Electronic Supplementary Material (ESI) for Environmental Science: Processes & Impacts.

Toxic Effects of Copper-based Nanoparticles or Compounds to Lettuce (Lactuca sativa) and Alfalfa (Medicago sativa

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Supplementary Information

Figure legends

Figure S1. Root elongation oflettuce (**A**) and alfalfa (**B**) plants exposed for 15 days in hydroponics to 0, 5, 10, 20 mg/L of Cu NPs/compounds. Plants were cultivated for 10 days in Hoagland nutrient solution before exposure to Cu treatments. Data are averages of four replicates $\pm$ SE. * stands for statistic differences at $p \leq 0.05$.

Figure S2. Shoot elongation of 10 day-old lettuce treated for 15 days in hydroponics with 0, 5, 10, 20 mg/L Cu NPs/compounds. Data are averages of four replicates ± SE. Different letters stand for statistic differences at $p \leq 0.05$.

Table S1. Major physicochemical properties of the particles used in this study

Property	***n*CuO**	**Bulk CuO**	***n*Cu**	**Bulk Cu**	**Kocide 3000**	**CuPRO 2005**
Primary particle size (nm)	$10^1 – 10^2$	$10^2 – 10^4$	$10^2 – 10^3$	$< 10^4$	$>10^4$	$10^4 – 10^6$
Hydrodynamic diameter (nm) [a]	280±15	376±26	2590±1138	4546±3940	1532±580	4779±4767
Zeta potential (mV) [a]	-34.4±0.5	-42.7±0.153±	-29.4±0.8	-35.4±1.27	-40.9±2.7	-47.8±1.1
Cu content (wt. %)	74.3	79.7	83.3	98.7	26.5	34.0
Other elements present	O, C	O	O, C	ND	C, O, Na, Al, Si, S, Cl	C, O, Na, Al, Si, P, Ca
Morphology	Rhombus, irregular	Prism, irregular	Irregular	Dendritic, plate-like, rhombus	Spherical	Spherical
Main Copper phase	CuO	CuO	Cu, Cu_2O	Cu	$Cu(OH)_2$	$Cu(OH)_2$
Crystal structure	Monoclinic	Monoclinic	Cubic	Cubic	Orthorhombic	Orthorhombic

[a]Measurement was done at pH 7

ND = Non-detect

Table S2. Element accumulation in lettuce roots (mg/kg). Data are averages of four replicates ± SE. Different letters stand for statistical differences at $p \leq 0.05$. * stands for statistic differences at $p \leq 0.05$.

	Treatments	5 mg/L	10 mg/L	20 mg/L
	*n*Cu	6338.5±731.3 c	9373.9±1061.3 b	13318.8±458.9 c
	Bulk Cu	5034.9±213.8 bc	7960.7±501.3 ab	11111.4±365.9 bc
	*n*CuO	3555.8±210.5 ab	9140.9±99.4 ab	9941.3±531.0 ab
	Bulk CuO	3156.2±394.4 ab	6992.6±790.9 a	6941.3±673.0 a
	CuPRO 2005	3427.0±265.3 ab	7313.4±490.9 a	8459.5±360.5 ab
	Kocide 3000	2136.5±54.3 a	7674.1±417.7 a	7505.3±161.5 a
	$CuCl_2$	5770.7±900.3 c	11796.1±214.9 c	20366.5±2440 d
	*n*Cu	1103.9±1103.8 ab	ND	ND
	Bulk Cu	ND a	ND	ND
	*n*CuO	4783.1±120.4 c	ND	ND
P	Bulk CuO	8573.5±281.2 d	ND	ND
	CuPRO 2005	3786.8±1787.2 bc	ND	ND
	Kocide 3000	4271.0±478.2 c	ND	ND
	$CuCl_2$	349.9±349.9 ab	ND	ND
	*n*Cu	570.1±89.6 a	640.1±74.1 a	457.4±126.5
	Bulk Cu	680.8±97.5 a	851.2±68.6 a	678.7±64.3
	*n*CuO	709.5±61.6 a	977.4±55.7 a	979.7±522.9
Fe	Bulk CuO	940.9±64.4 a	1088.1±63.8 ab	736.7±137.2
	CuPRO 2005	1209.7±104.1 ab	951.9±38.4 a	512.7±10.2
	Kocide 3000	550.3±7.85 a	1168.3±211.4 ab	902.5±94.4
	$CuCl_2$	1681.2±378.2 b	1750.1±351.2 b	1646.5±272.2 *
	*n*Cu	1583.5±63.3 abc	1568.0±30.8 a	1735.5±32.4 ab
	Bulk Cu	1863.6±71.2 bc	1614.8±16.1 a	1551.3±9.7 a
S	*n*CuO	1449.1±98.1 a	1579.4±36.6 a	1638.6±128 ab
	Bulk CuO	1560.6±67.6 ab	1811.7±90.5 ab	1652.1±31.5 ab
	CuPRO 2005	1804.5±78.6 bc	1604.1±48.2 a	1543.9±37.7 a
	Kocide 3000	1470.1±51.3 a	1950.8±94.6 bc	1860.4±44.5 b
	$CuCl_2$	1908.6±72.6 c	2179.8±70.7 c	2520.7±59.4 c

Table S3. Element accumulation in lettuce shoots (mg/kg). Data are averages of four replicates ± SE. Different letters stand for statistical differences at $p \leq 0.05$. * stands for statistic differences at $p \leq 0.05$.

	Treatments	5 mg/L	10 mg/L	20 mg/L
Cu	*n*Cu	30.2±1.4 bc	45.8±2.4 ab	78.6±1.8 *
	Bulk Cu	29.1±0.9 bc	23.3±0.6 a	27.5±0.6
	*n*CuO	23.3±0.9 ab	28.8±5.7 ab	25.6±3.8
	Bulk CuO	23.6±1.8 ab	31.1±2.2 ab	20.8±2.6
	CuPRO 2005	24.6±0.4 abc	33.3±3.5 ab	41.0±3.8
	Kocide 3000	20.0±1.4 a	52.4±9/7 b	34.6±5.1
	$CuCl_2$	31.6±3.2 c	49.0±9.7 ab	24.3±10.6
P	*n*Cu	4946.8±164.5 bc	5056.1±586.8	5139.4±263.4
	Bulk Cu	5883.0±233.5 c	5358.5±295.0	5172.5±160.2
	*n*CuO	5132.9±320.1 bc	4958.4±151.2	5610.6±92.7
	Bulk CuO	5735.0±240.7 c	4739.9±112.5	4878.9±258.6
	CuPRO 2005	5063.6±167.2 bc	4606.8±277.2	4615.0±239.0
	Kocide 3000	4244.4±316.0 ab	4129.6±43.7	4817.3±69.8
	$CuCl_2$	3848.1±217.2 a	4264.2±219.9	4803.1±403.9
Fe	*n*Cu	26.3±2.1 a	27.0±3.3 ab	35.6±1.9
	Bulk Cu	46.1±5.7 ab	25.7±0.6 a	35.3±0.9
	*n*CuO	31.9±3.7 a	25.6±1.6 a	50.6±8.7
	Bulk CuO	68.5±13.3 b	27.7±0.2 ab	28.5±2.7
	CuPRO 2005	35.2±2.6 a	37.2±1.5 bc	28.4±2.0
	Kocide 3000	26.1±0.3 a	31.5±3.7 abc	36.2±5.7
	$CuCl_2$	39.1±0.6 a	41.2±3.3 c	42.3±5.5
S	*n*Cu	441.7±26.9 a	441.6±13.0 b	401.4±10.1 ab
	Bulk Cu	497.3±31.8 ab	440.4±15.4 b	420.2±5.3 ab
	*n*CuO	500.9±73.9 b	391.4±6.7 ab	408.5±10.7 ab
	Bulk CuO	396.5±15.8 a	443.1±2.7 b	372.4±5.2 ab
	CuPRO 2005	355.0±16.3 a	357.3±7.8 a	358.6±8.9 a
	Kocide 3000	350.2±2.6 a	427.0±21.3 ab	434.8±2.3 b
	$CuCl_2$	424.2±20.7 a	367.5±29.3 ab	428.2±31.5 b

Table S4. Element accumulation in alfalfa roots (mg/kg). Data are averages of four replicates ± SE. Different letters stand for statistical differences at $p \leq 0.05$. * stands for statistic differences at $p \leq 0.05$.

	Treatments	5 mg/L	10 mg/L	20 mg/L
Cu	*n*Cu	1038.7±339.6 a	2995.3±765.8 a	6479.0±435.6 b
	Bulk Cu	1091.5±113.0 a	2106.1±145.0 a	1890.5±166.3 a
	*n*CuO	1882.8±95.2 bc	3977.7±141.6 ab	3463.7±83.9 a
	Bulk CuO	1341.4±102.6 ab	2964.4±215.1 a	4651.6±543.4 ab
	CuPRO 2005	2503.0±100.5 c	2286.1±736.7 a	1914.2±534.6 a
	Kocide 3000	2014.6±20.3 bc	2938.3±625.4 a	3348.0±155.7 a
	$CuCl_2$	1641.7±130.0 ab	5106.4±353.9 b	12391.6±1353.7 c
P	*n*Cu	2768.6±309.1	ND	ND
	Bulk Cu	3151.5±104.5	1267.4±63.3	1875.8±157.6
	*n*CuO	573.8±436.8	ND	ND
	Bulk CuO	2419.2±164.3	ND	ND
	CuPRO 2005	1458.0±1313.5	ND	ND
	Kocide 3000	369.7±152.4	ND	ND
	$CuCl_2$	2133.5±45.7	ND	ND
Fe	*n*Cu	369.6±85.6 ab	466.2±42.4 b	342.9±26.3
	Bulk Cu	262.8±3.3 a	470.6±15.4 b	365.3±51.9
	*n*CuO	377.6±22.4 ab	441.1±1.9 b	332.8±14.7
	Bulk CuO	340.0±91.1 ab	533.0±53.6 b	411.1±10.5
	CuPRO 2005	530.8±45.8 ab	544.3±30.7 b	283.6±17.3
	Kocide 3000	483.4±58.9 ab	206.8±13.5 a	203.3±13.5
	$CuCl_2$	577.2±42.1 b	844.4±58.7 c	1048.9±186.1 *
S	*n*Cu	1061.6±22.1 a	1445.1±99.9 a	1440.3±125.5 bc
	Bulk Cu	1101.4±16.9 a	1224.5±45.5 a	1035.5±24.1 a
	*n*CuO	962.1±65.9 a	1246.9±68.9 a	1171.4±17.1 ab
	Bulk CuO	1126.5±21.6 a	1244.7±47.1 a	1381.3±28.2 bc
	CuPRO 2005	1408.0±30.9 b	1444.6±120.2 a	1628.1±154.4 cd
	Kocide 3000	1492.3±23.7 bc	1632.3±127.5 ab	1828.5±57.2 d
	$CuCl_2$	1600.8±71.4 c	2013.9±154.7 b	2198.6±27.7 e

Table S5. Element accumulation in alfalfa shoots (mg/kg). Data are averages of four replicates ± SE. Different letters stand for statistical differences at $p \leq 0.05$. * stands for statistic differences at $p \leq 0.05$.

	Treatments	5 mg/L	10 mg/L	20 mg/L
	*n*Cu	123.3±41.7 ab	123.9±32.0	297.6±6.1 b
	Bulk Cu	44.2±4.0 a	51.5±8.1	102.5±19.0 a
	*n*CuO	147.1±5.6 b	70.4±17.9	125.6±5.3 a
Cu	Bulk CuO	48.9±10.0 a	74.6±12.7	96.2±11.7 a
	CuPRO 2005	153.9±22.2 b	146.1±55.1	263.1±70.5 ab
	Kocide 3000	175.3±6.5 b	181.9±13.9	159.8±37.3 ab
	$CuCl_2$	121.7±14.0 ab	325.0±66.3 *	576.1±37.2 c
	*n*Cu	4280.5±233.0 c	4573.5±256.7 c	3600.9±255.1 abc
	Bulk Cu	3384.9±160.0 ab	3317.0±26.3 ab	3243.2±212.5 ab
	*n*CuO	2995.9±310.0 a	3000.9±44.1 a	2802.8±63.6 a
P	Bulk CuO	3051.0±153.0 a	3187.7±124.9 ab	2783.9±181.6 a
	CuPRO 2005	3565.7±74.7 abc	3530.4±189.6 ab	3859.6±103.2 bc
	Kocide 3000	3575.9±74.0 abc	3780.7±239.7 b	4280.9±209.2 c
	$CuCl_2$	4014.4±113.8 bc	5350.8±68.5 d	5559.1±263.9 d
	*n*Cu	30.9±2.4 abc	26.6±2.9	25.0±1.2
	Bulk Cu	19.0±0.6 a	19.7±0.5	24.8±0.8
	*n*CuO	38.0±3.5 bc	18.7±2.2	19.8±1.3
Fe	Bulk CuO	18.9±0.9 a	20.3±1.2	25.4±1.4
	CuPRO 2005	25.3±1.5 ab	22.7±2.8	28.7±7.9
	Kocide 3000	31.6±0.5 abc	24.9±1.3	24.9±2.1
	$CuCl_2$	40.6±6.7 c	42.8±5.6 *	75.6±5.8 *
	*n*Cu	3593.9±81.6 e	5546.4±817.5 *	2609.0±187.4 bc
	Bulk Cu	2153.5±105.8 d	1867.0±171.7	1955.0±131.8 ab
	*n*CuO	1395.9±91.5 ab	1973.6±56.5	1638.6±22.2 a
S	Bulk CuO	1718.6±74.6 bc	1696.8±176.8	1846.6±41.5 ab
	CuPRO 2005	1568.1±79.3 ab	2271.3±93.2	2226.2±145.0 ab
	Kocide 3000	1297.4±89.6 a	1804.0±158.2	2384.6±256.1 abc
	$CuCl_2$	1954.9±98.8 cd	3169.3±160.2	3122.0±295.3 c

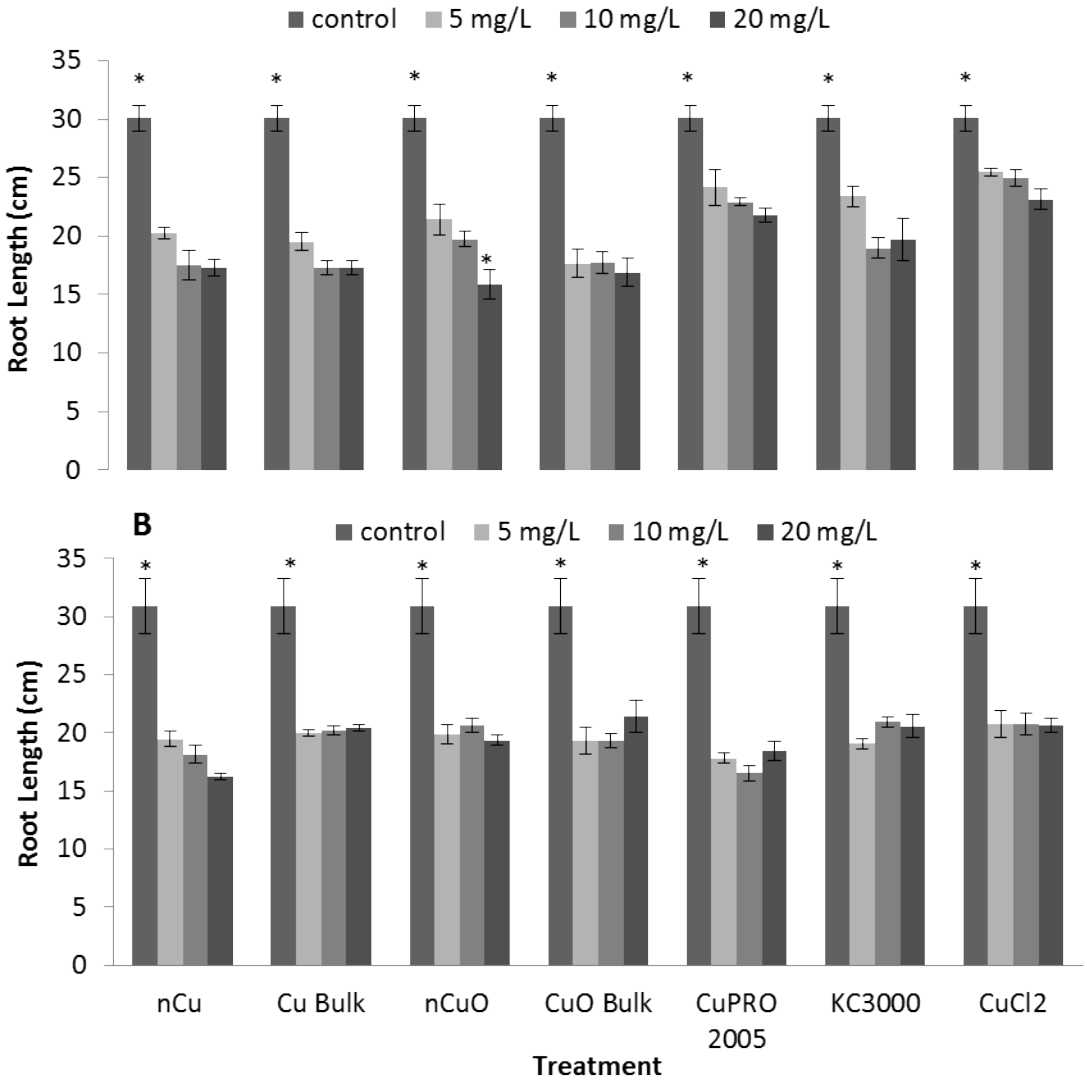
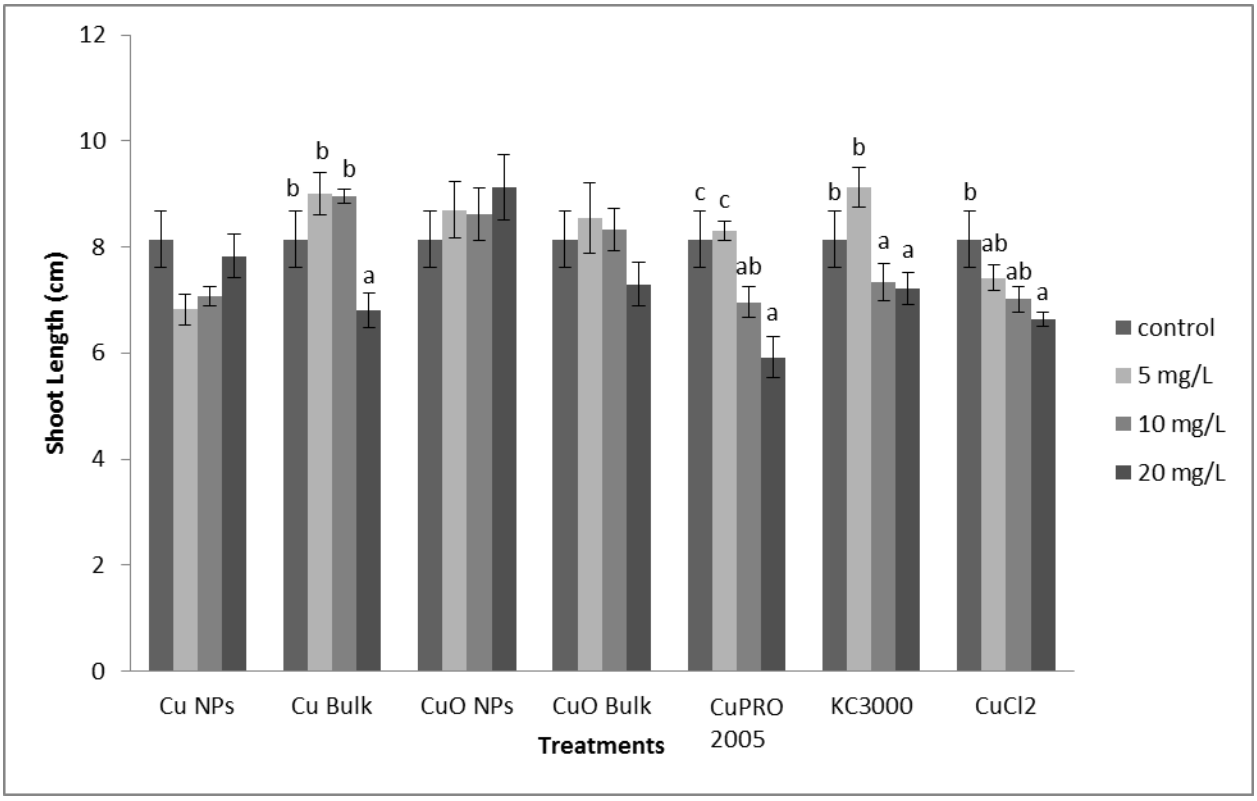

control
5 mg/L
10 mg/L
20 mg/L
Root Length (cm)
35
30
25
20
15
10
5
0
B
nCu
Cu Bulk
nCuO
CuO Bulk
CuPRO 2005
KC3000
CuCl2
Treatment

Figure S1.


12
10
8
6
4
2
0
Shoot Length (cm)
b
b
b
a
c
c
ab
a
b
b
a
a
b
ab
ab
a
Cu NPs
Cu Bulk
CuO NPs
CuO Bulk
CuPRO 2005
KC3000
CuCl2
Treatments
control
5 mg/L
10 mg/L
20 mg/L

FigureS2.