Are accumulated sulfide-bound metals metabolically available in the benthic Oligochaete *Tubifex tubifex*?

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Supporting information

Table S1: Location and coordinates of the different sampling sites.
All sampling sites are located in the Flemish part of Belgium.

Site	River	Basin	Location	XY-Coordinates	
1	Dommel	Meuse	Neerpelt	223907	213568
2	Dommel	Meuse	Neerpelt	223523	214512
3	Dommel	Meuse	Neerpelt	223533	217395
4	Dommel	Meuse	Neerpelt	223950	218080
5	Molse Nete	Nete/Scheldt	Mol	201657	207709
6	Zwarte beek	Demer/Scheldt	Beringen	207810	191320
7	Molse Nete	Nete/Scheldt	Mol	206536	208313
8	Asbeek	Nete/Scheldt	Olmen	210621	202812
9	Scheppelijke Nete	Nete/Scheldt	Mol	203131	209305
10	Kleine Nete	Nete/Scheldt	Dessel	199000	214560
11	Diepteloop	Nete/Scheldt	Beerse	179427	214118
12	Asbeek	Nete/Scheldt	Balen	210621	202812
13	Rijt	Dender/Scheldt	Ninove	124728	168918
14	Kruisbeek	Dender/Scheldt	Ternat	131472	174929
15	Molenbeek	Scheldt	Puurs	144568	196833

Table S2: General water and sediment characteristics.

Average values (n = 3) and standard deviations are presented for the sediment samples; pH, O_2, Electrical Conductivity (EC) and hardness were only measured once (n = 1). AVS: Acid Volatile Sulfides; LOI: Loss of Ignition (Organic Matter content); DOC: Dissolved Organic Carbon, the samples were filtered with a 0.20 µm membrane. Water hardness was calculated based on the formula: 2.5 * [Ca] + 4.1 * [Mg].

	Water					Sediment		
Site	**pH**	**O_2** (mg/l)	**EC** (µS/cm)	**DOC** (µg/l)	**Hardness** (mg/l)	**AVS** (µmol/g)	**LOI** (%)	**Clay content** (%)
1	7.11	9.48	335	9.09 ± 0.450	77.2	2.09 ± 2.98	1.12 ± 0.45	1.56 ± 0.04
2	7.12	8.81	1031	9.26 ± 0.008	96.6	4.01 ± 5.64	1.70 ± 1.22	1.15 ± 0.52
3	7.19	8.75	1057	8.42 ± 0.303	106	1.22 ± 0.17	2.04 ± 1.29	9.24 ± 6.24
4	7.23	8.76	1066	9.26 ± 0.008	109	1.18 ± 1.05	0.80 ± 0.59	0.78 ± 0.28
5	6.80	10.19	628	5.09 ± 0.013	142	6.59 ± 2.55	1.05 ± 0.34	2.54 ± 0.03
6	6.57	8.22	197,1	9.02 ± 0.062	41.3	29.5 ± 36.4	7.80 ± 5.45	8.76 ± 6.59
7	6.82	7.55	408	7.52 ± 0.645	91.1	2.42 ± 1.19	2.58 ± 0.25	4.05 ± 1.11
8	7.04	8.88	234	8.14 ± 0.132	55.5	0.05 ± 0.07	2.20 ± 0.93	3.22 ± 1.15
9	6.70	10.35	438	4.50 ± 0.031	123	120 ± 70.5	3.28 ± 2.74	4.47 ± 1.70
10	6.97	10.56	367	6.07 ± 0.041	88.8	0.20 ± 0.14	0.98 ± 0.59	1.23 ± 0.25
11	6.95	9.96	546	9.97 ± 0.081	86.9	0.01 ± 0.01	1.30 ± 0.62	1.34 ± 0.53
12	7.24	8.25	280	7.34 ± 0.054	125	0.56 ± 0.14	1.08 ± 0.26	1.84 ± 0.35
13	8.16	10.58	785	5.15 ± 0.218	241	10.4 ± 1.42	1.19 ± 0.06	0.50 ± 0.06
14	8.34	9.95	614	34.3 ± 0.184	200	0.20 ± 0.07	4.57 ± 2.25	4.79 ± 3.61
15	8.41	7.43	835	6.13 ± 0.043	265	14.3 ± 3.85	2.40 ± 0.54	5.75 ± 1.54

Table S3: Metal concentrations in environmental fractions.

Average concentrations (n = 15) and standard deviations of the sampling sites are presented. Each time the minimum (-) as well as the maximum (+) concentration found at the different sites is reported. $ToSW_{Me}$: Total metal concentration in the surface water; $DiSW_{Me}$: Dissolved metal concentration in the surface water; $PaSW_{Me}$: Particulate metal concentration in the surface water; $PaSW_{Me}$ were calculated by subtracting $ToSW_{Me}$ - $DiSW_{Me}$. SedMe: Total metal concentration in the sediment; SEM_{Me}: Simultaneously extracted metals, together with AVS; SEM_{Me}-AVS: Molar difference between SEM and AVS.

	Water			**Sediment**		
	$ToSW_{Me}$ (µmol/l)	**$DiSW_{Me}$ (µmol/l)**	**$PaSW_{Me}$ (µmol/l)**	**Sed_{Me} (µmol/g)**	**SEM_{Me} (µmol/g)**	**SEM_{Me}-AVS (µmol/g)**
Cd	0.010 ± 0.017	0.008 ± 0.015	0.002 ± 0.004	0.057 ± 0.103	0.045 ± 0.080	-12.9 ± 30.5
	(0.0001 - 0.053)	(0.00004 - 0.047)	(0.00002 - 0.014)	(0.036 - 0.405)	(0.0013 - 0.310)	(-119 - 0.018)
Cu	0.110 ± 0.161	0.089 ± 0.147	0.017 ± 0.017	0.268 ± 0.237	0.289 ± 0.235	-12.6 ± 30.5
	(0.027 - 0.678)	(0.020 - 0.613)	(0.0004 - 0.065)	(0.036 - 0.751)	(0.075 - 0.847)	(-119 - 0.824)
Zn	4.51 ± 8.82	3.50 ± 7.13	1.01 ± 1.77	9.50 ± 18.8	7.00 ± 12.9	-5.92 ± 19.0
	(0.443 - 35.5)	(0.129 - 28.4)	(0.067 - 7.04)	(0.633 - 75.1)	(0.859 - 51.4)	(-68.3 - 8.64)
Pb	0.025 ± 0.072	0.012 ± 0.036	0.013 ± 0.037	0.255 ± 0.223	0.230 ± 0.285	-12.7 ± 30.6
	(0.0002 - 0.285)	(0.0002 - 0.140)	(0.00002 - 0.145)	(0.014 - 0.890)	(0.042 - 1.20)	(-119 - 1.19)
Ni	0.234 ± 0.110	0.189 ± 0.093	0.045 ± 0.040	0.146 ± 0.086	0.117 ± 0.048	-12.8 ± 30.6
	(0.051 - 0.416)	(0.051 - 0.391)	(0.0009 - 0.133)	(0.053 - 0.371)	(0.044 - 0.180)	(-120 - 0.167)
As	0.047 ± 0.043	0.039 - 0.042	0.009 ± 0.006	0.539 ± 0.638	0.260 ± 0.295	-12.7 ± 30.6
	(0.015 - 0.177)	(0.009 - 0.171)	(0.002 - 0.022)	(0.027 - 2.03)	(0.048 - 0.991)	(-120 - 0.816)
Cr	0.027 ± 0.009	0.023 ± 0.006	0.004 ± 0.004	0.137 ± 0.072	0.123 ± 0.064	-12.8 ± 30.6
	(0.018 - 0.049)	(0.017 - 0.039)	(0.0002 - 0.012)	(0.063 - 0.299)	(0.051 - 0.238)	(-120 - 0.164)
Co	0.106 ± 0.076	0.085 ± 0.063	0.021 ± 0.024	0.074 ± 0.061	0.043 ± 0.036	-12.9 ± 30.6
	(0.010 - 0.258)	(0.009 - 0.245)	(0.001 - 0.080)	(0.022 - 0.256)	(0.009 - 0.153)	(-120 - 0.023)
Ag	0.0002 ± 0.00004	0.0002 ± 0.00003	0.00002 ± 0.00002	0.002 ± 0.002	0.0004 ± 0.0006	-12.9 ± 30.6
	(0.0001 - 0.0003)	(0.0001 - 0.0002)	(0.000001 - 0.00008)	(0.00009 - 0.012)	(0.00005 - 0.002)	(-120 - 0.013)

Table S4: Pearson correlations among metal concentrations in *T. tubifex* and environmental fractions.

r-values and significance level are presented. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$. Sed_{Me}: Total metal concentration in the sediment; Sed_{Me}/OM: Total metal concentration in the sediment normalized for organic matter content; Sed_{Me}/clay: Total metal concentration in the sediment normalized for clay content; SEM_{Me}: Simultaneously extracted metals, together with AVS; SEM_{Me}-AVS: Molar difference between SEM and AVS; SEM_{Me}-AVS/OM: Molar difference between SEM and AVS normalized for organic matter content; $ToSW_{Me}$: Total metal concentration in the surface water; $DiSW_{Me}$: Dissolved metal concentration in the surface water; $PaSW_{Me}$: Particulate metal concentration in the surface water. $PaSW_{Me}$ were calculated by subtracting $ToSW_{Me}$ - $DiSW_{Me}$.

Metal	Fraction	Sed_{Me}	Sed_{Me}/OM	Sed_{Me}/Clay	SEM_{Me}	SEM_{Me}-AVS	SEM_{Me}-AVS/OM	$ToSW_{Me}$	$DiSW_{Me}$	$PaSW_{Me}$
Cd	Total	0.543*			0.564*			0.604*	0.629*	
	Organelles + HDP					0.589*				
	Granules + HSP									
	Rest fraction									
Cu	Total									
	Organelles + HDP								0.618*	
	Granules + HSP									
	Rest fraction			0.568*						
Zn	Total	0.700**	0.629*	0.664**	0.636*					
	Organelles + HDP		0.593*	0.625*			0.511*			
	Granules + HSP									
	Rest fraction									
Pb	Total	0.525*	0.650**	0.568*	0.714**			0.675**	0.729**	0.696**
	Organelles + HDP				0.511*	0.539*			0.532*	
	Granules + HSP									
	Rest fraction		0.725**	0.729**						
Ni	Total									
	Organelles + HDP									
	Granules + HSP									
	Rest fraction						0.582*			
As	Total	0.586*	0.725**	0.779***	0.704**			0.736**	0.725**	
	Organelles + HDP		0.550*	0.625*	0.557*			0.696**	0.661**	
	Granules + HSP									
	Rest fraction		0.618*	0.782***	0.650**					
Cr	Total									
	Organelles + HDP					0.543*	0.568*			
	Granules + HSP									
	Rest fraction					0.618*	0.514*			
Co	Total									
	Organelles + HDP					0.782***	0.582*			
	Granules + HSP									
	Rest fraction									
Ag	Total									
	Organelles + HDP			-0.586*						
	Granules + HSP									
	Rest fraction		0.632*	0.543*						

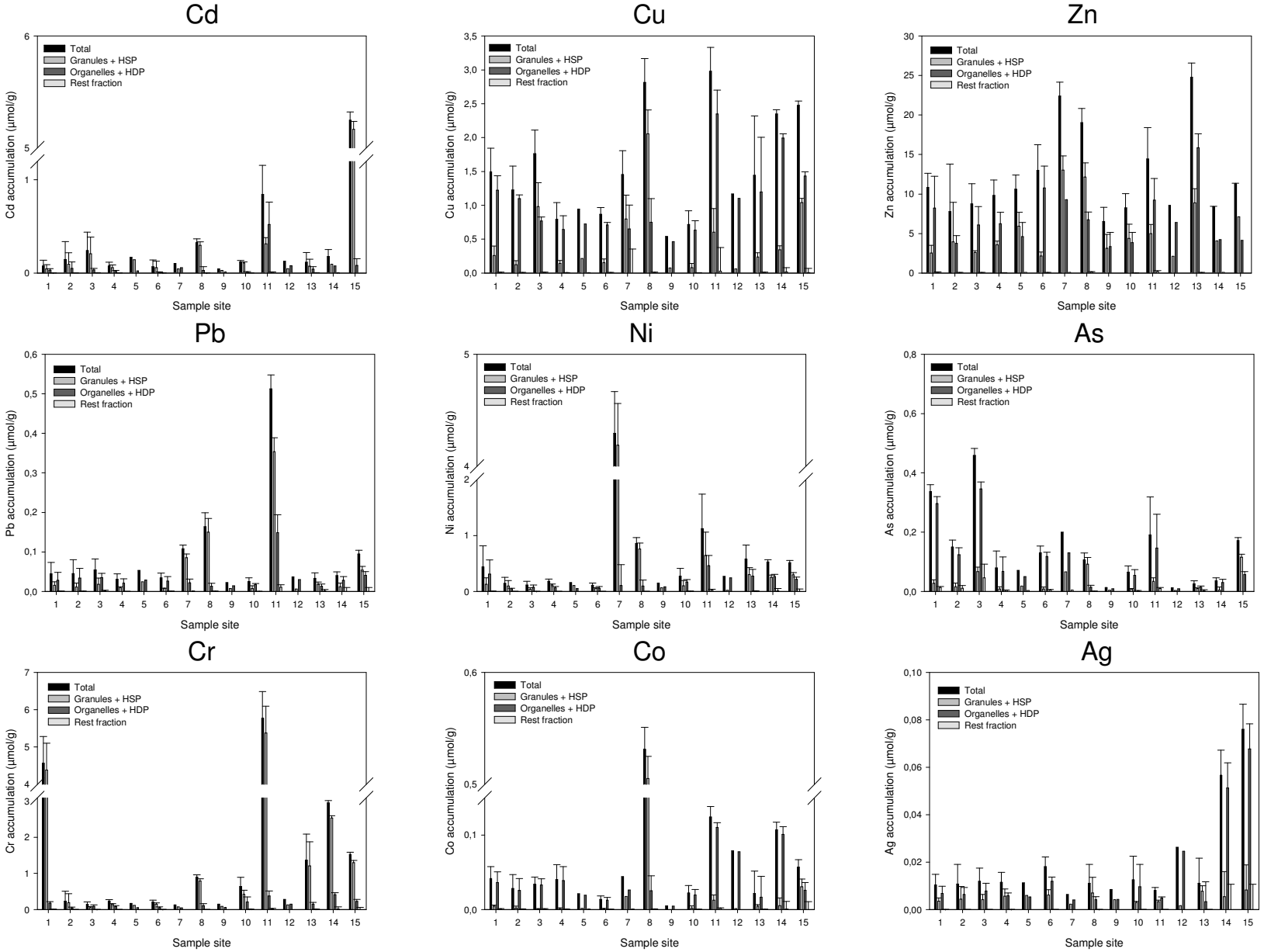

Fig. S1: Metal accumulation in whole tissue and subcellular fractions in *T. tubifex*.
Average concentrations (n = 3) and standard deviations are presented. The amount of metals in the metal sensitive fractions (MSF; organelles + HDP) and as biological detoxified metal (BDM; HSP + MRG) of the worms are calculated for all the sample sites separately.

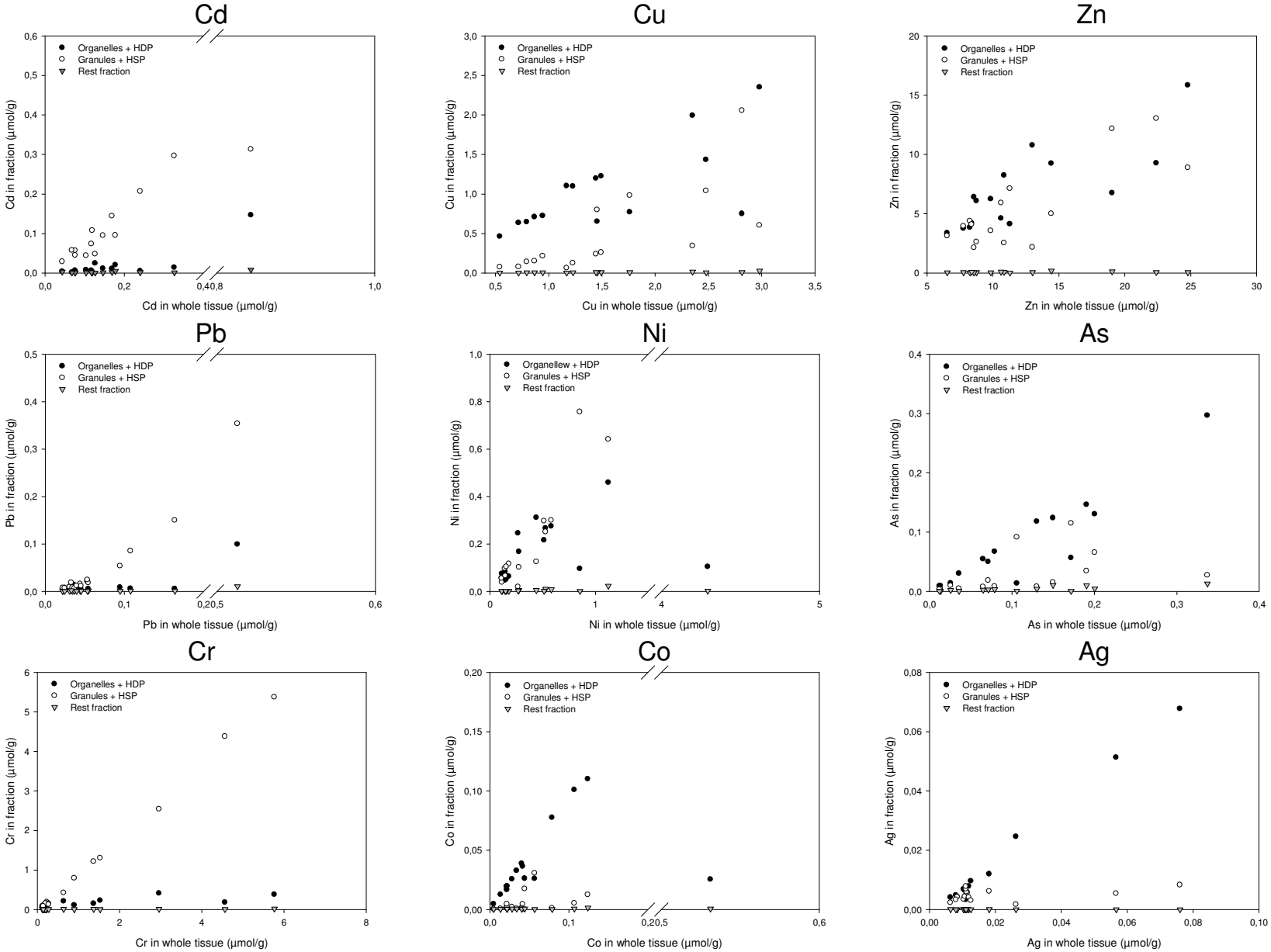

Fig. S2: Metal distribution in subcellular fractions as a function of whole tissue metal accumulation.

3 different fractions are presented: The metal sensitive fractions (MSF; organelles + HDP), the biological detoxified metal (BDM; HSP + MRG) and the rest fraction.

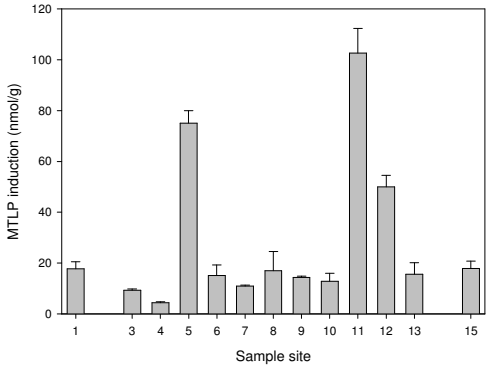

Fig. S3: MTLP induction in *T. tubifex*.
Average concentrations (n = 3) and standard deviations are presented.
Regarding sample sites 2 and 14 not enough worm tissue remained to determine MTLP concentrations.