EXPERIMENTAL STUDIES ON THE RESIDUAL MARINE AND VITICULTURAL BIORESOURCES VALORIZATION FOR NEW ORGANIC FERTILIZERS

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The paper presents some aspects regarding the obtaining of new ecological fertilizers for organic and classical agriculture by exploiting valuable residual bioresources, such as marine biomass and waste from viticulture and secondary vinification process. Biomass composed of marine green and brown algae, invertebrate shells, fermented and unfermented grape marc and chopped woody shoots of vines were combined in different proportions, resulting in eight experimental variants of fertilizer, which were incorporated into steppe chernozemic mollisols, specific to the Murfatlar area of Constanța County, Romania. In 2019, in the proposed mixtures of soil – residual biomass, under greenhouse conditions, different types of vegetal species were cultivated, namely, Vitis vinifera L. (Pinot Gris variety), Lolium perene L., Sinapis alba L., and Trifolium repens L. The comparative statistical interpretation of the obtained results indicated a significant increase of essential nutrients (phosphorus and potassium), classifying the fertilized soils into the category of those with very good nutrients supply. An improvement of the soil organic matter (humus) was observed, the increase compared to the control being of 40.9% in the case of the V5 variant. A moderate increase of the 3% $CaCO_3$ content was also observed. Considering the experimental results obtained under greenhouse conditions, we will continue the study on experimental lots cultivated with different white and black varieties of Vitis vinifera L, representative for the Murfatlar vineyard.

Keywords: residual marine biomass, shells, grape marc, biofertilizers

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1. Introduction

The fertilizer can be a natural or synthetic mineral and/or organic, simple or complex product, which is applied as liquid, semi-fluid or solid on the soil or at leaf level, being a nutritious source rich in mineral and organic elements for plants, which contributes to the improvement of the physical, chemical and biological characteristics of the soil [1, 2]. Knowing the agrochemical state of the soil, the organic matter (humus) supply, nitrogen, phosphorus and potassium, is essential, allowing the application of fertilizing solutions which complete the necessary nutrients in order to ensure the crops economic efficiency [3]. Organic waste used as soil fertilizer has beneficial effects on the macro- and micronutrients of the soil, reducing the need for chemical fertilizers [4]. Currently, there is a growing concern for the environment protection, and the recovery and valorisation of biomass could bring economic benefits [5 -7].

The wine industry generates a large amount of residual biomass, sweet and fermented marc, vine shoots, resulting from the cutting works, yeasts, which can be used in different fields such as agriculture [8], food industry [9], cosmetics, pharmacy, etc. [10]. The use of marc for soil fertilization is a sustainable and complete method of exploiting this resource [11]. Its bio-stimulatory activity on plants is provided by supplying mineral nutrients directly or indirectly (mainly N, but also P, S and other macro- and micronutrients), fibers, these substances being found in forms easily assimilable to the plant [12 - 14]. As regards the vine shoots resulted from cuttings, from each stock up to 2 kg of shoots are removed every year, about 3500 kg/ha. The shoots, chopped and incorporated into the soil, undergo a slow biological degradation, bringing an important contribution of organic and mineral substances into the soil. The use of algal biomass in different types of soil fertilization is already being studied, as the high content of fibers and mineral elements justifies its biostimulator and regenerator role [15, 16].

Along the Romanian Black Sea coast, macrophyte algae species, *Cystoseira barbata* (L.) C. Agardh, 1820, common littoral brown algae in the Phaeophyceae Class, Fucales (fucoids) Order and *Ulva lactuca* (L.), known as *sea lettuce*, an edible green macro algae in the Ulvophyceae Class, Ulvaceae family, abound in high quantities and on large areas [17, 18]. Excessive development of algal flora poses problems both in terms of affecting the marine ecosystem, but also has cascading effects on both the ecosystem functionality and tourism activities [19, 20]. However, it can be a renewable and cheap source of food, soil fertilizers, biofuels, pharmaceuticals, cosmetics, nutraceuticals [21 - 24]. The study consisted in obtaining some ecological fertilizers for application in organic as well as classical farming, or on soils for viticulture, based on the valorisation of organic and mineral components from residual marine biomass and bioresources from viticulture and the secondary vinification process.

2. Experimental

Sampling and storage of bioresources from viticulture and secondary vinification process

Grape marc is a by-product of the winemaking process, consisting of skins (55 - 65%), seeds (18 - 25%), and liquid residues (must, wine) depending on grape variety. From a grape harvest of 7 - 14 tones/ha, the obtained grape marc is in the range of 2 - 4 tones. In this study, the marc was dried in a thin layer, at ambient temperature. Every 24 hours it was aerated by palletation, to facilitate water evaporation and thus avoid the development of bacteria and fungi in the moist layer. The vine shoots resulting from the cutting works were stored in a specific area in a semi-open system [25].

Sampling and storage of marine residual bioresources

Three types of residual marine biomasses constituent species, such as macrophytic green algae *Ulva lactuca* (L.), brown algae species *Cystoseira barbata* (L.) C. Agardh and zoobenthos (invertebrates shell material) were used. The residual marine biomass samples have been collected from two representative locations of Romanian Black Sea coast, Mamaia - Pescarie Gulf and 2 Mai Gulf, Constanta County, in the June – September 2019 period, following the standard sampling of the marine biomass, total number of processed samples being 8 macrophyte algae and 8 marine invertebrates shells [26].

The quantitative sampling of macroalgae was performed using the squares method with frames of appropriate dimensions depending on the characteristics of the population and the substrate type. For the quantitative harvesting, wooden frames with a side of 10/10 cm were used. The fresh samples were washed from associated residual fauna, sorted on main groups (green algae, brown algae) and separated based on taxonomic groups identification (Fig. 1) [16, 26].

Invertebrates shell material was collected by the sample square method. The samples were subjected to a preliminary washing with sea water in plastic tanks while stirring in order to remove impurities, gravel and sand. Sea water was preferred because it does not change the characteristics of the native environment and cell lysis is avoided, a phenomenon that would lead to the loss of organic matter. After washing, the material was placed in a vat with a grate in order to drain the remaining water, followed by drying in dark conditions, at room temperature, for 72 hours. The marine biomass was grinded and mechanically sieved, combining the oscillating movements with the rectilinear and circular ones, the elliptical movements, perpendicular to the sieve or circular with vibratory movements. The shells were subjected to the following operations: mechanical crushing - grinding with an electric mill, separation through a no. 4 sieve and the retention of fractions smaller than 400 μm and a coarse white-cream powder was obtained. The procedure for the marine biomass sampling and processing in the lab is in accordance with Standard Methodology for Biology Samples Analyzes [16, 26].

Fig. 1. Residual marine biomass (brown algae, green algae, invertebrates shell) samples along south Romanian Black Sea coast

The bioresource from residual viticulture and secondary vinification process was obtained from the Research Station for Viticulture and Oenology Murfatlar, Constanta County, Romania.

After drying, the residual biomass samples were crushed, weighed and homogenized in the proportions established by the scheme (Fig. 2). In order to establish the appropriate doses of the six components (green algae, brown algae, shells, fermented marc, unfermented marc and chopped vine shoots) used in the analysed mixtures, several experimental variants were performed, namely: a variant made up of equal parts of each viticulture and marine constituent (35 g/ constituent) and 6 variants having a component in double quantity (70 g/constituent) [31].

The experiment took place under greenhouse conditions within the Research Station for Viticulture and Oenology Murfatlar, Constanta County, Romania, for a period of five months (June - November, 2019). The variants were placed in vegetation vessels in which 25 kg of steppe chernozemic molisols, specific to the Murfatlar vineyard area, for which the physicochemical properties are listed in Table 1, were mixed with the experimental biofertilizers.

In the vessels (surface 80 x 40 cm) filled with soil and a variant of organic fertilizer mixture (Fig. 2), four different vegetal species, such as, *Vitis vinifera* L. (Pinot Gris variety), *Lolium perene* L., *Sinapis alba* L., *Trifolium repens* L. were planted and cultivated. During the experimental period, soil moisture was maintained at a permanent level of 70% of field capacity. At the end of the observation period, soil samples were taken for agrochemical analysis of each experimental variant in order to observe the effect of the fertilizers. To obtain a homogeneous soil sample/experimental biofertilizer variant, the soil was collected from six distinct points of the vegetation vessel at a 0-20 cm depth interval, then transferred into paper bags, labelled and analysed.

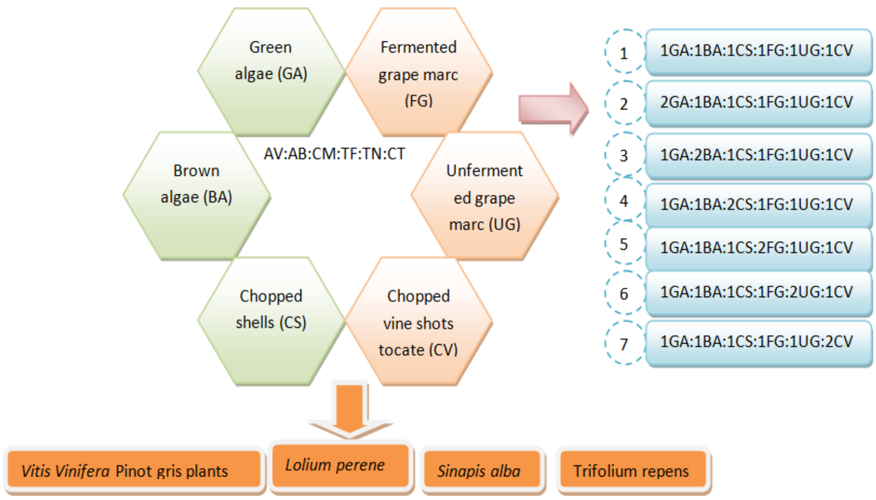

Fig. 2. Scheme of new organic fertilizers based on residual marine biomass, viticulture and secondary vinification process bioresources

Physical-chemical analyses

The physicochemical analyses carried out on soil samples were: *p*H, determined in aqueous suspension using a combined glass-calomel electrode, the humic substances content, determined by the Walkley-Black method, modified by Gogoasa; the mobile forms of phosphorus and potassium soluble in ammonium acetate lactate solution (AL) at *p*H 3.75, by the Egnèr-Rhiem-Domingo method, determined by spectrometry or flame photometry, respectively. The iron content by the AAS method, respectively the total soluble salts by conductometrically using a *p*H Cond707 glass electrode, were determined [32 - 34].

Statistical analysis

Data were expressed as the mean ± standard deviation. One-way analyses of variance of means (ANOVA) and the mean differences of the various samples were compared by a post-hoc test (Duncan multiple mean comparison test). A difference of $p \leq 0.05$ was considered as significant.

3. Results and discussion

The characteristics of untreated soil samples are listed in Table 1.

Table 1

Physical-chemical characteristics of untreated soil samples

Control sample	*p*H	Total soluble salts (mg/100 g)	$CaCO_3$ (%)	Phosphorus, as P_2O_5 (ppm)	Potassium, as K_2O (ppm)	Iron (ppm)	Humus (%)
	8.2	74.3	2.43	21.0	130	58.5	2.54

The results obtained are presented in Table 2 for *Vitis vinifera* L. (Pinot Gris variety), highlighting the improvement of the physico-chemical properties of the soil by exploiting the organic and mineral matter of the marine and vini-viticultural bioresources. The soil reaction under the influence of the applied organic fertilizers is low alkaline (*p*H 8.1-8.2), the values obtained in the experimental variants are in the optimum *p*H range for grapevine (5.5 - 8.2) [13]. The salt content classifies the fertilized soils in the category of low salinized, the values in the case of the experimental variants ranging between 136.3-170.3 mg/100 g soil. Soil salinity recommended for plants grown under greenhouse conditions, but also in the case of grapevines is 300 ppm [12, 27]. Regarding the calcium carbonate content, the obtained data classifies the fertilized soils in the category of soils with medium content (< 3 %), being in the range of 2.2-3.3% for the experimental variants cultivated with *Vitis vinifera* L. (Pinot Gris variety). The importance of mobile phosphorus in grapevines mineral nutrition is well-known, as it stimulates flower fertilization, helps fruit ripening, wood maturation and root branching [28]. The annual phosphorus consumption of grapevine is 20 - 40 kg/ha on average, and the optimum soil content is 10-20 mg P_2O_5/100g soil. In our study, the soil phosphorus level accessible for plants increased at the "very good" level of supply compared to the control (21.0 ppm), as the obtained values ranging between 138.3 and 281.4 ppm for *Vitis vinifera* L. (Pinot Gris variety). Regarding mobile potassium, significantly higher values were recorded in the case of V5 variant for *Vitis vinifera* L. (Pinot Gris variety). Potassium is an indispensable element for the growth and fruiting of grapevines and contributes, among other things, to iron absorption, intensifying the pigmentation of berries in red varieties. Potassium is considered the most beneficial element in terms of influence on grapevine's resistance to the attack of diseases and pests [29, 30]. The optimal

K_2O content in soil is 30-50 mg and the annual consumption is 70 - 150 kg/ha. Potassium content values in the experimental variants were in the range of 800 - 1000 ppm, with the V5 variant showing the best results, the fertilized soils being classified in the category of those with very good nutrients supply. Regarding microelements, iron was analysed, and the values were in the range of 120.5-147.1 ppm, classifying the soils in the medium supply category. The humus content for the fertilized soils ranged between 2.16 - 3.58%, the best results were obtained for V5 variant cultivated with *Vitis vinifera L.* (Pinot Gris variety), with an increase of 40.0 % compared to the control.

Table 2

Physical-chemical characteristics of the soil variants cultivated with *Vitis vinifera* L. (Pinot Gris variety)

Variant	*p*H	Total soluble salts (mg/100 g)	$CaCO_3$ (%)	Phosphorous, P_2O_5 (ppm)	Potassium, K_2O (ppm)	Iron, Fe (ppm)	Humus (%)
V1	8.2±0.1 (a)	137.4±2.8 (e)	2.2±0.4 (d)	149.5±3.4 (d)	800±20.4 (d)	147.1±4.5 (b)	2.16±0.3 (b)
V2	8.1±0.1 (a)	136.3±2.4 (e)	3.3±0.6 (ab)	261.8±4.5 (b)	940±29.8 (bc)	131.5±3.7 (c)	3.34±0.8 (ab
V3	8.1±0.2 (a)	161.8±3.5 (b)	2.9 ±0.2 (bc)	159.8±2.5 (c)	972±24.2 (ab)	144.8±2.5 (b)	2.86±0.5 (ab
V4	8.2±0.2 (a)	170.3±4.1 (a)	2.6±0.3 (cd)	138.3±3.5 (e)	932±23.8 (bc)	129.4±3.0 (c)	2.62±0.4 (ab)
V5	8.1±0.1 (a)	164.6±3.0 (b)	3.6±0.1 (a)	281.4±5.2 (a)	1000±30.7 (a)	156.5±5.0 (a)	3.58±0.9 (a)
V6	8.1±0.2 (a)	155.0±2.8 (c)	2.6±0.1 (cd)	164.8±4.0 (c)	906±27.4 (c)	120.5±2.5 (d)	2.62±0.5 (ab)
V7	8.2±0.1 (a)	143.5±2.2 (d)	3.3±0.1 (ab)	166.3±4.2 (c)	1000±30.5 (a)	143.6±3.0 (b)	3.34±0.7 (ab)

Note: Average values ± standard errors (n=3). The letters in the brackets show the statistical difference among results for $p < 0.05$. For the same compound, a common letter for 2 or more variants shows no significant difference among them.

In Table 3, the results obtained for the culture of *Lolium perene L.* are presented, where it is visible that the application of experimental fertilizers determined significant increases on the salt content, the values oscillating in the range of 123.8-146.9 mg/100g.

Table 3

Physical-chemical characteristics of the soil variants cultivated with *Lolium perene* L.

Var	*p*H	Total soluble salts (mg/100 g)	$CaCO_3$ (%)	Phosphorous, P_2O_5 (ppm)	Potassium, K_2O (ppm)	Iron, Fe (ppm)	Humus (%)
V1	8.2±0.2 (a)	146.9±3.9 (a)	2.6±0.6 (b)	143.5±4.1 (c)	880±22.8 (d)	156.9±5.1 (a)	2.62±0.5 (b)
V2	8.1±0.1 (a)	130.1±2.7 (c)	2.3 ±0.3 (b)	143.9±3.7 (c)	758±20.5 (f)	142.9±4.7 (b)	2.27±0.3 (b)
V3	8.3±0.2 (a)	123.8±2.0 (d)	3.1±0.7 (ab)	148.7±4.5 (c)	958±28.3 (ab)	133.1±3.7 (c)	3.1±0.7 (ab)
V4	8.2±0.1 (a)	136.3±3.1 (b)	2.9±0.6 (ab)	161.3±4.7 (b)	1000±30.2 (a)	161.1±5.5 (a)	2.86±0.3 (ab)
V5	8.2±0.2 (a)	134.4±2.9 (bc)	3.6±0.5 (a)	159.9±5.0 (b)	906±20.1 (cd)	125.5±3.0 (c)	3.58±0.4 (a)
V6	8.1±0.1 (a)	138.7±2.5 (b)	2.6±0.3 (b)	179.3±5.8 (a)	932±27.5 (bc)	130.8 ±3.2 (c)	2.62±0.3 (b)
V7	8.2±0.2 (a)	116.3 ±2.0 (e)	2.6±0.2 (b)	161.2±4.5 (b)	830±24.2 (e)	141.3±4.1 (b)	2.62±0.5 (b)

Note: Average values ± standard errors (n=3). The letters in the brackets show the statistical difference among results for $p < 0.05$. For the same compound, a common letter for 2 or more variants shows no significant difference among them.

The highest increases from a statistical point of view were registered in the case of variant V1, followed by variant V4, V6, V5, V2, V3, the lowest values being registered at variant V7, where the soils fall into the category of weakly salinized. The contribution of organic substances from the administered fertilizer variants led to significant changes in the soil and in the case of phosphorus and potassium content, the highest values in the case of phosphorus were recorded at variant V6 (179.3 ppm) and for potassium in the V4 variant (1000 ppm), the fertilized soils fall into the category of those with very good fertilization.

The contribution of fertilizers applied on the culture of *Sinapis alba L.*, are presented in Table 4, where it can be observed significant increases in the case of the studied variants. The content of phosphorus (113.3-224.4 ppm) and potassium (854-1000 ppm) includes fertilized soils in the category of those with very good fertilization. Significant increases are also observed in the case of the humus percentage, those being registered in the case of the V6 variant by 3.82%

Table 4

Physical-chemical characteristics of the soil variants cultivated with *Sinapis alba* L.

Var.	*p*H	Total soluble salts (mg/100 g)	$CaCO_3$ (%)	Phosphorous, P_2O_5 (ppm)	Potassium, K_2O (ppm)	Iron, Fe (ppm)	Humus (%)
V1	8.4±0.2 (a)	127.5±2.2 (b)	3.4±0.9 (a)	113.3±2.7 (e)	1000±33.2 (a)	147.4±4.7 (b)	3.4±0.5 (ab)
V2	8.4±0.3 (a)	107.4±2.0 (d)	3.1±0.5 (a)	165.2±5.1 (d)	854±27.3 (c)	136.6 ±4.0 (c)	3.1±0.4 (ab)
V3	8.3±0.2 (a)	142.1±3.1 (a)	3.3±0.8 (a)	218.9±6.2 (ab)	1000±35.2 (a)	118.6±3.2 (c)	3.34±0.3 (ab)
V4	8.4±0.1 (a)	98.9±1.8 (e)	3.1±0.5 (a)	198.3±3.3 (c)	1000±40.1 (a)	135.6±3.8 (c)	3.1±0.2 (ab)
V5	8.3±0.2 (a)	112.5±2.4 (c)	2.9±0.3 (a)	224.4±6.0 (a)	1000±38.2 (a)	130.8±3.5 (c)	2.86±0.2 (b)
V6	8.4±0.1 (a)	100.3±1.7 (e)	3.8±0.8 (a)	173.4±5.3 (d)	972±36.3 (ab)	136.5±3.7 (c)	3.82±0.6 (a)
V7	8.3±0.2 (a)	108.1±1.9 (d)	3.6±0.6 (a)	213.8±6.0 (ab)	932±32.1 (b)	194.9 ±5.0 (a)	3.58±0.5 (ab)

Note:Average values ± standard errors (n=3). The letters in the brackets show the statistical difference among results for p <0.05. For the same compound, a common letter for 2 or more variants shows no significant difference among them.

For *Trifolium repens L.*, the obtained results are highlighted in Table 5.

Table 5

Physical-chemical characteristics of the soil variants cultivated with *Trifolium repens* L.

Var	*p*H	Total soluble salts (mg/100 g)	$CaCO_3$ (%)	Phosphorous, P_2O_5 (ppm)	Potassium, K_2O (ppm)	Iron, Fe (ppm)	Humus (%)
V1	8.4±0.1 (a)	104.0±2.5 (f)	3.6±0.7 (a)	179.2±3.9 (cd)	940±23.8 (a)	116.9±2.2 (d)	3.58±0.3 (a)
V2	8.2±0.2 (ab)	159.5±1.8 (c)	2.9±0.3 (a)	175.9±4.2 (d)	1000±35.5 (a)	142.6±3.9 (b)	2.86±0.2 (bc)
V3	7.9±0.1 (b)	244.1±4.3 (a)	2.6±0.5 (a)	190.3±4.7 (b)	1000±38.2 (a)	141.9±4.0 (b)	2.62±0.2 (c)
V4	7.9±0.2 (b)	160.8±2.2 (c)	3.6±0.9 (a)	186.1±4.4 (bc)	744±29.5 (c)	138.8±3.3 (bc)	3.58±0.4 (a)
V5	7.8±0.2 (b)	220.7±4.0 (b)	2.6±0.6 (a)	227.1±5.2 (a)	1000±36.3 (a)	141.3±4.1 (b)	2.62±0.1 (c)
V6	8.0±0.1 (bc)	148.2±2.1 (d)	2.6±0.3 (a)	167.7±3.1 (e)	866±22.1 (b)	133.5±3.7 (c)	2.62±0.2 (c)
V7	8.3±0.1 (a)	110.5±1.8 (e)	3.1±0.8 (a)	161.6±3.3 (e)	1000±38.3 (a)	166.8±4.5 (a)	3.1±0.1 (b)

Note: Average values ± standard errors (n=3). The letters in the brackets show the statistical difference among results for p <0.05. For the same compound, a common letter for 2 or more variants shows no significant difference among them.

Significant increases are observed as a result of the application of fertilizers on salts, the values oscillating in the range of 104.0-244.1 mg / 100 g, statistically significant increases being registered in the case of variant V3, followed by V5, V4, the lowest values being quantified at variant V1. Regarding the phosphorus and potassium content, there are significant increases in the case of the V5 variant, the soils falling in this case also in the category of those with very good fertilization. The humus content showed significant increases in the case of variant V1 and V4, the lowest values being registered in variants V3, V5 and V6.

At the end of the experimental period, the vegetable mass from each type of crop and each variant (0,032 m^2) was harvested and weighed, the results being presented in Fig. 3. Significant increases were noticed in the case of *Vitis vinifera* L. (Pinot Gris variety) and *Sinapis alba* L., for the V5 variant (70 g). In the case of *Lolium perene* L. species, the best results were obtained for the V1, V6 and V7 variants. For *Trifolium repens* L. species, the best results were obtained for V1 and V6 variants. The results emphasized that new organic fertilizers administration had benefits on vegetal growth of studied crops.

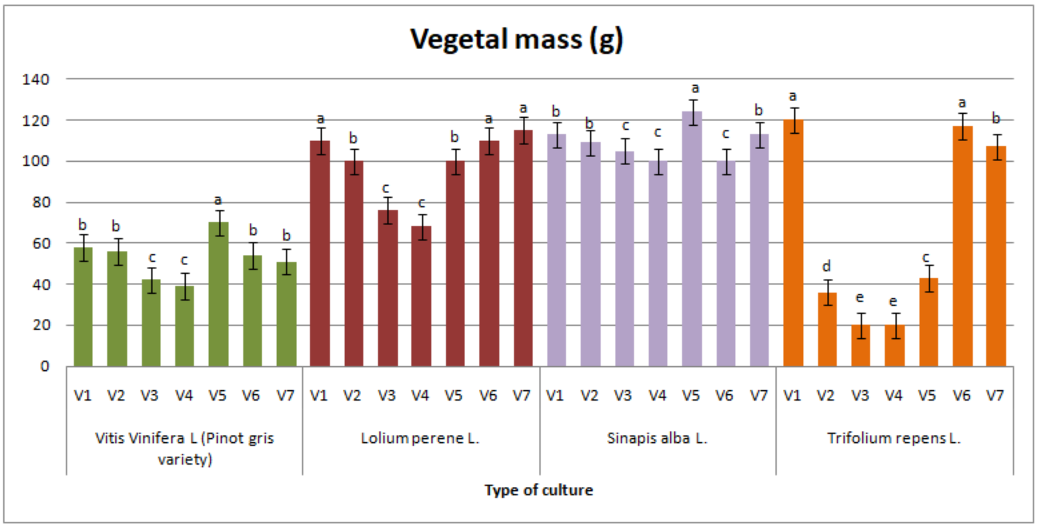

Fig. 3. Vegetable mass obtained for different crops after the addition to the soil of the new organic fertilizer experimental variants

4. Conclusions

The influence of new organic fertilizers made through mixing different bioresources from residual marine and vini-viticultural biomasses on properties of soils cultivated with *Vitis vinifera* L. (Pinot Gris variety), *Lolium perene* L., *Sinapis alba* L. and *Trifolium repens* L., revealed a significant increase of essential nutrients (phosphorus and mobile potassium), an improvement of organic matter (humus) and a moderate increase of the $CaCO_3$ content.

The experimental results obtained in greenhouse conditions and the effects of the residual biomass variants used as soil biostimulators and regenerators, encourage us to continue the study in field conditions on experimental lots, for different white or black varieties of *Vitis vinifera* L., important for the Murfatlar vineyard area.

Acknowledgements

The authors would like to express appreciation for the financial support of the Romanian Ministry of Research - UEFISCDI, for Complex Project no. 85PCCDI / 2018, Acronym INOBIOMAR, Project code PN-III-P1-1.2-PCCDI-2017-0701.

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