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Biological treatment of drinking water by chitosan based nanocomposites

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Nanotechnology is a promising interdisciplinary area that is likely to have wide ranging implications in all fields of science and technology. The rapid growth in nanotechnology has significant interest in the environmental application of nanomaterials. One of the latent applications of antimicrobial nanomaterials is their use in decentralized or point- of- use water treatment. The present study focuses on chitosan loaded nanoparticles and secondary metabolites (*Streptomyces* sp.) loaded chitosan nanoparticles which were synthesized by ionotrophic gelation method. The synthesized nanoparticles were proven by antimicrobial activity test. Then the nanoparticles were coated on 4 micron membrane by dipping method. A membrane filtration technique is used for the treatment of water to remove or kill the bacteria from drinking water sample. The characterization of synthesized nanoparticles was done by dynamic light scattering (DLS) and Fourier transform infrared spectroscopy (FTIR). The size of the chitosan loaded nanoparticles and secondary metabolites loaded chitosan nanoparticles were 164 and 177 nm, respectively and the zeta potential was highly stable and found to be 35 and 47 mV, respectively. The synthesized nanoparticles have a lot of surface areas contrasted to macro particles. They can be improved with a variety of reactor groups to raise their affinity to target compounds for removal of organic and inorganic pollutants from contaminated water. The quality of water is confirmed by membrane filtration method and multiple tube fermentation techniques.

Key words: Nanoparticles coated membrane, membrane filtration method, MPN techniques.

INTRODUCTION

Nanotechnology is an interesting field of science contributing materials showed designed features between atoms and large materials in the nano range (Patil et al., 2012; Ali, 2012). There is a need to develop new and powerful antibacterial agents because of increasing concerns of bacterial infections. Predominantly, nanoparticles were used in different fields of human life like food preservation, burn dressings, safe cosmetics, medical devices and water treatment (Pant et al., 2011) and other range of products.

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The wide range of bio-application of nanoparticles is due to their tremendous antibacterial activity on a number of both Gram positive and negative bacteria (Li et al., 2011). The bactericidal effect of nanoparticles was confirmed by means of their size, shape, size distribution, morphology, surface functionalization and stability. Water is essential for the existence of life on earth. About 75% of earth is covered by water beyond which 97% is salt water and just 3% is available for drinking, agriculture, domestic and industrial consumption, at the same time as the rest is confined in oceans and underground reservoirs like salt water, polar ice caps, glaciers and ground water. Due to increase in industrialization and human population, demands for water supply have also been increased (Dara, 1998).

Water pollution is caused by toxic metals and microbial contamination that has a serious environmental and public health issue. Bacterial contamination of water persists to be an extensive problem across the country and is a major source of illness and deaths among 37.7 million affected by waterborne diseases annually in India. The most important pathogenic organisms responsible for water borne diseases in India are bacteria (*E. coli, Shigella,* and *V. cholera*), viruses (*Hepatitis A, Polio Virus, Rota Virus*) and parasites (*E histolytica, Giardia, Hook worm*). Water treatment is the exclusion of suspended and colloidal particles, organic matter, and microorganisms rather than other material that are harmful to health, looking for the lowest cost of consumption, process and maintenance, and reduced environmental impact to the surrounding region (Ali and Gupta, 2007; Libanius, 2008; Ali, 2010; Ali et al., 2012 and Ali, 2014).

Nanoscaled chitosan has latent drinking water disinfection application as an antimicrobial agent in membranes, sponges or outside coating of water storage tanks. It has greater reward than other disinfections since it has a higher antibacterial activity, a broad range of activity against bacteria, viruses and fungi and a lower toxicity to higher order animals and humans. On the other hand, the efficacy of microbial control depends upon the material preparation method and presence of organics. Chitosan act as an effective disinfectant only at acidic pH because of its solubility and the accessibility of charged amino groups (Kavanagh, 1972; Rabea et al., 2003). The preparation of water- soluble derivatives of chitosan could ultimately conquer this limitation.

Nanofiltration (NF) is a relatively modern membrane process used most frequently with low total dissolved solids (TDS) waters such as surface water and fresh groundwater, with the purpose of softening (polyvalent cation removal) and elimination of disinfection by-product precursors such as natural organic matter and synthetic organic matter. Along with the surface charged character, NF membranes can be separated into charged and neutral NF membranes, respectively. A number of researches recommended that have better permeation, separation and fouling resistant properties in charged NF membranes than neutral NF membranes as they reject a solute not only by the steric hindrance effect, but as well as by the electrostatic repulsive effect. The present study focuses on the removal of bacteria from drinking water sample by using chitosan with bioactive compounds loaded (from *Streptomyces* sp.) nanocomposites coated membrane.

MATERIALS AND METHODS

Isolation of *Streptomyces* sp

The soil samples were collected from various agricultural areas which have been serially diluted up to 10^{-5}. The spread plate technique was followed and incubated at 28°C for seven days. The *Streptomyces* sp. isolates were maintained on starch casein agar slants.

Screening for antimicrobial activity by well diffusion method

The *Streptomyces* isolates were grown in starch casein broth at 28°C for 10 days and the activity were observed by well diffusion method. The culture filtrate was loaded into the wells which had been inoculated with test organism, *S. aureus*, *K. pneumonia, P. aeruginosa* and *E. coli* and incubated for overnight and measured the zone of inhibition (No et al., 2002).

Extraction of the secondary metabolites from culture supernatant using different solvents

The secondary metabolites from crude culture broth were extracted with different solvents like n-hexane, ethyl acetate, petroleum ether and chloroform. The solvents were proportionally mixed in the ratio of 1:1 and tested for the presence of secondary metabolites using well diffusion method (Augustine et al., 2005).

Thin layer chromatography

The secondary metabolites obtained after solvent extraction was spotted on silica gel. Thin-layer chromatography (TLC) plates were developed by using ethanol: water: chloroform (40: 40: 20) solvent system. The spotted TLC plates were exposed to iodine vapors for the development of spots concerning the presence of secondary metabolites.

Synthesis of chitosan loaded nanoparticles

Chitosan loaded nanoparticles were synthesized by ionotropic gelation method. Chitosan was dissolved in 1% (v/v) acetic acid to obtain a 0.3% (w/v) chitosan solution. Tripolyphosphate (TPP) was dissolved in Millipore water to a concentration of 1%. Chitosan loaded nanoparticles (CNP) were obtained by the 1 ml of TPP solution which was poured drop wise to the 5 ml chitosan solution. The solution was stirred for 15 min and then sonicated. The nanoparticles were collected by centifugation at 9000 rpm for 40 min and freeze dried at -70°C for further analysis (Ali et al., 2010).

The secondary metabolites loaded chitosan nanoparticles (SCNP) were attained by adding a extract of secondary metabolites (obtained from *Streptomyces* spp.) to the nanoparticle suspension during chitosan nanoparticle synthesis earlier than centrifugation and stirred for 1 h. Then it was further purified as described above.

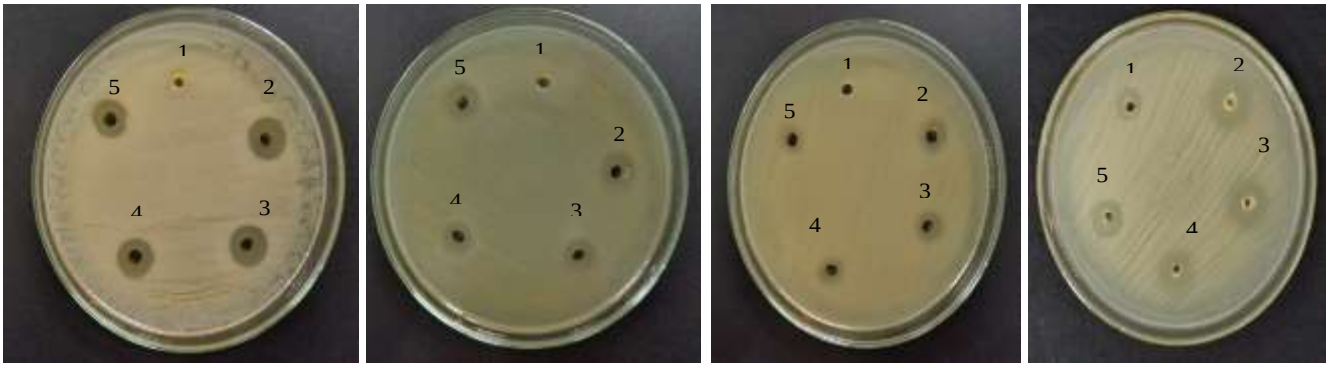

Figure 1. Antibacterial activity of *Streptomyces* sp. (1-10 µl, 2-20 µl, 3-30 µl, 4-50 µl and 5- control).

Table 1. Antibacterial activity of *Streptomyces* sp against various microorganisms by well diffusion method

Test organism	Zone of inhibition (mm)				
	10 µl	20 µl	30 µl	50 µl	Control
S. aureus	-	9	11	14	15
P. aeruginosa	-	8	10	12	13
K. pneumonia	-	9	11	13	10
E. coli	8	11	13	14	13

Characterization of nanoparticles

Dynamic light scattering (DLS)

The particle size and zeta potential for chitosan loaded nanoparticles (CNP) and secondary metabolites loaded chitosan nanoparticles (SCNP) were identified by using Zetasizer ver.620 (Malvern Instruments). The nanoparticles were performed on a scattering angle at 90° at a temperature of 25°C.

FTIR spectroscopy

FTIR spectroscopy is used to study the chemical characterization of the material. FTIR analyses were observed in the range of 400-4000 cm^{-1} with the help of KBr pellets. The diverse mode of vibrations were identified and consigned to conclude the functional groups in the sample.

Nanoparticles coated on membrane

The synthesized chitosan loaded nanoparticles (CNP) and secondary metabolites loaded chitosan nanoparticles (SCNP) were coated on 4 micron membrane by dipping method. The membrane was placed in synthesized nanoparticles and kept in a rotary shaker at 110 rpm for 12 h. Then the coated membrane was kept in a sterile closed container for drying at room temperature.

Membrane filtration technique

The membrane filtration techniques provide a direct count of total coliforms and faecal coliforms in the water sample. This test was carried out in a flow through membrane filtration system on a CNP coated membrane, SCNP coated membrane and nanoparticles non-coated membrane in a sterile condition. The water sample was collected in a sterile container for further analysis. It is clear that the permeability of nanoparticles coated membrane has slightly reduced contrast to non coated membrane. It is also observed that permeability reduced and removal of compound increased (Stafie, 2004).

Multiple tube fermentation (MPN) technique

The multiple tube fermentation technique had presumptive test, confirmed test and completed test was used to identify the presence of coliform in the filtered water sample (APHA, 1975).

RESULTS AND DISCUSSION

Isolation and screening of *Streptomyces* sp.

The study which was undertaken to "isolate and screen the *Streptomyces* sp. for secondary metabolites from various agricultural soil samples resulted in the following observations: the *Streptomyces* colonies were typically identified in the (10^{-3}) dilution in starch casein agar (SCA). Out of the 20 *Streptomyces* sp. that were subjected to screening process, some of them showed antimicrobial activity against test organisms like *S. aureus*, *K. pneumonia, P. aeruginosa* and *E. coli* (Figure 1 and Table 1). The best activity showed *Streptomyces* sp. was chosen for extraction of secondary metabolites using different solvents.

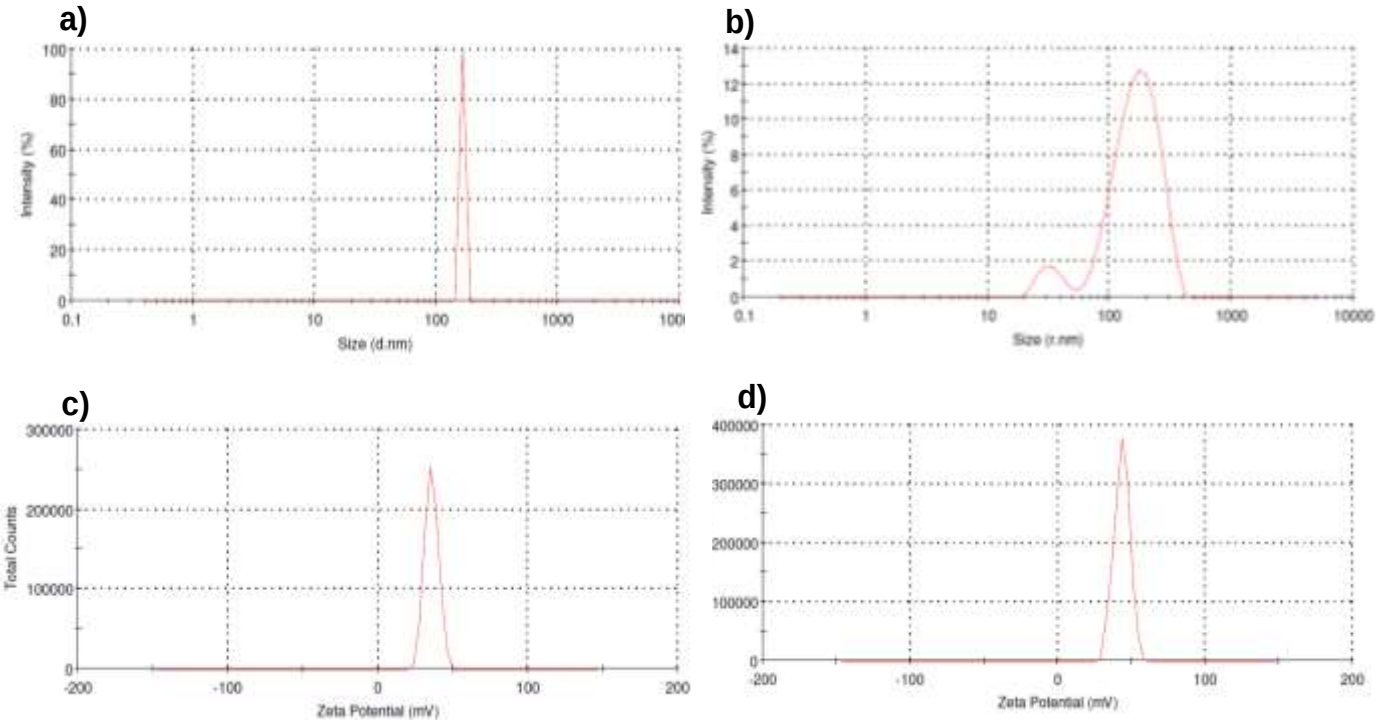

Figure 2. Size and zeta potential distribution of chitosan loaded nanoparticles (a & c) and secondary metabolites loaded chitosan nanoparticles (b & d).

Extraction of the secondary metabolites

The different solvents like chloroform, n-butanol, ethyl acetate and petroleum ether were used for the extraction of the secondary metabolites. The ethyl acetate extract showed higher antibacterial activity by well diffusion method and it was loaded into chitosan nanoparticles.

Thin layer chromatography

The partially purified secondary metabolites obtained after solvent extraction analyzed using pre-coated silica gel plates. The different solvent extracts were spotted on the TLC plate and exposed to iodine vapors for the development different bands. The different yellow color bands appeared at various R_f values of 0.68, 0.65, 0.72, 0.63, 0.52 of the partially purified product. The diverse range of Rf values were analytical of the association of secondary metabolites in the different solvent systems because of the variation in the solubility (Boer et al., 2005).

Characterization of nanoparticles

Dynamic light scattering

The size and zeta potential are the important features for nanoparticles. Chitosan nanoparticles (CNP) and secondary metabolites loaded chitosan nanoparticles (SCNP) size were 164 and 177 nm, respectively (Figure 2a and 2b). The zeta potential of chitosan nanoparticles (CNP) was 35 mV and secondary metabolites loaded chitosan nanoparticles (SCNP) was 43 mV (Figure 2c and 2d). The zeta potential was improved radically as a result of loading the secondary metabolites. The zeta potential is an essential parameter for the identification of stability in nanoparticles. The nanoparticles was physically stabilized by electrostatic repulsion, the minimum value of zeta potential has ±30 mV (Muller et al., 2001).

The zeta potentials were improved extensively due to the loading of secondary metabolites because the positive charge of amino groups in chitosan interacted with it. As a result of both chitosan loaded nanoparticles and secondary metabolites loaded, chitosan nanoparticles were very stable.

FTIR Spectroscopic analysis

The infrared spectra were recorded on Fourier Transform Spectrometer within the range (400-4000 cm^{-1}). The FTIR spectrums for chitosan loaded nanoparticles and secondary metabolites loaded nanoparticles are presented in Figure 3. The data presents a strong wide peak about 3600-3200 cm^{-1} that is related to hydrogen bounded O-H stretching vibration of alcohol and phenols at 3441 and

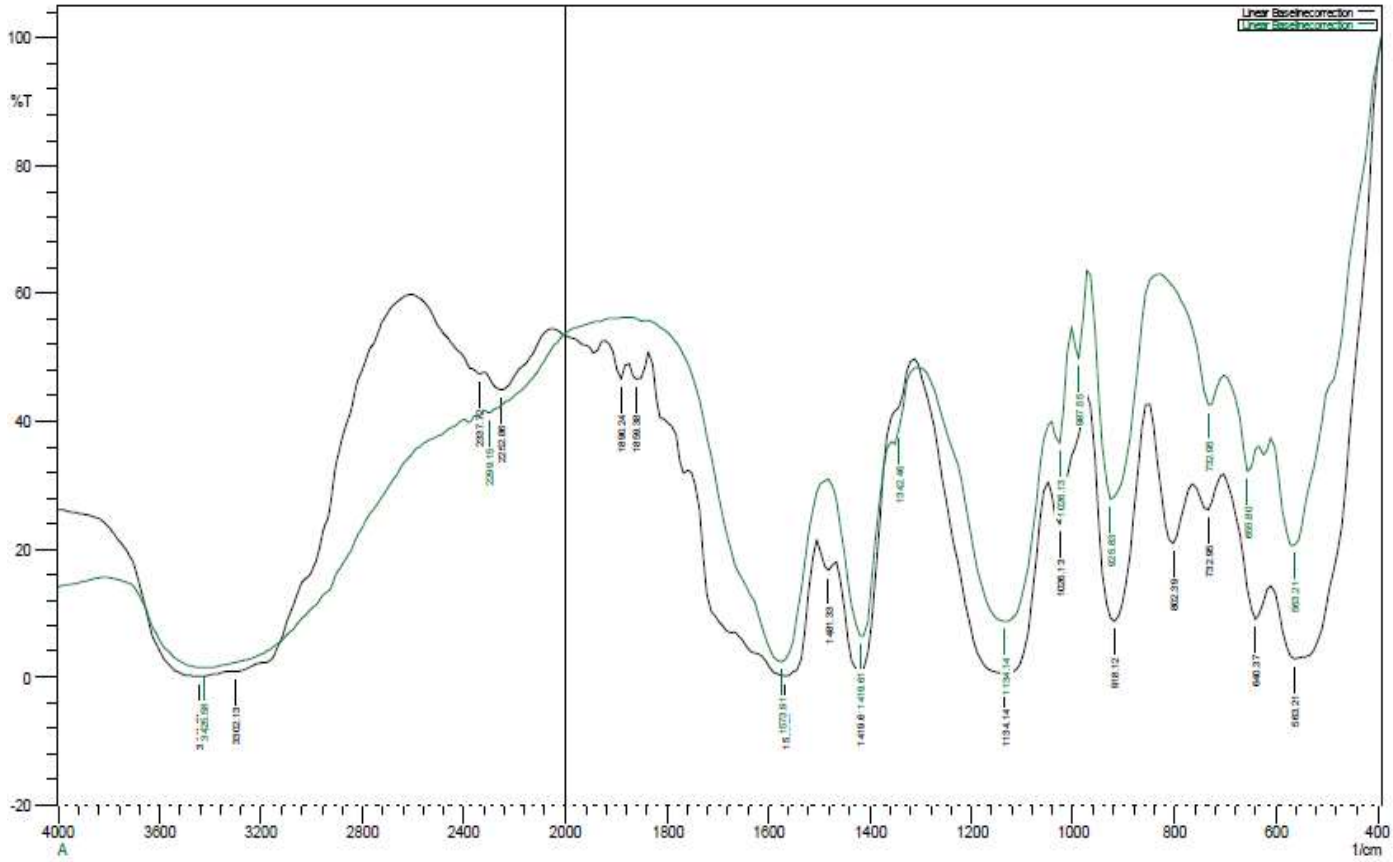

Figure 3. FTIR spectrum for chitosan loaded nanoparticles (black line) and secondary metabolites loaded nanoparticles (blue line). Black line: Chiotsan loaded nanoparticles; Green line: Bioactive compound loaded nanocomposites.

3302 cm^{-1} shifted to 3425 cm^{-1}. Primary amine N-H stretching vibration has the same absorption in this region that overlap with O-H stretching vibration peak. The peak of stretching vibration for C-N in primary amine is observed in 1481 cm^{-1}. The peaks at 1566 cm^{-1} shifted to 1573 cm^{-1} belong to N-H bending vibration in primary amine and carbonyl group stretching vibration in amide type II, respectively. An asymmetric stretching vibration peak of C-O-C is observed in 1134 cm^{-1} on CNP and SCNP. The peaks in N-H bending vibration and carbonyl stretch in amide type II have 1419 and 1566 cm^{-1} shifted to 1419 and 1573 cm^{-1}, respectively. The bending of O-H bond was shifted from 918 to 925 cm^{-1} of carboxylic acid. The formation of nitro compounds with N-O stretching was observed at 1342 cm^{-1} and C-H bending of alkenes observed at 987 cm^{-1} in secondary metabolites loaded nanoparticles whereas, chitosan loaded nanoparticles contain no peak. The peak of 640 cm^{-1} was shifted to 655 cm^{-1} due to C-Br stretching of alkyl halides of secondary metabolites (Balozet, 1971; Radmanesh, 1990). Therefore, we converse that the ammonium groups of chitosan are linked with tripolyphosphoric groups of TPP. The intra and inter- molecular actions are improved in chitosan nanoparticles. In addition to this, the interaction of chitosan and TPP to secondary metabolites to form a different some peak is also observed.

Membrane filtration technique

The filtered membrane was placed overturned on nutrient agar medium and incubated at 37°C for 24 h and observed the growth of colonies and it was counted directly. The chitosan loaded nanoparticles coated membrane hold the moderate amount of bacterial growth and secondary metabolites loaded chitosan nanoparticles has the highest amount of the bacterial growth and non coated membrane holds the less amount of bacterial growth. Mille et al. (2002) confirmed that the increase in external osmotic pressures to a bacterial cell, decline in cytoplasmic volume and as a result decrease in the cell volume can be seen.

Multiple tube fermentation (MPN) technique

The occurrence of coliforms was identified by MPN techniques. A large amount of positive combination and MPN index were attained from the non coated membrane

Table 2. MPN index for different combination of positive results while five tubes were used per dilution (10 ml, 1.0 ml, 0.1ml sample).

Types of membrane filtered water sample	5 /10 ml	5/1 ml	5/0.1 ml	No. of positive combination	MPN index
Non coated membrane filtered water sample	3	2	2	3-2-2	17
CNP coated membrane filtered water sample	2	1	1	2-1-1	9
SCNP coated membrane filtered water sample	1	0	1	0-1-1	4

filtered water sample. The SCNP coated membrane and CNP coated membrane showed maximum removal of coliforms identified by MPN techniques than non-coated membrane. The MPN index of the filtered water sample is shown in Table 2. The nanoparticles coated membrane has slightly charged surface because the pore size are less than individual sort of magnitude higher than the size of ions, charge interaction plays a dominant role (Rautenbach and Groschl, 1990). This effect can be used to remove the molecules.

Conclusion

Nanofiltration membranes (NF) are used in drinking water treatment or wastewater treatment. It was a low pressure membrane development with the intention of separate materials in micrometer range. It was a pressure-driven membranes with properties between those of reverse osmosis and ultra filtration membranes. It has the ability to remove turbidity, microorganisms and inorganic ions. They proved that they were very effective for removing bacteria from contaminated water. The *Streptomyces* sp. was isolated and screened for secondary metabolites by well diffusion method. The chitosan loaded nanoparticles and secondary metabolites loaded chitosan nanoparticles were successfully synthesized. They were characterized by DLS and FTIR and they proved the size and Zeta potential (stability) of the synthesized nanoparticles. Then the synthesized nanoparticles were coated on membrane by dipping method. The water sample is filtered through membrane filtration technique of nanoparticles coated membrane and non coated membrane and analysis by multiple tube fermentation (MPN) technique for the comparison of nanoparticles coated membrane and non coated membrane for the maximum removal of bacteria in the water sample.

Conflict of interests

The authors did not declare any conflict of interest.

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