# Exposure media and nanoparticle size influence on the fate, bioaccumulation, and toxicity of silver nanoparticles to higher plant *Salvinia minima*

## Supporting information

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Table S1: The Hoagland's Medium basal salt recipe used in this study.

| Chemical | Conc. (mg/L) |
|---|---|
| $NH_4H_2PO_4$ | 115.03 |
| $H_3BO_3$ | 2.86 |
| $Ca(NO_3)_2$ | 656.4 |
| $CuSO_4.5H_2O$ | 0.08 |
| $C_{12}H_{12}Fe_2O_{18}$ | 5.23 |

| | |
|---|---|
| $MnCl_2 \cdot 4H_2O$ | 1.81 |
| $MoO_3$ | 0.016 |
| $KNO_3$ | 606.6 |
| $ZnSO_4 . 7H_2O$ | 0.22 |
| $MgSO_4$ | 240.76 |

Table S2: freeze dryer settings used in the experimental setup.

| Shelf (°C) | Hold (min) | Vacuum (mTorr) |
|---|---|---|
| -40 | 360 | 80 |
| -30 | 360 | 80 |
| -20 | 360 | 80 |
| -10 | 360 | 80 |
| 0 | 120 | 400 |
| 20 | 120 | 600 |

Table S3: The achieved recovery rates for Ag obtained from analysis with ICP-MS.

| | Sequence Run | QC value | %Recovery | | | |
|---|---|---|---|---|---|---|
| | | | | | | |
| QC mix + Hg | 1st run | 2.33 | 93.29 | | | |
| Dig 2.5ppb QC | 1st run | 2.74 | 109.66 | | | |
| QC mix + Hg | 1st run | 2.45 | 97.91 | | | |
| Dig 2.5ppb QC | 1st run | 2.81 | 112.53 | | | |
| QC mix + Hg | 1st run | 2.52 | 100.62 | | | |
| Dig 2.5ppb QC | 1st run | 2.64 | 105.76 | | | |
| | | | | | | |
| QC mix + Hg | 2nd run | 2.28 | 91.27 | | | |
| Dig 2.5ppb QC | 2nd run | 2.81 | 112.28 | | | |
| Dig 2.5ppb QC new | 2nd run | 2.83 | 113.02 | | | |
| QC mix + Hg | 2nd run | 2.49 | 99.70 | | | |
| Dig 2.5ppb QC | 2nd run | 2.69 | 107.62 | | | |
| Dig 2.5ppb QC new | 2nd run | 2.74 | 109.56 | | | |
| QC mix + Hg | 2nd run | 1.95 | 78.10 | | | |
| Dig 2.5ppb QC | 2nd run | 2.45 | 98.06 | | | |
| Dig 2.5ppb QC | 2nd run | 2.65 | 106.05 | | | |
| | | | | | | |
| QC mix + Hg | 3rd run | 2.42 | 96.73 | | | |
| Digested 2.5ppb QC | 3rd run | 2.63 | 105.12 | | | |
| QC mix + Hg | 3rd run | 2.49 | 99.67 | | | |
| Digested 2.5ppb QC | 3rd run | 2.84 | 113.55 | | | |
| | | | | | | |
| NB : Digested 2.5ppb QC was digested following the same procedure for the samples | | | | | | |

Table S4: Comparison of whole plant Ag accumulation (μg/mg dry weight) between 10-Ag NPs and 40-Ag NPs under different water chemistries. In brackets are standard deviations, where n = 3. Student's t-test, $p < 0.05$.

| | **10-Ag NPs** | **40-Ag NPs** | ***p* value** |
|---|---|---|---|
| MHW | 0.163 (0.022) | 0.120 (0.004) | 0.076 |
| NOM | 0.154 (0.020) | 0.110 (0.020) | 0.056 |
| $Ca^{2+}$ | 0.193 (0.027) | 0.157 (0.0007) | 0.149 |

Table S5: Percentage growth reduction relative to respective controls.

| | **10-Ag NPs** | **40-Ag NPs** |
|---|---|---|
| MHW | 63.08 | 57.03 |
| NOM | 36.28 | 78.76 |
| $Ca^{2+}$ | 1 414.57 | 1 276.02 |

Table S6: Results of AgNPs sizes and concentrations under variant exposure media. The 48 h average particle size were obtained with DLS, modal particle size obtained using NTA over 48 h, and NTA was employed to determine particle concentration over 48 h. The given values are the mean ± standard error ($n$ = 3). Differing symbols indicate statistical difference ($p < 0.05$) within a specific AgNPs' size.

| | | 10-AgNPs | | | 40-AgNPs | | |
|---|---|---|---|---|---|---|---|
| Parameter | Time | MHW | $Ca^{2+}$ | NOM | MHW | $Ca^{2+}$ | NOM |
| DLS size (nm) | 48 h | 156.85 (±8.74) | 176.14 (±17.18) | 257.88* (±30.24) | 60.42* (±4.54) | 173.74# (±13.36) | 107.94^ (±24.48) |
| NTA size (nm) | 0 h | 38.33 (±2.30) | 46.66 (±4.16) | 68.0 (±36.42) | 38.33 (±1.52) | 41.66 (±1.15) | 39.66 (±0.57) |
| | 24 h | 76.33 (±5.77) | 88.66 (±3.51) | 54.33 (±8.50) | 47.66 (±1.52) | 52.66 (±4.04) | 37.00 (±2.00) |
| | 48 h | 86.33 (±7.50) | 88.66 (±3.51) | 64.50 (±6.36) | 52.00 (±2.64) | 58.33 (±6.42) | 38.66 (±0.57) |
| Particle concentration (particles/mL) | 0 h | $2.12^{8}$ ($\pm 1.55^{7}$) | $4.39^{8}$ ($\pm 1.01^{8}$) | $2.32^{8}$ ($\pm 7.21^{6}$) | $5.86^{8}$ ($\pm 1.47^{7}$) | $5.75^{8}$ ($\pm 9.54^{6}$) | $4.81^{8}$ ($\pm 1.31^{7}$) |
| | 24 h | $1.17^{9}$ ($\pm 4.65^{7}$) | $9.28^{8}$ ($\pm 5.51^{6}$) | $4.68^{8}$ ($\pm 8.0^{7}$) | $7.47^{8}$ ($\pm 5.39^{7}$) | $5.45^{8}$ ($\pm 2.51^{7}$) | $1.48^{9}$ ($\pm 1.24^{8}$) |
| | 48 h | $6.98^{8}$ ($\pm 6.27^{7}$) | $7.54^{8}$ ($\pm 1.17^{7}$) | $2.53^{8}$ ($\pm 2.12^{6}$) | $5.19^{8}$ ($\pm 3.01^{7}$) | $3.94^{8}$ ($\pm 7.71^{7}$) | $1.11^{9}$ ($\pm 4.13^{7}$) |

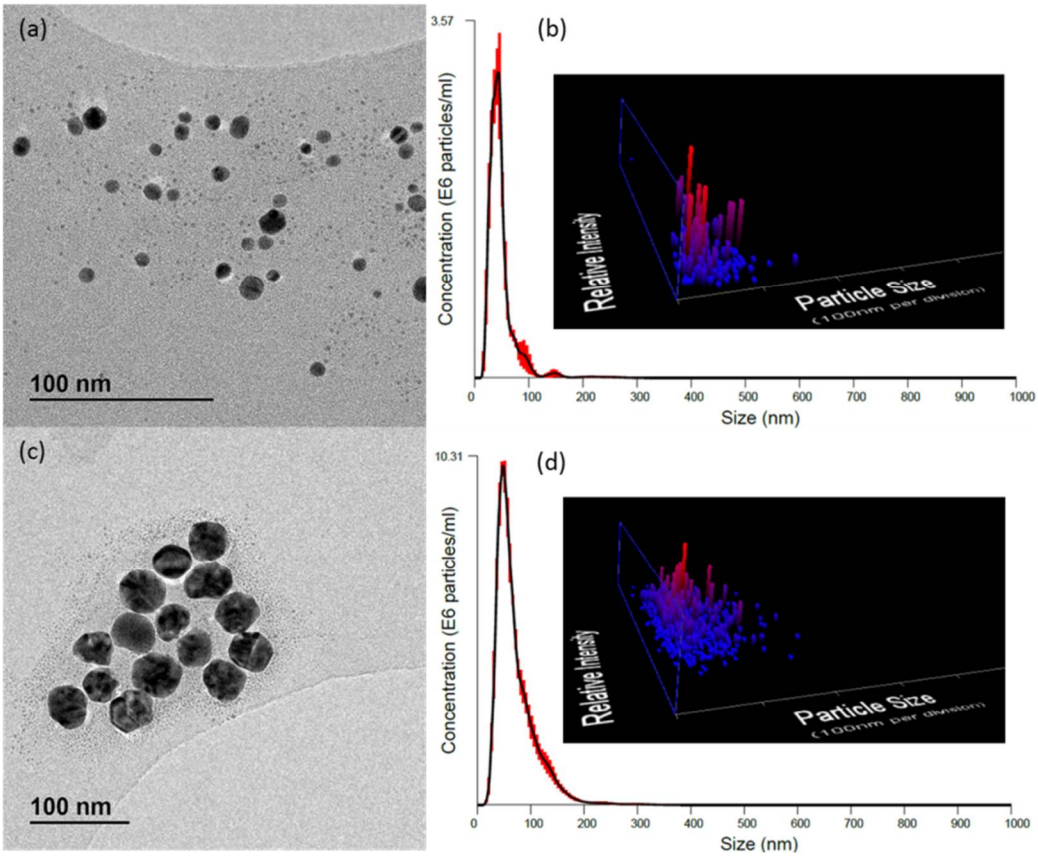


Figure S1: Ag NPs size obtained before testing for 10-Ag NPs with (a) TEM and (b) NTA, and 40-nAg NPs with (c) TEM and (d) NTA. Red bars denote the standard error. Inserts in (c) and (d) illustrate relative Ag NPs size intensities.

| MHW 10 nm | Wt% | Wt% Sigma |
|---|---|---|
| C | 52.01 | 0.60 |
| O | 39.93 | 0.54 |
| Na | 0.25 | 0.04 |
| Mg | 0.87 | 0.04 |
| Si | 0.23 | 0.02 |
| S | 0.99 | 0.04 |
| Cl | 1.47 | 0.04 |
| K | 3.12 | 0.06 |
| Ca | 1.02 | 0.04 |
| Ag | 0.11 | 0.06 |
| Total | 100.00 | |

| Ca 10 nm | Wt% | Wt% Sigma |
|---|---|---|
| C | 52.79 | 0.66 |
| O | 38.46 | 0.58 |
| Na | 0.21 | 0.04 |
| Mg | 0.52 | 0.04 |
| Si | 0.46 | 0.03 |
| P | 0.79 | 0.05 |
| S | 0.89 | 0.05 |
| Cl | 1.06 | 0.04 |
| K | 2.73 | 0.06 |
| Ca | 1.72 | 0.05 |
| Cu | 0.29 | 0.07 |
| Ag | 0.09 | 0.07 |
| Total | 100.00 | |

| NOM 10 nm | Wt% | Wt% Sigma |
|---|---|---|
| C | 52.76 | 0.73 |
| O | 39.36 | 0.66 |
| Mg | 0.46 | 0.04 |
| S | 0.77 | 0.05 |
| Cl | 1.49 | 0.05 |
| K | 3.14 | 0.08 |
| Ca | 1.91 | 0.07 |
| Ag | 0.10 | 0.09 |
| Total | 100.00 | |

| MHW 40nm | Wt% | Wt% Sigma |
|---|---|---|
| O | 64.63 | 0.52 |
| Na | 1.05 | 0.17 |
| Mg | 2.80 | 0.16 |
| Si | 1.07 | 0.11 |
| P | 0.61 | 0.13 |
| S | 3.76 | 0.15 |
| Cl | 5.58 | 0.16 |
| K | 13.39 | 0.25 |
| Ca | 5.79 | 0.19 |
| Cu | 0.90 | 0.27 |
| Ag | 0.42 | 0.26 |
| Total | 100.00 | |

| Ca 40 nm | Wt% | Wt% Sigma |
|---|---|---|
| C | 52.80 | 0.66 |
| O | 37.23 | 0.58 |
| Na | 0.32 | 0.05 |
| Mg | 0.75 | 0.04 |
| Si | 0.31 | 0.03 |
| P | 0.30 | 0.04 |
| S | 1.29 | 0.05 |
| Cl | 1.23 | 0.04 |
| K | 4.33 | 0.08 |
| Ca | 1.04 | 0.05 |
| Cu | 0.21 | 0.07 |
| Ag | 0.18 | 0.07 |
| Total | 100.00 | |

| NOM 40 nm | Wt% | Wt% Sigma |
|---|---|---|
| C | 57.19 | 0.48 |
| O | 37.05 | 0.45 |
| Na | 0.27 | 0.03 |
| Mg | 0.38 | 0.03 |
| Al | 0.54 | 0.03 |
| S | 0.64 | 0.03 |
| Cl | 0.62 | 0.03 |
| K | 2.52 | 0.05 |
| Ca | 0.69 | 0.03 |
| Ag | 0.10 | 0.05 |
| Total | 100.00 | |

Figure S2: The detected elemental analysis for samples exposed to 10-Ag NPs in MWH, Ca, and NOM (top row) and to 40- Ag NPs in MWH, Ca, and NOM (bottom row).

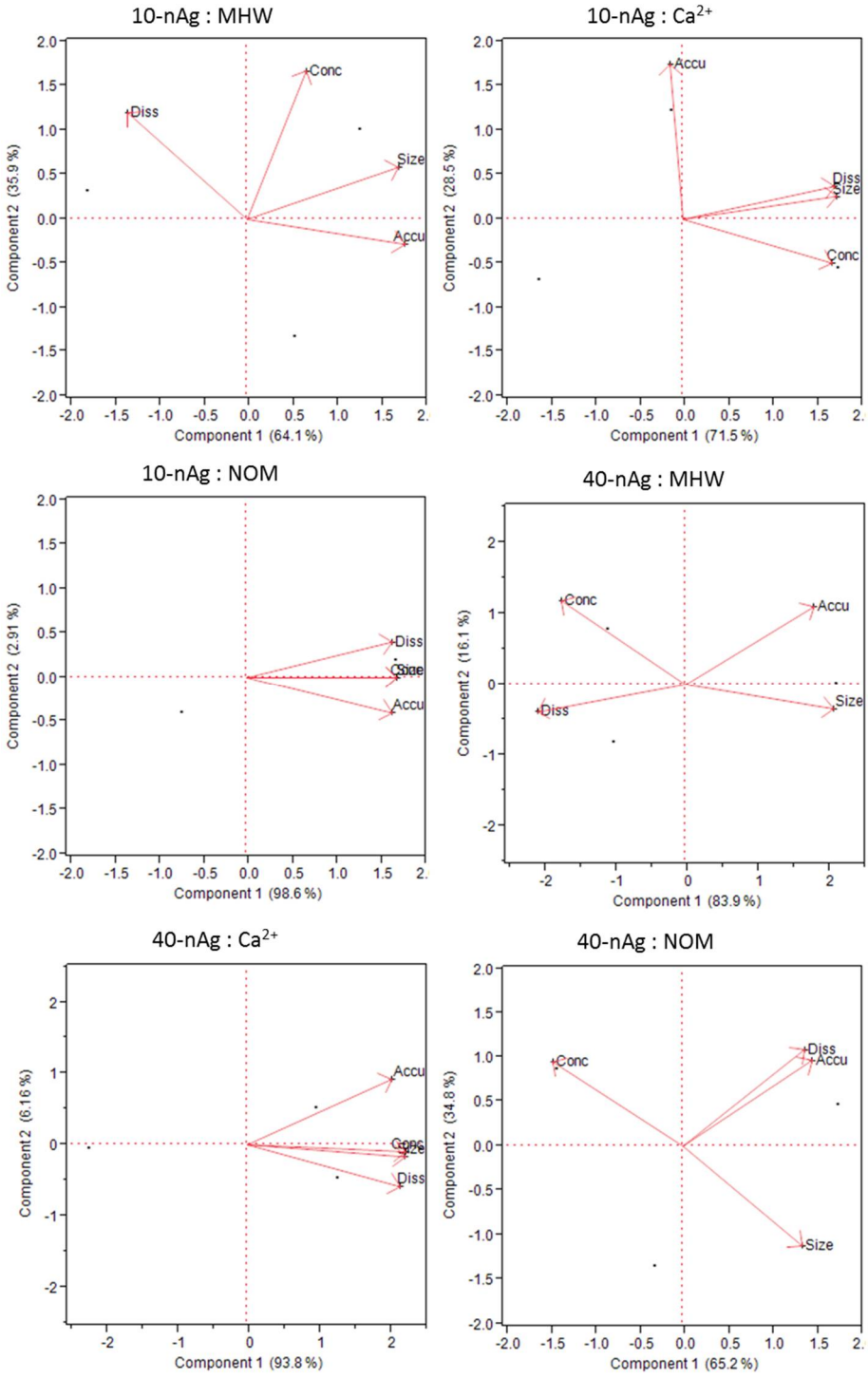


Figure S3: PCA plots illustrating the association of Ag accumulation (accu) to the Ag NPs size (size), dissolution (diss), and Ag NPs concentration (conc) p for 10-Ag NPs and 40-Ag NPs under different water chemistries (MHW, NOM, and $Ca^{2+}$).

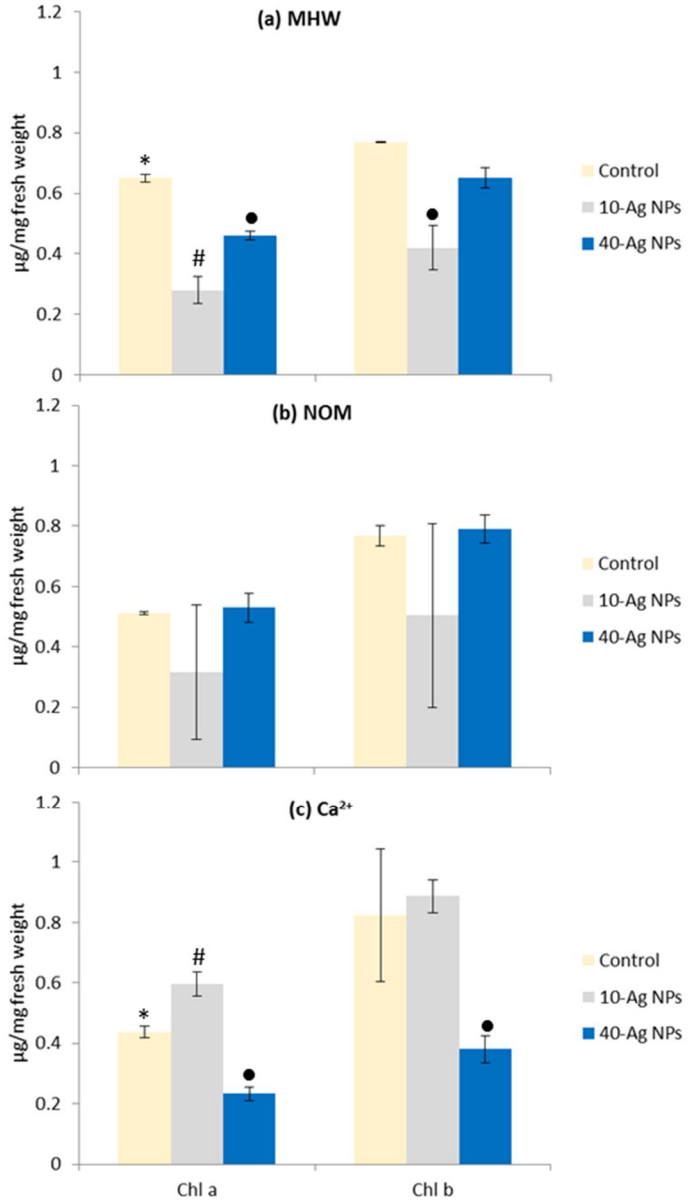


Figure S4: Quantification of chlorophyll pigments Chl *a*, Chl *b* and their ratios in *S. minima* after exposure to 10- and 40-AgNPs for 48 h. Bars denote standard error ($n$ = 3). Differing symbols on top of error bars indicate statistical difference within a specific photosynthetic parameter. Turkey Kramer HSD, $p < 0.05$.