

Ecological responses of *Holothuria sanctori* to metal contamination at La Punta del Hidalgo, Tenerife Island: two-year monitoring and analysis

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Abstract

This study investigates the variations in metal and trace element concentrations within the *Holothuria sanctori* species over two years, between 2021 and 2022, with a specific focus on differences between the "Cold" and "Warm" stations. A total of 80 specimens were collected during four sampling periods, each comprising 20 individuals in the months of January and August. The selection of Punta del Hidalgo as the sampling area was based on the presence of this species in the intertidal zone and the observation of a higher number of specimens in the vicinity of an underwater outfall. The analysis of metal contents (Zn, Cd, Pb, Cu, Ni, Cr, and Fe in mg/kg) revealed significant differences in concentrations between the "Cold" and "Warm" stations across the study years. The warm station consistently displayed higher metal levels, with notable increments observed in zinc (Zn), cadmium (Cd), lead (Pb), copper (Cu), nickel (Ni), chromium (Cr), and iron (Fe). Variations in metal concentrations within *H. sanctori* during the summer months at the warm station can be attributed to seasonal weather conditions, increased tourist activities, and ocean currents transporting contaminants. The

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study underscores the importance of monitoring and controlling exposure to toxic metals. Limits established for cadmium, lead, and nickel exposure provide crucial data for public health policies and environmental regulations, safeguarding against adverse effects of chronic metal exposure.

Keywords: metal; trace element; underwater outfall; bioindicator; environmental monitoring,

Respuestas ecológicas de Holothuria sanctori a la contaminación por metales en La Punta del Hidalgo, Isla de Tenerife: dos años de monitoreo y análisis

Resumen

En esta investigación se estudian las variaciones en las concentraciones de metales y oligoelementos dentro de la especie *Holothuria sanctori* durante dos años, entre 2021 y 2022, con un enfoque específico en las diferencias entre las estaciones "Frías" y "Cálidas". Se colectaron un total de 80 ejemplares durante cuatro períodos de muestreo, comprendiendo cada uno 20 individuos en los meses de enero y agosto. La selección de Punta del Hidalgo como área de muestreo se basó en la presencia de esta especie en la zona intermareal y la observación de un mayor número de ejemplares en las cercanías de un emisario submarino. El análisis de los contenidos de metales (Zn, Cd, Pb, Cu, Ni, Cr y Fe en mg/kg) reveló diferencias significativas en las concentraciones entre las estaciones "Frías" y "Cálidas" a lo largo de los años de estudio. La estación caliente mostró consistentemente niveles de metales más altos, con incrementos notables observados en zinc (Zn), cadmio (Cd), plomo (Pb), cobre (Cu), níquel (Ni), cromo (Cr) y hierro (Fe). Las variaciones en las concentraciones de metales dentro de *H. sanctori* durante los meses de verano en la estación cálida pueden atribuirse a las condiciones climáticas estacionales, el aumento de las actividades turísticas y las corrientes oceánicas que transportan contaminantes. El estudio subraya la importancia de monitorear y controlar la exposición a metales tóxicos. Los límites establecidos para la exposición al cadmio, el plomo y el níquel proporcionan datos cruciales para las políticas de salud pública y las regulaciones ambientales, salvaguardando contra los efectos adversos de la exposición crónica a metales.

Palabras clave: metal; oligoelemento; emisario submarino; bioindicator; monitoreo ambiental.

Introduction

The marine environment exhibits a high degree of sensitivity to anthropization, characterized by the human-induced influence and alteration of the marine ecosystem. This phenomenon encompasses a broad spectrum of impacts, including the contamination of oceans with plastic waste, chemicals, and oil spills, the depletion of commercial fish populations due to overfishing, and the degradation of coastal habitats like mangroves and coral reefs resulting from urbanization and coastal development (Pacyna et al. 2006; Durrieu de Madron et al. 2011; Wang et al. 2018; Morrison et al. 2019; Yuan et al. 2019; Corrias et al. 2020). The marine ecosystem reacts to these impacts in diverse ways.

To begin with, pollution and habitat alterations directly affect many marine species, leading to population declines, localized extinctions, and shifts in the composition of marine communities. Furthermore, ecological imbalances can occur within marine ecosystems, exemplified by harmful algal blooms and the proliferation of invasive species that exploit the modified conditions (Penha-Lopes et al. 2011; Kravchenko et al. 2014; Auger et al. 2015; Li et al. 2017; Ali et al. 2019; Zheng et al. 2022). These factors can exert a global-scale influence on marine ecosystems, potentially leading to catastrophic outcomes. In summary, the marine ecosystem plays a vital role in the overall health of our planet, supporting a substantial portion of Earth's biodiversity. Nevertheless, anthropization poses a grave threat to these ecosystems, underscoring the urgency of taking measures to mitigate its impacts and safeguard the diversity and resilience of marine ecosystems for future generations (Howarth et al. 2005; Pezzullo 2009; Dolenec et al. 2011; Verma and Dwivedi 2013; Zavodny et al. 2017).

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Human impact on the marine environment encompasses a range of activities that markedly augment the presence of metals and trace elements in seas and oceans. These trace elements encompass metals like lead, mercury, cadmium, and other metallic compounds that are crucial in minuscule quantities for the proper functioning of marine ecosystems but pose challenges when their concentrations increase due to human activities (Clark et al. 2001; Harrison 2001; Temsch et al. 2010; Vikas and Dwarakish 2015; Ardeshir et al. 2017).

Industrial pollution stands out as a principal contributor to the accrual of metals and trace elements in oceanic expanses. Factories and power plants emit a diverse array of metals and noxious chemicals, which eventually make their way to the sea through rivers and currents. Furthermore, deep-sea mining and mineral extraction can directly release metals and trace elements into the marine ecosystem (Barros et al. 2007; Gaudry et al. 2007; Shekhar et al. 2008; Karantininis et al. 2010). Agricultural practices also wield significant influence in introducing trace elements into marine domains. Runoff from rainfall carries fertilizers and pesticides from agricultural lands to rivers, which subsequently flow into the sea. These chemical agents may harbor metals and trace elements, and their release can disrupt the natural equilibriums within marine ecosystems (Jiang et al. 2006; Tomas et al. 2013; Ritchie and Roser 2018; Hossain et al. 2019; Kerfahi et al. 2020).

Holothurians, commonly known as sea cucumbers, have gained recognition as highly effective bioindicators of marine pollution owing to their capacity to accumulate and react to a diverse range of contaminants present in oceanic environments. A fundamental factor contributing to their efficacy as bioindicators is their filter-feeding behavior, which classifies them as detritivores and makes them susceptible to the accumulation of environmental contaminants (Tuwo and

Conand 1992; Xing and Chia 1997; Hamel et al. 2001; Parra-Luna et al. 2020). These remarkable organisms can amass various substances, including heavy metals, hydrocarbons, toxic chemicals, and microplastics within their tissues. Their role as non-predators, with their primary exposure to contaminants occurring through water filtration, allows their contaminant concentrations to serve as a direct reflection of water quality. Additionally, holothurians possess a biological defense mechanism that empowers them to detoxify and eliminate toxic substances.

This mechanism encompasses the capability to sequester harmful compounds in their tissues and subsequently expel them. Consequently, they function as authentic indicators of exposure, with their presence and contaminant levels offering insights into both current and historical pollution within a specific area (Warnau et al. 2006; Turk Culha et al. 2016; Boluda-Botella et al. 2023; González-Delgado et al. 2024). This study's primary goal is to evaluate the ecological condition of La Punta del Hidalgo on Tenerife Island in the Canary Islands, Spain. It entails a comprehensive two-year monitoring program to analyze metals and trace elements across both the warm and cold seasons in *Holothuria sanctori*.

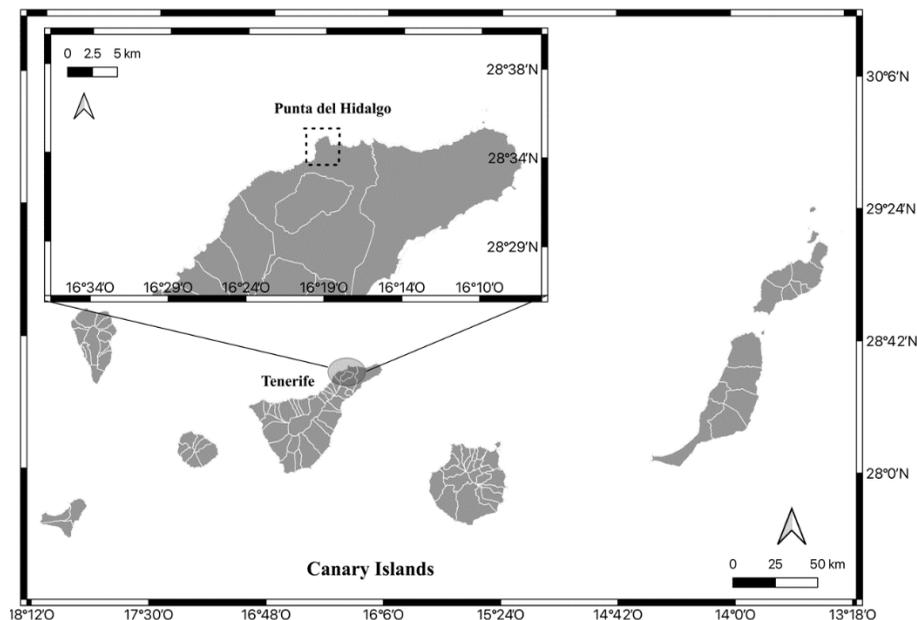
Material and methods

A total of 80 specimens of *Holothuria sanctori* were collected in a study conducted during four periods between 2021 and 2022. In each of these periods, 20 specimens were gathered in the months of January and August each year. Punta del Hidalgo was chosen as the sampling area because this species is found in the intertidal zone, and a higher number of specimens are observed in the vicinity of an underwater outfall (Fig. 1).

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Figure 1. Map of the sampling areas in the Punta del Hidalgo of the Canary archipelago



Sample preparation

The analytical samples comprised a segment of tissue, 5-8 grams for organisms. These samples were deposited in porcelain crucibles and subjected to a 24-hour drying process in an oven at 70°C. Subsequently, they were incinerated in a muffle oven for 48 hours at 450°C ± 25°C until they transformed into white ashes. Once the white ashes were obtained, they were filtered using a 1.5% HNO₃ solution, diluted to 25 mL for the subsequent determination of metal content via Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). This method was employed to assess the concentration of toxic metals and trace elements. To ensure the accuracy of the determinations, a quality control solution was applied every ten samples. Additionally, certified reference materials

(DORM-1 and DOLT 2) were utilized to validate the results. All data are presented in milligrams per kilogram of wet weight (mg/kg w.w.). The analysis included blanks and standard reference materials, and the following metals and trace elements were sampled: Cd, Pb, Cr, Cu, Fe, Ni, and Zn. By employing these reference materials, a recovery rate exceeding 97% was achieved. Both blanks and standard reference materials were analyzed concurrently with the samples (Lozano-Bilbao et al. 2023a, b; Thorne-Bazarra et al. 2023).

Statistical analysis

In order to examine possible differences in the content and relative composition of heavy and trace metals in the analyzed samples, a permutational multivariate analysis of variance (PERMANOVA) was performed using Euclidean distances. A two-way design was used, where the factor "year" was considered as a fixed factor with two levels of variation (2021 - 2022) and the factor "season" was considered as a fixed factor with two levels of variation (cold - warm). The variables included in the analysis were Cd, Cr, Cu, Fe, Ni, Pb and Zn. In order to conduct the statistical tests, 9999 permutations of interchangeable units were performed and post-hoc comparisons between pairs were carried out to verify the differences between the levels of the significant factors (p value < 0.05). Clusters were determined using principal coordinate analysis (PCoA) in which elements were represented as vectors (Anderson and Braak 2003; Anderson 2004). The statistical packages PRIMER 7 and PERMANOVA p v.1.0.1 were used to perform these statistical analyses.

Results

Table 1 displays the metal contents (Zn, Cd, Pb, Cu, Ni, Cr, and Fe in mg/kg) at the "Cold" and "Warm" stations during the years 2021 and 2022, revealing significant differences in the levels of these metals (Table 2), the PERMANOVA statistical analysis revealed significant differences in the levels of metals (Zn, Cd, Pb, Cu, Ni, Cr, and Fe) between the "Cold" and "Warm" stations over the two years of the study, but no significant differences were observed within the same stations over the two years. At the "Warm" station, higher concentrations were recorded in comparison to the "Cold" station for many of the analyzed metals in both years. Zinc (Zn) exhibited an increase from 51.3 ± 4.6 mg/kg in 2021 at the "Cold" station to 64.9 ± 7.7 mg/kg in 2021 at the "Warm" station, while in 2022, it varied from 50.8 ± 3.9 mg/kg to 67.4 ± 6.0 mg/kg (Fig. 2). Cadmium (Cd), lead (Pb), copper (Cu), nickel (Ni), chromium (Cr), and iron (Fe) also experienced similar increments in their concentrations at the "Warm" station compared to the "Cold" station. These findings suggest a greater accumulation of these metals at the "Warm" station over the years 2021 and 2022.

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Table 1. Mean concentrations of metal muscle sample (mg/Kg) with their standard deviation, according to the study area according to the study area

	2021		2022	
	Cold	Warm	Cold	Warm
Zn	51.3±4.6 (44.5-58.2)	64.9±7.7 (54.0-79.5)	50.8±3.9 (44.4-55.7)	67.4±6.0 (59.1-79.6)
Cd	0.531±0.081 (0.432-0.713)	0.691±0.089 (0.604-0.868)	0.532±0.073 (0.406-0.699)	0.716±0.08 (0.611-0.878)
Pb	1.61±0.11 (1.46-1.82)	1.98±0.23 (1.37-2.24)	1.59±0.098 (1.47-1.80)	2.05±0.18 (1.81-2.38)
Cu	6.07±0.37 (5.48-6.64)	8.05±0.918 (7.20-9.73)	6.07±0.35 (5.48-6.58)	8.30±0.83 (7.34-9.75)
Ni	2.81±0.25 (2.52-3.29)	3.64±0.47 (3.11-4.50)	2.78±0.19 (2.55-3.10)	3.87±0.52 (3.15-4.62)
Cr	1.48±0.34 (1.18-2.26)	2.39±0.50 (1.74-3.25)	1.46±0.38 (1.17-2.47)	2.51±0.46 (1.96-3.26)
Fe	133.1±11.9 (118.2-153.1)	169.1±16.3 (148.5-194.1)	134.7±12.2 (118.7-152.1)	173.2±15.1 (151.3-194.5)

Table 2. PERMANOVA analysis of year versus the factor "season" and the studied metals

	2021 vs. 2022		Cold vs. warm	
	Cold	Warm	2021	2022
Zn	0.833	0.403	0.001*	0.001*
Cd	0.826	0.474	0.002*	0.001*
Pb	0.697	0.262	0.001*	0.001*
Cu	0.986	0.511	0.001*	0.001*
Ni	0.83	0.295	0.001*	0.001*
Cr	0.884	0.549	0.001*	0.001*
Fe	0.859	0.569	0.001*	0.001*

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Figure 2. Line graph of each metal in years and seasons

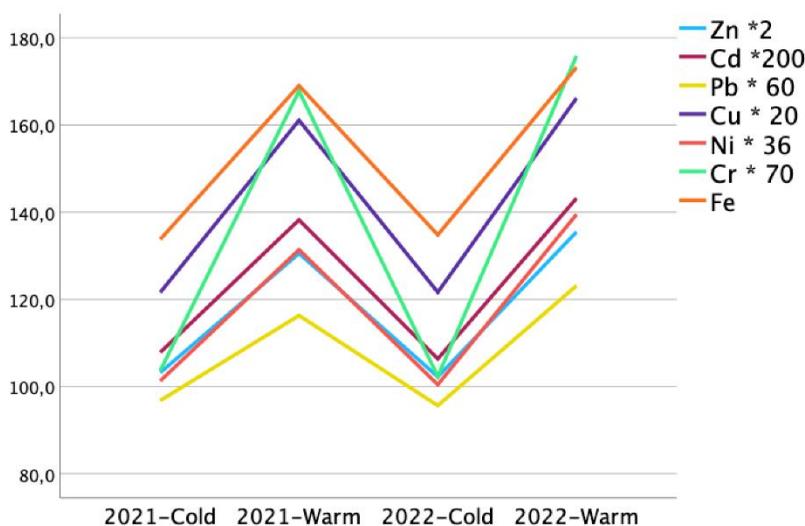


Table 3. Results of the mean metal concentrations in mg/kg and the maximum daily intake in grams that a 70 kg adult person could ingest during his/her lifetime without adverse effects

-	-	2021	2022
Cd 2.5 µg /kg /week	Mean	0.611	0.624
	Grams	40.10	39.26
Pb 0.5 µg /kg/ day	Mean	1.795	1.82
	Grams	19.78	19.51
Ni 2.8 µg/kg/ day	Mean	3.225	3.323
	Grams	60.78	58.98

Discussion and conclusions

The variation in the concentration of metals and trace elements in the *Holothuria sanctori* species within the same year, with an increase during the summer months at the warm station, can be attributed to a series of interconnected factors. Firstly, seasonal weather conditions play a fundamental role, as the summer months are typically associated with higher temperatures and increased solar radiation, influencing the metabolic activity and behavior of the species (Tuwo and Conand 1992; Morgan 2001; Sicuro et al. 2012b). Additionally, ocean currents and circulation patterns can transport contaminants and metals from various sources to the warm station, thereby increasing the concentrations in the water and, ultimately, in filter-feeding organisms like holothurians. Moreover, it's important to consider that during the summer months, there is often a higher influx of tourists and recreational activities in coastal areas, which can lead to the release of contaminants and metals through various human activities.

These pollutants can reach the marine environment and accumulate in marine organisms, including holothurians. The bioavailability of metals can also change due to biogeochemical factors such as water salinity and acidity, influencing the organisms' ability to absorb and accumulate these elements. *H. sanctori*, a species of holothurian, has emerged as a valuable bioindicator of heavy metal and trace element contamination in areas near underwater outfalls due to its detritivorous nature and its position in the marine food chain (Sicuro et al. 2012a; Navarro et al. 2013; Ahmed et al. 2017; Sroyraya et al. 2017).

These organisms primarily feed on decomposing organic matter that accumulates on the seabed, making them excellent accumulators of particles and contaminants present in the sediment. By inhabiting areas near underwater

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outfalls, where wastewater is often released into the ocean, holothurians can concentrate and accumulate heavy metals and trace elements found in this wastewater. By monitoring the concentrations of these pollutants in holothurians, scientists can assess the health of the marine ecosystem and the impact of pollutant release through underwater outfalls. The capacity of *H. sanctori* to reflect water quality and the presence of contaminants in its environment makes it a valuable tool for environmental management and monitoring in coastal and underwater regions (Warnau et al. 2006; Magdy et al. 2021). In the area, several studies have been conducted on contamination by heavy metals and trace elements in water and different organisms (Lozano-Bilbao et al. 2018, 2021, 2024; Lozano-Bilbao and Alcázar-Treviño 2023). This leads us to suggest that the species used in our study, *H. sanctori*, could be a good bioindicator of environmental contamination by heavy metals and trace elements in this specific zone.

Previous studies on contamination in water and organisms have revealed the presence of these pollutants, and *H. sanctori*, being a marine organism capable of accumulating and responding to the presence of heavy metals and trace elements in its environment, could reflect the environmental quality and extent of pollution in the study area. This capacity for accumulation and response to pollutants makes it a potential useful biological indicator for monitoring and evaluating the environmental health of this marine ecosystem concerning the presence of specific contaminants.

Mohammadizadeh et al. 2016, studied the concentrations of holothuria in the Persian Gulf, obtaining Cd values of 0.91-1.15 mg/kg, Zn 44.28 mg/kg and Pb 15.78. These are very high values compared to those found in our study, this may be due to the fact that the Persian Gulf is an area very frequented by commercial sea routes and in the neighboring countries there are no regulations and legislation

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as rigorous as in Spain. Turk Culha et al. 2016 studied the concentrations of holothuria in the Persian Gulf, obtaining Cd values of 1.19 mg/kg, Zn 56.73 mg/kg, Pb 4.31 mg/kg and Ni 11.06 mg/kg. These values are very high compared to those found in our study, this may be due to the fact that, like the Persian Gulf, the Bosphorus is an area frequented by commercial marine routes with high levels of pollutants.

The Table 3 provides significant data on exposure to different metals during the years 2021 and 2022, with limits established in micrograms per kilogram of body weight per week or day for cadmium (Cd), lead (Pb), and nickel (Ni). These limits are used to calculate the maximum amount in grams that an adult weighing 70 kg could ingest over their entire life without experiencing adverse effects. This type of analysis is crucial for assessing and mitigating potential risks associated with chronic exposure to toxic metals. The weekly exposure limit for cadmium in 2021 was 2.5 micrograms per kilogram of body weight per week ($\mu\text{g}/\text{kg}/\text{week}$), and it remained the same in 2022 at 2.5 $\mu\text{g}/\text{kg}/\text{week}$. The average exposure levels in the population were 0.611 $\mu\text{g}/\text{kg}/\text{week}$ in 2021 and slightly increased to 0.624 $\mu\text{g}/\text{kg}/\text{week}$ in 2022. These levels translate into cumulative exposure, and if we consider an adult weighing 70 kg, the calculated safety limit in grams that could be ingested without risks over a lifetime was 40.10 grams in 2021 and 39.26 grams in 2022. Regarding lead (Pb), the daily exposure limit was 0.5 $\mu\text{g}/\text{kg}/\text{day}$ in both 2021 and 2022. The average exposure levels were recorded at 1.795 $\mu\text{g}/\text{kg}/\text{day}$ in 2021 and slightly increased to 1.82 $\mu\text{g}/\text{kg}/\text{day}$ in 2022. Although this difference may not seem significant, when considering the maximum amount in grams that a 70 kg person could ingest over their entire life without risks, it would be 19.78 grams in 2021 and 19.51 grams in 2022. Finally, let's examine nickel (Ni). The daily exposure limit was 2.8 $\mu\text{g}/\text{kg}/\text{day}$ for both years. The average exposure levels

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were 3.225 µg/kg/day in 2021 and increased to 3.323 µg/kg/day in 2022. This indicates an increase in the population's average exposure over these two years. In terms of the maximum amount of nickel that a 70 kg person could ingest over their entire life without risks, this translates to 60.78 grams in 2021 and 58.98 grams in 2022. These calculations underscore the importance of monitoring and controlling exposure to toxic metals such as cadmium, lead, and nickel in the population. The established limits help define safe levels of ingestion over time, providing crucial data for public health policies and environmental regulations aimed at protecting people's health against the adverse effects of chronic exposure to heavy metals (Reglamento (CE) No 1881/2006 2006; Reglamento (CE) No 420/2011 2011; Reglamento (UE) No 488/2014 2014; Reglamento (UE)2015/1005 2015).

Credit authorship contribution statement

Sampling data and analysis were performed by all authors. **Int** ELB, AJG, SP; **MM:** ELB, CR, AH, DGW; **Res:** ELB, AJG, AH; **CD:** ELB, AH, CR, SP, DGW, AJG.

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

Data will be made available on request

Declarations

Ethics approval all authors declare that the use of animals for this research complies with the requirements of the European legislation on the use of animals for experimentation. All the f samples collected were provided by the fishermen in the fish markets, so these organisms were not slaughtered by the authors of this manuscript; therefore, we faithfully comply with the Code of Practice for Housing and Care of Animals Used in Scientific Procedures.

Consent to participate for the study, no animals had to be killed, so it is not applicable.

Consent for publication The authors consent the publication of this study.

Competing interests The authors declare no competing interests.

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Conflict of interest and originality declaration

As stipulated in the *Code of Ethics and Best Practices* published in *Ceres Journal*, the authors, *Lozano-Bilbao, Enrique; González-Weller, Dailos and Gutiérrez, Ángel* declare that they have no real, potential or evident conflicts of interest, of an academic, financial, intellectual or intellectual property nature, related to the content of the article: *Ecological responses of Holothuria sanctori to metal contamination at La Punta del Hidalgo, Tenerife Island: two-year monitoring and analysis*, in relation to its publication. Likewise, they declare that the work is original, has not been published partially or totally in another medium of dissemination, no ideas, formulations, citations or illustrations were used, extracted from different sources, without clearly and strictly mentioning their origin and without being duly referenced in the corresponding bibliography. They consent to the Editorial Board applying any plagiarism detection system to verify their originality. The authors declare that in preparing this manuscript they did not use generative artificial intelligence tools for text writing or data interpretation.