

Supplementary Information

Accumulation, biotransformation, and multi-biomarker responses after exposure to arsenic species in the earthworm *Eisenia fetida*

Zhifeng Wang, Zhaojie Cui*

School of Environmental Science and Engineering, Shandong University, No. 27 Shanda South
Road, Jinan 250100, PR China.

***Corresponding author.** E-mail address: cuiwj@sdu.edu.cn (Z. Cui). Tel/Fax: +86 531 88361176.

Table S1 Concentration (mg kg⁻¹ dry wt) of four As species and total As in *E. fetida* following 64 days exposure to As contaminated soils. Earthworms of group I, II, III, IV were exposed to arsenite [As (III)], arsenate [As (V)], monomethylarsonate (MMA) and dimethylarsinate (DMA), respectively.

Experimental group	Exposure days	As in <i>E. fetida</i> (mg kg ⁻¹ dry wt)				
		As(III)	As(V)	MMA	DMA	Total As
Group I	2d	14.36	6.13	N.D.	N.D.	22.27
	4d	18.64	8.35	N.D.	N.D.	29.45
	8d	20.12	11.05	N.D.	1.16	35.79
	16d	25.86	15.33	1.27	5.37	52.44
	32d	40.57	25.49	1.64	7.79	87.22
	64d	42.25	24.10	4.11	11.72	94.85
Group II	2d	6.49	18.50	N.D.	N.D.	26.78
	4d	10.13	23.67	N.D.	N.D.	36.56
	8d	14.29	22.43	N.D.	N.D.	40.32
	16d	17.20	25.36	3.22	4.31	60.59
	32d	28.17	39.89	1.28	10.21	95.14
	64d	27.02	42.14	2.64	10.71	99.40
Group III	2d	N.D.	5.50	60.49	22.31	97.32
	4d	1.42	6.78	140.32	45.77	210.58
	8d	2.68	7.12	166.28	59.17	265.82
	16d	3.81	6.42	123.95	45.31	221.34
	32d	5.13	11.47	82.75	31.56	181.18
	64d	7.67	14.22	68.72	25.95	167.39
Group IV	2d	N.D.	6.59	8.22	73.14	113.06
	4d	N.D.	6.80	15.79	182.61	269.47
	8d	2.01	7.02	18.10	205.90	295.55
	16d	2.65	7.20	14.35	144.76	229.80
	32d	6.19	12.12	18.04	83.06	145.37
	64d	7.00	13.94	11.73	57.51	106.74

N.D. = below limit of quantification or not detected.

Table S2 Multi-biomarker responses in *E. fetida* following 64 days exposure to As contaminated soils. Earthworms of group I, II, III, IV was exposed to As(III), As(V), MMA and DMA, respectively.

Biomarker parameter	Exposure days	Biomarker responses in <i>E. fetida</i>				
		Control	Group I	Group II	Group III	Group IV
SOD (U mg ⁻¹ prot)	2d	70.34	105.37	88.08	75.59	74.31
	4d	71.56	126.55	98.26	77.30	76.48
	8d	68.91	137.74	119.92	73.02	75.37
	16d	67.50	103.33	96.15	76.32	72.85
	32d	70.12	60.47	64.77	87.12	84.99
	64d	68.13	50.80	57.27	86.67	84.42

CAT	2d	319.52	363.63	357.33	335.40	326.18
(U g ⁻¹ prot)	4d	316.32	389.24	381.81	328.93	326.59
	8d	317.31	391.13	375.10	325.76	326.80
	16d	320.65	348.40	336.82	315.37	316.01
	32d	320.93	246.11	279.94	349.96	351.51
	64d	315.48	260.09	275.88	351.76	354.69
GPx	2d	9.10	14.68	11.25	9.49	9.03
(nmol•min ⁻¹ •mg ⁻¹ prot)	4d	9.02	13.72	12.07	9.56	9.38
	8d	9.24	11.67	10.39	9.44	9.20
	16d	9.15	8.13	9.26	9.37	8.98
	32d	8.97	6.06	7.74	9.54	9.69
	64d	8.92	6.90	7.47	10.22	10.13
GST	2d	75.79	114.33	93.67	82.18	80.66
(nmol•min ⁻¹ •mg ⁻¹ prot)	4d	75.44	121.48	109.74	86.62	82.31
	8d	78.38	103.26	87.21	80.02	79.65
	16d	76.25	82.22	78.26	77.55	77.13
	32d	74.83	58.09	64.48	78.50	81.36
	64d	73.21	60.28	63.30	79.46	82.11
GR	2d	6.08	7.25	6.67	6.31	5.99
(U g ⁻¹ prot)	4d	5.80	8.86	8.32	6.41	6.10
	8d	6.01	9.72	9.31	6.83	6.58
	16d	5.97	9.33	8.25	6.90	6.83
	32d	5.92	7.07	6.38	7.25	7.01
	64d	6.12	6.29	6.31	7.31	7.13
GSH	2d	23.12	14.35	17.33	23.78	23.42
(µmol g ⁻¹ prot)	4d	25.30	21.74	24.21	23.48	24.16
	8d	24.95	33.14	31.19	26.17	26.48
	16d	24.37	41.61	38.02	28.99	26.74
	32d	22.56	30.18	25.27	28.87	27.13
	64d	22.79	25.07	23.59	35.42	33.03
MDA	2d	1.17	1.44	1.39	1.16	1.13
(nmol mg ⁻¹ prot)	4d	1.11	1.59	1.43	1.24	1.17
	8d	1.18	1.43	1.42	1.29	1.21
	16d	1.20	1.75	1.66	1.31	1.25
	32d	1.09	1.97	1.78	1.12	1.13
	64d	1.14	1.66	1.51	1.15	1.17
MTs	2d	167.86	234.84	193.99	175.12	179.57
(nmol-SH g ⁻¹ prot)	4d	168.09	273.45	206.05	180.39	171.65
	8d	167.78	305.08	237.94	189.55	188.23
	16d	166.54	326.30	271.82	194.41	190.77
	32d	168.62	380.96	320.11	216.52	219.27
	64d	167.16	387.88	326.07	221.65	216.74

SOD = superoxide dismutase, CAT = catalase, GPx = glutathione peroxidase, GST = glutathione S-transferase, GR = glutathione reductase, GSH = reduced glutathione, MDA = malondialdehyde, MTs = metallothioneins.

Table S3 Integrated biomarker responses (IBR) values in *E. fetida* following 64 days exposure to As contaminated soils. Earthworms of group I, II, III, IV was exposed to As(III), As(V), MMA and DMA, respectively.

Exposure days	IBR values			
	Group I	Group II	Group III	Group IV
2d	33.77	20.23	11.76	13.93
4d	42.27	27.23	11.95	10.03
8d	39.72	27.47	10.97	10.76
16d	34.69	23.88	12.78	11.91
32d	41.73	27.51	13.66	13.39
64d	33.08	25.89	16.64	15.94