Starnes, Daniel; Unrine, Jason; Chen, Chun; Lichtenberg, Stuart; Starnes, Catherine; Svendsen, Claus; Kille, Peter; Morgan, John; Baddar, Zeinah Elhaj; Spear, Amanda; Bertsch, Paul; Chen, Kuey Chu; Tsyusko, Olga. 2019. **Toxicogenomic responses of Caenorhabditis elegans to pristine and transformed zinc oxide nanoparticles**. *Environmental Pollution*, 247. 917-926. https://doi.org/10.1016/j.envpol.2019.01.077

Supplemental Material

Supplemental Information

Summary: 20 pages including 9 figures and 7 tables

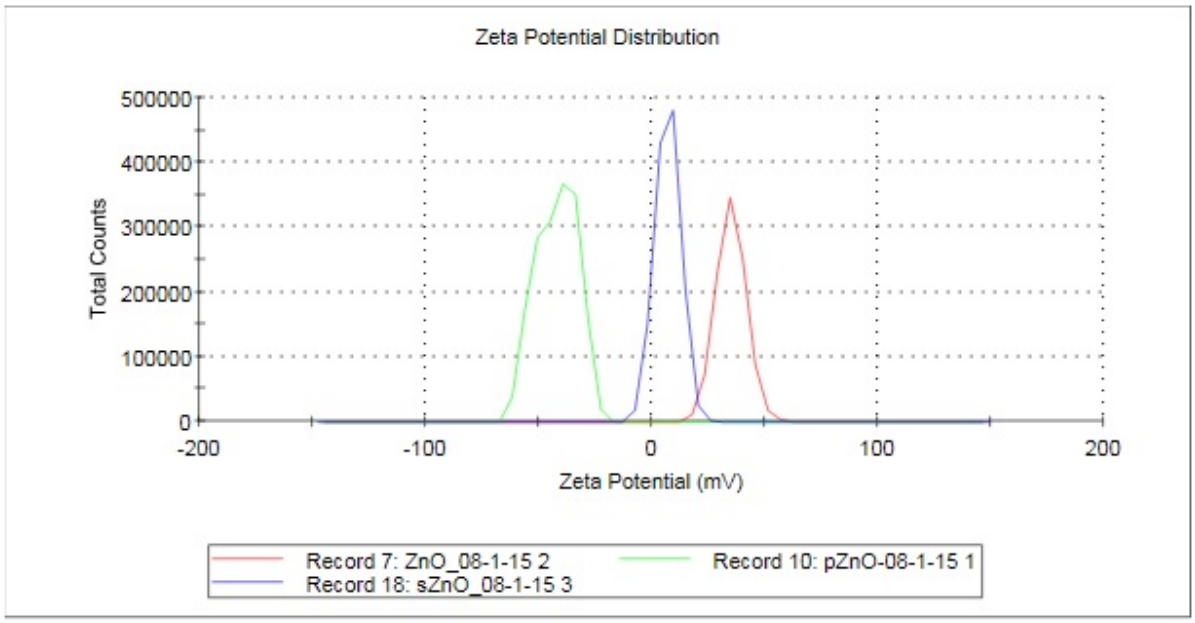

Fig. S1. Zeta Potential distribution of pristine Zinc Oxide manufactured nanoparticles (ZnO-MNPs), phosphatized pZnO-MNPs and sulfidized sZnO-MNPs, measured in synthetic soil pore water.

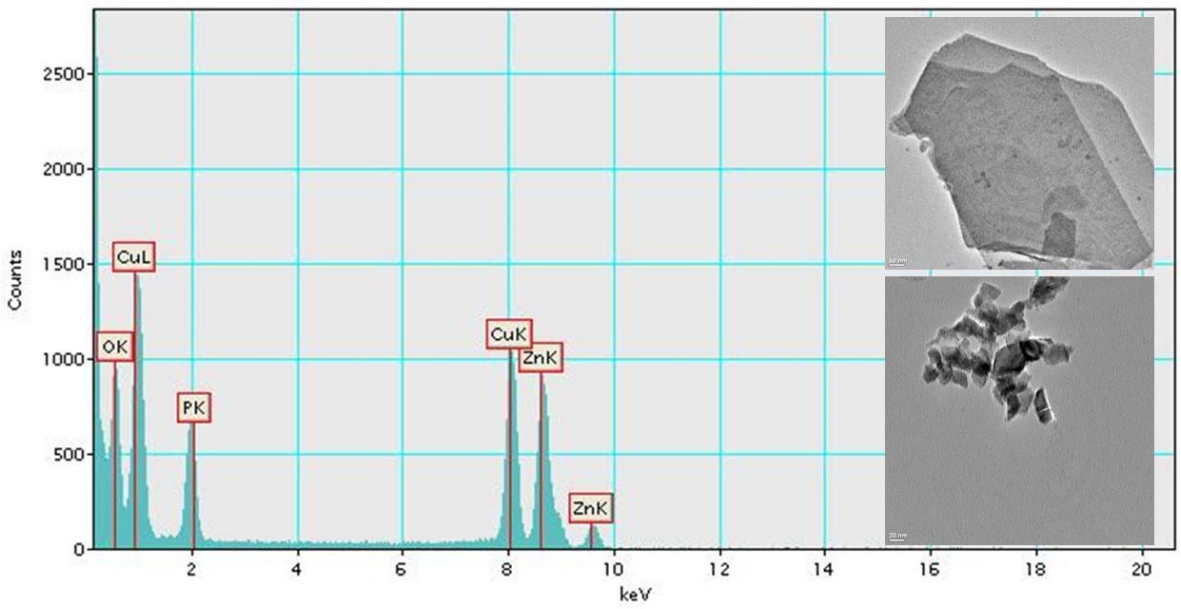

Fig S2. X-ray absorption spectroscopy EDS data and representative transmission electron micrographs of phosphatized Zinc Oxide manufactured nanoparticles (pZnO-MNPs). Top insert picture is pZnO-MNPs at 20k times magnification and bottom insert picture is pZnO-MNPs at 50k times magnification.

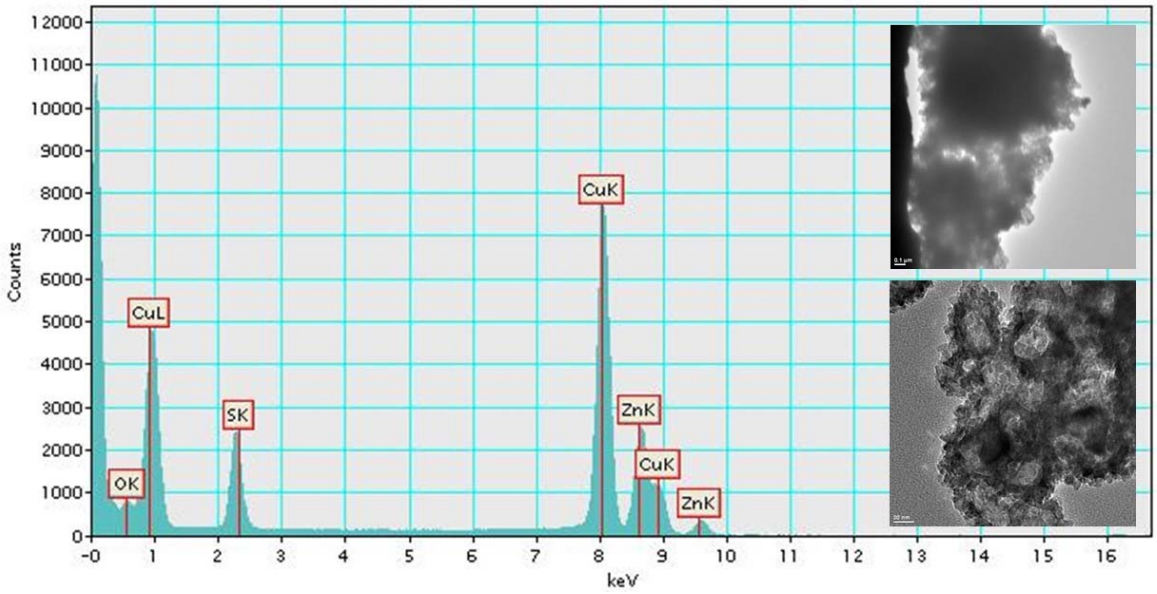

Fig. S3. X-ray absorption spectroscopy EDS data and representative transmission electron micrographs of sulfidized Zinc Oxide manufactured nanoparticles (sZnO-MNPs). Top insert picture is sZnO-MNPs at 8k times magnification and bottom insert picture is sZnO-MNPs at 80k times magnification.

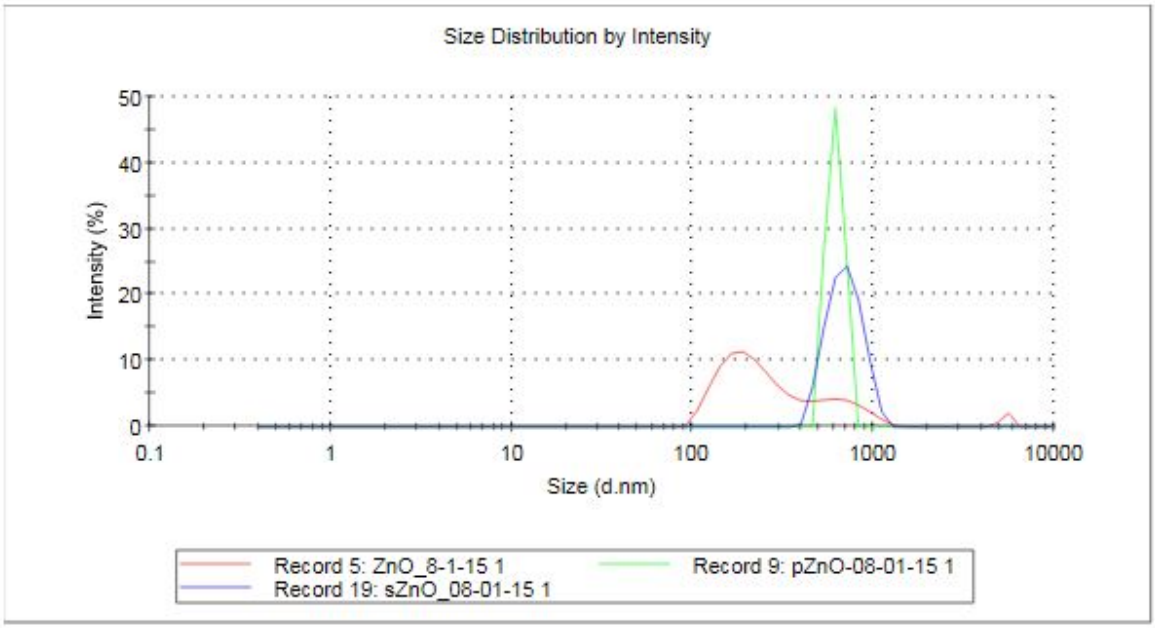

Fig S4. Intensity weighted Z-average hydrodynamic diameter distribution of pristine Zinc oxide manufactured nanoparticles (ZnO-MNPs), phosphatized pZnO-MNPs and sulfidized sZnO-MNPs, measured in synthetic soil pore water.

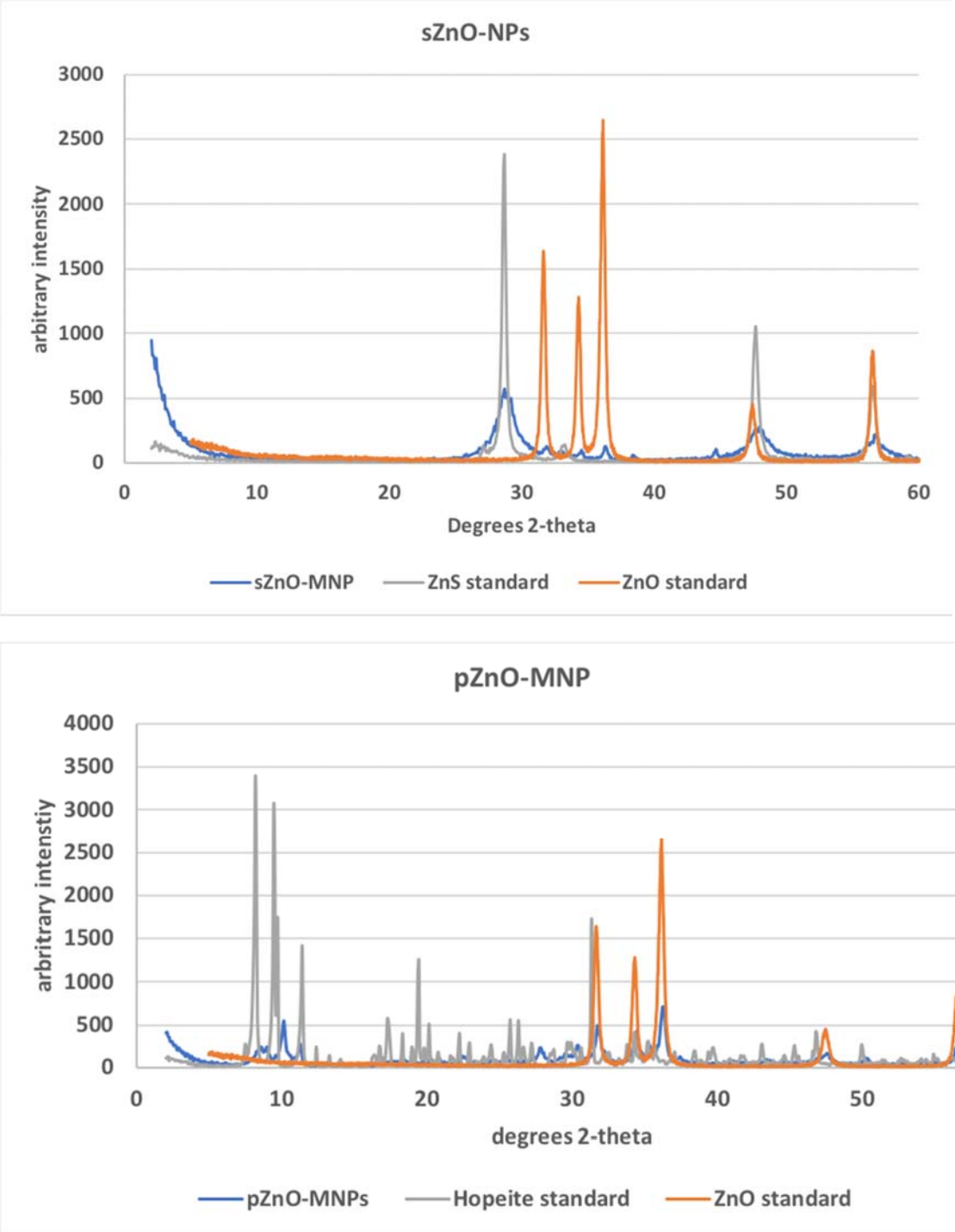

Figure S5. Powder X-ray fractograms for sulfidized ZnO nanoparticles (sZnO-MNPs; Top) and phosphatized ZnO nanoparticles (pZnO-MNPs) bottom.

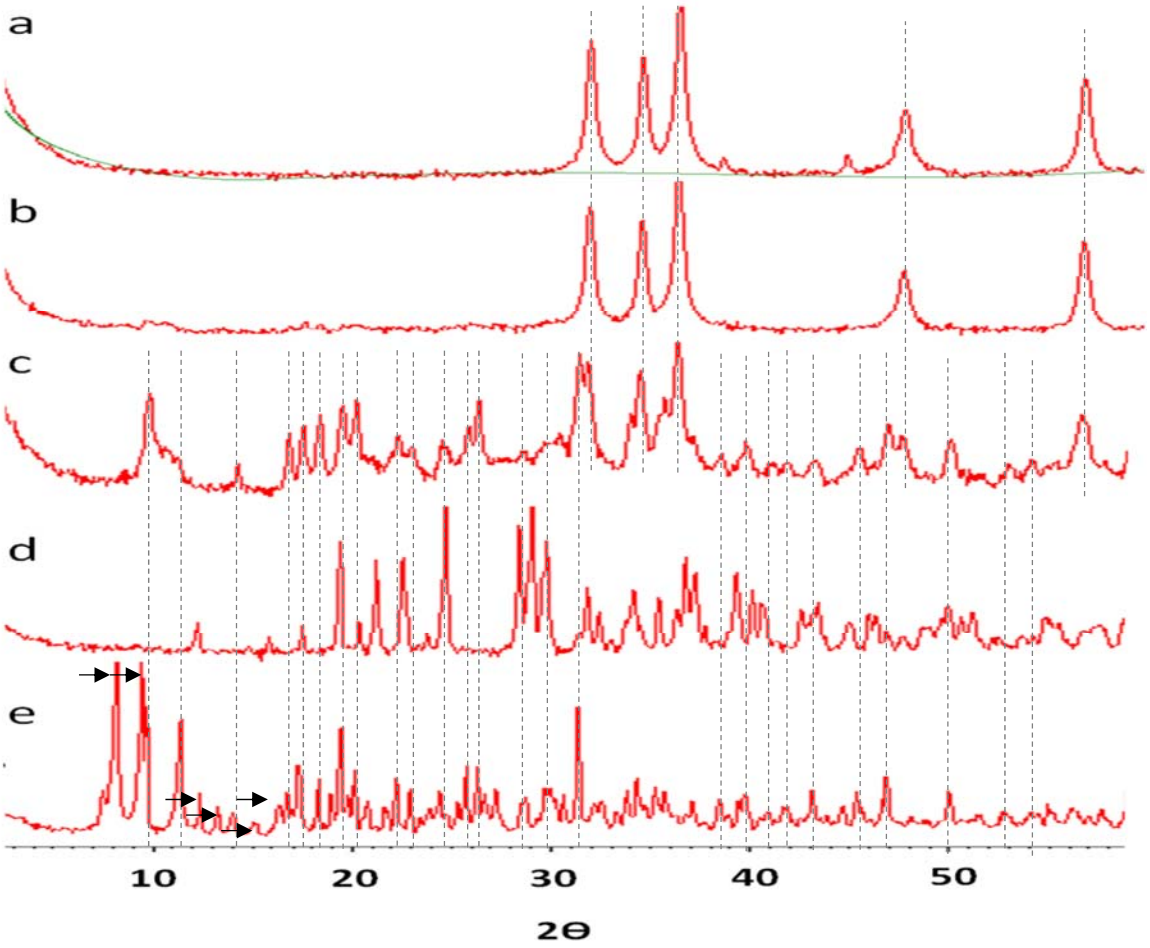

Figure S6: X-ray diffraction patterns for manufactured ZnO nanoparticles (a), particles transformed at 150 mg L^{-1} phosphate for 72 h at pH 8 (b) and pH 6 (c) anhydrous $Zn_3(PO_4)_2$ (d) and hopeite (e). Dashed lines have been added to show that all peaks in the aged samples correspond to peaks in either the ZnO or hopeite standards. The black arrows indicate peaks that should not be present in the hopeite standard (see reference XRD pattern in SI) and are probably due to impurities in the reference hopeite standard. Reprinted with permission from Rathnayake et al., 2014.

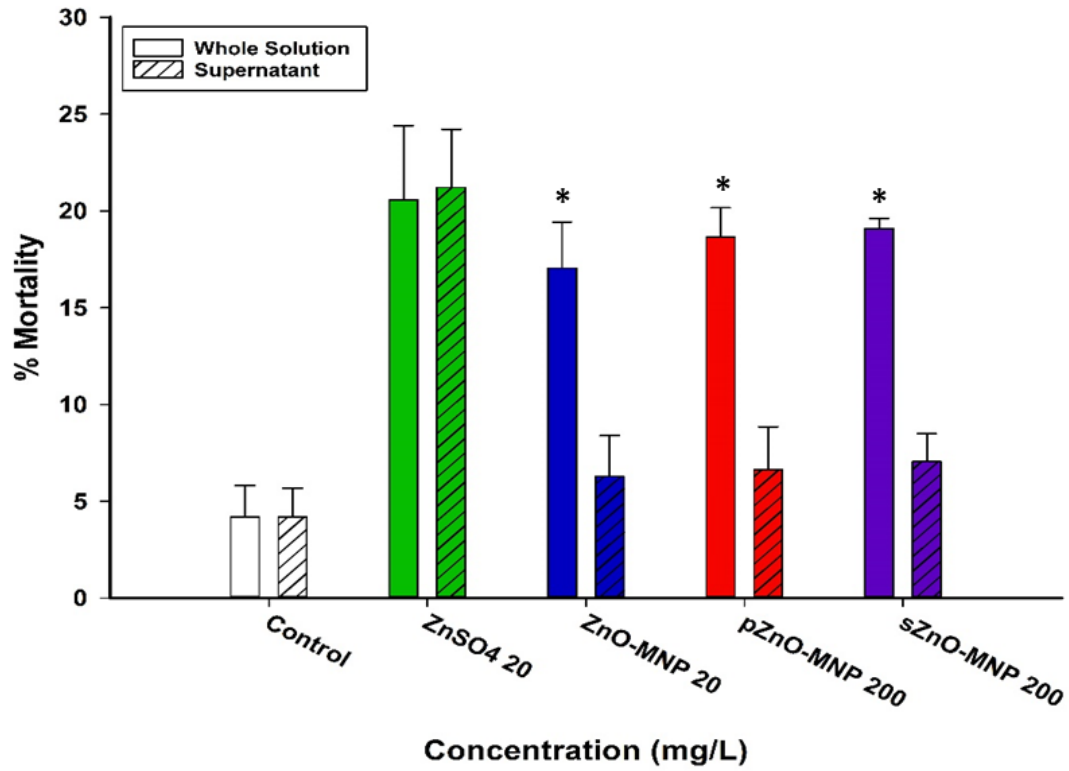

Figure S7. Mortality of *Caenorhabditis elegans* after 24 hours without feeding when exposed to particle free supernatants (Supernatant) versus whole solutions of $ZnSO_4$, pristine (ZnO-MNPs), phosphatized (pZnO-MNPs), and sulfidized (sZnO-MNPs) zinc oxide manufactured nanoparticles in synthetic soil pore water. Data are presented as mean percent mortality with error bars indicating standard error of the mean. An asterisk (*) indicates significantly different mortality between exposures in whole solution and supernatant at $\alpha \leq 0.05$ based on Student's t-test.

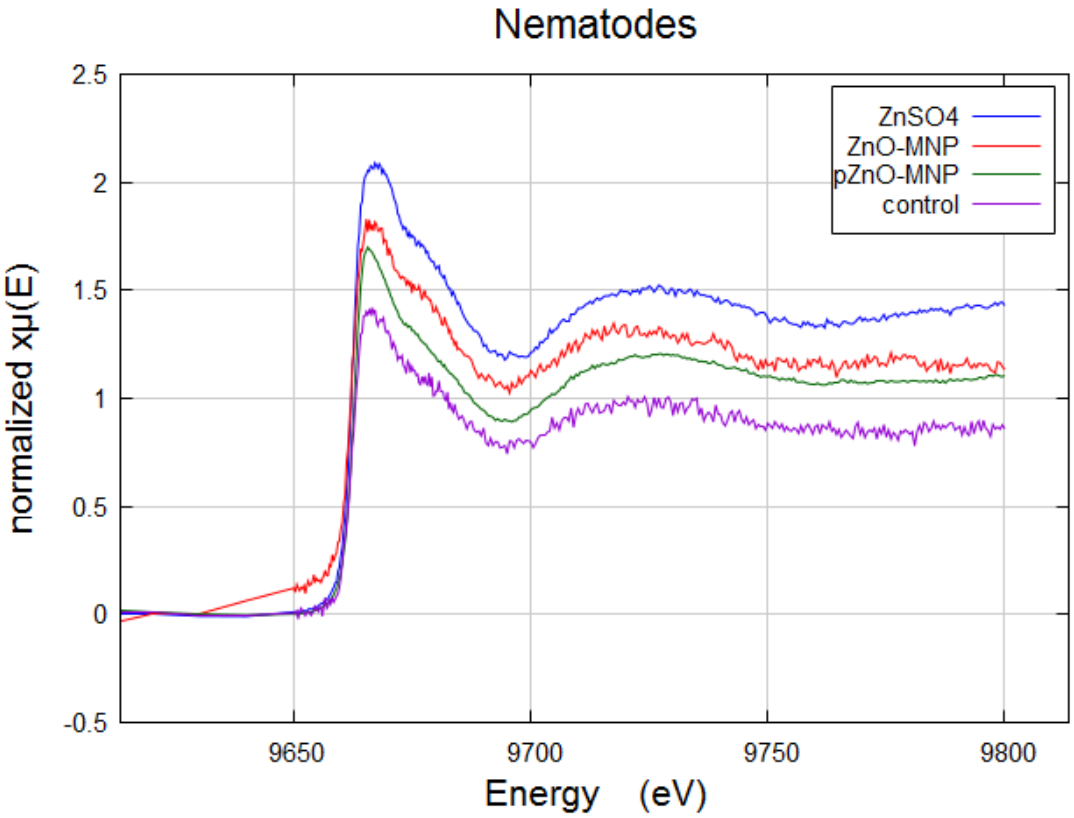

Figure S8. Zn K-edge X-ray absorption near-edge structure (XANES) spectra for *Caenorhabditis elegans* unexposed, and exposed to $ZnSO_4$, pristine ZnO-MNPs and phosphatized pZnO-MNPs.

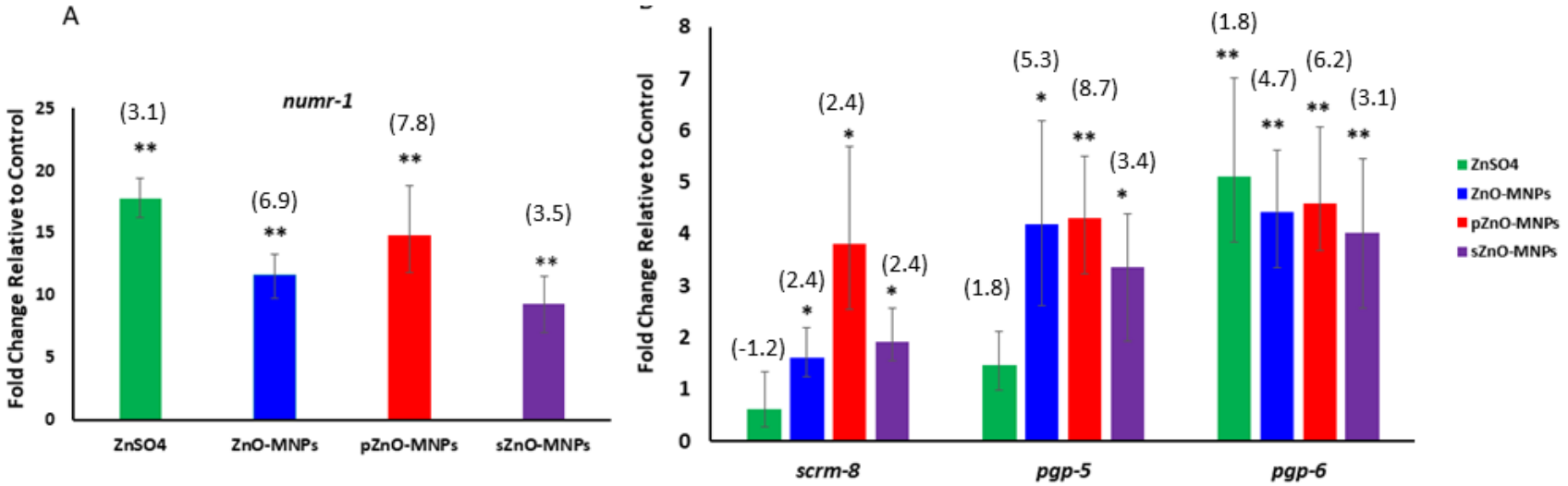

Figure S9. qRT-PCR confirmation from the independent experiment of the gene expression for A) nuclear localized metal responsive (*numr-1*) gene and B) scramblase (*scrm-8*) and p-glycoproteins (*pgp-5* and *pgp-6*) in *C. elegans* after exposure at EC_{30} for 48 hours to $ZnSO_4$, pristine ZnO-MNPs and transformed phosphatized pZnO- and sulfidized sZnO-MNPs. The significant differences from controls are shown as * at $p < 0.05$ and as ** at $p < 0.001$. Expression of all genes were significantly different from control, except for *scrm-8* and *pgp-5* in ZnO_4 treatment, which is in agreement with the microarray data.

Table S1. Characterization results of the pristine ZnO-MNPs, phosphatized (pZnO-MNPs), and sulfidized (sZnO-MNPs) in the exposure media (synthetic soil pore water).

Treatment	**Zeta Potential**		**Hydrodynamic diameter (volume weighted)**		**Z-average diameter, nm (intensity weighted)**
	Mean (mV)	Standard Deviation	Mean (nm)	Standard Deviation	
ZnO-MNPs	34.5	7.60	182.7	75.09	265.4
pZnO-MNPs	35.01	6.64	630.6	93.04	1715
sZnO-MNPs	33.9	6.96	749.6	171	1022

Table S2. ABI assay IDs used for qRT-PCR confirmation.

Gene Symbol	ABI Assay ID	Efficiency %	Forward Primer	Reverse Primer
pgp-5	**Ce02500335_g1**	**95.33**		
pgp-6	**Ce02500372_g1**	**96.01**		
scrm-8	**Ce02504322_g1**	**96.65**		
Y45F10D.4	**Ce02467252_g1**	**99.33**		
numr-1*	**A13951Q**	**97.81**	**CTCCACATCGTCGTCATTGTG**	**GCTTCGAGATCTTGAACGATCA**
All primer information is property of ABI, the primers and probes can be ordered using the ABI assay IDs. *** Indicates custom designed primers.**				

Table S3. Parameter estimates from linear regression analysis of concentration response curves for reproduction data along with endpoint estimates for EC_{30} and EC_{50} values. LCI= lower 95% confidence interval, UCI = upper 95% confidence interval. All slopes and intercepts significant at $p < 0.001$.

Treatment	Slope	LCI	UCI	Intercept	LCI	UCI	R^2	EC_{30}	LCI	UCI	EC_{50}	LCI	UCI
$ZnSO_4$	-76.47	-93.71	-59.23	187.87	171.51	204.23	0.83	0.74	0.59	0.89	1.23	1.03	1.42
ZnO-MNPs	-75.30	-99.18	-51.42	179.18	156.52	201.84	0.71	0.71	0.50	0.93	1.19	0.92	1.46
pZnO-MNPs	-8.02	-9.89	-6.15	185.53	167.78	203.27	0.82	6.94	5.39	8.50	11.57	9.65	13.49
sZnO-MNPs	-8.22	-10.30	-6.14	185.61	165.87	205.35	0.79	6.78	5.09	8.46	11.29	9.25	13.34

Table S4. List of Biological Process (BP) Gene Ontology terms that were identified by Partek using lists of significantly altered genes from N2 wild type nematodes when exposed to $ZnSO_4$, pristine zinc oxide manufactured nanoparticles (ZnO-MNPs), phosphatized aged (pZnO-MNPs) and sulfidized (sZnO-MNPs) at the EC_{30} for 48 hours.

GoTerm BP	Category	Genes in Category	P-Value	Fold Enrichment	Exposure
6952	defense response	13	5.37E-09	19.043	ZnSO4
6950	response to stress	19	5.61E-09	18.9988	ZnSO4
2376	immune system process	10	1.82E-08	17.8237	ZnSO4
50896	response to stimulus	20	3.24E-08	17.2451	ZnSO4
45087	innate immune response	9	2.31E-07	15.2818	ZnSO4
6030	chitin metabolic process	3	6.45E-06	11.9521	ZnSO4
46686	response to cadmium ion	4	6.95E-06	11.8767	ZnSO4
6040	amino sugar metabolic process	3	2.23E-05	10.7119	ZnSO4
10038	response to metal ion	4	0.00016	8.71762	ZnSO4
6022	aminoglycan metabolic process	3	0.00034	7.99598	ZnSO4
10035	response to inorganic substance	4	0.00062	7.38167	ZnSO4
9607	response to biotic stimulus	5	0.00065	7.33235	ZnSO4
98542	defense response to other organism	4	0.00502	5.29347	ZnSO4
40002	collagen and cuticulin-based cuticle development	4	0.00735	4.91265	ZnSO4
42335	cuticle development	4	0.00765	4.87318	ZnSO4
9605	response to external stimulus	5	0.00781	4.85224	ZnSO4
50829	defense response to Gram-negative bacterium	3	0.00803	4.82413	ZnSO4
42221	response to chemical	4	0.01578	4.14918	ZnSO4
9617	response to bacterium	3	0.0218	3.82591	ZnSO4
6952	defense response	18	2.02E-11	24.6242	ZnO
2376	immune system process	12	8.25E-09	18.6126	ZnO
6950	response to stress	23	1.26E-08	18.1931	ZnO
45087	innate immune response	11	8.18E-08	16.3188	ZnO
6955	immune response	11	8.18E-08	16.3188	ZnO
50896	response to stimulus	24	1.55E-07	15.6778	ZnO
46686	defense response	5	9.55E-07	12.94211333	ZnO
50829	immune system process	7	2.22E-06	11.41389333	ZnO
10038	response to stress	6	3.02E-06	9.885673333	ZnO
98542	innate immune response	8	8.00E-06	8.357453333	ZnO
51707	response to other organism	8	8.79E-06	11.642	ZnO

10035	response to inorganic substance	6	2.37E-05	10.651	ZnO
9617	response to bacterium	7	2.73E-05	10.5087	ZnO
1990170	stress response to cadmium ion	3	0.00012	9.04803	ZnO
9605	response to external stimulus	8	0.0005	7.6023	ZnO
42221	response to chemical	7	0.00057	7.46442	ZnO
51704	multi-organism process	10	0.02365	3.74451	ZnO
6952	response to stress	36	1.90E-15	33.8977	pZnO
6950	immune system process	45	1.90E-08	17.7784	pZnO
2376	defense response to bacterium	18	1.66E-07	15.6117	pZnO
42742	response to bacterium	14	4.50E-07	14.6146	pZnO
9607	innate immune response	15	6.26E-07	14.2836	pZnO
45087	immune response	17	7.45E-07	14.1096	pZnO
6955	response to cadmium ion	17	7.45E-07	14.1096	pZnO
46686	response to stimulus	7	8.65E-07	13.9604	pZnO
50896	stress response to cadmium ion	47	1.56E-06	13.3693	pZnO
1990170	stress response to metal ion	5	5.60E-06	12.0924	pZnO
97501	defense response to Gram-negative bacterium	5	1.22E-05	11.3154	pZnO
50829	response to metal ion	10	1.51E-05	11.0985	pZnO
10038	masculinization of hermaphroditic germ-line	8	3.17E-05	10.3584	pZnO
42006	response to inorganic substance	5	0.00011	9.12733	pZnO
10035	response to external stimulus	8	0.00041	7.80126	pZnO
9605	germ-line sex determination	15	0.00061	7.40502	pZnO
18992	defense response to Gram-positive bacterium	7	0.00089	7.02676	pZnO
50830	sex determination	6	0.00095	6.95599	pZnO
7530	hermaphrodite germ-line sex determination	7	0.00242	6.02417	pZnO
40021	aspartate family amino acid biosynthetic process	6	0.00265	5.93324	pZnO
34248	regulation of translation	6	0.00528	5.2435	pZnO
1901607	aspartate family amino acid metabolic process	4	0.00791	4.83971	pZnO
7276	male gamete generation	16	0.01249	4.38307	pZnO
48232	spermatogenesis	5	0.01312	4.33364	pZnO
46394	cellular amino acid biosynthetic process	6	0.01706	4.07113	pZnO

8652	sulfur compound metabolic process	4	0.0176	4.04	pZnO
6790	lipid catabolic process	5	0.02255	3.79208	pZnO
44272	positive regulation of meiotic cell cycle	4	0.02291	3.77626	pZnO
51446	serine family amino acid biosynthetic process	5	0.0273	3.60099	pZnO
42221	cell cycle switching, mitotic to meiotic cell cycle	9	0.0357	3.33271	pZnO
60184	positive regulation of cell cycle	4	0.03618	3.31914	pZnO
45787	meiotic nuclear division	5	0.04516	3.09757	pZnO
7126	fatty acid metabolic process	9	0.04541	3.09197	pZnO
6631	sulfur amino acid metabolic process	4	0.04852	3.02572	sZnO
6952	defense response	21	2.94E-14	31.1582	sZnO
50896	response to stimulus	28	6.91E-10	21.0932	sZnO
6950	response to stress	25	9.04E-10	20.8247	sZnO
2376	immune system process	13	1.03E-09	20.695	sZnO
45087	innate immune response	12	1.08E-08	18.3469	sZnO
6955	immune response	12	1.08E-08	18.3469	sZnO
46686	response to cadmium ion	5	1.12E-06	13.6993	sZnO
43207	response to external biotic stimulus	9	1.13E-06	13.6973	sZnO
98542	defense response to other organism	8	1.02E-05	11.4943	sZnO
9617	response to bacterium	7	3.37E-05	10.2971	sZnO
10038	response to metal ion	5	6.27E-05	9.6765	sZnO
9605	response to external stimulus	9	0.00012	9.07008	sZnO
97501	stress response to metal ion	3	0.00019	8.55572	sZnO
10035	response to inorganic substance	5	0.00033	8.01295	sZnO
50829	defense response to Gram-negative bacterium	5	0.00041	7.8068	sZnO
42221	response to chemical	7	0.0007	7.26866	sZnO
16042	lipid catabolic process	3	0.00561	5.18258	sZnO
50830	defense response to Gram-positive bacterium	3	0.00688	4.97962	sZnO
51704	multi-organism process	11	0.01167	4.451	sZnO

Table S5. List of Molecular Function (MF) Gene Ontology terms that were identified by Partek using lists of significantly altered genes from N2 wild type nematodes when exposed to $ZnSO_4$, pristine zinc oxide manufactured nanoparticles (ZnO-MNPs), phosphatized aged (pZnO-MNPs) and sulfidized (sZnO-MNPs) at the EC_{30} for 48 hours.

Go Term: MF	Category	Genes in Category	P-Value	Fold Enrichment	Exposure
42302	structural constituent of cuticle	15	6.07E-12	25.8273	ZnSO4
5198	structural molecule activity	16	6.44E-08	16.5579	ZnSO4
8061	chitin binding	3	6.45E-06	11.9521	ZnSO4
42302	structural constituent of cuticle	13	1.79E-07	15.5332	ZnO
42626	ATPase activity, coupled to transmembrane movement of substances	8	3.19E-06	12.6566	ZnO
5198	structural molecule activity	15	7.58E-05	9.48739	ZnO
42623	ATPase activity, coupled	8	0.00013908	8.88049	ZnO
17111	nucleoside-triphosphatase activity	9	0.010998	4.51004	ZnO
16462	pyrophosphatase activity	9	0.0141894	4.25526	ZnO
42302	structural constituent of cuticle	25	9.11E-09	18.5135	pZnO
42626	ATPase activity, coupled to transmembrane movement of substances	11	0.00011117	9.10449	pZnO
3730	mRNA 3'-UTR binding	5	0.00016339	8.71935	pZnO
5198	structural molecule activity	29	0.00045233	7.7011	pZnO
3729	mRNA binding	5	0.00080168	7.1288	pZnO
16758	transferase activity, transferring hexosyl groups	13	0.00227614	6.08527	pZnO
42623	ATPase activity, coupled	12	0.00286986	5.85349	pZnO
16757	transferase activity, transferring glycosyl groups	13	0.00624482	5.076	pZnO
22804	active transmembrane transporter activity	12	0.00805273	4.82174	pZnO
16887	ATPase activity	13	0.0199169	3.91619	pZnO
4497	monooxygenase activity	8	0.022407	3.79838	pZnO
46906	tetrapyrrole binding	11	0.023181	3.76442	pZnO
42302	structural constituent of cuticle	17	4.48E-11	23.8288	sZnO
5198	structural molecule activity	19	3.41E-07	14.8922	sZnO

42626	ATPase activity, coupled to transmembrane movement of substances	5	0.00251235	5.98654	sZnO
30246	carbohydrate binding	10	0.00406916	5.50432	sZnO
42623	ATPase activity, coupled	5	0.021161	3.8556	sZnO
16798	hydrolase activity, acting on glycosyl bonds	3	0.0228562	3.77853	sZnO
22804	active transmembrane transporter activity	5	0.034328	3.37179	sZnO

Table S6. List of Cellular Compartments (CC) Gene Ontology terms that were identified by Partek using lists of significantly altered genes from N2 wild type nematodes when exposed to $ZnSO_4$, pristine zinc oxide manufactured nanoparticles (ZnO-MNPs), phosphatized (pZnO-MNPs) and sulfidized (sZnO-MNPs) at the EC_{30} for 48 hours.

GoTerm: CC	Category	Genes in Category	P-Value	Fold Enrichment	Exposure
5581	collagen trimer	3	0.00409714	5.49747	ZnSO4
5581	collagen trimer	3	0.0123931	4.39062	ZnO
45121	membrane raft	7	5.99E-05	9.72213	pZnO
5764	lysosome	6	0.000636159	7.36006	pZnO
5581	collagen trimer	7	0.00063737	7.35816	pZnO
5773	vacuole	6	0.00264987	5.93324	pZnO
43186	P granule	6	0.0239935	3.72997	pZnO
36464	cytoplasmic ribonucleoprotein granule	6	0.0458981	3.08133	pZnO
45121	membrane raft	5	2.19E-05	10.7309	sZnO
5581	collagen trimer	5	0.000128716	8.9579	sZnO

Table S7. The list of differentially expressed genes at fold change (FC) ±2 and $p < 0.05$ and the direction of their expression from the Aminoacyl t-RNA biosynthesis pathway induced in response to all ZnO-MNP but not ZnSO4 treatments. NS indicates nonsignificant at the criteria used for the FC and/or p-value.

Gene ID	MNP Treatment		
	ZnO-MNPs	pZnO-MNPs	sZnO-MNPs
C03A3.8	up	up	up
C03B1.16	up	up	NS
C05C9.7	NS	up	NS
C06G4.7	NS	NS	up
C09B9.80	up	up	up
C16H3.6	up	up	up
C17D12.15	NS	up	up
C18A11.13	up	up	up
C27A2.10	up	up	up
C28A5.11	up	up	up
C28A5.12	up	up	up
C37A5.12	up	up	NS
C48C5.7	NS	up	NS
F02D10.11	up	up	up
F08F1.19	NS	up	NS
F08G5.18	up	up	up
F09B9.7	up	up	up
F12D9.13	up	up	up
F12D9.18	up	up	up
F12D9.5	NS	up	NS
F13G11.145	up	up	up
F14H8.10	up	up	up
F15B9.14	up	up	NS
F15G9.9	up	up	up
F22F1.8	up	up	up
F22G12.9	up	up	NS
F22H10.9	NS	up	up
F33D11.15	up	up	up
F39B1.4	up	up	up
F40H6.7	up	up	up
F42A10.11	up	up	up
F42A10.12	up	up	up
F52H2.10	up	up	up
F53F8.10	up	up	up
T14F9.11	up	up	NS
F57G8.12	NS	NS	up
F57G8.13	up	up	up

F59C12.9	up	up	up
K01A12.9	up	up	up
K02E2.15	up	up	up
K03E6.8	up	up	up
M03A1.12	up	up	up
M163.20	NS	up	NS
R04D3.14	up	NS	up
T01C1.9	up	up	up
T06H11.18	up	up	up
T25D1.4	up	up	up
Y37E11AL.16	up	NS	NS
Y38H6A.10	up	up	up
Y40C5A.16	up	NS	up
Y51H7BR.9	up	up	up
Y53C10A.18	up	up	up
Y65A5A.26	NS	up	NS
Y73B6BL.283	up	NS	up
Y73F8A.1164	up	up	up
Y73F8A.1171	up	up	up
Y7A5A.14	up	NS	NS
Y7A5A.15	up	up	up
Y95B8A.13	up	up	up
ZC412.12	NS	NS	up
ZK678.11	NS	up	up