Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Supplementary Information

Assessment of Cu and CuO nanoparticle ecological responses using laboratory small-scale microcosms

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5 Tables, 21 Figures

Table S1. Standardized information for determining nanoparticle zeta potential in NCM:

Surface functionalization	None
Shape	spherical
Model used to compute the zeta potential	Henry's Equation (Smoluchowski approximation)
Duration of measurement	90 seconds for single measurement
Applied voltage	148 V
Number of measurements made and averaged to determine each ZP	12
Total number of replicate measurements	3
pH	7.2 ± 0.2
Ionic strength	0.01 mol/L
Ionic composition	Detailed information in [50]
Temperature	25 C
Total number of replicate measurements	3

Table S2. *The dissolution rate constants and predicted maximum dissolved Cu released in the exposure media by fitting the measured data to a first order exponential model.*

Parameter	Cu NP	CuO NP
$[Cu_{diss}]_{max}$ (mg Cu/L)	3.20 ± 0.185	2.80 ± 0.073
k (hr^{-1})	0.0246 ± 0.0036	0.0334 ± 0.0025

Table S3. *Cu speciation in exposure media using measured dissolved Cu concentrations.*

0.44 mg Cu/L		
Speciation	**Concentration (mol/L)**	**Concentration (mg/L)**
Cu(H2BO3)2 (aq)	9.8E-14	6.3E-09
Cu(NO3)2 (aq)	5.7E-14	3.7E-09
Cu(OH)2 (aq)	1.0E-08	6.5E-04
Cu(OH)3-	6.8E-12	4.4E-07
Cu(OH)4-2	1.1E-17	7.3E-13
Cu+2	9.7E-07	6.2E-02
Cu2(OH)2+2	5.3E-09	6.8E-04
Cu2OH+3	3.2E-12	4.0E-07
Cu3(OH)4+2	4.7E-11	9.0E-06
CuCl+	6.2E-09	3.9E-04
CuCl2 (aq)	6.4E-12	4.1E-07
CuCl3-	2.7E-16	1.7E-11
CuCl4-2 1	7.2E-21	4.6E-16
CuEDTA-2	4.9E-06	3.2E-01
CuH2BO3+	9.7E-10	6.2E-05
CuH2EDTA (aq)	7.7E-15	4.9E-10
CuHEDTA-	8.2E-10	5.2E-05
CuHPO4 (aq)	3.1E-07	2.0E-02
CuHSO4+	6.5E-16	4.2E-11
CuNO3+	1.1E-09	6.9E-05
CuOH+	3.8E-07	2.4E-02
CuOHEDTA-3	1.1E-10	6.9E-06
CuSO4 (aq)	9.9E-09	6.3E-04
CuMoO4(s)	**2.9E-07**	1.8E-02
/	/	/
Totol Cu (sum)		0.443
% solid in the speciation		4.15%

1.97 mg Cu/L		
Speciation	**Concentration (mol/L)**	**Concentration (mg/L)**
Cu(H2BO3)2 (aq)	2.2E-13	1.4E-08
Cu(NO3)2 (aq)	1.3E-13	8.3E-09
Cu(OH)2 (aq)	2.3E-08	1.5E-03
Cu(OH)3-	1.5E-11	9.9E-07
Cu(OH)4-2	2.6E-17	1.6E-12
Cu+2	2.2E-06	1.4E-01
Cu2(OH)2+2	2.8E-08	3.5E-03
Cu2OH+3	1.6E-11	2.1E-06
Cu3(OH)4+2	5.5E-10	1.1E-04
CuCl+	1.4E-08	8.9E-04
CuCl2 (aq)	1.5E-11	9.3E-07
CuCl3-	6.1E-16	3.9E-11
CuCl4-2 1	1.6E-20	1.0E-15
CuEDTA-2	4.9E-06	3.2E-01
CuH2BO3+	2.2E-09	1.4E-04
CuH2EDTA (aq)	7.7E-15	4.9E-10
CuHEDTA-	8.2E-10	5.2E-05
CuHPO4 (aq)	6.0E-07	3.8E-02
CuHSO4+	1.5E-15	9.5E-11
CuNO3+	2.5E-09	1.6E-04
CuOH+	8.6E-07	5.5E-02
CuOHEDTA-3	1.1E-10	6.9E-06
CuSO4 (aq)	2.2E-08	1.4E-03
CuMoO4(s)	**4.1E-07**	2.6E-02
Cu3(PO4)2(s)	**7.3E-06**	1.4E+00
Totol Cu (sum)		1.984
% solid in the speciation		70.56%

2.69 mg Cu/L		
Speciation	**Concentration (mol/L)**	**Concentration (mg/L)**
Cu(H2BO3)2 (aq)	2.4E-13	1.5E-08
Cu(NO3)2 (aq)	1.4E-13	8.8E-09
Cu(OH)2 (aq)	2.4E-08	1.6E-03
Cu(OH)3-	1.6E-11	1.1E-06
Cu(OH)4-2	2.7E-17	1.8E-12
Cu+2	2.3E-06	1.5E-01
Cu2(OH)2+2	3.1E-08	4.0E-03
Cu2OH+3	1.8E-11	2.3E-06
Cu3(OH)4+2	6.6E-10	1.3E-04
CuCl+	1.5E-08	9.5E-04
CuCl2 (aq)	1.5E-11	9.9E-07
CuCl3-	6.5E-16	4.2E-11
CuCl4-2 1	1.7E-20	1.1E-15
CuEDTA-2	4.9E-06	3.2E-01
CuH2BO3+	2.3E-09	1.5E-04
CuH2EDTA (aq)	7.7E-15	4.9E-10
CuHEDTA-	8.2E-10	5.2E-05
CuHPO4 (aq)	5.8E-07	3.7E-02
CuHSO4+	1.6E-15	1.0E-10
CuNO3+	2.6E-09	1.7E-04
CuOH+	9.1E-07	5.8E-02
CuOHEDTA-3	1.1E-10	6.9E-06
CuSO4 (aq)	2.4E-08	1.5E-03
CuMoO4(s)	**4.1E-07**	2.6E-02
Cu3(PO4)2(s)	**1.1E-05**	2.1E+00
Totol Cu (sum)		2.709
% solid in the speciation		77.99%

Table S4. *Summary of the potential total D. magna and zebrafish uptake in each nanocosm exposure.*

Exposure	Concentrations (mg Cu/L)	In *D. magna* (mg Cu)	In Zebrafish (mg Cu)	In chorion (ZF) (mg Cu)	Total (mg Cu)	% of total exposed Cu
Ionic Cu	0.44	-	9.8E-06	-	9.8E-06	0%
	1.97	1.1E-06	6.2E-06	-	7.3E-06	0%
	2.69	4.3E-06	3.9E-06	3.3E-05	4.1E-05	0%
Cu	1	7.8E-05	7.7E-06	-	8.6E-05	2%
	5	2.7E-04	5.0E-06	-	2.8E-04	1%
	10	6.2E-05	1.8E-06	2.3E-03	2.3E-03	5%
CuO	1	1.9E-04	6.0E-06	-	2.0E-04	4%
	5	1.2E-03	8.9E-06	-	1.2E-03	5%
	10	1.4E-03	5.4E-06	7.5E-03	8.9E-03	18%

Table S5. *Summary of the organism responses in each nanocosm exposure.*

	NP exposure concentrations (mg Cu/L)	0	1	5	10	1	5	10
	$CuCl_2$ exposure concentrations (mg Cu/L)	0	0.44	1.97	2.69	Unweighted Sum		
Cu NP	Algae growth rate	100%	−11%	−16%	−42%	−45%	−76%	−167%
	Bacteria growth rate	100%	−2%	−16%	−20%			
	D. magna survival	100%	−7%	−40%	−60%			
	Zebrafish hatching	100%	−26%	−4%	−44%			
CuO NP	Algae growth rate	100%	+1%	−9%	−31%	+5%	−46%	−79%
	Bacteria growth rate	100%	+4%	−7%	−9%			
	D. magna survival	100%	−7%	−7%	−7%			
	Zebrafish hatching	100%	+7%	−22%	−33%			
$CuCl_2$	Algae growth rate	100%	−4%	−16%	−30%	+7%	−15%	−63%
	Bacteria growth rate	100%	−9%	−1%	−6%			
	D. magna survival	100%	+13%	+7%	−27%			
	Zebrafish hatching	100%	−11%	−4%	0%			

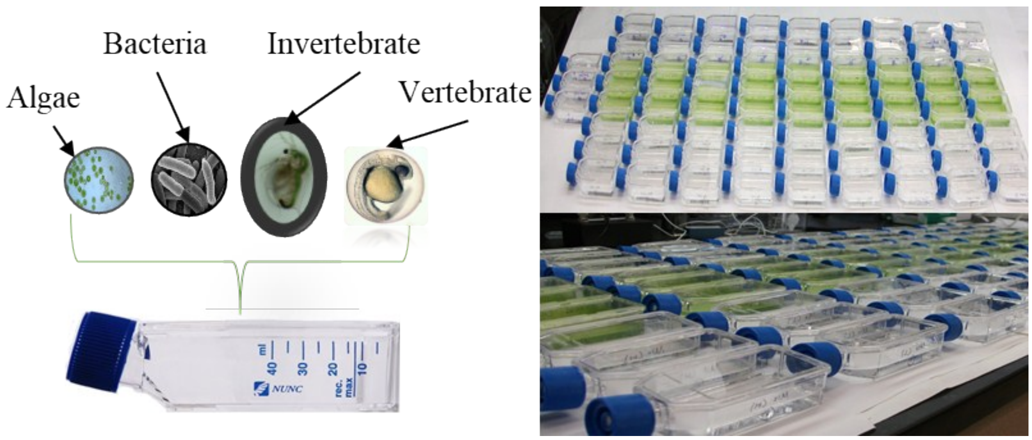

Figure S1. *Photographs of nanocosms.*

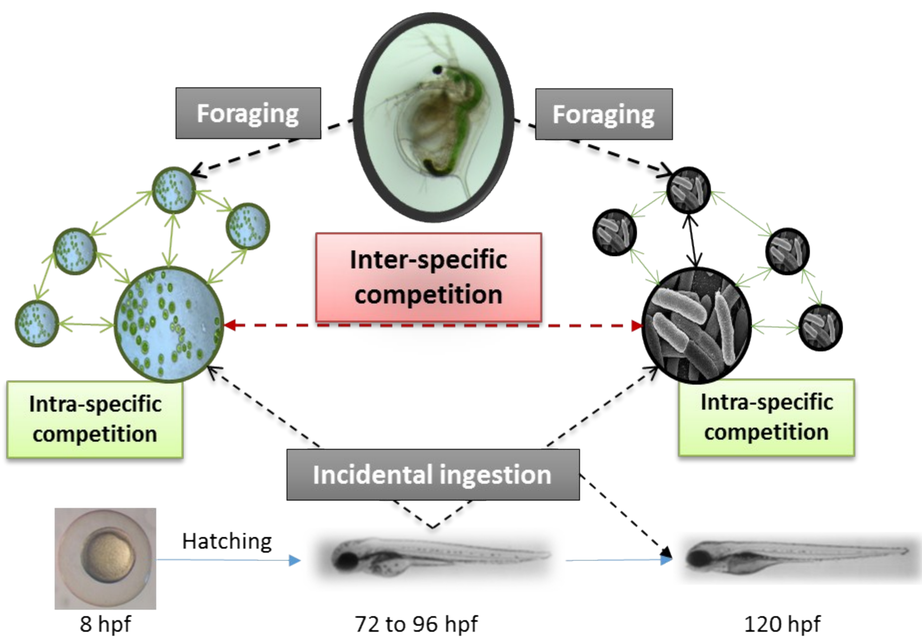

Figure S2. *Schematics of potential species interactions in the nanocosm.*

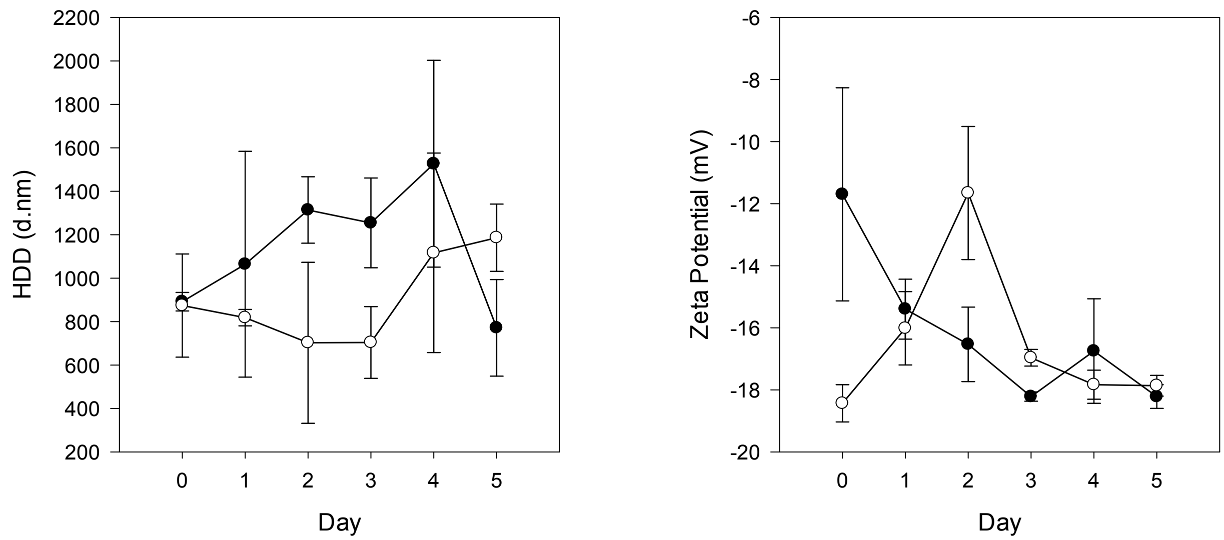

Figure S3. *Hydrodynamic diameter (a) and zeta potential (b) of Cu and CuO NP measured in nanocosm media at 10 mg Cu/L over 120 hours. Error bar represents standard deviation of three sample replicates.*

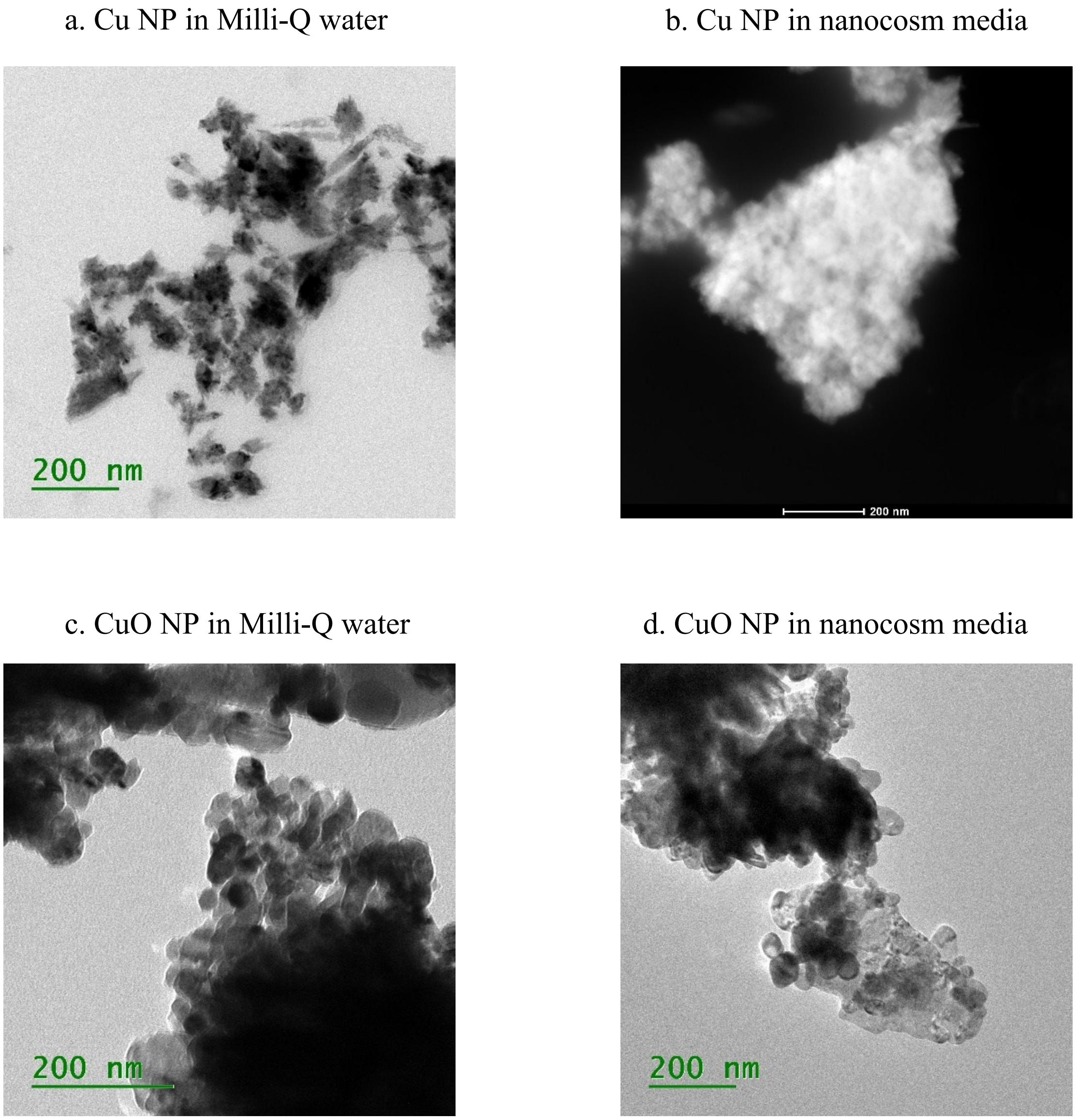

Figure S4. *TEM images of Cu and CuO NP after dried in Milli-Q water (a, c) and nanocosm media (b, d).*

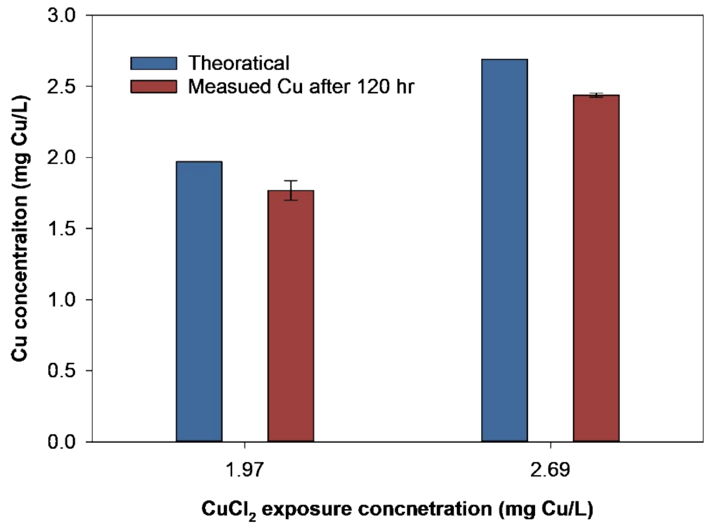

Figure S5. *Relationship between exposed ionic Cu (as* $CuCl_2$*) at 0 hour and the measured dissolved Cu concentration in nanocosm after 120 hours. Error bar represents standard error of three sample replicates.*

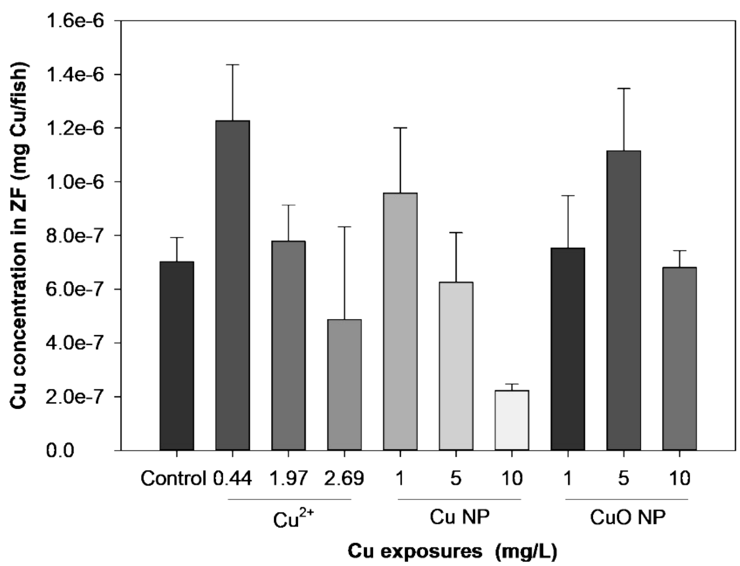

Figure S6. *Cu uptake in naturally hatched zebrafish after 120 hours. Error bar represents standard deviation of two sample replicates due to the low concentration can be quantified.*

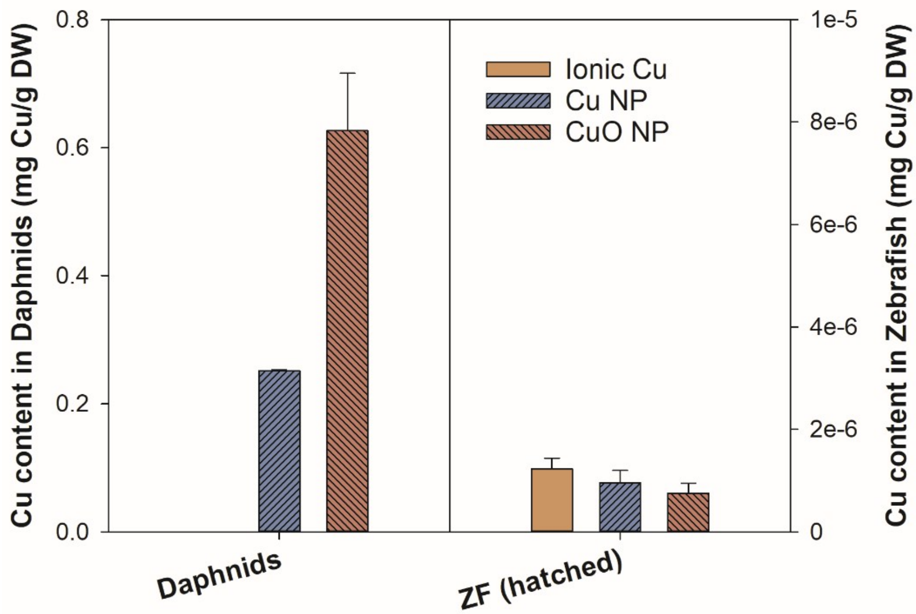

Figure S7*. Cu uptake in D. magna and zebrafish (hatched) with 1 mg Cu/L exposures after 120 hours (0.44 mg Cu/L for ionic Cu exposure).*

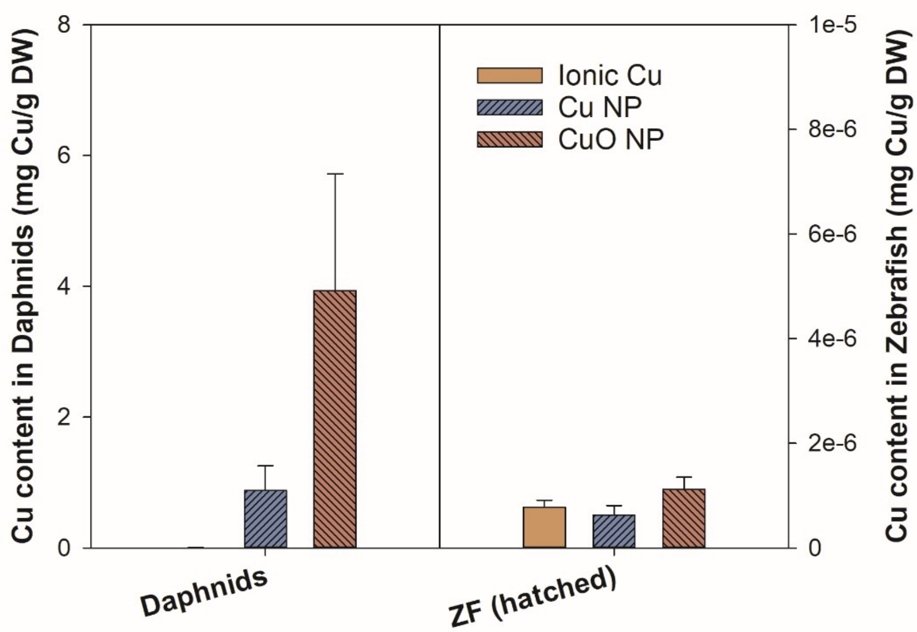

Figure S8*. Cu uptake in D. magna and zebrafish (hatched) with 5 mg Cu/L exposures after 120 hours (1.97 mg Cu/L for ionic Cu exposure).*

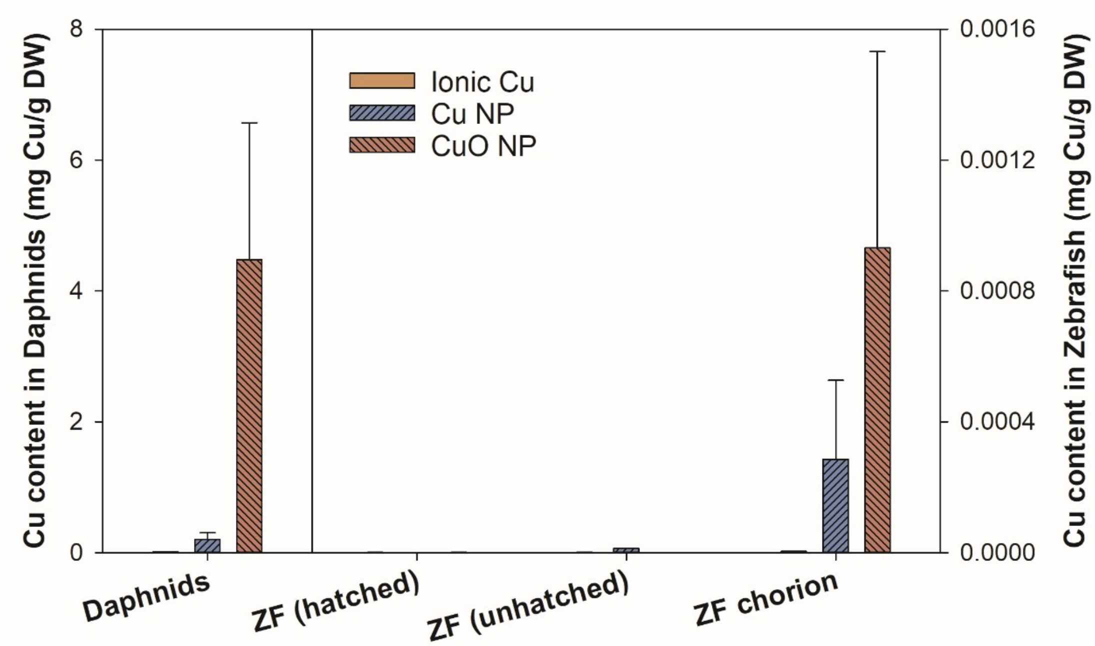

Figure S9. *Cu uptake in D. magna and zebrafish (hatched) with 10 mg Cu/L exposures after 120 hours (2.69 mg Cu/L for ionic Cu exposure).*

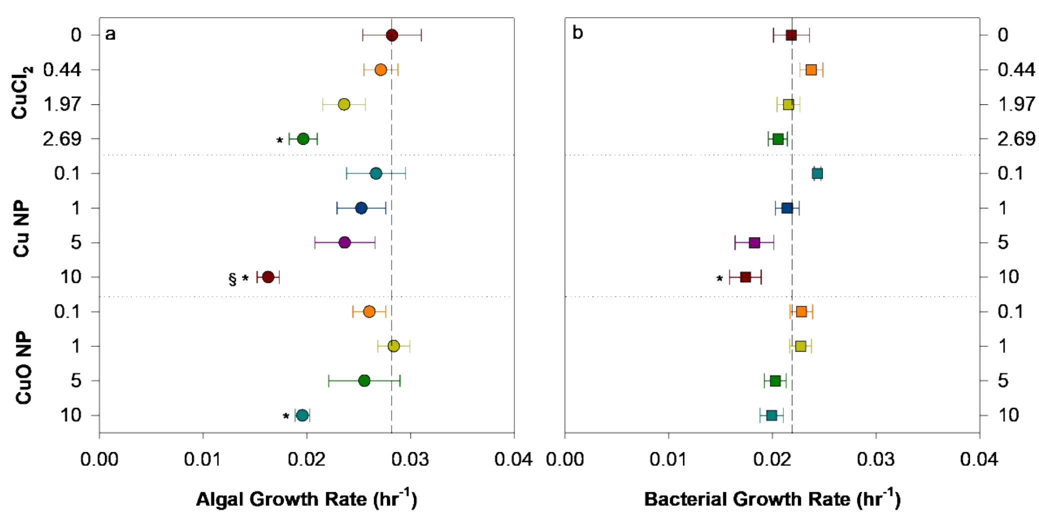

Figure S10. *Algal (a) and bacterial (b) growth rates in all exposure conditions over 120-hour exposure period. * indicates significant lower growth rates compared to the corresponding control. # indicates significant lower growth rates compared to other type of Cu exposure under the same concentration. Error bar represents standard deviation of four sample replicates.*

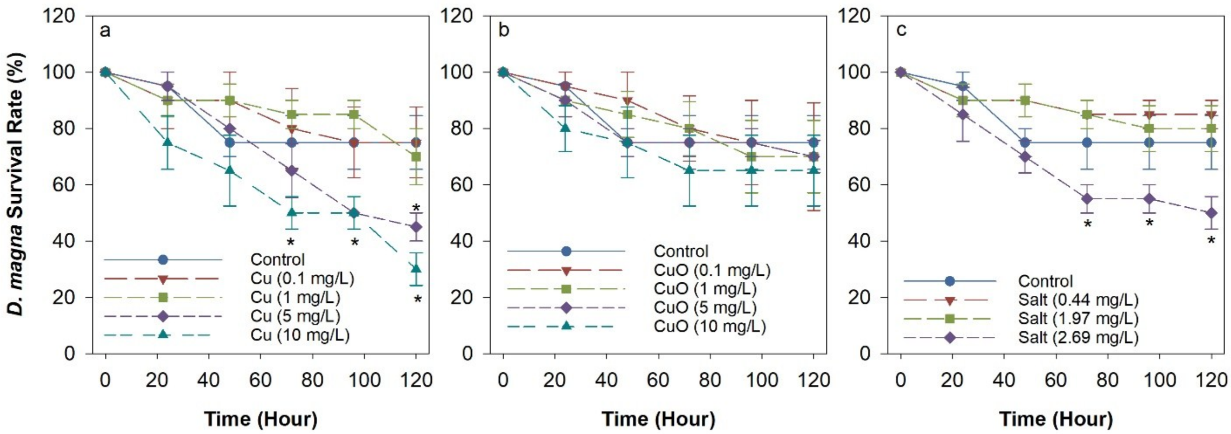

Figure S11. *D. magna survival rate in all exposure conditions over 120-hour exposure period. * indicates significant lower survival rates compared to the corresponding control. Error bar represents standard deviation of four sample replicates.*

Quality control and calibration of nanocosm

Dissolved oxygen

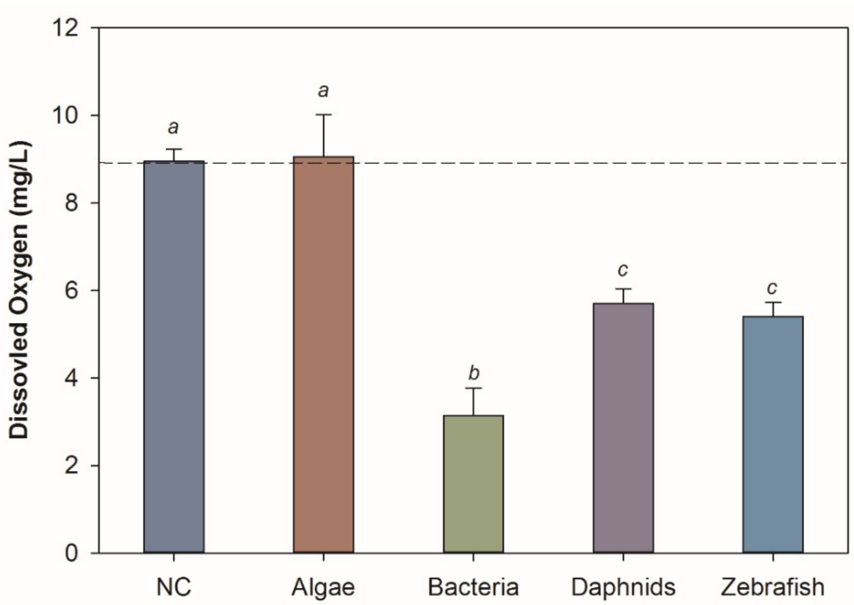

Figure S12. *Dissolved oxygen measured in each individual species and nanocosm after 120 hours (Dissolved oxygen measured in D. magna were measured at 48 hour). NC: nanocosm. Dashed line is the saturation dissolved oxygen at 21 °C. Lowercase letters indicate significant difference of measured dissolved oxygen among exposure scenarios. Error bars represent standard error of two experimental trials, each trial includes three sample replicates (n = 6 measures).*

A dissolved oxygen meter with a micro-oxygen electrode (Lazar Research Laboratories, Inc., Los Angeles, CA) was used to measure the dissolved oxygen in nanocosm and each individual species. Individual species was prepared in the same nanocosm media and with the same quantity of organisms but separately. The dissolved oxygen was measured in the nanocosm and each individual species at 120 hour of the experiment (48 hours for *D. magna*) and is presented in Figure S11. Nanocosm controls have demonstrated that the dissolved oxygen reached saturation measured at 120 hours. Thus, the dissolved oxygen is not the limiting factor for organismal growth in nanocosm controls. The high dissolved oxygen is due to production of oxygen through algae photosynthesis. Although other species can utilize oxygen in nanocosm, the small volume and the shallow depth of the exposure environment, helps the oxygen exchange readily.

Nanocosm quality control

The organism growth, survival, and zebrafish hatching success in the control nanocosms were compared to determine the consistency of the nanocosm assay. Control nanocosms were compiled the present and previous studies. There are no significant differences in algal growth rates or algal survival over the different exposure trials (Figure S12). Figure S13 shows the comparison of bacterial growth rates and bacterial survival after 120 hours. Figure 14 shows *D. magna* survival rates in different trials of experiments. There was no significant difference among exposure trials. The mean *D. magna* survival is at 80%. Using four control replicates as an example, 75% survival rate will elicit significant higher mortality compared to 100% survival rate. Thus, to consider the success of control nanocosm, it requires no more than one out of five *D. magna* mortality from each sample replicate. Figure S15 combines the zebrafish survival and hatching rate of controls from different trials. No significant difference was found in zebrafish survival among controls. However, there was a significant lower zebrafish hatching rate in trial 3, with 84% hatching rate compared to 100% and 2. In trial 3, two replicates had two out of eight embryos that had delayed hatching. The significantly lower hatching rate is likely due to the smaller sample size compared to other control trials. For quality control, we recommend to use seven out of eight fish for both survival and hatching rate to be consider a successful control for future reference. Overall, there was above 80% survivability in all organisms after 120 hours. The high survival rate and consistency throughout different trials of experiments indicates that the nanocosm assay is repeatable in our laboratory. To further validate and improve repeatability, more thorough control calibration and interlaboratory testing are necessary to move the research forward to next stage.

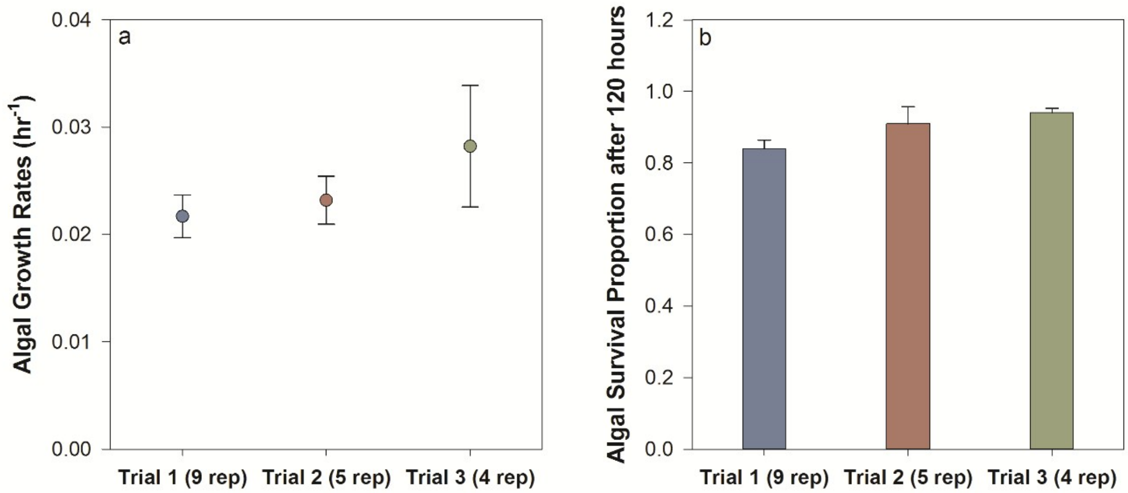

Figure S13. *Combined algal growth rates (a) and 120-hour algal survival probability (b) from different trials of experiments with multiple replicates. Error bar represents standard error of sample replicates.*

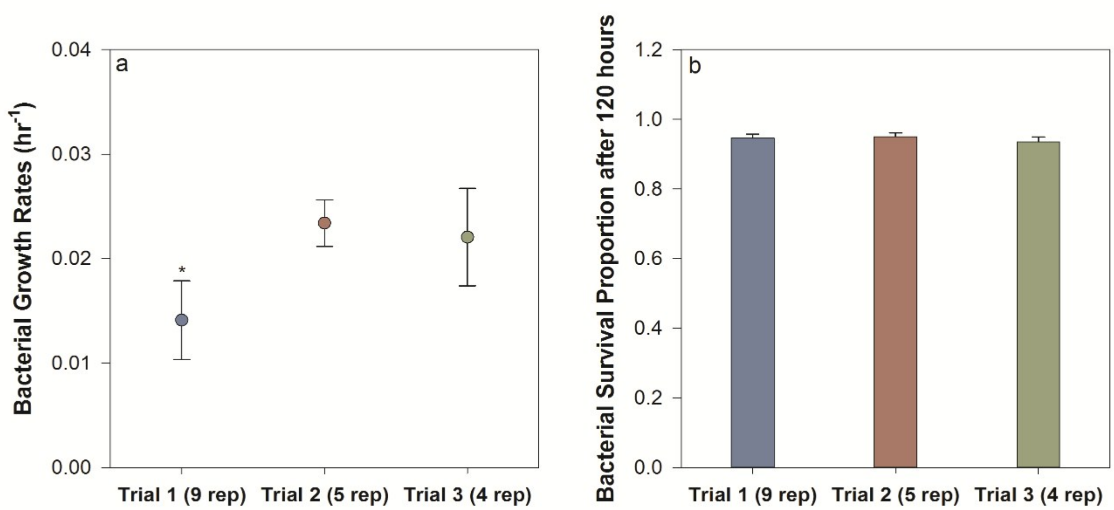

Figure S14. *Combined bacterial growth rates (a) and 120-hour bacterial survival probability (b) different trials of experiments with multiple replicates. Error bar represents standard error of sample replicates. * indicates significant difference in bacterial growth rates.*

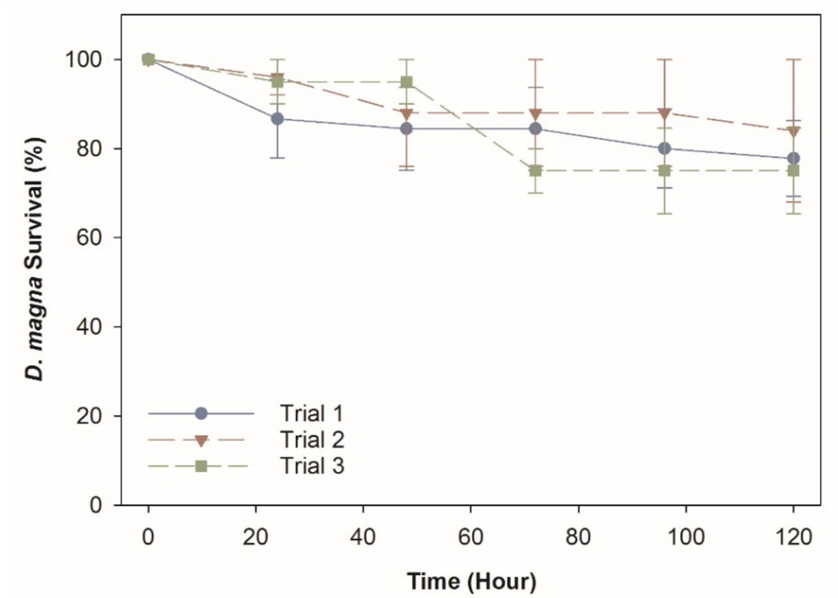

Figure S15. *Combined D. magna survival rate from different trials of experiments with multiple replicates. Error bar represents standard error of sample replicates.*

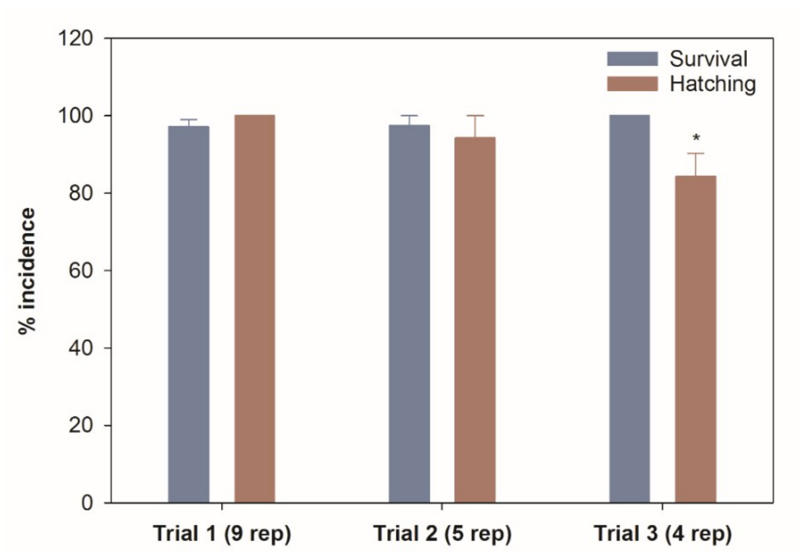

Figure S16. *120-hour zebrafish survival (blue bars) and hatching rate (red bars) from different trials of experiments with multiple replicates. Error bar represents standard error of sample replicates. * indicates significant difference in hatching rates among three experimental trials.*

EDS analysis

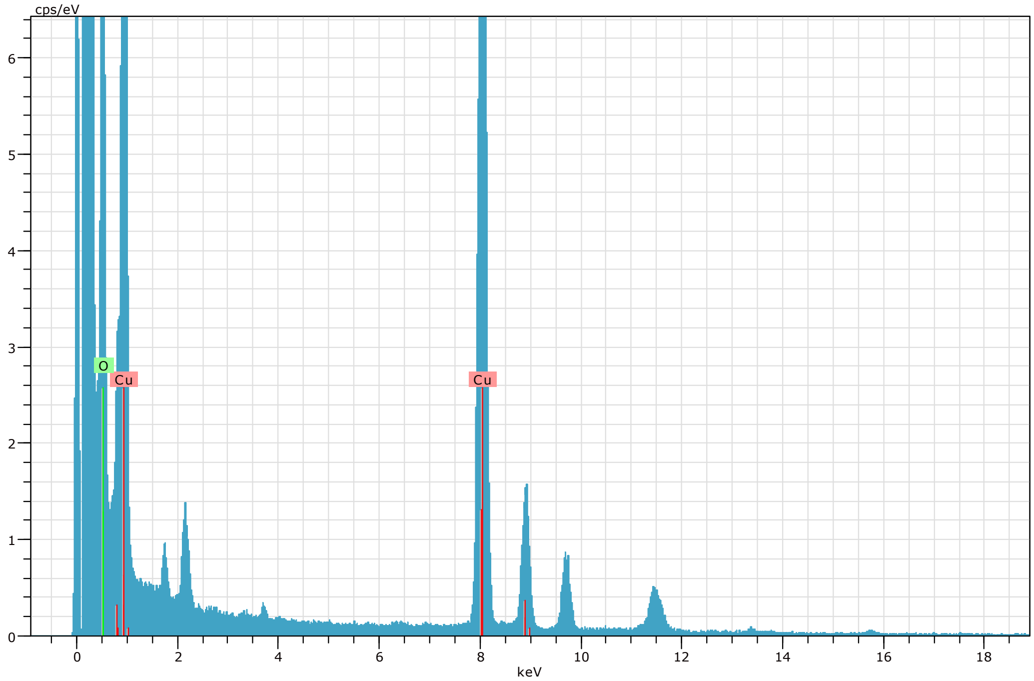

Figure S17. *EDS of Cu in Milli-Q water.*

Spectrum: B.spx

El	AN	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error (1 Sigma) [wt.%]
O	8	K-series	28.51	28.51	61.30	0.87
Cu	29	K-series	71.49	71.49	38.70	2.16
		Total:	100.00	100.00	100.00	

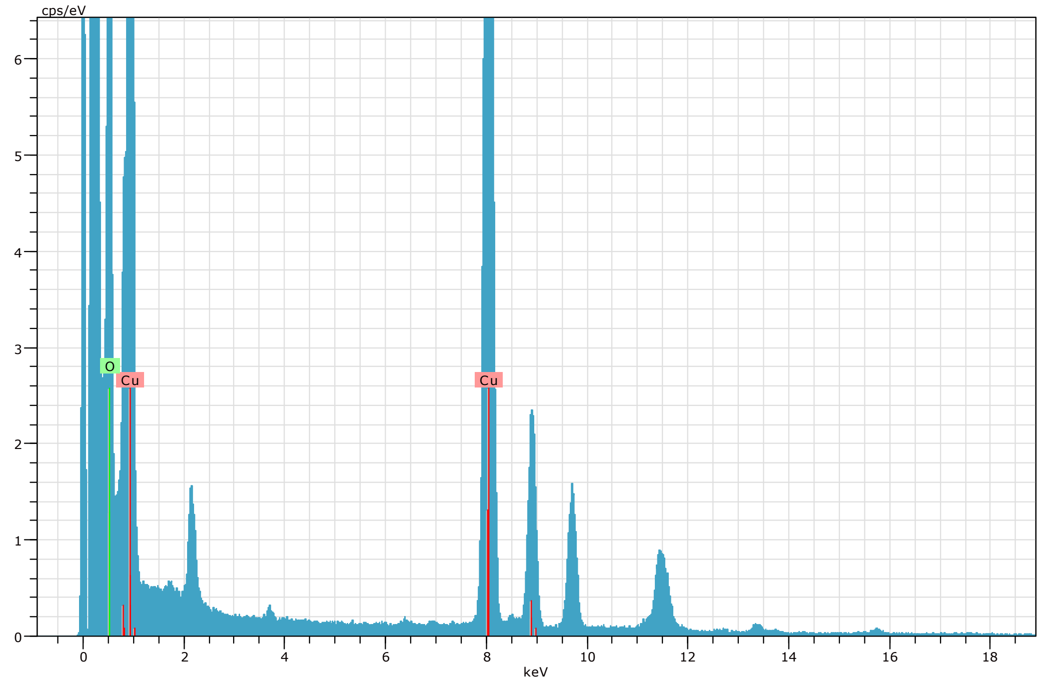

Figure S18. *EDS of CuO NP in Milli-Q water.*

Spectrum: 1

El	AN	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error (1 Sigma) [wt.%]
O	8	K-series	25.68	25.68	57.85	0.78
Cu	29	K-series	74.32	74.32	42.15	2.24
		Total:	100.00	100.00	100.00	

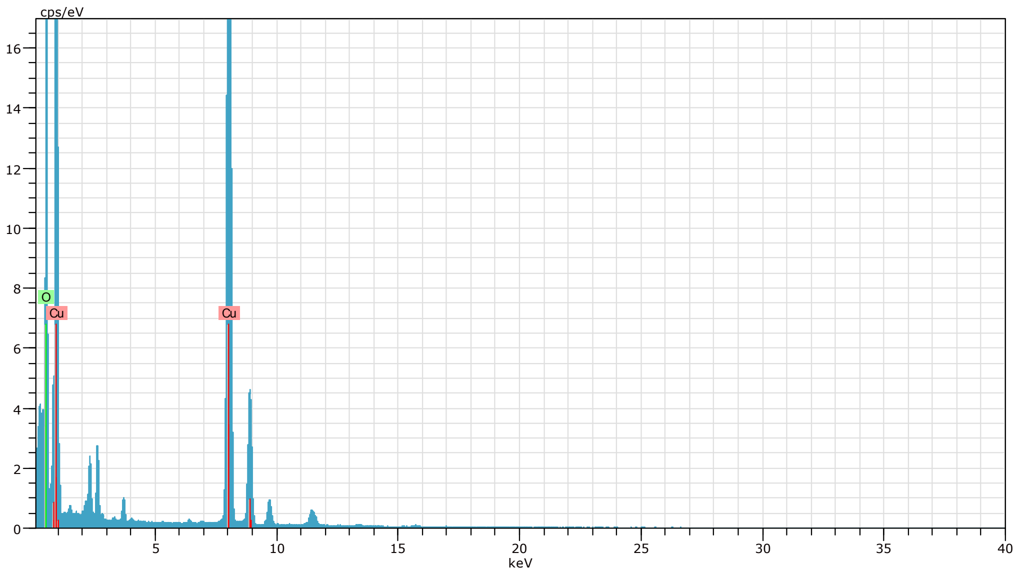

Figure S19. *EDS of Cu NP in nanocosm media.*

HV:200.0kV Puls th.:17.34kcps

El	AN	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error (1 Sigma) [wt.%]
Cu	29	K-series	74.67	74.67	42.60	2.27
O	8	K-series	25.33	25.33	57.40	0.79
		Total:	100.00	100.00	100.00	

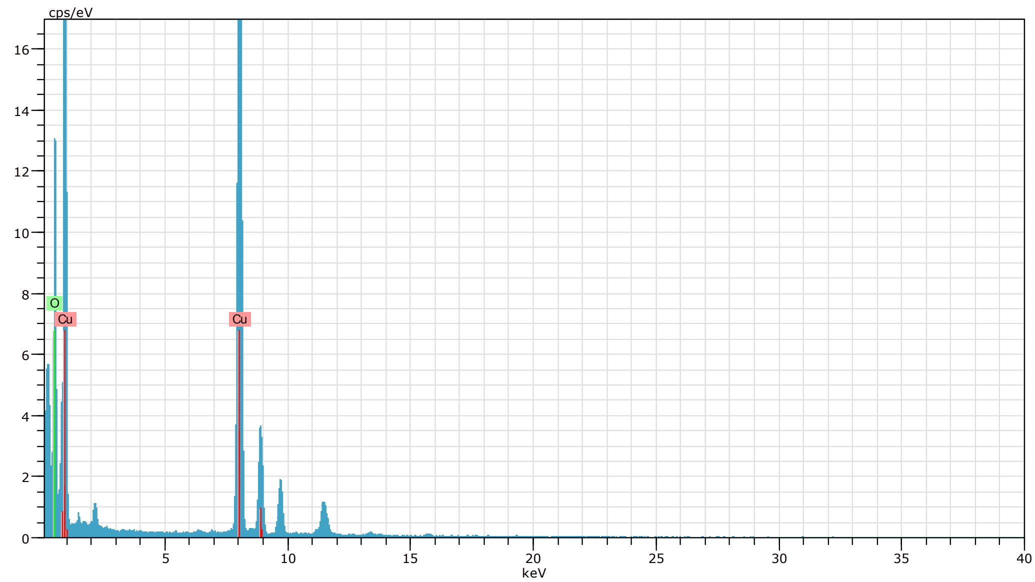

Figure S20. *EDS of Cu NP in nanocosm media.*

HV:200.0kV   Puls th.:15.37kcps

El	AN	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error (1 Sigma) [wt.%]
Cu	29	K-series	76.42	76.42	44.93	2.33
O	8	K-series	23.58	23.58	55.07	0.75
		Total:	100.00	100.00	100.00	

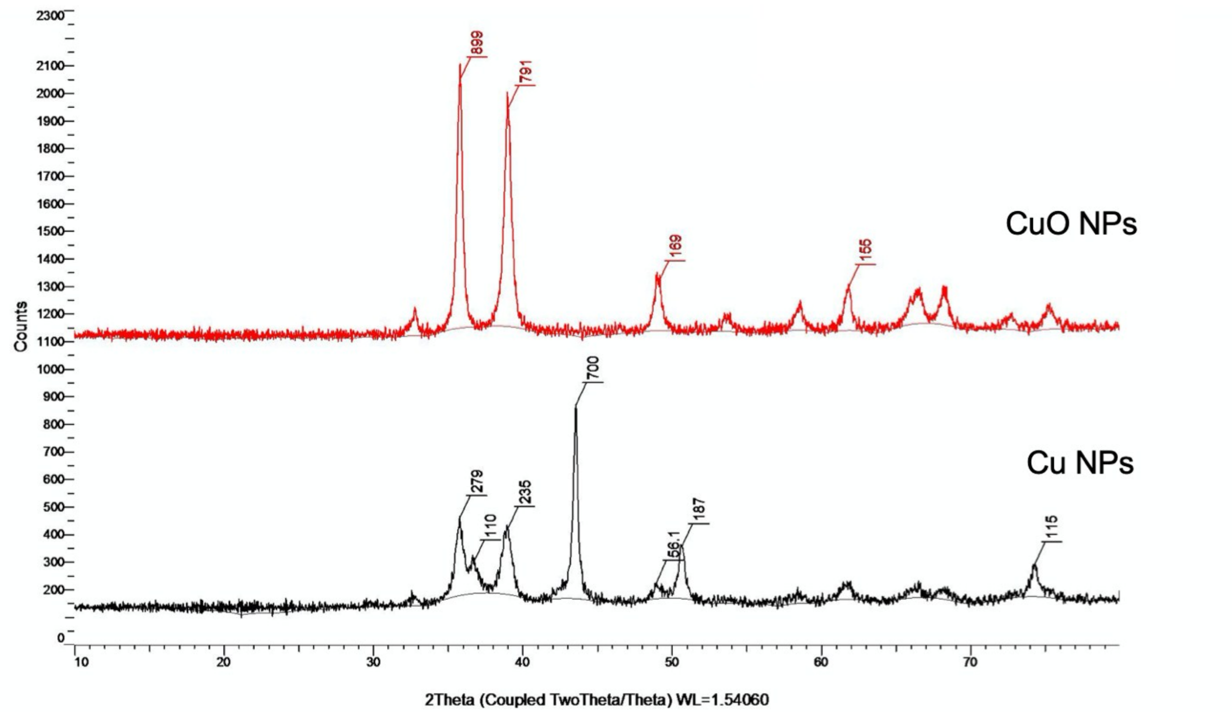

Figure S21. *XRD pattern of Cu and CuO NPs.*