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Heavy Metal Removal (Copper and Zinc) in Secondary Effluent from Wastewater Treatment Plants by Microalgae

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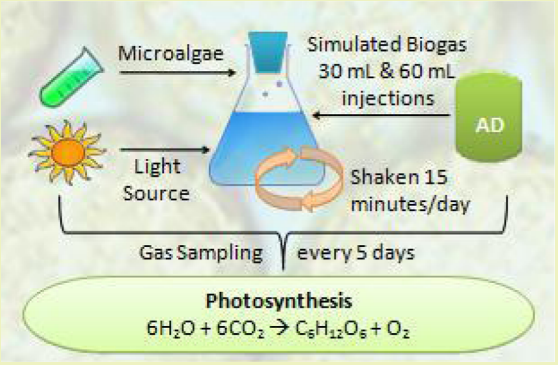
ABSTRACT: Microalgae is used for the removal of heavy metals from a wastewater treatment plant discharge. Laboratory-scale experiments are described that characterize the heavy metal uptake of copper and zinc by three microalgae strains: *Chlorella vulgaris*, *Spirulina maxima*, and a naturally growing algae sample found in the wastewater from a wastewater treatment plant (containing *Synechocystis* sp. (dominant) and *Chlorella* sp. (common) and a few cells of *Scenedesmus* sp.) Tests were conducted using untreated and autoclaved secondary effluent as a substrate. In the untreated secondary effluent trial, the microalgae removed up to 81.7% of the copper, reaching a lowest final concentration of 7.8 ppb after 10 days. Zinc was reduced by up to 94.1%, reaching 0.6 ppb after 10 days. The removal rates varied significantly with the microalgae strain. Higher heavy metal removal efficiencies were obtained in the autoclaved secondary effluent than the untreated secondary effluent, suggesting microorganisms already present in secondary effluent contribute negatively and compete with microalgae for nutrients, hindering microalgae growth and uptake of heavy metals. Inoculated samples showed decreased heavy metal concentrations within 6 h of initial inoculation, suggesting microalgae do not require long periods of time to achieve biosorption of heavy metals.



KEYWORDS: *Heavy metals, Microalgae, Chlorella vulgaris, Spirulina maxima, Wastewater treatment*

INTRODUCTION

Microalgae have unique characteristics that allow for them to potentially be utilized in broad and versatile ways in climate change technologies such as CO_2 sequestration for the production of biofuels. Compared to other photosynthetic plants, microalage are more productive carbon dioxide users and can fix larger amounts of carbon dioxide per unit area than terrestrial plants.[1] Further, as a part of their metabolic processes, microalgae uptake nutrients (such as nitrogen and phosphorus), which occur in high levels in secondary effluent from domestic wastewater treatment plants.[2] Additionally, they have the ability to adsorb heavy metals onto their cell surfaces through a process called "biosorption".[3] By removing both nutrients and heavy metals, microalgae have the potential to therefore provide significant tertiary treatment.

If tertiary treatment of wastewater and CO_2 sequestration is combined with utilizing the resulting biomass as feedstock to an anaerobic digester to create biogas, microalgae have the potential to serve many roles.[4−6] DeSchamphelaire and Verstraete designed a stand-alone closed-loop system with an algal growth unit to produce biomass as the feedstock to an anaerobic digester that produced biogas.[7] The effluent of the anaerobic digester was used to replace the recirculation liquid of the anode of the microbial fuel cell, and a suspension of living algae was recirculated over the cathode.[7] Hence, using solar energy and microalgae, a closed-loop system was created that produced both biogas and electricity. Alternatively, the biomass grown could be subsequently used as a biofuel (biodiesel or biohydrogen). The end use of microalgae after its removal of heavy metals can result in a more sustainable approach to wastewater treatment.

The present study focuses on the biosorption of heavy metals in wastewater that result from various industrial processes and domestic wastes. When discharged to receiving water bodies without removal, heavy metals may be harmful to both human and aquatic life as they are nondegradable and thus persistent.[8] Approximately 40 heavy metals have potential toxicity to humans, animals, plants, and microorganisms.[9] Therefore, it is important that these heavy metals are removed prior to discharge to surface waters.

Conventional methods of heavy metal removal such as ion exchange or lime precipitation are often ineffective or very expensive when used for the reduction of heavy metals at very low concentrations of 10−100 mg/L.[10,11] Other methods include chemical precipitation, solvent extraction, and adsorption that require high energy input, capital investment, and operational costs and may not substantially decrease heavy metal concentrations.[12] There has also been investigation into the use of electrochemical removal, photochemical degradation, and oxidation.[13−15] For the removal of many organic and

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Table 1. Various Heavy Metal Removal Achieved by Microalgae

I heavy metal	II algal strain and condition	III process	IV removal efficiency	V reference
selenium	combined algal−anaerobic bacteria; live algae	high rate ponds containing drainage water	94−100%	30
chromium	*Spirogyra condensate* and *Rchizoclonium hieroglyphicum*; dried algae	batch equilibrium in conical flasks; synthetic and tannery wastes	75% for low algae concentrations (<100 mg/L)	8
chromium	*Chlorella miniata*; dried algae; cultivated in domestic wastewater	250 mL conical flasks agitated at room temperature; synthetic solutions	at equilibrium, 75% and 100% removal for Cr(III) and Cr(VI), respectively	31
chromium and copper	*Sargassum* sp. (macroalgae) and *Chlorococcum* sp.); dried algae	batch sorption in 500 mL polyethylene bottles; synthetic solutions	*Sargassum* removes up to 87% Cu (1.0−30.0 mg/L Cu) , while *Chlorococcum* removed 43−75% Cu. *Sargassum* more efficient at higher Cr concentrations, while *Chlorococcum* removed 67% Cr at 2 mg/L solution	12
cadmium, copper, lead, and zinc	*Haslea ostrearia*, *Phaeodactylum tricornutum*, *Skeletonema costatum*, and *Tetraselmis suecica*; live algae	250 mL Erlenmeyer flasks at 14:10 light:dark cycle; 17 °C; salt water or enriched seawater	results show reduction in Cu and Cd	32
copper, zinc, cadmium, and mercury	*Cladophora fracta*; live algae	batch testing in 100 mL Erlenmeyer flasks; synthetic solutions	Cu^{2+}, Zn^{2+}, Cd^{2+}, and Hg^{2+} were 99%, 85%, 97%, and 98%, respectively	33
lead, cadmium, copper, and arsenic	Cyanophyta (*Oscillatoria princeps* 92%, *Oscillatoria subbrevis* 2%, and *Oscillatoria formosa* 1%) and Chlorophyta (*Spirogyra aequinoctialis* 3%, *Mougeta* sp. 1%, and others 1%); dried algae	batch testing in 250 mL Erlenmeyer flasks; synthetic solutions	metals were removed	34
cadmium, mercury, lead, arsenic, and cobalt	*Spirogyra hyaline*; dried algae	batch testing in 250 mL Erlenmeyer flasks; synthetic solutions	order of metal uptake for dried biomass was found to be Hg > Pb > Cd > As > Co	35
cadmium, nickel, lead, zinc, and copper	*Spirogyra neglecta*, *Pithophora oedogonia*, *Hydrodictyon reticulatum*, *Cladophora calliceima*, *Aulosira fertilizsima*; dried algae	batch testing in 100 mL Erlenmeyer flasks; synthetic solutions	*S. neglecta* and *P. oedogonia* can remove >75% of Cu^{2+} and Pb^{2+} from the multimetal solution	36
nickel	*Oedogonium hatei*; untreated and acid-treated dried algae	batch testing in 100 mL synthetic solution	maximum adsorption capacity of untreated and treated algal biomass was found to have greater or comparable values compared to other similar biosorbents	37

inorganic contaminants, however, adsorption is considered to be the preferred option as it is the most widely applicable.[16] As a result, significant research has been conducted on the topic of contaminant adsorption in wastewater. Several studies for example have examined the possibility of utilizing waste materials to adsorb both organic and inorganic contaminants.[17−28] Researchers are also currently investigating the use of carbon nanotubes and composites for adsorption of contaminants.[16] One such study tested the use of manganese dioxide supported on carbon nanotubes for lead removal from wastewater.[29]

As an alternative adsorption medium, heavy metal removal by microalgae has the potential to be very efficient and economical. Examples of applications reported in the technical literature are listed in Table 1 and demonstrate, as listed in column IV, that the removal efficiency of microalgae may be substantial.

While microalgae have the potential to be a versatile and economic option to treat secondary effluent and create a valuable byproduct, the suitability to serve these many purposes must be judged in part on the ability to remove heavy metals from secondary effluent. As seen in Table 1, few of the studies

related to algae and heavy metal removal have been completed using live algae and nonsynthetic domestic wastewater. Therefore, experiments described herein were designed to observe the influence of live microalgae on heavy metal removal, copper and zinc, specifically, on secondary effluent and the influence of symbiotic relationships of microalgae and bacteria together on heavy metal removal in secondary effluent.

MATERIALS AND METHODS

Microalgae. The microalgae strains used in this experiment are *Chlorella vulgaris* and *Spirulina maxima*, which are strains of green and blue-green microalgae, respectively.

The *Chlorella vulgaris* was purchased from the University of Waterloo Canadian Phycological Culture Centre (CPCC), formerly known as the University of Toronto Culture Collection (UTCC). The strain used was *C. vulgaris* CPCC 90 (formerly UTCC 90) collected by Dep. IAM as C-531. Isol. R. Pratt, U.S.A., pre1946. The *Spirulina maxima* was purchased from UTEX, The Culture Collection Center at the University of Austin, Texas. The strain used was UTEX LB 2342, which was collected from Natron Lake, Chad, in 1963.

Another microalgae was found in the wastewaters of a municipal wastewater treatment plant (WWTP) in Collingwood, Ontario, Canada, and was used as the third strain. Taxonomic analysis was performed on this third sample of microalgae by microscopic examination, and it was determined to be comprised of *Synechocystis* sp. (dominant) and *Chlorella* sp. (common). There was also *Scenedesmus* sp., but very few cells. This mixture will be referred to as "Mixture." The strains and their combinations have been renamed as follows: *Chlorella vulgaris* = CV, *Spirulina maxima* = SM, Mixture = MIX, *Chlorella vulgaris* and *Spirulina maxima* = CV/SM, *Chlorella vulgaris* and Mixture = CV/MIX, *Spirulina maxima* and Mixture = SM/MIX, and *Chlorella vulgaris*, *Spirulina maxima*, and Mixture = CV/SM/MIX.

Culture Medium. The synthetic culture medium used to culture stock solutions of the microalgae consisted of macronutrients (MN) and a trace elements (TE) solution. The MN solution used was based on the Zarrouk medium composed of the following composition in 1 L of deionized water: 18 g of $NaHCO_3$, 2.5 g of $NaNO_3$, 0.5 g of K_2HPO_4, 1 g of NaCl, 0.059 g of $CaCl_2 \bullet 2H_2O$, 0.88 g of $Na_2EDTA \cdot 2H_2O$, 0.2 g of $MgSO_4 \cdot 7H_2O$, and 0.01 g of $FeSO_4 \cdot 7H_2O$.[38] The composition of TE in 1 L of deionized water was 2.86 g of H_3BO_3, 0.21 g of $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 1.8 g of $MnCl_2 \cdot 4H_2O$, 0.08 g of $CuSO_4$, and 0.22 g of $ZnSO_4 \bullet 7H_2O$. Stock solutions of these two solutions were prepared and stored in the refrigerator to ensure availability of culture medium when needed. All chemicals used in this experiment were purchased from Cole Parmer.

Sample Preparation. The microalgae were grown in 250 mL Erlenmeyer flasks, each containing 150 mL of the culture medium after autoclaving. Culture medium was autoclaved at 121 °C for 15 min at 98−137 kPa. Stock solutions were created with 10% (v/v) inoculation from previous stock solutions. The flasks were kept in a chamber exposed to a light source with a 16:8 (L:D) cycle. Two sets of two bulbs provided 15 W each to the samples. Temperatures ranged from 26−28 °C throughout the duration of the experiment. Temperatures were dependent on the light source, as the temperature in the growth chamber was less than that of the ambient air in the laboratory.

Experimental Design. One of the two trials was performed using the secondary effluent from the City of Waterloo WWTP without amendment. The secondary effluent was collected after the secondary clarifier but prior to disinfection by chlorination. The average characteristics of the secondary effluent were as follows: total phosphorus, 0.148 mg/L; ammonia, 30.7 mg/L; copper, 56.6 mg/L; zinc, 31.6 mg/L; and pH 7.96.

In the first trial, secondary effluent was added to the autoclaved flasks, and inoculation was then added. This trial has been termed the "untreated" trial. In the second trial, secondary effluent was autoclaved in addition to the flask prior to adding inoculation, destroying any microorganisms in the effluent. This trial has been termed the "autoclaved" trial. This was done to observe changes without the influence of bacteria and microalgae naturally in the secondary effluent as there may exist symbiotic relationships between microalgae and bacteria.[39] Autoclaving the sample destroyed any of the naturally occurring microalgae, as well as any bacteria, in the secondary effluent.

Erlenmeyer flasks (250 mL) were filled with 150 mL of the secondary effluent and inoculated with 6 mL of microalgae grown previously in synthetic medium. Seven ratios were used in duplicate to represent all combinations of strains for a total of 14 flasks as listed in Table 2. Three sets of flasks were inoculated with single strains: CV, SM, and MIX. Four sets of flasks were inoculated with combined strains: CV/SM, CV/MIX, SM/MIX, and CV/SM/MIX. Secondary effluent without inoculation was used as the control. Averages were taken for each duplicate set of samples and were used in subsequent analysis.

Table 2. Volume of Inoculation for Combinations of Strains

	volume of inoculation (mL)		
combination	*Chlorella vulgaris* (CV)	*Spirulina maxima* (SM)	Mixture (MIX)
PE (control)			
CV	6		
SM		6	
MIX			6
CV/SM	3	3	
CV/MIX	3		3
SM/MIX		3	3
CV/SM/MIX	2	2	2

Heavy Metals Analysis. Measurements of Cu and Zn were performed using a Shimadzu AA-6300 atomic absorption spectrophotometer equipped with a GFA-EX7i graphite furnace atomizer and an ASC-6100 auto sampler. A normal graphite tube was used in the furnace. Background correction was accomplished by the deuterium lamp method (Shimadzu). The furnace temperature programs used were as recommended by the manufacturer (Shimadzu). The analytical conditions for each metal are as follows: wavelength—copper (324.8 nm), zinc 213.9 (nm); slit width—copper (0.7 nm), zinc (0.7 nm); and lamp current (mAmp)—copper (6 nm), zinc (10 nm).

Sampling was performed in a laminar flow fume hood to prevent contamination from ubiquitous microorganisms. Three mL samples were taken from the Erlenmeyer flasks every two days and centrifuged with a Beckman GS-6R centrifuge with a GH-3.7 horizontal rotor with a G force of 1425 for 15 min at 5 °C. The supernatant was used for analysis in the spectrophotometer, while the biomass remained in the centrifuge tube. The initial value for heavy metals used in calculations was taken after inoculation to account for any addition of heavy metals due to the synthetic medium or the microalgae itself. The heavy metal removal efficiency was calculated as per eq 1

$$\text{Removal efficiency (\%)} = \frac{\text{Initial concentration} - \text{Final concentration}}{\text{Initial concentration}} \times 100 \quad (1)$$

RESULTS AND DISCUSSION

The initial Cu and Zn concentrations as well as the date of sampling are listed in Table 3.

The variation in initial Zn concentration is a result of different conditions in the WWTP between the time periods as

Table 3. Initial Cu and Zn Concentrations

trial	date	initial Cu (ppb)	initial Zn (ppb)
untreated	July 8, 2010	56.8	12.8
autoclaved	June 2, 2010	56.4	50.3

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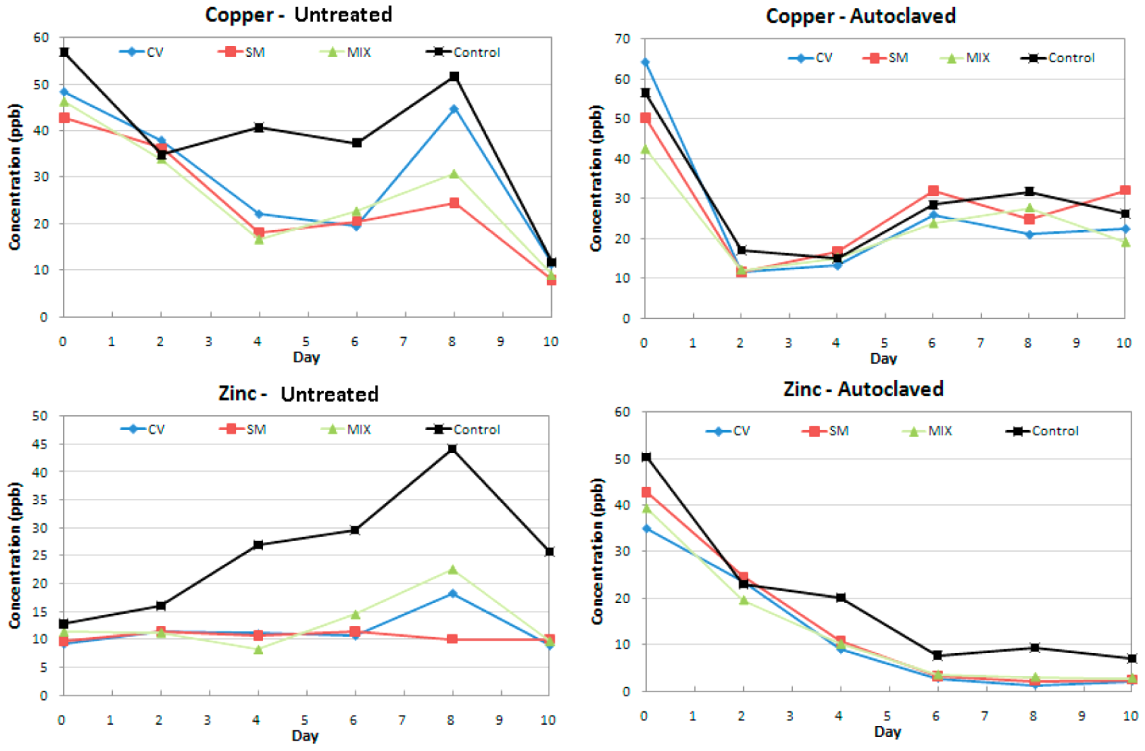

Figure 1. Zinc and copper removal in untreated and autoclaved secondary effluent for single strains.

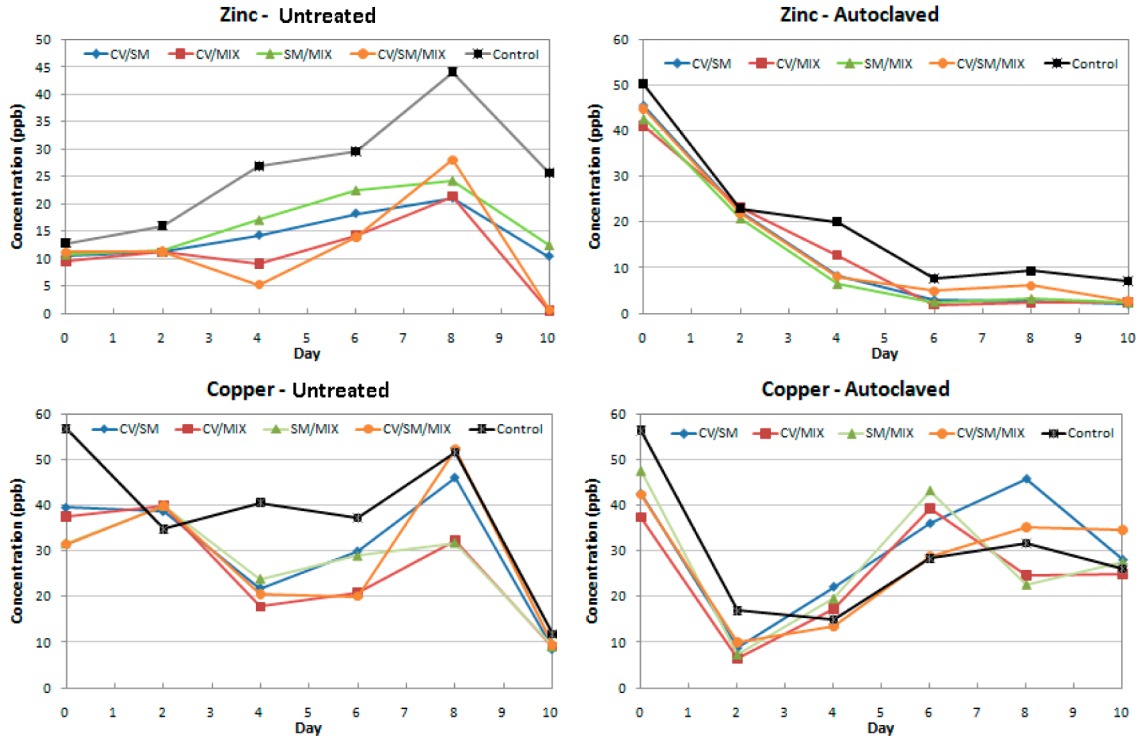

Figure 2. Zinc and copper removal in untreated and autoclaved secondary effluent for combined strains.

the trials were run consecutively. As a result of normal processes of a WWTP, the secondary effluent obtained at the beginning of each trial may differ in initial concentrations as apparent in Table 3. The secondary effluent contained some particles of soil and debris. In addition, it had an earthy odor resembling soil. It was observed that within a 48 h period after inoculation, the microalgae had already visibly begun to grow in their new medium in both the untreated and autoclaved trials. However, substantial visible growth in the autoclaved trial took longer, up to 72 h in comparison to just 24 h in the untreated trial. This was expected as the untreated trial would have contained an initial concentration of microalgae.

The controls were not inoculated with any algae strain, but visible growth appeared in the controls for the untreated trial.

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This growth is a result of naturally occurring microalgae, ubiquitous in the environment and within a WWTP. The visible growth in the controls took longer to appear than in the inoculated samples as expected due to a higher initial concentration. No visible growth was observed in the controls during the two week period for the autoclaved trial as expected.

The initial concentrations were recorded after inoculation to account for any addition of heavy metals due to the culture media from the stock solutions as both Cu and Zn are found in the PE solution. As a result, the initial concentrations varied throughout the samples on day 0 (Figures 1 and 2).

The controls in the autoclaved trial achieved heavy metal removal. It would be expected that because the biomass is dead that adsorption may not be possible, but this is not the case. Adsorption of heavy metals by dead microalgal biomass is not only possible but, as Ashour et al. suggest, it is beneficial.[40,41] Dead cells eliminate the possibility of metal toxicity limitations, eliminate the need for growth media, and increase the ease of repeated use of the biosorbent.[41] This is demonstrated by the studies outlined in Table 1 that used dead algae and were able to achieve significant heavy metal removal.

For Cu, the initial concentrations were similar, ranging from 31.5 to 56.8 ppb for the untreated trial and from 37.4 to 64.1 ppb for the autoclaved trial. However, Zn showed greater variation. The untreated trial had a range from 9.3 to 12.8 ppb, and the autoclaved trial had a much higher range from 35.0 to 50.3 ppb. A trend was still observed in the untreated trial results although the initial concentrations were lower.

The difference in initial concentrations between the inoculated samples and the controls are displayed in Table 4.

Table 4. Differences in Concentrations for Cu and Zn between Control and Inoculated Samples on Day 0 after Inoculation

	Cu		Zn	
microalgae	untreated (ppb)	autoclaved (ppb)	untreated (ppb)	autoclaved (ppb)
CV	8.5	−7.7	3.5	15.3
SM	14.1	6.2	3.1	7.5
MIX	10.6	14.1	1.4	10.9
CV/SM	17.2	13.8	2.3	4.9
CV/MIX	19.2	19.0	3.3	9.1
SM/MIX	25.0	8.9	2.0	7.5
CV/SM/MIX	25.3	14.0	1.6	5.4

With the exception of CV in the Cu autoclaved trial, all the initial concentrations of the inoculated samples were lower than the controls. The decrease in Cu concentration for CV may be a result of the culture media. The inoculums contain both a microalgae sample and the culture media, both of which contain Cu. In this particular case, the Cu concentration of one of the duplicate samples was very high, 76.0 ppb compared to 52.2 ppb. If considering only the lower concentration of 52.5 ppb, this value is also lower than the control of 56.4 ppb, which is consistent with the remaining data. The initial decreases in concentrations between inoculation and testing for Cu and Zn concentrations show that microalgae can uptake heavy metals in a short time period as there was a time lapse of at least 1 h but no more than 6 h.

Concentration differences between the control and inoculated samples were higher for the untreated trial than the autoclaved trial for both Cu and Zn as visibly indicated in Figures 1 and 2. In the untreated trial, a larger gap is observed between the controls and the samples compared to the autoclaved trial. This difference can be explained by the additional naturally occurring microalgae found in secondary effluent, which would not be found after autoclaving. As a result, the untreated trial has a higher initial concentration of microalgae to contribute to heavy metal removal.

Throughout the test, the control maintained higher concentrations for all strains in the untreated trial except for CV/SM/MIX on day 8 for Zn and on day 2 for all combination strains. In some trials, for example, the SM strain trial, the concentration differences are as high as 27.3 and 34.1 ppb for Cu and Zn on day 8, respectively. In comparison, in the autoclaved trial, SM produced only a concentration difference of 7.0 and 7.2 ppb for Cu and Zn on day 8, respectively.

Tables 5 and 6 show the highest percent removal for copper and zinc and the day found.

Table 5. Highest Percent Removal for Copper

	untreated		autoclaved	
microalgae	% removal	day	% removal	day
control	79.4	10	73.5	4
CV	76.5	10	81.9	2
SM	81.7	10	76.9	2
MIX	80.6	10	71.7	2
CV/SM	78.8	10	79.5	2
CV/MIX	76.2	10	82.8	2
SM/MIX	71.3	10	84.4	2
CV/SM/MIX	69.9	10	76.6	2

Table 6. Highest Percent Removal for Zinc

	untreated		autoclaved	
Microalgae	% removal	day	% removal	day
control	–	–	86.0	10
CV	2.9	10	96.3	8
SM	–	–	94.9	8
MIX	27.7	4	93.1	10
CV/SM	0.7	10	95.5	10
CV/MIX	94.1	10	95.4	6
SM/MIX	–	–	94.7	10
CV/SM/MIX	94.1	10	94.2	10

The performance of the autoclaved trial was better than the untreated trial in terms of removal efficiencies. Simeonova et al. suggest that dead cells uptake metal ions to an equal or greater extent than living cells, possibly explaining why the autoclaved trial would achieve similar or higher removal efficiencies in a shorter amount of time.[40]

Zn removal in the autoclaved trial was very high with removal efficiencies up to 96.3% and 94.9% for CV and SM, respectively. These efficiencies were close to 10% higher than that of the control at 86.0%. Between the single strains and the combined strains, there were not significant appreciable differences between the removal efficiencies. Cu removal in the autoclaved trial had high removal efficiencies as well, between 70−85% but not as high as Zn in the mid-90% range. Furthermore, these removal efficiencies were found on day 2 instead of day 10.

The decrease in Cu concentration was more rapid in the autoclaved trial than the untreated trial. The highest removal

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efficiencies obtained for all the inoculated samples, single and combined in the autoclaved trial, was on day 2. Table 7 shows the removal efficiencies for Cu on day 2 for both trials. CV/MIX, SM/MIX, and CV/SM/MIX did not achieve appreciable removal until day 4 and were not displayed.

Table 7. Day 2 Cu Removal Efficiencies for Untreated and Autoclaved Trials

strain	untreated	autoclaved
control	38.7	70.0
CV	21.5	81.9
SM	15.2	76.9
MIX	26.7%	71.7
CV/SM	2.1	79.5
CV/MIX	–	82.8
SM/MIX	–	84.4
CV/SM/MIX	–	76.6

In both trials, some mechanism caused desorption of Cu. Sandau et al. found the pH value to be the most decisive factor of heavy metal sorption by microalgae.[42] At low pH of 1−3, Cd removal by *Fucus vesiculosus* decreased. pH was not measured throughout this experiment to analyze change in pH for desorption of Cu. Zhou et al. successfully desorbed Cu from microalgae by passing a desorption solution of 10 mL of 0.10 EDTA through a glass column of *S. kjellmanianum* saturated with Cu.[43] Approximately 98% of Cu was recovered. EDTA is present in this experiment due to the addition of $Na_2EDTA{\cdot}2H_2O$ in the macronutrient solution of the culture medium. EDTA is added to the experiment when the inoculums are added. This could be a possible explanation for desorption of Cu after day 2.

Instead of an overall decrease in concentration in the untreated trial, as observed in the autoclaved trial, the control had an increase in concentration of Zn reaching a maximum of 44.1 ppb from an initial concentration of 12.8 ppb on day 8, an increase of 31.3 ppb. The inoculated samples followed this increase in concentration trend somewhat but did not increase as quickly or as severely. Using MIX and CV/MIX as an example, their concentrations reached 22.6 and 21.4 ppb, respectively, on day 8 from an initial concentration of 11.4 and 9.5 ppb, an increase of only 11.2 and 11.9 ppb, respectively. This spike was also observed for Cu on day 8, while most of the inoculated samples were able to remain below the control concentration. The exception was CV/SM/MIX that spiked to a concentration just 0.6 ppb higher than the control. Despite an increase in concentration, the inoculated samples were able to counteract the increase.

In addition to the upward trend, the highest Zn removal efficiencies were obtained by any combination that involved the mixture sample, MIX, CV/MIX, and CV/SM/MIX. The untreated removal efficiencies of the aforementioned strains were comparable to their autoclaved removal efficiencies, within a 2% range. This suggests that the mixture strain, consisting of *Synechocystis* sp. (dominant) and *Chlorella* sp. (common), combined with this secondary effluent, provides a good environment for microalgae growth and Zn uptake.

The Ontario Provincial Water Quality Objectives (PWQO) for Cu and Zn is 5 and 20 ppb, respectively. The lowest concentrations obtained by microalgae grown in secondary effluent are shown below in Tables 8 and 9.

Table 8. Lowest Cu Concentrations Obtained by Microalgae Grown in Secondary Effluent

	untreated (ppb)		autoclaved (ppb)	
microalgae	initial (day 0)	final	initial (day 0)	final
control	56.8	11.7	56.4	15.0
CV	48.3	11.4	64.1	11.6
SM	42.7	7.8	50.2	11.6
MIX	46.2	9.0	42.3	12.0
CV/SM	39.6	8.4	42.6	8.7
CV/MIX	37.6	9.0	37.4	6.4
SM/MIX	31.7	9.1	47.5	7.4
CV/SM/MIX	31.5	9.5	42.4	9.9

Table 9. Lowest Zn Concentrations Obtained by Microalgae Grown in Secondary Effluent

	untreated (ppb)		autoclaved (ppb)	
microalgae	initial (day 0)	final	initial (day 0)	final
control	12.8	–	50.3	7.0
CV	9.30	9.0	35.0	1.3
SM	9.67	–	42.7	2.2
MIX	11.4	8.2	39.4	2.7
CV/SM	10.5	10.4	45.4	2.0
CV/MIX	9.54	0.6	41.2	1.9
SM/MIX	10.8	–	42.7	2.3
CV/SM/MIX	11.2	0.7	44.9	2.6

No microalgae was able to achieve the PWQO for Cu of 5 ppb. Both the untreated and autoclaved trials achieved the PWQO of 20 ppb for Zn, but the autoclaved trial overall produced more consistent numbers well below the discharge limit. The range for the untreated trial was 0.6 to 16.0 ppb, whereas the autoclaved trial ranged from 1.3 to 7.0 ppb.

Trends in Cu uptake suggest that concentrations could continue decreasing for the untreated trial beyond day 10. However, this was not the case for the autoclaved trial, where the lowest Cu concentrations were achieved by day 2, and Cu concentrations increased beyond day 2. For Zn, the autoclaved trial reached lowest concentrations by day 6 and remained at these levels until day 10, suggesting any longer time period would not achieve lower concentrations. Because the untreated trial already had very low concentrations of Zn initially, it is difficult to draw conclusions about trends.

CONCLUSIONS AND RECOMMENDATIONS

The use of microalgae represents a feasible option to remove heavy metals in a WWTP. Overall, the *Mixture* and *Spirulina maxima* strains achieved highest removal efficiencies for Cu and Zn. In both the trials, untreated and autoclaved, all microalgae strains and combinations were able to demonstrate the potential for heavy metal uptake while being grown in secondary effluent from a WWTP. In the untreated trial, there were greater concentration differences between the inoculated samples and the control than that of the autoclaved trial as there is an initial concentration of microalgae. Conversely, the autoclaved trial reached higher removal efficiencies more quickly for Cu and did not display as large a concentration difference between the control and inoculated samples, suggesting a relationship between bacteria and microalgae exists.

Dead biomass is capable of adsorbing heavy metals with potentially higher performance. As autoclaving destroys the

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microorganisms, the microorganisms present in the secondary effluent are dead after autoclaving. The dead biomass can still uptake heavy metals, and as such, the autoclaved trial overall had a better performance in heavy metal removal compared to the untreated trial that consisted of living biomass. The combination of single strains, CV/SM, CV/MIX, SM/MIX, and CV/SM/MIX did not appear to significantly impact the efficiency of Cu removal between the untreated and autoclaved trials. However, the mixture strain performed better by itself or in combination with other strains to achieve removal efficiencies comparable to their Cu removal in the untreated trial of Zn. In general, the microalgae were able to remove between 69.9% and 81.7% of the Cu and between 0.7% and 94.1% of the Zn in the untreated trial depending on the strain. In the autoclaved trial, removals were higher from 71.7% to 84.4% and from 93.1% to 96.3% for Cu and Zn, respectively, depending on the strain.

Although the removal was lower in the untreated than the autoclaved trial, significant removal was achieved. With further study into the effect of initial microalgae vs metal concentration, optimizing process conditions, pretreatment, and controlling conditions that lead to desorption, final discharge limits may be achieved. Employing live algae in this application would represent an advantage over using dead biomass as regeneration is not required, nutrients can be removed, and a valuable feedstock for anaerobic digestion and biogas production or the health food market can be created to provide a more sustainable approach to wastewater treatment.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Li, Y.; Horsman, M.; Wu, N.; Lan, C. Q.; Dubois-Calero, N. Biofuels from microalgae. *Biotechnol. Prog.* **2008**, *24*, 815−820.

(2) Xin, L.; Hong-ying, H.; Jia, Y. Lipid accumulation and nutrient removal properties of a newly isolated freshwater microalga, *Scenedesmus* sp. LX1, growing in secondary effluent. *New Biotechnol.* **2010**, *27* (1), 59−63.

(3) Romera, E.; Gonzalez, F.; Ballester, A.; Blazquez, M. L.; Munoz, J. A. Comparative study of biosorption of heavy metals using different types of algae. *Bioresour. Technol.* **2007**, *98* (17), 3344−53.

(4) Rigoni-Stern, S.; Rismondo, R.; Szpyrkowicz, L.; Zilio-Grandi, F.; Vigato, P. A. Anaerobic digestion of nitrophilic algal biomass from the venice lagoon. *Biomass* **1990**, *23* (3), 179−99.

(5) Samson, R.; LeDuy, A. Biogas production from anaerobic digestion of *Spirulina maxima* algal biomass. *Biotechnol. Bioeng.* **1982**, *24* (8), 1919−24.

(6) Vergara-Fernandez, A.; Vargas, G.; Alarcon, N.; Velasco, A. Evaluation of marine algae as a source of biogas in a two-stage anaerobic reactor system. *Biomass Bioenergy* **2008**, *32* (4), 338−44.

(7) De Schamphelaire, L.; Verstraete, W. Revival of the biological sunlight-to-biogas energy conversion system. *Biotechnol. Bioeng.* **2009**, *103* (2), 296−304.

(8) Onyancha, D.; Mavura, W.; Ngila, J. C.; Ongoma, P.; Chacha, J. Studies of chromium removal from tannery wastewaters by algae biosorbents, *Spirogyra condensata* and *Rhizoclonium hieroglyphicum*. *J. Hazard. Mater.* **2008**, *158* (2−3), 605−14.

(9) Sandau, E.; Sandau, P.; Pulz, O. Heavy metal sorption by microalgae. *Acta. Biotechnol.* **1996a**, *16* (4), 227.

(10) Wilde, E. W.; Benemann, J. R. Bioremoval of heavy metals by the use of microalgae. *Biotechnol. Adv.* **1993**, *11* (4), 781−812.

(11) Mehta, S. K.; Gaur, J. P. Use of algae for removing heavy metal ions from wastewater: progress and prospects. *Crit. Rev. Biotechnol.* **2005**, *25* (3), 113−52.

(12) Jacinto, M. L. J. A. J.; David, C. P. C.; Perez, T. R.; De Jesus, B. R. Comparative efficiency of algal biofilters in the removal of chromium and copper from wastewater. *Ecol. Eng.* **2009**, *35* (5), 856−60.

(13) Karthikeyan, S.; Gupta, V.; Boopathy, R.; Titus, A.; Sekaran, G. A new approach for the degradation of high concentration of aromatic amine by heterocatalytic Fenton oxidation: Kinetic and spectroscopic studies. *J. Mol. Liq.* **2012**, *173*, 153−163.

(14) Gupta, V.; Jain, R.; Mittal, A.; Mathur, M.; Sikarwar, S. Photochemical degradation of the hazardous dye Safranin-T using TiO_2 catalyst. *J. Colloid Interface Sci.* **2007b**, *309*, 464−469.

(15) Gupta, V.; Jain, R.; Varshney, S. Electrochemical removal of the hazardous dye Reactofix Red 3 BFN from industrial effluents. *J. Colloid Interface Sci.* **2007c**, *312*, 292−296.

(16) Gupta, V.; Ali, I.; Saleh, T.; Nayak, A.; Agarwal, S. Chemical treatment technologies for waste-water recycling—An overview. *RSC Adv.* **2012**, *2*, 6380−6388.

(17) Mittal, A.; Kurup (Krishnan), L.; Gupta, V. Use of waste materials—Bottom ash and de-oiled soya, as potential adsorbents for the removal of Amaranth from aqueous solutions. *J. Hazard. Mater.* **2005**, *B117*, 171−178.

(18) Mittal, A.; Gupta, V.; Malviya, A.; Mittal, J. Process development for the batch and bulk removal and recovery of a hazardous, water-soluble azo dye (Metanil Yellow) by adsorption over waste materials (bottom ash and de-oiled soya). *J. Hazard. Mater.* **2008**, *151*, 821−832.

(19) Mittal, A.; Mittal, J.; Malviya, A.; Kaur, D.; Gupta, v. Adsorption of hazardous dye crystal violet from wastewater by waste materials. *J. Colloid Interface Sci.* **2010**, *343*, 463−473.

(20) Jain, A.; Gupta, V.; Bhatnagar, A.; Suhas.. A comparative study of adsorbents prepared from industrial wastes for removal of dyes. *Sep. Sci. Technol.* **2003**, *38* (2), 463−481.

(21) Jain, A.; Gupta, V.; Jain, S.; Suhas. Removal of chlorophenols using industrial wastes. *Environ. Sci. Technol.* **2004**, *38*, 1995−1200.

(22) Gupta, V.; Sharma, S. Removal of zinc from aqueous solutions using bagasse fly ash—A low cost adsorbent. *Ind. Eng. Chem.* **2003**, *42*, 6619−6624.

(23) Gupta, V.; Mittal, A.; Krishnan, L.; Mittal, J. Adsorption treatment and recovery of the hazardous dye, Brilliant Blue FCF, over bottom ash and de-oiled soya. *J. Colloid Interface Sci.* **2006a**, *293*, 16−26.

(24) Gupta, V.; Mittal, A.; Kurup, L.; Mittal, J. Adsorption of a hazardous dye, erythrosine, over hen feathers. *J. Colloid Interface Sci.* **2006b**, *304*, 52−57.

(25) Gupta, V.; Ali, I.; Aaini, V. Defluoridation of wastewaters using waste carbon slurry. *Water Res.* **2007a**, *41*, 3307−3316.

(26) Gupta, V.; Jain, R.; Varshney, S. Removal of Reactofix golden yellow 3 RFN from aqueous solution using wheat husk—An agricultural waste. *J. Hazard Mater.* **2007d**, *142*, 443−448.

(27) Gupta, V.; Gupta, B.; Rastogi, A.; Agarwal, S.; Nayak, A. A comparative investigation on adsorption performances of mesoporous activated carbon prepared from waste rubber tire and activated carbon for a hazardous azo dye—Acid Blue 113. *J. Hazard Mater.* **2011**, *186*, 891−901.

(28) Gupta, V.; Mittal, A.; Malviya, A.; Mittal, J. Adsorption of carmoisine A from wastewater using waste materials—Bottom ash and deoiled soya. *J. Colloid Interface Sci.* **2009**, *335*, 24−33.
(29) Saleh, T.; Gupta, V. Column with CNT/magnesium oxide composite for lead(II) removal from water. *Environ. Sci. Pollut. Res.* **2012**, *19*, 1224−1228.
(30) Gerhardt, M. B.; Green, F. B.; Newman, R. D.; Lundquist, T. J.; Tresan, R. B.; Oswald, W. J. Removal of selenium using a novel algal-bacterial process. *Res J. Water Pollut. Control Fed.* **1991**, *63* (5), 799−805.
(31) Han, X.; Yuk, S. W.; Ming, H. W.; Tam, N. F. Y. Feasibility of using microalgal biomass cultured in domestic wastewater for the removal of chromium pollutants. *Water Environ. Res.* **2008**, *80* (7), 647−53.
(32) Gagneux-Moreaux, S.; Cosson, R. P.; Bustamante, P.; Moreau, C. Growth and metal uptake of microalgae produced using salt groundwaters from the bay of bourgneuf. *Aquat. Living Resour.* **2006**, *19* (3), 247−55.
(33) Ji, L.; Xie, S.; Feng, J.; Li, Y.; Chen, L. Heavy metal uptake capacities by the common freshwater green alga *Cladophora fracta*. *J. Applied Phycol.* **2012**, *24*, 979−983.
(34) Sulaymon, A.; Mohammed, A.; Al-Musawi, T. Competitive biosorption of lead, cadmium, copper, and arsenic ions using algae. *Environ Sci. Res.* **2013**, *20*, 3011−3023.
(35) Kumar, J. I. N.; Oommen, C. Removal of heavy metal by biosorption using freshwater alga *Spirogyra hyalina*. *J. Environ. Biol.* **2012**, *33*, 27−31.
(36) Singh, A.; Mehta, S.; Gaur, J. Removal of heavy metals from aqueous solution by common freshwater filamentous algae. *World J. Microbiol. Biotechnol.* **2007**, *23*, 1115−1120.
(37) Gupta, V.; Rastogi, A.; Nayak, A. Biosorption of nickel onto treated alga (*Oedogonium hatei*): Application of isotherm and kinetic models. *J. Colloid Interface Sci.* **2010**, *342*, 533−539.
(38) Feng, D.; Wu, Z.; Wang, D. Effects of N source and nitrification pretreatment on growth of *Arthrospira platensis* in human urine. *J. Zhejiang Univ., Sci., A* **2007**, *8* (11), 1846−52.
(39) Park, Y.; Je, K.; Lee, K.; Jung, S.; Choi, T. Growth promotion of *Chlorella ellipsoidea* by co-inoculation with *Brevundimonas* sp. isolated from the microalga. *Hydrobiologia* **2008**, *598* (1), 219−28.
(40) Simeonova, A.; Godjevargova, T.; Ivanova, D. Biosorption of heavy metals by dead *Streptomyces fradiae*. *Environ. Eng. Sci.* **2008**, *25* (5), 627−33.
(41) Ashour, I.; Abu Al-Rub, F. A.; Sheikha, D.; Volesky, B. Biosorption of naphthalene from refinery simulated waste-water on blank alginate beads and immobilized dead algal cells. *Sep. Sci. Technol.* **2008**, *43* (8), 2208−24.
(42) Sandau, E.; Sandau, P.; Pulz, O.; Zimmermann, M. Heavy metal sorption by marine algae and algal by-products. *Acta. Biotechnol.* **1996b**, *16* (2−3), 103.
(43) Zhou, J. L.; Huang, P. L.; Lin, R. G. Sorption and desorption of cu and cd by macroalgae and microalgae. *Environ. Pollut.* **1998**, *101* (1), 67−75.