



HOKKAIDO UNIVERSITY

Title	Phytofiltration of arsenic and cadmium by using an aquatic plant, <i>Micranthemum umbrosum</i> : Phytotoxicity, uptake kinetics, and mechanism
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Supplementary Table 1 Composition of nutrient elements (oven dry basis) of *Micranthemum umbrosum* plant parts grown in As tainted water

Dose ($\mu\text{g L}^{-1}$)	Plant parts	K (mg g^{-1})	Ca (mg g^{-1})	Mg ($\mu\text{g g}^{-1}$)	Mn ($\mu\text{g g}^{-1}$)	Zn ($\mu\text{g g}^{-1}$)
0	Leaf	23.33 $\pm$ 0.59	3.54 $\pm$ 0.10	22.66 $\pm$ 0.37	225.09 $\pm$ 8.32	82.57 $\pm$ 1.46
	Stem	17.54 $\pm$ 0.73	2.89 $\pm$ 0.15	18.03 $\pm$ 1.11	120.05 $\pm$ 3.94	39.26 $\pm$ 1.88
	Root	8.38 $\pm$ 0.63	3.94 $\pm$ 0.08	4.87 $\pm$ 1.62	93.98 $\pm$ 5.65	37.94 $\pm$ 1.19
200	Leaf	24.63 $\pm$ 0.33	3.63 $\pm$ 0.10	**40.91 $\pm$ 0.72	**135.11 $\pm$ 2.00	**70.02 $\pm$ 1.84
	Stem	**25.73 $\pm$ 0.69	4.69 $\pm$ 0.26	**32.56 $\pm$ 1.89	143.95 $\pm$ 4.18	79.85 $\pm$ 1.95
	Root	10.75 $\pm$ 0.81	3.01 $\pm$ 0.12	9.01 $\pm$ 0.81	170.60 $\pm$ 2.05	99.69 $\pm$ 4.58
500	Leaf	21.88 $\pm$ 0.30	*3.38 $\pm$ 0.06	**32.50 $\pm$ 3.10	**83.58 $\pm$ 1.39	**63.27 $\pm$ 1.37
	Stem	**25.99 $\pm$ 0.31	4.17 $\pm$ 0.14	**28.16 $\pm$ 1.19	**92.79 $\pm$ 2.85	78.76 $\pm$ 4.30
	Root	12.13 $\pm$ 1.32	4.93 $\pm$ 0.20	6.26 $\pm$ 0.75	148.64 $\pm$ 7.73	93.65 $\pm$ 2.26
1000	Leaf	17.22 $\pm$ 0.65	**2.77 $\pm$ 0.05	*24.00 $\pm$ 1.51	**68.47 $\pm$ 3.49	**52.79 $\pm$ 2.65
	Stem	**22.47 $\pm$ 0.48	4.06 $\pm$ 0.03	15.91 $\pm$ 1.31	*89.84 $\pm$ 0.98	74.76 $\pm$ 2.74
	Root	9.50 $\pm$ 1.02	4.93 $\pm$ 0.24	3.77 $\pm$ 1.46	108.50 $\pm$ 6.90	89.21 $\pm$ 4.27

Each value indicated as Mean $\pm$ SEM (n=3); ** and * showed significantly difference against control or 0 ($\mu\text{g L}^{-1}$) at $p < 0.01$ and 0.05 , respectively.

Supplementary Table 2 Composition of nutrient elements (oven dry basis) of *Micranthemum umbrosum* plant parts grown in Cd contaminated water

Dose ($\mu\text{g L}^{-1}$)	Plant parts	K (mg g^{-1})	Ca (mg g^{-1})	Mg ($\mu\text{g g}^{-1}$)	Mn ($\mu\text{g g}^{-1}$)	Zn ($\mu\text{g g}^{-1}$)
0	Leaf	23.33 $\pm$ 0.59	3.54 $\pm$ 0.10	22.66 $\pm$ 0.37	225.09 $\pm$ 8.32	82.57 $\pm$ 1.46
	Stem	17.54 $\pm$ 0.73	2.89 $\pm$ 0.15	18.03 $\pm$ 1.11	120.05 $\pm$ 3.94	39.26 $\pm$ 1.88
	Root	8.38 $\pm$ 0.63	3.94 $\pm$ 0.08	4.87 $\pm$ 1.62	93.98 $\pm$ 5.65	37.94 $\pm$ 1.19
300	Leaf	23.64 $\pm$ 0.93	3.73 $\pm$ 0.08	**19.04 $\pm$ 0.11	**407.88 $\pm$ 21.71	*71.73 $\pm$ 2.43
	Stem	21.28 $\pm$ 1.35	4.55 $\pm$ 0.38	14.37 $\pm$ 1.61	**361.38 $\pm$ 22.37	67.18 $\pm$ 4.73
	Root	*12.53 $\pm$ 0.83	*3.56 $\pm$ 0.17	5.61 $\pm$ 0.65	*301.14 $\pm$ 30.53	48.04 $\pm$ 2.35
1000	Leaf	23.32 $\pm$ 1.09	3.33 $\pm$ 0.11	*20.16 $\pm$ 0.41	*301.67 $\pm$ 0.89	**67.39 $\pm$ 2.03
	Stem	18.66 $\pm$ 0.52	3.76 $\pm$ 0.06	20.30 $\pm$ 0.53	**168.87 $\pm$ 5.36	54.61 $\pm$ 2.62
	Root	**14.87 $\pm$ 0.73	**1.14 $\pm$ 0.01	7.00 $\pm$ 0.79	*155.77 $\pm$ 11.12	42.38 $\pm$ 1.04

Each value indicated as Mean $\pm$ SEM (n=3); ** and * showed significantly difference against control or 0 ($\mu\text{g L}^{-1}$) at $p < 0.01$ and 0.05 , respectively.

Supplementary Table 3 Linear regression model for uptake kinetic parameters of inorganic and organic As species; and Cd influx into *Micranthemum umbrosum*

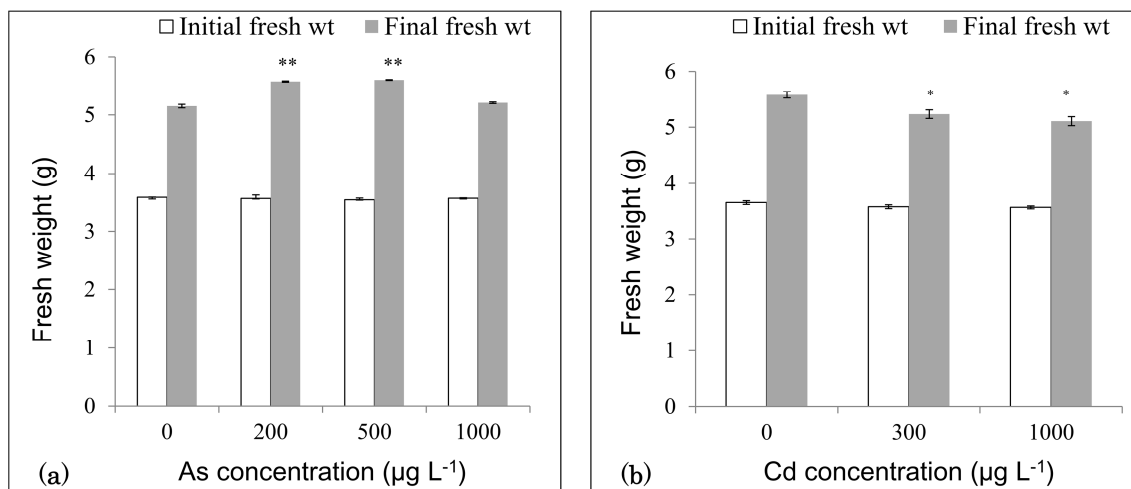
Species	Linear regression model				
	V_{max} ($\mu\text{g g}^{-1}$ DW 24h $^{-1}$)	K_m ($\mu\text{g L}^{-1}$)	R^2	a (slope)	b (Intercept)
Arsenite	420	454.31	0.9027	0.4191	19.599
MMAA	90	354.43	0.7679	0.0763	17.957
DMAA	55	378.6	0.7047	0.0375	13.325
Cadmium	390	481.62	0.9684	0.3484	27.204

Supplementary Table 4 Amino acids content in 1000 $\mu\text{g L}^{-1}$ Cd treated leaf (n=3)

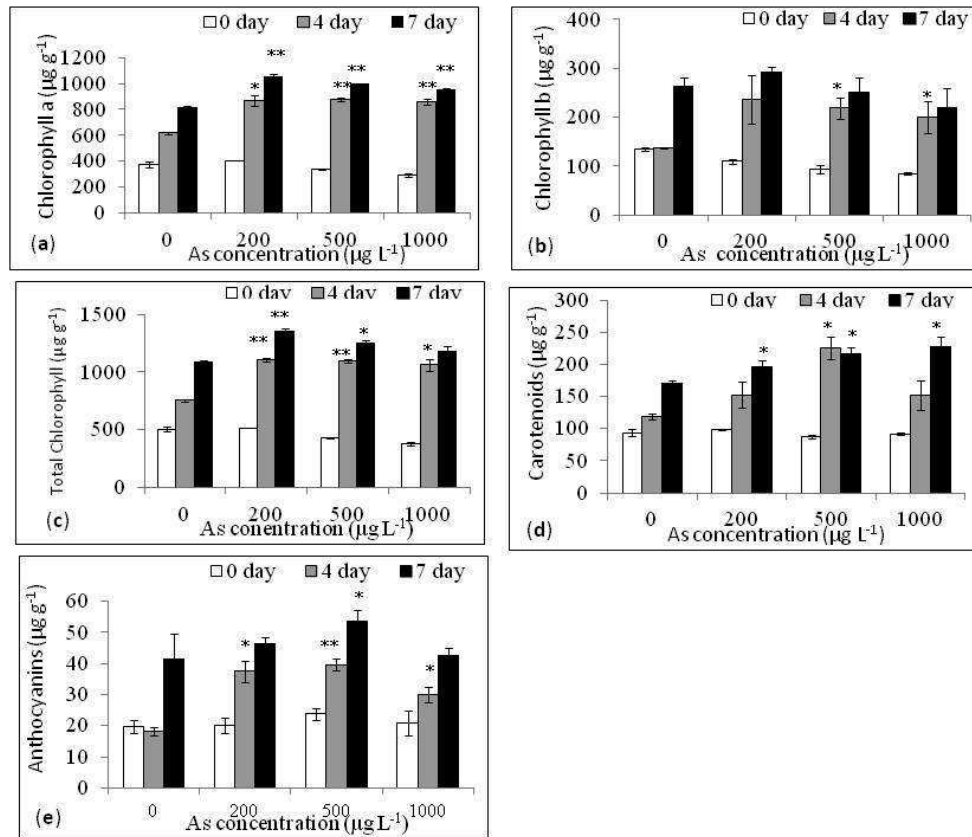
Amino acids	Mean (nmol $\pm$ SEM)	%	Amino acids	Mean (nmol $\pm$ SEM)	%
CysCOOH	0.95$\pm$0.06	2.13	Met	0.04 $\pm$ 0.01	0.08
Asp	5.87 $\pm$ 2.73	13.19	Ile	2.41 $\pm$ 1.00	5.43
Thr	2.98 $\pm$ 1.34	6.69	Leu	3.66 $\pm$ 1.52	8.23
Ser	3.09 $\pm$ 1.30	6.94	Tyr	0.05 $\pm$ 0.03	0.11
Glu	5.20 $\pm$ 2.20	11.69	Phe	1.53 $\pm$ 0.71	3.43
Gly	5.49 $\pm$ 1.96	12.34	Lys	3.09 $\pm$ 1.46	6.95
Ala	4.43 $\pm$ 1.90	9.96	His	0.75 $\pm$ 0.30	1.68
Cystine	0.11 $\pm$ 0.03	0.25	Arg	1.22 $\pm$ 0.51	2.74
Val	3.64 $\pm$ 1.57	8.17	Total	44.48$\pm$17.89	100

Supplementary Table 5 Aquatic plant that accumulate As and Cd from water environment

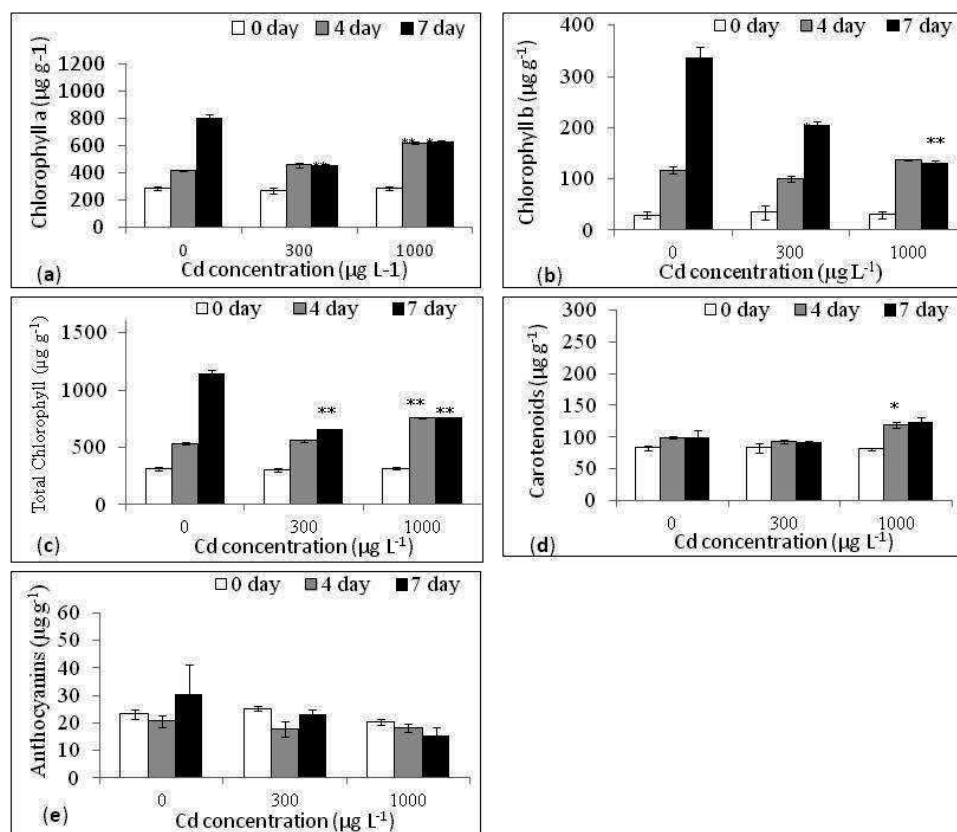
Scientific name	Element uptake	Amount uptake mg kg ⁻¹ (DW)	References
<i>Eleocharis acicularis</i> (L.) Roem. & schult.	As	1470	Sakakibara <i>et al.</i> , 2011
<i>Pteris vittata</i> L.	As	1400	Baldwin and Butcher,
<i>Pteris cretica</i> (L.) cv. <i>Mayii</i>	As	1200	2007
<i>Wolffia globosa</i> (Roxb.) Hartog & Plas	As	>1000	Zhang <i>et al.</i> , 2009
<i>Hydrilla verticillata</i> (L.f.) Royle	As	325	Lee <i>et al.</i> , 1991
<i>Azolla caroliniana</i> Willd.	As	280	Zhang <i>et al.</i> , 2008
<i>Spirodela polyrhiza</i> (L.) Schleid.	As	0.353 µmol g ⁻¹	Rahman <i>et al.</i> , 2007a
<i>Micranthemum umbrosum</i> (J.F.Gmel.) Blake	As	1219	Current study
<i>Limnocharis flava</i> (L.) Buchenau	Cd	>1000	Abhilash <i>et al.</i> , 2009
<i>Azolla pinnata</i> R. Br.	Cd	740	Rai, 2008
<i>Wolffia globosa</i> (Roxb.) Hartog & Plas	Cd	500	Xie <i>et al.</i> , 2013
<i>Echinochloa polystachya</i> (Kunth) Hitchc	Cd	230-300	Solis-Dominguez <i>et al.</i> , 2007
<i>Helianthus annuus</i> L.	Cd	40-330	Zhi-xin <i>et al.</i> , 2007
<i>Rorippa globosa</i> (Turcz. Ex Fisch. & C.A. Mey.) Hayek	Cd	>100	Wei <i>et al.</i> , 2008
<i>Micranthemum umbrosum</i> (J.F.Gmel.) Blake	Cd	799	Current study



Supplementary Fig. 1 Initial and final fresh weight of plant grown in 0, 200, 500, 1000 $\mu\text{g As L}^{-1}$ (a) and 0, 300, 1000 $\mu\text{g Cd L}^{-1}$ (b) water. Error bars indicate mean $\pm$ SEM (n=3). ** and * denote significant differences at $P < 0.01$ and 0.05 , respectively, compared with their respective control.



Supplementary Fig. 2 Chlorophyll a (a), chlorophyll b (b), total chlorophyll (c), carotenoids (d) and anthocyanins (e) content of *M. umbrosum* leaf grown in 0, 200, 500 and 1000 $\mu\text{g As L}^{-1}$ water at 0, 4, and 7 days interval. Error bars indicate mean $\pm$ SEM (n = 3). ** and * denote significant differences at $P < 0.01$ and 0.05 , respectively, compared with their respective control (day).



Supplementary Fig. 3 Chlorophyll a (a), chlorophyll b (b), total chlorophyll (c), carotenoids (d) and anthocyanins (e) content of *M. umbrosum* leaf grown in 0, 300 and 1000 $\mu\text{g Cd L}^{-1}$ water at 0, 4, and 7 days interval. Error bars indicate mean $\pm$ SEM (n=3). ** and * denote significant differences at $P < 0.01$ and 0.05 , respectively, compared with their respective control (day).