EXTRACTION OF CHLOROPHYLL A, B AND CAROTENOIDS FROM *NANNOCHLOROPSIS OCULATA* AFTER HEAVY METAL ADSORPTION

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Abstract
The main objective of the present study was to determine the most suitable solvent for photosynthetic pigments extraction from the *Nannochloropsis oculata* biomass after adsorption of Cu, Zn and Cd from mono and multicomponent solutions. The results revealed that the highest percentage removal of heavy metals from mono solutions was achieved for Zn 20 mg/L (96.2 %), followed by Cu 20 mg/L (92.6 %) and Zn 50 mg/L (92.1 %), respectively. Organic solvents, namely ethanol, methanol and acetone were tested for chlorophyll a, b and carotenoids extractions. It was observed that higher contents of *chlorophyll a* were obtained after extraction with methanol from algal biomass after Cd and Zn adsorption from single component solutions. After a comparative examination, the highest content of *chlorophyll b* was obtained after the extraction with acetone.

Introduction
Photosynthetic pigments, like *chlorophyll a, b* and carotenoids, are valuable bioactive compounds that can be extracted from microalgal biomass. These components play a vital role in sustaining life in both plants and animals and have beneficial effects on human health. Due to their usability in various fields, such as food, pharmaceutical or cosmetic industry, many studies have been carried out to enhance chlorophyll extraction and fractionation from microalgae [1]. In the extraction process the following parameters are important: type of solvent used, pressure, temperature and contact time. Each solvent has certain characteristics, more or less compatible, for an efficient extraction of photosynthetic pigments, taking into account the safety rules [2]. Acetone proved to be a poor extractant for chlorophyll from certain vascular plants and algae [3]. For algae and recalcitrant vascular plants, the most efficient extractant for chlorophylls was methanol [4], less volatile and flammable than acetone, but it is toxic if inhaled or absorbed through the skin [2]. For the chlorophyll analysis, ethanol was identified to be the most appropriate solvent due to its lower toxicity [2,5].
The aim of this study was to determine the contents of photosynthetic pigments (*chlorophyll a, b* and carotenoids) from *Nannochloropsis oculata* biomass after heavy metals adsorption using ethanol, methanol and acetone as solvents in the extraction process.

Experimental
Materials
The *Nannochloropsis oculata* biomass (powder) was purchased from Astaxa GmbH (Germany Milz Gerbergasse). The chemicals, such as nitric acid, hydrochloric acid, methanol, ethanol and acetone were of analytical grade and were purchased from Merck (Germany).

Batch adsorption experiments
The experiments were carried out in 250 mL Erlenmeyer flasks using 5 g of *Nannochloropsis oculata* biomass (powder) mixed with 100 mL synthetic heavy metals (Cu, Zn, Cd) solution at

various concentrations (20 - 300 mg/L) for 300 min and stirred at 75 rpm at room temperature. After adsorption, the biomass was separated from the liquid phase by filtration through Whatman filter paper with a diameter of 125 mm and dried in a universal oven (UFE 400, Memmert, Germany) for 6 hours at 60 ºC. 20 mL of liquid phase were measured and carefully transferred to a clean and conditioned beaker. 7 mL of 65 % HNO_3 and 21 mL of 37 % HCl were added. Samples were left overnight at room temperature for pre-digestion. After digestion at controlled temperature, the samples were filtered and set for metals determinations.

The heavy metals content (Cu, Zn, Cd) was determined by inductively coupled plasma - optical emission spectrometer (OPTIMA 5300 DV, Perkin Elmer, Norwalk, USA), assembled with an CETAC U-6000 AT ultrasonic nebulizer.

The heavy metal amount in the adsorbent phase and the removal efficiency were calculated using the equations mentioned by Mudyawabikwa et al. [6].

Pigments extraction

0.5 g of *Nannochloropsis oculata* biomass after heavy metals adsorption experiments were mixed with 10 mL of different solvents for pigment extraction. The samples were sonicated for 15 min. After extraction, the samples were centrifuged at 4000 rpm for 8 minutes.

The absorbance was measured using a Lambda 25 Perkin-Elmer UV/VIS spectrophotometer. The obtained values were used to calculate the chlorophyll a (µg/mL), chlorophyll b (µg/mL) and carotenoid (µg/mL) content applying the equations mentioned by Lichtenthaler and Buschmann [7].

Results and discussion

Effect of heavy metals removal by *Nannochloropsis oculata* biomass

Cu, Zn and Cd removal by *Nannochloropsis oculata* biomass starting from different initial concentrations and mixtures are presented in Figure 1.

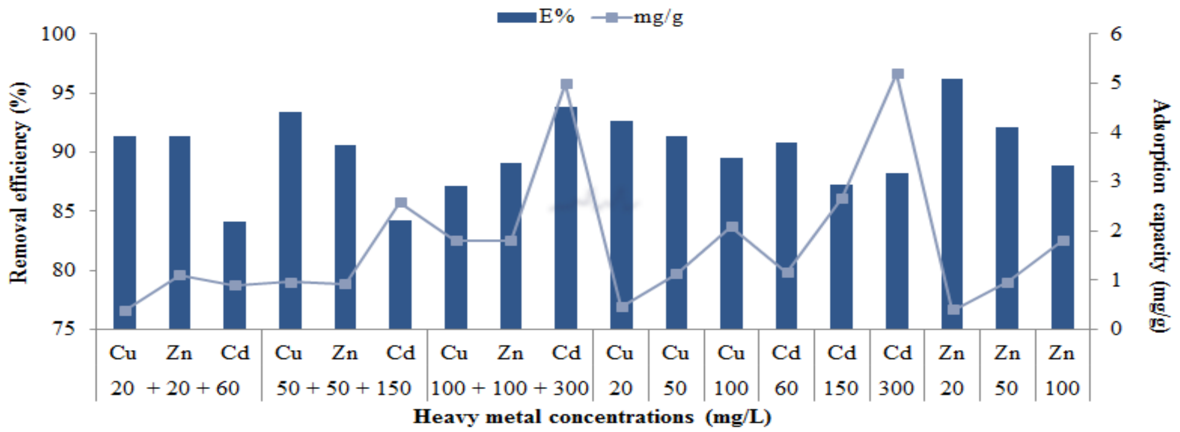


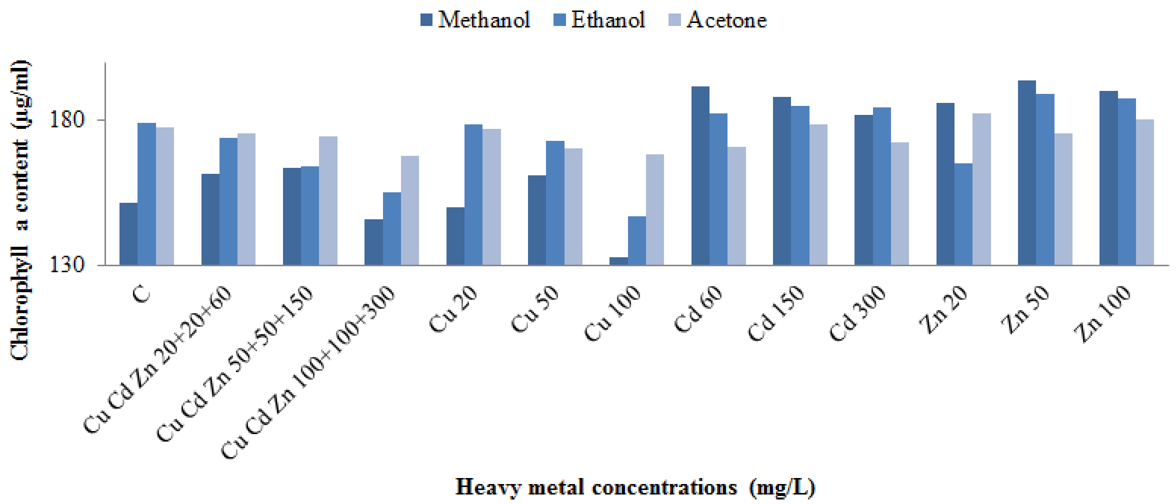
Figure 1. Influence of the initial heavy metal concentrations over the removal efficiency and amount in the adsorbent phase

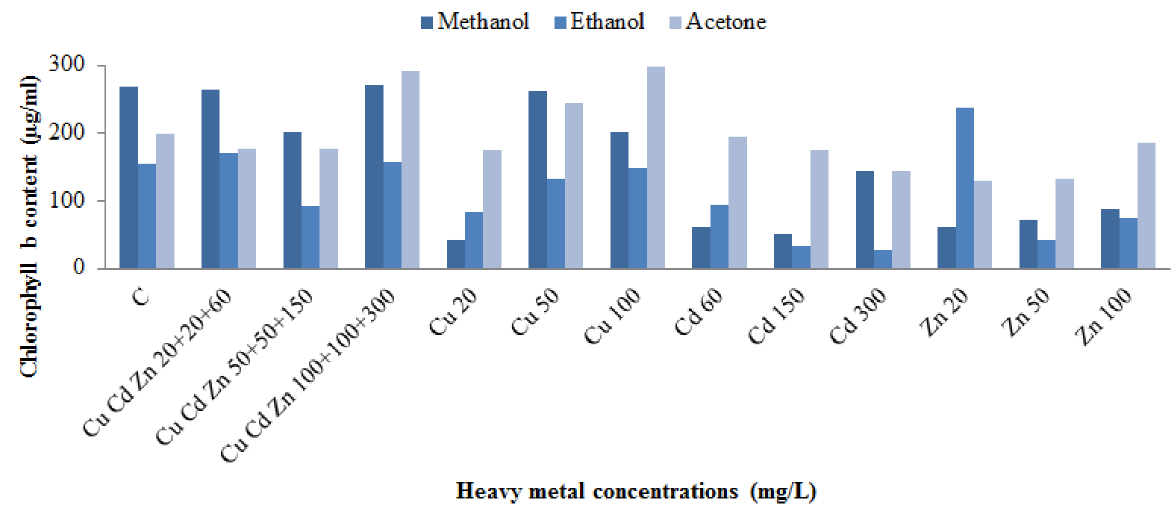
The biomass adsorption capacity improved with an increase of the initial concentrations of heavy metals. The results indicate that *Nannochloropsis oculata* biomass has a great capacity to remove high amounts of Cd from aqueous solutions, even in the presence of other metals, such as Cu and Zn. The removal efficiencies ranged from 84 % to 96 % for Cu, Zn and Cd, respectively. The highest removal efficiency and adsorption capacity was observed for Zn 20 mg/L (96 %) (mono component solutions) and Cd 300 mg/L (5.2 mg/g) (multicomponent solutions). A gradually decrease in removal efficiencies was observed in the multicomponent solutions, compared with mono component solutions.

Effect of solvents for extraction of photosynthetic pigments

The effect of methanol, ethanol and acetone for photosynthetic pigments extraction from *Nannochloropsis oculata* biomass after heavy metals adsorption is presented in Figure 2.

Spectrophotometric analysis of photosynthetic pigments from *Nannochloropsis oculata* biomass after heavy metals adsorption showed changes in their content compared to the control sample.





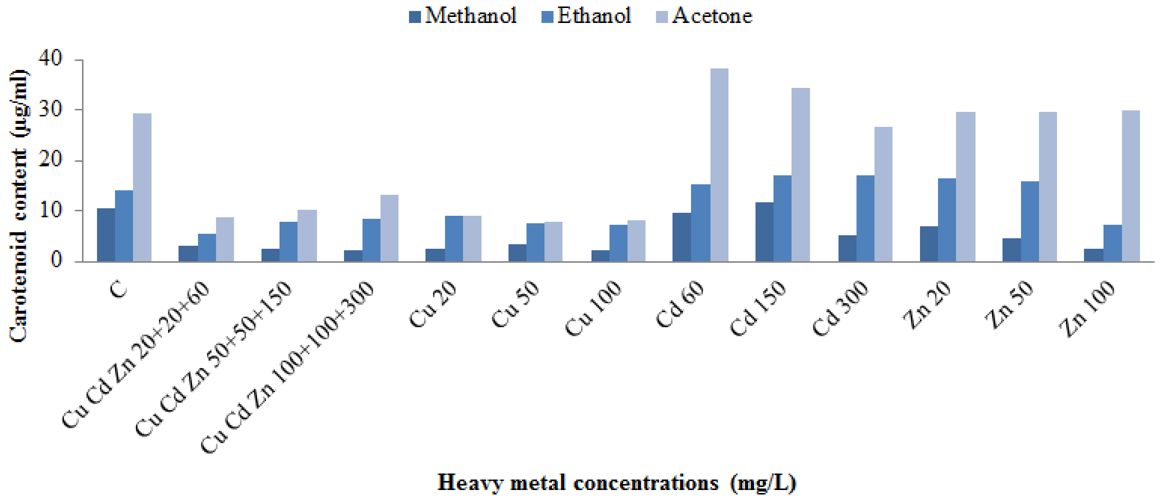


Figure 2. Chlorophyll a, b and carotenoid content after extraction with different organic solvents

The highest contents of *chlorophyll a* were achieved using methanol (193.7 µg/mL, 50 mg/L Zn) as an extraction solvent, followed by extraction with ethanol (189.0 µg/mL, 50 mg/L Zn) and acetone (182.2 µg/ml, 20 mg/L Zn) (Figure 2). The chlorophyll a content slowly increased with respect to the control sample after the extraction from the algal biomass used for Cd and Zn removal. Furthermore, lower *chlorophyll a* contents were extracted from *Nannochloropsis oculata* biomass after heavy metals removal from multicomponent solutions and Cu removal from monocomponent solutions.
The highest contents of *chlorophyll b* extraction (298.5 µg/ml, 100 mg/L Cu), were extracted with acetone, followed by methanol (271.0 µg/ml, Cu Cd Zn 100+100+300 mg/L) and ethanol (236.7 µg/ml, Zn 20 mg/L).
The highest *carotenoid* contents were achieved after extraction with acetone in all the samples used for heavy metal removal (Figure 2).

Conclusion

In the present study the influence of heavy metals on extraction of photosynthetic pigments in *Nannochloropsis oculata* biomass was investigated. The *Nannochloropsis oculata* biomass show a remarkable ability to remove higher concentrations of heavy metals from single (Zn 20 mg/L, 96.2 %) and multicomponent solutions (Cu Cd Zn 50+50+150 mg/L, 93.4% 90.6 % 84.2%). The obtained results showed that *Nannochloropsis oculata* biomass had a higher Cd removal capacity, than of Cu and Zn. The content of photosynthetic pigments varied by changing the concentrations of heavy metals. The highest measured chlorophyll a content was obtained using methanol. Acetone gave higher concentrations for carotenoide than methanol and ethanol in all the studied samples. Further studies will be conducted in order to determine the effect of metals on photosynthetic pigments from microalgae grown in a culture medium with an increased content of heavy metals.

Acknowledgements

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