

SUPPLEMENTARY INFORMATION

The *in vivo* effects of silver nanoparticles on terrestrial isopods, *Porcellio scaber*, depend on a dynamic interplay between shape, size and nanoparticle dissolution

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Nanoparticle synthesis

Ag spherical nanoparticles were prepared following the work of Komarneni et al.¹ with slight variations. In a typical procedure, 2.4 g of PVP were dissolved in 20 mL of ethylene glycol by stirring for 2 hours. After two hours, 0.16 g of silver nitrate were added and kept under stirring for 20 minutes in the dark. The colorless solution turned to yellow, characteristic of the formation of a silver nano-seed dispersion, due to the reductive ability of PVP. The resulting dispersion was then heated in a microwave oven (CEM, Discovery microwave) at 200 W for 12 or 23 seconds to produce 5–6 nm (AgNS_5-6) and 11–12 nm (AgNSs_11-12) in diameter spherical nanoparticles, respectively. After cooling down to room temperature, the nano-particulated solid was washed by centrifugation (21000 rpm, 30 min) and re-dispersed in water.

Silver nanocubes (AgNCs) were prepared by using an experimental process adapted from the polyol synthesis of NCs reported by Siekkinen et al.². In our work, the heating procedure and consequently the synthesis conditions were modified to an ultrafast microwave heating. Briefly, AgNCs were synthesized by reducing AgNO₃ with EG in the presence of sodium hydrosulphide and PVP. A commercial microwave oven with a magnetron frequency of 2.45 GHz, 1000 W at maximum power (Ethos Plus, Milestone) was used for the synthesis. In a typical procedure, 20 mL of ethylene glycol were heated for 10 minutes under microwave radiation at 195 °C. Afterwards, the ethylene glycol was cooled down to room temperature to prepare the solutions. A 10 mL ethylene glycol solution of sodium hydrosulfide was vigorously stirred after the addition of 5.4.10⁻⁴ M PVP. Then, the mixed solution was droplet injected using a syringe pump into 10 mL of a magnetically stirred ethylene glycol solution of AgNO₃ (0.05 M). The resulting solution became brownish due to the formation of Ag₂S. After 35 s of ultrafast synthesis in a Teflon-lined high-pressure autoclave, under microwave irradiation, the reaction media appeared green-ochre. To quench the reaction, the microwavable autoclave was kept in an ice-water bath. AgNCs were precipitated and purified by the addition of acetone, followed by the sonication and centrifugation at 9000 rpm for 5 min. The resulting nanoparticles were stored as lyophilized powders.

Changes in feeding parameters

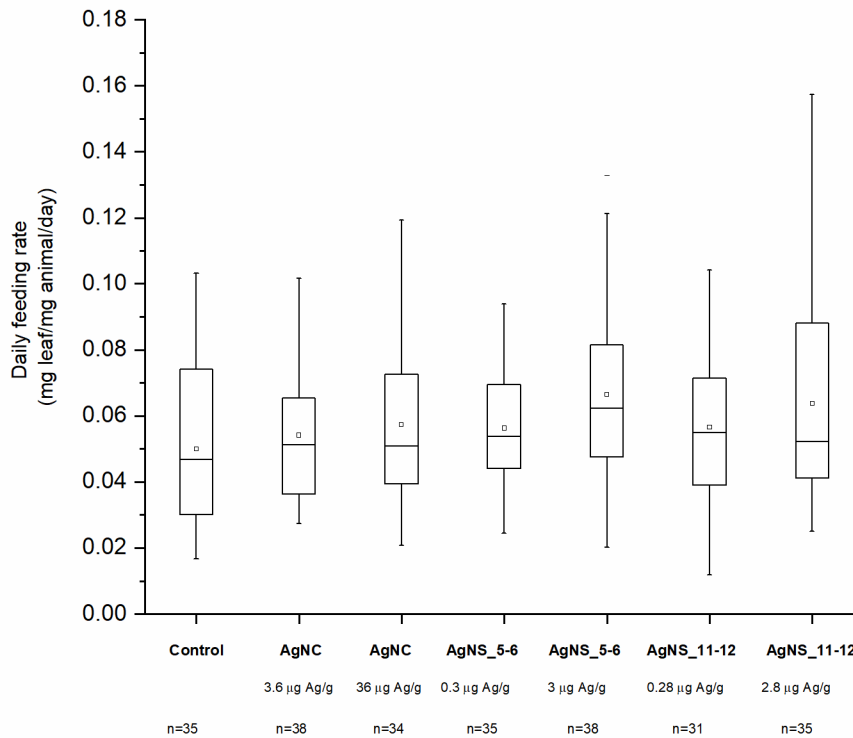


Figure S1: Feeding rate (mg of consumed leaf per mg of animal weight per day) of animals fed for 14 days on food dosed with AgNCs and AgNS_5-6 and AgNS_11-12. Symbols on the box plot represent minimum and maximum data values (whiskers), mean value (\square), 75th percentile (upper edge of box), 25th percentile (lower edge of box), median (line in box) and max and min value (-). There were no statistically significant differences between exposed and control animals. Nominal exposure concentrations (3.6 and 36 $\mu\text{g Ag/g}$ per leaf for AgNCs, 0.3 and 3 $\mu\text{g Ag/g}$ per leaf for AgNS_5-6 and 0.28 and 2.8 $\mu\text{g Ag/g}$ per leaf for AgNS_11-12), as well as the number of surviving animals and animals without marsupia per group, are shown on the x-axis (n). No statistically significant differences between controls were found therefore in each group three experiments are combined. Altogether 42 animals per group were exposed.

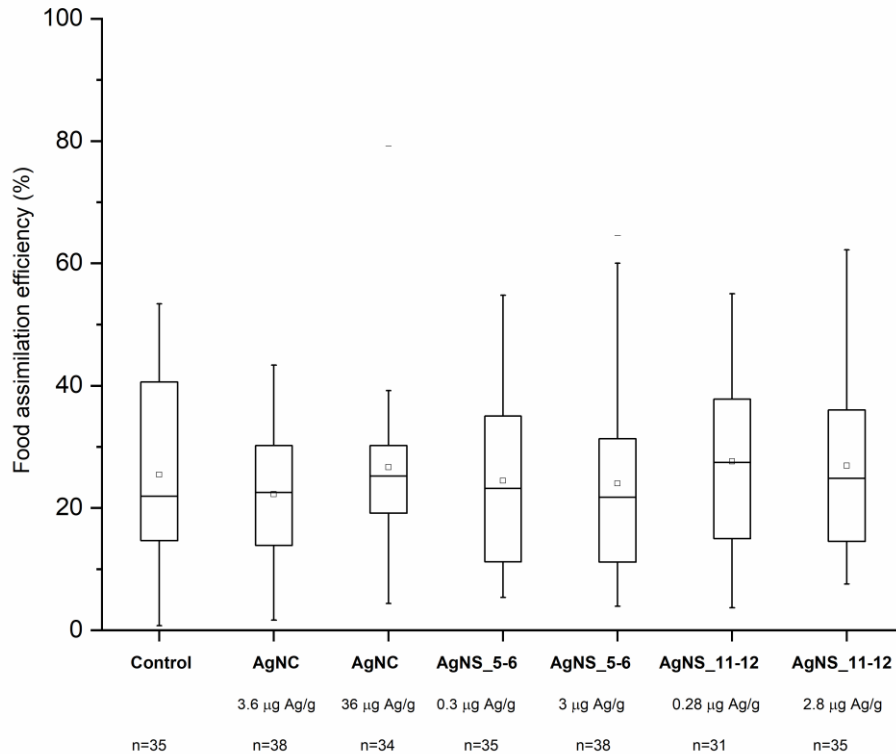


Figure S2: Percent of assimilated food (difference between the mass of consumed leaves and mass of faecal pellets divided by the mass of consumed leaf) of animals fed for 14 days on food dosed with AgNCs and AgNS_5-6 and AgNS_11-12. Symbols on the box plot represent minimum and maximum data values (whiskers), mean value (\square), 75th percentile (upper edge of box), 25th percentile (lower edge of box), median (line in box) and max and min value (-). There were no statistically significant differences between exposed and control animals. Nominal exposure concentrations (3.6 and 36 $\mu\text{g Ag/g}$ per leaf for AgNCs, 0.3 and 3 $\mu\text{g Ag/g}$ per leaf for AgNS_5-6 and 0.28 and 2.8 $\mu\text{g Ag/g}$ per leaf for AgNA_11-12), as well as the number of surviving animals and animals without marsupia per group, are provided on the x-axis (n). In each group three experiments are combined. Altogether 42 animals per group were exposed.

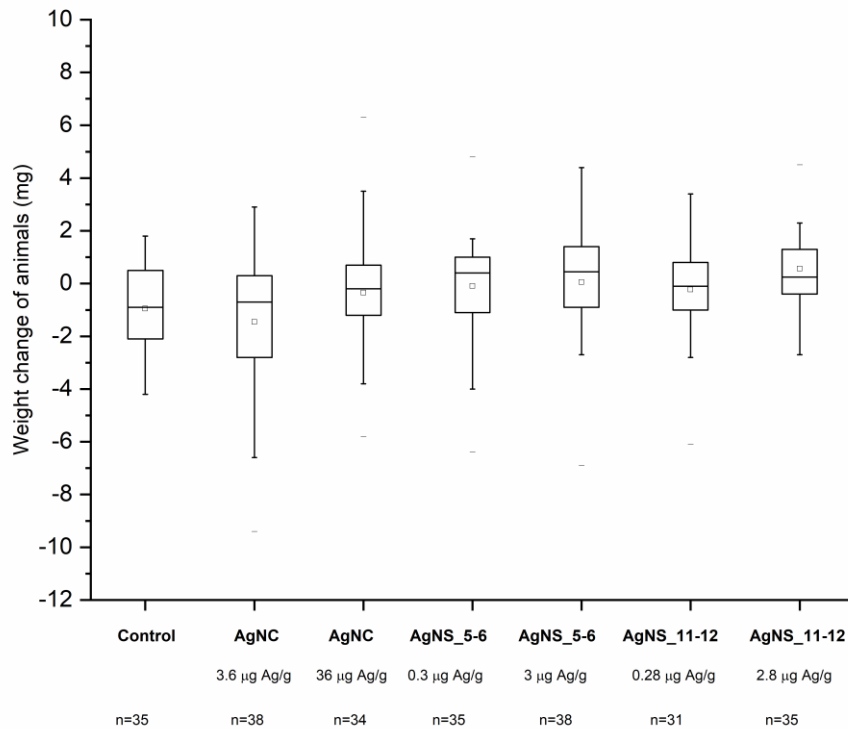


Figure S3: Weight change (difference between the mass at the end of the exposure period with the mass at the beginning of the exposure period) of animals fed for 14 days on food dosed with AgNCs and AgNS_5-6 and AgNS_11-12. Symbols on the box plot represent minimum and maximum data values (whiskers), mean value (\square), 75th percentile (upper edge of box), 25th percentile (lower edge of box), median (line in box) and max and min value (-). There were no statistically significant differences between exposed and control animals. Nominal exposure concentrations (3.6 and 36 $\mu\text{g Ag/g}$ per leaf for AgNCs, 0.3 and 3 $\mu\text{g Ag/g}$ per leaf for AgNS_5-6 and 0.28 and 2.8 $\mu\text{g Ag/g}$ per leaf for AgNS_11-12), as well as the number of surviving animals and animals without marsupia per group, are shown on the x-axis (n). In each group three experiments are combined. All together 42 animals per group were exposed.

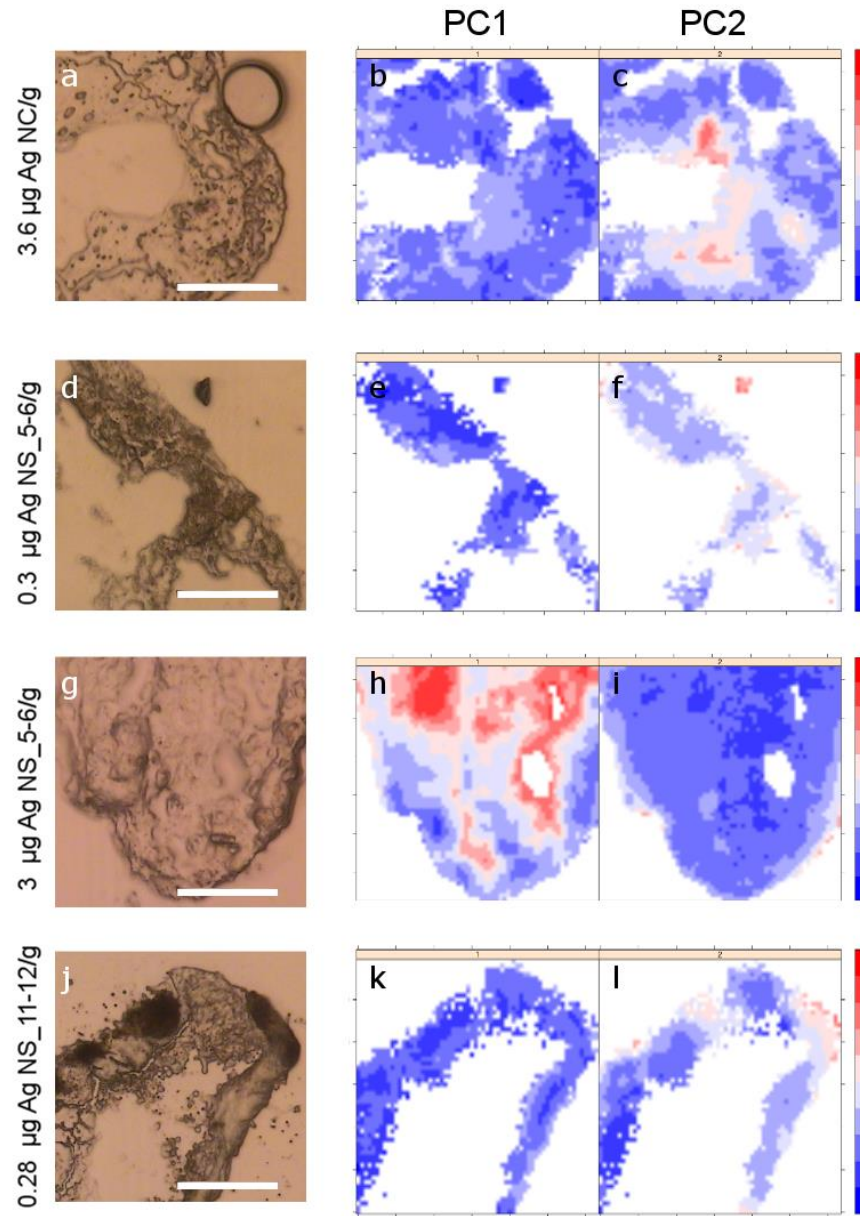


Figure S4: PCA heat maps of controls and exposed samples. a-c Light microscopy image and PC1 and PC2 images of a representative section of digestive gland of an animal fed with 3.6 μg AgNC/g per leaf; d-f Light microscopy image and PC1 and PC2 images of a representative section of digestive gland of an animal fed with 0.3 μg AgNS_5-6/g per leaf ; g-i light microscopy image and PC1 and PC2 images of sample a representative section of digestive gland of an animal fed with 3 μg AgNS_5-6/g per leaf /g per leaf; j-l light microscopy image and PC1 and PC2 images of sample a representative section of digestive gland of an animal fed with 0.28 μg AgNS_11-12/g per leaf. The white scale bar is 50 micron. PCA scale bar extends from -0.3 (blue) to 0.7 (red) a.u. for all the PCA images.

Univariate vibrational analysis

SPECTRAL INTENSITY \pm SD (A.U.)			
Sample name	Region 1	Region 2	Region 3
Control	4.69 \pm 0.60	2.79 \pm 0.60	0.5 \pm 0.1
Ag NC 3.6 μ g Ag/g of leaf	4.17 \pm 0.87	2.83 \pm 0.82	0.61 \pm 0.39
Ag NC 36 μ g Ag/g of leaf	4.55 \pm 0.79	2.28 \pm 0.47	0.62 \pm 0.23
Ag NS 5-6 nm 0.3 μ g Ag/g of leaf	4.23 \pm 0.77	3.11 \pm 0.75	0.58 \pm 0.10
Ag NS 5-6 nm 3 μ g Ag/g of leaf	2.73 \pm 0.65	5.78 \pm 1.27	0.46 \pm 0.05
Ag NS 11-12 nm 0.28 μ g Ag/g of leaf	3.98 \pm 0.90	2.58 \pm 0.61	0.53 \pm 0.12
Ag NS 11-12 nm 2.8 μ g Ag/g of leaf	2.91 \pm 1.08	5.14 \pm 1.76	0.47 \pm 0.06

Table S1: Average vibrational band intensity in the spectral Region 1 (1700-1480 cm^{-1}), diagnostic of cellular protein content, Region 2 (3100-2800 cm^{-1}), mainly diagnostic of cellular lipid content, and Region 3 (1260-1190 cm^{-1}), mainly diagnostic of cellular nucleic acids and phospholipids, in all analyzed samples for each exposure group with standard deviation (a. u. = absorbance units).

References

- 1 S. Komarneni, D. Li, B. Newalkar, H. Katsuki and A. S. Bhalla, *Langmuir*, 2002, **18**, 5959–5962.
- 2 A. R. Siekkinen, J. M. McLellan, J. Chen and Y. Xia, *Chem. Phys. Lett.*, 2006, **432**, 491–496.