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Original Scientific Paper

Bioaccumulation of Heavy Metals by Green Algae *Cladophora glomerata* in a Refinery Sewage Lagoon

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Green algae, especially *Cladophora* species, are generally considered as the best bioindicator of aquatic bodies contamination by nutrients as well as by heavy metals. Therefore, the object of this study was to investigate to which extent toxic metals (Ni, V, Cd, Pb, Cr) are present in algal biomass and to establish the bioaccumulation coefficients in correlated algae / water environments in a sewage lagoon, which represents the tertiary stage of a refinery waste water treatment plant (Slovnaft, Bratislava). Due to the orographic depression between the Eastern Alps and Western Carpathians and the characteristic atmospheric circulation with predominance of NW wind direction, the deposition of heavy metal aerosols from closed industrial activities is expected to contribute to bioaccumulation capacities of algae for these elements. The water of the refinery sewage lagoon has been monitored for BOD, COD, petroleum hydrocarbons, pH, BTX, halogenated hydrocarbons, some polyaromates, phosphates, ammonium, sulfates and suspended matter. From the viewpoint of heavy metal contents in agricultural composts, a management scenario for algal biomass reutilization, *i.e.* recycling nutrients and proteins back into the biosphere, *via* compost processing, is proposed.

Key words: green algae, *Cladophora glomerata*, heavy metals bioaccumulation.

INTRODUCTION

Chlorophyta, commonly known as green algae, are responsible for most of the primary productivity in some water bodies. They subsist on inorganic

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nutrients and produce organic matter from carbon dioxide by photosynthesis. In the absence of photosynthesis, the metabolic process consumes oxygen, causing oxygen depletion in the aquatic system.[1]

The role of algae in waste water treatment and their affinity for heavy metal cations, based on high negative surface charge, has been recognized for several decades.[2] Mono-species cultures of green algae contain protein (over 50% of dryweight), nutrients (nitrogen, phosphorus) and may contain various bioaccumulated toxic elements. This is an advantage from the viewpoint of tertiary sewage treatment but a disadvantage if the intent is to use waste-grown algae for fish or livestock feeding or composting.

The current anthropogenic metal emissions have resulted in soil and water toxic elements concentrations up to several orders of magnitude higher than estimated natural concentrations.

The most important source of atmospheric lead is the combustion of fuel containing tetraalkyllead antiknock agents. Cadmium and nickel enter the environment *via* three main routes: refining and use of cadmium, copper and nickel smelting and fuel combustion. Consequently, toxic elements concentrations in many living organisms, including vertebrates, may be approaching thresholds of toxicity for adverse effects of toxic metals.

Such thresholds are difficult to define as they vary with the chemical and physical form of metals, exposure regime and other elements present, and they also vary both within and between species.[3] Atmospheric deposition of heavy metal aerosols in the study area may originate from closed industrial activities such as waste incineration, heating plants and crude oil processing. Data on specific metals in Russian oil ash have been published as follows: vanadium 0.01–30 g/kg; nickel 0.01–20 g/kg.[4,5] The exposure of algae to atmospheric pollutants at an industrial site may be indicated by the prevailing wind direction as well as by the pollutant residues in the water body.

Cladophora glomerata is generally considered as the most proper bioindicator of heavy metals in aquatic bodies.[6,7,8] Therefore, the main objective of this study was to investigate to which extent toxic metals (Ni, V, Cd, Pb, Cr) are present in the algae-bioindicator *Cladophora glomerata* in the lagoon of a refinery waste water treatment plant (Slovnaft, Bratislava). The water quality of the refinery sewage lagoon has been monitored, besides the selected metals, for some other parameters, *e.g.* BOD, COD, petroleum hydrocarbons, pH, BTX, polyaromatic hydrocarbons, ammonium, phosphates, sulfates and suspended matter.

The attained results have been evaluated from the viewpoint of environmental contamination and compared to the highest tolerated toxic metal contents for agricultural food, compost and water quality. Bioaccumulation coefficients in correlated algae / waste water environments have been calculated to qualify the toxic elements accumulation efficiency of *Cladophora* species in approximately 1 month intervals.

EXPERIMENTAL

Study Area and Sampling Strategy

The sewage lagoon is situated on the left bank of the river Danube, at the northern border of the local wheat land. The mean annual temperature is 9.8 °C, the warmest month is July (mean temperature 21 °C) and the coldest month January (mean temperature –3 °C). The long-term average precipitation in the area is 550 mm. The continental climate with a mild zone typical of this area is determined by the wind conditions of the Danube Lowland and the Low Carpathian Mountains; the orographic situation with its characteristic air circulation results in the prevailing NW wind direction of mean velocity 5.2 m/s.

The sewage lagoon is a concrete basin of a total volume of 98 580 cubic meters, 186 meters long and 106 meters wide, 5 meters deep, divided in 2 sections, where the refinery waste water is discharged after mechanical, chemical and biological treatment. Six surface aeration devices of MX-302 type permanently provide aerobic conditions in the lagoon. The residence time of treated water in the sewage lagoon is about 10 days and the oxygen content in the water, depending on temperature, ranges from 3.5 to 10 mg/L. The water temperature during the winter remains within 10 °C and during the summer within 30–32 °C. Water transparency reaches 10 to 50 cm.

During the summer, the green algae from the lagoon were scraped once per month and during the winter at two-three months intervals. Samples of algae were collected from a variety of sites in the lagoon watershed, mostly from surface swimming mattresses, scraped from the wall or thrown out on the lagoon bank. Sampling of chlorophyta (algal biomass) was done manually in Petri dishes and transported to the laboratory in a 25 L-cooling box (Verano, Spain). Samples were dried for about 24 hours at 105 °C in the laboratory dryer (Premed, KBG, Poland) and then homogenized into a fine, less than 0.060 mm grain-sized fraction in an agate mill (Fritsch 200, Germany).

Acid decomposition of samples was performed in a mixture of 10 mL 65% HNO_3 (suprapure) and 10 mL deionized H_2O in closed PTFE digestion vessels for 50 minutes, using a microwave oven (CEM Corporation, Model MSD-2000, USA). The following program was selected for digestion: P = 70%, 5-step regime timed at 10 minutes intervals, and elevated pressure from 20 to 150 psi, tap 5 and fan 100. After the procedure, the solutions were transferred into 100 mL calibrated flasks and filled up to the mark with D.I. water.

Analytical Procedures

The toxic metal concentrations in all samples were analyzed using an ICP 2070 Spectrometer (Baird, U.S.A.) and AAS using a Varian SpectrAA10 Apparatus with Graphite Tube Atomizer GTA-95 (Victoria, Australia). The analytical accuracy of measurements was checked by means of certified reference materials CRM 1643c (National Institute of Standards, U.S.A.) for trace elements in water and CRM P-ACHK (Institute of Radioecology and Applied Nuclear Techniques, Slovak Republic) for green algae. Table I presents the operating conditions of heavy metal analyses in algal biomass using both AAS-ETA and ICP-AES methods.

Physico-chemical analyses of waste water (Table II), *i.e.* phosphates, ammonium, sulfates, pH, COD (dichromate method), BOD, phenol, suspended matter were done according to the Unified methods for water analysis, the total organic carbon concentration (TOC) was determined on a TOC Analyser (Model DC-190, U.S.A. Dohrmann) using the Shuttle Module with the sample heated to 800 °C and detected by IR spectrometry.[9]

Petroleum hydrocarbons, as extracts of nonpolar organic compounds in Freon (1,1,2-trichlorotrifluoroethane) were analyzed by IR spectrometry according to DIN 38 409/18, using the Specord 80 Apparatus, Zeiss-Germany, in the absorbance range from 3100 up to 2800 cm^{-1}. However, since Freon is an ecologically unacceptable chemical, as an extractant of nonpolar organic compounds, paraffin solvents may be used as an alternative.

The content of specific hydrocarbons (including halogenated hydrocarbons), aromates – benzene, toluene, xylene (BTX) as well as some polyaromates, *i.e.* α-benzopyrene and fluoranthene, was determined by means of GC/MS (Varian 3400, U.S.A) with an ECD detector and capillary column DB 5 (30 m/ 0.25 mm). Stripping gas was helium and the trap consisted of a Tenax-silica gel-active coke mixture. Extrac-

TABLE I

Operating conditions for trace elements analyses in algal biomass using AAS-ETA and ICP-AES methods[a]

	Cd	Pb	Ni[b]	V	Cr[b]
Wavelength / nm	228.8	283.3	231.6	318.5	267.7
Slit / nm	0.5	0.5	0.15	0.2	0.15
Background corr.	D_2	D_2	231.6	–	267.7
Drying temp. / °C (time / s)	120 (60)	120 (45)	–	110 (55)	–
Charring temp. / °C (time / s)	500 (10)	700 (25)	–	1100 (25)	–
Atomizing temp. / °C (time / s)	1900 (5)	2300 (5)	–	2600 (5)	–
Cleaning temp. / °C (time / s)	2300 (2)	2300 (2)	–	2700 (2)	–

[a] Peak height mode; Internal gas (Ar) flow 300 mL/min; Matrix modifier: $NH_4H_2PO_4$ – for Cd, Pb; $Mg(NO_3)_2 \cdot 6\ H_2O$ – for V; Batching by volume 20 μL sample and 10 μL matrix modifier.

[b] Elements determined by ICP-AES with adequate analytical accuracy checked by CRM.

TABLE II

Physico-chemical characteristics of lagoon waste water

Index	Value / mg L^{-1}	Index	Value / μg L^{-1}
BOD	4.9	Cr	8.1
COD	32.1	V	175.9
NH_4^+	1.5	Ni	32.1
PO_4^{3+}	1.0	Pb	22.5
suspended matter	4.1	Cd	0.5
TOC	14.7	BTX	0.7
phenol	0.03	α-benzopyrene	< 0.1
SO_4^{2-}	215.4	fluoranthene	< 0.1
petroleum hydrocarbons (by IR)	0.02	chlorinated hydrocarbons	1.1
pH	8.2		

[a] Sampling done monthly; the values represent the mean contents of mixed, every 2 hours sampled lagoon water on the sampling day.

tion of polyaromates was accomplished at laboratory temperature in benzene, of halogenated hydrocarbons in n-hexane on Tekator 1048 Apparatus (Sweden). Quantitative determination of hydrocarbons was provided by external calibration of assemblies with standard solutions (U.S. Polyscience and Swiss Supelco).

RESULTS AND DISCUSSION

Physico-chemical characteristics of the refinery sewage lagoon water and of the green algae species *Cladophora* found in it have been studied to verify whether the algal biomass matrix represents a typical bioindicator of polluted water bodies.

The presence of green algae – species *Cladophora glomerata* in the lagoon has been verified by SEM (Electron Probe Microanalyser) Jeol-JXA 840A, Japan.

Currently, the refinery sewage facility operates with a third of its capacity, *i.e.* at a loading rate of 300 L/s utilizing the sewage lagoon for tertiary waste water treatment. In the past, mostly due to technological problems and a higher loading rate, there were a few water quality oversteps, especially in BOD indexes, sulfates and petroleum compounds, which have been solved by equalizing the quality of the waste water treated in the lagoon.

Today, the final water quality in the tertiary stage of waste water treatment does not usually exceed the values of the surface water class, as verified by the analyses (Table II).

Cladophora glomerata abundantly growing on the surface of the lagoon primarily accumulates residual nutrients and heavy metals from both the aquatic and atmospheric environments. According to Table II, no significant nutrient overloading has been found in the lagoon water. Therefore, the next goal of the study was to investigate the heavy metal occurrence in both the water and algae environments. The phenomenon of atmospheric impact of heavy metal aerosols on the food chain and substance transfer at this highly industrial site was the subject of some other papers.[10,11]

Table III presents the computed statistical data on the measured element concentrations. Statistical data processing was performed separately with the STATGRAPHICS 7.0 software package. The Box-and-Whisker

TABLE III

Statistical data on toxic elements in sampled green algae. Concentrations in mg/kg

Variable	V	Ni	Pb	Cd	Cr
No. of samples	9	9	9	9	9
Arithmetic mean	37.6	15.7	7.9	0.1	1.7
Median	38.4	15.8	8.0	0.1	1.5
Mode	38.4	15.8	8.0	0.1	1.5
Standard error	6.0	2.5	5.6	0.05	0.6
Minimum	27.5	10.5	1.4	0.05	1.2
Maximum	43.4	19.3	18.1	0.2	3.0
Range	15.8	8.8	16.7	0.2	1.8
Lower quartile	32.6	15.3	4.3	0.1	1.4
Upper quartile	42.5	16.4	11.8	0.1	1.7
Interquartile range	9.8	1.1	7.4	0.02	0.4
Coefficient of variation	16.1	15.9	70.2	48.2	35.2
CRM P-ACHK [a] certified value	0.40[b]	1.23±0.10[b]	1.23±0.07	0.045±0.003	2.40±0.21
CRM P-ACHK [a] determined value	0.41	1.54	1.57	0.04	2.69

[a] CRM P-ACHK: certified reference material for essential and toxic elements in green algae, Institute of Radioecology and Applied Nuclear Techniques, Košice, Slovak Republic.

[b] Informative values.

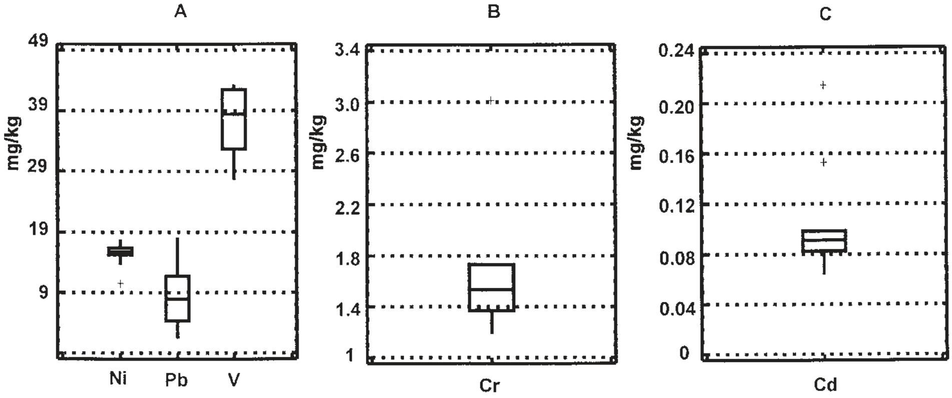

Figure 1. Multiple Box-and-Whisker diagram for the above elements determined in *Cladophora* algae.

plots for Ni, Pb, V, Cr and Cd (Figure 1) demonstrate the Exploratory data analysis for individual elements in which the same statistical software was used. The central box covers the middle, 50% of data values between the lower and upper quartiles. The crossed lines in each box are medians. The whiskers extend only to those points which are within 1.5 times of the box length (interquartile range). Outliers on the diagrams are indicated as single points outside the range of whiskers. It is supposed that several factors, *e.g.* lagoon site, hydraulic and air conditions, may influence inhomogeneous accumulation of especially Cd and Pb in the algae biomass (Table III).

According to the environmental legislation, the arithmetic mean values of algal biomass in mass fraction ($w \times 10^{3}$) exceed only for nickel content the agricultural feed maximum, but not the agricultural compost maximum (Figure 2). This may support future solid waste management at the petrochemical plant based preferably on the choice of alternative composting processes. From the viewpoint of water classification, the vanadium content of the mean value 175.9 μg/L points to enhanced surface water contamination (Figure 3).

This fact may indicate that the processing of Russian crude oil in the refinery is characteristically enriched by vanadium and nickel elements and point to the deficiency of the state-of-the-art air pollution control systems in closed incinerators. According to the attained analytical results, the algae significantly adsorb metal pollutant residues, however not sufficiently those of vanadium. Prolongation of the final waste water treatment in the lagoon would probably increase vanadium adsorption efficiency of the algae.

On the other hand, the water treated in the lagoon may not be classified according to the surface water quality, *i.e.,* after 10 days retention, this wa-

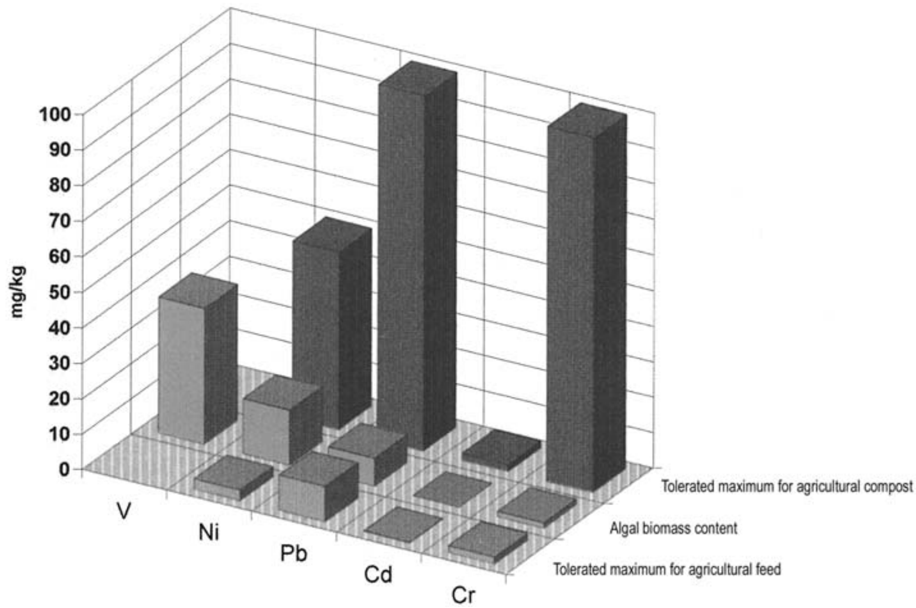

Figure 2. Arithmetic mean values of toxic elements in algal biomass, in mg/kg, compared to the tolerated maximum values of metals in agricultural feed and compost.

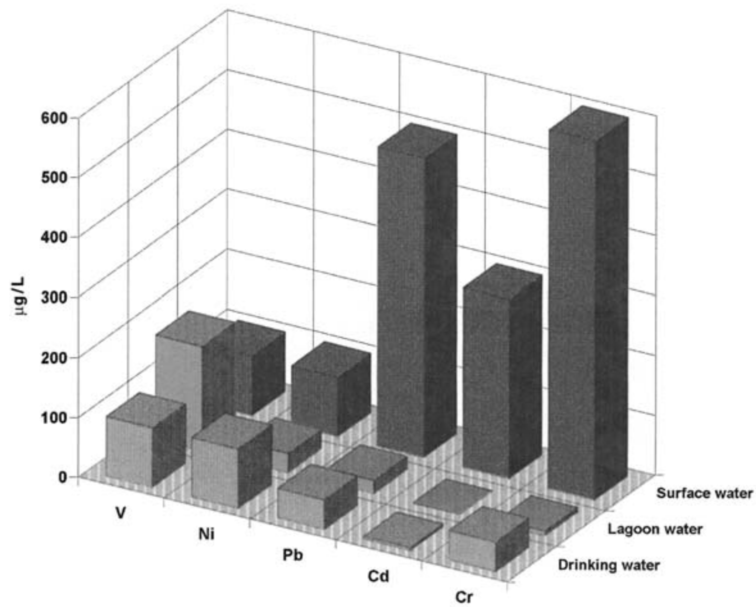

Figure 3. Toxic element contents in lagoon water, in μg/L, compared to the tolerated maxima for drinking and surface waters.

ter is discharged into the Danube river, whose water flow rate provides a huge dilution of elements and thus recipient water is usually attributed to the surface water class.

To compare heavy metal contents in the algal biomass and in the lagoon water, the bioaccumulation coefficients have been calculated (Table IV).

TABLE IV

Bioaccumulation coefficients in correlated algae / water environments

Metal	Metal content in lagoon water μg L^{-1}	Metal content in algal biomass mg kg^{-1}	Bioaccumulation factor in algae
Pb	22.5	7.9	351
Cd	0.5	0.1	200
Ni	32.1	15.6	486
Cr	8.1	1.7	210
V	175.9	37.6	214

The current waste management of the petrochemical company is based on independent, self-solid waste incineration, including combustion of the bank-discharged algae. Since the 1980s, approximately 40% of the produced hazardous waste of the company (sewage sludges, petroleum contaminated soil and concrete material) has been incinerated in the existing rotary kiln (Babkock, Germany) and storey-type combustion chamber (Polyma, Japan).

However, some alternative waste management scenarios, *e.g.* composting, especially for sludges and algae, have been recommended for the future, to relieve the present incineration of solid waste, which does not satisfy the updated criteria of air pollution control and investment costs for the construction of new incinerators are extremely high.

CONCLUSIONS

The high bioaccumulation abilities of *Cladophora* algae for selected metals have been confirmed. Waste water quality in the tertiary treatment stage, *i.e.* in the sewage lagoon, may satisfy the surface water criteria, except for the vanadium content.

Wide-spread algae species usually occur in eutrophicated aquatic conditions, primarily in oligo- up to beta-saprogenic water bodies. In the lagoon water, the ammonium concentration of 1.5 mg/L represents the tolerated surface water maximum. However, the total phosphorus concentration in this water exceeds tolerance (1 mg/L as phosphate). The treated waste water microbiology points to the presence of several species of microorganisms (protozoa – *Monas vulgaris*, *Pleuromonas jaculans*); ciliates were substituted abundantly by *Cyclidium citrulus* and *Vorticella microstoma* and diatomaceae mostly by *Rhoicophenia*. Predominance of *Cladophora* in the studied lagoon may result from the local conditions, *i.e.* water temperature, slightly enhanced phosphorus concentration and some petrochemical residues in the water (COD index equals to 32.13 mg/L).

To return sufficient nutrients and proteins back into the biosphere, the algal biomass is recommended on the basis of the analyzed and acceptable toxic metals content for compost processing.

ABBREVIATIONS

ICP-AES – inductively coupled plasma-atomic emission spectrometry
AAS-ETA – atomic absorption spectrometry-electrothermal atomization
BOD – biological oxygen demand
COD – chemical oxygen demand
TOC – total organic carbon
BTX – benzene, toluene, xylene

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SAŽETAK

Bioakumulacija teških metala zelenim algama *Cladophora glomerata* u sabirnoj laguni rafinerije

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Zelene alge, osobito vrsta *Cladophora*, smatraju se prikladnim bioindikatorima onečišćenja voda hranjivim solima i teškim metalima. Namjera je ove studije, da se istraži nakupljanje teških metala (Ni, V, Cd, Pb, Cr) u biomasi alga, te da se odrede koeficijenti bioakumulacije između alga i vodenog okoliša, u laguni s otpadnom vodom, koja predstavlja tercijarni stupanj uređaja za pročišćavanje otpadnih voda rafinerije (Slovnaft, Bratislava). S obzirom na orografsku depresiju, između istočnih Alpa i zapadnih Karpata, te karakteristično atmosfersko kruženje s pretežitim smjerom vjetra NW, očekuje se da taloženje aerosola teškim metalima, zbog industrijske aktivnosti, pridonosi kapacitetu algi za akumulaciju tih elemenata. Ispitivanja voda sabirne lagune otpada iz rafinerije obuhvaćala su određivanje BPK, KPK, naftnih ugljikovodika, pH, BTX, halogenugljikovodika, nekih poliaromata, fosfata, amonija, sulfata i suspendiranih tvari. Razmatrana je mogućnost ponovnog korištenja algalne biomase, tj. recikliranje hranjivih soli i proteina u biosferu, obradbom biokomposta.