# Nutrient recovery from olive by-products through vermicomposting with *Eisenia andrei*

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**Abstract**

In this study, *Eisenia andrei* potential for detoxification of the olive by-products was evaluated. A standard mixture containing 600 g of olive pomace (OP), 300 g of horse manure and 100 g of wheat straw was imbibed by various olive mill wastewater (OMW) ratios (0%, 10%, 25% and 50%) to prepare four mixtures (M1, M2, M3 and M4 respectively), then inoculated with adult earthworms. The biological parameters related to earthworm's biomass gain and reproduction rate were better in the mixture M1 which used as control mixture (10.7 mg worm$^{-1}$ day$^{-1}$ and 0.17 cocoons worm$^{-1}$ day$^{-1}$ respectively) followed by M2 and M3. When OMW ratio exceeded 50% of OMW (M4), mortality of earthworm occurred after 7 days of incubation. The reduction of phenol concentration was higher in mixture M1 (91%) compared to other mixtures (M2: 72%, M3: 54%). The C/N ratio decreased in all mixtures to reach values between 17 and 22 at the end of vermicomposting process. The phytotoxicity test revealed that the overall germination index was greater than 80% in M1, M2 and M3, which showed that vermicompost produced can be used as organic amendment for biological and sustainable agriculture.

**Keywords:** Olive pomace; Olive mille wastewaters; Vermicomposting; *Eisenia andrei*; Total phenols.

## 1. Introduction

In Morocco olive industry has an important socio-economic role, with a production of about 16 million tons of olive and 3 million tons of olive oil (FAOSTAT 2014). However this sector also causes serious environmental problems due to the production of large quantities of olive by-products; OP and OMW, during short periods (Roig *et al*. 2006). Indeed OMW present a major environmental challenge due to their high toxic organic loads, high chemical oxygen demand (COD) (110 g L$^{-1}$) (Stasinakis *et al*. 2008). Given this situation, several chemical physical and biological techniques have been developed to treat and recycle OMW but despite the efficiency of these processes, their high cost is the main drawback to their industrial application (Paraskeva *et al*. 2007). Vermicomposting is an alternative cost-effective and rapid biotechnological process for bioconversion into a stabilized humus-like product (Garg *et al*. 2006a).

The aim of the present study is to use vermicomposting at the laboratory scale as a means of recycling and treating olive by-product (OMW and OP), in order to reduce their toxicity, and process them into organic soil.

## 2. Materials and methods

### 2.1. Earthworms and collection of olive by-products

Earthworms (with clitellum) belonging to the species *Eisenia andrei* (Bouché 1972) have been chosen. Olive by-products (OMW and OP) were collected from three-phase centrifugation system.

### 2.2. Experimental set up

The experiments were carried out in 9 L plastic boxes with tight-fitting lids which referred to as vermireactors. The process of laboratory vermicomposting lasted 10 weeks.

The substrate mixture of 60% OP + 30% HM + 10% WS. This mixture was then imbibed with different ratios of OMW ie 0% (control) M1, 10% (M2), 25% (M3) and 50% (M4). Quantity of 3 kg of each mixture was partitioned between three compartments of vermicomposter (1 kg compartment$^{-1}$) and then inoculated with 40 adult earthworm kg$^{-1}$ of substrate. Each treatment was run in triplicate.

### 2.3. Physico-chemical analysis

The pH was measured in an aqueous solution 1 10$^{-1}$ (weight volume-$^{1}$) (Pansu *et al*. 2001). The C/N ratio was determined by the method of Dumas (1831). Total phenol content in different samples was calorimetrically estimated at 765 nm, using the Folin–Ciocalteu reagent (Folin and Ciocalteu 1927).

### 2.4. Phytotoxicity test

Vermicompost maturity was evaluated using phytotoxicity test on tomato seeds according to Zucconi *et al*. (1985). Aqueous extracts were prepared by stirring a mixture of vermicompost with distilled water in a ratio of 1: 4 (w v$^{-1}$) (Lima *et al*. 2008). The mixture was then centrifuged at 3000 rpm for 25 min, and then supernatant was diluted with distilled water to obtain aqueous extracts dilutions 25, 50, 75 and 100%. Tomato seeds were sterilized and placed in sterilized Petri dishes and soaked with 10 ml of different aqueous extract ratio of vermicompost 25%, 50%, 75%

and 100%. The Petri dishes were incubated at 25 ° C for 6 days. Distilled water was used as control. Each test was performed in three repetitions. The number of germinated seeds was counted and root length was measured. Germination index (Gi) was calculated using the following equation:

$$Gi = (G / G0) x (L / L0) x 100$$

Where, G and L are respectively the average germination percentage and the average root length obtained in each dilution. G0 and L0 are the germination percentage and the average root length of control respectively (100% distilled water). The overall germination index (GI) was calculated, it is the average germination index Gi of dilutions 50% and 75% (Zucconi *et al.* 1985).

### 2.5. Statistical analysis

The mean value of each treatment and the corresponding standard deviation were calculated taking into account the three repetitions performed. The data obtained were subjected to statistical analysis with STATISTICA 6.0 software. The variance analysis (ANOVA) was performed. In cases where significant differences were found ($p < 0.05$), means were separated using the Newman-Keuls test ($p < 0.05$).

## 3. Results and discussion

### 3.1. Earthworm growth and reproduction in different mixtures

*E. andrei* biomass patterns are reported in Table1. The mean earthworm biomass increased significantly during vermicomposting period M1, M2 and M3. However in M4 earthworm's biomass was slightly decreased. Indeed, the maximum biomass was recorded in M1 (37.1 g), followed by M2 (33.5 g) and M3 (30.9 g) after 6, 7 and 8 weeks respectively. After 8 weeks of laboratory vermicomposting, the total biomass decrease to reach a value of 35.5, 30.6 and 26.2 g in M1, M2 and M3 respectively. It has been suggested that decline of earthworms biomass at the end of the vermicomposting process was due to the conversion and depletion of substrate (Garg and Gupta 2011). The minimum average biomass was recorded in the M4 (20.7 g), a significant decrease was observed due to a high mortality rate (20 %). It was reported that raw materials with high phenols fraction and lignin concentration (such as OP and OMW) are not well adequate for growth and development of most earthworm's species (Ganesh *et al.* 2009). Moreover, the chemical composition of the feed substrate has an important role in feed rate of earthworms (Suthar 2008b) consequently the difference biomass gain between earthworm can be explained by the difference of phenol concentration of each mixture.

The maximum growth rate was achieved in M1 (10.7 mg $worm^{-1}$ $day^{-1}$), followed by M2 (8.12 mg $worm^{-1}$ $day^{-1}$), and M3 (7.24 mg $worm^{-1}$ $day^{-1}$). However M4 exhibited a loss of 0.79 mg $worm^{-1}$ $day^{-1}$, at the end of vermicomposting process (Table. 1). The difference in growth rate between mixtures can be attributed to antimicrobial and toxic effect of OMW, which inhibits microbial activity that is essential for organic matter degradation by earthworms (Suthar 2008a).

**Table 1:** Earthworms biomass during vermicomposting process (mean ± SD, n = 3).

| Mixture | Mean initial earthworm biomass (g) | Mean maximum biomass achieved (g) | Mean biomass achieved at the end (g) | Net biomass acquired $earthworm^{-1}$ at the end (mg) | growth rate mg $worm^{-1}day^{-1}$ |
|---|---|---|---|---|---|
| M1 | 11.5 ±0.1[a] | 37.1 ±0.4 [a] | 35.6 ±0.6 [a] | 602.5 ±0.3 [a] | 10.04±0.03 [a] |
| M2 | 11.2± 0.2[a] | 33.5 ± 0.7 [a.b] | 30.6 ±0.1[a.b] | 487.5±0.2 [a.b] | 8.12±0.03 [a.b] |
| M3 | 11.1 ±0.1 [a] | 30.9 ± 0.5[b] | 29.2 ±0.1 [b] | 452.5 ±0.1 [b] | 7.54±0.01 [b] |
| M4 | 11.2±0.4 [a] | 20.7 ±0.4[c] | 9.3±0.4[c] | -47.5 ± 0.2[c] | -0.79±0.02 [c] |

*Mean value followed by different letters is statistically different (ANOVA; Newman-Keuls t-test, $P < 0.05$).

### 3.2. Physico-chemical changes in waste mixture during vermicomposting

The aim of this work was to test earthworm's potential for OMW detoxification. Indeed according to figure 1, M4 has the highest phenols concentration (3.1 mg $g^{-1}$). Phenols concentration decreased in all the mixtures during vermicomposting process. Maximum reduction of phenol concentration was obtained in M1 (91%), followed by M2 (72%), M3 (55%), and the minimal decrease of phenol concentration was observed in M4 (23%). Although the phenolics compounds are resistant to biodegradation, a significant reduction in phenols concentration was measured (91 %) (M1). In addition Cayuela *et al* (2008) reported a disappearance of phytotoxicity of tow-phase olive wastes after 40 composting weeks. Zenjari *et al.* (2006) attributed the decrease of phenol concentration to the microbial bioconversion of polyphenolic compounds and their interaction with secondary metabolites contributing to the biosynthesis of humic substances

C / N ratio present an important parameter to evaluate vermicompost maturity. C / N ratio of different initial mixtures varies between 30.3 ± 0.3 and 35.8 ± 0.6 (fig. 2), (M1 and M4 respectively). During vermicomposting process C/N ration decrease in all mixture to reach final C/N ratio between 17.8 ± 0.1 and 22.1 ± 0.3 in M1 and M4 respectively (Fig. 2). The results of this study are in agreement with those obtained by Michailides *et al.* (2011), which indicated a final C/N ratio of 27. In addition Komilis and Tziouvaras (2009) reported a final C/N ratio of 27 after 548 days of composting. Indeed, C/N ratio decrease during vermicomposting has often been attributed to mineralization of organic matter by micro-organisms (Grigatti *et al.* 2004). The decrease in C/N ratio can be attributed to combined action of earthworms and microorganisms provoking mineralization and

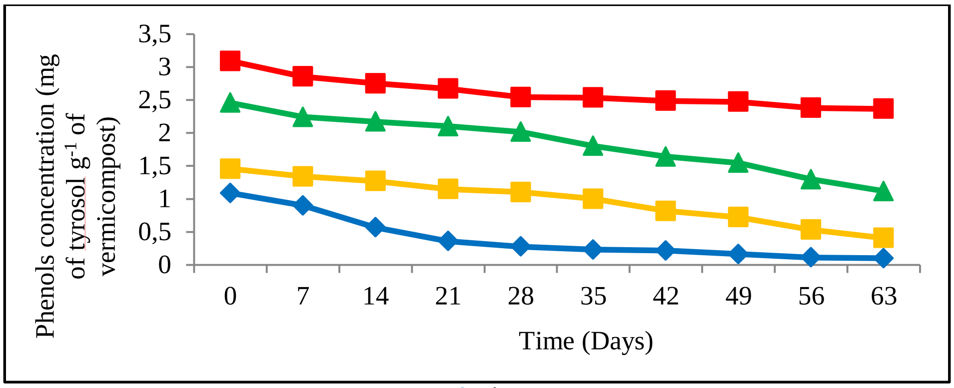


**Figure 1.** phenol concentration in different mixtures (M1), (M2), (M3) and (M4) during vermicomposting process.

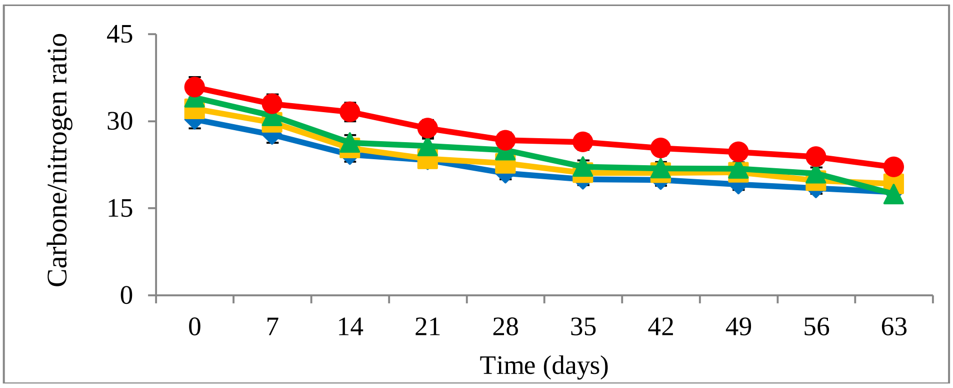


**Figure 2**. Changes in carbon/nitrogen ratio of different mixtures; (M1), (M2), (M3) and (M4) during vermicomposting process.

transformation of carbon compounds to simpler and available forms (Parkin and Berry 1999).

**3.3. Phytotoxicity test**

Tomato's seeds germination percentage in different dilutions of each mixture, are exposed in Figure 3. In the end of vermicomposting, germination indices increased significantly, mainly due to disappearance of OMW toxicity at the end of vermicomposting. Germination index obtained in this work was greater than 80% in the M1, M2 and M3 (86 %, 84% and 81 %) respectively (Fig. 3), while the germination index does not exceed 60% in M4. The important germination index obtained in M1, M2 and M3 can be attributed to a reduction of toxic organic substances contained in OMW and OP, and their transformation on nutritional elements for seed germination. However, the low germination index obtained in M4 may be due to phenolics compounds, especially tannins that reduce plant growth.

The difference between the germination percentages between the various mixtures may due to existence of a beneficial microorganism's community responsible for an enzyme activity or substances that are involved in the growth of roots (Tomati *et al*. 1995). In addition Zenjari *et al*. (2006) attributed the decrease of phenol concentration to the microbial bioconversion of polyphenolic compounds contributing to the biosynthesis of humic substances.

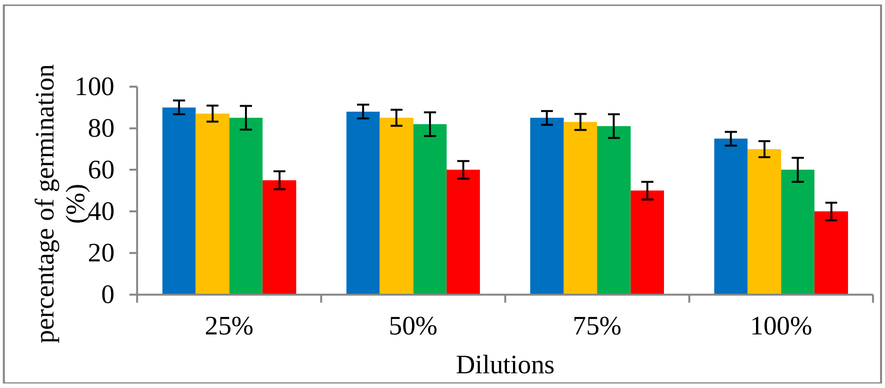


**Figure 3**. percentage of seeds germination in different dilutions of each mixture (M1), (M2), (M3) and (M4).

## 4. Conclusion

The results of this study support the potential of *Eisenia andrei* to grow and reproduced favorably in the OP mixed with HM, WS and imbibed with diluted OMW. Although OMW is a recalcitrant organic by-product for decomposition, the combined action of earthworms and micro-organisms enhances its biodegradation. Phytotoxicity test revealed that vermicompost displayed a high degree of stability, as is demonstrated by high germination index (more than 80%). Acclimatization of earthworms is recommended to enhance their adaptation to high OMW concentrations. Thermophilic pre-composting of olive by-products prior to vermicomposting process could be favorable to reduce OMW toxicity towards earthworms.


## Acknowledgements

This research work was financially supported by PHC Toubkal TBK/15/03 – Campus France N° 32548XH project.


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