Science of the Total Environment 786 (2021) 147501

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Natural community of macroalgae from chromium-contaminated site for effective remediation of Cr(VI)-containing leachates

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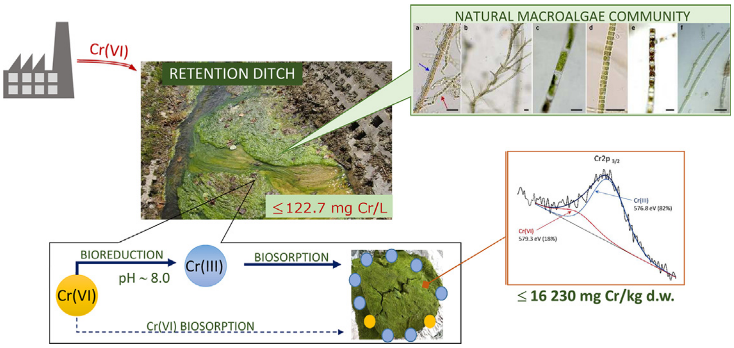
HIGHLIGHTS

- Macroalgal community grown in a highly Cr(VI)-polluted aquatic reservoir was studied.
- Taxonomic analysis revealed the presence of 13 algal species in the biological mat.
- The algae exhibited hyperaccumulative properties toward chromium (up to 16,230 mg/kg).
- It was found that Cr(VI) detoxification was reduction followed by Cr(III) biosorption.
- Ion exchange and complexation were confirmed as the main mechanisms of Cr(III) binding.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 20 February 2021
Received in revised form 28 April 2021
Accepted 29 April 2021
Available online 4 May 2021

Editor: Filip M.G. Tack

Keywords:
Algae
Phycoremediation
Cr(VI)
Industrial effluents

ABSTRACT

The natural macroalgal community, which developed in the unique and extremely Cr(VI)-polluted aquatic reservoir situated near a historical chromium-waste landfill, was studied in order to recognize the main mechanisms of Cr(VI) detoxification by the algal species. The conducted taxonomic analysis revealed mixed composition of the filamentous forms of algae and showed that three species of *Tribonema*, namely *T. vulgare*, *T. microchloron* and *T. viride*, which have not been studied before with regard to the mechanisms of Cr(VI) removal, are likely responsible for the effective bioremediation of this highly Cr(VI)-polluted habitat. The studied algal community, with the ability to grow in extremely high concentrations of Cr(VI), i.e. up to ca. 6150 times the upper limit for surface water, exhibited hyperaccumulative properties for chromium (max 16230 mg/kg dry weight) under the given environmental conditions. We found that the main mechanism of Cr(VI) detoxification was reduction followed by Cr(III) biosorption – feasibly by ion exchange and complexation mechanisms – and that the excellent efficiency of chromium reduction under the given, unfavorable weakly alkaline conditions indicates the biological origin of this process. It was concluded that the examined reservoir inhabited by the algal community can be used, after some modifications, as a simple cost-effective "bioreactor" allowing the reduction of chromium concentration to the desired level. Moreover, the conducted studies are also essential to obtain in-depth knowledge and should also be helpful in the relevance of the community for its further application as a potential biosorbent of Cr(VI) on a global scale.

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https://doi.org/10.1016/j.scitotenv.2021.147501

1. Introduction

Hexavalent chromium is a highly toxic and carcinogenic element present in various industrial effluents in concentrations exceeding thousands of times the allowable limits for natural waters. Many efforts have been made so far to develop effective technologies for its removal from aqueous solutions. Among them, especially phycoremediation (the application of algae in wastewater treatment) has recently attracted a lot of attention (Furey et al., 2017; Mantzorou et al., 2018; Salama et al., 2019). Algae exhibit some important advantages over other biomass:

- they grow under a wide range of environmental conditions including in heavy metal-polluted sites, therefore algae cultivation may be achieved in agricultural, municipal or industrial wastewater (Abou-Shanab et al., 2011; Salama et al., 2019);
- they show high growth rates because of the excellent photosynthetic activity (Sukla et al., 2013);
- harvested algal biomass can be further used for other applications such as biofuel generation (Abou-Shanab et al., 2011; Craggs et al., 2014; Gupta et al., 2017; Mantzorou et al., 2018; Tian et al., 2014), or production of metal nanoparticles (Priyadarshini et al., 2019) and bioplastic materials (Lutzu et al., 2021).

The effective removal of Cr(VI) from industrial effluents, by means of various micro- and macroalgae species, has been reported (Bwapwa et al., 2017; He and Chen, 2014; Lin et al., 2020; Sahmoune et al., 2011; Saravanan et al., 2017; Singh et al., 2021). However, despite the abovementioned advantages making algae attractive for "green" wastewater treatment technologies, phycoremediation is still hardly applied in industrial practice. The conventional methods of Cr(VI) remediation used in the chemical industry, such as ion exchange or reduction/precipitation, are often characterized by the required efficiency and also the technical know-how is readily available. Notwithstanding, most of these technologies are expensive and may be ineffective when the chromium concentration in wastewater is low (<100 mg/L) (Gupta and Rastogi, 2008). Moreover, precipitation of $Cr(OH)_3$ generates a large amounts of sludge that needs to be further processed. Therefore, the implementation of phycoremediation in industrial wastewater treatment can be beneficial for the chemical industry, but there are still many issues that have to be addressed to make this technology more common in industrial practice (Bwapwa et al., 2017; Furey et al., 2017; Sahmoune et al., 2011; Salama et al., 2019). Since phycoremediation depends on the biosorption and bioaccumulation capacities of algae, recent research undertaken worldwide have focused on two main areas: the use of dried or living algal biomass as the biosorbent in fixed bed adsorption systems, and the utilization of living organisms in purpose-built wetland systems such as high-rate algal ponds (HRAP) (Craggs et al., 2014; Fletcher et al., 2020; Young et al., 2017). It should be noted that biosorption, based on physicochemical properties of the biomass surface, can be considered as the initial stage of the whole bioremediation process; it is assumed that biosorption represents usually more than 80% of the total chromium uptake by algae (Mehta and Gaur, 2005). Bioaccumulation is a more complex, metabolism-dependent process and relates to metal detoxification after its permeation across the cell membrane and/or tonoplast (Mehta and Gaur, 2005; Saravanan et al., 2017; Volesky, 2007). It is noteworthy that the efficiency of living algae may be higher than that of dried biomass, as they can remove a greater quantity of metals using both biosorption and bioaccumulation mechanisms for a longer time (Salama et al., 2019). Living organisms are also especially beneficial in the treatment of wastewater with a lower load of pollutants (Cabatingan et al., 2001; Sahmoune et al., 2011). Moreover, the use of dried, ground algal biomass or living microalgae in biosorption systems has some serious disadvantageous (difficulties in sorbent separation, high hydraulic resistance in fixed-bed systems) which may need their immobilization within the structure of porous solid materials (Zeraatkar et al., 2016).

Irrespective of the form of the algal biomass used, much effort has been made to reveal the mechanisms of cell surface sorption/precipitation and intercellular accumulation of chromium (Saravanan et al., 2017). Since it may influence the metabolism of the cells (e.g. inhibition of photosynthesis, respiration and other enzymatically driven processes), and disrupt the membrane integrity owing to electrolyte leakage and deterioration of biomolecules'structure, the mechanisms protecting the cells from penetration by chromium are of the highest importance. They involve adsorption on extracellular polymeric substances (EPS) as well as adsorption or precipitation on the cell walls. Both EPS and cell walls are composed mainly from polysaccharides and their derivatives, e.g. alginic acid, proteins, glycoproteins, phospholipids, which contain various functional groups playing a vital role in Cr(III) and Cr(VI) binding (Davis et al., 2003). The relative ratios of functional groups may significantly differ for various algal strains (Priyadarshini et al., 2019; Salama et al., 2019), and some functionalities may bind chromium by various mechanisms, e.g. the carboxyl group – besides ion exchange – might be engaged in complexation with Cr(III) (Bertagnolli et al., 2014; Papageorgiou et al., 2010). Moreover, aside from the EPS, some low molecular compounds or enzymes may also be excreted from the cells so that they could react with Cr(VI), lowering its toxicity by reduction to less harmful Cr(III). The essential difference between these two forms of chromium is that Cr(VI) can easily penetrate the cell membrane, affecting its integrity or influencing the metabolic processes within the cells (Francisco et al., 2010). In contrast, the cell membrane is almost impermeable to trivalent chromium, because of which it is about 100 times less toxic and about 1000 times less mutagenic than Cr(VI) (Polti et al., 2010). Thus, the reduction of Cr(VI) to Cr(III) is thought to be the crucial process protecting the living cells from being damaged, and it may be either of enzymatic or non-enzymatic origin. Therefore, determination of the mechanisms responsible for chromium removal is of the highest importance and may be helpful in the implementation of phycoremediation under specific conditions.

Another prospective research area is the development of new algal strains characterized by higher resistance and greater capacity for chromium removal. It may be achieved at the molecular level by genetic engineering methods leading to highly effective biosorbents (Apandi et al., 2019; Rajamani et al., 2007). Because of the essential differences in characteristics of various effluents from the chemical industry, and the importance of algae acclimatization to the given conditions, the utilization of algae developed naturally in the polluted aquatic environment seems to be especially beneficial (Bakatula et al., 2014; Rehman and Shakoori, 2001; Yewalkar et al., 2007). Some cyanobacteria species commonly called *Spirulina* are useful in bioremediation of Cr-polluted water (Rezaei, 2016). Also different taxa of microalgae, such as *Chlorella*, *Chlamydomonas*, *Dunaliella*, as well as filamentous *Nitella* and *Ulothrix tenuissima* Kützing, show high capacity of Cr phytoextraction (Chabukdhara et al., 2017).

In this paper we present a study on the unique aquatic reservoir situated in the area of a chemical company which has been producing Cr(VI) compounds for almost one hundred years. In this area Cr(VI)-contamination of groundwater is a consequence of rainfall infiltration of the historical chromium-waste landfill. The production wastes were gathered on this landfill for about 40 years, until the 1960s. Since the 1990s the polluted groundwater has been collected in the reservoir for further Cr(VI) chemical detoxification. From the biological point of view the aquifer enabled the development of a unique, stable habitat — an aquatic ecosystem more than 20 years old. Therefore, the algal community found there – in the form of a characteristic biological mat – has created mechanisms enabling the algae to colonize and grow in extremely high concentrations of Cr(VI), up to approximately 6150 times the upper limit for surface water. Identifying the organisms present in the biological mat, as well as understanding the processes controlling the phycoremediation at the physicochemical level, is essential to obtain in-depth knowledge on the protection of the natural environment and thus of human health. It should also be helpful in determining the relevance of the community for its further application as a potential biosorbent of Cr(VI) on a global scale in a temperate zone. To sum up, it is of the highest importance – from both a scientific and a practical point of view – to identify the main mechanisms of Cr(VI) detoxification by the studied biocenosis.

2. Materials and methods

2.1. The algal community

The material was collected from the area of a chemical company situated in the southern Poland (Fig. 1a). The plants grew in a retention ditch collecting infiltrates from the nearby situated historical chromium-waste landfill (Fig. 1b, c). They created a community constituted by filamentous algae forming a characteristic biological mat (Fig. 1e). The 120 m length ditch crosses the aquifer to a depth of about 0.5 m and acts as a filter for Cr(VI)-polluted groundwater (Fig. 1d). It allows also surface drainage. Three groundwater mouths are situated there. The concrete slabs stabilized the slopes and the bottom of the ditch. The bottom is covered with gravel. Cr leachate (Fig. 1d) is collected in the ditch to the level c.a. 50 cm and then it is pumped by submersible pump into a retention tank, where hexavalent chromium is chemically reduced to trivalent form. For the analysis ca. 600 g of the biological mat was harvested in September 2019 from the stands adjacent to the groundwater mouths, immediately transported to the laboratory and washed several times with tap water and three times with distilled water. In the next step the biomass was frozen in liquid nitrogen and stored for 2 days at −20 °C. After that, the frozen algae were enclosed in a lyophilizer chamber (Alpha 1–4 Martin Christ Gefriertrocknungsan-lagen GmbH lyophilizer, Germany) and freeze dried for 120 h at 1.03 mbar and −20 °C. The freeze-dried material (Fig. 1f) was kept at −20 °C until analysis.

2.2. Chromium desorption

Desorption experiments were carried out by contacting the harvested, freeze-dried biomass contained chromium (Fig. 1f) with various eluents, namely 0.1–0.5 M HNO_3, 0.1–0.5 M NaOH, 0.1 M EDTA and 0.1 M $CaCl_2$. Thus, 0.1 g of the sample was shaken out for 24 h with 50 mL of the given solution in an Erlenmeyer flask using the laboratory shaker IKA HS 260 Control at 130 rpm. Afterwards the solutions were passed through syringe filters of porosity 0.20 μm (ChemLand, Poland), and analyzed in terms of pH, total chromium and Cr(VI) concentration. Determination of the total chromium content in the raw biomass was conducted after its mineralization in a Mars 5 Xpress microwave mineralization system (CEM, USA) using ultrapure HNO_3 (60%, Merck). The efficiency of chromium desorption by each eluent was made by comparing of the total chromium content in the biomass (determined after its mineralization) and chromium desorbed after contacting with the selected eluent. All desorption experiments were conducted in duplicate.

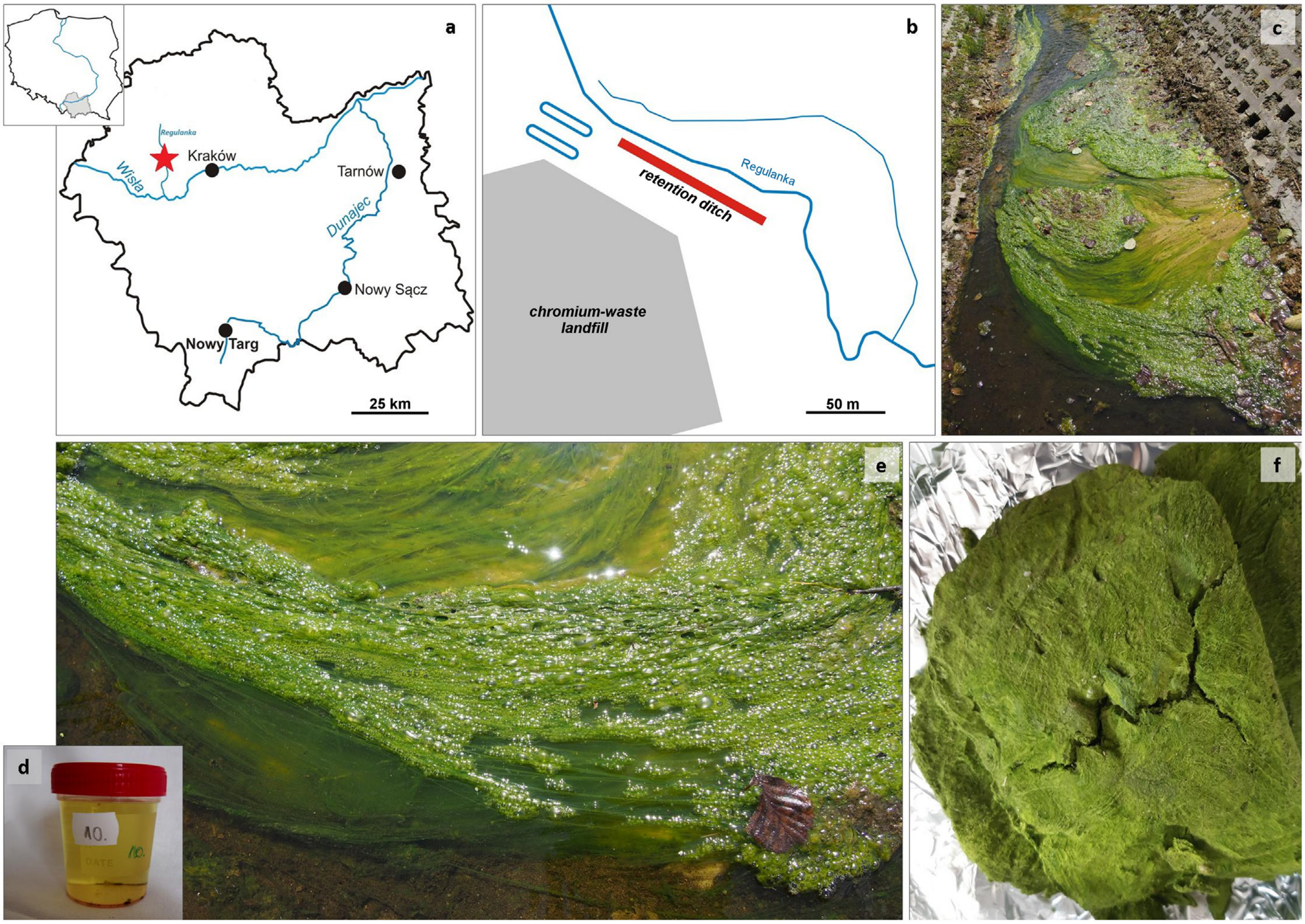

Fig. 1. The map of a study site (a) and localization of a Cr-waste landfill nearby a collecting ditch (b). The photographs of the ditch (c) collecting Cr(VI) leachate (d) and algal biological mat (e) used for the experiments after freeze drying (raw biomass containing chromium) (f).

2.3. Analytical methods

The taxonomic identification of the community was conducted four independent times in 2019 (June, July, August, September). For that reason samples were taken every 10 m over a distance of 30 m just near the three groundwater mouths, put into 100 mL plastic containers and transported to the laboratory. The composition of algae was determined using a Nikon ECLIPSE 600 light microscope with Nomarski phase contrast. The taxonomic analysis was performed according to Graham et al. (2009), John et al. (2011) and Wehr et al. (2014).

Total chromium concentration in eluents was analyzed using atomic absorption spectrometry with a graphite tube (Graphite Furnace AAS, GBC Scientific Equipment, Australia). Simultaneously, the content of Cr (VI) in solutions was determined by the spectrophotometric method with 1,5-diphenylcarbazide (DPC) using a UV–Vis spectrophotometer (Specord 210, Analytik Jena, Germany) at wavelength 540 nm (relative standard deviation RSD ≤ 2.87%). All the samples were acidified prior to analysis.

The FTIR spectra of the biomass surface were collected on a Fourier transform, vacuum spectrometer (Bruker VERTEX 70 V, Germany) equipped with an air-cooled DTGS detector. The data were recorded at the resolution of 4 cm^{-1} with collection of 64 scans and further processed using Bruker OPUS software. The surface of the biomass was also analyzed by X-ray photoelectron spectroscopy (XPS) using Al Ka radiation ($h\nu = 1486.6$ eV) with the hemispherical electron energy analyzer (Phoibos 100, Specs, Germany). C1 s peaks were used as the internal standard calibration peak at 284.7 eV.

The analysis of leachate water was performed twice: in July and October 2019. 100 mL water samples were collected at the same location as the biomass (for identification and sorption analysis), i.e. 3 samples every 10 m in each series. Chemical composition of water was analyzed by means of inductively coupled plasma mass spectrometry (ICP-MS) (ELAN 6100, Perkin Elmer, UK) and titration (PN-EN ISO 17294-1:2007, 2007; PN-ISO 9297:1994, 1994). The spectrometer was calibrated using ICP multi-element standard (Merck). SPS-SW1 batch 128 or 137 (LGC Standards) were used as the reference materials for elemental analysis of the solutions. Due to the color of samples the content of nitrate anions was analyzed with a nitrate ion selective electrode (Orion 92A+, Thermo Fisher Scientific, USA). Before measurement of metallic elements with ICP-MS, the water samples were acidified. The limits of quantitation (LOQ) for the elements analyzed by the abovementioned methods were as follows [mg/L]: Na 0.01; K 0.05; Li 0.001; Be 0.0005; Ca 0.05; Mg 0.001; Ba 0.0005; Sr 0.0003; Fe 0.01; Mn 0.003; Ag 0.001; Zn 0.001; Cu 0.001; Ni 0.001; Co 0.0002; Pb 0.0001; Hg 0.0001; Cd 0.0003; Se 0.01; Sb 0.0002; Al 0.005; Cr 0.005; Mo 0.0003; V 0.001; Zr 0.002; Ti 0.02; As 0.001; Tl 0.0001; W 0.0003; Cl 1.0; SO_4 3.0; HCO_3 24.4; NO_3 0.6; PO_4 0.02; BO_3 0.01; and HBO_2 0.01. The analyses of leachate were performed in the Laboratory of Hydrology, AGH University of Science and Technology, Kraków, Poland, certified by the Polish Centre for Accreditation (Certificate No. AB 1050, updated 27 May 2019). The results of water parameters were evaluated for normality by Shapiro-Wilk test based on Statistica 13.1.

3. Results and discussion

3.1. Aquatic system description

The composition and parameters of the water withdrawn from the retention ditch are presented in Table 1. None of the analyzed values related to the concentration of particular elements and physical parameters of water followed normal distribution, therefore we presented the range (min, max) and median values. The content of cations due to the method of analysis reflects total amount of an element with no discrimination between the speciation. However, in the case of Cr according to the both analysis of Cr speciation based on spectroscopy method and the physical parameters (Kotaś and Stasicka, 2000) of leachate: the weakly alkaline pH and a high positive redox potential, Cr(VI) was practically the only form of Cr detected in the water. The upper limit for Cr (VI) concentration in surface water is 0.020 mg/L (Journal of Laws of the Republic of Poland, 2019); thus the concentration of Cr was exceeded up to 6150 times. We also detected slightly elevated amounts of Ca and Cl in the leachate. The high content of nitrate anions indicates eutrophication, though the amount of phosphates was low; the high concentration of calcium ions likely caused phosphates' precipitation. The concentration of other than chromium hazardous elements did not exceed allowable limits for surface waters (Table 1) according to legal requirements (Journal of Laws of the Republic of Poland, 2019). The conductivity was typical for leachates and resulted from high concentration of ions (e.g. chromates, nitrates, chlorides). The specific concentration of particular elements in the leachate was a consequence of the past activity of the chemical company, which used Cr-ores for the production of Cr(VI) compounds: sodium chromate, chromic acid anhydride and potassium dichromate. Hexavalent chromium is very mobile in the wide range of pH (Kotaś and Stasicka, 2000), thus it can be easily infiltrated from the landfill unlike to Cr(III) and other heavy metals that precipitate under neutral pH. The higher level of nitrate ions may result from surface runoff from the nearby situated small municipal sewage treatment plant.

Table 1
Chemical composition and physical parameters of leachate. Various significant decimal places result from the different detection thresholds of the analysis. Two independent series of experiments were conducted with n = 3 independent replicates in each series. The upper limits for good (second class) quality of water are presented.

Cations, mg/L	Min	Max	Median	Limit
Na	178.40	443.50	245.45	–
K	3.56	8.26	4.45	–
Li	0.005	0.011	0.006	–
Be		<0.0005		0.0008
Ca	74.61	130.60	106.16	96.7
Mg	9.680	18.730	11.300	11.7
Ba	0.0170	0.0370	0.0280	0.05
Sr	0.1220	0.2190	0.1500	–
Fe	0.01	0.14	0.04	–
Mn	0.004	0.181	0.082	–
Ag		<0.001		–
Zn	0.002	0.010	0.006	0.1
Cu	0.001	0.008	0.004	0.01
Ni	0.001	0.004	0.002	0.034
Co	0.0002	0.0007	0.0004	0.05
Pb	0.0009	0.0028	0.0023	0.014
Hg		<0.0001		0.00007
Cd		<0.0003		0.00045
Se		<0.01		0.02
Sb		<0.0002		0.002
Al	0.009	0.018	0.012	0.4
Cr(VI)	**19.000**	**122.700**	**36.505**	0.02
Mo	0.0019	0.0250	0.0032	0.04
V	0.001	0.003	0.001	0.05
Zr		<0.002		–
Ti		<0.02		0.05
As		<0.001		0.05
Tl		<0.0001		0.002
W	0.0003	0.0020	0.0012	–
Anions, mg/L				
Cl	56.3	421.0	72.2	31.9
SO_4	262.2	747.0	390.7	89.4
HCO_3	384.0	553.0	468.0	–
NO_3	58.0	95.5	62.2	2.7
PO_3		<0.02		0.1
BO_3	0.19	0.60	0.39	2.0
HBO_2	0.14	0.46	0.29	2.0
Other parameters				
pH	8.13	8.44	8.31	7.5–8.3
Eh, mV	216	351	266	–
conductivity, μS/cm	1369	2780	1732	506

3.2. Algae community characterization

The plant biomass was a community of algae creating a characteristic biological mat. It was dark green and formed a rather loose structure floating above the bottom or floating on the surface. The analysis revealed mixed, relatively stable composition of the filamentous forms of algae (Fig. 2a–e) and one species of filamentous Cyanobacteria (*Dolichospermum* sp., formerly *Anabaena* sp.) (Fig. 2f). The list of the species is presented in Table 2 (taxon names according to Guiry and Guiry, 2021). The highest representation in the mat was exhibited by two species of *Tribonema*: *T. microchloron* and *T. vulgare*. The average proportions of the particular genera were as follows: *Tribonema* sp. 31% > *Klebsormidium* sp. 20% > *Stigeoclonium* sp. 14% > *Mougeotia* sp. = *Ulothrix* sp. = *Melosira* sp. (diatom) = *Dolichospermum* sp. 7% > *Spirogyra* sp. 5% > *Oedogonium* sp. 2%.

Some members of the community, in particular *Stigeoclonium tenue*, are often found in various types of polluted water systems (Wołowski, 1989). *Ulothrix tenuissima*, *Oedogonium* sp. and *Spirogyra* sp. were studied in terms of Cr(VI) sorption (Bakatula et al., 2014; Chabukdhara et al., 2017; Gupta et al., 2001; Onyancha et al., 2008), but as far as we know the data on the application of filamentous algae for Cr(VI) removal are limited compared to other heavy metals and unicellular forms of algae (Chabukdhara et al., 2017; Husien et al., 2019; Mohan and Pittman, 2006). *Tribonema* species have been previously studied in accordance with their capacity for biofuel production (Li-Beisson et al., 2019). The possibility of using *Tribonema* sp. to biomonitor pollution by toxic metallic elements (including Cr) in the territories exposed to contamination has been reported (Kuklin and Pomazkova, 2017). But as far as we know this species has been never mentioned in the context of the mechanisms of chromium removal. The maximal content of Cr in the studied algae community reached 16230 mg/kg dry weight (d.w.), while the median Cr content 4610.2 mg/kg d.w. under the given environmental conditions, what we proved in our earlier work (Augustynowicz et al., 2021). Bioconcentration factor (BCF): median Cr content in the plant biomass (4610.2 mg/kg d.w.) versus median Cr content in water (36.505 mg/L — compare Table 1) was 126.3 (Augustynowicz et al., 2021). According to the definition (van der Ent et al., 2013), the biomass of filamentous algae showed hyperaccumulation capacity for Cr — accumulation levels of Cr ≥ 300 mg/kg d.w., and BCF > 1.

In spite of the high potential of the biological mat for chromium bioremediation, the whole maintenance of the studied phycoremediation system has so far been limited to regular harvesting and disposal (without any pretreatment) of algae on the landfill site. It seems that in order to achieve its better utilization and to gain additional economic and environmental benefits, some operations should be undertaken. The potential application of the phytoextracted plant material and metals for the technology are described by Hunt (Hunt et al., 2014). The rebuilding of the retention ditch would enable better contact between the biological mat and the infiltrate, resulting likely in the higher efficiency of the remediation process. Moreover, the use of more advanced wetland systems, such as high-rate algal ponds (HRAP), might provide some additional benefits when combined with energy production from the biomass (Rodrigues de Assis et al., 2020). Also the safe disposal of chromium-loaded algae is of high importance, as it should be regularly harvested and processed to avoid release of the accumulated chromium back into their source. An worth considering solution would be liquefaction of the Cr-loaded algae in hydrothermal conditions or biofuels production (Tian et al., 2014), combined with chromium recovery. However, because such a complex technological solution would require high investment and might be economically not viable for small chemical plant, burning of the biomass combined with chromium recovery and energy production may be ultimately considered as an environmentally friendly and cost-effective solution.

Table 2
Species of algae occurring in the biological mat; (+) single, (1) sparse, (2) frequent, (3) very frequent, (4) in mass.

Taxa	Frequency
Dolichospermum cf. *affine* (Lemmermann) Wacklin, L.Hoffmann & Komárek	2
Klebsormidium subtile (Kützing) Mikhailyuk, Glaser, Holzinger & Karsten	4
Klebsormidium tribonematoideum (Skuja) Hindak	2
Maugeotia cf. *scalaris* Hassall	2
Melosira varians C. Agardh	2
Oedogonium sp.	+
Spirogyra cf. *circumlineata* Transeau	1
Spirogyra cf. *supervarians* Transeau	+
Stigeoclonium tenue (Aghard) Kützing	4
Tribonema viride Pascher	2
Tribonema vulgare Pascher	3
Tribonemna microchloron Ettl	4
Ulothrix tenuissima Kützing	2

3.3. Mechanisms of chromium phycoremediation

3.3.1. Desorption experiments

In the first step, the algal biomass containing chromium, collected from the natural habitat, was contacted with various eluents in order to desorb Cr(VI) and Cr(III) and to understand the binding pattern of chromium on algae. The varied efficiency of Cr(III) and Cr(VI)

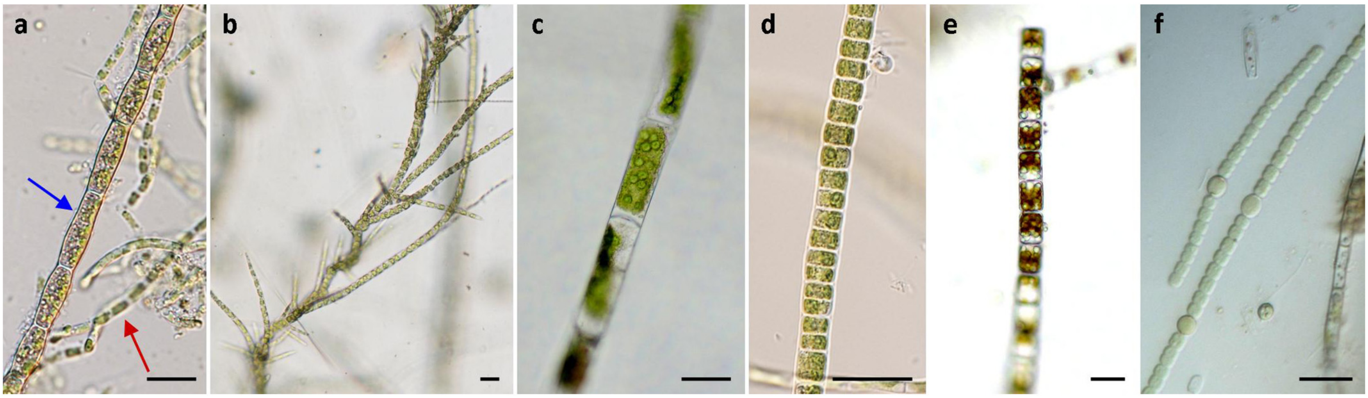

Fig. 2. Microphotographs of the predominant genera in the mat: *Tribonema* sp. (indicated by blue arrow) and *Klebsormidium* sp. (indicated by red arrow) (a); *Stigeoclonium* sp. (b); *Mougeotia* sp. (c); *Ulothrix* sp. (d); *Melosira* sp. (e); *Dolichospermum* sp. (f); scale bar = 20 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

desorption for different eluents (Fig. 3) allowed the preliminary identification of the main functional groups involved in the biosorption process. Moreover, rough distinction between chromium adsorbed on the biomass surface and intracellularly accumulated may be done using this simple method. Effective desorption of chromium using only diluted solutions that cause no disruption of the cell walls may indicate biosorption as the main mechanism of chromium detoxification, although partial removal of intracellular chromium is also inevitable during this process (Mehta and Gaur, 2005). As can be seen in Fig. 3, in spite of the presence of Cr(VI) solely in the studied aquatic system, the majority of chromium bound to the algae (>90%) was in the Cr(III) form and only low recovery yields of Cr(VI) by NaOH solutions were observed.

This is in accordance with other studies dealing with the removal of Cr(VI) from aqueous solution by means of algal biomass, indicating reduction as the important part of the remediation process (Pagnanelli et al., 2013; Samuel et al., 2015; Shen et al., 2010). The highest recovery of accumulated chromium was observed after the use of nitric acid, which might denote ion exchange as the dominating binding mechanism (Han et al., 2006; Yun et al., 2001), since the complexes of Cr(III) with carboxylic groups are not readily desorbed in mineral acids (Han et al., 2006; Yun et al., 2001). Chojnacka et al. studied adsorption/desorption of Cr(III) on *Spirulina* sp., and concluded that the biomass has characteristics similar to weakly acidic ion exchangers, and Cr(III) was bound to its surface mainly by carboxylate and phosphate functional groups (Chojnacka et al., 2005). Furthermore, since contact of the metal-loaded biomass with chelating agent (0.1 M EDTA) resulted in desorption of 13.3% of accumulated chromium, both ion exchange and complexation may be considered as the main mechanisms of Cr(III) binding. This was also confirmed by very low efficiency of chromium desorption in 0.1 and 0.5 M NaOH, which is a common method used for weak base anion-exchange resins regeneration, allowing desorption of only chromates(VI). Thus, it seems that passive biosorption might be mainly responsible for chromium accumulation. Meanwhile, the substantially higher desorption effectiveness of chromium in more concentrated HNO_3 and NaOH solutions, likely causing disruption of the cell walls (Kratochvil et al., 1998), may suggest the presence of chromium within the cells, and thereby confirming the minor involvement of metabolism-dependent processes in chromium accumulation by the described algal community.

3.3.2. FTIR and XPS studies

In order to study the interactions of the functional groups with Cr (III) and Cr(VI) more closely, the algal biomass collected from the natural habitat (raw biomass with adsorbed chromium (Fig. 1f)), as well as the biomass after chromium desorption by 0.1 M HNO_3 and 0.1 M NaOH, was characterized using infrared spectroscopy technique. As can be seen in Fig. 4, the spectra of the biomass contain peaks characteristic for the functional groups commonly present in biomolecules in algal cell walls (Table 3). More useful when studying the biosorption mechanisms are changes observed in spectra after contacting with HNO_3 and NaOH solutions (Fig. 4b–c). Evident changes in bands' intensity and frequency after chromium desorption in HNO_3 confirmed the role of carboxyl functional groups as the main functionalities in Cr(III) binding. The most noticeable changes were observed for bands at 1221, 1452 and 1740 cm^{-1}, which can be attributed to various carboxyl stretches (Table 3).

The newly formed bands at 1740 cm^{-1} (free COOH) and 1452 cm^{-1} (COO^-), as well as the increase in the band at 1221 cm^{-1} (C–O stretching in COOH) after desorption in HNO_3, confirmed the vital role of carboxylic groups in ion exchange with Cr(III) cations. Similar conclusions have also been presented by other authors (Godlewska et al., 2018; Lee and Chang, 2011; Popuri et al., 2007; Sari and Tuzen, 2008; Yun et al., 2001). The peak at 1651 cm^{-1}, which likely corresponds to C=O stretching in COOH and amide(I), became higher and shifted toward lower energy after chromium desorption in HNO_3, which might indicate some contribution of coordinate bonding between Cr(III) and oxygen in carbonyl groups.

This is in agreement with the suppositions made on the basis of desorption studies. Moreover, similar results were reported by Dittert et al. when studying Cr(III) biosorption by the brown macroalga *Laminaria digitate* (Dittert et al., 2012). Mechanisms of trivalent chromium binding to carboxylic groups by ion exchange and complexation have been described in detail by Papageorgiou et al. (2010) and Yun et al. (2001). It should also be noted that many authors have reported the evident enhancement in the intensity of the band at approximately 1630–1650 cm^{-1}, after Cr(VI) adsorption on the algal biomass, which had arisen from the increase in the amount of surface carbonyl groups formed as a result of alcohol and phenol oxidation by Cr(VI) (Pagnanelli et al., 2013; Shen et al., 2010). The results obtained in the present study (Fig. 3) suggest however that no significant chemical oxidation of biomass surface took place during Cr(VI) accumulation. The observed slight changes for bands at 1150–1160 cm^{-1} and 1370–1390 cm^{-1} reveal the small involvement in Cr(III) binding of sulfonate or phosphonate functionalities present in sulfonated polysaccharides, or phospholipids, respectively. Pagnanelli et al. reported binding of Cr(III) by labile electrostatic interactions to sulfonates present on the biomass surface of *Chlorella vulgaris* (Pagnanelli et al., 2013). The substantial shift of the band at 1537 cm^{-1} to 1516 cm^{-1} or 1517 cm^{-1} after chromium desorption in both HNO_3 and NaOH likely corresponds to Cr(VI) binding on glucosamine groups present in cell glycoproteins of both freshwater

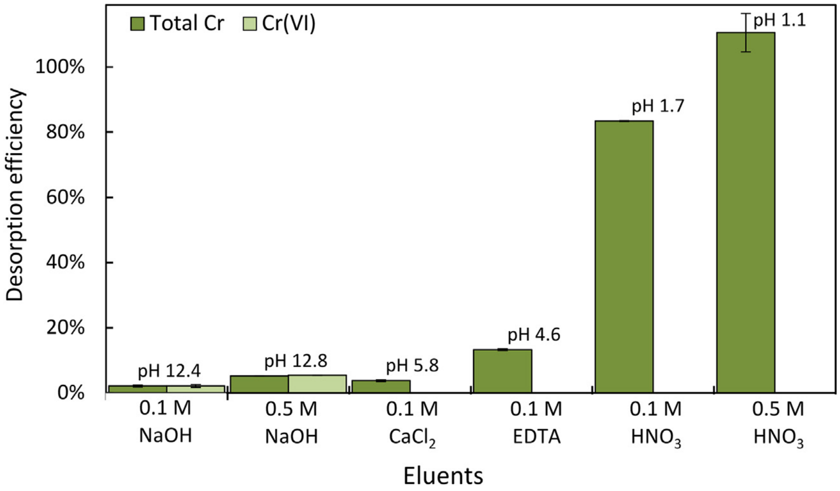

Fig. 3. Recovery yields of both total chromium and Cr(VI) from the algal biomass by various eluents.

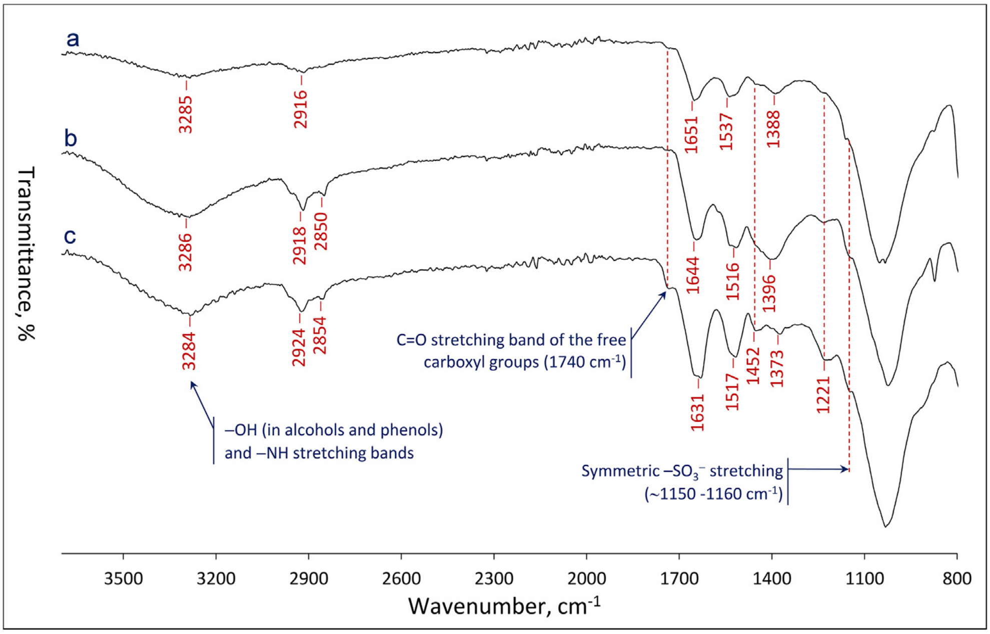

Fig. 4. FTIR spectra of raw algal biomass (a) and the biomass after chromium desorption by NaOH (b) and HNO_3 (c).

and sea algae (Table 3) (Kratochvil et al., 1998; Yalçın and Özyürek, 2018). Unlike the hydroxyl groups usually involved in Cr(VI) binding under acidic conditions, the glucosamine groups can react with chromates also at near neutral pH ($pk_a > 8$) (Han et al., 2006) according to the ion-exchange reaction characteristic for weak-anion exchangers:

$$R\text{-}NH_{2(s)} + H^{+}_{(aq)} + HCrO^{-}_{4(aq)} \rightarrow R\text{-}NH_3^{+}\cdots HCrO^{-}_{4(s)} \quad (1)$$

To confirm the abovementioned considerations, the X-ray photoelectron spectroscopy analysis of the biomass surface (raw biomass with adsorbed chromium (Fig. 1f)) was also conducted. Fig. 5 shows a deconvoluted Cr 2p spectra with two overlapping peaks at 576.8 eV (Cr(III)) and 579.3 eV (Cr(VI)). The results revealed the presence of mainly Cr(III) (~82%) bound on the algal surface and only a small amount of adsorbed Cr(VI), which is consistent with our previous findings from chromium desorption and FTIR analyses.

To sum up, the conducted studies indicated that chromium was bound mainly to the external site of the cells or to EPS, in the form of Cr(III), feasibly by ion exchange and complexation mechanisms. In view of the neutral or weakly alkaline pH of the studied aquatic system, precipitation of $Cr(OH)_3$ on the biomass surface is also very likely. However, since only Cr(VI) was present in the studied aquatic system, its highly effective reduction to Cr(III) under neutral conditions needs to be explained.

3.3.3. Mechanism of Cr(VI) reduction

It is commonly known that Cr(VI) is a powerful oxidizing agent, which can oxidize alcohols and phenols to corresponding ketones and carboxyl acids, while Cr(VI) is reduced to Cr(III) and then may be adsorbed on the freshly created functionalities. Polyphenols as well as hydroxyl groups in polysaccharides of algal cell walls and exopolysaccharides are considered as the main electron-donor groups in algal biomass responsible for Cr(VI) reduction (Elangovan et al., 2008; Yalçın and Özyürek, 2018). Especially the presence of

Table 3
Functional groups of studied algal biomass involved in biosorption process and the corresponding FTIR bands.

Assignment	Wavenumber, cm^{-1}			Examples of possible biomolecules
	Raw algae (with Cr)	After desorption		
		In 0.1 M HNO_3	In 0.1 M NaOH	
C=O stretching of the free COOH (Fourest and Volesky, 1996; Murphy et al., 2007)	–	~1740	–	Alginic acid, uronic acids, amino acids
C–O stretching of COOH (Murphy et al., 2007; Park et al., 2004)	–	1221	–	
Stretching of COO^{-} (Kang et al., 2007; Pagnanelli et al., 2013)	–	1452	–	
C=O stretching of COOH or amide(I) (Park et al., 2004; Yun et al., 2001)	1651	1631	1644	Amino acids (peptide bonds)
N–H stretching of amide(II)/amines (Pagnanelli et al., 2013; Zaccaroni and Scaravelli, 2008)	1537	1517	1516	Amino acids, glucosamine in glicoproteins
Asymmetric sulfate stretching (Murphy et al., 2007; Pagnanelli et al., 2013; Pankiewicz et al., 2016)	1388	1373	1396	Sulfated polysaccharides (heteropolysaccharides)
Symmetric sulfate or phosphate stretching (Chen et al., 2019; Dittert et al., 2012; Pankiewicz et al., 2016)	~1160	~1150	~1150	Sulfated polysaccharide or phospholipids

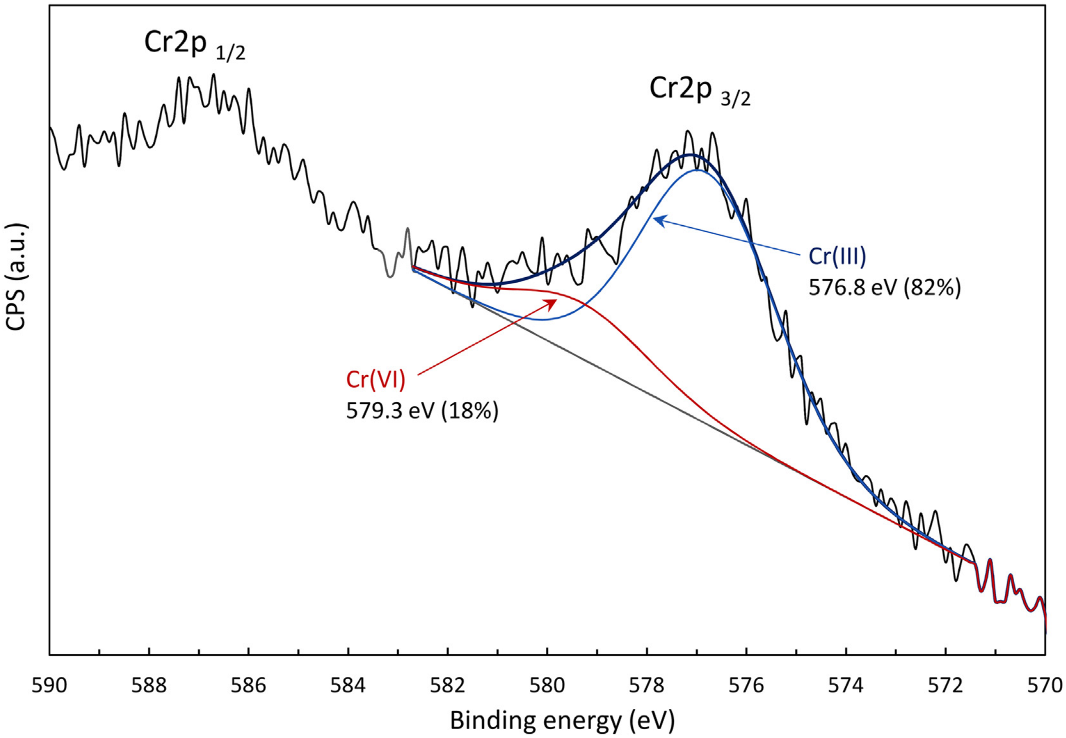

Fig. 5. Deconvoluted high resolution XPS Cr 2p spectra of the algal biomass surface.

exopolysaccharides (most often in the form of external slime) with a high number of hydroxyl groups may play an important role in the reduction of Cr(VI) to Cr(III) by algae (Hegde et al., 2016). Dittert et al. observed an increase in Cr(III) uptake capacity by the oxidized algal biomass (after Cr(VI) reduction) in comparison with the algae in their original form (Dittert et al., 2014). Moreover, the significant increase of the number of COOH groups during algal oxidation by Cr(VI) was confirmed by potentiometric titrations. Samuel et al. also observed the presence of new carboxylic groups on the algal surface after contacting with Cr(VI), explaining it by oxidation of the free aldehyde group present in reducing sugars created during polysaccharide hydrolysis (Samuel et al., 2015). Some authors have observed decolorization of dried algal biomass after oxidation by Cr(VI), which revealed the participation of the algal pigments in the reduction of hexavalent to trivalent chromium (Dittert et al., 2014; Han et al., 2006). Also the sulfhydryl groups, which are present mainly in peptides, may be oxidized to sulfonated groups (Han et al., 2006; Shevchenko et al., 2008), according to the reaction:

$$\text{R-SH}_{(s)} + 2\,\text{HCrO}^-_{4(aq)} + 8\text{H}^+_{(aq)} \rightarrow \text{R-SO}_3\text{H}_{(s)} + 2\,\text{Cr}^{3+}_{(aq)} + 5\,\text{H}_2\text{O}_{(aq)} \quad (2)$$

Notwithstanding, it should be noted that the reduction of Cr(VI) is a highly pH-dependent process, since not only electrons but also protons are necessary in this reaction:

$$\text{HCrO}_4^- + 7\text{H}^+ + 3\,\text{e}^- \rightarrow \text{Cr}^{3+} + 4\,\text{H}_2\text{O} \quad (3)$$

Thus, the process of biomass oxidation by Cr(VI) can be effective only under strongly acidic conditions, usually between pH 1 and 4. On the other hand, although low pH values substantially enhance the reduction rate, Cr(III) sorption is drastically reduced under acidic conditions as the biomass surface becomes protonated, resulting in an evident decrease in chromium removal efficiency (Al-Homaidan et al., 2018; Dittert et al., 2014). The increase in pH of the solution is beneficial for chromium sorption, but simultaneously it negatively influences Cr(VI) reduction. Even though the efficiency of this process under weakly acidic conditions (pH 4.5–5) is still high enough, the rate of Cr(VI) reduction drastically drops (Sawalha et al., 2005; Yalçın and Özyürek, 2018). A further increase in solution pH also results in suppression of the chromium reduction efficiency. Kratochvil et al. studied the removal of Cr(VI) from aqueous solutions by means of dried algal biomass and concluded that the reduction and uptake of hexavalent chromium at pH 7 were negligible (Kratochvil et al., 1998).

Meanwhile, the conditions in the studied aquatic system were weakly alkaline (pH 8.1–8.3), which implies that effective chemical reduction of Cr(VI) by the abovementioned mechanism is unlikely. Notwithstanding, the observed excellent chromium uptake by the algae suggests a different, metabolism-dependent mechanism of Cr(VI) reduction, feasibly biological or biologically induced chemical reduction. It should also be mentioned that the occurrence of adverse reduction of bound Cr(VI) only during desorption in mineral acids can be excluded in the studied system. The binding of Cr(VI) anions on the biomass surface, according to an “acid adsorption” mechanism, became effective only under acidic conditions when hydroxyl and phenolic groups became protonated. Also the amount of glucosamine groups on the biomass surface, which are able to bind chromium at neutral pH (reaction 1), is not sufficient to explain the observed excellent Cr(VI) uptake. The presence of mainly Cr(III) on the studied biomass surface was also confirmed in the desorption studies (Fig. 3), after washing of chromium-loaded algae with 0.5 M NaOH. This common method used for weak base anion-exchange resins regeneration (SenGupta, 2017) resulted in the desorption of only 5.2% of the total adsorbed chromium. Also the results from XPS studies revealed the presence of mainly Cr(III) on the biomass surface. Thus, and because of the presence of living algae in the studied system, the metabolism-dependent mechanism seems to be more likely responsible for Cr(VI) reduction. As has been previously mentioned, the process of Cr(VI) reduction by living organisms may be of either enzymatic or non-enzymatic origin. Although enzymatically driven processes are widely described mainly for bacteria, there are some studies reporting the ability of algae to reduce Cr(VI) via chromium reductase (Kamaludeen et al., 2003; Rehman and Shakoori, 2001; Yen et al., 2017; Yewalkar et al., 2007). However, the published data concern mainly microalgae. For example, Yen et al. reported complete reduction of Cr(VI), at pH 7.0, when contacting with living *Chlorella vulgaris* algae (Yen et al., 2017). Additionally, there are some important factors influencing both the rate and the effectiveness of Cr(VI) bioreduction. Yewalkar et al. revealed that Cr(VI) bioreduction by algae belonging to *Chlorella* sp. (isolated from an industrial waste disposal site) was stimulated by light and decreased in the dark (Yewalkar et al., 2007). Also the presence of Ca^{2+} and Mg^{2+} in the aquatic system facilitates Cr(VI) bioreduction under near neutral conditions (Opperman et al., 2008). Thus, it may be concluded that the highly effective reduction of Cr(VI) to Cr(III) by the studied community (under near neutral pH conditions and visible light, and in the presence of elevated concentration of Ca^{2+} ions) was of biological origin and may have resulted from e.g. enzymatic activity of the algae. Moreover, we can also predict that symbiotic bacteria that may also be present on the surface of the algal biological mat can play some role in the Cr(VI) bioreduction.

4. Conclusions

We revealed that the three *Tribonema* algal species, namely *T. vulgare*, *T. microchloron* and *T. viride*, dominating in the studied community, are likely responsible for the effective Cr(VI) bioremediation. It is worth drawing attention to the fact that these species have never been studied before with regard to the mechanisms of Cr(VI) removal. We found that the main mechanism of Cr(VI) detoxification by the studied biological mat was reduction followed by Cr(III) biosorption. The excellent efficiency of chromium reduction under the given weakly alkaline conditions indicates the biological origin of this process. However, further studies are needed to explain this phenomenon in detail.

We suggest that the examined retention ditch inhabited by the algal community can be used, after some modifications, as a simple, cost-effective "bioreactor" allowing the reduction of chromium concentration to the desired level. The vital benefits of the studied biological mat are:

- ability to grow in extremely high concentrations of Cr(VI), i.e. up to ca. 6150 times the upper limit for surface water,
- hyperaccumulative properties toward chromium present in the hexavalent form (accumulation levels ≥300 mg/kg d.w., and bioconcentration factor > 1),
- suitable physical form of filamentous macroalgae that does not require immobilization.

However, in order to better utilize the potential of the studied system, providing additional economic and environmental benefits, some operations should be undertaken. They include rebuilding of the retention ditch and solving the problem of safe disposal for further technological application of the chromium-loaded algae.

CRediT authorship contribution statement

Daniel Ociński: Conceptualization, Methodology, Formal analysis, Data curation, Visualization, Writing – original draft, Writing – review & editing. **Joanna Augustynowicz:** Conceptualization, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Konrad Wołowski:** Methodology, Data curation, Writing – original draft. **Piotr Mazur:** Formal analysis, Data curation. **Ewa Sitek:** Visualization. **Jerzy Raczyk:** Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We are grateful to Mrs. Beata Domnicz (University of Agriculture in Krakow) for the technical assistance in freeze-drying of the algae biomass. This work was financially supported by the grant "Incubator of Innovation 2.0" under the Intelligent Developmental Operational Program 2014–2020 (Measure 4.4) and subsidies (SUB/2020-050012-D017 and SUB/2020-050012-D011) of the Ministry of Science and Higher Education of the Republic of Poland.

References

Abou-Shanab, R.A.I., Hwang, J.H., Cho, Y., Min, B., Jeon, B.H., 2011. Characterization of microalgal species isolated from fresh water bodies as a potential source for biodiesel production. Appl. Energy 88, 3300–3306. https://doi.org/10.1016/j.apenergy.2011.01.060.

Al-Homaidan, A.A., Al-Qahtani, H.S., Al-Ghanayem, A.A., Ameen, F., Ibraheem, I.B.M., 2018. Potential use of green algae as a biosorbent for hexavalent chromium removal from aqueous solutions. Saudi J. Biol. Sci. 25, 1733–1738. https://doi.org/10.1016/j.sjbs.2018.07.011.

Apandi, N.M., Mohamed, R.M.S.R., Al-Gheethi, A., Kassim, A.H.M., 2019. Microalgal biomass production through phycoremediation of fresh market wastewater and potential applications as aquaculture feeds. Environ. Sci. Pollut. Res. 26, 3226–3242. https://doi.org/10.1007/s11356-018-3937-3.

Augustynowicz, J., Sitek, E., Latowski, D., Wołowski, K., Kowalczyk, A., Przejczowski, R., 2021. Unique biocenosis as a foundation to develop a phytobial consortium for effective bioremediation of Cr(VI)-polluted waters and sediments. Environ. Pollut. 273, 116506. https://doi.org/10.1016/j.envpol.2021.116506.

Bakatula, E.N., Cukrowska, E.M., Weiersbye, I.M., Mihaly-Cozmuta, L., Peter, A., Tutu, H., 2014. Biosorption of trace elements from aqueous systems in gold mining sites by the filamentous green algae (*Oedogonium* sp.). J. Geochem. Explor. 144, 492–503. https://doi.org/10.1016/j.gexplo.2014.02.017.

Bertagnolli, C., da Silva, M.G.C., Guibal, E., 2014. Chromium biosorption using the residue of alginate extraction from *Sargassum filipendula*. Chem. Eng. J. 237, 362–371. https://doi.org/10.1016/j.cej.2013.10.024.

Bwapwa, J.K., Jaiyeola, A.T., Chetty, R., 2017. Bioremediation of acid mine drainage using algae strains: a review. South Afr. J. Chem. Eng. 24, 62–70. https://doi.org/10.1016/j.sajce.2017.06.005.

Cabatingan, L.K., Agapay, R.C., Rakels, J.L.L., Ottens, M., Van der Wielen, L.A.M., 2001. Potential of biosorption for the recovery of chromate in industrial wastewaters. Ind. Eng. Chem. Res. 40, 2302–2309. https://doi.org/10.1021/ie0008575.

Chabukdhara, M., Gupta, S.K., Gogoi, M., 2017. Phycoremediation of heavy metals coupled with generation of bioenergy. In: Gupta, S.K., Malik, A., Bux, F. (Eds.), Algal Biofuels: Recent Advances and Future Prospects. Springer International Publishing, Cham, pp. 163–188 https://doi.org/10.1007/978-3-319-51010-1_9.

Chen, X., Song, L., Wang, H., Liu, S., Yu, H., Wang, X., Li, R., Liu, T., Li, P., 2019. Partial characterization, the immune modulation and anticancer activities of sulfated polysaccharides from filamentous microalgae *Tribonema* sp. Molecules 24, 1–11. https://doi.org/10.3390/molecules24020322.

Chojnacka, K., Chojnacki, A., Górecka, H., 2005. Biosorption of Cr^{3+}, Cd^{2+} and Cu^{2+} ions by blue-green algae *Spirulina* sp.: kinetics, equilibrium and the mechanism of the process. Chemosphere 59, 75–84. https://doi.org/10.1016/j.chemosphere.2004.10.005.

Craggs, R., Park, J., Heubeck, S., Sutherland, D., 2014. High rate algal pond systems for low-energy wastewater treatment, nutrient recovery and energy production. N. Z. J. Bot. 52, 60–73. https://doi.org/10.1080/0028825X.2013.861855.

Davis, T.A., Volesky, B., Mucci, A., 2003. A review of the biochemistry of heavy metal biosorption by brown algae. Water Res. 37, 4311–4330. https://doi.org/10.1016/S0043-1354(03)00293-8.

Dittert, I.M., Vilar, V.J.P., da Silva, E.A.B., de Souza, S.M.A.G.U., de Souza, A.A.U., Botelho, C.M.S., Boaventura, R.A.R., 2012. Adding value to marine macro-algae *Laminaria digitata* through its use in the separation and recovery of trivalent chromium ions from aqueous solution. Chem. Eng. J. 193–194, 348–357. https://doi.org/10.1016/j.cej.2012.04.048.

Dittert, I.M., de Lima Brandão, H., Pina, F., da Silva, E.A.B., De Souza, S.M.A.G.U., de Souza, A. Ô.A.U., Botelho, C.M.S., Boaventura, R.A.R., Vilar, V.J.P., 2014. Integrated reduction/oxidation reactions and sorption processes for Cr(VI) removal from aqueous solutions using *Laminaria digitata* macro-algae. Chem. Eng. J. 237, 443–454. https://doi.org/10.1016/j.cej.2013.10.051.

Elangovan, R., Philip, L., Chandraraj, K., 2008. Biosorption of chromium species by aquatic weeds: kinetics and mechanism studies. J. Hazard. Mater. 152, 100–112. https://doi.org/10.1016/j.jhazmat.2007.06.067.

van der Ent, A., Baker, A.J.M., Reeves, R.D., Pollard, A.J., Schat, H., 2013. Hyperaccumulators of metal and metalloid trace elements: facts and fiction. Plant Soil 362, 319–334. https://doi.org/10.1007/s11104-012-1287-3.

Fletcher, J., Willby, N., Oliver, D.M., Quilliam, R.S., 2020. Phytoremediation using aquatic plants. In: Shmaefsky, B.R. (Ed.), Phytoremediation. Concepts and Strategies in Plant Sciences. Springer International Publishing, pp. 205–260 https://doi.org/10.1007/978-3-030-00099-8_7.

Fourest, E., Volesky, B., 1996. Contribution of sulfonate groups and alginate to heavy metal biosorption by the dry biomass of *Sargassum fluitans*. Environ. Sci. Technol. 30, 277–282. https://doi.org/10.1021/es950315s.

Francisco, R., Moreno, A., Morais, P.V., 2010. Different physiological responses to chromate and dichromate in the chromium resistant and reducing strain *Ochrobactrum tritici* 5bvl1. BioMetals 23, 713–725. https://doi.org/10.1007/s10534-010-9338-9.

Furey, P.C., Liess, A., Lee, S., 2017. Substratum-associated microbiota. Water Environ. Res. 89, 1634–1675. https://doi.org/10.2175/106143017X15023776270610.

Godlewska, K., Marycz, K., Michalak, I., 2018. Freshwater green macroalgae as a biosorbent of Cr(III) ions. Open Chem. 16, 689–701. https://doi.org/10.1515/chem-2018-0075.

Graham, L.E., Wilcox, L.W., Graham, J.M., 2009. Algae. Pearson/Benjamin Cummings, San Francisco; Montreal.

Guiry, M.D., Guiry, G.M., 2021. AlgaeBase. World-wide electronic publication. [WWW Document]. National University of Ireland, Galway. URL. http://www.algaebase.org.

Gupta, S.K., Malik, A., Bux, F., 2017. Algal biofuels: recent advances and future prospects. Algal Biofuels: Recent Advances and Future Prospects. Springer International Publishing AG https://doi.org/10.1007/978-3-319-51010-1.

Gupta, V.K., Rastogi, A., 2008. Sorption and desorption studies of chromium(VI) from nonviable cyanobacterium *Nostoc muscorum* biomass. J. Hazard. Mater. 154, 347–354. https://doi.org/10.1016/j.jhazmat.2007.10.032.

Gupta, V.K., Shrivastava, A.K., Jain, N., 2001. Biosorption of chromium(VI) from aqueous solutions by green algae *spirogyra* species. Water Res. 35, 4079–4085. https://doi.org/10.1016/S0043-1354(01)00138-5.

Han, X., Wong, Y.S., Tam, N.F.Y., 2006. Surface complexation mechanism and modeling in Cr(III) biosorption by a microalgal isolate, *Chlorella miniata*. J. Colloid Interf. Sci. 303, 365–371. https://doi.org/10.1016/j.jcis.2006.08.028.

He, J., Chen, J.P., 2014. A comprehensive review on biosorption of heavy metals by algal biomass: materials, performances, chemistry, and modeling simulation tools. Bioresour. Technol. 160, 67–78. https://doi.org/10.1016/j.biortech.2014.01.068.

Hegde, S.M., Babu, R.L., Vijayalakshmi, E., Patil, R.H., Naveen Kumar, M., Kiran Kumar, K.M., Nagesh, R., Kavya, K., Sharma, S.C., 2016. Biosorption of hexavalent chromium from aqueous solution using chemically modified *Spirulina platensis* algal biomass: an ecofriendly approach. Desalin. Water Treat. 57, 8504–8513. https://doi.org/10.1080/19443994.2015.1019370.

Hunt, A.J., Anderson, C.W.N., Bruce, N., García, A.M., Graedel, T.E., Hodson, M., Meech, J.A., Nassar, N.T., Parker, H.L., Rylott, E.L., Sotiriou, K., Zhang, Q., Clark, J.H., 2014. Phytoextraction as a tool for green chemistry. Green Process. Synth. 3, 3–22. https://doi.org/10.1515/gps-2013-0103.

Husien, S., Labena, A., El-Belely, E.F., Mahmoud, H.M., Hamouda, A.S., 2019. Absorption of hexavalent chromium by green micro algae *Chlorella sorokiniana*: live planktonic cells. Water Pract. Technol. 14, 515–529. https://doi.org/10.2166/wpt.2019.034.

John, D.M., Whitton, B.A., Brook, A.J. (Eds.), 2011. The Freshwater Algal Flora of the British Isles. An Identification Guide to Freshwater and Terrestrial Algae, 2nd ed. Cambridge University Press.

Journal of Laws of the Republic of Poland, 2019. Regulation of the Polish Ministry of Maritime Economy and Inland Navigation as of 11 October 2019 item 2149.

Kamaludeen, S.P.B., Megharaj, M., Juhasz, A.L., Sethunathan, N., Naidu, R., 2003. Chromium-microorganism interactions in soils: remediation implications. Rev. Environ. Contam. Toxicol. 178, 93–164. https://doi.org/10.1007/0-387-21728-2_4.

Kang, S.Y., Lee, J.U., Kim, K.W., 2007. Biosorption of Cr(III) and Cr(VI) onto the cell surface of *Pseudomonas aeruginosa*. Biochem. Eng. J. 36, 54–58. https://doi.org/10.1016/j.bej.2006.06.005.

Kotaś, J., Stasicka, Z., 2000. Chromium occurrence in the environment and methods of its speciation. Environ. Pollut. 107, 263–283. https://doi.org/10.1016/S0269-7491(99)00168-2.

Kratochvil, D., Pimentel, P., Volesky, B., 1998. Removal of trivalent and hexavalent chromium by seaweed biosorbent. Environ. Sci. Technol. 32, 2693–2698. https://doi.org/10.1021/es971073u.

Kuklin, A.P., Pomazkova, N.V., 2017. Fresh-water macroalgae in monitoring of water pollution by toxic metals in near-border territories. Res. J. Pharm. Biol. Chem. Sci. 8, 812–820.

Lee, Y.C., Chang, S.P., 2011. The biosorption of heavy metals from aqueous solution by *Spirogyra* and *Cladophora* filamentous macroalgae. Bioresour. Technol. 102, 5297–5304. https://doi.org/10.1016/j.biortech.2010.12.103.

Li-Beisson, Y., Thelen, J.J., Fedosejevs, E., Harwood, J.L., 2019. The lipid biochemistry of eukaryotic algae. Prog. Lipid Res. 74, 31–68. https://doi.org/10.1016/j.plipres.2019.01.003.

Lin, Z., Li, J., Luan, Y., Dai, W., 2020. Application of algae for heavy metal adsorption: a 20-year meta-analysis. Ecotoxicol. Environ. Saf. 190, 110089. https://doi.org/10.1016/j.ecoenv.2019.110089.

Lutzu, G.A., Ciurli, A., Chiellini, C., Di Caprio, F., Concas, A., Dunford, N.T., 2021. Latest developments in wastewater treatment and biopolymer production by microalgae. J. Environ. Chem. Eng. 9, 104926. https://doi.org/10.1016/j.jece.2020.104926.

Mantzorou, A., Navakoudis, E., Paschalidis, K., Ververidis, F., 2018. Microalgae: a potential tool for remediating aquatic environments from toxic metals. Int. J. Environ. Sci. Technol. 15, 1815–1830. https://doi.org/10.1007/s13762-018-1783-y.

Mehta, S.K., Gaur, J.P., 2005. Use of algae for removing heavy metal ions from wastewater: progress and prospects. Crit. Rev. Biotechnol. 25, 113–152. https://doi.org/10.1080/07388550500248571.

Mohan, D., Pittman, C.U., 2006. Activated carbons and low cost adsorbents for remediation of tri- and hexavalent chromium from water. J. Hazard. Mater. 137, 762–811. https://doi.org/10.1016/j.jhazmat.2006.06.060.

Murphy, V., Hughes, H., McLoughlin, P., 2007. Cu(II) binding by dried biomass of red, green and brown macroalgae. Water Res. 41, 731–740. https://doi.org/10.1016/j.watres.2006.11.032.

Onyancha, D., Mavura, W., Ngila, J.C., Ongoma, P., Chacha, J., 2008. Studies of chromium removal from tannery wastewaters by algae biosorbents, *Spirogyra condensata* and *Rhizoclonium hieroglyphicum*. J. Hazard. Mater. 158, 605–614. https://doi.org/10.1016/j.jhazmat.2008.02.043.

Opperman, D.J., Piater, L.A., Van Heerden, E., 2008. A novel chromate reductase from *Thermus scotoductus* SA-01 related to old yellow enzyme. J. Bacteriol. 190, 3076–3082. https://doi.org/10.1128/JB.01766-07.

Pagnanelli, F., Jbari, N., Trabucco, F., Martínez, M.E., Sánchez, S., Toro, L., 2013. Biosorption-mediated reduction of Cr(VI) using heterotrophically-grown *Chlorella vulgaris*: active sites and ionic strength effect. Chem. Eng. J. 231, 94–102. https://doi.org/10.1016/j.cej.2013.07.013.

Pankiewicz, R., Łęska, B., Messyasz, B., Fabrowska, J., Sołoducha, M., Pikosz, M., 2016. First isolation of polysaccharidic ulvans from the cell walls of freshwater algae. Algal Res. 19, 348–354. https://doi.org/10.1016/j.algal.2016.02.025.

Papageorgiou, S.K., Kouvelos, E.P., Favvas, E.P., Sapalidis, A.A., Romanos, G.E., Katsaros, F.K., 2010. Metal-carboxylate interactions in metal-alginate complexes studied with FTIR spectroscopy. Carbohydr. Res. 345, 469–473. https://doi.org/10.1016/j.carres.2009.12.010.

Park, D., Yun, Y.S., Cho, H.Y., Park, J.M., 2004. Chromium biosorption by thermally treated biomass of the brown seaweed, *Ecklonia* sp. Ind. Eng. Chem. Res. 43, 8226–8232. https://doi.org/10.1021/ie049323k.

PN-EN ISO 17294-1:2007, 2007. Water Quality - Application of Inductively Coupled Plasma Mass Spectrometry (ICP-MS) - Part 1: General Guidelines. Polish Committee for Standardization, Warsaw, Poland.

PN-ISO 9297:1994, 1994. Water Quality - Determination of Chloride - Silver Nitrate Titration with Chromate Indicator (Mohr's Method). Polish Committee for Standardization, Warsaw, Poland.

Polti, M.A., Amoroso, M.J., Abate, C.M., 2010. Chromate reductase activity in *Streptomyces* sp. MC1. J. Gen. Appl. Microbiol. 56, 11–18. https://doi.org/10.2323/jgam.56.11.

Popuri, S.R., Kalyani, S., Kachireddy, S.R., Krishnaiah, A., 2007. Biosorption of hexavalent chromium from aqueous solution by using prawn pond algae (*Sphaeroplea*). Indian J. Chem. Sect. A Inorg. Phys. Theor. Anal. Chem. 46, 284–289.

Priyadarshini, E., Priyadarshini, S.S., Pradhan, N., 2019. Heavy metal resistance in algae and its application for metal nanoparticle synthesis. Appl. Microbiol. Biotechnol. 103, 3297–3316. https://doi.org/10.1007/s00253-019-09685-3.

Rajamani, S., Siripornadulsil, S., Falcao, V., Torres, M., Colepicolo, P., Sayre, R., 2007. Phycoremediation of heavy metals using transgenic microalgae. In: León, R., Galván, A., Fernández, E. (Eds.), Transgenic Microalgae as Green Cell Factories. Springer, New York, New York, NY, pp. 99–109 https://doi.org/10.1007/978-0-387-75532-8_9.

Rehman, A., Shakoori, A.R., 2001. Heavy metal resistance *Chlorella* spp., isolated from tannery effluents, and their role in remediation of hexavalent chromium in industrial waste water. Bull. Environ. Contam. Toxicol. 66, 542–547. https://doi.org/10.1007/s00128-001-0041-y.

Rezaei, H., 2016. Biosorption of chromium by using *Spirulina* sp. Arab. J. Chem. 9, 846–853. https://doi.org/10.1016/j.arabjc.2013.11.008.

Rodrigues de Assis, L., Calijuri, M.L., Assemany, P.P., Silva, T.A., Teixeira, J.S., 2020. Innovative hybrid system for wastewater treatment: high-rate algal ponds for effluent treatment and biofilm reactor for biomass production and harvesting. J. Environ. Manag. 274, 111183. https://doi.org/10.1016/j.jenvman.2020.111183.

Sahmoune, M.N., Louhab, K., Boukhiar, A., 2011. Advanced biosorbents materials for removal of chromium from water and wastewaters. Environ. Prog. Sustain. Energy 30, 284–293. https://doi.org/10.1002/ep.

Salama, E.S., Roh, H.S., Dev, S., Khan, M.A., Abou-Shanab, R.A.I., Chang, S.W., Jeon, B.H., 2019. Algae as a green technology for heavy metals removal from various wastewater. World J. Microbiol. Biotechnol. 35, 1–19. https://doi.org/10.1007/s11274-019-2648-3.

Samuel, J., Paul, M.L., Kumari, J., Ravikumar, K.V.G., Natarajan, C., Mukherjee, A., 2015. Removal of Cr(VI) by immobilized consortium of freshwater microalgae in batch and continuous system. Asian J. Chem. 27, 2161–2170. https://doi.org/10.14233/ajchem.2015.18003.

Saravanan, A., Kumar, P.S., Yashwanthraj, M., 2017. Sequestration of toxic Cr(VI) ions from industrial wastewater using waste biomass: a review. Desalin. Water Treat. 68, 245–266. https://doi.org/10.5004/dwt.2017.20322.

Sari, A., Tuzen, M., 2008. Biosorption of total chromium from aqueous solution by red algae (*Ceramium virgatum*): equilibrium, kinetic and thermodynamic studies. J. Hazard. Mater. 160, 349–355. https://doi.org/10.1016/j.jhazmat.2008.03.005.

Sawalha, M.F., Gardea-Torresdey, J.L., Parsons, J.G., Saupe, G., Peralta-Videa, J.R., 2005. Determination of adsorption and speciation of chromium species by saltbush (*Atriplex canescens*) biomass using a combination of XAS and ICP-OES. Microchem. J. 81, 122–132. https://doi.org/10.1016/j.microc.2005.01.008.

SenGupta, A.K., 2017. Ion exchange fundamentals. Ion Exchange in Environmental Processes. John Wiley & Sons, Ltd, pp. 50–129 https://doi.org/10.1002/9781119421252.ch2.

Shen, Y.S., Wang, S.L., Huang, S.T., Tzou, Y.M., Huang, J.H., 2010. Biosorption of Cr(VI) by coconut coir: spectroscopic investigation on the reaction mechanism of Cr(VI) with lignocellulosic material. J. Hazard. Mater. 179, 160–165. https://doi.org/10.1016/j.jhazmat.2010.02.073.

Shevchenko, N., Zaitsev, V., Walcarius, A., 2008. Bifunctionalized mesoporous silicas for Cr(VI) reduction and concomitant Cr(III) immobilization. Environ. Sci. Technol. 42, 6922–6928. https://doi.org/10.1021/es800677b.

Singh, P., Itankar, N., Patil, Y., 2021. Biomanagement of hexavalent chromium: current trends and promising perspectives. J. Environ. Manag. 279, 111547. https://doi.org/10.1016/j.jenvman.2020.111547.

Sukla, L., Nayak, M., Jena, J., Sekhar Panda, H., Pradhan, N., Panda, P., Mishra, S., Das, B., Sarika, C., Rao, B.V.S.K., Prasad, R.B.N., Mishra, B.K., 2013. Large scale cultivation of brackish water isolates *Scenedesmus* sp. in raceway pond for biodiesel production. In: Khanna, D.R., Chopra, A.K., Matta, G., Bhutiani, R., Singh, V. (Eds.), Environmental Technology. Daya Publishing House, New Delhi, pp. 79–91.

Tian, C., Li, B., Liu, Z., Zhang, Y., Lu, H., 2014. Hydrothermal liquefaction for algal biorefinery: a critical review. Renew. Sust. Energ. Rev. 38, 933–950. https://doi.org/10.1016/j.rser.2014.07.030.

Volesky, B., 2007. Biosorption and me. Water Res. 41, 4017–4029. https://doi.org/10.1016/j.watres.2007.05.062.

Wehr, J.D., Sheath, R.G., Kociolek, J.P. (Eds.), 2014. Freshwater Algae of North America. Ecology and Classification, 2nd ed. Academic Press.

Wołowski, K., 1989. The algae occurring in an uncovered trickling filter of sewage treatment plant in Cracow. Arch. Hydrobiol. Algol. Stud. 55, 207–239.

Yalçın, S., Özyürek, M., 2018. Biosorption potential of two brown seaweeds in the removal of chromium. Water Sci. Technol. 78, 2564–2576. https://doi.org/10.2166/wst.2019.007.

Yen, H.W., Chen, P.W., Hsu, C.Y., Lee, L., 2017. The use of autotrophic *Chlorella vulgaris* in chromium (VI) reduction under different reduction conditions. J. Taiwan Inst. Chem. Eng. 74, 1–6. https://doi.org/10.1016/j.jtice.2016.08.017.

Yewalkar, S.N., Dhumal, K.N., Sainis, J.K., 2007. Chromium(VI)-reducing *Chlorella* spp. isolated from disposal sites of paper-pulp and electroplating industry. J. Appl. Phycol. 19, 459–465. https://doi.org/10.1007/s10811-007-9153-z.

Young, P., Taylor, M., Fallowfield, H.J., 2017. Mini-review: high rate algal ponds, flexible systems for sustainable wastewater treatment. World J. Microbiol. Biotechnol. 33, 1–13. https://doi.org/10.1007/s11274-017-2282-x.

Yun, Y., Park, D., Park, J.M., Volesky, B., 2001. Biosorption of trivalent chromium on the brown seaweed biomass. Environ. Sci. Technol. 35, 4353–4358. https://doi.org/10.1021/es010866k.

Zaccaroni, A., Scaravelli, D., 2008. Toxicity of fresh water algal toxins to humans and animals. In: Evangelista, V., Barsanti, L., Frassanito, A.M., Passarelli, V., Gualtieri, P. (Eds.), Algal Toxins: Nature, Occurrence, Effect and Detection. Springer Netherlands, Dordrecht, pp. 45–89.

Zeraatkar, A.K., Ahmadzadeh, H., Talebi, A.F., Moheimani, N.R., McHenry, M.P., 2016. Potential use of algae for heavy metal bioremediation, a critical review. J. Environ. Manag. 181, 817–831. https://doi.org/10.1016/j.jenvman.2016.06.059.