

## VALORIZATION OF BLACK SEA GREEN MACROALGAE AS SUSTAINABLE BIOFERTILIZER SOURCES WITHIN THE CIRCULAR BIOECONOMY

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**Abstract.** In the context of sustainable agriculture and the valorization of natural biological resources, green macroalgae belonging to the genera *Ulva* and *Cladophora*, present in the algal biomass from the Romanian Black Sea coast, may represent a valuable source for obtaining products with biofertilizing and biostimulant potential. This potential is associated with the presence of bioactive compounds, photosynthetic pigments, polysaccharide fractions, and mineral elements that are important for plant nutrition and development. The aim of this study was to characterize the algal biomass and to evaluate selected biological properties of hydroalcoholic extracts obtained from these macroalgae, in order to assess their potential use in the formulation of environmentally friendly agricultural products. The characterization of the algal raw materials included determination of loss on drying, phytochemical screening, evaluation of antimicrobial activity, and determination of mineral elements and heavy metals. Loss on drying was determined in order to estimate the moisture content and to standardize the biomass used for extraction and formulation. Analysis of bioactive composition revealed the presence of several classes of biologically relevant compounds, which may contribute to the biostimulant properties of macroalgae. Antimicrobial activity of the hydroalcoholic extracts was evaluated using standard strains of *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*, with the aim of investigating the antibacterial and antifungal potential of the algal extracts. The results indicated the ability of the extracts to inhibit the growth of some tested microorganisms, suggesting the presence of metabolites with relevant biological activity. Determination of mineral profile confirmed the presence of important elements for plant nutrition, such as potassium, calcium, magnesium, and iron, supporting the potential agronomic value of the algal biomass. Heavy metal analysis was

necessary to assess the safety of using these raw materials in products intended for agricultural applications and to evaluate their compliance with requirements concerning contaminants in fertilizing products. Overall, the obtained results support the potential of macroalgae from *Ulva* and *Cladophora* genera as natural sources of bioactive compounds and nutrients for the development of biofertilizing or biostimulant products. However, confirmation of their agronomic applicability requires further plant-based tests, including the assessment of germination, growth, root development, biomass accumulation, and responses to abiotic stress.

**Keywords:** marine macroalgae; *Ulva lactuca*; *Cladophora vagabunda*; Black Sea coast; biostimulants; green fertilization; circular bioeconomy; sustainable agriculture.

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## 1. Introduction

Diversity and productivity of marine ecosystems are very important in preserving health of aquatic and terrestrial environment and provide important sources of food to humans and animals, additives in food and cosmetic industry, in medicine and bio-agriculture.

Marine algae are potential sources of highly bioactive secondary metabolites that might represent useful leads in the development of new pharmaceutical and cosmeceuticals requirements and for soil fertilization. Many studies are now being carried out on the chemicals which are being extracted from also marine algae for human benefits and welfare [1-4].

Marine algae biomass is an important resource of mineral elements, polysaccharides (agar-agar, alginats), proteins and vitamins. At the Romanian Black Sea Coast the macro-algae biomass envelopment is reported mainly in summer and is registered especially by the green macro-algae group; thus the largest deposits occur ashore after periods of storm especially, but also after bottom movement, when a large area of shallow coastline is "shaved" of vegetal carpet [5-9].

Distributed over the entire length of the Black Sea Romanian sector, from south of the Danube flowing mouths to the southern extremity of the littoral (Vama Veche), more developed in the Cape Singol-Constanta to Vama Veche sector, the marine biomass development of the macroalgal carpet in this sector is explained by the presence of limestone platforms which cover almost entirely the shallow waters and which represent the ideal substrate for their attachment. The group includes representatives of *Clorophyceae*, three orders: *Ulvales*, *Cladophorales* and *Bryopsidales*; the vegetative apparatus is diverse: lamellate, filamentous, tubular or cladomial [10 - 13].

Macrophytic algae—are multicellular, lamellar or filamentous, simple or branched, and perform photosynthesis using simple chlorophyll pigments or in

combination with other pigments. Green algae are highly diverse in terms of thallus organization: acellular (a single-celled body with multiple nuclei and no cell walls), unicellular, colonial, and multicellular. The thallus may be spherical, lamellar, ribbon-shaped, or filamentous. The thallus is green in color due to the predominance of chlorophylls *a* and *b* relative to other pigments [14].

Green algae of the genus *Cladophora* have a cosmopolitan distribution, being present in riparian ecosystems around the globe, in both freshwater and marine environments. They grow primarily attached to solid substrates, adapting effectively to habitats with strong currents, such as rivers or the littoral zones of lakes and seas. Their distribution in the form of small, localized clumps contributes to their increased resistance to the mechanical action of strong waves [14].

Regarding the other species of the genus *Ulva*, is a thin, flat green alga that grows in a disc-shaped cluster. It can reach a length of 18 to 30 cm. The thallus consists of two thick, soft, and translucent cells and grows attached, without a holdfast, to rocks or other algae via a small disc-shaped band. It is green to dark green in color and consists of two layers of irregularly arranged cells [14].

In the recent years there is a concern and an increased demand for organic agricultural fertilizer by valorification of various biological wastes. The processes for obtaining agricultural fertilizer from organic waste by mixing and stirring some components, achieving the final product in liquid form or suspension. This process raises issue adopts the too acidic pH of the products or packaging, storage and transport of manufactured products, which are likely due to the presence of water, to ferment and to degrade [15].

The aim of this study was to point out one part of the marine ecosystem on the Romanian seashore, seaweed species, which is not enough explored as useful resource in green fertilization purpose. In the present paper was studied the comparative antimicrobial activity, qualitative phytochemical screening of some fluids extracts from two species of multicellular green algae, *Ulva lactuca* Ag. - syn. *Ulva rigida* (L.), and *Cladophora vagabunda* (L.) Hoek, frequently encountered in the mixtures of thallophytes algae along the South Romanian Black Sea coast (Figure 1) the micro-, macroelements and heavy metals content in *Ulva rigida* L. and *Cladophora vagabunda* L. green macroalgae species, frequently present in residual marine biomass, as potential component of a new biostimulator-regenerator for soils poor in nutrients and organic substances, allowing recovery of marine algae biomass along Romanian Black Sea Coast, in the medio-littoral habitat.

In the context of sustainable agriculture and “green fertilization” practices, the use of algal biomass as a biofertilizer represents an environmentally friendly alternative to conventional chemical fertilizers. Green algae species of the genera *Ulva* and *Cladophora* are of particular interest due to their high content of bioactive compounds, macro- and microelements, polysaccharides, amino acids, and natural phytohormones that can stimulate plant growth and improve soil fertility.

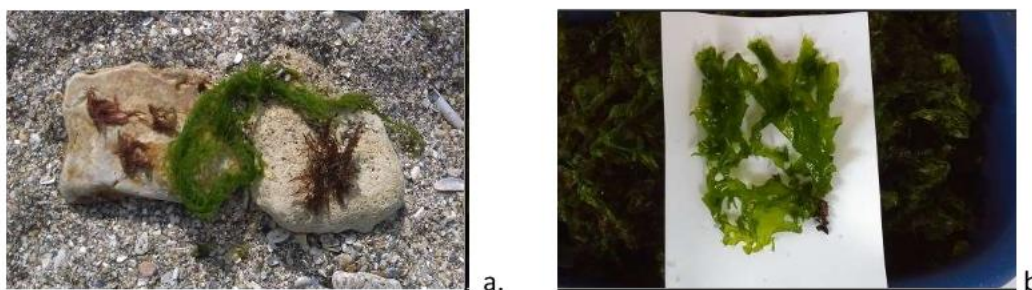


**Fig. 1.** Marine macroalgae residual biomass present along South Romanian Black Sea coast

Also, the legislation concerning marine ecosystem seaweed species used for green fertilization and biostimulant purposes has evolved significantly over the last decade, especially in the European Union. The regulatory framework now treats seaweed-derived products as part of the circular bioeconomy and sustainable agriculture sector.

## 2. Materials and Methods

Algal biomass intended for the extraction of fluid compounds was collected during the 2024–2025 period, between July and September, corresponding to the peak stage of algal development. Fresh marine algal biomass was transported to the laboratory, where it was cleaned of associated fauna and sorted into the main taxonomic groups. Manual harvesting was carried out in the shallow waters along the southern Romanian Black Sea coast, specifically in the Mangalia - 2 Mai - Vama Veche area, Constanta County. Particular attention was given to the occurrence of two common green algal species, *Cladophora vagabunda* L. Hoek and *Ulva lactuca* Ag. (syn. *Ulva rigida* L.), considered representative marine green algae and important bioindicator species. (Fig. 2 a and Fig. 2 b).



**Fig. 2.a** - *Cladophora vagabunda* (L.) Hoeck; **b** - *Ulva rigida* (L.)

The fresh samples were identified through both macroscopic and microscopic examination using field guides and specialized taxonomic literature [9-12]. Quantitative sampling of macrophytic algae was performed using the quadrat method, selected according to the characteristics of the algal population and the nature of the substrate [8].

For the evaluation of the quality and efficacy of algal biomass used in the formulation of a biofertilizer, the determination of moisture loss and phytochemical analysis are essential steps that enable the characterization of biomass composition, standardization of raw materials, and estimation of the final product's stability.

### ***2.1. Obtaining of marine green macroalgae powder material***

Following washing, the fresh algal biomass was dried at room temperature. Because algal material has a fragile structure, its chemical composition and biochemical properties can be altered by thermal treatment at temperatures exceeding 50 °C. After drying, the biomass was ground using a device equipped with two processing chambers: one for coarse fragmentation and another for fine grinding. The resulting material was separated using vibrating granulometric sieves fitted with mesh openings ranging from 0.045 to 6.3 mm. Particles larger than 1 mm were subjected to additional grinding. From the obtained algal powders, quantities ranging from 1 to 10 g of each species were collected for the preparation of hydroalcoholic extracts [16, 17].

### ***2.2 Extracts preparation.***

Marine green algal extracts from *Ulva* spp. and *Cladophora* spp. were prepared using 20 g dried algal material placed in 100 mL volumetric flasks and brought to volume with either 70:30 (v:v) ethanol, respectively 90:10 (v:v) ethanol. The extraction process was conducted over 12 days at room temperature, in darkness, while the mixtures were periodically agitated.

The extracts were subsequently filtered under normal pressure using Whatman blue-band filter paper, resulting in clear fluid algal extracts with concentrations of 20%, respectively. The extracts exhibited a characteristic algal odor and species-specific coloration, namely dark green for *Ulva* spp. and light green for *Cladophora* spp. The extract samples were stored at 4 °C to prevent oxidative degradation processes [17,18].

### ***2.3. Determination of Loss on Drying***

The determination of loss on drying is a standard quality control parameter for plant and algal raw materials. It allows for standardization of biomaterial batches, comparability of experimental results, assessment of product stability, prevention of microbiological contamination, reduction of the risk of fermentation or oxidative degradation.

In a biofertilizer, excessive moisture can promote the growth of bacteria and fungi, affecting agronomic efficiency and product safety. The optimal moisture content directly influences the concentration of nutrients and active metabolites. Properly dried biomass allows for a higher concentration of active compounds, increased stability during storage, precise formulation of application doses, and more efficient transport and handling.

For the two green macroalgae *Ulva* spp. and *Cladophora* spp., moisture loss is also important for evaluating biomass processing yield for the production of solid biofertilizers.

Determination of loss on drying is the procedure used to determine the mass of plant products or pharmaceutical preparations lost under specific conditions of temperature, pressure, and time, as specified in the monograph of the Romanian Pharmacopoeia, 10th Edition. The acceptable limits are expressed in grams and calculated as a percentage.

$$DLD \% = (\text{Weight of water in the sample} / \text{Total weight of the wet sample}) \times 100$$

#### **2.4. General qualitative phytochemical screening of extracts from marine green macroalgae**

Phytochemical screening is used to identify and quantify the main classes of bioactive compounds responsible for the biostimulatory and fertilizing effects of algae.

The two green macroalgae species, *Ulva* spp. and *Cladophora* spp., contain numerous secondary metabolites and nutrients, such as: sulfated polysaccharides, phenolic compounds, flavonoids, chlorophylls and carotenoids, amino acids, vitamins, natural phytohormones, and essential minerals [3, 6, 8, 15], which contribute to stimulating germination, root system development, increased nutrient uptake, increased resistance to abiotic stress, and improved soil microbial activity.

After selecting the green macroalgae species *Cladophora vagabunda* and *Ulva lactuca* syn. *Ulva rigida* from marine algal biomass, the unground plant sample is successively and selectively extracted using solvents of different polarities and by performing specific chemical reactions, through which various groups of bioactive compounds were identified [19, 20]. In the first stage, extraction is performed with a nonpolar solvent (ethyl ether p.a.), then with a solvent of medium polarity (methanol), and finally with distilled water. The following fractions were obtained: ether extract (A), alcoholic extract (B), and aqueous extract (C), in accordance with the provisions

of the current edition of the European Pharmacopoeia and the 10th edition of Romanian Pharmacopoeia [33, 34].

### **2.5. Determination of antimicrobial activity of marine green macroalgae extracts**

Determining the antimicrobial activity of hydroalcoholic extracts obtained from the macroalgae species *Ulva* spp. and *Cladophora* spp. represents an important step in the comprehensive characterization of “green fertilization” biofertilizers. This analysis highlights the biological potential of the extracted compounds and evaluates their ability to inhibit the growth of phytopathogenic microorganisms. Also, can contribute to: protecting plants against microbial agents; reducing microbiological contamination of the product; improving soil microbiological health; and reducing the use of chemical pesticides and fungicides.

The test microorganisms used in this study were two bacterial strains, *Escherichia coli* and *Staphylococcus aureus* and one fungal strain, *Candida albicans*. The use of these standard strains is justified by their relevance as representative microbiological models for evaluating the antibacterial and antifungal activity of the hydroalcoholic extracts obtained from the two macroalgal species *Ulva* spp. and *Cladophora* spp.

Each microbial strain was cultured for 24 hours on Nutrient Agar (for bacteria) and Sabouraud Dextrose Agar (for fungi); then, a 0.5 McFarland dilution of each strain was prepared in sterile physiological saline. From these dilutions, Petri dishes were inoculated using a sterile swab, spreading the solution across the entire surface of the solid culture medium (Mueller Hinton-Oxoid), specifically designed for antimicrobial testing. Then, wells were created using sterile, cut pipette tips (4 or 6 wells per plate). For the algal extracts, sterile cotton swabs were placed in the wells, onto which 100  $\mu$ L of sample was pipetted.

As controls, 70:30 (v:v) ethanol, respectively 90:10 (v:v) ethanol were used, as well as antibiotic discs (Cefuroxime CXM30 for Gram-negative bacteria and Sulfomethoxazole SXT25 for Gram-positive bacteria) or antifungal discs (Voriconazole VOR1 for fungi). Incubation was performed for 24-48 hours at 37 °C. The antimicrobial activity of the samples was evaluated by measuring diameter of inhibition zone, and the values represent the average of three replicates. The larger diameter of inhibition zone, the greater the sensitivity of the tested microorganism to the analyzed algal extracts [21].

## 2.6. Determination of marine green macroalgae mineral and heavy metals content

For each type of raw material, three samples with different dry masses were analyzed. For the determination of metal concentrations, the plant material was subjected to wet acid digestion using an oxidizing mixture consisting of concentrated nitric acid and hydrogen peroxide. The dried and homogenized samples were accurately weighed, treated with concentrated HNO<sub>3</sub>, and allowed to undergo pre-digestion at room temperature. The samples were then heated under controlled conditions until the organic matrix was decomposed. Hydrogen peroxide was subsequently added gradually, in successive aliquots, to ensure complete oxidation of the remaining organic matter. After cooling, the digests were filtered and quantitatively transferred to volumetric flasks, then brought to a known final volume with deionized water. The obtained solutions were further used for metal determination by spectrometric techniques. After complete digestion, the contents of the digestion vessels were transferred into 50 mL volumetric flasks for subsequent analysis.

Metal concentrations were determined by Atomic Absorption Spectrometry using Flame Atomic Absorption Spectrometry (FL-AAS). Apparatus used: High Resolution Continuum Source Atomic Absorption Spectrometer ContrAA-700, Analytik Jena AG, Germany, with autosampler for dilution sample, on acetylene flame technique, sequential analysis, at specific wavelengths, K ( $\lambda = 766.4$  nm), Ca ( $\lambda = 422.6$  nm), Mg ( $\lambda = 285.2$  nm), Cu ( $\lambda = 324.7$  nm), Zn ( $\lambda = 213.9$  nm), Cr ( $\lambda = 357.9$  nm), Mn ( $\lambda = 279.5$  nm), Fe ( $\lambda = 248.3$  nm), Pb ( $\lambda = 217$  nm), Cd ( $\lambda = 228.8$  nm) [19, 20].

## 3. Results and Discussions

Water loss through drying of marine green macroalgae species *Cladophora vagabunda* (L.) Hoek. and *Ulva rigida* C. Agardh (syn. *Ulva lactuca* L.), is the follows (Table 1): *DLD % Ulva rigida* = 8.3928 - 8.6743%; *DLD % Cladophora vagabunda* = 6.2964 - 6.8172%.

**Table 1.** Loss by drying of marine green macroalgae along south Romanian Black Sea Coast

Parameter	Algae biomass sample/ Location					
	<i>Cladophora/</i> Mangalia	<i>Cladophora/</i> 2 Mai Gulf	<i>Cladophora/</i> Vama Veche Gulf	<i>Ulva/</i> Mangalia	<i>Ulva/</i> 2 Mai Gulf	<i>Ulva/</i> Vama Veche Gulf
Loss by drying %g	6.4362	6.2964	6.8172	8.6743	8.3928	8.5972

The results of general phytochemical screening and identification tests for bioactive compounds from green macroalgae *Cladophora vagabunda* and *Ulva rigida* syn. *Ulva lactuca* extracts, at a concentration of 20% in 70:30 (v:v) ethanol and 90:10 (v:v) ethanol, respectively, are presented in Table 2.

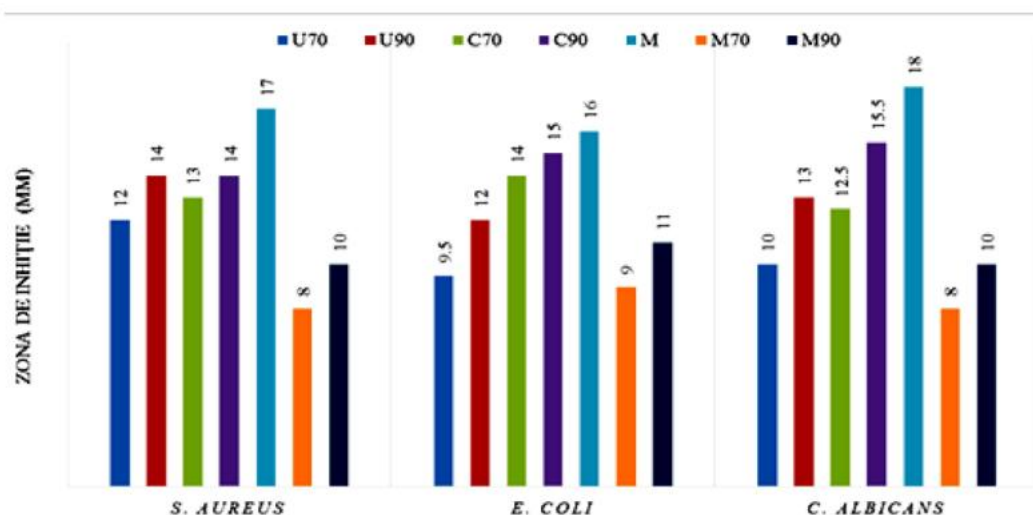
**Table 2.** Selective reactions used in general pharmacognostic analysis and the identification of active compounds in marine green macroalgae

Analyzed solutions	Qualitative reactions applied	Identified bioactive compounds
(A) Ether extract	Lieberman-Burchard	Sterols and triterpenes
	Fluorescent UV ( $\lambda = 365$ nm)	Cumarines
(B) Alcoholic extract	Reaction with ferrous chloride	Catechin tannin
	Fehling	Reducing agents
	Liebermann Bourchard	Triterpene heterosides
	Borntrager	Antracenosides
	Fluorescent UV ( $\lambda = 365$ nm)	Cumarines
	Fehling	Reducing agents
(C) Aqueous extract	H <sub>2</sub> SO <sub>4</sub> conc. + tymol	Oses and polyoses
	Foaming reaction	Saponosides
	FeCl <sub>3</sub>	Catechin tannin

With regard to the antimicrobial activity of hydroalcoholic extracts from marine green macroalgae *Cladophora vagabunda* and *Ulva rigida* (syn. *Ulva lactuca*), it was observed that activity varied depending on the microbial strain tested, algal species, and alcohol concentration used in the extraction process.

Antimicrobial agents selected as controls (Cefuroxime CXM30 for Gram-negative bacteria, Sulfomethoxazole SXT25 for Gram-positive bacteria, Voriconazole VOR1 for fungi) exhibited the highest inhibition (inhibition zone diameter ranging from 16–18 mm).

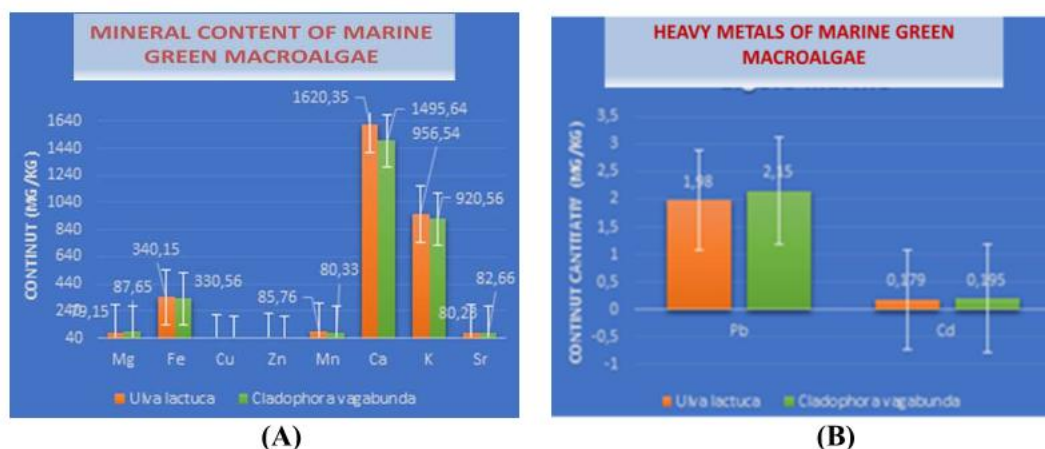
As for the tested algal hydroalcoholic extracts, slightly higher activity was observed in the 90:10 (v:v) ethanol samples for both *S. aureus* strains (inhibition diameter of 14 mm) and *E. coli* (inhibition diameter of 15 mm), as well as for the *Candida albicans* strain (inhibition diameter of 15.5 mm) (Figure 3).



**Fig. 3.** Inhibition zone diameter for the green macroalgae extracts: U70 = *Ulva lactuca* in 70:30 (v:v) ethanol; U90 = *Ulva lactuca* in 90:10 (v:v) ethanol; C70 = *Cladophora vagabunda* in 70:30 (v:v) ethanol; C90 = *Cladophora vagabunda* in 90:10 (v:v) ethanol; M = control (*Cefuroxime* CXM30 for Gram-negative, *Sulfomethoxazole* SXT25 for Gram-positive, *Voriconazole* VOR1 for *Candida*); M70 = 70:30 (v:v) ethanol and M90 = 90:10 (v:v) ethanol.

The residual marine biomass, consisting of a mixture of green macroalgae, was analyzed for the metals content. This biomass may be considered a potential natural resources as raw material for applications in green fertilization and as a biostimulant. The obtained values were compared with literature and the permissible heavy metal limits for soils established under the current legislation [23, 29].

Figure 4 (A) and (B), emphasize a comparative analysis of the variation in minerals concentrations (K, Ca, Mg, Fe, Cu, Zn, Mn, Sr) and heavy metals (Cd and Pb) for the marine green macroalgae species *Ulva rigida* syn. *Ulva lactuca* and *Cladophora vagabunda*. According to the results obtained, a high calcium content ( $1620.35 \pm 2.55$  mg/kg) was observed in the species *Ulva rigida* syn. *Ulva lactuca*. The mineral content (mg/kg) determined for the *Ulva rigida* syn. *Ulva lactuca*, varies in the following order: Ca > K > Fe > Mn > Sr > Mg > Zn > Cu (Figure 4).



**Fig. 4.** Metal content in the green macroalgae samples (mg/kg):  
(A) Minerals; (B) Heavy metals

Regarding to heavy metals content, samples of the green macroalgae *Ulva rigida* exhibited the lowest levels of heavy metals, specifically Pb (1.98 mg/kg) and Cd (0.179 mg/kg), compared to the green algae species *Cladophora vagabunda* (Fig. 4 B). The data obtained demonstrate that the green macroalgae of the studied species *Cladophora vagabunda* and *Ulva rigida* syn. *Ulva lactuca* do not exhibit a degree of heavy metal contamination that exceeds the permissible limit for this category of raw materials; therefore, they could be used to obtain bioextracts for fertilizers, hydrocolloids, and sustainable agriculture.

The correlation between low heavy metal content in algal extracts and legal standard limits for heavy metals in soils could be generally interpreted as a positive indicator of environmental safety and low contamination pressure in the ecosystem. In that way, standard legal limits for the heavy metals in soils, according to Romanian legislation in force, are presented in Table 3.

**Table 3.** Heavy metals limits in soil, [mg/kg] [29]

Soils limits	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
<b>Normal soil</b>	1	15	30	20	*	900	20	20	100
<b>Sensitive soil</b>	5	100	300	250	*	2000	200	250	700

\*Values not regulated yet

Low heavy metal content in algal extracts could suggest environmentally acceptable soil/sediment conditions and likely compliance with legal standards.

Metallic elements occur naturally in the environment and, under normal concentrations, do not pose a threat to ecosystems. However, when their levels exceed the threshold values established by environmental standards, they can

become pollutants capable of disturbing the balance of environmental components. Under such conditions, these elements may exert harmful effects on a wide range of living organisms and on the environment as a whole, thereby posing potential risks to humans who rely on the resources of the affected ecosystem.

Regarding this, the Main EU Legislation Governing Seaweed-Based Fertilizers is Regulation (EU) 2019/1009 on EU fertilising products, who entered into full application on 16 July 2022 and harmonized rules across EU Member States for: organic fertilizers, biofertilizers, soil improvers, plant biostimulants, seaweed extracts and algae-derived agricultural inputs [24].

Under the regulation, seaweed products are generally classified as: plant biostimulants, or organic soil improvers. The legislation directly affects: harvested wild macroalgae, cultivated seaweed and processed seaweed extracts.

In 2022, the European Commission launched the “Towards a Strong and Sustainable EU Algae Sector” which aims to: expand sustainable algae cultivation, reduce dependence on imported seaweed and support algae for fertilizers, carbon sequestration, circular economy applications, bioplastics and agriculture. The initiative identifies seaweed biostimulants as a strategic green sector [25, 31, 32].

Seaweed fertilizers may also fall under the Regulation (EU) 2018/848 (on organic production and labeling of organic products) which indicates that seaweed-based fertilizers can be authorized in organic farming if were sustainably harvested, free from contamination and processed according to approved methods (defined in Regulation (EU) 2018/848 and Implementing Regulation (EU) 2021/1165, and refers to only physical processing methods, means drying, dehydration, grinding or milling) [27, 28].

Also, seaweed fertilizers marketed in the EU must comply with the heavy metal limits, especially cadmium, lead, arsenic and mercury, pathogen controls, contaminant testing and labeling obligations.

The Food and Agriculture Organization recognizes seaweed as an important renewable biological resource used for fertilizers, animal feed, hydrocolloids and sustainable agriculture [26].

The results obtained for the metal content of residual marine algal biomass proposed for use in green fertilization and biostimulant applications, compared with the heavy metal limits established by current soil regulations, highlight the following conclusions:

- The heavy metals concentrations detected in the marine macroalgae biomass were low for this type of marine biological material and remained within the permissible limits established by the applicable standards for soils.

- From a sanitary perspective, the analyzed raw material complies with the required standards, as cadmium and lead concentrations were below the allowable limits.
- The concentrations of all analyzed minerals were found to be within the acceptable range for this category of samples.
- For both algal species, chromium and copper showed moderately elevated values, although all recorded concentrations remained within acceptable limits.
- Higher concentrations of calcium and potassium were identified in *Cladophora vagabunda* samples.
- Iron concentrations were found to be relatively similar in both analyzed algal species.
- Overall, *Cladophora vagabunda* exhibited a higher heavy metal content compared with *Ulva lactuca*.

Also, it was observed that the heavy metal concentrations identified are comparable with values reported in the literature [14, 15, 21, 30] and are consistent with current regulations regarding heavy metal concentrations in marine waters. This comparison is particularly relevant given the absence of specific regulatory limits for heavy metals in marine algal biomass. Reference values for marine waters include: Cu – 0.001 mg/L, Zn – 0.01 mg/L, Cd – 0.01 mg/L, and Pb – 0.03 mg/L.

A major concern for regulatory authorities is that, although seaweed-based fertilizers are natural products, they may contribute to the accumulation of persistent toxic elements in agricultural soils. Consequently, current European Union regulations for organic farming increasingly emphasize requirements related to heavy metal testing, product traceability, sustainable sourcing, and continuous contamination monitoring.

#### 4. Conclusions

- This study could be used for complex valorization of some commonly marine algae species according to the results which emphasize the possibility of using these marine resources in development of a new biofertilizer with biostimulatory and antimicrobial properties
- Loss on drying provides information regarding the stability, preservation, and standardization of biomass, while phytochemical analysis identifies the bioactive compounds responsible for its fertilizing and biostimulating effects.
- From an agronomic perspective, the antimicrobial properties of *Ulva* spp. and *Cladophora* spp. extracts may provide additional benefits for biofertilizer applications by limiting the proliferation of undesirable microorganisms and supporting healthier soil and plant development.

•Analyses were performed for determination of K, Ca, Mg, Cu, Zn, Cr, Mn, Fe, Cd, Pb metals concentration level from residual marine algae biomass samples proposed as nutrient component of a new biostimulator-regenerator, for agriculture improvement.

•The results highlight the potential of *Ulva* and *Cladophora* as promising natural resources for the development of environmentally friendly biofertilizers with complementary antimicrobial properties, supporting sustainable agricultural practices and marine algae biomass, containing *Ulva rigida* L. and *Cladophora vagabunda* L. species could be considered as reached sources of macro- and micro-elements, with valuable qualities to be used for obtaining a new biostimulator-regenerator for degraded soils.

Future legislation is expected to establish: algae-specific contaminant limits, marine biomass certification systems and standardized analytical methods.

**Conflict of interests.** The authors have no financial or proprietary interest to declare in any device presented in this article.

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