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Effects of silver adsorbed on fumed silica, silver phosphate glass, bentonite organomodified with silver and titanium dioxide in aquatic indicator organisms

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ABSTRACT

In order to reduce the level of transmission of diseases caused by bacteria and fungi, the development of antimicrobial additives for use in personal care, hygiene products, clothing and others has increased. Many of these additives are based on metals such as silver and titanium. The disposal of these products in the environment has raised concerns pertaining to their potential harmfulness for beneficial organisms. The objective of this study was to evaluate the influence of the shape, surface chemistry, size and carrier of three additives containing silver and one with titanium dioxide (TiO_2) on microcrustacean survival. *Daphnia magna* was used as a bioindicator for acute exposure test in suspensions from 0.0001 to 10,000 ppm. *Ceriodaphnia dubia* was used for chronic test in TiO_2 suspensions from 0.001 to 100 ppm. *D. magna* populations presented high susceptibility to all silver based additives, with 100% mortality after 24 hr of exposure. A different result was found in the acute experiments containing TiO_2 suspensions, with mortality rates only after 48 hr of incubation. Even on acute and chronic tests, TiO_2 did not reach a linear concentration-response *versus* mortality, with 1 ppm being more toxic than 10,000 ppm on acute test and 0.001 more toxic than 0.01 ppm on chronic assay. Silver based material toxicity was attributed to silver itself, and had no relation to either form (nano or ion) or carrier (silica, phosphate glass or bentonite). TiO_2 demonstrated to have a low acute toxicity against *D. magna*.

Introduction

Due to the known antimicrobial characteristics of silver (Ag) and titanium dioxide (TiO_2), the use of these elements is increasing in many industrial fields and consumer use materials (*e.g.*, surface coating, electronics, clothes, cosmetics, shoes, keyboards, toothpaste, sunscreen) (Dankovic et al., 2007; Kalbassi et al., 2011). However, while these elements may provide a manner to prevent infections by interfering in pathogenic microorganism proliferation, they may potentially impact the environment when released and transported into air, water, and soil ecosystems during their product life-cycle (Ribeiro et al., 2014). In this respect, an

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assessment of metal particles regarding their effects on human health and environment is necessary (Carlson et al., 2008).

In the aquatic environment, organisms can come into contact with substances that cause chromosomal aberrations, leading to impairment of cell division and tumor formation (Buzea et al., 2007). The researches have focused on the toxicity of Ag and TiO_2 particles to organisms, including algae (Navarro et al., 2008; Hartmann et al., 2010), bacteria (Binaeian et al., 2012; Heinlaan et al., 2008), invertebrates such as cladocerans (Heinlaan et al., 2008; Sakamoto et al., 2015) and vertebrates such as fish (Asharani et al., 2008; Zhu et al., 2008; Wehmas et al., 2015). *Daphnia magna* (*D. magna*) is recognized as a key organism in freshwater ecosystems by being important phytoplankton consumer, while they are preferentially preyed upon by fish (Persson et al., 2007). Also, because of their filter feeding mechanisms (Lovern and Klaper, 2006; Griffitt et al., 2008), sensitivity to environmental pollution, small body size and short life spans, *D. magna* is considered the most sensitive organism in the food web (Clément et al., 2013; Bondarenko et al., 2013), being used to assess the health of environments in ecotoxicological investigations (Lam and Wang, 2006; Flohr et al., 2012; Newton et al., 2013).

A wide range of silver modifications have been employed to improve silver antimicrobial capacity, like carbon nanotubes with silver (Rangari et al., 2010), silver/silica nanocomposites (Egger et al., 2009), zeolites doped with silver (Ferreira et al., 2012), and silver nanoparticles (Kong and Jang, 2008). The mechanisms reported about metal particles' toxicity have been variable depending on the carrier, surface coatings, size and system (Gatoo et al., 2014). Since nanoparticles (Nps) can interact in unpredicted ways with biological systems, a better understanding of the behavior of metal particles with different characteristics and interactions with key aquatic species is required (Albanese et al., 2012). Ag can be ranked after Hg (mercury) as having a high potential cumulative into daphnid bodies (Lam and Wang, 2006). Also, the ionic form Ag^+ is described as being the most harmful to aquatic organisms (Benn and Westerhoff, 2008). Silver nanoparticles (NpAg) are reported as more highly reactive and toxic (Allen et al., 2010). On the other hand, TiO_2 microparticles have been reported as being inert, non-toxic and non-migratory (Rosa, 2013), with a lethal concentration (LC_{50}) higher than 100 mg/L for nanoform and non-nanoform TiO_2 (Wiench et al., 2009). Though, studies with nano TiO_2 have described EC_{50}–48 hr around 8 mg/L in acute test (Dalai et al., 2013) and LC_{50}–48 hr of 7.75 mg/L (Das et al., 2013).

Reckoning with this fact, this study aims to investigate the toxicity effects of nanosilver, silver ions and titanium dioxide (TiO_2) particles toward aquatic crustaceans *Daphnia magna* (*D. magna*) and *Ceriodaphnia dubia* (*C. dubia*). The silver additives tested have three different forms: silver nanoparticles on fumed silica (NpAg_silica), silver phosphate glass (Ag^+_phosphate) and bentonite organomodified with silver (Ag^+_bentonite). The goal of comparing Ag and TiO_2 was to reveal potential differences in toxic mechanisms between these elements. Additionally, *C. dubia* reproduction was investigated after the exposition to TiO_2.

1. Material and methods

1.1. Characterization of the particles

Four additives were tested; nanosilver adsorbed on fumed silica ("NpAg_silica"), silver ions supported in phosphate glass ("Ag^+_phosphate"), bentonite organomodified with silver (Ag^+_bentonite") and a commercial rutile titanium dioxide ("TiO_2").

Determination of mineral composition was held by qualitative analysis by X-ray diffraction, in a PanAnalytical X'pert PRO (PanAnalytical, The Netherlands) and software X'PertHighScore (PanAnalytical, The Netherlands). Particle size distribution was determined by laser diffraction, the equipment used was a CILAS 1180 (Cilas, Orleans, France) particle size analyzer, with scanning range between 0.04 and 2500 μm. NpAg_silica, Ag^+_phosphate and TiO_2 were predispersed in deionized water using ultrasound (60 sec), Ag^+_bentonite was predispersed in isopropyl alcohol.

For transmission electron microscopy (TEM) G2 T20 (Tecnai), samples were dispersed in ethanol by ultrasound for 30 min. The samples were prepared by mounting a drop of the ethanol suspension containing the particles on a 300 mesh copper grid carbon film. Image acquisition was through acceleration voltage of 200 kV. The average particle diameter and scale distribution were calculated using ImageJ version 1.40 g software.

The specific surface area (SSA) was measured by nitrogen adsorption using BET method. Measurements were performed by a Quantachrome Nova 1000 (Quantachrome Instruments, Boynton Beach, FL, USA) and surface area analyzer. Samples were dried in an oven at 110°C for 24 hr and then vacuum at 200°C for 3 hr.

The zeta potential (ZP) measurements were carried out using a zeta scaler Zetasizer NanoZ (Malvern Instruments, Malvern, UK). Each sample was dispersed in deionized water to obtain suspensions at 1%. Suspension pH was adjusted to 3, 5, 7, 9 and 11 using NaOH 0.1 mol/L or HCl 0.1 mol/L.

1.2. Daphnia magna preparation to the acute toxicity assays

Acute toxicity tests were conducted according to ABNT NBR 12713:2009. The acute 24 hr and 48 hr toxicity tests were performed using neonate (2 hr and 26 hr old) *D. magna* (Landesamt Für Wasser und Abfall (LWA), Nordrhein-Westfalen, Düsseldorf, Germany). The animals were derived from the laboratory stock culture at the test facility, where they were reared in artificial fully defined M4 medium at 20°C. Culture medium was renewed twice weekly and the daphnids were fed with a suspension of the unicellular green algae *Desmodesmus subspicatus* and fish-yeast compost.

1.3. Preparation of solutions

For stock suspension preparation, appropriate amounts of Ag and TiO_2 particles were suspended in sterilized double distilled water and dispersed by shaking for 30 min (1500 r/min at 25°C) in a magnetic stirrer-SP-160 (Advantec MFS, Inc., Dublin, CA, USA). Working suspensions were made through serial dilution

followed by vigorous vortexing when required. During testing the suspensions were not shaken in order to avoid physically damaging the organisms. In the TiO_2 suspensions, further sedimentation of particles was visually observed.

1.4. Exposure

The test suspensions were prepared immediately before use by diluting the particle powder in eight different concentrations (0.001; 0.01; 0.1; 1; 10; 100; 1000; 10,000 ppm). Four replicates per concentration were used. A control test, without the added metal, was included for each treatment. The neonates were exposed to each particle/concentration in four replicates with five daphnids per replicate. A total of 32 acute tests were run. Tests were performed according to ABNT NBR 12713:2009 in a photoperiod of 16 hr under light (PHILIPS TLD 100–1000 lx 16w/840-NG) and 8 hr in the dark at a temperature of 20 ± 2°C.

D. magna was not fed during the testing period. Mortality status for the individuals in each container was checked after 24 hr and 48 hr of exposure. After an incubation period of 48 hr, immobilization of the daphnids was determined. The assay endpoint was death/immobilization. Oxygen content, temperature and pH of the control, first and last test dilutions were measured.

1.5. Ceriodaphnia dubia preparation to the chronic assay

Chronic toxicity test with the TiO_2 particle was conducted based on the ABNT NBR 13373:2011. The chronic seven-day test was performed using neonates of *C. dubia* (6–24 hr old at the start of the test) (Aplysia Soluções Ambientais LTDA, Vitória, Espírito Santo, Brazil), individually exposed per concentration. The animals were fed with the green algae *D. subspicatus* and a compost of fish and yeast, reared in artificial medium - Standard Methods for the Examination of Water and Wastewater.

1.6. Exposure

The test suspensions were prepared immediately before use by diluting appropriate amounts of the particle powder in six different concentrations (0.001; 0.01; 0.1; 1; 10; 100 ppm). A control test, without the added metal, was included for each treatment. The neonates were exposed to each particle/concentration in ten replicates with one daphnids per replicate. In total, 60 chronic tests were run. Chronic seven days test was performed in a photoperiod of 16 hr under light (PHILIPS TLD 100–1000 lx 16w/840-NG) and 8 hr in the dark at a temperature of 25 ± 2°C. Culture medium was renewed in an interval of 3 day. After the incubation period of 7 days, survival rate and the number of neonates produced by each female (reproduction) was recorded. Oxygen content and pH of the control, first and last test dilutions were measured.

1.7. Data analysis

Effective concentrations (EC) that cause an effect in 0 or 50% of test organisms (EC_0, EC_{50}) and no-observed-effect concentration (NOEC) were calculated with the help of the results obtained from the 7-day chronic toxicity tests.

Treatments were conducted with four (acute) and ten (chronic) replicates, and the results were presented as mean ± SD (standard deviation). All calculations were done with the aid of Microsoft Excel 2007.

2. Results

2.1. Particles characterization

Fig. 1 summarizes the characteristics of the different particles tested in this study, characterized by X-ray diffraction, transmission electronic micrograph, laser diffraction, specific surface area and zeta potential analyses. Diffraction X-ray analyses detected the presence of SiO_2 and Ag in the NpAg_silica (Fig. 1a) sample. The Ag^+ phosphate (Fig. 1b) sample featured an amorphous structure, not being possible to characterize its composition through this method. Ag^+_bentonite (Fig. 1c) sample was compounded by Montmorillonite-22A and cristobalite. TiO_2 (Fig. 1d) sample was characterized as pure rutile.

Fig. 1a indicates nanoparticles of silica (20 nm) and silver (10 nm). Composition was confirmed by energy dispersive X-ray (EDS) assay (result not shown). Laser diffraction assay determined an average size of 12.97 μm in the NpAg_silica sample. The size difference determined on TEM images and laser diffraction shows that particles have a high tendency to agglomeration. The SSA values found suggest that nanoparticles of silver and silica are irregular, which provides a high surface contact (Fig. 1a). In Fig. 1b is possible to see that the Ag^+_phosphate sample presents a planar form. The planar form found in Ag^+_phosphate and its non-crystalline shape characterized in the X-ray diffraction can be related to the preferences of silver ions for a tetrahedral molecular configuration, and creates a two-dimensional sheet composition including dimeric units (Suenaga et al., 2003). In TEM image the particles are 1 μm, similar to that determined by granulometric assay. The SSA value of Ag^+_phosphate was 6.16 m^2/g.

Ag^+_bentonite sample, shown in Fig. 1c, presents a typical platelet form found in montmorillonite clay (Kamena, 2010). The same size difference occurred with this sample, according to the laser diffraction assay, with an average size of 7.3 μm being observed, while in TEM the 1 μm size showed an agglomeration tendency. The SSA of 36.73 m^2/g was in agreement with the literature (20–90 m^2/g, Praus et al., 2010; Tian et al., 2014). The measure of SSA of montmorilonite by BET method was reported as non-effective, since nitrogen does not access the interlayer space (Praus et al., 2013). As seen in Fig. 1d, the TiO_2 sample features a spherical form, average size of 90 nm, lower than that determined by laser diffraction assay (0.29 μm), showing a low tendency to agglomeration.

The knowledge of the surface characteristic of the nanoparticles (NPs) is a pre-requisite to predict its stability in colloidal systems, and consequently its toxicity (Prathna et al., 2011). In that way, zeta potential (ZP) values, gives an indication about the NPs stability (Barrena et al., 2009). The amount of ZP provides a clue of potential stability of the particle, since particles with ZP below 30 mV or above –30 mV are deemed with high potential to form aggregates (Shieh et al., 2012; Tavares et al., 2014). ZP value from particles is not absolute and is dependent on the medium in which the

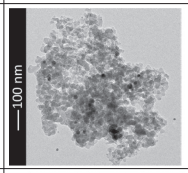
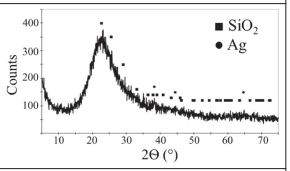
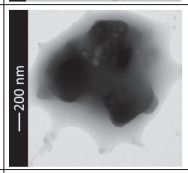
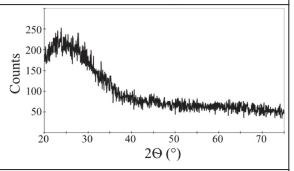
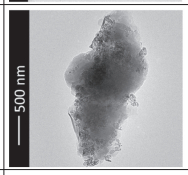
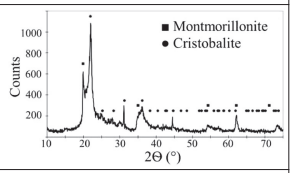
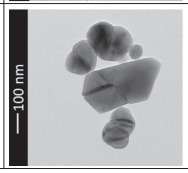
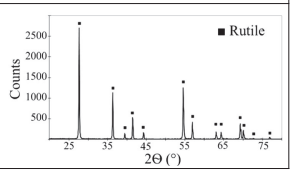
	Micrographs obtained by TEM	Average diameter by TEM, µm	Diameter by laser diffraction, µm		SSA, $m^2 g^{-1}$	Zeta potential at pH 5, mV	X-Ray diffraction graphics
a		0.02	D_{10}	4.70	293.90	-6.48	
			D_{50}	9.20			
			D_{90}	28.99			
			Average	12.97			
b		1.0	D_{10}	0.86	6.16	6.82	
			D_{50}	1.50			
			D_{90}	2.49			
			Average	1.61			
c		overlapping sheets	D_{10}	2.08	36.73	3.77	
			D_{50}	6.32			
			D_{90}	13.92			
			Average	7.30			
d		0.09	D_{10}	0.08	12.16	0.87	
			D_{50}	0.25			
			D_{90}	0.54			
			Average	0.29			

Fig. 1 – Micrographs obtained by TEM, diameter, specific surface area (SSA) and zeta potential of the additives: (a) NpAg_silica, (b) Ag^+_phosphate, (c) Ag^+_bentonite and (d) TiO_2. TEM: transmission electron microscopy; TiO_2: titanium dioxide.

particles are embedded, for example the substances present in the medium (Barrena et al., 2009; Orts-Gil et al., 2011; Giovanni et al., 2014) and its pH (Prathna et al., 2011). To verify sample stability, the zeta potential of particles in different pH ranges was investigated and shown in Fig. 2.

Except for the TiO_2 sample, the ZP value was between −30 and +30 mV in a wide range of pH, and was considered unstable with high tendency to agglomerate, in agreement with discrepancies of size determined by laser diffraction and TEM images (Fig. 1).

2.2. Abiotic variables

According to Ratte (1999), pH, organic carbon, cation exchange quality and the levels of silt and clay may be responsible for transforming the toxicity behavior of a determined substance. During all acute toxicity tests, the pH value remained within the range of 5.0 and did not vary by more than 6.2 units in any given test. The mean oxygen content of the test dispersions in acute toxicity tests was 5.96. During the chronic toxicity study the oxygen and pH content were 4.42 and 7.98, respectively (Table 1).

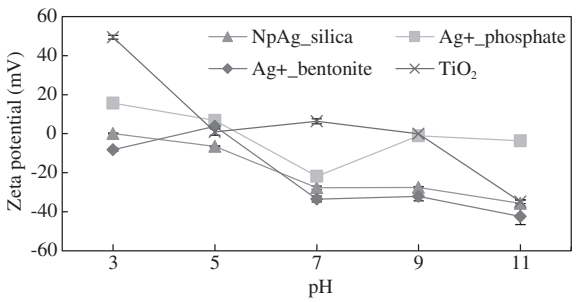


Fig. 2 – Zeta potential of NpAg_silica, Ag^+_phosphate, and Ag^+_bentonite and (–x-) TiO_2.

2.3. Acute toxicity

The results from the acute toxicity tests performed with NpAg_silica, Ag^+_phosphate and Ag^+_bentonite are summarized in Table 2. TiO_2 results are shown in Fig. 3. *D. magna* was found to be very sensitive to Ag based additives (NpAg_silica, Ag^+_phosphate, Ag^+_bentonite — 24 hr, 100% mortality), even at the low concentration of 0.0001 mg/L.

In all acute toxicity tests, the survival in the controls was 100%. This confirms that the validity criteria were fulfilled.

In the acute assays from TiO_2 sample, 1 ppm caused the high mortality percentage (75%) and 0.0001 ppm caused the

Table 1 – Mean values of the aqueous suspensions parameters.

Aqueous suspensions	24–48 hr *Daphnia magna*		7-day *Ceriodaphnia dubia*	
	Dissolved oxygen, mg/L	pH	Dissolved oxygen, mg/L	pH
NpAg_silica	5.76	5.00	–	–
Ag^+_phosphate	6.18	5.50	–	–
Ag^+_bentonite	5.71	6.00	–	–
TiO_2	6.20	5.00	4.42	7.98

Table 2 – Evaluation of metal aqueous suspensions (NpAg_silica, Ag^+_phosphate and Ag^+_bentonite) to *Daphnia magna*.

Aqueous suspensions		Treatment (ppm)								
		Control	0.0001	0.001	0.01	1	10	100	1000	10,000
NpAg_silica	Total individuals dead	0	20	20	20	20	20	20	20	20
	Mortality (%) after 24 hr	0	100	100	100	100	100	100	100	100
	Mortality (%) after 48 hr	–	–	–	–	–	–	–	–	–
Ag^+_phosphate	Total individuals dead	0	20	20	20	20	20	20	20	20
	Mortality (%) after 24 hr	0	100	100	100	100	100	100	100	100
	Mortality (%) after 48 hr	–	–	–	–	–	–	–	–	–
Ag^+_bentonite	Total individuals dead	0	20	20	20	20	20	20	20	20
	Mortality (%) after 24 hr	0	100	100	100	100	100	100	100	100
	Mortality (%) after 48 hr	–	–	–	–	–	–	–	–	–

low mortality percentage (0%) (Fig. 3). Calculation of EC_{50}–48 hr for TiO_2 was not possible due the non-homogeneity of the results through the Trimmed Spearman–Karber method.

2.4. Chronic toxicity

The chronic toxicity test (*C. dubia* reproduction) with TiO_2 showed the biggest neonates number with 0.01 ppm. Reproduction numbers decreased as the TiO_2 ratio increased (Fig. 4).

The chronic toxicity test (*C. dubia* reproduction) with TiO_2 showed the biggest neonates number with 0.01 ppm. Starting from 0.1 ppm the reproduction numbers decreased as the TiO_2 ratio increased (Fig. 4).

The highest concentration of TiO_2 that caused no observed deleterious effect (NOEC) in the *C. dubia* reproduction after 7-day chronic toxicity tests was 0.01 ppm. The effective concentration of TiO_2 that produces an effect in 0% (EC_0) and 50% (EC_{50}) of the tested organisms were 0.10 ppm and 0.063 ppm, respectively.

3. Discussion

In this study we assessed issues pertaining to the physic-chemical properties of four samples of metal particles and their relation to daphnids toxicity. Toxicity was observed in all Ag based acute assays (24 hr). On the other hand, TiO_2 was less toxic, leading to daphnia death only after 48 hr of the assay.

Below are some of the characteristics of the particles addressed in toxicological research and that can explain the differences in toxicity observed in the present study.

3.1. Size dependent toxicity

The first point of discussion about particle toxicity is their size. These concerns are related to the fact that small sized particles have the ability to traverse biological membranes (Oberdorster et al., 2004; Velzeboer et al., 2008). Moreover, the nanodesign provides physical and chemical characteristics that differ from the bulk materials of same chemical structure (Schwirn et al., 2014). In this way, nanoparticles may lead to the death of aquatic organisms due to accumulation of nanoparticles in the gut and disturbance of food intake (Zhu et al., 2010; Ates et al., 2013). In the case of daphnids, the activity of thoracic segments promotes a current of water, whereas particles (<50 μm in diameter) are directed to the mouth without any selective process (Hund-Rinke and Simon, 2006; Zhu et al., 2010), which is compounded by the difficulty in excreting the particulates (Petersen et al., 2009). In a study performed by Zhao and Wang (2012) more than 60% of NpAg was present in the gut of daphnids, indicating that ingestion was the prevalent route of consumption. Petersen et al. (2009) demonstrated that the carbon nanotubes captured by the daphnids remain mostly in the gut and were not incorporated by cell tissue. Once that nanoparticle (Np) are taken up by the cells, they may cause disorder in the protein structure (Rinna et al., 2015) and DNA damage (Asharani et al., 2008) with the production of reactive oxygen species (Dalai et al., 2013; Rinna et al., 2015). Fabrega et al. (2011) reviewed the effects of silver nanoparticles on algae, invertebrate, and fish species. Those authors support the idea that concentration as low as a few mg/L of NpAg can be harmful to various aquatic species.

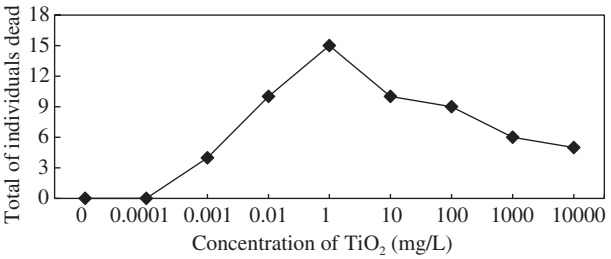


Fig. 3 – Total of dead *D. magna* after 48 hr in contact with TiO_2 suspensions.

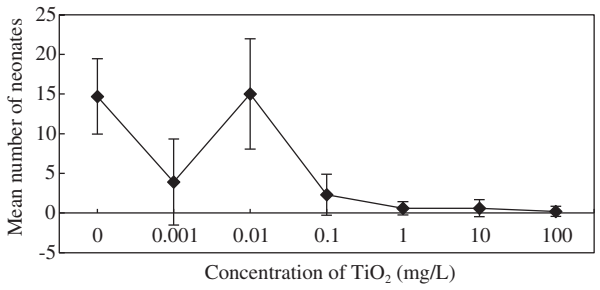


Fig. 4 – *C. dubia* neonates produced during the 7-day in contact with TiO_2 suspensions.

According to micrograph images, NpAg_silica and TiO_2 feature a nanoform, and both particles presented a different pattern of toxicity. In this way, the toxicity observed does not appear to be a response to exposure to nanoform particles; rather, it may be explained by the intrinsic metal properties (Griffitt et al., 2008).

3.2. Metal ion release and associated damage

Although the metal ion release from silver-based particles was not addressed in this study, this matter related to the metal toxicity is subject to discussion. There is no consensus as to which degree the NpAg toxicity originates from released silver ions and how much toxicity is related to NpAg *per se* (Beer et al., 2012). Nanoparticles require special attention because tiny particles have characteristics that increases metal ion release (Ivask et al., 2014). However, this release process can potentially be affected by the particles' agglomeration behavior (Allen et al., 2010). To some authors, the antimicrobial mechanism of silver loaded materials results from a long term release of the dissolved ion by oxidation of metallic silver in contact with water (Kumar and Münstedt, 2005; Allen et al., 2010). In this case, for aquatic organisms such as *D. magna*, the ion regular release is the most widely accepted mechanism to explain Ag toxicity (Bianchini and Wood, 2003; Zhao and Wang, 2013). Asharani et al. (2008) observed a higher toxicity of NpAg in the development of zebrafish (*Danio rerio*) embryos when compared to the Ag ions. The authors suggest that the penetration of nanoparticles through the skin and their interactions with tissues may have caused phenotypic defects in the treated embryos. Otherwise, Zhao and Wang (2011) observed no mortality in 48 hr when the daphnids were exposed up to 500 μg/L of NpAg, but harmful effects were detected when the substance of exposition was silver nitrate. Navarro et al. (2008) evaluated the toxic effect of NpAg and ionic Ag ($AgNO_3$) to photosynthesis process of *Chlamydomonas reinhardtii* and concluded that the inhibition on photosynthesis was mediated by the release of Ag ions. Zhang et al. (2015) also found that sunlight drive the reduction of Ag^+ to NpAg, decreasing acute toxicity of silver to *D. magna*, linking toxicity of silver to Ag^+ instead of AgNp or total silver.

3.3. Metal binding process and toxicity

Ag ions may be the highest risk factor from NpAg to aquatic organisms, and these ions have the binding ability with multiple substances, such as silver sulfide, silver chloride (Choi et al., 2009), cysteine (Navarro et al., 2008) and dissolved organic carbon (DOC) (Newton et al., 2013). These interactions between oxidized NpAg and ligants can modify the metal bioavailability and influence the toxicity of particles in natural aquatic environments (Allen et al., 2010; Newton et al., 2013). Also, the increase in organic matter (OM) concentration, can lead to a decrease in toxicity of the NpAg to daphnids (Erickson et al., 1998; Gao et al., 2012). In *D. magna* Ag and TiO_2 Np assimilation and depuration appeared to be more affected by the food concentration (Lam and Wang, 2006; Zhu et al., 2010). Ag can bind to OM from food residues or excreted by animals, and become less available to *D. magna* (Erickson et al., 1998). According to the standard protocol used it this study, *D. magna* was not fed during the acute toxic assays and no organic matter was added in the test suspensions. Also, other methods, such as the ISO 6341:2012 and OECD 202 follow the same standard. Therefore, we did not take into account this factor when evaluating the results.

In the present study, as all silver based additives with different physic-chemical properties were harmful to the microcrustaceans, the toxicity effect was linked to the silver itself, and not its form, size or characteristics of the carrier. In a broad view, the same pattern was reported by Carlson et al. (2008) who demonstrate that the toxicity of Ag may be connected to the material composition and not intrinsic to the nano size. Also, Griffitt et al. (2008) reported that Ag^+ did not explain all toxicity observed for NpAg exposure to *Danio rerio, Daphnia pulex* and *C. dubia*, and suggested that within the metals tested (silver, copper, aluminum, nickel, cobalt), some of them (copper and silver) had an intrinsic toxic characteristic.

3.4. TiO_2 properties and its toxic behavior

The acute assay with TiO_2 performed here showed heterogeneity in concentration–response, with 0.1 ppm being more toxic than 10,000 ppm. Hartmann et al. (2010) also reported a nonlinear characteristic in TiO_2 suspensions toward a freshwater green alga. Those authors observed that agglomerates in the suspension of 250 mg/L were smaller than in 2 mg/L dilution. This aggregate arrangement could be explained by the alterations in electrostatic repulsion or interactions with other materials with the dilution, which leads to the formation of larger and settleable agglomerates (Brant et al., 2008; Velzeboer et al., 2008).

During the toxicological experiments, agglomerated particles do not become readily available to the organisms contact and absorption, which lead to a less reactivity, and consequently, a less toxicity (Lovern and Klaper, 2006). Also, the agglomeration of nanoparticles reduces the surface area in which the metal oxidation reactions takes place (Allen et al., 2010). Dalai et al. (2013) reported a nonlinear result on *C. dubia* acute assay due to the agglomeration and sedimentation of TiO_2 nanoparticles at high concentration, which produced a lower bioavailability of the metal. However, the OECD (Organization for Economic Co-operation and Development), proposed finding the CL_{50} up to the concentration of 100 mg/L of TiO_2 Np, but this value was described as difficult to be dispersed (Wiench et al., 2009).

The evaluation of the effects of filtered and unfiltered but sonicated suspensions of TiO_2 was performed by Lovern and Klaper (2006). They found that the unfiltered/sonicated sample did not promote good dispersion of particles leading to less severe toxicity of TiO_2 (100–500 nm) to *D. magna* even at concentrations as high as 500 ppm. Hund-Rinke and Simon (2006) also observed the influence of sedimentation on low TiO_2 toxicity. For NpAg, the filtered suspensions were also more toxic than the unfiltered equivalent (Allen et al., 2010). In the acute tests, calculation of TiO_2 EC_{50}–48 hr was not possible due the heterogeneity of dose response values. The lower negative consequences observed in the TiO_2 acute test performed here, may be explained, at least in part, by the sedimentation of TiO_2 during the assay, which may have

provided a low availability of particles in suspension and ultimately reduced releases of metal. In the chronic assay, the concentrations that caused an effect of 0 or 50% in test organisms were EC_0 (0.10 ppm) and EC_{50} (0.063 ppm), respectively.

No appreciable effects of TiO_2 Np (100 mg/L) were observed in *Chydorus sphaericus*, *Pseudokirchneriella subcapitata* and *Vibrio fischeri* (Velzeboer et al., 2008). A study using *Danio rerio* embryos, in which TiO_2 Np was set at concentrations as high as 500 mg/L also did not show any toxic effects, but, instead provided a high hatching rate (Zhu et al., 2008). According to the previous studies, TiO_2 Np appears to exert a wide range of toxicological effects in bioindicator species. However, the non-toxic effect of TiO_2 found in short assays (48 hr and 7 days) cannot be repeated in an extended period of exposition. Wiench et al. (2009) and Zhu et al. (2010) performed a long term, acute (48 hr and 72 hr) and chronic (21 days) experiments, to analyze the *D. magna* response when exposed to nano and non-nano form of TiO_2. Those authors observed no particle size dependence and low acute toxicity with an EC_{50}–48 hr > 100 mg/L. However, in the acute test (72 hr) the EC_{50} was 1.62 mg/L and in the chronic test (21 days) the EC_{50} was 0.46 (Zhu et al., 2010) and 26.6 mg/L (Wiench et al., 2009). Ribeiro et al. (2014), when exposing *D. magna* to a 21-day test reported an EC_{50} of 0.38 μg/L and 1.0 μg/L for $AgNO_3$ and NpAg, respectively. It could be noted that the concentrations required to cause any damage to the *D. magna* health in the long term were much lower than that in the short (48 hr) acute test.

Anatase and rutile are two crystalline structures of TiO_2, with anatase being more chemically reactive (Warheit et al., 2007) and toxic (Clément et al., 2013). The photocatalyst properties of TiO_2 are related to its toxicity due the reactive oxygen species (ROS) production (Cai et al., 1992). Thus, the rutile structure is described as the less photocatalyst (Kakinoki et al., 2004) and non-cytotoxic form of TiO_2 crystal (Sayes et al., 2006; Turci et al., 2013). In the present study, TiO_2 features the rutile form that may be related to the non-acute toxicity.

In chronic toxicity experiments performed here, TiO_2 showed a reduction on neonate numbers with the increase in TiO_2 concentration (Fig. 4). Hund-Rinke and Simon (2006) found a TiO_2 NP dose-dependent toxicity to algae (*D. subspicatus*) (EC_{50} = 44 mg/L), but the effects to *D. magna* were less strong. In a toxicological study with TiO_2 nanoparticles toward *C. dubia*, Dalai et al. (2013) reported an EC_{50} on acute test of around 8.26 mg/L in experiments realized under photoperiod and 27.45 mg/L in the dark. Oxidative stress was the main factor related to the toxicity action under both dark and light situations.

In the present study, no relationship was apparent between the physical–chemical characteristics of the particles evaluated and toxicity. For example, sample Ag^+_phosphate featured a specific surface area (SSA) close to that of TiO_2 and high toxic effects were observed even at a low concentration of Ag^+_phosphate. On the other hand, NpAg_silica showed a nano scale similar to that of TiO_2, and it was even more toxic. Particles with high and low SSA values (NpAg_silica — 293.9 m^2/g) (Ag^+_phosphate — 6.16 m^2/g) respectively, produced the same toxicity results (100% mortality). Ag^+_bentonite have intermediate physical–chemical characteristics compared to the other analyzed particles, but also presented a high toxic behavior. Upon observation, the zeta potential (ZP) from TiO_2 presented the ZP value next to zero in the pH used in acute assay (0.87 mV in pH 5.0), and it was much less toxic than NpAg_silica (−6.48 mV in pH 5.0), Ag^+_phosphate (~7.0 mV in pH 5.5) and Ag^+_bentonite (~−20 mV in pH 6.0). Tavares et al. (2014) attributed the toxicity of Cr_2O_3 (15–30 nm) to *Daphnia similis* to the small particle sizes combined with the higher zeta potential value (−19.65 mV in pH 6.87). The more negative was the zeta potential values of carboxyl quantum dots, the greater the toxicity toward mice (Geys et al., 2008). Still, Arndt (2014) could not find a relation between the zeta potential and toxicity, after the study of carbon nanotubes with all charge types (positive, negative and neutral) which showed toxicity toward *D. magna*. Sha et al. (2015), following an extensive review, could not understand the pattern of toxicity from TiO_2 Np due to the lack of information about particle characteristics in the previous studies. In this study, considering that the TiO_2 ZP value was near zero and this sample presented no harmful effects against *D. magna*, assumptions can be made on the possibility of the low rate of electrons on the particle surface exerting an influence on the mode of interaction between TiO_2 and the daphnids.

The reported results indicate that undetermined processes are responsible for the toxic effects. In this regard, further studies are needed to verify the relation between the physical–chemical properties of particles and their toxic consequences.

4. Conclusions

The knowledge of the effects of novel particles to plants and animals is required in order to identify the most appropriate engineering to promote environmental preservation along with technological advance.

The present study considered four samples; three containing silver and one commercial titanium dioxide that can be used as antimicrobial additives. The objective was to investigate the toxicity effects of metal particles with different characteristics to daphnids. All silver samples were toxic to daphnids, even at a low concentration (0.0001 ppm), and was not possible to find a relation between the characteristics of the particles analyzed and its toxicity. On the other hand, TiO_2 showed little acute effect on *D. magna* and a dose-dependent response on *C. dubia*.

Despite no apparent relationship between the physical–chemical characteristics of the particles evaluated and toxicity, the TiO_2 zeta potential value next to zero may have influenced its biological response due to the low rate of particle surface electrons, on which can alter the interaction between TiO_2 particles and the daphnids.

Since there is a great variation between the encapsulation system, size, shape, and chemical composition of particles being used in materials; the heterogeneity of the new metal particles, hinders the comparison between our results and others studies. In this way, future investigations are needed to explain the toxic mechanism of silver and titanium, in a variety of particles features in a long-term assay.

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