Management of Olive Mill Waste Employing Vermicomposting Technology

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Abstract: This work illustrates the feasibility of vermicomposting technology to stabilize different type of olive wastes (Olive mill waste of discontinuous system OMW-D, OMW of three phase continuous system OMW-3P and OMW of two phase continuous system OMW-2P) by using Eisenia andrei .After 3month of vermicomposting the mature vermicompost was evaluated on the basis of agrochemical properties. The result exhibited that the vermicompost were characterized by slightly acidic to neutral pH (5.8–8.08), and optimal EC (2.63–4 dS.m⁻¹) and C/N ratios (38.26–12.99) and an increase in total N (6.05-16.23g.kg⁻¹).Furthermore the vermicompost content a considerable range of plant available forms phosphorus (5.23 g.kg⁻¹), potassium (22.19 g.kg⁻¹), calcium(18.44 g.kg⁻¹) and sodium(2.67 g.kg⁻¹). The polyphenol content decreased strongly between 82.49% and 97.98%. The phytotoxicity screening GI (~50%) clearly suggested the suitability of vermicomposted olive mill waste mixtures for agronomic purpose

Keywords: Olive Mill Waste, Vermicompost, Eisenia andrei, Agrochemical properties, Polyphenol degradation, Germination index

1. Introduction

The olive oil industry has a great importance in Mediterranean countries. They are the major world producers of olive oil with 2.205.300 t by EU member states in 2011 (Caputo et al., 2013). Morocco is the sixth largest producer of olive oil. The average annual of olive production is about 1.5 million tons, 65% are reserved for trituration 25% for table olives and the 10% r represents the consumption and losses. Olive oil is extracted in industrial or semi-industrial olive mills. The number of semi-modern and modern units is in the order of 565. They triturate about 600 000 tons of olives per year and this number is constantly increasing. The semi-modern units use the pressing technique. Their number is decreasing for the profit of modern units which use the technique of decantation and centrifugal separation. The three phase technique is still used, but now it's banned in order to be replaced by the twophase technique. The reason is mainly due to the pollution of rivers and groundwater by olive mill waste water OMWW. This two-phase technique does not need diluting water and improves oil yield of the process (El Mouhtadi et al., 2014).Although this new technique of trituration has produced a new solid waste called two phase olive mill waste (TPOMW) and it is engendered in huge quantities during a short period of time which caused serious management problems due to its phytotoxicity and semisolid texture (Fernández-Hernández et al., 2014).

Vermicomposting is a kindred process to compost and feature the addition of certain species of earthworms used to enrich the process. Furthermore, some species of earthworms are able to consume a wide range of organic wastes from sewage sludge, animal wastes, agricultural residues, domestic wastes and industrial wastes. (Yadav *et al.*, 2011). The viability of using vermicomposting to stabilize olive wastes, has proved. The olive mill waste OMW can support the reproduction and the growth of earthworm, especially when mixed with other waste nitrogen-rich (Melgar *et al.*, 2009). The subsequent application of vermicompost to soil demonstrates that they can be used as organic amendments to promote plant growth and regenerate degraded soils (Benitez *et al.*, 2002; Melgar

et al., 2009). In Morocco, where agriculture has a significant role, organic fertilizer production by vermicomposting of the olive-mill wastes can be the best and the cheapest solution for farmers.

This paper aims at studying the effect of the application of the vermicompost of the olive mill waste coming from different type of trituration (Discontinuous system, continuous three phase and continuous two phase system) with cattle manure for 3 months. Besides, we assess chemical quality, phytotoxicity and polyphenol degradation of residues composted.

2. Materials and Methods

2.1. Materials and sampling

All the olive oil mills waste (Olive mill waste of discontinuous system **OMW-D**, OMW of three phase continuous system **OMW-3P** and OMW of two phase continuous system **OMW-2P**) used in this study were obtained from three regions in Meknes province between November and December harvest season 2013–2014. The earthworm species *Eisenia andrei* (Bouché) were acquired from an earthworm's cultivator Pier Cristani, Temara-Rabat. Morocco. All the samples exhibited unfavorable composting properties, such as: high moisture content, total nitrogen content, acidic pH and unbalanced C/N ratio (Table 1).

2.2. Vermicomposting

Three different types of olive mill waste (**OMW-D**, **OMW-3P** and **OMW-2P**) were mixed with cattle manure in a ratio of 2:3 (P: P) and placed into six plastic square bins (30cm diameter \times 15 cm depth) were filled 4 kg of feed mixed. Also we put two bins as control, there is only cattle manure. The species of earthworm *Eisenia andrei* which we inoculate have in each a total biomass equivalent to 10% of the material dry weight. They are separately placed as indicated below:

- Bins 1: 75% OMW-D+ 25% Cattle manure+ Eisenia andrei
- Bins 2: 75% OMW-3P+ 25% Cattle manure+ Eisenia andrei
- Bins 3: 75% OMW-2P+ 25% Cattle manure+ Eisenia andrei
- Bins 4: 75% OMW-D+ 25% Cattle manure+ Eisenia andrei
- Bins 5: 75% OMW-3P+ 25% Cattle manure+ Eisenia andrei
- Bins C: Cattle manure+ Eisenia andrei

We cover the bins with a dark tissue in order to prevent light from getting in and to prevent the compost from drying out. Also we keep the outdoor temperature in the bin between 20°C and 25°C (Eliva et al., 1996a). The moisture was controlled daily to maintain the 75% moisture level. The bins 4 is weekly sprinkled with olive mill waste water of discontinuous system (OMWW-D), the same holds true for bins 5 with olive mill waste water of three phase continuous system (OMWW-3P). Other bins are sprinkled only with water. The bins are well-ventilated with several holes 100mm from the bottom (otherwise the worms will stay at the bottom of the bin and we may drown the worms), any excess "worm tea" or "leachate" will drain through these holes to the bottom bin and will collect on the upside-down plastic.

Table 1 : Main characteristics of the olive mill waste solid samples (dry weight).

Parameters			
	OMW-D	OMW-3P	OMW-2P
Moisture %	33.29	57.96	64.00
pH	6.21	5.11	5.50
Electrical Conductivity (dm.s ⁻¹)	3.59	4.22	5.33
Organic Matter (g.kg ⁻¹)	308.44	284.30	353.62
Total Organic Carbon (g.kg ⁻¹)	178.91	164.91	205.11
Total Nitrogen (g.kg ⁻¹)	6.14	2.11	5.61
C/N ratio	29.13	78.15	36.56
Total Phosphorus (g.kg ⁻¹)	1.20	1.16	1.34
The total phenolic compounds (g.kg ⁻¹)	9.85	12.32	26.62

Table 2 : Main characteristics of the olive mill waste water samples

Parameters		
	OMWW-D	OMWW-3P
Suspended solids (g.L ⁻¹)	10.79	2.88
Biochemical oxygen demand in 5 days $(g.L^{-1})$	112.66	175.33
Total chemical oxygen demand (g.L ⁻¹)	152.00	178.66
рН	5.50	5.47
Electrical Conductivity (dm.s ⁻¹)	15.47	10.36
Total Organic Carbon(g.L ⁻¹)	24.34	26.51
Total Nitrogen (g.L ⁻¹)	1.19	0.59
Nitrate(mg.L ^{-1})	0.02	0.03
Nitrite(mg.L ⁻¹)	0.49	0.55
Orthophosphate (mg.L ⁻¹)	0.13	0.12
Total Phosphorus(mg.L ⁻¹)	3.19	3.05
The total phenolic compounds(mg.L ⁻¹)	36.03	44.70

2.3. Analytical Methods

The pH and electrical conductivity (EC) were determined respectively on a mixture of compost/water (1:2.5 and 1:5) (Zenjari et al., 2001).Total organic carbon (TOC) and Organic matter (OM) were determined by using Walkley-Black method (Pansu et al., 2006) and total nitrogen (TN) was determined in 0.5 g samples by the Kjeldahl method and phosphorus(P) by colorimetry (Kiston et al., 1944). The total phenolic compounds (TPC) were determined according the method described by (Ait Baddi et al., 2009). All chemical analyses were carried out in triplicate and the standard deviation (SD) was calculated.

The phytotoxicity of compost extracts was determined according to the method by (Zucconi et al., 1981; Selim et al., 2012) by using seed germination technique Cress seeds (lepidum sativum). The percentage of seed germination, root elongation and germination index (GI) was calculated according to (Selim et al., 2012) as follows:

Seed germination (%): •

Seed germination(%)= $\frac{No \text{ of seed germinated in compost extract}}{No \text{ of seed germinated in compost extract}}$ ×100 No of seed germinated in control

Root elongation (%):

Root elongation(%)=
$$\frac{\text{Mean root length in compost extract}}{\text{Mean root length in control}} \times 100$$

2.4. Statistical Analyses

The experimental data were subjected to a one-way analysis of variance (ANOVA) by using the SPSS.20 program for Windows, the mean separations were performed by also the differences between means were determined using the Tukey's test at the level of significance of P < 0.05.

3. Results and Discussion

3.1. Vermicompost quality obtained

The vermicompost quality used to be characterized mostly by the pH and EC parameters. There were slights changes in the pH of vermicompost as compared to initial values in the all bins (Figure 1). The increase in pH could be caused by the degradation and consumption of organic acids by microorganisms (Hanc et al., 2014). However, the pH values of control (Cattle manure) was decreasing from 8.94±0.13 to 7.73±0.22. A decrease in pH (from 8.6 to 7.3) was also found in the study conducted by (Suthar, 2010) who vermicomposted agro-industrial sludge. The mineralization of nitrogen and phosphorus into nitrites/nitrates and orthophosphates, bioconversion of organic material into intermediate species of organic acid may have decreased the pH (Wani et al., 2013). Moreover, a decrease in pH might be an important factor in nitrogen retention as this elements is lost as volatile ammonia at high pH values (Eliva et al., 1998).

The EC increased slightly during the vermicomposting for all bins (Figure 1). This increase may be due to reduction of organic matter and release of different minerals salts: phosphate, ammonia, potassium, etc. (Suthar *et al.*, 2013). For the application of vermicompost to the land suggested that the maximum tolerance limits of plants for EC is 4.0 $d.Sm^{-1}$. The results of pH and EC show some difference between bins may be due to sprinkle system, bins4 and 5 were the louder of pH and the higher EC.



Figure 1 : The pH (A) and electrical conductivity (EC) (B) of vermicompost at the beginning (Initial) and after 3 month (Final). Results expressed as mean with standard deviation (n = 3). Vertical bars with different letter indicated significantly differences between treatments according to

Tukey test at a P < 0.05 level of probability

The results indicate that significant reduction in TOC and OM levels were observed during vermicomposting in all bins (Table 3). The association of earthworms (enzyme activities) and microbial communities (bacteria, fungi, actinomycets, protozoa, nematodes) are the answerable of mineralization of TOC (Suthar *et al.*, 2013). The maximum organic C loss 14.73% was in bins 3, while bin 4 exhibited the minimum 3.16 % organic C loss during the vermicomposting process, it is possible that the olive mill waste water reducing earthworms and microbial activity.

The TN in all bins had increased significantly (p ≤ 0.05) by the end of vermicompost, presumably because the mineralization of organic matter. The highest value was found in bin 5 (16.23±0.469g.kg⁻¹) also bin 4 (16.18±0.51 g.kg⁻¹) and the lowest was found in control (9.76±0.55 g.kg⁻¹). The results exhibited that bin5 which having the olive mill waste water as the damping agent showed the highest TN than those with water bin 2, there were also found in the study conducted by (Macci *et al.*, 2010). Maybe the olive mill waste water was responsible for increased nitrogen concentration (Aviani et *al.*, 2010).

The C/N ratio is one of the most widely indices for maturity of organic wastes. The C/N ration were in the range of 12.99 and 20.30 (Table 3). The loss of CO2 in the process of respiration and production of mucus and nitrogenous excrements are responsible for C/N ratio changes during vermicomposting (Prakash *et al.*, 2009). Senesi (1989) have reported that a decline in C/N ratio to \leq 20 indicates organic waste stabilization, its maturity and stability. The ratio of C/N is important for the proper growth of any plant. Furthermore, the decline in C/N ratio may be due to microbial respiration and the mineralization of the labile organic compounds, which reduced the weight and volume of the processed mass, and by the concentration effect the TN was increased (Hanc et *al.*, 2014).

Table 3: The total organic carbon (TOC), the organic matter (OM), the total nitrogen (TN) and ration Carbone/nitrogen (C/N	J)
of vermicompost at the beginning (I) and after 3 month (F). (g.kg ⁻¹ dry weight)	

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		Bin 1	Bin 2	Bin 3	Bin 4	Bin 5	Control
TOC	Ι	218.44±0.77d	230.67±3.47e	231.48±0.80e	218.44±0.77d	230.67±3.47e	203.35±2.32a
g.kg ⁻¹	F	210.95±0.95c	211.11±0.67c	197.37±4.66c	211.53±0.67c	210.96±0.41c	198.22±0.63b
OM	Ι	376.59±1.34d	397.67±5.98e	399.07±1.39e	376.59±1.34d	397.67±5.98e	350.59±4.01a
g.kg ⁻¹	F	363.67±1.64c	363.96±1.16c	367.00±2.12c	364.68±1.16c	363.24±0.71c	341.74±1.09b
TN	Ι	6.08±0.04a	6.83±0.07ab	6.05±0.03a	6.08±0.04a	6.83±0.07ab	7.82±0.83b
g.kg ⁻¹	F	15.84±0.05df	16.00±0.11df	15.11±0.38d	16.18±0.51df	16.23±0.49f	9.76±0.55c
C/N ratio	Ι	35.92 ≥20	33.77 ≥20	38.26 ≥20	35.92 ≥20	33.77 ≥20	26.00 ≥20
	F	13.31 ≤20	13.19 ≤20	13.06 ≤20	13.07 ≤20	12.99 ≤20	20.30 ≥20

Results expressed as mean \pm standard deviation (n = 3). Different letter indicated significantly differences between treatments according to Tukey test at p< 0.05 level of probability

The mature vermicompost produced was rich in nutrients in all bins. Phosphorus is a much-needed element for plant development and growth, the higher phosphorus (P) content was 5.230 ± 0.23 g.kg⁻¹report in bin 4. The level of Phosphorus in vermicompost might reflects the amount; of organic forms of phosphorus in waste mixture, but its mineralization, rate is directly affected by nature of

amendment material and activities of P mineralizing micro flora in decomposing wastes(Suthar 2010).Furthermore the nutrients such as Calcium (Ca) and potassium (K) concentrations of vermicompost were presented in Table 4. Both Ca and K concentration in vermicompost were significantly higher in bin 5(K) 22.19±1.05 g.kg⁻¹ (Ca) 18.44±0.77 g.kg⁻¹. These concentrations are comparable or higher than those reported by other studies (Suthar 2010; Hanc et *al.*, 2014). As a final point the mature vermicompost appears to contain sufficient nutrients (N, P, K, Ca, and Na) to be considered a high quality soil amendment.

Table 4 : The phosphorus (P), the potassium (K), the calcium (Ca), the sodium (Na) of vermicompost after 3 month (g.kg-1 dry weight)

dry weight)						
	Bin 1	Bin 2	Bin 3	Bin 4	Bin 5	Control
Р	4.81±0.10b	4.78±0.04b	4.82±0.03b	5.23±0.23b	4.19±0.12b	3.62±0.24a
Κ	18.36±0.67a	18.83±0.98a	18.19±1.14a	22.11±0.84b	22.19±1,05b	17.08±0.42a
Ca	12.18±0.64a	12.52±1.07ab	12.63±1.16ab	16.84±3.62bc	18.44±0.77c	10.95±0.15a
Na	1.67±0.30a	1.78±0.18a	1.73±0.08a	2.67±0.28b	2.45±0.32b	1.59±0.03a

Results expressed as mean \pm standard deviation (n = 3). Different letter indicated significantly differences between treatments according to Tukey test at a p< 0.05 level of probability

3.2. Polyphenol degradation

The total phenolic compounds is an important indicator of compost maturity of olive mill wastes, beside he is known to be responsible for the phytotoxicity of these waste (Ait Baddi et al., 2004; Alburquerque et al., 2006. Table 5 shows that total phenolic compound (TPC) was decreasing significantly (p< 0.001) throughout the vermicompost process. The total polyphenol degradation was about 82.49% and 97.98% these concentrations are similar or lower than those reported by other studies with compost process (Fernández-Hernández et al., 2014; Altieri et al., 2011; Alburquerque et al., 2006) which explain that the Eisenia andrei are able to degrade the polyphenol after 3month. Nevertheless the bin 5 showed lower degradation of TPC and bin 1 was the higher. According to (Domínguez et al.,2014), the earthworms act like mechanical mixers since they decompose organic matter, increasing the surface area exposed to microbes, and move fragments and bacteria-rich feces through the waste profile. The difference between bin 2 and bin 5 is about 11.45% and between bin 1 and bin 4 is about15.49% of polyphenol degradation which explain that the sprinkler system with olive mill waste is responsible for these difference, it's may be due to high phenolic content of olive mill waste water which reduces microbial activity (Melgar et al., 2009).

Table 5: The total phenolic compounds (g.kg⁻¹) of vermicompost at the beginning (Initial) and after 3 month

(Final).						
Treatments	Initial	Final	% of polyphenol			
	Mean±SD	Mean±SD	degradation			
Bin 1	7.94±0.32c	0.16±0.05a	97.98%			
Bin 2	11.68±0.34d	0.24±0.01a	97.94%			
Bin 3	13.59±0.16e	0.36±0.02a	97.35%			
Bin 4	7.94±0.32c	1.39±0.07b	82.49%			
Bin 5	11.68±0,34d	1.63±0.18b	86.04%			
Control	0.04±0.01a	0.01±0.01a	-			

Results expressed as mean \pm standard deviation (n = 3). Different letter indicated significantly differences between treatments according to Tukey test at a p< 0.001 level of probability

3.3. Phytotoxicity

Phytotoxicity is one of the most-important criteria for evaluating the suitability of organic materials for agricultural purposes (Zucconi et al., 1981). All the bins have the same GI ~ 100% to 70%. (Figure 2). In our study the phytotoxicity screening (GI) shows that the bin can be arranged as fallow: bin 5< bin 4< bin1<bin 3< bin 2 < Control. According to (Zucconi et al., 1981) the germination index ~50% has been used as an indication of phytotoxin-free compost. Therefore the result indicates the suitability of all vermicompost for agronomic purposes.



standard deviation (n = 3)

4. Conclusion

The study concludes that olive mill waste for all type of trituration are suitable feedstock for earthworms *Eisenia andrei* which is able to convert it into a value added product. The addition of cattle manure to olive mill waste enhance earthworm biomass during vermicomposting. The resulting vermicompost were characterized by slightly acidic to neutral pH, and optimal EC and C/N ratios. The mature vermicompost has higher nutrient content (Phosphorus, potassium, calcium, sodium). Moreover the *Eisenia andrei* are able to degrade the polyphenol. The phytotoxicity screening (GI) submitted the suitability of vermicomposting olive mill waste mixtures for agronomic purposes.

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