Supplementary information

Ecotoxicity Assessment of the Water Extracts from Metal-Contaminated and Hydrocarbon-Contaminated Soils

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Table S1 Ecotoxicological tests of the soils

Contact	Organism	Endpoint of the measurement	Standard bioassay	References
Direct contact (whole soil)	*Avena sativa* L.	Growth	ISO/DIS 15799, 1999	[1]
	Brassica rapa L.	Growth	ISO/DIS 15799, 1999	
	Eisenia fetida	Mortality	OECD 207, 1984	
	Eisenia fetida	Avoidance behavior	ISO/DIS 17512-1, 2008	
	Folsomia candida	Mortality and reproduction	OECD, 2009	[2]
		Chronic toxicity tests	ISO, 2014	[3]
	Radish (Raphanus lativus L.)	Germination	ASTM, 2014	[4]
	Vibrio fischeri (Microtox)	Bioluminescence inhibition	Environmental, 1998	[5]
	Brassica juncea	Seed germination and growth	OECD, 2006	
	Escherichia coli	SOS-Induction Factor	SOS-Chromotest kit	
	Vibrio fischeri (Microtox)	Bioluminescence inhibition	-	[6]
	Wheat (*Triticum aestivum* L.)	14-day root elongation	-	[7]
	Rape (*Brassica napus* L.)	Seed germination rate, seedling growth in 7-day seed germination tests.	-	[8]
	Cucumber (*Cucumis Sativus* L.)			
	Garden pea (*Pisum sativum*)	Root nodulation and leghaemoglobin assay	-	[9]
	Eisenia fetida	Acute, chronic and genotoxicity assasys	OECD, 1984, 2004	
	Wheat (Triticum aestivum)	5 d-shoot and root elongation assays and 14 d-shoot biomass measurement	ISO 11269-1; 11269-2, 2012	[10]
	Sorghum (Sorghum bicolor)			
	Tomato (Lycopersicon esculentum)			
	Lettuce (Lactuca sativa)			
	Garden cress (Lepidium sativum)			
	White mustard (Sinapis alba)			
	Lactuca sativa	Germination and growth	ISO, 2005	[11]

		inhibition (14 d)		
	Eisebua fetida	Survival (14 d)	ISO, 1993	
	Folsomia candida	Avoidance (20 min)	Not standardized	
	Folsomia candida	Reproduction (40 d)	ISO, 1999	
Indirect contact (Soil-water extract)	*Avena sativa* L.	Germination index (GI) GI (%)= [RSG×RRG]/100	Fuentes et al., 2004; Alvarenga et al., 2012	DIN 38414-S4 (1984).[1]
	Brassica rapa L.			
	Lepidium sativum L.			
	Vibrio fischeri	Bioluminescence inhibition	ISO 11348-2, 1998	
	Daphnia magna	Immobilization/mortality	ISO 6341, 1996	
	Vibrio fischeri (Microtox)	Bioluminescence inhibition	EN 12457-2 protocol, EC 2002.	Liquid-to-solid ratio of 100 g/L [2]
	Vibrio fischeri (Microtox)	Bioluminescence inhibition	-	The leaching solution comprised a 7.0 g soil sample mixed with 21 mL NaCl solution 2 % (w/v) heated to 50 °C and centrifuged at 160 rpm for 90 min. [5]
	Vibrio fischeri (Microtox)	Bioluminescence inhibition	Microbics Corporation, 1995.	Soil elutriates were obtained after mixing 15 g of soil and 30 g Milli Q water for 24 h. [6]
	Luctuca sativa L.	Germination, root and shoot elongation and fresh biomass	Filter paper test. OECD 208, 2006.	Solid-to-water ratio of 1:10 (m/V) according to DIN 38414-S4 (1984). [12]
	Avena sativa L.			
	Microalgae *(Pseudokirchneriella subcapitata)*	Growth inhibition	OECD 201, 1984.	
	Microcrustacean *(Daphnia magna)*	Immobilization	ISO 6341, 1996.	
	Azomonas agilis	Dehydrogenase activity	Hungarian standard (HS 21978-30)	For soil extraction 40 g of the soil sample was suspended in 80 ml deionised water. [13]
	Mustard seeds *Sinapis alba*	Length of germinated root and shoot	Hungarian standard (HS 21978-8)	

	Panagrellus redivivus	Reproduction inhibition	-	
	Tetrahymena pyriformis	Reproduction inhibition	-	
	Mustard seeds *Sinapis alba*	Root and shoot elongation	-	Czech legislation for characterization of waste and contaminated soils. Solid-to-water ratio of 1:10 (m/V) according to DIN 38414-S4 (1984). [14]
	Chlorococcal algae *Desmodesmus subspicatus*	Growth inhibition	ISO 8692, 2004	
	Brassica chinensis	Seed germination and root elongation	Wong et al., 2001	Sludge extracts were prepared by shaking 2 g fresh sewage sludge with 20 ml of milli-Q water. [15]
	Vibrio fischeri	Luminescence inhibition (30 min)	ISO 1998a, 1998b	[11]
	Micro-crustaceans Daphnia magna	Immobilization (48 h)	ISO, 1996	
	Algae Pseudokirchneriella subcapitata	Growth inhibition (72 h)	ISO, 2004	
	Rotifers Brachionus calyciflorus	Growth inhibition (48 h)	ISO 2008a, 2008b	
	Animals Mouse lymphoma cells	Chromosomic change (24 h)	Not standardized	
	Zebrafish larvae	Developmental neurotoxicity	-	The village soil leachate composed of 100 mL deionized water and 100 g village soil sample (1 g/mL) was shaken at 145 rpm on a thermostatic oscillator at 28°C (3d). [16]
	Zebrafish embryos	Developmental toxicity and thyroid hormone assay	-	100 g of village soil samples was added 100 mL of ultrapure water (1 g/mL). [17]
	Zebrafish embryos	Developmental toxicity	-	[18]

	Hepatocellular carcinoma HepG2 cell	Cancer cell metastasis	-	Following filtration with a 100-mesh sieve, a 100 g sample was added to a sealed beaker with 100 mL double-distilled water. [19]
	Vibrio fischeri	Bioluminescence inhibition	-	DIN 38414-S4 [20]
	Lactuca sativa	Germination; Root elongation	-	
	Raphanus sativus	Germination; Root elongation	-	
	Dictyostelium discoideum	Mortality; Endocytosis; Replication	-	

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