Technical report on ZEOWINE composting process

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Action B.1
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SOMMARIO

SUMMARY

Wine supply chain produces a considerable amount of organic wastes which remain as residues in the ecosystem. The environmental-friendly management of agricultural soils, usually involves the application of organic amendments. In addition, several recent researchers proposed the use of soil conditioners, such as zeolite, to improve the physical and chemical properties of agricultural soils.

In this context, with the aim of improving the agronomic value of compost, and simultaneously achieving sustainable recycling of organic wastes, we developed a vine management system based on the composting of winery organic wastes and zeolite to obtain a fertilizer to be used for vineyard soils.

The innovation of this composting process lies in the role of the natural zeolite on winery wastes valorization and recycling. In particular, we monitored the response of chemical and biochemical properties to different rates of zeolite on winery waste composting process.

The winery waste material (pomace and stems), was composted in presence of natural clinoptilolite zeolite at the following rates: 1) zeolite 0% : wastes 100% (0%, control); 2) zeolite 10% : waste 90% (ZEOWINE 1:10); 3) zeolite 30% : waste 70% (ZEOWINE 1:2.5).

The experimentation was set up in two areas in Tuscany (Central Italy): CMM-San Miniato and Col D'Orcia-Montalcino, characterized by a typically Mediterranean semiarid climate.

The presence of zeolite at the two rates (10% and 30%) during the composting of winery wastes enabled an higher retention of nutrients, enzymatic activity and humification of organic matter with respect to control compost. In addition, it decreased the electrical conductivity and increased the germination index, thus improving the agronomic quality of the compost.

INTRODUCTION

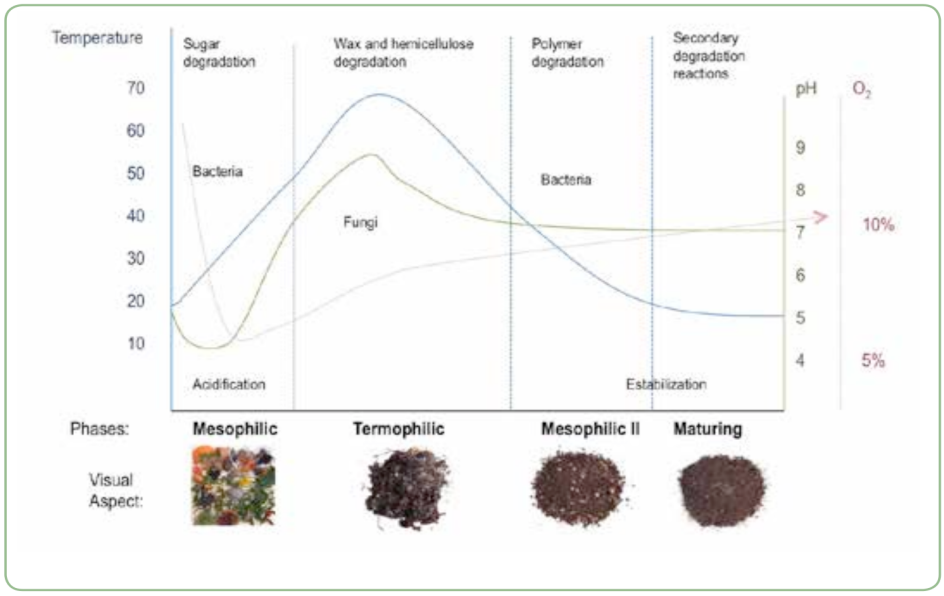
Grape is the leading fruit crop cultivated in the world (Food and Agriculture Organization of the United Nations, 2017). In 2018 the annual world production of wine was 292 million hectoliters and the world's agricultural land devoted to the production of wine was 7.4 kha (2019 Statistical Report on World vitiviniculture, 2019). The amount of solid organic wastes generated per wine's hectoliter is estimated to be about 25 kg (Lanfranchi et al., 2018), thus the yearly production of residues which requires to be treated and disposed of represents more than 7 million tons. In view of this, the wastes generated from the winery industry are addressed as a world growing environmental problem. The development of strategies to reduce the consumption of resources (energy, materials, chemicals) and the amount of waste released, by maximizing the recovery and recycling of by-products throughout a product's life cycle is acknowledged as an universal contemporary challenge (European parliament, June 2015).

However, in most grape growing states, grape marc, skin, stalk, and pomace from the winery supply chain are still treated as waste with little or no value; they are usually disposed of in open areas and are a source of environmental pollution due to the emanation of volatile organic compounds (VOC), the increase in chemical oxygen demand (COD), the presence of recalcitrant compounds and the free-run juices percolation (Rondeau et al., 2013).

Several studies described the suitability of composted winery residues as amendment for agriculture and for degraded soil restoration (Ferrer et al., 2001; Bertran et al., 2004; Gómez-Brandón et al., 2019; Pinter et al., 2019).

Composting of winery wastes and the integration of compost into the activities of the winery industry as amendment for vineyard soils have been also regarded as a recommendable waste management strategy to promote the increase in soil organic matter and to protect soil structure in usually poor vineyard soils (Paradelo et al., 2013).

Composting is based on the aerobic biological decomposition of fresh organic substrates, and the successive stabilization of the organic matter. During composting process it is possible to identify three different phases: a mesophilic phase, a thermophilic phase and a maturation phase. The initial mesophilic phase is characterized by a quick growth of fungi and bacteria, which mineralize simple organic compounds, such as sugars, producing CO_2, NH_3 and organic acids. The thermophilic phase is characterized by the biological produced heat and by the degradation of organic compounds, such as fats, cellulose and lignin. The high temperature in this phase (above 55 °C) allows to obtain a product free of pathogens and plant seeds. Finally, the maturation phase is characterized by the decrease in microbial activity, resulting from a decrease of biodegradable organic compounds, and by formation of stable humic substances (Martínez et al., 2016 and 2018). Despite of the benefits of composting process, there are also some negative aspects. N loss thorough ammonia volatilization is accounted for up to 50% of the initial total N and the released ammonia is considered as one of the main atmospheric pollutants during the composting process (Wang and Zeng, 2018; Chan et al., 2016).



MONITORING PARAMETERS

Since composting is a biological process carried out by microorganisms, parameters affecting their growth and reproduction should be taken into account. These factors include oxygen or aeration, substrate moisture, temperature, pH and the C:N ratio.

Externally, the composting process largely depends on environmental conditions, the method used, raw materials, and other elements, so that some parameters may vary. However, they must be under constant surveillance to always be within an optimal range. The parameters and their optimal ranges are listed below (FAO, 2015).

OXYGEN

Composting is an aerobic process and adequate ventilation should be maintained to allow respiration of microorganisms that release carbon dioxide (CO_2) into the atmosphere. Likewise, aeration prevents material to compact or fill with water. Oxygen requirements vary during the process, reaching the highest rate of consumption during the thermophilic phase.

Excessive aeration will cause a temperature drop and great loss of moisture by evaporation, causing the decomposition process to stop due to lack of water. The cells of microorganisms dehydrate, some produce spores and enzyme activity responsible for the degradation of the different compounds stops. Conversely, a low aeration prevents enough water evaporation, generating excessive moisture and an anaerobic environment. Odors and acidity are then produced by the presence of compounds such as acetic acid, hydrogen sulphide (H_2S) or methane (CH_4) in excess.

Aeration Percentage	Problem		Solutions
<5%	Low aeration	Insufficient water evaporation, generating excessive moisture and anaerobiosis environment	Volteo de la mezcla y/o adición de material estructurante que permita la aireación .
5% - 15% ideal range			
>15%	Excessive aeration	Drop in temperature and evaporation of water, causing the decomposition process to stop due to lack of water	Chop the material in order to reduce porous size and hence, aeration. Moisture should be regulated by adding water or fresh material or with more water content (fruit and vegetative scraps, grass, liquid manure and others).

MOISTURE

Moisture is a parameter closely related to microorganisms, because, like all living beings, they use water to transport nutrients and energy elements through the cell membrane.

The ideal moisture of the compost is around 50%, although it varies depending on physical condition, size of the particles and the composting system (see chapter on Particle Size). If moisture drops below 45%, microbial activity decreases, the degradation phases cannot be completed and hence, the resulting product is biologically unstable. If the moisture is too high (> 60%), water will saturate the pores and interfere oxygenation through the material.

The optimal moisture content for composting is 45% to 60% water by weight of the base material.

Moisture percentage	Problem		Solutions
<45%	Insufficient moisture	Can stop composting due to lack of water for microorganisms	Moisture should be regulated, either by adding water or fresh material with higher water content (fruit and vegetal waste, grass, liquid manure or others)
45% - 60% ideal range			
>60%	Insufficient oxygen	Too wet material, oxygen is displaced. Can develop zones of anaerobiosis.	Turn the mixture and/or add low moisture content material with high carbon content such as sawdust, straw or dry leaves.

TEMPERATURE

Ambient temperature has a wide range of variation depending on the phase of the process. Composting begins at ambient temperature that can rise up to 65°C with no need of human intervention (external heating). During the maturation phase temperature drops at ambient temperature.

It is desirable that temperature does not drop too fast, since the higher the temperature and the longer the time, the higher the decomposition rate and hygienization.

Temperature (°C)	Related causes		Solutions
Low temperature (ambient T < 35°C)	Insufficient moisture.	Low temperatures can occur by several factors, such as lack of moisture, so that microorganisms reduce the metabolic activity and therefore, temperature drops.	Wet the material or add fresh material with higher moisture percentage (fruit or vegetable waste or others)
	Insufficient material.	Insufficient material or inadequate pile shape to reach the appropriate temperature.	Add more material to the composting pile.
	Nitrogen deficit or low C:N ratio	The material has a high C: N ratio and hence, the microorganisms are lacking the necessary N to produce proteins and enzymes and slow down their activity. The pile takes more than week to increasing the temperature.	Add high N content material such as manure.
High temperature (ambient T >70°C)	Insufficient ventilation and moisture	The temperature is too high and the decomposition is inhibited, since most microorganisms are inactive and die.	Turn the mixture and/or add high C content material of slow degradation (wood or dry grass) to slow down the process.

PH

The composting pH depends on the source materials and varies in each phase of the process (from 4.5 to 8.5). In the early phases of the process, the pH was acidified by the formation of organic acids. In the thermophilic phase, due to the conversion of ammonium into ammonia, the pH rises, the medium is alkalised to finally stabilize at values close to neutral.

The pH determines the survival of microorganisms and each group has optimal pH for growth and multiplication. Most bacterial activity occurs at pH 6.0-7.5, while most fungal activity occurs at pH 5.5 to 8.0. The ideal range is from 5.8 to 7.2

pH	Related causes		Solutions
<4,5	Excess of organic acids	Plant materials such as kitchen waste, fruit, release many organic acids and tend to acidify the medium.	Add material rich in nitrogen until an appropriate C: N ratio is achieved.
4,5 – 8,5 ideal range			
>8,5	Excess of N	When there is excess of nitrogen in the source material, with poor C: N ratio related to moister and high temperatures, ammonia is produced and the medium is alkalised.	Add dry material with high carbon content (pruning, dry leaves, sawdust)

CARBON-NITROGEN (C: N) RATIO

The C: N ratio changes according to the parent material and the numeric ratio is obtained by dividing total C content (total C %) over the total N content (total N %), of the material to compost.
This ratio also varies throughout the process, with a continuous reduction from 35:1 to 15:1.

C:N	Related causes		Solutions
>35:1	Excess of Carbon	There is a large amount of carbon-rich materials in the mixture. The process tends to cool and to slow down.	Add nitrogen-rich material until an appropriate C: N ratio is achieved.
15:1 – 35:1 ideal range			
<15:1	Excess of Nitrogen	There is a higher amount of nitrogen-rich material in the mixture. The process tends to overheat generating odours from the ammonia released.	Add material with high carbon content (pruning, dry leaves, and sawdust).

ZEOLITE

Different compounds have been used as potential additives in order to decrease ammonia emission and improve the quality of compost. Several researchers showed that the use of natural minerals, such as zeolites, due to their unique physical and chemical properties, coupled with their abundance in sedimentary deposits and in rocks derived from volcanic parent materials, is an effective and practical approach for improving the quality of compost (Soudejani et al., 2019; Montalvo et al., 2020).
Zeolites are natural minerals belonging to the tectosilicates class, with a porous three-dimensional frameworks made of SiO_4 and AlO_4 tetrahedral. Due to their porous structure, high surface area, cation exchange and adsorption-desorption characteristics, zeolites are able to retain ammonium in a selective manner, thereby reducing losses of nitrogen to the environment (Ramesh and Reddy, 2011). Several studies have been showed the potential of zeolite in enhancing the rate of degradation and in reducing NH_3 losses during composting process (Jiwan et al., 2013; Chan et al., 2016)
As an example, Chan et al. (2016) observed a reduction in electrical conductivity, an improvement in compost maturity and an higher nitrogen content (reduction in ammonia volatilization of 18%) during "struvite" food waste composting by 10% zeolite amendment.
Similarly, in a composting process with sewage sludge, wheat straw and lime, the addition of zeolite at the rate of 30% drastically reduced the GHGs emissions, NH_3 loss and improved the maturity of compost

(Awasthi et al., 2016). Further, Wang et al. (2016) found that adding biochar and zeolite together can decrease the nitrogen loss and mitigate the emissions of ammonia and N_2O during pig manure composting. More recently, Peng et al. (2019) demonstrated that the addition of zeolite and superphosphate to windrow composting of chicken manure improved fertilizer efficiency and reduced greenhouse gas emission. Zeolite also increased porosity when used as a bulking agent in a domestic sewage sludge composting process (Villasenor et al.2011).
In addition, when applied to soil, compost modified with zeolite improved crop yield and water retention and prevented soil nutrient losses (Taheri-Soudejani et al., 2019).
However, even if previous works have focused on the use of zeolite and/or other additives in the composting process (Zorpas 2014; Tzia and Zorpas 2012), its application in the composting process has not been studied to date in winery waste composting.

COMPOSTING OBJECTIVES

One of the main objective of the LIFE ZEOWINE project was to demonstrate that the presence of zeolite into composting process of winery wastes is able of preserving nutrients content, stimulating the microbial activity and promoting humification, thus enhancing compost value.
In view of this, the present report is focused on investigating the evolution of physical, chemical and biochemical parameters during the composting process of winery supply chain residues in association with clinoptilolite-zeolite at different rates: 1) zeolite 0% : wastes 100% (0%, control); 2) zeolite 10% : waste 90% (ZEOWINE 1:10); 3) zeolite 30% : waste 70% (ZEOWINE 1:2.5).
The different rates of zeolite was tested in order to determine the combination that optimizes the performance of the composting process and improves the agronomic and environmental quality of the end-product.

MATERIALS AND METHODS

EXPERIMENTAL LAYOUT

In order to demonstrate the efficiency of zeolite-based compost in improving the composting process and the quality of the end product, in addition to the piles with zeolite and wastes (stalks, vine pruning and grape pomace) at the ratio 1:2,5 w/w foreseen by the project, 2 additional piles were set up at the first and second composting cycles at CMM (San Miniato): one with zeolite and wastes at the ratio 1:10 w/w and one with 100% of organic wastes as control without zeolite.
In particular, for the first composting cycle (start of composting 11/01/2019) the following piles were prepared at CMM:
- n. 3 piles of about 9 tons each with zeolite and vine wastes at the ratio 1:2.5 w/w;
- n. 1 pile of about 2 tons with zeolite and vine wastes at the ratio 1:10 w/w
- n. 1 control pile of about 2 tons with 100% of vine wastes.

On the basis of the results obtained from the first composting cycle, in the second composting cycle (start of composting 22/11/2019) the following piles were prepared at CMM:
- n. 2 piles of about 9 tons each with zeolite and vine wastes at the ratio 1:10 w/w;
- n. 1 piles of about 9 tons each with zeolite and vine wastes at the ratio 1:2.5 w/w;

- n. 1 control piles of about 9 tons with 100% of vine wastes.

Finally, in the last composting cycle at CMM (start of composting 12/12/2020) three piles of about 9 tons each with zeolite and vine wastes at the ratio 1:10 w/w were set up.

As envisaged by the project for each production cycle (2018, 2019 and 2020), 3 representative samples were collected from each pile. The samplings were carried out at the following times:
First composting cycle:
- beginning of the composting period (11 January 2019),
- end of the thermophilic phase (22 March 2019)
- end of the composting period (4 June 2019)
Second composting cycle:
- beginning of the composting period (22 November 2019),
- end of the thermophilic phase (22 February 2020)
- end of the composting period (21 May 2020)
Third composting cycle:
- beginning of the composting period (12 December 2020),
- end of the thermophilic phase (19 February 2021)
- end of the composting period (4 May 20121)

The composting protocol defined at CMM was transferred to the support partner Col D'Orcia where the following piles were prepared (start of composting 25/11/2019):
- n. 3 piles of about 30 tons each with zeolite and vine wastes at the ratio 1:10 w/w;
- n. 1 control pile of about 30 tons with 100% of vine wastes (stalk, vine pruning and **straw**)
At Col D'Orcia the pomace is used to produce spirit so that for the composting process it was replaced by straw.

As envisaged by the project, 3 representative samples were collected from each heap. The samplings were carried out at the following times:
- beginning of the composting period (25 November 2019),
- end of the thermophilic phase (21 January 2020)
- end of the composting period (23 April 2020)

Compost sampling:
Start of composting: 25/11/2019
End of termophilic phase: 21/01/2020
End of composting: 23/04/2020

The composting piles were prepared by mixing with mechanical equipment the organic residues with the zeolite with a granulometry 0.2-2.5 mm. This zeolite granulometry was selected to ensure better aeration of the piles during composting.
The characteristics of the zeolite used in the composting process are shown in Table 1. XRD analysis showed that the zeolite included about 85% clinoptilolite.

Table 1. Zeolite characteristics

	Zeolite
SiO_2 %	68.3±4.2
K_2O %	2.8±0.31
Na_2O %	0.75±0.07
Al_2O_3 %	12.3±1.03
Fe_2O_3 %	1.3±0.14
TiO_2 %	0.15±0.03
CaO %	3.9±0.25
MgO %	0.9±0.05
Loss on ingnition %	12.5±0.95
Ration Si/Al %	5.1±0.42
CEC $cmol_c\ kg^{-1}$	130±21

At each composting cycle, the composting processes were performed in a completely randomized factorial design. Periodical turning of each pile in order to increase the oxygen supply to microorganisms,

homogenize the materials and redistribute microorganisms, moisture and nutrients, was carried out at least twice a month, until the end of the composting process. Since microbial activity generates heat and evaporates water, the optimal moisture level of about 45% was maintained by sprinklers on top of each pile. Temperature and humidity were monitored every two days until the end of the composting process. A cover system of each pile was predisposed. The composting was carried until mature composts were obtained (about 150 days).
For each pile, five sub samples were collected at different heights at 60 cm depth from the pile surface to obtain a composite sample of about 1L. For each pile three composite samples were collected at the start of composting (T0), at the end of thermophilic phase (T1) and at the end of composting processes (T2). In laboratory, the samples were sieved (2 mm), homogenized and stored at room temperature until physical, chemical and biochemical analyses, and at 4 °C for inorganic N quantification. Each composite sample was analyzed in triplicate.

METHODS

Electrical conductivity (EC) and pH were measured on 1:5 (w/v) aqueous solutions using selective electrodes. Cation-exchange capacity (CEC) was determined by the method based on the saturation of the soil sample with 0.1 M $BaCl_2$-triethanolamine at pH 8.2 (Sumner and Miller, 1996). Volatile solids (VS) were measured as the loss on ignition at 550 °C for 24 h (EN 13039:2011). Dry matter was determined after 12 h at 105°C in order to express all data on a dry weight basis.
Total organic carbon (TOC) and Total Nitrogen (TN) content were measured with RC-412 Multiphase Carbon and FP-528 Protein/Nitrogen instruments, respectively (LECO Corporation, USA). The humic carbon (HC) was measured on 1:10 v/v sodium pyrophosphate solution 0.1 M pH 11, 4 h at 60 °C, according to the method of Ciavatta et al., 1990). Soil available P was extracted with 0.5 N $NaHCO_3$ (pH 8.5), while Total P was digested with HNO_3 and H_2O_2 in a microwave oven. Total phosphorus (TP) and available P (Pav) were determined using the procedure of Murphy and Riley (1962). Nitrate and ammonia were analyzed in a 1:5 (w/v) water extract by selective electrodes (Seven-Multi, Mettler Toledo).
Available potassium (Kav) was extracted with ammonium acetate (Helmke and Sparks, 1996). Macronutrients were determined by with ICP-OES (Agilent, Santa Clara, California, US) after HNO_3 and H_2O_2 digestion using a microwave oven.
The main physical properties of the mixtures [dry bulk density (BD), total pore space (TPS), particle density (PD), air capacity (AC), and water-holding capacity (WC)] were determined according to the UNI EN 13041 (2012) protocol. Briefly, the materials were equilibrated in water and then transferred in tubes made with two overlapping polyvinyl chloride rings (100 ± 1 mm diameter and 50 ± 1 mm height each). After having filled up, the double rings were saturated with water for 48 h and then transferred into a sandbox (Eijkelkamp Agrisearch Equipment, Giesbeck, Netherlands) at –10 cm pressure head (–1 kPa) for 48 h. Thereafter, the double rings were removed from the sandbox and separated. The lower rings were weighted and dried at 105 °C to constant mass. Easily available water (EAW) and water buffer capacity (WBC) were determined by increasing the values of suction pressure in the sandbox at –50 and –100 cm (–5 and –10 kPa, respectively).
Germination and initial root growth test was carried out on water extract 1:5 (w:v) with cress (*Lepidium sativum* L.) seeds (Hoekstra et al. 2002).
Enzyme activities were determined by the Marx et al. (2001) and Vepsäläinen et al. (2001) protocols using fluorogenic methylumbelliferyl (MUF) substrates. Samples were analyzed for β-glucosidase (EC 3.2.1.21), acid phosphatase (EC 3.1.3.2), arylsulphatase (EC 3.1.6.1) and butyrate esterase (EC 3.1.1.1) using 4-MUF-β-d-glucoside, 4-MUF-phosphate, 4-MUF-sulphate and 4-MUF–butyrate as substrates,

respectively. Fluorescence (excitation 360 nm; emission 450 nm) of the product 4-Methylumbelliferone was measured with an automated fluorimetric plate-reader (Infinite® F200PRO Tecan) at 0, 30, 60, 120, 180 min incubation times at 30 °C.
Escherichia coli and *Salmonella* spp. were assessed based on the US Compost Council methods (TMECC, 2004). All the evaluated end-products met with the specifications for *Salmonella* spp. And *E. coli*, presenting values less than 100 NMP (most probable number) g^{-1} (CQCC, 2001).
Pyrolysis-gas chromatography (Py–GC) was carried out by the Macci et al. (2012) method. Seven pyrolytic fragments were considered: acetonitrile (E_1), acetic acid (K), benzene (B), pyrrole (O), toluene (E_3), furfural (N), and phenol (Y). The area of each fragment was normalized, so that it referred to the percentage of the total of the selected seven peaks (relative abundances).

Statistical Analysis

The statistical analysis was performed by using Statistica 7.0 software (StatSoft Inc., Tulsa, Oklahoma, USA). Principal component analysis (PCA) was used to give possible patterns or clusters between the three composting processes (control, ZEOWINE 1:10 and ZEOWINE 1:2,5) and chemical and biochemical soil properties in the first composting cycle at CMM. All raw data were log transformed to reduce data heterogeneity and subsequently standardized. Only component loadings >0.70 were taken into consideration for interpretation of the PCs.

The chemical, biochemical and physical properties of the different composts at the three sampling times related to the first composting cycle at CMM and at Col D'Orcia are described in this deliverable. The results of the second and third composting cycles at CMM showed a similar trend and they are reported in this deliverable as annex.

RESULTS

Temperature profile

Figure 1 depicted the temperature variation in the demonstrative piles with and without natural zeolite at CMM-first cycle (a) and Col D'Orcia (b). A similar trend was also obtained at CMM second and third cycles.



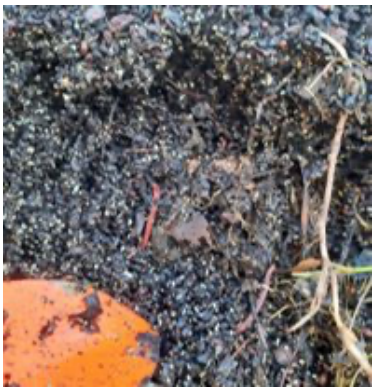
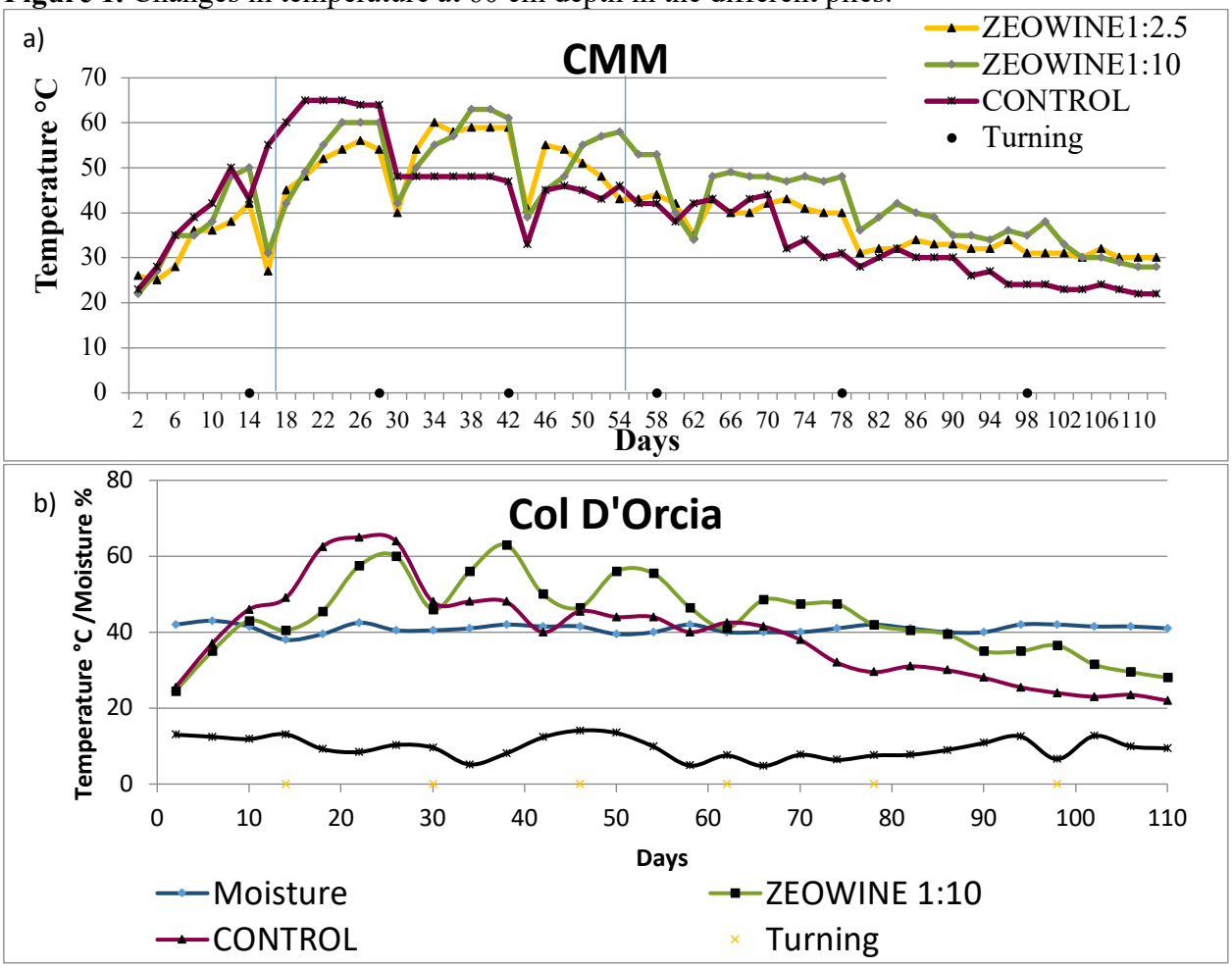


Figure 1. Changes in temperature at 60 cm depth in the different piles.



The temperature of all piles rapidly increased from the beginning of composting process. In the control piles the thermophilic phase (temperature higher than 55 °C) was reached after two weeks, while in the piles with zeolite it was recorded after three-four weeks. Temperature greater than 55 °C during this stage is extremely important to kill pathogens, thus achieving the sanitization of the raw material (Hou et al., 2017; Yang et al.2016).

The maximum temperature was measured in the control piles after 18 days (65 °C), while in the piles with zeolite it was reached after about 34-38 days from the beginning of composting (60-63 °C). The thermophilic phase was maintained for 12 days in the control pile (days from 16 to 28), for 24 days in the pile with 30% zeolite (ZEOWINE 1:2,5) (days from 24 to 48), and for 32 days in the pile with 10% zeolite (ZEOWINE 1:10) (days from 22 to 54).

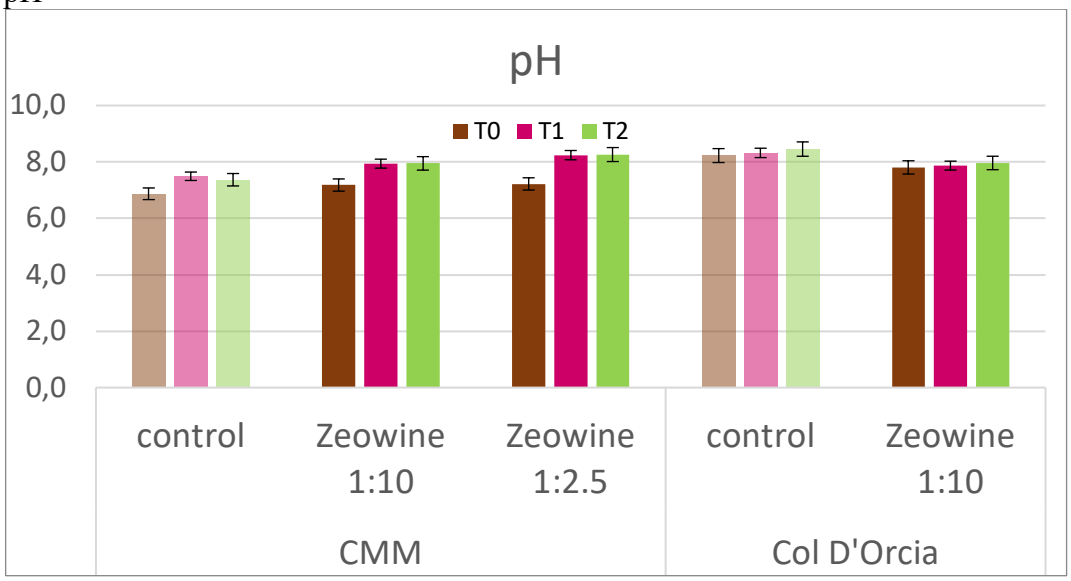
Similar results were also reported by Himanen and Hänninen (2009), who claimed that the duration of the thermophilic stage increased from 2 to 3 weeks following the addition of commercial products containing zeolite, kaolinite, chalk, ashes and sulfates to a mixture of biowastes and peat.

In the control piles the temperature decreased and reached the mesophilic stage (temperature lower than 50 °C) after 30 days from the beginning of composting process. However, this stage was reached after 52 and 64 days in the piles with 30% and 10% zeolite, respectively. During the mesophilic stage, the 10% and 30% zeolite piles showed a higher temperature with respect to the control pile. The presence of zeolite in the composting process, enhancing porosity of the compost, can enable a better aeration for metabolic heat generation by aerobic microorganisms with respect to control pile (Venglovsky et al., 2005).

CHEMICAL PROPERTIES

In each treatment, higher pH values were observed at T1 and T2 sampling times with respect to T0 (Figure 2).

Figure 2. pH

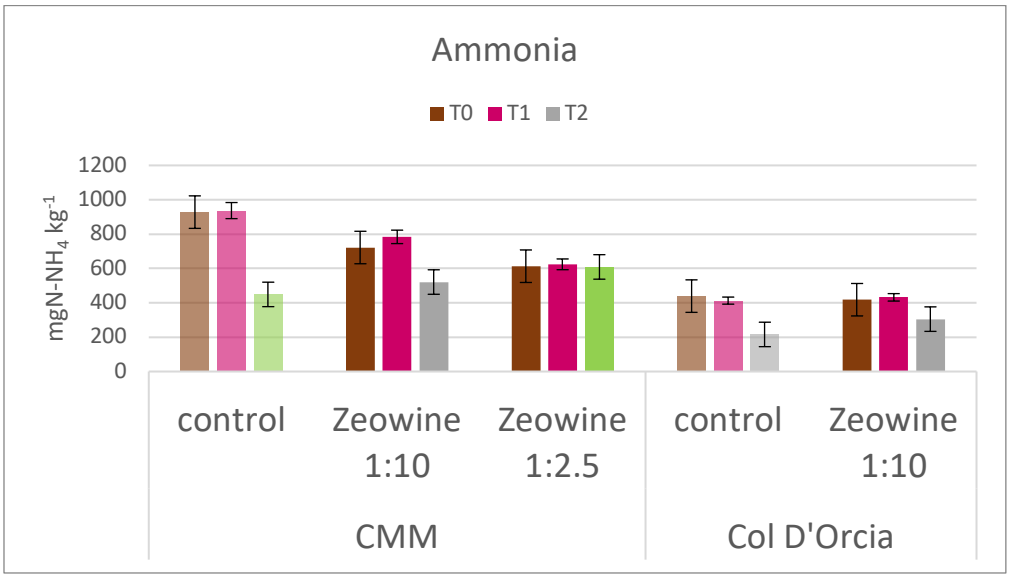


The lower pH at the beginning of the composting process (T0) can be due to the high level of short-chain organic acids in grape pomaces and to water-soluble polyphenol content in grape stalks (Bustamante et al., 2008).

The higher pH values in the ZEOWINE composts as compared with the control pile can be due to the increase in porosity of zeolite based composting piles, which improved the biological degradation of the organic substances and eliminated any anaerobic condition and accumulation of organic acids (Chan et al., 2016; Singh et al., 2013). Furthermore, the ammonification process, during organic matter degradation, and the higher retention of ammonia into zeolite lattices contributed to the higher pH in zeolite based composts with respect to control compost (Zabochnicka-Świątek and Malinska, 2010) (Figure 3).

In any case, the optimum pH from 7.0 to 8.5 to achieve efficient composting results has been obtained in all the compost piles (Makan et al., 2014; Wang et al., 2016).

Figure 3. Ammonia



The addition of zeolite greatly decreased the salinity, evaluated by measuring the electrical conductivity (EC) (Figure 4). Several studies confirmed that zeolite decreased the salinity of compost due to its high affinity for cations (Latifah et al., 2015) (Figure 5).

Figure 4. Electrical Conductivity

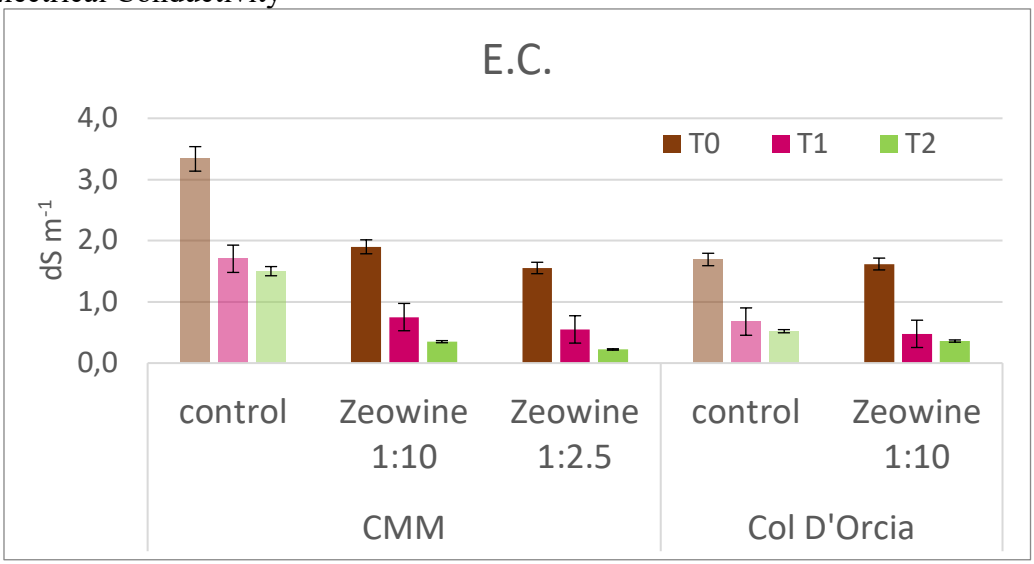
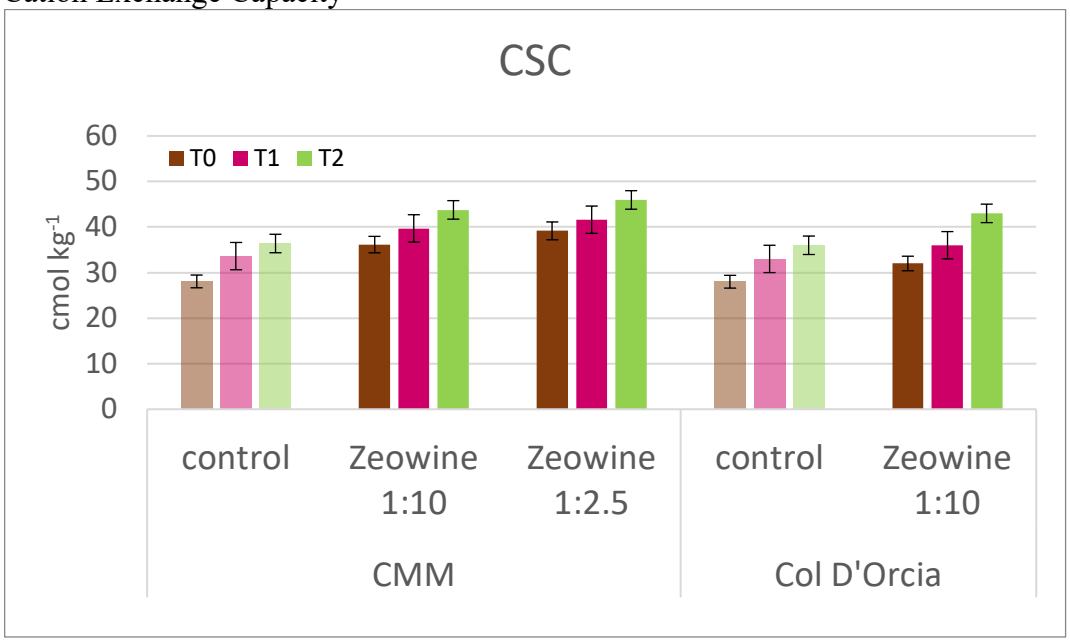
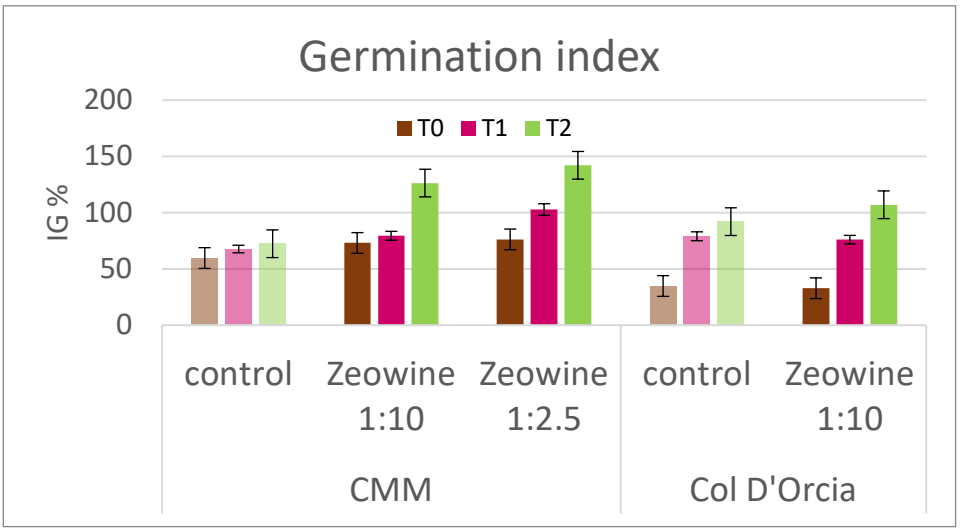


Figure 5. Cation Exchange Capacity



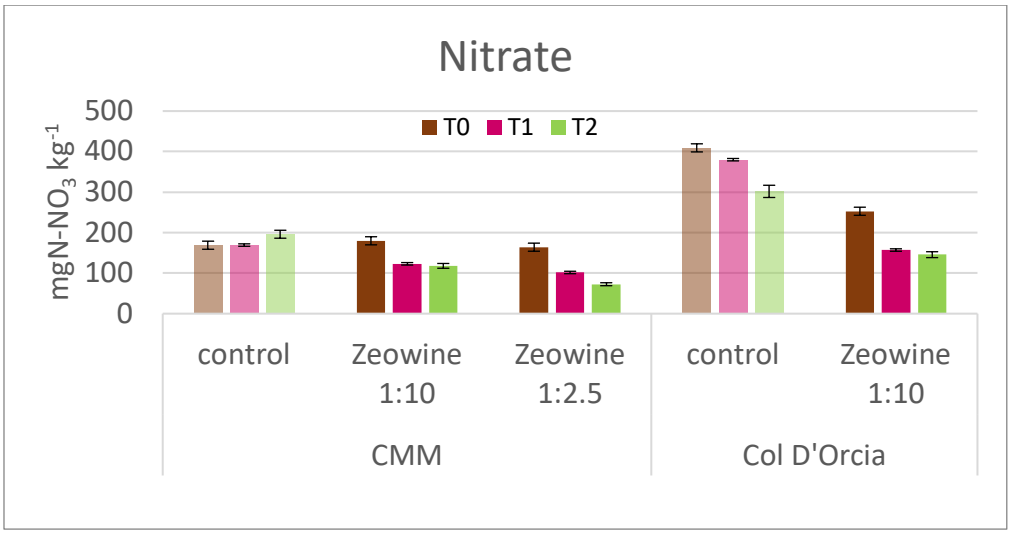
The higher salinity in the fresh compost without zeolite with respect to ZEOWINE 1:10 and 1:2,5 can be the reason for the lower germination rate (Figure 6). Makan et al. (2014) assumed that composts with EC content exceeding the limit content of 3 mS/cm would negatively affect the plant growth. Phytotoxicity decreased over time. According to Aggelis et al. (2002) all the composts obtained in our study, with and without zeolite, being characterized by a GI higher than 60% can be defined as non-phytotoxic. In addition, several authors reported a GI value for mature compost higher than 50% (Li et al.,2012; Wang et al., 2016). The values higher than 100% in the ZEOWINE composts suggested their possible biostimulant effect.

Figure 6. Germination Index



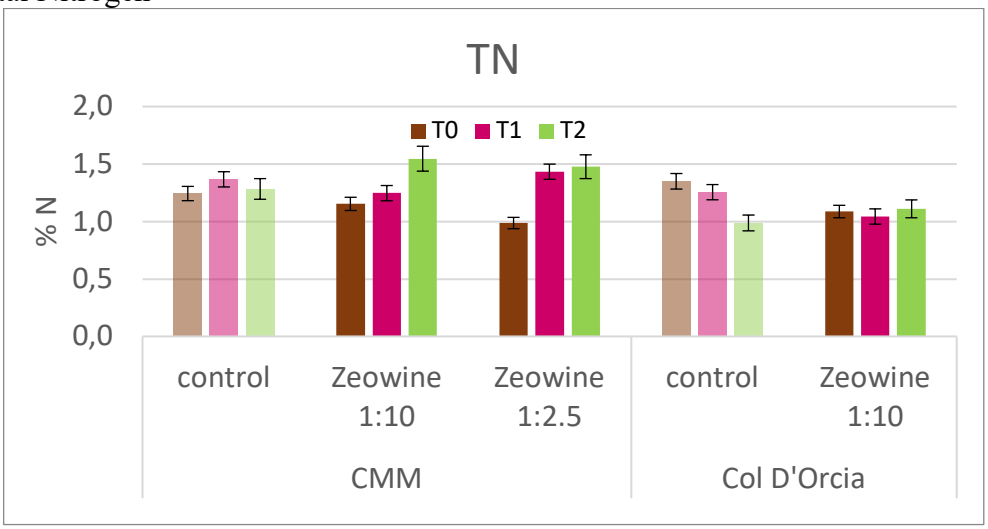
The addition of zeolite greatly decreased the N-NO_3^- (Figure 7) with respect to control piles at T1 and T2 sampling time. This can be explained by the fact that zeolite physically protects NH_4^+ ions against microbial nitrification (Ferguson and Pepper, 1987).

Figure 7. Nitrate



Total nitrogen decreased or did not change significantly over time in the control pile, while in ZEOWINE 1:10 and 1:2,5 composts a significant nitrogen increase was generally observed (Figure 8).

Figure 8. Total Nitrogen

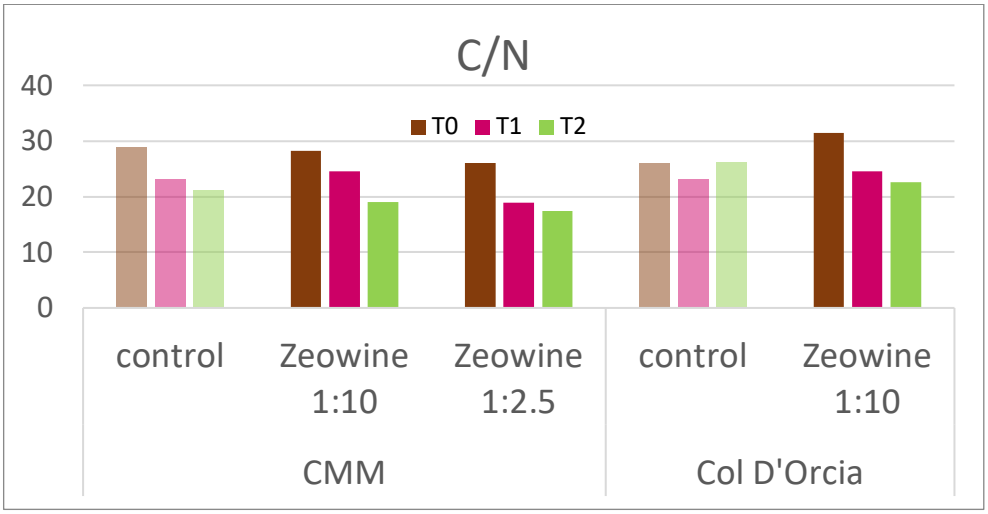


At the end of composting process (T2), the higher total nitrogen (TN) content in the ZEOWINE composts with respect to the compost without zeolite can be due to the adsorption of NH_4^+ into the negatively charged small size microporous of zeolite lattice.

Similar results were also reported by Zhang and Sun, 2016; these authors claimed that zeolite was able to retain the nitrogen in the end-product and decreased the ammonia volatilization and nitrification, thereby minimizing NO_3^- leaching.

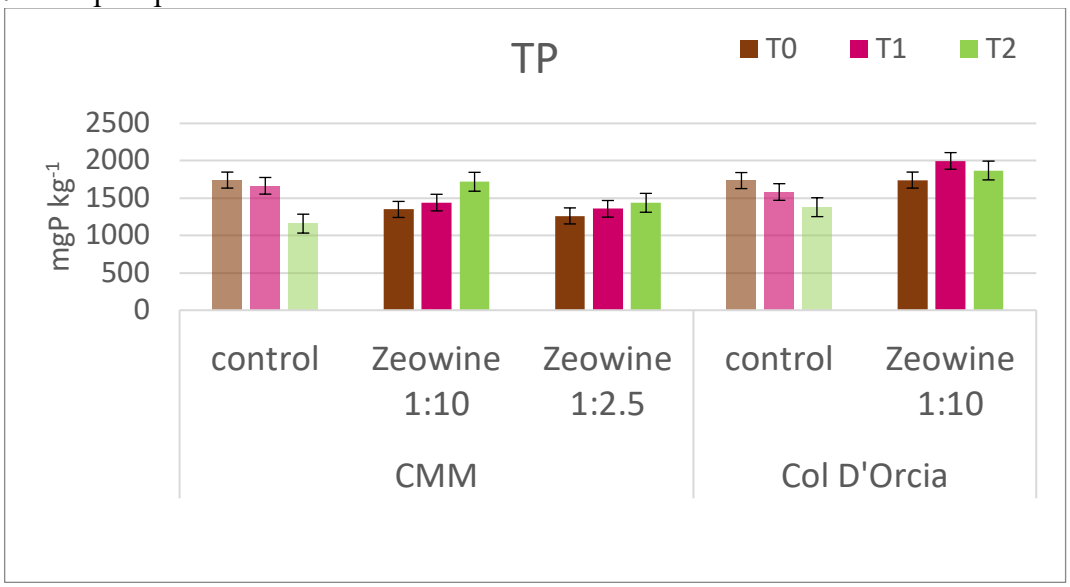
Zeolite addition leaded to a higher decrease in the C/N ratio with respect to the control compost (Figure 9).

Figure 9. C:N ratio



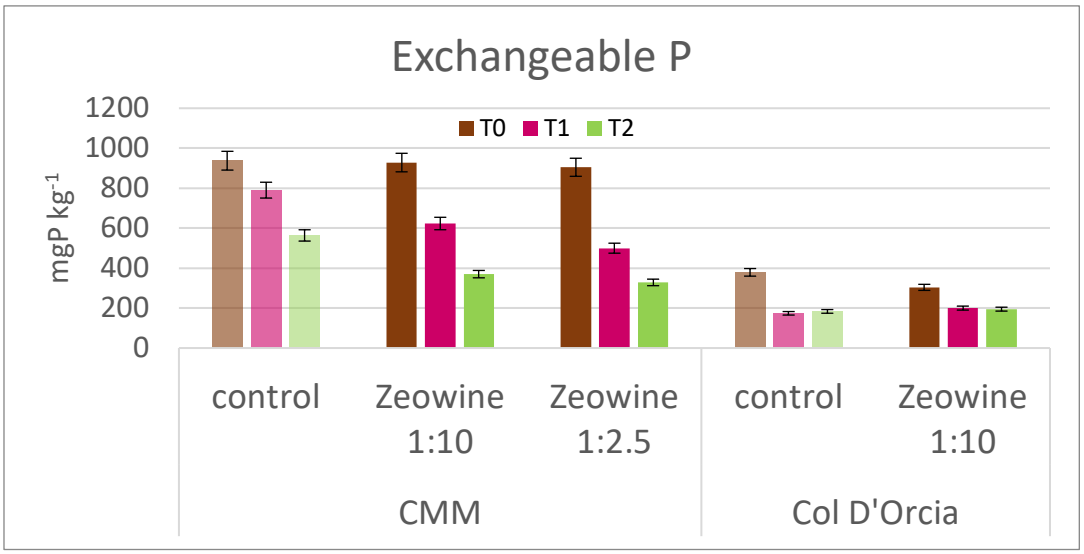
Brady and Weil (2010) recommended a range from 14 to 20 for mature compost. At the end of composting process, in the control compost this ratio was slightly higher than the suggested limit, while it was lower in ZEOWINE composts.
At the beginning of the composting process (T0), the lower nutrient content (TOC, TN and TP) in zeolite-based compost can be due to dilution effect of zeolite addition (Banegas et al. 2007).
An increase in total phosphorous in the ZEOWINE piles (1:10 and 1:2,5) was observed over time, while it decreased in the control pile (Figure 10).

Figure 10. Total phosphorus



Zeolite can adsorb or promote the formation of insoluble phosphate compounds, thus reducing compost-available P (Pav) content in comparison with the control compost without zeolite (Lefcourt and Meisinger 2001; Belyaeva and Haynes2012) (Figure 11).

Figure 11. Exchangeable phosphorus



Likewise, total potassium showed higher values in ZEOWINE composts (Hayawin et al. 2014), while its available form (Kav) decreased (Figure 12 and 13).

Figure 12. Total potassium

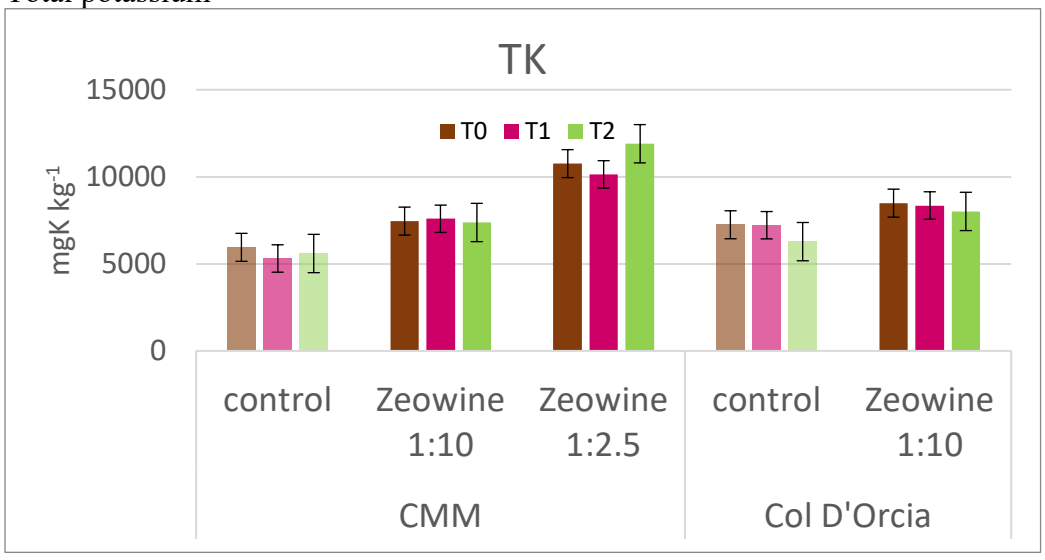
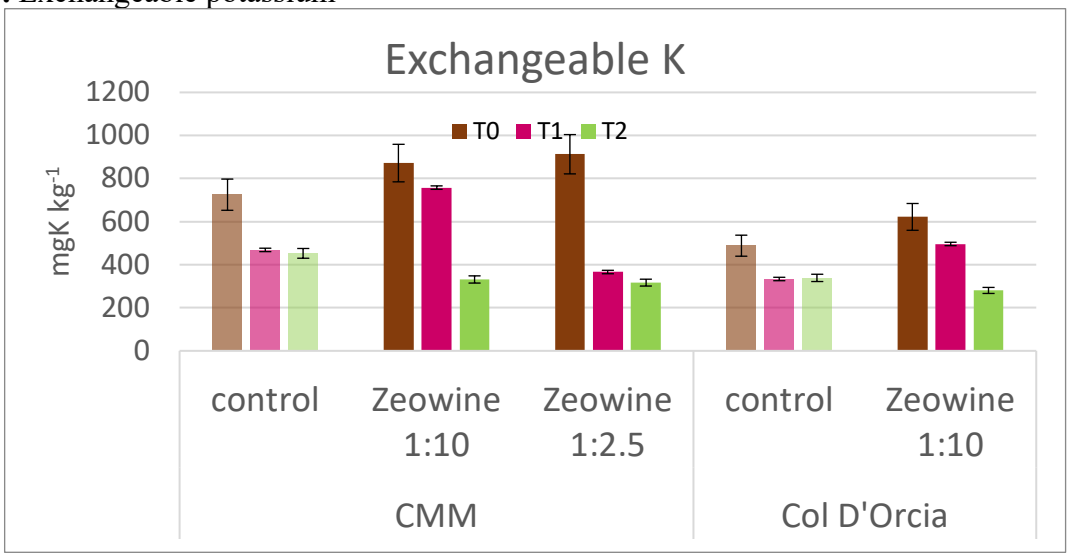
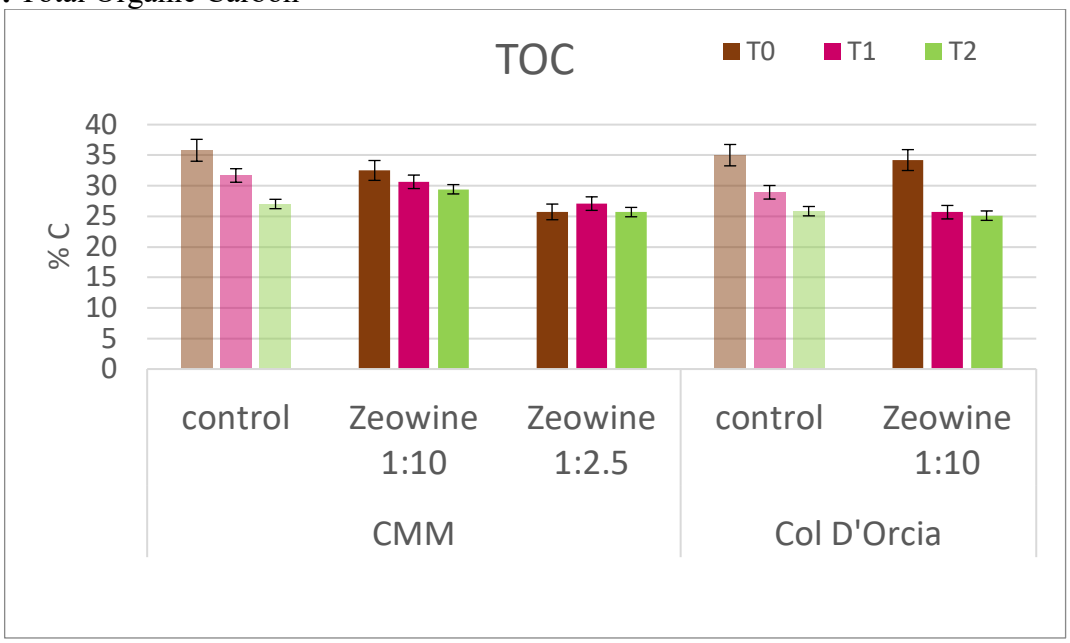


Figure 13. Exchangeable potassium



During composting process, TOC significantly decreased in the control pile, while it remains stable in the zeolite-based composts at CMM (Figure 14). Higher TOC values were observed at T2 sampling time in ZEOWINE 1:10 with respect to control compost at CMM.

Figure 14. Total Organic Carbon



It is reported that addition of 30% of zeolite and 1% lime (w/w, dry weight basis) to a mixture of biosolids and wheat straw, significantly enhanced OM degradation, humification and enzymatic activities during composting process (Awasthi et al., 2016).

PHYSICAL PARAMETERS

The physical properties of the different zeolite-based composts (control, ZEOWINE 1:10 and ZEOWINE 1:2,5) prepared at CMM are reported in Table 2, while the results of Col D'Orcia trails are reported in Table 3.
The moisture contents at the different suction pressure were reported in Figure 15 and Figure 16 (Water retention curve).

Table 2. Physical properties of CMM composts at T2 sampling time. Dry Bulk Density (BD, g/cm3); Particle Density (PD, g/cm3); Total Pore Space (TPS, % v/v); Air Capacity (AC, % v/v); Water Content (WC, % v/v); Easily Available Water (EAW, % v/v); Water Buffer Capacity (WBC, % v/v).

	ZEOWINE 1:2,5	ZEOWINE 1:10	Control	Reference values Green Waste Compost (Naddaf et al, 2011; Zhang and Sun 2016a)	Reference values Peat	Reference values growing media (Abad et al., 2001)
BD	0.86 ± 0.03	0.66 ± 0.03	0.46 ± 0.02	0.40	< 0.4	< 0.4
PD	2.13 ± 0.03	2.29 ± 0.05	1.94 ± 0.07		1.4-2.0	1.4-2.0
TPS	59.6 ± 1.16	71.2 ± 2.27	76.3 ± 3.55	70-80	85-90	>85%
AC	16.7 ± 2.39	33.2 ±2.50	39.2 ± 3.74	20-25	15-38	20-30
WC	42.9 ± 1.77	38.0 ± 2.55	42.0 ± 2.54	50	45-70	55-70
EAW	3.11 ± 0.33	7.37 ±1.45	8.43 ± 3.23	15	15-30	20-30
WBC	4.47 ± 0.24	1.05 ±1.02	0.96 ± 0.82	4	3-15	4-10

Figure 15. Water retention curve - CMM

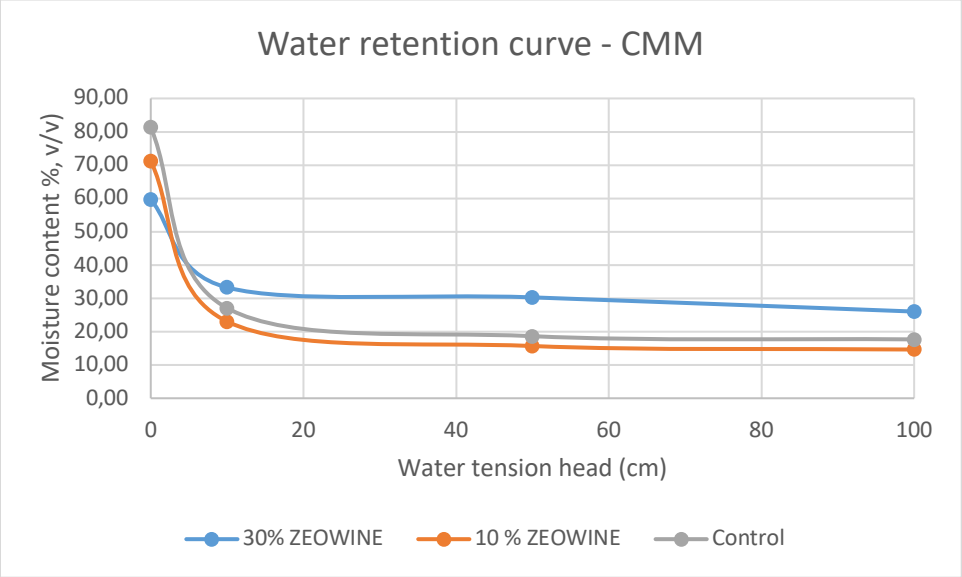
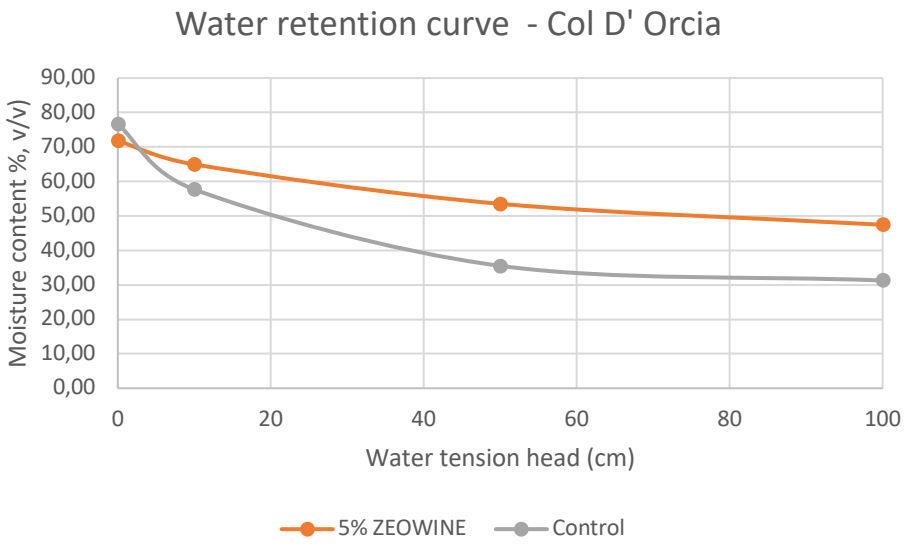


Table 3 Physical properties of Col D'Orcia composts at T2 sampling time. Dry Bulk Density (BD, g/cm^3); Particle Density (PD, g/cm^3); Total Pore Space (TPS, % v/v); Air Capacity (AC, % v/v); Water Content (WC, % v/v); Easily Available Water (EAW, % v/v); Water Buffer Capacity (WBC, % v/v).

	ZEOWINE 1:10	Control	References values Green Waste Compost (Naddaf et al, 2011; Zhang and Sun 2016a)	Reference values Peat	Reference values growing media (Abad et al., 2001)
BD	0.66 ± 0.05	0.53 ± 0.07	0.40	< 0.4	< 0.4
PD	2.35 ± 0.03	2.25 ± 0.03		1.4-2.0	1.4-2.0
TPS	71.8 ± 2.43	76.6 ± 4.33	70-80	85-90	>85%
AC	6.90 ± 1.39	18.9 ± 1.84	20-25	15-38	20-30
WC	64.9 ± 5.32	57.7 ± 4.63	50	45-70	55-70
EAW	11.4 ± 2.12	22.1 ± 3.23	15	15-30	20-30
WBC	6.08 ± 0.89	4.24 ± 0.32	4	3-15	4-10

Figure 16. Water retention curve – Col D'Orcia



As expected, the presence of zeolite in the ZEOWINE 1:10 and 1:2,5 composts, significantly affected these properties, given that all the physical properties were different with respect to the control. Moreover, the results were, in general, in line with the values of the ideal substrate proposed by Abad et al. (2001) for BD and PD.

The results of ZEOWINE 1:2,5 and ZEOWINE 1:10 prepared at CMM were comparable to Zhang and Sun (2015; 2016) findings for winery waste-based compost and clinoptilolite-based compost.

Notwithstanding that the ZEOWINE 1:2,5 mixture presented a relatively low level of water content;(WC); while water buffer capacity (WBC) responded to the threshold level proposed by Abad et

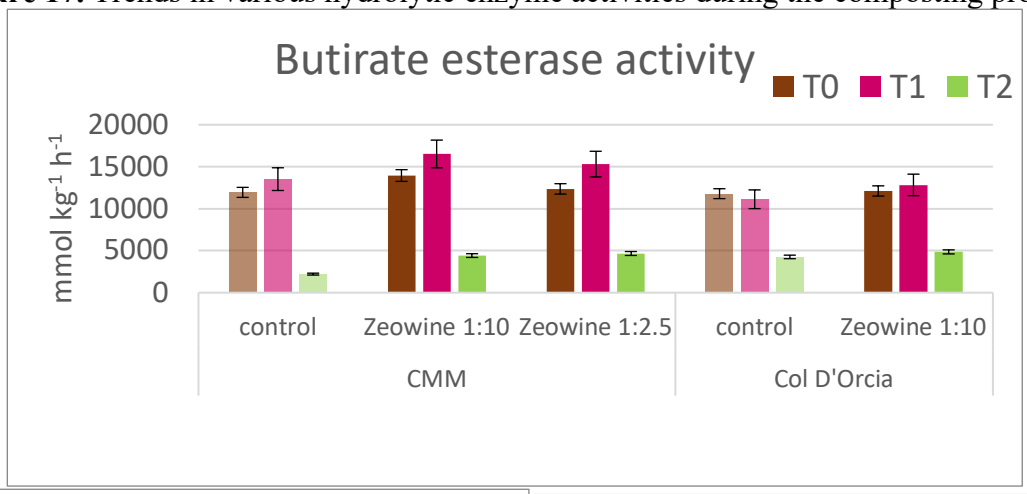
al. (2001) for an ideal growing media. This fact was probably due to the intrinsic effect of zeolite with respect to water adsorption (Zhang and Sun 2015).

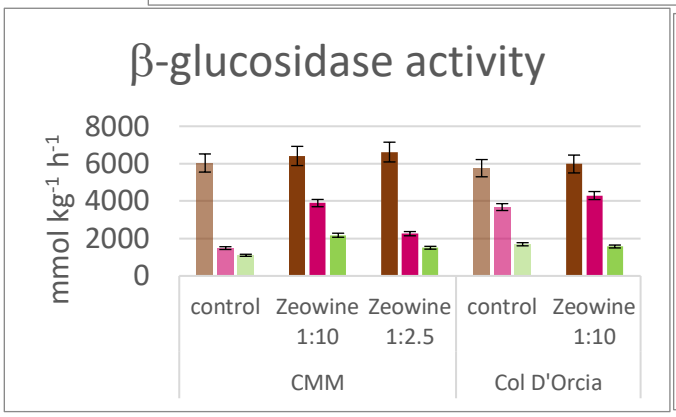
The different values found at Col D'Orcia for BD, PD and TPS in the control, with respect to CMM samples, were mainly due to the different starting material used in the composting process. Both control and ZEOWINE 1:10 responded to the threshold values for ideal growing media (Abad et al., 2001); moreover, the positive effect of zeolite in increasing the WBC was also confirmed in Col D'Orcia samples.

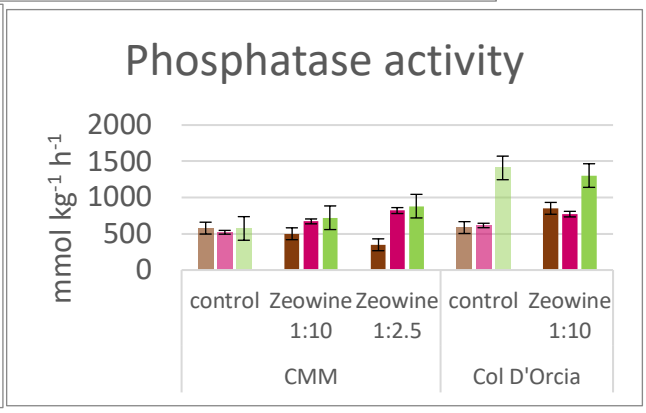
BIOLOGICAL AND BIOCHEMICAL PROPERTIES

Monitoring the presence and activity of various hydrolytic enzymes during composting process can help to evaluate the dynamics of nutritional elements and organic matter stabilization (Pecorini et al., 2020). Butyrate esterase is an unspecific lipophilic enzyme which may passively diffuse into the microbial cells and may therefore be cleaved by intracellular enzymes (Wittmann et al. 2004); for this reason, it can be considered related to the activity of living microorganisms.
From the beginning of composting process, butyrate esterase activity increased in all piles, while it decreased from the end of the thermophilic phase (Figure 17).

Figure 17. Trends in various hydrolytic enzyme activities during the composting process





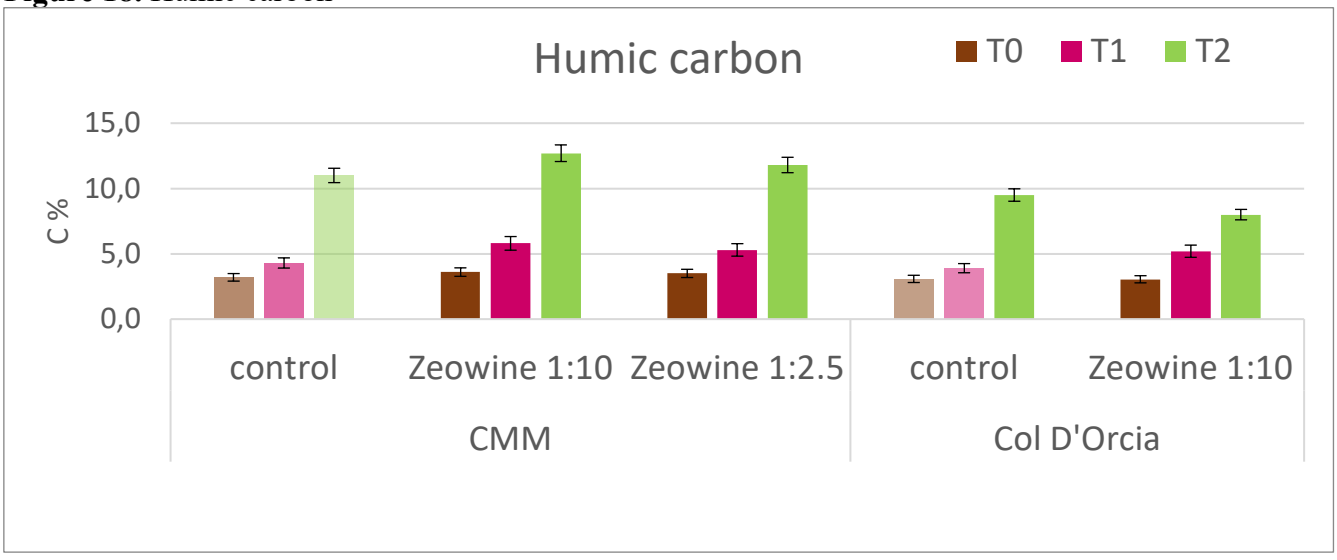


It can be assumed that at the end of the thermophilic stage maximum activity of butyrate esterase enzyme corresponded to a maximum microbial activity, which constantly decreased until the end of composting process. A similar situation was reported by Awasthi et al. (2018) who studied the evolution of microbial dynamics at the different stages of food waste composting process in presence of 10% zeolite.
In the control pile lower butyrate esterase activity was measured at the three sampling times of composting process with respect to the zeolite-based composts (Figure 17). It seems like that the presence of zeolite, due to its porous structure, could have provided optimum pH, moisture and aeration that favored microbial growth and activity. The effect of zeolite on microbial activity stimulation was also probably due to its role in microorganism protection by providing them a microbial habitat (Zhao et al., 2017).
β-glucosidase enzyme catalyzes the breakdown of cellobiose and other disaccharides to glucose and therefore it is involved in the carbon cycle (Nannipieri et al., 2002). This enzyme activity can be stimulated by the availability of organic substrates (Castillo et al.2013). β-glucosidase was higher at the beginning of the composting process due to the presence of labile organic matter easily degradable by microorganisms, while it progressively declined until the end of composting process due to the exhaustion of bioavailable organic substrates. At the end of composting process (T2) higher values were showed in the ZEOWINE 1:10 compost with respect to control compost (Figure 17).
The acid phosphatase enzyme hydrolyzes the organic phosphomonoesters to inorganic phosphorus and this enzyme activity is inhibited at high concentration of the product of reaction (orthophosphate) (Fernandez-Gomez et al., 2010). In the present study, acid phosphatase showed an opposite trend with respect to available P and a significant increase was noticed in ZEOWINE 1:10 and 1:2,5 final composts.

HUMIFICATION

During composting process, humic carbon significantly increased in each pile and higher values of this parameter were measured in the ZEOWINE 1:10 at CMM with respect to the control compost (Figure 18).

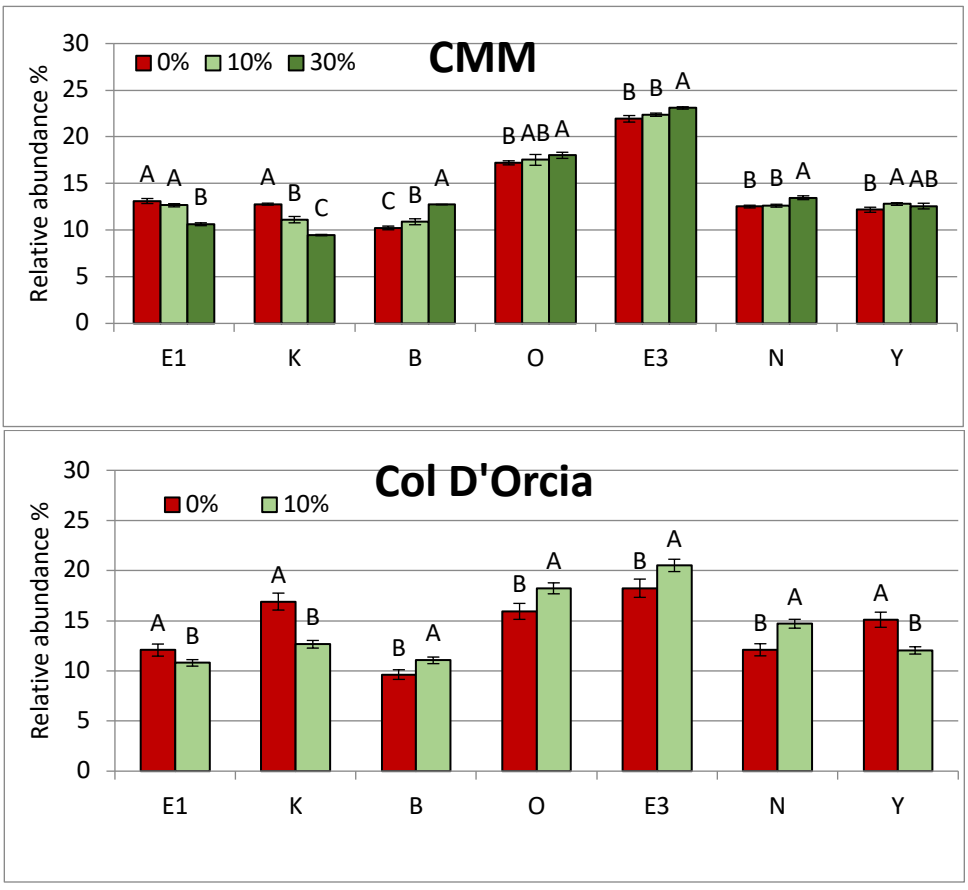
Figure 18. Humic carbon



At the end of the composting process (T2) the three obtained composts (control, ZEOWINE 1:10 and ZEOWINE 1:2,5) were analyzed by Py-GC in order to reveal differences in the structural composition

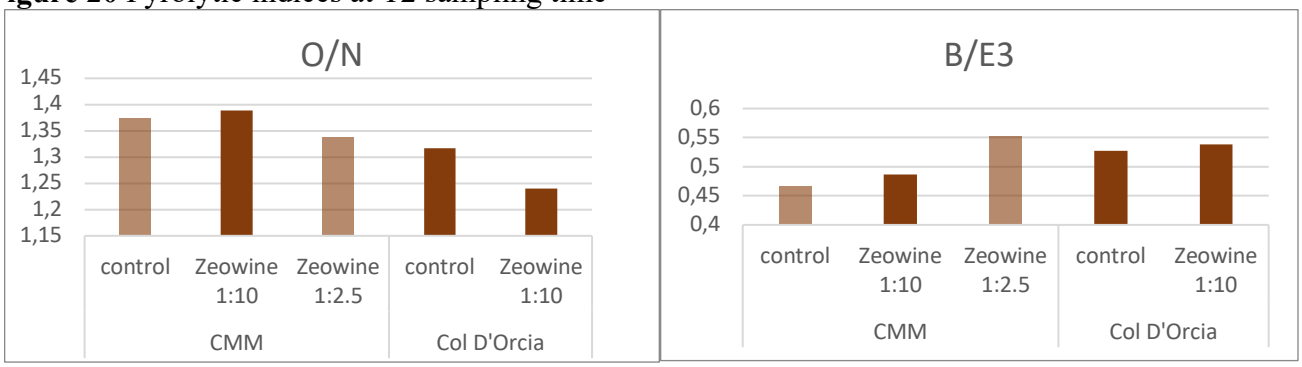
of organic matter. On the basis of the origin of the pyrolytic fragments it was possible to obtain information about the compost quality in terms of organic matter stability.
Acetonitrile (E_1) reflects the decomposition of aminoacids, proteins, and microbial cells; acetic acid (K), is mainly derived from fats and cellulose; benzene (B) is derived from condensed aromatic structures; toluene (E_3) reflects the degradation of aromatic uncondensed rings with aliphatic chains; pyrrole (O) is derived from N-containing compounds, such as proteins, nucleic acids and microbial cells; furfural (N) is a pyrolysate compound that is produced from polysaccharides, and phenol (Y) is derived from tannins condensed (humic) ligno-cellulosic structures (Doni et al., 2014).
The ratios between relative abundances of some of these pyrolytic compounds were used to interpret the results. In particular, the higher the ratio of B/E3, the higher the humification of organic matter, while the higher the ratio of O/N, the higher the extent of the mineralization of organic matter.
The amount of the pyrolytic fragment derived from the degradation of fats and cellulose (K) was affected by the presence of zeolite (Figure 19); the significant lower values in ZEOWINE 1:10 (10%) and 1:2,5 (30%) with respect to control (0%) indicated the lower "mineralizable" fraction of the organic matter.

Figure 19. Relative abundances of the main pyrolytic products (percentages of the total pyrogram area) at the end of composting process (T2) in the three piles at different rates of of zeolite.



An opposite trend was observed for the toluene pyrolytic fragment (E3) which derived from partially humified materials, such as ligno-cellulose and ligno-proteic compounds. Similarly, a higher amount of the pyrolytic fragment derived from the degradation of condensed aromatic structures (B) was found in both the ZEOWINE composts.
The higher relative abundance of aromatic pyrolytic products in the ZEOWINE composts with respect to the control compost could be indicative of the relatively higher degree of humification. The higher content of stabilized organic matter in zeolite-based compost, having carboxyl and phenolic functional groups with affinity for NH_4^+, can have contributed to the higher retention of nitrogen in comparison with the control pile.
The presence of zeolite also decreased the O/N ratio, indicating the lower organic matter mineralization state since furfural (N) is chemically and microbiologically less stable than pyrrole (O). Finally, the higher B/E_3 ratio in the zeolite-based composts indicated the higher degree of condensation of aromatic rings (Figure 20).

Figure 20 Pyrolytic indices at T2 sampling time



PRINCIPAL COMPONENT ANALYSIS

Principal component analysis was performed on standardized variables obtained from the first composting cycle at CMM to evaluate the correlations between the chemical and biochemical variables and identify the most important parameters that reflect the changes of the three zeolite-based composts (control, ZEOWINE 1:10 and ZEOWINE 1:2,5) at the three sampling times (T0, T1 and T2).
The variance contained in the first two components was 72.6% of the total variation (Table 4). The first PC (PC1 38.5% of variance) was closely correlated with the total nitrogen, available nutrients (K and P), and with the hydrolytic enzyme activities (phosphatase, β-glucosidase and arylsulphatase).
The negative correlation between available P and phosphatase activity (significant loadings on the PC1 with different sign) indicated that phosphatase activity was stimulated by the reduction in P availability.

Table 4 Principal component loadings.

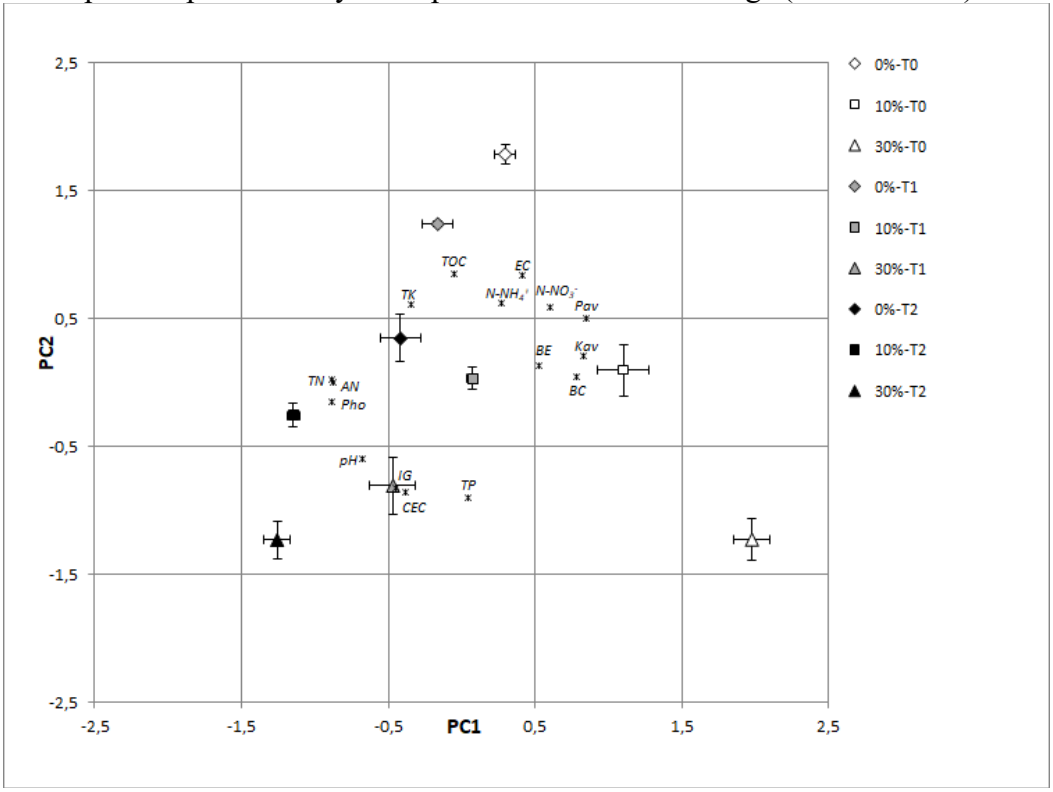
	PC 1	PC 2
pH	-0,675	-0,594
EC	0,419	0,841*
IG	-0,448	-0,823*
N-NO3-	0,602	0,588
Pav	0,847*	0,499
TOC	-0,052	0,852*
TP	-0,343	0,616
N-NH4+	0,267	0,623
TN	-0,888*	0,026
CEC	-0,381	-0,860*
TK	0,047	-0,897*
Kav	0,832*	0,205
Pho	-0,885*	-0,153
BE	0,531	0,130
BC	0,781*	0,051
AR	-0,872*	0,004
Var. Sp.	6,167	5,458
Prp.Tot.	0,385	0,341

*Parameters used for PCA interpretation.

The contribution of the second PC (PC2) was accounted for 34.1% of the total variance with electrical conductivity, total organic carbon, cation exchange capacity, total potassium and germination index being the most correlated variables. The negative correlation between EC and IG (significant loadings on the PC2 with different sign) indicated that the high EC negatively affected the seed germination and growth.

Fig. 21 provided the biplot of the PCA analysis obtained using the first two PCs. The three sampling times (T0, T1 and T2) were distributed throughout the PC1 and PC2 axes, thus indicating that the composting stage changed the chemical and biochemical properties of compost. The effect of zeolite addition on the composting process was clearly evident, since 30% and 10% zeolite-based composts were distant from each other and from the control compost in the plot, with a shift along both the PC axes, especially at the beginning of the composting process (T0). At the end of composting (T2), the effect of zeolite was still evident. The differentiation of the three end-composts (0%, 10% and 30%) was mainly on the physical-chemical parameters which showed a significant loading on the PC2. In particular, the composts with zeolite exhibited higher values of IG, TK and CEC and lower values of EC with respect to control compost.

Figure 21. Principal component analysis. Biplot of scores and loadings (PC 1 vs PC 2)



EC, Electrical Conductivity; IG, Index of Germination; N-NO3-, Nitrate; Pav, available Phosphorus; TOC, Total Organic Carbon; TP, Total Phosphorus; N-NH4+, Ammonia; TN, Total Nitrogen; CEC, Cation Exchange Capacity; TK, Total potassium; Kav, available possium; Pho, Phosphatase activity; BE, butyrate activity; BC, β-glucosidase activity; AR, arylsulphatase activity.

The significant parameters on the PC1 seemed to be related to the mineralization of OM during the composting process, playing the hydrolytic enzymes a key role in the mineralization of the organic matter, while the significant parameters on the PC2 seemed to be related to nutritional retention capacity of compost.

CONCLUSIONS

The presence of zeolite in composting process of winery wastes improved the quality of the final compost in terms of electrical conductivity, nutrient content, phytotoxicity, microbial activities and physical properties (water holding capacity, etc.). In particular, zeolite increased the adsorption of ammonium ions of compost, thus resulting in higher total nitrogen content in zeolite-based compost with respect to control compost without zeolite. The retention of ammonium when natural zeolite is added in the composting process is a very important aspect to increase the agronomic value of compost and reduce the environmental pollution. Finally, the py-GC results demonstrated that integration of zeolite in composting process offered the benefit of the higher carbon humification with respect to control compost.

The high agronomic value of the zeolite-based composts, both at ZEOWINE 1:10 and ZEOWINE 1:2,5, make them particularly suitable for the vineyard soils which generally have a very low organic matter content. The reintroduction of the compost into the production system, will also allow the closure of the residual material cycle.
By comparing the two zeolite-based composts (ZEOWINE 1:10 and ZEOWINE 1:2,5), we can conclude that the compost which contains 10% of zeolite (ZEOWINE 1:10) is the most suitable practical application for improving the winery wastes composting process and, at the same time, for saving on the cost of providing zeolite.

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ANNEX

Chemical properties

				Average		
				T0	T1	T2
%	TOC	CMM -I cycle	control	35,8	31,7	27,0
			Zeowine 1:10	32,5	30,6	29,4
			Zeowine 1:2.5	25,7	27,1	25,7
		CMM - II cycle	control	35,7	32,0	26,5
			Zeowine 1:10	33,6	29,5	28,9
			Zeowine 1:2.5	26,3	26,6	25,5
		CMM-III cycle	Zeowine 1:10	31,9	30,0	29,9
		Col D'Orcia	control	35,0	28,9	25,8
			Zeowine 1:10	34,2	25,7	25,1
%	TN	CMM -I cycle	control	1,24	1,37	1,28
			Zeowine 1:10	1,15	1,25	1,55
			Zeowine 1:2.5	0,99	1,43	1,48
		CMM - II cycle	control	1,24	1,26	1,12
			Zeowine 1:10	1,46	1,34	1,28
			Zeowine 1:2.5	1,12	1,17	1,20
		CMM-III cycle	Zeowine 1:10	1,41	1,38	1,32
		Col D'Orcia	control	1,35	1,26	0,99
			Zeowine 1:10	1,09	1,04	1,11
cmol/kg	CSC	CMM -I cycle	control	28,1	33,6	36,4
			Zeowine 1:10	36,1	39,7	43,8
			Zeowine 1:2.5	39,2	41,6	45,9
		CMM - II cycle	control	30,1	32,7	34,4
			Zeowine 1:10	35,1	41,0	45,3
			Zeowine 1:2.5	38,5	41,5	46,1
		CMM-III cycle	Zeowine 1:10	37,0	38,6	42,3
		Col D'Orcia	control	28,0	33,0	36,0
			Zeowine 1:10	32,0	36,0	43,0
dS/m	E.C.	CMM -I cycle	control	3,34	1,70	1,50
			Zeowine 1:10	1,90	0,75	0,35
			Zeowine 1:2.5	1,55	0,55	0,22
		CMM - II cycle	control	3,47	1,61	1,47
			Zeowine 1:10	2,10	0,73	0,34
			Zeowine 1:2.5	0,55	0,55	0,22
		CMM-III cycle	Zeowine 1:10	2,26	0,76	0,36
		Col D'Orcia	control	1,69	0,68	0,52
			Zeowine 1:10	1,62	0,48	0,36
	pH	CMM -I cycle	control	6,87	7,49	7,37
			Zeowine 1:10	7,18	7,94	7,95
			Zeowine 1:2.5	7,22	8,24	8,26
		CMM - II cycle	control	6,69	7,54	7,36
			Zeowine 1:10	7,23	7,85	8,02
			Zeowine 1:2.5	7,20	8,24	8,25
		CMM-III cycle	Zeowine 1:10	7,16	8,01	7,86
		Col D'Orcia	control	8,23	8,32	8,46
			Zeowine 1:10	7,81	7,87	7,96

Data reported as mean values, coefficient of variation of the three composite samples ranging from 2 to 10%.

				Average		
				T0	T1	T2
mg/kg	TK	CMM -I cycle	control	5949	5309	5595
			Zeowine 1:10	7458	7590	7376
			Zeowine 1:2.5	10758	10141	11899
		CMM - II cycle	control	5971	5510	5571
			Zeowine 1:10	7361	7500	7364
			Zeowine 1:2.5	10605	10253	11814
		CMM-III cycle	Zeowine 1:10	7521	7684	7421
		Col D'Orcia	control	7245	7219	6276
			Zeowine 1:10	8489	8354	8011
mg/kg	TP	CMM -I cycle	control	1742	1665	1159
			Zeowine 1:10	1349	1440	1719
			Zeowine 1:2.5	1262	1357	1437
		CMM - II cycle	control	1745	1555	1188
			Zeowine 1:10	1390	1560	1700
			Zeowine 1:2.5	1255	1294	1446
		CMM-III cycle	Zeowine 1:10	1310	1392	1760
		Col D'Orcia	control	1735	1582	1379
			Zeowine 1:10	1742	1998	1870
mg/kg	Ex-P	CMM -I cycle	control	938	790	564
			Zeowine 1:10	928	623	370
			Zeowine 1:2.5	905	500	328
		CMM - II cycle	control	962	776	588
			Zeowine 1:10	921	603	369
			Zeowine 1:2.5	902	524	334
		CMM-III cycle	Zeowine 1:10	938	624	380
		Col D'Orcia	control	379	174	183
			Zeowine 1:10	304	200	195
mg/kg	Ex-K	CMM -I cycle	control	725	469	453
			Zeowine 1:10	872	758	331
			Zeowine 1:2.5	913	366	317
		CMM - II cycle	control	714	478	434
			Zeowine 1:10	799	762	506
			Zeowine 1:2.5	875	380	330
		CMM-III cycle	Zeowine 1:10	868	788	553
		Col D'Orcia	control	488	334	339
			Zeowine 1:10	622	496	281
mg/kg	Cu-tot	CMM -I cycle	control	83	77	78
			Zeowine 1:10	72	73	70
			Zeowine 1:2.5	56	52	44
		CMM - II cycle	control	30	32	34
			Zeowine 1:10	38	36	35
			Zeowine 1:2.5	20	24	23
		CMM-III cycle	Zeowine 1:10	27	41	21
		Col D'Orcia	control	63	54	54
			Zeowine 1:10	45	42	44

Data reported as mean values, coefficient of variation of the three composite samples ranging from 2 to 10%.

				Average T0	T1	T2
%	Humic carbon	CMM -I cycle	control	3,20	4,30	11,0
			Zeowine 1:10	3,60	5,80	12,7
			Zeowine 1:2.5	3,50	5,30	11,8
		CMM - II cycle	control	3,51	4,58	11,0
			Zeowine 1:10	3,67	5,74	11,7
			Zeowine 1:2.5	3,42	5,41	7,9
		CMM-III cycle	Zeowine 1:10	3,84	5,69	10,9
		Col D'Orcia	control	3,1	3,9	10
			Zeowine 1:10	3,1	5,2	8
mg/kg	$N\text{-}NO_3$	CMM -I cycle	control	169	169	196
			Zeowine 1:10	180	123	118
			Zeowine 1:2.5	164	102	73
		CMM - II cycle	control	168	177	191
			Zeowine 1:10	184	129	120
			Zeowine 1:2.5	153	107	77
		CMM-III cycle	Zeowine 1:10	176	128	126
		Col D'Orcia	control	409	380	302
			Zeowine 1:10	253	157	146
mg/kg	$N\text{-}NH_4^+$	CMM -I cycle	control	928	937	449
			Zeowine 1:10	722	784	521
			Zeowine 1:2.5	613	624	609
		CMM - II cycle	control	952	960,5	479
			Zeowine 1:10	706	793	537
			Zeowine 1:2.5	630	625	613
		CMM-III cycle	Zeowine 1:10	742	769	562
		Col D'Orcia	control	439	413	216
			Zeowine 1:10	418	432	305
%	IG	CMM -I cycle	control	59,7	67,7	72,4
			Zeowine 1:10	73,0	79,4	126
			Zeowine 1:2.5	76,2	102,8	142
		CMM - II cycle	control	73,4	79,3	83,1
			Zeowine 1:10	70,6	80,9	123
			Zeowine 1:2.5	93,9	100,9	133
		CMM-III cycle	Zeowine 1:10	72,7	81,1	135
		Col D'Orcia	control	34,8	79,0	92,0
			Zeowine 1:10	32,8	76,0	107

Data reported as mean values, coefficient of variation of the three composite samples ranging from 2 to 10%.

Biochemical properties

			Average T0	T1	T2
b-glucosidase activity mmol kg^{-1} h^{-1}	CMM -I cycle	control	6036	1487	1103
		Zeowine 1:10	6413	3894	2175
		Zeowine 1:2.5	6621	2258	1507
	CMM - II cycle	control	6036	1484	1105
		Zeowine 1:10	6413	3894	2164
		Zeowine 1:2.5	6621	2264	1521
	CMM-III cycle	Zeowine 1:10	6413	3856	2184
	Col D'Orcia	control	5760	3680	1692
		Zeowine 1:10	5983	4296	1573
Phosphatase activity mmol kg^{-1} h^{-1}	CMM -I cycle	control	579	521	574
		Zeowine 1:10	501	671	721
		Zeowine 1:2.5	348	820	881
	CMM - II cycle	control	573	518	560
		Zeowine 1:10	527	684	736
		Zeowine 1:2.5	354	827	881
	CMM-III cycle	Zeowine 1:10	501	666	739
	Col D'Orcia	control	586	615	1409
		Zeowine 1:10	851	771	1304
Aril-Sulfatase Activity mmol kg^{-1} h^{-1}	CMM -I cycle	control	165	110	206
		Zeowine 1:10	133	190	295
		Zeowine 1:2.5	66	173	248
	CMM - II cycle	control	169	109	208
		Zeowine 1:10	157	198	287
		Zeowine 1:2.5	73	169	249
	CMM-III cycle	Zeowine 1:10	133	190	299
	Col D'Orcia	control	324	217	123
		Zeowine 1:10	377	276	160
Butirate Esterase Activity mmol kg^{-1} h^{-1}	CMM -I cycle	control	11944	13521	2215
		Zeowine 1:10	13952	16521	4418
		Zeowine 1:2.5	12360	15310	4666
	CMM - II cycle	control	11978	13511	2235
		Zeowine 1:10	13952	16508	4369
		Zeowine 1:2.5	12373	15146	4635
	CMM-III cycle	Zeowine 1:10	13925	16342	4498
	Col D'Orcia	control	11789	11123	4259
		Zeowine 1:10	12115	12833	4853

Data reported as mean values, coefficient of variation of the three composite samples ranging from 2 to 10%.