Electronic Supplementary Material (ESI) for Environmental Science: Processes & Impacts.

Contrasting effects of bioturbation on metal toxicity of contaminated sediments results in misleading interpretation of the AVS-SEM metal-sulfide paradigm

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Supplementary Information

Methods

General methods

All salts used for the preparation of solutions were of AR grade or higher. For physicochemical measurements, salinity was measured using a Mettler Toledo Seven2Go S3 conductivity meter fitted with an InLAB® 73X series conductivity probe; dissolved oxygen (DO) and pH were measured using WTW (Wissenschaftlich-Technische Werstätten) instruments (Multi 3410 with FDO® 925 probe and pH320 with SenTix 41 pH electrode). Dissolved ammonia was measured using a rapid test kit (API Fish Care, LR8600). All probes were pre-calibrated using the manufacturer's instructions.

Sediment characterisation

Total recoverable metals (TRM) were determined after low-pressure microwave-assisted (MARS 5, CEM) aqua regia digestion (3:1 HNO_3: HCl). Total organic carbon (TOC) analysis was determined as CO_2 evolution following a prescribed method.[1]. Dried, ground sediment samples were acid-treated to remove inorganic carbonates followed by high temperature combustion in an oxygen atmosphere (Leco TruMAC instrument) followed by infrared detection. Frozen sediment mini-cores were extruded within a nitrogen-filled glove box. Sections were taken from the surface (~0.5 cm from the top) and at depth (0.5-1 cm from the bottom) and three replicate core samples for each fraction homogenized before being analyzed for AVS and SEM.[2]

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(1) Matejovic I. Determination of Carbon and Nitrogen in samples of various soils by the dry combustion. Comm. Soil Sci. Plant Anal. 1997, 28, 1499-1511.

(2) Simpson, S. L. A rapid screening method for acid-volatile sulfide in sediments. Environ. Toxicol. Chem. 2001, 20 (12), 2657–2661.

Sulfidization procedure

Sulfidised sediment was prepared synthetically by the addition of a neutralized sulfide stock solution in molar excess to the sediment SEM concentration to convert the reactive iron and metals into their respective monosulfides (as shown in Equation S1).

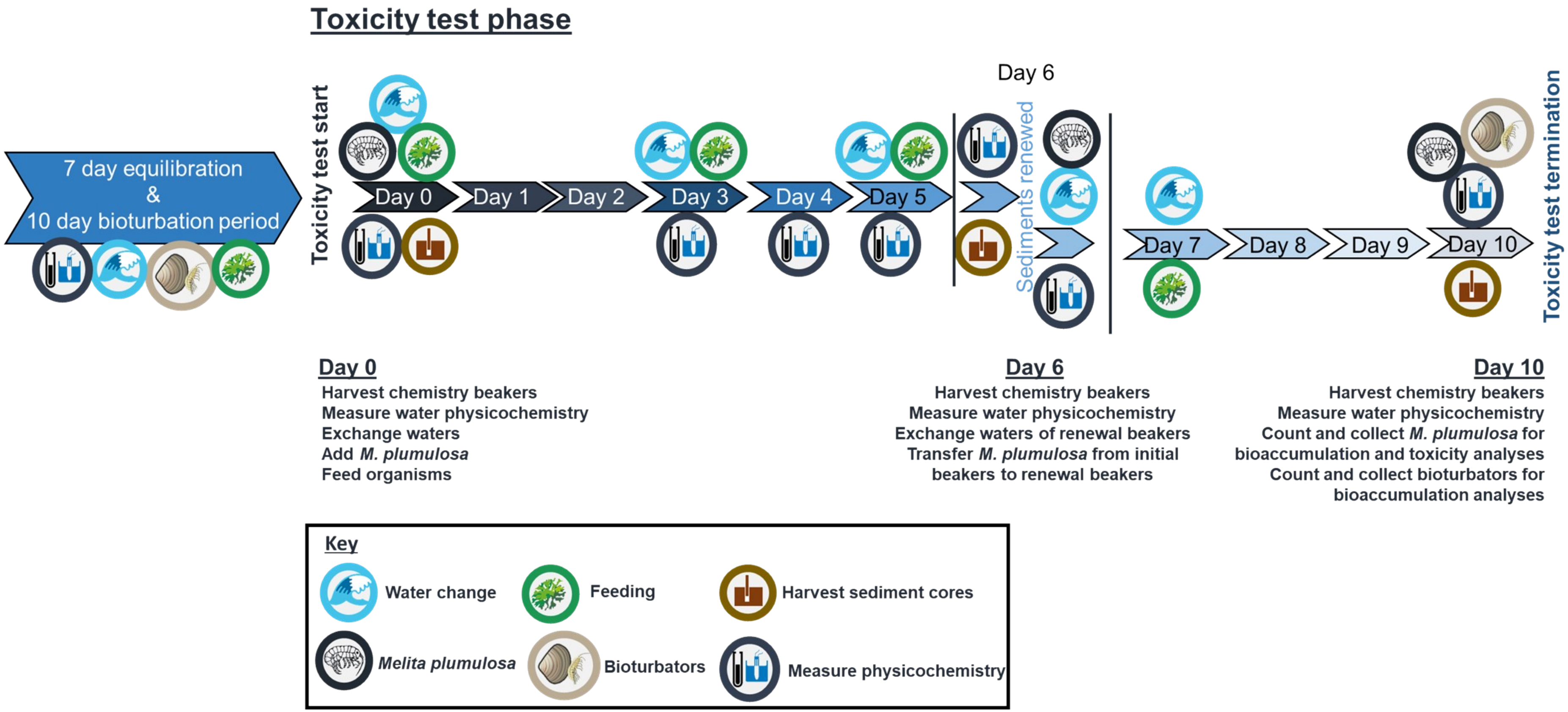
$$Fe(OH)_3 + 3HS^- \rightarrow FeS + 3H_2O \qquad \text{Equation S1}$$

A neutralized sulfide solution was used because a sulfide solution prepared using just $Na_2S.9H_2O$ resulted in a highly alkaline solution due to the excess of hydroxides produced during the reduction of $Fe(OH)_3$ (Equation S2).

$$8Fe(OH)_3 + 9S^{2-} \rightarrow 8FeS + SO_4^{2-} + 16OH^- + 4H_2O \qquad \text{Equation S2}$$

Sulfide stock solutions ($Na_2S.9H_2O$ (Sigma Aldrich) in deoxygenated deionized water) were neutralised with 2 mol L^{-1} of H_2SO_4 (BDH) until a pH of 7.3 was reached. In a bucket purged with N_2, neutralised (1.5 L, 200 mmol L^{-1}) sulfide solution was mixed into 10 kg wet weight sediment and the bucket sealed for 10 days, yielding a total AVS of 16 ± 0.1 mmol kg^{-1} dry weight. This was repeated using a second neutralised sulfide solution (150 mmol L^{-1}, yielding an AVS of 16 mmol kg^{-1} dry weight after 6 days); and then a third time using 1.0 L of 200 mmol L^{-1} sulfide solution and after 5 days, yielding an AVS of 33 ± 0.7 mmol kg^{-1} dry weight. The overlying water was removed (3.0 L) and the sediment was then washed with 1.0 L of deoxygenated seawater. To ensure consistency with sediment treatments, the reference sediment and non-sulfidized metal-contaminated sediments were washed with 2.5 L of deionised water then covered with 1.0 L of deoxygenated seawater a week prior to use.

Figure S1. Timeline for the toxicity testing phase



Results

Table S1. Final physicochemical properties of surficial sediments for the AVS_{Low} mesocosms

Treatment	Day	Simultaneously extractable metals (SEM) (1M HCl –extractable; mg kg^{-1} or % for Fe, dry weight)								SEM	AVS	∑SEM-AVS	∑SEM-AVS/ f_{OC}
		Fe	Mn	As	Cu	Ni	Pb	V	Zn	(µmol g^{-1})			
No AVS_{Low}	**10**	0.5	76 ± 24	10 ± 2.9	165 ± 45	2.5 ± 1.0	310 ± 103	19 ± 6.4	438 ± 131	18 ± 3.1	0.6 ± 0.3	17 ± 2.7	3.1 ± 0.5
Static AVS_{Low}	**0**	0.5	51 ± 32	6.8 ± 4.6	108 ± 65	1.2 ± 0.9	199 ± 153	13 ± 10	286 ± 208	7.1 ± 4.9	0.7 ± 0.7	6.4 ± 4.2	1.2 ± 0.8
	6	0.3	39 ± 11	4.9 ± 1.4	88 ± 25	1.5 ± 0.5	138 ± 43	9.0 ± 3.0	219 ± 61	5.4 ± 1.5	0.3 ± 0.1	5.1 ± 1.4	0.9 ± 0.3
	10	5.5	76 ± 24	10 ± 2.9	165 ± 45	2.5 ± 1.0	310 ± 103	19 ± 6.0	483 ± 131	18 ± 2.3	0.8 ± 0.2	17 ± 2.1	3.1 ± 0.4
Bioturbated AVS_{Low}	**0**	0.4	47 ± 4.0	7.1 ± 1.7	105 ± 6.0	1.4 ± 0.3	190 ± 7.1	13 ± 0.7	286 ± 14	7.0 ± 0.3	0.3 ± 0.3	6.6 ± 0.5	1.2 ± 0.1
	6	0.4	69 ± 26	11 ± 3.6	172 ± 64	3.2 ± 1.5	275 ± 100	19 ± 7.4	433 ± 159	11 ± 3.9	0.7 ± 0.3	10 ± 3.7	1.9 ± 0.7
	10	1	154 ± 15	15 ± 1.3	343 ± 28	5.3 ± 0.9	664 ± 63	37 ± 4.9	865 ± 88	22 ± 2.1	0.5 ± 0.1	21 ± 2.0	3.9 ± 0.4

For all results, n= 3 (mean ± SD). ∑SEM = 1 M HCl extractable metals. AVS = acid-volatile sulfide. f_{OC} = fraction of organic carbon, where the ∑SEM-AVS fraction is normalised by the fraction of organic carbon.

Table S2. Final physicochemical properties of deeper sediments for the *AVS_{Low}* mesocosms

Treatment	Day	Simultaneously extractable metals (SEM) (1M HCl –extractable; mg kg^{-1} or % for Fe, dry weight)								SEM	AVS	∑SEM-AVS	∑SEM-AVS/ f_{OC}
		Fe	Mn	As	Cu	Ni	Pb	V	Zn	(µmol g^{-1})			
No AVS_{Low}	**10**	0.5	59 ± 20	6.4 ± 2.1	83 ± 26	1.8 ± 0.4	228 ± 75	15 ± 5.0	337 ± 110	7.8 ± 3.0	0.2 ± 0.1	7.6 ± 2.9	1.4 ± 0.5
Static AVS_{Low}	**0**	0.5	27 ± 2.0	3.0 ± 0.8	48 ± 10	0.8 ± 0.2	108 ± 9.0	7.0 ± 0.2	163 ± 13	3.8 ± 0.4	0.4 ± 0.0	3.4 ± 0.4	0.6 ± 0.1
	6	0.4	93 ± 87	5.1 ± 0.4	89 ± 18	1.6 ± 0.5	162 ± 37	11 ± 3.0	253 ± 64	6.1 ± 1.4	0.3 ± 0.1	5.8 ± 1.4	1.1 ± 0.3
	10	8.5	124 ± 15	14 ± 0.8	327 ± 36	4.2 ± 0.6	507 ± 57	28 ± 4.0	674 ± 86	18 ± 5.0	0.9 ± 0.8	17 ± 5.8	3.1 ± 1.1
Bioturbated AVS_{Low}	**0**	0.3	43 ± 15	5.8 ± 1.4	93 ± 34	1.4 ± 0.6	189 ± 68	12 ± 4.5	277 ± 107	6.6 ± 2.5	0.5 ± 0.2	6.1 ± 2.3	1.1 ± 0.4
	6	0.3	69 ± 11	9.2 ± 3.7	164 ± 44	3.3 ± 1.1	282 ± 42	22 ± 1.2	487 ± 35	11 ± 1.4	0.9 ± 0.6	11 ± 2.1	2.0 ± 0.4
	10	8.4	126 ± 9.0	11 ± 0.3	263 ± 35	4.4 ± 0.3	562 ± 45	30 ± 2.0	722 ± 55	18 ± 1.6	0.4 ± 0.1	18 ± 1.6	3.3 ± 0.3

For all results, n= 3 (mean ± SD). ∑SEM = 1 M HCl extractable metals. AVS = acid-volatile sulfide. f_{OC} = fraction of organic carbon, where the ∑SEM-AVS fraction is normalised by the fraction of organic carbon.

Table S3. Final physicochemical properties of surficial sediments for the AVS_{High} mesocosms

Treatment	Day	Simultaneously extractable metals (SEM) (1M HCl –extractable; mg kg^{-1} or % for Fe, dry weight)								SEM	AVS	∑SEM-AVS	∑(∑SEM-AVS)/ f_{OC}
		Fe	Mn	As	Cu	Ni	Pb	V	Zn	(µmol g^{-1})			
No AVS_{High}	**10**	0.4	39 ± 1.8	3.3 ± 0.3	49 ± 3.0	0.8 ± 0.2	105 ± 9.9	11 ± 1.2	185 ± 13	4.1 ± 0.3	2.3 ± 0.4	2.6 ± 0.7	1.4 ± 0.4
Static AVS_{High}	**0**	0.4	27 ± 5.7	2.7 ± 0.7	35 ± 7.1	0.7 ± 0.1	91 ± 17	9.6 ± 1.5	168 ± 25	3.6 ± 0.6	4.7 ± 3.0	-1.1 ± 3.5	-0.6 ± 1.8
	6	0.8	66 ± 4.3	6.5 ± 1.3	97 ± 15	2.2 ± 0.5	198 ± 36	20 ± 4.1	378 ± 71	8.3 ± 1.5	6.5 ± 5.3	1.8 ± 4.1	0.9 ± 2.2
	10	0.5	38 ± 16	3.9 ± 0.9	58 ± 22	1.3 ± 0.8	127 ± 57	11 ± 4.9	226 ± 104	5.0 ± 2.2	1.0 ± 0.5	3.9 ± 1.7	2.1 ± 0.9
Bioturbated AVS_{High}	**0**	0.6	37 ± 0.9	6.5 ± 1.3	70 ± 19	1.7 ± 0.9	154 ± 30	16 ± 3.3	275 ± 57	6.1 ± 1.3	6.0 ± 2.1	0.1 ± 1.0	0.05 ± 0.5
	6	0.3	22 ± 4.6	2.8 ± 0.8	47 ± 12	1.0 ± 0.3	96 ± 21	9.7 ± 2.2	117 ± 8.2	3.9 ± 0.9	2.8 ± 1.1	1.2 ± 0.3	0.6 ± 0.2
	10	0.6	37 ± 5.7	3.0 ± 0.1	63 ± 9.2	1.7 ± 0.3	166 ± 24	16 ± 3.0	295 ± 37	6.3 ± 0.8	2.8 ± 1.2	3.5 ± 0.7	1.8 ± 0.4

For all results, n= 3 (mean ± SD). ∑SEM = 1 M HCl extractable metals. AVS = acid-volatile sulfide. f_{OC} = fraction of organic carbon, where the ∑SEM-AVS fraction is normalised by the fraction of organic carbon.

Table S4. Final physicochemical properties of deeper sediments for *AVS$_{High}$* mesocosms

Treatment	Day	Simultaneously extractable metals (SEM) (1M HCl –extractable; mg kg^{-1} or % for Fe, dry weight)								SEM	AVS	∑SEM-AVS	∑(∑SEM-AVS)/ f_{OC}
		Fe	Mn	As	Cu	Ni	Pb	V	Zn	(µmol g^{-1})			
No AVS$_{High}$	**10**	0.4	30 ± 1.5	1.1 ± 1.0	26 ± 6.7	0.8 ± 0.2	111 ± 7.5	13 ± 0.4	211 ± 14	4.2 ± 0.3	25 ± 6.4	-20 ± 6.2	-11 ± 3.3
Static AVS$_{Hight}$	**0**	0.5	31 ± 7.8	1.6 ± 0.5	31 ± 7.7	1.3 2) 0.2	108 ± 18	12 ± 2.8	225 ± 50	4.5 ± 0.9	7.4 ± 4.4	-2.9 ± 3.9	-1.5 ± 2.0
	6	0.6	44 ± 11	2.9 ± 1.4	42 ± 15	1.7 ± 0.3	156 ± 46	17 ± 4.6	316 ± 98	6.3 ± 2.0	14 ± 4.2	-7.2 ± 2.3	-3.8 ± 1.2
	10	0.6	39 ± 1.1	1.8 ± 0.3	34 ± 7.6	1.7 ± 0.1	149 ± 6.2	16 ± 0.2	302 ± 10	5.9 ± 0.2	19 ± 2.0	-12 ± 2.3	-6.3 ± 1.2
Bioturbated AVS$_{High}$	**0**	0.5	28 ± 2.8	2.9 ± 1.4	38 ± 3.9	0.9 ± 0.2	112 ± 7.8	12 ± 0.6	211 ± 20	4.4 ± 0.3	7.0 ± 1.9	-2.6 ± 1.7	-1.4 ± 0.9
	6	0.3	19 ± 1.6	2.4 ± 0.6	40 ± 11	0.7 ± 0.1	81 ± 14	7.4 ± 2.2	137 ± 19	3.1 ± 0.4	2.3 ± 0.5	4.1 ± 1.3	2.2 ± 0.7
	10	0.5	30 ± 3.1	2.8 ± 1.3	43 ± 9.4	1.5 ± 0.04	126 ± 16	12 ± 1.1	233 ± 24	4.9 ± 0.6	3.3 ± 0.6	1.6 ± 0.3	0.8 ± 0.2

For all results, n= 3 (mean ± SD). ∑SEM = 1 M HCl extractable metals. AVS = acid-volatile sulfide. f_{OC} = fraction of organic carbon, where the ∑SEM-AVS fraction is normalised by the fraction of organic carbon.

Table S5. Water chemistry and dissolved metal concentrations during the bioturbation (Bio) and Toxicity (Tox) test phases.

Sediment	Dissolved metal concentrations (0.45 µm-filtered, µg L^{-1})								Mean physicochemical Parameters				
	Fe	Mn	As	Cd	Cr	Cu	Pb	Zn	DO (%)	T (°C)	pH	NH_3 (ppm)	Salinity•
						Reference							
static^{Tox}	5.8 ± 2.1	2.1 ± 3.4	0.8 ± 0.2	0.1 ± 0.02	0.3 ± 0.1	2.3 ± 0.4*	0.1 ± 0.03	7.8 ± 5.3	97 ± 2.9	20 ± 0.5	7.8 ± 0.3	0.3 ± 0.6	32 ± 0.9
bioturb.^{Bio}	3.8 ± 1.4	2.9 ± 2.4	0.7 ± 0.0	0.1 ± 0.1	<0.2	1.1 ± 0.1	0.1 ± 0.1	8.5 ± 3.1	98 ± 1.3	20 ± 0.3	7.9 ± 0.1	1.2 ± 0.7	32 ± 0.6
bioturb.^{Tox}	42 ± 105	79 ± 76	2.1 ± 0.5	<0.1	<0.2	1.8 ± 1.9*	0.2 ± 0.2	4.8 ± 4.8	99 ± 0.6	21 ± 0.6	7.9 ± 0.1	0.0 ± 0.1	32 ± 0.6
						AVS_{Low}							
static^{NO}	2.5 ± 0.5	0.1 ± 0.2	2.3 ± 0.5	0.2 ± 0.1	0.4 ± 0.1	4.7 ± 1.1*	1.1 ± 0.3	15 ± 1.4	97 ± 1.2	20 ± 0.3	7.9 ± 0.1	0.1 ± 0.2	32 ± 0.8
static^{Tox}	2.1 ± 0.9	0.1 ± 0.1	2.2 ± 0.4	0.2 ± 0.1	0.3 ± 0.1	6.9 ± 2.3*	1.5 ± 0.5	19 ± 1.8	97 ± 2.4	20 ± 0.6	8.0 ± 0.2	0.0 ± 0.0	32 ± 0.6
bioturb.^{Bio}	2.8 ± 1.9	17 ± 15	2.8 ± 0.5	0.2 ± 0.1	<0.2	5.3 ± 2.2*	3.2 ± 0.6	18 ± 4.9	97 ± 1.7	20 ± 0.5	7.9 ± 0.2	0.7 ± 0.6	32 ± 0.6
bioturb.^{Tox}	3.3 ± 0.5	85 ± 28	5.1 ± 1.0	0.2 ± 0.1	<0.2	5.7 ± 1.6*	3.7 ± 1.5	12 ± 2.0	99 ± 0.7	21 ± 0.3	8.0 ± 0.1	0.6 ± 0.3	31 ± 0.4
						AVS_{High}							
static^{NO}	2.9 ± 0.9	0.2 ± 0.2	1.2 ± 0.5	<0.1	<0.2	1.5 ± 0.3*	0.1 ± 0.1	1.5 ± 0.5	96 ± 3.5	20 ± 0.4	8.2 ± 0.1	0.0 ± 0.1	32 ± 0.5
static^{Tox}	1.9 ± 0.4	6.2 ± 19	1.9 ± 0.4	<0.1	<0.2	3.2 ± 1.2*	0.2 ± 0.1	1.8 ± 0.8	94 ± 4.9	21 ± 0.4	8.2 ± 0.1	0.2 ± 0.3	32 ± 0.4
bioturb.^{Bio}	7.6 ± 5.6	123 ± 112	5.8 ± 3.3	<0.1	<0.2	1.0 ± 0.9	0.3 ± 0.1	3.5 ± 2.9	94 ± 5.0	20 ± 0.4	8.2 ± 0.1	1.3 ± 0.7	32 ± 0.4
bioturb.^{Tox}	12 ± 14	207 ± 76	3.4 ± 1.4	<0.1	<0.2	0.4 ± 0.3	0.4 ± 0.1	1.7 ± 0.7	97 ± 1.0	21 ± 0.4	8.0 ± 0.1	1.0 ± 0.6	32 ± 0.7
***WQGV* (µg L^{-1})**		*750*	*NV*	*5.5*	*4.4 (Cr(VI))*	*1.3*	*4.4*	*100*					

All data are mean ± SD (n= 12). The Water Quality Guideline values (WQGV) are reported at 95% species protection level. WQGVs for Cd, Cu, Pb, V and Zn were from ANZECC/ARMCANZ (2000). • WQGV for Mn is the recommended value currently under review Golding et al. (2016). * exceeded the WQGV. •salinity measured on the practical salinity scale (psu).

Table S6. Survival and sub-lethal toxicity data from the 10-day *M. plumulosa* bioassay

Sediment & treatment	Survival (% of control)						Reproduction (% of control)		
	Renewal			Termination					
	Males	Females	Total	Males	Females	Total	Average	Per female	Per surviving female
Reference									
static	89 ± 5.6	89 ± 5.6	89 ± 2.8	89 ± 5.6	83 ± 9.6	86 ± 5.6	100 ± 8.6	100 ± 8.6	100 ± 19
bioturb.	94 ± 5.6	94 ± 5.6	94 ± 2.8	89 ± 5.6	94 ± 5.6	92 ± 0.01	94 ± 22	94 ± 22	78 ± 15
AVS_{Low}									
static	106 ± 11	94 ± 11	100 ± 3.1	100 ± 13	93 ± 13	97 ± 0.01	4.1 ± 3.1	4.1 ± 3.1	4.6 ± 3.8
bioturb.	106 ± 6.2	88 ± 6.2	94 ± 5.4	94 ± 11	87 ± 6.7	90 ± 6.5	46 ± 7.5	46 ± 7.5	52 ± 11
AVS_{High}									
static	100 ± 6.2	106 ± 6.2	109 ± 3.1	94 ± 11	93 ± 26.7	94 ± 18	57 ± 14	57 ± 14	69 ± 21
bioturb.	75 ± 0.01	69 ± 6.2	72 ± 3.1	81 ± 6.3	73 ± 6.7	77 ± 5.6	23 ± 4.4	23 ± 4.4	31 ± 8.2

All data are expressed as mean ± standard error, where n=3.

Table S7. Organism bioaccumulation data following 10-day (*M. plumulosa*) and 27-day (*V. australiensis and T. deltoidalis*) exposures

Mesocosm	Organism	Metal accumulation (µg/g dw)						Toxicity Data	
								Number of organisms digested	Average Survival
		As	Cr	Cu	Ni	Pb	Zn	N#	(% control)
Reference									
static	***Melita***	10 ± 5.1	1.8 ± 1.4	72 ± 16	4.0 ± 0.3	6.1 ± 3.5	85 ± 26	6,5,5	89 ± 5.6
bioturb.	***Melita***	13 ± 3.0	2.5 ± 0.5	113 ± 32	5.1 ± 0.8	11 ± 1.7	163 ± 24	6,5,5	89 ± 5.6
	Victoriopisa	13 ± 5.1	0.7 ± 0.4	213 ± 99	1.4 ± 0.6	5.6 ± 2.7	137 ± 48	4,4,2	83 ± 17
	Tellina	15 ± 1.8	2.6 ± 0.8	173 ± 51	3.9 ± 0.8	37 ± 6.0	383 ± 35	6,6,6	100 ± 0.0
AVS_{Low}									
static	***Melita***	9.1 ± 3.1	3.2 ± 0.6	159 ± 7.4	16 ± 2.6	8.2 ± 1.5	145 ± 42	6.4.6	100 ± 13
bioturb.	***Melita***	9.1 ± 2.0	4.2 ± 1.2	100 ± 15	34 ± 10	5.2 ± 3.9	155 ± 60	5.6.4	94 ± 11
	Victoriopisa	8.8 ± 1.9	1.4 ± 0.3	172 ± 7.5	1.0 ± 0.2	29 ± 8.8	107 ± 10	4,4,2	100 ± 20
	Tellina	21 ± 3.3	7.8 ± 2.4	335 ± 126	5.8 ± 1.3	123 ± 30	614 ± 161	6,6,6	100 ± 0.0
AVS_{High}									
static	***Melita***	7.8 ± 3.2	1.8 ± 0.2	112 ± 18	8.1 ± 1.3	3.2 ± 0.6	113 ± 3.7	5.6.4	94 ± 11
bioturb.	***Melita***	9.9 ± 3.6	4.6 ± 2.4	94 ± 21	25 ± 8.1	6.7 ± 2.8	264 ± 30	5.4.4	81 ± 6.3
	Victoriopisa	12 ± 3.8	0.9 ± 0.3	148 ± 24	1.0 ± 0.4	7.4 ± 2.9	117 ± 38	4,4,4	120 ± 0.0
	Tellina	19 ± 4.0	5.7 ± 2.6	290 ± 51	5.3 ± 1.3	87 ± 31	585 ± 68	6,6,6	100 ± 10

Melita = amphipod *Melita plumulosa* (males only), *Victoriopisa*= amphipod *Victoriopisa australiensis*, = bivalve *Tellina deltoidalis*. Bioaccumulated concentrations are based on dry weight of tissue. N= 3, mean ± SE.

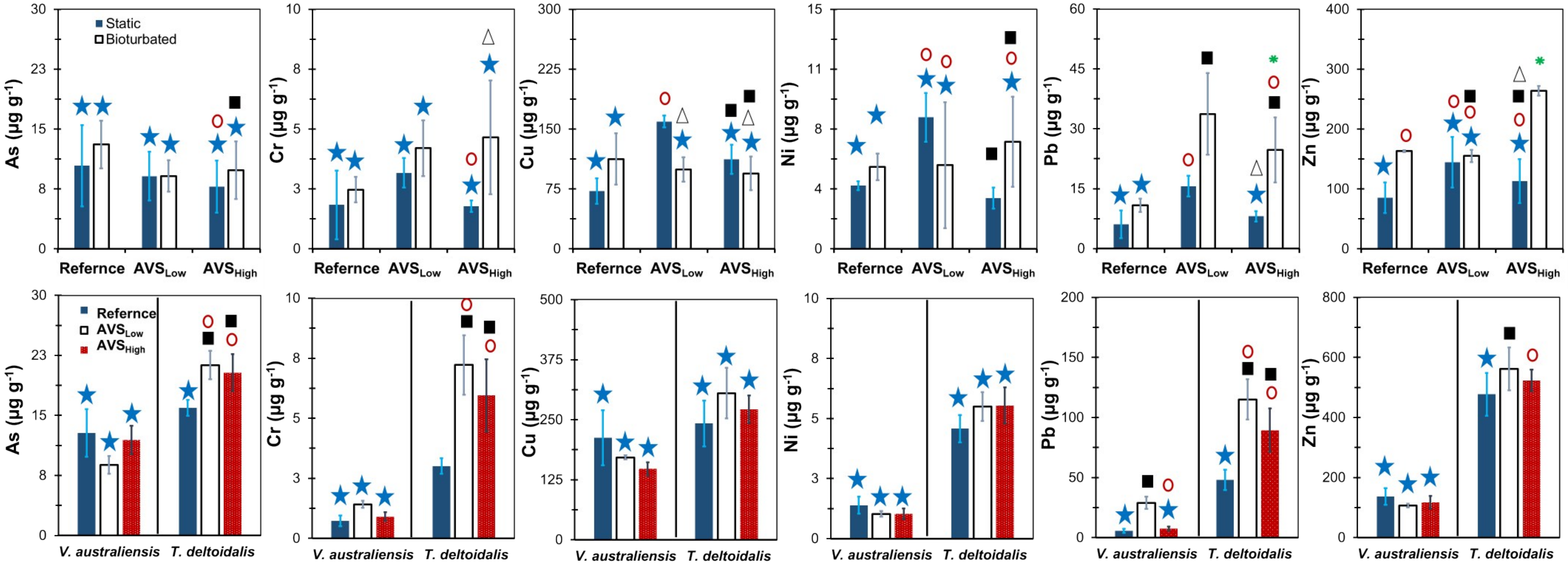


Figure S2. Metal accumulation data for *M. plumulosa* (top row) and bioturbators (bottom row) *V.australiensis* and *T. deltoidalis*. *Reference* = toxicity-control sediment, AVS_{Low}= metal- contaminated sediment, AVS_{High}= sulfidic metal-contaminated sediment. All data are mean ± SE (n=3). Different symbols indicate statistical differences between treatments ($p<0.05$).

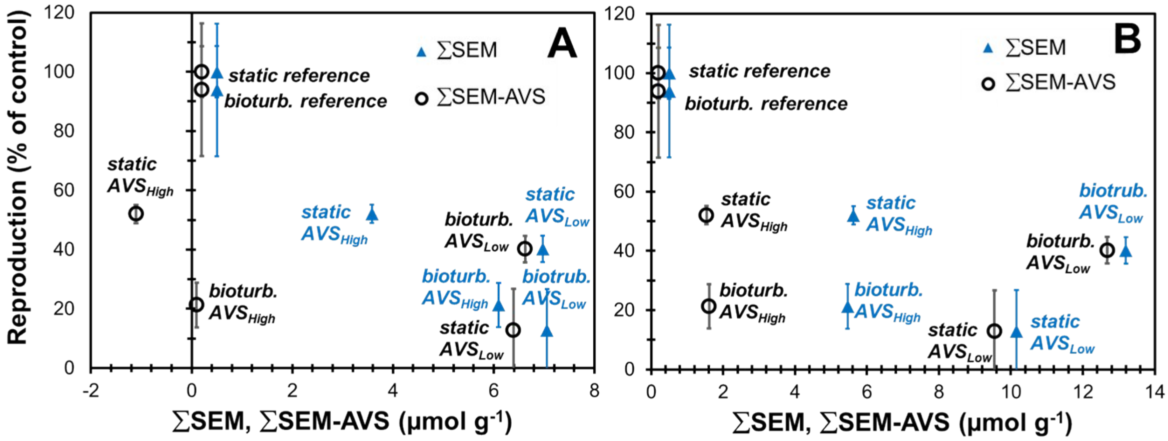


Figure S3. Relationship between sediment ∑SEM (blue triangles) and ∑SEM-AVS (open black circles) concentrations: A at the end of the bioturbation phase (Day 0 of toxicity testing phase, (n=3, mean ± SD)) and B: during the toxicity testing phase (average of Days 0, 6 and 10, (n=9, mean ± SD)) with reproductive toxicity to *M. plumulosa* (n=3 mean ± SE).

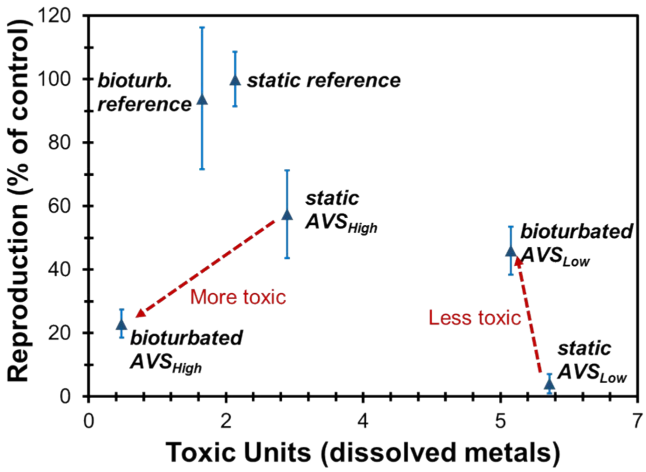


Figure S4. Relationship between water column dissolved metals (Cd, Cu, Ni, Pb and Zn) expressed as a toxic units (TUs) throughout the toxicity test phase and reproductive toxicity in *M. plumulosa* (n=3 mean ± SE). TU = ∑ [dM]/WQGV), where dM = Cd, Cu, Ni, Pb, and Zn. *Reference* = toxicity-control sediment, AVS_{Low}= metal-contaminated sediment, AVS_{High}= sulfidic metal-contaminated sediment.

Explanation for Figure S4:

A toxic unit (TU) approach based on water chemistry was used to assist in delineating relationships between dissolved metal (Cr, Cu, Ni, Pb, Zn) exposures and observed toxicity (Figure S4 of the SI). As expected for the AVS_{Low} sediment, lower toxicity in the *bioturbated* AVS_{Low} treatment corresponded with slightly lower TU (TU= 5.4) than in the undisturbed *static* AVS_{Low} (TU= 5.9) sediment. Conversely, the *static* AVS_{High} treatment was less toxic with higher TU (TU= 2.5), whereas the *bioturbated* AVS_{High} treatment was significantly more toxic with lower TU (TU= 0.4) than the *static* AVS_{Low} treatment. These predictions do not match the observed toxicity, and therefore correspond with the observations from the dissolved metal chemistry and suggests that perhaps bulk water samples were not adequate for predicting exposure and/or toxic effects.