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RESEARCH ARTICLE

Acute toxicity of nanoscale zeolitic imidazolate framework 8 (ZIF-8) to saltwater planktonic species *Artemia salina* and *Nannochloropsis oculata*

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Abstract

Zeolitic imidazolate framework-8 nanoparticles (ZIF-8 NPs) are metal–organic frameworks (MOFs) that have gained significant attention in various fields due to their unique properties. They have potential applications in drug delivery, gas storage, and catalysis. However, their increasing use raises concerns about their potential environmental impact. Our study evaluates the effects of ≈90 nm ZIF-8 NPs in two planktonic species, the green microalga *Nannochloropsis oculata* and the brine shrimp *Artemia salina*. After synthesis and characterization (SEM, EDS, BET, and DLS) of nanoporous ZIF-8 NPs, a growth inhibition test on microalgae (72 h) and acute immobilization test on instar I and II of *Artemia* nauplii (48 h) were conducted following, OECD 201 and ISO/TS 20787, respectively. The toxicity of ZIF-8 NPs to both species was time- and concentration-dependent. The 72-h median inhibitory concentration (IC_{50}) of ZIF-8 NPs for *N. oculata* based on average specific growth rate and yield were calculated as 79.71 ± 8.55 mg L^{-1} and 51.73 ± 5.16 mg L^{-1}, respectively. Also, the 48-h median effective concentration (EC_{50}) of ZIF-8 NPs on immobilization rate of instar I and II were calculated as 175.09 ± 4.14 mg L^{-1} and 4.69 ± 0.34 mg L^{-1}, respectively. Moreover, the swimming type of non-immobilized animals was affected by ZIF-8 NPs. These findings provide a good insight into the toxicity of nanoparticulate ZIF-8 to saltwater planktons and also confirm that instar II *Artemia* is more sensitive than instar I. This study demonstrated that ZIF-8 NPs, despite all their advantages, could have toxic effects on aquatic organisms. More studies are required to assess their potential environmental impact and develop strategies to mitigate their toxicity.

Keywords Aquatic nanotoxicology · *Artemia* · Brine shrimp · Metal–organic frameworks · Microalgae · Nanoporous

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Highlights
- ZIF-8 NPs were synthesized and well-characterized.
- Salina nauplii were immobilized following exposure to ZIF-8 NPs.
- Swimming type of non-immobilized nauplii was affected by ZIF-8 NPs.
- Instar II was more sensitive than instar I to ZIF-8 NPs.
- The growth of N. oculata was inhibited following exposure to ZIF-8 NPs.

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Introduction

Metal–organic frameworks (MOFs) are new porous materials which are composed of metal ions such as Zn, Fe, Ca, and Mg connected by various organic linkers such as imidazolates, pyridyl, amines, polycarboxylates, and phosphonates (Kitagawa et al. 2004; Carne et al. 2011; Duan et al. 2015). MOFs with nanoscale dimensions are called nano-MOFs (Ruyra et al. 2014) and are one of the latest developed materials in nanotechnology (Férey 2008; McKinlay et al. 2010). MOFs have important properties like high porosity, large surface area, tunable shapes and pore sizes, and different functional groups within the organic linker (Slater and Cooper 2015; Singco et al. 2016; Du et al. 2020). These characterizations enable them to be used in different applications, including catalysis (Falkowski et al. 2014), gas storage (Noro et al. 2000), ion exchange (Oh and Mirkin 2006), chemical sensors (Chen et al. 2008), drug delivery (Keskin

and Kizilel 2011) and biocidal materials (Wyszogrodzka et al. 2016). Some studies have evaluated MOFs as biocide material in water treatment (Prince et al. 2014; Quirós et al. 2015). MOFs also have great potential to degrade organic dyes in wastewater (Chandra et al. 2016; Fan et al. 2018). The widespread production and usage of MOFs, particularly in the environmental area (Fan et al. 2018), may inevitably lead to their entry and their by-product, into aquatic ecosystems (Moore 2002). Therefore, the environmental health and ecosafety of the MOFs must be evaluated before the practical use of these materials (Ruyra et al. 2014).

Zeolitic imidazolate frameworks (ZIFs), a subclass of MOFs, are composed of metal ions such as Zn or Co connected by imidazolate linkers to create unique structures (Nabipour et al. 2017). ZIF-8 crystals constructed from Zn^{2+} ions linked through four 2-methylimidazole rings (Arun et al. 2019) possess the characteristics such as high surface area, high porosity, high crystallinity, excellent biocompatibility, and ultrahigh thermal and chemical stability (Hayashi et al. 2007; Moggach et al. 2009; Fairen-Jimenez et al. 2011). The nanoscale ZIF-8, with a property of pH-sensitive behavior, is an ideal nanocarrier for drug delivery, especially for cancer therapy (Nordin et al. 2014; Zheng et al. 2015; Johari et al. 2021). Also Jing et al. (2014) proposed ZIF-8 as a potential photocatalyst for the degradation of organic pollutants, including methylene blue.

Despite the many advantages of ZIF-8, there is not much information about the safety of these crystals to humans and the environment. Vasconcelos et al. (2012) reported that half-maximal inhibitory concentration (IC_{50}) for ZIF-8 on the human promyelocytic leukemia (HL-60), human colorectal adenocarcinoma (HT-29) and mucoepidermoid carcinoma of human lung (NCI-H292) cell lines was above 25 µg mL^{-1}. Toxicity effects of ZIF-8 crystals on six different human cell lines emerged at concentrations above 30 µg mL^{-1}. Cytotoxicity of ZIF-8 was attributed to the release of zinc ions (Zn^{2+}) from ZIF-8 frameworks within the cell media, causing the mitochondrial reactive oxygen species (ROS) production, DNA damage, and ultimately initiating cellular apoptosis pathways (Hoop et al. 2018). MOFs, according to their structure, metal ions such as Zn^{2+}, Cu^{2+}, Ag^{+}, and organic ligands have various inhibitory effects on the growth of algal cells (Fan et al. 2018). Fan et al. (2018) found that MOFs containing Cu^{2}, Ag^{+} ions, and 2-methylimidazole organic ligands inhibited the growth of *Microcystis aeruginosa* algae compared to those containing Zn^{2+} ions and 2,5-dihydroxyterephthalic acid (DHTA) organic ligand. Experts suggest that the suppression of algal growth by nanostructured materials may be due to a variety of mechanisms. Release of metal ions from nanomaterials such as Cu^{2+} from Cu-MOF-74 (Fan et al. 2018) or Zn^{2+} from ZnNPs (Ji et al. 2011) inhibited the growth of *M. aeruginosa* and *Chlorella*, respectively. Nanomaterials, because of their high surface area, can interact with active groups on the surface of algae cells, causing damage and death of cells (Sadiq et al. 2011; Fan et al. 2018). The production of ROS and oxidative stress induced by nanomaterials are also responsible for inhibiting algal growth (Bhattacharya et al. 2010; Ji et al. 2011). Fan et al. (2018) reported that the ROS generation in cells of *M. aeruginosa* induced by Cu-MOF-74 destroyed cell structure. The toxicity of nanoscale MOFs on aquatic animals is poorly documented in the literature. Brine shrimp, *Artemia* sp., are aquatic microcrustaceans and often live in saltwater ecosystems. This marine invertebrate, as a non-selective filter feeder with digestibility of small size particles < 50 µm (Hund-Rinke and Simon 2006), is one of the first candidates for assimilation of nanomaterials (Ates et al. 2015). This zooplankton is also used as a model for ecotoxicology studies and toxicity assessment of manufactured nanomaterials (MNMs) (ISO TS 20787, 2017; Johari et al. 2019). Raju et al. (2020) assessed the cytotoxicity of different concentrations (25–150 µg mL^{-1}) of synthesized catechin encapsulated leaf-like morphology ZIF (ZIF-L) nanocomposite (CA@ZIF-L) on 24 old nauplii of *Artemia salina*. They found that CA@ZIF-L nanocomposite was not toxic at low concentrations, while mild toxicity was observed at high concentrations. Shi et al. (2021) showed that nanoparticulate ZIF-8 has limited lethal toxicity on embryo stages of zebrafish but may induce significant hyperactivity or bradycardia in hatched larvae. Also, Qiu et al. (2022) showed an increase of cetylpyridinium chloride toxicity in the presence of ZIF-8 nanoparticles.

Aquatic organisms such as microalgae and microcrustaceans are commonly used as model organisms to study the toxicity of chemicals (OECD 2004, 2011, ISO TS (2078)7 International Organization for Standardization 2017). Studying the toxicity of ZIF-8 nanoparticles on these organisms can provide valuable information on their potential impact on aquatic ecosystems. The present study focused on the fabrication of nanoscale zeolitic imidazolate framework 8 (ZIF-8) and the assessment of toxicity of different concentrations of synthesized ZIF-8 nanoparticles on marine microalgae (*Nannochloropsis oculata*) and brine shrimp (*Artemia salina*).

Materials and methods

Synthesis and characterization of nanoscale ZIF-8

Methanol, 2-methylimidazole ($C_4H_6N_2$), and zinc nitrate hexahydrate ($Zn(NO_3)_2{\cdot}6H_2O$) were purchased from Merck Co. The nanoscale ZIF-8 was synthesized, according to Zheng et al. (2015). Firstly, 1.2 g of zinc nitrate hexahydrate ($Zn(NO_3)_2{\cdot}6H_2O$) was dissolved in 40 mL of deionized water. 2-Methylimidazole ($C_4H_6N_2$) (2.64 g) was also

dissolved in 80 mL of methanol. Then, the prepared zinc nitrate solution was added dropwise into 2-methylimidazole solution under stirring. The color of the reaction solution changed from colorless to white, indicating the formation of ZIF-8 particles. Afterward, the suspension was sonicated for 20 min using a probe sonicator (FAPAN 150UT, FAPAN CO. Ltd.) and centrifuged at 8000 rpm for 15 min at 15 °C (Eppendorf 5804R, Germany) to obtain the ZIF-8 particles. The supernatant was carefully decanted, and the residue was washed three times with 90 mL of methanol, sonicated, and centrifuged. Subsequently, the ZIF-8 particles were dried using a freeze dryer (Dena Vacuum, FD-5005-BT, Iran).

The size, shape, and elemental composition of synthesized particles were determined using field emission scanning electron microscopy (FE-SEM; MIRA3 Tescan, Czech Republic) in conjunction with X-ray energy dispersive spectroscopy (EDS). To determine the average diameter and size distribution of particles, 180 individual particles in SEM images were measured using AxioVision software (Release 4.8.2.0, Carl Zeiss MicroImaging GmbH, Germany). The Brunauer–Emmett–Teller (BET) surface area analysis of the prepared sample was done by N_2 adsorption–desorption method and a BELSORP mini II (MicrotracBEL Corp., Osaka, Japan). The dynamic light scattering (DLS) analysis of the ZIF-8 suspension was conducted using a VASCO™ nanoparticle size analyzer (Cordouan Technologies, France). The SZ-100 (HORIBA, Japan) was used to measure the zeta potential of particles at a holder temperature of 25 °C. Furthermore, in our recently published paper by Salari Joo et al. (2023), we have reported the X-ray diffraction (XRD) analysis and X-ray photoelectron spectroscopy (XPS) results of an identical batch of ZIF-8 nanoparticles.

Microalgae toxicity test

An acute toxicity test for 72 h was performed on microalgae cells of *Nannochloropsis oculata* using different concentrations of ZIF-8 nanoparticles based on the standard protocol of the Organization for Economic Co-operation and Development test guideline (TG) number 201 (OECD 2011). The algae cells were cultured in 1 L glass Erlenmeyer flasks containing Walne's medium under a temperature of 26.5 ± 0.5 °C, salinity of 35 g L^{-1}, and light illumination provided by a combination of yellow and white fluorescent lamps. Concentrations of 0, 1, 5, 10, 30, 45, 60, 75, 90, 100, and 200 mg L^{-1} of ZIF-8 NPs were selected based on preliminary tests (data not shown). Aliquots of microalgae (1000 mL) in the exponential growth phase (initial biomass of 343.5 ± 28.2 cell mL^{-1}) were exposed to experimental concentrations for 72 h in triplicate. Then, 100 µL of algae cells was sampled at 24, 48, and 72 h in three replicates. The algal biomass was determined based on cell counting using a Neubauer chamber under a light microscope in triplicate. The cell viability was determined by trypan blue (Sigma Aldrich; C.I. 23,850 and CAS No: 72–57-1) staining at 0, 24, 48, and 72h of the experimental period according to the method described by Tayemeh et al. (2020). The average specific growth rate, the percent inhibition of algal growth, and percent inhibition of yield was calculated based on the TG 201 (OECD 2011). The median inhibitory concentration (IC_{50}) was also determined based on the average specific growth rate and the percent inhibition of yield using the EPA Probit analysis program (Version 1.5).

Artemia toxicity test

Artemia salina cysts were purchased from Binzhou Evergreen Aquaculture Co., Ltd. (China) and preserved at the refrigerator until use. Artificial seawater (ASW, 35 g L^{-1}) was prepared by dissolving 35 g of synthetic seawater salt (Delta Marine®, Inc., Iran) into 1 L of deionized water and vigorously aerated for 24 h. To obtain the nauplii (instar I stage), approximately 0.8 g of dry cysts was incubated for 24 h in a clear "V"–bottom glass incubator containing 800 mL of ASW at 29 ± 1 °C, under 1500 lx fluorescent light intensity.

The acute (48 h) toxicity tests were conducted on instar I and II naupliar stages of *A. salina* following ISO TS 20787 (2017). Based on the results of a series of preliminary tests (data not shown), the test concentrations of 100, 125, 150, 175, 200, 225, and 250 mg L^{-1}, as well as 2.5, 5, 7.5, 10, 12.5, 15, 17.5, and 20 mg L^{-1} of ZIF-8 NPs were chosen for instar I and II, respectively. To investigate the toxicity effect of ZIF-8 NPs, 6-well plates were filled with 10 mL of each tested concentration in ASWE, and five nauplii were transferred to each exposure vessel. The exposure beakers were incubated at 29 ± 1 °C in the dark with no aeration, and the nauplii were not fed during the exposure. Each test concentration was performed in triplicate. During the exposure, the immobilized nauplii were collected every 24 h and counted under a stereomicroscope. Furthermore, the non-immobilized *Artemia* were categorized according to their swimming type in normal swimming (Nor) or erratic swimming (ERR). The effective concentrations (ECs) of ZIF-8 NPs on immobilization rate were calculated using the EPA Probit analysis program.

Statistical analysis

Data were presented as mean value with standard deviation (*mean* ± *SD*). The statistical analysis was done using the SPSS software (Version 19; IBM SPSS, Armonk, NY, USA). The one-way ANOVA followed by the Duncan multiple range test was used to identify significant differences.

Results

Characterization of ZIF-8 particles

SEM micrograph and EDS analysis of ZIF-8 particles are shown in Fig. 1 (a, b). SEM micrograph indicated that the synthesized particles had hexagonal shapes with a mean diameter of 90.87 ± 14.55 nm, and size distribution ranged from 48.86 to 129.72 nm (Fig. 1 a). Accordingly, the majority of particles (87%) measured between 50 and 100 nm, while 8% were smaller than 50 nm, and 5% were larger than 100 nm. The EDS analysis indicated the presence of zinc (Zn) element as one of the components of the zeolitic imidazolate framework (Fig. 1 b). The results of BET surface area analysis based on the BET plot and BJH (Barrett–Joyner–Halenda) plot are shown in Table 1. These results indicate the high surface area (1587.9 $m^2\ g^{-1}$) and nanoporous structure of the synthesized particles. DLS technique based on cumulant analysis showed that the mean number diameter, Z-average, and Polydispersity Index (PDI) of ZIF-8 particles were 175.96 nm, 201.56 nm, and 0.19, respectively (Fig. 2a). The Sparse Bayesian Learning (SBL) algorithms on DLS data revealed that the mean number diameter of particles was 106.27 nm, and the number intensity of particles with 100.24 and 247.8 nm in the ZIF-8 suspension was 95.86 and 4.14%, respectively (Fig. 2 b). The zeta potential of ZIF-8 particles was measured to be – 26.8 mV.

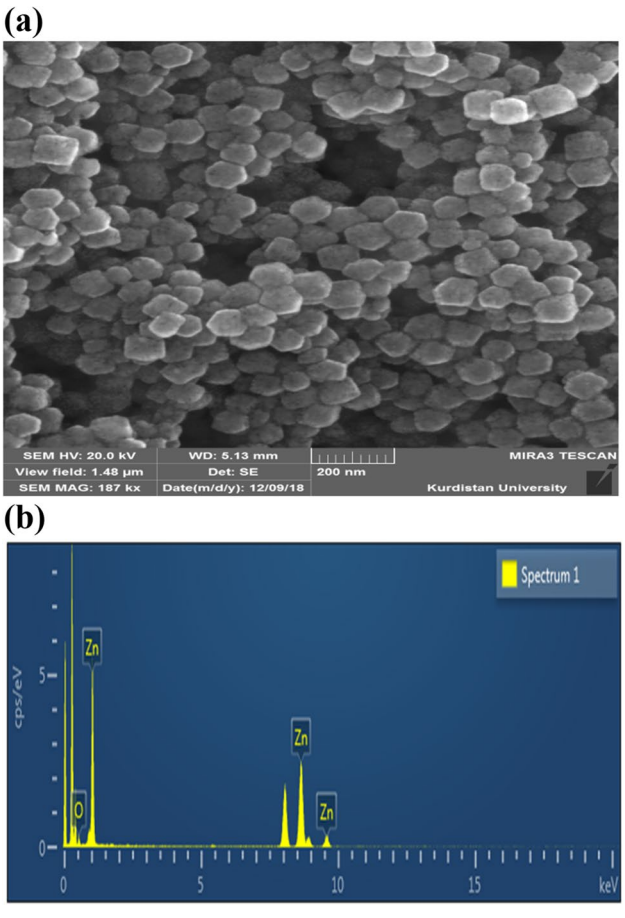

Fig. 1 SEM micrograph (**a**) and EDS spectrum (**b**) of ZIF-8 nanoparticles

Table 1 Results of BET surface area analysis of ZIF-8 nanoparticles

BET analysis	ZIF-8 NPs
Based on BET plot	
Total surface area ($m^2\ g^{-1}$)	1587.9
Mean pore diameter (nm)	2.5122
Total pore volume ($cm^{-3}\ g^{-1}$)	0.9973
Based on BJH plot	
Pore area ($m^2\ g^{-1}$)	73.235
Pore volume ($cm^{-3}\ g^{-1}$)	0.3778

Microalga toxicity

The IC_{50} of ZIF-8 NPs for *N. oculata* was determined to be 79.71 ± 8.55 mg L^{-1} and 51.73 ± 5.16 mg L^{-1} based on the average specific growth rate and yield, respectively. The biomass values of *N. oculata* exposed to different concentrations of ZIF-8 NPs are shown in Fig. 3. The trypan blue staining assay revealed that none of the exposed concentrations except 200 mg L^{-1} ZIF-8 caused cell death. The biomass of algae which exposed to 0, 1, 5, 10, 30, 45, and 60 mg L^{-1} of ZIF-8 NPs increased 7.3, 6.57, 5.53, 5.23, 5.37, 4.70, and 3.65-folds during 72 h of the exposure period. The biomass of algae exposed to 75, 90, and 100 mg L^{-1} of ZIF-8 NPs decreased during 24 h, but these values increased about 2.85, 2.43, and 2.07-fold up to 72 h. The algal biomass in 200 mg L^{-1} of ZIF-8 NPs decreased 3.21-fold during 72 h of exposure. The algal biomass decreased inversely with increasing exposure concentration from 0 to 200 mg L^{-1} after 72 h of the exposure period ($P < 0.05$).

The inhibition rates of 0–100% based on the specific growth and 0–100% for the yield of *N. oculata* exposed to 0–200 mg L^{-1} of ZIF-8 NPs were recorded (Fig. 4). In fact, the ascending trends in the percent inhibition rates of algae were observed with increasing ZIF-8 NPs from 0 to 200 mg L^{-1}.

Brine shrimp toxicity

The results of the effect of ZIF-8 nanoparticles on the survival and swimming type of *Artemia* nauplii (instar I and II) are shown in Fig. 5. Regarding the first instar, no immobilization was observed during the first 24 h of exposure except

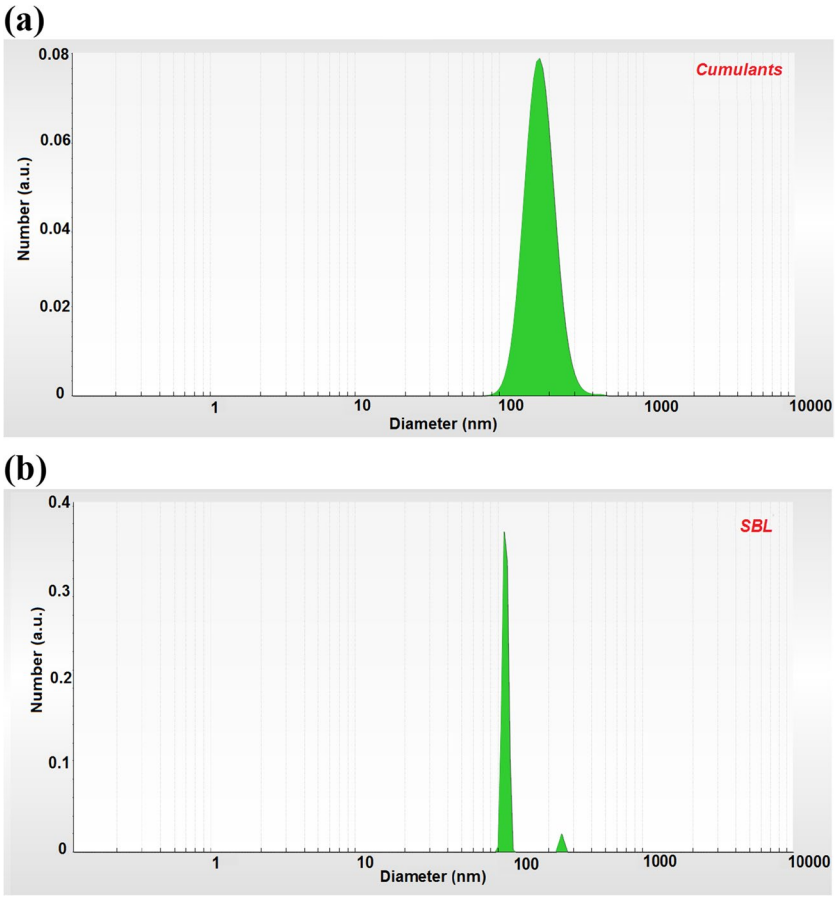

Fig. 2 Size distribution of ZIF-8 nanoparticles based on cumulants analysis (**a**) and Sparse Bayesian Learning (SBL) algorithms (**b**) of dynamic light scattering (DLS) technique

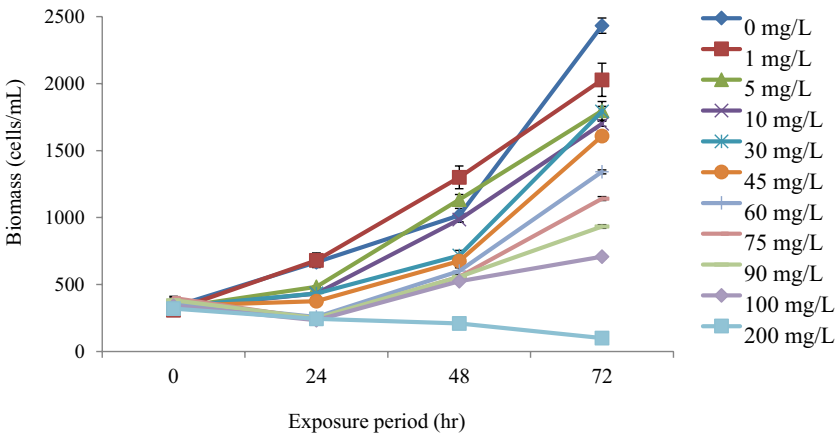

Fig. 3 Algal growth curves of *Nannochloropsis oculata* exposed to different concentrations of nanoscale zeolitic imidazolate framework 8 (ZIF-8), (*mean* ± *SD*)

in the highest concentration (i.e., 250 mg L^{-1}), and therefore, the ECs could not be calculated. Nevertheless, after 48 h of exposure, the immobilization started, and accordingly, the EC_{10}, EC_{50}, and EC_{90} of 95.20, 175.09, and 322.04 mg L^{-1} were calculated, respectively. In the case of the second instar, the 24-h EC_{10}, EC_{50}, and EC_{90} were 2.37, 14.99, and 94.80 mg L^{-1}; meanwhile, the 48-h EC_{10}, EC_{50}, and EC_{90} were 0.68, 4.69, and 32.09 mg L^{-1}, respectively. The findings of this study demonstrate that the toxicity of ZIF-8 to *Artemia* nauplii is both time- and concentration-dependent, with instar II being more sensitive than instar I.

Discussion

As with all other nanostructure materials, characterization of nanoscale ZIF-8 is essential in all scientific studies. The SEM results showed the formation of uniform hexagonal

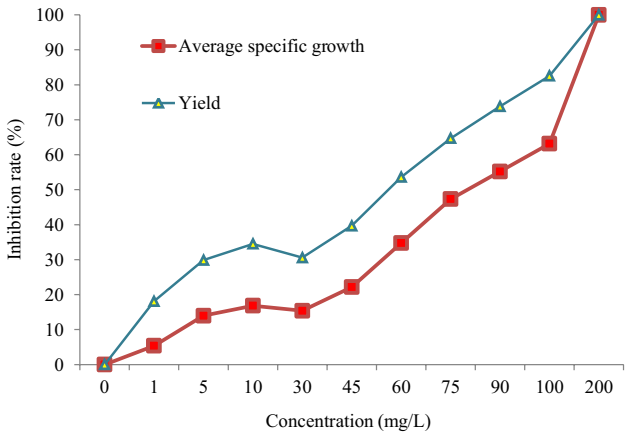

Fig. 4 The percent inhibition of growth for average specific growth and yield of *Nannochloropsis oculata* after 72-h exposure to different concentrations of nanoscale zeolitic imidazolate framework 8 (ZIF-8)

crystals with an average diameter of 90.87 ± 14.55 nm, which is in agreement with the findings of Luanwuthi et al. (2015), who synthesized the hexagonal crystals with an average particle size of 50–100 nm. Gross et al. (2012) also found from analysis of SEM images that the synthesized ZIF-8 particles were 99 ± 29 nm in diameter. Hoop et al. (2018) reported that rhombic dodecahedral crystals of ZIF-8 had an average diameter of 1.1 µm. The ZIF-8 crystals with a square shape and size of about 0.4 µm were also synthesized by Fan et al. (2018). These conflicting findings are probably due to the different methods of synthesizing ZIF-8 crystals. The results of BET analysis, including the surface area, mean pore diameter, and pore volume of ZIF-8, were consistent with those reported in the literature (Pan et al. 2011; Gross et al. 2012; Nabipour et al. 2017), indicating that the ZIF-8 was well synthesized with high surface area (1587.9 $m^2\ g^{-1}$) and nanoscale pores (2.5 nm). Researchers are advised to perform extra analysis of the materials following exposure and interaction with the organisms under investigation in future studies. To conduct such analyses, special devices and techniques may be necessary, which would require additional research to be conducted.

Recent advances in ZIF-8 have been explored by Abdelhamid (2021) for a variety of biomedical applications, including cancer therapy, anti-microbial activity, biosensing, and biocatalysis. Despite the many applications that have been introduced for these materials, the available ecosafety information of ZIFs remains very scarce. Abd El-Aziz et al. (2022) explored the toxic effects of ZIF-8 on arthropods, which are commonly used to decompose carrion. In the present study, we explored the potential toxicity effects of different concentrations of synthesized ZIF-8 on microalgae *Nannochloropsis oculata* and brine shrimp *Artemia salina.* The algal cells exposed to 0–100 mg L^{-1} ZIF-8 showed an ascending growth trend during 72 h; however, the growth rate of algae decreased with increasing exposure concentration. When the concentration increased to 200 mg L^{-1}, ZIF-8 reduced the algal biomass. The inhibition of growth for average specific growth and yield increased from 5.37 to 100% and from 18.09 to 100%, respectively, with the elevation of exposure concentration from 1 to 200 mg L^{-1}. These results indicated that the concentrations of 1–200 mg L^{-1} nanoscale ZIF-8 inhibited the growth of algal cells. Based on these results, the higher concentrations of ZIF-8 showed more significant toxicity effect on *N. oculata*, such that, the IC_{50} values based on average specific growth rate and yield for *N. oculata* were 79.71 ± 8.55 and 51.73 ± 5.16 mg L^{-1}. Fan et al. (2018) found that inhibition of the growth of *M. aeruginosa* began with increasing ZIF-8 crystal with the size of 0.4 µm to 1 mg L^{-1}, and the inhibition rate increased with the elevation of ZIF-8 concentration. The inhibitory effects of different MOFs mainly depend on the kind of metal ions (e.g., Ag^+, Cu^{2+}, and Zn^{2+}) released from their structure into media and the sensitivity of algal cells to these metal ions (Miao et al. 2009; Liu et al. 2017; Fan et al. 2018). Ji et al. (2011) reported that the inhibitory effect of nano-ZnO on *Chlorella* is mainly attributed to Zn^{2+} ions. Also it has been shown that particulate pollutants often lead to changes in oxidative stress, suggesting it to be a significant source of toxicity (Jin et al. 2023). The generation of ROS, oxidative stress, physical damage of direct contact, and bioassimilation of particles are also responsible for the toxicity of ZnO nanoparticles in living organisms (Lin and Xing 2008; Hou et al. 2018, Sarkheil et al. 2018). The algal cells can effectively eliminate the accumulated ROS through the activity of antioxidant enzymes (Xia et al. 2006; Li et al. 2015). However, antioxidant enzymes cannot remove excess ROS when algal cells are exposed to specific concentrations of ZnNPs (Wong et al. 2010). The research conducted by Fan et al. (2018) revealed that the concentration of ROS in *M. aeruginosa* increased by a remarkable 63% when the Cu-MOF-74 concentration was raised to 1 mg L^{-1}. A recent study has uncovered that the suppression of arginine biosynthesis pathway is responsible for the generation of ROS and energy metabolism disruption, which in turn enhances the anti-bacterial (and probably anti-algal) properties of ZIF-8 NPs (Liu et al. 2023). In the present study, the reduction of algal biomass at a concentration of 200 mg L^{-1} ZIF-8 NPs may be related to the release of more Zn^{2+} ions and, or excessive ROS production and, subsequently, oxidative stress leading to cell death. While the present study did not investigate any of these cases, it is recommended that future research focuses on measuring the release of Zn^{2+} ions into the exposure media at various time intervals, as well as assessing the production of ROS. Zhang et al. (2021) showed that at a concentration range of 0.01–1 mg L^{-1}, the adverse effects of ZIF-8 on *Phaeodactylum tricornutum* are frail and easily mitigated compared with those of ZIF-67.

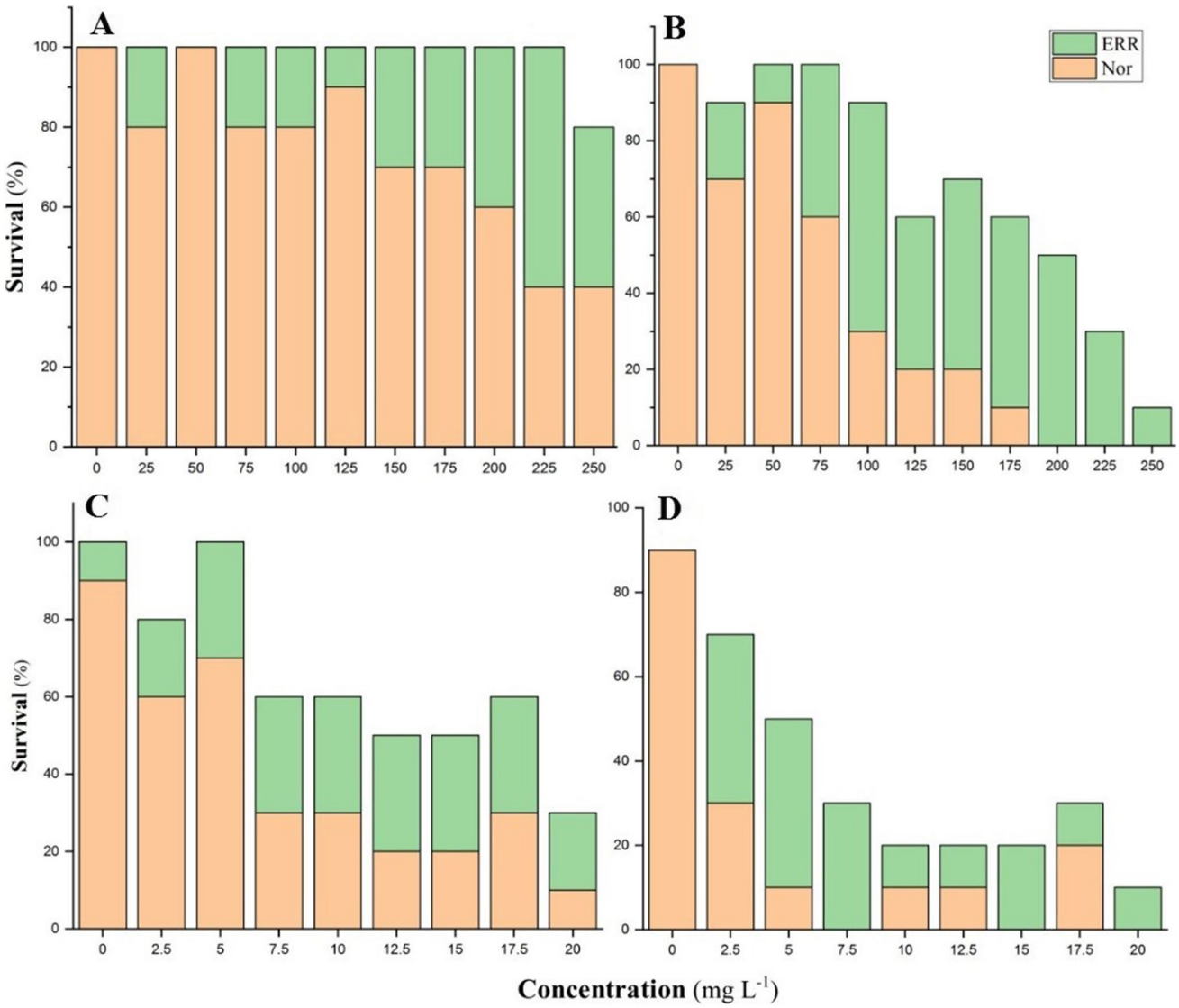

Fig. 5 The survival rate and swimming type of instar I (**A** and **B**) and II (**C** and **D**) of *Artemia salina* nauplii following 24-h (**A** and **C**) and 48-h (**B** and **D**) exposure to nanoscale zeolitic imidazolate framework 8 (ZIF-8)

To the best of the author's knowledge, there are very few studies on the effect of ZIFs on *Artemia*, and we found only three of them. Based on the results of Raju et al. (2020), the LC_{50} value of the CA@ZIF-L nanocomposite against *A. salina* nauplii was calculated to be 138.33 ± 3.72 mg L^{-1}. In another study by Raju and Natarajan (2023), the LC_{50} of fucoidan-loaded ZIF-L nanocomposite (FU@ZIF-L) was calculated at 108.43 ± 0.16 mg L^{-1}. In a separate study, the acute toxicity assay of *A. salina* revealed that the LC_{50} value of *Leucas aspera* extract-loaded ZIF-L nanoframeworks was 135.33 ± 2.00 mg L^{-1} (Raju et al. 2023).We should remind that, as stated in ISO TS 20787, the use of the term LC (lethal concentration), which is used in many similar articles on *Artemia* toxicity tests, does not seem to be correct, and the term EC (effective concentration) should be used instead because in this category of studies, it is not really death that is examined but immobilization. It should also be noted that, in none of these three studies, the life stage of nauplii was reported, and in addition, their ZIF type is different from the material used in the present study (ZIF-L vs. ZIF-8). Similar to what was shown in the present study, the higher sensitivity of the second instar compared to the first instar of *Artemia* nauplii has been previously reported in aquatic nanotoxicological studies (Zhu et al. 2017; Asadi Dokht Lish et al. 2019; de Paiva Pinheiro et al. 2023). This can be due to the closed alimentary tract in the first instar stage, which later opens in the instar II, and suspended particles can enter the body through the digestive tract and have a more significant impact.

According to the Globally Harmonized System of classification and labeling of chemicals (GHS 2011), any substance with a 72 h IC_{50} (for algae) or 48 h EC50 (for crustacea) of > 1 but ≤ 10 mg L^{-1} must be classified as "category acute 2" and any substance with a 72 h IC_{50} (for algae) or 48 h EC_{50} (for crustacea) of > 10 but ≤ 100 mg L^{-1} must be classified as "category acute 3." Therefore, according to the results, ZIF-8 NPs tested in the present study should be classified as "category acute 3" for *N. oculata* and instar I of *A. salina* as well as "category acute 2" for the instar II of *A. salina*. Moreover, based on the European Union Council Directive 67/548/EEC of 27 June 1967 (EC 1999) and European Union legislation (EC 2008), the corresponding values of "category acute 2" and "category acute 3" are equivalent to "toxic to aquatic organisms" and "harmful to aquatic organisms", both of which indicate that such substances "may cause long-term adverse effects in the aquatic environment."

It has been demonstrated that alterations in environmental conditions can significantly influence the toxicity of most pollutants (Fischer et al. 2013), yet there is a dearth of knowledge regarding the effect of environmental factors on the toxicity of MOFs. Consequently, further research is necessary in this area. Also, it has been shown that the physicochemical properties of MOFs are sensitive to environmental conditions, such as temperature and pH (Du et al. 2020) . Consequently, the stability of these materials in the exposure media should be taken into account, and it is advisable to conduct toxicological tests under various environmental conditions.

Future research on the ecotoxicology of MOFs, particularly ZIF-8, should focus on several key aspects to gain a better understanding of their environmental effects. Conducting acute and chronic toxicity tests (e.g., growth inhibition, reproductive impairment, and behavioral changes), determining the bioaccumulation potential and biomagnification through food chains, examining the influence of environmental factors such as pH, temperature, salinity, and presence of other contaminants on MOF stability, and devising strategies to minimize or mitigate any adverse effects associated with exposure to MOFs are all essential steps in the evaluation of MOFs.

Conclusion

This study was conducted to solve the lack of basic information about the toxicity of nanomaterials in saltwater ecosystems. Measured characterization confirmed the nanoscale size and nanoporous structure of synthesized ZIF-8. The results of toxicity experiments show that exposure to ZIF-8 NPs inhibits the growth of green microalgae (*N. oculata*) cells and decreases the survival of brine shrimp (*Artemia salina*) nauplii. Therefore, although from a technological point of view, zeolitic imidazolate frameworks have various positive practical features, the environmental safety of these materials should be given more attention by researchers and manufacturers, to use the necessary strategies to prevent unwanted pollution of aquatic environments. In the end, we recommend that the effect of ZIF-8 NPs toxicity on other aquatic organisms of different levels of the food chain be investigated in future studies and the mechanisms involved in their toxicity be identified.

Author contribution SAJ: conceptualization, funding acquisition, supervision, methodology, resources, project administration, validation, writing — original draft, and writing — review and editing; MBT: conceptualization, methodology, investigation, and formal analysis; ShV: investigation; MS: conceptualization, formal analysis, and writing — original draft.

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Declarations

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