

International Journal of Scientific Research in _ Biological Sciences Vol.8, Issue.1, pp.05-12, February (2021) DOI: https://doi.org/10.26438/ijsrbs/v8i1.512

E-ISSN: 2347-7520

Effect of Bisphenol-A on the Bioconversion of Tea Waste into Vermicompost by *Eudrilus Eugeniae* (Kinberg, 1867) at Different Intervals

A. Bhuvaneswari¹, K. Kalaivanan^{2*}, S. Durairaj³, G. Selladurai⁴

^{1,2,3,4}Dept. of Zoology, Arignar Anna Govt. Arts College, Cheyyar-604 407

*Corresponding Author: kalaik026@gmail.com

Available online at: www.isroset.org

Received: 05/Jan/2021, Accepted: 09/Feb/2021, Online: 28/Feb/2021

Abstract: Vermicompost has been appropriate alternative for the best hygienic and cost effective disposal of wastes. Since the vermitechnology is considered as one of the best methods, the largely available tea waste was taken to the present work to study the efficiency the Eudrilus eugeniae in the management of such waste by converting it into vermicompost. The efficiency of the conversion of Tea waste into compost in the form minerals N, P, K and Fe, Mn, Zn, Cu by Eudrilus eugeniae has been analyzed on 15th and 30th days. Physical characteristic were also analyzed simultaneously to understand the efficiency of earthworm in maintaining the physical status of the soil. Macro and micro nutrients were increased significantly on 15th and 30th days. FTIR spectrum was recorded for the control and experimental compost. The variation of spectrum was noted. The Spectrum of experimental consisted of nitrogen rich peak. The control system has no N-H stretching peak whereas tea waste spectra has N-H stretching peak. The peak at Bisphenol-A leads to large number of hydrogen group i.e., the mixture has more moisture than the control system. The tea waste has flavonoids which also exhibit the OH group at the same system. To understand the anatomy, histology of the different parts of the earthworm was studied. Histology of the tested anterior region of Earthworm reveals that the cuticle is not much damaged. The dorsal and ventral blood vessel ruined largely. The control animal of clitellar region showed intact structure of internal organs. The coelom and Blood vessels are well marked. In the experimental animal, the clitellar region showed slight modification of the anatomy. The pigment cells are prominent in the waste treatment with Bisphenol-A. In some places, the circular muscles are disorganized. In middle region, the cuticle and the epidermis are intact. The study reveals the impact of the Bisphenol on the conversion of tea waste into compost.

Keywords: tea waste, vermicompost, micro and macro nutrients, FTIR, histology of Earthworm

I. INTRODUCTION

The growing population and high level of industrialization have exacerbated the problem of solid waste management [1]. Large amounts of solid waste and mud produced by anthropogenic sources become a major problem [2]. Good waste management is very important and has become a major challenge in many countries [3]. Vermicomposting has become another effective way to dispose of safe and costly waste. Earthworm, by means of eco-biotechnological process, converts rich energy and complex matter into stable vermicompost [4]. Waste recycling using earthworm has become an important factor in sustainable agriculture that has a different impact on the use of safe waste disposal that prevents pollution without providing rich nutrients [5]. An environmentally friendly vermicomposting technology uses wormwood that can be effective in converting waste into waste [6].

The earthworms have been fortunate to degrade wastes and turn them into a fertilizer. It is the resourceful technique to renovate the biological wastes (human discarded, kitchen surplus, animal left-over, etc.) into a biological fertilizer [7]. Vermicompost has an abundant fine structure and it is highly saturated with nutrients that are freely available for plants intake.

TEA WASTE FOR COMPOST:

India is a world leader in the use, export, and production of tea. Tea production in India was 9,79,000 tons in 2009. Accounting for 31% of global tea production. India has retained its leadership in the tea industry for the past 150 years. 70% of its product is used within India. Many industries are currently dumping their tea waste in open areas or land areas that lead to land and water pollution. Also, tea leaves garbage in kitchens exposed to open spaces which also creates soil pollution. Proper disposal of tea leaves waste from factories, tea shops, hotels and kitchens is needed to maintain a healthy environment. Vermicomposting is a safe and inexpensive way to properly dispose of tea waste without harming the environment. Vermicomposting is a bio-oxidation process in which we find that the worm is very close to microorganism and other terrestrial organisms. The decay society affects the process of decomposition, accelerates the stability of the body, and completely transforms its

physical and chemical properties [8]. Micro-organisms that produce enzymes that cause chemical decay in living organisms and earthworms are important indicators of the process, as they involve the indirect reconstruction of living organisms by the fragmentation and introduction of new substances, leading to large colonies. [9].

Bisphenol -A

Bisphenol-A can occur during the chemical reactions, transport and processing. Consumer rear bins are primarily made up of pollutants from municipal waste water treatment plants, leaks from landfills, domestic waste burning, and environmental degradation of plastics in the environment. Bisphenol-A has low vapor pressure, high melting point, and moderate melting [10]. Bisphenol-A is considered to be low [11]. Hydrophobicity and thus has a limited concentration capacity. Based on these various factors, it is estimated that the major natural components of Bisphenol-A are abiotic and are associated with water and suspended solids (53%), soils (25%), or organisms (23%) [12,13].

Bisphenol - A used for single contaminant that can get into food from cans and polycarbonate plastic products such as baby bottles, tableware, and food containers. The use of Bisphenol-A in food and beverages causes daily exposure; The average human consumption of Bisphenol-A from epoxy-filled food cans was 6.6µg / day [14]. Heating plastic, as in a microwave, increases Bisphenol-A leakage into liquid; Temperature seems to be the most important factor in rewarding over the years of the vessel. The main source of Bisphenol-A in soil is land use for sewage or sewage [15]. Reported levels of Bisphenol-A in biosolids vary in size orders, ranging from 0.10 to 3.2×107 mg / kg dry weight [16]. The lifespan of Bisphenol-A in the soil is estimated at three days, up to 37 days. Bisphenol-Presence in the ground can cause great concern. As an environmental pollutant, Bisphenol-A interferes with nitrogen fixation in the roots of vegetable plants associated with bacterial symbiont Sinorhizo biummelilot. With the exception of half-life on soil for only 1-10 days, its availability makes it an important pollutant. As we consider bisphenol-A as one of the main pollutants in the soil, the effect of bisphenol-A on the conversion of bio wastes is necessary to understand the effectiveness of the earthworm in vermicomposting process.

II. MATERIALS AND METHODS

Biology of Earthworm

Eudrilus eugeniae, the elusion earthworm species was collected from Palani Earthworm Form Vanthavasi, Thiruvannamalai Dt, Tamil Nadu. The collected earthworms were brought to laboratory for acclimatization.

Experimental methods

In the experiment, predigested Tea wastes were mixed with soil. The experimental setup (expt-1) was maintained in the laboratory with the introduction of earthworm *Eudrilus eugniae.* In another pot the same mixture was taken with Bisphenol-A (expt-2) then released earthworm. Suitable control was maintained. The moisture content of the organic substrates in each pot was maintained between 60% and 65% throughout the study period by sprinkling water after every 24 hours. The experiment was conducted by randomized design with six replications. In the vermicompost materials the experimental set up was maintained till 30 days and the compost material was subjected to the analysis of micro and macro nutrient analysis such as total nitrogen (N), total phosphorus (P), total potassium (K) Zine (Zn), Manganese (Mn), Iorn (Fe), and Copper (Cu).

Control: Cow dung + Garden soil. **Experiment 1**: Garden soil + Cow dung + Tea waste. **Experiment 2**: Garden soil + Cow dung + Tea waste + Bisphenol-A.

III. RESULTS AND DISCUSSION

The conversion of Tea waste into vermicompost process was observed by using *Eudrilus eugeniae* in our laboratory at various intervals. The Tea waste was mixed with vermibed and seen the efficiency of the earthworm in the conversion of tea waste into minerals on 15^{th} day and 30^{th} day. Along with this, Bispenol-A was treated with the tea waste as an experiment.

The nutritional status of vermicompost depends on the type of impurities processed by the worm. Decreases were observed in the following parameters such as Total Nitrogen (N), and Total Potassium (K) compared with controls. Cow dung used as a decoction in the collection process has improved the quality of nutritional supplements that attract worms and accelerate waste disposal which has led to a reduction in certain nutrients.

The N value was decreased in the Bisphenol-A treated compost on 15th day whereas it was increased on 30th day analysis. All the values were statically significant P<0.01. The study indicated that the optimum levels of mineralization take only after 15th day. Similarly P was also increased only in the treatment where the level was 20% and Showed significance at P<0.05 (Fig-1-9). The analysis of Ferrous on 15thday indicated slight increase in all treatments. Statistical analysis also showed significance at P<0.01 level. The same experimental set up was kept for further analysis to study the efficiency of earthworm on 30th day where the nutrients were increased. The P and K levels were increased than the control value. The statistical analysis revealed significant at P<0.01 level. . It is obvious from the results that the vermicompost had comparatively higher quantities of total N, P, and K.

The analysis of Fe, P and Cu were also increased only in the treatment on 15^{th} day and all other treatments showed lower levels of Fe on 30^{th} day. The Fe in the experiment 2 the value was marginally increased than the control however it showed statistical insignificance whereas Cu

Int. J. Sci. Res. in Biological Sciences

showed significant increase. Mn also indicated the slight decrease in all the treatments on 15^{th} day.

PHYSICO-CHEMICAL PROPERTIES

Physico-chemical properties of the soil mixed with Tea waste were analyzed on 15^{th} and 30^{th} day along with control and experiment. There was no change in the electrical conductivity (EC) including bisphenol-A treated tea waste. The values in all the treatments were statistically insignificant. On 15^{th} day, the pH value was increased in all the treatments. In contrast to the analysis on 15^{th} day, the value was normal in the treatments after 30^{th} day when compared to control value.

In this study, was pH value reduction identified at the end of the trial.. During their study on vermicomposting certain biological residues can be produced that can lower the pH level at the end of the study.







Fig 4. Analysis Of Ferrous After The Conversion Of Tea Waste Into Compost By Eudrilus eugeniae



Fig 5. Analysis Of Manganese After The Conversion Of Tea Waste Into







Int. J. Sci. Res. in Biological Sciences

Fig 9. Analysis Of pH After The Conversion Of Tea Waste Into Compost By Fudrilus eug 7.6 7.4 7.2 7 PERCENTAGE 6.8 🖬 15 days 6.6 🖬 30 days 6.4 6.2 6 Control Ex control experiment TREATMENT

EX ; Experimental Control Confirmational Analysis of Functional Groups Using FTIR Spectrum (Table-1, Fig 1,2&3)

FTIR spectrum was recorded for the control and experimental compost. The experimental system consists of Tea waste which is considered to be nitrogen rich when decomposed in the soil. The control system has no N-H stretching peak. Whereas Tea waste decomposition FTIR spectra has N-H stretching peak at 3743 cm⁻¹. The peak at bisphenol-A leads to large number of hydrogen group and the mixture has more moisture than the control system. The hydroxy group at 3854 cm⁻¹ and C-O-C group at 1339 cm⁻¹ confirm the presence of anhydrates at 3860 cm⁻¹ which contains the presence of OH group in Alkaloids. The tea waste has flavonoids which also exhibit the OH group at the same system. The decomposition of bisphenol-A leads to large number of hydrogen group. ie., is the mixture has more moisture than the control system. The hydroxy group at 3854 cm⁻¹ and C-O-C group at 1339 cm⁻¹ confirm the presence of anhydrates

Table-3; FTIR-Band Assignment:					
S.No	Control wave number (cm ⁻¹)	Teawaste wave number (cm ⁻¹)	Tea waste +Bisphenol A	Band Assignmet	Functional group
1	3396(m)	3395(m)	3395(m)	N-H (St)	Amines
2	3090(m)	2864(m)	2925(m)	C-H (St)	Alkanes
3	2771(m)	2770(m)	2801(m)	H-C=O;C-H (St)	Aldehydes
4	2205(w)	2157(w)	2213(w)	-C=C- (St)	Alkanes
5	1693(s)	1694(s)	1692(s)	C=O (St)	Ketones
6	1550(s)	1516(s)	1548(s)	N-O	Nitro compound
7	1420(m)	1418(m)	1422(m)	(Asymmetric)	Aromatics
8	916(m)	918(m)	919(m)	C-C (in Ring)	Carboxylic acid
9	790(m)	784(m)	845(m)	O-H (St)	Alkyl halides
10	670(m)	670(m)	602(m)	C-CL (St)	Alkyl halides

≻m - medium, **w** - weak, **s** - strong, **St**-Stretching,



Page 1/1 Fig-1. FTIR Spectrum of Control

Vol.8, Issue.1, Feb 2021







Page 1/1

Fig- 3. FTIR Spectrum of Tea Waste with Bisphenol A

Histological Studies of Anterior, Clitellar, Middle and Posterior Regions of the Earthworm Eudrilus eugeniae exposed To Bisphenol-A(Plate1&2)

Anterior Region:

Histology of the tested internal region shows that the cuticle was not severely damaged. There is not much damage to the circular muscle area but some parts of the longitudinal muscles show signs of injury. The presence of the peritoneum from the longitudinal muscles can be seen elsewhere. The ventral nerve is slightly damaged.

Clitellar Region:

The control animal of clitellar region showed intact structure of internal organs. The coelom and Blood vessels are well marked. In the experimental animal, the clitellar region showed slight modification of the anatomy. The pigment cells are prominent in the waste treatment with Bisphenol-A. In some places, the circular muscles are disorganized.

PLATE 1: Cross Sections of Anterior and Clitellar regions of Earthworms, *Eudrilus eugeniae* exposed to Bisphenol -A





Middle Region:

In the middle region, the cuticle and epidermis are firm. Circular and long muscles are also unaffected. The peritoneum shows signs of suspension in several places. Typhlosole and pridiopore are clearly visible. The ventral spinal cord was not severely damaged there as the spinal cord and ventral vessel showed signs of damage. The cuticle of the posterior region is less visible. Minor damage is seen in other parts of the circular muscle. However, in the long-term muscles great damage and fractures are seen in a few places. The peritoneum covering the intestines is severely damaged.

Vermicomposting has become the safest and most costeffective way to clean up In present years, earthworms have been identified as one of the most important factors in processing organic waste into vermicompost. An important feature of vermicompost is that during the processing of various organic wastes by earthworms, many of the nutrients in the waste are converted into useful plant species [7]. Waste disposal. Earthworm is converts the energy of organic rich matter into stable vermicompost [4]. Waste recycling using earthworms has become an important factor in sustainable agriculture that has a different impact on the use of safe waste disposal that prevents pollution without providing rich nutrients [5]. An environmentally friendly vermicomposting technology uses wormwood that can be effectively used to convert waste into waste [6]. The current study analyzed the compost elements found in tea waste.

The body parts of the vermicompost of the tea plant have shown a variety of characteristics. The pH difference during tea tree infusion is shown in Fig (1 & 2) where there was a change in the pH of the vermicompost tea at 15 per day analysis but at the 30-day analysis there was no change in pH. Similarly with the disposal of cattle, there was no significant difference in pH values by the use of different numbers of people of Eudrilus eugeniae on the 30th day. By the 60th day, the pH of the 15- and 20-worm manure was the same, however, it was lower than the manure that decomposed by 5 and 10 worms. At the end of vermicomposting, the pH of 5 worm treatments was significantly higher than that of the treatment with 10, 15 and 20 earthworms. Without looking at the initial amount of wormwood, the pH became acidic and lower than pH at 30 and 60 days. The pH dropped as the number of earthworms increased during the application of sheep and poultry manure.

By day 30, treatment for 20 worms found a very low pH in sheep dung. At the end of vermicomposting, the pH treatment of 5 worms was significantly higher than other treatments. The caterpillar did not affect the pH of the chicken dung for 30 and 60 days. At 90 days, the pH of 5 worm treatment was higher than that of other treatments. At the end of the experiment, the pH of cattle, sheep, and chickens with 10, 15, and 20 worms was more acidic than litter made from 5 worms. The pH increases during pigmentation unlike other waste. At the end of vermicomposting, the pH deviated to neutral. The high pH of the acid content is probably due to the high mineral composition of N and P in nitrites / nitrate and orthophosphate due to the large size of earthworms. The conversion of organic matter into organic acid and organic

matter and the reduction of ammonia can also lead to lower pH [17]

A slight decrease in the values of electrical conductivity in vermicompost is probably due to the biological properties of C, Mg, and K .. We lose many natural substances in the early stages such as CO_2 at the end of the vermicomposting period. Vermicomposted materials were high in nitrogen. Including worms in the disposal, the material increases the value of N due to the soil worm, which is enriched with nitrogen mineralization of waste. It also suggested that the earth's fetus improve the nitrogen levels of the substrate by adding its foreign products, as well as the decaying tissue of dead worms to vermicomposting.

The nitrogen content of vermicompost in tea waste is reduced by day 15, but by day 30 they increase. The increase in nitrogen is probably due to the formation of organic minerals by earthworms during vermicomposting. Similarities between nitrogen content in the treatment of ten, 15, and 20 larvae in cattle, sheep, and poultry suggest that up to ten people in *the Eudrilus eugeniae* could be considered a global warming barrier to competition. Another explanation for the increase in nitrogen may be its addition of mucus, nitrogenous excretory substances, growth hormone, and enzyme-derived enzymes [18].

Phosphorus content also increased in this study during the increasing time of composting. This view was supported by [17]. The apparent effect of the number of earthworms on P content was observed only in the incubation of pig manure in 60 and 90 days and in the poultry manure for 90 days of the earth. With pig manure, within 90 days of fertilization, the effect of the earth's worm fluctuations varied while in poultry manure, P values were equivalent to 10 to 20 earthworms.

The increase in P content during vermicomposting is probably due to the addition of minerals and phosphorus stimulation by bacterial activity and phosphatase worms. The direct activity of the enzyme worm gut and the upliftment of small flowers can increase phosphorus [19]. The present study also agrees with that [20,21], which observed an increase in phosphorus during vermicomposting of waste and mud paper, respectively.

In contrast to other nutrients, potassium has shown a decrease in levels. Similarly, [22] reported a decrease in K in the production of coffee waste products. Contrary to our findings, [23], observed an increase in K content during sewage disposal. This variation can be caused by the chemical nature of the original green waste. Minor nutrients such as iron, manganese zinc and copper showed different levels on different days of vermicompost tea analysis.

FTIR SPECTRUM:

The control vermicast has no N-H stretching peak whereas Tea waste decomposition of FTIR spectra has N-H stretching peak at 3743 cm⁻¹. The peak at 3860 cm⁻¹ contains the presence of OH group in Alkaloides. The tea waste has flavonoids which also exhibit the OH group in the experiment then the control. The tea waste has rich nitrogen contents than the control system.

The decomposition of bisphenol-A leads to large number of hydrogen group and the mixture has more moisture than the control. The hydroxy group at 3854 cm¹ and C-O-C group at 1339 cm⁻¹ confirm the presence of anhydrates.

HISTOLOGICAL STUDIES:

The cross section of clitellum region of control and bisphenol-A treated tea waste were taken to study the anatomical features of Eudrilus eugeniae. In the control animal, the epidermis of the Eudrilus eugeniae consists of an epidermal epithelium and an overlying fibrous cuticle. Below the epidermis, circular and longitudinal muscles were intact. The coelom was clearly seen. Along with this, luman, and blood vessels were seen clearly. Below the epidermis, the pigment cells were numerous. The bisphenol-A treated section of clitellum region showed fewer changes when compared to control. The cuticle, epidermis and circular muscles were not changed in the experiment. The supposed to be the pigment cells were reduced to a greater extent. Similarly in the tea waste treated clitellar region showed very little changes in the luman, blood vessels and pigment cells. In the bisphenol-A treated and tea waste treated section were less affected. Inter vascular space was much reduced. Majority of the anatomical details were intact except few changes like the size of the luman, number of the pigment cells and the size of the coelom. Only in certain regions the thickness of the body wall was reduced.

IV. CONCLUSION

It is concluded that tea waste can be stabilized by vermicomposting in a short period of about 30 days. Percentage of total nitrogen showed a growth rate on the day of the 30-day test. It is suggested that in addition to nitrogen excrement and the accumulation of foreign products, body fluids and other biological fluids may be the cause of the increase in nitrogen levels. Species of *Eudrilus eugeniae* show better remodeling and increase the percentage of nutrients. Vermicomposting can be used as an alternative to various waste management technologies. The suitability of vermicompost produced as a living fertilizer was confirmed by studying the microscopic and microscopic elements.

The results also show that the Vermicompost produced by the earthworm has low electrical conductivity. It can therefore be used as important compost. Research also highlights the fact that a mixture of garbage cans and cow dung can be degraded at this time and provide a higher quality product. In the present study it has been proven that vermicomposting is introduced as an effective technology for converting tea waste. Environmental pollution

ACKNOWLEDGEMENT

The authors thank the college Principal and staff of Zoology Department for their encouragement and support.

REFERENCES

- [1] P.A, Beohar and R.K. Srivasta "journal of soil science, **1**, **04-11**, **2011.**
- [2] S. Suthar, "Vermistabilization of municipal sewage sludge amended with sugarcane trash using epigeic *Eisenia fetida* (Oligochaeta) *Journal of Hazardous materials*, (163):199-206 .2009.
- [3] T. Wanger and P. Arnold, Journal of cleaner production, 16(4): 410-421. 2018.
- [4] A. Nino, A. Rivera and Ramirez, "Production of organic fertilizer with Macro-Micronutrients from the solid waste generated at home" *European Journal of Experimental Biology*, 2 (1):199-205. 2012.
- [5] D.A. Cox, "Reducing nitrogen leaching-losses from containerized plant: the effects of controlled release fertilizers". J. Plant Nutr. 16: 533–545.1993.
- [6] C.J. Marlin, K.T. Rajeshkumar, "A study on sustainable utility of sugar mill effluent to vermi-compost". Adv Appl Sci Res 3: 1092–1097. 2012.
- [7] C.A. Edwards, I. Burrows, "The potential of earthworm compost as plant growth Media.In: Edwards C. A., Neuhauser E., (Eds) Earthworms in waste and environmental management "SPB Academic press, The Hague: The Netherlands, 21-32. 1988.
- [8] M. Aira, F. Monroy, J. Domínguez, "Eisenia fetida (Oligochaeta: Lumbricidae) modifies the structure and physiological capabilities of microbial communities improving carbon mineralization during vermicomposting of pig manure". Microb Ecol; 54(4):662-71, 2007.
- [9] C. Lazcano, M. Gmez-Brando, J. Dominguez, "Comparison of the effectiveness of composting and vermicomposting for the biological stabilization of cattle manure. Chosphere" 72 (7), 1013-1019, 2008.
- [10] A. Shareef, M.J. Angove, J.D. wells, B.B. Johnson,(2006) "Aqueous solubilities of estrone, 17b estrodiol, 17aethynylestradiol, and bisphenol A" J Chem Eng Data;51:879 -881.

- [11] I.T. Cousins, C.A. Stoples, G. M. Klecka and D.A. Mackay "Multimedia assessment of the environmental fate of bisphenol A". Hum Ecol Risk Assess; 8:107-1135. 2002.
- [12] J. Heinonen, V.K. Honkanen, I.J. Holopainen, "Bisphenol A accumulation in the freshwater clam pisidiumamnicum at low temperatures". Environ contamToxicol; 43:50-55.
- [13] Environmental Canada, "creening Assessment for the Challenge: Phenol, 4, 40- (1-Methylethylidene) bis-Bisphenol A. Chemical Abstracts Service Registry Number 80-05-7. 2008.
- [14] S.R. Howe, L. Borodinsky, "Potential exposure to bisphenol A from food-contact use of polycarbonate resine". Food Addit contam; 15: 370-5.1998.
- [15] E. Loffredo, N. Senesi, "Fate of anthropogenic organic pollutants in soils with emphasis on adsorption/ desorption processes of endocrine disruptor compounds pure" ApplChem; 78: 947-961.2006.
- [16] D.P. Mohapatra, S.K. Brar, R. Tyagi, R.Y. Surampalli, "Occurrence of bisphenol A in waste water and wastewater sludge of cucs treatment plant" J Xenobiotics; 1:9-16.2011.
- [17] S. Coulibaly. Sifolo, I. Kouadio, J. Kouassi Ebagnerin, I. Tondoh Bi, A. Zoro. "University of Abobo - Adjame, Department of Natural Sciences, Laboratory of Functional Genomics and Breeding, Philipp Agric Scientist" Vol. 94 No. 4, 88-95.2011.
- [18] B.K. Senapati, M.C. Dash, A.K. Rane, B.K. Panda. "Observation on the effect of earthworms in the decomposition process in soil under laboratory conditions". Comp Physiol Ecol 5: 140-142.1980.
- [19] C.A. Edwards, J.R. Lofty "Biology of Earthworms". London: Chapman and Hall; p. 283. 1972.
- [20] J.E. Satchell, K. Martin, "Phosphate activity in earthworm faeces. Soil" Biol Biochem 16: 191-194. 1984.
- [21] R. C. Le Bayon, F. Binet, "Earthworms change the distribution and availability of phosphorus in organic substrates". Soil Biol Biochem 38: 235-246. 2006.
- [22] F.H. Orozco, J. Cegarra, L.M. Trujillo, A. Roig, "Vermicomposting of coffee pulp using the earthworm *Eisenia fetida:* Effects on C and N contents and the availability of nutrients. Biol Fertil Soil 22: 162-166.1996.
- [23] M. Delgado, M. Bigeriego, I. Walter, R. Calbo, "Use of California red worm in sewage sludge transformation", *Turrialba*, 45, 33–4.1995.