Georgia Journal of Science

Volume 77 *Scholarly Contributions from the Membership and Others*

Article 1

2019

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Acknowledgements
The authors would like to thank Dr. Russell Goddard, Valdosta State University for help with SEM/EDX.

ACCUMULATION AND EFFECTS OF DISSOLVED AND NANOPARTICLE SILVER AND COPPER IN TWO SEAWEED SPECIES

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ABSTRACT

This study investigated the accumulation and effects of metal nanoparticles in two seaweed species, *Ulva lactuca* and *Agardhiella subulata*. Both seaweeds were exposed to silver nitrate ($AgNO_3$), silver nanoparticles, and copper oxide (CuO) nanoparticles for 48 h. Metal accumulation occurred in both seaweed species in a concentration-dependent manner after 48 h exposure to each form of metal. In several cases, seaweeds exposed to $AgNO_3$ (the dissolved form) accumulated comparatively higher tissue Ag concentration than seaweed exposed to Ag nanoparticles; and *A. subulata* had higher tissue Ag concentrations than *U. lactuca* after exposure to $AgNO_3$ for 48 h. Additionally, clear differences were observed in the regulation of Ag between the two seaweed species. Photosynthetic toxicity (primarily due to decreased maximum electron transport rate) was observed in *U. lactuca* after exposure to $AgNO_3$, Ag nanoparticles, and CuO nanoparticles. These results increase current knowledge about the differences in dissolved metal versus nanoparticle exposure in marine seaweeds and have implications in marine food webs.

Keywords: nanoparticles, silver, copper, seaweed, *Ulva lactuca, Agardhiella subulata*

INTRODUCTION

Metal nanoparticles are widely used because they are excellent conductors of electricity and have superior mechanical and optical properties (Klaine et al. 2008). Silver nanoparticles, in particular, are commonly used in medical industries due to their antibacterial and antifungal properties; and due to their utility in biosensing, spectroscopy, nanophotonics, and various other applications (Jin et al. 2001; Tao et al. 2007; Klaine et al. 2008; Dallas et al. 2011; Scholl et al. 2012; Wu et al. 2012; Chernousova and Epple 2013). Copper oxide (CuO) nanoparticles also have biocidal, antibacterial, antiviral, and antifungal properties in addition to a variety of industrial applications (Tilaki et al. 2007; Srivastava 2009; Grass et al. 2011; Santo et al. 2012). These metal nanoparticles can enter aquatic systems, bioaccumulate, and potentially exert toxicity to aquatic organisms (Luoma et al. 1999; Nowack and Bucheli 2007;

Fabrega et al. 2011; Bielmyer et al. 2012; Bielmyer-Fraser et al. 2014; Jarvis et al. 2013; Jarvis and Bielmyer-Fraser 2015; Jarvis et al. 2015; Miller et al. 2017).

The toxicity of dissolved metals has been well characterized; however, less is known about the toxicity of metal nanoparticles, particularly in marine systems (Navarro et al. 2008; Eisler 2010; Jarvis et al. 2013; Bielmyer-Fraser et al. 2014; Miller et al. 2017). Exposure to dissolved metals has been shown to inhibit chlorophyll production, photosynthesis, and growth in several seaweed species (Prasad and Strzalka 1999; Baumann et al. 2009; Jarvis and Bielmyer-Fraser 2015). Miller et al. (2017) showed reduced population growth in phytoplankton exposed to four types of nanoparticles, and suggested that population level effects could be predicted by declining photosynthetic efficiency. Determining the fate and effects of metal nanoparticles in aquatic organisms, especially primary producers, can allow better prediction of the risks of nanoparticles in aquatic environments (Nowack and Bucheli 2007).

Macroalgae are important primary producers; they serve as food for a variety of organisms; and they are considered efficient and reliable indicator organisms for metal pollution in the environment (Phillips 1977; Ho 1990; Misheer et al. 2006; Han et al. 2008; Wallenstein et al. 2009). Dissolved metals can be taken up by seaweed via adsorption of solutes to the seaweed surface, which is dependent on saturation state; concentration-dependent ion exchange; and via accumulation, in which solute enters the organism (Spooner 1949; Gutknecht 1961; Jarvis and Bielmyer-Fraser 2015). Dissolved silver (Ag) compounds (e.g. $AgNO_3$) can dissociate to Ag^+ ions and enter cells within organisms through cell membrane ion transporters, such as those regulating sodium and copper (Cu) transport in cells (Luoma 2008; Campbell 1995). Exposure of algae to dissolved Ag results in its distribution in the cell wall, cell membrane, cytosol, nucleus, chloroplasts, and mitochondria to varying degrees, depending on the silver speciation (Connell et al. 1991; Luoma et al. 1999; Bielmyer 2000; Leonardo et al. 2014). Metal nanoparticles could leach dissolved metal into solution to some extent and uptake could occur, as mentioned above; however, direct uptake of metal nanoparticles in macroalgae is also possible. Several studies have shown that nanoparticles can pass through cell membranes via diffusion, endocytosis, and phagocytosis (Jia et al. 2005; Limbach et al. 2005; Lynch et al. 2006; Rothen-Rutishauseret al. 2006; Moore 2006; Fabrega et al. 2011). Inside the cell, metal nanoparticles can interact with organelles and can be stored inside vesicles and other locations (Limbach et al. 2005; Rothen-Rutishauser et al. 2006; Bielmyer-Fraser et al. 2014). The smaller particle size and high surface area per unit mass of nanoparticles can increase their biological activity (Oberdoster et al. 2005), as compared to the dissolved metal forms. Bielmyer-Fraser et al. (2014) reported concentration-dependent metal accumulation and decreased population growth in the marine alga, *Thalassiosira weissflogii,* when it was exposed to ZnO, CuO, and Ag nanoparticles, as well as dissolved metals. Exposure to the two forms of metal resulted in similar toxicity, but there were substantial differences in cellular metal distribution in *T. weissflogii* (Bielmyer-Fraser et al. 2014). The authors suggest that the metal partitioning in algae was based on exposure to the different forms of metal, with more metal accumulating in the cell wall as a consequence of nanoparticles exposure (Jarvis et al. 2013; Bielmyer-Fraser et al. 2014).

The green alga, *Ulva lactuca*, and the red alga, *Agardhiella subulate*, are widely distributed (Gabrielson and Hommersand 1982, Zertuche-Gonzalez et al. 1995), commonly used in ecotoxicological and environmental biomonitoring studies, and have been shown to bioaccumulate metals (Burdin and Bird 1994; Kamala-Kannan et al. 2007; Han et al. 2008; Bielmyer et al. 2012; Jarvis and Bielmyer-Fraser 2015).

Additionally, dissolved metal absorption in seaweed is known to occur within 1–2 h, which makes seaweeds model organisms for acute toxicity bioassays (Sheng et al. 2004; Omar 2008; Areco and Afonso 2010). The objectives of this study were to measure tissue metal accumulation after exposure to $AgNO_3$, Ag nanoparticles, and CuO nanoparticles in the seaweeds, *U. lactuca* and *A. subulata,* and to assess the photosynthetic impairment in *U. lactuca* after exposure to the different forms of the metals.

MATERIALS & METHODS

Organisms

Ulva lactuca and *Agardhiella subulata* were shipped from the National Resource for Aplysia at the University of Miami's Rosenstiel School of Marine and Atmospheric Science (Miami, Florida) and immediately acclimated to testing conditions in a 50-L tank filled with 30 ppt synthetic seawater supplemented with f/10 nutrients (National Centre for Marine Algae and Microbiota, East Boothbay, Maine) under continuous aeration at a temperature of 26.1 ± 0.5 °C. Synthetic seawater was prepared 24 h before use by mixing Instant Ocean salt (Aquarium Systems Inc., Mentor, Ohio) with 18 mΩ Milli-Q water. The photoperiod was 12 h dark:12 h light with a light intensity of 33.2 µmol photons m^{-2} s^{-1}.

Experimental Solutions

Silver nanoparticles were obtained from QuantumSphere Inc. (Santa Ana, California); scanning electron microscopy (SEM; JEOL-6480_LV) showed that the Ag nanoparticles were 40–70 nm in diameter with no detectable impurities (Figure 1).

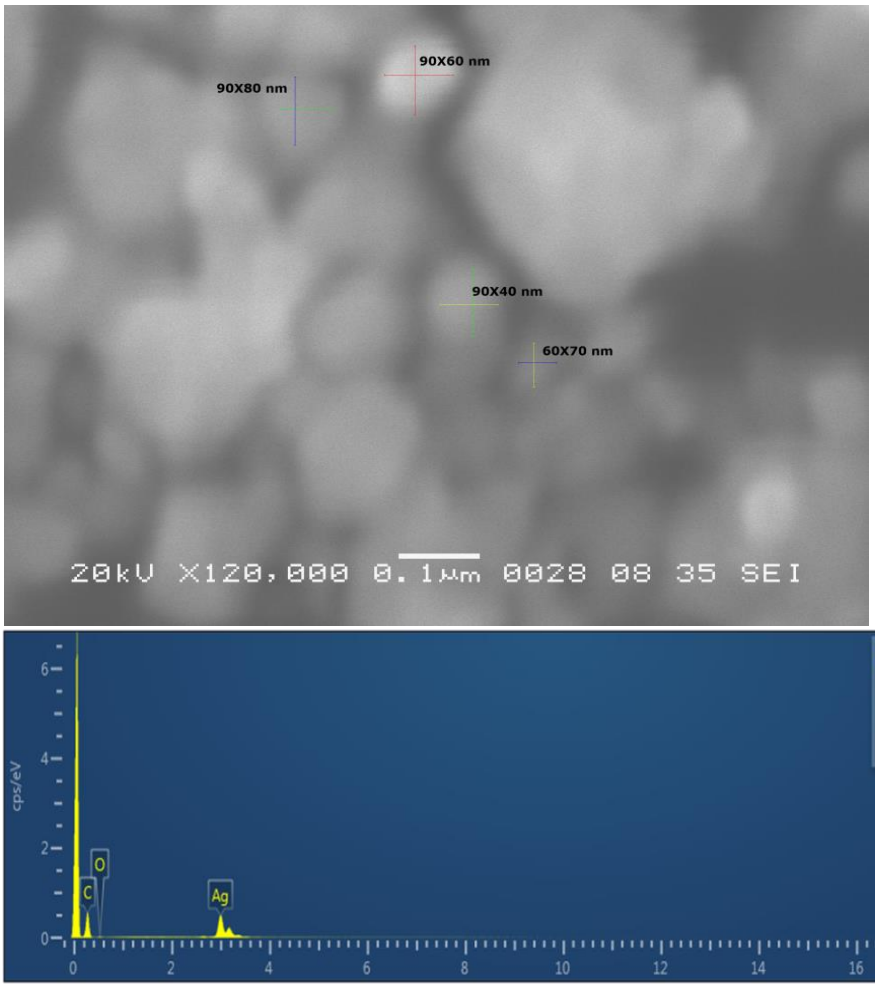

Figure 1 A) Scanning electron micrograph of AgO nanoparticles with their respective shapes and size and B) energy dispersive spectroscopy systems map spectrum confirming the presence of silver in the stock solution.

The nanocrystalline CuO nanoparticles were obtained from Nanophase Technologies Corporation (Romeoville, Illinois) and characterized as chemically pure, less than 100 nm in size, spherical, nonporous single crystals (Siddiqui et al. 2015).

All metal testing solutions were prepared 24 h prior to use and equilibrated in 200 ml glass culture bowls. The $AgNO_3$ testing solutions were prepared by adding a 10 mg/L Ag, as $AgNO_3$, stock solution to synthetic seawater (30 ppt). The nanoparticle stock solutions were prepared using established methods (Siddiqui et al. 2015). Briefly, nanoparticles were added to 18 mΩ ultrapure water, vortexed for 30 sec, sonicated for 30 min, and diluted with 30 ppt synthetic sea water (Siddiqui et al. 2015). The testing solutions were then made by mixing the stock solutions with 30 ppt synthetic saltwater.

Scanning electron microscopy was also used to characterize Ag nanoparticle and CuO nanoparticle stock solutions. Particle sizes and shapes were observed and photographed using high vacuum mode secondary electrons at a magnification of 120,000 and the analySIS imaging system GmbH. Energy dispersive spectroscopy (EDX; oxford Aztec, Inca; X-ford X-max 50 mm) was used to verify the presence of Cu or Ag.

Experimental design

U. lactuca and *A. subulata* (93 ± 0.06 mg) were exposed together in 2-L culture dishes to a control solution and solutions of 10, 100, and 1000 µg/L of each metal ($AgNO_3$, Ag nanoparticles, and CuO nanoparticles) for 48 h. A previous study in our laboratory characterized the accumulation and effects of dissolved Cu, as $CuNO_3$, in *U. lactuca* (Jarvis and Bielmyer-Fraser 2015). The results from that study were compared to the findings presented here. Each treatment had three replicates. Testing waters were measured daily for salinity and dissolved oxygen (DO); and mean ± standard deviation values remained within 30.0 ± 0.5 ppt salinity and 8.3 ± 0.66 mg/L DO. Temperature was maintained at 26.3 ± 0.45 °C. The average light intensity during the exposure period was 35.46 µmol photons m^{-2} s^{-1} with a photoperiod of 12 h light:12 h darkness. At 0 and 48 h, water samples from each replicate were collected with a syringe and filtered through a 0.45 µm filter into 15-ml polypropylene centrifuge tubes. Samples were acidified with trace metal grade nitric acid (Fisher Scientific, Pittsburgh, Pennsylvania) for later metal analysis. Seaweed samples for SEM were collected from the 1000 µg/L treatment of each metal after 48 h of metal exposure. Additional seaweed samples were collected at 24 and 48 h, dried in an oven at 80 °C for 12 h, and fully digested with trace metal grade nitric acid prior to metal analysis.

Imaging PAM fluorometry

Imaging pulse-amplitude modulated (PAM) fluorometry (Imaging-PAM, *M-Series,* Walz, Germany) was used at 24 and 48 h of exposure to measure maximum relative electron transport rate (rETR) and quantum yield of nonregulated energy dissipation (YNO) in *U. lactuca* from each treatment. The fluorometer uses light emitting diodes to measure photosynthetic efficiency of photosystem II (PSII) (Beer and Bjork 2000). The energy fraction that is passively dissipated as waste (heat and fluorescence) is represented by YNO (Bilger and Schreiber 1986; Juneau and Popovic 1999; Schreiber 2004; Klughammer and Schreiber 2008). In PAM fluorometry, YNO represents the PSII closed state, which does not contribute to electron transport and is therefore an indication of inefficiency of both photochemical energy conversion and protective regulatory mechanisms (Schreiber 2004; Juneau et al. 2005; Klughammer and Schreiber 2008). Alternatively, rETR represents photosynthetic efficiency as it approximates the rate of electron transfer through the photosystems (White et al.

2013). Increased YNO or decreased rETR are indicative of toxicity. This method could not be used with *A. subulata* accurately because of its highly branched structure.

SEM analysis

Ulva lactuca samples were cleaned with ultrapure water and then fixed with glutaraldehyde to maintain structural detail. The resulting sample was dried in an oven at 80 °C for 4 h. Samples were homogenized to a crude state with a pestle and mortar to prepare a slide. The samples were mounted on metal stubs and observed under the 20 kV, high pressure mode, 300× in SEM (JEOL-6480_LV). The presence of metal was confirmed by EDX (oxford Aztec, Inca; X-ford X-max 50 mm).

Metal analysis

Diluted water samples and digested seaweed samples were measured for silver or copper in triplicate using a graphite furnace atomic absorption spectrophotometer (GFAAS, Perkin-Elmer, AAnalyst 800). Standards for each metal were made using certified 1 g/ml metal standards dissolved in 3% nitric acid (Fisher Chemical, Fairlawn, New Jersey). Recalibration of the instrument was performed every 40 samples. Data are presented as micrograms per gram of metal dry weight (dw). Leaching of the metals from the nanoparticles into the solution could be measured using GFAAS; however, the concentration of nanoparticles in the solutions could not be quantified using this method.

Data analysis

Data were analyzed for normality and equal variance using a Shapiro-Wilk's test and Barlett's test, respectively. Significant differences ($p \leq 0.05$; n = 3) between treatments were identified by conducting a one-way ANOVA followed by a Tukey's test and using SigmaPlot software.

RESULTS

Mean metal concentrations in the exposure water after 48 h are presented in Table I. The measured dissolved Ag concentrations in the $AgNO_3$ solutions were 80–95% of the nominal (desired) values (Table I). The dissolved Ag and Cu concentrations in the Ag nanoparticles and CuO nanoparticle solutions were 5.4–37.99% of nominal and 11–38.97% of nominal, respectively, and are an indication of leaching from the nanoparticles (Table I). Therefore, the nanoparticles solutions provided a combination of nanoparticles and dissolved metal exposure, and it was assumed that the nanoparticle concentration was the difference between nominal and dissolved metal concentrations.

Table I. Dissolved metal concentrations (mean ± standard error) in testing waters after 48 h of exposure to $AgNO_3$, AgO nanoparticles, and CuO nanoparticles

Treatment (µg/L)	$AgNO_3$ (µg/L)	AgO nanoparticles (µg/L)	CuO nanoparticles (µg/L)
Control	0.80 ± 0.09	0.43 ± 0.03	0.10 ± 0.01
10	8.69 ± 0.39	0.54 ± 0.04	1.43 ± 0.05
100	95.0 ± 16.5	38.0 ± 2.02	39.0 ± 1.28
1000	902 ± 2.93	146 ± 15.8	116 ± 7.94

The presence of Ag nanoparticles and CuO nanoparticles in the stock solutions was verified by SEM/EDX. In both cases, spherical particles were observed, ranging from 40 to 90 nm and 90 to 120 nm in diameter, respectively (Figure 1; Siddiqui et al. 2015). Therefore, the nanoparticle exposure solutions contained a mixture of nanoparticles and dissolved Ag or Cu. The presence of CuO nanoparticles was detected in the seaweed samples by SEM analysis. Cu was located in the cell wall of *U. lactuca* (Figure 2). Seaweed samples were also prepared for Ag nanoparticle analysis; however, Ag was not detected on the samples using this method.

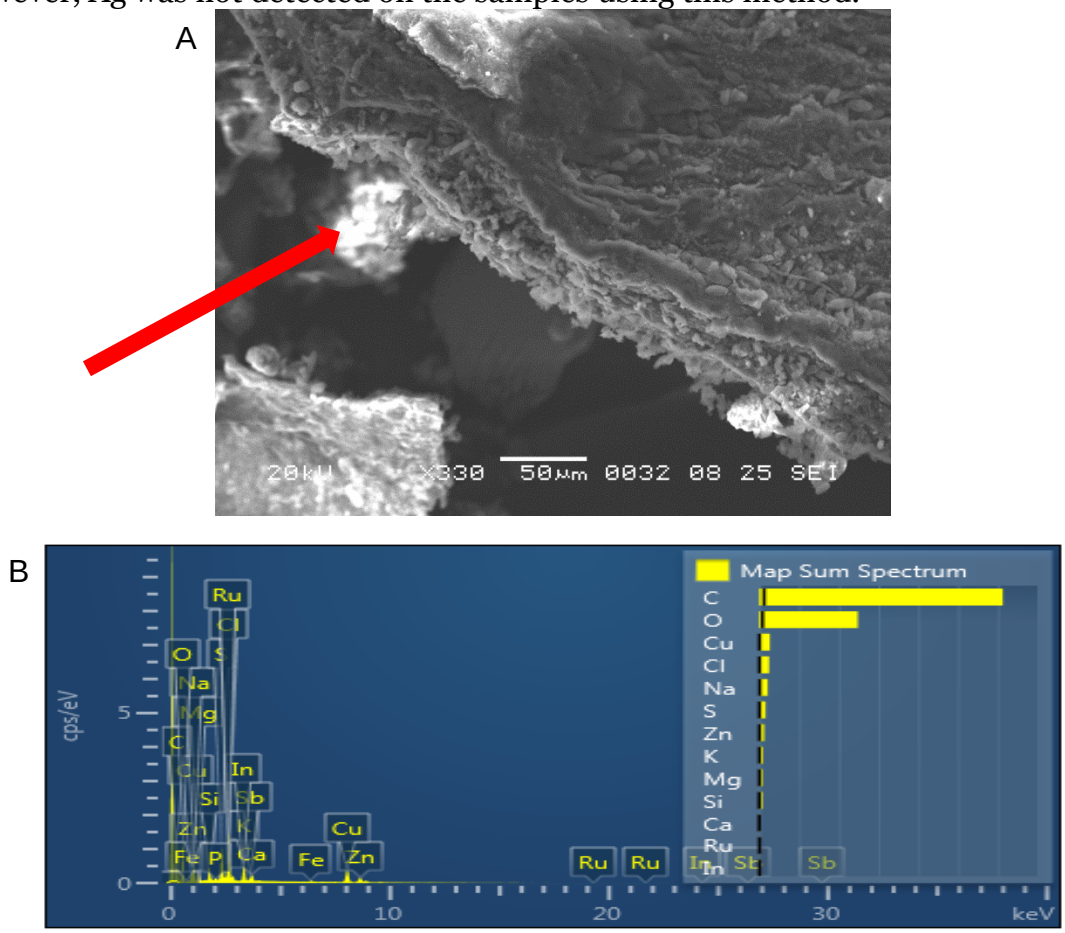

Figure 2. A) Scanning electron micrograph of CuO nanoparticles in the *U. lactuca* cell wall. B) Energy dispersive spectroscopy systems map spectrum confirming the presence of Cu.

All metal-exposed treatments contained significantly higher tissue metal concentrations than the controls (Figures 3, 4). Significant concentration-dependent Ag accumulation was observed in *U. lactuca* and *A. subulata* when they were exposed to Ag, as $AgNO_3$ or Ag nanoparticles for 24 and 48 h, as compared to controls (Figure 3). In some cases, *U. lactuca* and *A. subulata* accumulated more Ag, when exposed to $AgNO_3$, as opposed to Ag nanoparticles, especially as the concentration increased (Figure 3). Additionally, this occurred more frequently in *A. subulata* than in *U. lactuca*. In *U. lactuca,* there were no significant differences in Ag accumulation from 24 to 48 h after exposure to 10 µg/L Ag, as $AgNO_3$ or Ag nanoparticles (Figure 3A); however, tissue Ag decreased after exposure to 100 µg/L Ag, as $AgNO_3$ (Figure 3B), and after exposure to 1000 µg/L Ag, as $AgNO_3$ or Ag nanoparticles (Figure 3C). In *A. subulata*, tissue Ag decreased from 24 to 48 h with exposure to 10 µg/L Ag, as Ag nanoparticles, and did not significantly differ from 24 to 48 h with exposure to 100 and 1000 µg/L Ag, as Ag nanoparticles (Figure 3). Alternatively, tissue Ag increased

in *A. subulata* after exposure to 10 and 100 µg/L Ag, as $AgNO_3$ over 24–48 h (Figure 3A,B). No changes in Ag accumulation were observed between 24 and 48 h in *A. subulata* exposed to 1000 µg/L Ag (Figure 3C). After exposure of *A. subulata* to 10 and 100 µg/L Ag, as $AgNO_3$, for 48 h, a higher tissue Ag concentration was observed than that found in *U. lactuca* (Figures 3A,B).

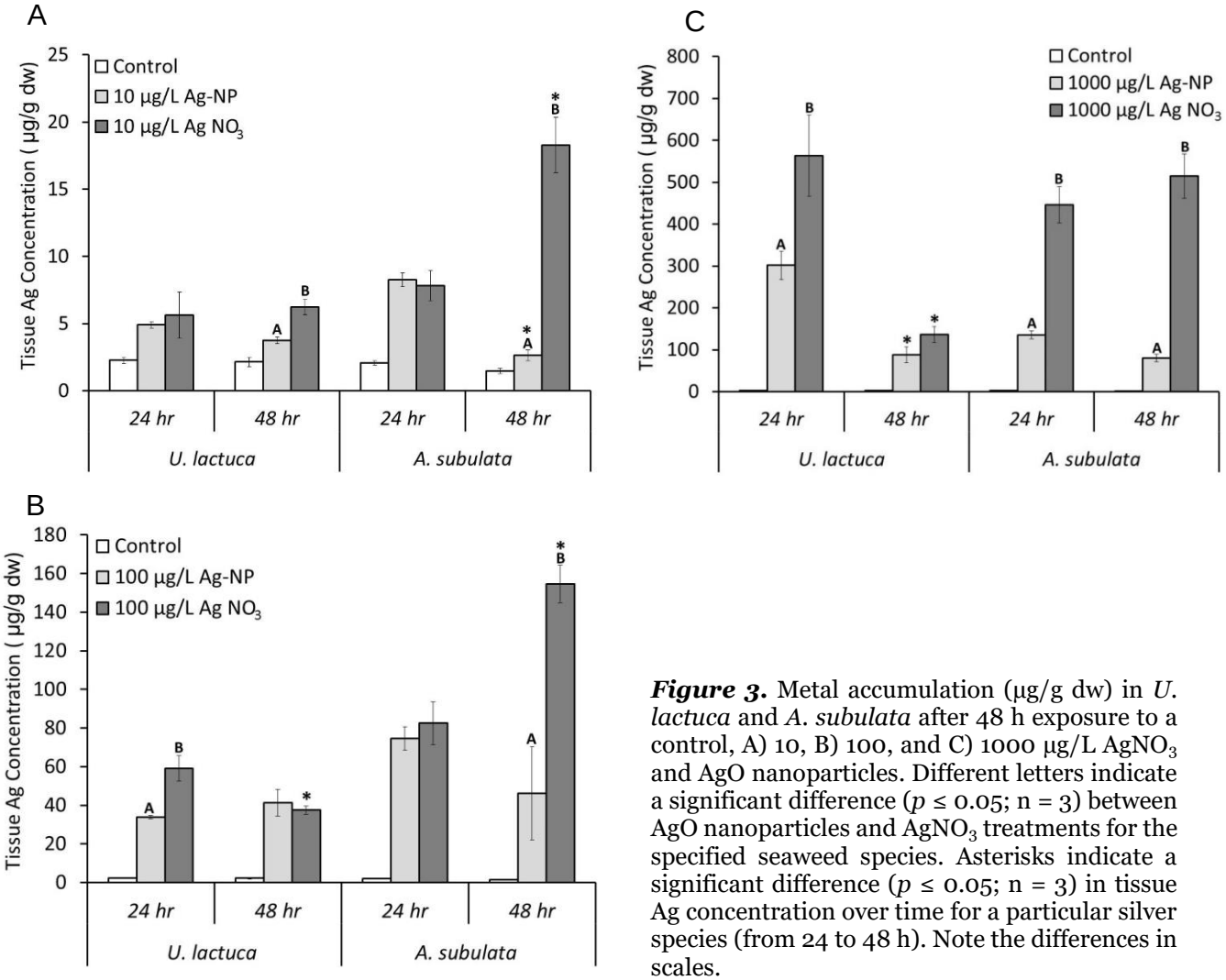

Figure 3. Metal accumulation (µg/g dw) in *U. lactuca* and *A. subulata* after 48 h exposure to a control, A) 10, B) 100, and C) 1000 µg/L $AgNO_3$ and AgO nanoparticles. Different letters indicate a significant difference ($p \leq 0.05$; n = 3) between AgO nanoparticles and $AgNO_3$ treatments for the specified seaweed species. Asterisks indicate a significant difference ($p \leq 0.05$; n = 3) in tissue Ag concentration over time for a particular silver species (from 24 to 48 h). Note the differences in scales.

Tissue Cu in *U. lactuca* and *A. subuluata* increased with increasing Cu exposure (Figure 4). Although not significant, a pattern of a time-dependent increase in tissue Cu was observed in both seaweeds exposed to the highest CuO nanoparticle concentration (Figure 4). *Ulva lactuca* and *A. subulata* demonstrated similar Cu accumulation patterns and concentrations throughout the experiment (Figure 4).

A significant decrease in the maximum rETR was observed in *U. lactuca* after exposure to $AgNO_3$, Ag nanoparticles, and CuO nanoparticles, as compared to respective controls (Figure 5). The maximum rETR was not concentration dependent, as the same magnitude of rETR inhibition was observed in all metal-exposed treatments (Figures 5D–F). The maximum rETR decreased over time (from 24 to 48 h) in *U. lactuca* exposed to 10 µg/L Ag as $AgNO_3$, and both 10 and 100 µg/L Ag, as Ag nanoparticles; whereas, no significant changes were observed in CuO nanoparticle

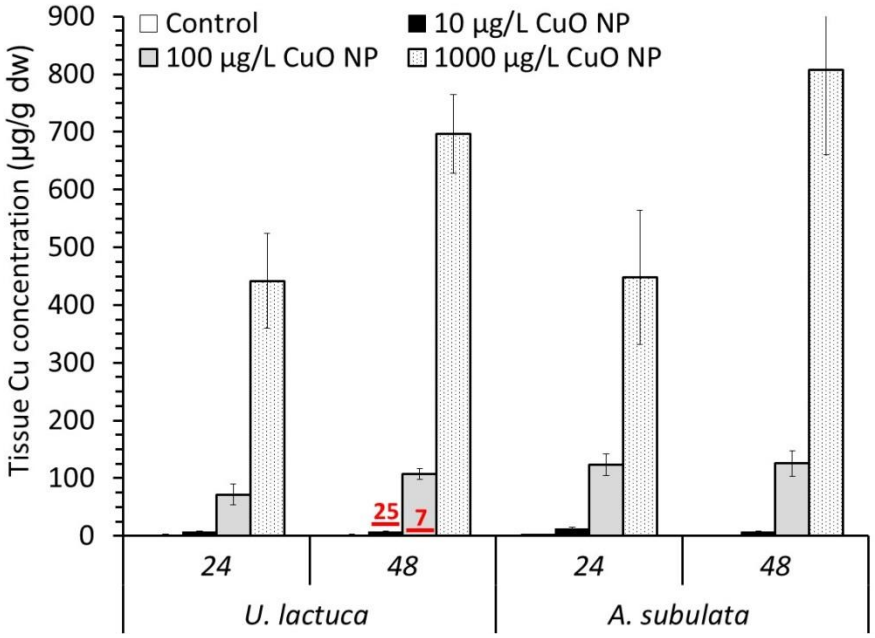

Figure 4. Copper accumulation (µg/g dw) in *U. lactuca* and *A. subulata* after exposure to a control and solutions of 10, 100, and 1000 µg/L CuO nanoparticles over 48 h. At each time point (24 or 48 h), all copper treatments were significantly different ($p \leq 0.05$; n = 3) from each other for each seaweed. Red lines and numbers indicate the copper accumulation (µg/g dw) in *U. lactuca* exposed to 10 and 100 µg/L $CuNO_3$ for 48 h from a previous study in our laboratory (Jarvis and Bielmyer-Fraser 2015).

treatments over time (Figures 5D–F). Significant increases in YNO were observed after exposure of *U. lactuca* to every concentration of $AgNO_3$ and CuO nanoparticles, as compared to concurrent controls (Figures 5A,C). Alternatively, no significant differences in YNO were observed in *U. lactuca* exposed to Ag nanoparticles over 48 h (Figure 5B). Quantum yield of nonregulated energy dissipation did not significantly increase with increasing exposure to 10–100 µg/L CuO nanoparticles (Figure 5C); however, there was an increased YNO in *U. lactuca* exposed to 100 µg/L Ag, as $AgNO_3$, as compared to *U. lactuca* exposed to 10 µg/L (Figure 5A). A time-dependent increase in YNO was observed in the 100 µg/L $AgNO_3$ treatment (Figure 5A); whereas, *U. lactuca* exposed to 10 and 100 µg/L Cu, as CuO nanoparticles, had a decreased YNO from 24 to 48 h (Figure 5F).

DISCUSSION

In this study, homeostatic regulation of Ag differed between seaweed species, over time, between the two forms of Ag (dissolved and nanoparticles), and by concentration. Exposure of *U. lactuca* to 100 and 1000 µg/L $AgNO_3$ and 1000 µg/L Ag nanoparticles resulted in more Ag accumulation at 24 h followed by a decrease in tissue Ag in those treatments by 48 h. Alternatively, exposure of *A. subulata* to $AgNO_3$ generally resulted in increased tissue Ag over time. These results suggest that *U. lactuca* regulates tissue Ag better than *A. subulata* and that Ag nanoparticles were less available for uptake or better regulated in both seaweed species, as compared to $AgNO_3$. Regulation of tissue Ag has been shown to occur via down regulation of membrane transport proteins in other studies (Jarvis and Bielmyer-Fraser 2015). Wang and Dei (1999) reported decreasing Cd, Se, and Zn uptake rate constants with increasing exposure concentration in *U. lactuca* and the red alga, *Gracilaria blodgettii,* which suggests down regulation of specific metal transporters.

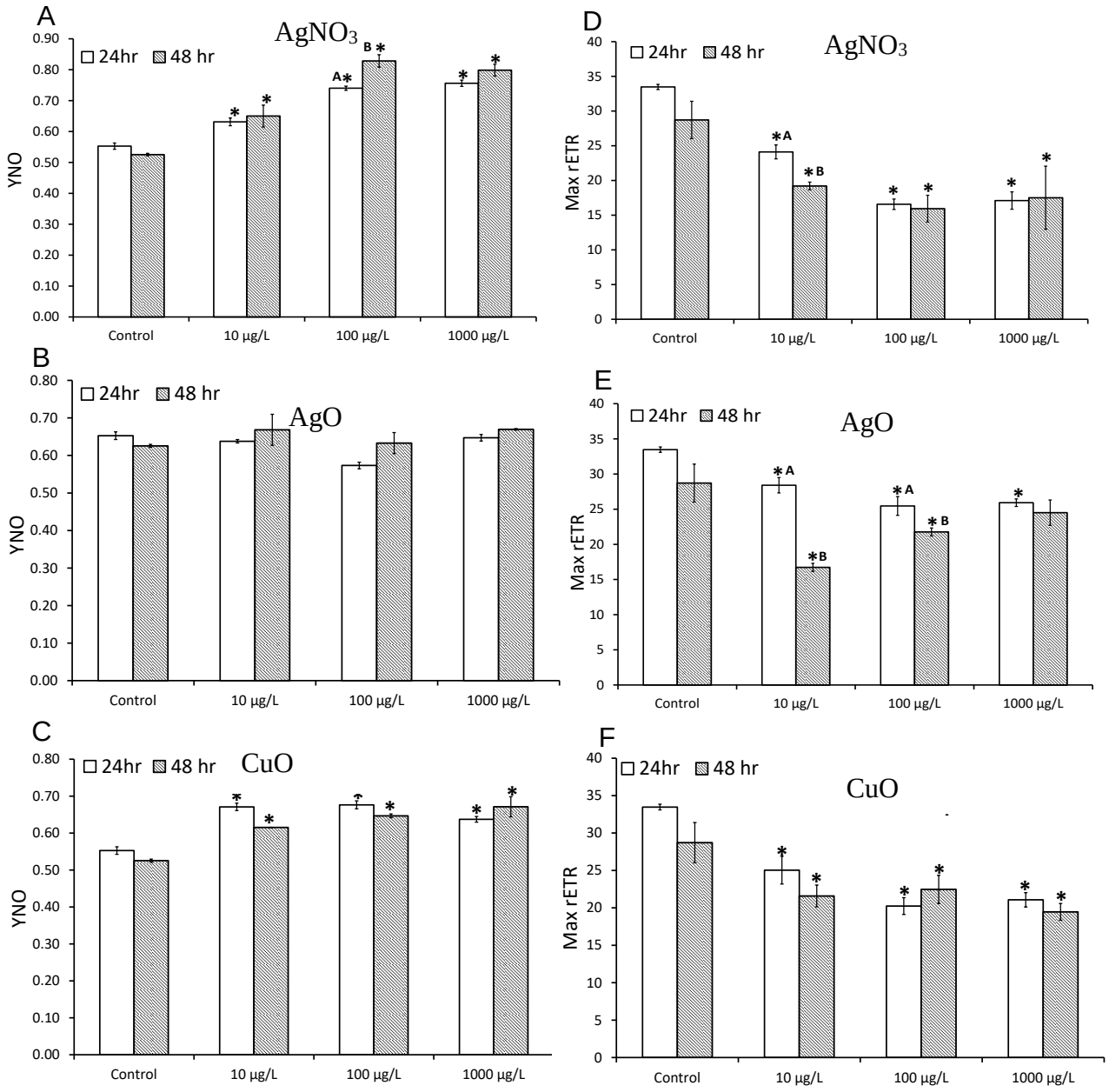

Figure 5. A.–C., Quantum yield of nonregulated energy dissipation (YNO) and D.–F., maximum relative electron transport rate (the maximum rETR) in *U. lactuca* after 24 and 48 h exposure to A) and D) $AgNO_3$; B) and E) AgO nanoparticles; and C) and E) CuO nanoparticles. *Represents a statistically significant difference ($p \leq 0.05$; n = 3) from the concurrent control in *U. lactuca*. Different letters indicate a significant difference ($p \leq 0.05$; n = 3) over time within the same treatment.

Algae have been shown to demonstrate a high biosorption capacity for metals in laboratory studies (Burdin and Bird 1994; Orduna-Rojas and Longoria-Espinoza 2006; Apaydin et al. 2010; Laib and Leghouchi 2012; Jarvis and Bielmyer-Fraser 2015), which is consistent with the findings here. In a previous study, *U. lactuca* exposed for 48 h to nominal concentrations ranging from 0 to 100 µg/L of $AgNO_3$ or Ag nanoparticles accumulated approximately 30–110 µg/g and 5–30 µg/g, respectively (Turner et al. 2012). These values are similar to those in our study where *U. lactuca* exposed to 10–1000 µg/L of $AgNO_3$ or Ag nanoparticles, accumulated approximately 7–150 µg/g and 5–100 µg/g, respectively; and, *A. subulata* accumulated approximately 3–500 µg/g and 3–100 µg/g, respectively. Silver accumulation in the seaweed over time was influenced by the form of Ag in *A. subulata*

more than in *U. lactuca*. Differences in structure, cellular components, and uptake transporters could account for the observed differences in tissue Ag between the two seaweed species. Additionally, differences in the abundance of metal binding proteins, such as phytochelatins, may have also played a role in the regulation and elimination of tissue Ag.

Changes in Ag toxicity resulting from the form of Ag used (with $AgNO_3$ being more toxic) were also observed in this study. Similarly, Turner et al. (2012) demonstrated that $AgNO_3$ has a higher toxicity (reduced chlorophyll *a* fluorescence quenching) in *U. lactuca* when compared to Ag nanoparticles at similar available exposure concentrations (0 to 100 µg/L $AgNO_3$ and Ag nanoparticles). Additionally, Ag nanoparticles were not as toxic to *U. lactuca* up to 15 µg/L Ag, and Ag nanoparticles accumulated at the algal surface in that study. *Ulva lactuca*, in our study, demonstrated a higher Ag accumulation when exposed to $AgNO_3$ compared to Ag nanoparticles in terms of accumulation factor, which is similar to the findings of Turner et al. (2012). However, it remains unclear whether the accumulation and toxicity in seaweed exposed to nanoparticles in our study resulted from dissolution of Ag or a combined exposure of dissolved Ag and Ag nanoparticles. The lowest treatment of 10 µg/L Ag nanoparticles had a measured dissolved Ag concentration of only 0.5 µg/L, which suggests that much of the toxic response (decreased maximum rETR) was due to nanoparticle exposure. Exposure to higher concentrations of Ag nanoparticles and $AgNO_3$ (500–10,000 µg/L) in the duckweed species, *Spirodela polyrhiza*, significantly decreased plant tissue nitrate–nitrogen content, chlorophyll *a*, chlorophyll *a*/*b*, and chlorophyll fluorescence (Jiang et al. 2012). Furthermore, $AgNO_3$ was more toxic than Ag nanoparticles, with EC_{50} values of 16.10 ± 0.75 vs 7.96 ± 0.81 mg/L, respectively, for chlorophyll *a* (Jiang et al. 2012). The mechanism of Ag uptake and toxicity from nanoparticles exposure is likely concentration-dependent. Silver uptake from 100 µg/L Ag nanoparticles exposure in the duckweed, *Lemna gibba*, was correlated with production of intracellular reactive oxygen species and reduction in plant cellular viability (Oukarroum et al. 2013). Furthermore, the authors suggest that the effects were due to direct contact with Ag nanoparticles. Silver toxicity from nanoparticle exposure in *U. lactuca* and *A. subulata* is more likely due to direct interaction with the nanoparticles at lower concentrations (with minimal leaching of the Ag ion), whereas, Ag toxicity at higher concentrations is more likely due to interaction of the seaweed with dissolved Ag which has leached into the solution.

Copper, as an essential trace metal, has been shown to bioaccumulate and has a high affinity for seaweeds (Ho 1990; Bielmyer-Fraser 2015). The tissue Cu concentrations in control *U. lactuca* in this study were 1.95 ± 0.79 to 2.7 ± 1.13 µg/g dw, which is similar to those reported in nonpolluted sites of Rabta Bay (2.37 ± 0.003 and 2.59 ± 0.002 µg/g dw, Western Mediterranean Sea, Algeria, Laib and Leghouchi 2012). Brown et al. (1999) reported a range of 0.1–3.0 µg/g dw Cu in *U. lactuca* from an uncontaminated site and 14–134 µg/g dw from a highly metal-contaminated site. The Cu concentrations reported in seaweeds collected from various sites range from 0.45 to 253 µg/g dw in green seaweed, 0.35 to 45.2 µg/g dw in red seaweed, and 1.0 to 103 µg/g dw in brown seaweed (Dutton et al. 1973; El-Sarraf 1995; Guisti 2001; Caliceti et al. 2002; Abdallah and Abdallah 2007; El-Nemr et al. 2012; Laib and Leghouchi 2012; El-Din et al. 2014; Bonanno and Orlando-Bonaca 2017, 2018). *Ulva lactuca*, in particular, has demonstrated a high Cu binding capacity in several studies (Ho 1990; Sheng et al. 2004; Misheer et al. 2006; Abdallah and Abdallah 2007; Gaudry et al. 2007; Omar 2008), due to comparatively high concentration factors of 0.47–0.6 × 10^4 (Seelinger and Edwaeds 1977). In this study, the tissue Cu concentrations in *U. lactuca* and *A. subulata* exposed to Cu nanoparticles were within

the range of those reported in the environment (at contaminated sites) and in laboratory studies (Burdin and Bird 1994).

Exposure of seaweeds to Cu as Cu nanoparticles, in the present study, resulted in an increased tissue Cu accumulation with increasing exposure concentration. A previous study in our laboratory showed a decrease in tissue Cu (25.6 µg/g dw to 6.82 µg/g dw) in *U. lactuca* with increasing exposure concentration from 10 to 100 µg/L dissolved Cu as $Cu(NO_3)_2$, which is similar to our results with $AgNO_3$ (Jarvis and Bielmyer-Fraser 2015). These results suggest that CuO nanoparticles may be less regulated in the seaweed than dissolved Cu. It is likely that other external factors can also affect the rate of Cu uptake (Hamdy 2000; Deng et al. 2007; Turner et al. 2009).

Several studies indicate that the metal binding capacity of algal cells can be determined by the distribution of polysaccharide, protein, and lipid functional groups in their cell walls (Veroy et al. 1980; Hamdy 2000; Deng et al. 2007). Results of our study indicate that at least some of the Cu was localized in the cell wall of the seaweed after exposure to CuO nanoparticles. Bielmyer-Fraser et al. (2014) reported higher Cu accumulation in the cell wall of the marine alga, *Thalassiosira weissflogii*, after exposure to 0.25–5 µg/L CuO nanoparticles, as compared to the same concentrations of dissolved Cu. Similarly, when the brown alga, *Sargassum filipendula*, was exposed to Cu and Ni, the metals were observed by SEM/EDX in the algal cell wall (Kleinübing et al. 2010). Raize et al. (2004) suggested that stronger cross-linking in the cell wall matrix occurs due to displacement of cations by metals. The distribution of Cu nanoparticles in the cell wall may be due to size aggregation and their tendency to agglomerate; and, may also protect the seaweed against Cu toxicity to some degree (Campbell et al. 2002). Bielmyer-Fraser et al. (2014) reported a higher percentage of metal in the organelle and endoplasmic reticulum fractions of *T. weissflogii* exposed to dissolved metals as compared to those exposed to metal oxide nanoparticles.

Differences in both Cu accumulation and toxicity were also observed when comparing the results of this study with the previous one in our laboratory that used dissolved Cu (Jarvis and Bielmyer-Fraser 2015). Copper nitrate accumulated more than CuO nanoparticles at 10 µg/L exposure; whereas, CuO nanoparticles accumulated to a greater extent at 100 µg/L (Jarvis and Bielmyer-Fraser 2015), likely due to better homeostatic regulation of the dissolved Cu. Photosynthetic impairment (decreased rETR) in *U. lactuca* was observed after exposure to a lower concentration (lowest observable effect; LOEC = 10 µg/L) of CuO nanoparticles than with $Cu(NO_3)_2$ (LOEC = 100 µg/L), possibly due to differences in Cu distribution. Photosynthetic toxicity has been a sensitive end point for seaweeds in other studies as well (Haglund et al. 1996; Baumann et al. 2009). The red seaweed, *Gracilaria tenuistipitata*, had reduced rETR (EC_{50} values of 50–170 µg/L Cu) and several species of green, red, and brown macroalgae had decreased chlorophyll fluorescence after 10 µg/L exposure of Cu and Cd (Haglund et al. 1996; Prasad and Strzalka 1999; Baumann et al. 2009). *Ulva lactuca* demonstrated lower photosynthetic activity after exposure to 4 mg/L Cu, which had leached from an antifouling paint particle mixture (Turner et al. 2009). Decreased growth and photosynthesis of *L. gibba* was reported after 48-h exposure to 0.1 to 0.4 g/L CuO nanoparticless (Perreault et al. 2010). Reduced growth rate, distribution of photosynthetic pigments, and morphology of *Landoltia punctate* was reported after exposure to 1.0 mg/L CuO nanoparticles (Lalau et al. 2015). Cu concentrations of 0.2 to 0.6 mg/L were reported to decrease growth within other macroalgal species (Zayed et al. 1998; Prasad et al. 2001; Kanoun-Boule et al. 2009). Reduced photosynthetic output could lead to decreased growth in seaweed and may also influence the quality of the seaweed for consumers.

CONCLUSION

A significant concentration-dependent metal accumulation was observed in the seaweeds, *U. lactuca* and *A. subulata*, when exposed to $AgNO_3$, Ag nanoparticles, and CuO nanoparticles for 48 h. Differences in tissue Ag accumulation in *U. lactuca* and *A. subulata* were observed based on the form of Ag (nanoparticles versus dissolved), the exposure concentration, and the seaweed species. Silver nitrate generally accumulated to a greater extent than did Ag nanoparticles and *U. lactuca* seemed to be better than *A. subulata* at regulating and eliminating tissue Ag concentrations over time. Additionally, $AgNO_3$ was more toxic than Ag nanoparticles to *U. lactuca*. When compared to previous studies from our laboratory (Jarvis and Bielmyer-Fraser 2015), CuO nanoparticles in this study were not as well-regulated as $Cu(NO_3)_2$, and CuO nanoparticles were more toxic than $Cu(NO_3)_2$ to *U. lactuca*. The concentration likely affects uptake and toxicity of Cu to *U. lactuca*. These results suggest that the mechanisms for metal uptake, accumulation, detoxification, and metal homeostasis may differ between dissolved Ag and Ag nanoparticles and dissolved Cu and CuO nanoparticles in seaweeds. Furthermore, these findings have important ecological implications as accumulated metal in seaweeds may be transferred to higher trophic levels (Volterra and Conti 2000; Eisler 2010; Jarvis et al. 2015).

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Russell Goddard, Valdosta State University, for help with SEM/EDX.

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