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Uptake of metals by live green macroalgae *Ulva reticulata* in industrial wastewater of Bayan Lepas, Penang, Malaysia

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A B S T R A C T

This study was conducted to investigate the ability of *Ulva reticulata* (Chlorophyta) to remove Cd, Co, Cr, Cu, Fe, Mn, Mg, Pb, V, and Zn from industrial wastewater. A 24 h experiment was performed under laboratory and *in situ* conditions, which were set up in two places: (1) the industrial area in Bayan Lepas and (2) the coastal area close to Jerejak Island. The initial amounts of metals in *U. reticulata* were ranked as follows: Mg > Fe > Zn > Mn > Ni > Cu > Cr > Co > Cd = Pb > V. However, after exposure to the experimental conditions for 24 h, the sequence amounts of the metals in the tissue changed. Fe showed the highest uptake *in situ* with a maximum uptake of 869.0 ± 84.1 $\mu g\ g^{-1}$ dw. Mg demonstrated the maximum uptake in the laboratory, which was 487.8 ± 130 $\mu g\ g^{-1}$ dw. Cd presented the lowest uptake under both *in situ* and laboratory conditions, which was 0.04 ± 0.027 $\mu g\ g^{-1}$ dw. The uptake capability of *U. reticulata* depended on the metal concentration in water and under experimental conditions. Overall, this study revealed that *U. reticulata* can improve the quality of water discharged from industrial areas.

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INTRODUCTION

Majority of land-based pollution from industrial, residential, and agricultural areas is ultimately discharged into the aquatic environment. Among the most important pollutants are metals, which are non-biodegradable and demonstrate high environmental persistence [1]. Various metals, such as cadmium, nickel, mercury, chromium, zinc, lead, copper, cobalt, and iron, were detected in industrial wastewater [2-9]. Some metals are toxic and carcinogenic agents [9]. Lead and cadmium are toxic even at low concentrations [10, 11], whereas copper is an essential metal at low concentrations but toxic at high concentrations [1].

Penang, which is located in the northern part of Malaysia, has experienced rapid growth in industry, aquaculture, tourism, and urbanization over the last three decades [12]. The Bayan Lepas Free Industrial Zone (FIZ) was established in southeastern Penang in 1972. FIZ industries are primarily involved in the production of electronics, fabrication of metal products, machinery, and precision tooling. Most factories in the mentioned area are related to electrical industries, particularly the semiconductor industry. Metals released from various industrial processes can cause environmental problems in the coastal area. Therefore, heavy metals in the industrial effluent must be treated before being discharged into coastal waters. Electrochemical and chemical precipitations, reverse osmosis, ion exchanges, coagulation, membrane separation, ozonation, and ion adsorption are conventional methods for metal elimination [13-15]. However, these methods present limitations, such as the production of secondary environmental damage from waste discharge, ineffectiveness at low concentrations (< 100 mg/L), and high cost [16, 17]. Hence, the main challenge is toimprove nonpolluting systems that can

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reduce the negative effects of these conventional methods [18]. Methods needed for the reomoval of heavy metals should only minimize the chemical uses; additionally, such methods should be cost effective, highly efficient, selective and environmentally friendly [19]. Various active (living) and inactive (dead) organisms were tested as biosorbents to separate metals from aqueous solutions. Among the living organisms, macroalgae can remove metals from water bodies. Malaysia, with an abundance and variety of seaweed, offers the potential to remove pollutants from wastewater. *Ulva reticulata* is local seaweed in Penang that grows well in the coastal areas near Bayan Lepas FIZ. Therefore, the current study aimed to assess the metal uptake of *U. reticulata* from the wastewater of Bayan Lepas FIZ.

MATERIALS AND METHODS

Ulva reticulata cultivation

Local seaweed *U. reticulata* was collected from the seashore of Penang Island (5° 21′ N, 100° 20′ E) during low tide (Figure 1). Collected samples were then placed in a cool box and transported to the Centre for Marine and Coastal Studies (CEMACS) Universiti Sains Malaysia in the month of February 2013.

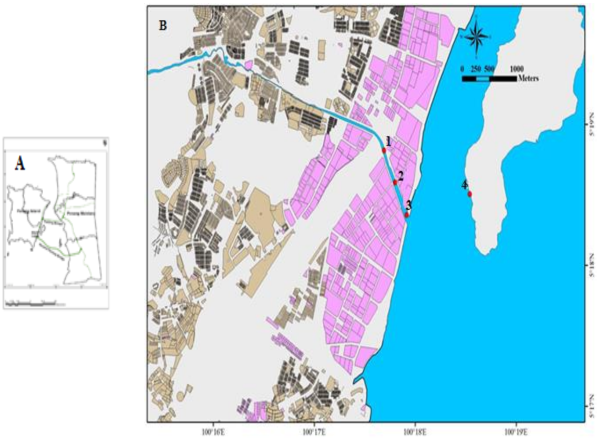


Figure 1. Penang (A) and Bayan Lepas FIZ (B)

Upon arrival at the CEMACS, the samples were washed with seawater until the seaweed was free from sand and epiphytic organisms. The washed samples were cultured with seawater of Teluk Bahang for four months in fiberglass tanks with dimensions of 5 m (L) × 2 m (W) × 1 m (H), which were covered with one layer of black orchid netting. After the cultivation period, the washed samples were transported to a laboratory for further experimentation. In the laboratory, several aquaria with dimensions of 1 m (L) × 0.60 m (W) × 0.45 m (H) were filled with 30ppt artificial seawater. The seawater in the laboratory was prepared by dissolving tap water with nutrient-free artificial salt (Instant Ocean, France). Subsequently, the collected *U. reticulata* was maintained in an aerated aquarium under a light intensity of 4 μmol photons m^{-2} s^{-1} and a photoperiod of 12L: 12D and was acclimatized for 5 days. Afterward, the sampleswere used for uptake experiments. Metal uptake was performed under two conditions: *in situ* (four stations) and in the laboratory.

In situ experiments

Industrial effluents from the Bayan Lepas FIZ are discharged several drainage canals and into the Keluang River, where they are finally released into the open sea across from Jerejak Island (Figure 1). The Keluang River was originated from Ara River and passed from housing area and then factories area in the Bayan Lepas FIZ. The Keluang River is located between phases III and IV of the Bayan Lepas FIZ, and some factories drain their wastewater into this rive, finally Keluang River discharged into the coastal area. The metal uptake by *U. reticulata* was evaluated at four stations. Stations 1, 2, and 3 were located on the Keluang River (factory area), and station 4 was near Jerejak Island (sea area). The experiment was performed in 250 mL perforated transparent mineral plastic bottles to allow water to flow in and out of the bottles. Each perforated bottle was made by punching holes around it by using a hot soldering iron (Figure 2a). Up to 4 g of fresh *U. reticulata* were weighed and placed inside each of the bottles (Figure 2b), and five bottles were joined together by a nylon rope. The bottles were fixed at 50 cm from the bottom of every station by using two wooden rods 150 cm in length (Figure 3). After 24 h, all the bottles were collected, and the *U. reticulata* was transported to a laboratory to determine its metal content.

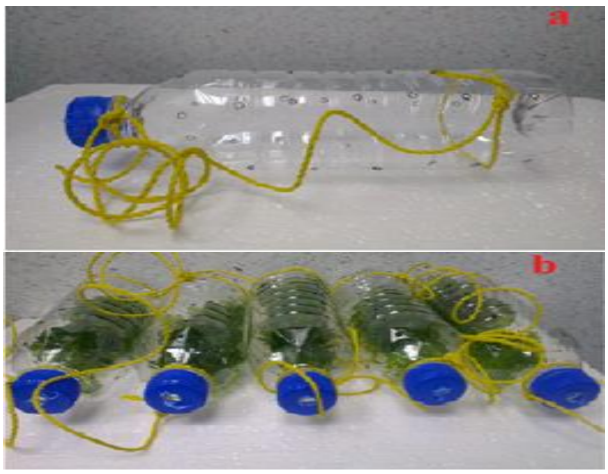


Figure 2. Perforated transparent plastic mineral water bottle 250 mL (a), perforated bottles with *U. reticulata* (b)

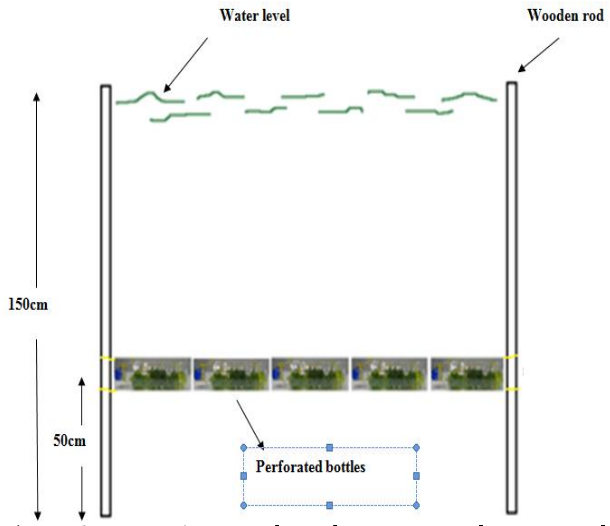


Figure 3. Five 250 mL perforated transparent plastic mineral water bottles containing *U. reticulata* fixed at 50 cm from the bottom using 2 wooden rods of 150 cm in length

Laboratory experiments

To compare the metal uptake *in situ* and under laboratory conditions, wastewater from the Bayan Lepas FIZ was collected from station 2 in the Keluang River (factory area) during low tide. Water samples were transferred into 1 L polypropylene bottles, which kept at cool box at −4 °C before being transported to the laboratory. During sample collection, physicochemical parameters, such as temperature, pH, dissolved oxygen, and salinity, were measured *in situ* by using a multi-parameter probe model YSI Pro Plus. To determine the metal uptake, 2 g of fresh *U. reticulata* was placed into a 250 mL conical flask containing 200 mL of wastewater with temperature 27ºC and was exposed to light intensity of 40 µmol photon m^{-2} s^{-1} under photoperiod of 12L: 12D for 24 h. Finally, *U. reticulata* was collected and prepared to determine the metal content.

Metal analysis

All fresh *U. reticulate* samples before and after exposure *in situ* and under laboratory conditions were dried using a freeze-drier (Labconco, USA) at −40 °C, with a pressure of −760 mmHg for 3 days. The metals were determined on the basis of microwave digestion. A mixture of 0.5 g *U. reticulata* was digested in closed XF 100-4 vessels with 9 mL of HNO_3 and exposed to microwave radiation for 10 min at 240 °C (Anton Paar, Multiwave 3000, Austria). When digestion was completed, the XF 100-4 vessels were allowed to cool to room temperature for 20 min, and the digested samples were made up to 25 mL with deionized water. Metals were analyzed by Inductively Coupled Plasma Optical Emission Spectrometry ICP-OES (PerkinElmer 4000, USA) and Graphite Furnace Atomic Absorption (GF-AAS) (Shimadzu 7000, Japan) [20]. Both ICP-OES and GF-AAS were calibrated using external standard metal solutions. Standard mixture components (21 elements) at a concentration of 100 mg/L for ICP-OES and individual standard solution of metals at a concentration of 1000 mg/L for GF-AAS were purchased from PerkinElmer.

RESULTS AND DISCUSSION

The metal contents (µg g^{-1} dw) in *U. reticulata* before and after exposure are presented in Table 1. The initial amounts of metal contents were ranked as follows: Mg > Fe > Zn > Mn > Ni > Cu > Cr > Co > Cd = Pb > V. The metal content after exposure was high. The highest metal content was 1131.2 ± 18.71 µg g^{-1} dw for Mg, and the lowest (not detected) was for V.

Macroalgae demonstrate a high ability to accumulate metals by thousands of times higher than the corresponding concentration of seawater [21]. A high concentration of Mg in seawater led to a large amount of Mg in *U. reticulata*. Fe content was the second highest in *U. reticulata*. Our finding is in agreement with the report of Billah et al. [21], who found high Fe content in *Bostrychia* sp. (red macroalgae) and *Chaetomorpha* sp. (green macroalgae) from Sarawak, Malaysia. High Fe concentration in Malaysian waters was also reported by Yap and Chen [22]. After *U. reticulata* was exposed to industrial wastewater and seawater containing metals at different stations and in the laboratory, the metal content in *U. reticulata* increased from 0.069 ± 0.003 µg g^{-1} dw to 1619 ± 130 µg g^{-1} dw. The highest and lowest contents were ascribed to Mg and V, respectively, which remained the same before and after *U. reticulata* exposure. However, some metal orders in *U. reticulata* were changed after exposure, indicating that the metal contents were significantly different ($p < 0.05$) before and after exposure (paired-sample *t*-test). Metal uptake (µg g^{-1} dw by *U. reticulata* at different stations during the 24 h experimentation is shown in Figure 4, in which the range of metal uptake was high. Metal uptake ranged from 0.022 ± 0.006 µg g^{-1} dw for Cd in station 4 to 868.9 ± 84.75 µg g^{-1} dw for Fe in station 1. Karez et al. [23] reported that metal content (Fe, Zn, and Pb) was high in algae because of the high metal concentration in water. As previously mentioned, Yap and Chen [22] found high Fe concentration in Malaysian water. In addition to metal concentration in the water column, metal bioabsorption depends on the cell wall components. Kratochvil and Volesky [24] reported that Fe^{2+} can compete for the binding sites better than other cations. A comparison of metal uptake by *U. reticulata* in FIZ wastewater (stations 1, 2, and 3) with seawater (station 4) showed that the amounts of Cr, Cd, Cu, Fe, Ni, and Zn uptake were higher in industrial wastewater stations than in the seawater station, whereas V, Co, Pb, and Mn uptake values were higher in the seawater station than in the industrial wastewater stations. The results indicated that Cd, Co, Cr, Cu, Fe, Mg, Mn, and Ni uptake showed significant difference (one-way ANOVA, $p < 0.05$), whereas Pb, V, and Zn uptake showed no significant difference (one-way ANOVA, $p > 0.05$) among the stations.

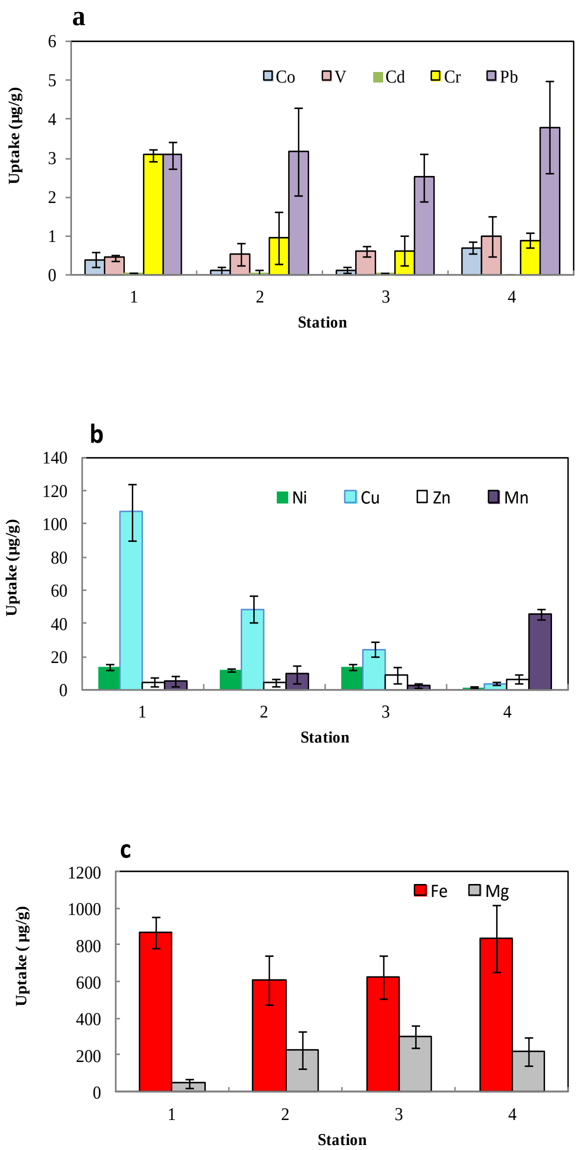
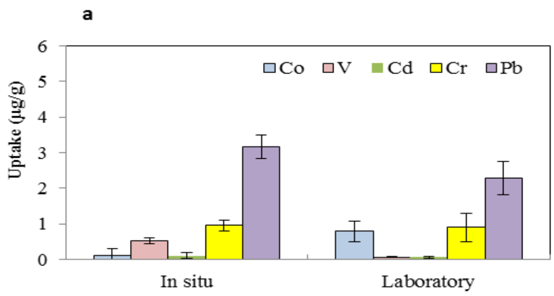


Figure 4. Metals uptake µg g^{-1}dw (mean ±SD) by *U. reticulata* at different stations during 24h experimentation duration (n=5) (a) Co, V, Cd, Cr and Pb; (b) Ni, Cu, Zn and Mn; (c) Fe and Mg.

Several authors revealed the linear relationship between the metal uptake by algae and the metal concentration in column water [22, 23]. The uptake of V and Pb by *U. reticulata* was higher in seawater (station 4) than in industrial wastewater, and this trend may be attributed to V and Pb in water that came from oil discharged by boats near Jerejak Island. The higher Cd, Ni, Cu, Fe, Cr, and Mg uptake by *U. reticulata* in FIZ wastewater can be attributed to a higher concentration of metals in FIZ wastewater. The metal contents of some macroalgae in different regions are presented in Tables 2 and 3. The metal contents of *Ulva* sp. in two Argentinean regions showed that the metal contents in the Arroya La Mata stream were greater than those in Punta Maqueda, except for Cd. We can conclude from the tables that Cd, Pb, and Co concentrations were low, whereas Fe and Mn concentrations were high. Pérez et al. [27] reported that *Ulva* sp. contains higher metal content in regions influenced by anthropogenic activities. Arroya La Mata was an area affected by contaminants derived from petroleum and other related industries.

In addition to metal concentration in the water column, factors such as pH, composition and wastewater characteristics can influence metal uptake [24]. Wastewater collected from FIZ exhibited a temperature of 27 °C, 0ppt salinity, 2.4 mg/L dissolved oxygen, and a pH of 7.2, whereas the seawater showed a temperature of 26.8 °C, 29ppt salinity, 5.6 mg/L dissolved oxygen, and a pH of 8.0. The cell walls of algae are responsible for most of the metal accumulation and metal binding in which biosorption occurs [22]. Among different metal uptake mechanisms, ion exchange was found to play a main role. Increased salinity leads to decreased metal biosorption because of competitive sorption between metals for functional groups. Therefore, 0ppt salinity and pH of 7.2 in wastewater led to an increase in metal uptake by *U. reticulata* in FIZ wastewater in Bayan Lepas. In addition to wastewater composition, metal biosorption depends on cell wall components. In multi-metal systems, cations compete to bind with functional groups in the algal cell walls. Some metal ions, such as Mg^{2+} (hard ion), prefer to bind ligands through oxygen, whereas metals such as Pb^{2+} (soft ion) form a strong bond with nitrogen and sulfur [25, 26].

A comparison of metal uptake (µg g^{-1} dw) by *U. reticulata* under *in situ* and laboratory conditions during the 24 h experiment is presented in Figure 5. All metal uptake values were higher under *in situ* condition than under laboratory condition except for Co, Mn, and Mg. This behavior can be explained by the *in situ* experimental condition, which demonstrated a continuous flow of wastewater; by contrast, non-continuous flow and a limited volume of wastewater were observed under laboratory conditions. Therefore, the metal uptake was lower under laboratory conditions. However, higher Mg, Mn, and Co uptake can be attributed to increased photosynthesis under laboratory conditions. The chlorophyll structure consists of Mg atoms linked with pyrrole rings, whereas Mn and Co are used as electron transport in photosystem II and photosynthesis pathway, respectively [27]. As such, an increase in light intensity and a decrease in turbidity under laboratory conditions led to an increase in Mg, Mn, and Co uptake.



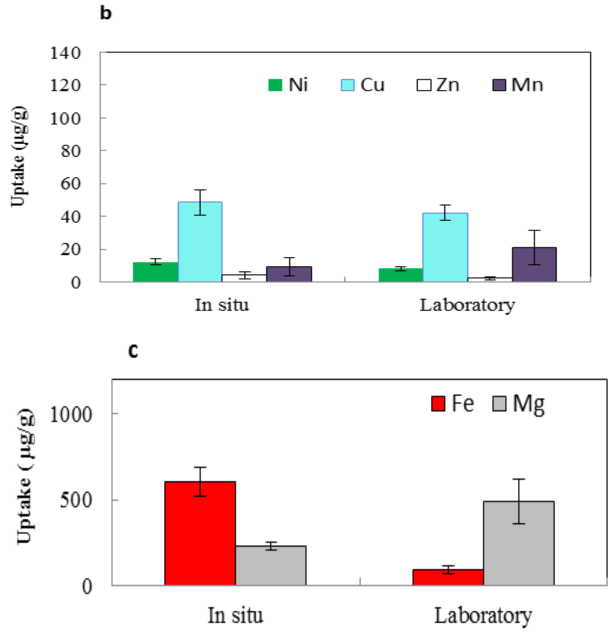


Figure 5. Compare metal uptake µg g-1dw (mean ±SD) by *U. reticulata* at in situ* and laboratory conditions during 24h experimentation duration

TABLE 1. Metals content (µg g^{-1} dw) in *U. reticulata* before and after 24h exposed to the industrial wastewater under two conditions: *in situ* (four stations) and in the laboratory

		After exposure				
		In situ (station)				
Metal	Before expose	1	2	3	4[I]	[II] Laboratory
Co	0.860±0.021	1.257±0.191	0.973±0.083	0.997±0.058	1.563±0.151	1.648±0.300
Cr	1.662±0.034	4.754±0.150	2.622±0.672	2.290±0.380	2.563±0.183	2.566±0.340
Ni	11.3±0.597	25.02±.1.875	23.58±0.946	25.059±1.822	12.70± 0.45	19.466±1.025
Cu	11.15±0.992	118.23±17.04	59.70±7.91	35.69±4.685	15.023±1.219	53.349±4.773
Zn	38.13±0.738	42.93±2.85	42.41±2.12	46.89±4.698	44.674±2.874	40.487±1.072
Fe	243.5±11.60	1112.4±87.75	850±133.9	870.8±116	1080±184.5	335.90±20.53
V	[III]BDL	0.455±0.080	0.526±0.290	0.615±0.128	0.988±0.518	0.069±0.003
Pb	0.087±0.003	3.172±0.336	3.256±1.138	2.597±0.598	3.889±1.187	2.373±0.475
Cd	0.088±0.034	0.198±0.001	0.189±0.082	0.178±0.027	0.110±0.007	0.166±0.035
Mn	29.28±0.879	34.727±3.15	38.60±5.44	31.948±1.415	74.956±2.933	50.427±10.622
Mg	1131.2±18.71	1179.6±23.98	1360.9±99.79	1434±62.79	1352.5±74.9	1619.07±130.9

where I=station 4 was near to Jerejak Island, II= Laboratory wastewater was from station 2, III= BDL (Below detection limit)

TABLE 2. Content of Co, V, Cd, Cr, Pb and Ni (µg g^{-1}dw) in macroalgae reported in different regions.

Species	Co	V	Cd	Cr	Pb	Ni	Location	Reference
Ulva sp.	0.4±0.12	5.57±1.73	0.17±0.05	1.14±0.30	1.33±0.72	1.22±0.44	Arroyo Mata, Argentina	[27]
Ulva sp.	0.27±0.04	4.30±1.14	1.03±0.11	0.84±0.19	0.82±0.32	0.99±0.99	Punta Maqueda, Argentina	[27]
Ulva sp.	0.4		0.1	4.2	1.4	3.90	Ireland	[14]
Ulva sp.	0.000-2.279	12.78-30.06	0.022-2.216	4.13-104.6	2.405-14.21	3-23.89	Thessaloniki, Greece	[28]
Gracilaria	BDL-3.077	9.470-13.29	0.009-0.148	1.89-4.424	1.829-9.79	1.646-8.507	Thessaloniki ,Greece	[28]
U. reticulata	0.86±0.02	BDL	0.088±0.03	1.66±0.03	0.087±0.003	11.3±0.60	CEMACS	Present study

where BDL= Below detection limit

TABLE 3. Content of Cu, Zn, Mn, Mg and Fe (µg g^{-1}dw) in macroalgae reported in different regions

Species	Cu	Zn	Mn	Mg	Fe	Location	Reference
Ulva sp.	3.21±2.22	17.4±7.0	41.8±4.4	32.9±4.2	532±245	Arroyo Mata, Argentina	[27]
Ulva sp.	1.74±1.44	21.8±2.7	31.7±5.1	27.2± 2.9	201±41	Punta Maqueda, Argentina	[27]
Ulva sp.	3.30	12.2	39.9	-	-	Ireland	[14]
Ulva sp.	2.5-15.42	82.95-240	10-321.8	-	-	Thessaloniki, Greece	[28]
Gracilaria	3.241-4.961	60.55-129.3	32.64-682	-	-	Thessaloniki, Greece	[28]
Bostrychia.Sp	62.1±0.2	120±1.07	75.3±3.05	-	3561±0.02	Sarawak, Malaysia	[21]
Chaetomorpha sp.	35.5±0.05	152±0.01	187.1±0.1	-	1312±0.3	Sarawak, Malaysia	[21]
U. reticulata	11.15±0.99	38.13±0.7	29.28±0.9	1131±18	243.5±11	CEMACS	Present study

CONCLUSION

The present study confirmed that *U. reticulata* can uptake Cu, Co, Cd, Cr, Fe, Mn, Mg, Ni, Pb, V, and Zn from the water column. Metal uptake by *U. reticulata* at different stations was influenced by metal concentration and water characteristics. Although *U. reticulate* was exposed for only 24 h, the amount of metal uptake was high; in some metals, such as Cu, the content increased by 10-fold after exposure compared with the content before exposure. High metal uptake confirmed that *U. reticulata* can efficiently remove metals from industrial wastewater. Furthermore, the results can facilitate further application studies by using *U. reticulata* for the remediation of industrial wastewater.

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چکیده

این مطالعه به منظور بررسی توانایی حذف فلزات سنگین (کادمیم، کبالت، کروم، مس، آهن، منگنز، منیزیم، سرب، وانادیم، و روی) توسط جلبک سبز *Ulva reticulata* در پساب صنعتی انجام گردید. آزمایشات به مدت یک شبانه روز تحت شرایط منطقه ای (محل) در دو منطقه صنعتی Bayan Lepas و دریایی (Jerejak Island) و همچنین آزمایشگاهی صورت گرفت. میزان اولیه فلزات در جلبک قبل از درمعرض گذاری با فلزات سنگین به ترتیب مقابل بود Mg > Fe > Zn > Mn > Ni > Cu > Cr > Co > Cd = Pb > V. اما میزان فلزات سنگین در بافت جلبک پس از معرض گذاری به مدت یک شبانه روز تغییر کرد. بیشترین میزان جذب فلزات مربوط به آهن با میزان جذب هشتصد و شصت و نه μg g^{-1} dw در محل و منیزیم چهارصد و هشتاد وهفت و هشت دهم μg g^{-1} dw در شرایط آزمایشگاهی بود. کادمیم کمترین میزان جذب چهار صدم μg g^{-1} dw تحت هر دو شرایط نشان داد. میزان توانیی جذب فلزات توسط بستگی به غلظت فلزات در آب ،شرایط محیطی و آزمایشگاهی داشت. به طور کلی این مطالعه نشان داد که *U. reticulata* می تواند کیفیت آب تخلیه شده از مناطق صنعتی را بهبود ببخشد.