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ENVIRONMENTAL IMPACT OF VITICULTURE: BIOFERTILIZER INFLUENCE ON PRUNING AND WINE WASTE

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Abstract

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The global wine and table grape industry, with annual sales exceeding US$100 billion, generates large quantities of cane pruning waste each year. This pruning is usually composted or burned as waste, often with a net cost incurred to the winery. Microorganisms, soil organisms or enzymes can degrade the "end-use" grapevine waste. In the European Waste Catalogue grapevine, waste is indexed as plant waste and as a part of biowaste. Since grapevine waste is subject to the aerobic treatment process, it is compost. Grapevine waste is subject to an anaerobic treatment process or treated by other methods, including mixtures with other materials like grape mark before/after treatment.

The aim of this paper is to investigate quality and quantity of the compost types made of pruning waste, grape mark, and the mixture of both. Preparation of microbial fertilizers consists of the *Bacillus* and *Pseudomonas* straining and their placement, at an adequate amount, in all assemblage. Results indicate that the microbial biomass causes the improvement of natural degradation.

Key words: viticulture, biofertilizer, waste

Introduction

Since agricultural secondary products/waste are barely used, highly-valuable substances, which separate during the natural or industrial procedures and which may be re-implemented in the production or as a supplement in human or animal nutrition, are thus ignored.

Efficient extraction and commercial implementation of these components may help carry out sustainability of the given production facilities, in this case vinyards and wine cellars (Das and Singh, 2004). So, for example, the total wine and table grapes production, with the basic value exceeding 100 billion dollars, guarantees a large quantity of pruned vine every year. However, the pruned mass is frequently burned, thus increasing expenses in the vineyard. In avereage, there is about a ton (USEPA, 1995) of the pruned vine mass while nearly 7,500,000 hectares are used for the world grape production each year (Prices STAT-FAOSTAT). Grapes are fruit crops grown widely in many areas of the world and 71% of the fresh grapes produced are used for wine production. During wine making, large quantities of wastes such as grape marc (the residue after pressing for white wines or vinification for red wines) and stalks are produced as by-products. The placing of the enormous amount of waste grape marc generated all over the world is an increasing problem. By processing 100 kg of grapevine approximately 20-25 kg of grape marc is produced. For example, in Europe, alone 112 million tons of grapes were processed by the wine industry in 1998, and an estimated 13% (14.5 million tons) of this amount corresponded to the by-product after pressing, consisting mainly of skins and seeds (Radovanovic et al., 2009).

Potential pruned vine utilization is very high, from compost to anti-phytopathogenic treatment extract, which contributes to a better grape production and processing sustainability. When decomposed, lignin, the major component of composite carbohydrates in agro-industrial waste, produces polycyclic aromatic hydrocarbon components such as benzopyrenes, catechols, hydroquinones, phenanthrenes and naph-

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thalenes if decomposed by heat. All these components can inhibit DNA synthesis caused by a carcinogen tumor of liver, lungs, throat and cervix in people and animals.

From the surface of 30 000 ha in Serbia, estimated as productive (www.stat.gov.rs), nearly 30 000 tons of pruned vine/ dry waste and about 74 690 t of wet waste are produced after grapes have been processed into wine, and that 62% marc, 14% lees, 12 % stalk and 12% waterwaste. Pomace or grape marc is made during the processing procedure, and it is mainly deposited in the open space. Grape marc can be used as fodder, especially during the winter when there are no pastures. A high price of labour and transportation expenses frequently limit utilization of direct advantages of this bioproduct (Sanchez et al., 2002). Plant material residue is recognized as biowaste (Europen Waste Catalogue, 2001). The best possible effect is attainable through its repeated intake in the soil, since that is the way to achieve optimum ballance among plant/vine growing, soil structure, and the supply of water, nutritions, oxygen and biomass of microorganisms. Soil of a good structure increases the number and diversity of organisms, decreases development of harmful units and promotes decomposing processes from organic substances to minerals (Raicevic et al., 2004). A stable organic mass is the leading factor for improvement of the soil structure and fertility (White, 2003; Sivcev et al., 2010). To make soil fertile would be the basis of organic system while organic substance supply might be the most important factor for the soil fertility preservation. Therefore, biodiversity of microorganisms, plant nutrients, sustainable water capacity, aggregate supply and land erosion control depend upon quality and quantity of the organic substance in the soil (Kandeler et al., 2005). Thus, it is necessary to determinate the abundance of microorganisms, because they are, after plants, most important biological agent in agricultural systems (Sarapatka, 2003). In addition, most important indicators of microbiological properties are microbial biomass, microbial activity and diversity, and enzyme activity (Sikora et al., 1995). Microorganisms supply most of the enzymes activity, due to their high metabolic activity, large biomass and fast reproduction (Speir and Ross, 1978)). Because of their role, one of the most studied enzymes is dehydrogenase and phosphomonoesterase. Dehydrogenase is good indicator of biological activity (Makoi and Ndakidemi, 2008) and act on alcohols and hemiacetals, while phosphomonoesterase catalyzed hydrolyze of esters and anhydrides of H_3PO_4 (Schmidt and Laskowski, 1961) and play key roles for fertility (Dick et al., 2000).

The present paper work is to investigate quality of the biowaste: pruned and chopped up vine, marc (pomace) after the grape processing and the mixture composed of the proportional amount of pruned vine and marc within natural conditions, including the activity of two groups of microorganisms *Bacillus* and *Pseudomonas.*

Materials and Methods

The experiment has been carried out near the vineyard, in three seedbeds of the size 10 x 2 x 0.2 m. Ripe pruned vine was put in one seedbed, marc in another (grape marc) deposited in the heap from the 2008 harvest, while mixed chopped up ripe vine and marc were, at the same proportion, put in the last seedbed. Each seedbed was provided with the mixture of microorganisms *Bacillus* (10^6 ml^{-1}) and *Pseudomonas*(10^6 ml^{-1}). The seedbeds were set in March 2009, within completely natural conditions. The mixture would be stirred from time to time to provide aerobic conditions for decomposition. There is a climatological station on the Faculty of Agriculture's Experimental School Estate «Radmilovac» (Altitude 130 m, Latitude 44°45′N, Longitude 20°35′E), near which the experiment has been set. Since the data for September and October are missing, data from the first-class meteorology station, 5 km away from the vinyard, have been used instead. Data on average monthly air temperature values, relative air humidity and precipitations are presented in Figures 1 and 2.

Seedbed samples were taken in Autumn 2009, Spring 2010, Autumn 2010, and then the analysis of microbiological presence was carried out. Microbial characterization of samples was carried out through the use of nutrient medium 0.1xTSA (for total bacteria) and Fyodorov`s medium for determination of *Azotobacter* sp. The samples were prepared by the dilution method. All experiments were performed in triplicate. The total microflora incubation period was five days at 30°C and two days at 30°C for *Azotobacter* sp. The population densities were recorded as colony forming units (CFU g^{-1}) of dry samples. In autumn 2010, the following characteristics were determined: pH value, the content of calcium, humus, the total content of ammonium and nitrate forms of nitrogen, the content of available phosporous and potassium as well as the proportion of carbon and nitrogen (C/N), all by means of the standard methods (Jakovljevic et al., 1998).

Dehydrogenase activity was determinate using by Casida et al. (1964) method, while activity of phosphomonoesterase according to Tabatabai (1994).

Results and Discussion

The wine industry uses large amounts of chemical fertilizers and organic substances (Kammerer et al., 2005; Arvanitoyannis et al., 2006). Prevention of possible vineyard crop diseases is a good way to present composting as

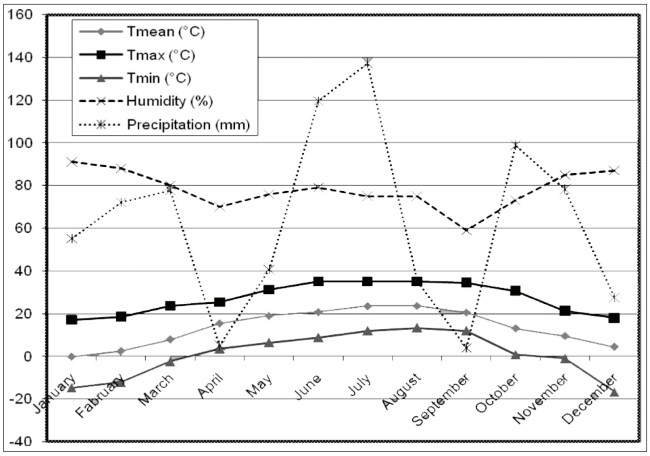

Fig. 1. Mean air temperature and sum of precipitation in the year 2009

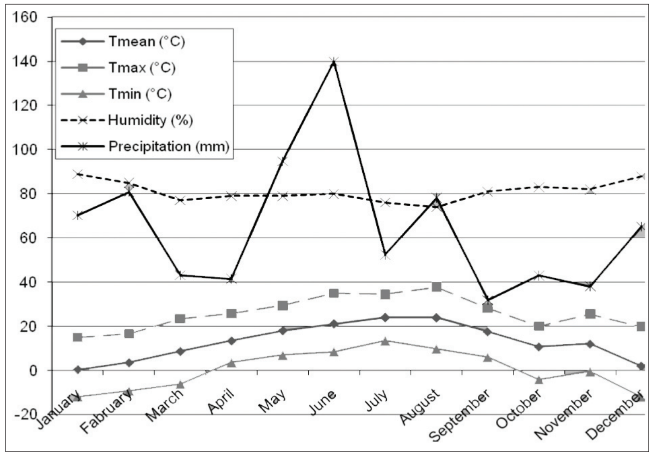

Fig. 2. Mean air temperature and sum of precipitation in the year 2010

the most suitable process for the organic waste reuse in the wine industry in vinayard crops (Ruggieri et al., 2009).

The experiment results indicate different presence of the total number of bacteria, depending on the transformation process duration. Bacteria were the most numerous in Spring 2010, which indicates that the most intensive transformation processes took place during that time (Table 1). Bacteria were the least numerous within the comminute pruning wood type of waste, while they were the most numerous within the pruning wood and grape marc mixture.

There were no considerable fluctuations in the number of *Azotobacter* among different treatments in Autumn 2009 and 2010, while the fluctuations were obvious in Spring 2010 (Table 2). *Azotobacter* were the least numerous within the comminute pruning wood type, while they were the most numerous within the 1:1 proportion of pruning wood and grape marc mixture. Bacteria were more numerous within the 1:1 proportion of pruning wood and grape marc mixture due to the larger quantity of available carbon and higher aeration. There was a gradual drop in pH value, which was mildly neutral. The carbonate content was low (Table 3).

There were no considerable fluctuations in the total nitrogen content among different treatments, while there was the highest presence of ammonium nitrogen and available phosporous within the 1:1 proportion of pruning wood and grape marc mixture. The highest content of nitrate nitrogen and available potassium was recorded within the grape marc type.

Table 1
Total bacterial number (CFUx10^6 g^{-1}) in different types of waste

Type of waste	Comminute pruning wood	Grape marc	Mixture 1:1 pruning wood and grape marc	Average
Autumn 2009	3.2	6.13	10.0	6.44
Spring 2010	5.8	31.6	75.33	37.58
Autumn 2010	4.23	4.88	5.96	5.02
Average	4.41	14.20	30.43	-

Table 2
Number of *Azotobacter* sp. (CFUx10^3 g^{-1}) in different types of waste

Type of waste	Comminute pruning wood	Grape marc	Mixture 1:1 pruning wood and grape marc	Average
Autumn 2009	57.3	66.5	62.9	62.2
Spring 2010	18.3	56.2	75.3	49.9
Autumn 2010	38.88	47.51	42.81	43.0
Average	38.16	56.7	85.4	-

As for the C/N proportion values, there were no considerable differences among the treatments, but the most important ones were recorded within the comminute pruning wood type. When organic matter passes through successive cycles of decomposition, the C/N ratio becomes smaller (White, 2003). The previous statement has been confirmed by our results: the C/N ratio of decomposers in well drained soils with pH~7 is close to 10. It includes the timely biodegradation with maintenance and continuous creation of high quality humus.

Dehydrogenase activity has been used as a parameter of compost stability (Garcia et al., 1992; Tiquia et al., 2002). As can be seen from Table 4, dehydrogenase activity is highest in mixture 1:1 type of waste during the composting process. The results were in accordance with data of total microflora. During the composting, increasing of dehydrogenase activity was recorded, except in comminute pruning wood between autumn 2009 and spring 2010. Wolna-Maruvka and Sawicka (2009) obtained similar values of dehydrogenase activity. However, curve of dehydrogenase activity changes during composting period differs from our results. During green waste composting, Gazi et al. (2007) were noticed the fluctuation of dehydrogenase activity values, which is in concordance with our results. In their research high values of dehydrogenase activity was observed at the end of maturity stages, which is similar with our investigation.

The results presented in Table 5 showed the fluctuation in phosphatase activity during composting. Values of acid phosphatase activity were highest in the end of composting period,

Table 3
Chemical characteristics of different types of waste

Type of waste		Comminute pruning wood	Grape marc	Mixture 1:1 pruning wood and grape marc
pH	H_2O	7.11	7.18	7.51
	KCl	6.26	6.43	6.93
$CaCO_3$ (%)		0.4	0.1	0.8
Humus (%)		2.71	2.37	2.12
Total N (%)		0.164	0.157	0.147
NH_4^+	mg kg^{-1}	126	147	161
NO_3^-	mg kg^{-1}	7.0	10.5	7.0
P_2O_5	mg 100 g^{-1}	13.8	17.0	21.4
K_2O	mg 100 g^{-1}	20.0	28.7	25.6
C/N		9.6	8.8	8.3

Table 4
Dehydrogenase activity of samples (µg TPF x g^{-1} x h^{-1})

Type of waste	Comminute pruning wood	Grape marc	Mixture 1:1 pruning wood and grape marc
Autumn 2009	4.42	2.97	5.60
Spring 2010	3.81	4.86	6.02
Autumn 2010	12.94	11.70	16.71
Average	7.06	6.51	9.44

Table 5
Phosphatase activity of samples (µg p-nitrophenol x g^{-1} x h^{-1})

Type of waste		Comminute pruning wood	Grape marc	Mixture 1:1 pruning wood and grape marc
Autumn 2009	acid	1.77	1.57	1.67
	alkaline	2.00	1.57	2.20
Spring 2010	acid	1.23	1.43	1.80
	alkaline	1.73	1.37	2.03
Autumn 2010	acid	7.07	7.67	3.07
	alkaline	1.33	2.60	2.23

while alkaline phosphatase activity values differs during the composting process and were controlled by type of samples.

In the comminute pruning wood was noticed decreasing of enzyme activity during composting, in grape mark increasing of its activity and in mixture 1:1 pruning wood and grape mark were recorded the minor changes in enzyme activity.

Composting helps manage large quantities of organic wastes in a sustainable manner. It is one of the technologies of integrated waste management strategies, used to recycle organic materials into a useful product (Giglotti et al., 2005).

Sustainable organic mass is the leading factor for improvement of the soil structure and fertility (Sivcev et al., 2006). The previous statement is confirmed by the content of humus, since its values get close to the vineyard optimum value in all three treatments. Generally, our results are in accordance with the ones acquired by Biala (2000). He has taken into account several results and showed the average vegetative yield and grape yield value obtained in Europe and Australia. The microorganisms decompose organic matter while their activity and enzymes, created during these processes, contribute to easier absorption of nutritious elements by vine and other plants.

Conclusions

It takes 16 months under the natural conditions to get comminute pruning wood and grape marc transformed into compost. It is characterized by high nutrient absorption capacity with nutrient availability. The highest compost/biofertilizer microbial activity is recorded in Spring, within the medium precipitation conditions in all three treatments. Chemical analysis of the compost has indicated the coherent relation of the examined components. Differences among the treatments/biofertilizers are not statistically relevant, and all three treatments have become important for the living environment preservation and conclusion of the grape and wine production cycle. The results indicate the possibility to obtain the products from viticulture applicable in agriculture.

Thus, it is possible to contribute to the global awareness of the municipal waste composting and to obtain different compost products from the Balkans agriculture.

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