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The effect of engineered iron nanoparticles on growth and metabolic status of marine microalgae cultures

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ABSTRACT

Synthetic zero-valent nano-iron (nZVI) compounds are finding numerous applications in environmental remediation owing to their high chemical reactivity and versatile catalytic properties. Studies were carried out to assess the effects of three types of industrially relevant engineered nZVI on phytoplankton growth, cellular micromorphology and metabolic status. Three marine microalgae (*Pavlova lutheri*, *Isochrysis galbana* and *Tetraselmis suecica*) were grown on culture medium fortified with the nano-Fe compounds for 23 days and subsequent alterations in their growth rate, size distribution, lipid profiles and cellular ultrastructure were assessed. The added nano Fe concentrations were either equimolar with the EDTA-Fe conventionally added to the generic f/2 medium (i.e. 1.17×10^{-5} M), or factor 10 lower and higher, respectively. We provide evidence for the: (1) broad size distribution of nZVI particles when added to the nutrient rich f/2 media with the higher relative percentage of the smallest particles with the coated forms; (2) normal algal growth in the presence of all three types of nZVIs with standard growth rates, cellular morphology and lipid content comparable or improved when compared to algae grown on f/2 with EDTA-Fe; (3) sustained algal growth and normal physiology at nZVI levels 10 fold below that in f/2, indicating preference to nanoparticles over EDTA-Fe; (4) increased total cellular lipid content in *T. suecica* grown on media enriched with uncoated nZVI25, and in *P. lutheri* with inorganically coated $nZVI_{powder}$, when compared at equimolar exposures; (5) significant change in fatty acid composition complementing the $nZVI_{powder}$-mediated increase in lipid content of *P. lutheri*; (6) a putative NP uptake mechanism is proposed for *I. galbana* via secretion of an extracellular matrix that binds nZVIs which then become bioavailable via phagocytotic membrane processes.

1. Introduction

Engineered Fe-rich nanoparticles (NPs) are key multifunctional material applied in many different fields of modern technology including environmental remediation (reviews of Zhang, 2003; Mueller and Nowack, 2010), the food industry (Fidler et al., 2004), and medical diagnostics (Cheng et al., 2005). Owing to their high chemical reactivity and capability to act as electron donors to catalyse a wide variety of reactions (Zhang, 2003) zero-valent iron nanoparticles (nZVI) in particular have been applied to decontamination of ground and surface waters, as well as industrial wastewaters polluted with a diverse group of compounds (Zhang, et al., 2010). In theory the use of nano-iron for in situ decontamination is more promising than in practise owing to issues regarding oxidation and aggregation and gelation, which however are being constantly improved by stabilising agents applied on the ZVI NPs (Mueller and Nowack, 2010). With such a widespread production/usage and with improved stability engineered nZVIs inevitably reach aquatic ecosystems (Readman et al., 2011), which already are naturally enriched with colloidal iron nanoparticles (Whang and Dei, 2003). Phytoplankton is therefore naturally exposed to elevated levels of NPs, both natural (via cloud processing of mineral dusts/volcanic ash) and engineered forms (from green technologies and various marine applications), but the mechanisms involved are not clear. Biological interactions especially in the case of the organic stabilising agents used to improve the mobility of nZVIs in the environment have been underestimated (Lerner et al., 2012) with potential negative impacts overlooked (Kadar et al., 2011, in press). Iron NPs can produce various reactive oxygen species (ROS) via Fenton-type reactions (LeBel et al., 1992) that cause oxidative injury to cells via lipid peroxidation and oxidation of thiol groups on proteins and DNA (Kadar et al., 2010a, b; Kadar et al., 2011, in press; Keenan et al., 2009; Li et al., 2009; Auffan et al., 2008; Fernaeus and Land, 2005; Zhou et al., 2003). Paradoxically, Fe is also an essential micronutrient for phytoplankton as it is required in fundamental cellular functions like photosynthesis and respiration and consequently its availability controls phytoplankton productivity, community structure, and ecosystem functioning in vast regions of the global ocean (reviewed by Gledhill and Buck, 2012). The biological availability of Fe is highly dependent on its chemical speciation as

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well as on the capabilities of organisms to extract Fe from various chemical species, whether by reduction or by dissolution via high affinity uptake mechanisms (Vraspir and Butler, 2009). The large number and complexity of interactions involved in the biogeochemical cycling of Fe in the marine environment are unresolved despite the concerted effort of scientists across several fields (Gledhill and Buck, 2012). There is a need for lab-scale experimental studies focussed on the metabolic role of trace elements as limiting nutrients and toxicants to phytoplankton. Hence, our small scale lab-based studies, in controlled conditions to enable direct observations of the physiological ecology of microalgae, which can then inform on the dynamics in natural assemblages (MacIntyre and Cullen, 2005). Here we propose culturing marine key microalgae (*Pavlova lutheri*, *Isochrysis galbana* and *Tetraselmis suecica*) with distinct ecological niche, evolutionary history (Eltgroth et al., 2005) and cell membrane constitution (Bendif et al., 2011), and thus putatively distinct Fe uptake mechanisms. We culture these algae on media amended with soluble- versus nano-iron in order to test the hypothesis that engineered NPs may be a more favourable source of iron to microalgae than the soluble form (applied here as EDTA-Fe). We will thus be able to make inferences on the larger scale implications of nano-scale mineral dust deposition on ocean productivity. In addition, we investigate how the lipid class and fatty acid composition of microalgal cells can change with variations in Fe substrate in culture media; understanding how bioavailability of Fe controls algal physiology may have both scientific and commercial impact as many marine phytoplankton species are receiving increasing interest because of their ability to produce lipids with high nutritional value for mariculture fed (Volkman et al., 1989), as human food ingredients (Khozin-Goldberg et al., 2011; Berge and Barnathan, 2005) or biodiesel (Mata et al., 2010). The species chosen here (*Tetraselmis*, *Isochrisis and Pavlova*) have been extensively studied regarding their favourable nutritional values (Muller-Feuga et al., 2003; Volkman et al., 1989). Furthermore, they are key components of the primary producers in brackish ecosystems that are particularly vulnerable to NP discharge (Readman et al., 2011).

The overall aim of this research was to collect data on the effect of nZVI on the growth and physiology of three key coastal marine microalgae (*I. galbana*, *P. lutheri* and *T. suecica*). We examine the effect of Fe supplementation via three environmentally relevant NPs that have distinct surface properties versus the conventionally applied EDTA-Fe. Cellular abundance and biometry, micromorphology and lipid production are examined and reported for each alga grown on different concentrations of the 3 types of nano-Fe amended media. We also determine the aggregation behaviour of NPs in the artificial algal growth medium to aid interpretation of the data. The larger scale implications of the particle-size mediated improvement in bioavailability are discussed with reference to the potential phytoplankton biomass increase in the open ocean following atmospheric iron NP input.

2. Materials and methods

2.1. Preparation of nanoparticle stock suspension and the behaviour of nZVIs in seawater

Dispersions of three different types of nZVIs were used, supplied as slurry or as powder from Nanoiron Ltd., Czech Republic. The selected compounds were: NANOFER 25S®, which is surface coated with a Na-acrylic copolymer, NANOFER 25® that contains no additives (uncoated), and NANOFER STAR® (Surface stabilized Transportable Air-stable Reactive) powder with an inorganic coating (referred hereafter as nZVI25S, nZVI25 and $nZVI_{powder}$, respectively). Details of the test materials are described elsewhere (Kadar et al., 2011 and 2012) and/or available from the product description sheets on the supplier's website, including information regarding the type and amount of stabilising agents (http://www.nanoiron.cz/images/stories/technical_data_sheet_nanofer_25s.pdf last accessed on the 30th of May 2012). Briefly, all three compounds are characterised by (manufacturer's information): mean particle size, 50 nm; specific surface area, >25 $m^2\ g^{-1}$; purity of iron in solid phase ~85%. Stock suspensions of 100 mg L^{-1} of each nanomaterial were made up in 100 mL filtered (0.2 μm) seawater (pH 8.15 ± 0.5), and the entire 100 mL volume of each dispersion was sonicated for 2 h in a Lucas Dawe Ultrasonics bath (model no. 6456-A1). Freshly sonicated suspensions were added to the autoclaved Fe-free trace metal mix and then to the f/2 media (devoid of iron) in the appropriate concentrations just before the inoculation with microalgae (see below).

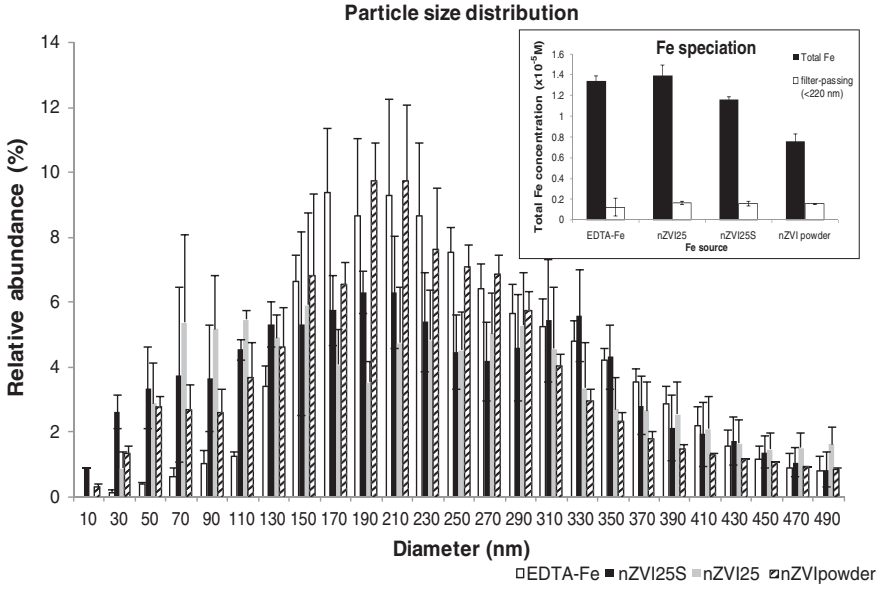

Fig. 1. Particle size-distribution in the stock suspensions (10 mg L^{-1}) of nZVI25 (uncoated), nZVI25S (organically coated using Na-acrylic copolymer), $nZVI_{powder}$ (inorganically coated) and the non-nano form for added as EDTA-Fe, 1 h after sonication. The bars represent averages ± STDEV ($n = 3$) of the relative percentages calculated by the Nanotrack software from 100 tracks/sample; figure insert shows total Fe concentration in experimental media both prior to and following filtration through 0.22 μm pore-size, polycarbonate membranes. The bars represent averages ± STDEV ($n = 3$).