Desalination and Water Treatment
www.deswater.com
doi: 10.5004/dwt.2022.28858

273 (2022) 139–148
October

Removal of cadmium from industrial wastewater using blue-green and green microalgae (*Aphanocapsa zanardinii* and *Chlorella vulgaris*)

Sabrina Dzizi[a,b,*], Nadjla Chaib[a,c], Faïza Noune[b,d], Hadjer Kaddeche[b,d], Nabil Charchar[e]

[a]*Department Process Engineering, Faculty of Technology, University of 20 August 1955 Skikda, Algeria, emails: dzizisabrina904@gmail.com/s.dzizi@univ-skikda.dz (S. Dzizi)*
[b]*Laboratoire de Recherche en Physico-Chimie des Surfaces et Interfaces (LRPCSI), University of 20 August 1955 Skikda, Algeria*
[c]*Laboratory of Catalysis, Bioprocesses and Environment (LCBE), University of 20 August 1955 Skikda, Algeria, email: n.chaib@univ-skikda.dz (N. Chaib)*
[d]*Department of Natural and Life Sciences, Faculty of Sciences, University of 20 August 1955 Skikda, Algeria, emails: F.noune@univ-skikda.dz (F. Noune), h.kaddeche@univ-skikda.dz (H. Kaddeche)*
[e]*Bioremediation Research Division: Biotechnology and Environment, Biotechnology Research Center (C.R.Bt), BP 73 UV 03, Nouvelle Ville Ali-Mendjli, Constantine, Algeria, email: n.charchar@crbt.dz (N. Charchar)*

Received 10 May 2022; Accepted 15 August 2022

ABSTRACT

Industrial activities produce large amounts of contaminated wastewater mainly with heavy metals. Due to its high potential for bioaccumulation for heavy metals, the use of microalgae biomass in their removal is an interesting technology. Indeed, through a variety of biological processes such as bio-sorption and bioaccumulation, microalgae have developed a big number of potential mechanisms to cope with heavy metal toxicity. The present study aims to study the heavy metal removal from industrial wastewater using two selected species *Chlorella vulgaris* and *Aphanocapsa zanardinii* isolated from the bay of Skikda. Samples were grown in a sterile medium Bold Basal until a sufficient biomass volume is obtained, then moved to new flasks that contain sterile wastewater. The bioremoval of cadmium using separately *C. vulgaris* and *A. zanardinii* was carried out by selecting three concentrations of cadmium (50, 100, and 250 mg·L^{-1}). The results showed that both microalgae have a significant ability to eliminate cadmium Cd^{2+} as a single source of contamination with significant removal rates using separately *A. zanardinii* (75.13%), and *C. vulgaris* (86.07%) as bioremediation tools.

Keywords: Chlorella vulgaris; *Aphanocapsa zanardinii*; Bioremoval; Heavy metals; Wastewater

1. Introduction

Aquatic environments contaminated by heavy metals (HMs) are of special concern because they are toxic, abundant and persistent in the environment, and accumulate over time in aquatic habitats [1]. Marine ecosystems are environments that are progressively more affected by human activity due to urban, agricultural, and tourist development in coastal cities [2–7]. Metallurgical and mining industries and oil refineries known to be sources of metallic contamination of the environment are generally located in coastal areas [8–10]. In addition, urban and industrial discharges use the sea as a dumping ground that can lead to high levels of pollution in marine and coastal ecosystems [4,11,12]. Indeed, the continuous discharges of wastewater produced mainly by anthropogenic activities, such as agricultural, urban, and in particular, industrial practices transport, and

* Corresponding author.

Presented at the First International Seminar on Pollution, Health, Environment and Bio-monitoring (SIPSEB-2021), 27–28 December 2021, Virtual Seminar, Skikda, Algeria

discharge of many pollutants (nitrogen, phosphorus, and mainly HMs) in coastal waters is a serious problem in the form of water pollution of aquatic environments and causes the degradation of ecosystems, thus promoting the bioaccumulation of toxic elements in marine organisms, including the transport along the trophic chain represents a danger to human health [2,13,14].

HMs pollution has become a major global concern in aquatic ecosystem rehabilitation because of its non-biodegradability, bioaccumulation, and biomagnifications through the food chain, resulting in adverse ecological and environmental effects [15,16]. The presence of HMs in wastewater seriously affects the environment, human health, and the ecosystem. Heavy metal bioremediation has gained considerable and increasing interest over time [17]. Many alternative technologies for the removal of HMs from wastewater exist, such as: electro-coagulation, precipitation, and the ion exchange, reverse osmosis, advanced oxidation, evaporation, and adsorption [18,19]. These various treatment techniques used to reduce or eliminate HMs in the environment have many disadvantages. However, bioremediation is more cost-effective and environment-friendly with fewer by-products compared with the aforementioned physicochemical methods [16]. This demonstrates the need for these effective treatment methods capable of effectively and completely reducing or eliminating heavy metal concentrations in wastewater before discharging it into natural resources. Phycoremediation uses microalgae to clean up water [20–23]. The present study aims to remove cadmium at both low and high concentrations using two isolated microalgae from a contaminated site: *Aphanocapsa zanardinii* (Hauck) Hansgirg and *Chlorella vulgaris* Beijerinck.

2. Experimental section

2.1. Samples collection

The algal samples were collected from Skikda Bay from three locations contaminated by wastewater discharged by the petrochemical industry. Blue-green and green microalgae were sampled using a phytoplankton net with a 20 μm mesh size [24]. The operation consists of filtering 1 L of seawater (30 cm below the surface of the water). The collected suspension is poured into two 125 mL glass sterilized flasks, one containing 5 mL of Lugol 10% for microscopic determination, and the second without conservation then transported in a dark cooler at 4°C. Algal species were then washed with distilled water to be ready for determination under a light microscope with a digital camera. Characteristics such as size, strands, shape, cell-layer thick, cell size, and filaments were used to identify the different species using keys for freshwater microalgae as Bellinger and Sigee [25], Serediak and Huynh [26], Van Vuuren et al. [27], and Canter-Lund and Lund [28].

2.2. Specific growing medium

Microalgae samples were initially inoculated in Bold Basal Medium (BBM) (1967) containing the following compounds: 10 mL of macronutrients: $CaCl_2{\cdot}2H_2O$, 2.5 $g{\cdot}L^{-1}$; $MgSO_4{\cdot}7H_2O$, 7.5 $g{\cdot}L^{-1}$; NaCl, 2.5 $g{\cdot}L^{-1}$; $NaNO_3$, 25 $g{\cdot}L^{-1}$; KH_2PO_4, 17.5 $g{\cdot}L^{-1}$; K_2HPO_4, 7.5 $g{\cdot}L^{-1}$ and 1 mL of micronutrients: ethylenediamine tetraacetic acid (EDTA), 50 $g{\cdot}L^{-1}$ + KOH 31 $g{\cdot}L^{-1}$; $FeSO_4{\cdot}7H_2O$, 4.98 $g{\cdot}L^{-1}$; H_3BO_3, 11.42 $g{\cdot}L^{-1}$ and 1 mL of this solution that contains $CuSO_4{\cdot}5H_2O$, 1.57 $g{\cdot}L^{-1}$; $ZnSO_4{\cdot}7H_2O$, 8.82 $g{\cdot}L^{-1}$; $MnCl_2{\cdot}4H_2O$, 1.44 $g{\cdot}L^{-1}$; $Na_2{\cdot}MoO_4{\cdot}2H_2O$, 0.71 $g{\cdot}L^{-1}$; $Co(NO_3)_2{\cdot}6H_2O$, 0.49 $g{\cdot}L^{-1}$ [29]. The BBM medium with EDTA improved its high capacity to chelate HMs. In addition to 2 g of sodium carbonate (Na_2CO_3) as a carbon source [30], then made up to one liter with sterile distilled water. Agar is added to the medium at this stage, to prepare a solid medium, by constant agitation at low temperature until boiling point, to ensure that the agar is completely dissolved. The media were then sterilized at 120°C for 120 min and stored at 4°C. The solid medium was used to enumerate, isolate, and transplant colonies for strain purification.

2.3. Experimental setup

The microalgae isolated and grown in the BBM medium were subsequently cultured with various concentrations of cadmium. The bold liquid culture medium was supplemented separately with a range of Cd^{2+} concentrations (50, 100, and 250 $mg{\cdot}L^{-1}$). The use of a gradient of increased cadmium concentrations was chosen based on the literature [31,32]. Cadmium chloride hydrate ($CdCl_2{\cdot}H_2O$) was used as a source of Cd^{2+}. Stock solutions of 1,000 ppm were prepared in Milli-Q water and sterilized by filtration (0.22 μm). The required concentrations of Cd^{2+} for the various experiments were prepared by the dilution method. A glass sterilized flask filled only with BBM served as a control. Both isolated species *C. vulgaris* and *A. zanardinii* were sampled from the mid-log phase culture as inoculum. All the experiments were carried out in triplicates and carried out in 250 mL glass sterilized flasks. All the flasks were incubated in an incubator shaker (Type Kuhner), maintained at 22°C ± 2°C, and 150 rpm agitation. The light was provided by continuous cool white fluorescent lamps with 4,000 LUX, and a light/dark photoperiod of 16 h/8 h. Media pH was controlled and adjusted at 5.7 ± 0.1, which is the optimum pH for cadmium removal [33]. All flasks were connected to a bubbling pump equipped with a flow rate meter under the pressure of mixed air (95%) and 5% of CO_2, ensuring the enrichment of cultures in CO_2 with the injection of 2 LPM.

2.4. Biomass growth measurements

Biomass growth for each culture was measured every 5 d. To improve the interpretation of the results, three methods of biomass growth measurements were performed: cell counts CC, optical density (OD), and dry biomass weight (DW) as a function of time. All tests were repeated 3 times in order to check the reliability of the results. The latter are reported as an average ± standard error for each measurement.

2.4.1. Cell counts

Cell counts were determined using a Thoma slide under a light microscope (Optika ×40), and expressed as the number of cells per μL. This method, allowed us to calculate cell densities taking into account dilutions, and using Eq. (1):

$$CC = \frac{n \times D}{V} \quad (1)$$

where n = number of counted cells, CC = number of cells per microliter (μL), D: dilution factor, V = counting volume: for Thoma, V = 0.1 μL (whole grid).

The log(CC) was then calculated to reduce the impact of high cell counts and facilitate plotting of growth curves. $\log(CC_0)$, $\log(CC_1)$, $\log(CC_2)$, and $\log(CC_3)$ referred respectively to the concentrations of Cd^{2+} (0, 50, 100, and 250 mg·L^{-1}). For each experiment and at each sampling time, growth rates (μ, expressed as divisions per day) and generation doubling times ($G = \ln(2)/\mu$) of the both *A. zanardinii* and *C. vulgaris* were calculated according to Guillard [34].

The number of divisions per day was calculated from the slope of the exponential curve between Day 5 and Day 21, illustrating log cell count as a function of time using Eq. (2).

$$\mu = \frac{\mathrm{Log}\left(CC_{21}\right) - \mathrm{Log}\left(CC_5\right)}{D_{21} - D_5} \quad (2)$$

where μ is the growth rate, and D for Day, CC_{21} is the cell number at Day 21 (cell·cm^{-2}), and CC_5 is the cell number at Day 5 (cell·cm^{-2}).

2.4.2. Optical density

The OD of both microalgae was measured by a HELIOS EPSILON UV-VIS 3SGN006008 at 620 nm every 5 d. Appropriate dilutions were required each time OD exceeded >0.7 [35]. The results are expressed in mg·L^{-1}.

2.4.3. Dry biomass weight

Dry biomass weights were determined by filtering 10 mL of the microalgal culture through 0.45 μm fiber filters. The collected biomass was then cleaned with distilled water to eliminate any residue, and dried at 105°C for 90 min. The results of DW were expressed in mg·L^{-1} using Eq. (3):

$$\mathrm{DW}\left(\mathrm{mg}\cdot\mathrm{L}^{-1}\right) = \frac{F_1 - F_0\left(\mathrm{mg}\right)}{V\left(\mathrm{mL}\right)} \times 1{,}000 \quad (3)$$

where F_1 is the filter's weight + dried biomass, and F_0 is the initial weight of the empty filter. V is the filtered volume (10 mL). DW_0, DW_1, DW_2, and DW_3 referenced to Cd concentrations (0, 50, 100, and 250 mg·L^{-1}).

2.5. Cd^{2+} concentrations determination

Samples of the media recovered after filtration for DW measurements every 5 d, were used to determine Cd^{2+} concentrations quantified by inductively coupled plasma-optical emission (ICP-OE) spectroscopy (PerkinElmer Optima 8300, American Laboratory Trading (ALT)) Water samples were filtered by a membrane filter, and analyzed individually using a special lamp with a specific wavelength. Heavy metal removal efficiencies were then calculated.

3. Results and discussion

3.1. Isolation of species

The isolated species were identified based on their morpho-cytology and growth response to the wastewater composition. *C. vulgaris* and *A. zanardinii* were the most abundant microalgae cells in the wastewater samples. The strains were isolated and purified on agar medium, then re-cultivated in liquid BBM medium for 20 d. A suspension of microalgae was collected from the exponential growth phase, where the concentration represents the initial measured concentration using three methods OD, DW, and CC (living cell counts).

3.2. Biomass growth development

Biomass concentration is one of the most important, but also one of the most challenging measurements. The number of live algal cells was log-transformed to a near normal distribution. The OD and DW measurements do not provide a true measurement of the number of live algal cells. This is because the OD method is based on light distribution not absorbance. The dry weight method fails if the sample contains any other insoluble particles. Likewise, the optical density measurement is of limited use if the solution is unclear. In addition, those methods do not distinguish viable cells from dead cells. In contrast, algal cell counts using a Thoma slide can detect viable cells among other solid particles. However, this method involves extensive preparations, and requires 24–48 h to incubate and count the cells. Therefore, cell counting is primarily used to cross-check other measurements, such as optical density and dry weight.

The growth curves obtained for OD, log(CC), and DW were congruent for both species *A. zanardinii* and *C. vulgaris* according to time of exposure for each concentration of Cd^{2+}. After an exponential phase of 21 d, the algae biomass reaches the stationary phase for almost the curves.

The results obtained from the OD allowed us to estimate the amounts of *A. zanardinii* and *C. vulgaris* respectively 68.53 ± 1.12 and 95.73 ± 1.61 mg·L^{-1} for [Cd]~50 mg·L^{-1}, 55.30 ± 0.80 and 97.07 ± 0.06 mg·L^{-1} for [Cd]~100 mg·L^{-1}, and 48.84 ± 0.45 and 59.13 ± 1.96 mg·L^{-1} for [Cd]~250 mg·L^{-1} (Fig. 1). These results were compared to OD_0 (initial optical density) for both species (12.10 ± 0.25 to 101 ± 0.33 mg·L^{-1}), in the same experimental conditions without any supply of cadmium (Table 1). The initial OD values at the beginning of the experiments were lower for both species for all Cd^{2+} concentrations.

The growth curves obtained from the DW measurements (Fig. 2) showed the same appearances of the curves obtained for OD.

Biomass obtained from an initial dry weight (DW_0) of 19.07 ± 0.04 mg·L^{-1}, at the issue of the experiments for each Cd^{2+} ion concentration were respectively for *A. zanardinii* and *C. vulgaris* 23.52 ± 0.32 and 24.27 ± 0.58 mg·L^{-1} for [Cd]~50 mg·L^{-1}, 20.88 ± 0.35 and 22.13 ± 0.86 mg·L^{-1} for [Cd]~100 mg·L^{-1}, and 17.94 ± 0.38 and 18.89 ± 0.26 mg·L^{-1} for [Cd]~250 mg·L^{-1} (Table 2).

Cellular enumeration of species using a Thoma slide under a light microscope is less affected by errors. Indeed, algal cell counts are more consistent based on the effective

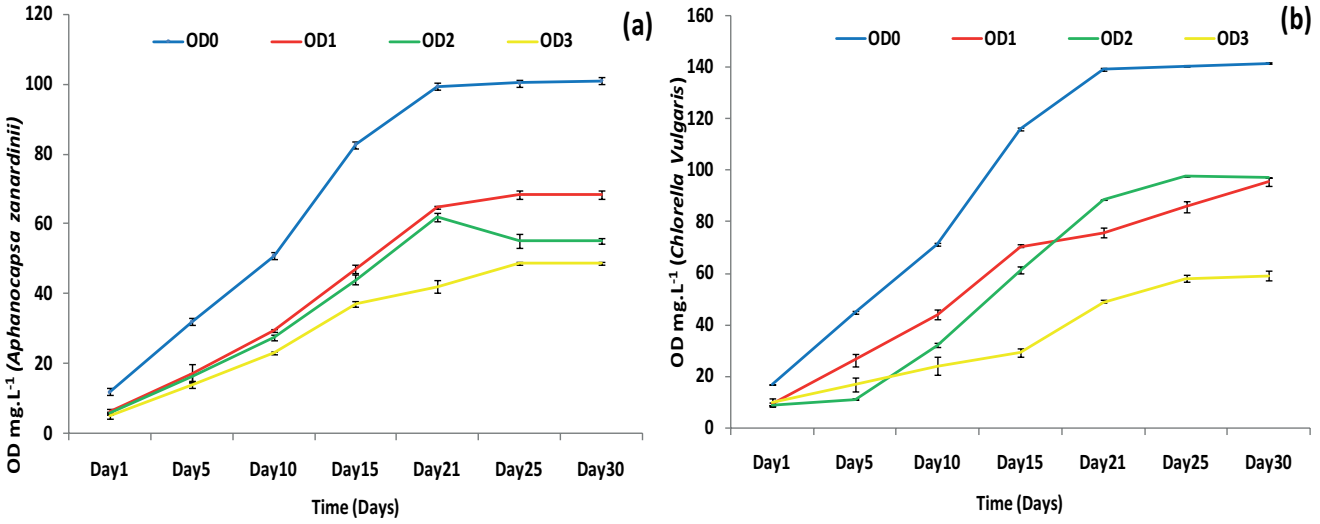

Fig. 1. Optical density evolution of both microalgae as a function of time: (a) *Aphanocapsa zanardinii* and (b) *Chlorella vulgaris*. OD_0, OD_1, OD_2, and OD_3 mentioned the concentrations of Cd^{2+} (0, 50, 100, and 250 mg·L^{-1}).

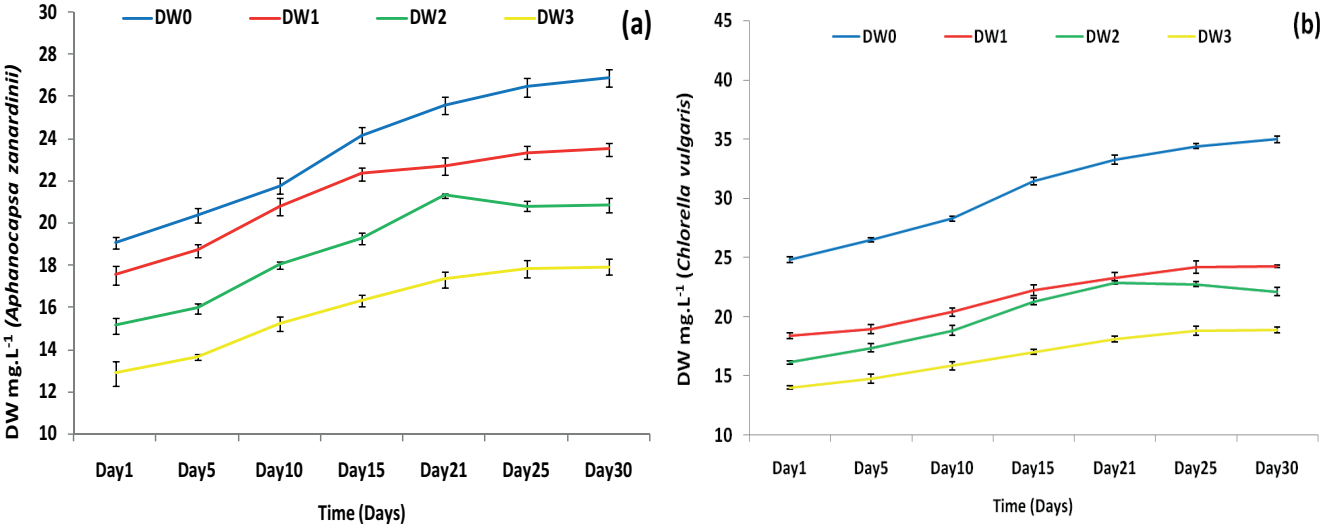

Fig. 2. Assessment of dry biomass weight growth as a function of time. (a) *Aphanocapsa zanardinii*, (b) *Chlorella vulgaris*. DW_0, DW_1, DW_2, and DW_3 referenced to Cd^{2+} concentrations (0, 50, 100, and 250 mg·L^{-1}).

values of the living cells (CC) in the liquid medium, with increasing Cd^{2+} concentrations. The counted living cell results were log transformed to log(CC) for each species to simplify the linearization of the projected points on growth curves. In living algae contact time has a greater effect on the biosorption capacity.

We recorded a gradually increase of cell counts for both species in comparison to each Cd^{2+} concentration from the first day of growth for four weeks (30 d). The log(CC) reach their highest values after 30 d of growth in the contaminated BBM medium (Fig. 3).

The initial log(CC_0) of 15.12 ± 0.44 mg·L^{-1} while the highest number was 31.29 ± 0.58 mg·L^{-1}. At the end of the experiments, for each Cd^{2+} ion concentration log(CC_1), log(CC_2), and log(CC_3) were respectively for *A. zanardinii* and *C. vulgaris* 26.08 ± 0.57 and 29.29 ± 0.22 mg·L^{-1} for [Cd]~50 mg·L^{-1}, 18.40 ± 0.31 and 20.23 ± 0.14 mg·L^{-1} for [Cd]~100 mg·L^{-1}, and 12.33 ± 0.25 and 17.07 ± 0.29 mg·L^{-1} for [Cd]~250 mg·L^{-1} (Table 3).

Fig. 3 shows the same experimental results in terms of living cell numbers in the cultures. Although, the relative position of the curves of exponential growth is the same as those obtained according to optical density and dry biomass weight (Figs. 1 and 2). We notice a decline in the number of cells at the end of the growth, since day 21. This apparent contrast (biomass increases while the cell population decreases) indicates that the growth of viable algae biomass is negatively affected by the long-time of exposure to the different concentrations of cadmium for both species investigated. Thus, it is on the basis of these outcomes that we can evaluate the growth rate of the cultures in the most reliable way.

Table 1
Optical density evolution for *Aphanocapsa zanardinii* and *Chlorella vulgaris* cultivated on liquid BBM with increased concentrations of Cd^{2+}

	Aphanocapsa zanardinii				*Chlorella vulgaris*			
Time (d)	OD_0~0 mg·L^{-1}	OD_1~50 mg·L^{-1}	OD_2~100 mg·L^{-1}	OD_3~250 mg·L^{-1}	OD_0~0 mg·L^{-1}	OD_1~50 mg·L^{-1}	OD_2~100 mg·L^{-1}	OD_3~250 mg·L^{-1}
Day 1	12.10 ± 0.13	6.28 ± 0.88	5.91 ± 0.42	5.18 ± 0.73	16.94 ± 0.05	9.30 ± 0.69	8.93 ± 0.12	10.03 ± 1.62
Day 5	32.20 ± 0.36	17.26 ± 2.62	16.20 ± 0.89	14.06 ± 0.79	45.08 ± 0.14	26.33 ± 2.31	11.20 ± 0.35	16.87 ± 2.89
Day 10	51.08 ± 0.41	29.61 ± 0.45	27.68 ± 0.89	23.10 ± 0.48	71.51 ± 0.16	44.00 ± 1.73	32.30 ± 0.87	24.27 ± 3.58
Day 15	82.08 ± 0.47	47.15 ± 1.31	44.12 ± 1.48	37.21 ± 0.97	115.96 ± 0.19	70.57 ± 0.58	61.40 ± 1.39	29.27 ± 1.50
Day 21	99.44 ± 0.50	64.83 ± 0.51	62.04 ± 1.02	42.09 ± 1.76	139.22 ± 0.20	75.87 ± 2.02	88.50 ± 0.00	49.13 ± 0.64
Day 25	100.40 ± 0.51	68.45 ± 1.25	55.21 ± 1.93	48.65 ± 0.39	140.56 ± 0.20	85.87 ± 1.96	97.83 ± 0.06	58.07 ± 1.15
Day 30	101.00 ± 0.52	68.53 ± 1.12	55.30 ± 0.80	48.84 ± 0.45	141.40 ± 0.21	95.73 ± 1.61	97.07 ± 0.06	59.13 ± 1.96

See Fig. 1 for codes.

Table 2
Evolution of the dry biomass weight of both microalgae grown on liquid BBM with different Cd^{2+} concentrations

	Aphanocapsa zanardinii				*Chlorella vulgaris*			
Time (d)	DW_0~0 mg·L^{-1}	DW_1~50 mg·L^{-1}	DW_2~100 mg·L^{-1}	DW_3~250 mg·L^{-1}	DW_0~0 mg·L^{-1}	DW_1~50 mg·L^{-1}	DW_2~100 mg·L^{-1}	DW_3~250 mg·L^{-1}
Day 1	19.07 ± 0.27	17.54 ± 0.44	15.13 ± 0.35	12.88 ± 0.60	24.80 ± 0.04	18.40 ± 0.25	16.14 ± 0.16	14.03 ± 0.12
Day 5	20.40 ± 0.31	18.71 ± 0.32	15.96 ± 0.24	13.66 ± 0.13	26.51 ± 0.01	18.95 ± 0.38	17.38 ± 0.33	14.77 ± 0.40
Day 10	21.77 ± 0.33	20.77 ± 0.40	18.02 ± 0.19	15.23 ± 0.31	28.31 ± 0.21	20.41 ± 0.36	18.85 ± 0.42	15.86 ± 0.36
Day 15	24.19 ± 0.35	22.34 ± 0.32	19.31 ± 0.28	16.33 ± 0.27	31.45 ± 0.05	22.24 ± 0.42	21.28 ± 0.27	17.04 ± 0.23
Day 21	25.59 ± 0.37	22.69 ± 0.42	21.33 ± 0.10	17.34 ± 0.39	33.27 ± 0.06	23.31 ± 0.47	22.88 ± 0.16	18.13 ± 0.26
Day 25	26.47 ± 0.38	23.37 ± 0.31	20.82 ± 0.22	17.84 ± 0.43	34.41 ± 0.22	24.20 ± 0.50	22.75 ± 0.20	18.81 ± 0.38
Day 30	26.91 ± 0.38	23.52 ± 0.32	20.88 ± 0.35	17.94 ± 0.38	34.99 ± 0.06	24.27 ± 0.12	22.13 ± 0.33	18.89 ± 0.22

See Fig. 2 for codes.

Table 3
Evolution of growth in algae biomasses cultivated on liquid BBM enriched or not with Cd at different concentrations

	Aphanocapsa zanardinii				*Chlorella vulgaris*			
Time (d)	$\log(CC_0)$~0 mg·L^{-1}	$\log(CC_1)$~50 mg·L^{-1}	$\log(CC_2)$~100 mg·L^{-1}	$\log(CC_3)$~250 mg·L^{-1}	$\log(CC_0)$~0 mg·L^{-1}	$\log(CC_1)$~50 mg·L^{-1}	$\log(CC_2)$~100 mg·L^{-1}	$\log(CC_3)$~250 mg·L^{-1}
Day 1	15.12 ± 0.35	12.60 ± 0.14	10.10 ± 0.13	6.65 ± 0.13	18.14 ± 0.26	14.54 ± 0.16	12.66 ± 0.08	10.13 ± 0.25
Day 5	17.30 ± 0.26	14.73 ± 0.04	11.78 ± 0.19	7.68 ± 0.15	20.76 ± 0.19	16.22 ± 0.25	14.13 ± 0.02	11.14 ± 0.16
Day 10	22.75 ± 0.32	16.83 ± 0.06	13.36 ± 0.14	8.11 ± 0.17	27.30 ± 0.24	19.04 ± 0.19	15.33 ± 0.46	12.48 ± 0.21
Day 15	27.17 ± 0.26	19.02 ± 0.05	15.14 ± 0.11	9.61 ± 0.18	32.60 ± 0.19	25.70 ± 0.13	17.89 ± 0.10	14.55 ± 0.34
Day 21	29.22 ± 0.33	24.61 ± 0.06	18.31 ± 0.14	11.02 ± 0.14	36.07 ± 0.25	27.85 ± 0.23	22.42 ± 0.14	16.75 ± 0.39
Day 25	28.41 ± 0.25	20.77 ± 0.04	14.34 ± 0.10	8.29 ± 0.22	29.70 ± 0.18	19.19 ± 0.17	17.82 ± 0.48	10.07 ± 0.24
Day 30	24.29 ± 0.25	16.08 ± 0.08	12.40 ± 0.08	5.33 ± 0.20	26.54 ± 0.19	16.29 ± 0.12	14.23 ± 0.14	6.07 ± 0.29

See Fig. 3 for codes.

Although essential to the understanding and accuracy of biomass growth, the determination of μ_{max} (maximum growth rate) is extremely delicate. Different mechanisms are involved: assimilation, variation of internal stocks, number of cells, etc. with numerous feedbacks from one factor to another, in order to obtain the most accurate value of this growth rate.

Generation doubling times *G* account less than 1 d for algae grown in BBM free of cadmium, and about 1 d for the initial concentration of cadmium (50 mg·L^{-1}), and up to 3.30 and 1.98 d respectively for *A. zanardinii* and *C. vulgaris* cultivated in BBM with 250 mg·L^{-1} of Cd^{2+} (Table 4).

The highest growth rates match the minimal generation doubling times. In other words, the optimal cadmium concentration would be selected based on the maximum growth rate value achieved and the lowest generation time.

This means that the best exponential growth rates retained from our results were those of *A. zanardinii* (μ = 0.62 d^{-1}, *G* = 1.12 d), and *C. vulgaris* (μ = 0.73 d^{-1}, *G* = 0.95 d) for the lowest Cd concentration (50 mg·L^{-1}).

The calculated growth rates (Table 4) for *A. zanardinii* and *C. vulgaris* are lower than those (2.08 d^{-1}) given by Findlay et al. [36] for green algae in their uncontaminated habitat. While we find other works that report *C. vulgaris* rates of 1.3 to 2.5 d^{-1} for waters not contaminated by metal pollution. On the other hand, weak growth rates of (0.207–0.187 d^{-1}) were recorded for this same species collected from Cu concentrations (200 and 500 μg·L^{-1}), compared to those without addition of Cu (0.216–0.203 d^{-1}) [37].

Hockaday et al. [38] compared the responses of *C. vulgaris* and *Scenedesmus obliquus* for removal of Cu/Cd and concluded that Chlorella was the more potent adsorbent since it was capable to adsorb the metals more rapidly from both a single metal solution and a mixed solution. *C. vulgaris* also had a higher binding capacity for both metals compared to *Scenedesmus obliquus*. When the metals are in a mixed solution, the presence of copper affected the cadmium adsorption of each algal species. This should be taken into consideration if *C. vulgaris* will be used for wastewater bioremediation, as the presence of other metals may reduce the efficiency of metal removal. However, the growth rates obtained from our experimental conditions are comparable to those found in the literature.

3.3. Removal rates of cadmium

Photosynthetic microalgae have the ability to consume pollutants like ammonia and phosphorus, as well as HMs [39,40], thereby qualifying them as good potential candidates for wastewater remediation. Their use in the phyco-treatment of wastewater has recently started [41,42]. They display high adaptability in various growing conditions, and effluent compositions, as they can tolerate HMs in excessive amounts.

Removal rates were calculated by comparing the final registered concentration and the initial concentration of cadmium (Table 5). The bioremoval of Cd^{2+} using *A. zanardinii* and *C. vulgaris* was stopped after 30 d in all the flasks by reaching the stationary phase of minimal [Cd^{2+}] from the 21st day (Fig. 4). The drop in Cd^{2+} content was improved by both species for all bioremoval treatments.

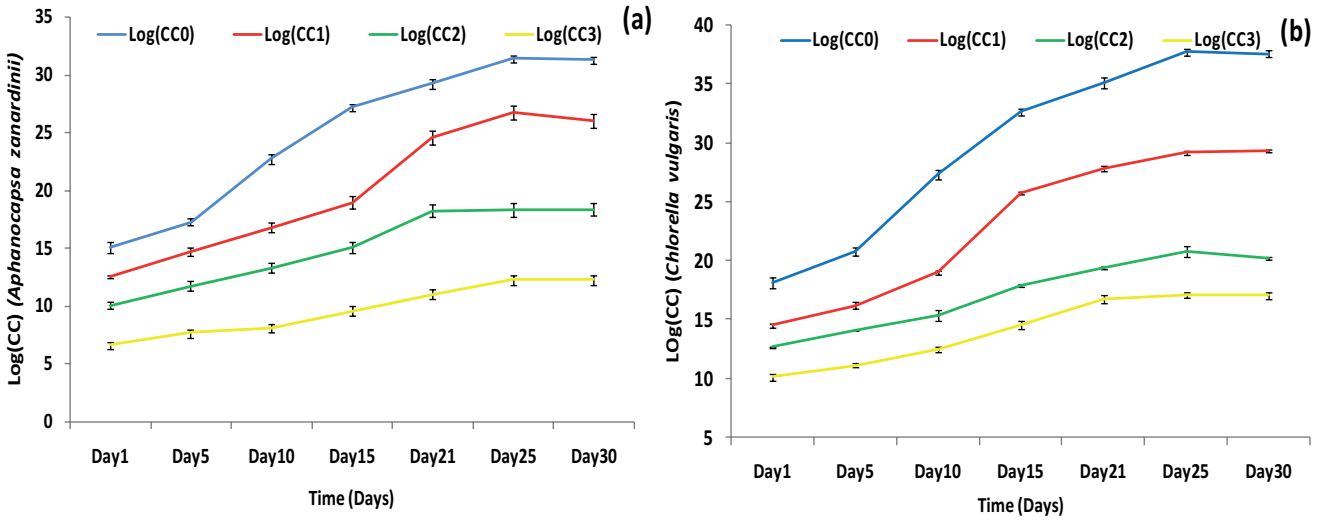

Fig. 3. Cell population growth in microalgae cultures as a function of time. (a) *Aphanocapsa zanardinii*, (b) *Chlorella vulgaris*. log(CC): log-transformed cell counts. log(CC_0), log(CC_1), log(CC_2), and log(CC_3) referred respectively to the concentrations of Cd^{2+} (0, 50, 100, and 250 mg·L^{-1}).

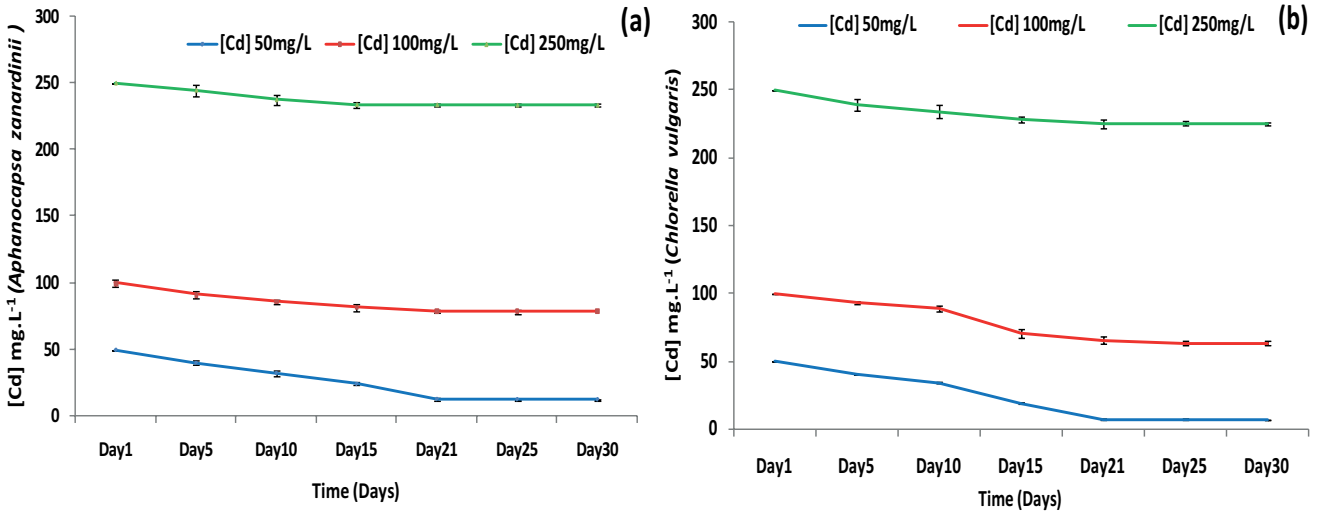

Fig. 4. Removal of Cd^{2+} using blue-green and green microalgae: (a) *Aphanocapsa zanardinii* and (b) *Chlorella vulgaris*.

The final concentrations were respectively 11.43 ± 0.55 and 6.97 ± 0.28 mg·L^{-1} for [Cd]~50 mg·L^{-1}, 72.37 ± 1.97 and 63.46 ± 1.49 mg·L^{-1} for [Cd]~100 mg·L^{-1}, and 233.09 ± 1.28 and 225 ± 1.25 mg·L^{-1} for [Cd]~250 mg·L^{-1}.

Significant bioremoval rates were recorded for *C. vulgaris* 86.07% [Cd]~50 mg·L^{-1}, 36.54% [Cd]~100 mg·L^{-1}, and 9.93% [Cd]~250 mg·L^{-1}. Bioremoval rates were also calculated for *A. zanardinii* 75.13% [Cd]~50 mg·L^{-1}, 21.63% [Cd]~100 mg·L^{-1}, and 6.65% [Cd]~250 mg·L^{-1}. The highest removal rates were obtained for [Cd]~50 mg·L^{-1} for both species, whereas the lowest rates were registered for [Cd]~250 mg·L^{-1} (Table 5). This leads to conclude that the higher Cd^{2+} concentrations the lower the removal rates will be.

The removal of HMs by living microalgae involves two phases [43]; (1 one is fast and mostly unaffected by cellular metabolism 'adsorption', and 2) the second is slower and relies on cellular metabolic activities 'absorption'.

Algae sampled from polluted sites are considered tolerant or resistant to HMs (Cu^{2+}, Cd^{2+}, Pb^{2+}, and Zn^{2+}) [44]. Biological removal is characterized by the accumulation of pollutants in aqueous solutions using living or non-living algae biomass, thereby enabling the removal of contamination in an environment-friendly manner [45,46].

A. zanardinii and *C. vulgaris* as all photosynthetic microalgae have also the ability to reduce by biosorption high amounts of HMs in water. *C. vulgaris* is able to remove 69% and 80% of Ni^{2+}, and Cu^{2+} ions, respectively at an initial concentration of 2.5 ppm. Whereas, by increasing the initial concentration to 10 ppm, the metal removal efficiency was further reduced to only 37% and 42%, respectively [47].

Table 4
Growth rates μ and generation doubling times *G* determined for both species *Aphanocapsa zanardinii* and *Chlorella vulgaris* for each Cd concentration

	Aphanocapsa zanardinii				*Chlorella vulgaris*			
	$\log(CC_0)$	$\log(CC_1)$	$\log(CC_2)$	$\log(CC_3)$	$\log(CC_0)$	$\log(CC_1)$	$\log(CC_2)$	$\log(CC_3)$
μ_{max} (d^{-1})	0.74	0.62	0.41	0.21	0.96	0.73	0.52	0.35
G (d)	0.93	1.12	1.70	3.32	0.72	0.95	1.34	1.98

See Fig. 3 for codes.

Table 5
Bioremoval rates of Cd^{2+} at different concentrations using microalgae *Aphanocapsa zanardinii* and *Chlorella vulgaris*

Time (d)	*Aphanocapsa zanardinii*			*Chlorella vulgaris*		
	[Cd] 50 $mg{\cdot}L^{-1}$	[Cd] 100 $mg{\cdot}L^{-1}$	[Cd] 250 $mg{\cdot}L^{-1}$	[Cd] 50 $mg{\cdot}L^{-1}$	[Cd] 100 $mg{\cdot}L^{-1}$	[Cd] 250 $mg{\cdot}L^{-1}$
Day 1	50.00 ± 0.00	100.00 ± 0.00	250.00 ± 0.00	50.00 ± 0.00	100.00 ± 0.00	250.00 ± 0.00
Day 5	40.11 ± 1.52	90.89 ± 2.82	244.68 ± 4.32	40.33 ± 0.52	93.58 ± 1.27	239.33 ± 3.90
Day 10	32.48 ± 1.94	85.93 ± 2.66	237.52 ± 2.46	34.07 ± 0.55	88.97 ± 2.38	234.33 ± 4.56
Day 15	24.16 ± 1.12	81.17 ± 1.58	233.88 ± 2.46	19.07 ± 0.48	70.70 ± 3.30	228.00 ± 2.13
Day 21	12.73 ± 0.84	78.47 ± 2.84	233.68 ± 0.0	7.47 ± 0.50	65.51 ± 2.56	225.00 ± 3.18
Day 25	12.48 ± 0.59	78.40 ± 1.27	233.38 ± 1.31	7.37 ± 0.34	63.66 ± 1.37	225.25 ± 1.68
Day 30	12.43 ± 0.55	78.37 ± 1.97	233.37 ± 1.28	6.97 ± 0.28	63.46 ± 1.49	225.19 ± 1.25
Bioremoval rates (%)	75.13	21.63	6.65	86.07	36.54	9.93

The efficiency of bioremediation depends on the selection of microorganisms [48]. For example, living *C. miniata* achieved around 85% removal rate of Ni^{2+} at an initial concentration of 30 mg, while *C. vulgaris* achieved only ~50% [49]. In another study, *Chlorella pyrenoidosa* achieved 70%–98% adsorption ability for Fe^{3+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, and Pb^{2+}, at an initial concentration of 5 mg [50]. Thus, higher bioremoval rates of single metals were compared with metal mixtures [51,52]. Bioremoval efficiency rates of cyanobacterial species as *Anabaena variabilis* and *Tolypothrix ceylonica* have the ability to adsorb/absorb HMs (Zn^{2+}, Fe^{3+}, Cu^{2+}, and Pb^{2+}), even at their high concentrations with selective preferences among them [53].

The removal rates in the continuous culture of *Aphanocapsa* (*Microcystis*) sp. for Cr^{+4} were better than in batch cultures [54]. *Microcystis* (*Aphanocapsa*) showed high biosorption potential from aqueous solution containing Pb^{2+}, Cu^{2+}, Cd^{2+}, and Zn^{2+} [55]. However, Rai and Tripathi [56] found that removal rates of Cd^{2+} when added solely in the growth medium were ~24%–34.2% removed. Although, when it comes to a mixture of Cd^{2+} and Cr^{4+}, the removal rates of Cd^{2+} were considerably higher (~65.2%). The mixture between Cd^{2+} and Cu^{+2} was also tested (~59.7%) [56].

Shanab et al. [57] demonstrated that *Phormidium ambiguum* was most sensitive to the three tested metal ions, albeit at low concentrations (5 and 10 $mg{\cdot}L^{-1}$), while both *Pseudochlorococcum typicum* and *Scenedesmus quadricauda* showed greater tolerance to high metal levels reaching concentrations of 100 $mg{\cdot}L^{-1}$. Bioremoval of heavy metal ions (Hg^{2+}, Pb^{2+}, and Cd^{2+}) using *Pseudochlorococcum typicum* from the aqueous solution exhibited that the highest rates occurred within the first 30 min of contact, recording 97% (Hg^{2+}), 86% (Cd^{2+}), and 70% (Pb^{2+}).

Heavy metal removal using microalgae biomass depends mainly on the initial metal content in the growth medium. Cadmium is the most poisonous heavy metal resulting from industrial wastewater [58,59]. Several studies reported that microalgae have different responses to cadmium and hence different bioremoval ways. However, the ability of *Chlamydomonas reinhardtii* to remove cadmium was significantly improved (69.8%–90.2% in 6 h) [60]. On the other hand, Ma et al. [61] studied the cadmium removal potential of suspended *Scenedesmus obliquus* and its biofilm, and recorded removal efficiencies of Cd^{2+} with an initial concentration of 3 $mg{\cdot}L^{-1}$ from 61.8% to 91.27%, and of 87.49% from 20 $mg{\cdot}L^{-1}$ as initial concentration.

Ye et al. [62] demonstrated that the highest bioremediation capacity of Cd^{2+} ion was 31.45 $mg{\cdot}g^{-1}$ for an initial dry biomass weight of 15 $g{\cdot}L^{-1}$ of *Porphyra leucosticta*, at pH~8.0 and time exposure of ~120 min from an initial concentration of 10.0 $mg{\cdot}L^{-1}$ for Cd^{2+} with bioremoval rate of 70%. Besides, the removal capacity for a real industrial effluent reached 75% from 7.6 $mg{\cdot}L^{-1}$ of Cd^{2+} ion concentration.

4. Conclusion

High heavy metal content in the aquatic environment resulting from industrial wastewater generates a serious problem because of their risks of toxicity. It is essential, to develop new bioremediation tools based on the exploitation of living microorganisms to eliminate or reduce effectively the high amounts of HMs

contaminating receptor environments, because of their effectiveness, and economic efficiency. Within this context, the present study aimed to test the capacity of two species *A. zanardinii* and *C. vulgaris* to remove cadmium starting from different initial increasing concentrations. The recorded results show higher removal rates using *C. vulgaris*, and much less by *A. zanardinii* from the initial concentrations of [Cd]~50 mg·L^{-1}. The low removal rates can be explained by the cadmium toxicity concentrations [Cd]~100 mg·L^{-1}, and [Cd]~250 mg·L^{-1} on the isolated freshwater microalgae. In fact, several living microalgae with innovative bioremediation properties have been the subject of numerous scientific studies carried out on the removal of HMs. More research in the domains of gene technology, pretreatments, biofilm techniques, and in combination with other microorganisms will enable the bioremoval processes of HMs using algae on a large scale.

CRediT authorship contribution statement

- ***Dzizi Sabrina***: Conceptualization, Investigation, Original manuscript writing.
- ***Chaib Nadjla***: Investigation, Project administration, Writing-Reviewing and Editing.
- ***Noune Faiza and Kaddeche Hadjer***: Resources, Investigation.
- ***Charchar Nabil***: Investigation, review and editing.

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

The present research was funded and supported by the Algerian General Directorate of Scientific Research and Technological Development (DGRSDT), and it fits into the university training research project (PRFU) A16N01UN210120180002. The authors express their thanks to the anonymous reviewers for their helpful comments on the paper.

References

[1] V. Memet, S. Bülent, Assessment of nutrient and heavy metal contamination in surface water and sediments of the upper Tigris River, Turkey, CATENA, 92 (2012) 1–10.
[2] D. Daby, Coastal pollution and potential biomonitors of metals in Mauritius, Water, Air, Soil Pollut., 174 (2006) 63–91.
[3] H. Huang, J.Y. Wu, J.H. Wu, Heavy metal monitoring using bivalved shellfish from Zhejiang coastal waters, East China Sea, Environ. Monit. Assess., 129 (2007) 315–320.
[4] J.V. Rao, P. Kavitha, K. Srikanth, P.K. Usman, T.G. Rao, Environmental contamination using accumulation of metals in marine sponges, *Sigmadocia fibulata* inhabiting the coastal waters of Gulf of Mannar, India, Toxicol. Environ. Chem., 89 (2007) 487–498.
[5] K. Sultan, A.S. Noor, P. Stefan, Distribution of Pb, As, Cd, Sn and Hg in soil, sediment and surface water of the tropical river watershed, Terengganu (Malaysia), J. Hydro-Environ. Res., 5 (2011) 169–176.
[6] C. Diop, D. Dewaele, A. Toure, M. Cabral, F. Cazier, M. Fall, B. Ouddane, A. Diouf, Study of sediment contamination by trace metals at wastewater discharge points in Dakar (Senegal), J. Water. Sci., 25 (2012) 277–285.
[7] G. Xuelu, C. Chen-Tung, Heavy metal pollution status in surface sediments of the coastal Bohai Bay, Water Resour. J., 46 (2012) 1901–1911.
[8] J.S. Ahn, Y.S. Park, J.Y. Kim, K.W. Kim, Mineralogical and geochemical characterization of arsenic in an abandoned mine tailings of Korea, Environ. Geochem. Health, 27 (2005) 147–157.
[9] M.R. Lee, J.A. Correa, Effect of copper mine tailings disposal on littoral meiofaunal assemblages in the Atacama region of Northern Chile, Mar. Environ. Res., 59 (2005) 1–18.
[10] A.N. Roychoudhury, M.F. Starke, Partitioning and mobility of trace metals in the Blesbokspruit: impact assessment of dewatering of mine waters in the East Rand, South Africa, Appl. Geochem., 21 (2006) 1044–1063.
[11] R. D'adamo, M. Di Stasio, A. Fabbrochini, Migratory crustaceans as biomonitors of metal pollution in their nursery areas. The Lesina Lagoon (SE Italy) as a case study, Environ. Monit. Assess., 143 (2008) 15–24.
[12] R. Smolders, L. Bervoets, V. Wepener, R. Blust, A conceptual framework for using mussels as biomonitors in whole efflent toxicity, Hum. Ecol. Risk Assess., 9 (2003) 741–760.
[13] A.L. Gonçalves, C.M. Pires-Jose, M. Simões, A review on the use of microalgale consortia for wastewater treatment, Algal Res., 24 (2017) 403–415.
[14] B. Olfa, Wastewater Treatment in Multitrophic Bioreactors Using Bacterial Microalgae Flocs Recoverable as Biogas, Thesis Jointly Supervised by the University of Carthage (Tunisia) and the University of Lorraine (France), Domain: Engineering of Processes and Products and Molecules, Doctoral Schools, Sciences and Engineering of Molecules, Processes, Products and Energy and Sciences and Technologies for Engineers, INSAT, 2018.
[15] C.D. Calvano, F. Italiano, L. Catucci, A. Agostiano, T.R.I. Cataldi, F. Palmisano, M. Trotta, The lipidome of the photosynthetic bacterium *Rhodobacter sphaeroides* R26 is affected by cobalt and chromate ions stress, Biometals: An Int. J. Role Metal Ions Biol. Biochem. Med., 27 (2014) 65–73.
[16] M. Aryal, M. Liakopoulou-Kyriakides, Bioremoval of heavy metals by bacterial biomass, Environ. Monit. Assess., 187 (2015) 1–26.
[17] M. CorraL-Bobadilla, A. Gonzalez-Marcos, E. Vergara-Gonzalez, F. Alba-Elias, Bioremediation of waste water to remove heavy metals using the spent mushroom substrate of *Agaricus bisporus*, Water, 11 (2019) 454–468.
[18] M. Fazlzadeh, R. Khosravi, A. Zarei, Green synthesis of zinc oxide nanoparticles using *Peganum harmala* seed extract, and loaded on *Peganum harmala* seed powdered activated carbon as new adsorbent for removal of Cr(VI) from aqueous solution, Ecol. Eng., 103 (2017) 180–190.
[19] I. Gajda, A. Stinchcombe, J. Greenman, C. Melhuish, I. Ieropoulos, Microbial fuel cell: a novel self-powered wastewater electrolyser for electrocoagulation of heavy metals, Int. J. Hydrogen Energy, 42 (2017) 1813–1819.
[20] E. Daneshvar, R.J. Wicker, P.L. Show, A. Bhatnagar, Biologically-mediated carbon capture and utilization by microalgae towards sustainable CO_2 biofixation and biomass valorization – a review, Chem. Eng. J., 427 (2022) 130884, doi: 10.1016/j.cej.2021.130884.
[21] G. Sibi, Factors influencing heavy metal removal by microalgae-a review, J. Crit. Rev., 6 (2019) 29–32.
[22] A.J. Bora, R.K. Dutta, Removal of metals (Pb, Cd, Cu, Cr, Ni, and Co) from drinking water by oxidation-coagulation-absorption at optimized pH, J. Water Process Eng., 31 (2019) 100839, doi: 10.22159/jcr.2019v6i6.35600.
[23] T. Kim, T.-K. Kim, K.-D. Zoh, Removal mechanism of heavy metal (Cu, Ni, Zn, and Cr) in the presence of cyanide during electrocoagulation using Fe and Al electrodes, J. Water Process Eng., 33 (2020) 101109, doi: 10.1016/j.jwpe.2019.101109.
[24] S.N. Francoeur, S.T. Rier, S.B. Whorley, In: J.T. Anderson, C.A. Davis, Eds., Methods for Sampling and Analyzing Wetland Algae Steven, Wetland Techniques, Volume 2: Organisms, Springer Science+Business Media, Dordrecht, 2013, pp. 1–58.

[25] E.G. Bellinger, D.C. Sigee, A Key to the More Frequently Occurring Freshwater Algae, Freshwater Algae: Identification and Use as Bioindicators, John Wiley & Sons, Ltd., Hoboken, 2010, pp. 137–244.
[26] N. Serediak, M.-L. Huynh, Algae Identification - Field Guide: An Illustrative Field Guide on Identifying Common Algae Found in the Canadian Prairies, Agriculture and Agri-Food, Agri-Environment Services Branch, Canada, 2011.
[27] S. Van Vuuren, J.C. Taylor, A. Gerber, C. Van Ginkel, Easy Identification of the Most Common Freshwater Algae, North-West University and Department of Water Affairs and Forestry, Pretoria, South Africa, 2006, pp. 1–200.
[28] H. Canter-Lund, J.W.G. Lund, Freshwater Algae: Their Microscopic World Explored (1995) (No. 582.26 CAN).
[29] K. Bajwa, N.R. Bishnoi, A. Kirrolia, J. Sharma, S. Gupta, Comparison of various growth media composition for physio-biochemical parameters of biodiesel producing microalgal species (*Chlorococcum aquaticum*, *Scenedesmus obliquus*, *Nannochloropsis oculata* and *Chlorella pyrenoidosa*), Eur. J. Biotechnol. Biosci., 5 (2017) 27–31.
[30] K. Ga-Yeong, R. Kosan, H. Jong-In, The use of bicarbonate for microalgae cultivation and its carbon footprint analysis, Green Chem., 21 (2019) 5053–5062.
[31] A. Verma, M. Agarwal, S. Sharma, N. Singh, Competitive removal of cadmium and lead ions from synthetic wastewater using *Kappaphycus striatum*, Environ. Nanotechnol. Monit. Manage., 15 (2021) 100449, doi: 10.1016/j.enmm.2021.100449.
[32] S. Li, S. Li, N. Wen, D. Wei, Y. Zhang, Highly effective removal of lead and cadmium ions from wastewater by bifunctional magnetic mesoporous silica, Sep. Purif. Technol., 265 (2021) 118341, doi: 10.1016/j.seppur.2021.118341.
[33] M. Oves, M.S. Khan, A. Zaidi, Biosorption of heavy metals by *Bacillus thuringiensis* strain OSM29 originating from industrial effluent contaminated north Indian soil, Saudi J. Biol. Sci., 20 (2013) 121–129.
[34] R.R.L. Guillard, 'Division Rates', J.R. Stein, Ed., Handbook of Phycological Methods: Culture Methods and Growth Measurements, Cambridge University Press, London, 1973, pp. 289–311.
[35] P.S. Chandrashekharaiah, D. Sanyal, S. Dasgupta, A. Banik, Cadmium biosorption and biomass production by two freshwater microalgae *Scenedesmus acutus* and *Chlorella pyrenoidosa*: an integrated approach, Chemosphere, 269 (2021) 128755, doi: 10.1016/j.chemosphere.2020.128755.
[36] D.L. Findlay, S.E.M. Kasian, L.L. Hendzel, G.W. Regehr, E.U. Schindler, J.A. Shearer, Biomanipulation of Lake 221 in the experimental lakes area (ELA): effects on phytoplankton and nutrients, Can. J. Fish Aquat. Sci., 51 (1994) 2794–2807.
[37] S. Li, Y. Yu, X. Gao, Z. Yin, J. Bao, Z. Li, R. Chu, D. Hu, J. Zhang, L. Zhu, Evaluation of growth and biochemical responses of freshwater microalgae *Chlorella vulgaris* due to exposure and uptake of sulfonamides and copper, Bioresour. Technol., 342 (2021) 126064, doi: 10.1016/j.biortech.2021.126064.
[38] J. Hockaday, A. Harvey, S. Velasquez-Orta, A comparative analysis of the adsorption kinetics of Cu^{2+} and Cd^{2+} by the microalgae *Chlorella vulgaris* and *Scenedesmus obliquus*. Algal Res., 64 (2022) 102710, doi: 10.1016/j.algal.2022.102710.
[39] S. Morin, T.T. Duong, A. Dabrin, A. Coynel, O. Herlory, M. Baudrimont, F. Delmas, G. Durrieu, J. Schäfer, P. Winterton, G. Blanc, M. Coste, Long-term survey of heavy-metal pollution, biofilm contamination and diatom community structure in the Riou Mort watershed, South-West France, Environ Pollut., 151 (2008) 532–542.
[40] S. Morin, N. Gómez, E. Tornés, M. Licursi, J. Rosebery, Benthic Diatom Monitoring and Assessment of Freshwater Environments: Standard Methods and Future Challenges, A.M. Romaní, H. Guasch, M. Dolors Balaguer, Eds., Aquatic Biofilms: Ecology, Water Quality and Wastewater Treatment, Caister Academic Press, UK, 2016, pp. 111–124.
[41] N. Chaib, S. Dzizi, H. Kaddeche, F. Noune, Performance of a fixed-bed bioreactor using diatom biofilms for wastewater bioremediation, Clean-Soil, Air, Water, 49 (2021) 2000282, doi: 10.1002/clen.202000282.
[42] A. Tiwari, T.K. Marella, Potential and Application of Diatoms for Industry-Specific Wastewater Treatment, S.K. Gupta, F. Bux, Eds., Application of Microalgae in Wastewater Treatment, Springer, Cham, 2019, pp. 321–339.
[43] S. Dwivedi, Bioremediation of heavy metal by algae: current and future perspective, J. Adv. Lab. Res. Biol., 3 (2012) 195–199.
[44] A.K. Zeraatkar, H. Ahmadzadeh, A.F. Talebi, N.R. Moheimani, M.P. McHenry, Potential use of algae for heavy metal bioremediation, a critical review, J. Environ. Manage., 181 (2016) 817–831.
[45] B. Volesky, Biosorption of Heavy Metals, 1st ed., Published Auguesr 15, CRC Press, Boca Raton, 1990, 408 p.
[46] G.M. Gadd, Heavy metal accumulation by bacteria and other microorganisms, Experientia, 46 (1990) 834–840.
[47] S.K. Mehta, J.K. Gaur, Characterisation and optimization of Ni and Cu sorption from aqueous solution by *Chlorella vulgaris*, Ecol. Eng., 18 (2001) 1–13.
[48] R. Dixit, D. Malaviya, K. Pandiyan, U.B. Singh, A. Sahu, R. Shukla, D. Paul, Bioremediation of heavy metals from soil and aquatic environment: an overview of principles and criteria of fundamental processes, Sustainability, 7 (2015) 2189–2212.
[49] N.F.Y. Tam, J.P.K. Wong, Y.S. Wong, Repeated use of two *Chlorella* species, *C. vulgaris* and WW1 for cyclic nickel biosorption, Environ. Pollut., 114 (2001) 85–92.
[50] C.A. Mahan, V. Majidi, J.A. Holcombe, Evaluation of the metal uptake of several algae strains in a multicomponent matrix utilizing inductively coupled plasma emission spectrometry, Anal. Chem., 61 (1989) 624–627.
[51] P.R. Pascucci, A.D. Kowalak, Public health benefits of using algae for simultaneous multiple metal extraction from waters, Rev. Environ. Health, 11 (1996) 205–212.
[52] Z. Lin, J. Li, Y. Luan, W. Dai, Application of algae for heavy metal adsorption: a 20-year meta-analysis, Ecotoxicol. Environ. Saf., 190 (2020) 110089, doi: 10.1016/j.ecoenv.2019.110089.
[53] E. El-Bestawy, Efficiency of immobilized cyanobacteria in heavy metals removal from industrial effluents, Desal. Water Treat., 159 (2019) 66–78.
[54] L. Travieso, R.O. Canizares, R. Borja, F. Benıtez, A.R. Domınguez, R. Dupeyron, V. Valiente, Heavy metal removal by microalgae, Bull. Environ. Contamin. Toxicol., 62 (1999) 144–151.
[55] S. Singh, S. Pradhan, L.C. Rai, Metal removal from single and multimetallic systems by different biosorbent materials as evaluated by differential pulse anodic stripping voltammetry, Process Biochem., 36 (2000) 175–182.
[56] P.K. Rai, B.D. Tripathi, Removal of heavy metals by the nuisance cyanobacteria *Microcystis* in continuous cultures: an eco-sustainable technology, Environ. Sci., 4 (2007) 53–59.
[57] S. Shanab, A. Essa, E. Shalaby, Bioremoval capacity of three heavy metals by some microalgae species (Egyptian Isolates), Plant Signaling Behav., 7 (2012) 1–8.
[58] U.K. Singh, B. Kumar, Pathways of heavy metals contamination and associated human health risk in Ajay River basin, India, Chemosphere, 174 (2017) 183–199.
[59] M. Danouche, N. El Ghachtouli, H. El Arroussi, Phycoremediation mechanisms of heavy metals using living green microalgae: physicochemical and molecular approaches for enhancing selectivity and removal capacity, Heliyon, 7 (2021) e07609, doi: 10.1016/j.heliyon.2021.e07609.
[60] R. Piña-Olavide, L.M. Paz-Maldonado, M.C. Alfaro-De La Torre, M.J. García-Soto, A.E. Ramírez-Rodríguez, S. Rosales-Mendoza, R.F. García De la-Cruz, Increased removal of cadmium by *Chlamydomonas reinhardtii* modified with a synthetic gene for γ-glutamylcysteine synthetase, Int. J. Phytorem., 22 (2020) 1269–1277.
[61] X. Ma, Y. Chen, F. Liu, S. Zhang, Q. Wei, Enhanced tolerance and resistance characteristics of *Scenedesmus obliquus* FACHB-12 with K3 carrier in cadmium polluted water, Algal Res., 55 (2021) 102267, doi: 10.1016/j.algal.2021.102267.
[62] J. Ye, H. Xiao, B. Xiao, W. Xu, L. Gao, G. Lin, Bioremediation of heavy metal contaminated aqueous solution by using red algae *Porphyra leucosticte*, Water Sci. Technol., 72 (2015) 1662–1666.