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Supplementary material

Does natural organic matter increase the bioavailability of cerium dioxide nanoparticles to fish?

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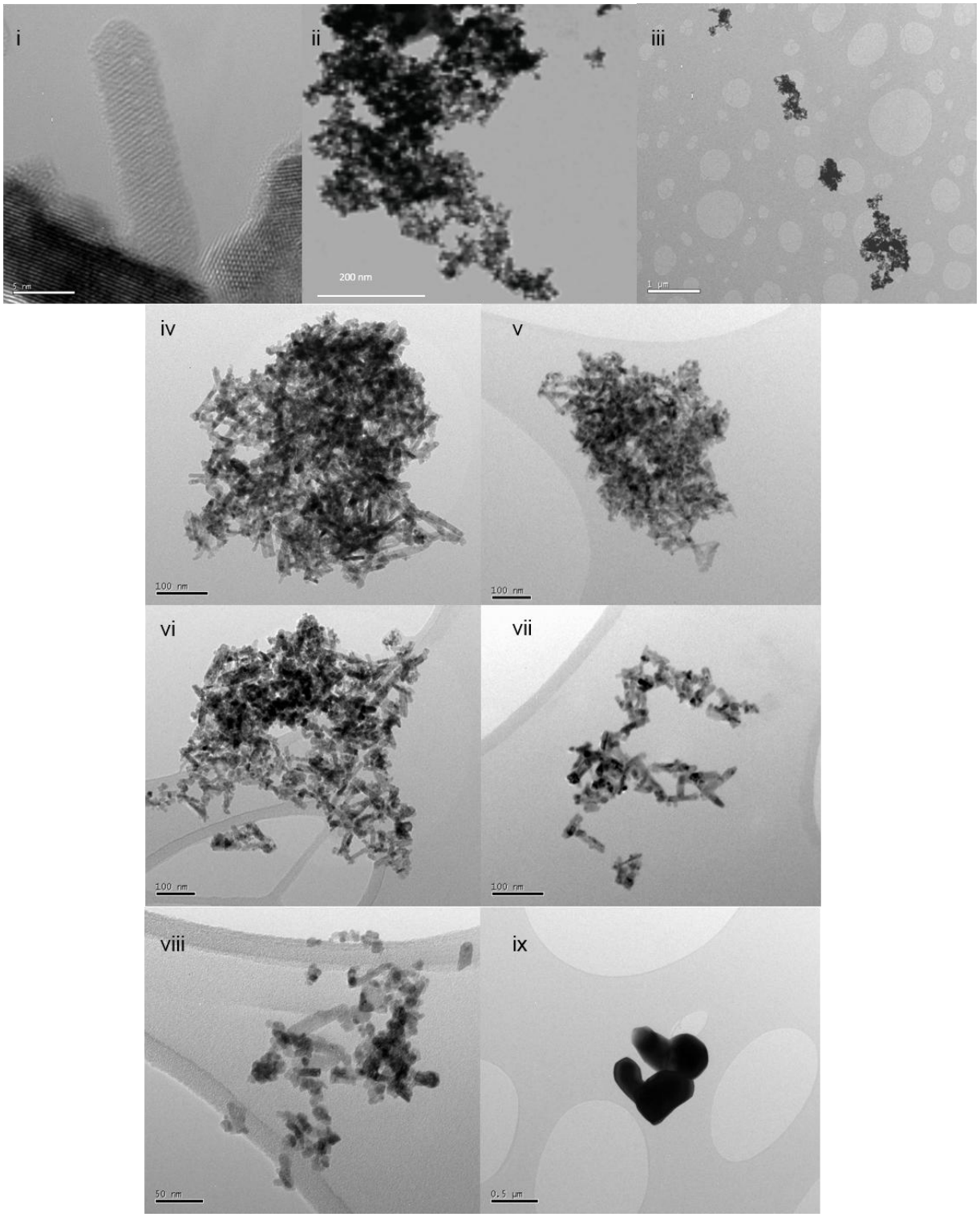

Fig. S1. Transmission electron microscopy (TEM) micrograph. TEM images of CeO_2 (Alfa Aesar) nanoparticles used in the exposure study (nominal size 20–30 nm): dry powder (i, ii) and from the various treatment tanks; (iii) uncoated CeO_2 in aquarium water, (iv) 20 µg citrate-coated CeO_2 L^{-1}, (v) 20 µg citrate-coated CeO_2 + 50 µg FA L^{-1}, (vi) 200 µg citrate-coated CeO_2 L^{-1}, (vii) 200 µg citrate-coated CeO_2 + 50 µg FA L^{-1}, (viii) 200 µg citrate-coated CeO_2 + 250 µg FA L^{-1}, (ix) 20 µg citrate-coated CeO_2 bulk particles + 50 µg FA L^{-1}.

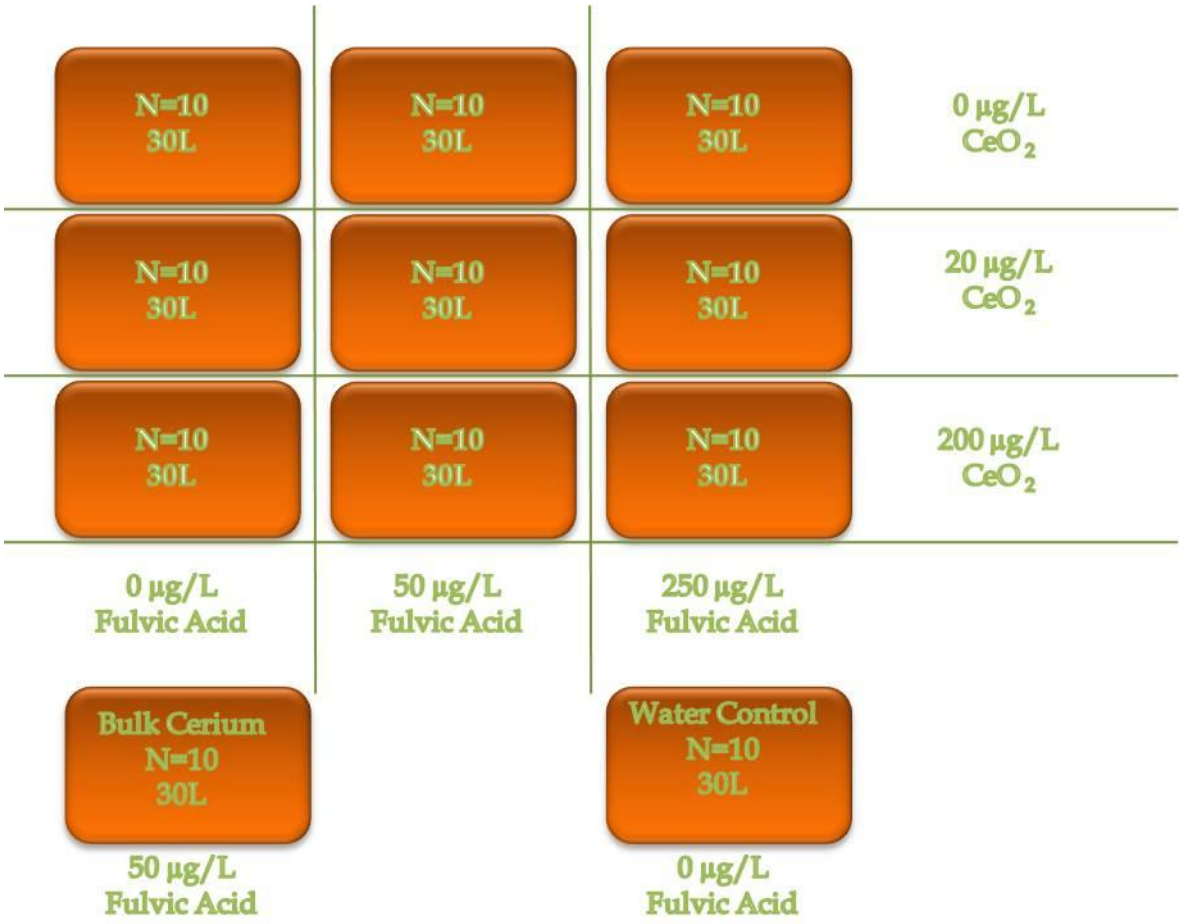

Fig. S2. Schematic of experimental set-up for exposing carp (*Cyprinus carpio*) to CeO_2 nanoparticles in the presence of fulvic acid. Fish numbers and exposure regime for both Experiment I and II were the same except for the exposure period. The study utilised a semistatic dosing system over a period of 32 days with a 50 % water change every second day. Bulk treatments were dosed at 20 µg L^{-1}.

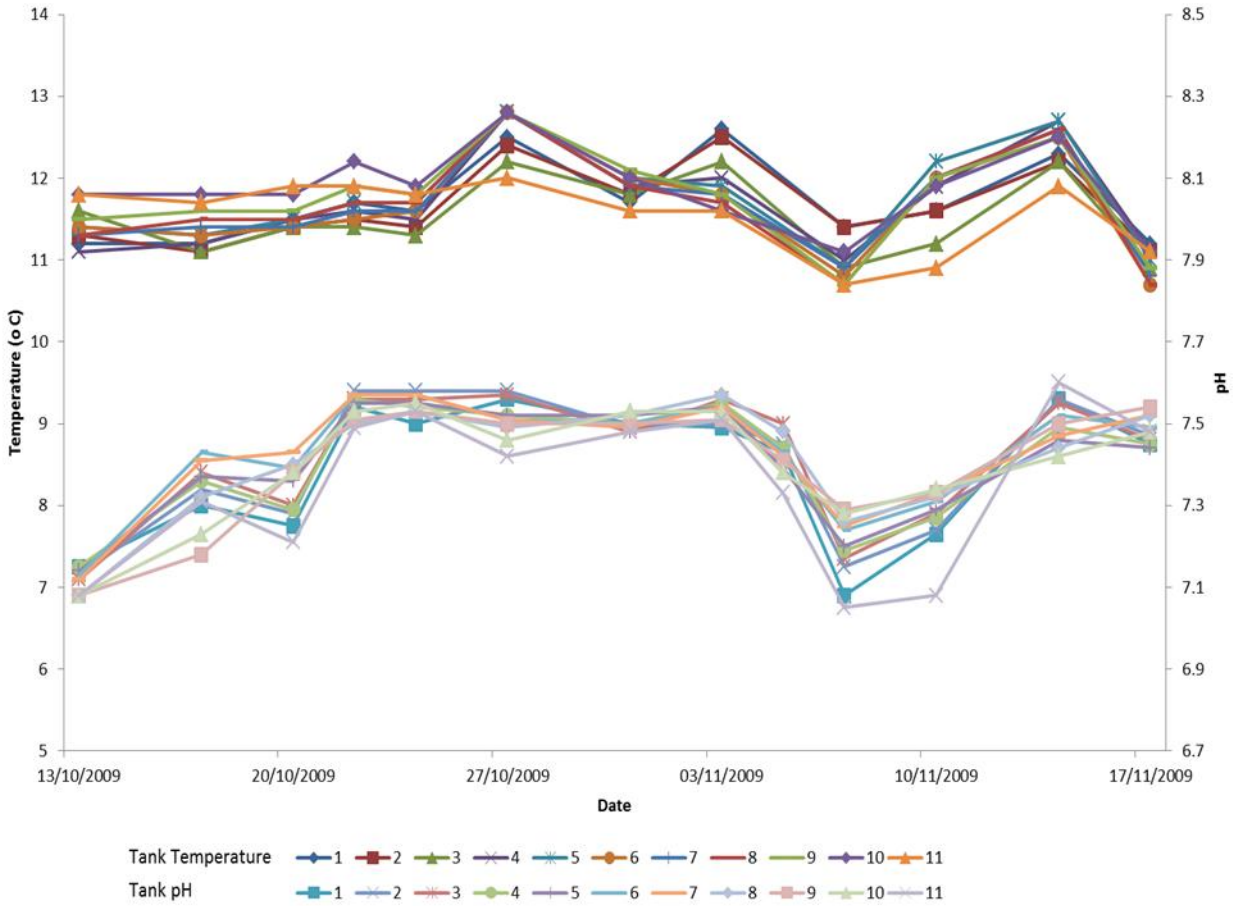

Fig. S3. pH and temperature in the treatment tanks for the carp (*Cyprinus carpio*) exposures to CeO_2 nanoparticles in the presence of fulvic acids. The pH and temperature measurements taken during the study were highly consistent between the treatments, with no major fluctuations throughout. Treatments are (1) citrate, (2) 50 µg FA L^{-1}, (3) 250 µg FA L^{-1}, (4) 20 µg nano CeO_2 L^{-1}, (5) 20 µg nano CeO_2 L^{-1} + 50 µg FA L^{-1}, (6) 20 µg nano CeO_2 L^{-1} + 250 µg FA L^{-1}, (7) 200 µg nano CeO_2 L^{-1}, (8) 200 µg nano CeO_2 L^{-1} + 50 µg FA L^{-1}, (9) 200 µg nano CeO_2 L^{-1} + 250 µg FA, (10) 20 µg bulk CeO_2 L^{-1} + 50 µg FA L^{-1} and (11) RO water.

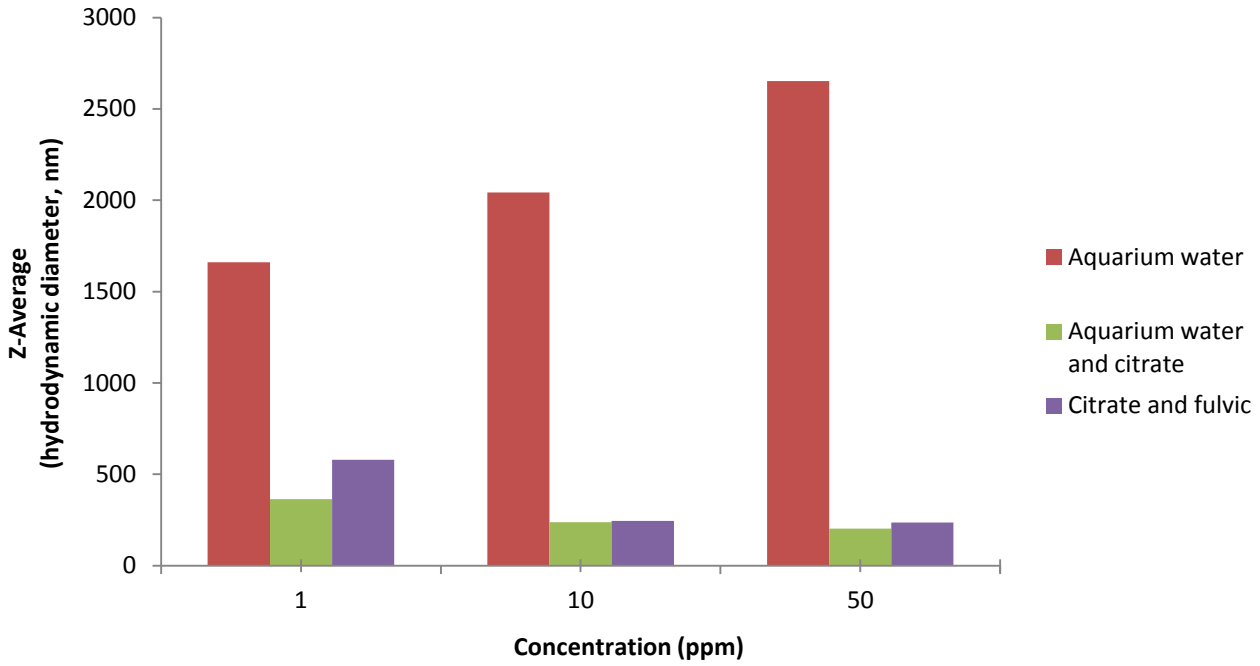

Fig. S4. Particle sizes measured by dynamic light scattering. Hydrodynamic diameter of nanoparticle aggregates in the presence of the citrate and citrate with fulvic acids were consistently smaller than aggregates formed in aquarium water alone. Particles were prepared and measured identically with those for the fish exposure studies, described in the *Methods* section. Data indicate a reduction in aggregate formation in the presence of citrate and fulvics, similarly to that noted in the fish exposure study. Ce concentrations adopted here were higher than in the fish exposure study to allow accurate measurement by DLS.

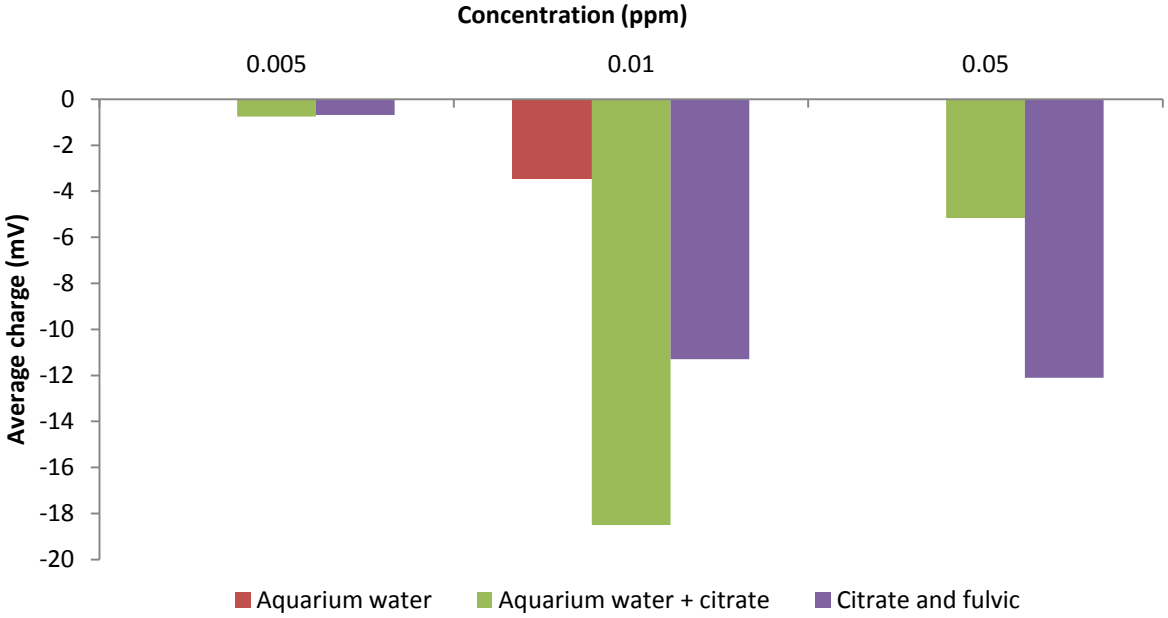

Fig. S5. Zeta potential (ζ) measurements of CeO_2 suspensions. The zeta potential of the nanoparticles was variable across the different CeO_2 concentrations; however, there was a trend for a reduced aggregation in the presence of citrate and citrate in combination with fulvic acid (FA) at the test concentrations most similar to those used in the fish exposures. Particles were prepared and measured identically with those for the fish exposure studies, described in the *Methods* section. Zeta potential values were not greater than ±30 mV, indicating the suspensions were not entirely stable.

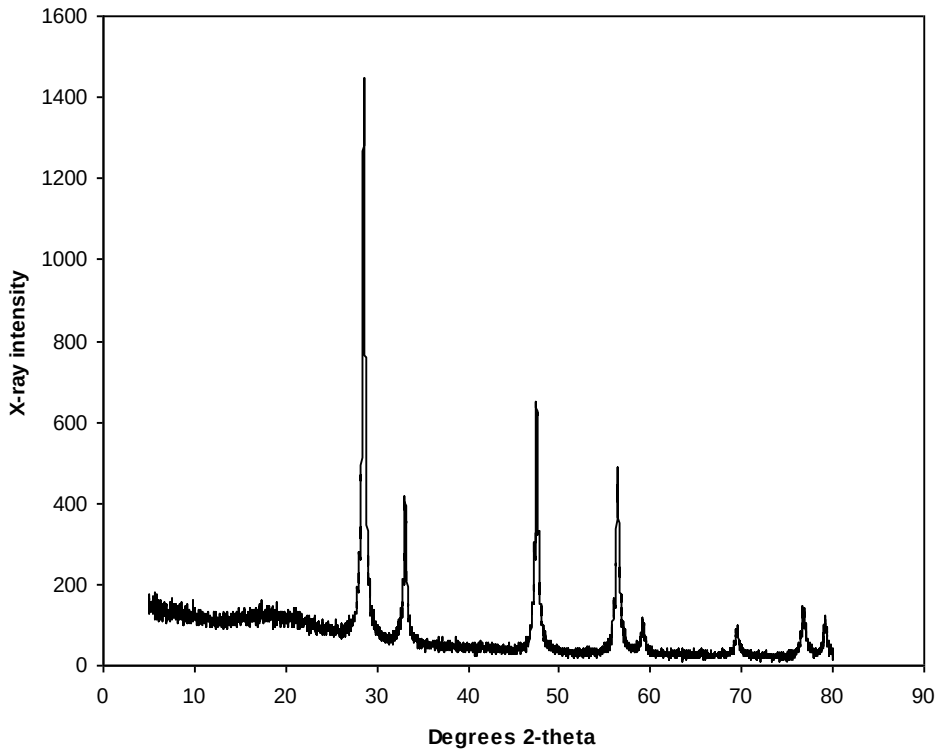

Fig. S6. X-Ray diffraction (XRD) diffractogram. XRD analysis of the particles collected from the exposure media onto the TEM grids verified that the aggregates we measured were ceria. This diffractogram of nano-ceria was obtained at room temperature and is representative of all CeO_2 treatments and identical to the diffractogram obtained from the dry CeO_2 nanoparticle powder (data not shown).