Effects of Iron-Oxide Nanoparticles (Fe_3O_4) Released From Synthesized Iron-based Thiourea Catalyst on the Growth, Cell Density, and Pigment Content of *Chlorella Vulgaris*

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Abstract

This study investigated the effects of Fe_3O_4 nanoparticles released from synthesized Thiourea catalyst to *Chlorella vulgaris* as an essential primary producer in aquatic systems. A range of Fe_3O_4 concentrations (0, 10, 100, 250, 500, 750, and 1000 mg L^{-1}) was applied for the exposure test. Biological parameters of *C. vulgaris*, including cell density, cell viability, and pigment content were assessed. Bioconcentration factor and bioaccumulation were evaluated for contaminated microalgae. Non-carcinogenic risks were then assessed using target hazard quotient (THQ) for potential human consumptions. Findings showed that *C. vulgaris* cell numbers increased from 0 to 500 mg L^{-1} of Fe_3O_4 . Chlorophyll a represented a time-dependent response, and greatest values were detected in 250 and 500 mg L^{-1} Fe_3O_4 at 4.2 and 4 mg/g , respectively. Chlorophyll b content showed a time-related manner in exposure to Fe_3O_4 with the highest values recorded at 250 mg L^{-1} after 96 h. Moreover, bioaccumulation displayed a dose-dependent response as bioaccumulated iron was in the largest amount at 15000 $\mu\text{g/g dw}$ in 1000 mg L^{-1} , whereas the lowest one was in the control group at 1700 $\mu\text{g/g dw}$. The bioconcentration factor showed a concentration-relevant decrease in all iron treatments and 10 mg L^{-1} of Fe_3O_4 represented the greatest BCF at 327.3611. Non-carcinogenic risks illustrated negligible hazard ($\text{THQ} < 1$) in a dose-response pattern and the largest EDI and THQ were calculated in 1000 mg L^{-1} at $7.4332\text{E-}07$ ($\text{mg kg}^{-1} \text{day}^{-1}$) and $1.06189\text{E-}09$, respectively. In essence, iron is an essential trace element for biological aspects in aquatic systems, but in exceeding concentrations could impose toxicity effects in *C. vulgaris* populations.

1. Introduction

Organocatalysts have received a great deal of attention over the last decade due to their low cost, availability, high yield, short reaction time, simplicity of product isolation, clean reaction profile, environmental benignity, recyclability, and reusability [1]. Having a strong hydrogen bonding capability, organic-based catalysts can significantly promote chemical reactions in environmental studies [2]. Urea has been recently applied as an organic base for organocatalysts used in wastewater management and air pollution to remove heavy metals [3]. Thiourea catalyst is generated from magnetic nanoparticles because of their high surface area, low toxicity and superparamagnetic behavior, and potential applications in many fields [4]. Many metals have been used as the metallic base of Thiourea catalyst, including Al, Fe, Cu, Si, and Zn; however, functionalized iron nanoparticles (Fe_3O_4) are more interesting due to their easily separable, reusable, non-toxic, low cost, and flexible design [5]. In organic chemistry, Fe_3O_4 catalyst coated with thiourea is used for the Knoevenagel reaction where formation of C-C double bonds and synthesis of α , β -unsaturated carbonyl compounds from active methylene and carbonyl compounds may occur [7]. Knoevenagel reaction is mainly used in the chemical processes to mitigate the human interference and secure operators from dangerous and carcinogenic solvents [6]. Fe_3O_4 nanomaterials play an essential role as a functionalized base for Thiourea catalyst, and is greatly used in such reactions in numerous industrial activities [8]. Such iron-based catalyst can release into the aquatic ecosystems and affect the organisms like microalgae as primary producers in these environments [9].

The environmental concentration of iron-based materials has been increased in the biota, including soil, air, and water [10]. Literature have reported the fate, biotransformation, bioaccumulation, and toxicity of such elements in various ecosystems [11, 12]. In particular, aquatic environments are the most important destinations for iron-based compounds through wastewater drainage and landfills in populated areas. Fe_3O_4 , in general, possess various behaviors when enter to the water systems, and undergo some processes, including aggregation, dissolution, redox reaction, and interaction with potential macromolecules [13]. These iron-based products can act as the best iron resource for microalgae, and in highly-contaminated systems lead to eutrophication and algal bloom [14]. Furthermore, iron-based materials in overloaded-conditions cause bioaccumulation and bioconcentration in the aquatic species, and thus, oxidative stress could result in through reactive oxygen species (ROS) production [15]. Aquatic flora and fauna are more sensitive to toxic materials compared with terrestrial species, and related compounds of iron can easily pass the cell membrane and cause severe damages to enzymes, proteins, and DNA integrity of organisms[16]. Lei et al. (2016) examined the toxicity of iron-based NPs to green algae in terms of fate, particle size, and oxidation effects [17]. *Chlorella vulgaris* (*C. vulgaris*) was used as an aquatic biological model to investigate iron-based oxidative stress [18]. The toxicity effects of iron oxide nanoparticles were assessed using Zebrafish (*Danio rerio*) in early life stages, that mortality, hatching delay, and malformation occurred in exposing to these nanomaterials [19]. The toxicity of nanoparticles to aquatic species has been investigated in numerous studies as Behzadi Tayemeh et al. (2020) examined the toxic effects of silver nanoparticles and ions on *C. vulgaris* biological responses [20]. Sayadi et al. (2020) used blackfish (*Capoeta fusca*) to study exposure effects of iron oxide nanoparticles and iron salts in causing toxicity, bioaccumulation, and tissue histopathology [21].

Microalga play a vital role in forming the primary production and energy base for all species inhabited in aquatic ecosystems[22, 19]. Such valuable photosynthetic species ranged from microscopic to macro sizes and are the main cause of the food and oxygen production in both freshwater and marine systems. Over the past decade, microalgae have gained significant attention among environmentalists due to their advantageous properties in aquatic ecological balance [23]. Many studies have been conducted concerning the toxic effects of metal contaminants on the biological aspects of microalgae, including growth rate, yield rate, pigment content, reproduction, and nutrient content [24]. Copper nanoparticles caused growth inhibition in *Skeletonema costatum* in exposing to microplastics [23]. Nutritional characteristics of microalgae examined through exposing to metals and metallic NPs, and pigments, biological macromolecules, and phenolic compounds reduced in the presence of these deleterious materials [26]. For this, microalgae have been always considered as ideal candidates and indicators in biological and ecological monitoring of the aquatic biota [27]. Moreover, phytoplanktons are being consumed as functional foods and reliable supplementary ingredients in human diet [26, 27, 29]. Having the richest source of unsaturated fatty acids, antioxidants, proteins, and pigments, microalgae have played a pivotal role in supplying aquatic-based bioactive compounds for consumers [30].

C. vulgaris is classified as unicellular green microalgae and known as an essential functional food in the world [19]. *C. vulgaris* is being sold in many countries like Japan, China, Germany, and India as the most

important algal nutrient in human health and aquaculture activities[31]. This freshwater microalga, in addition, is known for its anticancer, anti-inflammatory, antioxidant, and antibacterial merits, which is widely used in pharmaceutical and food industries [32]. *C. vulgaris* has a great potential of biosorption in binding with toxic materials and metals dissolved in the water[33, 34]. Having intracellular metal binding proteins such as metallothioneins, *C. vulgaris* has been identified as a viable microalga in alleviating the adverse effects of xenobiotics in aquatic ecosystems[35]. In addition, *C. vulgaris* has been an ideal biological model for primary producers to investigate the toxicological effects of metals and nanoparticles released into the aquatic environments. Therefore, this study intended to investigate the effect of iron-based nanoparticles released from synthesized Thiourea catalyst to *C. vulgaris*, as a pivotal primary producer in aquatic systems, in terms of bioaccumulation, bioconcentration, cell growth, pigment content, and risk assessment for potential human consumptions.

2. Materials And Method

2.1. Chemicals and instruments

All chemicals and reagents were purchased from Merck Company (Germany). Infrared (IR) spectra is applied to analyze the spectral experiment using spectrometer (Shimadzu FT-IR-8400S). Following the chemicals preparation, all first-made products were examined using ^1H NMR (500 MHz) spectra and Bruker DRX-500 to identify the chemical characteristics. To solve the chemicals, an advanced spectrophotometer equipped with CDCl_3 (as the solvent) and tetramethylsilane (TMS) (as the internal standard) at ambient temperature was applied. Zeiss-Sigma (VP 500) was used to obtain SEM images and EDX spectra was recorded on an Oxford Instrumental® version. A vibrating magnetometer was used to identify the magnetic properties of the synthesized catalyst (MD Co., Iran, www.mdk-magnetic.com). To analyze the thermogravimetric properties, thermal analyzer with a heating rate of $20^\circ\text{C min}^{-1}$ was used over a temperature range of 25 to 1100°C under flowing compressed nitrogen.

2.2. Catalyst preparation

To synthesize the catalyst, 5 mmol $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and 2.5 mmol $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ were dissolved in 100 mL deionized water under vigorous stirring (800 rpm). Then, NH_4OH solution (25%, w/w, 30 mL) was added to the prepared mixture at room temperature until adjusting the pH above 11. To form a black suspension, NH_4OH solution was continuously added to maintain the pH value between 11 and 12. The resulting black dispersion was continuously stirred for 1 h at room temperature and then refluxed for 6 h. Following the methodology, ethanol (40 mL) was applied at 40°C for 1 h to purify Fe_3O_4 nanoparticles for coating a layer of silica on the surface of the catalyst. Subsequently, tetraethylorthosilicate (TEOS, 10 mL) was charged to the reaction vessel, and the mixture was continuously stirred for 24 h. The silica-coated nanoparticles were collected by a magnet, followed by washing five times with EtOH, diethylether and drying at 100°C in vacuum for 12 h. A sample of MNPs- SiO_2 (1 g) dispersed in a mixture of 50 mL of dry toluene containing (3-chloropropyl) trimethoxy-silane (1 ml) as the effective linker with two different electrophile heads. The mixture was refluxed for 48 h. The final product was separated by filtration,

washed with toluene, and dried under vacuum for 24 h at 150°C. The prepared MNPs@SiO₂@-Si-(CH₂)₃-Cl (1g) and KI (1.66 g, 0.01 mol) were added to a solution of thiourea (0.76 g, 0.01 mol) and K₂CO₃ (1.38 g) dissolved in acetonitrile (50 mL) transferred to a round-bottom flask and the mixture was stirred under reflux condition for 8 h. The obtained solid was then magnetically collected from the solution and washed abundantly with water/ethanol followed by drying at 80°C for 12 h.

2.3. Characterization of synthesized Thiourea catalyst

To identify the characteristics of Synthesized catalyst, a range of optico-chemical techniques, including FT-IR, VSM, XRD, SEM, EDX, and TGA were applied. Results relating to the FT-IR spectra of magnetic NPs (Fe₃O₄@SiO₂) and Fe₃O₄@SiO₂@SiO₃-(CH₂)₃-thiourea are demonstrated in figure 1 (IV). The peaks at 568 and 801 cm⁻¹ are assigned to the Fe⁺³-O⁻ and Fe⁺²-O⁻ symmetrical stretching vibrations, respectively. The covalent bond between silicon and magnetite surface is underlined by the appearance of the band at 1092 cm⁻¹ for Si-O groups. Moreover, absorption bands at 800-950 cm⁻¹ are associated with vibrating bands for Si-O-Si groups (Fig. 1-IV). These results indicated that the SiO₂ layer was formed on the surfaces of Fe₃O₄. Signals appeared at 3421 cm⁻¹ regions can be assigned to the N-H bending, and 1634 cm⁻¹ are attributed to (C=S) stretching of thiourea. Magnetic hysteresis measurements were done in an applied magnetic field at r.t, with the field sweeping from -10000 to +10000 Os using a vibrating sample magnetometer (VSM) as a scientific instrument for measuring magnetic properties (Fig. 1-II). The magnetic saturation value of thiourea functionalized MNPs@SiO₂@SiO₃(CH₂)₃NHCSNH₂ is 23 emu/g. In addition, the XRD patterns of Fe₃O₄@SiO₂@SiO₃ (CH₂)₃NH(CS)NH₂ are demonstrated in figure 1-III. XRD contains seven characteristic peaks (2θ = 21.25°, 37.29°, 43.73°, 52.56°, 65.09°, 69.73°, 76.81°) which match with the peaks of Fe₃O₄ and this confirms that the crystalline structure of Fe₃O₄ does not change during functionalization of MNPs (Fig. 1-III).

The morphology of Fe₃O₄@SiO₂@SiO₃(CH₂)₃NH(CS)NH₂ before (Fig. 1-VII A), and after (Fig. 1-VII B) reaction was visualized using scanning electron microscopy (SEM) images as depicted in figure 1-VII. Based on the SEM analysis, the catalyst was made up of nanometer-sized iron particles shaping spherical morphology. Energy-dispersive X-ray spectroscopy (EDX) method was used to characterize the elemental composition of Fe₃O₄@SiO₂@SiO₃(CH₂)₃NHCSNH₂ (Fig. 1-VI). According to the EDX analysis, the well-dispersion of Fe₃O₄ nanoparticles was obtained through the preparation process. Thermal Gravimetric Analysis (TGA) and Differential Thermal Analysis (DTA) were applied in three steps (Fig. 1-V). The first rupture is associated with the loss of water 1% wt around 60°C, and the second and main loss in weight was recorded at 5% wt, 0.083 mol/100 g catalyst, associated with the elimination of thiourea and its propyl spacer between 200 to 300°C. The final thermal destruction, approximately 1 % wt, was observed from 350 to 700 °C, corresponding loss of silica portion of nanoparticles.

The doublet peak was observed in the low field relating to the H¹, near electron donor group. In addition, the unit singlet peak was related to the H³ and another doublet peak was for H² that it depended on the

group connecting to the aromatic bond which is the same for electron donor group, but the peak H² had shifted to NO₂ as this group had responded and shifted to the high/low field.

2.4. Algal cultivation

C. vulgaris was cultivated using the sterilized Bold's Basal Medium (BBM) as prescribed by Behzadi Tayemeh et al. (2020b). The cultivation was performed for 96 h, and an initial density of 10⁷ cells mL⁻¹ in 1.5 L-glass vessels was used as a stock solution.

2.5. Toxicity tests and exposure experiments

Exposure tests were conducted in the media using a range of concentrations, including 0, 10, 100, 250, 500, 750, and 1000 mg L⁻¹ Fe₃O₄ NPs. Exposure concentrations were selected according to the coated magnetic Fe₃O₄ NPs on a synthesized *Theurea* catalyst. For each treatment, three replicates were considered, and exposure tests were carried out using OECD 201 (OECD, 2011) protocols and guidelines. During the incubation period, cool white fluorescent lights (40W FL T10 230 V G13; 12 h light: 12 h dark regime) were used with constant intensity of 1000 μmol. photons.m⁻².s⁻¹ on the glass vessels aerated from the bottom and the temperature was adjusted at 26 ± 1°C.

2.6. Algal growth

During the exposure time, the density of algal cells was daily evaluated at 24, 48, 72, and 96 h after exposure test. For this, two procedures, including optical density (OD) and visible light microscopy were used to calculate the cell numbers. Spectrophotometric method (PerkinElmer Lambda 25 UV-VIS Spectrometer) at the absorbance of 680 nm was used for OD method, and light microscope equipped with a Neubauer chamber was applied to perform the visual count in triplicates for each treatment (OECD, 2011).

2.7. Pigment analysis

Pigments, including Chlorophyll a, Chlorophyll b, and carotenoid were considered as microalgae pigments and evaluated using a standard protocol prescribed by Silkina et al. (2015) with some modifications [36]. Following the pigment analysis, a volume of 13 ml medium was centrifuged for obtaining cultivated algal cells using 20,000 rpm for 5 min at 4°C. The supernatant was rejected and then the maintained cells were preserved at -80°C until experiments. Pigments were extracted according to the method stated by Tsiola et al. (2017) who used 1.5 ml of 90 % ethanol added to the collected cells and then vortexed severely for 2 min and kept for 24 h in a dark condition at room temperature [37]. A microplate reader (BioTek, 800 TS Absorbance Reader; USA) was used to read the pigments in each treatment using 150 μl in triplicates at 470, 666, and 653 nm.

2.8. Bioaccumulation and bioconcentration factor

To measure intracellular Fe₃O₄ concentrations, algal cells were first collected from the water medium and then were thoroughly washed with cultured medium (without heavy metal ion) to remove the potential

extracellular Fe_3O_4 ions. Digestion was conducted using $\text{HNO}_3:\text{HClO}_4$ at 80°C and 130°C for 1 and 3 h, respectively, based on the method prescribed by Qian et al. (2011) with some modifications [28]. Digested material was diluted with de-ionized water, and Fe_3O_4 concentrations were detected using flame atomic absorption spectrophotometer (Model 670G, USA). Bioconcentration factor (BCF) was calculated using equation 1 as suggested by Kuppu et al. (2018).

$$\text{BCF} \left(\text{Lkg}^{-1} \right) = \frac{\text{CMTD}}{\text{CSMW}}$$

1

Where CMTD is concentration of metal in dry tissue (mg kg^{-1}), and CSMW is the concentration of same metal in water (mg L^{-1}).

2.9. Health risk assessment of contaminated *C. vulgaris*

The estimated daily intake (EDI) and target hazard quotient (THQ) were used to evaluate the non-carcinogenic risk of Fe_3O_4 accumulated in *C. vulgaris*. Based on the method prescribed by Alipour and Banagar (2018) EDI and THQ were calculated using equations 2 and 3, respectively.

$$\text{EDI} \left(\text{mgkg}^{-1}\text{day}^{-1} \right) = \frac{\text{EF} \times \text{ED} \times \text{AIR} \times \text{C}}{\text{BW} \times \text{ATn}}$$

2

$$\text{THQ} = \frac{\text{EF} \times \text{ED} \times \text{AIR} \times \text{C}}{\text{RfD} \times \text{BW} \times \text{ATn}} \times 10^{-3}$$

3

where EF is the exposure frequency ($365 \text{ days year}^{-1}$); ED is the exposure duration (70 years, average lifetime for adults); AIR is the algal ingestion rate ($0.0035 \text{ Kg person}^{-1} \text{ day}^{-1}$); C is the Fe_3O_4 ions in algae (mg g^{-1}); RfD is the oral reference dose ($\text{Fe} = 0.7 \text{ mg kg}^{-1} \text{ day}^{-1}$); Bw is the average adult body weight (70 kg); and ATn is the average exposure time for non-carcinogenic risk ($365 \text{ days/year} \times \text{number of exposure years, assuming 70 years}$). Non-carcinogenic risk grouping was performed using the method prescribed by Cheng et al. (2018). For target hazard quotient, $\text{THQ} \geq 1$ showed the presence of non-carcinogenic risk, and $\text{THQ} < 1$ represented negligible hazard for people.

2.10. Statistical analysis

All of the analyses and comparisons were conducted using SPSS software version 19.0 (SPSS, Chicago IL, USA). Kolmogorov-Smirnov test was performed to assess the normality of data, and one-way ANOVA was used to determine possible differences between treatments. In all analyses, data were presented as mean $\pm$ SD, and differences were considered significant at $P < 0.05$. For data management and drawing

graphs, Microsoft Excel (version 2016, windows 7) was applied to define and calculate the risk assessment analysis.

3. Results

3.1. Cell density and algal growth

Cell density analysis is represented in figure 2. Based on the findings, cell density of *C. vulgaris* showed that the number of cells increased with the elevation of iron-based NPs from 0 to 100 mg L⁻¹ while in higher concentrations (i.e. 250, 500, 750, and 1000) demonstrated a decrease, especially after 72 and 96 h exposure period. Results showed that cell density elevated with the increase of nanomaterial concentration from 0 to 500 mg L⁻¹ after 0, 24, and 48 h exposure time (Fig. 2).

3.2. Pigment analysis

Results relating to the chlorophyll a content is depicted in figure 3. It is apparent that the content of chlorophyll a was in the lowest level after 24 h exposure time, whereas in a 96-h period the highest chlorophyll a was recorded in all treatments. Indeed, chlorophyll a content showed a time-dependent response to the concentration of iron nanoparticles. Moreover, in treatments exposed to 250 and 500 mg L⁻¹, chlorophyll a had 4.2 and 4 mg/g, respectively (Fig. 3). Chlorophyll b, in addition, demonstrated a time-relevant response to Fe₃O₄ NPs as chlorophyll a content increased from 0 to 96 h after exposure period in all concentrations (Fig. 4). The greatest pigment content was detected at the end of the experiment (96 h) while the lowest one was observed in the first day of the test. In terms of Fe₃O₄ NPs exposure doses, algal populations exposed to 250 and 1000 mg L⁻¹ illuminated the highest and lowest chlorophyll b content, respectively. Findings demonstrated that carotenoid underwent a concentration-dependent decrease in all tested groups (i.e. 0, 10, 100, 250, 500, 750, and 1000 mg L⁻¹). Furthermore, a time-relevant manner was observed between Fe₃O₄ NPs concentrations, and carotenoid content increased with the elevation of exposure time from 24 to 96 h in control, 10, and 100 mg L⁻¹ iron nanomaterials. However, *C. vulgaris* populations exposed to 250, 500, 750, and 1000 mg L⁻¹ showed the lowest carotenoid content in comparison with the lower concentrations and control group (Fig. 5).

3.3. Bioaccumulation and bioconcentration factor

Bioaccumulated Fe₃O₄ in *C. vulgaris* populations exposed to different concentrations is represented in figure 6. Based on the outcomes, bioaccumulation factor exhibited a concentration-related pattern in the tested groups. The highest bioaccumulation recorded at 15072.9 µg/g dw in exposing to 1000 mg L⁻¹ and the lowest one identified at 1592.32 µg/g dw in the control group where algal populations did not expose to Fe₃O₄ concentration (Fig. 5) and Table 1). Findings regarding the bioconcentration factor (BCF) illustrated that 10 mg L⁻¹ of Fe₃O₄ had the greatest BCF at 327.3611, whereas other concentrations (0, 100, 250, 500, 750, and 1000) demonstrated BCF no more than 50 (Fig. 7).

Table 1
Detected concentrations of Fe₃O₄ in contaminated *C. Vulgaris*

Fe ₃ O ₄ concentration (mg L ⁻¹)	Bioaccumulation (µg/g dw) Mean ± SD
Control	1592.32 ± 312.714
10	3273.61 ± 244.298
100	4086.32 ± 1343.91
250	4965.49 ± 1668.21
500	8696.36 ± 514.39
750	11203.9 ± 2152.33
1000	15072.9 ± 1928.64

3.4. Health risk assessment

Risk assessment analysis presented in Table 2. Outcomes suggested that estimated daily intake (EDI) increased with the elevation of Fe₃O₄ concentration, and the highest EDI was calculated at 7.4332E-07 for *C. vulgaris* populations who were exposed to 1000 mg L⁻¹. Target hazard quotient (THQ) revealed a concentration-dependent response to iron concentrations, and the largest THQ was obtained in exposing to 1000 mg L⁻¹ at 1.06189E-09. THQ in all contaminated populations of *C. vulgaris* to Fe₃O₄ was below 1 (i.e. THQ < 1), and therefore, cultivated microalgae represented negligible hazard for algal consumers.

Table 2
Estimated daily intake (EDI) and target hazard quotient (THQ) of contaminated *C. vulgaris*.

mg kg ⁻¹ day ⁻¹	EDI	THQ
0	7.85253E-08	1.12179E-10
10	1.61438E-07	2.30626E-10
100	2.01517E-07	2.87882E-10
200	2.44873E-07	3.49819E-10
500	4.28861E-07	6.12659E-10
750	5.5252E-07	7.89314E-10
1000	7.4332E-07	1.06189E-09

4. Discussion

Iron is one of the most abundant metal in the earth's crust (34.6 %) and would be naturally available in the environment [42]. This vital trace element plays a crucial role in producing materials in the photosynthetic organisms as well as cell division processes. There are a variety of iron-based compounds in the environment, but the most common forms are Fe_2O_3 , Fe_3O_4 , FeSO_4 , FeCl_3 , and Fe_2NO_3 . Iron in certain concentrations is essential for living-beings in the environment, but at higher concentrations, it can be toxic. In microalgae population, iron involves in metabolism pathways, including photosynthesis, pigment, DNA synthesis, nitrogen fixation, and respiration. Furthermore, iron-based materials are constructed through a wide range of human-made activities which result in iron pollution in the biota. Synthesized catalysts are designed to functionalize chemical reactions via reducing the cost and increasing the speed of processes. For this, many industries have applied organocatalysts containing metals as a reliable and viable base. The ever-increasing application of such catalysts has resulted in environmental pollution and aquatic toxicity for many organisms. Literature reported that wastewater from industrial developments contains 10 to 120 mg L^{-1} iron [43]. Iron content of wastewater and sediment were estimated to be 50 to 3500 $\mu\text{g L}^{-1}$ and 4300 to 7000 mg kg^{-1} in a lake located in Western Australia. Copper smelter companies are one of the most important sources of iron-based materials that cause 882 mg kg^{-1} iron in the sediment. Our findings revealed that the bioaccumulation of Fe_3O_4 NPs in *C. vulgaris* has a concentration-dependent manner in response to iron-based catalyst used in this study. Fe_3O_4 accumulated increasingly from $3273.61 \pm 244.298 \mu\text{g/g dw}$ in populations exposed to 10 mg L^{-1} to $15072.9 \pm 1928.64 \mu\text{g/g dw}$ detected in 1000 mg L^{-1} concentration. To support this, *C. vulgaris* has a great potential of absorption and bioaccumulation of environmental metals and is known as an accumulator used in toxicological studies. This bioindicator possesses a hard and thick cell wall containing a considerable amount of fiber that results in absorbing metal ions released into the aquatic biota. Metals such as iron are more desire to absorb and accumulate in the body of aquatic species, rather than being dissolved in the water. Trace elements could be adsorbed by the fish body and its organs in aquatic systems [44]. Another study was conducted using four species of marine algae to evaluate their bioaccumulated iron and its risk for human consumption. Results showed that *Gracilariopsis sp.* and *Sargassum sp.* contained larger amount of iron at 1960 and 1570 $\mu\text{g/g dw}$, respectively, compared to other species. BCF demonstrated that there is a concentration-relevant decrease among populations exposed to Fe_3O_4 , and the highest value was calculated for 10 mg L^{-1} treatment at 327.3611, whereas algae exposed to 1000 mg L^{-1} showed the lowest BCF at 15.07287 after 96 h of exposure. BCF has indirect relationship with the metal concentration exposed to the water medium, but with the increase of bioaccumulated Fe_3O_4 in algal body, this factor elevates. In general, microalgae are classified as good metal accumulators in aquatic systems, and $\text{BCF} > 1000$ suggested that the studied species has a great ability to bioconcentrate metals. Researchers claimed that marine diatom *Phaeodactylum tricornutum* could actively adsorb Cd (II) ions to remove such toxic materials from the water. They stated *P. tricornutum* showed BCF is a stronger cadmium bioconcentrator as its BCF was above 1000 in most tested concentrations [45]. In the present study, there was no BCF higher than 327.3611 (10 mg L^{-1}) and most tested concentrations displayed BCF below 50. To support this, a complete biosorption can occur by microalgae cells in case they expose to lower Fe_3O_4 concentrations,

and in turn, higher BCF could be obtained. With the increase of Fe_3O_4 concentration in the water, binding sites in the cell wall of the *C. vulgaris* are rapidly saturated, which inhibit the binding of more metal ions [45]. Moreover, in highly metal-contaminated environments, intracellular mechanisms in *C. vulgaris* regulate the gradient concentration between the water and organism [46].

According to outcomes emerged to this study, cell density of *C. vulgaris* populations increased in lower concentrations of Fe_3O_4 at 10, 100, and 250 mg L^{-1} after 72 and 96 h exposure period, and the highest cell growth was observed in populations exposed to 100 mg L^{-1} iron. However, in higher concentrations (i.e. 250, 500, 750, and 1000 mg L^{-1}) cell density was inhibited. It is investigated that the cell density in *C. vulgaris* populations exposed to silver nanoparticles (AgNPs) and silver ions (AgNO_3) during a 72-h exposure time increased firstly with the elevation of metal nanoparticles and ions, but in higher exposure concentrations and times, it remained stable due to inhibitory effects of such materials [20]. Aquatic microalgae possess limitations to use micronutrients and absorb trace elements in their inhabitants, and in higher concentrations these essential elements act as growth inhibitors. These findings mirrored those reported by Naorbe and Serrano (2018) who stated that microalgae *Tetraselmis tetrathele* showed inhibitory effects in exposing to Hg and Cd ions. They added that Hg and Cd in lower concentrations (i.e. 0, 0.1, 0.3, 0.5, 1, and 2 mg L^{-1}) showed no change in the cell density, while in higher doses at 3, 4, and 5 mg L^{-1} after 72 h of exposure time inhibitory effects were observed [48]. Furthermore, our results demonstrated that in the control group as well as after 24 and 48 h of exposure experiment cell density increased even at higher Fe_3O_4 concentrations (i.e. 500 mg L^{-1}); however, cell growth remained stable in facing 750 and 1000 mg L^{-1} . This may mean that microalgae such as *C. vulgaris* has their own growth regulatory mechanisms that act as inhibitory factors to adjust their populations based on the environmental conditions. The suppression of *C. vulgaris* growth in exposing to cadmium, lead, and copper stress was studied, and results concerned that the growth and chemical compositions decreased during the first 48 h of exposure time, and copper was more effective inhibitory factor than lead and cadmium to prevent cell growth [49]. Increasing on iron bioavailability above 200 μM could mitigate the growth rate of *C. vulgaris* populations exposed to Fe and increased the amount of lipid radical content in the intracellular space [18].

Pigment analysis, in this study, showed that chlorophyll a content increased over the exposure period (i.e. 0, 24, 48, 72, and 96 h) in all tested concentrations and the highest amount of this pigment was recorded after 96 h of exposure test. Obtained results indicated that *C. vulgaris* could produce chlorophyll a in a concentration-relevant increase up to 250 mg L^{-1} , but in further Fe_3O_4 concentrations it decreased to 1000 mg L^{-1} . Metal toxicity can cause a serious reduction in the production of chlorophyll a due to its adverse effects on protein construction. *C. vulgaris* pigment content decreased in exposing to supermagnetic iron oxide nanoparticles (Fe_3O_4) in a range of concentrations from 12.5 to 400 $\mu\text{g ml}^{-1}$ [39]. Pigment content of microalgae can be used as a reliable biomarker in investigating the health status of the organism, and in whole, community and ecosystem. Literature reported that the most important reason regarding the pigment reduction in microalgae is due to adaptation to unsuitable environmental

conditions through changing the pigments to supply organic nitrogen [51]. According to our results, chlorophyll b content showed a similar pattern as chlorophyll a, and a significant increase was observed during 96-h exposure time. The highest and lowest chlorophyll b were identified at 250 and 1000 mg L⁻¹, respectively. Carotenoid content of the studied microalgae showed a concentration-related decrease from 0 to 1000 mg L⁻¹. Literatures have proven that the loss of pigment content during the exposure period to metals is mainly because of damage in chloroplast ribosome as well as inhibition in the electron transport chain in the donor center of the *C. vulgaris* cells [52]. In toxic concentration, Fe₃O₄ interfere in the pigment production process and alter the essential enzymes and proteins accounted for pigment synthesis [41]. It is highly possible that with the ever-increasing applications of iron-based catalysts in chemical reactions used for various industries, the environmental concentration of Fe₃O₄ increase in the aquatic biota, including freshwater rivers, marine environments, lakes, and wetlands.

The importance of microalgae as functional foods and supplementary ingredients in human diet has been proven in many studies [54–57]. Having a diverse range of unsaturated fatty acids, antioxidants, omega-3, proteins, and pigments, *C. vulgaris* has engrossed many attentions among nutrition scientists and aquaculture companies. However, the health status of the *C. vulgaris*, in terms of bioaccumulated metals, is vital for those who consider such valuable ingredients in their daily diets. Because of this, carcinogenic and non-carcinogenic risk assessment tests are applied to examine the potential risk of edible microalgae for consumers. In this study, estimated daily intake (EDI) and target hazard quotient (THQ) were used to calculate non-carcinogen risk of contaminated *C. vulgaris*. In all contaminated *C. vulgaris* exposure tests, THQ was below 1 (the highest value: 1.06189E-09) and calculated as THQ < 1 meaning that iron-based catalysts had no non-carcinogenic risk for people in exposing to used Fe₃O₄ concentrations in this study.

Conclusion

This study investigated the toxicity of Fe₃O₄ concentrations used in Thiourea synthesized catalyst to *C. vulgaris* as an essential primary producer in aquatic ecosystems. In response to Fe₃O₄ concentrations, cell density, pigment analysis, bioaccumulation, and bioconcentration factor were assessed in *C. vulgaris* populations. Non-carcinogenic risk of contaminated microalgae, moreover, was calculated using EDI and THQ indices. According to the findings, Fe₃O₄ in lower concentrations (i.e. 10, 100, and 250 mg L⁻¹) was pleasant for the cell growth, whereas in higher doses (i.e. 500, 750, and 1000 mg L⁻¹) showed inhibitory effects for cell density, especially after 72 and 96 h. Chlorophyll a and b content increased in 10, 100, and 250 concentrations, while carotenoids represented a dose-relevant decrease to Fe₃O₄ concentrations. Although iron is a vital micronutrient for algal viability and reproduction, inhibitory and toxicity effects may occur in its exceeding concentrations above 500 mg L⁻¹. As a strong accumulator in aquatic systems, *C. vulgaris*, could desire to absorb released Fe₃O₄ ions, that led to increasing bioaccumulation with the elevation of iron concentrations. *C. vulgaris* possesses a great capacity to bioconcentrate iron nanoparticles, and in this regard, this species plays an essential role in biomagnification through food

web in aquatic environments. EDI and THQ revealed that non-carcinogenic risk of Fe_3O_4 was at negligible risk ($\text{THQ} < 1$) for people. Taken together, although iron-based organocatalysts used in industrial applications are green and safe, in over-used conditions act as inhibitor for primary producers like *C. vulgaris* and threaten the health status of consumers as aquatic functional food.

Declarations

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions

Taherh Ebrahimi Yazdanabdad: Conception and design, investigation and research, drafting of the article, **Ali Forghaniha:** critical revision of the article for important intellectual content, methodology and supervision, **Mozhgan Emtiazjoo:** final approval of the article, collection and assembly of data, **Majid Ramezani:** language revisions and finalizing the manuscript.

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References

1. Dekamin MG, Alikhani M, Javanshir S (2016) Organocatalytic clean synthesis of densely functionalized 4H-pyrans by bifunctional tetraethylammonium 2-(carbamoyl)benzoate using ball milling technique under mild conditions. *Green Chem Lett Rev* 9:96–105. <https://doi.org/10.1080/17518253.2016.1139191>
2. Karlsson S, Högberg HE (2003) Organocatalysts promote enantioselective 1,3-dipolar cycloadditions of nitrones with 1-cycloalkene-1-carboxaldehydes. *European J Org Chem* 2782–2791. <https://doi.org/10.1002/ejoc.200300172>
3. Roy Chowdhury A, Maiti S, Mondal A, Das AK (2020) Picolinohydrazide-Based Covalent Organic Polymer for Metal-Free Catalysis and Removal of Heavy Metals from Wastewater. *J Phys Chem C* 124:7835–7843. <https://doi.org/10.1021/acs.jpcc.0c00664>
4. Arefi M, Kazemi Miraki M, Mostafalu R et al (2019) Citric acid stabilized on the surface of magnetic nanoparticles as an efficient and recyclable catalyst for transamidation of carboxamides, phthalimide, urea and thiourea with amines under neat conditions. *J Iran Chem Soc* 16:393–400. <https://doi.org/10.1007/s13738-018-1523-8>

5. Roto R, Yusran Y, Kuncaka A (2016) Magnetic adsorbent of Fe₃O₄@SiO₂ core-shell nanoparticles modified with thiol group for chlorauric ion adsorption. *Appl Surf Sci* 377:30–36.
<https://doi.org/10.1016/j.apsusc.2016.03.099>
6. Khare R, Pandey J, Smriti S, Ruchi R (2019) The Importance and Applications of Knoevenagel Reaction (Brief Review). *Orient J Chem* 35:423–429. <https://doi.org/10.13005/ojc/350154>
7. Fallah-Mehrjardi M (2017) Fe₃O₄@Silica Sulfuric Acid: An Efficient and Heterogeneous Nanomagnetic Catalyst in Organic Reactions. *Mini Rev Org Chem* 14:122–129.
<https://doi.org/10.2174/1570193x14666170206144158>
8. Ghorbani-Choghamarani A, Darvishnejad Z, Norouzi M (2014) Cu(II)-Schiff base complex-functionalized magnetic Fe₃O₄ nanoparticles: A heterogeneous catalyst for various oxidation reactions. *Appl Organomet Chem* 29:170–175. <https://doi.org/10.1002/aoc.3266>
9. Zhang N, Xian G, Li X et al (2018) Iron Based Catalysts Used in Water Treatment Assisted by Ultrasound: A Mini Review. *Front Chem* 6:1–6. <https://doi.org/10.3389/fchem.2018.00012>
10. Fan D, Lan Y, Tratnyek PG et al (2017) Sulfidation of Iron-Based Materials: A Review of Processes and Implications for Water Treatment and Remediation. *Environ Sci Technol* 51:13070–13085.
<https://doi.org/10.1021/acs.est.7b04177>
11. Chu C, Lu C, Yuan J, Xing C (2020) Fate of Fe₃O₄@NH₂ in soil and their fixation effect to reduce lead translocation in two rice cultivars. *Food Sci Nutr* 8:3673–3681. <https://doi.org/10.1002/fsn3.1651>
12. Garner KL (2016) Assessing the Risk of Engineered Nanomaterials in the Environment: Modeling Fate, Exposure, and Bioaccumulation. 118
13. Hartmann NB, Skjolding LM, Hansen SF et al (2014) Environmental fate and behaviour of nanomaterials
14. Kadar E, Rooks P, Lakey C, White DA (2012) The effect of engineered iron nanoparticles on growth and metabolic status of marine microalgae cultures. *Sci Total Environ* 439:8–17.
<https://doi.org/10.1016/j.scitotenv.2012.09.010>
15. Ali H, Khan E, Ilahi I (2019) Environmental chemistry and ecotoxicology of hazardous heavy metals: Environmental persistence, toxicity, and bioaccumulation. *J Chem* 2019:.
<https://doi.org/10.1155/2019/6730305>
16. Yousefi N, Emtiazjoo M, Sepehr MN et al (2020) Green synthesis of *Pseudomonas aeruginosa* immobilized Fe₃O₄-multiwalled carbon nanotubes bio-adsorbent for the removal of 2,4,6-trinitrophenol from aqueous solution. *Environ Technol Innov* 20:101071.
<https://doi.org/10.1016/j.eti.2020.101071>
17. Lei C, Zhang L, Yang K et al (2016) Toxicity of iron-based nanoparticles to green algae: Effects of particle size, crystal phase, oxidation state and environmental aging *. *Environ Pollut* 4–11.
<https://doi.org/10.1016/j.envpol.2016.07.030>
18. Estevez MS, Malanga G, Puntarulo S (2001) Iron-dependent oxidative stress in *Chlorella 6 ulgaris*. 161:9–17

19. Zhu X, Tian S, Cai Z (2012) Toxicity Assessment of Iron Oxide Nanoparticles in Zebrafish (*Danio rerio*) Early Life Stages. 7:1–6. <https://doi.org/10.1371/journal.pone.0046286>
20. Behzadi Tayemeh M, Esmailbeigi M, Shirdel I et al (2020) Perturbation of fatty acid composition, pigments, and growth indices of *Chlorella vulgaris* in response to silver ions and nanoparticles: A new holistic understanding of hidden ecotoxicological aspect of pollutants. *Chemosphere* 238. <https://doi.org/10.1016/j.chemosphere.2019.124576>
21. Sayadi MH, Mansouri B, Shahri E et al (2020) Exposure effects of iron oxide nanoparticles and iron salts in black fi sh (*Capoeta fusca*): Acute toxicity, bioaccumulation, depuration, and tissue histopathology. *Chemosphere* 247:125900. <https://doi.org/10.1016/j.chemosphere.2020.125900>
22. Bavandi R, Emtiazjoo M, Saravi HN et al (2019) Study of capability of nanostructured zero-valent iron and graphene oxide for bioremoval of trinitrophenol from wastewater in a bubble column bioreactor. *Electron J Biotechnol* 39:8–14. <https://doi.org/10.1016/j.ejbt.2019.02.003>
23. Krichen E, Rapaport A, Le Floc'h E, Fouilland E (2019) Demonstration of facilitation between microalgae to face environmental stress. *Sci Rep* 9:1–11. <https://doi.org/10.1038/s41598-019-52450-9>
24. Xu H, Lee U, Coleman AM et al (2020) Balancing Water Sustainability and Productivity Objectives in Microalgae Cultivation: Siting Open Ponds by Considering Seasonal Water. <https://doi.org/10.1021/acs.est.9b05347>. -Stress Impact Using AWARE-US
25. Zhu X, Zhao W, Chen X et al (2020) Growth inhibition of the microalgae *Skeletonema costatum* under copper nanoparticles with microplastic exposure. *Mar Environ Res* 158:105005. <https://doi.org/10.1016/j.marenvres.2020.105005>
26. Xiao S, Liang T, Wong LS (2020) Toxicity of Metals and Metallic. Nanoparticles on Nutritional Properties of Microalgae
27. Wu N, Dong X, Liu Y et al (2017) Using river microalgae as indicators for freshwater biomonitoring: Review of published research and future directions. *Ecol Indic* 81:124–131. <https://doi.org/10.1016/j.ecolind.2017.05.066>
28. Andrade LM (2018) *Chlorella* and *Spirulina* Microalgae as Sources of Functional Foods, Nutraceuticals, and Food Supplements; an Overview. *MOJ Food Process Technol* 6. <https://doi.org/10.15406/mojfpt.2018.06.00144>
29. Nia MV, Emtiazjoo M, Chamani M (2020) Algae usage in producing functional food: A case study on *Spirulina platensis* for enrichment quail egg. *Res Mar Sci* 5:2020–2814
30. Bule MH, Ahmed I, Maqbool F et al (2018) Microalgae as a source of high-value bioactive compounds. *Front Biosci - Sch* 10:197–216. <https://doi.org/10.2741/s509>
31. Wells ML, Potin P, Craigie JS et al (2017) Algae as nutritional and functional food sources: revisiting our understanding. *J Appl Phycol* 29:949–982. <https://doi.org/10.1007/s10811-016-0974-5>
32. Ravi Kiran B, Venkata Mohan S (2021) Photosynthetic transients in *Chlorella sorokiniana* during phycoremediation of dairy wastewater under distinct light intensities. *Bioresour Technol* 340:125593. <https://doi.org/10.1016/j.biortech.2021.125593>

33. Cho DY, Chung AS, Lee ST, Park SW (1994) Studies on the biosorption of heavy metals onto *Chlorella vulgaris*. J Environ Sci Heal Part A Environ Sci Eng Toxicol 29:389–409.
<https://doi.org/10.1080/10934529409376043>
34. Goher ME, El-Monem AMA, Abdel-Satar AM et al (2016) Biosorption of some toxic metals from aqueous solution using non-living algal cells of *Chlorella vulgaris*. J Elem 21:703–714.
<https://doi.org/10.5601/jelem.2015.20.4.1037>
35. Huang Z, Li L, Huang G et al (2009) Growth-inhibitory and metal-binding proteins in *Chlorella vulgaris* exposed to cadmium or zinc. Aquat Toxicol 91:54–61.
<https://doi.org/10.1016/j.aquatox.2008.10.003>
36. Silkina A, Flynn K, Llewellyn C, Bayliss C (2015) Standard Operating Procedures for Analytical Methods and Data Collection in Support of Pilot-Scale Cultivation of Microalgae. Energ Algae Output WP1A301:395
37. Tsiola A, Pitta P, Giannakourou A et al (2017) Ocean acidification and viral replication cycles: Frequency of lytically infected and lysogenic cells during a mesocosm experiment in the NW Mediterranean Sea. Estuar Coast Shelf Sci 186:139–151.
<https://doi.org/10.1016/j.ecss.2016.05.003>
38. Qian H, Li J, Pan X et al (2011) Combined effect of copper and cadmium on heavy metal ion bioaccumulation and antioxidant enzymes induction in *Chlorella vulgaris*. Bull Environ Contam Toxicol 87:512–516. <https://doi.org/10.1007/s00128-011-0365-1>
39. Kuppu R, Manoharan S, Uthandakalaipandian R (2018) A study on the impact of water quality on the murrel fish *Channa striata* and *Channa punctata*. 11375–11387.
<https://doi.org/10.1039/c7ra13583a>
40. Alipour H, Banagar GR (2018) Health risk assessment of selected heavy metals in some edible fishes from Gorgan Bay, Iran. Iran J Fish Sci 17:21–34. <https://doi.org/10.22092/IJFS.2018.115582>
41. Cheng Z, Chen LJ, Li HH et al (2018) Characteristics and health risk assessment of heavy metals exposure via household dust from urban area in Chengdu, China. Sci Total Environ 619–620:621–629. <https://doi.org/10.1016/j.scitotenv.2017.11.144>
42. Cadmus P, Brinkman SF, May MK (2018) Chronic Toxicity of Ferric Iron for North American Aquatic Organisms: Derivation of a Chronic Water Quality Criterion Using Single Species and Mesocosm Data. Arch Environ Contam Toxicol 74:605–615. <https://doi.org/10.1007/s00244-018-0505-2>
43. Razmovski R, Šćiban M (2008) Iron(III) biosorption by *Polyporus squamosus*. African J Biotechnol 7:1693–1699. <https://doi.org/10.5897/ajb2008.000-5026>
44. Feng W, Wang Z, Xu H et al (2020) Species-specific bioaccumulation of trace metals among fish species from Xincun Lagoon, South China Sea. Sci Rep 10:1–11. <https://doi.org/10.1038/s41598-020-77917-y>
45. Torres E, Mera R, Herrero C, Abalde J (2014) Isotherm studies for the determination of Cd (II) ions removal capacity in living biomass of a microalga with high tolerance to cadmium toxicity. Environ Sci Pollut Res 21:12616–12628. <https://doi.org/10.1007/s11356-014-3207-y>

46. Sekabira K, Origa HO, Basamba TA et al (2011) Application of algae in biomonitoring and phytoextraction of heavy metals contamination in urban stream water. *Int J Environ Sci Technol* 8:115–128. <https://doi.org/10.1007/BF03326201>
47. Marissa C, Naorbe AESJ (2018) Effects of heavy metals on cell density, size, specific growth rate and chlorophyll a of *Tetraselmis tetrathele* under controlled laboratory. 11:589–597
48. Naorbe MC, Serrano AE (2018) Effects of heavy metals on cell density, size, specific growth rate and chlorophyll a of *tetraselmis tetrathele* under controlled laboratory conditions. *AACL Bioflux* 11:589–597
49. Bajguz A (2011) Suppression of *Chlorella vulgaris* growth by cadmium, lead, and copper stress and its restoration by endogenous brassinolide. *Arch Environ Contam Toxicol* 60:406–416. <https://doi.org/10.1007/s00244-010-9551-0>
50. Barhoumi L, Dewez D (2013) Toxicity of superparamagnetic iron oxide nanoparticles on green alga *Chlorella vulgaris*. *Biomed Res Int* 2013:. <https://doi.org/10.1155/2013/647974>
51. Metzler DM, Erdem A, Tseng YH, Huang CP (2012) Responses of algal cells to engineered nanoparticles measured as algal cell population, chlorophyll a, and lipid peroxidation: Effect of particle size and type. *J Nanotechnol* 2012:. <https://doi.org/10.1155/2012/237284>
52. Sendra M, Blasco J, Araújo CVM (2017) Is the cell wall of marine phytoplankton a protective barrier or a nanoparticle interaction site ? Toxicological responses of *Chlorella autotrophica* and *Dunaliella salina* to Ag and CeO₂ nanoparticles. *Ecol Indic* 0–1. <https://doi.org/10.1016/j.ecolind.2017.08.050>
53. Sendra M, Yeste MP, Gatica JM et al (2017) Direct and indirect effects of silver nanoparticles on freshwater and marine microalgae (*Chlamydomonas reinhardtii* and *Phaeodactylum tricornutum*). *Chemosphere* 179:279–289. <https://doi.org/10.1016/j.chemosphere.2017.03.123>
54. Ötles S, Pire R (2001) Fatty acid composition of *Chlorella* and *Spirulina* microalgae species. *J AOAC Int* 84:1708–1714. <https://doi.org/10.1093/jaoac/84.6.1708>
55. Ferrazzano GF, Papa C, Pollio A et al (2020) Cyanobacteria and microalgae as sources of functional foods to improve human general and oral health. *Molecules* 25:1–17. <https://doi.org/10.3390/molecules25215164>
56. Katiyar R, Arora A (2020) Health promoting functional lipids from microalgae pool: A review. *Algal Res* 46:101800. <https://doi.org/10.1016/j.algal.2020.101800>
57. Fields FJ, Lejzerowicz F, Schroeder D et al (2020) Effects of the microalgae *Chlamydomonas* on gastrointestinal health. *J Funct Foods* 65:103738. <https://doi.org/10.1016/j.jff.2019.103738>

Figures

Figure 1

Schematic of synthesized catalyst $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{SiO}_3(\text{CH}_2)_3\text{thiourea}$ (I), magnetization curve of $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{SiO}_3(\text{CH}_2)_3\text{thiourea}$ (II), XRD pattern of Fe_3O_4 (III-A) and $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{SiO}_3(\text{CH}_2)_3\text{thiourea}$ (III-B), FT-IR spectra of Fe_3O_4 (IV-blue curve), $\text{Fe}_3\text{O}_4@\text{SiO}_2$ (IV-red curve) and $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{SiO}_3(\text{CH}_2)_3\text{thiourea}$ (IV-green curve), thermogravimetric analysis of $\text{Fe}_3\text{O}_4@\text{SiO}_2@\text{SiO}_3(\text{CH}_2)_3\text{thiourea}$

(CH₂)₃-thiourea (V), EDX spectrum of Fe₃O₄@SiO₂@SiO₃-(CH₂)₃-thiourea (VI), SEM Analysis of the Fe₃O₄@SiO₂@SiO₃-(CH₂)₃-thiourea (VII-A), Fe₃O₄@SiO₂@SiO₃-(CH₂)₃-thiourea after reaction (VII-B).

Figure 2

Cell density of *C. vulgaris* exposed to different concentrations of Fe₃O₄ in a 96-h period.

Figure 3

Chlorophyll a content of *C. vulgaris* exposed to different concentrations of Fe_3O_4 in a 96-h period.

Figure 4

Chlorophyll b content of *C. vulgaris* exposed to different concentrations of Fe3O4 in a 96-h period.

Figure 5

Carotenoid content of *C. vulgaris* exposed to different concentrations of Fe3O4 NPs in a 96-h period.

Figure 6

Bioaccumulation of Fe_3O_4 in *C. vulgaris* during a 96-h exposure period (* indicates significant differences).

Figure 7

Bioconcentration factor of Fe₃O₄ in *C. vulgaris* during a 96-h exposure period.