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Efficiency of Earthworm, *Eudrilus Eugeniae* (Kinberg, 1867) in the Conversion if Orange Peel (*Citrus Sinensis*) Waste Mixed with Diclofenac into Compost

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Abstract: The efficiency of the earthworm *Eudrilus eugeniae* was studied in the present work where the earthworm was subjected to the conversion of Orange peel waste mixed with diclofenac into compost in the form of micro and macro minerals on 15th and 30th days. Micro and Macro nutrients such as N, P, K and Fe, Mn, Zn, Cu were analyzed in the Orange peel waste and mixed with diclofenac along with control. Similarly physical characteristics were also analyzed to understand the efficiency of earthworm in maintaining the physical status of the soil. The analysis revealed that the P and Fe were increased significantly on 15th and 30th days where as the Physical property i.e., EC was increased only in the orange peel waste conversion where as in the diclofenac contaminated waste, the EC was stable in the present study. The pH was slightly increased on 15th and stable on 30th day. FTIR Study was undertaken to identity the chemical group present in the control and experiment. Histological changes were compared with that of experiment and the efficiency of the earthworm has been understood in the present work within a short period of 15th to 30th days.

Key words: *vermicompost, FTIR, Eudrilu eugniae, Orange peel waste, Macro and micro Nutrients, histological studies.*

I. INTRODUCTION

Solid waste comprises countless different materials including both combustible and noncombustible wastes. They have to be properly managed otherwise they cause insanitation conditions which are hazardous to human being. Vermitechnology, one of the few techniques, helps to manage the waste by converting it into compost by using earthworms. Vermicomposting helps to process wastes simultaneously by giving bio fertilizers and proteins [1]. Thus vermitechnology could successfully be used to clean the environment as it uses wastes as raw material to change polluted environmental conditions into useful substances necessary for the sustainable agriculture [2]. Fruit wastes consist of fruits unsuitable for marketing due to bruising, inappropriate size, rotting, or insect attack, as well as marketable fruits which could be converted into a nutrient enriched bio fertilizer and used for agricultural purposes or land restoration. Vermicomposting, a process involving the bio stabilization of organic wastes by the joint action of earthworms and microorganisms, has proven to be a low-cost and rapid technique for the efficient management of vegetable wastes [3]. Several epigeics (*Eisenia fetida, Eudrilus eugeniae, and Perionyx excavatus*) have been identified as potential candidates to decompose organic waste materials [4, 5]. Traditionally vermicompost has been generated with animal manure as the substrate and has been recognized as a good soil conditioner and fertilizer [6]. In recent years, other organic substrates have also been vermicomposting and the products have been found to be as good as the manure based vermicomposting [7]. The earthworm is capable of decomposing large quantities of organic wastes rapidly and incorporating them into the topsoil. The vast utilization of several drugs for human and cattle therapeutics finally pollute the soil. The carcass of birds as well as cattle contains several drug residues and may affect the soil quality which indirectly affects the several biological processes including composting process. Since, earthworms are considered as bio indicators of the environmental pollution, the present study is aimed to select the worm and attempted to study the efficiency of the selected earthworm on the conversion of fruit waste into nutrients. The species of *Eudrilus eugeniae* exhibit better in converting the waste and increasing the percentage of NPK. Vermicomposting could be applied as an alternative technology for the management of waste. The suitability of the vermicompost produced as an

organic fertilizer was confirmed by the analysis of micro and macro nutrients.

II. MATERIALS AND METHODS

Collection of Earthworm

Eudrilus eugeniae, the elusion earthworm species was collected form chithathur, Vembakkam Tk, T.Vmalai DT, Tamil Nadu. The collected earthworms were brought to laboratory for acclimatization.

Diclofenac

In recent years pharmaceuticals have been seen as an emerging environmental protection problem due to their presence in water and soil ecosystems [8]. Diclofenac (DCF, (2-[(2, 6-dichlorophenyl) amino] benzene acetic acid) is a synthetic non-steroidal anti-inflammatory drug (NSAD), mostly used as its sodium salt in medical care as an analgesic, ant arthritic, and ant rheumatic. The medicine has been applied in human medicine for over 30 years [9]. Diclofenac sodium 3% gel gained US approval for the treatment of actinic keratosis more than 10 years ago. It has been proven that, applied in this form for over 10 years, the medicine is effective and safe. Diclofenac acts via inhibition of prostaglandin synthesis by inhibiting cyclooxygenase-1 (COX-1) and COX-2 with relative equipotency. Moreover, diclofenac may inhibit thromboxane-prostanoid receptor affecting arachidonic acid release and uptake, as well as inhibiting lipoxygenase enzymes, and activate the nitric oxide-cGMP ant nociceptive [10].

Experimental methods:

In the experiment, predigested Orange peel wastes were mixed with soil. The experimental set up was maintained in the laboratory with the introduction of earthworm *Eudrilus eugeniae.* In another pot the same mixture was taken with Diclofenac then released earthworm. Suitable control was maintained. Similarly Orange peel waste was also taken separately in another pot. The waste mixtures were allowed to pass through earthworm guts for vermicomposting. 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 three replications. In the vermicompost materials the experimental set up was maintained till 15 and 30 days. After that the compost material was analyzed for the micro and macro nutrients. The obtained compost material was subjected to the FTIR analysis.

Histological Studies

The histology of clitella region of earthworm was studied by adopting the routine paraffin method. The control and experimental animals were blotted free of mucus, washed thoroughly in physiological saline, cut into pieces of clitellar region of desired size and fixed in the fixative immediately after autopsy. Fixation was carried out at room temperature for 24hr, after which the tissues were transferred to 70% alcohol. Several changes of 70% alcohol were given until the yellow color disappeared from the tissues. The tissues were then dehydrated by passing through ascending graded of alcohol and cleared in xylene then infiltrated with molten paraffin. Finally the processed tissue was embedded in the paraffin wax.

Tissue section of 5-µm thick of cross sections were obtained using a rotary microtome. The section, thus obtained, was stained in hematoxylene and eosin, dehydrated using alcohol, cleared in xylene and mounted using dihydroxy phthalate xylol (DPX). The stained slides were observed in a Qasmo research microscope.

III. RESULTS AND DISCUSSION

The conversion of Orange peel waste into vermicompost process was observed by using *Eudrilus eugeniae* in our laboratory at various intervals. The Orange peel waste was mixed with diclofenac in the vermi bed and seen the efficiency of the earthworm in the conversion of Orange peel waste into minerals on15^{th} and 30^{th} day. The analysis showed the various levels of micro and macro nutrients. Among the nutrients, the important nutrient is the N which was decreased significantly on the 15^{th} day analysis in the diclofenac treated experiment where as in the other treatments; the level of N was same as that of control value. In the 30th day analysis, the nitrogen level of control was 89 ± 10.2 whereas in the diclofenac treatment the level of nitrogen was increased by 127.6 ± 10.5. The study indicated that the optimum levels of mineralization takes nearly after 15 days. Similarly P on 15^{th} day was also increased only in the treatment where the level was 6.3 ± 1.2 and showed significance at $P<0.05$ whereas on 30^{th} day the level was decreased by 12.3± 2.2 when compared to control. In contrast to the N and P, the K level was decreased on 15^{th} and 30^{th} day. Fe level was increased significantly only on 15^{th} day whereas there was a decrease on 30^{th} day. The analysis on 30^{th}day indicated gradual increase except the level of K and Mn in the conversion of orange peel waste experiment whereas in the diclofenac treated waste, the nutrients level were decrease except N, and Mn. The Statistical analysis also showed significance at $P<0.01$ level.

Fig 1. Analysis of Macro Nutrients after the conversion of Fruit Waste into compost by *Eudrilus eugniae* (15 days)

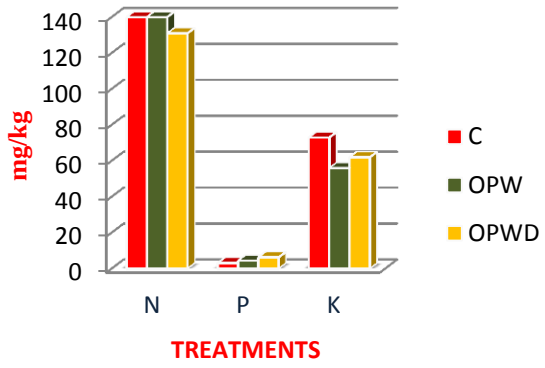


Fig 2. Analysis of Maicro Nutrients after the conversion of Fruit Waste into compost by *Eudrilus eugniae* (15 days)

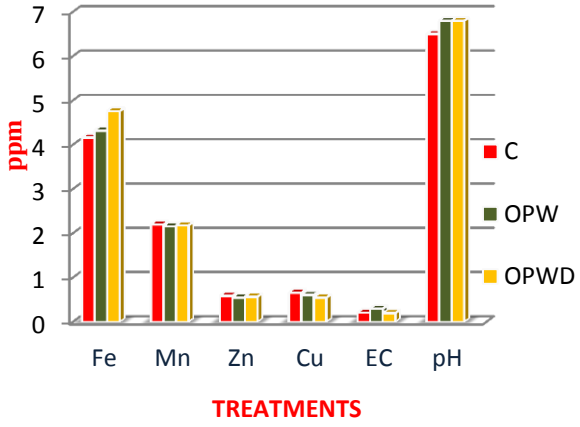


Fig 3. Analysis of Macro Nutrients after the conversion of Fruit Waste into compost by Eudrilus eugniae (30 days)

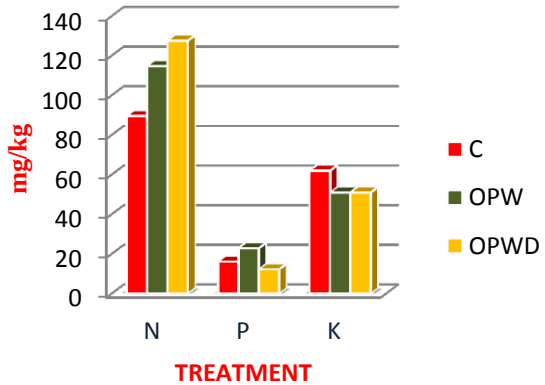
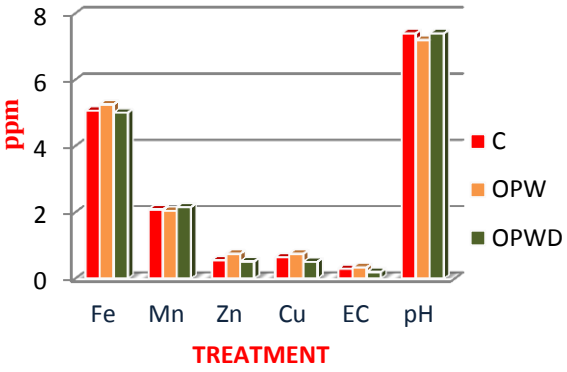


Fig 4. Analysis of Maicro Nutrients after the conversion of Fruit Waste into compost by Eudrilus eugniae (30 days)



The vermicompost was dark brown in colour and homogenous after 45 days of earthworm's activity. The changes in worm biomass for all the treatments over experimentation period were observed. At the end of the 30 days, the earthworm biomass had increased in all the treatments. It is obvious from the results that the vermicompost had comparatively higher quantities of total N and P then the raw Orange peel waste

Physico- Chemical Properties

Physico-chemical properties of the soil mixed with different weights of Orange peel waste were analyzed on 15th and 30th day along with control and experiment.

The Electrical Conductivity (EC) was increased by 0.30 ± 0.01 in Orange peel waste treatment where as in diclofenac treated Orange peel waste the level was decreased on 15th day and the pH value was slightly increased and no change was observed in rest of the treatment. In contrast to the analysis on 15th day, the value was as that of control in the case of pH whereas the EC was decreased slightly.

In this study, the pH value was recorded at the end of the experiment. The variability in pH could be due to the production of organic acids during organic waste decomposition. The analysis on 15th day showed increase of pH value in all the treatment. The 't'test analysis showed significant in the orange peel waste conversion and diclofenac mixed waste conversion. In rest of the treatments, the analysis showed insignificance.

Histological studies of Anterior regions of the earthworm *Eudrilus eugeniae* treated with Diclofenac (Plate 1&2):-

Anterior Region: Histology of the anterior region reveals that the cuticle is untouched with the damage. Not much

spoilage in the area of circular muscles but in some part of the longitudinal muscle can see the signs of injury. Detachment of peritoneum from longitudinal muscles can also be seen in some of the areas. The lumen of the intestine is not much harmed and the epithelial lining. The ventral nerve cord is damaged to a smaller extend. The dorsal and ventral blood vessel ruined largely.

Clitellar Region:

In the control, the cuticle, epidermis, longitudinal, circular muscle are very intact where as in the experimental, the intact nature is disturbed. The circular muscles are loosened. Number of pigment cell are absent in the experiment. Blood vessels are not seen clearly. Coelom is much reduced in the experimental clitellar region.

Middle Region:

In middle region, the cuticle and the epidermis are intact. The circular and longitudinal muscles are also unaffected. The peritoneum shows the signs of disconnect at several places. The typhlosole and the Nephridiopore are clearly visible. The chlorachogen cells crumble to a

PLATE 1. Histological studies of Anterior and Clitellar region of the Earthworm Eudrilus eugeniae

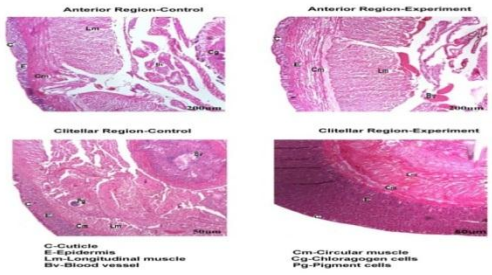
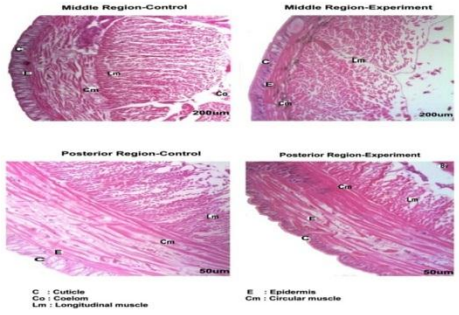


PLATE 2. Histological studies of Middile and Posterior region of the Earthworm Eudrilus eugeniae



Considerable extent. The ventral nerve cord is not much damaged whereas the dorsal blood vessel and ventral blood vessel have shown the signs of damage.

Posterior Region:

The cuticle of the posterior region is faintly visible. Light destruction is visible in some parts of the circular muscles. However, in the longitudinal muscles more damage and fissures are seen in few areas. The peritoneum covering the intestine is injured largely. The intestinal epithelial lining, the lumen of the intestine are also damaged. Greater degree of injury to the dorsal and ventral blood vessel is observed. Ventral nerve cord is damaged beyond recognition.

Conformational Analysis of Functional Group using FTIR Spectrum (Table 1 & Chart 1, 2 and 3)

The FTIR spectrum was recorded for the control, Orange peel waste and Orange peel waste with Diclofenac. The aim of the work is to find out the effect of diclofenac present, in the soil, on the decompose of Orange peel waste. The recorded FTIR spectrums for this control with experimental values have been compared and discussed (FTIR table 1 and chart 1-3). The peak of medium band at 1470 cm^{-1} in control system has shifted in both experimental system of control with Orange peel waste at 1422 cm^{-1} and Orange peel waste with diclofenac at 1420 cm^{-1}. It is observed that the diclofenac has decomposed in both experimental systems at C-N-C functional group.

The weak peak at 3223 cm^{-1} in the control system confirms the presence of NH stretching band (Amide band) and is totally absent in both the experimental systems and conforms that the diclofenac decomposed in these system at C-N-C chain linage.

The diclofenac exhibit a strong C=O group around 1750 to 1790 cm^{1} whereas this peak was absent in both experimental spectra confirms that the diclofenac decomposed on COOH group as C=O, whereas the peak is not noticed in control system.

Table 1. FTIR – Band Assignment.

S.No	Control Wave number (cm^{-1})	Orange peel waste Wave number (cm^{-1})	Orange peel waste + Dicofenac Wave number (cm^{-1})	Band assignment	Functional group
1.	3396 (m)	3394 (m)	3395(m)	N-H(st)	Amines, amides
2.	3273 (m)	3280 (m)	3278(m)	C=C-H;C-H (st)	Alkynes
3.	2471(m)	2467(m)	2530(m)	H-C=O;C-H (st)	Aldehydes
4.	2471(w)	2174(w)	2251(w)	C=C (st)	Alkynes
5.	1693(s)	1756(s)	1745(s)	C=O (st)	Ester
6.	1550(s)	1551(s)	1517(s)	N-O (st)	Nitro compound
7.	1036(m)	1040(m)	1039(m)	C-N (st)	Alphatic amines
8.	916(m)	918(m)	919(m)	O-H (ipb)	Carboxylic acids
9.	790(m)	792(m)	790(m)	C-CL (st)	Alkyl halides
10.	670(m)	692(m)	692(m)	C=C-H;C-H (st)	Alkynes

*m= Medium, w= Weak, s= Strong, st= Stretching, ipb= In Plane bending

Chart- 1.FTIR Spectrum of Control:-

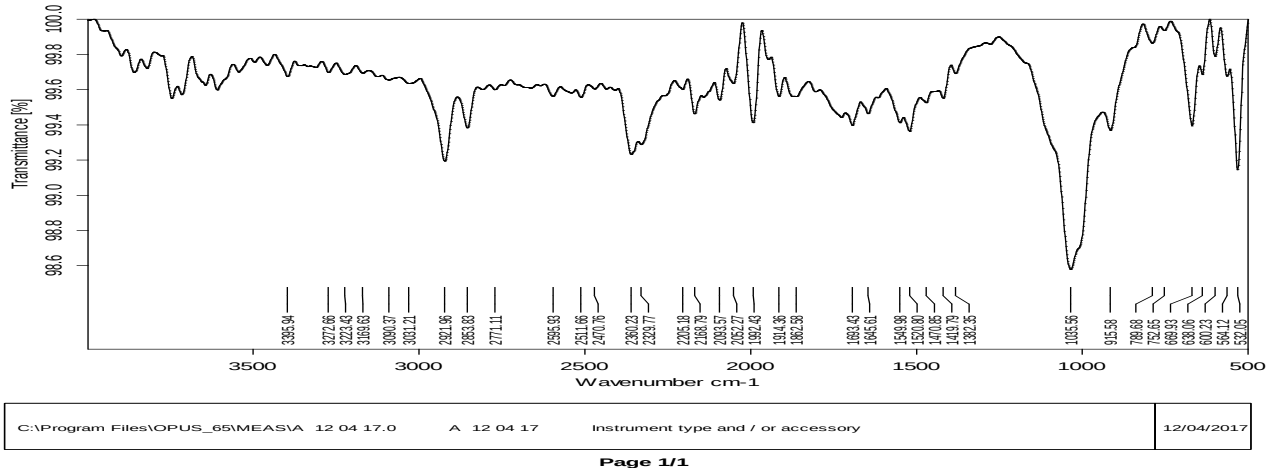


Chart-2. FTIR Spectrum of Orange peel waste Compost:-

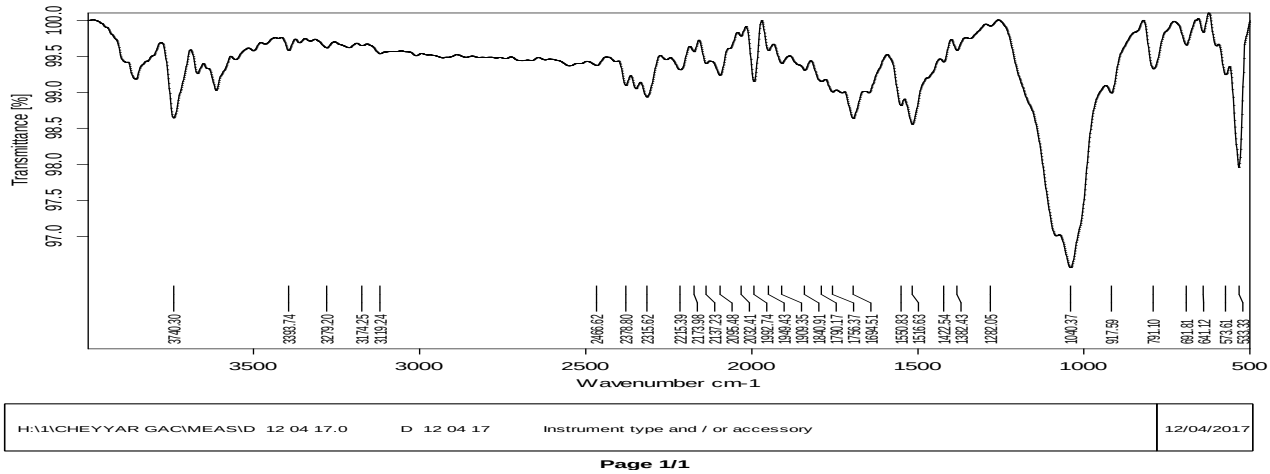
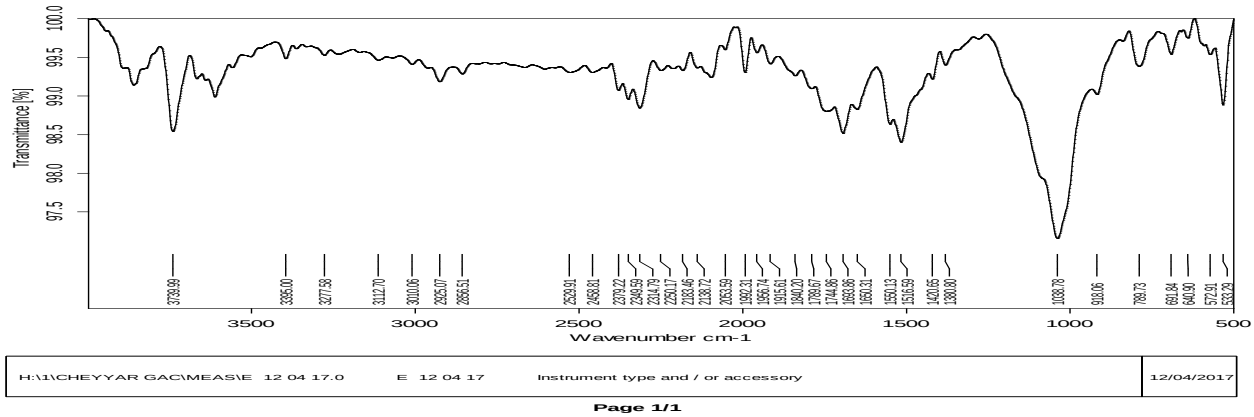


Chart-3. FTIR Spectrum of compost of the Orange peel waste with Diclofenac:-



DISCUSSION

In the present analysis, enhanced phosphorous level in vermicompost was observed and suggested that there is a phosphorous mineralization taking place during vermicomposting process. The passage of organic matter through the gut of earthworm results in the conversion of phosphorus which is more available to plants [11]. The increase in P content during vermicomposting could have been induced by the mineralization and the mobilization of phosphorus through bacterial and faucal phosphatase activity of the earthworms. The direct action of worm gut enzymes and the stimulation of micro flora could increase phosphorus. Our result is in agreement with those of [12]. [13] Who observed an increase in phosphorus during the vermicomposting of paper waste sludge and organic substrates, respectively. In contrast, [14] observed a decrease in P in the casts of *Pontoscolex corethrurus*. This difference may be due to the initial characteristics of the animal wastes used in our experiment and those of the soil used in their experiment.

The significant increase in the total nitrogen on 30^{th} day analysis is probably due to mineralization of the organic matter. The increase in the available phosphorous is due to the passage of ingested material through earthworm and due to the stimulation of microbial flora in the intestine. The microbial flora also increases the available ferrous in the final product.

Vermicompost is a potential organic manure which is rich in plant nutrients compared to farmyard manure or other organic manures in respect to supply of N, P, and K fertilizers The activities of dehydrogenase, Nitrogenize, Phosphatase, and urease were found higher in process of vermicomposting. It is fast growing in popularity as a tool of reclamation of waste and is used for reclamation of waste land [4, 15].

During vermicomposting, the total N concentration was declined (Fig 1). This differs from the study of vermicomposting where the N concentration remained stable or increased [16, 17]. The increase in pH observed during waste decomposition could have led to NH_3 volatilization because this process occurs in alkaline conditions. Also, $NH\beta_4$ is soluble and tends to volatilize in the form of NH_3 as the waste loses moisture. In addition, frequent watering of the vermin digesters could stimulate leaching of NO_3–N or gaseous N loss via de-nitrification. N content increased with an increase in composting time.

The increase in nitrogen was probably due to the mineralization of organic matter by earthworms during vermicomposting. The similarity between nitrogen content for the treatments with 10, 15 and 20 earthworms in cow, sheep and chicken wastes suggest that up to 10 individuals of *Eudrilus eugeniae* can be considered as the limit for earthworm density to prevent competition. For pig waste, it can be up to 15 earthworms. It is also suggested that the increase in nitrogen could be its addition in the form of mucus, nitrogenous excretory substances, growth-stimulating hormones and enzymes from earthworms [18]. which may be more important after 90 days. The enhanced mineralization of nitrogen could be attributed to the enhanced activity of earthworms.

The pH of the present study was increased on 15^{th} day whereas on 30^{th} day it was decreased. The bioconversion of organic material into intermediate substances of organic acids, the formation of organic acids and the volatilization of ammonia could also account for the decrease in the pH. Similar observations were reported by [5]. The soil pH is important because it affects the availability of nutrients in the soil. Many plant nutrients are not readily available to plants in highly alkaline or acidic soils.

These essential nutrients are most available to plants at a pH between 6 and 7.5 [19]. The results revealed (Figure 1) that the pH of the final vermicompost was 7.4 for orange peel waste. The lower pH recorded in the final products might have been due to the production of CO_2

and organic acids by microbial metabolism during decomposition of the substrate in the feed mixture. The decline in pH also might be due to the higher mineralization of nitrogen and phosphorous into nitrate/nitrates and orthophosphate [20]. Decrease in pH is an important factor in nitrogen retention as this element is lost as volatile ammonia at higher pH [21]. A decrease in pH was recorded in all the treatments during vermicomposting. The pH of the control showed the value of 6.5 ± 0.09 and it was increased to 7.4 ± 1.5 in feed materials on 30th day. The shift in pH in the study could be due to microbial decomposition during the process of vermicomposting [22].

Histology: The cross section of clitellar region of control, diclofenac treated and Orange peel waste treatments 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, the circular and longitudinal muscles were intact and form the body wall. The coelom was clearly seen. Along with this, lumen, blood vessels chloragogan cells were seen clearly. Below the epidermis, the pigment cells were numerous. The diclofenac treated section of clitellar region showed fewer changes when compared to control. The size of the blood vessel was reduced.

The cuticle, epidermis and circular muscles were not changed in the experiment. However, the coelom was enlarged. The supposed to be the pigment cells were reduced to a greater extent. Similarly in the Orange peel waste treated clitellar region showed very little changes in the luman, blood vessels and pigment cells. In the above diclofenac treated and Orange peel 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. The Orange peel waste and diclofenac were not much impact on the anatomy of the *Eudrilus eugeniae*.

FTIR Spectrum;- The recorded FTIR spectrums for the control with experimental values have been compared and discussed (FTIR chart 1-3). From the spectrum it is noticed that the experimental spectra show no variation with the each other.

The peak of medium band at 1470 cm^{-1} in control system has shifted in both experimental system of control with orange peel waste at 1422 cm^{-1} and orange peel waste with diclofenac at 1420 cm^{-1}. It is observed that the diclofenac has decomposed in both experimental systems at C-N-C functional group. The weak peak at 3223 cm^{-1} in the control system confirm the presence of NH stretching band (Amide band) is totally absent in both the experimental systems and conforms that the diclofenac decomposed in these system at C-N-C chain linage.

The diclofenac exhibits a strong C=O group around 1750 to 1790 cm^{-1}where as this peak was absent in both experimental spectra and confirms that the diclofenac decomposed on COOH group as C=O, whereas the peak is not noticed in control system.

In control and Experimental, the hydrogen bond due to –OH stretch was observed at 3935.40 and the appearance of new peaks in the region of 3831.59 at C2 indicates that there are some chemical changes in –OH stretch. There is an increase in the intensity of peaks from 3407.58 to 3419.18 which may be due to C-H bonds and OH groups. The appearance of new band at 2353.82 and 1352.70 may be due to formation of CH_2 and CH_3 acid group. Further there is an increase in the intensity of peaks from 2926.88 to 2928.20 which may be due to aliphatic C-H stretching. And the appearance of new peaks at 1594.60 indicates the formation of amides. Further there is a decrease in the intensity of peaks from 1050.83-1048.28 which indicates the formation of OH ions.

IV. CONCLUSION

Vermicomposting technique helps in converting decomposable organic wastes into valuable vermicompost through earthworm's activity and it is a faster and better process when compared with the conventional methods of composting. The accumulation of toxic chemicals and its effects on the growth, reproduction and life cycle of a dominant earthworm species was described by many researchers in Indian crop fields. Hence in the present study it could be concluded that *Eudrilus eugeniae exhibited* by a way of showing minor pathological symptoms and observed that the contaminant reduced the vermicomposting ability of the earthworm. Which are proved to be important criteria for monitoring the efficient and quick degradation of waste into high quality organic manure, thus vermicomposting by earthworm which promises to play a significant role both in cleaning the environment and building up of soil fertility for sustainable agriculture.

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