

Supplemental Information

Table S1. Analytical methods used to determine selected characteristics of the three reference soils.

Property	Detail	Reference
pH	1:5 (w/v) soil suspension using 0.01 M CaCl ₂ .	[1]
CEC	Cobalt hexamine method, using 0.0166 M cobalt(III) hexamine chloride ([Co(NH ₃) ₆]Cl ₃) as extracting solution at a 1:20 soil:liquid ratio (w/v).	[2]
Fe-ox	Oxalic acid-oxalate extracts of 1:50 (w/v) extraction systems, at buffered pH ≈ 3.	[3]
TC	Total carbon content of the soils was determined by dry combustion using a CN analyzer (VarioMax).	[4]
Inorganic carbon	CO ₂ preasure after the addition of a HCl:FeSO ₄ mixture to the soils sample in a sealed container	[5]
Total metal concentrations	Hot-acid digestion of 50 mg (dry mass) of each sample using a mixture of 1:3 HNO ₃ to HCl concentrated acids	[1]

Table S2. MixTox–model parameters corresponding to different deviation patterns. Associated deviation function and practical meaning of their values are included. Adapted from Jonker et al., 2005.

* $x=1$ for CA; $x=2$ for IA.

Deviations	Parameter	CA G function	IA G function	Value	Interpretation
Synergism or Antagonism	a^*	$G = e^{az_iz_j}$	$G = az_iz_j$	>0	Antagonism
				<0	Synergism
Dose Ratio related (DR)	b_i	$G_i = e^{(a+bz_i)z_iz_j}$	$G_i = (a + bz_i)z_iz_j$	>0	Antagonism with effects mainly caused by i
				<0	Synergism with effects mainly caused by i
Dose Level related (DL)	b_j	$G_i = e^{(a+bz_j)z_iz_j}$	$G_j = (a + bz_j)z_iz_j$	>0	Antagonism with effects mainly caused by j
				<0	Synergism with effects mainly caused by j
	a	$G = e^{az_iz_j}$	$G = az_iz_j$	>0	Low dose level: Antagonism
				<0	Low dose level: Synergism
	b^*	$G = e^{a(1-b(q_i+q_j))z_iz_j}$	$G = a(1 - b(1 - q_i q_j)) z_iz_j$	>x*	Inflexion point < EC50
				=x	Inflexion point = EC50
				x>b>x-1	Inflexion point > EC50
				<x-1	Magnitude of deviation is dose or effect level dependent

* z_i : relative toxic unit of component i (Equation (6)); * q_i : individual toxic effect of component i in the mixture (Equation (7)).

Table S3. Zinc and arsenic averaged total concentrations (mg kg^{-1}) for all treatments and all soils. Standard errors (SE) are given between brackets. Bold values correspond to the variable component and treatment standard error (SE, mg kg^{-1}) are between brackets. Dose levels are those of Figure 1.

		Single Zn		Single As		Fixed Zn + Increasing As		Fixed As + Increasing Zn		Ray	
Soil	Dose Level	Zn	As	Zn	As	Zn	As	Zn	As	Zn	As
		mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}				
S1	0	1400 (6)	240 (23)	1400(6)	240 (23)	1600 (31)	300 (5)	1400 (40)	530 (15)	1400 (6)	240 (23)
	1	1400 (37)	170 (25)	1400 (13)	380 (14)	1600 (29)	350 (21)	1300 (29)	420 (47)	1400 (7)	370 (2)
	2	1500 (28)	300 (28)	1300 (35)	400 (17)	1600 (50)	400 (18)	1400 (65)	360 (21)	1400 (18)	420 (2)
	3	1600 (31)	300 (5)	1400 (40)	530 (15)	1800 (158)	430 (23)	1800 (158)	430 (29)	1800 (160)	430 (23)
	4	2100 (28)	250 (35)	1500 (57)	604 (4)	1600 (21)	620 (68)	2200 (83)	400 (29)	2600 (29)	670 (150)
	5	3700 (3)	300 (51)	1400 (30)	850 (41)	1700 (14)	1000 (13)	3600(25)	400 (54)	4400 (129)	1500 (240)
S2	6	8700 (130)	300 (19)	1400 (61)	1300(200)	1700 (120)	1700 (10)	8500 (76)	430 (200)	9700 (0.5)	3300 (270)
	0	87(3)	22 (2)	87 (3)	22 (2)	360 (22)	39 (4)	87 (2)	80 (5)	87 (3)	22 (2)
	1	130 (10)	45 (8)	87 (2)	52 (0.5)	290 (5)	50 (1)	110 (0.1)	80 (3)	150 (9)	100 (3)
	2	170 (2)	48 (5)	85 (1)	55 (0.4)	280 (2)	64 (6)	170 (6)	47 (21)	230 (16)	200 (13)
	3	360 (22)	39 (12)	87 (2)	80 (5)	n.d.	n.d.	n.d.	n.d.	500 (12)	510 (30)
	4	670 (14)	60 (7)	96 (12)	150 (8)	270 (3)	100 (4)	1000 (122)	51 (16)	1300 (111)	1500 (120)
S3	5	2100 (99)	40 (4)	95 (1)	250 (6)	360 (15)	120 (1)	1700 (31)	46 (4)	3800 (3)	4600 (75)
	6	6800 (39)	59 (3)	98 (3)	470 (3)	320 (27)	370 (47)	6200 (95)	52 (10)	9600 (44)	13000 (400)
	0	350 (8)	72 (2)	350 (8)	72 (2)	700 (46)	73 (3)	320 (2)	130 (0.1)	350 (8)	72 (2)
	1	420 (25)	97 (22)	300 (3)	81 (21)	630 (23)	61 (2)	380 (5)	130 (7)	390 (1)	130 (3)
	2	450 (26)	83 (15)	300 (21)	95 (6)	710 (38)	82 (10)	420 (3)	140 (1)	450 (48)	130 (6)
	3	700 (46)	73 (3)	320 (2)	130 (0.1)	n.d.	n.d.	n.d.	n.d.	730 (21)	360 (27)
S4	4	1300 (2)	86 (4)	280 (9)	180 (7)	670 (31)	98 (2)	1200 (30)	120 (3)	1500 (25)	810 (5)
	5	3400 (7)	81 (5)	340 (18)	470 (35)	610 (19)	200 (2)	3100 (85)	140 (1)	3500 (70)	2200 (80)
	6	8300 (730)	130 (53)	320 (13)	1100 (25)	640 (31)	1100 (25)	7900 (44)	130 (3)	9800 (82)	7400 (170)

Table S4. Total Zn and As measured concentrations (mg kg^{-1}) and corresponding Net Root Elongation (NRE, cm) response for all treatments. Reported NRE values are treatment averaged values with associated standard deviations (cm) between brackets.

SOIL	Single Zn		Single As		Fixed Zn + Increasing As			Zn + Fixed As			Ray		
	Zn (mg kg^{-1})	NRE (cm)	As (mg kg^{-1})	NRE (cm)	Zn (mg kg^{-1})	As (mg kg^{-1})	NRE (cm)	Zn (mg kg^{-1})	As (mg kg^{-1})	NRE (cm)	Zn (mg kg^{-1})	As (mg kg^{-1})	NRE (cm)
S1	1400	9.6 (1)	240	9.6 (1)	1600	300	8.6 (1)	1400	530	9.1 (1)	1400	240	9.6 (1)
	1400	10 (1)	380	9.3 (1)	1600	350	9.1 (1)	1300	420	9.9 (1)	1400	370	9.1 (1)
	1500	9.6 (1)	400	9.1 (1)	1600	400	9.4 (1)	1400	360	9.4 (1)	1400	420	9.0 (1)
	1600	8.6 (1)	530	9.1 (1)							1800	430	8.1 (2)
	2100	7.6 (1)	604	5.4 (2)	1600	620	7.2 (1)	2200	400	9.6 (1)	2600	670	1.9 (2)
	3700	5.7 (2)	850	1.4 (0.2)	1700	1000	1.8 (1)	3600	400	5.8 (1)	4400	1500	1.2 (1)
	8700	1.1 (0.2)	1300	1.0 (0.1)	1700	1700	0.9 (0.1)	8500	430	0.9 (0.1)	9700	3300	0.7 (0.1)
S2	87	11 (1)	22	11 (1)	360	39	10 (0.4)	87	80	8.6 (1)	87	22	11 (1)
	130	11 (1)	52	9.6 (1)	290	50	9.7 (1)	110	80	4.7 (1)	150	100	6.7 (1)
	170	9.8 (1)	55	9.5 (1)	280	64	9.4 (0.1)	170	47	8.3 (0.3)	230	200	3.9 (0.4)
	360	10 (0.4)	80	8.6 (1)							500	510	0.9 (0.1)
	670	10 (1)	150	2.5 (0.4)	270	100	4.9 (0.3)	1000	51	8.6 (0.1)	1300	1500	0.9 (0.1)
	2100	8.9 (1)	250	0.8 (0.4)	360	120	2.0 (0.3)	1700	46	8.6 (1)	3800	4600	0.7 (0.1)
	6800	0.9 (0.1)	470	0.9 (0.1)	320	370	0.9 (0.1)	6200	52	0.8 (0.3)	9600	13000	0.9 (0.1)
S3	350	9.1 (2)	72	9.1 (2)	700	73	8.3 (1)	320	130	1.0 (0.3)	350	72	9.1 (2)
	420	8.4 (1)	81	7.3 (1)	630	61	7.3 (1)	380	130	8.5 (1)	390	130	5.7 (1)
	450	7.9 (1)	95	4.3 (2)	710	82	5.2 (1)	420	140	7.4 (1)	450	130	6.1 (1)
	700	8.3 (1)	130	1.0 (0.3)							730	360	6.6 (0.3)
	1300	6.8 (1)	180	0.8 (0.3)	670	98	2.2 (1)	1200	120	9.1 (2)	1500	810	3.1 (2)
	3400	3.0 (1)	470	1.0 (0.2)	610	200	0.6 (0.4)	3100	140	5.5 (2)	3500	2200	1.3 (0.5)
	8300	0.8 (0.1)	1100	0.8 (0.1)	640	1100	0.6 (0.3)	7900	130	0.4 (0.5)	9800	7400	0.8 (0.1)

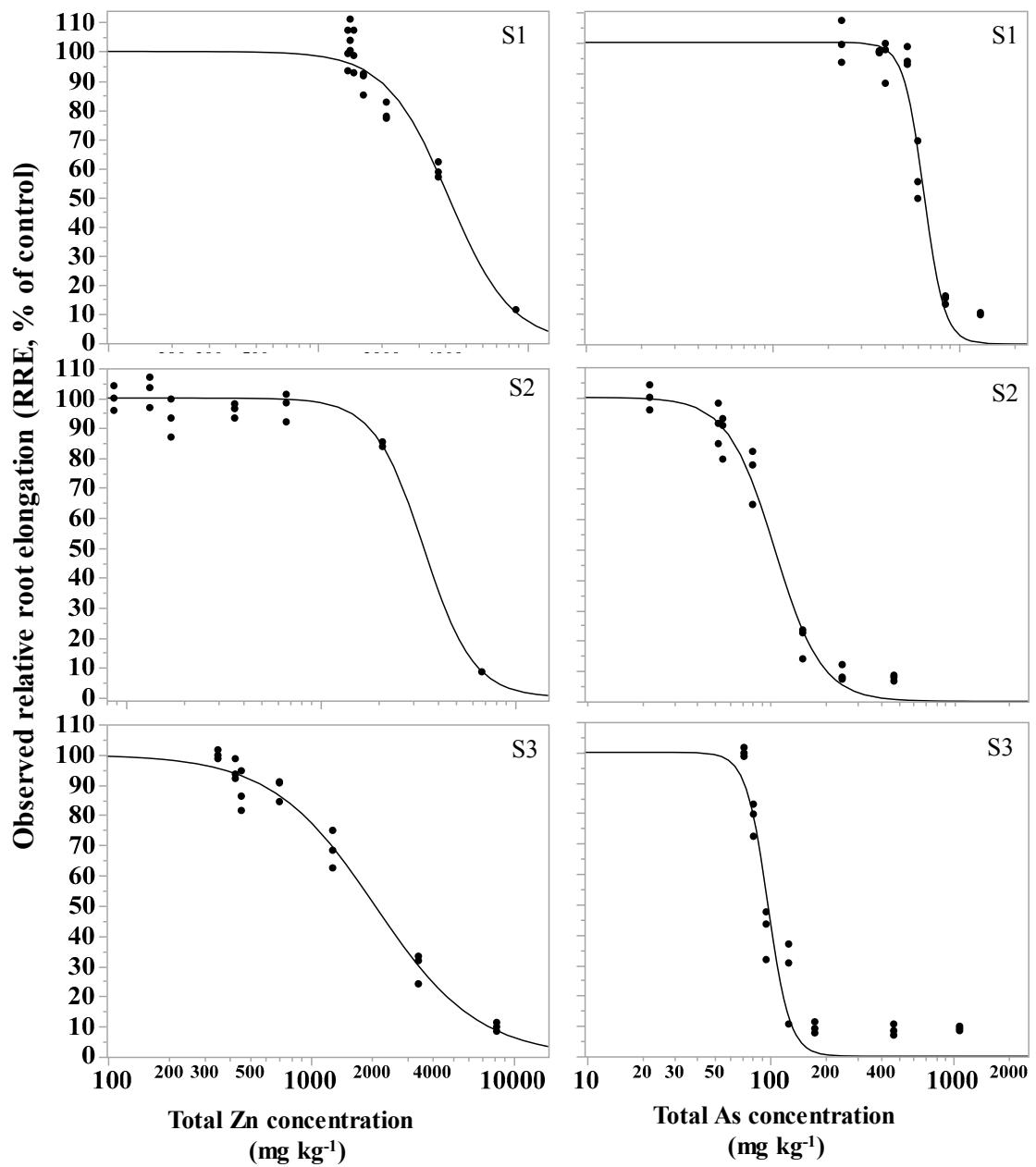


Figure S1. Dose–response curves for total zinc (left) and total arsenate (right) when applied singly to 3 different Mexican soils: S1, Zimapán; S2, Pozos; S3, Taxco. RRE, relative root elongation.

Table S5. Zinc and arsenic EC50 values using different concentration expressions.

Zn EC50						As EC50						
Total Zn Soil Concentration		Soil Solution Zn Concentration		Soil Solution Zn EC50 Referred to Soil Dry Mass ^a		Total As Soil Concentration		Soil Solution As Concentration		Soil Solution As EC50 Referred to Soil Dry Mass ^a		
mg kg ⁻¹	mmol kg ⁻¹	mg L ⁻¹	mmol L ⁻¹	mg kg ⁻¹	mmol kg ⁻¹	mg kg ⁻¹	mmol kg ⁻¹	mg L ⁻¹	mmol L ⁻¹	mg kg ⁻¹	mmol kg ⁻¹	
S1	4200	64	12	0.18	4	0.06	650	9	8	0.11	2.64	0.04
S2	3400	52	18	0.28	6	0.09	110	1	3	0.04	0.96	0.01
S3	2100	32	2	0.03	1	0.01	100	1	0.26	4E-03	0.08	1E-03

^a Considering a solid: liquid ratio of 25 g: 8mL.

Table S6. Zinc and arsenic concentrations ($\mu\text{mol L}^{-1}$) in the pore waters of selected single treatments for each experimental soil, dose levels are given in Table S2 and correspond to those of Figure 1. Standard errors (SE) are given in brackets.

Soil	Single Zn		Single As		
	Dose level	Zn	As	Zn	As
		$\mu\text{mol L}^{-1}$	$\mu\text{mol L}^{-1}$	$\mu\text{mol L}^{-1}$	$\mu\text{mol L}^{-1}$
S1	0	3 (1)	1.8 (0.2)	3 (1)	1.8 (0.2)
	2	5 (0.1)	1.2 (0.1)	5 (0.2)	5.8 (0.1)
	4	17 (0.4)	1.0 (0.1)	8 (1)	75 (4)
	6	3500 (140)	0.5 (<0.1)	11 (0.2)	4200 (120)
S2	0	3.1 (0.1)	0.6 (0.1)	3 (0.1)	0.5 (0.1)
	2	6.8 (0.1)	0.5 (0.1)	3 (<0.1)	8 (1)
	4	12 (1)	0.3 (<0.1)	2 (0.3)	123 (2)
	6	3000 (16)	0.1 (<0.1)	1 (0.3)	2400 (7)
S3	0	1.4 (0.1)	0.3 (<0.1)	1.4 (0.1)	0.3 (<0.1)
	2	6.7 (0.3)	0.3 (<0.1)	2.0 (0.2)	4.3 (0.1)
	4	16 (0.1)	0.3 (0.1)	1.4 (0.2)	79 (4)
	6	2400 (80)	0.4 (0.1)	0.8 (0.1)	5600 (80)

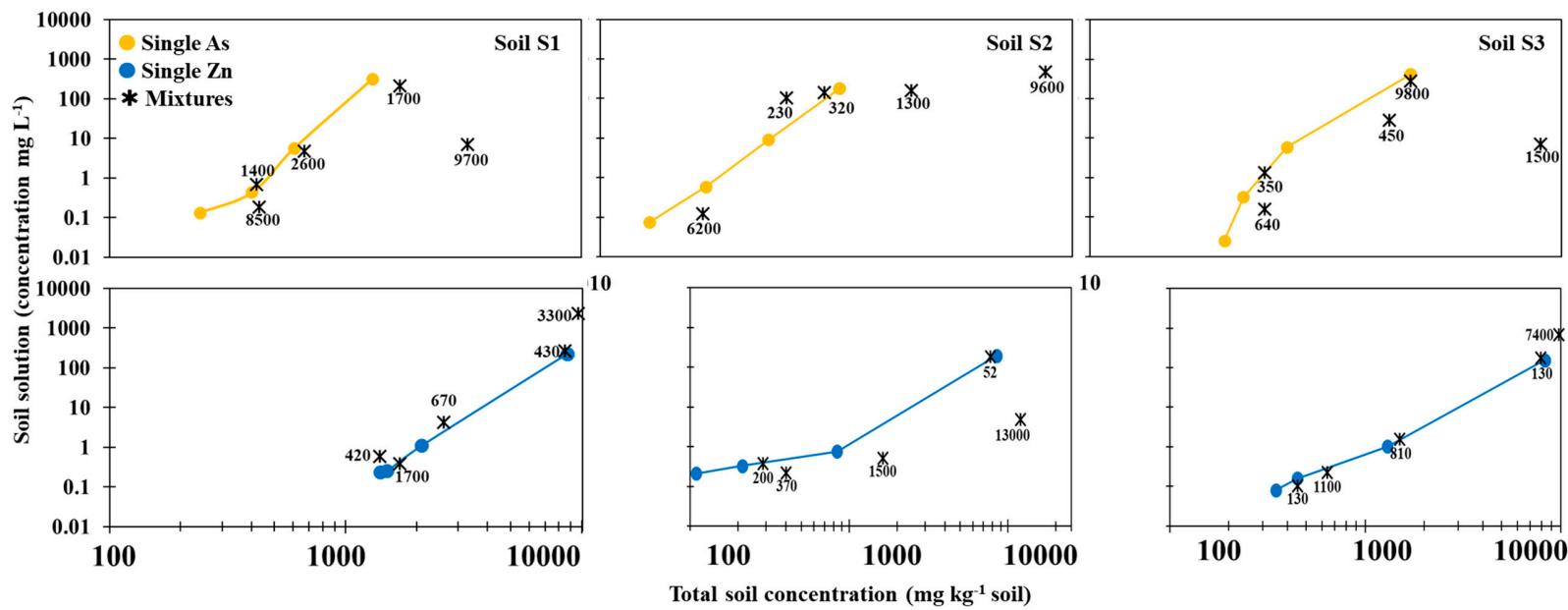


Figure S2. Soil solution concentration as a function of total soil concentration for As (top) and Zn (bottom) in single and mixture treatments. Data labels in the top panel present the Zn total concentration (mg Zn kg^{-1} soil) in each mixture. Data labels in the bottom panel present the As total concentration (mg As kg^{-1} soil) in each soil. Total and soil solution concentrations can be consulted in Table S7.

Table S7. Total (mg kg^{-1}) and soil solution (mg L^{-1}) concentrations of As and Zn for selected single (-) and mixture (+) treatments.

Soil	Dose level	As Concentration (Total: mg kg^{-1} ; Dissolved: mg L^{-1})				Zn Concentration (Total: mg kg^{-1} ; Dissolved: mg L^{-1})			
		Single Treatments (-Zn)		Mixture Treatments (+Zn)		Single Treatments (-As)		Mixture Treatments (+As)	
		Total	Dissolved	Total	Dissolved	Total	Dissolved	Total	Dissolved
S1	0	240	0.1	--	--	1400	0.2	--	--
	2	400	0.4	420	0.7	1500	0.3	1400	0.6
	4	604	5.6	670	5	2100	1	2600	4
	6	1300	311.8	3300	7	8700	226	9700	2293
	6	1300	311.8	1700	210	8700	226	1700	0.4
	3	--	--	430	0.2	--	--	8500	265
S2	0	22	0.1	--	--	87	0.2	--	--
	2	55	0.6	200	102	170	0.3	230	0.4
	4	150	9.2	1500	158	670	0.8	1300	0.5
	6	470	180.9	13000	462	6800	199	9600	5
	6	470	180.9	370	143	6800	199	320	0.2
	3	--	--	52	0.1	--	--	6200	185
S3	0	72	0.0	--	--	350	0.1	--	--
	2	95	0.3	130	1	450	0.2	450	0.1
	4	180	5.9	810	29	1300	1	1500	2
	6	1100	416.6	7400	7	8300	155	9800	689
	6	1100	416.6	1100	281	8300	155	640	0.2
	3	--	--	130	0.2	--	--	7900	175

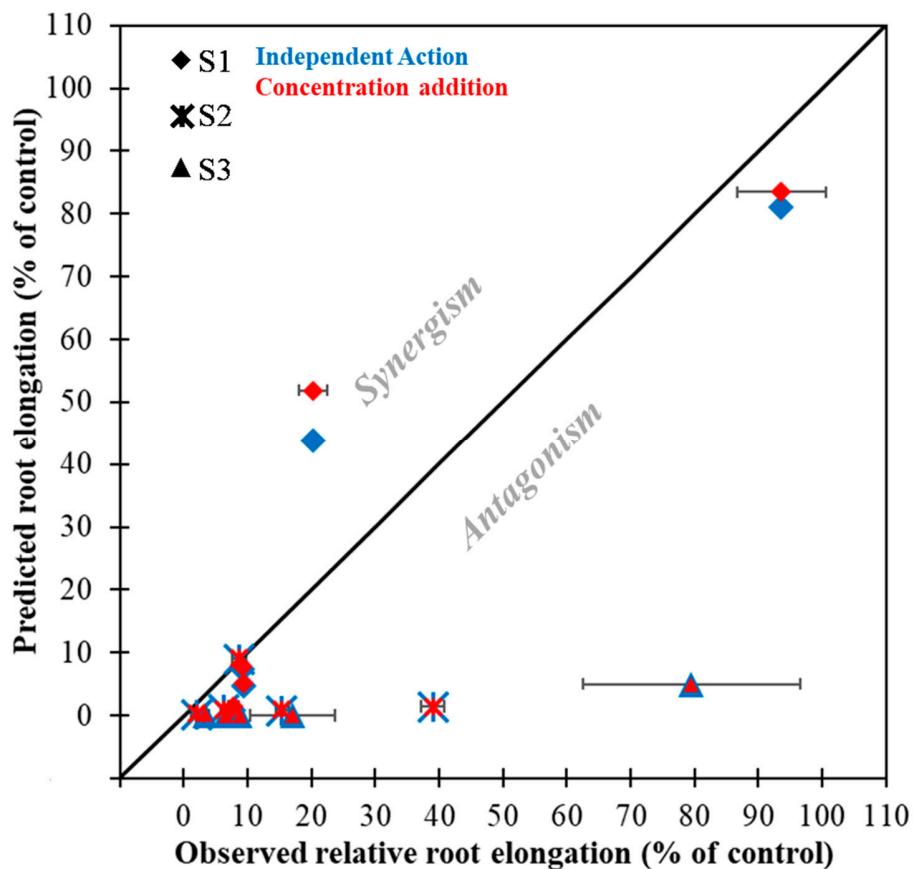


Figure S3. Predicted vs observed root relative elongation using soil solution Zn and Arsenic concentrations as dose expression. Predicted responses were obtained from CA (red) and IA (blue) reference models. Errors bars correspond to the 95% confidence interval (CI) for each treatment observation.

Table S8. Log transformed ion activity products (log IAP) of the solid species that could precipitate from those components with high soil solution concentration at highly dosed treatments. The IAP given in bold suggest saturation and potential precipitation. The solubility products (log Ksp) are given as reference.

Soil	Dose Level		Zn ($\mu\text{mol L}^{-1}$)	AsO ₄ ($\mu\text{mol L}^{-1}$)	Ca (mmol L^{-1})	Soil Solution pH	log IAP		
	Zn	As					As ₂ O _{5(s)}	Ca ₃ (AsO ₄) ₂ 4H ₂ O _(s)	Zn ₃ (AsO ₄) ₂ 2.5H ₂ O _(s)
S1	0	0	3.6	1.8	7.7	6.9	-64	-29	-39
	2	0	3.9	1.2	8.9	7.5	-64	-29	-39
	4	0	17	1	12		-65	-29	-38
	6	0	3449	0.5	137	7	-66	-28	-33
	0	2	4.5	6	6.5	7.6	-63	-29	-39
	0	4	0.6	75	6.6		-61	-26	-41
	0	6	9	4163	5.3	7.6	-57	-23	-31
	2	2	9	9	8.3	7.5	-63	-28	-36
	4	4	64	63	27		-61	-25	-33
S2	6	6	35056	93	111	6	-63	-26	-28
	0	0	3.3	1	4.4	6.8	-64	-30	-40
	2	0	5	0.5	4.3	7.5	-65	-31	-39
	4	0	12	0.5	8.9		-65	-30	-39
	6	0	3043	0.1	50	7.6	-67	-30	-34

S3	0	2	7.7	8	4.7	7.6	-63	-28	-37
	0	4	1.7	123	6.1	7.7	-60	-26	-36
	0	6	0.7	2415	4	7.8	-58	-24	-35
	2	2	6	1364	8.2	7.7	-58	-23	-33
	4	4	8	2103	10	7.4	-58	-23	-32
	6	6	75	6166	36	6.3	-57	-21	-29
	0	0	1.3	0.3	7.4	6.9	-66	-31	-42
	2	0	4.7	0.3	4.1	7.4	-65	-31	-40
	4	0	16	0.2	16		-66	-30	-39
	6	0	2362	0.4	103	7	-66	-28	-33
	0	2	1.2	4.2	5.8	7.6	-63	-28	-40
	0	4	1.2	79	4.9		-61	-26	-37
	0	6	1	5562	4.5	7.6	-57	-23	-34
	2	2	1.6	18	7.3	7.6	-62	-27	-38
	4	4	24	385	19		-59	-24	-32
	6	6	10530	4332	99	6.2	-61	-23	-28
log Ksp:						-35	-19	-28	

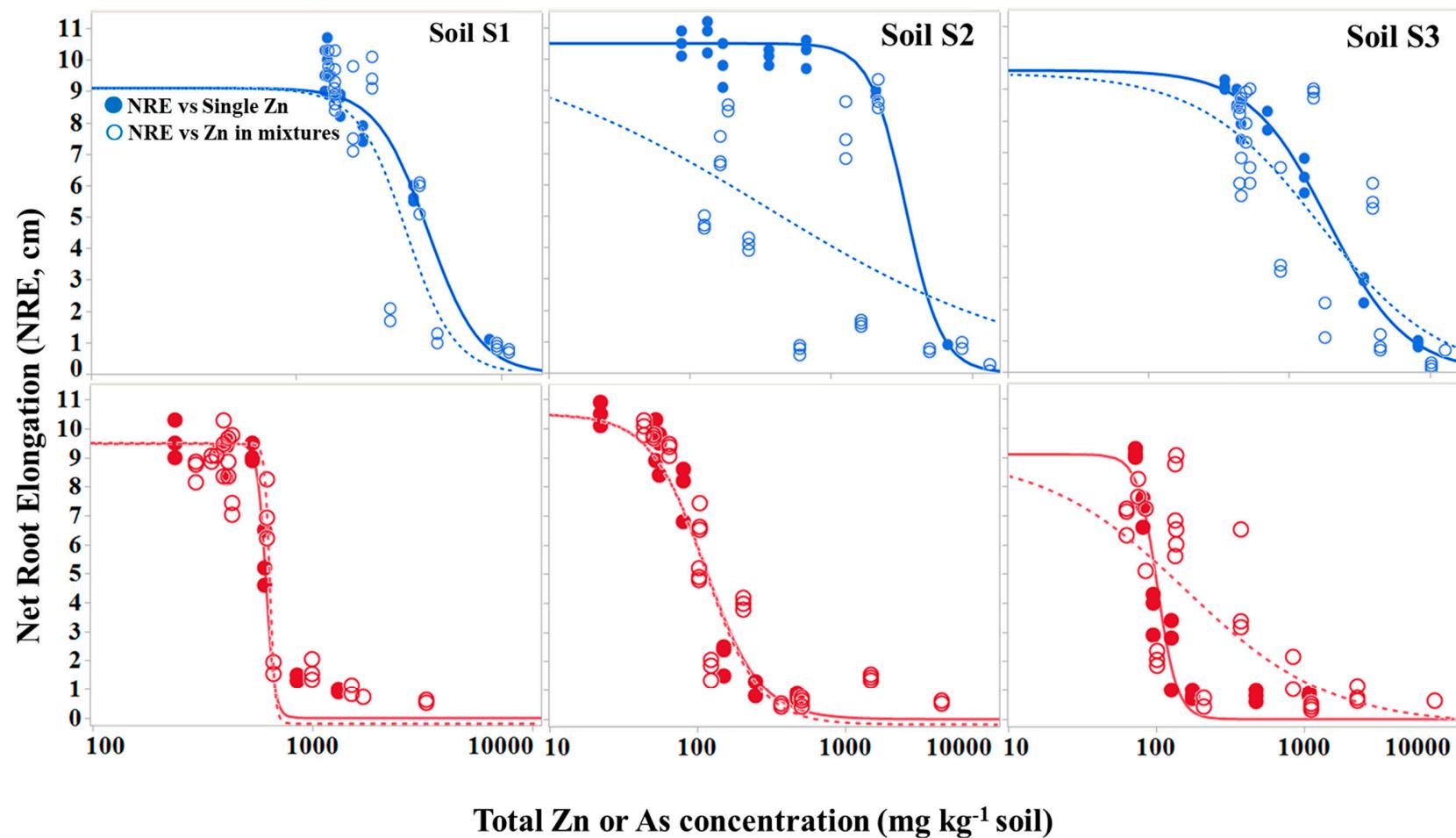


Figure S4. Barley net root elongation (NRE, cm) response to total Zn (top; in blue) or As (bottom, red) soil concentrations (mg kg^{-1}) in single and mixture treatments.

References

1. Houba, V.J. Soil Analysis Procedures. In *Soil and Plant Analysis, A Series of Syllabi. Part 5*; Department of Soil Science and Plant Nutrition: Wageningen, The Netherlands, 1985.
2. ISO 23470. *Soil Quality: Determination of Effective Cation Exchange Capacity (CEC) and Exchangeable Cations Using a Hexamminecobalt Trichloride Solution*; International Organization for Standardization: Geneva, Switzerland, 2007.
3. Schwertmann, U. The differentiation of iron oxides in soils by extraction with ammonium oxalate solution. *Z. Pflanz. Bodenk.* **1964**, *105*, 194–202.
4. Matejovic, I. Determination of carbon and nitrogen in samples of various soils by the dry combustion. *Commun. Soil Sci. Plant Anal.* **1997**, *28*, 1499–1511.
5. Sherrod, L.A.; Dunn, G.; Peterson, G.A.; Kolberg, R.L. Total inorganic carbon analysis by modified pressure-calcimeter method. *Commun. Soil. Sci. Plant. Anal.* **2002**, *33*, 2437–2438.

