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Research Article

BIOCHEMICAL STUDY OF CONTROL AND FIRE WOOD ASH (ELECTRICITY PRODUCTION WASTE) TREATED EXPERIMENTAL GROUP OF LOCAL SPECIES OF EARTHWORM *(LAMPITO MAURITII)* IN CHEYYAR TALUK, THIRUVANNAMALAI DISTRICT

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ABSTRACT

To understand the effect of firewood ash on vital organs of locally available earthworm *Lampito mauritti* was selected.The biochemical studies like protein, Lipid and Glycogen content in fore region, middle region and hind region of both control and wood ash treated experimental group of earthworm have been carried out.LC_{50} value was calculated before the experiment by the following method of Saptami Moitra and Verma (1997).The earthworm are divided into two groups, one is control (N=50) group another one is called experimental (N=50) group. The control group of earthworms was cultured in native soil with cow dung. The experimental group of earthworm cultured in $^{1}/_{10}$ of LC_{50} value (50g/kg of soil) of firewood ash with native soil and cow dung. The experimental period was one month (30 days).After 30 days of experiment earthworms from each group were collected and dissected out for the analysis of biochemical and histological studies. After 30 days of experiment the concentration of protein in the control group earthworm showed 11.4±0.22 µg/mg in fore region, 32.7±0.16 µg/mg in mid region and 12.6±0.10 µg/mg in hind region. But in the case of experimental group the earthworm showed highly reduced level of protein that is 6.4±0.10 µg/mg in fore region, 15.5±0.12 µg/mg in middle region and 11.3±0.11 µg/mg in hind region. After 30 days of experiment the Lipid content in both group showed variation. The glycogen content after 30 days of experiment. The control earthworm showed 24.9±0.22 µg/mg in fore region, 19.7±0.10 µg/mg in middle region and high amount of glycogen in hind region (29.5±0.28 µg/mg) were noticed. The experimental group of earthworm showed highly decreased level of glycogen content when compared to control group. This may be due to electricity production waste that is firewood ash contain the large amount of chemical substance called potassium oxide this may enter in to the body via food and alter the biochemical parameters.

INTRODUCTION

The earthworms are a group of invertebrates belonging to the phylum Annelida and class Oligochaeta and represented by more than 1000 species. Earthworm is a face organism and it is present in moist and dark places in mud. Earthworms are of great economic value to mankind because they improve the soil quality by their action. Earthworms ingest organic material and facilitate the redistribution of crop residues and organic matter throughout the soil profile (Timothy *et al.,* 1999). In the Indian subcontinent earthworms are represented by 509 species in 67 genera under 10 families (Julka, 1993). Vermiculture is gaining popularity as a means of converting various organic wastes into fertilizer.

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This earthworm-mediated decomposition process could provide new opportunities for improving soil fertility in India. Soils in the region are highly weathered and traditional strategies for soil fertility management such as fallowing are not practical, considering land and population pressures. The situation is exacerbated by the rapid decomposition of organic matter and the fact that farmers often do not apply enough organic fertilizer to maintain soil organic matter pools. Ironically, urban centers generate a waste stream that is mostly organic byproducts that could be recycled and reapplied to agricultural lands, but these wastes are often disposed in a haphazard manner that contributes to poor urban sanitation and public health. There is an urgent and compelling need to implement organic waste recycling technologies, such as vermicomposting. There is very little information on the feasibility of vermicomposting in the India context, although this technology could be a way to transform organic wastes into a fertilizer that could maintain or build soil organic matter pools. (Bhatnagar and Palta, 1996).

Introducing vermicomposting in locales where the technology has not previously been tested comes with its own set of challenges. The most important inputs (earthworms and organic wastes) have to be selected and managed with care. Earthworms appropriate for vermicomposting can be imported at a significant cost, although at the risk of local ecology. In most cases, earthworms do not even survive the voyage. Local earthworms may be used for vermicomposting, but they must be present and capable of transforming organic waste into bio-fertilizer in a reasonable amount of time. Organic wastes also have to possess characteristics (i.e. moisture, temperature and pH) conducive to the activity of the earthworm selected. Finally, there are social issues that need to be addressed, especially concerning farmers' perceptions of the technology and their willingness to use vermicompost as a fertilizer. Many innovative solutions to India's soil fertility problems have been rejected because local farmers were not included in the research and development stages. Success therefore hinges on the inclusion of farmers' wisdom in the research process. (Ismail, 1997). Earthworms are generally classified as saprophages, but based on their feeding habits they are classified into detritivores and geophages (Lee, 1985). Detritivores feed at or near the soil surface. They feed mainly on plant litter or dead roots and other plant debris in the organic matter rich surface soil horizion or on mammalian dung. These worms are called "humus formers" and comprise the epigic and anecic forms. *Perionyx excavates, Lampito mauritii, Eisenia fetida* and *Octochaetona serrata* are a few examples of detritivorous earthworms.

Earthworms are originally distributed in equatorial West Africa, presently found distributed in most parts of the world having been introduced for various purposes in vermin technology by vermiculturists. In parts of America and Europe, this species is more commonly and popularly known as "Night Crawler". In India, it is found to be common in many vermiculture establishments, particularly in Southern India. Since 1930, this species is reported to be distributed in Travancore, Pune and North Konkan areas. Edaphic factors such as soil moisture, pH, aeration, soil texture and organic matter determine the distribution of earthworms (Waters, 1951; Barley, 1961; Kaleemurrahman and Ismail, 1981 and Ismail, 1997). Soil temperature and moisture directly affect the rate of cocoon production, incubation time and hatching rates in earthworms (Dash and Senapati, 1980, 1982). Anecic earthworms which are predominantly responsible for making vertical burrows, cast liberally on the soil surface especially in compost soils (Habibullah and Ismail, 1985; Krishnamoorthy, 1989). It is interesting to note that surface casts play a significant role in soil profile development. Earthworm casts develop as soil aggregates by forms of gums those results from microbial digestion of organic components (Waksman and Martin, 1939) or by the binding effects of fungal hyphae (Parle, 1963a). Earthworms to a large extent alter soil porosity and it has been reported by (Wollny, 1980) that soils with earthworms show an increase in soil air volume from 8% to 30%. These characteristics relate earthworms to water infiltration and water holding capacity of soils. The hope of maintaining local varieties of anecic earthworms in difficult environments has been realized through *L. mauritii*. This species cannot only be maintained easily but also can be bred and cultured in sands and sandy soils amended with easily 5 available local inputs. Such cultures do not only serve as insitu soil conditioners but also gradually support plant growth (Anne Grace, 1996).

Earthworms affect the chemical composition of soils and are responsible for the distribution of plant nutrients and their transport, because they consume large quantities of surface litter and organic material. Earthworms process the transport of organic matter to the sub-surface layer of the soils and during this process much matter is ingested, macerated and execreted. Earthworms however seem to favour nitrification (Keller, 1983; Puh, 1941). And they increase bacterial population (Parle, 1963b) and soil aeration (Heymons, 1923; Anstett, 1951). As the protein content of earthworm tissue is high (Alawdeen and Ismail, 1986), on decomposition it also yeilds nitrate nitrogen (Russell, 1910). (Lee, 1983) summarized the influence of earthworms on soil nitrogen and nitrogen cycling. According to him nitrogenous products of earthworm metabolism are returned to the soil through casts, urine, mucoprotiens and dead tissues of earthworms. Other sources of nitrogen include decaying roots, excreta from invertebrates, dead plant and animal material and nitrogen fixation by microbes. Ammonia which forms a large proportion of the nitrogenous matter excreted by earthworms (Cohen and Lewis, 1949) may cause a temporary rise in the soil p^H. Earthworms however, are more sensitive to the components, which are required to make a particular pH than to the pH as such (Magdoom and Ismail, 1986). The C: N ratios of casts are; however, a little higher than that of the surrounding soil (Lavelle, 1978) as the quantity of carbon and nitrogen execrated in the cast is sometimes very large (Syers *et al*., 1979).

Earthworms feed on soil and organic waste resources like vegetable wastes, leaf litter, agro waste, fish waste and other biodegradable substances. As they pass through the gut these get mixed with enzymes and are egested as casts. Casts of earthworms alter the physical, chemical and biological properties of soils. Antibiotic substances produced by actinomycetes in the intestine of earthworms can inhibit the growth of fungi and gram positive bacteria and may explain why some actinomycetes and antibiotic resistant gram negative bacteria predominate in the gut (Ravasz *et al*., 1986; Kristufek *et al*., 1993). Casts are usually rich in ammonia and partially digested organic matter and thus provide a good substrate for the growth of microorganisms. Some of the intestinal mucus secreted during passage through the earthworm gut is egested with the casts where it continues to stimulate microbial activity and growth (Barois and Lavelle, 1986). There is more recent evidence that the microbial communities and activity associated with contents of fresh earthworm casts begins to change quite soon after the casts are deposited by the worms.

Earthworms have a major role in the breakdown of organic matter and the release and recycling of nutrients. They remove partially decomposed plant litter and crop residues from soil surface, ingest it, fragment it and transport it to the subsurface layers. The casts tend to be much more microbially active than the surrounding soil (Parle, 1963 a, b) and have plant nutrients in a form that can be readily utilized. The final process in organic matter decomposition is known as humification and this is basically the breaking down of large particles of organic matter into a complex amorphous colloid containing phenolic materials. Anecic earthworms, which are predominantly responsible for making vertical burrows, cast literally on the soil surface, especially in 7 compost soils (Habibullah and Ismail, 1985).

There are also reports that certain metabolites produced by earthworms may be responsible to stimulate plant growth (Gavrilov, 1962; Nielson, 1965). First of all, earthworm gut performs a unique environment subsystem of soil environment. The earthworm gut has stable conditions different from surrounding environment: permanent anoxia, the pool of free amino acids, organic acids, alcohols, sugars and of hydrogen, the products of organic oligo and polymer degradation (Karsten, and Drake, 1995; Horn *et al*., 2003). The gut mucosa could be used as an environment and nutrition by microorganisms and gut-derived enzymes of earthworms could affect the microbes on the soil particles (Brown, 1995). On the other hand, the earthworm is migrating in the soil and thus soil (or another substrate as leaf litter or manure composts) flows through earthworm gut. In the gut of the earthworms *Aporrectodea caliginosa* and E. *fetida* the microbial composition was changed towards the increasing numbers of non-spore forming bacteria and decreasing numbers of spore-forming bacteria, which also resulted in enhanced levels of nitrogen fixation (Tereschenko and Naplekova, 2002). A number of novel N2O –producing species of bacteria from genera *Dechloromonas (Betaproteobacteria), Flavobacterium (Cytophaga-Flavobacteria* group *Bacteroidetes*), and *Paenibacillus* (class Bacilli) were isolated from the gut of *A. caliginosa* (Horn *et al*., 2005).

Earthworms are extremely important in soil formation, principally through activities in consuming organic matter, fragmenting it, and mixing it intimately with soil mineral particles to form water-stable aggregates. During feeding, earthworms promote microbial activity by an order of magnitude, which in turn also accelerates the rates of breakdown and 8 stabilization of humic fractions of organic matter. Different species of earthworms do not affect soil formation in the same way because of very different behavior patterns. Some species consume mainly inorganic fractions of soil, whereas others feed almost exclusively on decaying organic matter. They can deposit their feces as casts either on the soil surface or in their burrows, depending on the species concerned, but all earthworm species contribute in different degrees to the comminution and mixing of the organic and inorganic components of soil and decrease the size of not only organic particles, but also mineral particles (Shrickhande and Pathak, 1951; Joshi and Kelkar, 1952).

Earthworms are only one of the numerous range of burrow organisms which improve soils (Pereira, 1993). Fertile soil harbours bacteria, fungi, actinomycetes, protozoans, proturans, symphylids, pauropods, spring tails, pseudoscorpions, insects and their larvae, millipedes, centipedes, slugs, snails, snakes, rodents and earthworms. This fauna along with diverse flora contribute to soil quality. Among the meso and macrofauna, earthworms seem to be the most soft-bodied animals living in the soil. Others either have a thick cuticle or some form of exoskeleton or protective device for their defense in the rather difficult subterranean ecosystem. So, the presence of earthworms in soils is indicative of the presence of other faunal representatives contributing to soil fertility. Earthworm species in a particular soil can also be indicators of the soil type and its properties (Lee, 1959; Ghilarov, 1965; Kaleemurrahman and Ismail, 1981). It would therefore be certainly appropriate to describe the earthworm as an indicator of soil health and referred as the 'pulse' of soil health. Healthier the pulse, healthier the soil. Soil quality is affected by soil aggregates and these aggregates often determine the retention and movement of water, diffusion of gases, growth and development of roots in the soil (Gupta and Sundararaman, 1991) and also provide large surface areas to which plant nutrients adhere (Reganold *et al.,* 1990). The capacity of the soil to hold such nutrients is an indication of the potential fertility of the soil (Pereira, 1993). Soil organic matter is an essential component of the soil and, in association with the soil fauna, contributes to soil fertility. Soil faunal density and diversity is also partly due to the desired C: N availability in soils. Mulching promotes and supports the density and diversity of a wide range of soil organisms. Sources of carbon in the form of cellulose, for example, are mainly contributed by plants and hence forests have a natural mulch cover during the fall to protect the soil and its inhabitants for the rest of the seasons. Mulching also has the added advantage of keeping the soil cool, thus preventing evaporation and erosion. According to Edwards and Lofty (1977), earthworms seem to consume very large amounts of litter, and the amount they turn over seems to be more dependent on the total amount of suitable organic matter available rather than on other factors.

The earthworms feed on fairly large amount of protein material from decomposing plant and animals. An adult *Glossoscolex giganteus* (Leuckart, 1836), a terrestrial Oligochaete, found in South Brazil ranges from 30 to 35 cm in length when contracted but when narcotized can measure more than 90 cm long. The worm is cylindrical, with a diameter of 1.5-1.8 cm, with a slightly larger diameter in the segment near to the clitellum. Because of its larger size and high amount of food consumption and composting ability, this is the first Oligochaete subjected to the biochemical and physiological studies (De Jorge *et al.,* 1965). Most of the studies carried out to evaluate the chemical composition of earthworms deal with the general, *Lumbricus, Polypheretima* and *Allolobophora,* all belonging to Lumbricidae (Laverack, 1963).

These include works of Bahl (1947) on *Pheretima posthuma* and of Heidermanns (1937) on *Lumbricus terreritis.* Needham's works of 1957 and 1960 dealt, respectively, with nitrogenous excreta and a rginase activity in *Lumbricus terrestris* and *Eisenia fetida*. Osmatic relations in earthworms were studied by Ramsay (1949) in *Lumbricus terrestris* and De Jorges *et al.* (1965) in *Glossoscolex giganteus* (Leuckart). In recent years the work on chemical composition of earthworms are carried out by Nguekam 1993; in *Eudrilus eugeniae,* Segbesan and Ugwukumba (2008) in *Hyperiodrilus euryauloz* and Md. Hasanuzzaman *et al.* (2010) in *Perionyx excavates.* Earthworms have been found to be a good source of protein (Guererro, 1983; Hilton, 1983; Tacon *et al.,* 1983 Kostecka and Paczka, 2006; Segbesan and Ugwumba, 2008) and its usage as fish bait it well known (Segun, 1978, Omorinkoba *et al.,* 1985). Earthworms, because of their high protein component, are fed to chickens, pigs and rabbits and as dietary supplement for fish species (Akiyama *et al.,* 1984; Stafford and Tacon *et al.,* 1985; Sabine, 1986; Mason *el al.,* 1990). Among 36 earthworm species found in Bangladesh, *Perionyx excavates* an epigeic earthworm is almost found throughout the year and is a suitable potential species for feeding roosters and fished (Ali, 2002). The high reproductive rate and biomass production of this tropical earthworm species makes it ideally suited for fish meal production (Edwards and Niederer, 1988).

Incorporation of the earthworms in fish/animal feeds can be considered as Non-conventional feeds. Such earthworm incorporated feeds are not usually common in the markets and are not the traditional ingredients used for commercial fish feed production (Devendra, 1988; Madu *et al.,* 2003). Due to their non-competitiveness, such feeds prove cheaper. The relationship of soil and earthworms are interrelated. Earthworms are important soil organisms much appreciated by fishermen, gardeners and agriculturists. Role of earthworms in agriculture is well known and several workers have studied the various aspects of the impact of earthworms on the agro eco system. (Dash 1978, Senapathi and Dash 1982). Earthworms increases available nutrients in soil they aerate and stir the soil which allows better water infiltration and earlier root penetration. The beneficial influence of earthworms in promoting soil structure and fertility is well known. Recelty their potential value in waste management and as a some of protein of animal feed has been recognized. This has prompted various researchers to conduct further studies on earthworm biology.

The distribution of earthworms is controlled by various soil factors like physico-chemical and availability of food. The sensitivity of earthworms to chemical stimulation has ecological significance. It influences their choice of habitat (Arrhenius, 1921: Bodenhelimer, 1935; Satchell, 1955). Sense organs which react to chemical stimuli are found on the prostomium or the buccal epithelium which comes into contact with substances when the buccal chamber is everted. The reactions of earthworms to salts, acids bases and their distribution in the soil in relation to pH have also been documented by Gates (1978). The world's best immediate hope for rapid increase in food and fiber production lies in wise use of fertilizers, which can increase yields worldwide by at least 50 percent (Hannah, 1978). In recent years, the easy availability and low price of chemical fertilizers have led to rapid increase in their use. Soil fertility has become one of the most important jargons of a conventional agronomist (Balaraman, 2005). This term has been directly correlated to fertilizers. The traditional concept to evaluate the soil had been its quality or health. Soil health is a more appropriate term as it reflects the entire system and not just the chemical status of the soil. Soil health includes the physical and chemical characteristics of the soil and also the biotic components of the soil. It is the "living" soil. Though multitudes of soil organisms are related to soil health, earthworm is the pulse of the soil. Thus, healthier the pulse, healthier the soil.

Earthworms are generally called the biological indicators of soil fertility. Since they support the healthy populations of bacteria, fungi, actinomycetes, protozoans, insects, spiders, millipedes and a host of others essential for sustaining a healthy soil (Sharma, 2003). Earthworms improve the soil in several ways. Earthworm acts as an aerator, crusher, mixer, grinder, chemical degrader and biological stimulator in soil (Murugappan, 2005). They mix organic matter with mineral soil, release nutrients and make available to the plants, aerate the soil and improve infiltration of water through burrowing and contribute to the formation of stable soil aggregates, producing the crumbly texture of a fertile soil by the intimate mixing of organic matter, microorganisms, mineral soil and secretions from the worm skin and gut (Ramesh *et al*., 2000). Earthworms are well known to help the soil in respiration, nutrition, excretion and stabilization. They cause tunneling, show buffering action, regulate soil 18 temperatures and thus stimulate useful activity of aerobic microorganisms (Kannaiyan and Lilly, 1999).

MATERIALS AND METHODS

In the present study electricity production wood waste (fire wood ash) with different concentration have been employed to study the effect of wood ash on biochemical and histological changes in the megacolecid earthworm, *Lampito mauritii*, a dominant species in the agricultural fields collected from cheyyar taluk, Thiruvannamalai District.

Experimental Animal

The earthworm *Lampito mauritii* collected from the Sandy Loam soils of Cheyyar Taluk, were maintained in the dark in native soil with about 20 g % moisture. Worms chosen for the experiment measuring of 15±2 cm in length with an average weight of 0.8 gm. After estimating the LC_{50}. The sublethal concentration 250 gm per 1kg of soil was used for culturing of experimental animal. The earthworms are grouped in to two (group I and group II). Each Group contains 50 No. of earthworms in a pot. Group I is called control earthworm (untreated) maintained in the native soil with cowdung. Group II is called experimental earthworm treated with fire wood ash (sublethal concentration) along with soil and cowdung. The experimental period was one month. After one month the earthworms from both control and experimental group were collected and dissected out the fore region(foregut), middle region(midgut) and hind region(hindgut) for biochemical studies.

Biochemical Study

Biochemical studies were carried out in control and fire wood ash treated experimental group of earthworms to know the content of protein, Lipid and glycogen, concentration. The estimation of the total protein was done by method described Lowery *et al.* (1951). The total lipid content was estimated by the method of Zollner and Kirsch (1962). The estimation of total glycogen was made by Glucose – Anthrone method as per Carrol *et al.* (1956). Tissue weighing 500 mg was taken from the anterior part, middle part and posterior part of the earthworms so as to cover the clitellar region for the estimation of chemical contents. The analysis was made five times for each group during the experimental period and the average value is presented.

RESULTS

Biochemical parameters

Control and Experimental group of Earthworms (*Lampito mauritii*) (fore region, Middle region and hind regions) the biochemical parameters are observed in the entire region (Foregut, Midgut and Hindgut). The results are given in Table1, Table 2 and Table 3, Figure 1, Figure 2 and Figure 3.

Protein content

The protein, Lipid and glycogen content of the earthworm tissues showed greater variation in control and Experimental group of Earthworms. The average protein content in control group showed 17.4±0.24 µg/mg in fore region, 32.7±0.16

µg/mg in middle region and 12.6±0.10 µg/mg in hind region. The protein content was significantly reduced from 17.4±0.24 to 6.4±0.10 µg/mg in for region. From 32.7±0.16 to 15.5±0.12 µg/mg in middle region and from 12.6±0.10 to 11.3±0.011 µg/mg in hind region respectively in experimental group of earthworm treated with firewood ash. The results are given in Table 1 and Figure 1.

Lipid content

Likewise the lipid content was also showed variation in control and experimental group of earthworms. The lipid content in control group showed 11.4±0.22 µg/mg in fore region, 11.5±0.17 µg/mg in middle region and 5.5±0.15 µg/mg in Hind region. But in the case of experimental group treated with electricity production waste (fire wood ash) showed slightly increased lead of lipid content from 11.4±0.22 to 15.02±0.15 µg/mg in fore region, slightly decreased from 11.5±0.17 µg/mg to 8.2±0.23 µg/mg in middle region of significant reduction of Lipid content from 5.5±0.15 to 0.6±0.09 µg/mg in hind region. The results are given in Table 2 and Figure 2.

Glycogen Content

The glycogen content in both control and experimental group of earthworms are given in Table 3 and Figure 3. From the table the glycogen content in control group showed 24.9±0.22 µg/mg in fore region, 19.7±0.10 µg/mg in middle region and 29.0±0.28 µg/mg in hind region. In control group among the regions absorbed the hind region showed high amount of glycogen content but in the case of experimental group showed remarkably reduced level of glycogen content in the entire region. (11.2±0.23 µg/mg in fore region, 4.9±0.27 µg/mg in middle region and 6.0±0.22 µg/mg hind region). The reduced level of glycogen content in all the regions of experimental group of earthworms where may be due to fire wood ash treatment.

Table 1.Shows the amount of protein (µg/mg) present in different region (Forgut, Midgut and Hindgut) of control and experimental group (treated with electricity production waste wood ash) of earthworm *(Lampito mauritii)*

Sl. No	Organ	Protein Concentratin (µg/mg)	
		Co006Etrol	Experiment
1	Foregut	17.4±0.24	6.4±0.10
2	Midgut	32.7±0.16	15.5±0.12
3	Hindgut	12.6±0.10	11.3±0.11

X±SD

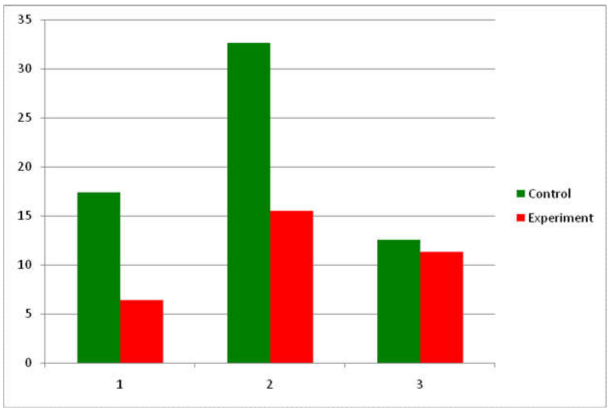

Figure 1.Shows the amount of protein (µg/mg) present in different region (Forgut, Midgut and Hindgut) of control and experiment group (treated with electricity production waste fire wood ash) of earthworm *(Lampito mauritii)*

Table 2. Shows the amount of Lipid (µg/mg) present in different region (Forgut, Midgut and Hindgut) of control and experimental group (treated with electricity production waste fire wood ash) of earthworm *(Lampito mauritii)*

Sl. No	Organ	Lipid Concentratin (µg/mg)	
		Control	Experiment
1	Foregut	11.4±0.22	15.02±0.15
2	Midgut	11.5±0.17	8.2±0.23
3	Hindgut	5.5±0.15	0.6±0.09

X±SD

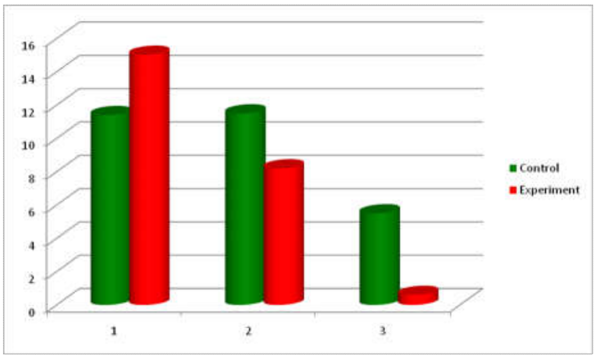

Figure 2. Shows the amount of Lipid (µg/mg) present in different region (Forgut, Midgut and Hindgut) of control and experimental group (treated with electricity production waste fire wood ash) of earthworm *(Lampito mauritii)*

Table 3. Shows the amount of Glycogen (µg/mg) present in different region (Forgut, Midgut and Hindgut) of control and experimental group (treated with electricity production waste fire wood ash) of earthworm *(Lampito mauritii)*

Sl. No	Organ	Glycogen Concentratin (µg/mg)	
		Control	Experiment
1	Foregut	24.9±0.22	11.02±0.23
2	Midgut	19.7±0.10	4.9±0.27
3	Hindgut	29.5±0.28	6.0±0.22

X±SD

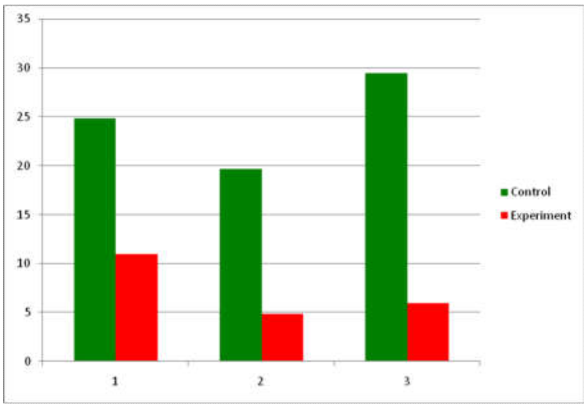

Figure 3. Shows the amount of Glycogen (µg/mg) present in different region (Forgut, Midgut and Hindgut) of control and experimental group (treated with electricity production waste fire wood ash) of earthworm *(Lampito mauritii)*

DISCUSSION

There is an worldwide upsurge of revaluation for forest biomass as an sustainable energy resource. Statistics says that we have eight million tons of wood waste annually in Japan. It partly derivers from sawmill, but mainly from demolition and construction process.

These wastes are chipped and utilized as pulping material, reprocessed material (e.g. particle board) and alternative energy resource to half amount produced. The reset of half are burnt uselessly. Energy purpose is the dominant usage, but, the fatal problem appears when wood is burnt in the direct burning boiler system. The problem is plenty of clinker. Ash contains potassium oxide, much, which lowers the fusing point of ash that produces clinker, difficult to deal with. Wood contains many kinds of mineral at certain balance that accelerate the plant's growth, but modern agricultural land lacks it. In direct burning system, burnt at high temperature, minerals become clinker, insolubles, which cannot assimilate to plants or soil microorganism. Minerals are to be the treasure, but currently it's only useless waste.

One of the benefits of earthworm culture is the production of a valuable protein source (Lieberman, 2002). It is used a good nutritional feed for live stock and fish production. The growth and weight of two groups of earthworms and the tissue components (protein, lipid and glycogen) vary from control and experiment and from region to region. Mason *et al.* (1990) and Pennino *et al.* (1991) have observed that this variation is proximately associated with specific ecology, food, season, life style, reproductive state, etc. From the results it is clear that the control group showed highest protein, lipid and glycogen content when compared to experimental group of earthworms treated with firewood ash. Protein content is considered to be the building material and involved in the alteration of almost every physiological function. It is always proportional to the growth of worm. The protein content, and reproductive activities of control group*(Lampito mauritii)* are highest in fore region. When the growth and reproductive activities of the worms are gradually reduced in experimental group. The protein content is also reduced in experimental group.

The information regarding the variation of protein, lipid and glycogen content due to fire wood ash containing high amount of potassium oxide. And protein content of earthworm is scanty. However, a comparative account on protein among control and experimental group. It is highest in foregut region and lowest in hind region. (Guerrero, 1983; Sogbesan *et al.,* 2008; Md. Hasanuzzaman *et al.,* 2010). Edwards (1985) and Ghatneker (1995, 2000) reported that the dry matter of an earthworm body contains 60 to 70% protein, 7 to 10% fat, 8 to 20% carbohydrate, 2.3% minerals and variety of vitamins. In the present study the variation in protein content is made it is highest in fore region slightly reduced in hind region in two groups of earthworms. But it is drastically reduced in all the region of experimental group treated with fire wood ash the variation of protein content is mainly due to the effect of fire wood ash because it contains high amount of potassium oxide. The lipid content of both groups of earthworms is reported to vary. The results are in agreement with those of Sogbesan *et al.,* (2008) and Guerrero, (1983). Lipid content is mainly responsible for reproductive activity of earthworm this lipid content was changed in experimental group due to fire wood ash treatment leads to decrease in the reproductive activity. The glycogen content in the tissue indicates the energy source for the metabolic activities including reproduction. There are reports that the dry matters of earthworm body contain 8 to 20% carbohydrate (Edwards, 1985; Ghatneker, 1995, 2000). In the present study the glycogen content of the body is found to be highest in fore region lowest in hind region, which is proportionate with the rate of growth of the worms and their reproductive activities. It appears that biochemical studies on the composition of the earthworms in relation to different regions. It is interesting to note that a number of animals are used as a protein source by the human populations in some regions of the world. The earthworm species *Andiorrhinus motto* and *Andiorrhinus kuru,* commonly referred as *motto* and *kuru*, respectively are known to be widely consumed in Venezuela. The analysis of the whole body of the earthworms contain large amount of proteins (64.5% and 72.9% dry weight), essential amino acids, lipids, carbohydrates and minerals, indicating that these worms contain potentially useful quantities of nutrients that are critical to the health of human beings (Paoletti *et al.,* 2003). Md. Hasanuzzaman *et al.,* (2010) worked on the nutritional composition of wild earthworm *Perionyx excavates* and found that it contains 46.57±0.97%, protein and 8.03±0.44% lipid. They concluded that this earthworm species has almost similar nutritional values to fish meal and thus would be an animal protein in supplementing fish meal. Further, they opined that year round production of this earthworm species through standard mass culture system and its radical use could be pivotal role in sustainable fisheries and aquaculture production. In all the above mentioned reports, the biochemical composition study of earthworms has been aimed towards its food value. Therefore, it can be concluded that earthworm can be used as replacement for fish meal and also a potential source of protein essential amino acids, lipids, minerals and trace elements. Therefore, they are widely consumed in Venezuela (Munnoli *et al.,* 2007). This nutritional value of earthworm is altered by fire wood ash treatment.

The changes in the biochemical parameters like protein lipid and glycogen may reflect in the histology also. The histology of foregut region, midgut region and hindgut region showed normal configuration with body wall, cuticle, epidermis, musculature, gizzard and coelom. But in the case of experimental group of earthworm treated with electricity production waste of fire wood ash disturbed the normal histology when they feed. The fire wood ash even though contains high amount of minerals but it contains the toxic substance called potassium oxide. This chemical accumulates in body tissue and reduce the protein, lipid and glycogen content. These reflect in the histology. The fore region, middle region and hind region of experimental group are totally damaged due to potassium oxide accumulation. The changes in the histology by a chemical substance may lead to toxicity of vermiculture and also vermicompost.

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