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Evaluation of microbial catabolic patterns and substrate induced respiration in various vermicomposting designs of organic waste by *Eisenia fetida*

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ABSTRACT

In our study we focused our work on the treatment of food waste by vermicomposting in the presence of Eisenia fetida. Furthermore, we have used 4 types of beddings for earthworms: dead chestnut leaves, vermiculite, soil, and commercial vermicompost. During this experiment we followed changes of catabolic capacity (biolog ecoplate) and biomass (SIR) of microflora we have also made the counting of the number of individuals of Eisenia fetida. From different stages of maturation. We found that the Eisenia fetida decreases strongly the substrate induced respiration (SIR) in all types of vermibeds used, moreover the the biolog ecoplate showed an increase of substrates utilization patterns , accept in amino acids and amines utilization in treatment witch we used vermiculite, the growth and fecundity of earthworms were higher in microcosms with vermiculite and vermicompost.

Key words: vermicomposting, *Eisenia fetida*, Microorganisms, Biolog Ecoplate, SIR, waste.

INTRODUCTION

Landfill and incineration were until now the most used methods for treating solid waste worldwide but in this last years recycling methods are under development in several countries, its about conversion of biodegradable organic matter from various sources into humic substances by the process of composting and vermicomposting [1-4].

In our study we are interested into vermicomposting which the most attention devoted to epigics, has been concentrated on the role of species such as *E fetida*; *E Andrei*, and others, when fed pure organic matter sources such as animal manures, sewages sludge, coffee or paper mill pulp, sugar can bagasse other organic residues these are rapidly transformed into stable fecal pellets, these pellets are then gathered and used as organic fertilizer with dramatic effects on horticultural crops and ornamental plants [5-7].

The vermicomposting is the result of action of many chemical and biological process that involves the interactions of earthworms and microbes for regulation of organic matter decomposition and humification, earthworms as a drivers of vermicomposting system could modify the microbial activity and population and these stimulate the decomposition of organic matter [8-10].

Lavelle and al [11] mentioned, when earthworms ingest dormant microorganisms these microorganisms may became activated by priming effect in the gut, which after continues for a short time in the casts because of abundance of soluble carbon and other nutrient resources.

The short time priming of microorganisms increases nutrient mineralization rates, releasing more plant available N and P, according to Brown and al [12] the mixture of mucus with ingested organic matter and high water content and neutral pH of foregut promoted the development of micro flora that could digest cellulose and other recalatrant

which earthworms can not digest by them selves, it also reported that earthworms prefer and partially digest the rapid growing fungi species metabolizing cellulose and carbohydrates during decomposition of organic matter.

The capacity of epigeic earthworms to consume considerable amount of row organic matter, have a broad range of enzymatic capacities probably mainly originating from ingested microorganisms [13].

Earthworms in many studies, like [14-16] increase microbial activity and reduce microbial biomass, these by decreasing overall nutrient availability

Keeping in view the above facts, the present study was conducted to access the ability of *E fetida* as epigeic earthworms in decomposition of organic waste and their action on catabolic profile of microorganisms and biomass.

MATERIALS AND METHODS

Forty plastic containers of 14 L with perforated lids, witch twenty we have put 76 adult specimens of *Eisenia fetida*, we used 2, 5 L of four types of vermibeddings, as indicated in the Table, the chemical composition of the waste and vermibeddings are described in table1

Table1: the Experimental design

Treatment	Vermibeding type	Abbreviation	Incubation time	Weight of the food waste used	Number of replicates of each vermibeding
With *Eisenia fetida*	Vermiculite Vermicompost Soil Chest nut leaves	V VC S F	120 days	1500g	5
control	Vermiculite Vermicompost Soil Chest nut leaves	V cont VC cont S cont F cont	120days	1500g	5

After 120d days the earthworms are removed and counted, the sub samples of the treated waste are used to determine, the catabolic ability of microorganisms, substrate induced respiration SIR.

The catabolic ability of microbial communities was assessed on the basis of patterns community level sol source utilization by using Biolog Ecopalte (Biolog Inc; Hayward,CA,USA).

The 31 wells Ecoplate comprises 30 substrate-containing well and control well without carbon source. The carbon sources are grouped as in the table2.

Table2: Biochemical categories of 30 substrates used in Biolog Ecoplate.

Simple sugars	polymers	Carboxylic acides	Amines acides	amines	Cyclic compound
Mannitol D2 N-Acetyl-D-glucoseamine E2 D-Xylose B2 i-Erithritol C2 β-Methyl-D-glucoside A2 D-Cellobiose G1 A-D-Lactose H1 Glucose-1-phosphate G2 D,L-Glycerol phosphate H2	Tween40 C1 Tween80 D1 α-Cyclodextrine E1 Glycogen F1	Pyruvic acid Methyl ester B1 D-galactonic acid A3 D-galacturonic acid B3 γ-hydroxybutyric acid E3 Itaconic acid F3 α-Ketobutyric acid G3 D-Malic acid H3 Glycyl-L-Glutamic acid F4 D-glucosaminic acid F2	L-Argenine A4 L-asparagine B4 L-phenylalanine C4 L-serine D4 L-threonine E4	Phenylethyl-amine G4 PutriscineH4	4-Hdroxy benzoic acid D3 2-Hydroxybenzoic acid C3

Dilutions of $10^{-4}$of samples were prepared for inoculating wells with 150 µl, and incubated at 20°C the optical densities were read in microplate reader at 595nm after incubation for, 24, 48, 96, 120 hours.

The 120h absorbance (Abs) data were used for analysis, this was the time showed the highest well response and used for the calculation of the AWCD (average well color development) as recommended by Garland [17], then the Abs are corrected by dividing each value by AWCD [18], [19], negative values of corrected Abs , were coded as zero[19], and after , for each group of substrates (Table2) we used the average of the corrected Abs

SIR was measured using a modified method of Aira and al [21] the rate of CO2, evolution during 5 hours incubation after adding 0,75 ml of glucose solution (80mg/100ml) to the waste, the CO2 evolved was trapped by 60 mmol NaOH precipitated with 3N BaCl2 and then titred with 30 mmol HCl with phenolphthalein.

Statistical analysis:
One way analysis of variance and separation of the means based on the least significant difference (LSD, P≤ 0,05) to determine significant difference between treatments,

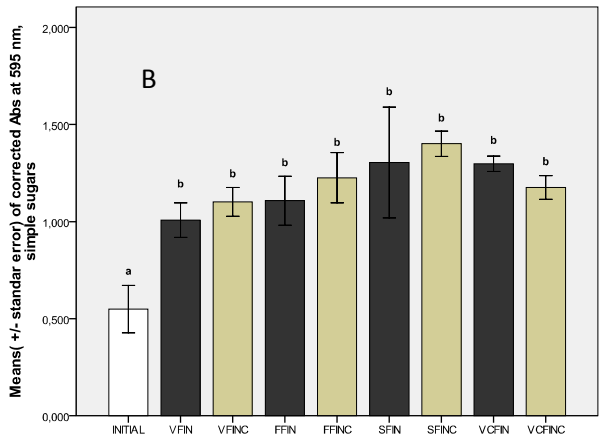
RESULTS AND DISCUSSION

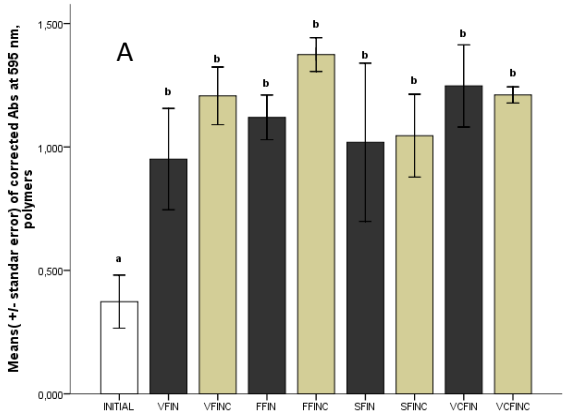
Biolog ecoplate:
Polymers:
Over time we noticed an increase in the use of polymers in all microcosms, it is noticed also, that there is no significant difference between the Abs values between microcosms with or without earthworm (Fig1.A).

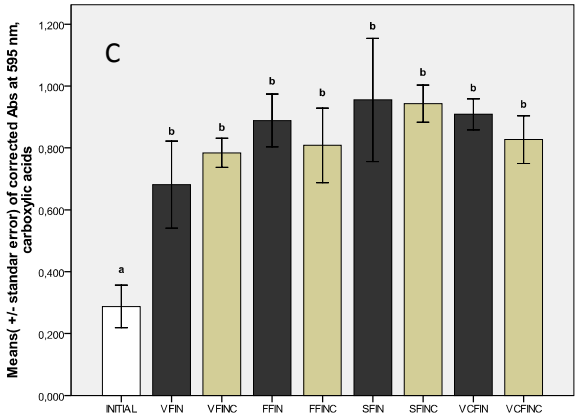
Simple sugars and carboxylic acids:
The Abs increase over time in all microcosms, but no difference between the microcosms according to the vermibedding used and also the presence or absence of earthworms(Fig1.B,C)..

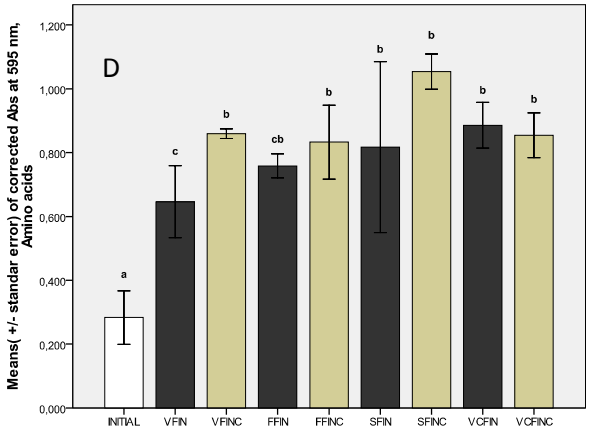
Amino acids:
A significant increase in Abs observed in in all microcosms, however, and only in microcosms with vermiculite and earthworms the Abs is lower than those of the control ones (Fig1.D).

Amines:
The use of amines increased over time at microcosms with vermicompost and soil, as it was noticed that the Abs measured of microcosms with vermiculite, are lower than those measured in microcosms with the soil. (Fig1.E).









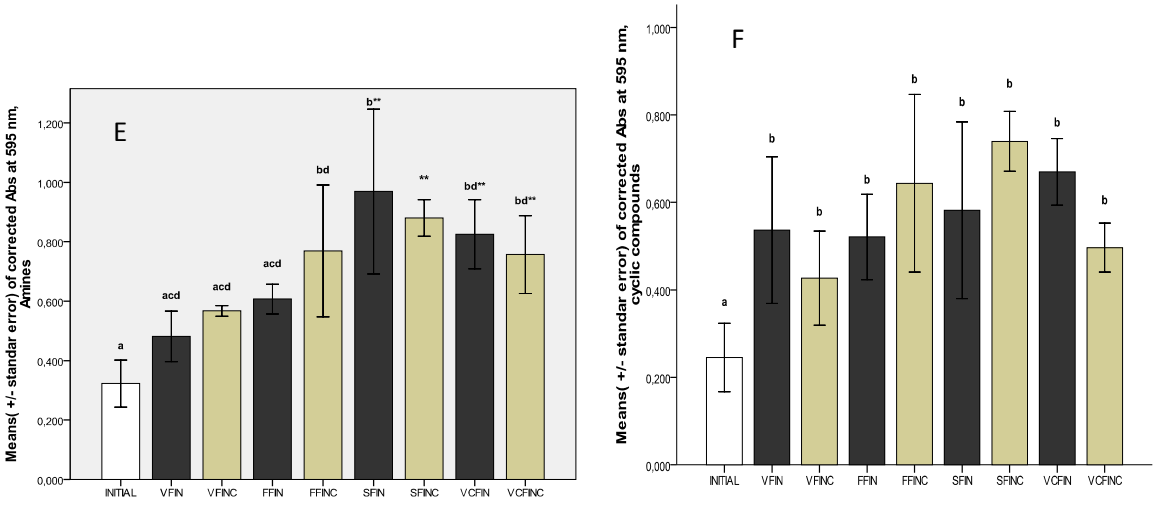


Figure 1: changes in the potential of carbon sources use in the food borne waste after 120 days of vermicomposting, initial: waste before treating, VFIN, FFIN, SFIN, VCFIN after treating with earthworms , using vermiculite, chestnut leaves, soil and vermicompost as vermibeddings respectively. VFINC, FFINC, SFINC, VCFINC are the controls without earthworms, after treating
Values are means ± standard error, different letters indicate significant differences between samples LSD test at p< 0,05

Cyclic compounds:
The Abs varies over time only at certain treatments like microcosms with vermicompost and soil in the presence of earthworms, and at control microcosm with, leaves, soil and vermicompost (Fig1.F).

SIR results:
In this work the SIR varies depending on the vermibedding used, and the presence or absence of earthworm, During treatment there was a decrease in the SIR in all microcosms with or without earthworms, but the decrease was greater in the presence of earthworms, we also note that treatment with leaves of chestnut has the largest SIR value than the others in the presence of earthworms, where the control microcosms with vermiculite and vermicompost have the highest SIR levels.

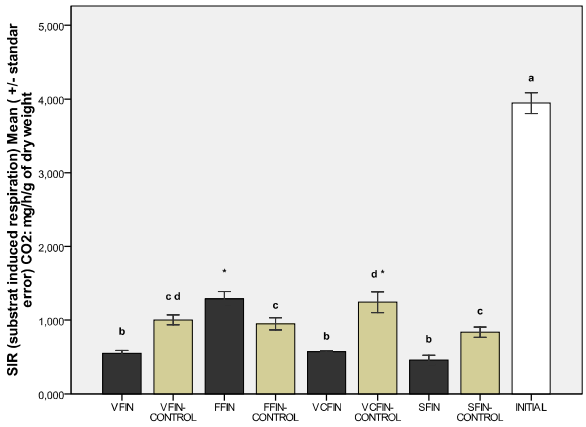


Figure.2: changes substrate induced respiration (SIR) in the food borne waste after 120 days of vermicomposting, initial: waste before treating, VFIN, FFIN, SFIN, VCFIN after treating with earthworms , using vermiculite, chestnut leaves, soil and vermicompost as vermibeddings respectively. VFINCONTROL, FFINCONTROL, SFINCONTROL, VCFINC ONTROL are the controls without earthworms, after treating

Earthworms counting:
The counting of the individuals of *Eisenia fetida* (Table.3) showed that the number of adults decreased significantly over time and in all treatments, but with higher production of cocoons in treatment with vermicompost as vermibed. The highest number of hatchlings was observed in treatment with vermiculite as vermibed, and the number of juveniles was higher in the treatments with the vermicompost and vermiculite as vermibed. The sub adults' numbers were similar in all treatments.

Table3: mean (±SE) of number of individuals from different stages of maturation of *Eisenia fetida* after 120 days of vermicomposting
Values in the same column followed by the same letter are note significantly different at P< 0, 05 using LSD test

Traitement	adults	cocons	hatchlings	juveniles	Sub-adult
F	13,40 ± 4,512**a**	86,00± 31,643**a**	45,60± 38,979**a**	10,40 ±8,286**a**	34,20 ±9,932**a**
S	21,40±8,708**ac**	151,40 ±100,353**ab**	39,6 ± 25,268**a**	29,00 ± 5,518**ac**	10,00 ±3,286**b**
V	30,40±2,502**bc**	203,6 ±38,570**ab**	614,80 ± 83,376**b**	58,60 ± 8,801**bc**	23,8 ±6,492**ba**
VC	38,8±2,746**b**	334,80 ±98,786**b**	13,059 ± 56,80**b**	56,80 ± 1,960**bc**	22,6± 2,249**ba**

DISCUSSION

In this study we evaluated the effect of epigeic earthworms on the catabolic capacity of microorganisms and their biomass during the decomposition of waste food. Microorganisms are involved in the degradation of organic matter; however earthworms are known to decrease the rate of organic matter in decomposition [22], which can lead to competition between earthworms and microorganisms for some organic compounds necessary to energy needs and growth.

In other studies it was found that earthworms involved in a reduction of the use of carbohydrate and some carboxylic acids and promote the development of microbial populations with the capacity to use polymers and amino acids [9], [5] In this study the potential use of polymers and sugars and carboxylic acids are not affected in microcosms with earthworms because we observed the same capacity utilization in the absence of earthworms, while the use of amino acids by microorganisms was reduced in microcosms containing vermiculite as vermibidding in the presence of earthworms compared those in the control treatments(fig), this can be explained by the high rate of cocoons and hatchlings at this treatment (Table3), which explains the decrease of the potential use of microorganisms for amino acids such as Asparagin necessary for growth. In microcosms containing soil and vermicompost bedding, using amines by microbes is higher compared to other microcosms, because of the presence of a previous microbial flora in these vermiddings.

Reduction of microbial biomass measured by SIR during the decomposition is due to the depletion of nutrient source by the action of earthworms, some authors have found that microorganisms are also a source of food for earthworms [23], which explains the decrease in SIR in treatments with earthworms.

According to other studies, the microbial biomass decreased in the presence of earthworms, during decomposition of vegetable wastes [8], and according to some authors the decrease is dependent on the earthworm species, density, and also the nature of the waste used [24], [25]. In the study carried by [15], microbial biomass decreases when the density of earthworms increases during the decomposition of manure in the soil. This may explain the values of microbial biomass in microcosms using the leaves where is recorded the lowest density of earthworms (Table3).

CONCLUSION

In this work we investigated the effect of earthworms on microflora during the decomposition of Organic residues, growth and fertility of Eisenia *fetida* in different materials used as vermibedding.

Biolog ecoplate in this study showed that the microbial flora involved in the decomposition of organic residues are microorganisms capable mainly of hydrolyzing the polymers, and the single carbon sources, in addition to other compounds, this catabolic potential is due to the nature of the residues used for vermicomposting, which are foodborne, glycogen, starch, proteins, and lipids. Which promotes colonization by specialized floras this type of metabolism, however, the direct effect of earthworms is detected In certain treatment as in microcosms with vermiculite where the presence of earthworms affected the potential use of amino acids, also the influence of the litter as vermicompost and soil where we see the contribution of nature of the medium used for vermicomposting in the biodegradation of some substance such as amines by microorganisms. While the action of earthworms is clearly seen in the reduction of microbial biomass measured by SIR,

The growth and fecundity of *E. fetida* depended on the type of vermibed used in the microcosms, which vermiculite and vermicompost showed favorable habitats for growth and reproduction by comparing to other vermibeds used.

REFERENCES

[1] M de Bertoldi; G Vallini; A Pera, *Waste Manage. Res*, **1983**, 1,157-172.
[2] CA Edwards; J E Bater, *Soil Biol. Biochem*, **1992**, 24, 1683-1689.
[3] F Shadanpour; A Mohammadi Torkashvand; K Hashemi Majd, *Ann of Biol Res*, **2011**, 2 (6): 109-115

[4] Lallawmsanga; D J Mukesh Kumar1; M D Balakumaran1; M Ravi Kumar; J Jeyarathi; P T Kalaichelvan, *Ann of Biol Res*, **2012**, 3 (5):2268-2274.
[5] G G Brown; I Barois; P Lavelle, *Eur. J. Soil Biol*, **2000**, 36, 177-198
[6]P Garg; A Gupta; S Satya, *Bioresour.Technol*, **2006**, 97 391-395.
[7] A Yadav; VK Garg, *Pedosphere*, **2013**, 23(5), 668-677.
[8] K Huang; F Li; Y Wei; X Chen; X Fu, *Bioresour.Technol*, **2013**,150, 235-241.
[9] M Gómez-Brandón; C Lazcano; M Lores; J Domínguez, *Appl. Soil Ecol*, **2010**, 44, 237-244.
[10] M Gómez-Brandón; C Lazcano; J Dóminguez, *Chemosphere*, **2008**, 70, 436-444.
[11] P Lavelle; C Lattaud; D Trigo; I Barois, *Plant and Soil*, **1995**, 170, 23-33.
[12] G G Brown; B M Doube; Earthworm Ecology, 2nd ed., CRC Press, Boca Raton, **2004**, 213-224
[13] J P Curry; O Schmidt, *Pedobiologia*, **2007**, 50, 463-477
[14] P J Bohlen; CA Edwards; Q Zhang; R W Parmelee; M Allen, *Appl. Soil Ecol*, **2002**, 20, 255-261
[15] C Sheehan; L Kirwan; J Connolly; T Bolger, *Eur. J. Soil Biol*, **2008**, 44, 65-70.
[16] P Jun- zhu; Q Yu-hui; S Zhen –jun; Z Shuo-xin; L Yun-le; Z Rui-qing, *Pedosphere* **2012**, 22 (5), 726-735.
[17] JL Garland, *FEMS Microbiol. Ecol*, **1997**, 24, 289-300.
[18] E Glimm; H Huer; B Engelen; K Smalla; H Bachhaus, *J. Microbiol. Meth*, **1997**, 30, 71-80.
[19] JA Groove; H Kautola; S Javadpour; M Moo-young; W.A Anderson, *Biochem. Eng. J*, **2004**, 18, 111-114.
[20] K P Weber; J A Grove; M Gehder; W A Anderson; R L Legge, *J. Microbiol. Meth*, **2007**, 69, 461-469.
[21] M Aira; J Dominguez, *Bioresour. Technol*, **2010**, 101, 7173-7176.
[22] A Yadav; VK Garg, *Bioresour. Technol*, **2011**, 102, 2874-2880.
[23] M Aira; F Monroy; J Dominguez; S Mato, *Eur. J. Soil Biol*, **2002**, 38 7-10.
[24] M Aira; F Monroy; J Dominguez, *Eur. J. Soil Biol*, **2006**, 42, s127-s131.
[25] M Aira; J Dominguez, *J. Hazard. Mater*, **2009**, 191 1234-1238.