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Adsorbent effect of *Chlorella vulgaris* and *Scenedesmus* sp. (Chlorophyta) for the removal of some heavy metals and nutrients

Bazı ağır metal ve nutrient gideriminde *Chlorella vulgaris* ve *Scenedesmus* sp. (Chlorophyta)'nin adsorbent etkisi

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Abstract: Objective: The aim of present manuscript is to evaluate the efficiency of two microalgae strains -*Chlorella vulgaris* and *Scenedesmus* sp- in removal of some inorganic nutrients and heavy metals.

Methods: For this aim the green microalgae (Chlorophyta), grown in controlled laboratory conditions, were used as biosorbent for the removal of six different concentrations of Antimony (Sb^{3+}), Manganese (Mn^{2+}), Copper (Cu^{2+}), Nickel (Ni^{2+}), Phosphate (PO_4^{3-}) and Nitrate (NO_3^-) which are prepared in aqueous solutions at conc. 0.1–1 mM for PO_4^{3-}; 5–30 mM for NO_3-; 2,5–100 ppm for heavy metals. Besides, the effects of heavy metals and inorganic nutrients on the cell's total carbohydrate and chlorophyll a-b content were investigated during 24 hours exposure.

Results: According to the results, the average removal efficiency of Phosphate, Nitrate, Antimony, Manganese, Copper and Nickel on *C. vulgaris* biomass was determined 95.91%, 21.63%, 28.64%, 49.41%, 33.38% and 29.96% while 98.15%, 14.28%, 10.05%, 8.52%, 30.18% and 20.62% on *Scenedesmus* sp. cells, respectively. On the other hand, the average adsorption capacities of Sb^{3+}, Mn^{2+}, Cu^{2+}, Ni^{2+}, PO_4^{3-} and NO_3^- ions were confirmed 0.18, 0.38, 0.28, 0.15, 1.18 and 9.30 mg g^{-1} by *C. vulgaris* and 2.99, 0.96, 11.50, 11.33, 0.75 and 4.56 mg g^{-1} by *Scenedesmus* sp. biomass.

Conclusion: Result of this study suggests that *Chlorella vulgaris* and *Scenedesmus* sp. have a remarkable ability on removal of excessive nutrients and heavy metals at laboratory conditions.

Keywords: Heavy metal, *Chlorella vulgaris*, *Scenedesmus* sp., adsorption, nutrient

Özet: Amaç: Mevcut çalışmanın amacı bazı inorganik besin ve ağır metallerin uzaklaştırılmasında iki mikroalg suşunun-*Chlorella vulgaris* ve *Scenedesmus* sp- etkinliğini değerlendirmektir.

Metod: Bu amaç için, kontrollü laboratuar koşullarında yetiştirilen yeşil mikroalgler (Chlorophyta), Fosfat 0.1–1 mM; Nitrat 5–30 mM; Ağır metaller 2,5–100 ppm'lik sulu çözeltilerde 6 farklı konsantrasyonda hazırlanan Antimon (Sb^{+3}), Mangan (Mn^{+2}), Bakır (Cu^{+2}), Nikel (Ni^{+2}), Fosfat (PO_4^{-3}) ve nitratın (NO_3^-) gideriminde biyosorbent olarak kullanılmıştır. Ayrıca, 24 saatlik deney süresince ağır metal ve nutrientlerin hücrelerin total karbonhidrat ve klorofil a-b içeriği üzerine etkisi araştırılmıştır.

Bulgular: Analiz sonuçlarına göre, *C. vulgaris* bioması tarafından fosfat, nitrat, antimon, mangan, bakır ve nikelin giderim verimliliği sırasıyla %95.91, %21.63, %28.64, %49.41, %33.38 and %29.96 olarak belirlenirken *Scenedesmus* sp. hücrelerinde %98.15, %14.28, %10.05, %8.52, %30.18 and %20.62 olarak belirlenmiştir. Bununla birlikte, Sb^{+3}, Mn^{+2}, Cu^{+2}, Ni^{+2}, PO_4^{-3} ve NO_3^- iyonlarının *C. vulgaris* bioması tarafından ortalama adsorbsi-

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yon kapasitesi sırasıyla 0.18, 0.38, 0.28, 0.15, 1.18 ve 9.30 mg g^{-1} ve *Scenedesmus* sp. bioması tarafından 2.99, 0.96, 11.50, 11.33, 0.75 ve 4.56 mg g^{-1} olarak saptanmıştır.

Sonuç: Çalışmanın sonucunda *Chlorella vulgaris* ve *Scenedesmus* sp. nin laboratuvar şartlarında aşırı nutrient ve ağır metal giderimi üzerinde olağanüstü bir yeteneğinin olduğunun kabul göreceğini düşündürmektedir.

Anahtar Kelimeler: Ağır metal, *Chlorella vulgaris*, *Scenedesmus* sp., adsorbsiyon, nutrient

Introduction

The environmental pollution caused by the industrial waste has been increased due to the rapid growth of global industries. Most of the industrial waste contain heavy metals with certain concentration. So, the presence of heavy metals in water and wastewater is increasing due to the industrial development-disposal in the sewerage or in the water bodies [1].

The heavy metal and nutrient pollution in soils and aquatic environments is a serious ecological problem. The presence of heavy metals in aquatic environment is known to cause severe damage to aquatic life. Most of the heavy metals are soluble in water and form aqueous solutions and consequently cannot be separated by ordinary physical and chemical means of separation. Biological methods such as biosorption/bioaccumulation for the removal of heavy metal ions may provide an attractive alternative to physico-chemical methods. The biomass is capable of absorbing and adsorbing metal ions from aqueous solution [2].

Microalgae are universally acknowledged as playing a very important role in natural water purification process [3,4]. Microalgae have become relatively popular as biosorbent of heavy metals due to the fact that microalgae, are a rich source in the ocean and other water bodies, relatively cheap to process and able to accumulate high metal content [5]. Thus, the use of microalgae for removal of heavy metals and nutrients from different wastes has been described by a number of authors [6–16].

This investigation was focused on the tolerance of the two microalgae; *Chlorella vulgaris* and *Scenedesmus* sp. (Chlorophyta) to heavy metal and nutrient treatments. The ability of a microalga species for bioremoval of heavy metals and inorganic nutrients has been tested under the effect of different concentration in aqueous solution.

Materials and Methods

Organisms, culture conditions and Preparation of metal and nutrient solutions

The *Chlorella vulgaris* and *Scenedesmus* sp. strains originated from an algae culture collection maintained at the Ege University Science and Technology Research and Application Center, Izmir/Turkey (EBILTEM). A standard initial inoculum of the isolated algae was inoculated to culture flasks (500 mL each) that contained 200 mL of sterile Bold Basal Medium [17] and incubated at 28±1°C under 12 h light (20 E m^{-2} s^{-1}±20%)/12 h dark regimen, with aeration (1.2 L min^{-1}) and magnetic stirring (110 rpm). The pH value was adjusted to 6–7 using 1 M NaOH and 1 M HCI. Continuous stirring of the culture was achieved using a 110 rpm magnetic plate. At the end of the incubation period (80–90 days) cultures were filtered and washed several times by distilled water. The algal stock was stored at 4°C in dark until use. At least three replicates for each sample and controls were used.

Antimony (Sb^{3+}), Manganese (Mn^{2+}), Nickel (Ni^{2+}), Copper (Cu^{2+}), Phosphate (PO_4^{3-}) and Nitrate (NO_3^-) stock solution (1000 mgL^{-1}) were prepared by diluting $C_8H_4K_2O_{12}Sb_2 \cdot H_2O$ (pH 5); $MnSO_4 \cdot H_2O$ (pH 4-6); $NiSO_4.6H_2O$ (pH 4-6); $CuSO_4.5H_2O$ (pH 5-7); KH_2PO_4 (pH 6) and $NaNO_3$ (pH 6) in deionized water. The working solutions were prepared by serial dilution of stock solution. The pH of heavy metal and nutrient solutions was adjusted to 6.5-7 with 1M NaOH and 1M HCI. The final solutions of the heavy metals were prepared, from which concentrations 0, 5, 10, 20, 40, 60, 80 and 100 ppm; and nutrients (PO_4^{3-} and NO_3^-) were used from which concentrations 0,1;0,2;0.4;0,6;0,8;1 mM and 5;10;15;20;25;30 mM, respectively.

For batch experimental, 10 ml microalgae biomass was added to 40 mL heavy metal and inorganic nutrient solution in erlenmeyer flask. The mixtures were shaken in orbital shaker at 110 rpm for 24 h. Then, the mixture was centrifuged at 4000 rpm for 15 min to separate the biomass and washed twice with distilled water and were used for the adsorption tests. The residual concentration of heavy metals in supernatant was determined by ICP-MS (Inductively Coupled Plasma–Mass Spectrometer-Agilent 7700) and the residual concentration of nutrients in supernatant was determined by Ion Chromatography (Dionex 5000 Thermo Scientific).

The metal uptake per gram of adsorbent, q (mg g^{-1}), was calculated using the equation:

$q=(ci-ct)*V/m$

Where V is the volume of the solution (mL) and m is the mass of biosorbent (g). ci and ct are the metal concentrations initially and in the terminally (mg mL^{-1}), respectively.

The efficiency of the removal was calculated using the following equation:

Removal efficiency=100*(*ci–ct)*/ci

Determination of dry weight

A definite volume (10 mL) of algal suspension was filtered through cellulose acetate filter membrane (47mm in diameter, 0.45 µm in pore size) and dried overnight in an oven at 105°C. Data were given as mg mL^{-1} algal suspension.

Determination of carbohydrate content

Total carbohydrate contents were measured using the phenol-sulfuric acid assay and using glucose as a standard. 1 mL aliquots of the cultures were used to quantified spectrophotometrically the total carbohydrate content by the phenol-sulfuric acid assay [18].

Determination of Chlorophyll pigment assay

10 mL sample of each culture was collected to analyze the chlorophyll content after 24 h of all metals and nutrients exposure, and acetone and magnesium carbonate were used to extract the chlorophyll from *C. vulgaris* and *Scenedesmus* sp.

Spectrophotometry was used to measure the content of chlorophyll a (Chl a) and chlorophyll b (Chl b) according to the method of Parsons and Strickland, 1963 [19]. Pigment content in the filtered extract were determined by the absorbance at 630, 645, 665 and 750 nm in a 1cm quartz cell against a blank of 80% aqueous acetone.

Statistics

All experiments were performed in 3 replicates. The data are presented as the mean±standard deviation of the mean (SDM).

Result and Discussion

Total carbohydrate and chlorophll a-b contents

Chlorophyll is the most important pigment in algal and cyanobacterial cells for collecting solar energy for photosynthesis [20,21]. Otherwise, photosynthesis is generally inhibited by heavy metals [22].

Chlorophyll 'a' concentration was recorded to be decreased simultaneously from an initial value of 0.7844 µg/L to 0.5146 µg/L for *C. vulgaris* and 0.1832 µg/L to 0.1635 µg/L for *Scenedesmus* sp. on the final hour of experiment (24 h). Sb^{3+}, Ni^{2+} and Cu^{2+} showed a strong inhibition of chlorophyll "a" biosynthesis even at the lower concentrations (2.5–10 ppm) on *C. vulgaris* biomass while Sb^{3+} and Mn^{2+} showed a strong stimulation of chlorophyll 'a' biosynthesis on *Scenedesmus* sp. (Table 1,2). On top of that, the data in Fig. 1 illustrated that, *C. vulgaris* biomass tolerated the toxicity of NO_3^- even at higher concentrations (15–30 mM), moreover the lower concentration of NO_3^- (5–15 mM) induced a pronounced stimulation of chlorophyll 'a'. Chlorophyll 'b' concentration was increased from an initial value of 0.2168 µg/L to 0.4214 µg/L on *C. vulgaris* and 0.0421 µg/L to 0.0643 µg/L on *Scenedesmus* sp. cell after 24 h of exposure. PO_4^{3-}, NO_3^-, Mn^{2+} and Cu^{2+} showed a strong stimulation of chlorophyll 'b' biosynthesis even at the lower concentrations (2.5–10 ppm) on *C. vulgaris* biomass while Sb^{3+}, Ni^{2+} showed a strong inhibition at the lower and higher concentrations (2.5–100 ppm) (Fig. 1). This means that the efficiency of the photosynthetic apparatus seemed to be more affected by Sb^{3+}, Ni^{2+}, $NO_3^-$and Cu^{2+}.

The total carbohydrate contents of *C. vulgaris* and *Scenedesmus* sp. were observed to be completely inhibited by initial and final concentration of all metals and nutrients which is similar to the reports [23]. Total carbohydrate concentration was recorded to be decreased simultaneously from an initial value of 0.7488 mg/mL to 0.1828 mg/mL on *C. vulgaris* and 0.4048 mg/mL to 0.2278 mg/mL on *Scenedesmus* sp. on the final hour of experiment (24 h). The obtained results in this investigation (Fig. 2) revealed that, the exposure to low and high concentration of Mn (2.5–100 ppm) reduced total carbohydrate significantly from an initial value of 0.7488 mg/mL to 0.0670 mg/mL and reduced to about 91.05% of the control. In terms of carbohydrate, Mn^{2+} seemed to exert high toxicity to *C. vulgaris* species even at its lower concentration (ppm<20).

This might be linked with the synthesis of carbohydrates (the most building material) and consequently the growth and survival of the two algae under investigation. This means that the efficiency of photosynthetic apparatus and the production of carbohydrates were closely associated with nitrogen-metabolism. This leads us to conclude that the regulation between carbohydrate and N-metabolism was associated with the heavy metal tolerance whereas the toxicity of these metabolic inhibitors disturbed by both components (carbohydrate and chlorophyll) [24].

Table 1: Metal and nutrient ion binding capacities, percent of metal and nutrient ion removal, total C.H. and Chlorophyll a-b content of *C. vulgaris* biomass. (Data represent mean values±SDM (n=3).

	Cons.	Metal and nutrient removal (mg g^{-1})	% Ion removal efficiency (100%)	Total C.H. (mg mL^{-1}) control: 0.7488±0.0001 mg mL^{-1}	Chl a (µg L^{-1}) control: 0.7844±0.0001 µg L^{-1}	Chl b (µg L^{-1}) control: 0.2168±0.0002 µg L^{-1}
Phosphate	0.1 mM	0.21±0.0001	88.84±0.0001	0.1326±0.0001	0.3399±0.0001	0.1444±0.0001
	0.2 mM	0.44±0.0002	94.10±0.0001	0.1956±0.0001	1.0541±0.0002	0.8888±0.0002
	0.4 mM	0.91±0.0000	97.08±0.0002	0.1309±0.0001	0.5843±0.0001	0.4250±0.0001
	0.6 mM	1.38±0.0002	98.07±0.0001	0.1527±0.0002	0.2799±0.0001	0.1022±0.0002
	0.8 mM	1.86±0.0001	98.57±0.0001	0.1412±0.0002	0.3762±0.0002	0.2084±0.0000
	1 mM	2.32±0.0001	98.79±0.0001	0.1907±0.0001	0.3476±0.0004	0.2148±0.0004
Nitrate	5 mM	3.45±0.0003	29.33±0.0002	0.5464±0.0003	1.6536±0.0003	1.8652±0.0002
	10 mM	2.65±0.0001	11.27±0.0002	0.5331±0.0001	1.3320±0.0001	1.1271±0.0002
	15 mM	3.85±0.0002	10.90±0.0002	0.4926±0.0002	0.9917±0.0001	0.8522±0.0001
	20 mM	12.46±0.0001	26.47±0.0001	0.5335±0.0002	0.4780±0.0001	0.2872±0.0003
	25 mM	15.94±0.0001	27.11±0.0001	0.5057±0.0001	1.2728±0.0002	1.0916±0.0003
	30 mM	17.45±0.0001	24.72±0.0001	0.5712±0.0001	0.8613±0.0001	0.6382±0.0001
Antimony	2.5 ppm	0.02±0.0003	40.99±0.0002	0.0707±0.0001	0.3973±0.0000	0.2404±0.0002
	5 ppm	0.03±0.0003	24.76±0.0002	0.1281±0.0000	0.3900±0.0001	0.1885±0.0002
	10 ppm	0.07±0.0001	32.78±0.0003	0.0897±0.0002	0.1901±0.0002	0.0316±0.0002
	25 ppm	0.16±0.0001	27.74±0.0001	0.1504±0.0001	0.4974±0.0002	0.3144±0.0001
	50 ppm	0.25±0.0002	21.68±0.0001	0.1495±0.0002	0.2013±0.0002	0.0507±0.0001
	100 ppm	0.57±0.0001	23.88±0.0001	0.0499±0.0003	0.2747±0.0001	0.1152±0.0001
Nickel	2.5 ppm	0.02±0.0001	38.90±0.0002	0.2189±0.0002	0.2579±0.0001	0.0601±0.0001
	5 ppm	0.05±0.0001	42.84±0.0003	0.1789±0.0001	0.1947±0.0001	0.0206±0.0001
	10 ppm	0.11±0.0001	45.78±0.0004	0.1141±0.0001	0.3636±0.0001	0.1748±0.0001
	25 ppm	0.08±0.0002	14.83±0.0002	0.0626±0.0001	0.2459±0.0001	0.0720±0.0002
	50 ppm	0.24±0.0001	20.75±0.0002	0.1017±0.0001	0.6543±0.0002	0.0855±0.0001
	100 ppm	0.40±0.0001	16.68±0.0001	0.1099±0.0001	0.2707±0.0001	0.1140±0.0001
Manganese	2.5 ppm	0.03±0.0002	45.17±0.0002	0.0608±0.0001	0.2892±0.0003	0.1426±0.0002
	5 ppm	0.08±0.0003	54.62±0.0001	0.0869±0.0000	0.9484±0.0001	0.8626±0.0001
	10 ppm	0.12±0.0001	45.55±0.0001	0.0888±0.0002	0.8209±0.0001	0.7485±0.0001
	25 ppm	0.38±0.0001	54.87±0.0003	0.0951±0.0003	0.6023±0.0002	0.4998±0.0002
	50 ppm	0.70±0.0001	53.55±0.0001	0.0664±0.0004	0.3524±0.0001	0.3015±0.0001
	100 ppm	0.96±0.0002	42.70±0.0001	0.0037±0.0001	0.5005±0.0002	0.3697±0.0002
Copper	2.5 ppm	0.02±0.0002	35.84±0.0002	0.0941±0.0001	0.0417±0.0001	1.8578±0.0001
	5 ppm	0.01±0.0001	31.24±0.0001	0.1086±0.0001	0.2522±0.0001	0.2282±0.0001
	10 ppm	0.05±0.0001	22.88±0.0003	0.0383±0.0002	0.3401±0.0001	0.3262±0.0001
	25 ppm	0.20±0.0001	34.08±0.0004	0.0857±0.0002	0.1790±0.0002	0.0827±0.0001
	50 ppm	0.38±0.0001	33.39±0.0002	0.1367±0.0001	0.3248±0.0003	0.1801±0.0002
	100 ppm	1.02±0.0002	42.85±0.0002	0.1650±0.0001	0.3663±0.0002	0.2581±0.0002

Heavy metal and nutrient removel-efficiency

Several green algal species appearing in polluted sites are tolerant or resistant to Cu^{2+}, Cd^{2+}, Pb^{2+} and Zn^{2+} [25–28]. Bioremoval is defined as the accumulation and concentration of pollutants from aqueous solutions by the use of biological material, thus allowing the recovery and/or environmentally acceptable disposal of the pollutants [29,30]. Microalgae were reported to be more efficient in sequestering metal species from solution than bacterial and fungal biomass [31].

24 h experiment showed that the concentration of metals such as antimony, manganese, copper, nickel and inorganic nutrients such as phosphate, nitrate were reduced by tested *C. vulgaris* in the percentages of 28.64, 49.41, 33.38, 29.96 and 95.91, 21.63, and by *Scenedesmus* sp. in the percentages of 10.05, 8.52, 30.18, 20.62 and 98.15, 14.28, respectively (Table 1,2).

The rate of accumulation of PO_4^{3-} was higher at lower and higher concentrations for two tested algae (Fig. 3,4). However, at higher concentrations (25–100

Table 2: Metal and nutrient ion binding capacities, percent of metal and nutrient ion removal, total C.H. and Chlorophyll a-b content of *Scenedesmus* sp. biomass. (Data represent mean values±SDM (n=3).

	Cons.	Metal and nutrient removal ($mg\ g^{-1}$)	% Ion removal efficiency (100%)	Total C.H. ($mg\ mL^{-1}$) control: 0.4048± $mg\ mL^{-1}$	Chl a ($\mu g\ L^{-1}$) control: 0.1832± $\mu g\ L^{-1}$	Chl b ($\mu g\ L^{-1}$) control: 0.0421± $\mu g\ L^{-1}$
Phosphate	0.1 mM	0.14±0.0001	95.58±0.0001	0.2292±0.0001	0.0894±0.0001	0.0069±0.0002
	0.2 mM	0.29±0.0002	97.53±0.0001	0.3808±0.0001	0.1771±0.0001	0.0047±0.0002
	0.4 mM	0.58±0.0002	98.71±0.0001	0.2424±0.0002	0.0692±0.0001	0.0068±0.0001
	0.6 mM	0.88±0.0001	99.47±0.0001	0.2312±0.0001	0.0571±0.0001	0.0180±0.0001
	0.8 mM	1.18±0.0001	99.41±0.0001	0.1552±0.0001	0.0552±0.0001	0.0294±0.0002
	1 mM	1.45±0.0001	98.20±0.0002	0.1544±0.0001	0.0656±0.0002	0.0099±0.0003
Nitrate	5 mM	2.60±0.0001	26.00±0.0001	0.1672±0.0002	0.0247±0.0002	0.0193±0.0003
	10 mM	1.40±0.0002	8.61±0.0001	0.5164±0.0001	0.1068±0.0001	0.0016±0.0001
	15 mM	0.42±0.0001	1.86±0.0001	0.3441±0.0002	0.0171±0.0001	0.0275±0.0001
	20 mM	3.95±0.0001	11.77±0.0002	0.4649±0.0001	0.0408±0.0001	0.0081±0.0001
	25 mM	14.28±0.0001	27.83±0.0001	0.4817±0.0001	0.0433±0.0001	0.0041±0.0002
	30 mM	4.73±0.0001	9.62±0.0002	0.1433±0.0001	0.0259±0.0001	0.0146±0.0001
Antimony	2.5 ppm	0.63±0.0002	16.93±0.0001	0.1664±0.0001	0.1129±0.0001	0.0069±0.0001
	5 ppm	0.15±0.0003	2±0.0002	0.1953±0.0002	0.2816±0.0001	0.1306±0.0001
	10 ppm	1.79±0.0001	12.1±0.0001	0.1869±0.0004	0.1526±0.0001	0.0079±0.0001
	25 ppm	6.96±0.0001	18.8±0.0001	0.2837±0.0001	0.5687±0.0002	0.3537±0.0003
	50 ppm	7.11±0.0002	9.6±0.0001	0.1757±0.0002	0.1050±0.0001	0.0134±0.0001
	100 ppm	1.30±0.0001	0.87±0.0001	0.1817±0.0004	0.4491±0.0001	0.3631±0.0002
Nickel	2.5 ppm	0.89±0.0002	23.91±0.0001	0.1305±0.0004	0.0416±0.0002	0.0027±0.0001
	5 ppm	1.12±0.0001	15.1±0.0002	0.1605±0.0002	0.0942±0.0002	0.0146±0.0001
	10 ppm	1.41±0.0002	9.5±0.0001	0.1103±0.0001	0.0756±0.0001	0.0038±0.0002
	25 ppm	9.86±0.0002	26.61±0.0001	0.1593±0.0001	0.0952±0.0001	0.0025±0.0001
	50 ppm	17.26±0.0001	23.3±0.0001	0.1543±0.0001	0.0828±0.0004	0.0152±0.0001
	100 ppm	37.48±0.0001	25.3±0.0004	0.1517±0.0003	0.1217±0.0002	0.0348±0.0002
Manganese	2.5 ppm	0.94±0.0002	25.38±0.0002	0.2113±0.0001	0.3615±0.0003	0.1729±0.0002
	5 ppm	1.16±0.0002	15.6±0.0004	0.2392±0.0001	0.2201±0.0002	0.1735±0.0002
	10 ppm	1.07±0.0001	7.225±0.0002	0.4775±0.0001	0.2558±0.0003	0.1518±0.0002
	25 ppm	0.5±0.0004	1.34±0.0004	0.1557±0.0002	0.5067±0.0003	0.3143±0.0001
	50 ppm	0.22±0.0003	0.3±0.0004	0.1940±0.0001	0.2534±0.0002	0.1417±0.0001
	100 ppm	1.85±0.0001	1.25±0.0003	0.1500±0.0002	0.2578±0.0002	0.0970±0.0004
Copper	2.5 ppm	1.88±0.0001	50.84±0.0004	0.4615±0.0002	0.0594±0.0002	0.0041±0.0003
	5 ppm	2.39±0.0002	32.25±0.0001	0.1576±0.0001	0.0192±0.0001	0.0005±0.0003
	10 ppm	3.01±0.0002	20.3±0.0001	0.1618±0.0001	0.0891±0.0001	0.0355±0.0002
	25 ppm	10.49±0.0001	28.32±0.0002	0.1298±0.0001	0.1203±0.0001	0.0567±0.0002
	50 ppm	21.85±0.0001	29.5±0.0001	0.1720±0.0003	0.1143±0.0001	0.0423±0.0001
	100 ppm	29.41±0.0002	19.85±0.0001	0.1240±0.0004	0.6752±0.0001	0.0242±0.0001

ppm) the uptake of manganese was lower and at the end of 24 h the absorption was almost stagnant at 25–50 ppm due to the toxicity caused to *Scenedesmus* sp. biomass (Table 2).

High levels of nitrogenous compounds in wastewater can be effectively removed only by algae [11,32,33]. *C. vulgaris* shows best adsorption capacity of nitrate, nickel and copper from different concentration of metal and nutrient solutions than *Scenedesmus* sp.

C. vulgaris and *Scenedesmus* sp. biomass removed 95.91% and 98.15% of phosphate in nutrient solutions during the 24 h, while the maximum capability of removal was 98.79% and 99.47% of experiment, respectively. These results similar to that reported by Okmen et al., (2007) [34] who concluded that *Chlorella* and *Scenedesmus* were the most efficient algal strains to eliminate phosphate from mixtures of municipal and refinery wastes. Phosphate removal by *C. vulgaris* during remediation is due to the utilization of phosphorus for growth [16].

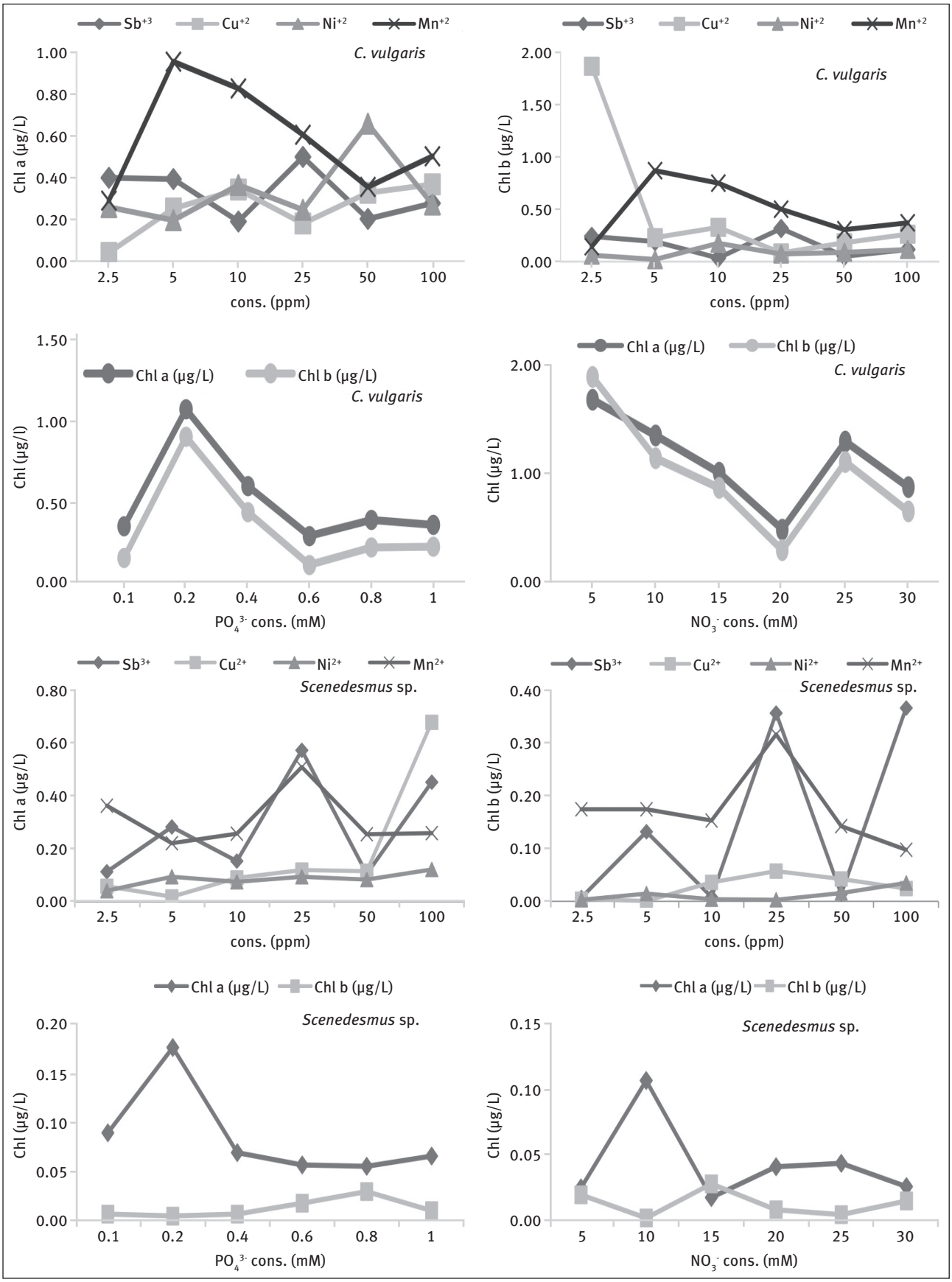

Figure 1: Effect of heavy metal and nutrients on chlorophll a-b contents in *C. vulgaris* and *Scenedesmus* sp.

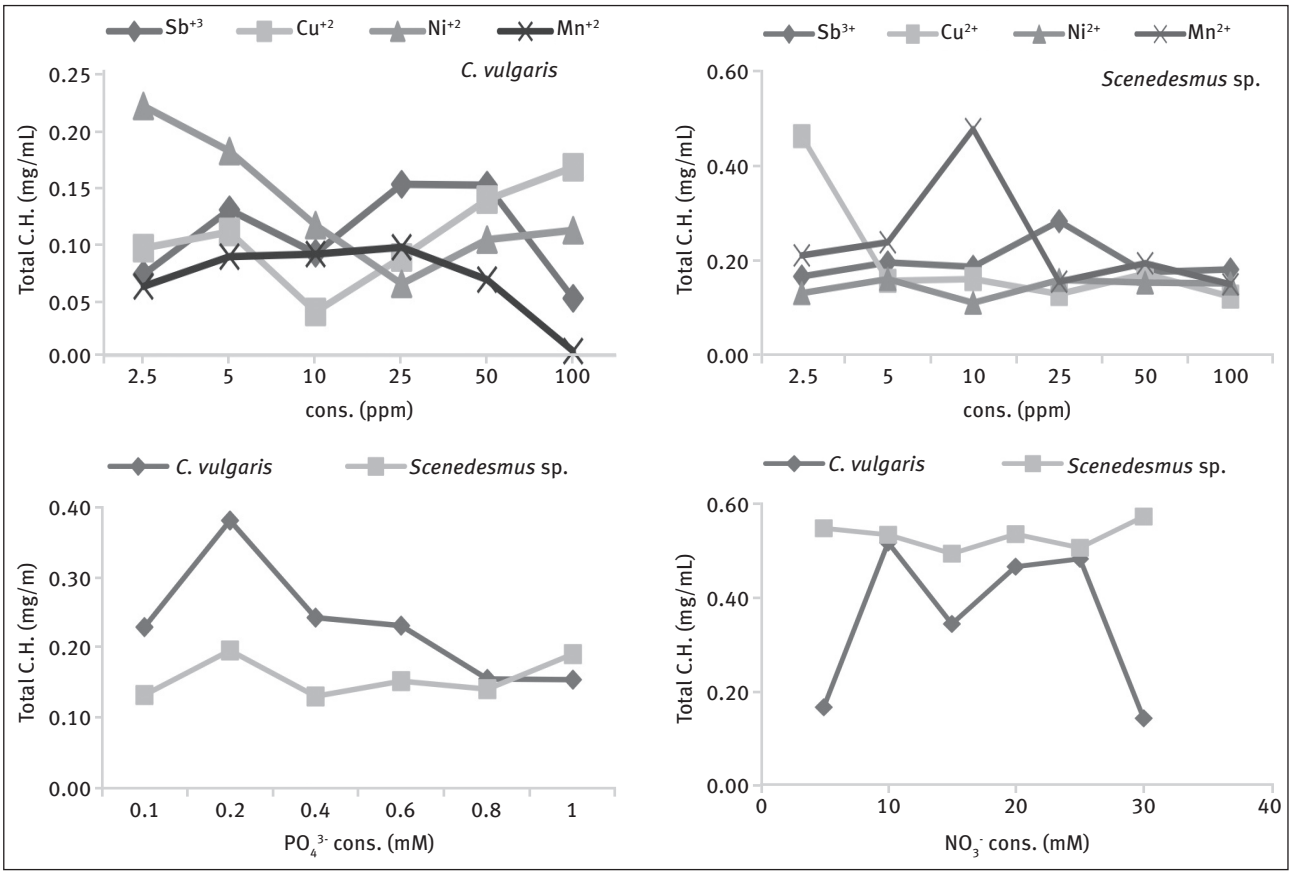

Figure 2: Effect of heavy metal and nutrients on total carbohydrate (C.H.) contents in *C. vulgaris* and *Scenedesmus* sp.

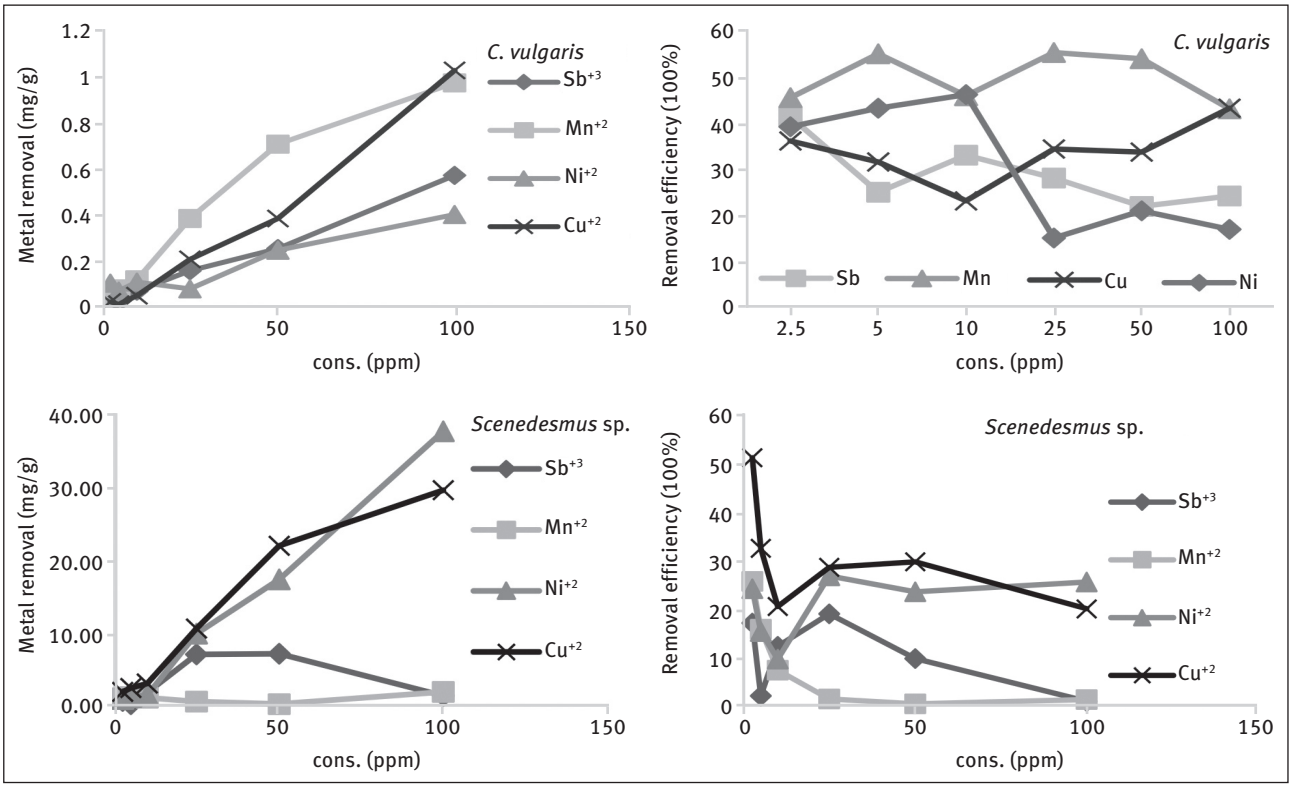

Figure 3: Heavy metal removal and efficiency of *C. vulgaris* and *Scenedesmus* sp.

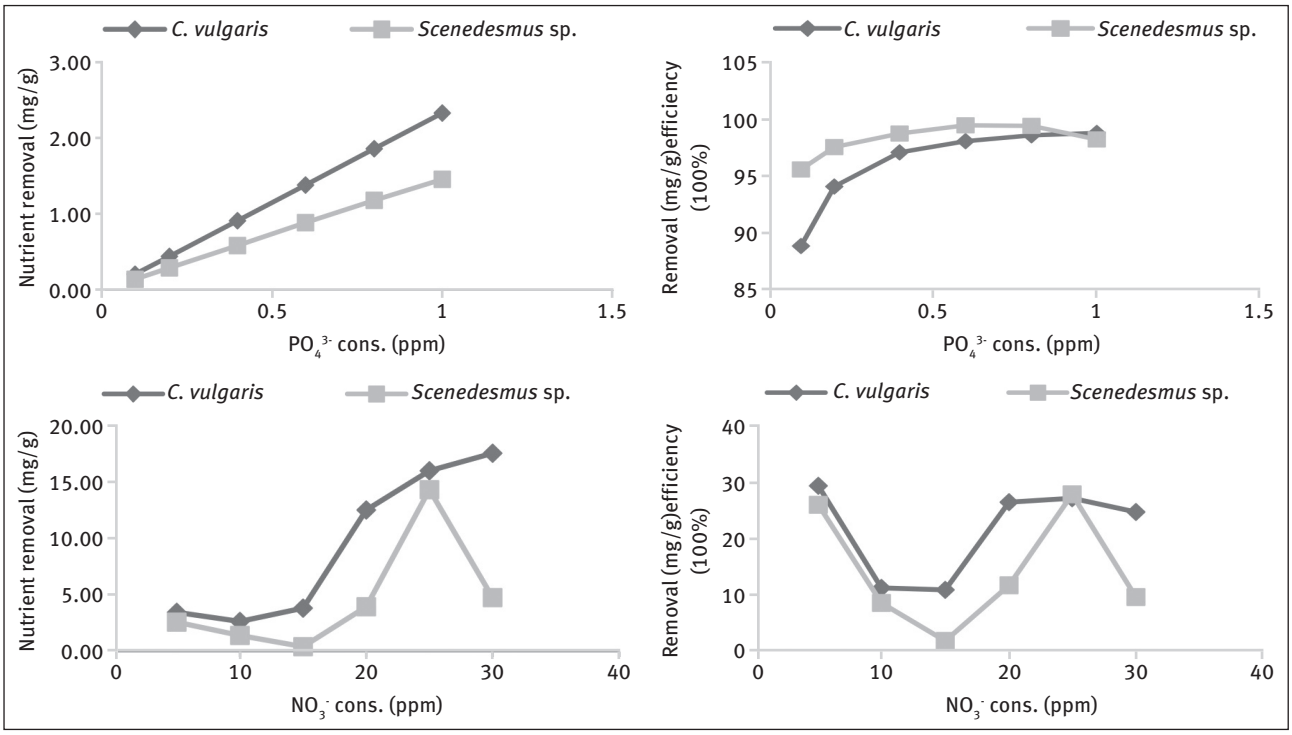

Figure 4: Nutrient removal and efficiency of *C. vulgaris* and *Scenedesmus* sp.

Conclusion

In conclusion, our results indicate that exposing *Chlorella vulgaris* and *Scenedesmus* sp. to different concentrations of Sb, Mn, Cu and Ni, results in an decrease in chlorophyll 'a' pigment and total carbohydrate content at lower and higher concentrations. Nevertheless, chlorophyll 'b' concentration was increased simultaneously at lower and higher concentrations of Mn^{2+} solutions on two tested algae. In this study, the removal effect for Ni, Mn, Cu, Sb heavy metals of *C. vulgaris* was Mn>Cu>Sb>Ni (0.38, 0.28, 0.18, 0.15 mg g^{-1}) while the removal efficiency was Mn>Cu>Ni>Sb (49.41%, 33.38%, 29.96%, 28.64%) and of *Scenedesmus* sp. was Cu>Ni>Mn>Sb (11.35, 11.34, 3.20, 2.99 mg g^{-1}) while the removal efficiency was Cu>Ni>Mn>Sb (20.95%, 20.62%, 13.40%, 10.05%), respectively. Microalgal remediation may contribute in the treatment of various sites contaminated with heavy metals and inorganic nutrients. *C. vulgaris* and *Scenedesmus* sp. can be used to reclaim the water bodies polluted with heavy metals even if an excessive amounts.

Result of this study suggests that *Chlorella vulgaris* and *Scenedesmus* sp. have a remarkable ability on removal of excessive nutrients and heavy metals at laboratory conditions.

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