HILL PUBLISHING

International Journal of Food Science and Agriculture, 2020, 4(2), 148-155
http://www.hillpublisher.com/journals/jsfa/
ISSN Online: 2578-3475
ISSN Print: 2578-3467

Potential of Acclimated Earthworm (*Eisenia Andrei*) for Detoxification of Olive Mill Wastewater

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How to cite this paper: Barhoum Kharbouch, Hicham Lakhtar, Sandrine Amat, Sevastianos Roussos, Nathalie Dupuy, and Abdelhamid El Mousadik. (2020) Potential of Acclimated Earthworm (*Eisenia Andrei*) for Detoxification of Olive Mill Wastewater. *International Journal of the Science of Food and Agriculture*, 4(2), 148-155.
DOI: 10.26855/ijfsa.2020.06.006

Received: November 22, 2019
Accepted: March 22, 2020
Published: April 24, 2020

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Abstract

The present study was conducted to evaluate the potential of acclimated and unacclimated earthworm (*Eisenia andrei*) for decomposition and detoxification of olive mill wastewater (OMWW). Although, they stimulate biological reactions at low concentrations, OMWW are toxic to earthworms and microorganisms at moderate concentrations and can cause negative effects on the vermicomposting processes. After two months of laboratory vermicomposting, the growth rate (21.83±0.01 mg $worm^{-1}$ day^{-1}) of earthworms and phenols reduction (72%), were higher with acclimated earthworms. However, unacclimated earthworms showed lower growth rate (4.67±0.01 mg $worm^{-1} day^{-1}$) and phenols reduction (41%). Moreover, the vermicompost produced with acclimated earthworm is characterized by alkaline pH (9.9), optimal electrical conductivity (78.20 dS cm^{-1}), optimal C/N ratios (17.40-18.00) and an increase in total nitrogen (1.7-2.0 %). The data reveal that the acclimated earthworms are well adapted in the moderate OMWW concentration and their utilization in vermicomposting process could be a promising alternative for OMWW detoxification. Additionally, vermicomposting can be a suitable technology for olive mill wastewater treatment.

Keywords

Olive Pomace, Olive Mill Wastewater, Vermicomposting, *Eisenia Andrei,* Total Phenols, Enzymatic Activities, Acclimatization

1. Introduction

Olive oil production causes serious environmental problems due the production of high quantity of by-products, namely olive pomace (OP), olive mill wastewater (OMWW) (from three-phase systems), and two-phase olive mill waste (TPOMW) (from two-phase systems) during short production season [1]. Worldwide annual production of OMWW is estimated at 3 × 10^6 m^3 [2], with an organic load equivalent to about 22 million people per year [1]. Morocco is the six olive oil-producing countries (130,000 t) [3]. However, this activity produces a huge quantity of OP and olive OMWW causing serious environmental problems. Indeed, OMWW present a major environmental challenge due to their highly toxic organic loads and their high chemical oxygen demand (COD)

(110 g L^{-1}) [4]. Nowadays, recycling of solid olive residues is generally limited to their use as a cheap and local source of fuel for ovens and water heating [5]. In Morocco, OMWW was simply dumped directly into the sewage system, kept in evaporation lagoons or just spread on land, what frequently results in an important environmental pollution [6]. Consequently, a direct application of OMWW in soil affects its infiltration capacity, with negatively affect groundwater quality [7]. The difficulty of treating OMWW is due to their high levels of recalcitrant organic compounds [8], and to technical constraints related to seasonal production and to the scattering of the olive-mill units over the whole territory.

Given this situation, several chemical, physical and biological techniques were developed to treat and recycle OMWW. Despite the efficiency of these processes, their high cost is the main drawback to their industrial application (example in Morocco) [9]. Vermicomposting is a cost-effective alternative and a rapid biotechnological process to convert organic substances into a stabilized humus-like product [10]. Furthermore, some species of earthworms are able to consume a wide range of organic wastes from sewage sludge, animal wastes, and agricultural residues, domestic and industrial wastes [11]. The viability of using vermicomposting to stabilize olive wastes was proved. OMWW can support reproduction and growth of earthworms, especially when mixed with other nitrogen-rich wastes [12]. The subsequent application of vermicompost to soil demonstrates that it can be used as organic amendments to promote plant growth and regenerate degraded soils [12]. In fact, vermicompost was considered as an excellent organic amendment because it contains plant growth hormones and a larger microbial population as well as it tends to contain more nutrients without negative impact on the environment than traditional compost [13].

In the literature, acclimation of earthworms prior to vermicomposting has never been studied. The aim of the present study was to test the effect of acclimatization on potential of earthworms for detoxification of OMWW at laboratory vermicomposting scale.

2. Material and Methods

2.1. Earthworms and collection of olive by-products

Earthworms (with clitellum) belonging to the *Eisenia* species [14] were used in this work. This specie was chosen thanks to its high activity, a short time of growth, high rate of reproduction and be handled easily [15].

Olive by-products (OMWW and OP) (Table 1) were collected from a three-phase centrifugation system in Oulad Teïma (30° 23 '40.8 "North, 9° 12' 32.3" West) province of Agadir, Morocco. Horse Manure (HM) was provided by a horse farm (Agadir). Wheat Straw (WS) obtained from the same farm was added as bulking agent. All substrates were air-dried in a greenhouse and stored before launching the vermicomposting experiments.

2.2. Experimental design

Two groups of earthworms (*Eisenia andrei*) have been acclimated differently. The first group of earthworms has been acclimated to the OMWW; by raising them in a mixture composed of 60% OP, 30% HM and 10% WS soaked with 10% OMWW for six months before our experimentation. The second group of earthworms was raised in the same mixture without OMWW. To initiate our experiment, a mixture of OP (60% D.W), HM (30% D.W) and WS (10% D.W) was prepared, then soaked by 80% of OMWW and pre-composted for one month. The laboratory vermicomposting study was carried out in a 2.5 L plastic box. To test the effect of acclimatation, 1 kg of pre-composted mixture was inoculated with 5 adult and acclimated earthworms [16]. Another, 1 kg of pre-composted mixture was inoculated with 5 adult and unacclimated earthworms. Each treatment was run in triplicate.

The change in individual biomass of earthworms was measured every 10 days in each experiment. Earthworms were separated from the substrate material by hand sorting, after which worms were washed in tap water to remove adhering material from their body, and subsequently weighed on a live weight basis. No correction for gut content was applied to any of the data. Then all measured earthworms were returned to the container. The following observations were made during experimentations: the maximum biomass achieved, net biomass gain, maximum growth rate (mg $worm^{-1}$ day^{-1}).

Samples were handily homogenized and collected from each experiment every ten days during two months and they were air-dried and stored for further analysis; pH, electrical conductivity, carbon/nitrogen (C/N) ratio and total phenol content. The moisture level of each mixture was maintained about 75–80% throughout the study period by periodic sprinkling of adequate quantity of water.

2.3. Statistical analysis

The data analyses were carried using STATISTICA, version 6, and one-way ANOVA was used to analyze the differences between treatments. A Newman-Keuls test was also performed to identify the homogeneous type of the data sets among different treatments for different biological parameters of earthworms and chemical and biochemical parameters (*i.e.*, pH, EC, phenol and C/N ratio) in each experiment.

3. Results and Discussion

3.1. Biomass and Growth Rate of Earthworms

As shown in Figure 1, the biomass of acclimated earthworms increased significantly from the beginning of the laboratory vermicomposting and their maximum biomass (ANOVA, F= 11.05, $P< 0.05$) reached was 3.58 g ± 0.3 after 40days. After this, a slight decrease of biomass was observed to reach 3.44 ± 0.24 g. However, unacclimated earthworms presented a decrease in average biomass during10 days of laboratory vermicomposting and a slight increase in the average biomass was recorded to reach 2.45±0.13 g at the end of the experiment.

The growth rate expressed in milligrams of biomass acquired per earthworm per day was considered as a very good indicator for comparing earthworm growth in different substrates [17]. The maximum growth rate was achieved in mixture with acclimated earthworms (21.83±0.02 mg worm^{-1}day^{-1}) (Fig. 2). However, the growth rate of unacclimated earthworms (4.67±0.03 mg worm^{-1}day^{-1}) was 4.67 lower than recorded with acclimated earthworms. Ganesh et al. [18] deduce that raw materials with high phenols fraction and lignin concentration (such as OP and OMWW) are not well adequate for the growth and the development of most species of earthworms. In this study, the difference in growth rate between earthworms could be attributed to the effect of the acclimatization of earthworms to OMWW. El Hajjoujiet al. [6] reported antimicrobial and toxic effects of OMWW. Suthar conclude that microbial activity is essential for organic matter degradation by earthworms [19].

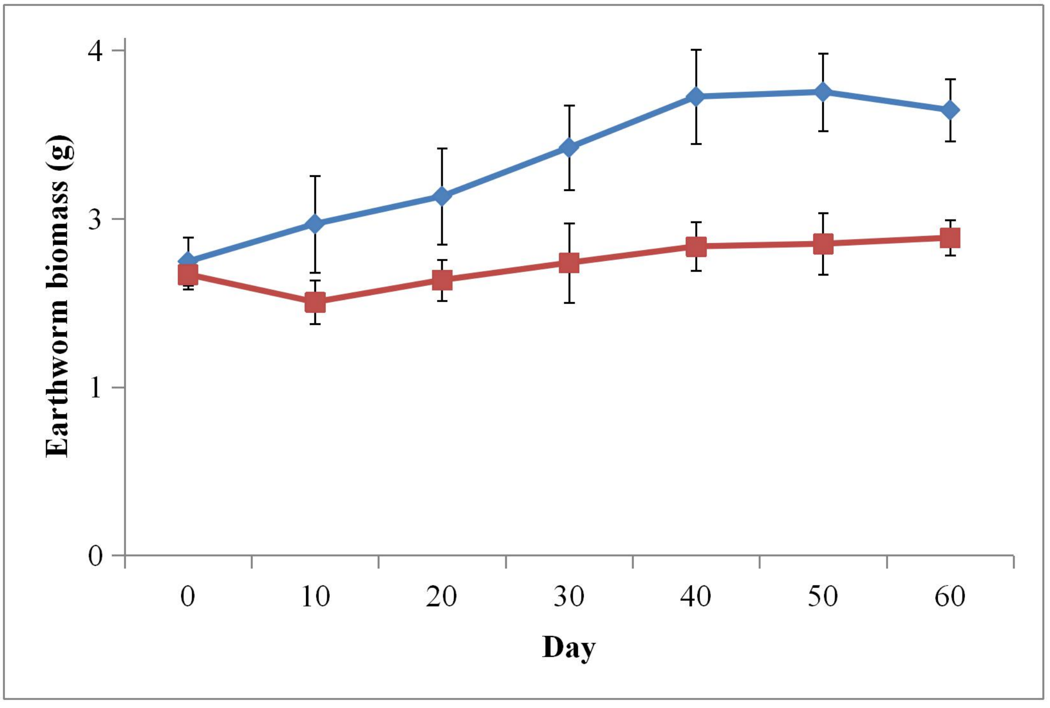

Figure 1: Biomass changes of acclimated ( ) and unacclimated ( ) earthworms during vermicomposting experiments. Vertical bars indicate standard deviation.

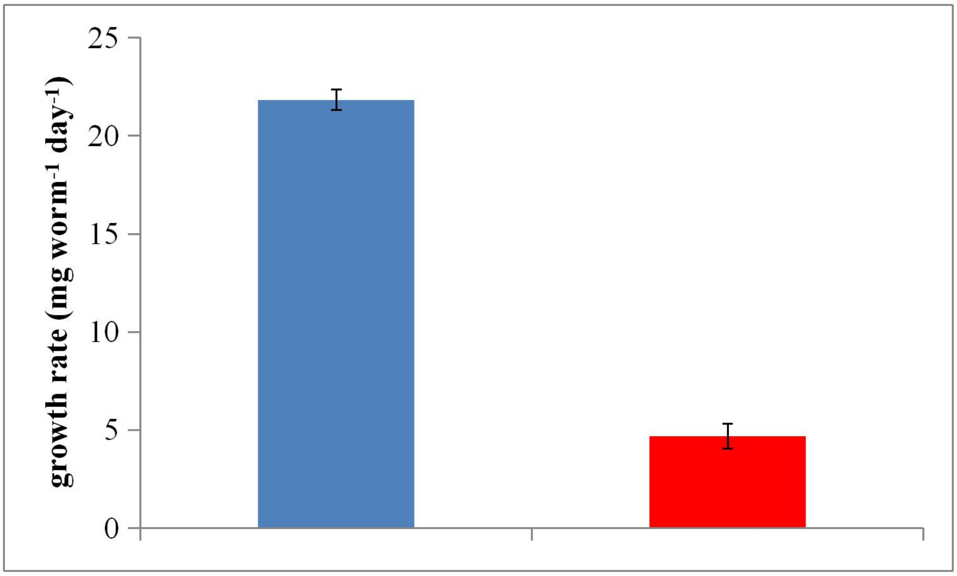

Figure 2: Growth rate of acclimated ( ) and unacclimated ( ) earthworms. Vertical bars indicate standard deviation.

3.2. pH

pH was a good indicator of bio-oxidation progress, microbial development as well as development of earthworm in vermicomposting process [17]. In fact, earthworms can survive in a pH range of 5 to 9. OMWW are acid pH [20], which caused serious environmental problems. Earthworms cannot support OMWW toxicity, for this reason, pre-composting was carried out one month prior to vermicomposting process. During vermicomposting period, a progressive increase of pH was observed in two mixtures, reaching a maximum of 9.9with acclimated earthworms (Table 2). At the end of vermicomposting, no significant difference was detected between experiments concerning this parameter (ANOVA, Newman-Keuls test, $P > 0.05$). The change of pH is strongly dependent on the starting materials. For example, no significant changes of pH are reported for these substrates: manures [21] and sorghum bagasse [22]. However, Benitez et al. [23] and Tiquia and Tam [24] reported a decrease of pH for sewage sludge and pig manure respectively, while increases were monitored in pruning wastes [25] rice straw and greenhouse waste [26]. Dias et al. [27] conclude that pH changes was affected by several factors: initial decarboxylation of organic acids, formation of ammonium from protein degradation, mineralization of nitrogen followed by nitrification (NH_4^+ is transformed into NO_3^-) and production of humic acids. During vermicomposting process, the mineralization of proteins, amino acids and peptides lead to the release of ammonium or volatile ammonia and contribute to the increase of pH. Tognettiet al. [28] reported that this increase could be due to the degradation of short-chain fatty acids and intensive nitrogen mineralization by microorganisms. Plaza et al. [29] noted an increase of pH (6 to 8) at the end of vermicomposting olive pomace added to horse manure. Asses et al. [30] reported same result during co-composting of sewage sludge with olive mill wastes or green residues. Additionally, earthworms did not affect the pH of organic substrates but they secrete intestinal calcium and eliminate NH_4^--N to maintain a neutral pH in their digestive tract [31].

3.3. Electrical Conductivity (EC)

Electrical conductivity (EC) of feed was a limiting factor for survival and growth of earthworms [32]. Mitchell [33] explained that earthworms were unable to survive in cattle solids with electrical conductivity of 5.0 dS m^{-1}. In addition, OMWW have negative effects on the soil because of its high salinity, low pH and the presence of toxic substances. Soluble salts, as estimated by electrical conductivity determination, are generally fairly high throughout the vermicomposting process, with initial values in the range of 229.4 mS cm^{-1}. Electrical conductivity (EC) decreased in two experiments to reach values between 76.2-129.4 mS $cm^{-1}$with acclimated and unacclimated earthworms respectively. The final values are significant (ANOVA, Newman-Keuls test $F = 813.62$, $P < 0.05$) (Table 2). Indeed, the maximum reduction of EC varied between 43% - 65%with acclimated and unacclimated earthworms respectively. The decrease in EC during the vermicomposting process can be attributed to the drop in soluble ion concentrations, because soluble ions are leached due to the irrigation of beds during the vermicomposting process. Soluble ion was immobilized by the prolific microorganisms or earthworms, or they are precipitated in the form of non-soluble salts [34], representing the biotransformation of waste mixture into more stabilized form of vermicompost [35].

Table 1: chemical properties of initial organic waste used for vermicomposting. Mean of three replicates ± standard deviation.

Substrate	N (%)	C (%)	C/N	pH	Phenols g L^{-1}
Olive pomace	1.07±0.26	50.86±0.10	49.53±1.17	6.4±1.3	0.21± 0.03
OMWW	0.97±0.05	43.69±1.58	45.32±2.71	4.6±1.1	5.62 ± 0.11
Horse manure	1.28±0.23	27.26±1.23	21.31±1.19	8.8±1.2	-
Wheat straw	0.38±0.11	41.77±1.81	107.95±3.13	7.2±0.5	-

Table 2: Changes in physicochemical characteristics mixtures with different groups of earthworms. Mean of three replicates ± standard deviation.

Day	N (%)	C (%)	O.M%	C/N ratio	pH	EC mS cm^{-1}
Acclimated						
0	1.7±0.06^{a}	41.9±0.07^{a}	72.1±0.17^{a}	25.3±0.67^{a}	9.5±0.27^{a}	229.4±1.16^{a}
10	1.7±0.09^{a}	39.7±0.07^{a}	68.3±0.17^{a}	23.8±0.94$^{a.b}$	9.6±0.13^{a}	185.2±5.05^{b}
20	1.8±0.10$^{a.b}$	41.1±0.06^{a}	70.7±0.14^{a}	23.3±0.88$^{b.c}$	9.7±0.02^{a}	148.0±1.78^{c}
30	1.6±0.06$^{a.b}$	36.7±0.09^{a}	63.1±0.20^{a}	22.6±0.58$^{b.c}$	9.8±0.01^{a}	137.3±2.44^{d}
40	1.8±0.06$^{a.b}$	37.1±0.01^{a}	63.8±0.02^{a}	20.4±0.49^{d}	9.8±0.02^{a}	122.3±2.89^{e}
50	2.0±0.01^{c}	39.1±0.05^{a}	67.2±0.12^{a}	19.7±0.11^{d}	9.8±0.01^{a}	108.0±4.89^{f}
60	2.1±0.05^{c}	36.1±0.08^{a}	62.0±0.19^{a}	17.4±0.28^{e}	9.9±0.05^{a}	78.2±2.00^{g}
Unacclimated						
0	1.7±0.06^{a}	41.9±0.10^{a}	72.1±0.11^{a}	25.3±0.67^{a}	9.5±0.27^{a}	229.4±1.16^{a}
10	1.6±0.06$^{a.b}$	39.4±0.12^{b}	67.7±0.15^{b}	25.1±0.67^{a}	9.6±0.13^{a}	195.8±4.10^{b}
20	1.5±0.06^{b}	36.8±0.06^{c}	63.3±0.08^{c}	24.9±0.76^{a}	9.7±0.08^{a}	177.5±1.87^{c}
30	1.6±0.06$^{a.b}$	39.8±0.12^{b}	68.4±0.15^{b}	24.8±0.73^{a}	9.8±0.05^{a}	156.1±4.26^{d}
40	1.5±0.06 ^{b}	35.3±0.12^{d}	60.6±0.15^{d}	24.2±0.78^{a}	9.8±0.04^{a}	140.3±1.77^{e}
50	1.7±0.06$^{a.b}$	34.3±1.10^{e}	58.9±1.30^{e}	19.9±0.94^{b}	9.9±0.02^{a}	131.4±4.30^{f}
60	1.9±0.10^{c}	34.3±1.10^{e}	58.9±1.30^{e}	18.0±0.67^{b}	9.9±0.08^{a}	129.4±1.19^{f}

*In each column, mean values followed by different letters are statistically different (ANOVA; Newman-Keuls t-test).

3.4. Carbon/Nitrogen ratio (C/N)

Carbon/ nitrogen ratio (C/N) was an important parameter to evaluate vermicompost maturity. Indeed, this ratio is one of the most important factors affecting vermicomposting process and the properties of the final product [35]. C/N ratio depends on the optimal physicochemical characteristics of the starting substrate, with an optimum ratio between 30 and 35 [36]. This value is confirmed and qualified by other authors [35- 37]. Golueke [38] reported that ideal initial C/N ratio for vermicomposting is 30. In the presence of woody substrates (wheat straw example) this ratio increases to values between 35 and 40, as a large proportion of carbon is not readily available to microorganisms. In this study, the initial C/N ratio was 35. After one of pre-composting C/N ratio decreased to 25, which is an optimal ratio for development and reproduction of earthworms [39] (Table 2).

After two months of vermicomposting, C/N ratio decreased gradually in both experiments starting from 25 and reaching values around 18 and 23 with acclimated earthworms and unacclimated earthworms respectively. These values are consistent with well-stabilized vermicompost that would not alter the microbial equilibrium when applied to soil [40]. Grigatti et al. [41] attributed the decrease of C/N ratio during vermicomposting process to mineralization of organic matter by microorganisms. Moreover, Parkin and Berry [42] confirmed that combined action of earthworms and microorganisms provoked mineralization and transformation of carbon compounds to simple and available forms consequently decrease C/N ratio.

3.5. Phenols

The aim of this work was to test effect of acclimatization of earthworms on their potential for OMWW detoxification. Ganesh et al. [18] noted that raw materials with high phenols fraction and lignin concentration (such as OP and OMWW) are not well adequate for growth and development of most species of earthworms. Besides, the chemical composition of the feed substrate has an important role in feed rate of earthworms [43]. Indeed, according to Figure 3, acclimated earthworms showed highest reduction of phenol concentration (72%), indicating that acclimatization of earthworms affect positively the adaptive potential of earthworms to OMWW and well accept their culture medium. Galliou et al. [44] obtained reduction of 75% phenol compounds in OMWW by combining solar greenhouse drying and composting. Zenjari and Nejmeddine [7] attributed the decrease of phenol concentration to microbial bioconversion of phenolic compounds and their interaction with secondary metabolites, contributing to the biosynthesis of humic substances. However, experiment with unacclimated earthworms did not lead to a significant reduction in the phenols concentration (41%).These results demonstrate the positive effect of the acclimatization of earthworms towards OMWW in order to obtain a high rate of detoxification. In another study done by Madani et al. [45], using Fenton oxidation processes was obtained 98.6% in phenol reduction, but they found that pH affect the efficiencies of Fenton process.

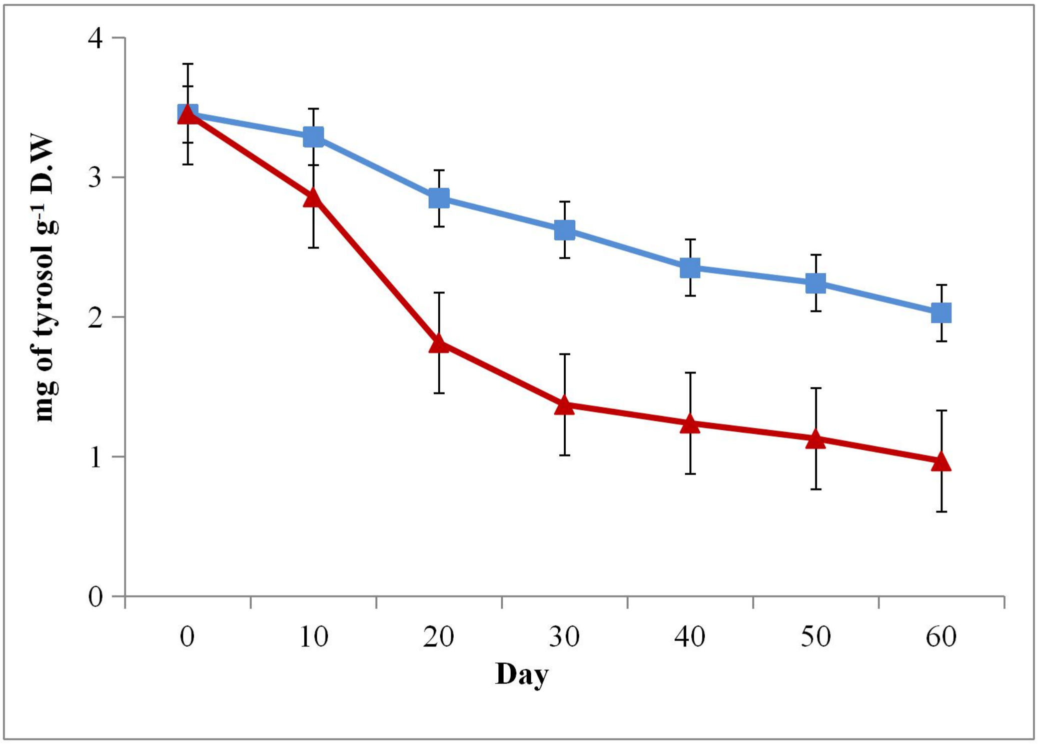

Figure 3: Changes in phenols' concentration with unacclimated (■) and acclimated (▲) earthworms during vermicomposting experiments. Vertical bars indicate standard deviation.

4. Conclusion

The results of this work confirm that acclimatization of *Eisenia andrei* allows a higher reproductive rate and a higher rate of OMWW detoxification than unacclimated earthworms. The rate of phenols reduction (72%) obtained with acclimatized earthworms demonstrates the positive effect of acclimatization. Although OMWW are a recalcitrant organic by-product for decomposition, acclimatization of earthworms can enhance their potential adaptation to high OMWW concentrations (80%). Physico-chemical characteristics pH, EC, C/N and phenols reduction rate obtained indicate a good level of maturation and stabilizationof vermicompost produced with acclimated earthworm.On the other hand, the decrease of enzyme activities at the end of vermicomposting could indicate a rapid tendency to stability of the vermicompost organic matter. Thus, the period requiredfor the acclimatization of earthworms must be adjusted thanks to concentrations of OMWW lower than their threshold concentrations to achieve an optimal achievement of vermicomposting process.

5. Acknowledgements

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

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