ZEOWINE characteristics

Caratteristiche di ZEOWINE

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Summary

Following the UE laws, the Italian waste management legislations is built on the "4R principle": Reduction, Reuse, Recycling and, in the end, material and energy Recovery according to the "Decreto Ronchi" D.Lgs. n. 22/1997. In addition, the Waste Framework Directive (WFD, 2008) introduced another important goal to be achieved within 2020 by member states, stating that at least 50% of waste generated has to be reused or recycled.

Composting is defined (FAO Term Portal, FAOTERM) as the mixture of organic matter digested aerobically that is used to improve soil structure and provide nutrients. The composting process includes several phases that must be met to obtain quality compost.

The vast majority of organic materials are compostable, including organic wastes from viticulture activities. Compost contains available nutrients of slow release and a high content of organic matter.

The use of natural zeolite during the process of winery wastes composting was aimed at improving the quality of compost in terms of chemical-nutritional, biological and physical properties.

The 11 January 2019 the field demonstration experiments of composting of winery wastes and zeolite were organized at CMM premise.

The wastes from vine prunings and wine processing (stalks and grape pomace) were collected (total amount of 19,5 tons) and shredded (4-5 cm). The zeolite was almost 85% clinoptilolite with a granulometry 0.2-2.5 mm.

Three piles of about 9 tons each were set up, the piles proportion was 1 zeolite : 2.5 wastes w/w (**ZEOWINE 1:2.5**). The piles were prepared by mixing the two components (Zeolite and winery wastes) with mechanical mixing equipment.

Taking into account the economic, commercial and agronomical aspects related to ZEOWINE marketing, the hypothesis of composting with a lower concentration of zeolite was analyzed. In view of this, an additional pile prepared by mixing zeolite and winery wastes at the ratio 1:10 w/w (**ZEOWINE 1:10**) was set up. Finally, one pile without zeolite was used as a control; this was to obtain more scientific evidences on the impact of zeolite concentrations on the ZEOWINE quality.

Periodical **turning** of each pile in order to promote aeration was carried out at least once a month, until the end of the composting process. **Irrigation,** in order to optimize water level by sprinklers on top of each pile, was aimed at moisture increase to 50% and maintenance. **Temperature** and **humidity** were monitored every day until the end of the thermophilic phase, successively every week. A cover system of each pile was predisposed.

At the end of composting process (22 June 2019), the obtained winery wastes and zeolite-based composts (**ZEOWINE 1:2.5, ZEOWINE 1:10**) were compared to the Italian legislation for growth substrates and fertilizers (Legislative Decree 75/2010).

Methods

Moisture In order to determinate the moisture of the samples, ceramic cresol were used, in wide the samples were introduced and then weighed in fresh. After that, crisols are introduced in an oven at 105°C for at least 4 hours. Then, the crisols are putting in a dryer until the sample is cooled avoiding moisture. After, the crisols have to be weighed to estimate the dry weight. The difference between both samples (fresh and dry) will be the moisture.

pH and CE was extracted by shaking for 2 h a mixture of sample and distilled water, at a 1:5 solid:liquid ratio, and then measured in a pH-meter and CE-meter respectively, after centrifuging and filtering through ash-less filter paper.

N-NH_3 (Ammonia nitrogen) and **N-NO_3** (Nitric nitrogen): ammonia and nitrates were determined on aqueous extracts by selective electrode.

Volatile solids (VS) were measured at 550°C as the loss on ignition.

Total organic C (TOC) and total Nitrogen (Nt) were determined by pre-treatment with HCl to eliminate carbonates followed by combustion at 1020°C and measurement in a Elemental Analyzer.

C:N It is the reflection of organic matter (OM) decomposition and its increase is a positive sign of humification.

Humic carbon was determined by compost analysis methods (Ciavatta et al., 1990).

Micro and macronutrients was analyzed by **ICP-OES** (Inductively coupled plasma-optical emission spectrometer) quantitative determination, is an elemental analysis technique.

Cation exchange capacity (CEC) was determined using the BaCl2 compulsive exchange method (Ozdemir et al. 2017).

Germination Index was determined in Petri dishes with filter paper and 15 seeds of rye-grass (*Lollium perenne*), 2ml of distilled water extracts (1:5 solid:liquid ratio) were added and dishes were put in a germination chamber at 28°C, 75% relative humidity and darkness. After germination, the number of germinated seeds was recorded and the length of the seedling roots and shoots was measured. All treatments were carried out by quadruplicate, and Petri dishes with 2 ml of distilled water instead of samples were used as control (Hoekstra et al., 2002).
Germination index (GI) was calculated according to the following equation: $GI = \% GS (L_R/L_{RC})$; where GI = Germination index in percentage; %GS = Percentage of germinated seed with respect to the control; L_R = Average root length in the treated seedling; and L_{RC} = Average root length in the control seedling.

Germination index is a widely accepted indicator of potential phytotoxicity for organic amendments. It combines the effect of the studied material on both, seed germination capacity and root elongation.

GI values lower than 50% indicate phytotoxicity; IG values between 80 and 50% indicate moderate phytotoxicity; GI higher than 80% indicate absence of phytotoxicity and values higher than 100% indicate a bio-stimulant effect.

Heavy metals were determined by ICP-OES after microwave digestion with nitric acid and hydrogen peroxide.

Physical properties [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).

Enzyme activities have been determined following the methods of Marx et al. (2001), and Vepsalainen et al. (2001), based on the use of the fluorogenic substrate methylumbelliferyl (MUB). The enzymes analyzed are: butyrate esterase (EC 3.1.1.1), β-glucosidase (EC 3.2.1.21), acid phosphatase (EC 3.1.3.2), arylsulphatase (EC 3.1.6.1). A moist sample (equivalent weight to 2 g oven-dry material) was weighed into a sterile jar and 50 ml of water are added. A homogenous suspension is obtained by homogenising with sonication for 1 min. Aliquots of 100 μl were withdrawn and dispensed into a 96 well microplate (three analytical replicates sample). Finally, 100 μl of 1 mM substrate solution were added. Fluorescence (excitation 360 nm; emission 450 nm) of the product 4-Methylumbelliferone was measured with an automated fluorimetric plate-reader (Infinite® F200PRO Tecan) after 0, 30, 60, 120, 180 min of incubation at 30 °C.

Microbiological analyses were performed according to the procedures described by Martinez et al. (2010).

Pyrolysis-gas chromatography (Py–GC) was carried out by the Macci et al. (2012) method. Seven pyrolytic fragments were considered: acetonitrile (E1), acetic acid (K), benzene (B), pyrrole (O), toluene (E3), 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).

ZEOWINE compost samples were collected to a 60-cm depth from the pile surface. For each pile, a composite sample consisting of 5 sub-samples was taken for a total of about 1L per sample. 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.

ZEOWINE characteristics

The values of physical-chemical properties of ZEOWINE products respected the threshold values proposed in Italian regulation (National legislation DL 75/2010 and subsequent amendments) for mixed plant growth substrate (TOC > 4% ds; pH range 4.5-8.5; EC < 1.0 dS/m), and for base growth substrate (TOC > 8% ds; pH range 3.5-7.5; EC < 0.7 dS/m).

The same national Law (D.Lgs 75/2010 and subsequent amendments) defined the standards for **fertilizers**; these can be divided into agronomical parameters (pH, moisture content, Carbon and organic Nitrogen, etc...), environmental parameters (heavy metals, physical impurities) and sanitization parameters (Salmonella spp., E.coli) (**Table 1**).

Our results highlighted how ZEOWINE (**Table 2**) reached a composition in line with the thresholds established by the Italian fertilizers legislation for a green compost (National legislation DL 75/2010).

The pH values in ZEOWINE 1:10 and ZEOWINE 1:2.5 were 7.97 and 8.28, respectively, in comparison with 7.39 in the control compost without zeolite. In any case, the optimum pH from 7.0 to 8.5 to achieve efficient composting has been obtained in all the compost piles.
The addition of zeolite greatly decreased the salinity, here approximated by measuring the electrical conductivity (EC) (**Table 2**).
The GI is a sensitive indicator of maturity and phytotoxicity. The level of germination index found in ZEOWINE (Table 2) was not phytotoxic for seed germination; in fact, all values of the piles were higher than 70%. A GI of more than 70% indicates phytotoxic-free and mature compost (Rui et al, 2012). Germination index (GI) values of 72%, 126% and 142% in control, ZEOWINE 1:10 and 1:2.5, respectively, were observed at the end of composting. The values higher than 100% in the zeolite-based composts suggested its possible biostimulant effect.
At the end of composting process, higher total nitrogen (TN) content in the compost with zeolite (ZEOWINE 1:10 and 1:2.5) with respect to the compost without zeolite was observed. It can be due to the adsorption of NH_4^+ into the negatively charged small size microporous of zeolite lattice.
Zeolite addition, retaining NH_4^+ leaded to a higher decrease in the C/N ratio with respect to the control compost. Brady and Weil (2010) recommended a range from 14 to 20 for mature compost. At the end of composting, in the control compost this ratio was slightly higher than the suggested limit (21), while it was 19 and 17.4 in ZEOWINE 1:10 and 1:2.5, respectively. The C:N ratio found in ZEOWINE composts fulfilled the threshold values (C/N <50) proposed by National legislation DL 75/2010 for compost.
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 (**Table 2**). Likewise, total potassium increased in ZEOWINE 1:2.5 zeolite based compost, while its available form (Kav) decreased.

In the control pile significantly lower butyrate esterase activity was measured with respect to the ZEOWINE composts. 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.

At the end of composting process higher β-glucosidase activity values were showed in the ZEOWINE 1:10 compost with respect to control and ZEOWINE 1:2.5 composts.

The physical properties of the two Zeowine composts (1:2.5 and 1:10) and of the control are reported in **Table 3**.

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

The results of ZEOWINE composts were comparable to Zhang and Sun (2015; 2016b) 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), the 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 characteristics of zeolite with respect to water adsorption (Zhang and Sun 2015). The moisture contents at the different suction pressure were reported in **Figure 1** (Water retention curve).

At the end of the composting process the three obtained composts 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 (E1) 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 (E3) 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. Finally, the ratio between the sum of K, N and E1 (aliphatic compounds) and B, E3 and Y (aromatic compounds) expresses the energetic reservoir of compost.

The amount of the pyrolytic fragment derived from the degradation of fats and cellulose (K) was affected by the presence of zeolite (**Figure 2**); the significant lower values in ZEOWINE 1:10 and 1:2.5 composts with respect to control indicated the lower "mineralizable" fraction of the organic matter. 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 composts where zeolite was added. In addition, the higher toluene fragment

(O) in the zolite-based composts with respect to control indicated the higher incorporation of nitrogen into the humified portion of the organic compounds.

The ZEOWINE 1:10 and 1:2.5 composts contained 63.6% and 66.4% of aromatic components (B, O, E3 and Y), respectively, while in the control they were 61.6%. The higher relative abundance of aromatic pyrolytic products in the zeolie-based 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 ZEOWINE compost, having carboxyl and phenolic functional groups 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/E3 ratio in the ZEOWINE composts indicated the higher degree of condensation of aromatic rings.

The concentration of Cr, Cu, Ni, Pb, Cd and Zn present in ZEOWINE at the end of the maturing process had lower values to the limit levels proposed by the Italian fertilizers legislation (National legislation DL 75/2010).

The human pathogens and fecal indicators were measured to complement the description of maturity compost. In all the evaluated ZEOWINE piles there was absence of Salmonella sp. and E. coli concentration was less than 100 colony forming unit (CFU) per gram. Furthermore, the fecal coliforms concentrations in all ZEOWINE piles showed lower values than <1000 colony forming unit (CFU) per gram.

Table 1. The thresholds established by the Italian fertilizers legislation (Decree n.75/2010) for green compost.

Moisture (%)	⩽50	Legislative Decree No. 75/2010
pH	6-8,8	Legislative Decree No. 75/2010
Organic Carbon (%)	⩾20	Legislative Decree No. 75/2010
Humic substances (%)	⩾2,5	Legislative Decree No. 75/2010
C:N	⩽50	Legislative Decree No. 75/2010
Cr (mg kg^{-1})	<100	Eco-label to soil improvers (2006/799/EC)
Pb (mg kg^{-1})	140	Legislative Decree No. 75/2010
Cd (mg kg^{-1})	1,5	Legislative Decree No. 75/2010
Ni (mg kg^{-1})	100	Legislative Decree No. 75/2010
Zn (mg kg^{-1})	500	Legislative Decree No. 75/2010
Cu (mg kg^{-1})	230	Legislative Decree No. 75/2010
Hg (mg kg^{-1})	1,5	Legislative Decree No. 75/2010
Cr(VI) (mg kg^{-1})	0,5	Legislative Decree No. 75/2010
Salmonella	absent	Legislative Decree No. 75/2010
Escherichia Coli	≤1000 UFC per g	Legislative Decree No. 75/2010
Germination Index	>60%	Legislative Decree No. 75/2010

Table 2. Characteristics of ZEOWINE1:2.5, ZEOWINE 1:10 and control compost.

		ZEOWINE 1:2.5	ZEOWINE 1: 10	CONTROL	Decreto legislativo No. 75/2010
pH		8,26	7,95	7,37	6-8,8
EC	dS m^{-1}	0,22	0,35	1,51	
CSC	Cmolc kg-1	45,9	43,8	36,4	
TOC	C %	25,68	29,41	27,01	⩾ 20
TN	TN%	1,48	1,55	1,28	
$N\text{-}NO_3$	mg kg-1	73	118	196	
$N\text{-}NH_4$	mg kg-1	611	540	469	
C/N		17,35	18,98	21,1	⩽50
Humic carbon	C%	3,5	3,6	3,2	⩾ 2,5
TK	%	1,19	0,738	0,559	
TP	%	0,144	0,172	0,116	
Available K	mgK kg^{-1}	317	531	453	
Available P	mgP kg^{-1}	328	370	568	
Cu	mgCu kg-1	44	70	78	<230
Zn	mgZn kg-1	35	45	49	<500
Cd	mgCd kg-1	<0,1	<0,1	<0,1	<1,5
Ni	mgNi kg-1	13	23	27	<100
Pb	mgPb kg-1	7,9	8,84	8,26	<140
Cr	mgCr kg-1	21	33	46	<100
Germination Index	%	142	126	72	>60%
Salmonella	CFU g-1	absent	absent	absent	absent
Escherichia Coli	CFU g-1	100	100	100	≤1000

Table 3 Physical properties. 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	References values Green Waste Compost (Naddaf et al, 2011; Zhang and Sun 2016a)	References values Peat	References values growing media (Abad et al., 2001)
BD	0.86 ± 0.03	0.66 ± 0.03	0.36 ± 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	81.3 ± 3.55	70-80	85-90	>85%
AC	44.0 ± 2.39	48.2 ±2.50	54.2 ± 3.74	20-25	15-38	20-30
WC	15.6 ± 1.77	23.0 ± 2.55	27.0 ± 2.54	50	45-70	55-70
EAW	2.70 ± 0.33	7.37 ±1.45	8.43 ± 3.23	15	15-30	20-30
WBC	3.87 ± 0.24	1.05 ±1.02	0.96 ± 0.82	4	3-15	4-10

Figure 1. Water retention curve.

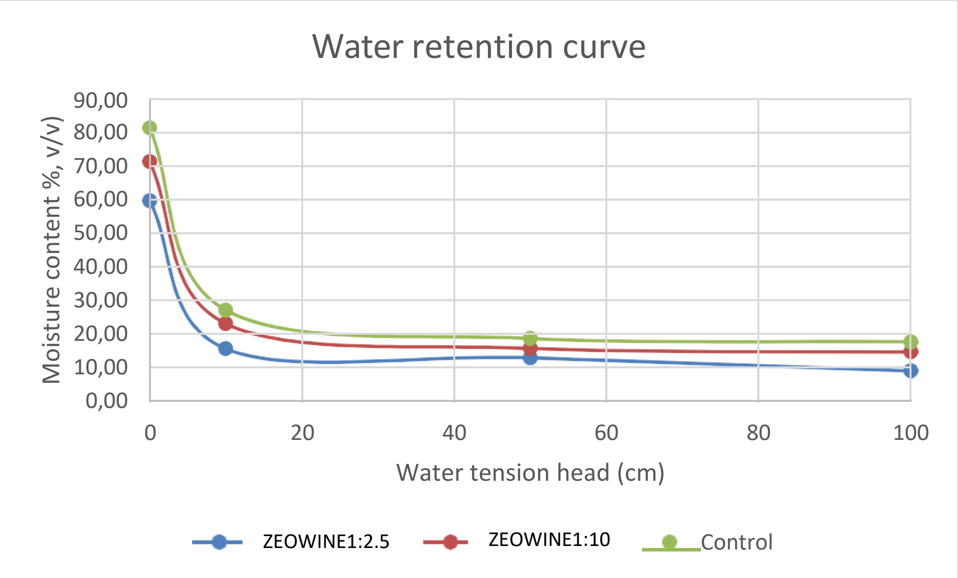
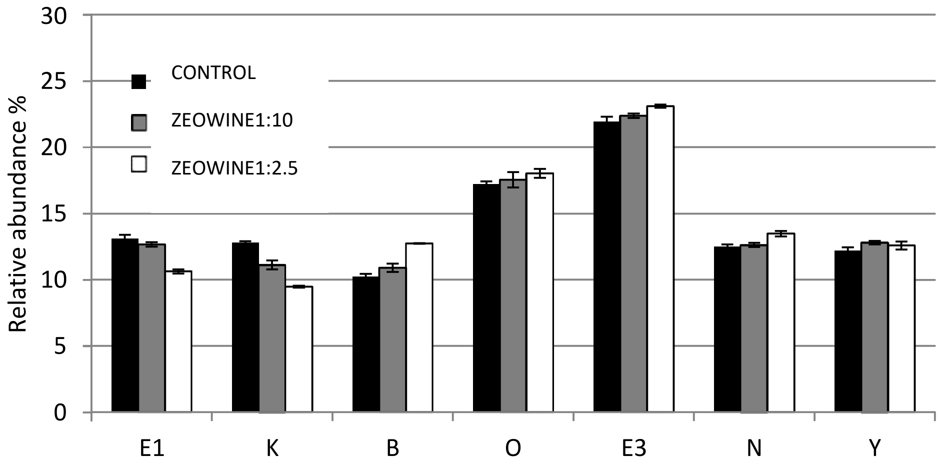


Figure 2. Relative abundances of the main pyrolytic products (percentages of the total pyrogram area) at the end of composting process in the three piles at different rates of of zeolite (0%, 10% and 30%).



	O/N	B/E3	AL/AR
Control	1,374	0,467	0,624
Zeowine 1:10	1,389	0,487	0,572
Zeowine 1:2.5	1,337	0,552	0,505

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 ZEOWINE 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 ZEOWINE composts, both 1:10 and 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 ZEOWINE composts (1:10 and 1:2.5), we can conclude that the ZEOWINE 1:10 compost 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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