Research regarding the valorization of marine and wine by-products for obtaining organic fertilizers

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ABSTRACT

The theme approached in this study is part of the state-of-the-art concerns regarding the valorization of by-products that represent a problem for the environment, due to the constant production and limited exploitation. The purpose of this paper was to render useful marine and wine by-products, in order to obtain fertilizers with applicability in the cultivation of the grapevine (*Vitis vinifera* L.). Green algae, brown algae, shells, fermented pomace, unfermented pomace and vine canes were combined in different proportions and applied to an experimental lot in the Murfatlar wine center, cultivated with the red wine cultivar, 'Feteasca neagra'. The high content in mineral elements of these by-products has led to significant increases in soil quality, especially regarding essential nutrients (phosphorus and mobile potassium) and organic matter (humus). Improvements have also been observed in the accumulation of biochemical compounds involved in plant growth and development, but also the quality and production of grapes. The obtained results encourage us to apply these fertilizers in the following years, diversifying the vine cultivars for white and red wines.

Keywords: grape pomace, marine biomass, biofertilizers, harvest improvement, essential nutrients

INTRODUCTION

In order to promote sustainable agriculture, organic waste can be considered an important resource. By-products of agricultural processing, manure, and other organic residues can indeed be used to increase soil fertility, as they are a source of important nutrients useful for crop growth and they induce an overall improvement in soil quality (Davies and Lennartsson, 2005; Erana *et al.*, 2019). Grape pomace is an abundant by-product (solid organic waste), consisting of the remaining skins, seeds, and stalks, representing around 15-25% of the total grape weight used in the winemaking process, depending on the cultivar and procedure (Oliveira and Duarte, 2016). The moisture after pressing is around 20-30% w/w, and the material is characterized by a C:N ratio ranging from 40 to 45:1, a pH ranging from 3 to 6, high organic matter content and organic forms of micro and macro-nutrients, which are mineralized through time (Lomillo and San, 2017). The compost-based conditioning influences the soil's physicochemical properties, by improving its structure, increasing the organic matter content, modifying the nutrient availability and inducing beneficial effects on the microbial load of the soil (Saha *et al.*, 2008). Applying organic

matter to the soil results in increased biomass of soil fauna, which plays a substantial role in organic matter decomposition, causing a constant nutrient release (Breure, 2014). Contrasting with chemical fertilizers, grape pomace and dried seaweed are biodegradable, they do not pollute the environment and pose no threat to the health of humans and other life forms (Chbani *et al.*, 2015). Grape pomace as an organic fertilizer has a certain value, mainly due to low content in phosphoric acids and nitrogen. Chemical analyses of the compost based on winery waste reveal a cultivar of different elements, such as Ca, Mg, Fe, Zn. Grapevine canes could also pose as an important source of nutrients, considering their significant quantities of minerals and amino-acids (Balasubrahmanyam *et al.*, 1978). Along the Romanian Black Sea shore, algal species such as *Cystoseira barbata* and *Ulva lactuca* can be found in great quantities (Marin and Timofte, 2011). Studies have shown that these seaweeds contain notable amounts of minerals, both macronutrients and trace elements (Panayatova and Stancheva, 2013) and their extracts stimulate plant growth and tolerance to stress factors, as well as nutrient uptake from the soil (Rathore *et al.*, 2009). Algae are not the only kind of abundant marine organism that can have a potential purpose as a biofertilizer: the snail genus *Rapana*. has a mineral-rich shell (Sereanu *et al.*, 2017), that can serve as a good calcium source, provided that it is finely shredded. The biomass can be used as a sustainable source of organic fertilizer, returning both nutrients and carbon to the soil, potentially improving soil quality, along with crop growth and nutrition. Moreover, research in large-scale algal biomass production has increased in recent years, for diverse applications including biofuels, animal feed and nutrient scavengers in wastewater treatment processes (Zhu *et al.*, 2013). This has also created opportunities for the development of by-products such as algal-based fertilizer, that could contribute to a more sustainable circular-economy for nutrients in arable farming systems. Results concerning the mineral composition of grape pomace (Bojan *et al.*, 2020), *Cystoseira barbata* (Panayatova and Stancheva, 2013) and *Ulva lactuca* (Breure, 2014) algal mass that has been highlighted in several studies are presented in Table 1.

Table 1. Proximate chemical composition of grape pomace and marine mass (Bojan *et al.*, 2020; Panayatova and Stancheva, 2013; Breure, 2014)

Compound mg/100 g	Quantity identified in grape pomace	Quantity identified in <i>Cystoseira barbata</i>	Quantity identified in <i>Ulva lactuca</i>
Na	87-244	188-196	552-4570
K	1184-2718	942-1444	630-1970
P	4-3157	79.28-131.12	93-150
Mg	92-961	343-485	940-3900
Ca	91-644	1900-2380	1700-2700
Mn	6-1356	442-512	-
Fe	5-5468	3000-3686	250-820
Zn	2-2254	114-172	-
Cu	39-130	67-87	-

The purpose of this paper was to capitalize on wine and marine by-products to obtain organic fertilizers with applicability in viticulture. After drying and shredding, green algae, brown algae, *Rapana sp.* shells, fermented pomace, unfermented pomace and grapevine canes were combined in different proportions (Table 2). In 2019, these mixtures were tested in the greenhouse, being applied on four types of crops: *Vitis vinifera* L. (Pinot Gris cultivar), *Lolium perenne* L., *Sinapis alba* L. and *Trifolium repens* L. From the 7 variants, 2 were selected based on the performances obtained on the soil quality, V2 and V5, which were applied in an experimental field cultivated with the grapevine cultivar for red wines

with Protected designation of origin Murfatlar, 'Feteasca neagra' (Artem *et.al.*, 2021) in the conditions of the year 2020.

Table 2. Proportion of marine and wine by-products in the component of organic fertilizers

Variant	Marine by-products			Wine by-products		
	Green algae (g)	Brown algae (g)	Shredded shells (g)	Fermented pomace (g)	Unfermented pomace (g)	Grapevine canes (g)
V1	35	35	35	35	35	35
V2	70	35	35	35	35	35
V3	35	70	35	35	35	35
V4	35	35	70	35	35	35
V5	35	35	35	70	35	35
V6	35	35	35	35	70	35
V7	35	35	35	35	35	70

As the quality and quantity of the harvest are determined to a large extent by the annual climatic conditions, the upward trend of the air thermal values observed in the last two decades has determined changes in the metabolism and processes of growth and development of the grapevine. During the study year 2020, the average air temperatures were 2.5°C higher than normal (average temperatures over the last 20 years). Among all ecological factors, water plays a key role, being the most important constituent of the vegetative organs of the grapevine, ensuring numerous metabolic functions, participating in biochemical reactions and the transport of the synthesized products. During the studied period, the distribution of precipitation was uneven, but the average amount reached values close to normal, as there was an alternation of months with heavy rains (June) with periods of severe drought (January, April, May, July). It should be noted that during the vegetation period (April, May, June) the grapevine's need for water is higher, especially in the budding, shoot development, flowering and fruit set phenological stages. In 2020, during the mentioned period, the amount of precipitation was very small, which led to the weak development of the shoots and grape bunches.

MATERIALS AND METHODS

Sampling

The experimental plots are placed on a parcel with a N-S exposition, a slope of 3-5%, and soil of chalky chernozem type, with clay texture. The vine is planted on the rootstock *Vitis berlandieri* x *V. riparia* Oppenheim 4 selection, clone SO₄, and trained with trunks of 70 cm and mid-wire bilateral cordons, with cane pruning. The density of the plantation is 4132 vines/ha, with 2.2 m between rows and 1.1 m between trunks. Each experimental plot consisted of 3 rows of vines for each variant and repetition. There were 3 experimental variants: control variant (unfertilized), V2 and V5, which are presented in table 2. Fertilizers were placed in the soil at a depth of 0-20 cm at each trunk at the end of February, for about 21 trunks/variant, in three repetitions. Climatic data were recorded with the meteorological station (Weather Master 2000, Envirodata, Australia) owned by the Murfatlar Research Station for Viticulture and Oenology, and encompass daily observations regarding maximum and minimum temperatures, as well as precipitation. For three months (May-July) weekly measurements were made on the length of the shoots, calculating the average growth on each shoot/trunk/variant. The biosynthesis of chlorophyll and carotenoids pigments was measured and starting with August, determinations were made in the dynamics of grape maturation, by determining the content of the soluble substance (Brix degrees), the total

acidity (g/l H₂SO₄), and the weight of 100 berries. In October, after the grapes were harvested, soil samples were taken and physicochemical determinations were made at OSPA Constanta.

Determination of soil

The analyses carried out on soil samples were: pH, determined in aqueous suspension using a combined glass-calomel electrode; the content of the humic substance, determined by the Walkley-Black method modified by Gogoasa; the mobile forms of phosphorus and potassium soluble in ammonium acetate lactate solution (AL) at pH 3.75 by the Egnèr-Rhiem-Domingo method, determined by spectrometry or flamephotometry, respectively. The total soluble salts were determined by the Juste Pouget method and conductometrically, using a pH Cond7.07 glass electrode and a conductometer, respectively.

Determination of chlorophyll

Leaf samples were prepared with a laboratory homogenizer, using about 0.1 g fresh material. Extraction solutions were used for each sample: 80% acetone. The extraction ratio was 1:10. Homogenized mixture is separated by centrifugation at 3000 rpm, for 10 minutes. The analytical determination was performed with Helios α spectrophotometer at the following wavelengths: 663 and 645 nm, for chlorophyll a and b (according to each extraction solvent) and 470 nm for total carotenoid (Sikandar *et.al.*, 2018). Equations used for calculation (Arnon, 1949) are presented below:

$$\text{Chlorophyll a } (\mu\text{g/g}) = (12.75 * A_{663}) - (2.6 * A_{645}) * Q \quad (1)$$

$$\text{Chlorophyll b } (\mu\text{g/g}) = (22.9 * A_{645}) - (4.68 * A_{663}) * Q \quad (2)$$

$$\text{Total carotenoid content (Cx+c)} = 1000 A_{470} - 1.90 \text{ Chll a} - 63.14 \text{ Chll b}/214 \quad (3)$$

$$\text{Total Chll} = \text{Chll a} + \text{Chll b} \quad (4)$$

$Q=V/W$, where V is the volume of acetone in ml, W is the weight of sample in mg and A is the solution absorbance at a given wavelength.

Determination of grapes

The dynamics of grape ripening were observed starting with August 18, every 7 days until harvest, determining three analytical parameters: weight of 100 berries (g), soluble content (° Brix) and total acidity (g/L sulfuric acid). The soluble solids content (°Brix) was obtained by direct reading using an electronic refractometer (ATAGO, model SMART). Titratable acidity (g /l H₂SO₄) was determined by titration with 0.1 N NaOH. The weight of berries was obtained by using a technical balance and reporting the result for 100 berries.

Statistical analysis

Data were expressed as the mean $\pm$ standard deviation. One-way analyses of variance of means (ANOVA) have been performed using SPSS Version 16.0 Statistical Package for Windows (SPSS Corporation, Chicago, IL), 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 significant.

RESULTS AND DISCUSSION

The values regarding the growth of the vine shoots are conditioned by the climatic factors from the vegetation period, the vigor of the cultivar and by the applied fertilizers. Analyzing the obtained data represented in figure 1, the influence of the applied fertilizers on the development of the vine can be observed. The highest values recorded regarding the length of the shoots were for V2 (43.3 cm) and V5 (39.7 cm) at the end of July, higher values compared to the untreated control, which recorded lengths of 30.4 cm. Similar results, in which the length of the shoots increased following the application of biofertilizers based on

algae, were obtained by Popescu G. and Popescu M. (2014). We observe a more obvious increase in shoots length starting with June, which is due to the 73.4 mm of precipitation, which facilitated the access of fertilizers to the vine roots. It should be noted that in April and May, the cumulative rainfall was only 29.1 mm, compared to the normal 83.7 mm, which led to a slowdown in the growth process for the shoots.

Figure 1. Graphical representation of the growth of vine shoots on experimental variants

The nutrients from the organic fertilizers have improved the biosynthesis of the main compounds of primary metabolism (chlorophyll pigments and carotenoids) in the leaves, as can be seen in Table 3.

Table 3. Dynamics of the biosynthesis of chlorophyll pigments and carotenoids on fertilized variants

Sample collection date	Variant	Chlorophyll a (µg/g)	Chlorophyll b (µg/g)	Total Chlorophyll (µg/g)	Carotenoid pigments (µg/g)	Total assimilatory pigments (µg/g)
03 June	Control	139.5	50.7	190.2	603.7	793.9
	V2	163.7	59.0	222.6	698.7	921.4
	V5	139.8	49.4	189.1	570.5	759.6
03 July	Control	207.4	101.0	308.4	867.8	1176.3
	V2	212.9	106.0	318.9	889.9	1208.7
	V5	208.8	105.5	314.2	885.3	1199.5
03 Aug.	Control	219.4	126.7	346.0	970.3	1316.4
	V2	223.4	135.3	358.7	1015.7	1374.4
	V5	222.2	138.4	360.5	1019.9	1380.5
03 Sept.	Control	197.5	90.1	287.6	819.2	1106.7
	V2	219.7	129.6	349.3	996.7	1346.1
	V5	208.8	106.9	315.7	896.03	1211.73

The content of chlorophyll varied throughout the studied period in the range of 163.7-223.4 µg/g in variant V2, between 139.7-222.2 µg/g for variant V5 and between 139.5-219.4 µg/g for untreated control. Higher values were recorded for all variants in August, falling between 219.4-223.4 µg/g. Regarding the evolution of carotenoid pigments, they showed values in the range of 570.5-1019.9 µg/g, the highest values being recorded in August at variant V5, followed by a very small difference from variant V2. The total content of photosynthetic pigments in the last month of the determinations showed the highest values in variant V2, followed by V5, both with significantly higher values compared to the

unfertilized control. A study by Nistor *et al.*, (2014) also reported an improvement in chlorophyll pigments in vines following the application of grape pomace as a biofertilizer.

Data on the dynamics of grape ripening are presented in Figure 2. The increase in sugar content was intense in August for all the studied variants, and the accumulation of sugars was different, depending on the applied fertilizer and the climatic conditions. Thus, the period of sugar accumulation was very hot, in which maximum temperatures were frequently recorded, with an average between 32 and 33°C, justifying the high values of sugars content at harvest time, of over 24°Brix for all the studied variants. The decrease of the total acidity in the maturation process started and evolved rapidly, being conditioned, also, by the climatic conditions. At the beginning of the period, the values of the total acidity were situated between 7.91 and 8.63 g/L sulfuric acids, in a very short time (at harvest), the values of this parameter decreased very much, reaching values between 2.73 and 3.85 g/L sulfuric acid.

The weight of 100 berries, along with sugars content and total acidity complete the characterization of the quality of the grapes at harvest. The highest values for the weight of 100 berries were recorded at harvest in variant V5 with 111.2 g, with a 23.28% increase compared to the unfertilized control, followed by variant V2 with 102.9 g, with a 14.08% increase compared to the control variant. Colapietra and Alexander, (2006) obtained similar results following the application of an algae-based biofertilizer, namely an improvement in grape quality in terms of sugar content and average grape weight.

Figure 2. The quality of grapes on experimental variants: C (Control, unfertilized), V2, V5

The average grape weight represents a very important parameter that constitutes an element of productivity and quality for the vine, being one of the determining factors in the production of grapes per trunk and, implicitly, of the production per unit area. Grape production was variable, as can be seen in Table 4; a significant increase was registered in the case of both fertilizing variants, the increases being 54.76% for the V2 variant (6.5 t/ha) and of 33.3% in the case of the V5 variant (5.6 t/ha). Analyzing the changes produced by the application of the two organic fertilizers on the physicochemical properties of the soil, compared to the unfertilized control, the beneficial effect is easily observed, being statistically significant.

Table 4. The characteristics of the technological indices on the studied variants

Variant	Average bunch weight (g)	Average 100 berries weight (g)	Average number of berries/bunches	Average rachis weight (g)	Yield (t/ha)
C	90.91±8.2 (b)	85.82±6.2 (b)	103.63±5.9 (a)	5.09±1.1 (a)	4.2±0.5 (b)
V2	110.83±9.7 (a)	105.66±5.7 (a)	98.77±4.2 (a)	5.14±1.5 (a)	6.5±0.8 (a)
V5	100.45±7.6 (ab)	95.61±3.1 (ab)	86.32±4.8 (b)	4.84±1.0 (a)	5.6±0.4 (a)
Fertilizer influence	*	*	*	ns	**

Average values $\pm$ 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. Significance regarding the location and maceration time factors is noted with stars, in accordance to the p -value used for calculation: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns= not significant.

The two fertilizers applied did not produce any negative effect on the soil pH, which remained at neutral values (7.7). Regarding the salt content, we observe a significant increase compared to the control, the increase is 58.8% in the case of variant V2 and 93.6% in the case of variant V5. Regarding phosphorus and potassium, two essential elements necessary for the development of the vine, it can be stated that the application of fertilizers has led to positive effects on the soil (Table 5). For both nutrients, there was an increasing trend compared to the control, classifying the soils in the category of those with very good nutrient supply. The increase for phosphorus was 135.5% for V2 and 68.2% for V5. In the case of potassium, the same growth trend of 76.7% compared to the control in the case of V2 and 35.4% in the case of the V5 variant can be observed. The values for humus registered slight increases, on average of 10.2%, insignificant from a statistical point of view.

Table 5. Physico-chemical characteristics of the soil under the influence of organic fertilizers

Experimental variant	pH (H ₂ O)	Salts (mg/100 g soil)	Humus content (%)	P ₂ O ₅	K ₂ O	CaCO ₃ (%)
				mobile form mg/100 g soil		
Control	7.7±0.1 (a)	41.1±2.8 (c)	2.65±0.3 (a)	58.5±4.2 (c)	155±10.3 (c)	1.1±0.1 (b)
V2	7.7±0.1 (a)	65.3 ±2.0 (b)	2.79±0.4 (a)	137.8±12.5 (a)	274±18.2 (a)	1.9±0.2 (a)
V5	7.8±0.1 (a)	79.6±2.2 (a)	3.05±0.6 (a)	98.4±10.4 (b)	210±15.1 (b)	0.5±0.1 (c)
Fertilizer influence	ns	***	ns	***	***	***

Average values $\pm$ 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. Significance regarding the location and maceration time factors is noted with stars, in accordance to the p -value used for calculation: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns= not significant

CONCLUSIONS

The research conducted in 2020 on the influence of organic fertilizers obtained by combining marine and wine by-products on viticultural soils has shown, on the one hand, significant increases in biochemical compounds involved in plant growth and development, as well as grape yield and quality, and on the other hand, it led to the improvement of soil in terms of essential nutrients (phosphorus and mobile potassium) and organic matter (humus). The combination of wine and marine by-products in the form of organic fertilizers

is an effective alternative to chemical fertilization but also a valuable tool for achieving a double goal: improving soil quality and reducing environmental impact.

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REFERENCES

1. Artem, V., Negreanu-Pirjol, T., Ranca, A., Ciobanu, C., Abduraman, A., Coroiu, V. and Negreanu Pirjol B.S. (2021). Experimental studies on the residual marine and viticultural bioresources valorization for new organic fertilizers. U.P.B. Sci. Bull., Series B, vol. 83, Nr.2, pp:65-76.
2. Balasubrahmanyam V.R., Eifert J. and Diofasi L. (1978). Nutrient reserves in grapevine canes as influenced by cropping levels. *Vitis*, 17(1): 23–29.
3. Bojan, A., Jančíková, S., Dordevi, D. and Tremlová, B. (2020). Grape Pomace Valorization: A Systematic Review and Meta-Analysis. *Foods* 9 (1627): 2-20.
4. Breure, M.S. (2014). Exploring the potential for using seaweed (*Ulva lactuca*) as organic fertilizer. Doctoral dissertation, MSc Thesis Plant Production Systems. PPS-80436, Wageningen University. Netherlands.
5. Chbani, A., Majed, S. and Mawlawi, H. (2015). Mineral content of mediterranean seaweeds, *Padina pavonica* L. (*Pheophytae*, *Ulva lactuca* L. and *Ulva linza* L. (*Chlorophytae*) for fertilizing use. *International Journal of Horticultural Science and Technology*, 2(2): 133-140.
6. Colapietra, M. and Alexander, A. (2000). Effect of foliar fertilization on yield and quality of table grapes. In V International Symposium on Mineral Nutrition of Fruit Plants 721, pp. 213-218.
7. Davies, G. and Lennartsson, M. (2005). Organic vegetable production. A complete guide. Henry Doubleday Research Association, London
8. Erana, F.G., Tenkegna, T.A. and Asfaw, S.L. (2019). Effect of agro industrial wastes compost on soil health and onion yields improvements: study at field condition. *Int J Recycl Org Waste Agric* 8:1–11.
9. García-Lomillo, J. and Gonzalez-San, J. (2017). Applications of wine pomace in the food industry: approaches and functions. *Compr. Rev. Food Sci. Food Saf.* 16:3-22.
10. Marin, O. and Timofte, F. (2011). Macrophytes on the Romanian Sea Shore. Edit. Boldaş, Constanța
11. Nistor, E., Dobrei, A., Dobrei, A., Kiss, E. and Ciolac, V. (2014). Grape pomace as fertilizer. *Journal of horticulture, forestry and Biotechnology*, 18(2):141-145.
12. Oliveira, M., Duarte, E. (2016). Integrated approach to winery waste: waste generation and data consolidation. *Front. Environ. Sci. Eng.* 10 (1): 168-176.
13. Panayotova, V. and Stancheva, M. (2013). Mineral composition of marine macroalgae from the Bulgarian Black Sea Coast. *Scripta Scientifica Medica*, 45(1):42-45.
14. Popescu, G.C., Popescu, M. (2014). Effect of the brown alga *Ascophyllum nodosum* as biofertilizer on vegetative growth in grapevine (*Vitis vinifera* L.). *Curr. Trends.Nat. Sci.*, 3(6):61-67.
15. Rathore, S.S., Chaudhary, D.R., Boricha, G.N., Ghosh, A., Bhatt, B.P., Zodape, S.T., Patolia, J.S. (2009). Effect of seaweed extract on the growth, yield and nutrient uptake of soybean (*Glycine max*) under rainfed conditions. *South African Journal of Botany*, 75(2): 351-355.
16. Saha, S., Mina, B., Gopinath, K., Kundu, S., Gupta, A. (2008). Relative changes in phosphatase activities as influenced by source and application rate of organic composts in field crops. *Bioresource Technology* 99: 1750-1757.
17. Sereanu, V., Feraru, D.L., Predeanu, G., Meghea, A. (2017). Microstructure and chemical characterization of *Rapana thomasiana* shell. *Scientific Bulletin Series C-Electrical Engineering and Computer Science*, 79(1): 81-92.
18. Sikandar, H., Husain A., Kaili, R., Muhammad, A., Zhihui, C. (2018). Response of tomato growth to foliar spray and root irrigation of aqueous garlic extract; a cocktail of antioxidative defenses, chlorophyll, carotenoid and soluble sugar contents. *International Journal of Agriculture & Biology*, Vol 20 (6): 1251-1259.
19. Zahira, Y., Ehsan, A., Afifi, Z., Masita M., Mohd, S.T. (2014). An overview: biomolecules from microalgae for animal feed and aquaculture. *Journal of Biological Research-Thessaloniki* volume 21, (6):113-123.
20. Zhu, L., Wang, Z., Shu, Q., Takala, J., Hiltunen, E., Feng, P., Yuan, Z. (2013). Nutrient removal and biodiesel production by integration of freshwater algae cultivation with piggery wastewater treatment. *Water Res.* 47, 4294–4302.