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Nanoscale Interactions: Evaluating the Ecotoxicity of Engineered Nanoparticles on the Dynamics of Commercially Important Microalgal Strains

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***Abstract*-**Society has long been exposed to naturally-occurring nanoparticles. Due to their ubiquitous nature, biological systems have adapted and built protection against their potential effects. However, for the past decades, there have been onslaughts of newly engineered nanoparticles being released in the environment with no known effects on ecosystems. Although these materials offer distinct advantages in manufacturing processes, such as odor-free fabric or controlled drug delivery, their fate in nature has yet to be thoroughly investigated. As the size of an already-large NPs market is expected to grow, due to advances in synthetic biology, it is vital that we increase our understanding of their impacts on human, food and natural ecosystems. Recent studies have shown that NPs affect phytoplankton biomass and diversity in the ocean, solely by regulating micronutrients bioavailability. These types of changes could ultimately impact several biogeochemical cycles, as phytoplankton are responsible for almost half of the primary production on earth. Consequently, this study was designed to evaluate the impact of various concentrations (0µM, 20µM, 40µM, 80µM and 100µM) of several manufactured nanoparticles (gold, carbon and iron) on the dynamics of four economically important microalgae strains. Responses, such as chlorophyll content, protein, lipid content, lipid profile, biomass and cell morphology were monitored over a period of two weeks. No significant acute toxicity was exhibited within the first 24 hours of exposure. However, after 4 days, a remarkably high mortality rate was detected with increasing NPs concentrations of Fe60, C80 and Au60. Iron suspensions were found to be more toxic to the microalgae strains tested than those of Gold and Carbon under comparable regimes. Further investigations with other, either positively or negatively charged nanoparticles, should provide a deeper understanding on the impacts on these engineered materials in our ecosystems.

Keywords- *Nanoparticles, Microalgae, Ecosystems Impacts, Industrial Processes, Algal Dynamics*

I. INTRODUCTION

Nanoparticles (NPs) are ubiquitous in nature, where they are found in varied composition. Both at a temporal and spatial scale, nanoparticles tend to display broad particle sizes, shapes and distribution. Commonly derived from natural and anthropogenic sources, some nanoparticles are known to interact directly with environmental pollutants, thereby improving environmental quality. In fact, studies by Erick [1] and Shawn [2] have demonstrated that nanoparticles have the capacity to enhance nutrient storage in soils. Another paper published by Thomas [3] also described nanoparticles ability to absorb contaminants, thereby protecting water quality. Luis [4] thoroughly investigated the involvement of nanoparticles in disease diagnostics for plant, while Paul [5] research dealt with human and animal health. The list of positive interactions of natural nanoparticles with the environment is non-exhaustive, as certain nanoparticles appear to form aggregates with completely new properties when interacting with living organisms (Team of Northwestern University chemists and colleagues from the national Center for Sustainable Nanotechnology) [6]. Over the years, scientists have gained some valuable insight into the molecular mechanisms by which nanoparticles interact with biological systems. Studies with cellular organisms have helped understand and predict why some nanomaterial/ligand coating combinations are detrimental to the cell while others are not. This knowledge has been used to design and engineer benign nanomaterials.

Nanoscience studies have identified a wide array of nanoparticles with unknown impacts on living organisms in terms of exposure and possible toxicity [7, 8, 9]. Other nanoparticles, however, have shown to represent potential environmental hazards [10, 11, 12]. By entering the food chain, water and air, their interactions with nutrients often triggered unintended or sometimes adverse environmental responses [13]. Using bioassays, countless studies have been performed to demonstrate the toxicity associated with nanoparticles [14, 15, 16]. From an eco-toxicological point of view, titanium oxides (TiO2), Silver (Ag) and Zinc (Zn) nanoparticles are the

most widely or extensively studied [17, 18, 19]. For example, minute concentrations of Zinc Oxides (less than 0.05 mg/L) have shown to suppress the growth of several microalgae strains [17]. Tests with widely used TiO2 (<5m g/L) led to similar results [18]. Evaluation of nanoparticles on microalgae dynamics is one widely used method to determine various chemicals toxicity levels [20]. Especially, when it comes to the effects of particles introduced into aquatic ecosystem, where algae play a crucial role in the food web. Microalgae affordability and high sensitivity to chemicals have prompted them to become the model organism in studies examining nanoparticles toxicity [20, 21]. Countless studies on the ecotoxicity of a variety of nanoparticles (carbon, iron, platinum) are currently underway using both bacteria and animal models and a range of toxicity mechanisms (e.g., dissolved ions, interactions with algae, entrapment of algal cells in NP aggregates) are now surfacing [22, 23]. These findings, altogether, point out to a series of highly complex interactions between nanoparticles and their surrounding biota in the environment.

Over the past few decades however, an additional onslaught of engineered nanoparticles has found their way into our aquatic and terrestrial ecosystems, thereby provoking seismic shifts in the environment [24, 25]. These nanomaterials originate from industries as broad as textiles, medical, cosmetics and electronics [24, 25]. Zinc and Copper oxides nanoparticles (ZnO and CuO) have been heavily used in dentistry, as antibacterial and in sunscreens [26, 27]. Upon use or consumption of these consumer products, these nanomaterials are constantly being released into the environment, either accidentally or intentionally [28]. And due to their distinct chemical properties, these nanoparticles are often associated with unforeseen health hazards [28, 29]. Characteristics such as their reactivity and most importantly their size have shown to increase their propensity to trigger negative ecological toxic effects in the environment [30]. Therefore, engineered nanomaterials are a focus of concern. Given synthetic NPs distinct structures and chemical properties, such as high mobility and enhanced chemical reactivity, compared to naturally-occurring nanoparticles, most organisms may not have appropriate defense mechanisms against them [31].

Nanoparticles are known to play essential roles not only in the earth global biogeochemical cycles but also in nutrients bioavailability and ecotoxicity [32]. Interactions with their abiotic surroundings have also been shown to impact their fate in nature including their ecotoxicological potential [33]. It has become therefore essential to assess the toxicity of an ever-increasing number of nanoparticles using various testing models. Using appropriate test organisms and methods, including proper endpoints, one can more accurately determine the impacts of engineered nanoparticles in aquatic and terrestrial ecosystems. Also, understanding the effect of mixtures of nanoparticles on environmental biota requires further investigation, as most toxicological studies have been performed on individual particles. Therefore, to reflect natural interactions of nanoparticles more realistically, this study was conducted to assess the toxicity of not only individual NPs but also mixtures of NPs on algal dynamics. We hypothesized that the toxicity of a mixture of manufactured nanomaterials can be predicted based on the behavior of individual particles regardless of biotic or abiotic interactions.

II. MATERIALS AND METHODS

To examine the toxic effects of both individual and mixtures of nanoparticles on microalgae, *Chlorella vulgaris*, *Scenedesmus dimorphus*, *Dunaliella tertiolecta* and *Neochloris oleoabundans*, four economically important microalgae, were exposed to manufactured iron (Fe), carbon (C) and gold (Au) NPs for a period of eight weeks. The toxicity of these NPs was evaluated based on endpoint responses such as chlorophyll, protein, and lipid content, lipid profile, biomass and morphology (microscopic observation). Different concentrations of the NPs (0µM, 20µM, 40µM, 80µM and 100µM) were tested. The whole experimental design is listed in figure 1.

A. Study System

A mix of freshwater and saltwater algae including four commercially important algae strains were used in this study. The two saltwater strains were *Dunaliella tertiolecta* and *Neochloris oleoabundans.* The two freshwater strains were *Chlorella vulgaris* and *Scenedesmus dimorphus* These microalgae have been fairly-well studied on various grounds [34, 35]. *D. tertiolecta* belongs to the *Chlorellaceae* family *and* has a high reproduction rate and low oil content [36] with a preferred temperature of 30℃ up to even 42℃ [37]. *N. oleoabundans* belongs also to the *Chlorophyceae* family, and has a fairly fast reproduction rate, a preferred temperature range of 28-32℃ and a normal oil yield capacity, although some studies have shown that it can be manipulated to produce more oil [38]. *Chlorella vulgaris* displays several favorable biological characteristics, which make it a highly regarded species [39]. The growth of Chlorella under different growth conditions and regimes resulting in high biomass for biofuels production is one of these features. The optimal temperature for *Chlorella vulgaris* is about 30°C, in which the maximum biomass productivity is achieved [39]. Finally, *S. dimorphus* belongs to the Chlorophyta family and grows best at temperature of 28℃ and 30℃, where it produces the most biomass for downstream processing [40].

B. Study Sites

This study was conducted at Center for Renewable Energy and Sustainability (CRES-Center) of the University of Puerto Rico, Rio Piedras, College of Natural Sciences, Department of Environmental Sciences.

C. Experimental Design

The experimental design was loosely based on the work by Tang [41], with the goal of evaluating how these strains respond to various concentrations of nanoparticles. A 96-well plate was used in this experiment. Each experimental well in the 96-well plate contained 340$\boldsymbol{\mu}$L of BBM (Bold's Basal Medium) for freshwater algae or F/2 for saltwater. Nanoparticles at a specific concentration (0µM, 20µM, 40µM, 60µM and 80µM) were added to each well. These treatments were all run in triplicates. Negative (only BBM or F/2 algal

growth medium) and positive (algal cultures without nanoparticles) controls were also run in triplicates.

D. Synthesis and characterization of nanomaterials

1) Synthesis of gold nanoparticles

Gold nanoparticles were synthesized using the citrate reduction method [42]. An aliquot of 20 mL of 1mM gold (III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$) was heated and stirred until the solution was boiling. Immediately, 5mL of a 1 % sodium citrate solution was added. The mixture was continuously heated and stirred until a change in color was observed from yellow to red.

2) Characterization of gold nanoparticles

The citrate reduction method is known to give spherical nanoparticles [42]. Microscopy images taken with a FEI F20 TEM confirmed the spherical morphology. Computer program ImageJ was used to determine the average size of the nanoparticles to be 10-15 nm. UV-Vis spectroscopy was used to measure the resonance plasmon of the AuNPs which was consistent with the average size and morphology.

3) Magnetite nanoparticles synthesis

Magnetite nanoparticles were synthesized via the co-precipitation method as presented by S. I. Shanmuga et al. [43]. In brief, a 100 mL solution containing 0.1 M of ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) and 0.2 M ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) was concocted in a round flask using nanopure water. The contents of the resulting solution were mixed vigorously with the use of a magnetic stirrer at a constant temperature of 85^{o}C and kept under inert atmosphere. At the same time, 80 mL of a 1.0M solution of sodium hydroxide (NaOH) was added to the previous solution in a dropwise manner to serve as the reducing agent. In the final stages of reaction, the solution changed from brown to black and was removed from the heat. A black precipitate with a translucent supernatant confirmed the formation of the magnetite nanoparticles. These nanoparticles were then washed via centrifugation with nanopure water a total of 4 times to remove excess NaOH and the remaining salts. The resulting nanoparticles were dried at 70^{o}C under inert atmosphere. Ultimately, the black precipitate was pulverized and stored in a vial under vacuum.

4) Characterization of magnetite nanoparticles through SEM-EDS

Morphology, size, and elemental composition of the synthesized magnetite nanoparticles were assessed through Scanning Electron Microscopy (SEM) and Energy-Dispersive X-ray Spectroscopy (EDS) with a JEOL JSM-6480-LV microscope. The analysis was carried out at 20 kV and under 20,000X magnification. The characterization by S. I. Shanmuga et al [43] shown average size range from 70 to 100 nm, and the arrangement of the nanoparticles are cubic centered.

E. Algal Culture and Cells Exposure

Chlorella vulgaris, *Scenedesmus dimorphus*, *Dunaliella tertiolecta* and *Neochloris oleoabundans* purchased from NCMA (The National Center for Marine Algae and Microbiota - Bigelow Laboratory for Ocean Sciences, Maine - USA) were cultured in BBM/F/2 media (pH 8.2) supplemented with vitamins B1 and B12 at 30 °C under light–dark cycles of 12hr each, in an incubator shaker (New Brunswick™ I24 shaker) at 100 rpm. Cell numbers and volume were measured with a spectrophotometer (Thermo Scientific™ NanoDrop™ 2000/2000c Spectrophotometers) on a daily basis for a period of 40 days.

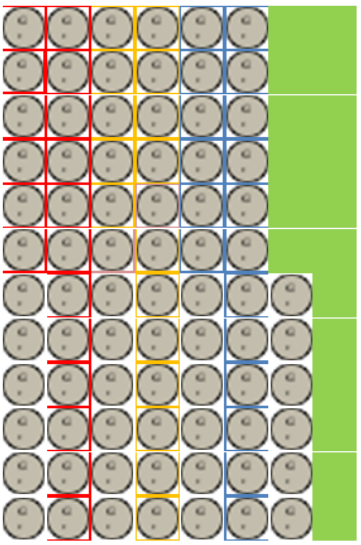

Figure 1. Experimental Setup using 96-well plates with different treatment arrangements. Saltwater Algae – Mixed Cultures (90-Well-Plate Set up) – F2

In order to determine the initial inoculum, *Chlorella vulgaris*, *Scenedesmus dimorphus*, *Dunaliella tertiolecta* and *Neochloris oleoabundans* were cultured in Erlenmeyer flasks containing 30ml of BBM / F/2. When the cultures reached a certain optical density (OD of 1.0), they were vortexed to obtain a homogenized solution. Before exposure to AuNP, CNP and FeNP, exponentially grown algae were centrifuged at 2000×*g* for 10min and then resuspended in MOPS. The final cell density for toxicity assessment was 1.5×10^4 cell/mL.

96-well plates were used for the inoculation of algal solutions for each species. Two plates were designed for mixed culture grown on BBM (freshwater algae) and F/2 (marine algae) (Figure 1). The plates were set up in a similar fashion, with the only difference being the algae strains used. In the Figure 1 schematics, each well is labelled. These labels represent either the controls or the specific nanoparticle and concentration used. Each well contains a total of 340 microliters, this volume is divided between the specific nanoparticle concentration and the rest with the corresponding media. The nanoparticles were classified as C (Carbon), G (Gold), F (Iron1) and I (Iron2). Then each of these nanoparticles was added at a specific concentration into the wells. B represent blanks and the subscripts on each of the letters correspond to the specific nanoparticles' concentration (2=20μM, 4=40μM, 6=60μM and 8=80μM).

Using the schematic below, the plates were filled with the appropriate culture media and nanoparticles. Triplicates were

done on each concentration and control. Afterwards, plates were sealed with parafilm and labelled accordingly. Initial measurement (T_0) was taken in a plate reader or spectrophotometer (iMark Microplate Absorbance Reader from Bio-Rad) at a wavelength of 450nm, and the plates were incubated under controlled temperature of 30°C and humidity. After exposure to AuNP (0–80μM) CNP (0–80μM) and FeNP (0–80 μM) to study their toxic effects, the growth and photosynthetic yield were measured every 24hr for a period of 6 weeks. The former was performed using the spectrophotometer and the latter by fluorometry using a PHYTO-PAM (Heinz Walz GmbH, Germany). The values were presented as percentage of controls and were plotted as a function of measured total NP and cell-associated NP (*supplemental data*).

F. Extraction and Determination of chlorophyll

Chlorophyll content was determined by acetone extraction. 1.5ml of algal biomass was added to 90% acetone and homogenized by vortexing. The mixture was then incubated in a 50°C water bath for 30 minutes. Following incubation, the mixture was centrifuged at 3000 rpm for 10 minutes and the supernatant transferred to a clean centrifuge tube. The solution was allowed to stand for a short period of time prior to an additional 10min of centrifugation. This procedure was completed in subdued lighting. The chlorophyll content of the samples was determined by using the spectrophotometric methods. In order to determine the chlorophyll content of the extract, the absorbance of the sample was measured at several wavelengths, between the range of 250 and 800 nm against the solvent (acetone) blank to obtain the total spectrum. The concentration of Chlorophyll a and Chlorophyll b were evaluated according to the following SCOR-UNESCO (1966) equations [44]:

Chlorophyll a -μg chlorophyll/mlmedium =

$$(11.64A663 – 2.16\ A645 + 0.10A630)v/\ lV$$

Chlorophyll b -μg chlorophyll/mlmedium =

$$(-3.94A663 + 20.97\ A645 - 3.66A630)v/\ lV$$

Axxx = the absorbance at xxx nm, after removing the sample absorbance at 750 nm against a blank of the solvent used V = l = V = the volume of acetone used (ml) I = the spectrophotometric cell length (cuvette) (cm) V = the sample volume (ml)

G. Protein Extraction

Protein extraction was based on a modified method developed by Price [45]. For each strain, 5 mg (±10%) of freeze-dried micro-algae material was weighed out. Samples were resuspended by vortexing in 250 μL 6% (w/v) TCA (Trichloroacetic acid). Homogenates were incubated in a water bath at 65 °C for 15 min, in screw-capped micro-centrifuge tubes and allowed to cool to RT. The homogenates were centrifuged at 15,000*g* for 20 min at 4 °C (Microcentrifuge 5415 R, Eppendorf AG, Hamburg, Germany) and their supernatants discarded. The pellets were resuspended in 0.5 mL Lowry Reagent D by repeated pipetting or vortexing and incubated over a series of time-points (10 min to 22 h) at 55 °C. Samples were then cooled to RT, spun at 15,000*g* for 20 min RT and the supernatant retained; samples were then frozen at −20 °C for further analysis.

H. Protein quantification

Protein quantification followed the method of Lowry [46] as modified by Price (1965) [45]. A stock of Lowry Reagent D was made up daily in a 48:1:1 ratio of Lowry Reagents A (2% (w/v) Na_2CO_3 (anhydrous) in 0.1 N NaOH); B (1% (w/v) NaK Tartrate tetrahydrate) and C (0.5% (w/v) $CuSO_4.5H_2O$ in H_2O), respectively. Reagents A, B, and C were stored at RT. The Lowry assay also employed Folin-Ciocalteu phenol reagent (Sigma). A stock of a 1:1 ratio of 2 N Folin-Ciocalteu phenol reagent: ultra-pure water was made daily. An appropriate volume (up to 50μL) of the above protein extract was added to individual 1.5mL microfuge tubes in triplicate, followed by the addition of 950μL of Lowry Reagent D. microfuge tubes were immediately mixed by inversion. Samples were then incubated for 10min at RT. Next, 0.1mL of the diluted Folin-Ciocalteu phenol reagent was added to each tube and vortexed immediately. After 30min at RT, the absorbance of each sample was read at 600nm (Nicolet Evolution 300 spectrophotometer, Thermo Electron Corporation, Madison, WI) using VisionPro™ software (Thermo Electron Corporation) (Findlay, 1990, Walker, 2002). Calibration curves were prepared for each assay with a bovine serum albumin (BSA) stock solution (200 mg/mL; Sigma P5369) and using a polynomial line of best fit generated in Microsoft Excel 2017.

I. Lipid extraction

A modified protocol of Folch et al. [46] was adapted to provide a final solvent system volume of 10ml for extraction of about 200mg of wet weight microalgal biomass. Microalgal paste was homogenized in a glass Potter-Elvehjelm homogenizer together with solvents. Cell debris was removed by means of vacuum filtration through a Whatman grade GF/C glass microfiber filter (1.2 μm) into a glass centrifuge tube. Phase separation was facilitated by centrifugation at 350g for 2min and the organic, lower phase was placed in an aluminum foil cup for overnight solvent evaporation at room temperature followed by gravimetrical determination of the lipid extract. In the Folch method, microalgal paste was homogenized in a 2:1 chloroform:methanol (v/v) mixture and cell debris was removed by filtration. The homogenizer and collected cell debris were rinsed with fresh solvent mixture and the rinse was pooled with the previous filtrate prior to the addition of a 0.73% NaCl water solution, producing a final solvent system of 2:1:0.8 chloroform:methanol:water (v/v/v).

J. Chemical analysis of lipid extracts

For a general screening of extracted compounds, solvents were removed by evaporation and the dry extracts were dissolved in dichloromethane prior to a full scan analysis by GC/MS for m/z fragments up to 700 (DB50 column, EI 35 eV, quadrupole). Qualitative and quantitative FA (fatty acids) profile analyses were performed at the Department of Chemistry. FA were methylated as previously described [23] and analyzed with a gas chromatograph with an FID detector (GC-FID) [24]. The GC-FID analyses were done with two and three replicates for qualitative analyses and with

three replicates with internal standards (461 standard reference mixture from Nu-Chek Prep Ink. USA) for quantitative analyses.

K. Microscopic analyses

At the end of the experiment, the impact of nanoparticles on the shape of algal cells was analyzed using light fluorescence microscopy (BX-51; Olympus, Tokyo, Japan). To obtain clear images of the algal mucilaginous sheath, samples were negatively stained with nigrosine solution (100 g/L, formalin 5 mL/L in water) (Sigma-Aldrich), based on a method modified from Guedes *et al*. [47]. A drop of algal suspension was placed near the edge of a glass slide and mixed gently with a drop of nigrosin. A smear was drawn and dried for 1sec. Using this method, the algal mucilaginous sheath is unstained (white) while the background becomes black when using a light microscope.

L. Data analysis

All data were analyzed by GraphPad Prism (version 5.02 for Windows, USA). Fluorescent units obtained in the cell assays were converted to percent viability of control cells. Concentrations leading to 10% and 50% effect (EC10s, EC50s) were determined by nonlinear regression sigmoidal dose–response curve fitting using the Hill slope equation and were presented as the mean of three independent experiments, with a 95% confidence interval. Data are presented as mean ± standard deviation, n = 3.

III. RESULTS AND DISCUSSION

The toxicity of Au, Fe and C nanoparticles on various algal strains was investigated (Figs. 2-5). In this study, several endpoints, such as growth, photosynthetic capacity, morphology and permeability of the algal cells were monitored over a 40-day period. The experimental results indicated that 60µM of FeNP could completely inhibit the growth of 10^7 cfu/mL algal cells in liquid F/2 medium. In the biochemical study, it has been observed that, FeNP resulted in the leakage of proteins. Lowry assay proved the extracellular release of proteins into the growth media. When the algal cells were exposed to FeNP, many pits were observed, and the cells were found to be fragmentary. In other studies, released Fe from NPs monitored by AAS, suggested that FeNP can destroy the permeability of algal membranes. Our study demonstrated that FeNP damaged the structure of algal cell membrane and depressed the activity of some membranous enzymes which caused the algal cells to die eventually.

A. Algal Cells Toxicity

The microalgae responded differently to the various treatments, in terms of growth. However, the 60uM concentration of iron NPs seems to be fatal to most strains, as it caused algal death after less than a week exposure (Figs. 2-5).

By decreasing phytoplankton biomass and their concomitant diversity in oceans and lakes, NPs will automatically help regulate micronutrients bioavailability.

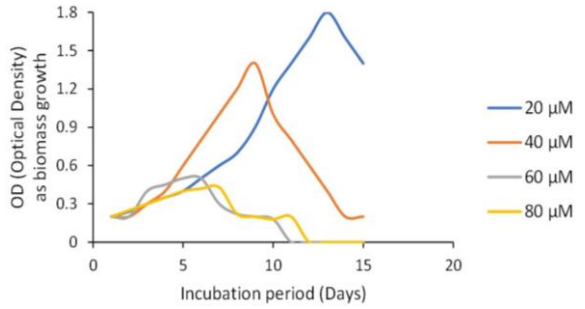

Figure 2. Impact of various Concentrations of FeNP on Dunaliella Growth

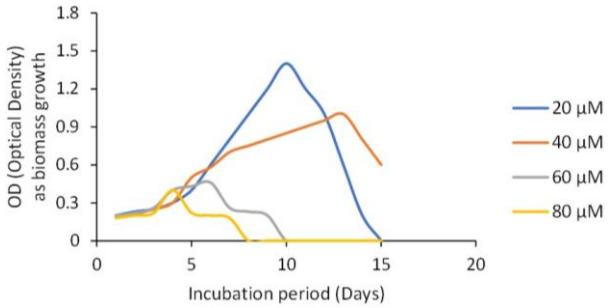

Figure 3. Impact of various Concentrations of FeNP on Chlorella Growth

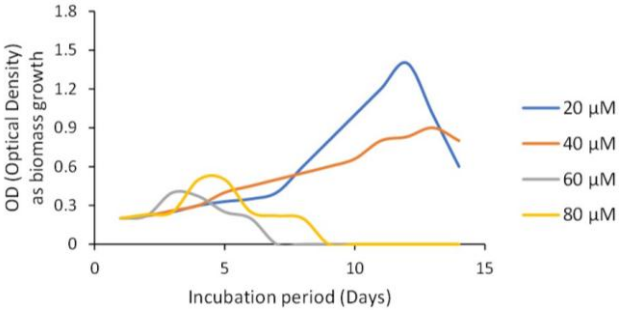

Figure 4. Impact of various Concentrations of FeNP Scenedesmus Growth

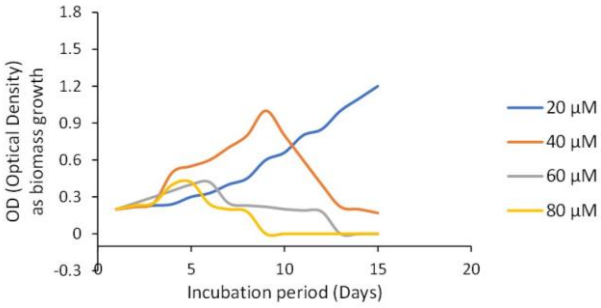

Figure 5. Impact of various Concentrations of FeNP on Neochloris Growth

These micronutrients, such as Cu, Co, Fe, Mn, Mo, Ni and Zn are known to be involved in important enzymatic processes within aquatic ecosystems. Therefore, such changes could have

profound consequences on the Carbon and Nitrogen biogeochemical cycles, Food-web interactions can also be affected, as almost 50% of primary production on earth is driven by phytoplankton, which are at the base of the aquatic food chain. Such findings highlighted the vulnerability of our ecosystems to NPs. Further studies should shed lights on additional impacts and assist regulatory bodies in dealing with these nanomaterials.

Photosynthetic capacity is indeed one of the most direct parameters which can shed light on algal cells viability, as these are considered autotrophic organisms. Consequently, to determine the toxicity of Fe, Au and Carbon nanoparticles on algal cells, the photosynthetic yield was assessed over an extended period of time (2 weeks). Effective concentrations (EC50s) causing a 50% decrease in photosynthetic yield were calculated from dose–response curves derived from various algal cells exposed to different concentrations of NPs. The EC50s was about 1.5µM and 0.09µM for algae exposed to Au and C-NPs respectively. These values were in fact comparable with EC50 values reported for other algal species [3].Therefore, it is safe to conclude that gold and carbon NPs at certain concentrations could induce toxicity in algae.

B. Proteins Binding

Findings from experiments conducted on the effects of NPs on algae have shown that algal cells do not take up NPs [8,9]. The interactions happen only at the extracellular interface. Several studies have demonstrated that alkaline phosphatase, an enzyme produced by a variety of organisms in aquatic environments, is stimulated [8] by AuNP and remained unaffected by C-NPs. However, it was unclear whether the said effects were due to lack of interactions with NPs or were not able to be measured correctly [8]. On the other end, several reports have demonstrated the inhibitory effects of NPs on extracellular enzymes [8]. β-glucosidase was shown in the same study on periphyton cited above [8], to be inhibited by dissolved gold particles. The same enzyme has displayed inhibitory effects in heterotrophic biofilm, when exposed to Ti-NPs [9]. It is therefore of utmost importance, that the interactions of extracellular enzymes be studied further, and their mechanisms of interaction better understood.

Studies have also shown that since the behavior of NPs is affected by the growth medium, this seems to directly impact their toxicity. [10] AuNP toxicity to algae was mediated by dissolved gold, which was adsorbed onto the surface, thereby inhibiting extracellular enzyme activity. Results from our work showed that algal cell wall seems to be a barrier to NPs uptake. Yet, NPs seems to affect extracellular enzymes secreted by algae as well. Further studies need to be conducted, focusing on dissolution and aggregation of NPs, which will shed light on the localization of these particles, a factor that seems to impact their bioavailability and toxicity.

IV. CONCLUSIONS

The summary of the side by side comparison of CNP, FeNP and AuNP-cell interactions for algae revealed that the growth media seems to influence NP behavior and toxicity. Such findings highlight the importance of characterizing NPs, when evaluating possible risks of these materials. The cell membrane provides a barrier preventing the uptake of NPs, which ended up being toxic just by adsorbing to the surface. This is a clear indication that the exposure media is playing an important role in the whole process of course, any toxicity studies should take the cell type and structure into account. Via specific effects, NPs have the ability to inhibit enzymes activities by binding to proteins extracellularly and intracellularly. The work presented here provides clear examples of NPs interactions with microorganisms in aquatic ecosystems. Future research should not only focus on understanding the mechanism of interactions but also help design better and safer nanomaterials

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