Supplementary Information

Release of silver from nanoparticle-based filter paper and the impacts to mouse gut microbiota

Authors:

Jingtao Wu[a], Cui Li[a, c], Jie Zhang[a], Neal W. Menzies[a], Paul M. Bertsch[a, d], Peng Wang[b*], Peter M. Kopittke[a]

[a]*The University of Queensland, School of Agriculture and Food Sciences, St Lucia, Queensland, 4072, Australia*

[b]*Nanjing Agricultural University, College of Resources and Environmental Sciences, Nanjing, 210095, China*

[c]*Northwestern Polytechnical University, Research Centre for Ecology and Environmental Sciences, Xi'an 710072, China*

[d]*Commonwealth Scientific and Industrial Research Organisation (CSIRO), Land and Water, 41 Boggo Road, Ecosciences Precinct, Dutton Park, 4102, Queensland, Australia*

*Corresponding author: Peng Wang, Phone: +86 25 8439 9562, Email: p.wang3@njau.edu.cn

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Effects of NOM and Cl on Ag release

The effects of NOM (0 and 5 mg C L^{-1}) and Cl (0, 20 and 18,000 mg L^{-1}) on Ag release were investigated individually and in combination to evaluate their specific impact on Ag release from the Ag-NP paper. Different concentrations of NOM [Suwannee River Fulvic Acid (SRFA, Standard II, IHSS, USA)] and Cl (NaCl) were prepared in deionized water to obtain six water treatments with the desired concentrations of NOM and Cl. The six water samples were filtered through the Ag-NP paper without sunlight exposure in 25 mL increments until a total of 400 mL of water had been filtered. Additionally, we used filter paper that had been exposed to sunlight, with the high Cl (18,000 mg L^{-1}) water samples filtered through this paper. The filtrates were collected after 25, 50, 75, 200 and 400 mL and were acidified with 1 mL of 70% HNO_3 before measurement for Ag using inductively coupled plasma optical emission spectrometery (ICP-OES).

XAS analyses of the filter paper and filtrate

The X-ray absorption near edge structure (XANES) spectra were collected in fluorescence mode using a 100-element solid-state Ge detector with each energy spectrum calibrated from the simultaneous measurement of a metallic foil reference in transmission. All analyses were performed in a liquid He cryostat at ca. 10 K.

In addition to the samples, we examined six solid standards ($AgNO_3$, AgCl, Ag_2CO_3, Ag_3PO_4, Ag-NPs and bulk Ag_2S) and three aqueous standards (Ag-humic acid, Ag-cysteine and Ag-glutathione). The solid standards were prepared by diluting with cellulose to ca. 200 mg kg^{-1}. The aqueous standards were prepared by mixing 1 mL of 20 mM $AgNO_3$ with 1 mL of 200 mM of corresponding ligands (humic acid, cysteine or glutathione). The mixed solutions were then adjusted to ca. pH 6 with 0.1 M NaOH and more deionized water was

added to obtain a volume of 7 mL. Finally, the three solutions were mixed with 3 mL glycerol to minimize the formation of ice-crystals.

Spectra were collected on two replicate locations of the samples and reference compounds in pellet form. The obtained data were merged and normalized using Athena (version 0.8.056). Principal component analysis (PCA) and target transformation (TT) were performed to identify the likely species in samples by SixPack (version 9). Linear combination fitting (LCF) of the sample spectra was used to identify the speciation component in each sample with the fitting range from -30 to 100 eV with Athena.

Bacterial 16S rRNA gene quantification and sequencing

The V4-V5 regions of 16S rRNA gene was amplified with MJ Mini™ thermal cycler equipped with MiniOpticon™ (Bio-Rad). The quantitative real time polymerase chain reaction of 16S rRNA gene of faeces was performed using gene primers 519F and 907R.[1] The final reaction volume was 20 μL of mixture containing 10 μL SYBR Premix Ex Taq II (TaKaRa, China), 7.2 μL RNase free Ultra-Pure water, 2 μL DNA template (10 ng μL^{-1}) and 0.4 μL of each primer (25 mmol L^{-1}). The amplification program was initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturing at 95 °C for 30 s, annealing at 56 °C for 30 s and extension at 72 °C for 30 s.

For 16S rRNA gene sequencing, the V4-V5 region was amplified using primers 515F and 907R and followed the protocol as aforementioned. The amplified product was purified using the GeneJET Gel Extraction Kit (Thermo Scientific) and then quantified using a Promega Quantifluor® ST fluorometer (Promega, USA). A sequencing library was generated by NEBNext® UltraTM DNA Library Preparation Kit (NEB, USA) and run on the Agilent Bioanalyzer 2100 system to assess size and quality. Finally, the pooled DNA products were sequenced on an Illumina MiSeq platform to obtain a paired end reading of 250 bp/300 bp.

Operational taxonomy units (OTUs) were clustered using UPARSE at 97% similarity and singletons were re-moved (version 10 http://drive5.com/uparse/). Then, the taxonomy of each OTU were obtained using the RDP Classifier (http://rdp.cme.msu.edu/). The raw sequence data has been deposited in the NCBI SRA database under accession number PRJNA587355 (Effects of silver-containing filtrate on mice microbiota).

Table S1. Chemical properties of six water samples, including Cl, dissolved organic C (DOC), Fe, P, pH and electrical conductivity (EC). The first six water samples (Australia) were used in the filtration experiment and the remaining sample (Yangtze River, China) was used for the mouse microbiota experiment.

	Cl	**DOC**	**Fe**	**P**	**pH**	**EC**	**Location**
	mg L^{-1}	**mg L^{-1}**	**mg L^{-1}**	**mg L^{-1}**		**dS m^{-1}**	
Deionized water	0.11	0.07	0.02	0.06	6.0	0.0002	-
Brisbane River, freshwater	61	5.2	1.83	0.15	7.5	0.37	27°33'S 152°44'E
Brisbane River, tidal	1100	7.4	2.2	0.26	6.9	3.4	27°29'S 153°00'E
UQ Lake	13	6.6	0.16	0.17	6.6	0.19	27°29'S 153°00'E
Brown Lake	20	5.2	0.06	0.07	5.2	0.1	27°29'S 153°25'E
Seawater	18000	1.7	0.06	0.21	7.9	56	27°25'S 153°31'E
Yangtze River	69	5.6	0.86	0.31	7.3	0.21	32°09'N 118°49'E

Table S2. Results of the linear combination fitting of the Ag K-edge XANES spectra of filter paper. The proportion of the various silver species are given as percentages followed by a percentage variation in brackets. The goodness of fitting is indicated by the R-factor.

Treatment		Standards					R factor[a]
		AgCl (%)	Metallic Ag (%)	$AgNO_3$ (%)	Ag-cysteine (%)	Ag_3PO_4 (%)	
Deionized water	Paper without sunlight exposure	10 (0.6)	80 (0.4)	9.5 (0.5)			0.00006
Brisbane River, freshwater		12 (0.5)	82 (0.3)	6.9 (0.4)			0.00004
Brisbane River, tidal		32 (0.8)	58 (0.5)			10 (0.7)	0.0001
University Lake		10 (0.6)	79 (0.3)			11 (0.4)	0.00005
Brown Lake		20 (0.6)	69 (0.4)			11 (0.4)	0.00005
Seawater		33 (1.2)	46(0.7)	21 (0.9)			0.00022
Deionized water	Paper with sunlight exposure		94 (0.5)			7.7 (0.5)	0.00001
Brisbane River, freshwater		5.6 (0.1)	84 (0.6)	11 (0.8)			0.00015
Brisbane River, tidal		11 (1)	75(0.6)			14(0.8)	0.00016
University Lake			86 (0.7)			15 (0.7)	0.00018
Brown Lake		1.6 (1.3)	77 (0.8)			21 (0.1)	0.00025
Seawater			73 (0.7)	9.8 (0.4)	17 (0.8)	11 (0.7)	0.00006
Control, no sunlight			64 (0.7)	36 (0.7)			0.00026
Control, sunlight			96 (0.5)	6.3 (0.5)			0.0001

[a] R factor $=\Sigma i(\text{experimental} - \text{fit})^2/\Sigma i(\text{experimental})^2$

Table S3. Results of the linear combination fitting of the Ag K-edge XANES spectra for the filtrate samples. The proportion of the various silver species are given as percentages followed by a percentage variation in brackets. The goodness of fitting is indicated by the R-factor.

Treatment		Standards					R factor[a]
		AgCl (%)	Metallic Ag (%)	Ag_2S (%)	$AgNO_3$ (%)	Ag-cysteine (%)	
Deionized water					96 (0.6)	5.3 (0.6)	0.00011
Brisbane River, freshwater	Paper without sunlight exposure	75 (1.2)			11 (0.9)	14 (0.8)	0.00017
Brisbane River, tidal		67 (1.7)		17 (1.1)	16 (1.3)		0.00032
University Lake		79 (1.5)			12 (1.1)	9.2 (0.9)	0.00025
Brown Lake		78 (1.4)			13 (1.1)	9 (0.9)	0.00022
Seawater		32 (2.4)		43(1.6)	25 (1.9)		0.0006
Deionized water	Paper with sunlight exposure	63 (0.3)	23 (0.2)		14 (0.3)		0.00001
University Lake		44 (1.1)	44 (0.6)		12 (1)		0.00018
Seawater		39 (2.1)		35 (1.3)	26 (1.6)		0.0004

[a] R factor $=\Sigma i(\text{experimental} - \text{fit})^2/\Sigma i(\text{experimental})^2$

Table S4. Body weight of female/male mice from each cage after oral gavage of sterile river water, Ag-containing filtrate, $AgNO_3$ and Ag-NPs during incubation. Different letters indicate significant differences among different treatments ($P < 0.05$)

Group	Sex	Incubation time (d)				
		0	**7**	**15**	**30**	**45**
River water	Female	51.3±0.4c	54.4±0.3c	57.9±0.4c	61.7±1.1c	62.4±1.1c
Filtrate	Female	51.6±0.8bc	53.6±0.6c	58.1±0.3c	61.8±0.6c	61.6±0.2c
$AgNO_3$	Female	51.5±0.2c	53.8±0.2c	59.8±0.7c	62.4±0.2c	63.8±0.5c
Ag-NPs	Female	56.2±2.4abc	53.4±0.6c	60.8±1.4c	63±1.2c	63.9±1c
River water	Male	57.9±1a	65.5±1.2a	76.2±0.3a	78.8±0.3a	81.7±1.2a
Filtrate	Male	57.2±1.1a	64.3±1.2ab	74.5±0.9a	77.8±0.8a	78.7±1.1a
$AgNO_3$	Male	56.7±0.5ab	63.8±1.5ab	72.6±1.4ab	75.5±1.2ab	77.3±1.6ab
Ag-NPs	Male	56.5±0.7abc	61.1±0.6b	69.1±0.5b	72.1±0.7b	73.4±0.9b

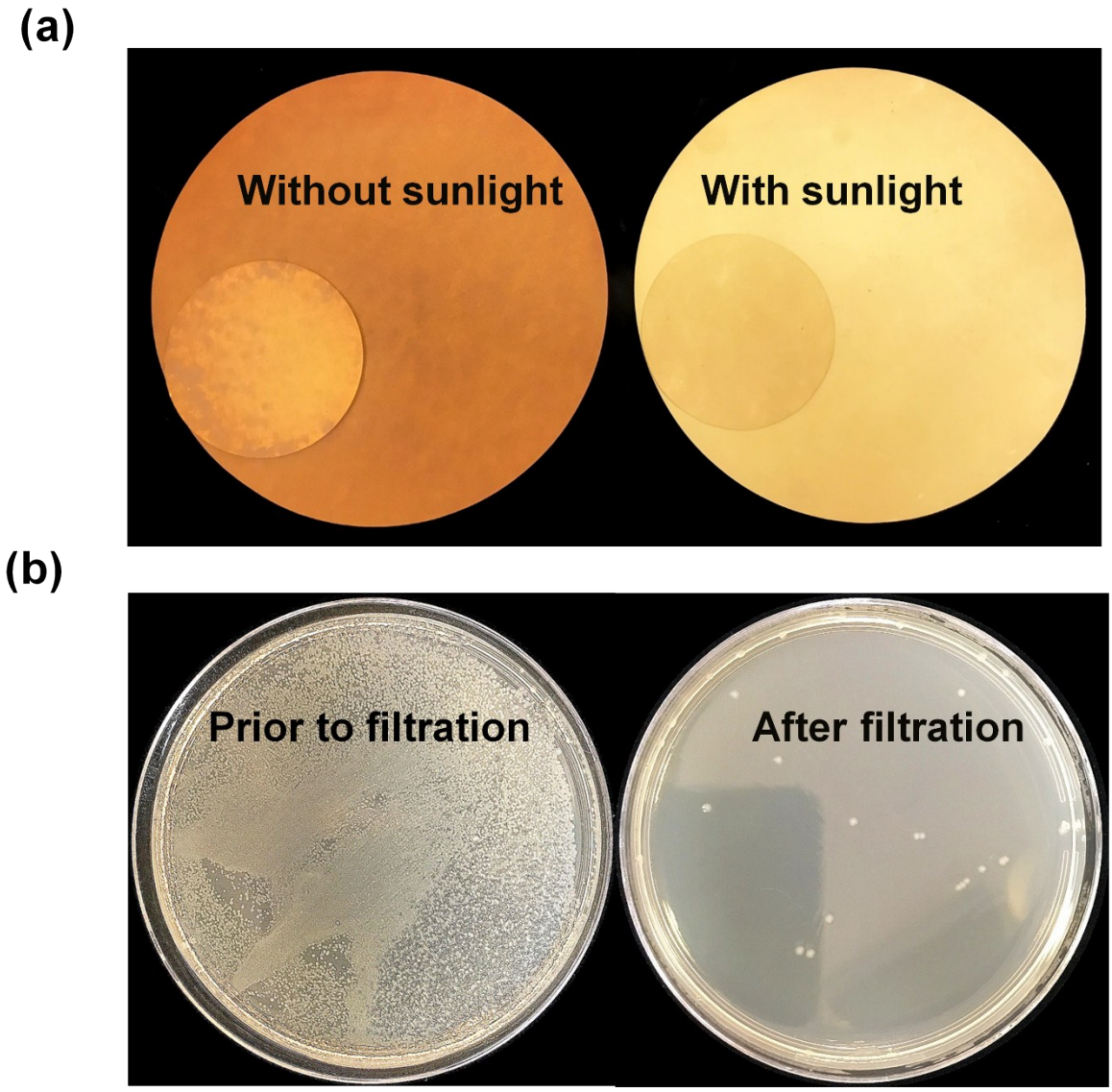

Fig. S1 The Ag-NP-containing filter papers and their antibacterial effectiveness. (a) The Ag-NPs paper either with sunlight exposure (left) or without sunlight exposure (right). Large circles are original papers whilst the small circles were used for the filtration experiment. (b) *E. coli* colonies on tryptone broth spread with *E. coli* suspension (deionized water) either before filtering through the original Ag-NP paper (left) or after filtering through the original Ag-NP paper (right).

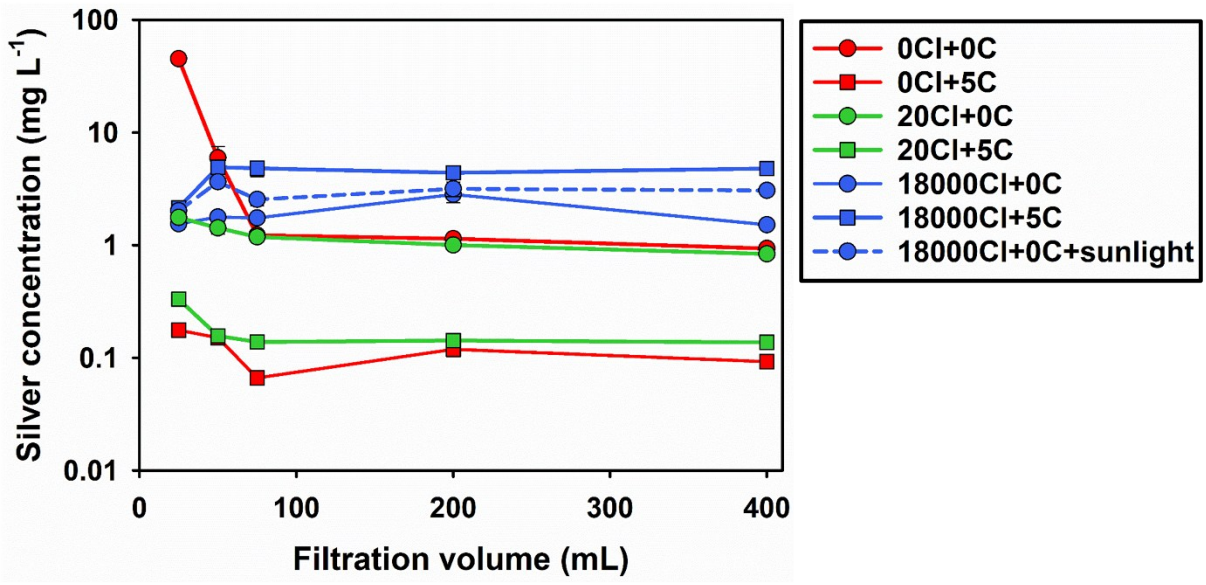

Fig. S2. Effects of natural organic matter (NOM) and Cl on the release of Ag from filter paper. The Cl was added at concentrations of (mg L^{-1}): 0 (0Cl), 20 (20Cl) and 18,000 (18,000Cl). The NOM was added at C concentrations of (mg L^{-1}): 0 (0C) and 5 (5C). One treatment of the filter papers were exposed to sunlight prior to filtering the water (sunlight, blue dashed line).

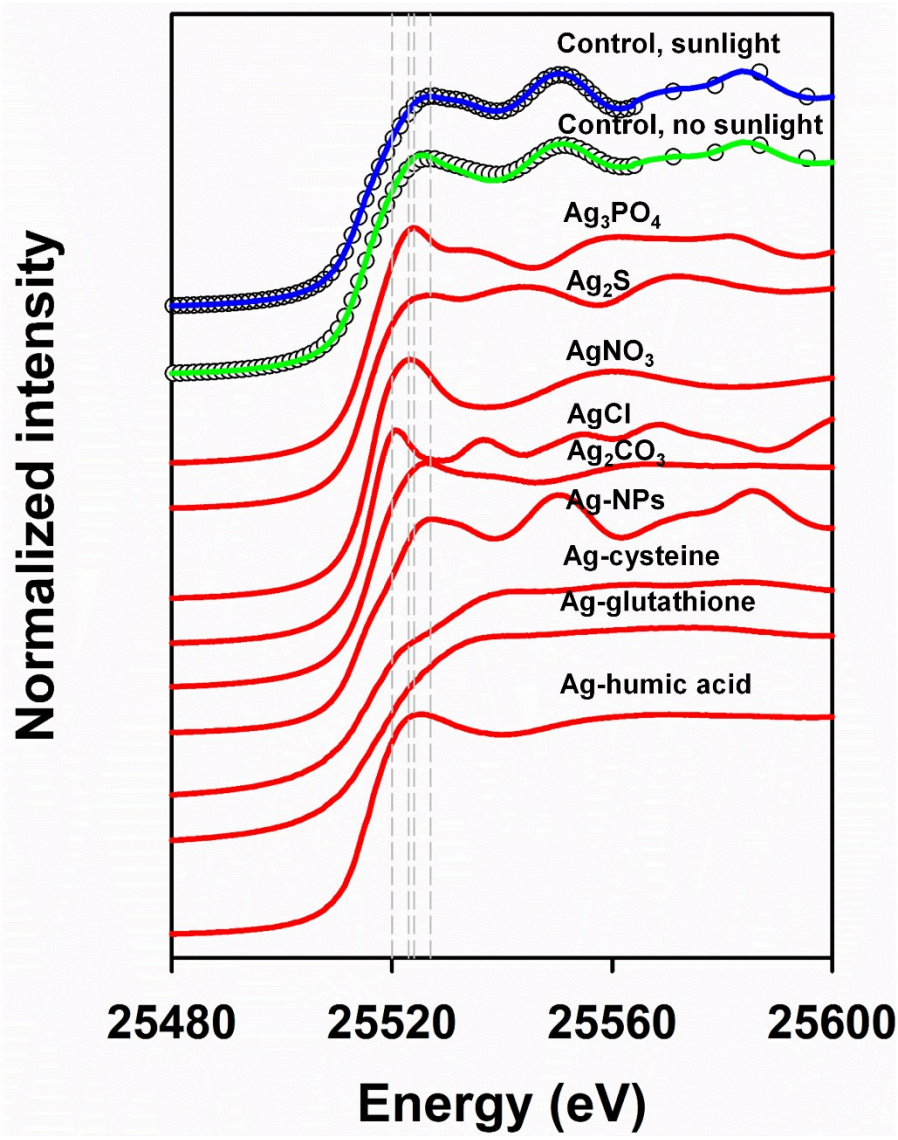

Fig. S3 Normalized Ag K-edge XANES spectra for standards (red lines) and two control filter paper samples. The filter paper samples were unused (had not been used to filter any water) and were either not exposed to sunlight or were exposed to sunlight. The black circles show the raw data whilst the green and blue lines are the fitted curves predicted by linear combination fitting (LCF). For reference, the grey vertical lines correspond to the white line peaks of AgCl (25,520 eV), $AgNO_3$ (25,523 eV), Ag_3PO_4 (25,524 eV) and Ag-NPs/Ag_2S (25,527 eV).

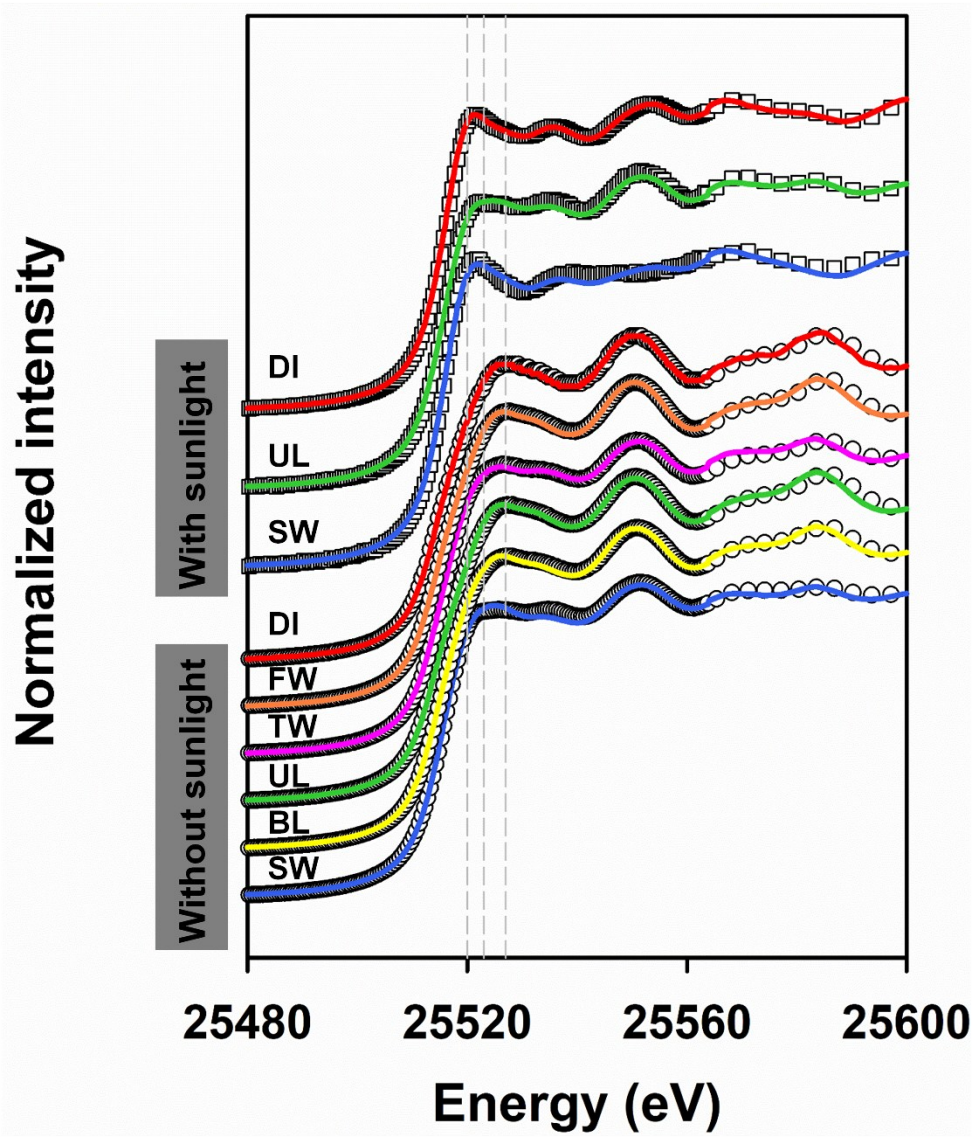

Fig. S4 Normalized Ag K-edge XANES spectra for selected filtrates. A total of six waters were filtered with paper, being deionized water (DI), fresh river water (FW), tidal river water (TW), UQ Lake water (UL), Brown Lake water (BL) and seawater (SW). The filter papers were either not exposed to sunlight or were exposed to sunlight. The black symbols show the raw data and the coloured lines show the fitted curves predicted by linear combination fitting (LCF). For reference, the grey vertical lines from left to right correspond to the white line peaks of AgCl (25,520 eV), $AgNO_3$ (25,523 eV), and Ag-NPs/Ag_2S (25,527 eV).

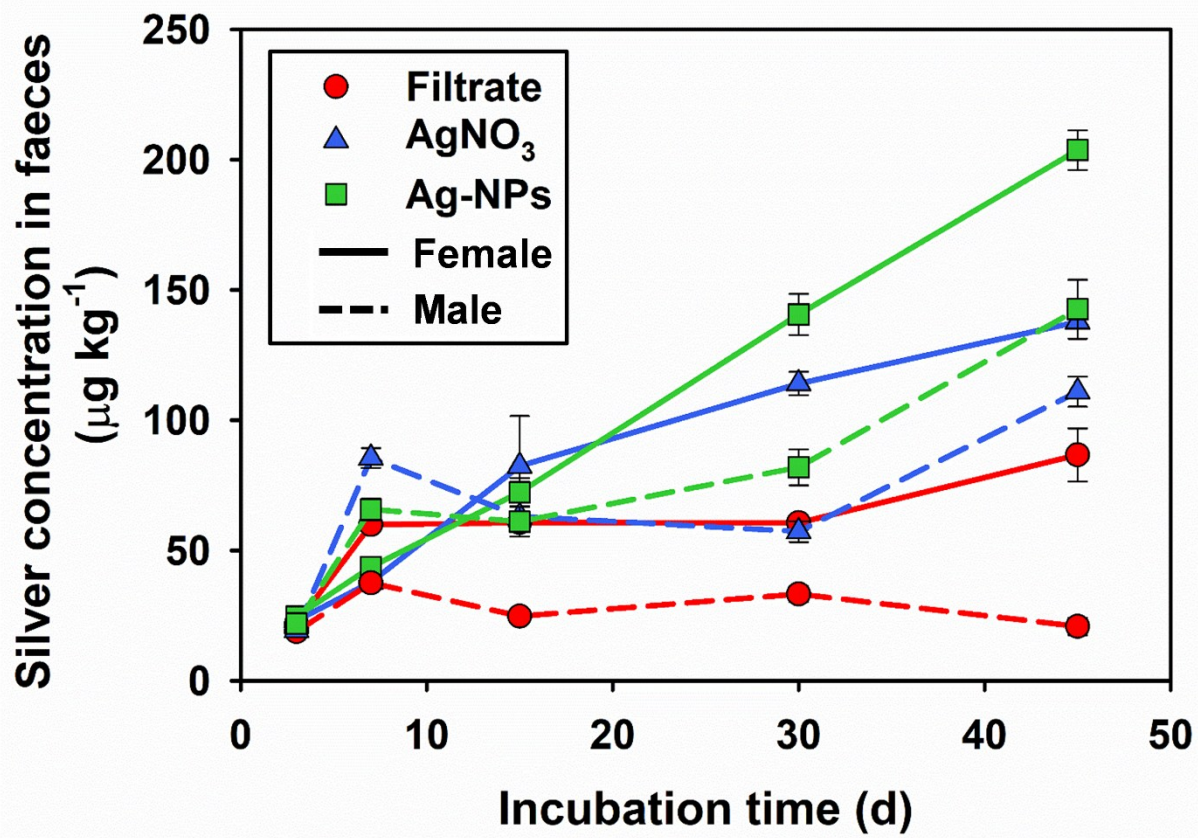

Fig. S5 Silver concentration in the faeces of male and female mice after oral gavage of sterile river water, Ag-containing filtrate, $AgNO_3$ and Ag-NPs for 45 d.

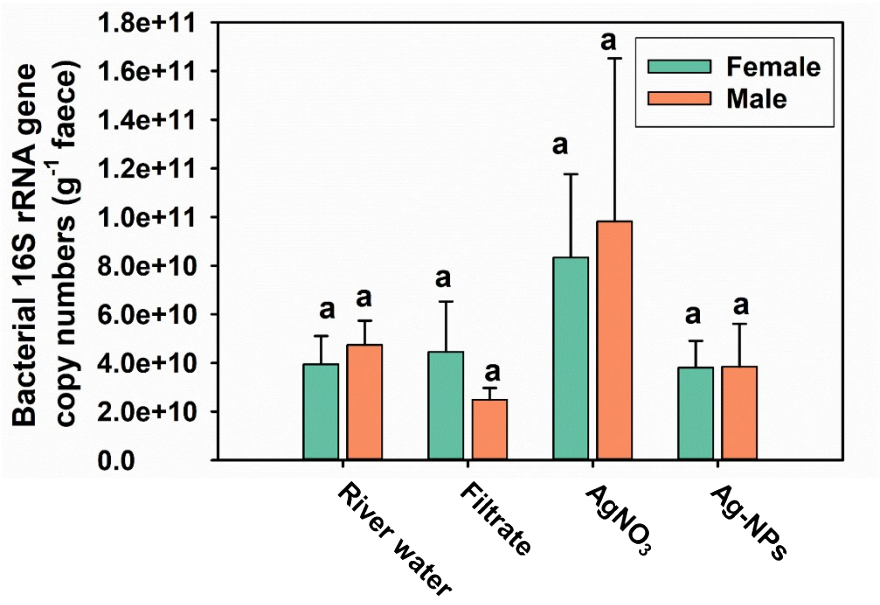

Fig. S6 Bacterial 16S rRNA gene copy numbers in faeces. Significant differences between the treatments of either female or male are indicated by different lowercase letters ($P < 0.05$).

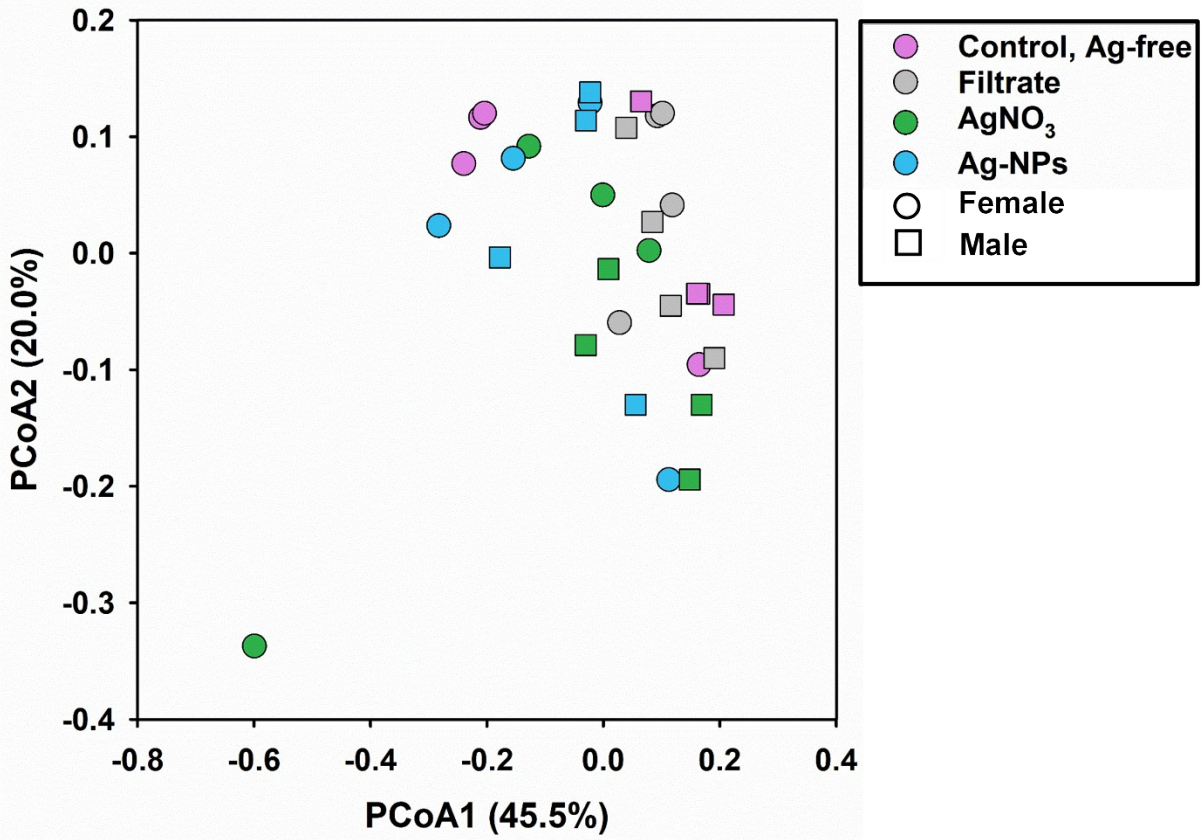

Fig. S7 Principal coordinates analysis (PCoA) of bacteria in faecal samples of female and male mice after oral gavage of sterile river water for 45 d, with the water being control (Ag-free) or containing Ag from one of three sources (Ag-containing filtrate, $AgNO_3$ and Ag-NPs). The PCoA plot was based on the weighted UniFrac distance matrix and relative abundances of bacterial OTUs were used as the dataset. Microbial communities were significantly different between groups (AMOVA, $P = 0.004$).

References

1. C. Chen, K. Huang, W.Y. Xie, S.H. Chen, Z. Tang, and F.J. Zhao, 2017, Microbial processes mediating the evolution of methylarsine gases from dimethylarsenate in paddy soils. *Environ. Sci. Technol.* 51(22), 13190-13198.