

# The effect of silica and maghemite nanoparticles on remediation of Cu(II)-, Mn(II)- and U(VI)-contaminated water by *Acutodesmus* sp.

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Received: 22 July 2014 / Revised and accepted: 25 February 2015 / Published online: 26 March 2015  
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**Abstract** The effect of silica and maghemite nanoparticles (NPs) on the sequestration of Cu, Mn and U by *Acutodesmus* sp. was investigated with the aim of quantifying the influence of NPs on the remediation efficiency of the alga. Metal removal was thus quantified in NP-only, algae-only and NP-algae batch treatments. Results showed that adsorption in NP-only systems was rapid, attaining equilibrium within 5 min. Removal of Cu was higher with maghemite NPs, while more Mn and U were removed with silica NPs. Reaction kinetics were better described by the pseudo-second-order rate model, and isotherm data were fitted by the Freundlich model. Metal removal in NP-algae systems was ~12–27 % higher than in algae-only or NP-only systems due to the greater number of sorption sites in NP-algae treatments. NPs also modified algae-metal partitioning: extracellular concentrations were higher and intracellular fractions lower in the presence of NPs relative to controls (without NPs). NP agglomeration in metal solutions was quantified in order to determine the potential for NP absorption by algal cells. Results showed that NPs coalesced to form agglomerates 300 ( $\pm$ 100) nm in

diameter, which were unlikely to be absorbed through algal cell walls. As some studies have shown metal toxicity to be related to intracellular metal fractions in algae, a combination of NPs and algae for phycoremediation can therefore improve the efficiency of operations both by increasing removed metal fractions and by protecting algal cells from metal toxicity.

**Keywords** Biosorption · Phycoremediation · Aquatic metal contamination · Algae · Nanomaterials

## Introduction

The contamination of aquatic systems by metal and radionuclide ions as a result of mining and other anthropogenic activities has elicited increasing concerns globally. This is because excessive metal concentrations are deleterious to both humans and other organisms, extending beyond individual species to entire communities (Niyogi et al. 2002). As such, research is increasingly focused on finding novel and efficient methods for the remediation of metal-contaminated water, not only to avert toxicity in ecosystems but also to meet the growing human demand for water.

Various techniques including chemical precipitation, reverse osmosis, nanofiltration, adsorption, and ion exchange are currently applied for the removal of metal ions from contaminated water (Kurniawan et al. 2006). The high costs of some of these conventional technologies has, however, spurred interest in lower-cost technologies that make use of plants (phytoremediation) and algae (phycoremediation). These are relatively new approaches to the treatment of metal-contaminated water that exploit the potential of algae and higher plants to accumulate metal ions in their biomass (Rajamani et al. 2007). The use of microalgae, e.g. *Scenedesmus*, *Acutodesmus* and *Desmodesmus* spp., for phycoremediation is particularly attractive due to their large

**Electronic supplementary material** The online version of this article (doi:10.1007/s10811-015-0555-z) contains supplementary material, which is available to authorized users.

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surface area to volume ratios. These organisms also possess high-affinity metal binding functionalities on their cell walls, viz. hydroxyl, sulphhydryl, carboxyl and amino groups that enable microalgae to bind up to 10 % of their biomass as metals (Tien et al. 2005; Rajamani et al. 2007; Monteiro et al. 2008).

The adsorptive removal of metal ions from contaminated water is also of considerable interest due to the ease of the adsorption process and the availability of a wide range of adsorbents (Siao et al. 2007). In keeping with recent advances in nanoscience, nanoparticles are increasingly investigated as adsorbents for the remediation of metal-contaminated water. Because of their high reactivities and relatively large surface areas, nanoparticles (NPs) sequester contaminants more efficiently than larger adsorbents, while also generating smaller volumes of contaminated wastes (Engates and Shipley 2011; Hua et al. 2012).

The aim of this study was to explore the combined use of NPs and algae for the remediation of metal-contaminated water. Based on the high efficiency of both of these adsorbents, metal removal should be enhanced relatively to NP-only or algae-only systems due to biologically mediated uptake, higher sorptive surface areas and the high reactivity of NPs. Recent evidence also points to a reduction in the toxicity of metal ions to algae in the presence of NPs (Hartmann et al. 2010; Yang et al. 2012a, b; Dalai et al. 2014). As such, besides increasing sorptive surface areas and the efficiency of phycoremediation, NPs may also increase the viability of algae in remediation systems.

Cu, Mn and U are common metal contaminants in freshwater bodies receiving wastewater from mining and industrial effluent. Their uptake by species of the Scenedesmaceae which are easily cultured freshwater green microalgae with a high-metal removal capacity (Zhang et al. 1997; Terry and Stone 2002; Monteiro et al. 2008) was studied in the presence and absence of silica and maghemite NPs in order to determine the influence of NPs on metal removal by *Acutodesmus* sp. The influence of NPs on the partitioning of metal ions between extracellular and intracellular fractions of algal cells was also quantified. The information generated from this study, besides informing the optimization of phycoremediation, may also be applied for delineating the potential effects of NPs on algae-contaminant interactions, especially in light of the expected increase of anthropogenic NPs in the environment (Wilson Centre 2014).

## Materials and methods

Analytical grade  $\text{Cu}(\text{NO}_3)_2 \cdot 2.5\text{H}_2\text{O}$  and  $\text{Mn}(\text{NO}_3)_2 \cdot \text{H}_2\text{O}$  (Sigma-Aldrich, Germany) and  $\text{UO}_2(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$  (Ace Chemicals, South Africa) were used for experiments. Commercially prepared silica and maghemite NPs were purchased

from Sigma-Aldrich. All metal solutions, NP suspensions and culture media were prepared using deionised water. NP suspensions were prepared by 30-min sonications in a water bath. This was done immediately before use to minimise losses through particle settling and adhesion to container walls. Metal solutions were prepared 1 day in advance to allow for equilibration. The pH of metal solutions was adjusted to 6.5 using 0.01 M NaOH. This pH was chosen as it was found to be favourable for the survival of the algae.

The algae strain used in this work was *Acutodesmus dimorphus* isolated from a freshwater source in Johannesburg, South Africa. The cells were grown in modified Bold's Basal media (Appendix 1 in [Electronic supplementary material](#)) in 250-mL Erlenmeyer flasks at  $25 \pm 1$  °C and with continuous bubbling with air. Light ( $150 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) was provided in a 18:6-h light/dark cycle using cool-white fluorescent lamps, and the pH of cultures was maintained at 6–8. All glassware and plastic ware were soaked overnight in 0.2 M  $\text{HNO}_3$  and rinsed three times with deionised water before culturing. Glassware and culture media were sterilised by autoclaving at 121 °C for 15 min before use.

## Particle characterisation

The primary particle size of NPs was determined using a Tecnai G2 Spirit transmission electron microscope at an acceleration of 120 kV. NPs were suspended in deionised water, sonicated in a water bath for 30 min and placed on lacey copper grids to dry before analysis. The specific surface area, pore volume and pore sizes of particles were determined using a Micrometrics Tristar 3000 (Micrometrics Instruments, USA) following  $\text{N}_2$  adsorption-desorption for 4 h at 150 °C. Particle phase (crystalline or amorphous) was determined by X-ray diffraction (XRD) using a Bruker D8 diffractometer ( $\text{Cu K}\alpha$ ).

In order to understand the state of particles in test media, their hydrodynamic sizes in metal solutions were determined by dynamic light scattering (DLS) using a Zetasizer NanoZS (Malvern Instruments, UK). Measurements were taken as soon as NPs were dispensed into metal solutions ( $t_0$ ) and after 4 h ( $t_4$ ; the experimental duration of adsorption reactions). Thus, 3 mg of NPs were suspended in 1 L of deionised water and sonicated for 30 min. An aliquot of this suspension (10 mL) was then dispensed into a 50-mL polyethylene terephthalate (PET) jar containing 10 mL metal solution and light scattering readings taken immediately (10–15 s lag time) and after 4 h. The hydrodynamic sizes of NPs after the 30 min sonications in deionised water were also determined.

## Metal uptake experiments

The first step in studying the effect of NPs on algae-metal uptake was the investigation of NP-metal interactions. Thus, the kinetics and isotherms of Cu, Mn and U adsorption by NPs

were investigated at pH 6.5 ( $\pm 0.2$ ). This was followed by quantification of metal adsorption by algae alone and eventually by algae in the presence of NPs.

Adsorption kinetics were determined at reaction durations ranging from 10 s to 1 h using 1.42, 3.30 and 0.99 mg L<sup>-1</sup> of Cu, Mn and U, respectively. Isotherms were studied at concentrations ranging from 0.66 to 5.41 mg L<sup>-1</sup> for Cu, 1.36–5.65 mg L<sup>-1</sup> for Mn and 0.55–1.57 mg L<sup>-1</sup> for U. Ten millilitres of NP suspension (3 mg L<sup>-1</sup>) was added to each metal ion solution in a PET jar and allowed to react for 5, 10, 15, 30, 45, 60, 300, 600, 900, 1800, 2700 and 3600 s. Isotherm experiments were conducted for 60 min. All experiments were conducted in duplicate and at ambient temperature ( $\sim 25$  °C).

At the end of the incubations, NPs were separated from mixtures using 100 kDa Amicon ultracentrifugal filters at 2808×g for 30 min. Filtrates were acidified with 3 % HNO<sub>3</sub>, and their metal concentrations (non-adsorbed fraction) were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES; Spectro Instruments, Germany). Metal removal was quantified by mass balance calculations. Nanoparticle metal loading at a given time ( $q_t$ , mg g<sup>-1</sup>) and at equilibrium ( $q_e$ , mg g<sup>-1</sup>) was calculated using Eqs. 1 and 2, respectively.  $C_i$  and  $C_e$  are the initial and equilibrium metal ion concentrations (mg L<sup>-1</sup>), respectively;  $m$  is the mass of adsorbent (g), and  $V$  is the volume of adsorbent solution used (L).

$$q_t = \frac{(C_i - C_e)V}{m} \quad (1)$$

$$q_e = \frac{(C_i - C_e)V}{m} \quad (2)$$

Kinetics data were fitted to the pseudo-second-order model ( $q_t = q_e(1 - \exp(-kt))$ ) (Ho and McKay 2004) and isotherm data to the Freundlich adsorption isotherm ( $\log q_e = 1/n \times \log C_e + \log K_F$ ) (LeVan and Vermeulen 1981). Data fit was determined by linear regression.

Experiments were then conducted to investigate the effects of NPs on metal adsorption by *Acutodesmus* sp. Metal uptake by algae was quantified at initial metal concentrations varying from 0.66 to 1.42 mg L<sup>-1</sup> for Cu, 1.36–3.30 mg L<sup>-1</sup> for Mn and 0.55–0.99 mg L<sup>-1</sup> for U, in the presence or absence of silica and maghemite NPs. Cu and U concentrations were selected based on average concentrations in acid mine drainage (AMD)-contaminated surface water from Johannesburg (Etale et al. 2014a), but those of Mn were lower than field concentrations to prevent toxicity.

Algae cells in the exponential growth phase were harvested by centrifugation for 20 min at 2808×g and 4 °C. The supernatant was discarded, and the algae pellet was rinsed twice with deionised water and re-suspended in deionised water. One millilitre of harvested cells ( $1.69 \times 10^8$  cells mL<sup>-1</sup>) was

then added to 10 mL metal ion solutions in PET jars simultaneously with 10 mL of silica or maghemite NP suspensions (3 mg L<sup>-1</sup> in deionised water). Control experiments, i.e. without NPs, were also conducted under the same conditions. Experiments were conducted in triplicate, at pH 6.5 ( $\pm 0.2$ ), for 4 h. They were run for this duration due to the rapid rate of metal adsorption by both NPs (as determined in the treatments run in the presence of NPs alone as well as in previous experiments; *personal observation*) and algae (Monteiro et al. 2008). This short duration also obviates significant pH increments due to CO<sub>2</sub> depletion by photosynthesis (Vogel et al. 2010).

At the end of the exposure period, algae or algae-NP pellets were separated from the aqueous fraction as in NP-only experiments. Aqueous fractions were acidified using 3 % HNO<sub>3</sub>, and their metal concentrations (un-adsorbed metal fraction) were determined by ICP-OES.

To quantify absorbed (intracellular) and surface-adsorbed (extracellular) metal concentrations, algal pellets (from controls) and algae-NP pellets (from treatments) were processed following the method described by Franklin et al. (2000). Metal concentrations in all fractions were then determined by ICP-OES. An aliquot of un-exposed algae was processed in the same manner in order to facilitate the calculation of actual adsorbed and absorbed concentrations.

#### Statistical analyses

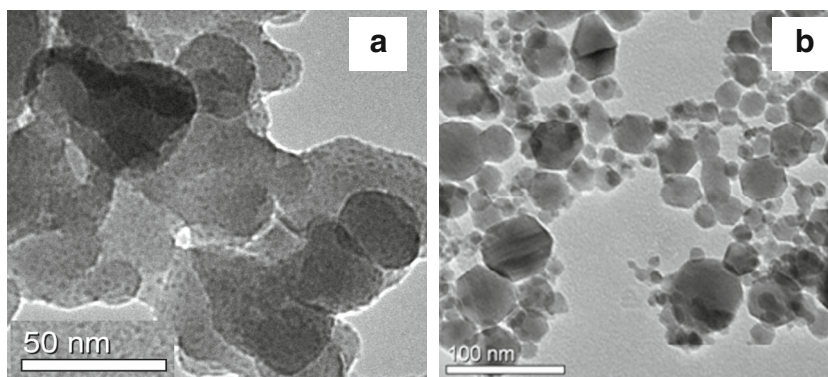
Variations in agglomerate size with metal concentrations and algae-metal uptake in the presence and absence of NPs were analysed for significant differences ( $p < 0.05$ ) based on results of a one-way analysis of variance with Tukey's post hoc comparisons. The relationship between intracellular and extracellular metal concentrations was analysed using the Pearson correlation test. Statistical analyses were conducted using SAS (version 9.2).

## Results

### Particle characterisation

The primary particle size of silica NPs was determined to be in the <20–50 nm range, while that of maghemite NPs was determined to be in the <10–80 nm range (Fig. 1). The specific surface area of silica NPs was, however, much higher than that of maghemite (Table 1), and X-ray diffractograms (Appendix 2 in [Electronic supplementary material](#)) showed that silica NPs were amorphous, while those of maghemite were crystalline. Light scattering measurements, however, revealed that both silica and maghemite NPs agglomerated considerably in water (no metal), attaining hydrodynamic diameters of 280 and 283 nm, respectively (Fig. 2).

**Fig. 1** Transmission electron micrographs of silica (a) and maghemite (b) NPs



Four observations were made based on the light scattering data. First, NP agglomerates were significantly larger ( $p < 0.05$ ) in metal solutions than in deionised water. For example, in  $1.42 \text{ mg L}^{-1}$  Cu solutions, Si NPs had a mean hydrodynamic diameter of  $368 (\pm 27) \text{ nm}$ , but only  $280 (\pm 29) \text{ nm}$  in deionised water. Second, although agglomerate sizes of both NP types were comparable in deionised water, in metal solutions, maghemite NP agglomerates were in some cases larger than those of silica. The third observation was that NP agglomerate sizes were significantly lower after the 4-h incubation ( $t_4$ ) compared to  $t_0$ . Lastly, although the presence of metal ions increased NP agglomerate sizes (relative to in deionised water), there was no additional increase in agglomerate size with further increases in metal concentrations.

#### NP-metal adsorption

The adsorption of Cu, Mn and U to silica and maghemite NPs was rapid, and equilibrium was attained within 5 min (Fig. 3). Slightly more Cu was adsorbed by maghemite than silica, i.e. 65 versus 62 %, but Mn and U were more efficiently adsorbed by silica, i.e. 65 versus 61 % for Mn and 68 versus 65 % for U. Nonetheless, adsorption kinetics for all the three ions to both NP types were fitted by the pseudo-second-order model ( $R^2 \geq 0.99$ ) (Table 2).

Isotherm studies showed that metal adsorption by NPs increased with initial concentrations, and data were best fitted by the Freundlich model (Table 2).  $1/n$  values for Cu and U were  $> 1$ , implying that the removal of these ions at the test pH was via both adsorption and precipitation (Foo and Hameed 2010). Mn, on the other hand, was removed by adsorption

only. Based on  $K_F$  values, adsorption affinities of ions for both NP types were in the order  $\text{Cu} > \text{U} > \text{Mn}$ .

#### Modification of algae-metal removal by NPs

The influence of NPs on the removal of Cu, Mn and U from solution by algal cells was assessed in terms of (i) total removed concentrations and (ii) extracellular (bound to cell walls) and intracellular partitioning.

The algal cellular content of all the three ions increased with initial concentrations of Cu, Mn and U. Further, metal content was significantly higher in the presence of NPs than in their absence ( $p < 0.05$ ) (Fig. 4). For instance, for  $1.42$ ,  $3.30$  and  $0.99 \text{ mg L}^{-1}$  solutions of Cu, Mn and U, respectively, algae-metal content in the absence of NPs was  $4.71$ ,  $9.98$  and  $3.64 \times 10^{-9} \text{ } \mu\text{g cell}^{-1}$  for Cu, Mn and U, respectively. However, these concentrations increased to  $6.94$ ,  $14.2$  and  $4.11 \times 10^{-9} \text{ } \mu\text{g cell}^{-1}$  in the presence of silica NPs and to  $7.40$ ,  $13.4$  and  $4.02 \times 10^{-9} \text{ } \mu\text{g cell}^{-1}$  in the presence of maghemite NPs. Notably, metal uptake by algae in the presence of the two NPs followed trends suggested by isotherm calculations (Table 2), i.e. Cu uptake was higher with maghemite than silica, while Mn and U uptake was higher with silica than maghemite.

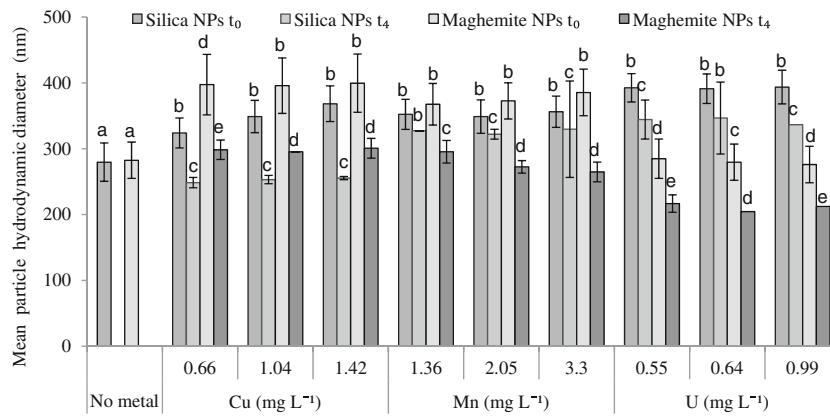
Also noteworthy was the fact that adsorption efficiencies in combined NP-algae systems did not attain efficiencies greater than 85 %, even though such an expectation would not be unreasonable, considering their individual adsorption efficiencies. For example, while maghemite NPs and algae took up 65 and 56 %, respectively, of Cu from  $1.42 \text{ mg L}^{-1}$  Cu solutions, only 85 % was taken up in NP-algae systems. Similarly, 65 % of Mn was adsorbed by silica NPs and 51 % was taken up by algae from  $3.30 \text{ mg L}^{-1}$  Mn solutions, but only 73 % was removed by the NP-algae system.

Extracellular and intracellular concentrations of all the three test ions increased with initial metal concentrations (Fig. 5). For example, at an initial U concentration of  $0.99 \text{ mg L}^{-1}$ , mean extracellular U concentrations were  $3.79 \times 10^{-9} (\pm 7.39 \times 10^{-11}) \text{ } \mu\text{g cell}^{-1}$  in the presence of silica

**Table 1** Structural properties of silica and maghemite NPs as determined by BET measurements

NP type	Surface area ( $\text{m}^2 \text{ g}^{-1}$ )	Pore size (nm)	Pore volume ( $\text{cm}^3 \text{ g}^{-1}$ )
Silica	611.71	4.12	0.63
Maghemite	40.84	11.27	0.11



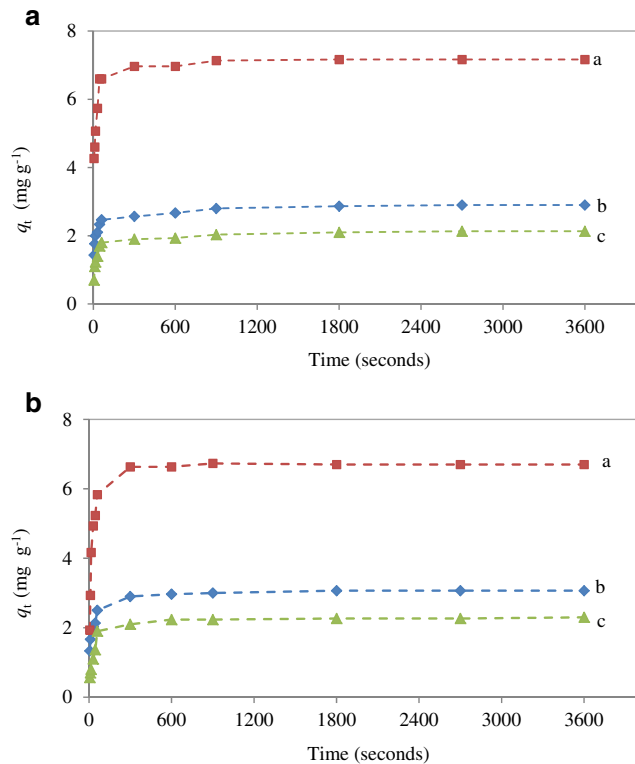


**Fig. 2** Silica and maghemite particle size in the absence of metal ions and in the presence of various concentrations of Cu, Mn and U. *t*<sub>0</sub> values correspond to aggregate diameters immediately after NPs were added to metal solutions and *t*<sub>4</sub> to diameters after 4 h. Initial NP concentrations

were 3 mg/L, and data are mean±standard deviation (*n*=3). Different letters above columns within a concentration indicate statistically significant differences (*p*<0.05) in mean hydrodynamic size of NP agglomerates within that particular concentration

NPs and  $3.62 \times 10^{-9}$  ( $\pm 4.75 \times 10^{-11}$ )  $\mu\text{g cell}^{-1}$  in the presence of maghemite NPs, but only  $3.17 \times 10^{-9}$  ( $\pm 2.66 \times 10^{-10}$ )  $\mu\text{g cell}^{-1}$  in controls. Intracellular concentrations, on the other hand, were not significantly different in the presence and absence of NPs, except for U (Fig. 5a(ii), b(ii) and c(ii)). However, there were no correlations between extracellular and intracellular algae-metal concentrations, except for a weak correlation in U (*r*=0.54; *p*=0.004).

Regarding the effects of individual NPs, extracellular Cu concentrations were significantly higher from solutions containing silica than maghemite NPs, while the opposite was true for Mn and U. Taken together, these results suggest that (i) except for U, NPs did not affect intracellular metal uptake; (ii) extracellular uptake followed trends seen in metal removal using NPs only; and (iii) the additional metal uptake due to the presence of NPs was localised to external surfaces of algal cells and that, as a result, intracellular uptake was reduced.



**Fig. 3** Adsorption kinetics of Mn (a), Cu (b) and U (c) to silica (a) and maghemite (b) nanoparticles at pH 6.5. Data are mean±standard deviation (*n*=2)

**Discussion**

Particle characterisation by XRD, TEM and BET confirmed the chemical composition and physical characteristics of silica and maghemite NPs. Silica, which was amorphous, had a surface area 15 times greater than that of crystalline maghemite due to its greater porosity (Table 1). Despite this, light scattering measurements revealed that both types of NPs agglomerated considerably in metal solutions, with maghemite agglomerates being larger than those of silica (Fig. 2). Further, although the presence of metal ions in solution increased NP agglomerate sizes (relative to agglomerate sizes in deionised water), this increase was not exponential with metal concentrations. Dalai et al. (2014) reported similar observations for titania NPs, i.e. that although NP hydrodynamic sizes were greater in the presence of Cr(VI) ions than in their absence, increasing metal concentrations from 0.5 to 1 mg L<sup>-1</sup> did not significantly increase the hydrodynamic size of titania agglomerates.

NPs agglomerate in solution due to (i) inherent properties such as high surface energies and (ii) solution characteristics including pH, temperature, ionic strength and the nature of cations in solution (Guzman et al. 2006). Divalent cations like Cu<sup>2+</sup>, Mn<sup>2+</sup> and UO<sub>2</sub><sup>2+</sup> enhance NP agglomeration by decreasing the Debye length of the NP hydration sheath. This

**Table 2** Kinetic and isotherm parameters obtained after fitting adsorption data to the pseudo-second-order kinetics model and the Freundlich isotherm model

NP type	Metal	Pseudo-second-order model			Freundlich model		
		$R^2$	$q_e$ (mg g <sup>-1</sup> )	$k^2$ (g mg <sup>-1</sup> s <sup>-1</sup> )	$R^2$	$K_F$ (mg g <sup>-1</sup> ) (L mg <sup>-1</sup> ) <sup>1/n</sup>	1/n
Silica	Cu	1	2.91	0.018	0.99	8.74	1.77
	Mn	1	7.18	0.020	0.99	3.77	0.99
	U	0.99	2.24	0.064	0.99	7.98	1.26
Maghemite	Cu	0.99	3.08	0.022	0.99	10.13	1.62
	Mn	1	6.73	0.017	0.95	3.68	0.96
	U	0.99	2.15	0.032	0.99	9.91	1.18

leads to less electrostatic repulsion and the domination of attractive forces between particles, hence agglomeration (Navarro et al. 2008). Solution pH also affects agglomerate formation; particles in solutions with a pH close to their  $pH_{PZC}$  (pH at which particle charge equals zero) coalesce due to reduced repulsive surface charges. As such, in the present study, maghemite NPs with a  $pH_{PZC}$  of 6.3 (Vayssieres 2009) agglomerated more than those of silica with a  $pH_{PZC}$  of 2–3 (Kosmulski 2009).

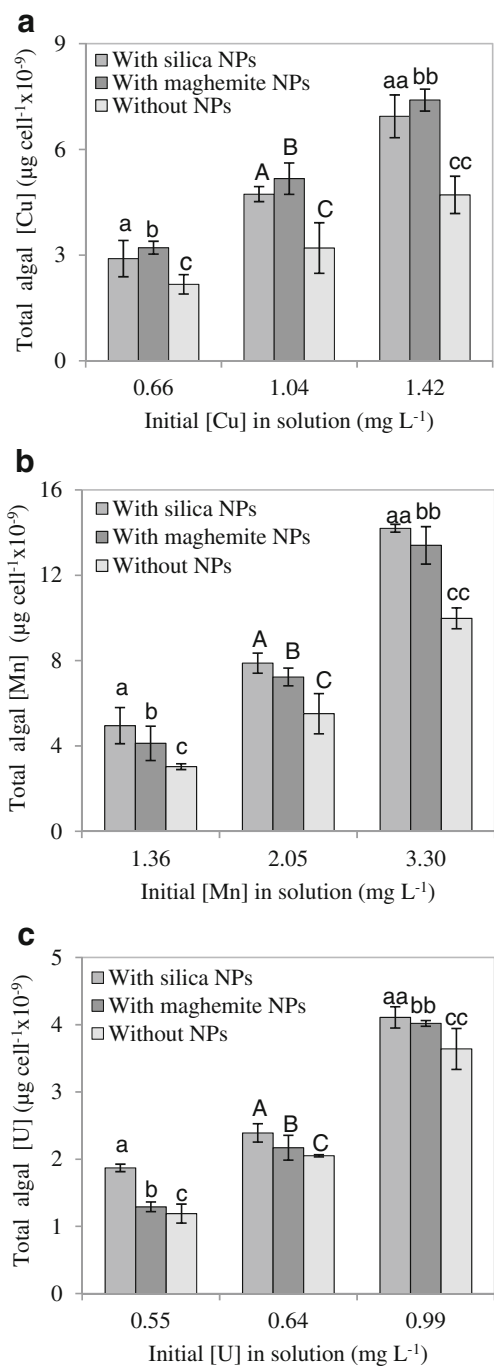
Time-related decreases in agglomerate size were likely the result of incomplete adsorption at  $t_0$ , i.e. immediately after introducing NPs to metal solutions. With time, metal ions experienced the full attractive force of the NP surface and were held closer, resulting in slightly smaller agglomerates at  $t_4$ . Nonetheless, the particle size distribution for both NP types was narrow (data not shown) and hydrodynamic diameters were all in the range of 300 ( $\pm 100$ ) nm.

In order to better describe the effects of NPs on metal uptake by algae, metal adsorption was first studied in NP-only systems (i.e. in the absence of algae). The rapid adsorption (within 5 min) seen in these systems (Fig. 3) is a characteristic of adsorption to NPs and has been reported previously for various NPs (Muresanu et al. 2008; Hartmann et al. 2010; Grover et al. 2012; Yang et al. 2012b). It is simply the result of a greater number of atoms and reactive surface features like kinks and edges located at the surface of particles (Brown et al. 1999). The  $>1$  values of  $1/n$  for Cu and U (Table 2) imply that the removal of these ions was by a combination of adsorption and precipitation. This is explained by the fact that these two ions existed in solution primarily in the insoluble forms, i.e. CuO and  $UO_2(OH)_2 \cdot H_2O$ . Finally, in comparison to studies conducted at similar pH, silica's capacity for U binding was higher than that reported for tin oxide NPs at pH 6 (4.42 mg g<sup>-1</sup>) by Nilchi et al. (2013) and maghemite's capacity for Cu was lower than that of maghemite nanotubes (111 mg g<sup>-1</sup>), likely due to the higher surface area of the nanotubes (Roy and Bhattacharya 2012).

Having described NP-metal interactions, NP effects on metal uptake by algae were then investigated. Metal removal in NP-algae systems was higher than in NP-only or algae-only systems, a result attributed to the additional sorption sites

provided by NPs (Yang et al. 2012a, b; Dalai et al. 2014). However, the observed metal removal efficiencies did not match expectations based on uptake in NP-only and algae-only systems. A number of factors may be responsible for such outcomes, including the sequestration of NPs by the cell wall and exopolymeric substances (EPSs) before metal adsorption to the NPs was complete. Reinhardt (2004) showed that these polysaccharide-rich anionic colloids (EPS) produced by algae in response to high metal concentrations also induce NP aggregation. Nevertheless, compared to algae-only systems, removal efficiencies in NP-algae systems were  $>20\%$  higher for Cu and Mn and  $>10\%$  higher for U. Such increases represent significant gains and make a case for the combined use of NPs and algae. Successful operations will, however, hinge on determining the optimal NP concentration as concentrations that are too high can reduce efficiencies due to (i) diminished sorptive surface areas as a result of NP aggregation, (ii) algal cellular damage by reactive oxidative species (ROS), (iii) reduced nutrient uptake or (iv) impeded photosynthesis due to encapsulation of cells by particles (Hartmann et al. 2010). In addition, it is recommended that NPs are first introduced into metal solutions and given a chance to adsorb metal ions before algae are introduced into the treatment system. This would counter *premature* sequestration of NPs by EPS and algal cell walls which we think curtailed NP and overall adsorption efficiency in this study.

Assessments of the effects of NPs on algae-metal partitioning facilitate an understanding of modifications in algae-metal interactions brought about by NPs and may provide some information for the prediction of physiological responses. In this work, extracellular and intracellular algae-metal fractions increased with solution metal concentrations in both test and control experiments. Our findings are consistent with other studies of green algae, including Cu and U uptake by *Chlorella* sp. (Franklin et al. 2000), Cu uptake by *Desmodesmus* (*Scenedesmus*) *subspicatus* (Ma et al. 2003) as well as Zn and Cu accumulation by *Chlorella pyrenoidosa* and *Scenedesmus obliquus* (Zhou et al. 2012). The addition of NPs, however, changed uptake dynamics. In the presence of NPs, extracellular metal concentrations were higher and intracellular fractions were lower, relative to controls. This



**Fig. 4** Removal of Cu (a), Mn (b) and U (c) by *Acutodesmus* sp. in the presence and absence of silica and maghemite NPs after 4 h. Data are mean ± standard deviation (n=3). Different letters above columns indicate statistically significant differences in the means of metal concentrations (p<0.05)

can be explained both by the rapid metal complexing action of NPs which reduced the bioavailable fraction for intracellular uptake, and the fact that NP hydrodynamic size (300 ± 100 nm) was too large for transport through algal cell wall pores (5–20 nm; Navarro et al. 2008). Thus, unless cell membranes are damaged, e.g. by reactive oxidative species

(Hartmann et al. 2010), or absent such as in *Ochromonas danica* where CdTe quantum dots were internalised by macropinocytosis (Wang et al. 2013), intracellular metal uptake by algae is likely to be reduced in the presence of NPs.

Concentrations in extracellular matrices, on the other hand, are likely to increase due to the localisation of metal-laden NPs on external surfaces of algae. However, these concentrations, which were higher than those reported by Franklin et al. (2000), may have been overestimated because they were a combination of metal ions adsorbed to (i) algal surfaces and exudates, (ii) NPs bound to algal surfaces and (iii) unbound NPs that precipitated on algal surfaces over the duration of the experiment or during the first centrifugation cycle. Although this last fraction should not be included in extracellular concentrations, there was no way of eluting it separately from the actual algal-bound fraction. Nevertheless, this technicality is not relevant to remediation operations where higher total sequestration is the primary consideration.

NPs modify extracellular and intracellular metal uptake in various ways, the most obvious being the depletion of the bioavailable metal pool by adsorption. A few other mechanisms analogous to those facilitated by organic matter are also plausible. (i) The adsorption of NPs to algal cells, for instance, may increase the population of negatively charged sites on algal cell walls and, subsequently, cation uptake (Slaveykova et al. 2003). (ii) NPs may form ternary surface complexes linking metal ions to algal functional groups, thus increasing the adsorption of ions that have a low affinity for algal binding sites, but a high affinity for NPs (Lamelas and Slaveykova 2007). (iii) NPs may also damage algal membranes via oxidative action, allowing access for metal-laden NPs (Hartmann et al. 2010).

To our knowledge, silica and maghemite NP effects on metal uptake by algae have not been reported in the peer-reviewed literature, and so the results presented here are novel. Furthermore, most studies to date have been conducted on multicellular organisms like daphnids (Zhang et al. 2007) and fishes (Fan et al. 2011) which have different modes of metal uptake than algae. Nonetheless, the findings reported here are comparable to those using the more commonly studied titanium NPs (Hartmann et al. 2010; Yang et al. 2012a; Yang et al. 2012b). Yang and co-workers, for example, reported reduced intracellular Cd<sup>2+</sup> concentrations in *Chlamydomonas reinhardtii* in the presence of bare titanium NPs (Yang et al. 2012b) and polyacrylate-coated titanium NPs (Yang et al. 2012a).

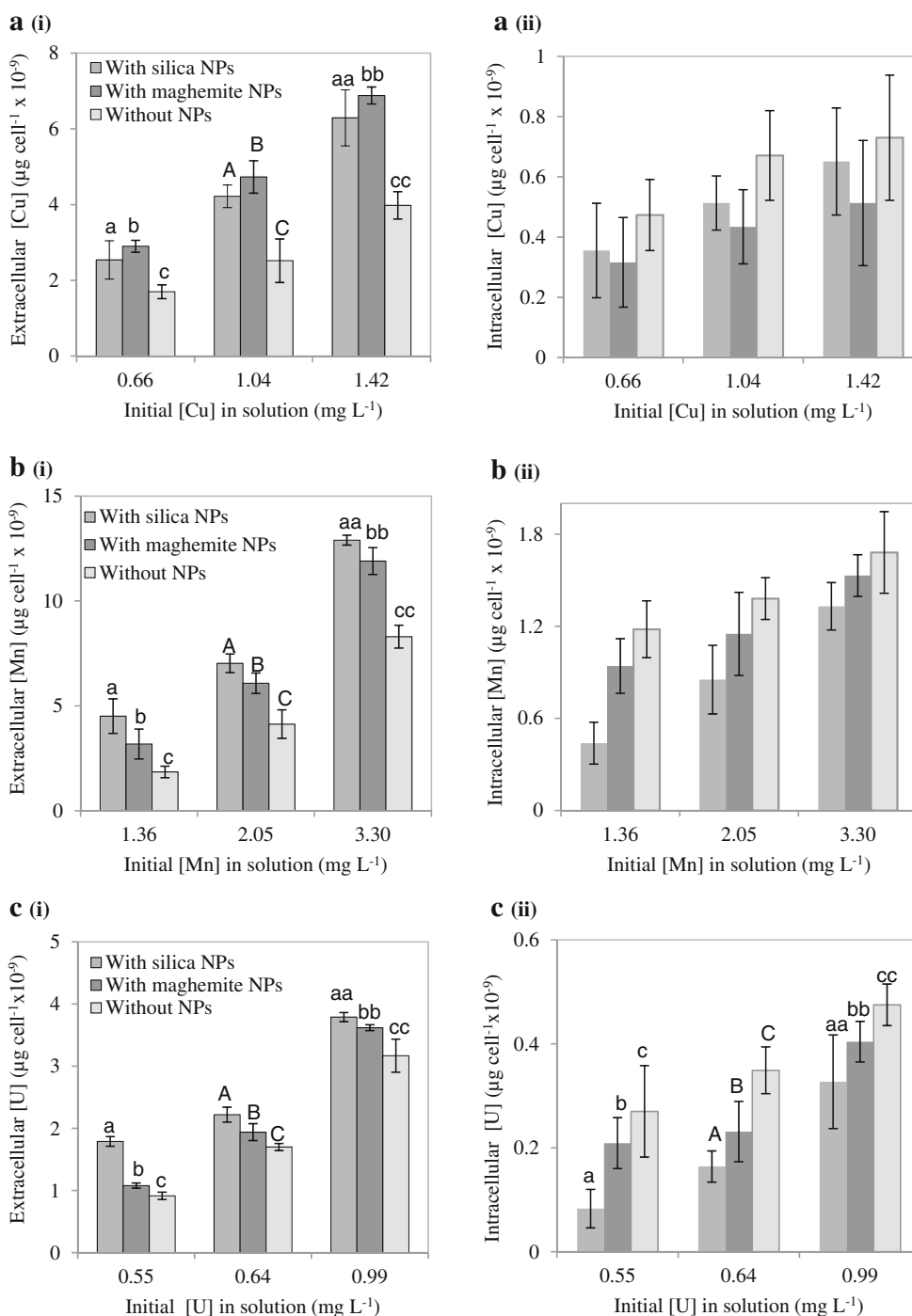
For the treatment of AMD-contaminated waters, factors including pH, metal concentrations and sulphates need also to be considered. Our previous studies have shown ferric, manganese and sulphate ions to increase metal adsorption to NPs (Etale et al. 2014b). These ions may, therefore, also increase the efficiency of remediation systems combining NPs and algae. However, these factors (pH, metal ions) also affect

the viability of algae. The pH of mine drainage, for example 2–5, may induce physiological stress and affect the metal uptake efficiency of algal cells, although some studies have shown aquatic species to be more tolerant to low pH than metal ions. Nonetheless, we surmise that the addition of NPs prior to introduction of algae may counter, to some extent, metal toxicity to algae. Ultimately, however, the use of nanoparticles in nano-bioremediation remains to be a subject to a number of factors among their cost and environmental safety.

The current high cost of these materials limits their large-scale application, and their fate in the aquatic environment is not yet fully understood (Wang et al. 2011).

Finally, concerning the correlation between intracellular and extracellular U concentrations and the significant effect of NPs on the former (Fig. 5c(ii)), the interpretation of this result requires knowledge on the speciation of the test ions at the experimental pH. At pH 6.5, U existed largely as the uncharged  $UO_2(OH)_2 \cdot H_2O$  species (schoepite), alongside a

**Fig. 5** Extracellular (i) and intracellular (ii) concentrations of Cu (a), Mn (b) and U (c) of *Acutodesmus* sp. cells in the presence and absence of silica and maghemite NPs. Legends in i are for both graphs in the set, and data are mean±standard deviation (n=3). Different letters above columns within a concentration indicate statistically significant differences (p<0.05) in the means of metal concentrations





smaller fraction of charged polymeric species such as  $\text{UO}_2\text{OH}^+$ ,  $(\text{UO}_2)_3(\text{OH})_5^+$  and  $(\text{UO}_2)_2(\text{OH})_3\text{CO}_3^-$  (Franklin et al. 2000). Unlike ions, uncharged species easily traverse biological membranes (Vigneault et al. 2000), hence the correlation between extracellular and intracellular U concentrations. This transport was, however, decreased in the presence of NPs because of the additional surface areas for schoepite deposition. A similar rationale explains the significantly lower intracellular fraction with silica relative to maghemite, i.e. the higher surface area of the former provided greater depositional surface areas for schoepite.

In conclusion, the removal of Cu, Mn and U was higher in NP-algae treatments than in NP-only or algae-only treatments due to greater surface area in the NP-algae system. Cu removal increased from 56 % in an algae-only system to 85 % in an algae-maghemite system. Mn removal increased from 51 to 73 %, and U removal went from 59 to 68 % in algae-silica systems. Further work is, however, required to determine optimal parameters with respect to NP concentrations that will not be inhibitory to algal growth and performance or result in aggregation substantial enough to negate the effects of additional sorption surface areas.

**Acknowledgments** Funding for this study was provided by the Global Change and Sustainability Research Institute of the University of Witwatersrand. We are indebted to Prof. Stuart Sym for the starter culture, access to his culturing facility and for helpful comments on the manuscript; Mr. Siphon Mhlambi for identifying the algal specie; and Dr. Lisa Du Toit for her help with light scattering measurements.

**Conflict of interest** The authors declare that they have no conflict of interest.

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