

Supplemental online material to the article:

Comparison of fitness effects in the earthworm *Eisenia fetida* after exposure to single or multiple anthropogenic pollutants

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Table S1. Regression table for the generalized linear mixed model on earthworm survival after 4 weeks of exposure to pollutants. The intercept represents the control treatment.

	Estimate	SE	z	p
Intercept(Control)	-3.58	0.873	-4.10	<0.001
Brake dust 0.5	3.58	1.004	3.56	<0.001
Brake dust 2	6.75	1.218	5.54	<0.001
Soot 0.5	1.06	1.058	1.00	0.315
Soot 2	1.51	1.030	1.46	0.144
MP fibers 0.5	0.46	1.119	0.41	0.678
MP fibers 2	-0.70	1.376	-0.51	0.612
MP fragments 0.5	0.00	1.192	0.00	>0.999
MP fragments 2	-0.74	1.386	-0.53	0.595
Mix 0.5	1.17	1.051	1.11	0.267
Mix 2	-0.70	1.376	-0.51	0.612
Mix 8	5.02	1.049	4.79	<0.001

Notes. Model formula: Survival ~ treatment + (1 | GlassID); Family: binomial(logit); SE: Standard error.

Table S2. Regression table for the generalized linear mixed model on earthworm survival after 8 weeks of exposure to pollutants. The intercept represents the control treatment.

	Estimate	SE	z	p
Intercept(Control)	-3.13	0.764	-4.09	<0.001
Brake dust 0.5	3.13	0.916	3.42	<0.001
Brake dust 2	7.46	1.394	5.36	<0.001
Soot 0.5	0.82	0.965	0.85	0.396
Soot 2	1.20	0.942	1.27	0.202
MP fibers 0.5	0.64	0.980	0.66	0.511
MP fibers 2	-1.21	1.332	-0.91	0.365
MP fragments 0.5	-0.47	1.128	-0.41	0.679
MP fragments 2	-0.06	1.058	-0.06	0.952
Mix 0.5	0.70	0.978	0.71	0.475
Mix 2	0.91	0.962	0.95	0.344
Mix 8	7.34	1.359	5.40	<0.001

Notes. Model formula: Survival ~ treatment + (1 | GlassID); Family: binomial(logit); SE: Standard error.

Table S3. Regression table for the linear model with known sampling variance on earthworm weight. The intercept represents the control treatment. Interactions with time represent earthworm growth per week over eight weeks of exposure. Estimates and p-values are for comparisons with the control.

	Estimate	SE	z	p
Intercept(Control)	0.31	0.018	17.44	<0.001
Brake dust 0.5	-0.04	0.026	-1.37	0.169
Brake dust 2	-0.02	0.024	-1.02	0.307
Soot 0.5	-0.02	0.027	-0.78	0.435
Soot 2	-0.01	0.025	-0.21	0.833
MP fibers 0.5	-0.04	0.022	-1.79	0.074
MP fibers 2	-0.01	0.022	-0.35	0.727
MP fragments 0.5	-0.03	0.023	-1.13	0.258
MP fragments 2	0.01	0.025	0.32	0.749
Mix 0.5	-0.01	0.027	-0.43	0.666
Mix 2	-0.02	0.024	-0.85	0.395
Mix 8	-0.03	0.028	-1.07	0.285
Week (Growth in control)	0.05	0.004	11.04	<0.001
Brake dust 0.5:Week	-0.04	0.006	-6.67	<0.001
Brake dust 2:Week	-0.07	0.014	-5.30	<0.001
Soot 0.5:Week	0.01	0.006	1.12	0.263
Soot 2:Week	0.01	0.006	1.06	0.291
MP fibers 0.5:Week	0.01	0.006	1.64	0.101
MP fibers 2:Week	0.00	0.006	0.10	0.922
MP fragments 0.5:Week	0.00	0.005	0.79	0.429
MP fragments 2:Week	0.00	0.006	0.39	0.695
Mix 0.5:Week	0.00	0.006	0.38	0.706
Mix 2:Week	-0.06	0.005	-10.31	<0.001
Mix 8:Week	-0.09	0.007	-12.33	<0.001

Notes. Model formula: Weight ~ treatment * week; SE: Standard error.

Table S4. Regression table for the generalized linear model on the number of cocoons produced by earthworms after 8 weeks of exposure to pollutants. Juveniles were given 4 more weeks to hatch before counting. The intercept represents the control treatment.

	Estimate	SE	z	p
Intercept(Control)	3.69	0.092	40.07	<0.001
Brake dust 0.5	-5.88	1.007	-5.84	<0.001
Brake dust 2	-5.88	1.007	-5.84	<0.001
Soot 0.5	-0.71	0.142	-4.99	<0.001
Soot 2	-0.78	0.144	-5.44	<0.001
MP fibers 0.5	-0.85	0.145	-5.86	<0.001
MP fibers 2	-0.06	0.131	-0.47	0.639
MP fragments 0.5	-0.72	0.142	-5.03	<0.001
MP fragments 2	0.01	0.130	0.05	0.962
Mix 0.5	-2.21	0.206	-10.74	<0.001
Mix 2	-5.07	0.717	-7.08	<0.001
Mix 8	-3.37	0.324	-10.41	<0.001

Notes. Model formula: Cocoons ~ treatment; Family: nbinom2(log); SE: Standard error.

Table S5. Regression table for the generalized linear model on the number of juveniles produced by earthworms after 8 weeks of exposure to pollutants. Juveniles were given 4 more weeks to hatch before counting. The intercept represents the control treatment.

	Estimate	SE	z	p
Intercept(Control)	3.13	0.181	17.33	<0.001
Brake dust 0.5	-5.33	1.028	-5.18	<0.001
Brake dust 2	-5.33	1.028	-5.18	<0.001
Soot 0.5	-5.33	1.028	-5.18	<0.001
Soot 2	-4.52	0.748	-6.04	<0.001
MP fibers 0.5	-4.11	0.627	-6.56	<0.001
MP fibers 2	0.22	0.253	0.85	0.395
MP fragments 0.5	-5.33	1.028	-5.18	<0.001
MP fragments 2	0.05	0.255	0.21	0.835
Mix 0.5	-5.21	1.029	-5.06	<0.001
Mix 2	-5.21	1.029	-5.06	<0.001
Mix 8	-5.33	1.028	-5.18	<0.001

Notes. Model formula: Juveniles ~ treatment; Family: nbinom2(log); SE: Standard error.

Table S6. Regression table for the generalized linear mixed model on catalase (CAT) activity (U/mg protein) in earthworms after 8 weeks of exposure to pollutants. The intercept represents the control treatment.

	Estimate	SE	z	p
Intercept(Control)	1.99	0.116	17.13	<0.001
Brake dust 0.5	0.54	0.166	3.26	0.001
Soot 0.5	0.31	0.155	2.01	0.045
Soot 2	0.26	0.165	1.57	0.116
MP fibers 0.5	0.24	0.155	1.57	0.117
MP fibers 2	0.12	0.158	0.74	0.458
MP fragments 0.5	0.19	0.150	1.24	0.216
MP fragments 2	0.39	0.159	2.45	0.014
Mix 0.5	0.48	0.160	2.98	0.003
Mix 2	0.35	0.159	2.21	0.027

Notes. Model formula: $CAT \sim \text{treatment} + (1|\text{GlassID})$; Family: gamma(log); SE: Standard error.

Table S7. Regression table for the generalized linear mixed model on glutathione S-transferase (GST) activity (nmoll/min/mg protein) in earthworms after 8 weeks of exposure to pollutants. The intercept represents the control treatment.

	Estimate	SE	z	p
Intercept(Control)	16.80	4.613	3.64	<0.001
Brake dust 0.5	-2.91	6.738	-0.43	0.666
Soot 0.5	5.37	6.625	0.81	0.418
Soot 2	16.25	6.738	2.41	0.016
MP fibers 0.5	-0.51	6.433	-0.08	0.937
MP fibers 2	-4.81	6.433	-0.75	0.455
MP fragments 0.5	11.47	6.142	1.87	0.062
MP fragments 2	-3.27	6.524	-0.50	0.616
Mix 0.5	15.06	6.524	2.31	0.021
Mix 2	-4.97	6.625	-0.75	0.453

Notes. Model formula: $CAT \sim \text{treatment} + (1|\text{GlassID})$; Family: gaussian(identity); SE: Standard error.

Table S8. Regression table for the generalized linear mixed model on malondialdehyde (MDA) activity (U/mg protein) in earthworms after 8 weeks of exposure to pollutants. The intercept represents the control treatment.

	Estimate	SE	z	p
Intercept(Control)	55.33	4.527	12.22	<0.001
Brake dust 0.5	-8.43	6.613	-1.27	0.202
Soot 0.5	-9.65	6.502	-1.48	0.138
Soot 2	-10.90	6.613	-1.65	0.099
MP fibers 0.5	-13.11	6.313	-2.08	0.038
MP fibers 2	-2.21	6.403	-0.35	0.730
MP fragments 0.5	-16.38	6.028	-2.72	0.007
MP fragments 2	-3.99	6.403	-0.62	0.533
Mix 0.5	-6.57	6.403	-1.03	0.305
Mix 2	-9.68	6.502	-1.49	0.137

Notes. Model formula: $\text{MDA} \sim \text{treatment} + (1|\text{GlassID})$; Family: gaussian(identity); SE: Standard error.

Table S9. Composition of the different soil mixtures used in the chronic toxicity experiment with *Eisenia fetida*. Treatment soils were prepared by weighing the respective amounts of pollutants and soils to reach the targeted % v/v (volume pollutant/ volume soil) concentrations. In order to be able to convert the targeted concentration (% v/v) into weights, the bulk density (g/ml) of the four pollutants was determined by calculating the mean value from the weight measurements of three 0.5 ml samples each. 100 g of soil was determined to correspond to a volume of 110 ml. For each component, the weight (in g) and respective volume (in ml; within brackets) is given. In addition, the concentration in % w/w (weight pollutant/ weight soil) is given for each treatment.

Treatment	Concentration (% v/v)	Concentration (% w/w)	PS fragments (density 0.47 g/ml)	PS fibers (density 0.08 g/ml)	Break dust (density 1.89 g/ml)	Soot (density 0.14 g/ml)	Soil (density 0.91 g/ml)
<i>Control</i>	0	0	-	-	-	-	100 g (110 ml)
<i>Single stressor</i>							
PS fragments	0.5	0.26	0.259 g (0.55 ml)	-	-	-	99,600 g (109,45 ml)
PS fibers	0.5	0.04	-	0.044 g (0.55 ml)	-	-	99,600 g (109,45 ml)
Brake dust	0.5	1.04	-	-	1.039 g (0.55 ml)	-	99,600 g (109,45 ml)
Soot	0.5	0.08	-	-	-	0.077 g (0.55 ml)	99,600 g (109,45 ml)
PS fragments	2	1.05	1.034 g (2.2 ml)	-	-	-	98.098 g (107.8 ml)
PS fibers	2	0.18	-	0.176 g (2.2 ml)	-	-	98.098 g (107.8 ml)
Brake dust	2	4.24	-	-	4.158 g (2.2 ml)	-	98.098 g (107.8 ml)
Soot	2	0.31	-	-	-	0.308 g (2.2 ml)	98.098 g (107.8 ml)
<i>Multiple stressor</i>							
Mix	0.5	0.35	0.065 g (0.1375 ml)	0.010 g (0.1375 ml)	0.258 g (0.1375 ml)	0.019 g (0.1375 ml)	99,600 g (109,45 ml)
Mix	2	1.45	0.259 g (0.55 ml)	0.044 g (0.55 ml)	1.039 g (0.55 ml)	0.077 g (0.55 ml)	98.098 g (107.8 ml)
Mix	8	6.16	1.034 g (2.2 ml)	0.176 g (2.2 ml)	4.158 g (2.2 ml)	0.308 g (2.2 ml)	92.092 g (101.2 ml)