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Toxicity Assessment of MXene in *Scenedesmus quadricauda* Physicochemical Parameters

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Abstract MXenes are a family of 2D materials, gaining popularity in various fields of life due to their potential applications. Toxicology study on MXene related to water systems with algae has not yet been conducted. Stress response of *S. quadricauda,* a reliable bioindicator for freshwater bodies in terms of changes in algal growth, photosynthetic pigments, protein thiol group, and morphology were monitored by varying MXene concentrations (0.0001–10 mg/l). The results revealed a relatively low toxicity on the algal specific growth rate (SGR) of up to 0.1 mg/l MXene, but at higher concentrations, the SGR observed was significantly higher. It is proposed that the reason for this anomaly is that MXene, like other 2D nanomaterials, tends to form agglomerates with algae in the media confirmed by microscopic images demonstrating agglomeration of coenobium. Changes in the protein thiol groups were stable, indicating moderately higher oxidative stress, but the decrease in photosynthetic pigment production was significant even at the lowest applied MXene (0.001 mg/l). The correlation matrix showed an insignificant MXene toxicity. Upon exposure to even the lowest MXene concentration 0.0001 mg/l, increase in cell size and flagella shortening was noted. Higher concentrations resulted in cell agglomeration, shading effect, cell damage, change in coenobium structure, and even cell death. It is presumed that this agglomeration was the main reason for MXene toxicity, inhibiting light and nutrients, decreasing the growth and pigments,

Statement of Environmental Implication MXene is an emerging 2D nanomaterial with various field applications, including water purification. For all we know ecotoxicological study of MXene in freshwater ecosystem is not yet reported apart from zebrafish embryo. As per OECD guidelines algae are a reliable tool for such toxicity assessments. We confirm in our study that due to shading effect of MXene, photosynthetic pigments decreased in *S. quadricauda* along with slight increased protein thiols content. Protein thiols are an acceptable parameter for indication of higher oxidative stress. For the stated reasons we believe this study will be a novel and interesting one for your journal.

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especially at higher concentrations. As algae and MXene are potential candidates for water bioremediation, making this toxicity assessment more relevant and needed.

Keywords MXene toxicity · 2D materials · Algal agglomeration · Oxidative stress

1 Introduction

Nanomaterials are a dynamic class of materials sought after for a variety of real-world applications. Based on the source used, nanomaterials can be divided into three types, such as carbon-based nanomaterials (graphene, carbon nanotubes, etc.), metal-based nanomaterials (nano-TiO_2 and nano-Al_2O_3, layered double hydroxides) and hybrid nanomaterials (MXene, transition-metal dichalcogenides) (Joudeh & Linke, 2022). In past decades, MXene has intrigued researchers due to its graphene-like characteristics and classification as a new type of 2D layered material. The first MXene ($Ti_3C_2T_x$) was discovered by the (Naguib et al., 2011) and symbolized MXene with the general formula $M_{n+1}X_nT_z$ (where n = 1, 2, 3), where M represents an early transition metal element, X represents carbon or nitrogen, and T_z represents functional groups such as -OH, -O or -F. The value originates from its exceptional qualities, including mechanical strength, surface area, and conductivity, which have gained importance in exploding them in a variety of sectors, including batteries, super capacitors, heavy metal adsorption, biomedical engineering, organic pollutant degradation, and cancer (Atri et al., 2024; Ganesan et al., 2022; Khatami & Iravani, 2021; Kusumkar et al., 2023; Yu et al., 2022). Among MXene materials, $Ti_3C_2T_x$ has been extensively researched in aquatic environmental remediation (Jatoi et al., 2023; Lv et al., 2024), and therefore, it is important to understand their toxicity to aquatic organisms, also for safe disposal of MXene into water bodies.

Environmental concern about the presence and disposal strategies of nanomaterials in natural systems, including aquatics, has increased in recent years (Ding et al., 2018). Chlorophytes have been widely used as model organisms in phytotoxicity assessments and are potential candidates for phytoremediation (Udaiyappan et al., 2017). Different species of *Scenedesmus* genus have been explored for phytoremediation to remove metals and pollutants from wastewater (Mondal et al., 2019). The members of the *Scenedesmus* genus usually form miniature colonies called coenobia or autocolonies. These coenobia consist of 4–8 cells connected in a row (Chia et al., 2015; Pickett-Heaps & Staehelin, 1975). The exact mechanism responsible for the decrease in coenobium size is unknown, but much research has shown that in stress conditions the ability of the genus *Scenedesmus* to form coenobium becomes compromised (Lombardi et al., 2007). This variation in the structure of coenobium can be used as a biomarker for environmental toxicity or stress conditions in water systems. Several studies on potential adverse effect to microalgae in water systems monitoring consider variations in cell growth, morphology, pigment production, oxidative stress, biomass production, and cell motility (Bere et al., 2012; Liu et al., 2015; Mohammed & Markert, 2006; O'Neill & Rowan, 2022; Pillai, 2010). Manufactured nanomaterials are capable of entering the algal cell causing subcellular damage; for example, reduced graphene oxide and multilayer graphene 2D nanomaterials in *Chlorella pyrenoidosa* destroyed the cell membrane and penetrated within the cell (Zhao et al., 2017). Nanomaterials are also capable of adsorbing in an algae cell, encapsulating them, and binding to the surface inducing physical stress (Middepogu et al., 2018; Wang et al., 2019). Several authors have discussed the adsorption of nanoparticles on the algal cell wall (shading effect), which can prevent their entry into the cell, thereby reducing toxic effects, but the adsorbed particles also reduce the availability of nutrients and light for algae. Adsorption depends mainly on the shape of the algal cell, the dose of nanoparticles, the physiochemical properties of the algae, and growth conditions.

Changes in the thiol group in stress state are a conventional and widely used parameter to conclude the toxicity of any material toward algal cells (Filová et al., 2021). Glutathione and phytochelatin are vital tools in algal cell detoxification against stress conditions. When this detoxifying capacity of the cell is depleted by radicals (e.g. reactive oxygen species, ROS), one of the first signs of the high oxidative stress is depletion of these thiol-rich molecules. These substances are included among thiols and their production was observed in our investigation by determining the level of reduced protein -SH groups.

da Costa et al. (2016) explained that the toxicity of nanoparticles is mainly exerted on algae by the metal ions released from them, which can cause serious oxidative stress (Da Costa et al., 2016). Mainly, toxicity depends on the particle size, the metal ions in it and the algal species used in the test (Ji et al., 2011), and manifests itself mostly as inhibition of photosynthesis, a barrier in the intake of nutrients, and increased production of ROS (Long et al., 2012). There are several reports in which nanoparticles, as Multi-walled carbon nanotubes, (MWCNT), Al_2O_3, TiO_2 nanotubes and layered double hydroxides induce stress effects on distinct algal species and resulted in decreased growth, oxidative stress, morphological alterations, agglomeration, and internalization (Zhang et al., 2015).

Since MXene can be potentially toxic to human cells, it is very crucial to access its toxicity before using it in multiple fields of life. In one of the in vitro studies with human cells Ti_3C_2 MXene (up to 500 mg/l) exhibited effects of oxidative stress in two normal cells lines, MRC-5 (fibroblasts) and HaCaT (keratinocyte cell line) and two cancerous cell lines A549 (alveolar basal epithelial cells) and A375 (melanoma cells). Human mesenchymal stem cells exposed to MXene up to 100 µg/ml showed significant decline in cellular proliferation (Jang & Lee, 2021). Wu et al. (2020) observed neural cell damage and even apoptosis at 25 µg/ml concentration of $Ti_3C_2T_x$ nanosheets. To date, there is only one study to assess MXene toxicity in aquatic systems conducted on zebrafish model (Nasrallah et al., 2018). $Ti_3C_2T_x$ was exposed to zebrafish embryos and a dose-dependent uptake was determined after a 96-h experiment. The EC50 value calculated from this study was 257.46 µg/ml. Up to 50 mg/l of $Ti_3C_2T_x$ no stress effect or mortality was observed in embryos. The application of MXene above 100 mg/l caused an increase in mortality rate and stress responses (heart edema, abnormal yolk size, neurotoxicity, and escoliosis). According to the Acute Toxicity Rating Scale of the Fish and Wildlife Service (FWS), MXene was placed in a practically nontoxic group of chemicals, and it was suggested that up to 100 mg/l of $Ti_3C_2T_x$ is safe to be disposed of in water systems. In a relevant study, modified $Ti_3C_2T_x$ with Au was used in zebrafish, reducing the toxicity in embryos. The observed LC50 was around 1 mg/ml with no teratogenic effects observed on the embryos (Hussein et al., 2019). Recently, Jakubczak et al. (2022) explored the incorporation of Nb-based MXenes with phytoremediation using the algae *P.* (or *Raphidocelis*) *subcapitata*. to track the bioremediation mechanism of algae (Jakubczak et al., 2022). The study concluded that there was no toxicity of MXene for green algae (Jakubczak et al., 2022).

As explained by Rasool et al. (2019), $Ti_3C_2T_x$ derived from Ti_3AC_2 (where A is Al, Ga, Si and Zn) has been extensively explored in water purification from organic pollutants, heavy metals, and others (Rasool et al., 2019). The increasing usage and potential application of MXene in environmental remediation calls for the need to address its environmental effects and toxicity assessment. However, the environmental impact of $Ti_3C_2T_x$ (MXene), especially its potential risks to aquatic biota and ecosystems, has never been explored using the primary autotrophic model organism, algae.

Microalgae are the most sensitive autotrophic part of the aquatic food chain, making them a reliable bioindicator for aquatic toxicological investigations. The safety assessment of MXene that will be disposed of in water systems or used in water purification was the main objective of this research. Therefore, we tested the effect of MXene on the growth, photosynthetic pigment content (chlorophyll a and chlorophyll b), and protein thiol groups of *S. quadricauda* together with microscopic observations to determine whether some morphological changes will relate to the use of this new promising chemical for cleaning or decontamination of the environment, especially aquatic bodies.

2 Materials and Methods

2.1 Preparation of MXene Material

MXene ($Ti_3C_2T_x$) was prepared using the acid-salt etching route as previously reported procedure (Kusumkar et al., 2023). For this, 2.94 g of ammonium fluoride (NH_4F, Merck, Germany ˃98.0%) was added to a plastic bottle containing 20 ml hydrochloric acid (HCl, Merck, 37.0–38.0%) and dispersed by ultrasonication for 15 min. Subsequently, 0.5 g of titanium aluminium carbide (Ti_3AlC_2, Merck, Germany ˃90.0%, 40 µm mesh size) was added slowly to the acid-salt reaction mixture, placed in an ice bath to avoid exothermic reactions. The reaction mixture was

then kept on a magnetic stirrer for 48 h at 25 ^{0}C, and the solution was decanted followed by washing with 2 mol/l HCl for 15 min to remove additional impurities. Furthermore, the obtained product was washed several times with distilled water to achieve a natural pH, and then dried using a vacuum-assisted filtration system.

The purity of the MXene was identified using a PANalytical X-ray diffractometer equipped with CuKα radiation in a 2θ range of 3–65^{0}. Morphological and structural studies were carried out using scanning electron microscopy (SEM, Tescan Lyra III), transmission electron microscopy (TEM, JEOL JEM ARM 200 cF), including high-resolution transmission electron microscopy (HRTEM), and analysis of selected area electron diffraction patterns (SAED). The surface chemistry of the MXene was manifested using X-ray photoelectron spectroscopy (XPS, Omicron multiprobe system) with hemispherical mirror analysers and monochromatic Al Kα radiation.

2.2 Management of Algae Cultures and Composition of Test Medium

In this investigation, the green freshwater alga *Scenedesmus quadricauda* (Turpin) Brébisson Greifswald/15 from the collection of the Institute of Botany of the Czech Academy of Sciences in Třeboň (Czech Republic) was used. The algae stock culture was cultivated in modified Bristol modif. Bold Medium (BBM) (Bischoff, 1963; Bold, 1949). The test medium was prepared according to (STN EN ISO 8692, 2012) and the OECD norm (OECD, 2011) and is mentioned as the STN medium. The management was the same as explained by Filová et al. (2021). The culture STN medium comprises of macronutrients—$NaHCO_3$: 595.17 µmol/l (50 mg/l), NH_4Cl: 280.37 µmol/l (15 mg/l), $MgCl_2.6H_2O$: 59.02 µmol/l (12 mg/l), $CaCl_2.2H_2O$: 122.38 µmol/l (18 mg/l), $MgSO_4.7H_2O$: 60.85 µmol/l (15 mg/l), K_2HPO_4: 9.16 µmol/l (1.6 mg/l); and micronutrients—$FeCl_3.6H_2O$: 0.30 µmol/l (0.08 mg/l), $Na_2EDTA.2H_2O$: 0.27 µmol/l (0.1 mg/l), H_3BO_3: 2.99 µmol/l (0.185 mg/l), $MnCl_2.4H_2O$: 2.10 µmol/l (0.415 mg/l), $ZnCl_2$: 0.02 µmol/l (3×10^{-3} mg/l), $CoCl_2.6H_2O$: 0.01 µmol/l (1.5×10^{-3} mg/l), $CuCl_2.2H_2O$: 6×10^{-5} µmol/l (1×10^{-5} mg/l), $Na_2MoO_4.2H_2O$: 0.03 µmol/l (7×10^{-3} mg/l). K_2HPO_4 is from Centralchem (Slovak Republic); $FeCl_3.6H_2O$ is from Slavus Ltd. (Slovak Republic); all other chemicals were purchased from Lachema-Chemapol (Czech Republic). The *S. quadricauda* culture was kept for 72 h under constant conditions, with illumination 1700 lx (22.1 µmol s^{-1} m^{-2}), temperature 24 ± 1 °C and light/dark period of 18/6 h (STN EN ISO 8692, 2012). The flasks were shaking during the whole experimental time (Biosan Multi Shaker PSU 20, Riga, Latvia).

All concentrations were tested in three parallels and the experiment period was 72 h (3 days). At the start and end of each experiment, the pH was recorded to monitor any pH variation. The pH ranged from 7.9 ± 0.40. *S. quadricauda,* calibration curve for the number of ceonobia (cells) was prepared from dependence of the number of cells in the Bürker chamber at different dilutions and spectrophotometrically at 750 nm against distilled water. The test alga was inoculated into the 50 ml Erlenmeyer flask containing 2.10^4 coenobia/1 ml of *S. quadricauda* in cultivation medium. MXene was tested at concentrations of 0.0001, 0.001, 0.01, 0.1, 1 and 10 mg/l after preparing stock solutions in distilled and sterilised water.

2.3 Measurement of the Algal Specific Growth Rate (SGR) Parameter

During the experiment, algal growth was measured spectrophotometrically (UV-1600PC, VWR, China) on a first and last day at the wavelength of 750 nm against distilled water (Bolier and Donze, 1989). After determining the number of algae cells for *S. quadricauda, the* specific growth rate (SGR) was calculated using the following equation (STN EN ISO 8692, 2012);

$$\mu(hours) = \frac{\ln\left(N_L\right) - \ln\left(N_0\right)}{t_L - t_0} \quad (1)$$

here, μ = SGR is the specific growth rate (h^{-1}), N is the number of algal cells at time L of the test, N_0 is the number of algal cells at time 0, t_L is the time of the end of the experiment (72 h) and t_0 the time at the start of the experiment.

Afterwards, the SGR was calculated using the equation (Filova et al., 2021).

$$I(SGR - Tm) = 100 \times \frac{SGR(control) - SGR(Tm)}{SGR(control)} \quad (2)$$

Here, *I* is the inhibition of SGR (in %), *SGR* is the specific growth rate, and *Tm* is the tested concentration of MXene.

2.4 Photosynthetic Pigment (PP) Determination

The algae suspension (5 ml) was removed from each test sample and placed in plastic test tubes. The tubes were centrifuged for 15 min at 2,900 g. The supernatant was removed and 3 ml of 95% denatured ethanol with glass beads were added. The test tube was vortexed to disrupt the cells and release their chlorophyll content into the added ethanol. After the vortex, the plastic caps were placed on the test tubes to prevent the ethanol from evaporating. The test tubes were placed in dark conditions for 30 min. The test tubes after 30 min were vortexed and then centrifuged for 5 min at 2,900 g. The supernatant was removed into clean tubes. The algal homogenate was prepared for further measurements.

The content of photosynthetic pigments (chlorophyll a, chlorophyll b) was measured spectrophotometrically at 649 and 665 nm, where 95% ethanol was used as a reference (Filová et al., 2021; Lichtenthaler & Wellburn, 1983). Pigment concentrations were calculated with the following equations by Fargašová (Fargasova, 1999; Fargašová, 2001):

$$\text{chlorophyll a} = \left(13.7 \times A_{665}\right) - \left(5.76 \times A_{649}\right) \quad (3)$$

$$\text{chlorophyll b} = \left(25.8 \times A_{649}\right) - \left(7.60 \times A_{665}\right) \quad (4)$$

The values obtained were displayed and compared with the control and statistically evaluated using the unpaired two-tailed Student's *t*-test in MS Excel (Microsoft Corporation, USA).

2.5 Protein thiol (-SH) Group Determination

Protein thiol groups (-SH) act as parameter of protein oxidation during increased oxidative stress in *S. quadricauda* here because higher level of –SH are indicative of higher level of oxidation stress in algae (Nowicka, 2022). There are peptides as reduced glutathione (GSH) among the protein thiol groups and in plants are common spread also phytochelatins, and this parameter is obviously changed during higher oxidative stress because protein thiol groups play an important role as antioxidants in the cell against any stress condition (Halliwell, 2006). This parameter is not under the recommendations of standardized tests of OECD and ISO norms and is novelty of our research (STN EN ISO 8692, 2012; OECD, 2011). For protein thiol group changes in algal samples, an ethanol algal homogenate of 0.5 ml with 0.1 ml of 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) solution from Sigma-Aldrich, Germany (20 mmol dm^{-3}) solution was measured spectrophotometrically at a wavelength of 412 nm against 95% ethanol. The samples after the vortex were placed in the dark for 45 min at laboratory temperature before spectrophotometric measurement. The protein thiol (-SH) group concentration was calculated using the following equation (Ellman et al., 1961; Viner et al., 1997):

$$\varepsilon(-\mathrm{SH}) = 13{,}600\ \left(mol.L^{-1}\right)^{-1} x(0.5\ cm)^{-1}$$

$$c(-SH)\left(mol.L^{-1}\right) = \frac{A(412\,nm)}{13600\left(mol.L^{-1}\right)^{-1} x(1cm)^{-1} x0.5\ cm} x \frac{V(hom)(L)}{V(reaction\ mixture)(L)} x1000 \quad (5)$$

where ε is the extinction coefficient, V(hom.) is the volume of the algal homogenate and V(reaction mixture) is the final volume.

2.6 Microscopic Analysis

A microscopic study under the Leica DM2500 LED (Leica Microsystems, Germany) was also conducted to determine the morphological implications induced in *S. quadricauda* cells at different concentrations of applied MXene on the last day of the experiment. Evans blue cell dye (Thermo-Fisher, Slovakia) was used for the microscopic examination (Vijayaraghavareddy et al., 2017). From the algal samples, 1 ml was taken in an Eppendorf tube with 50 µl Evans blue, prepared as 0.025 g Evans blue in 10 ml 0.1 mol/l $CaCl_2$ (pH 5.6). Tubes were centrifuged for 20 min, and the supernatant was removed using a pipette. With the help of the pipette, the algal pallets were washed 3–5 times with distilled water to

remove excess dye. The samples were placed on a clean glass slide for microscopic analysis using oil immersion differential interference contrast (DIC) microscopy.

2.7 Statistical Analysis

Statistical significance was calculated using of unpaired two-tailed Student's *t*-test in MS Excel 2016 (Microsoft Corporation, USA) for the groups tested groups against the control. Statistical significance at the level of $^{*}p<0.05$; $^{**}p<0.01$; and $^{***}p<0.001$ and evaluated for the graphs, where the mean with their mean standard deviation (SD) is shown. Correlation analyses among all parameters were estimated in Jamovi v. 2.3.18 (The Netherlands; https://www.jamovi.org/).

3 Results and Discussion

Figure 1a shows the appearance of sharp and intense *00 l* reflections at lower angle in the PXRD pattern of the product obtained after acid-salt etching treatment. These observations are consistent with the reported literature and confirmed monophasic formation of MXene (Kusumkar et al., 2023). SEM images demonstrated clear evidence for the stabilization of multilayered MXene (Fig. 1b). Low magnification TEM image showed the plan-view of microscale multilayered bulk structure and SAED pattern acquired from the selected area revealed single crystalline nature of MXene (Figure, 1c, d). Bright spot in SAED pattern suggested hexagonal motif, which is oriented along [0001] direction. Indeed, the presence of functional groups such as C-Ti-O_x, C-Ti-F_x, C-Ti-OH_x on the surface of MXene was evidenced by their surface study based on XPS measurements (Figure S1) (Kusumkar et al., 2023).

The specific growth rate of *S. quadricauda* was observed to be not much influenced by the applied MXene concentrations (0.0001–0.1 mg/l). Higher values of applied MXene 1 and 10 mg/l slightly, but not significantly, increased algae SGR.

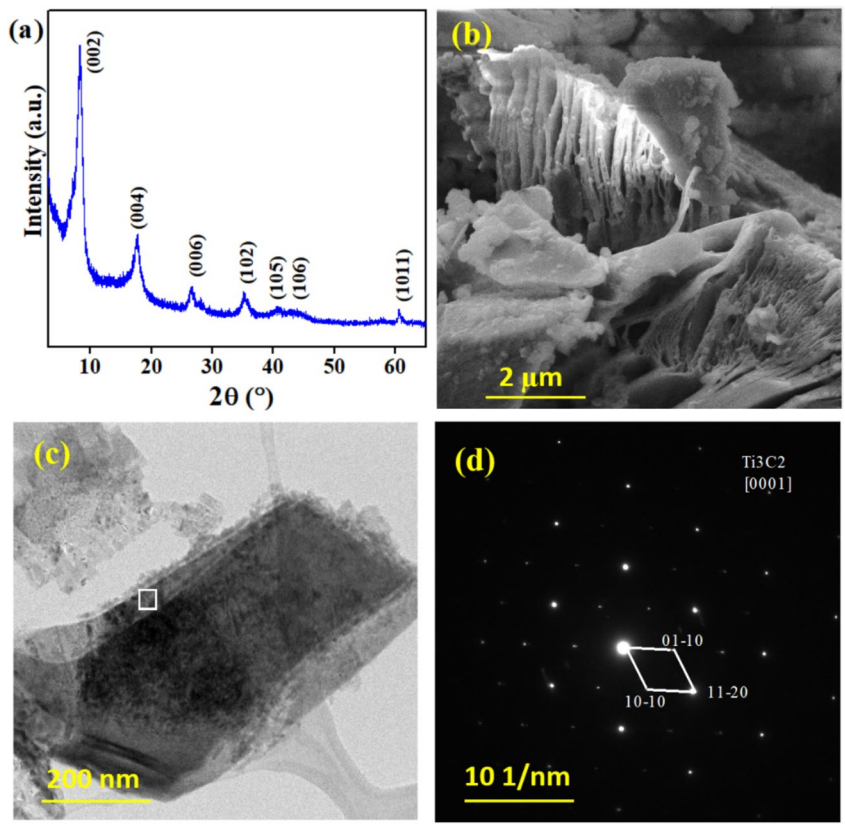

Fig. 1 L(**a**) XRD pattern of $Ti_3C_2T_x$ obtained by salt-acid etching of the Ti_3AlC_2 MAX. (**b**) SEM, (**c**) TEM image and (**d**) SAED pattern of $Ti_3C_2T_x$

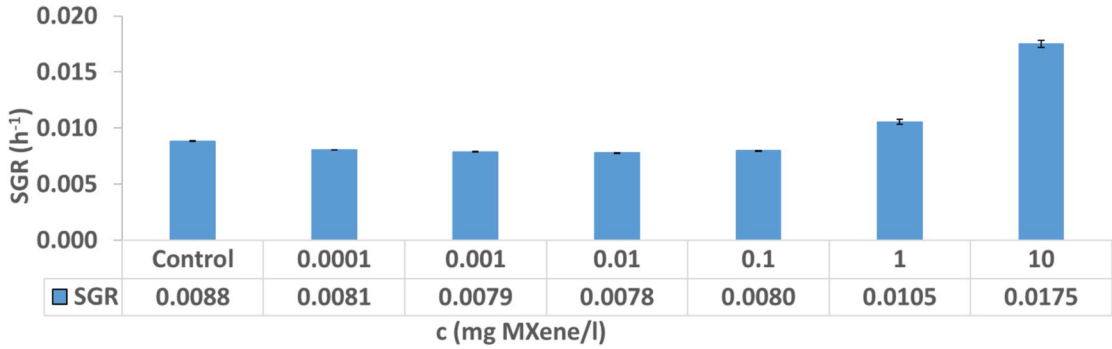

Fig. 2 Dependence of the specific growth rate (SGR) of *S. quadricauda* after 72 h in the presence of different concentrations of MXene

The values in Fig. 2 indicate a relatively low toxicity of the applied MXene to the studied algae. However, the growth tends to non-significantly decrease with applied MXene up to 0.1 mg/l. indicated the interaction of MXene with the algal media, which can be a possible explanation for high spectrophotometric determination of SGR for 1 and 10 mg/l MXene concentrations. Figure 3 demonstrated that MXene is forming agglomerates with *S. quadricauda* and resulting in clumps (Fig. 3c) as a well as prolonged and enlarged ceonobia of *S. quadricauda* compared to control cells with smaller flagella (Fig. 3a, b) that hinder cell motility. Evans blue dye is an indicator of dead cells, and we observed them in agglomerates dyed in blue (Fig. 3c) and separately as coenobium (Fig. 3d). Since MXene contains Ti ions that could interact with the lipopolysaccharide layer on the cell membrane of algae and could play some role in the aggregation of algae into larger agglomerates (Fig. 3c). The interaction of MXene with STN media makes it less suitable for algae growth.

MXene significantly decrease both chlorophylls level if its concentration was higher than 0.01 mg/l (Fig. 4). As seen in Fig. 3c, the agglomeration of MXene with algae cultured in STN medium was significant and could interfere with both algal growth and photosynthetic pigment production due to a reduction in the amount of light available for photosynthesis. Based on this, it can be assumed that smaller MXene particles became part of agglomerates, in which they remained attached even after centrifugation of the samples to determine the production of pigments and SGR. Their binding to agglomerates also reduced the growth rate and pigments production in algae at higher concentration of MXene (1 and 10 mg/l).

The results of the evaluation of the production of photosynthetic pigments shown in Fig. 4 show that the substance tested reduced the production of chlorophyll a more than chlorophyll b. The intensity of the anti-inflammatory effect increased with increasing concentration of MXene. Therefore, the comparison between their relations as sum (a + b) and ratio (a/b) was calculated in Fig. 5.

Many previous studies with algae, especially *S. quadricauda*, have explained the inhibition of growth and pigments'content in the presence of various toxins, such as: toxic metals, e.g. Cd (up to 0.5 mg/l), Cu (up to 2 mg/l) and Pb (up to 30 mg/l) (Mohammed & Markert, 2006), organic pollutants such as imidazolium chloride ionic liquids (Liu et al., 2015), nanoparticles such as Ag^+ with 0.2 nm particle size (Zouzelka et al., 2016), chemicals such as triphenyltin (TPT) up to 0.064 mg/l (Xu et al., 2011), plastics such as polyurethane, polypropylene and high-density polyethylene up to 3 mg/l (Lazareva et al., 2021). As for MXene, its toxicity to algae has not yet been evaluated. Our study confirmed that MXene inhibits both the growth and the production of photosynthetic pigments in algae, similar to other toxicants (Fig. 3, 4).

The decrease in the level of pigments in algae was more pronounced than the inhibition of their growth, especially at higher concentrations of MXene (1 and 10 mg/l). Therefore, it can be concluded that the nature of the effect of MXene can be compared to the effect of the concentration of other metals on the growth and production of pigments in algae (Čypaitė et al., 2014).The results showed that, unlike most

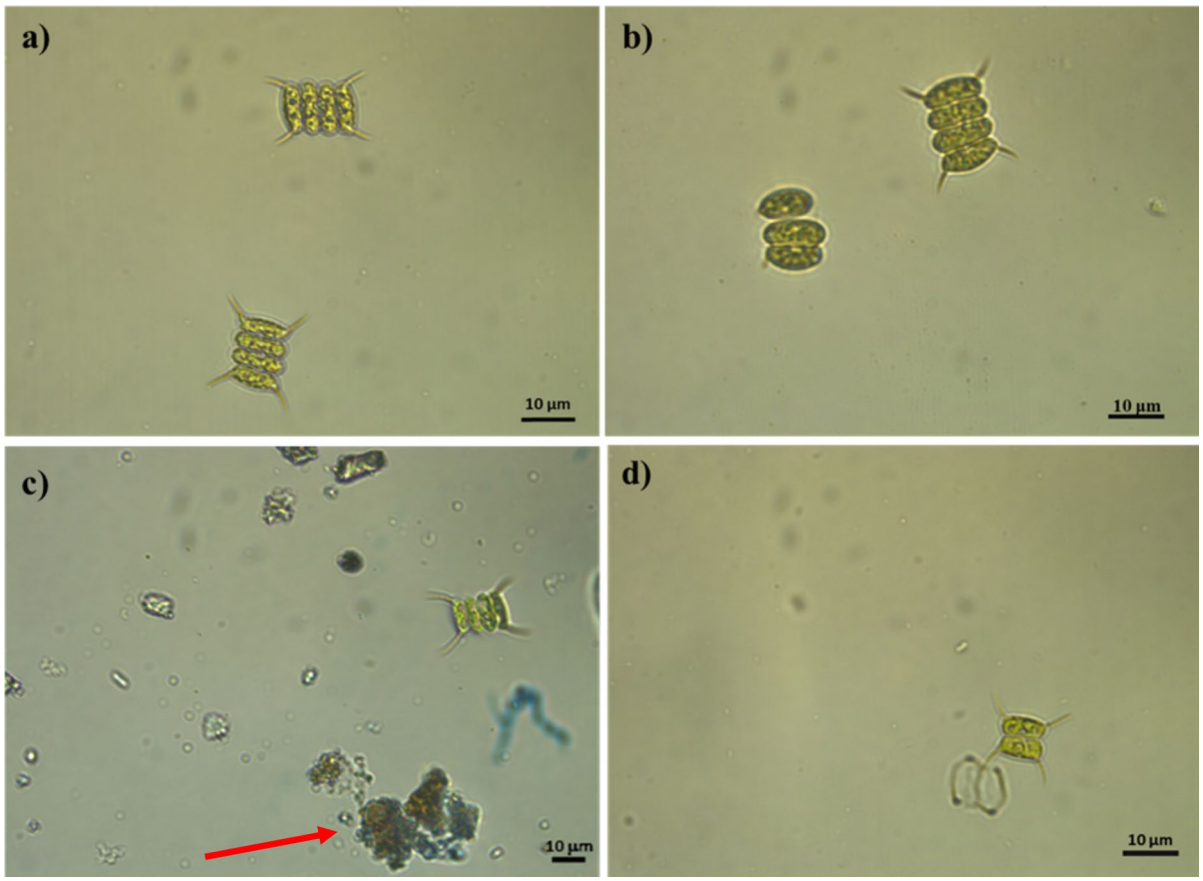

Fig. 3 Morphological changes in *S. quadricauda* after 72 h after exposure to different concentrations of MXene. Evans blue was used for staining and observation were made by DIC microscopy. **A**) Control; **b**) 0.0001 mg/l MXene; **c**) 1 mg/l MXene; **d**) 10 mg/l MXene. The magnification used was 100 × for a), **b**) and **d**), and 63 × for **c**). The red arrow shows the aggregation of algae cells in the presence of MXene

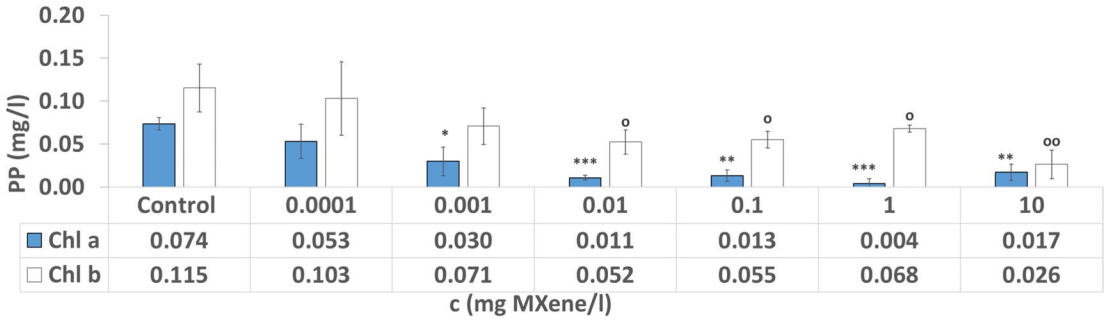

Fig. 4 Photosynthetic pigments content (PP) in *S. quadricauda* after 72 h of exposure to MXene (n = 3). Legend: Chl a – chlorophyll a, Chl b – chlorophyll b. The arithmetic averages with their standard deviations (SD) are shown in the graph. Statistical significance was evaluated using a two-tailed Student's *t*-test, where *$p < 0.05$; **$p < 0.01$ and ***$p < 0.001$ is compared to the control. The asterisk (*) is for Chl a and the circle (o) is for Chl b

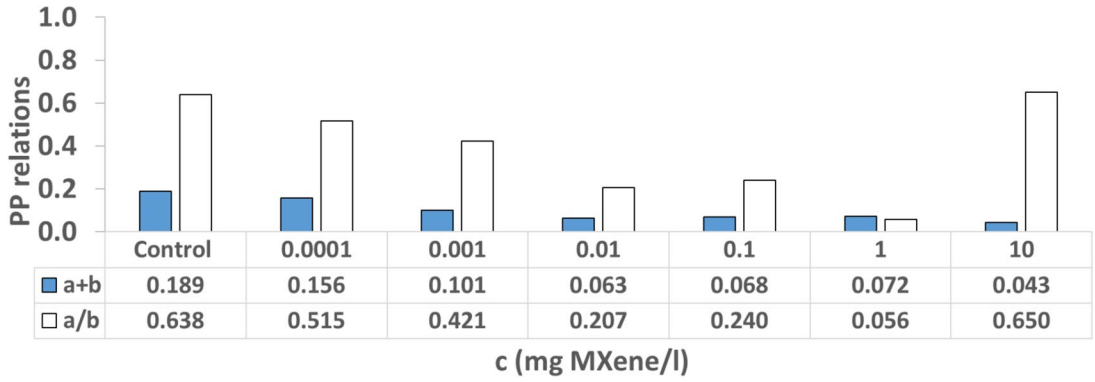

Fig. 5 Photosynthetic pigments (PP) relations in *S. quadricauda* after 72 h of exposure to MXene (n = 3)

metals, MXene inhibits the production of Chl a more than Chl b, which is the dominant photosynthetic pigment (Aggarwal et al., 2012).

The growth inhibition and significant photosynthetic pigment production decrease seen in *S. quadricauda* (Fig. 3,4) could be due to the interference of Ti present in the MXene used. As nanomaterials are capable of releasing heavy metal ions from them and these metal ions can cause toxicity to living cells (Da Costa et al., 2016). Ti can exhibit ionic forms Ti^{4+}, Ti^{3+} and Ti^{2+} which are capable of causing damage to living cells (Prokopiuk et al., 2023) including algae as reported by Fu et al. (2015) we found that metal nanoparticles after a 96-h exposure increase the level of ROS, oxidative stress, and the formation of agglomerates in the alga *P. subcapitata* and lead to a decrease in growth. In the alga *P. subcapitata*, the toxic effect of modified Al_2O_3, SiO_2 and TiO_2 nanoparticles is also confirmed (Metzler et al., 2012). The observed toxic trend was $TiO_2 > Al_2O_3 > SiO_2$. The high toxicity of TiO_2 nanoparticles may be a consequence of the aggregation of nanoparticles with the surface of algae cells, which we also observed in our experiments.

Change in protein thiol groups is a reliable parameter of algal cell toxicity toward any pollutant or toxin. In this context, in the presence of MXene a statistically significant increase was observed in the concentrations of the protein thiol group (Fig. 6). There are several studies using animal and bacterial cells (Jastrzębska et al., 2020; Rozmysłowska-Wojciechowska et al., 2020; Szuplewska et al., 2022), which explained MXene toxicity as increased cellular oxidative stress. In our study a significant increase in the protein thiol (–SH) groups was also manifested already at the lowest concentration applied to MXene (Fig. 6). The thiol content increased with increasing concentration of MXene,

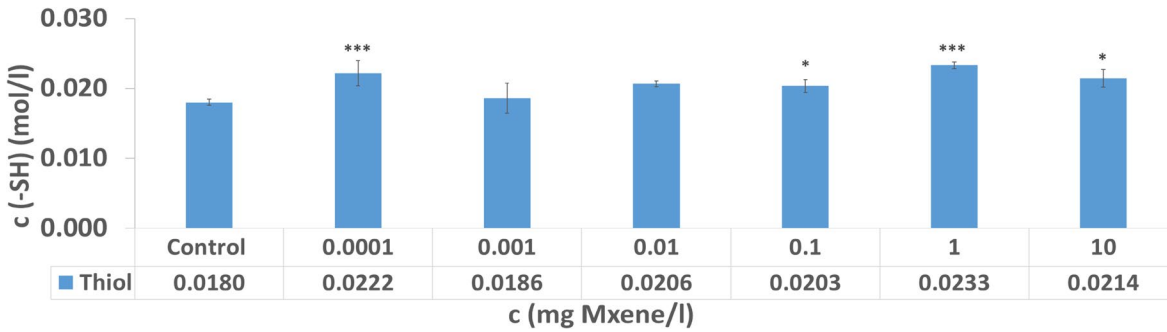

Fig. 6 The effect of MXene on protein thiol (–SH) groups of *S. quadricauda* after 72 h of exposure. The arithmetic means with their standard deviation (SD) (n ≥ 2) are shown on the graph. Statistical significance was evaluated using a two-tailed Student's *t*-test, where *p < 0.05 and ***p < 0.001 is compared to the control

indicating increased oxidative stress. An increase in oxidative stress and thiol groups has been reported in various types of algae in the presence of metals (Kaplan et al., 1995; Suárez et al., 2010).

The current study showed an agglomeration, a change in cell morphology, a loss of coenobium structure, a decrease in flagella cells, and even cell death (Fig. 3b, c, d). Evans blue staining showed that, at a low concentration of MXene, there is a change in the morphology of *S. quadricauda* cells, which is reflected in the shortening of the flagella and the enlargement of the cells. The same observations were introduced for *Scenedesmus obliquus* in the presence of TiO_2 (Dalai et al., 2013). At higher concentrations, MXene with STN medium and/or algae produced agglomerates, the formation of which in the presence of nanoparticles is reported by several authors (Karwowska et al., 2012; Long et al., 2012; Zhang et al., 2015). The highest concentration of MXene caused the death of algae cells (Fig. 3d). Based on the results reported by Love et al. (2012), it can be concluded that agglomeration of MXene with algae in the STN medium may be a reason of comparatively low toxicity effects on growth inhibition and thiol production, but significantly adverse on photosynthetic pigments'content as the agglomerates dispersed in the medium reduce the access of light and or nutrients to the algae cells. Although the observation of changes in the morphology of algae exposed to nanoparticles is common, quantification of the effect of agglomeration on algae is problematic. During aglomeration, the cell wall is damaged, which is then reflected in the increased intake of nanoparticles or metals released from them (Karwowska et al., 2012; Love et al., 2012; Zhao et al., 2016). All this causes stress in algal cells.

The high optical density of MXene is responsible for blocking the access of light to algal cells (Long et al., 2012), leading to a reduction in their ability to capture light for photosynthesis and consequently to a reduction in growth and pigment production (Fig. 3). Correlation analysis showed that the chemical did not statistically influence the parameters evaluated (Table 1). This indicates the suitability of using lower concentrations of MXene for the bioremediation of the aquatic environment, as the safety limits for aquatic organisms will not be exceeded. No statistical dependence was confirmed in the correlation matrix in Table 1. However, we observed growth (Fig. 2) and photosynthetic pigments' content (Fig. 4) inhibition together with protein thiol groups (Fig. 6) and morphological changes (Fig. 3) in the tested algae that was concentration dependent of MXene. Many authors observed similar changes and inhibitions in the studied parameters in the presence of different nanomaterials (Karwowska et al., 2012; Metzler et al., 2012; Mohammed & Markert, 2006).

Table 1 MXene correlation matrix for the observed parameters with the Spearman correlation coefficient *r* and the statistical significance p compared to the control (* for $p<0.05$; ** for $p<0.01$; *** for $p<0.001$). Legend: chl a, chlorophyll a; chl b – chlorophyll b; –SH, protein thiol groups; SGR – specific growth rate

MXene	SGR	Chl a	Chl b	–SH
SGR	-			
Chl a	−0.169	-		
Chl b	−0.204	0.329	-	
–SH	0.278	−0.353	−0.014	-

For comparison, the results we obtained show (Fig. 2) that the interaction of TiO_2-nanomaterial MXene with the alga *S. quadricauda* is intense as also reported by Arze et al. (2019) for the alga *P. subcapitata* against CeO_2 and TiO_2 nanomaterials.TiO_2-nanomaterial displayed greater interaction towards *P. subcapitata* than CeO_2. Our microscopic observations confirmed the physical attachment of the TiO_2-based nanomaterial (MXene) to algal cells that other authors observed too (Middepogu et al., 2018; Wang et al., 2019). Subsequently, the created agglomeration (Fig. 3) inhibited the supply of light and nutrients to algal cells, which was reflected in the inhibition of monitored parameters and increased stress factors in our study. Similar conclusions regarding the toxicity of TiO_2 anatase nanoparticles, titania (3–196 mg/l with 21 nm particle size) such as growth inhibition and chlorophyll decline on *Scenedesmus* sp. and *Chlorella* sp. were also declared by Sadiq et al. (2011).

MXene has been used for water purification in recent decades. The ability of MXene and its modified versions to absorb heavy metals is well documented (Gan et al., 2020). To further use MXene for water remediation, it is essential to study its potential toxicity on aquatic species, especially in a reliable bioindicator such are algae. Furthermore, the newly obtained ecotoxicological results presented by us can be used in further analyses of the safe use of MXene for water purification in real time.

4 Conclusion

In many freshwater ecotoxicological assessments, *S. quadricauda* has been used as a reliable bioindicator, and therefore we also used it in our tests to evaluate the impact of nanomaterials called MXene on freshwater biota. Parameters such as inhibition of growth, photosynthetic pigments content, changes in protein thiol groups production and morphological impairments were used to understand toxicity of MXene applied in concentrations 0.0001–10 mg/l. The decrease in growth and microscopic images taken after Evans blue staining show the ability of MXene to form agglomerates in the algal STN medium, which prevent the access of light and nutrients necessary for photosynthesis and growth. The intensity of the adverse effect increased with the concentration of the tested nanomaterial. In addition to growth, MXene significantly inhibited the production of Chl a and Chl b already at a low concentration (0.001 mg/l), and at a concentration of 10 mg/l, a change in the structure of coenobia, flagella shortening, change in size and cell death was observed using Evans blue dye. Although MXene is widely used for water purification and bioremediation, its toxicity to aquatic algae has only been observed very sporadically. The study presented by us points out, depending on the concentration used, the potential toxicity of this product to algae, as it induces oxidative stress in them and disrupts their basic production and biochemical parameters. However, the correlation matrix shows that lower concentrations of MXene may not show toxicity and can be a suitable and safe material used in water purification and remediation.

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Data Availability Data will be made available upon request.

Declarations

Conflict of interest There the authors declare that they have no conflict of interest.

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