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Fate of silver nanoparticles in wastewater and immunotoxic effects on rainbow trout

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ABSTRACT

Silver nanoparticles (AgNPs) are currently used in technology, medicine and consumer products, even though the fate and the ecotoxicological risks on aquatic organisms of these new materials are not well known. The purpose of this study was to investigate the fate, bioavailability of AgNPs and their effects on fish in presence of municipal effluents. Juvenile rainbow trout were exposed for 96 h to 40 μg/L of AgNPs or 4 μg/L of dissolved silver ($AgNO_3$) in diluted (10%) municipal wastewater. Silver (Ag) concentrations were measured both on water samples and fish tissues (liver and gills). Toxicity was investigated by following immunological parameters in the pronephros (viability, phagocytosis) and biomarkers in liver and gills (cyclooxygenase activity, lipid peroxidation, glutathione-S-transferase, metallothioneins, DNA strand breaks and labile zinc). Results indicated that AgNPs appeared as small non-charged aggregates in wastewaters (11.7 ± 1.4 nm). In gills, the exposure to AgNPs induced morphological modifications without visible nanoparticle bioaccumulation. Dissolved Ag^+ was bioavailable in diluted effluent and induced oxidative stress (lipid peroxidation), labile zinc and a marginal decrease in superoxide dismutase in fish gills. Ag^+ also increased significantly metallothionein levels and inhibited the DNA repair activity in the liver. Finally, the two silver forms were found in liver and induced immunosuppression and inflammation (increase in cyclooxygenase activity). This study demonstrated that both forms of Ag produced harmful effects and AgNPs in wastewater were bioavailable to fish despite of their formation of aggregates.

1. Introduction

Silver nanoparticles (AgNPs) are widely used in technology, medicine and consumer products. They are manufactured for their antimicrobial properties (Dos Santos et al., 2014). Their own properties, with a high surface area ratio, could modify Ag toxicity and class them in a specific contaminant category (NRC, 2012). In a 2010 survey, 1000 consumer products included AgNPs (Massarsky et al., 2014a; Project on Emerging Nanotechnologies, 2013). Although there is a customary use of silver (Ag), the increasing use of AgNPs raises new safety concerns. AgNPs can enter the aquatic environment through washing of treated clothes and the release from products, such as clothes, cosmetics, toothpaste, soaps, and food containers (Benn and Westerhoff, 2008; Ribeiro et al., 2014). Because of their increasing commercial use, legitimate concerns about the release and impacts of AgNPs to aquatic ecosystems are warranted.

Few studies on the behavior of AgNPs in wastewater treatment plants (WWTPs) are found in the literature. Most of AgNPs based consumer products inputs occurs via the release in wastewaters which reaches sewage treated plants (Kaegi et al., 2011). In Germany, intentional or accidental release of nanoscale silver particles Ag-NPs were measured in treated effluent (12 ng/L) for an estimated AgNP load of 4.4 g/day (Siripattanakul-Ratpukdi and Fürhacker, 2014; Li et al., 2013). According to Blaser et al. (2008) Ag residues from Europe, Asia and North America reached 190–410 t/year and between 11.5 to 31.7% of those residues passed through WWTPs and were found in receiving natural water. A significant proportion (about 10%) of the AgNPs which enter in the WWTPs passed through the treatment process and are released by their effluent (Gottschalk et al., 2009; Limbach et al., 2008).

In natural water, AgNPs could be transformed through different processes, such as oxidation, reduction, dissolution, sulfidation, aggregation and adsorption (Lowry et al., 2012). These transformations have an influence on the persistence, mobility and bioavailability of the AgNPs (Lowry et al., 2012). In natural water, the natural organic matter (NOM) could stabilize the nanoparticles (NPs) thus maintaining them in non-aggregated state (Lowry et al., 2012; Cumberland and Lead, 2009). Monovalent Ag^+ could

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bind to organic polyanions, such as fluvic and humic acids (King and Jarvie, 2012). Moreover, the humic substances could also provide long-term reservoir for NPs and maintain them in the column or surface water for over large distance (King and Jarvie, 2012). In wasterwaters, AgNPs are expected to be sulfidized and form Ag_2S in non-aerated system,while in aerated systems they would be unstable with a degradation half-life of days to weeks (Kaegi et al., 2011; Lowry et al., 2012). Kaegi et al. (2011) also found that 10% of metallic Ag (Ag^0) from AgNPs was found in the effluent.

AgNPs were shown to caused cytotoxicity, oxidative stress, damage (lipid peroxidation), reduced mitochondrial activity, and genotoxicity (Gagné et al., 2012; Scown et al., 2010; Braydich-Stolle et al., 2005; Arora et al., 2008; Fabrega et al., 2011; Bruneau et al., 2015). AgNPs can also bind onto cell membranes and affect permeablity and respiratory function of the cells by decreasing the Na^+/K^+ ATPase activity (Morones et al., 2005). The toxicity of AgNPs was partly associated to the release of ionic Ag and NPs induced steric hindrance effects that could lead to protein degradation, DNA damage and lipid peroxidation (Gagné et al., 2013).

The first line of the fishes immune system is the mucus layer found on the surface of the gills, skin and intestines (Jovanović and Palić, 2012). It acts as a barrier shielding the fish from microbial invasion by using lysozyme, lectins, immunoglobulin M (IgM) and other proteolytic enzymes (Bols et al., 2001). This layer can also trap NPs, modify their surface charge properties and decrease their penetration rate (Handy et al., 2008; Jovanović and Palić, 2012). The second line of the immune system of the fish is the pronephros. It is involved in hematopoiesistic i.e., involved in maturation of neutrophils and macrophages and serves as a neutrophil depot (Zapata, 1979). NPs could be internalized in the endolysosomal compartment and engulfed though different processes according to their size. Small NPs (up to 100 nm) are preferentially internalized though calveolae-mediated and clathrin-mediated endocytosis, whereas NPs with a size ≥500 nm are engulfed though receptor mediated phagocytosis or macropinocytosis (Dobrovolskaia and McNeil, 2007; Bartneck et al., 2010; Jovanović and Palić, 2012). Macropinocytosis could be the main uptake route of aggregated NPs (Bartneck et al., 2010). Moreover, the surface charge promotes the engulfment of NPs. Walker and Parsons (2012) demonstrated that NPs with either positive or negative surface charge but less so for neutral NPs activated the phagocytosis activity (Dobrovolskaia et al., 2008; Zahr et al., 2006).

When cells are exposed to AgNPs, reactive oxygen species (ROS) are produced. This process is partly caused by the released ionic Ag form and inflammation process. The ROS such as superoxide anions, hydrogen peroxide and the hydroxyl radical cause oxidative damage, cytotoxicity and DNA damage (Griffitt et al., 2013). During the oxidative burst, the organism's defense mechanism and free metal ligands such as thiols are increased. Also, thiols binding and metallothioneins that are cysteine rich proteins, have been recognised to increase the sequestration of Ag^+ from AgNPs in the liver of trout exposed to surface waters (Gagné et al., 2012).

The purpose of this study was to determine the fate and effects of AgNPs and Ag^+ (as silver nitrate—$AgNO_3$) on rainbow trout, as animal model, after in vivo exposure in diluted wastewater. The Ag concentrations were determined in water and in fish tissues to assess the bioavailability of each silver form. Immunological parameters and biomarkers in liver and gills were performed to evaluate the toxicity of the two Ag forms after an environmental exposure. $AgNO_3$ was chosen to compare the effects of the dissolved form with AgNPs. Dissolved Ag is lethal for aquatic organisms at a concentration of 20 μg/L (Griffitt et al., 2008). A sublethal concentration of dissolved Ag^+ was chosen in this study corresponding approximatively to the labile Ag from the AgNP.

Table 1
Physical characteristics and concentration of total organic carbon (TOC) and dissolved organic carbon (DOC) in the control water utilized for experiments exposure test. The samples were non-exposed to silver.

Control water	TOC (mg/L)	DOC (mg/L)	pH	Conductivity (μS/cm)
Tap water	2.33	2.03	7.45	290
Wastewater	30.3	21.4	7.7	683
10% effluent, T = 0H	7.7	3.44	7.55	273
10% effluent, T = 96H	10.3	8.4	8.45	340

2. Materials and methods

2.1. Silver

A stock solution of PELCO® NanoXact™ AgNPs from Ted Pella Inc (California, USA) was used. According to the manufacturer's specifications, the AgNPs have a mean size of 22 ± 2 nm, are supplied in 2 mM citrate buffer, pH 7.4 and have a zeta potential of −50 mV. For the exposure experiments with AgNPs, a concentration of 40 μg/L total Ag was prepared in 10% of the municipal effluent physically and chemically treated. Silver nitrate ($AgNO_3$) (Sigma–Aldrich, ON, Canada) was dispersed directly in the fish tank at a concentration of 4 μg/L.

2.2. Fish

Juvenile female rainbow trout (*Oncorhynchus mykiss*) (mean head to fork length 121.3 ± 5.5 mm; mean weight 24.3 ± 2.8 g) were provided by a local hatchery (Pisciculture des Arpents-Vert, Ste-Edwidge, Qc), maintained in 1000-L tanks at 15 °C, fed daily with a commercial trout chow during 2 weeks and held under a natural photoperiod (12 h light:12 h dark) prior to exposure experiments.

2.3. Exposure to diluted municipal effluent and silver

A composite wastewater sample was obtained in November 2013 form the Montreal WWTP. The exposure wastewater was diluted 1:10 (v:v) in tap water originating from the St. Lawrence River that was dechlorinated and UV treated (Table 1). Total organic carbon (TOC) and dissolved organic carbon (DOC) concentrations, as well as pH and conductivity were measured at the beginning and the end of the exposure (Table 1). Metal contamination of the Montreal effluent was previously documented by Gagnon et al. (2006) and copper and zinc were the most accumulated metals in caged mussels exposed to the wastewater dispersion plume.

Eight trout were placed in each 20 L containers lined with polyethylene bags and exposed to 10% wastewater with AgNPs (40 μg/L), dissolved silver ($AgNO_3$) (4 μg/L) or without contaminant added. Controls are 10% of effluent water diluted in tap water. The fish were monitored daily for any signs of distress or changes in swimming and breathing behaviour. Dissolved oxygen was maintained above 80%, pH between 7.5–8.6, and temperature at 15 °C during the exposure. The water was not renewed during the experiment. After a 96 h exposure period, the fish were euthanized with 0.1% of MS-222 (Sigma–Aldrich, ON, Canada) using the Canadian Council on Animal Care methods. Pronephros was kept for immune parameter measures. Liver and gills were immediately collected, weighed and stored at −80 °C for subsequent chemical and biochemical analyses.

2.4. AgNP characterization

2.4.1. Transmission electron microscopy (TEM) and electron-dispersive X-ray analysis (EDS)

A sample of AgNPs in 10% wastewater was collected after 96 h and kept at 4 °C. The samples as well as the stock solution were