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Hazardous effects of silver nanoparticles for primary producers in transitional water systems: The case of the seaweed *Ulva rigida* C. Agardh

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ABSTRACT

The acute toxicity of citrate capped silver nanoparticles (AgNP) and silver nitrate was evaluated on the marine macroalga *Ulva rigida* C. Agardh (1823). Silver bioaccumulation, ultrastructural chloroplast damages verified by TEM microscopy, inhibition of primary production, neutral lipid production and oxidative stress were observed after 24 h of exposure to AgNP. The toxic effects of silver nitrate in artificial seawater started from a concentration of 0.05 ppm and was more toxic than AgNP that produced effects from a concentration of 0.1 ppm. However only AgNP induced lipid peroxidation in *U. rigida*. The addition of natural organic and inorganic ligands, represented by transparent exopolymer particles (TEP) and clay, drastically reduced AgNP acute toxicity in a ratio AgNP:ligand of 1:100 and 1:200, respectively. The findings suggest a marked toxicity of Ag on marine macroalgae which however should be mitigated by the high natural ligand concentrations of the transitional environments.

1. Introduction

The development of nanotechnology led to a rapid expansion of nanomaterials that are likely to become a source of many different nanoparticles (NP) discharged into the environment (Gambardella et al., 2015). Different unique properties of NP like high specific surface area and mobility, could potentially lead to unexpected health or environmental hazards (Maynard et al., 2006). The use of silver colloids and nanoparticles have become a topic of interest in many biotechnological applications, attributed to their consolidated antimicrobial and biocidal properties (Fabrega et al., 2011; Rai et al., 2009). A survey on the patent search engine "Espacenet" (European Patent Office, 2019) for "silver nano" reveals an exponential trend in the release of > 5000 patents from the year 2000 and the 52% of these were recorded in the last 5 years. The same exponential trend can be found in the increasing scientific interest on silver nanoparticles documented in peer review journal publications from the early 2000s (McGillicuddy et al., 2017).

Various applications of AgNP in many domestic and industrial products include: healthcare and cosmetics, clothing, food packaging, paints, laundry additives, home appliances and medical devices(Zhang et al., 2015). Moreover 53% of the EPA (Environmnetal Protection Agency)-registered biocidal silver products are reported to contain AgNP (Nowack et al., 2011). The steady increase in consumer products released to the market, containing silver nano-compounds, could reflect in an increasing dispersion and diffusion of silver into the environment. AgNP can be discharged during synthesis, manufacturing and use of these products as well as the recycling and disposal processes (Köhler et al., 2008).

Little systemic toxicity was reported in vivo toward humans for the doses reported to be toxic for microbes and parasites (Hadrup and Lam, 2014; Le Ouay and Stellacci, 2015; Stoddard et al., 2013; van der Zande et al., 2012). This probably contributed to the widespread use of AgNP and at the same time increased the concern of researchers for the final receiving environments. In fact, Ag is considered the second most toxic metal (after Hg) for aquatic organisms (Kennedy et al., 2010).

The possible adverse effects of increased environmental exposure to AgNP include the development of silver-resistant bacteria (Gupta et al., 1999; Percival et al., 2005; Silver, 2003) and the impairment and

Abbreviations: AgNP, silver nanoparticles; CTRL, control; LPO, lipid peroxidation; MDA, malondialdehyde; NP, nanoparticles; PP, primary production; TEP, transparent exopolymer particles; TEM, transmission electron microscopy; TWS, transitional water systems

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deterioration of aquatic organisms and environments, especially primary producers (Asharani et al., 2008; Choi et al., 2008; Griffitt et al., 2008; Navarro et al., 2008). AgNP toxicity mechanisms include: membrane adhesion and disruption; alterations in proton-pump function; lipopolysaccharide degradation; enzymes inactivation; cellular phosphate management disruption; inhibition of DNA synthesis, ribosome denaturation and ROS production (Moreno-Garrido et al., 2015). To date, the vast majority of research involving Ag and AgNP toxicity on primary producers has been focused mainly on freshwater species and microalgae with little concern toward both marine species and macroalgae (Moreno-Garrido et al., 2015; Navarro et al., 2008). Macroalgae in transitional water systems (TWS) are important marine primary producers, directly affecting the following levels of the trophic chain and consequently the final quality state of many coastal environments (Sfriso et al., 2009, 2014).

The present study aims to investigate the interactions of AgNP and $AgNO_3$ with the most common primary producer of TWS, the seaweed *Ulva rigida* C. Agardh (1823) utilizing biochemical and physiological biomarkers. Moreover, we investigate the effects of TWS natural organic and inorganic ligands on the toxicity of AgNP.

2. Material and methods

2.1. Experimental design

We evaluated the effects of silver in the form of citrate capped AgNP on a model primary producer organism of TWS: the macroalga *U. rigida,* a biological indicator species for metal bioaccumulation (Favero et al., 1996). For comparison the effects of $AgNO_3$ as source of Ag^+ was also tested in parallel in order to identify AgNP specific toxic behaviors. The reactions of *U. rigida* were examined by lipid peroxidation (LPO), primary production (PP), ultrastructural cellular alterations (by TEM imaging) and epifluorescence microscopy. A second experiment was performed to evaluate the effects of AgNP on *U. rigida,* examining PP and LPO, in presence of two common natural compounds of TWS: transparent exopolymer particles (TEP) and clays.

2.2. Reference study area

The Venetian Lagoon (Italy) was selected in this study as reference site to simulate the TWS conditions for the toxicity test. This lagoon is influenced by Venice town, where there is no sewer system and drains convey waters to canals. In this lagoon, metal pollution was found in sediments mainly due to the pollution released in the past by the Porto Marghera industrial area and the town discharges (Frignani et al., 1997; Masiol et al., 2014). Two rivers bring freshwater near the town from the mainland (Osellino and Dese rivers) in areas with high water residence time that can reach up to 60 days (Guerzoni and Tagliapietra, 2006).

2.3. Nanoparticles synthesis and characterization

AgNP were synthesized with the citrate reducing method reported in Ratyakshi and Chauhan (2009). The plasmon resonance peak maximum was measured with an UV-VIS spectrophotometer (Jenway, Germany). The size distribution of the nanoparticles was characterized through a transmission electron microscope (TEM) FEI Tecnai G2 (Hillsboro, Oregon) and three images of AgNP from TEM were processed by ImageJ software for the evaluation of particle size by Feret diameter.

2.4. Tested seaweeds and experimental setup

Artificial seawater (ASW) was prepared dissolving in one litter of Milli-Q water: NaCl: 24.6 g, KCl: 0.67 g, $CaCl_2$:1.36 g, $MgSO_4x7H_2O$: 6.29 g, MgCl: 4.66 g, $HNaCO_3$: 0.18 g, according to Kester et al. (1967). All the glassware was cleaned with “Contrad” detergent, soaked overnight in HNO_3 1%, to remove any trace metal elements, and eventually rinsed with milli-Q water and $HNaCO_3$ 1% buffer solution. Small (young) thalli of the seaweed *U. rigida* (max. 5 cm long) were collected in spring from the artificial rocky shores of the Lagoon of Venice in an area with high water renewal of the Lido island. The seaweed thalli were kept in seawater for 3 hours (h) for acclimation until their employment in the experimental tests.

The effects of both $AgNO_3$ and AgNP were tested in artificial seawater in concentration ranges from 0.01 to 120 ppm for LPO and from 0.1 to 20 ppm for PP. The toxicity on *U. rigida* was examined after 24 h by PP and LPO analyses, employing for each tested concentration, three flasks (100 mL) with 500 mg of fresh seaweed, equivalent to an infield standing crop of 1 kg m^{-2}. The experiments were carried out at 22 °C.

2.5. Lipid peroxidation

Levels of MDA in *U. rigida* were measured according to Wahsha et al. (2012). A 100-mg fresh macrophyte sample was homogenized in 5 mL solution of 0.25% thiobarbituric acid in 10% trichloroacetic acid, and incubated at 95 °C for 30 min, followed by quick cooling and centrifuged at 10,000*g* for 10 min. The absorbance of the clear supernatant was read spectrophotometrically at 532 nm and correction for unspecific turbidity was done by subtracting the absorbance of the sample at 600 nm. The LPO was expressed as malondialdehyde (MDA) millimols formed per gram of fresh tissue (mmol g^{-1} fw), using a molar extinction coefficient of 155 mM^{-1} cm^{-1}. Experiments were performed in triplicate.

2.6. Primary production

Primary production was measured as changes in dissolved oxygen (DO) concentration at 22 °C after 24 h of AgNP/$AgNO_3$ exposure (Odum, 1956) by an oximeter (model Oxi 196, Wissenschaftlich–-Technische Werkstatten GmbH), equipped with a battery stirrer BR190. Two sets of three replicates for each concentration were tested: one set was kept in dark, where respiration depleted oxygen. The other set was kept under continuous light of 30 μmol (red and blue LED ratio 2:1), where photosynthesis produced oxygen. The sum of photosynthesis and respiration returns the primary production score, which was expressed as mg of elemental oxygen per gram of dry tissue produced in 1 h during the process (mg O_2 g^{-1} dw^{-1} h^{-1}).

2.7. Transmission electron microscopy (TEM)

To investigate the ultrastructural alterations of *U. rigida* induced by AgNP and $AgNO_3$, control and treated thalli were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 6.9) for 2 h at 4 °C. After fixation, thalli were treated according to Moro et al. (2002). Samples embedded in an Epon-Durcupan ACM mixture were cut through a Reichert Ultracut S ultramicrotome and the ultrathin sections were poststained with lead citrate and examined with a transmission electron microscope (TEM - FEI Tecnai G2) operating at 100 kV.

2.8. Epifluorescence microscopy

Nile red lipid staining protocol reported in Storms et al. (2014) was applied to the samples treated with different AgNP and $AgNO_3$ concentrations. Chlorophyll-*a* autofluorescence and lipid fluorescence were visualized by fluorescent microscope (Olympus BX51 Microscope) and images were processed by ImageJ software.

2.9. Metal analysis

U. rigida thalli were rinsed with artificial seawater, dried with a tissue and maintained in oven at 50 °C for 48 h. The samples were homogenized and 100 mg were digested in acid-treated Teflon vessels

by 3 mL of HNO_3, 3 mL of $HClO_4$ and 4 mL of Milli-Q water for 2 h at 130 °C (Rigollet et al., 2004). The volume of the extracts was brought to 50 mL, centrifuged at 5000 rpm for 20 min, transferred in polyethylene containers and maintained at 4 °C until analysis. Silver analysis was carried out by Perkin Elmer ICP-OES 5300DV. All the analyses were done in triplicate.

2.10. TEP and turbidity effect

The effect of TEP and turbidity on the toxicity of AgNP was evaluated using two concentrations of AgNP (0.5 and 5 ppm) by addition of sulphated agar (anionic polysaccharide) and clay. The TEP were synthesized by sulphated agar purified according to Sfriso et al. (2017) from *Gracilariopsis longissima* (S.G. Gmelin) Steentoft, L.M. Irvine et Farnham then dissolved in boiling water ad poured in cold ASW. Pharma-grade purified clay was purchased from the market and tested in the range from 10 to 100 ppm.

2.11. Statistical analysis

All treatments were done in triplicates. Means and standard deviations were calculated for each treatment. The Shapiro-Wilk test was used to check for normality and significant differences between control samples and AgNP/$AgNO_3$ exposed algal samples were determined by Mann-Whitney *U* test where *p*-value of 0.05 or less was considered to be significant. All the analyses were performed by using Statistica version 10.1 (Statsoft, Inc. Tulsa, USA).

3. Results

3.1. Nanoparticle characterization

The citrate capped AgNP displayed a surface plasmon resonance peak within the range from 412 to 416 nm. The AgNP mean size and distribution, determined by ImageJ particle analysis on TEM microscopic images, showed a mean Feret diameter of 35 ± 9.1 nm (Fig. 1a). The particle size distribution, reported in histogram (Fig. 1b), showed that > 90% of the AgNP ranged from 20 to 50 nm in size. Results about behavior and fate of AgNP and $AgNO_3$ in ASW were included in Supplementary material.

3.2. Silver uptake and toxicity

Silver nanoparticles were bioaccumulated in *U. rigida* tissues from the solution. The metal analysis showed that the uptake of silver follows the Langmuir isotherm equation (Fig. 2) with a saturation point in *U. rigida* of 11.3 µg Ag g^{-1} fw^{-1} from a 5 ppm AgNP solution. A relatively high accumulation was found in *U. rigida* already from 1 ppm AgNP solution reaching 8.50 µg Ag g^{-1} fw^{-1} in the seaweeds.

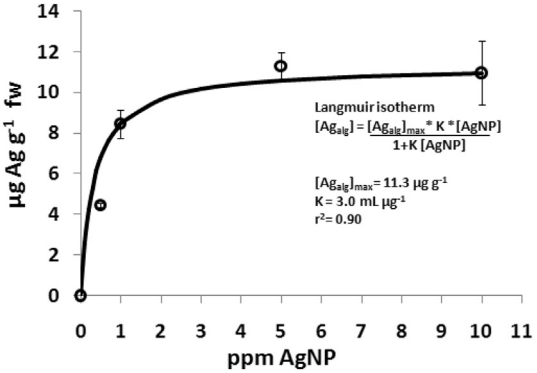

Fig. 2. Silver bioaccumulation in *U. rigida* from AgNP solutions. Data are mean ± s.d. (n = 3).

The MDA levels are presented in Fig. 3a, in agreement with the bioaccumulation results, displayed for AgNP, the lowest observed adverse effect level was at 1 ppm, the first concentration significantly different from control (Test U Mann-Whitney: d.f. 9, $p = 0.01$).

The oxidative stress generated by AgNP steadily increased up to concentrations of 30 ppm AgNP, then started decreasing in conjunction with cell death, as ascertained by light microscope (data not shown). For comparison, the oxidative stress generated by $AgNO_3$ was also investigated from 0.01 to 120 ppm, but the MDA values did not diverge from the control. However, the $AgNO_3$ produced a toxic effect, reducing the PP by 68%, starting from the concentration of 0.05 ppm (**Fig. 3b**). Negative values of PP were found from 0.1 to 5 ppm, corresponding to consumption of oxygen due to respiration to cope with the toxic effect of silver. This also highlighted that respiration processes were still working up to 1 ppm. AgNP generated instead an eutrophication effect from 0.01 to 0.07 ppm. The adverse effects manifested only from 0.1 ppm with the complete inhibition of PP at 5 ppm $AgNO_3$ in conjunction with the maximum bioaccumulation of silver in the seaweed. The oxidative stress measured by MDA started for AgNP after primary production was inhibited but this increase in MDA was found only for AgNP and not for $AgNO_3$. The $AgNO_3$ at 5 ppm induced cell lysis and death. Conversely, AgNP completely inhibited PP but did not induce lysis, as verified by epifluorescence, and produced a steady increase of oxidative stress.

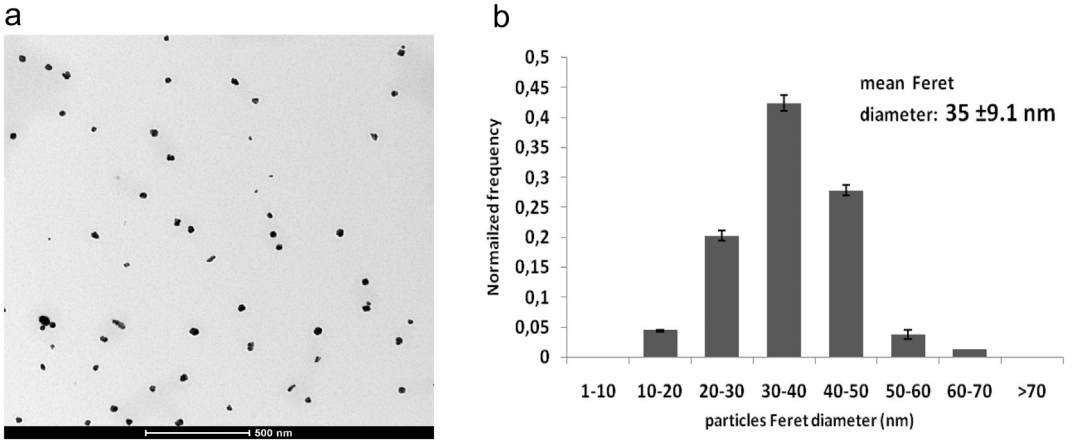

Fig. 1. a: TEM image of AgNP.
b: size distribution of AgNP. Data are mean ± s.d. ($n = 3$).

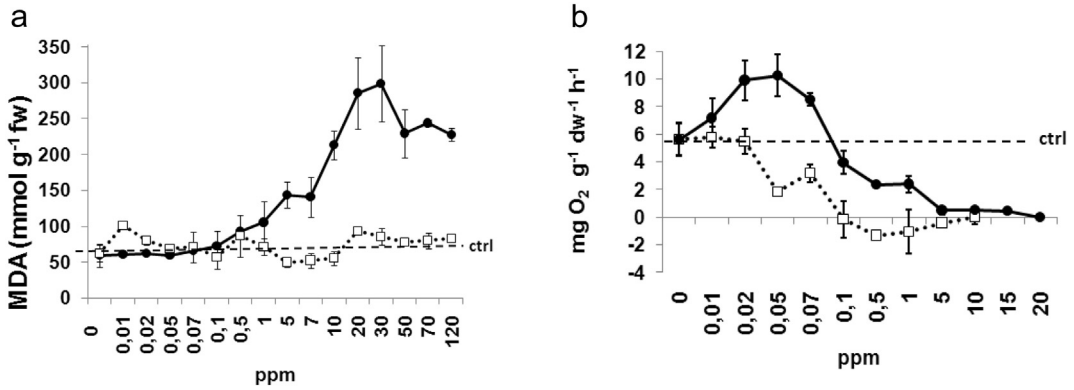

Fig. 3. a: MDA levels in *U. rigida* at different concentrations of AgNP (•) and $AgNO_3$ (□). Data are mean ± s.d. ($n \geq 3$).
b: PP in *U. rigida* at different concentrations of AgNP (•) and $AgNO_3$ (□). Data are mean ± s.d. (n ≥ 3).

3.3. Epifluorescence imaging

The microscope investigation results comply with the effects of AgNP and $AgNO_3$ on PP. The control (CTRL) images displayed healthy cells and the respective red auto-fluorescence of chlorophyll-*a* (Fig. 4a and b). At 0.05 ppm of AgNP no significant effects were noticed and only chlorophyll-*a* auto-fluorescence was observed (Fig. 4c). Conversely, for the same concentration of $AgNO_3$ (Fig. 4d) small yellow dots appeared inside the cells due to Nile-red binding neutral lipids. At 0.5 ppm both AgNP (Fig. 4g and e) and $AgNO_3$ (Fig. 4f) displayed an

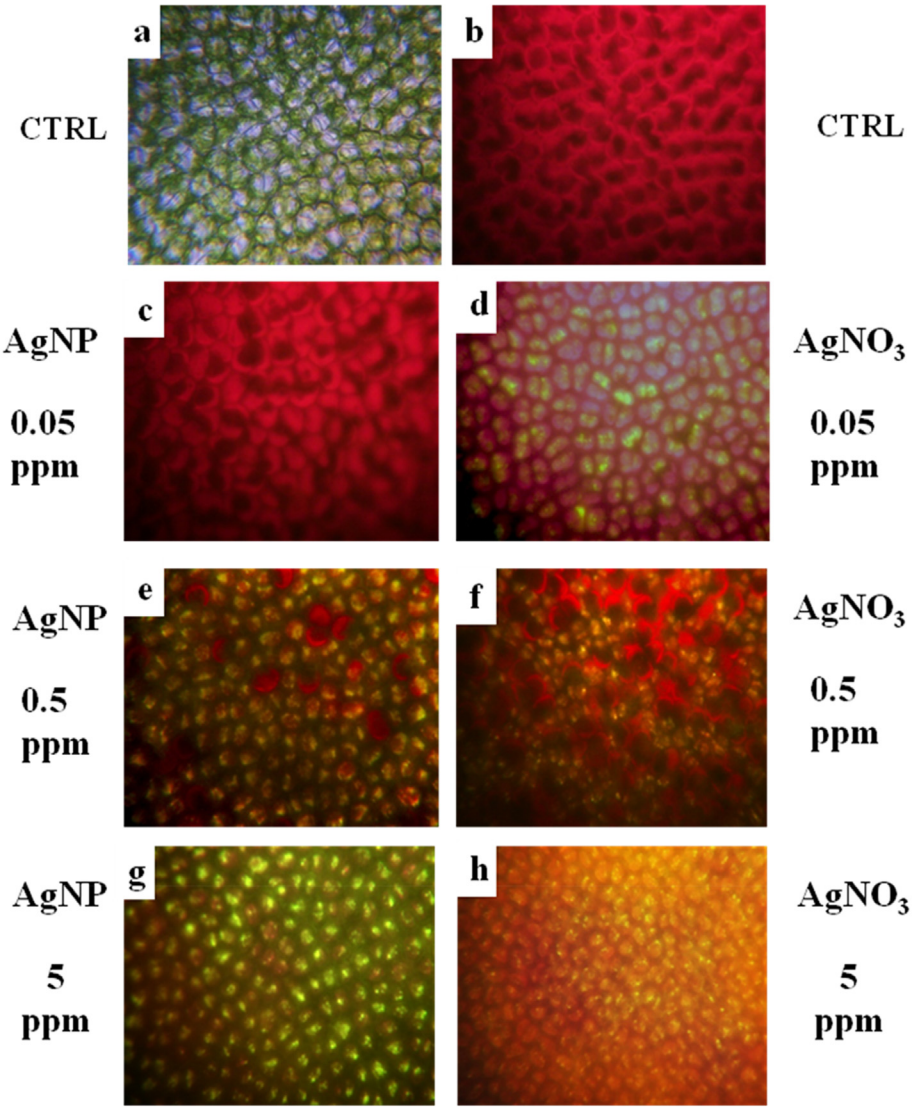

Fig. 4. Light and epifluorescence microscope (400×) images of samples treated with AgNP and $AgNO_3$ (0.05, 0.5, 5 ppm) in comparison with control without toxicants (CTRL).

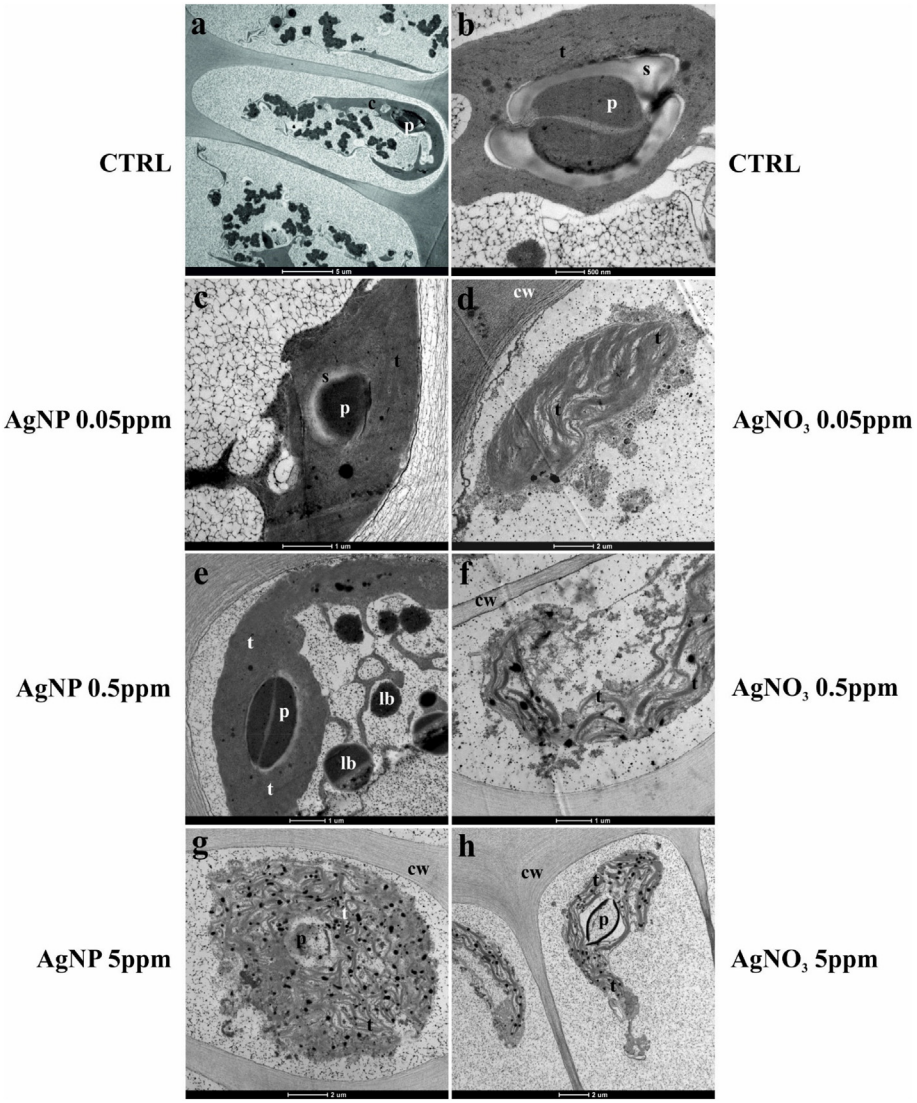

Fig. 5. Transmission electron microscope images of samples treated with AgNP and $AgNO_3$ (0.05, 0.5, and 5 ppm) in comparison with control without toxicants (CTRL). (c = chloroplast; t = thylakoid; p = pyrenoid; cw = cell wall; lb. = lipid body).

increased number of stained cells, which density seemed higher for AgNP solutions. Eventually, at 5 ppm all the cells were stained (Fig. 4g), but for $AgNO_3$ the effect was more marked with cell disruption, lysis (Fig. 4h) and lipid dispersion, creating a misty effect onto the algal tissues.

3.4. Ultrastructural analysis

Transmission electron microscope analysis carried out to investigate the ultrastructural modifications of *U. rigida* induced by AgNP and $AgNO_3$, confirmed the results obtained at light microscope. CTRL images showed cells with a chloroplast characterized by regular thylakoids and with an evident pyrenoid surrounded by starch (Fig. 5a and b). At 0.05 ppm of AgNP no relevant modifications were noticed (Fig. 5c), but at 0.05 ppm of $AgNO_3$ thylakoidal membranes appeared swollen (Fig. 5d). At 0.5 ppm of both AgNP (Fig. 5e) and $AgNO_3$ (Fig. 5f) some ultrastructural changes were evident. In particular, in the AgNP treated thalli, cells showed chloroplasts with stacked thylakoids, complete pyrenoids and some small electron-dense inclusion bodies, probably neutral lipids, as detected by Nile-red staining at the epifluorescence microscope (Fig. 5e). Instead, in the $AgNO_3$, chloroplasts exhibited swollen thylakoids and some osmiophilic granules (Fig. 5f). At AgNP 5 ppm the cells showed chloroplasts characterized by a large number of osmiophilic granules, remaining pyrenoids and swollen thylakoids (Fig. 5g). This was more evident at $AgNO_3$ 5 ppm, where more damaged chloroplasts showed disorganized thylakoidal membranes with many osmiophilic globules surrounding a residual of pyrenoid (Fig. 5h). Moreover, TEM analysis did not highlight a bioaccumulation of silver nanoparticles inside the cells of *U. rigida*. There was, in fact, a very impenetrable cell wall (**Fig. S1**), besides to an external exopolymeric substance layer (**Fig. S2**), that constituted a barrier, preventing the access of larger particles, and consequently a direct internalization of Ag nanoparticles.

3.5. TEP and turbidity effect

The effect of TEP addition on AgNP toxicity was evaluated by MDA and PP. MDA values verged toward the control with increasing TEP concentrations (Fig. 6a and b). The tests showed a 50% reduction of the effects of 0.5 ppm AgNP with the addition of 10 ppm of TEP and a complete detoxification at a TEP concentration of 50 ppm. The same TEP concentrations employed in the test did not produce any significant variations on toxicity of 5 ppm AgNP. Eventually TEP produced detoxification in a ratio AgNP:TEP of 1:100.

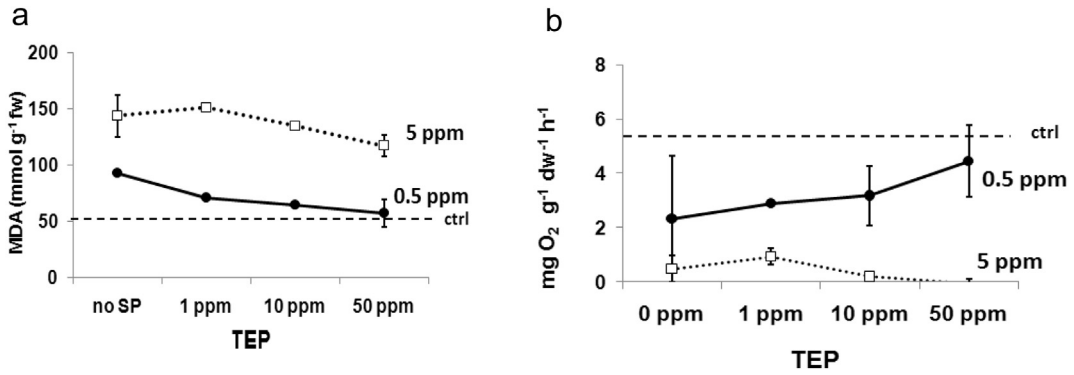

Fig. 6. a: MDA concentration in *U. rigida* for 5 ppm (□) and 0.5 ppm AgNP (•) at different concentrations of TEP. Data are mean ± s.d. (n = 3).
b: PP in *U. rigida* for 5 ppm (□) and 0.5 ppm AgNP (•) at different concentrations of TEP. Data are mean ± s.d. (n = 3).

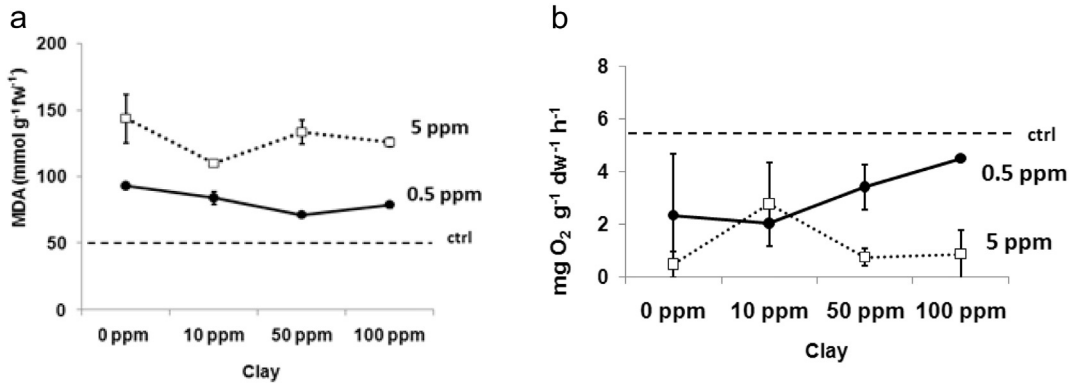

Fig. 7. a: MDA concentration in *U. rigida* for 5 ppm (□) and 0.5 ppm AgNP (•) at different clay concentrations. Data are mean ± s.d. (n = 3)
b: PP in *U. rigida* for 5 ppm (□) and 0.5 ppm AgNP (•) at different clay concentrations. Data are mean ± s.d. (n = 3).

A similar behavior was found for the effect of clay on AgNP toxicity, evaluated by MDA (Fig. 7a). The values displayed only a small reduction of the adverse effects due to clay addition to the solutions. PP values displayed instead an almost complete detoxification of 0.5 ppm AgNP by 100 ppm of clay with an AgNP:clay ratio of 1:200. Conversely, the toxic effects of a 5 ppm AgNP solution could not be reduced even by 100 ppm of clay in solution (Fig. 7b). Eventually both clay and TEP at high concentration greatly reduced AgNP toxicity, in terms of PP inhibition, but only TEP also reduced the oxidative stress.

4. Discussion

Despite the high ecological and economical importance and high productivity of TWS, they represent a sink for many pollutants including metals (Guerzoni and Tagliapietra, 2006; Ridgway and Shimmield, 2002). However, until recently, little attention was paid to silver contamination despite the increased level of production and release of AgNP based products. Different marine organisms were examined for AgNP toxicity, but only few articles discuss the effects of silver and AgNP on marine macroalgae, which are the dominant primary producers in TWS (Moreno-Garrido et al., 2015).

The results of this study confirm the high phytotoxicity of silver from both AgNP and $AgNO_3$ on the marine macroalga *U. rigida* as previously reported for microalgae (He et al., 2012). The toxicity of silver from $AgNO_3$ was tenfold higher in comparison with AgNP (Boenigk et al., 2014) moreover $AgNO_3$ produced a severe inhibition of primary production in *U. rigida* starting from 0.05 ppm. This result was in accordance with the previously reported value of 0.045 ppm, the lowest available silver concentration at which a toxic response was measured in *Ulva* (Turner et al., 2012). At this concentration AgNP did not produce any PP impairment, but an eutrophication effect was measured up to 0.070 ppm and PP inhibition due to AgNP was found at higher concentrations (0.100 ppm).

The toxic effect of silver from $AgNO_3$ was higher than that from AgNP as confirmed by the epifluorescence microscopic examination. The increase of neutral lipids, highlighted by the nile red staining, was associated with membrane destabilization by metal pollution (Kumar et al., 2010). The destabilization of the thylakoidal membrane system was confirmed by observations at the transmission electron microscope, where chloroplasts, of both AgNP and $AgNO_3$ treated cells, appeared strongly damaged, with reduced thylakoidal membranes and evident dilatations, besides a large number of osmiophilic globules. These morphological changes usually are correlated with functional alterations in the chloroplasts, measured by photosystem I and II activities (Hall et al., 1972).

Negative PP values were found for *U. rigida,* caused by the respiration process in the absence of photosynthetic activity, for $AgNO_3$ concentrations between 0.1 and 1 ppm. This suggests that mitochondrial activity was still acting at $AgNO_3$ concentrations up to 1 ppm, while the chloroplast photosystem in the range 0.045–0.075 ppm was already inhibited (Turner et al., 2012). Complete inhibition of both photosynthesis and respiration activities occurred at 5 ppm as found by Teodoro et al. (2011), who reported impairment of mitochondrial activity between 2 and 5 ppm. Eventually, both AgNP and $AgNO_3$ at 5 ppm produced the complete PP inhibition, but only AgNP produced an oxidative stress. Conversely, $AgNO_3$ induced cell death and lysis but no oxidative stress measurable by MDA. These differences are probably due to the routes by which toxicity is carried out. Copper transporter proteins were reported to mediate the transport of Ag^+ into the cells (Blaby-Haas and Merchant, 2012) and could be an important route of silver uptake and toxicity. In regard to AgNP we have no evidence of direct internalization of the particles. Many other studies report no

evidence of AgNP direct uptake (Zheng et al., 2019), though we proved that silver was bioaccumulated reaching at 24 h the saturation point from an AgNP solution of 5 ppm. The same concentration induced a complete PP inhibition both in $AgNO_3$ and AgNP solutions, highlighting that silver definitely produced toxic effects inside the cell. AgNP was reported to accumulate mainly on the cell surface (Hu et al., 2018) with little internalization in microalgae (Zheng et al., 2019). The pore size of the cell wall mat in algae was estimated to be of about 7-20 nm, excluding the access of larger particles (like the ones tested in this study) to the cell surface (Navarro et al., 2008; Zemke-White et al., 2000). However, the measure of oxidative stress in *U. rigida* reveals membrane destabilization and reactive oxygen species production. The oxidative stress effect was not produced by Ag^+ from $AgNO_3$ but only by the AgNP and could be related to the effect of Ag bulk forms or to AgNP oxidation and dissolution with the production of both Ag^+ and free radicals in proximity of the cell surface (Batchelor-mcauley et al., 2014; Oukarroum et al., 2012; Zewde et al., 2016). The free Ag^+ released in proximity of the cell wall could be complexed by citrate (available at a concentration 10 times higher than Ag in the AgNP solution) or by the abundant Cl^- in ASW. This would create soluble $AgCl_x^{(1-x)}$ complexes (Batchelor-mcauley et al., 2014; Levard et al., 2012) able to pass through the cell wall mat, reaching the membrane where Ag^+ can be internalized. These hypotheses are supported by the fact that no particles were observed inside the cell wall in the TEM micrographs at larger magnifications; moreover, a barrier effect due to exopolimeric substances produced by algae was reported hindering the AgNP internalization (Zheng et al., 2019).

On the large scale the behavior and fate of AgNP in the aquatic environments are the result of the complex interactions between the chemistry of nanoparticles and the physical, chemical and ecological properties of the receiving environments (Le Ouay and Stellacci, 2015). Some investigations reported the role of different kinds of natural organic ligands, such as the adsorption of dissolved organic substances to the surface of AgNP, changing their superficial properties (e.g. redox potential, charge, coating) and Ag^+ release (El Badawy et al., 2010; Levard et al., 2012; Liu and Hurt, 2010; Xiu et al., 2011). The role of dissolved organic carbon/matter was reported to alter dissolution, colloidal stability and toxicity of citrate-capped AgNP (Pokhrel et al., 2013). Conversely, few data are available on the effects of natural particulate matter, both organic (TEP) and inorganic (clay).

In the Lagoon of Venice, average values of particulate suspended matter of 40 ppm were reported with peaks of 115 ppm (Sfriso and Sfriso, 2017). Moreover, the northern Adriatic Sea is also characterized by high TEP concentrations, ranging from 2 to 44 ppm with peak values over 400 ppm (Engel, 2001; Passow, 2002).

We observed a distinct effect, of both TEP and clay (with average concentrations of the Venice Lagoon) on the detoxification of AgNP up to 0.5 ppm. A similar detoxification of AgNP due to external exopolymeric substances was reported (Miao et al., 2009; Zheng et al., 2019) and anionic polysaccharides such as k-carrageenan and xanthan gum (Elsupikhe et al., 2015; Xu et al., 2014) were used as capping agents for AgNP due to their affinity for the particles. Concurrently, Dong et al. (2009) used the clay for AgNP immobilization during synthesis ascertaining the clay binding action on the particles. Although both clay and TEP greatly reduced AgNP toxicity as PP only TEP reduced the oxidative stress. This could be explained by the antioxidant properties of the sulphated polysaccharides (Francavilla et al., 2013; Souza et al., 2012), that could reduce the reactive oxygen species produced by AgNP dissolution.

Some hypothesis and models on AgNP release in the environment were proposed and different amounts of AgNP into the aquatic ecosystems were predicted and measured in the part per trillion range. Gottschalk et al. (2009) model predicted silver concentration ranges for surface waters and sewage treatment plant effluents of 0.07–0.11 ppt and 21–42 ppt, respectively. Moreover, predicted environmental concentrations of AgNP in water compartment were estimated in the range of 30 to 80 ppt (Mueller and Nowack, 2008). Similar values, in the ppt range, were measured by Liang-Saw et al. (2002) in Colorado surface waters and sewage effluents (1.8–537 ppt). Eventually, for a risk estimation Blaser et al. (2008) took into account silver concentration values in the range from 40 to 320 ppt from sewage effluent. The estimated concentrations for freshwater in the parts per trillion range were lower than the values hazardous for seaweeds and aquatic organisms. The NP released into the environment are expected to display brief residence times in the water column and are expected to aggregate and accumulate in sediments and benthic communities, especially when salinity increases near the sea decreasing the stabilizing effect of the dissolved organic matter on AgNP (Burgess et al., 2014). Indeed, Chinnapongse et al. (2011) reported agglomeration of 80% of the particles within 1 h and the complete disappearance after 10 h in accordance with our results. However, the results we provided (Supplementary material) also highlighted the transformation of AgNP to AgCl in ASW eventually dissolving in 24 h for ca. 60–70% probably leading to the formation of the highly mobile forms $AgCl_x^{(1-x)}$ complexes. In this context, estuaries and lagoons with high water residence time (> 15–30 days) could accumulate silver that may generate toxic effects in the long term.

It is unlikely that concentrations lower than 0.5 ppm could pose a threat to primary producers in TWS, where the combined effect of both TEP and turbidity (usually high in these environments) should neutralize the adverse effects on primary producers. The few data found in literature on silver environmental contamination report very low concentrations (in the ppt range) in the water column, under the values found to be toxic for *U. rigida,* but the silver bioconcentration factors can range from 10^4 to slightly beyond 10^5 (Toni, 1999). Moreover, silver concentration values from 3.5 to 10.3 $\mu g\, g^{-1}$ dw were reported for sediments and mussels in the Venice Lagoon (Giusti and Zhang, 2002), warning about the presence of this element, not so negligible. Eventually data on long-term exposition to low levels of silver and AgNP is still missing and the sequestration of NP by TEP and clays could extend the lifespan of NP into the environment.

5. Conclusions

The obtained results from different lines of evidence converge in describing the acute toxicity of AgNP and $AgNO_3$ in *U. rigida.* Silver bioaccumulation, altered chloroplast, inhibition of primary production and lipid peroxidation were assessed in the seaweed after 24 h of exposure. The natural organic and inorganic ligands present in transitional environments were also found to detoxify the AgNP at concentrations up to 0.5 ppm. AgNP concentrations under this value do not seem to pose a threat for primary producers in transitional waters. However, further long-term investigations are recommended to examine the response of seaweeds at very low AgNP concentrations (ppb/ppt) that can be bioaccumulated in confined water bodies with high water residence time.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2019.104942.

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