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# 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[1,2]. This intricate web of life spans various habitats, forming a complex and dynamic ecosystem crucial for the planet's health and all its inhabitants[3,4]. 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[5,6]. Additionally, climate change is also a significant contributor to the long-term decline of kelp ecosystems, exacerbated by other regional environmental stresses[7]. This is worrying, as aquatic ecosystems are exploited for food, transportation, industrial activities, and recreation[8]. 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[9], yet suffer from heavy pollution[8]. 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[10–13].

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 Chillon rivers[11]. 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[14,15]. 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[16–18]. 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.[19], 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[11,20,21]. 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[22–24]. 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[25–27]. 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 área, 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[13]. 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[28]. 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[11]. 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[29,30], and the number of organisms used followed the principles of the three "r's"[31]. 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.

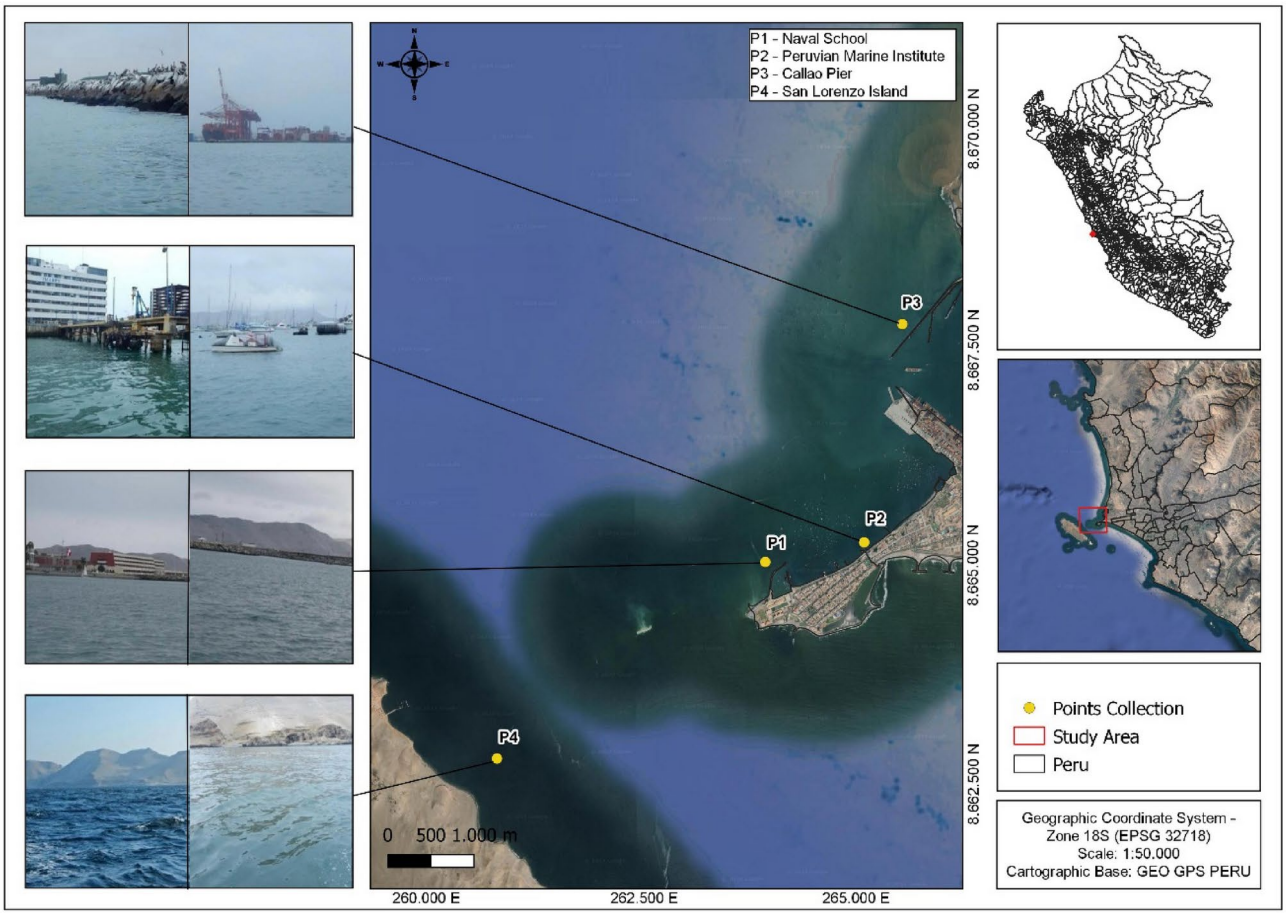


**Fig. 1**. The geographic location of sampling stations-areas in the coastal zone of Callao, Peru. PA1: Naval School (12° 4′3.30"S; 77°10′8.60"O); PA2: Peruvian Marine Institute (12° 3′56.20"S; 77° 9′30.70"O); PA3: Callao Pier (12° 2′34.10"S; 77° 9′15.40"O); PA4: San Lorenzo Island (12° 5′16.57"S; 77°11′52.03"O). 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 pollution discharge, biological processes, and overall water quality[32–34]. 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[35,36].

## 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 °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.[11].

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)[37].

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[38].

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° ± 1 °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 °C ± 1°℃, 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°28′48.37"S 76°48′2.30"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[39]. 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 µm, as indicated by US EPA[37]. 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 Sedgewick-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 ± 1 °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[37], in a randomized complete block design (RCBD) of 6 × 4. To each tube, 100 µL 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[37].

## 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[40].

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

where:

I = Population growth inhibition (%)

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

Nc = 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[41]. 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[42,43], 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 $LC_{50}$ 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 $LC_{50}$, NOEC, and LOEC. The Schneider-Orelli's formula[44] 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-Wilks 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 $IC_{50}$. 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[45].

## 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.

| | Fall | | Winter | | Spring | | Summer | |
|---|---|---|---|---|---|---|---|---|
| Parameters | 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[46–48]. 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[49]. 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[34]. 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[50].

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[51–53]. 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 $IC_{50}$, 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 $IC_{50}$ 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[54,55]. 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 |
| $IC_{50}$ (%) | 43.85 | 51.48 | 67.38 | 52.61 |
| PA2 | | | | |
| NOEC (%) | 50 | 6.25 | < 6.25 | 100 |
| LOEC (%) | 100 | 12.5 | 6.25 | > 100 |
| $IC_{50}$ (%) | 31.81 | 88.6 | 60.09 | 43.17 |
| PA3 | | | | |
| NOEC (%) | 50 | 100 | 25 | < 6.25 |
| LOEC (%) | 100 | > 100 | 50 | 6.25 |
| $IC_{50}$ (%) | 43.85 | 38.42 | 49.82 | 56.19 |
| PA4 | | | | |
| NOEC (%) | 100 | 6.25 | 100 | 6.25 |
| LOEC (%) | > 100 | 12.5 | > 100 | 12.5 |
| $IC_{50}$ (%) | 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[56]. 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[57,58].

The $LC_{50}$, 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 $LC_{50}$ (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[59,60]. 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[57,58], 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 |
| $LC_{50}$ (%) | > 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 |
| $LC_{50}$ (%) | > 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 |
| $LC_{50}$ (%) | > 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 |
| $LC_{50}$ (%) | 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. $LC_{50}$: 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 |
| $IC_{50}$ (%) | > 100 | > 100 | 85.32 | > 100 |
| PA2 | | | | |
| NOEC (%) | < 6.25 | 25 | < 6.25 | < 6.25 |
| LOEC (%) | 6.25 | 50 | 6.25 | 6.25 |
| $IC_{50}$ (%) | 1.11 | > 100 | 8.62 | > 100 |
| PA3 | | | | |
| NOEC (%) | < 6.25 | 6.25 | 100 | < 6.25 |
| LOEC (%) | 6.25 | 12.5 | > 100 | 6.25 |
| $IC_{50}$ (%) | 1.14 | 68.55 | > 100 | > 100 |
| PA4 | | | | |
| NOEC (%) | < 6.25 | 25 | 100 | < 6.25 |
| LOEC (%) | 6.25 | 50 | > 100 | 6.25 |
| $IC_{50}$ (%) | > 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. $IC_{50}$: 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[61].

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[62–64]. 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[65]. Additionally, the toxicity of metals varies with changes in hydrochemical properties like calcium, pH, and organic matter content (Hong et al., 2020[66]). Temperature and suspended solids also affect dissolved oxygen and electrical conductivity[35], while eutrophic conditions, exacerbated by sewage deposits, further increase turbidity[35].

The $IC_{50}$, 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 IC50 values below 100%, highlighting increased organism sensitivity to these conditions. These findings align with Ramírez[67], 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[68,69]. In this study, LOEC, NOEC, and $IC_{50}$ 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.[22] 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[27,70,71].

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[72]. Banaee et al.[73] 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[73]. 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[74,75]. 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[27].

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[76].

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[77–79].

## 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.

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## 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

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