IMPACTS OF *Lumbricus terrestris* (LINNAEUS, 1758) IN THE VERMICOMPOSTING BED OF *Eisenia fetida* (SAVIGNY, 1826)

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Submitted to
Central Department of Zoology
Institute of Science and Technology
Tribhuwan University
Kirtipur, Kathmandu

DECLARATION

I hereby declare that the work presented in this thesis has been done by myself and has not been submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to the author(s) or institution(s).

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RECOMMENDATION

This is to recommend that the thesis entitled "Impacts of *Lumbricus terrestris* (Linnaeus, 1758**)** in the vermicomposting bed of *Eisenia fetida* (Savigny, 1826)" has been carried out by Shreeram Ghimire for the partial fulfillment of Master's Science in Zoology with special paper Ecology and Environment. This is his original work and has been carried out under my supervision. To the best of my knowledge, this thesis work has not been submitted for any other degree in any institutions.

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LETTER OF APPROVAL

On the recommendation of supervisor "Dr. Tej Bahadur Thapa", this thesis submitted by Shreeram Ghimire entitled "Impacts of *Lumbricus terrestris* (Linnaeus, 1758**)** in the vermicomposting bed of *Eisenia fetida* (Savigny, 1826)" is approved for the examination and submitted to the Tribhuwan University in partial fulfillment of the requirements for Master's Degree of Science in Zoology with special paper Ecology and Environment.

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CERTIFICATE OF ACCEPTANCE

This thesis work submitted by Shreeram Ghimire entitled "Impacts of *Lumbricus terrestris* (Linnaeus, 1758**)** in the vermicomposting bed of *Eisenia fetida* (Savigny, 1826)" has been accepted as a partial fulfillment for the requirements of Master's Degree of Science in Zoology with special paper Ecology and Environment.

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ABSTRACT

Vermicomposting is being used by farmers to produce high quality organic manure for farm practices in Nepal. *Eisenia fetida* is generally used in vermicomposting process in a windrow bed just above soil. Direct contact of vermicomposting bed with soil always opens possibility of mixing of anecic worms such as *Lumbricus terrestris* from soil. The study was conducted with the prime objective of assessing impacts of such mixing of *L. terrestris* in vermicomposting bed of *E. fetida* comparing monoculture and polyculture beds. Two polyculture treatments were designed as one with 33.33% and another with 50% *L. terrestris*. It focused on comparing nutritional value of vermicompost and population growth of *E. fetida* with proportion of *L. terrestris* in each bed. Nitrogen, Potassium and Phosphorus were analyzed by using Kjeldahl method, Flame photometry method and Vanadate-molybdate method respectively while population of *E. fetida* was counted manually. Rates of both population growth and biomass growth were compared between monoculture and polyculture treatments.

Correlation coefficient analysis showed proportion of *L. terrestris* had strong negative (-0.94) relation with nitrogen content, moderate negative (-0.78)relation with phosphorus content, weak positive (0.39) with potassium content and weak negative (-0.15) with population of *E. fetida* . p-value of correlation showed significant correlation ($p<0.05$) of proportion of *L. terrestris* with nitrogen and phosphorus content of vermicompost but not significant correlation with final population of *E. fetida* and potassium value of vermicompost. Average population multiplication rate of *E. fetida* was 39.63 times in monoculture bed followed by 38.98 times and 39.35 times in two polyculture bed respectively. Estimated nitrogen content, phosphorus content and final population of *E. fetida* were 186.27% (2.92% in monoculture and 1.02% in polyculture), 15.52% (0.67% in monoculture and 0.58% in polyculture) and 1.12% (813 in monoculture and 804 in polyculture) higher in monoculture bed compared to polyculture bed but potassium was 2.56% (1.17% in monoculture and 1.20% in polyculture) lower in monoculture bed.

The study indicated that *L. terrestris* greatly affected nutritional value of vermicompost rather than population growth process based on facts that nitrogen percentage reduced when *L. terrestris* was introduced in vermicomposting bed of *E. fetida*. Monoculture bed was better than polyculture bed for vermicomposting in terms of quality of vermicompost and population growth of *E. fetida*. So, contamination of *L. terrestris* in the vermicomposting bed of *E. fetida* should be avoided to preserve nutrient value of vermicast produced by *E. fetida* and population growth of the species. Microbiological and other chemical parameters like heavy metal concentration should be studied to investigate more impacts.

1 Introduction

1.1 Background

1.1.1 Earthworm

Earthworm is one of major group of phylum Annelida living in soil rich in moisture and organic content. Among diverse families of earthworms, lumbricidae and eudrilidae family have greater importance in agriculture due to their ability of improving soil quality (Gajalakshmi and Abbasi, 2003). Charles Darwin has mentioned earthworm as nature's ploughman in his book Earthworms in 1881 (Satchell, 1983; Feller *et al.*, 2003). Earthworms need moist environment essential for their life processes and to meet moisture need, they hibernate during dry season (Gates, 1961).

Earthworms are cylindrical in shape with body divided into muscular segments making them easier for movement. Digestive tract runs through entire length of body with gizzard as major digestive organ where enzymatic activity accelerates and several chemical changes initiate (Curry and Schmidt, 2007). Coelomic fluid secreted by earthworm is antipathogenic in nature (Cameron, 1932) and helps in lubrication during movement in burrows (Zhang *et al*., 2016). Earthworms are hermaphrodite animals. After fertilization, a mucous covering is shed to soil which ultimately develops into cocoon. Cocoon gives hatchlings without clitellum which later becomes clitellate one.

Some earthworms use organic matter while other use minerals and humus present in the soil. So, based on soil strata, the variability of earthworm species occurs according to phyto-geo-chemical status (Reich *et al.*, 2005). In other way, it is accepted that biological activity of soil is modified by presence of earthworm like other fauna (Satchell, 1983). Some earthworms act by producing feces (epigeics) while other affect by mucous lining in burrows (anecics) (Binet and Curmi, 1992). Earthworms induce microbial behavior of soil by increasing population of microbes (Loquet *et al*., 1977). Microbial activity stabilizes soil nutrition and more than that microbes provide life to soil and earthworms generally make soil suitable for microbes (Lee, 1985). Earthworms not only affect microbial status of soil but also directly affect soil organic carbon cycle by increasing carbon storage in soil and decreasing carbon turnover (Don *et al.*, 2008), therefore, population of earthworm plays significant role in global carbon emission to atmosphere. Interaction of plant and earthworm is surprising fact of nature where earthworm largely affects plant growth and above all, lumbricid worms are important for plant nitrogen intake (Springett and Gray, 1997). Earthworm is also source of nutrient to various animals like Badger (Kruuk and Parish, 1981). Earthworms are also affected by modernization of farming practices such as use of chemical fertilizers and tillage practices. Use of chemical fertilizers largely alters abundance of earthworms (Bengtsson *et al.*, 2005) and use of modern tillage practices also decreases earthworm abundance by 2-9 times (Chan, 2001).

The invasive property of earthworms has been largely studied in the world. Bohlen *et al.* (2004) reported that forest of North America has been invaded by earthworm species from Europe and Asia affecting soil C and N along with effects on understory plant species where some herbal species are disappearing from forests. Urban areas are more vulnerable to invasion of exotic species in compare to less disturbed areas (Winsome *et al.*, 2006).

1.1.2 Diversity of Earthworm

Carolus Linnaeus in 1758 firstly described *Lumbricus terrestris*, after which nearly 26 new earthworm species are added annually in the list with estimation of more than 6000 species in the world (Reynolds, 1994). But Edwards and Lofty (1972) estimated 1800 species while Bouche (1977) estimated 3000 species of earthworm around world. Zoological Survey of India (2016) have mentioned 21 new species recorded inside Indian territories between 100 years of survey from 1916 to 2015. Nepal being one of rich country in biodiversity, earthworm diversity of Nepal is an unanswered question yet (Tamrakar, 2005).

Diversity of earthworm is largely affected by geological features, plant diversity and climate (Stojanovic *et al.*, 2013). Bouche (1977) classified earthworms into three different ecological groups: epigeic, anecic and endogeic. Epigeic and anecic earthworms mainly live on fresh or little decomposed plant residue which live above mineral soil in litter layer (epigeic species) or which are collected and concentrated in middle at the entrance of their vertical burrows (anecic species) (Marhan and Scheu, 2005). Of the three main categories of earthworms – epigeics (humus feeders, surface dwellings), anecics (geophytophagous, soil dwelling which construct vertical tunnels) and endogeics (geophagous, soil-dwelling which construct horizontal branching burrows) – the epigeics are most suited to vermicomposting (Abbasi *et al.*, 2009). Among families of Oligochaeta, Eudrilidae and Lumbricidae have greater economic value for soil enhancement (Fragoso *et al.*, 1997).

1.1.3 Vermicomposting

Tompkins and Bird (2004) described application of earthworm in soil management specially in enhancing soil fertility and soil pest management started before the time of Christ and they mentioned some species like *Eisenia sp.* and *Bismatus sp.* as soil builder. Vermicomposting is a simple and low cost, an environment friendly biotechnology system for the processing or treatment of organic wastes (Hand *et al.*, 1988), in which certain species of earthworms are used to accelerate the breakdown of organic matter and stabilization of soil aggregates (Dindal, 1985) to enhance the process of conversion of waste to a useful byproduct. Earthworms in vermicompost produce casts which are fine and look like tea grains (Jayakumar *et al.*, 2009). Since vermicast contains water-soluble nutrients, it is an excellent, nutrient-rich organic fertilizer and soil conditioner (Ravichandran *et al.*, 2001). The vermicomposting process includes two distinct phases regarding the activity of earthworms: (i) an active phase during which earthworms process the organic waste, thereby modifying its physical state and microbial composition (Lores *et al.*, 2006), and (ii) a maturation- like phase marked by the displacement of the earthworms towards fresher layers of undigested waste, during which the microbes take over the decomposition of the waste processed by the earthworms (Aira *et al.*, 2007). Vermicomposting technique is applied from urban areas as tool for municipal waste management to rural areas as tool for high quality organic manure production useful in organic farming. Vermiculture is becoming famous and receiving considerable attention in recent years internationally for its potential role in organic farming and sustainable development.

Monoculture and polyculture practice of vermicomposting is familiar among researchers. *Eisenia fetida*, *Perionyx excavatus* and *Eudrilus eugeniae* are prescribed for better result from polyculture practice while *E. fetida* is regarded as good species for monoculture practice (Suthar and Singh, 2008; Khwairakpam and Bhargava, 2009; Hayawin *et al*., 2014). Some researchers have also mixed some anecic species like *Lampito mauritii* with epigeic species (Suthar and Singh, 2008) where they found better result in polyculture bed rather than from monoculture bed.

1.1.3.1 Worms used in Vermicomposting

Lumbricid worms inhabit in variable climate ranging from cold sub-temperate climate to tropical hot climate. The worms such as *Eudrilus eugeniae* (Kinberg) are applied in tropical and evergreen warm climatic region while worms such as *Eisenia fetida* are useful in region with turbulent climatic features due to its better adaptive ability (Gajalakshmi and Abbasi, 2003). *E. fetida* is one of the species widely used for this purpose due to its property easy-to-raise. The *E. fetida* can decompose organic waste, excrete the so-called worm cast and synthesize earthworm biomass (Kumar *et al.*, 2010). *E. fetida* can use different solid wastes as foods, including legume litter, sewage sludge, activated sludge, rabbit manure, cattle manure, pig manure, and sheep manure (Garg *et al.*, 2006). *E. fetida* is commonly known as: the “compost worm”, “manure worm”, “red worm”, and “red wriggler” which has the capacity for very rapid reproduction and can be expected to double every 60 to 90 days, but only if the following conditions are met (Munroe 2011):

- Adequate food (must be continuous supply of nutritious food)
- Well aerated bedding with moisture content between 70% and 90%
- Temperatures maintained between 15 and 30 ^{o}C

Anecic worms are also used in vermicomposting. Some practitioners use *Lampito mauritii* by mixing with other epigeic species (Suthar and Singh, 2008). Another anecic worm *Lumbricus terrestris* is not used in vermicomposting bed but it is abundant in top soil of Kathmandu valley. *Lumbricus terrestris* basically feeds on post decomposing leaf litters and humus rich soil (Wright, 1972). Ehlers (1975) recorded this worm in surface of soil with living in vertical burrows with poor efficiency of digestion of fresh organic matter and poor reproduction compare to other epigeic species recorded by Wright (1972).

1.1.3.2 Bed of vermicomposting

Vermicomposting is practiced on the top soil layer by making bed of gray colored cellulose rich materials like hay, leaf litters, saw dust, rice bran etc. with properties like high absorbency, good bulking potential and high C: N ratio to provide stable habitat to worm (Munroe, 2011). Bed can be made at land called windrow technique or above ground called raised bed (Gajalakshmi and Abbasi, 2003). Windrows range from 1 to 2.5 m wide and can be as long as 0.5 km which requires a well-drained soil as a base, or a sloped concrete pad to prevent

accumulation of water and anaerobic decomposition at the bottom of the windrow (Edwards, 1998). Possibility of contamination of other worms present in soil to vermicomposting bed remains open in Windrow method (Lim *et al.,* 2015). Some researchers claimed the bedding materials like leaf, paper, saw dust etc. as major cause of attraction of anecic worms in bed of epigeic worms (Edwards, 1988; Gajalakshmi and Abbasi, 2003; Munroe, 2011).
Bottom of bed is always filled with cellulosic matters like saw dust, rice bran, coconut fiber, leaf litter, jute or paper with 1 inch to 4 inches thickness depending on size of vermicomposting plant. Strong cellulosic fiber takes long time to decompose and absorbs unwanted moisture. This bottom bedding materials make warm in cold season and cool hot environment providing uniformity in quality of bed.

1.1.3.3 Nature of food for Earthworms in vermicomposting

Epigeic worms are widely used in vermicomposting due to their voracious nature and rapid production of cast (Ravindran *et al.*, 2014). Cast production and feeding potential depend on length of alimentary canal (Ibrahim *et al.,* 2016). Shorter the size of alimentary canal, cast will pass out faster and vice-versa. Epigeic worms like organic rich food materials. Researches in Rothamsted experimental station, United Kingdom have shown that various animal wastes like poultry wastes and cattle wastes can be converted to organic manure by use of earthworms with more efficiency in cattle dung (Edwards and John, 1992). Sharma *et al.* (2005) have found cattle manure as ideal medium for vermiculture practice. Fine particle size of cattle manure is better for digestion which improves growth rate and fecundity of earthworm (Lowe and Butt, 2003). Nutrient content of feed is also an important aspect because earthworms prefer Protein and Carbohydrate rich material (Satchell, 1967). Cow manure is perfect for worms in terms of protein and carbohydrate combination. Hartenstein and Hartenstein (1981) have recorded 1 kg cow manure per kg worm as best rate of input with highest output in terms of cast production and fecundity.

1.1.4 Vermicomposting scenario of Nepal

Vermicomposting is being widely talked and discussed among local people of Kathmandu valley with the objective of reducing household wastes and producing manure for rooftop garden by Non-Governmental Organizations and Governmental organizations (Tamrakar, 2005). On the other hand, Nepal government has started initiative of subsidies in vermicompost among farmers since 2008 (Pokhrel and Panta, 2009). Some commercial farms are producing vermicompost in Terai region but exact figure of vermicompost production and consumption around Nepal has not been assessed yet (Devkota *et al.*, 2014).
Farmers are using windrows bed to produce vermicompost where they recorded a lot of anecic worms like *L. terrestris* in vermicompost. So mixing of these worms has developed deep concern over farmers but the effect of mixing of naturally occurring worms like *L. terrestris* is not assessed yet.

1.2 Statement of the Problem

There is vast disparity between demand and supply of fertilizer in Nepal where government import chemical fertilizer from India and other countries (Shrestha, 2010). So, people are practicing vermicompost technology as alternate source of fertilizer required for farming practices (Pradhan and Tamrakar, 1999). Exotic worms such as *E. fetida* is used in vermicomposting but native anecic worms are also recorded in the bed. Farmers and entrepreneurs of vermicomposting are unaware of such contamination of *L. terrestris* in bed of *E. fetida*. Population of *E. fetida* increases by two to four folds within three months of time under normal condition (Loh *et al.*, 2005). But if *L. terrestris* is mixed, the interaction between these worms create impacts on multiplication and also on quality of vermicompost. So, the impacts on multiplication of *E. fetida* and quality of vermicompost should be identified and the mixing of anecic worms in the bed of epigeic and exotic worms needs assessed.

1.3 Objectives

General objective of this study was to determine the impacts of *L. terrestris* on population multiplication of *E. fetida* and vermicompost quality produced by *E. fetida*. Specific objectives were:

(i) To assess the population growth of *Eisenia fetida* in different treatment

(ii) To determine nutrient quality of vermicompost in different treatment.

(iii) To examine quality of bed in terms of worms multiplication and quality of compost.

1.4 Justification

Farmers in Nepal are using traditional windrow method of vermicomposting as usual method above top soil layer using *Eisenia fetida* (epigeic worm) as major species to produce vermicast (Baral *et al.*, 2012). Top soil in Nepal is abundant with *L. terrestris* (anecic worm) which is recorded in bed of epigeic worms by farmers. *L. terrestris* is humus lover and easily moves to bed of *E. fetida*. On the other hand, several researchers have used other anecic species like *Lumbricus rubellus* in vermicomposting process in polyculture bed with epigeic species like *E. fetida*. As *L. terrestris* is abundant in Nepalese soil, its interaction with epigeic worms need to be known by vermicomposting concerned people. This study has tried to simplify these two queries of impacts on vermicomposting and nature of interaction with epigeic species. In this study, native species of earthworm like *L. terrestris* has been cultured below the bed of exotic epigeic species like *E. fetida* and impacts has been studied. Soil and leaf litter was used to make bed for *L. terrestris* just below the bed of *E. fetida* made of saw dust and cow dung. Impacts were measured in terms of NPK value of vermicompost and multiplication rate of epigeic worms used in vermicomposting. When anecic worms were introduced in the bed of epigeic worms, rate of multiplication of *E. fetida* i.e. population growth was not significantly affected. Chemical analysis of vermicompost showed that nitrogen value of vermicompost has been largely negatively affected and phosphorus affected less in the bed with higher number of *L. terrestris* while only potassium value has been positively affected. So for betterment of vermicomposting method, mixing of native species specially *L. terrestris* should be avoided to preserve population growth rate and quality of vermicast produced by *E. fetida*.

1.5 Limitations

This study has used *L. terrestris* only as anecic species. Due to limitation of technical resources, microbial and other chemical parameters like heavy metal content in the vermicompost sample was not carried out.

2 Literature Review

2.1 Diversity and Ecology of Earthworm

Earthworm is a soil macro fauna responsible for providing life to soil affecting physical, biological and chemical parameters. Among 23% constituent of total living organisms of this globe as soil organisms (Decaens *et al.*, 2006), earthworms are larger players to make soil specific in nature according to specific group of earthworms present there (Loranger *et al.*, 1998). With estimation of earthworm diversity being 1,800 species by Edwards and Lofty (1972), based on habitat ecology, this invertebrate has been classified into three categories as: epigeic, anecic and endogeic. Epigeic earthworms live above soil surface and are phytophagous. Epigeics don't play any role in changing soil structure. Anecic earthworms are geophytophagous and live in vertical burrow but never live in too depth as they feed on dead leaves dragging in burrows. They come out from burrow at night. Endogeic earthworms are purely geophagous and live in horizontal burrow in considerable depth (Bouche, 1977; Gajalakshmi and Abbasi, 2003; Marhan and Scheu, 2005). Earthworms are becoming problems too for example; native species of earthworms are replaced by exotic species (Hendrix and Bohlen, 2002) affecting soil ecosystem of that place. In these regards, ecological roles of earthworms have been greatly revealed by studies and are taken as one of most important aspects of ecosystem (Edwards, 2004).

2.2 Economic Importance of Earthworms

With emergence of biotechnology as modern science, earthworms and their application are discussed extensively. Applications are studied in fields like organic manure production, poultry (Khan *et al.*, 2016), fish (Pucher *et al.*, 2014) and piggery feed ingredients, biomedical value (Bergé and Vulliet, 2015), etc. Organic fertilizer production by using earthworms is widely used around world. It is a low-cost technology with better output for farmers and for reducing solid wastes of municipalities (Dindal, 1985; Ravichandran *et al.*, 2001; Baral *et al.*, 2012). Three major characters like voraciously feeding, high rate of reproduction and high affinity of feeding any type of biodegradable organic waste, epigeics are used extensively in vermicomposting (Gajalakshmi and Abbasi, 2003; Abbasi *et al.*, 2009). Major six species are used such as: *Eisenia fetida, Eisenia andrei, Perionyx excavatus, Perionyx fovatus, Eudrilous eugeniae,* and *Lumbricus rubellus*. In context of Nepal, *Eisenia fetida* is used in majority of commercial farms for vermicomposting purpose (Pradhan and Tamrakar, 1999; Baral *et al.*, 2012) and it is exotic species for Nepalese soil (Tamrakar, 2005).

Vermicompost is used as major source of nutrients for plants in organic farming, hydroponics, aquaponics, and plant nurseries and even in fish culture to increase phytoplankton production. Vermicompost is converted to vermitea and is used as major substrate in aquaponics system to introduce and increase nitrogenous bacterial population in the system (Kumar and Singh, 2001). Kaur and Ansal (2010) has recorded higher fish yield in a pond supplied with vermicompost compare to pond supplied with cow manure. Vermicompost application in soil is familiar among organic farmers which reduces pest infestation in plants and other soil borne diseases (Edwards *et al.*, 2004).

Eisenia fetida, due to its ability of digestion, adaptation to local climate and fast reproducing efficiency, is widely used in vermicomposting (Mitchell, 1997; Suthar, 2009). Tamrakar (2005) compared nutrient (NPK) content of cast produced by exotic species (*Eisenia fetida*) with local species (*Perionyx fovatus*) and found higher value in cast of exotic species. Anecics worm are considered as geo-phytophagous consuming leaf litters and soil minerals (Hendriksen, 1990) while epigeics worms like *E.fetida* prefer food with more organic matter like kitchen waste and cow dung. Hendriksen (1991) observed that both epigeics and anecics worms ingest food in the rate of their body weight per day in moderate condition showing greater importance to reduce carbon emission from organic waste by digesting kitchen waste and municipal wastes.

2.3 Population Dynamics of Earthworms

Dominguez *et al.* (2000) fed sewage sludge mixing with paper and cardboard dust to *Eisenia andrei* and recorded cocoon production rate of 3 cocoons per earthworm per week. Similarly, Bhattacharjee and Chaudhury (2002) estimated cocoon production rate per year for 7 tropical earthworm species of India and found highest rate in *Perionyx excavatus* (156 cocoon per worm per year) with highest rate of hatchling success (53%) compared to other anecic worms. Edwards (1988) recorded highest value (3.3) of mean hatchlings per cocoon for *E. fetida* among other epigeic species by feeding animal, agricultural and industrial wastes. Before that, Hartenstein *et al.*, (1979) studied reproductive potential of *E. fetida* by feeding horse manure and recorded 500 progenies from 8 worms in 300 cc density with ages 5-27 weeks. Venter (1988) studied lifecycle of *E. fetida* and recorded 121 cocoons per worm per year with 1 to 9 hatchlings per cocoon (2.7 offspring per cocoon in average) having 73% success hatchling rate of cocoons. Some researchers studied ecological interaction between different earthworm species to find factors responsible for population change. Capowiez (2000) studied interspecific and intraspecific relation between different ecological groups of earthworms. He studied burrowing behavior and movement of different species where he recorded different extent of length and size of burrow along with different pattern of use of burrow. Zirbes et al. (2010) studied intraspecific interaction between individuals of *E. fetida* by setting olfactometer who recorded common movement of worms in a common drilosphere based on influences to each other favoring cooperation. Jegou *et al.* (2001) studied interaction between *Lumbricus terrestris*, *Aporrectodea giardi* and *A. caliginosa* in terms of burrowing activity and found that burrow length was larger along with more branching made by *L. terrestris* when paired with other.

2.4 Nutrient Value of Vermicast

Garg *et al.* (2006) recorded high nitrogen value in vermicast produced by *E. fetida* when fed with textile sludge compared to institutional waste (majority component as paper) and kitchen waste. Bansal and Kapoor (2000) studied vermicompost quality produced when fed with mustard residues, sugarcane trash and cattle manure and did not find significant difference in NPK values. Loh *et al.* (2005) studied NPK value of vermicast produced by *E. fetida* fed with cattle and goat manure and found high nitrogen value in vermicast fed with cattle manure and high phosphorus and potassium value fed with goat manure. Fosgate and Babb (1972) used *L. terrestris* in vermicomposting of cow manure and found less NPK value compared to *E. fetida* and very less juveniles were recorded. Kladivko (1993) described feeding nature of anecic worms such as *L. terrestris* found in north temperate hemisphere which pulls plant residues and even fecal matter of some epigeics several centimeters below surface and feeds after softening by microbes. Kladivko (2001) studied tillage relation with soil macro fauna abundance in soil and found *L. terrestris* as one of major macro fauna with greater mobility and significant soil drilling properties. Another aspect of *L. terrestris* attraction on humus rich material is presence of fungi. Edwards and Fletcher (1988) studied interaction of micro fungi and earthworm and found positive interaction as earthworms largely feed upon micro fungi. The phenomenon has been largely proved by study of Tiunov and Scheu (2000) who have recorded large portion of mycoflora after examination of casts. Study of Tiunov and Scheu (2000) revealed fungi loving properties of *L. terrestris* who studied burrow wall of earthworms which usually construct its wall with fecal matters and Coelomic fluid. Yami *et al.* (2003) studied vermicast and gut of *E. fetida* after 2 months of feeding this worm with agricultural and kitchen wastes who recorded 9 different fungi species including 3 actinomycetes species. So *L. terrestris* is attracted by cast of *E. fetida* rich in fungi.

Daniel and Anderson (1992) studied gut content of *Lumbricus rubellus* and found that the microbial biomass and microbial respiration increased in cast of worms indicating increased bacterial activity in gut which in turn affects NPK value of cast. Fischer at al. (1995) also studied gut content of *L. terrestris* who recorded highest activity of bacteria between foregut and hind gut. Aira and Dominguez (2009) studied bacterial activities in gut of epigeic and anecic earthworm who recorded higher consumption of bacteria by anecic worms to reach their nutrient need while epigeics had higher bacterial count in casts. Another study of Edwards and Lofty (1972) mentioned about time of food passage from gut which showed 4-5 times slower for *L. terrestris* in compare to *E. fetida* concluding vast difference in bacterial digestion in gut in these two worms. Van Gansen (1962) studied function of food tube of *Eisenia fetida* and found that nitrogen absorption increased on going towards posterior end due to bacterial and enzymatic activities. Edwards and Fletcher (1988) studied microbial and earthworm interaction extensively. They fed *E. fetida* with 18 different bacteria and recorded highest yield. They concluded that earthworm activity is largely affected by bacterial activities in terms of nutrition in casts and growth of worms. Another study of Trigo *et al.* (1999) about mutualism between earthworm and micro flora showed higher percentage of gut mucus in epigeics than anecic earthworms. Sivasankari (2016) studied vermicompost to identify nutritional importance where he recorded role of hormones like Indole-3-acetic acid which promotes growth of plant directly and promotes germination multiplying effect of NPK value.

2.5 Comparative Study of Monoculture and Polyculture Bed

Loehr *et al.* (1985) studied monoculture and polyculture bed to find temperature and moisture range suitable for process. *Dendrobaena veneta, Eisenia fetida*, *Eudrilus eugeniae, Perionyx excavatus* and *Pheretima hawayana* were used which were fed with municipal waste sludge. *E. fetida* produced large number of young worms after 20 weeks of experiment but polyculture produced less compare to monoculture beds. Suthar (2008) studied polyculture and monoculture vermicomposting bed feeding municipal sewage sludge. He used *E. fetida* (epigeic) and *Lampito mauritii* (anecic) in polyculture bed and *Eisenia fetida* in monoculture bed. Polyculture bed had better result in terms of nutrient value of compost and enzymatic and bacterial activity. Khwairakpam and Bhargava (2009) studied polyculture bed against monoculture bed of epigeic worms. They used *E. fetida*, *Eudrilus eugeniae* and *Perionyx excavatus* for polyculture bed along with individual species for monoculture bed feeding sugarcane filter mud. They found no any specific differentiation between different cultures. Munnoli and Bhosle (2011) studied water holding capacity of vermicompost produced in monoculture and polyculture beds. *E. fetida*, *Eudrilus eugeniae* and *Megascolex megascolex* were used. Water holding capacity of vermicompost of monoculture bed of *Megascolex megascolex* was higher than all other monoculture and polyculture beds.

3 Materials and Methods

3.1 Materials

Following materials were used for study.

- Plastic tray (32 c.m.*27 c.m.*6.5 c.m.)
- Jute cover
- Cow dung
- Leaf litter
- Saw dust
- Sprayer
- Digital weighing machine
- Forceps
- Hand lens

3.2 Experimental site

The experiment on monoculture (*E. fetida*) and polyculture (*E. fetida* and *L. terrestris)* vermicomposting was conducted in the laboratory of the Central Department of Zoology, Tribhuwan University from 2016 March 28 to 2016 May 28.

3.3 Data Collection Methodology

3.3.1 Experimental design

The experiment consisted of the Randomized Block Design (RBD) to compare impacts of monoculture and polyculture systems in terms of impacts of *L. terrestris* on the *E. fetida* with three replications. Three treatments were used as first with monoculture of *E. fetida*, second and third with polyculture bed containing *E. fetida* and *L. terrestris* in the ratio of 66.67/33.33 and 50/50 % respectively all consisting 3 replications (Table 1, Table 2).R1, R2 and R3 referred to three consecutive replications of three treatments T1, T2 and T3 respectively.

Table 1 Treatments for Monoculture and Polyculture bed

Treatment no.	**Treatment detail Number/Proportion(%) of each worm**		**Total number of worms**
	E. fetida	*L. terrestris*	20
T1	20/100	0/0	30
T2	20/66.67	10/33.33	40
T3	20/50	20/50	

Table 2 Lay Out of the Experiment

T1R1	T1R2	T1R3
T2R1	T2R2	T2R3
T3R1	T3R2	T3R3

T1R1 – T1R3: monoculture bed with 100% *E. fetida*
T2R1 – T2R3: polyculture bed with 66.67% *E. fetida* and 33.33% *L. terrestris*
T3R1 – T3R3: polyculture bed with 50% *E. fetida* and 50% *L. terrestris*

3.3.2 Experiment

E. fetida (Plate 1) and *L. terrestris* (Plate 2) were used in this study because the *E. fetida* is extensively used in vermicomposting in Nepal while *L. terrestris* is abundant in Kathmandu valley (Tamrakar, 2005). *E. fetida* was purchased from local private farms while *L. terrestris* was collected from Coronation Garden of Tribhuwan University. *L. terrestris* was collected in garden by spraying white mustard powder solution and soap solution (Lawrence and Bowers, 2002).

Plate 1 *Eisenia fetida*

Plate 2 *Lumbricus terrestris*

Poly-culture bed (*E. fetida* and *L. terrestris*) was made by soil and leaf litter while mono-culture bed (*E. fetida*) was made by saw dust (Plate 3). For each culture, bed of 2-inch thickness was made.
E. fetida was fed with cow dung in the proportion of their weight collected from nearby cattle farm after moistening with water (Hendriksen, 1991) in the interval of 10 days. *E. fetida* in first bed was 18 grams and 1080 grams of cow dung was fed for 60 days. Same input was given to all other beds. *L. terrestris* was fed with leaf litter and soil.

In polyculture setup, *L. terrestris* was introduced in the bed and after they disappeared from surface, cow dung was spread and then *E. fetida* was then introduced (Plate 4). But in monoculture, *E. fetida* was introduced above cow dung in the bed. In both cases, beds were covered with moist jute sheet (Plate 5). Cow dung was used as food for earthworm at the interval of 10 days (Plate 6) and water was used for moistening in every 2 days interval. Vermicompost, earthworms and cocoons were harvested after 60 days.

3.4 Population count

All the vermicompost of each bed was poured and spread above paper sheet. The small heaps were made and left for 1/2 hours in day light to settle down all worms in the bottom. The compost was separated thoroughly to separate cocoons and worms. *E. fetida* and *L. terrestris* were separated (Plate 7). The process was repeated for all nine beds. Mature *E. fetida* was counted first to find mortality rates (Plate 8). Juveniles are counted with the help of forceps and hand lens.

3.5 Analysis of physio-chemical properties of vermicompost

Vermicompost was harvested in May 28, 29 and 30 with reaching of two months period (Plate 9). Harvested vermicompost was analyzed to assess NPK status in Agriculture Technological Center (ATC), Pulchowk, Lalitpur.

3.5.1 Moisture content

Moisture content of vermicompost was analyzed by comparing dry and wet weight after dried at 105°C for 24 hours in hot air oven. To analyze moisture content, 100 gm vermicompost sample was taken in crucible and placed inside oven at 105°C. The sample was then cooled to room temperature in desiccators. Dried weight was determined by weighing oven-dried sample.

$$Moisture\ content\ (\%) = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}} \times 100\ \%$$ (Tchobanoglous *et al.*, 1993)

3.5.2 Nitrogen analysis

Nitrogen in the vermicompost was analyzed using Kjeldahl method (Estefan *et al.*, 2013). Kjeldahl method is based on volumetric analysis where unreacted acid is calculated against acid reacted to find how much ammonia is neutralized.

In this method, organic substance present in vermicompost is decomposed by heating with sulphuric acid to release ammonium sulphate as major indicator of available nitrogen in vermicompost. The solution is heated to make it colorless from dark black color of compost and is neutralized against sodium hydroxide to get ammonia. The amount of nitrogen was calculated in percentage as Total Kjeldahl Nitrogen (TKN) which is sum of organic nitrogen, ammonia (NH_3) and ammonium (NH_4^+).

3.5.3 Phosphorous analysis

Phosphorus present in vermicompost was analyzed by Vanadate-molybdate method (Kitson and Mellon, 1944). The sample was mixed with Sodium carbonate in the ratio of 1:5 by weight which was then dissolved in Hydrochloric acid solution and heated. Solution was then cooled and diluted to 10 times that of original solution. Diluted solution was then mixed with Vanadate-molybdate reagent in the ratio of 4:1 and then placed in the colorimeter. The concentration of phosphate was estimated in ppm (parts per million) which was then converted to percentage.

3.5.4 Potassium analysis

Potassium contents of the vermicompost was analyzed by flame photometry method (Toth and Prince, 1949). Flame photometry is based on this principle of sensitivity to light of certain wavelength where atoms become excited on getting light energy and finally emit radiation of different color. In this method, the sample was mixed with Sodium carbonate in the ratio of 1:5 by weight which was then dissolved in Hydrochloric acid solution and heated. Solution was then cooled and diluted to 10 times that of original solution. The solution was sprinkled through flame and the reading was then recorded in photometer. The concentration was obtained in mM (milimoles) which was then converted to percentage concentration.

3.6 Data Analysis

The data were analyzed using Karl Pearsonian's Correlation coefficient. The proportion of *L. terrestris* in each bed was correlated with nitrogen, phosphorus and potassium value of vermicompost to assess impacts on quality of vermicompost. Similarly, the proportion of *L. terrestris* was correlated with the final count of *E. fetida* to assess impact on population of *E. fetida* in vermicomposting.

Karl Pearsonian's Correlation Coefficient (r) = $\frac{\sum(X-\bar{X})(Y-\bar{Y})}{\sqrt{\sum(X-\bar{X})^2}\sqrt{\sum(Y-\bar{Y})^2}}$

Significance of correlation was studied using p-value calculation which was calculated with help of microsoft excel.

3.7 Bed quality

Two parameters such as quality of vermicompost and multiplication rate of population of *E. fetida* were used to evaluate bed quality. Value of correlation coefficient determined nature of impact of *L. terrestris* to *E. fetida* in terms of vermicompost quality and population growth. Impact in population growth of *E. fetida* due to presence of *L. terrestris* was evaluated by calculating multiplication rate in each bed comparing initial and final population.

$$Multiplication\ rate\ of\ E.fetida = \frac{Final\ population - Initial\ population}{Initial\ population} = \frac{y-x}{x}$$

$$Biomass\ increase\ rate\ of\ E.fetida = \frac{Final\ wight - Initial\ weight}{Initial\ weight} = \frac{b-a}{a}$$

Nutrient value of compost was obtained from NPK analysis of vermicompost.

4 Result

4.1 Rate of Multiplication of population and Biomass Increase of *E. fetida*

E. fetida population multiplied significantly ranging from 37.35 times to 40.85 times while biomass increased from 3.33 times to 4.94 times. Highest multiplication rate (40.85 times) was found in polyculture bed T3R2 followed by monoculture bed (40.7 times) T1R1. The polyculture bed T3R3 had lowest multiplication rate (37.35 times). The rate of multiplations among monoculture and polyculture bed had less variability (Table 3).
The trend in biomass increase rate for total population was similar as population growth trend. Highest growth (4.94 times) in biomass of population was found in bed T3R2 followed by bed T2R2 (4.25 times). Lowest rate (3.33 times) of biomass growth was found in bed T3R3. The variability in growth of biomass of population was less as similar to population multiplication rate (Table 3).

Table 3 Rate of Multiplication of population and Biomass Increase of *E. fetida* in different beds

Bed	Population growth				Biomass growth		
	Initial pop^n	Final pop^n	Multiplication rate (times)		Initial Weight(g)	Final Weight (g)	Biomass increase rate (times)
			Value of each bed	Average Value (± errors)			
T1R1	20	834	40.7	39.63± 0.55	18g	93g	4.17
T1R2	20	797	38.85		17g	84g	3.94
T1R3	20	807	39.35		18g	87g	3.83
T2R1	20	817	39.85	38.98± 0.44	18g	89g	3.94
T2R2	20	793	38.65		16g	84g	4.25
T2R3	20	789	38.45		18g	83g	3.61
T3R1	20	817	39.85	39.35± 1.04	17g	88g	4.17
T3R2	20	837	40.85		16g	95g	4.94
T3R3	20	767	37.35		18g	78g	3.33

Multiplication rate of *E. fetida* was highest (39.63 times) for monoculture bed (T1) followed by polyculture bed (T3) (39.35 times) and lowest value (38.98 times) was reported in polyculture bed (T2). Variability in multiplication rate among monoculture and polyculture bed was low indicating weak impact of *L. terrestris* on population growth of *E. fetida* (Figure 1).

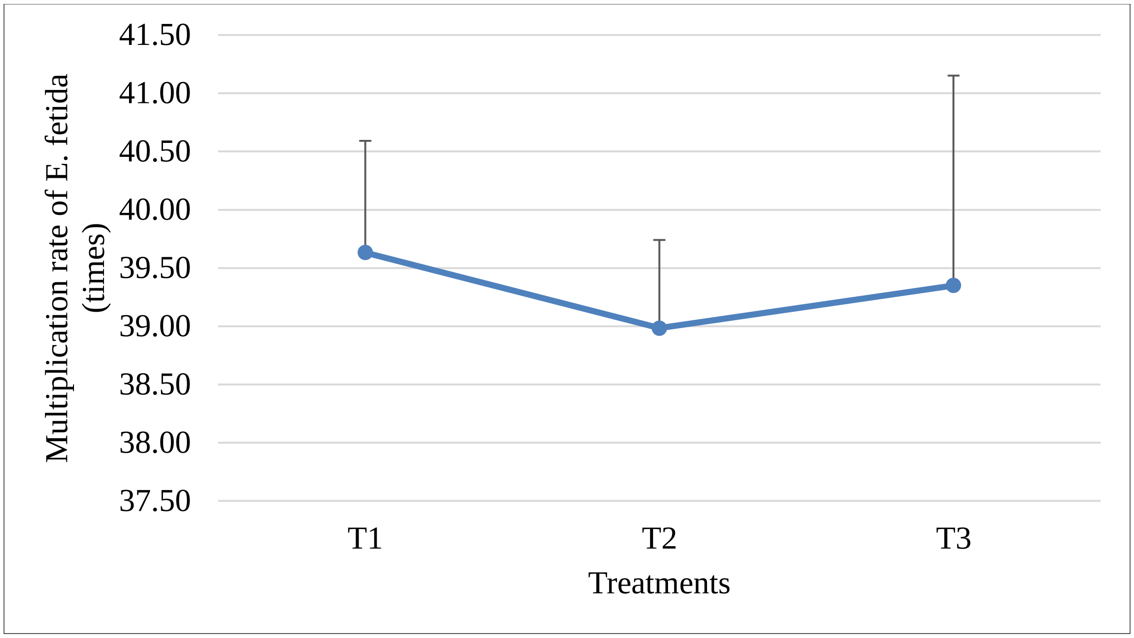

Figure 1 Multiplication rate of *E. fetida* in different treatments (s.d.± errors)

Biomass increase rate of *E. fetida* was highest (4.94 times) for polyculture bed (T3R2) followed by polyculture bed (T2R2) (4.25 times) and lowest value (3.33 times) was reported in polyculture bed (T3R3). Less difference of biomass increase rate was reported between monoculture and polyculture bed indicating less impact on fecundity of *E. fetida* due to presence of *L. terrestris* in vermicomposting bed (Figure 1).

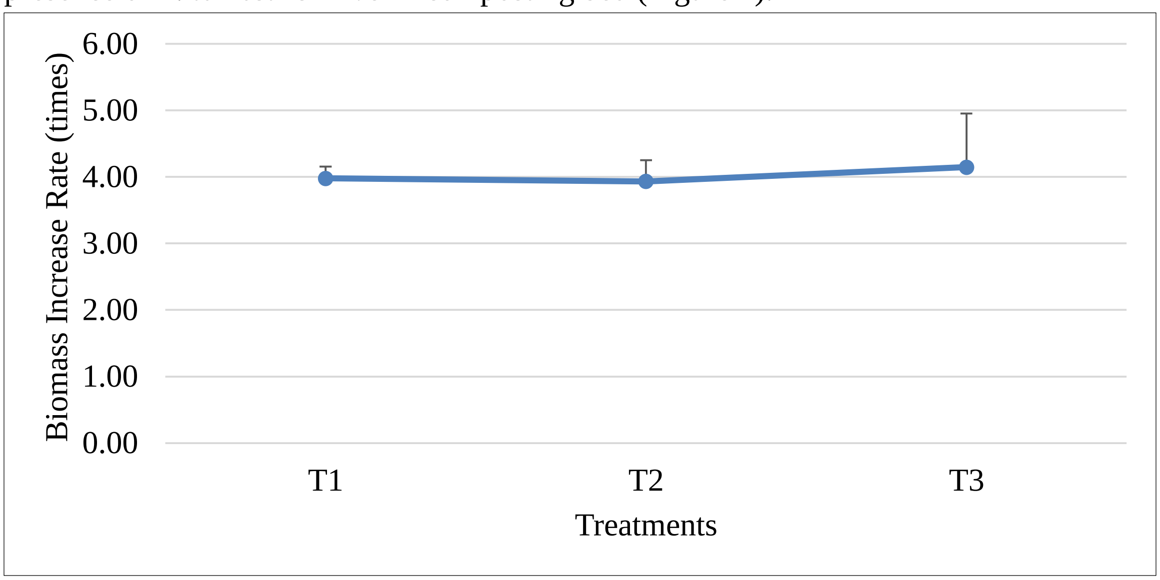

Figure 2 Biomass increase rate of *E. fetida* in different treatments (s.d.± errors)

4.2 Physio-Chemical Characters of Vermicompost

4.2.1 Moisture content (in %)

The moisture content in vermicompost samples was recorded as 64.30%, 62.62% and 62.30% in average in treatments T1, T2 and T3 respectively (Figure 3). The result revealed that moisture content was highest in monoculture bed T1 followed by polyculture bed T2.

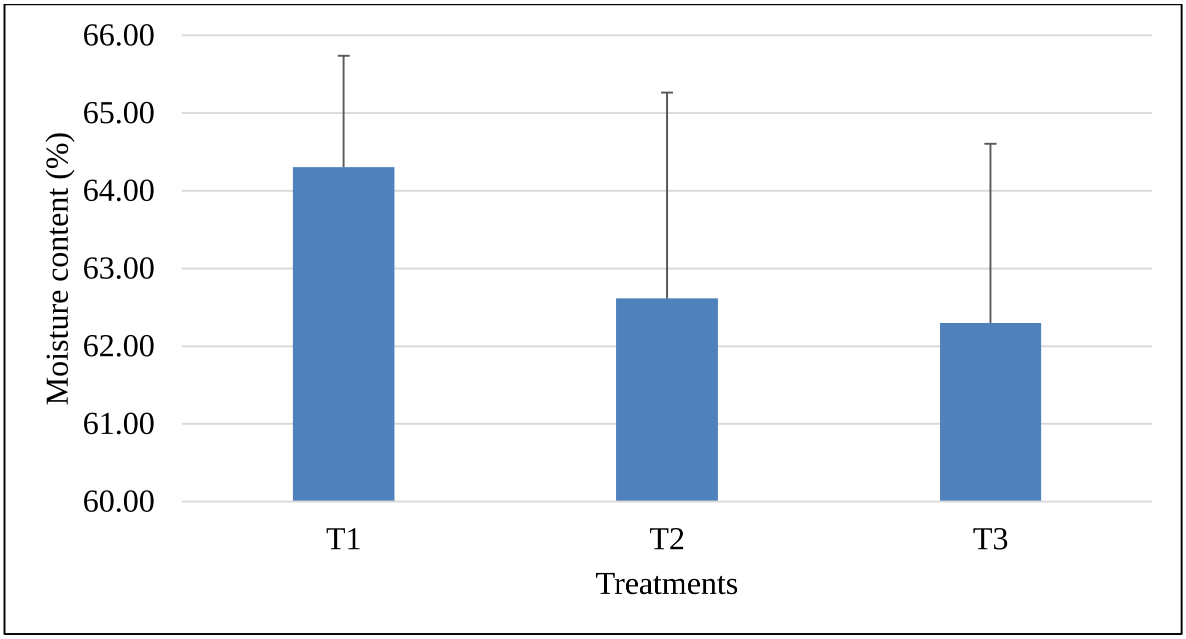

Figure 3 Moisture content of different treatments (s.d. ±errors)

4.2.2 Total Nitrogen (in %)

The total nitrogen content in harvested vermicompost from different beds were found to be 2.92%, 1.00% and 1.04% in treatments T1, T2 and T3 respectively (Figure 4). The result revealed that bed T1 (monoculture bed with *E. fetida*) has maximum nitrogen content (2.92%) followed by polyculture bed T3 (1.04%) and lowest amount (1.00%) of nitrogen was found in bed polyculture T2.

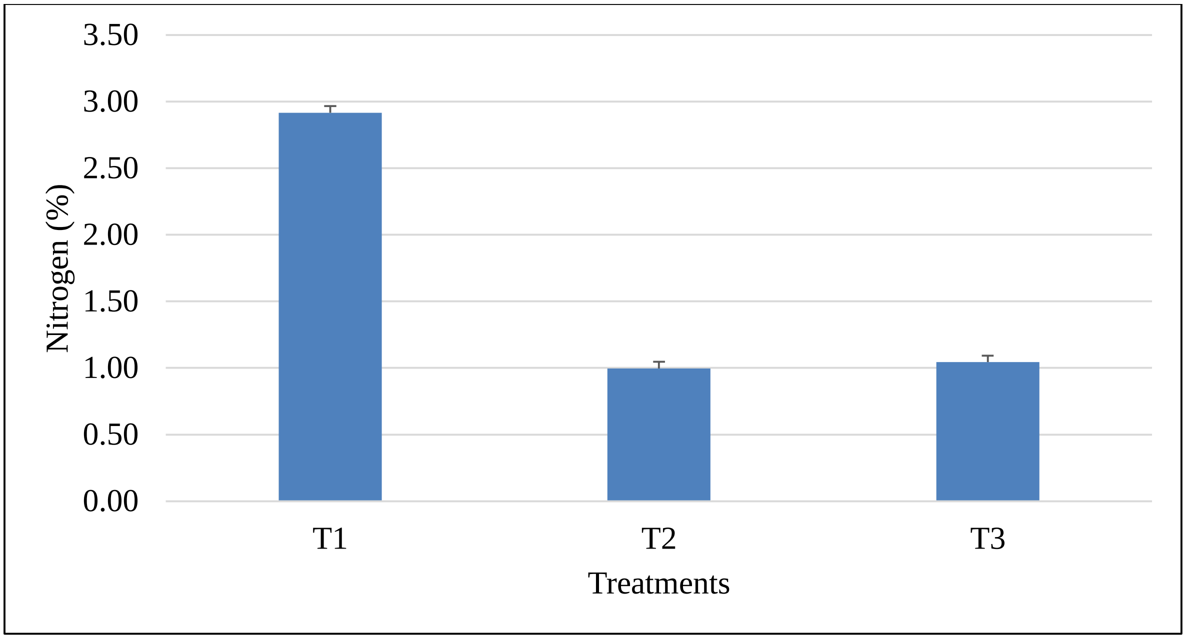

Figure 4 Total nitrogen content of different beds (s.d. ±errors)

4.2.3 Phosphorus (in %)

The phosphorus contents of harvested vermicompost from different beds were found to be 0.67%, 0.58% and 0.58% in treatments T1, T2 and T3 respectively (Figure 5). The result revealed that monoculture bed T1 has maximum phosphorus content (0.67%) followed by polyculture bed T2 and T3 (0.58%) The polyculture beds have comparatively low phosphorus content than in monoculture bed.

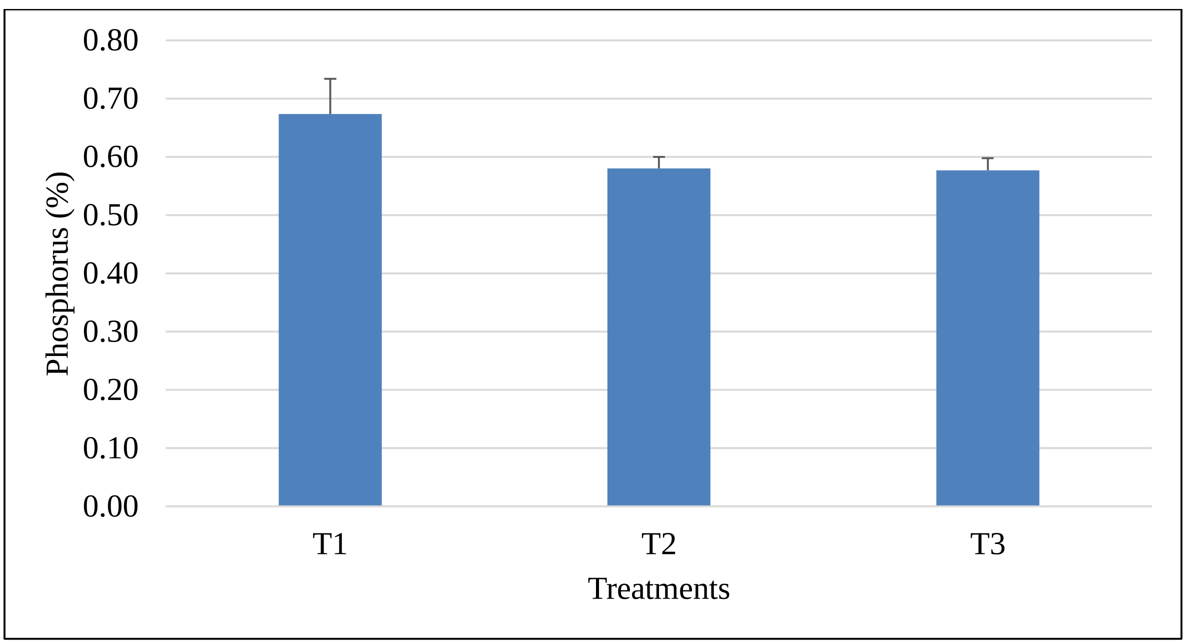

Figure 5 Phosphorus content of different types of treatments (s.d. ±errors)

4.2.4 Potassium (in %)

The Potassium contents of harvested vermicompost from different beds were found to be 1.17%, 1.20% and 1.20% in treatments T1, T2 and T3 respectively (Figure 6). The result revealed that the potassium content of polyculture treatments T2 and T3 (1.20%) are same and higher than monoculture treatment T1 (1.17%).

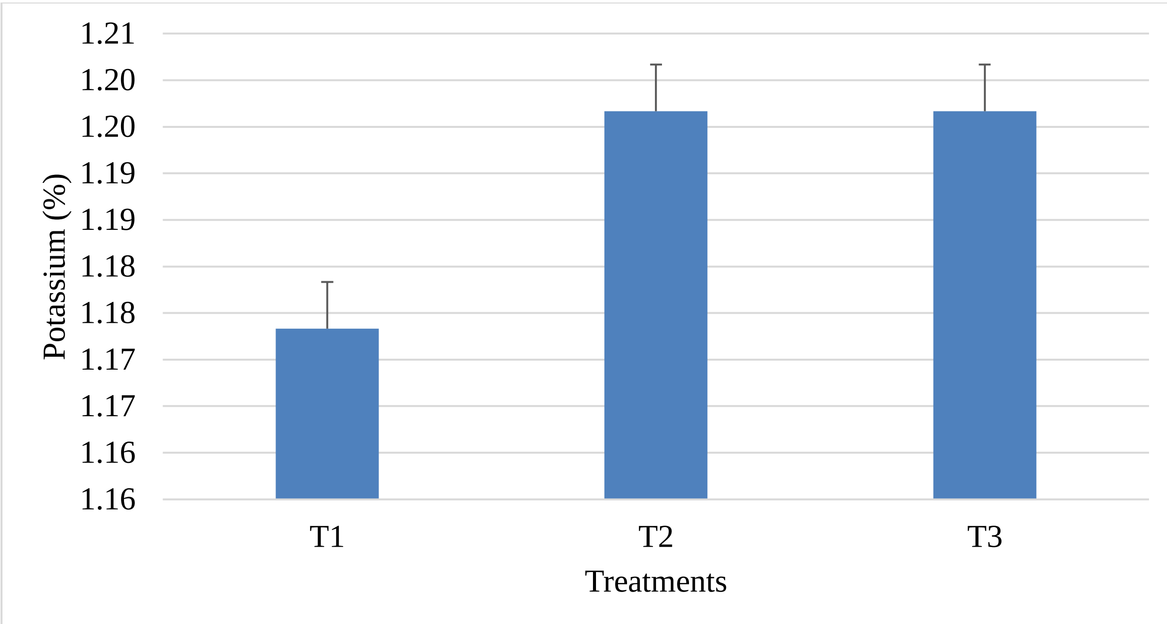

Figure 6 Potassium content of different types of treatments (s.d.±errors)

4.3 Statistical Analysis

Correlation coefficient between proportion of anecic worm and final population of *E. fetida*, was found to be weakly negative (-0.15). Similarly correlation with nitrogen percentage and phosphorus percentage of vermicompost were strongly negative (-0.94) and moderately negative (-0.78) respectively. Weak but positive correlation cofficient (0.39) was reported between proportion of anecic worms and potassium content of vermicompost (Table 4). P-value analysis showed that there was significant correlation ($p<0.05$) between proportion of anecic worms with Nitrogen and Phosphorus but not significant with final population of *Eisenia fetida* and potassium of vermicompost.

Table 4 Correlation between proportion of L. terrestris and final population counts of *E. fetida*, Nitrogen, Potassium and Phosphorus % of vermicompost

Bed	Proportion of *L. terrestris* by total population(%) (A)	Final Population of *E.fetida* (B)	Nitrogen % (C)	Potassium % (D)	Phosphorous % (E)
T1R1	0	834	2.95	1.15	0.61
T1R2	0	797	2.87	1.21	0.73
T1R3	0	807	2.93	1.16	0.68
T2R1	33.33	817	0.91	1.21	0.58
T2R2	33.33	793	1.1	1.22	0.56
T2R3	33.33	789	0.98	1.16	0.60
T3R1	50	817	1.12	1.17	0.60
T3R2	50	837	1.08	1.21	0.57
T3R3	50	767	0.93	1.21	0.56
	Correlation coefficient between A and B ($\boldsymbol{r_1}$)= -0.15, p-value=0.84 ⟷				
	Correlation coefficient between A and C ($\boldsymbol{r_2}$)= -0.94, p-value=0.0014 ⟷				
	Correlation coefficient between A and D ($\boldsymbol{r_3}$)= 0.39, p-value= 0.64 ⟷				
	Correlation coefficient between A and E ($\boldsymbol{r_4}$)= -0.78, p-value = 0.0034 ⟷				

4.4 Bed quality

Population growth rate of *E. fetida* in monoculture bed and polyculture bed showed that final population in monoculture bed was 1.15% higher than in polyculture bed. Similarly, nitrogen and phosphorus value were 65.07% and 13.43% higher in vermicompost collected from monoculture bed compared to polyculture bed. But potassium value was 1.7% low in monoculture bed compared to polyculture bed. Based on these values, nitrogen and phosphorus were highly and negatively affected due to presence of *L. terrestris* in polyculture bed indicating monoculture bed of *E. fetida* as productive bed compared to polyculture bed.

5 Discussions

Epigeic worms (example *E. fetida*) when mixed with anecic worms (example *L. terrestris)*, affects efficiency of vermicomposting process. In this study, the impacts were measured in terms of increase rate of population and biomass of *E. fetida* as one major aspect and nutrient value of vermicompost as another aspect. Population growth rate and biomass increase rate both were affected negatively by introducing *L. terrestris* but in less extent. The impact is mainly due to difference in burrow structure and niche characters of these two species of earthworms (Jegou, 1999). NPK value was also affected by presence of anecic worms in bed of *E. fetida*. Among three chemical parameters, nitrogen was highly but negatively affected due to digestion of nitrogen bacteria in large extent by *L. terrestris* in compare to *E. fetida* which affected nitrogen value of vermicompost(*Zhang et al., 2000).* Since *L. terrestris* stores large amount of protein in body tissue and is larger in size than *E. fetida*.

5.1 Population growth

Population growth rate of *E. fetida* at monoculture bed was 39.63 times with nearly same values 38.98 and 39.35 for two polyculture beds represented 1.6% and 0.7% change from monoculture population respectively. Final population of *E. fetida* decreased in small proportion (1.12%) in polyculture with *L. terrestris. L. terrestris* as anecic worm in polyculture bed with *E. fetida* was first experiment in Nepal with the studies, using *Lampito mauritii* in polyculture bed as anecic worm mixed with epigeic worms (Suthar and Singh, 2008). Gajalakshmi *et al.* (2001) recorded high difference between monoculture (*Eudrilus eugeniae*) and polyculture bed (*Eudrilus eugeniae, Perionyx excavates*, *Lampito mauritii* and *Drawida willsi*) with 40% more offspring from polyculture bed after 15 days of experiment. Similarly Suthar and Singh (2008) found 42.9% more offspring in polyculture bed of *E. fetida* and *Lampito mauritii* than in monoculture bed of *E. fetida*. The difference may be due to differences in nature between *L. terrestris* and *Lampito mauritii*. Regarding multiplication rate of *E. fetida*, similar result was found by Venter (1988) as 797 offspring produced by 20 worms in 2 months period compared to this study with 795 offspring under similar conditions.

A weak negative correlation between proportions of *L. terrestris* with final population of *E. fetida* indicates moderately negative impact on population growth of *E. fetida*. The impact probably related to the differences in burrow structure and function as well as with difference in niche structure. Similar study done by Jegou (1999) using X-Ray method concluded that the *L. terrestris* makes vertical permanent burrow structure but *E. fetida* makes temporary structures for common movement individuals. Mixing of these two species in a bed create disturbance in movement, consequently affects on the mating and cocoon production process. *L. terrestris* is highly responsive to tactile stimulus than *E. fetida* (Jegou, 1999) affecting peaceful and solitary behavior. Foraging behavior becomes more uniform and active in monoculture bed but it is affected when two different worms are mixed due to difference in preference of food (Suthar and Singh, 2008). When *L. terrestris* is mixed with *E. fetida*, it moves upward abruptly to consume freshly decomposed manure like cast of *E. fetida* rather than older manure or soil which disturbs activities of *E. fetida* (Fosgate and Babb, 1972). The differences in burrow structure, foraging behavior and food preferences create impacts on population growth but in a weaker extent which can be due to strong reproducing potential of *E. fetida*.

5.2 Nutrient value of vermicompost

Average values of NPK among all 9 beds (nitrogen 1.65%, phosphorous 0.61% and potassium 1.18%) indicate effects of the substrates that worms feed and probably to the physiological activities in gut. Edwards *et al.* (2004) reported that the oxidized and organic matter present in vermicompost plays vital role in development of balanced nutrients. Enzymatic and bacterial activities in vermicompost are essential for accelerating nutrient formation process which also suppresses parasite and nematode activities in soil (Edwards *et al.*, 2004). NPK value (nitrogen 2-3%, potassium 1.85-2.25% and phosphorus 1.55-2.25%) recorded by Sinha *et al.* (2009) is higher as the substrate used in their research was kitchen waste while cow dung was used in this study.

Among three chemical nutrients, nitrogen value decreases strongly in vermicompost collected from bed with *L. terrestris* than in monoculture bed which shows nitrogen diminishing effect of *L. terrestris* by consumption. Similar effect has been seen in case of phosphorus but less than nitrogen. Potassium was weakly but positively affected which indicates potassium is less consumed by *L. terrestris*.

5.2.1 Nitrogen

The higher value of nitrogen (2.92% in average) in the vermicompost collected from monoculture beds than in polyculture beds (1.02% in average) was due to differences in bioaccumulation of protein in body tissue (Rault *et al.*,2007), bacterial and enzymatic activities in gut (Fischer *et al.*, 1995) between two species. Monoculture bed of *E. fetida* contained 186.27% more nitrogen than that recorded in polyculture bed of *E. fetida* and *L. terrestris*. This estimation of higher concentration of nitrogen in vermicast from bed with *E. fetida* than in bed of *E. fetida* mixed with anecics worms indicate negative impact on vermicompost quality with presence of *L. terrestris*. Opposite result was reported by some researchers after using *Lampito mauritii* in polyculture bed as *L. terrestris* has been used in this study. Suthar and Singh (2008) found 20.41% higher nitrogen in vermicompost of polyculture bed (*E. fetida* and *Lampito mauritii*) than monoculture bed of *E. fetida* after feeding cow dung for 90 days. Similarly Suthar (2008) recorded increasing value of Nitrogen in casts of epigeic, anecic and polyculture bed of *E. fetida* and *Lampito mauritii* as 7.46g/kg, 7.73g/kg and 9.18g/kg respectively which shows 23.06% more nitrogen in polyculture bed than monoculture bed. But Tripathi and Bhardwaj (2004) reported 52.94% higher nitrogen value in vermicompost produced by *E. fetida* than by *Lampito mauritii* when feeding with kitchen waste for 150 days.

Bacterial and enzymatic activities are responsible for nitrogen content of vermicompost. Anecic worms like *L. terrestris* are thicker in diameter storing protein in body tissue extracting from substrate they feed (Paoletti *et al.*, 2003) compare to *E. fetida* which is shorter in length and thinner in diameter supported by study of Rault *et al.* (2007) who reported 2-3 times more nitrogen in body tissue of *L. terrestris* than other epigeic worms. In windrow bed system, *L. terrestris* extracts nitrogen to assimilate protein when it feeds on cast of *E. fetida* reducing nitrogen available in cast. The major fact of consuming vermicast of *E. fetida* by *L. terrestris* is due to less cellulose in it as cellulose digestion in gut of *L. terrestris* is poor compared to *E. fetida* to convert cellulosic substrates into organic matter (Fosgate and Babb, 1972; Aira *et al.*, 2006). So nitrogen discharge in fecal mass becomes high in *E. fetida* than in *L. terrestris*. In-situ analysis of bacteria present in gut of *L. terrestris* has shown that number of bacteria decreases towards posterior end (Fischer *et al.*, 1995) indicating decreased bacterial activity in casts of this anecic worm but bacterial population does not change instead nutrient and microbial stabilization occurs in gut of *E. fetida* (Aira *et al.*, 2009). But phosphatase and protease activities in gut of anecic worms is higher, essential for protein formation which in turn decreases nitrogen content in cast of *L. terrestris* (Zhang *et al.*, 2000). Nutrient extraction from substrates to store in body tissue, poor bacterial and enzymatic activities in gut make less nitrogen in cast produced by *L. terrestris* than that in *E. fetida*. Polyculture bed containing *Lampito mauritii* produced more nitrogen than monoculture bed in these researches dissimilar to this research which is due to differences between *Lampito mauritii* and *L. terrestris.*

5.2.2 Phosphorous

Phosphorus value of vermicompost collected from monoculture beds was 15.52% higher than in polyculture bed possibly affected by bacterial and enzymatic activities in gut. In this sense, phosphorus was moderately but negatively correlated (-0.78) with proportion of *L. terrestris* indicating negative impact on quality of vermicompost. Some international research practices have reported different result with higher phosphorus in polyculture bed using anecic species other than *L. terrestris*. Suthar and Singh (2008) recorded 9.55% higher phosphorus content in vermicast collected from polyculture bed (*E. fetida* and *L. mauritii*). But Tripathi and Bhardwaj (2004) found 36.14% more phosphorus in vermicompost produced by *E. fetida* than anecic worms when fed with cow dung separately.

Phosphorus is absorbed in gut of anecic worms as Adenosine Tri-Phosphate (ATP) storage in body tissue which is higher in *L. terrestris* due to intense protease and phosphatase activities compared to *E. fetida* (Zhang *et al.*, 2000). *E fetida* is shorter in length than *L. terrestris* which results in more phosphorus solubilizing bacterial (PSB) activities in gut resulting in higher population of PSB in cast of *E. fetida* (Kumar and Singh, 2001). So less phosphorus in vermicompost was recorded due to bacterial and enzymatic activities in gut when *L. terrestris* was mixed with *E. fetida*.

5.2.3 Potassium

Potassium value of vermicompost collected from monoculture beds (1.17% in average) was 2.56% lower than in polyculture beds (1.19% in average). The correlation (0.39) between proportion of *L. terrestris* and potassium content of vermicompost indicates weak positive

impact on potassium concentration due to presence of *L. terrestris* in vermicomposting bed affected by soil ingesting properties of *L. terrestris.* Similar result was obtained by Suthar and Singh (2008) who recorded 9.55% more potassium produced by monoculture bed of *E. fetida* and polyculture bed of *E. fetida* and *Lampito mauritii* respectively. Suthar (2007) recorded Potassium value of casts as 5.95g/kg, 6.0g/kg and 6.15g/kg produced by epigeic, anecic and polyculture bed of *E. fetida* and *Lampito mauritii* respectively feeding mixture of humus rich soil and cow dung for polyculture and cow dung only for monoculture bed. Similarly, Tripathi and Bhardwaj (2004) reported that potassium content in vermicast increased by 38% and 42% produced by *Eisenia fetida* and anecics worms respectively feeding cow manure separately.
Potassium due to its less or no role in digestion and nutrition assimilation process, it is less affected that presence in substrate worms feed upon. Suthar (2007) reported higher potassium level in agricultural wastes like cattle manure as one of reason behind higher potassium in cast even in presence of anecic worms compared to other nutrients like nitrogen and phosphorus (Suthar, 2007). *L. terrestris* feeds on soil humus as it is geophytophagous, which influences increment of potassium ion concentration in casts (Basker *et al.*, 1993), but *E. fetida* does not feed soil and it is just phytophagous. So, potassium level already present in substrate and soil inhaling properties of *L. terrestris* resulted more potassium in polyculture bed than in monoculture bed.

5.3 Bed quality analysis

Population growth rate of *E. fetida* and nutritional value of vermicompost were two parameters to analyze vermicomposting bed quality. Strong negative impact to nitrogen, moderate negative impact to phosphorus, weak positive impact to potassium of vermicompost and weak negative impact to population growth of *E. fetida* due to presence of *L. terrestris* in the bed of *E. fetida* indicates polyculture bed of *E. fetida* and *L. terrestris* counterproductive. Monoculture bed was better in terms of two basis taken in this study compared to polyculture bed different from experience of other researchers as *Lampito mauritii* was used in polyculture bed. Suthar (2008) took two criteria to identify best bed for vermicomposting as microbial activity and decomposition activity who found better performance in bed containing *E. fetida* and *Lampito mauritii* than that in monoculture bed. Another study of Suthar (2008) used two criteria to identify best reactor among monoculture and polyculture bed as nutrient analysis of vermicompost and metal concentration. He found better result of polyculture reactor containing *E. fetida* and *Lampito mauritii* fed with sewage sludge. Polyculture beds in these studies perform better result as *L. mauritii* was used as anecic worms. Nutritional value and population growth is always counted to evaluate quality of vermicomposting system by farmers. So criteria like nutrition value of vermicompost and population growth of worms are proper to analyze quality of bed.

6 Conclusion and Recommendations

6.1 Conclusion

The multiplication rate of *E. fetida* in monoculture bed was higher than that in polyculture bed. When proportion of *L. terrestris* in bed was increased to 33% to 50%, the multiplication rate decreased by little bit with weak negative correlation (-0.15) showing negative impact in population growth of *E. fetida* with presence of *L. terrestris* in vermicomposting bed affected by differences in burrow structure, response to tactile stimulus, foraging behavior and niche structure between these two species.

Similarly presence of *L. terrestris* in the bed of *E. fetida* also affected vermicompost quality. When proportion of *L. terrestris* was increased in the bed of *E. fetida*, nitrogen percentage and phosphorous percentage decreased while potassium percentage increased. Correlation between proportion of anecic worms and Nitrogen was -0.94 showing strong negative impact due to presence of *L. terrestris* in bed of *E. fetida* affected by differences between bio-accumulation of protein, bacterial and enzymatic activities in gut between these two species. Similarly correlation of proportion of *L. terrestris* with phosphorous was -0.78 showing same impact as in nitrogen affected by bacterial and enzymatic activities in gut differing in these two species. But correlation with potassium was 0.39 affected showing weak positive impact of presence of *L. terrestris* in bed of *E. fetida* affected by potassium ion consumption by *L. terrestris* present in soil.

Combining all these findings, bed quality was analyzed using two parameters as population growth of *E. fetida* and NPK value of vermicompost to identify best bed. Polyculture bed was counterproductive in terms of these circumstances compare to monoculture bed.

So contamination of *L. terrestris* in windrow bed of *E. fetida* in vermicomposting seems to be more negative rather than positive. Based on these findings, contamination of *L. terrestris* in bed of *E. fetida* should be avoided to preserve population growth rate of *E. fetida* and quality of vermicompost produced by *E. fetida.* It is concluded that Polyculture bed of *E. fetida* and *L. terrestris* is not beneficial for farmers doing vermicomposting.

6.2 Recommendations

Based on results and discussions, the following points are recommended:

- Farmers doing vermicomposting in windrow bed should check entrance of anecic worms or naturally occurring worms specially *L. terrestris* in bed by avoiding use of agricultural wastes like weeds inhabited by *L. terrestris* and by making bed clean from weeds and other wastes.
- Specific enzymes and their role in NPK value of vermicast should be studied in different species of earthworms used in vermicomposting.

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Appendices

Appendix 1 Plates

Plate 3 Bed preparation for *Eisenia fetida* using saw dust

Plate 4 Introducing *Eisenia fetida* and *Lumbricus terrestris* to bed after bed filling with cow dung

Plate 5 Covering bed with jute sheet

Plate 6 Weighing cow dung to feed worm

Plate 7 Separating worm from vermicompost after experiment

Plate 8 Counting *E. fetida*

Plate 9 Collecting vermicompost for chemical analysis