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Technical Article

Toxicity of leachates from pilot reactors simulating a landfill with different concentrations of AgNP

Toxicidade dos lixiviados de reatores-piloto simulando um aterro com diferentes concentrações de NPAg

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ABSTRACT

This work's objective was to verify the leachates toxicity from pilot reactors (PR) simulating a landfill containing different concentrations of silver nanoparticles (AgNP). Acute toxicity tests (48 h) with *Daphnia magna* were carried out in leachates containing 50, 150 and 450 mg AgNP.kg^{-1}, in addition to a blank for control. Toxicity tests with the pure solution of silver nanoparticle and leachates resulted by the reactors were performed. The acute toxicity tests performed with *D. magna* confirmed the toxicity of the leachates, as well as confirmed that the silver nanoparticles are toxic, presenting EC50 (48 h) of 0.63 μg.L^{-1} of pure nanoparticle and ranging from 1.52 to 3.37% for the leachates. Overall, the results from the present study indicate that exposures of aquatic invertebrates to silver nanoparticles could have important ecological effects on lower trophic levels in aquatic ecosystems. The results may contribute to a better understanding of the quality of municipal solid waste (MSW) landfill leachates, with reference to nanoparticle interference and consequent treatment efficiency.

Keywords: municipal solid waste; leachate; toxicology; silver nanoparticles.

RESUMO

O objetivo deste trabalho foi verificar a toxicidade de lixiviados de reatores piloto (RP) simulando um aterro contendo diferentes concentrações de nanopartículas de prata (AgNP). Testes de toxicidade aguda (48 h) com *Daphnia magna* foram realizados em lixiviados contendo 50, 150 e 450 mg de AgNP.kg^{-1}, além de um branco para controle. Foram realizados testes de toxicidade com a solução pura de nanopartículas de prata e lixiviados resultantes dos reatores. Os testes de toxicidade aguda realizados com *D. magna* confirmaram a toxicidade dos lixiviados, bem como confirmaram que as nanopartículas de prata são tóxicas, apresentando EC50 (48 h) de 0,63 μg.L^{-1} de nanopartículas puras e variando de 1,52 a 3,37% para os lixiviados. No geral, os resultados do presente estudo indicam que a exposição dos invertebrados aquáticos a nanopartículas de prata pode ter importantes efeitos ecológicos nos níveis tróficos inferiores nos ecossistemas aquáticos. Os resultados podem contribuir para a melhor compreensão da qualidade dos lixiviados de aterros sanitários de resíduos sólidos urbanos (RSU), com referência à interferência de nanopartículas e consequente eficiência de tratamento destes.

Palavras-chave: resíduos sólidos urbanos; lixiviado; toxicologia; nanopartículas de prata.

INTRODUCTION

The increasing interest in nano-materials and the study of their characteristics has been one of the greatest scientific challenges since the popularization of nanotechnology. Because they are versatile, metallic nanoparticles are important as a field of research with use in several areas. They have attracted attention in areas such as nanoelectronics and medical studies. For the most part, studies are made with silver nanoparticles or colloidal silver, which has action against a wide variety of bacteria, fungi and viruses (RANGEL, 2014).

The use of nanometric particles in solution (KEY; MASS, 2001) is reported in 18th century documents, but the usage of these nanoparticles was further intensified in the early 20th century (GIBBS, 1999). Many methods have been studied to test their toxicity against microorganisms and the following scale of toxicity has been done: Ag > Hg > Cu > Cd > Pb > Co > Au > Zn > Fe > Mn > Mo > Sn (BERNI NETO; RIBEIRO; ZUCOLOTTO, 2008). The AgNP can be very toxic (YANG *et al.*, 2013a).

There are also lack of knowledge about these nanomaterials and concern about the risks of these particles and their impacts on different ecosystems, both in ecotoxicology and in human health. Studies have shown that silver nanoparticles can kill liver cells and rat brain cells, in addition to cell death, through the binding of the AgNP to the membrane surface, interfering with cellular respiration

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and permeability (JU-NAM; LEAD, 2008; BYSTRZEJEWSKA-PIOTROWSKA; GOLIMOWSKI; URBAN, 2009; STANLEY, 2014; ROY *et al.*, 2014; BOUR *et al.*, 2015; DWIVEDI *et al.*, 2015; CANESI; CIACCI; BALBI, 2015; MATIAS *et al.*, 2015). The use of these materials generates nano-residues, which can exhibit various behavior such as agglomeration, aggregation and coating of biomolecules, yet unknown, which regulate the properties of their toxicity (LYNCH; WEISS; VALSAMI-JONES, 2014). These very fine particulate nano-residues can break the cell membrane and cause damage (PINTO, 2009).

Taking into account the high microbicidal potential of AgNP and its good applicability in the industry, it is necessary to further verify the effects that such nanoparticles can cause in living beings (YANG *et al.*, 2013b), since the continuous ingestion of silver can lead to liver and stomach problems, in addition, there is an inadequate disposal of the packaging of these products.

Considering the increase in the use of nanoparticles in several sectors and the lack of knowledge about possible impacts on the environment, this research proposes to contribute with results to help to understand the possible risks to the environment and/or health. The general objective of this work is to evaluate the toxicity of the leachate coming from reactors filled with MSW and inoculated with different concentrations of AgNP, as well as to correlate a toxicity with physicochemical variables of the leachates.

METHODOLOGY

The infrastructure used during the project was in the Department of Environmental Engineering of Santa Catarina Federal University, including the Solid Waste Laboratory (LARESO), the Environmental Toxicology Laboratory (LABTOX) and the Integrated Environmental Laboratory (LIMA). A specific methanogenic activity (SMA) using the Automatic Methane Potential Test System (AMPTS II) was performed to verify the concentrations for the assembly of the pilot reactors. At the end of this first experiment, four pilot reactors simulating landfill were constructed, identified as PR1, PR2, PR3 and PR4, with dimensions of 2 m height and 200 mm diameter. The pilot reactors has two acrylic windows (78 × 13 cm) to allow visual access to the inside. The upper parts of the pilots were closed with a polyvinyl chloride (PVC) lid. A rain simulation system and a gas outlet to a processing system were attached to this cover. At the bottom, a layer of pebble support was placed to facilitate the percolation of leachate and to avoid clogging. The structure was closed with a PVC lid, in which a tap was installed to collect the leachate.

The support substrate used into the PR was a mixture of organic matter following the qualitative–quantitative standard prescribed by Pinto (2000) denominated as FORSUp (organic fraction of standard solid waste). The mixture was treated (cut, crushed and homogenized) manually and with a domestic multiprocessor, in order to increase the specific surface area and facilitate the microbial action. The inoculation was done with a sludge collected at the treatment station of the Santa Catarina Sanitation Company (CASAN). The substrate was mixed with the sludge and three different concentrations of silver nanoparticles, in addition to a blank for control and the PR were filled. After the PR were filled up, a rain simulation system was activated, introducing ultrapure water weekly, according to the average monthly rainfall of the last 10 years for the region of Florianopolis.

Three samples were collected in the months of August, October and December, referring to collections number 1, 11 and 21. The collections were taken at one-month intervals so that there was some significant difference between the results of the toxicological tests. The leachate was sampled taking 500 mL of leachate from each pilot using a polyethylene bottle. The standards methods (APHA; AWWA; WPCF, 2005) were used to determine pH, chemical oxygen demand (COD), total solids (TS), total volatile solids (TVS) and total fixed solids (TFS). Trace elements were determined by atomic absorption flame atomization and graphite furnace according to USEPA Methods 3020A and 3010A (USEPA, 1992a; 1992b; 1995), with some modifications. The dynamic light scattering (DLS) technique was used to measure the size of the AgNP, considering the particle size distribution as a function of intensity, area, volume and quantity. The silver nanoparticle used in this work is of commercial origin without encapsulation and polyvinyl acetate (PVA) stabilizer.

The measurements obtained using the DLS technique were also used to evaluate the stability of the nanoparticles. Their size was measured by the LUMiSizer 6110-87 under the following conditions: refractive index of silver 1,390, particle absorption coefficient of 0.002, refractive index of dispersant 1,361, viscosity 1,200 cP, equilibrium time of 1 minute and temperature of 25ºC. About 50 measurement cycles of 10 seconds each were performed. The measurements were performed in triplicates and the mean particle size distributions were considered. A solution of 10,000 ppm de AgNP was used to reach the desired concentrations. The selected concentrations were based on the already existing results of Yang *et al.* (2012).

Considering that the results of these first concentrations showed a low influence in the methane production, we chose to do an experiment with 10 times higher, being 50 mg AgNP.kg^{-1}, 150 mg AgNP.kg^{-1} and 450 mg AgNP.kg^{-1} (for a more extrapolated concentration), so 125, 275 and 1,125 mL of silver nanoparticles were added in each PR, respectively, besides the white for control, to reach the desired concentrations.

To start the toxicity tests, a sensitivity one was performed using potassium dichromate ($K_2Cr_2O_7$), as standard, in which each batch of *Daphnia magna* is tested to determine if the organisms are suitable for the acute toxicity test, following the Brazilian Standard (Norma Brasileira — NBR) No. 12713 (ABNT, 2016). This method consists of exposure of *D. magna* neonates (< 24 h old) to the sample (qualitative) or to several sample dilutions (quantitative) for up to 48 h. During this assay, five test organisms per replicate were used and 16 replicates were

performed, where the standard value of $K_2Cr_2O_7$ was 0.83 ± 0.10 mg.L^{-1} and dilutions of 0.5; 0.7; 0.9 and 1.1, for the 24-hours period.

The acute toxicity test was performed according to NBR 12713 (ABNT, 2016), at the LABTOX. *Daphnia magna* neonates at 2 to 26 hours of life were exposed to a series of different dilutions of the same sample over a period of 48 hours. For the acute toxicity tests, samples of the leachates from the PR and the pure silver nanoparticle solution, with concentration of 10,000 ppm, were used. For each test, different dilution factors were used in triplicate, besides control vials (without addition of AgNP).

For each concentration, the immobility and/or the mortality of the organisms were observed after the exposure period of 24 and 48 hours, closing the test after 48 hours. The results of the acute toxicity tests were expressed as percentages of dead and/or immobilized organisms at each concentration, such as LC50 and/or EC50, which is the mathematical expression of the dose and/or concentration of the substance causing death/immobilization of 50% of the population exposed.

RESULTS AND DISCUSSION

The DLS data analysis showed that the average size of the silver nanoparticles is 40 nm and the Polydispersity Index (PDI) value was 0.277. The single peak obtained indicated that silver nanoparticles do not have polydispersity in size, demonstrating a good synthesis.

An acute toxicity test using only the pure nanoparticle, with concentration of 10,000 ppm, was carried out and it presented EC50 of 0.63 µg.L^{-1}, proving how toxic this pure substance is.

Some significant differences were observed in the toxicological behavior in relation to the different concentrations of nanoparticles. As expected, the concentration of 450 mg.kg^{-1} was highly toxic, with an average value of EC50 of 1.52%, as can be seen in Table 1.

Comparing the average values of toxicity between the leachates of all the pilot reactors, it was expected, according to the EC50 (48 h) average, that the leachate produced in the PR4 samples were the most toxic, with a mean of 1.52%. The low value of the standard deviation indicates that the results are close to the expected value. In the same table, it can be observed that the PR1 sample was shown to be less toxic than the other ones. However, the average value of EC50 was 3.37%, showing that even without the addition of AgNP, the leachate appears to be toxic, as we can see, according to Marsalek *et al.* (1999): non toxic EC50 > 100; potentially toxic 100 > EC50 > 40; toxic 40 > EC50 > 10; highly toxic EC50 < 9.

According to Restrepo (2013), the high toxicity of this leachate can be related by mixing the organic and synthetic part, which produces chemical reactions, facilitating the solubilization of toxic substances from the solid matrix to the aqueous phase.

Table 2 shows that the pH values found in the samples varied between 3.43 and 5.46, considered low, probably due to the production

Table 1 – Average values and standard deviation EC50 (48 h) for samples from pilot reactors.

Samples	EC50 (48h) (%)	Standard deviation
(PR1) Control	3.37	0.075
(PR2) 50 mg AgNP.kg^{-1}	2.16	0.3
(PR3) 150 mg AgNP.k g^{-1}	2.01	0.175
(PR4) 450 mg AgNP.k g^{-1}	1.52	0.234

PR: pilot reactor.

Table 2 – Results of physical-chemical analysis of leachates.

Parameters	Unity	PR1			PR2			PR3			PR4		
		Day			Day			Day			Day		
		1	11	21	1	11	21	1	11	21	1	11	21
pH		5.4	5.04	5.46	3.7	5.07	5.21	3.43	4.55	5.43	3.78	4.76	5.2
COD	mg.L^{-1}	963.74	976.89	980.01	976.59	997.58	970.64	1003.2	964.28	985.6	964.25[1]	879.84[1]	962.59
TS	mg.L^{-1}	730	1500	1430	0	1590	770	630	1040	1010	0	1750	880
TVS	mg.L^{-1}	630	1060	850	0	1150	770	490	830	640	0	1410	600
TFS	mg.L^{-1}	100	440	580	0	440	0	140	210	370	0	340	280
Ni	mg.L^{-1}	1.178	0.336	0.259	1.339	0.607	0.737	0.92	0.373	0.335	0.891	0.307	0.86
Pb	mg.L^{-1}	[2]	0.673	0.481	0.246	0.723	1.06	0.41	1.583	1.263	0.01	1.366	0.413
Cr	mg.L^{-1}	0.152	0.696	1.039	0.151	1.797	2.755	0.375	0.281	0.252	1.079	[2]	[2]
Cu	mg.L^{-1}	[2]	0.174	0.288	[2]	0.053	0.054	[2]	0.043	0.03	0.201	[2]	0.048
Cd	mg.L^{-1}	[2]	[2]	[2]	[2]	[2]	[2]	[2]	[2]	[2]	[2]	[2]	[2]
Fe	mg.L^{-1}	1.992	2.472	3.036	1.992	3.193	5.350	0.937	0.832	2.004	2.88	4.036	5.029
Ag	mg.L^{-1}	0.02	[2]	[2]	0.017	[2]	[2]	0.016	0.01	0.079	0.013	0.011	0.013

PR: pilot reactor; COD: chemical oxygen demand; TS: total solids; TVS: total volatile solids; TFS: total fixed solids; [1]dilution 1.200^{-1}. The other samples were diluted in 1.20^{-1}; [2]the values are very close to the detection index. They were identified but not quantified.

of mineral acids and acid salts, since the generally is between 6.5 and 7. The leachate from these reactors are complex mixtures, which contain considerable concentrations of macronutrients, inducing a high COD.

Correlations of the results of the physical-chemical variables with the results of the toxicological variable EC50 (48 h) were carried out, in an attempt to identify the main variables responsible for the toxic effect of the leachate. Table 3 shows the correlations between the variables analyzed and the toxicity.

Few parameters had a low correlation with toxicity. The TVS and COD variables were the ones that most correlated with the toxicological variable EC50 (48 h). In addition, the PR1 presented a very strong or strong correlation with all variables except pH, evidencing that the addition of AgNP may have influenced this variable, and silver may have been complexed with other elements and/or organic compounds. This behavior also suggests that the presence of recalcitrant organic compounds, as already studied by Kjeldsen *et al*. (2002) and Thomas *et al*. (2009), may have influenced the toxicity (GIANNIS *et al*., 2008).

Among the trace elements, Ni, Pb, Cr, Cu and Ag presented a strong correlation with the toxicity variable. Pb had a high correlation with the toxicity and Cu was the element that presented the highest correlation with the toxicity among trace elements. Cd presented low concentration, which may be related to the fact that it has been complexed with organic compounds, increasing toxicity. However, Fe presented high concentrations in the analyzes, but its correlation with the toxicity was low, except in the PR1, which had very strong correlation with the toxicity, evidencing that, in the other pilots, Fe may have been complexed together with the AgNP.

Table 3 – Correlation between analyzed variables and toxicity.

Variables	Pilot reactor			
	PR1	PR2	PR3	PR4
pH	0.25	-0.89	-0.09	0.76
COD	-0.99	-0.63	0.90	-1
TS	-0.97	-0.98	-0.57	0.99
TVS	-0.79	-0.99	0.90	0.97
TFS	-0.98	-0.78	0.19	0.97
Ni	0.99	0.97	0.47	-0.82
Pb	-0.92	-0.69	-0.72	0.93
Cr	-0.96	-0.73	0.32	-0.86
Cu	-0.99	-0.92	-0.71	-0.99
Cd	0.83	0.02	0.97	-0.69
Fe	-0.90	-0.48	0.54	0.63
Ag	0.92	0.98	0.54	-0.79

PR: pilot reactor; COD: chemical oxygen demand; TS: total solids; TVS: total volatile solids; TFS: total fixed solids.

It is also noted that samples PR4-11 and PR3-21 were less toxic than PR1-11 and PR1-21, and it may be assumed that, over the weeks and stabilization of chemical and biochemical reactions, the toxicity levels of the silver nanoparticles were insufficient to overcome that of the leachate without the addition of AgNP. In the collections of day 21, a decrease of the values of COD and an increase of pH was noticed. Metcalf and Eddy (1991) state that, as pH neutralizes, COD declines due to the degradation of organic matter, thus, reducing toxicity. However, this fact occurred only with samples from PR4 and PR3. Yet, for Pablos *et al*. (2011), this correlation should not be considered a cause and effect relationship, but an association between the physico-chemical variables and the total content of toxic substances in the leachate.

According to Restrepo (2013), low concentrations of trace elements in the four pilot reactors are characteristic of new leachates. The author also states that, in general, Cr, Cu and Ni do not present significant correlation with toxicity. Pb presented a high correlation with it, and it can be said that this was due to the fact that, in some samples, this element was above the concentration that causes acute effect (0.45 $mg.L^{-1}$ — CETESB, 1990). Like Cu, it was the trace element that showed the highest correlation with toxicity. Its concentration was above the values that cause acute effect in aquatic organisms (0.009 $mg.L^{-1}$ — CETESB, 1990).

As the trace elements are subjected to strong adsorption to soil particles, complexation and chelation (transport) with organic and inorganic binders, they can cause slow movement, decrease in their availability and toxicity (XIAOLI *et al*., 2007; RESTREPO, 2013). In general, the correlations showed that the addition of AgNP in the pilot reactors adds to the toxicity of the leachates, and the results of toxicological analyzes lead one to believe that AgNP is very toxic to the organism *Daphnia magna*. However, in the statistical analysis of the data, p value was 0.49, indicating that, statistically, the means of EC50 (48 h) values did not present a significant difference.

CONCLUSION

Physical-chemical variants of leachates can provide strong evidence of acute toxicity, in addition to the acute toxicity test with pure AgNP. *Daphnia magna*, being a sensitive organism, was effective in determining the MSW toxicity and proved to be a suitable organism for toxicity testing in this type of sample.

Among the leach samples from the four PR evaluated, the one with the highest toxicity values was the one collected in PR4, suggesting that this occurred due to the high concentration of silver nanoparticles (450 $mg.kg^{-1}$) and the low pH, although, statistically, the averages of toxicological analyzes did not differ among themselves.

In general, more studies should be carried out together with other physico-chemical parameters and more and be analyzed together for a longer period in order to find ways to minimize the possible impacts that silver nanoparticles and nanowaste can cause.

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