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Impact of heavy metals (As, Hg and Pb) on phytoplankton community structure in the coastal waters of Campeche, southeastern Gulf of Mexico

Impacto de los metales pesados (As, Hg y Pb) en la estructura de la comunidad fitoplanctónica de las aguas costeras de Campeche, sureste del Golfo de México

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ABSTRACT

To determine the potential impact of As, Hg, and Pb on the phytoplankton structure along the central coast of Campeche, water samples were collected monthly from October 2022 to July 2023 at six sites with different anthropogenic activities. The abundance of cyanobacteria showed a positive correlation with silicates, nitrates, and ammonium. Diatoms and nanoflagellates exhibited a positive association with phosphates and dissolved oxygen, factors that favored their increase. Dinoflagellates were positively associated with pH, temperature, salinity, and Hg variables, all of which were linked to increased abundance. Regarding the relationship between phytoplankton groups and heavy metals, a moderate positive correlation (r ≈ 0.47) was observed. Although the overall correlation between the main phytoplankton groups and the studied metals was weak (r ≈ 0.26), the CCA revealed specific patterns of association between phytoplankton groups and certain heavy metals (p > 0.005). Diatoms and nanoflagellates appeared better adapted to environments with Pb and Hg, respectively, while dinoflagellates showed a strong association with Hg. In contrast, cyanobacteria, being more vulnerable to these contaminants, exhibited a negative relationship with these metals, especially with As.

KEYWORDS: Anthropogenic activities, environmental impact, heavy metals, seawater, planktonic microalgae.

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RESUMEN

Con la finalidad de determinar el impacto potencial de As, Hg y Pb sobre la estructura del fitoplancton a lo largo de la costa central de Campeche. Se recolectaron muestras de agua mensualmente de octubre de 2022 hasta julio de 2023 en seis sitios con diversas actividades antropogénicas. La abundancia de cianobacterias mostró una correlación positiva con los silicatos, nitratos y amonio. Las diatomeas y los nanoflagelados presentaron una asociación positiva con los fosfatos y el oxígeno disuelto, factores que favorecieron su incremento. Los dinoflagelados se asociaron positivamente con el pH, la temperatura, la salinidad y el Hg, variables vinculadas a un aumento en su abundancia. En cuanto a la relación entre los grupos de fitoplancton y los metales pesados, se observó una correlación positiva moderada ($r \approx 0.47$). Aunque la correlación general entre los principales grupos de fitoplancton y los metales estudiados es débil ($r \approx 0.26$), la CCA reveló patrones específicos de asociación entre los grupos de fitoplancton y ciertos metales pesados ($p > 0.005$). Las diatomeas y los nanoflagelados parecen adaptarse mejor a ambientes con Pb y Hg, mientras que los dinoflagelados mostraron una fuerte asociación con Hg. En cambio, las cianobacterias, al ser más vulnerables a estos contaminantes, mostraron una relación negativa con estos metales, especialmente con el As.

PALABRAS CLAVE: Actividades antropogénicas, impacto ambiental, metales pesados, agua de mar, microalgas planctónicas.

Introduction

In recent decades, global environmental problems have worsened, mainly due to the ongoing accumulation of various pollutants. This has caused significant changes in the atmosphere, hydrosphere, and lithosphere, disturbing biogeochemical cycles and the balance of many ecosystems (Masindi & Muedi, 2018). These changes create a possible threat to the survival, growth, and reproduction of many species, both animals and plants, due to the widespread distribution of contaminants and the ecological damage they can cause (Yu *et al.,* 2024).

Heavy metals are among the most critical contaminants in aquatic ecosystems due to their high toxicity even at trace concentrations. Elements such as arsenic (As), barium (Ba), bismuth (Bi), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), manganese (Mn), mercury (Hg), nickel (Ni), lead (Pb) and selenium (Se), although naturally occurring, become environmentally significant through anthropogenic inputs associated with industrial processes and urban and agricultural activities (Szymańska-Walkiewicz *et al*., 2022; Adnan *et al*., 2024; Xia *et al*., 2024; Mai *et al*., 2025). Once released into aquatic systems via industrial effluents, municipal runoff, and urban discharge, these metals accumulate predominantly in sediments. Hydrodynamic resuspension can subsequently return them to the water column, facilitating their entry into the aquatic food web (Shumilin *et al*., 2011; Yao *et al.,* 2024).

Heavy metal pollution along Mexico's coasts, as in other parts of the world, is a major environmental concern due to its severe ecological effects, public health risks, and potential economic consequences. These pollutants originate from various sources, including industrial, agricultural, and urban activities, and their presence in coastal ecosystems can devastate biodiversity and ecosystem functioning (Covarrubias & Peña-Cabriales, 2017). This issue is exacerbated in developing countries, where wastewater treatment infrastructure is notably lacking (WWAP, 2017). Consequently, wastewater generated by various industrial processes often flows into coastal areas due to insufficient treatment facilities in these regions (Zhou *et al*., 2022).

To address this problem, Mexican governmental authorities have implemented regulations, by Official Standards, such as NOM-001-SEMARNAT-2021 and NOM-147-SEMARNAT/SSA1-2004, to regulate pollution in wastewater and soil, respectively (SEMARNAT, 2007, 2021). The proper management of hazardous waste, as mandated by NOM-004-SEMARNAT-2002, is directly relevant to the purpose of this study (SEMARNAT, 2003). This regulation not only defines the operational requirements for handling such materials but also serves as a critical preventive framework for limiting the release of toxic metals into the environment. Its enforcement establishes the minimum control conditions that are necessary to assess, mitigate, and reduce environmental risks associated with these contaminants, thereby supporting the study's objective of safeguarding environmental integrity and ensuring compliance with established technical standards.

Among the chemicals of greatest concern for human health and the environment are As, classified as acutely toxic and harmful to the environment; Hg, which can be highly toxic to aquatic organisms and cause long-lasting harmful effects; and Pb, known for its carcinogenic properties (PNUD, 2018). Pb is of paramount concern for human health. Its notoriety stems from potent and irreversible neurotoxicity, with even low-level exposure capable of impairing cognitive development, particularly in children (Zahir *et al*., 2005). The mechanistic basis for Pb's toxicity involves the disruption of neurotransmitter regulation and the induction of oxidative stress via generation of reactive oxygen species (ROS) and depletion of antioxidant reserves (Flora *et al*., 2012). Furthermore, Pb's environmental persistence, its propensity to bioaccumulate, and its legacy from widespread use in gasoline, paint, and industrial applications have led to pervasive global distribution (Wuana & Okieimen, 2011). The continued mobility of Pb in aquatic systems, driven by sediment resuspension and biogeochemical cycling, underscores the critical need for advanced monitoring strategies that account for its dynamic behavior to accurately evaluate long-term risks to ecosystem integrity and public health.

The increase in pollution of aquatic systems consequently reduces water quality, causing stress to the surrounding aquatic biota (Trujillo-Jiménez *et al*., 2011). This degradation directly affects various populations of marine organisms, disrupting the food web and impacting primary, secondary, and tertiary consumers - especially primary producers, which form the base of the marine food web (Domingues *et al*., 2008; D'Costa & Naik, 2019). Primary producers are also

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responsible for approximately 70 % of the planet's total photosynthesis (Pal & Choudhury, 2014). Microalgae, unicellular organisms that constitute a dominant group within marine phytoplankton, are crucial to the proper functioning of aquatic ecosystems. They serve as important bioindicators because their presence and abundance provide valuable information about the health and water quality of these systems (Vuorio *et al*., 2007). Thus, any alteration in environmental dynamics caused by pollutants could have a drastic impact on the ecosystem (Wells *et al*., 2015; Yang *et al*., 2024). Recent literature has documented that contamination by these specific metals can alter the composition and diversity of phytoplankton communities (Manic *et al*., 2024). The objective of this study was to determine the potential impact of As, Hg, and Pb on the phytoplankton structure along the central coast of the State of Campeche in the southeastern Gulf of Mexico.

Material y Methods

Sampling

Water samples were collected monthly from October 2022 to July 2023 at six sites along the central coast of Campeche, each with diverse anthropogenic activities (Figure 1). The sampling locations were chosen to encompass a spectrum of anthropogenic pressures along the coastline. Station S1 was established in the metropolitan area of Campeche city, within the town of Lerma, at the local tourist beach, Playa Bonita. Notably, a thermoelectric plant situated approximately 1.5 km inland discharges its processed coolant water directly into the marine environment. The primary economic activities in the towns of Seybaplaya and Champotón are artisanal and coastal fishing. This has led to the development of informal piers and the establishment of seafood processing plants along the shore. Surface seawater samples were collected with 1 L plastic bottles, and 100 mL aliquots were used for cell counting of phytoplankton taxa (n = 10). Additionally, horizontal tows were carried out for 5 min using a conical hand net with a 20 μm mesh size (n = 10). Both types of samples were fixed *in situ* with a 1 % neutral Lugol's solution and subsequently preserved by adding neutralized formalin to a final concentration of 4 % (Andersen & Throndsen, 2004).

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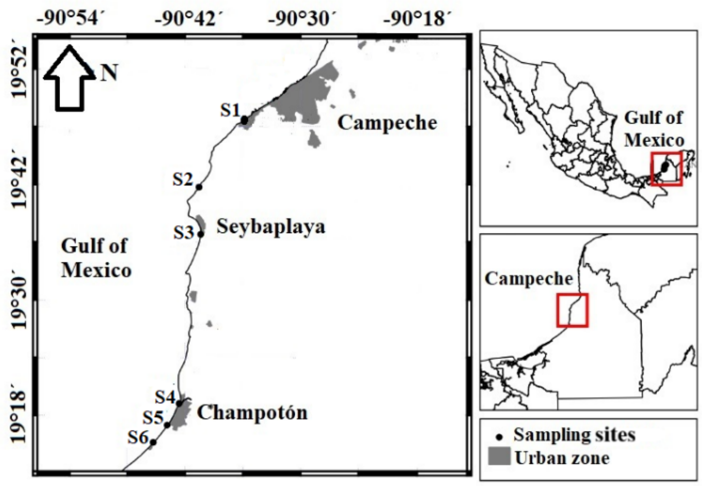

Figure 1. Sampling sites (S1–S6; marked by black circles) in the coastal waters of the State of Campeche in 2022-2023.

Temperature (°C), salinity, pH, and dissolved oxygen (DO; mg L^{-1}) were measured on-site with a HANNA multiparameter probe, model HI9828 (Woonsocket, RI, USA), with a sensor model HI769828 and a Hach multiparameter probe, model HQ40d (Hach Company, Ames, IA, USA). Nutrient determination in water was performed by direct sampling using an amber bottle with a 2 L capacity, containing enough water for nutrient and heavy metal analysis. Subsequently, the samples were preserved at 4°C. Analyses of nitrites, nitrates, ammonium, orthophosphates, and silicates were performed using standard chemical methods for marine environmental monitoring (UNEP, 1991).

Sample analysis

For the determination of As, Hg, and Pb in water samples, a modified version of the procedure proposed by U.S. EPA (2007) and Bhaskar *et al*. (2010) was applied. For the analysis, 15 mL of filtered seawater was used, and the samples were transferred to a Teflon-lined digestion vessel, adding 5 mL of an acid mixture of 4 mL of 65 % HNO_3 and 1 mL of 37 % HCl. Digestion of the samples was carried out in a MARS 5 microwave digestion system (CEM). The microwave oven conditions were heating to 150°C for 20 min at a maximum pressure control of 200 psi. Metals and metalloids were determined using differential pulse anodic extraction voltammetry (DPASV) VA Computrace 797 Metrohm (Switzerland). For the determination of Pb, the working electrode HMDE (mercury drop electrode), platinum auxiliary electrode, and reference electrode Ag/AgCl (3M KCl) were used. For the determination of Hg and As, the working electrode (rotating gold electrode), auxiliary electrode (glassy carbon electrode), and the reference electrode (Ag/AgCl (3M KCl) electrolyte vessel) were used. For metal determinations, Sigma-Aldrich TraceCERT

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certified standards of Pb (16595), As (39436), and Hg (16482) were used. Method validation was performed by measuring the percentage recovery for each analyzed metal: 95 % for Pb, 90 % for As, and 91 % for Hg. The limits of detection (LOD) for each element were 0.03 ng/g for Pb, 0.02 ng/g for As, and 0.02 ng/g for Hg.

The counting of phytoplankton cells was carried out according to the Utermöhl's technique (1958), using 10 mL sedimentation cylinders and a Zeigen brand inverted microscope (Xiamen, China) equipped with 10x/0.25 Ph1 ADL and phase contrast LD 25x/0.30 Ph1 objectives. The identification of the species from unfixed samples and documentation with photomicrographs were performed using a Motic compound microscope, model BA210 (Xiamen, China) equipped with the built-in planarchromatic objectives 5x/0.10, 10x/0.25, 20x/0.40, 40x/0.65 and 100x/1.25. With the use of specialized taxonomic keys, phytoplankton species were identified (Hallegraeff *et al*., 2004; Licea *et al*., 2004, 2016; Lundholm, 2009; UNESCO, 2009; Lassus *et al*., 2016; Steidinger & Meave del Castillo, 2018).

Statistical analysis

The normality of the recorded data was examined using the Kolmogorov-Smirnov test, and homoscedasticity was assessed with the Barlet test. The possible differences among the sampling stations were analyzed using an ANOVA, and Tukey's honestly significant difference test was applied at the 0.005 significance level (Daniel, 1993). The calculation routine was carried out with the Statgraphics Centurion 18 program, version 18.1.1. Canonical correspondence analysis (CCA) was used to examine the relationships between phytoplankton groups, environmental factors, inorganic nutrients, and heavy metals (Ter Braak, 1986). The abundance of the species was transformed to the square root and the environmental data to Log (x +1) before the analysis because the phytoplankton counts do not follow a normal distribution, and there was a large difference in magnitude between the values of biological data and environmental data. The significance of the CCA axes was assessed using a Monte Carlo analysis with 499 permutations.

Results and Discussion

Physicochemical variables

At the six study sites (S1-S6; Figure 1), seasonal variations in both temperature and salinity were observed. However, only salinity showed significant differences between the sampling sites (F = 2.64; $p < 0.005$). Sampling site S6 recorded the minimum value of temperature (24.89 ± 1.83°C) and salinity (26.76 ± 2.43), while the maximum values of temperature (34.07 ± 2.61°C) and salinity (36.10 ± 1.59) were observed at sampling site S2.

In contrast, although pH and DO values varied between sampling sites, no significant differences were observed in either parameter (see Table 1). The pH range extended from a minimum at sampling site S6 (7.09 ± 0.31) to a maximum at sampling site S5 (8.48 ± 0.31). For DO, the minimum value was recorded at station S6 (2.84 ± 1.78 mg L^{-1}) and the maximum at sampling site S5 (12.12 ± 2.68 mg L^{-1}).

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Table 1. Physicochemical variables from six sampling sites along the Campeche coast, Gulf of Mexico, in 2022–2023 (range and mean ± standard deviation).

Sampling sites	t°C	Salinity	pH	DO (mg L^{-1})
Playa Bonita (S1)	25.37-32.82 28.42±2.51	29.70-35.89 33.36±1.72	7.55-8.24 7.90±0.25	4.31-8.36 6.38±1.27
Sombreron (S2)	25.33-34.07 28.17±2.61	30.25-36.10 33.98±1.59	7.44-8.41 7.84±0.27	3.59-8.76 6.49±1.53
Payucan (S3)	25.56-30.92 28.12±1.98	30.78-34.49 32.60±1.35	7.36-8.02 7.69±0.21	3.54-10.61 6.29±2.46
Villamadero (S4)	25.26-30.64 27.89±1.89	33.18-35.53 34.27±0.91	7.50-8.17 7.82±0.21	4.82-10.26 7.28±1.80
Tecnológico (S5)	25.76-30.59 28.04±1.67	31.42-34.89 33.44±1.08	7.42-8.48 7.95±0.31	4.52-12.12 7.08±2.68
Villamar (S6)	24.89-30.81 27.89±1.83	26.76-35.30 32.10±2.43	7.09-8.10 7.63±0.31	2.84-8.33 5.06±1.78
Differences between sampling sites	F = 0.09 $p > 0.005$	F = 2.64 $p < 0.005$	F = 2.14 $p < 0.005$	F = 1.55 $p > 0.005$

The physicochemical variables measured, such as temperature, salinity, and pH, align with the findings reported by Poot-Delgado *et al.* (2021, 2022, 2025) and Gómez-Figueroa *et al*. (2023), who state that these values are typical for the central coast of Campeche, as they are influenced by the shallowness of the study area. Additionally, the DO levels are influenced by water movements generated by rain and wind, as well as by the photosynthesis of photoautotrophic organisms (De la Lanza & Gómez, 1999).

Inorganic nutrients

The levels of nitrites, nitrates, and ammonium in marine coastal waters exceed the maximum permitted limits. Nitrites presented values that ranged between 0.06 and 0.12 mgL^{-1}. Despite these differences, no significant variation was found between the sampling sites (F = 0.53; $p > 0.005$). Nitrates varied between 0.01 and 3.26 mgL^{-1}. Sampling site S5 presented the highest nitrate concentration with an average of 0.91 ± 0.95 mgL^{-1}, while sampling site S1 recorded the lowest level with an average of 0.54 ± 0.40 mgL^{-1}. However, no significant differences were observed between the sampling sites (F = 0.52; $p > 0.005$). Ammonium ranged between 0.21 and 13.08 mgL^{-1}. Sampling site S4 recorded the highest concentration with an average of 5.44 ± 4.41 mgL^{-1}, while sampling sites S2 and S1 recorded the lowest concentrations with averages of 3.79 ± 2.63 and 3.90 ± 2.69 mgL^{-1}, respectively. However, the difference between the sampling sites was not significant (F = 0.41; $p > 0.005$).

In contrast, phosphate levels remained within established limits. Despite the observed variations (not detectable at 0.51 mgL^{-1}), no significant differences were found between the sampling sites ($F = 0.81$; $p > 0.005$), as detailed in Table 2. Sampling site S1 recorded the highest concentrations, with an average of 0.11 ± 0.16 mgL^{-1}, while sampling site S4 recorded the lowest level with an average of 0.04 ± 0.03 mgL^{-1}. Silicates ranged between 0.16 and 3.13 $mg\ L^{-1}$. Sampling site S5 recorded the highest concentration with an average of 2.15 ± 0.39 mgL^{-1}, while sampling site S3 recorded the lowest concentration with an average of 1.86 ± 0.62 mgL^{-1}. However, the difference between the sampling sites was not significant ($F = 0.85$; $p > 0.005$).

Human activities primarily drive fluctuations in inorganic nutrient concentrations in coastal waters. While sources such as electricity generation and aquaculture contribute (Ortiz-Lozano *et al*., 2005), the most significant input is often untreated urban wastewater, leading to the characteristically high levels of nitrogen compounds observed in this and other studies (Alpuche-Gual, 2014; Poot-Delgado *et al*., 2021, 2022, 2025; Gómez-Figueroa *et al*., 2023). Research on coastal phytoplankton dynamics frequently examines their response to such nutrient influxes and local hydrographic conditions (Smith, 2006; Reed *et al*., 2016; Paczkowska et al., 2020). These episodic inputs can trigger transient but ecologically critical shifts in nutrient stoichiometry, often favoring nitrogen over phosphorus (Lu *et al*., 2023; Ristea *et al*., 2025).

Table 2. Inorganic nutrient content ($mg\ L^{-1}$) from six sampling sites along the Campeche coast, Gulf of Mexico, in 2022–2023 (range and mean ± standard deviation); n = 10 for each sampling site.

Sampling sites	Nitrites	Nitrates	Ammonium	Phosphates	Silicates
Playa Bonita (S1)	0.06-0.11	0.01-1.30	0.49-10.43	0.02-0.51	1.86-2.19
	0.10±0.02	0.54±0.40	3.90±2.69	0.11±0.16	2.09±0.10
Sombreron (S2)	0.07-0.11	0.06-3.15	0.21-9.47	0.02-0.37	0.16-2.19
	0.10±0.02	0.78±0.90	3.79±2.63	0.09±0.12	1.86±0.62
Payucan (S3)	0.07-0.12	0.25-3.26	0.43-11.28	0.00-0.08	1.73-3.13
	0.09±0.01	0.91±0.95	5.44±4.41	0.04±0.03	2.15±0.39
Villamadero (S4)	0.07-0.11	0.11-1.26	0.38-10.43	0.02-0.11	1.37-2.19
	0.09±0.01	0.61±0.37	3.98±3.20	0.06±0.03	2.01±0.25
Tecnológico (S5)	0.07-0.11	0.22-1.09	0.38-13.08	0.05-0.17	1.59-2.18
	0.10±0.01	0.64±0.34	3.89±3.55	0.08±0.04	2.00±0.19
Villamar (S6)	0.05-0.11	0.23-1.30	0.49-10.60	0.02-0.12	1.86-2.18
	0.09±0.02	0.56±0.40	3.43±2.86	0.07±0.03	2.05±0.13
Differences between sampling sites	F = 0.53 $p > 0.005$ 0.002*	F = 0.52 $p > 0.005$ 0.04*	F = 0.41 $p > 0.005$ 0.01*	F = 0.81 $p > 0.005$ 5*	F = 0.85 $p > 0.005$

*Upper limits established for marine coastal waters (CONAGUA, 2016).

As, Hg, and Pb levels in seawater

The minimum concentration of As was recorded at sampling site S3 (0.024±0.100 μg mL^{-1}), while the maximum concentration was found at sampling site S2 (0.372±0.046 μg mL^{-1}). However, no significant differences were detected between the sampling sites (F = 0.55; $p > 0.005$). In contrast, the maximum Hg concentration was recorded at sampling site S4 (0.036±0.011 μg mL-1), and in this case, significant differences were found among the sampling sites (F = 3.07; $p < 0.005$). The minimum Pb concentration was recorded at sampling site S1, with 0.028±0.094 μg mL^{-1}, and the maximum value at sampling site S1, with 0.332±0.094 μg mL^{-1}; no significant differences were observed between the sampling sites for this element (F = 0.25; $p < 0.005$). It is important to mention that all the values recorded at the stations exceeded the limits established in the aquatic life criteria for toxic chemicals (Table 3).

This fluctuation is environmentally relevant because it suggests that S1 receives intermittent Pb inputs, likely associated with rainwater runoff, urban discharges, or localized increases in the metal's mobility through resuspension (Zhou *et al*., 2022). Since even the minimum values exceed permissible limits for aquatic life, this monthly variability during the studied year cycle implies that coastal organisms are continuously exposed to hazardous concentrations, with potential cumulative effects and risks to the local food web (Yao *et al*., 2024).

However, a wide range of xenobiotics, including heavy metals, has been released into the environment (Miglani *et al*., 2022). Heavy metals play a crucial role in the degradation of coastal ecosystems (Alabssawy & Hashem, 2024). Phytoplankton is particularly susceptible to the impacts of metal pollution due to its high growth rate and biomass production (Permana & Akbarsyah, 2019), as well as its capacity for bioaccumulation, which makes it a useful bioindicator of metal contamination in marine ecosystems (Rauf *et al*., 2019).

When studying the presence of heavy metals in seawater, it is crucial to consider several factors, as the toxicity of these metals is closely related to their chemical speciation and bioavailability (Ansari *et al*., 2004). Multiple factors simultaneously regulate the concentration and speciation of heavy metals in seawater, potentially interfering with accurate measurements. Our study did not address all these factors comprehensively, which gives our work a preliminary character in these aspects.

The heavy metals analyzed (As, Hg, and Pb) were selected because they have been previously reported in the study area in sediments (Poot-Delgado *et al*., 2021). However, studies on the effects of toxic metals and the response of marine phytoplankton in Mexican coastal waters are scarce. This study is the first report of anthropogenic toxic-metal contamination in the seawater of the central coast of Campeche. The relative contribution of metal concentrations was As > Pb > Hg, which could vary with the levels of metals and metalloids discharged at the different sampling sites.

Table 3. Statistics for As, Hg, and Pb concentrations (µg mL^{-1}) from six sampling sites along the Campeche coast, Gulf of Mexico, in 2022–2023 (range and mean ± standard deviation); n = 10 for each sampling site.

Sampling sites	As	Hg	Pb
Playa Bonita (S1)	0.234-0.356 0.292±0.045	0.003-0.022 0.011±0.005	0.028-0.332 0.148±0.094
Sombreron (S2)	0.226-0.372 0.270±0.046	0.003-0.018 0.009±0.004	0.088-0.273 0.143±0.061
Payucan (S3)	0.024-0.341 0.260±0.100	0.003-0.015 0.009±0.003	0.104-0.166 0.125±0.021
Villamadero (S4)	0.220-0.341 0.271±0.038	0.009-0.036 0.020±0.011	0.062-0.300 0.138±0.065
Tecnológico (S5)	0.011-0.314 0.249±0.94	0.004-0.015 0.010±0.003	0.100-0.177 0.138±0.025
Villamar (S6)	0.205-0.313 0.260±0.041	0.004-0.015 0.011±0.003	0.105-0.226 0.153±0.039
Differences between sampling sites	F = 0.55 $p > 0.005$	F = 3.07 $p < 0.005$	F = 0.25 $p < 0.005$
U.S. EPA recommended aquatic life criteria (µg mL^{-1}; Buchman, 2008; US EPA, 2022)	0.01	0. 0009	0. 001

Major phytoplankton groups

The abundances of the nanoflagellates fluctuated between a minimum value of $2.0x10^3$ ± $2.12x10^5$ cells L^{-1} at sampling site S2 and a maximum value of $1.3x10^6$ ± $4.2x10^5$ cells L^{-1} at sampling site S1 (Figure 2a). However, no significant differences were observed between the sampling sites (F = 0.98; $p > 0.005$). The minimum diatom abundance was recorded at sampling site S2 (846 ± $1.2x10^5$ cells L^{-1}), and the maximum value was $2.3x10^6$ ± $7.8x10^5$ cells L^{-1} at sampling site S1 (Figure 2b). Despite this, no significant differences were observed (F = 1.36; $p > 0.005$). The mean dinoflagellate abundances were on the order of 10^3 cells L^{-1}, with a minimum value of 76 ± $1.9x10^3$ cells L^{-1} at sampling site S5 and a maximum value of $1.3x10^4$ ± $4.8x10^3$ cells L^{-1} (Figure 2c). In this case, significant differences were observed between the sampling sites (F = 2.39; $p < 0.005$). Cyanobacteria, although absent at some stations, showed mean abundances ranging between 10^3 and 10^4 cells L^{-1} (Figure 2d), without presenting significant differences between sampling sites (F = 1.52; $p > 0.005$).

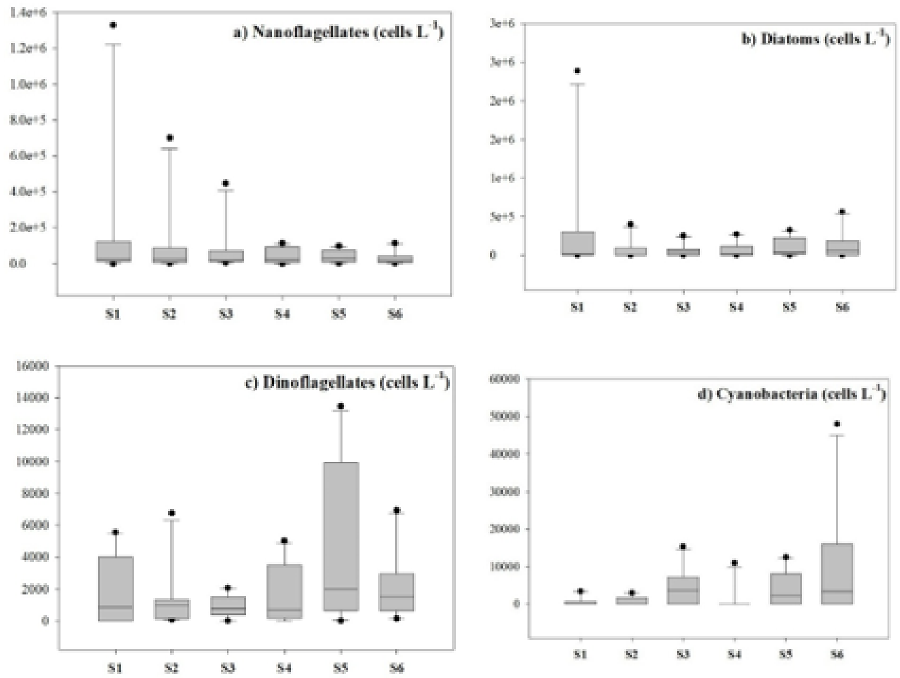

Figure 2. Abundances of major phytoplankton groups from six sampling sites. Cell abundances obtained from the period 2022–2023 (n = 10 for each box plot). Box plots indicate the range (vertical bars) and median (line). The outliers are indicated with a dot.

The elevated concentrations of nitrogenous and phosphorus compounds recorded in this study (Table 2) are consistent with the observed structure of the phytoplankton community, which was dominated by nanoplankton and diatoms and followed a seasonal pattern influenced primarily by climatic forcing (Figure 3). This aligns with previous characterizations of the Campeche coast, which document phytoplankton communities marked by high cell abundance, species richness, and diversity (Herrera-Silveira *et al*., 2009; Gómez-Figueroa et al., 2023; Poot-Delgado & Pérez-Morales, 2023; Poot-Delgado *et al*., 2025). Such nutrient-rich conditions, however, can also predispose the ecosystem to shifts in phytoplankton structure, including the proliferation of harmful microalgal species (Anderson, 1989; Smayda, 2002; Glibert, 2017; Glibert & Burkholder, 2018). These blooms pose significant risks, potentially leading to fish mortality, degraded water quality (Hallegraeff *et al*., 2021), and human intoxication from consuming contaminated seafood.

A key finding was the significant spatial variation exclusively observed in dinoflagellate abundance, a pattern attributable to their unique ecophysiology and the heterogeneous environmental gradients across the sampling sites. Dinoflagellates are notably sensitive to subtle

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changes in water quality, enhancing their utility as bioindicators for localized environmental conditions (Smayda, 2002; Wells *et al*., 2015). The CCA analysis (Figure 3a) confirmed this, revealing a strong association between dinoflagellates and variables such as pH, temperature, salinity and Hg, all of which exhibited discernible spatial variation (Table 1). Their motility allows active niche partitioning within the water column, enabling a more responsive and localized abundance pattern in response to chemical and physical gradients (Glibert & Burkholder, 2018). Furthermore, the common trait of mixotrophy among dinoflagellates (Jeong *et al*., 2010; Stoecker *et al*., 2017) provides a versatile nutritional strategy, making their populations particularly responsive to site-specific perturbations. These include inputs of organic matter or localized contaminant loads (e.g., Hg at sampling site S4), which varied with anthropogenic pressure, such as near the thermoelectric discharge at sampling site S1 versus the fishing zones of Champotón (Glibert, 2020). In contrast, the broader environmental tolerances and faster growth rates of diatoms and nanoflagellates likely facilitated a more uniform distribution across the study area, damping spatial signatures in their abundance (Litchman *et al*., 2007). Similarly, the lack of significant variation in cyanobacteria may reflect the homogeneously high levels of key nutrients such as nitrogen across all sites, which did not create a strong selective gradient for this group (Paerl *et al*., 2016).

Temporal and seasonal dynamics of phytoplankton and metal interactions

Although the primary focus of the CCA was on spatial patterns, the monthly sampling regime revealed notable temporal trends in the phytoplankton community structure, influenced by seasonal climatic forcing. A marked increase in the total phytoplankton biomass, particularly of diatoms and nanoflagellates, was observed during the warmer months (April-July 2023), coinciding with higher solar radiation and water temperatures (e.g., exceeding 30 °C at sampling site S2, Table 1). This period of elevated productivity aligns with the seasonal nutrient enrichment patterns previously reported for the Campeche coast (Poot-Delgado *et al*., 2022; Gómez-Figueroa *et al*., 2023). Conversely, during the northerlies (October-February), characterized by lower temperatures (down to 24.9 °C at sampling site S6) and increased water mixing, the community showed a relative decrease in overall abundance but a higher relative proportion of dinoflagellates. This seasonal succession is consistent with the known physiological preferences of these groups, where diatoms often thrive in turbulent, nutrient-rich conditions, while some dinoflagellates can better exploit stratified, warmer waters.

More critically, these seasonal shifts in hydrographic conditions directly modulate the bioavailability and potential toxicity of the heavy metals studied. The warmer, more stratified water column during summer can promote hypoxic conditions near the sediments, especially in nutrient-enriched areas. As discussed earlier, such hypoxia facilitates the reductive dissolution of Fe/Mn oxyhydroxides, potentially explaining the higher concentrations of bioavailable As and Pb measured in the water column during this period (Table 3). Thus, the apparent tolerance of diatoms and nanoflagellates to Pb and Hg, as indicated by the CCA, may be tested seasonally during these summer months, when metal mobilization peaks. Similarly, the strong association of dinoflagellates with Hg and higher temperatures (Figure 3a) may reflect a seasonal co-occurrence rather than a direct physiological preference, highlighting the complex interplay between seasonal environmental drivers and contaminant dynamics. Therefore, the phytoplankton community's

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response to heavy metal pressure is not static but is intrinsically linked to the seasonal pulse of the regional climate, which controls both microbial growth cycles and geochemical processes that regulate metal speciation.

Integrated CCA interpretation: relationships among physicochemical variables, inorganic nutrients, heavy metals, and phytoplankton

The CCA effectively captured the relationships between phytoplankton groups and environmental variables, with the first two axes explaining 99 % of the total variance (Axis 1: 34.3 %; Axis 2: 64.7 %). The model's validity was confirmed by Monte Carlo tests, which showed that both the first canonical axis and the combined axes were significant (p < 0.005). The species-environment correlation for the first axis was high (r ≈ 0.66), indicating a robust linear relationship. The distribution of phytoplankton groups in the ordination space revealed distinct environmental affinities (Figure 3a). Cyanobacteria exhibited a strong association with silicates, nitrates, and ammonium, a finding consistent with this group's documented preference for nitrogenous compounds, particularly ammonium (Ochoa-de Alda *et al*., 1996; Von Rückert & Giani, 2004). In contrast, diatoms and nanoflagellates were more closely linked to phosphates and DO. This pattern may result from the high nutrient concentrations promoting self-limitation and intense competition, leading to rapid nutrient uptake, with these groups potentially benefiting more from phosphorus availability under such conditions (Makareviciute-Fichtner *et al*., 2024). Dinoflagellates, however, were primarily influenced by a different suite of variables, showing a strong association with pH, Hg, temperature, and salinity. This specific niche separation underscores the unique ecophysiological adaptations of dinoflagellates compared to the other phytoplankton groups studied.

CCA with physicochemical variables and toxic metals

The response of the microalgal species to the physicochemical variables examined is explained primarily by the first two axes of the CCA (axis 1: 17.6 %; axis 2: 80.6 %; total: 98.8 %). The correlation between the major phytoplankton groups and the physicochemical variables was moderate (r ≈ 0.47). Nonetheless, a significant overall relationship exists between the taxa and the variables included in the analysis. The Monte Carlo test showed that the first canonical axis is marginally significant (F = 10.677; p < 0.005), whereas all canonical axes combined are significant (F = 2.048; p < 0.005), indicating a meaningful relationship between phytoplankton groups and environmental variables (Table 4b). The CCA plot (Figure 3b) shows that diatoms and nanoflagellates cluster closely and are negatively correlated with DO but positively correlated with pH. Dinoflagellates exhibit a positive correlation with temperature and a slight positive correlation with pH. Cyanobacteria, in contrast, correlate positively with As and slightly with Pb, and negatively with DO. Additionally, Hg and salinity are positively correlated, suggesting that higher salinity is associated with higher Hg concentrations. As and Pb show negative correlations with DO, indicating that elevated concentrations of these metals are associated with lower DO levels.

This pattern suggests that the apparent association between cyanobacteria and the studied metals is likely indirectly mediated by eutrophication. Nutrient enrichment promotes cyanobacterial blooms, and the subsequent decay of this biomass reduces oxygen levels. The resulting hypoxic conditions favor the reductive dissolution of sediment-bound Fe/Mn oxyhydroxides, releasing sequestered As and Pb into the water column, an established geochemical mechanism supported by studies in similarly impacted systems (Frisbie *et al*., 2024).

Regarding the relationship between the major phytoplankton groups and heavy metals, when environmental variables such as temperature, salinity, pH, and DO are considered, a moderate positive correlation is observed (r ≈ 0.47; Table 4b). However, the CCA graph (Figue 3b) suggests that high concentrations of As and Pb may favor cyanobacterial growth, although these conditions also coincide with lower DO levels. The negative DO metal correlation indicates that As and Pb may contribute to DO depletion, potentially degrading aquatic ecosystem quality, as also reported for the lower coastal zone of Bangladesh (Frisbie *et al*., 2024). Moreover, the positive relationship between salinity and Hg implies that more saline environments tend to exhibit higher Hg concentrations. This aligns with the findings of Ramírez-Rochín *et al*. (2021), which identify salinity as a key factor influencing Hg toxicity in seawater, potentially posing additional risks to marine life under such conditions.

CCA with heavy metals only

CCA analysis showed that the first three canonical axes explain 100 % of the species–environment relationship, with the first axis accounting for most of the variance (81.2 %). However, the eigenvalues were small, and the overall correlation between phytoplankton groups and metals was weak (r ≈ 0.26), indicating only a weak positive association. Species–environment correlations across the first three axes were low, and Monte Carlo tests confirmed that neither the first axis nor all axes combined were statistically significant ($p > 0.005$; Table 4c). This suggests the absence of a strong linear relationship between the environmental variables and the species.

Table 4. Eigenvalues and percentage of the total variance explained by the temporal canonical correspondence analysis of the main phytoplankton groups from 2022-2023 sites along the Campeche coast, southeastern Gulf of Mexico

a) Physicochemical variables, toxic metals, and phytoplankton

Axes	Eigenvalues	Species-environmental correlations	Cumulative percentage variation
1	0.062	0.663	34.3
2	0.011	0.553	99
3	0.001	0.453	100
4	0.080	0.000	0.000
Test of significance of first canonical axis		F = 23.492	$p < 0.005$
Test of significance of all canonical axes		F = 2.588	$p < 0.005$

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Continuation

Table 4. Eigenvalues and percentage of the total variance explained by the temporal canonical correspondence analysis of the main phytoplankton groups from 2022-2023 sites along the Campeche coast, southeastern Gulf of Mexico

b) Toxic metals and phytoplankton

Axes	Eigenvalues	Species-environmental correlations	Cumulative percentage variation
1	0.032	0.475	17.6
2	0.008	0.741	98.8
3	0.000	0.368	100
4	0.011	0.000	0.000
Test of significance of first canonical axis		F = 10.677	$p > 0.005$
Test of significance of all canonical axes		F = 2.048	$p > 0.005$

c)Toxic metals and phytoplankton

Axes	Eigenvalues	Species-environmental correlations	Cumulative percentage variation
1	0.009	0.265	18.8
2	0.002	0.218	98.9
3	0.000	0.162	100
4	0.133	0.000	0.000
Test of significance of first canonical axis		F = 2.947	$p > 0.005$
Test of significance of all canonical axes		F = 1.225	$p > 0.005$

Although overall correlations were weak, the CCA ordination revealed distinct group-specific patterns (Figure 3c). Diatoms and nanoflagellates showed positive associations with Pb and Hg, while dinoflagellates were more strongly related to Hg. In contrast, cyanobacteria were negatively associated with these metals, particularly As. These patterns imply that diatoms, nanoflagellates, and dinoflagellates may exhibit greater tolerance to metal-contaminated environments, whereas cyanobacteria appear more sensitive to these contaminants.

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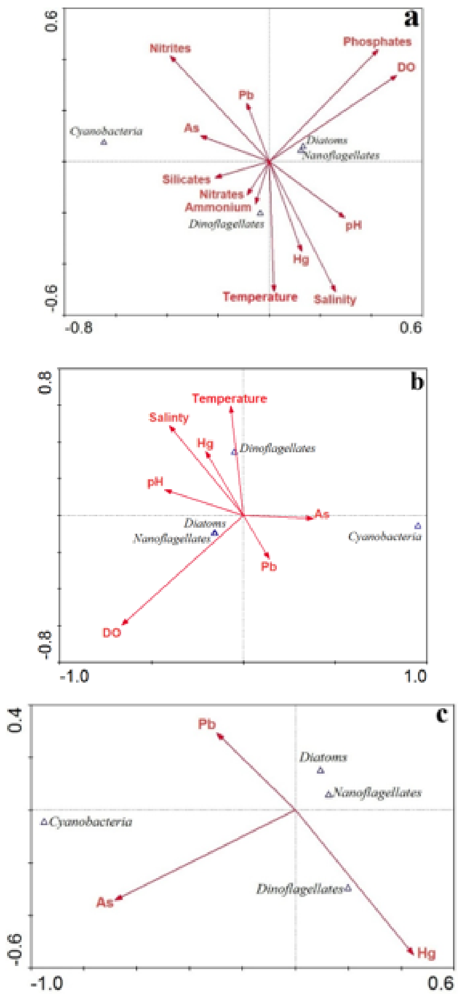

Figure 3. Relationships between major phytoplankton groups, a) physicochemical variables, inorganic nutrients, and toxic metals; b) physicochemical variables, toxic metals, and c) toxic metals, by CCA analysis.

Data were obtained during the period 2022–2023.

Conclusion

These results provide a snapshot of how the studied variables converge at a given moment but do not allow a detailed characterization of the metals' ecotoxicity in isolation. Multiple toxic metals released into the aquatic environment create chemical mixtures and reciprocal effects. However, current toxicological assessments mainly focus on the toxicity of individual metals. The findings underscore the complexity of interactions between nutrients, heavy metals, and phytoplankton, and highlight the need to consider multiple environmental factors when assessing the impact of pollution. The absence of a strong linear relationship does not preclude specific effects, and the differentiated responses of major phytoplankton groups may have significant implications for marine ecosystem health. Future studies should address these factors more integratively to understand ecotoxicity in real-world contexts better, considering contaminant mixtures and their synergistic effects.

Authors' contributions

Research concept and design: C.A.P.D. Data analysis: C.A.P.D., J.R.V.O., M.M.B.R. Project administration: C.A.P.D., J.R.V.O Data collection, statistical analysis: C.A.P.D., J.R.V.O., M.M.B.R. Writing: C.A.P.D., J.R.V.O., M.M.B.R., Y.B.O., A.P.M. Literature review, critical revision: C.A.P.D., J.R.V.O., M.M.B.R., Y.B.O., A.P.M. Technical supervision, final manuscript editing: C.A.P.D., J.R.V.O., M.M.B.R., Y.B.O., A.P.M.

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Ethics statement

Not applicable

Acknowledgments

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Conflict of interest

The authors declare that they have no conflicts of interest.

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