

## Determination of Biochemical Changes in Cow Manure During the Process of Vermicopost with the Usage of Earthworms (*Eisenia Fetida*)

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### ABSTRACT

Nowadays, the waste problem is one of the global concerns. Since the main part of the wastes is the organic materials, the vermicompost technology is considered as one of the most indispensable and effective strategies to solve the problem. In vermicompost technology earthworms as biological reactors together with microorganisms cause the organic materials to decompose and stabilize and also to produce biological nutrient fertilizers. The cattle waste is the most proper nutrition for the earthworms in which the amount of organic material, organic carbon and nitrogen alter during the process of decomposition and stabilization. This study was carried out with the aim of determination of biochemical changes in cow manure with the usage of earthworms (*Eisenia fetida*) being kept in the cow manure and natural soil surroundings for 28 days and the control samples were measured in the absence of earthworms. The amount of organic materials, organic carbon, nitrogen, C/N, moisture, pH and EC were determined at the beginning and end of the experiment. The obtained results clarify that the earthworms' activities in the cow manure surroundings are the reason for the reduction of organic material from 32.3% to 24.6% and C / N from 23.4 to 14.6 and the changes in moisture level, pH and EC.

**Key words:** Earthworm, *Eisenia fetida*, vermicompost, cow manure, decomposition, biochemical changes.

### Introduction

The decomposition process is the regulator of ecosystem food chain, which is done by microorganisms. The decomposers of food chain are highly complex and they are mainly consisted of protozoa, nematodes and earthworms [2].

Earthworms are important group of soil organisms. They form ninety percent of the soil biomass and have an important role in food chain. The earthworms are assumed as a main food source for various animals such as birds, fish, insects, mammals and reptiles. Earthworms have interaction with other soil organisms and have effects on primary production, decomposition, energy flow and nitrogen cycle [16].

It is more than 600 million years that earthworms are known as the managers of ecosystems in waste management and soil improvement and plant growth. Until now, about 4,400 different species of worms have been identified. However, few of them are compatible with being waste consumers and biodegradable and

some of them are biological accumulators and biotransmitters of toxic chemicals [21].

Among many species of earthworms, the species *Eisenia fetida* has high birth rate, high temperature tolerance range and this species is capable of reproducing in the various organic waste with a wide range of moisture [19]. In addition, it is the most significant species for the decomposition of organic waste and vermicompost production. Vermicompost technology means the effective management of urban and industrial organic solid wastes by the use of the biodegradation, stabilization and conversion of the waste into useful materials (bio-nutrient fertilizers) [23].

The Vermicompost caused by earthworms metabolize and disposal mixture of soil and organic matter are the advance form of the compost. On the other hand, this is responsible for the transfer of some organic compounds (proteins, acid Nucleic, fats and carbohydrates) to a stabilized composition in the worms and microorganisms digestive system [18].

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Many researchers mention the capability of vermicompost for using in horticultural and agricultural industries. Over the past few years the application of earthworms has increased as a powerful and perfect ecological system for manure management [19].

To understand the key role of earthworms in the soil, they should be intended as remarkable natural biological reactors which are proficient for various operations in the soil [10].

Worms are as the aerator, grinder, crusher and decomposer of chemical materials and waste. After ingestion of nutrition waste by earthworm's muscle crop which is a colloidal mill, the nutrition waste is grinded very delicately into smaller size of 2 and 4 microns which provides huge special surface for the microbe invasion and then it passes through the intestines for the enzyme operation. In the earthworms' crops and intestines, some protein enzymes such as Lipase (enzyme that breaks down the lipid), Amylase (enzyme that breaks down starch), Cellulase and Kytynaz are existed which cause a relative fast biochemical changes of Cellulase and protein substances found in organic waste and across the earthworms intestine, the pH range of change is only from 6.3 to 7.3.

Earthworms are helpful for the growth of beneficial microbes such as bacteria and fungi and actinomycete which are found in the wastes biomass [7]. Earthworms are the host for millions of decomposer microbes in their internal guts being described as a bacteria factory. They swallow microbes and excrete them out (several more times to digest). This process in soil is completed together with the excreting of nutrients nitrogen and phosphorus in earthworms' wastes [22].

The number of bacteria and actinomycete increases up to 1000 times in the digested materials after passing through the inner guts of earthworms [8]. Earthworms and microbes live together in order to accelerate the decomposition of organic materials in the waste [15].

In the earthworms' stomach, the denitrification phenomenon occurs which makes the nitrate resuscitation and N<sub>2</sub>O production by the assistance of stomach microflora [9].

The ultimate process of vermicompost and the decomposition of organic materials is humic making where a large part of organic materials convert to a complex and formless colloids with the phenolic content. Nearly 25% of the organic material becomes humic. Humic in the soil is essential for plants growth and their survival [23]. The required energy in all these processes is the result of highly efficient oxidation of biological waste [10]. The earthworms play critical role in the global biochemical cycles.

In the present study *Eisenia fetida* species has been selected to determine the cow manure biochemical changes through the vermicompost

process. It is on the grounds that *Eisenia fetida* is one of the best of earthworms for making vermicompost.

## Material and Methods

### *The tested earthworms:*

*Eisenia fetida* species was prepared from a company of breeding E.f species and vermicompost named Talieh Sabz in Tehran, Iran and they were raised with OECD, 1984 method. Those earthworms which were mature in terms of physiological matters were selected for the test [17].

### *Natural soil and cow manure tests:*

The natural soil and cow manure were collected from the gardens in north of Iran where the earthworms were native to this region. Then it was exposed to air for a while thereafter it passed through a 5 mm sieve until it became homogeneous [12]. At the beginning of the experiment, the percentage of clay, silt, sand and soil texture were determined using the hydrometer method [4].

At the beginning and the end of the test, biochemical characteristics of natural soil and cow manure such as moisture, pH, EC, organic matter, organic carbon, total amount of nitrogen (N) and C/N were determined in the samples containing the earthworms and the control samples (without earthworms).

The moisture level with drying the samples for 48 hours at a temperature of 80 degrees Celsius, pH with material suspension in distilled water with 1:5 ratio, EC, organic matter (550 ° C ignition temperature for 4 hours), organic carbon (total organic matter = organic carbon × 1.724), the total amount of nitrogen (N) by Kjeldahl( method and C / N ratio with the division of organic carbon to nitrogen were measured.

One hundred grams of natural soil and cow manure (fifty grams of each) poured in glass containers and the content moisture was brought to 50% with distilled water. Then ten mature earthworms put in each container and they were covered with clear plastic lid with some holes for air and the samples were kept for 28 days at 2 ± 24 temperature. Ten samples were prepared and the control samples were measured without the presence of worms in the soil. For data analysis, SPSS software (ver.17) was used and Excel (office, 2010) was used to draw graphs

## Results and Discussion

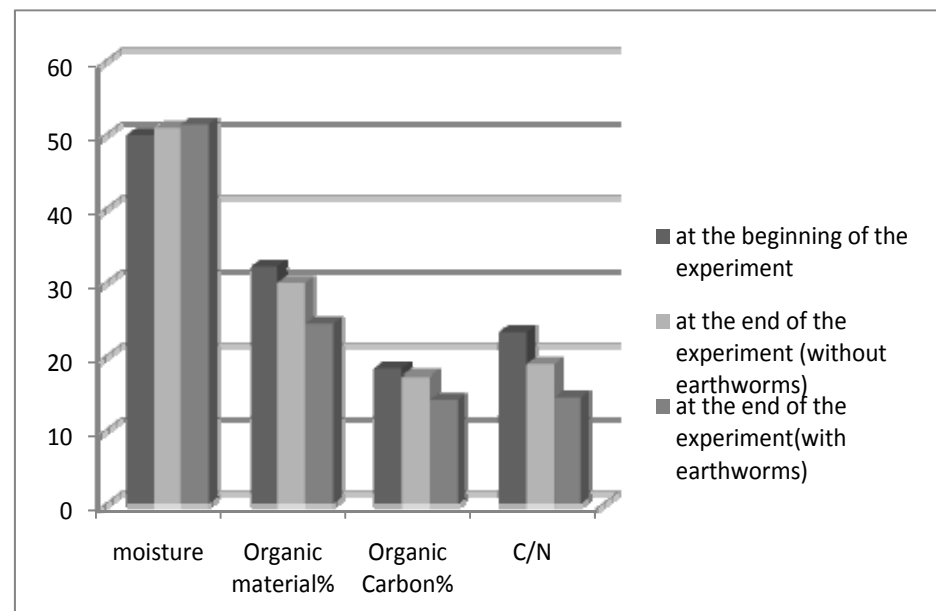
The tested natural soil had 5% clay, 40% silt and 55% sand with sandy loam texture which is a suitable soil for earthworms. Biochemical characteristics of natural soil and cow manure at the beginning of the experiment and the analysis results of the samples

containing earthworms and control samples without earthworms at the end of the experiment (28 days)

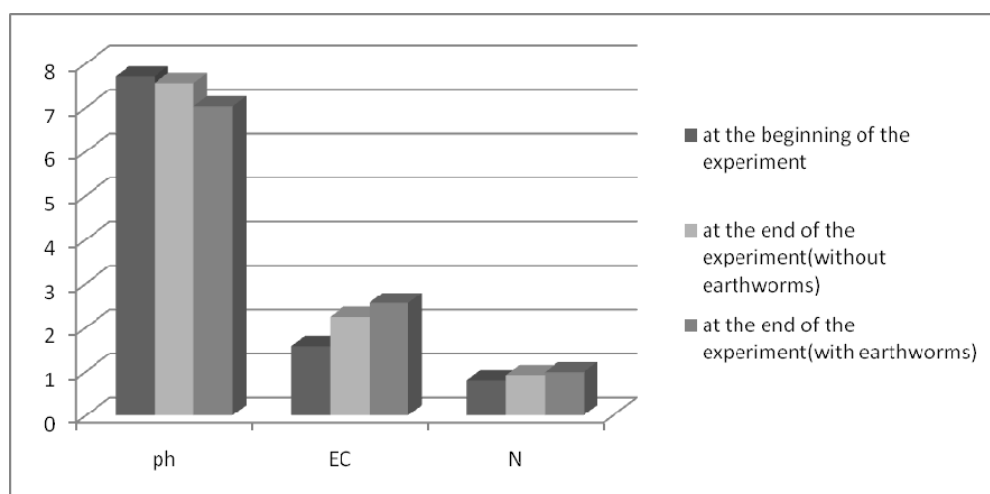
are reported in table 1 and are shown in charts 1 and 2.

**Table 1:** Comparison between the biochemical characteristics of the samples at the beginning and end of the experiment (28-day period)

sample	moisture	pH	EC ms/cm	Organic material%	Organic Carbon%	Nitrogen%	C/N
SOIL AND COW MANURE (at the beginning of the experiment)	50	7.7	1.571	32.3	18.7	0.8	23.4
SOIL AND COW MANURE (at the end of the experiment)	51	7.55	2.24	30	17.4	0.91	19
VERMICOMPOST (AT THE END OF THE EXPERIMENT)	51.5	7.02	2.55	24.6	14.3	0.98	14.6



**Fig. 1:** Moisture percentage, organic material, organic carbon and C / N at the beginning and end of the experiments with and without the presence of earthworms in the soil and cow manure (28-day period).



**Fig. 2 :** The amount of pH, EC and nitrogen percentage at the beginning and end of the experiments with and without the presence of earthworms in the soil and cow manure (28-day period).

The results showed that the earthworms and microorganisms play a chief role in the process of cow manure decomposition. In all samples, there was a slight increase in moisture level after 28 days. As the moisture level increased in samples with earthworms from 50% to 51.5% and in samples without earthworms from 50% to 51%.

Similar results have been reported by [3,19] that studied the cow manure biochemical changes by using earthworms for 4 months in laboratory conditions. In [19] test the moisture level increased in samples with earthworms from 77% to 81% and it rose up to 84% in samples without earthworms [3, 19].

On the Contrary, the results of the experiments done by [1] revealed that the deduction in the moisture level of organic materials. It owned to this fact that the samples were kept with no cover as a lid in his experiment [1]. However it was covered with clear plastic in this experiment which made the evaporation rate very low.

The electrical conductivity increased subsequently from 1.571 to 2.55 and 2.24 in sample with earthworms and in sample without earthworms. In addition pH level decreased from 7.7 to 7.02 and 7.55 at the end of the experiment and after 28 days.

These results are totally in accordance with the achievements of [3,19] that showed the decrease in cow manure pH by earthworms' activities in the 4-month period and [19] who reported the highest rate of decline in the first 4 weeks of experiment [3,19].

According to [6,1] reports, the main reason of pH decrease can be due to the accumulation of organic acids from microbial metabolism or the fulvic production and humic acids during the decomposition process [1,6].

Comparison between the amount of organic material and organic carbon at the beginning and end of the experiment indicated that after 28 days the amount of organic material in samples with earthworms and control samples without earthworms changed from 32.3% to 24.6% and 30% and the amount of organic carbon declined from 18.7 % to 14.3 % and 17.4 % .

The key reasons of organic material reduction were consumption of organic material by microorganisms and earthworms and the rapid decomposition of carbon compounds [19]. The total nitrogen in samples with earthworms rose up from 0.8% to 0.98% and it reached 0.91% in control samples.

Earthworms are the crucial issues to transformation of nitrogen and the quick mineralization process. The C/N amount in samples with earthworms declined from 23.4 to 14.6 and it reduced to 17.4% in control samples.

It is worthy to mention that the process of decomposition and stabilization of organic matter and nitrogen mineralization in samples with earthworms are much quicker than the control

samples without earthworms due to the coexistence and cooperation of earthworms and microorganisms.

Several studies provided similar results with the findings of this research. For example, [13] reported the C/N reduction of the barn yard manure, after a period of 3 months and [24] identified the C/N reduction of pruning and garden waste from 62 to 27 after a period of 8 months [13,24].

By passing time the activities of earthworms and microorganisms caused the mineralization of nitrogen and the increase in nitrogen percentage especially in the first few weeks of experiments and at the end of the experiment the C/N decreased from 36 to 20.

The findings of [3] studies on cow manure for 4 months together with [12] studies on different organic waste indicated that the activities of earthworms caused faster decomposition of carbon compounds, mineralization of nitrogen compounds and stabilization of waste materials [12,3].

All these studies have parallel results with the present research which all of them emphasize on the critical and sensitive effect of earthworms and microorganisms on the decomposition of organic waste and energy cycle.

#### *Results:*

The results of this study revealed that the cow manure biochemical characteristic of the samples with earthworms and control samples without earthworms was changed after 28 days. The moisture level increased in samples with earthworms from 50% to 51.5% and also in samples without earthworms from 50% to 51%. The pH level decreased in samples with earthworms from 7.7 to 7.02 and in samples without earthworms from 7.7 to 7.55. Further reduction of pH in samples containing earthworms was due to further degradation of organic material, microbial metabolism and humic acids production. The amount of organic material in samples with earthworms and control samples without earthworms changed from 32.3% to 24.6% and 30% and the amount of organic carbon declined from 18.7 % to 14.3 % and 17.4 % . The total nitrogen in samples with earthworms rose up from 0.8% to 0.98% and it reached 0.91% in control samples and also the C/N amount in samples with earthworms declined from 23.4 to 14.6 and it reduced to 17.4% in control samples.

The results indicate that the process of decomposition and stabilization of organic material and nitrogen mineralization in samples with earthworms in comparison with control samples is much higher and quicker because of the earthworms and microorganisms cooperation. The results reveal that earthworms together with decomposer microorganisms such as bacteria, fungi and ectinomists make the organic wastes decompose and stabilize faster. During this process the organic

materials become biological fertilizer (Vermicompost) which is a suitable method for recycling organic waste, food waste and wastewater sludge. Thus, earthworms are used for organic and garbage waste management and they produce nutritious bio-fertilizers by decomposing and stabilizing organic material and this is a very useful and effective method to improve soil fertility and plant growth.

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