Residual Marine Algae Biomass - An Important Raw Material for Obtaining a Soil Biostimulator-Regenerator

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Abstract

This paper highlight a study regarding the valorification of residual marine algae biomass along Romanian Black Sea Coast, which recorded in the last summer period an accumulation of a large seaweed biomass quantities. The valorization of this waste was conceived as a result of theirs nutritional potential for improvind degraded soils of Dobrudja region, Romania and as complex capitalization of this biomass. It was establish the biotechnological process for the new biosolid nutrient composite obtaining. The parameter physical-chemical results of raw material used for biofertilizer obtaining, emphasized an increased organic charge compared with the inorganic compounds. Also, an increased value of total organic nitrogen and an optimum pH limits, was registered. Regarding the nutrients concentrations values, it could be noticed that the new biosolid fertilizer contains organic matter and valuable nutritive elements (N, P) could be contributed to the enhancement of the soil quality. The new biosolid biostimulator-regenerator obtained from natural residues bring a complex of nutrients for degraded soils by the presence of organic residual compounds, through the proteins, lipids, nutrients (nitrogen, phosphorus) and mineral salts content, necessary for plants nutrition and improvement of soils quality.

Keywords: residual marine algae biomass, soil, biostimulator and regenerator

Introduction

In the recent years there is a concern and an increased demand for organic agricultural fertilizer by valorification of various biological wastes. It is known that the purpose of developing, natural biofertilizers for agriculture using mixtures of grass and manure or high nitrogen binding bacteria *Azospirillum brasilense*, vegetable waste or *Bacillus megaterium*, *Bacillus macerans*, protein additives undergo fermentation or waste of skin, as organic mass soil nutrient (Lacatusu R., 2008).

In order to meet new demands for organic waste fertilizers solutions began to emerge based on different compost vegetable waste, sewage sludge, food waste, etc. (Lacatusu R., 2008; Nastac M., 2015).

There are also known the processes for obtaining agricultural fertilizer from organic waste by mixing and stirring the components, achieving the final product in liquid form or suspension. This process raises issue adopts the too acidic *p*H 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 (Negreanu-Pirjol T., 2017).

Use of algae in agriculture is focused on the fact that fertilizers based on seaweed have some properties considered to be very valuable: they mellow the soil, absorb and retain moisture, they are not attacked by pests and they contain large amounts of nutrients, nitrogen, sodium, potassium.

In the vegetative bodies, in addition to macroelements, micronutrients like Mn, Zn, Cu, Fe and other complex substances such as vitamins, auxins are present as well. Application of seaweed as fertilizer can contribute in this way to the reinclusion of these elements in the biological circuit (Negreanu-Pirjol B., 2011; Negreanu-Pirjol T., 2011; Zăgan S., 2011).

Algae administration can be done in several ways: by direct introduction into the soil layers alternating with other natural fertilizers, introduction after washing or as extracts or powders. But the algae products do not totally replace the organic or natural fertilizers, therefore, usually they are administered along with these; currently complex fertilizers achievement is wanted (Negreanu-Pirjol T., 2011; Gomoiu M.T., 1978).

Talophytic macroalgals algae spread from the Romanian Black Sea coast as brown and red algae have the most important role, green algae having a more reduced one (Bavaru A., 1977).

Ulva rigida is widespread in the Romanian Black Sea area, being more abundant between Costineşti and Mangalia. This algae's vegetative body has a light green to dark green color, with foliaceous blade looks of irregular shape, sometimes with numerous breaks in the middle, fixed on the substrate through a fixation system consisting of dark color rizoids. It can reach sizes of 5 to 30 cm or even more. Maximum development appear to be in winter-spring, at low water depth. It is encountered as well in polluted areas and sometimes in abundance in nutrients rich areas (Bavaru A., 1977).

Enteromorpha intestinalis is a multicellular alga, with a hollow vegetative body at the beginning, usually simple, sometimes very little branched, and then it detaches from the substrate and becomes lamellar. It may reach up to 1 m high and 1 mm to 10 cm wide. The vegetative body is fixed to the substrate, and its base issues fixation rizoids that unite, resulting a disk. It is a eurihalin species – it tolerates environments with salinities ranging from very low (fresh water), medium (brackish water) to very high (seas and oceans) salinity. It is able to rapidly colonize different unpopulated environments, often being the first of algae species to be established on the coasts stones. It stands in highly contaminated waters, even polluted (Bavaru A., 1977).

Ceramium rubrum, the most popular red alga in the Black Sea is presented as a filamentous clump, of dark red color, attached to substrate through rizoids. The filaments have dichotomic ramification. This species is noted by a marked polymorphism. It is an annual species, sometimes largely colonizing the rocky substrates in the mid-and infralitoral and it may be epiphytic (on other larger algae). On our coast it is encountered along the entire coastal area, all year long, with greater development during spring and summer (Bavaru A., 1977).

Algae, like all communities of marine organisms, are subject to all conditions of the marine environment, being a number of ecological factors with great importance for the development of macrophytes algae as: substrate, light, hydrodinamics, temperature, and salinity (Fig. 1) (Bavaru A., 1977; Sîrbu R., 2014; Baweja P., 2016).

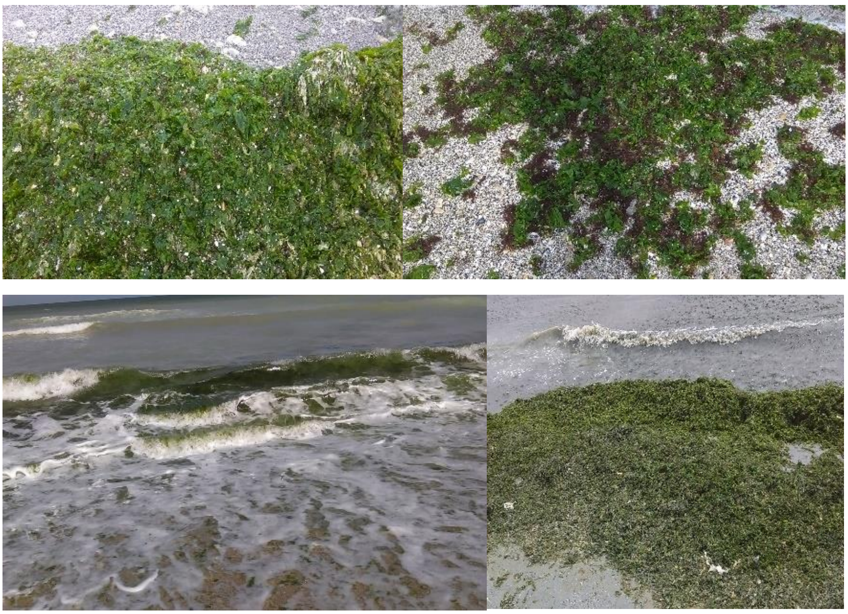

Fig. 1. Residual marine biomass along the south Romanian Black Sea Coast

The present paper emphasize the obtaining process of a new biosolid biostimulator-regenerator, as result of a mixture between residual marine biomass, macrophytobenthos: green, red and brown algae (such as *Clorophyta, Rodophyta, Phaeophyta*), in order to restore degraded soils poor in nutrients and organic substances, allowing recovery of marine algae biomass along Romanian Black Sea Coast, in the medio-littoral habitat (Biris-Dorhoi E.-S, 2018).

This new biosolid biostimulator-regenerator will be used as an innovative environmental non-conventional technology for agriculture, forestry, land reclamation and regeneration in relation to the benefits of marine waters protection. Through the biotechnology applied, the new bio-fertilizer will keep some of the qualities of residual marine biomass.

Material and Methods

Marine biomass sampling and analysis

The collecting of marine biomass samples was carried out between June - September 2018 - 2020, with minimum two samples per month; the sampling were from the Romanian beaches of Black Sea seaside in the Mamaia - Pescarie – 2 May Gulf – Vama Veche, Constanta County. All sample types were analyzed after collection in laboratory, in maximum 5 days (for all determined parameters we used averaged value, triple work samples were taken for each collecting point). The last decade of July - September, was characterized by the presence of abundant residual green macroalgae biomass, from which representative samples were taken. In the studied period, high atmosphere temperatures were recorded, over 35 °C and for sea water over 26°C. Residual marine biomass from Romanian Black Sea coast was dominated by green macroalgae (*Cladophora vagabunda*, *Ulva* and *Enteromorpha* species group), relative abundant were red species *Ceramium rubrum* and few pieces of *Porphyra leucosticta* and *Phylophora*; brown species *Cystoseira barbata* appeared only in the south littoral (Vasiliu F., 1984; Vasiliu F., 1996; Sava D., 2007; Sava D., 2006; AOAC 2016).

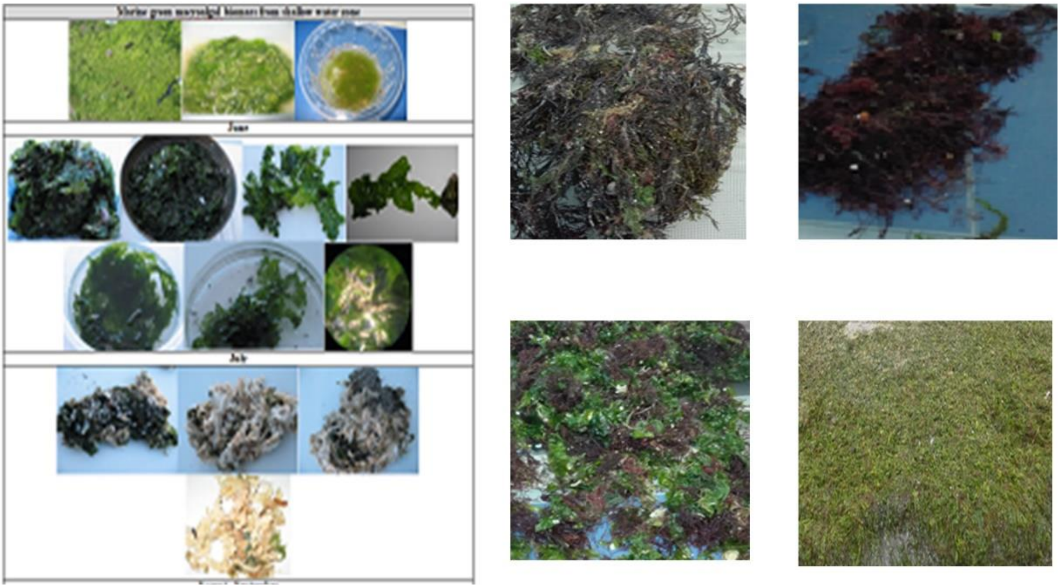

Fig. 2. Benthic macroalgal species from the shoreline of Romanian Black Sea Coast

In a first stage was carried out the separation of vegetal/macroalgae biomass and the triage and proper identification of organisms, made on the basis of taxonomic characters emphasized by stereomicroscope and Nikon microscope; considered quantitative parameters were occurrence frequency and dominance of an increased number of species (Fig. 2).

For physical-chemical and biochemical characterization of marine biomass, were used the follows methods:

Determination of *p*H was achieved using a Consort electronic *p*H-meter with temperature sensor.

Total phosphorus / phosphate, Kit Aquaquant1.14445.0001 (Merck)

Chlorides, Kit Aquaquant1.14401.0001 (Merck)

Nitrates, Portable UV-vis spectrophotometer Spectroquant, Merck

Nitrite, Kit Aquamerck1.11170.0001 (Merck)

Sulphides, Kit Aquaquant1.14416.0001 (Merck)

Sulfates, Portable spectrophotometer Spectroquant, Merck

Ammonium, Kit Aquaquant1.14428.0001 (Merck)

Total salts dissolved, WTW Portable TDS-meter

Determination of loss by drying was done under the provisions of the applicable European Pharmacopoeia (PhE 10.0 Ed.)

Determination of residue on ignition was performed under the provisions of the applicable European Pharmacopoeia (PhE 10.0 ED.)

Determination of total protein by Kjeldahl method was performed according to the official method of analysis for plant products European Pharmacopoeia (PhE 10.0 Ed.)

The quantitative determination of soluble proteins was done by Lowry method (Lowry O.H., 1951)

The quantitative determination of carotenoids was done by HPLC in ranversed stage (Dietz J.M. 1988; Hui Ni, 2005)

Determination of ascorbic acid in algae extracts was done by iodometric method described in European Pharmacopoeia (PhE 10.0 Ed.)

Sample preparation for heavy metals determination, AAS method

Heavy metals (Cu, Zn, Cr, Mn, Pb, Cd), by Atomic Absorption Spectrophotometry was analyzed.

For each raw material, a number of 3 samples with different dried masses were analyzed. A mixture of concentrated acids H_2SO4 96%, H_3PO4 85%, HF 40%, HNO_3 65%, was used for mineralization. After the complete digestion, the content of the digestion vessels was decanted in 50 mL flasks to be analyzed. For the metal content determination, measurements were performed by Atomic Absorption Spectrometry in both Graphite Furnace (GF-AAS) and Flame (FL-AAS) (Welz, 1999; Welz, 2005).

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, Cu (λ = 324.7 nm), Zn (λ =213.9 nm), Cr (λ = 357.9 nm), Mn (λ = 279.5 nm), Pb (λ =217 nm), Cd (λ = 228.8 nm) (Bucur Arpenti M., 2014, Cadar E., 2019).

For biochemical determinations, raw material consisted of a mixture of macrophyte algae collected along Black Sea coast, were stored in air for 48 hours for pre-drying. Aqueous extracts of algae were obtained as scheme in Fig. 3, Fig. 4. All determinations were made with double samples.

Cold aqueous extract:

Algae were suspended in distilled water, in relation 1:5 (m:m) and were kept cold (9 °C) for 84 h. Then the liquid was decanted and then clarified by filtration.

FRESH ALGAE

↓

SUSPENSION IN DISTILLED WATER (1 : 5) 48 hours at 4 °C

↓

DECANTING

↓

FILTRATION

Fig. 3. Scheme on how to obtain algal extracts (cold method)

Warm aqueous extract:

Algae were suspended in distilled water in relation 1:5 (m:m) and were maintained at 48°C for 4 hours. After cooling, the liquid was decanted, then clarified by filtration. The scheme to obtain the two extracts

FRESH ALGAE

↓

SUSPENSION IN DISTILLED WATER (1 : 5)

↓

WARMING (48 °C, 4 h)

↓

DECANTING

↓

FILTRATION

Fig. 4. Scheme on how to obtain algal extracts (warm method)

Results

Physical-chemical and biochemical characterization of residual marine biomass

The residual marine biomass (mixture between different algae species) was analized regarding the physical-chemical parameters (Table 1). Aqueous algal extracts was analized regarding the physical-chemical and biochemical parameters (Table 2).

Codification of sample type/ collected location: *1* - Total dried residual vegetal marine biomass / Romanian Littoral; *2* – Fresh macro algal green biomass/ Mamaia-Pescarie; *3* - Fresh macro algal green biomass / 2 May; *4* - Fresh macro algal green biomass / Vama Veche; *5* - Mixture residual marine macroalgae (green, brown, red with vegetative body) / 2 May; *6* - Mixture residual marine macroalgae (green, brown, red with vegetative body) / Vama Veche.

In the Table 1 are presented the average values for the mineral content of marine biomass for different collecting stations, as follows:

Table 1. Physical-chemical parameters of residual marine biomass, in dried substance

Parameter	Samples					
	1	2	3	4	5	6
*p*H	7.31	7.19	7.63	7.13	7.28	7. 34
Temperature (°C)	29	30	30	32	30	32
Total phosphorus (mg /g)	0.0024	0.0024	0.0024	0.114	0.0016	0.0018
PO_4^{3-} (mg/g)	0.0076	0.0076	0.0070	0.0070	0.068	0.073
Cl^- (mg/g)	0.0030	0.0014	0.0035	0.0012	0.0044	0.0048
NO_3^- (mg/g)	2.230	2.250	1.487	1.198	2.432	2.133
NO_2^- (mg/g)	0.01	0.001	0.011	0.011	0.130	0.120
S^{2-} (mg/g)	0.001	0.032	0.006	0.066	0.051	0.033
SO_4^{2-} (mg/g)	0.014	0.03	0.012	0.046	0.068	0.050
NH_4^+ (mg/g)	0.015	0.005	0.025	0,005	0.005	0.021
Total dissolved salts (mg /g)	3. 33	2.5	3.33	3.17	3.8	2.31
Cu (mg/kg)	3,023	3,640	3,432	3,028	4,326	4,280
Zn (mg/kg)	16,15	17,31	17.98	16,19	18,95	18,93
Cr (mg/kg)	2,041	1,370	1,620	1,467	1,506	1,880
Mn (mg/kg)	0,129	0,130	0,144	0,006	0,146	0,158
Pb (mg/kg)	1,112	1,270	1,198	1,010	1,113	1,126
Cd (mg/kg)	0,081	0,092	0,083	0,088	0,112	0,230

In the Table 2, are presented the average values for the physical-chemical and biochemical parameters of the aqueous algal biomass extracts (*p*H, dry substance, β-caroten, ascorbic acid, soluble protein, total Kjeldahl protein).

Table 2. Physical-chemical and biochemical determinations for aqueous algal biomass extracts

Sample	*p*H	Dry substance (%)	β-caroten (µg/dL)	Ascorbic acid (mg/mL)	Soluble protein (µg/mL)	Total Kjeldahl protein
Cold algae extract	5,60	0,560	112,3	1,890	0,948	1,966
Warm algae extract	6,62	0,577	68,3	0,862	1,048	2,130

Discussions

Regarding physical-chemical determinations of residual marine biomass:

Not significant differences between residual marine biomass collected from all stations, Mamaia-Pescarie - 2 May - Vama Veche, Constanta County were observed;

*p*H values recorded are within acceptable limits, without significant variations;

Total phosphorus content was registered in normal variation interval, greater quantity in samples 5 and 6 (mixture of algae), but all samples are included in the normal values;

Samples containing brown and red algae (5 and 6), shows higher values for other parameters: phosphates, nitrates, nitrites, sulphides, sulfates, total dissolved salts;

Sulfate ion shows values closer to the standard rules, but in larger quantities in sample 5;

The amount of ammonium ions shows slight variations in observation;

The heavy metal content has been low for this type of marine biological samples and are within the limits imposed by the standards values for surface water;

Were not observed significant differences between marine biomass residues collected from all three stations;

Higher zinc content, without exceeding the admissible limits for biological samples from the marine environment, was observed.

Regarding biochemical determinations of residual marine biomass:

Simple and reproducible methods were used to prepare cold and warm maceration extracts from the harvested algae biomass;

The values of biochemical parameters obtained for residual marine biomass in generally were comparable and not recorded a significant variation; all values parameters are in the acceptable limits for this type of samples.

Increased quantities of proteins in mixture algae biomass were registered (Table 2).

Technological process for obtaining the biosolid biostimulator-regenerator of soils

The process for obtaining the new biosolid nutrient composite, as mixture variants no. 1- 4, (Table 3), involved the following steps: The marine algae biomasses were conditioned by dehydration, at ambient temperature, for 72 hours. All biomass components were sprayed with a grinding machine, powders obtained were sieved through a sieve with mesh diameter of 90 µm. *p*H potentiometric method of each formula was determined, resulting in values ranging from 7.09 to 7.89. It was wetted with distilled water, with volumes of 75-95 mL, then left to macerate for 24 hours at room temperature. After 24 hours, the solid product was passed through the meshes of a sieve with diameter of 1.25 μm, when non-uniform grains were obtained, which were subjected to the drying process, the oven at 50 °C. Dry granules were sieved again for uniformity. Circular shaped granules were obtained, whose color intensity was correlated to the mineral charcoal content. For specific smell elimination, mineral coal, granular sawdust, dry powder strongly odoriferous plant vegetation or dry powder citrus fruit peel, granulated in a granulator were used (Negreanu-Pîrjol B.-S., 2012; Negreanu-Pirjol T., 2019). It was obtained a biosolid nutritive composite, granular powder and homogeneous, different colours, from green-brownish until brown, with physical-chemical characteristics of the Table 4.

Table 3. Variants of new biosolid nutrient composite

Raw material	Variant 1	Variant 2	Variant 3	Variant 4
	Weight parts, g			
Green marine algae biomass	30	60	10	20
Brown marine algae biomass	30	20	60	10
Red marine algae biomass	30	10	20	60
Mineral charcoal and other absorbents	10	10	10	10

Table 4. General physical-chemical average parameters of biosolid nutrient composite, in dried substance

Parameter	Mixture of marine algae biomass
*p*H	7,09 - 7,43
Total soluble salts	1 - 20%
Phosphates	traces
Chlorides	0,2 %
Nitrates	0,5%
Sulphates	0,1%
Ammonium	traces

Conclusions

Regarding physical-chemical parameters analyzed of residue marine biomass, we conclusion that no significant differences between marine biomass collected from all three stations were observed. The values of physical-chemical parameters obtained for the three categories of residual marine macroalgae biomass generally were comparable and without recorded a significant variations; all values parameters are in the acceptable limits for this type of samples;

The new biosolid nutrient composite as organic fertilizer can replace some classical solid fertilizers by direct application to soil;

The biocomposite has a *p*H close to soil *p*H, being neutral to slightly alkaline, with low chlorine content, valuable for salty soils and have good stability (in time and light) of the physical-chemical and biochemical properties;

Used as raw material, the organic residue proposed are easily accessible, with minimal costs for their collection, which gives a low-price end product biofertilizer;

The biostimulator-regenerator effect of new biocomposite for strengthening and start up for plant protection, plant nutrition and growth promotion, unconditional soil features and could be used in agriculture, horticulture, viticultura for soil quality improvement.

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