ORIGINAL PAPER

STUDIES REGARDING POLLUTING AGENTS IN BLACK SEA ALGAE

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Abstract. *The capitalization of marine resources for therapeutic purposes is a desideratum which can bring about numerous advantages. Black Sea algae represent an important marine bioresource. They have represented an object of study in numerous published papers. The main prerequisite condition for the use of algae as a marine bioresource is the absence of contamination by polluting substances. The present paper studies polluting agents heavy metal content with a potential toxic effect (Pb, Cd, Cu and Zn).The method for heavy metal content determination was atomic absorption spectrometry. The studied algae included Cystoseira barbata, Ceramium rubrum, Ulva lactuca, Enteromorpha intestinalis, and Cladophora vagabunda, harvested from the Black Sea during June-August in two different years: 2016 and 2017. Comparisons in what regards the heavy metal content between the two years were made. The results obtained outline the low level of pollution of the algae biomass studied, in what regards heavy metal content.*

Keywords: *polluting agents, Cystoseira barbata, Ceramium rubrum, Ulva lactuca, Enteromorpha intestinalis, Cladophora vagabunda.*

1. INTRODUCTION

The marine resources for therapeutic purposes offered by the Black Sea have been harnessed due to the quality of the maritime habitats alongside the Romanian shore [1]. The Black Sea ecosystem has been studied in order to outline opportunities for harvesting marine resources [2, 3]. Of particular importance is the seaweed that can be harnessed, as they are an important resource in the medical and pharmaceutical fields [4]. The antioxidant activity of Black Sea algae has been outlined [5]. Bioactive compounds of pharmaceutical interest from the algae of the Romanian shore have been studied [6]. *Ceramium rubrum* alga has been studied in order to test the existence of active principles [7, 8]. A series of vitamins has been identified in the content of Black Sea algae, amongst which the most important are vitamins D2 and D3 (Ergocalciferol and Cholecalciferol) [9]. The Black Sea marine bioresource can represent an important nutritional source [10, 11]. According to the degree of contamination with various pollutants and residual waste generated by sand or other marine organisms, algae can be capitalized in the pharmaceutical field [12] or even in agriculture, in order to obtain fertilizers [13, 14].

Studies regarding the microbiological characterization of algae have been performed, especially for those that have been utilized with various purposes [15, 16]. These important purposes have led to the need to consider all legal aspects regarding protection against pollutant agents in the Black Sea, which can contaminate the marine bioresource [17, 18]. For use in the

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pharmaceutical field, the utilization of solely uncontaminated resources has been necessary. Comparative studies regarding the influence of pollutant agents on the contamination of marine ecosystems have been performed in literature on the matter [19].There are also studies regarding water quality monitoring on the Romanian shore, which refer to heavy mental content [20]. This paper presents a research on heavy metal content of the algae found on the Romanian shore of the Black Sea. Heavy metals are polluting agents resulting from the contamination of the sea water.

The sample lot is composed of marine algae with representatives from different species: green algae (*Ulva lactuca* syn. *Ulva rigida, Enteromorpha Intestinalis, Cladophora vagabunda*), brown algae (*Cystoseira barbata*), and red algae (*Ceramium rubrum*). They were harvested from the Romanian coast of the Black Sea in two different years, in June-August of 2016 and 2017. The study was represented by the determination of heavy metal content (Pb, Cd, Cu and Zn).

2. MATERIALS AND METHODS

2.1. ALGAE SAMPLE

Determination of heavy metal content was performed on all samples taken into study, with representatives from various species: green algae (*Ulva lactuca* also known as *Ulva rigida, Enteromorpha intestinalis, Cladophora vagabunda*), brown alga (*Cystoseira barbata*) and red alga (*Ceramium rubrum*).

2.2. PRELIMINARY WASHING AND DRYING

Preliminary washing was performed with the use of sea water, in plastic vats, with the help of a mixing device. This step was aimed to remove impurities, gravel and sand from the prime material. Sea water was preferred because it does not modify the characteristics of the native environment and because it avoids cellular lysis, a phenomenon which would lead to the loss of organic substance. After washing, the material was set in vats with grills, in order to drain residual water. The material was then dried at room temperature. The algae mass has a fragile structure and a chemical and biochemical structure, whose bioactive attributes are affected by temperatures above 50 °C.

2.3. GRINDING AND SIEVING

The dried material was grinded with the use of a ROBOT type grinding device, which is frequently used in the food industry. The device has two working compartments, one for gross grinding and the other for fine grinding. The resulting material was then separated after granulation with a vibrating device for granulometric sieves. The device contains a set of sieves from 0.045 to 6.3 mm. Fractions above 1 mm were once again grinded. 50 g from each algae specie was collected for determination.

2.4. ANALYTICAL TECHNIQUE

2.4.1. Atomic Absorption Spectrometry – Acetylene flame technique (HR-CS AAS-Flame) – for the analysis of samples in which analysis concentrations are expressed as mg/L [ppm]

Atomic absorption spectrometry (AAS) is one of the UV – Vis optical methods and is based on measuring radiant power absorbed by a population of free atoms. Because at normal temperature, only metallic mercury can provide free atom vapors, the samples must be atomized through heating at intervals between 1200 – 2000 °C, according to the specific metal. In the case of HR-CS AAS-Flame, the liquid sample is introduced in the flame as an aerosol with the use of a nebulizer. Due to the temperature of the flame, the solvent evaporates and the sample is decomposed to an atomic state. According to the temperature of the flame, the atoms can either remain on a fundamental energetic level or they can undergo an excitation process, situation in which they can pass on one or several excited energetic levels. The radiation source – usually a hollow cathode lamp (HCL) – releases a narrow spectral line, characteristic for the analyzed element. The released light beam, which is either mechanically or electronically modulated, passes through the flame, which contains atomic vapors of the analyzed element. The atoms, which are found on the fundamental energetic level, absorb part of the source's radiation, which leads to a decrease in the radiant power transmitted through the flame. The flame is obtained through a burning process which involves a fuel (acetylene) and an oxidizer (air, nitrous oxide), as these generate powerful exothermic reactions. By analogy with molecular absorption spectrophotometry, the flame can be considered a dynamic „atom vat", in which free atoms are formed continuously. The light beam transmitted through the flame is transformed by a photodetector into an electric signal, which is then amplified. After demodulation, the signal is recorded and displayed.

Heavy metal content in water samples and sediments were measured with the use of atomic absorption spectrometry methods. Control for AAS methods consists of: a sample of concentrated acids (with varying volumes, according to the type of sample analyzed), subjected to a digestion process. The sample is represented by the following mixture: 2 mL H_2SO_4 96 %, 2 mL H_3PO_4 85 %, 2 mL HF 40 % and 1 mL HNO_3 65%.

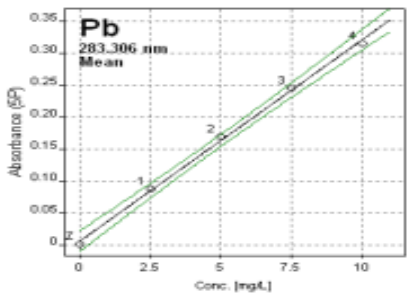
The devices used are: ContrAA-700 Analytik Jena AG, Germany high resolution atomic absorption spectrometer (Fig. 1), with a dilution specimen autosample, based on the acetylene flame technique and sequential analysis at specific wavelengths: Pb (283.306 nm), Cd (228.8018 nm), Cu (324.754 nm) and Zn (213.857 nm); Mettler Toledo analytical scale; • Thermoadjustable electric water bath with a temperature domain of 100 °C; • Thermoadjustable drying stove.

Fig. 1 HR-CS AAS ContrAA® 700, Analytik Jena, Germany.

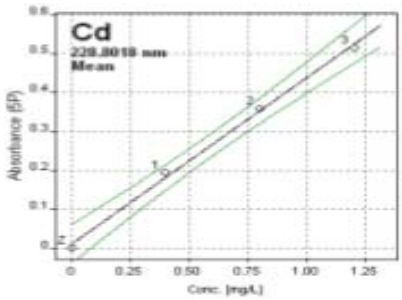
2.4.2. Sample preparation and calibration procedure

Solid samples were dried up till 105°C, in order to reach a constant mass. For mineralization after decantation, the samples were filtered on quantitative Whatman filter paper. After drying, the algae samples from the biomass were mineralized with concentrated acids in order to determine the presence and concentration of metallic elements at temperatures and pressures controlled in the digestion system.

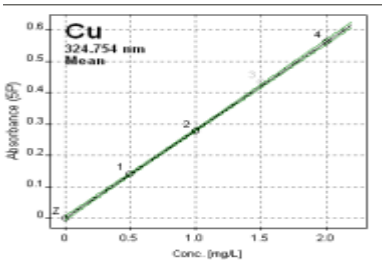
After this process, the content of digestion dishes was introduced into flasks graded at 25 mL and brought to volume with deionized distilled water. Figs. 2 a)-d), and d present the calibration curves registered for Pb, Cd, Cu and Zn and the detection limits of the AAS device in the case of the four analyzed metals.



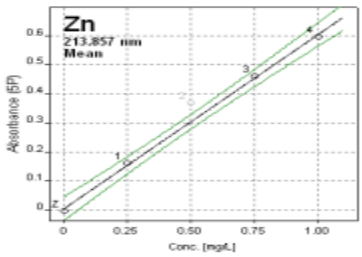
a) Pb calibration curve
(λ = 283.306 nm)
Relationship: Linear equation of the calibration curve:
A = 0.0063773C+0.0314956
correlation coefficient
(r):0.9980672
$(r)^2$:0.996138267



b) Cd calibration curve
(λ = 228.8018 nm)
Relationship: Linear equation of the calibration curve:
A = 0.0111676C+0.4275206
correlation coefficient
(r): 0.9963396
$(r)^2$:0.992692622



c) Cu calibration curve (λ = 324.754 nm)
Relationship: Linear equation of the calibration curve:
A = 0.0004863C + 0.2803596
correlation coefficient
(r): 0.99984
$(r)^2$: 0.999870418



d) Zn calibration curve (λ = 231.857 nm)
Relationship: Linear equation of the calibration curve:
A = 0.0043069C + 0.5990307
correlation coefficient
(r): 0.97070
$(r)^2$: 0.996652771

Figure 2. Calibration curves for Pb. Cd, Cu and Zn.

3. RESULTS AND DISCUSSION

Heavy metal determination was made by using the following formula:

$$[conc] = \frac{C_{curve} \cdot V_{sample}}{m_{sample}} \ [mg/kg] \quad (1)$$

The analyzed heavy metals were Pb, Cd, Cu and Zn. For each metal, gauge solutions were prepared. For each calibration curve, the linear relationship and correlation coefficients are presented (r) and $(r)^2$.

The results obtained for heavy metal content are presented in Tables 1 and 2 and Figs. 3 and 4.

Table 1. Heavy metal content for algae studied in 2016.

Type of alga	Pb [mg/kg]	Cd [mg/kg]	Cu [mg/kg]	Zn [mg/kg]
Green alga - *Ulva Lactuca* (syn. *Ulva rigida*)	3.05	0.201	3.01	19.28
Green alga - *Enteromorpha Intestinalis*	3.30	0.337	3.61	14.16
Green alga - *Cladophora vagabunda*	4.97	0.381	5.02	6.78
Brown alga - *Cystoseira barbata*	3.51	0.281	4.19	1.93
Red alga - *Ceramium rubrum*	5.04	0.391	6.58	6.72

Table 2. Heavy metal content for algae studied in 2017.

Type of alga	Pb [mg/kg]	Cd [mg/kg]	Cu [mg/kg]	Zn [mg/kg]
Green alga - *Ulva Lactuca* (syn *Ulva rigida*)	2.95	0.266	4.30	19.40
Green alga - *Enteromorpha Intestinalis*	3.25	0.375	4.45	14.95
Green alga - *Cladophora vagabunda*	4.87	0.378	5.08	6.76
Brown alga - *Cystoseira barbata*	5.51	0.330	4.66	2.55
Red alga - *Ceramium rubrum*	5.95	0.395	6.90	7.25

In green algae (*Ulva* and *Enteromorpha*), the highest concentration of zinc was found for the years 2016 and 2017. Higher heavy metal content can be observed for the *Ceramium rubrum* red alga, compared to that of the green algae (*Ulva Lactuca Enteromorpha Intestinalis* and *Cladophora vagabunda)* or that of the brown alga *Cystoseira barbata,* with the exception of zinc. Green and brown algae can be found in shore area, at shallow depths, where sunrays can penetrate; consequently, part of these metals enter the biogeochemical circuit and are used in photosynthesis, while the *Ceramium rubrum* red alga is found at higher depth, where sunrays cannot penetrate and photosynthesis cannot be performed and where, due to the currents specific to the Black Sea, heavy metal deposits are formed.

The comparative analysis of heavy metal content for the species of macroalgae outline the fact that the *Ulva lactuca* green alga is very resistant to polluted environments and presents the lowest content of Pb, Cd, Cu and the highest content of Zn. It has been observed that the concentration limits for heavy metals in the studies alga biomass are comparable to data obtained in literature and are in accordance to current legislation which regulates heavy metal concentration, taking into account that there are no concentration limits for heavy metal content in the algae biomass [19]. Alga biomass samples collected in July 2016-July 2017 show higher concentration of heavy metal content compared to data in literature [20, 21].

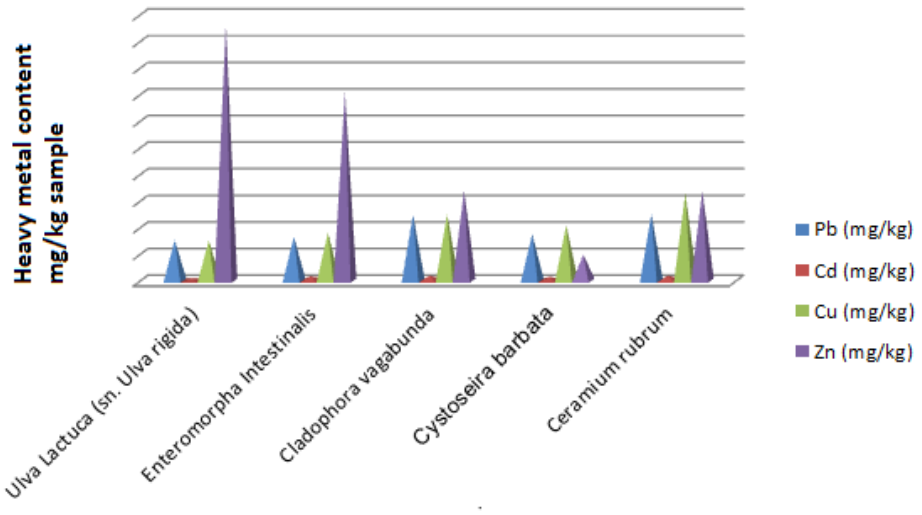


Figure 3. Variation of heavy metal content in algae studied in 2016.

In what regards the algae species, research has demonstrated that both the alginic acid and the metal content of the algae vary with species. Also, the chemical composition of algae show differences even within the same plant, registering important variations according to the isolated anatomical and morphological element isolated after harvest. Experimental data demonstrates that the influence of the harvest area (water composition, climate, and so on) is very high on algae compounds. Consequently, the high quantity of metal in seaweed is determined by ionic changes between the sea water and the alginate from the plant [23].

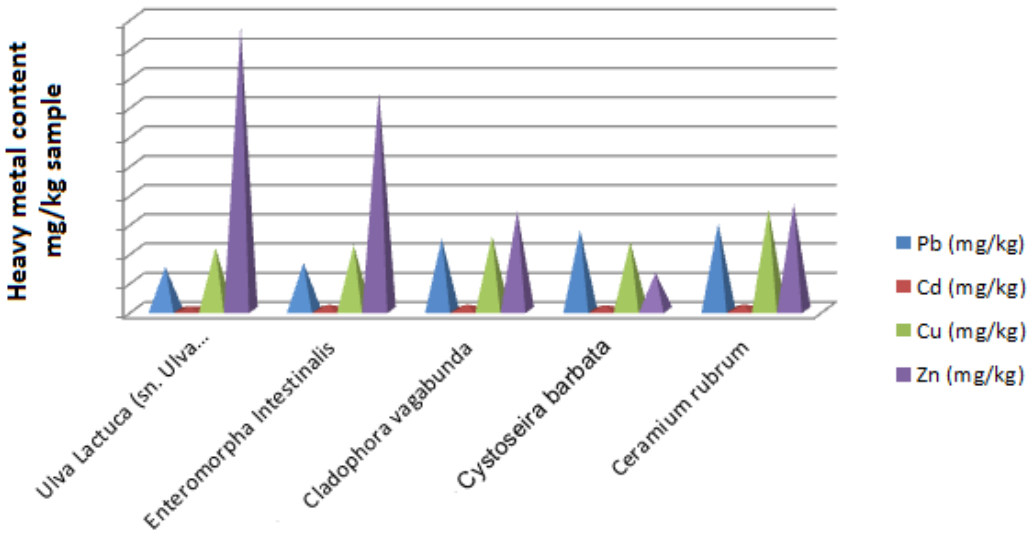
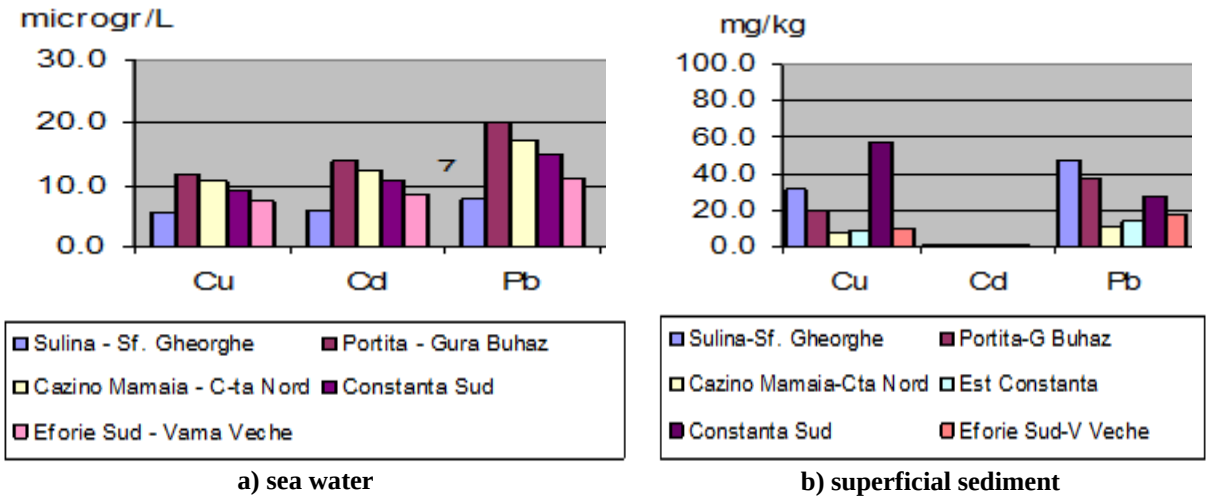


Figure 4. Representation of heavy metal content for algae studied in 2017.

Biochemical modifications which intervene during the vegetative period according to season are rigorously reflected in the chemical variation of algae composition: a maximum content in fats, proteins, alginic acid and ash is registered in spring, while autumn registers a maximum content in matine and laminarin.

The length and method of seaweed storage also lead to such modification, reflected in the structure and compound concentration of algae. Based on these notions and taking into account the chemical composition of algae, which is influenced by all of the above described factors, outlines the importance of choosing the species, the morphological element and the harvest season for an efficient capitalization.



a) sea water **b) superficial sediment**

Figure 5. Distribution of average heavy metal concentrations in abiotic components of the marine ecosystem in the Romanian shore in 2016.

A comparison between reported heavy metal content in sea water and superficial sediment can also be made [24, 25]. Fig. 5 presents average heavy metal concentration values

for abiotic components of the marine ecosystem of the Romanian shore in 2016. Compared to the data presented in Figs. 3 and 4, it can be noted that seaweed have a much lower heavy metal content for cooper, cadmium and lead compared to that of the sediments. This can be explained by the annual life cycle of the algae, which do not accumulate as much heavy metals as the sediment. By comparisons made between seaweed and sea water heavy metal content, however, a more significant accumulation can be observed for algae.

The highest content is presented by red algae, the analyzed representative being *Ceramium rubrum,* which shows significant lead concentrations for lead (5.04 mg/kg sample and 5.95 mg/kg sample respectively). The values are higher compared to those of the sea water, but lower compared to the superficial sediment.

4. CONCLUSIONS

The biological role of macrophytic algae is well known in all water basins, as they help maintain the biological equilibrium and they represent the foundation of primary productivity in these basins.

From this study, the following conclusions can be drawn:

- Heavy metal content, analyzed through atomic absorption spectroscopy, is under currently permitted limits and is comparable with data presented in literature [21, 22].
- Marine algae accumulate heavy metals in their composition, more than that existent in the sea water, but under the limits registered for superficial marine sediment [25].
- Experimental data demonstrates that the harvest area for seaweed biomass is a factor that influences heavy metal content.
- Algae, which are highly developed in the Black Sea due to the eutrophication phenomenon, can represent a valuable raw material source, with a low content of polluting agents, which can be used in the pharmaceutical industry [26-28].

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