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Toxicity of selenium nanoparticles on *Poterioochromonas malhamensis* algae in Waris-H culture medium and Lake Geneva water: Effect of nanoparticle coating, dissolution, and aggregation

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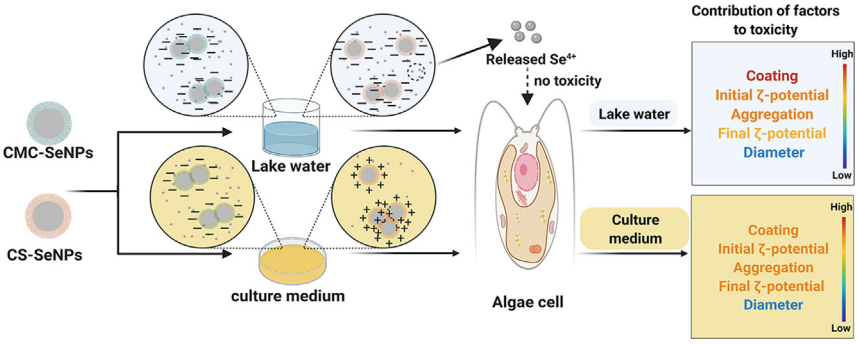
HIGHLIGHTS

- Exposure media of SeNPs should include culture and natural environmental media.
- SeNPs are 5–10 times more toxic in Lake Geneva water compared to culture medium.
- Surface coating is the most influential toxicity SeNPs factor in Lake Geneva water.
- SeNPs themselves are the main toxicity driver on algae but not released ions.

GRAPHICAL ABSTRACT



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ABSTRACT

Understanding the algal toxicity of selenium nanoparticles (SeNPs) in aquatic systems by considering SeNPs physicochemical properties and environmental media characteristics is a concern of high importance for the evaluation and prediction of risk assessment. In this study, chitosan (CS) and sodium carboxymethyl cellulose (CMC) coated SeNPs are considered using Lake Geneva water and a Waris-H cell culture medium to investigate the effect of SeNPs on the toxicity of algae *Poterioochromonas malhamensis*, a widespread mixotrophic flagellate. The influence of surface coating, z-average diameters, ζ-potentials, aggregation behavior, ions release, and medium properties on the toxicity of SeNPs to algae *P. malhamensi* was investigated. It is found that SeNPs are 5–10 times more toxic in Lake Geneva water compared to the culture medium, suggesting that the traditional algal tests in Waris-H culture medium currently underestimate the toxicity of NPs in a natural water environment. Despite significant dissolution, it is also found that SeNPs themselves are the toxicity driver, and dissolved ions have only a marginal influence on toxicity. SeNPs diameter is found a minor factor in toxicity. Based on a principal component analysis (PCA) it is found that in Lake Geneva water, the nature of the surface coating (CMC versus CS) is the most influential factor controlling the toxicity of SeNPs. In the culture medium, surface coating, ζ-potential, and aggregation are found to contribute at the same level. These results highlight the importance of considering in details both NPs intrinsic and media properties in the evaluation of NPs biological effects.

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1. Introduction

Selenium (Se) is an essential element for life, existing in nature in four forms: selenate (+VI, SeO_4^{2-}), selenite (+IV, SeO_3^{2-}), elemental selenium (0, Se^0), and selenide (−II, Se^{2-}) (Pyrzynska, 2002; Xu et al., 2021). The ratio of these selenium species in nature depend on pH, the presence of complexing agents or dissolved gases (especially oxygen), and suspended matter (Pyrzynska, 2002; Sharma et al., 2015). In aquatic environments and soil under most natural redox conditions, the Se (VI) and Se (+IV) are dominant inorganic Se species (El-Ramady et al., 2014; Fordyce, 2007). Selenide mainly exists in organic compounds (such as, Selenomethionine, Selenocysteine) in organisms and stable as metal selenides in strongly reducing conditions (Tan et al., 2016). Se^0 are the main Se species in sediment of lake, and comprised about 30%–60% of the total Se in sediment (Pettine et al., 2015; Wiramanaden et al., 2010). The toxicity of Se is related to dose and chemical forms; high levels of Se are toxic and cause environmental problems (Lemly, 2004; Santos et al., 2015). Selenium nanoparticles (SeNPs) with zero-value state are currently at the forefront of materials research, attracting much attention in antitumor, antibacterial, and antioxidant ability (Jamroz et al., 2018; Palomo-Siguero et al., 2017; Song et al., 2020; Zhai et al., 2017). In addition, from an environmental point of view, SeNPs have been applied to remediate elemental mercury (Hg^0) contaminated groundwater, air and soil to form mercuric selenide (HgSe) (Fellowes et al., 2011; Wang et al., 2017; Wang et al., 2018). SeNPs are naturally present in the environment and biological organisms such as plants, fungi, or soil bacteria can reduce selenite (SeO_3^{2-}) or selenate (SeO_4^{2-}) ions to biogenic SeNPs due to the presence of reducing enzymes in organisms (Hosnedlova et al., 2018; Li et al., 2021). Synthesized and biogenic SeNPs are then expected to be present and transported in various aqueous systems, and for risk assessment, the toxicity and stability of SeNPs in these systems has to be evaluated.

With bioaccumulation, concentrations of Se are also expected to increase through food chains (Santos et al., 2015). Algae, which is at the bottom of the aquatic food chain, play a vital role in the ecotoxicological risk of Se owing to the fact that they biologically transform inorganic Se into organic selenide compounds (Fan et al., 2002; Ponton et al., 2020). It was also found that SeNPs exhibit greater toxicity than selenite on aquatic organisms (such as Zebrafish embryos, Medaka fish, and *Vibro fischeri*) (Afzal et al., 1999; Li et al., 2008; Mal et al., 2017). However, few studies have paid attention to the toxicity of SeNPs in algae cells.

Water chemistry is expected to have significant effects on algal toxicity of NPs (Joonas et al., 2019). Most researches related to algae toxicity have been conducted in culture medium without considering the difference between natural water environments and culture media. Indeed, cellular damages caused by NPs may be reduced by dissolved natural organic and inorganic compounds present in natural aquatic systems at variable concentrations resulting in lower toxicity (Eigenheer et al., 2014). Moreover, previous studies showed that NPs exposed in standard *Daphnia* salt-only media overestimates the toxicological effects to *Daphnia magna* compared to natural aquatic environment (Ellis et al., 2020). Therefore, it is important to evaluate the potential toxicity of SeNPs to algae cells not only in culture media but also in natural waters.

It should also be noted that natural aquatic parameters have significant effects on the NPs aggregation behavior and NPs ion release (dissolution) hence playing an important role in determining their toxicity (Jung et al., 2018; Oriekhova and Stoll, 2018). Aggregated NPs are less mobile, less bioavailable, and potentially less toxic than dispersed NPs (Booth et al., 2015; Ramirez et al., 2019). In addition, depending on media oxidation reduction potential, SeNPs have the possibility to be oxidized to Se^{4+} in the aquatic environment (Zhang et al., 2004b). Therefore, to assess the risk of SeNPs, detailed information on aggregation and dissolution characteristics of SeNPs in different aquatic environments are also needed.

Surface coating is often necessary to improve the stability of SeNPs because of the high surface energy of bare SeNPs causing fast aggregation (Song et al., 2021). However, the coating of NPs is also expected to influence the physicochemical properties and ecotoxicological characteristics of NPs (Saavedra et al., 2019; Wu et al., 2020). Polysaccharides have been widely used as common coating polymers to prepare stable and uniformly dispersed SeNPs with low toxicity and biocompatibility (Chen et al., 2015; Zhang et al., 2004a). Sodium carboxymethyl cellulose (CMC) is composed of D-glucose molecules, whose hydroxyl groups are substituted with carboxymethyl groups; while chitosan (CS) is composed of glucosamine and *N*-acetyl glucosamine. CMC and CS are common polysaccharides used to prepare SeNPs and have been considered in the treatment of cancer, bacterial infections and application in aquaculture (Hegerova et al., 2017; Song et al., 2020; Xia et al., 2019). Unfortunately, limited information is available on the behavior and impact of coated SeNPs in the environment. In particular, there is a lack of research on the toxicity of these coated nanoparticles to algae cells, which will provide a better understanding of ecotoxicological effect of engineered SeNPs on aquatic organisms.

Since the toxicity of NPs in aquatic environments is driven by intrinsic and extrinsic factors, including their intrinsic properties, stability regarding aggregation, and the properties of the surrounding aquatic environment, such as pH and ionic strength, comprehensive and statistical assessments are required to isolate the important factors related to the NPs toxicity (Nagy et al., 2012; Zhang et al., 2016). In such conditions, principal component analysis (PCA) can be used to reduce the dimensionality of data and calculate the contribution of variables to a component (Wang et al., 2014b).

In the light of all these considerations, in this study, CS and CMC were first used as stability agents to prepare coated CS-SeNPs and CMC-SeNPs. Then, the aggregation behavior, surface charge modifications in presence of natural organic matter and ions, dissolution, and algae toxicity of CMC-SeNPs and CS-SeNPs were investigated to gain insight into the effect of different surface coatings, dissolution, and aggregation of SeNPs on algae toxicity in a natural water environment (Lake Geneva water). A comparison was then made with culture medium of *P. malhamensis*, a widespread mixotrophic flagellate (Wei et al., 2020), which was exposed to different concentrations of CMC-SeNPs and CS-SeNPs in Lake Geneva water and culture medium for 24 h and 48 h. The contribution of hydrodynamic diameters, surface charge, aggregation behavior, and coating to toxicity were compared in Lake Geneva water and culture medium using PCA.

2. Materials and methods

2.1. Chemicals

Selenite (H_2SeO_3) was purchased from Chemie Brunschwig (Basel, BS, Switzerland). Ascorbic acid was purchased from Sigma Aldrich (Buchs, SG, Switzerland). Chitosan with a molecular weight of 700 kDa was purchased from Jinan Haidebei Co., Ltd. (Shandong, China), and the deacetylation degree was over 85%. Sodium Carboxymethylcellulose with a molecular weight of 600 kDa was purchased from Shanghai Macklin Biochemical Co. Ltd. (Shanghai, China), and substitution degree was 0.9. Diluted sodium hydroxide (NaOH) and hydrochloric acid (HCl) were purchased from Merck (Titrisol®, Zoug, ZG, Switzerland). *Poterioochromonas malhamensis* (CCAC 3498 strain) was purchased from Central collection of algal cultures (Essen, Germany). Hydrogen peroxide solution (≥30 Wt%) and nitric acid (65 Wt%) for analysis were purchased from Fluka company (Washington, American).

2.2. Preparation of SeNPs

SeNPs were prepared through the chemical reduction of selenite by ascorbic acid (Zhang et al., 2015). Selenite (20 mmol/L, 10 mL) was mixed with 10 mL CMC or CS solution (500 mg/L) used as a stabilizer, then stirred 5 min at 600 rpm. 10 mL of 80 mmol/L ascorbic acid and 70 mL water were added to the above solution. After stirring for 3 h at room temperature, 30 mL of SeNPs solution was dialyzed in a dialysis bag with a 3000 Da molecular weight cut-off for 48 h, then SeNPs solution was transferred to a 50 mL volumetric flask and make up to 50 mL. The pH was adjusted to 3.0 for CS-SeNPs and 10.0 for CMC-SeNPs adding

small amounts of NaOH and HCl (0.5 M and 0.05 M) respectively. The stock suspension of SeNPs was stored in the dark at 4 °C.

2.3. Characterization of SeNPs

The hydrodynamic diameters of SeNPs in stock solution were obtained with dynamic light scattering (DLS) and nanoparticle tracking analysis (NTA) technique. DLS measurements were performed with a Zetasizer Nano ZS (Malvern Instruments Ltd., UK) at 25 °C. Three measurements of ten runs were performed for each sample. NTA measurements were performed with a NanoSight instrument (LM14, Nanosight Ltd., Amesbury, UK). SeNPs stock solution was diluted 100 times, and was then measured for 60 s at room temperature. The analytical Software (NTA 2.3) was used to obtain and analyze the data. Transmission electron microscopy (TEM) analysis was performed using a Hitachi JEM 1200EX (Hitachi and High Technologies America, Inc. USA). The concentration of the SeNPs stock solution was measured by Inductively Coupled Plasma Mass Spectrometry (ICP-MS, 7500 Series, Agilent, USA). 0.1 mL SeNPs stock solution mixed with 0.8 mL nitric acid and 1.0 mL hydrogen peroxide was heated for 60 min at 100 °C. The solution was made up to 5 mL, then was measured by ICP-MS.

2.4. ζ-potential and z-average hydrodynamic diameter variation of SeNPs as a function of pH in ultrapure water

12.5 mg of SeNPs /L was prepared by diluting 10 mL of stock solution in 90 mL ultrapure water. NaCl at a concentration of 1 mM was used as the background electrolyte. The suspension pH of SeNPs was changed by adding small volumes of diluted NaOH and HCl (0.01 M) to reach pH values in the range of 3–11. The pH was determined by a pH meter (Mettler Toledo, Columbus, USA). ζ-potentials and z-average hydrodynamic diameters of SeNPs solution with different pH were measured in triplicate with a Malvern Zetasizer Nano ZS at 25 °C after adjusting the pH of the suspension.

2.5. ζ-potential and z-average hydrodynamic diameters of SeNPs in ultrapure water, Geneva Lake water, and Waris-H culture medium

5 mg of SeNPs/L was dispersed in ultrapure water, Lake Geneva water (pH 8.5 ± 0.1, conductivity 279 ± 1 μs/cm) and culture media (pH 7.0 ± 0.2, conductivity 672 ± 1 μs/cm). The pH of ultrapure water was adjusted to the same values as Lake Geneva water and culture medium respectively to use as a comparison. The composition of Lake Geneva water and culture medium are given in supplementary Tables S1 and S2. Lake Geneva water was collected from Lake Geneva (Geneva, Switzerland), and was then filtered through a 0.2 μm filter (Millipore, Switzerland) in triplicate. ζ-potentials and z-average hydrodynamic diameters were measured every 20 min for 2 h. Various concentrations of SeNPs (0.5, 1.0, 5.0, 10.0 mg/L) were used in the culture medium and Lake Geneva water. The z-average hydrodynamic diameter and ζ-potential variations were measured at 0, 2, 24, and 48 h.

2.6. Dissolved Se^{4+} ions concentration

5.0 mg of SeNPs/L was chosen for the dissolution study because in such conditions NPs exhibited better stability in culture medium and Lake Geneva water. 5.0 mg of SeNPs /L was then prepared by diluting the stock solution in ultrapure water, Lake Geneva water, and culture medium. The concentration of the dissolved Se in the SeNPs suspension was determined at 0, 24, and 48 h. To this end, a 3 mL sample was transferred to an Ultra-15 centrifugal filter device (Millipore Corp., Ann Arbor, MI, USA) with a 3000 Da molecular weight cut-off (Song et al., 2020; Zhang et al., 2015). During centrifugation (Ultracentrifuge Beckman Coulter® Optima L-100 XP) at 7500 ×g for 30 min, 2 mL free Se solution was mixed with 0.1 mL nitric acid and 0.5 mL hydrogen peroxide, then was heated for 1 h at 100 °C (Xiao et al., 2021; Zhang and Tang, 2020). The solution was completed to 5 mL and analyzed using ICP-MS.

2.7. Algal viability assay

P. malhamensis were maintained in a modified Waris-H culture media at 25 °C with a light illumination (5 μmol photons m^{-2} s^{-1}, light: dark of 12: 12 h) in a specialized incubator (MIR 253, Sanyo, Japan). *P. malhamensis* was pre-cultivated for 6 days before testing. The cells were harvested at the mid-exponential phase and redispersed in the Waris-H culture medium and Lake Geneva water. Before dispersing into Lake Geneva water, the cells were washed twice with Lake Geneva water to remove the culture medium and collected by centrifugation at 3059 ×*g* for 10 min.

SeNPs stock suspension was added to Waris-H culture medium and Lake Geneva water with cell density of 1×10^6 cells mL^{-1} for the final concentration ranging from 0.05 to 10.0 mg/L. At the end of 24 and 48 h incubation time, the mixture was analyzed using a BD Accuri C6 flow cytometer (BD Biosciences, San Jose, CA). Further, the concentration inducing the mortality in 50% of the algal (EC_{50}) was calculated from probit analysis. The concentrations of the selenite solutions at 1.0 and 10.0 mg/L were used to evaluate the potential toxic contribution of Se ions dissolved from SeNPs.

2.8. Statistical analysis

All results and data were obtained through at least three independent experiments. All data is unit variance scaled and centered with the mean. Statistical significance was performed using one-way analysis of variance (ANOVA) using Origin software (Version 9, Originlab Corporation, USA). On the other hand, Principle component analysis (PCA) was performed with RStudio software (Version 1.4.1106). To obtain the contribution of parameters to toxicity in Lake Geneva water or culture medium, CMC-SeNPs and CS-SeNPs each of four concentrations and two-incubation time (24 h or 48 h) with total 16 samples were used to carry out the analysis. We assumed the data to evaluate aggregation to correspond to the difference between SeNPs hydrodynamic diameters in the aquatic environment at 0 h and the hydrodynamic diameters at 24 or 48 h. The parameters corresponding to coating were respectively set to 10.0 and − 10.0 for CMC-SeNPs and CS-SeNPs. Initial ζ-potentials of SeNPs corresponded to the ζ-potentials of SeNPs measured at 0 h in aquatic environment, and the final ζ-potentials corresponded to the ζ-potentials of SeNPs measured at 24 or 48 h. All data was first scaled and mean centered for PCA. A level of $P < 0.05$ was considered significant and statistically significant differences were indicated with an asterisk.

3. Results and discussion

3.1. Nanoparticle characterization

CMC and CS were chosen as stabilizers and capping agents to synthesize CMC-SeNPs and CS-SeNPs. The pH of CMC-SeNPs and CS-SeNPs stock suspension was adjusted to 10.0 and 3.0 to obtain stabilized SeNPs, respectively. The z-average hydrodynamic diameters of CMC-SeNPs and CS-SeNPs in the stock suspensions measured by DLS are found equal to 180.7 ± 4.5 nm and 80.5 ± 2.8 nm (Fig 1(a)). However, the major drawback of DLS is that small amounts of aggregation can overestimate sizes (Filipe et al., 2010). Therefore, for the determination of accurate sizes, hydrodynamic diameters of SeNPs were also measured by NTA. Results show one peak for CS-SeNPs at 73.0 nm in good agreement with the result of DLS, indicating that CS-SeNPs are monodispersed. On the other hand, three peaks of CMC-SeNPs at 73.0, 157.0, and 197.0 nm indicate the presence of monomers, dimers, and trimers of CMC-SeNPs at pH 10.0 (Fig. 1b). These findings, which were also supported by TEM measurements (Fig. 1c and d), indicate the presence of dimers and trimers in CMC-SeNPs stock solution and are explaining the significant increases in the hydrodynamic

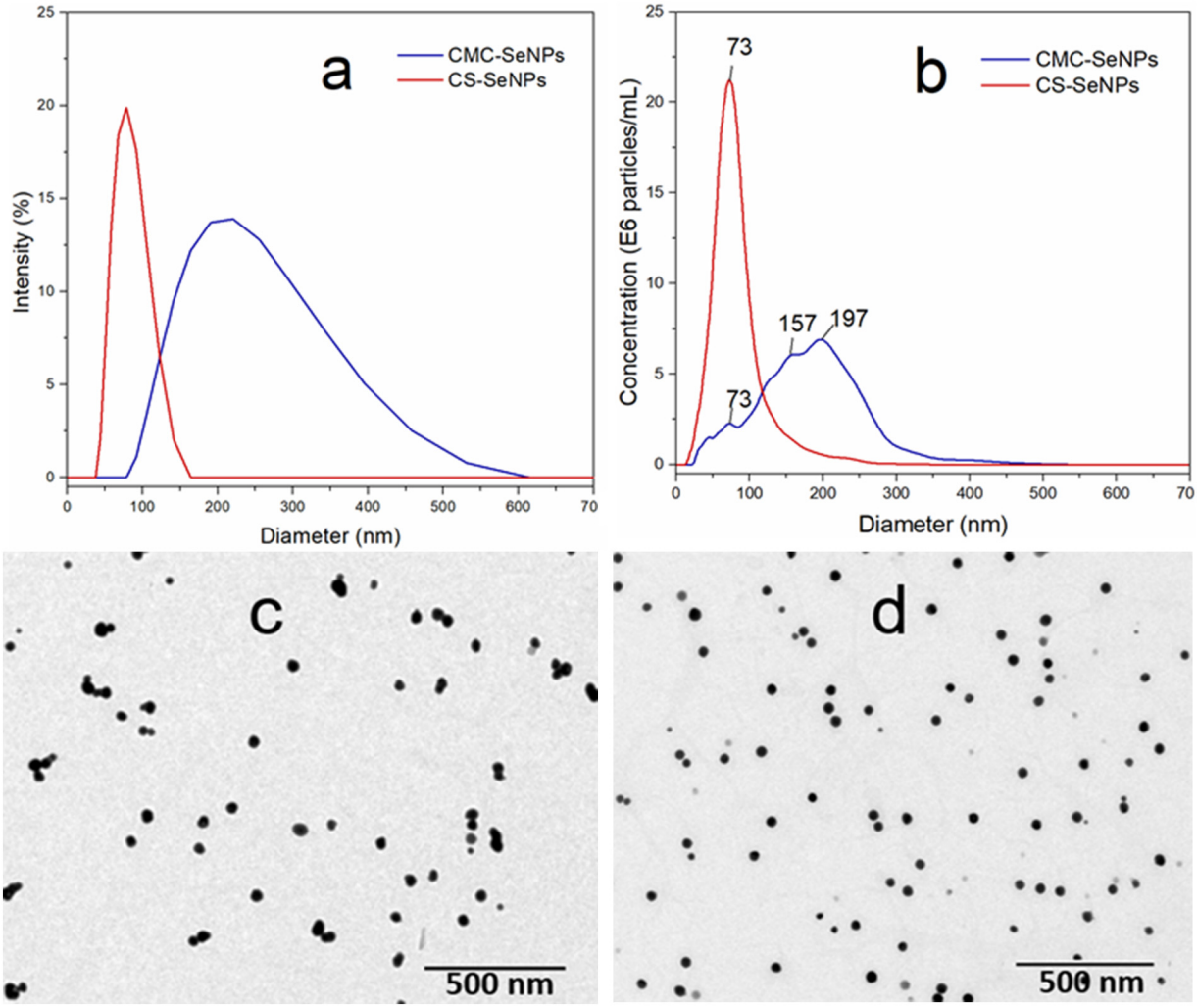

Fig. 1. Particle size distributions and TEM images of CMC-SeNPs and CS-SeNPs. The particle size distributions of CMC-SeNPs and CS-SeNPs are obtained by (a) Dynamic Light Scattering (DLS) and (b) Nanoparticle Tracking Analysis (NTA). TEM images of CMC-SeNPs and CS-SeNPs are given in (c) and (d) respectively.

diameters of CMC-SeNPs obtained from DLS. No great difference is found in the primary particle size of CMC-SeNPs and CS-SeNPs. According to the total selenium content measured by ICP-MS, stock dispersion concentrations of both CMC-SeNPs and CS-SeNPs are found equal to 127.7 ± 0.9 mg/L and 125.5 ± 3.9 mg/L, respectively.

3.2. Influence of pH on the ζ-potential and z-average hydrodynamic diameter of SeNPs in ultrapure water

To investigate the behavior of CMC-SeNPs and CS-SeNPs in the different media, both z-average hydrodynamic diameters and ζ-potential were measured from pH 3.0 to pH 11.0 at a concentration of 12.5 mg/L. Ultrapure water was first considered as a reference case. As shown in Fig. 2, the ζ-potential of CMC-SeNPs is equal to −15.1 ± 0.6 mV at pH 3.0 and increases to −41.1 ± 0.5 mV at pH 6.0 due to the presence of ionized carboxymethyl groups on the surface of the CMC. CMC-SeNPs are found stable from pH 3.0 to pH 11.0 due to the presence of negative charges resulting in electrostatic repulsions. The slow and limited increase of z-average hydrodynamic diameters from pH 3.0 to pH 6.0 is here expected to be caused by the swelling of carboxymethyl groups (Yadollahi et al., 2015). On the other hand, CS-SeNPs, at pH 3.0, exhibit positive charges due to the presence of NH_4^+. ζ-potentials of CS-SeNPs continuously decrease to ~0.0 mV from pH 3.0 to pH 8.0 by converting NH_4^+ to NH_3. The pH of the point of zero-charge (pH_{PZC}) for CS-SeNPs is obtained at pH 7.75 ± 0.25. When the pH is further increased (pH > pH_{ZCP}), the surface charge of CS-SeNPs is reversed to negative values due to deprotonation processes of NH_4^+ and negative charge of hydroxyl surface groups (Beurer et al., 2012).

3.3. Behavior of SeNPs in Lake Geneva water and culture medium

To help in the evaluation of the important factors playing a role in SeNPs toxicity, detailed information on the stability of SeNPs in the different media is needed. The pH of Lake Geneva water and culture medium are respectively 8.5 ± 0.1 (> pH_{ZCP} of CS-SeNPs) and 7.0 ± 0.2 (< pH_{ZCP} of CS-SeNPs). The pH of ultrapure water was respectively adjusted to the same value with Lake Geneva water and culture medium to use as a comparison. As shown in Fig. 3a, CMC-SeNPs at 5 mg/L are negatively charged in all samples with z-average diameters of CMC-SeNPs equal to 164.9 ± 6.6 nm for 2 h indicating stability in both Lake Geneva water and culture medium. The ζ-potentials of CMC-SeNPs in the culture medium and Lake Geneva water are found lower than in ultrapure water at similar pH values because of the specific adsorption of positively charged ions (such as Ca^{2+}, Mg^{2+}) on the NPs surface.

In Lake Geneva water, CS-SeNPs exhibit a hydrodynamic diameter of 97.6 ± 2.9 nm, and no significant change of the diameters is observed after 2 h (Fig. 3b). CS-SeNPs display a negative charge in Lake Geneva water similarly to ultrapure water (pH 8.5), and in agreement with the results obtained in Fig. 2. The z-average diameters of CS-SeNPs in culture medium increase from 106.6 nm at 0 h to 133 nm at 2 h. Since no change of z-average diameters is observed in ultrapure water (pH 7), the slow increase

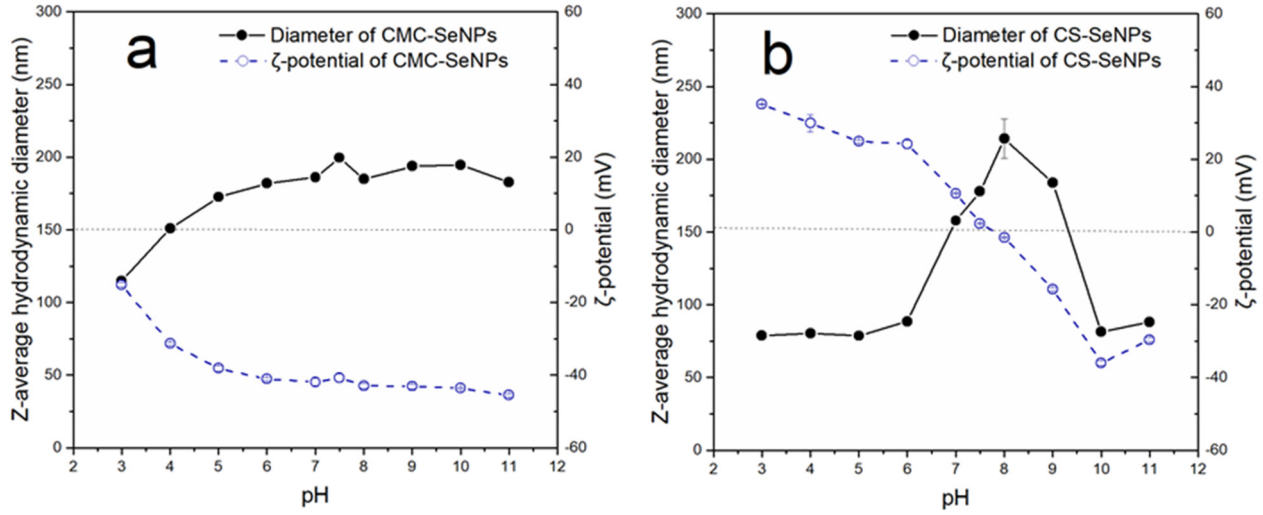

Fig. 2. Effects of pH in ultrapure water on z-average hydrodynamic diameters and ζ-potentials of (a) CMC-SeNPs and (b) CS-SeNPs.

in culture medium is likely due to the presence of suspended organic polymer and/or dissolved ions in the culture medium, which promotes the formation of small aggregates. A negative charge of CS-SeNPs in the culture medium is not in agreement with the fact that the pH of the culture medium is lower than the pH_{ZCP} of CS-SeNPs. Adsorption of organic molecules including protein and amino acids present in the culture medium promote the formation of negative charge surface of the CS-SeNPs in culture medium (Ortelli et al., 2017).

Owing to the fact that the toxicity of NPs is both concentration and time-dependent, the stability of SeNPs in exposure media at various NPs concentrations and at 0, 2, 24, and 48 h incubation time were also considered. As shown in Fig. 4a and b, in Lake Geneva water at 0.5 mg/L, z-average hydrodynamic diameters of CMC-SeNPs increase from 143.4 ± 4.3 nm at 0 h to 256.5 ± 31.4 nm at 48 h, and z-average hydrodynamic diameters of CS-SeNPs increase from 106 ± 4.5 nm at 0 h to 445.0 ± 22.8 nm at 48 h. The ζ-potential values of CMC-SeNPs and CS-SeNPs at 0.5 mg/L are around −5 mV. Aggregation of SeNPs in Lake Geneva water at 0.5 mg/L is related to a decrease of the electrostatic repulsions between NPs which is likely due to the divalent cation adsorption to the negatively charged CMC-SeNPs and CS-SeNPs. At 5.0 and 10 mg/L, z-average hydrodynamic diameters of CMC-SeNPs and CS-SeNPs remain constant for 48 h in Lake Geneva water, and the ζ-potentials of both CMC-SeNPs and CS-SeNPs are higher than −13 mV.

In the culture medium, all diameters of CMC-SeNPs remain constant. The ζ-potentials increase by increasing concentration, and decrease with time due to the absorption of positively charged ions. CS-SeNPs remain stable at 0.5 and 1.0 mg/L. When the concentration of CS-SeNPs is higher than 5 mg/L, the z-average diameters of CS-SeNPs increase with time, and the diameters reach about 400 nm at 48 h. Such a behavior is due to concentration effects accelerating the kinetics of aggregation.

In general, it was found that NPs concentration is a factor determining the aggregation of SeNPs in exposure media. Low concentrations of SeNPs in Lake Geneva water easily aggregate, while aggregation of CS-SeNPs occurs at higher concentration levels in culture medium. Also, it was found that CMC-SeNPs present significantly better stability than CS-SeNPs due to higher negative surface charge.

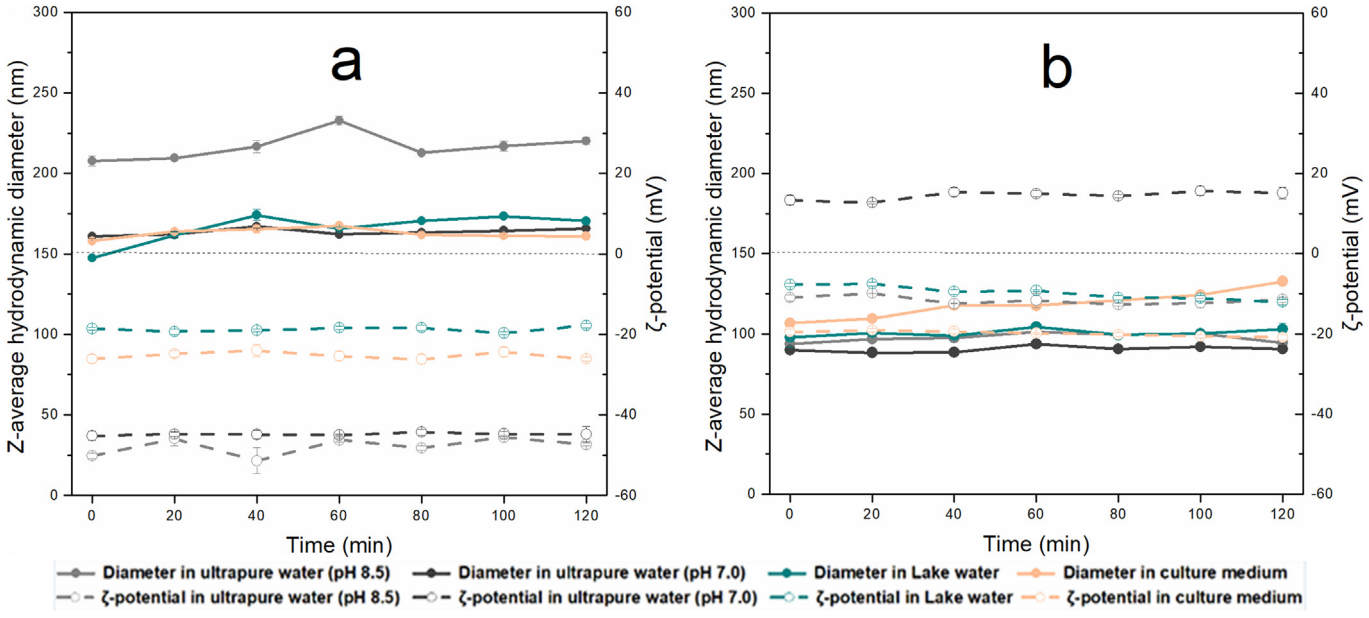

Fig. 3. *Z*-average hydrodynamic diameters and ζ-potentials of (a) CMC-SeNPs and (b) CS-SeNPs in ultrapure water (pH 8.5 and 7.0), Lake Geneva water (pH 8.5) and culture medium (pH 7.0) at 5.0 mg/L as a function of time.

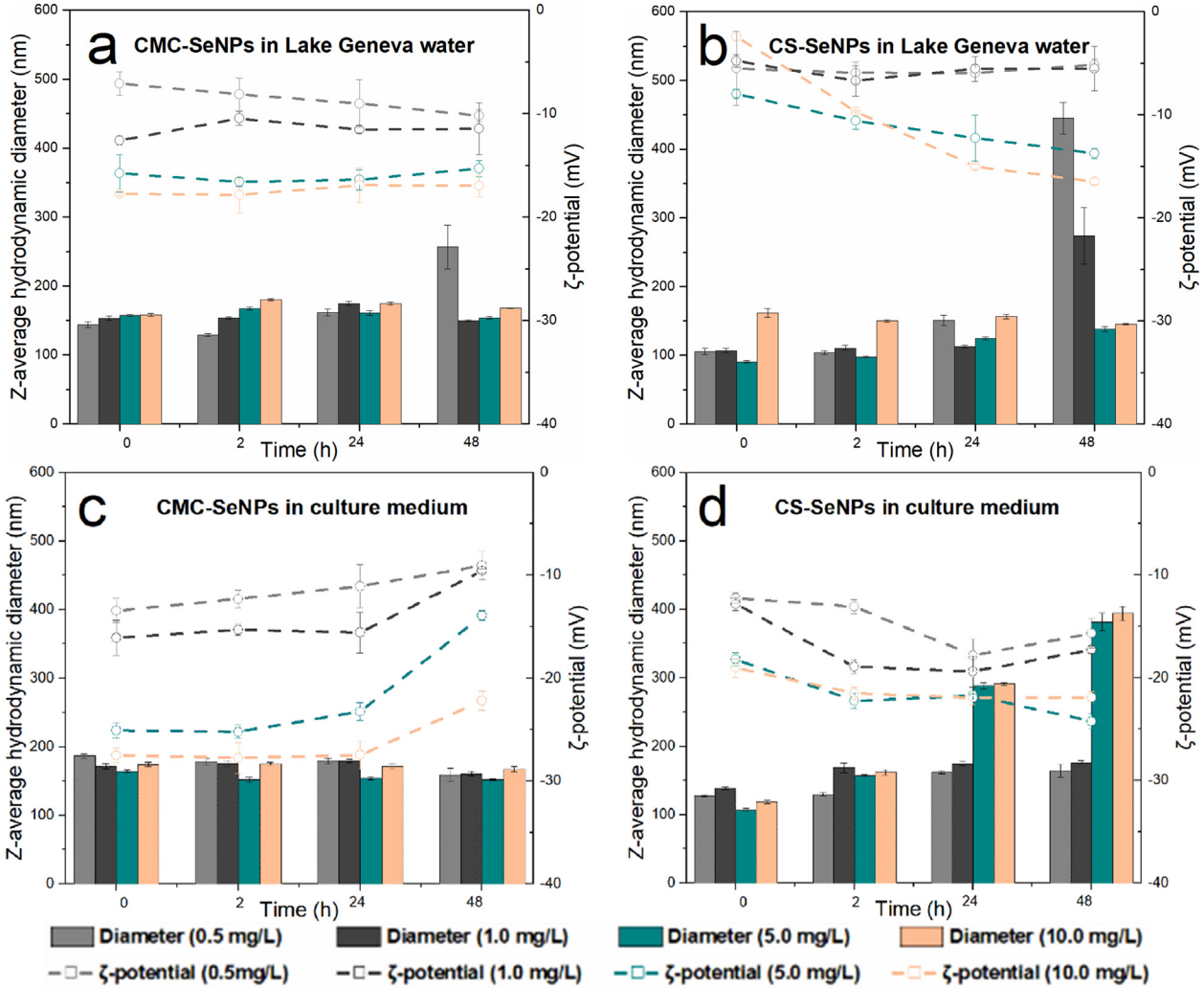

Fig. 4. Z-average hydrodynamic diameters and ζ-potentials of (a) CMC-SeNPs and (b) CS-SeNPs in Lake Geneva water at concentration 0.5, 1.0, 5.0, and 10.0 mg/L at 0, 2, 24, 48 h. Z-average hydrodynamic diameter and ζ-potentials of (c) CMC-SeNPs and (d) CS-SeNPs in culture medium at concentration 0.5, 1.0, 5.0, and 10.0 mg/L at 0, 2, 24, and 48 h.

3.4. Release of selenium ions from SeNPs in Lake Geneva water and culture medium

Dissolved selenium concentration was determined at 0, 24, and 48 h at 25 °C which were the same incubation times and temperature than algae toxicity experiments. As presented in Fig. 5, a time-dependent dissolution of SeNPs is observed in ultrapure water, culture medium, and Lake Geneva water. After 48 h, CMC-SeNPs (Fig. 5a) exhibit the higher dissolution rate in the Lake Geneva water (35.3 ± 1.4%) and culture medium (33.9 ± 2.2%) compared to ultrapure water (20.8 ± 1.3%). No significant difference in the dissolution rate of CMC-SeNPs between Lake Geneva water and culture medium was observed. CS-SeNPs in the Lake Geneva water have a higher dissolution behavior (31.03 ± 1.7% at 48 h) than in ultrapure water (20.9 ± 0.3% at 48 h). The higher percentage of selenium ion release is expected to be due to the formation of Selenium-NH_3, Selenium-OH, or Selenium-COOH complexes with suspended natural organic matter present in Lake Geneva water (such as humic residues, and polysaccharides) and culture medium polymers (such as proteins, amino acids, vitamins and carbohydrates) favoring the increase of Se^{4+} ions release from SeNPs (Johnston et al., 2018). However, it should be noted that CS-SeNPs in the culture medium provide significantly lower dissolution rates (11.5 ± 0.7% at 48 h) than in Lake Geneva water and ultrapure water, which is due to the adsorption of negatively polymers at the positively charged surface of CS-SeNPs hence limiting the dissolution of CS-SeNPs. The effect of dissolved selenium on the algal toxicity of SeNPs is discussed in the next section.

3.5. Adverse effects of SeNPs on algal cell viability in Lake Geneva water and culture medium

To determine whether the observed differences in particle stability and dissolution behavior result in a toxicity potential to algal, the cell viability was determined into Lake Geneva water and culture medium at 24 and 48 h incubation (Table 1). As shown in Fig. 6, two types of SeNPs induce statistically significant diminution of cell viability with a dose-time response relationship. EC_{50} of CMC-SeNPs and CS-SeNPs in the Lake Geneva water at 24 h is 0.60 ± 0.05 mg/L and 0.50 ± 0.01 mg/L, respectively. The EC_{50} of CMC-SeNPs and CS-SeNPs in culture medium is 6.20 ± 0.11 mg/L and 2.50 ± 0.12 mg/L, respectively. Compared with CMC-SeNPs, CS-SeNPs in the culture medium show significantly higher toxicity via positive charge connecting with the negatively charged cell surface. Interestingly, in Lake Geneva water, CS-SeNPs with negative surface charge still have higher toxicity than negative charged CMC-SeNPs, but the toxicity difference is not as significant as in culture medium, and no difference was found between CMC-SeNPs and CS-SeNPs at a concentration of 5 mg/L at 24 h. Besides, SeNPs in Lake Geneva water exhibit 5–10 times higher toxicity than that in the culture medium, probably own to rich biomolecules from the culture medium which obstructed cellular damages caused by NPs (Ellis et al.,

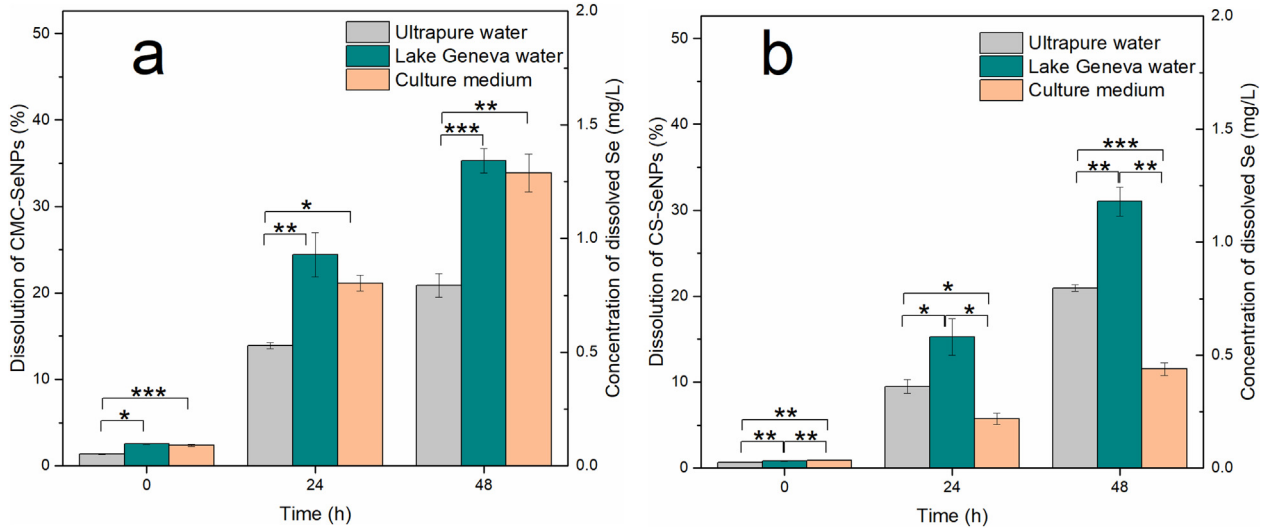

Fig. 5. Dissolution percentage of (a) CMC-SeNPs and (b) CS-SeNPs dispersed in ultrapure water (pH 7), Lake Geneva water, and culture medium for the 0, 24, and 48 h period at a concentration of 5.0 mg/L. Asterisks denote a significant difference between release results in different aquatic systems: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

2020; Joonas et al., 2019). The pH was the dominant factor in toxicity of NPs by influencing the adsorption of natural organic matter to the surface of NPs (Van Hoecke et al., 2011). Therefore, the traditional algal tests in Waris-H culture medium currently here underestimate the toxicity of NPs compared to the realistic water environment.

We also tested the toxicity of Se^{4+} on algae at concentrations 1.0 and 10.0 mg/L in Lake Geneva water and culture medium at 24 and 48 h incubation period to understand the toxicity potential of the dissolved fraction from SeNPs. The highest toxicity were observed from 10 mg/L H_2SeO_4 in lake water at maximum incubation period (48 h), and only caused 16.7% decrease in cell viability (Fig. S1). However, the maximum concentration of the dissolved Se ions from SeNPs at the concentration of 5 mg/L were less than 1.5 mg/L (Fig. 5a). Therefore, this absence of toxicity of the dissolution ions in algae cell demonstrates that the toxicity results observed above (Fig. 6) are predominantly due to the NPs themselves and not released ions.

3.6. Principal component analysis (PCA)

PCA is considered here to summarize the relationships between the NPs intrinsic properties and stability (aggregation rate) in test solutions and bioassays responses. PCA indicates that 80.8% and 97.4% of the overall variance is explained by the two principal components in Lake Geneva water and culture medium respectively (Fig.S2). The importance of each components in the two working media is listed in Table S3.

In Lake Geneva water, the nature of surface coating (CMC or CS), with highest contribution (Fig. 7a), is found the most influential toxicity factor to drive the toxicity of SeNPs. Initial ζ-potentials of SeNPs corresponded to the ζ-potentials of SeNPs measured at 0 h in aquatic environment, and the final ζ-potentials corresponded to the ζ-potentials of SeNPs after incubation. The initial ζ-potentials constitute a secondary factor and have a higher impact on toxicity than final ζ-potentials. The higher contribution of coating than ζ-potentials is in line with the toxicity results: CS-SeNPs with negative surface charge in lake water still have higher toxicity than negative charged CMC-SeNPs. Previous research showed that chitosan interacts with the surface of cell membrane not only by electrostatic interactions but also via its functional uncharged groups (Pavinatto et al., 2010). In the culture medium (Fig. 7b), coating, aggregation behavior, and ζ-potential exhibit identical contribution levels. The stronger difference in aggregation behavior and the opposite surface charge between CMC-SeNPs and CS-SeNPs in culture medium leads to contribution of aggregation and ζ-potentials at the same level with coating.

Comparing to other parameters, the influence of initial hydrodynamic diameters on toxicity is less important in both Lake Geneva water and culture medium. These results are consistent with previous studies in which, for example, the size of AgNPs is the minor factor determining the toxicities to *Daphnia magna* compared to surface coating (Hou et al., 2017); Hydrodynamic diameters of Chitosan/pDNA NPs have marginal effect in toxicity, while zeta potentials of these NPs have a high impact and correlation with their cytotoxicity (Loretz and Bernkop-Schnuerch, 2007). In addition, previous study showed that: although mass-based exposure metrics supported the hypothesis that smaller nanoparticles would be more toxic than larger particles, however surface area-based metrics revealed that toxicity was independent of size, and the increased toxicity observed with smaller nanoparticle may be results of increasing in ions release (Bonventre et al., 2014; Wang et al., 2014a). Therefore, although CS-SeNPs showed a lower hydrodynamic diameter than CMC-SeNPs, it cannot be deemed as a principal reason to induce higher toxicity of CS-SeNPs.

Table 1
EC_{50} of CMC-SeNPs and CS-SeNPS on *P. malhamensis* cells in Lake water and culture medium for the 24 h and 48 h period evaluated.

EC_{50} (mg/L)	24 h	48 h
CMC-SeNPs in lake water	0.60 ± 0.05	0.100 ± 0.031
CS-SeNPs in lake water	0.50 ± 0.01	0.060 ± 0.004
CMC-SeNPs in culture medium	6.20 ± 0.11	1.30 ± 0.02
CS-SeNPs in culture medium	2.50 ± 0.12	1.10 ± 0.08

4. Conclusion

In this study, the influence of surface coating, z-average diameters, ζ-potentials, aggregation behavior, ions release, and medium properties on the toxicity of SeNPs to algae *P. malhamensi* has been investigated. SeNPs are found to induce a statistically significant diminution of cell viability with a dose-time response relationship, and exhibit 5–10 times higher toxicity in Lake Geneva water than that in the culture medium. This is an important outcome indicating that the traditional algal tests in culture medium currently underestimate the toxicity of NPs in realistic water environment. Moreover, results reveal that the SeNPs themselves are the main toxicity driver but not the released ions. Regarding the nature of the coating, CS-SeNPs exhibit higher toxicity in Lake Geneva water and cell culture medium to algae cells than CMC-SeNPs. Surface coating is found the most influential toxicity factor of SeNPs in both media whereas SeNPs diameters are found a minor factor in toxicity. These results contribute to the understanding of the fate and toxicity of SeNPs in different aquatic environments

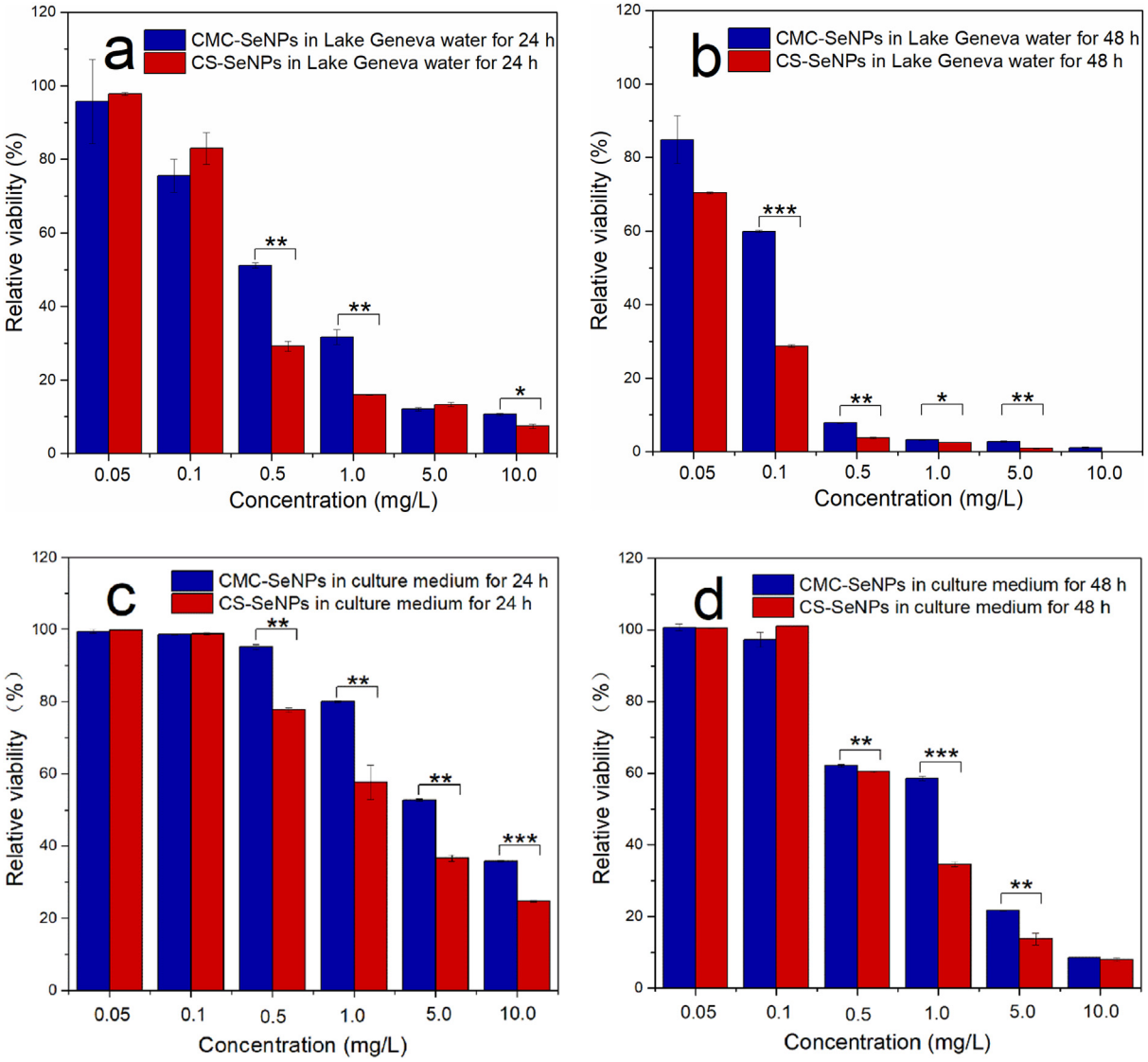

Fig. 6. Toxic effects of SeNPs on *P. malhamensis*. Effects on Algal cells viability exposed to CMC-SeNPs and CS-SeNPs in Lake Geneva water after (a) 24 h and (b) 48 h, and in culture medium after (c) 24 h and (d) 48 h. Asterisks denote a significant difference between toxicity results of CMC-SeNPs and CS-SeNPs: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

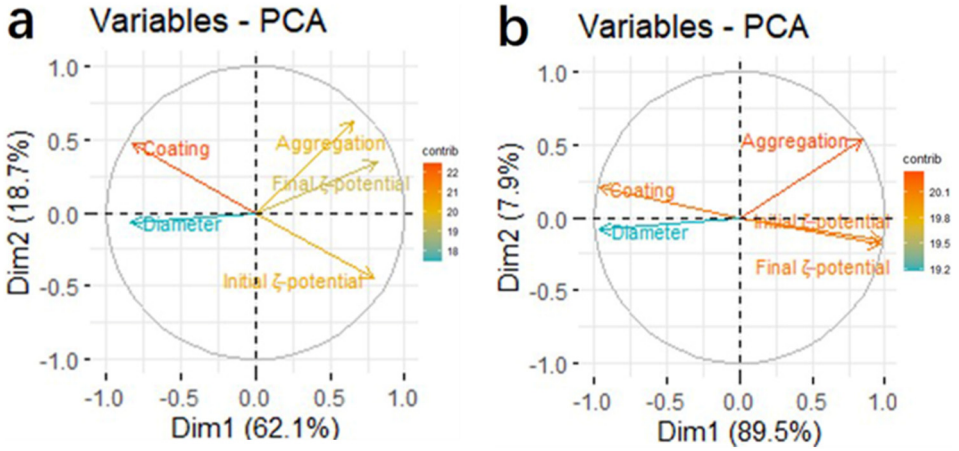

Fig. 7. Principal component analysis (PCA) relating coating, aggregation, initial ζ-potentials (0 h), final ζ-potentials (24 or 48 h), and hydrodynamic diameter response of the 24 h and 48 h algae toxicity exposed to CMC-SeNPs and CS-SeNPs in (a) Lake Geneva water and (b) culture medium.

and help predict the toxicity of NPs according to their characteristics and stability. This study also raises the question of the environmental risk evaluation of SeNPs on freshwater organisms, methods and parameters to consider for that purpose.

CRediT authorship contribution statement

Yuying Chen: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization, Funding acquisition. **Wei Liu:** Conceptualization, Methodology, Validation, Formal analysis, Writing – review & editing, Resources, Supervision. **Xiaojing Leng:** Conceptualization, Validation, Methodology, Resources, Writing – review & editing, Funding acquisition, Supervision, Project administration. **Serge Stoll:** Conceptualization, Validation, Methodology, Resources, Writing – review & editing, Supervision, Project administration.

Declaration of competing interest

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.

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Appendix A. Supplementary data

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