



## OPEN Use of multispecies (*Nannochloropsis oceanica*, *Artemia franciscana*, and *Arbacia nigra*) approach to assess the quality of marine water from Callao Bay, Peru

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Multi-species tests in bioassays offer a holistic view of the ecosystem's response to toxicity, as different species display varying sensitivities to pollutants. This research aimed to assess the ability of toxicity tests' to distinguish contamination levels, examine site-specific effects, and investigate seasonal variability. Using a multispecies approach (*Nannochloropsis oceanica*, *Artemia franciscana*, and *Arbacia nigra*), bioassays evaluated marine water quality from Callao Bay in Peru across four sampling areas (Naval School: PA1, Peruvian Marine Institute: PA2, Callao Pier: PA3, and San Lorenzo Island: PA4). These species, with varying sizes and morphologies, are relevant to marine systems and ideal for multispecies toxicity testing, contributing to broader environmental impact discussions. To conduct toxicity bioassays, seasonal evaluations were performed in fall, winter, spring, and summer. Brine shrimp displayed seasonal variations in toxicity values, with notable mortality rates during winter. *Nannochloropsis oceanica* was the most sensitive species, showing moderate toxicity across seasons. Areas impacted by pollution sources, such as wastewater and maritime traffic, exhibited the highest toxicity levels (PA3 and PA4). These fluctuations underscore the need to consider seasonal and local conditions when assessing organism sensitivity to seawater contaminants. Additionally, they reveal the complex interplay between environmental factors, water quality, and organism responses in marine ecosystems.

**Keywords** Acute bioassay, Aquatic toxicity, Ecotoxicology, Heavy metals, Pollution

The ocean, as the largest continuous planetary ecosystem, hosts a vast array of organisms spanning different trophic levels, from primary producers like phytoplankton to apex predators such as fish, sharks, and whales<sup>1,2</sup>. This intricate web of life spans various habitats, forming a complex and dynamic ecosystem crucial for the planet's health and all its inhabitants<sup>3,4</sup>. Preserving this biodiversity is vital for ensuring the stability and resilience of marine ecosystems in the face of ongoing environmental challenges.

Pollution of aquatic ecosystems is ongoing, with many human-made pollutants significantly increasing in marine environments, posing a major global concern in recent decades<sup>5,6</sup>. Additionally, climate change is also a significant contributor to the long-term decline of kelp ecosystems, exacerbated by other regional environmental stresses<sup>7</sup>. This is worrying, as aquatic ecosystems are exploited for food, transportation, industrial activities, and recreation<sup>8</sup>. Addressing these challenges requires assessing how contaminants and environmental changes

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impact marine organisms, especially those at the base of the food web. This understanding is key to protecting aquatic ecosystems and ensuring their resilience against growing human activities and environmental pressures. Coastal areas, though just 7% of marine environments provide over 50% of ocean ecosystem food due to high primary productivity<sup>9</sup>, yet suffer from heavy pollution<sup>8</sup>. Pollutants in the environment can accumulate in organisms through direct or indirect routes, with the latter primarily occurring through the consumption of organisms at lower trophic levels and biomagnification through the food chain. Excessive levels of potentially toxic elements, such as heavy metals, have been detected in the coastal region of Callao Bay in water, sediments, and three native fish species<sup>10–13</sup>.

The area along the Peruvian coast is a significant hotspot for marine pollution by heavy metals such as Cd, Cr, Hg, Ni, Ag, Pb, and Se, primarily due to wastewater from domestic, agricultural, and industrial sources originating from the Rímac and Chillón rivers<sup>11</sup>. These nonessential metals interact with cellular components, leading to the generation of reactive oxygen species (ROS), transcriptional changes, and DNA damage, ultimately contributing to diseases like multiorgan damage and cancer<sup>14,15</sup>. The Callao Port Terminal handles 90% of the country's maritime traffic, both commercial and military, amidst an urban environment consisting of adjacent private land for residential and industrial purposes. Additionally, the Callao sea receives about three times more domestic wastewater than what is produced by the province.

Assessing toxicity in this context is essential for sustainable development, protection of marine resources, and human health risk assessment. Evaluating toxicity in Callao Bay helps gauge the overall health of the aquatic ecosystem in the region. Understanding the presence and effects of pollutants can inform management strategies to mitigate human-induced impacts and preserve biodiversity<sup>16–18</sup>. Findings from these assessments can guide regulatory agencies and policymakers in implementing measures to reduce pollution and protect marine habitats. This may include improving wastewater treatment and enforcing pollution control measures.

Using a variety of marine species in bioassays provides a more holistic view of the ecosystem's response to toxicity. Multi-species tests, as suggested by Schuijt et al.<sup>19</sup>, incorporate interactions among species, such as competition and predation, and food chains, which result in bioaccumulation. Different species offer distinct perspectives on ecosystem health, broadening our understanding of how pollutants impact marine environments at multiple trophic levels<sup>11,20,21</sup>. Microalgae like *Nannochloropsis oceanica* are part of the phytoplankton and a primary producer value used in ecotoxicity testing due to their rapid growth and role as primary producers<sup>22–24</sup>. In addition, invertebrates such as *Artemia franciscana* which is part of the zooplankton and, is a primary consumer and, *Arbacia nigra* which is part of the macrozoobenthos and, is an omnivore and carnivore species have demonstrated potential as bioindicators in various toxicity assays<sup>25–27</sup>. These species' varying ecological roles provide a comprehensive framework for evaluating pollutant impacts in the coastal region of Callao Bay.

This study aimed to assess the toxicity of marine water from Callao Bay, Peru, using a multispecies bioassay approach with *N. oceanica*, *A. franciscana*, and *A. nigra*. By focusing on species from different trophic levels and ecological functions, this research sought to identify contamination levels across various sampling sites and explore the seasonal variability of water toxicity. We hypothesized that contamination would vary by site and season, with higher levels of toxicity expected in areas closer to industrial and urban zones, and that the multispecies approach would provide a more detailed understanding of the marine ecosystem's response to pollution. The findings will contribute to broader discussions on environmental impacts, offering insights for managing and protecting marine resources.

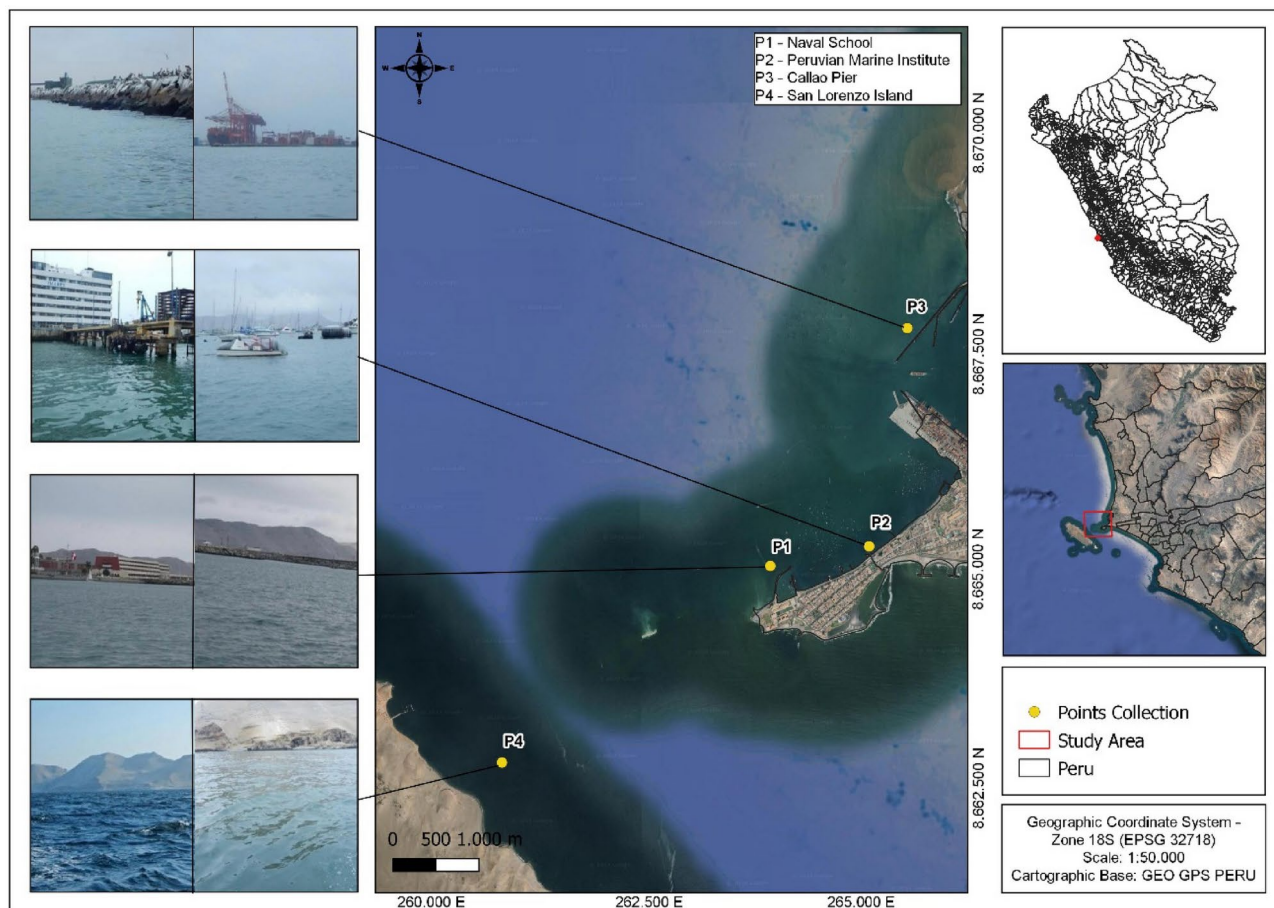
## Material and methods

### Study area, sampling, and ethical aspects

The research was conducted in the coastal zone of the marine Bay of Callao, Peru, a region renowned for its strategic significance across various domains, including industrial, tourist, recreational, commercial, and naval activities, as well as its vital role as a port. Four sampling areas were considered, georeferenced with a GPS (GARMIN model map 4215) and spatially located on a map with the QGIS 3.30.3 (Fig. 1).

The region faces challenges from rapid urbanization, inadequate sanitation, and industrial waste discharge, which result in emissions into the atmosphere and aquatic systems. This has negative impacts on marine life and the quality of life for residents<sup>13</sup>. According to Peru's coastal-marine water bodies classification, PA1 and PA3 are located in areas with marine port, industrial, or sanitation activities, while P2 is in a zone designated for primary contact recreation. The PA3 sampling station, due to its proximity to the Rímac and Chillón Rivers, and the collectors of wastewater: Comas, Taboada, Centenario, and Bocanegra Height El Camotal Zone receives the greatest impact of pollution from various economic activities such as domestic, agricultural, industrial, and mining waste that is dumped into its riverbed in an uncontrolled manner<sup>28</sup>. On the other hand, San Lorenzo Island (PA4) and its surroundings have a well-preserved biota due to minimal human activity for many years. Additionally, part of the island is dedicated to mollusk extraction and cultivation<sup>11</sup>. PA1 to PA3 are classified as Special Treatment Zone—Ecological Economic Unit with environmental degradation, and P4 is classified as an Ecological Economic Unit of Production Core with High Hydrobiological Potential.

To conduct toxicity bioassays, four seasonal evaluations were carried out in Callao Bay: fall (May 30, 2015), winter (August 22–23, 2015), spring (November 22–23, 2015), and summer (January 5–6, 2016). The study adhered to all national and international ethical standards of ecotoxicology. The use of invertebrates in laboratory studies is permitted in ecotoxicological research without ethical restrictions<sup>29,30</sup>, and the number of organisms used followed the principles of the three “r's”<sup>31</sup>. For proper management of *N. oceanica*, *A. franciscana*, and *A. nigra* culture, reagents and seawater samples, as well as their disposal, the “Safety Plan for Laboratories and Workshops (SSST-PLC-01)” was followed the Rectoral Resolution No. 10026-2009 -UNFV and the “Security Protocol for Engineering, Architecture and Natural Sciences Laboratories and Workshops (SSST-PS-02)”. National permits were obtained from the relevant authority for collecting water samples in Callao Bay. The three marine species do not have a maximum limit of permissible collection in the Peruvian sea.



**Fig. 1.** The geographic location of sampling stations-areas in the coastal zone of Callao, Peru. PA1: Naval School ( $12^{\circ} 4' 3.30''\text{S}$ ;  $77^{\circ} 10' 8.60''\text{O}$ ); PA2: Peruvian Marine Institute ( $12^{\circ} 3' 56.20''\text{S}$ ;  $77^{\circ} 9' 30.70''\text{O}$ ); PA3: Callao Pier ( $12^{\circ} 2' 34.10''\text{S}$ ;  $77^{\circ} 9' 15.40''\text{O}$ ); PA4: San Lorenzo Island ( $12^{\circ} 5' 16.57''\text{S}$ ;  $77^{\circ} 11' 52.03''\text{O}$ ). QGis (version 3.30.3).

The rationale behind selecting the four specific sampling seasons (fall, winter, spring, and summer) is based on the distinct environmental conditions and potential variability in pollutant levels observed throughout the year in Callao Bay. These seasons are critical for capturing temporal variations in temperature, water circulation, water flow, and human activities, which can influence pollutant discharge, biological processes, and overall water quality<sup>32–34</sup>. In coastal ecosystems like Callao Bay, pollutant levels can fluctuate due to seasonal changes in industrial activity, maritime traffic, and rainfall patterns, which affect the distribution and concentration of contaminants. Therefore, seasonal assessments allow for a more comprehensive understanding of how these factors contribute to the spatial and temporal dynamics of aquatic pollution<sup>35,36</sup>.

### Ecotoxicological bioassays with marine water

Four liters of water were collected from each sampling site in plastic containers for conducting ecotoxicological bioassays. These samples were kept within a cold chain (maintained at  $-04^{\circ}\text{C}$  for preservation and transfer) until they were utilized at the Laboratory of Ecology and Animal Biodiversity (LEBA) at Federico Villarreal National University (UNFV). Physicochemical parameters, including surface temperature, pH, salinity, and conductivity, were measured in situ using an EXO 2 multiparameter probe at the beginning and end of each bioassay.

#### *Bioassays with microalgae (Nannochloropsis oceanica)*

Toxicity assays were conducted to inhibit the growth of microalgae following the EPA (Environmental Protection Agency) protocol OPPTS 850.5400, OECD 201 (Organization for Economic Co-operation and Development), and ISO 8692 from 2002. The bioassay was static, with no renewal of the test solution, and the pH, temperature, and electrical conductivity of the samples were measured at the beginning and end of the test. The experimental design included five concentrations of water samples with a dilution factor of 0.5 (100, 50, 25, 12.5, and 6.25%), along with a negative control (Cn) in test tubes, each with 4 replicates. Concentrations for seawater were selected as indicated in standard protocols of the EPA, OECD, and ISO, previously cited and used also by Osorio et al.<sup>11</sup>.

The total test volume was 2.5 mL, with illumination ranging from 4.500 to 5.000 lx, permanent and constant, for up to 96 h of exposure. The strain of *N. oceanica* was obtained from the Algae Culture Laboratory at the Faculty of Marine Biology of the Southern Scientific University (UCSUR). Regarding the microalgae cultivation

method, it was initially seeded on Agar–Agar in Petri dishes and then transferred to test tubes in liquid phase in a modified Guillard “f/2” medium with UV-filtered seawater (0.22u) containing necessary salts, following the proposal of the U.S. Environmental Protection Agency (US EPA)<sup>37</sup>.

The initial inoculum in all cases was 10.000 cells/mL. The evaluated response was the cell density determined at 24, 48, 72, and 96 h in all replicas of all concentrations and the control series, using a Neubauer chamber or hemocytometer and a compound microscope at 40X magnification.

#### *Bioassays with brine shrimp (Artemia franciscana)*

Ecotoxicological assays with *A. franciscana* were not conducted at a fixed age of the nauplii but at a specific developmental stage. This was done to avoid physiological differences in early developmental stages that could mask sensitivity differences among nauplii populations. Larval instar II was chosen, which, while not completely impermeable, still allows toxicity testing without the need for feeding individuals<sup>38</sup>.

For the toxicity test, 250 mg of *Artemia* cysts were used, which were incubated in 500 ml of seawater with a salinity of 35 g/L, in a 500 ml beaker, at a temperature of  $23^{\circ} \pm 1^{\circ} \text{C}$  and illumination ranging from 500 to 1000 lx for one hour during the hydration phase. The cysts were kept in suspension with light and continuous aeration, resulting in a population of nauplii at 22–24 h predominantly in the instar II phase. The nauplii used for the test were healthy, without anybody malformations.

The acute toxicity tests were conducted in multiwell plates (Multiwell®), with each test comprising five concentrations of seawater and a control group, each replicated four times. Each well contained a total volume of 2 mL, consisting of artificial seawater (Fluval Sea®) for the control groups (with physicochemical characteristics of pH = 8.3; EC = 0.86 dS/m), and seawater solutions at 100, 50, 25, 12.5, and 6.25% concentrations (dilution factor of 0.5). Subsequently, ten instar II nauplii were added to each well using a small, elongated Pasteur pipette to avoid adding additional volume to each well, which could dilute the solutions. Finally, the plates were incubated at  $23^{\circ} \text{C} \pm 1^{\circ} \text{C}$ , under constant illumination, with no food provided to the nauplii.

#### *Bioassays with sea urchins (Arbacia nigra)*

The specimens of *A. nigra* were collected on Pucusana Beach, south of the city of Lima, Peru ( $12^{\circ}28'48.37''\text{S}$   $76^{\circ}48'2.30''\text{W}$ ). They were extracted from the rocky intertidal zone and kept cold during transportation to the laboratory. Stimulation to obtain sperm and eggs was performed by injecting them with 0.5 M KCl through the peristome<sup>39</sup>. The sea urchins were then left to rest supported on their oral surface, and they began to expel gonadal material through the aboral surface; males were identified by the expelled substance being white, while females expelled a red-colored substance.

Subsequently, using a Pasteur pipette, a small drop of gonadal material from both sexes was deposited onto a microscope slide and observed under a microscope (Olympus at 40X) to verify the quality of the gametes. Sperm should exhibit high mobility, and oocytes should have a diameter close to 10  $\mu\text{m}$ , as indicated by US EPA<sup>37</sup>. Once the gamete quality was confirmed, sperm expelled from four males was collected in a 15 mL glass tube, using a Pasteur pipette in the absence of seawater, and placed on ice until the start of the bioassay.

Eggs from four females were also collected in 10 mL glass tubes. A solution of 2.000 eggs/mL was prepared using a Sedgwick-Rafter counting chamber (Wildlife Supply Company, Gridded Sedgwick-Rafter cell, USA), and sperm was diluted to  $5 \times 10^7$  cells/mL using artificial seawater (Fluval Sea®) at a pH of 7.8, using a hemocytometer (Marienfeld®, Neubauer Improved, Germany).

The assay was conducted at a temperature of  $14 \pm 1^{\circ} \text{C}$ . For the bioassay, 20 mL centrifuge tubes were used to prepare solutions at five different concentrations of seawater samples: 100, 50, 25, 12.5, and 6.25%, along with a control, with a total volume of 5 mL, each having four replicates per point<sup>37</sup>, in a randomized complete block design (RCBD) of  $6 \times 4$ . To each tube, 100  $\mu\text{L}$  of sperm suspension was added. After 1 h, 1 mL of egg suspension was added.

A validity criterion for the bioassay was set at 75% fertilization in the controls after 30 min of adding the egg suspension. Finally, 0.5 mL of formalin was added to each tube to preserve the material for later reading. To evaluate the percentage of fertilization, 1 mL of each test tube was transferred to a Sedgwick-Rafter chamber. The number of fertilized and unfertilized eggs was counted, distinguished by the absence of the fertilization membrane<sup>37</sup>.

### Statistical analysis

#### *Bioassays with microalgae (Nannochloropsis oceanica)*

The dose–response relationship was derived from concentration data as the independent variable and the percentage of population growth inhibition as the dependent variable. Population growth inhibition percentage was calculated at 96 h of exposure using Eq. 1 ref<sup>40</sup>.

$$I = 100 \left[ 1 - \frac{N_e}{N_c} \right] \quad (1)$$

where:

I = Population growth inhibition (%)

$N_e$  = Cell density exposed to the toxicant (cells/mL)

$N_c$  = Cell density of the control (cells/mL)

Using the inhibition data, the median inhibitory concentration (IC50) was determined through the Probit test with a 95% confidence level; which calculates the concentration of the contaminant that results in a 50%

inhibition of population growth<sup>41</sup>. In addition, the No Observed Effect Concentration (NOEC), which is the highest concentration at which no significant effects are observed compared to the control group, and the Lowest Observed Effect Concentration (LOEC), the lowest concentration at which significant effects are detected<sup>42,43</sup>, were also determined.

To determine the NOEC and LOEC of seawater, the Shapiro–Wilk normality test, Levene’s test for homogeneity of variances, one-way ANOVA, and Tukey’s post hoc test were conducted. Significant differences between heavy metal treatments were determined using the non-parametric Mann–Whitney test for seasons and the Kruskal–Wallis test to compare the four sampling points. All calculations were performed using IBM SPSS Statistics 22.

#### *Bioassays with brine shrimp (Artemia franciscana)*

The calculation of LC<sub>50</sub> was assessed using Probit analysis, and the significance between concentrations per sampling area was evaluated with the Tukey significance test. For both calculations, the statistical package SPSS version 22.0 was used. Pearson correlation was found between the values of LC<sub>50</sub>, NOEC, and LOEC. The Schneider-Orelli’s formula<sup>44</sup> was used for mortality percentage correction. Data were transformed using the logarithm plus 1 before analysis.

#### *Bioassays with sea urchins (Arbacia nigra)*

The data for fertilized eggs were transformed to the arcsine of the square root, and a Shapiro–Wilk test for normality and Levene’s test for homogeneity of variances was conducted. In cases where the results did not meet the assumptions of normality and/or homoscedasticity, the Kruskal–Wallis test was performed to determine the LOEC, NOEC, and IC<sub>50</sub>. Analysis was conducted using the SPSS 22.0 statistical program. In case of significant differences between concentrations, a Tukey’s Honestly Significant Difference (HSD) test was conducted<sup>45</sup>.

## Results and discussion

### Ecotoxicological bioassays using marine water: assessing the impact on microalgae (*N. oceanica*)

The pH varied from 6.99 (PA2 in Summer) to 9.87 (PA3 in Winter). Based on initial readings, the pH sequence across sampling areas was: PA2 > PA1 > PA3 > PA4 (Fall), PA3 > PA1 > PA2 > PA4 (Winter), PA2 > PA3 > PA1 > PA4 (Spring), and PA2 > PA1 > PA3 > PA4 (Summer). Variations were observed across seasons, primarily due to seasonal precipitation, which dilutes seawater, leading to fluctuations in pH and salinity. Physicochemical parameters were assessed at the beginning and end of each bioassay, as outlined in Table 1.

The PA3 and PA4 sampling areas consistently exhibited the highest conductivity, peaking at 54.3 mS/cm during the spring (Table 1). In winter, PA3 recorded the highest salinity value, reaching 36.5 g/L by the end of the sampling period. High concentrations of PA3 can be linked to various contamination sources and environmental factors. The area’s proximity to port activities may contribute to seawater intrusion, especially during dry seasons with reduced freshwater flow.

Parameters	Fall		Winter		Spring		Summer	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
PA1								
pH	8.24	9.87	8.13	9.53	8.33	8.49	7.86	9.42
Temperature (°C)	21.3	24	23.3	23.9	22.6	21	21.6	21.3
Conductivity (mS/cm)	53.1	53.3	53.4	53.6	53.1	53.6	53.7	50.3
Salinity (g/L)	34.9	35	35.2	35.3	34.9	35.3	35.4	36
PA2								
pH	8.09	8.23	8.19	8.37	7.52	8.55	6.99	9.42
Temperature (°C)	23.4	21.5	23.2	21.5	23.5	22.6	21.6	21.3
Conductivity (mS/cm)	53.5	53.4	53.3	53.5	53.1	53.6	53.7	50.2
Salinity (g/L)	35.2	35.1	35.1	35.3	34.9	35.3	35.6	36
PA3								
pH	8.27	9.09	7.91	9.87	8.21	8.49	7.98	9.42
Temperature (°C)	22.8	23.8	22.9	23.9	23.5	21.3	21.7	21.3
Conductivity (mS/cm)	53.5	53.3	53.2	52.9	53.1	53.6	54.3	50.1
Salinity (g/L)	35.2	36.5	35.1	34.9	34.9	35.3	35.5	36
PA4								
pH	8.35	9.09	8.24	9.23	8.62	9.23	8.98	9.42
Temperature (°C)	23.5	24	23.5	23.6	21.7	23.6	21.7	21.3
Conductivity (mS/cm)	52.6	52.8	53.1	53.6	53.1	53.6	54.3	50.1
Salinity (g/L)	34.6	34.8	34.9	35.3	35.5	35.3	35.5	36

**Table 1.** Physicochemical parameters of the water taken at the beginning and end of the bioassay with microalgae *Nannochloropsis oceanica*. PA1: Naval School; PA2: Peruvian Marine Institute; PA3: Callao Pier; PA4: San Lorenzo Island.

These results are crucial, as physicochemical parameters like conductivity and salinity have direct implications for the survival and growth of *N. oceanica*, a species known for its sensitivity to environmental fluctuations<sup>46–48</sup>. The high salinity and conductivity may have created unfavorable conditions for the microalga, hindering its ability to absorb nutrients and carry out photosynthesis efficiently.

The temporal and spatial variations in the physicochemical parameters are essential for evaluating aquatic pollution, influenced by both natural and anthropogenic sources<sup>49</sup>. For instance, the increase in water temperature has been correlated with faster decomposition of organic matter and pollutants due to intensified microbial activity at higher temperatures<sup>34</sup>. In Callao Bay, the elevated temperatures during spring and summer may have facilitated increased biodegradation of organic compounds, temporarily improving water quality during these seasons. Furthermore, the temperature and salinity values recorded across the four seasons were consistently correlated with water quality parameters, such as turbidity and nutrient concentrations<sup>50</sup>.

These discussions are significant because elevated salinity can affect the solubility of dissolved oxygen in water, which in turn impacts the respiration of marine organisms. In highly saline waters, such as PA3, the lower availability of oxygen may represent an additional environmental stressor for species evaluated in this research, affecting their ability to survive in polluted environments. Another critical factor to consider is pH, which directly influences the solubility of heavy metals and nutrients in water. In Callao Bay, the pH remained within a range that could potentially alter the toxicity of certain pollutants, negatively impacting the development and physiology of aquatic organisms.

The combination of pH, elevated temperature, salinity, and conductivity contributes to a series of cumulative stresses affecting the aquatic community, including the species tested in the bioassays<sup>51–53</sup>. These results suggest that the interaction between physicochemical parameters and the bioassays conducted should be further explored in future studies. The observed variations in salinity, conductivity, and temperature play a crucial role in determining the health of marine organisms and highlight the need for continuous monitoring to assess how water quality in Callao Bay may continue to affect its biodiversity and local ecological dynamics.

The IC<sub>50</sub>, NOEC, and LOEC values at 96 h of exposure for *N. oceanica* in water samples from Callao Bay (Table 2) indicate a clear pattern of toxicity. Water from sites PA4 and PA3 showed the highest toxicity levels, significantly inhibiting algal growth. This is likely due to their proximity to the industrial zone. In contrast, sites PA1 and PA2, located further from the industrial activity in more open sea areas, exhibited much lower toxicity levels. These results suggest that distance from pollution sources is a key factor influencing the toxicity observed in the bay.

The lowest toxicity IC<sub>50</sub> value was observed in PA2 during winter (88.6%), while the highest was recorded in PA4 during spring (22.66%) (Table 2). Fall and spring were the seasons most significantly associated with increased toxicity, contrasting with winter, which exhibited lower toxicity across most sites. This seasonal variation could be influenced by environmental factors such as the consistently high conductivity and salinity levels observed in PA3 and PA4, which may be linked to industrial effluents and natural seawater influxes. Microalgae have demonstrated greater sensitivity to these conditions compared to invertebrates and fish, especially when exposed to natural products, domestic, and industrial effluents<sup>54,55</sup>. Further studies could clarify how these abiotic factors contribute to the seasonal toxicity patterns observed.

Parameters	Fall	Winter	Spring	Summer
PA1				
NOEC (%)	12.5	< 6.25	6.25	100
LOEC (%)	25	6.25	12.5	> 100
IC <sub>50</sub> (%)	43.85	51.48	67.38	52.61
PA2				
NOEC (%)	50	6.25	< 6.25	100
LOEC (%)	100	12.5	6.25	> 100
IC <sub>50</sub> (%)	31.81	88.6	60.09	43.17
PA3				
NOEC (%)	50	100	25	< 6.25
LOEC (%)	100	> 100	50	6.25
IC <sub>50</sub> (%)	43.85	38.42	49.82	56.19
PA4				
NOEC (%)	100	6.25	100	6.25
LOEC (%)	> 100	12.5	> 100	12.5
IC <sub>50</sub> (%)	28.18	46.81	22.66	46.81

**Table 2.** Assessment of marine water toxicity and its effects on the growth inhibition of *Nannochloropsis oceanica* in Callao Bay, Peru. PA1: Naval School; PA2: Peruvian Marine Institute; PA3: Callao Pier; PA4: San Lorenzo Island. NOEC: Maximum concentration at which no adverse effects are observed compared to the control. LOEC: Minimum concentration where adverse effects are observed compared to the control. IC50: median inhibitory concentration.

Drawing upon these insights, microalgae like *N. oceanica* have become instrumental in ecological models for assessing the toxicity of environmental samples. The toxicity data obtained not only provide a snapshot of species-specific responses but also offer critical insight into potential cascading effects throughout the marine ecosystem. Microalgae, being primary producers, play a foundational role in food webs<sup>56</sup>. A decline in their population due to exposure to contaminants can disrupt the energy flow to higher trophic levels, affecting species like zooplankton, fish, and even marine mammals.

Moreover, species such as *A. franciscana* and *A. nigra* are key components of marine food chains. Toxicity affecting these species can lead to a reduction in the availability of food for predators, ultimately impacting ecosystem services like fisheries, which are vital for human communities. Furthermore, changes in the abundance and health of these species can disrupt nutrient cycling and habitat structure, leading to broader ecological consequences. Therefore, the findings highlight the need for ongoing monitoring and mitigation of contaminant sources to protect marine biodiversity and sustain ecosystem services.

### Ecotoxicological bioassays using marine water: assessing the impact on brine shrimp (*A. franciscana*)

The pH varies between sampling points and seasons. However, the highest values were recorded at PA4 in all seasons, and the lowest at PA3 in all seasons except winter. Other physicochemical variables are shown in Table 3. Overall, the recorded pH values generally fall within the World Health Organization's recommended range of 7.0 to 8.5 for water quality<sup>57,58</sup>.

The LC<sub>50</sub>, NOEC, and LOEC values for *A. franciscana* at 24 and 48 h of exposure, across different seasons (Table 4), reveal seasonal variations in toxicity. In fall, spring, and summer, no mortality was observed at 24 h, and there were no significant differences between sampling sites, except for PA4, which had a notable LC50 of 48.08%. However, in winter, toxicity increased significantly, with the highest LC50 recorded at PA1 (81.85%), followed by PA4 (62.91%) and PA2 (54.45%). After 48 h, fall and spring continued to show no mortality across all sites, but in winter, toxicity persisted, with PA2 showing the highest LC<sub>50</sub> (27.83%), followed by PA1 (41.44%) and PA4 (55.14%). These results highlight the increased toxicity during winter and the pronounced effect at PA4 throughout the year.

These results suggest a potential seasonal influence on the species' response to water exposure, with winter showing increased sensitivity to higher contaminant concentrations<sup>59,60</sup>. These findings are important for assessing the potential impacts of environmental conditions and water quality on aquatic life, providing valuable insights for the management and conservation of aquatic ecosystems. However, it is important to emphasize the need for further research to fully understand the underlying mechanisms of the observed effects and their relevance to the health and well-being of aquatic organisms and ecosystems as a whole.

### Ecotoxicological bioassays using marine water: assessing the impact on sea urchins (*Arbacia nigra*)

The physicochemical parameters of the assay, as depicted in Table 5, revealed instances where pH levels exceeded recommended thresholds for water quality standards<sup>57,58</sup>, except for PA2 during the summer season, where it

Parameters	Fall	Winter	Spring	Summer
PA1				
pH	8.1	7.9	8.5	8.7
Temperature (°C)	21.5	18.1	20.8	21.5
Conductivity (mS/cm)	53.4	53.7	53.8	53.1
Salinity (g/L)	35.1	35.5	35.3	34.7
PA2				
pH	7.9	8.0	8.1	8.1
Temperature (°C)	20.4	16.9	20.1	20.8
Conductivity (mS/cm)	53.7	53.9	53	53.2
Salinity (g/L)	35.2	35.1	34.8	34.8
PA3				
pH	8.0	8.0	8.2	8.2
Temperature (°C)	20.1	17.2	19.6	21.2
Conductivity (mS/cm)	53.8	53.7	53.5	53.4
Salinity (g/L)	35.2	34.9	34.9	35.3
PA4				
pH	8.2	8.1	8.6	9.1
Temperature (°C)	24.6	18.4	19.9	23
Conductivity (mS/cm)	53.9	53.7	53.3	53.6
Salinity (g/L)	35	35.1	34.9	35.3

**Table 3.** Physicochemical parameters of the water taken at the beginning and end of the bioassay with *Artemia franciscana*. PA1: Naval School; PA2: Peruvian Marine Institute; PA3: Callao Pier; PA4: San Lorenzo Island.

Parameters	24 h of exposure				48 h of exposure			
	Fall	Winter	Spring	Summer	Fall	Winter	Spring	Summer
PA1								
NOEC (%)	100	50	100	100	100	50	100	100
LOEC (%)	> 100	100	> 100	> 100	> 100	100	> 100	> 100
LC <sub>50</sub> (%)	> 100	81.85	> 100	> 100	> 100	41.44	> 100	> 100
PA2								
NOEC (%)	100	50	100	100	100	50	100	25
LOEC (%)	> 100	100	> 100	> 100	> 100	100	> 100	50
LC <sub>50</sub> (%)	> 100	54.45	> 100	> 100	> 100	27.83	> 100	90
PA3								
NOEC (%)	100	100	100	100	100	100	100	100
LOEC (%)	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
LC <sub>50</sub> (%)	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
PA4								
NOEC (%)	100	100	100	100	100	100	100	100
LOEC (%)	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
LC <sub>50</sub> (%)	48.08	62.91	> 100	> 100	> 100	55.14	> 100	> 100

**Table 4.** Assessment of marine water toxicity and its effects on the mortality of *Artemia franciscana* in Callao Bay, Peru. PA1: Naval School; PA2: Peruvian Marine Institute; PA3: Callao Pier; PA4: San Lorenzo Island. NOEC: Maximum concentration at which no adverse effects are observed compared to the control. LOEC: Minimum concentration where adverse effects are observed compared to the control. LC<sub>50</sub>: Median lethal concentration.

Parameters	Fall		Winter		Spring		Summer	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
PA1								
pH	9.06	9.00	8.67	8.61	8.21	8.51	8.38	8.38
Temperature (°C)	28.7	26.4	28.5	25.1	26.1	29.8	26.8	23.6
Conductivity(mS/cm)	35.3	41.8	35.4	45.5	53.5	39.8	53.4	40.1
Salinity(g/L)	53.1	26.9	53.4	29.5	35.4	25.4	35.5	25.6
Dissolved oxygen (mg/L)	2.49	6.34	2.28	5.88	1.72	6.24	2.15	6.37
PA2								
pH	8.71	8.49	8.88	8.58	8.18	8.51	6.86	8.48
Temperature (°C)	28.7	26.4	28.3	25.2	26.2	24.6	26.8	23.5
Conductivity(mS/cm)	35.4	42.2	35.5	44.3	53.6	40.4	53.5	40
Salinity(g/L)	53.2	27.1	53.4	28.6	35.4	25.6	35.4	25.5
Dissolved oxygen (mg/L)	1.35	6.12	2.21	5.93	0.9	6.03	1.33	5.97
PA3								
pH	8.74	8.34	8.77	8.33	8.17	8.56	8.32	8.42
Temperature (°C)	28.2	25.6	28.8	25	26.9	24.8	26.7	25.8
Conductivity(mS/cm)	35.5	38.8	35.4	42.2	53.4	40.6	53.3	39.9
Salinity(g/L)	53.4	24.7	53.5	27	35.5	25.9	35.3	25.5
Dissolved oxygen (mg/L)	1.74	6.35	1.44	5.77	0.89	5.98	0.65	6.46
PA4								
pH	8.79	8.31	8.77	8.33	8.14	8.64	8.35	8.68
Temperature (°C)	28.5	25.9	28.6	25	26.5	25.2	26.7	27.7
Conductivity(mS/cm)	35.5	41.3	35.5	42.2	53.6	41	53.3	40.5
Salinity(g/L)	53.4	26.4	28.6	27	35.6	26.2	35.5	25.9
Dissolved oxygen (mg/L)	3.61	6.01	3.11	5.77	2.4	6.10	4.32	7.81

**Table 5.** Physicochemical parameters of the water taken at the beginning and end of the bioassay with sea urchins *Arbacia nigra*. PA1: Naval School; PA2: Peruvian Marine Institute; PA3: Callao Pier; PA4: San Lorenzo Island.

Parameters	Fall	Winter	Spring	Summer
PA1				
NOEC (%)	< 6.25	100	100	< 6.25
LOEC (%)	6.25	> 100	> 100	6.25
IC <sub>50</sub> (%)	> 100	> 100	85.32	> 100
PA2				
NOEC (%)	< 6.25	25	< 6.25	< 6.25
LOEC (%)	6.25	50	6.25	6.25
IC <sub>50</sub> (%)	1.11	> 100	8.62	> 100
PA3				
NOEC (%)	< 6.25	6.25	100	< 6.25
LOEC (%)	6.25	12.5	> 100	6.25
IC <sub>50</sub> (%)	1.14	68.55	> 100	> 100
PA4				
NOEC (%)	< 6.25	25	100	< 6.25
LOEC (%)	6.25	50	> 100	6.25
IC <sub>50</sub> (%)	> 100	76.56	1.08	> 100

**Table 6.** Assessment of marine water toxicity and its effects on the fertilization of *A. nigra* in Callao Bay, Peru. PA1: Naval School; PA2: Peruvian Marine Institute; PA3: Callao Pier; PA4: San Lorenzo Island. NOEC: Maximum concentration at which no adverse effects are observed compared to the control. LOEC: Minimum concentration where adverse effects are observed compared to the control. IC<sub>50</sub>: median inhibitory concentration.

measured 6.86. Such deviations from recommended pH ranges could be concerning, as they may signal changes in water quality that could potentially impact aquatic organisms<sup>61</sup>.

Dissolved oxygen levels in PA1, PA2, and PA3 indicated hypoxic conditions (2–3 mg/L), raising concerns about the health of these aquatic ecosystems (Table 5). Such conditions are particularly troubling as salinity and dissolved organic matter can influence the toxicity of Cu and other heavy metals<sup>62–64</sup>. These water quality parameters are interdependent, for example, rising temperatures often reduce pH, which negatively impacts aquatic life, while lower dissolved oxygen levels can limit nutrient availability<sup>65</sup>. Additionally, the toxicity of metals varies with changes in hydrochemical properties like calcium, pH, and organic matter content (Hong et al., 2020<sup>66</sup>). Temperature and suspended solids also affect dissolved oxygen and electrical conductivity<sup>35</sup>, while eutrophic conditions, exacerbated by sewage deposits, further increase turbidity<sup>35</sup>.

The IC<sub>50</sub>, NOEC, and LOEC values from the fertilization bioassays using *A. nigra* across various concentrations of Callao Bay seawater (Table 6) generally indicate low toxicity. However, specific sites—PA1 (spring), PA2 (fall and spring), PA3 (fall and spring), and PA4 (fall and summer)—showed IC<sub>50</sub> values below 100%, highlighting increased organism sensitivity to these conditions. These findings align with Ramírez<sup>67</sup>, who observed over 50% mortality in Chilean silverside (*Odontesthes regia*) exposed to Callao Port waters, particularly when concentrations included 25% ballast water, with mortality becoming more pronounced after 24 h of exposure.

The ecotoxic mortality observed based on the LOEC values could be attributed to a complex mix of contaminants rather than a specific group like heavy metals<sup>68,69</sup>. In this study, LOEC, NOEC, and IC<sub>50</sub> values varied significantly among sampling points and seasons. This variation highlights the importance of considering seasonal and local conditions in evaluating organism sensitivity to different seawater concentrations. Furthermore, there are very few studies on the effects of toxic metals on marine species in the region, indicating a need for further research in this area.

Comparing the sensitivity of the three evaluated species to contaminants, *N. oceanica* exhibited the highest sensitivity, with moderate toxicity consistently observed across seasons, while PA4 and PA3 showed the highest levels of toxicity. López et al.<sup>22</sup> noted that this represents one of the earliest reports on the subject, stating that no reports have yet been made regarding the use of the microalga *N. oceanica* for toxicity testing in any environmental matrix of Peruvian bays. In contrast, *A. franciscana* demonstrated lower sensitivity during the fall, spring, and summer seasons.

*Arbacia nigra* exhibited high levels of toxicity, particularly in PA2 (fall and spring), PA3 (fall), and PA4 (spring). This species is one of the most commonly used model organisms in studies of fertilization and early embryonic development. Several studies have demonstrated its sensitivity as a bioindicator of effect for various xenobiotic chemicals, including pharmaceuticals, pesticides, heavy metals, and Polycyclic Aromatic Hydrocarbons<sup>27,70,71</sup>.

The recorded physicochemical parameters are key in determining water quality and their impact on marine organisms. Variations in pH, temperature, conductivity, and salinity directly influence the survival, growth, and physiological responses of species like *N. oceanica*, *A. franciscana*, and *A. nigra*. These parameters not only reflect the current environmental conditions but also provide insight into the potential stressors affecting marine life, emphasizing the importance of ongoing monitoring to understand their ecological implications and manage aquatic ecosystems effectively.

Metal bioavailability from marine pollution by heavy metals is influenced by factors such as pH, salinity, organic matter, temperature, dissolved oxygen, and alkalinity<sup>72</sup>. Banaee et al.<sup>73</sup> summarize the adverse effects of well-studied metals (As, Cd, Cu, Cr, Co, Ni, Pb, Hg, and Zn), their presence in waters, and their impact on crustaceans. They note that salinity can influence Cu and Cr bioavailability<sup>73</sup>. In marine environments, high salinity reduces metal availability through complexation and precipitation, while alkaline pH causes metals to precipitate, lowering availability. Acidic pH increases solubility and toxicity, and higher temperatures enhance metal solubility in water.

The identification of both highly and minimally impacted sites by water toxicity is essential for managing and conserving marine ecosystems, underscoring the need for targeted mitigation strategies in vulnerable areas<sup>74,75</sup>. This is particularly relevant in regions directly affected by maritime traffic, such as the Callao Port Terminal, and the resulting urban development. A study examined pollutant transfer to the herbivore *A. nigra* in an industrialized coastal zone in Central Chile, showing that these organisms are vulnerable to contamination and can transfer pollutants to higher trophic levels<sup>27</sup>.

Recommending a specific species for use depends on the study's objectives and environmental conditions. *Nannochloropsis oceanica* may be more suitable for long-term assessments due to its moderate sensitivity throughout the year. Meanwhile, *A. nigra* may be better suited for short-term assessments, given its high sensitivity during the fall and spring seasons. It's crucial to acknowledge that hindrances in organism development or spawning in each new generation could lead to a decrease in population size or even the extinction of sensitive species<sup>76</sup>.

Employing all three species enables a comprehensive assessment of contaminant impacts across different seasons, offering a thorough understanding of environmental quality and risks to aquatic ecosystems. It is essential to establish a robust monitoring program for regular evaluations of water quality and species health. Targeted mitigation strategies should be implemented in vulnerable areas, particularly near industrial and high-traffic zones. Furthermore, increasing public awareness and engaging local communities in conservation efforts are critical, as environmental degradation often results from insufficient environmental responsibility and ineffective public policies<sup>77–79</sup>.

## Conclusions

*Nannochloropsis oceanica* was the most sensitive species, showing moderate toxicity across seasons. Areas impacted by pollution sources, such as wastewater and maritime traffic, exhibited the highest toxicity levels (PA3 and PA4). *Artemia franciscana* showed lower sensitivity during the autumn, spring, and summer seasons. Toxicity levels fluctuated across seasons, with fall and spring displaying the highest toxicity. *A. nigra* exhibited high toxicity in PA2, PA3, and PA4. This variation underscores the importance of considering seasonal and local conditions when evaluating organism sensitivity to seawater concentrations. Few studies address the impact of toxic metals on marine species in the region, highlighting the need for further research. These findings reveal the complex interplay between environmental factors, water quality dynamics, and organism responses in marine ecosystems. Implementing a continuous monitoring program and mitigation strategies in vulnerable areas, especially near industrial zones, is essential.

## Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Received: 20 June 2024; Accepted: 30 December 2024

Published online: 07 January 2025

## References

- Elgarahy, A. M., Akhdhar, A. & Elwakeel, K. Z. Microplastics prevalence, interactions, and remediation in the aquatic environment: A critical review. *J. Environ. Chem. Eng.* **9**(5), 106224. <https://doi.org/10.1016/j.jece.2021.106224> (2021).
- Kolipinski, M., Subramanian, M., Kristen, K., Borish, S. & Ditta, S. Sources and toxicity of mercury in the San Francisco Bay area, spanning California and beyond. *J. Environ. Public Health* <https://doi.org/10.1155/2020/8184614> (2020).
- Laffoley, D. et al. Eight urgent, fundamental and simultaneous steps needed to restore ocean health, and the consequences for humanity and the planet of inaction or delay. *Aquat. Conserv. Mar. Freshw. Ecosyst.* **30**(1), 194–208. <https://doi.org/10.1002/aqc.3182> (2020).
- Lloret, J. et al. The roses ocean and human health chair: a new way to engage the public in oceans and human health challenges. *Int. J. Environ. Res. Public Health* **17**(14), 5078. <https://doi.org/10.3390/ijerph17145078> (2020).
- Zamani-Ahmadmahmoodi, R., Malekabadi, M. B., Rahimi, R. & Johari, S. A. Aquatic pollution caused by mercury, lead, and cadmium affects cell growth and pigment content of marine microalga *Nannochloropsis oculata*. *Environ. Monitor. Assess.* **192**, 1–11. <https://doi.org/10.1007/s10661-020-8222-5> (2020).
- Muruganandam, M. et al. Impact of climate change and anthropogenic activities on aquatic ecosystem—A review. *Environ. Res.* <https://doi.org/10.1016/j.envres.2023.117233> (2023).
- Smale, D. A. et al. Marine heatwaves threaten global biodiversity and the provision of ecosystem services. *Nat. Clim. Change* **9**(4), 306–312. <https://doi.org/10.1038/s41558-019-0412-1> (2019).
- Häder, D. P. et al. Anthropogenic pollution of aquatic ecosystems: Emerging problems with global implications. *Sci. Total Environ.* **713**, 136586. <https://doi.org/10.1016/j.scitotenv.2020.136586> (2020).
- Alexander, D. E., & Fairbridge, R. W. (Eds.). *Encyclopedia of environmental science*. Springer Science & Business Media. (1999).
- Alvaríño, L., Saez, G., Acioly, T. M. D. S., Viana, D. C. & Iannacone, J. Biochemical indicators of contamination in the coastal area of Callao, Peru. *Lat. Am. J. Aquat. Res.* **51**(3), 351–362. <https://doi.org/10.3856/vol51-issue3-fulltext-2946> (2023).
- Osorio, M. et al. Use of *Brachionus plicatilis* (Rotifera) to assess the quality of marine water in Callao Bay, Peru. *J. Integr. Coast. Zone Manag.* **22**, 103–116. <https://doi.org/10.5894/rgci-n441> (2022).

12. Guabloche, A., Alvarino, L., Acioly, T. M. D. S., Viana, D. C. & Iannacone, J. Assessment of essential and potentially toxic elements in water and sediment and the tissues of *Sciaena deliciosa* (Tschudi, 1846) from the Coast of Callao Bay, Peru. *Toxics* **12**(1), 68. <https://doi.org/10.3390/toxics12010068> (2024).
13. Alvarino, L., Guabloche, A., da Silva Acioly, T. M., Viana, D. C. & Iannacone, J. Assessment of potentially toxic metals, metalloids, and non-metals in muscle and liver tissue of Two Fish Species (*Mugil cephalus* Linnaeus, 1758 and *Odontesthes regia* (Humboldt, 1821) from the Coastal Area of Callao, Peru. *Reg. Stud. Mar. Sci.* <https://doi.org/10.1016/j.rsma.2024.103423> (2024).
14. Dasharathy, S. et al. Mutagenic, carcinogenic, and teratogenic effect of heavy metals. *Evid. Based Complement. Altern. Med.* <https://doi.org/10.1155/2022/8011953> (2022).
15. Sambu, S., Hemaram, U., Murugan, R. & Alsofi, A. A. Toxicological and teratogenic effect of various food additives: an updated review. *BioMed Res. Int.* <https://doi.org/10.1155/2022/6829409> (2022).
16. Chen, D. et al. Biogenic elements-informed assessment of the impact of human activities on river ecosystems. *J. Environ. Manag.* **353**, 120276. <https://doi.org/10.1016/j.jenvman.2024.120276> (2024).
17. Hale, R., Swearer, S. E., Sievers, M. & Coleman, R. Balancing biodiversity outcomes and pollution management in urban stormwater treatment wetlands. *J. Environ. Manag.* **233**, 302–307. <https://doi.org/10.1016/j.jenvman.2018.12.064> (2019).
18. Sievers, M., Hale, R., Parris, K. M. & Swearer, S. E. Impacts of human-induced environmental change in wetlands on aquatic animals. *Biol. Rev.* **93**(1), 529–554. <https://doi.org/10.1111/brev.12358> (2018).
19. Schuijt, L. M. et al. (Eco) toxicological tests for assessing impacts of chemical stress to aquatic ecosystems: facts, challenges, and future. *Sci. Total Environ.* **795**, 148776. <https://doi.org/10.1016/j.scitotenv.2021.148776> (2021).
20. Clements, W. H., Hickey, C. W. & Kidd, K. A. How do aquatic communities respond to contaminants? It depends on the ecological context. *Environ. Toxicol. Chem.* **31**(9), 1932–1940. <https://doi.org/10.1002/etc.1937> (2012).
21. Xin, Z. et al. Species sensitivity analysis of heavy metals to freshwater organisms. *Ecotoxicology* **24**, 1621–1631. <https://doi.org/10.1007/s10646-015-1500-2> (2015).
22. López, H., Miglio, M. C. & Paredes, C. Pruebas de toxicidad con elutriados de sedimento marino de las bahías los chimus, tortugas y casma en Perú empleando las microalgas *Isochrysis galbana* y *Nannochloropsis oceanica*. *Ecol. Apl.* **20**(2), 169–177. <https://doi.org/10.21704/rea.v20i2.1807> (2021).
23. Seoane, M., Cid, A. & Esperanza, M. Toxicity of bisphenol A on marine microalgae: Single- and multispecies bioassays based on equivalent initial cell biovolume. *Sci. Total Environ.* **767**, 144363. <https://doi.org/10.1016/j.scitotenv.2020.144363> (2021).
24. Stone, S., Adams, M. S., Stauber, J. L., Jolley, D. F. & Warne, M. S. J. Development and application of a multispecies toxicity test with tropical freshwater microalgae. *Environ. Pollut.* **250**, 97–106. <https://doi.org/10.1016/j.envpol.2019.03.058> (2019).
25. Donohoe, R. M. et al. Toxicity of Refugio Beach Oil to Sand Crabs (*Emerita analoga*), Blue Mussels (*Mytilus* sp.), and Inland Silversides (*Menidia beryllina*). *Environ. Toxicol. Chem.* **40**(9), 2578–2586. <https://doi.org/10.1002/etc.5148> (2021).
26. Guaralde, A. D. R. et al. An *Artemia franciscana* bioassay for the monitoring of lipophilic phycotoxins in marine bivalve mollusc cultures: An alternative to screening testing?. *Rev. Ambient. Água* <https://doi.org/10.4136/ambi-agua.2549> (2020).
27. Latorre-Padilla, N., Meynard, A., Rivas, J. & Contreras-Porcía, L. Transfer of pollutants from *Macrocystis pyrifera* to *Tetrapygus niger* in a highly impacted coastal zone of Chile. *Toxics* **9**(10), 244. <https://doi.org/10.3390/toxics9100244> (2021).
28. MINAGRI – Ministerio de Agricultura y Riego. *Protocolo Nacional para el Monitoreo de la Calidad de los Recursos Hídricos Superficiales*. Resolución Jefatural N° 010–2016. MINAGRI, ANA (Autoridad Nacional del Agua) Available in: [https://www.ana.gob.pe/sites/default/files/publication/files/protocolo\\_nacional\\_para\\_el\\_monitoreo\\_de\\_la\\_calidad\\_de\\_los\\_recursos\\_hidricos\\_superficiales.pdf](https://www.ana.gob.pe/sites/default/files/publication/files/protocolo_nacional_para_el_monitoreo_de_la_calidad_de_los_recursos_hidricos_superficiales.pdf). (Accessed 22 March 2024). (2016).
29. Passantino, A., Elwood, R. W. & Coluccio, P. Why protect decapod crustaceans used as models in biomedical research and in ecotoxicology? Ethical and legislative considerations. *Animals* **11**(1), 73. <https://doi.org/10.3390/ani11010073> (2021).
30. Pastorino, P., Prearo, M. & Barceló, D. Ethical principles and scientific advancements: In vitro, in silico, and non-vertebrate animal approaches for a green ecotoxicology. *Green Anal. Chem.* **8**, 100096. <https://doi.org/10.1016/j.greac.2024.100096> (2024).
31. Crespi-Abril, A. C. & Rubilar, T. Moving forward in the ethical consideration of invertebrates in experimentation: Beyond the Three R's Principle. *Rev. Biol. Trop.* **69**, 346–357. <https://doi.org/10.15517/rbt.v69isuppl.1.46366> (2021).
32. Le, T. V. et al. Spatial and temporal analysis and quantification of pollution sources of the surface water quality in a coastal province in Vietnam. *Environ. Monitor. Assess.* **195**(3), 408. <https://doi.org/10.1007/s10661-023-11026-x> (2023).
33. Naimae, R. et al. Long-term water quality monitoring: Using Satellite images for temporal and spatial monitoring of thermal pollution in water resources. *Sustainability* **16**(2), 646. <https://doi.org/10.3390/su16020646> (2024).
34. Yang, S. et al. A novel assessment considering spatial and temporal variations of water quality to identify pollution sources in urban rivers. *Sci. Rep.* **11**(1), 8714. <https://doi.org/10.1038/s41598-021-87671-4> (2021).
35. Araújo, K. S. S. et al. Biomonitoring of waters and tambacu (*Colossoma macropomum* × *Piaractus mesopotamicus*) from the Amazônia Legal, Brazil. *Water* **16**(18), 2588. <https://doi.org/10.3390/w16182588> (2024).
36. Deng, Y., Zhang, Y., Pan, D., Yang, S. X. & Gharabaghi, B. Review of recent advances in remote sensing and machine learning methods for lake water quality management. *Remote Sens.* **16**(22), 4196. <https://doi.org/10.1016/j.marpolbul.2024.116732> (2024).
37. US EPA. *Methods for Chemical Analysis of Water and Wastes* (Environmental Protection Agency, 1994).
38. Sleeter, R. B. & Brendel, K. Homogeneous populations of *Artemia* nauplii and their potential use for in vitro testing in developmental toxicology. *Teratog. Carcinog. Mutag.* **5**(1), 41–54. <https://doi.org/10.1002/tcm.1770050106> (1985).
39. Olaechea, P., Panéz, J. J. & Gonzáles-Figueroa, H. Desarrollo embrionario de *Tetrapygus niger* (Molina, 1782) “Erizo Negro” en diferentes temperaturas. *Biotempo* **6**, 27–31. <https://doi.org/10.31381/biotempo.v6i0.879> (2006).
40. Joubert, G. A bioassay application for quantitative toxicity measurements, using the green algae *Selenastrum capricornutum*. *Water Res.* **14**(12), 1759–1763. [https://doi.org/10.1016/0043-1354\(80\)90111-6](https://doi.org/10.1016/0043-1354(80)90111-6) (1980).
41. Meynard, A. et al. Synergistic, antagonistic, and additive effects of heavy metals (copper and cadmium) and polycyclic aromatic hydrocarbons (PAHs) under binary and tertiary combinations in key habitat-forming kelp species of Chile. *Environ. Sci. Pollut. Res.* **28**, 18300–18307. <https://doi.org/10.1007/s11356-021-13261-6> (2021).
42. Capela, R. et al. Development of a *Lymnaea stagnalis* embryo bioassay for chemicals hazard assessment. *Sci. Total Environ.* **908**, 168061. <https://doi.org/10.1016/j.scitotenv.2023.168061> (2024).
43. Mitchell, H. C. et al. Ecotoxicity threshold values for 4-hydroxychlorothalonil, carbendazim, dimethoate and methoxyfenozide in fresh and marine waters: Part I. Derivation of threshold values. *Sci. Total Environ.* **948**, 174578. <https://doi.org/10.1016/j.scitotenv.2024.174578> (2024).
44. Puntener, W. *Manual for Field Trials in Plant Protection* 2nd edn. (Agricultural Division, 1981).
45. Lane, D., & Salkin, N. Tukey's honestly significant difference (HSD). *Encyclopedia of research design*, 1565–1570 (2010).
46. Barera, S. & Forlani, G. The role of proline in the adaptation of eukaryotic microalgae to environmental stress: An underestimated tool for the optimization of algal growth. *J. Appl. Phycol.* <https://doi.org/10.1007/s10811-023-03017-9> (2023).
47. Lemley, D. A. et al. Effective management of closed hypereutrophic estuaries requires catchment-scale interventions. *Front. Mar. Sci.* **8**, 688933. <https://doi.org/10.3389/fmars.2021.688933> (2021).
48. Mohy El-Din, S. M. & Abdel-Kareem, M. S. Effects of copper and cadmium on the protein profile and DNA pattern of marine microalgae *Chlorella salina* and *Nannochloropsis salina*. *Environ. Process.* **7**, 189–205. <https://doi.org/10.1007/s40710-019-00419-1> (2020).
49. Wang, L. et al. Impacts of land uses on spatio-temporal variations of seasonal water quality in a regulated river basin, Huai River, China. *Sci. Total Environ.* **857**, 159584. <https://doi.org/10.1016/j.scitotenv.2022.159584> (2023).

50. VanLandeghem, M. M. et al. Spatial and temporal patterns of surface water quality and ichthyotoxicity in urban and rural river basins in Texas. *Water Res.* **46**, 6638–6651. <https://doi.org/10.1016/j.watres.2012.05.002> (2012).
51. Pinho, J. V. et al. Effects of salinity on naphthalene adsorption and toxicity of polyethylene microparticles on *Artemia salina*. *Chemosphere* **362**, 142718. <https://doi.org/10.1016/j.chemosphere.2024.142718> (2024).
52. Velasco, J. et al. Effects of salinity changes on aquatic organisms in a multiple stressor context. *Philos. Trans. Royal Soc. B* **374**(1764), 20180011. <https://doi.org/10.1098/rstb.2018.0011> (2019).
53. Zhang, L. et al. Transcriptomic analysis reveals the mechanism of low/high temperature resistance in an outstanding diet alga *Nannochloropsis oceanica*. *Aquac. Rep.* **27**, 101365. <https://doi.org/10.1016/j.aqrep.2022.101365> (2022).
54. García-Galán, M. J., Matamoros, V., Uggetti, E., Díez-Montero, R. & García, J. Removal and environmental risk assessment of contaminants of emerging concern from irrigation waters in a semi-closed microalgae photobioreactor. *Environ. Res.* **194**, 110278. <https://doi.org/10.1016/j.envres.2020.110278> (2021).
55. Han, P., Lu, Q., Fan, L. & Zhou, W. A review on the use of microalgae for sustainable aquaculture. *Appl. Sci.* **9**(11), 2377. <https://doi.org/10.3390/app9112377> (2019).
56. Martínez-burgos, W. J. et al. The role of microalgae as bioindicators of aquatic contamination. En algae as a natural solution for challenges in water-food-energy nexus: Toward carbon neutrality. *Singap. Springer Nat. Singap.* [https://doi.org/10.1007/978-981-97-2371-3\\_13](https://doi.org/10.1007/978-981-97-2371-3_13) (2024).
57. WHO. *Guidelines for Drinking-Water Quality*, 4th ed., WHO: Geneva, Switzerland, 155 (2011). Available in: <https://www.who.int/publications-detail-redirect/9789241549950> (Accessed 22 January 2024).
58. WHO. *Health Guidelines for Use of Wastewater in Agriculture and Aquaculture* (World Health Organization, 1989).
59. Cailleaud, K. et al. Seasonal variation of hydrophobic organic contaminant concentrations in the water-column of the Seine Estuary and their transfer to a planktonic species *Eurytemora affinis* (Calanoid, copepod). Part 2: Alkylphenol-polyethoxylates. *Chemosphere* **70**(2), 281–287. <https://doi.org/10.1016/j.chemosphere.2007.06.012> (2007).
60. Fahd, F., Veitch, B. & Khan, F. Risk assessment of Arctic aquatic species using ecotoxicological biomarkers and Bayesian network. *Mar. Pollut. Bull.* **156**, 111212. <https://doi.org/10.1016/j.marpolbul.2020.111212> (2020).
61. Saalidong, B. M., Aram, S. A., Otu, S. & Lartey, P. O. Examining the dynamics of the relationship between water pH and other water quality parameters in ground and surface water systems. *PLoS ONE* **17**(1), e0262117. <https://doi.org/10.1371/journal.pone.0262117> (2022).
62. Gao, Y., Zhu, J. & He, A. Effect of dissolved organic matter on the bioavailability and toxicity of cadmium in zebrafish larvae: Determination based on toxicokinetic–toxicodynamic processes. *Water Res.* **226**, 119272. <https://doi.org/10.1016/j.watres.2022.119272> (2022).
63. Zhang, H. et al. Effects of dissolved organic matter on distribution characteristics of heavy metals and their interactions with microorganisms in soil under long-term exogenous effects. *Sci. Total Environ.* **947**, 174565. <https://doi.org/10.1016/j.scitotenv.2024.174565> (2024).
64. Zhu, M. et al. Effects of moisture and salinity on soil dissolved organic matter and ecological risk of coastal wetland. *Environ. Res.* **187**, 109659. <https://doi.org/10.1016/j.envres.2020.109659> (2020).
65. Vasistha, P. & Ganguly, R. Water quality assessment of natural lakes and its importance: An overview. *Mater. Today Proc.* **32**, 544–552. <https://doi.org/10.1016/j.matpr.2020.02.092> (2020).
66. Hong, Y. J. et al. Progress in the research of the toxicity effect mechanisms of heavy metals on freshwater organisms and their water quality criteria in China. *J. Chem.* <https://doi.org/10.1155/2020/9010348> (2020).
67. Ramirez, A. Efectos ecotoxicológicos de las aguas de lastre de buques sobre *Odontesthes regia regia* (pejerrey) y la calidad de las aguas del puerto del Callao – Perú. Tesis para optar el grado de Magister en Ciencias Ambientales con mención en Gestión y Control de la Contaminación. Unidad de Posgrado, Facultad de Ingeniería Geológica, Minera, Metalúrgica y Geográfica, Universidad Nacional Mayor de San Marcos, Lima, Perú. Available in: <https://hdl.handle.net/20.500.12672/15320> (Accessed 27 February 2024). (2020).
68. Di Poi, C., Costil, K., Bouchart, V. & Halm-Lemeille, M. P. Toxicity assessment of five emerging pollutants, alone and in binary or ternary mixtures, towards three aquatic organisms. *Environ. Sci. Pollut. Res.* **25**, 6122–6134. <https://doi.org/10.1007/s11356-017-9306-9> (2018).
69. Gosset, A., Durrieu, C., Orias, F., Bayard, R. & Perrodin, Y. Identification and assessment of ecotoxicological hazards attributable to pollutants in urban wet weather discharges. *Environ. Sci. Process. Impacts* **19**(9), 1150–1168. <https://doi.org/10.1039/C7EM00159B> (2017).
70. Espejo, G. Z. et al. Effect of Albendazole at different concentrations on fertilization and early cleavage in *Tetrapygus niger* “sea urchin”. *Sci. Agropecu.* **4**(3), 251–256 (2013).
71. Zavaleta-Espejo, G., Saldaña-Jiménez, J., Blas-Cerdán, W. & Meléndez-Rodríguez, D. Efecto del Diclofenaco sobre la fecundación de *Tetrapygus niger* “erizo negro de mar”. *Rebiol* **38**(1), 62–71 (2018).
72. Biswas, A., Chandra, B. P. & Prathibha, C. Highly efficient and simultaneous remediation of heavy metal ions (Pb(II), Hg(II), As(V), As(III) and Cr(VI)) from water using Ce intercalated and ceria decorated titanate nanotubes. *Appl. Surf. Sci.* **612**, 155841. <https://doi.org/10.1016/j.apsusc.2022.155841> (2023).
73. Mahdi, Banaee Amir, Zeidi Nikola, Mikušková Caterina, Faggio (2024) Assessing Metal Toxicity on Crustaceans in Aquatic Ecosystems: A Comprehensive Review. *Biological Trace Element Research* **202**(12) 5743–5761 [10.1007/s12011-024-04122-7](https://doi.org/10.1007/s12011-024-04122-7)
74. Cordes, E. E. et al. Environmental impacts of the deep-water oil and gas industry: a review to guide management strategies. *Front. Environ. Sci.* **4**, 58. <https://doi.org/10.3389/fenvs.2016.00058> (2016).
75. Thacharodi, A. et al. Microplastics in the environment: a critical overview on its fate, toxicity, implications, management, and bioremediation strategies. *J. Environ. Manag.* **349**, 119433. <https://doi.org/10.1016/j.jenvman.2023.119433> (2024).
76. Rodrigues, S. K. et al. Sediment quality in a metal-contaminated tropical bay assessed with a multiple lines of evidence approach. *Environ. Pollut.* **228**, 265–276. <https://doi.org/10.1016/j.envpol.2017.05.045> (2017).
77. Acioly, T. M. D. S., da Silva, M. F., Barbosa, L. A., Iannacone, J. & Viana, D. C. Levels of potentially toxic and essential elements in water and estimation of human health risks in a river located at the interface of Brazilian savanna and Amazon biomes (Tocantins River). *Toxics* **12**(7), 444. <https://doi.org/10.3390/toxics12070444> (2024).
78. da Silva, M., Acioly, T., Francisco da Silva, M., Iannacone, J. & Viana, D. C. Levels of potentially toxic and essential elements in Tocantins River sediment: Health risks at Brazil’s Savanna-Amazon interface. *Sci. Rep.* **14**(1), 18037 (2024).
79. Rauh, E. & Hughes, S. Collaboration for source water protection in the United States: Community water systems engagement in nitrate pollution reduction. *Wiley Interdiscip. Rev. Water* **11**(2), e1682. <https://doi.org/10.1002/wat2.1682> (2024).

### Author contributions

Lorena Alvariño: Conceptualization; Formal analysis; Investigation; Methodology; Visualization; Roles/Writing—original draft; Writing—review & editing. Luz Castañeda: Data curation; Formal analysis; Investigation; Validation; Roles/Writing—original draft; Writing—review & editing. Grober Panduro: Formal analysis; Investigation; Validation; Roles/Writing—original draft; Writing—review & editing. Thiago Machado da Silva Acioly: Methodology; Roles/Writing—original draft; Writing—review & editing. Diego Carvalho Viana: Resources; Supervision; Writing—review & editing. José Iannacone: Project administration; Supervision; Validation; Conceptualization; Formal analysis; Investigation; Methodology.

### Declarations

#### Competing interests

The authors declare no competing interests.

#### Additional information

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1038/s41598-024-85025-4>.

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