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

Supplementary Material

Determination of the bioaccessible fraction of cupric oxide nanoparticles in soils using an *in vitro* human digestibility simulation.

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Supplemental Table S1 Chemical components of the synthetic human digestive fluids: saliva, gastric, duodenal and bile, as sourced from INERIS[23].

	Reagents (Supplier)	Saliva fluid	Gastric fluid	Duodenal fluid	Bile fluid	Volume (ml)
Inorganic	KCl, CAS 7447-40-7 (Sigma-Aldrich P3911-1KG, Lot 096K0040)	448 mg	412 mg	282 mg	188 mg	250
	$NaH_2PO_4.H_2O$, CAS 10049-21-5 (BDH Laboratory Supplies, Lot A894021)	444 mg	133 mg	-	-	
	KSCN, CAS 333-20-0 (Sigma-Aldrich P2713-250G, Lot BCBQ3455V)	100 mg	-	-	-	
	Na_2SO_4, CAS 7757-82-6 (Sigma S9627-500G, Lot 068K0119)	285 mg	-	-	-	
	NaCl, CAS 7647-14-5 (Sigma-Aldrich 71380-500G, Lot SZBD2810V)	149 mg	1376 mg	3506 mg	2630 mg	
	$CaCl_2.2H_2O$, CAS 10035-04-8 (Sigma-Aldrich 223506-500G, Lot BCBK4048V)	-	200 mg	-	-	
	NH_4Cl, CAS 12125-02-9 (Fisher Scientific UK A/3920/53, Lot 1479160)	-	153 mg	-	-	
	$NaHCO_3$, CAS 144-55-8 (Sigma S5761-500G, Lot 088K0144)	-	-	2803.5 mg	2893 mg	
	KH_2PO_4, CAS 7778-77-0 (Fisher Scientific UK P/4800150, Lot 1671699)	-	-	40 mg	-	
	$MgCl_2.6H_2O$, CAS 7791-18-6 (Sigma-Aldrich M2670-500G, Lot 026K01421)	-	-	25 mg	-	
	NaOH 1M, CAS 1310-73-2 (Sigma-Aldrich 06203-1KG, Lot SZBC1660V)	0.9 ml	-	-	-	
	HCl 37%, CAS 7647-01-0 (Fisher Scientific UK, Lot 1554937)	-	4.15 ml	90 µl	90 µl	
Organic	Urea, CAS 57-13-6 (Acros Organics 140750010, Lot A0365884)	100 mg	42.5 mg	50 mg	125 mg	250
	D-(+)-Glucose, CAS 50-99-7 (Sigma-Aldrich G7528-1KG, Lot SLBK8673V)	-	325 mg	-	-	
	D-Glucuronic acid, CAS 6556-12-3 (Sigma-Aldrich G5269-10G, Lot SLBJ7931V)	-	10 mg	-	-	
	D-(+)-Glucosamine hydrochloride, CAS 66-84-2 (Sigma-Aldrich G4875-25G, Lot BCBK8296V)	-	165 mg	-	-	
Enzymes	Alpha amylase, CAS 9000-90-2 (Sigma-Aldrich A6814-1MU)	72.5 mg	-	-	-	250 + 250 = 500
	Mucin, CAS 84082-64-4 (Sigma-Aldrich M2378-100G, Lot SLBH9969V)	25 mg	1500 mg	-	-	
	Uric acid, CAS 69-93-2 (Sigma-Aldrich U2625-25G, Lot BCBM8832V)	7.5 mg	-	-	-	
	Bovine Serum Albumin, CAS 90604-29-8 (Sigma-Aldrich A3912-10G, Lot SLBM6041V)	-	500 mg	500 mg	900 mg	
	Pepsin, CAS 9001-75-6 (Acros Organics 417070200, Lot A0366774)	-	500 mg	-	-	
	$CaCl_2.2H_2O$, CAS 10035-04-8 (Sigma-Aldrich 223506-500G, Lot BCBK4048V)	-	-	100 mg	111 mg	
	Pancreatin, CAS 8049-47-6 (Sigma-Aldrich P3292-25G, Lot SLBJ0255V)	-	-	1500 mg	-	
	Lipase, CAS 9001-62-1 (Sigma-Aldrich L3126-25G, Lot SLBJ1166V)	-	-	250 mg	-	
	Bile bovine, CAS 8008-63-7 (Sigma-Aldrich B3883-25G, Lot MKBQ6668V)	-	-	-	3000 mg	
pH	Inorganic + organic + enzymes	6.5 ± 0.5	1.1 ± 0.1	7.4 ± 0.2	8 ± 0.2	

Supplemental Table S2 Total copper concentration measurements in soil at day 14 in the earthworm tests.

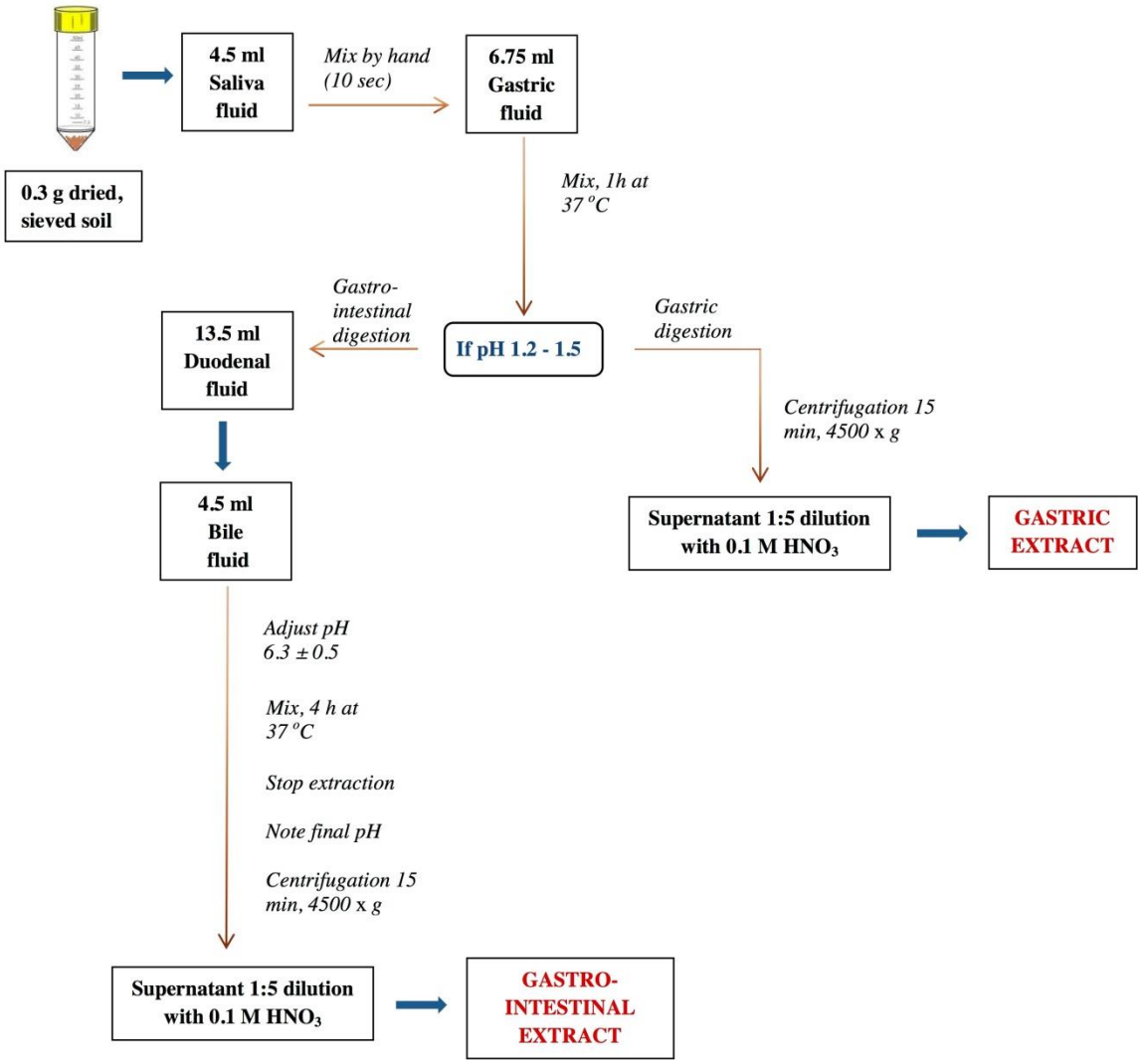
Soil exposure	Nominal soil copper concentration (mg Cu kg^{-1} dry soil)	[1]Total measured copper concentration (mg Cu kg^{-1} dry soil)				[2]Mean ± SD	[2]S.E.M.	[3]Between-replicate box CV (%)
		Soil box 1	Soil box 2	Soil box 3	Soil box 4			
[$,^]BGS 102 soil	*26.0 ± 1.5	16.52	16.52	16.18	17.54	16.69 ± 0.59	0.29	3.53
[$]LUFA 2.2 soil	**1.37 ± 0.2	3.60	2.99	2.47	2.79	2.96 ± 0.47	0.24	16.00
$CuSO_4$	200	183.82	197.66	179.55	163.64	181.17 ± 14.01	7.01	7.73
CuO Bulk	200	186.24	164.99	184.40	233.61	192.31 ± 29.16	14.58	15.16
	1000	1124.50	960.81	1004.15	975.65	1016.28 ± 74.36	37.18	7.32
Uncoated CuO NPs	200	154.54	159.26	159.43	145.66	154.72 ± 6.45	3.23	4.17
	1000	855.16	820.49	883.08	838.64	849.34 ± 26.57	13.29	3.13
CuO NPs COOH-coated	200	74.29	104.15	79.94	91.78	87.54 ± 13.25	6.63	15.14
	1000	470.10	366.40	534.38	446.75	454.41 ± 69.40	34.70	15.27
CuO NPs NH_4^+-coated	200	138.06	105.25	149.97	124.64	129.48 ± 19.18	9.59	14.82
	1000	574.60	520.38	574.76	669.94	584.92 ± 62.19	31.10	10.63
CuO NPs PEG-coated	200	63.96	90.82	81.37	67.67	75.95 ± 12.42	6.21	16.35
	1000	202.01	372.15	354.28	346.30	318.69 ± 78.53	39.27	24.64

[1] Data are means (n = 2 technical replicates *per* soil box) of total measured metal concentration by ICP-OES, following *aqua regia* acid digestion; [2] Data are means ± standard deviation (SD) and standard error of mean (S.E.M.) determined from the four separate soil boxes *per* treatment; [3] Between-replicate box coefficient of variation (CV), expressed as a percentage, refers to the total procedural variation from the soil acid digestion *per* treatment; [$] Control soil boxes, with no copper soil dosing; [^] BGS 102 soil was not used in the earthworm tests, but solely included to validate the analytical chemistry; * Certificate of analysis from Wragg[28]; ** Measured total copper concentration in fresh soil (day zero) in the earthworm tests from Tatsi *et al.*[24]

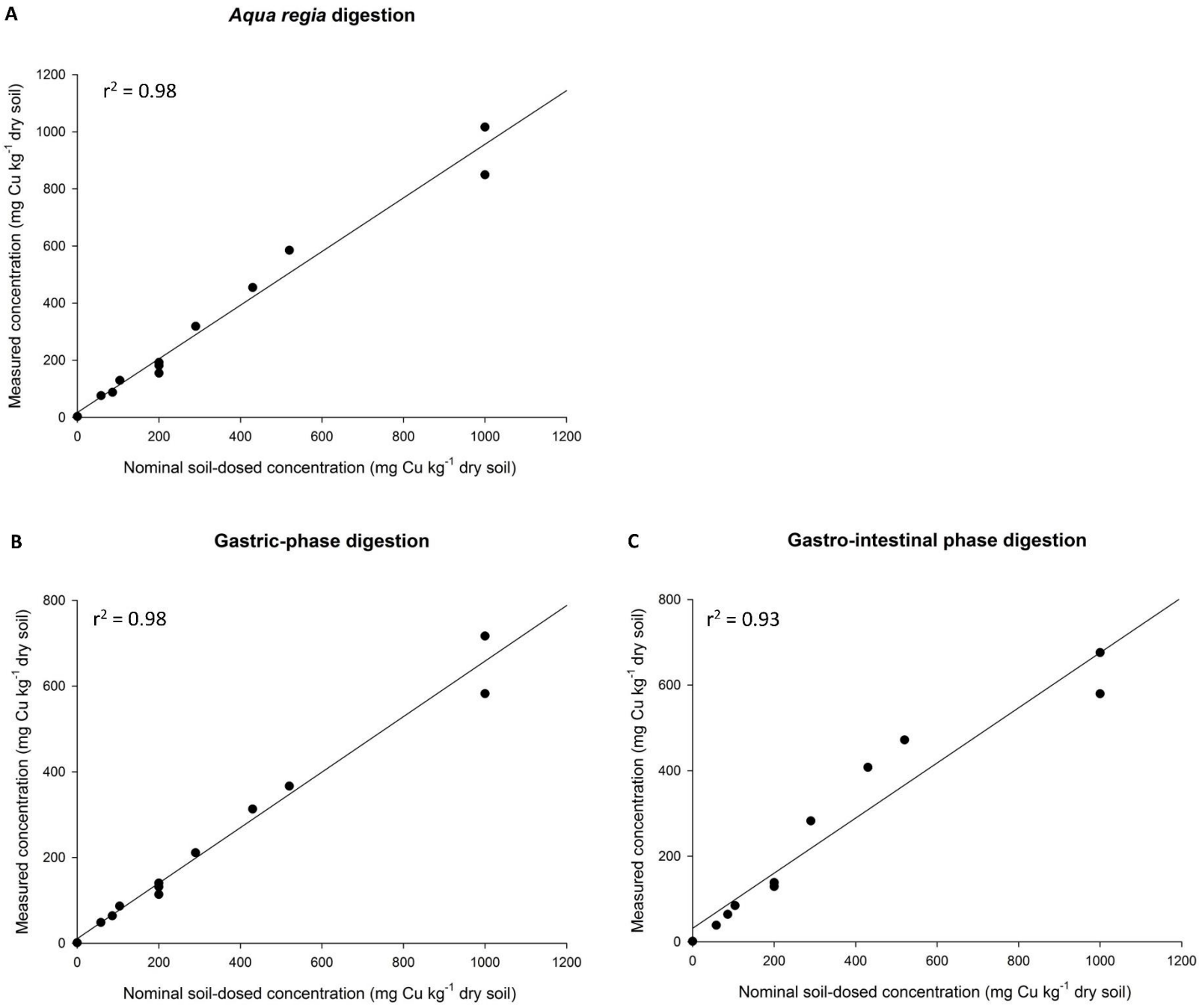
Supplemental Table S3 Calculated bioaccessible fractions.

Soil exposure	Nominal soil copper concentration (mg Cu kg^{-1} dry soil)	[1]Gastric phase BAF (%)	[2]CV (%)	[1]Gastro-intestinal phase BAF (%)	[2]CV (%)
[$,^]BGS 102 soil	*26.0 ± 1.5	35.3 ± 1.6	9.9	40.2 ± 0.9	4.8
[$]LUFA 2.2 soil	**1.37 ± 0.2	38.8 ± 6.4	33.2	31.9 ± 6.6	41.3
$CuSO_4$	200	73.2 ± 4.9	13.3	76.6 ± 3.3	8.5
CuO Bulk	200	74.1 ± 9.7	26.1	72.7 ± 7.3	20.1
	1000	70.5 ± 2.5	7	66.8 ± 2.4	7.3
Uncoated CuO NPs	200	73.6 ± 3.4	9.4	83.4 ± 1.5	3.6
	1000	68.6 ± 1.9	5.4	68.2 ± 3.9	11.3
CuO NPs COOH-coated	200	73.5 ± 3.6	9.8	73.4 ± 4.0	11
	1000	69.2 ± 2.0	5.8	90.2 ± 3.0	6.6
CuO NPs NH_4^+-coated	200	66.7 ± 2.1	6.4	65.4 ± 2.2	6.8
	1000	63.2 ± 3.3	10.5	81.0 ± 3.0	7.4
CuO NPs PEG-coated	200	62.7 ± 4.4	13.9	49.4 ± 6.6	26.7
	1000	67.2 ± 2.6	7.8	89.5 ± 2.9	6.5

[1] Bioaccessible fraction (BAF) calculated as a percentage from *n* = 2 technical soil samples *per* box, and data then presented as means ± S.E.M percentage BAF from *n* = 4 separate boxes *per* treatment or control; [2] Percentage coefficient of variation (CV) amongst the BAF values determined from the separate soil boxes (*n* = 4) *per* treatment or control; [$] Control soil boxes, with no copper soil dosing; [^] BGS 102 soil was not used in the earthworm tests, but solely included to validate the analytical chemistry; * Certificate of analysis from Wragg[28]; ** Measured total copper concentration in fresh soil (day zero) in the earthworm tests from Tatsi *et al.*[24]



Supplemental Fig. S1 Step-wise depiction of the validated Bioaccessibility Research Group of Europe (BARGE), Unified Bioaccessibility Method (UBM), as adapted from INERIS.[23]



Supplemental Fig. S2 Relationship for the different test materials between their nominal initial soil dosing copper concentration in mg Cu kg^{-1} dry soil (*x-axis*) against mean total measured copper concentration (n = 4 replicate soil boxes *per* treatment) in mg Cu kg^{-1} dry soil (*y-axis*) following (A) *aqua regia* acid digestion (B) gastric digestion and (C) gastro-intestinal digestion.