Phytoremediation of arsenic- and molybdenum-contaminated alkaline wastewater by *Eleocharis acicularis* in winter in Japan

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Abstract. Phytoremediation using aquatic plants is a sustainable, low-cost measure for remediating water contaminated by toxic heavy metals. In this study, we conducted a channel experiment using *Eleocharis acicularis* in heavy metal-contaminated mildly alkaline wastewater under unfavorable plant habitat conditions in winter in northeastern Japan. The wastewater from an embankment consisting of Neogene marine sediments had a temperature of 10-15 °C and a pH of about 9, and it contained ~0.02 mg/L of As and ~ 0.23 mg/L of Mo. About 16 kg (fresh weight) of *E. acicularis* was laid in a plastic channel measuring 30 cm in width by 20 m in length, and the channel was enclosed in a tunnel greenhouse. The experiment was conducted for the 3 months from November 2015 at an average flow rate of 0.3 L/min and an air temperature of -4 to 19 °C. No reductions in As or Mo concentrations in the outflow were detected. However, at 3 months, the *E. acicularis* showed accumulations of ~7 mg/kg As and ~18 mg/kg Mo as dry weight, indicating that this remediation method is workable in an unfavorable low-temperature, mildly alkaline environment.

1. Introduction

Toxic heavy metal elements, such as arsenic (As) and cadmium (Cd), in drinking water are a key issue of concern to human and animal health [1-2]. In light of this, a sustainable, low-cost remediation method is required for the treatment of contaminated wastewater that is drained from soil and rock excavated from building sites, tunnels and mines. Phytoremediation is method of remediating contaminated soil and water by using plants that are able to hyperaccumulate organic and inorganic contaminants, including heavy metal elements [3]. Phytoremediation is considered to be more sustainable than methods that use chemical media or physical removal and sealing of the excavation site [4]. Phytoremediation using aquatic plants has advantages in that some species can be grown in sized sheets in free-floating, submerged or emergent conditions, and can take up elements directly through their roots and leaves from flowing or pooled water [5]. The sized sheets are easy to install, replace and dispose of, making them convenient in terms of treatment site design and management. Some aquatic macrophytes can be grown in or on water without soil. This enables complex factors such as surface adsorption and desorption of heavy metal elements in a soil medium to be excluded, which is advantageous in simplifying the evaluation of element uptake efficiency from wastewater to

aquatic macrophytes. However, a disadvantage is that the range of growth conditions favorable for aquatic plants in wastewater (e.g., chemistry, temperature and flow rate of wastewater) is more limited than in a soil environment and there is no buffering of pH and nutrients by soil material. Therefore, many case studies under various conditions of water and weather are needed before phytoremediation can be used for the various types of contaminated wastewater.

This paper focuses on the phytoremediation of wastewater contaminated with heavy metals by using the aquatic macrophyte *Eleocharis acicularis*. This species is known as a hyperaccumulator of elemental heavy metals such as As, cupper (Cu), zinc (Zn), lead (Pb), indium (In), silver (Ag) and Cd [6-10] and it is also good at accumulating antimony (Sb) [11]. *E. acicularis* grows as an autochthon worldwide, including in northern Japan, which has snowfall and subzero air temperatures. It can grow in submerged, emergent and soil conditions in nature, and in sheets it can grow in a water pool with freely flowing water. Many studies using aquatic plants have demonstrated its effectiveness at accumulating toxic heavy metal elements in the field and in the laboratory [5]. To our knowledge, no studies have addressed the phytoremediation of actual wastewater flow in a channel with aquatic macrophytes under winter weather conditions, where the low temperatures and shorter day length would be unfavorable for plant growth and element uptake. The main objectives of the present study are to understand a phytoremediation efficiency in a 90-day experiment conducted in the winter from November 2015 to February 2016 using *E. acicularis* in mildly alkaline wastewater contaminated with As and molybdenum (Mo) in a flow-controlled channel at a wastewater treatment site in Japan at about 38° North latitude.

2. Data and method

2.1 Site description and experimental design

In this study, an experiment channel was set up at a wastewater treatment site about 150 meters above sea level in Sendai City, northeastern Japan. At this site, an embankment mainly consisting of about 800,000 m^3 of Neogene (late Miocene to early Pliocene) marine sedimentary rocks steadily drains mildly alkaline wastewater contaminated with As at a flow rate of about 20 L/min. The wastewater from the embankment is first collected in a drainage tank before an As treatment plant.

2.2 Experimental design

The experiment channel, with a total length of 20 m, is set adjacent to the drainage tank. The experimental channel was constructed from two small channels that were 10 m in length (figure 1). The channels are made of 1 cm thick PVC resin plates with 30 cm in width and 10 cm in sidewall

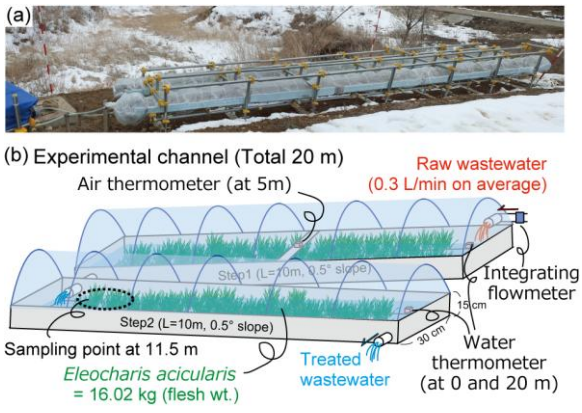

Figure 1. (a) The experimental channel set at a wastewater treatment site. (a) Photo. (b) Diagram.

Table 1. Cumulative volume of inflow, temperature and pH in raw and treated wastewater during the experiment.

Time	Cumulative	Raw (0m)		Treated (20m)	
(day)	flow vol. (L)	T(°C)	pH	T(°C)	pH
0.00	0	15.5	8.94	Unreached	
0.05	188	16.3	-	-	6.11
0.10	300	13.6	-	12.9	6.74
0.23	466	11.1	-	6.6	7.12
0.79	898	11.5	-	6	7.39
0.98	1061	15.2	9.02	-	7.46
1.06	1120	14.7	9.00	14.8	7.4
1.13	1148	13.4	9.08	14	7.36
42	29428	17.1	9.32	20.9	7.89
89	39371	12.5	-	14.8	-
90	40098	10.1	-	5.9	-

Table 2. The results of chemical analysis of the certified reference material and the list of analytical methods for *E. acicularis* and water samples (M1 and M2). Abbrebiations: NA, not analyzed; MS: ICP-MS, AES: ICP-AES, Mo: spectrophotometric determination using molybdeum blue.

Reference materials		Al	Mn	Fe	Zn	As	Se	Mo	Cd	Sb	Pb	Sr	Mg	Na	Ca	K	P
	Method	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
TMDA-64.2	Reference	290	295	306	310	162	155	290	266	128	288	0.641	-	-	-	-	-
	M1	300	297	303	306	159	156	287	274	133	306	0.654	5.14	8.94	21.4	0.99	0.023
	M2	302	NA	329	314	158	141	270	253	120	283	0.699	6.58	10.53	24.9	NA	NA
CRM-7202b	Reference	-	-	-	-	-	-	-	-	-	-	-	1.23	3.64	4.51	0.83	-
	M1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1.03	3.42	4.39	0.99	3.35
	M2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.04	1.19	3.63	4.48	0.88	NA
Analytical method		Al	Mn	Fe	Zn	As	Se	Mo	Cd	Sb	Pb	Sr	Mg	Na	Ca	K	P
E. acicularis.		MS	AES	AES	MS	MS	MS	MS	MS	MS	MS	MS	AAS	AAS	AAS	AAS	Mo
	M1	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	AAS	AAS	AAS	AAS	Mo
	M2	MS	-	MS	MS	MS	MS	MS	MS	MS	MS	AES	AES	AES	AES	AES	-

height. The two channels were connected by hosepipe and placed with slope angle of 0.5° (figure 1b). To protect plants in the channel from low temperatures and snowfall in winter, the channel was covered with a tunnel greenhouse of 0.2-mm-thick clear plastic sheeting and the sidewalls and bottom were covered with 20-mm-thick urethane foam (figure 1). The rate of inflow of the raw wastewater could be roughly controlled by faucet handle at the tank and was measured by an integrating flowmeter (NW05-NTN of Aich Tokei Denki Co., Ltd.) at the inflow point.

2.3 Sample preparation

Sheets of *E. acicularis* with a plant height of 10-15 cm, a width of 30 cm and a length of 60 cm were weighed for fresh weight after being hung out for 1-2 hours to dry off the water that was added for shipping. The fresh weight of each sheet ranged from 261 to 906 g, and the total fresh weight in the channel was 16.02 kg. After weighing, the sheets were laid in the channel so as to leave 50 cm free of plants at the uppermost and lowermost parts of each channel, in order to keep clearance for water flow. The area density of the *E. acicularis* was 29.74 kg/m^2 as fresh weight.

2.4 Sampling and measurement

During the channel experiment, 7 inflow and 17 outflow samples were collected using a PP-bottle that was rinsed with ultrapure water after being soaked in 1M nitric acid for at least a week. At sampling, the water temperature and the pH of inflow and outflow were measured by portable tester (HI98121 Hanna Inc.). As pre-treatment, 0.6 ml of nitric acid (69% HNO_3 for ultratrace analysis from Wako Pure Chemical Industries, Ltd.) was added to a 50-ml sample after filtration using a syringe filter with a pore diameter of 0.45 µm in the laboratory.

Several pinches of *E. acicularis* were sampled from around 11.5 m from the upstream end of the channel 13 times during the experiment (table 3). The sample was dried at 50°C in a drying oven for at least 24 hours and then was measured for dry weight. The sample was powdered as whole plant using sample mixer (IKA A11 basic) with stainless steel blade. Acid digestion of *E. acicularis* was performed, according to the following steps. 0.1 g of dried powder was mixed with 5 ml ultrapure-grade HNO_3 and 2 ml H_2O_2 in a closed, 100-ml PTFE vessel, and then the mixture was digested with the assistance of a microwave (Titan MPS PerkinElmer Co., Ltd.), with the sequence of 5 minutes at 150°C, 15 minutes at 190°C and 10 minutes at 50°C. The analytical solution was prepared by dilution up to 50 ml with ultrapure water.

The chemical components in the wastewater and the *E. acicularis* samples were analyzed using one of four methods: Inductively-coupled plasma mass spectrometry (ICP-MS), Inductively-coupled plasma atomic emission spectrometry (ICP-AES), atomic adsorption spectroscopy (AAS) and adsorption spectroscopy using molybdenum blue. Table 2 lists the method that was used for each element and sample type. The methods for water samples are subdivided into M1 and M2. The

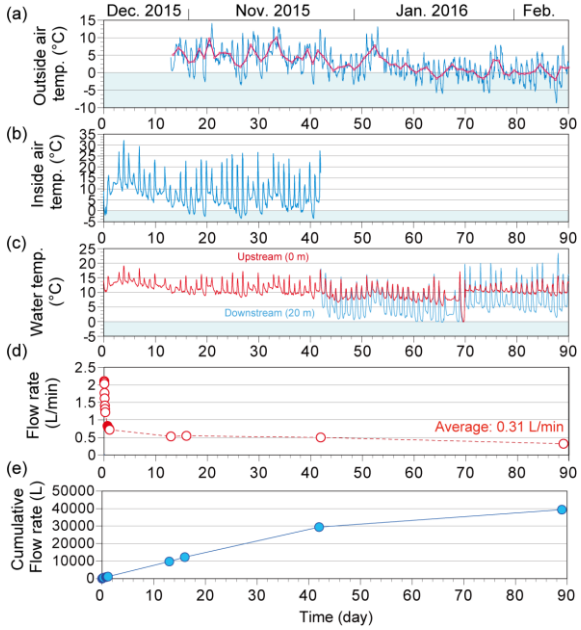

Figure 2. (a) Outside air temperture (blue: raw data; red: daily average), (b) inside air temperature, (c) water temperature, (d) flow rate and (e) cumulative flow volume during the experiment.

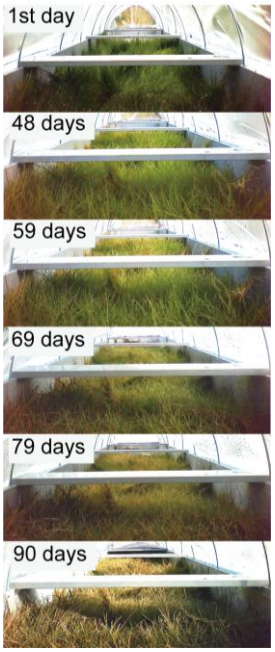

Figure 3. Photos taken by fixed-point camera downstream-ward at 16 m from the inflow point.

analytical accuracy of each element was evaluated by using certified reference materials of TMDA 64.2 (National Water Research Institute of Environment Canada) and CRM-7202b (National Institute for Environmental Studies, Japan). The elements Al, Mn, Fe, Zn, As, Se, Mo, Cd, Sb, Pb and Sr were measured within ±10% error, and the elements Sr, Mg, Na, Ca and K were measured within ±6%. The elements of Mg and K as determined by the M1 method show greater errors within ±20%.

3. Results

3.1 Experimental conditions and plant growth

The change in air temperature outside of the channel from day 12 to day 90 is graphed in Figure 2a. The outside temperature ranged from -8.7 to 14.1°C. On successive days between day 44 and day 51 and after day 56, the daily minimum air temperature was below zero at midnight and the daily mean was below 5°C. The air temperature inside the tunnel greenhouse in the upper channel ranged from -3.5 to 32.1°C from the start to day 43 (figure 2b). The tunnel greenhouse's contribution to the increase in inside air temperature can be estimated as +1.8 to +17.2°C from the daytime temperature difference between the inside and outside of the channel (figure 2b). The water temperature at the point of inflow during the 90 days was 11.6°C on average and ranged from 5.4-19.1°C. At the point of outflow, the range of water temperature was greater than at inflow. Between day 60 and day 69, the water temperature of outflow was almost 0°C. The total volume of flow into the experiment channel during the 90 days was 40,098 L. The flow rate decreased from about 2 L/min at day 1 to 0.5 L/min at day 43. The average flow rate during the experiment was 0.31 L/min (table 1 and figure 2d). Throughout the experiment period, the inflow showed no turbidity and the pH was roughly stable, at the mildly alkaline values of 9.0-9.3 (table 1). However, the treated wastewater showed pale green turbidity during the first several hours and turned transparent after that. The pH of the treated wastewater gradually changed from 6.11 at the start to 7.89 at day 43 (table 1). The recorded photos of *E. acicularis* growth at 16 m from the inflow point represent a lowering of water depth at day 61 and a further lowering at days 65-66, which is recorded as a decrease in flow rate between days 40 and 90

Table 3. The chemical compositions of the *Eleocharis acicularis*, the raw wastewater and the treated wastewater. Values with "<" were below the detection limit.

E. acicularis		Al	Mn	Fe	Zn	As	Se	Mo	Cd	Sb	Pb	Sr	Mg	Na	Ca	K	P
Time (day)		mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
0		1050	85.9	1727	149.5	0.51	0.38	4.82	11.09	5.47	3.00	29.5	1230	504	4357	17705	6323
0.30		792	95.1	1716	176.5	0.72	0.38	7.78	7.55	3.77	2.74	31.6	971	555	5614	15568	6142
0.73		305	99.7	803	164.9	0.53	0.25	6.61	7.31	5.50	1.86	27.1	1088	559	4816	23524	6880
1.13		1915	123.1	2486	206.3	1.34	0.26	9.80	7.55	6.59	4.10	31.1	1138	645	5602	16501	6183
4		1114	125.7	1463	141.0	1.28	0.22	12.46	5.92	0.62	1.72	28.7	1076	1021	5268	24247	6860
6		4543	170.2	3325	164.1	2.72	0.19	10.68	6.25	0.84	1.77	27.9	1224	1417	4903	25110	7132
8		373	173.0	435	139.6	0.98	0.13	9.74	4.25	0.31	0.60	23.4	1063	1588	4635	28545	7196
12		1129	250.0	824	132.8	1.65	0.10	11.66	4.26	0.93	0.69	25.4	1133	1679	4683	27189	6975
16		2947	240.6	2076	148.5	2.29	0.24	11.77	3.83	0.62	1.71	31.3	1109	1701	5887	21340	6259
32		154	708.4	461	117.9	2.64	0.18	17.37	3.25	1.27	0.73	32.8	991	2407	6275	22908	5539
42		177	511.8	283	109.7	2.71	0.14	16.54	2.74	0.34	0.59	29.5	970	2697	6040	22210	5419
63		1774	884.9	1811	109.6	9.51	0.22	26.62	1.80	0.73	1.33	35.5	942	2703	8058	14731	4350
89		5410	443.6	3877	105.0	8.79	0.33	17.84	1.18	0.67	1.96	36.8	1051	1187	8244	8134	4027
Raw wastewater		Al	Mn	Fe	Zn	As	Se	Mo	Cd	Sb	Pb	Sr	Mg	Na	Ca	K	P
Time (day)	Method	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
0	M1	<2	<2	<2	<1	26.6	<0.2	253	0.06	0.413	<0.2	0.860	3.98	88.4	259	11.6	0.045
1.13	M1	<2	<2	<2	1.6	26.1	<0.2	251	0.06	0.403	<0.2	0.859	4.05	89.5	263	12	0.043
13	M2	<30	NA	<32	<8	26.0	<0.2	239	<0.1	0.333	<0.7	0.818	3.80	82.3	237	12.4	NA
16	M2	<30	NA	<32	<8	25.0	<0.2	218	<0.1	0.402	<0.7	0.813	4.30	81.0	235	11.6	NA
42	M1	<2	38.7	<2	<1	27.1	<0.2	258	<0.06	0.411	<0.2	0.865	3.97	89	257	12.1	0.045
89	M2	<30	NA	<32	8.1	26.2	0.813	256	0.12	0.451	0.99	0.875	3.79	91.0	255	10.9	NA
90	M2	<30	NA	<32	<8	25.2	0.654	252	<0.1	0.333	<0.7	0.884	3.86	82.5	255	11.7	NA
Treated wastewater		Al	Mn	Fe	Zn	As	Se	Mo	Cd	Sb	Pb	Sr	Mg	Na	Ca	K	P
Time (day)	Method	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
0.05	M2	76	NA	65.6	548	19.4	<0.2	115	43.92	0.543	0.79	NA	NA	NA	NA	NA	NA
0.10	M2	<30	NA	<32	76.0	23.4	<0.2	211	7.38	0.364	<0.7	NA	NA	NA	NA	NA	NA
0.13	M2	<30	NA	<32	29.8	24.2	<0.2	222	3.61	0.363	<0.7	NA	NA	NA	NA	NA	NA
0.18	M2	<30	NA	<32	26.4	22.8	<0.2	223	2.85	0.373	<0.7	NA	NA	NA	NA	NA	NA
0.30	M1	2.6	137	5.25	15.8	24.1	<0.2	239	2.06	0.421	<0.2	0.859	4.21	90.4	258	13.5	0.445
0.73	M2	<30	NA	<32	8.5	24.7	<0.2	232	0.79	0.37	<0.7	NA	NA	NA	NA	NA	NA
0.89	M2	<30	NA	<32	<8	24.7	<0.2	242	0.71	0.34	<0.7	NA	NA	NA	NA	NA	NA
1.13	M1	<2	115	5.27	8.7	24.8	<0.2	249	0.95	0.455	<0.2	0.864	4.14	90.6	261	12.5	0.329
4	M2	<30	NA	<32	<8	25.3	<0.2	227	0.11	0.381	<0.7	NA	NA	NA	NA	NA	NA
6	M2	<30	NA	<32	<8	25.9	<0.2	232	0.13	0.33	<0.7	NA	NA	NA	NA	NA	NA
8	M2	<30	NA	<32	<8	25.5	<0.2	231	0.10	0.303	<0.7	NA	NA	NA	NA	NA	NA
12	M2	<30	NA	<32	<8	21.6	0.28	196	<0.1	0.252	<0.7	NA	NA	NA	NA	NA	NA
16	M2	<30	NA	<32	<8	15.3	<0.2	125	<0.1	0.259	<0.7	NA	NA	NA	NA	NA	NA
32	M2	<30	NA	<32	<8	28.3	<0.2	232	<0.1	0.388	<0.7	NA	NA	NA	NA	NA	NA
42	M1	<2	<2	<2	2.5	29.5	<0.2	262	0.19	0.618	<0.2	0.864	3.84	90.5	259	12.4	0.070
63	M1	<2	92.7	<2	<1	26.5	<0.2	267	<0.06	0.372	<0.2	0.869	3.84	90.8	269	12.3	0.038
89	M2	<30	NA	<32	<8	28.0	0.21	256	b.d.	0.382	<0.7	NA	3.98	98.3	297	NA	NA

(figure 2). After the lowering, the blighting of shoot tops was observed and it progressed to the whole body by day 90 (figure 3). The blighting is considered to have been caused by low-temperature damage associated with water depth lowering as the result of decreased water inflow. A similar low-temperature event also occurred between days 43 and 51. At that time, growth hindrance was not observed in photos because the water level did not decrease at that time.

3.2 Chemical compositions of wastewater

The timing of sampling and the chemical compositions of the raw and treated wastewater and *E. acicularis* are listed in table 3 and graphed in figure 4. The chemical compositions of the raw wastewater show no notable variations during the experiment. They averaged 26.0±0.7 µg/L for As and 247±14 µg/L for Mo, with a ±1σ error. These concentrations of As and Mo exceed the WHO guideline values for drinking water of 10 and 70 µg/L, respectively [2]. Most of the Cd concentrations are below the detection limit, and their maximum values are 0.12 µg/L. The maximum value of Mn concentration is 38.7 µg/L. The concentrations of Cd and Mn are far lower than the guideline values of 3 and 500 µg/L, respectively. The elements Se and Pb were not detected. The other elements showed the average concentrations of 0.853±0.027 mg/L for Sr, 0.044±0.001 mg/L for P, 3.96±0.18 mg/L for

Mg, 86.2±4.1 mg/L for Na, 252±11 mg/L for Ca and 11.8±0.5 mg/L for K. The elements Al, Mn, Fe and Zn were almost below their detection thresholds (table 3).

The treated wastewater immediately after the start (~day 0.3) shows notably higher concentrations of Cd, Zn, Mn, Fe, Pb, Sb, P and Al than the concentrations in the raw wastewater (table 3 and figure 4). These enrichments could be explained by mixing of groundwater and fertilizer attached to the *E. acicularis*, which were used for growing it in sheet form before the experiment. From day 1 to day 89, there were no noticeable differences in the element concentrations between the raw and treated wastewaters, except for Cd and for temporary decreases in As, Mo and Sb concentrations at day 12 and day 16. The concentration of Cd showed a rapid decrease from day 0.05 to day 0.3, and the decrease continued gently to day 8.

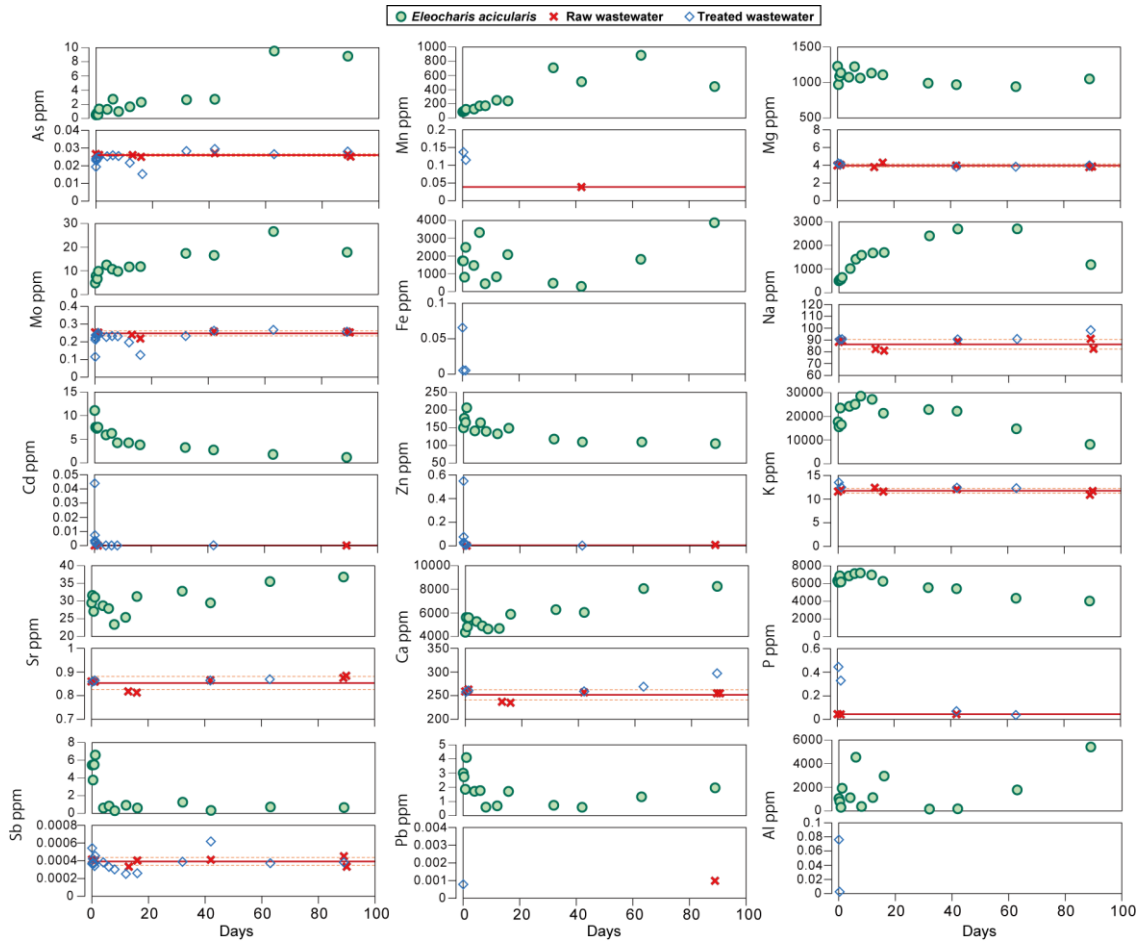

Figure 4. The graphs of measurement chemical compositions of *E. acicularis* (upper graph in each element) and raw and treated wastewaters (bottom). The unit of ppm means mg/kg for *E. acicularis* and mg/L for wastewater. Red solid and dashed lines in the bottom graphs indicate the average values and one standard diviation for the raw wastewater samples.

3.3 Chemical compositions of E. acicularis

Concentrations of As, Mo and Mn in *E. acicularis* during the experiment all show a remarkable linear increase from day 0 to day 63 and then a slight decrease from day 89 (table 3 and figure 4). From day 0 to day 63, As increased from 0.51 to 8.79 mg/kg dry wt., Mo from 4.82 to 26.62 mg/kg dry wt. and Mn from 85.9 to 884.9 mg/kg dry wt. Although Na also tended to increase from day 0 to day 63, it did

not show a linear trend. The rate of increase gradually slowed toward day 63. The elements Sr and Ca increased slightly from 29.5 to 35.5 mg/kg dry wt. and from 4357 to 8058 mg/kg dry wt., respectively. The concentrations of Cd, Zn, Se and Sb decreased with time from 11.1 to 1.8 mg/kg dry wt., 150 to 110 mg/kg dry wt., 0.38 to 0.22 mg/kg dry wt. and 5.47 to 0.73 mg/kg in dry wt., respectively. The concentrations of K and P in plants are responsive to the addition or depletion of fertilizer in the growth environment. These elements increased slightly until day 8 and then decreased linearly. The variations of Fe and Al concentrations did not show any systematic tendencies.

4. Discussion

The nonsystematic decreases in As, Mo, Mn and Na concentrations observed in the sample of *E. acicularis* at day 89 suggests that the lowering of water level and flow rate at day 65 with low temperature from day 60 to day 66 caused to the plant shoots to wither in the channel. Therefore, this study evaluates the phytoremediation capability of *E. acicularis* for As, Mo and Mn, whose concentrations showed linear increases from the start to day 63 in the winter experiment.

The bio-concentration factor (BCF), a factor for estimating phytoremediation potential, is defined as the ratio of the concentration of a target element in plant tissue to that in the growth solution or soil [12]. In the most environments, the BCF for solution is higher than those for soil due to the small portion of the element responsible for uptake from soil materials and pore water to plant root. Thus, using BCFs for the comparison of phytoremediation potentials between soil and solution environments should be carefully considered. The translocation factor, which is defined as the ratio of an element's concentration in the shoot to its concentration in the root, is also a key factor in evaluating of phytoremediation ability [13]. In this study, *E. acicularis* was analyzed in terms of the whole plant, including the roots. Thus, the BCF value in this study was calculated by using the concentration in the whole plant (BCF_{whole}). The preliminary measurement for a sample with shoots separated from roots shows a translocation factor of 0.74 for As, 1.18 for Mo and 1.77 for Mn. These translocation factors are near 1. Thus, BFC_{whole} did not greatly differ from BCF for shoots and is comparable to the BCF obtained in a previous study.

4.1 Arsenic (As)

In our experiment from day 0 to 63, the As concentrations of *E. acicularis* increased from 0.51 to 8.79 mg/kg dry wt. in the wastewater flow with 0.026 mg/L and flow velocity about 0.31 L/min. The average uptake volume per day for As from the wastewater is calculated as 0.143 mg/kg dry wt./day at day 63. The BCF_{whole} for As in this study is ranges from 18-85 up to day 42 and then increases to 346 at day 69. A previous channel experiment in air temperature of 24.6-31.9°C in summer using *E. acicularis* in the same type of sloped channel with a length of 50 m and three 100-L inflows, with temperature of 26.2-34.5°C, pH 7.5, flow volume of 0.4-0.6 L/min and As concentration of 0.234 mg/L, found an increase in As concentration of *E. acicularis* from 0.14 to 4.16 mg/kg dry wt. [14]. The BCF_{whole} for solution were calculated from 0.37 to 17.8, for 2 days. The uptake rate for this 50 m channel experiment was calculated about 2 mg/kg dry wt./day, which is over 10 times higher than 0.145 mg/kg dry wt./day of this study. This corresponds to the increase in BCF_{whole} with time in our 63 days study. The lower As uptake efficiency in this study is considered as the difference of growth condition

In other case of soil environment, the two month cultivation of *E. acicularis* in the mine tailing site from September in Japan has been reported As concentration of 29.2-872 mg/kg dry wt. in shoots. These *E. acicularis* were grown in the contaminated sediment site with 188-1470 mg/kg dry wt. of As along a small river with flow velocity of 10 cm/s and As concentration of 0.039 mg/L. The BCF are estimated as 0.21-7.39 for the contaminated sediments and 1003-29966 for the river water. The BCF_{whole} for As in our flow-controlled channel experiment was lesser than 10-100 times as high as the BCF for the river water in the soil environment. This suggests that As taken up from the As and Mo contaminated wastewater in the adverse growth season is accumulated less efficiently than the As take up from the solutions in favorable growth media and season. Previous study for three other As

hyperaccumulating aquatic macrophytes (*Egeria densa, Elodea canadensis* and *Lagarosiphon major*) from natural riverside with similar As concentrations in river water (0.01-0.02 mg/L) to our channel experiment reported high BCFs of 4987-24847 [15]. These BCFs are also one-two order higher than the BCF_{whole} of *E. acicularis* in our channel experiment. Laboratory batch experiments at 24°C using *E. acicularis* of 100 g as flesh weight in a solution with an As concentration of 0.057 mg/L (i.e., about twice the concentration of the present study) found an uptake rate of 0.377 mg/kg dry wt./day and BCF of 113 for 10 days [8]. The solution volume is 1L and replaced it each days. The daily water volume to the plant flesh weight was assumed as 10 L/kg fresh wt./day. This volume was smaller than the roughly estimated as 32.7 L/kg fresh wt./day for 63 days. Our uptake rate of 0.143 mg/kg dry wt./day was lower than that of the laboratory experiment. However, our BCF results were much higher, indicating the possibility that water flow would be a key to effective element uptake or that a long-term experiment would be needed in order to appropriately evaluate phytoremediation by BCF in *E. acicularis* and other aquatic macrophytes. If the contribution of transpiration from the shoots to the decrease in As concentration is not considered, then the As treatment capability through day 63 of the experiment was 3.3%, calculated as the average daily uptake mass of 0.369 mg in *E. acicularis* divided by the daily flow mass of 11.23 mg in the raw wastewater flow. This low capability of As accumulation is corresponding to the result that there is little to distinguish the As concentrations in the treated wastewater from those in inflows thought the experiment.

4.2 Molybdenum (Mo)

Our experimental results may be the first scientific report of Mo transportation from contaminated wastewater to *E. acicularis*. The average uptake ratio of Mo was 0.346 mg/kg dry wt./day, and its efficiency is evaluated as 0.8% to 106.7 mg/L/day of the daily volume in wastewater flow. The BCF for Mo increases with time from 20.2 at day 1.13 to 50.8 at day 32 and on to 88.3 at day 63. However, the rate of increase tended to slow with time. This implies that the Mo concentration of 26.62 mg/kg dry wt. in *E. acicularis* at day 63 has almost reached the maximum accumulating concentration for the wastewater with the Mo concentration of 0.247 mg/L. The Mo accumulation by *E. acicularis* in this study did not exceed the hyperaccumulation value of 1,000 mg/kg dry wt. for typical heavy metal elements [5]. However, our results demonstrate that *E. acicularis* has an uptake performance for Mo that is similar to that for As, and that *E. acicularis* has a BCF_{whole} >1 for Mo accumulation. Thus, *E. acicularis* can be considered a good candidate as an accumulator of Mo from contaminated wastewater. Further experiments on accumulation and tolerance of Mo in *E. acicularis* should be carried out under various conditions.

4.3 Other element

The accumulation of Mn was 884.9 mg/kg dry wt. at day 63, which is lower than the lower limit concentration for Mn of hyperaccumulator plants at 10,000 mg/kg [15]. However, this Mn content exceeds the typical range for non-hyperaccumulators of 20-500 mg/kg dry wt. [16]. The average uptake rate was determined as 12.68 mg/kg dry wt./day. If use the maximum value of 0.0387 mg/L in the raw wastewater as the concentration in solution, then the BCF_{whole} for Mn is calculated as 240-20640. *E. acicularis* would be evaluated as having enough capability to accumulate Mn from wastewater. However, a previous report on Mn accumulation in *E. acicularis* concluded that this species is inadequate as an accumulator of Mn because the Mn concentration was higher in the roots than in shoots [8]. The preliminary analysis for separated root and shoot gives a translocation factor of 1.78, indicating that *E. acicularis* can also be a candidate as an accumulator of Mn.

4.4. Improvement point for this experimental design

The observations of the green colored shoots and increasing of As and Mo concentrations in plant body to day 63 indicate that *E. acicularis* has significant potential of As and Mo uptake from a contaminated wastewater in low air temperature condition fall below 5°C for the case that inflow water keep over 10°C in temperature and stable flow rate above 0.3 L/min. These element uptakes

appear not to be caused by plant growth but by basal metabolism with stable uptake rate at equilibrium with water temperature, flow rate and element concentration of solution, because of the almost linearly increasing of As and Mo with time and As and Mo concentrations in plant tend to decrease after shoots change to non-green colored. As a future experimental design, the estimation of element uptake efficiency of *E.acicularis* should be investigated by the comparison with the water and air temperature and growth rate, with or without several types of fertilizer.

5. Conclusions

The 90-day monitoring results of weather, water temperature, flow volume and chemical compositions in water and *E. Acicularis* showed a relationship between the temperature of the wastewater flow and the uptake of heavy metal elements. While the temperatures of the inside air and the water flow in the channel were continuously kept above 0°C by the tunnel greenhouse in the channel, the bio-concentration factors of As and Mo were found exceed 1. This demonstrates that phytoremediation using *E. acicularis* is workable for the elements As and Mo in moderately contaminated wastewater flow, even in low-temperature conditions that are inhospitable to aquatic macrophytes.

6. Acknowledgements

The authors would like to thank Mr. Mito of Transportation Bureau City of Sendai for allowing us to conduct experiment in this site and Mr. Endo, Mr. Naruse and Mr. Oishi of OYO Corporation for their contributions to the setting up of the experimental channel. Mr. Sugawara of Sugawara Inc. gave us assistance with sampling.

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