

**BIOREMEDIATION OF PESTICIDE CONTAMINATED AGRICULTURAL SOIL  
BY VERMITECHNOLOGY AND TOXICITY ASSESSMENT BY *ALLIUM CEPA***

A  
Thesis

Submitted to



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**DOCTOR OF PHILOSOPHY (Ph.D)**  
in  
**Zoology**

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**PUNJAB**  
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### **DECLARATION**

I hereby declare that the work for the thesis entitled, “**Bioremediation of Pesticide Contaminated Agricultural soil by Vermitechnology and toxicity assessment by *Allium cepa***” submitted to **School of Bioengineering and Biosciences**, Lovely Professional University, Phagwara for the award of degree of Doctor of Philosophy in the subject of Zoology is entirely my own work and has not been submitted in part or full for any other degree/diploma at this or any other University/Institution. All the ideas and references have been duly acknowledged.

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### CERTIFICATE

It is hereby certified that the work for the thesis entitled “**Bioremediation of Pesticide Contaminated Agricultural soil by Vermitechnology and toxicity assessment by *Allium cepa***” submitted to **School of Bioengineering and Biosciences**, Lovely Professional University, Phagwara, for the award of degree of Doctor of Philosophy was carried out in the Department of Zoology by Ms. Shivika Datta under our guidance and supervision. To the best of our knowledge, the present work is the result of her original investigation and study. No part of this thesis has ever been submitted for any other degree/ diploma at this or any other University/Institution. The thesis is fit to be considered for the award of degree of Ph.D.

## ABSTRACT

Biodiversity plays an invincible role in maintenance of ecological equilibrium and forms the basis of cropland system. Earthworms, the ecological engineers, significantly influence the dynamics of soil and organic matter and play an important role in retention and cycling of nutrients. Intensification of agriculture by indiscriminate use of pesticide has caused a steep fall in the number of soil biodiversity and soil infertility. Pesticides easily complex with metal ions. Most pesticides have chelating sites where free metal ions easily bind and form stable complexes. This leads to a decrease in free metal ions that can be readily used by plants. Vermicomposting is being adopted as an innovative green technology for conversion of organic wastes into vermicompost. Earthworms help to release nutrients rapidly back to nature in plant available forms that were locked. Also it is a low cost technology and more efficient in comparison to other conventional methods.

In this study, the effect of pesticides, atrazine and acephate on two different species of earthworms was taken into account, one exotic *Eisenia fetida* and another indigenous species *Metaphire posthuma*. The earthworm species were utilized to transform and degrade the pesticides atrazine and acephate in the soil medium by the process of vermiremediation. The genotoxic effect of pesticides and vermicompost on *Allium cepa* was also studied *in vivo* and *in vitro*. The results suggest the toxicity of acephate over atrazine. Also the sensitivity of *E. fetida* towards both the pesticides was found to be more than *M. posthuma* and thus *M. posthuma* was more resistant and adaptive to pesticides. However, the physico-chemical analysis revealed better activity of *E. fetida* than *M. posthuma*. More nutrient content was observed after vermicomposting by *E. fetida*. The degradation studies also revealed that the activity of *E. fetida* was most efficient in degradation of pesticide over vermicomposting by *M. posthuma* and aerobic composting. The *Allium cepa* studies also reveal that pesticides cause genotoxic effects and that vermicompost on the other hand is non-cytotoxic in nature and helps in efficient cell growth. The result indicates that addition of vermicompost in agriculture field acts as soil ameliorator and plays an important role in promotion of cell division and proliferation, hence good for the plant health and crop productivity and also the activity of earthworms in agricultural soil leads possibly to efficient degradation of pesticides in the soil.

**Keywords:** Acephate, Atrazine, Earthworm, Toxicity, Vermiremediation

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# **1. INTRODUCTION**

## 1. Introduction

Soil biodiversity plays a critical role in increasing the sustainability of agriculture. Soil organisms include a tremendous diversity and facilitate maximum exploitation of the resources available in the different habitats at various levels of resolution (Ferris and Tuomisto 2015). The Green Revolution that dates back to 1960s joined hands with the increasing population and thus takes away the credit to fulfil the enormous demand of food supply but totally ignores the way it impedes with the services of ecosystem. During this revolution, an extensive quantity of chemical pesticides, insecticides and fertilizers were used to increase crop yield. During initial periods of application, it resulted in good harvest and productivity but during the last four decades, the productivity of the soil is getting reduced (Gupta et al. 2014a; Vanita et al. 2014). Soil organisms also provide wider collective benefits such as maintenance of biodiversity above and below the ground, increasing permeation of water for prevention of pollution in water courses, organic waste clearance, degradation of pollutants, and a colossal source of genetic and chemical diversity with many other prospective applications (Altieri 1999). Microbial biodiversity include plant growth promoting rhizobacteria, N<sub>2</sub>-fixing cyanobacteria, plant disease suppressive bacteria, fungi and soil toxicant-degrading microbes that enrich the soil by improving the soil quality, promoting soil health, growth, quality, and productivity of crops (Singh et al. 2011). Although organic farming is gaining importance, the application of synthetic pesticides is still practiced in agriculture and for sanitation purposes; pesticide residual prevalence in environmental matrices is always an environmental concern (Srimurali et al. 2015). These pesticides do not degrade naturally unlike other organic contaminants and are retained in the soil for longer period. Some pesticides leach into the ground but usually run with surface water and enter into the growing food crops as well as in larger water bodies (Janos et al. 2010). Decomposition of pesticides caused either through higher temperature conditions or accidental fire cases lead to numerous by-products that may cause disastrous consequences for the environment and the population (Laane et al. 2012). Such agricultural practices can hamper the ecosystem services rendered by biodiversity. The use of fertilizers and pesticides can increase nutrient level in soil but can also increase toxins in ground and surface water leading to health and water purification costs and affect aquatic fauna and other biodiversity in soil.

Most of the metal in the transition series of the periodic table form complexes with pesticides. Many studies have been reported till date depicting the strong adsorption of pesticides with metals ions (Kumar et al. 2015b). An increase in Cu concentration increases



complexation of the pesticides. Pesticide adsorption is enhanced by three ways, firstly Copper is coordinated with pesticide, Secondly, pesticide copper complexes have higher ability to be adsorbed on soil rather than free pesticide and finally copper acting as a bridge between the pesticides and soil. Low pH also enhances metal chelation with pesticides (Rojas et al. 2015). There are also other studies that report the adsorption of other metals ions such as Zinc. Low pH influences the zinc adsorption on sites of goethite through pesticides (Kaur et al. 2017).

Wide range of terrestrial and aquatic habitats is also contaminated by anthropogenic activities. The ecological equilibrium is skewed due to industrialization and urbanization, increasing population pressure and the problem is compounded by the limited stock of natural resources. The magnitude and nature of the problem is dynamic, bringing new challenges and creating a constant lacuna in the need for developing appropriate and effective technologies.

Earthworm is an important soil organism in development and maintenance of nutrient value of soil by converting biodegradable material and organic waste into nutrient rich vermicast (Jansirani et al. 2012). They are also known as ecological engineers (Jones et al. 1994). Importance of earthworm in the ecosystem was documented very early by Aristotle, the Greek philosopher who called them 'intestines of the earth' and then in 1881, Charles Darwin highlighted their role in breakdown of dead plant as well as animal matter. Earthworms can consume a wide range of unstable organic matter such as animal waste, industrial waste, sewage sludge, etc. (Saranraj and Stella 2012; Wu et al. 2014; Lim et al. 2016). The burrowing activity of earthworms enhances decomposition, formation of humus, development of soil structure, and cycling of nutrients. The product obtained by the modulation of organic waste in the earthworm gut is quite different from its parent waste material and is also known as black gold or vermicast (Patangray 2014; Lim et al. 2015a). Vermicompost increases the water holding capacity, porosity, and softness of soil thus requiring less tillage and irrigation. It is also rich in microbial diversity, nutrients, plant growth regulators (PGRs) and has properties of inhibiting pathogenic microbes (Gupta et al. 2014a; Mosa et al. 2015). Addition of earthworms and vermicompost to soil also maintains an optimum level of soil media in terms of metal concentration, soil porosity and aeration, pH, and electrical conductivity (Weber et al. 2007; Pathma and Sakthivel 2012)

Vermicomposting is a low cost technology, environmental friendly process to treat organic waste (Lazcano and Dominguez 2011). It is also rich in microbial diversity, nutrients, plant growth regulators (PGRs) and has properties of inhibiting pathogenic microbes (Gupta et al. 2014a; Mosa et al. 2015). In a short period of time, vermicomposting actually synergizes the microbial degradation with earthworm activity for reducing, reusing and

recycling waste materials. Although microorganisms mortify the organic matter biochemically, earthworms are the decisive drivers of the process as they aerate condition and splinter the substrate, thus radically altering the microbial activity. Earthworms scrap the organic matter acting as mechanical blenders and amend its physical and chemical status by progressively reducing the ratio of C/N and increasing the surface area exposed to microorganisms, thus making it much more encouraging for microbial activity and further decomposition (Dominguez et al. 2010). Earthworms have a significant role in carbon regulation in the tropical forests, hence their diversity in natural ecosystems of the tropical areas have received renewed attention in recent years (Fragoso et al. 1997; Jimenez and Decaens 2000; Hendrix et al. 2006; Feijoo et al. 2011).

The present study has embarked on two major aspects: (i) the effects of pesticides on earthworms and (ii) the use of vermicompost to increase soil quality and its role in sustainable agriculture. Many authors report the use of earthworms and the process of vermicomposting for the bioremediation of different types of wastes to produce vermicompost (organic gold) (Kaur et al. 2010; Bhat et al. 2014; Singh et al. 2014). This compost can be used to increase the quality of soil and crops. But, the enormous use of pesticides has severely affected the invertebrate biodiversity including earthworms. Earthworms help in a number of tasks that support many ecosystem services that favour agrosystem sustainability but exhaustive practices such as the use of pesticides affect earthworms largely (Altieri 1999). Taking into consideration, the impact of agricultural practices on earthworm population in cropping system, broader outlook should focus toward incorporating and undertaking such cropping systems and practices that favour their population and functions which are proven to be important for soil fertility and plant production. The pesticides affect directly the soil structure by its supplementation and the soil structure indirectly by causing biodiversity loss. Thus on one hand, the toxicity of pesticides on earthworms forms a critical aspect; on the other hand role of earthworms to alter and better the physiochemical properties of soil affected with pesticides is equally pivotal (Datta et al. 2016).

**2. REVIEW  
OF  
LITERATURE**

## **2. Review of Literature**

In the present review an attempt has been made to document the work done in past on the effect of pesticides on earthworms; role of biodiversity in sustainable development; mechanism of action of vermicompost on plants; role of earthworms in bioremediation of bio-solid wastes and for enhancement of bioavailability of the nutrients present in them. Along with this the literature on use of vermicompost as a biofilter has also been compiled.

### **2.1 Relationship between soil and soil biodiversity**

The soil biota forms an obligatory constituent of terrestrial ecosystems and is known as the “biological engine of the earth” (Ritz et al. 2004). Soil organisms such as earthworm play a significant role in ecosystem and help in the detoxification of noxious elements (Altieri 1999). Other functions include nutrient supply for plant growth, water regulation, carbon sequestration, nutrient cycling, support for biodiversity, etc. (Turbe et al. 2010). Microbial biodiversity include plant growth promoting rhizobacteria, N<sub>2</sub>-fixing cyanobacteria, plant disease suppressive bacteria and fungi, and soil toxicant-degrading microbes that enrich the soil by improving the soil quality, promoting soil health, growth, quality, and productivity of crops (Singh et al. 2011). However, environmental pollution such as acid rain may decrease the soil microbial activity and change the metabolic capability of the soil microbial community (Wang et al. 2014). The soil biota plays a key role in a number of environmental processes that are important in sustenance of terrestrial life and is fundamental within agricultural systems (Brussaard et al. 2007). Escalating functional biodiversity in agricultural ecosystems is a foremost ecological approach to fetch sustainability to production (Altieri 1999). Various reports from scientific literature state that for benefit of all agricultural systems, the protection and enrichment of biodiversity and biomass of soil biota is imperative. The functions of soil organisms for the benefit of agricultural productivity are reviewed hereafter.

### **2.2 Functions of Soil-biodiversity**

#### **2.2.1 Organic matter decomposition and soil aggregation**

Organic matter decomposition is a catabolic process of recycling by the earthworm and can be considered as important as the fabrication of new organic material. Earthworms accounts for the physical fragmentation, chemical degradation, transformation, and translocation of organic matter (Barrios 2007). Soil macrofauna, especially earthworms, significantly influence the soil dynamics, organic matter, retention and cycling of nutrients.

These factors are determined by their density and behavior of earthworms (Thiele-Bruhn et al. 2012; Baumann et al. 2013). Endogeic earthworms shape bacterial functional communities and affect organic matter mineralization in a tropical soil (Bernard et al. 2011).

#### 2.2.2 Availability of nutrients

The availability of nutrients plays a very critical role in chemical fertility of the soil. It was reported that an appropriate management of the organic materials by earthworms in the soil can increase the overall yield and profitability of the crop (Senapati et al. 1999). The evidences show that earthworms also accelerate the process of weathering for soil structure maintenance (Carpenter et al. 2007). As weathering occurs, essential elements are released which aids the growth within the soil biota. Fungi (saprotrophic and mutualistic) have been thought as an important aspect of weathering as they increase the rate of mineralization (Hoffland et al. 2004). The degradation of plant and animal matter for the release and binding of nutrients and trace elements is considered to be one of the most important functions of soil biodiversity (Schinner et al. 2012).

#### 2.2.3 Fixation of nitrogen in usable forms

A major part of organisms including plants are unable to utilize gaseous nitrogen (Bernhard 2010). Thus, the task of fixing nitrogen is performed by microorganisms, by free living ones such as cyanobacteria and other genera of bacteria and actinomycetes or by symbiotic bacteria which form root nodules in legumes (Gordon and Wheeler 2012; Paul 2014; Llorens-Marès et al. 2015; Gresshoff et al. 2015).

#### 2.2.4 Breakdown of toxic products

Soil organisms are also known for converting toxic substances into less toxic products. Akhtar et al. (2013) reported that bioremediation by microbes could be an efficient way to reduce the level of heavy metal contaminants such as arsenic and lead from soil. Microbes and other biological systems are used to degrade environmental hazards (Vidali 2001). Vermicomposting also leads to chemical transformations by altering the physico-chemical parameters of the medium. The process of vermicomposting can be taken up at commercial level using municipal and other sources of industrial wastes and the product be marketed as good-quality organic manure (Lim et al. 2011; 2014; 2015a; Shak et al. 2014; Bhat et al. 2013). Various species of earthworms contribute to removal of contaminants such as polychlorinated biphenyls (PCBs) (Luepromchai et al. 2002), polycyclic aromatic hydrocarbons (PAHs) (Sun et al. 2011; Hernández-Castellanos et al. 2013), pesticides (Shan

et al. 2011), herbicides (Kelsey et al. 2011; Tejada et al. 2011), and crude oil (Fernández et al. 2011) from soils.

#### 2.2.5 Soil and water relationships

The structure of soil is mediated by organic and inorganic substances that constitute the microstructures formed by activities of living organisms which involves the burrowing action of macrofauna and mesofauna, growth of root, etc. or by structures such as roots and fungal hyphae (Bronick and Lal 2005). The burrowing activities of earthworms influence water and nutrient dynamics (Mando and Miedema 1997). The termites helps in promotion of water infiltration in crusted soils which helps in rehabilitation of soil and regeneration of vegetation cover (Pardeshi and Prusty 2010; Dawes 2010). The earthworms and termites are also known to reduce soil erosion by formation of burrows and production of water stable aggregates (earthworm cast and termite nests) which limits the runoff from surface and protect soil from erosion (Evans et al. 2011; Jouquet et al. 2012). Earthworms help in raising the soil pH by consuming the litter and its incorporation in the soil humus layer (Ampoorter et al. 2011). The roots, bacteria, hyphae, and fauna such as earthworms influence mucilages that are involved in lining of biopores (Brussaard and Kooistra 2013). These create holes, prevent run off and aid infiltration, aeration, and proper tillage, improving the physical characteristics of the soil.

#### 2.2.6 Pest and disease control

Pest control can be achieved by utilising natural predators, which are living organisms that directly kill insects (pest) or weaken them reducing the damage potential of significant pests (Pedigo and Rice 2014). By restoration of beneficial organisms that can attack, fend off, or antagonize pathogens, a vigorous soil structure could be maintained (Wafaa and Haggag 2002). Soil suppressed from diseases can be obtained naturally by utilization of biodiversity. Plants grown in such soil type tend to be healthier than those growing in soil having less diversity. Thus, beneficial organisms can be directly added to the soil environment and be made favorable for them through correct agronomic management. However the unmarked use of pesticides causes a decline in soilbiota and thus affects the natural services of the soil biodiversity for ecosystem maintenance.

### 2.3 Pesticides: Brief history and status

The use of synthetic pesticides came into being during World War II to get rid from hunger and diseases (Zadoks and Waibel 2000). The credit for this goes to the introduction of “Green Revolution”. The marked tools for green revolution were the synthetic pesticides like organochlorines, organophosphates, triazines and carbamates. At that time pesticide was considered a major boon for the society as it increased the food production many folds and also saved the population from dreaded diseases (Carvalho 2017). Today a number of consequences particularly environmental and health issues have aroused public concerns because of the pesticides that had been sprayed since last six decades (EEA 2013). The problem of the overuse of pesticides in relation to the health effects and environment is a major concern. Pesticides are targeting the pests, but about 98% of the pesticides end up reaching the water, air and soil in place of their target species resulting in undesired effects (Hurley et al. 1998). Pesticides reach the people indirectly after they are washed into streams, lakes, rivers etc. The development of pesticide resistance was observed in important pests due to excessive and indiscriminate use of pesticides (Jayaraj 1989). The pathways of degradation of pesticides and their kinetics depend on abiotic and biotic factors (Laane et al. 2012) which are specific to a particular pesticide. Pesticides and herbicides sprayed in agricultural fields may affect the non-target organisms like earthworms and damage the ecosystem. The effect of pesticidal chemicals may also affect adversely the soil microorganisms (Wasim et al. 2009) and may affect soil fertility (Andreu and Pico 2004). The fate and behavior of these chemicals in soil ecosystem is significant in view of the fact that they are degraded by various factors and have the probability to be in the soil, water etc. (Gavrilescu 2005). The use of modern agricultural techniques has caused a steep fall in the number of biodiversity associated with cropland ecosystem (Thrupp 2002). In an indirect way the aggravated use of pesticides has affected the quality of soil and undoubtedly thence the production of the crops. The removal of toxic metabolites is now a day’s the primary concern to the environmentalist.

India ranks second in Asia after China and twelfth globally in the manufacture of pesticides (Agnihotri 2000; Mathur 2010). India is the third largest consumer of pesticides in the world and the highest among the South Asian countries. Punjab accounts for the highest usage of these pesticides. Though the state accounts for 1.5% landmass of the country, it consumes about 17% of pesticides used in India. The per hectare pesticide use is highest in Punjab (923 g/ha) as compared to other agriculturally advanced states like Haryana, Andhra Pradesh, Tamil Nadu, Karnataka and Gujrat (Agnihotri 2000; Tiwana et al. 2009). According to Kheti Virasat (2007), Malwa’s cotton belt account for 0.5% of total geographical area of Punjab but it consumes 75% of the total pesticides used in Punjab (Misra 2007).

## 2.4 Effect of pesticides on earthworms

Due to the exposure of pesticides to the earthworms, they are less able to perform their valuable and critical functions in the soil ecosystem (Rathore and Nollet 2012). The pesticide may either affect mortality rates of earthworms by directly distressing them or in an indirect way by affecting reproduction, neurological functions, or by causing behavioral changes (Table 2.1). Possible effect of pesticides and insecticides on earthworms in soil also depends upon earthworm species, type of contaminants, its concentration, soil characteristics, etc. (Rodriguez-Campos et al. 2014). Epigeic and anecic species are affected mostly due to their presence on the upper surface of soil while endogeic species are less affected due to surface application of pesticides (Singh et al. 2016a). The Organization for Economic Cooperation and Development (OECD) proposed *Eisenia fetida* as a reference earthworm for toxicity testing because it can be easily cultivated in a laboratory, mature in few weeks, and has a high reproduction rate (OECD 2004). Oluah et al. (2016) studied the toxicity and the histopathological effects of atrazine on earthworms, *Nsukkadrilus mbae*, and reported significant adverse effects. Many enzymatic activities have been well considered as biomarkers of environmental pollution. These enzymes of living organisms possess antioxidant activities and can defend cells against adverse effects of reactive oxygen species (ROS) (Rahman 2007). Accumulation of ROS such as H<sub>2</sub>O<sub>2</sub> and superoxide radical causes damage to components of cells such as DNA, proteins, and lipids (Lopez et al. 2006). Atrazine forms ROS, which cause single and double-strand breaks in DNA and thus is genotoxic (Song et al. 2009). Alkaline single-cell gel electrophoresis (SCGE, comet assay), developed by Singh et al. (1988), is a widely used technique to detect DNA damage due to environmental stress. Zhou et al. (2011) reported a decrease in growth and reproduction in earthworm *E. fetida andrei* when exposed to concentration of 5 mg/kg of mixture of cypermethrin and chlorpyrifos. Glyphosate and 2,4-D cause severe effects on the development and reproduction of *E. fetida* as reported by Correia and Moreira (2010). Inhibition in superoxide dismutase (SOD) levels, induction of oxidative stress, and olive tail moments of single-cell gel electrophoresis of coelomocytes indicate DNA damage. Sorour and Larink (2001) reported that benomyl caused noticeable effect in *E. fetida* on the development and appearance of many spermatozoa; spermatids showing various degrees of hypodevelopment that included some without acrosomes and some with abnormal acrosome development. According to Wang et al. (2015), neo-nicotinoid insecticides such as imidacloprid, acetamiprid, nitenpyram, clothianidin, and thiacloprid are toxic to earthworms and can significantly inhibit fecundity and cellulase activity in *E. fetida* and also damage the



epidermal and midgut cells of earthworm. Benomyl is also known for its teratogenic properties, the most common being the disruption of microtubules and thus it is also known as a microtubule poison (Hess and Nakai 2000). In case of carbaryl, cholinesterase (ChE) inhibition was observed even at the lowest dose and the shortest duration of exposure to *E. fetida*. In addition, the biotransformation enzyme activities are also inhibited by this pesticide, but to a lesser extent. The biochemical responses investigated were sensitive, with evident effects observed even at the lowest concentration (Riberaa et al. 2001). Shi-ping et al. (2007) reported that chlorpyrifos has an adverse impact on growth and reproduction in earthworms, but this is largely dependent upon pesticide concentration and exposure period. Lydy and Linck (2003) reported the exposure of *E. fetida* to chlorpyrifos in combination with atrazine or cyanazine, in which the resulting toxicity was greater than the additive. Leena et al. (2012) reported a profound change in the testes of *E. kinneari* after 20 day exposure of dimethoate, suggesting a disturbance in the cellular enzyme system which in turn interferes with the process of normal gametogenesis. Gobi and Gunasekaran (2010) concluded that intoxication of herbicide butachlor consumes the reserve energy from the chloragogen tissue which leads to reduced production of biomass and cocoon production. Capowiez et al. (2010) observed that pesticide application causes variation in cast production and nutrient content with varying concentration of pesticides. According to Suthar (2014), the pesticide-exposed worms produced less cocoons than the control, but in *Lampito mauritii*, an unusual reproduction (hormesis) was recorded. Jordaan et al. (2012) reported the effect of pesticide azinphos-methyl on maturation, growth, reproduction, burrowing activity, and ChE inhibition in *E. andrei* and indicated that shorter spraying intervals are expected to be further detrimental to these non-target organisms than longer exposure intervals. Further, he concluded that higher sublethal pesticide concentrations, coupled with shorter exposure intervals, can have the same effect over time as acutely toxic concentrations. Ahmed (2013) studied the influence of four pesticides (Cyren, Ridomil, Triplen, and Mamba) on *Lumbricus terrestris* earthworm and indicated a loss of weight. Profenofos not only caused direct toxicity but also exhibited significant histological and morphological changes in the body wall of *E. fetida* (Reddy and Rao 2008). It can thus be concluded that pesticides lead to loss in biodiversity and that of beneficial organisms such as earthworms etc. leading further a loss in ecosystem services and degradation of land quality.

**Table 2.1 Effect of pesticides on different earthworm species**

S. No.	Pesticide	Earthworm Species	Response	References
1.	Atrazine	<i>Nsukkadrilus mbae</i>	Chloragogenous layer and epithelial tissue damage; prominent vacuolations and pyknotic cells.	Oluah et al. (2010)
2.	Cypermethrin and Chlorpyrifos	<i>Eisenia fetida</i>	Decrease in growth and reproduction	Zhou et al. (2011)
3.	Glyphosate and 2,4-D	<i>Eisenia fetida</i>	Severely affect development and reproduction	Correia and Moreira. (2010)
4.	Benomyl	<i>Eisenia fetida</i>	Hypodeveloped sperms; sperms with abnormal acrosome development	Sorour and Larink (2000)
5.	Imidacloprid, acetamiprid, nitenpyram, clothianidin and thiacloprid	<i>Eisenia fetida</i>	Significantly inhibit fecundity and cellulase activity and also damage the epidermal and midgut cells of earthworm.	Wang et al. (2015)
6.	Carbaryl	<i>Eisenia fetida</i>	Cholinesterase (ChE) inhibition observed even at the lowest dose	Riberaa et al. (2001)
7.	Chlorpyrifos	<i>Eisenia fetida</i>	Adverse impact on growth and reproduction in earthworms	Shi-ping et al. (2007)
8.	Chlorpyrifos, Atrazine, Cyanazine	<i>Eisenia fetida</i>	Binary mixtures of chlorpyrifos with atrazine and cyanazine demonstrated greater-than-additive toxicity	Lydy and Linck (2003)
9.	Dimethoate	<i>Eisenia kinneari</i>	A disturbance in the cellular enzyme system which led to profound changes in testes	Leena et al. (2012)
10.	Butachlor	<i>Eisenia fetida</i>	The reserve energy from the chloragogen tissue is consumed which leads to reduced production of biomass and cocoon production.	Gobi and Gunasekaran (2010)
11.	Chlorpyrifos	<i>Eisenia fetida</i>	Melting and break down of earthworm body was observed on higher concentration	(Pawar and Ahmad 2014)
12.	Azinphos-methyl	<i>Eisenia andrei</i>	Affects maturation, growth, reproduction, burrowing activity and inhibits ChE	Jordaan et al (2011)
13.	Cyren, Ridomil, Triplen and Mamba	<i>Lumbricus terrestris</i>	Coiling, swollen body, sluggish movements and a significant decrease in total sperm numbers. Cyren being most toxic; Triplen and Mamba moderately toxic and Ridomil	Ahmed (2013)

			the least	
14.	Carbendazim, dimethoate, and glyphosate	<i>Eisenia fetida</i>	independently in combination is detrimental to the growth and reproduction	Yasmin and D'Souza (2007)
15.	Profenofos	<i>Eisenia fetida</i>	Causes direct toxicity and significant histological and morphological changes in the body wall.	Reddy and Rao (2008)
16.	Chlorpyrifos and Azinphos methyl	<i>Aporrectodea caliginosa (Savigny)</i>	Cholinesterase (ChE) inhibition	Reinecke and Reinecke (2007)
17.	Endosulphan and Aldicarb	<i>Lumbricus terrestris</i>	Loss in weight, Reduction in the growth rate. Aldicarb more toxic than endosulfan	Mosleh et al. (2003)
18.	Carbaryl and Dieldrin	<i>Eisenia fetida</i>	Inhibition of growth and cocoon production	Neuhauser and Callahan (1990)
19.	Chlorpyrifos, Carbofuran and Mancozeb	<i>Perionyx excavatus</i>	Toxicity decreased in the order- carbofuran > chlorpyrifos > mancozeb	De Silva et al. (2010)
20.	Malathion	<i>Eisenia fetida</i>	Significant reduction in body weight and decreased spermatocytic viability	Navarro and Obregon (2005)
21.	Acetochlor	<i>Eisenia fetida</i>	Growth and numbers of juveniles per cocoon were affected significantly.	Xiao et al. (2006)
22.	Cypermethrin	<i>Eisenia fetida</i>	Significant reduction in cocoon production. Juveniles more sensitive than adults	Shi-ping et al. (2008)
23.	Benomyl	<i>Eisenia fetida</i>	Toxicity of benomyl was lower in tropical than temperate artificial soils No reproduction in tropical natural soil.	Rombke et al. (2007)
24.	Lindane and deltamethrin	<i>Eisenia fetida</i>	Lindane proved to be more toxic than deltamethrin. Significant effects on growth and cellulase activity.	Shi et al. (2007)
25.	Imidacloprid	<i>Aporrectodea nocturna and Allolobophora icterica</i>	No significant avoidance by earthworms but significant weight loss. Lesser and slower burrowing activity.	Capowiez and Berard (2006)
26.	Glyphosate and Chlorpyrifos	<i>Eisenia fetida andrei</i>	Glyphosate reduced cocoon production. NRRT (Neutral red retention test) and Comet assays revealed alterations at a subcellular level.	Casabe et al. (2007)

27.	Imidacloprid	<i>Lumbricus terrestris</i> and <i>A. caliginosa</i>	Decrease in body mass and cast production at higher concentrations	Dittbrenner et al. (2010)
28.	Imidacloprid	<i>Lumbricus terrestris</i> and <i>A. caliginosa</i>	Burrowing effects on <i>A. caliginosa</i> even at lower concentrations but burrowing effects for <i>L. terrestris</i> observed only at higher concentrations.	Dittbrenner et al. (2011)
29.	Azinphos-methyl	<i>Eisenia andrei</i>	Reduction in burrowing activity and inhibition in cholinesterase activity	Jordaan et al. (2012)
30.	Chlorpyrifos and Fenvalerate	<i>Eisenia fetida</i>	Cellulase and SOD (Superoxide dismutase) activity inhibited whereas CAT (Catalase) activity first increased and then decreased.	Wang et al. (2012)
31.	Fomesafen	<i>Eisenia fetida</i>	Low doses could not lead to oxidative stress and peroxidation.	Zhang et al. (2013)
32.	Carbaryl	<i>Eisenia andrei</i>	Dose-dependent inhibition of AChE activity	Gambi et al. (2007)
33.	Monocrotophos	<i>Eisenia fetida</i>	Dose-dependent inhibition of AChE activity. Co-relation between AChE activity and morphological damage.	Rao and Kavitha (2004)
34.	Butachlor, malathion and carbofuran	<i>Drawida willsi</i>	Butachlor did not alter AChE activity. Maximum AChE inhibition after 9 days of malathion and after 12 days of carbofuran exposure.	Booth and O'Halloran (2001)
35.	Imidacloprid	<i>Aporrectodea nocturna</i> and <i>A. icterica</i>	The continuity of the burrow systems made by both species was altered. Gas diffusion through the <i>A. nocturna</i> soil cores was reduced but no difference in gas diffusion in <i>A. icterica</i> soil cores.	Capowiez and Berard (2006)
36.	Imidacloprid	<i>Aporrectodea nocturna</i> and <i>Allolob ophora icterica</i>	The LC <sub>50</sub> for <i>A. nocturna</i> and <i>A. icterica</i> was between 2 and 4 mg kg <sup>-1</sup> dry soil. Also, significant decrease in weight was observed.	Capowiez et al. (2005)
37.	Mixture of Ni and Chlorpyrifos	<i>Lumbricoid</i>	Combinations of Ni and chlorpyrifos cause additive toxicity for earthworms. Worms accumulate Ni and chlorpyrifos in their tissues.	Lister et al. (2011)
38.	R-metalaxyl	<i>Eisenia fetida</i>	Enantioselective	Xu et al.

	and <i>rac</i> -metalaxyl		bioaccumulation of metalaxyl in earthworm observed with preferential accumulation of S-enantiomer.	(2011)
39.	Imidacloprid	<i>Pheretima posthuma</i>	Increase in protein content in clitellum, inhibition of some proteins in head and no change in abdomen after exposure.	Faheem and Khan (2010)
40.	Atrazine and chlorotoluron	<i>Eisenia fetida</i>	Atrazine was more toxic to earthworm than chlorotoluron. Combination showed synergistic effect. SOD activity shows increase.	Xu et al. (2006)
41.	Cypermethrin, endosulfan, carbaryl, chlorpyrifos, aldicarb, monocrotophos	<i>Perionyx excavatus</i>	Order of toxicity- Cypermethrin > endosulfan > carbaryl > chlorpyrifos > aldicarb > monocrotophos	Gupta et al. (2010)
42.	Carbofuran	<i>E. fetida</i>	Protein content and TChE (Total Cholinesterase activity) increased in low level and vice-versa. SOD reduced when carbofuran concentration increased and vice versa.	Ling (2006)
43.	Dichlorovos	<i>E. fetida</i>	Weight of earthworm decreases. Reproduction and avoidance behavior significantly affected.	Farrukh and Ali (2011)

## 2.5 Vermitechnology

The unmarked and constant use of chemical fertilizers and pesticides at large scale to increase the crop output have led to the deterioration of soil by heavy withdrawal of macronutrients (Prasad and Singh 1981), deficiency of micronutrients (Kanwar and Randhawa 1978), nutrient imbalance (Singh et al. 1989) and reduction in organic matter content (Padamja et al. 1996) and by causing a loss in soil biota. This in turns leads to reduction of plant growth and yield. The researchers have thus shifted their focus on evolving organic farming practice which could maintain higher crop output by restoring fertility of soil. The process of vermicomposting has been a breakthrough in the field of agriculture to maintain the soil and entire ecosystem free from contamination. The term “vermicompost” originated from latin word “vermes” meaning “worms” and the process of composting of organic residues using earthworms is known as vermicomposting. Earthworms

influences soil microbial community, physical and chemical properties and are popularly known as the 'farmer's friend' or 'nature's plowman'. Earthworms are voracious eaters of cow dung and organic matter and move downwards in the bed in search of food and this capability makes them efficient for soil structure maintenance (Durai 2017). According to Tapiador (1981) 100 tons of organic matter can be changed over into 30 tons of manure after worm action. Earthworms would breakdown to an extensive variety of organic matter bringing about the creation of vermicompost which is rich in plant supplements.

## 2.6 Vermitechnology - International/Indian status

The concept of vermitechnology dates back to the middle of 20<sup>th</sup> century. Holland in 1970 was the first country to setup vermicompost plant followed by England and Canada. Thereafter, it also spread in USA, Italy, Philippines, Thailand, China, Korea, Japan, Brazil, France, Australia and Israel (Edward 1988). In many countries like Australia and New Zealand, vermicomposting of food trimmings at household level is becoming popular. The process of vermiculture has been implemented from home worm bins to large scale composting of municipal wastes and biosolids. The largest vermiculture operation is done by the Hobart city council in Tasmania, which uses worms to digest around sixty-six cubic yards per week of municipal biosolids (Applehof et al. 1996). Worm composting also is becoming more popular as an educational activity in schools.

However, in India vermitechnology is still developing. Though it has been more than a decade farmers and agro based industries are culturing earthworms and many research laboratories are experimenting with this, still a lacuna exist in proper promotion of techniques for culturing of earthworms (Kale 2002). With the amount of waste produced in India, 400 million metric tons of plant nutrients can easily be extracted from it (Gupta 2001).

## 2.7 Factors affecting vermicomposting

### 2.7.1 Moisture

Earthworms prefer a moist and aerated habitat and are much more active in moist soils in comparison to dry. The body of earthworm constitutes 75-90% of water and they lack a mechanism for maintenance of stable internal water content (Grant 1955a; Kretzschmar and Bruchou 1991). Their water content is greatly influenced by the water potential of the soil. The maximum number of earthworms occurs in soil containing moisture between 12-30% (Olson 1928); being 23.3% to be most favorable for them to fabricate casts. Tomlin and

Miller (1980) also reported *E. fetida* development and reproduction at 82% of moisture content level. Dominguez and Edwards (2004) reported delay in sexual development of earthworms at low moisture conditions but Tripathi and Bharadwaj (2004) and Lee et al. (2016) reported 70-80% moisture content as optimum for *E. fetida* in cow manure. A solid positive connection between earthworm biomass and expanded soil dampness content has been observed (Beylich and Graefe 2009). Earthworms do not thrive in dry soil and migrate to lower layers to avoid drought. For some species such as *L. rubellus* (Parmelee and Crossley 1988) and *O. serrata* (Ismail 1983) cocoons act as survival stage.

### 2.7.2 Temperature

Temperature has significant influence on reproductive activities (Butt 1991; Fayolle et al. 1997) and determines composition and structures of earthworm communities (Lavelle et al. 1999). According to Lee (1985) and Edward and Bohlen (1992), the optimum temperature for temperate species are in the range 10-20°C, and 20-30°C for tropical and subtropical species. In case of *E. andrei* and other *Eisenia* species, the growth and reproduction occur between 20-25°C whereas temperature lower than 5°C and higher than 30°C often leads to mortality (Hartenstein and Bisesi 1989; Kaneda et al. 2016). According to Reinecke (1974), the lower higher lethal temperatures after 48 h contact, for *L. terrestris* is 28°C, for *A. caliginosa* is 26°, for *P. hupiensis* and *E. fetida* 25°C (Grant 1955b) and 29.7°C for *A. rosea* (Reinecke 1974). Frederickson and Howell (2003) compared population of earthworms in unheated (temperature 6.3 ± 2.3°C) and heated beds (temperature 13.7 ± 0.8°C) and found greater earthworm biomass and higher number of cocoons and hatchlings in heated bed. But Giraddi et al. (2008) reported that in *E. eugeniae* and *P. excavatus* lower temperature (20-24°C) was associated with higher hatchling percentage in comparison to higher temperature (27-30°C). This could lead to the conclusion that different earthworm species show diverse response with temperature. Higher temperature in vermicomposting systems leads to loss of nitrogen in the form of NH<sub>3</sub> volatilization (Tiquia and Tam 2000) and increase in the chemical and microbial activity in the food mixture, which leads to the reduction of oxygen level and thus had negative effects on earthworms (Dominguez and Edwards 2004). Moisture and temperature are usually inter-related. Dry and high surface temperature soils are much more restrictive to earthworms than water logged and low temperatures soils (Gerard 1967; Nordstrom and Rundgren 1974; Bolton and Phillipson 1976). Earthworms tend to migrate away at unsuitable temperatures. Growth activity, reproduction, respiration and metabolism of earthworms are all prejudiced greatly by temperature (Edwards and Bohlen 1996). Food

utilization of *Lumbricus terrestris* increases directly with temperature up to 20°C yet decreases over 22°C (Daniel 1991). Fecundity is additionally influenced especially by various temperatures. The ideal temperature for cocoon formation by *Lumbricus terrestris* is 15°C with 25.3 cocoons produced per annum (Butt 1991). Cocoon generation by *E. fetida* is reported to linearly increase with rise in temperature from 10-25°C although the number of hatchlings per cocoon is lower at 25°C than at 20°C (Reinecke and Kriel 1981).

### 2.7.3 pH

Earthworms are very sensitive to pH. The pH of soil is an important factor that limits the number and distribution of species in a particular soil. Earthworms are known to be absent in very acidic soil (pH < 3) and scarce in soil with pH < 4. The majority of temperate climate species prefer a pH of 5.0-7.4 (Satchell 1967; Bouche 1972). Most species of earthworms prefer neutral soil pH (Abbasi and Ramasamy 2001; Edwards et al. 2004). *E. fetida* prefer soils with a pH in the range 7.0-8.0 (Rivero-Hernandez 1991). A significant positive correlation between the pH and the seasonal abundance of juvenile and young adult *Octochaetona phylloti* was observed in semi-dry tropical fields in India (Reddy and Pasha 1993). The decomposition of organic matter during vermicomposting, leads to production of intermediates like ammonium and humates that alters the pH to neutral or acidic depending on the negatively and positively charged groups (Pramanik et al. 2007).

### 2.7.4 Organic Matter

The distribution of organic matter in soil also forms an important factor affecting distribution of earthworms. High organic matter reduces the activity of worms, therefore enhancing anaerobic activity of microorganisms which creates anaerobic and foul odor conditions (Hartenstein and Bisesi 1989). The size of earthworm population, type of species present, rate of growth and fecundity are dependent on the kind and amount of feed material. Earthworms like *E. fetida* are highly attracted to dung animal and droppings (Stephenson 1930) which has made this species a potential in waste management. A strong positive correlation was observed between earthworm number and biomass with the organic matter content of the soil (Hendrix et al. 1992).

### 2.7.5 Soil Type

Earthworms are moreover influenced by texture and soil type although this aspect is relatively less studied. The soil texture affects the activity of earthworm by factors like cation-exchange capacity, moisture relationships and nutrient status. Medium textured soils appear to be more favorable to earthworms than sandy soils (Guild 1948). Nordstrom and



Rundgren (1974) found an increase in earthworm abundance with increase in clay content. Only few species of earthworms can survive in semi-deserts and deserts (Kollmannsperger 1956) and some worms can inhabit the cold and arid soil of north-eastern Russia. El-Duweini and Ghabbour (1965) showed that population of *Apporectodea caliginosa* is negatively correlated with gravel and sand content in soils. Severely and moderately eroded sandy clay loams sustain more earthworms and better biomass than little eroded soil with high sand content (Hendrix et al. 1992). Fragoso and Lavelle (1992) showed that earthworm communities dominated by geophagous species are characteristic of nutrient rich soils as compared to litter feeding epigeic species which are normally present in nutrient poor soils.

#### 2.7.6 Stocking Density

The density of earthworms is influenced by several factors including initial substrate quality and quantity, temperature, moisture, and soil structure and texture (Edwards and Bohlen 1996; Wever et al. 2001; Smetak et al. 2007). The copulation frequency of earthworms is high at low population density, whereas it decreases when the density approaches the carrying capacity of the substrate (Rodriguez-Canche et al. 2010). It has been reported that the optimum stocking density of worms fit for vermicomposting is 1.60 kg worms/m<sup>2</sup> (Ndegwa et al. 2000b). Dominguez et al. (1997) reported that *E. andrei* grow slow in higher population densities with lower final body weight.

### 2.8 Effect of earthworms on soil

Earthworms play an important role in the decomposition of organic matter and enriching soil nutrients. Their contribution is mainly through physical processes such as feeding, fragmentation, aeration, turnover and dispersion, chemical processes such as digesting organic substrate and releasing the bioavailable form of nutrients to soil (Senapati 1993).

#### 2.8.1 Physical effects

The burrowing activities of earthworm tend to alter the soil physically. According to Krishnamoorthy (1989) and Habibullah and Ismail (1985) anecic earthworms make vertical burrows and liberally casts on surface of soil whereas endogeic earthworms are responsible for flat burrows and deposit their casts inside the burrows. Earthworm casts are developed as soil aggregates because of mixing of mineral particles with organic matter or by the binding effects of fungal hyphae (Parle 1963a; Abbasi and Ramasamy 2001). According to Edwards

et al. (2004), earthworm move and turn over large amounts of soil from deeper layers to upper strata. Almost 2-250 ton/hectare/year may move in this way equivalent to bringing a layer of soil between 1 mm and 5 cm thick to the surface every year, creating a stone free layer in soil surface. Some species make permanent burrows whereas others randomly move through the soil leaving cracks and crevices. Both types are important in soil aeration, porosity and drainage. Highly efficient water conducting channels are known to be created by anecic and endogeic species.

Earthworm activity makes a significant contribution to soil aeration by creating channels that allow air to penetrate into deeper layers of soil minimizing the incidence of anaerobic layers (Edwards et al. 2004). A study depicted an increase in water infiltration rate from 15 to 27mmh<sup>-1</sup> after 10 years of earthworm inoculation (Clements et al. 1991). Positive correlation was observed between water percolation and earthworm biomass; burrow length and burrow surface (Bouché and Al-Addan 1997). The infiltration rate increased by 150 mmh<sup>-1</sup> per 100 gm<sup>-2</sup> of earthworms. Endogeic species increases soil macro porosity and water infiltration which helps in reduction of run off. Also the cast formed by endogeic and anecic species on the soil surface improves structural stability and gives better resistance to erosion (Le Bayon et al. 2002). 50 % reduction in soil erosion was marked because of the increased infiltration rate due to anecic earthworm burrows (Shuster et al. 2002). Biogenic aggregates of *Amyntas khami* were responsible for a 75 % decrease in runoff on an experimental field with 40% slope in Vietnam (Jouquet et al. 2007).

Earthworms thus contribute to soil structure and formation through humus formation, mineral weathering, and mixing of these components to create stable aggregates i.e. organo-mineral complexes, which are deposited either on the soil surface or within the soil profile (Le Bayon et al. 2002).

### 2.8.2 Chemical effects

Earthworms affect the chemical composition of the soil. They play a vital role in distribution and transport of nutrients by consumption of large amount of organic matter and surface litter and after ingestion, maceration and excretion transports to sub surface layer of soil (Hairiah et al. 2006). Anecic and epigeic earthworms directly ingest poorly decomposed litter at the soil surface, while endogeics ingest soil and assimilate a small fraction of the organic matter it contains (Bhadoria and Saxena 2010). After ingestion, the unassimilated fraction is further fragmented during digestion and mixed with soil. In the end the soil organic matter (SOM) along with the undigested litter is returned back to the soil in the form

of cast. Fresh casts possess active bacteria and high mineralization rates, at least transiently, and these nutrient cycling processes decline with the age of casts (Bertrand et al. 2015). Earthworms enhance nitrification (Xu et al. 2013; Ramadass et al. 2015) by increasing bacterial communities (Parle 1963b) and aeration of soil. Earthworm tissue is also rich in protein content which thus on decomposition also yields nitrate-nitrogen (Alawdeen and Ismail 1986). The products of metabolism of earthworm are backed into the soil through urine, casts, muco-proteins and dead cells, tissues of earthworms (Lee 1983). Eriksen-Hamel and Whalen (2007) reported that the availability of soil mineral N, and subsequently the N concentration in soybean grain, is increased with the abundance of earthworms, *A. caliginosa*. Ammonia excreted by the earthworms may cause an increase in pH. According to Abbasi and Ramasamy (2001), casts are richer in total exchangeable bases, manganese, total organic matter, calcium, potassium, and phosphorus. Earthworms favor nitrification since they increase bacterial population and soil aeration. The most important effect of earthworms may be the stimulation of microbial activity that occurs in cast (Hoang et al. 2016; Huang and Xia 2018) which enhance the transformation of soluble nitrogen into microbial protein preventing their loss by leaching. 40% of the total aerobic nitrogen fixers 13% of total anaerobic nitrogen fixers and 16% of total denitrifiers are present in the drilosphere with the maximum population being within 20-40 cm depth of the surface soil. Senapati and Dash (1982) suggested that of the nitrogen added from the decomposed worm tissue 25% is in the form of nitrate, 45% as ammonia, 3% as soluble organic compounds and 27% undecomposed remains of setae, and cuticle. Earthworms contribute to carbon cycling through several complementary mechanisms (Snyder et al. 2016; Lubbers et al. 2017). The availability of some of the water-soluble nutrients (K, Ca, Mg) is also enhanced as SOM and litter pass through earthworm gut, because these nutrients are solubilized and dissolved from soil minerals during the grinding/rearrangement of organo-minerals during gut transit (Carpenter et al. 2007).

### 2.8.3 Biological effects

Earthworms turnover the sub soil to the surface and expose it to bacterial action which helps in the decomposition of cellulose which otherwise is difficult to decompose. Through the act of depositing vermicast on the surface, earthworms bring the subsoil to the surface and expose it to the bacterial action. Bacteria help in the decomposition of cellulose which otherwise doesn't decompose easily (Jairajpuri 1993). The organic matter when pass through the gut of earthworms, gets mixed with enzymes and egested as casts. Some of the

intestinal mucus secreted during passage through the earthworm gut is egested with the casts where it continues to stimulate microbial activity and growth (Barois and Lavelle 1986; Scheu 1991). Actinomycetes present in the intestine of the earthworm inhibit the growth of fungi and gram positive bacteria (Jayasinghe et al. 2009). Vermicast produced by earthworm is known to play antagonizing role for pathogenic fungi by production of siderophores, chitinase, antibiotics, fluorescent pigments, and cyanide (Han et al. 2005). Casts are usually rich in ammonia and partially digested organic matter and thus provide a good substrate for the growth of microorganisms. In the presence of earthworms, the above ground production has been increased significantly in 79% of cases while it decreased in 9% of cases (Scheu 2003). Earthworms also play a role in control of weeds by modification of seed germination by burial, ingestion and maternal effects (Laossi et al. 2010). The spreading of symbionts (mycorrhizae) carried by earthworms colonizing new fields was shown by Gange (1993). Earthworms are also known to increase the nodulation of legume plants by *Rhizobium* (Doube et al. 1994). Earthworms also suppress plant parasitic nematode population by the production of enzyme chitinase which breaks down the chitin in insect's exoskeleton (Munroe 2007). This was also supported by Arancon (2007). Vermicast produced by earthworms also exert suppressive effect on plant pathogens such as *Pythium*, *Rhizoctonia*, *Verticillium*, and *Plectosporium* (Edwards et al. 2006). Production of plant growth regulators (PGRs) such as auxins, gibberellins, cytokinins, ethylene, and ascorbic acids are also associated with vermicompost (Frankenberger and Muhammad 1995). Pesticide spray was significantly reduced where earthworms and vermicompost were used in agriculture (Rao et al. 2007; Suhane 2007).

## 2.9 Earthworms for composting

Earthworms occur all over the world but only rarely in deserts, areas under constant snow and ice, mountain ranges and areas almost entirely lacking in soil and vegetation. Their distribution depends upon the soil type and other physio-chemical characteristics of soil (Satchell 1967). The edaphic factors play a vital role in the distribution of earthworms (Kumar et al. 2018). Native earthworms contribute for better activity and have greater effect on nutrient dynamics as they have altered themselves to local soil and climatic conditions (James 1991). Earthworms are classified based on their feeding habits and ecology.

### 2.9.1 Trophic classification

Earthworms are saprophages, but they are classified into detritivorous and geophagous based on their feeding habits (Lee 1985).

#### 1) Detritivorous earthworms

They feed on litter or dead roots and other plant debris present on the soil surface or on mammalian dung. They are regarded as “humus formers” due to the presence of their epigeic and anecic forms. Example: *E. eugeniae*, *E. fetida*, *L. mauritii*, and *P. excavatus*.

#### 2) Geophagous earthworms

They are known as “humus feeders” comprised of endogeic earthworms that feed deep soil which is beneath surface of topsoil and consumes organic rich soil in large quantity. Example: *M. posthuma*, *O. thurstoni*

### 2.9.2 Ecological classification

Bouche (1977) classified earthworms into epigeics, anecics and endogeics laying stress on ecological strategies.

#### 1) Epigeic earthworms

The epigeic species lives on the leaf litter that is present on the soil surface. They are phytophagous and do not effect texture of the soil structure as they are unable to dig the soil. They are often smooth and bright red or reddish-brown in colour.

Examples: *D. rubida*, *E. fetida*, *E. eugeniae* and *P. excavatus*.

#### 2) Endogeic earthworms

Endogeic earthworms feed on and live in the soil. They are geophagous which forms horizontal burrows within the soil to move around and to feed. Endogeic earthworms are generally of pale, grey, pale pink, green or blue colours.

Examples: *A. longa*, *L. mauritii* and *L. terrestris*.

#### 3) Anecic earthworms

Anecic earthworms forms permanent vertical burrows in the soil. They feed on the leaf litter as well as on the top soil and hence are geophytophagous. They have dark coloured head end (red or brown) and pale tail.

Examples: *A. caliginosa*, *A. rosea*, *M. posthuma*, *O. cynaeum* and *O. thurstoni*.

### 2.9.3 Characteristics of earthworms for vermicomposting

Dominguez and Edwards (2010) described the following characteristics of earthworms suitable for the process of vermicomposting

- Wider range of tolerance to environmental factors; capable of survival in changing moisture and temperature.
- Feeding preference and malleability to wide range of organic materials (high and rich organic matter).
- High multiplication rate, low gestation period, high cocoon-forming and reproduction production rate.
- High biomass consumption, digestion and assimilation efficiency for organic matter decomposition.
- Easy to culture.

Different species of earthworms produce vermicompost that have varied nutrient composition. Thus, selection of earthworm species for vermicomposting is crucial. It is well established that epigeic species of earthworms are reliable for vermicomposting of different organic wastes (Ismail 2005). There are several advantages of using local varieties of earthworms. The introduction and establishment of the foreign earthworm species (Stockdill 1982) is time taking and has been justified by few scientists (Murphy 1993; Lavelle et al. 1999) though it is unnecessary and undesirable to interfere with the local biodiversity (Ismail 1995). Vermitechnology includes use of 20 different species found in India, but application of only 5 of them is majorly involved (Garg and Kaushik 2005; Garg et al. 2006; Suthar and Singh 2008a). These are namely, *P. excavatus* (Perrier 1872), *L. mauritii* (Kinberg) and *P. elongata* (Erseus) belonging to Megascolecidae; *E. eugeniae* (Kinberg) belonging to Eudrilidae; *E. fetida* (Savigny) belonging to Lumbricidae. The former three species being local and the later two are exotic. The epigeic species viz., *E. eugeniae*, *E. fetida* and *P. excavatus* are suitable for vermicomposting as they are voracious waste eaters and biodegraders (Sinha et al. 2010). Kale et al. (1982) have reported *P. excavatus* to be the Indian earthworm equivalent of *E. fetida*.

Epigeics are predominantly employed for biosolid waste management as these earthworms can accelerate decomposition process to a significant level and yield high quality composts, in comparison to those synthesized via traditional methods (Tripathi and Bhardwaj 2004). *E. fetida* is predominantly used in the world for this purpose as it is ubiquitous, can tolerate a wide range of temperature and can survive in wastes with good moisture content (Reinecke et al. 1992). *E. eugeniae* and *P. excavatus* are the other commonly used worms. *E.*

*Eugeniae* have large size, multiplies rapidly but lack temperature tolerance, thus used in the areas which has steady temperature such as tropical areas. Some species which retain themselves in Indian climates are *L. mauritii*, *D. bolani*, *D. willsi* and *P. elongata* (Dash and Senapati 1985; Shinde et al. 1992; Singh 1997). There is species specificity according to difference in food preference and time taken for bioremediation process. A comparative study between exotic *E. fetida* and local *L. mauritii* species of earthworms for evaluation of their efficiency in vermicomposting of municipality solid waste showed that *E. fetida* was superior to *L. mauritii* in terms of TOC reduction, C/N ratio reduction, increase in EC and TK but *L. mauritii* was also able to amend the characteristics of soil (Kaviraj and Sharma 2003). Tripathi and Bhardwaj (2004) also examined that both the species caused an increase in N, P, K and a decrease in C/N and C/P ratios after 150 days. *E. fetida* showed rapid decomposition in comparison to moderate decomposition shown by *L. mauritii*, when kitchen waste was amended with cow dung. Moreover, the mean number of cocoons and adults produced by *E. fetida* were more than *L. mauritii*. Finally, they concluded that *E. fetida* was well-adapted species for decomposition of the kitchen waste blended with cow dung under tropical conditions. Dominguez et al. (2001) stated that *E. eugeniae* can grow rapidly over the animal waste thus it can also serve as a suitable candidate for vermicomposting.

Out of all the Indian species, *P. excavatus*, *D. modigilani*, *D. nepalensis* and *L. Mauritii* can be exploited for vermicomposting because of their characteristics like continuous breeding, high yield of cocoon, short growth span and high hatching success rate (Bhattacharjee and Chaudhuri 2002). Earthworm species *E. eugeniae*, *E. fetida*, *P. sansibaricus*, *P. corethrurus* and *M. chinensis* were evaluated for their efficiencies for degrading organic wastes and out of which *E. eugeniae* was found to be superior amongst all (Padmavathamma et al. 2008). Some scientists recommend that vermicomposting via polyculture delivers quick results in comparison to monoculture (Dash and Senapati 1985; Suthar 2008). On conducting the experiment with three earthworm species, i.e. *E. fetida*, *P. excavatus* (epigeic) and *L. mauritii* (anecic), the reactor containing polyculture showed effective result in comparison to reactor containing the traditional monoculture (Suthar and Singh 2008b). On the other hand, Elvira et al. (1996) reported that mixed cultures of epigeics i.e. *E. fetida*, *L. rubellus* and *D. rubida* did not illustrate any benefits over pure cultures. *E. andrei* exhibited high multiplication rates in mixed cultures, whereas the multiplication rate of *L. rubellus* and *D. rubida* declined slightly in mixed cultures on comparing it with pure cultures. Ismail (1993) favored the in-situ soil community especially earthworms

encompassing the epigeic and anecic varieties for the conjoined practice of litter and soil management.

#### 2.10 Earthworms used in the current study

There are certain challenges and controversies which are associated for selecting the earthworms for vermiculture in India. The researchers who favor and want to promote indigenous earthworms for vermiculture process discourage the exotic species of foreign origin. However the exotic species, *E. fetida* is widely used in the decomposition process of different organic wastes in India due to its epigeic feeding habit, tolerance to varied temperature, climatic fluctuations, very high reproductive ability and less sensitive to density pressure (Reinecke and Kriel 1981; Haimi and Huhta 1990). The local species *L. mauritii* is also generally used for the same purpose due to its ability to survive in varied temperature, moisture and other physical factors (Bakthavathsalam and Uthayakumar 2007). Summary of biology and life history of *E. fetida* and *L. mauritii* has been reviewed by Reinecke et al. (1992) and Ramalingam (1997).

#### 2.11 Biology of *E. fetida* (experimental animal)

*Eisenia fetida* belongs to the family of lumbricidae and genus *Eisenia*. It belongs to phylum annelida (ringed animals) and is hermaphroditic. *E. fetida* is epigeic, red in colour (also known as red wiggler) which survives in a wide range of climatic conditions and active in all seasons (Haimi and Huhta 1990; Fadaee 2012). Its length varies from 22-130 mm and number of segments 80-110. The adult worms weigh around 1.5-4 gm. During puberty, the genital belt reaches in between 24 25 or 26 or 32 segment. Sexual maturity is usually reached by the time when the worm is 4-6 weeks old. They create cocoons at a rate of about one per 14-21 days. About 8-20 eggs are contained in the cocoon out of which only one-third hatch. Cocoons change colour, very light in the beginning to becoming darker with a reddish tinge before hatchlings emerge. The food consumption and reproduction rate in these worms is high and they thrive better in environments that are rich in organic matter (Tohidinejad et al. 2011).

These worms are tough which can be readily controlled and endured in mixed cultures. Being closely related to *E. andrei*, this species is generally used in United States of America, Europe and Australia by the name *L. rubellus*. *E. fetida*, the 'composting gorilla worms' are also known as 'African red wigglers'. These are found to convert the organic wastes into vermicompost faster than the burrowing earthworms, *P. elongate* and *P. asiatica*. *E. fetida* can tolerate wide range in temperatures ranging from 0 to 40° C but the regeneration



capacity is more at 25-30°C and at 40-45% moisture level. Mathur et al. (2006) have suggested that the biodegradable constituent present in the waste could get cleansed during the process of vermicomposting by *E. fetida* without causing any adverse effect on the humans and environment. *E. fetida* is found to feed on organic waste equivalent to its own body weight every day, thus, 64 million worms having weight 64 tons would consume 64 tons of waste everyday and yield 30-32 tons of vermicompost each day with conversion rate of 40-50% (Pandit et al. 2012).

### 2.12 Physiology of *E. fetida*

Vermicomposting encompasses bio-oxidative process in which earthworm play crucial role. The conversion of organic matter into useful product take place in two stages; the first stage (physio-mechanical) which involves airing, mixing and milling of organic matter whereas in second (biochemical) the labile substrates such as, sugar, amino acids, lipids and cellulose gets decayed by bacteria. In this second stage, the maturation of carbon sources and recalcitrant substances along with other materials like hemicelluloses and lignin as well as the formation of stable humic substances (Moreno et al. 2005) takes place. According to Edwards and Bohlen (1996), these earthworm feeds on the organic matter as a food source and require the help of protozoans, rotifers, nematodes, bacteria and fungi to obtain its nutriments. According to Sharma et al. (2005) 100-300 mg/g of dry weight food is consumed by earthworm each day. Out of the total amount of material eaten by the worm, only 5-10% is used for growth and metabolic purpose whereas the other 85% is excreted as vermicompost which also comprises of urine (as ammonia) and mucoproteins (Blair 1997). According to Sharma et al. (2005), the elementary requirements of the worm encompass four basic elements i.e. green residues (high nitrogen content), brown residues (high carbon content), water (moisture) and air (oxygen and ventilation).

### 2.13 Earthworms, vermicomposting, soil, and plant health

Vermicomposting is a cost-effective and ecologically friendly practice subjected for treatment of organic waste for conversion into vermicompost (Lazcano and Dominguez 2011; Lim et al. 2016). Vermicompost can be stated as the nutrient-rich microbiologically active substance, i.e. organically modified having high porosity, water holding capacity, and a low C/N ratio and have most nutrients which can readily be taken up by plants (Dominguez et al. 2004). There has been an ample rise in sustainable agricultural practices because of increasing concerns of consumers regarding issues of food quality, environmental safety, and

conservation of soil (Lazcano et al. 2011). Vermicompost is produced under mesophilic conditions, in which microorganisms biochemically alters the organic matter. Earthworms plays vital role in this process, as they control the aeration and splintering of the substrate, as a resultant it radically alters the functioning of microbes. Earthworms degrades the organic matter, by acting as the mechanical blenders and alters its physio-chemical properties by subsequently reducing the C/N ratio and increasing the exposed surface area for microbes, thus making it available for microbial action and further decomposition (Dominguez et al. 2010). Vermicompost helps in maintaining the soil structure healthy by altering the physicochemical properties of soil or any medium where the earthworms are planned to cultivate. The use of biofertilizers, compost, or vermitechnology have been exposed to make a larger assistance to the environment by attributing less use of chemical fertilizers and hence reduced environmental pollution (Joshi et al. 2015). Earthworm plays important role in cropping systems that can boost agricultural sustainability practices (Bertrand et al. 2015). With time, vermicomposted materials are being considered as organic fertilizers for sustainable agriculture, and a major part of study has been shifted towards the effect of vermicompost on plant growth and soil properties (Lazcano and Dominguez 2011). Compost and vermicompost produces significantly greater soil organic carbon with addition to other plant nutrients in comparison to mineral fertilizers (Weber et al. 2007).

#### 2.14 Destructive chemical fertilizers versus protective compost

The chemical fertilizers boosted the food productivity to enormous scale but in return it affects the environment as well as society (Tilman et al. 2002). Suhane (2007) conducted the comparative study of the farm soil under organic farming and chemical farming and comparison is illustrated in **Table 2.2**. The quantity of food produced dramatically increased but the quality of nutrition and fertility of soil subsequently shattered. Chemical fertilizers were stated to be the slow poison (Sinha et al. 2010) for the soil as it decreases the biological resistance in crops which make it susceptible to pests and diseases and also eradicates the valuable soil organisms which help in regaining the fertility of soil naturally (Adhikary and Gantayet 2012). The excessive use of nitrogen fertilizers elevated the inorganic nitrogen content in ground water via leaching and due to entry in human food it led to severe consequences on human health (Sinha et al. 2009). On the other hand, organic farming practices in which various nutrients of biological origin like compost offers the perception of food safety and security for future. The compost is not only a good source of macronutrients and micronutrients but also contains plenty of beneficial soil microbes which

help in fertility improvement, soil regeneration, promotion of growth in plants, and many more (Alvarez et al. 1995). Further, the source for compost being of biological origin is thus a renewable one. On the contrary, the source of chemical fertilizers is petroleum products which have the capability to deplete and thus are non-renewable (Sinha et al. 2010; Adholeya and Das 2012). By the use of compost (including vermicompost), the environment and society is benefited in all the stages (Jalil 2010). Thus, soil biodiversity and sustainable agriculture can be positively correlated for maintenance of ecological equilibrium.

### 2.15 Compost versus vermicompost

Composting as well as vermicomposting is stated as the two best procedures for stabilizing the solid organic wastes biologically (Vivas et al. 2009; Wu et al. 2014; Lim et al. 2016). They can be used as a source of organic matter amendment for the soil (Gonzalez et al. 2010). A brief comparison of compost versus vermicompost is given in **Table 2.3**. Composting is regarded as an aerobic practice which requires both thermophilic and mesophilic conditions that involves the action of microbes. It is composed of two phases: first, the thermophilic phase, which is the active phase of composting where decomposition is intense. Second, the maturation phase, which is marked by mesophilic temperature where the remaining organic matter is decomposed slowly. Vermicomposting is also an aerobic process, requires mesophilic conditions exclusively, and involves the action of earthworms along with the microbes. It is composed of two phases: first, the active phase where earthworms process organic matter and alters the physical and microbial state of material. Second, the maturation phase which involves displacement of earthworm to undigested fresh layers of matter where microbes take over the role of decomposition (Dominguez and Edwards 2010). The end product of composting is heterogeneous when compared to uniformly divided vermicompost material (Tognetti et al. 2005). Compost is well established on industrial scale but vermicompost is yet to be adapted at such level. Tognetti et al. (2005) reported greater nutrient quality and microbial activity in vermicompost than in compost.

**Table 2.2 Farm soil properties under organic farming and chemical farming**  
(Suhane 2007)

<b>Chemical and biological properties of soil</b>	<b>Organic farming (Use of composts)</b>	<b>Chemical farming (Use of chemical fertilizers)</b>
Availability of nitrogen (kg/ha)	256.0	185.0
Availability of phosphorus (kg/ha)	50.5	28.5

Availability of potash (kg/ha)	489.5	426.5
<i>Azotobacter</i> (1000/gm of soil)	11.7	0.8
Phospho bacteria (100,000/kg of soil)	8.8	3.2
Carbonic biomass (mg/kg of soil)	273.0	217.0

## 2.16 Effects of vermicompost on plant growth

Vermicompost significantly increases yield of spinach, onion, and potato (Ansari 2008). Plant height, number of leaves and fruit weight was higher in the vermicompost-treated field compared to the control (Mamta et al. 2012). Dhanalakshmi et al. (2014) reported an increase in root length, shoot length, and branch and leaf number in the seeds of okra, brinjal, and chili sown in vermicompost containing soil. The influence of vermicompost on branch and leaf number was high when compared to untreated control; almost all the growth, yield, and quality parameters such as mean stem diameter, mean plant height, yield/plant, marketable yield/plant, mean leaf number, and total plant biomass increased significantly in vermicompost medium compared to control in case of tomato, *L. esculentum* L. as reported by Joshi and Vig (2010). A high length in seedling, high amount of photosynthetic pigments such as chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoid content, high protein content, amino acid and sugar were recorded in groundnut seedlings (Mathivanan et al. 2012). Vermicompost is also known to stimulate growth of various plant species including horticultural crops such as strawberry (Arancon et al. 2004), groundnut (Kumar et al. 2014), chilli (Adhikary and Gantayet 2012), garlic (Suthar 2009), tomato (Abduli et al. 2013), and sweet corn (Lazcano et al. 2011). Vermicomposting has also shown positive effects on aromatic and medicinal plants (Prabha et al. 2007) and fruit crops such as banana and papaya (Reddy et al. 2014) and ornamentals plants such as geranium (Chand et al. 2007), marigolds (Shadanpour et al. 2011), and chrysanthemum (Hidalgo and Harkess 2002). Lazcano et al. (2010a,b) reported the positive effects of vermicompost on forestry species such as acacia, eucalyptus and pine tree. Vermicompost also positively stimulates seed germination in several plant species such as green grams (Karmegam et al. 1999) and tomato plants (Zaller 2007). Edwards et al. (2004) reported that vermicompost shows positive effect on vegetative growth, stimulating shoot and root development. There have been some reports that also mention an increase in nutritional quality with vermicomposting in tomato (Abduli et al. 2013), chinese cabbage (Wang et al. 2010), spinach (Peyvast et al. 2008), strawberries (Singh et al. 2008), and sweet corn (Lazcano et al. 2011).

There is no doubt that a larger part of scientific study evidences the positive effect of vermicompost on plant growth and yield still, there is also strong evidence that these effects are not constant and vary in magnitude (Lazcano and Dominguez 2011). In fact, few studies mention a decrease in growth and even plant death on vermicomposting (Roberts et al. 2007). This variability may depend on the physical, chemical, and biological characteristics of vermicompost, which vary depending on the earthworm species used, the production process, and the age of vermicompost (Rodda et al. 2006; Roberts et al. 2007; Warman and AngLopez 2010).

### 2.17 Plant growth-regulating mechanisms

Vermicompost may stimulate the growth and yield of plants by a direct mechanism or may follow an indirect one (**Table 2.4**).

**Table 2.3 Comparison of compost and vermicompost**

S.No	Parameter	Compost	Vermicompost
1.	Temperature	Thermophilic 45C-65°C	Mesophilic : 25C-40°C
2.	Phases	1. Thermophilic phase: Active phase of composting; intense decomposition 2. Maturation Phase: Decrease in temperature at mesophilic range; slower decomposition	1. Active Phase: Earthworms process, modify physical and microbial composition of waste. 2. Maturation Phase: Displacement of earthworms towards fresh layers of organic matter where microbes take over the role of decomposition.
3.	Action	Only microbes	Microbes and earthworms
4.	End product	Slightly heterogenous, stabilized humus-like material	Stabilized, homogenous, finely divided peat-like material
5.	Use	Well established on industrial scale	Not fully adapted on industrial scale
6.	Drawbacks/Limitations	Volatilization of NH <sub>3</sub> during thermophilic process.	Requirement of maintenance of mesophilic temperature; neutral pH and high humidity.
7.	Nutritional quality	Low nutrient content and low microbial activity	High nutrient content and microbial activity

8.	Economy	Low price	Triple that of compost
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### 2.17.1 Direct mechanisms

As far as direct mechanism of plant growth is concerned, vermicompost forms a source of plant macronutrients and micronutrients. Many nutrients are present in readily available forms (inorganic) to the plants; others are released by slow, gradual, and constant mineralization of organic matter (Chaoui et al. 2003). An increase in total porosity and water holding capacity after addition of vermicompost has been significantly reported (Hidalgo et al. 2006; Campitelli and Ceppi 2008; Lim et al. 2015a). A significant elevation in pH as well as total organic carbon of soil and declination in soil bulk was reported after conducting the study using vermicompost practice in two consecutive growing seasons (Gopinath et al. 2008). Ferreras et al. (2006) found that on adding 20 ton of vermicompost per hectare in the agricultural soil for two consecutive years, improved porosity and aggregate stability of the soil was obtained. Marinari et al. (2000) and Asghari et al. (2009) reported a significant increase in the number of large and elongated soil pores after application of vermicompost. These changes together improve the availability of water and aeration which increases the rate of seedling emergence and growth of root.

Vermicompost directly hinders the plant growth by regulating the plant growth hormones (PGHs). Many reports approve the role of hormonal activity triggered via earthworms (Suthar 2010). Neilson (1965) and Zhang et al. (2015) have reported that earthworms secrete growth-stimulating hormone such as auxins, cytokinins, and gibberellins as verified by studies conducted on vermicompost. Grappelli et al. (1987) and Tomati et al. (1988) demonstrated that addition of aqueous solution extracted from vermicompost demonstrated similar pattern of growth as shown on the addition of auxins, gibberellins, and cytokinins in the soil for growing ornamental plants. By stimulating and promoting the microbial activity within organic substrates, earthworms come up to be the imperative agents competent of influencing the production of PGRs by microorganisms (Grappelli et al. 1987). But, some researchers emphasize that micro-organisms aid in the plant growth hormone (PGHs) production and not earthworms. Neilson (1965) provided the first evidence of indole compounds present in the tissues of *A. caliginosa*, *L. rubellus*, and *E. fetida*. El Harti et al. (2001) substantiated the fact of rooting in bean seeds in a crude extract of earthworm because of the presence of indole compounds of endogenous origin. Atiyeh et al. (2002) speculates that the growth responses by addition of vermicompost appear as ‘hormone induced activity’ that may be associated with the high level of humic acids in vermicompost. Another report

also suggested humic acids enhanced nutrient uptake by the plants by stimulating growth of the root, increasing the proliferation of root hairs and increasing the permeability of root cell membrane (Pramanik et al. 2007). Canellas et al. (2002) isolated humic acids from vermicompost suggesting that humic acid accelerates the elongation of the root and of lateral root formation in maize roots. Aguiar et al. (2013) reported the promotion of lateral root emergence and induction of proton pumps by humic acid after application of vermicompost for 60 days. Arancon et al. (2006) also reported more fruits and flowers in pepper plant treated with humic acid extracted from food waste vermicompost than commercially produced humic acid.

#### 2.17.2 Indirect mechanisms

- a. Vermicompost also works to benefit plants via indirect mechanisms. Firstly, vermicompost guards against various diseases and pests by induction of biological resistance in plants or by executing pesticidal action (Al-Dahmani et al. 2003; Mamta et al. 2012). Vermicompost has the capability to induce biological resistance in plants as it contains some antibiotics and actinomycetes. Earthworms and vermicompost resulted in significant decrease in pesticidal spray usage in agriculture (Rao et al. 2007; Suhane 2007).
- b. Secondly, reports speculate a decrease in pest population during vermicomposting. Edwards and Arancon (2004) reported decrease in plant damage, such as tomato, cabbage and pepper, in trials in which 20 and 40% of vermicompost was added in the soil and significant reduction in arthropod populations. Munroe (2007) explained that this is due to the production of enzyme chitinase during vermicomposting which breaks down the chitin in the insect's exoskeleton. Vermicomposts can also suppress plant parasitic nematode populations. Arancon et al. (2002; 2003; 2005; 2007) demonstrated significant suppression in plant parasitic nematode populations by the application of solid vermicompost to tomatoes, peppers, strawberries, and grape plants.
- c. Vermicompost also indirectly affects plant growth by mitigation of plant diseases (Noble and Coventry 2005; Termorshuizen et al. 2006; Trillas et al. 2006). In regard to fungal diseases, addition of vermicompost extracts to ornamental plant decrease the sporulation of *P. cryptogea* (Orlikowski 1999). Infection induced by *F. lycopersici* (Szczech 1999) and *P. nicotianae* (Szczech and Smolinska 2001) was significantly reduced on the addition of vermicompost in solid form to the seeds of tomatoes. Vermicompost is known for its antagonistic property against pathogenic fungi which

produces antibiotics, chitinase, cyanide, fluorescent pigments and siderophores (Han et al. 2005). Edwards et al. (2006) observed that vermicompost exert suppressive effect against various plant pathogens like *Pythium*, *Rhizoctonia*, *Verticillium*, and *Plectosporium* and disappearance of these effects after sterilization of the vermicompost led to the conclusion that disease suppression takes place due to the presence of biological suppressive agents in vermicompost.

**Table 2.4 Plant growth regulating mechanisms stimulated by vermicomposting**

<b>DIRECT MECHANISM</b>			
<b>S.No</b>	<b>Mechanism Regulated</b>	<b>Mode by which mechanism is regulated by Vermicompost</b>	<b>Reference</b>
1.	Source of plant macro and micro nutrients	Vermicompost helps in gradual and efficient release of nutrients	Chaoui et al. (2003); Bhat et al. (2013)
2.	Soil porosity and moisture holding capacity	Causes an increase in total porosity and water holding capacity of soil.	Hidalgo and Harkess (2002); Hidalgo et al. (2006); Campitelli and Ceppi (2008)
		Leads to an improved soil porosity and aggregate stability in soil.	Ferreras et al. (2006)
		Significant increase in number of large and elongated soil pores	Marinari et al. (2000)
3.	Soil bulk density, pH	Significant decrease in soil bulk density and increase in pH.	Gopinath et al. (2008)
4.	Supply of PGRs and plant hormones	First evidence of presence of indole compounds in tissues of <i>A.a caliginosa</i> , <i>L. rubellus</i> and <i>E. fetida</i> .	Nielson (1965)
		Influence growth by direct uspply of plant growth regulators(PGRs)	Tomati et al. (1988); Tomati and Galli (1995)
		Secretion of plant growth hormones like auxins, cytokinins, gibberlins	Grappelli et al. (1987); Tomati et al. (1988)



		Rooting in bean seeds observed in crude extract of earthworms because of presence of indole compounds	El Harti et al. (2001a; 2001b)
5.	Stimulation of microbial activity	Vermicompost also stimulate and promote microbial activity in organic substrates, thus competent of influencing the production of PGRs by microbes	Grappelli et al. (1987); Tomati et al. (1988); Tomati and Galli (1995)
6.	Humic acid concentration in soil	Growth responses were associated with high level of humic acids in vermicompost	Atiyeh et al. (2000; 2002)
		Humic acid isolated from vermicompost suggested that humic acids enhance root elongation and formation of lateral roots in maize	Canellas (2000)
		Humic acid help in enhanced nutrient uptake by stimulating growth of root, increasing proliferation of root and permeability of root cell membrane	Pramanik et al. (2007)

#### INDIRECT MECHANISM

1.	Increase in biological resistance	Vermicompost protects plants against various diseases and pests by induction of biological resistance in plants or by killing them.	Al-Dahmani (2003); Mamta et al. (2012)
		Pesticide spray significantly reduced by use of earthworms and vermicompost as they release some antibiotics and actinomycetes and increase biological resistance	Singh (1993); Suhane (2007)
2.	Decrease in Pest population	Statistically significant decrease in arthropods (aphids, buds, mealy bug, spider mite) populations after addition of vermicompost.	Edwards & Arancon (2004)
		The decrease in number of pests could be related to the production of enzyme chitinase which breaks down the insect's exoskeleton.	Glenn (2007)
3.	Mitigation of plant diseases	Vermicomposting indirectly affect plant growth by mitigation of plant diseases	Noble and Coventry (2005); Termorshuizen et al. (2006); Trillas et al. (2006)
		Reduced sporulation of the pathogen <i>P. cryptogea</i> ; Reduced infection by <i>F. lycopersici</i> and <i>P.</i>	Orlikowski (1999); Szczech (1999) and Szczech and Smolinska

		<i>nicotianae</i>	(2001)
		Vermicompost exert suppressive effect on several plant pathogens such as <i>Pythium</i> , <i>Rhizoctonia</i> , <i>Verticillium</i> , and <i>Plectosporium</i>	Edwards et al. (2006)
4.	Bio-availability of nutrients for the plants	Vermicompost leads to high bio-availability of nutrients for the plants like N, P, K, Mg, Ca. Also large surface area of vermicompost expose greater sites for microbial action for stronger retention of nutrients	Edwards and Burrows (1988); Edwards et al. (1988; 2004); Edwards et al. (2004); Arancon et al. (2004; 2006)
5.	Increase in microbial population	Vermicompost tends to increase the microbial diversity	Tiwari et al. (1989); Frankenberger and Muhammad (1995); Pramanik et al. (2007); Singh (2009)

- d. Vermicompost leads to high bioavailability of nutrients for the plants such as nitrates (N), phosphates (P), soluble potassium (K), magnesium (Mg), exchangeable phosphorus (P) and calcium (Ca) (Edwards et al. 2004). Kumar et al. (2015a) also reported about the presence of micronutrient in high concentration in *Eichhornia* mediated vermicompost. Large surface area of vermicompost, promotes microbial activities as exposed area leads to strong retention of nutrients (Arancon 2004). During the process of vermicomposting, nutrients such as N, P, Ca and Mg, and K are released and tossed by microbial activity and converted into a more soluble and easy to assimilate forms (Martin-Gil et al. 2008)
- e. It tends to increase the microbial diversity thus promoting plant growth. Vermicomposts are rich in microbiota particularly bacteria, fungi, and actinomycetes (Scheu 1987; Tiwari et al.1989; Singh 2009). A large number of evidence depict that fungi, actinomycetes, yeasts, and algae also produce PGRs such as auxins, gibberellins, cytokinins, ethylene, and ascorbic acids in appreciable quantities, and because earthworms boost up their population, larger numbers of PGRs are available in vermicompost (Frankenberger and Muhammad 1995). It was reported that the microbial population is higher in vermicompost prepared from cow dung in comparison to municipal solid wastes (Pramanik et al. 2007). Plant growth-promoting bacteria (PGPB) directly stimulate growth by fixation of nitrogen, solubilization of nutrients,

and production of growth hormones such as aminocyclopropane-1-carboxylate (ACC) deaminase (Glick 2014).

## 2.18 Earthworm and bioremediation

Earthworms help in continuous mixing of soil and maintain aerobic conditions which favor degradation of contaminants. The role of earthworm in bioremediation of soil may help in promotion of agricultural sustainability (Wen et al. 2004). Earthworms not only facilitate the microbial and biochemical soil activity on the substrate but also partially stabilize the earthworm's excretions reducing environmental risks (Coutino-González et al. 2010). It has also been reported that earthworms play a vital role in bioaccumulation of poly-aromatic hydrocarbons (PAHs) (Matscheko et al. 2002; Contreras-Ramos et al. 2006). Yong-Ping et al. (2013) suggested that earthworms can help in the bioremediation process of contaminated soils by enhancing plant growth and micro-organisms development. The effect of individual and combination of earthworms and ryegrass (*L. multiflorum Lam.*) for the removal of fluoranthene from a sandy-loam alluvial soil were examined in a 70-day microcosm experiment. Earthworms are well thought-out bioindicators of soil fertility (Dickinson et al. 2005), and they have also been revealed to perk up the soil quality of degraded land. Because earthworms engage in such an essential task in soil processes, any practice that creates a complimentary environment for earthworms in the long run will augment soil fertility and safeguard biodiversity. Regrettably, earthworms down with other beneficial soil microorganisms have turned into the non-target recipients of pesticides (Booth et al. 2003). Besides, earthworms accumulate pesticide residues and can potentially broadcast these residues to predators higher up in the food chain (Huang and Iskandar 1999).

## 2.19 Vermicomposting in Bioremediation

### 2.19.1 Garden, Kitchen and Agro waste

According to Bouwman (2007), a number of tests have been developed to determine the effect of pollutants on earthworms, the reason being that earthworms are vital ecological component for maintaining soils fertility. A lot of focus has been deviated upon the potential role of vermiculture in the amelioration of problems associated with waste disposal. Garden waste can be converted into manure by vermicomposting (Mohan 2014; Shah et al. 2015). Vermicomposting can work as a proficient technology which can convert hollow fruit bunches to nutrient-rich organic fertilizers if it is mixed in appropriate ratio with cow dung (Lim et al. 2014). According to another study agricultural waste digested by earthworms

undergoes bio-chemical transition which leads to the formation of the cast that contains plant nutrients and growth stimulating hormones in an integrated form. This increased level of nutrients can be attributed to enzymatic and microbial activity of earthworms and the results advocate that vermicompost samples prepared from agro-wastes recorded a fairly higher level of major and micro nutrients in comparison to the initial levels of nutrients. The high availability of nutrients is also due to the enzymatic as well as microbial activity of the earthworms. Yadav and Garg (2009) and Suthar (2009a) reported the vermicomposting of residues of post-harvested crops such as wheat, millets and pulses should be done so that agricultural wastes could be changed into valuable products like vermicompost which indicates the possibility of using agricultural waste for sustainable crop production. Singh et al. (2013) also compared the recycling of the temple waste comprising mainly the floral offerings; kitchen waste and farmyard waste for conversion to vermicompost by *E. fetida*. The highest worm biomass yielded from temple waste. Moreover the vermicompost produced from temple waste showed higher germination index, enhanced shoot and root length, high number of secondary roots and higher total biomass in chickpea in comparison to vermicompost derived from kitchen waste and farmyard waste. Suthar (2007) vermicomposted agriculture waste, farm-yard manure and urban solid with earthworm, *P. sansibaricus*. The declination in organic carbon content, C:N ratio and increase in N, P, K and plant metabolites in the end product and growth pattern of *P. sanibaricus* on varied organic waste reveals about the application of this species on waste recycling operations at low input basis.

#### 2.19.2 Heavy Metal Reduction from sewage

Vermicompost may be promoted to be used in sustainable agriculture and for safe management of agricultural, industrial, domestic and hospital wastes which otherwise may be very risky for life and environment (Pathma and Sakthivel 2012). Mishra et al. (2014) reported that municipal sewage waste by vermicomposting can be effectively converted to nutrient rich, ecofriendly biofertilizer. The sewage sludge has high nutritive value for plants and after adequate treatment of eliminating heavy metals it is applied as fertilizers (Bettiol 2004; Suthar 2009b). He et al. (2016) also evaluated the content and type of heavy metals during vermicomposting of sewage sludge and found out that additives like straw, soil, sawdust, flyash help stabilize sewage sludge and eliminates its toxicity. Soobhany et al. (2015) also compared the efficacy of composting and vermicomposting of municipal solid waste. Vermicomposting caused significant reduction in Cd (43.3-73.5%), Cr (11.3-52.8%), Cu (18.9-62.5%), Co (21.4-47.6%), Zn (34.6%) and Ni (19.9-49.6%). The heavy metal was

found to be concentrated in the tissue of the earthworm after vermicomposting ranging from 6899.5 to 6901.4 mg/kg for Cd 2251.5 to 2261.0 mg/kg for Cr 2050.8 to 2067.8 mg/kg for Cu, 601.8 to 616.1 mg/kg for Co, and 42.9 to 74.6 mg/kg for Ni and 620.6 mg/kg for Zn (Soobhany et al. 2015). The bioconcentration factor (BCF), is the ratio of the metal concentration in earthworm tissue to the total metal concentration in the medium (Lukkari et al. 2006). The BCFs of *E. fetida* for various heavy metals in sewage sludge followed the order Cr > Zn > Cd > Pb > Cu (Shahmansouri et al. 2005). The difference in the percentage decrease of heavy metals is mainly due to the competition between heavy metals and earthworms accumulation (Soobhany et al. 2015).

Singh and Kalamdhad (2013) reported the feasibility of earthworms to mitigate metal toxicity and to enhance the nutrient profile in water hyacinth vermicompost for sustainable land improvement practices. The bioavailability and leachability was marginally reduced by the vermicomposting of water hyacinth by *E. fetida*. Vermicomposting of sewage sludge showed decrease in C: N ratio and total organic carbon (TOC) whereas increase in EC, total nitrogen, potassium, calcium, phosphorus was observed, which favors the recycling of sewage sludge as the fertilizer. Khwairakpam and Bhargava (2009) also vermicomposted sewage sludge and observed an increase in EC, N, K, Ca, Na, P; also the heavy metals Cu, Mn, Pb and Zn were now in permissible limits thus indicating that recycled sewage sludge through vermicompost can prove to be a good quality fertilizer.

### 2.19.3 Sugarcane Industry

Sugar industry is seasonal industry which is functional for 120-200 days in the year (Kolhe and Ingale 2011). India holds the second position as sugar manufacture in the world (after Brazil), maintaining about 10–12% sugar production of the world. Pressmud, bagasse and sugarcane residues like sugar beet pulp, sugar beet mud are produced as by-products during the production (Zeyer et al. 2004). A number of chemicals are being added to sugarcane (raw material) in order to increase the price of end products. Sugarcane industry uses large amount of water which leads to the generation of the larger amount of waste water which is contaminated with oil and grease (Kolhe and Ingale 2011). Sangwan et al. (2008) stated pressmud as a rich reservoir of micronutrients like Zn, Fe, Cu, Mn and macronutrients like N, P and K, organic carbon, sugar, proteins and enzymes. Thus, due to such rich constituents, it can serve as manure that can be used as soil additives. Sen and Chandra (2007) also successfully studied the transformation of organic constituents and humification of bagasse pressmud, and trash (sugar industry wastes) during the vermicomposting process. According to Bhat et al. (2014), vermicomposting could be an important tool to reduce the

genotoxicity of pressmud. Percent aberration in root meristems of *Allium cepa* was highest (30.8 %) after exposure to 100 % pressmud extract after 6 h but was reduced to 20.3 % after vermicomposting. The post vermicompost matter can be used as soil additive as it is a good source of plant nutrients. Reddy and Shantaram (2003) evaluated the efficacy of *E. fetida* to regulate the sugar industry wastes. The waste like bagasse, pressmud and trash yielded by sugar-cane industry, have been exposed to vermicomposting and results unruffled the production of a compost that is nutrient rich and has demonstrated the high crop yield, minimal soil depletion and its role value adding disposal of waste constituents (Kumar et al. 2010).

#### 2.19.4 Distillery

Distillery is recognized as the polluting unit that generates large amounts of foul smell and colour waste water known as spent wash. Generally, the waste water is discharged in the waste water either untreated or partially treated. Techniques are being tried for improvisation of spent wash for the growth of earthworms that are soil conditioners and thus may find a role in agriculture. Suthar and Singh (2008a) reported the results from vermicomposting of aerobically-treated sludge obtained from distillery by *E. fetida*. The high amount of decomposition and mineralization was observed in the vermicompost of distillery sludge (40%) having less amount of bedding, which aided in biomass gain and reproduction rate of earthworm. A declination in pH (7.81–19.20%), organic C (8.50–25.80%) content, and an elevation in N (130.40–170.70%), P (22.20–120.80%), K (104.90–159.50%), Ca (49.10–118.10%), and Mg (13.60–51.20%) content was enumerated at the end of the experiment which advocates that potential of earthworm can be retarded by higher proportions of distillery sludge. In another study earthworm, *E. andrei* were chosen for vermicomposting of various type of waste generated by winery industry. Decrease in biomass of earthworms in all winery wastes were recorded in comparison to manure. The reduction in C:N ratio, electrical conductivity and phytotoxicity where as elevation in humic materials, nutrient contents and pH was observed in all cases depicting an improvement in the agronomic value thus winery waste could be utilized as raw substrate in vermicomposting (Nogales et al. 2005). The stabilization of distillery sludge composition in comparison to other waste constituents (organic) together with pressmud, water hyacinth, litter of plant and cow dung in several proportions by vermicomposting (by *E. eugeniae*) was reported along with reduction in metal content by Senappa et al. (1995). Suthar and Singh (2008a) also vermicomposted sludge of distillery blended with cow dung via *P. excavatus* and showed significant reduction in pH (10.50–19.50%) organic carbon content (12.80–27.20%) and an upsurge in total N (128.80–

151.90%), P (19.50–78.30%), K (95.40–182.50%), Ca (45.90–115.60%), and Mg (13.20–58.60%). All these studies imply that for the conversion of distillery sludge into nutrient rich manure, the use of earthworms can be predicted to be highly effective.

#### 2.19.5 Food and Beverage Industry

Food industry generates a large amount of solid wastes, which is a good source of organic material and soil nutrients. Yadav and Garg (2009), vermicomposted food industry sludge along with cow dung. The results reported a decrease in organic matter, organic carbon content, C:N ratio, pH and increase in potassium, nitrogen, phosphorus content, ash content and EC. Nitrogen content increased in the range of 12.20–28.70 g/kg after vermicomposting. C:N ratio was 1.60-5.25 folds lesser in final vermicompost than initial raw substrate. Another study reported the vermicomposting of food industry waste water mixed with biogas plant slurry employing *E. fetida*. The *E. fetida* was unable to survive in 100% sludge. There was no adverse effect recorded on the quality of vermicompost on the addition of sludge in the range of 20-30%. An increase of 1.5-fold to 5.0-fold in nitrogen content was observed (Yadav and Garg 2010). Therefore, vermicomposting technology can not only be used for reducing the toxicity of the sludge but also for the recovery of nutrients from food industry wastewater sludge. Another study evaluated the vermicomposting of food industry sludges and a significant elevation in total N (60-214%), P (35.8-69.6%), Na (39-95%), and K (43.7-74.1%), where as reduction in pH (8.45-19.7%), total organic C (28.4-36.1%) and C:N ratio (61.2-77.8%) was documented. The results propose that good quality compost can be generated in composition with other organic waste from food industry sludge (Garg et al. 2012). Singh et al. (2010) reported the vermicomposting of beverage industry bio-sludge alone and mixed with cattle manure. Worms inoculated @ 25g/kg of substrate were capable of degradation of 50:50 mixtures in 75 days. In 105-110 days, the best quality product was obtained when worms were inoculated @ 25g/kg of substrate. Thus, vermistabilization of beverage industry waste could be achieved in 110 days, provided that it is mixed with cow dung as 100% sludge is toxic to worms.

#### 2.19.6 Paper And Pulp Industry

The paper industry discharges high volumes of extremely colored and toxic waste water in the environment and due to this it has been contemplated as one of the noxious pollutants causing pollution of land (soil), air and water (Martin 1998). Disposal of waste paper is a major issue for this industry; its discharge in the river or stream results in serious problems for aquatic life (Kesalkar et al. 2012). The effluent is generally alkaline having high

BOD and COD and rich in chlorides, sulphates of Na, Ca, trace metals like Hg, Pb, Cr etc. The waste water after proper treatment and proper precautions could be effectively used to increase the soil productivity (Chhonkar et al. 2000). Presence of the structural polysaccharides and low N content in the sludge of paper mill are two limiting factors for effective biodegradation (Elvira et al. 1997). Vermicomposting of paper mill sludge along with sewage sludge by *E. fetida* was performed and the outcome depicted that mixture in 1:6 ratio was found to be highly effective for obtaining maximum gain in biomass of earthworms and decrease in heavy metal content in the endproduct (Elvira et al. 1995). According to Elvira et al. (1996), 3:1 ratio of paper mill sludge and primary sewage sludge was found to be best among other composition for ideal development and reproduction of *E. fetida* during vermicomposting. *E. andrei* was also employed to manage the mixture of sludge obtained from paper mill and dairy industry mixed with pig and poultry slurry and concluded that this mixture could be utilized as a food source for vermicomposting (Elvira et al. 1997). According to Elvira et al. (1998), Vermicomposting of sludges from a paper mill mixed with dairy industry waste (cattle manure) were studied in a six-month pilot-scale experiment. The total biomass and number of earthworms increased. The vermicomposts were rich in N and P and had good confirmation, low levels of heavy metals, low EC, high humic acid contents and good stability and maturity. Similar studies of vermiconversion were also reported with another species of earthworm, *L. rubellus* (Kavian et al. 1998). Banu et al. (2001) employed an indigenous anecic and two exotic epigeic species of earthworm and described about the biotransformation of sludge produce in paper mill. They reported that 25% of paper mill sludge with standard bedding material [containing *M. indica* foliage (40%) + cow dung (40%) + Sawdust (20%)] was optimum mixture and *E. fetida* was verified to be the superior worm which can be used for biotransformation of paper mill sludge out of three-earthworm species. Moreover, a study revealed that the vermicomposting of tomato-plant waste along with sludge obtained from paper-mill facilitated the recycling of both the wastes, also aiding in the improvement of greenhouse crops for the development of sustainable environment (Fernández-Gómez et al. 2013). Vermicomposting of sludges from a paper mill mixed with manure of cattle were also studied and a 22- and 36- fold increase in number of earthworms was observed and a 2.2 and 3.9 fold increase in biomass was observed in addition to vermicompost that was rich in nitrogen and phosphorus, low level of heavy metal, high humate content and good stability. This clearly indicated that these sludges could potentially act as substrate in commercial production of vermicompost (Elvira et al. 1997).

#### 2.19.7 Textile Industry



The textile industry is the backbone of Indian economy accounting for 14% of industrial production and constitutes third part of total exports. It is the single largest employer in the industrial sector, employing about 35 million people (Chavan 2001). Kaushik and Garg (2003), studied the chief characteristics of sludge produced by textile industry i.e. total solids 192 g/kg; pH, 8.4; total organic carbon (TOC) 138 g/kg; Total nitrogen (TKN), 0.66 g/kg and C:N ratio 230. Various experiments employing vermicomposting for the conversion of textile mill sludge mixed with cow dung into value added products have been performed. Textile mill sludge mixed with 30% cow dung, slightly degrades the quality of vermicompost but if higher percentage of sludge is used then it affects the growth and sexual maturity negatively and results in a lower NPK (Kaushik and Garg 2003; 2004). Similarly, Garg and Kaushik (2005), also successfully utilized *E. fetida* for vermistabilization of textile mill sludge spiked with poultry droppings and reported that mixture of poultry droppings (70%) and sludge of textile mills (30%) is favorable for growth and reproduction of earthworms (Garg and Kaushik 2005). Garg et al. (2009) produced vermicompost by employing *E. fetida* on sludge of textile mill mixed with cow dung and horse dung. Temperature variation came out to be a major factor that significantly affected the growth and fecundity of *E. fetida*. In controlled temperature environment, hatchling and cocoon production was more. Vermicomposting resulted in increase in nitrogen and phosphorus contents and lowering of potassium, C:N ratio, pH, and electrical conductivity. Garg et al. (2006) determined the feasibility of vermicomposting to change the solid sludge produced by textile mill mixed with slurry (anaerobically treated) obtained from biogas plant into vermicompost and check the possibility to use vermicomposting practices in industries for managing the synthesized waste. Vermicomposting leads to acidic pH, upsurge in N, P and K and significant decrease in C:N ratio.

#### 2.19.8 Thermal Power Plant Waste

Fly ash is used in building construction and is composed of silica, aluminium, oxides of iron, calcium, magnesium, arsenic, chromium, lead, zinc, nickel and other metals. It is generated in large amounts by coal fired thermal power plants and its disposal is a very challenging problem in different parts of the world including India. India is declared to be the producer of 150 million tons of fly ash yearly (Gupta et al. 2005). Gupta et al. (2005) evaluated the vermicomposting of fly ash blended with cow dung in four different proportions, i.e. 20, 40, 60, and 80%. The maximum growth of earthworms was observed in 60% proportion mixture which was more or less similar to control whereas 80% proportion mixture revealed the marked decrease in worm development. Moreover, 40% proportion

mixture of substrate showed highest output of vermicompost, high number of worm growth whereas highest biomass gain by earthworm was found in 20% proportion mixture. At the end of the experiment substrate showed a 30–50% reduction in heavy metals in up to 60% fly ash containing substrates and 10–30% reduction in 80% fly ash containing substrates. The study also revealed the considerable bioaccumulation of heavy metals by earthworms as depicted by the results of metal analysis of earthworms. It was concluded that up to 60% fly ash–cow dung mixtures can be used for sustainable and efficient vermicomposting. Bhattacharya and Chattopadhyay (2006) reported that during vermicomposting of fly ash a considerable amount of insoluble plant nutrients (Fe, Mn, Cu, and Zn) and some heavy metals (Pb, Cd, and Cr) from fly ash was transformed into more soluble form. Fly ash and cowdung in different proportions were treated with an epigeic earthworm (*E. fetida*) and the solubility of different trace elements were estimated periodically. The results revealed that the inclusion of epigeic earthworm *E. fetida* in different combinations of fly ash and cow dung converted a considerable amount of the micronutrients into bioavailable forms. Venkatesh and Eevera (2008) also reported vermicomposting of fly ash with cow dung in different combinations. Fly ash was mixed with cow dung in 1:3 1:1, and 3:1 ratios and *E. eugeniae* worms were allowed to feed on this for 60 days. The concentration of macro and micronutrients in the final composition of fly ash and cow dung increased in comparison to fly ash alone transforming nitrogen, phosphorus, potassium and micronutrients (Mg, Cu, Zn, Fe, B, Mo and Mn) from fly ash into more soluble forms and thus increasing the bioavailability of nutrients. The electrical conductivity increased in the beginning of vermicomposting up to 30 days in 1:3 mixture, and then steadily decreased till end of vermicomposting.

## 2.20 Adoption/extension parameters

During the mid 1960s, agriculture had made a remarkable progress but at present it has reached a plateau stage beyond which sustaining the growth is an uphill task. There is an urgent requirement to develop strategy for sustainable agriculture to curb the adverse effects led by green revolution on the natural resources of Punjab. Organic farming is an option to sustain agricultural growth (Bajwa 2003) and is associated with the promotion of health of soil, better environment and production of clean and quality foods. Even consumers are now prepared to pay a premium price for such products and thus demand is increasing at national and international level (Mahadevappa 2004). Now farmers have started avoiding the use of agrochemicals and have adopted the use of biocontrol inputs like mechanical control, use of

light trap, pheromones trap (Bhattacharya and Pandey 2003). There is an emphasis on developing a strategy for promoting sustainable agriculture with organic farming emerging as the foremost options. Earthworms are the most important component of soil biota and well known for their abilities improve soil structure, fertility and agricultural production.

Biodiversity plays an important role in sustainable agriculture. Due to deforestation, use of excessive fertilizers and pesticides, many species are destroyed. Adoption of agro-forestry would maintain ecological balance and protect soil health (Patil 2003a). Burning of crop residue as practiced by farmers results in great loss of solar energy, moisture and carbon; instead crop residues being agricultural by products, should be used to prepare compost (Patil 2003b). Vashishtha (2003) reported that sustainable agriculture helped to cut down the external cost of seeds, manures and pesticides. The problem in organic farming is that farmyard manure (FYM) had become a rare product as a result of decreasing animal population. Due to over-exploitation of shrubs and trees, manure produce via green leaf has also decreased. Moreover, high cost of labor and other requirements, reusing of farm wastes as manures was never considered as an option. Under vermicompost many beneficial heterotrophic micro-organisms could not survive in the soil due to very low amount of organic matter in the soil. The most common waste found in environment is of organic origin. The conversion of organic waste is required to maintain the fertility of the soil and prevent the environmental pollution. The converted product aids in the improvement of physio-chemical and biological properties of soil and further improves the quality of the soil (Ismail 2005).

Ronquillo (2006) explained that vermiculture has bright prospects from a simple technology going organic. People adopt vermiculture because it will significantly contribute in solving their garbage problems; detoxify the soil, make it naturally fertile to increase crop yield and income of farmers and provide employment for those who will produce bio fertilizer instead of spending scarce government finances on importing chemical fertilizers and high tech equipment. There is a need for change in farm practices with focus on organic farming and vermiculture. Stressing the need for organic farming and vermiculture, the farming community must give importance to agriculture-allied activities and save the country from the ongoing agricultural crisis (Zinia 2007). In general, research on vermiculture and vermicomposting is leading towards appreciation of some of the soil entity as well as adding to the other benefits derived from agriculture. In general research on vermiculture and vermicomposting is leading towards appreciation of some of the finer and physical aspects of soil entity as well as adding to other benefits derived from vermiculture. Dissemination of

information on organic farming in the country has enlightened the farmer to engage in and adopt vermitechnology as a part of organic farming (Kalra et al 2008).

# **3. HYPOTHESIS**

### **3. HYPOTHESIS**

With the fast mounting population, there has been a lag in the check of pesticide usage. At one place we acknowledge the use of pesticide and on the other place our greed for more productivity leads to the misuse of pesticides which further leads to adverse effects on the living beings. Pesticides are used to protect the agricultural crops from various pests, weeds etc but the persistence of these pesticides in the soil effects the life cycle of non-targeted species. When pesticides are mixed with the soil they get adsorbed on the soil surface and form stable complexes hence the effectiveness of soil and pesticides itself reduces. The time has come when we strictly need to verify the scenario of pesticide use and the environmental effects. In the lure to achieve higher production human beings are killing themselves and other living beings. The soil biodiversity helpful in maintenance of the optimal properties and fertility of the soil have been affected markedly with the use of such chemicals. Thus, we are involving ourselves into the purposeful work of investigating the effect of the pesticides on earthworms and how the technology of vermicomposting (which is widely used now days to convert toxic wastes into value added products) be beneficial in remediating the pesticide contaminated soil to a fertile one.

# **4. AIMS AND OBJECTIVES**

#### **4. AIMS AND OBJECTIVES**

1. To study the effect of different pesticides on the exotic species *Eisenia fetida*.
2. To study the effect of different pesticides on the indigenous species of earthworms of Jalandhar region.
3. To study the effect of soil (affected with pesticides) procured from farm lands by genotoxicity assessment (*Allium cepa* test).
4. To study the effect of different pesticides and vermicompost by *Allium cepa* test invitro.
5. To study the physicochemical quality of vermicompost and the soil.



**5. MATERIALS  
AND  
METHODS**

## 5. MATERIALS AND METHODS

Experiments were conducted to determine the toxicity and genotoxic potential incurred by the pesticides on the important soil biota (earthworms). Along with the soil biota, it also directs to establish the efficiency of vermicomposting for remediation of soil contaminated with pesticides leading to reduction in their use and maintenance of soil fertility.

### 5.1 Earthworm species

#### 5.1.1 *Eisenia fetida*

Young non-clitellate earthworms were procured from the vermifarm of Khalsa College, Amritsar and its culture was maintained.

#### 5.1.2 *Metaphire posthuma*

Clitellated earthworms were collected from fields and its culture was maintained.

### 5.2 Pesticides

#### 5.2.1 Acephate

Acephate used is in the form of Asataf (75 SP formulation of acephate, Tata Rallis) and was obtained from Nagpal Sales Corporation, Jalandhar city.

#### 5.2.2 Atrazine

Atrazine is used in the form of Atrataf (50 WP formulation of atrazine, Tata Rallis). It was procured from Nagpal Sales Corporation, Jalandhar city.

### 5.3 Test Medium

Two types of test medium were considered for toxicity experiments

#### 5.3.1 Artificial test medium (OECD Artificial Soil)

The OECD artificial soil is composed of three components:

- a) Coco peat (substitute of sphagnum peat) - 10%
- b) Kaolin clay (kaolinite content preferably above 30 per cent) -20%
- c) Industrial sand (fine sand should be dominant with more than 50 per cent of the particles between 50 and 200 microns) -70%

All these components were procured from Natraj Enterprises, Jalandhar City. The dry constituents are thoroughly mixed and moisture content of soil is determined by drying a small sample at 105°C and re-weighing. De-ionised water was used to moisten the soil to

give an overall moisture content of 35% of the dry weight. pH was adjusted to  $6.0 \pm 0.5$  by addition of calcium carbonate.

### 5.3.2 Natural test medium

For both the species of earthworm, respective natural medium was considered. For *E. fetida*, natural medium was cow dung and for *M. posthuma*, natural medium taken was garden soil.

#### a) Cow dung (CD)

Fresh cow dung was procured from a dairy farm situated in the vicinity of Lovely Professional University, Phagwara, Punjab

#### b) Garden soil (GS)

Garden soil was procured from the garden of Lovely Professional University, Phagwara, Punjab.

### 5.4 Vermireactors

Circular plastic trays with an area of  $2211.72 \text{ cm}^2$  were taken for toxicity tests and vermi-remediation experiment.

### 5.5 Experimental Design

The experiments for toxicity, genotoxicity and bioremediation of soil were carried out separately.

### 5.6 Acute Toxicity test

#### 5.6.1 Filter paper test

Glass petriplates 10 cm in diameter were taken. The petriplates were lined with filter paper cut to a suitable size such that it does not overlap. Aqueous solutions of acephate and atrazine were prepared to form a range of concentrations. The treatments were taken in geometric series for both acephate (10, 20, 40, 80, 160, 320  $\mu\text{g}$ ) and atrazine (100, 200, 400, 800, 1600  $\mu\text{g}$ ). 1 ml of pesticide solution is pipetted onto the filter paper on each petriplate and evaporated to dryness. After drying 1ml of deionised water is added to each petriplate to moisten the filter paper. Each petriplate is sealed with plastic film with small ventilation holes. A preliminary range-finding test was first performed for a more precise screening test.

Ten replicates were taken per treatment and each replicate consist of one worm per petriplate. 10 control petriplates were setup along with the treatment range levels. Worms should be kept on moist filter paper for few h before being placed onto the petriplates to void

their gut content. They are washed and dried before placement on filter paper. The tests are performed in the dark for a period of 24 h. Worms are classified as dead when they do not respond to a gentle mechanical stimulus to the front end. The mortality was recorded. This test was performed to check the toxicity of pesticides, atrazine and acephate on exotic species, *E. fetida* and indigenous species, *M. posthuma*.

#### 5.6.2 Artificial soil toxicity test

A preliminary range finding test was performed in different concentration (3.90, 7.81, 15.62, 31.25, 62.5, 125, 250, 500, 1000, 2000 mg) of acephate and atrazine. Artificial soil (1 kg) was mixed uniformly with aqueous suspensions of acephate and atrazine. This test was applied on *E. fetida* and *M. posthuma* to check the toxicity of atrazine and acephate in artificial soil. Earthworms need to be conditioned in artificial soil for 24 h before the experiment. 1 kg of the artificial soil is placed in a tub and 10 earthworms (pre-conditioned) are placed on the surface of test medium (OECD artificial soil, in this case) for 14 days in triplicates. Three control dishes, treated with the same solvent (but without any chemical) are also considered. 10 g of feed mixture (finely ground cow dung) was added in the middle of soil (Van Gestel et al. 1989). The containers were covered with jute mats to prevent the test medium from drying and retain the moisture level. At intervals the level of moisture in the medium was checked and water was supplemented if moisture content decreased. The artificial soil was again supplemented with feed mixture (cow dung) after it was consumed. The mortality was recorded after 7 and 14 days. The final endpoint i.e adult mortality was used to calculate lethal dose (LD<sub>50</sub>) for 7 and 14 days both using probit analysis.

#### 5.6.3 Modified soil toxicity test

This toxicity test was conducted in a natural test medium (cow dung for *E. fetida* and garden soil for *M. posthuma*) similar to artificial soil toxicity test to check the toxicity of pesticide atrazine and acephate.

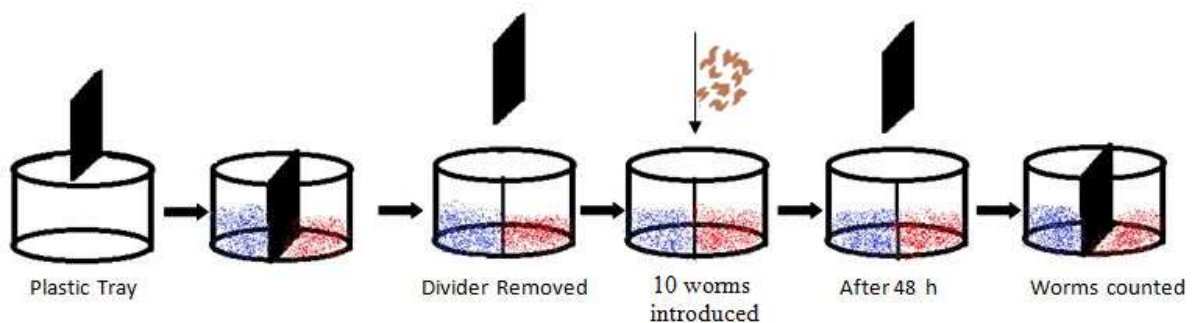
### 5.7 Sub-lethal Toxicity

To study sub-lethal toxicity, a range-finding test to avoid mortality was performed. This test is invalid if more than one worm per vessel is dead or missing by the end of the experiment.

#### 5.7.1 Avoidance Test

Ten adult earthworms (*E. fetida* and *M. posthuma*) were exposed simultaneously to a control soil (without any pesticide) and pesticide treated soil. A test vessel was taken which

was divided into two equal sections by means of a vertically introduced divider. Soil was spiked with various concentrations of acephate and atrazine (3.90, 7.81, 15.62, 31.25, 62.5, 125, 250 mg/kg). The test was run with 5 replicates per treatment. Artificial soil (OECD soil) and cow dung/garden soil was used as the test medium. The selected worms were acclimatized for 24 h before being transferred to the test substrates. The soil was then air dried and sieved (2mm) to obtain a homogenous soil mixture. Vessels were filled with sieved soil up to height of 6 cm. One half of vessel was filled with test soil (section A) and other half with control soil (section B). The separator (vertical divider) was then removed and 10 worms are placed on to the separating line of each test vessel and thus are presented with a choice between the test soil and the control soil (**Fig 5.1**). The plastic test unit was wrapped with tin foil (with small ventilation holes) to eliminate light and preventing worms from escaping. No feeding of the earthworms is required during the test. The avoidance tests were validated by dual control test to check if the worms in the absence of a contaminant, do not congregate but distributes themselves randomly in both sides of vessel and do not display behavior that might be mistaken for avoidance (Yeardley 1996).



**Figure 5.1 Schematic representation of the procedure followed in earthworm avoidance test**

After the test period of 48h, the separator (vertical divider) is introduced separating the control and treated soil. The number of worms is then counted for both the sections of the vessel. The worms found on the separating line were counted as 0.5. The effective concentration ( $EC_{50}$ ) was determined for avoidance of atrazine and acephate by both earthworm species *E. fetida* and *M. posthuma* in artificial soil and garden soil. The soils were considered to be toxic (habitat function reduced or limited) if >80% of the worms stayed in the control soil (Hund-Rinke and Wiechering 2000). Avoidance response at various concentrations was used to determine the median effective concentration ( $EC_{50}$ ) using a probit analysis model was used.

For each replicate the avoidance response was calculated using Eq. 1

$$NR = \left[ \frac{C-T}{N} \right] * 100 \dots \dots \dots (Eq. 1)$$

NR = net avoidance response (%)

C = number of worms in control soil

T = number of worms in pesticide-amended soil

N = total number of worms exposed

## 5.8 Genotoxicity studies in vivo

### 5.8.1 Site for collection of samples

The soil samples were collected from various sites of agriculture field in district Phagwara, Punjab, India (31° 13' 4" N latitude and 75° 46' 9" E longitude) under maize cultivation where pesticide Atrataf 50 WP (atrazine) @ 1kg/acre was sprayed. This was referred to as pesticide treated soil (PTS). Another soil sample was taken from an organic farmland where pulses (mung bean) were grown and vermicompost was applied @ 500 kg/acre. This was referred as vermicompost treated soil (VTS). Soil samples were collected at a depth of 15-20 cm from 4-5 sites of each agricultural field and pooled together to make a single composite sample. Samples were brought to laboratory and dried at room temperature for 72 h. It was then finally ground to fine powder, sieved (Cabrera et al. 1999) and saved for further investigation.

### 5.8.2 *Allium cepa* for genotoxicity tests

Equal sized bulbs of *A. cepa* were purchased from the local market. Dried and mould attack onion bulbs were discarded and also that had started shooting green leaves. The onions were submerged in water for 10-12 h so as to soften their scales. The outer scales were removed carefully without damaging the root primordials. After the removal of outer scales, the bulbs were kept into fresh water to prevent the root primordial from drying. Ten onion bulbs were set up for each concentration. The experiment was performed at about 20 ± 2° C and was protected against direct sun light. The bulbs were placed in distilled water and kept undisturbed for 3 days till roots of 1-2cm length were obtained. The bulbs were then treated with various concentrations of PTS and VTS for 24 h and 48 h. After 24 h and 48 h, the root length was measured according to Fiskesjo (1985). The treatment solution was changed after every 24 h.

### 5.8.3 Preparation of extract

The extracts of vermicompost treated soil (VTS) and pesticide treated soil (PTS) were prepared according to the French Standardized Method (Ferrari et al. 1999). 1 L of water was added to 100g of soil sample which was then subjected to continuous shaking for 24 h. After that the suspension was filtered using a Whatman Filter Paper 42 (pore size 2.5  $\mu\text{m}$ ). The filtrate was evaluated further for genotoxic effects immediately. Various concentrations (10%, 20%, 40%, 60%, 80% and 100%) of extracts along with negative control (distilled water) were used for treatment. The root tips of *A. cepa* were exposed to various concentrations of extracts. The root length was measured after 24h and 48 h and root tips were fixed in fixative i.e farmer's fluid (glacial acetic acid and ethanol in 1:3 ratio) after 24h and 48h.

### 5.8.4 Fixation, slide preparation and scoring

After treatment, the root tips were excised, washed and then fixed in Farmer's fluid. After 24 h of fixation, the root tips were hydrolyzed in 1N HCl for 2-3 min and then squashed in aceto-carmin and 1N HCl (9:1) in water bath (60°C) for 12-15 min. The meristematic zone was removed and immersed in 45% glacial acetic acid for 1 min and then transferred to a clean slide, squashed under a cover slip and sealed with mountant DPX (a mixture of distyrene, a plasticizer (tricresyl phosphate) and xylene). All slides were coded and examined under a light microscope.

### 5.8.5 Genotoxicity studies

Root length was estimated in whole root bundles. The mean value was calculated from ten measurements and relative growth value was expressed as percent of the control value. Other signs of toxicity like change in colour of roots, consistency of roots, presence of root hooks, twists, or crochets were also examined. The comparison of toxic effects were analyzed by calculating the mitotic index (MI), which is the ratio of dividing cells to total cells analyzed. The mitotic index (MI) was determined by the examination of about 3000-4000 dividing cells for each concentration. The Chromosomal aberrations (CA) were also observed and classified as physiological (c-mitosis, stickiness, vagrant chromosomes, laggard chromosomes) and clastogenic (chromatin bridges and chromosomal breaks) aberrations and percent chromosomal aberration frequency was calculated out of 1000 cells examined.

## 5.9 Bioremediation of pesticide spiked soil

In this phase, experiments were conducted to find the amount of pesticide mixed with soil test media for supporting maximum population build up of worms and giving a product with best genotoxic and physico-chemical characteristics.

### 5.9.1 Setting up of vermireactors

Cow dung and artificial soil (1:1) was taken as the soil test medium. The recommended dose for both atrazine and acephate is 800 g/acre (Farm operations, PAU). Five concentrations of pesticides i.e recommended dose,  $\frac{1}{2}$  of recommended dose,  $\frac{1}{4}$  of recommended dose, 2X of recommended dose and 4X of recommended dose were taken for experimentation. According to our area of vermireactor, the recommended dose is 44 mg and thus a series of 11, 22, 44, 88, 176 mg of pesticide in the soil test media was used. Acephate and atrazine as aqueous suspension in different concentration were added to 2 kg soil test media. After 24 h, a small proportion of test soil from each vermireactor was taken and subjected to air-drying and then sealed in polythene bags for physicochemical and genotoxic analysis. These proportions of soil were taken to be the 'pre-vermicompost' soil samples. Thereafter 20 worms of *E. fetida* (non-clitellate) and *M. posthuma* (clitellate) were released in a vermireactor containing atrazine and acephate pesticides. Vermireactors with test medium were also subjected to traditional aerobic composting (without worms) for comparative evaluation of efficiency of vermicomposting and aerobic composting.

Data were recorded for percent mortality, biomass, cocoon production, rate of hatching and number of hatchlings at 15 day intervals through 90 days. Earthworms, cocoons and hatchlings were sorted out by hand from the feed mixtures for recording their numbers. At the end of the experiment the products of vermicomposting were sieved and air dried, packed in polythene bags and stored in a dry cool place for genotoxic and physico-chemical analysis. These samples were tagged as 'post-vermicompost' samples. Same was followed for aerobic composting samples.

## 5.10 Genotoxic Assessment

The extracts were prepared (as described in section 3.8.3) from pre-vermicompost and post-vermicompost samples. A series of concentration (10%, 20%, 40%, 80% and 100%) of the extracts were prepared and root tips of *A. cepa* were exposed to these concentrations. The root lengths were estimated after 24h and 48h and the root tips were excised and fixed in farmer's fluid after 24h and 48h. Slides were prepared (as described in section 3.8.4). The mitotic index (MI) and the chromosomal aberrations (CA) were observed and calculated.



**Table 5.1 Representation of various doses of atrazine and acephate subjected to different processes**

Treatment	Control	11mg	22mg	44mg	88mg	176mg
<b>ATRAZINE</b>						
Pre-Vermicompost	C <sub>AT</sub>	AT1	AT2	AT3	AT4	AT5
Post-Vermicompost by <i>E. fetida</i>	C <sub>ATE</sub>	ATE1	ATE2	ATE3	ATE4	ATE5
Post-Vermicompost by <i>M. posthuma</i>	C <sub>ATM</sub>	ATM1	ATM2	ATM3	ATM4	ATM5
Post aerobic composting	C <sub>ATC</sub>	ATC1	ATC2	ATC3	ATC4	ATC5
<b>ACEPHATE</b>						
Pre-vermicompost	C <sub>AC</sub>	AC1	AC2	AC3	AC4	AC5
Post-Vermicompost by <i>E. fetida</i>	C <sub>ACE</sub>	ACE1	ACE2	ACE3	ACE4	ACE5
Post-Vermicompost by <i>M. posthuma</i>	C <sub>ACM</sub>	ACM1	ACM2	ACM3	ACM4	ACM5
Post aerobic composting	C <sub>ACC</sub>	ACC1	ACC2	ACC3	ACC4	ACC5

### 5.11 Physico-chemical analysis

For physico-chemical analysis of pre-vermicompost and post-vermicompost samples, pH, EC, TDS, organic carbon, nitrogen, phosphorus, sodium, potassium, calcium, lithium were estimated at the beginning (pre-vermicompost) and at the end (post-vermicompost) of the experiment.

#### 5.11.1 pH

5 g air dried sample was dissolved in 50 ml distilled water (1:10 w/v) and shaken on an orbital shaker for 40 minutes. Then supernatant was taken and pH of the supernatant was recorded by using digital meter (Eutech Instruments, PCSTestr 35 series).

#### 5.11.2 Electrical conductivity (EC)

5 g air dried sample was dissolved in 50 ml distilled water (1:10 w/v) and shaken on an orbital shaker for 40 minutes. Then supernatant was taken and EC of the supernatant was recorded using digital meter (Eutech Instruments, PCSTestr 35 series). The results were expressed in mS/cm.

### 5.11.3 Total Dissolved Solids (TDS)

5 g air dried sample was dissolved in 50 ml distilled water (1:10 w/v) and shaken on an orbital shaker for 40 minutes. Then supernatant was taken and TDS of the supernatant was recorded using digital meter (Eutech Instruments, PCSTestr 35 series).

### 5.11.4 Nitrogen (TKN)

The method of Bremner and Mulvaney (1996) was used for estimation of Total Kjeldhal Nitrogen.

#### (i) Reagents

a) Digestion mixture:  $K_2SO_4$ ,  $CuSO_4$  and  $SeO_2$  in the ratio of 10:4:1 was taken as the digestion mixture.

b) Boric acid indicator solution: Twenty gram boric acid was dissolved in about 700 ml of hot distilled water. The solution was cooled and then transferred to one liter volumetric flask containing 20 ml of mixed indicator solution (prepared by dissolving 100 mg bromo-cresol green and 50 mg of methyl red in 100 ml of ethanol). After thoroughly mixing the contents of the flask, final volume was made up to one liter with distilled water.

c) Sulphuric acid: Concentrated sulphuric acid.

d) 0.01N HCl (for titration).

#### (ii) Digestion of sample

a) 0.5g dried sample was taken in a 100 ml flask and 15 ml of digestion acid mixture (1g digestion mixture in 15ml concentrated sulphuric acid) was added to it.

b) The contents in the flask were then heated at low temperature until frothing stopped and then temperature was raised to boiling point.

c) The flask was rotated in between and the digestion continued till the contents in the flask turned light yellow green.

d) The digest was cooled and final volume was made 50 ml with distilled water.

#### (iii) Distillation

a) An aliquot of 10 ml was taken from the digested sample and run in micro Kjeldhal apparatus with 10 ml of 40% NaOH.

b) 5 ml boric acid indicator was taken into a flask and placed below the condenser taking care that the tip of the condenser dipped into it. After this distillation was started and about 50 ml condensate was collected in the flask. The flask was removed before stopping the heat to prevent back sucking of the liquid. The indicator in condensate turned greenish-blue due to

dissolution of ammonia, which was titrated with 0.01N HCl. At the end point the colour changed from greenish-blue to permanent light pink

(iv) Calculation

$$\% \text{ Nitrogen} = S (a - b) \times N \times 4.1$$

where, a = volume of HCl used for sample (ml),

b = volume of HCl used for blank (ml),

S = weight of sample taken (g),

N = Normality of HCl,

1.4 = multiplication factor

5.11.5 Organic Carbon (OC)

This was estimated by Walkley-Black method (1996).

(i) Reagents

- a) Potassium Dichromate solution
- b) Sulphuric acid
- c) Phosphoric acid
- d) Barium diphenylamine sulphonate (Indicator)
- e) Ferrous sulphate (1M): For titration

(ii) Titration

- a) 0.5g of soil was taken in a flask and 5 ml of dichromate solution was added to it.
- b) After that 10 ml of conc. sulphuric acid was added to the flask and swirled.
- c) Allow the contents in the flask to stand for 30 min.
- d) After 30 min, 125ml of distilled water and 5ml of phosphoric acid is added to the contents in the flask. Allowed to cool.
- e) 0.5 ml of indicator (Barium diphenylamine sulphonate) was added to the flask.
- f) The contents in the flask were then titrated with ferrous sulphate (1M) which is brown in colour.
- g) The color changes to purple and end point is green.

(iii) Calculation

$$\text{Organic Carbon\%} = M \times [(V_1 - V_2)/S] \times 0.39 \times \text{mcf}$$

where, M = Molarity of ferrous sulphate solution (from blank titration)

V<sub>1</sub> = ml ferrous sulphate solution required for blank

$V_2$  = ml ferrous sulphate solution required for sample

S = weight of air-dry sample in grams

$0.39 = 0.3 \times 10^{-3} \times 100\% \times 1.3$  (3 = equivalent weight of Carbon)

mcf = moisture correction factor

#### 5.11.6 Organic Matter (OM)

Calculation

Organic matter % = 2 x % Carbon

#### 5.11.7 Phosphorus

Phosphorus was estimated by the method of John (1970).

##### (i) Reagents

a) Stock Solution: 20 g ammonium molybdate was dissolved in 300 ml of distilled water. 450 ml of 10N  $H_2SO_4$  was added to it slowly with constant stirring then 100 ml of 0.5% antimony potassium tartarate was added. Final volume was made to one liter with distilled water and stored away from direct light in a dark colored glass bottle.

b) Working Reagent: 1.5 g ascorbic acid was added to 100 ml of stock solution. This reagent was always prepared fresh.

c) Standard solution: Standard stock solution of 1000 mg/l (1000 ppm) was prepared by dissolving 0.439 g  $KH_2PO_4$  in 100 ml of distilled water and standard curve was prepared in the range of 0.2, 0.4, 0.6, 0.8 and 1.0  $\mu g$  /ml.

d) Diacid mixture: It was prepared by mixing concentrated nitric acid ( $HNO_3$ ) and perchloric acid ( $HClO_4$ ) in the ratio of 4:1 (v/v).

##### (ii) Procedure

a) 0.5 g soil was taken in a 250 ml digestion flask and 15 ml of diacid mixture was added to it. The mixture was digested in a digestion chamber till it became colourless. The contents were diluted to about 30 ml with distilled water, filtered through watman filter paper no.1 and transferred to a 50 ml volumetric flask, final volume was made 50 ml with distilled water.

b) From each flask 1 ml aliquot was taken in a 50 ml volumetric flask and 5 ml of freshly prepared mixed reagent was added to it. Final volume was made 50 ml with distilled water. After 30 min, absorbance of the solution was measured at 880 nm using a UV-Visible spectrophotometer-117.

#### 5.11.8 Potassium, sodium, lithium and calcium

Potassium, sodium, lithium and calcium were measured according to APHA (1998) with the help of Systronics Flame photometer-117 in the diacid digest of the samples prepared in section 3.11.7.2(a). Standard stock solution of 1000 mg/l (1000 ppm) Na and K was prepared by dissolving 0.2543 g of NaCl and 0.191g of KCl in 100 ml distilled water each and standard curve was prepared in the range of 20, 40, 60, 80 and 100 mg/l for Na and K.

#### 5.12 Genotoxic analysis in vitro

The microscopic and macroscopic parameters were also compared for pre and post vermicompost soil (recommended dose) after vermicomposting by *E. fetida* and *M. posthuma*.

The procedure of carrying out these genotoxic studies was same as described for PTS and VTS extracts in section 3.8.

#### 5.13 Pesticide content analysis

The amount of pesticide present in the soil media pre and post vermicompost was compared and the vermin-degradation of pesticides were studied.

##### 5.13.1 Acephate extraction

1. 10g pre and post vermicompost soil samples were taken separately.
2. In each soil sample, 50 ml of methanol was added.
3. Shake for 1h at 270 rpm. Evaporated all extracts to about 3ml on rotary evaporator.
4. Centrifuge at 4000 rpm.

The solvents were analysed by ESI-MS

##### 5.13.2 Atrazine extraction

Column was packed with a mixture of 10 g soil sample and 10 g of anhydrous sodium sulphate, 0.4 g florisil, 0.3 g charcoal having a layer of 15 g anhydrous sodium sulphate upper and lower side of this packing. One hundred twenty milliliters of ethyl acetate:hexane solution (1:1) was taken as an eluate.

The solvents extracted were further analysed by GCMS.

#### 5.14 Statistical Analysis

One way ANOVA was used to calculate the differences among various mixtures. Pearson's correlation coefficient was used to calculate the relationship between the concentrations and chemical parameters. Student's t-test was used to evaluate the differences between initial and final values of various chemical parameters. Statistical analysis was done with the help of Minitab 14 computer software programme. Probit analysis (Vincent 2008) was performed for calculation of lethal doses ( $LD_{50}$ ) and effective concentration of avoidance ( $EC_{50}$ ). JMP Software was also used for statistical analysis.

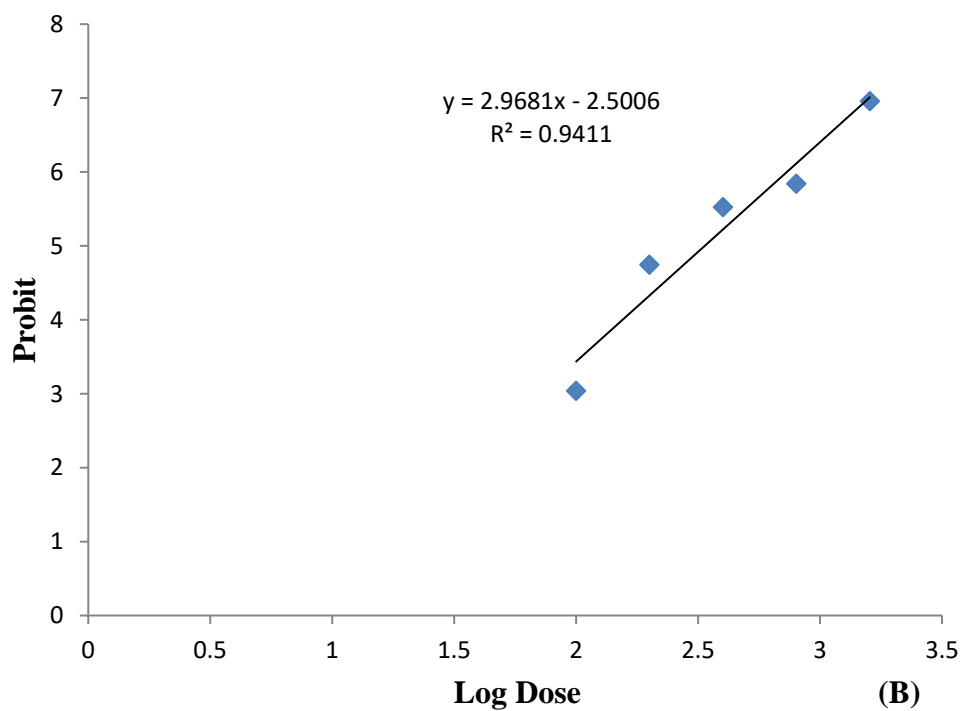
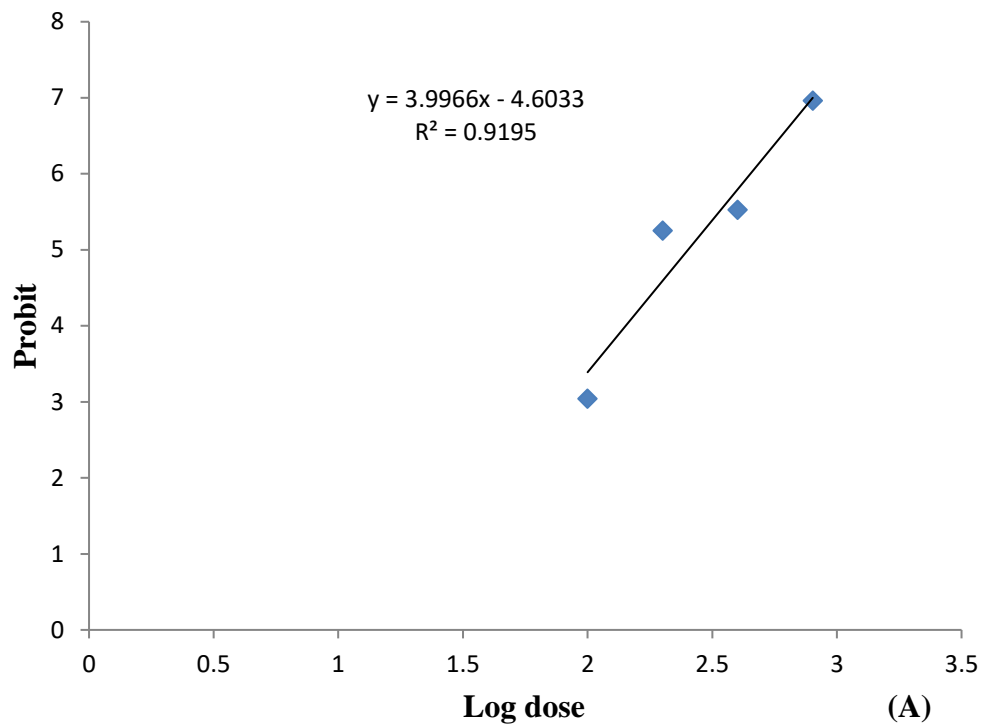
## 6. Results and Discussion

### 6.1 Atrazine toxicity

#### 6.1.1 Atrazine toxicity on earthworm species by filter paper contact test

The filter paper contact test is an initial screening technique to determine the relative toxicity of chemicals on earthworms in which the chemical is mainly absorbed by the skin. As per the guidelines of OECD and ISO, *E. fetida* is utilized for the evaluation of toxicity through contact filter paper test. However, we replicated the same experiment for our indigenous tropical variety, *M. posthuma*. The toxicity of atrazine towards *E. fetida* by filter paper method was found to be dose dependent. According to contact filter paper test, the pesticides are classified as supertoxic ( $<1 \mu\text{g}/\text{cm}^2$ ), extremely toxic ( $1\text{--}10 \mu\text{g}/\text{cm}^2$ ), very toxic ( $10\text{--}100 \mu\text{g}/\text{cm}^2$ ), moderately toxic ( $100\text{--}1000 \mu\text{g}/\text{cm}^2$ ) or relatively nontoxic ( $>1000 \mu\text{g}/\text{cm}^2$ ) based on the resulting  $\text{LC}_{50}$  values (Roberts and Dorough 1984). The lethal dose ( $\text{LD}_{50}$ ) for *E. fetida* exposed to atrazine on filter paper was found out to be  $253.01 \mu\text{g}/\text{cm}^2$  after 24h (**Fig 6.1**). This indicates that atrazine is ‘moderately toxic’ to *E. fetida*. Morphological abnormalities like coiling and twisting were observed in our study. Our results were found to vary with the findings of Wang et al. (2016) which depict the  $\text{LC}_{50}$  values of atrazine toxicity in *E. fetida* as  $22.75 \mu\text{g a.i}/\text{cm}^2$  (filter paper contact test) after 24 h. This result could be accredited to the fact that the formulation of atrazine considered for toxicity studies was Atrataf 50 WP and its toxicity would be lower than the active ingredient.

Wang et al. (2015) reported the toxicity with respect to active ingredient while in our study 50 WP formulation of atrazine was used. Chen et al. (2014) calculated the median lethal dose for atrazine with respect to *E. fetida* by filter paper contact test. The  $\text{LD}_{50}$  was found to be 143.2 mg/L after 48h using active ingredient while in our study with the use of formulation the lethal dose ( $\text{LD}_{50}$ ) was found to be  $253.01 \mu\text{g}/\text{cm}^2$  after 24h. Yang et al. (2015) also investigated the median lethal dose for earthworm *E. fetida* for atrazine via filter paper test and artificial soil toxicity test after 48h. The  $\text{LD}_{50}$  determined from filter paper test was  $143.03 \mu\text{g}/\text{ml}$ . The reason for the lower lethal dose found by Yang et al. (2015) may be again ascertained to the use of active ingredient of pesticide. Lydy and Linck (2003) reported the  $\text{LD}_{50}$  of  $2.9 \mu\text{g}/\text{cm}^2$  for *E. fetida* towards atrazine after 96h of exposure. Our contrasting results with Lydy and Linck (2003) can be attributed to the use of different formulation of herbicide and majorly to the difference in exposure of time. The



**Figure 6.1 Dose-response plots for calculation of LD<sub>50</sub> for (A) *E. fetida* (B) *M. posthuma* exposed to atrazine on filter paper test**



median lethal concentration for another similar triazine herbicide, azodrin was observed to be  $460 \pm 0.2 \mu\text{g}/\text{cm}^2$  for filter paper contact test. Coiling and curling were also observed for the same species (Rao and Kavitha 2004). In our study, *E. fetida* were reportedly found to be sluggish, few segments were swollen and secretion of coelomic fluid was observed at higher concentration after 10h. Worms were comparatively much less active in comparison to control. Significant anatomical changes were observed in the  $\text{LC}_{50}$  exposed worms during exposure of 24 h. Development of constriction and swelling below clitellar region was observed. Body had developed breaks and the lesions which led to fragmentation of the body.

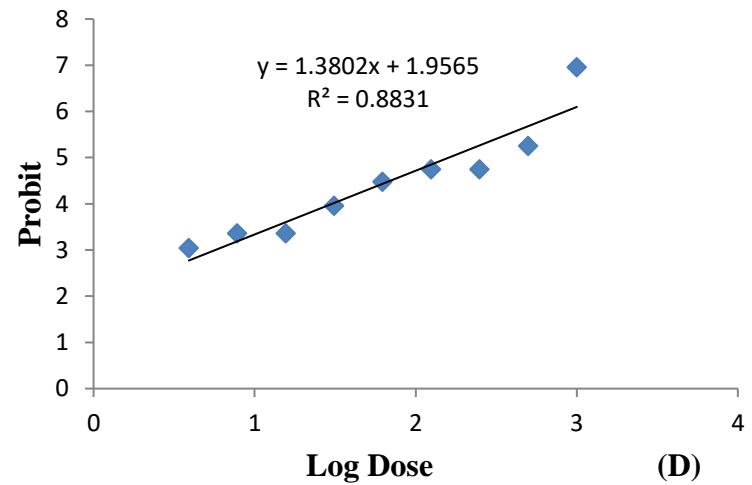
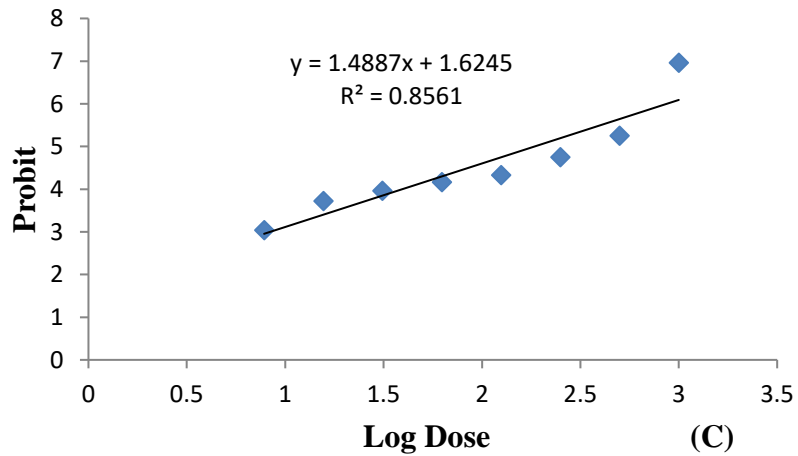
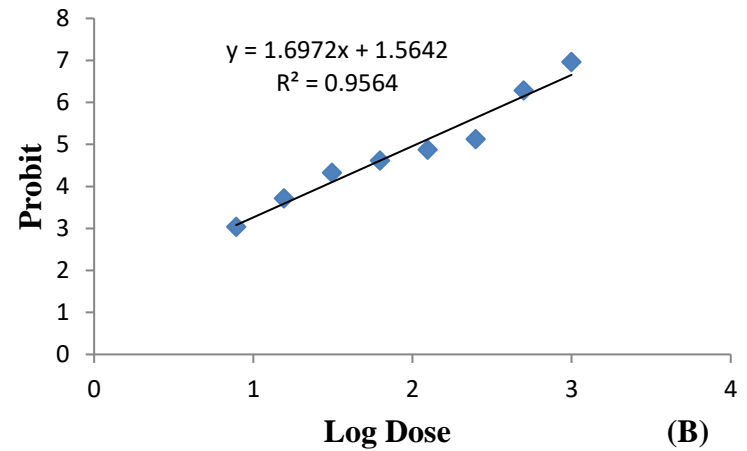
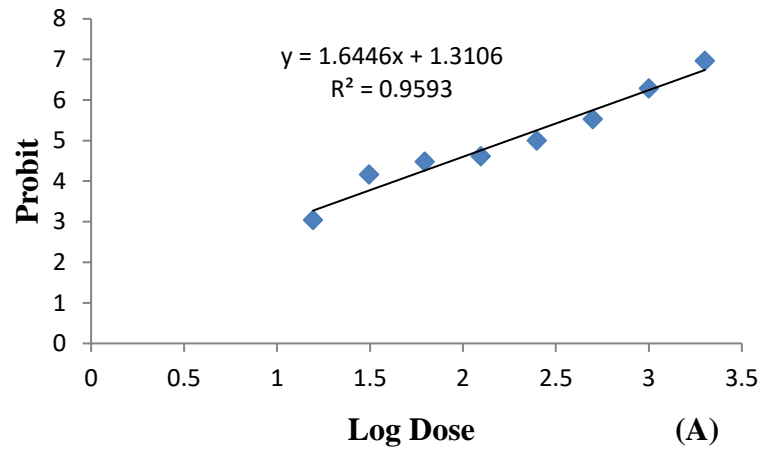
The lethal dose ( $\text{LD}_{50}$ ) for *M. posthuma* for atrazine on filter paper was found to be  $331.13\mu\text{g}/\text{cm}^2$ . Symptoms of coiling and curling were shown profusely. Segments were found to be swollen and constricted. Bloody lesions ultimately caused death. The reason for mortality on filter paper test might also be ascertained to physical stress on the earthworms as they were kept on filter paper in a glass petriplate without food for 24h with toxicant (Canas et al. 2011). There have been a number of data that use the filter paper test as a toxicity test for earthworm. But there is equally large number of researchers that doubt the validity of this test (De Silva et al. 2009b). Grumiaux et al. (2010) and Tripathi et al. (2010), ascertain that contact filter paper test poorly reflects the situation in the soil ecosystem. On the other hand, the artificial soil test mimics the exact natural environment of earthworms and in that, the pesticides are mainly absorbed by the gut (De Silva et al. 2009; Udovic and Lestan 2010). In our study, if we compare both the test species with respect to atrazine toxicity, we found that *E. fetida* was comparatively more sensitive than *M. posthuma* as it showed lower  $\text{LD}_{50}$  values.

### **6.1.2 Atrazine toxicity on earthworm species evaluated by OECD artificial soil toxicity test**

The toxicity of atrazine was also evaluated by OECD artificial soil toxicity method. The lethal concentration ( $\text{LC}_{50}$ ) atrazine in OECD recommended artificial soil for *E. fetida* was found to be 175.60 mg/kg after 7 days and 105.86 mg/kg after 14 days (**Table 6.1**). Our results corroborated with the findings of Chen et al. (2014); Wang et al. (2015) and Wang et al. (2016) with respect to  $\text{LD}_{50}$ . The lethal concentration ( $\text{LC}_{50}$ ) of atrazine in OECD recommended artificial soil for *M. posthuma* was found to be 184.00 mg/kg after 7 days and 160.32 mg/kg after 14 days (**Fig 6.2**). Chen et al. (2014) and Yang

et al. (2015) also found LD<sub>50</sub> for atrazine which came out to be 168.20 mg/kg after 14 day of OECD artificial soil test in *E. fetida*. In case of atrazine also, *E. fetida* was found to be sensitive with respect to *M. posthuma*, in OECD soil. After 7 days of exposure to atrazine both the species were found to exhibit similar toxicity to the herbicide while after 14 days exposure of atrazine *E. fetida* was found to be 1.5 times more sensitive than *M. posthuma*.

Our results can be compared with the findings of Wang et al. (2016) which depict the LC<sub>50</sub> values of atrazine toxicity in *E. fetida* as 204.8 and 180.4 mg a.i kg<sup>-1</sup> after 7 and 14 day of OECD artificial soil test. The results cannot be exactly compared as we used the pesticide formulations while Wang et al (2016) considered active ingredient (a.i) and also because of the different test species taken. The median lethal concentration for another similar triazine herbicide, azodrin was observed to be 171±21 mg/kg and 132±20 mg/kg, for 7 and 14 days respectively (Rao and Kavitha 2004). Atrazine is known to induce the P450 system, and converts chlorpyrifos to oxon metabolite (Miota et al. 2000). Such enzymes are also known to be found in invertebrate species (Oleksiak et al. 2000). Oluah et al. (2010) also studied the toxicity and histopathological effects of the herbicide atrazine on earthworm, *N. mbae*. The LC<sub>50</sub> was found to be 8.60, 7.05, 7.37, 7.23 after 24, 48, 72, 96 h. Forney and Davis (1981) also stated the inhibition of electron transport of photosystem II due to atrazine. This may induce general esterase activity in addition to cytochrome P450 in *E. fetida* (Lydy and Linck 2003). Such induction of enzymes is further responsible for pesticide breakdown which results in either increase or decrease of the toxicity of other pesticides depending on whether the resulting metabolites are more or less toxic than their parent compounds (Anderson and Zhu 2004). *E. fetida* was also tested for toxicity with respect to two herbicides, atrazine and butachlor and an insecticide k-cyhalothrin. The order of toxicity was found to be ranked as atrazine > k-cyhalothrin > butachlor (Chen et al. 2014). In many reports, atrazine have been reported to show synergistic effects when taken in a cocktail of pesticides. In combination with three organophosphate insecticides viz., chlorpyrifos, methyl parathion, and diazinon, atrazine was found to cause a significant increase in toxicity to *H. azteca* compared with individual pesticides (Anderson and Lydy 2002). The toxicity of chlorpyrifos was also known to be enhanced by atrazine and cynazine in the fourth instar larvae of the aquatic midge, *Chironomus tentans* (Clark et al. 2002). In *C. tentans*, the LD<sub>50</sub> determined from OECD artificial soil toxicity test was found to be 168.20 mg/kg which corroborated with our LD<sub>50</sub> 175.60 mg/kg although the comparison is not appropriate because of different species.



**Figure 6.2** Dose-response plots for calculation of LD<sub>50</sub> for *E. fetida* exposed to atrazine in OECD artificial soil after (A) 7 days (B) 14 days and *M. posthuma* exposed to atrazine in OECD artificial soil after (C) 7 days (D) 14 days

In case of atrazine toxicity in OECD soil, *E. fetida* was found to be as sensitive as *M. posthuma* after 7 days whereas after 14 days *E. fetida* was found to be 1.5 times more sensitive than its indigenous counterpart, *M. posthuma*.

### **6.1.3 Atrazine toxicity on earthworm species evaluated by a modified soil toxicity test**

Another modified version of soil toxicity test was considered for evaluation of atrazine toxicity. The artificial soil was replaced by natural test medium (cow dung and garden soil). This was performed to establish relation between earthworms and realistic environmental conditions and as such how does the test actually vary with the artificial soil toxicity test recommended by OECD. The lethal concentration (LC<sub>50</sub>) of atrazine in cow dung for *E. fetida* was found to be 197.88 mg/kg after 7 days and 80.61 mg/kg after 14 days (**Table 6.1**). The lethal concentration (LC<sub>50</sub>) of atrazine in garden soil for *M. posthuma* was found to be 372.34 mg/kg after 7 days and 210.62 mg/kg after 14 days (**Fig 6.3**). *E. fetida* was found to be almost twice as sensitive with respect to *M. posthuma* in respective natural test medium whereas after 14 days the sensitivity of *E. fetida* as compared to *M. posthuma* rose to 2.6 times. Thus, the tropical indigenous species were found to be more tolerant species in comparison to temperate exotic species, *E. fetida*, which is best known for the process of vermicomposting. This could be ascertained to the fact that the local species that were used for the experiment was collected from agricultural fields and as such they are adapted to the exposure by pesticides whereas *E. fetida* is not found in agricultural soils of Punjab. The exotic species is being cultivated in medium of cow dung for industrial bioremediation or research purpose. Thus, the difference in their sensitivity. This was also supported by De Silva and Gestel (2009) who also reported greater sensitivity of *E. fetida* in comparison to local worms of Sri Lanka. Singh et al. (2016a) also reported that *M. posthuma* was found to be maximum in all field types and that *M. posthuma* has completely adapted to physical disturbance, intensive use of insecticide and pesticide and also human interventions. Their endogeic nature is another reason for their abundance (Ernst and Emmerling 2009; Jouquet et al. 2010).

Concluding all the three test results (filter paper, OECD artificial soil and modified soil toxicity test) performed to evaluate atrazine toxicity, it was inferred that OECD artificial soil toxicity method was found to be the most sensitive of all. However, the significance of modified soil toxicity test cannot be ignored as it gives the first hand comparison of toxicity testing under natural habitats. Also in natural medium, the toxicity was found to be less than OECD artificial soil depicting that natural medium forms the optimum condition for

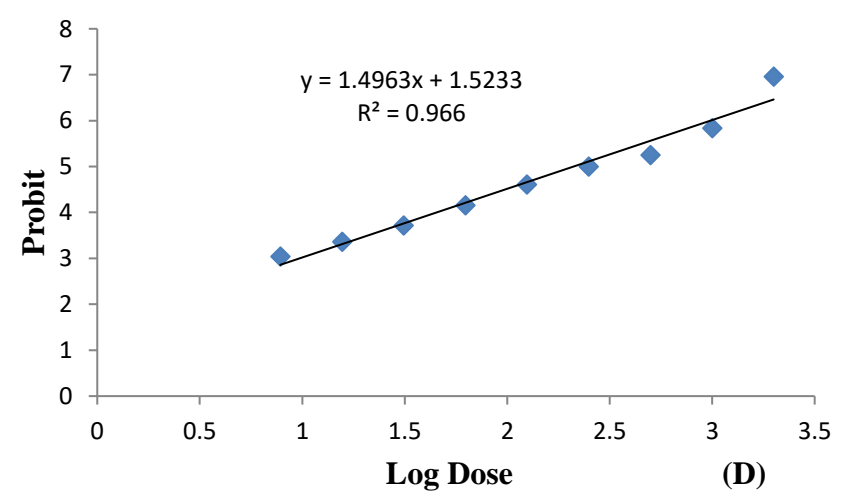
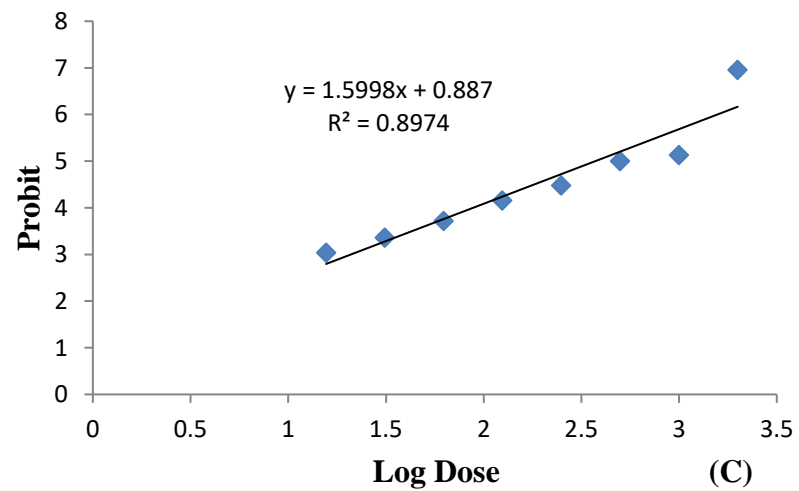
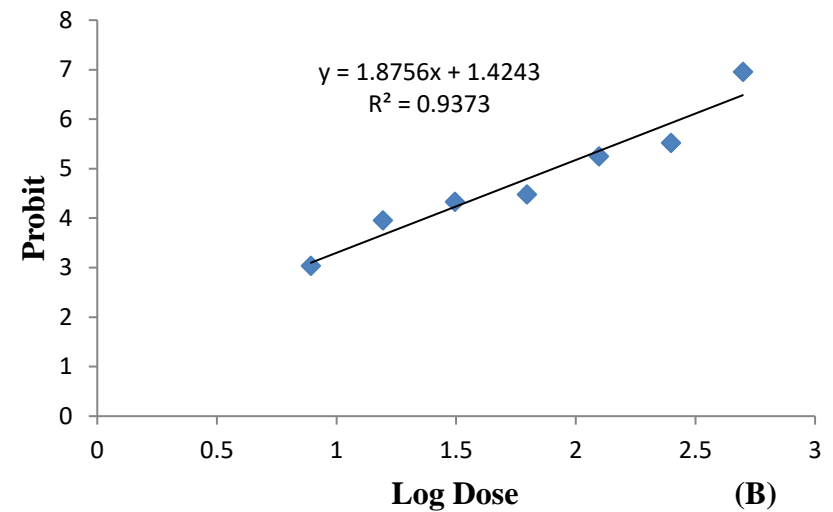
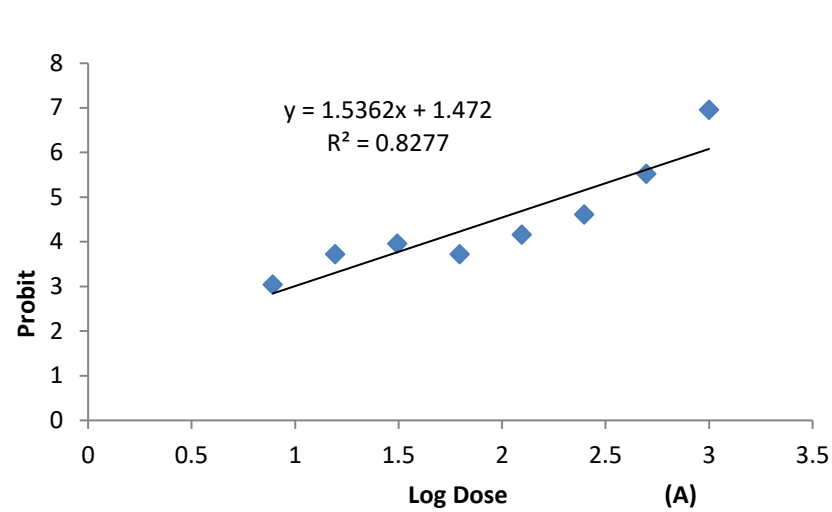


Figure 6.3 Dose-response plots for calculation of LD<sub>50</sub> for *E. fetida* exposed to atrazine in cow dung after (A) 7 days (B) 14 days and *M. posthuma* in garden soil after (C) 7 days (D) 14 days

**Table 6.1 Comparative account of toxicity of atrazine and acephate evaluated from various toxicity tests in various mediums**

Species	Test type	Soil type/medium	Calculated value	Time period	Acephate conc.	Atrazine conc.
<i>E. fetida</i>	Filter paper test		LD <sub>50</sub> (µg/cm <sup>2</sup> )	24 h	54.65	253.01
	OECD artificial soil toxicity test	OECD	LD <sub>50</sub> (mg/kg)	7 d	67.31	175.60
				14 d	50.73	105.86
	Modified soil toxicity test	Natural (CD)	LD <sub>50</sub> (mg/kg)	7 d	143.90	197.88
				14 d	92.93	80.61
	Avoidance test	OECD	EC <sub>50</sub> (mg/kg)	48 h	26.37	49.78
		Natural (CD)	EC <sub>50</sub> (mg/kg)	48 h	52.55	74.61
	<i>M. posthuma</i>	Filter paper test		LD <sub>50</sub> (µg/cm <sup>2</sup> )	24 h	75.85
OECD artificial soil toxicity test		OECD	LD <sub>50</sub> (mg/kg)	7 d	153.33	184.00
				14 d	74.58	160.32
Modified soil toxicity test		Natural (GS)	LD <sub>50</sub> (mg/kg)	7 d	168.60	372.34
				14 d	95.40	210.62
Avoidance test		OECD	EC <sub>50</sub> (mg/kg)	48 h	49.88	112.09
		Natural (GS)	EC <sub>50</sub> (mg/kg)	48 h	85.03	110.71

earthworm activity and survival and as such also the tolerant capacity of earthworm is found better in natural conditions owing to the fact that they are use to it.

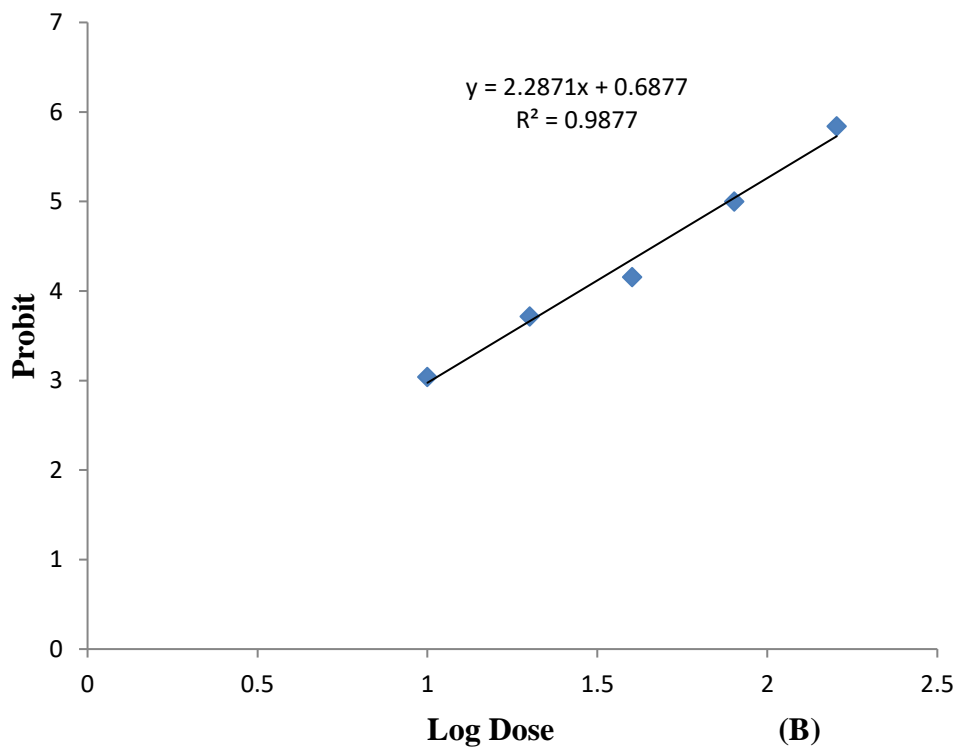
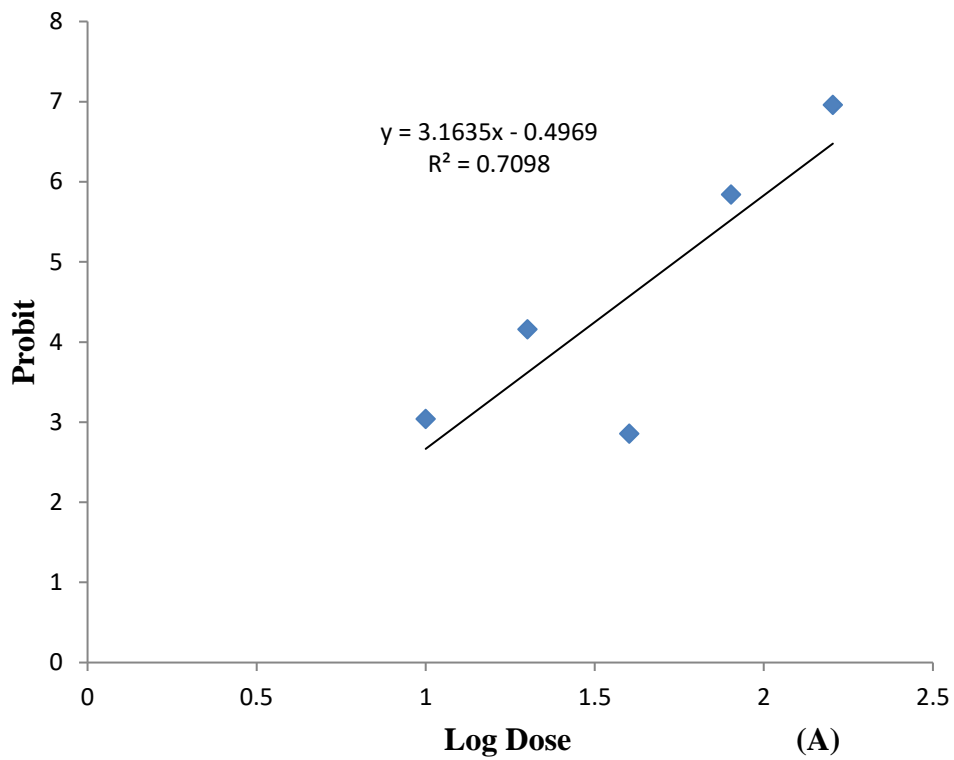
## **6.2 Acephate toxicity**

### **6.2.1 Acephate toxicity on earthworm species evaluated by filter paper contact test**

The toxicity of acephate towards *E. fetida* by filter paper method was found to be dose dependent. The lethal dose (LD<sub>50</sub>) for *E. fetida* exposed to acephate on filter paper was found out to be 54.65µg/cm<sup>2</sup>(**Fig 6.4**). This indicates that acephate is “very toxic” to earthworm species according to the classification by Roberts and Dorough (1984). This also leads us to the fact that acephate when absorbed even through the skin, produces acute toxic effects. The morphological abnormalities did not show any development of coiling and twists. Mucus secretion was also observed by 10 h. After 14 to 20h of exposure, worms exhibited surface lesions, extrusion of coelomic fluid resulting in lesions and mortality aftermath. This observation was similar to the findings of Reddy and Rao (2008) while studying the toxicity of another organophosphorus insecticide, profenofos. The earthworm bodies in higher concentration appear to be thin, slimy and melted after 24 h. The earthworms at lower concentrations show retarded movement at 10h. Significant anatomical changes included breaks in body and bloody lesion on the body of earthworm. No swelling was observed but extrusion of excessive coelomic fluid was observed. Reddy and Rao (2008) also observed release of excessive coelomic fluid while studying the toxicity of a similar organophosphorus insecticide, profenofos on *E. fetida*. The LC<sub>50</sub> value of profenofos was found to be 4.56±0.14 µg/cm<sup>2</sup> for 24 h which is comparatively much lower for what we found for acephate.

Similar observations like, retarded activity, surface lesions, coelomic fluid release in addition to ruptures in body wall were seen in filter paper contact toxicity tests in *E. fetida* exposed to organophosphorous herbicide, glyphosate (Correia and Moreira 2010). Another organophosphorus insecticide, Chlorpyriphos incurred lethal toxicity on earthworm species, *E. fetida* with an LC<sub>50</sub> value of 0.047±0.006 µg/cm<sup>2</sup>and 0.037±0.006 µg/cm<sup>2</sup> for 24 and 48 h respectively (Rao et al. 2003).

This test has sought its application on *E. fetida* species as recommended by ISO and OECD. But here we replicate the entire test on *M. posthuma*. In case of *M. posthuma*, the lethal dose (LD<sub>50</sub>) for acephate was found to be 75.85µg/cm<sup>2</sup>. There have been absolutely nil data that we have come across with respect to filter paper contact test studied in *M. posthuma* species. If we compare the tolerant ability of both the species, we found that *E. fetida* was comparatively more sensitive than *M. posthuma* for acephate.



**Figure 6.4 Dose-response plots for calculation of LD<sub>50</sub> for (A) *E. fetida* (B) *M. posthuma* exposed to acephate on filter paper test**



### 6.2.2 Acephate toxicity on earthworm species evaluated by OECD artificial soil toxicity test

The lethal concentration (LC<sub>50</sub>) of acephate in OECD recommended artificial soil for *E. fetida* was found to be 67.31 mg/kg after 7 days and 50.73 mg/kg after 14 days. In case of *M. posthuma*, the lethal dose (LD<sub>50</sub>) was found to be 153.33 mg/kg after 7 days and 74.58 mg/kg after 14 days exposure to acephate in OECD artificial soil (**Fig 6.5**). The indigenous species was found to be significantly more tolerant species than *E. fetida* which is best known for the process of vermicomposting. *E. fetida* was found to be 2.3 times more sensitive than *M. posthuma* after 7 days of acephate exposure. However, after 14 days *E. fetida* was found to be 1.48 times more sensitive than *M. posthuma*. There have been many studies on the effects of pesticides on earthworms. However, the data on formulations of atrazine and acephate is very rare. Chen et al. (2014) carried out toxicity experiments with butachlor, imidacloprid, and chlorpyrifos on *E. fetida*. Acephate is also shown to have DNA damaging properties. The nucleophilic attack on phosphorus moiety in organophosphorous pesticides, like acephate leads to DNA damage and alkylation of DNA bases directly or in an indirect way via protein alkylation leading to disintegration of DNA (Bhinder and Chaudhary 2014). A connection between exposure to acephate and abnormal metabolism including glucose, nucleic acid and protein metabolism was also found (Hao et al. 2012). Bhinder and Chaudhary (2013) concluded that 75% SP formulation of acephate forms to be a potential mutagen for the genome of mosquito, *Culex quinquefasciatus* as they induce dominant lethality and that the usage of these pesticides can be deleterious to the genome of other living systems. Many other researchers report of acephate being a mutagenic and carcinogenic (Garrett et al. 1986; Perocco et al. 1996). However, Preetha et al. (2009) compared the toxicity of insecticides, thiamethoxam, acephate, chlorantraniliprole and endosulfan on *Trichogramma chilonis* and found out that acephate was safest out of all having lowest LD<sub>50</sub> in comparison to other insecticides. Booth and O'Halloran (2001) studied the influence of an organophosphorus insecticide chlorpyrifos on the chronic toxicity of earthworm species, *A. caliginosa* at different concentrations. It was found that growth and fecundity was significantly affected even at lower dose of 28 mg/kg. Yang et al. (2015) also investigated the LD<sub>50</sub> for chlorpyrifos, another organophosphorus insecticide and classified as moderately toxic as its median lethal concentration was found to be 377mg/kg.

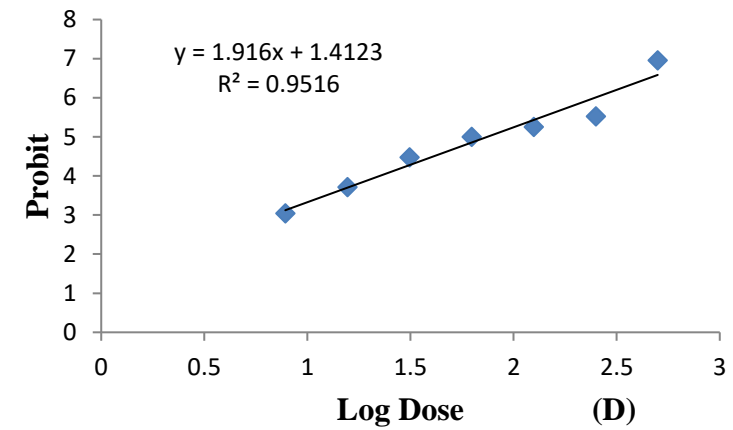
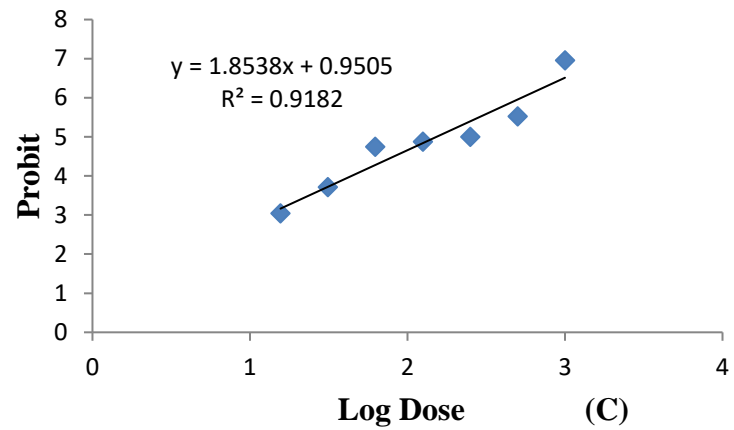
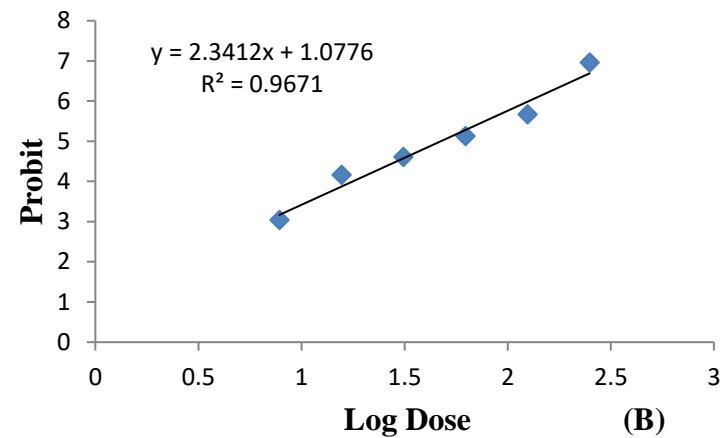
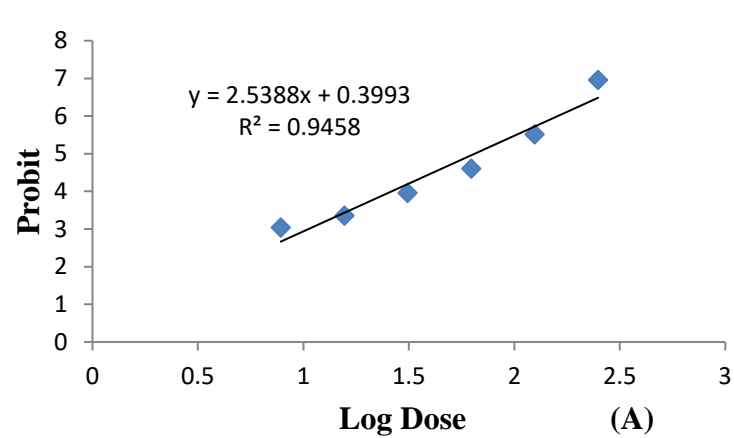


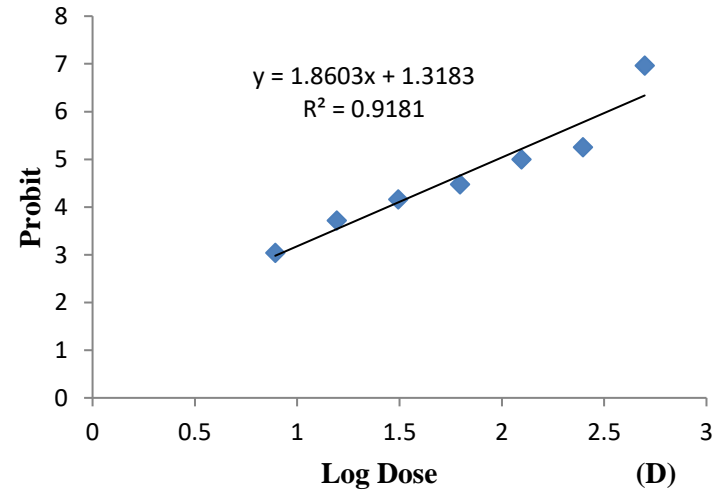
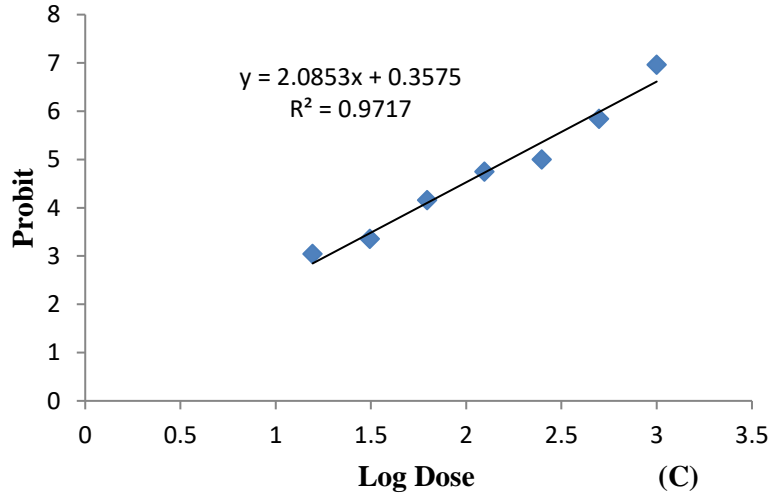
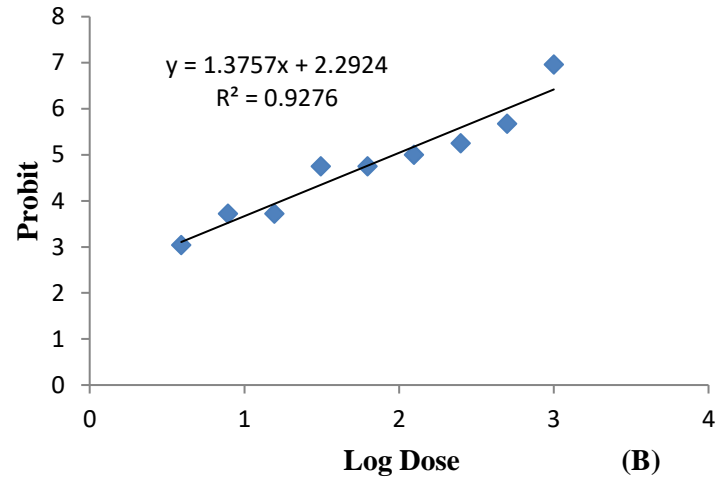
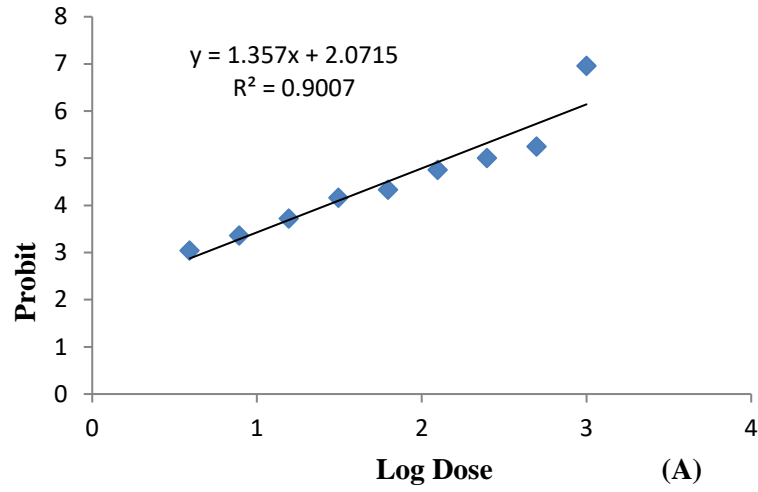
Figure 6.5 Dose-response plots for calculation of LD<sub>50</sub> for *E. fetida* exposed to acephate in OECD artificial soil after (A) 7 days (B) 14 days and *M. posthuma* exposed to acephate in OECD artificial soil after (C) 7 days (D) 14 days

### **6.2.3 Acephate toxicity on earthworm species evaluated by a modified soil toxicity test using natural test medium**

The toxicity of acephate was evaluated after 7 and 14 days for *E. fetida* and *M. posthuma* in respective natural medium. For *E. fetida* pre-composted cow dung was used and for *M. posthuma*, garden soil was utilized. This was performed to establish relation between earthworms and realistic environmental conditions and as such how does the test actually vary with the artificial soil toxicity test recommended by OECD. The lethal concentration (LC<sub>50</sub>) of acephate in cow dung for *E. fetida* was found to be 143.90 mg/kg after 7 days and 92.93mg/kg after 14 days. In case of *M. posthuma*, the lethal dose (LD<sub>50</sub>) was found to be 168.60 mg/kg after 7 days and 95.40 mg/kg after 14 days exposure to acephate in OECD artificial soil (Fig 6.6). *E. fetida* was found to be more sensitive than *M. posthuma* after 7 and 14 days of acephate exposure.

Basically, the organophosphates are alkylating agents that either directly disintegrate DNA or indirectly via protein alkylation (Mohn 1973; Wild 1975). The nucleophilic attack on the phosphorus moiety in organophosphates causes phosphorylation of the DNA (Mohn 1973). Acephate is known to be more potential genotoxicant than chlorpyrifos. The reason for this could be attributed to the difference in their chemical structure. Chlorpyrifos has a bulky side chain unit containing aromatic ring and the substitution of the oxygen atom by a sulfur atom in P=O bond whereas acephate molecule containing the P=O bond may be the reason for the reduced genotoxic activity seen with chlorpyrifos. Sulphur-containing organophosphates, to which acephate belongs, are S-oxidized to highly reactive intermediates within cells and tissues, which may even lead to mutagenic changes in the body cells and tissues (Farang et al. 2000).

Acephate, belonging to this group of organophosphates, has been implicated as a potent generator of free radicals, their basic mechanism of action, causing oxidative stress and damage to tissues (Rao et al. 1991).



**Figure 6.6 Dose-response plots for calculation of LD<sub>50</sub> for *E. fetida* exposed to acephate in cow dung after (A) 7 days (B) 14 days and *M. posthuma* in garden soil exposed to acephate in garden soil after (C) 7 days (D) 14 days**

Concluding the toxicity of acephate with all the three tests performed it was observed that filter paper test in case of *E. fetida* and OECD artificial soil test in case of *M. posthuma* was found to be the most sensitive. The natural test medium in vermireactors mimics the natural environment condition and forms natural miniature surroundings for the test species. This forms the reason as to why the toxicity is comparatively lower in natural test medium than OECD for both test species. Comparing the two species in terms of toxicity of acephate, it was found that *E. fetida* was found to be more sensitive than *M. posthuma*.

Also natural medium condition support earthworm survival and tolerant capacity. The reason of this could be attributed to the fact that local species are perpetual with the pesticide spray and thus are resistant to the same. Because of their natural conditions, *M. posthuma* are more tolerant to pesticides than *E. fetida*.

Though many literature supports that *E. fetida* are capable to tolerate highly toxic substances, etc (Saxena et al. 2014) but our study dignified *M. posthuma* having more tolerant capacity than *E. fetida*.

### **6.3 Effect of Atrazine and Acephate on the avoidance behavior of earthworm species**

#### **6.3.1 Validity of tests**

According to ISO (2008) guidelines (17512-1), two validity criteria are fulfilled for the avoidance test to be considered valid.

- (i) The experiment should be considered invalid if the number of dead or missing worms is  $> 10\%$  per treatment.
- (ii) The avoidance behavior should be validated by a dual control test.

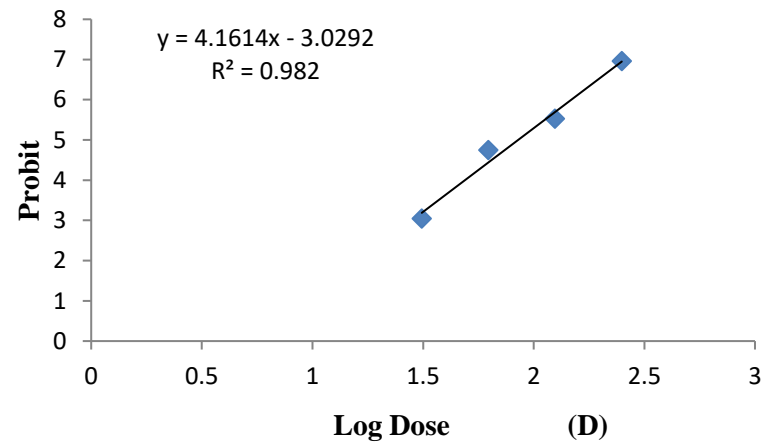
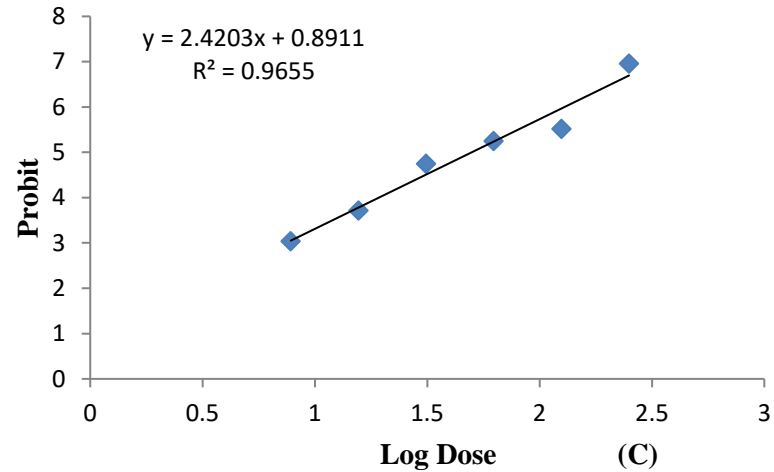
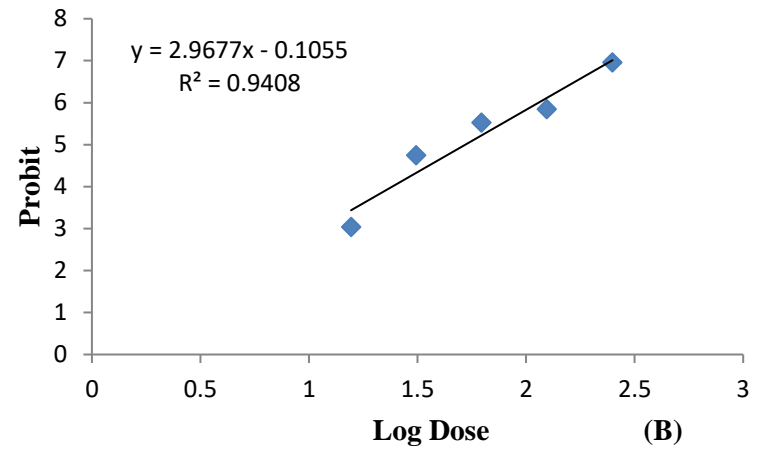
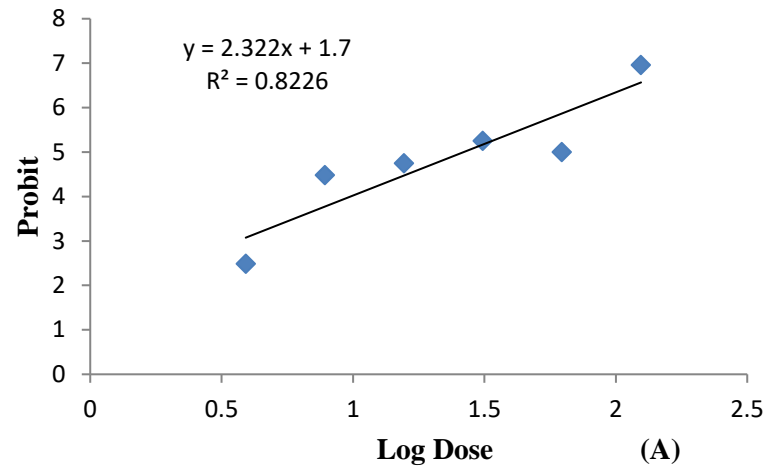
The first criterion was fulfilled in all the tests with OECD artificial soil and natural test medium. The avoidance tests were also validated by dual control test to check if the worms in the absence of a contaminant, do not congregate but distributes themselves randomly in both sides of vessel and do not display behavior that might be mistaken for avoidance (Yeardley 1996). For this, control soil was used in both the sections of the vessel and earthworm avoidance behavior was analysed. The proportion of earthworms on both sides in the dual control test with OECD soil and natural medium (CD and GS) were found to be non-significant (student t-test,  $p > 0.05$ ). The concentration at which 100% mortality was recorded was eliminated from the statistical analysis of the avoidance behavior.

### 6.3.2 Avoidance Behaviour

A positive (+) net response indicates avoidance and a negative (-) net response indicates negative avoidance or attraction towards the chemical tested. In the lower doses of atrazine and acephate, hormesis effect was observed. Hormesis is a biphasic response phenomenon whereby a positive (beneficial) effect results from exposure to low doses of a chemical which otherwise is toxic or lethal at higher concentration. Within the hormetic zone, a favorable biological response to low doses is observed. Hormetic effects can usually be observed in a low-dose zone, below the no observed effect levels (Calabrese 2005). In the lower doses of atrazine and acephate, the number of earthworms was found more in the test soil than control. Thus, non-avoidance i.e. attraction (form of hormesis in this case) was observed in both the species for both the pesticides in both types of soil media.

#### 6.3.2.1 Effect of insecticide acephate

The effective concentration (EC<sub>50</sub>) of avoidance for acephate by *E. fetida* was found to be 26.37 mg/kg and 52.55 mg/kg in OECD and CD respectively. While in case of avoidance by *M. posthuma*, the effective concentration (EC<sub>50</sub>) was found to be 49.88 mg/kg and 87.57 mg/kg in OECD and GS respectively. Another organophosphate insecticide dimethoate was also evaluated for its toxicity by earthworm avoidance test. The EC<sub>50</sub> values were found to be 24.06 and 9.73 mg/kg in OECD and natural medium depicting its high toxicity (De Silva and Amarasinghe 2008). It was found that *E. fetida* was 1.9 times more sensitive than *M. posthuma* with respect to acephate in OECD artificial soil. However, in natural medium, *E. fetida* was found to be 1.7 times more sensitive than *M. posthuma* for acephate (**Table 6.2**). Habitat function (i.e. 80% of the worms in the control soil or  $\geq 60\%$  avoidance response) was reduced at acephate concentration of  $\geq 62.5$  mg/kg in CD whereas in OECD it was reduced at 125mg/kg dry soil for *E. fetida*. For *M. posthuma*, habitat function was reduced at 62.5 mg/kg dry soil in OECD and  $\geq 125$ mg/kg dry soil in GS (**Table 6.2**). The negative avoidance behavior (attraction) with respect to acephate was observed in the lowest concentration (3.90 mg/kg) for both soil types in *M. posthuma*. *E. fetida* also showed negative avoidance (attraction) in the lowest concentration (3.90 mg/kg) in OECD artificial soil but in case of CD two lower most concentrations (3.90, 7.80 mg/kg) depicted negative avoidance. Such type of negative avoidance (hormesis) was also observed by De Silva and Gestel (2009) while studying the sensitivity of *E. fetida* and *P. excavatus* towards chlorpyrifos and carbofuran by avoidance test.



**Figure 6.7** Dose-response plots for calculation of  $EC_{50}$  for avoidance exhibited by *E. fetida* in (A) OECD artificial soil (B) cow dung and *M. posthuma* in (C) OECD artificial soil (D) garden soil, exposed to acephate

**Table 6.2 Avoidance response of *E. fetida* and *M. posthuma* to acephate in artificial OECD soil and natural medium**

Concentration (mg)	Mean net response			
	<i>E. fetida</i>		<i>M. posthuma</i>	
	OECD	CD	OECD	GS
3.90	-60	-60	-20	-40
7.81	30	-20	0	0
15.62	40	20	10	-20
31.25	60	0	40	10
62.5	50	60	60	40
125	100	80	70	70
250	100	100	100	100

**Table 6.3 Avoidance response of *E. fetida* and *M. posthuma* to atrazine in artificial OECD soil and natural test medium**

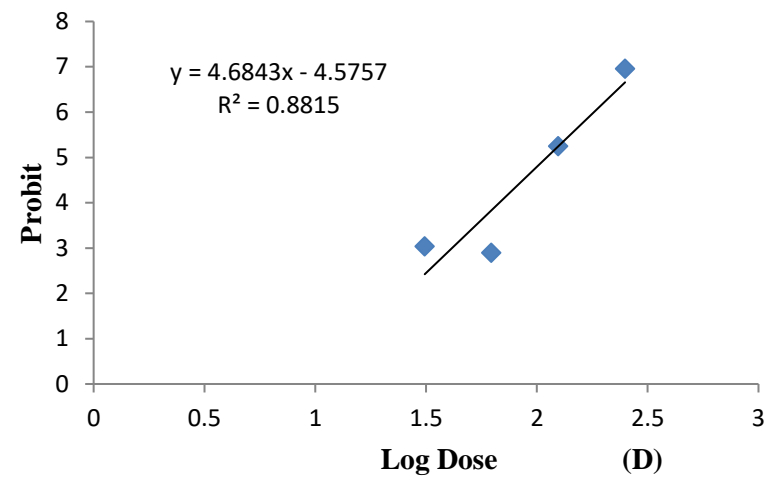
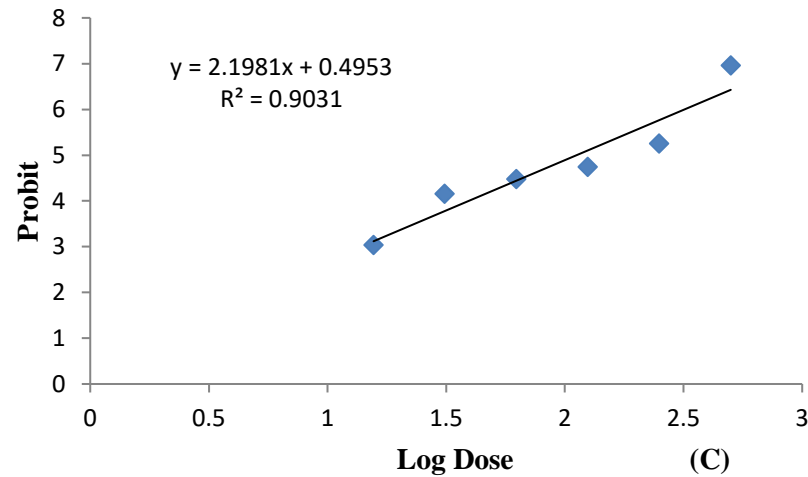
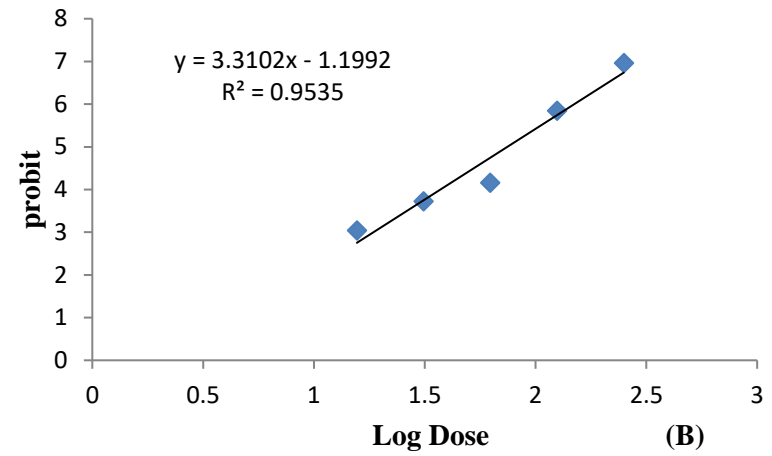
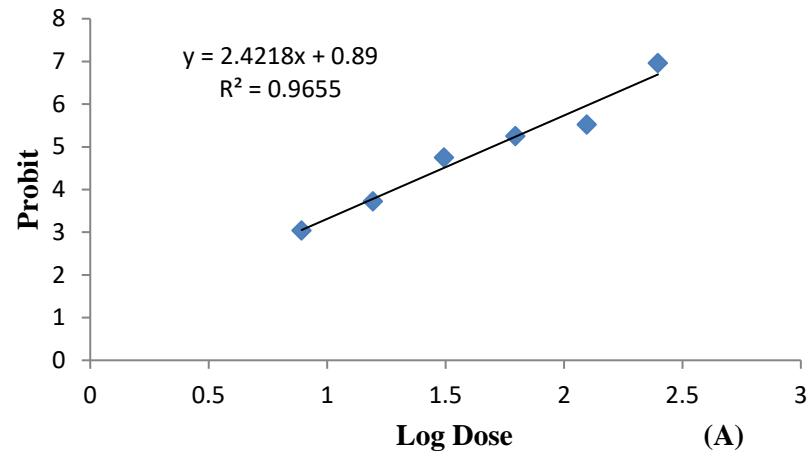
Concentration (mg)	Mean net response			
	<i>E. fetida</i>		<i>M. posthuma</i>	
	OECD	CD	OECD	GS
3.90	-20	-20	-10	0
7.81	0	-60	0	-20
15.62	10	-10	20	-20
31.25	40	10	30	0
62.5	60	20	40	20
125	70	80	60	60
250	100	100	100	100



### 6.3.2.2 Effect of herbicide atrazine

The effective concentration ( $EC_{50}$ ) of avoidance for atrazine by *E. fetida* was found to be 49.78 mg/kg and 74.61 mg/kg in OECD and CD respectively. While in case of avoidance by *M. posthuma*, the effective concentration ( $EC_{50}$ ) was found to be 112.09 mg/kg and 110.71 mg/kg in OECD and GS respectively. The avoidance of *E. fetida* for atrazine in OECD artificial soil was 2.3 times more than *M. posthuma*. In natural medium, *E. fetida* was found to be 1.5 times more sensitive than *M. posthuma* for atrazine. Habitat function (i.e. 80% of the worms in the control soil or  $\geq 60\%$  of avoidance response) was reduced at atrazine concentration of  $\geq 62.5$  mg/kg soil for *E. fetida* in OECD soil while in CD the habitat function was reduced to  $\geq 125$  mg/kg dry soil. For both soil types in *M. posthuma* the habitat function was found to be reduced at  $\geq 125$  mg/kg dry soil. The negative avoidance behavior or attraction with respect to atrazine was observed in few lower doses as depicted in **Table 6.3**. Other pesticides like benomyl and carbendazim were also found to be avoided by the earthworms (Loureiro et al. 2005; Garcia et al. 2008).

The avoidance test is known as the first and foremost tier of risk analysis of contaminated sites with various chemicals. It can be considered as a sensitive tool as earthworms detect and respond to a wide range of contaminants like hydrocarbons, heavy metals, explosives, crude oil and pesticides (ISO 2007). In the present study we report the avoidance of *E. fetida* and *M. posthuma* towards acephate and atrazine in natural medium and OECD soil. According to ISO (200), the experiment should be considered invalid if the number of dead or missing worms is  $>10\%$  per treatment. Acephate was more toxic to both the species in both the soil types with lower  $EC_{50}$  values in comparison to atrazine. This avoidance test also supports the results of our toxicity analysis with respect to both test species type; both pesticides and both the soil types. The results of avoidance tests with respect to pesticides have been found to vary. According to Garcia et al. (2008) and Loureiro et al. (2005), benomyl and carbendazim was found to be avoided by earthworms. This was also supported by Reinecke et al. (2002) and Garcia et al. (2008) who reported the avoidance behavior of earthworms towards mancozeb and lambda-cyhalothrin respectively. However Hodge et al. (2000) reported a lack of avoidance response by *Apporectodea caliginosa* towards diazinon and chlorpyrifos both in fields and laboratory analysis. According to Garcia et al. (2008) the avoidance test is clearly more sensitive than the acute toxicity tests and at least as sensitive as the chronic one.



**Figure 6.8 Dose-response plots for calculation of EC<sub>50</sub> for avoidance exhibited by *E. fetida* in (A) OECD artificial soil (B) cow dung and *M. posthuma* in (C) OECD artificial soil (D) garden soil exposed to atrazine**

If we compare both the species type, we infer that *E. fetida* was much more sensitive than *M. posthuma* because the EC<sub>50</sub> was found to be much higher in case of the latter. De Silva et al. (2009b) also found *E. fetida* being more sensitive than local earthworms from Sri Lanka. Singh et al. (2016a) also reported that *M. posthuma* was found to be maximum in all field types and that *M. posthuma* has completely adapted to physical disturbance, intensive use of insecticide and pesticide and also human interventions. Their endogeic nature is another reason for their abundance (Ernst and Emmerling 2009). *E. fetida* is an epigeic species and *M. posthuma* is an endogeic species. Both of them have relatively different ecological niches and therefore the difference in their sensitivities.

According to Mather and Christen (1998), field application of pesticides leads to surface migration of earthworms which can be viewed as a consequence of avoidance. Capowiez and Berard (2006) reported endogeic species, *Allolobophora icterica* more sensitive than anecic *Apporrectodea nocturna* in terms of avoidance behaviour to imidacloprid. The sensitivity of earthworms depends on the species (Edwards and Coulson 1992) and on the ecological types (Tomlin 1992). The sensitivity of various species differs due to the characteristic differences in chemoreceptors (Stephenson et al. 1998), physiological and morphological (Edwards and Bohlen 1996) and ecological differences (Lukkari and Haimi 2005). In contrast, Owojori and Reinecke (2009), reported that irrespective of soil properties and ionic constitution, *A. caliginosa* was found to more sensitive than *E. fetida*. This was also contrary to the results of Pearce and Pearce (1979) who found *E. fetida* to be more sensitive than *A. caliginosa* in avoidance test taking sand as substrate. The differences in substrates could be ascertained for this differed response as soil properties on their own have the capability to affect behavioural response (Amorim et al. 2008). Ellis et al. (2007) compared the avoidance behaviour of *E. fetida* in carbendazim in two artificial soils; one containing kaolin clay and other comprised of bentonite clay. Both the soil types showed significant differences in mortality indicating that clay type also play a role in influencing toxicity. However, De Silva et al. (2009b) found that temperature plays a bigger role in avoidance behaviour than soil type. The avoidance tests can therefore have clear advantages as the first and foremost screening tool for risk assessment and secondly in soil quality criteria studies, warranting quantitative assessment of the contaminants bioavailability and toxicity. The laboratory tests though account for limitations for avoidance toxicity testing. According to Ellis (2007), avoidance tests are unable to account for the influence of soil conditions such as moisture on earthworm behaviour and subsequent exposure to pollutants. The avoidance

behaviour of earthworms are also influenced by organic matter content and the texture of soils (acting singly or cumulative) (Natal-da-Luz et al. 2008). Field studies or terrestrial model ecosystem experiments (Burrows and Edwards 2004) are required to estimate the modification of earthworm behaviour with respect to soil conditions.

In our study, the tropical species, *M. posthuma* was found to be less sensitive than *E. fetida* in avoidance tests with herbicide, atrazine and insecticide, acephate under tropical conditions. This variation may lead to a wrong estimate of toxicity of contaminants in soil. Thus, *E. fetida* could still be recommended well out of two because of its greater sensitivity. This study also depicted that the soil medium plays a crucial role in the avoidance behaviour which ultimately influences the toxicity of the contaminants found in the medium. The organic matter and soil texture needs to be noticed for conducting such tests. Predictive models for the influence of pedological properties of soil on the avoidance response needs to be validated further by testing more natural and manipulated soils. These cumulative present and future aspects of research could then be correctly utilised for analysis of real site specific avoidance. Further a clear evaluation of ecological range for specific species when defined can lead to segregation of usage of specific species for specific soil type.

Avoidance tests are undoubtedly useful screening tools for examination of potential contaminants in the soil (Schaefer and Achazi 2004). Magnanimous amount of literature depicts the correlation of avoidance and mortality and therefore the avoidance test is an easy, time saving alternative to long term tests like survival and reproduction tests. The results in this study try to make a possible contribution to overcome a lack of data on the effect of pesticides under tropical conditions.

#### **6.4 Effect of soil (affected with pesticides) procured from farm lands by genotoxicity assessment (*A. cepa* test)**

Physico-chemical analysis of both the soil types was performed. Toxic effects of pesticide sprayed soil (PTS) and vermicompost sprayed soil (VTS) were evaluated in terms of macroscopic and microscopic parameters.

##### **6.4.1 Physico-chemical analysis**

Significant difference was observed between the two soil types with respect to physico-chemical parameters (**Table 6.4**). In VTS the pH ( $7.63 \pm 0.011$ ) was recorded to be significantly higher ( $p < 0.05$ ) than the PTS ( $7.32 \pm 0.018$ ) although both were alkaline in nature. The increase in pH may be the results of the decomposition of nitrogenous substances

during vermicomposting (Muthukumaravel et al. 2008). Also earthworms support the population build up of catabolically active microbes (Aira et al. 2007; Kaur et al. 2010) which results into degradation of short chain fatty acids and precipitation of calcium carbonate which may have lead to increase in pH of VTS. However it has also been reported that pH is dynamic and substrate dependent (Ndegwa and Thompson 2000a). Humus is also reported to bind free cations and raise pH of the soil (Brady and Weil 2002) which may be another reason for a higher pH in the products of vermicomposting.

The electrical conductivity (EC) reflects the salinity of a material and a good indicator of applicability and utility of compost or vermicompost in agricultural purposes. The electrical conductivity decreased significantly ( $p < 0.001$ ) from PTS to VTS. This decrease can be attributed to the stabilization of the mixtures and reduction of ions. This was corroborated with the findings of Singh et al. (2014) and Singh et al. (2016b) while studying the vermicomposting of beverage industry sludge and comparison of vermicast and various other soils respectively. Total dissolved salts (TDS) also showed similar trend as EC ( $p < 0.001$ ). The total organic matter was also significantly different ( $p < 0.001$ ). The TOM in case of VTS was 1.5 folds higher than PTS. The total organic carbon (TOC) was found to be significantly higher ( $p < 0.001$ ) in VTS in comparison to PTS. TOC in case of VTS was  $4.91 \pm 0.02$  while in that of PTS was  $3.35 \pm 0.01$ . Several studies support these results explaining about carbon loss from substrates in the form of  $\text{CO}_2$  brought out by the combined action of earthworms and microbes (Tognetti et al. 2007; Hait and Tare 2011; Singh and Suthar 2012; Dominguez and Edwards (2004). Kaviraj and Sharma (2003); Bhat et al (2014); Prakash and Karmegam (2010) have also reported reduction of TOC as  $\text{CO}_2$  during vermicomposting in sugar industry waste; municipal waste; sugar mill sludge respectively.

Total available phosphorus (TAP) showed no significant difference with respect to the samples VTS and PTS. The gut enzymes of earthworms stimulate the phosphate solubilizing microbes thereby promoting the release of phosphorus in vermicast (Suthar 2009; Prakash and Karmegam 2010; Sangwan et al. 2010). Krishnamoorthy (1990); Tripathi and Bhardwaj (2004); Bayon and Binet (2006), observed that an increase in phosphate content of vermicompost was due to presence of acid and alkaline phosphatases in the worm gut. However the level of phosphorus in PTS can be attributed to the use of phosphate solubilizing fertilizers and organophosphorus pesticides. Total potassium (TK) significantly ( $p < 0.001$ ) declined from PTS to VTS. In VTS, TK was decreased by 53.11%. Garg and Gupta (2009) and Ravindran et al. (2008) also reported similar decline in level of potassium during the

vermicomposting of textile mill sludge and solid waste from leather industry respectively. Singh et al. (2010); Kumar et al. (2010) predicted this decline due to the use of potassium by earthworms during metabolic activity. Sangwan et al. (2010) reported higher potassium content in the vermicompost produced from the distillery sludge. Total Sodium (TNa) decreased significantly ( $p < 0.001$ ) from PTS to VTS. The percent decrease was (48.90%). This was supported by Bhat et al. (2013); Kaur et al. (2010) but Subramanian et al. (2010) observed that sodium content remained unchanged during the vermicomposting of sago industry solid wastes. Total Calcium (TCa) level was found to be significantly ( $p < 0.001$ ) higher in VTS than PTS. The TCa level in VTS was 94.07% higher than PTS. These results corroborated with the findings of Garg and Kaushik (2005); Oyedele et al. (2006) and Yadav and Garg (2011) but Chaudhari et al. (2009) reported low content of Ca in cast compared to surrounding soil. Earthworms promote the process of mineralization converting a proportion of bound form of calcium to free forms, resulting in its enrichment (Garg and Kaushik 2005). There was no significant difference in the level of Total lithium (TLi) with respect to VTS and PTS.

**Table 6.4 Comparative account of physico-chemical properties (mean  $\pm$  SE) of Vermicompost treated soil (VTS) and Pesticide treated soil (PTS)**

S.No.	Parameter	Vermicompost treated soil (VTS)	Pesticide treated soil (PTS)
1.	Soil texture	Sandy Loam	Sandy Loam
2.	pH	7.63 $\pm$ 0.011	7.32 $\pm$ 0.018*
3.	EC ( $\mu$ S/cm)	524.23 $\pm$ 0.01	576.19 $\pm$ 0.16***
4.	TDS (ppm)	373.16 $\pm$ 0.08	406.21 $\pm$ 0.26***
5.	TOC (%)	4.91 $\pm$ 0.02	3.354 $\pm$ 0.01***
6.	TOM (%)	9.92 $\pm$ 0.06	6.69 $\pm$ 0.01***
7.	TAP(g/kg)	0.257 $\pm$ 0.05	0.273 $\pm$ 0.03
8.	TNa (g/kg)	0.585 $\pm$ 0.02	1.145 $\pm$ 0.07***
9.	TK (g/kg)	2.047 $\pm$ 0.39	4.366 $\pm$ 0.20***
10.	TCa (g/kg)	11.342 $\pm$ 3.19	5.844 $\pm$ 1.94***
11.	TLi (g/kg)	1.227 $\pm$ 0.28	1.126 $\pm$ 0.19

The level of significance was determined by Student's t-test: \* $p \leq 0.05$ , \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.001$

#### 6.4.2 Macroscopic parameters

*A. cepa* test is standard test for rapid and sensitive screening of chemicals and pollutants that represent environmental hazards. Root tip is often the first and foremost part of a plant that comes into contact with chemicals/pollutants found in water or soil. Root tip system of *A. cepa* has particularly shown sensitivity to harmful effects of such environmental hazards (Fiskesjo 1995). The effects were observed after 24 h and 48 h. However there are several studies that dictate toxic effects of various chemicals with the help of *A. cepa* test in less than 24 h even in just 3 h (Grover and Kaur 1999; Yuzbasioglu et al. 2009; Katnoria et al. 2011; Soodan et al. 2012; 2015). The effects may be observed by analyzing macroscopic parameters like root growth and root shape or consistency or by microscopic parameters like mitotic index and frequency of chromosomal aberrations. The root lengths of *A. cepa* after exposure to various concentrations of VTS and PTS were significantly different after 24 h and 48 h (**Table 6.5**). The maximum root length was observed in 10% PTS after 24 h and then in 10% VTS concentration after 48 h. The root length and concentration were negatively correlated in both cases of PTS after exposure of 24 h ( $r = -0.74$ ,  $n = 6$ ,  $P < 0.05$ ) and 48 h ( $r = -0.99$ ,  $n = 6$ ,  $P < 0.05$ ) and in case of VTS after 24 h ( $r = -0.96$ ,  $n = 6$ ,  $P < 0.05$ ) and 48 h ( $r = -0.90$ ,  $n = 6$ ,  $P < 0.05$ ). The average decrease in root length was far more prominent in PTS extracts concentrations than in VTS and significant difference in root lengths were found in 20%, 40% and 80% concentrations of PTS and VTS. It was observed that PTS extracts suppressed root growth when compared with VTS extracts. Thus, VTS shows ameliorated effects than PTS. The root forms like the presence of twists (crochet, hooks) were noticed in higher concentration of PTS after 48 h whereas VTS showed normal growth of roots in all concentrations. The regulation of root growth is brought together by independent events that lead to the process of cell division in the mitotically active meristematic zone and cell elongation in proximal region of the root tip (Shishkova et al. 2008). Inhibition of root development and the appearance of stunted roots are indicators of growth retardation and cytotoxicity (Herrero 2012; Goujon 2014; Liman 2015).

#### 6.4.3 Microscopic Parameters

Growth retardation in *A. cepa* is explained by cytotoxicity and chromosomal anomalies as genotoxicity. Toxicity is not always related to genotoxicity (Kovalchuk 1998). The parameter of cytotoxicity is reliable, quick and sensitive enough for monitoring even slightly polluted surface waters (Linnainmaa et al. 1978). Our study evaluated the potential application of plant genotoxicity tests for scanning mutagens in agricultural soils. Several

other studies have been carried out using this bioassay to evaluate the genotoxicity of glyphosate (Rank et al. 1993), diuron (Chauhan et al.1998), cypermethrin and fenvalerate (Chauhan et al. 1999), monocrotophos and chlorpyrifos (Sinha and Kumar 2014), atrazine (Sharma and Vig 2012; malathion (Bianchi et al. 2015), industrial waste (Katnoria et al. 2011), agricultural residue (Christofoletti et al. 2013).

The data of mutagenicity assays and plant genotoxicity is considered to be of limited value by regulatory agencies when extrapolated to humans; although they do accept data of non-mammalian systems like bacteria, yeast, drosophila etc. But it has been found out that certain chemicals give comparable results in terms of genotoxicity in plant and animal systems. Hence, *A. cepa* bioassay may prove to be a useful alternative for animal experimentation for analyzing cytotoxicity and genotoxicity (Kovalchuk 1998).

**Table 6.5 Comparative root length of *A. cepa* after exposed to various concentrations of VTS and PTS extracts after 24 h and 48 h**

Concentration	Exposure time (h)	VTS		PTS	
		Root length (cm)	% Root length	Root length (cm)	% Root length
Control	24h	3.51 ± 0.34	100	3.51 ± 0.34	100
	48h	4.22 ± 0.40	100	4.22 ± 0.40	100
10%	24h	3.34 ± 0.50	95.10	3.52 ± 0.58	100.28
	48h	4.35 ± 0.96	103.08	3.80 ± 0.48	90.04
20%	24h	3.49 ± 0.80	99.43	2.68 ± 0.47**	76.35
	48h	4.11 ± 1.16	97.39	3.74 ± 0.56	88.62
40%	24h	3.18 ± 0.85	90.59	2.51 ± 0.54*	71.50
	48h	4.03 ± 1.20	95.49	3.56 ± 0.51**	84.36
60%	24h	3.01 ± 0.51	85.75	2.84 ± 0.61	80.91
	48h	3.79 ± 1.23	89.81	3.26 ± 0.29	77.25
80%	24h	2.59 ± 1.06	73.78	2.47 ± 0.43	70.37
	48h	3.80 ± 1.12	90.04	3.03 ± 0.31*	71.80
100%	24h	2.25 ± 0.78	64.10	2.28 ± 0.33	64.95
	48h	2.94 ± 1.48	69.66	2.64 ± 0.36	62.55

The level of significance was determined by Student's t-test: \*p≤0.05, \*\*p≤0.01



#### 6.4.3.1 Mitotic index (MI)

MI was calculated by the total number of dividing cells in the cell cycle. The mitotic index of the negative control is  $26.14 \pm 1.60$  after 24 h of exposure and  $26.16 \pm 1.31$  after 48 h. These figures were similar to the results of negative control (distilled water) as studied by Liman et al. 2010; Sharma and Vig 2012; Kuchy et al. 2016; but in some studies the mitotic index for control (distilled water) is even higher than these values (Konuk et al. 2007; Liman et al. 2011 ). The mitotic index was reduced to  $10.30 \pm 0.91$  and  $09.75 \pm 0.62$  after 24 and 48 h of exposure in case of 100 % PTS while in 100% VTS the mitotic was found to be  $24.40 \pm 1.74$  and  $25.40 \pm 0.80$  after 24 h and 48 h respectively. This is the first clear indication of soil enriched with vermicompost being a better alternative. The mitotic index is significantly more in VTS than PTS. A concentration dependent inhibition of MI was observed with increasing concentration of PTS. MI observed after exposure of 10% VTS ( $26.04 \pm 0.15$ ) after 48 h was similar as that of control ( $26.16 \pm 1.31$ ). The lowest concentration (10%) of PTS produced minimum negative impact and highest concentration (100%) produced maximum negative impact on mitotic activity of meristematic cells of roots of *A. cepa* (**Table 6.6**).

The reduction in mitotic index was dose and duration dependent. A decrease in MI in *A. cepa* has been previously related to the exposure of pesticides (Saxena et al. 2005). The decrease in MI can be related to the cytotoxic potential of the test compound (Samka-kincl et al. 1996; Kaymak and Goc-Rasgele 2009; Radic et al. 2010). Fiskesjo (1988) also reported a significant decline in MI due to the effect of toxic chemicals on spindle apparatus. The mitotic index is contemplated to be reliable for identification of cytotoxic pollutants present in the environment (Grover and Kaur 1999; Chandra et al. 2005). The mitotic index in case of VTS is consistent and in PTS it subsequently decreased. The MI in both PTS and VTS were significantly different for all the concentrations at both 24 and 48 h of exposure except 10% at 24 h. This suggests a highly cytotoxic behavior of pesticides. In our studies, we have found a high negative correlation between mitotic index and concentration ( $r = -0.953$ ,  $n = 6$ ,  $P < 0.05$ ) after 24 h and ( $r = -0.947$ ,  $n = 6$ ,  $P < 0.05$ ) after 48 h of treatment for extracts of PTS while MI from VTS extracts showed positive correlation with concentration ( $r = 0.083$ ,  $n = 6$ ,  $P < 0.05$ ) after 24 h and ( $r = 0.082$ ,  $n = 6$ ,  $P < 0.05$ ) after 48 h of treatment. The linear relationship between concentrations (10% ,20%, 40%, 60%, 80%, 100%) and mitotic index of PTS and VTS was obtained by regression analysis (**Fig. 6. 9**). On the other hand, it was found that the addition of vermicompost plays an important role in promotion of cell division and proliferation. The MI for VTS can be related to previous reports that tribute an increase in

plant height, number of leaves, fruit weight higher in vermicompost treated field than control (Mamta et al. 2012; Dhanalakshmi et al. 2014). For the evaluation of cytotoxic and toxic potential of contaminants for environmental pollution monitoring, the reduction or increase in mitotic index are principal indicators (Hoshina 2002). Vermicompost is known to provide impetus to growth of various plant species like strawberry, groundnut, chilli, garlic, tomato, sweetcorn (Arancon et al. 2004; Suthar 2009; Lazcano et al. 2011; Kumar et al. 2012; Adhikary and Gantayet 2012; Abduli et al. 2013).

#### 6.4.3.2 Chromosomal aberrations (CA)

The occurrence of chromosomal bridges, breaks, vagrants, C-mitosis, laggards and stickiness were more prominent in 100% concentration of PTS, both after 24 h and 48 h of exposure whereas mild frequency of aberrations are observed in and after 24 h and 48 h of exposure in VTS. The lowest frequency of CA was observed to be 2% in control after 24 h and in 10% VTS after 24 h. The highest frequency of CA (54.50%) was found to be in 100% of PTS extract after 48 h (**Table 6.7**). PTS significantly ( $P < 0.05$ ) induced higher chromosomal aberrations than VTS. The percentage of chromosomal aberration showed positive correlation with concentration of extract after 24 h of treatment in PTS extract ( $r = 0.989$ ,  $n = 6$ ,  $P < 0.05$ ) and after 48 h of treatment in PTS extract ( $r = 0.931$ ,  $n = 6$ ,  $P < 0.05$ ); also after 24 h ( $r = 0.945$ ,  $n = 6$ ,  $P < 0.05$ ) and 48 h ( $r = 0.983$ ,  $n = 6$ ,  $P < 0.05$ ) of treatment with VTS extract (Fig. 6.10). Thus, the rate of chromosomal aberrations increased as the concentration of extracts increased. Chromatid breaks give rise to bridges and fragments. The formation of bridges may also be ascribed to unequal exchanges that lead to formation of dicentric chromosomes which are equally pulled at both poles in anaphase stage (Sax and Sax 1968). The breakage and fusion of chromosomes and chromatids or changing activation of replication enzymes are also responsible for formation of bridges (Badr et al. 1992; Luo et al. 2004). Spindle anomalies lead to vagrant chromosomes. The failure of chromosomes or acentric fragments to move to either pole is responsible for formation of laggards (Evseeva 2005). The unequal translocation, inversions of chromosome segment, the formation of chromatin bridges attribute to stickiness and results in non-separation of chromosomes at anaphase (El-Najjar and Soliman 1982). Entanglement of inter chromosomal chromatin fibres or affected peripheral proteins such as DNA topoisomerase II also leads to stickiness. C-mitosis occurs when a cell or its progeny becomes polyploid (McGill et al. 1974; Klasterska et al. 1976; Gaulden 1987).

**Table 6.6 Effect of Pesticide treated soil (PTS) and Vermicompost treated soil (VTS) on mitotic index and chromosomal aberrations of the root meristem cells of *A. cepa***

Concentration	Exposure time (h)	Mitotic Index <sup>a</sup>	
		VTS	PTS
Control	24 h	26.14 ± 1.60	26.14 ± 1.56
	48 h	26.16 ± 1.31	26.06 ± 1.31
10%	24 h	24.57 ± 2.28	24.10 ± 0.23
	48 h	26.04 ± 0.15	21.95 ± 0.79***
20%	24 h	25.10 ± 1.89	19.58 ± 1.35**
	48 h	24.23 ± 0.96	19.71 ± 0.51***
40%	24 h	27.22 ± 3.37	16.62 ± 0.55***
	48 h	27.10 ± 1.39	15.19 ± 0.68***
60%	24 h	28.17 ± 0.53	12.72 ± 0.52***
	48 h	26.41 ± 0.41	10.82 ± 0.35***
80%	24 h	26.27 ± 0.51	12.20 ± 0.30***
	48 h	25.93 ± 0.90	09.58 ± 1.00***
100%	24 h	24.40 ± 1.74	10.30 ± 0.91***
	48 h	25.40 ± 0.80	09.75 ± 0.62***

<sup>a</sup>out of 4000-6000 cells examined for Mitotic index

The level of significance was determined by Student's t-test: \*p≤0.05, \*\*p≤0.01, \*\*\*p≤0.001

The term 'c-mitosis' was coined by Levan (1938) so as to depict the effect of a chemical which prevents spindle microtubule assembly by dissociation of disulphide bonds the way colchicine acts. Stickiness is an irreversible chromosome abnormality that leads to cell death (Fiskesjo 1997; Goujon 2014). Chemicals can produce genotoxic effects either directly or indirectly by inhibiting the DNA repair system; for example by competing with certain ions essential for DNA polymerases (Hartwig 1994; Cebulska-Wasilewska et al. 2005). The aberrations induced by direct genotoxic effects may be repaired by intervention of DNA repair mechanisms to maintain the integrity of the genome (Vodicka et al. 2004). But indirect genotoxic effects prove to be exponentially toxic. Glyphosate is one such pesticide which decreases DNA repair (Cavas and Konen 2007). In the present study, (PA) anaphase lags were found to be maximum in PTS treatment for both 24 h (44.34%) and 48 h (27.63%) of exposure out of total aberrations. This was followed by C-mitosis in both 48 h (22.68%)

and 24 h (17.81%). Anaphase lags show two groups of anaphasic chromosomes that lay close to each other near the equatorial plate. With respect to clastogenic aberrations (CGA) in PTS the chromosomal breaks in 24 h (10.12%) and 48 h (10.07%) was more than chromosomal bridges.

In case of VTS, out of total aberrations, chromatin breaks were found to be maximum in 24 h (21.59%) and 48 h (20.80%) followed by C-mitosis in 24 h (20.07%). Vagrants were found to be the least (9.93%). The marked number of chromosomal aberrations in PTS depicts the genotoxic effects of pesticides on *A. cepa*. These results are in accordance to reports describing the vegetable extracts showing chromosomal aberrations in *A. cepa* because of pesticide residues (Feretti et al. 2007) and also with some previous study of Dragoeva et al. (2009); Soodan et al. (2012), who also reported various chromosomal abnormalities like vagrant chromosomes, chromosomal fragments at anaphase and telophase and multipolar anaphases depicting genotoxicity of agricultural soil. Leme et al. (2012) also reported various chromosomal abnormalities while assessing the genotoxicity of a soil matrix contaminated with bio-diesel. Similar studies from other parts of the world reported various mitotic and chromosomal abnormalities predicting the genotoxic and clastogenic potential of contaminated soil (Masood and Malik 2013; Souza et al. 2013). However, vermicompost delineate positive results based on a number of agricultural studies. Gopinath et al (2008) reported an improvement in water and air availability, encouraging root growth and seedling emergence when vermicompost was applied @ 60 kg/ha for two consecutive growing seasons. In case of tomato, vermicompost amended soil pots (20tn/ha) showed better growth than plants grown in inorganic fertilizer amended soil (Kashem et al. 2015). A significant increase in the growth and productivity was found in strawberries cultivated with 5 and 7.5 tn/ha of vermicompost in comparison with strawberries cultivated with equivalent doses of mineral fertilizers (Lazcano and Domínguez 2011). The present study indicated the genotoxic potential of PTS extract and also an inclination towards the practicability of vermicompost to ameliorate the toxicity/genotoxicity. Thus, it can be concluded that vermicompost might be overall beneficial in terms of soil health, plant health and crop productivity ensuring safe standards for the biodiversity that help maintain the eco-equilibrium.

**Table 6.7 Different chromosomal aberrations (physiological and clastogenic) in the root meristem cells of *A. cepa* exposed to pesticide treated soil (PTS) extract for 24 h and 48 h**

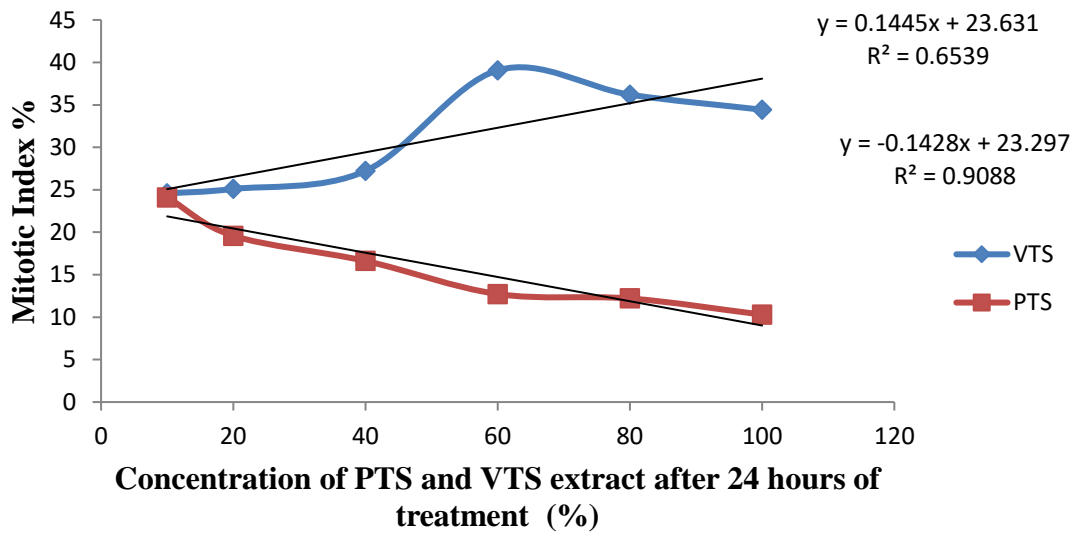
Type of Chromosomal aberration	Time of exposure	No. of aberrant cells <sup>a</sup>						
		Control	10%	20%	40%	60%	80%	100%
<b>Physiological Aberrations (PA)</b>								
C-Mitosis	24 h	-	-	20	27	42	58	80
	48 h	15	28	75	82	80	115	105
Anaphase lag	24 h	7	15	25	115	123	145	135
	48 h	5	57	95	97	85	135	135
Stickiness	24 h	-	5	22	20	17	30	42
	48 h	10	20	70	65	70	67	100
Vagrants	24 h	5	5	13	15	28	42	50
	48 h	-	15	40	53	47	60	112
<b>Total PA</b>	24 h	12	25	80	177	210	275	307
	48 h	30	120	280	297	282	377	452
<b>Clastogenic Aberrations (CGA)</b>								
Chromosomal Bridges	24 h	3	-	5	3	10	13	25
	48 h	-	10	15	16	30	33	40
Chromosomal Breaks/ Fragments	24 h	5	12	15	17	20	27	33
	48 h	15	22	20	17	33	62	53
<b>Total CA</b>	24 h	8	12	20	20	30	40	58
	48 h	15	32	35	33	63	95	93
Total Aberration (PA+CGA)	24 h	20	37	100	197	240	315	365
	48 h	45	152	315	330	345	472	545
<b>Percent Aberration</b>	24 h	<b>2</b>	<b>3.7</b>	<b>10</b>	<b>19.7</b>	<b>24</b>	<b>31.5</b>	<b>36.5</b>
	48 h	<b>4.5</b>	<b>15.2</b>	<b>31.5</b>	<b>33</b>	<b>34.5</b>	<b>47.2</b>	<b>54.5</b>

<sup>a</sup>out of 1000 cells examined

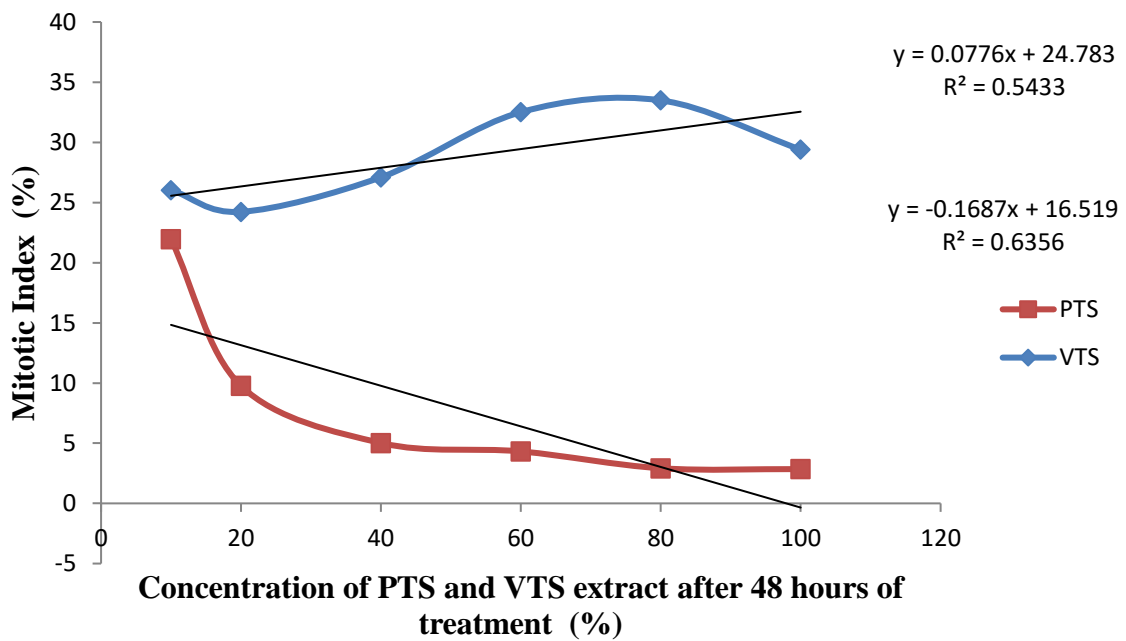
**Table 6.8 Different chromosomal aberrations (physiological and clastogenic) in the root meristem cells of *A. cepa* exposed to vermicompost treated soil (VTS) extract for 24 h and 48 h**

Type of Chromosomal aberration	Time exposure of	No. of aberrant cells <sup>a</sup>						
		Control	10%	20%	40%	60%	80%	100%
<b>Physiological Aberrations (PA)</b>								
C-Mitosis	24 h	-	5	5	5	7	17	14
	48 h	5	8	5	10	10	13	10
Anaphase lag	24 h	7	5	5	7	5	8	5
	48 h	5	5	7	8	7	10	7
Stickiness	24 h	-	5	3	5	10	12	10
	48 h	5	4	5	7	10	5	7
Vagrants	24 h	5	-	-	3	8	8	8
	48 h	-	-	3	5	3	7	8
<b>Total PA</b>	24 h	12	15	12	20	30	45	37
	48 h	15	17	20	30	30	35	32
<b>Clastogenic Aberrations (CGA)</b>								
Chromosomal Bridges	24 h	3	5	8	5	7	7	13
	48 h	10	10	5	7	10	12	10
Chromosomal Breaks/ Fragments	24 h	5	-	5	10	10	10	17
	48 h	2	5	10	10	12	15	13
<b>Total CA</b>	24 h	8	5	5	15	17	17	30
	48 h	12	15	15	17	22	27	35
Total Aberration (PA+CGA)	24 h	20	20	25	35	47	62	55
	48 h	27	32	35	47	52	62	67
<b>Percent Aberration</b>	24 h	<b>2</b>	<b>2</b>	<b>2.5</b>	<b>3.5</b>	<b>4.7</b>	<b>6.2</b>	<b>5.5</b>
	48 h	<b>2.7</b>	<b>3.2</b>	<b>3.5</b>	<b>4.7</b>	<b>5.2</b>	<b>6.2</b>	<b>6.7</b>

<sup>a</sup>out of 1000 cells examined

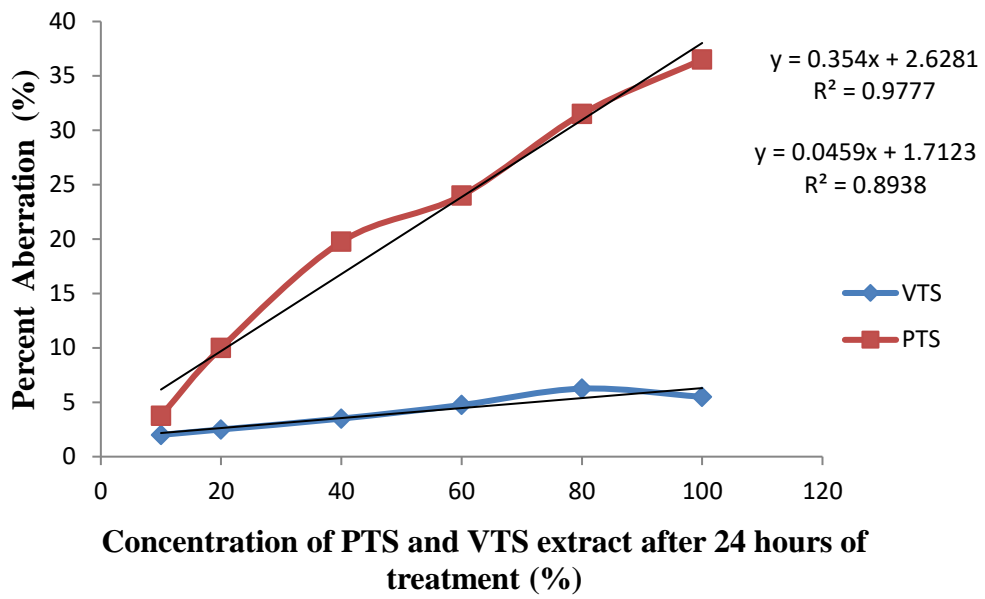


(A)

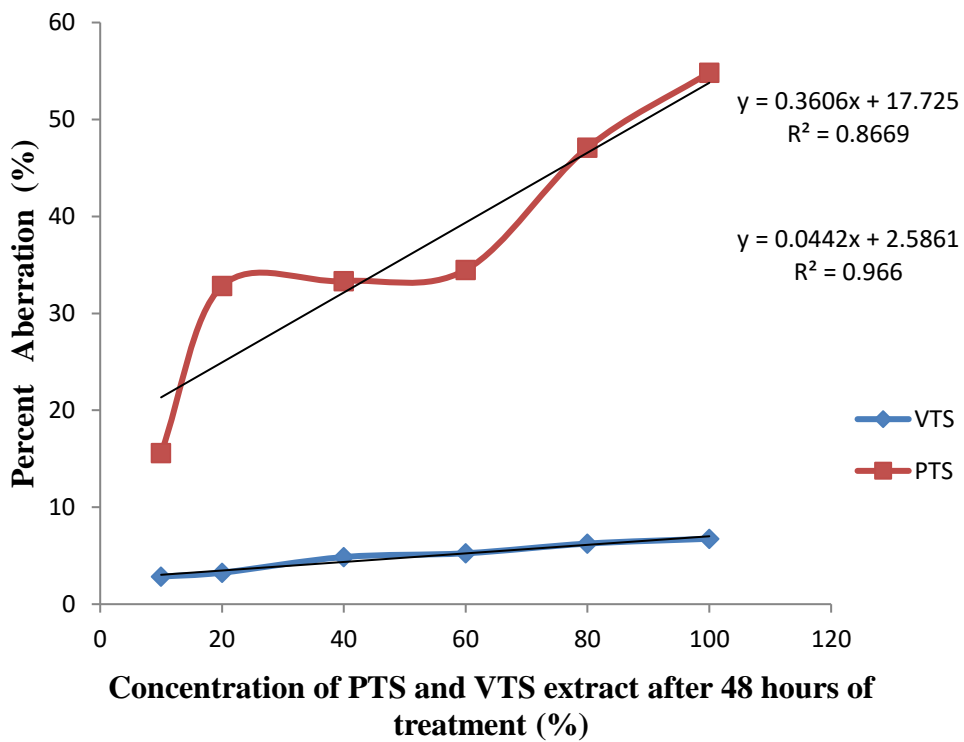


(B)

**Figure 6.9** Relationship between different concentrations of PTS and VTS extract and mitotic index after (A) 24 h and (B) 48 h of treatment in *A. cepa* root chromosomal aberration assay



(A)



(B)

**Figure 6.10 Relationship between different concentrations of PTS and VTS extract and percentage aberration after (A) 24 h and (B) 48 h of treatment in *A. cepa* root chromosomal aberration assay**



## 6.5 Effect of different pesticides and vermicompost by *A. cepa* test in vitro

### 6.5.1 Genotoxic studies on pre and post vermicompost Atrazine extracts (AT) by *E. fetida* and *M. posthuma*

The genotoxic effects of pre (AT extract) and post vermicomposted soil extracts of atrazine after activity of *E. fetida* (ATE extract) and *M. posthuma* (ATM extract) on the meristematic cells of *A. cepa* were compared based on mitotic index and chromosomal aberrations.

#### 6.5.1.1 Macroscopic Parameters

In case of vermicomposting by *E. fetida*, the results of root length when compared from pre to post vermicompost extracts and were found to be significantly different after vermicomposting by *M. posthuma* (**Table 6.9**). It was found that exposure to pre vermicompost AT extract significantly ( $p < 0.05$ ) suppressed the growth of roots of *A. cepa*. The average root length in control (distilled water) was found to be  $6.6 \pm 0.36$  cm. The root length was found to be least ( $4.2 \pm 0.85$ ) in pre-vermicompost 100% AT extract while after vermicomposting by *E. fetida* it increased to be  $6.5 \pm 0.82$ . The root length and concentration are negatively correlated in both pre-vermicompost AT extract ( $r = -0.982$ ,  $n = 6$ ,  $P < 0.05$ ) and post vermicompost ATE extract ( $r = -0.935$ ,  $n = 6$ ,  $P < 0.05$ ) after exposure of 48 h.

However, in case of vermicomposting by *M. posthuma*, the results of root length when compared from pre to post vermicompost extracts and were also found to be significantly different ( $p < 0.05$ ). It was found that exposure to 100% pre-vermicompost ATM extract significantly ( $p < 0.05$ ) suppressed the growth of roots. The root length was found to be least ( $4.2 \pm 0.85$ ) in pre-vermicompost 100% AT extract while after vermicomposting by *E. fetida* it increased to be  $5.9 \pm 0.29$ . Thus, we can conclude that vermicomposting reduces the toxic effect of atrazine. The root length and concentration are negatively correlated in both pre ( $r = -0.982$ ,  $n = 6$ ,  $P < 0.05$ ) and post vermicompost ATM extract ( $r = -0.850$ ,  $n = 6$ ,  $P < 0.05$ ) after exposure of 48 h (**Table 6.10**).

On comparison it was found that vermicomposting through *E. fetida* resulted in maintaining higher length of *A. cepa* roots in comparison to post vermicompost by *M. posthuma*. *A. cepa* test is a standard test for rapid and sensitive screening of chemicals and pollutants that represent environmental hazards. Root tip is often the first and foremost part of a plant that comes into contact with chemicals/pollutants found in water or soil. Root tip

**Table 6.9 Comparative root length of *A. cepa* exposed to pre vermicompost AT extract and post vermicomposted (recommended dose) ATE extract after vermicomposting by *E. fetida***

Concentration	Pre Vermicompost	Post Vermicompost
	Root length (cm)	Root length (cm)
Control	6.6±0.36	
10%	7.16±1.14	7.9±1.26*
20%	6.44±0.38	8.2±0.94**
40%	5.96±0.58	7.3±2.03*
80%	5.16±0.82	7.08±0.57***
100%	4.2±0.85	6.5±0.82***

The level of significance was determined by Student's t-test: \*p≤0.05, \*\*p≤0.01, \*\*\*p≤0.001

**Table 6.10 Comparative root length of *A. cepa* exposed to pre vermicompost AT extract and post vermicomposted (recommended dose) ATM extract after vermicomposting by *M. posthuma***

Concentration	Pre Vermicompost	Post Vermicompost
	Root length (cm)	Root length (cm)
Control	6.6±0.36	
10%	7.16±1.14	8.1±0.47*
20%	6.44±0.38	7.1±0.49*
40%	5.96±0.58	7.9±0.63**
80%	5.16±0.82	6.8±0.87***
100%	4.2±0.85	5.9±0.29**

The level of significance was determined by Student's t-test: \*p≤0.05, \*\*p≤0.01, \*\*\*p≤0.001.

system of *A. cepa* has particularly shown sensitivity to harmful effects of such environmental hazards (Fiskesjo 1995).

#### 6.5.1.2 Microscopic Parameters

The microscopic analysis of *A. cepa* exposed to AT extract revealed that the results obtained after exposure to various concentrations of pre and post-vermicomposted concentrations were significantly different.

##### 6.5.1.2.1 Mitotic index

The mitotic index is basically the number of cells in division stage to the total number of cells. The MI in the control was found to be  $15.55 \pm 0.62$  and  $15.07 \pm 2.17$  and it was reduced to  $7.15 \pm 0.26$  in 100% pre-vermicompost AT extract. The MI in 100% post-vermicompost ATE extract was found to be  $15.81 \pm 1.18$  and  $15.47 \pm 0.86$  after 24h and 48h. Similarly, with increase in the concentration the MI in pre-vermicompost AT extract reduced significantly. Thus, a concentration dependent inhibition in mitotic index was found with increasing concentration of pre vermicompost AT extract. The post extracts after vermicomposting by *E. fetida* in all the concentrations had significantly higher ( $p < 0.05$ ) MI than their respective pre-extracts (**Table 6.11**).

In case of post vermicompost by *M. posthuma*, the MI in the control was significantly ( $p < 0.05$ ) reduced to  $7.5 \pm 0.98$  and  $7.15 \pm 0.26$  in pre-vermicompost 100% AT extract after 24 and 48h respectively in pre-vermicompost extracts. But after vermicomposting the MI of 100% ATM improved to  $12.06 \pm 1.30$  and  $11.28 \pm 0.38$ . A concentration dependent inhibition in mitotic index was found with increasing concentration of pre vermicompost ATM extract. The post extracts after vermicomposting by *M. posthuma* in all the concentrations had significantly higher ( $p < 0.05$ ) MI than their respective pre-extracts (**Table 6.12**).

We compare the mitotic index in *A. cepa* exposed to post vermicompost extracts for both the species of earthworms and observed that vermicomposting through *E. fetida* resulted in significantly ( $p < 0.05$ ) higher mitotic index when compared with its indigenous counterpart, *M. posthuma*. A decrease in MI in *A. cepa* has been previously related to the exposure of heavy metals (Jain et al. 2004; Liu et al. 2009), etc. In this study the decrease in MI could be ascertained with the presence of pesticides in the soil extracts. It is well known and understood that the body tissues of earthworms especially chloragocytes in addition to the intestinal microflora are capable to detoxify and reform most of the obnoxious elements be it

heavy metals, waste products and other toxic products. The percentage of chromosomal aberrations was concentration and duration dependent. Vermicomposting by *E. fetida* and *M. posthuma* is capable of transforming highly toxic forms to non toxic forms with *E. fetida* showing better efficiency. This was also supported by Arillo and Melodia (1991); Fischer and Koszorus (1992), who stated that mitochondrial and cytoplasmic fractions in earthworms like *E. fetida*, play an important role in metabolic conversion of highly toxic forms of heavy metals to nontoxic forms. The mitotic index is considered to be a powerful indicator in monitoring environmental pollution, especially for the evaluation of contaminants that present toxic and cytotoxic potential (Hoshina 2002).

The observations infer to the conclusion that vermicomposting could be an important tool for reducing the toxicity of atrazine as evidenced by our results of MI indicating better vermicomposting efficiency of *E. fetida*. Our study was also supported by a similar study by Bhat et al. (2014) who reported an increase in MI after vermicomposting of pressmud by *E. fetida*. An increase in MI was also noticed after vermicomposting of sugar beet waste by *E. fetida* (Bhat et al. 2015). The mitotic index in the roots of *Vicia faba* was also found to be elevated after vermicomposting of flyash by *E. fetida* in comparison to pre-vermicompost flyash (Jain et al. 2004). Srivastava et al. (2005) also observed an increase in MI in roots of *A. cepa* after vermicomposting of municipal sludge by *E. fetida*. Bhat et al. (2016) also depicted an increase in MI in roots of *A. cepa* after vermicomposting of bagasse waste of sugar industry. Datta et al. (2018) also compared the genotoxicity of pesticide treated soil and vermicompost treated soil and found higher MI in the latter.

#### 6.4.1.1.2 Chromosomal Aberrations

Various types of chromosomal aberrations were studied as physiological and clastogenic aberrations. The highest frequency of CA was found in 100% pre-vermicompost extract of AT (13.1% and 14.6%) after 24 and 48 h. But after vermicomposting by *E. fetida*, the CA in 100% ATE extract significantly reduced to 4.8% and 4.6% after 24 and 48h. In all the concentrations of extracts, the CA was found to be significantly reduced ( $p < 0.05$ ) in post-vermicompost (ATE) extracts when compared to pre-vermicompost extract after 24 and 48 h respectively. The percentage of CA showed positive correlation with concentration of pre vermicompost AT extract after 24 h ( $r = 0.980$ ,  $n=5$ ,  $P < 0.05$ ) and after 48 h ( $r = 0.920$ ,  $n = 5$ ,  $P < 0.050$ ); also after 24 h ( $r = 0.964$ ,  $n = 5$ ,  $P < 0.05$ ) and 48 h ( $r = 0.919$ ,  $n = 6$ ,  $P < 0.05$ ) of post

**Table 6.11 Mitotic index values (mean  $\pm$  SE) in control and different concentrations of pre-vermicompost AT extract and post-vermicompost ATE extract after vermicomposting by *E. fetida***

Concentration	Time (h)	Mitotic index in pre-vermicompost	Mitotic index in post-vermicompost
Control	24 h	15.55 $\pm$ 0.62	
	48h	15.07 $\pm$ 2.17	
10% - 44	24 h	14.52 $\pm$ 0.71	15.36 $\pm$ 0.76*
	48h	12.28 $\pm$ 2.27	15.92 $\pm$ 1.25***
20% - 44	24 h	10.92 $\pm$ 3.19	16.43 $\pm$ 0.28***
	48h	9.63 $\pm$ 0.91	15.56 $\pm$ 0.96***
40% - 44	24 h	9.3 $\pm$ 0.62	16.66 $\pm$ 0.77***
	48h	8.82 $\pm$ 0.29	16.20 $\pm$ 1.08***
80% - 44	24 h	8.67 $\pm$ 0.58	16.09 $\pm$ 0.32***
	48h	8.25 $\pm$ 0.38	16.04 $\pm$ 0.49***
100% - 44	24 h	7.5 $\pm$ 0.98	15.81 $\pm$ 1.18***
	48h	7.15 $\pm$ 0.26	15.47 $\pm$ 0.86***

The level of significance was determined by Student's t-test: \*p $\leq$ 0.05, \*\*\*p $\leq$ 0.001

**Table 6.12 Mitotic index values (mean  $\pm$  SE) in control and different concentrations of pre-vermicompost and post-vermicompost ATM extract after vermicomposting by *M. posthuma* 24 and 48 h**

Concentration	Time (h)	Mitotic index in pre-vermicompost	Mitotic index in post-vermicompost
Control	24 h	15.55 $\pm$ 0.62	
	48h	15.07 $\pm$ 2.17	
10% - 44	24 h	13.52 $\pm$ 0.71	14.08 $\pm$ 1.59*
	48h	12.28 $\pm$ 2.27	14.79 $\pm$ 0.30**
20% - 44	24 h	10.92 $\pm$ 3.19	13.29 $\pm$ 0.53***
	48h	9.63 $\pm$ 0.91	13.78 $\pm$ 1.00***
40% - 44	24 h	9.3 $\pm$ 0.62	14.85 $\pm$ 0.91***
	48h	8.82 $\pm$ 0.29	13.86 $\pm$ 0.43***
80% - 44	24 h	8.67 $\pm$ 0.58	12.74 $\pm$ 0.22***
	48h	8.25 $\pm$ 0.38	12.26 $\pm$ 0.73***
100% - 44	24 h	7.5 $\pm$ 0.98	12.06 $\pm$ 1.30***
	48h	7.15 $\pm$ 0.26	11.28 $\pm$ 0.38***

The level of significance was determined by Student's t-test: \*p $\leq$ 0.05, \*\*p $\leq$ 0.01, \*\*\*p $\leq$ 0.001

vermicompost ATE extract. The rate of CA increased as the concentration of ATE extracts increased. In case of vermicomposting by *M. posthuma*, CA in 100% ATM extract significantly reduced to 8.2% and 8.5% after 24 and 48h compared to significantly higher CA in pre-vermicompost AT extract (13.1% and 14.6%) after 24 h and 48 h respectively. In all the concentrations of ATM extracts, the CA was found to be significantly reduced ( $p < 0.05$ ) in post-vermicompost ATM extracts when compared to pre-vermicompost extract after 24 and 48 h respectively. The percentage of CA showed positive correlation with concentration of extract after 24 h of treatment in ( $r=0.980$ ,  $n=5$ ,  $P < 0.05$ ) and after 48 h of treatment in pre-vermicompost AT extract ( $r=0.920$ ,  $n = 5$ ,  $P < 0.050$ ); also after 24 h ( $r=0.921$ ,  $n=5$ ,  $P < 0.05$ ) and 48 h ( $r = 0.957$ ,  $n = 6$ ,  $P < 0.05$ ) of post vermicompost ATM extract. The rate of CA increased as the concentration of extracts increased. Our study was also supported by a similar study by Bhat et al. (2014), who reported a decrease in CA after vermicomposting of pressmud by *E. fetida*. Vermicomposting of sugar beet waste by *E. fetida* also leads to reduction in chromosomal abnormalities (Bhat et al. 2015). The chromosomal anomalies in the roots of *Vicia faba* was also found to decrease after vermicomposting of flyash by *E. fetida* in comparison to pre-vermicompost flyash (Jain et al. 2004). Shrivastava et al. (2005) also observed a decrease in CA in roots of *A. cepa* after vermicomposting of municipal sludge by *E. fetida*. Bhat et al. (2016) also depicted a decrease in CA in roots of *A. cepa* after vermicomposting of bagasse waste of sugar industry.

The data obtained from the chromosomal analysis of pre and post extracts of atrazine by *E. fetida* and *M. posthuma* species depict that vermicomposting by *E. fetida* species help in the significantly higher reduction of chromosomal abnormalities when compared to *M. posthuma*.

**Table 6.13 Different chromosomal aberrations (physiological and clastogenic) in the root meristem cell of *A. cepa* exposed to pre vermicompost AT extract for 24h and 48 h**

Type of chromosomal aberration	Time of exposure	No. of aberrant cells <sup>a</sup>					
		Control	10%	20%	40%	80%	100%
<b>Physiological Aberrations (PA)</b>							
C-Mitosis	24 h	3	15	17	16	21	17
	48 h	6	14	20	15	8	22
Anaphase lag	24 h	5	10	24	25	16	21
	48 h	0	17	22	23	28	32
Stickiness	24 h	0	10	14	11	22	27
	48 h	5	12	11	21	20	18
Vagrants	24 h	8	11	10	15	13	18
	48 h	8	7	8	10	16	20
Total PA	24 h	16	46	65	67	75	83
	48 h	19	50	61	69	72	92
<b>Clastogenic Aberrations (CGA)</b>							
Chromosomal Bridges	24 h	7	18	12	12	18	20
	48 h	5	16	21	11	12	22
Chromosomal Breaks/ Fragments	24 h	0	14	9	25	24	28
	48 h	1	12	11	31	35	32
<b>Total CA</b>	24 h	7	32	21	37	42	48
	48 h	6	28	32	42	44	54
<b>Total Aberration (PA+CGA)</b>	24 h	23	72	86	104	115	131
	48 h	25	78	93	111	119	146
Percent Aberration	24 h	2.3	7.2	8.6	10.4	11.5	13.1
	48 h	2.5	7.8	9.3	11.1	11.9	14.6

<sup>a</sup>out of 1000 cells examined

**Table 6.14 Different chromosomal aberrations (physiological and clastogenic) in the root meristem cells of *A. cepa* exposed to post vermicompost ATE extract after vermicomposting by *E. fetida* for 24h and 48 h**

Type of chromosomal aberration	Time of exposure	No. of aberrant cells <sup>a</sup>					
		Control	10%	20%	40%	80%	100%
<b>Physiological Aberrations (PA)</b>							
C-Mitosis	24 h	3	4	2	4	5	11
	48 h	6	3	5	5	5	5
Anaphase lag	24 h	5	5	4	3	5	8
	48 h	0	3	3	5	8	8
Stickiness	24 h	0	2	7	6	7	6
	48 h	5	2	5	6	5	11
Vagrants	24 h	8	2	0	3	4	7
	48 h	8	3	2	3	4	7
<b>Total PA</b>	24 h	16	13	13	16	21	32
	48 h	19	12	13	19	22	31
<b>Clastogenic Aberrations (CGA)</b>							
Bridges	24 h	7	3	5	6	5	10
	48 h	5	4	6	5	7	11
Breaks	24 h	0	3	6	4	5	7
	48 h	1	5	3	5	5	6
<b>Total CA</b>	24 h	7	6	11	10	10	16
	48 h	6	9	9	12	12	17
Total Aberration (PA+CGA)	24 h	23	19	24	26	31	48
	48 h	25	21	22	31	34	46
<b>Percent Aberration</b>	24 h	2.3	2.9	2.4	2.6	3.1	4.8
	48 h	2.5	3.1	2.2	3.1	3.4	4.6

<sup>a</sup>out of 1000 cells examined



**Table 6.15 Different chromosomal aberrations (physiological and clastogenic) in the root meristem cells of *A. cepa* exposed to post-vermicompost ATM extract after vermicomposting by *M. posthuma* for 24h and 48 h**

Type of chromosomal aberration	Time of exposure	No. of aberrant cells <sup>a</sup>					
		Control	10%	20%	40%	80%	100%
<b>Physiological Aberrations (PA)</b>							
C-Mitosis	24 h	3	10	10	11	16	15
	48 h	6	12	9	10	14	16
Anaphase lag	24 h	5	4	6	12	12	19
	48 h	0	3	12	14	16	14
Stickiness	24 h	0	7	12	12	15	12
	48 h	5	8	14	17	11	17
Vagrants	24 h	8	11	8	11	14	15
	48 h	8	12	9	13	10	12
<b>Total PA</b>	24 h	16	32	36	46	57	61
	48 h	19	35	44	54	51	59
<b>Clastogenic Aberrations (CGA)</b>							
Bridges	24 h	7	8	9	13	9	14
	48 h	5	7	5	10	14	15
Breaks	24 h	0	6	7	8	12	7
	48 h	1	4	8	9	18	11
<b>Total CA</b>	24 h	7	14	16	18	21	21
	48 h	6	11	13	19	32	26
Total Aberration (PA+CGA)	24 h	23	43	52	64	78	82
	48 h	25	46	57	73	83	85
<b>Percent Aberration</b>	24 h	2.3	4.3	5.2	6.4	7.8	8.2
	48 h	2.5	4.6	5.7	7.3	8.3	8.5

<sup>a</sup>out of 1000 cells examined

### 6.5.2 Genotoxic studies on pre and post vermicompost Acephate extracts (AC) by *E. fetida* and *M. posthuma*

The genotoxic effects of pre (AC extract) and post vermicomposted soil extracts of acephate after activity of *E. fetida* (ACE extract) and *M. posthuma* (ACM extract) on the meristematic cells of *A. cepa* were compared based on mitotic index and chromosomal aberrations.

#### 6.5.2.1 Macroscopic Parameters

In case of vermicomposting by *E. fetida*, the root lengths of *A. cepa* after exposure to various concentrations of pre and post ACE extracts were significantly different after 24 h and 48 h (**Table 6.16**). It was found that exposure to pre vermicompost AC extract significantly ( $p > 0.05$ ) suppressed the growth of roots. The average root length in control was found to be  $6.6 \pm 0.36$  cm. The root length was found to be least ( $4.10 \pm 0.63$ ) in pre-vermicompost 100% AC extract while after vermicomposting it increased to be  $6.52 \pm 0.93$ cm. The root length and concentration are negatively correlated in both pre vermicompost AC extract ( $r = -0.904$ ,  $n = 6$ ,  $P < 0.05$ ) and post vermicompost ACE extract after exposure of 48 h ( $r = -0.936$ ,  $n = 6$ ,  $P < 0.05$ ). Thus, we can conclude that vermicomposting by *E. fetida* reduces the toxic effect of atrazine.

In case of vermicomposting by *M. posthuma*, the root length was found to be least ( $4.10 \pm 0.63$ ) in pre vermicompost 100% AC extract while after vermicomposting it increased to be  $5.81 \pm 0.79$  cm (**Table 6.17**).The root length and concentration are negatively correlated in both pre vermicompost AC extract ( $r = -0.972$ ,  $n = 6$ ,  $P < 0.05$ ) and post vermicompost ACM extract after exposure of 48 h ( $r = -0.789$ ,  $n = 6$ ,  $P < 0.05$ ). Thus, we can conclude that vermicomposting by *M. posthuma* also reduces the toxic effect of atrazine.

The vermi-remediation significantly improves the root length of *A. cepa* exposed to atrazine and acephate soil extracts. However on comparison of root length from post vermicompost extracts for *E. fetida* and *M. posthuma* we conclude that vermicomposting by *E. fetida* significantly promotes significant root length elongation in comparison to *M. posthuma*.

**Table 6.16 Comparative root length of *A. cepa* exposed to pre vermicompost AC extract and post vermicompost ACE extract after vermicomposting by *E. fetida***

Concentration	Pre Vermicompost	Post Vermicompost
	Root length (cm)	Root length (cm)
Control	6.6±0.36	
10%	6.02 ± 1.56	6.80 ± 0.79*
20%	5.21 ± 1.14	6.72 ± 2.37**
40%	4.82 ± 0.39	6.40 ± 0.49***
80%	4.17 ± 0.62	6.79 ± 1.25***
100%	4.10 ± 0.63	6.52 ± 0.93***

The level of significance was determined by Student's t-test: \*p≤0.05, \*\*p≤0.01, \*\*\*p≤0.001

**Table 6.17 Comparative root length of *A. cepa* exposed to pre vermicompost AC extract and posts vermicompost ACM extract after vermicomposting by *M. posthuma***

Concentration	Pre Vermicompost	Post Vermicompost
	Root length (cm)	Root length (cm)
Control	6.6 ± 0.36	
10%	6.02 ± 1.56	6.1 ± 1.25
20%	5.21 ± 1.14	6.52 ± 0.24
40%	4.82 ± 0.39	6.25 ± 0.88*
80%	4.17 ± 0.62	5.69 ± 0.59***
100%	4.10 ± 0.63	5.81 ± 0.79**

The level of significance was determined by Student's t-test: \*p≤0.05, \*\*p≤0.01, \*\*\*p≤0.001

### 6.5.2.2 Microscopic Parameters

The microscopic analysis of *A. cepa* to AC extracts revealed that the results obtained after exposure to various concentrations of pre and post-vermicompost concentrations were significantly different.

#### 6.5.2.2.1 Mitotic index

The MI in the control was found to be 15.55 ± 0.62 and 15.07 ± 2.17 which was significantly (p < 0.05) reduced to 6.8 ± 0.27 and 6.24 ± 0.93 in 100% pre vermicompost AC extract after 24 and 48h respectively. However, after vermicomposting by *E. fetida*, the MI

for 100% ACE rose to  $14.37 \pm 1.36$  and  $14.61 \pm 2.24$  which was significantly similar to the control values of MI. A concentration dependent inhibition in mitotic index was found with increasing concentration of pre-vermicompost AC extract. The post extracts in all the concentrations had significantly higher ( $p > 0.05$ ) MI than their respective pre-extracts (**Table 6.18**).

**Table 6.18 Mitotic index values (mean  $\pm$  SE) in control and different concentrations of pre-vermicompost AC extract and post-vermicompost ACE extract after vermicomposting by *E. fetida***

Concentration	Time (h)	Mitotic index in pre-vermicompost	Mitotic index in post-vermicompost
Control	24 h	$15.55 \pm 0.62$	
	48h	$15.07 \pm 2.17$	
10%- 44	24 h	$8.9 \pm 0.8$	$16.62 \pm 1.86^{**}$
	48h	$8.75 \pm 1.62$	$15.23 \pm 0.28^{**}$
20%- 44	24 h	$8.5 \pm 0.52$	$16.24 \pm 2.17^{**}$
	48h	$8.24 \pm 0.81$	$15.16 \pm 0.62^{***}$
40% - 44	24 h	$7.8 \pm 0.42$	$14.67 \pm 1.08^{***}$
	48h	$7.52 \pm 2.61$	$15.92 \pm 1.84^{***}$
80% - 44	24 h	$7.2 \pm 0.83$	$15.20 \pm 0.91^{***}$
	48h	$7.11 \pm 0.23$	$14.26 \pm 0.77^{***}$
100% - 44	24 h	$6.8 \pm 0.27$	$14.37 \pm 1.36^{***}$
	48h	$6.24 \pm 0.93$	$14.61 \pm 2.24^{***}$

The level of significance was determined by Student's t-test:  $^{**}p \leq 0.01$ ,  $^{***}p \leq 0.001$

After vermicomposting by *M. posthuma*, the MI for 100% ACM rose to  $11.08 \pm 0.33$  and  $10.86 \pm 0.22$  which was significantly different from MI in 100% pre vermicompost AC extract ( $6.8 \pm 0.27$  and  $6.24 \pm 0.93$ ). The post extracts after vermicomposting by *M. posthuma* in all the concentrations had significantly higher ( $p > 0.05$ ) MI than their respective pre-extracts (**Table 6.19**). Our study was also supported by a similar study by Bhat et al. (2014) who reported an increase in MI after vermicomposting of pressmud by *E. fetida*. An increase in MI was also noticed after vermicomposting of sugar beet waste by *E. fetida* (Bhat et al. 2015). The mitotic index in the roots of *Vicia faba* was also found to be elevated after vermicomposting of flyash by *E. fetida* in comparison to pre-vermicompost flyash (Jain et al. 2004). Shrivastava et al. 2005 also observed an increase in MI in roots of *A. cepa* after vermicomposting of municipal sludge by *E. fetida*. Bhat et al. (2016) also depicted an

increase in MI in roots of *A. cepa* after vermicomposting of bagasse waste of sugar industry. The percentage of chromosomal aberrations was concentration and duration dependent. Thus *E. fetida* is capable of transforming highly toxic forms to non toxic forms. This was also supported by Arillo and Melodia (1991); Fischer and Koszorus (1992), who stated that mitochondrial and cytoplasmic fractions in *E. fetida* play an important role in metabolic conversion of highly toxic forms of heavy metals to nontoxic forms. The mitotic index is considered to be a powerful indicator in monitoring environmental pollution, especially for the evaluation of contaminants that present toxic and cytotoxic potential (Hoshina 2002). The observations infer to the conclusion that vermicomposting could be an important tool for reducing the toxicity of atrazine as evidenced by our results of MI.

On comparison of MI of acephate and atrazine, we conclude that formulation of acephate inhibited the mitotic index of meristem cells of *A. cepa* more in comparison to formulation of atrazine.

**Table 6.19 Mitotic index values (mean  $\pm$ SE) in control and different concentrations of pre-vermicompost AC extract and post-vermicompost ACM extract after vermicomposting by *M. posthuma***

Concentration	Time (h)	Mitotic index in pre-vermicompost	Mitotic index in post-vermicompost
Control	24h	15.55 $\pm$ 0.62	
	48h	15.07 $\pm$ 2.17	
10% - 44	24h	8.9 $\pm$ 0.8	12.89 $\pm$ 2.29***
	48h	8.75 $\pm$ 1.62	12.53 $\pm$ 0.89***
20% - 44	24h	8.5 $\pm$ 0.52	12.81 $\pm$ 1.18***
	48h	8.24 $\pm$ 0.81	12.14 $\pm$ 0.74***
40% - 44	24h	7.8 $\pm$ 0.42	11.96 $\pm$ 0.29***
	48h	7.52 $\pm$ 2.61	11.57 $\pm$ 3.29***
80% - 44	24h	7.2 $\pm$ 0.83	12.28 $\pm$ 1.15***
	48h	7.11 $\pm$ 0.23	11.14 $\pm$ 0.84***
100% - 44	24h	6.8 $\pm$ 0.27	11.08 $\pm$ 0.33***
	48h	6.24 $\pm$ 0.93	10.86 $\pm$ 0.22***

The level of significance was determined by Student's t-test: \*\*\*p $\leq$ 0.001.

#### 6.5.2.2.2. Chromosomal Aberrations

Various types of chromosomal aberrations were studied as physiological and clastogenic aberrations. The lowest frequency of CA was found in control (2.3 and 2.5%) after 24 and 48 h respectively. The highest frequency of CA was found in 100% pre-vermicompost extract of AC (23.1 and 27.6%) after 24 and 48 h (**Table 6.20**). But after vermicomposting by *E. fetida* the CA in 100% ACE extract significantly reduced to 6.2 and 6.8% after 24 and 48h (**Table 6.21**). In all the concentrations of extracts, the CA was found to be significantly reduced ( $p > 0.05$ ) in post-vermicompost ACE extracts when compared to pre-vermicompost AC extract after 24 and 48 h respectively.

The percentage of CA showed positive correlation with concentration of pre-vermicompost extract of AC after 24 h ( $r = 0.970$ ,  $n = 5$ ,  $P < 0.05$ ) and 48 h ( $r = 0.958$ ,  $n = 5$ ,  $P < 0.050$ ) of treatment; also after 24 h ( $r = 0.910$ ,  $n = 5$ ,  $P < 0.05$ ) and 48 h ( $r = 0.950$ ,  $n = 6$ ,  $P < 0.05$ ) of post vermicompost ACM extract. After vermicomposting by *M. posthuma*, the CA in 100% ACM extract significantly reduced to 13.4 and 14.4% after 24 h and 48h (**Table 6.22**). Our study was also supported by a similar study by Bhat et al. (2014) who reported a decrease in CA after vermicomposting of pressmud by *E. fetida*. Vermicomposting of sugar beet waste by *E. fetida* also leads to reduction in chromosomal abnormalities (Bhat et al. 2015).

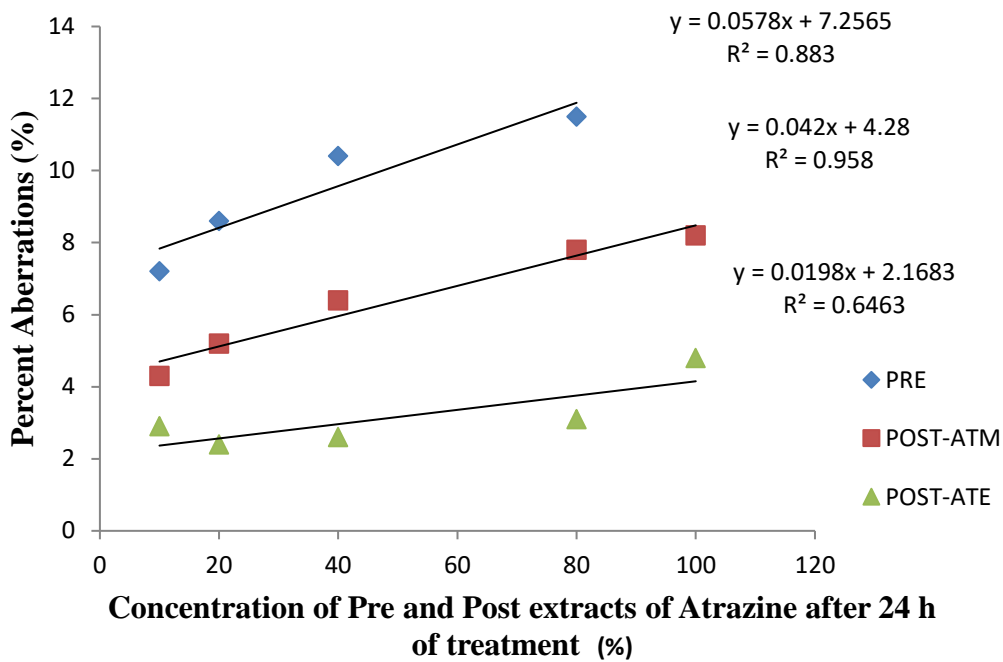
The chromosomal anomalies in the roots of *V. faba* were also found to decrease after vermicomposting of flyash by *E. fetida* in comparison to pre-vermicompost flyash (Jain et al. 2004). Shrivastava et al. 2005 also observed a decrease in MI in roots of *A. cepa* after vermicomposting of municipal sludge by *E. fetida*. Bhat et al. (2016) also depicted a decrease in CA in roots of *A. cepa* after vermicomposting of bagasse waste of sugar industry. The vermiremediation of soils contaminated with acephate and atrazine by *M. posthuma* thus reduces the potential genotoxic effects incurred by these two pesticides significantly. The comparison of chromosomal aberrations also suggests that vermicomposting by *E. fetida* significantly ( $p < 0.05$ ) reduced the chromosomal abnormalities in comparison to *M. posthuma*.

Chromatid breaks give rise to bridges and fragments. The formation of bridges may also be ascribed to unequal exchanges that lead to formation of dicentric chromosomes which are equally pulled at both poles in anaphase stage (Sax and Sax 1968). The breakage and fusion of chromosomes and chromatids or changing activation of replication enzymes are also

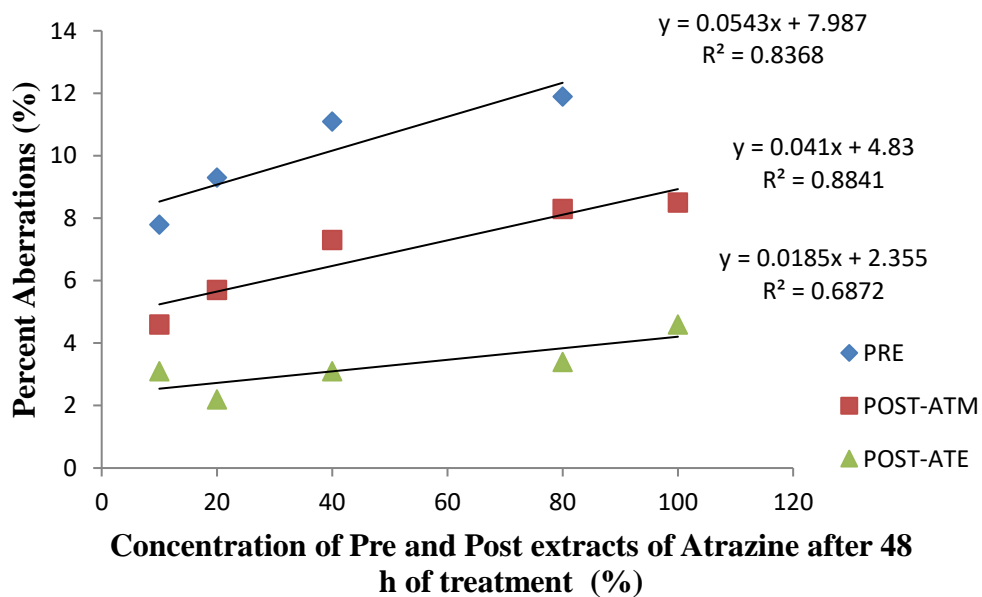
responsible for formation of bridges (Luo et al. 2004). Spindle anomalies lead to vagrant chromosomes. The failure of chromosomes or acentric fragments to move to either pole is responsible for formation of laggards (Evseeva et al. 2005). The aberrations induced by direct genotoxic effects may be repaired by intervention of DNA repair mechanisms to maintain the integrity of the genome (Cebulska-Wasiewska et al. 2005). But indirect genotoxic effects prove to be exponentially toxic. Glyphosate is one such pesticide which decreases DNA repair (Vodicka et al. 2004).

The marked number of chromosomal aberrations in pre-vermicompost extracts of ACE and ATE depicts the genotoxic effects of pesticides on *A. cepa*. These results are in accordance to reports describing the vegetable extracts showing chromosomal aberrations in *A. cepa* because of pesticide residues (Feretti et al. 2007) and also with some previous study of Dragoeva et al. (2009); Soodan et al. (2012), also reported various chromosomal abnormalities like vagrant chromosomes, chromosomal fragments at anaphase and telophase and multipolar anaphases depicting genotoxicity of agricultural soil. Leme et al. (2012) also reported various chromosomal abnormalities while assessing the genotoxicity of a soil matrix contaminated with bio-diesel.

Similar studies from other parts of the world reported various mitotic and chromosomal abnormalities predicting the genotoxic and clastogenic potential of contaminated soil (Masood and Malik 2013; Souza 2013). However, vermicompost delineate positive results based on a number of agricultural studies. Gopinath et al. (2008) reported an improvement in water and air availability, encouraging root growth and seedling emergence when vermicompost was applied for two consecutive growing seasons.



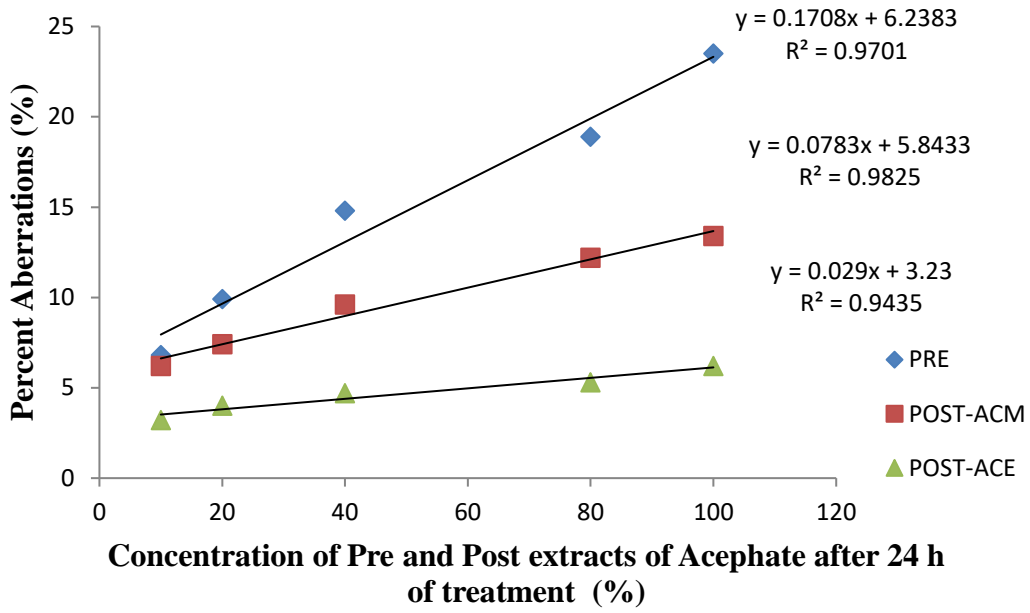
(A)



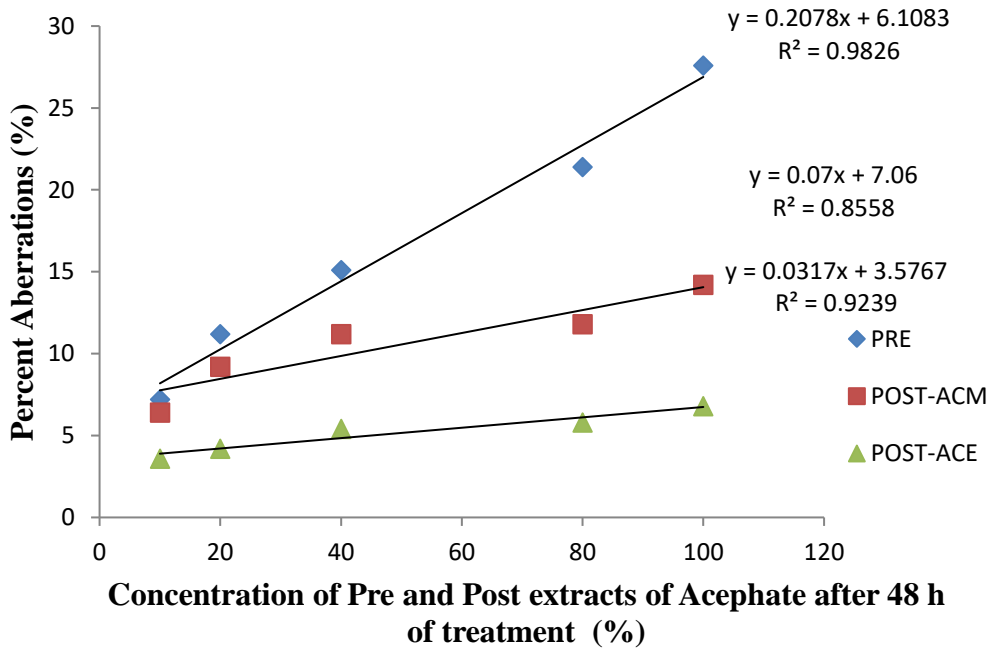
(B)

**Figure 6.11 Relationship between different concentrations of pre-vermicompost and post-vermicompost atrazine extract and percentage aberration in *A. cepa* root chromosomal aberration assay after vermicomposting by *E. fetida* and *M. posthuma* after (A) 24 h and (B) 48 h**





(A)



(B)

**Figure 6.12** Relationship between different concentrations of pre-vermicompost and post-vermicompost acephate extract and percentage aberration in *A. cepa* root chromosomal aberration assay after vermicomposting by *E. fetida* and *M. posthuma* after (A) 24 h and (B) 48 h

**Table 6.20 Different chromosomal aberrations (physiological and clastogenic) in the root meristem cells of *A. cepa* exposed to pre vermicompost AC extract after 24h and 48h**

Type of chromosomal aberrations	Time of exposure	No. of aberrant cells <sup>a</sup>					
		Control	10%	20%	40%	80%	100%
<b>Physiological Aberrations (PA)</b>							
C-Mitosis	24 h	2	12	11	23	40	46
	48 h	5	12	22	34	32	66
Anaphase lag	24 h	6	10	16	21	38	39
	48 h	5	10	20	32	39	41
Stickiness	24 h	6	14	24	36	38	31
	48 h	5	13	25	27	43	47
Vagrants	24 h	3	12	22	29	30	52
	48 h	4	12	14	21	28	40
<b>Total PA</b>	24 h	17	48	73	109	146	168
	48 h	19	47	81	114	142	194
<b>Clastogenic Aberrations (CGA)</b>							
Bridges	24 h	2	11	14	20	23	35
	48 h	3	13	12	18	38	42
Breaks	24 h	4	9	12	19	20	32
	48 h	3	12	19	19	34	40
<b>Total CA</b>	24 h	6	20	26	39	43	67
	48 h	6	25	31	37	72	82
Total Aberration (PA+CGA)	24 h	23	68	99	148	189	235
	48 h	25	72	112	151	214	276
<b>Percent Aberration</b>	24 h	2.3	6.8	9.9	14.8	18.9	23.1
	48 h	2.5	7.2	11.2	15.1	21.4	27.6

<sup>a</sup>out of 1000 cells examined

**Table 6.21 Different chromosomal aberrations (physiological and clastogenic) in the root meristem cells of *A.cepa* exposed to post vermicompost ACE extract after vermicomposting by *E. fetida* for 24h and 48 h**

Type of Chromosomal aberration	Time of exposure	No. of aberrant cells <sup>a</sup>					
		Control	10%	20%	40%	80%	100%
<b>Physiological Aberrations (PA)</b>							
C-Mitosis	24 h	2	5	3	8	6	4
	48 h	5	5	5	7	8	8
Anaphase lag	24 h	6	4	9	11	9	9
	48 h	5	7	7	6	7	12
Stickiness	24 h	6	6	6	8	11	11
	48 h	5	5	8	10	10	7
Vagrants	24 h	3	7	10	8	9	11
	48 h	4	9	12	15	12	8
<b>Total PA</b>	24 h	17	22	28	35	35	35
	48 h	19	26	32	38	37	35
<b>Clastogenic Aberrations (CGA)</b>							
Bridges	24 h	2	6	6	6	10	15
	48 h	3	5	5	7	9	17
Breaks	24 h	4	4	6	6	8	12
	48 h	3	5	5	9	12	16
<b>Total CA</b>	24 h	6	10	12	12	18	27
	48 h	6	10	10	16	21	33
Total Aberration (PA+CGA)	24 h	23	32	40	47	53	62
	48 h	25	36	42	54	58	68
<b>Percent Aberration</b>	24 h	2.3	3.2	4	4.7	5.3	6.2
	48 h	2.5	3.6	4.2	5.4	5.8	6.8

<sup>a</sup>out of 1000 cells examined

**Table 6.22 Different chromosomal aberrations (physiological and clastogenic) in the root meristem cells of *A. cepa* exposed to post vermicompost ACM extract after vermicomposting by *M. posthuma* for 24h and 48 h**

Type of Chromosomal aberration	Time of exposure	No. of aberrant cells <sup>a</sup>					
		Control	10%	20%	40%	80%	100%
<b>Physiological Aberrations (PA)</b>							
C-Mitosis	24 h	2	12	10	12	12	17
	48 h	3	18	14	16	14	25
Anaphase lag	24 h	4	18	16	20	14	18
	48 h	4	22	16	22	20	22
Stickiness	24 h	7	12	10	16	22	28
	48 h	5	10	18	14	16	24
Vagrants	24 h	2	10	14	14	18	16
	48 h	0	8	16	17	16	15
<b>Total PA</b>	24 h	15	52	50	62	66	79
	48 h	12	58	64	69	66	86
<b>Clastogenic Aberrations (CGA)</b>							
Chromosomal Bridges	24 h	3	6	14	20	32	31
	48 h	3	6	18	18	28	32
Chromosomal Breaks/ Fragments	24 h	2	4	10	14	24	24
	48 h	0	0	10	25	24	26
<b>Total CA</b>	24 h	5	10	24	34	56	55
	48 h	3	6	28	43	52	58
Total Aberration (PA+CGA)	24 h	20	62	74	96	122	134
	48 h	15	64	92	112	118	144
<b>Percent Aberration</b>	24 h	2.3	6.2	7.4	9.6	12.2	13.4
	48 h	2.5	6.4	9.2	11.2	11.8	14.4

<sup>a</sup>out of 1000 cells examined

## 6.6 Physicochemical quality of vermicompost and the soil

For this phase of experiment, mixture of soil along with pesticides was taken and after that vermicomposting was performed on the mixture with two different species *E. fetida* and *M. posthuma*. Followed is a comparison of rate of degradation and physico-chemical characteristics of pre and post vermicompost product.

### 6.6.1 Atrazine: Physico-chemical Parameters

Physico-chemical parameters showed significant changes with varying concentration of atrazine and in the soil mixtures.

#### 6.6.1.1 pH and EC

After vermicomposting of pesticide supplemented soil by *E. fetida* the pH of the medium was found to increase in the final product significantly ( $p < 0.05$ ) over initial. The increase in pH showed a positive correlation with the concentration of atrazine (46% in C<sub>ATE</sub>, 59% in ATE1, 87% in ATE2 128% in ATE3, 37% in ATE4 and 47% in ATE5). Among all the soil mixtures the maximum increase in pH was found in ATE3(128%) while control showed the least increase (46%). The electrical conductivity was found to increase in the final vermicomposted product in all the concentrations. The maximum increase was found in C<sub>ATE</sub> (217%) followed by ATE4 (173%) and ATE5 (118%) and minimum was found in ATE2 in (66%). The order of increase in EC followed the order C<sub>ATE</sub>>ATE4>ATE3>ATE5>ATE1>ATE2 (**Table 6.23**).

After vermicomposting of pesticide supplemented soil by *M. posthuma* pH of the medium was found to increase in final product significantly ( $p < 0.05$ ) over initial (**Table 6.23**). The percentage increase was found to be more in initial three concentrations (116% in ATM1 102% in ATM2 and 113% in ATM3) but was not found much in higher concentrations (1% in ATM4 and 6% in ATM5). Among all the soil mixtures the maximum increase in pH was found in the dose ATM1(116%) while ATM4 showed the least increase (1%). The order of increase in pH followed ATM1>ATM3>ATM2>C<sub>ATM</sub>>ATM5>ATM4. The electrical conductivity was found to increase in the final vermicomposted product except in ATM1 (86%). The maximum increase was found in ATM3 (200%) and minimum was found in ATM5 in (7%). The increase in EC after vermicomposting by *M. posthuma* followed the order ATM3>C<sub>ATM</sub>>ATM4>ATM2>ATM5. In C the percentage increase in EC was 106%.

When the initial substrate was subject to aerobic composting, i.e the mixture of soil along with pesticides without earthworms the pH was found to decrease with the exception of

ATC1 and ATC3. The pH increased by 110% in ATC3 and by 47% ATC1. The maximum reduction was found in ATC2 (47%) followed by ATC4 (20%) and ATC5 (14%). On the other hand electrical conductivity was also found to be reduced in ATC2 (73%) and ATC4 (45%). However the increase in ATC1, ATC3 and ATC5 was also significantly less than their respective counterparts after vermicomposting by *E. fetida* and *M. posthuma*.

Comparing the efficiency of vermicomposting by *Eisenia*, *Metaphire* and aerobic composting the increase in pH was found in the order ATE3> ATM1> ATM3> ATM2> ATE2> ATE1> ATE5> C<sub>ATE</sub>> C<sub>ATM</sub>> ATE4> ATM5> ATM4. In the initial two concentrations, the pH in ATM was found to be significantly higher ( $p < 0.05$ ) than ATE whereas in last three concentrations, pH in ATE was found to be significantly higher ( $p < 0.05$ ) than ATM. The decomposition of nitrogenous substances during the process of vermicomposting forms the basis of increase in pH (Muthukumaravel et al. 2008). According to Aira et al. (2007), earthworms augment a mutualistic relationship with the microbes and play a vital role in increasing the population of catabolically active microbes. The resultant is faster degradation of short chain fatty acids and precipitation of calcium carbonate which may be the reason of increase in pH of vermicompost products (Tognetti et al. 2007). Microbes are also known to release excess of nitrogen in the form of ammonia which when dissolved in water leads to an increase in the pH (Beck-friis et al. 2003; Mainoo et al. 2009). Binding of humus to free cations also results in increase in pH of the soil (Brady and Weil 2002). Increase in pH during vermicomposting has been reported by Datar et al. (1997). Fernandez-Gomez et al. (2010) ascertains that this increase in pH is due to the increase in concentration of  $\text{NH}_4^+$  ions. The pH shift is however considered to be dynamic and substrate dependent (Ndwega and Thompson 2000). The capability of increasing pH in higher concentrations of pesticides makes *E. fetida* more efficient than *M. posthuma*. Aerobic composting on the other side depicts decrease in pH and hence infers to being less efficient than vermicomposting. On the other hand, the electrical conductivity reflects the salinity of any material and is a good indicator of the applicability and utility of a compost or vermicompost for agricultural purposes. The activity of *E. fetida* significantly ( $p < 0.05$ ) showed higher EC than *M. posthuma*. The aerobic composting showed reduced EC than vermicompost substrates. This leads to the conclusion that activity of *E. fetida* leads to higher mineralization of salts comparative to *M. posthuma*. According to Khwairakpam and Bhargava (2009); Yadav and Garg, (2009) loss in organic matter with a corresponding mineralization of salts is responsible for the increase in EC. Kaviraj and Sharma (2003) also related the increase in EC due to the

loss of organic matter and release of various mineral salts like phosphate, potassium, ammonia, etc. The increase in EC was also attributed to increase in soluble salts level because of mineralization by microbes and earthworms (Karmegam and Daniel 2009). Correspondingly increase in sodium and potassium content in the mixtures may also be responsible for the rise in EC of the products of vermicomposting (Guoxue et al. 2001). Thus, the significant increase in level of EC after activity of *E. fetida* is better than that of *M. posthuma*.

#### 6.6.1.2. Nitrogen

After vermicomposting of pesticide supplemented soil by *E. fetida*, Nitrogen was found to significantly ( $p < 0.05$ ) increase in all the concentrations in post-vermicompost substrate. The order of increase was found to be  $C_{ATE} > ATE1 > ATE3 > ATE2 > ATE4 > ATE5$ . In  $C_{ATE}$  an increase of 85.4% was recorded.

After vermicomposting of pesticide supplemented soil by *M. posthuma*, the nitrogen was again found to significantly ( $p < 0.05$ ) increase in  $C_{ATM}$ , ATM1, ATM2, ATM3. However a decrease in nitrogen was found to be observed in ATM4 and ATM5. This could be ascertained to the fact that due to higher pesticide concentration, there may be reduced earthworm activity and hence reduction in nitrogen content. The order of increase in nitrogen was found as  $C_{ATM} > ATM3 > ATM2 > ATM1$ . In  $C_{ATM}$  the increase in TKN was found to be 22.7%. In case of aerobic composting, the TKN content was found to increase with the exception in highest concentration (ATC5) in which a loss of 2.6% was seen. The order of increase in TKN in other concentrations was found to be  $C_{ATC} > ATC4 > ATC1 > ATC2 > ATC3$ .

According to Viel et al. (1987), losses in organic carbon might be responsible for nitrogen addition. Earthworms also have a great impact on nitrogen mineralization so that mineral nitrogen was retained in the nitrate form (Atiyeh et al. 2000). According to Cynthia and Kumar (2012), increase in TKN could be attributed to the decomposition brought about by the worms accelerating mineralization of nitrogen content. Suthar (2007) also backs this trend explaining that earthworms are responsible for enrichment of nitrogen content of vermicompost through decaying tissues of dead earthworms and microbial mediated nitrogen transformation in vermicomposting systems. The decay of the dead worms also contributes to an increase in N as a large portion of dry weight of a worm is protein (Tripathi and Bhardwaj 2004). A loss in organic carbon is also responsible for nitrogen addition (Viel 1987). The

increase in nitrogen content after vermicomposting is also attributed to the mineralization of C-rich materials and due to the activity of N-fixing bacteria (Plaza et al. 2007; Crusmey et al. 2014). But Chauhan and Joshi (2010) explained that the final N content of vermicompost depends on the initial N in the medium and on the extent of decomposition. This was also backed by Sangwan et al. (2010) who reported an increase in TKN due to nitrogen mineralization by earthworms. The decay of the dead worms also contributes to an increase in N as a large portion of dry weight of a worm is protein (Tripathi and Bhardwaj 2004). Fernandez-Gomez et al. (2010) have reported 96% increase in nitrogen content after vermicomposting of wastes. The reason for this is ascertained to the concentration effect caused by the degradation of the labile organic compounds, which reduces the volume of the composting mass due to the release of CO<sub>2</sub> and the mineralization of nitrogen during decomposition of organic matter resulting in increased N in the vermicompost. This was also supported by Parthasarathi and Ranganathan (2000) who inferred that N, P and K contents in vermicompost increases due microbial enzyme activities while passing through the gut of the earthworms. However, the decline in nitrogen in aerobic composting was found. And this was supported by Brady and Weil (2002); Singh et al. (2010) who stated that nitrogen content declines in traditional aerobic composting because of the utilization of nitrogen by the rapidly multiplying heterotrophic bacteria. Das et al. (2015) also used *M. posthuma* for vermicomposting and observed that *M. posthuma* increases nitrogen content in the end product significantly and very efficiently.

#### 6.6.1.3. Organic Carbon

After vermicomposting of pesticide supplemented soil by *E. fetida*, the TOC was found to significantly decrease in the final vermicompost. The maximum decrease was found to be ATE3 (290.6%) and minimum in ATE2 (93.7%). The percent decrease in OC in vermicomposted products followed the order ATE3>ATE4>ATE1>C<sub>ATE</sub>>ATE5>ATE2. Post-vermicompost by *M. posthuma* also indicates a significant decline ( $p < 0.05$ ) in organic carbon (OC) in the products of vermicomposting was found. The decline in final products over initial was found to be maximum in ATM2(257.4%) and minimum in ATM5 (140.4%). The percent decrease in OC in vermicomposted products in case of *M. posthuma* followed the order ATM2>ATM1>ATM3>ATM4>C<sub>ATM</sub>>ATM5.

In case of aerobic composting, the decrease in TOC in final vermicomposting medium was found to be significant than initial medium. The maximum decrease was found



in ATC1. The order of decrease followed the order  $C_{ATC} > ATC1 > ATC5 > ATC3 > ATC4 > ATC2$ . However, the decrease in TOC was comparatively less than found in the process of vermicomposting.

According to Edwards (2004), earthworm work by fragmenting and homogenising the ingested material through muscular action of their foregut and additional extracellular enzymes provided by their gut microbes. The increase in surface area and addition of mucus and enzymes to the ingested material further leads to enhanced microbial action. This additive action of earthworm and microbes lead to loss of carbon from the substrates in the form of microbial respiration ( $CO_2$ ) (Tognetti et al. 2007; Hait and Tare 2011; Singh and Suthar 2012). Kaviraj and Sharma (2003) have reported a 20-40% reduction of TOC as  $CO_2$  during vermicomposting of municipal or industrial wastes. Higher loss of carbon during vermicomposting can be corroborated to higher rate of mineralization by catabolically active microbes the activity of which is enhanced by the worms (Aira et al. 2007, Prakash and Karmegam 2010). The present findings are supported by earlier worker who reported up to 67% C loss during the vermicomposting process of different organic waste using different earthworm species (Suthar 2009). *E. fetida* leads to higher reduction in TOC content in the process of vermicomposting in comparison to *M. posthuma*.

#### 6.6.1.4. Phosphorus

After vermicomposting of pesticide supplemented soil by *E. fetida*, the phosphorus content increased significantly ( $p < 0.05$ ) in all the products of vermicomposting. Percent increase was found to be maximum in ATE3 (8%) and minimum in ATE5 (2%). The increase in phosphorus content in post-vermicompost samples were found to be in order  $ATE3 > ATE1 > ATE2 > C_{ATE} > ATE4 > ATE5$ .

After vermicomposting of pesticide supplemented soil by *M. posthuma*, the phosphorus content increased significantly ( $p < 0.05$ ) in the products of vermicomposting with the exception in higher concentrations like ATM4 and ATM5. Percent increase was found to be maximum in ATM3 (5.6%) and minimum in ATM2 (0.3%) and unchanged in ATM5. In the initial concentrations of atrazine (ATM1-ATM3), the level of phosphorus increased significantly while in the lower concentrations (ATM4 and ATM5), the level of phosphorus decreased and remained unchanged respectively. The increase in phosphorus content in post-vermicompost samples were found to be in order  $ATM3 > C_{ATM} > ATM1 > ATM2$ .

In aerobic composting, the phosphorus content was found to increase but the increase was not significant. However in ATC4 exceptionally, a decrease in phosphorus content was found. Our results corroborated with the findings of Kaviraj and Sharma (2003), Tognetti et al. (2005), Liu and Price (2011) depicting the stimulatory effect of earthworms on phosphorus availability in soil. Worm casts are also rich in alkaline phosphatase and hence lead to rise in content of phosphate (Bayon and Binet 2006; Prakash and Karmegam 2010). Sangwan et al. (2010) also reported a 1.3-1.5 fold increase in phosphorus content in the vermicomposting of pressmud. Phosphate solubilising microbes in the presence of worms are responsible for conversion to soil phosphorus in mineral form to available forms (Kumar et al. 2015c). Vermicomposting was reported to be an efficient way for transformation of unavailable phosphorus to available forms for the plant (Ghosh et al. 1999). The increase in phosphorus content after vermicomposting was also supported by Makin et al. (2014); Das et al. (2015). Pramanik et al. (2007) have reported that acid production during organic matter decomposition by the microorganisms is the major mechanism for solubilization of insoluble phosphorus, which subsequently results in increase in phosphorus content in vermicomposts.

#### 6.6.1.4 Potassium

After vermicomposting of pesticide supplemented soil by *E. fetida*, the potassium level decreased significantly ( $p < 0.05$ ) in the final products of vermicomposting with the exception in ATE5 which showed an increase of 27%. Percent decline was maximum in ATE4 (278.5%) and minimum in ATE2 (44%). Percent decline in potassium in the products of vermicomposting was in the order of ATE4>ATE3>ATE1>ATE2>C<sub>ATE</sub>.

After vermicomposting of pesticide supplemented soil by *M. posthuma*, the potassium decreased significantly ( $p < 0.05$ ) in the final products of vermicomposting. Percent decline was maximum in ATM3 (27.1%) and minimum in ATM4 (5%). Percent decline in potassium in the products of vermicomposting was in the order of ATM3>C<sub>ATM</sub>>ATM2>ATM1>ATM5>ATM4. The aerobic composting also witnessed a decrease in potassium content in final products from initial. However, the decrease was not found to be as much as observed during the process of vermicomposting. The maximum decrease was found in ATC2 (13.4%). However, ATC5 showed an increase of 0.8%.

Comparing the potassium level decrease with respect to both the species, the potassium level was found to significantly decrease more after the activity of *E. fetida* in comparison to *M. posthuma*. This depicted the higher activity of *E. fetida* in comparison to *M.*

*posthuma*. A decrease in potassium was also reported by Sangwan et al. (2010) during the vermicomposting of sugar industry waste. The decline in level of potassium was attributed to leaching in the process of vermicomposting and subsequent utilization of potassium by the earthworms (Orozco et al. 1996; Benitez et al. 1999; Garg et al. 2006 and Kumar et al. 2010). Higher pH is also known to make potassium ions more susceptible to fixation by colloids and thus a rise in pH can also be responsible for decline in level of potassium (Brady and Weil 2002). Utilization of potassium by earthworms for their metabolic activity was also confirmed by Singh et al. (2010); Vig et al. (2011); Yadav and Garg (2011); Bhat et al. (2013). Adi and Noor (2009) added that the solubilisation of insoluble potassium is because of formation of acid during decomposition by microbes. However, Delgado et al. (1995), Suthar (2008), Pramanik (2007), Liu and Price (2011) reported higher potassium content in the vermicompost produced from the different feed mixtures.

#### 6.6.1.5 Sodium

Vermicomposting by *E. fetida* brought an increase in the level of sodium in the final product. Maximum increase in sodium was found in ATE4 (581%) while a decrease in level of Na was found in ATE1 (20%). The percent increase in sodium in final products followed the order ATE4>ATE2>ATE5>ATE3 >C<sub>ATE</sub>(Table 6.24).

Vermicomposting by *M. posthuma* brought an increase in the level of sodium in the product. Maximum increase in sodium was found in ATM3 (17.9%). The percent increase in sodium in final products followed the order ATM3>ATM2>C<sub>ATM</sub>>ATM1>ATM4>ATM5. In case of aerobic composting decrease in sodium level was found in final vermicompost products when compared to initial with exception in ATC3 that recorded an increase of 14.7%. The maximum decrease was found in ATC2 (36.2%). The results of aerobic composting in this case were found to be completely contrasting to what found in the case of vermicomposting. On comparison of increase in sodium content post vermicompost, it was found that the increase in sodium content after vermicomposting by *E. fetida* was significantly higher ( $p < 0.05$ ) than that of *M. posthuma*. Our finding corroborated with the studies of Yadav and Garg (2011) who observed increase in sodium content in the final products of vermicomposting of vegetable wastes. The above trend was supported by Khawairakpam and Bhargava (2009); Vig et al. (2011); Singh et al. (2010); Bhat et al. (2013) and However the sodium content was found unchanged in all treatment combinations during the vermicomposting of sago industry soild wastes (Subramanian et al. 2010).

#### 6.6.1.6 Lithium and Calcium

Lithium and calcium increase from pre-vermicompost sample to final products with the exception of highest concentration (ATE5) after vermicomposting by *E. fetida*. The maximum increase in Lithium was found in C<sub>ATE</sub> (6.6%) followed by ATE1 (5.8%). The order of increase of Lithium in the post vermicompost products were found in the order C<sub>ATE</sub>>ATE1>ATE3>ATE2>ATM4. On the other hand Calcium was also found to increase markedly in the products of vermicomposting in comparison to initial substrate. The order of increase in the products of vermicomposting in comparison to initial substrate was found to be C<sub>ATE</sub>>ATE1>ATE3>ATE2>ATE4. The maximum increase was found in C<sub>ATE</sub> (65.8%) followed by ATE1 (40.2%) and minimum in ATE4 (26%). In ATE5 however a decline of 19.3% was recorded. After vermicomposting by *M. posthuma*, the lithium and calcium decrease from pre-vermicompost sample to final products; however this decrease was found to be significant ( $p < 0.05$ ) only in the lowest concentration (ATM1). The maximum decline in Lithium was found in ATM3 (6%) and exceptionally ATM5 recorded an increase of 0.6%. The order of decline of Lithium in the post vermicompost products were found in the order ATM3>ATM1=ATM2=ATM4. On the other hand Calcium was also found to decrease in the products of vermicomposting in comparison to initial substrate. The order of decrease in the products of vermicomposting in comparison to initial substrate was found to be ATM2>ATM3>ATM1>C<sub>ATM</sub>>ATM5>ATM4. The maximum decrease was found in ATM2 (100.1%) and minimum in ATM4 (4.2%).

In case of aerobic composting, the Li was found to increase in three concentrations ATC1 (1.3%), ATC3 (1.9%) and ATC4 (0.5%) as well as C<sub>ATC</sub> (3.3%). However, in ATC2 and ATC5 decrease was observed in content of Li. In case of Ca, a decrease in ATC3 (41.6%), ATC4 (19%), ATC5 (89.6%) was observed. However, in ATC1 and ATC2 an increase of 57.1% and 17.7% was observed. C<sub>ATC</sub> showed an increase of 27.5%

Our results corroborated with the findings of Oyedele et al. (2006) who observed significantly higher calcium content in vermicast than surrounding soil like we observed for post vermicompost by *E. fetida*. Both the species here showed contrasting results. Post vermicompost the level of Ca and Li increased with *E. fetida* however a decline in the content of Ca and Li was observed after vermicomposting with *M. posthuma*.

**Table 1.23 Initial and final nutrient content (mean ± SE) and percent change over initial nutrient content of different proportions of atrazine in the soil with earthworms *M. posthuma* (ATM) and *E. fetida* (ATE) and aerobic composting (ATC)**

Parameter	pH		EC		TKN		TOC		TAP	
Conc.	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
<b>Control ATE</b>	7.12±0.03d	7.58±0.61a (46)	1.19±0.89d	3.36±0.24a (217)	0.74±0.01c	1.59±0.07a (85.4)	7.48±0.31a	5.65±0.040d (-183.3)	0.10±0.05b	0.13±0.01a (3.1)
<b>Control ATM</b>		7.50±0.88b (38)		2.25 ±0.74b (106)		0.96±0.05b (22.7)		6.06±1.05c (-142.6)		0.12±0.01ab (2)
<b>Control ATC</b>		7.39±0.38c (27)		2.01±0.37c (82)		0.87±0.03bc (12.8)		6.46±0.89b (-102.5)		0.11±0.04ab (1.6)
<b>ATE1</b>	7.23±0.07c	7.82±0.17b (59)	1.48±0.27b	2.56±0.18a (108)	0.81±0.06c	0.90±0.08a (9.7)	7.28±0.064a	5.15±0.12c (-213.3)	0.15±0.01b	0.22±0.07a (7)
<b>ATM1</b>		8.39±0.02a (116)		0.62±0.16c (-86)		0.86±0.02b (5.2)		4.83±0.04d (-245.7)		0.15±0.01b (0.5)
<b>ATC1</b>		7.7±0.03b (47)		2.35±0.12a (87)		0.83±0.32c (1.9)		6.30±0.46b (-98.4)		0.15±0.01b (0.7)
<b>ATE2</b>	7.39±0.06c	8.26±1.08b (87)	1.59±0.39b	2.25±0.73a (66)	0.66±0.02d	0.71±0.05b (4.2)	7.68±1.482a	6.74±0.15b (-93.7)	0.18±0.01b	0.24±0.012a (6)
<b>ATM2</b>		8.41±0.10a (102)		2.23±0.31b (64)		0.78±0.03a (11.2)		5.10±0.50d (-257.4)		0.19±0.01b (0.3)
<b>ATC2</b>		6.92±0.22d (-47)		0.86±0.44c (-73)		0.68±0.07c (2)		7.37±0.05b (-31.1)		0.20±0.04b (2.1)
<b>ATE3</b>	7.31±0.59d	8.59±0.82a (128)	2.04±0.18d	3.49±0.62b (145)	0.60±0.19c	0.66±0.07b (6.5)	7.75±0.18a	4.85±0.17d (-290.6)	0.14±0.04c	0.22±0.02a (8)
<b>ATM3</b>		8.52±0.09b (113)		4.04±1.46a (200)		0.74±0.03a (13.9)		5.53±0.27c (-222.3)		0.20±0.02ab (5.6)
<b>ATC3</b>		8.41±0.03c (110)		2.19±0.05c (15)		0.617±0.04c (1.5)		7.335±0.31b (-42.3)		0.194±0.03b (5.4)
<b>ATE4</b>	7.34±0.23b	7.71±0.44a (37)	2.4±0.22c	4.13±0.57a (173)	0.62±0.04b	0.66±0.08a (3.9)	7.91±0.22a	5.10±0.59d (-280.7)	0.15±0.06b	0.18±0.03a (3)
<b>ATM4</b>		7.35±0.05b (1)		3.48±0.35b (98)		0.46±0.01c (-15.4)		6.04±0.23c (-187.2)		0.11±0.01c (-3.1)
<b>ATC4</b>		7.14±0.06c (-20)		1.95±0.07d (-45)		0.66±0.31a (4.5)		7.53±0.53b (-38)		0.12±0.03c (-2.4)
<b>ATE5</b>	7.56±0.27c	8.03±0.28a (47)	1.07±0.84d	2.25±0.38a (118)	0.70±0.06a	0.72±0.06a (2.3)	7.45±0.77	5.85±0.52 (-159.40)	0.16±0.02a	0.18±0.15d (2)
<b>ATM5</b>		7.62±0.05b (6)		1.14±0.78c (7)		0.34±0.03d (-36.4)		6.04±0.04 (-140.4)		0.16±0.01c (0)
<b>ATC5</b>		7.42±0.02d (-14)		1.77±0.26b (70)		0.67±0.61c (-2.6)		6.99±0.22 (-45.8)		0.18±0.02b (2.3)

Mean values followed by different letters in a same parameter per concentration (Initial and Final) are significantly different (one-way ANOVA; Tukey's test,  $p < 0.05$ )

**Table 6.24 Initial and final nutrient content (mean±SE) and percent change over initial nutrient content of different proportions of atrazine in the soil with earthworms, *M. posthuma* (ATM) and *E. fetida* (ATE) and aerobic composting (ATC)**

	TK		TNa		TLi		TCa	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
<b>Control</b>	1.27±0.02a	1.02±0.08d	1.67±0.19b	1.78±0.66a	1.11±0.035b	0.17±0.09d	3.04±0.05d	3.69±0.058a
<b>ATE</b>		(-24.7)		(10.5)		(6.6)		(65.8)
<b>Control</b>		1.07±0.64c		1.76±0.82a		1.09±0.14c		3.45±1.06b
<b>ATM</b>		(-19.2)		(9.3)		(2)		(41.5)
<b>Control</b>		1.12±0.49b		1.65±0.31b		1.14±0.58a		3.31±0.92c
<b>ATC</b>		(14.3)		(-2)		(3.3)		(27.5)
<b>ATE1</b>	1.82±0.35a	1.25±0.16d	1.81±1.15b	1.61±0.82c	0.12±0.07c	0.18±0.09a	2.23±0.32ab	2.63±0.09a
		(-57)		(-20)		(5.8)		(40.2)
<b>ATM1</b>		1.70±0.01c		1.90±0.03a		0.09±0.01d		1.36±0.09b
		(-115)		(9.1)		(-3)		(-86.7)
<b>ATC1</b>		1.74±0.35b		1.79±0.01b		0.13±0.08b		2.80±0.08a
		(-8)		(-1.9)		(1.3)		(57.1)
<b>ATE2</b>	1.85±0.18a	1.41±0.31d	1.46±0.19c	1.98±0.36a	0.17±0.058b	0.21±0.020a	3.33±0.12c	3.59±0.224a
		(-44)		(52)		(4.1)		(26.6)
<b>ATM2</b>		1.60±0.14c		1.58±0.04b		0.14±0.04c		2.33±0.48d
		(-24.6)		(12.1)		(-3)		(-100.1)
<b>ATC2</b>		1.72±0.05b		1.09±0.08d		0.12±0.06d		3.50±0.33b
		(-13.4)		(-36.2)		(-4.7)		(17.7)
<b>ATE3</b>	1.21±0.90a	0.48±0.07d	1.41±0.19c	1.86±0.18a	0.151±0.02c	0.19±0.037a	2.511±0.54b	2.82±0.24a
		(-73)		(45)		(4.55)		(31.8)
<b>ATM3</b>		0.93±0.35c		1.58±0.04b		0.09±0.01d		1.61±0.01d
		(-27.1)		(17.9)		(-6)		(-89.6)
<b>ATC3</b>		1.14±0.04b		1.55±0.07b		0.17±0.01b		2.09±0.14c
		(-6.7)		(14.7)		(1.9)		(-41.6)
<b>ATE4</b>	1.43±0.04a	1.35±0.02c	2.53±0.15	8.34±0.01	0.14±0.03b	0.16±0.01a	2.41±0.01b	2.67±0.02a
		(-278.5)		(581)		(2.35)		(26)
<b>ATM4</b>		1.38±0.08b		2.61±0.04*		0.11±0.01c		2.36±0.21c
		(-5)		(8)		(-3)		(-4.2)
<b>ATC4</b>		1.40±0.04b		2.50±0.04*		0.14±0.01b		2.22±0.08d
		(-2.8)		(-2.2)		(0.5)		(-19)
<b>ATE5</b>	1.98±0.13b	2.25±0.86a	1.52±0.13c	1.98±0.39a	0.13±0.01ab	0.08±0.01c	2.62±0.04a	2.42±0.05c
		(27)		(45.5)		(-4.17)		(-19.3)
<b>ATM5</b>		1.89±0.03d		1.57±0.01b		0.13±0.01a		2.57±0.04b
		(-9)		(5.3)		(0.6)		(-5)
<b>ATC5</b>		1.98±0.05b		1.36±0.03d		0.12±0.01b		1.72±0.04d
		(0.8)		(-16.5)		(-0.8)		(-89.6)

Mean values followed by different letters in a same parameter per concentration (Initial and Final) are significantly different (one-way ANOVA; Tukey's test, p < 0.05)

6.6.2 Pesticide content analysis: Atrazine

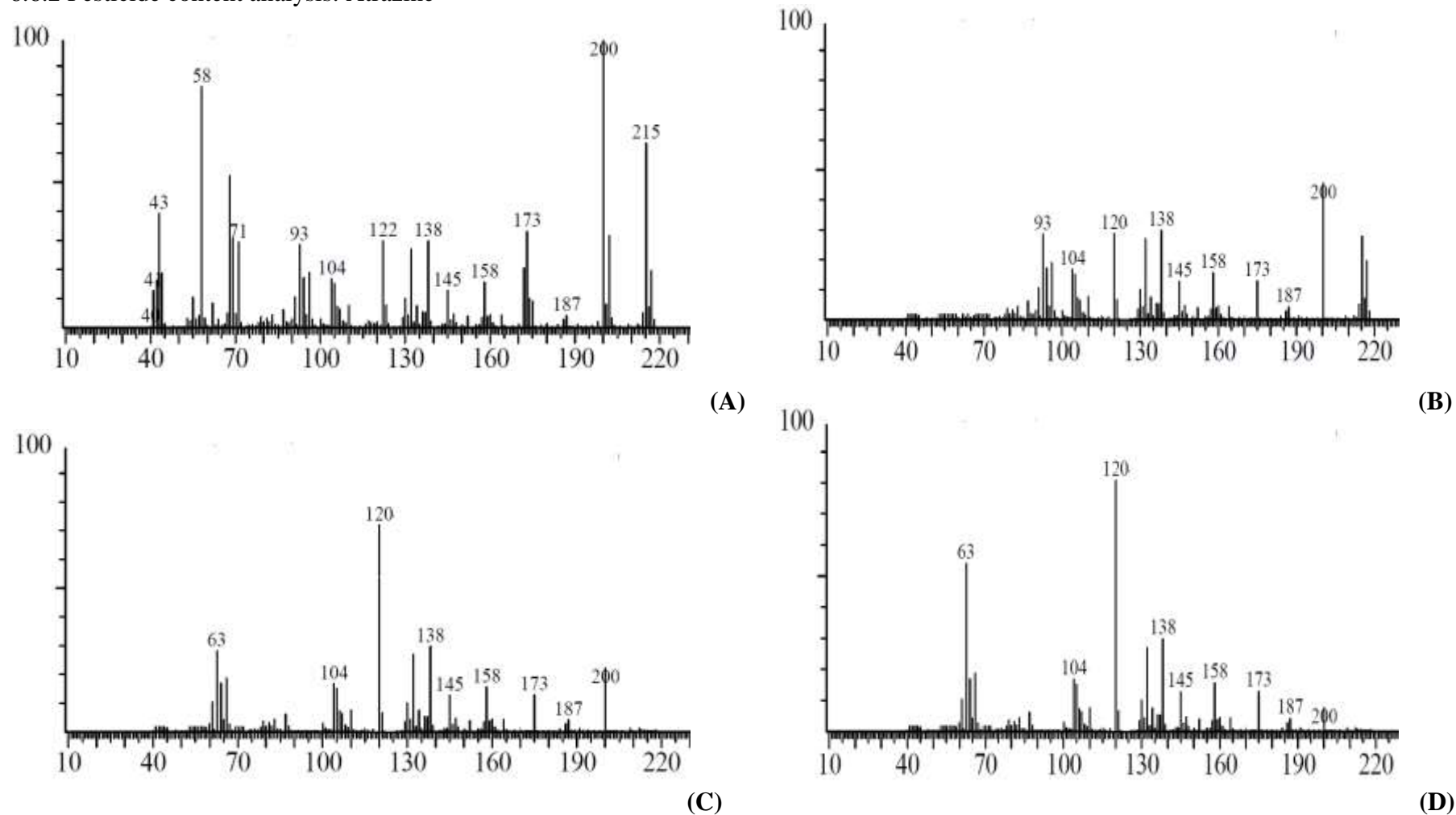


Figure 6.13 The mass spectrum of (A) Atrazine (B) soil medium after aerobic composting (C) soil medium after vermicomposting by *M. posthuma* (D) soil medium after vermicomposting by *E. fetida*

The mass/ charge ratio (m/z) of atrazine observed at m/z 200 i.e. {[M+H]<sup>+</sup>} with retention time of 19.363 min. Two different metabolites were formed by all the treated groups after 90 days of degradation and were identified as ((ethylamino) methylamino) methanediol m/z at 120, and aminomethanediol at m/z 63. During ionization process the oxidation takes place which showed successive increase for 90 days followed by peak declination at m/z 200. Percentage decrease in peak at m/z 200 has confirmed that upto 90 days 60% atrazine was decomposed in aerobic composting groups. In case of vermicomposting by *M. posthuma*, it was observed that on the 90<sup>th</sup> day of the decomposition, atrazine was 78% decomposed into ((ethylamino) methylamino) methanediol at m/z 120 (72%), and phosphoramidate at m/z 63 (27%). In case of *E. fetida*, on 90<sup>th</sup> day, from mass analysis studies maximum decomposition (94%) of atrazine was observed, that is atrazine at m/z 200 (94%) was decomposed into m/z 120 (84%) and m/z 63 (54%).

The ability of organic amendments for enhancement of atrazine and metatiron degradation in soil was also studied previously by different methods. The soil samples were treated with manure, compost and vermicompost at rates of 0, 0.5 and 2% (w/w). After the incubation period of 20, 40 and 60 days the concentration of atrazine and metatiron were determined by HPLC. Residual concentration of atrazine after 20, 40 and 60 days of incubation was found out to be 46.5, 38.9, and 36.2 mg/kg. However concentration of metatiron was found to be 2.9, 1.0, and 0.6 mg/kg after 20, 40 and 60 days respectively. More than 90% of chlorophenol, which is another organochlorine pesticide was degraded when chlorophenol contaminated soil was composted with a pile composed of straw compost (Laine and Jorgensen 1997). Introduction of composted manure is also known to enhance the degradation of methyl isothiocyanate and methyl bromide by 100% and 12% respectively lowering their emissions marginally (Gan et al. 1998). It was also found that degradation of atrazine is dependent on soil texture. The residual concentration of herbicides was more in sandy loam than in silty clay soil (Forouzangohar et al. 2005). Moorman et al. (2001) improved the removal of herbicide atrazine, trifluralin and metolachlor by using organic amendments like compost, corn fermentation product, manure, peat, saw dust and corn stalks. Atrazine degradation was enhanced by addition of 0.5% manure, 5% cornstalk and 5% peat in soil. On the contrary Delgado-Moreno and Pena (2009) found out no improvement in the removal of simazine, cyanazine, terbutylazine and prometryn after the addition of compost, vermicompost and olive cake in soils. Different organic amendments (mushroomspent compost, biogas slurry, farmyard manure and sodium citrate) were compared for their



effectivity in the removal of atrazine from contaminated soils. The biogas slurry showed highest atrazine removal (34.14%) followed by a combination of sodium citrate and farmyard manure (31.8%), mushroom spent (29.17%) and farmyard manure (22.07%) (Kadian et al.2008). Composted municipal sewage waste and composted straw was also evaluated to bioremediate atrazine-contaminated soil. However the residual atrazine concentration was found to be higher in soil treated with these organic amendments. The reason being that both these amendments promoted the production of bound residues of atrazine. The municipal sewage waste accelerates the sorption of atrazine which in turn causes a reduction in its bioavailability for its degrading microbes whereas composted straw was correlated with the formation of hydroxyatrazine (Houot et al. 1998). Humic acid from vermicompost and humic acid from peat were compared for the degradation of another triazine pesticide, metribuzin and it was found out that humic acid from vermicompost degraded the pesticide, metribuzin significantly more than humic acid derived from peat (Landgraf et al. 1998). This study supported our experiments. Reduction in diuron concentration in the soil was observed after the incorporation of vermicompost from winery and distillery waste (Fernandez-Bayo et al. 2008). Vermicompost addition was also known to reduce diuron availability. The degradation of diuron was further boosted by the addition of urea (Romero et al. 2010).

Our study thus depicts that soil amended by the process of vermicomposting help in degradation of atrazine in comparison to traditional aerobic composting. *E. fetida* was found to be more effective than *M. posthuma*. The comparison of nutritional status and physico-chemical properties are also suggestive of the fact that *E. fetida* is more effective species than *M. posthuma*.

### 6.6.3 Population build up of *E. fetida* in atrazine

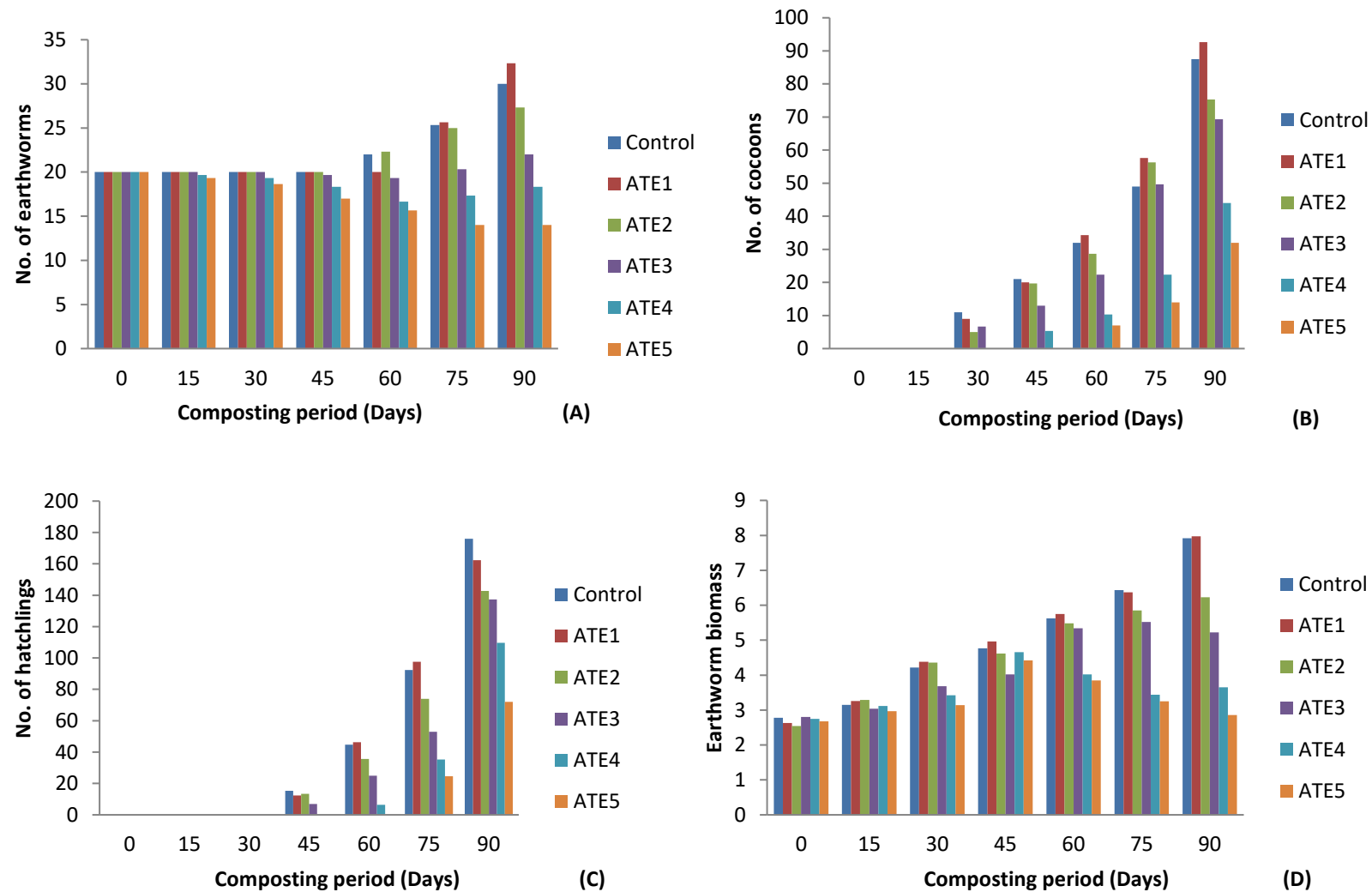
Based upon the recommended doses of atrazine, and toxicology studies carried out with the same, five concentrations of pesticides were spiked with the artificial soil for carrying out the experiment. Cow dung was also added to increase the acceptance of pesticide spiked artificial soil and to provide nourishment to earthworms for their survival for 3 months in the substrate. Cow dung not only increases the acceptance of pesticide spiked substrate as feed but also help to enhance the population build up of worms (Bhat et al. 2015). The addition of nutrient rich organic waste to fresh coffee ground increases the worm survival rates and earthworm biomass production (Liu and Price 2011). The ideal harvest time was found to be 90 days for atrazine spiked substrate, as compost granulated completely by this

time. Population build up in the form of number of worms, cocoons and hatchlings was measured to evaluate the acceptance of particular level of pesticides in the soil. These parameters were found to be significantly different at different concentration of pesticides. The numbers of worms at different time intervals in different concentrations of pesticides in soil substrate were found to be statistically different ( $p < 0.05$ ). After 30 days and 45 days, the number of earthworms in ATE4 and ATE5 were found to statistically decrease from rest of the substrate mediums. In the present study, the clitellum appeared between 15<sup>th</sup> to 30<sup>th</sup> day in all the substrate mixtures except ATE4 and ATE5 of pesticide spiked substrate between 30<sup>th</sup> to 45<sup>th</sup> day. The number of earthworms was found to increase on 60<sup>th</sup> day in control, ATE1, ATE2. The maximum number of earthworms was noticed in ATE1 ( $32.33 \pm 2.33$ ) on 90<sup>th</sup> day of experiment followed by C<sub>ATE</sub> ( $30 \pm 1$ ) and ATE2 ( $27.33 \pm 1.33$ ) and ATE3 ( $22 \pm 0.57$ ). The minimum number of earthworms was found in ATE5 ( $14 \pm 0.57$ ) followed by ATE4 ( $18.33 \pm 0.33$ ) on 90<sup>th</sup> day of experiment (**Fig 6.14**). The reason of this decrease in number in higher concentrations could be attributed to the non-palatability of food and toxicity of pesticide. The highest mortality was witnessed on 90<sup>th</sup> day in ATE5 (30%). Changes in chemical composition of substrate, production of toxic or foul smell of gases (nitrogen oxide, ammonia, carbon dioxide, etc.), high C:N ratio of the initial substrate are some of the factors responsible for earthworm mortality (Flegel and Schreder 2000). The survival, biomass production, and reproduction of earthworms are the best indicators to evaluate the vermicomposting process (Bhat et al. 2014). According to Ndegwa and Thompson (2000b), Tripathi and Bhardwaj (2004); Gajalakshmi et al. (2005), survival, growth rate and potential of reproduction for earthworms is highly dependent upon kind, palatability and quality of food (in terms of chemistry). The number of cocoons with different pesticide concentrations was also found to be significantly different ( $p < 0.05$ ). Cocoon formation was first observed on the 30<sup>th</sup> day of experiment in C, ATE1, ATE2, ATE3 except in ATE4 and ATE5 in which the cocoons were observed in 45<sup>th</sup> and 60<sup>th</sup> day respectively. The maximum number of cocoons on 90<sup>th</sup> day was observed in ATE1 ( $92.66 \pm 2.40$ ) followed by C<sub>ATE</sub> ( $87.5 \pm 1.5$ ) and minimum in ATE5 ( $32 \pm 3.46$ ). Cocoon production was significantly less in substrate with higher concentration of pesticide suggesting that higher concentration prove to be toxic for development of earthworms. The biochemical quality of the feed and supporting medium forms to be an important factor that influences the production of cocoons (Flack and Hartenstein 1984; Edwards et al. 1998). Fayolle et al. (1997) have also pointed out that food source play an important role on cocoon production rate. Quality of food influencing the

growth of worms; onset and rate of cocoon production is also depicted by Suthar (2008). The substrates having lower C:N ratio usually display higher rate of cocoon production (Kumar et al. 2010). The earthworms size was small to medium and hence it seemed that energy that otherwise is used to increase the biomass was utilized in reproduction (increasing number of cocoons). This was supported by a number of researchers who observed a higher rate of cocoon production by small to medium sized epigeic earthworms (Kale et al. 1992; Chaudhuri and Bhattacharjee 2002; Barne and Striganova 2005; Suthar 2008). However, in contrast to this, Lavelle (1981) found a positive relationship between size of an adult worm and cocoon production. Higher C:N ratio in higher concentration of pesticides may be the reason of delayed clitellum development and thence cocoon production as low level of nitrogen in the substrate is considered to be a limiting factor for growth of worms (Butt 1993). So as to infer, we can say that higher concentration of pesticide in soil media can significantly affect the cocoon production, delays sexual maturity and reproduction of *E. fetida*.

Significant difference ( $p < 0.05$ ) in the number of hatchlings was also observed with different concentrations of pesticides in substrate medium. The number of hatchlings was also negatively correlated with the concentration of pesticides spiked substrate. Hatchlings were first observed for the first time on 45<sup>th</sup> day in C<sub>ATE</sub>, ATE1, ATE2 and ATE3 while ATE4 and ATE5 showed hatchlings for the first time on 60<sup>th</sup> and 75<sup>th</sup> day respectively (**Fig 6.14**). The maximum number of hatchlings ( $176 \pm 4.35$ ) observed on 90<sup>th</sup> day in C<sub>ATE</sub> and minimum in ATE5 ( $72 \pm 5.03$ ). Hatchlings number was significantly lower in substrates with higher concentrations of pesticides (ATE4 and ATE5) as compared to substrates with lower concentrations of pesticide (ATE1, ATE2, ATE3). This could be corroborated to the higher production of cocoons in substrates with lower concentrations of pesticides and vice-versa. Reinecke and Reinecke (1997) also reported ultra-structural damage in sperms of earthworms exposed to toxic elements and associate it with lower fertility and viability of cocoons.

The biomasses of the earthworms in different concentrations of pesticides were found to vary significantly ( $p < 0.05$ ). An increase in biomass was recorded in all the concentrations. This was supported by Rathinamala et al. (2008) and Bhat et al. (2015). The biomass was found to be negatively correlated with the concentration of atrazine. Maximum biomass was attained by ATE1 ( $7.92 \pm 0.31$ ) on 90<sup>th</sup> day whereas ATE5 showed minimum biomass on 90<sup>th</sup> day ( $2.86 \pm 0.43$ ). Presence of fungus during the process of vermicomposting becomes additional food to the worms which contributed to the higher weight of the worms (Pramanik and Chung 2011).



**Figure 6.14 Population build up of *E. fetida* in various concentrations of atrazine (A) No.of earthworms (B) No.of cocoons (C) No.of hatchlings (D) Earthworm biomass at different time durations (days)**

#### 6.6.4 Acephate:Physico-chemical Parameters

Physico-chemical parameters showed significant changes with varying concentration of atrazine and in the soil mixtures.

##### 6.6.4.1 pH and EC

After vermicomposting by *E. fetida*, the percent difference in pH significantly increased from pre to post samples. The maximum increase was found in ACE3 (106%) and the minimum in C<sub>ACE</sub> (46%). The order of increase was found as ACE3>ACE1>ACE2>ACE4>ACE5. The electrical conductivity reflects the salinity of any material and is a good indicator of the applicability and utility of a compost or vermicompost for agricultural purposes. The electrical conductivity was found to significantly ( $p < 0.05$ ) increase in the final vermicomposted products. The maximum increase was found in C<sub>ACE</sub> (217%) and minimum was found in ACE1 in (83%). The increase in EC in post-vermicompost samples were found to be in order C<sub>ACE</sub>>ACE2>ACE3>ACE4>ACE5>ACE1 (**Table 6.25**).

After vermicomposting by *M. posthuma*, the percent difference in pH in case of acephate does not show a common gradation. In C<sub>ACM</sub> and in case of ACM1, ACM2 the pH increased by 38%, 9% and 7% respectively while in other subsequent concentrations the pH was found to decrease. The pH was found to be positively correlated with initial three lower concentrations while for the next three concentrations it thereafter declined. The maximum decrease was found in the highest concentration (ACM5). The order of decline in pH was ACM5>ACM3>ACM4. The electrical conductivity was found to significantly ( $p < 0.05$ ) increase in the final vermicomposted products. The maximum increase was found in ACM1 (121%) followed by C<sub>ACM</sub> (106%) and minimum was found in ACM4 in (12%). The increase in EC in post-vermicompost samples were found to be in order ACM1>C<sub>ACE</sub>>ACM2>ACM3>ACM5>ACM4.

In case of aerobic composting, ACC1 and ACC3 recorded an increase in pH by 1% and 13% respectively. However ACC2, ACC4, ACC5 recorded a decrease of 34%, 36% and 35% in pH. The EC in this case was found to increase in ACC1 and ACC4 by 4% and 28%. However a decline in EC was found in ACC2 (33%), ACC3 (6%) and ACC5 (54%).

On comparison of the pH level of ACE and ACM post vermicompost we conclude that *E. fetida* increases the pH level significantly in comparison to *M. posthuma*. Our results corroborate with the findings of Togenetti et al. (2007); Muthukumaravel et al. (2008);

Mainoo et al. (2009). The increase in pH has also been reported by Datar et al. (1997). According to Aira et al. (2007), the mutual role of earthworms and microbes play a vital role in enhanced degradation of short chain fatty acids and precipitation of calcium carbonate which leads to an eventual increase in pH. According to Khwairakpam and Bhargava (2009); Yadav and Garg, (2009) loss in organic matter with a corresponding mineralization of salts is responsible for the increase in pH. Kaviraj and Sharma (2003) also related the increase in EC due to the loss of organic matter and release of various mineral salts like phosphate, potassium, ammonia, etc. The increase in EC was also attributed to increase in soluble salts level because of mineralization by microbes and earthworms (Karmegam and Daniel 2009). Correspondingly increase in sodium and potassium content in the mixtures may also be responsible for the rise in EC of the products of vermicomposting (Guoxue et al. 2001).

#### 6.6.4.2 Nitrogen

The nitrogen content after vermicomposting by *E. fetida* was found to increase in all the groups in final products of vermicompost. The increase was found to be highest in C<sub>ACE</sub> (85.4%) followed by ACE4 (39.7%) and the least in ACE5 (1.6%). The order of increase in nitrogen was found in C<sub>ACE</sub>> ACE4> ACE1> ACE2> ACE3> ACE5.

The nitrogen after vermicomposting by *M. posthuma* was found to increase in all the groups except for ACM5. The order of increase in nitrogen content was found to follow C<sub>ACM</sub>>ACM3>ACM4>ACM2>ACM1. The decrease in nitrogen content in ACM5 (14%) may be assumed because of lower earthworm activity because of higher pesticide concentration.

In case of aerobic composting, the final products showed an increase in nitrogen content compared to final with the exception in ACC4 and ACC5. The increase was found to be maximum in C<sub>ACC</sub> (12.8%) followed by ACC2 (11%) followed by ACC1 (5.2%) and 4.9% in ACC3. A reduction of 19.9% and 9% was recorded in ACC4 and ACC5.

According to Viel et al. (1987), losses in organic carbon might be responsible for nitrogen addition. Earthworms also have a great impact on nitrogen mineralization so that mineral nitrogen was retained in the nitrate form (Atiyeh et al. 2000). According to Cynthia and Kumar (2012), increase in TKN could be attributed to the decomposition brought about by the worms accelerating mineralization of nitrogen content. Suthar (2007) also backs this trend explaining that earthworms are responsible for enrichment of nitrogen content of vermicompost through decaying tissues of dead earthworms and microbial mediated nitrogen

transformation in vermicomposting systems. The decay of the dead worms also contributes to an increase in N as a large portion of dry weight of a worm is protein (Tripathi and Bhardwaj 2004). A loss in organic carbon is also responsible for nitrogen addition (Viel 1987). The increase in nitrogen content after vermicomposting is also attributed to the mineralization of C-rich materials and due to the activity of N-fixing bacteria (Plaza et al. 2007; Crusmey et al. 2014). But Chauhan and Joshi (2010) explained that the final N content of vermicompost depends on the initial N in the medium and on the extent of decomposition. This was also backed by Sangwan et al. (2010) who reported an increase in TKN due to nitrogen mineralization by earthworms. The decay of the dead worms also contributes to an increase in N as a large portion of dry weight of a worm is protein (Tripathi and Bhardwaj 2004).

#### 6.6.4.3 Organic Carbon

After vermicomposting by *E. fetida*, significant decline in TOC in the products of vermicomposting was found. The maximum decline was found in ACE4 (280.5%) and minimum decline in ACE3 (114.5%). The decrease in TOC follows the order ACE4 > ACE2 > ACE1 > C<sub>ACE</sub> > ACE5 > ACE3.

The significant decline ( $p < 0.05$ ) in organic carbon (OC) in the products of vermicomposting was also found after vermicomposting by *M. posthuma*. The decline in final products over initial was found to be maximum in ACM3 (179.4%) and minimum in ACM5 (3.9%). The percent decrease in OC in vermicomposted products followed the order ACM4 > ACM3 > C<sub>ACM</sub> > ACM2 > ACM1 > ACM5.

In case of aerobic composting the significant decline in TOC in the products of final vermicomposting was found except in ACC1 which showed an increase. The maximum decline was found in ACC3 (41.5%) and ACC1 recorded an increase of 18.8%. The order of decline in TOC follows as C<sub>ACC</sub> > ACC3 > ACC4 = ACC5 > ACC2. The decline in TOC in aerobic composting was however insignificant when compared to its counterparts in vermicomposting processes.

According to Edwards (2004), earthworm work by fragmenting and homogenising the ingested material through muscular action of their foregut and additional extracellular enzymes provided by their gut microbes. The increase in surface area and addition of mucus and enzymes to the ingested material further leads to enhanced microbial action. This additive action of earthworm and microbes lead to loss of carbon from the substrates in the form of microbial respiration (CO<sub>2</sub>) (Tognetti et al. 2007b; Hait and Tare 2011; Singh and Suthar

2012). Kaviraj and Sharma (2003) have reported a 20-40% reduction of TOC as CO<sub>2</sub> during vermicomposting of municipal or industrial wastes. Higher loss of carbon during vermicomposting can be corroborated to higher rate of mineralization by catabolically active microbes the activity of which is enhanced by the worms (Aira et al. 2007, Prakash and Karmegam 2010).

#### 6.6.4.4 Phosphorus

The level of phosphorus after vermicomposting by *E. fetida* was found to increase significantly ( $p < 0.05$ ) in final products of vermicomposting when compared with initial. The maximum increase in the level of phosphorus was found to be in ACE4 (3.7%) and maximum decline of 3.2% was recorded in ACE5. The increase in phosphorus followed the order ACE4 > C<sub>ACE</sub> > ACE2 > ACE1 = ACE3.

The phosphorus content after vermicomposting by *M. posthuma* increased significantly ( $p < 0.05$ ) in the products of vermicomposting with the exception in ACM4 and ACM5. The decrease in higher doses may be corroborated to the reduced earthworm activity in the same. These results corroborate with the gradation found in pH mentioned above. Percent increase was found to be maximum in ACM1 (5.5%) and minimum in ACM3 (1.2%). The increase in phosphorus content in post-vermicompost samples were found to be in order ACM1 > C<sub>ACM</sub> > ACM2 > ACM3. The maximum decrease was found in ACM4 (3.2%) followed by ACM5 (2.5%). In control and in the initial concentrations of atrazine (ACM1-ACM3), the level of phosphorus increased significantly while in the lower concentrations (ACM4 and ACM5), the level of phosphorus decreased.

In case of aerobic composting, decrease in phosphorus content was recorded in final products with the exception in ACC1 which recorded an increase in 1%. ACC4 recorded a decrease of 10% while ACC2, ACC3 and ACC5 recorded a decline of 1% each. However the results of aerobic composting were completely contrasting than that of the vermicomposting. Our results corroborated with the findings of Kaviraj and Sharma (2003), Tognetti et al. (2005) and Liu and Price (2011) depicting the stimulatory effect of earthworms on phosphorus availability in soil. Worm casts are also rich in alkaline phosphatase and hence lead to rise in content of phosphate (Bayon and Binet 2006; Prakash and Karmegam 2010). Sangwan et al. (2010) also reported a 1.3-1.5 fold increase in phosphorus content in the vermicomposting of pressmud. Phosphate solubilising microbes in the presence of worms are responsible for conversion to soil phosphorus in mineral form to available forms (Kumar et al.



2015c). Vermicomposting was reported to be an efficient way for transformation of unavailable phosphorus to available forms for the plant (Ghosh et al. 1999). The increase in phosphorus content after vermicomposting was also supported by Makin et al. (2014); Das et al. (2015).

#### 6.6.4.5 Potassium

After vermicomposting by *E. fetida*, the level of potassium decreased significantly ( $p < 0.05$ ) in the final products of vermicomposting with the exception in control. Percent decline was maximum in ACE2 (206%) and minimum in ACE1 (57%). Percent decline in potassium in the products of vermicomposting was in the order of ACE2>ACE3>ACE4>ACE5>ACE1>C<sub>ACE</sub>.

Potassium decreased significantly ( $p < 0.05$ ) in the final products after vermicomposting by *M. posthuma*. Percent decline was maximum in ACM2 (31.5%) and minimum in ACM5 (16.5%). Percent decline in potassium in the products of vermicomposting was in the order of ACM2>ACM3>ACM1>C<sub>ACM</sub>>ACM4>ACM5. Higher pH makes potassium ions more susceptible to fixation by colloids and thus a rise in pH can also be responsible for decline in level of potassium (Alexander 1983; Brady and Weil 2002). A decrease in potassium was also reported by Sangwan et al. (2010) during the vermicomposting of sugar industry waste.

In case of aerobic composting, decline in the content of potassium was recorded in the final products of composting. The maximum decline was found in ACC1 and minimum decline was found in ACC5. The decline in potassium in this case followed the order ACC1>ACC3>ACC2>C<sub>ACC</sub>>ACC4>ACC5. In C<sub>ACC</sub> an increase of 14.3% was recorded (**Table 6.26**). The decline in case of aerobic composting was comparatively far less than that of its counterparts in case of vermicomposting.

Higher pH makes potassium ions more susceptible to fixation by colloids and thus a rise in pH can also be responsible for decline in level of potassium (Brady and Weil 2002). An increase in potassium was also reported by Sangwan et al. (2010) during the vermicomposting of sugar industry waste. The decline in level of potassium was attributed to leaching in the process of vermicomposting and subsequent utilization of potassium by the earthworms (Orozco et al. 1996; Benitez et al. 1999; Garg et al. 2006 and Kumar et al. 2010). Utilization of potassium by earthworms for their metabolic activity was also confirmed by Singh et al. (2010); Vig et al. (2011); Yadav and Garg (2011); Bhat et al. (2013). Adi and

Noor (2009) added that the solubilisation of insoluble potassium is because of formation of acid during decomposition by microbes. However, Delgado et al. (1995), Suthar (2008), Liu and Price (2011) reported higher potassium content in the vermicompost produced from the different feed mixtures.

#### 6.6.4.6 Sodium

A decrease in the level of sodium was observed in the product after vermicomposting by *E. fetida*. Maximum decrease in sodium was found in ACE5(101%) and minimum decrease in ACE2 (6.5%). The percent increase in sodium in final products followed the order ACE5>ACE4>ACE3>ACE1>ACE2. C<sub>ACE</sub> recorded an increase of 10.5%.

Activity of *M. posthuma* leads to an increase in the level of sodium in the post vermicompost product. Maximum increase in sodium was found in ACM1(17.7%) and minimum increase in ACM2 (1.2%). The percent increase in sodium in final products followed the order ACM1>C<sub>ACM</sub>>ACM5>ACM4>ACM3>ACM2. Our finding corroborated with the studies of Yadav and Garg (2011) who observed increase in sodium content in the final products of vermicomposting of vegetable wastes.

In case of aerobic composting a decrease in content of sodium was found in all the final products of composting. The maximum decline was found in ACC3 and minimum decline in ACC4. The order of decrease was found in the order ACC3> ACC4> ACC2> ACC1> ACC5.

Our finding corroborated with the studies of Subramanian et al. (2010) who found decrease in all treatment combinations during the vermicomposting of sago industry soild wastes. However, Yadav and Garg (2011) who observed increase in sodium content in the final products of vermicomposting of vegetable wastes. The above trend was supported by Khawairakpam and Bhargava (2009); Vig et al. (2011); Yadav and Garg (2011); Bhat et al. (2013) and Singh et al. (2010). However the sodium content was found unchanged in all treatment combinations during the vermicomposting of sago industry soild wastes (Subramanian et al. 2010).

#### 6.6.4.7 Lithium and Calcium

Lithium increased from pre-vermicompost sample to final products significantly ( $p < 0.05$ ) after vermicomposting by *E. fetida*. The maximum increase in lithium was found in ACE2 (100.5%) and minimum decline was found in ACE5 (4%). The order of increase of lithium in the post vermicompost products were found in the order

ACE2>ACE3>ACE1>ACE4>ACE5. On the other hand calcium was also found to increase in the products of control vermicomposting in comparison to initial substrate with the exception of highest concentration. The order of increase in the products of vermicomposting in comparison to initial substrate was found to be ACE2>ACE3>ACE1>ACE4>C<sub>ACE</sub> whereas in ACE5 decline of 32% was observed.

Lithium decreased from pre-vermicompost sample to final products significantly ( $p < 0.05$ ) after vermicomposting by *M. posthuma*. The maximum decline in lithium was found in ACM1 (3.2%) and minimum decline in was found in ACM2=ACE5 (0.9%). The order of decline of Lithium in the post vermicompost products were found in the order C<sub>ACM</sub>>ACM1>ACM3>ACM4>ACM2=ACM5. On the other hand Calcium was also found to increase in the products of control and lower two after vermicomposting in comparison to initial substrate. In case of calcium content, the order of increase in the products of vermicomposting in comparison to initial substrate was found to be C>ACM1>ACM2 whereas the decline in calcium was found to be maximum in ACM3>ACM4>ACM5. The lowering of Ca content in higher doses again can be related to the reduced activity of earthworms. The maximum increase was found in ACM1 (59.2%) and maximum decrease was found in ACM5 (27.3%). Our results corroborated with the findings of Oyedele et al. (2006) who observed significantly higher calcium content in vermicast than surrounding soil. However, Chaudhari et al. (2009) and Singh et al. (2016) reported low calcium content in vermicast as compared to surrounding soil.

In case of aerobic composting the Li content was found to decrease in ACC1, ACC4 and ACC5. However, in ACC2 and ACC3 the the Li content was found to increase. The Ca content in case of aerobic composting was found to decrease in all the products of composting except in ACC1 (81%). The order of decline of Ca was ACC3> ACC5> ACC2> ACC4. The lowering of Ca content in higher doses again can be related to the reduced activity of earthworms. The maximum increase was found in ACE2 (1093.5%). Our results corroborated with the findings of Oyedele et al. (2006) who observed significantly higher calcium content in vermicast than surrounding soil. However, Chaudhari et al. (2009) and Singh et al. (2016) reported low calcium content in vermicast as compared to surrounding soil.

**Table 6.25 Initial and final nutrient content (mean±SE) and percent change over initial nutrient content of different proportions of acephate in the soil with earthworms, *M. posthuma* (ATM), *E. fetida* (ATE) and aerobic composting (ACE)**

	pH		EC		TKN		TOC		TAP	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
<b>Control ACE</b>	7.12±0.03d	7.58±0.61a (46)	1.19±0.89d	3.36±0.24a (217)	0.74±0.01c	1.59±0.07a (85.4)	7.48±0.31a	5.65±0.04d (-183.3)	0.10±0.05b	0.13±0.01a (3.1)
<b>Control ACM</b>		7.50±0.88b (38)		2.25±0.74b (106)		0.96±0.05b (22.7)		6.06±1.05c (-142.6)		0.12±0.01ab (2)
<b>Control ACC</b>		7.39±0.38c (27)		2.01±0.37c (82)		0.87±0.03c (12.8)		6.46±0.89b (-102.5)		0.11±0.04ab (1.6)
<b>ACE1</b>	7.38±0.85c	8.25±0.81a (87)	1.45±0.36d	2.28±0.94b (83)	0.62±0.27d	0.96±0.13a (33.7)	7.25±0.02b	5.34±0.12d (-190.7)	0.10±0.05b	0.12±0.05b (2)
<b>ACM1</b>		7.47±0.05b (9)		2.66±1.42a (121)		0.69±0.03b (7)		6.78±0.36c (-46.8)		0.15±0.02a (5.5)
<b>ACC1</b>		7.39±0.25c (1)		1.49±0.64c (4)		0.67±0.02c (5.2)		7.44±0.28a (18.8)		0.11±0.02b (1)
<b>ACE2</b>	7.5±1.15b	8.19±0.08a (69)	1.52±0.61c	3.49±0.39a (197)	0.82±0.12d	0.96±0.84a (14)	7.25±0.82a	5.21±0.08d (-203.9)	0.14±0.06c	0.17±0.03a (3)
<b>ACM2</b>		7.57±0.06b (7)		2.23±0.20b (71)		0.923±0.05c (9.8)		6.67±0.36c (-58.5)		0.15±0.01ab (1.6)
<b>ACC2</b>		7.16±0.03c (-34)		1.19±0.08d (-33)		0.93±0.01b (11)		7.04±0.55b (-20.8)		0.13±0.03c (-1)
<b>ACE3</b>	7.39±0.10c	8.45±1.28a (106)	1.15±0.57c	2.82±0.14a (167)	0.88±0.04d	0.91±0.07c (3.3)	6.24±0.42a	5.10±0.136c (-114.5)	0.17±0.05b	0.15±0.07c (2)
<b>ACM3</b>		7.27±0.10d (-12)		1.53±1.25b (38)		1.218±0.52a (33.6)		4.452±0.238d (-179.4)		0.182±0.01a (1.2)
<b>ACC3</b>		7.52±0.23b (13)		1.09±0.06d (-6)		0.93±0.072b (4.9)		5.83±0.36b (-41.5)		0.16±0.05ab (-1)
<b>ACE4</b>	7.54±0.9b	8.14±0.71a (60)	1.71±1.12d	2.96±0.78a (125)	0.92±0.05c	1.32±0.02a (39.7)	7.52±0.07a	4.72±0.03d (-280.5)	0.11±0.04b	0.14±0.03a (3.7)
<b>ACM4</b>		7.52±0.01b (-2)		1.83±0.80c (12)		1.08±0.19 (16)b		5.61±0.03c (-191.1)		0.07±0.02c (-3.2)
<b>ACC4</b>		7.18±0.08 (-36)c		1.99±0.02b (28)		0.72±0.29d (-19.9)		7.120.07b (-40)		0.01±0.12d (-10)
<b>ACE5</b>	7.64±0.06b	8.18±0.24a (54)	1.34±0.82c	2.42±0.11a (108)	0.98±0.31a	0.99±0.04a (1.6)	7.02±0.235a	5.86±0.10d (-115.3)	0.15±0.02a	0.11±0.06c (-3.2)
<b>ACM5</b>		7.51±0.05c (-13)		1.59±0.64b (25)		0.84±0.07b (-14)		6.98±0.10b (-3.9)		0.12±0.01bc (-2.5)
<b>ACC5</b>		7.29±0.01d (-35)		0.8±0.07d (-54)		0.89±0.19b (-9)		6.62±0.48c (-40)		0.14±0.03ab (-1)

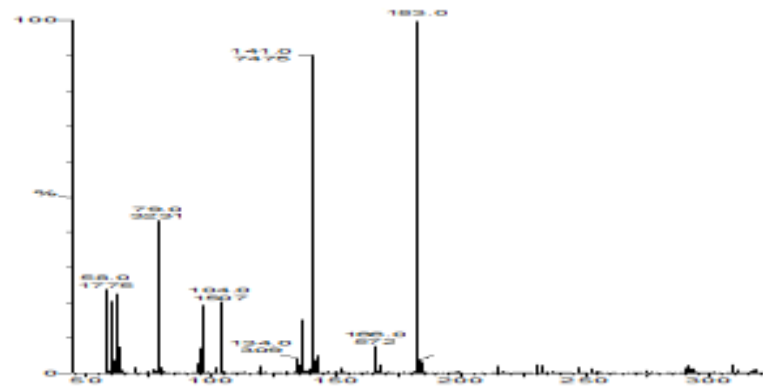
Mean values followed by different letters in a same parameter per concentration (Initial and Final) are significantly different (one-way ANOVA; Tukey's test, p < 0.05)

**Table 6.26: Initial and final nutrient content (mean±SE) and percent change over initial nutrient content of different proportions of acephate in the soil with earthworms, *M. posthuma* (ATM), *E. fetida* (ATE) and aerobic composting (ACE)**

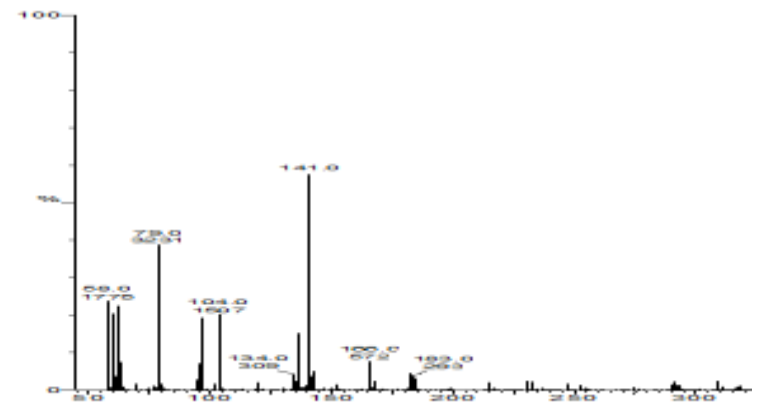
	TK		TNa		TLi		TCa	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
<b>Control ACE</b>	1.27±0.02a	1.02±0.08d (-24.7)	1.67±0.19b	1.78±0.66a (10.5)	1.11±0.03b	0.17±0.09d (6.6)	3.04±0.05d	3.69±0.05a (65.8)
<b>Control ACM</b>		1.07±0.64c (-19.2)		1.76±0.82a (9.3)		1.09±0.14c (2)		3.45±1.06b (41.5)
<b>Control ACC</b>		1.12±0.49b (14.3)		1.65±0.31b (-2)		1.14±0.58a (3.3)		3.31±0.92c (27.5)
<b>ACE1</b>	1.43±0.35a	0.86±0.16d (-57)	1.81±1.15a	1.61±0.82d (-20)	1.26±0.72b	1.84±0.97a (58)	1.47±0.86d	7.14±0.89a (567)
<b>ACM1</b>		1.14±0.09b (-28.2)		1.98±0.08a (17.7)		1.22±0.06b (-3.2)		2.06±0.85c (59.2)
<b>ACC1</b>		1.09±0.09c (-34)		1.61±0.04b (-20)		1.16±0.02b (-10)		2.28±0.07b (81)
<b>ACE2</b>	2.57±0.09a	0.51±0.42d (-206)	1.90±0.41a	1.84±0.04b (-6.5)	1.05±0.12bc	2.06±0.07a (100.5)	1.42±0.36c	12.35±1.15a (1093.5)
<b>ACM2</b>		2.25±0.01c (-31.5)		1.91±0.04a (1.2)		1.04±0.02c (-0.9)		1.69±0.08b (27.1)
<b>ACC2</b>		2.28±0.01b (-29)		1.66±0.08c (-24)		1.21±0.02b (16)		1.27±0.08d (-15)
<b>ACE3</b>	2.51±0.42a	0.61±4.39b (-190)	2.09±0.25a	1.33±1.41c (-76)	2.31±0.02a	2.99±0.53a (68)	2.16±0.28b	8.95±3.37a (679.5)
<b>ACM3</b>		2.21±0.02a (-29.3)		2.10±0.10a (1.7)		2.28±0.01a (-2.5)		1.60±0.16c (-56)
<b>ACC3</b>		2.19±0.02a (-32)		1.56±0.10b (-53)		2.43±0.04ba (12)		0.75±0.32d (-141)
<b>ACE4</b>	2.4±0.09a	1.10±0.11c (-130)	2.79±0.04a	1.9±0.12c (-89.5)	1.60±0.02b	1.88±0.18a (27.5)	1.78±0.36b	4.52±1.37a (274)
<b>ACM4</b>		2.21±0.26b (-18.1)		2.81±0.07a (2.8)		1.58±0.01c (-1.6)		1.42±0.14d (-35.9)
<b>ACC4</b>		2.21±0.26b (-19)		2.53±0.03b (-26)		1.29±0.02d (-31)		1.70±0.73c (-8)
<b>ACE5</b>	2.85±1.74a	1.92±0.34c (-93)	2.12±0.12b	1.11±0.42d (-101)	1.74±0.33a	1.78±0.31a (4)	2.18±0.01a	1.86±0.18b (-32)
<b>ACM5</b>		2.68±0.08b (-16.5)		2.16±0.02a (4.1)		1.73±0.01a (-0.9)		1.91±0.43b (-27.3)
<b>ACC5</b>		2.7±0.08b (-15)		1.96±0.04c (-16)		1.70±0.73a		1.8±0.29b (-34)

Mean values followed by different letters in a same parameter per concentration (Initial and Final) are significantly different (one-way ANOVA; Tukey's test,  $p < 0.05$ )

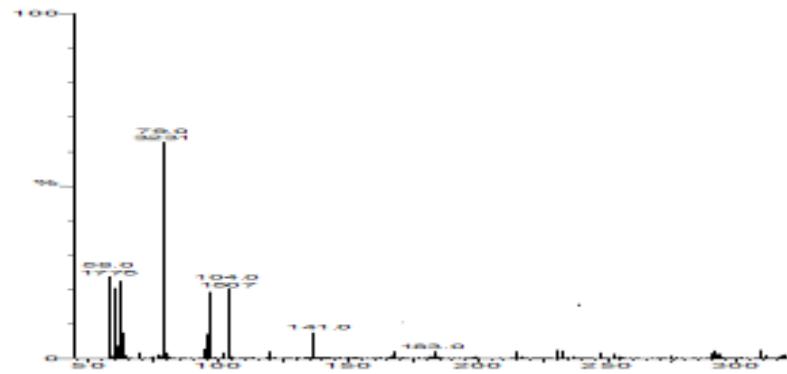
6.6.5. Pesticide content analysis: Acephate



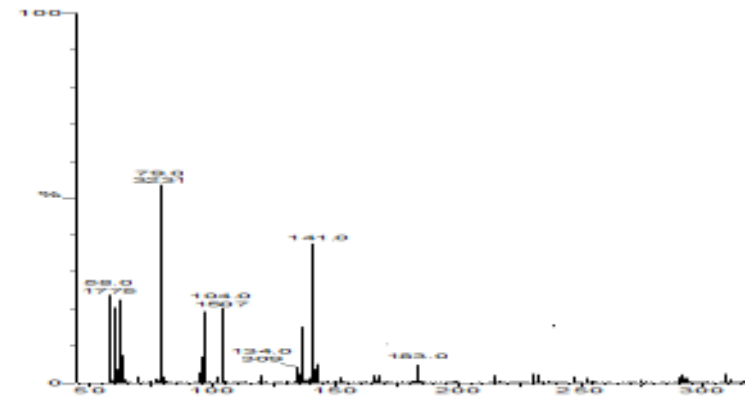
(A)



(B)



(C)



(D)

Figure 6.15 The mass spectrum of (A) acephate (B) soil medium after aerobic composting (C) soil medium after vermicomposting by *M. posthuma* (D) soil medium after vermicomposting by *E. fetida*

The mass/ charge ratio (m/z) of acephate is observed at m/z 183 i.e. {[M+H]<sup>+</sup>} and [M+H]<sup>+</sup>H<sub>2</sub>O at m/z 198. Four other metabolites of acephate are characterized at metamidophos m/z at 141, phosphoramidic acid m/z ratio at 96, phosphoramidate at m/z 78 and unknown at m/z 62. In addition, oxidation of the protonated acephate molecule at m/z 183 occurred during the ionization process resulting in successive increase for 90 days followed by decrease in peak at m/z 165 (**Fig 6.15**) Percentage decrease in peak at m/z 183 has confirmed that upto 90 days more than 90% acephate was decomposed in aerobic composted groups. In case of *M. posthuma*, it was observed that on the 90<sup>th</sup> day of the decomposition, acephate was 95% decomposed into methamidophos at m/z 141 (68%), and phosphoramidate at m/z 78 (57%). In case of *E. fetida*, on 90<sup>th</sup> day, from mass analysis studies maximum decomposition of acephate was observed, that is acephate at m/z 183 (98%) was decomposed into m/z 141 (92%).

Degradation studies on acephate with organic amendments are rare. Similar organophosphate, chlorpyrifos and other pesticides like cypermethrin, fenvalerate and trichlorpyr butoxyethyl ester was analysed for its bioremediation with cow dung slurry. Chlorpyrifos, also an organophosphate was rapidly hydrolysed to 3,5,6 trichloro-2-pyridinol in 25 and 50 mg/kg chlorpyrifos amended soil but in 100 mg/kg chlorpyrifos amended soil it was present till the 3<sup>rd</sup> day of the experiment. In case of 25 and 50 mg/L cypermethrin, it was hydrolysed to 3-phenoxy benzaldehyde and 3-phenoxybenzyl alcohol respectively by 7<sup>th</sup> day. Trichlorpyr butoxyethyl degraded to form trichlorpyr acid and 3,5,6 trichloro pyridinol as principal metabolites within 24 h. The soil-pesticide mix, nutrient availability, microbial population of the cow dung slurry is the factors affecting bioremediation of pesticides (Geetha and Fulekar 2008). *Pseudomonas plecoglossicida* and *Pseudomonas aeruginosa* are organisms for bioremediation of cypermethrin (Boricha and Fulekar 2009) and chlorpyrifos (Fulekar and Geetha 2008) respectively. Fenvalerate was degraded and intermediates like 4-chloroalpha benzene acetic acid and 3-phenoxy-benzoic acid are formed over a period of seven days (Geetha and Fulekar 2010). These intermediates are less toxic than parent compound. Vermicompost was analysed for its adsorption capacity for the removal of another organophosphate pesticide methylparathion. The maximum adsorption capacity was found to be 0.17 mg/g depicting vermicompost as an economical adsorbent minimizing environmental impacts of methylparathion (Mendes et al. 2012). This supports our study that depicts that degradation of acephate by vermicomposting was more than traditional aerobic composting and that *E. fetida* helps in better vermin-degradation than *M. posthuma*.

#### 6.6.6 Population build up of *E. fetida* in acephate

Based upon the recommended doses of acephate, and toxicology studies carried out with the same, five concentrations of pesticides were spiked with the artificial soil for carrying out the experiment. Cow dung was also added to increase the acceptance of pesticide spiked substrate and to provide nourishment to earthworms for their survival for 3 months in the substrate. (Bhat et al.2015). The addition of nutrient rich organic waste to fresh coffee ground increases the worm survival rates and earthworm biomass production (Liu and Price 2011). The ideal harvest time was found to be 90 days for atrazine spiked substrate, as compost granulated completely by this time.

Population build up in the form of number of worms, cocoons and hatchlings was measured to evaluate the acceptance of particular level of pesticides in the soil. These parameters were found to be significantly different at different concentration of pesticides. The number of worms at different time intervals in different concentrations of pesticides in soil substrate were found to be statistically different ( $p < 0.05$ ). The number of earthworms was found to decrease in ACE5 after 15 days only. After 30 days the number of earthworms in ACE5 was also found to statistically decrease from rest of the substrate mediums. In the present study, the clitellum appeared between 30<sup>th</sup> to 45<sup>th</sup> day in all the substrate mixtures except ACE4 and ACE5 of pesticide spiked substrate in which the clitellum appeared between 45<sup>th</sup>-60<sup>th</sup> and 60<sup>th</sup> -75<sup>th</sup> day respectively (**Fig 6.16**). The number of earthworms was found to increase on 60<sup>th</sup> day in C<sub>ACE</sub>, ACE1, and ACE2. The maximum number of earthworms was noticed in C<sub>ACE</sub> ( $30 \pm 1$ ) on 90<sup>th</sup> day of experiment followed by ACE1 ( $22.66 \pm 1.20$ ), ACE2 ( $22 \pm 1.15$ ) and ACE3 ( $19.33 \pm 0.33$ ). The minimum number of earthworms was found in ACE5 ( $13 \pm 0.57$ ) followed by ACE4 ( $16.33 \pm 0.33$ ) on 90<sup>th</sup> day of experiment. The reason of this decrease in number in higher concentrations could be attributed to the non-palatability and toxicity of pesticide. The highest mortality was witnessed on 90<sup>th</sup> day in ACE5 (35%). Changes in chemical composition of substrate, production of toxic or foul smell of gases (nitrogen oxide, ammonia, carbon dioxide, etc.), high C:N ratio of the initial substrate are some of the factors responsible for earthworm mortality (Flegel and Schreder 2000). The survival, biomass production, and reproduction of earthworms are the best indicators to evaluate the vermicomposting process (Bhat et al. 2014). According to Ndegwa and Thompson (2000b), Tripathi and Bhardwaj (2004); Gajalakshmi et al. (2005), survival, growth rate and potential of reproduction for earthworms is highly dependent upon kind, palatability and quality of food (in terms of chemistry).

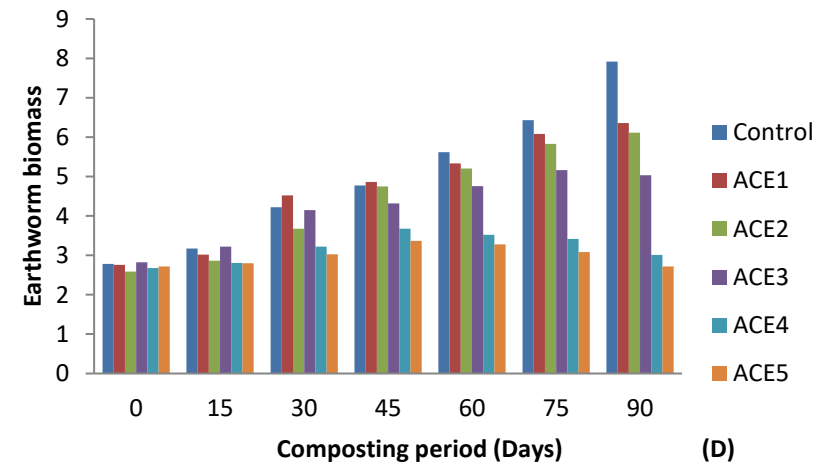
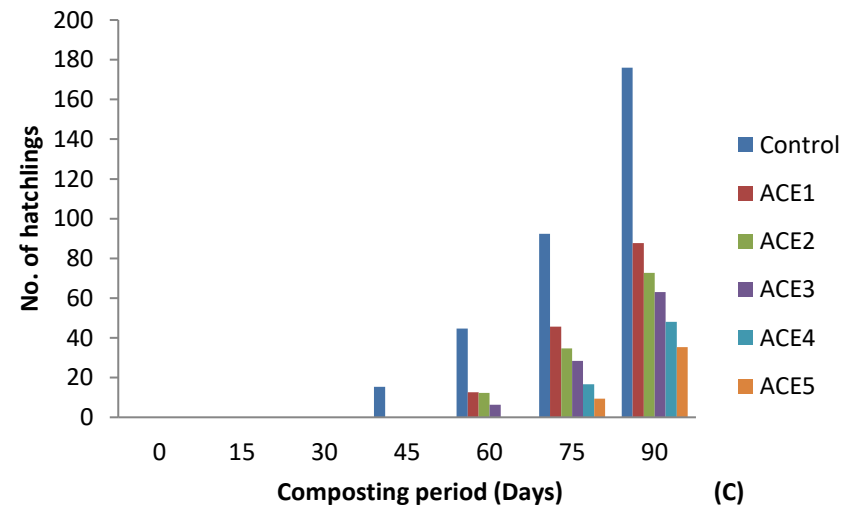
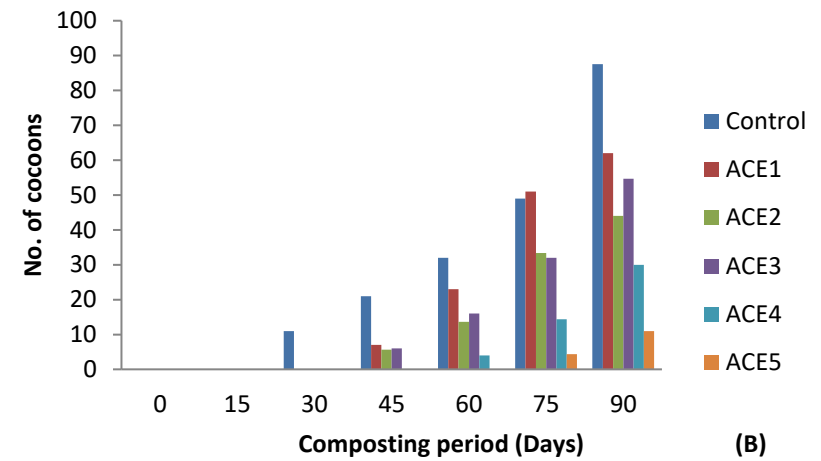
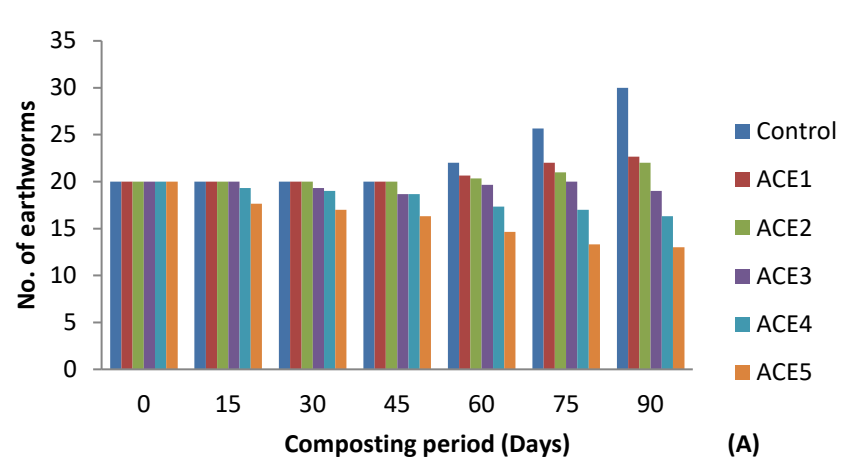


The number of cocoons with different pesticide concentrations was also found to be significantly different ( $p < 0.05$ ). Cocoon formation was first observed on the 30<sup>th</sup> day in C<sub>ACE</sub> only and thereafter on 45<sup>th</sup> day of experiment in ACE1, ACE2, and ACE3. However, in ACE4 and ACE5 the cocoons were observed on 60<sup>th</sup> and 75<sup>th</sup> day respectively. The maximum number of cocoons on 90<sup>th</sup> day was observed in C<sub>ACE</sub> ( $87.5 \pm 1.5$ ) and minimum in ACE5 ( $11 \pm 1.20$ ). Cocoon production was significantly less in substrate with higher concentration of pesticide suggesting that higher concentration prove to be toxic for development of earthworms. The biochemical quality of the feed and supporting medium forms to be an important factor that influences the production of cocoons (Flack and Hartenstein 1984; Edwards et al. 1998). Fayolle et al. (1997) have also pointed out that food source play an important role on cocoon production rate. Quality of food influencing the growth of worms; onset and rate of cocoon production is also depicted by Suthar (2008). The substrates having lower C:N ratio usually display higher rate of cocoon production (Kumar et al. 2010). The earthworms size was small to medium and hence it seemed that energy that otherwise is used to increase the biomass was utilized in reproduction (increasing number of cocoons). This was supported by a number of researchers who observed a higher rate of cocoon production by small to medium sized epigeic earthworms (Kale et al. 1982; Senapati and Sahu 1993; Chaudhuri and Bhattacharjee 2002; Barne and Striganova 2005; Suthar 2008). However, in contrast to this, Lavelle (1981) found a positive relationship between size of an adult worm and cocoon production. Higher C:N ratio in higher concentration of pesticides may be the reason of delayed clitellum development and thence cocoon production as low level of nitrogen in the substrate is considered to be a limiting factor for growth of worms (Butt 1993). So as to infer, we can say that higher concentration of pesticide in soil media can significantly affect the cocoon production, delays sexual maturity and reproduction of *E. fetida*.

Significant difference ( $p < 0.05$ ) in the number of hatchlings was also observed with different concentrations of pesticides in substrate medium. The number of hatchlings was also negatively correlated with the concentration of pesticides spiked substrate. Hatchlings were first observed for the first time on 45<sup>th</sup> day in C while on 60<sup>th</sup> day hatchlings appeared in ACE1, ACE2 and ACE3 while ACE4 and ACE5 showed hatchlings for the first time 75<sup>th</sup> day respectively. The maximum number of hatchlings ( $176 \pm 4.35$ ) observed on 90<sup>th</sup> day in C<sub>ACE</sub> and minimum in ACE5 ( $35.33 \pm 1.45$ ). Hatchlings number was significantly lower in substrates with higher concentrations of pesticides (ACE4 and ACE5) as compared to substrates with lower concentrations of pesticide (ACE1, ACE2, ACE3). This could be

corroborated to the higher production of cocoons in substrates with lower concentrations of pesticides and vice-versa. These results were supported by Kaur et al. (2010) and Chauhan and Singh (2013). Reinecke and Reinecke (1997) also reported ultra-structural damage in sperms of earthworms exposed to toxic elements and associate it with lower fertility and viability of cocoons.

The biomass of the earthworms in different concentrations of pesticides were found to vary significantly ( $p < 0.05$ ). The biomass was found to increase in all the concentrations. This was supported by Bhat et al. (2015) and Rathinamala et al. (2008). However, the biomass was found to be negatively correlated with the increasing concentration of pesticide. Maximum biomass was attained by control ( $7.92 \pm 0.31$ ) on 90<sup>th</sup> day whereas ACE5 showed minimum biomass on 90<sup>th</sup> day ( $2.62 \pm 0.31$ ). Presence of fungus during the process of vermicomposting becomes additional food to the worms which contributed to the higher weight of the worms (Pramanik and Chung 2011).



**Figure 6.16 Population build up of *E. fetida* in various concentrations of acephate (A) No.of earthworms (B) No.of cocoons (C) No.of hatchlings (D) Earthworms biomass at different time durations (days)**



## 7. SUMMARY AND CONCLUSION

Punjab has a very rich agricultural land and thus use of pesticides in this state is inevitable. The usage of pesticides no doubt improves the productivity of crop but it also affects the non target biodiversity. The excessive usage of pesticides is known to degrade the soil quality which also affects quality of crop production. Keeping these views in mind, we tried to establish the relationship between pesticides and soil eco-engineers i.e earthworms.

Two pesticides, namely atrazine, a herbicide and acephate, an insecticide was taken up and their toxicity was evaluated on an exotic species, *E. fetida* which is best known for vermicomposting and on *M. posthuma*, which is an indigenous species and found natively in Punjab. Four tests: Filter paper test, OECD artificial soil test, Modified soil test and Avoidance test were performed to evaluate the toxicity. Out of the two pesticides, Acephate was found to be more toxic than atrazine probably due to the reason that Asataf, the formulation for acephate is 75% SP and Atrataf, the formulation for atrazine is 50% WP. Filter paper test provides a first hand information as this test gives us insight to the reaction of earthworms when the pesticides is absorbed through the skin. However, the validity of test is still not taken at par with others and thus is not considered very reliable. This test is only considered as a preliminary one for toxicity determinations. The OECD artificial soil is considered to be a reliable test for toxicity evaluations. The OECD test provides an artificial soil medium free from any other contaminants and pollutants so that test species predict the actual toxic effects of the chemical/test compound. The modified soil toxicity includes a modification of the previous test with the replacement of artificial soil with natural medium. This was considered to further mimic the exact environmental conditions for test organisms to evaluate pesticide toxicity. Another test according to ISO standards, avoidance test was performed to check the relationship between toxicity and avoidance behaviour of earthworms. The basic idea of this test was to check the concentration/dose at which the earthworms start avoiding the pesticides. Out of the two species, *E. fetida* was more sensitive than local species *M. posthuma*. The reason may be ascertained to the fact that *M. posthuma* are already found in agricultural lands abundantly and as such they are use to the usage of pesticides and thus are more tolerant than *E. fetida*.

In vivo study, considering the effect of pesticides and vemicompost on the genotoxicity of *A.cepa* was also performed. The soil was collected from two different farmlands, one where pesticide was used and one where vermicompost was used. The soil was then subject to evaluate the genotoxicity of pesticide and vermicompost exerted on *A.cepa*. It was concluded that pesticides are cytotoxic and have the ability to damage DNA

and thus also show genotoxic effects. Vermicompost on the other hand is non-cytotoxic in nature and helps in efficient cell growth. Also, the physico-chemical analysis reveal lower TOC, EC and higher pH and more nutrient content in vermicompost treated soil in comparison to pesticide treated soil. By the use of vermicompost in lieu of pesticides, the biodiversity and useful organisms do not perish which further tends to elevate the physical and nutritional status of crops.

Two different earthworm species were evaluated for their efficiency to ameliorate the soil contaminated with the pesticides. The pre-vermicompost and post vermicompost mediums were analysed in terms of physico-chemical quality and genotoxicity. Both the earthworm species were compared for better efficiency of transformation and degradation of pesticide in the soil. This process of vermiremediation was also compared with the process of aerobic composting for remediation of pesticide contaminated agricultural soil. It was concluded that in comparison to aerobic composting (i.e soil + Pesticide: without earthworms), vermicomposting (i.e soil + pesticide + earthworm) was found to be better in terms of nutritional status, physico-chemical parameters and genotoxic aspects. Vermicomposting was very effective in reduction of chromosomal abnormalities and maintains mitotic index. Also after vermicomposting the physico-chemical characteristics were found to be better than pre vermicompost soil-pesticide samples.

Comparing the two species, it was found out that physico-chemical and genotoxic characteristics were better observed in post vermicompost samples by *E. fetida* in comparison to *M. posthuma*. Also, literature supports *E. fetida* as a very good species for vermicomposting; here in our study too we dignify the same over *M. posthuma* for vermiremediation of pesticide contaminated soil. But, *M. posthuma* are more tolerant species than their exotic counterpart *E. fetida* depicted from the toxicity tests performed. Thus in comparison to aerobic composting, vermicomposting give far better results and vermiremediation of pesticide contaminated soil is better performed by exotic species, *E. fetida* than *M. posthuma*.

## **8. BIBLIOGRAPHY**

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# **9. APPENDICES**

## ABBREVIATIONS

S. No.	Abbreviation	Expansion
1.	%	Percentage
2.	µg	Microgram
3.	a.i	Active ingredient
4.	AC	Pre-vermicompost acephate extract
5.	ACC	Acephate extract post aerobic composting
6.	ACE	Acephate extract post-vermicompost by <i>E. fetida</i>
7.	ACM	Acephate extract post-vermicompost by <i>M. posthuma</i>
8.	ANOVA	Analysis of variance
9.	AT	Pre-vermicompost atrazine extract
10.	ATC	Atrazine extract post aerobic composting
11.	ATE	Atrazine extract post-vermicompost by <i>E. fetida</i>
12.	ATM	Atrazine extract post-vermicompost by <i>M. posthuma</i>
13.	B	Boron
14.	BCF	Bio concentration factor
15.	C	Celsius
16.	CA	Chromosomal aberrations
17.	Ca	Calcium
18.	CAT	Catalase
19.	CD	Cow dung
20.	Cd	Cadmium
21.	CGA	Clastogenic aberrations
22.	ChE	Cholinesterase
23.	cm <sup>2</sup>	Square centimeter
24.	Co	Cobalt
25.	Cr	Chromium
26.	Cu	Copper
27.	CuSO <sub>4</sub>	Copper sulphate
28.	EC	Electrical conductivity
29.	Fe	Iron
30.	FYM	Farmyard manure
31.	GS	Garden soil
32.	H	Hours
33.	ISO	International Organization for Standardization
34.	K	Potassium
35.	K <sub>2</sub> SO <sub>4</sub>	Potassium sulphate
36.	kg	Kilogram
37.	Kg/ha	Kilogram per hectare
38.	M	Molarity
39.	mg	Miligram
40.	MI	Mitotic Index
41.	Min	Minute
42.	mm	Millimeter
43.	Mn	Manganese

44.	Mo	Molybdenum
45.	N	Nitrogen
46.	Ni	Nickel
47.	NRRT	Neutral red retention test
48.	OECD	Organization for Economic Co-operation and Development
49.	P	Phosphorus
50.	PA	Physiological aberrations
51.	Pb	Lead
52.	PGRs	Plant growth regulators
53.	Ppm	Parts per million
54.	PTS	Pesticide treated soil
55.	SCGE	Single cell gel electrophoresis
56.	SE	Standard error
57.	SeO <sub>2</sub>	Selenium dioxide
58.	SOD	Superoxide dismutase
59.	TAP	Total available phosphorus
60.	TCa	Total calcium
61.	TDS	Total dissolved solid
62.	TK	Total potassium
63.	TKN	Total kjeldahl nitrogen
64.	TLi	Total lithium
65.	TNa	Total sodium
66.	TOC	Total organic carbon
67.	TOM	Total organic matter
68.	Ton	Tonnes
69.	v/v	Volume by volume
70.	VTS	Vermicompost treated soil
71.	w/v	Weight by volume
72.	Zn	Zinc
73.	NaOH	Sodium hydroxide
74.	M	Molarity
75.	N	Normality
76.	H <sub>2</sub> SO <sub>4</sub>	Sulphuric acid
77.	HNO <sub>3</sub>	Nitric acid
78.	HClO <sub>4</sub>	Perchloric acid
79.	EC	Effective concentration
80.	LD	Lethal dose
81.	ESI-MS	Electrospray ionisation mass spectrometry
82.	GCMS	Gas chromatography-mass spectrometry
83.	nm	Nanometer
84.	rpm	Rotations per minute
85.	µg/ml	Microgram per milliliter

## APPENDIX- II

## LIST OF SCIENTIFIC NAMES AND THEIR ABBREVIATIONS MENTIONED IN THE THESIS

S.No.	Scientific Name	Abbreviated Name
1.	<i>Allolobophora icterica</i>	<i>A. icterica</i>
2.	<i>Aporrectodea longa</i>	<i>A. longa</i>
3.	<i>Aporrectodea nocturna</i>	<i>A. nocturna</i>
4.	<i>Aporrectodea rosea</i>	<i>A. rosea</i>
5.	<i>Aporrectodea caliginosa</i>	<i>A. caliginosa</i>
6.	<i>Dendrobaena rubida</i>	<i>D. rubida</i>
7.	<i>Dichogaster bolani</i>	<i>D. bolani</i>
8.	<i>Dichogaster modigliani</i>	<i>D. modigliani</i>
9.	<i>Drawida willsi</i>	<i>D. willsi</i>
10.	<i>Drawida nepalensis</i>	<i>D. nepalensis</i>
11.	<i>Eisenia andrei</i>	<i>E. andrei</i>
12.	<i>Eisenia fetida</i>	<i>E. fetida</i>
13.	<i>Eudichogaster kinneari</i>	<i>E. kinneari</i>
14.	<i>Eudrilus eugeniae</i>	<i>E. eugeniae</i>
15.	<i>Fusarium lycopersici</i>	<i>F. lycopersici</i>
16.	<i>Lampito mauritii</i>	<i>L. mauritii</i>
17.	<i>Lolium multiflorum</i>	<i>L. multiflorum</i>
18.	<i>Lumbricus terrestris</i>	<i>L. terrestris</i>
19.	<i>Lumbricus rubellus</i>	<i>L. rubellus</i>
20.	<i>Lycopersicum esculentum</i>	<i>L. esculentum</i>
21.	<i>Megascolex chinensis</i>	<i>M. chinensis</i>
22.	<i>Octochaetona phylloti</i>	<i>O. phylloti</i>
23.	<i>Octochaetona serrata</i>	<i>O. serrata</i>
24.	<i>Octochaetona thurstoni</i>	<i>O. thurstoni</i>
25.	<i>Octolasion cyaneum</i>	<i>O. cyaneum</i>
26.	<i>Perionyx excavatus</i>	<i>P. excavates</i>
27.	<i>Perionyx sansibaricus</i>	<i>P. sansibaricus</i>
28.	<i>Pheretima elongata</i>	<i>P. elongate</i>
29.	<i>Pheretima hupiensis</i>	<i>P. hupiensis</i>
30.	<i>Pheretima posthuma</i>	<i>P. posthuma</i>
31.	<i>Phytophthora cryptogea</i>	<i>P. cryptogea</i>
32.	<i>Phytophthora nicotianae</i>	<i>P. nicotianae</i>
33.	<i>Pontoscolex corethrurus</i>	<i>P. corethrurus</i>
34.	<i>Vicia faba</i>	<i>V. faba</i>



## LIST OF PUBLICATIONS

**Research papers from the present thesis**

1. **Datta,S.**, Singh, J., Singh, J., Singh, S., Singh, S. (2018). Assessment of genotoxic effects of pesticide and vermicompost treated soil with *Allium cepa* test. Sustainable Environment Research 28(4):171-184
2. **Datta,S.**, Singh, J., Singh, J., Singh, S. (2016). Earthworms, pesticides and sustainable agriculture: a review. Environmental Science and Pollution Research 23(9): 8227-8243

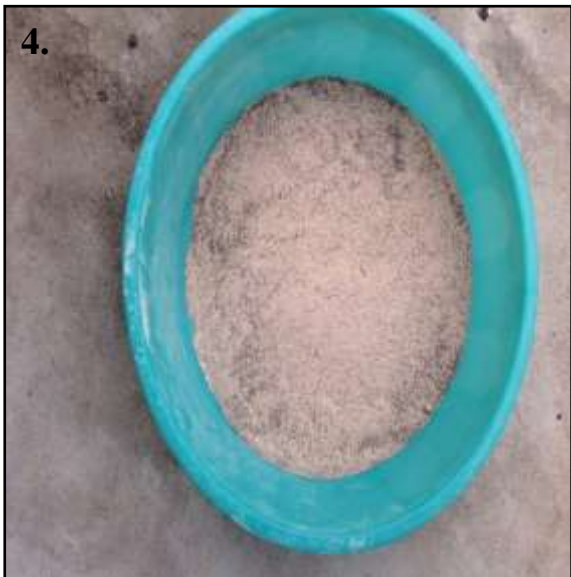
**Other Papers**

1. Gill, J. P. K., Sethi, N., **Datta, S.**, Girdhar, M., Mohan, A. (2017). Glyphosate toxicity for animals. Environmental Chemistry Letters 1-26.
2. Singh, S., Kumar, V., Chauhan, A., **Datta, S.**, Wani, A. B., Singh, N., Singh, J. (2017). Toxicity, degradation and analysis of the herbicide atrazine. Environmental Chemistry Letters 1-27.
3. Singh, S., Kumar, V., Upadhyay, N., Singh, J., Singla, S., **Datta, S.**(2017). Efficient biodegradation of acephate by *Pseudomonas pseudoalcaligenes* PS-5 in the presence and absence of heavy metal ions [Cu(II) and Fe(III)], and humic acid 3 Biotech 7(4): 262.
4. Singh, S., Singh, N., Kumar, V., **Datta, S.**, Wani, A. B., Singh, D., Singh, K., Singh, J. (2016). Toxicity, monitoring and biodegradation of the fungicide carbendazim. Environmental Chemistry Letters 14(3): 317-329
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6. Kumar, V., Singh, S., Bhadrecha, P., Kaur, P., Bhatia, D., Singla, S., **Datta, S.**, Chandel, V., Bhat, M. A., Kashyap, N., Kalia, A., Singh, J. (2015) Bioremediation of Heavy Metals by Employing Resistant Microbial isolates from Agricultural Soil Irrigated with Industrial Waste Water. Oriental Journal of Chemistry 31(1):357-361

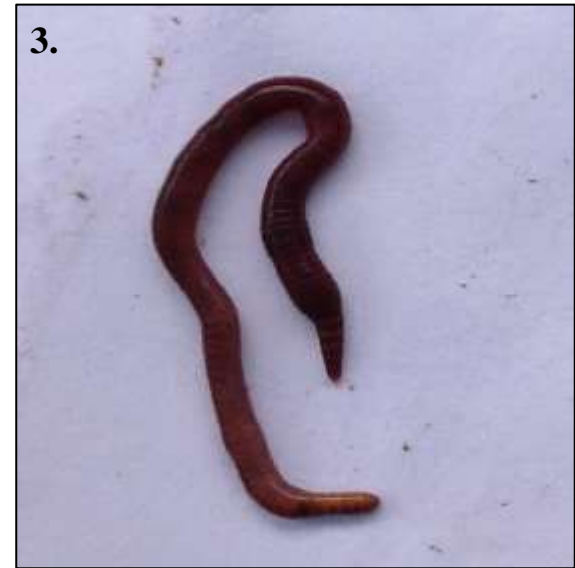
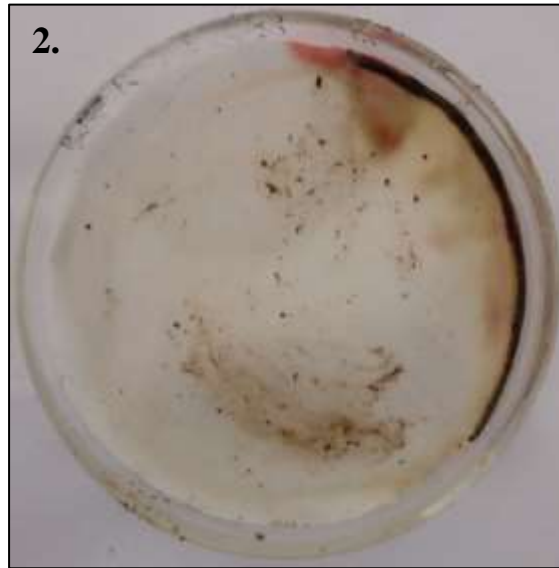
7. Kumar, V., Upadhyay, N., Kumar, N., Kaur, S., Singh, J., Singh, S., **Datta, S.** (2014) Environmental Exposure and Health Risks of the Insecticide Monocrotophos-A Review. *Journal of Biodiversity and Environmental Science* 5:111-120.
8. Kumar, V., Singh, S., Manhas, A., Singh, J., Singla, S., Kaur, P., Bhadrecha, P., **Datta, S.**, Negi, P., Kalia, A. (2014) Bioremediation of Petroleum hydrocarbon by using *Pseudomonas* species isolated from Petroleum contaminated soil. *Oriental Journal of Chemistry*. 30(4):1771-1776.
9. Kaur, A., Kumar, V., Singh, S., Singh, J., Upadhyay, N., **Datta, S.**, Singla, S. (2014) Toll like receptor associated keratitis and strategies for its management. *3 Biotech* 5(5):611-619.

#### **Poster/Abstract Presentation**

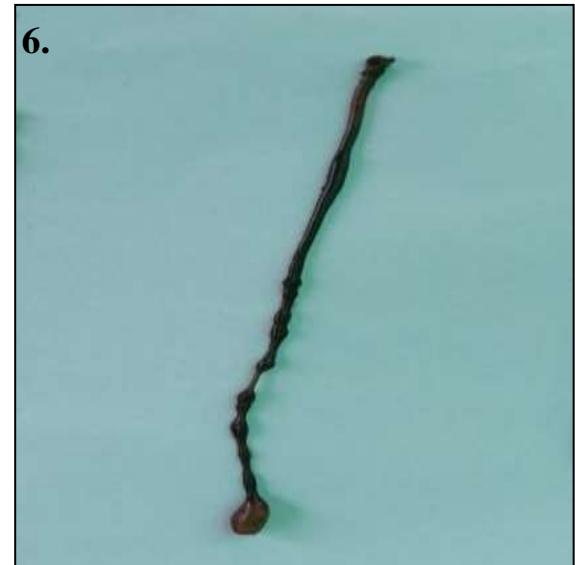
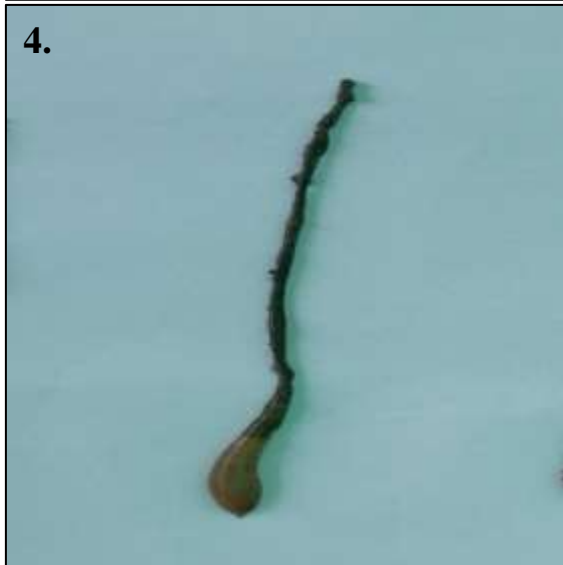
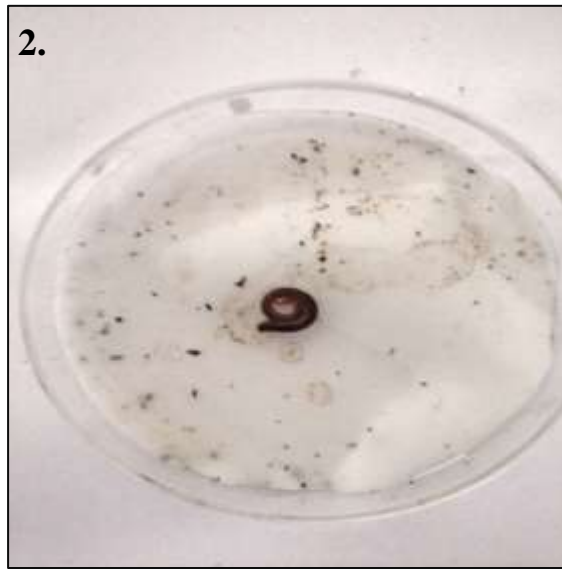
1. **Datta, S.**, Singh, J., Singh, J. (2016) Comparative sensitivity of earthworm avoidance tests and various acute toxicity tests for toxicity determination. National conference on emerging trends in biotechnology for agriculture, medicine and environment. 18-19, Mahila PG Mahavidyalaya, Jodhpur.
2. **Datta, S.**, Singh, J., Singh, J. (2017). Assessment of Acephate Toxicity on Exotic Earthworm *Eisenia fetida* by Earthworm Avoidance Test. Recent trends in Plant and Environmental Sciences. 9-10 Feb, Guru Nanak Dev University, Amritsar
3. **Datta, S.**, Singh, J., Singh, J., Singh, S. (2017) Effect of Sutlej river water on physiochemical parameters on two different varieties of *Tritium aestivum*. Innovative strategies for sustainable water management. 17-18<sup>th</sup> Nov, Lovely Professional University, Phagwara.
4. **Datta, S.**, Singh, J., Singh, J. (2018) Assessment of Atrazine and Acephate Toxicity on different Earthworm Species. 21<sup>st</sup> Punjab Science Congress. 7-9 Feb, Punjab Agricultural University, Ludhiana.
5. **Datta, S.**, Singh, J., Singh, J. (2018) Effect of herbicide atrazine on exotic species, *Eisenia fetida* and indigenous species, *Metaphire posthuma* by acute toxicity tests. Challenging and Emerging dimensions of medicinal plants and their products of Thar desert. 19-20 Feb, Mahila PG Mahavidyalaya, Jodhpur.
6. **Datta, S.**, Singh, J., Singh, J. (2018) Evaluation and comparison of genotoxic potential of pesticide and vermicompost treated soil with *Allium cepa* test. 3<sup>rd</sup> Green and Sustainable Chemistry. 13-16 May, Elsevier & Leuphana University, Berlin, Germany



**Plate (A)** 1. Industrial sand 2. Kaolin clay 3. Coco peat 4. OECD Artificial soil 5. Garden soil 6. Cow dung

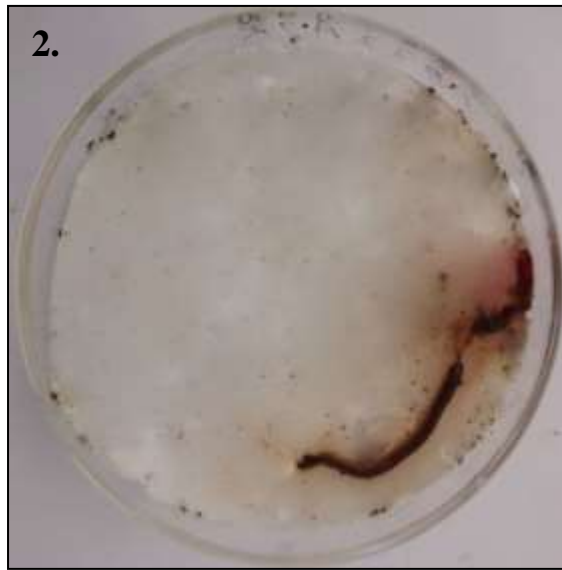


**Plate (B) Effect of atrazine on *E. fetida*:** 1. Filter paper (control) 2. Filter paper test (treated) 3. Control worm 4. Curling and coiling (treated) 5. Lesions (treated) 6. Segmental constrictions (treated)

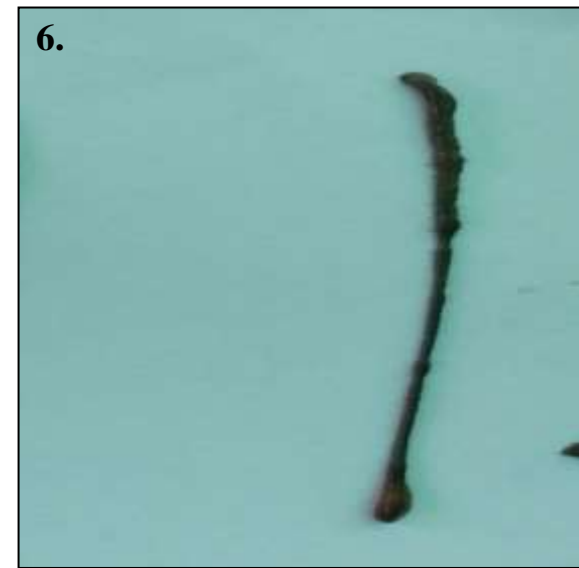
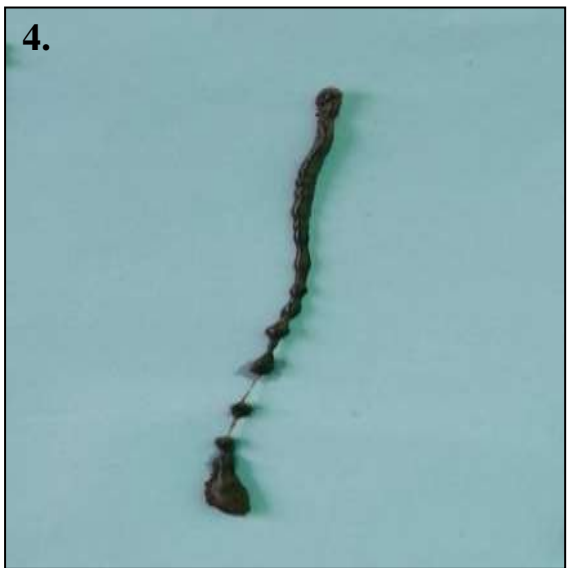


**Plate (C) Effect of atrazine on *M. posthuma*:** 1. Filter paper (control) 2. Filter paper test (treated) 3. Control worm 4. Bleeding sores at post-clitellar region (treated) 5. Segmental swelling (treated) 6. Constricted segments and melted body (treated)

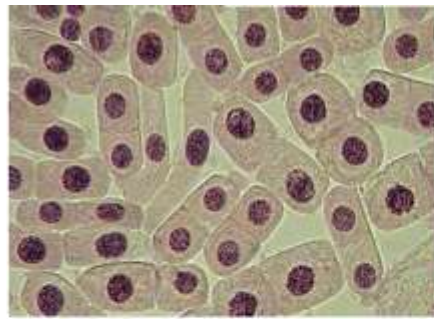




**Plate (D) Effect of acephate on *E. fetida*:** 1. Filter paper (control) 2. Filter paper test (treated) 3. Control worm 4. Severe constriction (treated) 5. Bloody Lesions (treated) 6. Body divided into two parts (treated)



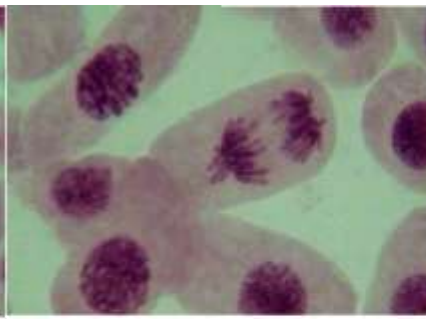
**Plate (E) Effect of acephate on *M. posthuma*:** 1. Filter paper (control) 2. Filter paper test (treated) 3. Control worm 4. Severe constriction with segmental breakdown (treated) 5. Segmental thinning (treated) 6. Melted post-clitellar region (treated)



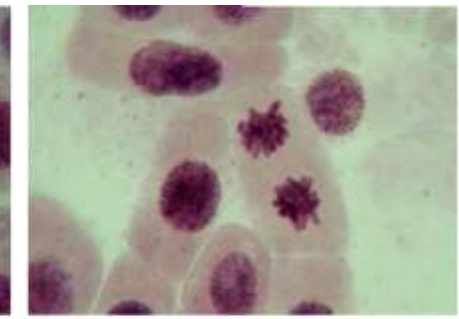
a) Prophase



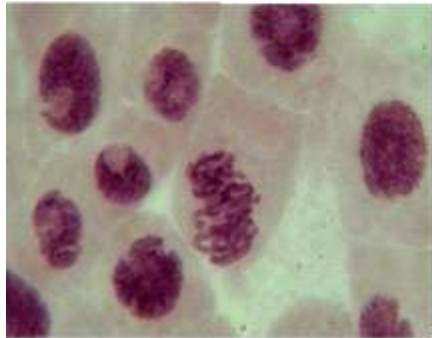
(b) Metaphase



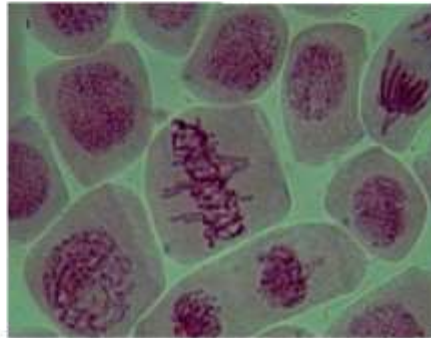
(c) Anaphase



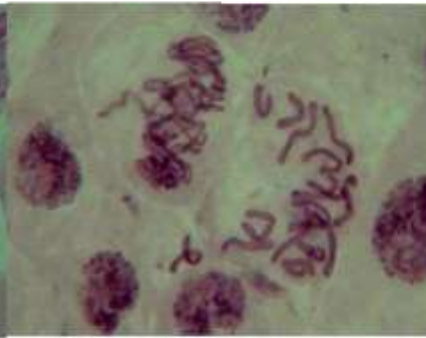
(d) Telophase



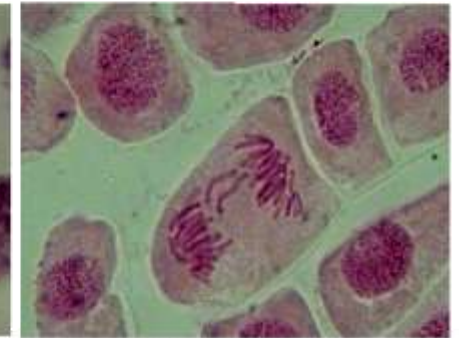
(e) Stickiness



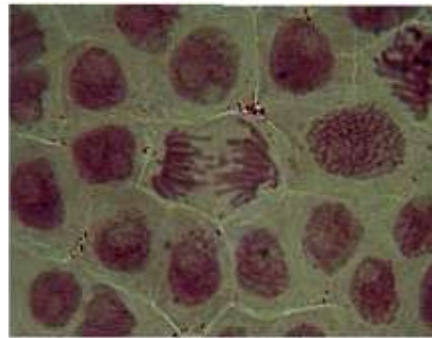
(f) Laggards



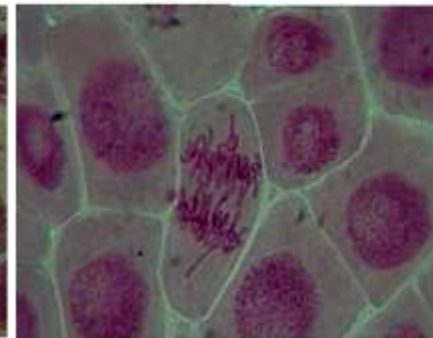
(g) C-mitosis



(h) Vagrants



(i) Fragments



(j) Chromosomal Bridges

**Plate (F)** Root tip cells of *Allium cepa* showing normal stages of mitosis (a-d) and chromosomal aberrations (e-j)