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Tatsi, K

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Supplementary Information

Consequences of Surface Coating and Soil Ageing on the Toxicity of Cadmium Telluride Quantum Dots to the Earthworm *Eisenia fetida.*

Kristi Tatsi[1], Thomas Hutchinson[2] and Richard D. Handy[1,3].

[1]School of Biological Sciences, Plymouth University, Drake Circus, Plymouth, PL4 8AA, UK.

[2]School of Environmental Sciences, Plymouth University, Drake Circus, Plymouth, PL4 8AA, UK.

[3]Visiting Professor, Department of Nutrition, Cihan University, Kurdistan Region, Iraq.

* To whom correspondence should be addressed. email: R.Handy@plymouth.ac.uk

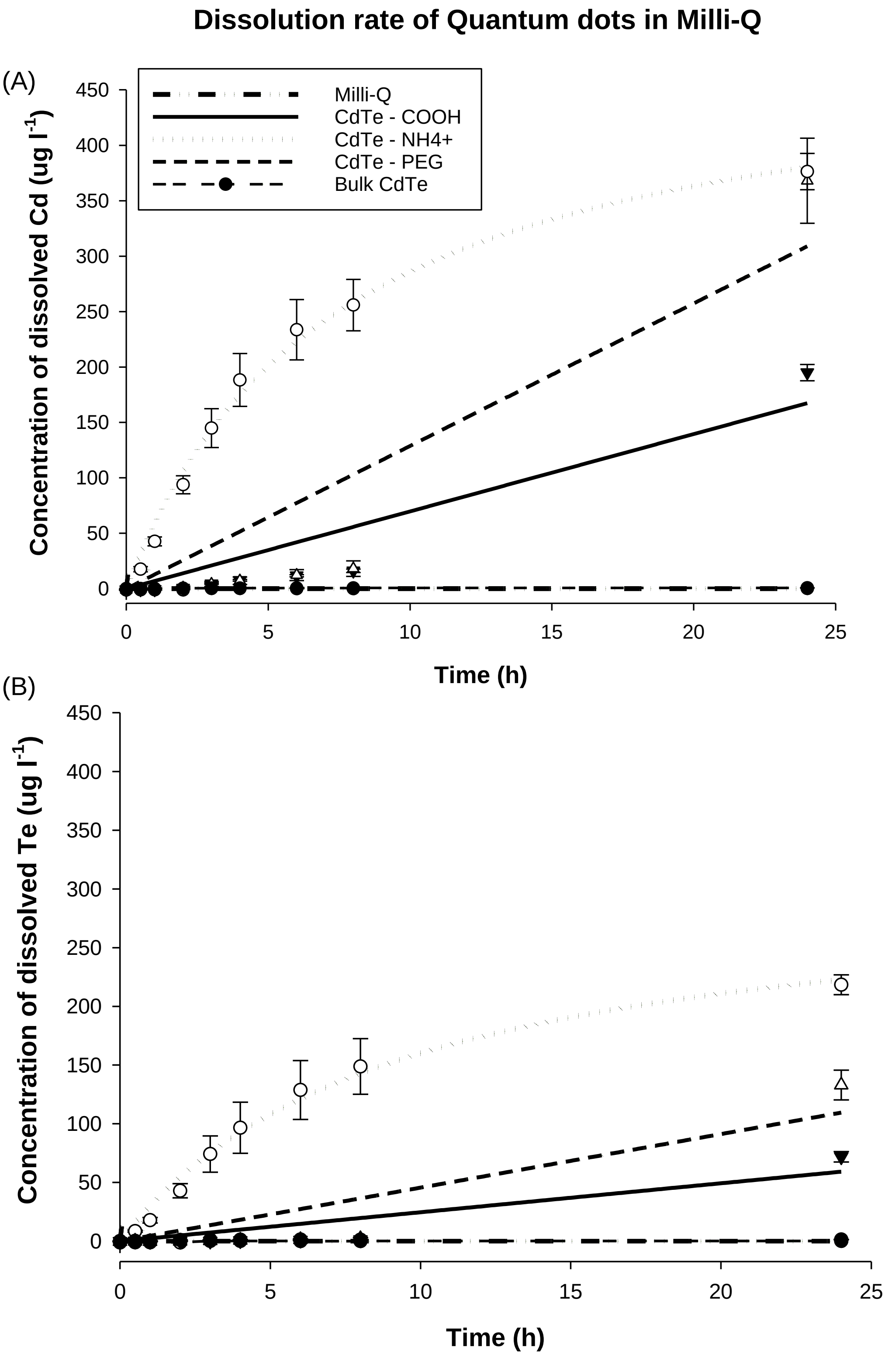

Fig. S1 Dissolution by dialysis of (A) total dissolved Cd and (B) total dissolved Te from CdTe quantum dots with different surface coatings compared to a CdTe micron-sized powder (bulk material). Milli-Q water alone was used as a blank. Note, the dialysis bags contained 100 mg l^{-1} of each material and only µg amounts are released, and with negligible release from the bulk material. Data are means ± SD, *n* = 3 replicates, fitted to a rectangular hyperbola using SigmaPlot.

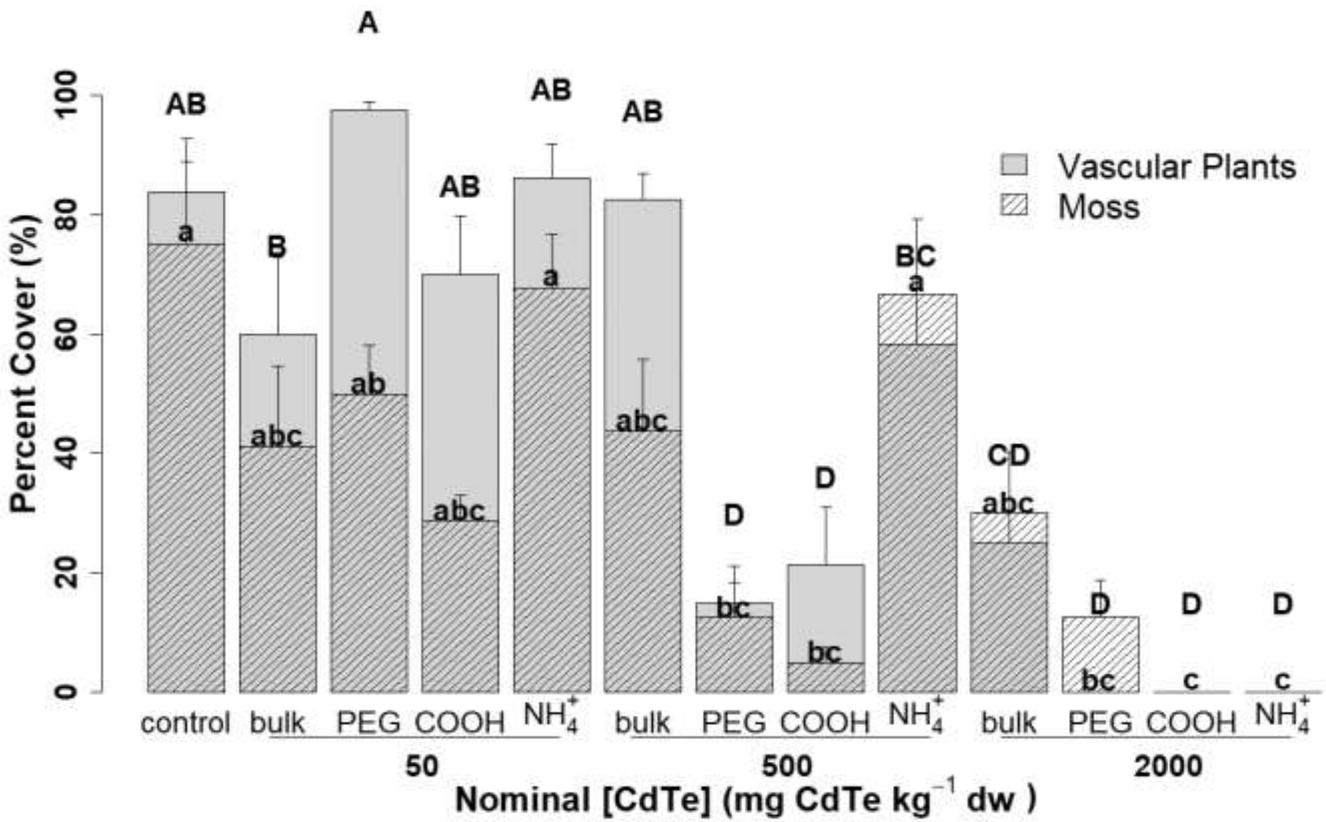

Fig. S2 The total cover of vascular plants (solid bars) and moss (dashed bars) on the soil from exposure to controls, CdTe-bulk ('bulk'), and differently coated CdTe QD ENMs (PEG, COOH and NH_4^+ coated) at 50, 500 and 2000 mg kg^{-1} dw nominal concentrations of CdTe QDs. Data shown as the percent cover of 4 replicate boxes of soil (mean ± SEM, $n = 4$). Different letters denote statistically significantly different treatments across nominal test concentrations ($P < 0.05$, ANOVA). Capital letters represent differences between vascular plant cover and non-capital letters represent differences between moss cover.

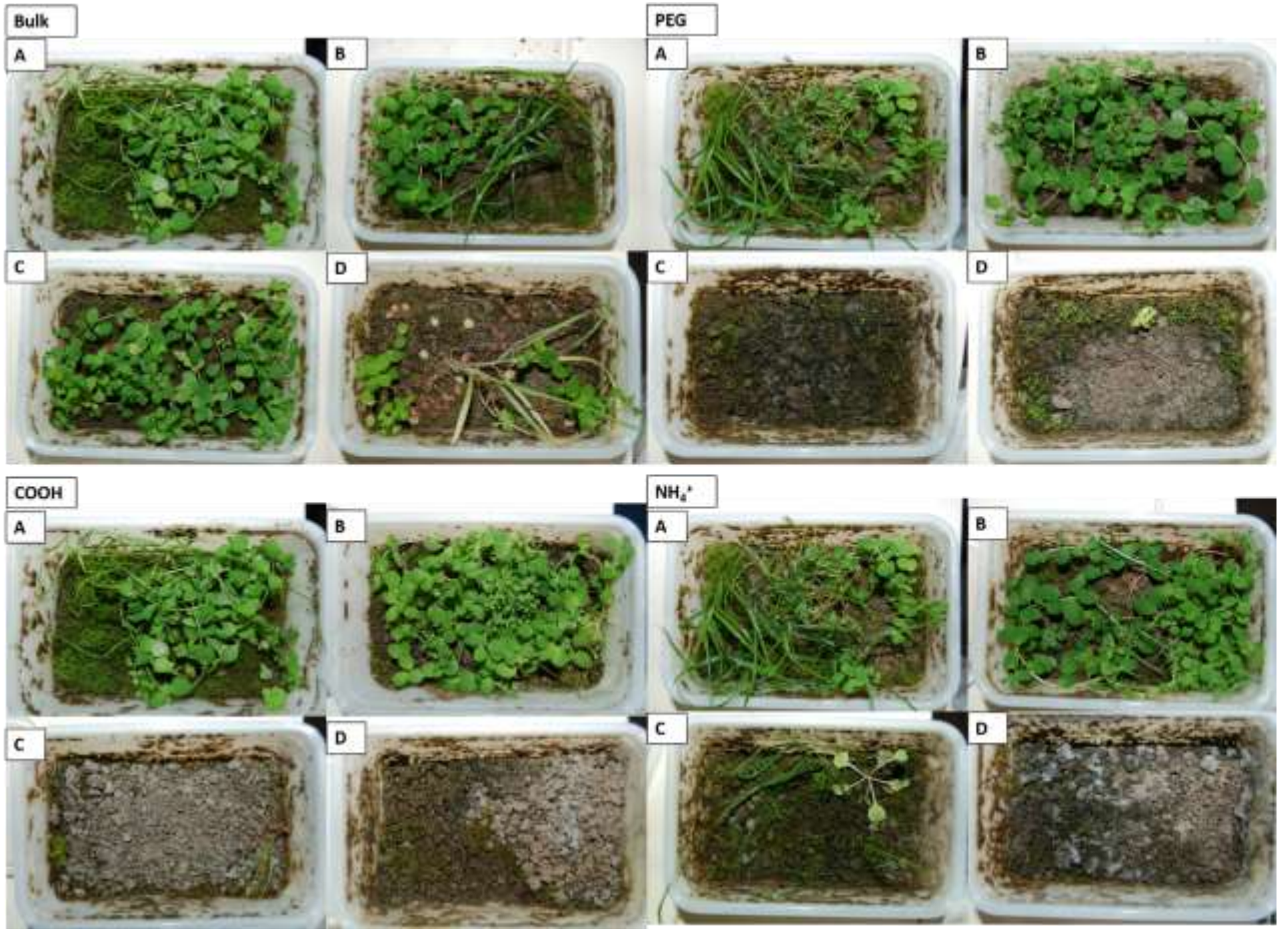

Fig. S3 Example images of plants from the (A) four control treatments and CdTe-bulk, CdTe PEG, -COOH and -NH_4^+ QD exposures at nominal (B) 50 mg CdTe kg^{-1} dw (C) 500 mg CdTe kg^{-1} dw and (D) 2000 mg CdTe kg^{-1} dw taken after 6 months since the QDs were mixed into the soil. Note the limited growth in the highest exposures (C,D) and possible mould and presence of a bacterial film on images across nanoscale treatments at 500 and 2000 mg CdTe QD nominal exposures (C, D)

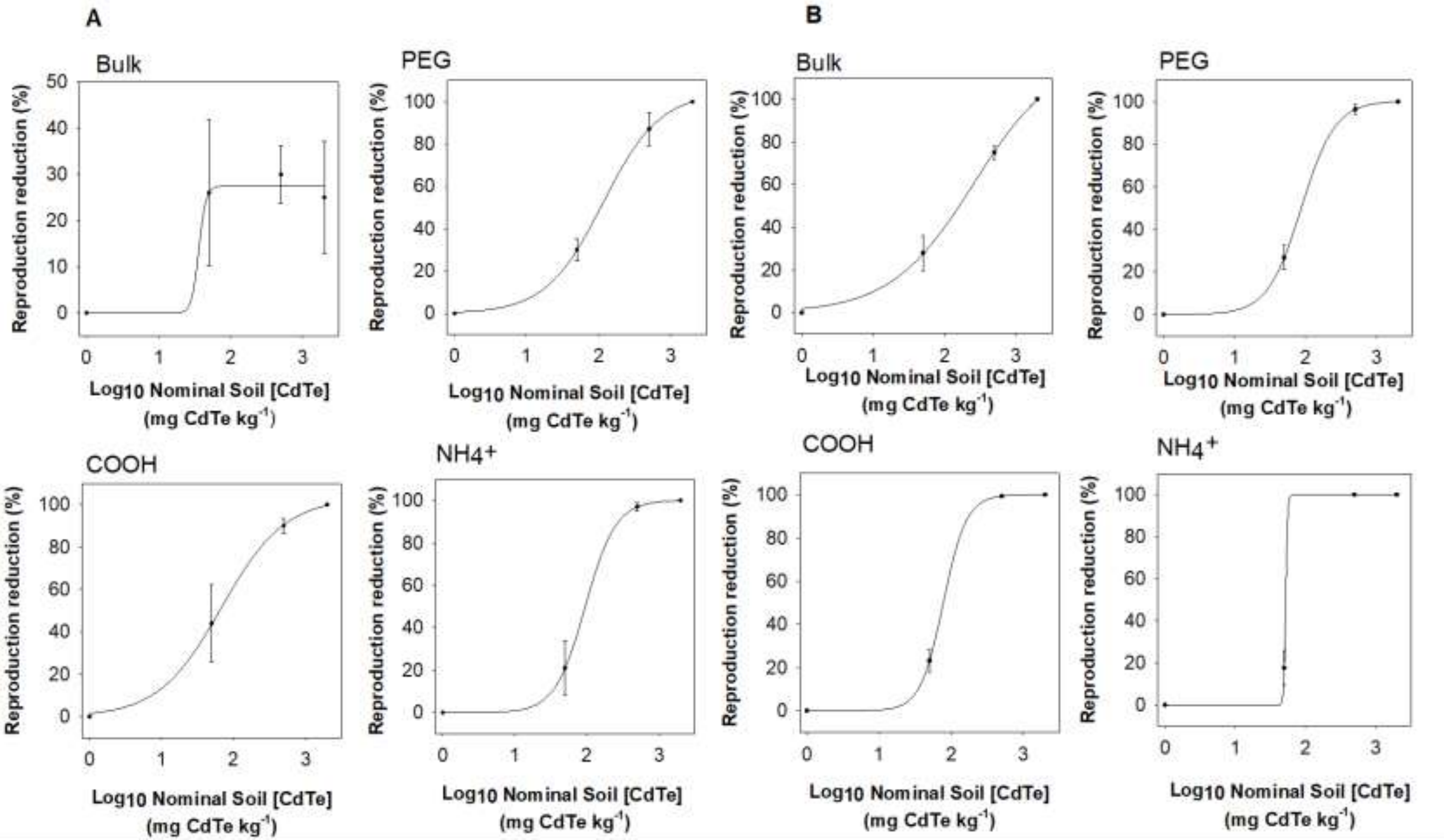

Fig. S4 Reproduction curves derived from the number of juveniles produced in each treatment in the (A) fresh and (B) aged soils measured on day 56. The Log_{10} transformed concentrations 1.7, 2.6 and 3.3 equate to nominal concentrations 50, 500 and 2000 mg CdTe QD kg^{-1}dw, respectively. The sigmoid curves were fitted to raw data ($r^2 > 0.8$ for curve fits, excluding fresh soil CdTe-bulk data, $r^2 = 0.3$) while the mean ± SEM (n = 4) is shown.

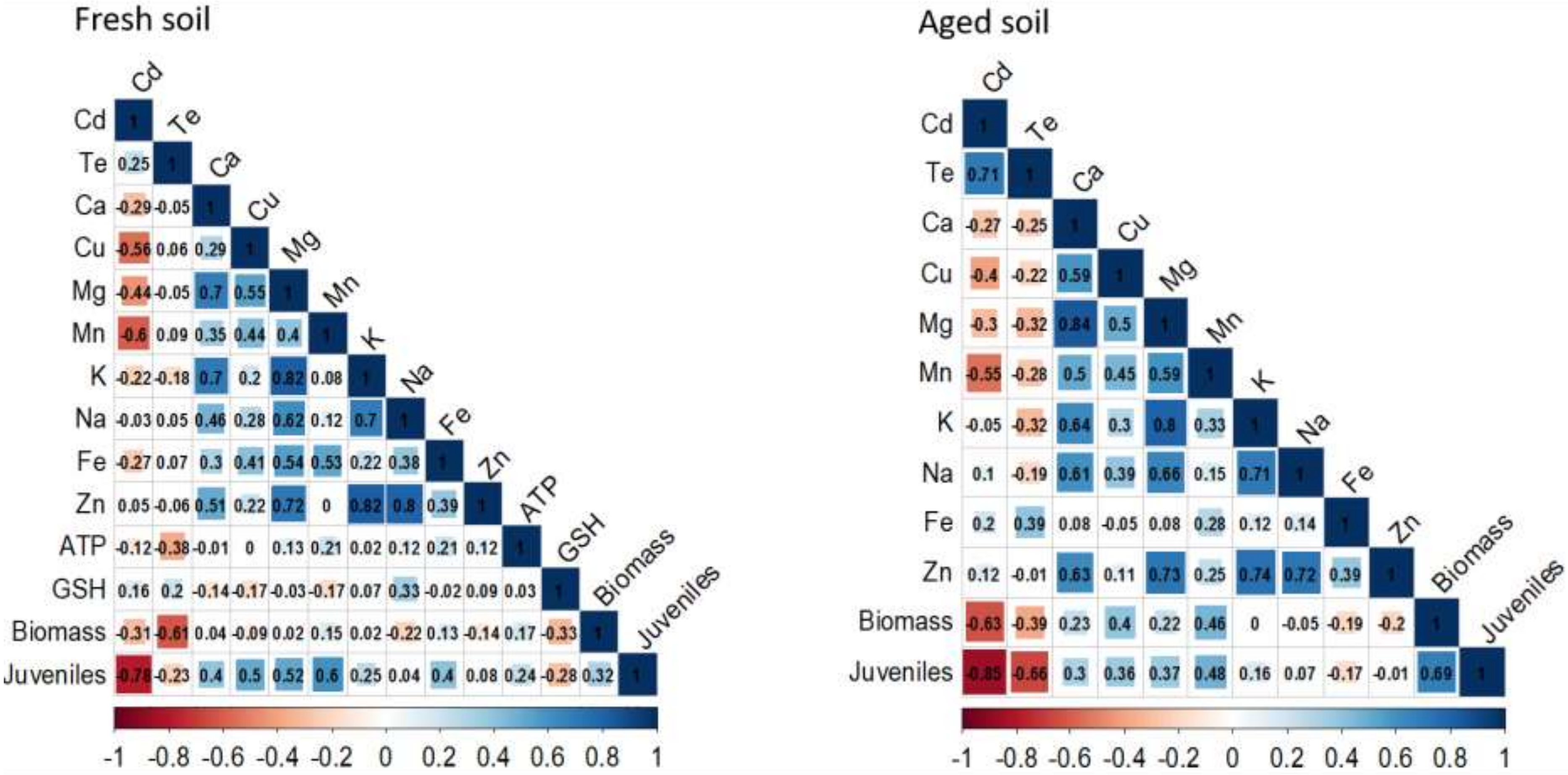

Fig. S5 Correlation matrices based on Spearman's rank analyses of all data together for electrolytes or metals in earthworm tissue, biochemistry, and reproductive endpoints within fresh (left) or aged (right) soil experiments. ATP = sodium pump activity; GSH = total glutathione in earthworm tissue.

Table S1 Soil pH in the beginning and end of the fresh and aged CdTe QD experiment.

Soil [CdTe], nominal mg CdTe kg^{-1} dw	Time (days)	Control	CdTe-bulk	CdTe-PEG	CdTe-COOH	CdTe- NH_4^+
				pH		
Fresh soil						
50	0	5.59 ± 0.04^{ab}	5.61 ± 0.05^{ab}	5.52 ± 0.01^{bc}	5.53 ± 0.02^{abc}	5.38 ± 0.05^{cfg}
500	0		5.28 ± 0.06^{fg}	5.89 ± 0.03^{d}	5.36 ± 0.02^{cfg}	5.71 ± 0.08^{ae}
2000	0		5.87 ± 0.09^{de}	5.52 ± 0.04^{bc}	5.21 ± 0.04^{g}	5.44 ± 0.05^{bcf}
50	28	5.52 ± 0.01^{ab}	5.39 ± 0.02^{b}	5.78 ± 0.1^{ac}	5.57 ± 0.02^{a}	5.51 ± 0.01^{ab}
500	28		5.39 ± 0.01^{b}	5.51 ± 0.02^{ab}	5.60 ± 0.01^{ab}	5.48 ± 0.02^{ab}
2000	28		5.70 ± 0.01^{a}	5.76 ± 0.01^{a}	5.70 ± 0.01^{a}	5.78 ± 0.02^{a}
Aged soil						
50	0	5.50 ± 0.03^{abc}	5.38 ± 0.01^{c}	5.61 ± 0.14^{abc}	5.60 ± 0.03^{abc}	5.44 ± 0.06^{bc}
500	0		5.42 ± 0.04^{bc}	5.71 ± 0.13^{a}	5.63 ± 0.03^{ab}	5.70 ± 0.1^{a}
2000	0		5.64 ± 0.05^{ab}	5.70 ± 0.01^{a}	5.59 ± 0.05^{abc}	5.49 ± 0.02^{abc}
50	28	5.53 ± 0.06^{ab}	5.38 ± 0.04^{a}	5.60 ± 0.22^{ab}	5.61 ± 0.03^{ab}	5.41 ± 0.05^{ac}
500	28		5.48 ± 0.04^{ab}	5.55 ± 0.13^{ab}	5.58 ± 0.06^{ab}	5.56 ± 0.03^{ab}
2000	28		5.56 ± 0.04^{ab}	5.69 ± 0.04^{b}	5.65 ± 0.03^{bc}	5.57 ± 0.04^{ab}

Data presented as mean ± SEM (n = 4). Values that do not share a letter are statistically significantly different across treatments within each time point and test concentration ($P < 0.05$, Kruskal-Wallis, Dunn's test). There were no statistically significant differences between the control soils and the treatments in the beginning and the end of the aged soil experiment ($P > 0.05$, Kruskal-Wallis, Dunn's test). The aged soil pH, both at the beginning and the end of the experiment, did not statistically significantly differ from the pH in the fresh soil experiment ($P > 0.05$, Student's t-test).

Table S2 Total concentration of electrolytes in CdTe-bulk or CdTe QD ENM exposed earthworms in the fresh and aged soil experiments after 28 days of exposure.

Soil [CdTe]		Measured concentration (mg kg^{-1} dw)				
Nominal, mg Cu kg^{-1} dw	Electrolytes	Control	CdTe-bulk	CdTe-PEG	CdTe-COOH	CdTe-NH_4^+
Fresh soil, day 28						
50	Na	5001 ± 289[bcd]	5077 ± 264[abcd]	5130 256[abcd]	4932 ± 218[cd]	4334 ± 314[cd]
	K	8166 ± 386[ab]	8060 ± 491[abc]	8434 ± 482[abcd]	7815 ± 350[ab]	7115 554[abcd]
	Ca	4020 ± 327[a]	3839 ± 220[a]	4266 ± 351[a]	4215 ± 150[a]	3689 ± 287[ab]
	Mg	751 ± 35[ab]	737 ± 30[ab]	741 ± 38[ab]	718 ± 33[ab]	672 ± 41[abc]
	Fe	554 ± 85[a]	624 ± 80[a]	329 ± 47[ab]	468 ± 85[ab]	662 ± 113[a]
	Mn	43 ± 6[ab]	49 ± 7[ab]	27 ± 5[bc]	34 ± 4[abc]	52 ± 5[ab]
	Zn	98 ± 5[bcd]	98 ± 4[bcd]	104 ± 5[bcd]	102 ± 6[bcd]	95 ± 6[bcd]
	Cu	10 ± 0[ab]	11 ± 0[a]	8 ± 0[abc]	8 ± 0[bc]	8 ± 0[bc]
500	Na		5628 ± 342[abcd]	5211 ± 362[abcd]	4129 ± 207[d]	3503 ± 432[cd]
	K		8296 ± 419[ab]	8495 ± 451[ab]	6632 ± 259[bcd]	5990 ± 494[cd]
	Ca		4262 ± 244[a]	4542 ± 267[a]	3753 ± 308[ab]	3328 ± 325a[b]
	Mg		789 ± 42[a]	754 ± 38[ab]	593 ± 34[bcd]	485 ± 32[d]
	Fe		592 ± 68[a]	652 ± 120[a]	485 ± 73[ab]	246 ± 24[b]
	Mn		56 ± 6[a]	45 ± 6[ab]	37 ± 5[abc]	13 ± 3[c]

	Zn		111 ± 5ab^{c}	108 ± 3^{bc}	83 ± 3^{d}	80 ± 5^{cd}
	Cu		11 ± 0^{ab}	9 ± 0a^{bc}	5 ± 0^{c}	6 ± 0^{c}
2000	Na		5416 ± 207^{abc}	7533 ± 479^{a}	6640 ± 335^{ab}	4630 ± 691^{abcd}
	K		8648 ± 193^{ab}	8909 ± 547^{a}	8757 ± 196^{ab}	5806 ± 715^{d}
	Ca		4276 ± 168^{a}	4602 ± 384^{a}	4026 ± 264^{a}	2388 ± 349^{b}
	Mg		806 ± 20^{a}	762 ± 36^{ab}	747 ± 32^{ab}	529 ± 56^{cd}
	Fe		572 ± 97^{a}	653 ± 123^{a}	586 ± 60^{a}	374 ± 63^{ab}
	Mn		44 ± 5^{ab}	14 ± 2^{c}	15 ± 3^{c}	14 ± 4^{c}
	Zn		108 ± 2^{b}	130 ± 5^{ab}	135 ± 3^{a}	91 ± 11^{abcd}
	Cu		10 ± 0^{ab}	8 ± 0^{abc}	6 ± 0^{c}	9 ± 1^{abc}
Aged soil, day 28						
50	Na	6176 ± 435	7281 ± 297 *	7344 ± 413 *	6227 ± 776	5569 ± 556
	K	8646 ± 527^{ab}	10937 ± 719^{a} *	9445 ± 185^{ab}	9889 ± 692^{ab}	8523 ± 791^{ab}
	Ca	3413 ± 203^{abc}	4751 ± 244^{ab} *	5105 ± 447^{a}	4238 ± 203^{bcd}	3801 ± 455^{bcd}
	Mg	915 ± 61^{ab}	1153 65^{a} *	1141 44^{a} *	961 49^{ab} *	887 89^{ab}
	Fe	432 ± 54	440 ± 78	672 ± 76 *	565 ± 104	463 ± 78
	Mn	54 ± 8^{ab}	65 ± 8^{a}	68 ± 9^{a} *	59 ± 8^{ab}	50 ± 4^{ab}
	Zn	114 ± 7	144 ± 5 *	143 ± 3 *	124 ± 10	124 ± 13
	Cu	10 ± 1^{a}	9 ± 0^{a}	10 ± 0^{a}	4 ± 0b^{cd} *	4 ± 0^{bcd} *
500	Na		6463 ± 283	8127 ± 531 *	5880 ± 371 *	6290 ± 639 *
	K		8788 ± 379^{ab}	9817 ± 316^{ab}	9639 ± 600^{ab} *	10593 ± 1113^{ab} *
	Ca		4436 ± 218^{abc}	4563 ± 460^{ab}	4197 ± 337^{abc}	3999 ± 443^{abc}

	Mg	969 ± 31^{ab} *	1028 ± 50^{ab} *	921 ± 56^{ab} *	985 ± 107^{ab} *
	Fe	526 ± 92	359 ± 36	540 ± 67	579 ± 85 *
	Mn	55 ± 7^{ab}	43 ± 5^{ab}	53 ± 10^{ab}	45 ± 11^{ab}
	Zn	126 ± 4	129 ± 5 *	139 ± 10 *	144 ± 13 *
	Cu	7 ± 0^{ab} *	6 ± 0^{abc}	3 ± 0^{cd} *	4 ± 0^{bcd}
2000	Na	6053 ± 668	7591 ± 645	6694 ± 747	6969 ± 470 *
	K	7570 ± 705^{b}	8107 ± 646^{ab}	9381 ± 713^{ab}	9944 ± 457^{ab} *
	Ca	3249 ± 217^{bc} *	3378 ± 551^{abc}	3130 ± 316^{bc} *	2632 ± 263^{c}
	Mg	741 ± 66^{b}	836 ± 69^{ab}	844 ± 60^{b}	845 ± 37^{b} *
	Fe	437 ± 34	558 ± 64	533 ± 64	578 ± 42 *
	Mn	24 ± 5^{b} *	28 ± 8^{ab}	19 ± 4^{c}	18 ± 3^{c}
	Zn	109 ± 7	135 ± 9	137 ± 6	141 ± 3 *
	Cu	4 ± 0^{bc} *	5 ± 1^{abcd}	2 ± 0^{d} *	3 ± 0^{bcd} *

Data expressed as mean ± SEM (*n* = 8) mg element kg^{-1} dw and is rounded to the nearest mg. Different letters denote statistically significant differences for each element within exposure concentration and experiment ($P < 0.05$, ANOVA). Asterisk (*) denotes statistically significant difference between measured concentrations of a given element in earthworms from the fresh and aged soil ($P < 0.05$, T-test, unpaired), labelled on the aged soil data.