Supplemental Information

Multi-step Method to Extract Moderately Soluble Copper Oxide Nanoparticles from Soil for Quantification and Characterization

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Summary of Supplemental Information

Characterization of CuO NPs

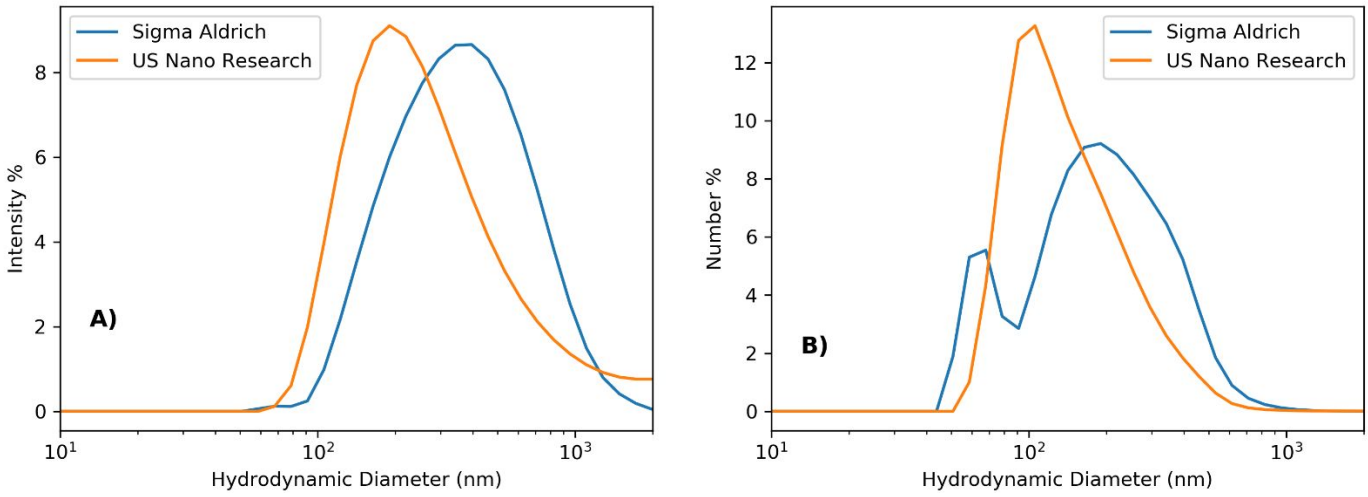

Figure S1: Hydrodynamic diameter of US Research Nanomaterials and Sigma Aldrich CuO NPs by intensity (A) and number (B). CuO NPs were suspended in DI water at a concentration of 10 mg l^{-1}, pH = 5.7.

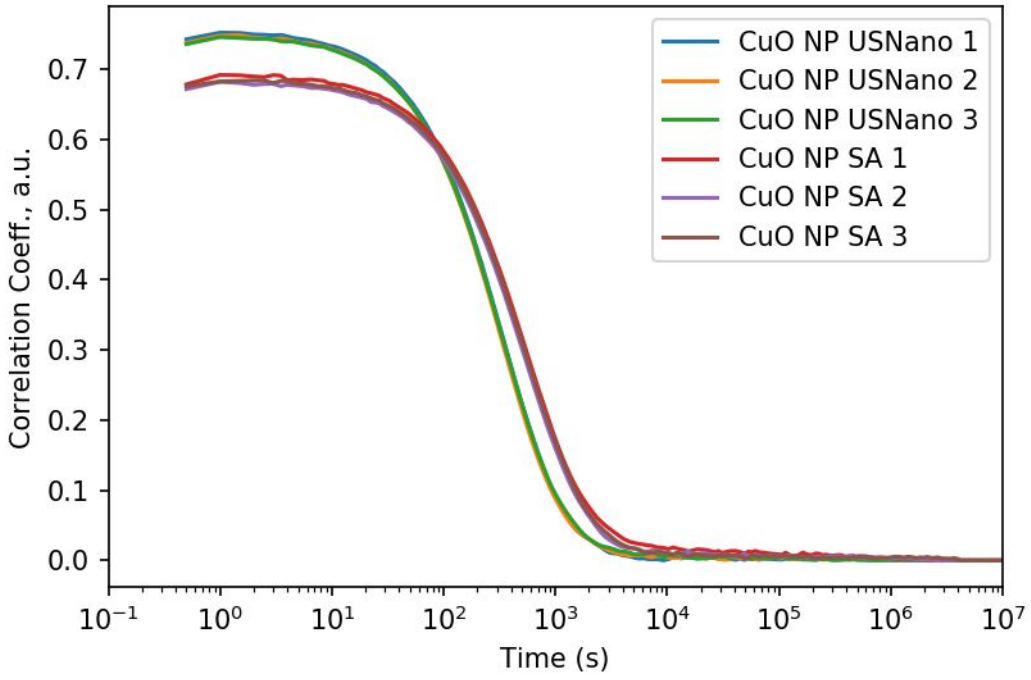

Figure S2: Correlograms of US Nano Research and Sigma Aldrich CuO NPs in triplicates.

Table S1: Hydrodynamic diameter average and standard deviation (triplicate measurements)

Sample ID	Intensity Based Average (Standard dev) (nm)	Number Based Average (Stdev) (nm)
CuO NP SA	401 (182)	267 (1)
CuO NP US Nano	293 (36)	160 (12)

Table S2: Zeta potential of Sigma Aldrich and US Nano Research CuO NPs at a concentration of 10 mg l^{-1} and 5 mM NaCl. pH of suspensions was 5.7 and there was no further alteration.

Sample ID	Zeta Potential Average (Stdev) (mV)
Sigma Aldrich	-15.9 (1.4)
US Nano	4.8 (1.8)

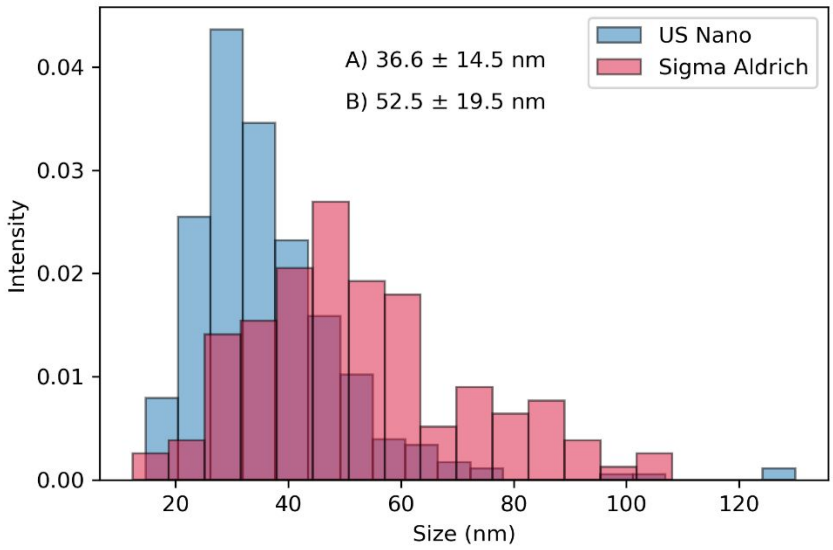

Figure S3: TEM size distribution of Sigma Aldrich CuO NPs (A, number of particles measured = 122) and US Nano Research CuO NPs (B, number of particles measured = 306). Particle sizes were measured by imaging software, ImageJ.

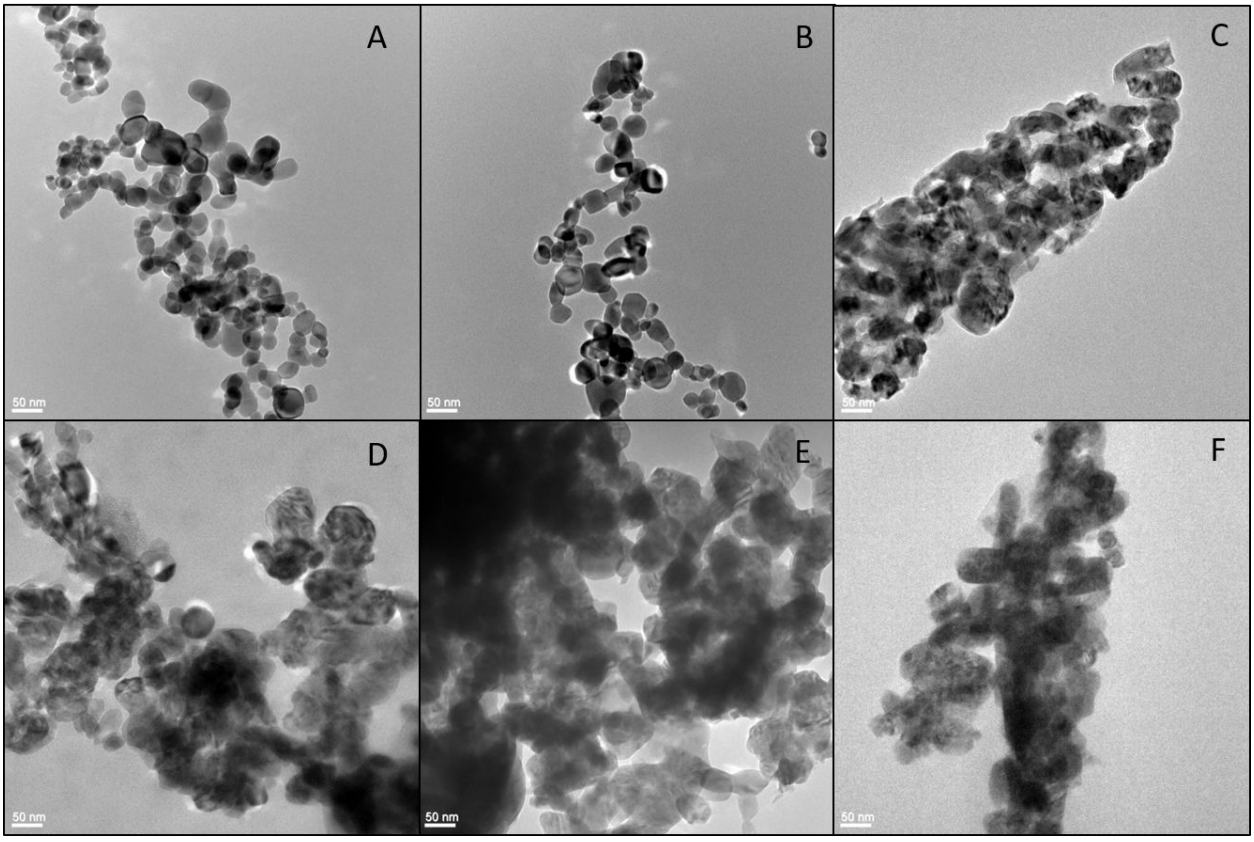

Figure S4: Example TEM images of US Nano Research CuO NPs (A and B), PVP-coated Sigma Aldrich CuO NPs (C and D), and uncoated Sigma Aldrich CuO NPs (E and F).

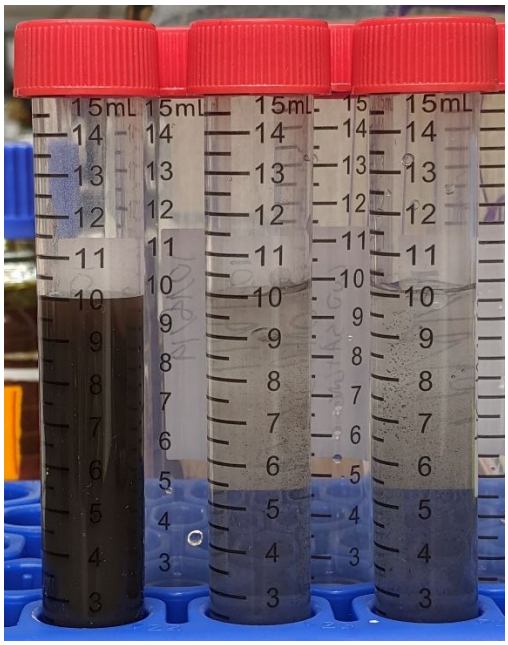

Figure S5: CuO NP suspensions at a nominal concentration of 1 g L^{-1} in DI water after 1 hour of settling. US Nano (left), Sigma Aldrich (middle), Sigma Aldrich-PVP coated (right). US Nano CuO NPs remained in suspension whereas Sigma Aldrich (coated and uncoated) settled.

Effect of Sonication for the size of Colloid and CuO NP extract

To further understand the influence of sonication on the soil colloids and CuO NPs extracted, the hydrodynamic size distribution of each extract was determined by DLS (Figure S6). The average size of the NP/colloids suspensions decrease based upon the output energy level. Probe sonication for 1 minute had the highest average size of about 702 ± 44 nm while the probe sonication for 30 minutes had the smallest average size of 492 ± 35 nm. This is probably due to physical abrasion leading to breaking the primary soil particles. While the dissolution of CuO NPs can alter the particle size, this is a minor component compared to the overall composition of the colloidal extract.

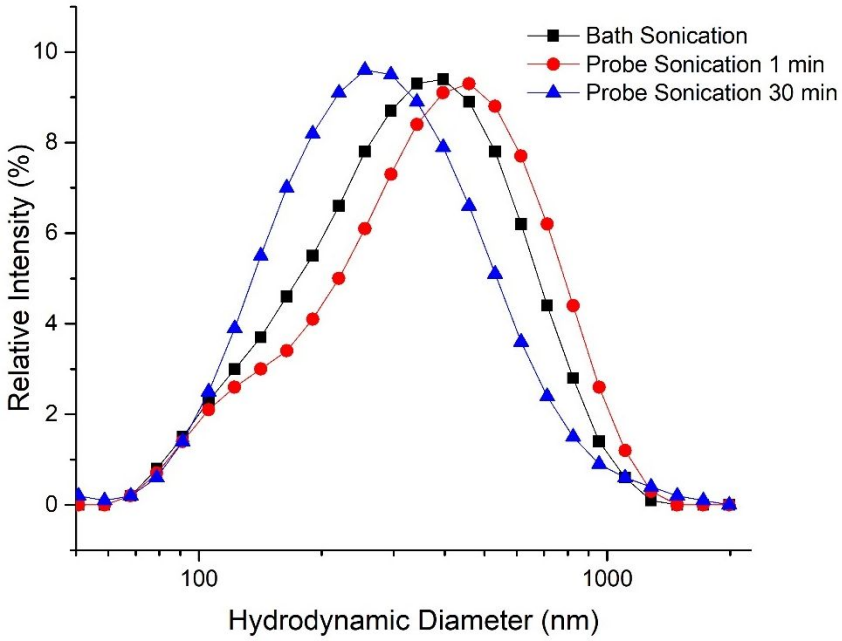

Figure S6: The DLS size distribution of the < 1 μm surfactant extract from soil for comparing different sonication steps (Probe sonication for 1 minute (702 ± 44nm, 6.6 joules), Bath Sonication for 8 minutes (576 ± 81nm, 38.4 joules), and Probe sonication for 30 minutes (492 ± 35 nm, 198 joules).

Testing Cloud Point Extraction Efficiency

For this study, it is assumed that cloud point extraction completely separates the particulate Cu and soluble Cu. To verify this assumption, a 3 kDa Millipore centrifugation tube was used to filter the surfactant and liquid phase at the end of an extraction experiment with baseline parameters and a spike concentration of 250 mg-Cu kg^{-1} of uncoated SA CuO NPs. Each phase was centrifuge at 4000 rpm for 30 mins. The Cu that remained in the filtered supernatant is identified as dissolved Cu while particulate Cu remaining in the retentate. Surfactant phase was diluted with ultrapure to dissolve micelles and ensure there is no interferences the filtration process. (**Figure S7).** For the solution phase, a total of 14.7% is dissolved Cu and 4.6% is particulate Cu while for the surfactant phase, 1.6% is dissolved Cu and 25.7% is particulate. These results clearly show that most of the particulate Cu fraction reside in either the soil or the surfactant phase and the dissolved fraction is in the solution phase.

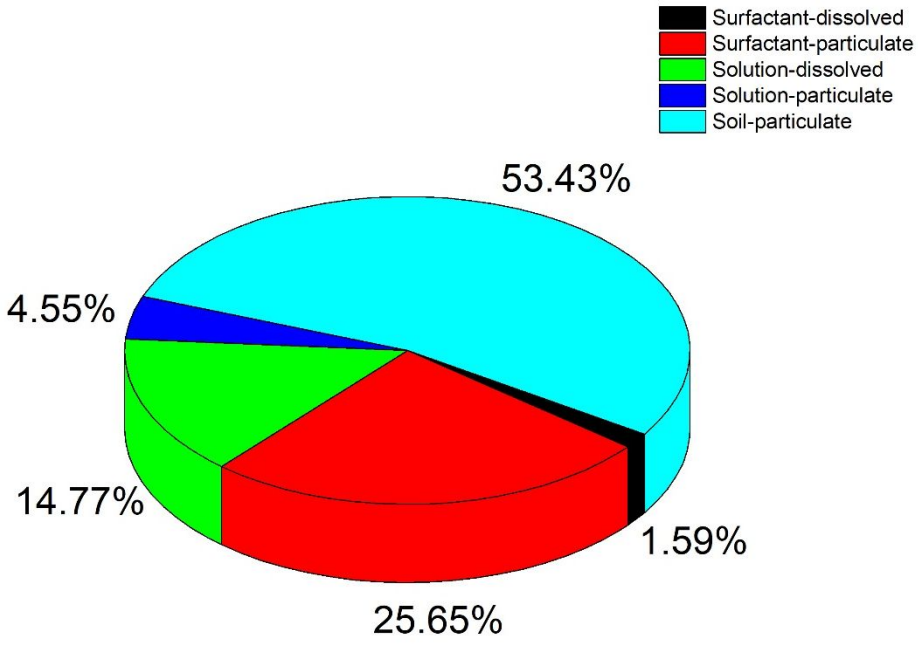

Figure S7: Cu distribution after extraction and after filtration for solution and surfactant phase. Cu that remained in the filtrate is labeled as dissolved and what is removed is labeled as particulate.

Recovery of Copper for each trial

Recovery of copper in each experiment is vital to determine if there is substantial loss throughout the extraction process. To do this, copper concentration was determined for each batch of experiments, then following equation was used to calculate the recovery.

$$Recovery\ of\ Copper\ (\%) = \frac{C_{solution} + C_{surfactant} + C_{soil}}{C_{dosed}} \times 100$$

Where $C_{solution}$, $C_{surfactant}$, and C_{soil} is the soil copper concentration residing in the solution, surfactant (extracted), and soil phase respectively after the extraction process, and C_{dosed} is the soil copper concentration initially measured. The recovery of copper is calculated for each trial and shown below (Figure S7). Most trials are within the range of 75-125% with 15% of the total sample being outliers. Each outlier was investigated and found that there is negligible effect on the compositional data set shown in the manuscript. The fraction with the most variation is the soil phase. This could mean that the outliers exist due to heterogeneity Cu concentration in the dosed soil.

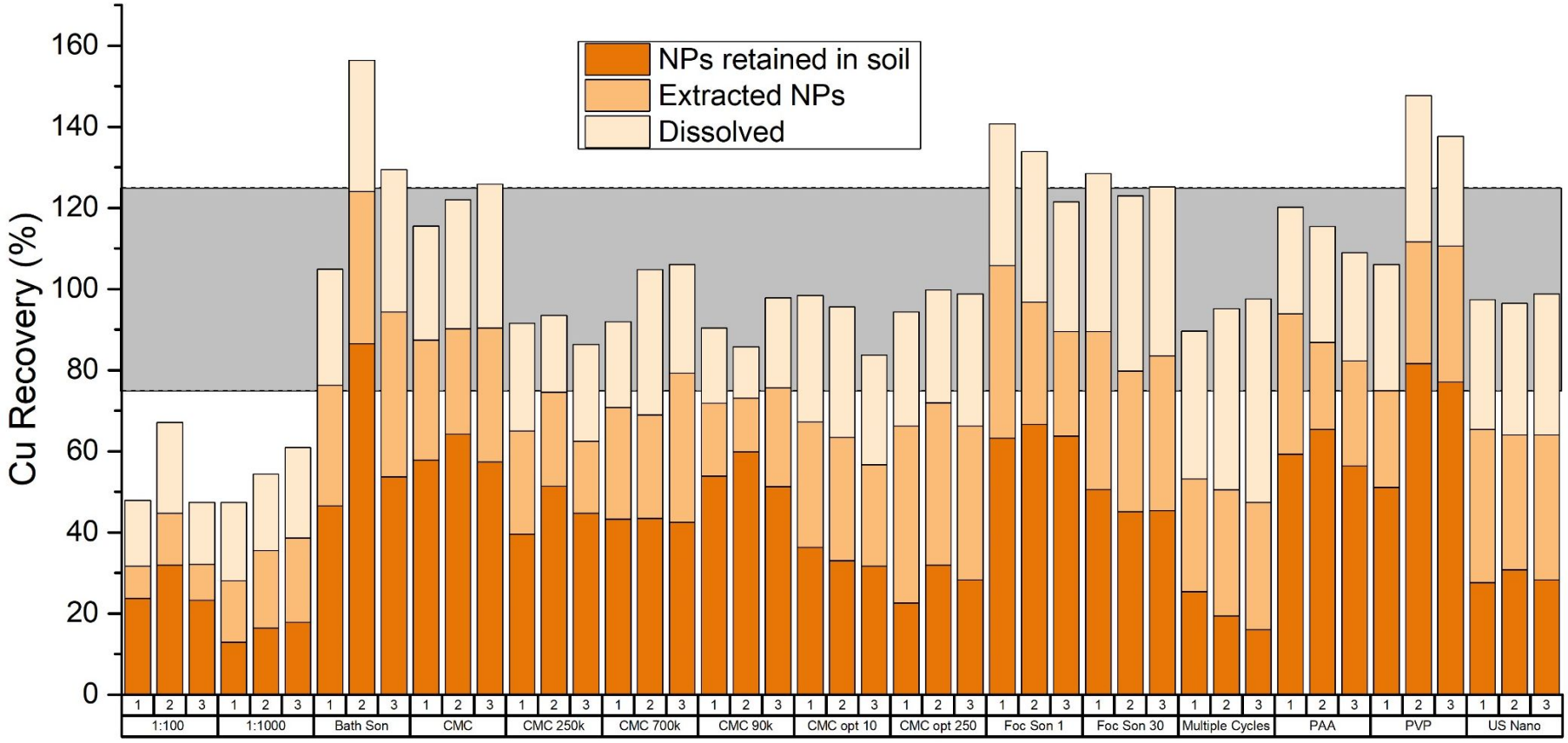

Figure S8: Recovery of Cu with the sum of all three phases (dissolved, extracted, and soil) in each trial. Cu concentration for dosed soil was measured for each batch of experiments. Gray region represents a range of 75-125% recovery.

Table S3: Comparing statistical significance of Cu-content results within each trial for each compartment (soil, dissolved, and extracted NPs). Statistical differences based on one-way ANOVA and Tukey test ($p < 0.10$) are indicated by capital letters.

Dispersing Agent and DTPA	Soil	Dissolved NPs	Extracted NPs
DTPA	A	A	N/A
CMC	B	B	A
PAA	B	B	A
Molecular Weight	**Soil**	**Dissolved NPs**	**Extracted NPs**
CMC 90k	A	A	A
CMC 250K	AB	A	AB
CMC 700k	B	A	B
Sonication	**Soil**	**Dissolved NPs**	**Extracted NPs**
Probe 1 min	A	A	A
Bath 8 min	AB	A	A
Probe 30 min	B	B	A
SLR	**Soil**	**Dissolved NPs**	**Extracted NPs**
1 10	A	A	A
1 100	A	B	B
1 1000	B	B	C
Extraction Cycles	**Individually**	**Accumulating**	
1	A	A	
2	B	AB	
3	B	B	
NP Coating and Stability	**Soil**	**Dissolved NPs**	**Extracted NPs**
SA	A	A	A
SA PVP Coated	A	A	A
US Nano	B	B	B
Dosage Concentration	**Soil**	**Dissolved NPs**	**Extracted NPs**
250 mg kg^{-1}	A	A	A
10 mg kg^{-1}	B	A	B

Variation and Correlation coefficient matrices

Table S4: Correlation coefficient and covariance matrix for each experiment comparing soil, solution, and surfactant Cu content.

Sample ID		Correlation coefficient matrices			Covariance matrices		
		Soil	Solution	Surfactant	Soil	Solution	Surfactant
CMC	Soil	1	-0.0381	-0.789	0	0.018	0.032
	Solution	-0.0381	1	-0.267	0.018	0	0.016
	Surfactant	-0.789	-0.267	1	0.032	0.016	0
PAA	Soil	1	-0.0381	-0.789	0	0.018	0.032
	Solution	-0.0381	1	-0.267	0.018	0	0.016
	Surfactant	-0.789	-0.267	1	0.032	0.016	0
CMC 90k	Soil	1	-0.971	-0.978	0	0.133	0.146
	Solution	-0.971	1	0.9	0.133	0	0.004
	Surfactant	-0.978	0.9	1	0.146	0.004	0
CMC 250k and Control	Soil	1	-0.686	-0.391	0	0.092	0.061
	Solution	-0.686	1	-0.401	0.092	0	0.062
	Surfactant	-0.391	-0.401	1	0.061	0.062	0
CMC 700k	Soil	1	-0.466	-0.018	0	0.687	0.042
	Solution	-0.466	1	-0.876	0.687	0	0.136
	Surfactant	-0.018	-0.876	1	0.042	0.136	0
Probe Sonication 1 min	Soil	1	0.833	-0.972	0	0.003	0.071
	Solution	0.833	1	-0.94	0.003	0	0.055
	Surfactant	-0.972	-0.94	1	0.071	0.055	0
Probe Sonication 30 mins	Soil	1	-0.834	0.324	0	0.014	0.002
	Solution	-0.834	1	-0.792	0.014	0	0.013
	Surfactant	0.324	-0.792	1	0.003	0.13	0
PVP	Soil	1	-0.849	0.335	0	0.076	0.015
	Solution	-0.849	1	-0.783	0.076	0	0.066
	Surfactant	0.335	-0.783	1	0.015	0.66	0
US Nano	Soil	1	-0.182	-0.958	0	0.006	0.014
	Solution	-0.182	1	-0.107	0.006	0	0.007
	Surfactant	-0.958	-0.107	1	0.014	0.007	0
Optimum 10 mg kg^{-1}	Soil	1	-0.782	-0.812	0	0.005	0.005
	Solution	-0.782	1	0.27	0.005	0	0.001
	Surfactant	-0.812	0.27	1	0.005	0.001	0
Optimum 250 mg kg^{-1}	Soil	1	-0.507	-0.791	0	0.035	0.055
	Solution	-0.507	1	-0.126	0.035	0	0.022
	Surfactant	-0.791	-0.126	1	0.055	0.022	0
1:100 soil liquid	Soil	1	0.741	-0.783	0	0.0125	0.02
	Solution	0.741	1	-0.998	0.0125	0	0.063
	Surfactant	-0.783	-0.998	1	0.02	0.063	0
1:1000 soil liquid	Soil	1	-0.973	0.266	0	0.117	0.025
	Solution	-0.973	1	-0.48	0.117	0	0.042
	Surfactant	0.266	-0.48	1	0.025	0.042	0