EXPLORING POTENTIAL EARTHWORM SPECIES FOR SOIL NUTRIENT ENHANCEMENT AND VERMICOMPOSTING IN SUB-TROPICAL FOREST OF MOKOKCHUNG, NAGALAND

by

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Dedicated to my father, late Chonyimong Sangtam, my mother Yangthsala Sangtam, and all my siblings

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CERTIFICATE

This is to certify that the thesis entitled "**Exploring potential earthworm species for soil nutrient enhancement and vermicomposting in sub-tropical forest of Mokokchung, Nagaland**" is an original research work carried out by **Mr. Lirikum** (Regd. No. Ph.D./ZOO/00290 Dated 28/08/2018) under my supervision in the Department of Zoology, Nagaland University, Lumami. He has fulfilled all the requirements of Ph.D. regulations for submission of the thesis. The content of this thesis is original and has not been submitted elsewhere for the award of any degree or distinction. The thesis is, therefore forwarded for adjudication and consideration for the award of the degree of Doctor of Philosophy (Ph.D) in Zoology under Nagaland University.

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DECLARATION

I, Mr. Lirikum, do hereby declare that the thesis entitle "**Exploring potential earthworm species for soil nutrient enhancement and vermicomposting in sub-tropical forest of Mokokchung, Nagaland**" is an original work done by me. The content of this thesis has not been submitted elsewhere for the award of a degree in any University/Institution.

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVATIONS

AAS	:	Atomic Absorption Spectrophotometer
NH_4F	:	Ammonium Fluoride
ANOVA	:	Analysis of variance
Av. K	:	Available Potassium
$C_2H_4O_2 \cdot H_3N$	:	Ammonium acetate
Av. N	:	Available Nitrogen
Av. P	:	Available Phosphorus
BD	:	Bulk Density
CD	:	Cow Dung
C:N	:	Carbon: Nitrogen
$CaCl_2$	:	Calcium Chloride
Cu	:	Copper
$C_{12}H_{11}N$	:	Diphenylamine
DTPA	:	diethylenetriaminepentaacetic acid
$CuSO_4$	:	Copper sulfate
$NH_4.2(FeSO_4)_2 \cdot 6H_2O$	:	Ferrous Ammonium Sulphate
HCl	:	Hydrochloric Acid
HSD	:	Honestly Significance Difference
Fe	:	Iron
KS	:	Kitchen Scrap
MF	:	Mixed forest
Mn	:	Manganese

MX	:	Mixed substrate
OC	:	Organic Carbon
PL	:	Plantation
K_2SO_4	:	Potassium sulphate
$K_2Cr_2O_7$	:	Potassium Dichromate
RH	:	Relative Humidity
RS	:	Rice Straw
NaOH	:	Sodium Hydroxide
H_2SO_4	:	Sulphuric Acid
TN	:	Total Nitrogen
TR	:	Total Rainfall
$C_6H_{15}NO_3$	:	Triethanolamine
Zn	:	Zinc

Chapter 1

Introduction

CONTENTS

1.1 General introduction

Soil is home to diverse fascinating organisms, including bacteria, fungi, protozoa, and variety of invertebrate animals. These organisms are critical decomposers of decayed plants and animals, enrich the soil nutrients which in turn, taken up by plants for better growth and development (Garlet *et al.,* 2019). Among diverse forms of life in the soil, earthworms represent about 80% of the soil invertebrate biomass in temperate, tropical, and sub-tropical ecosystems (Fragoso and Lavelle, 1992; Nainawat and Nagendra, 2001). Earthworms belonging to class Oligochaeta under the phylum Annelida are bilaterally symmetrical with a segmented body. They are cosmopolitan in distribution and present in aquatic and terrestrial ecosystems (Paliwal, 2014) except in sea, desert, and areas devoid of vegetation and permanent snow.

Earthworm population study is an important step toward the assessment of density and biomass in relation to various soil physico-chemical parameters and ecosystems service they provide. The number and diversity of earthworms in soil are considered an important criterion of soil fertility. Diversity, distribution, and abundance of earthworms vary depending on the habitat, local and regional climate (Bhadauria *et al.,* 2012), and physico-chemical properties such as moisture, temperature, bulk density, pH, conductivity, aeration, texture, etc. (Singh *et al.,* 2016; Singh *et al.,* 2022). Because of their sensitivity to land use systems, earthworms can also be used as ecological indicators of soil degradation (Li *et al.,* 2021). Thus, understanding the influence of abiotic and biotic factors on species composition, population dynamics, and distributional pattern of earthworms is essential for developing management strategies for improving soil fertility and plant growth in different sub-systems. Also, exploring and studying earthworm

diversity offer valuable insights into the intricate relationships between these organisms and their surrounding environment.

Earthworms are one of the essential groups of fauna that affect soil fertility (Mardiani *et al.,* 2022). They are considered keystone species as they provide habitat through tunnelling for other non-burrowing organisms (Shipitalo and Le, 2004; Medina-Sauza *et al.,* 2019) and create macropores facilitating better aeration and water infiltration. They consume organic matter, such as decomposing plant material and animal waste, and break it down through their digestive system. As they pass the ingested material through their bodies, it undergoes physical and chemical transformations, resulting in nutrient-rich castings or vermicompost. These castings contain higher levels of essential nutrients, such as nitrogen, phosphorus, and potassium, than the original organic matter (Bhat *et al.,* 2013; Ahmed and Al-Mutair, 2022). Additionally, earthworm activities promote the development of soil aggregates, which improve soil structure, porosity, and water-holding capacity. The cumulative effect of these processes leads to improve soil fertility, nutrient availability, and overall plant health (Scheu, 2003; Brown *et al.,* 2004). Thus, earthworms exhibit a unique concept of taking part in soil function, a combination of earthworms, physical structures, and whole microbial and invertebrate communities in soil.

Many agricultural soils lack one or other forms of nutrients that are required for optimal plant growth. Farmers often use synthetic fertilizers (Glick, 2012) to elevate food production; however continuous usage of such fertilizers leads to harmful environmental effects (Adesemoye and Kloepper, 2009). In this context, use of vermicompost as biofertilizer is considered important for sustainable agricultural practices while restoring

natural balance. Earthworms, as nature's ploughman and having important association with beneficial microorganism are key players in this process (Nair *et al.,* 1997; Gopal *et al.,* 2009) and play an essential role in soil nutrient enhancement (Domínguez *et al.,* 2010). However, exhibiting variable characteristics among the different species, earthworms play different types of role in the soil systems. Therefore, identifying their ecological categories, selections of ideal species and understanding their biology are required for the effective utilization of earthworms (Edwards and Bohlen, 1996**).**

1.2 Ecological Categories of Earthworm

Earthworms are also called "intestine of the earth", "farmer's friends" and "ecosystem engineers" due to their unique role in changing the soil's physico-chemical structure. On the basis of their niche, burrowing, feeding, and casting activities, earthworms are classified into three different ecological categories viz. Epigeic, endogeic, and Anecic (Edwards and Bohlen, 1996; Thakuria *et al.,* 2010). (1) **Epigeic** species include *Perionyx excavatus* Perrier, *Eisenia fetida* Savigny, *Eudrilus eugeniae* Kingberg, etc. They are small to medium size, active, surface dwellers, deeply pigmented, highly resistant to environmental fluctuations, high regeneration capacity and mainly live on organic matter. Epigeic species of earthworms are good bio degraders that feed mainly on leaf litter, and animal excreta and convert decayed plants and animals to stabilize manure (Lavelle, 1988). High growth rate, short life cycle, high fecundity, regeneration capacity, etc. are some important characteristics of epigeic species (Domínguez and Edwards, 2011).

(2) **Endogeic** species are shallow soil dwellers, weakly pigmented, small to medium size, and tolerant to some extent of disturbances. These species of earthworms live in horizontal burrows; they are known to have mutualistic relationships with soil microflora

(Barois and Lavelle, 1986). Endogeic earthworms feed chiefly on the rhizosphere in the subsoil and usually produce a good amount of cast on the soil's surface. They are more active during rainy seasons and generally undergo a resting state during dry seasons. During dry seasons endogeic species usually remain in the burrow, whorl up like a twisted rubber band, and produce mucus to keep them moist. *Kanchuria sp, Eutyphoeus assamensis* Stephenson and *E. gigas* Stephenson are some of the examples of endogeic species. (3) **Anecic** earthworms are deep soil dwellers, lightly pigmented, and medium to large in size. They are comparatively less active and live inside vertical burrows. Anecic species mainly feeds on soil mixed with organic matter from upper soil strata. Through burrowing and casting activities, anecic earthworms play a very important role in modifying soil structures and degrading plant debris (Lavelle, 1988). *Drawida papillifier papillifier* Stephenson, *Lampito mauritii* Kinberg and *Amyanthas alexandri* Beddard are a few example of anecic species.

Each category of earthworms plays an equally important role in soil and their response to environmental disturbances may vary. However, distinctions of such ecological categories of earthworms in the tropics are not possible, because most of the earthworms in the region are geophagous, i.e. endogeic and only a few species of earthworms are detrivores (Kale and Krishnamurthy, 1978).

1.3 Studies on earthworm growth and reproduction

Effective utilization of earthworms for biodegradable waste, manure production, and plant growth requires an understanding of their biology (Edwards and Bohlen, 1996). Knowledge of the reproductive strategies of earthworms is mostly known in temperate

regions but in tropic regions, information is very limited (Elvira *et al*., 1996; Nair and Bennour, 1998; Jimenez *et al*., 1999). Olive and Clark (1978) distinguish three basic reproduction types in earthworms-(a) monotelic: species that breed only once during a lifetime (b) polytelic: breeding occurs at several times and (c) semi-continuous/continuous: breeds several times in a year and release gametes in small broods over the extended breeding season. To promote vermitechnology, it is essential to study the growth and reproduction of earthworms. A basic understanding of earthworm reproduction may help select suitable species, and bio-indication properties, and predict the population status.

Recent studies indicated that under Indian conditions epigeic species, such as *P. excavatus, P. ceylanensis* Mich, *E. eugeniae, E. fetida,* and *E. Andrei* Bouché, complete their life cycle in organic waste and produce vermicompost (Chaudhuri and Debnath, 2020). *L. mauritii, Polypheretima elongate* Perrier, *D. nepalensis* Michaelsen, *D. willsi* Michalsen, *Metaphire posthuma* Vaillant and *M. houlleti* Perrier can also degrade organic matter in municipal sewage sludge which contains considerable amounts of sand particles. Reinecke and Viljoen (1990) reported the influence of feeding patterns on the reproduction of *E. fetida.* The reproductive biology of these vermicomposting earthworms has been studied by many in the past (Senapati and Sahu, 1993; Chaudhuri and Bhattacharjee, 2011; Ali and Kashem, 2018; Coulibaly *et al.,* 2019). Some of the characteristics of earthworms suitable for vermicomposting include (1) adaptability with respect to environmental factors, (2) capability of inhabiting a high percentage of organic matter, (3) prolific breeder (high fecundity) with high hatching success, and (4) short life cycle (Chaudhri and Debnath, 2020). Among the various species of earthworms, *P.*

excavatus is considered one of the most highly efficient and potential earthworms for vermicomposting and is commonly found in tropical Asia (Gates, 1972). This species, commonly known as the blue worm or Indian blue worm, has garnered significant attention for its ability to convert organic waste into nutrient-rich vermicompost. Thus understanding the growth and reproduction patterns of *P. excavatus* is crucial for optimizing vermicomposting processes and harnessing their ecological benefits. Under different biodegradable organic waste (leaf litter, straw waste, coir pith, and pressmud) combines with cow dung, Birundha *et al*. (2013) performed growth and reproduction studies of *P. excavatus.* Differences in growth rate, cocoon/worm/day, and hatching success were observed depending on the substrate used; indicating that food substrate directly affects earthworm growth and reproduction. Although little information on its life cycle and reproduction under laboratory conditions is available from India, fundamental information on its growth rate, suitable substrate, and potential application in vermicomposting for waste management, nutrient enhancement, and plant growth is scanty (Sadia *et al.*, 2020). Leaf litter was found to be the most suitable substrate for earthworms to thrive on. The feeding habits and nutritional requirements of earthworms significantly impact their growth rates. *P. excavatus* is known to be a voracious feeder, consuming a wide range of organic materials, including kitchen waste, agricultural residues, and animal manure. The nutritional composition of the substrate was found to influence the earthworm's growth and reproductive performance significantly. Therefore, a comprehensive understanding of their biology, particularly their reproductive strategies is necessary to advance vermitechnology and utilize these organisms effectively.

1.4 Earthworm: a potential resource for phosphate solubilizing bacteria (PSB)

The mutualistic interactions of earthworms and microorganisms are called the "sleeping beauty paradox" (Lavelle *et al.,* 1995; Brown *et al.,* 2000), where dormant microorganisms are activated by the suitable environment created by the secretion of easily assimilable glycoproteins in the form of intestinal or cutaneous mucus in drilosphere. Phosphate solubilizing bacteria **(**PSB) along with plant growth-promoting rhizobacteria (PGPR) could decrease synthetic fertilizer usage by 50% without any adverse effect on crop yield (Jalili *et al.,* 2009). Among many changes that are brought to soil physico-chemical parameters, earthworms are known to harbor diverse species of phosphate solubilization bacteria (PSB) and enzymes in their gut that play a vital role in the release of a soluble form of phosphate (Bhat *et al.,* 2017). Recently, a study on earthworm-associated bacteria is gaining attention because most bacteria are metabolically active and have multi-beneficial abilities (Singh *et al.,* 2015).

Through the involvement of phosphate solubilizing microorganisms and enzymes such as phosphatase, phosphotriesterases, etc., insoluble forms of phosphorus like iron phosphate (Fe_3PO_4), aluminum phosphate ($AlPO_4$), or tricalcium phosphate ($Ca3PO_4)_2$ in the soil are reported to be hydrolyzed to available form through the process of solubilization (Inorganic phosphate) and mineralization (organic phosphate) (Sharma *et al.,* 2013; Khan *et al.,* 2014; Koch *et al.,* 2018). Phosphate solubilization bacteria (PSB) may increase the solubility of the precipitated form of phosphorus like Ca_3-$(PO_4)_2$ through the release of the proton, phenolic compound (Illmer *et al.,* 1995), organic (Ryan *et al.,* 2001), mineral acids (Chen *et al.,* 2006) and liberation of extracellular enzymes (McGill & Cole, 1981). The availability of micronutrients like Fe^{2+} and Zn^{2+} and the process of

biological nitrogen fixation may also be enhanced by PSB and its associated microbes (Kucey, 1988). Recently, considerable attention has been given to the study of earthworm-associated PSB as inoculum to promote plant growth and yield, such practices may serve as an alternative to minimize the usage of chemical fertilizer in agronomy. Isolation and application of PSB can reduce the dependence on expensive and synthetic chemical fertilizers, thereby promoting the environmentally approachable means of agronomy (Manzoor *et al.,* 2017). However, these microbes' potential and applicability as bio-fertilizers for human welfare are not fully explored.

1.5 Earthworms: Key factor for plant growth enhancement

To feed the increasing human population, food production is generally increased by enlarging cultivable areas or by increasing fertilizer doses, however, both have a negative effect on the ecosystems (Chojnacka *et al.,* 2020). In intensive agricultural practices, the continuous use of chemical fertilizers damages the soil system irreversibly. In this context, earthworms are considered one of the important soil macroinvertebrates that contribute to plant growth enhancement by enriching soil nutrients and other microbial faunal activities. Earthworms can modify habitat, and convert soils into specialized functional domains, called driloshphere that regulate soil nutrient fluxes well beyond their life span (Bouché *et al.,* 1975).

Earthworms employ various mechanisms to stimulate plant growth, encompassing various effects on soil physical properties, extending from the macroscopic scale to the microsite level. Earthworms increase plant growth through increased microbial activity and increased soil nutrient availability (Scheu, 2003; Paliwal, 2020).

Bio-control of pests and disease, and stimulations of plant growth-regulating substance are some of the possible ways through which earthworm increases plant growth (Brown *et al.,* 2004).The potential effects of earthworms on ecosystem modification, plant community composition, and productivity are well demonstrated when earthworms are introduced to areas that were previously earthworm-free (Frelich *et al.,* 2006; Mudrák and Frouz, 2018).

1.6 Vermicomposting

Solid waste management has been an integral part of a sustainable society; however, the increasing volume and complexity of waste associated with the modern economy have become an issue of global concern as it is posing a serious risk to ecosystems and human health (Bhat *et al.,* 2018; UNEP, 2021). The accumulation rate of organic waste from industries, domestic households, and agricultural sectors imposes excessive burdens which need a holistic approach to treat this waste without harming the environment. Globally, an estimated 11.2 billion tonnes of solid waste are produced yearly, of which 1.2 billion tons are non-hazardous industrial waste (Bhat *et al.,* 2018; UNEP, 2021). In recent times, with better collection systems and advanced technologies, developed countries have adopted the most useful solid waste management systems like waste segregation, efficiently treating, reusing, and recycling solid waste (Lim *et al.,* 2015).

During composting of organic wastes, nitrogen loss occurs through ammonia, nitrogen oxides, or other forms leading to the loss of fertilizing value of composts as well as promoting greenhouse gases (GHG) emissions (Rini *et al.,* 2020). Many factors are known to affect GHG emissions during traditional aerobic composting, such as moisture

content, additives, bulking agent, temperature, pile scale, and aeration conditions (Lv *et al.,* 2018). The practices of uncontrolled dumping and sanitary landfilling emit significant amounts of greenhouse gases (Samal *et al.,* 2019). In this context, vermicomposting, a reliable, efficient, and environmentally friendly method is gaining great interest among many researchers across the globe. Though studies regard that vermicomposting also generates GHGs, it is reported that a controlled vermicomposting process reduces GHG emissions (Rini *et al.,* 2020).

Vermicomposting is the process of organic waste degradation (Composting) using certain species of earthworms (epigeic). It is a mesophilic process utilizing microorganisms and earthworms. Earthworms feed the organic waste materials and pass it through their digestive system as casts, resulting in the formation of manure known as vermicompost. Vermicomposting creates a window of opportunity for microbes and earthworms to act together for waste degradation and nutrient enrichment. In view of its eco-friendly nature, vermicomposting is widely used around the world for decomposing different organic materials into environment-friendly products (manure). Suthar and Singh (2008) reported that phosphorus concentration in vermicompost are usually higher and may contain 64.1–112.8% more that initial values. Increased concentration of potassium was also reported by Hussain *et al.* (2016) during vermicomposting of vegetables waste and rice straw. During bioconversions of *Lantana camara* using vermicomposting, Devi and Khwairakpam (2020) reported the major changes occurs in nutrients. Similarly, other researchers have also reported that earthworm activities stabilize the macro nutrient content in the waste biomass (Mago *et al.*, 2021). With increasing challenges of waste management in urban and rural areas, the use of

earthworms gets more attention for a healthier society with more scope of going organic and today, vermitechnology in waste management and nutrient enhancement is embraced by many across the country (Sharma and Garg, 2018; Gupta *et al.,* 2019; Balachandar *et al.,* 2021). While total available phosphorus increased from intial 6.23–7.2 g/kg to 7.23–9.8 g/kg.

While biochemical reactions during the degradation of organic waste are done mainly by the microorganism, earthworms are the crucial drivers of the process as they aerate, condition, and fragment the substrate, thereby drastically altering the microbial activity (Sharma *et al*., 2009; Kiyasudeen *et al.,* 2014). Vermicomposting accelerates the rate of waste degradation into manure thus modifying the physico-chemical properties of the waste (Devi *et al.,* 2020). Microorganism present in the earthworm gut has also significant role in the nutrient transformation and degradation of waste into useful manure (Edwards and Bohlen, 1996; Sun *et al.,* 2020). The capability to accumulate heavy metals from industrial wastes/sludge by earthworms is reported; wherein the gut microbes and the chloragocyte cells of earthworms detoxify heavy metals (Bhat *et al.,* 2018). For instance, during the vermicomposting process by *E. fetida*, the toxicity and total concentration of heavy metals reduced, while bacterial composition and diversity changed greatly (Wang *et al.,* 2017). Because of the efficient and eco-friendly means of waste management and nutrient stabilization, it is also been categorized as a rewarding discipline of biology (Anand and Sinha, 2020).

Due to the high rate of feeding, assimilation of organic matter, tolerance to a wide range of environmental fluctuations, short life cycles, high fecundity, and resistance during handling, epigeic earthworms are preferred over endogeic and aneceic for

vermicomposting. Two tropical epigeic species viz., African night crawler, *E. eugeniae* and Oriental earthworm, *P. excavatus* and two temperate species namely red earthworm, *E. andrei* and *E. fetida* are extensively used in vermicomposting (Graf, 1981; Beetz, 1999; Sinha *et al.*, 2002). European species such as *D. nepalensis* and *Dendroba enaveneta* are less successful because of their slow growth and high moisture content requirement (Kaushal and Bisht, 1992; Muyima *et al.*, 1994). *P. excavatus* withstands a wide range of moisture and temperature fluctuation than *M. posthuma* and *Eisenia sp.* and is considered as a reliable earthworm species for vermicomposting (Shanthi *et al.*, 1993). However, Karmegam and Daniel (2008) suggested the suitability of *L. mauritii* and *P. ceylanensis* for vermicomposting and further emphasized that earthworm species capable of inhibiting a high percentage of organic matter have a greater range of adaptability to environmental change. Most of the studies on vermicomposting species are based on exotic species (*E. fetida/E. eugeinae*) of earthworms (Dominguez and Edwards, 2011; Gajalaxmi *et al.*, 2005; Kapoor *et al.*, 2015).

1.7 Effects of abiotic factors on earthworms during vermicomposting

Earthworms have a well-defined range of tolerance for environmental factors such as temperature, moisture, pH, etc. However, the rate of cocoon production, developmental activities, and rate of vermicast production is critically affected by the increasing level of tolerance range of these factors (Domínguez and Edwards, 2011). Because of the higher risk from predators and harsh environmental conditions, epigeic species are known to have a wider range of environmental tolerance. Above the critical environmental factors, earthworms (aneceic species) move deep down the burrow and undergo hibernation where their growth rate get reduced and undergo very limited activities.

Temperature: Optimum temperature for suitable earthworm species used in vermicomposting ranges from 15 to 30 °C (Garg and Gupta, 2011). At temperature below 10 °C, feeding and other developmental activities get reduced and further below 4 °C, cocoon production and growth rate of earthworm stops. For example, *E. eugeniae* and *P. excavatus* has an optimum range of 25 °C but they die at temperature below 9 °C and above 30 °C (Edwards, 1988). Also, at temperatures above 30 °C, microbial activities of the casts and the surrounding soil increase which leads to depletion of the oxygen availability for the earthworms. Cocoon production is mostly affected by the temperature compared to growth rate and other developmental activities.

Moisture: The moisture content is very essential for the distribution and occurrence of various species of earthworms which is evident from higher population density during the rainy season (Kale and Karmegam, 2010). As the water content of earthworms is greatly influenced by the surrounding soil water potential (Kretzschmar and Bruchou, 1991), the survival of earthworms directly depends on adequate soil moisture, and the vermicomposting process is affected once the moisture level falls below 50%. Suthar (2007) maintained the optimum moisture content of 65–75% in vermibin for successful vermitechnology. While Dominguez *et al.* (1997) found a suitable range of 80–90% with the 85% optimum, researchers from Nova Scotia recorded the best growth and reproductive response of earthworms at 75–80% of moisture content (Georg, 2004).

Aeration: During vermicomposting, aerating vermibin is necessary to enhance waste degradation and better mobility of worms (Senthilkumar *et al.,* 2016) and therefore vermibin has to be ploughed manually at regular interval. However high rate of aeration leads to loss of moisture and increases the temperature which is lethal for the earthworm. Palaniappan *et al.* (2017) observed that aeration at the rate of 0.62 L/min/kg for 4–6 h in

an artificially engineered condition of pre-processed vegetable waste was ideal for vermicomposting.

pH: Epigeic species can tolerate a wide range of pH (5–9) but when vermibin gets too acidic or alkaline earthworm tends to leave the bin. Kaur (2020) observed that the pH of vermbin often drops during the process of vermicomposting however needs to be maintained at 6.5–7.5. As pH also depends on the food material used, the best choice of pH for successful vermicomposting is around neutral to slightly alkaline and acidic.

1.8 Review of literature

1.8.1 International

Fragoso *et al.* (1999) recorded 20l2 species of earthworms from humid tropics agro-ecosystem. Blackmore and Paoletti (2006) enlisted 715 species in Australia, most of which are endemic and adapted to regional climatic conditions. Nguyen (2016) recorded the presence of 212 species belonging to eight families in Vietnam. Rutgers *et al.* (2016) developed a model to map earthworm diversity in Europe, utilizing existing datasets from various locations. Despite differences in collection methods across countries, the authors observed that land use and geographical factors predominantly influenced the diversity and population dynamics of earthworms in the region. Philip *et al.* (2019) created global maps depicting the distribution, diversity and abundance of earthworms collected from 9212 sites in 57 countries and revealed that earthworm diversity and abundance reached their peak in mid-latitude regions while in tropical regions, biomass was found to be highest. However, James *et al.* (2021) presented a counterargument, disagreeing with the notion of a positive relationship between earthworm abundance and richness along the latitudinal gradient and suggested that in Asia and the western Pacific regions, species

richness actually peaks between 0° to 30°N. Cameron *et al.* (2021) reported that endogeic category of earthworm was found to be more compared to epigeic and anecic both in eastern Canada and Central Europe. Mulia *et al.* (2021) reported that earthworm biomass is higher in natural forests compared to agroforestry (anthropogenically disturbed habitats) in Quang Nam Province, Vietnam and also observed that the earthworm population in natural and regenerated forests is much denser compared to agroforestry, annual croplands, and home gardens. Hoeffner *et al.* (2021) while working in the temperate grasslands of Brittany, France, reported that earthworm density and biomass were 517.0±57 ind.m^{-2} and 219.4±20 g m^{-2} respectively and emphasized that variation and abundance of different earthworm category (epigeic, endogeic ad anecic) is largely explained by the soil properties e.g. soil organic matter, alkalinity and soil pH rather than grassland management and landscape diversity. From the Poonch division of Pakistan, Khan *et al.* (2021) reported that earthworms' diversity, distribution, and abundance are affected by the soil temperature, pH, moisture contents, soil texture, organic matter content, available phosphorous, potassium, and nitrogen. Narayanan *et al.* (2021) updated the earthworm checklist with total number of 81 species and subspecies belonging to 20 genera and 8 families from Sri Lanka. Denier *et al.* (2022) also reported that microbial metabolic activity and earthworm communities and diversity are sensitive to land-use systems and are affected by tillage. Lam *et al.* (2022) have also reported the presence of 41 earthworms belonging to 12 genera and six families from the south-eastern part of Vietnam. Knowledge on the reproductive strategies of earthworms is mostly known in temperate regions (Elvira *et al.,* 1996; Nair and Bennour, 1998; Jimenez *et al.,* 1999). Notable contributors to understanding the biology of earthworms along with the vermicomposting potential include Neuhausetr *et al.* (1984), Domínguez *et al.* (2000),

Siddique *et al.* (2005), Nfor *et al.* (2022). Reinecke and Hallatt (1989) reported a slower growth rate of *P. excavatus* at 25°C under urine-free cattle manures as compared to other vermicomposting worms.

Mapile *et al.* (2020) also isolated and identified many strains of bacteria and fungi, including *Aeromonas caviae* and *Bacillus xiamenensis* from the African night crawler, *E. eugeinae* that showed high phosphate solubilization index (PSI). Houida *et al.* (2021) also isolated six main bacteria genera, namely *Enterobacter*, *Citrobacter*, *Aeromonas*, *Pseudomonas*, *Bacillus*, *Terribacillus* from the chloragogenous tissue of the earthworm *A. molleri*, through 16s gene sequencing having plant promoting traits.

Laossi *et al.* (2009) reported significant effect of earthworm's species i.*e. A. caliginosa* (endogeic species) and *Lumbricus terrestris* Linnaeus (anecic species) on increased plant biomass such as *Poa annua*, a grass; *Trifolium dubium*, a legume; *Veronica persica*, a forb. Jana *et al.* (2010) observed the earthworm influence on the above-ground biomass productivity of plants (*Arabidopsis thaliana*).Van Groenigen (2014) reported that, on average, earthworms increase crop yield and above-ground biomass productivity by 25% and 23% respectively. Xiao *et al.* (2017) also reported that the presence of earthworms increased plant growth by 20%, while soil nutrients such as nitrogen content increased by 11%. In a microcosm experiment on plant community succession, Mudrák and Frouz (2018) showed that the effects of earthworms (*L. rubellus* Hoffmeister and *A. caliginosa* Savigny) on young soil were proportionately more compared to developed soil, indicating that earthworm activity is more important in undeveloped than in developed soil. Kabi *et al.* (2019) reported that reproduction, growth rate, and off take of African night crawler *E. eugeniae* was influenced by the type of

organic substrate used as feed, and inadequately aged substrate negatively affects the cocoon production. Sadia *et al.* (2020) reported better growth and reproduction of *P. excavatus* in chopped banana plant trunk than water hyacinth, vegetable scrap, paddy straw, and sugarcane bagasse used as feed.

Hallam *et al.* (2020), through an assessment of earthworms' effects on soil physical-hydraulic properties, herbage production, and wheat growth, reported that earthworms increased water holding capacity of soil by 9%, organic matter by 9%, total nitrogen by 3.5%, and shoot biomass by 58%.The effects of earthworms on plant-available soil phosphorus and the subsequent effect on plant growth were reported by Trap *et al.* (2021). Wang *et al.* (2022) reported the improvement of fertility and microbial communities of cadmium-contaminated soil by earthworms and Arbuscular mycorrhiza fungi.

The pioneering practices of vermiculture and its uses for vermicomposting started during 1970 in Holland and subsequently followed in England. In USA, the vermicomposting farm was started in 1978–79 on an industrial scale with an average vermicompost production of 500 tons in a month (Edwards, 1988) and many other countries such as Japan, Thailand, China, Brazil, France, etc. practices vermiculture in large scale for the degradation of organic wastes (Sinha *et al.*, 2002). Australia is also a major country actively practicing the vermiculture for eco-friendly management of wastes. The Redland Shire Council in Queensland, Australia is operating a vermiculture plant since 1998 with a capacity of 20,000 tons/annum (Lotzof, 2000). There are around 3000 vermicomposting plants in Japan with a vermicast production of 5–50 tons/month (Aalok *et al.*, 2008) of which company such as Aoka Sangyo Co. Ltd. produces 10 tons

of live earthworm and 400 tons of vermicompost in a month (Kale, 1991). An innovative discipline of vermiculture biotechnology *i.e.*, the breeding and propagation of earthworms and the use of its castings has become an important tool of waste recycling all over the world (Aalok *et al.,* 2008). Subsequently, vermitechnology is widely used for the conversion of solid waste into manure from the municipal wastes, paper mills and food industries (Ceccanti and Masciandaro, 1999; Edwards and Arancon, 2004; Bhat *et al.*, 2018; Ganguly and Chakraborty, 2020; Falco *et al.*, 2021). Vermicomposting facility and its practices have developed at a rapid rate and vermicasts produced by earthworms are presently used as a nutrient supplement for organic food production and in agricultural sector (Mahmud *et al.*, 2020; Messiga *et al.*, 2020).

The macronutrients directly affected by the earthworms are Nitrogen, Phosphorus, Potassium, and Organic Carbon. During the degradation of rice straw and kitchen waste through vermicomposting, Zhi-Wei *et al.* (2019) reported a significant increase in phosphorus (31.38–55.89%) and potassium. (33.40–63.15%) while a reduction in total organic carbon (TOC) (38.24–43.49%) and Total nitrogen (TN) (9.01–32.52%) was observed. With a high percentage of TOC reduction, substantial amount of C:N gets reduced and stabilized manures were formed. Lv *et al.* (2018) performed vermicomposting of sewage sludge and reported that earthworm increased the rate of organic carbon degradation and nitrogen mineralization.

1.8.2 National: India in General

The pioneering work of earthworm studies in the Indian subcontinent dates back to the 18th century (Templeton, 1844) by describing the new earthworm species *Megascolex caeruleus* Templeton, from Sri Lanka. While evaluating the effect of

deforestation and degradation of natural forest on earthworm community in central Himalayas, Bhaudaria *et al.* (2000) observed that loss of climax natural forest lead to loss of endemic species and results in dominance of exotic species. Around 89 % of the recorded Indian earthworm species are endemic (Julka and Paliwal, 2005). Julka *et al.* (2009) recorded the presence of 590 species from India. Suthar (2009) reported six species of earthworms from three differently managed agro-ecosystems having the maximum number of earthworms recorded from mixed farming systems, followed by organically managed and conventional agro-ecosystems. In Kashmir valley, Najar and Khan (2011) reported eight earthworm species belonging to the family Moniligastridae, Megascolecidae and Lumbricidae of which *Aporrectodea caliginosa, Octolasion cyaneum* Savigny and *E. fetida* were recorded as the first report. In the Trans-Gangetic plains of eastern Himalayas, Sharma and Poonam (2014), recorded nine earthworm species, namely *Amynthas morrisi* Kingberg, *A. robustus* Perrier, *L. mauritii, M. posthuma, E. incommodus* Beddard, *E. waltoni* Perrier, *E. nicholsoni* Beddard, *Octocheatona beatrix* Beddard and *D. nepalensis* the distribution of which were affected by soil physico-chemical factors and food quality. Singh *et al.* (2016) reported that in addition to vegetation type and other abiotic factors, soil physico-chemical parameters also determine the earthworm abundance. Mubeen and Hatti (2018) reported fourteen species of earthworm belonging to Octochaetidae, Megascolicidae, Ocnerodrilidae, and Eudrilidae family from the Southern part of India. Similarly, Ahmed *et al.* (2022) while studying the influence of climate, soil, and cropping pattern on earthworm diversity and abundance in Western Himalaya observed that earthworm density positively correlated with rainfall, soil moisture, and organic matter while a negative relationship was observed with soil pH. Mubeen and Hatti (2022) also reported 33 species and sub-species belonging

to the families Benhamiidae, Eudrilidae, Megascolecidae, Ocnerodrilidae, Octochaetidae, and Moniligastridae from the long-term survey of earthworm species in the arid regions of Hyderabad-Karnataka of which73% species were endemic and the remaining 23 % were exotic to the region.

Dash and Senapati (1980) studied morphological characters of *L. mauritii, D. willsi,* and *Octochaetona surensis* and cocoon characters with respect to soil temperature and moisture. Bhattacharjee and Chaudhuri (2002), performed a laboratory studies on cocoon production and fecundity of seven tropical earthworm species namely *P. elongata, P. excavatus, L. mauritii, Pontoscolex corethrurus* Muller, *Dichogaster modiglianii* Rosa and *D. nepalensis* and reported that cocoon production and incubation period were affected by variations in temperature. It has been noted that high fecundity, low incubation period, a short period of developmental time, continuous nature of breeding of epigeic earthworms (*P. excavatus* and *D. modiglianii*) and the top soil endogeic species (*P. corethrurus, D. nepalensis* and *L. mauritii*) indicates their potential application in vermitechnology. While studying the life assessment of *P. excavatus, D. nepalensis* and *M. houlleti* under laboratory conditions using cow manure and oak litter, Joshi and Dabral (2008) observed higher growth rate of *P. excavatus* in comparison to the other two species. Karmegam and Daniel (2009) suggested that due to high reproduction and short life cycle, *P. ceylanensis* can be utilized in vermiculture practice. Chaudhuri and Bhattacharjee (2011) studied the reproductive biology of tropical geophagous earthworms, viz., *P. corethrurus, D. assamensis, D. papillifer papillifer, E. comillahnus, M. houlleti, D. affinis* Stephenson, *O. beatrix* and *Lennogaster chittagongensis* Stephenson under laboratory conditions. Biswas *et al.* (2018) isolated

three strains of PSB (*Bacillus megaterium*, *Staphylococcus haemolyticus*, and *Bacillus licheniformis*) from the earthworm gut, *M. posthuma* Vaillant and confirmed that with high concentration of heavy metal such as Zinc and Copper, earthworm associated PSB solubilizes the phosphorus and making it available for plant absorption. Through biochemical characterization and 16s RNA gene sequencing, Banerjee *et al.* (2019) isolated and identified phosphate solubilizing strains of bacteria such *Bacillus safensis* (MF 589718), *Bacillus flexus* (MF 589717) and *Staphylococcus haemolyticus* (MF 589719) from the earthworm, *M. posthuma*, among which the Bacillus strains appeared to be significantly more potent than the Staphylococcus strain in promoting plant growth and removing heavy metals (Cromium, Copper and Zinc) from aqueous media. Additionally these strains also exhibited several plant growth promoting traits (e.g., indole acetic acid (IAA), gibberellic acid (GA) and ammonium ion production, 1-aminocyclopropane- 1-carboxylic acid (ACC) deaminase activity). Chaudhuri and Datta (2020) observed that cocoon sizes vary depending on the size of the earthworm clitellum and recorded maximum fecundity and shortest incubation period in *P. ceylensis* than *P. excavatus* and *E. eugeniae.* Bhakta *et al*. (2022) isolated and characterized the potential phosphate solubilizing bacteria from the earthworm *M. posthuma.* The application of isolates showed a higher rate of seed germination (10–50%) and plant growth (shoot length — 21% and leaf number — 77%) compared to that of the control in *Abelmoschus esculentus*. The productions of bioavailable forms of Phosphorus in the rhizosphere zone by the isolates were responsible for the better plant growth characteristics.

In the context of a tropical country like India, specific earthworm species, such as *P. corethrurus* and *D. willsi*, have demonstrated great promise for promoting plant

growth, with a biomass of approximately 30 g m^{-2} or more. Studies have shown that these earthworms can increase the grain yield of agriculturally important plants by over 40% (Brown *et al*., 2004). Mago et al. (2021) reported that earthworm activities significantly reduce pH, OC and C:N, and C:P ratio of the waste. While total available phosphorus was increased to 7.23–9.8 g/kg from initial concentration of 6.23–7.2 g/kg. Also, 13.1–25.85% increase in potassium concentration was observed.

1.8.3 Northeast Region

Ramanujam and Jha (2011) observed that earthworm population's dynamics were significantly correlated with rainfall and the physical characteristics of the soil in agroforestry ecosystem in Mizoram and recorded that earthworm density and biomass ranged from 6 to 243 ind.m^2 to 3.2 - 677.64 g.m^2 in high altitude and from 0 to 176 ind.m^2 to 0 - 391.36 g.m^2 respectively. Haokip and Singh (2012) made a comparative study on earthworm diversity and distribution from the natural forest and disturbed agro-forestry in Manipur. Rajkhowa *et al.* (2014) reported the diversity of earthworm in different soil habitats of Assam. From the west Khasi hills of Meghalaya, Kharkongor (2018) reported the taxonomic and ecological studies of earthworm. Earthworm resources, and casting activities from different agro ecosystems and rubber plantations in Tripura are reported by Chaudhuri and Nath (2011), Jamatia and Chaudhuri (2017). Dhar and Chaudhuri (2020) reported seven earthworm species with an average density and biomass of 163 ind.m^2 and 56 g/m^2 respectively in paddy fields and plantations of West Tripura. In traditional slash and burn cultivation of Mizoram, Lalthanzara and Zodinpuii (2021) recorded nine species of earthworm belonging to three families, with a record of least number of earthworms during the cultivation phase compared to the pre-cultivation and

post-cultivationphases. Saikia *et al.* (2021) reported eight species namely *L. maruitii, P. excavatus, P. pulvinnatus* Stephenson, *M. posthuma,* and *Amyathas diffringens* Baird from different soil habitats in Golaghat district of Assam.

During the laboratory studies of tropical earthworms in Tripura, Bhattacharjee and Chaudhuri (2002) reported that cocoon production and incubation period were affected mainly by variations in temperature. The reproductive biology of tropical geophagous earthworms, viz. *P. corethrurus, D. assamensis, D. papillifer papillifer, E. comillahnus, M. houlleti, D. affinis, O. beatrix* and *Lennogaster chittagongensis* under laboratory conditions were studied by Chaudhuri and Bhattacharjee (2011).

1.9 Present scope of study in Nagaland

Nagaland covers a geographical area of 16579 sq. km. and lies between 25°60´´ and 27°40´´N and 93°20´´ and 95°15´´ E. The state offers tropical, sub-tropical and temperate forest ecosystems spreading in different altitudes and harbors different types of plants and animals. With increased developmental activities and urbanization, there has been a random obliteration of natural forest ecosystems and land use patterns which result in the habitat destruction of many animals, including soil fauna. Along with these, slash-and-burn systems of agriculture practices severely affect the diversity of earthworms and reduction of original forest species of earthworms (Bhadauria and Ramakrishnan, 1991; Darlong and Alfred, 1991). It is therefore necessary to conduct an elaborate study on documentation, diversity assessment, and population dynamics of earthworms in Nagaland to have a comprehensive understanding of the ecological functions they perform and their importance in maintaining the overall soil health and productivity of the forests. Mokokchung district of Nagaland, the present study area is a

hilly terrain with sub-tropical forest ecosystems and located at an altitude ranging from 1,312 feet to 8,428 feet. While considerable information on earthworm studies is available from the different Northeast states like Sikkim (Subedi *et al.*, 2018), Mizoram (Ramanujam and Jha, 2011; Zodinpuii and Lalthanzara, 2019), Tripura (Chaudhuri and Bhattacharjee, 2011; Chaudhri and Debnath, 2020), Manipur (Haokip and Singh, 2012; Agrawal and Sarangthem, 2023), Meghalaya (Kharkongor, 2018; Thakur *et al.*, 2020), Assam (Rajkhowa *et al.,* 2015; Saikia *et al.*, 2021) and Arunachal Pradesh (Tasung *et al.*, 2023), studies on earthworm from Nagaland is very scanty (Thyug and Kakati, 2018). The basic information on diversity of earthworms and study on biology and reproduction of local species; and their potential application in vermicomposting, nutrient stabilization, and plant growth enhancement is considered as crucial research gap in Nagaland. As reports on vermicomposting efficiencies of native species are scanty, exploration of local earthworm species and evaluation of beneficial microbes associated with earthworms needs immediate attention. Hence the present study has been undertaken in two different but adjacent forest ecosystems i.e. mixed forest and managed mono plantation area under the Minkong forest in Mokokchung, district, Nagaland with the following objectives.

1.10 Objectives of the study

1. To study temporal variation in density, diversity, biomass, and population dynamics of earthworms from two different subtropical forest.
2. To study the biology of dominant earthworm species on different food substrates in laboratory condition and assessment of associated phosphate solubilizing bacteria (PSB).
3. To evaluate the effects of selected earthworms on soil properties, nutrient stabilization and plant growth enhancement and vermicomposting.

Chapter 2

Earthworm community characteristics and temporal variation of density and biomass in Minkong forest, Mokokchung, Nagaland

Contents

2.1 Introduction

Earthworms constitute about 80% of the total soil invertebrate's biomass in temperate, tropical and sub-tropical ecosystems and play an important role in sustaining above-ground biodiversity (Fragoso and Lavelle, 1992). Their role in litter degradation, nutrient cycling and improvement of physico-chemical properties of soil such as structure, density, porosity and moisture has been studied in many parts of the world (Carey *et al.*, 2009; Zhi-Wei *et al.*, 2019; Tamartash and Ehsani, 2021). They are sensitive to habitat change and in recent times, mass destruction of forest for intense agriculture management and other anthropogenic activities has detrimentally affected the earthworm diversity (Singh *et al.,* 2020). Works like Gonzalez *et al*. (1999); Bhaudaria *et al*. (2000); Chaudhuri and Nath (2011) have shown that even under similar climatic condition, earthworm community structure and distribution patterndepend on land use system, plant species composition and availability of organic matter.

The feeding behaviour and burrowing activities of earthworms play an essential role in changing the nutrient conversion, organic carbon stabilisation, and bulk density of soil (Blouin *et al.,* 2013). Earthworms are also known for enhanced decomposition by facilitating microorganisms such as fungi and bacteria and increasing nutrient availability to plants (Blouin *et al*., 2013; Castro *et al.,* 2019). Due to its stabilizing effect on the soil ecosystem, considerable attention has been given to studies on population dynamics, diversity, distribution and community structures of earthworm (Cardinael *et al.,* 2019; Mcinga *et al.,* 2021; Cameron *et al.,* 2021; Cremonesi *et al.,* 2021). While a few reports are available on different aspects of earthworm studies from different climatic zone of North Eastern India like Tripura (Chakraborty *et al.,* 2020), Assam (Tiwari *et al.,* 2020), Manipur (Singh *et al.,* 2020) and Mizoram (Lalthanzara and Zodinpuii, 2021),

information on earthworm in Nagaland is very scanty (Thyug and Kakati, 2018). Hence, the present chapter focuses on the population dynamics, community characteristics and temporal variations of earthworm from two different sites i.e. mixed forest and mono plantations of *Daubanga grandiflora*, in Minkong forest under Mokokchung district, Nagaland.

2.2 Materials and Methods

2.2.1 Study area

The present study was conducted (on the monthly basis) from January, 2019 till February, 2020 at Minkong forest (**Fig. 2.1**) which is located at 15 km away from Mokokchung Town. The two study sites viz., mixed forest (MF) and plantation (PL) lying at 1325 m above sea level are characterised by steep slopes. The most common tree species found in the mixed forest (MF) are *Atrocarpus chaplasha* Roxb, *Ficus semicordata* Buch-Ham. ex SM, *Schima wallichii* (DC.) Korth. *Trema orientalis* (L.) Blume, etc., Shrubs like *Tephrosia candida* DC, *Pavetta indica* L, *Styrax serrulata* Roxb, and *Crotalaria cytisoides* Roxb are also commonly found. While plantation area (PL) is a mono plantation of *Daubanga grandiflora* (Roxb. Ex DC) Walper Grasses like *Panicum* sp., *Saccharum arundinaceum* (Retz.) Welker, Vorontz. & E.A. Kellogg, *Digitaria* sp., *Musa markkuana* H. Wendl. & Drude are commonly found. The ground surface of the plantation area is cleared about 3 times a year, while the mixed forest remains fairly undisturbed (**Fig. 2.2**).

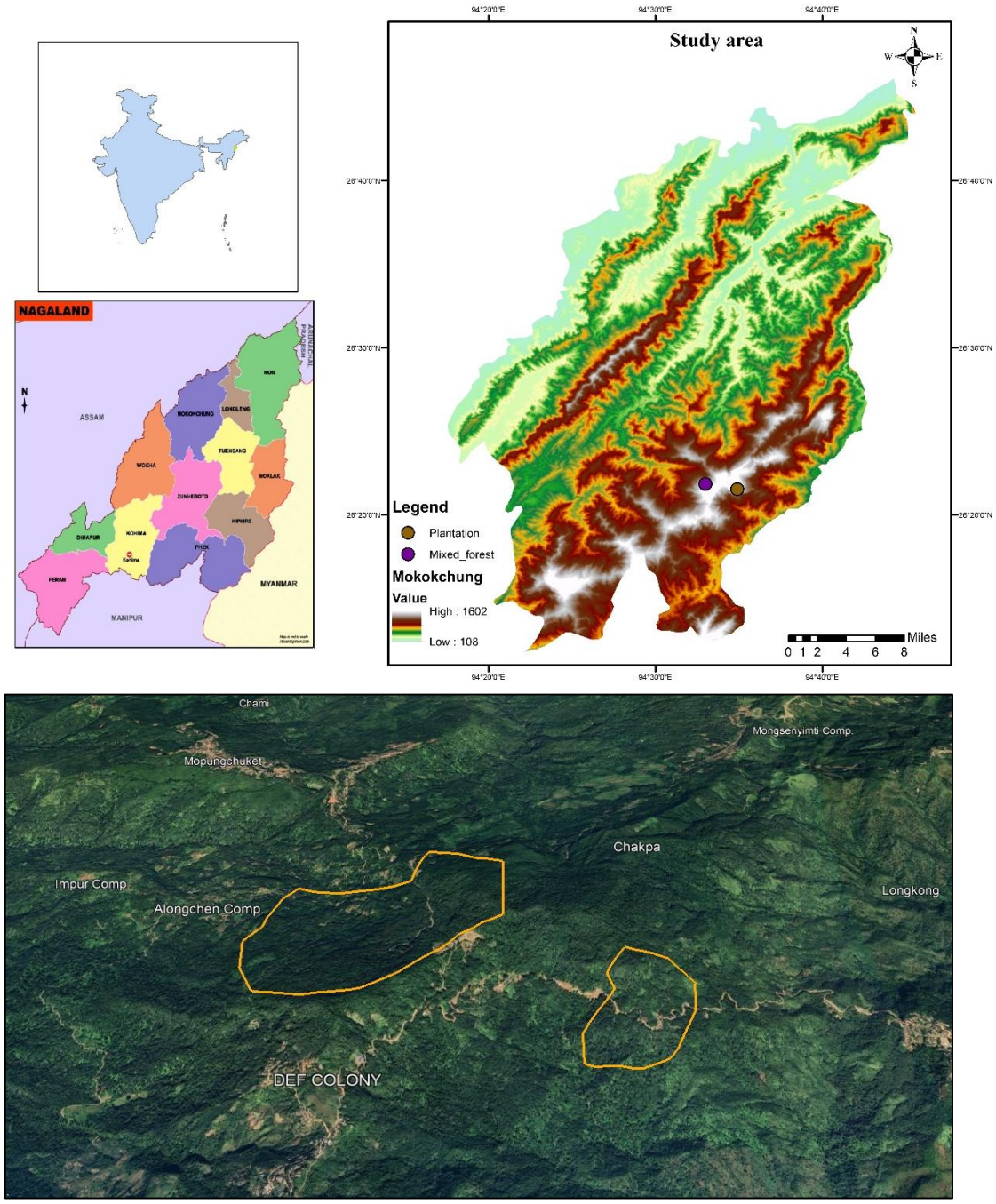

Fig 2.1 Study area in Minkong forest under Mokokchung district Nagaland, India.

A **B**

Fig. 2.2 Study area within the Minkong forest, showcasing two distinct regions. Panel A represents the designated plantation area (PL), while Panel B showcases the mixed forest.

2.2.2 Climate

The climate of the study area is monsoonal with warm moist summers and a chilly dry winters. The meteorological data of 2019 (**Fig. 2.3**) shows mean maximum air temperature varied from 25.64°C (January) to 30.8°C (May) and the mean minimum air temperature varied from 5.68°C (January) to 23.03°C (July). The maximum relative humidity (83.21%) was recorded in the month of August while minimum was recorded in the month of March (63%). Total rainfall ranges from 0-14.96mm where highest rainfall was observed in the month of July followed by September (10.07mm), and June (7.93mm). While minimum rainfall was observed in the month of December followed by January (0.13mm), followed by February (1.47mm), and March (2.16mm), respectively.

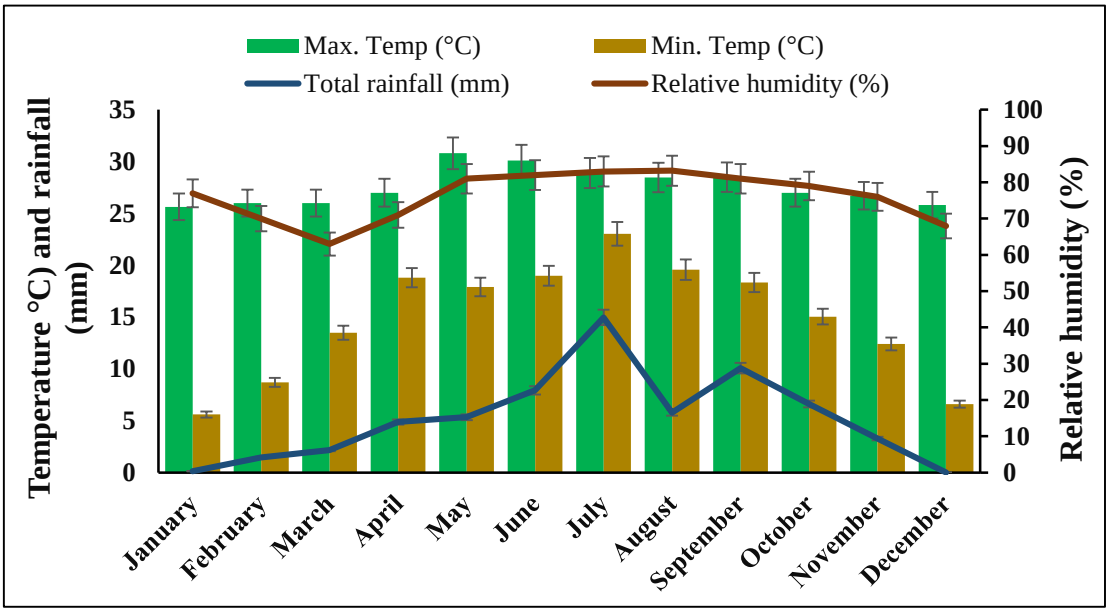

Fig. 2.3 Climatic variations in the study area during 2019-2020.

2.2.3 Earthworm Sampling

To uniformly sample earthworm specimens from all the sites in the study area, a grid of 9 plots (5m×5m^2) were demarcated in both MF and PL (**Fig. 2.4**), and earthworm sampling was done by digging three (3) subplots (25×25×30 cm^3) in each of 9 plots. So a total of twenty seven (27) soil monoliths were sampled for earthworm collection on monthly basis (**Fig. 2.5**). Earthworm samples were collected at monthly intervals from 8:00 to 9:00 a.m. after which they were washed, dried (with the help of blotting paper) and weighed immediately for biomass estimation. Specimen's maturity was determined based on the presence/absence of clitellum. Ecological categories of earthworms were studied following Hendrix and Bohlen (2002). Earthworms were separated into different categories based on the clitellum development viz., juveniles (weight ≤ 20 mg), young (>20 mg but without distinct clitellum) and adults (distinct clitellum).

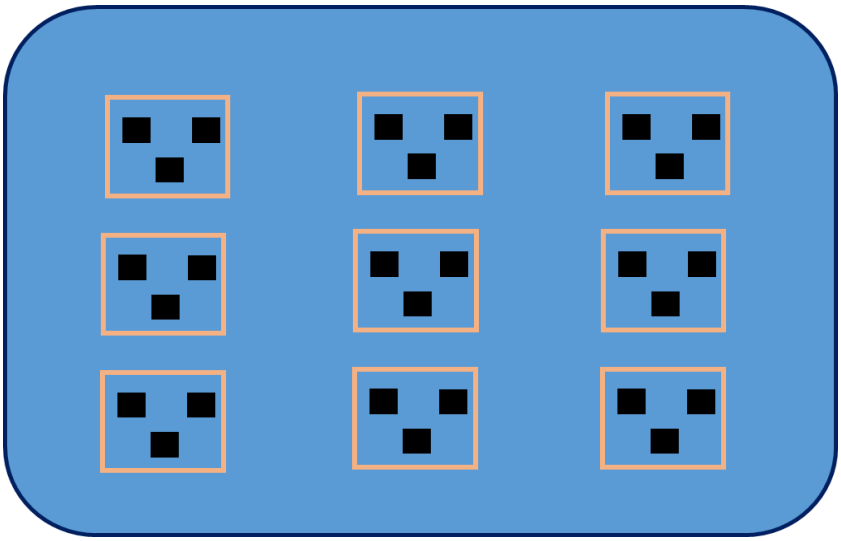

Fig. 2.4 Pictorial representation of sampling design.

A **B**

Fig. 2.5 (A) Soil thermometer and record book used in the filed survey (B) Earthworm sampling.

2.2.4 Soil Samples collection and physico-chemical analysis

From each experiment sites, composite soil samples to a depth of 15 cm were collected at monthly intervals and the average soil temperature was recorded *in situ*. Soil moisture and pH were determined in fresh soil samples at 1:20 ratio of soil and distilled water. Soil bulk density was determined by bottle weighing method (Pawar *et al.*, 2009).

Soil samples were air dried, ground and passed through a 1 mm mesh size sieve and used for subsequent chemical analysis *viz.,* organic carbon (Walkley and Black, 1934), total and available nitrogen (Kjeldahl, 1883) using kel plus nitrogen estimation system, available phosphorus (Bray and Kurtz, 1945) and available potassium was estimated by ammonium acetate method (Hanway and Heidel, 1952).

2.2.5 Data analysis

Earthworm density and biomass were expressed in terms of individuals/m^2 and fresh weight $g.m^{-2}$. Earthworm communities were analysed for relative abundance, index of dominance (Engelmann, 1973), Species richness index (Menhinick, 1964), index of general diversity (Shannon and Weiner, 1963), Simpson diversity (Simpson, 1949) and Bray-Curtis species similarity index. For the analysis of data collected the following formula were used

Density (ind.m^{-2}) = $\frac{\text{Total number of individuals in the quadrats}}{\text{Total number of quadrats}} \text{x}16$

Biomass (g.m^{-2})= $\frac{\text{Total biomass of individuals of a species in all the quadrats}}{\text{Total number of quadrats studied}} \text{x}16$

Relative abundance = $\frac{\text{Total number of individuals of cocnerned species}}{\text{Total number of individuals of all species}} \text{x}100$

Species dominance- Calculated based on relative abundance (RA) using Engelman's scale given below

Eudominant species	=	RA 31.7%-100%
Dominant species	=	RA 10.1%-31.6%
Subdominant species	=	RA 3.2%-10.0%
Recedent species	=	RA 1.1%-3.1%
Subrecedent species	=	RA<1.0%

Shannon-Weiner diversity (H′): Taking as a function of both richness and evenness, H′ index aids in assessing how proportionately the individuals are distributed among species in a community. Shannon-Weiner diversity (H′) index was calculated using the following formula

$H' = -\sum P_i \ln P_i$, where pi = proportion of total sample belonging to i^{th} species, ln = natural logarithm.

Evenness or Equitability (J): Evenness is the measure of how different the abundances of the species in a community are from each other. A community where every species had the same abundance would be perfectly even. All natural communities are highly uneven, so evenness is a relative statement and the value varies from 0 (maximally uneven) to 1 (perfectly even). The logic behind Shannon evenness is that if diversity is a mixture of richness and evenness, then removing richness should produce evenness. It was calculated as:

$J = H'/H_{max} = H'/\ln S$, where, H′ is the Shannon-Weiner Index and lnS is the natural logarithm of the number of species observed.

Simpson diversity (D): Simpson diversity is based on the probability that two individuals drawn at random from an infinite community would belong to different species. It is also considered as index of dominance and it was calculated as:

$C = \Sigma P_i^2$; where Pi = ni/N (1-D), ni = proportion of total sample belonging to i^{th} species, N- total number of individuals.

2.2.6 Statistical analysis

The mean differences of earthworm density, biomass and other soil parameters was tested using independent sample t-test (**Fig. 2.6**). One-way ANOVA (p<0.05)

followed by Post hoc tukey test to determine significant difference in seasonal variations of earthworm density and biomass. All data were analysed using SPSS (Version 22).

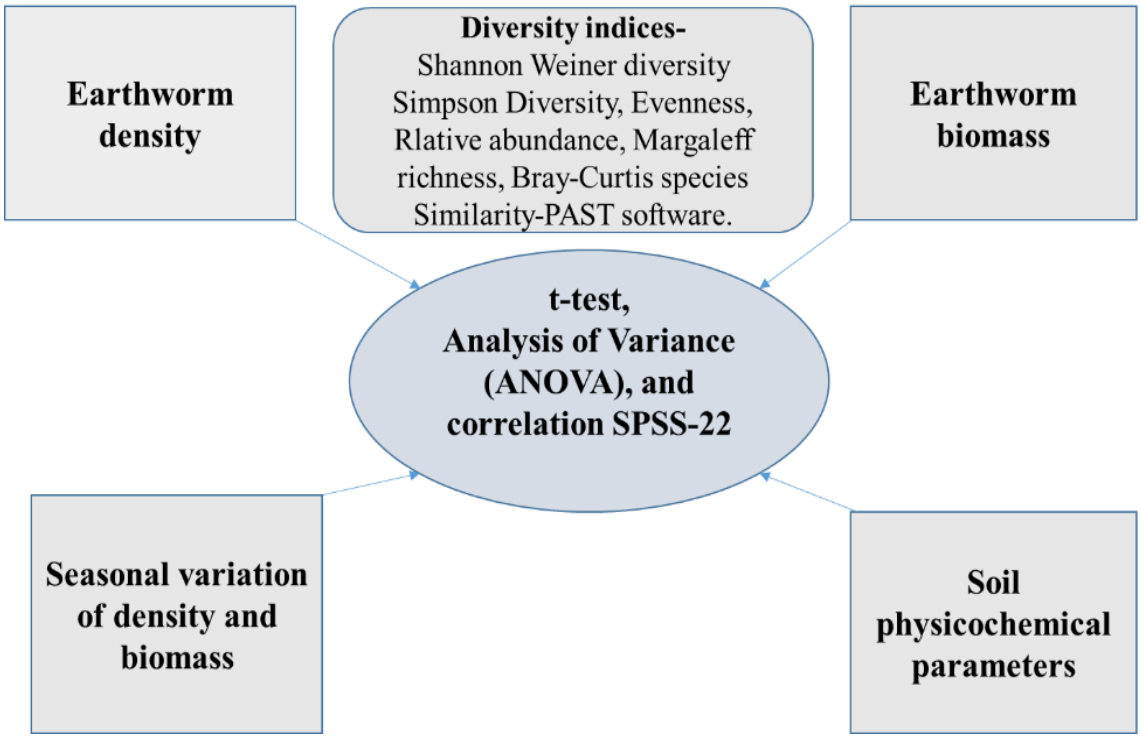

Fig. 2.6 Pictorial representation of statistical analysis performed.

2.3 Results

2.3.1 Earthworm community characteristics

A total of 389 and 467 individuals of earthworm were sampled from mixed forest (MF) and plantation area, respectively. Twelve earthworm species *viz.*, *Amynthas cortices* (Kinberg), *Amynthus gracilis* (Kinberg), *Drawida assamensis* (Gates), *Drawida hodgarti* (Stephenson), *Drawida nepalensis* (Michaelsen), *Drawida* sp., *Eutyphoeus assamensis* (Stephenson), *Eutyphoeus festivus* (Gates) *Metaphire houlleti* (Perrier), *Perionyx excavatus* (Perrier), *Perionyx simlaensis* (Michaelsen) and *Pontoscolex corethurus* (Müller) were recorded from the two study sites (**Fig. 2.7**). Out of 12 species, 5 species (*A. corticis*, *A. gracilis*, *M. houlleti*, *P. excavatus* and *P. simlaensis*) belong to family Megascolecidae, 4 species (*Drawida* sp. *D. assamensis*, *D. hodgarti* and *D. nepalensis*)

from Moniligastridae, 2 species (*E. assamensis*, *E. festivus*) from Octochaetidae and 1 species (*P. corethurus*) from Glossoscolecidae.

In MF, ten (10) earthworm species were present, highest density was recorded for species such as *D. nepalensis*> *P. excavatus*> *Drawida sp.*> *E. festivus*> *D. hodgarti*> *P. corethurus*> *P. simlaensis*> *E. assamensis*> *M. houlleti*> *A. gracilis* with the relative abundance (RA %) of 19.53, 19.28, 13.11, 10.79, 7.45, 6.68, 5.69, 4.88, 4.62 (**Fig. 2.8-A**). As per their RA, three species were found to be dominant (*P. excavatus*, *D. nepalensis*, *Drawida sp*. *E. festivus*) in MF. The sampled earthworm species belong to three ecological categories such as Epigeic, Epianecic, and endogeic categories (**Table 2.1**).

In PL, nine (9) earthworm species were found and density of recorded earthworms were in the order: *D. assamensis*> *P. excavatus* > *D. nepalensis*> *E. festivus*> *Drawida sp.*> *P. corethurus*> *P. simlaensis*> *E. assamensis*> *A. cortices* (**Table 2.2**). Based on their RA (**Fig. 2.8-B**), one recedent, three subdominant, and five dominant earthworm species were classified. Similar to MF, earthworms from PL belongs to three ecological categories such as epigeic, epianecic, and endogeic (**Table 2.2**). The maximum density of earthworms was contributed by *D. nepalensis* (45.03 ind.m^{-2}) and *D. assamensis* (50.96 ind.m^{-2}) in mixed forest and plantation area, respectively. Total earthworm biomass was higher in plantation (338.47 g.m^{-2}) compared to mixed forest (254.55 g.m^{-2}). However, no significant mean difference (p>0.05) was observed.

Earthworm species in MF were found to be more diverse with a Shannon Weiner diversity index (H´) of 2.01±0.24, Simpson diversity (D)-0.31±0.07, Evenness -0.9±0.04, and Margalef (S) richness-3.29±0.26. While in PL, the diversity index was 1.56±0.37 and

0.21±0.06 for H′ and D with Evenness 0.83±0.04 and Margalef (S) richness. 1.14±0.07 respectively. Although differences in diversity indices was observed between the study area (**Table 2.3**), except Margalef (S), independent sample t- test shows no significant differences between sites (p<0.05). Also, two studies sites showed high species similarity. Bray-Curtis similarity index shows that MF and PL share 73% species similarity.

Fig. 2.7 Earthworm specimens collected from the study area and earthworms undergoing adaptations during dry seasons.

Table 2.1 Earthworm population characteristics and dominance from Mixed forest (MF)

Family	Species name	Density (ind./m^2)	Relative Abundance-RA (%)	Dominance	Ecological category
Glossoscolecidae	*P. corethurus*	17.18±0.54	7.45	Sub Dominant	Endogeic
Megascolecidae	*A. gracilis*	10.66±0.49	4.62	Sub Dominant	Epianecic
	M. houlleti	11.25±0.63	4.88	Sub Dominant	Epianecic
	P. excavatus	44.44±1.78	19.28	Dominant	Epigeic
	P. simlaensis	15.40±0.74	6.68	Sub Dominant	Epigeic
Moniligastridae	*D. hodgarti*	18.37±0.65	7.96	Sub Dominant	Endogeic
	D. nepalensis	45.03±1.13	19.53	Dominant	Endogeic
	Drawida sp.	30.22±1.43	13.11	Dominant	Endogeic
Octochaetidae	*E. festivus*	24.88±1.09	10.79	Dominant	Endogeic
	E. assamensis	13.03±0.54	5.69	Sub Dominant	Endogeic

Table 2.2 Earthworm population characteristics and dominance from Plantation (PL)

Family	Species	Density (ind.m-2) (± SE)	Relative Abundance-RA (%)	Dominance	Ecological category
Glossoscolecidae	*P. corethurus*	26.07±0.92	9.42	Sub Dominant	Endogeic
Megascolecidae	*A. corticis*	7.70±0.34	2.78	Recedent	Epianecic
	P. excavatus	48.59±1.37	17.55	Dominant	Epigeic
	P. simlaensis	14.22±1.04	5.13	Sub Dominant	Epigeic
Moniligastridae	*D. nepalensis*	43.85±1.26	15.84	Dominant	Endogeic
	D. assamensis	50.96±0.98	18.41	Dominant	Endogeic
	Drawida sp	30.22±0.87	10.92	Dominant	Endogeic
Octochaetidae	*E. assamensis*	12.44±0.51	4.49	Sub Dominant	Endogeic
	E. festivus	42.66±0.88	15.41	Dominant	Endogeic

Table 2.3 Comparison of earthworm diversity indices in the study area

Parameters	**Mixed forest**	**Plantation**	*t*	Df	*p*-value
Shannon-Weiner diversity (H')	2.01±0.24	1.56±0.37	2.00	6	0.09
Simpson diversity (D)	0.31±0.07	0.21±0.06	2.09	6	0.08
Evenness	0.9±0.04	0.83±0.04	1.68	6	0.14
Margalef (S)	3.29±0.26	1.14±0.07	15.57	6	0.001
Bray-Curtis similarity index (%)	73				

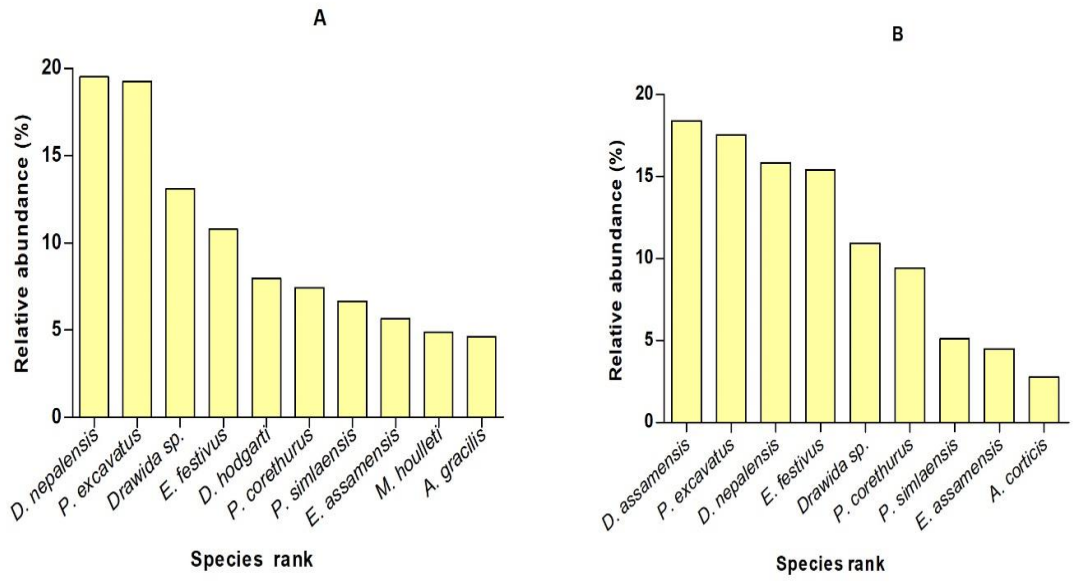

Fig. 2.8 (A) Relative abundance of earthworm species in mixed forest (MF), (B) Relative abundance of earthworm species in Plantation (PL).

2.3.2 Monthly variation of earthworm density and biomass

In MF, density of Juvenile, young and adult increased sharply in the month of March and continued till late October with fluctuations. For instance, the density for juveniles ranges from 0-7.7±0.12 ind.m^{-2} (Nov-May), for young it was 3.55±0.1-10.07±1.2 ind.m^{-2} (April-July), and for adult it was 0-24.88±6.5 ind.m^{-2} (February-May). The total density ranges from 3.77±1.69 - 48.57±8.99 ind.m^{-2} (December-July) as shown in **Fig. 2.9 (A-D)**. Similarly, earthworm biomass also varied widely with juveniles showing a range of 0-1.84±0.12 g.m^{-2} (November-May), for young it ranged from 0.67±0.1-10.56±1.2 g.m^{-2} (December-July) and for adult, it ranged from 0-41.1±3.4 g.m^{-2} (February-May). The total biomass ranged from 1.21±0.35 to 46.96±22.65 g.m^{-2} (December-May) (**Fig. 2.10-A-D**).

In PL, density of juveniles ranged from 0.59±0.23-15.40±3.1 ind.m^{-2} (December-August), for young, 0.59±0.1-33.18±5.5 ind.m^{-2} (March-August), and for adult, it ranged from 0-26.07±5.89 ind.m^{-2} (March-August) (**Fig. 2.11-A-C**). Total earthworm density in PL ranges from 3.55±0.69-74.66±10.19 ind.m^{-2} (February-August) as shown in the **Fig. 2.11(D)**. Similarly earthworm biomass varied with the juvenile showing a range of 0.14-15.8±1.78 (March-August), for young 0.41±0.1-16.77±5.2 g.m^{-2} (November-August), and for adult 0-59.07±10.56 g.m^{-2} (March-August). The total maximum biomass was recorded during August with 91.64±15.45 g.m^{-2} while minimum was recorded in the month of March with 2.21±0.75 g.m^{-2} (**Fig. 2.12-D**). Although variations were observed, earthworm density ($t_{(70)}$=-0.18, p=0.85) and biomass ($t_{(70)}$=-0.73. p=0.46), they were not differ significantly between the two study areas (**Table 2.4**).

Table 2.4 Results of independent sample t-test for earthworm density and biomass between study sites.

Source of variations		Levene's Test for Equality of Variances		t-test for Equality of Means		
		F	Sig.	t	df	Sig. (2-tailed)
Density	Equal variances assumed	0.47	0.49	-0.18	70	0.85
	Equal variances not assumed			-0.18	68.41	0.85
Biomass	Equal variances assumed	2.59	0.11	-0.73	70	0.46
	Equal variances not assumed			-0.73	63.86	0.46

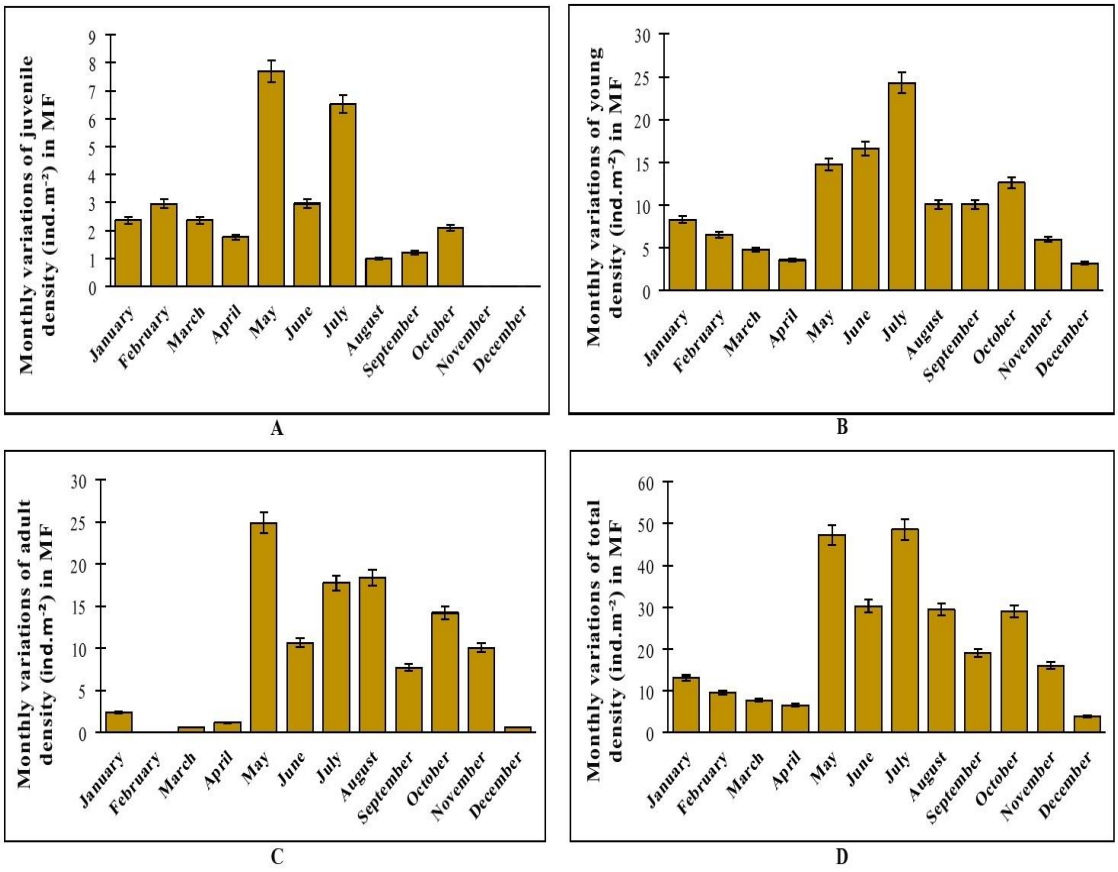

Fig. 2.9 Monthly variations of Juvenile (A) young (B) Adult(C), and Total (D) earthworm density in MF.

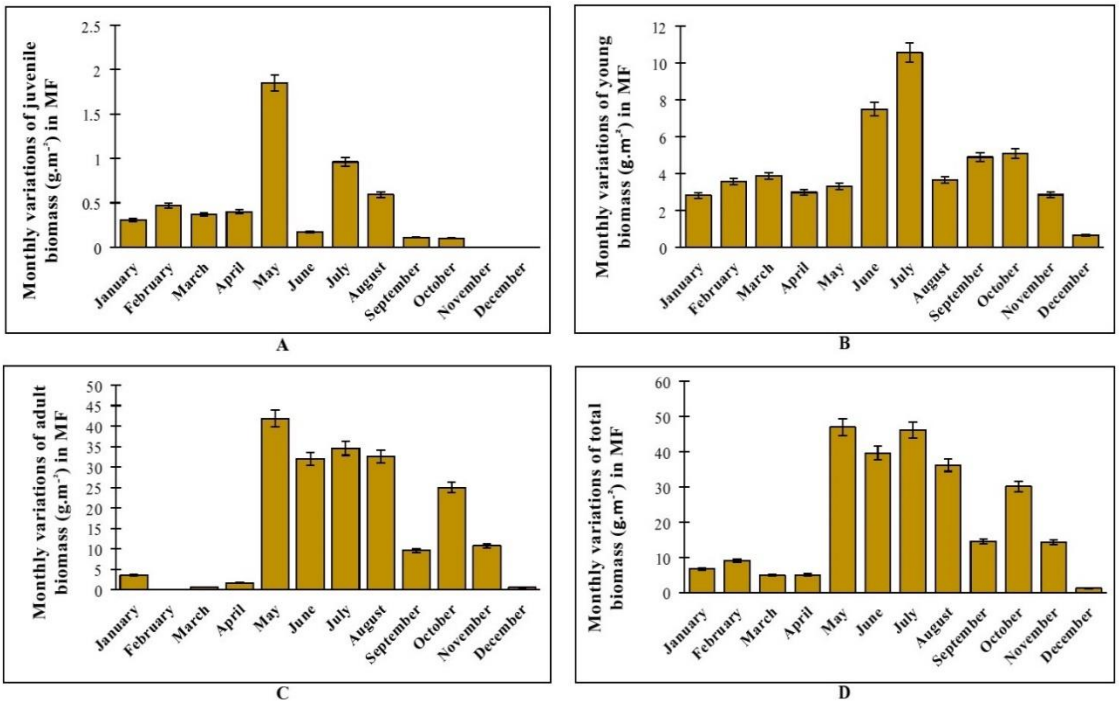

Fig. 2.10 Monthly variations of Juvenile (A) young (B) Adult(C), and Total (D) earthworm biomass in MF.

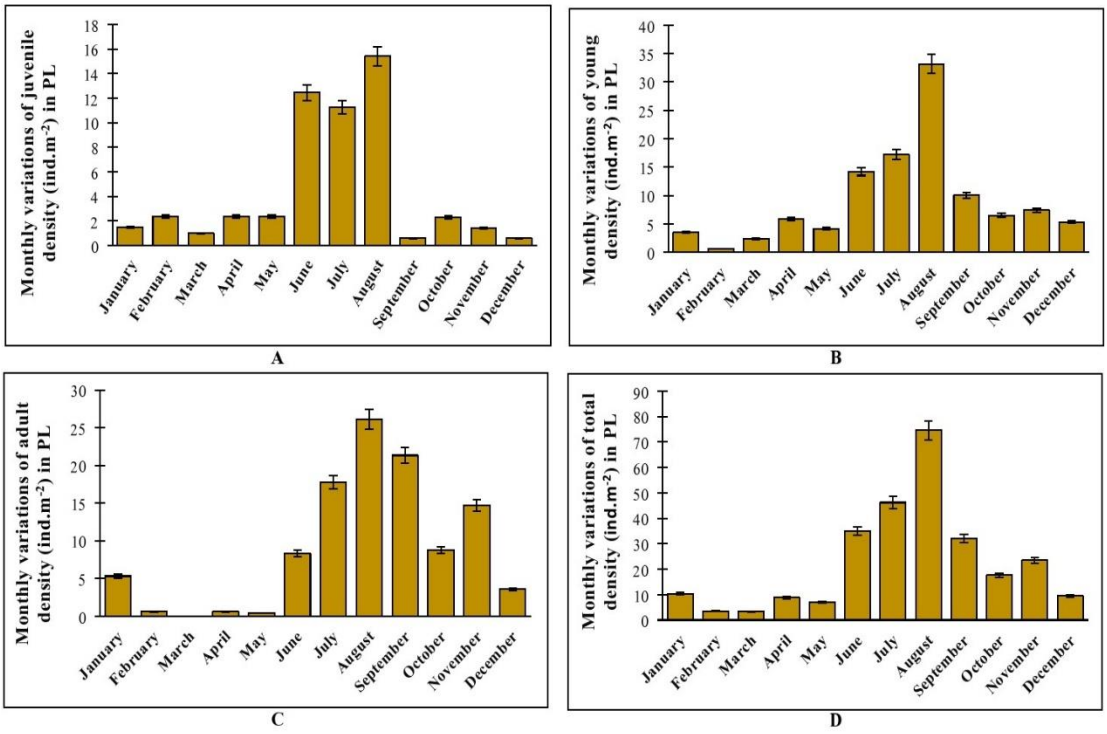

Fig. 2.11 Monthly variations of Juvenile (A) young (B) Adult(C), and Total (D) earthworm density in PL.

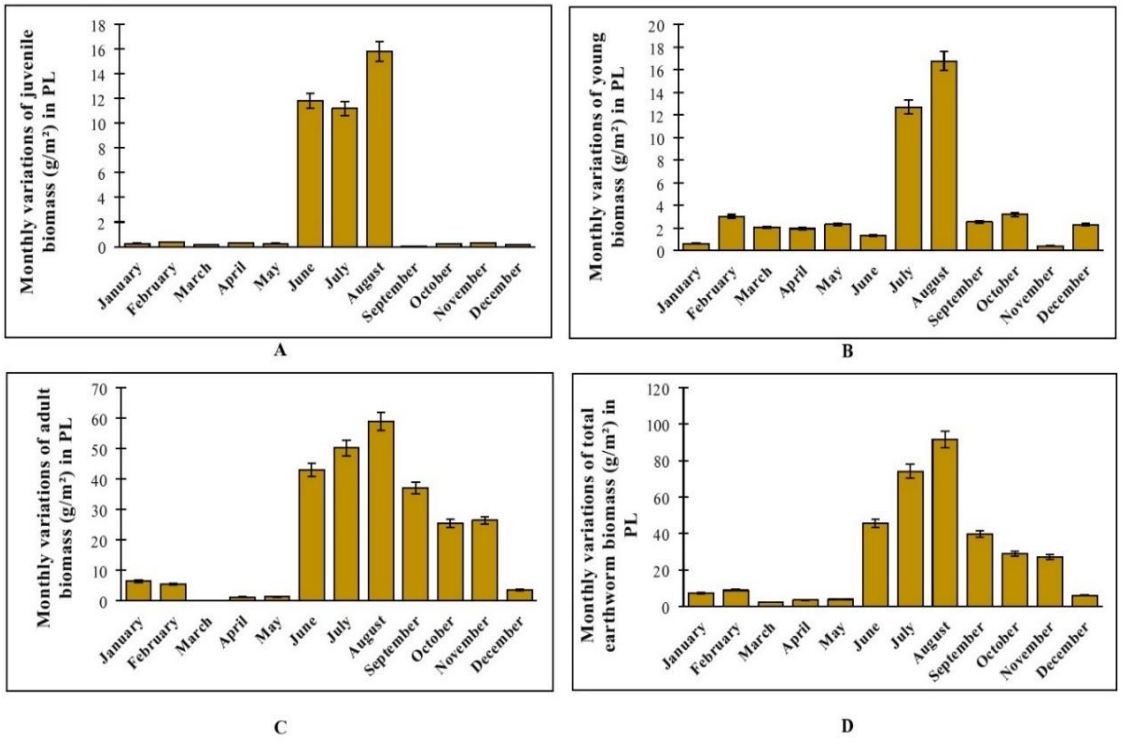

Fig. 2.12 Monthly variations of (A) Juvenile (B) young (C) Adult, and (D) Total earthworm biomass in PL.

2.3.3 Seasonal variations of density and biomass

In MF, total juvenile density varied from 2.37 (winter) to 17.18 55.62 ind.m^{-2} (monsoon). Young population density vary from 14.8 to 55.62 ind.m^{-2}, exhibiting minimum and maximum during spring and monsoon respectively. Similarly adult group of earthworm was recorded maximum during monsoon (53.31 55.62 ind.m^{-2}) and minimum in spring (55.62 ind.m^{-2}) (**Fig. 2.13-A-C**). Having shown significant difference among the seasons (**Table 2.5**), total density of earthworm was recorded maximum and minimum during monsoon (126.12±21.5 55.62 ind.m^{-2}) and spring (23.67±6.55 55.62 ind.m^{-2}) respectively. Population density of juveniles ($F_{(3,8)}$=6.68, p=0.014), young ($F_{(3,8)}$=12.8, p=0.002), and adult ($F_{(3,8)}$=7.1, p=0.012) varied significantly in different seasons (Table 2.6). Multiple comparison test showed that population density in all groups were

significantly higher in monsoon (p<0.05) than all other seasons, however the difference was not significant among post monsoon, spring and winter seasons. Two-way ANOVA test shows that, in MF, regional seasons ($F_{(3,6)}$= 18.48, p=0.000002), earthworm age category ($F_{(2,6)}$=13.86, p=0.0001), and the interaction ($F_{(6,36)}$=3.57, p=0.01) of the two factors have a significant effect on earthworm density (**Table 2.7**).

Similarly, seasonal biomass of Juvenile, young and adult varied from 0.9 (post monsoon) to 5.46 g.m^{-2} (monsoon); from 6.35 (Winter) to 21.36 g.m^{-2} (Monsoon) and from 3.01 (Spring) to 108.36 g.m^{-2} (monsoon) respectively. (**Fig. 2.14-A-C**). Maximum and minimum total biomass of earthworm were recorded during monsoon (132.7±22.14 g.m^{-2}) and spring (18.93±3.79 g.m^{-2}) respectively. Analysis of variance (one-way ANOVA) shows that total earthworm biomass varied significantly ($F_{(3,35)}$=8.1, p=0.001) depending on the seasons (**Table 2.5**). Multiple comparison test shows young biomass in monsoon was significantly (p<0.05) higher than winter but no significant (p>0.05) differences was observed compared to spring and post monsoon. Two-way ANOVA test indicate that earthworm biomass are significantly affected by seasons ($F_{(3,6)}$=17.69, p=0.000003), age category ($F_{(3,6)}$=43.35, p=1.0782E-8), and interactions of the two factors ($F_{(3,6)}$=13.79, p=9.5244E-7).

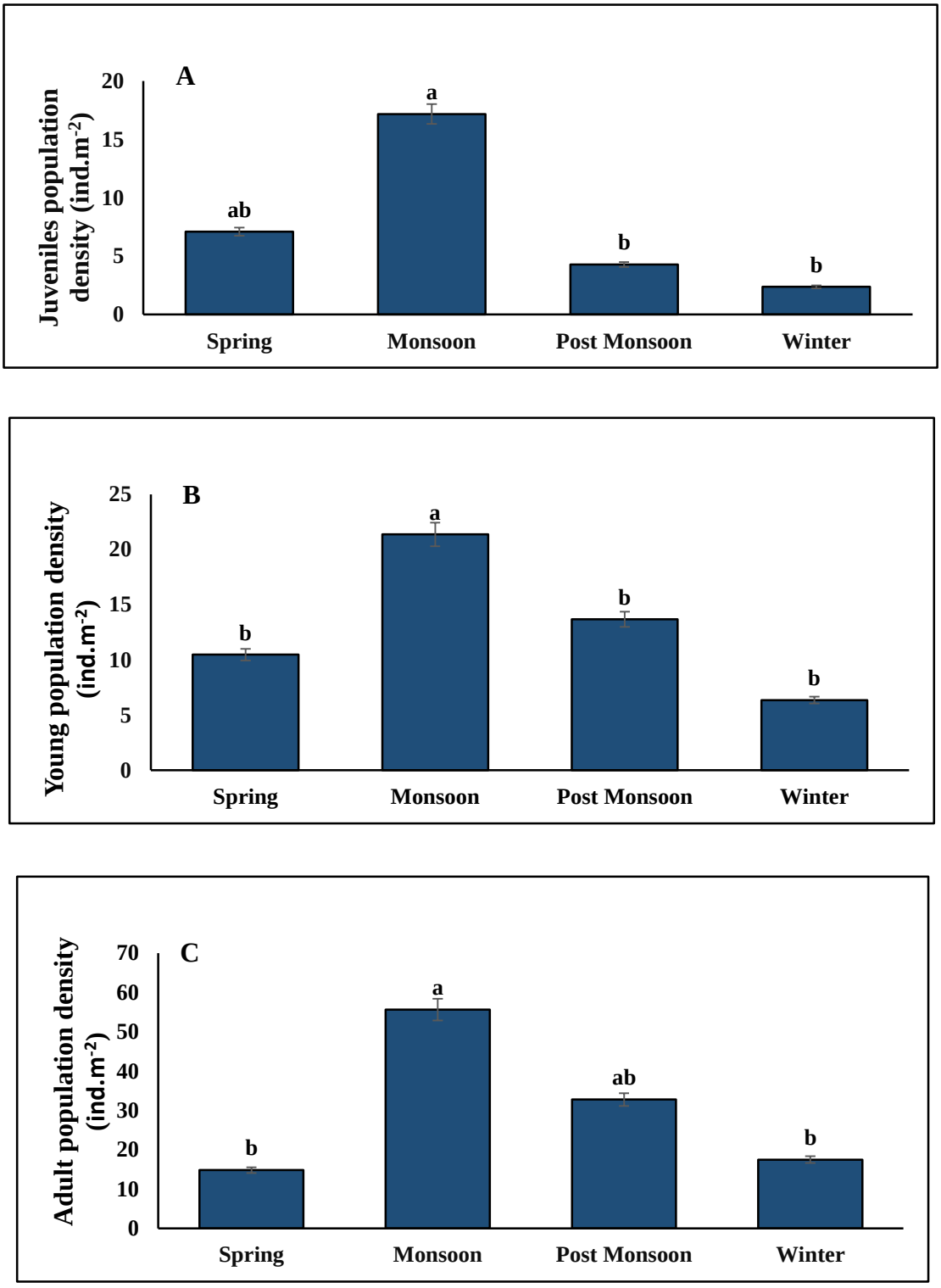

Fig. 2.13 Seasonal variations of (A) Juvenile (B) Young (C) Adult earthworm density in MF. Different superscript in the bar indicates statistically significant difference at $p<0.05$ by the multiple comparisons Tukey test.

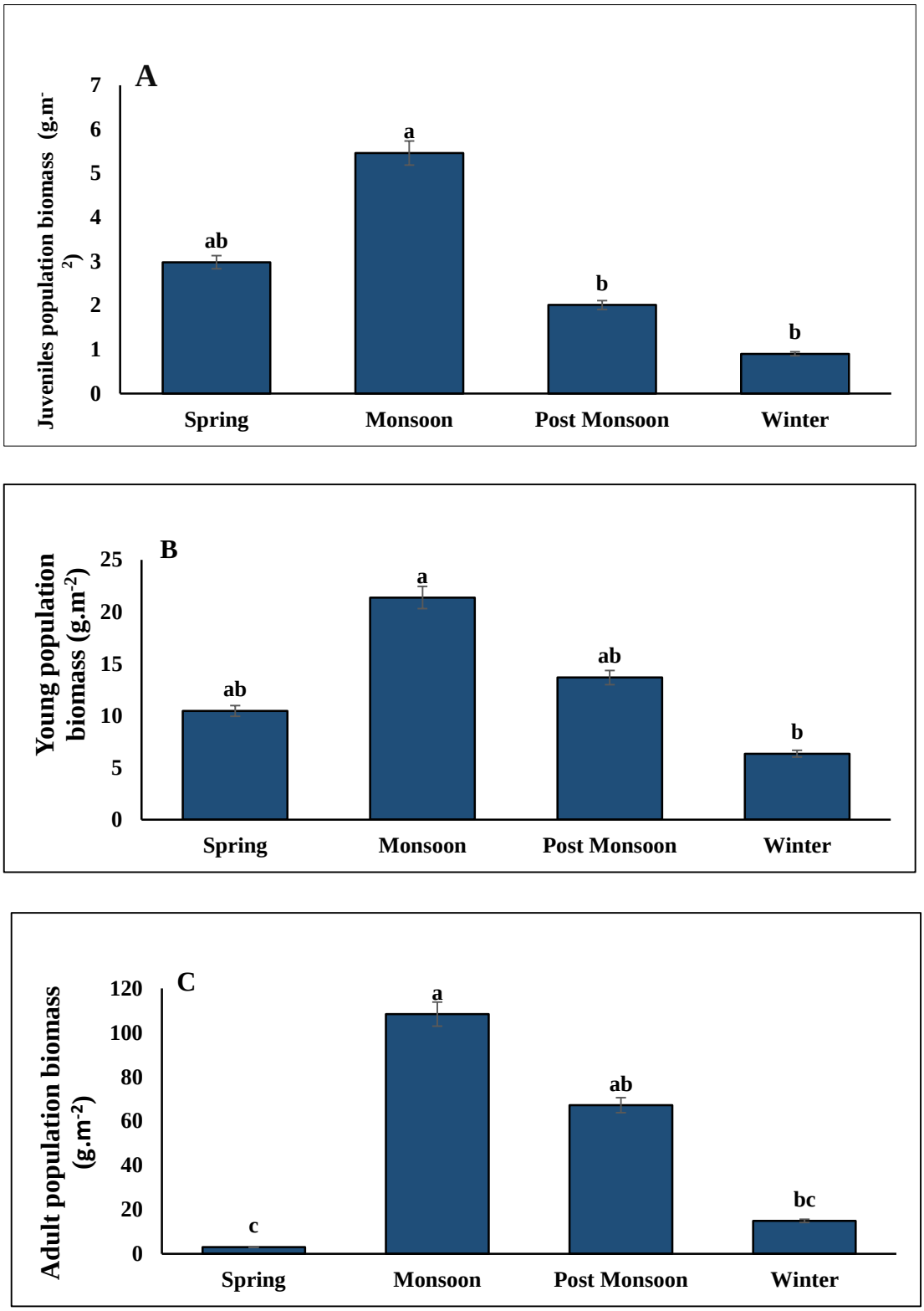

Fig. 2.14 Seasonal variations of Juvenile (A), Young (B) and Adult (C) biomass in MF. Different superscript in the bar indicates statistically significant difference at $p<0.05$ by the multiple comparisons Tukey test.

Table 2.5 One-way ANOVA test results for seasonal variations of total earthworm density and biomass in MF.

Source of variations		Sum of Squares	df	Mean Square	*F*	Sig.
Total earthworm density	Between Groups	394.146	2	197.073	5.154	.011
	Within Groups	1261.918	33	38.240		
	Total	1656.064	35			
Total earthworm biomass	Between Groups	1547.713	2	773.856	8.960	.001
	Within Groups	2850.276	33	86.372		
	Total	4397.989	35			

$p<0.05$ indicate statistical significant difference at 95% confidence interval.

Table 2.6 One-Way ANOVA tests results for seasonal variations of different earthworm age category density and biomass in MF.

Source of variations		Sum of Squares	df	Mean Square	*F*	Sig.
Juvenile Density	Between Groups	43.440	3	14.480	6.686	0.014
	Within Groups	17.325	8	2.166		
	Total	60.764	11			
Young Density	Between Groups	350.790	3	116.930	12.804	0.002
	Within Groups	73.058	8	9.132		
	Total	423.848	11			
Adult Density	Between Groups	566.793	3	188.931	7.180	0.012
	Within Groups	210.513	8	26.314		
	Total	777.306	11			
Juvenile Biomass	Between Groups	45.582	3	1.861	6.68	0.014
	Within Groups	13.948	8	1.743		
	Total	19.530	11			
Young Biomass	Between Groups	41.351	3	13.450	4.446	0.022
	Within Groups	31.222	8	3.903		
	Total	71.573	11			
Adult Biomass	Between Groups	2377.412	3	792.471	16.620	0.001
	Within Groups	381.453	8	47.682		
	Total	2758.865	11			

$p<0.05$ indicate statistical significant difference at 95% confidence interval.

Table 2.7 Two-Way ANOVA tests result for variations of total earthworm density influenced by regional seasons and earthworm age category in MF.

Dependent Variable: Earthworm density					
Source	Type III Sum of Squares	df	Mean Square	*F*	Sig.
Corrected Model	1346.022[a]	11	122.366	9.472	0.000003
Intercept	1849.397	1	1849.397	143.160	1.3279E-11
Seasons	715.307	3	238.436	18.457	0.000002
Age Category	358.188	2	179.094	13.863	0.000100
Seasons*Age Category	277.339	6	46.223	3.578	0.011252
Error	310.042	24	12.918		
Total	3533.251	36			
Corrected Total	1656.064	35			

a. R Squared = .813 (Adjusted R Squared = .727)
$p<0.05$ indicate statistical significant difference at 95% confidence interval.

Table 2.8 Two-Way ANOVA tests result for variations of total earthworm biomass influenced by regional seasons and earthworm age category in MF.

Dependent Variable: Earthworm Biomass					
Source	Type III Sum of Squares	df	Mean Square	*F*	Sig.
Corrected Model	3966.083[a]	11	360.553	20.173	1.6694E-9
Intercept	1782.657	1	1782.657	99.739	5.0425E-10
Seasons	948.999	3	316.333	17.699	0.000003
Age Category	1549.630	2	774.815	43.351	1.0782E-8
Seasons*Age Category	1479.187	6	246.531	13.793	9.5244E-7
Error	428.957	24	17.873		
Total	6198.201	36			
Corrected Total	4395.040	35			

a. R Squared = .902 (Adjusted R Squared = .858)
$p<0.05$ indicate statistical significant difference at 95% confidence interval.

In PL, the seasonal fluctuation of juvenile, young and adult density ranged from 4.29 (post monsoon) to 39.1104 ind.m^{-2} (monsoon), 9.422 (winter) to 64.5922 ind.m^{-2} (monsoon) and 0.99 (spring) to 52.1443 ind.m^{-2} (monsoon) (**Fig. 2.15-A-C**). Total earthworm density was found to be maximum in monsoon (155.84±12.74), followed by

post monsoon (73.09±20.27), winter (23.32±2.87) and spring (19.17±5.76) having showed significant difference among the seasons (Table 9). One way ANOVA indicated that with changing seasons, density of juvenile ($F_{(3,8)}$=58.28, p=0.00001), young ($F_{(3,8)}$=7.41, p=0.01), adult ($F_{(3,8)}$=7.41, p=0.01), and total ($F_{(3,\ 35)}$=15.88, p=0.000002) density varied significantly (**Table 2.10**). Two-way ANOVA showed that regional seasons ($F_{(2,6)}$=4.14, p=0.02), different earthworm age categories ($F_{(3,6)}$=21.79, p=4.9141E-7) significantly affect the variations of density in PL, however, the interaction of these two factors ($F_{(2,36)}$=1.93, p=0.11) does not have significant effect on variations of density (**Table 2.11**).

Biomass of juvenile, young and adult varied from 0.625 (post monsoon) to 28.1 g.m^{-2} (monsoon), 6.0 (winter) to 30.82 g.m^{-2} (monsoon), and 2.42 (spring) to 152.29 g.m^{-2} (monsoon). Highest total biomass was observed during monsoon with 211.21±17 g.m^{-2} followed by post monsoon (95.69±9.45 g.m^{-2}), winter (22.08±3.5 g.m^{-2}) and spring (9.51±2.8 g.m^{-2}) (**Fig. 2.16-A-C**). Among different seasons, biomass of juvenile (F(3,8)=4.42,p=0.041), young ($F_{(3,11)}$=18.26, p=0.001), adult ($F_{(3,11)}$=59.35, p=0.00001), and total ($F_{(3,35)}$=5.54, p=0.004) varied significantly in PL (**Table 2.10**). Multiple comparison test shows that young biomass in monsoon was significantly higher (p<0.05) than other seasons, however, difference was not significant among spring, post monsoon, and winter. With the minimum recorded spring, adult biomass of earthworm significantly increased (p<0.05) during Monsoon, thereafter it decreased gradually in post monsoon and winter season. In PL, earthworm biomass variations were significantly affected by changing seasons ($F_{(2,6)}$=48.93, p=2.2257E-10), different earthworm age categories

($F_{(2,6)}$=68.96, p=1.1233E-10), and interactions of these two factors ($F_{(6, 36)}$=20.07, p=2.884E-10) (**Table 2.12**).

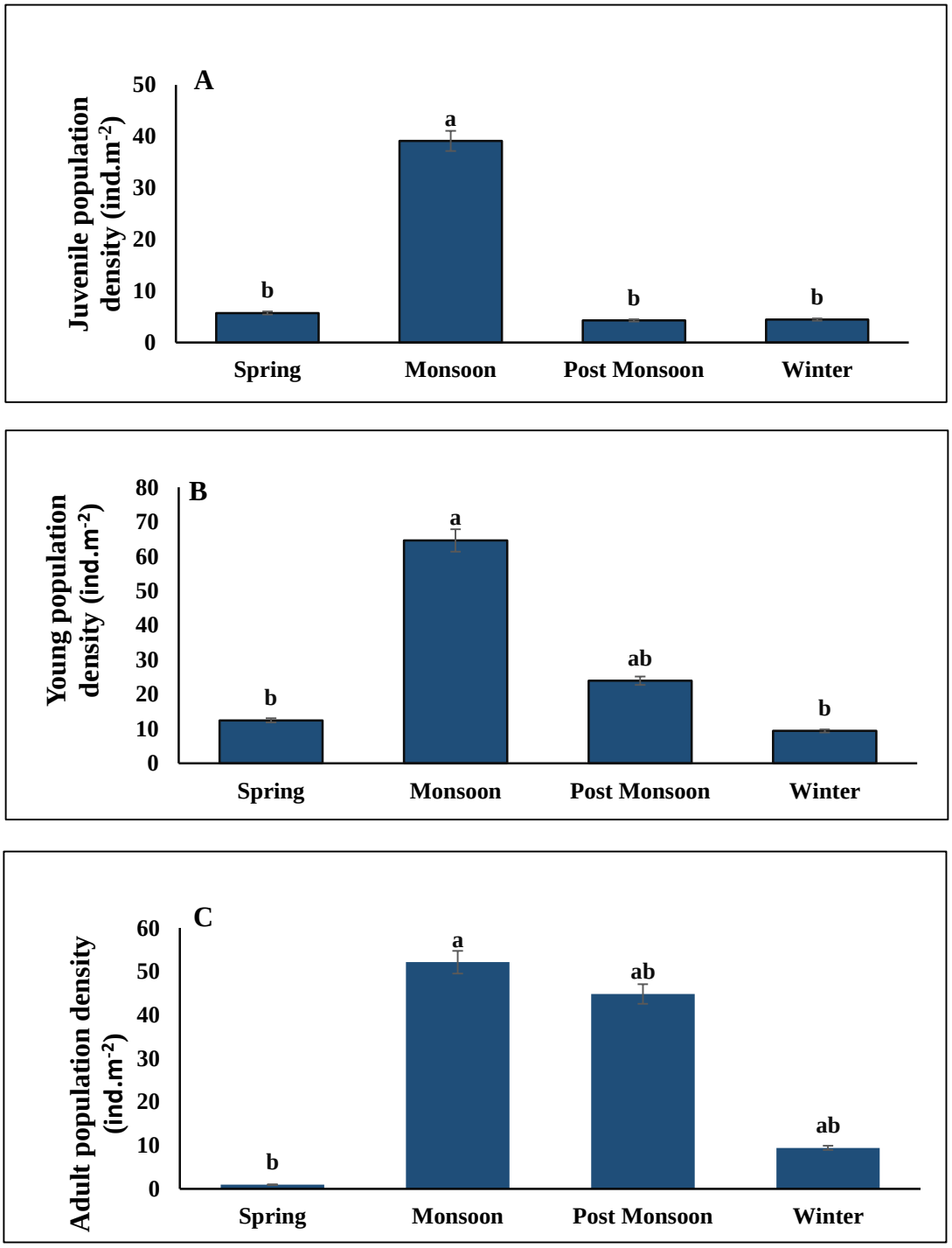

Fig. 2.15 Seasonal variations of Juvenile (A), Young (B) and Adult (C) density in PL. Different superscript in the bar indicates statistically significant difference at p<0.05 by the multiple comparisons Tukey test.

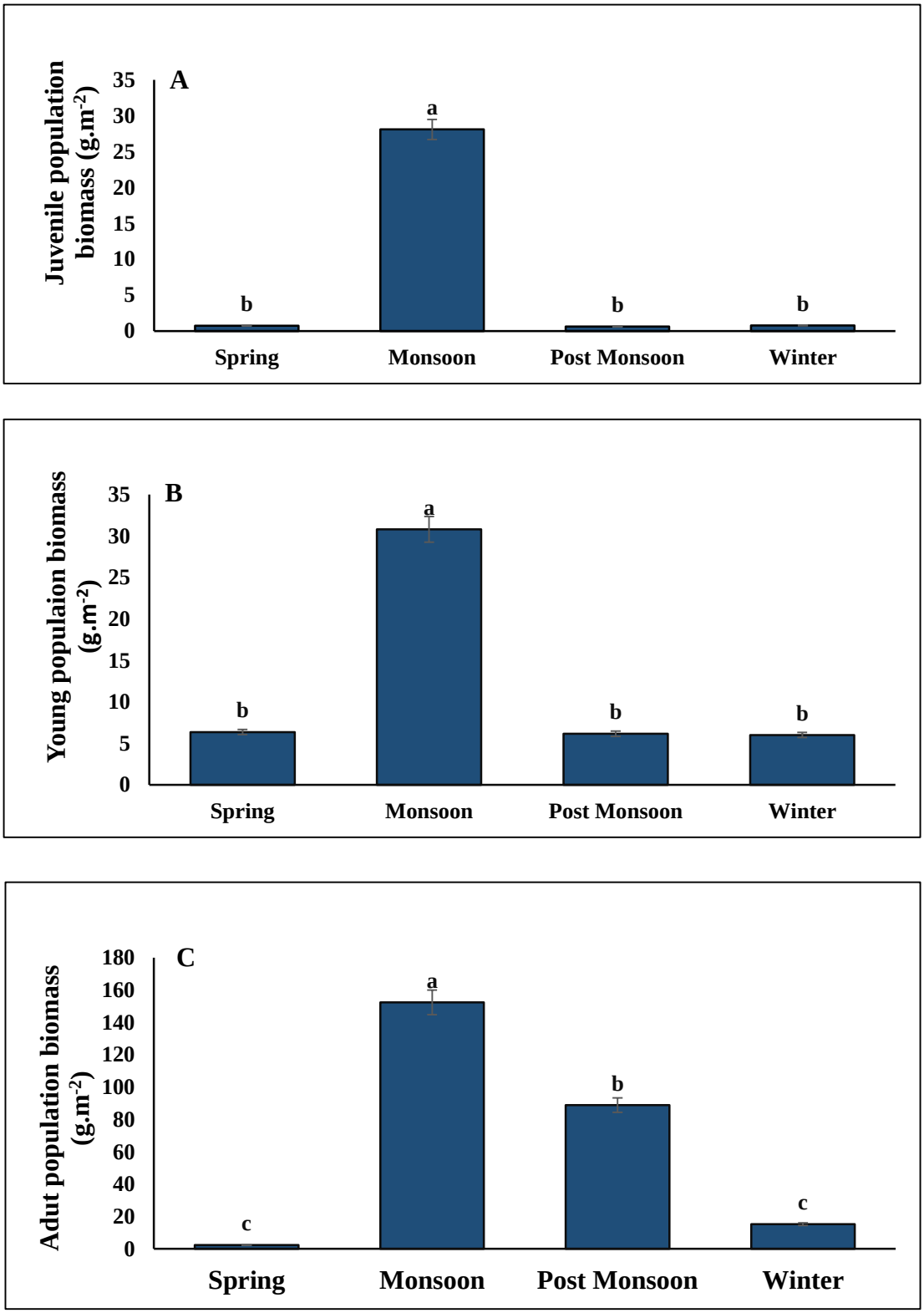

Fig. 2.16 Seasonal variations of Juvenile (A), Young (B) and Adult (C) biomass in PL. Different superscript in the bar indicates statistically significant difference at $p<0.05$ by the multiple comparisions Tukey test.

Table 2.9 One-way ANOVA test results for seasonal variations of total earthworm density and biomass in PL.

Source of variations		Sum of Squares	df	Mean Square	F	Sig.
Density	Between Groups	1346.968	3	448.989	15.886	.000002
	Within Groups	904.428	32	28.263		
	Total	2251.396	35			
Biomass	Between Groups	2855.499	3	951.833	5.545	.004
	Within Groups	5492.700	32	171.647		
	Total	8348.199	35			

$p<0.05$ indicate statistical significant difference at 95% confidence interval.

Table 2.10 One-Way ANOVA tests results for seasonal variations of different earthworm age category density and biomass in PL.

Source of variations		Sum of Squares	df	Mean Square	*F*	Sig.
Juvenile density	Between Groups	294.296	3	98.099	58.297	.000
	Within Groups	13.462	8	1.683		
	Total	307.758	11			
Young density	Between Groups	646.995	3	215.665	7.416	.011
	Within Groups	232.646	8	29.081		
	Total	879.641	11			
Adult density	Between Groups	645.012	3	215.004	6.924	.013
	Within Groups	248.400	8	31.050		
	Total	893.412	11			
Juvenile biomass	Between Groups	187.543	3	62.514	4.420	.041
	Within Groups	113.155	8	14.144		
	Total	300.698	11			
Young biomass	Between Groups	151.850	3	50.617	18.269	.001
	Within Groups	22.165	8	2.771		
	Total	174.014	11			
Adult biomass	Between Groups	4859.154	3	1619.718	59.354	.000001
	Within Groups	218.312	8	27.289		
	Total	5077.466	11			

$p<0.05$ indicate statistical significant difference at 95% confidence interval.

Table 2.11 Two-Way ANOVA tests result for variations of total earthworm density influenced by regional seasons and earthworm age category in PL.

Dependent Variable: Earthworm density					
Source	Type III Sum of Squares	df	Mean Square	*F*	Sig.
Corrected Model	1756.888[a]	11	159.717	7.752	0.000016
Intercept	2046.750	1	2046.750	99.335	5.2462E-10
Age category	170.585	2	85.292	4.140	0.028542
Seasons	1346.968	3	448.989	21.791	4.9141E-7
Age category * Seasons	239.336	6	39.889	1.936	0.115811
Error	494.508	24	20.604		
Total	4298.146	36			
Corrected Total	2251.396	35			

a. R Squared = .780 (Adjusted R Squared = .680). $p<0.05$ indicate statistical significant difference at 95% confidence interval.

Table 2.12 Two-Way ANOVA tests result for variations of total earthworm biomass influenced by regional seasons and earthworm age category in PL.

Dependent Variable: Earthworm biomass					
Source	Type III Sum of Squares	df	Mean Square	*F*	Sig.
Corrected Model	7881.390[a]	11	716.490	36.837	2.5148E-12
Intercept	3182.973	1	3182.973	163.646	3.2891E-12
Age category	2682.847	2	1341.424	68.966	1.1233E-10
Seasons	2855.499	3	951.833	48.936	2.2257E-10
Age category * Seasons	2343.043	6	390.507	20.077	2.884E-8
Error	466.809	24	19.450		
Total	11531.172	36			
Corrected Total	8348.199	35			

a. R Squared = .944 (Adjusted R Squared = .918). $p<0.05$ indicate statistical significant difference at a 95% confidence interval.

2.3.4 Soil physico-chemical characteristics of the study area

In both the study sites, significant (p<0.05) variations of soil temperature was observed among the seasons. Soil temperature ranged from 14.42±2.55 (winter) to 22.63±1.7 (monsoon) in MF and 15.39±1.44 (winter) to 22.98±1.8 (monsoon) in PL (Table 2.13). Similarly, soil moisture, pH, Av. P varies significantly (p<0.05) depending on the seasons. Soil moisture ranged from 28.23±2.54 (winter) to 50.09±5.59 (monsoon) in MF and 29.7±6.64 (winter) to 51.48±3.07 (monsoon) in PL. Bulk density ranges from 0.79±0.04 (winter) to 0.87±0.015 (spring) in MF, while in PL, it ranges from 0.76±0.03 (spring) to 0.82±0.02 (post monsoon). The soil pH ranged from 4.75±0.07 (post monsoon) to 5.75±0.32 (monsoon) in MF and 5.4±0.42 (post monsoon) to 6.13±0.85 (monsoon) in PL. Seasonally OC ranged from 0.15±0.04 (winter) to 2.02±2.2 (monsoon) in MF and 2.02±1.37 (post monsoon) to 2.96±1.60 (winter) in PL. Av. N ranges from 146.34±68.4 (monsoon) to 190.6±3.02 (winter) in MF and 148.7±18.01 (monsoon) to 189.3 ±25.1 (winter) in PL. TN varies between 0.50±0.25 (monsoon) to 0.62±0.12 (spring) in MF while in PL it ranges from 0.49±0.05 (post monsoon) to 0.77±0.14 (winter). In MF, AV. P ranges from 8.68±0.18 (post monsoon) to 28.3±8.1 (winter) and in PL, it ranges from 27.09±0.54 (post monsoon) to 68.72±8.37 (spring). Av. K ranges from 149.33±7.5 (winter) to 163±3.6 (monsoon) in MF and 140.3±22.4 (winter) to 149±4.35 (spring) in PL.

Table 2.13 Edaphic factor characteristics of four different seasons in MF and PL.

Parameters	Study sites	Spring	Monsoon	Post Monsoon	Winter	*p*-value
Temp (˚C)	MF	20.06±0.90	22.63±1.7	16.02±0.26	14.42±2.55	0.012*
	PL	20.73±0.98	22.98±1.8	20.35±2.68	15.39±1.44	0.014*
Moisture (%)	MF	39.32±12.8	50.09±5.59	36.65±3.77	28.23±2.54	0.0033*
	PL	41.78±5.38	51.48±3.07	40.37±8.71	29.7±6.64	0.002**
Bulk density	MF	0.87±0.015	0.79±0.06	0.85±0.06	0.79±0.04	ns
	PL	0.76±0.03	0.8±0.07	0.82±0.02	0.8±0.1	0.036*
pH	MF	5.46±0.3	5.75±0.32	4.75±0.07	5.4±0.26	0.013*
	PL	6±0.34	6.13±0.85	5.4±0.42	5.8±0.26	ns
OC (%)	MF	1.65±0.42	2.02±2.2	0.69±0.007	0.15±0.04	ns
	PL	2.51±0.55	2.64±0.55	2.02±1.37	2.96±1.60	ns
Av. N (mg/kg)	MF	154.7±65.4	146.3±68.4	188.1±28.3	190.6±3.02	ns
	PL	156.4±32.6	148.7±18.0	156.3 ±23.7	189.3 ±25.1	ns
TN (%)	MF	0.62±0.12	0.50±0.25	0.56±0.14	0.51±0.06	ns
	PL	0.6±0.15	0.64±0.06	0.49±0.05	0.77±0.14	ns
Av. P (mg/kg)	MF	19.6±4.78	21.8±8.53	8.68±0.18	28.3±8.1	0.0002**
	PL	68.72±8.37	37.57±6.67	27.09±0.54	32.84±6.59	0.019*
Av. K (kg/ha)	MF	151±7	163±3.6	162.6±13.5	149.33±7.5	ns
	PL	149±4.35	144.33±11.5	147.66±7.3	140.3±22.4	ns

** indicates a statistically high significant difference, * indicates a statistically significant difference, ns-not significant.

2.3.5 Correlations of earthworm density with soil physico-chemical parameters

Pearson correlation analysis shows that, in MF, density of young (r=0.62, p=0.03) and adult (r=0.72, p=0.008), and biomass of young (r=0.59, p=0.04) and adult (r=0.75, p=0.005) shared significant positive relationship with soil temperature. Similarly, density and biomass of young and adult earthworm also shared significant positive relationship with total rainfall of the area (**Table 2.14**). Throughout the season, variations of total earthworm density were significantly correlated with soil temperature (r=0.78, p=0.002), moisture (r=0.84, p=0.001), bulk density (r=0.63, p=0.02), and total rainfall (r=0.71, p=0.009) (**Fig. 2.17-A-F**). Total biomass also shared positive relationship with soil temperature, moisture, RH, and TR (**Table 2.15**).

In PL, the density of juvenile (r=0.61, p=0.03), young (r=0.65, p=0.02), and adult (r=0.58, p=0.04) and biomass of adult (r=0.69, p=0.01) earthworm's exhibit positive relationships with soil temperature (**Fig. 2.18-A-F**). Similarly, density and biomass of juvenile (r=0.68, p=0.01; r=0.63, p=0.02), young (r=0.0.76, p=0.004; r=0.62, p=0.03), and adult (r=0.63, p=0.02; r=0.68, p=0.01) earthworm exhibit significant positive relationship with soil moisture. Earthworm density and biomass also shared positive relationship with pH, OC, and Av. P, TR, humidity (**Table 2.16**). Similarly, total earthworm density was positively correlated with temperature (r=0.62, p=0.02), moisture (r=0.67, p=0.01) and OC (r=0.64, p=0.02). While total biomass shared significant positive correlations with soil temperature (r=0.68, p=0.01), moisture (r=0.71, p=0.009), and rainfall (r=0.67, p=0.01) (**Table 2.17**).

Table 2.14 Results of the correlation studies of earthworm density and biomass with abiotic factors in MF.

Parameters		Juvenile density (ind./m^2)	Young density (ind./m^2)	Adult density (ind./m^2)	Juvenile biomass (g/m^2)	Young biomass (g/m^2)	Adult biomass (g/m^2)
Temp (°C)	Pearson Correlation	0.347	0.625*	0.720**	-0.219	0.595*	0.751**
	Sig. (2-tailed)	0.269	0.030	0.008	0.493	0.041	0.005
	N	12	12	12	12	12	12
Moist (%)	Pearson Correlation	0.149	0.468	0.563	-0.130	0.558	0.550
	Sig. (2-tailed)	0.644	0.125	0.057	0.686	0.059	0.064
	N	12	12	12	12	12	12
pH	Pearson Correlation	-0.377	-0.428	-0.594*	0.553	-0.331	-0.548
	Sig. (2-tailed)	0.227	0.165	0.042	0.062	0.293	0.065
	N	12	12	12	12	12	12
OC	Pearson Correlation	0.247	0.379	0.118	-0.204	0.393	0.190
	Sig. (2-tailed)	0.438	0.224	0.715	0.526	0.206	0.554
	N	12	12	12	12	12	12
TN	Pearson Correlation	-0.142	-0.152	-0.073	-0.495	-0.310	-0.029
	Sig. (2-tailed)	0.660	0.638	0.821	0.101	0.326	0.929
	N	12	12	12	12	12	12
C:N	Pearson Correlation	0.324	0.433	0.046	0.148	0.565	0.091
	Sig. (2-tailed)	0.304	0.159	0.886	0.646	0.055	0.777
	N	12	12	12	12	12	12
BD	Pearson Correlation	-0.070	0.105	0.180	-0.128	0.274	0.147
	Sig. (2-tailed)	0.830	0.746	0.577	0.691	0.389	0.649
	N	12	12	12	12	12	12
Av. P	Pearson Correlation	-0.014	-0.199	-0.316	0.249	-0.266	-0.140
	Sig. (2-tailed)	0.967	0.535	0.317	0.436	0.404	0.664
	N	12	12	12	12	12	12
Av. K	Pearson Correlation	0.022	0.470	0.521	-0.342	0.404	0.560
	Sig. (2-tailed)	0.945	0.123	0.082	0.276	0.192	0.059

Table 2.14. Conti..

	N	12	12	12	12	12	12
RH	Pearson Correlation	0.548	0.346	0.263	0.333	0.389	0.437
(%)	Sig. (2-tailed)	0.065	0.271	0.408	0.290	0.211	0.156
	N	12	12	12	12	12	12
TR	Pearson Correlation	0.451	0.825**	0.585*	-0.147	0.875**	0.634*
(mm)	Sig. (2-tailed)	0.141	0.001	0.046	0.649	0.000	0.027
	N	12	12	12	12	12	12

** Correlation is significant at the 0.01 level (2-tailed).

*Correlation is significant at the 0.05 level (2-tailed).

Temp-Temperature, Moist-Moisture, OC-Organic carbon, TN-Total Nitrogen, C:N-carbon:nitrogen, BD-Bulk density, Av. P-Available phosphorus, Av. K- available potassium, RH-relative humidity, TR-Total rainfall.

Table 2.15 Results of the correlation studies of total earthworm density and biomass with abiotic factors in MF.

		Temp (°C)	Moist (%)	pH	OC	TN	C:N	BD	Av. P	Av. K	RH (%)	TR (mm)
Total density (ind.m^{-2})	Pearson Correlation	0.786**	0.841**	-0.342	-0.173	-0.472	0.029	0.634*	-0.417	0.429	0.102	0.713**
	Sig. (2-tailed)	0.002	0.001	0.276	0.591	0.121	0.928	0.027	0.178	0.165	0.753	0.009
	N	12	12	12	12	12	12	12	12	12	12	12
Total biomass (g.m^{-2})	Pearson Correlation	0.697*	0.739**	-0.26	-0.247	-0.362	-0.101	0.620*	-0.29	0.402	-0.008	0.587*
	Sig. (2-tailed)	0.012	0.006	0.414	0.439	0.247	0.754	0.032	0.361	0.195	0.98	0.045
	N	12	12	12	12	12	12	12	12	12	12	12

*Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Temp-Temperature, Moist-Moisture, OC-Organic carbon, TN-Total Nitrogen, C:N-carbon:nitrogen, BD-Bulk density, Av. P-Available phosphorus, Av. K- available potassium, RH-relative humidity, TR-Total rainfall.

Table 2.16 Results of the correlation studies of earthworm density and biomass with abiotic factors in PL.

Variables		Juvenile density ($ind.m^{-2}$)	Young density ($ind.m^{-2}$)	Adult density ($ind.m^{-2}$)	Juvenile biomass ($g.m^{-2}$)	Young biomass ($g.m^{-2}$)	Adult biomass ($g.m^{-2}$)
Temp (°C)	Pearson Correlation	0.618*	0.659*	0.581*	0.494	0.489	0.693*
	Sig. (2-tailed)	0.032	0.020	0.048	0.102	0.107	0.012
	N	12	12	12	12	12	12
Moist (%)	Pearson Correlation	0.682*	0.760**	0.632*	0.633*	0.621*	0.685*
	Sig. (2-tailed)	0.015	0.004	0.028	0.027	0.031	0.014
	N	12	12	12	12	12	12
pH	Pearson Correlation	-0.326	-0.556	-0.727**	-0.575	-0.638*	-0.611*
	Sig. (2-tailed)	0.302	0.060	0.007	0.051	0.026	0.035
	N	12	12	12	12	12	12
OC	Pearson Correlation	0.753**	0.758**	0.617*	0.894**	0.929**	0.715**
	Sig. (2-tailed)	0.005	0.004	0.033	0.000	0.000	0.009
	N	12	12	12	12	12	12
TN	Pearson Correlation	0.422	0.126	0.001	0.189	0.163	0.274
	Sig. (2-tailed)	0.172	0.696	0.998	0.556	0.614	0.390
	N	12	12	12	12	12	12
CN	Pearson Correlation	-0.065	-0.157	-0.347	-0.186	-0.210	-0.298
	Sig. (2-tailed)	0.842	0.625	0.269	0.562	0.513	0.346
	N	12	12	12	12	12	12
BD	Pearson Correlation	0.014	0.013	0.029	0.357	0.363	-0.065

Table 2.16. Conti..

	Sig.(2-tailed)	0.965	0.968	0.928	0.254	0.246	0.841
	N	12	12	12	12	12	12
Av. P	Pearson Correlation	-0.375	-0.501	-0.653*	-0.324	-0.273	-0.632*
	Sig. (2-tailed)	0.230	0.097	0.021	0.303	0.390	0.027
	N	12	12	12	12	12	12
Av. K	Pearson Correlation	-0.061	-0.015	0.157	0.127	0.056	0.035
	Sig. (2-tailed)	0.851	0.963	0.625	0.695	0.863	0.914
	N	12	12	12	12	12	12
RH	Pearson Correlation	0.492	0.190	-0.232	0.183	0.185	0.071
(%)	Sig. (2-tailed)	0.104	0.554	0.468	0.569	0.565	0.826
	N	12	12	12	12	12	12
RF	Pearson Correlation	0.522	0.494	0.578*	0.467	0.488	0.711**
(mm)	Sig. (2-tailed)	0.082	0.103	0.049	0.126	0.108	0.010
	N	12	12	12	12	12	12

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Temp-Temperature, OC-Organic carbon, TN-Total Nitrogen, C:N-carbon:nitrogen, BD-Bulk density, Av. P-Available phosphorus, Av. K- available potassium, RH-relative humidity, TR-Total rainfall.

Table 2.17 Results of the correlation studies of total earthworm density and biomass with abiotic factors in PL.

		Temp	Moist	pH	OC	TN	CN	BD	Av. P	Av. K	RH (%)	TR (mm)
Density (ind.m^{-2})	Pearson Correlation	0.626*	0.676*	-0.49	0.644*	0.34	-0.28	-0.23	-0.5	-0.21	0.095	0.543
	Sig. (2-tailed)	0.029	0.016	0.104	0.02	0.29	0.371	0.466	0.098	0.509	0.769	0.068
	N	12	12	12	12	12	12	12	12	12	12	12
Biomass (g.m^{-2})	Pearson Correlation	0.682*	0.717**	-0.46	0.57	0.39	-0.26	-0.25	-0.56	-0.15	0.124	0.675*
	Sig. (2-tailed)	0.015	0.009	0.128	0.05	0.22	0.409	0.428	0.061	0.652	0.701	0.016
	N	12	12	12	12	12	12	12	12	12	12	12

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Temp-Temperature, Moist-Moisture, OC-Organic carbon, TN-Total Nitrogen, C:N-carbon:nitrogen, BD-Bulk density, Av. P-Available phosphorus, Av. K- available potassium, RH-relative humidity, TR-Total rainfall.

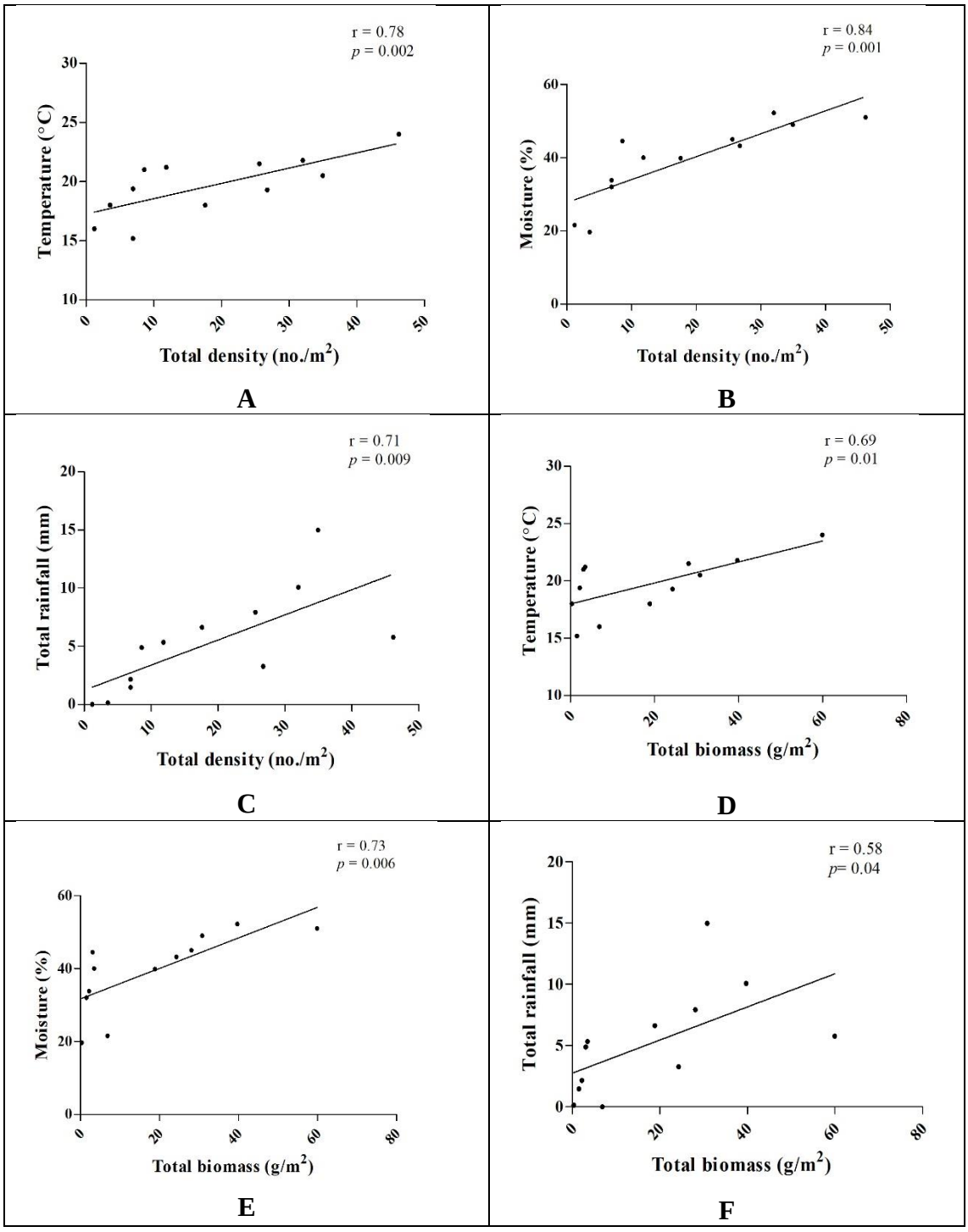

Fig. 2.17 Significant positive correlation of density with (A) Soil temperature (°C) (B)-soil moisture (C) total rainfall and biomass with (D)-with Soil temperature(E)- soil moisture (F) total rainfall in MF.

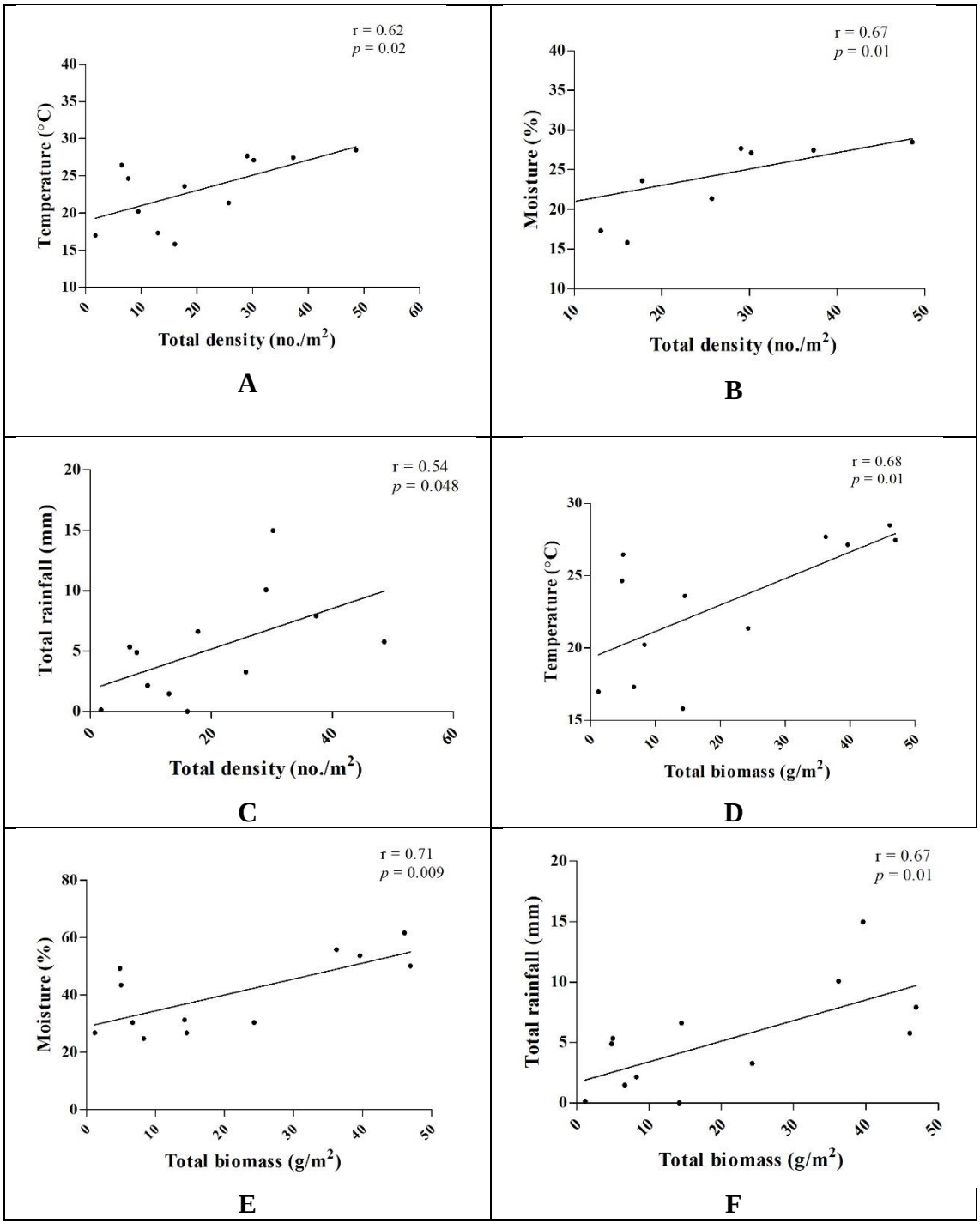

Fig. 2.18 Significant positive correlation of density with (A) Soil temperature (°C) (B)-soil moisture (C) total rainfall and biomass with (D)-with Soil temperature(E)- soil moisture (F) total rainfall in PL.

2.4 Discussion

The present study reports 9-10 species of earthworms in mono plantation and mixed forest, which is well within the range of 1-15 species in an earthworm community (Edwards and Bohlen, 1996). Depending on the land used, species richness of earthworm vary considerably; example Mohan *et al.* (2013) and Singh *et al.* (2016)[a] reported 1-5 species in different agro-ecosystems in Punjab. Kaushal and Bisht (1994), Kaushal *et al.* (1995) reported 3-8 species in cultivated land, Ahmed *et al.* (2022) reported 3-12 species along the different elevation gradient and land used types in Western Himalaya. Sharma *et al.* (2022) reported 17 species from forest area, grassland, land near shallow water, roadside, vegetative garden, cultivation land and orchard. Dey and Chaudhuri (2016) reported 13 species from a 30-35 year old pineapple plantation in Tripura. Soil degradation and habitat loss, unsustainable management of soil, and invasive species are considered to have negative impact on earthworm diversity (Dewi and Senge, 2015). In the present study, most of the recorded species are endemic to the Indian region, except *P. corethrurus* which is mostly found in human-inhabited area (Grosso *et al.,* 2006). Earthworm population density negatively correlates with species diversity (Shakir and Dindal, 1997). In plantation area higher density with more dominant species was associated with lower species diversity. Also, high diversity in mixed forest is attributed to less anthropogenic activities and greater habitat heterogeneity whereas the lesser number of earthworm species in plantation area could be due to homogeneity of ecological niche and less canopy cover (Dey and Chaudhuri, 2014).

The earthworm community in the present study consist of exotic and peregrine species. Habitat fragmentation is one of the key reasons for the dominance of exotic species at the place initially occupied by native species (Kalisz and Wood, 1995). In terms

of both species richness and density, the endogeic group of earthworm dominates both mixed forest and mono plantation, which is consistent with reports from different ecosystems (Hackenberger and Hackenberger, 2014; Singh *et al*., 2016; Ahmed *et al*., 2022). Jouqet, *et al.* (2010) reported that endogeic earthworms are most resistant to a disturbance in impacted habitats. Previous researchers have found that the conversion of forest to agro ecosystems reduces the diversity and density of anecic earthworms, however on the contrary endogeic species increases (Zou *et al*., 2006; Gonz´alez *et al*., 2008). Usually, epigeic species are found in the nutrient-rich leaf litter (Schelfhout *et al*., 2017), however, the rare occurrence of epigeic earthworm was found in the present study, and this could be attributed to the transportation along with the dung manure (Ahmed *et al*., 2022). The present study reports the occurrence of epigeic, endogeic, and anecic species, and confirms that earthworm communities in ecosystems consist of one or two types of epigeic, two to four types of endogeic, and zero to two kinds of anecic species (Ahmed *et al*., 2022; Pop, 1997). The vertical distribution of earthworms in the soil is determined by abiotic factors and earthworm activities. Usually, the highest density of earthworm is present in 0-10 cm depth. Gonz´alez *et al.* (2007) opined that the availability of food resources and abiotic factors determined the high diversity and abundance in the upper layer of the soil.

The present study shows, maximum density of earthworms is contributed by *D. nepalensis* (45.03 ind.m^{-2}) and *D. assamensis* (50.96 ind.m^{-2}) in MF and PL areas, respectively. The dominant characteristic of *D. assamensis* with respect to biomass, density, and relative abundance is also reported from different climatic regimes in India (Tiwari *et al*., 1992; Chuadhuri and Nath, 2011; Dey *et al*., 2012; Dey and Chaudhuri, 2016). The epigeic species, *P. excavatus* was also found in abundance in MF and PL

which may be due to high percentage of soil moisture and organic carbon (Kaushal *et al*., 1995; Bisht *et al*., 2003; Lalthanzara and Ramanujam, 2014). Even though the land use system is different, both the study sites exhibited high percentage of species similarity as the study areas share similar climate and geography. As the endogeic earthworms are least affected by disturbance (Mariotte *et al.,* 2016; Singh *et al.,* 2020), a comparatively higher abundance of this group was present in the study area. Coexistence of both exotic (*P. corethurus*) and native species (such as *D. nepalensis* and *P. excavatus*) in two distinct habitats indicates the unique characteristics of a biodiversity hotspot zone (Dey and Chaudhuri, 2016).

Total earthworm density and biomass were significantly ($p<0.05$) higher during monsoon season in both the study areas. Higher rainfall along with favourable relative humidity during monsoon might result in higher population density and biomass in comparison to winter and spring season (Joshi and Aga, 2009). Chaudhuri *et al.* (2009) also observed that earthworms remain active for about 6 months (May to October) and is predominantly found during monsoon season in a subtropical forest in the North-eastern states of India. The pre-monsoon showers during spring coupled with good retention of moisture in the area result in an increased earthworm population density which continue up to the monsoon season. Potapov *et al.* (2021) have also opined that increased density and biomass of earthworm in the monsoon season could be due to fast litter decomposition, easy availability of food, and shelter that protects them from other predators.

Generally, monsoon season with optimum temperature acts as a cue for neurosecretion favouring cocoon production and higher breeding rate of earthworm (Bhattacharjee and Chaudhuri, 2002). In the present study, with the arrival of monsoon,

the population increased significantly (p<0.05) at both the study sites. It was observed that clitellate and non-clitellate density increased alternatively. While young population density in mixed forest remained fairly constant with significant increase (p<0.05) during monsoon season, in plantation area all age categories (juvenile, young and adult) showed significant variation with highest density recorded during monsoon (juvenile= 17.18 ind.m^2, young= 21.36 ind.m^2 and adult= 55.62 ind.m^2). No significant differences were observed in biomass and density among different age categories (juvenile, young and adult) during winter and spring seasons. In both study areas, it has been observed that regional seasons, earthworm age category and interaction of two independent factor have significant effect on earthworm density and biomass.

High percentage of soil moisture and organic carbon influence the abundance of epigeic species such as *P. excavatus*. Similar findings have been reported from different forest ecosystems in Mizoram (Lalthanzara and Ramanujam, 2014). Good canopy cover in mixed forests provides suitable range of moisture and temperature for the sustenance of earthworms, as tree diversity and earthworm diversity are found to have a positive relation (Cesarz *et al.*, 2007). As shown by a dramatic increase in the number of juveniles, young, and adult populations, earthworm populations are heavily reliant on moisture, temperature, relative humidity, and rainfall. Low earthworm density and biomass was seen during dry season, which coincided with a drop in soil moisture and temperature. The difference in earthworm distribution between the two study locations also suggests that habitat variability creates a larger niche for the lower macro invertebrate.

Earthworm diversity and density are known to be affected directly by the physico-chemical properties of the soil. Yvan *et al.* (2012) reported that soil physical property impacts earthworms burrowing activities and growth. Many researchers (Chan and

Barchia, 2007; Jänsch *et al.,* 2013; Bartz *et al.,* 2013) in the past have reported that soil organic carbon also effects the distribution and density of earthworm emphasised that soil organic carbon determines the availability of food for earthworms and is a critical factor for earthworm distribution. Because of the cutaneous mode of respiration, soil moisture plays a very important role in the availability of earthworms (Sharma and Poonam, 2014; Walsh *et al*., 2019). Tiwari and Joshi (2023) noted that various abiotic factors, including moisture positively impact the earthworm and further emphasised that variations of soil parameters and land use pattern effects the distribution of earthworms. Higher population density and dominance of certain endogeic earthworm species *viz., D. assamensis* and *D. nepalensis* in the study area may be due to the retention of a sufficient amount of moisture at lower soil depth (10-20cm) to uphold the maximum earthworm density (Sarlo, 2006). Because the two study sites fall under same climatic conditions, soil moisture and temperature (p>0.05) did not differ significantly. Also, seasonally, no significant differences were noted for bulk density, OC, TN, Av.N, TN, and Av. K. in the study sites. Available phosphorus varied significantly (p>0.05) among the seasons in both the study areas. Correlation studies indicates that, density and biomass of juvenile, young, and adult earthworm share positive and significant relationship with soil temperature, moisture, and relative humidity indicating that availability and dominance of epigeic and epianecic earthworm species depends on the temperature and moisture.

Chapter 3

Reproductive performance of *Perionyx excavatus* Perrier and isolation of associated phosphate solubilizing bacteria

Contents

3.1 Introduction

Earthworm, one of the macro-invertebrates, constitute the majority of soil fauna and plays a vital role in soil processes. With sufficient food and moisture, they are found in all aquatic and terrestrial habitats (Paliwal, 2014). Earthworms follow a heterogeneous distribution pattern, and their number fluctuates depending on the soil's physico-chemical properties (Singh *et al.,* 2016[b]). A high range of adaptations and the ability to disperse and reproduce in various ecosystems also help the earthworm increase their diversity (Phillips *et al.,* 2021). Many earthworm studies from India, including north-eastern states, are available (Ahmed *et al*., 2022; Anuja *et al.,* 2022; Gudeta *et al.,* 2022). And today, earthworms' resource explorations aim to assess their ability to mitigate organic waste problems through vermicomposting and soil nutrient enrichment. However, a study on their availability, growth, reproductions, and associated beneficial microbes is significant for the efficient usage of earthworms.

P. excavatus is an epigeic earthworm that feeds mainly on organic waste and requires high moisture to establish its populations (Edwards *et al.,* 1998; Sadia *et al.,* 2020). The prospect of *P. excavatus* in waste management has been exhibited by its ability to vermicompost various organic substrates such as household waste (Suthar and Singh, 2008), fruit waste (Dey *et al.*, 2021), vegetable waste, food waste, paper waste, ash waste, and cow dung (Rupani *et al.,* 2023). The increased amount of macronutrients (nitrogen, phosphorus, potassium; calcium, and magnesium) in manures produced by earthworms are aided by the activities of plant growth-promoting bacteria associated with it (Parthasarathi *et al.,* 2007). However, despite proving its potential to accomplish eco-friendly means of waste management and nutrient enrichment, fundamental knowledge

on growth and reproduction and the possibility of phosphate solubilizing bacteria associated with *P. excavatus* found in this region of the country is lacking.

Few studies of different earthworm species on their growth and reproduction have been conducted worldwide (Reinecke and Hallat, 1989; Hallat *et al.,* 1990; Karmegam and Daniel, 2009; Fernandez *et al.*, 2010). Under a limited supply of substrates, Debnath and Chaudhuri (2020) reported that the growth rate of *P. excavatus* was significantly higher in cow dung. While cocoon production was higher in acacia: cow dung mixture, the lowest growth rate, and cocoon production were observed in the mikania-cow dung mixture. Except for scanty reports made by Joshi and Dabral (2008), and Sadia *et al.* (2020), where authors highlighted helpful information on the growth rate and cocoon production, long-term studies on survival, fecundity, and cocoon production are lacking. As epigeic species, adaptability is very high in *P. excavatus* (Lirikum *et al.,* 2022), so with differences in biogeography, climates, and varying food substrates, the reproductive behaviour and associated beneficial microbes are expected to vary. Therefore, the fundamental aspects of reproductive behaviours under different substrates in different regions are critical to promoting the mass production of *P. excavatus*.

Phosphorus (P) is the second most crucial macronutrient required for the growth and development of plants (Illmer *et al.,* 1995). It is a naturally occurring element found in rocks and soils. Limitations of P significantly determine ecosystem productivity (Bhattacharjee *et al.,* 2021). Although naturally occurring P in soil is high, water-soluble forms (orthophosphates, HPO_4^{2-} or $H_2PO_4^{1-}$) are often low (<1 mg/kg) (Ghosh *et al.,* 2015). So, to overcome the low concentrations of available P, synthetically produced phosphate fertilizers are applied in the soil to enhance the plant's productivity. However,

applications of P fertilizers have several limitations, such as deterioration of soil physical, chemical, and biological properties (Bhakta *et al.,* 2022). Also, since chemical phosphate fertilizers contain a high amount of heavy metals, excessive usage of phosphate fertilizers promotes heavy metal build-up in soil (Ulén *et al.,* 2007; Atef *et al.,* 2023). Therefore, it is imperative to search for ecologically appropriate phosphate fertilizers to limit the adverse effect of chemical fertilizers (Singh and Reddy, 2011).

As rich microhabitats of several soil bacteria, earthworms are reported to harbor diverse forms of plant growth-promoting bacteria; hence earthworms and their associated bacteria attracted the interest of many researchers. Houida *et al.* (2021) reported several bacteria in *Aporrectodea molleri* having phosphate solubilization potential, Indole Acidic (IAA) productions, siderophores, and nitrogen fixation ability. Similarly, in *Metaphire posthuma*, Bhakta *et al.* (2022) also isolated and characterized bacteria (*Pseudomonas aeruginosa*) with a solubilizing phosphate index of 4.8 ± 0.5. Application of isolated bacteria also increased seed germination (10-50%) and plant growth (shoot length 21% and leaf number 77%). However, such studies in promising epigeic earthworm like *P. excavatus* is lacking.

Food substrate affects not only the size of an earthworm but also its reproduction rate (Domínguez *et al.,* 2000; Kabi *et al.,* 2020). Irrespective of the ecological categories of earthworms, cow dung is considered the best food additive (Lowe and Butt, 2005). Other preferred substrates for earthworms include domestic organic waste, livestock waste (Rini *et al.,* 2020), agro waste (Kamalraj *et al.,* 2017), paper mill waste (Ganguly and Chakraborty, 2019), etc. Also environmental factors such as pH, temperature, moisture, and aeration also influence the growth rate and cocoon production. Hence, from the above

fact, under a limited supply of food substrate aided by cow dung as food additives, the present study was undertaken to investigate the reproductive performance of *P. excavatus* in food substrates such as kitchen scrap, rice straw, and cow dung. Also, based on the increased level of available phosphorus in vermicompost manure (Zhi-wei *et al.*, 2019) and plant growth enhancement ability of earthworms (Pathma and Sakthivel, 2012), the isolation and characterization of *P. excavatus*-associated bacteria that solubilize the phosphate was performed.

3.2 Materials and Method

3.2.1 Earthworm collection

Earthworms were collected using the quadrat method followed by hand sorting during field exploration on diversity studies in the Minkong forest (26°21'43.34"N and 94°33'23.42"E) under Mokokchung district Nagaland, India. The collected specimens were kept in a vermicompost chamber (60x50mm) in the Zoology Department, Nagaland University, Lumami, India, to adapt to the local environment and multiply their populations. The locality of the study area experiences chills, windy winter, and warm, humid summer with an average temperature ranging between 8.2 °C to 21.46 °C in winter and 17 °C to 25.24°C in summer. Humidity varies between 74.4% in winter and 86% in summer.

In the mass cultured, earthworms were fed a mixture of half-decomposed cow dung and other domestic waste such as potato peel, banana, and other vegetable scraps. From the stock sample, healthy, young, non-clitellate worms weighing 174.15±11.27mg were sorted out to study their growth and reproduction under different substrates.

3.2.2 Collection of raw materials and preparations of bedding combinations

Cow dung and rice straw were collected from a local cattle farm and one (1) year old abandoned Jhum field located in Zaphumi village (26°13'56.70"N and 94°28'22.02"E), two km away from Nagaland University, Lumami, India. At the same time, domestic kitchen waste, a mixture of different vegetable scraps, was collected daily for 20 days. Collected raw materials were chopped into 10-20mm using a machete and decomposed for fifteen (15) days with proper moisture (60-70%) before the experiment began for softening of a substrate, thermal stabilization, and hastening the degradation process. In an earthen pot of 2.5 Ltr. Capacity, three different substrate treatments were prepared as food for earthworms. Cow dung is known to be preferred by earthworms as food. Therefore, one pot was prepared with only cow dung and kept as T1.

Cow dung (CD) only (T1).

Soil: Kitchen Scrap (KS): CD (T2) at a 7:3:1 ratio.

Soil: Rice Straw (RS): CD (T3) at a 7:3:1 ratio.

Three sets of substrate combinations were prepared, each with a total substrate weight of 2200g. In each set, a pair of pre-weighed young, non-clitellate earthworms (weighing 174.15±11.27mg) were added to all replicates. The experimental pot was covered with a moistened jute bag to keep it dark, moist, and cool. 60-70 % moisture was maintained by sprinkling water at regular intervals.

After every seven days, each experimented pot was inspected, hand-sorted out the worm and its weight was taken using a digital weighing instrument (Oblivion-OBSF400A) to determine the changes in its biomass and growth rate calculations. During

the experimentation, once the earthworms developed their clitellum distinctly, cocoons and hatchlings were searched manually in the experimented pot with the help of a spatula at regular intervals of seven days. The collected hatchlings and cocoons were separated from the experimental pot, and cocoon incubation was done separately. Freshly collected cocoons were incubated in batches of 3 in a petri dish and covered with moistened cotton to maintain the moisture requirement for the cocoons. The experiment was conducted at room temperature (18-25°C).

The growth rate/worm/day was calculated as $= \frac{\text{Maximum weight} - \text{Initial weight}}{\text{No. of days to attain maximum weight}}$

Rate of cocoon productions/week$= \frac{\text{No.of cocoons produced}}{\text{No.of adults}} \; x \; 7.$

3.2.3 Sample preparation for bacterial isolation

Healthy, clitellated earthworms were collected from the stock culture, and their body surfaces were rinsed with distilled water multiple times to cleanse soil samples attached to its body. The cleaned earthworms were starved for 24 hours in a clean beaker and allow them to empty the ingested soil samples in their gut (**Fig. 3.1**). After 24 hours, earthworms were further cleansed with double distilled water followed by narcotizing and disinfected with 70% alcohol. The sanitized earthworms were placed within a laminar air flow, where they were smeared with mortar and pestle to create a paste-like, which was then carefully transferred into an airtight container. The prepared mixture was subsequently earmarked for additional experimentation and was utilized within a span of 24 hours.

3.2.4 Isolation of earthworm-associated bacteria

0.1g of the sample (earthworm-smeared paste) was suspended in 0.9 ml of saline water at a 1:9 ratio. The suspended sample was vortex to mix the samples thoroughly and

suspended for 5 minutes. An aliquot of the suspension was serially diluted from 10^{-1}-10^{-5}. With the help of a loop, samples were streaked in nutrient agar containing10g glucose, 5g $MgCl_2.6H_2O$, 0.25g $MgSO_4.7H_2O$, 0.2g KCl, 0.1g $(NH_4)2SO_4$, and 15g agar per liter. The plates were incubated for 24 hours, and the colony formed was isolated and further cultured separately (Pure culture) in nutrient agar. The isolated colonies were assigned the names colony-1 (C1), colony-2 (C2), colony-3 (C3), colony-4 (C4), and colony-5 (C5).

The colony forming unit (cfu/ml) of the mixed culture was calculated as

$$\text{Cfu} = \frac{\text{No. of colonies} \times \text{Dilution factor}}{\text{Vol. of culture plated}}$$

3.2.5 Morphological and biochemical characteristics of isolates

For morphological characterization, colonies formed were examined for shape, margin, elevation, texture, color, and optical properties. A nitrate reduction test was conducted to examine the ability of bacteria to hydrolyze nitrate to nitrite based on their ability to produce nitrate reductase enzymes. The capacity of bacterial isolates to utilize citrate as a carbon and energy source was assessed through a citrate utilization test. A positive diagnostic test was indicated by the transformation of the medium from green to blue color. Also, a qualitative assessment of indole acetic acid (IAA) productions was conducted using Luria Bertani (LB) media supplemented with 0, 2, and 5 mg mL−1 of L-tryptophan and incubated at 32±2 °C for 12 days. The development of the pink color indicated the positive results of the IAA productions.

3.2.6 **Screening of phosphate solubilizing bacteria (PSB)**

The isolated bacterial colonies to solubilize phosphate were tested in pikovskaya agar media (containing dextrose 10 g; tricalcium phosphate (TCP) 5 g; yeast extract 0.5

g; ammonium sulphate 0.5 g; potassium chloride 0.2 g; sodium chloride 0.2 g; magnesium sulphate 0.1 g; ferrous sulphate trace; manganese sulphate trace; agar 15 g; distilled water 1 L) following pour plate technique. Spot inoculation of bacteria was done in triplicates for each colony and kept in an incubator at 30°C for 120 hours. Bacteria showing clear zone around the colony were considered positive for phosphate solubilization. The diameter of the clear zone formed around the colony was measured at 72, 96, and 120 hours to determine the phosphate solubilization index (PSI). Phosphate solubilization index (PSI) was calculated following Edi-Premono *et al.* (1996).

$$PSI = \frac{\text{Colony diamter+Clear zone diamter}}{\text{Colony diameter}}$$

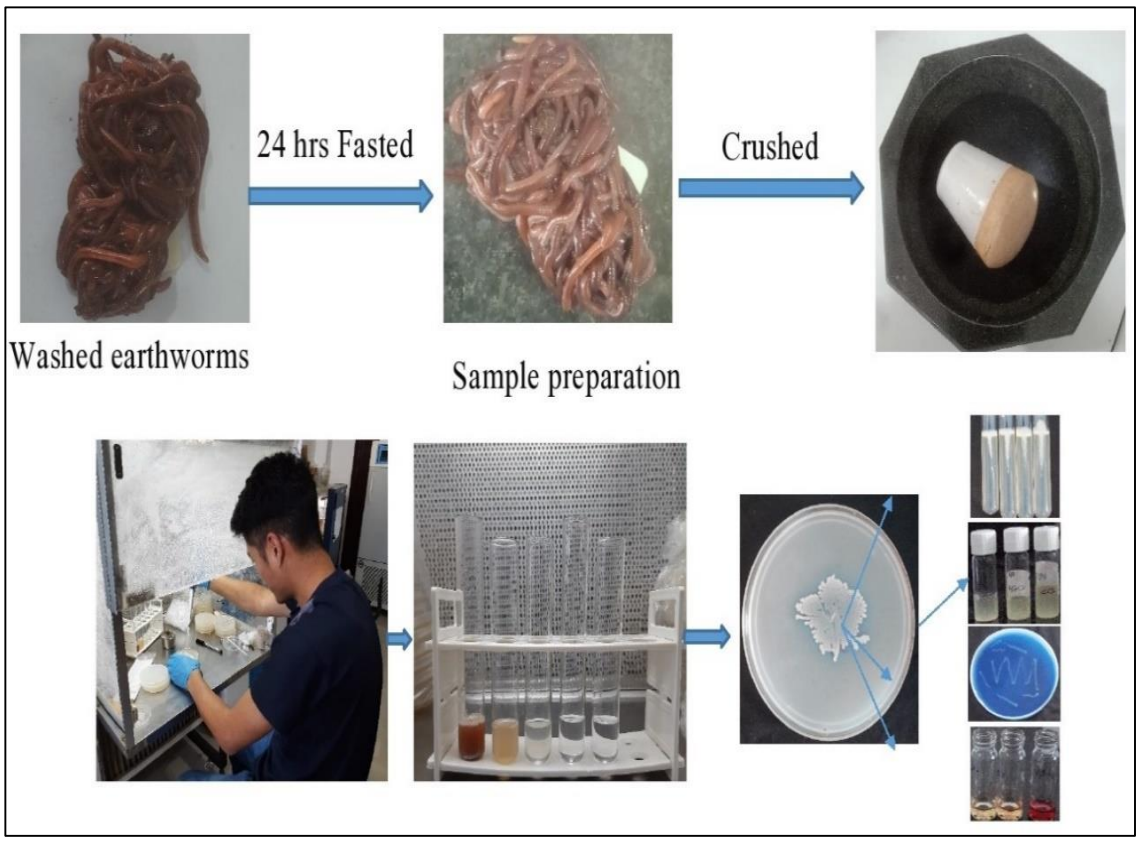

Fig. 3.1 Schematic representation of bacterial isolation from earthworm and biochemical test performed in the laboratory.

3.2.7 Molecular characterization

DNA was isolated from the culture, its quality was evaluated on 1.0 % agarose gel, and a single band of high-molecular-weight DNA was observed. The 16S region was amplified by 27F and 1492R primers. After PCR amplification, a quality check for the samples was carried out by gel electrophoresis (2% agarose gel, **Fig. 3.11**) and purification was performed using QIAGEN QIAquick PCR Purification Kit (cat. No. 28104). Purified samples were taken for sequencing. The sequencing PCR reaction was set up in Applied Biosystems™ MiniAmp™ Plus Thermal cycler using Big Dye ™ Terminator V3.1 kit. The consensus sequence of the 16S rRNA gene was generated from forward and reverse sequence data using aligner software. The 16S rRNA gene sequence was used to carry out BLAST with the NCBI GenBank database. Based on the maximum identity score, a few selected sequences were selected and downloaded in FASTA format which was further used for the phylogenetic tree analysis using MEGAX 9 (**Fig. 3.9** and **Fig. 3.10**). The 16S rRNA gene sequence of strains isolated was submitted to Gene Bank under accession numbers OQ927064, OQ927066, OQ875851, and OQ927062.

3.2.8 Statistical analysis

Analysis of variance (ANOVA) at a 95% confidence level ($p<0.05$) was tested using SPSS (version 22). Results of the significant differences were further analyzed for multiple comparisons test (Tukey test) to assess the differences in earthworm growth characteristics and PSI of different bacteria.

3.3 Results

3.3.1 Biomass of earthworm under different food substrates

The biomass gain of *P. excavatus* under different food substrates is shown in **Fig. 3.2**. Differential net weight gain and time required to gain maximum weight were also observed. Due to available food materials, earthworm biomass increased rapidly at the beginning, but with limited food materials due to the decaying of organic matter, earthworm biomass started to decline. In the T2 treatment, earthworm biomass increased exponentially for 21 days with a maximum weight gain of 415.66 mg, after which it started to decline (**Fig. 3.2**). The final biomass of the earthworm was 193.5 mg. In T1 and T3, as indicated by their biomass gain, earthworms took a longer time to acclimatize to the new environment. Biomass increase was gradual for about 42 days, after which it started to decline. The average biomass of *P. excavatus* was higher in T2 (285.96±63.16), followed by T1 (222.86±39.86) and T3 (205.94±28.5). Earthworm biomass in different treatments varies significantly ($F_{(2,37)}$=10.27, p<0.05). Multiple comparisons test shows T2 was significantly higher (p<0.05) compared to T1 and T3; however, no significant differences (p>0.05) were observed between T1 and T3. Higher biomass in T2 indicates the suitability of organic waste for its body mass development. With increasing biomass, earthworms initially showed a positive growth rate; the highest was observed in T2, followed by T1 and T3. Although the average growth rate was highest in T2 (9.53±4.4), followed by T1 (4.44±1.83) and T3 (3.63±2.52), with no significant ($F_{(2,15)}$=2.48, p<0.05) mean differences were observed.

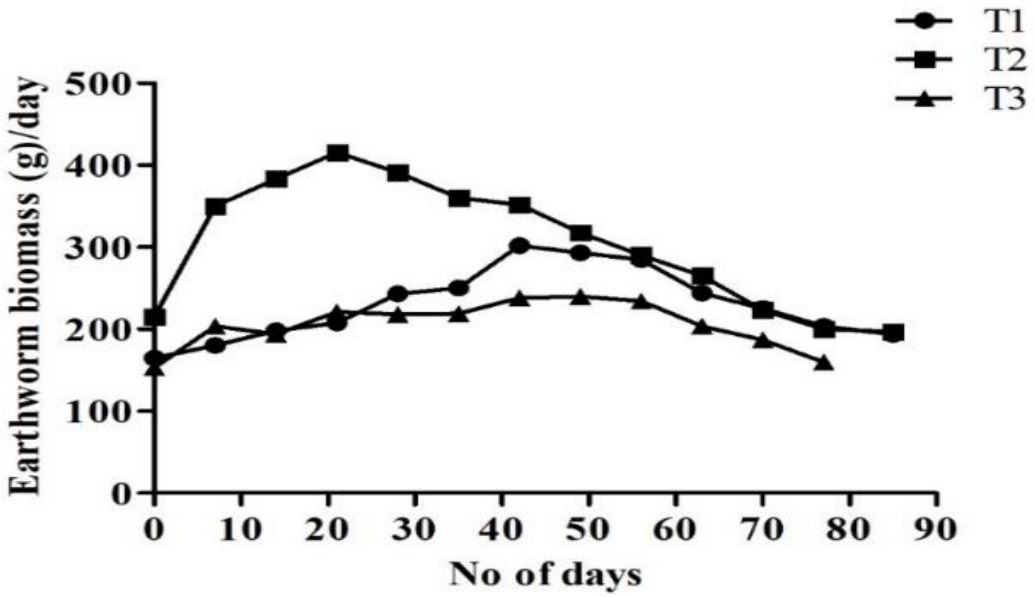

Fig. 3.2 Earthworm biomass gain curve under limited food supply in different experimental setups-T1-Cow dung (CD), T2- Soil: Kitchen Scrap (KS): CD, and T3- Soil: Rice Straw (RS): CD.

Key biological parameters of *P. excavatus* observed under different experimental food diets (T1, T2, and T3) are shown in **Table 1**. Initially, earthworm biomass varies between 161mg to 165mg. With different substrates used as feed, the highest weight gain was observed in T2, followed by T1, and the lowest weight gain was observed in T3 (**Table 3.2**). Variations in net weight gain of earthworms were observed under various organic waste treatments, but differences were statistically insignificant ($F_{(2,11)}$=0.69, p>0.05). In *P. excavatus*, depending on the food substrate, the time taken to gain maximum weight differ significantly ($F_{(2,11)}$=13.31, p<0.05). In the T2 treatment, the least number of times (weeks) was required to gain maximum weight, followed by T3 and T1. Tukey test shows that in T2, the time it takes to achieve maximum weight was significantly (p<0.05) less than in T3. However, no significant difference (p>0.05) was observed between T3 and T1.

Table 3.1 Earthworm biomass under three experimental treatments (Mean±SE).

Substrate	Initial weight (mg)	Max. weight (mg)	Net weight gain (mg)	Max. Weight achieved on (weeks)	Final weight (mg)
T1	165.16±9.9[a]	320.33±39.87[ab]	122.33±39.0[a]	6.33±0.57[ab]	184.33±10.21[a]
T2	192.5±38.15[a]	415.66±63.31[a]	142.4±22.5[a]	3.16±0.57[b]	191±16.57[a]
T3	170.50±22.07[a]	256.40±12.07[b]	94.5±10.39[a]	6.33±2.08[a]	159.66±29.5[a]
F	1.85	16.16	0.69	13.31	0.58
p-value	0.19	0.002*	0.52	0.002*	0.59

Data represent mean ± SD. Mean with different superscripts within the same raw differ significantly ($p<0.05$) by Tukey's HSD at a 95 % confidence level ($p<0.05$) *Represents statistically significant at $p<0.05$.

3.3.2 Reproduction

Differences in reproduction under different organic substrates provided as food are highlighted in **Table 3.2**. The clitellum development of earthworms shows the sexual maturity of earthworms. In kitchen scrap earthworms, it takes 3.2 weeks to develop distinct clitellum indicating the suitability of the organic substrate. In T1, T2, and T3, clitellum development in *P. excavatus* takes 3.66±0.57, 3.2±0.5, and 6.0±1.00 weeks (**Table 3.2**), and the analysis of variance shows that time taken for clitellum development in different food treatment were significantly ($F_{(2,9)}=14.52$, $p<0.05$) different.

The cocoon production was first observed in T2, followed by T1 and T3, and the time taken for cocoon production was significantly different ($F_{(2,10)} = 5.81$, $p<0.05$) depending on the treatment. In T1 treatment, cocoon production started on the 28th day,

and two different peaks of cocoon production were observed (35th and 70th day). Also, in T2 treatment, the first cocoon was observed on the 28th day, and cocoon production peaks at two different intervals *viz.* 35th and 49th day (**Fig. 3.3**). Whereas in T3 treatment, cocoon production started later compare to T2 and T1, it was observed first on the 42nd day and it gradually declined. In T1 treatment, cocoon production ceased at 9.33 weeks, followed by 9.25 and 11.66 weeks in T2 and T3. The average number of cocoon productions/worm/day in T1, T2, and T3 were 0.26±0.01, 0.23±0.08, and 0.20±0.07. Although variations in cocoons produced per worm were observed under different feeds, no significant differences ($F_{(2,18)}$= 0.93, p>0.05) in the number of cocoons produced per worm were observed. The cocoon incubation period lasted 25.6±5.5 days at ambient temperature (14-20 °C), and hatchlings produced per cocoon were 0.92.±0.28 (**Fig. 3.4**). In the T2 treatment, the number of hatchlings was highest on 42nd day, whereas in T1 and T3, hatchlings were more on 56th and 70th day. The length of freshly emerged hatchlings varies in the range of 10.8±1.92 mm and breadth of 0.3mm.

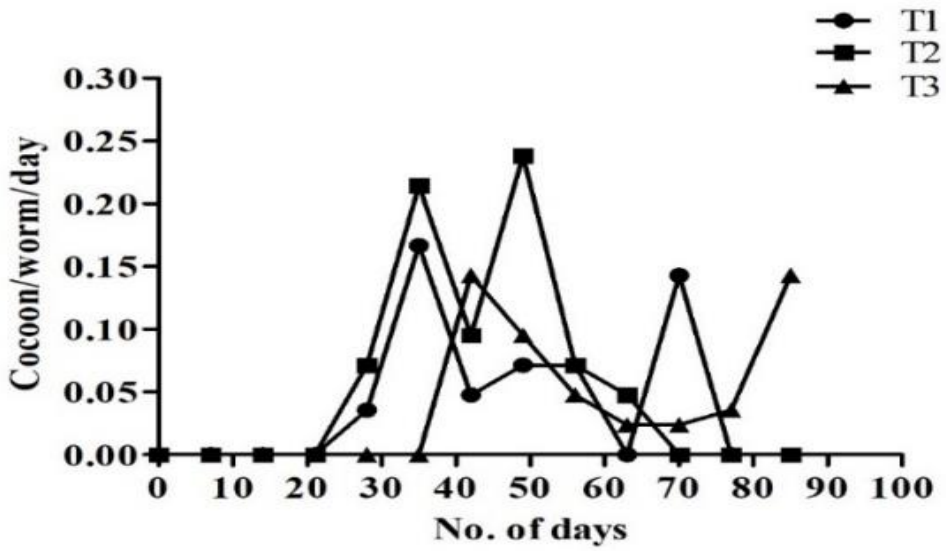

Fig. 3.3 Earthworm cocoon laying under three different experimental food additives-T1, T2, and T3.

Table 3.2 Earthworm weight, maturation, and their hatchlings observation under three experimental treatments.

Substrate	Clitellum development (Weeks)	Cocoon production started on (weeks)	No. of cocoon produced/worm/day	Cocoon production ceased after (weeks)
T1	3.66 ± 0.57^{b}	4.66 ± 0.5^{b}	0.26 ± 0.01^{a}	9.33 ± 1.52^{a}
T2	3.25 ± 0.5^{b}	5.33 ± 0.5^{ab}	0.23 ± 0.08^{a}	9.25 ± 0.5^{a}
T3	6 ± 1.0^{a}	7.33 ± 0.5^{a}	0.2 ± 0.07^{a}	11.66 ± 1.52^{a}
F	14.52	5.81	0.93	4.13
p-value	0.003*	0.02*	0.41	0.65

Data represent mean ± SD. Mean with different superscripts within the same raw differ significantly ($p<0.05$) by Tukey's HSD at a 95 % confidence level ($p<0.05$) *Represents statistically significant at $p<0.05$ one-way ANOVA.

3.3.3 Cocoon morphology

Freshly hatched, oval-shaped, greenish colored with tapering-pointed at both ends with a 14.2 x 6 mm diameter. With an increased incubation period, the cocoon color changed to brownish and became less rigid. The posterior end is pointed and shortened, while the anterior end becomes flattened with sharp edges. Hatchlings emerge from one end of the cocoon (**Fig. 3.4**).

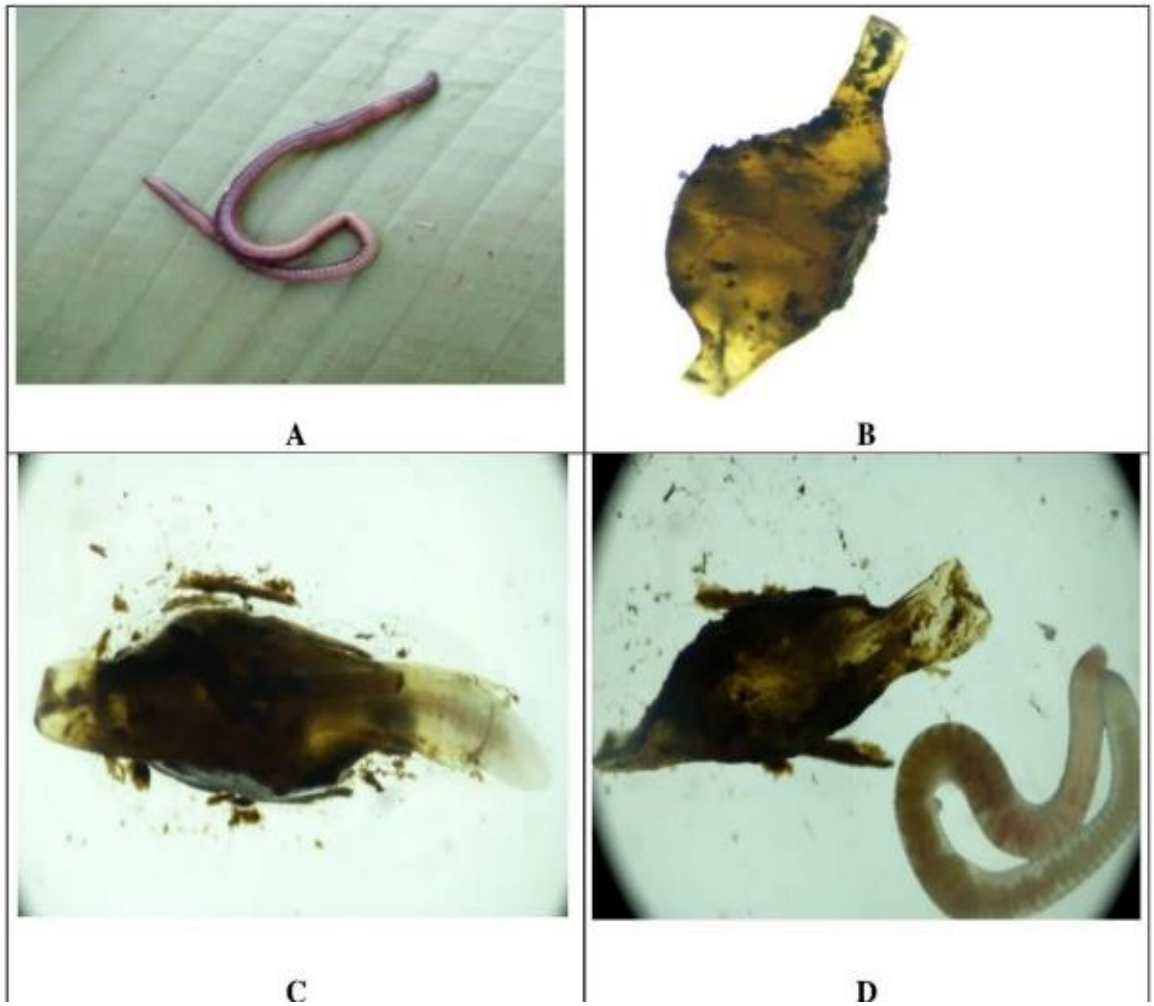

Fig. 3.4 (A) Adult earthworm (*P. excavatus*) (B) typical cocoon under microscope (C) Hatchling starting to emergeout of one end of cocoon (D) hatchling fully emerged out of the cocoon.

3.3.4 Earthworm-associated phosphate solubilizing bacteria

As shown in **Table 3.3**, the isolate's morphology varies after 24 hours of incubation. The microbial load was quantified by calculating the colony-forming unit (cfu). The cfu of bacteria in the sample was $9.4x10^{-5}$ cfu/ml. After serial dilution, most of the plates with 10^{-4} dilution showed confluent growth of aerobic bacteria, after which the population density decreased up to 10^{-5} dilution, where only a countable amount of Cfu was observed (**Fig. 3.5**).

Table 3.3 Morphological characteristics of bacteria isolated from *P. excavatus*

Colony	Shape	Margin	Elevation	Texture	Color	Optical property
C1	Round	Entire	Raised	Shiny	Light	Opaque
C2	Irregular	Irregular	Gown into medium	Dull	Off white	Translucent
C3	Irregular	Irregular	Raised	Shiny	Light orange	Opaque
C4	Irregular	Irregular	Gown into medium	Dull	Off white	Translucent
C5	Round	Entire	Raised	Shiny	Light	Opaque

Table 3.4 Results of the biochemical test conducted for bacteria isolated from *P. excavatus.*

Biochemical characteristics	C1	C2	C3	C4	C5
Citrate utilization	+	+	-	+	+
Methyl red	-	-	-	-	-
Nitrate reduction test	-	-	-	-	-
Motility	-	-	-	-	-
Phosphate solubilization	+	-	+	+	+
Indole productions	+	+	+	+	+

- indicate negative results, + indicate positive results

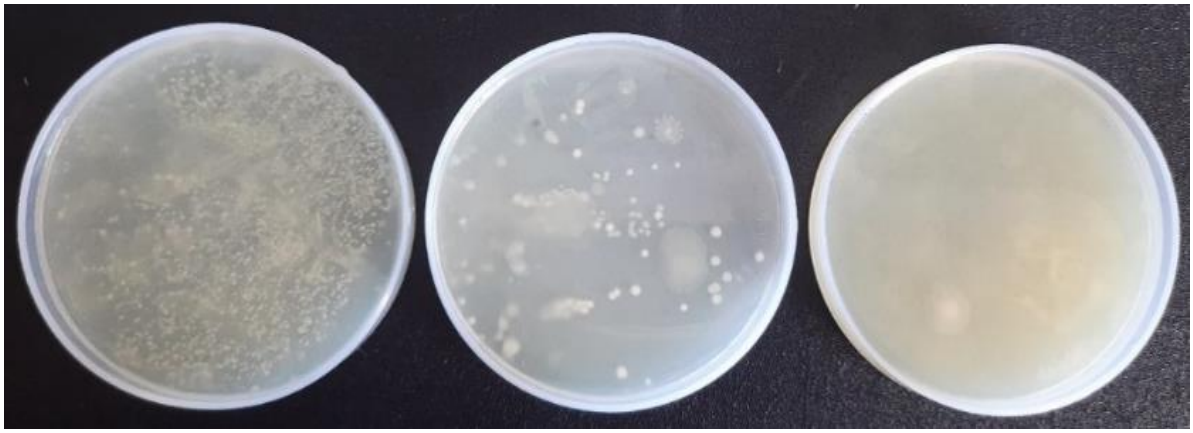

Fig. 3.5 Serially diluted (10^{-3}, 10^{-4}, and 10^{-5}) earthworm sample for cfu count.

3.3.5 Phosphate solubilization Index (PSI)

The appearance of a hollow zone around the colony indicates phosphate solubilization. Depending on the bacteria's ability, the diameter of the hollow zone varies (**Fig. 3.7**). The present study shows that four isolates were positive for phosphate solubilization. In contrast, one isolate showed negative results (**Table 3.5**). The PSI of isolates varies depending on the species. The colony diameter (CD) and hollow zone diameter (HZD) increased gradually with time. In C1, on the 7th day, CD and HZD were 16.6±1.0mm and 27.82±1.67mm, with a calculated PSI value of 16.8±1.7mm. C2 showed negative for the phosphate solubilization, but for C3, CD, and HZD varied between 31.5

to 33.0mm and 36.2 to 36.5mm with PSI of 11.2±2.1mm on the 7th day. C4 diameter and HZD were 13.25±3.4mm and 17.87±0.85mm with PSI of 14.0±2.7mm. While for C5, CD and HZD were 24.5±3.1mm and 34.2±2.6mm with PSI of 12.9±1.8mm. The calculated PSI of colonies varied from 11.1 to 16.8mm on different days, and colonies as shown in **Fig. 3.6**, the PSI of bacteria colonies showed significant differences ($F_{(4, 18)}$ =7.9, p<0.05). The highest PSI was observed in C1, followed by C4, C5, and C3. Except in C4, in other colonies, PSI values were observed to be more on the 7th day.

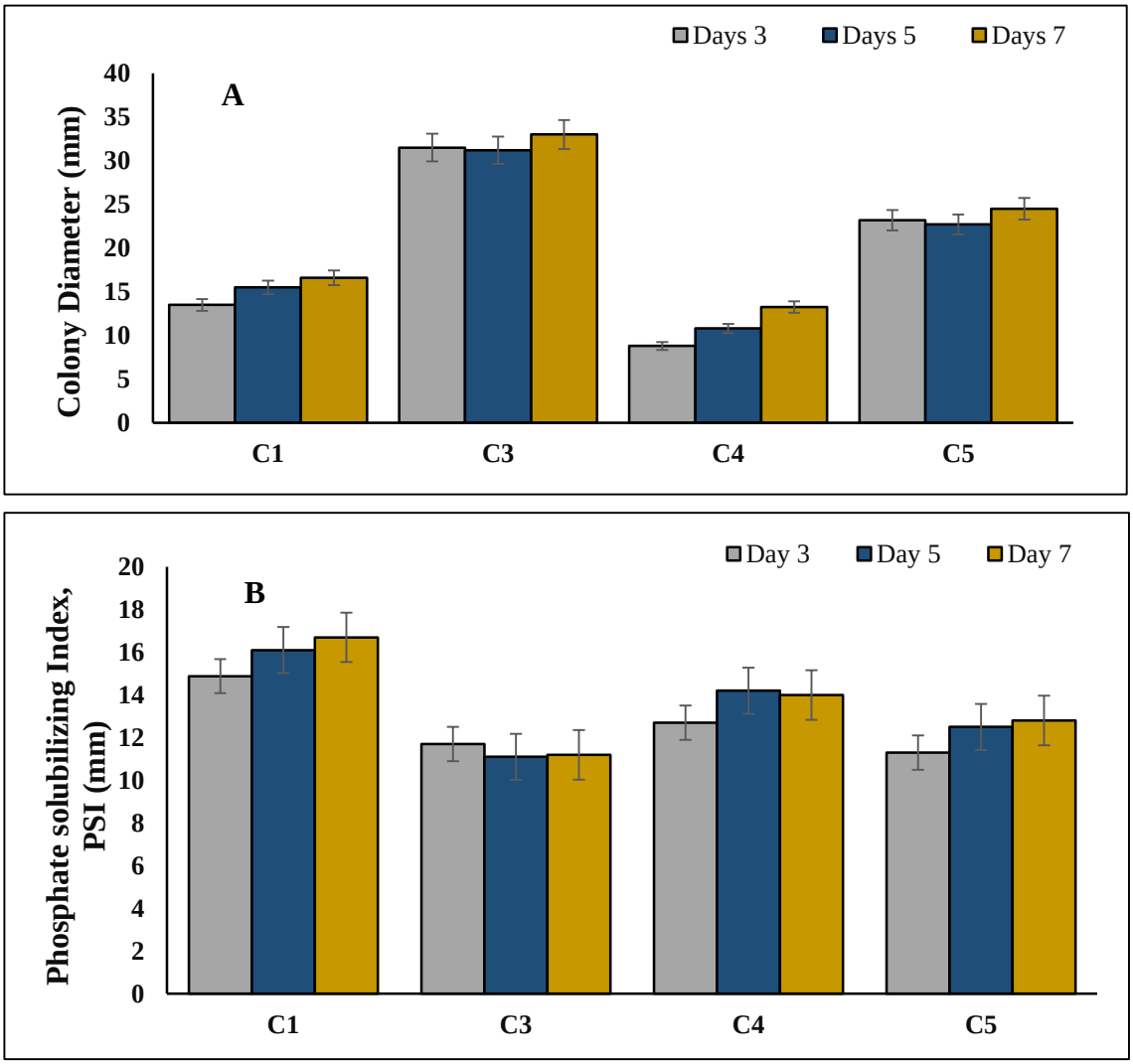

Fig. 3.6 (A) Bacteria colonies diameter over a period of seven (7) days (B) PSI of bacteria at the final stage of incubation. Different superscripts indicate statistically significant differences at p<0.05.

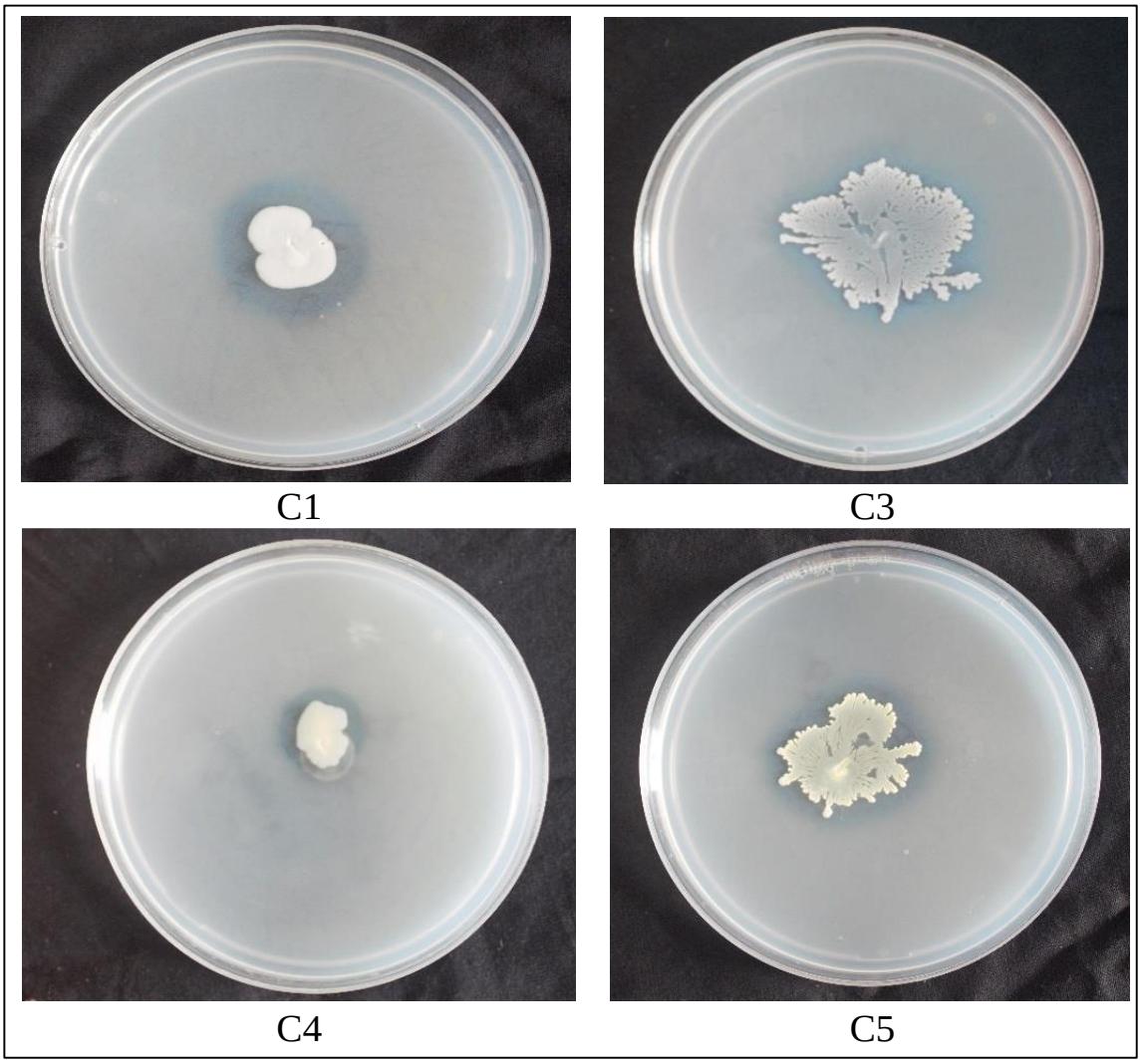

Fig. 3.7 Phosphate solubilizing potential of bacteria colonies (C1, C3, C4, and C5) isolated from *P. excavatus.*

3.3.6 IAA productions

The IAA production of the isolate was proportional to the incubation period. After seven days of incubation, all the isolates showed positive for IAA productions, and indicated by the intensity of red color, the amount of IAA productions differed among the colonies (**Fig. 3.8**). It was observed that better results of IAA productions were observed in C2>C1>C4>C3>C5. Biochemical tests were conducted for citrate utilization, methyl red, nitrate reduction, motility, phosphate solubilization, and indole production. The

results of the biochemical test are shown in **Table 3.5**. Positive results of the citrate utilization are indicated by the conversion of forest green to dark blue color or Simmon's citrate slant. Out of the five colonies isolated, citrate utilization was positive in four colonies, and one colony showed negative. Positive results indicate the presence of citrate enzymes in the selected colonies. The enzymes hydrolyze the citrate to oxaloacetic acid and acetic acid (Schneider *et al.,* 2000). This test identifies the capability of isolates to utilize citrate as a carbon source (Max *et al*., 2010). Diffused growth of bacteria along the stabbed line indicates positive for bacteria motility test, but all isolates show negative results. Also, the isolates showed negative results for methyl red and nitrate reduction.

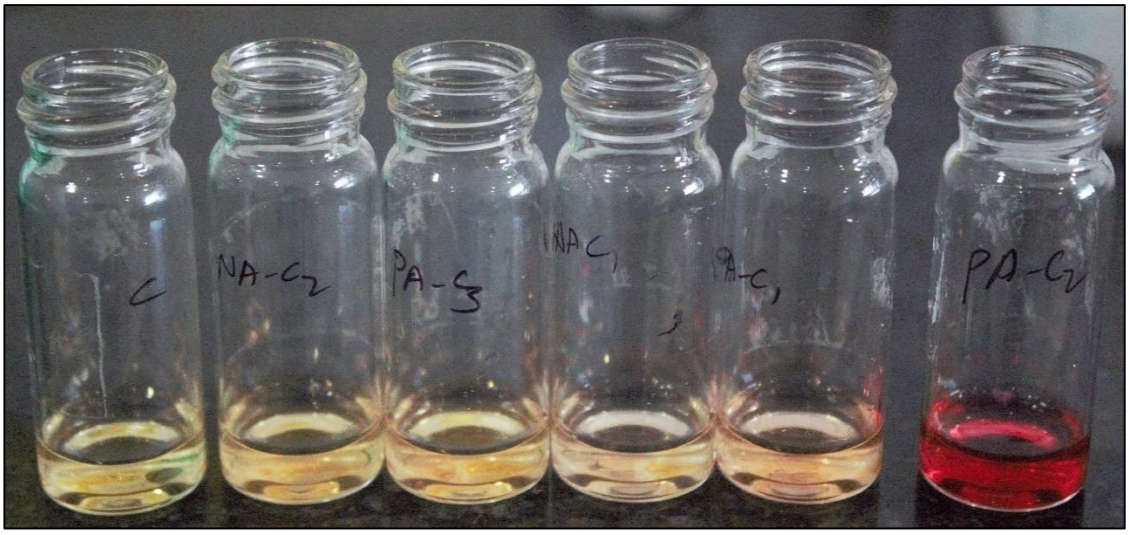

Fig. 3.8 Positive test results of the IAA production test- the difference in colour intensity indicates the different potential of bacteria to produce IAA.

3.3.7 Phylogenetic analysis

The taxonomic position through 16s rDNA sequence similarity revealed that C1 shared 99.8% similarity with *Pseudomonas alcaliphila* JABI (CP016162.1). The next closest homologue was *P. chengduensis* strain BC1815 chromosome, complete genome

(CP111110.1), *P. chengduensis* strain T1624 chromosome, complete genome (CP095766.1). C3 showed proximity of 98.85% with *Citrobacter freundii* ATCC 8090 = MTCC 1658 = NBRC 12681 strain ATCC 8090 chromosome, complete genome (CP049015.1 2). The next closest homologue was found to be *C. freundii* strain R47 chromosome R47, complete sequence (CP040698.1) and *C. freundii* strain FDAARGOS_549 chromosome, complete genome (CP033744.1). C4 showed proximity of 99.73% with *Achromobacter* sp. Marseille-Q4954 partial 16S rRNA gene, strain (OX265244.1). C5 showed proximity of 99.53% with *Citrobacter farmeri* strain CCRI-24236 chromosome, complete genome Sequence (CP081314.1). The next closest homologue was found to be *C. farmeri* strain AFS006815 16S ribosomal RNA gene, partial sequence (OP986118.1) and *C. farmeri* strain AUSMDU00008141 complete genome (CP022695.1) (**Table 3.5**). Therefore the bacterial strains C1, C3, C4, and C5 were identified as *Pseudomonas alcaliphila* OQ927064, *Citrobacter freundii* OQ927066, *Achromobacter* sp. OQ875851, and *Citrobacter farmer* OQ927062, respectively.

Table 3.5 Taxonomic affiliation of bacteria isolated from the epigeic earthworm, *P. excavatus*.

Accession number	Nearest relative	The accession number of the nearest relative	Sequence percentage identity
OQ927064	*Pseudomonas alcaliphila*	CP016162.1	99.8%
OQ927066	*Citrobacter freundii*	CP049015.1 2	98.85%
OQ875851	*Achromobacter* sp.	OX265244.1	99.73%
OQ927062	*Citrobacter farmeri*	CP081314.1	99.53%

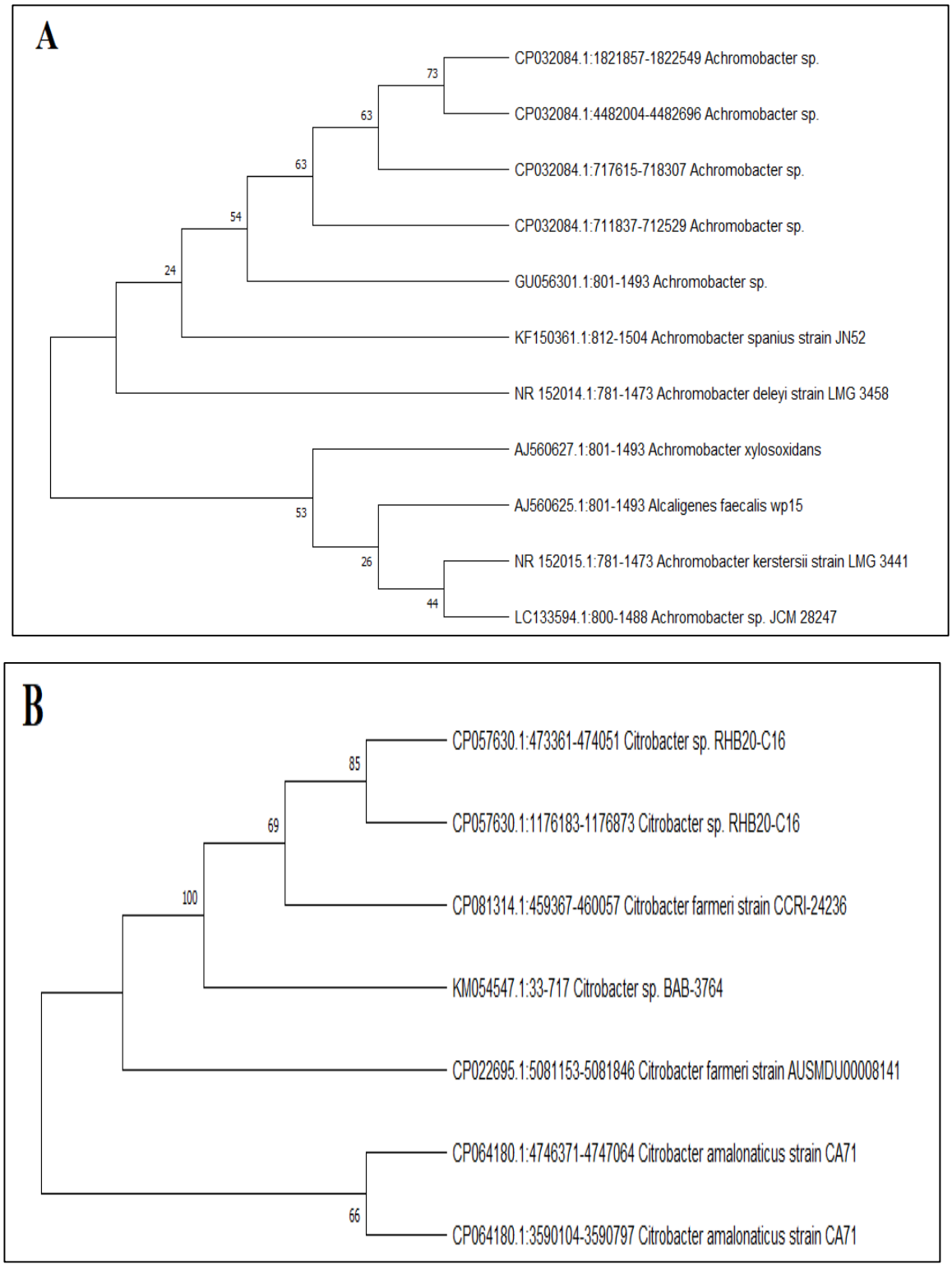

Fig. 3.9 Neighbour-joining phylogenetic tree drawn by 16S rDNA sequence of *Achromobacter* sp. Marseille-Q4954 (A) and *Citrobacter farmeri* strain CCRI-24236 (B) isolated from *P. excavatus* showing their position and relationship with other strains.

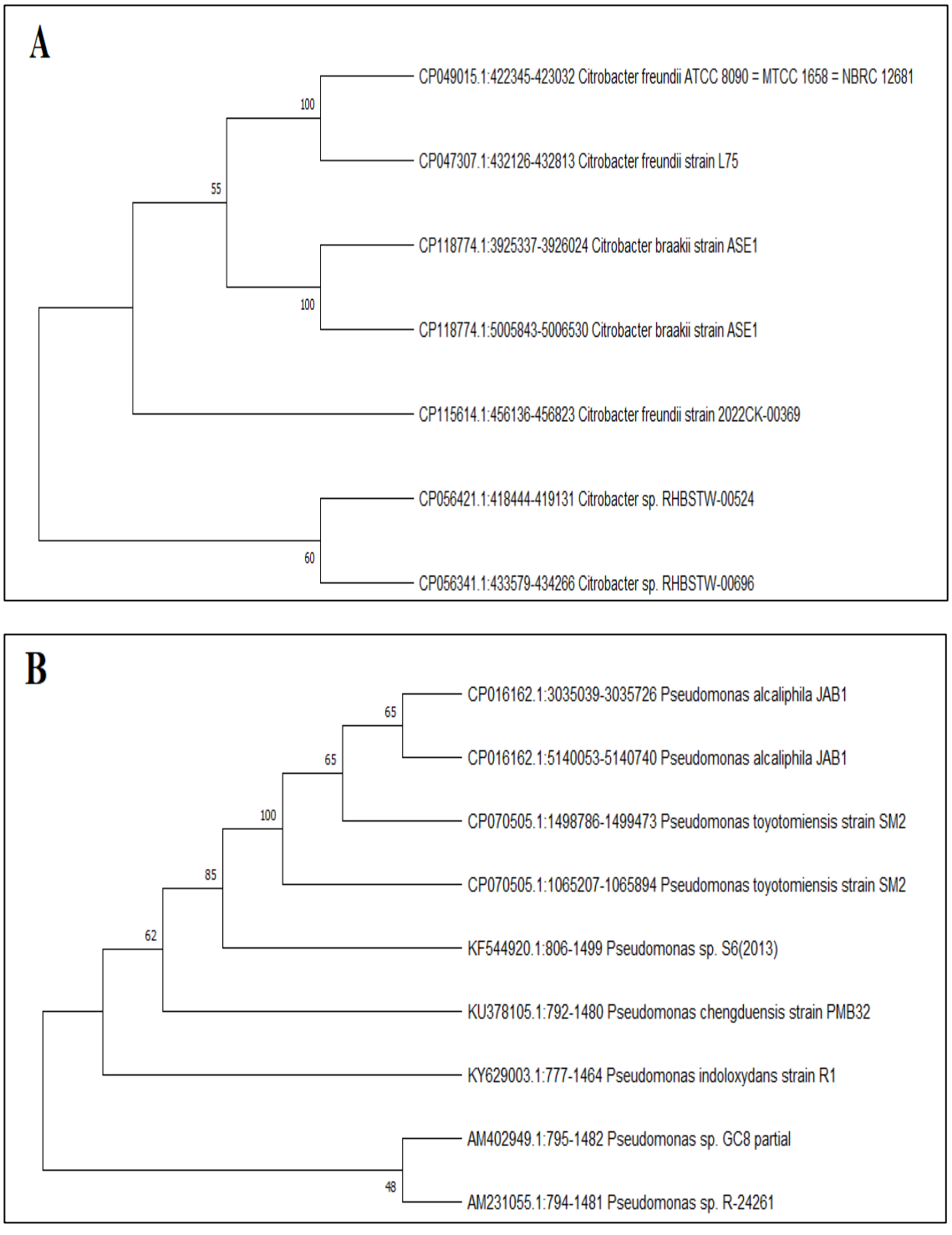

Fig. 3.10 Neighbour-joining phylogenetic tree drawn by 16S rDNA sequence of *Citrobacter freundii* ATCC 8090 = MTCC 1658 = NBRC 12681 (A) and *Pseudomonas alcaliphila* JABI (B) isolated from *P. excavatus* showing their position and relationship with other strains.

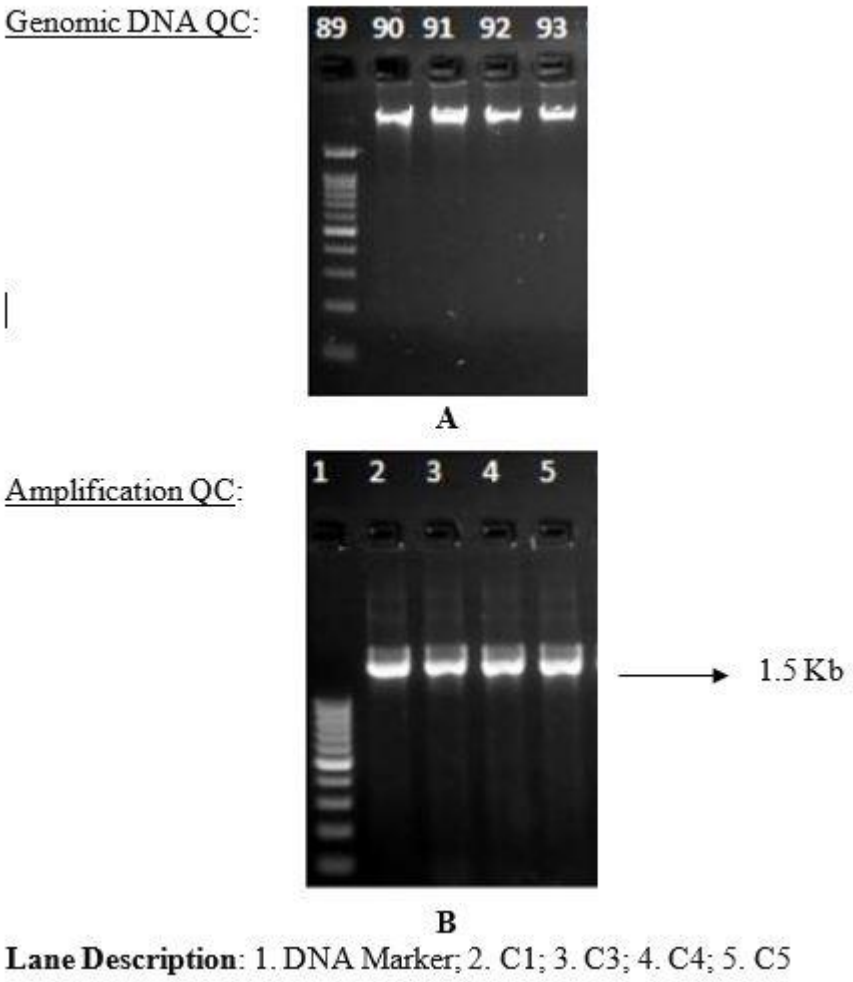

Fig. 3.11 DNA loaded in 1% Agarose gel (A) PCR products loaded on 1% Agarose gel (B).

3.3 Discussion

In addition to soils, earthworms feed upon various organic materials and extract sufficient nourishment required for their growth and reproduction. Availability and types of food influence the size of earthworm populations and their species diversity and reproduction (Edwards and Bohlen, 1996). In the present study, the influence of food availability on earthworms was seen in biomass accumulation and growth rate. Earthworm biomass gain varies depending on the kind of substrate used as feed. Although

no significant differences (p>0.05) were observed, net biomass gain in T2 (142.4±7.23) was higher compared to T1 (123.33±39.00) and T3 (107.66±18.58), indicating higher nutrient assimilation efficiency in T2 which could also be due to better macronutrient content of the substrate.

It is argued that earthworm biomass gain is associated with microbial load in its environment. Suthar (2007)[b] reported that earthworm growth and reproduction are subjective to microbial biomass, nutrient quality, and decomposition activities of microbes. Kabi *et al.* (2020), while assessing the growth rate of *E. eugeinae*, another epigeic earthworm, reported that maximum growth rate coincided with sexual maturity, which also has a firm reliance on nutrient supply and other environmental factors such as aerations and optimum moisture. Accelerated biomass accumulation and growth rate during the pre-reproductive phase were more or less comparable with Kabi *et al.* (2020) and Viljoen and Reinecke (1989), who observed a curvilinear growth pattern in the early days of the hatchlings till they attained sexual maturity. A generalized decrease in earthworm biomass was observed after attending sexual maturity (**Fig. 3.2**), which could be due to changes in their physiological condition before the onset of cocoon production. Many researchers in the past (Mba, 1983; Garg *et al.*, 2005) opined that the decrease in biomass could be due to the large amount of energy required for the elaborate partitioned process of copulation, cocoon production, and reproduction. Since earthworm biomass gain depends on food availability, combinations of organic matter in bedding are significant. In the present study, the ratio of organic matter in the combinations of food substrates must be lesser than required, which might be attributed to lesser biomass gain than in other studies like Suthar (2009)[b].

Due to easily assimilable organic matter, cow dung is the most preferred diet of earthworms irrespective of species (Loh *et al.,* 2005; Suthar, 2007). However, in the present study, the growth rate in T2 (46.33±19.7) was higher compared to T3 (30.13±14.18) and T1 (26.66±11.71). Also, earthworm growth rate ($F_{(2,15)}$=2.48, p<0.05) and cocoon production ($F_{(2,18)}$=0.93, p>0.05) showed no significant differences (p>0.05) among the treatments. Lesser biomass gain in rice straw could be due to an excess amount of flavonoids, lignin, and polyphenols content (Chaudhuri *et al.,* 2013).

Clitellium development is an essential feature in earthworm reproduction and life cycle. Depending on the substrate used and nutrient availability, the number of days required for clitellum development varies. In T2 treatment, *P. excavatus* takes 22.75±3.5 days for clitellum development, which is less than T1, which took 24.5±4.95 days. While in T3, clitellum development takes 42±4.94 days which is significantly (p<0.05) higher than T1 and T2. The observed duration of clitellum development in the present study was in agreement with Karmegam and Daniel (2009) observation where *P. ceylanensis* takes 15-35 days for clitellum development. However, the present study contradicts the findings of Garg *et al.* (2005), where clitellum development of another epigeic species, *Eisenia fetida,* requires 21 days when fed with matured cattle manure. In the clitellum, a glandular non-segmented body, earthworms secrete a viscid sac to make a cocoon where eggs are stored post-copulation. Fertilization and development of the embryo take place inside the cocoon until hatching. Earthworm clitellum has an important implication on cocoon size as its diameter determines the size of the cocoon (Chaudhuri and Datta, 2020). Similarly, Senapati and Sahu (1993) also reported that the diameter of the earthworm clitellum and its cocoons are directly correlated with one another.

The ability of clitellated earthworms to produce viable cocoons from different treatments indicates the suitability of species for vermicomposting and other applied experimentation. Besides the biochemical quality of the substrate, cocoon production is determined by microbial biomass and decomposition activities (Dominguez *et al.,* 2003; Suthar, 2006). Based on the quantity of organic matter present, the total number of cocoons produced varies, where the highest cocoon/worm/day was observed in T2 (0.26) followed by T1 (0.23) and T3 (0.20). Higher than the present findings, Debnath and Chaudhuri (2020) reported that *P. excavatus* cocoon production ranges from 0.13 to 0.3 cocoon/worm/day in a different form of organic waste (**Table 3.6**). Lower cocoon production in the present study could be due to the lesser amount of organic matter in the feed preparations (high substrate-to-soil ratio).

Combinations of cattle manure and other plant materials facilitate the fermentation of substrate in the worm gut, which provides metabolites needed to meet energy and protein requirements for cocoon production in earthworms (Kabi *et al*., 2020). According to Suthar (2007), the nitrogen content of the substrate has positive effects on earthworm cocoon production and further development by influencing the dietary need for protein. Lower cocoon production in T3 and T2 could be due to high phytochemicals and various essential oils having antimicrobial and antifungal activities that inhibit earthworm reproduction rate (Baral *et al.,* 2011; Rufatto *et al.,* 2012). In addition to microbial activities and various physico-chemical parameters, cocoon production in earthworms is affected by biological (biomass) and ecological factors (temperature). Interestingly, Satchel (1967) reported a different relationship between cocoon production and the soil profile where it lives. Thus, surface dwellers are more likely to produce more

cocoons as they are more prone to predation and high mortality rate in early life (Lee and Piearce, 1987).

In natural conditions, cocoon production peaks during monsoon and post-monsoon seasons, which are attributed to optimum moisture and temperature. Temperature beyond the optimum range acts as a cue for the neurosecretory activity that declines cocoon production in earthworms (Olive and Clerk, 1978). Also, Satchel (1967) pointed out that substrate moisture content and cocoon production have a direct relationship. Following Bhattacharjee and Chaudhuri (2002), moisture was maintained at 70-80% in the present study. The low rate of cocoon production per earthworm in the present study could be due to lower temperatures compared to other regions of the country. Irrespective of the substrate, a drastic decrease in cocoon production was observed towards the end, indicating that *P. excavatus* is a continuous feeder, one quality that can put this earthworm species ahead of others in vermicomposting and waste management through organic matter degradation.

Temperature is an essential regulatory factor in the incubation period of the earthworm cocoon. The incubation period of the cocoon was 25.6±5.5 days which is very high compared to other species, such as *E. eugeniae* where the incubation period ranges from 10.79 to 12.41 days (Kabi *et al.,* 2020). While in *P. ceylanensis,* Karmegam and Daniel (2009) reported an incubation period of 12 to 26 days with hatching success (%) of 74.67, 82.67%, and 82.67% in worms cultured singly, in batches of four and eight. A higher incubation period could be attributed to a lower temperature as the present study was conducted in between 18-25°C. Chaudhuri and Datta (2020) also reported a decrease in the incubation period of *P. excavatus* and *P. ceylanensis* cocoons within a temperature

range of 22-31°C. While on the other hand, Chaudhuri and Bhattacharjee (2011) reported that under laboratory conditions, an increase in temperature increases the incubation period of species such as *Eutyphoeus comillahnus* and *Octochaetona beatrix.*

P. excavatus hatching rate was 55.6% which is very close to 53% as reported by Chaudhuri and Bhattacharjee (2002) but lower than 63% as reported by Hallat et al. (1990) and higher than 49% by Chaudhuri and Datta (2020). In the present study, low temperature and lesser microbial load might be attributed to the low hatching efficiency of the cocoon. Also, laying the cocoon by newly matured and nonmated (parthenogenetic) earthworms, as reported by Hallat *et al.* (1990), might contribute to the low hatching percentage of the cocoon. It is reported that the largest cocoon size with a more extended incubation period produces more hatchlings in *E. eugeinae* than smaller earthworms such as *P. excavatus.* Bhattacharjee and Chaudhuri (2002) reported that the incubation period and the number of hatchlings per cocoon share a positive relationship in *Lampito mauritii*. Chaudhuri and Datta (2020) also reported that hatchlings in *E. eugeinae* (2.58) were highest and lowest in *P. excavatus* (1), while in the present study, hatchlings observed per cocoon were 0.92.±0.28.

Table 3.6 Growth and reproduction of epigeic earthworm species under different food substrate

Earthworm species	Culture materials	Growth rate (mg per worm per day)	Reproduction rate		References
			Cocoon /worm/ day	Juvenile/ adult/ week	
	Cow dung	2.86	-	2.45	
	Cow dung-Kitchen waste	2.47	-	1.37	Chaudhuri & Bhattacharjee (2002)
	Cow dung-Straw	4.75	-	14	
	Cow dung-Leaf litter	4.02	-	11.7	

	Rubber leaf litter	5.04	-	0.2	Chaudhuri *et al*. (2003)
	Pressmud-Cow dung	4.81	0.79	-	Birundha *et al*. (2013)
	Cow dung	22.91	0.25	1.39	
	Acacia leaf-Cow dung	3.47	0.3	3.31	Debnath & Chaudhuri (2020)
P. excavatus	Bamboo leaf litter-Cow dung	12.6	0.23	2.22	
	Mikania micrantha-Cow dung	9.47	0.13	0.66	
	Cow dung		0.23±0.08		Present study
	Kitchen scrap		0.26±0.01		
	Rice straw		0.20±0.07		
Dichogaster modiglianii	Pasture soil	-	0.19	-	Bhattacharjee & Chaudhuri (2002)
	Rubber leaf litter	6.2	-	1.3	Chaudhuri *et al*. (2003)
E. fetida	Cow dung	16.3	0.39	-	
	Goat waste	16.5	0.32	-	Garg *et al*. (2005)
	Sheep waste	26.2	0.44	-	
	Kitchen waste-*Mangifera indica* leaf litter	3.77	0.25	-	Suthar (2007)
P. sansibaricus	Cattle solid waste	8.00	0.22	-	Suthar (2009)
P. ceylanensis	Cow dung	1.34	0.94	-	Karmegam & Daniel (2009)
	Rubber leaf litter	28.8	-	1.4	Chaudhuri *et al*. (2003)
E. eugeinae	*Diospyrosa meanoxylon* leaf litter	68.00	0.54	-	Kadam (2015)

Using microorganisms as biofertilizers is one of the most sustainable approaches towards enhancing agricultural output. Indeed, it is one of the paradigm shifts that emphasizes the use of biological amendment instead of conventional chemical fertilizers. Quite a number of microorganisms are there to solubilize the insolubilize form of phosphorus, making it available for the plant to absorb. Therefore the application of these microorganisms as biofertilizers is a promising strategy to increase crop production.

Some of the bacteria that are known to solubilize phosphorus through solubilization and mineralization include *Azotobacter*, *Enterobacter*, *Erwinia*, and *Paenibacillus*, etc. (Chakraborty *et al.,* 2009; Babalola and Glick, 2012; Kumar *et al.,* 2014). Genus *Pseudomonas* having plant growth-promoting characteristics is also been reported by other researchers (Linu *et al.,* 2019; Bhakta, 2022). It is suggested that through the application of major PSB such as *Pseudomonas, Bacillus, Micrococcus, Aspergillus, Fusarium*, etc. crop production can be increased up to 200–500 kg/ha (Saritha and Prasad-Tollamadugu, 2019). In the present study, four bacterial colonies were found positive for phosphate solubilization, implying that the *P. excavatus* body is an important habitat for PSB. The beneficial attributes of such bacteria could be a potential source for exploring microbial as biofertilizers. PSI (mm) of bacterial colonies varies from 1.13±0.03 to 1.59±0.09mm. Increasing incubation period leading to higher PSI value in the present study were in conformity with the other reports where isolated *Bacillus sp*. showed maximum PSI at 96 hr incubation (Banerjee *et al.,* 2010). This might be due to the cellular growth of the strains that reached their exponential phase. The principal cause of phosphate solubilization is the acidification of the culture medium by low molecular mass organic acids secreted by microorganisms (Khan *et al.,* 2013). Similarly, Ma *et al.* (2009) reported that various organic acids like gluconic acid, 2-ketogluconic acid, oxalic acid, isobutyric acid, lactic acid, acetic acid, citric acid, isovaleric acid, etc. produced by phosphate solubilizing bacteria enhance solubilization of insoluble phosphates.

Following the spread plate method using Pikovskaya's agar media, Bhakta *et al.* (2022) identified eight PSB colonies from the earthworm gut; of total bacterial colonies, *Pseudomonas aeruginosa* EGM8 strain was considered the potential PSB with the highest PSI (4.8± 0.5) and inorganic phosphate solubilization activity (1.053 ± 0.18 mg/l) and

concluded that earthworm would be a potential source for microbial biofertilizer for high crop production. In the present study, the PSI (mm) of bacteria was higher than the previous record, with the highest being observed in C1 (15.81±0.92), C4 (13.63±0.81), C5 (12.8±0.79), and C3 (11.33±0.32). Biswas *et al.* (2022) also isolated three strains of bacteria from *Metaphire posthuma*, namely *Bacillus megaterium* (MF 589715), *Staphylococcus haemolyticus* (MF 589716), and *Bacillus licheniformis* (MF 589720). The isolated strains solubilized phosphate even in the presence of metals (Cu and Zn), showing resistance to significant concentrations. Also, the strains were able to produce IAA in the presence of L-tryptophan and possessed ammonium ion production potential.

In addition, the isolated strains of bacteria also showed positive for IAA productions, one of the most critical plants growth-promoting phytohormones. IAA productions in the present study were tryptophan dependent. IAA production by earthworm-associated bacteria conformed with Biswas *et al.* (2018). The IAA-producing microorganism stimulates the plant cell elongation or divisions, especially in roots, thereby providing greater surface area for soil nutrient absorption and enhancing root growth and length (Glick, 2012).

Chapter 4

Plant growth and soil nutrient enhancement

Contents

4.1 Introduction

The rapid increase in the world's population and unprecedented human consumption necessitate increased food production. This has been achieved by the intensive use of chemical fertilizers and pesticides in agricultural practices but. But these unsustainable practices to maximize productivity has resulted in land degradation, human health hazards, interrupted ecological nutrient cycling, and destruction of healthy biotic communities (Shah *et al.,* 2018). Therefore, it is vital to emphasize on cost-effective and eco-friendly methods of agriculture for a healthier future.

In soil, large-bodied macroinvertebrates such as earthworms play a crucial role in plant productivity. Through ingestion and microbial priming activities, solubilization of inaccessible forms of soil nutrients is anchored by earthworms making it available for plants (Prescott, 2005; Yoshitake *et al.*, 2014; Van Groenigen *et al.,* 2019). Burrowing activities of earthworms aid aerations and improve biochemical properties providing stability and resilience to the soil ecosystem (Singh and Gupta, 2018; Wurst *et al.,* 2018). Earthworms can process up to 250 tons per hectare of soil yearly (Zaller *et al.,* 2013) and provide optimum soil conditions for plant growth. Earthworms also increase soil organic carbon by incorporating organic materials into the soil (Fahey *et al.*, 2013) and generate macropores that increase the water flow, which protects the soil surface against erosion (Sharma *et al.,* 2017). With proliferative reproduction, the earthworm is an economically affordable, environmentally sustainable, and socially acceptable potential candidate for improving soil properties for better plant productivity (Hallam *et al.*, 2020).

It is well understood that in the natural environment, different ecological categories of earthworms (epigeic, endogeic, and anecic) and their interactions with other soil biota

improve the soil's physico-chemical properties. Earthworms initiate numerous mechanisms for plant growth stimulation ranging from large-scale effects on soil physical properties to the microsite level. The potential effects of earthworms on ecosystem modification, plant community composition, and productivity are well demonstrated when earthworms are introduced to areas that were previously earthworm-free (Frelich *et al.,* 2006; Mudrák and Frouz, 2018). The benefits of earthworms are highlighted through increased levels of microbial activity, nutrient availability, and rhizosphere processes (Scheu, 2003; Paliwal, 2020). With the introduction of earthworms in soil with sandy textures, poor in organic matter, and with a moderately acidic pH, plant shoot and grain biomass increases to 56.3% and 35.8%, respectively (Brown *et al.,* 2004).

Studying earthworms' influence on soil properties is essential to develop management strategies for improving soil fertility and plant growth in different subsystems of tropical areas. Although positive effects of earthworms on plant growth have been described in agroecosystems (Brown *et al.,* 1999; Edwards and Arancon, 2022), quantitative studies on the role of earthworms in augmenting plant biomass productivity have not been satisfactorily established. In a tropical country like India, earthworm species such as *Pontoscolex corethrurus* and *Drawida willsi* with biomass of around 30 g m^{-2} or more, are considered promising in plant growth and shown to increase the grain yield (> 40%) of agriculturally important plants (Brown *et al.,* 1999). While single species of earthworms under laboratory conditions resulted in more significant improvements in soil physico-hydraulic properties, however such studies on the most important horticultural plants such as *Capsicum chinense* (King chili) and *Zea mays* are lacking.

C. chinense (King chili) one of the hottest chili in the world, is commonly grown in Indian states of Assam, Manipur, and Nagaland; and besides being appreciated for its taste and pungency, it is also rich in vitamins, minerals, and nutrients (Medina-Lara *et al.*, 2019). King chili is one of the key ingredients in Naga cuisine and plays a vital role in the region's culinary traditions and cultural identity. The state has favorable climatic conditions for growing this chili variety, and the demand for it has also increased in recent years. Therefore employing efficient means of farming could provide windows of opportunities for entrepreneurs to gain significant economic importance. *Z. mays* (Corn, locally known as Makai) is a vital staple food crop in Nagaland and Northeast India. It plays a crucial role in the region's food security and sustains a large population. Corn-based dishes are integral to traditional cuisine, and their cultivation and consumption have deep cultural and social significance. Corn adds dietary diversity and nutritional value to the local diet with a rich amount of carbohydrates, dietary fiber, vitamins (such as thiamine and niacin), and minerals (such as phosphorus and magnesium). Also, corn cultivation is a vital livelihood source for many farmers in Nagaland. It provides employment opportunities, especially for small-scale farmers, and contributes significantly to the rural economy. The sale of corn and its by-products, such as corn flour, corn flakes, and corn oil, generates income for farmers and local entrepreneurs. It is necessary to formulate ideas to design of agricultural strategies that facilitate an increase in the growth and yield of these economically potential plants while conserving the concept of organic farming and sustainability.

P. excavatus and *E. fetida* are the most promising earthworm species used for vermitechnology. Being epigeic, these macroinvertebrates dwell in the organic carbon-rich soil and play a vital role in nutrient turnover. Apart from a few studies supporting the

usage of *P. excavatus* and *E. fetida* in vermicomposting (Das *et al.,* 2022; Pottipati *et al.,* 2022), information on the role in plant growth, productivity, and soil macronutrient enhancement is lacking. Therefore to abide by the initiatives to implement the practice of organic farming and sustainability, exploitation of these potential macroinvertebrates (*E. fetida* and *P. excavatus*) is vital.

Nagaland (Northeast India) is a hilly state where indigenous people depend on agricultural practices for their sustenance. There is a need to further encourage organic farming practices without decreasing the quality and quantity of productivity for viable and healthier living. In this context, the application of epigeic earthworm species like *P. excavatus* and *E. fetida* is considered sustainable, especially for *C. chinense* (Naga king chili) and *Z. mays*, popularly used by local inhabitants for consumption as well as vending in the local market. Therefore, the present study focuses on improving the biomass and yield of *C. chinense* and *Z. mays* by inoculating *P. excavatus* and *E. fetida* in the soil. We hypothesize that earthworms act as soil nutrient facilitators by acting as critical agents in nutrient turnover and enhancing the plants' growth and productivity.

4.2 Materials and Method

4.2.1 Pre-experimental preparations

Two different earthworm species, *E. fetida* and *P. excavatus* were used for the experiment (a) *E. fetida*, a commercially available species, was procured from a local vermicomposting farm in Wokha, Nagaland, India, and (b) *P. excavatus*, was sampled during an earthworm resource exploration study in Minkong forest (26°21'43.34"N and 94°33'23.42"E) under Mokokchung district, Nagaland. Both the earthworm species were mass cultured separately in the vermicomposting chamber in Zoology Department,

Nagaland University, for adaptation to the local environment and multiplication of their population. Moisture in the vermiculture setup was maintained at 60%-70% by sprinkling water regularly, and temperature ranged between 28°C to 34.6°C. Earthworms were fed with a mixture of urine-free pre-decomposed cow dung collected from a local farm and domestic organic waste collected from the households.

4.2.2 Experimental design

Two commonly available and preferred plants *C. chinense* (locally known as Naga king chili) and *Z. mays* (Makai) were selected for experiment. Loose topsoil (0-10 cm depth), rich in organic matter, was collected from the University campus for plant growth experiments. Before the experimental setup, seeds were sprouted by soaking in water for 36 hours.

For *C. chinense*, the experiment was performed in triplicates using 5 L capacity plastic pots with a dimension of 250mm in length and 200mm in diameter. The nine pots were filled with soil, sprinkled with water, and 150 g of urine-free half-decomposed cow dung was put in each pot as feed for earthworms; the same amount of cow dung was also applied to the control to maintain the uniformity of nutrients in the pot. Ten (10) healthy, clitellate individuals of *P. excavatus* with an average weight of 350.34±7.4 mg were introduced in the first three-pots. Similarly, ten (10) healthy *E. fetida* with an average weight of 410.12±8.5 mg were introduced in the following three pots. The third set of three pots was kept as the control without any earthworms. For *Z. mays*, nine plastic pots (in triplicate for each group) of 15 L capacity (Length- 390mm and diameter- 320mm) were used due to the larger plant size. The pots were filled with soil, labeled, and regularly sprinkled to maintain optimum moisture (40-50%). 300 g of urine-free half-decomposed

cow dung was put in each pot as feed for earthworms. Twenty (20) healthy clitellated earthworms, *P. excavatus* (average weight 350.34±7.4), and *E. fetida* (average weight 410.12±8.5) were inoculated each in the first and second sets of the pots, respectively, and the remaining three pots were kept as control with no earthworm.

In all the pots, earthworms were allowed to settle and observed for three days to ensure normal survival and growth. Once the earthworms settled and moved inside the soil, a pair of sprouted *C. chinense* and *Z. mays* seeds were planted into their respective pots. In all the experimental pots, optimum moisture (40-50%) was maintained by sprinkling water regularly. Because the experiment was performed under a greenhouse, pest attacks were not observed. The temperature ranged between 28-34.6°C and humidity 40-80%, depending on the weather fluctuations. From day one till the plants fully matured and began to bloom, the growth of plants was monitored at a regular interval of 7 days, measuring the stem length and counting the number of leaves and fruits. For *C. chinense*, once the plant started to bear fruits and matured, harvesting was done weekly, and fruits were weighed using a portable digital weighing machine (Oblivion-OBSF400A) to record the amount of fruit harvested per plant/week.

4.2.3 Soil physico-chemical analysis

Soil samples were analyzed at two intervals, i.e., before and after the experiment. Before analysis, soil samples were air-dried, sieved through a 1mm mesh size, and kept in an airtight plastic bag. pH was measured using a digital pH meter (Lab junction-111) at a 1:20 soil-water ratio**. (a)** Organic carbon: This was analyzed by a modified form of the wet oxidation method (Walkley and Black, 1934). Samples were digested with potassium dichromate using sulphuric acid and titrated with ferrous ammonium sulfate in

the presence of diphenylamine as an indicator- the endpoint, indicated by green in color, was noted and calculated for organic carbon (%). (**b**) Total nitrogen (TN): This was done by Kel plus instrument (Pelican equipment- Classic- DX VAT-E). In this the sample is digested and amino nitrogen is converted into ammonium radicals in the presence of strong acid (H_2SO_4) aided by potassium sulfate and copper sulfate as a catalyst. Further, separation and isolation of nitrogen from the digestion tube were processed through distillation in the presence of sodium hydroxide (NaOH), during which ammonium radicals are converted into ammonia which was collected by trapping in 4% boric acid (H_3BO_3). Lastly, total nitrogen determination was done by titrating with 0.1N hydrochloric acid (HCl) in the presence of methyl red and bromocresol green (8:10) as an indicator. (**c**) Available phosphorus (Av. P) was determined spectrophotometrically (Systronic spectrophotometer-166) using a modified method initially described by Bray and Kurtz (1945). The bound form of phosphorus and acid-soluble phosphorus was extracted using a bray reagent containing 0.03N ammonium fluoride (NH_4F) and 0.025N HCl. The amount of available phosphorus was determined by the intensity of blue color development when treated with a molybdate-ascorbic acid reagent. (**d**) Available Potassium (Av. K): This was determined following a modified form of the ammonium acetate method described by Hanway and Heidel (1952), where neutral ammonium acetate (NH_4OA) was used to extract exchangeable potassium ions, which was further determined by a flame photometer (Systronic flame photometer-130).

4.2.4 Statistical analysis

The mean significant differences in the number and biomass of leaves, length, and biomass of stem, length and biomass of root, growth rate, and total fruit harvest among

the treatment were evaluated using one-way ANOVA at a 95% confidence level (p<0.05). Each analysis was followed up with multiple comparison tests (Tukey test) to find the mean differences between treatments. Based on the eigenvalues and factors loading of the principal component, a dimensional reduction technique, Principal component analysis (PCA), was evaluated to comprehend the relationship of soil physico-chemical parameters with plant morphology. The statistical analysis was computed using SPSS (Version 22) and OriginPro 22.

4.2 Results

4.3.1 Earthworm effects on *C. chinense*

In *P. excavatus* inoculated soil, the average growth rate (cm/day) was maximum (8.3±2), followed by the control and *E. fetida* inoculated soil with 7.5±3.5 and 5.7±1.4 respectively (**Fig. 4.1-A**). The average growth rate in *P. excavatus* inoculated soil was 12.59% higher than the control, while in *E. fetida* inoculated soil, it was 9.65% lower. A significant difference in the number of leaves recorded from earthworm inoculated soil (163.66±43.88 and 91.25±13.43 in *P. excavatus* and *E. fetida* respectively), and control (46.5±19.94) ($F_{(2, 9)}$= 8.9, p<0.05) was recorded (**Table 4.1**). A high percentage in the number of leaves was recorded in both *P. excavatus* and *E. fetida* inoculated soils (250.40% and 142.85%, respectively). Mean leaf biomass (g) also varies significantly ($F_{(2,12)}$=7.89, p<0.05) depending on the treatment, and maximum biomass resulted from *P. excavatus* (69.16±20.73)> *E. fetida* (28.87±0.82)> control (35.85±6.77). With 92% and 10.06% increase over control, biomass increase in *P. excavatus* was significantly (p<0.05) higher. The average stem length (mm) at the time of harvest was higher in *P. excavatus* (410±49.7) than in control (398.7±70.2) and *E. fetida* (368.7±36.6) (**Fig. 4.1-B**), showing an increase and decrease of 7.66% and 2.77% respectively over the control.

Stem length was substantially higher in the presence of *P. excavatus* (**Table 4.1**). While stem biomass (g) in *P. excavatus* inoculated soil was increased by 47.30% (89.32±24.27) over the control (63.00±26.78), while in *E. fetida* (46.42±21.27), it was decreased by 16.43% (**Fig. 4.2**).

Table 4.1 Morphological characteristics of *C. chinense* in the presence and absence of earthworms.

Treatment	*P. excavatus*	*E. fetida*	Control	*H*	*p*-value
No. of leaves	163.66±43.88^{a}	91.25±13.43^{ab}	46.5±19.94^{b}	8.95	0.007**
Leaf biomass (g)	69.16±20.73^{a}	28.87±0.82^{c}	35.85±6.77^{bc}	7.89	0.006*
Stem length(mm)	41±4.97^{a}	36.87±3.66^{a}	39.87±7.02^{a}	0.26	0.87
Stem biomass(g)	89.32±24.27^{a}	46.42±21.27^{b}	63.00±26.78^{a}	2.68	0.12
Root length (mm)	40.5±8.21^{a}	25.12±2.01^{b}	30.00±8.04^{ab}	8.12	0.01*
Root biomass(g)	41.8±12.75^{a}	31.39±12.7^{a}	29.2±8.87^{a}	2.55	0.11

Data represent mean ± SD. * indicate a significant difference at $p<0.05$. Mean with different superscripts within the same row differ significantly ($p<0.05$) by the Tukey test at a 95% confidence level.

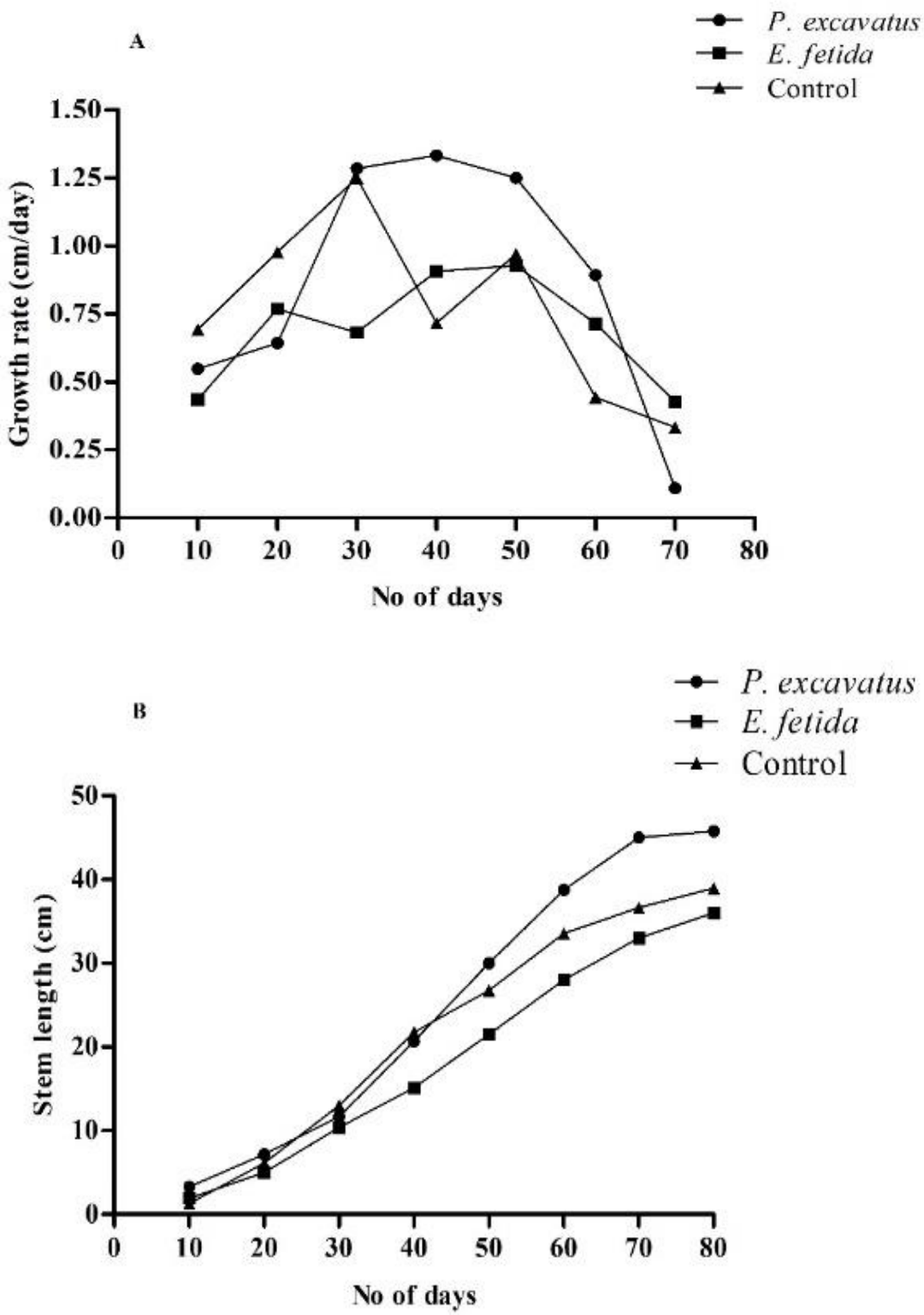

Fig. 4.1 (A) Growth rate of *C. chinense* under earthworm treatment and control (B) Increasing pattern of *C. chinense* stem under earthworm-mediated soil and control.

Root length (cm) in *P. excavatus* (405.00±82.1), *E. fetida* (251.2±20.1), and control (300.0±80.4) differ significantly ($F_{(2,9)}$=8.12, p<0.05). Due to fungal infection, the root length from *E. fetida* inoculated soil was decreased by 2.42%, while in *P. excavatus,* the root length was 30.14% higher. Also, root biomass (g) was recorded to be maximum in *P. excavatus* (41.8±12.75) with a 58.04% increase over control, while in *E. fetida* (31.39±12.7), it was increased by 20.22% (**Fig. 4.2**). Along with the plant morphological characters, significant variations ($F_{(2,10)}$=5.24, p<0.05) in total fruit yield (g) per plant were also recorded. Total harvest was observed to be maximum in *P. excavatus* (573.27g), followed by control (266.8g) and *E. fetida* (112.99g). In *P. excavatus* inoculated soil, ripened chili was harvested at 17.59 g per week with an average of 108.13±37.93g per plant. In control and *E. fetida*, ripened chili was harvested at 13.91g and 9.24g per week. While the quantity of harvest per week does not differ significantly ($F_{(2,18)}$=1.21, p>0.05) among the treatments, it was 26.49% higher (*P. excavatus)* and 33.53% lesser (*E. fetida)* over the control. It was observed that during the initial 30-35 days, plants grew successfully in the earthworm-treated soil and control. However, fungal infection occurs in *E. fetida* inoculated soil. 50% of the plant roots in *E. fetida*-inoculated soil (**Fig. 4.3A-F**) did not survive till maturity, and the average growth rate was negatively affected (**Fig. 4.3-F**)

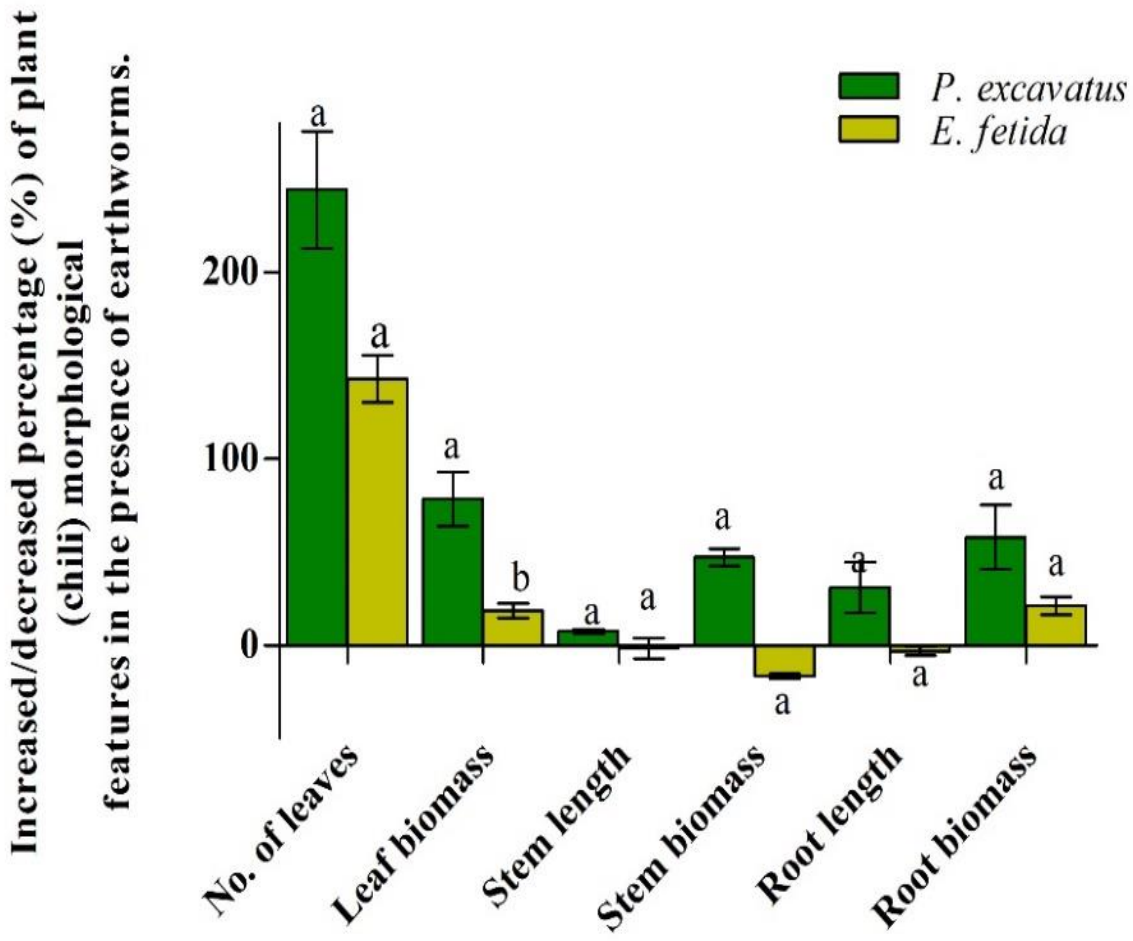

Fig. 4.2 Increased/ decrease in *C. chinense* morphological characters in the presence of *P. excavatus* and *E. fetida***.** Data represent mean ± SD. Different superscripts between earthworm treatments differ significantly (p<0.05) by the Tukey test at a 95% confidence level.

Fig. 4.3 (A)Flowering of *C. chinense* observed on day 50 in *P. excavatus* treated soil (B) Ripen fruit from *P. excavatus* treated soil observed on 78th day (C) Fully ripen chili in*E. fetida* treated soil (D) One-time harvest from single plant grown in *P. excavatus* treated soil (E) Freshly harvested plant taken for root length and biomass estimation (F) Fungal infected root and stem causing rotting of plants starting from underground roots to stem resulting into the black in color, observed in *E. fetida* treated soil.

4.3.2 Earthworm effects on *Z. mays*

Growth peaks between 70-80 days, the highest average growth rate (cm/day) was recorded in *E. fetida* inoculated soil (13.6±8.5), followed by *P. excavatus* (13.0±5.3) and control (12.7±2.5) indicating the positive effect of earthworms (**Fig. 4.4-A**). The average number of leaves per plant was maximum (14.5±0.95) in *E. fetida* soil, showing the increasing percentage of 9.93% over control. The maximum leaf biomass (g) was recorded in *P. excavatus* (82.6±3.5) followed by control (76.32±4.45) and *E. fetida* soil (63.25± 4.07) (**Table 4.2**). In *P. excavatus* treated soil, leaf biomass was 11.52% higher than in control, while in *E. fetida*, biomass was 16.42 % lower (**Fig. 4.5**). With broader leaf diameter, plants were found to be healthy, and no infestation of plants by insects was observed as the experiment was conducted in a greenhouse (**Fig. 4.6A-F**). Also, unlike *C. chinense*, no pathogenic infections occurred in the plants; all the seedlings grown, matured, and successfully bore corn of varying sizes, depending on the treatments. The average stem length (**Fig. 4.4B**) at the final harvest was maximum in *E. fetida* (246±19.05), followed by *P. excavatus* (222±12.28) and control (206± 13.22), exhibiting an increasing trend of 19.24% and 7.82%, respectively, over the control. Maximum stem biomass was recorded in *E. fetida* (592.16±7.06), followed by *P. excavatus* (517.24±15.44) and control (517.71± 7.12) showing significant differences ($F_{(2,11)}$ = 79.78, p<0.05). Multiple comparisons test shows that the mean stem biomass from *E. fetida* was significantly (p<0.05) higher than *P. excavatus* and control pot. Depending on the treatment, root length also differs significantly ($F_{(2,13)}$=21.41, p<0.05), where the maximum was observed in *P. excavatus* (87.9±5.43), followed by *E. fetida* (69.45±4.1) and control (68.15±1.95) (**Table 4.2**). Multiple comparisons test indicates that the mean root length from *P. excavatus* was significantly higher than that of *E. fetida* and control.

In *P. excavatus,* 28.9% increase in root length over the control was observed, while in *E. fetida*, root length was increased by only 1.9% (**Fig. 4.5**).

Table 4.2 Morphological characteristics of *Z. mays* plants in the presence and absence of earthworm.

Treatment	*P. excavatus*	*E. fetida*	Control	*F*	*p*-value
No. of leaves	13.5±0.95[a]	14.5±0.95[a]	13.5±1.29[a]	2.21	0.16
Leaf biomass (g)	82.6±3.51[a]	63.25±4.07[b]	76.32±4.45[ab]	23.07	0.0002*
Stem length (cm)	222±12.28[a]	246±19.05[a]	206±13.22[a]	14.13	0001*
Stem biomass (g)	517.24±15.44[a]	592.16±7.06b	517.71±7.12[a]	79.78	2.821E-7*
Root length(cm)	87.9±5.43[a]	69.45±4.1[b]	68.15±1.95[b]	21.41	0.000077*
Root biomass (g)	107.55±4.84[b]	126.49±5.69[a]	104.59±4.81[b]	30.11	0.000035*

Data represent mean ± SD. * indicate a significant difference at $p<0.05$. Mean with different superscripts within the same row differ significantly ($p<0.05$) by the Tukey test at a 95% confidence level.

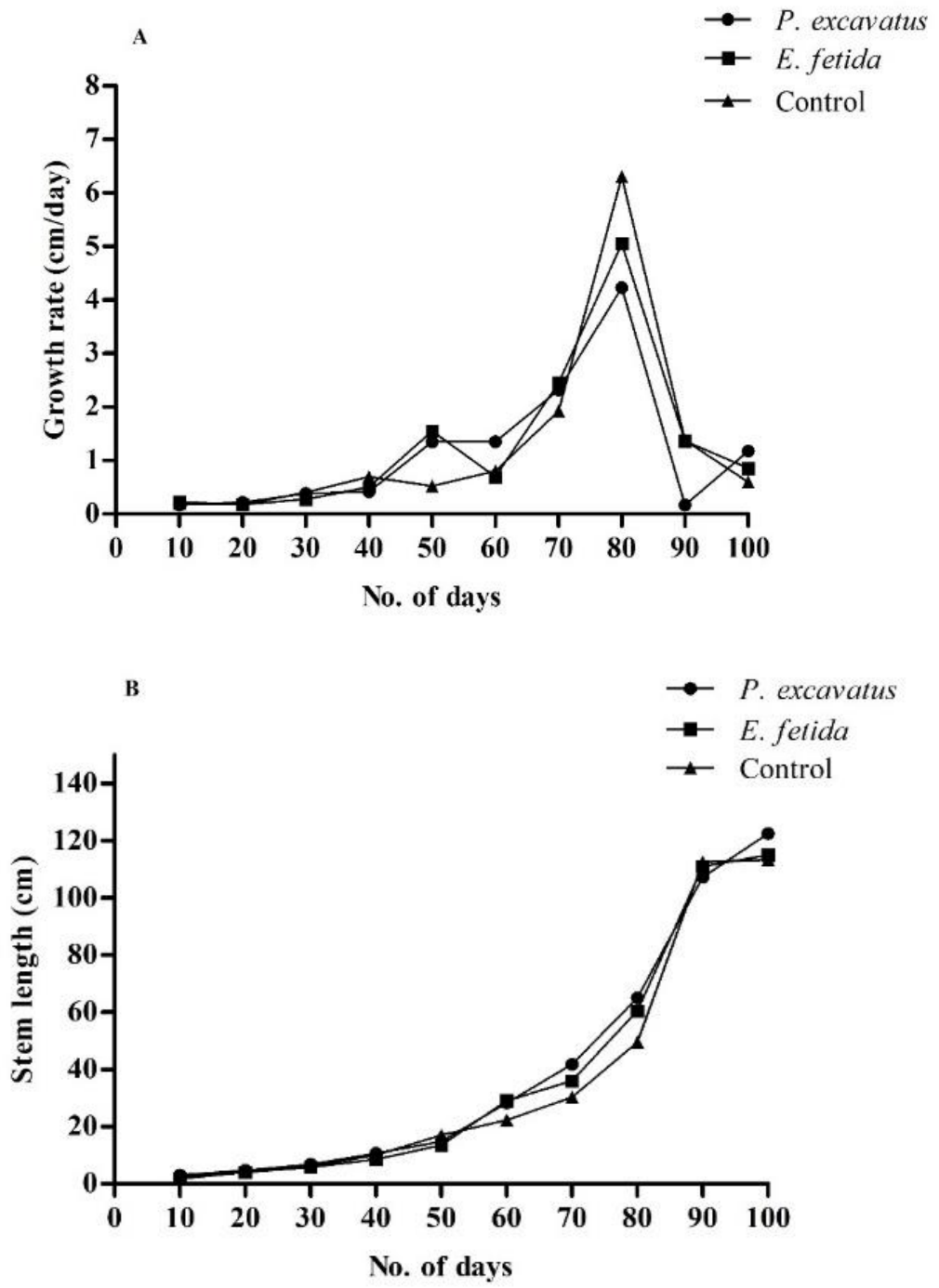

Fig. 4.4 (A) The growth rate of *Z. mays* in the presence and absence of earthworms. (B) Stem length of *Z. mays* under control and earthworm treatment.

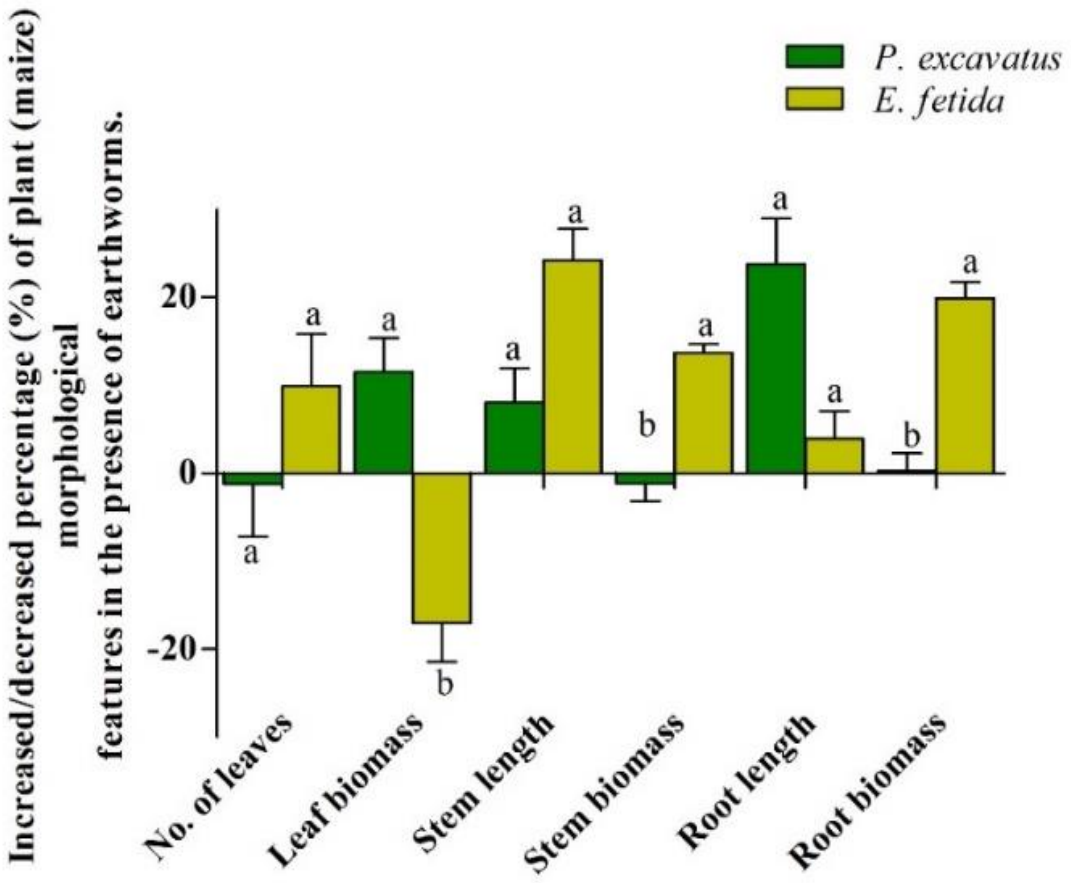

Fig. 4.5 Increased/ decrease of *Z. mays* morphological characters in the presence of *P. excavatus* and *E. fetida***.** Data represent mean ± SD. Different superscripts between earthworm treatments differ significantly (p<0.05) by the Tukey test at a 95% confidence level.

A significant variation of root biomass ($F_{(2, 11)}$ = 30.11, p<0.05) was also recorded with a maximum in *E. fetida* treated soil (126.49±5.69) followed by *P. excavatus* (107.55±4.84) and control (104.59±4.81), showing the increasing trend of 20.93% and 2.82% respectively over control (**Fig. 4.5**). Post hoc test shows root biomass from earthworm-treated soil was significantly (p<0.05) higher compared to control, however, no significant differences (p>0.05) were observed between the two earthworm species. Among the treatments, the total kernel count per corn was significantly different

($F_{(2,9)}$=37.78, p<0.05). The highest number of kernels per corn was present in *P. excavatus* (333.5±13.5), followed by *E. fetida* (261.5±16.5) and control (235±22), showing 41.91% and 11.27% increase over control. Similarly, there was also a significant difference ($F_{(2,9)}$=7.92, p<0.05) in average kernel weight with maximum production from *P. excavatus* (104.6±14.9) followed by *E. fetida* (66.68±6.78) worked soil and control (56.32±10.38) exhibiting 95.05% and 24.34% increase over control.

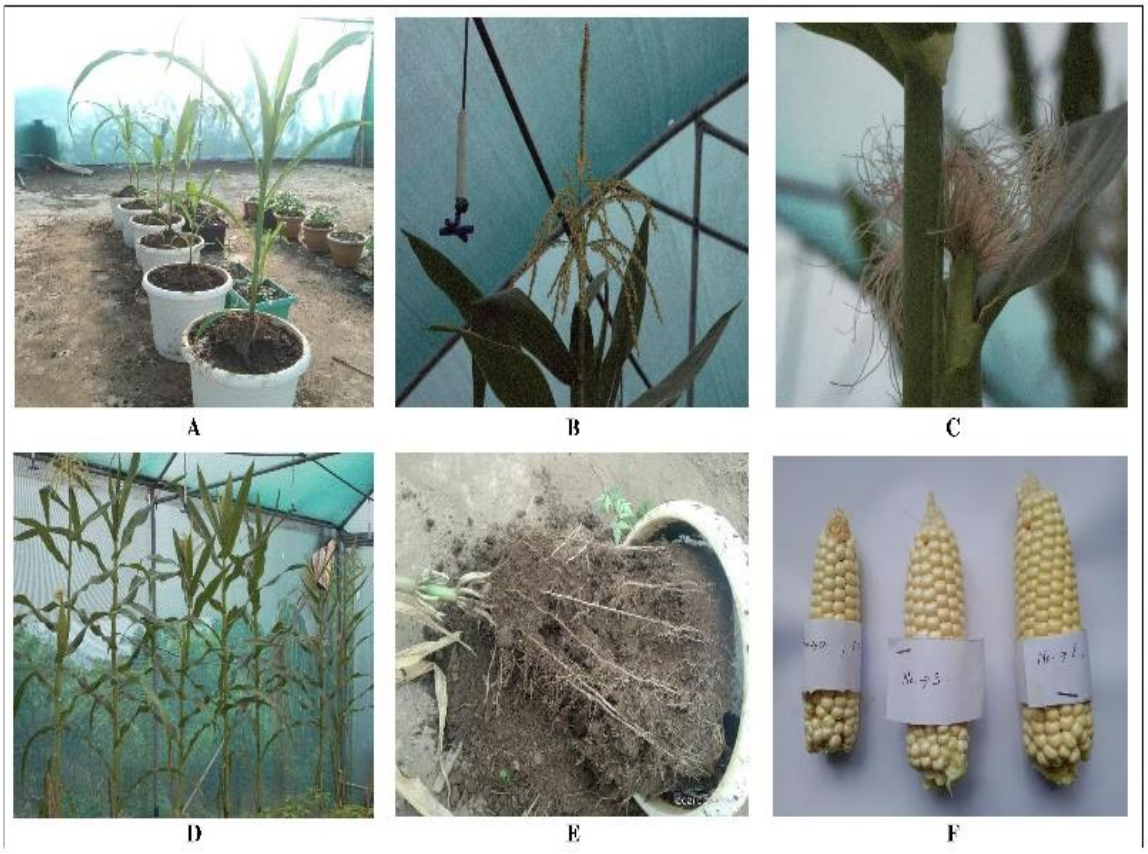

Fig. 4.6 (A)Initial days (Day 63) of *Z. mays* grown in *P. excavatus* treated soil (B) Tassel bearing spikelet pairs, first observed on day 108 in *E. fetida* treated soil (C) First silk appears on the 110th day in *E. fetida* treated soil (D) Fully matured corn bearing plant as observed under greenhouse (E) Removing of roots from the experimented pot for plant biomass estimation and measurement of stem and root length (F) final harvested corn with healthy kernel obtained from control, *P. excavatus* and *E. fetida* treated soil.

4.3.3 Earthworm effect on soil nutrients

Table 4.3 Initial and final physico-chemical characteristics of soil obtained from *Z. mays* and *C. chinense* grew pot treated with *P. excavatus*, *E. fetida*, and control

			A- *C. chinense*			
	Initial	*P. excavatus*	*E. fetida*	control	*F*	*p*-value
pH	5.6±0.04^{a}	6.14±0.34^{a}	6.09±0.15^{a}	6.12±0.28^{a}	3.43	0.07
OC (%)	2.63±0.25$^{\mathbf{b}}$	3.24±0.24$^{\mathbf{ab}}$	3.29±0.32^{a}	3.04±0.06$^{\mathbf{ab}}$	4.61	0.03*
TN (%)	0.42±0.02^{b}	0.51±0.04^{ab}	0.46±0.03^{ab}	0.6±0.04^{a}	13.71	0.002*
C:N	6.17±0.62^{a}	6.56±1.02^{a}	7.42±1.17^{a}	5.17±0.62^{a}	3.27	0.08
Av. P (mg/kg)	33.15±2.31^{c}	41.96±3.84^{a}	41.03±1.00^{ab}	37.93±1.15^{ab}	8.41	0.007*
Av. K (mg/kg)	185.75±18.1^{b}	263.16±15.3^{a}	208.53±9.7^{ab}	202.88±12.7^{ab}	21.78	0.000038*
			B- *Z. mays*			
	Initial	*P. excavatus*	*E. fetida*	control	*F*	*p*-value
pH	5.6±0.04^{a}	5.79±0.65^{a}	6.26±0.26^{a}	5.95±0.08^{a}	1.84	0.21
OC (%)	2.63±0.25^{b}	3.34±0.22^{ab}	3.4±0.38^{a}	3.11±0.2^{ab}	4.81	0.03*
TN (%)	0.42±0.02^{a}	0.32±0.01^{b}	0.34±0.02^{b}	0.3±0.02^{b}	23.9	0.000239*
C:N	6.17±0.62^{b}	10.21±0.21^{a}	9.94±1.41^{a}	10.35±0.04^{a}	19.6	0.000481*
Av. P (mg/kg)	33.15±2.31^{c}	43.38±2.85^{a}	42.54±0.61^{ab}	40.19±1.53^{bc}	16.4	0.001*
Av. K (mg/kg)	185.75±18.1^{b}	218.63±7.3^{a}	215.68±4.5^{ab}	206.46±9.95^{ab}	7.03	0.006*

Data represent mean ± SD. * indicate a significant difference at $p<0.05$. Mean with different superscripts within the same row differ significantly ($p<0.05$) by the Tukey test at a 95% confidence level**.**

4.3.3.1 Soil pH

Changes observed in various physico-chemical parameters such as pH, OC, TN, Av. P, and Av. K reflects the effects of earthworms on soil nutrient mineralization. Initially, soil pH was slightly acidic (5.6±0.04), but at the end of the experiment, it increased marginally in both *C. chinense* and *Z. mays*-grown soil in the presence of *E. fetida*, *P. excavatus*, and control (**Table 4.3**). In *C. chinense*-grown soil, in the presence of *P. excavatus*, *E. fetida*, and control, the final pH was 6.14±0.34, 6.09±0.15, and 6.12±0.28 with an increased percentage of 9.6%, 8.5%, and 9.18% respectively (**Fig. 4.7-A**). In *Z. mays* soil, the final soil pH was 5.79±0.65, 6.26±0.26, and 5.95±0.08 in the *P. excavatus and E. fetida* soil and control with an increasing percentage of 3.30%, 11.73%, and 6.12% respectively (**Fig. 4.7-B**). The increase in soil pH was the least affected by the earthworm's activities and did not vary significantly ($F_{(2,\ 6)}$= 0.03, p>0.05) among different treatments.

4.3.3.2 Organic carbon (%)

Initially, the OC of the soil samples used for the experiment was 2.63±0.25 (**Table 4.3**). At the end of the experiment, OC increased to 3.04±0.06 to 3.4±0.38 depending on the plant grown and the presence and absence of earthworms. In *C. chinense*-grown soil, the maximum amount of OC was present in the presence of *E. fetida* (3.29±0.32), followed by *P. excavatus* (3.24±0.24) and control (3.04±0.06) with 25.76%, 23.47%, and 16.24% increase over the initial concentration (**Fig. 4.7-A**). Similarly, in *Z. mays*-grown soil, the highest concentration of OC was recorded in the presence of *E. fetida* (3.4±0.38), *P. excavatus* (3.34±0.22), and control (3.1±0.2) with 30.48% 27.36% and 18.4% increase (**Fig. 4.7-B**).

4.3.3.3 Total Nitrogen (TN) (%)

Contrary to other parameters, total nitrogen showed an uneven increase and decrease. Initially, the average amount of TN was 0.42±0.02; however, at the end of the experiment, in *C. chinense*-grown soil, TN was increased to 0.51±0.04 and 0.46±0.03 in the presence of *P. excavatus* and *E. fetida*, respectively. While in control, the final concentration of TN was increased to 0.6±0.04. Variations of TN among the treatment were significantly ($F_{(2,6)}$=9.9, p<0.05) different. Depending on the presence and absence of earthworms, the increased percentage of TN over the initial value were significantly ($F_{(2,6)}$=25.61, p=0.001) different with the highest being observed in control (41.86%), *P. excavatus* (20.81%), and *E. fetida* (8.19%) (**Fig. 4.7-A**). Post hoc test shows TN increased in the control pot was significantly (p<0.05) higher compared to *P. excavatus* and *E. fetida*. While in *Z. mays*-grown soil, TN decreased (**Table 4.3**) in all the treatments, and the final concentration was 0.32±0.01, 0.34±0.02, and 0.3±0.02 in *P. excavatus*, *E. fetida*, and control, respectively. The maximum reduction was recorded in control (29.72%), followed by *P. excavatus* (23.42%), and *E. fetida* (19.48%) (**Fig. 4.7-B**).

4.3.3.4 C:N

C:N ratio is another essential parameter to indicate the nutrient stability of the soil. C:N 15-20 is considered an acceptable range for agronomy (**FAO, 2020**). In the present study, substantial variations of C:N were observed depending on the treatment but well within the suggested required range. Having the initial ratio of 6.17±0.62 in the *C. chinense* grown soil, C:N ratio in the presence of *E. fetida* and *P. excavatus* were increased to 7.42±1.17 and 6.56±1.02 with 21.92% and 7.48% increase. While in control, the final C:N ratio was decreased by 15.39% (5.17±0.62). However, in *Z. mays* grown-

soil, C:N ratio was 10.35±0.34, 10.21±0.21, and 9.94±0.41 in control, *P. excavatus*, and *E. fetida*, respectively exhibiting a maximum increase of C:N ratio in control (68.68%), followed by *P. excavatus* (66.58%), and *E. fetida* (63.46%). Analysis of variance study shows that in *Z. mays* grow soil, the increased percentage of C:N varies significantly ($F_{(2, 6)}$= 47.17, p<0.05) among treatments.

4.3.3.5 Av. P (mg/kg)

In *C. chinense*-grown soil, although no significant ($F_{(2,6)}$=2.33, p>0.05) differences were observed among the treatment, Av. P was increased to 41.96±3.84, 41.03±1.00, and 37.93±1.15 in the presence of *P. excavatus*, *E. fetida*, and control, respectively. The increased percentage of Av. P in *P. excavatus, E. fetida*, and control was 31.03%, 28.67%, and 21.59% (**Fig. 4.7-A**). Similarly, in *Z. mays*-grown soil, a higher percentage of increase was recorded in the presence *of P. excavatus* (31%), *E. fetida* (28%), and control (21%), respectively, and the final concentration of Av. P was 43.38±2.85, 42.54±0.61, 40.19±3.10.

4.3.3.6 Av. K (mg/kg)

The initial amount of Av. K was 185.75±18.1 (**Table 4.3**). In *C. chinense*-grown soil, Av. K concentration was increased at the final and showed significant differences ($F_{(2, 9)}$=26.86, p<0.05) among the treatment. The highest concentration was present in *P. excavatus* (263.16±15.39), *E. fetida* (208.53±9.75), and control (202.88±12.76), with increasing trend of 41.67%, 12.26%, and 9.22%, respectively over the initial value. Increased percentage of Av. K in treatment also shows significant differences ($F_{(2,6)}$=15.23, p<0.05). The post hoc test shows an Av. K increased in *P. excavatus* inoculated soil was significantly (p<0.05) higher compared to *E. fetida* and control. In *Z.*

mays-grown soil also, although the final amount of Av. K varies insignificantly among the treatment ($F_{(2,9)}$=2.78, p>0.05), substantially higher amount of Av. K was recorded in the presence of earthworms *P. excavatus* (218.63±7.34), *E. fetida* (215.68±4.53), and control- 206.46±9.95). In *P. excavatus* inoculated soil, Av. K was increased by 18.44% over the initial value, while in *E. fetida* and control, 14.28%, and 7.75% increase over the initial concentration was observed.

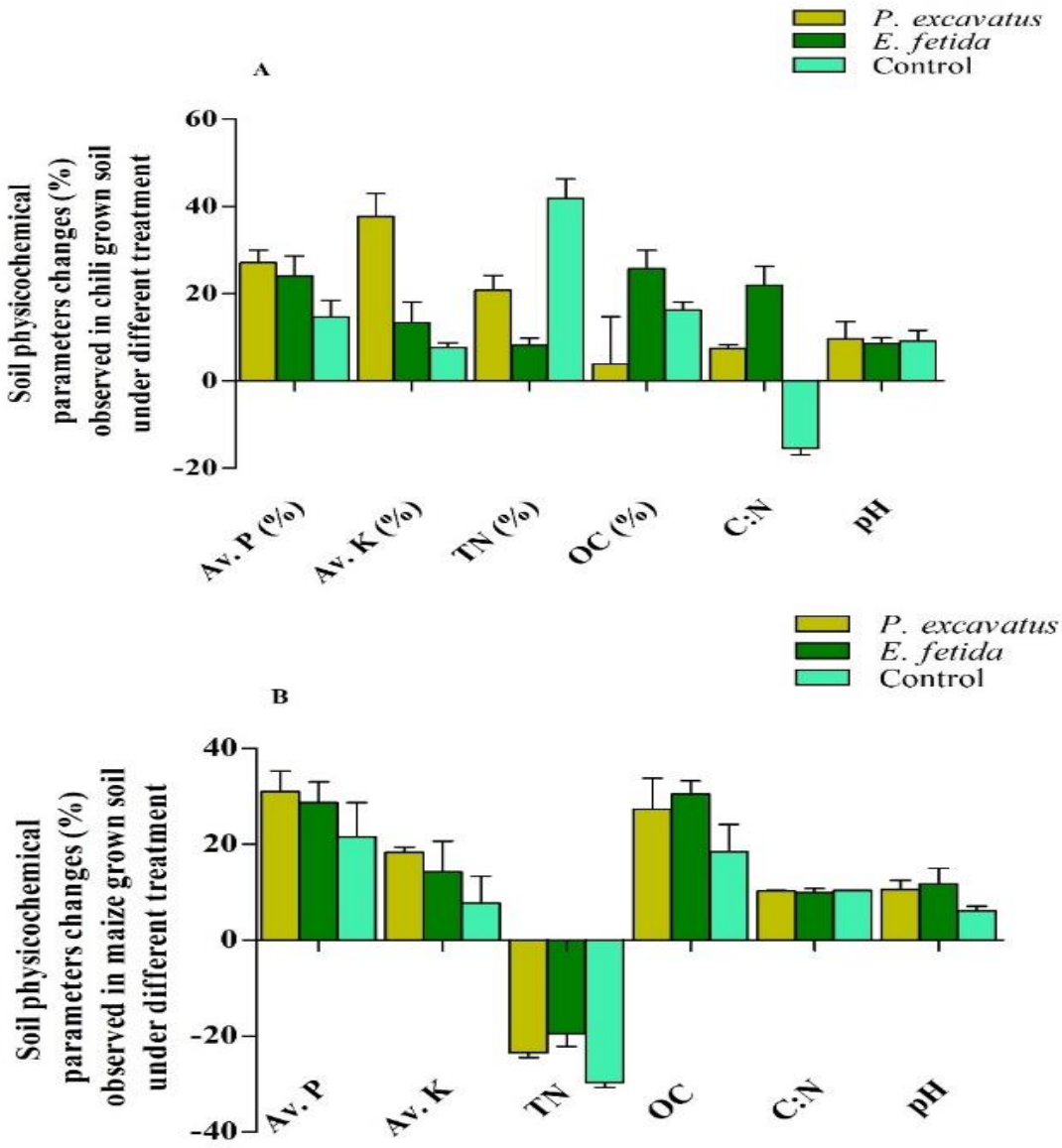

Fig. 4.7 Increased percentage (%) of soil physico-chemical parameters over initial value in *C. chinens* (A), and *Z. mays* (B) grown soil in the presence and absence of earthworm.

4.3.4 Principal component analysis (PCA)

Six soil physico-chemical parameters, namely TN, OC, C: N ratio, Av. P, Av. K, and pH, and seven plant morphological characters such as number of leaves, stem length, stem biomass, leaf biomass, number of nodes, root length, and root weight, were subjected to PCA to comprehend the relationship among the plant's morphological characteristics and soil parameters. PCA resulted in two principal components, PC 1 and PC 2- the results of the PCA loading score plots are shown in **Fig. 4.8 (A-B)**. For *C. chinense*, PCA 1, with eigenvalues 5.81, accounted for 49.01 % of the variance, while PCA 2 accounted for 33.31% of the variance. For PC 1 positive loading score was observed in TN, Av. P, Av. K, pH, number of leaves, leaves biomass, and stem length. While in PC 2 positive loading score was observed for root length, stem length, leaves biomass, number of nodes, and root length. As indicated by the overlapping of an eclipse in **Fig. 4.8 (A-B)**, average loading scores for earthworm treatment and control for both plants do not differ significantly (p>0.05). For *Z. mays*, PCA 1 with an eigenvalue of 4.75 explains 36.58% of the total variance and positive loading scores for soil parameters such as TN, Av. P, Av. K, pH, and plant characteristics like leaves, stem length, biomass, and root weight. While negative loading scores were observed for OC and C:N. PCA 2 showed a positive loading score for TN, OC, C:N, Av. P, Av. K, root length, and negative loading score for the number of leaves, stem length, stem biomass, number of nodes, and root biomass. Similar to the *C. chinense*, for *Z. mays* also, average loading scores of plants and soil parameters from the earthworm (*P. excavatus, E fetida*) inoculated soil and control do not differ significantly (p>0.05) from one another (**Fig. 4.8-B**).

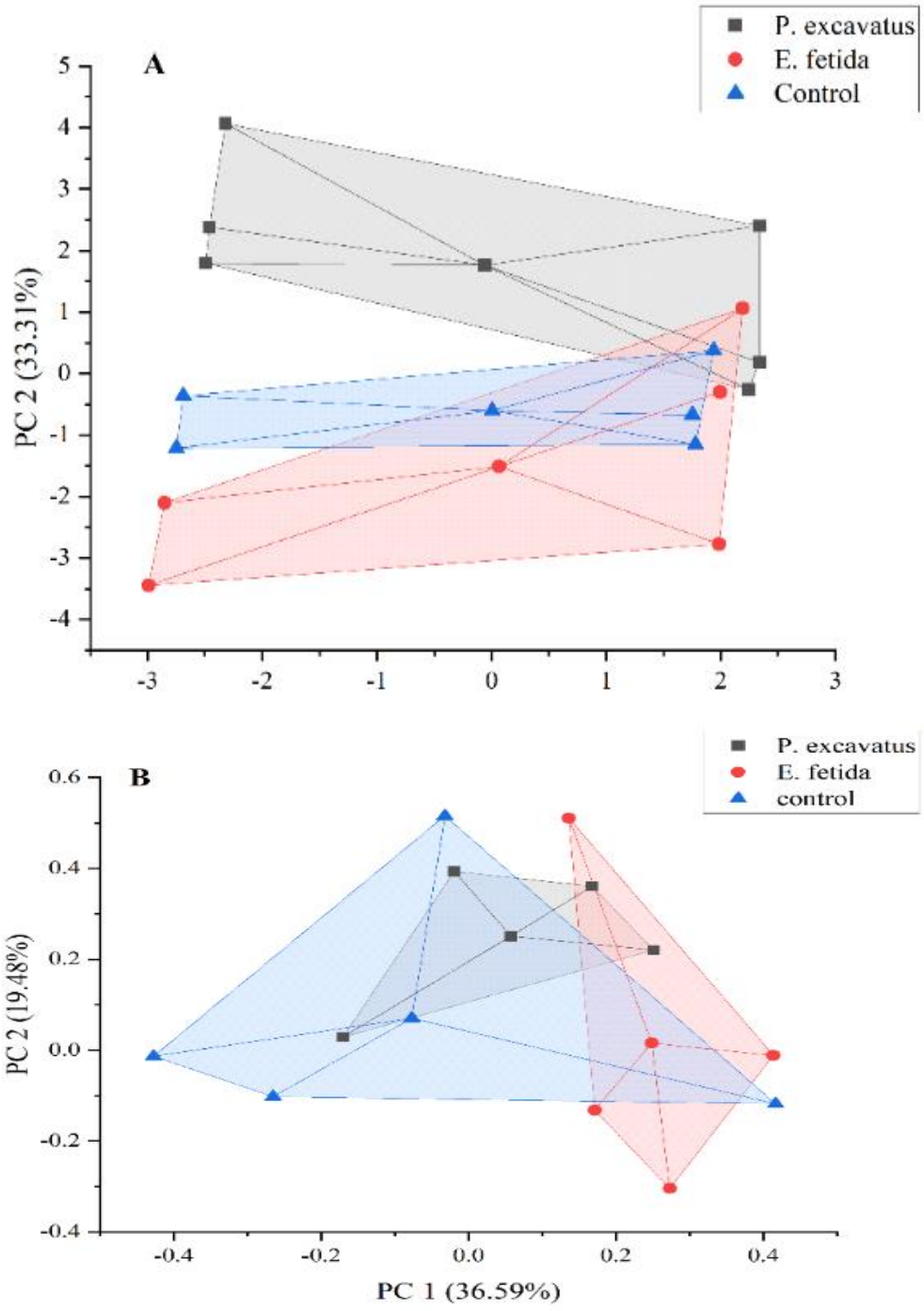

Fig. 4.8 Loading scores of principal components analysis (PC 1 and PC 2) for soil physico-chemical parameters and plant morphological characteristics obtained from control, *P. excavatus*, and *E. fetida* for (A) *C. chinense* experimented pot and (B) *Z. mays* experimented pot.

4.4. Discussion

Sustainable agriculture encompasses food production from plants or animals using different techniques without adverse impacts on humans, environment, and animals. Extensive use of fertilizers and pesticides boosts food production but also deteriorates the biodiversity (above and below the ground) associated with cropland. As an essential component of the soil, earthworms maintain soil fertility and play a key role in sustainability. The presence of earthworms enhanced the soil nutrient, plant biomass (leaves, stem, root), and fruit yield (ripened chili, kernel count, and kernel weight), which is consistent with the notion that earthworms increase plant growth (Scheu, 2003). Similarly, in the present findings, plants grown in earthworm treatment *(P. excavatus* and *E. fetida*) resulted in better morphological characteristics such as shoot biomass, stem length, number of leaves, coloration, biomass, and fruit yield.

In *P. excavatus* treated soil, average growth rate of *C. chinense* was 11.43% higher than control (**Fig. 4.1-A**). However, in *E. fetida* inoculated soil, *C. chinense* resulted in a lower growth rate (-9.65%), stem length (-2.77%), stem biomass (-16.43%), and root length (-2.42%) (**Fig. 4.2**). In *P. excavatus* soil, 100% of plants survived, and no bacterial/fungal infections were observed. Therefore it is less likely that earthworm-associated pathogens cause disease in plants. Fungal infections could be due to excess waterlogging in the root systems because chili plants are sensitive to the waterlogging in the soil where they are grown. Also, unsterilized seeds may cause fungal infection in the *E. fetida* inoculated soil. In *C. chinense*, fungal infection negatively impacted total fruit harvest, plant growth rate, leaf biomass, and root biomass. The average growth rate of *Z. mays* was higher in *E. fetida* (13.6±8.5cm/day) *and P. excavatus* (13.0±5.3cm/day) than in the control (12.7±2.5cm/day). Apart from the microbial effects, concentrations of salts

in the soil, mainly sodium chloride (NaCl), are responsible for the reduction in the productivity of economically important crops such as chili (*C. annuum*), tomato (*Solanum lycopersicum*), and potato (*S. tuberosum*) (Maas and Hoffman, 1977; Medina-Lara, 2018). Also, Aktaş *et al.* (2006) and Niu *et al.* (2010) considered chili plants very susceptible to abiotic factors.

Xiao *et al.* (2018) reported that the presence of earthworms increased plant growth by 20% and further emphasized that earthworms' effect on plant growth is more effective when mixed earthworm species are inoculated simultaneously. In the present study, distinct differences in leaf coloration were also observed, with leaves acquiring dark green color in earthworm-worked soil, which could be due to more chlorophyll and carotenoid content (Usmani *et al.*, 2018). Brown *et al.* (2004) suggested that there are five possible ways through which earthworms positively affect plant productivity. (i) biocontrol of pests and diseases (ii) stimulation of plant microbial associations (iii) production of plant growth regulating substances (iv) changes in soil physico-chemical structures and (v) enhanced nutrient availability for absorption through roots. In *C. chinense*, the effects of *P. excavatus* were higher, as evident from the increasing number of leaves (250.4%), leaf biomass (92%), stem length (11.6%), stem biomass (41.77%), root length (35%) and root biomass (43.15%) over the control. Van Groenigen (2014) reported that the presence of earthworms increased the aboveground plant biomass productivity by 23% and suggested that earthworms enhanced plants' growth mainly through their ability to release nitrogen trapped in organic matter. Similarly, Trap *et al.* (2021) also reported that the presence of endogeic earthworms significantly increased the shoot biomass (26%) of rice (*Oryza sativa*). Increased plant biomass in the presence of earthworms is attributed to releasing inaccessible forms of nutrients in the soil, making it available for plant absorption. Along

with the other plants' morphological characteristics, the present study has shown the positive effect of earthworm (*P. excavatus*), recording 71.3% higher chili fruit production per plant. Gong and Gao (2019) opined that shoot biomass is less sensitive to changes in soil fertility. Roubíčková (2013) rreported that once earthworms processed soils, their effects on plant growth get reduced. Similarly, earthworms' role in plant growth enhancement is more impactful in developing soil than in developed soil and further emphasized that during early to a late succession of plants community, earthworm activities play a significant role (Mudrák and Frouz, 2018).

In the present study, both the earthworm species have a fair share of effects on *Z. mays*, with *P. excavatus* with more perceptible effects in leaf biomass (11.52%) and root length (28.9%). At the same time, *E. fetida* effects were more in morphological characters, such as the number of leaves (9.93%), stem length (24.63%), stem biomass (14.39%), and root biomass (20.93%). The effect on kernel count and weight was more with *P. excavatus* (41.91% and 95.07%) than *E. fetida* (11.27% and 24.35%). Bacteria such as *Bacillus safeness, B. flexus*, and *Staphylococcus haemolyticus* associated with earthworm gut show plant growth-promoting potentials with the production of indole acidic acid (IAA), gibberellic acid (GA), ammonia, aminocyclopropane-1-carboxylic acid (ACC) deaminase activity, and phosphate solubilizing activities (Banerjee *et al.*, 2019). The higher percentage of plant growth characteristics in the presence of earthworms could be attributed to associated bacteria that release the insoluble form of soil nutrients into a soluble form, making it available for plants to absorb (Biswas et al., 2018; Houida *et al.*, 2022). The better plant morphological characteristics, growth, and yield of *C. chinense* and *Z. mays* may be owed to the improved nutrient content (N, Av. P, Av. K) due to earthworm activities irrespective of species. The PCA analysis shows

soil parameters such as TN, N, and Av. P, Av. K and pH were positive, indicating that these soil nutrients are critical for developing the number of leaves, biomass, stem length, nodes, and root length of both plants. Earthworms improve plant growth by enhancing organic matter mineralization and nutrient stabilization, increasing the soil porosity and nutrient retention capacity. However, the mechanism connecting earthworms-soil promoting plants' growth response is more complex due to the involvement of multiple factors interaction (Braga *et al.,* 2016).

In *C. chinensis* grown soil, Av. P was increased by 27.12% (*P. excavatus* inoculated soil) and 24.11% (*E. fetida* inoculated soil), while in *Z. mays* grown soil, 31.03% (*P. excavatus*) and 28.67% (*E. fetida*) increase were observed. In *C. chinense* grown soil, Av. K was observed to increase by 42.39% (*P. excavatus*) and 12.70% (*E. fetida*), while in *Z. mays* grown soil, an 18.38% and 14.27% increase was observed. The increased concentration of phosphorus in the earthworm worked soil may be attributed to earthworm activity conducive to phosphate-dissolving bacteria in the soil (Ramnarain *et al.,* 2019). The activity of earthworm gut enzymes, phosphatase, formation of organic acids, and discharge of total phosphorus from a complex form of humic acid mediated by microbial activity might contribute to the increased concentration of Av. P in the soil (Sharma and Garg, 2018_b; Gusain and Suthar, 2020). Many earthworm gut-associated bacteria are reported to have phosphate and potassium-solubilizing bacteria. Yakkou *et al.* (2022) observed that out of 16 bacteria isolated from earthworm gut, six bacteria, namely *Pseudomonas aeruginosa, Pantoea vagans, Buttiauxella gaviniae, Raoultella planticola, Aeromonas sp. Aeromonas drosophila* have the potential to solubilize insoluble forms of Potassium. Therefore, apart from the other biochemical process, an

increased Av. P and Av. K could be attributed to earthworm-associated bacteria that solubilize the bound form of macronutrients (Bhakta *et al.,* 2022).

Irrespective of earthworm species, the increased amount of macronutrients in the soil is more distinct, indicating its role in nutrient turnover, making it more readily available for plant absorption. An increase in organic carbon and inconsistent changes in total nitrogen (Table 3) might be attributed to the decomposition of leaves from plants themselves. Plant-available water, water-holding capacity, bulk density, and soil organic matter were also reported to increase earthworm presence (Hallam *et al.,* 2020). Zhao *et al.* (2018) reported an 11% increase in nitrogen in the presence of earthworms. Enhanced plant productivity increases the organic matter input to the soil, increasing the earthworm's food supply.

Despite higher soil fertility in earthworm-treated soil, a lack of significant differences in *Z. mays* stem length and its biomass from earthworm-treated soil and control were observed (Table 2). Also, for *C. chinense*, no significant differences were observed in stem length from earthworm-treated soil and control. It is important to note that our study was short-term, conditioned for optimum earthworm activities through proper moisture maintenance, and conducted under greenhouse conditions. Earthworm affects plant biomass productivity more in soil with no earthworm legacy than in earthworm-mediated soil (Mudrák and Frouz, 2018). Therefore, it is essential to elucidate the long-term effects of earthworms on soil physico-chemical parameters, plant productivity, and associated microorganisms that bring about various changes in soil systems in many different natural environments.

Chapter 5

Vermicomposting: organic waste degradation, analysis of macronutrient stabilisation and heavy metal concentration

Contents

5.1 Introduction

Solid waste management is one of the most challenging environmental issues faced by all nations today. Several strategies are adopted to valorize solid organic waste and agricultural residues. These are composting, production of the board, binder-less board paper, or converting this organic waste to clean fuels and petrochemical substitutes via pyrolysis. Organic waste may also be recycled by enhanced hydrolyzed urine, hydrolysis to sugar, which may be fermented to give bioethanol (Fahmy & Mobarak, 2013; Fahmy *et al.*, 2017, 2020; Alemayehu *et al.*, 2022a, 2022b). Effective management of solid waste is essential to build a sustainable society and contributes to the mitigation of environmental pollution. However, it is one of the most ignored and substandard services provided by the government and local authorities (Ostad-Ali-Askari, 2022; Letcher & Vallero, 2019). Biomass from agriculture waste, household products, and livestock waste has potential benefits, especially for the rural economy. Without recycling, waste disposal to water bodies, agricultural soil, and open field dumping creates a burden of heavy metal contamination, nutrient loss, atmospheric pollution, and health hazards. Therefore, it is imperative to assess the waste management system considering the three core waste management hierarchy systems (3CWMHS), prevention and reduction, recycling and recovery of energy, and alleviating the health risk to residents (Doaemo *et al.,* 2021).

The traditional aerobic composting of organic waste leads to the loss of nitrogen through ammonia gas and nitrogen oxides, reducing the fertilizing value of manures and contributing to the Greenhouse Gases (GHGs) in the atmosphere (Javadinejad *et al.*, 2019; Zhu-Barker *et al.,* 2017). However, these limitations can be bridged by vermicomposting, a reliable, efficient, and environmentally friendly method of waste management, and this is gaining interest among many researchers across the globe. Some

studies suggest that vermicomposting also generates GHGs into the atmosphere, but recent studies have shown that a controlled process reduces emissions (Rini *et al.,* 2020). During vermicomposting, earthworms ingest, grind, and digest the waste, and its gut-associated microorganisms play a significant role in nutrient transformation (Edwards & Bohlen, 1996; Sun *et al.,* 2020). The joint action of earthworms and microorganisms convert waste to high-quality manure with a rich amount of nutrients such as nitrogen, phosphorus, potassium, and calcium available for plant absorption (Rajkhowa *et al.,* 2015). Besides, the product (manure) of vermitechnology is known to harbor a higher number of phosphate solubilizing bacteria (PSB) which further enhances phosphorus availability (Lirikum *et al.,* 2022). Vermicomposting changes the bacterial diversity prominently and reduces heavy metals from organic waste (Wang *et al.,* 2017). However, depending on the substrate used, compost and vermicompost still contain heavy metals. Hence it is essential to quantify heavy metal content to avoid soil contamination, metal toxicity to soil biota, bio-concentration in crop yield, and meet the legal regulations.

India is one of the fastest-growing developing countries in the world, but with the advancement in the growing economy, serious measures need to be addressed as far as sustainability and circular economy is concerned. Considering a large amount of agricultural (about 350 million tonnes) and other domestic waste generation in India, the conversion of waste into fertilizing manure through eco-friendly management is shallow (ICAR, 2020). The enormous volume of waste biomass is either disposed of in the agricultural fields, burnt on site, or disposed of along the roads or railway tracks which causes environmental problems and loss of valuable nutrients in the plant biomass (Thomas *et al.,* 2019). Hence, an environmentally friendly approach to solid waste containment, especially in urban areas, has become vital.

Earthworms are the most abundant animals in the terrestrial ecosystem and are considered keystone species (Li *et al.,* 2022); exploration of multiple species with better efficiency in waste management needs to be carried out for a cleaner and healthier ecosystem. Some of the standard features upon which earthworms are considered suitable for vermicomposting include- Surface or litter dwelling, ability to colonize in an organic-rich substrate, high rate of organic matter consumption, digestion and assimilation, adaptations to a wide range of environmental conditions, and high reproduction and short life cycle (Dominguez & Edwards, 2011). The biology and physiology of epigeic earthworms *Eisenia fetida* and *Perionyx excavatus* fulfil the above-listed characteristics; these earthworm species have the potential to be used for minimizing waste disposal problems (Hasan *et al.,* 2022). *E. fetida*, also known as red-wrigglers, are commercially available earthworms used for vermicomposting, while *P. excavatus* or blue Indian worm is another preferred species for vermitechnology in the Indian subcontinent.

Recently vermicomposting has been studied using many different types of wastes, including agricultural and food waste (Wang *et al.,* 2022), coir pith (Jayakumar *et al.,* 2022), and eucalyptus leaves (Bhagat *et al.,* 2022). In India, several researchers have experimented the nutrient recovery process through vermicomposting of domestic waste and weeds using *E. fetida* and *P. excavatus* (Devi & Khwairakpam, 2020; Mago *et al.,* 2021; Kaladhar & Srinivasan, 2022). In Nagaland (India), scanty records of the characterization of macronutrients obtained from vermicomposting of agricultural wastes using *E. fetida* are available (Borang *et al.,* 2016; Chatterjee et al., 2016) but efficiency of *P. excavatus* in nutrient stabilization and heavy metal remediation through vermitechnology is lacking. Therefore to abide by the initiatives and governmental regulations to implement the practice of organic farming practices, waste management,

and mitigation of pollutants in the environment, exploitation of these potential macroinvertebrates is vital.

Nagaland, situated in the easternmost corner of India, agriculture is a significant economic activity, out of which a large amount of domestic and agricultural waste is generated. But knowledge of wastes' bioconversion using earthworms into nutrient-rich manure and reduced heavy metal toxicity is minimal. Therefore, the present study, being the first of its kind, was conducted to assess manure production and changes in physico-chemical parameters like pH, organic carbon (OC), Total nitrogen (TN), Available phosphorus (Av. P), and Available potassium (Av. K) using locally available earthworm *P. excavatus* and *E. fetida* in an aerobic vermicomposting set up. Also, mitigation of heavy metal viz., Iron (Fe), copper (Cu), Manganese (Mn), and Zinc (Zn), in organic biomass such as kitchen waste, rice straw, cow dung, and a mixture of all organic waste was tested in the present study.

5.2 Materials and methods

5.2.1 Earthworm species

The experiment was conducted using two earthworm species, i.e., *P. excavatus* and *E. fetida*. The *P. excavatus* was collected from the Mingkong forest (**Fig. 5.1**) of Nagaland (26° 21′ 50.18″ N and 94° 33′ 37.20″ E) and authenticated at ZSI (Zoological Survey of India), Kolkata. The commercially available species *E. fetida* was procured from a local vermicomposting farm, Wokha town (26° 05′ 26.82″ N and 94°15′ 31.33″ E), Nagaland, India. Both species were maintained separately in the vermicomposting chamber as stock culture using cow dung as food substrate for subsequent experiments.

5.2.2 Collection of raw materials

Rice straws were collected from fve months old Jhum feld (26° 13′ 42.89″ N and 94°28′ 24.70″ E) near Nagaland University Lumami, Zunheboto, (**Fig. 5.1**), and brought to the laboratory. The required amount of domestic kitchen scraps were collected daily for ten days from the Kamnoi, Research Scholar Hostel (Nagaland University, Lumami). Before setting up the experiment, rice straws and kitchen scraps were chopped into 1–2 inches and pre-decomposed for 15 days with regular sprinklings of water to facilitate decomposition. From a local farm, urine-free cow dung of *Bos indicus* (Vechur) fed with green plants was collected. Fresh cow dung generally generates heat and toxic compounds that cause mortality to the earthworm therefore the collected dung was pre-decomposed for 15 days.

5.2.3 Experimental design

The vermicomposting was performed in a square plastic container of 20 × 20 × 16 cm. For the vermibed, a combination of rice straw, kitchen waste, and mixed substrate was prepared with cow dung at a 3:2 (w/w) ratio. Since the earthworm belongs to the epigeic species category and requires high moisture to establish its populations, the vermibed was maintained at 70–80% moisture (**Table 5.1**). Each experimental setup (*P. excavatus, E. fetida,* and control) contained 2 kg of raw materials and was established in triplicates. The materials in all the setup were allowed to decompose for three weeks to control the moisture and eliminate volatile toxic gases for better earthworm and microorganism activity (Yadav & Garg, 2016; Sharma & Garg, 2017). Due to its high organic matter and rich nutrients, cow dung was used as a standard medium for all the treatments (Reinecke & Hallatt, 1989; Reinecke *et al.,* 1992). In each setup, 20 healthy clitellated *P. excavatus* and *E. fetida* (average biomass 3.29±0.03 g and 4.08±0.41 g) were

inoculated except control. The vermibeds were kept in the dark, and the adequate moisture was maintained by sprinkling water. The vermicomposting pot were covered with jute bags to prevent moisture loss and turned once a week. After 60 days, the vermicompost was collected from all the experimental setups. The collected samples were air-dried and stored in airtight plastic bags for a comparative study of physico-chemical parameters. Final weight of the vermibed was weighed at the end of the experiment, and comparisons were made among the treatments.

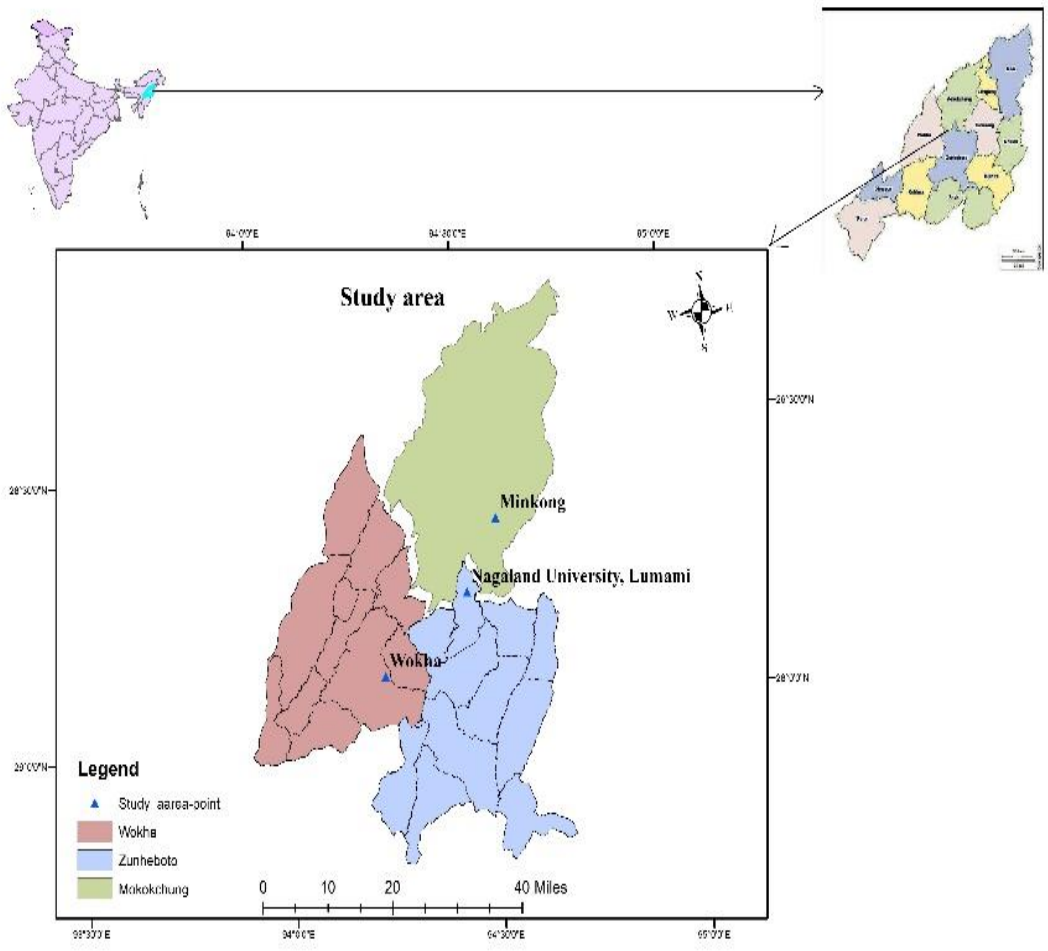

Fig. 5.1 Map shows earthworms' collection area and raw materials under Mokokchung, Wokha, and Zunheboto district, Nagaland, India.

Table 5.1 Experimental setup of vermicomposting using different substrate combinations and earthworm species.

Substrate used	Earthworm species	Feedstock composition ratio
Kitchen scrap (KS)	*P. excavatus*	KS+CD (3:2)
Cow dung (CD)	*P. excavatus*	CD ONLY
Rice straw (RS)	*P. excavatus*	RS+CD (3:2)
Mixed (MX)	*P. excavatus*	MX+CD (3:2)
Kitchen scrap (KS)	*E. fetida*	KS+CD (3:2)
Cow dung (CD)	*E. fetida*	CD ONLY
Rice straw (RS)	*E. fetida*	RS+CD (3:2)
Mixed (MX)	*E. fetida*	MX+CD (3:2)
Kitchen scrap (KS)	No earthworms	KS+CD (3:2)
Cow dung (CD)	No earthworms	CD ONLY
Rice straw (RS)	No earthworms	RS+CD (3:2)
Mixed (MX)	No earthworms	MX+CD (3:2)

KS=Kitchen scrap; CD=Cow dung only; RS=Rice straw, MX= Mixture of kitchen scrap, rice straw and cow dung.

5.2.4 Physico-chemical analysis

Physiochemical analysis was performed at two interval i.e. before the start of experiment and at the end of experiment. Initially, substrates were dried, crushed, and sieved through a 1 mm mesh size sieve and analyzed for pH, OC, TN, Av. P, Av. K, Cu, Fe, Mn, and Zn. pH was measured using a digital pH meter at 1:25 sample-water solution. The modified wet oxidation method initially described by Walkley & Black (1934) was used to determine OC. Samples were digested with potassium dichromate ($K_2Cr_2O_7$)

using sulphuric acid (H_2SO_4), and digested samples were further titrated with ferrous ammonium sulphate $(NH_4)2Fe(SO_4)_2 \cdot 6H_2O$) in the presence of diphenylamine ($C_{12}H_{11}N$) as an indicator. The endpoint, indicated by green color, was noted and calculated for organic carbon (%). TN was determined using Kel plus instrument (Pelican equipment-Classic- DX VATE). Through digestion, amino nitrogen in the sample is converted into ammonium radicals in the presence of strong acid (H_2SO_4) aided by potassium sulphate (K_2SO_4) and copper sulfate ($CuSO_4$) as a catalyst. Further, separation and isolation of nitrogen from the digestion tube were processed through distillation in the presence of sodium hydroxide (NaOH), during which ammonium radicals get converted into ammonia gas which was collected by trapping in 4% boric acid. At last, TN determination was done by titrating with 0.1N hydrochloric acid (HCl) in the presence of methyl red and bromocresol green (8:10) as an indicator. Av. P was determined spectrophotometrically (Systronic spectrophotometer-166) using a modifed form of Bray & Kurtz (1945). The bound form of phosphorus and acid-soluble phosphorus was extracted using a bray reagent containing 0.03N ammonium fluoride (NH_4F) and 0.025N HCl acid, and the amount of phosphorus was determined by the intensity of blue colour development when treated with a molybdate-ascorbic acid reagent. Av. K was determined by a modifed procedure of the one initially described by Hanway & Heidel (1952). Neutral ammonium acetate ($C_2H_4O_2 \cdot H_3N$) solution was used as extracting solution for exchangeable potassium ions, which was further determined by a fame photometer (Systronicfalme photometer-130). Heavy metals (Fe, Cu, Mn, and Zn) were estimated following the DTPA method (Lindsay & Norvell, 1978) using Atomic absorption spectrophotometer (SHIMADZU CORP, AA-6880). Soil samples were mixed with extractant containing 0.005M diethylenetriaminepentaacetic acid (DTPA), 0.1M

triethanolamine ($C_6H_{15}NO_3$), and 0.01M Calcium chloride ($CaCl_2$) at a 1:2 ratio (10g of soil in 20 ml of extractant) and shaken for 2 h at 25 °C. Samples were filtered through Whatman No.1 filter paper, and against the standard solutions for each metal, the filtrates were measured at atomic absorbtion spectrophotometer (AAS) using the respective lamp.

5.2.5. Statistical analysis

The data is presented in mean±SD. Analysis of variance (One-way ANOVA) at 95% intervals (p<0.05) was conducted to find the mean significant difference among treatment. While two-way ANOVA was conducted to find the effects of the interaction of independent variables (treatment and substrate) and also the main effects on final soil nutrient parameters. Each test was followed by a multiple comparisons test (Tukey test) to find the mean significant difference between the variables. The statistical analysis was conducted using IBM SPSS- 22 software.

5.3 Results

5.3.1 Substrate degradation

The final products of the vermicompost were dark, homogeneous, and finely textured which could retain more moisture. In *P. excavatus* treated pot, final weight of the substrate was lowest in MX (781.8±13.42 g) followed by CD (825.08±12.05 g), RS (862.63±28.39 g) and KS (914.46±17.88g). Similarly, in *E. fetida*, the lowest substrate weight was observed for CD (616.85±123.64 g), followed by KS (666.8316.62 g), MX (753.45±17.16 g), and RS (779.66±11.5 g) (**Fig. 5.2**). The total weight of vermibed (g) in the presence and absence of earthworms were significantly different ($F_{(2,\ 33)}$=45.17, p<0.05), indicating high substrate degradation in the earthworm-treated pot (**Fig. 5.3**). Higher rate of substrate decomposition was found in *E. fetida* (64.78%), followed by *P.*

excavatus (57.53%) and control (46.85%) which resulted into the lower weight of total vermibed.

In all the treatments, the final weight of the vermibed differ significantly depending on the substrate used (**Table 5.2**). Two-way ANOVA was performed to analyze the effect of earthworm species and substrate used on final weight of vermibed. The results show that the interaction effects of earthworm species and substrates were insignificant ($F_{(6, 24)}$=2.09, p>0.05). But there was significant main effects of earthworm species ($F_{(2, 24)}$=71.01, p<0.05) and substrate ($F_{(2, 24)}$=4.98, p<0.05) on manure productions (**Table 5.3**).

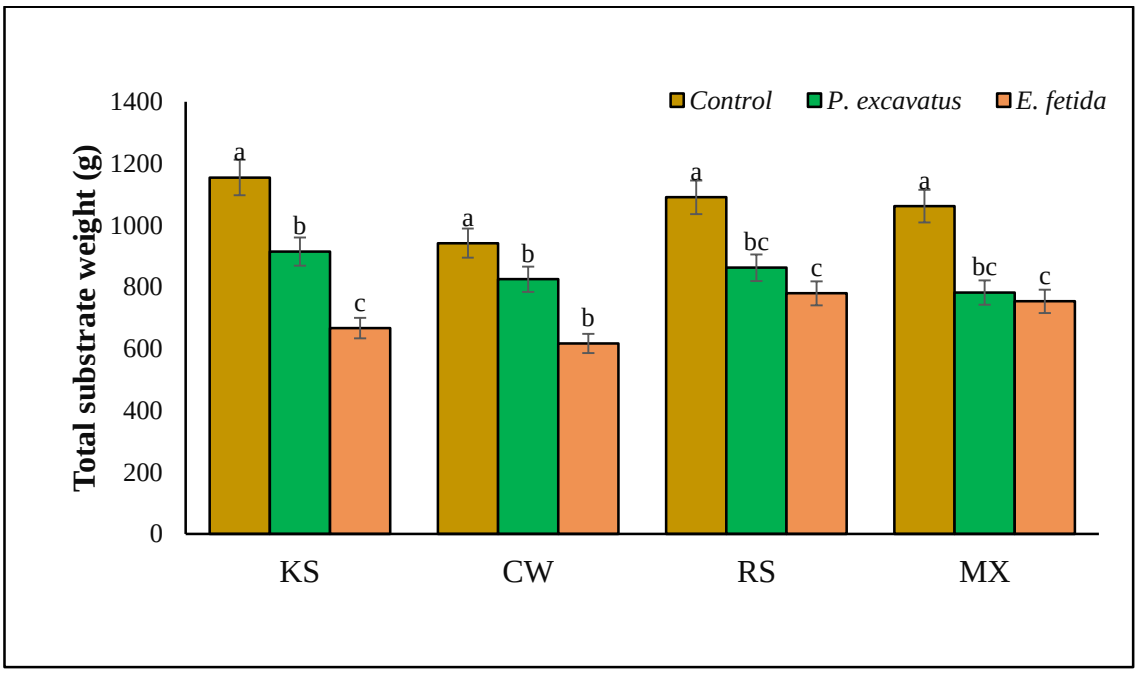

Fig. 5.2 Final weight of vermibed in control, *P. excavatus* and *E. fetida*. Mean with different superscripts among the treatment differ significantly (p<0.05) by Tukey's HSD at a 95% confidence level.

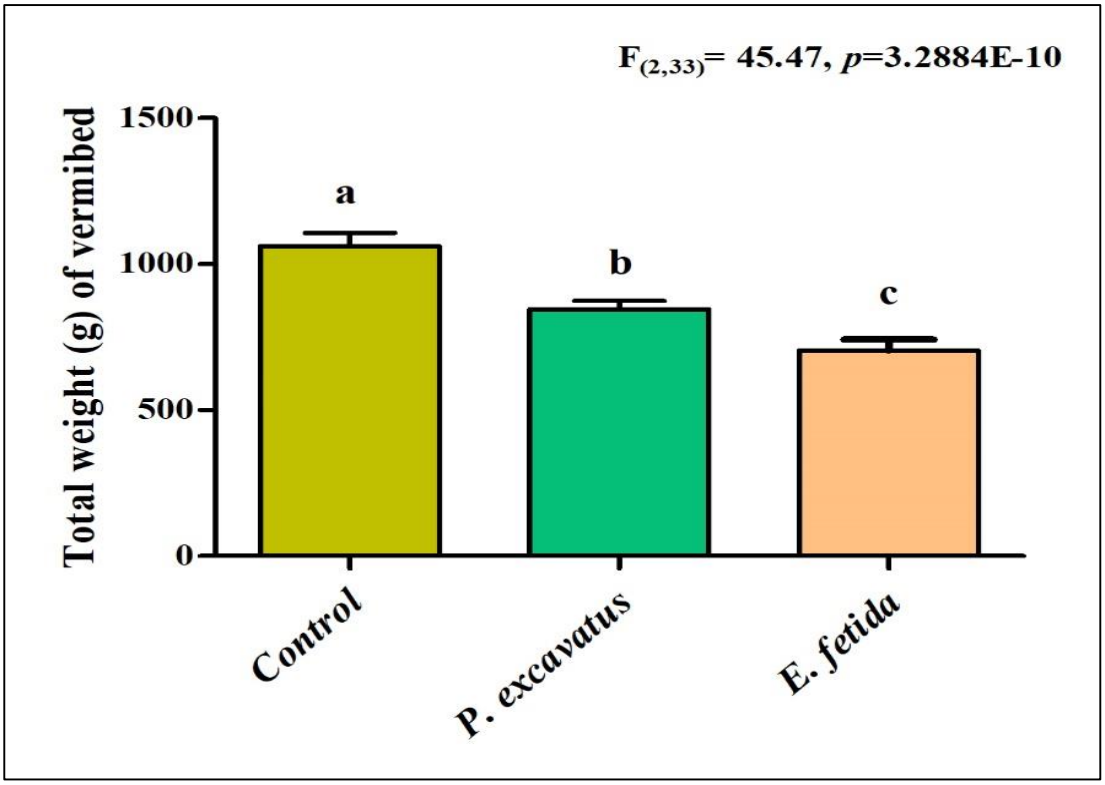

Fig. 5.3 Final weight of vermibed in control and earthworm treatment. Mean with different superscripts among the treatment differ significantly by Tukey's HSD test at a 95% confidence level (p<0.05).

Table 5.2 One-way ANOVA test results for final weight of vermibed in different treatment.

		Sum of Squares	df	Mean Square	*F*	Sig.
Final substrate weight (KS)	Between Groups	357266.682	2	178633.341	43.664	.00001
	Within Groups	24546.728	6	4091.121		
	Total	381813.410	8			
Final substrate weight (CW)	Between Groups	162870.463	2	81435.231	6.589	.031
	Within Groups	74150.896	6	12358.483		
	Total	237021.359	8			

Table 5.2 conti..

Final substrate weight (RS)	Between Groups	155646.795	2	77823.397	19.246	.002
	Within Groups	24261.525	6	4043.587		
	Total	179908.319	8			
Final substrate weight (MX)	Between Groups	174663.553	2	87331.777	57.909	.00001
	Within Groups	9048.558	6	1508.093		
	Total	183712.112	8			

Table 5.3 Two-way ANOVA test results appraising the effect of substrate used and earthworm treatment on the final weight of the vermibed. Earthworm species and substrate used are independent factor while manure productions are dependent factor.

Dependent Variable: Manure					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	932671.016[a]	11	84788.274	15.415	8.3222E-11
Intercept	27303779.249	1	27303779.249	4964.042	2.4869E-29
Treatment	781219.479	2	390609.740	71.016	8.3222E-11
Substrate	82223.264	3	27407.755	4.983	0.007919
Interaction	69228.273	6	11538.046	2.098	.0.091170
Error	132007.479	24	5500.312		
Total	28368457.745	36			
Corrected Total	1064678.495	35			

a. R Squared = .876 (Adjusted R Squared = .819)

5.3.2 Changes in macronutrients after vermicomposting

Table 5.4 Physiochemical parameters of the substrate before the start of the experiment (mean±SD).

Parameters	KS	CD	RS	MX
pH	6.79±0.2	7.6±0.24	7.9±0.18	7.4±0.4
Organic carbon (%)	23.5±1.72	23.13±1.06	23.99±1.43	29.92±1.13
Total Nitrogen (%)	1.35±0.10	1.86±0.01	1.54±0.16	2.07±0.03
C:N	17.76±0.35	16.07±0.52	43.46±2.2	21.68±0.7
Available Phosphorus (mg/kg)	65.97±3.56	43.46±2.2	32.69±0.42	39.64±1.82
Available potassium (mg/kg)	227.19±13.91	234.49±7.05	226.67±19.82	183.36±12.81

5.3.2.1 pH

Initially pH ranges from 6.79 to 7.9 (**Table 5.4**), after vermicomposting, reduction of pH was observed in all the substrate. In control, *P. excavatus* and *E. fetida*, pH was reduced to 6.51, 7.38, 6.17-7.21, and 5.67-7.29 showing a substantial reduction at the end of vermicomposting (**Fig. 5.4**). Average amount of pH in the final vermicomposted manure was reduced to 7.08±0.41, 6.86±0.48, and 6.77±0.74 in control, *P. excavatus* and *E. fetida* (**Fig. 5.5**). In control reduction percentage of pH ranges from 1.17 to10.49% with an average reduction of 5.58 %. While in *P. excavatus* and *E. fetida*, reduction percentage ranges between 5.64-9.07% and 4.24-16.43% with an average reduction percentage of 7.59% and 9.03% (**Fig. 5.6 and 5.7**).

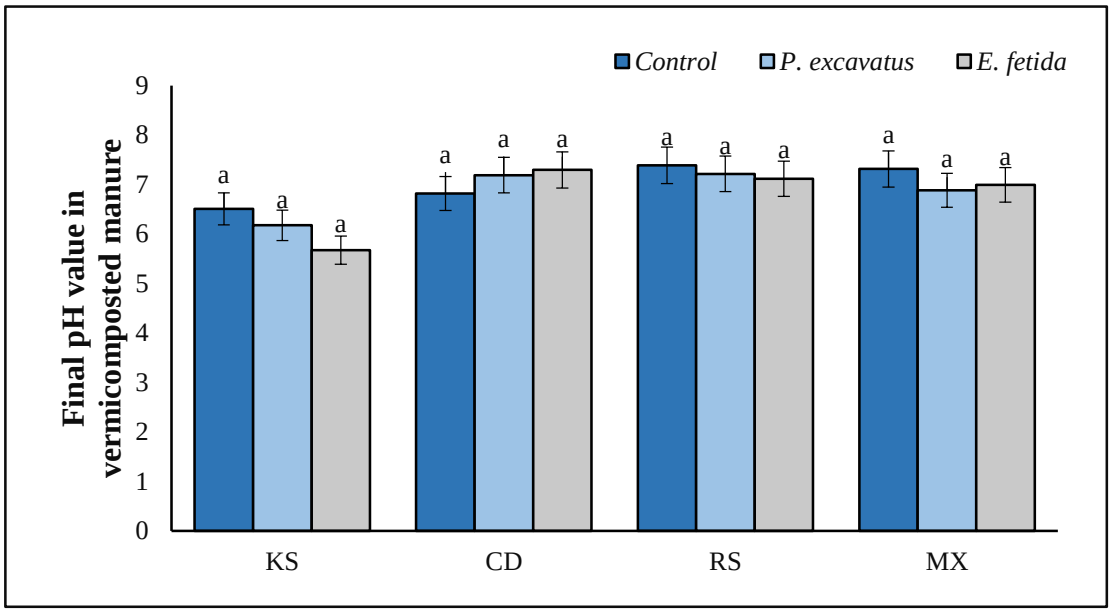

Fig. 5.4 Final amount of pH at the end of vermicomposting in different substrate used as vermibed. Mean with different superscripts among the treatment differ significantly (p<0.05) by Tukey's HSD at a 95% confidence level.

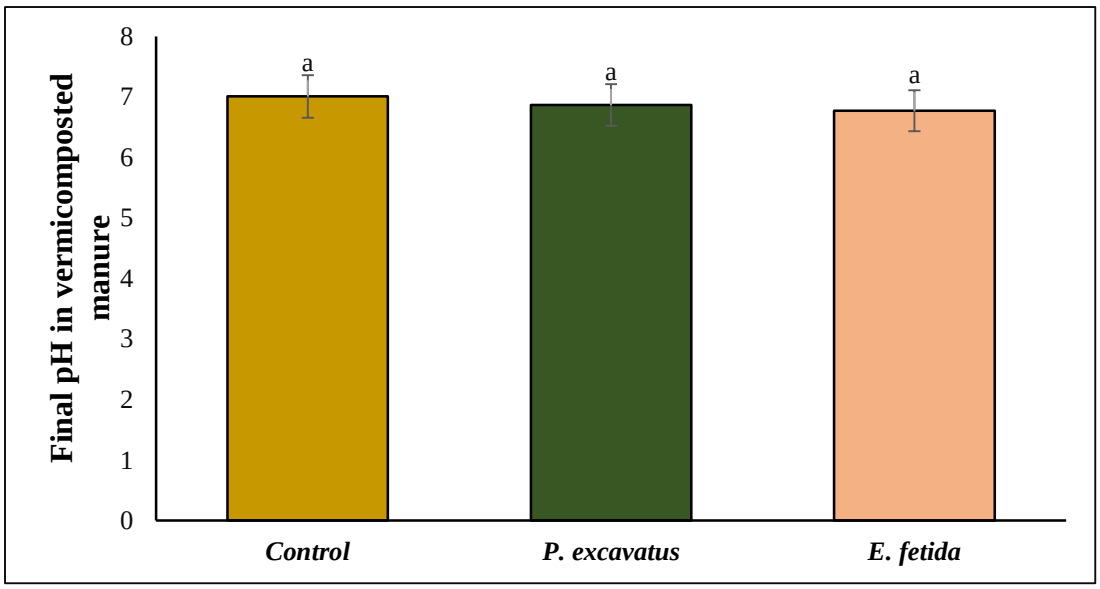

Fig. 5.5 Average amount of pH in different treatment at the end of vermicomposting. Mean with different superscripts among the treatment differ significantly (p<0.05) by Tukey's HSD at a 95% confidence level.

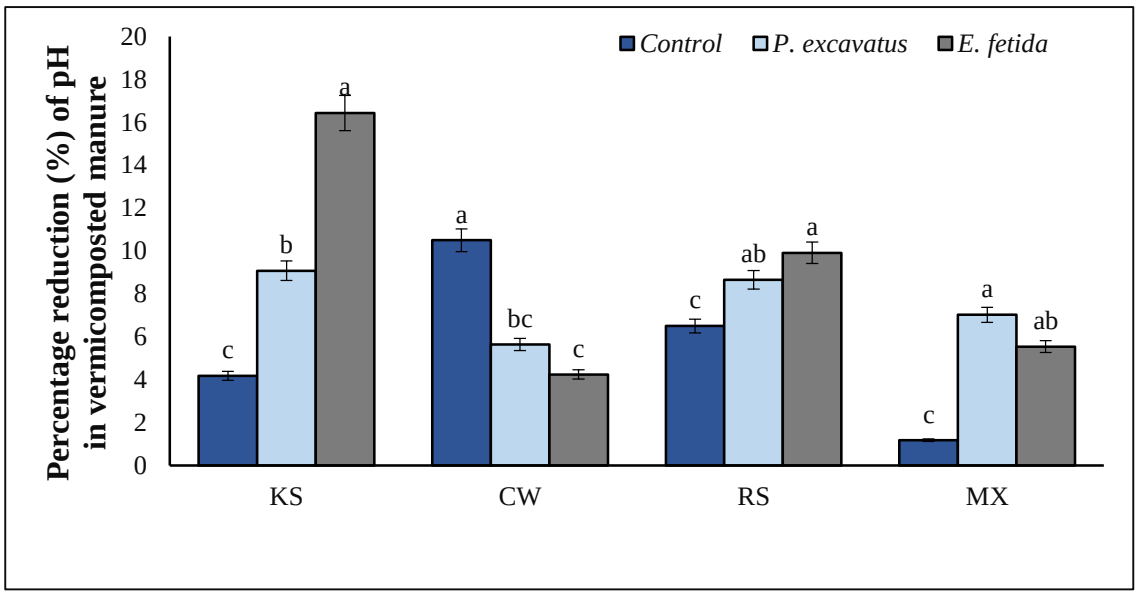

Fig. 5.6 Percentage reduction of pH in different substrate at the end of vermicomposting. Mean with different superscripts among the treatment differ significantly (p<0.05) by Tukey's HSD at a 95% confidence level.

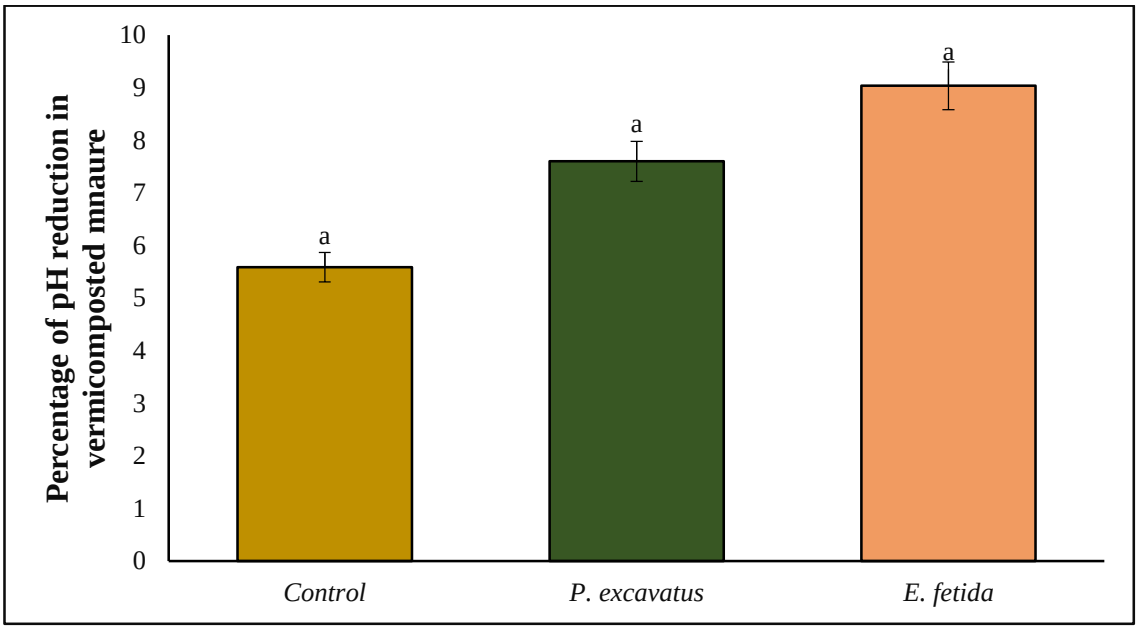

Fig. 5.7 Average percentage reduction (%) of pH in different treatments at the end of vermicomposting. Mean with different superscripts among the treatment differ significantly (p<0.05) by Tukey's HSD at a 95% confidence level.

5.3.2.2 Organic Carbon (OC %)

The amount of OC recorded initially in all the substrates (23.5±1.72, 23.13±1.06, 23.99±1.43, and 29.92±1.13 in KS, CD, RS, and MX, respectively) was reduced subsequently at the end of the experiment. In control, OC in KS, CD, RS, and MX was reduced to 11.31±2.12, 16.56± 1.79, 15.13± 1.28, and 15.85± 2.65. In *P. excavatus*, OC was reduced to 9.41±0.51, 8.46±1.23, 13.57±1.13, and 14.19±0.75. Similarly, in *E. fetida* treatment it was reduced to 11.34±2.2, 16.49±2.69, 10.08±0.51, and 14.39±1.53 (**Fig. 5.8**). Mean significant differences of OC in different substrate treated with earthworms and control are given in **Table 5.5**. The final mean value of OC was found to be higher in control (14.71±2.73), followed by *E. fetida* (13.07±3.1*)* and *P. excavatus* (11.4±2.74*),* exhibiting significant differences among the treatments ($F_{(2, 33)}$=3.99; p<0.05). Multiple comparison tests (Tukey's) show the mean value of OC in control and *P. excavatus* differ significantly (p<0.05) however, no significant difference (p>0.05) was observed between the two earthworm species used (**Fig. 5.9**).

On analysing two-way ANOVA, the interaction effect of species and substrate has a significant impact on OC dynamics ($F_{(2, 24)}$=6.71, p<0.05). Also, main effect of earthworm species ($F_{(2, 24)}$=11.29, p<0.05), substrates ($F_{(3, 24)}$=9.64, p<0.05) were significant (**Table 5.10**). In the control pot, OC reduction was maximum in KS (51.84±10.62%) and minimum in CD (28.53±4.73%). In *P. excavatus* inoculated pot, the reduction of OC was more in CD (63.22±6.91%), followed by KS (59.72±5.09%), MX (52.58±1.73%), and RS (43.18±6.9%). While in *E. fetida*, the maximum reduction was observed in RS (57.94±1.03%), followed by MX (51.71±6.94%), KS (51.34±11.34%), and CD (28.28±14.64%) (**Fig. 5.10**). The average reduction percentage of OC was maximum in *E. fetida* (54.67±9.31%) followed by *P. excavatus* (47.31±5.88%) and

control (41.12±3.91%) (**Fig. 5.11**). Multiple comparison tests show percentage reduction of OC in *P. excavatus* was significantly higher (p<0.05) compared to the control, however, no significant difference was observed between the compared to *E. fetida*.

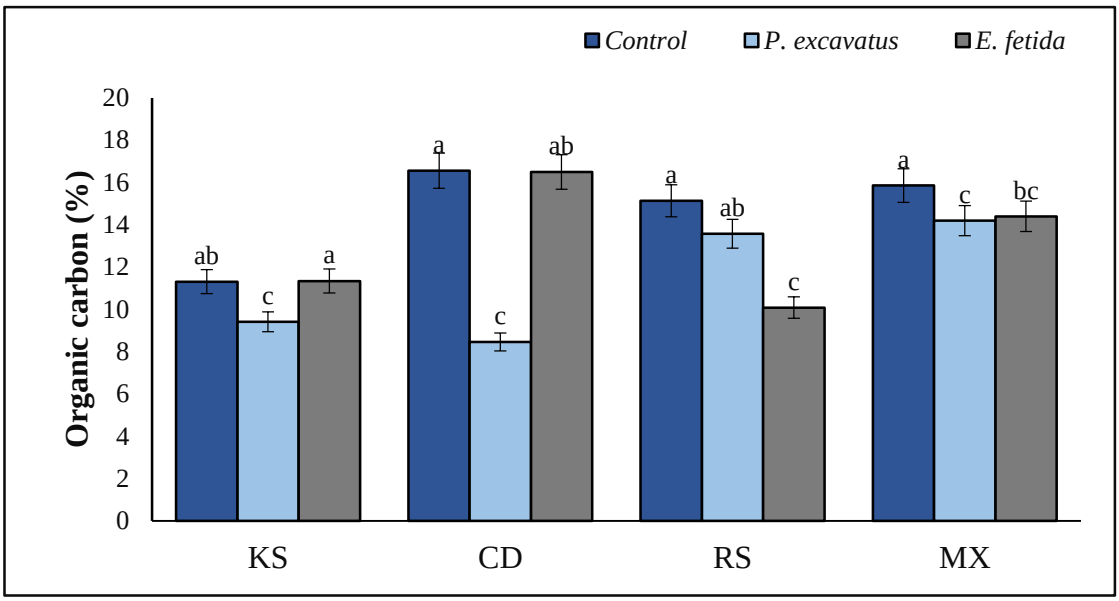

Fig. 5.8 Final amount of OC at the end of vermicomposting in different substrate used as vermibed. Mean with different superscripts among the treatment differ significantly (p<0.05) by Tukey's HSD at a 95% confidence level.

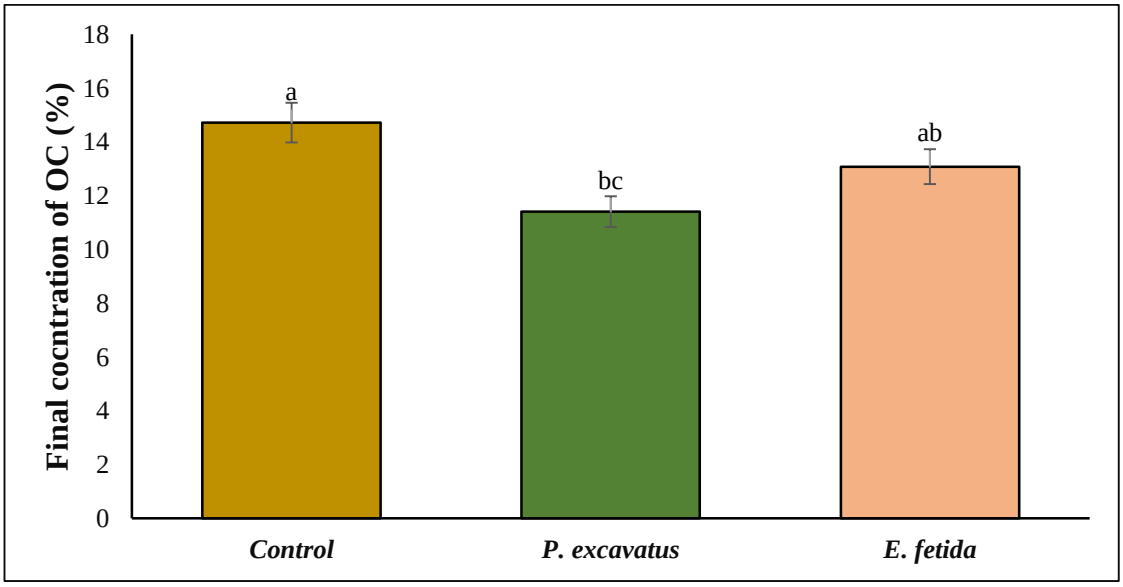

Fig. 5.9 Average amount of OC in different treatment at the end of vermicomposting. Mean with different superscripts among the treatment differ significantly (p<0.05) by Tukey's HSD at a 95% confidence level.

Table 5.5 One-way ANOVA test results for the final concentration of OC the in the different substrates used as vermibed.

Source of variations		Sum of Squares	df	Mean Square	F	Sig.
KS	Between Groups	7.336	2	3.668	1.134	.382
	Within Groups	19.415	6	3.236		
	Total	26.751	8			
CD	Between Groups	130.202	2	65.101	16.312	.004
	Within Groups	23.946	6	3.991		
	Total	154.148	8			
RS	Between Groups	40.072	2	20.036	18.783	.003
	Within Groups	6.400	6	1.067		
	Total	46.472	8			
MX	Between Groups	4.931	2	2.466	.743	.515
	Within Groups	19.904	6	3.317		
	Total	24.835	8			

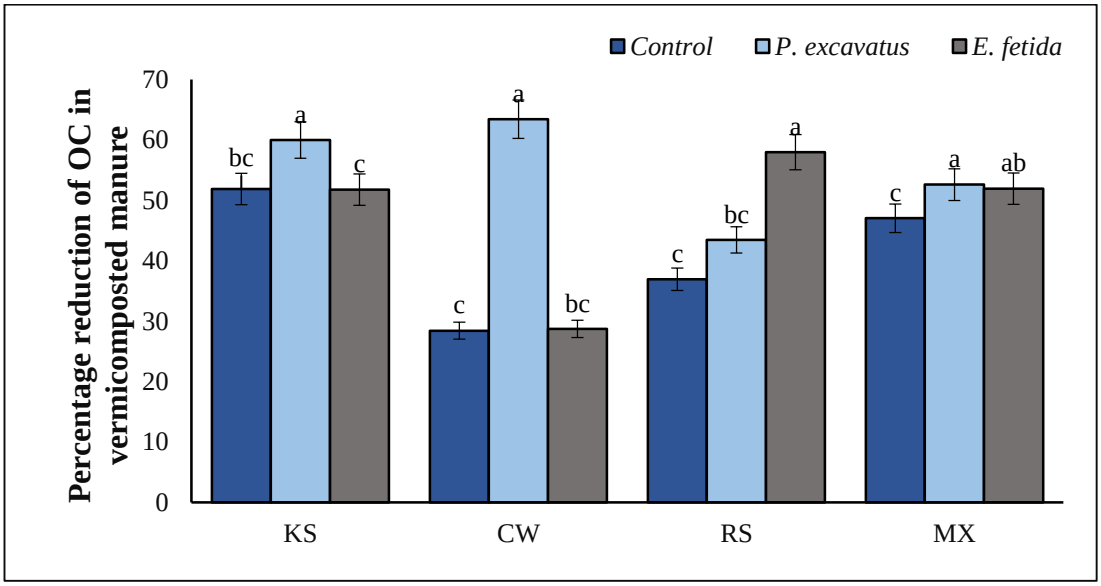

Fig. 5.10 Percentage reduction of OC in different substrate used as vermibed. Mean with different superscripts among the treatment differ significantly (p<0.05) by Tukey's HSD at a 95% confidence level.

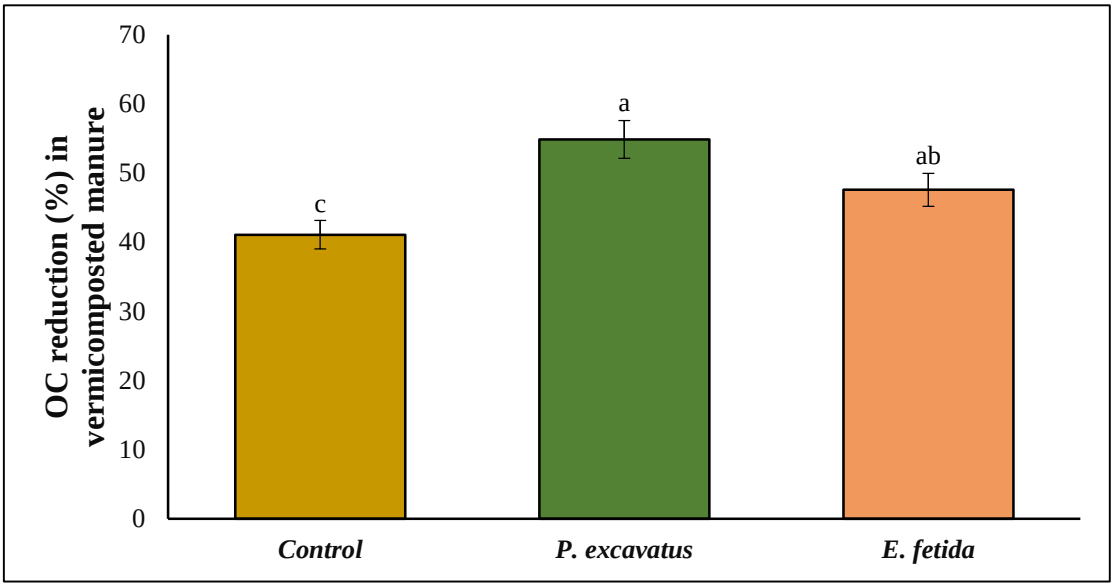

Fig. 5.11 Mean percentage reduction of OC in final vermicomposted manures produced from earthworm treatment and control. Mean with different superscripts among the treatment differ significantly (p<0.05) by Tukey's HSD at a 95% confidence level.

5.3.2.3 Total Nitrogen (TN%)

Similar to OC, the initial concentration of TN (Ranging from 1.35 to 2.06%) was considerably reduced in all substrates. In control, TN was reduced to 1.21±0.2, 1.50±0.1, 1.12±0.20, and 1.31±0.23 in KS, CD, RS, and MX. While in *P. excavatus*, the final concentration of TN was 0.74±0.04, 0.5±0.03, 1.05±0.02, and 1.09±0.03 in KS, CD, RS, and MX. Similarly, in *E. fetida* treated pot, TN concentration was 0.93±0.07, 1.06±0.01, 0.86±0.05, and 1.07±0.05 in KS, CD, RS, and MX (**Fig. 5.12**). In KS and CD, depending on the treatment TN concentration varies significantly, however, no significant differences were observed in RS and MX (**Table 5.6**). The mean value of TN in control, *P. excavatus* and *E. fetida* was 1.30±0.22, 0.84±0.25, and 0.98±0.1. Multiple comparisons test shows the average amount of TN in *P. excavatus* (0.84±0.25% and *E. fetida* (0.98±0.1%) were significantly (p<0.50) lower compare to control (1.29±0.22%) (**Fig. 5.13**).

The percentage reduction of TN among different substrates over the initial value ranges from 16.75 to 29.2%, 21.86 to 75.49%, and 35.82 to 48.38 % in control, *P. excavatus*, and *E. fetida*, respectively (**Fig. 5.14**). The highest reduction percentage of TN was observed in *P. excavatus* (47.56%), *E. fetida* (41.36%), and control (23.82%) (**Fig. 5.15**). Two way ANOVA shows that interaction of earthworm species and substrate had significant influence on the variations of TN in final vermicomposted manures (**Table 5.10**).

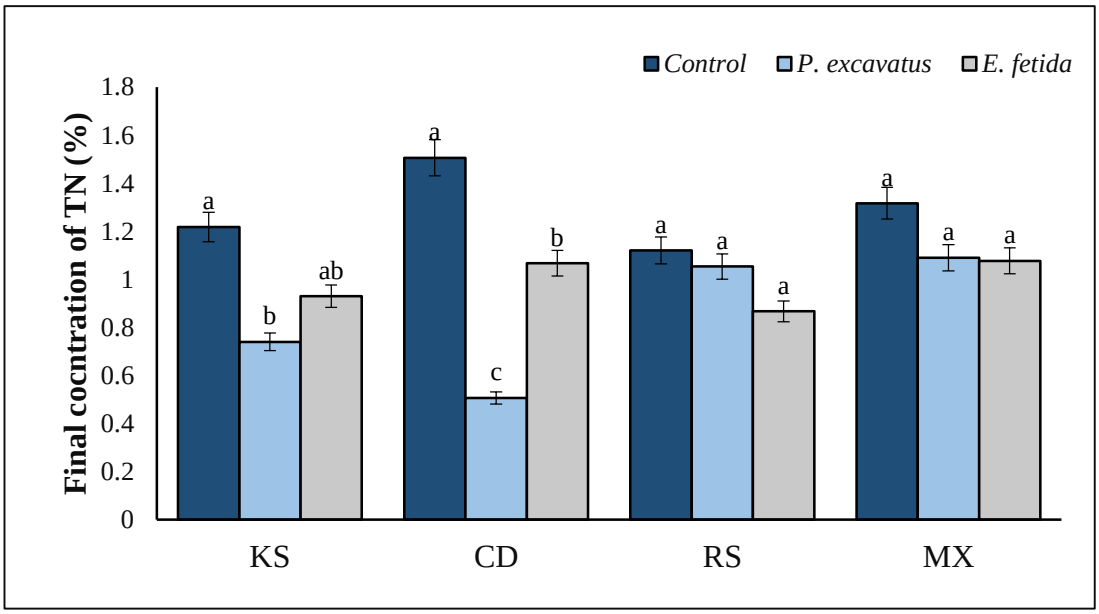

Fig. 5.12 Final amount of TN at the end of vermicomposting in different substrate used as vermibed. Mean with different superscripts among the treatment differ significantly (p<0.05) by Tukey's HSD at a 95% confidence level.

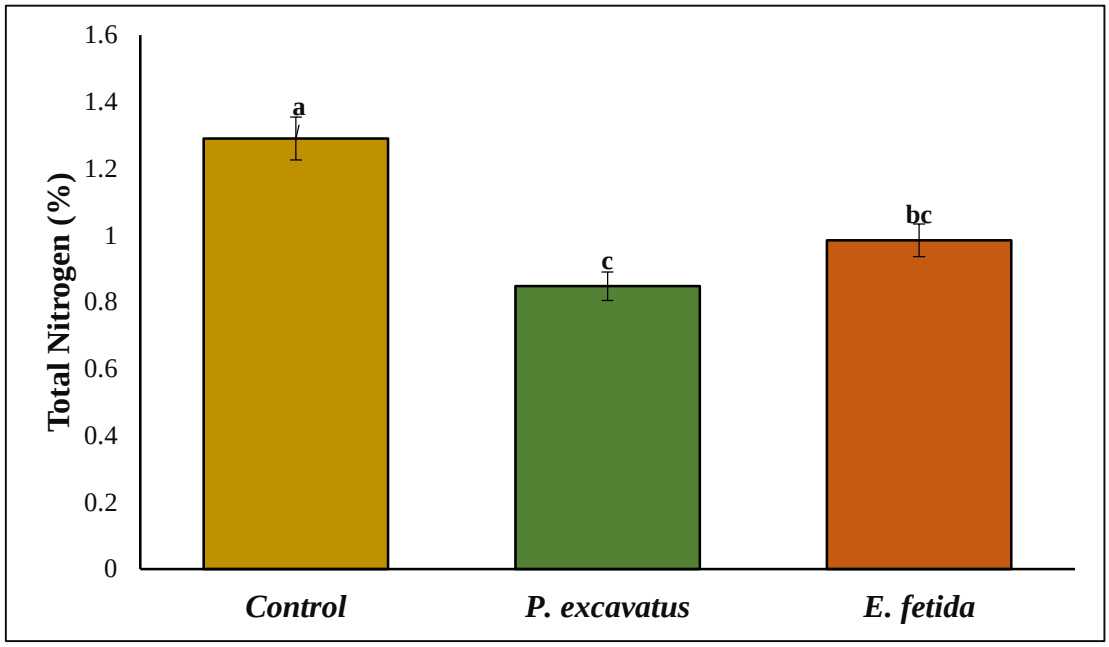

Fig. 5.13 Average amount of TN in different earthworm treatment and control at the end of vermicomposting. Mean with different superscripts among the treatment differ significantly (p<0.05) by Tukey's HSD at a 95% confidence level.

Table 5.6 One-way ANOVA test results for the final concentration of TN the in the different substrates used as vermibed.

Source of variations		Sum of Squares	df	Mean Square	*F*	Sig.
KS	Between Groups	1436.778	2	718.389	6.351	.033
	Within Groups	678.695	6	113.116		
	Total	2115.473	8			
CD	Between Groups	3526.605	2	1763.302	168.398	.000005
	Within Groups	62.826	6	10.471		
	Total	3589.431	8			
RS	Between Groups	584.638	2	292.319	2.870	.133
	Within Groups	611.016	6	101.836		
	Total	1195.654	8			
MX	Between Groups	316.965	2	158.483	2.612	.153
	Within Groups	364.036	6	60.673		
	Total	681.001	8			

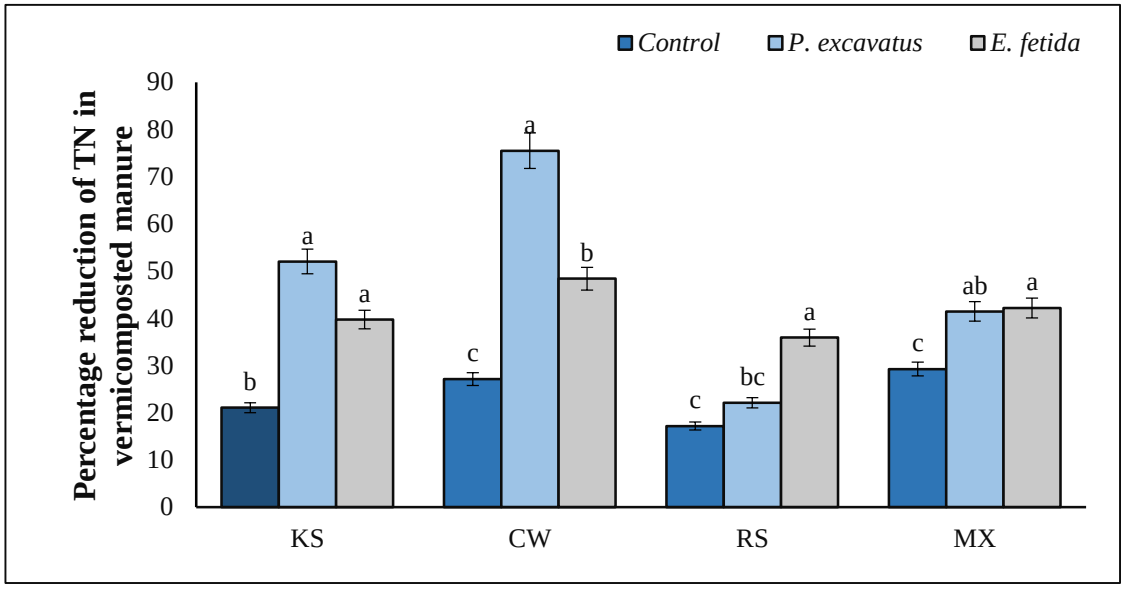

Fig. 5.14 Percentage reduction of TN in different substrate used as vermibed. Mean with different superscripts among the treatment differ significantly (p<0.05) by Tukey's HSD at a 95% confidence level.

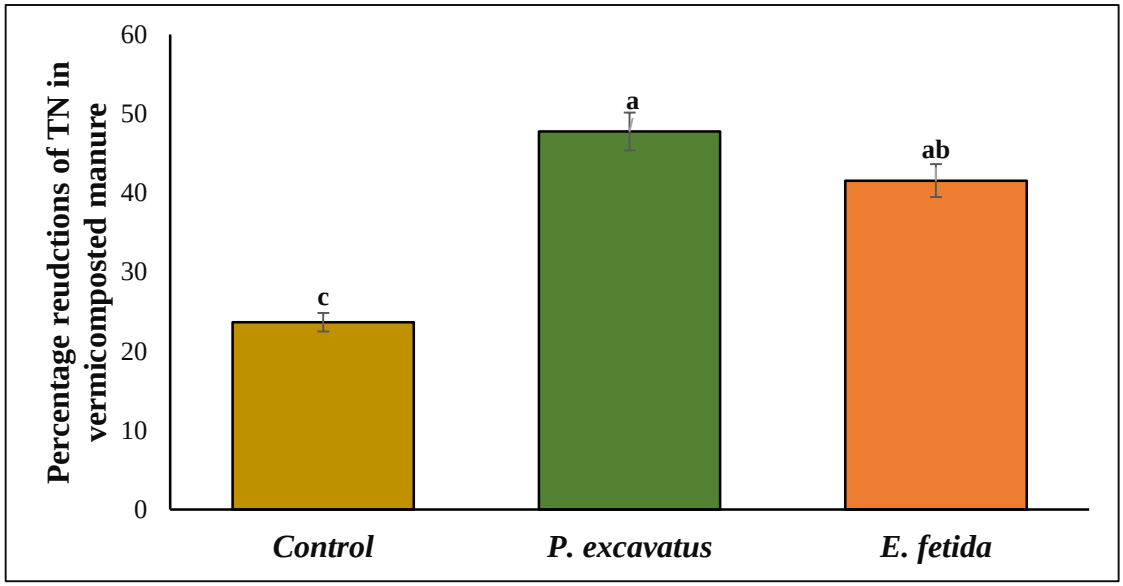

Fig. 5.15 Mean percentage reduction (%) of TN in final vermicomposted manures produced from earthworm treatment and control. Mean with different superscripts among the treatment differ significantly (p<0.05) by Tukey's HSD at a 95% confidence level.

5.3.2.4 C:N ratio

Decomposition rate and compost maturity depend on the C: N ratio, which is an essential factor for earthworm survival, reproduction, and other microbes in the vermicomposting process. C:N ratio in different food substrates are given in **Fig. 5.16.** In the final vermicomposted manures, the average amount of C:N ratio does not differ significantly ($F_{(2,\ 33)}$=2.26, p>0.05) among *P. excavatus* (13.88±2.51), *E. fetida* (13.14±2.1) and control (11.69±2.99) (**Fig. 5.17**). But compared to an initial ratio of 15.37±1.79 to 21.68±0.7 depending on the substrate, C: N reduced from 22.74% to 49.56%, 17.04% to 27.38%, and 16.96% to 34.44% in control, *P. excavatus*, and *E. fetida* (**Fig. 5.18**). The highest reduction percentage was observed in control (33.01±13.12), *E. fetida* (25.28±7.75) and *P. excavatus* (21.41±4.54) (**Fig. 5.19**).

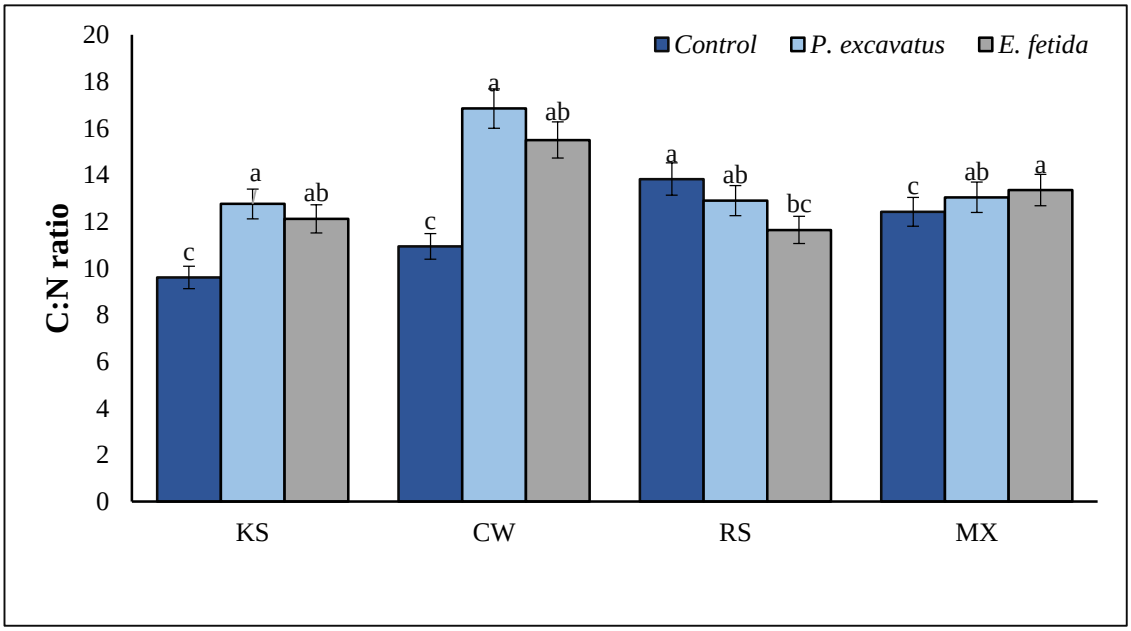

Fig. 5.16 Final amount of C:N at the end of vermicomposting in different substrate used as vermibed. Mean with different superscripts among the treatment differ significantly (p<0.05) by Tukey's HSD at a 95% confidence level.

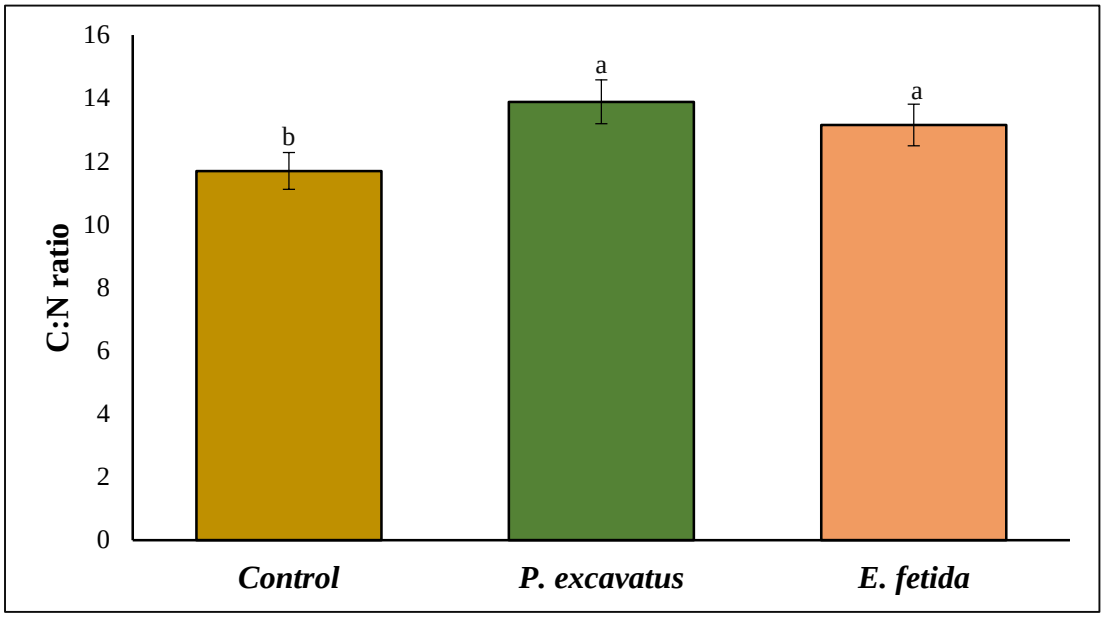

Fig. 5.17 Average amount of C:N in different earthworm treatment and control at the end of vermicomposting. Mean with different superscripts among the treatment differ significantly (p<0.05) by Tukey's HSD at a 95% confidence level.

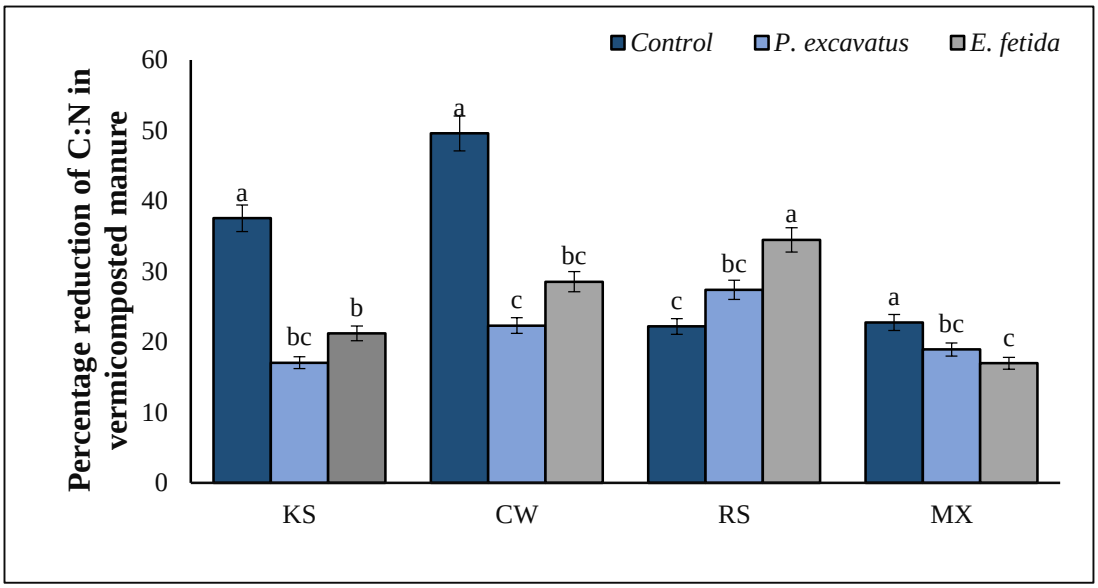

Fig. 5.18 Percentage reduction of C:N in different substrate used as vermibed. Mean with different superscripts among the treatment differ significantly (p<0.05) by Tukey's HSD at a 95% confidence level.

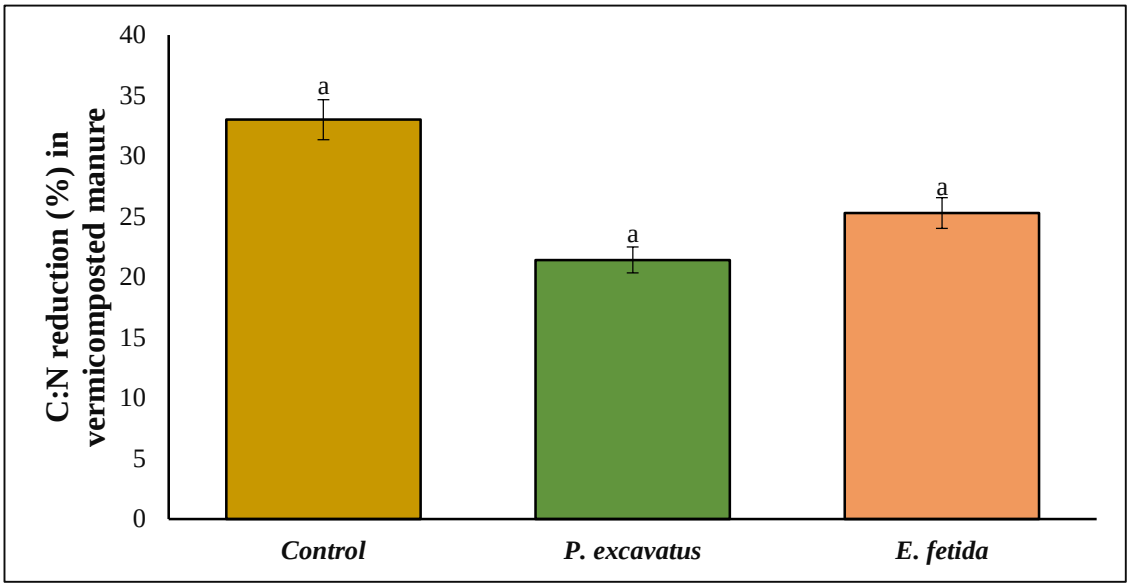

Fig. 5.19 Mean percentage reduction (%) of C:N in final vermicomposted manures produced from earthworm treatment and control. Mean with different superscripts among the treatment differ significantly (p<0.05) by Tukey's HSD at a 95% confidence level.

5.3.2.5 Available Phosphorus (Av.P mg/kg)

With an initial record ranging from 32.69±0.42 to 65.97±3.56, the total concentration of Av. K increased from 41.58±0.6 to 86.54±0.6 (RS>MX>CD>KS) in *P. excavatus*, 47.66±0.56 to 86.55±0.74 (RS>MX>KS>CD) in *E. fetida* and 38.55±1.64 to 79.14±2.09 (RS>MX>CD>KS) in control (**Fig. 5.20**) showing an average of 59±19.27, 61.2±17.5 and 54.32±17.51 in *P. excavatus, E. fetida,* and control respectively (**Fig. 5.21**). One way ANOVA test for the variations of Av. P in vermibed under different treatments are shown in **Table 5.7.**

Effects of earthworm species and different substrate used on Av. P was evaluated using two-way ANOVA, and results show interaction effects of earthworm and substrate were insignificant ($F_{(6, 24)}$=2.05, p>0.05). But simple main effects of earthworm ($F_{(2, 24)}$=13.28, p<0.05) and substrate used ($F_{(3, 24)}$=, p<0.05) have significant effects on Av. P concentration (**Table 5.10**). The increased percentage of Av. P over the initial

concentration was observed as 17.89 to 20.72%, 27.21 to 32.88%, and 20.54 to 51.83% in control, *P. excavatus, and E. fetida*, respectively (**Fig. 5.22**). The mean increased percentage was higher in *E. fetida*, *P. excavatus*, and control with 35.80±13.34%, 29.51±2.26, and 35.8±13.34 showing a significant difference ($F_{(2,\ 33)}$=5.52, p<0.05) among the treatment, and Tukey test shows, in *E. fetida* worked manure, an increased percentage of Av. P was significantly (p<0.05) higher compared to the control. However, no significant differences were observed between *E. fetida* and *P. excavatus* (**Fig. 5.23**).

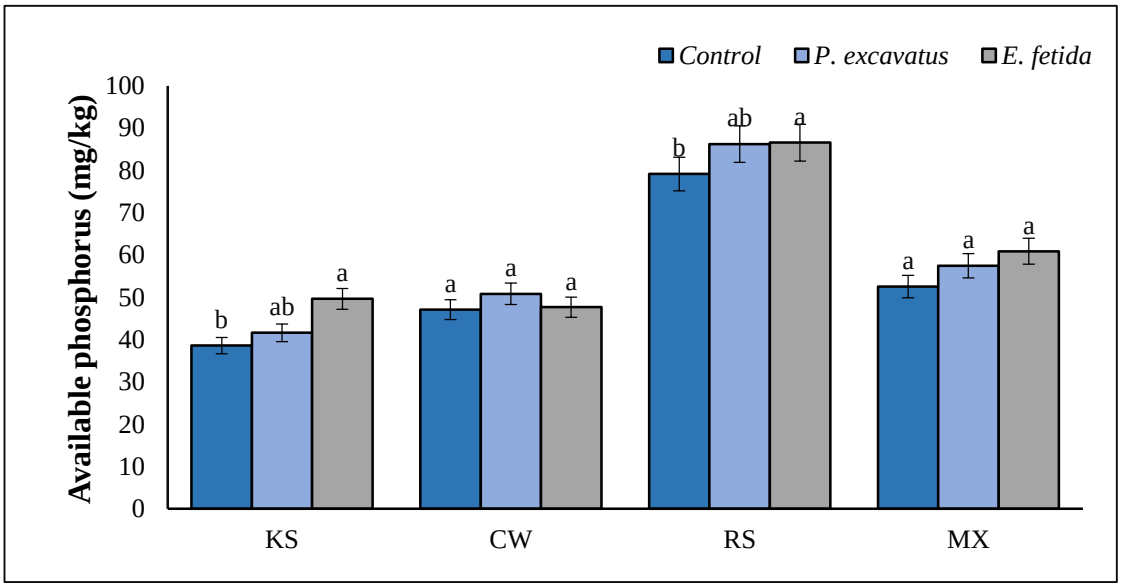

Fig. 5.20 Final concentration of available phosphorus at the end of vermicomposting in different substrate used as vermibed. Mean with different superscripts among the treatment differ significantly (p<0.05) by Tukey's HSD at a 95% confidence level.

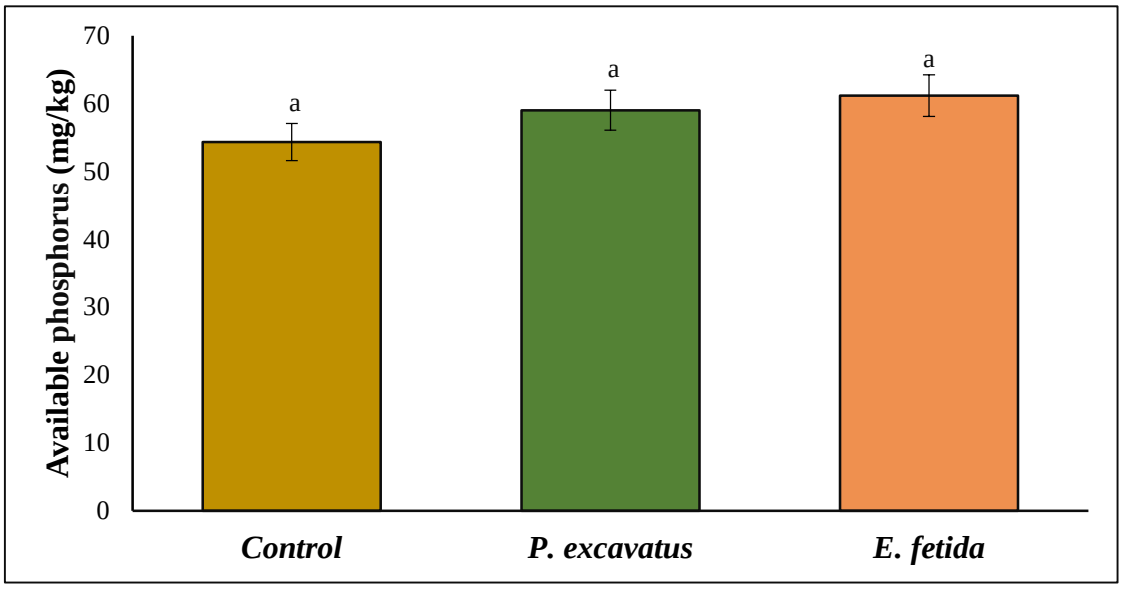

Fig. 5.21 Average amount of Av. P in different earthworm treatment and control at the end of vermicomposting. Mean with different superscripts among the treatment differ significantly (p<0.05) by Tukey's HSD at a 95% confidence level.

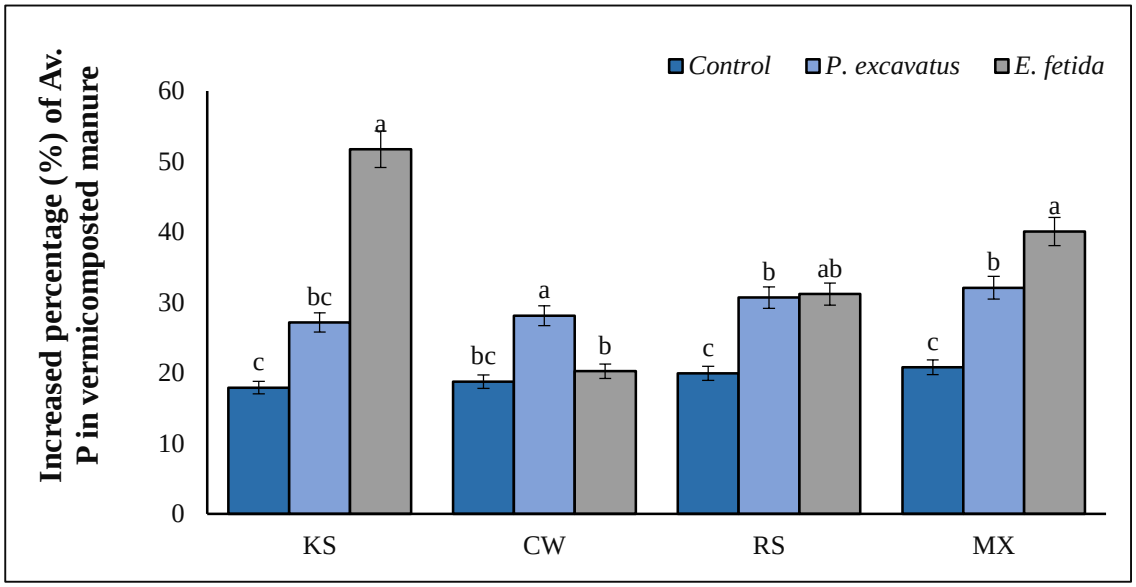

Fig. 5.22 Increased percentage of available phosphorus in different substrate used as vermibed. Mean with different superscripts among the treatment differ significantly (p<0.05) by Tukey's HSD at a 95% confidence level.

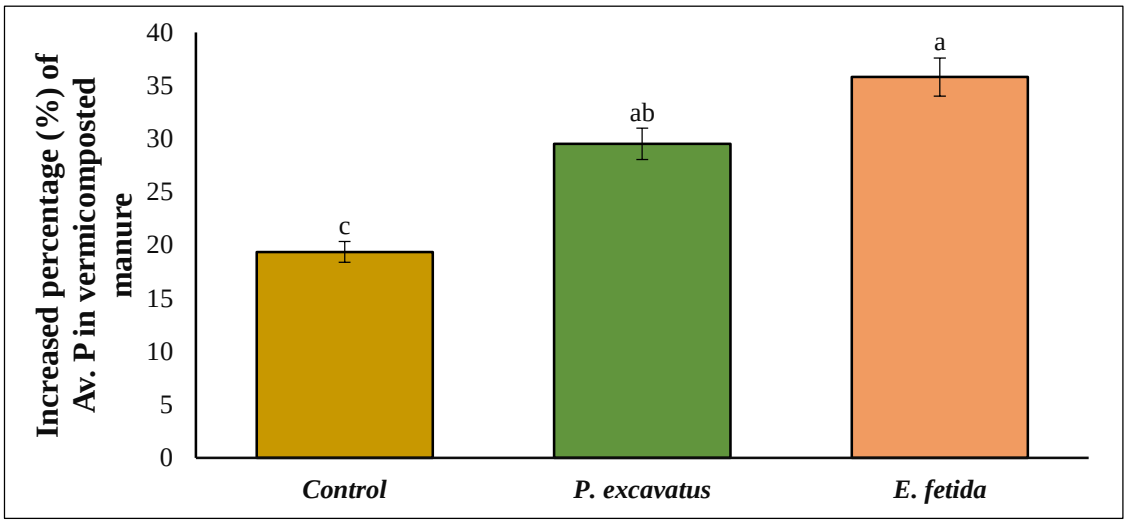

Fig. 5.23 Mean increased percentage of available phosphorus in final vermicomposted manures produced from earthworm treatment and control. Mean with different superscripts among the treatment differ significantly (p<0.05) by Tukey's HSD at a 95% confidence level.

Table 5.7 One-way ANOVA test results for the final concentration of available phosphorus in different substrates used as vermibed.

Source of variations		Sum of Squares	df	Mean Square	*F*	Sig.
KS	Between Groups	195.764	2	97.882	19.843	.002
	Within Groups	29.597	6	4.933		
	Total	225.361	8			
CD	Between Groups	23.938	2	11.969	1.054	.405
	Within Groups	68.108	6	11.351		
	Total	92.046	8			
RS	Between Groups	105.002	2	52.501	12.013	.008
	Within Groups	26.222	6	4.370		
	Total	131.224	8			
MX	Between Groups	105.862	2	52.931	2.237	.188
	Within Groups	141.988	6	23.665		
	Total	247.849	8			

5.3.2.6 Available potassium (Av. K mg/kg)

With an initial concentration ranging from 183.36±12.81 to 226.67±19.82, in control, the concentration of Av. K increased to 201.24±10.25 to 263.18±13 (MX>RS>KS>CD), in *P. excavatus* it increased to 229.24±14.11 to 288.09±9.45 (KS>RS>MX>CD) and in *E. fetida* 244.4±19.7 to 301±15 (KS>MX>RS>CD) (**Fig. 5.24**) exhibiting significant differences in KS ($F_{(2,8)}$=7.83, p=0.02) and CD ($F_{(2,8)}$=6.31, p=0.035) (**Table 8**). The highest amount of Av. K was observed in *P. excavatus (*270.2±29.32), followed by *E. fetida* (276.0417±27.33), and control (243.74±29.56) (**Fig. 5.25**).

One-way ANOVA shows that Av. K vary significantly depending on the treatment ($F_{(2, 33)}$=4.29, p<0.05) (**Table 5.9**). Multiple comparisons show that Av. K in *E. fetida* was significantly (p<0.05) higher compared to the control, while no significant difference was observed in comparisons to *P. excavatus* (p>0.05). Earthworm treatment ($F_{(2, 35)}$=12.7, p=0.0001) and substrate ($F_{(3,35)}$=21.73, p=5.0171E-7) used have significant effect on the Av. K , however interaction effect ($F_{(6,35)}$=1.45, p=0.23) of the two factors does have significant effect on Av. K concentration (**Table 5.10**). The increased percentage of Av. K among different substrates over the initial value was recorded to be 10.24 to 27.21%, 20.07 to 27.57%, and 16.72 to 34.17% in control, *P. excavatus,* and *E. fetida*, respectively (**Fig. 5.26**). Depending on the presence and absence of earthworms, the mean increased percentage of Av. K in the manure was significantly different ($F_{(2, 33)}$=6.24, p<0.05) among all the treatments, with a maximum in *E. fetida* (27.42%) followed by *P. excavatus* (24.36%) and control (14.67%) (**Fig. 5.27**). Tukey test shows that *P. excavatus* and *E. fetida* worked manures have significantly (p<0.05) higher percentages than the control.

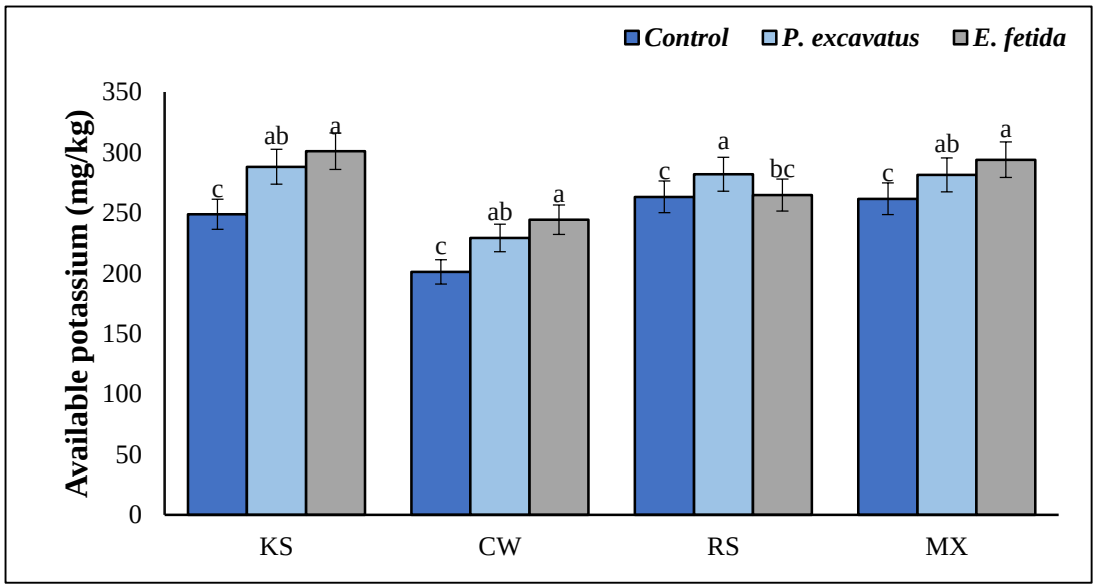

Fig. 5.24 Final concentration of available potassium at the end of vermicomposting in different substrate used as vermibed. Mean with different superscripts among the treatment differ significantly (p<0.05) by Tukey's HSD at a 95% confidence level.

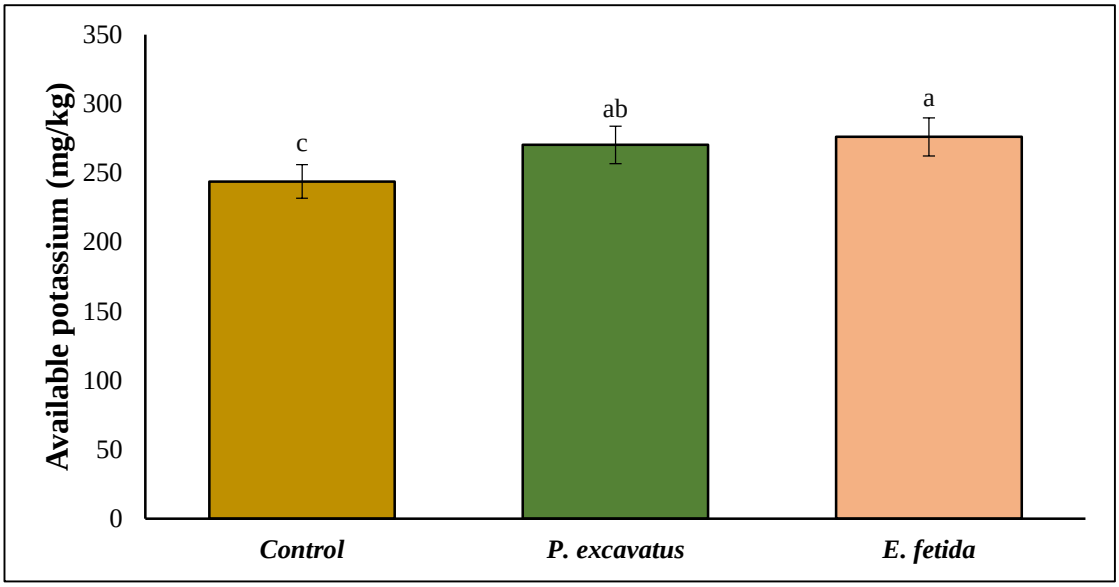

Fig. 5.25 Average amount of Available potassium in different earthworm treatment and control at the end of vermicomposting. Mean with different superscripts among the treatment differ significantly (p<0.05) by Tukey's HSD at a 95% confidence level.

Table 5.8 One-way ANOVA test results for the variations of available potassium in different substrates used as vermibed.

Source of variations		Sum of Squares	df	Mean Square	*F*	Sig.
KS	Between Groups	4424.582	2	2212.291	7.835	.021
	Within Groups	1694.094	6	282.349		
	Total	6118.676	8			
CD	Between Groups	2876.697	2	1438.348	6.210	.035
	Within Groups	1389.629	6	231.605		
	Total	4266.325	8			
RS	Between Groups	653.634	2	326.817	.844	.475
	Within Groups	2323.208	6	387.201		
	Total	2976.843	8			
MX	Between Groups	1592.977	2	796.488	3.735	.088
	Within Groups	1279.576	6	213.263		
	Total	2872.553	8			

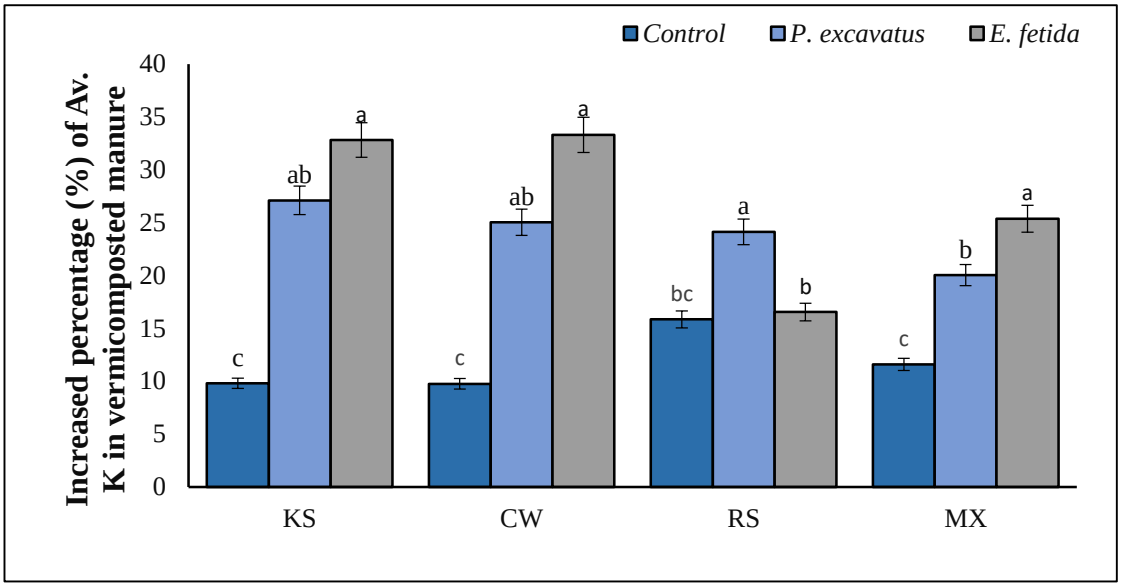

Fig. 5.26 Increased percentage of available potassium in different substrate used as vermibed. Mean with different superscripts among the treatment differ significantly (p<0.05) by Tukey's HSD at a 95% confidence level.

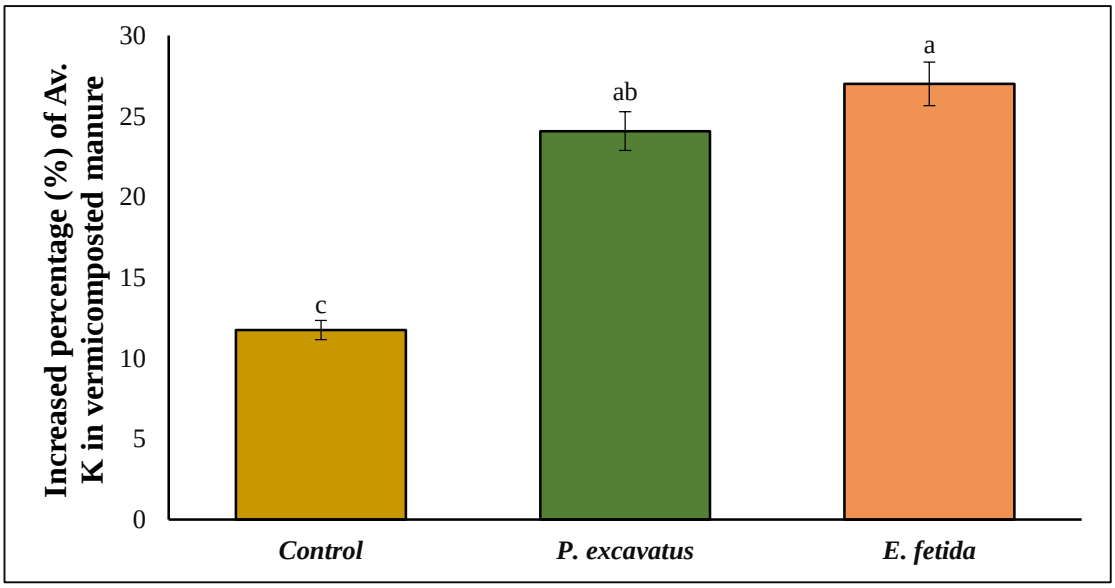

Fig. 5.27 Mean increased percentage of available potassium in final vermicomposted manures produced from earthworm treatment and control. Mean with different superscripts among the treatment differ significantly (p<0.05) by Tukey's HSD at a 95% confidence level.

Table 5.9 One-way ANOVA test results for the variations of various physico-chemical parameters in earthworm treatment and control.

Source of variations		Sum of Squares	df	Mean Square	F	Sig.
pH	Between Groups	.343	2	.171	.536	.590
	Within Groups	10.555	33	.320		
	Total	10.898	35			
OC	Between Groups	65.541	2	32.770	3.996	.028
	Within Groups	270.641	33	8.201		
	Total	336.182	35			
TN	Between Groups	1.231	2	.615	14.946	0.000024
	Within Groups	1.359	33	.041		
	Total	2.590	35			
C:N	Between Groups	29.779	2	14.889	2.263	.120
	Within Groups	217.157	33	6.581		
	Total	246.936	35			
Av. P	Between Groups	294.282	2	147.141	.525	.596
	Within Groups	9250.479	33	280.318		
	Total	9544.761	35			
Av. K	Between Groups	7109.442	2	3554.721	4.298	.022
	Within Groups	27294.458	33	827.105		
	Total	34403.900	35			

Table 5.10 Two-way ANOVA test results appraising the effect of substrate used and earthworm species on the variations of various physiochemical parameters in earthworm treatment and control. Treatment and substrate used are the independent factor while physico-chemical parameters are the dependent factor.

Parameters	Among the treatment		Among substrate		Interaction	
	F-value	P-value	F-value	P-value	F-value	P-value
pH	2.10	.14	29.12	3.6147E-8	3.08	.022
OC	11.29	0.00035	9.64	0.00023	6.71	0.00028
TN	44.50	8.4144E-9	4.67	.010	10.03	0.000014
C:N	2.85	.07	2.48	.085	1.68	.16
Av. P	13.28	0.00013	266.19	1.512E-18	2.05	.09
Av. K	12.75	0.000168	21.73	5.0171E-7	1.459	.234

5.3.3 Changes in heavy metals concentration after vermicomposting

Table 5.11 Initial concentration of heavy metals in in the substrate used.

Parameters	KS	CD	RS	MX
Copper (Cu mg/kg)	5.65±0.36	4.74±0.04	5.1±0.06	5.39±0.05
Iron (Fe mg/kg)	258.66±2.68	220.64±5.54	179.03±2.5	127.98±16.38
Manganese (Mn mg/kg)	56.02±1.33	51.22±2.05	54.11±1.49	56.01±0.88
Zinc (Zn mg/kg)	15.17±0.64	14.1±0.06	14.54±0.38	14.83±0.35

Data represent mean ±SD. KS-Kitchen scrap, CD- Cow dung, RS- Rice straw, MX-Mixed.

Table 5.12 Final concentrations of heavy metals in various substrate treated with earthworms.

Cu (mg/kg)					
Substrate	Control	*P. excavatus*	*E. fetida*	*F*	*P*-value
KS	3.36±0.26^{a}	1.68±0.29^{b}	1.55±0.31^{b}	36.023	0.00045
CD	2.25±0.24^{a}	1.66±0.00^{b}	1.86±0.16^{ab}	9.378	.014
RS	2.19±0.27^{a}	1.49±0.03^{b}	1.45±0.03^{b}	20.338	.002
MX	2.95±0.43^{a}	1.92±0.04^{b}	1.84±0.01^{b}	18.209	.003
Fe (mg/kg)					
KS	151.33±11.2^{a}	120.68±0.51^{b}	143.53±0.36^{a}	18.023	.003
CD	147±9.53^{a}	132.35±2.94^{b}	146.32±3.02^{ab}	5.651	.042
RS	163.6±13.57^{a}	151.86±2.14^{ab}	132.95±0.16^{b}	11.433	.009
MX	105.4±15.83^{a}	97.25±1.89^{a}	112.76±5.57^{a}	1.896	.230
Mn (mg/kg)					
KS	12.24±2.8^{a}	5.54±0.33^{b}	5.34±0.46^{b}	16.910	.003
CD	27.66±6.15^{a}	11.39±1.19^{b}	7.32±0.31^{b}	26.461	.001
RS	18.81±2.88^{a}	7.43±0.44^{b}	4.54±0.45^{b}	58.822	0.00011
MX	21.88±6.91^{a}	9.90±0.20^{b}	5.04±0.24^{b}	14.096	.005
Zn (mg/kg)					
KS	12.80±0.91^{a}	10.32±0.26^{b}	11.46±0.39^{ab}	13.113	.006
CD	13.51±0.46^{a}	10.99±0.26^{b}	11.17±0.07^{b}	60.838	0.000104
RS	12.1±0.61^{a}	9.49±0.37^{b}	10.53±0.18^{b}	28.062	.001
MX	13.22±0.23^{a}	11.91±0.13^{b}	12.08±0.03^{b}	60.740	0.000104

Data represent mean ±SD. KS-Kitchen scrap, CD- Cow dung, RS- Rice straw, MX-Mixed. Mean with different superscripts among the column differ significantly (p<0.05) by Tukey's HSD at a 95% confidence level.

Earthworms directly affect heavy metals through absorption in their tissue, known as bioaccumulation (Sizmur & Hodson, 2009). Heavy metals could induce the synthesis of metallothionein isoform in earthworms' intestines that bind metal ions, forming

organometallic ligands and thus reducing the exchangeable fractions of metals (Goswami *et al.,* 2014). The bioaccumulation factors (BAF) of earthworms for heavy metals are in order of Cadmium (Cd) > Zinc (Zn) > Copper (Cu) > Nickel (Ni) > Lead (Pb) (Rorat et *al.*, 2016). However, Bernard et al. (2010) argued and demonstrated that *E. fetida* could eliminate Pb but not Cd when exposed to contaminated soils.

The amount of Cu (mg/kg) in the initial stage ranged from 4.74 to 5.65, with a maximum concentration in KS followed by MX, RS, and CD, which was considerably reduced on vermicomposting (Table 5.11). Cu concentration in *P. excavatus* inoculated pot was reduced to 1.49±0.03, 1.66±0.007, 1.68±0.29, and 1.92±0.04 in RS, CD, KS, and MX, respectively, with an average concentration of 1.68±0.2 (**Table 5.12**). In contrast, in *E. fetida,* it was reduced to 1.45±0.03, 1.55±0.31, 1.84±0.01 and 1.86±0.16 in RS, KS, MX, and CD, respectively, with an average concentration of 1.68±0.24. The reduction of Cu concentration in control was comparatively less, showing a mean amount of 2.69±0.58. In the final manures produced, Cu concentration varies noticeably, depending on the earthworm treatment and substrates used. Two-way ANOVA was analyzed to study the effects of substrate and earthworm species on Cu concentration. The results show that the interaction effects of independent variables (substrates and treatment) were significant ($F_{(6, 24)}$=6.28, p<0.05). Also, the main effects show earthworm species ($F_{(2, 33)}$=80, p<0.05) and substrate ($F_{(3, 33)}$=11.04, p<0.05) significantly affect Cu concentration.

In *P. excavatus,* the percentage reduction (%) of Cu was maximum in RS (70.78±1.04), followed by KS (70.01±7.06), CD (64.88±0.17), and MX (64.38±1.13). While in *E. fetida* treated pot, there was a gradual decline of 72.15±7.39%, 71.49±0.97%, 65.72±0.18%, and 60.73±3.39%, in KS, RS, MX, and CD, respectively. However, in the

control pot, reduction (%) was comparatively less, as observed in RS (57.08±5.46), CD (52.55±5.02), MX (45.15±8.21), and KS (40.3±5.87) (**Fig. 5.28**). Further, the mean reduction of Cu in *P. excavatus, E. fetida*, and control over initial readings were 67.51±4.32%, 67.52±5.98%, and 48.77±8.62%, respectively. One-way ANOVA shows mean reduction percentage of Cu differs significantly ($F_{(2,\ 33)}$=32.71, p<0.05) in *P. excavatus, E. fetida*, and control. Multiple comparisons test show no significant differences (p>0.05) between the two earthworm species, but the amount of Cu in earthworm in worked manures were significantly (p<0.05) lower than the control.

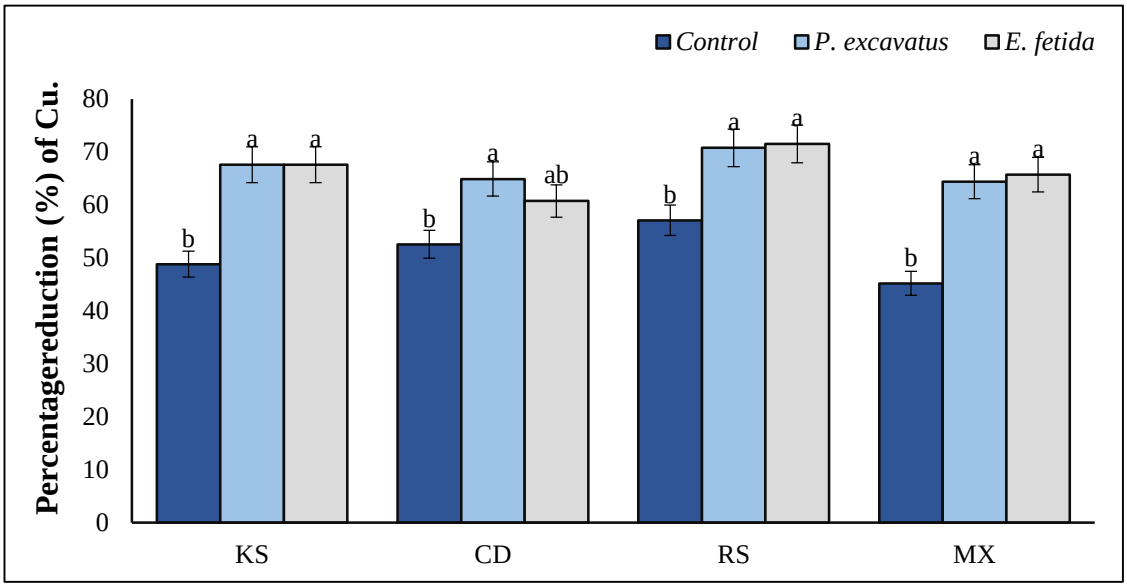

Fig. 5.28 Decreased percentage of Cu in different substrate used as vermibed at the end of vermicomposting. Mean with different superscripts among the treatment differ significantly (p<0.05) by Tukey's HSD at a 95% confidence level.

Having the initial amount of Fe (mg/kg) ranging from 258.66±2.68 in KS, 220.64±5.54 in CD, 179.93±2.5 in RS, 127.98±16.38, (**Table 5.11**), the concentration was reduced to 97.25±1.89 to 151.86±2.14, 112.76±5.57 to 146.32±3.02 and 105.4±15.83 to 163.66±13.57 in *P. excavatus, E. fetida* and control pots respectively in all substrates

(**Table 5.12**). The final Fe concentration in control, *P. excavatus*, and *E. fetida* were 141.85±25.34, 125.53±20.70, and 133.89±14.03. Unlike other parameters, Fe concentration in different earthworm treatments does not differ significantly ($F_{(2,33)}$=1.88, p>0.05). However, significant ($F_{(3,33)}$=21.52, p<0.05) differences among the substrate were observed.

The percentage reduction (%) of Fe with *P. excavatus* was maximum in CD (55.47±2.67) followed by KS (53.34±0.59), MX (23.21±9.49), and RS (15.6±0.14). While in *E. fetida* treated pot, there was a decline of 11.3±7.2%, 26.1±0.94%, 33.62±3.03%, and 44.5±0.43% in MX, RS, CD, and KS, respectively. In the control pot, reduction (%) was comparatively less, as observed in RS (9.07±6.74), MX (17.75±4.02), CD (33.27±5.98), and KS (41.51±3.85) (Fig. 5.29). Further, the highest mean reduction (%) of Fe was observed in *P. excavatus, E. fetida*, and control with 36.9±22.11%, 28.88±13.03%, and 25.4±14.02% but no significant ($F_{(2, 33)}$=1.46, p>0.05) differences have resulted among the treatment (**Fig. 5.32**).

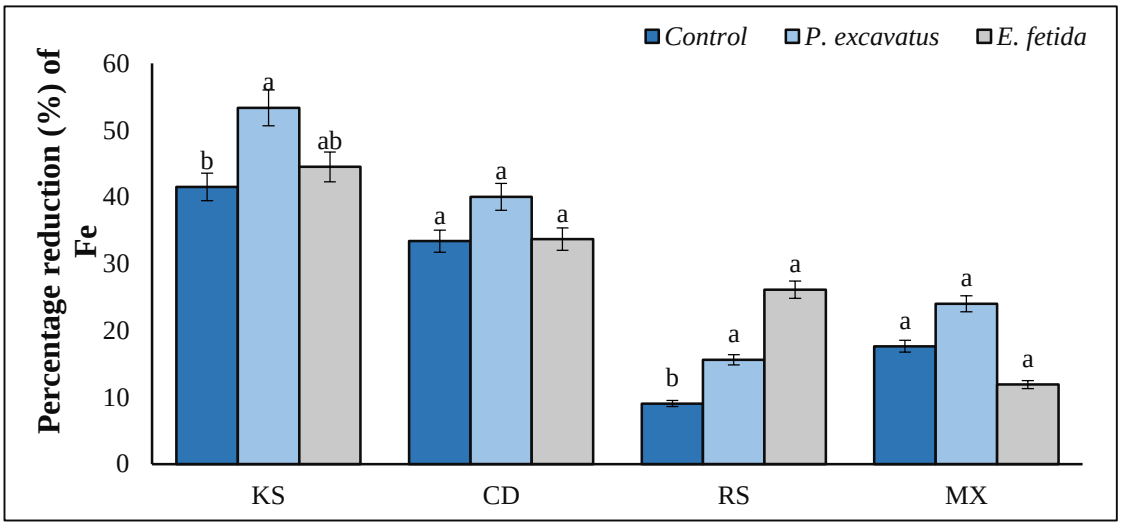

Fig. 5.29 Decreased percentage of Fe in different substrate used as vermibed at the end of vermicomposting. Mean with different superscripts among the treatment differ significantly (p<0.05) by Tukey's HSD at a 95% confidence level.

Initial concentration of Mn (mg/kg) ranged from 51.22±2.05 to 56.02±0.88 in the different substrates, with the maximum amount in CD, followed by RS, KS, and MX (**Table 5.11**). However, the concentration was substantially decreased at the end of the experiment in all treatments. With the treatment of *P. excavatus,* the Mn concentration was reduced to 5.54±0.33 to 11.39±1.19 showing the maximum reduction in KS (90.08%), followed by RS (86.26%), MX (82.32%), and CD (77.74%). In *E. fetida* treated pot also, there was a decline of 95.45%, 94.65%, 94.95%, and 92.67% in RS, KS, MX, and CD, respectively, having a final concentration ranges of 4.54±0.45 to 7.32±0.31. In the control pot also, Mn was reduced considerably (12.24±2.8 to 27.66±6.15), highlighting the maximum reduction (%) in KS (78.06±5.47%) followed by RS (65.3±4.63%), MX (61.05±11.76%), and CD (46.17±10.49%) (Fig. 5.30). The average amount of Mn in *P. excavatus*, *E. fetida*, and control were 8.56±2.41, 5.56±1.14, and 20.14±7.22, respectively. Two-way ANOVA shows that the interaction effect of earthworm species and substrate impact Mn concentration significantly ($F_{(6,\ 13)}$=2.99, p<0.05). While the main effects show earthworm species treatment ($F_{(2,\ 33)}$=81.92, p<0.05) and substrate used ($F_{(3,\ 33)}$=11.09, p<0.05) also have a significant effect on Mn concentration. Tukey test shows Mn concentration in *P. excavatus* and *E. fetida* were significantly (p<0.05) lower compared to the control, however, no significant (p>0.05) differences were observed between the two earthworm species. Further, mean variation in the reduction percentage of Mn in *E. fetida* (94.43±0.1%), *P. excavatus* (84.1±0.95%), and control (62.64±3.56%) over initial were significantly ($F_{(2,\ 33)}$=42.73, p<0.05) different and showed better efficiency of earthworm species.

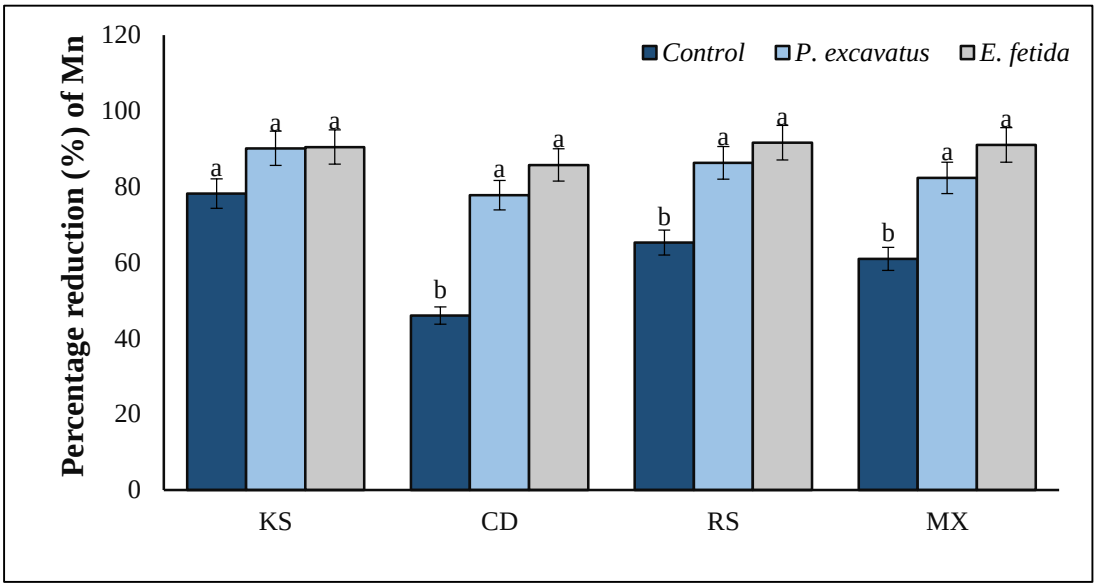

Fig. 5.30 Decreased percentage of Mn in different substrate used as vermibed at the end of vermicomposting. Mean with different superscripts among the treatment differ significantly (p<0.05) by Tukey's HSD at a 95% confidence level.

The initial concentration of Zn (mg/kg) ranged from 14.1±0.06 to 15.17±0.64 in different substrates, and the concentration of Zn at the end of vermicomposting was found to be 9.49±0.37 to 11.91±0.13, 10.53±0.18 to 12.08±0.03 and 12.1±0.61 to 13.51±0.46 in *P. excavatus, E. fetida* and control respectively. The average amount of Zn in *P. excavatus, E. fetida*, and control were 10.68±0.95, 11.31±0.61, and 12.91±0.75 showing significant ($F_{(2,33)}$=25.5, p<0.05) differences among earthworm treatment and control. Multiple comparisons test indicate no significant (p>0.05) differences between two earthworm species in minimizing the Zn concentration but both *P. excavatus* and *E. fetida* showed significant (p<0.05) effects on Zn. In *P. excavatus*, maximum reduction (%) of Zn concentration was recorded in RS (34.75±1.02%) followed by KS (31.95±1.36%), CD (22.02±2.25%), and MX (19.682.17%), it was noticed to be comparatively less in *E fetida* treated substrates of RS (27.54±1.2%), KS (24.35±4.68%), CD (20.80±0.2%), and MX (18.54±1.71%). In control, the maximum reduction was observed in RS>KS> MX>CD

with 15.68±2.99, 16.75±3.82, 10.84 ±3.52, and 4.22±0.34 (**Fig. 5.31**). Analysis of variance shows significant variations in the mean reduction percentage of Zn were observed depending on the treatment ($F_{(2,33)}$=22.26, p<0.05) and substrate ($F_{(3,33)}$=4.63, p<0.05). Tukey test shows reduction (%) of Zn concentration in *P. excavatus* and *E. fetida* were significantly higher (p<0.05) compared to the control, while no significant difference was observed between earthworms (p>0.05) (**Fig. 5.32**).

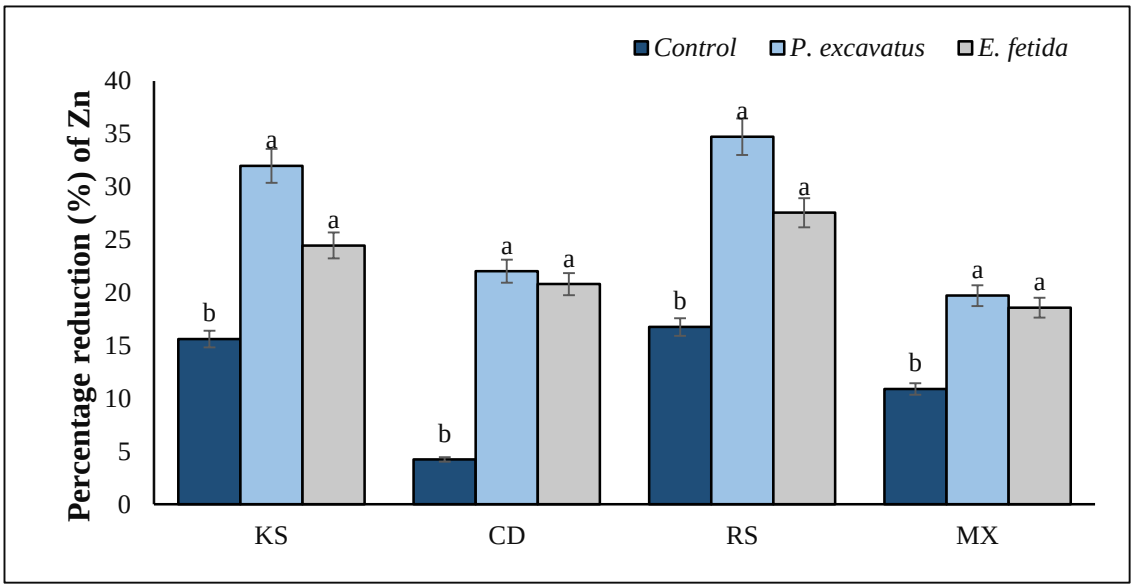

Fig. 5.31 Decreased percentage of Mn in different substrate used as vermibed at the end of vermicomposting. Mean with different superscripts among the treatment differ significantly (p<0.05) by Tukey's HSD at a 95% confidence level.

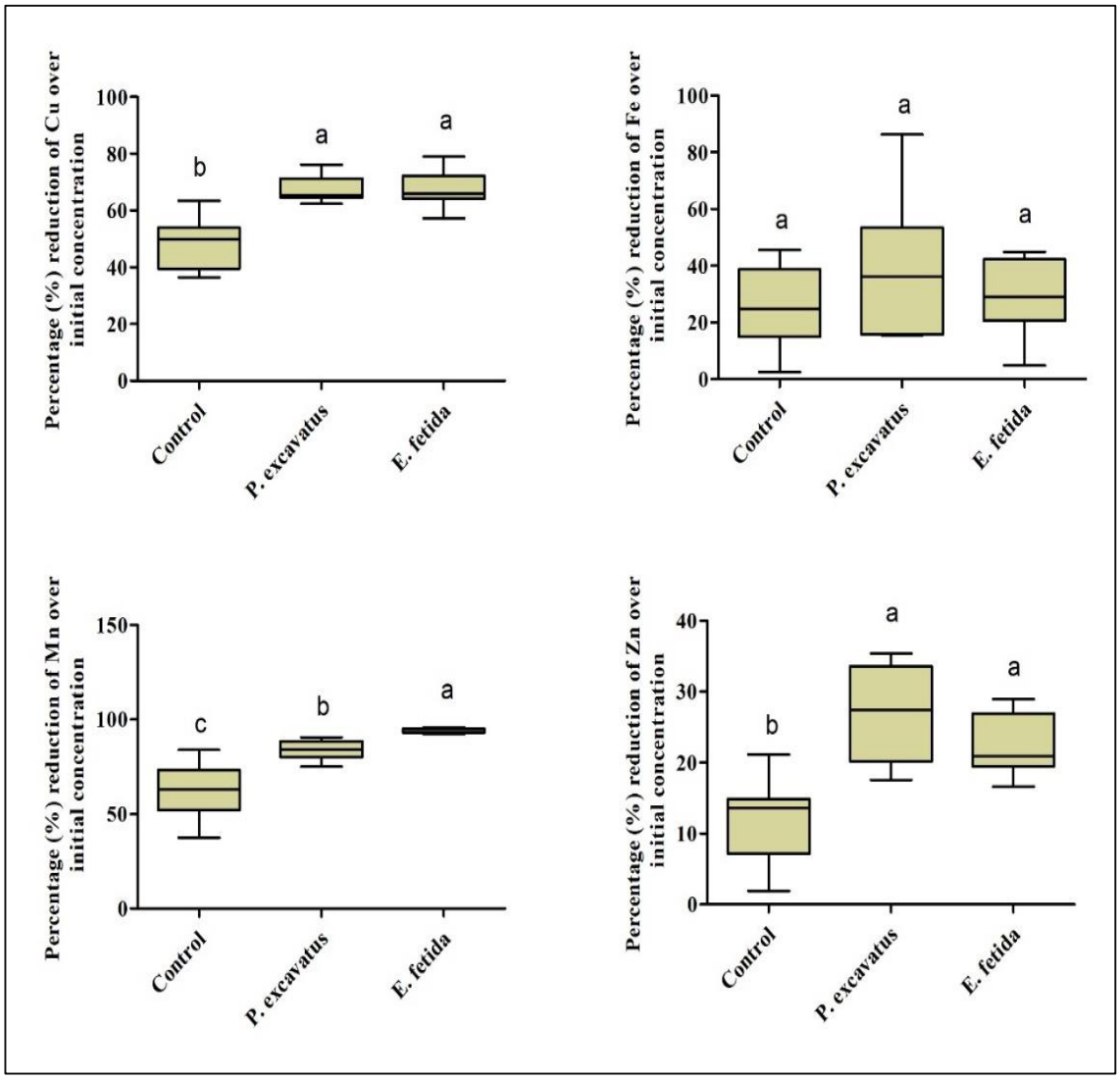

Fig. 5.32 Mean percentage reduction of heavy metal concentration over initial value in earthworms worked manure and control manure. Mean with different superscripts among the treatment differ significantly (p<0.05) by Tukey's HSD at a 95% confidence level.

5.3.3 Cocoon counting

Suitability of waste for worms and their reproduction rate can be indicated by the cocoon production. For *E. fetida* maximum cocoon production was observed in MX (212±13) followed by KS (205±30.4), CD (168.33±5.13) and RS (115±26.62), whereas for *P. excavatus* maximum production was observed in KS (90.5±14.5) followed by MX

(87±14.18), CD (71.33±6.8) and RS (61.66±19.75) (**Fig. 5.33**). Total cocoon produced by *E. fetida* (2101±44.1) was significantly higher (p<0.05) compare to *P. excavatus* (972±19.97). In the present study with twenty (20) matured individuals of earthworm introduced, average cocoon production was 175 for *E. fetida* (**Fig. 5.34**) and 77.25 for *P. excavatus.* Similarly, Balachandar *et al.* (2020) also reported maximum of 170.85 cocoon per vermibed inoculated with 30 individuals of earthworms from Pressmud, cow dung and *Leucaena leucocephala* mixed at 2:1:1 ratio. Various factors affect cocoon production including quality of organic waste, food palatability, stocking density of worms etc. Analysis of two way ANOVA indicate that interaction of earthworm species and substrate used as vermibed affects cocoon production significantly (p<0.05). With further degradation of organic matter, cocoon production decreased, indicating importance of food availability for growth and reproduction of earthworms. Similarly, Negi and Suthar (2013) emphasised that earthworm biomass and reproduction depends on substrate mixture provided as feed for earthworms.

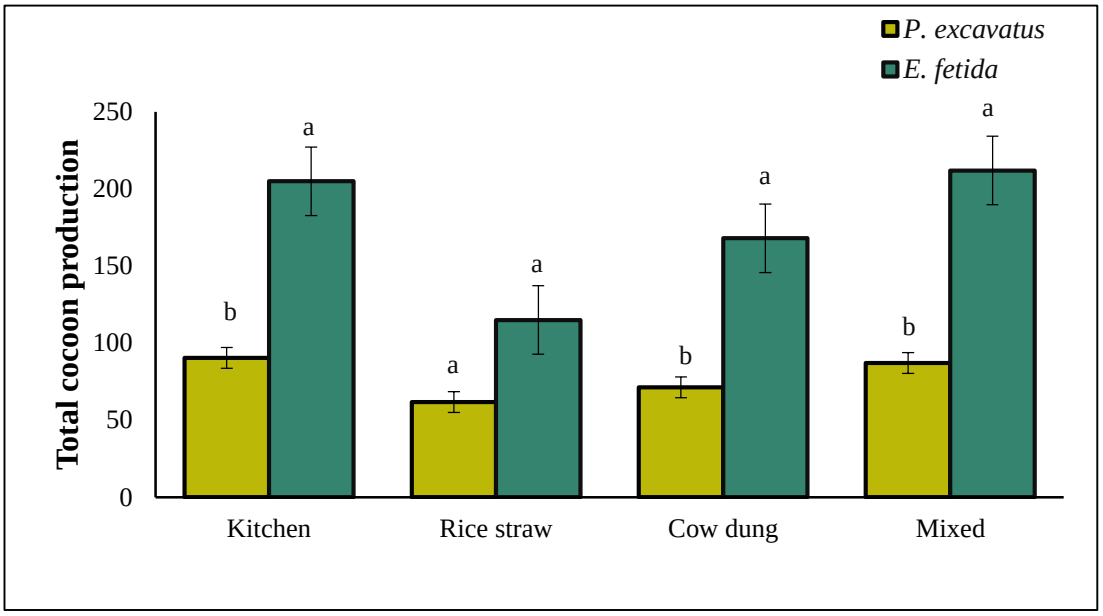

Fig. 5.33 Total cocoon productions of earthworms in different substrate during vermicomposting. Mean with different superscripts among the treatment differ significantly (p<0.05) by Tukey's HSD at a 95% confidence level.

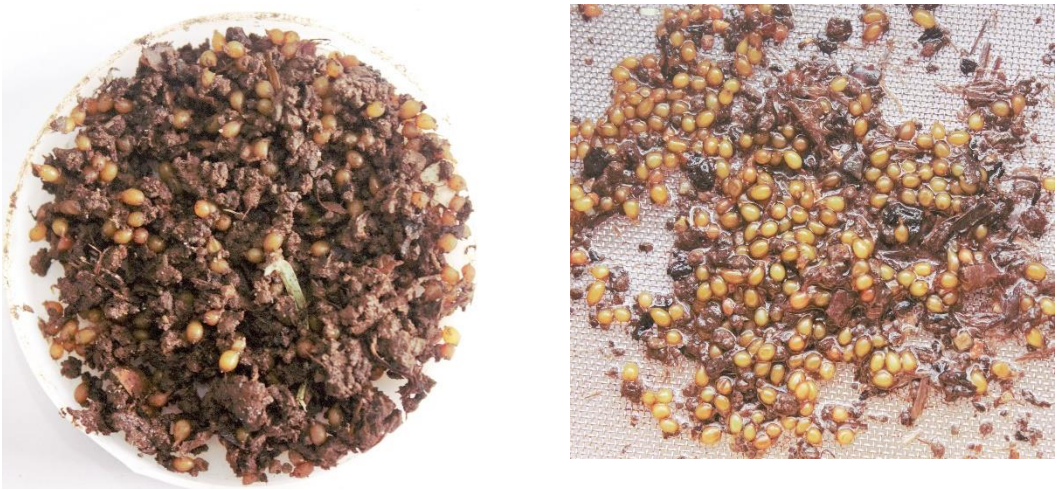

Fig. 5.34 Cocoon production of *E. fetida* during vermicomposting.

5.4 Discussion

Circular economy encompass recycling, reusing, and reducing the loss of resources, and Engaging in vermicomposting for organic waste management problems is a win–win option that not only deals with the pollution of organic waste but also recovers the necessary nutrients. Vermicomposting is an important alternative to composting for nutrient cycling, and resource recovery that promotes sustainable development. Sharma & Garg $(2018)_b$ suggested that the decomposition rate depends on the efficiency of earthworm species and the nature of organic materials used during vermicomposting. Similar to the present finding, Paul *et al.* (2011) reported 70.48% biomass reduction of municipal wastes during vermicomposting using *P. ceylensis*. Similarly, Huntley & Ansari (2021), during vermicomposting of vegetable wastes using *P. excavatus*, the final amount of manure produced was 51.54% (515.45g out of 1000g) initial substrate used. In the present study, nutrient stabilization of the substrate was observed viz. reduced OC, TN, C:N and increased Available phosphorus, and available potassium. During vermicomposting, assimilation and respiratory activity of earthworms, in association with microbes, utilize biodegradable organic matter resulting to a rapid decrease in organic carbon (Lv *et al.,* 2018). Sharma & Garg (2018) have reported 17-58% of total organic carbon reduction from rice straw, paper waste, and cow dung mixtures during vermicomposting. In contrast, Mago *et al.* (2021) reported a 40.09 to 64.06% reduction of organic matter from banana crop waste. Also, the decomposition and humification of carbonaceous materials in the substrate led to reduced OC (Negi & Suthar, 2018). OC reduction could also be attributed to the emission of CO_2 from vermibin (Lv *et al.*, 2018).

With the joint action of microorganisms, earthworms bring about numerous changes in biodegradable waste ranging from physical, chemical, and biological

characteristics. The decline in nitrogen could be due to the utilization of organic matter by heterotrophic microorganisms and the release of ammonia gas. The average reduction of TN in *P. excavatus* (47.56%), *E. fetida* (41.36%), and control (23.82%) were significantly different ($F_{(2,\ 33)}$=9.2, p<0.05), indicating that nitrogen mineralization depends on the nature of substrate and type of earthworm employed. Zhi-Wei (2019) observed a significant reduction in the final vermicomposted organic matter (1.01% to 0.54%). Contrary to the present finding, Suthar (2009)[c] reported a higher amount of TN (range 2.49–3.17%) in controls and emphasized that earthworms increase nitrogen levels by adding their excretory materials and mucus body fluid and decaying tissues of dead worms in the bin. C: N ratio from 15-20 is considered acceptable for vermicompost applications in agronomy (FAO, 2020). In the present study, the ratio that microbes consume OC is more with little nitrogen, leading to a decline in C: N ratio. The rapid decline of organic carbon and C: N ratio during the initial stage of vermicomposting indicates the conversion of biodegradable waste into a stable end product (Lv *et al.,* 2018; Gusain & Suthar, 2020; Pandit *et al.,* 2020) and is also attributed to the consumption of organic matter, cellulose, and hemicellulose by earthworms (Sharma & Garg, 2019).

Available phosphorus increased in all the substrates at the end of the experiment, however it varies depending on the substrate used and earthworm species present. The increased phosphorus concentration in vermicompost may be attributed to earthworm activity conducive to phosphate-dissolving bacteria in the feedstock. The activity of earthworm gut enzymes, phosphatase, formation of organic acids, and discharge of total phosphorus from a complex form of humic acid mediated by microbial activity might contribute to the increase of phosphorus in vermicompost (Sharma & Garg, 2018; Gusain & Suthar, 2020). The increased activity of phosphate solubilizing enzymes in the

earthworm gut may also contribute to a higher concentration of available phosphorus (Ramanarian *et al.,* 2019). Ghosh *et al.* (2018) reported that in the first 50 days, phosphatase and phytase enzymes are responsible for phosphorus mineralization. Balachandar *et al.* (2020) also reported a 98% increase in phosphorus concentration using epigeic species, *Eudrilus eugeinae,* from green manure. At the same time, Nayak *et al.* (2013) reported a 29.1 to 46.9 % increase in phosphorus concentration from vermicomposting sewage sludge. In addition to other nutrients potassium is also aother essential nutrient that play critical role in plant physiological process such as tolerance to water stress. Suthar (2007)c reported an enhanced level of exchangeable potassium in the manure from 26.3 to 125.2% using *P. sansibaricus* and recorded higher microbial activity during the vermicomposting process of agriculture waste, farmyard manure, and urban solid waste. Zziwa *et al.* (2021) recorded a 74.0 to 81.3% increase in total potassium from vermicompost produced from pineapple waste. The enhanced level of exchangeable potassium from an insoluble state in the vermicomposted products is due to earthworms as they increase the number of microorganisms and their activity (Kaviraj & Sharma, 2003). Many earthworm gut-associated bacteria are reported to have phosphate and potassium-solubilizing bacteria. Yakkou *et al.* (2022) observed that out of 16 bacteria isolated from earthworm gut, six bacteria, namely *Pseudomonas aeruginosa*, *Pantoeavagans, Buttiauxella gaviniae, Raoultella planticola, Aeromonas sp. Aeromonas hydrophila* have the potential to solubilize insoluble forms of potassium. Therefore, apart from the other biochemical process, an increased Av. P and Av. K could be attributed to earthworm-associated bacteria that solubilize the bound form of Phosphorus and potassium.

Earthworm has the ability to absorb heavy metal and its prospects in mitigation of heavy metal toxicity have been reported in the past. Lv *et al.* (2016) reported a significant reduction of exchangeable Cu during vermicomposting. They emphasized that earthworm bodies might take up the heavy metals, which leads to a decrease in Cu concentration. Similarly, Suthar *et al.* (2014) reported that vermistabilization significantly reduced the amount of Cu (68.8–88.4 %) and demonstrated that bioaccumulation of heavy metals by earthworms was in the order Cd>Cr>Pb>Cu. Pattanaik & Reddy (2010) reported that earthworms remediate heavy metals from the waste by bioaccumulating in their body tissue, resulting in a decline in its concentration in vermicomposted manure. In the present study, 64.38% to 70.78% reduction of Cu concentration was observed in *P. excavatus* and a 60.73% to 72.15% reduction was observed in *E. fetida.* Differences in the reduction percentage of heavy metals could be due to the differences in bioaccumulation potential of earthworms and microbiological community variations in the substrates. During vermicomposting, a substantial reduction of Fe concentration (13.1 to 19.9%) was also observed by Suthar & Singh (2008). Likewise, Hobbelen *et al.* (2006) reported that heavy metal availability in earthworm tissues increases while its concentration decreases in vermicompost. Singh & Kalamdhad (2013) reported that high percentage reduction of Mn in the earthworm-treated pot could be due to the absorption of most available fractions of Mn through the epithelial tissue of earthworms during vermistabilizations. Similar to the present finding, Singh and Kalamdhad (2013) reported 42.6 to 84.6% reduction in heavy metals during vermicomposting. However, in contrast to the present study, Soobhany *et al.* (2015) have shown that vermicomposting increases Mn concentrations considerably and asserted that formations of the organically bound complex rather than augmentation in the total content might lead to increased Mn concentrations.

While studying the remediation of heavy metals from urban waste using earthworms, Pattanik & Reddy (2011) observed a significant concentration of metals increase in earthworm tissue, and 56% of Zn was removed from the biodegradable waste within 60th days. Dominguez *et al.* (1997) also confirmed that in 60 days, the bioavailability of heavy metals (Zn and Cu) decreased by 35 to 55%. Pattanaik & Reddy (2010) reported that earthworms remediate heavy metals from the waste by absorbing their body, resulting in a decreased concentration in the manures. The present findings indicate the potentiality of *P. excavatus* and *E. fetida* to reduce heavy metal toxicity and remediate the polluted landscape. Among the different ecological categories of earthworms, epigeic earthworm such as *E. fetida* has shown better bioaccumulation capability of heavy metals compared to endogeic and anecic earthworms (Turgay *et al.*, 2011). It is suggested that phosphorus treatment significantly reduces the bioavailability of metals (Cd, Zn, and Pb), probably due to the formation of metal phosphate complexes in the soil (Maenpaa *et al.*, 2002). Similarly, due to the joint action of earthworms and bacteria (primarily PSB), an increased amount of phosphorus in the vermicomposted manure might decrease heavy metal availability.

Chapter 6

Summary and conclusions

Earthworm constitutes a majority of soil fauna and plays a vital role in regulating the dynamics of soil systems. They are one of the keystone species and are also called ecosystem engineers. The physical and chemical changes brought about by the earthworm to the soil significantly affect the biological properties, including the suitability of soil as a habitat for other organisms. However, the overall effect of earthworms in the soil process depends on their ecological category. For instance, epigeic or litter-dwelling species such as *Perionyx excavatus* and *Eisenia fetida* are important in litter degradation and early decomposition stages. In contrast, endogeic earthworms such as *Aporrectodea caliginosa* and *A. trapezoids* are characteristically soil inhabitants that pronounced the changes in the soil's physical structures. Anecic species are characterized by deep vertical burrowing activities, mainly feeding on soil mixed with organic matter from upper soil strata. Earthworms also play an important role in modifying soil structures and decomposing plant debris. Because of their significance in ecosystems, waste degradation, plant growth, mutualistic relationships with other plant growth-promoting microbes, and soil fertility, considerable attention has been given to the scientific studies of earthworms. India, being a geographically diverse tropical country, provides congenial habitat for earthworms and harbors about 3% of the world's earthworm species. Across the country, earthworm resource explorations have been carried out in different ecosystems, such as plantations, agroecosystems, jhum fallow, and urban habitats. Similarly, soil nutrient enhancement and isolation and characterization of plant growth-promoting bacteria associated with different species of earthworms have also been reported. Considering the functional attributes of earthworms and their potential applications in nutrient enhancement, plant growth, and waste management, a research

programme has been undertaken in Mokokchung district, Nagaland with following the objectives:

4. To Study the temporal variation of density, biomass, and population dynamics of earthworms in the subtropical forest under the Mokokchung district, Nagaland.
5. To study the biology of dominant earthworm species and their associations with phosphate solubilizing bacteria (PSB).
6. To evaluate the earthworm effects on soil nutrients and plant growth, vermicomposting efficiency and nutrient analysis of the vermicompost.

Earthworm studies in subtropical forest ecosystems were conducted in two different types of vegetation viz. mixed forest and monoplantations (*Daubanga grandiflora*) located in Mingkong reserve forest area, Mokokchung district, Nagaland. The present study was carried out from January, 2019 till February, 2020 at Minkong forest, Nagaland during spring, monsoon, post monsoon and winter season of the year. The two study sites viz., mixed forest and plantation lying at an altitude of 1325 m above sea level are characterised by gentle to steep slopes. The most common tree species in mixed forest are *Atrocarpus chaplasha* Roxb., *Ficus semicordata* Buch. ex J.E. Smith, *Schima wallichii* (DC.) Korth. *Trema orientalis* (L.), etc., while, plantation area is a mono plantation of *Daubanga grandiflora* (Roxb. ex DC.) Walp. The ground surface of the plantation area is cleared about 3 times a year, while mixed forest remains fairly undisturbed. The climate is monsoonal with warm moist summer and cool dry winter, and receives an average annual rainfall of 1001.6 mm. The earthworm species were collected using the quadrat method, and soil samples were collected from 0-15 cm layers. 389 and 467 individuals of earthworms were sampled from the mixed forest (MF) and plantation (PL), respectively. In MF, ten (10) earthworm species belonging to family

Glossoscolecidae (*P. cothurnus*),Megascolecidae (*A. gracilis*, *M. houlleti*, *P. excavatus*, and *P. simlaensis*), Moniligastridae (*D. hodgarti*, *D. nepalensis*, and *Drawida* sp.), and Octochaetidae (*E. festivus* and *E. assamensis*) were recorded, while in PL, nine (9) earthworm species belonging to the family Glossoscolecidae (*P. corethurus*), Megascolecidae (*A. cortices*, *P. excavatus*, *P. simlaensis*), Moniligastridae (*D. nepalensis*, *D. assamensis*, *Drawida sp.* and *E. assamensis*), and Octochaetidae (*E. festivus*) were found. Species such as *P. excavatus*, *D. nepalensis*, *Drawida* sp., and *E. festivus* were found to be dominant with high density and relative abundance in the study area. Shannon-Weiner diversity (H') was 2.01±0.24 and 1.56±0.37 in MF and PL; Simpson diversity (D) was 0.31±0.07 and 0.21±0.06 MF and PL, while evenness was 0.9±0.04 and 0.83±0.04 respectively. Both the study area shared 73% species similarity.

In MF, total earthworm density ranged from a minimum of 3.77±1.69 ind.m^{-2} (December) to a maximum of 48.57±8.99 ind.m^{-2} (July). Total earthworm biomass in MF ranges from 1.21±0.35 to 46.96±22.65 g.m^{-2} having the minimum during December and May respectively. In PL, total earthworm density ranges from 3.77±1.69 to 48.57±8.99 ind.m^{-2}. With the arrival of dry seasons, earthworm density and biomass decreased significantly in both the study area. Seasonal variations also showed that earthworm density and biomass increased significantly with the arrival of monsoon season (p<0.05). In MF, juvenile, young, and adult density varies from 2.37-17.18 ind.m^{-2}, 6.35-21.36 ind.m^{-2}, and 14.8-55.62 ind.m^{-2}, while in PL, juvenile, young, and adult density varies from 29-39.11 ind.m^{-2}, 49.42-64.59 ind.m^{-2}, and 0.99-52.14 ind.m^{-2} respectively. Correlations analysis showed that density and biomass of different earthworm age categories were positively correlated with environmental factors such as temperature,

moisture, and rainfall, indicating that earthworm survival, abundance, and activities depend on these factors.

Considerable variations of earthworm density, biomass, and species richness in the study sites indicate the dependence of earthworms on land use systems and environmental factors. Higher species richness in MF was attributed to the homogeneity of habitat and less anthropogenic disturbance compared to PL, where it was cleared periodically to maintain the plantations. The presence of exotic and native species also indicates the unique characteristic of biodiversity hotspots zone. Also, the dominance *of P. corethurus* and *D. nepalensis* in PL indicate that anthropogenically modified ecosystems are more vulnerable to invasion by peregrine species.

Epigeic earthworm, *P. excavatus,* one of the dominant earthworm species, was studied for its reproductive strategies and its associations with microbes having the potential for phosphate solubilization. The life assessment was conducted in an earthen pot (2.5 ltr capacity) with substrate preparations as T1 (Cow dung (CD) only), T2 (Soil: Kitchen waste: Cow dung at a 7:3:1 ratio), T3 (Soil: Rice straw: Cow dung at a 7:3:1 ratio). Initially, earthworm biomass increased rapidly in all the food substrates, showing the maximum in kitchen scrap (T2), followed by cow dung (T1) and rice straw (T3). Maximum weight gain of earthworm was recorded in T2 with 415.66 mg compared to the initial weight of 192.5±38.15. In T1 and T3, earthworm biomass increased gradually for about 42 days, after which it started to decline. The average biomass of *P. excavatus* was higher in T2 (285.96±63.16), followed by T1 (222.86±39.86) and T3 (205.94±28.5), and statistical analysis shows that earthworm biomass in different treatments varies significantly ($F_{(2,37)}$=10.27, p<0.05) depending on the food substrate used. Key biological

parameters such as clitellum development and initiations of cocoon production were also significantly different. Number of cocoon productions were 0.26±0.01, 0.23±0.08, and 0.2±0.07 in T1, T2, and T3.

On the assessment of phosphate solubilizing bacteria (PSB) associations with *P. excavatus*, three bacteria were found to be positive for phosphate solubilisation. The isolated bacteria were also positive for biochemical tests such as citrate utilization and indole production. At the same time, the negative test resulted for nitrate reductions and motility tests. The bacteria's phosphate solubilization index (PSI) (C1, C3, C4, and C5) varies depending on the isolates. The PSI was 16.8±1.7mm, 11.2±2.1mm, 14.0±2.7mm, and 12.9±1.8mm. Molecular characterization through 16s rDNA sequencing revealed that isolates positive for phosphate solubilisation (C1, C3, C4, and C5) were *Pseudomonas alcaliphila*OQ927064, *Citrobacter freundii* OQ927066, *Achromobacter* sp. OQ875851, and *Citrobacter farmer*, OQ927062, with sequence percentage identity of 99.8%, 98.85%, 99.73%, and 99.53%.

High reproduction and survival rates were observed in all substrates under a limited food source. This indicates that *P. excavatus* feeds on a wide range of food and has a good range of adaptations for their survival. The positive attributes of *P. excavatus* indicate their suitability for vermiculture and use in vermicomposting. The presence of beneficial microbes further complemented the ideal characteristics of *P. excavatus* for vermicomposting and nutrient enhancement.

A pot experiment for plant growth enhancement using earthworm *P. excavatus* and *E. fetida* was performed for two commonly used plants, *Capsicum chinense* (locally known as Naga king chili) and *Zea mays* (maize-Makai). Also, the effect of earthworms

on soil nutrients such as Nitrogen (N), Phosphorus (P), and Potassium (K) was analyzed. For *C. chinense* average growth rate (mm/day) was 8.3±2 and 7.5±3.5 and 5.7±1.4 in the presence of *P. excavatus, E. fetida*, and control. In earthworms inoculated pot, number of leaves and leaf biomass was significantly higher (p<0.05). Similarly, the stem length and biomass of *C. chinense* were substantially higher in the presence of earthworms. Along with the better morphological features in the presence of earthworms, the total fruit harvest was maximum in *P. excavatus*, followed by control and *E. fetida* with 573.27g, 266.8g 112.99g, respectively. Infections in the roots of *C. chinense* were observed in *E. fetida*-treated pot, affecting the fruit yield. For *Z. mays*, *the* average stem growth rate (mm/day) was highest in *E. fetida* soil (13.6±8.5), followed by *P. excavatus* (13.0±5.3) and control (12.7±2.5). Also, the number of leaves, leaves biomass, stem length, stem biomass, root length, and root biomass were substantially higher in *P. excavatus* and *E. fetida* inoculated pot compared to the control, indicating the positive effects of earthworm. The highest number of kernels per corn was observed in *P. excavatus* (333.5±13.5), followed by *E. fetida* (261.5±16.5) and control (235±22), showing 41.91% and 11.27% increase over control. Similarly, a significant difference ($F_{(2, 9)}$=7.92, p<0.05) in average kernel weight was observed with the maximum in *P. excavatus* (104.6±14.9) followed by *E. fetida* (66.68±6.78) worked soil exhibiting 95.05% and 24.34% increase over control.

A higher amount of macronutrients in the presence of earthworms supported better plant growth. Substantial increase in the concentration of Av. P and Av. K was observed both in *P. excavatus* and *E. fetida.* Initially, soil pH was acidic, and pH increased marginally in both the plant-grown soil and the presence of earthworms. In *C. chinense*-grown soil, in the presence of *P. excavatus, E. fetida*, and control, the final pH was

increased by 9.6%, 8.5%, and 9.18%, respectively. While in *Z. mays*-grown soil, soil pH was increased by 3.30%, 11.73%, and 6.12% in *P. excavatus and E. fetida* soil and control, respectively. A substantial amount of increase in Organic carbon (OC) was observed. In the presence of *E. fetida*, *P. excavatus* and also in control, 25.76%, 23.47%, and 16.24% increases over the initial concentration were observed in *C. chinense*-grown soil. Similarly, in *Z. mays*-grown soil, the OC was increased by 30.48% and 27.36% in the presence of *E. fetida* and *P. excavatus.*

Contrary to other parameters, TN showed an uneven increase and decrease. In *C. chinense*-grown soil, TN was lower in *P. excavatus* (0.51±0.04) and *E. fetida* (0.46±0.03) compared to the control (0.6±0.04).While in *Z. mays*-grown soil, TN was lowest in control, *P. excavatus* and *E. fetida* with 29.72%, 23.42%, and 19.48% reduction over control. Av. P and Av. K concentration in the final soil samples was substantially higher compared to the control. In addition to the increased soil nutrients and other properties, plants growth enhancement could be attributed to secretions of growth-promoting substances such as indole acidic acid (IAA), gibberellic acid (GA), ammonia, aminocyclopropane-1-carboxylic acid (ACC) deaminase activity, and phosphate solubilizing activities by the earthworm associated bacteria. The study demonstrates that with the inoculation of earthworms, pot culture of seasonal vegetables could be done organically without adding chemical fertilizer. The study concluded that *P. excavatus* and *E. fetida* are promising epigeic earthworms that are essential in soil nutrient enhancement and plant productivity. With increasing practices of kitchen gardens, especially in urban areas with limited space for plant growth, miniature plants such as *C. chinense* can be grown as pure organic by inoculating earthworms as mediators for soil nutrient requirements.

Compared to commercially used epigeic earthworm *E. fetida*, potential applications of *P. excavatus* in vermicomposting, nutrient stabilization, and heavy metal concentration reductions were assessed. The experiment was conducted in a 20 × 20 × 16 cm container. The results show that the final weight of the vermibed bed differs depending on the substrate used and earthworm species. In *E. fetida* and *P. excavatus* inoculated pot, substrate degradation was significantly higher (p<0.05) with 64.78% and 57.53%. Macronutrient stabilization was observed in the form of reduced OC, TN, and increased Av. P and Av. K. Av. P was significantly (p<0.05) increased to 59±19.27 and 61.2±17.5 in *P. excavatus* and *E. fetida* over initial concentration—similarly, the final concentration of Av. K was increased to 270.2±29.32 and 276.0417±27.33 in *P. excavatus* and *E. fetida.* C:N is one of the critical parameters for assessing nutrient stabilization. Compared to an initial ratio of 15.37±1.79 to 21.68±0.7 depending on the substrate used, the percentage of C:N reduction observed in *P. excavatus* and *E. fetida* were17.04% to 27.38%, and 16.96% to 34.44%.

Mitigation of heavy metal concentration in different substrates by the presence of earthworms was also assessed. From an initial concentration of 4.74 to 5.65 mg/kg, Cu concentration in *P. excavatus* and *E. fetida* was reduced to 1.68±0.2, similarly in *E. fetida* inoculated setup, Cu concentration was reduced to 1.68±0.24 with a mean reduction percentage of 67.51±4.32% and 67.52±5.98% which is significantly higher compared to control. After vermicomposting, other metals such as Fe, Mn, and Zn also reduced significantly (p<0.05) compared to their initial concentration. Differences in the reduction percentage of heavy metals could be attributed to the differences in the bioaccumulation potential of earthworms and microbiological community variations in the substrates.

Both *E. fetida* and *P. excavatus* exhibited significant substrate degradation, with macronutrient stabilization observed in the form of reduced organic carbon (OC) and total nitrogen (TN), as well as increased available phosphorus (Av. P) and available potassium (Av. K). The C:N ratio, a critical parameter for nutrient stabilization, was also improved, falling within acceptable ranges for vermicompost applications in agronomy. Additionally, the presence of earthworms contributed in mitigating heavy metal concentrations in the substrates. Copper (Cu) concentrations were significantly reduced, and other metals such as iron (Fe), manganese (Mn), and zinc (Zn) also showed significant reductions compared to their initial concentrations. The variations in heavy metal reduction could be attributed to the different bioaccumulation potentials of earthworms and variations in the microbiological communities within the substrates. These findings suggest the potential of *P. excavatus* and *E. fetida* to reduce heavy metal toxicity and remediate polluted landscapes. Notably, epigeic earthworms like *E. fetida* and *P. excavatus* have demonstrated efficient bioaccumulation capabilities for heavy metals compared to other ecological categories of earthworms. Overall, this study highlights the valuable contributions of earthworms in vermicomposting processes, nutrient stabilization, and the remediation of heavy metal pollution, paving the way for their practical applications in sustainable waste management and environmental restoration.

Earthworms play a crucial role as significant bioindicators of environmental well-being. By observing their growth and reproduction, valuable information about soil pollution and the overall ecosystem status can be obtained. Keeping track of their population dynamics can aid in the detection of potential environmental concerns and support conservation endeavors. In the context of sustainable waste management and

organic farming, earthworms have shown promise in vermicomposting processes. Studies on different earthworm species, such as *P. excavatus* and *E. fetida*, reveal their potential to enhance soil nutrient mineralization and improve plant biomass productivity. Utilizing earthworms as mediators for soil nutrient requirements could lead to increased interest in eco-friendly practices like vermitechnology, promoting organic waste conversion and nutrient enrichment. Moreover, ongoing research on earthworm interactions with various plant species and their specific effects on plant growth can provide valuable insights for agricultural practices and ecosystem management. Understanding the mechanisms behind earthworms' positive impact on plant growth can lead to more efficient and sustainable agricultural practices.

Overall, the future holds numerous opportunities for earthworm research to contribute to various fields, including biodiversity conservation, waste management, soil remediation, and sustainable agriculture. The integration of earthworms into environmental management and agricultural practices could significantly enhance ecological sustainability and promote a healthier and more resilient environment.

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List of Publications

Jing, L., Kiewhuo, P., Ao, B., & Kakati, L. N. (2023). Nutrient stabilization and heavy metal reduction in organic wastes using Eisenia fetida (Savigny) and Perionyx excavatus (Perrier). *Environment, Development and Sustainability*, 1-20. https://doi.org/10.1007/s10668-023-03088-1

Jing, L., Mozhui, L., Kakati, L. N., & Thyug, L. (2022). Earthworm community structure and population dynamics at Minkong forest of Mokokchung, Nagaland. *Journal of Environmental Biology*, *43*, 810-817.

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Kiewhuo, P., Mozhui, L., Kakati, L. N., **Lirikum** & Meyer-Rochow, V. B. (2022). Traditional rearing techniques of the edible Asian giant hornet (*Vespa mandarinia* Smith) and its socio-economic perspective in Nagaland, India. *Journal of Insects as Food and Feed*, *8*(3), 325-335.

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Conferences/Seminar/Workshops presented/attended

Oral presentation in NMHS-sponsored National Conference on Reviving Traditional Knowledge for Biodiversity Conservation (RTKBC-23). Organized by Department of Zoology, Nagaland University, Lumami-798627 on 31st March 2023.

Oral presentation in International Conference on "Impacts & Consequences of Environmental Degradation on Animal Health and Human Wellbeing, organized by Abhayapuri College in association with Department of Zoology, Guwahati University and Aaranyak, Assam on 2nd to 4th September, 2021.

Oral presentation in International E-Conference on Sustainable and Futuristic Materials (SFM-2021) Organized by International Research Centre and Department of Chemistry, Kalasalingam Academy of Research and Education, Krishnankoil, Department of Chemistry, J. M. Patel Arts, Commerce & Science College, Bhandara, and Department of Chemistry, Kamla Nehru Mahavidyalaya, Nagpur on 29-30th November, 2021.

Oral presentation "in International Conference on Multidisciplinary aspects of Environment and Sustainable Development organized by Department of Zoology at Raj Rishi Govt. Autonomous College from 15th – 18th Dec. 2021.

Oral presentation "In voice-virtual 3rd International Conference on Environmental, Agricultural, Chemical, and Biological Science ICEACBS 2022 Unitedx Nations SDGS Jan 22 to 26 2022.

Oral presentation "Two day National Seminar on Biodiversity for Human Welfare: Current and Future Trends India (BHWCFTI-2019)- Sponsored by National Biodiversity authority and India Academic Researchers Associations on 7-8th Feb 2019 organised by Department of Zoology St. Joseph University, Dimapur, Nagaland.

Attended workshop on Mapping the Future with GIS, organised by division of Geoinformatics, Faulty of Natural Science, JSS academy of Higher Education and Research Mysuru on 19th Nov. 2020.

Attended Workshop on Digital Modules of Bioinformatics from 25-15th Nov 2021 organized by Decode life.

Attended E-workshop on Data- Analysis with "R" programming on 25th to 26th June 2022-Certificate ID : JIWTU9EJYFNNDI-Organised by COMMACAD.

Attended One Day Workshop on Importance of IPR in Academic Institutions" organized by IPR Cell, Nagaland University on 29th May, 2019.

Attended ICSSR Sponsored National Seminar on Writing Quality Research Papers: Preparation, Presentation, and Production on 19th February 2019.

Attended DBT Sponsored National Workshop on Newer Frontiers in Bioinformatics and Research Methodology organized by Bioinformatics Infrastructure Facility (BIF) Centre, Nagaland University, Lumami on 13-19th Nov. 2019.

Academic Awards/achievements

1. CSIR-UGC NET-JRF (2019) (Life Science)

2. Best oral Presentation (3rd Prize) in Two day National Seminar on Biodiversity for Human Welfare: Current and Future Trends India (BHWCFTI-2019)- Sponsored by National Biodiversity authority and India Academic Researchers Associations on 7-8th Feb 2019 organised by Department of Zoology St. Joseph University, Dimapur, Nagaland.

3. Young Scientist Best Oral Presentation in voice-virtual 3rd International Conference on Environmental, Agricultural, Chemical, and Biological Science ICEACBS 2022, United Nations SDGS, 22-26th Jan. 2022.