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Impacts of copper oxide nanoparticles in bell pepper (*Capsicum annum* L.) plants: A full life cycle study

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Fig. S1: Plant biomass in (a) root (b) stem (c) leaves (d) fruit in bell pepper plants grown under the nCuO, bCuO, and $CuCl_2$ treatments. Data are averages of 4 replicates ± SE

Fig. S2: Total foliar area in bell pepper plants grown under the nCuO, bCuO, and $CuCl_2$ treatments. Data are averages of 4 replicates ± SE.

Table S1. Root elemental concentration

Concentration of elements in the bell pepper root samples measured in mg of the essential element/kg of the dried sample. Plants were cultivated for 90 days in natural soil amended with Cu based chemicals at 0, 62.5, 125, 250, and 500 mg/kg concentrations. Statistically significant differences are being denoted by different letters between samples, as per the Tukey's test ($p \leq 0.05$). Concentration is an average of 4 replicates ± SE. Comparisons were made for one element at a time.

		nCuO	bCuO	$CuCl_2$
P	**Control**	2766.55 ± 196.36 a	2766.55 ± 196.36 a	2766.55 ± 196.36 a
	62.5 mg/kg	2012.87 ± 171.14 ab	2379.56 ± 70.05 ab	2679.21 ± 64.20 ab
	125 mg/kg	1937.15 ± 132.50 ab	2811.81 ± 294.37 ab	2086.57 ± 65.74 ab
	250 mg/kg	2070.44 ± 199.17 ab	2180.46 ± 85.08 ab	2634.74 ± 374.46 ab
	500 mg/kg	1919.39 ± 121.71 ab	1781.52 ± 138.55 b	2463.43 ± 270.95 ab
S	**Control**	2639.81 ± 167.81 b	2639.81 ± 167.81 b	2639.81 ± 167.81 b
	62.5 mg/kg	2332.20 ± 129.49 b	2888.01 ± 248.95 b	2741.03 ± 182.14 b
	125 mg/kg	2524.06 ± 168.61 b	2859.90 ± 120.62 b	2991.83 ± 270.92 ab
	250 mg/kg	2328.76 ± 140.98 b	2599.18 ± 65.87 b	2940.35 ± 146.32 ab
	500 mg/kg	2231.42 ± 209.86 b	2452.11 ± 95.26 b	4505.87 ± 1064.18 a
Se	**Control**	0.19 ± 0.11 b	0.19 ± 0.11 b	0.19 ± 0.11 b
	62.5 mg/kg	0.02268 ± 0.023 b	2.39237 ± 2.001 ab	0.919795 ± 0.920 b
	125 mg/kg	0.5989 ± 0.396 b	6.878 ± 2.34 a	0.924 ± 0.924 b
	250 mg/kg	0.170 ± 0.1699 b	3.52 ± 1.670 ab	0.043987 ± 0.05 b
	500 mg/kg	1.69 ± 1.0 ab	2.43 ± 1.335 ab	2.580 ± 2.043 ab
Mo	**Control**	2.83 ± 0.823 ab	2.83 ± 0.823 ab	2.83 ± 0.823 ab
	62.5 mg/kg	2.604 ± 0.54 ab	0	2.9 ± 0.46 ab
	125 mg/kg	1.60 ± 0.527 ab	0	4.87 ± 1.0055 a
	250 mg/kg	1.95 ± 0.52 ab	0	2.538 ± 0.2297 ab
	500 mg/kg	0.603 ± 0.28 b	0	3.05 ± 0.78 ab
Cu	**Control**	47.67 ± 2.01 c	47.67 ± 2.01 c	47.67 ± 2.01 c
	62.5 mg/kg	96.50 ± 11.70 bc	93.93 ± 6.0 bc	92.77 ± 10.98 bc
	125 mg/kg	102.07 ± 14.16 bc	85.61 ± 10.45 bc	127.40 ± 29.23 bc
	250 mg/kg	141.20 ± 10.42 b	135.57 ± 45.65 b	136.51 ± 100.50 b
	500 mg/kg	161.48 ± 117.51 b	118.73 ± 76.34 bc	256.31 ± 127.89 a

Table S2: Stem elemental concentration

Concentration of elements in the bell pepper stem samples measured in mg of the essential element/kg of the dried sample. Plants were cultivated for 90 days in natural soil amended with Cu based chemicals at 0, 62.5, 125, 250, and 500 mg/kg concentrations. Statistically significant differences are being denoted by different letters between samples, as per the Tukey's test ($p \leq 0.05$). Concentration is an average of 4 replicates ± SE. Comparisons were made for one element at a time.

		nCuO	bCuO	$CuCl_2$
S	**Control**	4223.59 ± 279.21 bc	4223.59 ± 279.214 bc	4223.59 ± 279.214 bc
	62.5 mg/kg	4333.158 ± 564.44 bc	4692.72 ± 846.66 abc	4144.46 ± 254.12 bc
	125 mg/kg	3351.12 ± 180.02 c	4107.61 ± 361.92 bc	4461.53 ± 331.34 bc
	250 mg/kg	3531.76 ± 353.71 c	4659.57 ± 503.14 abc	6769.61 ± 640.58 a
	500 mg/kg	3369.25 ± 209.04 c	3929.77 ± 107.08 c	6240.69 ± 672.15 ab
Zn	**Control**	15.21 ± 1.034 ab	15.21 ± 1.034 ab	15.21 ± 1.034 ab
	62.5 mg/kg	10.37 ± 0.358 ab	17.05 ± 4.4979 a	14.19 ± 0.859 ab
	125 mg/kg	7.88 ± 1.233 ab	13.19 ± 2.39ab	14.74 ± 2.009 ab
	250 mg/kg	8.62 ± 0.6459 ab	15.52 ± 1.67 ab	12.70 ± 2.56 ab
	500 mg/kg	7.11 ± 0.74 b	12.56 ± 1.2149 ab	10.56 ± 1.72 ab

Table S3: Leaf elemental concentration

Concentration of elements in the bell pepper leaf samples measured in mg of the essential element/kg of the dried sample. Plants were cultivated for 90 days in natural soil amended with Cu based chemicals at 0, 62.5, 125, 250, and 500 mg/kg concentrations. Statistically significant differences are being denoted by different letters between samples, as per the Tukey's test ($p \leq 0.05$). Concentration is an average of 4 replicates ± SE. Comparisons were made for one element at a time.

		nCuO	bCuO	$CuCl_2$
P	**Control**	3115.54 ± 121.87 ab	3115.54 ± 121.87 ab	3115.54 ± 121.87 ab
	62.5 mg/kg	2569.57 ± 217.10 ab	3640.06 ± 798.28 ab	3494.75 ± 128.15 ab
	125 mg/kg	2256.22 ± 112.07 b	3887.245 ± 506.83 a	3009.73 ± 168.41 ab
	250 mg/kg	2710.68 ± 167.20 ab	3356.70 ± 361.70 ab	3168.13 ± 186.12 ab
	500 mg/kg	2501.91 ± 137.63 ab	2929.86 ± 156.26 ab	3110.98 ± 4.874 ab
S	**Control**	4201.58 ± 120.895 b	4201.58 ± 120.895 b	4201.58 ± 120.895 b
	62.5 mg/kg	4179.03 ± 187.44 b	4523.07 ± 363.20 ab	4495.43 ± 185.92 ab
	125 mg/kg	3647.26 ± 149.00 b	4216.67 ± 249.61 b	4071.82 ± 175.37 b
	250 mg/kg	4051.73 ± 178.42 b	4276.00 ± 103.51 b	5865.53 ± 823.57 a
	500 mg/kg	4127.46 ± 122.5 b	3836.93 ± 349.27 b	4175.82 ± 254.84 b
R Mg	**Control**	10090.66 ± 320.379 b	10090.66 ± 320.380 a	10090.66 ± 320.379 a
	62.5 mg/kg	10270.49 ± 733.14 ab	10612.32 ± 841.623ab	11020.78 ± 223.48 ab
	125 mg/kg	10681.7 ± 761.570 ab	11121.33 ± 931.22 ab	11907.54 ± 477.66 ab
	250 mg/kg	10558.65 ± 878.28 ab	11425.45 ± 348.98 ab	11499.91 ± 296.13 ab
	500 mg/kg	11125.93 ± 557.41 ab	13078.47 ± 836.227 a	12704.06 ± 640.66 a
Se	**Control**	3.956 ± 1.005 ab	3.956 ± 1.005 ab	3.956 ± 1.005 ab
	62.5 mg/kg	1.25 ± 0.70 b	5.0333 ± 0.912 ab	1.445 ± 0.8996 b
	125 mg/kg	1.69 ± 0.587 b	8.9637 ± 2.218 a	0.80 ± 0.476 b
	250 mg/kg	1.13 ± 0.379 b	3.75 ± 0.968 ab	0.5088 ± 0.294 b
	500 mg/kg	0.88 ± 0.62 b	5.53 ± 1.686 ab	2.37 ± 1.215 b
Zn	**Control**	31.06 ± 2.88 a	31.06 ± 2.88 a	31.0589 ± 2.88 a
	62.5 mg/kg	17.42 ± 1.80 abc	28.93 ± 3.45 ab	30.84 ± 1.273 a
	125 mg/kg	15.97 ± 1.63 bc	28.9 ± 3.84 ab	24.23 ± 2.13 abc
	250 mg/kg	16.62 ± 1.11 abc	27.16 ± 2.43 abc	21.65 ± 1.73 abc
	500 mg/kg	13.95 ± 0.64 c	26.45 ± 0.92 abc	18.62 ± 2.54 abc
Cu	**Control**	11.30 ± 1.21 b	11.30 ± 1.21 b	11.30 ± 1.21 b
	62.5 mg/kg	17.88 ± 1.92 ab	17.88 ± 3.32 ab	19.79 ± 1.356 ab
	125 mg/kg	16.49 ± 1.11 ab	22.2897 ± 3.26711 a	20.1874 ± 2.88297 ab
	250 mg/kg	20.81 ± 1.80 ab	21.3892 ± 1.50597 a	25.8583 ± 2.15487 a
	500 mg/kg	21.17 ± 1.44 ab	20.7797 ± 0.83864 ab	24.4618 ± 1.80751 a

Table S4: Fruit elemental concentration

Concentration of elements in the bell pepper fruit samples measured in mg of the essential element/kg of the dried sample. Plants were cultivated for 90 days in natural soil amended with Cu based chemicals at 0, 62.5, 125, 250, and 500 mg/kg concentrations. Statistically significant differences are being denoted by different letters between samples, as per the Tukey's test ($p \leq 0.05$). Concentration is an average of 4 replicates ± SE. Comparisons were made for one element at a time.

		nCuO	bCuO	$CuCl_2$
P	**Control**	4499.16 ± 176.14 ab	4499.16 ± 176.14 ab	4499.16 ± 176.14 ab
	62.5 mg/kg	4243.46 ± 499.98 ab	4919.697 ± 203.74 a	4925.72 ± 482.01 a
	125 mg/kg	3923.18 ± 124.15 ab	5042.77 ± 326.038 a	4454.77 ± 302.37 ab
	250 mg/kg	3877.87 ± 208.63 ab	4656.89 ± 158.59 ab	4641.70 ± 303.59 ab
	500 mg/kg	3292.66 ± 130.14 b	4047.35 ± 147.997 ab	4614.67 ± 420.61 ab
Ca	**Control**	1326.83 ± 68.01 ab	1326.83 ± 68.01 ab	1326.83 ± 68.01 ab
	62.5 mg/kg	1322.74 ± 243.15 ab	1404.07 ± 69.87 ab	1439.03 ± 324.04 ab
	125 mg/kg	1247.51 ± 185.70 ab	1068.435 ± 30.56 ab	1352.40 ± 145.40 ab
	250 mg/kg	1212.29 ± 154.40 ab	1534.693 ± 87.75 ab	1104.66 ± 79.67 ab
	500 mg/kg	1026.36 ± 130.58 b	1314.35 ± 63.43 ab	1893.41 ± 360.38 a
Se	**Control**	0.41 ± 0.32 b	0.41 ± 0.32 b	0.41 ± 0.32 b
	62.5 mg/kg	0	1.113 ± 0.643 b	0.1996 ± 0.20 b
	125 mg/kg	0.26 ± 0.26 b	0.94 ± 0.76 b	0.11 ± 0.067 b
	250 mg/kg	0.20 ± 0.20 b	3.38 ± 0.67 a	0
	500 mg/kg	0.26 ± 0.26 b	1.06 ± 0.614 b	0.629 ± 0.28 b
Zn	**Control**	13.135 ± 0.88 abc	13.135 ± 0.88 abc	13.135 ± 0.88 abc
	62.5 mg/kg	10.88 ± 1.32 abcd	15.01 ± 0.44 a	12.38 ± 1.19 abc
	125 mg/kg	8.96 ± 0.54 bcd	13.48 ± 1.05 ab	10.31 ± 0.74 bcd
	250 mg/kg	8.33 ± 0.32 ab	14.37 ± 0.36 a	11.76 ± 0.46 abcd
	500 mg/kg	6.97 ± 0.296 d	11.40 ± 0.40 abcd	11.27 ± 1.30 abcd
Cu	**Control**	8.22 ± 0.32 b	8.22 ± 0.32 b	8.22 ± 0.32 b
	62.5 mg/kg	10.76 ± 1.21 ab	10.63 ± 0.52 ab	10.84 ± 0.51 ab
	125 mg/kg	10.70 ± 0.86 ab	10.56 ± 0.998 ab	9.35 ± 0.48 ab
	250 mg/kg	10.69 ± 0.81 ab	12.40 ± 0.84 a	11.80 ± 0.66 ab
	500 mg/kg	9.57 ± 0.83 ab	9.80 ± 0.58 ab	10.60 ± 0.93 ab
Ni	**Control**	0.23 ± 0.042 cd	0.23 ± 0.042 cd	0.23 ± 0.042 cd
	62.5 mg/kg	0.595 ± 0.05 abc	0.73 ± 0.11 a	0.45 ± 0.089 abcd
	125 mg/kg	0.20 ± 0.051 cd	0.713 ± 0.099 a	0.18 ± 0.061 d
	250 mg/kg	0.44 ± 0.129 abcd	0.68 ± 0.126 ab	0.44 ± 0.095 abcd
	500 mg/kg	0.52 ± 0.053 abcd	0.57 ± 0.058 abcd	0.28 ± 0.069 bcd

Table S5: Physical characteristics of the natural soil used in the study as analyzed by Hanna pH/EC/TDS/°C meter (HI 9811-5)

	Soil sample in water	**Soil sample in $CaCl_2$**
pH	7.4	7.5
Electrical conductivity µS/cm	1740	3170
Total dissolved solid mg/L	860	1590

Table S6: The background concentration of elements in pristine soil used for experiments. The concentrations are averages of 3 replicates ± SE.

Element	**Concentration (mg/kg)**
K	3250.65 ± 92.60
S	398.48 ± 51.20
Mg	6723.40 ± 147.53
Ca	30317.15 ± 593.61
Fe	15133.04 ± 221.88
Zn	49.79 ± 0.68
Cu	17.34 ± 0.53
Mn	454.70 ± 8.33
Al	11791.76 ± 341.10
Cr	12.72 ± 0.33
Pb	20.53 ± 0.56
Ni	12.75 ± 0.25
P	835.21 ± 23.78
Si	1219.44 ± 38.37

Table S7: Translocation factors for Cu (TF = C_{leaf}/C_{root}), ratio of concentration of Cu in leaf vs that in root.

The TF are averages of 4 replicates ±SE.

Cu Species → Concentration ↓	Control	nCuO	bCuO	$CuCl_2$
Control	0.24±0.02			
62.5		0.20±0.04	0.20±0.05	0.22±0.03
125		0.17±0.03	0.26±0.03	0.18±0.04
250		0.15±0.02	0.18±0.04	0.19±0.02
500		0.14±0.02	0.18±0.02	0.10±0.02

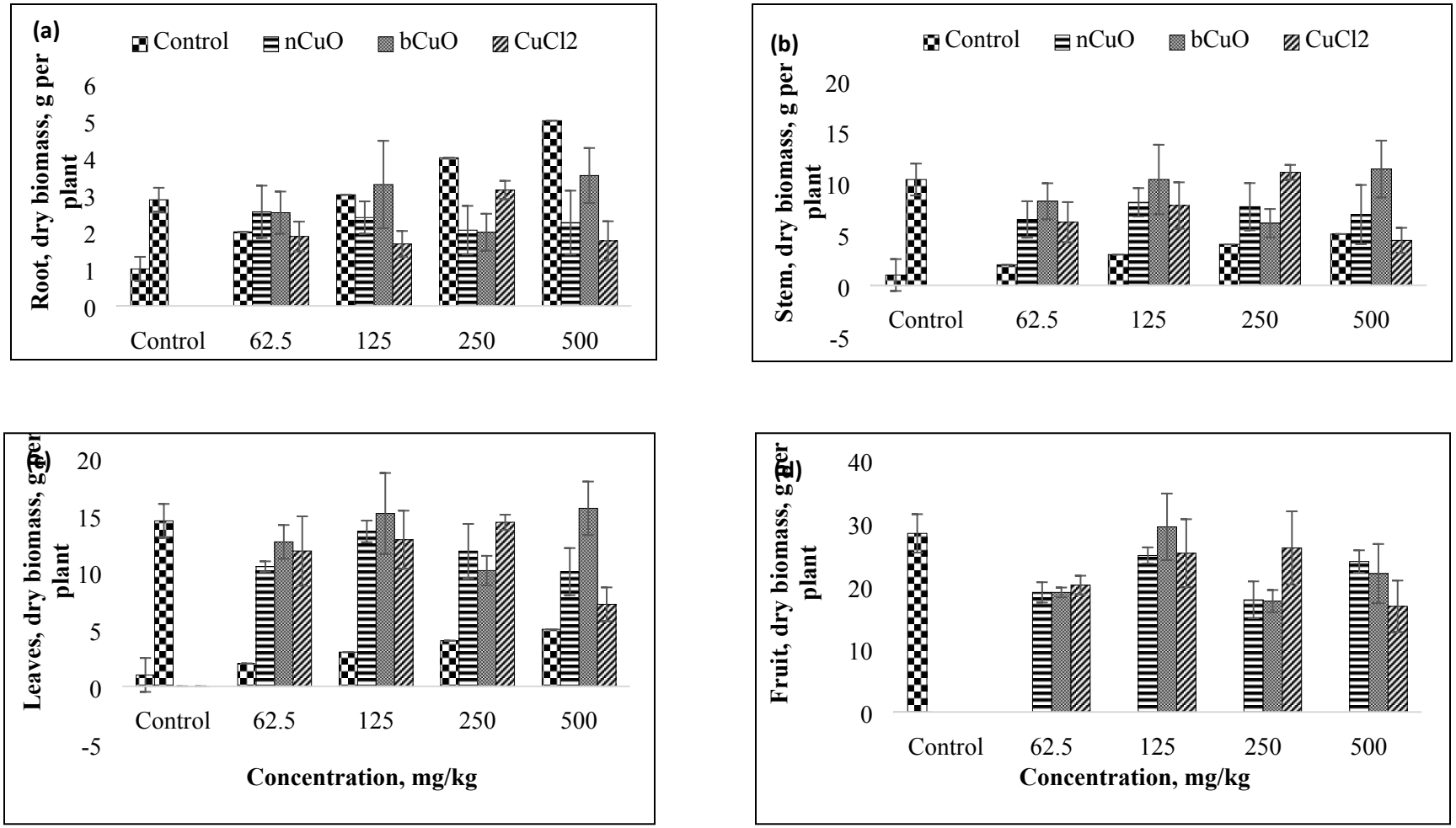
(a)
Control
nCuO
bCuO
CuCl2
Root, dry biomass, g per plant
0
1
2
3
4
5
6
Control
62.5
125
250
500
(b)
Control
nCuO
bCuO
CuCl2
Stem, dry biomass, g per plant
-5
0
5
10
15
20
Control
62.5
125
250
500
(c)
Leaves, dry biomass, g per plant
-5
0
5
10
15
20
Control
62.5
125
250
500
Concentration, mg/kg
(d)
Fruit, dry biomass, g per plant
0
10
20
30
40
Control
62.5
125
250
500
Concentration, mg/kg

Fig. S1

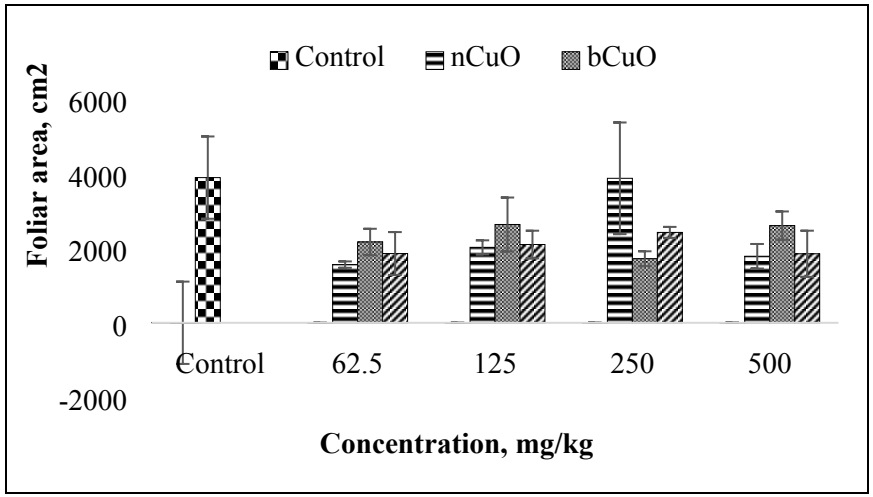
Control
nCuO
bCuO
Foliar area, cm2
6000
4000
2000
0
-2000
Control
62.5
125
250
500
Concentration, mg/kg

Fig. S2