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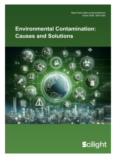

Article

Bioaccumulation and Ecotoxicity of Silver Nanoparticles in Simple Food Chain Algae-Microcrustaceans in the Presence of Natural Organic Matter

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Supplementary Information

Values of *in-situ* measurements of the Sorocabinha River are in Table S1.

Table S1. Data from *in-situ* measurements of the Sorocabinha River.

Parameters	Mean
Electric conductivity /$\mu S \cdot cm^{-1}$	136.0 ± 0.1
BOD_5 / $mg \cdot L^{-1}$	0.54 ± 0.01
DO / $mg \cdot L^{-1}$	1.91 ± 0.01
pH	5.8 ± 0.1
Temperature / °C	23.5 ± 0.1
TOC / $mg \cdot L^{-1}$	26.1 ± 0.1

Exposure Medium Composition

The composition of both freshwater solutions is as follows. For the algae medium exposure solution (Table S2), USEPA (2002) was adopted as a protocol.

Table S2. Composition of the Algae medium exposed solution.

Reagent	V_{Stock} /L	V_{Total} /L	C_{teoric} /M
$Ca(NO_3)_2 \cdot 4H_2O$	0.001	1	1.69×10^{-4}
KNO_3	0.001	1	9.89×10^{-4}
$MgSO_4 \cdot 7H_2O$	0.001	1	1.22×10^{-4}
K_2HPO_4	0.001	1	2.30×10^{-4}
$CuSO_4 \cdot 5H_2O$	0.0005	1	6.01×10^{-8}
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.0005	1	2.43×10^{-8}
$ZnSO_4 \cdot 7H_2O$	0.0005	1	1.04×10^{-7}
$CoCl_2 \cdot 6H_2O$	0.0005	1	1.26×10^{-7}
$Mn(NO_3)_2 \cdot 4H_2O$	0.0005	1	1.20×10^{-7}
H_3BO_3	0.0005	1	4.85×10^{-7}
$C_6H_5FeO_7 \cdot 5H_2O$	0.0005	1	2.43×10^{-6}
$FeCl_3 \cdot 7H_2O$	0.0005	1	1.08×10^{-6}
$FeSO_4 \cdot 7H_2O$	0.0005	1	1.12×10^{-6}
$NaHCO_3$	0.001	1	1.79×10^{-4}

Sensitivity Test (Raphidocelis Subcapitata)

Sensitivity tests were performed on the organism *R. subcapitata* according to ABNT (2018). The tests evaluated the sensitivity of the organism during tests that were performed throughout the year based on a known reference substance, in this case, sodium chloride (NaCl). To this end, the EC50 was established through algal biomass produced by cell counting. Five NaCl concentrations (0.4, 0.8, 1.6, 3.2, 6.4 g·L^{-1}) were established based on the sensitivity values for the same organism by Santos, Vicensotti, and Monteiro (2007).

One week before the n the week preceding each test, Pre-culture preparation was performed. It is a cell culture that guarantees the exponential growth phase of the algae. Thus, under the same cultivation conditions as the organisms, a sufficient amount of solution was prepared (in this case, it was decided to use 2 flasks (Erlenmeyer with capacity for 500mL) containing 300 mL Oligo medium), starting from the initial concentration of seaweed 10^4 and 10^5 cells mL^{-1} to obtain algal solution for further testing. The day before the test, the cell number was counted to ensure the minimum initial number of cells required. On the day of the test, the algal inoculum was prepared by centrifuging the pre-culture solution (4500 rpm, t = 15 minutes) and counting the number of cells.

In properly decontaminated and previously sterilized conical flasks, 100 mL of test solution prepared according to the established concentration and an aseptic culture inoculum of known algal concentration were added according to Table S3.

Table S3. Preparation of test solutions from a NaCl 128 g·L^{-1} stock solution.

Concentration (g·L^{-1} NaCl)	Stock$_{NaCl}$ (mL)	Water$_{ultrapure}$ (mL)	Oligo Medium (mL)	Inocule (mL)
Control	0	6.00	94	0.1–1.0
0.4	0.31	5.69	94	0.1–1.0
0.8	0.63	5.37	94	0.1–1.0
1.6	1.25	4.75	94	0.1–1.0
3.2	2.50	3.50	94	0.1–1.0
6.4	5.00	1.00	94	0.1–1.0

The volume of the inoculum to be inserted (Equation (S1)) as well as the cell concentration determination equation for Fuchs-Rosenthal chamber counting (Equation (S2)), shown in Figure 7 with the aid of a biological microscope, are described below.

Equation (S1) Formula for determining the volume of algae to be inoculated for culture maximization.

$$V_i = \frac{C_i * V_f}{N} \tag{S1}$$

where:

V_i = inocule volume (mL)

V_f = solution volume (mL)

C_i = initial concentration (cells mL^{-1})

N = cells in suspension (cells mL^{-1})

Equation (S2) Determination of algal solution concentration in Fuchs-Rosenthal chamber.

$$C_f = \frac{N * 160.000}{Q} \tag{S2}$$

where:

C_f = algal concentration (cells/mL)

N = number of cells counted.

Q = number of squares counted.

Study Involving Cells Counting by Spectrophotometry.

As an alternative method to obtain the algae concentration after the maximization process, the spectrophotometry UV-VIS was used. The method consists of obtaining a relation between the absorbance value *versus* cellular concentration (cell/mL) based on the method proposed by Souza [1]. The author proposes a counting relation based on the absorbance value in order to facilitate the counting procedure due to time demand, chambers, and/or specific microscopes to obtain the concentration.

The selection of the working wavelength was performed using a Varian spectrophotometer, for scanning along the wavelengths in the region between 200 and 800 nm using an algal solution of *R. subcapitata* in the concentration of 10^7 cells mL^{-1}, previously determined using a counting chamber (Fuchs-Rosenthal chamber). According to the spectrum obtained (Figure S1), the working λ = 685 nm was chosen for algae concentration, the same wavelength determined by Souza [1].

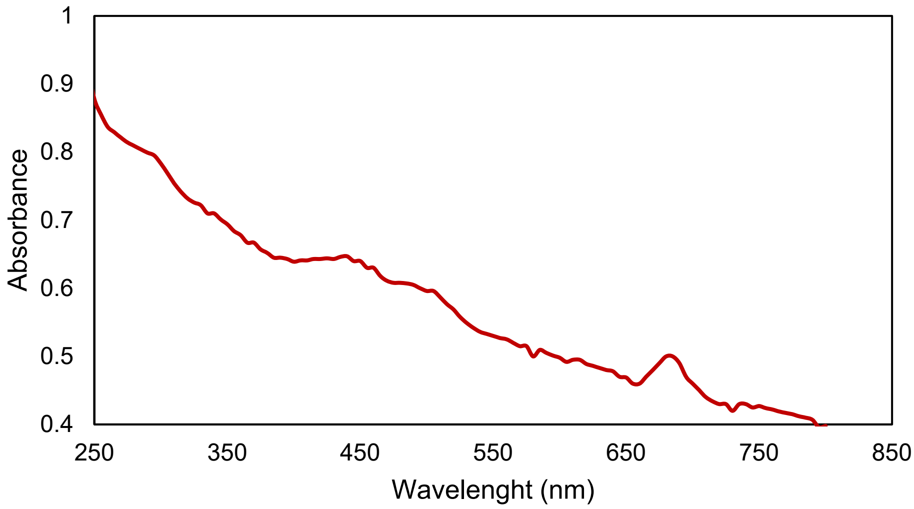
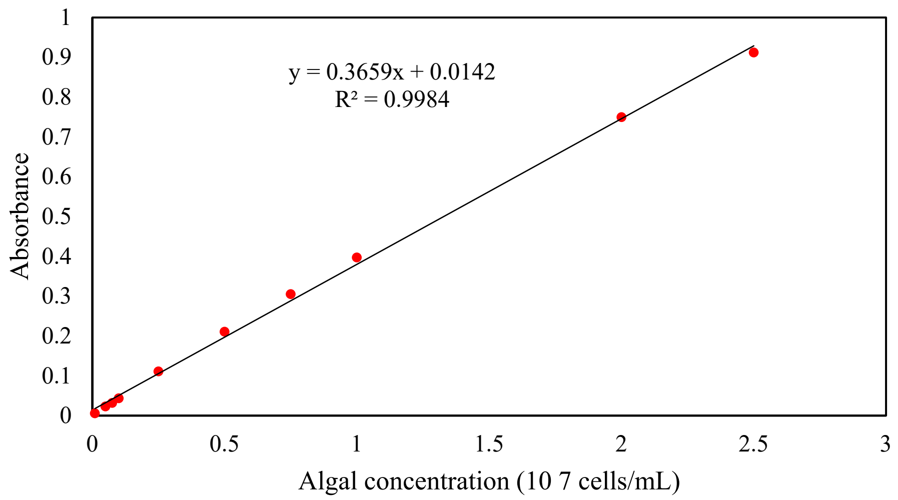
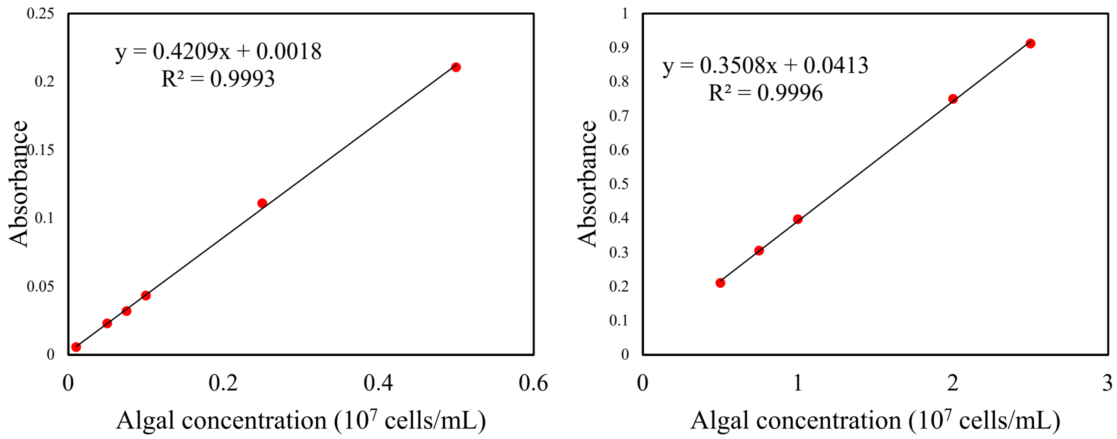


Figure S1. Absorbance obtained by Varian spectrophotometer.

The results obtained by the relation between absorbance and algal concentration using linear regression were organized as graphs to obtain the calibration curve in two ways, as shown in Figure S2. The first curve (Figure S2a) was constructed with all the concentration measurement points. In contrast, two distribution point curves of lower concentration values were plotted at a minimum of 5 points (Figure S2b) and higher concentrations, as seen in Figure S2c.



(a)



(b) (c)

Figure S2. Calibration curve using cell concentration and absorbance values measured in Femto Spectrophotometer (wavelength 685 nm). a) curve containing all measured points b) curve with low concentrations (0.01 to 0.5 × 10^7 cells mL^{-1}) c) curve with high concentrations (0.5 to 2.5 × 10^7 cells mL^{-1}).

Thus, by checking the correlation coefficient obtained through the equation of the line obtained by constructing a linear curve, it is possible to observe the best coefficients and, consequently, the best curves are those separated into two concentration curves. Souza [1] also compares the construction of two curves and observes the same behavior. To do so, the use of Absorbance and concentration ratios for a growth curve construct of *R. subcapitata* was performed, to optimize counting of homogeneous algae solution (e.g., internal production and control of cultures, as food for organisms like *D. similis*, also cultivated at this laboratory).

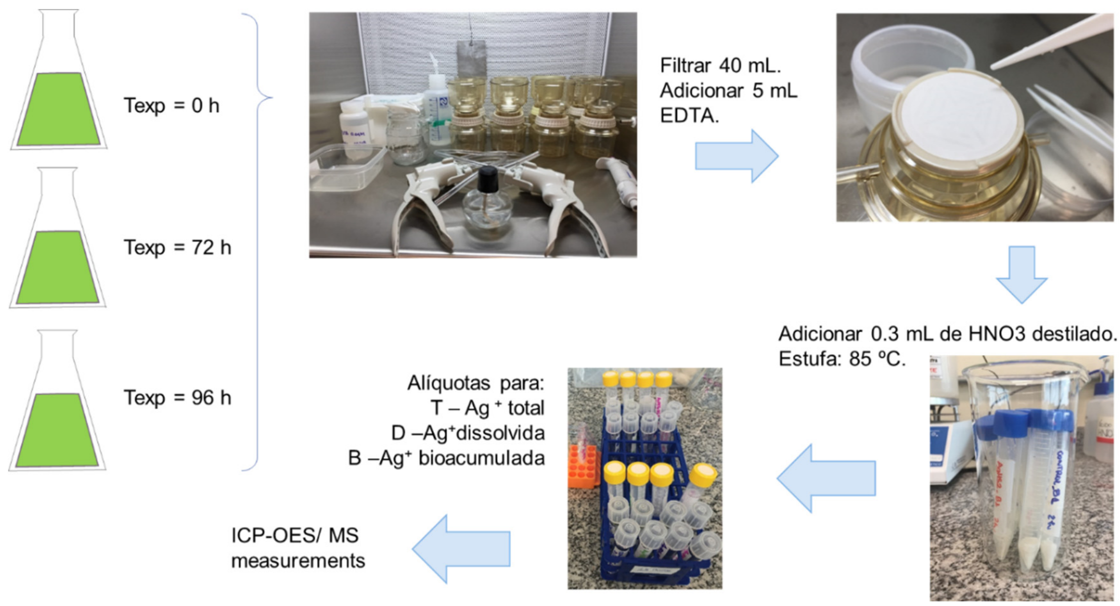


Figure S3. *R. subcapitata* bioaccumulation assay schedule.

For Daphnia medium exposure solution (Table S4), soft reconstituted water (hardness) was adopted from OECD 202.

Table S4. Composition of Daphnia medium exposed solution.

Reagents	V_{Stock} /L	V_{Total} /L	C_{teoric}/M
$Ca(SO)_4{\cdot}2H_2O$	0.022	1	1.92 × 10^{-4}
KCl	0.011	1	2.95 × 10^{-5}
$MgSO_4{\cdot}7H_2O$	0.011	1	2.72 × 10^{-4}
$NaHCO_3$	0.011	1	6.29 × 10^{-4}

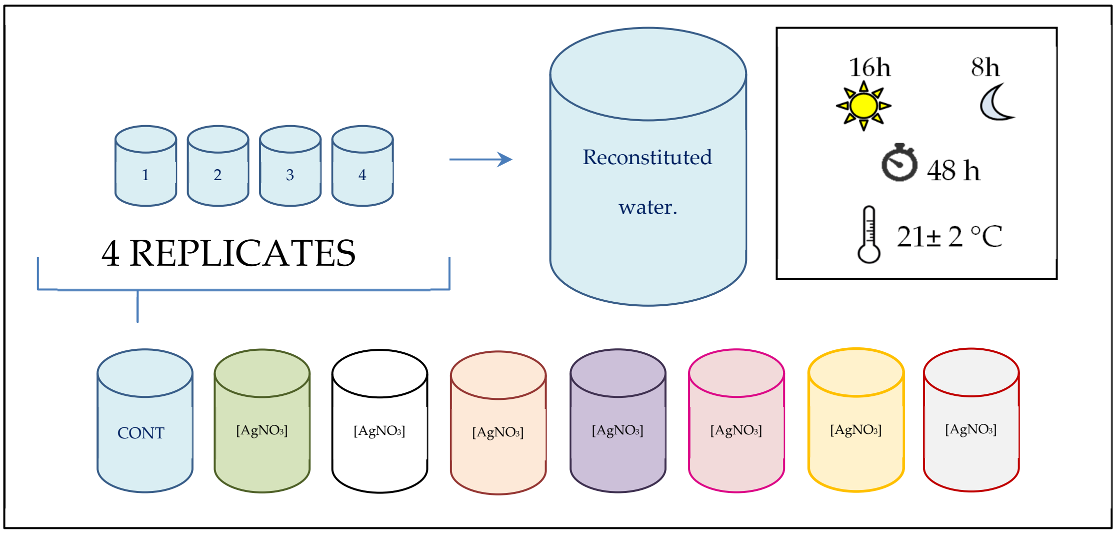


Figure S4. Definitive test layout for D. similis. Five concentrations ($AgNO_3$) plus one Control under temperature, photoluminescence, and experiment time controlled conditions.

Sensitivity test using reference toxicant (Daphnia similis)

The data of the sensitivity test expressed in EC_{50} obtained using NaCl as a reference toxicant is available in Figure S5. The value is similar to the reference values for the same substance from the original laboratory, attesting to its health and good acclimatization.

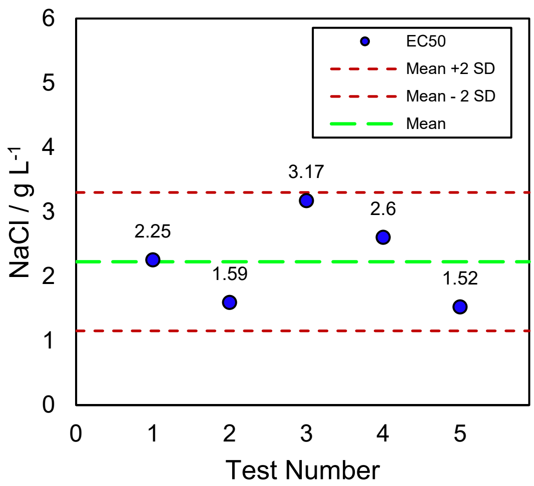


Figure S5. Control chart of EC_{50} Daphnia similis using NaCl as toxicant reference substance.

Table S5. General results of toxicity assays using *Daphnia similis*.

$AgNO_3$ – EC_{50}

Concentration	Survival/%	Offspring/neonate female^{-1}	SDOffspring	pHMean	pHSD	ODMean	ODSD	Conductivity	ConductivitySD
Control	90	17	3	7.58	0.02	-	-	-	-
1	90	17	3	7.55	0.01	-	-	-	-
2	100	9	3	7.62	0.00	-	-	-	-
5	70	4	1	7.52	0.01	-	-	-	-
10	10	0	0	7.63	0.00	-	-	-	-
25	0	0	0	7.66	0.00	-	-	-	-
50	0	0	0	-	-	-	-	-	-
100	0	0	0	-	-	-	-	-	-

AgCit – EC_{50}

Concentration	Immobility / %	pHMean	pHSD	ODMean	ODSD	Conductivity	ConductivitySD
0	0	7.74	0.02	5.63	0.23	187.65	0.85
1	0	7.60	0.07	4.45	0.23	187.15	4.23
5	10	7.72	0.01	4.25	0.18	188.80	0.50
10	10	7.73	0.01	4.08	0.14	189.50	0.75
50	100	7.59	0.21	3.73	0.11	189.63	0.04
100	100	7.71	0.01	3.68	0.13	191.58	1.83

AgPeg – EC_{50}

Concentration	Immobility / %	pHMean	pHSD	ODMean	ODSD	Conductivity	ConductivitySD
0	0	7.74	0.02	5.63	0.23	187.65	0.85
10	0	7.60	0.02	3.58	0.18	190.80	1.35
50	10	7.60	0.02	3.50	0.20	264.43	111.84
100	5	7.46	0.01	2.85	0.23	190.70	1.35
250	95	5.64	0.23	2.70	0.20	193.60	0.90
500	100	3.90	0.01	2.88	0.04	237.48	0.43

AgNP 1 µg·L^{-1}

Concentration	Survival / %	Offspring / neonate female-1	SDOffspring	pHMean	pHSD	ODMean	ODSD	Conductivity	ConductivitySD
Control	97	79	11	7.56	0.02	5.82	0.52	-	-
AgCit1	80	99	6	7.45	0.17	5.43	0.03	-	-
AgCit1HS1	87	94	9	7.49	0.12	4.75	0.42	-	-
AgCit1HS2	97	80	11	7.47	0.08	4.80	0.37	-	-
AgPeg1	97	78	17	7.44	0.09	5.00	0.33	-	-
AgPeg1HS1	97	90	9	7.45	0.07	5.28	0.55	-	-
AgPeg1HS2	83	84	26	7.43	0.07	5.37	0.20	-	-

AgNP100 µg·L^{-1}

Concentration	Survival / %	Offspring / neonate female-1	SDOffspring	pHMean	pHSD	ODMean	ODSD	Conductivity	ConductivitySD
Control	95	48	12	5.58	2.79	3.08	2.06	-	-
AgCit100	0	0	0	3.89	3.89	0.00	0.00	-	-
AgCit100HS1	0	3	0	5.05	3.37	4.45	4.45	-	-
AgCit100HS2	15	5	2	5.61	2.80	3.64	2.43	-	-
AgPeg100	50	31	18	5.48	2.74	3.41	2.28	-	-
AgPeg100HS1	30	33	2	5.38	2.69	4.80	3.20	-	-
AgPeg100HS2	70	45	15	5.31	2.65	3.26	2.17	-	-

References

1. Souza, M.B. Avaliação da toxicidade aguda de um herbicida comercial e dos componentes químicos Diuron e Hexazinona em *Ceriodaphnia dubia*. PhD Thesis, Universidade de Ribeirão Preto, Ribeirão Preto, Brazil, 2012.