

Silica and Maghemite Nanoparticles Enhance the Phycoremediation of Hg-Contaminated Water by *Scenedesmus sp.*

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Abstract. Mercury contamination of aquatic resources is a subject of global concern due to the detrimental effects of the metal on aquatic and terrestrial life even at very low concentrations, necessitating the removal of this ion from contaminated water. In this work, a novel technique involving the combined use of NPs and algae for bioremediation was investigated. Thus, the removal of Hg from aqueous media using *Scenedesmus sp.* - a fresh water alga with high metal uptake efficiency, and NPs of silica and maghemite was quantified. Results showed that Hg removal was up to 16-26% higher in NP-algal treatments than in algae-only systems. NPs also modified algal metal partitioning: extracellular concentrations were higher and intracellular fractions lower in the presence of NPs relative to controls (without NPs). As some studies have shown metal toxicity to be related to intracellular metal fractions in algae, a combination of NPs and algae for phycoremediation may therefore improve remediation efficiency both by increasing removed metal fractions and protecting algal cells from Hg toxicity.

Key words: biosorption, mercury, algae, bioremediation, metal-contaminated water.

1. Introduction

Despite considerable efforts to limit its use and subsequent release into water bodies, gold-mining remains a significant source of aquatic mercury contamination, globally [1]. In Johannesburg, South Africa, the effects of more than a century of gold mining on water resources are increasing as ever greater volumes of decant from decommissioned mines contaminate streams and rivers [2]. Mercury contamination is of particular concern because it is linked to several diseases in humans, as well as the decimation of aquatic species and birds [1]. Its removal from contaminated water is therefore a priority.

Although a number of techniques including ultrafiltration and electrolysis have been tested for the removal of Hg from wastewater, these involve high capital and running costs [3]. As a result, bioremediation using fungi [4], algae [5,6] and bacteria [7] has attracted considerable interest due to the simplicity of operations and the lower capital and operational costs [8]. The growth of nanoscience has also resulted in the application of nanoparticles (NPs) as adsorbents. Relative to bulk counterparts, NPs are more efficient adsorbents because of their higher sorption surface areas and higher reactivities which mean that less adsorbent masses are required and less contaminated wastes are produced [9].

In this work, a novel technique involving the combined use of algae and NPs for the removal of Hg ions from aqueous media has been explored. The hypothesis is that combined NP-algal systems will have higher removal efficiencies than NP-only or algae-only systems due to (i) increased sorptive surface areas and (ii) reduced Hg toxicity to algae [10]. The information generated from this study may be applied for informing

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both the optimisation of phycoremediation and the effects of NPs on natural phycoremediation of metal-contaminated waters.

2. Materials and Methods

Analytical grade mercury nitrate ($\text{Hg}(\text{NO}_3)_2 \cdot \text{H}_2\text{O}$) and commercially prepared silica and maghemite NPs were purchased from Sigma Aldrich (Schnelldorf, Germany). Mercury solutions and NP suspensions were prepared using deionised water. NP suspensions were prepared by sonicating NPs in a water bath for 30 minutes. A starter culture of *Scenedesmus sp.* was acquired from a stock isolated from a fresh water source in Johannesburg and cultured in modified Bold's Basal media under continuous bubbling with air and $150 \mu\text{mol photons/m}^2/\text{s}$ of light provided in a 18:6-h light:dark cycle. The pH of cultures was maintained at 6-8 as this was determined to be optimal for growth of the algae. All glassware used for culturing was soaked overnight in 0.2 M HNO_3 , rinsed several times with deionised water and sterilized, along with the culture media, by autoclaving at $121 \text{ }^\circ\text{C}$ for 15 minutes before use.

The effect of NPs on mercury uptake by *Scenedesmus sp.* cells was then investigated by quantifying uptake in, algae only and NP-algal treatments at pH 6.5 (± 0.2). In a typical experiment, algae in the exponential growth phase (1.69×10^8 cells), were exposed for four hours to 0.4, 0.6 and 0.8 $\mu\text{g/L}$ mercury solutions in the presence or absence of NPs. At the end of incubations, algae or algae-NP pellets were separated by ultracentrifugation and mercury concentrations in the filtrate (non-adsorbed fraction) determined by inductively coupled plasma-optical emission spectrometry (ICP-OES; Spectro Instruments, Kleve, Germany). The pellet was then processed following the method described by Ma et al. [11] in order to determine the contributions of intracellular and extracellular binding to overall mercury removal, and the relationship between these concentrations assessed by the Pearson correlation test using SAS statistical software.

3. Results and Discussion

Hg removal by *Scenedesmus sp.* cells increased with initial solution metal concentrations, both in solutions with and without NPs (Fig. 1). However, metal removal was higher in solutions containing NPs relative to those without. For example, mercury removal from 0.8 $\mu\text{g/L}$ solutions was 56 and 66% in solutions containing silica and maghemite NPs, respectively, but only 40% in algae-only treatments. This is due to the higher sorptive surface areas available for mercury ion sorption in NP-algal systems [12]. Notably, mercury removal at all concentrations was higher with maghemite than silica NPs, despite the ~ 15 times greater surface area of the latter [13]. This highlights the effect of NP properties, some of which are not yet clearly understood, on metal adsorption [14].

An important finding was that mercury removal in combined treatments did not match expectations based on efficiency in NP-only and algae only treatments. That is, while mercury removal was as high as 71% in NP-only treatments (removal by maghemite from 0.8 $\mu\text{g/L}$ solutions, data not shown) and 40% in algae-only treatments, maximum efficiency in NP-algal treatments was only 66% (Fig. 1). This may be due to the sequestration of NPs by algal exopolymeric substances (EPS) before NP adsorption had attained its optimal efficiency. This phenomenon may be circumvented in batch phycoremediation operations by 'staggering' the introduction of NPs and algae into the contaminated water in order to allow ample time for NP adsorption before their sequestration by EPS.

Removal of metal ions from aqueous media by algae involves adsorption to cell walls (extracellular) as well up intracellular uptake [15]. Assessments of metal concentrations in extracellular and intracellular algal matrices revealed that NPs influenced metal partitioning in algal cells. Extracellular Hg concentrations were higher and intracellular concentrations lower in solutions containing NPs relative to those without NPs. Furthermore, although there was no correlation between these concentrations ($r = 0.46$; $p = 0.015$), they were significantly different at higher concentrations (Fig. 2). This implies that although NP-metal binding did not attain its full potential due to EPS sequestration, it nonetheless reduced the available metal pool for intracellular uptake. This finding is corroborated by (i) the rapid rate of NP-metal adsorption [15] and (ii) studies of the hydrodynamic diameter of NPs (data not shown) which revealed that in mercury solutions, NPs

formed agglomerates >300nm in diameter. At this size range, NPs were unlikely to traverse algal membranes [17] and were therefore likely localised, along with adsorbed mercury ions, on the algal cell wall.

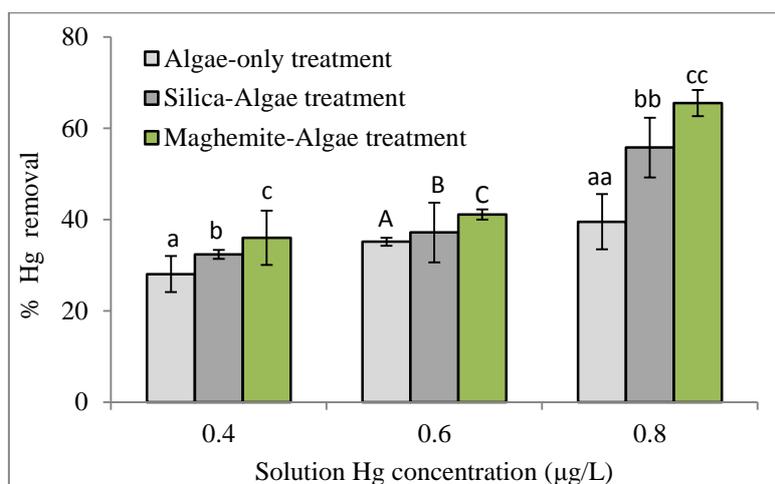


Fig. 1: The effect of silica and maghemite NPs on mercury removal efficiency of *Scenedesmus sp.* cells.

Silica and maghemite NPs may therefore be applied to enhance the efficiency of mercury removal by *Scenedesmus sp.* Further, the viability of *Scenedesmus sp.* cells may be enhanced by the presence of NPs [12]. Its phycoremediation efficiency will therefore increase in the presence of NPs not only due to the addition of sorption sites, but also as a result of increased algal viability.

Successful operations will, however, hinge on determining optimal NP concentrations as concentrations that are too high can reduce efficiencies due to NP aggregation and subsequently, diminished sorptive surface areas or encapsulation of cells by particles which would then hinder photosynthesis [18]. In addition, for the treatment of AMD-contaminated waters, factors including pH, metal concentrations and sulphates need to be considered as these also affect algal viability. Future efforts therefore need to be aimed at determining optimal operational conditions.

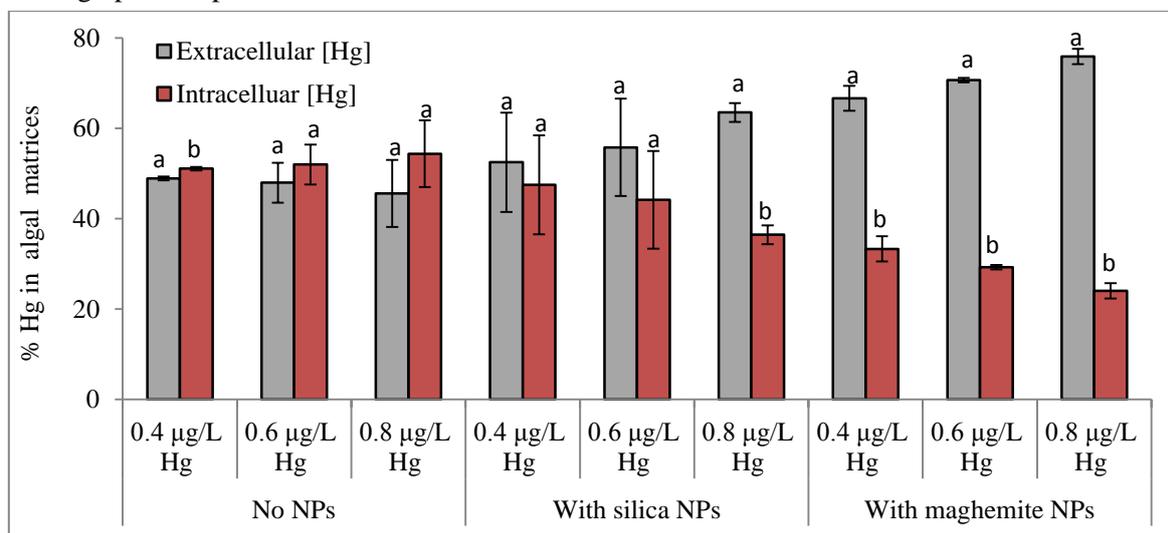


Fig. 2: The effect of silica and maghemite NPs on partitioning of mercury in *Scenedesmus sp.* cells.

4. Acknowledgements

Funding from the Global Change and Sustainability Research Institute (GCSRI) of the University of Witwatersrand is gratefully acknowledged.

5. References

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