Research Article

Received: 23 December 2020 Revised: 25 February 2021 Accepted article published: 2 March 2021 Published online in Wiley Online Library:

(wileyonlinelibrary.com) DOI 10.1002/jctb.6710

Silver nanoparticle filter for domestic wastewater reuse

Ana M Gagneten,[a*] Natalí Romero,[a,b] Ulises Reno,[a,b] Luciana Regaldo,[a,b] Silvina V Kergaravat,[a,b,c] Boris E Rodenak-Kladniew[d] and Guillermo R Castro[e,f*]

Abstract

BACKGROUND: Under scarcity of freshwater, the reuse and low-cost technological solutions applied to wastewaters seek to reduce contamination to the users and freshwater biota.

RESULTS: A low-cost cellulose membrane was doped with silver nanoparticles to filter urban wastewater (UW) from a city in Argentina. The total amount of coliforms and *Escherichia coli* in the filter decreased by 99.6% and 99.9%, respectively. The leak of silver from the filter was 275 ng L^{-1}, analyzed by square wave anodic stripping voltammetry. Silver nanoparticles tested on HepG2 and A549 mammalian cell lines showed no toxicity in a broad concentration range. Calculation of the organic matter provided by dead bacteria post-filtration was 347 µg L^{-1} proteins, 148 µg L^{-1} nucleic acids, 57 µg L^{-1} lipids, and 53 µg L^{-1} polysaccharides, indicating high availability of organic matter. The retention of inorganic salts in the filter was 78.5% ammonia, 6.2% nitrates, 97.6% nitrites, and 19.2% phosphates. In post-filtered UW, the *Lactuca sativa* germination test showed early seed germination between 90% and 95% in all the dilutions tested. In the range of 6.25% to 50.0%, filtered UW showed no significant differences in the hypocotyl but the difference was significant in the radicle length (mm) compared to the control made of synthetic media ($P < 0.05$).

CONCLUSION: The development of a low-cost filter based on cellulose membranes doped with silver nanoparticles allowed the reuse of wastewater for domestic purposes and garden irrigation.

Keywords: silver nanoparticles; biocide filter; remediation; wastewater treatment; *E. coli*. coliforms

INTRODUCTION

Freshwater scarcity is among the greatest environmental problems currently being faced worldwide. It has been predicted that the number of people living with limited water access will increase to 3.5 billion by 2025. Domestic reclaimed water is an option that is being studied and increasingly adopted in areas with water shortages, arid regions, or far away from cities.[1] Novel strategies must be developed to deal with this problem, and wastewater reuse is thought to play a key role, especially in peri-urban, semirural, or rural areas where municipal sewage systems and tap water are not available, and in developing countries where central services have yet to reach most of the population.[2] Finally, small units for recycling water could be used for domestic purposes and gardening. Reusing water is not the only option to balance supply and demand, but in many cases, it is also a cost-effective solution. Urban and domestic wastewaters usually contain a high bacterial concentration that should be diminished before the water can be discarded into the environment or reused for different purposes. According to the World Health Organization (WHO) guidelines, the number of total coliforms employed for crop irrigation (including those expected to be eaten raw and those used in public parks or sports fields) must be lower than 1000 per 100 mL.[3]

---

* Correspondence to: AM Gagneten, Laboratorio de Ecotoxicología, Facultad de Humanidades y Ciencias, Santa Fe, Argentina, E-mail: amgagneten@gmail.com; or GR Castro, Laboratorio de Nanobiomateriales, CINDEFI, Departamento de Química, Facultad de Ciencias Exactas, Universidad Nacional de La Plata – CONICET (CCT La Plata), Buenos Aires, Argentina. E-mail: grcastro@gmail.com

a Laboratorio de Ecotoxicología, Facultad de Humanidades y Ciencias, Universidad Nacional del Litoral, Santa Fe, Argentina

b Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Santa Fe, Argentina

c Laboratorio de Sensores y Biosensores, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina

d Facultad de Ciencias Médicas, Instituto de Investigaciones Bioquímicas de La Plata (INIBIOLP), CONICET-UNLP, CCT-La Plata, La Plata, Argentina

e Laboratorio de Nanobiomateriales, CINDEFI, Departamento de Química, Facultad de Ciencias Exactas, Universidad Nacional de La Plata – CONICET (CCT La Plata), Buenos Aires, Argentina

f Max Planck Laboratory for Structural Biology, Chemistry and Molecular Biophysics of Rosario (MPLbioR, UNR-MPIbpC), Partner Laboratory of the Max Planck Institute for Biophysical Chemistry (MPIbpC, MPG), Centro de Estudios Interdisciplinarios (CEI), Universidad Nacional de Rosario, Rosario, Argentina

In recent years, the need to develop efficient and economically convenient methods for the remediation and reuse of urban and household wastewaters has motivated the study of new technologies. For this purpose, current wastewater technologies require either the development of several steps in artificial ponds to stabilize and degrade microbes and viruses or the use of complex systems that need to be supervised, maintained, and are time-consuming. Some wastewater treatments contain chemical disinfectants (such as chlorine or UV lights) and require strong aeration by micro- and nanobubble systems, in addition to open decanter tanks and many filtration units. Classical approaches are based on membrane technology, such as ultrafiltration and inverse osmosis.[4] However, these technologies are particularly expensive for domestic applications or intensive use and they require further treatment of the water before disposal because of the high number of microbes and viruses. A critical and comprehensive review of emerging trends in sustainable membrane-based wastewater treatment was published elsewhere.[5]

The biocidal activity of silver has been described since the beginning of human society. More recently, the synthesis of silver nanoparticles (AgNPs) has opened a new spectrum of applications in many therapeutic and sanitizing devices because of their high antimicrobial and virucidal activity.[6] Recently, AgNPs have been used for water treatment in suspension, with the disadvantage of the potentital silver release to the environment along with the treatment.[7] Another strategy was to immobilize AgNPs in inorganic supports, such as graphene[8] or polyelectrolytes cross-linked with glutaraldehyde.[9]

Cellulose and its derivatives are being used for different technological purposes, including nanofibers, nanocrystals, and membrane filters of different types.[10] The main advantages of cellulose devices are the lack of toxicity, ease of scale-up, low cost, and biodegradability.

The toxicity of wastewater is mainly evaluated by acute and chronic bioassays through algae, higher plants, and aquatic invertebrates as sentinels. Microalgae are unicellular organisms and key components as primary producers of aquatic food web structure and functionality, so their damage may impact the entire ecosystem. *Chlorella vulgaris* is a member of eukaryotic green microalgae that multiplies asexually within 24 h. It is also extremely sensitive to changes in its environment, allowing the evaluation of toxic effects of pollutants over several generations. *C. vulgaris* is widely used as a model aquatic organism for toxic studies, to evaluate technological processes, and in regulatory tests.[11]

The advantages of cladocerans in toxicological assays are parthenogenetic reproduction without genetic modifications, short life cycle, ease of cultivation under laboratory conditions, and high sensitivity to toxic substances. For these reasons, top regulatory agencies and international organizations developed standard toxicological methods based on cladocerans. The cladoceran *Ceriodaphnia dubia* has been used extensively in aquatic toxicity tests for decades.[12]

The present work aimed to develop a cellulose filter doped with silver nanoparticles for the treatment of urban wastewater (UW) effluent to be used at a domestic scale for gardening or household applications, intensive use in sanitary units, etc. AgNPs were synthesized, and their toxicity was tested on mammalian cell cultures. The UW effluent was physicochemically characterized by pH, salinity, conductivity, dissolved oxygen, total and dissolved suspended solids; the amount of silver in the filtered UW sample and the ammonium, nitrates, nitrites, and phosphates of the effluent were also determined. The Ag-filter biocidal activity was evaluated on total coliforms, and *E. coli* and biomolecules released to the medium were estimated. Post-filtered UW samples were studied for toxicity using freshwater biological models (i.e., *C. vulgaris* and *C. dubia*), and *Lactuca sativa* was used to analyze early seed germination and growth.

MATERIALS AND METHODS

Reagents and media

Silver nitrate (99.9%), polyvinylpyrrolidone (PVP, MW ≈ 40 kDa, $BiNO_3$, and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals, media, and reagents were of analytical grade from Merck, Oxoid, Gibco, or similar and used without further purification. A buffer composed of 100 mM NaCl and 100 mM acetic acid/acetate (pH = 4.0) was utilized in the electrochemical measurements.

Wastewater sampling

Urban wastewater samples were collected from the wastewater treatment plant located in San Justo city, Santa Fe province, Argentina (30°78′93″ S, 60°62′09″ W). The sampling was performed from a secondary facultative lagoon of UW treatment that is 50 m wide, 100 m long, and 2.20 m deep. A 20 L sample was taken in two 10 L drums from the outlet of the lagoon. The sampling was carried out at the manhole about 5 cm under the lagoon surface. One 10 L sample was used for filtration and microbiological analysis, cell toxicity tests, and *L. sativa* growth. The other intact 10 L sample was kept at 4 °C for further analysis involving physicochemical characterization, the chemical composition of major components, and microbiological analysis of the effluent. All assays were performed in triplicate on each sample.

Effluent physicochemical characterization

Temperature, pH, salinity (ng L^{-1}), conductivity ($\mu\text{S cm}^{-1}$), dissolved oxygen (DO, mg L^{-1}), total suspended solids (TSS, mg cm^{-1}), and total dissolved solids (TDS, mg L^{-1}) were measured with a YSI Professional Plus, 6050000 water quality multiparameter instrument (Ohio, USA).

Determination of *Escherichia coli* and total coliforms

The microbial quantification of total coliforms and *E. coli* was performed by the most probable number (MPN), as outlined in Standard Methods.[13] Briefly, samples inoculated in lauryl tryptose (LST) broth medium were diluted 100- and 1000-fold for MPN in five test tubes and incubated at 35 °C for 24–48 h. A positive result was indicated by the presence of acid and gas production in the Durham tube. *E. coli* was quantified in LST supplemented with the fluorogenic substrate 4-methylumbelliferyl-ß-D-glucuronide (MUG) incubated at 35 °C for 18–24 h.[13]

Culture conditions and toxicity test with *Chlorella vulgaris*

Chlorella vulgaris CLV2 (BCN, Mx.) was cultivated in 2 L Erlenmeyer flasks under sterile conditions using Bold´s Basal Medium (BBM), 8000 lx/lx, and 100 rpm at 23 ± 1 °C.[14] The algal growth inhibition test was performed as recommended by OECD.[15] The experiments were carried out with 10^4 cells/mL. Five UW dilutions (6.2%, 12.5%, 25.0%, 50.0%, and 100.0%) plus a negative control were evaluated in triplicate. Three replicates of 100 µL per sample were counted in the Neubauer chamber at 24, 48, and 72 h. In all instances, at least 25 squares were counted to ensure errors lower than 10%.[14] The considered endpoints were the cell density and algal growth inhibition (percentage of inhibition, 1%), estimated according to OECD.[15]

Culture conditions and toxicity test with *Ceriodaphnia dubia*

Ceriodaphnia dubia was collected from the Parana River alluvial valley and individually maintained with 30 mL of synthetic medium in glass beakers. Animals were fed three times a week with a drop of algal suspension (*Scenedesmus obliquus*, A_{650} = 1.5) for each culture chamber. Twenty neonates (<24 h) of *C. dubia* were divided into five groups of individuals for each treatment and negative control (without effluent). The test organisms were exposed to five UW dilutions: 6.2%, 12.5%, 25.0%, 50.0%, and 100.0%. Results were considered acceptable when mortality in the control group was lower than 10% at 48 h.

Nanoparticle synthesis

Silver nanoparticles were synthesized by a chemical reduction method using PVP in the presence of NaOH, as previously reported.[16]

Mammalian cell lines and culture conditions, cell viability, and proliferation

Human hepatoma (HepG2) (ATCC® HB-8065™) and human alveolar adenocarcinoma (A549) (ATCC CCL-185) cells were cultured as previously reported.[17] After confluence, cells were incubated in DMEN supplemented with different concentrations of AgNPs, and later, the cytotoxic effect was determined with MTT. The results were expressed as a percentage of cell viability compared to the control without AgNPs.

Filter device and filtration

The 412 mL filter was made of cellulose (pore diameter < 10 μm) coated with phenolic and polyurethane resins and was immersed in 500 mL AgNPs at 25 °C with stirring for 4 h. Later, the filter was dried at room temperature for 24 h and washed 10 times with Milli-Q water before use. Three UW effluents of 3-l subsamples were filtered and kept at 4 °C for further analysis.

Quantification of Ag(I) by SWASV

The electrochemical determinations of Ag(I) were performed with a voltammetric analyzer Epsilon BAS, Bioanalytical Systems Inc. (West Lafayette, IN, USA). The method is based on Ag(I) detection on SPE modified with bismuth film (BiFE) by SWASV. BiFE was plated *in situ* by deposition of a drop containing 0.1 μg L^{-1} of Bi(III) and the Ag(I) solution in working buffer, as previously reported.[18] Triplicates of the effluent were tested for the presence of silver ions (Ag(I)) before and after filtration.

Lactuca sativa assays

The post-filtered UW samples were diluted in serial concentrations: 6.2%, 12.5%, 25.0%, 50.0%, and 100.0% with proper control.[14] For each triplicate experiment, 20 seeds were placed in a 9 cm diameter glass Petri dish with filter paper in the bottom; 4 mL of the different dilutions was poured into the plate and then incubated in darkness at 20 ± 2 °C for 120 h. The endpoints were: (i) seed germination (SG, % of germinated seeds after the experiment); (ii) root length (RL), and (iii) hypocotyl length (HL).[19]

Table 1. Physicochemical parameters of San Justo city urban wastewater

Parameters	Values
Temperature (°C)	18 ± 2
pH	8.1 ± 0.1
Salinity (ng L^{-1})	0.53 ± 0.03
Dissolved oxygen (mg L^{-1})	8.2 ± 0.1
Conductivity (μS cm^{-1})	958 ± 6
TSS (mg L^{-1})	1140 ± 103
TDS (mg L^{-1})	739 ± 76

TSS, total suspended solids; TDS, total dissolved solids.

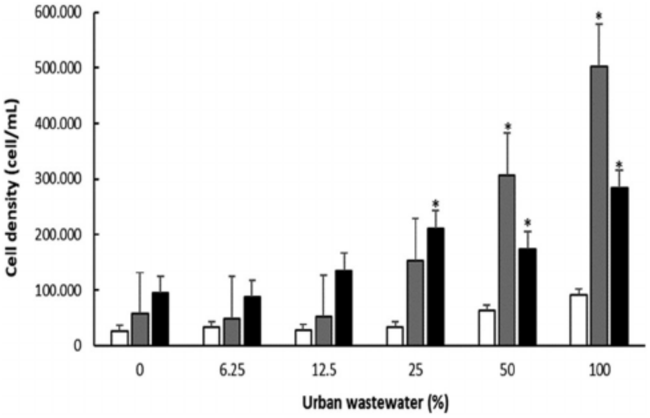

Figure 1. Effect of urban effluent concentration on *Chlorella vulgaris* growth at times 24 (▭), 48 (▨), and 72 (■) h. Error bars indicate the standard deviation (three replicates per treatment and control (without UW). An asterisk (*) denotes significant differences to the control ($P < 0.05$).

Statistical evaluation of the data

One-way ANOVA or the Kruskal-Wallis test was carried out after checking the normality and homoscedasticity of the data. Statistical analyses were carried out using the software SigmaPlot 12.0.

RESULTS AND DISCUSSION

The physicochemical characteristics of the UW effluent from the San Justo city wastewater treatment plant are summarized in Table 1. The effluent temperature and pH were 18 ± 2 °C and 8.1 ± 0.1, respectively, both of which are in the range of Santa Fe state limits for wastewater discharge (pH range 7.5 to 8.5).[20] The high average value of dissolved oxygen was 8.2 ± 0.1 mg L^{-1}, which is enough for the aerobic degradation of the organic matter and suitable for aquatic organisms. Salinity and electric conductivity values were 0.53 ± 0.03 ng L^{-1} and 958.33 ± 6.51 μS cm^{-1}, respectively, and the TSS concentration of the effluent was about 1140 ± 103 mg L^{-1}.

Chlorella vulgaris growth was not inhibited in the presence of 6.25% to 100% effluent. No significant differences in algae growth between the two lowest effluent concentrations (6.2% and 12.5%) and the control were found ($P > 0.05$). Conversely, effluent concentrations higher than 25% and up to 100% produced a significant stimulation of cell proliferation ($P < 0.05$) between 48 and 72 h (Fig. 1). Consequently, the effluent was identified not only as nontoxic to the microalgae but also as a stimulant of *C. vulgaris* growing at 25% and higher concentrations. Similar results on

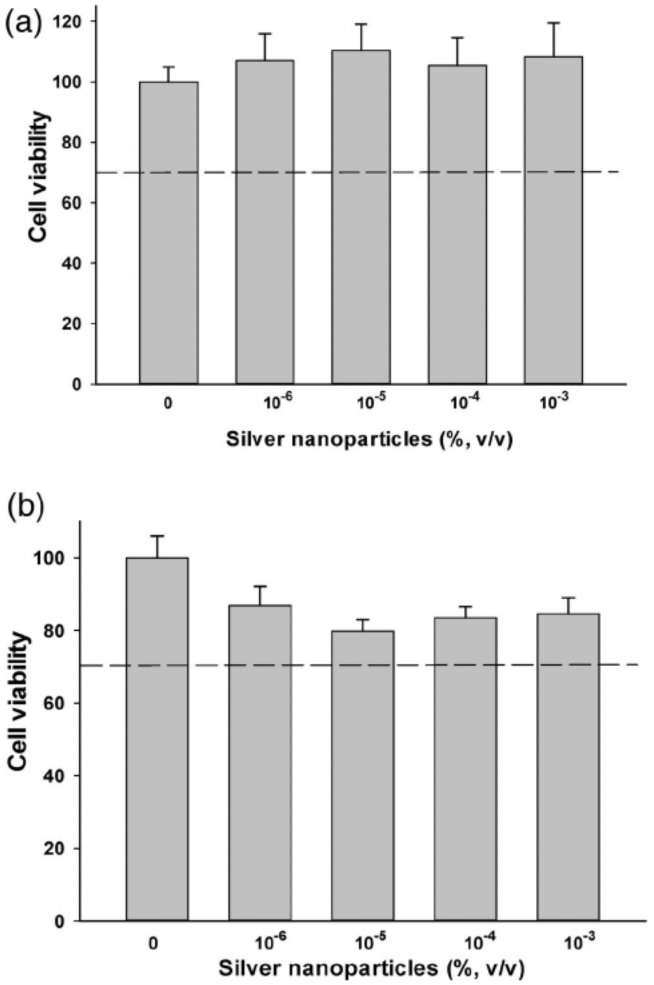

Figure 2. Effect of silver nanoparticle concentrations on HepG2 (a) and A549 (b) cells viability at 48 h. Dotted lines indicate the 70% of viability; below that value, the formulation is considered as cytotoxic. Concentration of 1×10^{-3}% (v/v) AgNPs is equivalent to 0.345 mg L^{-1} Ag.

C. vulgaris growth in the presence of 25% to 100% UW were reported elsewhere.[21] The *C. vulgaris* mixotrophic growth conditions generated more biomass than the autotrophic conditions.[22] In mixotrophic cultures, carbon assimilation occurs from organic compounds; however, in photoautotrophic cultures carbon dioxide (CO_2) is the unique source of carbon.[14] Similar results were found for the cladoceran *C. dubia* in treatments with the effluent at 48 h. Besides the lack of effluent toxicity to the microalgae and the cladoceran, the microbial content of the prefiltered UW effluent was in the order of 10^6 cells/mL (Table 2). Regulatory organizations, such as the Environmental Protection Agency (Guidelines for Water Reuse AR-1530. EPA/600/R-12/618, US) and the European Union (Towards a legal instrument on water reuse at EU level), have established a concentration of *E. coli* below 200 CFU/100 mL and 100 CFU/100 mL for domestic uses, respectively.[23,24] Also, the EU recommends a concentration of *E. coli* lower than 1000 CFU/100 mL for drip irrigation. [24]

The synthesis of AgNPs in alkaline PVP was previously reported,[16] and toxicity tests of AgNPs on the human liver (HepG2) and lung (A549) cell lines showed no toxicity up to 0.365 mg L^{-1} in HepG2 cells (Fig. 2(a)). Similar results were previously reported using AgNPs capped with polyethyleneimine,

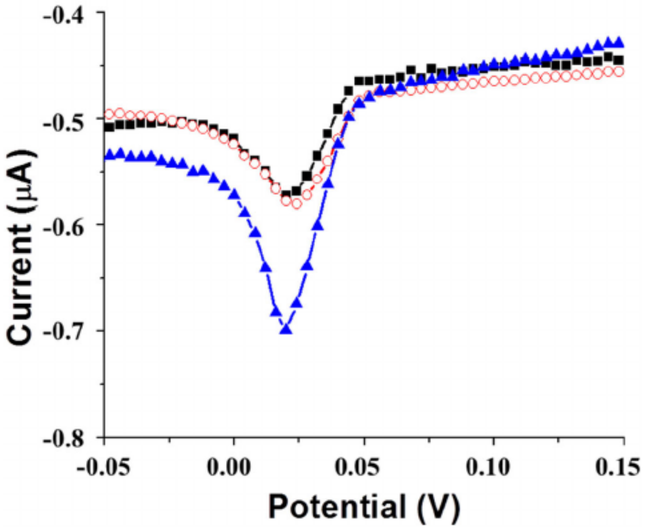

Figure 3. SWASV of the Ag(I) response on BiFE of the blank solution (■), pre-filtered (○), and post-filtered (▲) UW. Experimental conditions: 100 mM NaCl 100 mM acetic acid/acetate buffer (pH = 4.0). Parameters: accumulation at −1.278 V for 60 s, equilibration step of 2 s, square-wave anodic stripping voltammetry scan with potential ranging from −0.6 to 0.2 V, frequency of 25 Hz, amplitude of 25.0 mV, and potential step of 5 mV.

reaching the toxicity limit at concentrations lower than 1 mg mL^{-1}.[25] Besides, AgNPs slightly but not significantly diminished A549 cell growth in a concentration-independent manner according to the ISO 10993-5 standard,[26] establishing the cytotoxicity limit at 30% of cell death (Fig. 2(b)). These results demonstrate that AgNPs are nontoxic to the mammalian cells tested in a wide range of concentrations.

Later, AgNP suspension containing 365 mg L^{-1} Ag(I), measured by SWASV (CV = 7%, n = 3), was used to dope the filter. Due to possible environmental and human health effects from silver exposure, the silver content of the effluent post-filtration was also analyzed (Fig. 3). The leaked Ag(I) concentration in filtered UW was 275 ± 34 ng L^{-1} Ag(I) (CV = 12%, n = 3), which is only 0.007% of the original silver concentration and less than the WHO (2008) guideline limits for drinking water (<0.10 and 0.05 mg L^{-1}, respectively) and the EPA surface water quality criteria ($\leq$0.07 mg L^{-1}). The analysis of urban wastewater effluents in the US and Europe displayed a wide range of AgNPs, from 1.0 ng L^{-1} to 0.2 μg L^{-1}.[27] Also, cellulose is a nontoxic and environmentally biodegradable polymer.[28] Furthermore, the resin of the cellulose filter do not pose a health risk, as many industries use them for multiple purposes. Since many resins are frequently used for dental, pharmaceutical applications, and drinking-water treatment .[29] These results strongly suggest that the silver-treated effluent is nontoxic and make it possible to consider the reuse of the effluent for domestic purposes.

The antimicrobial activity of the AgNPs-filter showed a reduction of 99.9% of total coliforms and 99.6% of *E. coli* in the effluent (Table 2). Particularly, the *E. coli* concentration in the effluent post-filtration was 8.69, 86.95, and 867.56 folds lower than the maximum levels allowed by the EPA and the EU (Table 2). Notably, the reduction in the *E. coli* concentration in the effluent was obtained without any chemical treatment, therefore avoiding the use of toxic substances, such as chlorine. Besides, in the treatment with chlorine, the treated UW exceeded 0.6 times the

Table 2. Filter efficiency on bacterial removal of San Justo city urban wastewater

	Bacteriological counts (CFU/100 mL)		
Microorganisms	Pre-filtration	Post-filtration	Removal Efficiency (%)
Total Coliforms	2.1×10^6	330	99.9
Escherichia coli	3.0×10^3	11	99.6

Table 3. Average and standard deviation of root length and hypocotyl length in UW dilutions and the control

	Average and standard deviation	
UW dilutions (%)	Root length (mm)	Hypocotyl length (mm)
100.00	15.9 ± 3.9	28.0 ± 6.1
50.00	20.6 ± 7.0	28.3 ± 6.2
25.00	20.7 ± 5.4	28.6 ± 5.1
12.50	19.9 ± 4.4	27.7 ± 4.6
6.25	20.9 ± 7.5	26.0 ± 7.3
0.00 (Control)	21.0 ± 11.0	28.0 ± 8.7

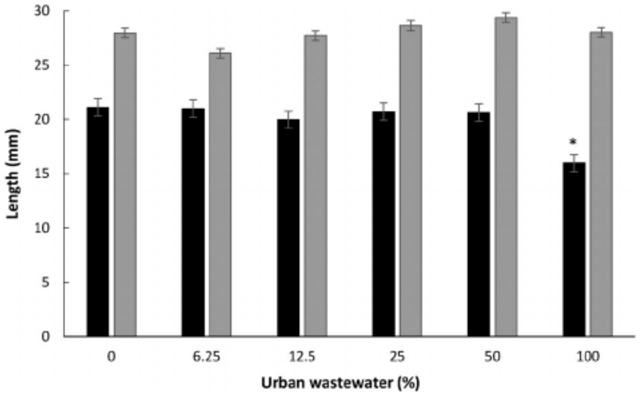

Figure 4. Effect of filtered urban effluent concentrations on the length (mm) of the root (■) and hypocotyl (▨) of *Lactuca sativa*. Error bars indicate the standard deviation (three replicates per treatment and control. Asterisk (*) denotes significant differences to the control ($P < 0.05$).

guideline levels of total fecal coliforms. In this context, a filter containing AgNPs as a biocide was developed to test the potential reuse of the effluent for household uses.

Similar results were reported using cellulose blotting sheets doped with AgNPs to purify drinking water with a log decay of 6 and 3 for *E. coli* and *E. faecalis*, respectively.[30] Similarly, AgNPs adsorbed on a film made of weak electrolytes cross-linked with glutaraldehyde showed a reduction of 93% coliforms in industrial wastewater after 60 h of treatment.[9]

The interaction of AgNPs with *E. coli* observed by TEM showed the attachment of the nanoparticles to the outer membrane, followed by cell internalization and bacterial lysis.[29] In another work, AgNPs tested on the Gram-positive *S. aureus* and *B. cereus*, and on the Gram-negative *E. coli* and *P. aeruginosa* showed high antimicrobial activity; particularly, TEM images revealed the incorporation of AgNPs inside *P. aeruginosa* cells, producing lysis.[31]

The lack of toxicity of filtered UW effluent in plants was tested in *L. sativa*. Early seed germination in post-filtered UW ranged from 90% to 95% in all the dilutions tested. Similar results were observed in the control, with a seed germination percentage above 90% (Table 3). The results showed that the growth of *L. sativa* roots was sensitive to undiluted post-filtered effluent (100%) with significant differences compared with the control made in minimal synthetic media ($P < 0.05$) (Fig. 4). This could be because the root system is more susceptible to environmental conditions than the sprouting system.[32] Similarly, half UW dilution used for watering *Solanum lycopersicum*, *L. sativa*, and *Raphanus sativus* showed a significant root decrease.[33] On the contrary, no differences were observed in the hypocotyl length (mm) in the presence of UW dilutions and the control (Fig. 4). The positive effects on *L. sativa* germination and growth, as in the control group, could be due to the high concentrations of inorganic nutrients (such as nitrogen and phosphorus) and organic molecules from bacteria in the post-filtered effluent. The high nutritional potential of wastewater stimulated seed germination and growth, measured as root and hypocotyl elongation. In previous work, municipal wastewater used to water *L. sativa* was found to be nontoxic.[34]

The removal efficiency of major inorganic components present in the effluent by the AgNP doped filter was: 78.5% ammonium, 6.2% nitrate, 97.6% nitrite, and 19.2% phosphate (Table 4). The high content of nitrate (93.8%) and phosphate (80.8%) in the post-filtered wastewater suggests the effective application of AgNPs-filter for the treatment of wastewaters. Similarly, UW treated with soil/sand filters showed 0.06 mg L^{-1} ammonium and 1.48 mg L^{-1} nitrate in the filtrate of *Likovrisis* plant (Attica, Greece).[35]

Table 4. Filter performance of inorganic components removal

	Concentration (mg L^{-1})		
Components	Pre-filtration	Post-filtration	Removal Efficiency (%)
Ammonium	3.4	0.7	78.5
Nitrate	3.1	2.9	6.2
Nitrite	24.5	0.6	97.6
Phosphate	14.6	11.8	19.2

Additionally, the biocidal effect of AgNPs is well known; they cause cell lysis and the subsequent release of biological molecules to the media, which are an excellent source of varied organic nutrients that can be easily assimilable by cells and/or biodegradable, improving the soil quality. The theoretical macromolecular composition and cell weight of *E. coli* were reported previously; it is composed of 55.0% proteins, 20.5% RNA, 3.1% DNA, 9.1% lipids, and 8.4% of various polysaccharides (i.e., lipopolysaccharides, peptidoglycan, and glycogen), with a unitary mass of 3×10^{-13} g/bacterial cell.[36] Considering these parameters and the bacterial cell concentration in the pre-filtered and post-filtered UW, and assuming that total coliforms (which include members of the genera *Escherichia*, *Citrobacter*, *Enterobacter*, and *Klebsiella*) have similar macromolecular composition and cell weight, it is possible to estimate the number of macromolecules in the post-filtered UW. The metabolites contributing to post-filtered UW were roughly 347 $\mu g\ L^{-1}$ of proteins, 148 $\mu g\ L^{-1}$ of nucleic acids, 57 $\mu g\ L^{-1}$ of lipids, and 53 $\mu g\ L^{-1}$ of polysaccharides. These biological molecules and the inorganic components present in the filtrate can potentially be used for many purposes, including watering vegetables and gardening, as previously suggested.[37] Among the advantages of AgNPs-filter for UW treatment are reuse and the provision of essential organic nutrients for plant growth. Particularly, the low application of fertilizers and chemical compounds can be remarked.

The treated wastewater addition to soil increases the concentration of nitrogen, phosphorus,[37] and other compounds (K, Ca, Na, and Mg, among others),[38] and could be beneficial for both soil and plants by reducing the need for chemical fertilizers and improving soil fertility and crop productivity.[39] Also, wastewater reuse is a way to reduce pressure on freshwater resources.

CONCLUSIONS

This preliminary work emphasizes: (i) the toxicity testing of effluent on two biological models, (ii) the antibacterial efficiency of AgNPs doped in a cellulose filter, and (iii) the evaluation of its potential to be reused as a liquid fertilizer. The effluent of San Justo city does not show any toxicity to the microalgae *C. vulgaris* and the cladoceran *C. dubia*, but it has a high microbial content. The AgNPs-filter reduces the microbial concentration by 99% in coliforms and *E. coli*. The AgNPs-filtered UW effluent showed low silver leakage and was able to support the growth of *L. sativa* (lettuce). These promising results showed that the AgNP doped cellulose filter could be a simple and fast alternative for wastewater treatment with high biocidal activity and scalability. More experiments must be performed to confirm the advantages of AgNPs-filter for the treatment of UW.

ACKNOWLEDGEMENTS

This study was supported by grants from the *Secretaría de Políticas Universitarias* (Grant VT42-UNL11805), *Universidad Nacional del Litoral* (Grant CAI+D Or./1-11), *Universidad Nacional de La Plata* (Grant X-815), and *Consejo Nacional de Investigaciones Científicas y Técnicas*. We especially thank Ms. Julieta Arias for experimental assistance, *Cooperativa de Servicios Públicos*, *Sociales*, *de Asistencia Social y Vivienda* Ltd. (CSP), and Municipality of San Justo city for the provision of wastewater effluents.

CONFLICT OF INTERESTS

None.

REFERENCES

1 Munoz I, Tomas N, Mas J, Garcia-Reyes JF, Molina-Diaz A and Fernandez-Alba AR, Potential chemical, and microbiological risks on human health from urban wastewater reuse in agriculture. Case study of wastewater effluents in Spain. *J Environ Sci Health B* **45**: 300–309 (2010). https://doi.org/10.1080/03601231003704648.

2 Qadir M, Wichelns D, Raschid-Sally L, McCornick PG, Drechsel P, Bahri A *et al.*, The challenges of wastewater irrigation in developing countries. *Agric Water Manag* **97**:561–568 (2010). https://doi.org/10.1016/j.agwat.2008.11.004.

3 Blumenthal UJ, Mara DD, Peasey A, Ruiz-Palacios G and Stott R, Guidelines for the microbiological quality of treated wastewater used in agriculture: recommendations for revising WHO guidelines. *Bull WHO* **78**:1104–1116 (2000).

4 Ciardelli G, Corsi L and Marcucci M, Membrane separation for wastewater reuse in the textile industry. *Resour Conserv Recycl* **31**:189–197 (2001). https://doi.org/10.1016/S0921-3449(00)00079-3.

5 Yusuf A, Sodiq A, Giwa A, Eke J, Pikuda O, de Luca G *et al.*, A review of emerging trends in membrane science and technology for sustainable water treatment. *J Clean Prod* **266**:121867 (2020). https://doi.org/10.1016/j.jclepro.2020.121867.

6 Durán N, Marcato PD, Conti RD, Alves OL, Costa F and Brocchi M, Potential use of silver nanoparticles on pathogenic bacteria, their toxicity, and possible mechanisms of action. *J Braz Chem Soc* **21**:949–959 (2010). https://doi.org/10.1590/S0103-50532010000600002.

7 Bolaños-Benítez V, McDermott F, Gill L and Knappe J, Engineered silver nanoparticle (Ag-NP) behavior in domestic on-site wastewater treatment plants and in sewage sludge amended-soils. *Sci Total Environ* **722**:137794 (2020). https://doi.org/10.1016/j.scitotenv.2020.137794.

8 Firouzjaei MD, Shamsabadi AA, Sharifian Gh M, Rahimpour A and Soroush M, A novel nanocomposite with superior antibacterial activity: a silver-based metal organic framework embellished with graphene oxide. *Adv Mater Interfaces* **5**:1701365 (2018). https://doi.org/10.1002/admi.201701365.

9 Zarpelon F, Galiotto D, Aguzzoli C, Carli LN, Figueroa CA, Baumvol IJR *et al.*, Removal of coliform bacteria from industrial wastewaters using polyelectrolytes/silver nanoparticles self-assembled thin films. *J Environ Chem Eng* **4**:137–146 (2016). https://doi.org/10.1016/j.jece.2015.11.013.

10 Mokhena T and John M, Cellulose nanomaterials: new generation materials for solving global issues. *Cellul* **27**:1149–1194 (2020). https://doi.org/10.1007/s10570-019-02889-w.

11 Romero N, Visentini FF, Márquez VE, Santiago LG, Castro GR and Gagneten AM, Physiological and morphological responses of green microalgae *Chlorella vulgaris* to silver nanoparticles. *Environ Res* **189**: 109857 (2020). https://doi.org/10.1016/j.envres.2020.109857.

12 Kosmala A, Charvet S, Roger M-C and Faessel B, Impact assessment of a wastewater treatment plant effluent using instream invertebrates and the *Ceriodaphnia dubia* chronic toxicity test. *Water Res* **33**: 266–278 (1999). https://doi.org/10.1016/S0043-1354(98)00176-6.

13 Baird R, Eaton AD, Rice EW and Bridgewater L, *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, Aurora, CO (2017).

14 Reno U, Regaldo L and Gagneten AM, Circular economy and agro-industrial wastewater: potential of microalgae, in *Bioremediation Processes. Valorisation of Agro-Industrial Residues – Volume I: Biological Approaches*. New York: Springer, pp. 111–129 (2020).

15 OECD, Freshwater alga and cyanobacteria, growth inhibition Test (No 201). OECD Guidelines for the Testing of Chemicals (2011).

16 Kim J-S, Reduction of silver nitrate in ethanol by poly (N-vinylpyrrolidone). *J Ind Eng Chem* **13**:566–570 (2007).

17 Rodenak-Kladniew B, Islan GA, de Bravo MG, Durán N and Castro GR, Design, characterization, and in vitro evaluation of linalool-loaded solid lipid nanoparticles as potent tool in cancer therapy. *Colloids Surf B Biointerfaces* **154**:123–132 (2017). https://doi.org/10.1016/j.colsurfb.2017.03.021.

18 Kergaravat SV, Romero N, Regaldo L, Castro GR, Hernandez SR and Gagneten AM, Simultaneous electrochemical detection of ciprofloxacin and Ag (I) in a silver nanoparticle dissolution: application to ecotoxicological acute studies. *Microchem J* **162**:105832 (2020). https://doi.org/10.1016/j.microc.2020.105832.

19 Bagur-González MG, Estepa-Molina C, Martín-Peinado F and Morales-Ruano S, Toxicity assessment using *Lactuca sativa* L. bioassay of the metal (loid)s as, cu, Mn, Pb and Zn in soluble-in-water saturated

soil extracts from an abandoned mining site. *J Soils Sedim* **11**: 281–289 (2011). https://doi.org/10.1007/s11368-010-0285-4.

20 Anonymous. Regulation of quality control of sewage effluent discharge and disposal of biosolids (in Spanish). ENRES 0324. Santa Fe, Argentina, (2011).

21 Znad H, Al Ketife AM, Judd S, Al Momani F and Vuthaluru HB, Bioremediation and nutrient removal from wastewater by *Chlorella vulgaris*. *Ecol Eng* **110**:1–7 (2018). https://doi.org/10.1016/j.ecoleng.2017.10.008.

22 Miao M-S, Yao X-D, Shu L, Yan Y-J, Wang Z, Li N *et al.*, Mixotrophic growth and biochemical analysis of *Chlorella vulgaris* cultivated with synthetic domestic wastewater. *Int Biodeterior Biodegrad* **113**: 120–125 (2016). https://doi.org/10.1016/j.ibiod.2016.04.005.

23 US EPA. U.S. Environmental Protection Agency. Guidelines for Water Reuse AR-1530. EPA/600/R-12/618 (2012).

24 Alcalde-Sanz L, Gawlik B, Minimum quality requirements for water reuse in agricultural irrigation and aquifer recharge. Towards a legal instrument on water reuse at EU level (2017). https://doi.org/10.2760/804116.

25 Kawata K, Osawa M and Okabe S, In vitro toxicity of silver nanoparticles at noncytotoxic doses to HepG2 human hepatoma cells. *Environ Sci Technol* **43**:6046–6051 (2009). https://doi.org/10.1021/es900754q.

26 ISO 10993–5: 2009, *Biological Evaluation of Medical Devices - Part 5: Tests for In Vitro Cytotoxicity*. International Organization for Standardization, Geneva (2009).

27 US EPA, *U.S. Environmental Protection Agency*. Water Quality Standard, Washington, DC (2018).

28 Dankovich TA and Gray DG, Bactericidal paper impregnated with silver nanoparticles for point-of-use water treatment. *Environ Sci Technol* **45**:1992–1998 (2011). https://doi.org/10.1021/es103302t.

29 Dror-Ehre A, Mamane H, Belenkova T, Markovich G and Adin A, Silver nanoparticle-*E. coli* colloidal interaction in water and effect on *E. coli* survival. *J Colloid Interface Sci* **339**:521–526 (2009). https://doi.org/10.1016/j.jcis.2009.07.052.

30 Wang D and Chen Y, Critical review of the influences of nanoparticles on biological wastewater treatment and sludge digestion. *Crit Rev Biotechnol* **36**:816–828 (2016). https://doi.org/10.3109/07388551.2015.1049509.

31 Islan GA, Mukherjee A and Castro GR, Development of biopolymer nanocomposite for silver nanoparticles and ciprofloxacin-controlled release. *Int J Biol Macromol* **72**:740–750 (2015). https://doi.org/10.1016/j.ijbiomac.2014.09.020.

32 Žaltauskaitė J and Vaisiūnaitė R, Evaluation of municipal effluent toxicity using higher plants and invertebrates. *Environ Res Eng Manag* **53**: 17–23 (2010).

33 Belhaj D, Jerbi B, Medhioub M, Zhou J, Kallel M and Ayadi H, Impact of treated urban wastewater for reuse in agriculture on crop response and soil ecotoxicity. *Environ Sci Pollut Res* **23**:15877–15887 (2016). https://doi.org/10.1007/s11356-015-5672-3.

34 Castillo GC, Vila IC and Neild E, Ecotoxicity assessment of metals and wastewater using multitrophic assays. *Environ Toxicol* **15**:370–375 (2000). https://doi.org/10.1002/1522-7278(2000)15:5<370:AID-TOX3>3.0.CO;2-S.

35 Tsigoida A and Argyrokastritis I, The effect of sub-irrigation with untreated and treated municipal wastewater on organic matter and nitrogen content in two different soils. *Global NEST J* **1**: 389–398 (2019). https://doi.org/10.30955/gnj.002810.

36 Phillips R, Kondev J, Theriot J and Garcia H, Physical biology of the cell, in *Garland Science*, 2nd edn. Taylor and Francis Group LLC, New York, NY (2012).

37 Abegunrin T, Awe G, Idowu D and Adejumobi M, Impact of wastewater irrigation on soil physico-chemical properties, growth, and water use pattern of two indigenous vegetables in Southwest Nigeria. *Catena* **139**:167–178 (2016). https://doi.org/10.1016/j.catena.2015.12.014.

38 Galavi M, Jalali A, Ramroodi M, Mousavi SR and Galavi H, Effects of treated municipal wastewater on soil chemical properties and heavy metal uptake by sorghum (*Sorghum bicolor* L.). *J Agric Sci* **2**: 235–241 (2010). https://doi.org/10.5539/jas.v2n3p235.

39 Matheyarasu R, Bolan NS and Naidu R, Abattoir wastewater irrigation increases the availability of nutrients and influences on plant growth and development. *Water Air Soil Pollut* **227**:253–269 (2016). https://doi.org/10.1007/s11270-016-2947-3.