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Heavy metal pollution and algal sustainability in manzala lake: an integrated ecotoxicological and geospatial approach

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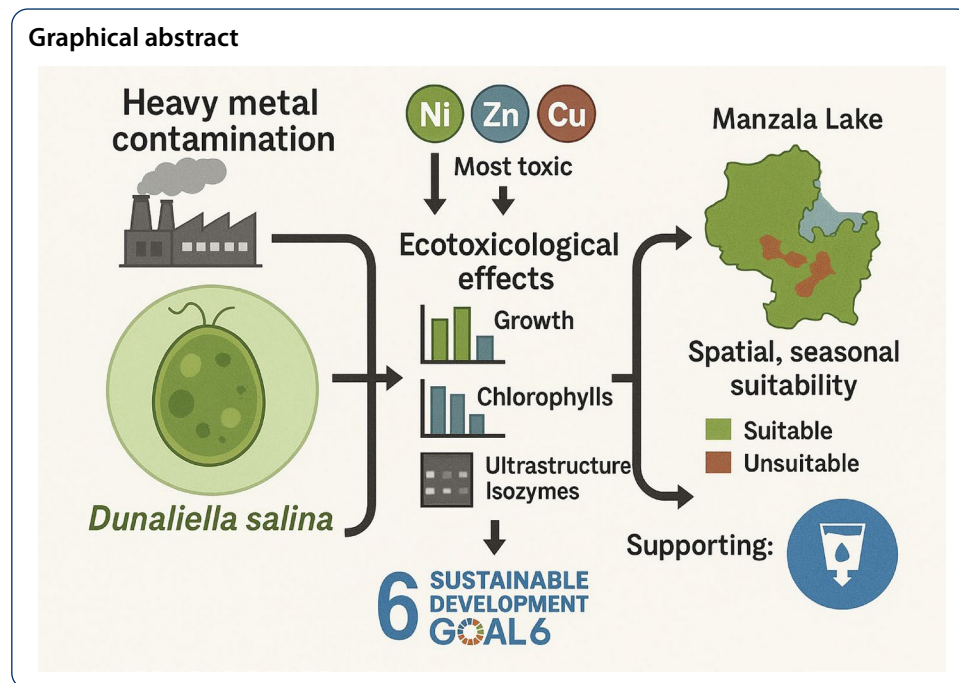
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Abstract

Heavy metal contamination is considered one of the most serious environmental threats, posing significant risks to human health, aquatic ecosystems, and animal life. The marine microalga *Dunaliella salina* is of high nutritional and ecological importance and serves as a valuable bioindicator for assessing aquatic pollution. This study investigates the spatial distribution and risk assessment of three heavy metals (Ni, Zn, and Cu) in Manzala Lake, Egypt, and examines their ecotoxicological effects on *D. salina*. The spatial suitability of Manzala Lake for algal growth was evaluated using Landsat imagery and geospatial analyses based on the obtained results. The alga was employed as a biological monitor to evaluate the stress induced by metal exposure through measurements of growth rate, chlorophyll content, cellular ultrastructure, and antioxidant enzyme activities (peroxidase, superoxide dismutase, and catalase). The results revealed that the EC₅₀ values for the three metals were approximately 15 mg/L. Lower concentrations of Ni, Zn, and Cu stimulated algal growth and chlorophyll content, whereas higher concentrations inhibited both parameters, with the extent of inhibition varying according to the metal type and dosage. Among the three analyzed metals, copper exhibited the highest toxicity under all tested concentrations, followed by zinc and nickel. Ultrastructural examination of *D. salina* cells revealed distinct morphological alterations, further confirming the greater toxicity of copper compared with the other metals. The southern parts of the lake exhibited the highest concentrations of all investigated metals, primarily due to the inflow of wastewater from multiple drainage sources. Seasonal variations significantly influenced metal concentrations, resulting in fluctuating suitability between summer and winter. Nevertheless, the analysis indicated that nearly 70% of Manzala Lake remains suitable for *D. salina* growth with respect to Ni, Cu, and Zn levels, except for a few scattered unsuitable patches. Overall, the study aligns with Sustainable Development Goal (SDG) 6 by contributing to scalable solutions aimed at reducing pollution, protecting aquatic ecosystems, and promoting the sustainable management of water resources.

Keywords Spatial analyses, Manzala lake, Heavy metals contamination, *Dunaliella salina*, Ultrastructure, Isozymes, Sustainability





1 Introduction

1.1 Heavy metals in aquatic systems: sources, general toxicity, and global problem

Heavy metals, often referred to as trace metals, comprise a diverse group of elements, some of which are physiologically essential for living organisms [1]. A new term used to reflect the harmful properties of these metals is “potentially toxic elements” and this seems to gain acceptance. These elements are also known as micronutrients because certain of these elements, such as Cu, Mo, Mn, and Zn, are hazardous at high quantities but necessary in small but crucial amounts. An increase in the flux of metal compounds in aquatic environments has unavoidably been caused by the enormous rise in the use of heavy metals over the past few decades. Such metals can enter aquatic environments through both diffuse sources—such as road and agricultural runoff and atmospheric deposition—and point sources, including industrial discharges and effluents from sewage treatment plants [2]. The use of marine environments, particularly coastal and lagoonal lakes, for waste disposal should be permitted only after a comprehensive assessment of potential environmental impacts. Such evaluations must rely on scientifically sound methodologies that consider the local ecological conditions and predict the potential effects of these activities on both the environment and human well-being [3].

Metal compounds contamination severely harms aquatic ecosystems and human health [4]. Industrial activity is a major source of these hazardous metals. While conventional methods like precipitation, filtration, electrolysis, ion exchange, and evaporation are effective for several metal removal from aqueous solutions [5], they are often hindered by high cost and poor economic efficiency. Addressing the complex environmental pollution stemming from human activities, researchers are increasingly turning to biological solutions, specifically using various types of algae, to manage toxic metal poisoning [6, 7]. However, a key challenge in this biological wastewater treatment is that excessive metal ion levels can kill or impair the activity of the purifying microorganisms [8].

Ni^{2+} ions are widely recognized to be necessary cofactors for algal and bacterial enzymes [9]. Freshwater and marine algae's like (*Chlorella salina*) photosynthesis, growth, and nitrogenase activity are all decreased by nickel toxicity, for example in cyanobacterium *Anacystis nidulans* which was a good indicator of environmental pollution [10]. The effect of nickel as heavy metal pollutant on *Chlorella pyrenoidosa* was studied by Wong and Wong [11] who found that algal growth was suppressed by the high concentration of nickel used. Zinc regarded as an essential mineral nutrient for plant and algae growth since it is a component of many different enzymes such as carboxypeptidase, carbonic anhydrase, superoxide dismutase, and a number of dehydrogenases [12]. Zinc deficiency in plants limits growth, resulting in low dry weight and a significant reduction in electron transfer [13].

Copper is a vital nutrient for organisms such as plants and algae since it is found in numerous proteins and enzymes involved in several metabolic pathways, however, when utilized in amounts that exceed its specific level, it can be harmful [14, 15]. High copper concentrations inhibited the synthesis of chlorophylls a and b in the green alga *Chlorella vulgaris* [16]. In general, copper's particularly hazardous physical and chemical forms are its free ion [17]. A number of antioxidant enzymes, including as catalase, glutathione, peroxidase, and ascorbate peroxidase, as well as the production of low-molecular-weight compounds like carotenoids and glutathione, can be activated by algae in response to heavy metals [18]. A wide range of mechanisms to improve heavy metal tolerance in algae have been documented, including metal chelation by organic acids, amino acids, peptides, or proteins (metallothionein and phytochelatin conjugates) or metal compartmentalization away from metabolic activities by transport into vacuoles [19, 20].

1.2 Role of algae as bioindicators: adaptive capacity, antioxidant enzymes, and biomarkers

Bioindicators include living organisms such as animals, microorganisms, and plants that can provide data regarding the health of the environment, ranging from deep oceanic ecosystems to high altitude terrestrial environments [21]. Bioindicators are key instruments for monitoring environmental changes and have the power to assess ecosystem health before it is affected by giving biological reactions that can assist makers and environmental managers. The increasing diversity of pollutants in marine lakes poses a growing threat, endangering the health of various habitats and the entire biota, from benthic to surface ecosystems [22]. Analytical evaluations of different organic and inorganic pollutants as well as microalgae *Dunaliella* are frequently utilized in environmental monitoring. To offer a global assessment of the potential risks associated with the studied areas, such tests are conducted at various trophic levels [23, 24]. Several types of photosynthetic cyanobacteria and green microalgae can produce antioxidants in response to oxidative stress induced by metals, acidity, pollution, ultraviolet radiation, and nutritional constraints [25, 26].

1.3 Why *Dunaliella salina* and Manzala Lake: justification for the model organism and study site

The majority of the microalgae in highly saline water conditions is *Dunaliella*, which is also used as a supplement in both human and animal diets as an important source of vitamin A. The particularly halotolerant eukaryotic species known as *Dunaliella* can be found in a range of marine environments, such as seas, saltwater lakes, swamps, and

salt canals close to the Sea [27]. Due to its high β -carotene content (up to 10% of its dry cell weight), *D. salina* is the most extensively grown strain in the genus *Dunaliella*. Salt lakes drier as salinity increases. Due to these modifications, the species *D. salina* is able to produce large amounts of β -carotene, which can turn lakes red. Halophilic bacteria are thought to be successfully used in *D. salina* culture. It may facilitate different cellular interactions that preserves ecological balance and helps recycle organic materials in the culture. Natural β -carotene derived from *D. salina* has a variety of applications, involving biomass powder as a feed pigment and cosmetics. violaxanthin, Zeaxanthin, and neoxanthin are among the other carotenoids found in *D. salina* cells [28].

Manzala Lake is the largest Egyptian Lake which is widely exposed to different influencing natural and anthropogenic processes. Therefore, the lake receives water whether clean, saline, contaminated from multiple sources including Mediterranean Sea, drains and River Nile [29]. Due to this variation, the Lake is selected to investigate its suitability for the growth of *D. salina* using spatial analyses. Further, the present study investigates the toxicity of Ni, Zn and Cu on metabolic responses of *D. salina*.

Water quality and ecosystem health are seriously threatened by the growing levels of heavy metal contamination in Manzala Lake, which is caused by runoff from industry and agriculture. This emphasizes the urgent need for long-term rehabilitation techniques. This is in line with Sustainable Development Goal 6 (SDG 6), which emphasizes pollution reduction and aquatic environment conservation in order to guarantee universal access to and sustainable management of water and sanitation by 2030 [30]. According to research of Shahi et al. [31], on designed macroalgal and microalgal adsorbents, algal bioremediation provides an environmentally benign way to reduce heavy metal pollution, assisting SDG 6's goal of enhancing water quality.

1.4 Aim of the work and research gap

The purpose of this research was to learn more about the spatial distribution and risk assessment of heavy metals (Ni, Zn, and Cu) in Manzala Lake, and to assess their effects on the ecotoxicological sensitivity of *D. salina*, which was used as a biological monitor, by measuring stress on algal growth, chlorophyll, cell ultrastructure and isozyme activities (peroxidase, superoxide dismutase, and catalase). Utilizing the marine alga *D. salina* as a bioindicator provides vital information for understanding the spatial and temporal presence of heavy metal contaminants in the lake. The present study connects these water quality efforts—centered on *D. salina*—to SDG 6 goals concerning ecosystem health in Lake Manzala. Although there are still a number of research gaps, the current study offers insightful information on heavy metal contamination in Manzala Lake. To capture temporal variability and climatic impacts on metal factors, multi-season, long-term monitoring is required.

2 Materials and methods

2.1 Algal culture

The unicellular microalga *D. salina* was collected from the Botany and Microbiology department's culture collection at Alexandria University's Faculty of Science. Muller Hinton (MH) media was used to cultivate axenic cultures of *D. salina* [32]. Optical density (O.D.) is very useful for determining growth when using a Perkin Elmer (Lambda 1)

ultra violet spectroscopy with a wavelength of 450 nm. The optical density of the material was estimated using Robert [33] equation

$$\text{O.D.} = \log(I_0) - \log(I)$$

where I = the sample's transmission. I_0 = the blank transmittance has been adapted to read 100%.

The spectrophotometer method is used to estimate chlorophyll concentrations. Pellets are extracted using 90% acetone, stored in a refrigerator, and then transferred to a cuvette. Absorption at 647nm and 664nm is measured, and the values of the concentrations of chlorophylls "a" and "b" are approximated using Jeffrey and Humphrey's [34] trichromatic equation:

$$\text{Chl. a (mg/L)} = 11.93 E_{664} - 1.93 E_{647}$$

$$\text{Chl. b (mg/L)} = 20.36 E_{647} - 5.50 E_{664}$$

where E_{664} = absorbance at 664 nm. E_{647} = absorbance at 647 nm.

2.2 Heavy metal bioassay

Ni, Zn, and Cu are three significant heavy metal pollutants in the aquatic ecosystem originating from industrial waste water that have harmful impacts on ecosystems; thus, they were chosen for this study. As documented by Wong and Pak [35], an earlier study used a wide range of metal solutions—including ZnCl_2 , NiCl_2 , and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ —to establish the alga's tolerance limits for these heavy metal salts. EC_{50} for the three tested elements obtained around 15 mgL^{-1} and we selected higher and lower concentrations to compare the results. These results are going with those obtained by El Agawany [36].

2.3 Cell ultrastructure

This was performed by the use of electron microscopy. 100 ml algal culture samples have been collected and vigorously centrifuged for a period of 20 min. Before being fixed in the same solution with 2% glutaraldehyde, the pellet was washed twice with 50 mM phosphate buffer (pH 7.2) with 0.25 M sucrose. Following 90 min of post-fixation in osmium tetroxide (OSO_4) at 4°C [37], the samples were serially transferred via a gradual aqueous—ethanol series before incorporating them in Spurr's resin [38] with uranyl acetate [39], and finally, with lead citrate [40]. Sections were seen using a Jeol TEM $1200\times$ at 80 kv.

2.4 Estimation of isozymes

2.4.1 Peroxidase

Using a flat-bed electrophoresis apparatus (LKB [Bromma, Sweden] (Multiphor 2117), IEF was utilized to separate peroxidase isozymes. 10% v/v glycerol, 3% (v/v) Pharmalytes (Sigma), and 3% (v/v) glycerol were incorporated in the 2-mm gel (5%T, 3%C). Using GelBond PAG film (Pharmacia) as support, pH 3–10, 1% (v/v) Pharmalytes pH 5–8, and 1% (v/v) Pharmalytes pH 8–10.5 were tested. The anode strip was treated with 1 M phosphoric acid, whereas the cathode strip was treated with 1 N sodium hydroxide. Gels were operated at 10°C for 5 h at 20 W power throughout the experiment, with sample applicators removed after 45 min. After electrophoresis, the gel was submerged in PBS

(10 mM sodium phosphate, pH 6.0, 150 mM sodium chloride) for 45 min to equalize pH. The gel was then colored with 9.25 mM p-phenylenediamine, 90.1 M sodium citrate and 3.92 mM H₂O₂, for 10–15 min [41]. A gel was split and run for varying lengths of time between 1 and 2 h since the cathode strip had noticeable peroxidase activity. Although isozymes with pH values higher than the pH range of the gel were visible and allowed for comparison of activity levels between treatments, bands were under focused.

2.4.2 Superoxide dismutase

On nondenaturing polyacrylamide gels (10%T, 3%C), superoxide dismutase isozymes were isolated at 80 V for 19.5 h at 4 °C using the Laemmli [42] procedure. The -Mercaptoethanol was absent from the loading buffer. After electrophoresis, gels colored using a method akin to Beauchamp and Fridovich [43]. Putting the gels in a solution of 50 mM sodium phosphate, pH 7.5, and 2.45 mM 2,2'-di-p-nitrophenyl-5,5'-diphenyl-3,3'-(3,3'-dimethoxy-4,4'-diphenylene) ditetrazolium chloride containing 26.5 mM N,N,N',N'-tetramethylethylenediamine and 26.5 M riboflavin was added after soaking in 50 mM sodium phosphate, pH 7.5, for 20 min. The gels were then switched to 1% (v/v) acetic acid to stop the process after being subjected to low light intensity (9 mol m⁻² s⁻¹) for around 50 min.

2.4.3 Catalase

Catalase isozymes separated at 80 V for 22 h at 4 °C on nondenaturing polyacrylamide gels (7%T, 3%C, notation of Righetti, 1983) using Laemmli [42] methods. The 3X loading buffer contained no β-mercaptoethanol (which was found to inhibit CAT3) but was made to 60 mM DTT. Gels were then soaked in 3.27 mM H₂O₂ for 25 min, rinsed in water, and stained in a solution of 1% (w/v) potassium ferricyanide, 1% (w/v) ferric chloride (equal volumes of 2% [w/v] solutions of each component, added sequentially) in a method similar to that of Woodybury, et al. [44].

2.4.4 Statistical analysis

The gathered data were statistically analysed using two-way ANOVA (Analysis of Variance). Duncan's Improved Multiple Range Test (*P* 0.05) used to examine the variance between mean probability vaglutradlues. F-tests also performed, with the least significant difference (LSD) set at 0.05. All experimental measurements were conducted in triplicate to ensure data accuracy and reproducibility. Statistical analysis of the three biological replicates showed consistent trends across treatments, with mean values ± standard deviations demonstrating significant differences among metal concentrations as confirmed by one-way ANOVA (*p* ≤ 0.05).

2.4.5 Suitability of Manzala Lake for *Dunaliella salina*

Manzala Lake, the largest coastal lake in Egypt, occupies an area of 404.69 km² and has a mean depth of 1.15 m (with variations ranging from 0.7 to 1.5 m). Geographically, it is located in the northeastern Nile Delta, positioned between latitudes 31°00' and 31°35' N and longitudes 31°45' and 32°22' E. A thin sandy barrier separates the lake from the Mediterranean Sea. The lake maintains critical hydrological connections through the Boughaz inlets to the Mediterranean Sea, to the Suez Canal via the east, the Damietta Branch of the Nile to the west, and the El-Salam Canal to the south (Fig. 1).

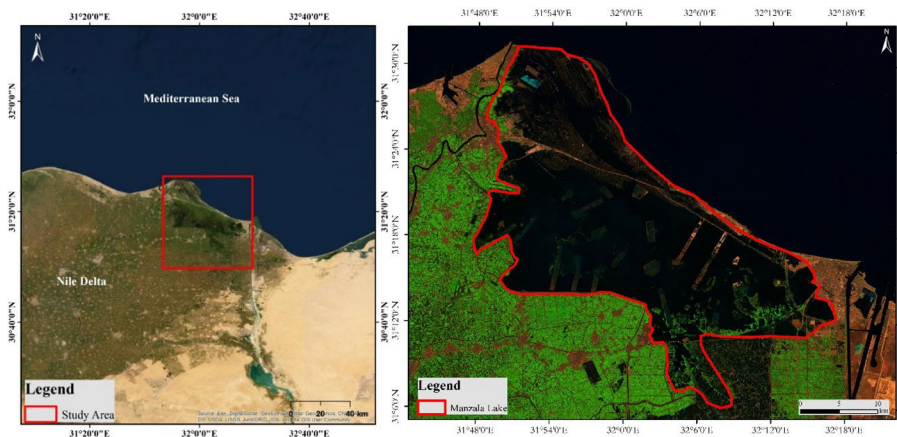


Fig. 1 Location map showing Manzala lake, its surrounding area, and main inflow sources

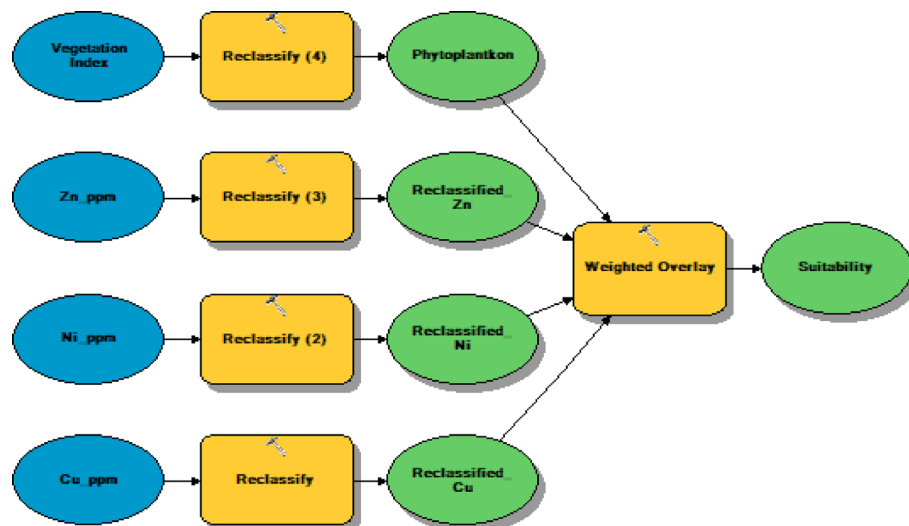


Fig. 2 Cartographic model design for the suitability analyses in Manzala Lake

The lake receives saline water at the North from the Mediterranean Sea and freshwater comes from the eastern Part from the River Nile and wastewater from multiple drains at the southern parts [29, 45].

The spatial distribution maps of the three investigated heavy metals (Zn, Ni and Cu) were generated using the geostatistical analyses in ArcGIS software. The analyses of eleven sites of metals in winter and summer were used according to Elmorsi et al. [29] for the generation of spatial maps. Further, multispectral Landsat image dated 2021 was processed to generate Normalized Difference Vegetation Index (NDVI) which represents a spectral index specific for separation of algae and phytoplankton in aquatic environment. The vegetation index and metals raster layers were reclassified based on the suitability analyses according to the experiment findings to identify the potential suitability maps in the two seasons according to the developed cartographic model identified in Fig. 2.

The spatial distribution maps of the three investigated heavy metals (Zn, Ni, and Cu) were generated using geostatistical analyses in ArcGIS software. Concentration data from eleven sampling sites during winter and summer, following Elmorsi et al. [29], were

interpolated using the Inverse Distance Weighted (IDW) method to produce continuous spatial surfaces. In addition, a multispectral Landsat 8 image (acquired in 2021) was processed to derive the Normalized Difference Vegetation Index (NDVI), which served as a spectral indicator for identifying areas of aquatic vegetation, algae, and phytoplankton growth.

For the suitability assessment, both the heavy metal concentration layers and the NDVI raster were reclassified into five suitability classes: lowest, low, moderate, high, and highest. The classification thresholds were determined according to the observed concentration ranges and ecological risk levels. Areas characterized by low heavy metal concentrations and higher NDVI values were assigned to the high and highest suitability classes, reflecting healthier aquatic conditions, whereas zones with elevated metal concentrations and low NDVI were classified as low or lowest suitability. The reclassified layers were integrated using a weighted overlay analysis based on the relative ecological importance of each parameter to generate the final seasonal suitability maps (Fig. 2).

3 Results and discussion

3.1 Effect of different Ni^{2+} , Zn^{2+} , and Cu^{2+} concentrations on growth of *Dunaliella salina* measured as optical density

Data displayed in a Table 1 (Supplementary data) and illustrated in (Fig. 3) clearly demonstrated in the normal culture condition a steady increase in optical density with increasing culturing duration. Normal growth was shown by the control cultures' optical density (O.D.), which gradually rose from 0.21 ± 0.02 on day 4 to 0.47 ± 0.03 on day 16. When exposed to Ni^{2+} , the O.D. was highest at 5 mg/L (0.45 ± 0.02) and lowest at 25 mg/L (0.29 ± 0.03). Zn^{2+} showed a similar pattern, with growth being increased by

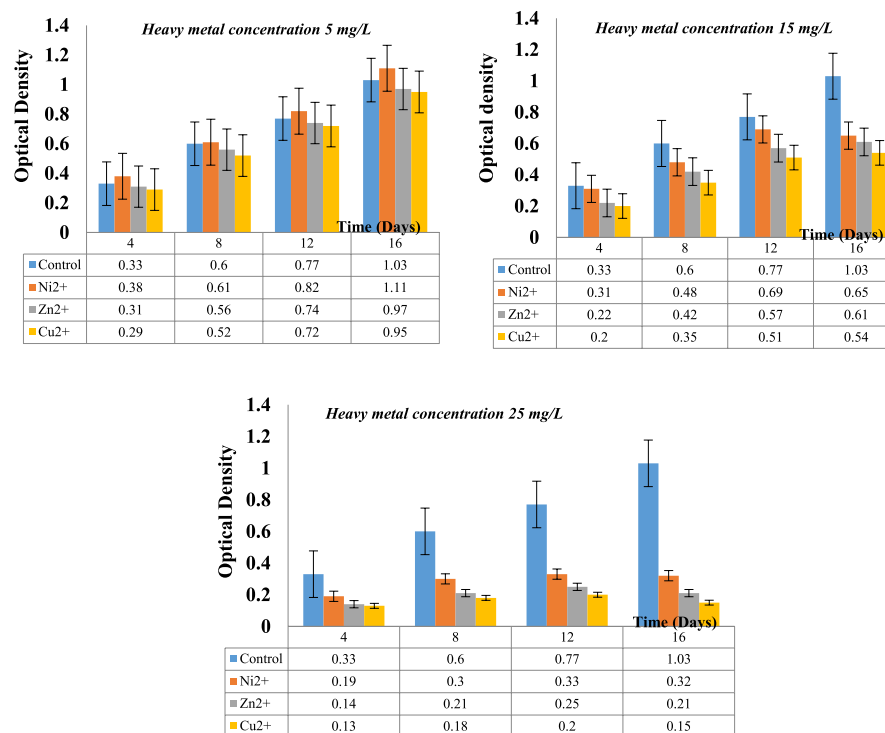


Fig. 3 The impact of various Ni^{2+} , Zn^{2+} , and Cu^{2+} concentrations (mg L^{-1}) on the growth of *D. salina* cells grown for 16 days as assessed by optical density

5 mg/L (0.43 ± 0.02) and decreased to 0.27 ± 0.04 by 25 mg/L. The greatest hazardous impact caused by Cu^{2+} , which decreased O.D. from 0.39 ± 0.02 at 5 mg/L to at least 0.22 ± 0.03 at 25 mg/L ($p < 0.001$). These results demonstrate that Cu^{2+} , Zn^{2+} , and Ni^{2+} all significantly suppressed algal growth.

This pattern is consistent with the work of El-Agawany and Kaamoush [46], who reported that while lower Zn^{2+} concentrations benefited *Dunaliella tertiolecta*, higher levels caused dose- and time-dependent growth inhibition. According to availability of zinc, it could potentially have a stimulatory or inhibitory effect. Low concentration of zinc promoted the growth of some algae but high concentration retarded the growth and caused a reduction of cell division [3]. The function of proteins, enzymes, and membrane integrity can be affected by too much copper, which can impede plant growth [24, 47]. Kaamoush and El-Agawany [48] confirmed that concentration of 25 mgL^{-1} of Cu^{2+} had an inhibitory effect on growth and protein content in *D. tertiolecta* and induced a high toxic effect on the alga. Another significant finding was made by Kaamoush et al. [24] who demonstrated that the inhibitory impact of copper on *Spirulina platensis* growth as examined by optical density, growth rate, and mean growth rate was more apparent than that of nickel and zinc at different doses studied. When the toxicity of zinc and nickel was compared, it was discovered that Zn^{2+} ions were more destructive than Ni^{2+} ions. Also, El-Agawany and Kaamoush [49]. Demonstrated that after 4 days of cultivation, the optical density of a *D. tertiolecta* culture at a concentration of 10 mgL^{-1} soluble nickel was lower than that of a culture at a concentration of 5 mgL^{-1} . During the 16 day culture period, *D. salina* growth was considerably impacted by metal concentration, as confirmed by the statistical analysis (ANOVA), which showed extremely significant differences ($p < 0.001$) across treatments. According to the findings, which showed a distinct concentration-dependent pattern. These variations were corroborated by LSD values, which showed that Cu^{2+} had the most potent inhibitory impact on cell growth.

3.2 The impact of various Ni^{2+} , Zn^{2+} , and Cu^{2+} concentrations (mgL^{-1}) on the concentration of chlorophyll (mgL^{-1}) in *Dunaliella salina* cells grown for 16 days

Data documented in Tables (2, 3 and 4, Supplementary data) and displayed in (Fig. 4) verified that total chlorophylls content at normal culture (control) showed progressive increase till the end of the experiment. The ratio of chlorophylls “a” and “b” remained practically constant until the 12th day. On the 16th day of growth. The effect of varying Ni^{2+} , Zn^{2+} , and Cu^{2+} concentrations on chlorophyll content in *D. salina* demonstrated that the intensity of toxicity depends on the type of metal, Cu^{2+} was more toxic than Zn^{2+} and Ni^{2+} under all tested concentrations. Under normal growth conditions, on day 16, total chlorophylls in control cultures had risen consistently to $12.1 \pm 0.6 \text{ }\mu\text{g/mL}$. Despite Ni^{2+} stress, the pigment content was lowest at 25 mg/L ($7.2 \pm 0.4 \text{ }\mu\text{g/mL}$) and most significant at 5 mg/L ($11.4 \pm 0.5 \text{ }\mu\text{g/mL}$). Similar dose-dependent trends were seen in Zn^{2+} , with chlorophyll peaking at $10.9 \pm 0.3 \text{ }\mu\text{g/mL}$ (5 mg/L) and falling to $6.8 \pm 0.5 \text{ }\mu\text{g/mL}$ (25 mg/L). The strongest inhibition was brought on by exposure to Cu^{2+} , as total chlorophylls decreased from $9.5 \pm 0.4 \text{ }\mu\text{g/mL}$ (5 mg/L) to the lowest value ever measured, $5.3 \pm 0.3 \text{ }\mu\text{g/mL}$ at 25 mg/L. In cells treated with Cu^{2+} , the chlorophyll a/b ratio dropped from 2.1 in the control group to 1.4, indicating photosynthetic stress and pigment degradation.

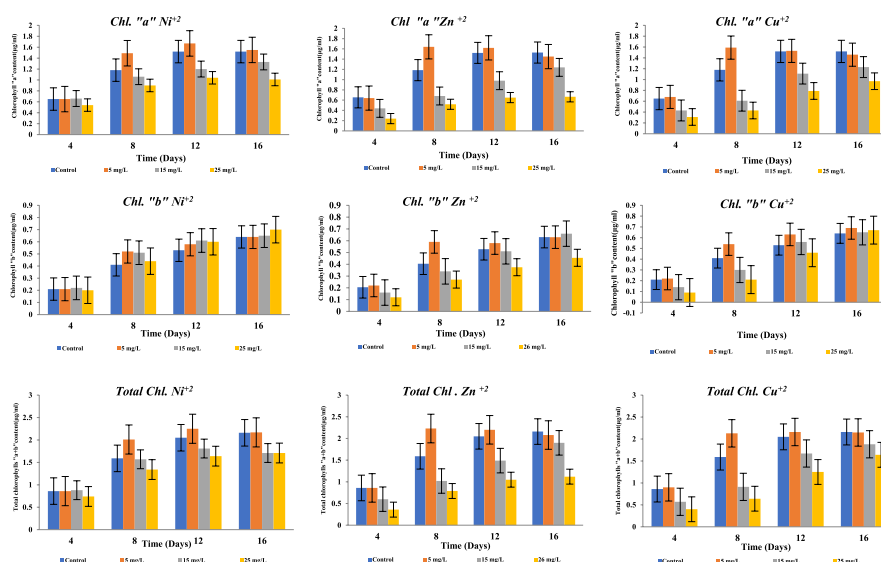


Fig. 4 Effect of different concentrations of Ni^{2+} , Zn^{2+} and Cu^{2+} (mg/L^{-1}) on chlorophylls concentration ($\mu\text{g/ml}$) of *D. salina* during culturing period 16 days

Kaamouch et al. [50] explained the same result and substantiated that copper's stimulatory effect on *Spirulina platensis* during the initial two days of cultivation at lower concentrations can be clarified through the creation of an organic molecule or by the requirement for copper in algal metabolism that lessens metal toxicity. Statistical analyses (ANOVA, $p < 0.001$) showed that the presence of Ni^{2+} , Zn^{2+} , and Cu^{2+} had a significant impact on *D. salina*'s chlorophyll a, b, and total pigment levels. In every instance, chlorophyll synthesis was enhanced by lower metal concentrations (5–15 mg/L), but it was significantly reduced by higher concentrations (25 mg/L), suggesting that stress inhibited photosynthetic activity. As metal levels rose, the a/b ratio steadily decreased, indicating chloroplast damage and pigment deterioration. The most drastic decrease in pigment content was produced by Cu^{2+} among the studied metals, followed by Zn^{2+} and Ni^{2+} . This supported the concentration-dependent effect of heavy metals on algal photosynthetic efficiency and confirmed the toxicity hierarchy ($\text{Cu} > \text{Zn} > \text{Ni}$).

The oxidative potential of copper (II), which results in the loss of chlorophyll, may be the reason of copper's toxic action at greater doses. El-Agawany and Kaamouch [46] observed that low concentrations of zinc metal increased the content of chlorophyll biosynthesis in *D. tertiolecta*, higher zinc concentrations hindered chlorophyll biosynthesis, which could be related to the heavy metal's detrimental influence on the enzyme activity involved in chlorophyll biosynthesis.

Many studies evaluated the toxicity of copper and zinc, Kaamouch et al. [50], indicated that lower Cu^{2+} concentrations improved *D. salina* growth performance whereas copper concentrations reduced algal growth more effectively than zinc. Furthermore, copper at elevated levels ($> 5 \text{ mg/L}$) was found to suppress the development of *Nitzschia closterium* by 50% more than the control [51, 52]. According to findings of El-Agawany and Kaamouch, [49] concentration of total chlorophylls in *D. tertiolecta* cultures exposed to 5 mg/L^{-1} of dissolved nickel steadily rose up to the 12th day. At a concentration of 10 mg/L^{-1} of dissolved nickel, there was a small increase in the content of total chlorophylls from the beginning till the end of the culturing period. However, at dissolved

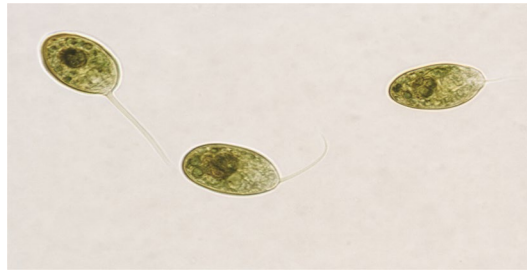


Fig. 5 Morphological structure of *D. salina* cells under control conditions showing normal pear-shaped morphology with intact flagella and uniform pigmentation

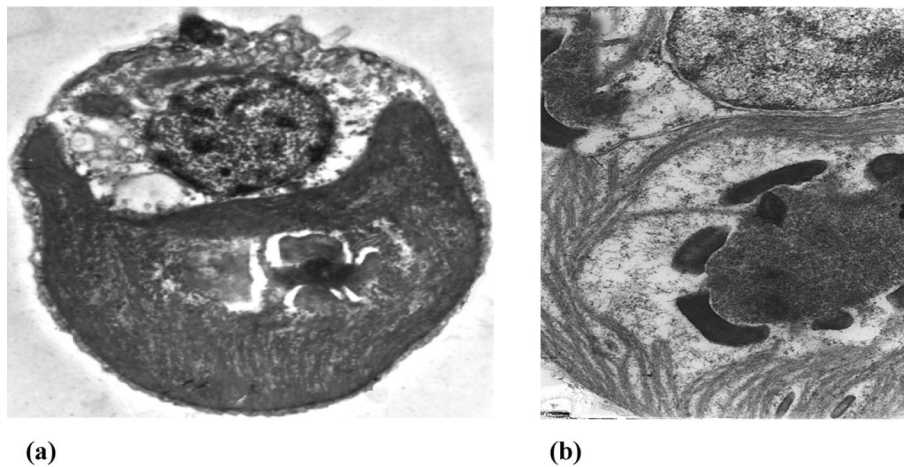


Fig. 6 Transmission electron micrographs of *D. salina* under control conditions: **a** whole cell showing normal ultrastructure and organized cup-shaped chloroplast; **b** enlarged section illustrating the regular arrangement of thylakoid membranes within the chloroplast

nickel concentrations of 15, 20, and 25 mgL⁻¹, there was a significant drop in total chlorophyll content.

3.3 Effect of 25 mg/L of Ni²⁺, Zn²⁺ and Cu²⁺ on the ultrastructure of *Dunaliella salina* cells

Under normal culture conditions, the cells of *D. salina* exhibit a spherical to pear-shaped morphology, being characteristically broader at the posterior end than the anterior end. Their dimensions range from 5 to 18 µm in length and 4.5–14 µm in width. The cytoplasm is filled with minute, colorless, refractive granules that are distributed evenly. In older cells, large starch grains accumulate, typically localized in the anterior portion. The two flagella are generally straight near their base before curving downward. The eyespot globules appear to be suspended freely within the chloroplast matrix (Figs. 5, 6).

- The cavity of the chloroplast is nearly concave especially at the margin of the chloroplast between the two arms of the cup shaped.
- The anterior globule of the eyespot can be hardly observed.
- The rim between the two arms of the cup shaped chloroplast is nearly straight.
- The cytoplasmic organelles are clear.

Considering 25 mg.L⁻¹ for each examined element was the highest hazardous concentration that had a definite detrimental effect on the organism's development and metabolic processes, the ultrastructure for each element was assessed at 25 mg/L.

Cells of *D. salina* cultured on 25 mg.L^{-1} of Ni^{2+} for 12 days were recorded in Fig. 7. A quick check at this plate reveals that the cells' morphology and ultrastructure of cell inclusions have changed somewhat. The cup-shaped chloroplast seemed normal, with two arms. Starch grains were plainly visible surrounding the pyrenoids, while β -carotene granules and other cell inclusions were uniformly distributed inside the protoplast.

Cells were uniformly pigmented and brilliant green under normal conditions, but treatment to 25 mg/L of Ni^{2+} resulted in a faint yellowish-green hue, suggesting modest stress. Due to partial chlorophyll degradation, cultures treated with Zn^{2+} had a dull green to light yellow hue, whereas cultures exposed to Cu^{2+} showed a noticeable brownish or bleached appearance, which was indicative of substantial pigment loss and chloroplast disarray. These apparent color changes are consistent with ultrastructural damage and a decrease in chlorophyll content, indicating that Cu^{2+} had the most toxic effect of all the metals studied.

The chloroplast lamellae could also be observed, and the pyrenoid is apparent. The nucleus could also be observed within the cup-shaped chloroplast's two arms. A small contraction of the cell's exterior thin membrane also detected. Ling [53] suggested that, during the growth period, the percentage cell viability of *D. tertiolecta* cultures exposed to Ni^{2+} remained identical to the control cultures, but Cu induced ultrastructural changes in microalga, including vacuolization, thylakoids structure distortion, slight condensation of chromatin, and cell lysis. After exposing to Cu^{+2} for 24 h, the pyrenoid and its starch granules blebbed and changed shape in *D. tertiolecta*.

Figure 4 that has been obtained from the electron microscope for the effect of 25 mg/L Zn^{2+} cleared that Zn^{2+} is more toxic than Ni^{2+} . The exterior uneven form of the cells derived from 25 mg/L Zn^{2+} seemed less wavy, and the chloroplast lamellae could be seen clearly but somewhat separated. There are fewer starch grains than in the case of Ni^{2+} . The mitochondria looked to be unevenly dispersed inside the cell, and the nucleus's wall is similarly irregularly shaped. The carotene granules looked to be dispersed throughout the protoplasm. The contents of the cell shrunk somewhat and moved away from the cell's outer membrane. The two arms of the cup-shaped chloroplast are separated by a large distance. Our outcomes are consistent with those of Landi et al. [54], who demonstrated that Zn exposure generated intracellular stress in *Caulerpa racemose* due to membrane degradation and a decrease in cell wall surface area, and a loss of selective permeability of cell compartments. Other bioindicators, such as moss and liverwort, have already demonstrated same behavior [55, 56].

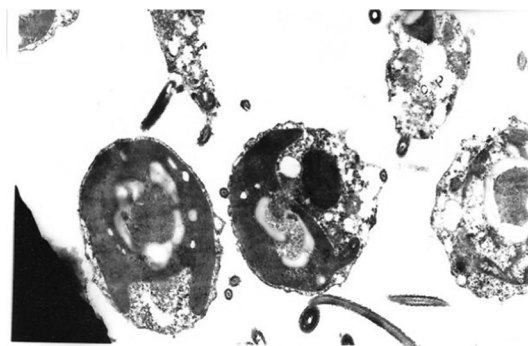


Fig. 7 Transmission electron micrograph of *D. salina* cell exposed to 25 mg/L Ni^{2+} showing minor structural alterations with intact chloroplast and pyrenoid

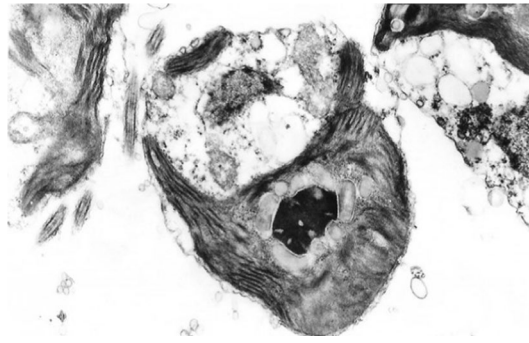


Fig. 8 Transmission electron micrograph of *D. salina* cell exposed to 25 mg/L Zn^{2+} illustrating partial separation of chloroplast lamellae and irregular mitochondrial distribution

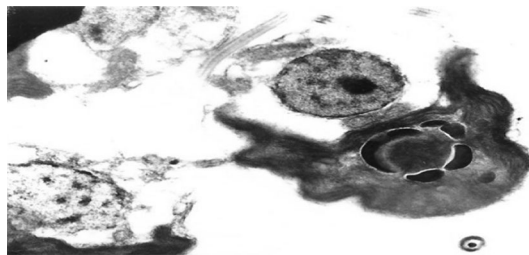


Fig. 9 Transmission electron micrograph of *D. salina* cell exposed to 25 mg/L Cu^{2+} showing pronounced chloroplast disorganization, pigment loss, and nuclear membrane disruption

The influence of $25 \text{ mg.L}^{-1} \text{ Cu}^{2+}$ on the ultrastructure of *D. salina* cultured for 12 days revealed by (Fig. 9). This plate demonstrated evident separation of chloroplast lamellae and loss of the nuclear membrane, accompanied by partial inclusion of the nucleus and a reduced or absent nucleolus. When compared to the normal condition, the anomalies inside the cell could be detected where the distance between the two arms of the cup-shaped chloroplast was far apart. The pigments began to detach off the lamellae in large numbers. The cell membrane, like the cup-shaped chloroplast, seemed wavy. Starch granules were readily visible surrounding the pyrenoids. Mitochondria are dispersed irregularly inside the cell. The nucleus became weak, and the majority of the pigment fractions detached from the lamellae. The outside cell membrane became visible, and the cup-shaped chloroplast became wavy. Mitochondria and starch granules were distributed irregularly inside the cells. Almost all of the nucleus inclusions seemed to disintegrate concomitant with the eventual removal of the nucleolus wall and carotene granules.

The same observations were also recorded in case of 25 mg/L Zn^{2+} and Ni^{2+} but the destructive effect of Zn^{2+} was less than Cu^{2+} , while in case of Ni^{2+} a slight change could also be seen in the internal structure of the organism. The destructive effect of Ni^{2+} on the ultrastructure of *D. salina* was less than both Zn^{2+} and Cu^{2+} indicating that Cu^{2+} is more toxic than both Zn^{2+} and Ni^{2+} and at the same time Zn^{2+} is more toxic than Ni^{2+} . The destructive effect of $25 \text{ mg.L}^{-1} \text{ Cu}^{2+}$, Zn^{2+} and Ni^{2+} on the lamellae structure of the chloroplast and the disappearance of the green color could in turn affect the cells photosynthesis (Figs. 7, 8, 9).

Morales-loo and Gautx [57] arrived at the same conclusion who noticed that the toxic impact may disrupt the biosynthesis mechanism required for the functional

photosynthetic apparatus. Also, Zachleder and Tukaj [58] explained that pigment production in stressed *Scenedesmus armatus* cells was observed till the end of culturing period, the cell was then bleached after a rapid breakdown of all pigments. Solomonova et al. [59] investigated the influence of varying CuO nanoparticle concentrations on the growth, functional, and morphological properties of *D. salina* and *Prorocentrum cordatum* and concluded that the cells were poisonous and that the cell envelopes were mechanically damaged. The pollutant's toxic effect resulted in a progressive increase in reactive oxygen species (ROS) synthesized in algal cells, resulting in oxidative stress in the context of lower specific growth rates, this was most likely caused by the release of Cu^{2+} ions from the oxide to the cells. The mechanical impact of the CuO nanoparticles caused distortion and rupture of the cell envelopes, they also collected on the outer surface of the algal cells.

3.4 Isozyme pattern of *Dunaliella salina* under different concentrations of Ni^{2+} , Zn^{2+} and Cu^{2+}

Cytological characteristics are among the most commonly used indicators for distinguishing an organism's response to environmental stress. The application of isozyme electrophoresis in this field enhances analytical efficiency and resolution. Isozymes represent different molecular forms of the same enzyme that occur within a species under various physiological or environmental conditions [60]. The resulting banding pattern, or zymogram, serves as a biochemical marker that can be used to characterize and differentiate species or assess stress-induced metabolic variations. Many variations of an enzyme may be recognised under these settings (stresses or any other cause). However the reactive oxygen species (ROS) action of common microalgae was not tested, the ROS scavenging effect demonstrated and compared using superoxide dismutase and catalase [61]. The activity of isozymes could be employed as a predictor and sensitive biomarker of heavy metal bioavailability in the marine environment [36]. In the present study, three isozymes pattern namely peroxidase, superoxide dismutase, and catalase were studied by gel electrophoresis. The results obtained were represented in (Figs. 6, 7 and 8; Supplementary data) and zymogram (Figs. 5, 6, 7, 8, 9, 10, 11, 12; Supplementary data). At control experiment the studied *D. salina* gave 7 bands for peroxidase and superoxide dismutase while catalase isozyme gave 6 bands only. These statistical and visual differences confirm a strong correlation between metal exposure intensity and biochemical stress indicators in *D. salina*. Its strong redox activity and high affinity for sulfhydryl and carboxyl groups in proteins provide a molecular explanation for the reported greater toxicity of Cu^{2+} relative to Zn^{2+} and Ni^{2+} . The excessive production of reactive oxygen species (ROS), including hydrogen peroxide and superoxide anions, is encouraged by excess Cu^{2+} and upsets cellular redox equilibrium. Under electron microscopy, these ROS cause peroxidation and structural damage to membrane lipids. Moreover, Cu^{2+} easily attaches itself to enzymes that contain thiols, changing their structure and impairing vital antioxidant and metabolic processes. On the other hand, Ni^{2+} exhibits reduced reactivity and restricted ROS formation, whereas Zn^{2+} serves as a structural cofactor for several enzymes and mostly causes oxidative stress through indirect processes.

3.4.1 Peroxidase isozymes

Concerning the effect of Ni^{2+} ions, the results obtained in Figs. 5, 6 indicated that at 5&15 mg/L Ni^{2+} , the remaining, the vanished, and the newly established bands (7, 0, and 3 bands respectively) are the same under these two concentrations. On the contrary, results recorded in Tables 5, 6 clarified that, at concentration 25 mg/L Ni^{2+} , the total number of bands decreased to 9 and the unchanged bands decreased to 5 while the disappeared and the newly formed bands increased to 2 and 4 bands respectively compared to the other two concentrations.

The obtained results showed that at 5 mg/L Zn^{2+} the sum of bands increased to 10 bands, 5 of these bands are originally found in the control and 5 bands newly formed, while 2 bands disappeared. These 2 bands are bands number 8 and 11. The majority of the new bands were discovered between moderate and low molecular weights, while the majority of the lost bands were identified between moderate and low molecular weights. At 15 and 25 mg/L Zn^{2+} , the sum of bands increased from 10 appeared at 5 mg/L to 12 bands. In case of Zn^{2+} ions the number of unchanged and disappeared bands (compared to control) are the same under all concentrations used (5 and 2 bands respectively). However, at 15 and 25 mg/L Zn^{2+} the newly formed bands were 7 in both concentrations. The whole map may explain the fact that *D. salina* can acclimatize itself to Zn^{2+} ions.

The appearance of bands on the gel represents the level of stress experienced by the organism [62]. Peroxidase, superoxide dismutase, and catalase zymograms demonstrated that the stressors caused by Ni^{2+} , Zn^{2+} , and Cu^{2+} had an effect on these three isozymes. The zymograms also show that several bands shared by the control and the three tested concentrations. Others have vanished, while others have emerged. For the three isozymes studied, as shown in (Tables 5, 6, 7: Supplementary data), there were seven different bands found in the control samples. Under Zn^{2+} (15–25 mg/L), the total number of bands rose to 12, indicating enzyme activity, but Ni^{2+} (25 mg/L) decreased the number to 9. Strong enzymatic modification was indicated by the Cu^{2+} treatments, which yielded the most newly created bands (5–6) and the fewest unmodified bands (4). Cu^{2+} was confirmed as the most powerful stressor when the maximum enzymatic diversity and lowest stability were seen under 25 mg/L Zn^{2+} and 25 mg/L Cu^{2+} , respectively.

The enhanced activity of peroxidase enzymes caused by Pb (II) treatment may lower peroxide and H_2O_2 concentrations, minimizing the danger of oxidative stress [63]. Danouche et al. [64] reported a significant increase in the level of free radical scavenging activity, specifically peroxidase, in cells cultivated on Pb (II)-medium comparable to control media. Overall, the elevated level of activity of peroxidase enzymes following Pb (II) treatment may lower peroxide and H_2O_2 concentrations, lowering the danger of oxidative stress.

3.5 Superoxide dismutase isozyme

Superoxide dismutase (SOD), a vital anti-oxidant enzyme, is assumed to be the first line of protection against the formation of reactive oxygen species (ROS). When under stress, superoxide anions rise, but SOD dismutase them into hydrogen peroxide and oxygen molecules [26]. The influence of varying Ni^{2+} , Zn^{2+} , and Cu^{2+} concentrations on Superoxide dismutase isozyme the number of bands that remained unchanged and the number of disappeared bands are the same at 5 and 15 mg/L Ni^{2+} . The findings

established that the only difference recorded in the number of total bands and those newly formed. At 25 mg/L Ni^{2+} , the overall number of bands fell dramatically from 7 to 4, and the unchanged bands decreased to 3, while the disappeared bands increased from 2 to 4 and only 1 band newly appeared. This may indicate that concentrations of 5 and 15 mg/L Ni^{2+} are less toxic than 25 mg/L Ni^{2+} . Regarding the impact of various Zn^{2+} concentrations on superoxide dismutase isozyme, the results recorded indicated that Zn^{2+} is more toxic than Ni^{2+} on *D. salina*. Data could clearly explain this idea. The number of unchanged bands at 5 and 15 mg/L Zn^{2+} decreased from 7 to 5, while at 25 mg/L the number decreased to 4 bands compared to control (7 bands). Taking into consideration the effect of 5, 15, and 25 mg/L Cu^{2+} , the results clearly show that Cu^{2+} ions are more toxic than the other two elements on protein pattern of the superoxide dismutase isozyme. In a comparable manner with mild toxicity of Zn metal, SOD activity in *Arthrospira platensis*-M2 was elevated, potentially to deal with the cells' fast production of superoxide radicals [65]. The antioxidant activity of *Chlorella vulgaris* became active after 96 h of exposure to heavy metals on microplastics, with the degree of activation being proportional to the type and dosages of microplastics [61].

3.6 Catalase isozyme

As far as the effect of Ni^{2+} , Zn^{2+} , and Cu^{2+} ions on catalase isozymes, the results cleared also that Ni^{2+} is still less toxic than both Zn^{2+} and Cu^{2+} . The findings on the effect of the levels of 5, 15, and 25 mg/L Ni^{2+} affecting the pattern of protein catalase isozymes confirmed that Ni^{2+} is less toxic than both Zn^{2+} and Cu^{2+} . At 5 and 15 mg/L Ni^{2+} , the complete number of bands and the unaffected, the vanished, and the newly produced bands are all the same, while at 25 mg/L Ni^{2+} only 2 bands disappeared and no bands appeared new. In case of Zn^{2+} , toxicity on catalase isozymes was more than that in case of Ni^{2+} . At 5 mg/L Zn^{2+} the sum of bands that appeared were 8. This number decreased gradually by increasing the concentration of Zn^{2+} reaching 4 bands at 25 mg/L. Also the unchanged bands were the same at 5 and 15 mg/L Zn^{2+} but greatly decreased at 25 mg/L. The same phenomena also recorded for the disappeared bands. In terms of the influence of 5, 15, and 25 mg/L Cu^{2+} , the findings showed the whole number of bands were present in the case of Cu^{2+} were 9, 7, and 7 at concentrations 5, 15, and 25 mg/L Cu^{2+} respectively, while the newly formed bands were 4, 2, and 3 at concentrations 5, 15, and 25 mg/L Cu^{2+} respectively. Our findings are consistent with those of Elsayed [66] who found that Catalase activities were dramatically boosted in both Zn and Cu-treated algae *Scenedesmus Obliquus*. Also, Liu et al. [61] verified that in inhibition studies by different heavy metals in *Chlorella vulgaris*, both the SOD and CAT indices were substantially different from the control; particularly, both the SOD and CAT concentrations were significantly higher than the control. ROS-induced membrane oxidation improved the algal antioxidant system, leading to enhanced SOD and CAT activity. According to our outcomes, the increased activity of the antioxidant isozymes catalase, peroxidase, and superoxide dismutase indicates an adaptive metabolic reaction to mitigate these oxidative effects.

Algae with high antioxidant levels believed to be capable to regulate the redox equilibrium inside their cells, making them more resilient to oxidative damage [67]. The fact that total antioxidant levels—which comprise both enzymatic and non-enzymatic antioxidants—generally rose in tandem with stress levels indicates that cells may be able to

tolerate the stress. This finding can be validated if the stress level is not high enough to surpass the concentration threshold that could endanger or kill the organism. For *D. bardawil*, a similar result has been with higher Irgarol 1051 concentrations causing a decline in total antioxidant activity [25].

The toxicity of heavy metals is largely determined by how they interact with biological proteins. Because of its high affinity for imidazole and sulfhydryl ($-SH$) groups, copper (Cu^{2+}) easily attaches to structural and functional proteins, changing their shape and impairing enzyme activity as well as electron transport routes. The cell's resistance to oxidative stress is weakened as a result of this protein–metal interaction, which disrupts the regular folding and operation of antioxidant enzymes including catalase, peroxidase, and superoxide dismutase. Although zinc (Zn^{2+}) is a structural and catalytic cofactor that is necessary for many enzymes, high concentrations of zinc can displace other metal cofactors, such as Mg^{2+} or Fe^{2+} , disrupting protein structure and metabolic balance [63–65].

4 Potential of Manzala lake suitability for *Dunaliella salina*

The potential suitability of Manzala Lake assessed and monitored in winter and summer using the three tested metals and vegetation index as shown in (Figs. 10, 11, 12). Seasonal changes grammatically affected the levels of Cu, Ni and Zn thus showed a great

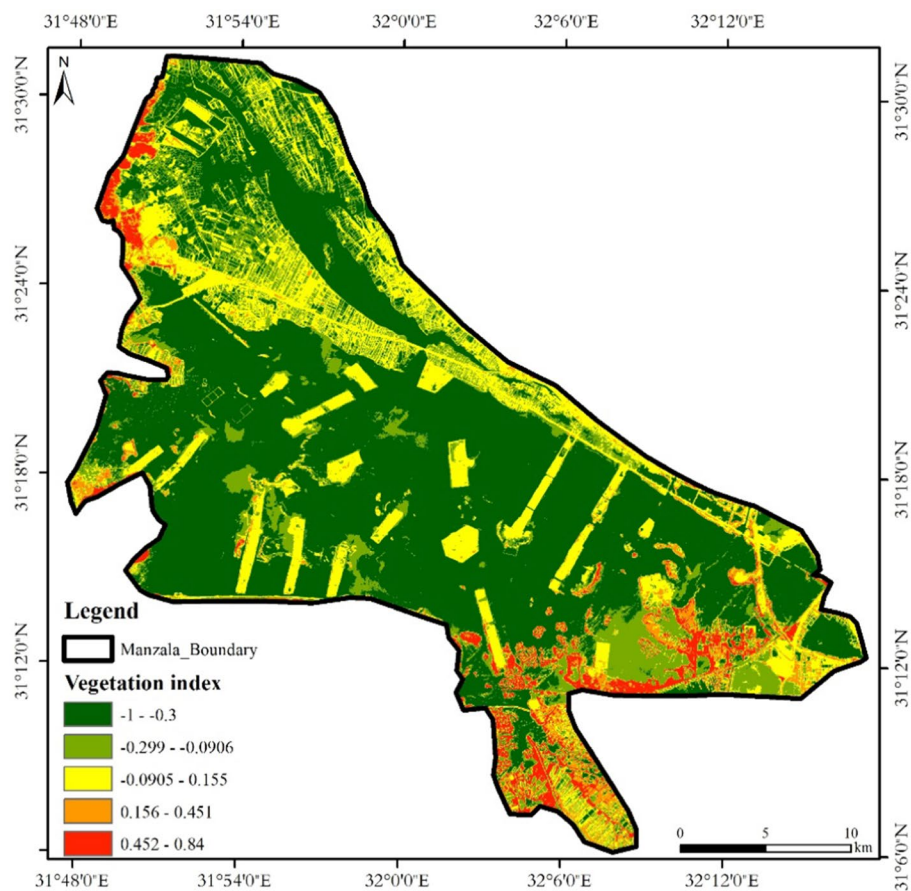


Fig. 10 Spatial variation of the vegetation index (NDVI) across Manzala Lake, where different colors represent variations in vegetation density/phytoplankton; high values (red–orange) indicate dense/ moderate vegetation or phytoplankton content, while low values (yellow–green) indicate sparse or no vegetation

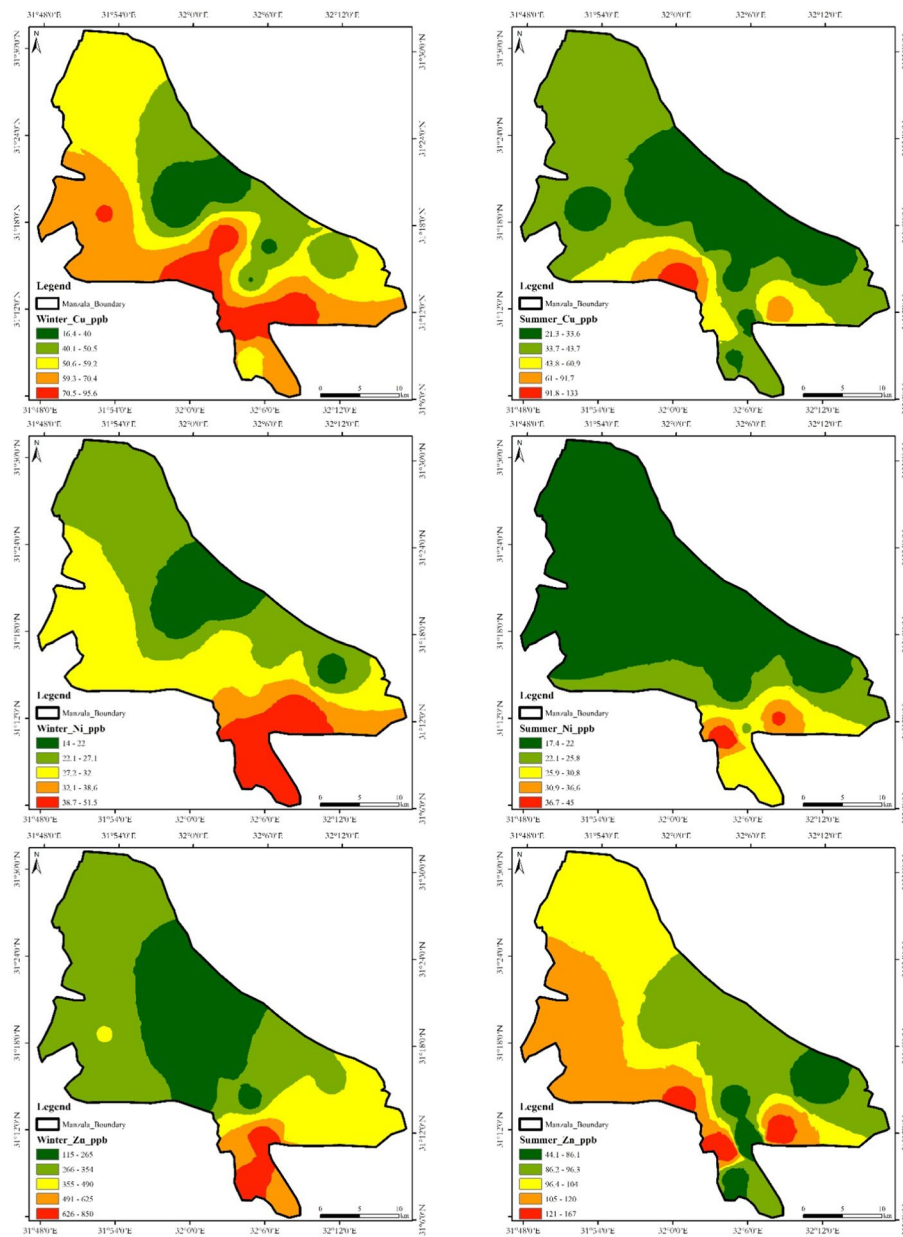


Fig. 11 Spatial Distribution maps of heavy metals ($\mu\text{g/L}$) at winter (left maps) and summer (right maps)

spatial fluctuation within the area of study. Winter showed higher mean values for all metals than summer reflecting the influence of environmental factors such as lower temperatures, increased surface runoff, and reduced biological uptake by aquatic vegetation on metal accumulation; Ni, Cu and Zn reported 31.10, 59.32 and 372.32 $\mu\text{g/L}$, respectively at winter and 24.25, 41.52 and 99.49 $\mu\text{g/L}$ respectively at summer season.

The higher mean concentrations of Cu, Ni, and Zn observed in winter can be attributed to a combination of hydrological, biological, and physicochemical factors. During winter, lower water temperatures and reduced biological activity (including photosynthesis and microbial uptake) limit metal assimilation by aquatic organisms. Additionally, lower evaporation rates and limited water exchange reduce dilution, causing metals to accumulate in the water column. Seasonal runoff from agricultural and industrial

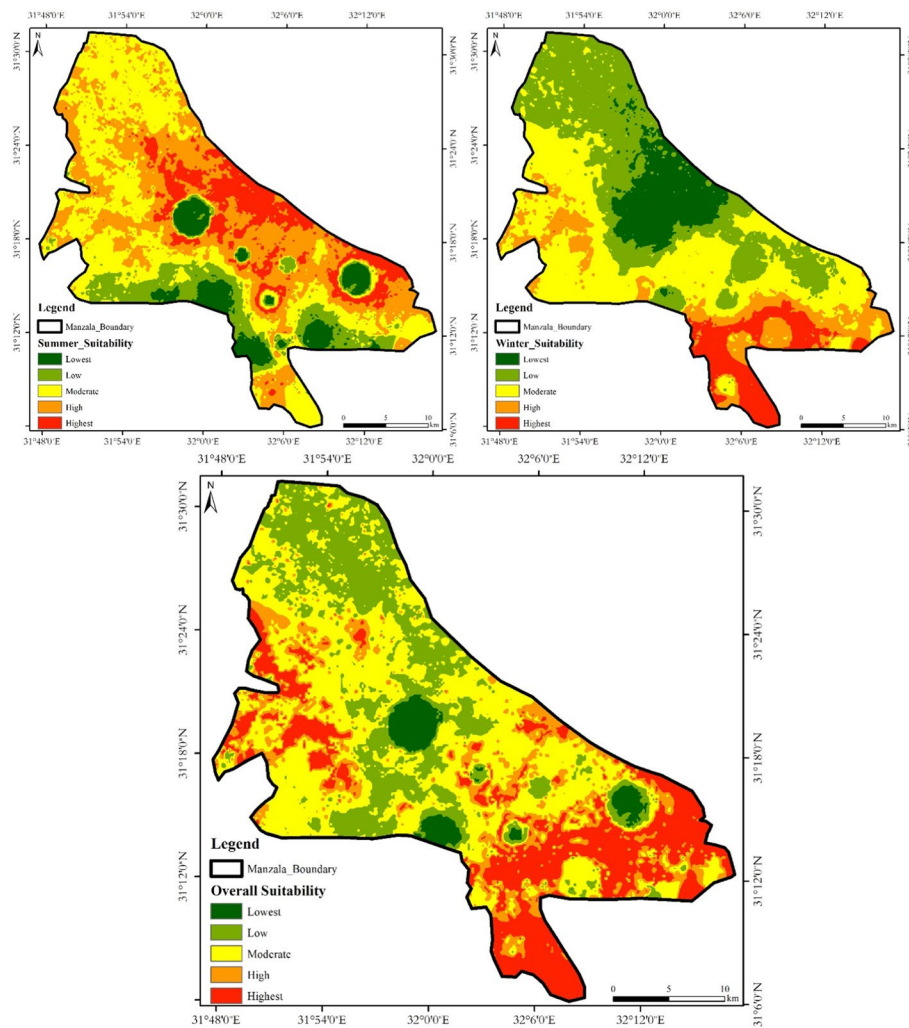


Fig. 12 Spatial suitability of Manzala lake winter (top right), summer (top left) and overall suitability (bottom maps) for the growth of *Dunaliella salina*

sources can also increase during winter rains, introducing additional metal loads. In contrast, during summer, higher temperatures enhance biological uptake, algal growth, and precipitation of metals as hydroxides or carbonates, which collectively contribute to lower dissolved concentrations.

Generally, the southern parts of Manzala Lake reported the highest levels of all investigated metals due to receiving wastewater from different drains at these sites [45]. Due to the obvious variation in levels of metals between summer and winter, the seasonal suitability of the Lake is changeable (Figs. 11). However, the final suitability map considered the common sites in the two seasons indicating the potential permanent sites. Investigations showed that almost 70% of Manzala Lake is suitable for *D. salina* growth in regard to levels of Ni, Cu and Zn except few scattered patches in the lake. This shows promising results that should be investigated in detail to support the biological economy of such algal species. El Agawany et al. [68] disclosed that the Ni, Zn, and Cu abundances in Qaroun lake were favorable for the growth of *D. tertiolecta* (less than 5 mg/L).

Algal bioremediation helps achieve SDG 6, which focuses on sanitation and clean water. These methods enhance water quality, safeguard aquatic habitats, and guarantee

safe water supplies for nearby populations who depend on Manzala Lake for irrigation and fishing by lowering heavy metal pollution. Long-term water resource management has been aided by the sustainable nature of algal-based techniques, which use renewable biomass and are in line with the goal's emphasis on eco-friendly solutions.

The Manzala Lake suitability analysis provides an essential spatial framework that complements the experimental toxicity findings. While the laboratory assays using *D. salina* quantified the biological responses to varying heavy metal concentrations, the geospatial suitability model translates these outcomes into a lake-wide environmental context. This integration bridges controlled ecotoxicological data with real spatial conditions, illustrating how zones of higher metal concentrations correspond to areas of lower ecological suitability. The combined interpretation enhances the understanding of ecosystem health by linking biological sensitivity with spatial contamination patterns, thereby supporting a more comprehensive environmental risk assessment for Manzala Lake.

Several sustainable pollution-control measures have been implemented in Lake Manzala, as summarized below:

- Engineered wetlands: Construction of treatment wetlands (e.g., Bahr El-Baqar system) to purify drain water before discharge into the lake.
- Dredging and restoration: Periodic dredging to improve water circulation and reduce sediment contamination.
- National rehabilitation programs: Inclusion of Lake Manzala in Egypt's national lakes restoration initiative led by EEAA and NWRC.
- Monitoring and research: Continuous water-quality monitoring and academic studies assessing ecological recovery and pollution trends.
- Improved local practices: Adoption of sustainable aquaculture, regulated water reuse, and pilot bioremediation using aquatic plants and microbes.

5 Conclusion

This study combined ecotoxicological evaluation with geospatial analysis to assess the extent of heavy metal contamination (Ni^{2+} , Zn^{2+} , and Cu^{2+}) in Manzala Lake, Egypt, and its biological impact on the halotolerant microalga *Dunaliella salina*. Clear knowledge of the geographical distribution of metals and the biological reaction of algae to different stress levels was made possible by the integrated method. The experimental results revealed a distinct toxicity hierarchy among the tested metals, following the order $\text{Cu}^{2+} > \text{Zn}^{2+} > \text{Ni}^{2+}$. At lower concentrations, these metals stimulated algal growth and enhanced chlorophyll content, whereas higher concentrations exerted inhibitory effects on both parameters. These results demonstrate the crucial point in aquatic ecosystems where the benefits of micronutrients are outweighed by toxic excess.

Its dual role as a bioindicator for pollution monitoring and a bioremediator that can tolerate and metabolically adapt to metal stress is confirmed by the ultrastructural and isoenzymatic changes seen in *D. salina* under heavy metal exposure, which show its physiological sensitivity and adaptive potential. These results highlight how excessive buildup of heavy metals endangers primary producers, interferes with photosynthetic efficiency, and eventually impacts ecological stability and biodiversity in aquatic ecosystems.

The study creates a scalable methodology for evaluating and controlling metal pollution in aquatic ecosystems through integrating geographical mapping with laboratory testing. The method gives decision-makers a scientific framework for determining pollution hotspots, setting remedial priorities, and putting in place long-term monitoring initiatives.

The study contributes to the advancement of water quality assessment tools and promotes the sustainable management of natural water resources. It aligns with national strategies for pollution mitigation and ecosystem restoration in the Nile Delta region, thereby supporting Sustainable Development Goal 6 (SDG 6) “Clean Water and Sanitation.” By addressing water pollution and ecosystem health, the findings provide a robust foundation for future research focused on developing scalable and site-specific applications. Future investigations should emphasize field validation and spatial mapping to enhance implementation in Manzala Lake. Overall, the results underscore the importance of integrating biological indicators with geospatial technologies to establish data-driven, ecologically sound strategies for sustainable water resource management.

6 Limitation of the work

- **Controlled Laboratory Conditions:** Because the ecotoxicological studies were carried out in a lab, they might not accurately represent the intricate and changing environmental circumstances of Manzala Lake.
- **Limited Metal Scope:** Only three heavy metals— Ni^{2+} , Zn^{2+} , and Cu^{2+} , were examined; Pb^{2+} , Cd^{2+} , Hg^{2+} , and organic pollutants were not included since they might be hazardous.
- **Short-Term Exposure:** Without evaluating long-term, chronic ecological effects on *D. salina* populations, the study mainly examined the effects of short-term exposure.
- **Single Bioindicator Species:** Because responses might vary among species and trophic levels, depending just on *D. salina* as a bioindicator may restrict ecological representativeness.
- **Limitations on Spatial Resolution:** Despite the use of geospatial analysis, the resolution of Landsat imagery might not be sufficient to identify microhabitat heterogeneity or small-scale pollution gradients.

7 Environmental implications

The current work emphasizes *Dunaliella salina*'s dual function as an efficient bioindicator of heavy metal pollution in aquatic environments and as a valuable marine resource. Particularly in the southern regions of Manzala Lake, the identification of higher levels of Ni^{2+} , Zn^{2+} , and Cu^{2+} highlights the pressing need for better wastewater treatment in order to lower pollution inputs from industrial and agricultural drains. A possible threat to aquatic biodiversity and the integrity of the food web indicated by copper's strong toxicity and notable effects on cellular shape and enzyme function. This study shows a scalable method for monitoring and controlling heavy metal contamination by combining ecotoxicological studies with geospatial tools. This directly supports SDG 6's goals for the sustainable use and preservation of water resources. For Manzala Lake and related ecosystems, the results also offer vital baseline data that may direct pollution prevention efforts, environmental policies, and restoration plans.

Stricter management of industrial and agricultural wastewater flows is desperately needed, according to our findings from Manzala Lake, especially in the southern areas where metal deposition was highest. By combining geospatial analysis with ecotoxicological evaluation, policymakers may create monitoring programs, prioritize cleanup efforts, and locate pollution hotspots in a useful framework. These results bolster regional environmental management plans that promote better water quality and ecosystem conservation and are in line with SDG 6 and national sustainability targets.

8 Future work

- Long-Term Monitoring: To identify temporal trends in pollution, set up ongoing, seasonal monitoring programs utilizing *D. salina* and other bioindicators.
- Multi-metal Interaction Studies: To find out more about the cumulative pollution consequences and metal–metal interaction processes, investigate the combined toxicological and synergistic effects of various heavy metals (such as Cu, Zn, Cd, Pb, and Ni) on *Dunaliella salina* and related microbial communities.
- Field Validation and Expansion: To verify laboratory toxicity limits and evaluate ecosystem-level effects, conduct in situ field studies.
- Advanced Geospatial Modelling: To forecast the spread of contaminants and seasonal fluctuation, improve spatial mapping using high-resolution satellite data, hydrodynamic modelling, and machine learning.
- Pollution Source Tracking: Use isotopic and chemical fingerprinting together to find out where heavy metals are coming from in Manzala Lake.

Supplementary Information

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Supplementary Material 1.

Author contributions

Equal contributions made to the manuscript's preparation by M.K., N.E.A. and A.E.Z. The experiment created and the laboratory analyses were carried out by M.K. and N.E.A. A.E.Z. completed the suitability evaluations, created the spatial distribution maps, and processed satellite pictures. The statistical analyses were completed and the study findings tabulated by M.K., N.E.A. and A.E.Z. The paper was written by M.K., N.E.A. and A.E.Z. who also made revisions to the final draft. The submitted manuscript read and approved by M.K., N.E.A. and A.E.Z.

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Data availability

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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