Journal of Water & Health

Journal of Water and Health Vol 22 No 4, 785 doi: 10.2166/wh.2024.035

Physicochemical and ecotoxicological approaches for Moknine Continental Sebkha in Tunisia

Afifa Belaid[a], Karima Bekir[a], Asma Beltifa[a], Mouncef Sedrati[b], José Juan Santana Rodríguez[c] and Hedi Ben Mansour[a,c,*]

[a] UR Analysis and Process Applied on the Environment (UR17ES32), Higher Institute of Applied Sciences and Technology, University of Monastir, Mahdia, Tunisia
[b] Geo-Ocean UMR 6538, CNRS, Ifremer, UBO – UBS, Plouzane, France
[c] Instituto Universitario de Estudios Ambientales y Recursos Naturales (i-UNAT), Universidad de Las Palmas de Gran Canaria, Las Palmas de Gran Canaria 35017, Spain
*Corresponding author. E-mail: hedi.benmansour@ulpgc.es

HBM, 0000-0001-7936-9828

ABSTRACT

Degradation of water quality is an emerging issue in many developing countries. In this context, industrial and domestic effluents heavily contaminate the coast of Moknine Continental Sebkha in Tunisia. The present study aimed to biomonitor the seawater quality of the Moknine Continental Sebkha coast using physicochemical and ecotoxicological approaches. The ecotoxicological assessment was performed using three species representing different trophic levels, namely *Vibrio fischeri*, *Selenastrum capricornutum*, and *Lepidium sativum*. In the physicochemical analysis such as BOD (biochemical oxygen demand), COD (chemical oxygen demand), TSS (total suspended solids), TOC (total organic carbon), NO_3^- (nitrate), AOX (adsorbable organic halogen), the recorded levels of pH and total suspended solids did not comply with the Tunisian standard (NT.09.11/1983). The ecotoxicological data confirmed that the tested water samples displayed toxicity to two test indicators *L. sativum* and *S. capricornutum*. A targeted chemical screening of the Moknine Continental Sebkha coast previously performed revealed the presence of total mercury, four phthalate acid esters, and one non-phthalate plasticizer, a fact that could explain the observed ecotoxicological effects and therefore might harm the biotic area and the health of the surrounding population.

Key words: biomonitoring, ecotoxicity, Moknine Continental Sebkha, plasticizers

HIGHLIGHTS

- Water quality biomonitoring of the coast of Moknine Continental Sebkha in Tunisia, which is heavily contaminated by industrial and domestic effluents.
- Water quality assessment included physicochemical and ecotoxicological approaches.
- Ecotoxicity for two target organisms (*Selenastrum capricornutum* and *Lepidium sativum*) was detected.
- Industrial output affects the water quality and biodiversity.

1. INTRODUCTION

Contamination of water resources has reached an alarming level in many developing countries. Human activities, particularly those related to coastal development and industrialization, continuously introduce toxic contaminants and increase the inputs of nutrients (cultural eutrophication) into the aquatic environment, threatening the living organisms and affecting food security as well as drinking water quality. In this context, the Tunisian coast is under increasing pressure due to socio-economic development and inefficient management of coastal environment and associated waste. Traditionally, the environmental impact of the anthropogenic pressures on aquatic ecosystems has been mainly assessed by monitoring consistently general water quality parameters such as physicochemical (e.g., heavy metal, organic matter, hydrocarbon contents, etc.) and microbiological-related parameters (e.g., faecal indicators of water contamination) (Ilter Turkdogan Aydinol *et al.* 2012; Riani *et al.* 2014; Zrelli *et al.* 2018). Although the physicochemical approach provides insight into the overall pollutant load of a water matrix, it may fail to make hazard investigations and predictions of possible toxic potentials and to understand

the complex interactions between structurally different chemicals and their transformation products as well as their chemical bioavailability occurring in water samples (Tavakoly Sany *et al.* 2015). Therefore, physicochemical approaches should be combined with toxicological bio-tools in order to deeply evaluate the overall potency of the micropollutant burden and to identify the main pathways of their influence in multi-stressed water bodies (Anderson & Phillips 2016).

In this context, ecotoxicological tests with species from different trophic links in the food chain may be used to biomonitor the water quality as they offer more knowledge of the complexity of various environmental factors and the chemicals dynamic in water matrices and they may help to understand the hazards of natural stressors and of emerging pollutant mixtures considering the combined interactions between stressors (Dummee *et al.* 2012; Serpa *et al.* 2014; De Souza Celente *et al.* 2020). In addition, standardized toxicity bioassays help to target sites with environmental vulnerability without any preknowledge of what contaminants might be present. To date, different species of bacteria, fish, crustaceans, algae and plants have been frequently used for this purpose, namely for marine and estuarine water quality assessments (Anderson & Phillips 2016). In fact, the luminescence inhibition biotest with marine photobacterium *Vibrio fischeri* has often been applied as a simple, quick and reliable biotool for evaluating the acute toxicity of numerous chemicals, surface water and wastewater (Lechuga *et al.* 2016; Methneni *et al.* 2021a, 2021b).

A unicellular freshwater algal bioassay (for example the use of *Selenastrum capricornutum*, renamed as *Pseudokirchneriella subcapitata*), which represents a significant primary food producer in aquatic environment, had also been applied in water pollution biomonitoring (Silva *et al.* 2009).

Among several bioassays using higher plants, the *Lepidium sativum* phytotoxicity test is frequently used for testing toxicity of chemical compounds (Favier *et al.* 2019) or water matrices (Žaltauskaitė & Čypaitė 2008; Methneni *et al.* 2021b) due to their fast seed germination and growth (roots and shoots), low cost and sensitivity to low pollutant levels (Favier *et al.* 2019). Thus, phytotoxicity bioassays constitute an essential element of ecotoxicological investigation because it represents the action of a toxic chemical at the first interface of the developing and growing plant (seed) and its environment (Lyu *et al.* 2018).

The central Moknine Continental Sebkha (MCS) is one of the most important resources of water and biodiversity. However, it is becoming vulnerable and experiencing noticeable deterioration of ecosystem health as it continuously receives massive volumes of treated or partially treated effluents dumped by a nearby wastewater treatment plant (WWTP) and by industrial sectors such as dairy, medical and fish industries, in addition to agricultural drainage water (Archiplan-Dgat 2019; Jebara *et al.* 2021c). Many publications have reported the spatial and seasonal distribution and sources of phthalate acid esters (PAEs), non-phthalate plasticizers (NPPs), mercury (Hg), pharmaceutically active compounds, paraben preservatives and UV filters in the coastal area of Tunisia (Afsa *et al.* 2020; Jebara *et al.* 2021a, 2021b; Fenni *et al.* 2022). The occurrence of these toxic chemicals in aquatic environments can adversely affect the aquatic ecosystem health and the food web equilibrium.

Therefore, the main goals of this research study are to (i) biomonitor the water quality of the MCS coast through the analysis of physicochemical properties combined with an ecotoxicological screening using a battery of bioassays with multiple trophic levels test systems (*V. fischeri*, *S. capricornutum*, and *L. sativum*), (ii) determine whether ecotoxicity is related to different sampling sites, and to (iii) investigate the contribution of some contaminants to the ecotoxicity of the water samples. To date no attempt has been made to assess the ecotoxicological profile and the environmental scenario of this area. This research study will contribute to a debate on future strategies for biomonitoring and sustainable management of pollution hotspots in water ecosystems MCS.

2. MATERIALS AND METHODS

2.1. Study area and sample collection

MCS is a saline aquatic ecosystem in the eastern region of Tunisia, Monastir-Mahdia provinces. Five sites (S1–S5) were selected as sampling points as they are located near environmental pollution sources including urban and industrial areas and tourism industries (Table 1, Figure 1). The main sources of contamination of the coast of MCS are the processing industries of fishing products, the dairy industries, the textile industries and the wastewater treatment plants. Surface water samples at each site were collected by divers using Niskin bottles.

Sampling was carried out monthly (at the first week of each month) for 9 months (from March 2022 to November 2022) using the composite sampling methodology. Once collected, water samples (10 L) were immediately transported to the laboratory by cooler boxes, prior to measuring the physicochemical properties, and/or kept at −20 °C until toxicological testing. For toxicity assessment, water samples representing each site were mixed.

Table 1 | The co-ordinates of the study areas located at Moknine Continental Sebkha

Coastal study areas	GPS co-ordinates
Moknine Continental Sebkha (S1)	35°37′28.9″N 10°55′33.5″E
Moknine Continental Sebkha (S2)	35°37′10.3″N 10°55′21.3″E
Moknine Continental Sebkha (S3)	35°32′38.8″N 10°58′37.7″E
Moknine Continental Sebkha (S4)	35°34′55.8″N 10°59′15.8″E
Moknine Continental Sebkha (S5)	35°34′01.2″N 10°55′54.4″E

N, north; E, East; GPS, Global Positioning System.

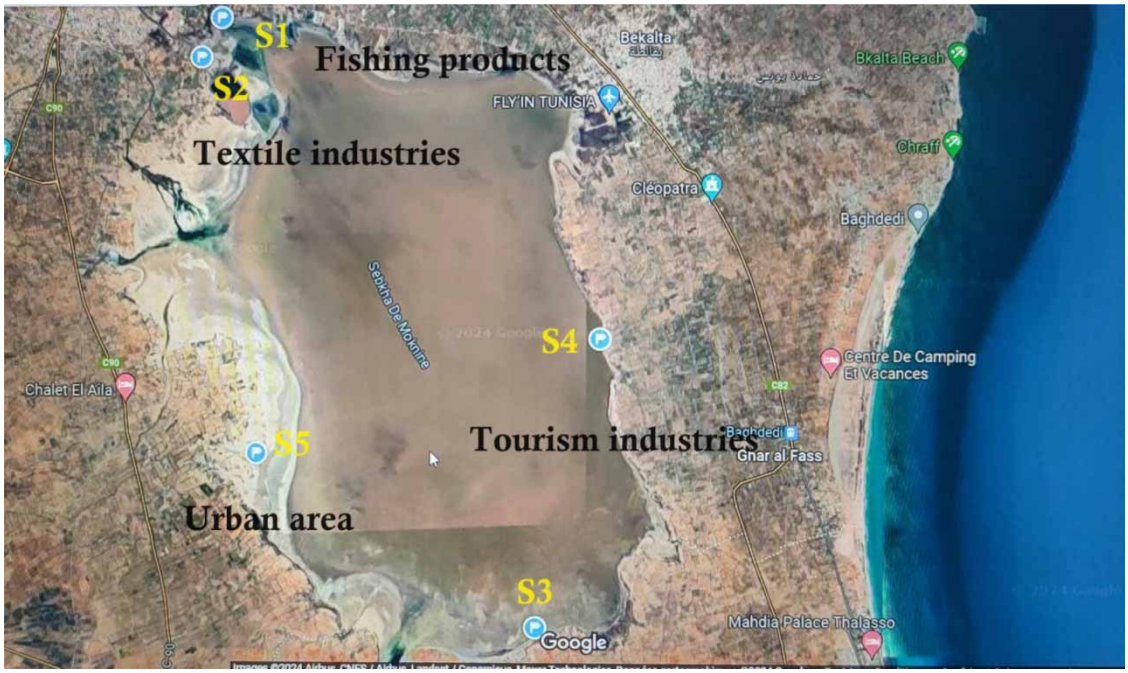

Figure 1 | Map illustrating the geographical location of the MCS (Tunisia) and of the sampling sites (S1–S5).

2.2. Physicochemical and chemical analysis of water samples

Physicochemical indicators of water quality including chemical oxygen demand (COD), biochemical oxygen demand (BOD), total organic carbon (TOC), total suspended solids (TSS), nitrate (NO_3^-) and adsorbable organic halogens (AOX) were measured using a portable UV analyzer (Pastel UV, Secomam, Alès, France). A turbidimeter AQUALITIC® (Dortmund, Germany) was used to measure turbidity parameters and conductivity was determined using conductimeter WTW 315i apparatus. The pH measurements of water samples were carried out using a WTW pH meter.

2.3. Biological approach: ecotoxicological analysis

2.3.1. *V. fischeri* luminescence test (Microtox®)

The procedure for carrying out the bioluminescence assay was based on Microtox® protocols (UNE-EN ISO 11348-2 2009). In this bioassay, the evaluated endpoint was the decrease in luminescence of the marine bacteria *V. fischeri* strain NRRL-B-11177, before and after incubation in different dilutions of seawater samples of CSM (100, 50, 25, 12.5, 6.2, and 3.1%). Bacteria were reconstituted from a lyophilized state by adding a reconstitution solution provided by Dr Lange (Dr Bruno Lange GmbH Co., Dusseldorf, Germany). Negative control samples (i.e., bacterial suspensions to which NaCl (2%) had been added instead of tested samples) were treated parallel to tested samples. The *V. fischeri* luminescence was measured after 15 and 30 minutes of incubation at 15 °C. Tests on all water samples were conducted in duplicate. The averages of *V. fischeri*

bioluminescence were calculated and then used to determine the concentration capable of inhibiting half of the bacterial bioluminescence (EC_{50}), which was calculated as a previous study by Jurado *et al.* (2012).

2.3.2. Microalgae growth test (Algaltox f™)

S. capricornutum was used to evaluate the chronic toxicity of seawater samples according to the UNE-EN ISO 8692 (2012) standard (UNE-EN ISO 8692 2012). Six test sample dilutions (100, 50, 25, 12.5, 6.25, and 3.1%) in triplicate were prepared. *S. capricornutum* in the flask was adjusted to 10^4 cells/mL by dilution with the ISO freshwater algal test medium. 25 mL aliquots of the diluted water samples were transferred to extra cells that were incubated at 23 ± 1 °C and under continuous illumination. Optical density of *S. capricornutum* at a wavelength of 670 nm was determined at the beginning of the bioassay and every 24 h until 72 h. The averages of *S. capricornutum* growth rates were determined and then used to calculate the effective concentration capable of inhibiting 50% of algal growth (EC_{50}), which was calculated as previous studies of Jurado *et al.* (2012) and Fernández-Serrano *et al.* (2014).

2.3.3. Phytotoxicity test: *L. sativum*

Phytotoxicity bioassay was performed following the method outlined by Methneni *et al.* (2021a). Seawater samples were diluted to the concentrations: 100, 50, 25, 12.5, 6.25, and 3.1%. Tests were carried out in three replicates. A total of 25 healthy seeds of the garden cress (*L. sativum*) plant were put on filter paper that was placed into Petri dishes and treated with 5 mL of the test solution. After exposure for 72 h, root and shoot lengths (cm) were measured. The inhibition of the root and shoot elongation was calculated compared to the control and was used to calculate the EC_{50} values, which represent the concentrations capable of inhibiting 50% of the root and shoot growth. EC_{50} values were determined using a calculation method outlined by Jurado *et al.* (2012) and Fernández-Serrano *et al.* (2014).

3. RESULTS

3.1. Physicochemical characterization of MCS seawater samples

Table 2 presents a complete overview of the physicochemical characteristics of seawater samples taken from the MCS coastal stations during a period of 9 months. The highest contents of COD, BOD, TOC, and TSS were observed in S1, while the highest levels of conductivity, turbidity and nitrate were observed in S5. The lowest values of pH, COD, BOD, nitrate, and TSS were found in S3 and the lowest levels of TOC and turbidity were detected in S4. It should be noted that the levels of all physicochemical parameters were below the Tunisian standard limit (NT.09.11/1983), except for the pH (in S4 and S5) and the TSS (in S1).

3.2. Ecotoxicological assessment of MCS coastal seawater samples

3.2.1. Toxicity to *S. capricornutum*

Figure 2 presents the ecotoxicity results, obtained when exposing *S. capricornutum* for 72 h to the MCS coastal seawater samples at different concentrations. A strong inhibition of algal growth was observed in all the studied coastal sites exceeding a percentage inhibition of 80% after exposure to 100% of seawater concentration in S2, S4, and S5.

3.2.2. Toxicity to *V. fischeri*

In this study, the short-term toxicity of seawater samples from the MCS coastal areas was measured by the Microtox® assay. Bioluminescence is determined in *V. fischeri* after exposure to various concentration percentages of the tested seawater samples for 15 and 30 min. Toxic effects (bioluminescence inhibition) were not observed at all studied coastal sites (results not shown).

3.2.3. Toxicity to *L. sativum*

The chronic toxicity of the MCS coastal water samples for *L. sativum* is presented in Figure 3. Inhibition of *L. sativum* root and shoot elongation was observed in all tested seawater samples. A strong inhibition of root growth (96%) was observed after exposure to 100% of seawater concentration in S1, meanwhile, the higher inhibition of shoot growth (93%) was shown in S2.

Table 2 | Physicochemical properties of the seawater samples from the MCS coastline during the study period (2022)

Study coastal areas Physicochemical properties	S1		S2		S3		S4		S5			Tunisian Acceptable Limits NT.09.11/1983
	Min–Max	Mean–SD	Min–Max	Mean–SD	Min–Max	Mean–SD	Min–Max	Mean–SD	Min–Max	Mean–SD	Mean–SD	
pH	6.59–8.35	7.66 ± 0.79	7.04–8.37	7.7 ± 0.64	7.3–8.27	7.3 ± 0.552	7.85–8.51	8.21 ± 0.33	7.71–8.62	8.16 ± 0.45	7.80 ± 0.38	6,5–8,5
COD (mg/L)	3.5–50.83	14.62 ± 2.82	3.8–7.83	6.09 ± 1.92	3.2–5.76	4.73 ± 1.35	4.1–10.2	6.26 ± 3.41	8.53–13.1	10.27 ± 2.46	8.39 ± 4.05	90
BOD (mg/L)	1.6–24	6.738 ± 1.08	1.8–7.3	4.05 ± 2.31	1.4–2.66	2.15 ± 0.66	1.39–4.66	2.71 ± 1.72	3.96–6.03	4.74 ± 1.25	4.08 ± 1.80	30
TOC (mg/L)	1.06–17.36	4.73 ± 0.79	1.2–4.8	2.67 ± 1.51	0.9–5.3	2.6 ± 2.36	1.26–3.13	1.93 ± 1.04	3.96–6.03	4.74 ± 1.25	3.01 ± 1.05	–
Conductivity (μS/cm)	500.3–652.66	618.19 ± 73.84	495–652.66	610.83 ± 77.29	644.33–651	648.33 ± 3.52	654–716	686.66 ± 31.13	2.63–4.03	3.16 ± 0.75	662.58 ± 56.74	–
Turbidity (NTU)	0.38–3.86	2.12 ± 2.46	1.67–2.32	2.07 ± 0.28	1.09–6.23	3.18 ± 2.69	0.63–1.63	1.04 ± 0.52	3.35–7.48	4.82 ± 2.30	2.65 ± 1.43	–
NO_3^- (mg/L)	5.6–10.4	6.846 ± 2.25	6.03–6.43	6.23 ± 0.28	5.46–6.43	5.79 ± 0.54	5.93–7.26	6.49 ± 0.68	7.1–7.43	7.21 ± 0.19	6.51 ± 0.54	90
AOX (mg/L)	< 0.5		< 0.5		< 0.5		< 0.5		< 0.5		<0.5	1
TSS (mg/L)	6.9–98.66	27.85 ± 4.7	7.5–15.1	11.815 ± 3.66	6.2–11.2	9.17 ± 2.63	8–18.86	11.92 ± 6.02	16.13–24.46	19.30 ± 4.50	15.75 ± 7.91	30

BOD, biochemical oxygen demand; COD, chemical oxygen demand; TSS, total suspended solids; TOC, total organic carbon; NO_3^-, nitrate; AOX, adsorbable organic halogen; SD, standard deviation; Min, minimum; Max, maximum.

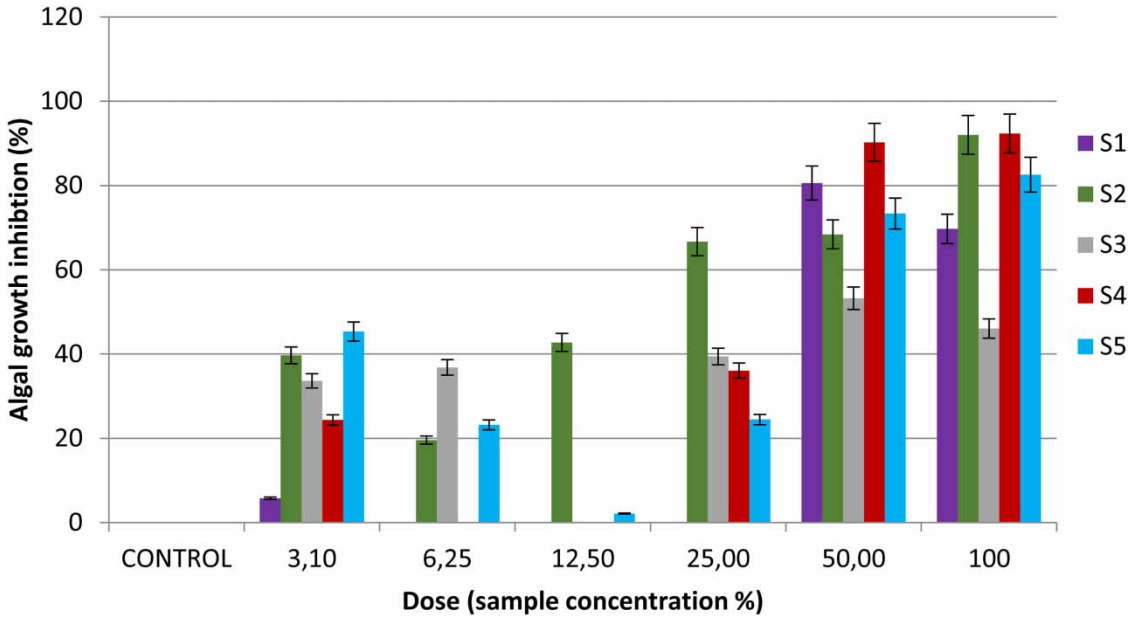

Figure 2 | Growth inhibition percentage of algae *S. capricornutum* exposed for 72 h to different doses of water from the coast of MCS. Error bars represent the standard deviation of the mean ($n = 3$).

3.3. Comparison between the ecotoxicological bioassays

Table 3 presents the results expressed as EC_{50} of all performed bioassays. The inhibition of *S. capricornutum* growth and the inhibition of the root and shoot elongation of *L. sativum* were more pronounced in S5 than the other areas. The shoot growth rate of *L. sativum* was the most sensitive bioassay when assessing the toxicity of water samples from the MCS coast (CMS).

4. DISCUSSION

The Moknine Sebkha (Tunisia) is a highly saline aquatic system located in the Sahel of Tunisia. Many studies are taken in this system: sedimentology, organic geochemistry of sediment and geochemical organic and mineral of the water. All studies show that this system is oligotrophy and is subdivided into microareas governed by the topography, the climatology and the hydrology (Chairi 2018).

This environment constitutes one of the most coveted wetlands in the Sahel, undergoing different forms of degradation, but at the same time presenting potential for sustainable development. It is time to design Sebkha as a support for economic development and not a dumping ground for waste and clearance for harmful activities. This cannot succeed without the contribution of water and land planning stakeholders to find a balance between socio-economic issues and environmental objectives.

In general, the levels of physicochemical characteristics, namely organic pollution-related indicators, gradually decreased from the outer areas (S1–S5) of the MCS coastline. The spatial variation of physicochemical parameters may be explained by (1) the proximity to maritime and industrial activities, which constitute the main contributory activities to pollutant emissions and (2) the speed of movement of water masses and the atmospheric wind flow variability (Kim *et al.* 2020). The station of MCS (S1), sheltering one of the most important fishing and trade ports in Tunisia with its own food canning, mechanical, metallurgical and textile industries, revealed a higher pollution level as a result of pronounced anthropogenic pressures. Station S4 benefits from an active fishing port with 725 fishing units participating in 40% of the total governorate production and numerous seafood-canning industries. Station S3 has a fishing port; nevertheless, it contributes by a low total fish production (0.4%) of the governorate, as well as a much lower degree of industrialization. A 'dilution effect' induced by the speed of circulation of coastal currents can contribute to the low contamination degree observed in S3 and S4 stations (Jebara *et al.* 2021a). In addition, intertidal movements have a significant impact on the hydrodynamic pattern of existing chemical compounds in the water, a factor that can elucidate the variability of the obtained results (Gurgel *et al.* 2016). In

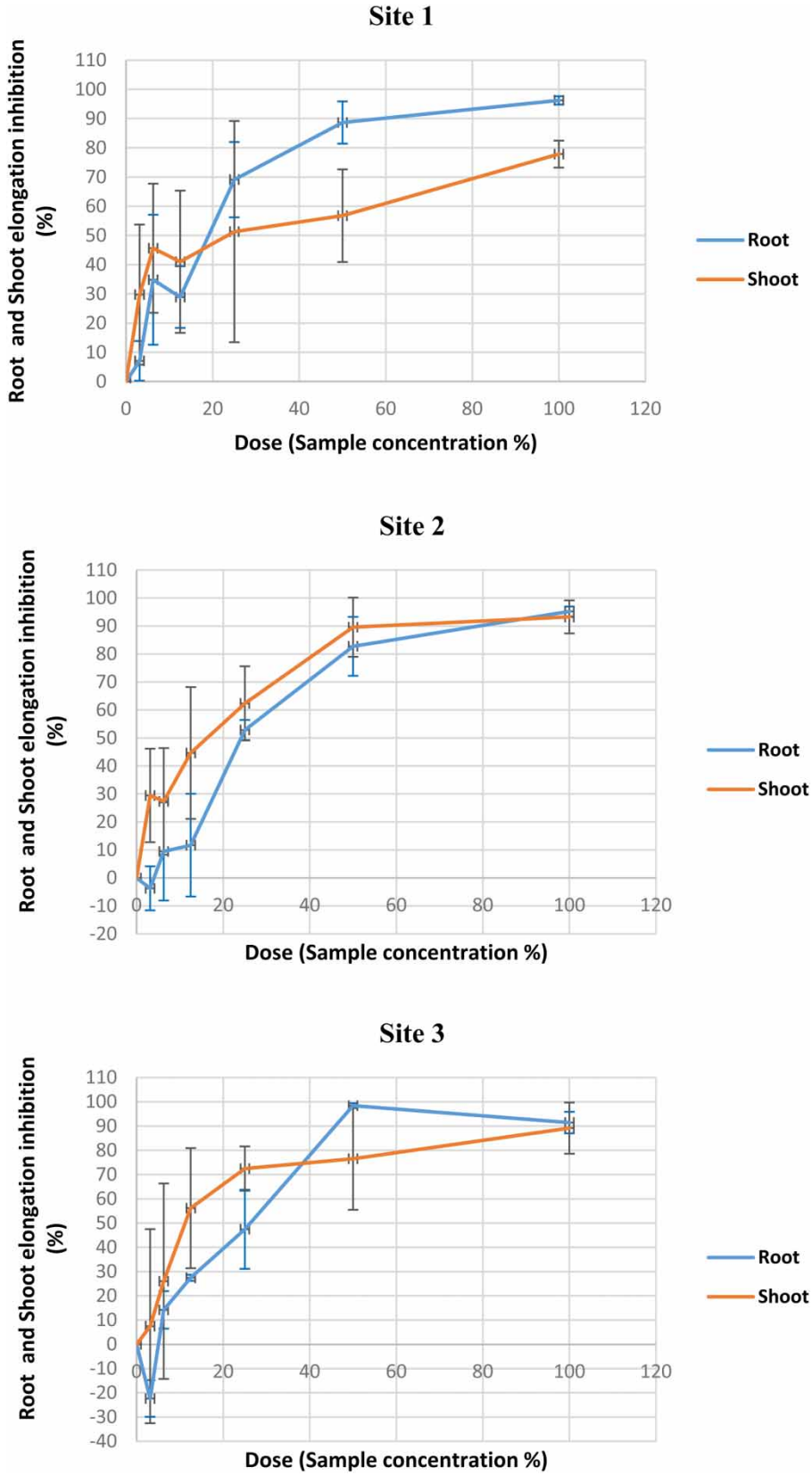

Figure 3 | Root and shoot elongation inhibition percentage of *L. sativum* exposed for 72 h to different doses of seawater from the coast of MCS. Error bars represent the standard deviation of the mean ($n = 3$). (*continued.*).

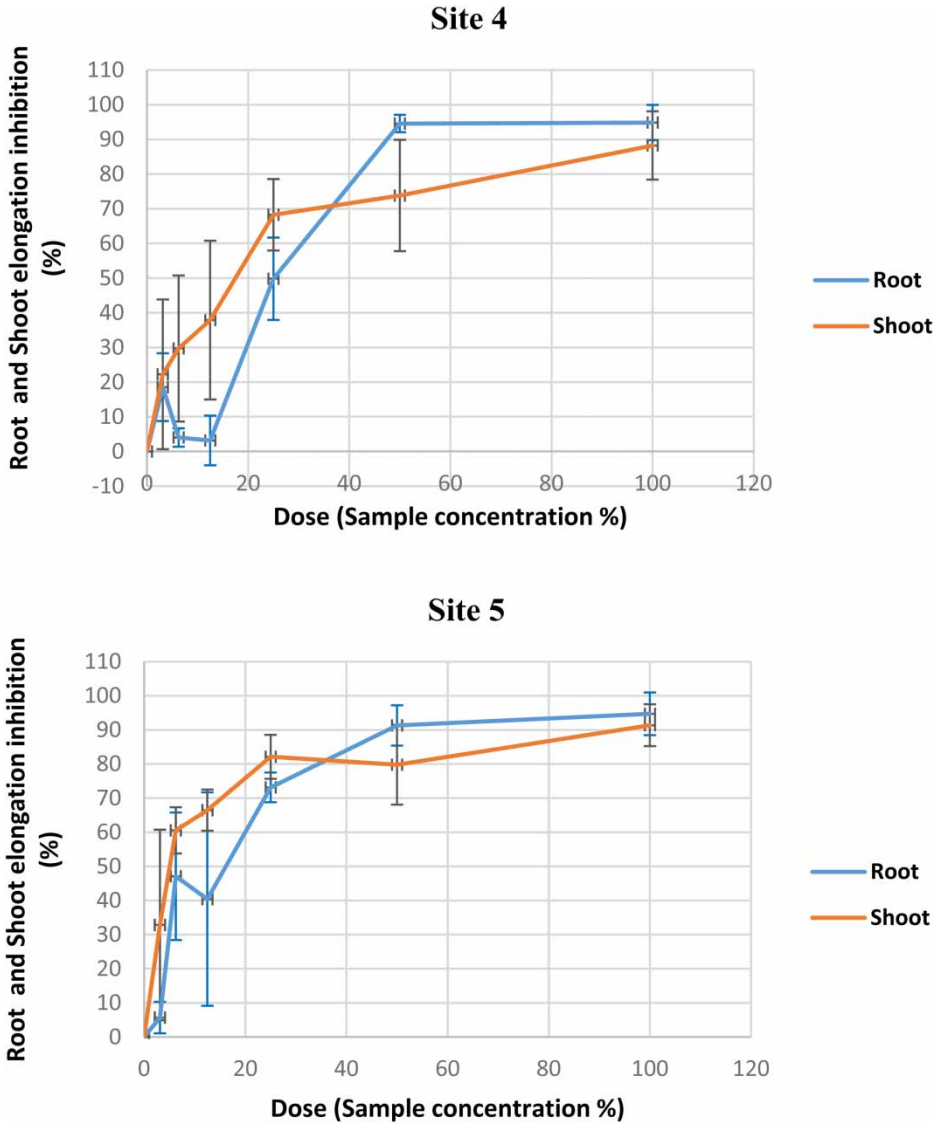

Figure 3 | Continued.

Table 3 | Toxicity of MCS seawater samples towards *V. fischeri, L. sativum* and *S. capricornutum* expressed as EC_{50} (%)

Sampling sites	S1	S2	S3	S4	S5
S. capricornutum					
72 h EC50	34,6	22,1	26,2	24,1	21,8
V. fischeri					
15 min EC50 *(%)*	nd	nd	nd	nd	nd
30 min EC50	nd	nd	nd	nd	nd
L. sativum					
72 h EC50 , Root	23	20,6	20,6	20,9	12,8
72 h EC50 , Shoot	12,6	12,5	15	6	5,8

EC_{50} refers to the effective concentration of water samples causing adverse effects for 50% of exposed species.
nd, not detected.

regard to the Tunisian authorities (NT.09.11/1983), we observed that the majority of parameters in seawater samples exceeded the permitted concentrations in the seawater for SM, COD, BOD, turbidity.

When it occurs, the deterioration of the water body cannot be explained uniquely based on a physicochemical screening as these analyses do not give an insight into the ecological and biological effects of a water matrix. Evaluation of the toxicity of water samples can only be done by determining the effect on multiple model species. In this study, the toxicity of seawater samples from the MCS coastal areas was assessed using three organisms belonging to multiple trophic levels: the *S. capricornutum*, *V. fischeri*, and the *L. sativum*. Obvious toxicological effects were found on the two species: *S. capricornutum* and *L. sativum* after exposure to the seawater samples, whereas *V. fisheri* was the only species that was not affected at all.

The toxic effect of our tested samples against the model species was compared to that of other aquatic environments worldwide (based on the EC50 values). Our seawater samples showed effects similar to those of the surface water rivers in Morocco which have been receiving untreated industrial wastewater from textile, tanneries, canneries, oil and pottery (10–40% inhibition for *S. capricornutum* and absence of toxic effect for *V. fischeri* Koukala *et al.* 2004). Our seawater samples were found severely toxic to the algae *S. capricornutum*, higher than that from the surface water of the Portuguese river (96.6 to >100%), continuously receiving runoff water and untreated sewage from livestock (Serpa *et al.* 2014).

The toxic effect of our water samples on *L. sativum* (if we consider shoot growth rate as a study parameter) in this study was higher than that of the surface water from Khniss and Hamdoun rivers in Tunisia (37 and 42.1%), heavily contaminated by heavy metals and toxic textile dyes and showing signs of poor water quality (Methneni *et al.* 2021b). The different responses between the biomarkers observed in this study may be due to the different levels of sensitivity to environmental stress and the different biological levels and time scales at which these biomarkers act (Handy *et al.* 2003). It is worth noting that inhibition of growth of *L. sativum* (considering the shoot growth rate as an endpoint) was the most sensitive parameter towards tested seawater samples.

A targeted chemical screening of the MCS coastline revealed the presence of total mercury (Hg), four PAEs (DiBP, DEP, DEHP, and DBP) and one NPP (DEHT), with obvious quantity differences and under different temporal and spatial conditions (Jebara *et al.* 2021a, 2021b), a reason that could explain the obtained toxic effects.

The mercury has been reported to induce toxic effects to marine and freshwater algae (*Microcystis aeruginosa, Nannochloropsis oculata*, and *Chlamydomonas reinhardtii)* by inhibiting their cell growth and photosynthesis and by inducing physiological alterations (Samadani & Dewez 2018; Zamani-Ahmadmahmoodi *et al.* 2020; Tang *et al.* 2023). Even at low applied concentrations, this metal may exercise toxic effects to a wide range of plants by inducing growth retardation (Ahammad *et al.* 2018), inhibiting photosynthesis activity (Assad *et al.* 2016), generating reactive oxygen species (ROS) (Kim *et al.* 2017), oxidizing lipid content (Zhou *et al.* 2008) and inducing genotoxic responses (Azevedo *et al.* 2018; Sun *et al.* 2018).

The acute toxicity of PAEs to *S. capricornutum* by inhibiting their growth and reducing their pigments is well documented in the literature (Gledhill *et al.* 1980; Suggatt & Foote 1981; Bionomics 1984; Jonsson & Baun 2010). Suggatt & Foote (1981) have exposed the algae *S. capricornutum* to the DEP compound during 96 h and based on the chlorophyll a content and the cell growth inhibition rate they found EC_{50} values of 85.6–90.3 mg/L. Bionomics (1984) measured effects on *S. capricornutum* upon exposure to DBP and DEP between days 6 and 10 and recorded EC_{50} values of 7 and 132 mg/L, for both compounds, respectively.

PAEs may act as an environmental stressor for terrestrial and aquatic plants by inducing various morphological, physiological, metabolic and cellular alterations in plants. Indeed, PAEs are reported to affect seed germination, roots elongation, seedlings growth, biomass, photosynthetic pigments and metabolic activities of different plant species, namely perennial ryegrass, radish, wheat, alfalfa, oat, cucumber and onion (Ma *et al.* 2015; Zhang *et al.* 2016; Ge *et al.* 2020). Nevertheless, the toxicity of DEHT is rarely documented. DEHT is known to have toxic effects on rats (Barber & Topping 1995; Ball *et al.* 2012).

Previous studies have proven the contamination of MCS coast by pharmaceutically active compounds, UV filters and paraben preservatives (Afsa *et al.* 2020; Fenni *et al.* 2022). Excessive exposure to these compounds would have adverse toxicological effects on vertebrates, invertebrates and plants (Li & Randak 2009; Prakash & Anbumani 2021; Maia *et al.* 2023).

Nevertheless, mixtures of toxic chemicals present in a water matrix (even at trace concentrations) can exert joint effects on living organisms, which can be additive, synergistic or antagonistic. The contamination of MCS is undoubtedly the consequence of intense industrial activity. However, the solution does not lie in the closure of these industries, which constitute a driving force not only of the economy of the Monastir city region but also of the Tunisian economy. The solution then lies in the development of wastewater treatment techniques initially by adopting environmentally friendly processes and therefore working in harmony with sustainable development objectives. This will also avoid the growing concern of the population living very intensely around the Sebkha, especially with the bad smell and the massive death of migratory birds. The diversity in plant groups in MCS undoubtedly contributes to the diversification of habitats and landscapes. They represent a

varied food source for wildlife: herbivorous or carnivorous waterbirds, domestic or wild herbivorous mammals. The existence of a few mammalian species is reported, including the rat, the jerboa, the hedgehog (*Paraechinus aethiopicus*) and the hare (*Lepus kabilicus*); species of amphibians (*Rana saharica* and *Buffo viridis*) and reptiles. The flora is also quite diverse. There were 52 species in the area. Some have an appreciable economic value: *Fhragmites communis and Juncus maritimus*. Others are characterized by their abundance: *Arthrocnmum indicum*, *Solanum sodamaeum*, and *Salicornia arabica* (Landolsi & Rejeb 2014). This diversity is today threatened by pollution by these emerging contaminants which risks disrupting the aquatic ecosystem and in certain cases the massive death of living beings.

5. CONCLUSION

This study clearly showed that biomonitoring based on biological indicators and markers is effective in predicting contamination and toxicity of the coast of MCS. Assessment was performed using three species representing different trophic levels, namely *V. fischeri*, *S. capricornutum* and *L. sativum*. It also turned out that the coast of MCS is heavily contaminated with plasticizers. A targeted chemical screening of the MCS coast previously performed revealed the presence of total mercury, four PAEs and one non-phthalate plasticizer, a fact that could explain the observed ecotoxicological effects and therefore might harm the bioma and the human health of the surrounding population. It is important to implement a physicochemical treatment of the coagulation flocculation type in each industry and at the outlet of the WWTPs, because this process makes it possible to reduce turbidity and therefore the pollutant load including plasticizers, textile dyes pharmaceutical residues, etc.

In this study, the limited volume of samples analyzed as well as the limited coverage of the study area, which only covers the coasts of Sebkha with a short sampling periodicity, leaves this study preliminary. For this, we propose in the perspective of carrying out a complete study by expanding the study areas and to vary the periods of sample collection to have a more correct overview of the water quality and the risks incurred.

ACKNOWLEDGEMENT

Dr H.B.M. thanks the Spanish government for his Maria Zambrano grant.

FUNDING

None declared.

AUTHOR CONTRIBUTIONS

A.B. did data analysis and investigation; wrote the original draft; K.B. did the investigation; M.S. and A.B. did the statistical analysis and guided the study; H.B.M. conceptualised, supervised, and validated the study.

DATA AVAILABILITY STATEMENT

Data cannot be made publicly available; readers should contact the corresponding author for details.

CONFLICT OF INTEREST

The authors declare there is no conflict.

REFERENCES

Afsa, S., Hamden, K., Lara Martin, P. A. & Mansour, H. B. 2020 Occurrence of 40 pharmaceutically active compounds in hospital and urban wastewaters and their contribution to Moknine Continental Sebkha coastal seawater contamination. *Environmental Science and Pollution Research* **27** (2), 1941–1955. https://doi.org/10.1007/s11356-019-06866-5.

Ahammad, S. J., Sumithra, S. & Senthilkumar, P. 2018 Mercury uptake and translocation by indigenous plants. *Rasayan Journal of Chemistry* **11** (1), 1–12.

Anderson, B. & Phillips, B. 2016 Saltwater toxicity tests. In: *Marine Ecotoxicology: Current Knowledge and Future Issues* (Blasco, J., Chapman, P. M., Campana & Hampel, M., eds.) Academic Press, Cambridge, MA, pp. 167–197.

Archiplan-Dgat 2019 Rapport définitif de la 1ère phase de l'étude du schéma directeur d'aménagement et du développement du gouvernorat de Mahdia. *Janvier* :1–347.

Assad, M., Parelle, J., Cazaux, D., Gimbert, F., Chalot, M. & Tatin-Froux, F. 2016 Mercury uptake into poplar leaves. *Chemosphere* **146**, 1–7. https://doi.org/10.1016/j.chemosphere.2015.11.103.

Azevedo, R., Rodriguez, E., Mendes, R. J., Mariz-Ponte, N., Sario, S., Lopes, J. C., de Oliveira, J. M. P. F. & Santos, C. 2018 Inorganic Hg toxicity in plants: A comparison of different genotoxic parameters. *Plant Physiology and Biochemistry* **125**, 247–254. https://doi.org/10.1016/j.plaphy.2018.02.015.

Ball, G. L., McLellan, C. J. & Bhat, V. S. 2012 Toxicological review and oral risk assessment of terephthalic acid (TPA) and its esters: A category approach. *Critical Reviews in Toxicology* **42** (1), 28–67. https://doi.org/10.3109/10408444.2011.623149.

Barber, E. D. & Topping, D. C. 1995 Subchronic 90-day oral toxicology of di (2-ethylhexyl) terephthalate in the rat. *Food and Chemical Toxicology* **33** (11), 971–978. https://doi.org/10.1016/0278-6915(95)00060-F.

Bionomics, S. & Bionomics, S. 1984 *Toxicity of Fourteen Phthalate Esters to Freshwater Green Alga (Selenastrium Capricornutum) Final Report (Report No. BP-84-1-4)*. Chemical Manufacturers Association, Washington, DC, USA.

Chairi, R. 2018 Biomarkers on sediments in a highly saline aquatic ecosystem: case of the Moknine Continental Sebkha (Eastern Tunisia). *Journal of Coastal Zone Management* **21**, 2.

De Souza Celente, G., Colares, G. S., da Silva Araújo, P., Machado, Ê. L. & Lobo, E. A. 2020 Acute ecotoxicity and genotoxicity assessment of two wastewater treatment units. *Environmental Science and Pollution Research* **27** (10), 10520–10527. https://doi.org/10.1007/s11356-019-07308-y.

Dummee, V., Kruatrachue, M., Trinachartvanit, W., Tanhan, P., Pokethitiyook, P. & Damrongphol, P. 2012 Bioaccumulation of heavy metals in water, sediments, aquatic plant and histopathological effects on the golden apple snail in Beung Boraphet reservoir, Thailand. *Ecotoxicology and Environmental Safety* **86**, 204–212. https://doi.org/10.1016/j.ecoenv.2012.09.018.

Favier, L., Rusu, L., Simion, A. I., Hlihor, R. M., Pacala, M. L. & Augustyniak, A. 2019 Efficient degradation of clofibric acid by heterogeneous photocatalytic oxidation process. *Environmental Engineering and Management Journal* **18** (8), 1683–1692. Available from: http://www.eemj.icpm.tuiasi.ro; http://www.eemj.eu

Fenni, F., Sunyer-Caldú, A., Mansour, H. B. & Diaz-Cruz, M. S. 2022 Contaminants of emerging concern in marine areas: First evidence of UV filters and paraben preservatives in seawater and sediment on the eastern coast of Tunisia. *Environmental Pollution* **309**, 119749. https://doi.org/10.1016/j.envpol.2022.119749.

Fernández-Serrano, M., Jurado, E., Fernández-Arteaga, A., Ríos, F. & Lechuga, M. 2014 Ecotoxicological assessment of mixtures of ether carboxylic derivative and amine-oxide-based non-ionic surfactants on the aquatic environment. *Journal of Surfactants and Detergents* **17** (6), 1161–1168. https://doi.org/10.1007/s11743-014-1621-2.

Ge, J., Cheng, J., Li, Y., Li, Q. X. & Yu, X. 2020 Effects of dibutyl phthalate contamination on physiology, phytohormone homeostasis, rhizospheric and endophytic bacterial communities of *Brassica rapa var. chinensis*. *Environmental Research* **189**, 109953. https://doi.org/10.1016/j.envres.2020.109953.

Gledhill, W. E., Kaley, R. G., Adams, W. J., Hicks, O., Michael, P. R., Saeger, V. W. & LeBlanc, G. A. 1980 An environmental safety assessment of butyl benzyl phthalate. *Environmental Science & Technology* **14** (3), 301–305.

Gurgel, P. M., Navoni, J. A., de Morais Ferreira, D. & do Amaral, V. S. 2016 Ecotoxicological water assessment of an estuarine river from the Brazilian Northeast, potentially affected by industrial wastewater discharge. *Science of The Total Environment* **572**, 324–332. doi: 10.1016/j.scitotenv.2016.08.002.

Handy, R. D., Galloway, T. S. & Depledge, M. H. 2003 A proposal for the use of biomarkers for the assessment of chronic pollution and in regulatory toxicology. *Ecotoxicology* **12** (1), 331–343. https://doi.org/10.1023/A:1022527432252.

Ilter Turkdogan Aydinol, F., Kanat, G. & Bayhan, H. 2012 Sea water quality assessment of Prince Islands' Beaches in Istanbul. *Environmental Monitoring and Assessment* **184** (1), 149–160. doi:10.1007/s10661-011-1954-5.

Jebara, A., Albergamo, A., Rando, R., Potortì, A. G., Turco, V. L., Mansour, H. B. & Di Bella, G. 2021a Phthalates and non-phthalate plasticizers in Tunisian marine samples: Occurrence, spatial distribution and seasonal variation. *Marine Pollution Bulletin* **163**, 111967. https://doi.org/10.1016/j.marpolbul.2021.111967.

Jebara, A., Lo Turco, V., Faggio, C., Licata, P., Nava, V., Potortì, A. G., Crupi, R., Mansour, H. B. & Di Bella, G. 2021b Monitoring of environmental Hg occurrence in Tunisian coastal areas. *International Journal of Environmental Research and Public Health* **18** (10), 5202. https://doi.org/10.3390/ijerph18105202.

Jebara, A., Turco, V. L., Potortì, A. G., Bartolomeo, G., Mansour, H. B. & Di Bella, G. 2021c Organic pollutants in marine samples from Tunisian coast: Occurrence and associated human health risks. *Environmental Pollution* **271**, 116266. https://doi.org/10.1016/j.envpol.2020.116266.

Jonsson, S. & Baun, A. 2010 Toxicity of mono-and diesters of o-phthalic esters to a crustacean, a green alga, and a bacterium. *Environmental Toxicology and Chemistry: An International Journal* **22** (12), 3037–3043. https://doi.org/10.1897/02-548.

Jurado, E., Fernández-Serrano, M., Núñez Olea, J., Lechuga, M., Jiménez, J. L. & Ríos, F. 2012 Acute toxicity of alkylpolyglucosides to *Vibrio fischeri, Daphnia magna* and microalgae: A comparative study. *Bulletin of Environmental Contamination and Toxicology* **88** (2), 290–295. https://doi.org/10.1007/s00128-011-0479-5.

Kim, Y. O., Bae, H. J., Cho, E. & Kang, H. 2017 Exogenous glutathione enhances mercury tolerance by inhibiting mercury entry into plant cells. *Frontiers in Plant Science* **8**, 683. https://doi.org/10.3389/fpls.2017.00683.

Kim, S., Lee, Y. S. & Moon, H. B. 2020 Occurrence, distribution, and sources of phthalates and non-phthalate plasticizers in sediment from semi-enclosed bays of Korea. *Marine Pollution Bulletin* **151**, 110824. https://doi.org/10.1016/j.marpolbul.2019.110824.

Koukala, B., Dominika, J., Vignatia, D., Arpagausa, P., Santiagoc, S., Ouddaned, B. & Benaabidate, L. 2004 Assessment of water quality and toxicity of polluted rivers Fez and Sebou in region of Fez (Morocco). *Environmental Pollution* **131**, 163–172. https://doi.org/10.1016/j.envpol.2004.01.014.

Landolsi, S. & Rejeb, H. 2014 Milieux aquatiques et Urbanisation : La Sebkha de Moknine. Milieux aquatiques et Urbanisation : La Sebkha de Moknine, pp 1-20

Lechuga, M., Fernández-Serrano, M., Jurado, E., Núñez-Olea, J. & Ríos, F. 2016 Acute toxicity of anionic and non-ionic surfactants to aquatic organisms. *Ecotoxicology and Environmental Safety* **125**, 1–8. https://doi.org/10.1016/j.ecoenv.2015.11.027.

Li, Z. H. & Randak, T. 2009 Residual pharmaceutically active compounds (PhACs) in aquatic environment–status, toxicity and kinetics: A review. *Veterinarni Medicina* **54** (7), 295–314. doi:10.17221/97/2009-vetmed.

Lyu, J., Park, J., Pandey, L. K., Choi, S., Lee, H., De Saeger, J., Depuydt, S. & Han, T. 2018 Testing the toxicity of metals, phenol, effluents, and receiving waters by root elongation in *Lactuca sativa* L. *Ecotoxicology and Environmental Safety* **149**, 225–232. https://doi.org/10.1016/j.ecoenv.2017.11.006.

Ma, T., Teng, Y., Christie, P. & Luo, Y. 2015 Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2-ethylhexyl) phthalate. *Frontiers of Environmental Science & Engineering* **9** (2), 259–268. https://doi.org/10.1007/s11783-014-0652-2.

Maia, C., Sousa, C. A., Sousa, H., Vale, F. & Simões, M. 2023 Parabens removal from wastewaters by microalgae–Ecotoxicity, metabolism and pathways. *Chemical Engineering Journal* 139631. https://doi.org/10.1016/j.cej.2022.139631.

Methneni, N., Morales-González, J. A., Jaziri, A., Mansour, H. B. & Fernandez-Serrano, M. 2021a Persistent organic and inorganic pollutants in the effluents from the textile dyeing industries: Ecotoxicology appraisal via a battery of biotests. *Environmental Research* **196**, 110956. https://doi.org/10.1016/j.envres.2021.110956.

Methneni, N., González, J. A. M., Van Loco, J., Anthonissen, R., Van de Maele, J., Verschaeve, L., Fernandez-Serrano, M. & Mansour, H. B. 2021b Ecotoxicity profile of heavily contaminated surface water of two rivers in Tunisia. *Environmental Toxicology and Pharmacology* **82**, 103550. https://doi.org/10.1016/j.etap.2020.103550.

Prakash, V. & Anbumani, S. 2021 A systematic review on occurrence and ecotoxicity of organic UV filters in aquatic organisms. *Reviews of Environmental Contamination and Toxicology* **257**, 121–161. https://doi.org/10.1007/398_2021_68.

Riani, E., Sudarso, Y. & Cordova, M. R. 2014 Heavy metals effect on unviable larvae of Dicrotendipes simpsoni (Diptera: Chironomidae), a case study from Saguling Dam, Indonesia. *AACL Bioflux* **7**, 76–84. Available from: http://www.bioflux.com.ro/aacl.

Samadani, M. & Dewez, D. 2018 Effect of mercury on the polyphosphate level of alga *Chlamydomonas reinhardtii*. *Environmental Pollution* **240**, 506–513. https://doi.org/10.1016/j.envpol.2018.04.141.

Serpa, D., Keizer, J. J., Cassidy, J., Cuco, A., Silva, V., Gonçalves, F., Cerqueira, M. & Abrantes, N. 2014 Assessment of river water quality using an integrated physicochemical, biological and ecotoxicological approach. *Environmental Science: Processes & Impacts* **16** (6), 1434–1444. https://doi.org/10.1039/C3EM00488K.

Silva, A., Figueiredo, S. A., Sales, M. G. & Delerue-Matos, C. 2009 Ecotoxicity tests using the green algae *Chlorella vulgaris* – a useful tool in hazardous effluents management. *Journal of Hazardous Materials* **167** (1–3), 179–185. https://doi.org/10.1016/j.jhazmat.2008.12.102.

Suggatt, R. H. & Foote, K. 1981 *Comprehensive review of acute aquatic toxicity data on phthalate esters. Contract SRC TR, 81, p.537. Final Report*. Syracuse Research Corporation, Syracuse, NY, USA.

Sun, Y., Chao, J., Gu, W., Wang, M. & Zhang, W. 2018 Photosynthesis characteristics and physiological indexes of *Atracty lodeslancea* (Thunb.) DC. under mercury stress. *Journal of Southern Agriculture* **49** (3), 448–453.

Tang, W., He, M., Chen, B., Ruan, G., Xia, Y., Xu, P., Song, G., Bi, Y. & Hu, B. 2023 Investigation of toxic effect of mercury on *Microcystis aeruginosa*: Correlation between intracellular mercury content at single cells level and algae physiological responses. *Science of The Total Environment* **858**, 159894. https://doi.org/10.1016/j.scitotenv.2022.159894.

Tavakoly Sany, S. B., Hashim, R., Rezayi, M., Rahman, M. A., Razavizadeh, B. B. M., Abouzari-lotf, E. & Karlen, D. J. 2015 Integrated ecological risk assessment of dioxin compounds. *Environmental Science and Pollution Research* **22** (15), 11193–11208. https://doi.org/10.1007/s11356-015-4511-x.

UNE-EN ISO 11348-2 2009 Water quality-Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Luminescent bacteria test)-Part 2: Method using liquid-dried bacteria (ISO 10348-2: 2007).

UNE-EN ISO 8692 2012 Water quality-Fresh water algal growth inhibition test with unicellular green algae (ISO 8692:2012).

Žaltauskaitė, J. & Čypaitė, A. 2008 Assessment of landfill leachate toxicity using higher plants. *Environmental Research, Engineering & Management* **46** (4), 42–47.

Zamani-Ahmadmahmoodi, R., Malekabadi, M. B., Rahimi, R. & Johari, S. A. 2020 Aquatic pollution caused by mercury, lead, and cadmium affects cell growth and pigment content of marine microalga, Nannochloropsis oculata. *Environmental Monitoring and Assessment* **192** (6), 1–11. https://doi.org/10.1007/s10661-020-8222-5.

Zhang, Y., Zhang, H., Sun, X., Wang, L., Du, N., Tao, Y., Sun, G., Erinle, K. O., Wang, P., Zhou, C. & Duan, S. 2016 Effect of dimethyl phthalate (DMP) on germination, antioxidant system, and chloroplast ultrastructure in *Cucumis sativus* L. *Environmental Science and Pollution Research* **23** (2), 1183–1192. https://doi.org/10.1007/s11356-015-5855-y.

Zhou, Z. S., Wang, S. J. & Yang, Z. M. 2008 Biological detection and analysis of mercury toxicity to alfalfa (*Medicago sativa*) plants. *Chemosphere* **70** (8), 1500–1509. https://doi.org/10.1016/j.chemosphere.2007.08.028.

Zrelli, E., Rabaoui, R., Ben Alaya, L., Daghbouj, M., Castet, N., Besson, S., Michel, P., Bejaoui, S. & Courjault-Radé, N. 2018 Seawater quality assessment and identification of pollution sources along the central coastal area of Gabes Gulf (SE Tunisia): Evidence of industrial impact and implications for marine environment protection. *Marine Pollution Bulletin* **127**, 445–452. https://doi.org/10.1016/j.marpolbul.2017.12.012.

First received 21 January 2024; accepted in revised form 6 March 2024. Available online 19 March 2024