

ORIGINAL RESEARCH PAPER

Impact of silver ions and silver nanoparticles on the plant growth and soil microorganisms

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ABSTRACT: There is a growing consumer market for products that proclaim to decrease microorganism counts to prevent infections. Most of these products are loaded with silver in its ionic or nanoparticle form. Through use or during production, these particles can find their way into the soil and cause an impact in microbial and plant communities. This study aims to evaluate the impact of silver based particles in *Avena byzantina* (oat), *Lactuca sativa* (lettuce) and *Raphanus sativus* (radish) development and in the soil microorganism abundance. Oat, lettuce and radish plants were cultivated in soil contaminated with particles of bentonite organomodified with silver (Ag⁺_bentonite), silver phosphate glass (Ag⁺_phosphate) and silver nanoparticles adsorbed on fumed silica (AgNp_silica). Plant development and microorganisms' abundance were evaluated. To some degree, Ag⁺_bentonite impacted plants development and AgNp_silica causes an adverse effect on microbial abundance. The impact on plants and microorganisms was contradictory and varied according to soil and particles physicochemical characteristics.

KEYWORDS: *Microbial population; Nanoparticles; Silver particles; Soil health; Terrestrial ecotoxicity.*

INTRODUCTION

The use of silver (Ag) in functionalized products became widespread because of its biocidal effect (Wakshlak *et al.*, 2015). Silver ions released from loaded materials can end up in the environment and have a negative impact (Nowack *et al.*, 2011). A study accomplished by Sun *et al.* (2016) highlights that in the European Union silver nanoparticles (AgNps) released from consumer goods have as final destination landfills (79 µg/kg) and sewage treatment plants (61 µg/kg). The action of AgNps can vary depending on particle sizes, characteristics and transformation pattern in the environment. Regarding the size, the AgNp damage relies on its ability to enter

cells and be oxidized, generating reactive oxygen species (ROS) (Pokhrel and Dubey, 2013; Singh and Kumar, 2015). The chemical characteristics of particle coating will influence the agglomeration and dissolution properties (Furtado *et al.*, 2016). Moreover, the interaction of AgNp with the soil can modify physical and chemical characteristics which will influence stability, availability and, in turn, impact the toxicity of nanoparticles (Anjum *et al.*, 2013). For example, the conversion of silver to Ag-sulfide has greater impact on its toxicity because of the lower solubility of this modified silver specie (Doolette *et al.*, 2015). Microbial communities are responsible for the maintenance of soil health. This way, an impact on these communities will affect agricultural production safety (McGee *et al.*, 2017). Plant growth based bioassay has been used to verify metal particle toxicity

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on the terrestrial environment (Dimkpa *et al.*, 2009; De Oliveria *et al.*, 2016). Soil ensure the life on earth through the ecosystem services (Mol and Keesstra, 2012; Brevik *et al.*, 2015). In this ecosystem, the concern rests on the fact that these particles can affect the composition of microbial communities (Zhai *et al.*, 2016), plant growth (Dimkpa *et al.*, 2013), reproduction of earthworms (Tsyusko *et al.*, 2012) and furthermore, can accumulate in the food chain (Servin *et al.*, 2013). Concerns related to the degradation of soil ecosystem prompted the development of monitoring and remediation projects (Keesstra *et al.*, 2016). With that in mind, it is important to understand the toxic aspects related to soil communities and potential pollutants to achieve the balance between the technological development and ecosystem health. Thus, the aim of this study is to evaluate the impact of three commercial silver-based biocide additives for use in polymers, being them: silver ions (silver phosphate glass, bentonite organomodified with silver) and silver nanoparticles (silver nanoparticles adsorbed on fumed silica), in the development of three plant species (oat, radish and lettuce), as well as the effects of such materials on soil microorganism abundance. This study has been carried out in South of Brazil in 2015-16.

MATERIAL AND METHODS

For the studies soil samples were experimentally contaminated with particles of bentonite organomodified with silver (referred to here as “Ag⁺_bentonite”), silver phosphate glass (referred to here as “Ag⁺_phosphate”) and silver nanoparticles adsorbed

on fumed silica (referred to here as “AgNp_silica”). Particle characteristics were previously determined in Tomacheski *et al.* (2016), Fig. 1 shows particles morphology determined by Transmission Electron Microscopy (TEM), Table 1 presents the average size determined by TEM, specific surface area (SSA) and zeta potential (ZP).

It was noted that in Ag⁺_phosphate at pH 7, occurred a decrease in zeta potential and then at pH 9 it rises again. One explanation is that at pH 9 an oxidation of silver ions occurred forming silver oxide (Ag₂O). Thus, considering that the oxide surface has compatibility with the phosphate (Wu *et al.*, 2008), the increase in ZP at pH 9 may be due to the rise in surface charge density on Ag₂O promoted by phosphate ions. However, particles with zeta potential below 30 mV or above -30 mV are deemed instable and with high capacity to form aggregates (Shieh *et al.*, 2012; Tavares *et al.*, 2014) and, in this study, all three particles presented ZP between or near to 30 mV and -30 mV.

Germination assay

Germination assay was conducted using seeds of three plant species: *Avena byzantine* (oat), *Lactuca sativa* (lettuce) and *Raphanus sativus* (radish). The seeds were obtained commercially and sorted according to size and appearance and kept in dark, at room temperature before use. The mean germination rates of all plant seeds were greater than 90% as tested in a preliminary study (results not shown). The germination assay was performed using vessels (15 cm x 45 cm) containing 2,000 g of Organosol. It was

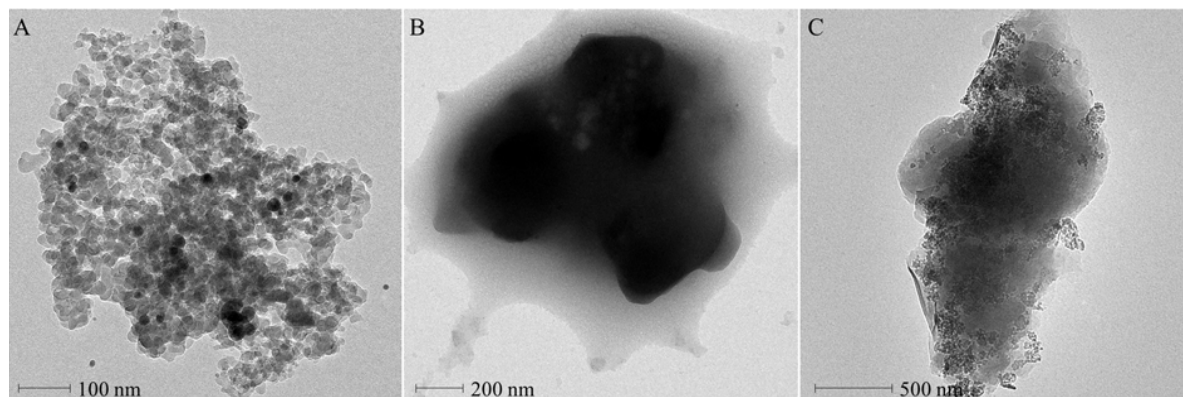


Fig. 1: Transmission electron microscopy of the additives evaluated: A) AgNp_silica, B) Ag⁺_phosphate and C) Ag⁺_bentonite

used three vessels per additive. The particles were added in three vessels at a final concentration of 10 g/kg. Three vessels were used as control (no additive). Sowing was carried out in July 2015 (winter in the south of Brazil-29°40'54"S and 51°03'25"W). The plants were watered with 100 mL of water collected from rain every other day. A seed was considered as having germinated when shoots were evident above the soil surface. Fig. 2 shows the apparatus used for the germination assay. After a period of 35 days the plants were carefully removed from the soil and root length and dry weight measurements were taken after drying (48 h at 58 °C) the washed plants. The percentage of relative seed germination was calculated considering the total germination in the control sample as 100%. The lengths of the seedling roots were measured with a digital caliper rule. Each treatment was conducted with 25 seeds of lettuce, 25 seeds of radish, and 5 seeds of oat, and the results were presented as mean

± SD (standard deviation). Differences between treatments were analyzed using Analysis of Variance (ANOVA) followed by Tukey comparison tests using PAST version 3.14 software (Hammer *et al.*, 2001). Each of the experimental values was compared to its corresponding control. The level of significance was set at (*p*) less than 0.05.

Soil characterization

After harvesting, a composite sample of each additive type was prepared by mixing the three vessels of soil with same additive. For verify the concentration of silver a composite soil was acid digested based on the standard Environmental Protection Agency 3050B and the Inductively Coupled Plasma Optical Emission Spectrometry was performed using a ICP-OES, Thermo Scientific iCAP 6300 Model. Total nitrogen was measured by the Kjeldahl process based on British Standard 1309/7 and the International Union

Table 1: Physical-chemical characteristics of the additives evaluated

Additive	Main information	Average diameter by TEM (µm)	Specific surface area (m ² /g)	Zeta potential at pH (mV)	
AgNp_silica	Silver nanoparticles adsorbed on fumed silica (SiO ₂)	0.02	293.90	3	0.12
				5	-6.48
				7	-27.70
				9	-27.53
				11	-35.77
Ag ⁺ _phosphate	Silver ions supported on phosphate glass	1.0	6.16	3	15.7
				5	6.82
				7	-21.80
				9	-1.06
				11	-3.64
Ag ⁺ _bentonite	Bentonite compounded by cristobalite and montmorillonite 22A, organomofied with silver ions.	1.5	36.73	3	-8.31
				5	-3.77
				7	-33.53
				9	-32.17
				11	-42.30



Fig. 2: Germination assay
A) greenhouse used in the test and B) vessels arrangement within the greenhouse.

of Research Commission 10. Soil pH was measured using the potentiometric method with 1:10 dilution of soil, deionized water. Total phosphorus was measured by colorimetric assay, based on 4500 system from Standard Methods for the Examination of Water and Wastewater (Rice *et al.*, 2012).

Isolation of soil microorganisms

Four composite soil samples were used for microbial isolation (three contaminated samples and one control sample). For the enumeration of microorganisms, 25 g of each composite soil sample was suspended in 225 mL peptone salt solution (0.1 %) and homogenized in Stomacher for 60 s (10⁻¹ dilution). For the enumeration of mesophilic aerobic microorganisms 1 mL (10⁻⁴) of soil suspension was placed in sterile Petri dishes and molten Plate Count Agar (Oxoid) was added to these plates and incubated for 48 h at 36 ± 1 °C. For yeast and fungi isolation the dilution (10⁻⁴) was placed in Potato Dextrose Agar (MERCK) for 7 days at 25 ± 1 °C. *Pseudomonas aeruginosa* (*P. aeruginosa*) counts were performed according to the Most Probable Number test. Soil

suspension (10⁻¹) was inoculated in *Pseudomonas* Asparagine Broth (BIOLOG) and incubated for 24–48 h at 35–37 ± 1 °C. The positive test was confirmed with acetamide broth after the incubation for 24–36 h at 35–37 ± 1 °C. For the enumeration of *Bacillus* sp. 10 g of each composite soil sample was suspended in 90 mL of dilution water and homogenized in Stomacher for 60s. Then, 10 mL of diluted samples (10⁻⁴) were heat-shocked at 80 °C for 12 min and cooled at 4 °C for 5 min. After this process, the suspension was placed in nutrient agar (oxid) and incubated for 24 h at 30 °C.

RESULTS AND DISCUSSION

It is known that the soil physicochemical characteristics have an influence on the availability and consequently the toxicity of pollutants (Schlich and Hund-Rinke, 2015). Table 2 shows the chemical characteristics of the soil contaminated and not contaminated used in this study. The addition of Ag⁺ phosphate reduced the soil pH, while Ag⁺ bentonite made it more alkaline and AgNp_silica had no effect compared to the control. The percentage of nitrogen (N) was greater than the control in all soil samples,

Table 2: Physicochemical characteristics of metal loaded soil

	Control	Ag ⁺ _phosphate	Ag ⁺ _bentonite	AgNp_silica
pH	7.9	7.4	8.4	8.0
Nitrogen (%)	0.33	0.37	0.38	0.77
Phosphorus (mg/kg)	109	141	1082	175
Silver (mg/kg)	0	176.0	65.1	49.9

Table 3: Oat, lettuce and radish germination (%), relative germination (%), comparing loaded soil with the control), root growth (mm) and dry mass (g)

	Control	Ag ⁺ _phosphate	Ag ⁺ _bentonite	AgNp_silica
Oat				
Germination (%)	80	120	40	120
Relative germination (%)	-	150	50	150
Root growth (mm)	133 ± 25 ^a	233 ± 68 ^a	362 ± 47 ^a	103 ± 56 ^b
Dry mass (g)	0.026 ± 0.003 ^a	0.046 ± 0.015 ^a	0.042 ± 0.013 ^a	0.034 ± 0.006 ^a
Lettuce				
Germination (%)	80	64	36	40
Relative germination (%)	-	80	45	50
Root growth (mm)	23 ± 0.00 ^a	31 ± 7 ^a	24 ± 9 ^a	28 ± 7.85 ^a
Dry mass (g)	0.07 ± 0.00 ^a	0.06 ± 0.00 ^a	0.06 ± 0.00 ^a	0.04 ± 0.00 ^a
Radish				
Germination (%)	88	84	88	92
Relative germination (%)	-	95%	100%	105%
Root growth (mm)	92 ± 39 ^a	81 ± 23 ^a	52 ± 14 ^b	79 ± 27 ^a
Dry mass (g)	0.025 ± 0.01 ^a	0.035 ± 0.01 ^b	0.016 ± 0.01 ^c	0.031 ± 0.01 ^a

Values are given as mean ±SD. Averages that sharing the same superscript letter (a, b or c) are not significantly different from each other (p>0.05, Tukey's test).

mainly in AgNp_silica. The phosphorus (P) content was detected in high amounts in all soil samples. However, the concentration of P in Ag⁺_bentonite soil was fairly high, showing a concentration ten-fold higher than the control. The high amount of P in soil with Ag⁺_bentonite can be related to capability of clays to retain P in soil (Ulén and Etana, 2014). According to ICP-OES results, high amounts of silver were found in the sample loaded with Ag⁺_phosphate followed by Ag⁺_bentonite and AgNp_silica.

It has been reported that the reaction of plants to metal present in soil can vary depending on plant species and characteristics of the particle tested (De Oliveira *et al.*, 2016, Tariq *et al.*, 2015, Abdel-Ghani *et al.*, 2016). In this study the toxicity of each additive was manifested in distinct ways. Table 3 shows germination; relative germination; root growth and dry mass values of plants cultivated in the control soil (without additive) and in the metal contaminated soil. Pictures of oat, lettuce and radish after harvest are shown in Figs. 3, 4 and 5 respectively.

Oat plants had lower germination rate in the soil with Ag⁺_bentonite, however low root growth was observed in the soil loaded with AgNp_silica ($p < 0.05$) (Table 3 and Fig. 3). Root growth and dry mass values of lettuce presented no difference among the additives tested; however the germination was lower in the soil with Ag⁺_bentonite (Table 3 and Fig. 4). Root growth and dry mass of radish varied between the additives ($p < 0.05$), with lower values found in the soil loaded with Ag⁺_bentonite (Table 3 and Fig. 5). Moreover, in Ag⁺_phosphate and AgNp_silica soils it was verified enhance of dry mass values of radish plants compared to the control. Plant development was significantly lower in the soil loaded with Ag⁺_bentonite, which presented high P levels. Phosphorus uptake is regulated according to plant growth rate and thus its concentration will vary in different plant species (Narang *et al.*, 2000). Nevertheless, the imbalance of phosphorus concentration causes the depletion of organic matter and nutrients and may have reflected in plant growth in Ag⁺_bentonite loaded soils.

The main theory to explain silver toxicity is that silver ions generate reactive oxygen species (ROS, such as oxygen superoxide and hydrogen peroxide) which injures cell membrane and impacts DNA (Kumari *et al.*, 2009). In the case of nanoparticles (AgNps), silver can be oxidized as shown in Eq. 1, resulting in the liberation of silver ions as shown in Eq. 2 (Xiu *et al.*, 2012; Kaveh *et al.*, 2013).



Fig. 3: Oat cultivated in soils containing
A) Control, B) Ag⁺_bentonite, C) Ag⁺_phosphate and D) AgNp_silica

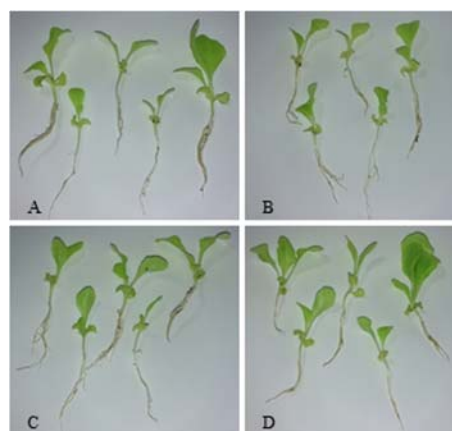
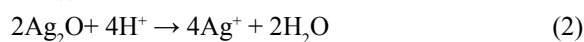


Fig. 4: Lettuce cultivated in soils containing
A) Control, B) Ag⁺_bentonite, C) Ag⁺_phosphate and D) AgNp_silica



Fig. 5: Radish cultivated in soils containing
A) Control, B) Ag⁺_bentonite, C) Ag⁺_phosphate and D) AgNp_silica



Soil pH influences the oxidation of Ag nanoparticles and the release of ions (Schlich and Hund-Rinke, 2015). The modification of pH can change the potential zeta of particle (as seen in Table 1) modifying particle agglomeration (Prathna et al., 2011), hence the pH of the medium and the nanosilver coating influences its toxicity by making them unstable (El badawy et al., 2010; Dimkpa et al., 2013).

Soil pH suitable for oat and lettuce growth is near 5.5-6.5. So, neutral to alkaline pH observed in the soil with Ag⁺_bentonite (pH 8.4) and AgNp_silica (pH 8.01) may have contributed to the low relative germination rates in oat (50% - Ag⁺_bentonite), lettuce plants (45% - Ag⁺_bentonite and 50% - AgNp_silica) and the root growth of radish (52 mm-Ag⁺_bentonite).

In oat plants, low root growth was observed in the soil loaded with AgNp_silica (p<0.05). Joshi et al. (2012) suggest that silver nanoparticles are toxic due to their ability to relocate to the shoots, entering the cell membrane and being oxidized within the cell. After exposure to AgNp, plant roots showed a differential expression of proteins related to plant defense system against oxidative stress (Vannini et al., 2013). Coutris et al. (2012) has shown that some forms of AgNp can be more dangerous, since in this format Ag is more promptly available than Ag ions.

In contrast, previous studies have shown that silver nanoparticles presented lower toxicity to plants than Ag⁺ (Singh and Kumar, 2015). Besides that, studies revealed growth promotion in different species of plants (Judy et al., 2015; Mustafa et al., 2015). Particularly in the case of AgNps, Mustafa et al. (2015) depict these nanoparticles as beneficial to the growth of soybean exposed to flooding. During the experiments carried out by these authors, the weight of the plant increased when cultivated with particles of 15 nm at a concentration of 2 ppm. Proteomic analysis suggested that the soybeans would suffer less with the absence of oxygen when treated with AgNps. Thus, plants growing in flooding

conditions could have their development favored by the ROS generated from silver. Pokhrel and Dubey (2013) studies revealed that the development and root growth of maize and cabbage was less affected by metal nanoparticles than the ionic form. Kaveh et al. (2013) evaluated the action of AgNps and ionic silver on *Arabidopsis thaliana*. The exposure to 1.0 and 2.5 mg/L AgNps (20 nm) allowed for biomass gain; the same concentration of Ag⁺ did not affect growth. However, by increasing the concentration to 5.0 and 20 mg/L of both AgNp and Ag⁺ the weight of the plants presented lower values compared to the control. These divergent effects in plants exposed to nanoparticles or ionic silver can be explained by the hormesis mechanism. This process is characterized by enhanced growth at low concentrations of a toxic substance and an inhibition in higher doses (Poschenrieder et al., 2013). In addition, the binding of sulfur and silver (Ag₂S - Ag sulfidation) or other sulfur-bound forms may be related to the low toxicity of AgNp to plant development (Levard et al., 2012; Doolette et al., 2015). The sulfidation of AgNp renders these particles less bioavailable due to the insolubility property of sulfidized nanoparticles (Reinsch et al., 2012; Levard et al., 2013). Once that the susceptibility of plants to toxic metals can vary not merely between plant species, but either between cultivars, it becomes difficult to make a connection among the presence of silver in soil and its toxic effects in plants. Priac et al. (2017) observed different ecotoxicological responses from lettuce cultivars after be irrigated with the same metal-loaded wastewater.

Microorganisms perform key role in soil biological processes (Judy et al., 2015). Due to silver's well-documented toxicity for microbes (Calder et al., 2012; He et al., 2016), negative effect to both bacteria and fungi was expected upon Ag-exposure. Interestingly, despite the varying levels of negative effects of metal based additives on plant development, Ag⁺_phosphate and Ag⁺_bentonite improved the proliferation of *Bacillus*, mesophilic bacteria and fungi population. Table 4 shows the abundances of microorganisms in different metal loaded soil.

Table 4. Soil microbiota after harvest

	Control	Ag ⁺ _phosphate	Ag ⁺ _bentonite	AgNp_silica
<i>Bacillus</i> sp. (CFU/g)	2.47 x 10 ⁵	2.82 x 10 ⁵	4.18 x 10 ⁵	7.76 x 10 ⁴
Mesophilic bacteria (CFU/g)	2.47 x 10 ⁶	4.47 x 10 ⁶	3.12 x 10 ⁶	3.14 x 10 ⁵
Fungi and yeasts (CFU/g)	1.39 x 10 ⁴	1.68 x 10 ³	3.74 x 10 ⁴	2.08 x 10 ³
<i>Pseudomonas aeruginosa</i> (MPN/g)	<0.2	<0.2	<0.2	<0.2

Note: CFU/g: Colony Forming Units per gram; MPN/g: Most Probable Number per gram

According to Schlich and Hund-Rinke (2015) soil pH between 5.5 and 7.5 was found to have weak microbial toxicity. In the present study, the lowest bacterial toxicity was associated with acid (Ag^+ _phosphate, pH 7.4) and alkaline (Ag^+ _bentonite, pH 8.4) soils. The growth of bacteria and fungi populations in Ag^+ _bentonite soil may be related to the ability of bentonite (clay) to absorb silver ions (Magaña *et al.*, 2008; Calder *et al.*, 2012). Given that ionic silver has an inclination to interact with inorganic ligands and organic matter (Yang *et al.*, 2013), the high concentration of phosphorus in Ag^+ _bentonite soil (Table 2) may have provided a decrease in Ag toxicity for microorganisms. Moreover, extracellular polymeric substances produced by bacteria provide protection for the cells (Joshi *et al.*, 2012). Judy *et al.* (2015) reported that Ag_2S forms, which are produced in soil and wastewater environments, are less toxic to Gram-negative, Gram-positive bacteria and fungi populations than ionic Ag and polyvinylpyrrolidone coated Ag nanomaterials. Reinsch *et al.* (2012) observed that sulfidation decreases the toxic potential of AgNps against the *Escherichia coli* bacteria.

In contrast, in AgNp_silica loaded soils negative effect was noted on microbial development, oat and radish growth. This toxicity may be related to Ag connection with thiol groups found in enzymes and proteins of bacteria (Lemire *et al.*, 2013). Furthermore, the AgNp_silica additive analyzed in this study has a specific surface area which provides high contact with microorganism cells in soil; besides of the described action in inhibit the plant growth by dropping chlorophyll content (Qian *et al.*, 2013).

There was no detection of *P. aeruginosa* in the standard neither in metal loaded soil, which was expected once that this bacterium occurs in aquatic environment (Selezska *et al.*, 2012), being also reported in soil contaminated with untreated waste or subjected to hydrocarbon (Deredjian *et al.*, 2014).

At the same time that plants and soil microorganisms are sensible to the presence of metals, some groups of inedible plants and microbes can be used for soil remediation. Researchers have reported the capability of Rhodes grass (*Chloris gayana*) to uptake antimony, arsenic, cadmium, lead, silver and zinc (Keeling and Werren, 2005), and also *Jatropha curcas* and *Puccinellia frigida* to remove mercury (Marrugo-Negrete *et al.*, 2015) and boron (Rámila *et al.*, 2015) from soil, respectively. Alaribe and Agamuthu

(2015) mentioned the use of *Lantana camara* for phytoremediation of soil contaminated with lead. Moreover, some bacteria can be used to mitigate harmful effects of metals in soil, such as *Pseudomonas tolaasii*, *Pseudomonas fluorescens*, *Alcaligenes* sp. and *Mycobacterium* sp. (Dell'Amico *et al.*, 2008).

Despite the natural filtration process that occurs when the effluent permeates the soil layers, attention must be taken in the metal-contaminated wastewater discard, once these xenobiotics substances can still reach groundwater (Keesstra *et al.*, 2012).

CONCLUSION

It can be concluded that the responses of plants and microorganisms to the Ag present in soil vary according to size and chemical characteristics of the particle as well as to soil characteristics and the sensitivity of the plant. Silver nanoparticles and silver ions can be used as antimicrobial additives to avoid microbial proliferation in everyday use products, but attention must be taken on disposal of these materials in the environment.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

ABBREVIATIONS

%	Percentage
°C	Centigrade degree
$\mu\text{g}/\text{kg}$	Microgram per kilogram
μm	Micrometer
Ag	Silver
Ag^+	Silver ion
Ag^+ _bentonite	Bentonite organomodified with silver
Ag^+ _phosphate	Silver phosphate glass
Ag_2O	Silver oxide
Ag_2S	Silver sulfide
AgNp	Silver nanoparticle
AgNp_silica	Silver nanoparticle adsorbed on fumed silica

ANOVA	Analysis of variance
CFU/g	Colony forming unites per gram
cm	Centimeter
g	Gram
g/kg	Grams per kilogram
H	Hydrogen
h	Hour
H ₂ O	Water
ICP-OES	Inductively coupled plasma optical emission spectrometry
mg/kg	Milligram per kilogram
mg/L	Milligram per liter
min	Minute
mL	Milliliter
mm	Millimeter
MPN/g	Most probable number per gram
mV	Millivolts
N	Nitrogen
nm	Nanometer
O	Oxigen
P	Phosphorus
p	P-value
<i>P. aeruginosa</i>	<i>Pseudomonas aeruginosa</i>
ppm	Parts per million
ROS	Reactive oxygen species
S	South
SD	Standard deviation
SiO ₂	Silica (silicon dioxide)
SSA	Specific surface area
TEM	Transmission electron microcopy
W	West
ZP	Zeta potential

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