar et al., 2022a), sea cucumbers were sampled and analysed together with seafloor sediments for the transfer and accumulation of heavy metals in IMTA. In brief, the IMTA system was set up at increasing distances from a commercial fish farm (Fig. 1B). In October 2018, sea cucumbers of similar initial mean weight ( $\pm$  standard deviation) ( $24.6 \pm 2.1$  g) were cultured in cylindrical cages ( $1 \times 0.2$  m (d  $\times$  h)) made of 0.8 cm galvanised mesh wiring and a synthetic rope mesh bottom. Sea cucumber cages were stocked randomly with 10 individuals at an initial mean stocking biomass of  $313 \pm 6.6$  g m<sup>-2</sup> and set in replicates of three cages on the seafloor at 8 m water depth directly below a fish cage of gilthead sea bream (*Sparus aurata*) at 0 m (E0), another three cages at 10 m (E10) and then again at 25 m (E25) from the centre of the fish cage (Fig. 1C).

### 2.2. Sampling and heavy metal analysis

Sediment corers of  $5 \times 10$  cm (d  $\times$  h) were used to collect ten seafloor sediment samples within 2 m from the sea cucumber cages at E10 and E25, and another ten from the reference site, over 1 km from the fish farm facilities. In addition, ten sea cucumbers were sampled across the three cages deployed on the seabed at E10 and E25 that had been set up as part of the IMTA study, but not at E0 due to mass sea cucumber mortalities within the first month of the study (for further information see Cutajar et al., 2022). Similarly, ten sea cucumbers of similar final weight were sampled from natural populations at a reference site (35°50'2.20" N, 14°32'54.09" E), where no aquaculture activity was present (Fig. 1B). Wet body weights of *H. poli* samples were  $50.1 \pm 3.7$  g at the IMTA site and  $58.1 \pm 12.3$  g at the reference site. Sediment and sea cucumber samples were transported to the laboratory in a cool box.

At the laboratory, the top 3 cm layer of sediment core samples was extracted, dried at 60 °C to constant weight, and stored. Prior to metal analysis, the dried sediment samples were ground to fine powder using pestle and mortar. Sea cucumbers were washed, weighed, and processed to extract the body wall tissue, predominantly consisting of connective tissue, and sampled analysed collectively with muscle tissue. Processed sea cucumber samples were frozen at -20 °C and then freeze-dried (ALPHA 1-4 LDplus, Martin-Christ) before being ground to a fine powder using a ball mill (MM 200 Retsch).

The sediment concentrations of arsenic (As), Cd, Cr, Cu, Fe, Hg, Ni, Pb and Zn, measured as total metal content, were determined using inductively coupled plasma mass spectrometry (ICP-MS) (ThermoFisher ICAQ RQ). Sediments and blanks were acid-digested (5 mL HNO<sub>3</sub> 69 %) in the Microwave Digestion System (MarsXpress, CEM). Digested samples were treated with MilliQ deionized water, 200  $\mu$ l gold solution (10 ppm) for Hg determination and diluted further with HNO<sub>3</sub> before analysis. Multi-element standard solutions were used to prepare calibration curves and these were accepted at  $R^2 > 0.999$  for concentration calculation. Samples were assessed using an internal quality approach and validated when criteria for quality assurance were met. The analytical procedure was tested using the CRM recovery, which ranged from 85 % to 99 %, at a significance level of 0.05. All samples were analysed in triplicates to avoid batch-specific errors.

Sea cucumber tissue samples, blanks and Certified Reference Material (CRM) were acid-digested (5 mL HNO<sub>3</sub> 67–70 %, 1 mL H<sub>2</sub>O<sub>2</sub> 30 % and 4 mL MilliQ deionized water) in a microwave system (MARS 5, CEM), after which the total content of As, Cd, Cu, Fe, Hg, Pb and Zn was determined using inductively coupled plasma optical emission spectrometry (ICP-OES) (Optima 8000, PerkinElmer) coupled with a hydride-generation system for Hg determination. The analytical

## Figures and Tables



**Fig. 1.** A. Location of the study area, Marsaxlokk Bay, in southeast Malta. B. Locations of the fish farm, represented by the red marker, and the reference site, represented by the yellow marker, in Google Earth (Scale bar: 1 km). C. Sea cucumber cage positions at the experimental sites (E0, E10 and E25) near a gilthead sea bream fish cage as part of an integrated multi-trophic aquaculture system (Scale bar: 100 m).

procedure was tested using the CRM recovery, which ranged from 88 % to 98 % at significance level of 0.05.

To assess bioaccumulation of heavy metals in sea cucumbers from sediment, the bioconcentration factor (BCF) sea cucumbers – sediment was expressed as the ratio of metal concentration in the sampled tissue of *H. poli* to the mean concentration in sediments, separately for the IMTA and reference site. The BCF was estimated for metal concentration data of sediments and sea cucumber tissue according to Aydin-Onen et al. (2015). The bioconcentration of metals by *H. poli* occurs when  $BCF > 1$  (Aydin-Onen et al., 2015; Islam et al., 2017).

### 2.3. Data analysis

Metal concentrations in sea cucumber tissue were derived from pooled samples collected from sites E10 and E25, as mandated by the observed mortalities at the end of the IMTA study (Cutajar et al., 2022a). This approach was adopted due to the absence of significant variance in metal concentrations in *H. poli* tissue across these sites to allow a reliable comparison of heavy metal contamination between the IMTA and reference sites amidst varied industrial sources of contaminants in the bay.

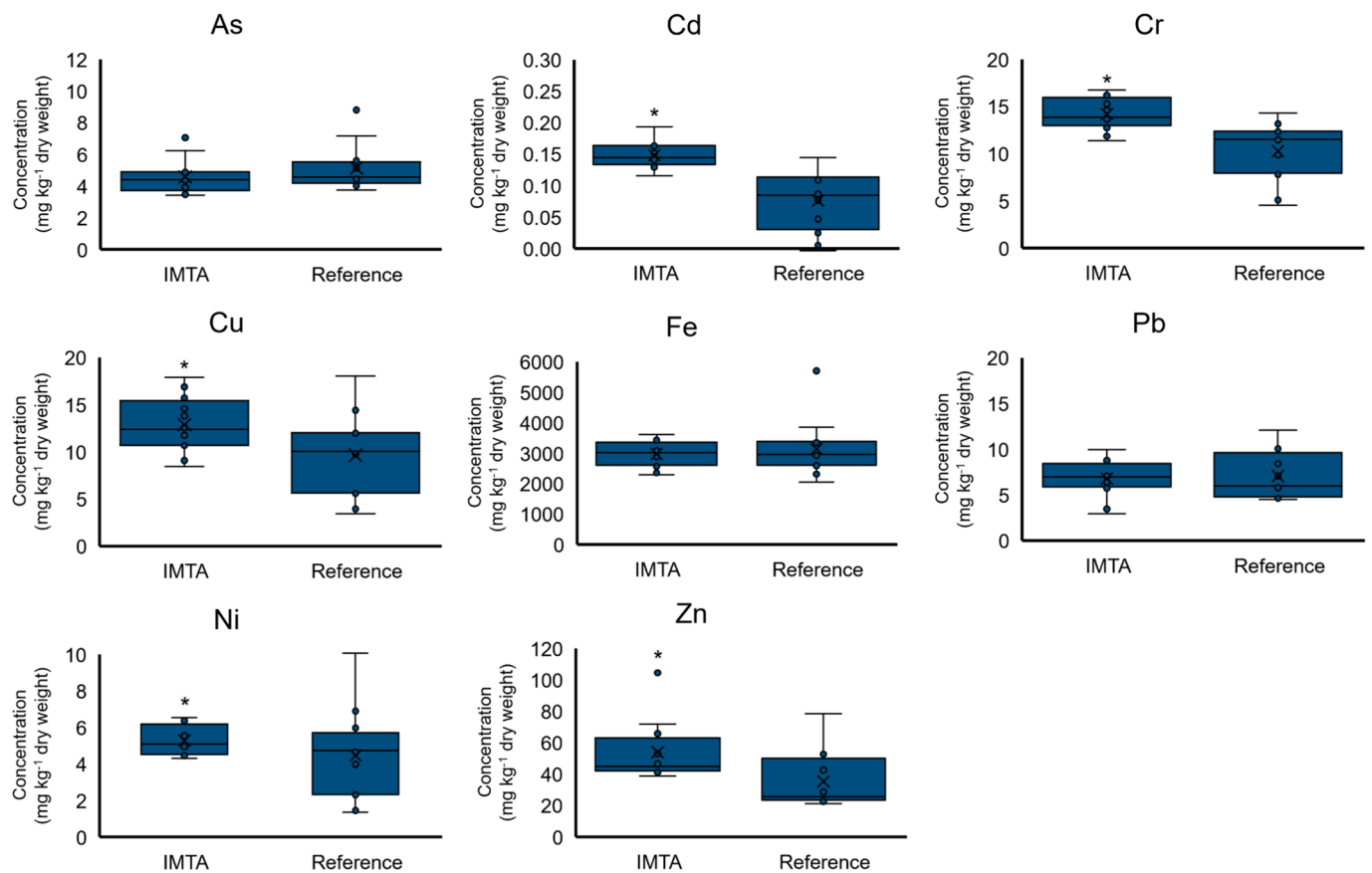
Data for each sediment sample were expressed as the mean of replicates taken. Data for each sediment sample collected and pooled across sites E10 and E25, were expressed as the mean of replicates taken. Assumptions of normality and homogeneity of variances were assessed using Shapiro-Wilk and Levene's tests, respectively. Box plots were used to identify outliers for each individual heavy metal for sediment and sea cucumber samples. An independent samples t-test was used to assess

differences in mean metal concentrations between the IMTA and reference sites for both sediment and sea cucumbers. The non-parametric test, Mann-Whitney U, was used when assumptions of normality were violated, particularly when outliers were identified. Statistical analysis was performed using SPSS v26.0 for Windows (SPSS Inc., Chicago, USA). Statistical significance criterion was set at  $p < 0.05$  level.

## 3. Results and discussion

### 3.1. Heavy metal concentration in sediments

The concentrations of Cd ( $U = 116.5$ ,  $n = 72$ ,  $p < 0.001$ ), Cr ( $U = 438.5$ ,  $n = 72$ ,  $p = 0.018$ ), Cu ( $U = 345$ ,  $n = 72$ ), Ni ( $U = 169$ ,  $n = 72$ ), and Zn ( $U = 259$ ,  $n = 72$ ) ( $p < 0.001$ ) were significantly higher near fish cages than the reference site (Fig. 2). Given the proximity of the fish cages, this could be due to localised enrichment from marine aquaculture. Higher concentrations of Cd, Cu and Zn in sediments below fish cages have been ascribed to their use in aquaculture feeds and anti-fouling net coatings (Belias et al., 2003; Dean et al., 2007; Sutherland et al., 2007; Basaran et al., 2010). However, for a thorough evaluation of the distribution and impact of metals in sediments, it is imperative to take into account the variability and origin of heavy metals in coastal sediments. It is essential to understand the natural abundance of metals or whether these are derived from anthropogenic activities particularly in heavily industrialised environments. No significant spatial variation was observed in sediment concentrations of Pb ( $U = 613$ ,  $n = 72$ ,  $p = 0.693$ ), As ( $U = 814.5$ ,  $n = 72$ ,  $p = 0.061$ ), and Fe ( $U = 642$ ,  $n = 72$ ,  $p = 0.946$ ) between the IMTA site and reference site. Additionally, the



**Fig. 2.** Heavy metal concentrations ( $\text{mg kg}^{-1}$  dry weight) in sediments at the integrated-multi trophic aquaculture site and the reference site, at the end of the study (October 2019). Values are given as mean  $\pm$  standard deviation ( $n = 10$ ). All results for Hg concentrations were below the limit of quantification so were not included. \*denotes statistically significant difference ( $p < 0.05$ ) between data for sites.

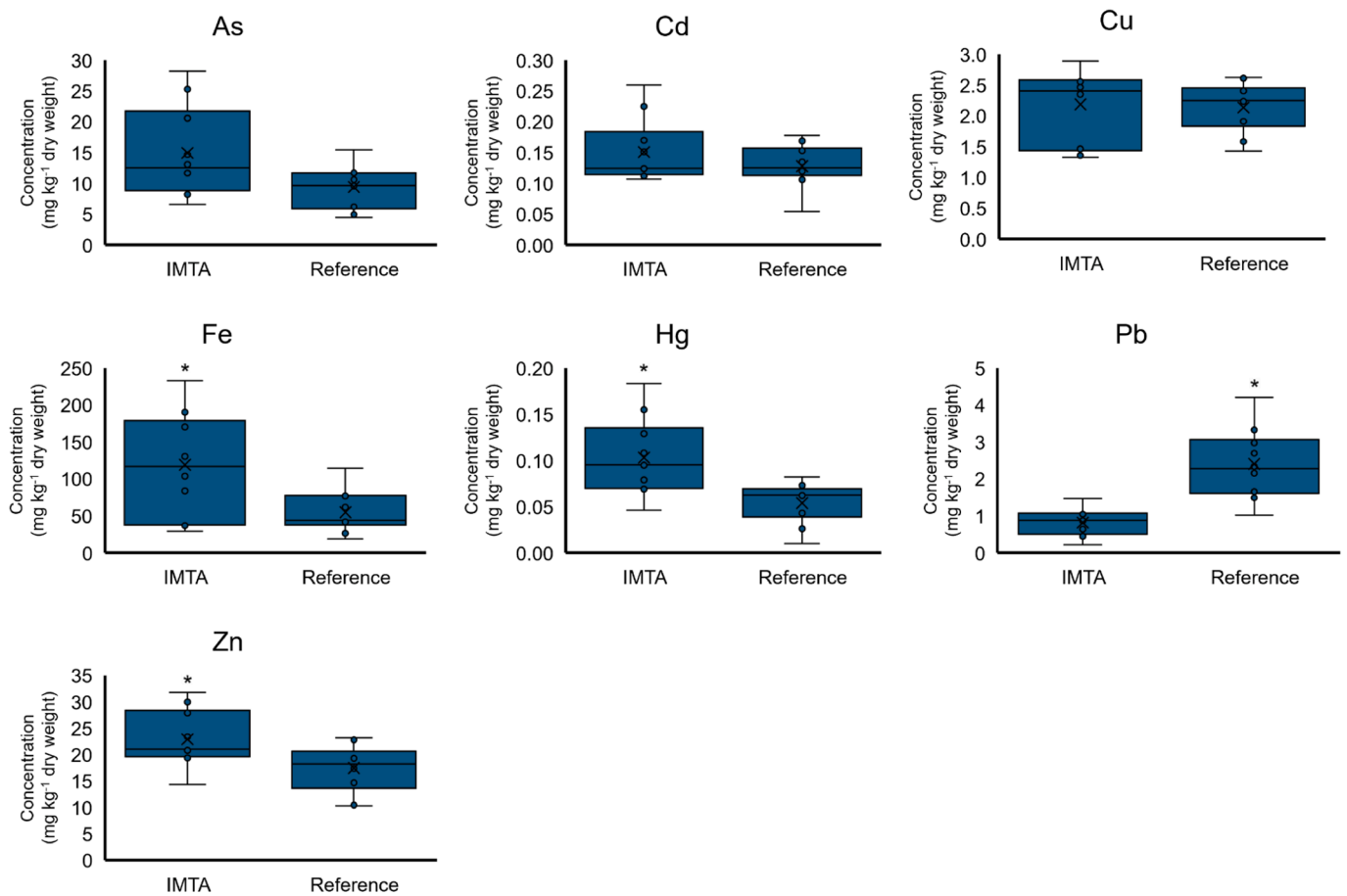
concentrations of Hg in sediments were below limits of quantification at both sites. These findings indicate that metals like Pb and As, which are not typically present in commercial fish diets, are unlikely to accumulate below cages from fish cage waste deposits. Instead, their presence may be more closely associated with broader anthropogenic sources prevalent throughout the bay, such as industrial effluents and maritime activities. While there is evidence to suggest that Fe concentrations in surface sediments near fish cages could be influenced by inputs from fish feed (Belias et al., 2003; Sutherland et al., 2007), it is important to consider that natural background levels also play a significant role in metal accumulation. This is particularly relevant given that Fe levels in commercial diets for sea bass and sea bream can be relatively low (160.13–249.03  $\text{mg kg}^{-1}$  DW) (Kalantzi et al., 2016). Given the various natural and anthropogenic factors that can influence the IMTA and reference sites within this type of bay and that can contribute to the observed levels, it remains challenging to attribute accumulation of specific contaminants to single point sources.

In terms of environmental quality, the levels of metals except Ni near fish cages and elsewhere in the bay are within maximum concentration limits set for good environmental status of contaminants in sediments listed as priority substances that present significant risks to the aquatic environment (Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013). Metal levels in sediments at the IMTA site and reference site were below reference values for Cd (0.3  $\text{mg kg}^{-1}$  DW), Hg (0.3  $\text{mg kg}^{-1}$  DW), and Pb (30  $\text{mg kg}^{-1}$  DW) but not for Ni (0.03  $\text{mg kg}^{-1}$  DW) in non-industrial marine sediments (ERA, 2020). However, reference values and quality standards for sediments in industrial areas would be more comparable and applicable especially since exceedances in metal concentration in sediments have been reported in the port area in this study (ERA, 2015). Still, threshold values for

contaminant levels are not always available for nearshore industrial environments and consideration for the applicability of thresholds that have been adopted is recommended.

### 3.2. Heavy metal levels in sea cucumbers

The sea cucumber tissue revealed high concentrations of Fe and Zn at both sites (Fig. 3). In the Mediterranean region, the body wall of *H. poli* in the natural environment reportedly contains high levels of Fe (19.4–40.6  $\text{mg kg}^{-1}$  DW) and Zn (8.9–14.9  $\text{mg kg}^{-1}$  DW) that vary according to the spatial distribution of metals in sediments and the distinct geographical origins (Sicuro et al., 2012; González-Wangüemert 2021). Essential metals, including Fe and Zn, play important physiological functions in sea cucumbers as reported for *Holothuria floridana* in Marugo et al. (2022). Expectedly, these metals are found in the highest concentrations in sea cucumber body wall tissue. In sea cucumber tissue, significantly higher mean concentrations of Fe ( $t(1,12) = -2.612, p = 0.023$ ) and Zn ( $t(1,18) = -2.477, p = 0.023$ ) were recorded at the IMTA site when compared to those at the reference site. The Zn levels in *H. poli* in IMTA reflect the higher concentrations of the metal in sediments near fish cages, which spatial differences in metal accumulation would not only be influenced by a variety of environmental factors but also the physiological traits of the organism (Warnau et al., 2006). The Pb levels in sea cucumbers cultured in IMTA were comparable with those recorded in *H. poli* elsewhere in the Mediterranean, which were reported as 1.3  $\text{mg kg}^{-1}$  DW (Storelli et al., 2001) and 0.88  $\text{mg kg}^{-1}$  DW (Storelli et al., 2001). Moreover, Pb levels in *H. poli* in IMTA were significantly lower than those recorded at the reference site in this study ( $t(1,12) = 4.985, p < 0.001$ ). Elsewhere, González-Wangüemert et al. (2018b) reported higher levels of Pb in *H. poli* tissue (3.1  $\text{mg kg}^{-1}$  DW) than those



**Fig. 3.** Heavy metal concentrations ( $\text{mg kg}^{-1}$  dry weight) in the body wall/muscle tissue of *H. poli* cultured at the integrated-multi trophic aquaculture (IMTA) site and the reference site, at the end of the study (October 2019). Values are given as mean  $\pm$  standard deviation ( $n = 10$ ). \*denotes statistically significant difference ( $p < 0.05$ ) between data for sites.

reported for the same species near fish cages in this study and attributed the accumulation of Pb in the sea cucumber to historic anthropogenic activities. Hg concentration in sea cucumbers cultured in IMTA was higher ( $t(1,18) = -3.251, p = 0.004$ ) than the reference site, however, Hg levels were lower at both sites than those reported in *H. poli* along the Southern Adriatic coast ( $0.96 \text{ mg kg}^{-1}$  DW) (Storelli et al., 2001). No significant spatial differences ( $p > 0.05$ ) were recorded for As ( $t(1,13) = -2.156, p = 0.051$ ), Cd ( $t(1,18) = 1.147, p = 0.268$ ) and Cu ( $t(1,18) = -0.223, p = 0.827$ ). The concentrations recorded for Cd in sea cucumbers in IMTA and the reference site in this industrial bay were higher than those observed in *H. poli* in studies from Storelli et al. (2001) ( $0.04 \pm 0.01 \text{ mg kg}^{-1}$  DW), Sicuro, et al. (2012) ( $0.07 \text{ mg kg}^{-1}$  DW), González-Wangüemert et al. (2018b) ( $0.09 \pm 0.01 \text{ mg kg}^{-1}$  DW) and Montero et al. (2021) ( $0.03 \text{ mg kg}^{-1}$  DW), which metal found in *H. poli* González-Wangüemert et al. (2018b) ascribed to historic anthropogenic activities. Similarly, the concentrations of As and Cu in the body tissue of *H. poli* align with previous findings for this species. Specifically, As levels in *H. poli* in the industrialised Gulf of Cagliari (Sardinia) were recorded at  $33.0 \text{ mg kg}^{-1}$  (DW) in Sicuro et al. (2012) and  $22.9 \text{ mg kg}^{-1}$  (DW) in *H. poli* from the Southern Adriatic region in Montero et al. (2021). For Cu, the levels were  $2.5 \text{ mg kg}^{-1}$  (DW) and  $3.1 \text{ mg kg}^{-1}$  (DW) in the respective studies. These levels are also comparable to those of As, found in seafood commonly consumed throughout the Mediterranean, as reported by Ferrante et al. (2019).

As bottom-dwellers, a close association between the metal concentrations of holothuroids and sediments would be expected. The bio-concentration ratios recorded in sea cucumbers for Hg at the IMTA site ( $9.12 \pm 3.73$ ) and at the reference site ( $4.78 \pm 1.97$ ) were the highest

among the metals, followed by As in sea cucumbers near fish cages ( $3.58 \pm 1.77$ ) and at the reference site ( $2.26 \pm 0.82$ ) (Table 1). Generally, the BCF values for Hg and As indicated bioaccumulation in *H. poli* in the bay to confirm the high affinity for these metals in sediments. Albeit the most abundant metals in sediments and the sea cucumber tissue, the lower concentrations reported for Fe and Zn in *H. poli*, when compared to levels in sediment reveal that these metals are regulated without bioaccumulation beyond metabolic and physiological needs, corroborating Storelli et al. (2001). Average BCF values for Cd, Cu, Fe, Pb and Zn in *H. poli* revealed that bioaccumulation did not occur in the body wall/muscle tissue of sea cucumbers over the one-year period of the study.

Despite the increasing demand for Mediterranean sea cucumbers, commercial aquaculture production of *H. poli* has yet to be launched and presently, work is still limited to research efforts (González-Wangüemert and Domínguez-Godino, 2016; González-Wangüemert et al., 2018a; Rakaj et al., 2019; Cutajar et al., 2022a, b). For this reason, production data for *H. poli* during grow-out especially as part of open-water IMTA are not available. However, additional evidence from a complete harvest cycle would provide valuable complementary information on the bioaccumulation of contaminants in the body wall/muscle tissue of *H. poli* and the implications for fish-sea cucumber IMTA. Considering that *H. poli* doubled from an initial average weight of 24 g at an approximate growth rate of  $0.2 \% \text{ day}^{-1}$  during a 12-month experimental period (Cutajar et al., 2022a), stocking open-water cages with smaller juveniles and harvesting at a market size of 70–110 g demand a longer grow-out period than that reported in this study and that would have probable effects on metal bioaccumulation throughout production to consider.

**Table 1**Mean ( $\pm$  standard deviation, SD) and range of bioconcentration factors for metals at the integrated multi-trophic aquaculture and reference sites.

		As	Cd	Cu	Fe	Hg	Pb	Zn
IMTA site	Mean (SD)	3.58 (1.77)	0.99 (0.34)	0.16 (0.04)	0.04 (0.02)	9.12 (3.73)	0.11 (0.05)	0.41 (0.01)
	Range	1.58–6.77	0.70–1.70	0.10–0.21	0.01–0.07	4.07–16.20	0.03–0.20	0.25–0.56
Reference site	Mean (SD)	2.26 (0.82)	0.84 (0.23)	0.16 (0.03)	0.02 (0.01)	4.78 (1.97)	0.32 (0.12)	0.31 (0.08)
	Range	1.07–3.71	0.35–1.16	0.10–0.19	0.01–0.04	0.89–7.26	0.14–0.56	0.18–0.41

### 3.3. Implications of metal contamination

After one year of open-water culture, sea cucumbers are evidently vulnerable to contamination when placed in sediments near commercial fish cages. Bioaccumulation of As and Hg reveals greater propensity of *H. poli* to bioconcentrate toxic contaminants in tissue when closer to fish cages. Measured metal concentrations and bioconcentration in sea cucumber tissue reflect the bioavailability of contaminants where *H. poli* is exposed to waste deposition under fish cages. This has implications for the performance of extractive species in open-water IMTA and reveals the need to monitor and understand how the distribution of particulate wastes below and near fish cages changes as a function of cage production.

This study reveals a snapshot of metal contamination during production of sea cucumbers in open-water IMTA. It shows that the promising role of sea cucumbers in IMTA can be threatened by exposure to contaminants under fish cages. However, if this system is to be scaled up to be an efficient solution for benthic waste management and value-added production it is important to understand how patterns of waste distribution around fish cages influence metal bioaccumulation in sea cucumber tissue over representative production periods. Throughout production, farm-level practices can add to local site-specific complexities and lead to variable waste distribution patterns and sedimentary conditions around commercial fish cages (Cutajar et al., unpub. data). This temporal variation in food availability and quality affects the transfer of organic material in fish-sea cucumber IMTA (Cutajar et al., 2022b). In addition, changes in metabolic processes that can include aestivation at higher water temperatures and hibernation in winter described in holothuroids may influence feeding and the quantities of nutrients, organic matter and contaminants absorbed. This can add to temporal changes in the uptake and bioaccumulation of heavy metals in sea cucumber tissue. Irregular trends of waste deposition can possibly influence the bioavailability of metals and the exposure of sea cucumbers to these contaminants. Since this potentially affects the biomitigation and production efficiency of extractive organisms in IMTA, producers need to be able to capture this variability in the bioavailability of metals in sediments and bioconcentration in sea cucumber tissue. This requires detailed and finer resolution monitoring over a longer time scale for a more representative account of e.g. complete production cycles of the fish farm, different feeding regimes, and local hydrographic variabilities. Moreover, this requires a shift from mono-specific considerations for cage production towards a better appreciation of the implications these activities could have on the physiological activities of sea cucumbers, and their growth and biomitigative performance in IMTA. In an integrated system, viability and profitability may depend on the efficient recapture of feed and energy and therefore, the environmental and economic benefits of sea cucumbers need to be understood and reassuringly consistent.

In the broader perspective, the possibilities and challenges for IMTA development are multifaceted and require further research. In terms of contamination, multiple stressors can influence the contamination of sea cucumber tissues and the performance of extractive organisms in IMTA. The performance of extractive species feasibility of IMTA requires an understanding of single and combined effects of contaminants, and environmental and anthropogenic complexities, especially in industrialised areas. Tank-based trials should provide additional evidence for the transfer of other important contaminants in sediments (e.g.,

organometallic compounds, aromatic organics, and halogenated hydrocarbons) and the effects of exposure over representative production timescales. Essentially, this is a complex system and unless knowledge gaps and uncertainties are addressed in consideration of real-world aquaculture processes, scaling up of IMTA will remain a challenge.

### 4. Conclusion

Sea cucumbers cultured under near commercial fish cages offer the possibility to extract organic waste associated with intensive aquaculture however, the bioconcentration of toxic metals demand careful monitoring over entire production cycles and with consideration for farm and site complexities that could influence the bioavailability of contaminants in sediments. Research is needed to understand the growth response and waste mitigation efficiency of these extractive organisms when exposed to different levels and types of contaminants and the implications for the scalability and viability of fish-sea cucumber IMTA in these environments.

### CRediT authorship contribution statement

**Karl Cutajar:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Lynne Falconer:** Writing – review & editing, Supervision, Conceptualization. **Trevor C. Telfer:** Writing – review & editing, Supervision, Conceptualization.

### Declaration of Competing Interest

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

### Data availability

No data was used for the research described in the article.

### Acknowledgements

This research received funding from the Tools for Assessment and Planning of Aquaculture Sustainability (TAPAS) project within the EU H2020 program (Grant Agreement No 678396) and the ENDEAVOUR Scholarships Scheme from the Ministry of Education and Employment, Malta. We are grateful for the support from the Department of Earth and Marine Sciences (DiSTeM) at the University of Palermo and the National Interuniversity Consortium for Marine Sciences (CoNISMa) in Rome, and special thanks to Mr. Graeme McWhinnie at the University of Stirling for the laboratory assistance. Our appreciation to our colleagues at AquaBioTech Group, and the owners of the fish farm (MFF Ltd.), Mr. Saviour Ellul and Mr. Giovanni Ellul.

### References

- Andral, B., Stanisiere, J.Y., Sauzade, D., Damier, E., Thebault, H., Galgani, F., Boissery, P., 2004. Monitoring chemical contamination levels in the Mediterranean based on the use of mussel caging. *Mar. Pollut. Bull.* 49 (9–10), 704–712. <https://doi.org/10.1016/j.marpolbul.2004.05.008>.
- Aydin-Onen, S., Kucuksezgin, F., Kocak, F., Acik, S., 2015. Assessment of heavy metal contamination in *Hediste diversicolor* (O.F. Müller, 1776), *Mugil cephalus* (Linnaeus,

- 1758), and surface sediments of Bafa Lake (Eastern Aegean). *Environ. Sci. Pollut. Res.* 22 (11), 8702–8718.
- Aydin, M., Tunca, E., Alver Şahin, Ü., 2017. Effects of anthropological factors on the metal accumulation profiles of sea cucumbers in near industrial and residential coastlines of Izmir, Turkey. *Int. J. Environ. Anal. Chem.* 97 (4), 368–382.
- Barbieri, E., Paes, E.T., 2011. The use of oxygen consumption and ammonium excretion to evaluate the toxicity of cadmium on *Farfantepenaeus paulensis* with respect to salinity. *Chemosphere* 1 (84), 9–16. <https://doi.org/10.1016/j.chemosphere.2011.02.092>.
- Basaran, A.K., Aksu, M., Egemem, O., 2010. Impacts of the fish farms on the water column nutrient concentrations and accumulation of heavy metals in the sediments in the Eastern Aegean Sea (Turkey). *Environ. Monit. Assess.* 162 (1), 439–451.
- Belias, C.V., Bikas, V.G., Dassenakis, M.J., Scoullou, M.J., 2003. Environmental impacts of coastal aquaculture in eastern Mediterranean bays: the case of Astakos Gulf, Greece. *Environ. Sci. Pollut. Res.* (10), 287–295.
- Benali, I., Boutiba, Z., Merabet, A., Chèvre, N., 2015. Integrated use of biomarkers and condition indices in mussels (*Mytilus galloprovincialis*) for monitoring pollution and development of biomarker index to assess the potential toxic of coastal sites. *Mar. Pollut. Bull.* 1 (95), 385–394. <https://doi.org/10.1016/j.marpolbul.2015.03.041>.
- Cutajar, K., Falconer, L., Massa-Gallucci, A., Cox, R.E., Schenke, L., Bardócz, T., Andolina, C., Signa, G., Vizzini, S., Sprague, M., Telfer, T.C., 2022b. Stable isotope and fatty acid analysis reveal the ability of sea cucumbers to use fish farm waste in integrated multi-trophic aquaculture. *J. Environ. Manag.* 318, 115511.
- Cutajar, K., Falconer, L., Massa-Gallucci, A., Cox, R.E., Schenke, L., Bardócz, T., Sharman, A., Deguara, S., Telfer, T.C., 2022a. Culturing the sea cucumber *Holothuria polii* in open-water integrated multi-trophic aquaculture at a coastal Mediterranean fish farm. *Aquaculture* 550, 737881.
- Dean, R.J., Shimmield, T.M., Black, K.D., 2007. Copper, zinc and cadmium in marine cage fish farm sediments: an extensive survey. *Environ. Pollut.* 1 (145), 84–95. <https://doi.org/10.1016/j.envpol.2006.03.050>.
- Environment and Resources Authority, 2015. Contamination by Hazardous Substances: Descriptors 8 & 9 of the Marine Strategy Framework Directive (2008/56/EC). *Initial Assessment, Good Environmental Status and Environmental Targets for Malta*. Available at: (<https://era.org.mt/wp-content/uploads/2019/05/MSFD-InitialAssessment-Contamination.pdf>) (Accessed on 6<sup>th</sup> March 2023).
- Environment and Resources Authority, 2020. Contamination (in environment and seafood) – Descriptors 8 & 9 of the Marine Strategy Framework Directive (2008/56/EC). *Update on Articles 8, 9, and 10 of the Marine Strategy Framework Directive (2008/56/EC) in Malta's Marine Waters*, 211pp. Available at: ([https://era.org.mt/wp-content/uploads/2020/06/MSFD-Art.-17-Update-Malta\\_FINAL.pdf](https://era.org.mt/wp-content/uploads/2020/06/MSFD-Art.-17-Update-Malta_FINAL.pdf)) (Accessed on 6<sup>th</sup> March 2023).
- Ferrante, M., Napoli, S., Grasso, A., Zuccarello, P., Cristaldi, A., Copat, C., 2019. Systematic review of arsenic in fresh seafood from the Mediterranean Sea and European Atlantic coasts: A health risk assessment. *Food Chem. Toxicol.* 126, 322–331.
- González-Wangüemert, M., Domínguez-Godino, J., 2016. Sea cucumbers as new marine resource in Europe. *Front. Mar. Sci.* 3.
- González-Wangüemert, M., Domínguez-Godino, J.A., Cánovas, F., 2018a. The fast development of sea cucumber fisheries in the Mediterranean and NE Atlantic waters: From a new marine resource to its over-exploitation. *Ocean Coast. Manag.* 151, 165–177.
- González-Wangüemert, M., Roggatz, C.C., Rodrigues, M.J., Barreira, L., da Silva, M.M., Custódio, L., 2018b. A new insight into the influence of habitat on the biochemical properties of three commercial sea cucumber species. *Int. Aquat. Res.* 10 (4), 361–373.
- Grosso, L., Rakaj, A., Fianchini, A., Morroni, L., Cataudella, S., Scardi, M., 2021. Integrated Multi-Trophic Aquaculture (IMTA) system combining the sea urchin *Paracentrotus lividus*, as primary species, and the sea cucumber *Holothuria tubulosa* as extractive species. *Aquaculture* (534), 736268. <https://doi.org/10.1016/j.aquaculture.2020.736268>.
- Islam, M., Al-Mamun, A., Hossain, F., Quraishi, S., Naher, K., Khan, R., Das, S., Tamim, U., Hossain, S.M., Nahid, F., 2017. Contamination and ecological risk assessment of trace elements in sediments of the rivers of Sundarban mangrove forest, Bangladesh. *Mar. Pollut. Bull.* 124 (1), 356–366.
- Israel, D., Lupatsch, I., Angel, D.L., 2019. Testing the digestibility of sea bream wastes in three candidates for integrated multi-trophic aquaculture: Grey mullet, sea urchin and sea cucumber. *Aquaculture* (510), 364–370. <https://doi.org/10.1016/j.aquaculture.2019.06.003>.
- Kalantzi, I., Shimmield, T., Pergantis, S., Papageorgiou, N., Black, K., Karakassis, I., 2013. Heavy metals, trace elements and sediment geochemistry at four Mediterranean fish farms. *Sci. Total Environ.* (444), 128–137. <https://doi.org/10.1016/j.scitotenv.2012.11.082>.
- Lafabrie, C., Pergent-Martini, C., Pergent, G., 2008. Metal contamination of *Posidonia oceanica* meadows along the Corsican coastline (Mediterranean). *Environ. Pollut.* 151 (1), 262–268.
- Li, L., Tian, X., Yu, X., Dong, S., 2016. Effects of acute and chronic heavy metal (Cu, Cd, and Zn) exposure on sea cucumbers (*Apostichopus japonicus*). *BioMed. Res. Int.* <https://doi.org/10.1155/2016/4532697>.
- Marrugo-Negrete, J., Pinedo-Hernández, J., Marrugo-Madrid, S., Navarro-Frómata, E., Díez, S., 2021. Sea cucumber as bioindicator of trace metal pollution in coastal sediments. *Biol. Trace Elem. Res.* 199 (5), 2022–2030.
- Montero, N., Atzori, M., Marras, B., Bettoschi, A., Nurchis, P., Coroneo, V., Sanna, C., Schintu, M., 2021. Trace metal levels in the edible tissues of sea cucumbers (*Holothuria tubulosa* and *Holothuria polii*) from Sardinia (Western Mediterranean). *Ital. J. Food Saf.* 10 (3), 9576.
- Neofitou, N., Lolas, A., Ballios, I., Skordas, K., Tziantziou, L., Vafidis, D., 2019. Contribution of sea cucumber *Holothuria tubulosa* on organic load reduction from fish farming operation. *Aquaculture* 501, 97–103. <https://doi.org/10.1016/j.aquaculture.2018.10.071>.
- Parra-Luna, M., Martín-Pozo, L., Hidalgo, F., Zafrá-Gómez, A., 2020. Common sea urchin (*Paracentrotus lividus*) and sea cucumber of the genus *Holothuria* as bioindicators of pollution in the study of chemical contaminants in aquatic media. A revision. *Ecol. Indic.* 113, 106185.
- Pillet, M., Dabrowski, M., Marengo, M., Fullgrabe, L., Leduc, M., Fontaine, Q., Le Floch, S., Huet, V., Churlaud, C., Lejeune, P., 2023. Preliminary inter-port study of the quality of environments using physiological responses of invertebrates exposed to chronic trace element and organic contamination in Corsica (Mediterranean Sea). *Ecotoxicology* 2 (32), 243–260. <https://doi.org/10.1007/s10646-023-02635-w>.
- Rabeh, I., Telahigue, K., Bejaoui, S., Hajji, T., Chouba, L., EL Cafsi, M., Soudani, N., 2019. Effects of mercury graded doses on redox status, metallothionein levels and genotoxicity in the intestine of sea cucumber *Holothuria forskali*. *Chem. Ecol.* 3 (35), 204–218. <https://doi.org/10.1080/02757540.2018.1546292>.
- Rakaj, A., Fianchini, A., Boncagni, P., Scardi, M., Cataudella, S., 2019. Artificial reproduction of *Holothuria polii*: a new candidate for aquaculture. *Aquac. Environ. Interact.* 498, 444–453.
- Rosa, J., Lemos, M.F., Crespo, D., Nunes, M., Freitas, A., Ramos, F., Pardal, M.Á., Leston, S., 2020. Integrated multitrophic aquaculture systems—potential risks for food safety. *Trends Food Sci. Technol.* 96, 79–90.
- Sadoul, B., Caprioli, J., Barrier-Loiseau, C., Cimiterra, N., Laugier, T., Lagarde, F., Chary, K., Callier, M.D., Guillermand, M., d'Orbecastel, E.R., 2022. Is *Holothuria tubulosa* the golden goose of ecological aquaculture in the Mediterranean Sea? *Aquaculture* (554), 738149. <https://doi.org/10.1016/j.aquaculture.2022.738149>.
- Sakianakis, D.G., Renieri, E., Kentouri, M., Tsatsakis, A.M., 2015. Effect of heavy metals on fish larvae deformities: a review. *Environ. Res.* (137), 246–255. <https://doi.org/10.1016/j.envres.2014.12.014>.
- Sicuro, B., Piccinno, M., Gai, F., Cesarina, A.M., Danieli, A., Daprà, F., Mioletti, S., 2012. Food quality and safety of Mediterranean sea cucumbers *Holothuria tubulosa* and *Holothuria polii* in Southern Adriatic Sea. *Asian J. Anim. Vet. Adv.* 7, 851–859.
- Storelli, M., Storelli, A., Marcotrigiano, G., 2001. Heavy metals in the aquatic environment of the Southern Adriatic Sea, Italy: Macroalgae, sediments and benthic species. *Environ. Int.* 26 (7–8), 505–509.
- Sutherland, T., Petersen, S., Levings, C., Martin, A., 2007. Distinguishing between natural and aquaculture-derived sediment concentrations of heavy metals in the Broughton Archipelago, British Columbia. *Mar. Pollut. Bull.* 54 (9), 1451–1460.
- Telahigue, K., Rabeh, I., Bejaoui, S., Hajji, T., Nechi, S., Chelbi, E., El Cafsi, M., Soudani, N., 2020. Mercury disrupts redox status, up-regulates metallothionein and induces genotoxicity in respiratory tree of sea cucumber (*Holothuria forskali*). *Drug Chem. Toxicol.* 3 (43), 287–297. <https://doi.org/10.1080/01480545.2018.1524475>.
- Tolon, M.T., Emiroglu, D., Gunay, D., Ozgul, A., 2017. Sea cucumber (*Holothuria tubulosa* Gmelin, 1790) culture under marine fish net cages for potential use in integrated multi-trophic aquaculture (IMTA). *Indian J. Geo-Mar. Sci.* 4 (46), 749–756.
- Wang, J., Ren, T., Han, Y., Zhao, Y., Liao, M., Wang, F., Jiang, Z., 2015. The effects of dietary lead on growth, bioaccumulation and antioxidant capacity in sea cucumber, *Apostichopus japonicus*. *Environ. Toxicol. Pharmacol.* 2 (40), 535–540. <https://doi.org/10.1016/j.etap.2015.08.012>.
- Warnau, M., Dutrieux, S., Ledent, G., Rodriguez y Baena, Alessia, M., Dúbois, P., 2006. Heavy metals in the sea cucumber *Holothuria tubulosa* (Echinodermata) from the Mediterranean *Posidonia oceanica* ecosystem: Body compartment, seasonal, geographical and bathymetric variations. *Environ. Bioindic.* 4 (1), 268–285.
- Yucel-Gier, G., Pazi, İ., Kucuksezgin, F., 2013. Spatial Analysis of Fish Farming in the Gulluk Bay (Eastern Aegean). *Turk. J. Fish. Aquat. Sci.* 4 (13), 737–744. [https://doi.org/10.4194/1303-2712-v13\\_4\\_19](https://doi.org/10.4194/1303-2712-v13_4_19).