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The stochastic association of nanoparticles with algae at the cellular level: Effects of NOM, particle size and particle shape

Fazel Abdolahpur Monikh [a,b,*,1], Latifeh Chupani [c], Zhiling Guo [d], Peng Zhang [d], Gopala Krishna Darbha [e], Martina G. Vijver [a], Eugenia Valsami-Jones [d], Willie J.G. M. Peijnenburg [a,f]

[a] Institute of Environmental Sciences (CML), Leiden University, P.O. Box 9518, 2300 RA Leiden, the Netherlands
[b] Department of Environmental & Biological Sciences, University of Eastern Finland, P.O. Box 111, FI-80101 Joensuu, Finland
[c] University of South Bohemia in Ceske Budejovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Zatisí 728/II, 389 25 Vodňany, Czech Republic
[d] School of Geography, Earth and Environmental Sciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK
[e] Environmental Nanoscience Laboratory, Department of Earth Sciences & Centre for Climate and Environmental Studies, Indian Institute of Science Education and Research Kolkata, Mohanpur, West Bengal 741246, India
[f] National Institute of Public Health and the Environment (RIVM), Center for Safety of Substances and Products, Bilthoven, the Netherlands

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ABSTRACT

Association of nanoparticles (NPs) with algae likely plays a critical role in their transfer in aquatic food chains. Although our understanding of the ecotoxicity and fate of NPs in the environment is increasing, it is still unclear how the physicochemical properties of NPs influence their interaction with algae at cellular levels and how this is reflected at a population level. This is due to the limitation in the existing analytical techniques to quantify the association of NPs with cells. To fill this data gap, we applied the novel technique of single-cell inductively coupled plasma mass spectrometry to quantify the cellular association of gold (Au)-NPs with algal cells (*Pseudokirchneriella subcapitata*) as a function of particle size, shape (spherical 10 nm, spherical 60 nm, spherical 100 nm, rod-shaped 10 × 40 nm, and rod-shaped 50 × 100 nm), and surface chemistry [citrate and natural organic matter (NOM) coating] on a cell-by-cell basis. The association of Au-NPs with algal cells was found to be a random probability following a so-called stochastic process; after 72 h of exposure, less than 45% of the cell population accumulated NPs on their surface. The number of Au-NPs per cell was found to be heterogeneously distributed as some cells were associated with a significantly higher number (e.g. up to 600 spherical 10 nm particles per cell) of Au-NPs than other cells present in the medium. The presence of NOM on the surface of the particles decreased the percentage of cells containing NPs except for the spherical 60 nm Au-NPs. We conclude that some algae within a population can accumulate NPs on their surface and this accumulation is influenced by the size, shape, and surface chemistry of NPs. It is important to understand how NPs may enter aquatic food chains to assess the possible risk.

1. Introduction

Nanoparticles (NPs) can enter the environment and it is highly likely that they interact with microorganisms such as bacteria and algal cells. Algae play a vital role in aquatic ecosystems by forming the energy base of the aquatic food web. Internalization of NPs in algal cells, which are typically covered with extracellular polymeric substances (EPS) and have a protective membrane (Chen et al., 2019), is likely to be low (Abdolahpur Monikh et al., 2019a; Chen et al., 2019; Navarro et al., 2008). However, it is reported that NPs are associated with algal cells and accumulate in their EPS (Chen et al., 2019). This implies that NPs internalization in algae is not the only way through which NPs can enter aquatic food chains, because it is possible that the first consumers digest the accumulated NPs present in the EPS of algal cells. Thus, it is of paramount importance to shed light on NPs association with algal cells and the factors influencing this association.

* Correspondence to: Van Steenis Building, Einsteinweg 2, 2333 CC Leiden, the Netherlands.
E-mail address: f.a.monikh@cml.leidenuniv.nl (F. Abdolahpur Monikh).
[1] ORCID: 0000-0001-9500-5303

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The association of NPs with algae could be influenced by the physicochemical properties of NPs, e.g. particle size and shape (Abdolahpur Monikh et al., 2019a). For example, a previous study (Taylor et al., 2016) reported that heterogeneity in uptake among similar cells could lead to a cell-to-cell variance in the cellular uptake within a population. The cellular heterogeneity is related to physiological features and to the so-called bio-environment of the cells as well as to the cell life cycle (Chattopadhyay et al., 2014; Taylor et al., 2016). Nevertheless, it is still unknown if the association of NPs of the same size and shape to algal cells is following a uniform pattern or rather is stochastic among the cells of a given population. This can dramatically change our understanding of the interactions of NPs with microorganisms and, thus, of their transfer in food chains and the possibly resulting environmental risk.

In natural conditions, algae are scarcely exposed to pristine NPs. When NPs are released into the environment, they are immediately coated with natural organic matter (NOM) (Wang et al., 2016). NOM consists of a heterogeneous mixture of e.g. humic substances, hydrophilic acids, proteins, lipids, carbohydrates, and carboxylic acids. Sorption of NOM on the surface of particles not only offers the particles a highly diverse surface chemistry, but it also increases the stability of the particles against aggregation. This keeps the particles in the dispersed phase for a longer time (Delay et al., 2011) and increases the likelihood of algal exposure to a dispersed NPs with different surface chemistries despite having a similar core particle (Röhder et al., 2014). In turn, the presence of NOM on the surface of NPs may influence the interaction of the particles with the EPS and, consequently, the association of the particles to the algal cells (Chen et al., 2019). In our previous study we showed that NOM on the surface of NPs determines the strength of NP association with algae cells (loosely or strongly attachments) (Abdolahpur Monikh et al., 2019a). We hypothesize that the presence of NOM on the surface of NPs influences the quantity of NPs attached to the surface of each cell.

Investigating the association of NPs with cells at the cellular level is a challenging task due to the limitations in the existing protocols and analytical techniques. The existing methodologies are based on acid, alkaline, or enzymatic digestions in which NPs are, first, isolated from algae while the cells are completely digested. This process is followed by the measurement of the total mass of the ions forming the NPs (Abdolahpur Monikh et al., 2019b). These approaches offer the average mass of the particles accumulated on/in cells at the population level of algae rather than at the cellular level. By averaging the metal concentration over a population of cells, it is not possible to understand the association of nanoparticles with each cell. Some methodologies rely on the application of fluorescently labeled NPs. However, leaching of the labels can easily lead to artefacts, and the fluorescent materials may be mistakenly considered to be associated with the cells even though no longer part of the NPs (Salvati et al., 2011).

Operating inductively coupled plasma mass spectrometry (ICP-MS) in a single-cell mode, known as scICP-MS, allows quantification of metals at the cellular level. The technique was applied to quantify the accumulated metallic NPs within algae cells (Abdolahpur Monikh et al., 2019c) and provides information about the mass of metals in individual cells (Ho and Chan, 2010; López-Serrano Oliver et al., 2018; Mueller et al., 2014; Wang et al., 2015). The technique is operating based on the assumption that one cell is introduced into the plasma at a time. In the plasma, ion plumes of the cells and the metals within the cells are generated and the events are detected (Miyashita et al., 2014). Accordingly, the quantity of metals within the cells is measured at levels as low as an attogram. Wang et al. (2015) have applied scICP-MS to determine essential elements, including Fe, Cu, Zn, Mn, P, and S in two types of cancer cells (Hela and A549) and one type of normal cells (16HBE). Merrifield et al. (2018) used scICP-MS to measure the uptake and attachment of dissolved Au and gold (Au)-NPs in algae. López-Serrano Oliver et al. (2018) employed scICP-MS to study the uptake of 50 nm silver NPs by THP-1 cells. The authors reported that the technique provides a potential tool for monitoring elements at a single-cell level.

In this study, scICP-MS was applied to quantify the association of Au-NPs to algal cells on a cell-by-cell basis as a function of particle size and shape in the presence and absence of NOM. The objective of this study is to reveal how the variation in particle size, shape, and surface chemistry due to the presence of NOM influences the association of NPs with cells at the cellular level.

2. Materials and methods

2.1. Materials

The used chemicals in this study were of analytical grade and purchased from Sigma-Aldrich (Zwijndrecht, the Netherlands) unless otherwise mentioned. We purchased citrate-coated Au-NPs of different sizes and shapes from Nanopartz™ (Nanopartz Inc., the US). In this study, we used spherical (10, 60 and 100 nm) Au-NPs and rod-shaped (diameter × length) 10 × 40 and 50 × 100 nm Au-NPs. Suwannee River NOM (1R101N) was purchased from the International Humic Substances Society. $AuCl_3$ was purchased from Sigma-Aldrich (Zwijndrecht, Netherlands).

2.2. Characterization of the Au-NPs in different media

The Au-NPs were characterized in Milli-Q (MQ) water and in the algal culture media. The concentration of all Au-NPs of different sizes and shapes were 10 mg/L for measuring the size of the NPs. The particles dispersed in MQ (10 mg/L) and sonicated using a SONOPULS ultrasonicator (BANDELIN electronic. Berlin, Germany) at a power of 40 W for 10 min. The dispersions for each size and shape were used as stock dispersions to prepare the exposure tests. The dispersion stability of the particles in the algal culture medium was also tested by dispersing 10 mg/L of the Au-NPs in the medium. The particles were mixed with a 10 mg/L NOM solution to allow the NOM to attach to the surface of the particles for 24 h (see S1, Supporting Information). This concentration of the NOM is an environmentally relevant concentration reported for natural surface water in the EU.

A JEOL 1010 transmission electron microscope (TEM) operating at 70 kV accelerating voltage was used to determine the shape and size of the particles. A Zetasizer Nanodevice (Malvern Panalytical, Netherlands) was used to determine the hydrodynamic size and zeta potential of the particles in different samples. To determine the dissolution profile and the number of the Au-NPs in the culture media, a single-particle (sp)ICP-MS (PerkinElmer NexION 300D ICP-MS) was applied. The operation conditions and the sample preparation for the spICP-MS are reported in S2, Supporting Information.

2.3. Algal exposure test

The algal culture was prepared following the OECD guideline (OECD 201) (OECD, 2011). A density of $5 \times 10^3 \pm 200$ cells/ml of *Pseudokirchneriella subcapitata* was used as the initial concentration as recommended by the OECD guideline. The number of the algal cells was measured using an Aquafluor Meter (TURNER DESIGNS, San Jose, CA, the USA). Before exposure, we determined the growth rate of the algae. The exposure was performed when the algal growth rate reached a steady state (after 6 days) to minimize the effect of the cell doubling on the association of the particles with the cells. The algae were exposed to 1 mg/L of citrate-coated and or NOM-coated Au-NPs of different sizes and shapes separately. Citrate coated Au-NPs are used in ecotoxicological studies due to the low toxicity of citrate to organisms (Abdolahpur Monikh et al., 2019a). Although this concentration could be higher than what is expected in the environment, we selected the concentration of the Au-NPs to facilitate the quantification of the particles. We are aware that 1 mg/L of Au-NPs of different sizes and shapes contains a different

Table 1
Single cell ICP-MS parameters.

Sample Uptake Rate	0.02 ml/min
Nebulizer	MEINHARD HEN (High Efficiency Quartz Concentric Nebulizers)
Spray Chamber	Asperon
Injector	2.0 mm id Quartz
RF Power	1600 W
Sample Uptake Rate	0.02 ml/min
Dwell time	50 μs
Acquisition time	40 s
Replicates per sample	1
Transport Efficiency	41%

number of particles in the systems (Abdolahpur Monikh et al., 2019c), we nevertheless performed the study on a mass basis because most of the available studies in literature use mass as a dose metric. Along with the samples, several control treatments were used, including algae exposed to ionic Au, algae exposed to NOM, and algae without any exposure.

The flasks containing algae and NPs as well as the controls were constantly shaken at 80 rpm using a G10 Gyratory Shaker (Washington, the US) to prevent sedimentation of the algae. The exposures were carried out in a climate chamber (22 °C) at a light intensity of 70 mE/(m^2 s) for 72 h. After exposure, the algal cells were kept at 4 °C for 24 h to sediment. After 24 h, the pellet of the algal cells was separated from the supernatant by gently discarding the supernatant. The supernatant was kept for further analysis. The algal pellets were washed by diluting the pellets with 10 ml of a Phosphate-Buffered Saline (PBS, 0.1 mol, pH 7.4) solution to remove the unbound particles from the samples. The algal samples were centrifuged at 2300*g* for 10 min (Sorvall RC 5B plus centrifuge, Fiberlite F21-8). This washing step of algae was repeated twice to assure that the unbound particles were not transferred to the next step. The resulting pellets were dispersed with 10 ml of PBS solution and immediately sent for scICP-MS measurements.

2.3.1. Algal cell integrity

It is important to assure that the concentration of 1 mg/L of Au-NPs is not lethal to the algae. Moreover, it is required to understand whether the ions released from the Au-NPs are taken up by the algae cells. Our previous study showed that Au-NPs induce no lethal toxicity to algae at concentrations lower than 10 μg/L (Abdolahpur Monikh et al., 2019a). The lethal toxicity is not the only possible adverse effect that can be induced by Au-NP. It is possible that exposure to Au-NPs leads to membrane damage in algae (Abdolahpur Monikh et al., 2019a). To verify that the particles do not cause membrane damage at the used concentration in the current study, propidium iodide (PI) was used to dye the cells following the method reported previously (Abdolahpur Monikh et al., 2019a). Accordingly, PI can penetrate the cells with a damaged membrane and produce red fluorescence upon observation using a Confocal Microscope system (Leica TCS SPE) with available laser lines of 488, 532, and 633 nm.

In a separate experiment (as a control), we conducted a test using $AuCl_3$ to determine the cellular uptake of ionic Au. Accordingly, algal cells were exposed to $AuCl_3$ at a concentration of 1 mg/L for 72 h under the same conditions as used for the NPs exposure.

2.4. ScICP-MS measurements

The algal cells were dispersed in PBS and 500 μL of the samples were diluted to reach a final volume of 50 ml (0.1 mol, pH 7.4). Two ml of each sample was used for scICP-MS measurements. A PerkinElmer NexION 300D ICP-MS operating in Single Cell mode was used to measure the quantity of NPs in the algal cells. The conditional set up of the scICP-MS is given in Table 1. A single Cell Micro DX Autosampler, a high efficiency nebulizer (HEN), and an Asperon spray chamber were used for the sample introduction. The autosampler is applying a syringe pump for accurate, low-flow delivery of the cells to the nebulizer which aspirates the cell suspensions, without rupturing the cells. The Asperon spray chamber provides a laminar flow for maximum delivery of the cells to the plasma, for maximum efficiency it is a full consumption nebulizer meaning that all the samples are delivered to the plasma, thus minimizing loss of sample.

2.5. Data analysis

The IBM SPSS Statistics 25 software was used to run the statistical analyses of the data. Kolmogorov-Smirnov and Levene tests were performed to check the normality and homogeneity of variances, respectively. Significant differences between the citrate-coated and NOM-coated NPs were calculated using the *T*-test. The $p < 0.05$ was taken as a significant cut-off. Results are reported as mean and standard deviation of 15 replicates. All the graphs were plotted using the software OriginLab 9.1.

3. Results and discussion

3.1. Characterization of Au-NPs

Before performing the exposure test, we characterized the Au-NPs in MQ water and in the algal exposure medium to assess the behavior of the particles during the exposure test. The stability behavior of the particles can dramatically influence their exposure concentration in the media over time and consequently their interaction with algae. The TEM images of the particles are reported in Fig. S1 in the Supporting Information. The shape of the particles agreed with what has been reported by the supplier. The physicochemical properties of the Au-NPs are reported in Table 2. The TEM measured morphology of the particles was in agreement with the morphology reported by the supplier. The citrate-coated particles had a negative zeta potential and the presence of NOM significantly ($P < 0.05$) decreased the zeta potential of the particles. The attachment of NOM to the surface of the particles was confirmed by the decrease in the value of the zeta potential towards a more negative value after incubation of the particles in the NOM suspension. This agrees with what has been reported in the literature (Abdolahpur Monikh et al., 2019a). The attachment of NOM to the surface of the particles can change the zeta potential of the particles.

Table 2
Physicochemical characteristics of Au-NPs as measured in MQ water and in algal culture media without algae.

Au-NPs	TEM measured size (nm)		Zeta potential			
	Reported by the producer	Measured in this study	Reported by the producer	Measured in this study in MQ water	Measured in this study in the exposure media	NOM-coated particles in the exposure media
Spheric	10	10 ± 3	-20	-23 ± 3	-17 ± 4	-30 ± 3
	60	60 ± 8	-20	-23 ± 4	-16 ± 3	-29 ± 3
	100	100 ± 14	-20	-24 ± 2	-17 ± 2	-28 ± 4
Rod Au-NPs	10 × 45	10 × 47 ± 2 × 3	-22	-22 ± 3	-19 ± 4	-30 ± 2
	50 × 100	56 × 140 ± 7 × 38	-22	-24 ± 5	-20 ± 3	-27 ± 3

TEM measured size: the average of 100 particles. The size of rod-shaped is presented as diameter × length.

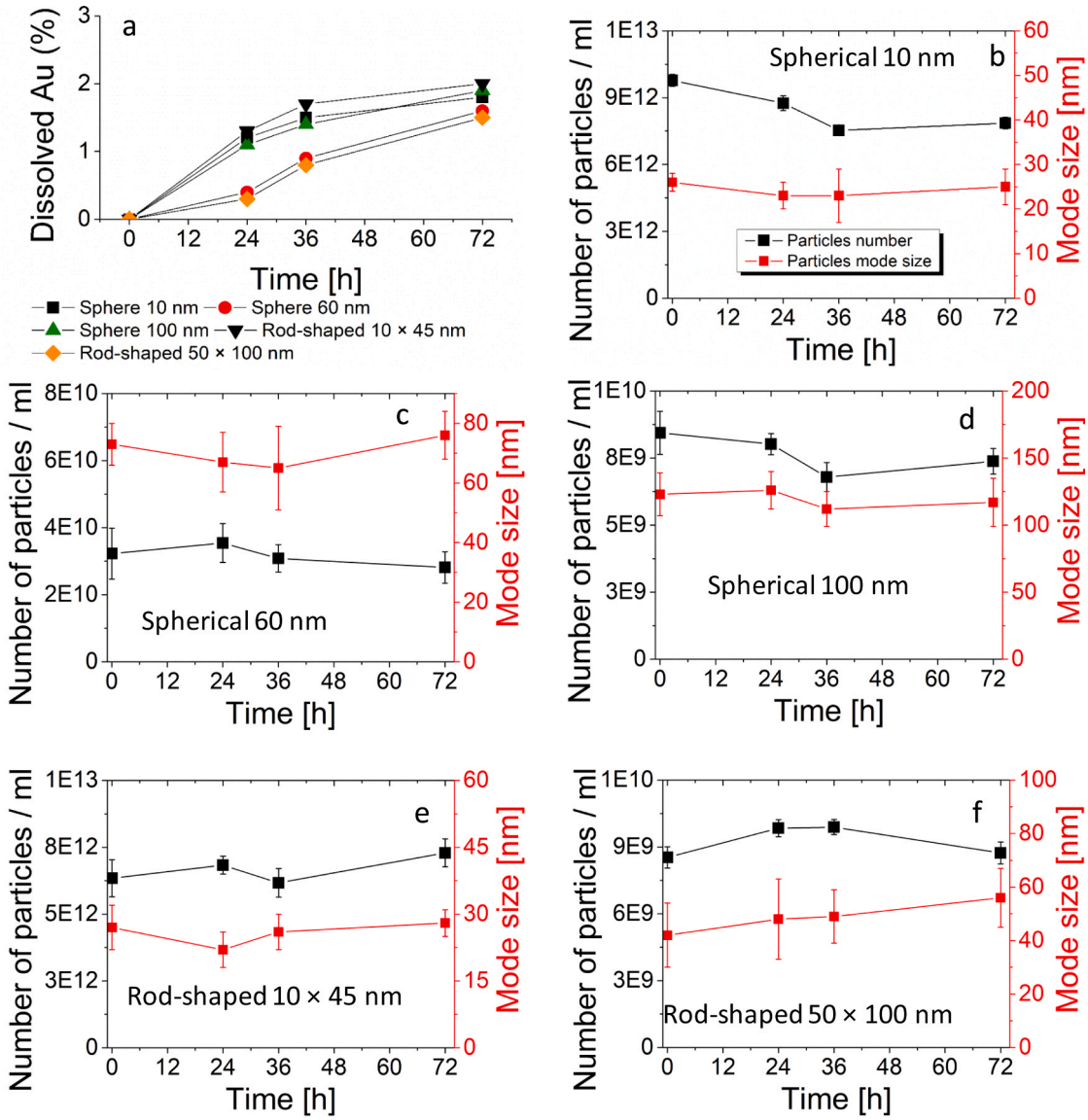

Fig. 1. a) The percentage of dissolved Au in the exposure media over 72 h incubation time. b-f) The particle number of Au-NP (black line) and the measured mode size of the size distribution (red line) over the duration of the exposure in the exposure media without algae cells. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

This influences the interaction of the negatively charged particles with the cells (Delay et al., 2011). Moreover, NOM changes the surface chemistry of the particles and, in turn, the chemistry of the surface of NPs, which is in direct contact with the cell membrane, influences the way cells interact with the particles (Lynch et al., 2009).

It is documented that the composition of different media may have a different influence on the aggregation and dissolution of NPs (Bian et al., 2011; Walters et al., 2013). The size and shape of NPs influence their aggregation and dissolution, and in turn, aggregation and dissolution influence the interactions of the particles with microorganisms. We measured the number and the dissolution rate of the particles in the exposure media (without algae) over 72 h using spICP-MS to obtain an overview of the conditions to which the algae are exposed. The data for particle dissolution (Fig. 1a) showed that all the Au-NPs have a low extent of dissolution in the exposure media. The released ions from the particles constituted less than 2% of the total Au mass, which is in agreement with the results of our previous study (Abdolahpur Monikh et al., 2019a).

Fig. 1b-f (black lines) shows the number of particles in the exposure media over time. The number of particles did not change significantly over 72 h incubation time. This was also confirmed by the measured mode (hydrodynamic size) of the particle size distribution over 72 h incubation time (Fig. 1b-f, red lines). These findings indicated that the algal cells were exposed to relatively stable dispersions of Au-NPs rather than to aggregates and Au ions.

3.2. Assessment of cellular mortality and integrity

To assure that the exposure to 1 mg/L of the Au-NPs did not induce any toxicity to algal cells, we investigated the lethal toxicity and cell membrane damage of algae due to the exposure to the Au-NPs of different sizes and shapes. No significant cellular mortality was observed when compared to mortality in the control groups (Fig. S2, supporting information). This agrees with previously reported results (Moreno-Garrido et al., 2015). However, it is also possible that Au-NPs cause membrane damage to the cells without inducing lethal toxicity (Abdolahpur Monikh et al., 2019a). Damage in the cell membrane may influence the cellular uptake of the particles or even the secretion of EPS by the cells and in turn, affect the accumulation of NPs in the EPS. We investigated whether the exposure to Au-NPs of different sizes and

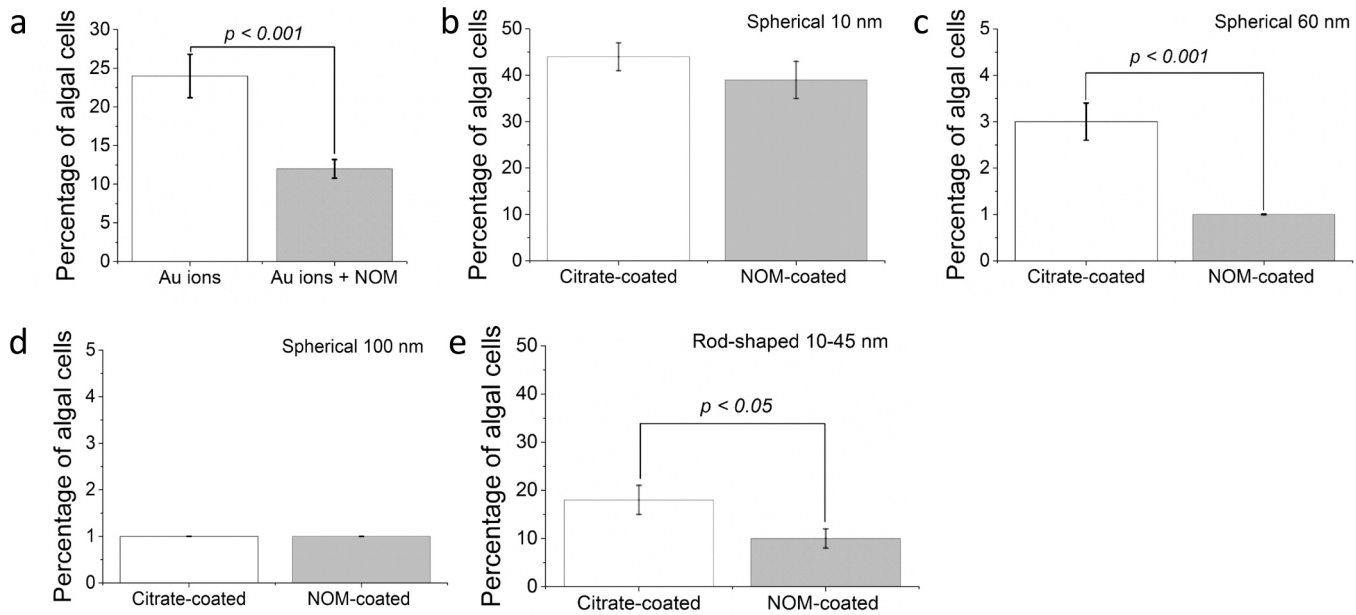

Fig. 2. a) The percentage of algal cells containing Au ions in the medium with and without NOM. The percentage of algal cells accumulated citrate-coated or NOM-coated spherical 10 nm (b) spherical 60 nm (c) spherical 100 nm (d) or rod-shaped 10 × 45 nm (e) Au-NPs on their surfaces.

shapes could cause membrane damage to the algal cells. The confocal microscope pictures (Fig. S3, Supporting Information) showed that there is no difference between the control cells and treated cells. It is thus clear that the cells were intact (no membrane damages) and not stained, as can be deduced from the absence of the PI inside the cells. It was reported (Abdolahpur Monikh et al., 2019a) that if cellular membrane damage occurs due to the physical toxicity of NPs, the affected cells would show red fluorescence due to the uptake of the PI. Our data indicated that the cells could function normally under the exposure conditions, which allows further investigations of the association of the Au-NPs with the cells.

3.3. Association of Au-NPs with algal cells

The reported results are the average of 15 replicates. Fig. 2 shows the percentage of algal cells in the algae population that contained either Au ions ($AuCl_3$) or Au-NPs. We did not differentiate between associated and internalized particles in the cells. However, as previously reported, it is highly likely that the particles are associated with the cells rather than internalized in the cells (Hoecke et al., 2009) due to the protective membrane of the algae and the presence of EPS. The graphs show that citrate-coated spherical 10 nm Au-NPs were associated with a higher percentage of the algae when compared to ionic Au. This is an interesting finding because it shows that a larger population of algae may transfer Au when Au is present in its particulate form rather than when it is present in the ionic form. This can be further investigated in future studies to support the risk assessment of NPs because a considerable amount of metallic NPs is dissolvable in aquatic systems. It implies that algae could transfer metallic NPs associated with their surfaces to the first consumer in addition to transferring metallic ions. It can thus not be ruled out that dissolution decreases the cellular uptake of NPs and this may be applicable for other soluble NPs as well.

The association of the spherical Au-NPs with cells was different across different sizes. The spherical 10 nm NPs were associated with almost half of the algae population, while the 60 nm and 100 nm Au-NPs were associated with only 1% of the population.

Regarding the shape-related differences, we found (Fig. 2) that the percentage of algae that accumulated bare rod-shaped 10 × 45 nm Au-NPs on their surface was lower than the percentage of algae that accumulated bare spherical 10 nm Au-NPs and higher than the percentage of algal cells that accumulated spherical 60 nm and 100 nm Au-NPs. The rod-shaped 50 × 100 nm particles did not associate with the algal cells. EPS consist of a complex mixture of high-molecular-weight biopolymers, which influence the physicochemical properties of algae such as the surface charge, structure, and interaction (Xiao and Zheng, 2016). It was reported that NPs may penetrate the EPS layer around the algal cells (Adeleye and Keller, 2016; Chen et al., 2019) and the penetration is determined by the shape and size of the particles (Abdolahpur Monikh et al., 2019a). Our findings suggest that NPs may penetrate the EPS of only some cells within a given population and this penetration changes upon changing particle size and shape. The EPS of algal cells varies from cell to cell depending on the triggering factors and the life cycle of the algae (Xiao and Zheng, 2016). EPS also can protect cells against toxic substances (Zhou et al., 2016). For example, when algae are exposed to NPs, they may shed their EPS as a defense mechanism against NPs. As a result, this EPS release could vary from cell to cell and this variation could influence the association of Au-NPs with the cells of a given population. It is likely that the rod-shaped 50 × 100 nm did not penetrate the EPS, and they were thus removed from the surface of the EPS layer upon the washing step performed in our study. This implies that the association of Au-NPs to algal cells could be a size-specific phenomenon.

When coated by a NOM, the association of the particles with the algae decreased [significantly for Au ions ($p < 0.001$) and rod-shaped 10–45 nm Au-NPs ($p < 0.05$)] regardless of the size and shape of the particles, except for the spherical 60 nm particles. Coverage of NPs by NOM increases the negative value of the zeta potential of the particles. Consequently, the repulsion between the NOM-coated NPs and the anionic EPS reduces the attachment efficiency of the particles to the cells (Chen et al., 2019), which may lead to a reduction of cellular association. However, the association of NOM-coated spherical 60 nm Au-NPs was significantly higher than the association of citrate-coated 60 nm Au-NPs.

3.4. Number of Au-NPs associated with algal cells

Knowledge of the geometry and the density of Au corresponding to the mass of each of the Au-NPs used in this study (see S5, Supporting

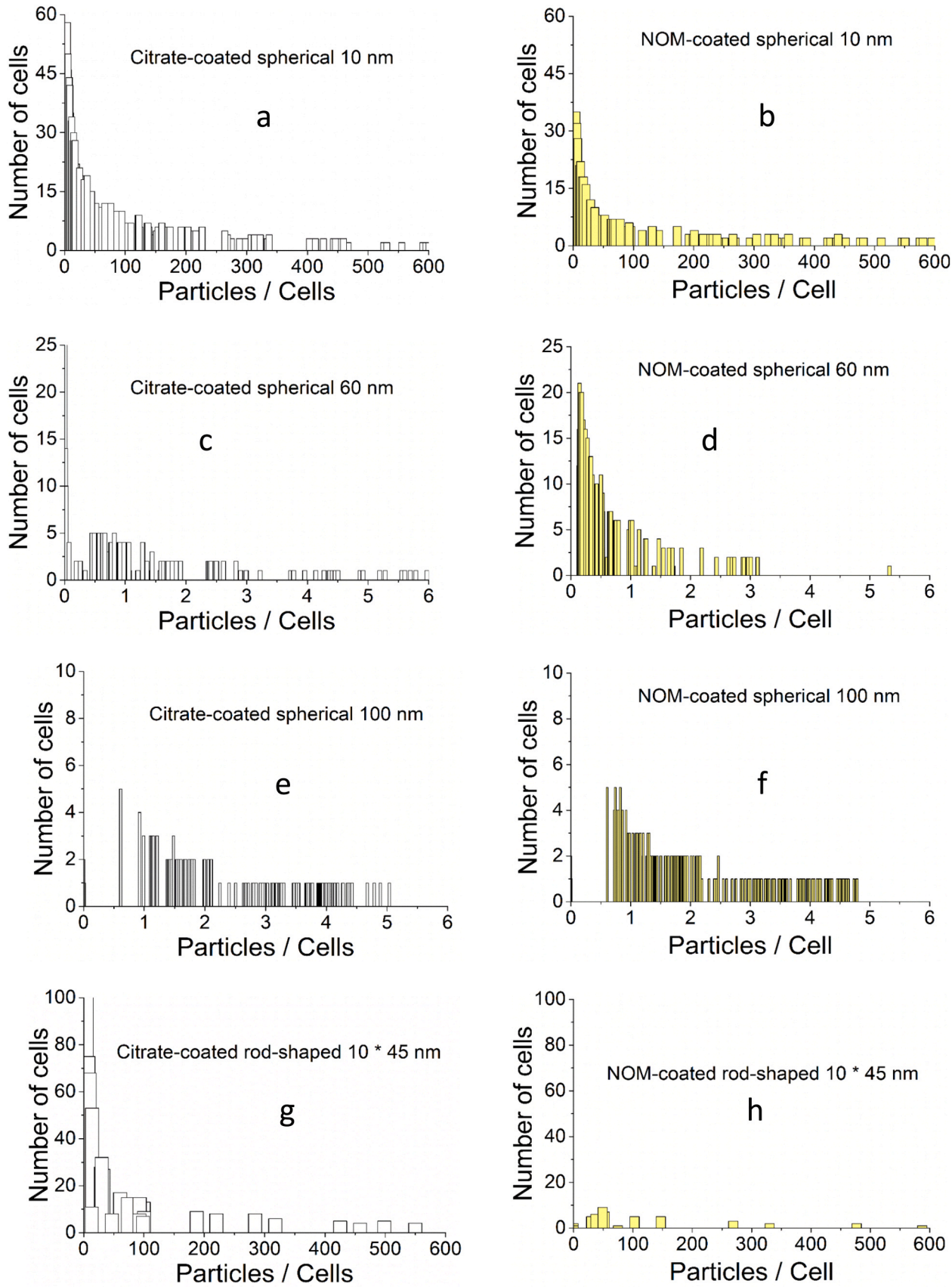

Fig. 3. The number of Au-NP accumulated on the surface of each algal cell within a given population as a function of particle size and shape and in the presence and absence of NOM. The number distribution of the spherical 10 nm Au-NPs (a-b), the spherical 60 nm Au-NPs (c-d), the spherical 100 nm Au-NPs (e-f), and the rod-shaped 10 × 45 nm Au-NPs (g-h).

Information), allowed us to convert the measured mass of the particles to the particle number. After determining the number of Au-NPs per cell, we plotted these data to show the numbers of Au-NPs in each cell as a function of particle shape, size, and presence/absence of NOM (Fig. 3).

The results obtained for algal cells exposed to spherical 10 nm Au-NPs are reported in Fig. 3a-b. Some algal cells accumulated up to 600 citrate-coated and NOM-coated Au-NPs per cell on their surfaces, although the number of these cells is low in the population and most of the cells accumulated < 200 Au-NPs per cell on their surfaces. Fig. 3c-d shows that most of the population accumulated less than 5 particles/cell on their surface when the population was exposed to citrate-coated and NOM-coated spherical 60 nm Au-NPs. The number distribution of the spherical 100 nm Au-NPs is shown in Fig. 3e-f. Some of the algal cells accumulated 1–5 particles/cell on their surface when they were exposed to citrate-coated and NOM-coated spherical 100 nm Au-NPs. Fig. 3g-h shows the number distribution of the rod-shaped 10 × 45 Au-NPs. Some cells accumulated up to 600 citrate-coated and NOM-coated rod-shaped 10 × 45 Au-NPs per cell on their surface.

Single-cell analysis has already shed light on various cellular differences in uptake between individual human immune system cells and cancer cells in the same population (Ascoli et al., 2008; Irish et al., 2004; Sachs et al., 2005). Our study for the first time documents this phenomenon for algal cells as a function of NP physicochemical properties. We exposed the algae cells to different numbers of NPs, and although this can lead to different uptake in cells as observed in our previous study (Abdolahpur Monikh et al., 2019c), the current study clearly shows that similar algal cells within a single population contain different numbers of particles even when they are exposed for a certain period of time to particles of the same size and shape. Some cells can associate with up to hundreds of particles while others did not associate with any particle at all (or lower than the detection limit of the instrument, which is attogram). Two explanations can be put forward for this observation. First, although we exposed the cells when they were at a steady state of their growth, cell division could still take place to some extent, which leads to the division of a single algal cell into more than two daughter cells (Sun et al., 2020). During division, the associated NPs could be shared between the mother and daughters. Redistribution of internalized NPs during cell mitosis is also assumed to be random in mammalian cells (Ascoli et al., 2008; Bergeland et al., 2001). The stochastic distribution of the Au-NPs between the mother and the daughters may cause the cell-by-cell variation in the number of the associated particles in the algal population. But we believe that the association of NPs with algae is most likely controlled by the surface physicochemical properties of the cells and the NP combined, following random collisions and successful attachments. The number of NPs associated with algae determines the number of particles entering aquatic food chains. If the first consumer feeds on algae, which contain a high number of NPs, the particles cannot only influence the organisms themselves but may also transfer across aquatic food chains.

4. Conclusions

In summary, our results showed that the spherical 10 nm Au-NPs were associated with a higher percentage of the algal population in comparison to larger spherical Au-NPs, followed by the rod-shaped 10 × 45 nm NPs. Our findings showed that NPs associate with only a percentage of the cell in an algal population. In general, the mass and the number of Au-NPs per cell are heterogeneously distributed and some cells within the algae population are associated with a significantly higher mass and number of Au-NPs than other cells. We can conclude that the association of NPs to algal cells is following a stochastic process resulting in a random distribution of the associated NPs to algal cells. This may complicate the estimation and modeling of trophic transfer of NPs in aquatic ecosystems, challenging the risk assessment of NPs. The methodology developed in our study can assist to better understanding of the processes and mechanisms underlying the toxicity of NPs to microorganisms at the cellular level. For example, this new approach can be used to quantify the cellular association of different metallic NPs by other microorganisms and cells such as bacteria which are critical for not only environmental science but also for nanomedicine and nanobiotechnology. The applied methodology in this study facilitates understanding how NPs influences the life-cycle span of microorganisms (e.g. viability of cells at different time endpoints, cell division processes, and differences between the cells).

Author contributions

F.A.M. conceptualized, supervised, wrote, and reviewed the study. F.A.M. and L.C. performed the experiment of NPs exposure and organisms handling. F.A.M. Z.G., P.Z., and G.K.D. contributed performed the statistical analysis and contributed to editing the paper. W.J.G.M.P., M.G.V., I.L., and E.V.J. contributed to conceptualizing, supervising, and editing the paper.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2021.112280.

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