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Interaction of zero valent copper nanoparticles with algal cells under simulated natural conditions: Particle dissolution kinetics, uptake and heteroaggregation

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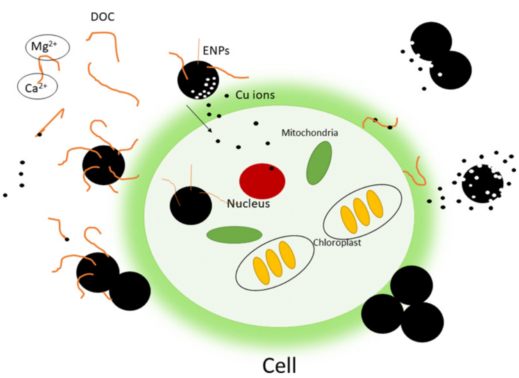
HIGHLIGHTS

- Algal cells decreased dissolution of Cu^0-ENPs simulated natural water.
- DOC increased the dissolution of Cu^0-ENPs by increasing the particle aggregation.
- DOC increased the heteroaggregation of the particles with algae.
- In the presence of the cell, DOC decreased the particle dissolution.

GRAPHICAL ABSTRACT



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ABSTRACT

Some metal-based engineered nanoparticles (ENPs) undergo fast dissolution and/or aggregation when they are released in the environment. The underlying processes are controlled by psychochemical/biological parameters of the environment and the properties of the particles. In this study, we investigated the interaction between algal cells and zero valent copper nanoparticles (Cu^0-ENPs) to elucidate how the cells influence the dissolution and aggregation kinetics of the particles and how these kinetics influence the cellular uptake of Cu. Our finding showed that the concentration of dissolved Cu ($[Cu]_{dissolved}$) in the supernatant of the culture media without algal cells was higher than the $[Cu]_{dissolved}$ in the media with algal cells. In the absence of the cells, dissolved organic matter (DOC) increased the dissolution of the particle due to increasing the stability of the particles against aggregation, thus increasing the available surface area. In the presence of algae, Cu^0-ENPs heteroaggregated with the cells. Thus, the available surface area decreased over time and this resulted in a low dissolution rate of the particles. The DOC corona on the surface of the particles increased the heteroaggregation of the particles with the cells and decreases the uptake of the particles. Our findings showed that microorganisms influence the fate of ENPs in the environment, and they do so by modifying the dissolution and aggregation kinetics of the Cu^0-ENPs.

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1. Introduction

In the last decades, the application of engineered nanoparticles (ENPs) in a range of consumer and industrial products is rapidly increasing. As a consequence, the environmental release of these ENPs upon production, manufacturing and application is practically inevitable (Bihan et al., 2013). This has generated an increase in the concern about the behaviour and fate of these ENPs in the environment, which are still not well-known, although considerable information regarding these topics have been generated during the last years (Chen et al., 2016; Ehret et al., 2014; Monikh et al., 2018).

Copper (Cu) ENPs are increasingly used ENPs in different fields, such as cosmetic, electronic, biomedical and environmental (Pu et al., 2016). As a consequence of their high production and use, these ENPs (and metal ENPs in general) may be released to the environment and affect the ecosystems, mainly aquatic systems and sediments (Peijnenburg et al., 2015; Wiesner et al., 2006). Currently, there are many studies focusing on studying the physicochemical behaviour and fate of Cu ENPs and/or their toxicity in different organisms (Dobrochna et al., 2018; Griffitt et al., 2007; Pu et al., 2016; Sharma et al., 2015; Song et al., 2015; Wang et al., 2014; Xiao et al., 2018; Zhu et al., 2017), although more studies focused specifically on the effects of the different physicochemical water properties are needed to understand the toxicity effects of Cu ENPs. As it is known, dissolution and aggregation of ENPs can both be affected by physicochemical parameters of aquatic systems, such as pH, ionic strength and natural organic matter (NOM) (Keller et al., 2010; Li et al., 2012, 2010), and also by the physicochemical properties of the ENPs, such as size, shape and chemical composition (Cornelis et al., 2014; Lead et al., 2018; Lowry et al., 2010). Thus, it is critical to understand the aggregation and dissolution processes of ENPs and the influence of the different physicochemical parameters on them to their influence on their uptake and toxicity in the aquatic environment (Keller et al., 2010). For example, NOM can stabilise particles and prevent their aggregation in suspension (Abdolahpur Monikh et al., 2018). By increasing the stability of the ENPs, a large surface area of the ENPs is available for dissolution (Wang et al., 2015; Wang et al., 2011; Arenas-Lago et al., 2019). Thus, the NOM corona (formation of a shell of NOM molecules on the surface of NP) may catalyse the dissolution of ENPs (Arenas-Lago et al., 2019). According to the basic principles of colloidal science, increasing ionic strength in a system leads to aggregation of particles due to the screening of the double layers as described by DLVO theory (Everett, 1988). Aggregation, as a result, decreases the specific surface area of the particles and subsequently reduces the rate of dissolution of the particles (Adeleye et al., 2014; Zhang et al., 2010).

Hence, to date, the focus of most of the studies that investigated dissolution and aggregation of ENPs is shifted towards understanding the influence of physicochemical parameters of the system on the behaviour of particles under simplified mono-parameter conditions. It remains largely unknown what is the joint effect of physicochemical parameters on the aggregation/dissolution behaviour of ENPs. The role of microorganisms on the underlying process is also largely disregarded despite the fact that microorganisms are ubiquitous. Moreover, microorganisms have very high surface-to-volume ratios (Baker et al., 2014), which may increase the possibility of cell-particle interactions. Literature showed that cell-particle interactions may lead to aggregation, known as heteroaggregation (Ge et al., 2015), or particle uptake by the cells (Ma et al., 2015). It is, however, unexplored how and to which extent cell-particle interactions influence the fate of ENPs, particularly the dissolution of ENPs.

This study is based on two main hypotheses extracted from the literature after reviewing most of the existing papers in the field of ENPs fate/behaviour in the environment. On the bases of the existing data, it is first expected that ENPs in natural ecosystems are subject to heteroaggregation with microorganisms such as algae and bacteria (Ge et al., 2015). We demonstrate that heteroaggregation of ENPs with algae decreases the particle specific surface area and reduces particle dissolution. Secondly, it is expected that the presence of NOM will hinder the aggregation of ENPs (Arenas-Lago et al., 2019), while NOM-catalysed dissolution may still take place. We demonstrated that algae accumulate the ions which are released from the surface of NOM-stabilised particles. In the presence of algae, thus, dissolution of NOM-stabilised particles increased.

The main objectives of this study were to investigate the algae-catalysed dissolution, heteroaggregation and uptake of Cu^0-ENPs (as a representative of ENPs) in a model system mimicking natural surface waters using the micro-algae *Pseudokirchneriella subcapitata* (a representative of microorganism). Dissolution was assessed as a function of the joint effects of algae, NOM and ionic strength at pH 7.5.

2. Material and methods

2.1. Materials

All chemicals used in this study were reagent grade. Optima grade hydrochloric acid (HCl 30%) and nitric acid (HNO_3 65%) were purchased from Merck (Suprapure®, USA). Sodium hydroxide (NaOH) and copper nitrate ($CuNO_3$) were purchased from Sigma-Aldrich (Sigma-Aldrich Corp., St. Louis, MO, USA).

In this study, 25 nm spherical Cu^0-ENPs with a specific surface area of 30–50 m^2/g were purchased from IoLiTec-Ionic Liquids Technologies GmbH. Suwannee River NOM was supplied by the International Humic Substances Society (1R101N).

2.2. Characterization of the Cu^0-ENPs

The hydrodynamic size and the zeta potential of the Cu^0-ENPs dispersed in Milli-Q (MQ) water were measured using a Zetasizer Nanodevice (Malvern Panalytical, NL) with a He—Ne laser 633 nm. A JEOL 1010 Transmission Electron Microscopy (TEM) was used to measure the particle size and to observe the particle shape and the interaction of the particles with cells.

2.3. Test medium preparation

A stock dispersion of Cu^0-ENPs (250 mg/L) was prepared by dispersing the ENPs in MQ water. The dispersions were sonicated using a SONOPULS ultrasonicator (BANDELIN electronic. Berlin, Germany) at 100% amplitude tip with for 10 min. A 1000 mg/L stock solution of ionic Cu ($CuNO_3$) was prepared and stored for further use. A stock solution (500 mg/L) of Suwannee River NOM was prepared with MQ water (Supporting Information). The obtained suspension, which was reported as dissolved organic carbon (DOC) in this study, was pH-adjusted (pH 8) to represent the natural conditions and stored at 4 °C until use. Concentrations of $CaCl_2$ and $MgSO_4$ used to set the ionic strength were selected in a fixed content molar ratio of 4:1 (Ca^{2+}/Mg^{2+}) according to the method reported by Arenas-Lago et al. (2019). A ratio of 4:1 (Ca^{2+}/Mg^{2+}) is mimicking natural conditions, as reported in the literature (Abdolahpur Monikh et al., 2018). We used either 0.1 M NaOH or 0.1 M HCl to change the pH of the solution.

2.4. Assessment of Cu^{2+} ions fate and dissolution of Cu^0-ENPs in the culture media

To each medium, DOC was added in different concentrations of 0, 5, 20 or 50 mg/L and Ca^{2+}/Mg^{2+} in total concentrations of 0, 2.5 or 10 mM to mimic natural conditions at pH 7.5 as reported by Arenas-Lago et al. (2019). Aliquots of the sonicated stock dispersion were taken and added to each testing culture medium to reach a final concentration of 1 mg/L of Cu^0-ENPs. Ionic Cu were also taken and added to each medium, separately, to test the interactions between Cu^{2+} ions and algae. The dissolution kinetic experiment was performed over 32 h (the duration was arbitrarily selected), establishing different sampling times: 0, 4, 8, 24 and 32 h after preparation of the suspensions. All tests were performed in triplicate. The total Cu in the medium was measured over time by taking an aliquot from each medium and digesting using HNO_3. The samples were analysed using a flame atomic absorption spectrometer (FAAS; Perkin Elmer AAnalist 100) to measure total Cu. To separate dissolved Cu ($[Cu]_{dissolved}$) and particulate Cu, aliquots of each dispersion were taken from the supernatant (top 5 cm) at each time point (0, 4, 8, 24 and 32 h) and centrifuged at 4000 rpm for 30 min at 4 °C according to the method reported by Arenas-Lago et al. (2019). The supernatants were taken and analysed using a FAAS after acid digestion. Changes in the $[Cu]_{dissolved}$ concentrations in the samples allow examining the dissolution kinetics within 32 h of exposure.

2.5. Influence of algae cells on the fate of cu ions and on dissolution kinetics of Cu^0 ENPs

The unicellular algae *Pseudokirchneriella subcapitata* was cultured (see the Supporting Information) and used as the test microorganism. To study the dissolution kinetics and the uptake of Cu^0-ENPs by algae in the systems, the method reported by Wang et al. (2011) was used after modification. Along with the samples, six samples were tested as control; three containing no DOC, electrolytes and Cu^0-ENPs and three containing no Cu^0-ENPs but DOC and electrolytes. To monitor dissolution, aliquots were carefully taken from the supernatant at each time point (after 0, 4, 8, 24 and 32 h of incubation) and centrifuged at 4000 rpm for 30 min at 4 °C. After centrifugation, the supernatants were separated, digested with HNO_3, and analysed for $[Cu]_{ion}$ using FAAS. The total Cu in the samples (exposure media) was measured after acid digestion.

2.6. Observing and measuring the cell wall bound and intracellular Cu^0-ENPs

To assess the heteroaggregation between ENP and algal cells and the particles bound to the algae, a test medium containing no DOC and Ca^{2+}/Mg^{2+} was used to culture the algal cells. Culture media containing DOC was not used because the presence of DOC complicates the observation of ENP-cell interactions using TEM. About 200 μL of the dispersion of the particles and algal cells were pipetted onto copper grids. The grids were kept in darkness at room temperature for 24 h to allow the samples to dry. The images were obtained at 70 kV accelerating voltage.

The resulting pellets of algal cells from the previous section (Section 2.5) were treated with 5 mL of 0.02 M EDTA to complex the Cu bound to the cell walls (Wang & Xing, 2011). After 20 min, the suspensions were centrifuged at 4000 rpm for 10 min. The supernatants were collected and after acid digestion, the concentration of Cu was measured using FAAS.

After separation of the Cu bound reversibly to the cell walls, the remaining algal pellets were acid-digested for 3 h. MQ water was added to the residues to reach a volume of 20 mL. The Cu concentration in the resulting samples was measured using FAAS. This provides the intracellular and hence the bioavailable Cu content.

2.7. Statistics and data analysis

Data were analysed statistically with the statistical program SPSS v. 19. Data are expressed as the average ± standard deviation (SD) of three replicates. Kolmogorov-Smirnov and Levene tests were applied to check the normality and homogeneity of variances, respectively. ANOVA and Duncan's multiple range tests were used to compare the differences between groups ($p < 0.05$). Pearson correlation coefficients were applied to examine the relationship between DOC concentration, electrolyte concentration, and the amount of Cu attached to the algal cells.

3. Results and discussion

3.1. Characterization of Cu^0 ENPs

The Cu^0-ENPs were characterised in dispersions containing only MQ water. We measured the hydrodynamic (D_h) and the TEM-measured size immediately after sonication to reduce the aggregation time or fast transformation of the particles. The DLS data showed that D_h increased over time. After 1 h, the particle D_h was between 278 nm and 425 nm. The averaged zeta potential value of about -3.4 ± 0.4 mV was measured by electrophoretic mobility and indicates that the particles are prone to aggregation as the value of the zeta potential is close to zero (Monikh et al., 2018). The obtained TEM picture showed an immediate aggregation of the particles (Fig. S1, Supporting Information).

3.2. Influence of algal cells on particle dissolution in culture media

We measured the $[Cu]_{dissolved}$ in the culture media (with and without algal cells) at different contents of DOC and Ca^{2+}/Mg^{2+} (Fig. 1) to determine how the presence of algae affects the dissolution of Cu^0-ENPs. The initial concentration of the Cu^0-ENPs in the exposure media was 1 mg/L. The controls showed that there were no differences in the number of cells cultured in the testing media due to the presence of DOC and Ca^{2+}/Mg^{2+}.

The $[Cu]_{dissolved}$ in the supernatant of the culture media without algal cells were higher than the $[Cu]_{dissolved}$ observed in the media containing algae (Fig. 1). In the culture media without algae, the $[Cu]_{dissolved}$ increased over 32 h in all conditions, while the $[Cu]_{dissolved}$ decreased in the presence of algae, except for media containing 50 mg/L DOC and 0 mM Ca^{2+}/Mg^{2+}. Three explanations can be put forward: i) the $[Cu]_{dissolved}$ during the 32 h exposure period are taken up by algae. ii) dissolution did not take place or was lower than in the media without algae. As previously reported by Adeleye et al. (2016), lower dissolution of ENPs in culture media with high algae densities could be attributed to a high ENP-cell heteroaggregation. Heteroaggregation between Cu^0-ENPs and the cells is described in detail in the next section. iii) The decrease in the $[Cu]_{dissolved}$ over time may result from the growth of the algae population by time as this also results in a low concentration of dissolved oxygen available for the oxidation of the Cu particles (Reinsch et al., 2010). Because the dissolution of Cu^0-ENPs in water is an oxidative process (Wang et al., 2016) following the stoichiometry below (Eq. (1)):

$$Cu^0(s) + 1/4\, O_2\,(aq) + H^+\,(aq) \leftrightarrow Cu^+\,(aq) + 1/2\, H_2O \quad (1)$$

In a similar study with iron oxide, Gonzalez et al. (1989) reported that oxidation rates of Fe^{2+} decreased as the mass concentration of algal cells increased. However, after 14 days of exposure, there was no obvious difference in the level of dissolved Fe in all the conditions (Adeleye et al., 2016).

In the media without algal cells, increasing the concentration of DOC also increased the $[Cu]_{dissolved}$ in the supernatant. In our previous study, we showed that DOC can increase the dissolution of Cu^0-ENPs in MQ water (Arenas-Lago et al., 2019). In this study, we observed that the DOC can also catalyse the dissolution of Cu^0-ENPs in culture media. As it was expected, DOC increases the stability of the Cu^0-ENPs, favouring the dissolution of the Cu^0-ENPs due to the large available surface area (Wang et al., 2015; Wang et al., 2011; Arenas-Lago et al., 2019). It is also possible that the complexation of functional groups of DOC with Cu^0-ENPs surfaces weakens the surface Cu—Cu and Cu—O bonds (Aiken et al., 2011; Korshin et al., 1998; Wang et al., 2015; Arenas-Lago et al., 2019). Thus, the detachment of the DOC from the surface of the particles may lead to enhanced dissolution through ligand promoted processes (Misra et al., 2012). In the media containing 10 mM of Ca^{2+}/Mg^{2+} and no DOC, the $[Cu]_{dissolved}$ is higher compared to media containing 0 and 2.5 mM Ca^{2+}/Mg^{2+}. The $[Cu]_{dissolved}$ in some cases is even higher than in the media containing DOC, where DOC-

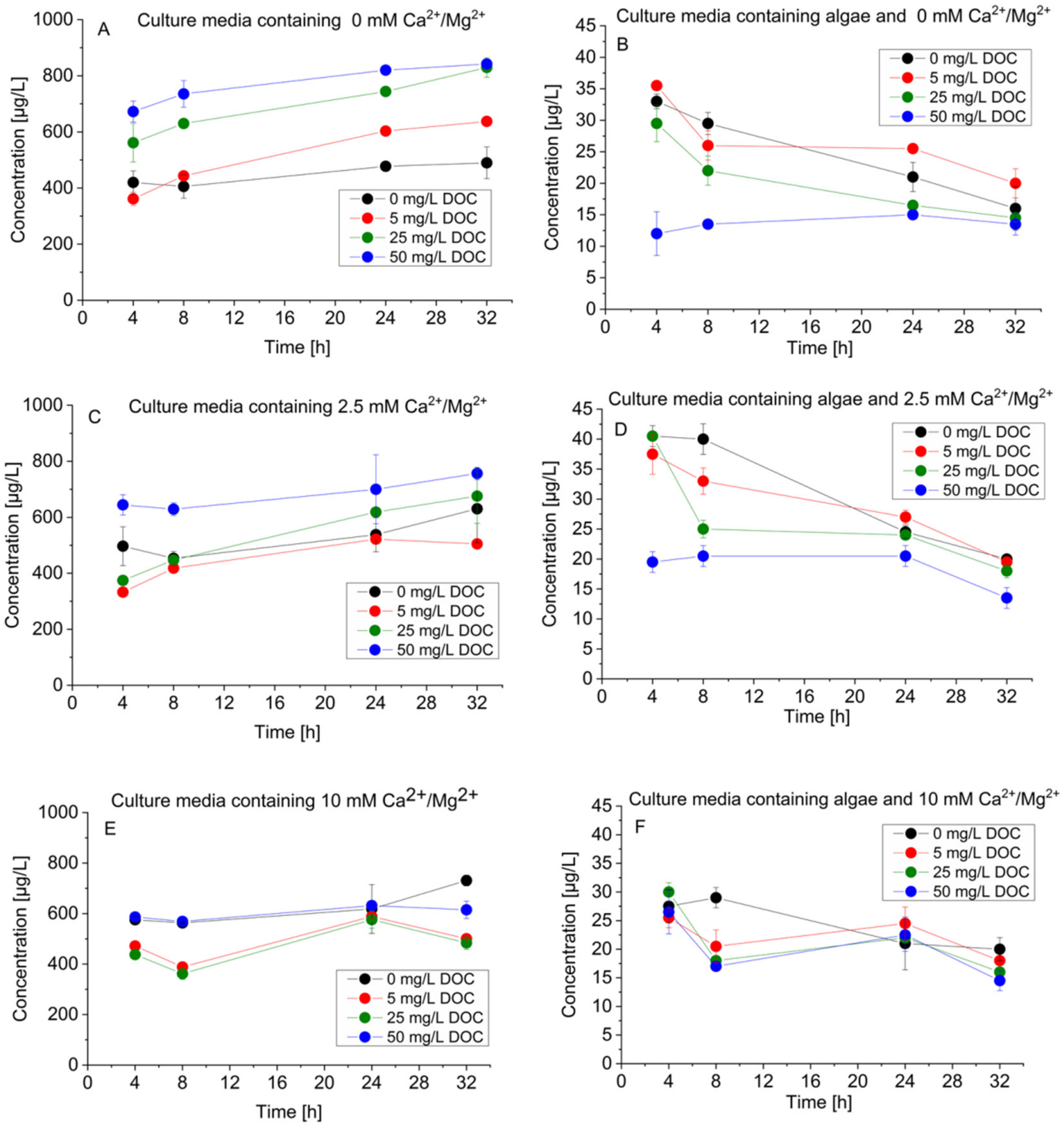

Fig. 1. Concentration of Cu dissolved $[Cu]_{dissolved}$ from Cu^0 ENPs in the culture media without (A,B and C) and with (D,E and F) algal cells as a function of time with different DOC and Ca^{2+}/Mg^{2+} contents. The culture media are mimicking natural surface waters and contain different DOC concentrations and different ionic strength at pH 7.5.

catalysed dissolution takes place (Fig. 1). In the media without DOC, the particles are positively charged (Table S1, Supporting Information). As we observed in our previous study (Arenas-Lago et al., 2019), an increase in the concentration of Ca^{2+}/Mg^{2+} increases the positive charge of the Cu^0-ENPs (Table S2, Supporting Information) due to specific interactions of Ca^{2+} with the Cu^0-ENPs (Monikh et al., 2018). Due to the repulsive electrostatic force between the particles, Cu^0-ENPs remain stable against aggregation and, consequently, dissolution of the Cu^0-ENPs increases.

When algae were present in the culture media, an increase in the concentration of the DOC reduced the concentration of $[Cu]_{dissolved}$ in the culture media (Fig. 1). The concentration of $[Cu]_{dissolved}$ as a function of DOC in all media containing algae was as follows: 5 mg/L > 25 mg/L > 50 mg/L DOC. These results are opposite to those indicated by (Wang et al., 2011), who found that DOC (fulvic acids) increases the $[Cu]_{dissolved}$ in the culture media. This disagreement could be due to the different types of DOC used in these two studies.

We calculated the dissolution rates ($k_{dissolution}$) of the Cu^0-ENPs in the culture media without algal cells and the data are reported in units of $ng/cm^2/h$ in Table 1. We observed that as the concentration of the Ca^{2+}/Mg^{2+} increases in the media, the $k_{dissolution}$ decreases. The influence of DOC on the $k_{dissolution}$ is less pronounced at 10 mM Ca^{2+}/Mg^{2+}.

3.3. Attachment of Cu^{2+} and Cu^0-ENPs to algal cells

In this section, the adsorption of Cu^{2+} to the surface of the algae over time was investigated to study the differences between Cu^{2+} and Cu^0-ENPs attachments. The quantities of Cu^{2+} attached to the surface of

Table 1
Dissolution rates ($k_{dissolution}$) of Cu^0-ENPs in the culture media without algae up to 32 h.

Concentration of Ca^{2+}/Mg^{2+} — DOC added	Culture media containing no algae
	($ng\ cm^{-2}\ h^{-1}$) | R^2
0 mM–0 mg/L	7.5 | 0.91
0 mM–5 mg/L	24 | 0.96
0 mM–25 mg/L	22 | 0.98
0 mM–50 mg/L	14 | 0.94
2.5 mM–0 mg/L	12.5 | 0.80
2.5 mM–5 mg/L	15 | 0.82
2.5 mM–25 mg/L	26 | 0.98
2.5 mM–50 mg/L	10 | 0.92
10 mM–0 mg/L	13 | 0.82
10 mM–5 mg/L	9 | 0.4
10 mM–25 mg/L	11 | 0.43
10 mM–50 mg/L	4.5 | 0.65

the algae are reported in Fig. 2. The amount of Cu^{2+} attached to the algae showed two distinct adsorption patterns. Increasing the concentration of DOC in the medium decreased the concentration of Cu^{2+} attached to the algae. Previous studies, (Ma et al., 2003; Wang et al., 2011) reported that NOM increased the cell-wall-bound Cu^{2+} which subsequently increased the toxicity of Cu^{2+} to algae. Although they are in disagreement with our findings, this disagreement could be related to the type of algae and/or the NOM used in the studies as we have filtered the NOM to obtain the DOC fraction of the NOM.

To study the heteroaggregation of ENPs with algae, the algae exposed to Cu^0-ENPs were analysed using TEM (Fig. 3 and Fig. S2 in the Supporting Information). The figure shows that aggregates and unbound particles are attached to the surface of algal cells after 4 h of exposure.

As shown in Fig. 4, in general, the $[Cu]_{total}$ attached to the algal cells exposed to Cu^0-ENPs increased over time in all media. The concentration of attached Cu to algal cells showed a clear correlation with the amount of DOC in the media as increased concentrations of DOC were accompanied by increased $[Cu]_{total}$ attached to the algal cells ($R^2 = 0.99$, $p < 0.05$). The highest concentration of the attached $[Cu]_{total}$ to algal cells was in the media with 50 m/L DOC and followed by media containing 25, 5 and 0 mg/L DOC. Likewise, Ma et al. (2003) indicated that NOM favoured the cell-wall-bound Cu^{2+}, which is consistent with the explanation above. This pattern was observed in all media regardless of the concentration of Ca^{2+}/Mg^{2+} in the media. This can explain our finding in the previous section, where increasing the level of DOC in the culture media with algal cells reduced the dissolution of the Cu^0-ENPs. The DOC corona on the surface of the particles increases the heteroaggregation of the particles with the cells. This is different from the pattern observed for Cu^{2+} attachment, where a higher concentration of DOC decreases the level of attached Cu to the surface of the cells. Future studies may focus on this topic that how DOC can increase the heteroaggregation of ENPs with algal cells despite the fact that DOC are reported to act as natural stabilizer of ENPs against aggregation (Abdolahpur Monikh et al., 2018).

In all media with 50 mg/L DOC, the $[Cu]_{total}$ attached to algal cells showed an increase even at the last sampling point (32*h*). Similar trends were observed for media containing 25 mg/L DOC at 2.5 and 10 mM Ca^{2+}/Mg^{2+}. However, in the media with ≤5 mg/L DOC, regardless of the concentrations of Ca^{2+}/Mg^{2+}, the level of cell-bound $[Cu]_{total}$ reached a peak at 24 h. After 24 h, the $[Cu]_{total}$ decreased. It is likely that the Cu^0-ENPs are taken up by the algal cells after 24 h and the presence of DOC on the surface of the particles decreases the particle uptake, as was also reported previously by Mensch et al. (2017). The uptake of the particles by algae is described in the next section.

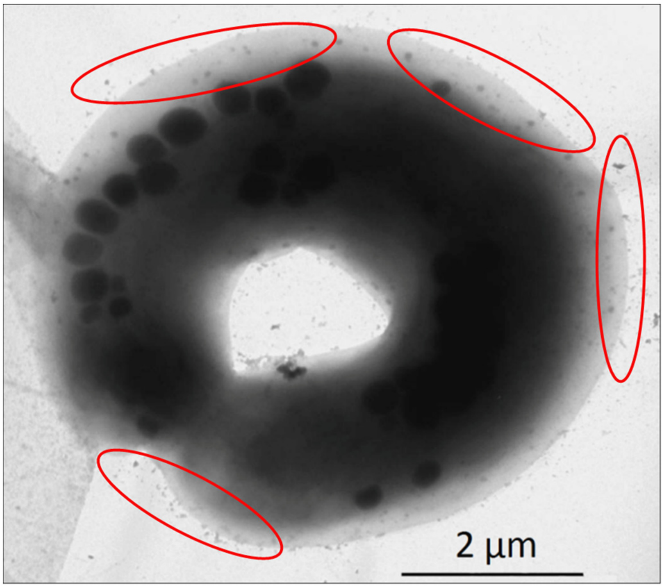

Fig. 3. TEM image of an algal cells which was exposed to Cu^0-ENPs (1 mg/L) in the absence of DOC. The picture shows that the cell is surrounded by single particles and aggregates of Cu^0-ENPs of different sizes. Some Cu^0-ENPs and aggregates are attached to the surface of the cell showing the heteroaggregation of these ENPs with the cells (e.g. red circles).

3.4. Internalization of total cu by algae exposed to Cu^{2+} or Cu^0-ENPs

In order to understand the internalization of Cu^0-ENPs by algae, it is first of all important to understand the uptake of the ionic form of Cu by the algae, and therefore to differentiate between the uptake of particulate and ionic form of the Cu. Accordingly, algal cells were exposed to

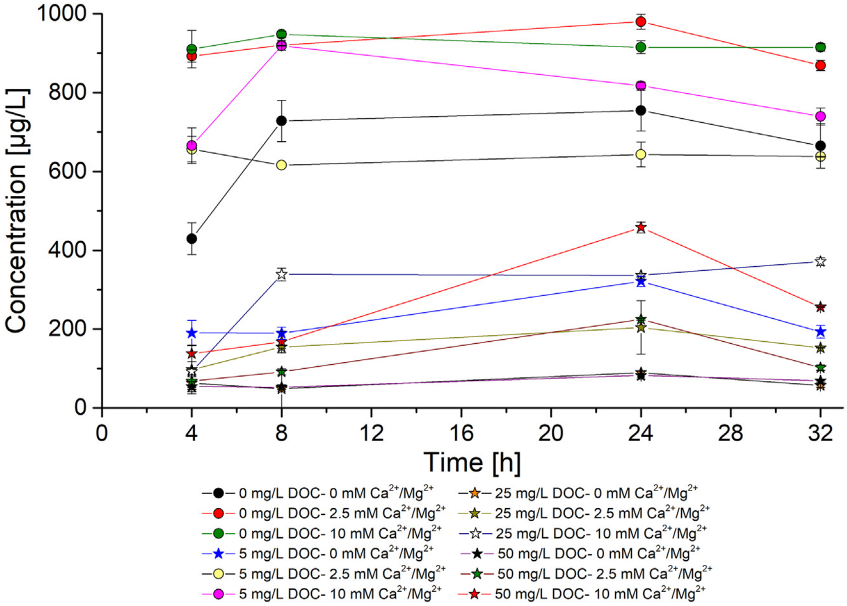

Fig. 2. Total concentration of Cu adsorbed to the surface of the algal cells during 32 h of exposure of the cells to Cu^{2+} in the media culture as a function of DOC concentration and ionic strength.

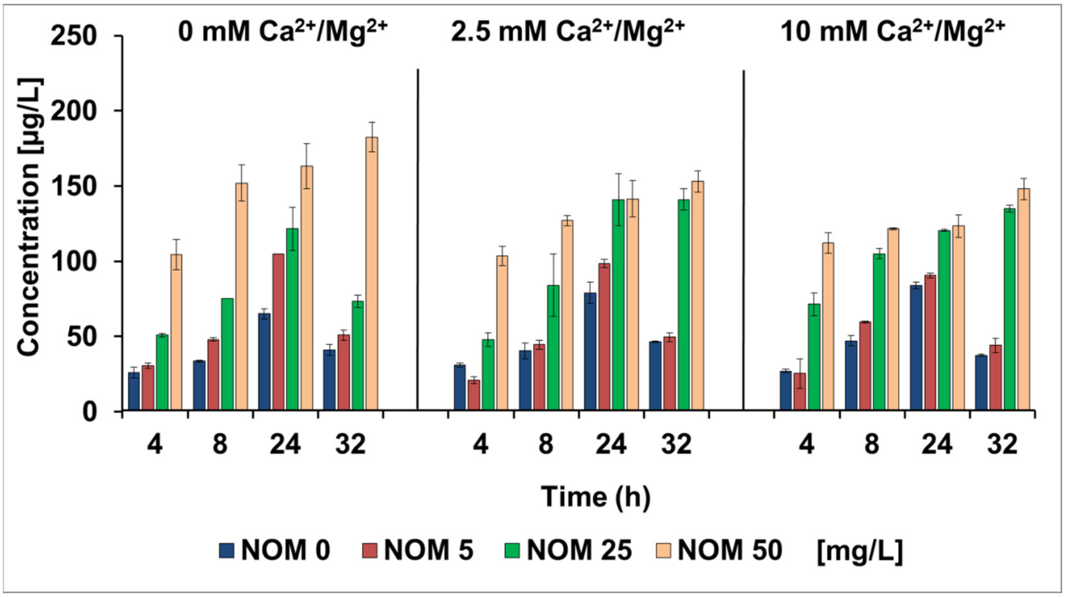

Fig. 4. The concentration of total Cu attached to the surface of algal cells exposed to Cu^0-ENPs in different culture media contain 0, 5, 25 or 50 mg/L DOC and 0, 2.5 or 10 mM Ca^{2+}/Mg^{2+} during 32 h of exposure.

Cu^{2+} under the same conditions as used for the Cu^0-ENPs. The concentration of Cu in the supernatant (Fig. 5a) in units of µg/L and the $[Cu]_{total}$ accumulated in the cells (Fig. 5b) in units of µg Cu per mg (µg/mg) of dry biomass were measured, respectively. The results show that the concentration of Cu^{2+} in the suspension decreases over 32 h, except in a few cases (Fig. 5a), since the algae are accumulating Cu over time. This is confirmed by the results reported in Fig. 5b, where the concentration of Cu in the algae increases over time.

Two distinct scattering patterns in the data were observed which were a function of the composition of the medium (Fig. 5a). In the presence of DOC, the concentration of Cu^{2+} in the supernatant is high. It is reported that Cu^{2+} has a high affinity for organic ligands to form insoluble complexes (Mudunkotuwa et al., 2012). When DOC is present, Cu^{2+} complexes with carboxylic and phenolic functional groups available on the DOC. By increasing the DOC concentration, the concentration of Cu^{2+} complexed with functional groups increases, and ultimately Cu^{2+} becomes unavailable to algae. The results also showed that by increasing the ionic strength in the medium, the concentration of Cu^{2+} in the supernatant decreases. This is also expected because Ca^{2+}/Mg^{2+} added to the system competes with Cu^{2+} for sorption to the functional groups available on the surface of DOC (Davis, 1984). This renders Cu^{2+} available to algae. Rippner et al. (2018) reported that DOC forms complexes with Cu and decreases the toxicity of both CuO NPs and free ionised Cu to duckweed. These authors concluded that DOC changes the Cu speciation and therefore the toxicity of Cu in natural systems as predicted by speciation modelling software.

The $[Cu]_{total}$ in the cells exposed to Cu^0-ENPs is reported in Fig. 6. The pattern of uptake of the Cu^0-ENPs was different in comparison to the pattern observed for the uptake of Cu^{2+}. After 4 h of exposure, Cu in the media with 25 mg/L DOC treatment was found to accumulate more inside the algae than in any other media; 53, 55 and 44 µg Cu/mg dry weight for media containing 0, 2.5, and 10 mM Ca^{2+}/Mg^{2+}, respectively. After 24 h, the internalised $[Cu]_{total}$ increases in algae incubated in media with 0 and 5 mg/L DOC. These data confirmed that the attached Cu on the algae incubated in media with ≤5 mg/L DOC are taken up by the cells after 24 h. This shows that the DOC corona reduces the uptake of Cu^0-ENPs by cells.

4. Conclusions

We have investigated the joint effects of NOM (as DOC), ionic strength and algal cells, as a representative of microorganisms, on the

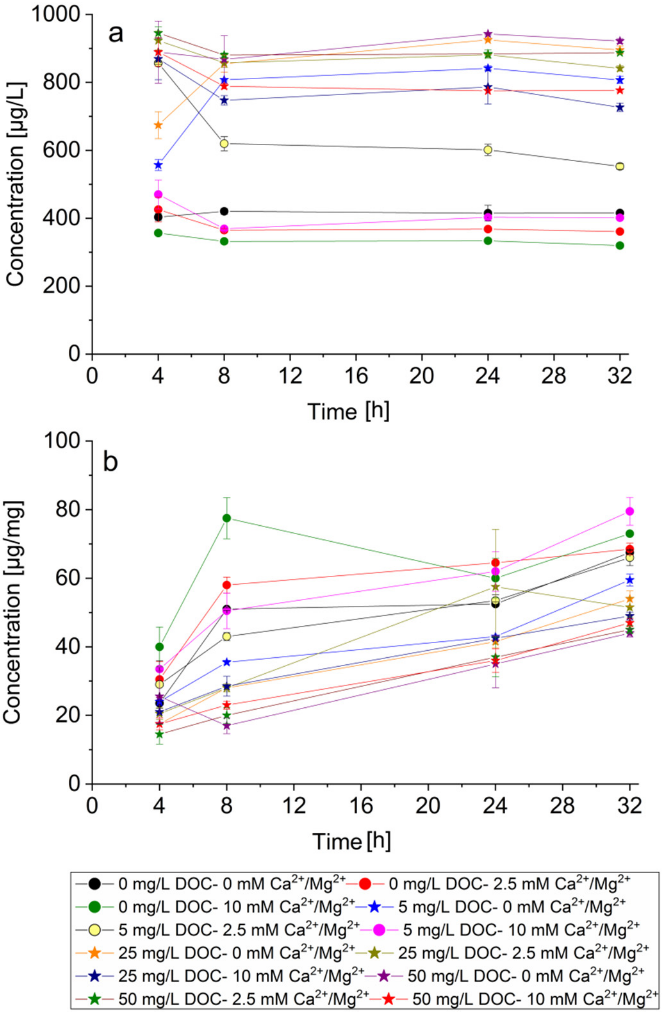

Fig. 5. The interaction of algal cells with Cu^{2+} in the media culture as a function of DOC concentration and ionic strength during 32 h of exposure. The amount of Cu (µg/L) in the supernatant in the presence of algal cells and the concentration of Cu (µg/mg) accumulated in the algal cells are reported in graphs a and b, respectively.

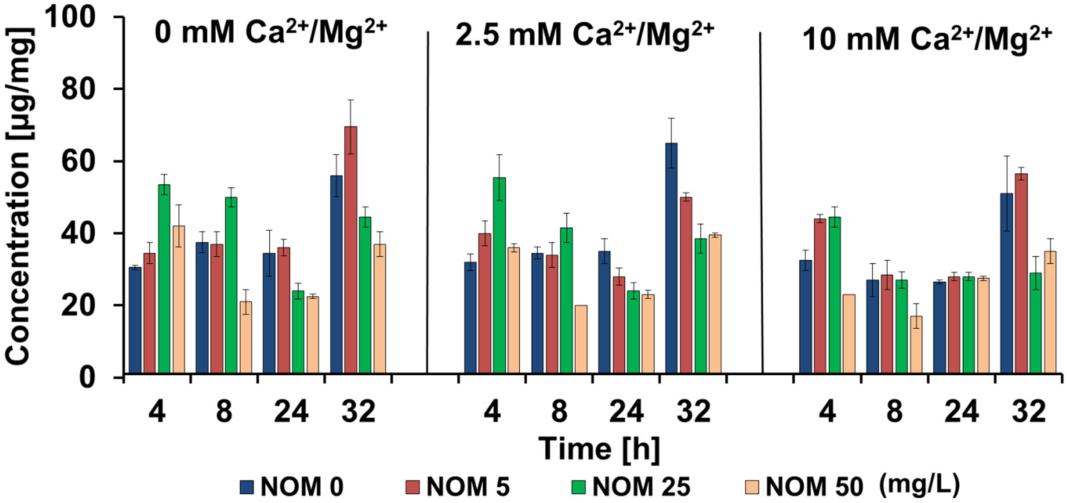

Fig. 6. The concentration of total Cu (μg Cu/mg dry weight) accumulated in algal exposed to Cu^0-ENPs in different culture media mimicking natural surface waters.

dissolution, heteroaggregation and the uptake of Cu^0-ENPs in natural surface water. Our finding showed that the presence of algae cells decreases the dissolution of the Cu-ENPs. DOC increases the dissolution of Cu^0-ENPs by reducing the aggregation of the particles and also through ligand-promoted dissolution. However, in the presence of algal cells, increasing the concentration of DOC decreased the amount of the dissolved Cu in the media. The particles were heteroaggregate with the cells and the heteroaggregation increased with enhancing the level of DOC in the media. As a consequence, the particles dissolution decreases. The uptake of the Cu-ENPs by algal cells showed a dynamic pattern. Increasing the concentration of DOC decreases the amount of Cu taken up (25 mg/L) and increase the uptake of Cu in other cases (≤ 5 mg/L). The finding of our study showed the importance of considering the microorganisms in investigating and modelling the fate of ENPs because they, directly and indirectly, influence the stability behaviour of the ENPs in the environment. It also shows that studies performed in simplified condition without microorganisms are depicting the general and unrealistic processes occurring in natural condition. Our study can facilitate the movement towards a more complex condition with respect to microorganisms as seen under natural conditions.

Declaration of Competing Interest

There are no conflicts to declare.

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Appendix A. Supplementary data

Preparation of the NOM and the cell culture. Fig. S1: A Transmission Electron Microscope (TEM) picture shows the immediate aggregation of the Cu0-ENPs particles. Table S1: size and zeta potential of the Cu ENPs in MQ water. Table S2: Zeta potential and the standard deviation for the Cu0-ENPs in different media. Heteroaggregation of Cu_0-ENP and algal cells. Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.06.388.

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