World of earthworms with pesticides and insecticides

Rashi Miglani and Satpal Singh Bisht
Department of Zoology, D.S.B Campus, Kumaon University, India

ABSTRACT
Earthworms are important organisms in soil communities and are known for sustaining the life of the soil. They are used as a model organism in environmental risk assessment of chemicals and soil toxicology. Soil provides physical and nutritive support to agricultural system by regulating biogeochemical cycles, nutrient cycle, waste degradation, organic matter degradation etc. The biggest threat to soil health are pesticides and synthetic chemicals including fertilizers. Earthworms are most severely hit by these xenobiotic compounds leading to a sizeable reduction of their population and adversely affecting soil fertility. Earthworms are incredible soil organisms playing a crucial role in maintaining soil health. Pesticides used in crop management are known to be most over-purchased and irrationally used soil toxicants, simultaneously, used insecticides contribute to a quantum of damage to earthworms and other non-target organisms. LC50 and LD50 studies revealed that earthworms are highly susceptible to insecticides causing immobility, rigidity and also show a significant effect on biomass reduction, growth and reproduction by disrupting various physiological activities leading to loss of earthworm population and soil biodiversity.

KEY WORDS: earthworms; insecticide; non-target organism; soil macrofauna; xenobiotics

Introduction
Agricultural expansion and indiscriminate use of pesticides often lead to affect soil ecosystem causing heavy population damage, toxicity and soil pollution (Hole et al., 2005; Mangala et al., 2009). An estimate has been made globally that $38 billion are spent on pesticides each year (Pan-Germany, 2012). The pesticides applied to the agricultural field should only be toxic to the target organisms, biodegradable and eco-friendly to some extent (Rosell et al., 2008). But unfortunately most of the pesticides are non-specific and kill organisms that are harmless and very useful to the various ecosystems. This concern got attention just after the publication of Silent Spring by Rachel Carson in 1962, which brought environmental issues to concern to the general public. Along the developmental scale, the advance farming practices caused bioaccumulation in humans as well as in many other animals.

The pesticides used in agriculture land cause morphological, behavioral and physiological changes in reproductive, nervous, respiratory and osmoregulatory organs of many soil organisms and contaminate the soil which exerts a harmful impact on various invertebrates (Fingerman, 1984; Mangala et al., 2009., de Silva P.M.C.S. 2009). Depending upon the chemical nature of pesticides and soil properties organs undergo a series of chemical pathways, transport, adsorption and desorption processes (Thapar et al., 2015, Baishya, 2015). Among
the different classes of pesticides, insecticides are found to be most lethal toxic class of pesticides and pose risk to non-target organisms (Aktar et al., 2009, Mahmood, 2016). The insecticide residues have been reported from agriculture systems along with many other ecotypes such as cropping fields, estuaries, oceans and even in the many urban settlements (Sánchez-Bayo, 2011; Guruge & Tanabe, 2001).

There are more than 8300 species in Oligochaetes, out of which more than half are terrestrial earthworms (Reynolds & Wetzel, 2004). The earthworm diversity of India represent 11.1% out of total earthworm diversity in the world. There are more than 505 species and sub-species of earthworms belonging to 67 genera and 10 families (Julka, 2001; Kathireswari, 2016). Earthworms are the supreme component of soil macrofauna and are the most important soil invertebrates responsible for developing and maintaining the nutritive value of soil by converting biodegradable material and organic waste into nutrient-rich vermicast (Kaushal et al., 1995).

Vermicast obtained by modulation of organic waste through earthworm gut is different from its parental waste material and popularly known as black gold (Lim et al., 2015b; Patangray, 2014). Earthworms are acknowledged as ‘ecosystem engineers’ as they extensively influence physical, chemical and biological properties of soil (Pelosi et al., 2014). Earthworms boost soil physical properties such as hydraulic conductivity, porosity, bulk density, infiltrability, aggregate stability etc. (Devkota et al., 2014). Earthworms improve nutrient availability by ingesting organic residues of different C:N ratios (Patnaik & Dash, 1990). Activities of earthworms also help in enhancing beneficial soil microbes. The gut mucus secretion and excretion from earthworms are known to enhance the activity of microorganisms (Bhaduria & Saxena 2010). The incredible services provided by the earthworms to the ecosystem are somehow at risk and recent research findings are now mainly focused on understanding earthworms and their responses to different pesticides.

### World of pesticides

Historians have traced the use of pesticides to the time of Homer around 1000 B.C. but the earliest records of insecticides are associated with the burning of brimstone (Sulfur) as a fumigant. The insecticide selection was limited during the onset of World War II and by the end of it, it got a new concept of insect control with the modern era of chemicals. The first synthetic organic insecticide introduced was DDT. Traditionally, insecticides are chemical or biological agents meant for the control the insects. The control may be by killing of the insects or by preventing them from engaging in their destructive activities. Insecticides may be natural or manmade and applied to target pests by applying with various delivery systems such as spray, baits, slow-release, diffusion etc. (Ware & Whitacre, 2004).

### Classification of pesticides

Pesticides are classified as insecticides, fungicides, herbicides, rodenticides, nematicides, molluscicides and plant growth regulators. Each group is specifically designed to target pests, but they put undesired toxic effects on non-target organisms. (Cortet et al., 2002; Jänsch et al., 2005; Lo, 2010; Zhang et al., 2010; Yasmin & D’Souza, 2010; Wang et al., 2012a; Milanovic et al., 2014).

The major classes of pesticides are summarized in Table 1. Among the different classes of pesticides, insecticides are known as one of the major class that contributes greatly to pest control and are further divided into different groups. The insecticide groups are classified on the basis of their chemical nature as per Insecticide Resistance Action Committee IRAC 2016 (Table 2).

### Toxicity and lethality of pesticides

The effect of toxic chemicals in a biological system is dose-related. The LC$_{50}$ (Fifty percent lethal concentration) is the amount of pesticide dispersed in the air and the value is measured in milligrams per liter. The lower the LC$_{50}$ value, the more lethal is the pesticide. Whereas LD$_{50}$

### Table 1. Major classes of pesticides*

| Types of Pesticides | Use and Action | Examples |
|---------------------|----------------|----------|
| Insecticides        | A substance used to control or eliminate or to prevent the attack of the insects that destroys/kill/mitigate plant/animal. | DDT, Methyl Parathion, Phorate, Chloropyrifos, Imidacloprid, Cypermethrin, Dimethoate |
| Herbicides          | Substances which are used to control the noxious weed and other vegetation that is growing with the desired species causing poor plant growth. | Acetochlor, Butachlor, Terbis, Glyphosate, 2,4-D, and 2,4,5-T. |
| Fungicides          | Substances used to destroy or inhibit the growth of fungi/diseases that infect plants/animal. | Carbendazim, Ampropylflos, Carboxin |
| Rodenticides        | Chemicals used to kill rodents i.e. mice, rat etc. | Warfarin, Arsenous oxide |
| Nematicides         | Substances used to repel or inhibit the nematodes damaging various crops. | Aldicarb, Carbofuran |
| Molluscicides       | Substances used to inhibit the growth and kills snails and slugs and small black sans-culottes. | Gardene, Fentin, Copper sulfate. |
| Plant growth regulators | A substance that causes the retardation or accelerates the rate of growth or rate of maturation. | Acibenzolar, Probenazole |

*As per Pesticide Action Network 2010 (PAN 2010)
(Fifty percent lethal dose) is calculated under controlled laboratory conditions by administration of the specific dose within a particular time to estimate the toxicity of the pesticides to an organisms (Table 3 and Table 4). The LD\textsubscript{50} values are expressed as milligram per kilogram of body weight (Canadian center for occupational health and safety 2018).

**Production and consumption of pesticides worldwide and Indian scenario**

The rise in population has increased the demand for agricultural products and to meet the demand the agriculture practices are commercialized into agribusiness. This practice facilitated the growth of crop protection by formulating agrochemicals on a large scale. The pesticide market scenario seems to be export-oriented rather than import-oriented (Table 5). The pesticide market of India is expected to grow by 12% to 13% per annum to reach $6.8 billion by 2017 and export demand by 15% to 16% (Surana et al., 2012). In the year 2018, as per India pesticide industry analysis, the CAGR (compound annual growth rate) observed 14.7% rendering the predicted size of the market at Rs. 2, 29, 800 million whereas on other side the global insecticide market is valued at USD 15.30 billion in 2016 and is likely to reach USD 20.82 Billion by 2022, at a CAGR of 5.27 from 2016 to 2022 respectively (Agro pages 2015).

The Indian pesticide industry is the biggest in Asia and the 12\textsuperscript{th} in the world and ranks fourth among global suppliers and it is expected to increase its growth till 2026.

---

**Table 2. Classification of insecticides based on their chemical nature (IRAC 2016)***

| Main Groups        | Action                                                                 | Basic Structure                                                                 | Examples                                  |
|--------------------|------------------------------------------------------------------------|---------------------------------------------------------------------------------|-------------------------------------------|
| Organophosphates   | Inhibit AChE in nervous system of target organisms                     | ![Organophosphates Structure](image)                                            | Chlorpyrifos, Dichlorovos, Triazophos, Profenofos, Parathion, Phorate, Diazinon |
| Organochlorines    | Binds at GABA site Inhibit chloride flow in the nervous system of target organisms | ![Organochlorines Structure](image)                                             | Chlordane, Endosulfan                     |
| Carbamates         | Inhibit AChE in nervous system of target organisms                     | ![Carbamates Structure](image)                                                 | Aldicarb, Carbaryl, Carbofuran, Isopropcar |
| Pyrethroids        | Acts on Nervous system which cause changes in nerve membrane permeability to sodium and potassium ions | ![Pyrethroids Structure](image)                                                 | Acrinathrin, Allethrin, Bioallethrin, Cycloprothrin, beta-Cyfluthrin, Cyhalothrin, lambda-Cyhalothrin, gamma-Cyhalothrin, Cypermethrin, alpha-Cypermethrin, beta-Cypermethrin, theta cypermethrin, zeta-Cypermethrin, Pyrethrins (pyrethrum) |
| Neonicotinoids     | Acts as an agonist of acetylcholine and is therefore effective on many insects | ![Neonicotinoids Structure](image)                                              | Acetamiprid, Clothianidin, Dinotefuran, Imidacloprid, Nitenpyram, Thiacloprid, Thiamethoxam |

* As per Insecticide Resistance Action Committee

---

**Table 3. Toxicity range of pesticides (CCOHS 2018).**

| S.No. | Category    | LD\textsubscript{50} oral mg/kg (ppm) | Example      |
|-------|-------------|--------------------------------------|--------------|
| 1     | Extremely toxic | 1 mg/kg(ppm) or less                  | Parathion, aldicarb |
| 2     | Highly toxic  | 1–50 mg/kg(ppm)                       | Endrin       |
| 3     | Moderately toxic | 50–500 mg/kg(ppm)                    | DDT, Carbofuran |
| 4     | Slightly toxic | 500–1000 mg/kg(ppm)                  | Malathion    |
| 5     | Non-toxic (practically) | 1–5 gm/kg                          | –            |

**Table 4. Acute toxicity range of pesticides according to the Environment Protection Agency (2009).**

| Class | Category    | Oral LD\textsubscript{50} (mg/kg) | Dermal LD\textsubscript{50} (mg/kg) | Inhalation LC\textsubscript{50} (mg/l) |
|-------|-------------|----------------------------------|----------------------------------|---------------------------------------|
| I     | Danger      | <50                              | <200                             | <0.2                                  |
| II    | Warning     | 50–500                           | 200–2,000                        | 0.2–2.0                               |
| III   | Caution     | 500–5,000                        | 2,000–20,000                    | 2.0–20                                |
| IV    | Caution (Optional) | >5,000                          | >20,000                         | >20                                   |

---
The pesticide market is likely to display a CAGR of 7.04% in value terms by the year 2026 (Agro pages 2015). Among different classes of pesticides (Figure 1 and Figure 2) the insecticides dominate the other classes of pesticides and accounts for 60% of total market value and are used in major crops like rice and cotton, whereas herbicides and fungicides account for 16% and 18% respectively (FICCI 2015). Globally the consumption of herbicide is found to be highest followed by insecticides, fungicides and other pesticides (Arnab et al., 2014). Consumption of agrochemicals in India is one of the lowest in the world with per hectare consumption of just 0.6 kg/ha compared to US (4.5 kg/ha) and Japan (11 kg/ha) (FICCI, 2014). This practice of pesticide usage in India needs to focus on high yield of bio-pesticides to promote eco-friendly and sustainable methods of agriculture.

Globally, the pesticides cover only 25% of the cultivated land area and consumption of pesticide worldwide is 2 million tons per year including India while comparing with Korea and Japan where it is 6.6 and 12.0 kg/ha respectively whereas Indian consumption is 0.5 kg/ha.

**Pesticides and soil environment**

Soil has the center position for the existence of organisms and ensures their survival, the term soil health and soil environment are used to describe the soil property which holds soil physical, chemical, biological characteristics, those maintain productivity and environment quality which promote the health of plants and animals (Doran., 1994). Soil is a mandatory component for terrestrial environment and is acknowledged as “Biological engine of the earth” (Ritz et al., 2004). Before the era of industrial revolution, i.e. early to mid-1900’s, farming practices were environment-friendly and the connection between agriculture and ecology was very strong. Immediately after this, the ecology and farming linkage was ignored resulting in high productivity at the cost of the environmental quality. Therefore the agro-ecosystem safety becomes a daunting challenge and is adversely affected the soil health.

Use of pesticides has become an integral part of our modern life in order to meet the demand of a growing population which is expected to be 10 billion by 2050 (Saravi & Shokrzadeh, 2011). As per an estimate of the last decade nearly $38 billion was spent on pesticides globally (Pan-Germany, 2012). The major fraction of pesticides accumulated in the soil and further repeated use of pesticides may cause lethal effects. The accumulation of pesticides in organo-mineral components of complex structures greatly influence the processes like mobilization, immobilization, bioavailability and transport (Gevao et al., 2003; Piccolo et al., 1998). The degraded pesticides alter microbial diversity, biochemical reactions and enzymatic activity (Hussain et al., 2009; Munoz-Leoz et al., 2011). The enzymatic pool of soil comprises of free enzymes, immobilized extracellular enzymes and the enzymes secreted by the microorganism well known...
as bioindicators of soil health (Mayanglambam et al., 2005; Hussain et al., 2009). The change in enzymatic activity demonstrates the effect of pesticides on soil biological functions (Garcia et al., 1997; Romero et al., 2010). Pesticides channel themselves through various biophysical pathways in soil ecosystems (Figure 3).

Animals thriving in soil are always under the threat of various chemicals used in agricultural practices, more specifically the pesticides. It is well established that these xenobiotic products are usually difficult to degrade by soil microbes therefore there is always a chance of their entry to various food chains and food webs resulting bioaccumulation and bio-concentration (Maurya & Malik 2016; Dureja & Tanwar, 2012; Edward & Bolen, 1992; Paololetti, 1999). Earthworms bio-accumulate organic pollutant (Jager et al., 2005), heavy metals (Nahmani et al., 2007) and nanoparticles (Canesi & Prochazkova, 2014) through skin and via soil ingestion. The effect of these pesticides applied to soil has effect on earthworm mortality (Roberts & Dorough, 1984; Panda S & Sahu, 2002), reproduction (Senapati et al., 1991; Schaefer 2004), metabolism (Brown et al., 2004) and also enhance the mechanism of bioamplification (Stephenson et al., 1997; Johnson et al., 1999). Earthworms experience inadvertent toxicity from terrestrially applied pesticides (Edward & Bolen, 1992) and this uptake of chemical increases bio-concentration of pesticides in earthworms.

Therefore the knowledge of the toxico-kinetics of terrestrially applied pesticides in earthworms is necessary to predict the risks of bio-concentration and bio-accumulation (Van Gestel & Weeks, 2004) on earthworm populations and ecological communities. The bio-accumulation of insecticides in earthworms may not lead to a significant effect on the animal to that extent but may produce serious damage to higher trophic level, but with long-term exposure to these pesticides, earthworms get acclimatized and accumulated (Huang & Iskandar, 1999). The increase in the concentration of pesticides and their non-biodegradable nature make them persist in the tissue of the organism at each successive level of food chain through the process of bio-amplification which cause greater harm to those of higher trophic level compared to those of lower levels. Several studies have been undertaken and demonstrated that at each trophic level the laethality of these pesticide increases (Gill & Garg, 2014).

**Pesticide toxicity and non-target organism**

The effect of pesticides on non-target organisms has been a matter of debate for researchers worldwide. There are many reports on the non-target killing of various species (Ware, 1980; Aktar et al., 2009; Datta et al., 2016; Dutta & Dutta, 2016; Stanley et al., 2016). Pesticides show the extreme effect on the aquatic ecosystem, animal and plant biodiversity and terrestrial food webs. It is estimated that less than 0.1% of pesticides applied to crop reach to the target pest (Pimental, 1995) and more than 99% of applied pesticide have the potential to impact non-target organisms and it percolates deep into the soil ecosystems including the water-table.

In India, 76% of the pesticides used are insecticides whereas globally the insecticide consumption is 44% (Mathur, 1999). The insecticidal effects on non-target species are categorized as per Nasreen et al. (2000) harmless (<50% mortality), slightly harmful (50–79% mortality), moderately harmful (80–89% mortality) and harmful (>90% mortality) when tested as per the field recommended dose. The categorization standards are used by the International Organization for Biological Control, West Palaeartic Regional Section (IOBC/WPRS) working group, to assess the insecticidal effects on non-target organisms (Hassan, 1989). The insecticides also act as a potential neurotoxicant on non-target species as it inhibits the essential enzyme, acetylcholinesterase (ACHE) in the nervous system of insects and other animal species (Gambi et al., 2007; Caselli et al., 2006). There are reports that toxicity of chemical pesticides used not only affects the target pests but also other species in different degrees (Sanchez-Bayo, 2012). Such as, natural insect enemies e.g., parasitoids and predators are most susceptible to insecticides and are severely affected (Aveling, 1981; Vickerman, 1988). Along with natural enemies, the population of soil arthropods is also drastically disturbed because of indiscriminate pesticide application in agricultural systems. Soil invertebrates are essential for the maintenance of soil structure, transformation, nutrient dynamics and mineralization of organic matter severely affecting the food chain and food webs.

In some cases, the concentrations of pesticides residue have been shown to be sufficiently high to affect many non-target species, including very important soil macrofauna, such as earthworms which are known to deliver ecosystem good and services (Frampton et al., 2006; Daam et al., 2011; Bertrand et al., 2015). Insecticides alter the eco-physiology of the earthworms (Liang et al., 2007) and there are studies on toxicological effect of carbaryl in different earthworm species such as Eisenia Andrei, P. Excavatus, Ph erotima Posthuma and Metaphire Posthuma (Lima et al., 2015; Saxena et al., 2014). Few studies have also shown the toxic effect of Imidacloprid, a common neonicotinoid insecticide, on earthworms

| Import/Export  | Category | Country | 2010–11 | 2011–12 | 2012–13 | 2013–14 | 2014–15 | 2015–16 | 2016–17 |
|---------------|----------|---------|---------|---------|---------|---------|---------|---------|---------|
| Import        | Pesticides | India   | 53996   | 58647   | 65018   | 77375   | 95361   | 71029   | 100238  |
| Export        | Pesticides | India   | 173171  | 207948  | 228790  | 252747  | 285209  | 307368  | 379852  |

*Q.T in Metric Tonnes (Technical Grade)*
(Capowiez et al., 2005, 2006). Majority of work has been carried out on the potential risk of organophosphorus pesticides like fenitrothion, malathion, monocrotophos, phorate in tropical agro-ecosystem using earthworm as test organism (Panda & Sahu, 1999, 2004; Patnaik & Dash, 1990). Much more investigations are needed to study various insecticides and their level of toxicity to non-target soil macro-fauna including various earthworm species.

**World of Earthworms**

The earthworms thrive almost all soil types and are known as the indicator of soil health and toxicity including various soil pollutants and pesticides. Lee (1985) categorized earthworms based on their feeding habit as detritivores (feed near the surface on decomposing litter and on dead roots) and geophagous (remain on the subsurface which consumes large quantities of soil). According to Lavelle (1983), geophagous earthworms are further categorized into polyhumic (feed on topsoil and occupy different soil strata), oligohumic (feed on the soil of low organic matter) and mesohumic (feed on humus and soil) and are abundantly found in the tropical regions.

Edward & Bohlen (1992) reported that earthworms are highly susceptible to pesticides such as insecticides, therefore they are considered as a model organism to evaluate the effects of insecticides. There are certain pesticide families that are considered as harmful to earthworms i.e. neonicotinoids, strobilurins, sulfonylureas, triazoles, carbamates and organophosphates (Pelosi et al., 2014). The pesticides affect mortality of earthworms by directly distressing them or by altering their physiology (Sabra & Mehana, 2015). Pesticides have a negative effect on the survival and reproduction of earthworms especially at higher concentration (>25mg/kg). Possible effects of pesticides and insecticides on earthworms in the soil are also dependent on earthworm species, type of contaminant and its concentration, soil characteristics etc. (Roriguez-Campos et al., 2014).

The organization for economic cooperation and development (OECD) proposed *Eisenia Fetida* (*Oligochaeta*) as a reference earthworm species for toxicity testing because it can easily be cultivated in the laboratory, mature in few weeks and has a high reproductive rate (OECD, 1984, 2004, 2015; ISO, 1993). The different insecticides classes had different toxic effects on *Eisenia Fetida*. Earthworm growth, reproduction (cocoon production, number of hatchlings per cocoon and incubation period) is also influenced by use of pesticide in a dose-dependent manner (Yasmin & D’Souza, 2010).

**Earthworms Morpho groups and their exposure to pesticides**

Earthworms are classified into four ecological groups, each group is described by different traits in the soil system (Bouché, 1977; Edwards & Bohlen, 1996) including their exposure to various types of pesticides. **Epigeic** worms are represented by *Lumbricus rubellus*, *Dendrobaena octedra*, *Lumbricus castaneus* and usually found in upper 10–15 cm soil layer and feed on decaying organic matter present in the litter. The species that belong to this group are highly exposed to pesticides while ingesting litter. **Endogeic** worms are represented by *Aporrectodea caliginosa*, *Allolobophora chlorotica* or *Allolobophora icterica* and are of a bigger size ranging from 1 to 20 cm. They feed upon the organic matter which is incorporated and mixed with minerals in the soil where the pesticides have already reached and mixed with soil. **Anecic** worms include *Lumbricus terestis*, *Aporrectodea longa* and are usually bigger and pigmented. They reflect strong muscles with great burrowing activity and some species reaches to the giant size such as 10 to 110 cm. They feed upon the surface litter mainly during the night and create long sub-vertical burrows (1 to 6 m) and thus ingest more amount of soil and get exposed to pesticides by ingesting contaminated soil. **Compost** worm is represented by *Eisenia fetida* and *Dendrobaena veneta* commonly used in vermicomposting practices. Compost worms are bright red in color and stripy and are commonly called ‘tiger worms’. These worms are usually kept in controlled soil pits therefore are less exposed to soil toxicants.

**Effect of insecticides on earthworms**

Solaimalai et al. (2004) investigated effect of various pesticides and their sub-lethal effect on earthworms and demonstrated that the sub-lethal effects cause rupturing of cuticle, oozing out of coelomic fluid, swelling, paling of body and softening of body tissues. Other studies include the cellular autolysis (Luo et al., 1999), damage to male reproductive system (Sorour & Larnik, 2001), swelling (Bharath & Subbarao, 1984) and coiling of tail (Espinoza-Navarro & Bustos-Obregon, 2004). The higher and the lower dose of insecticides cause physiological damage (cellular dysfunction and protein catabolism) to earthworms (Schreck et al., 2008).

Temperature also plays an important role in degree of pesticide toxicity. Bindesbol et al. (2009) investigated effects of freezing temperatures on toxicities of abamectin and carbendazim. De Silva et al. (2009) investigated influence of temperature and soil type on the toxicities of chlorpyrifos and carbofuran. Lima et al. (2015) investigated effects of carbaryl under low and high temperatures and Garcia et al. (2008) assessed effects of three pesticides on the avoidance behavior under temperate and tropical conditions. These investigations showed that change in temperature may influence the pesticide toxicity, but the results obtained from these studies were not definite and substantiated by any other studies.

There are many studies on neurotoxicity caused by various insecticides namely neonicotinoid imidacloprid, oxadiazine indoxacarb, pyrethroids alpha-cypermethrin and lambda-cyhalothrin and the combination of organophosphate chlorpyrifos and pyrethroid cypermethrin. All these insecticides primarily affect nervous system – neonicotinoids interfere with the transmission of stimuli in the nervous system causing irreversible blockage of acetylcholine receptors, oxadizines act as voltage-gated
sodium channel blockers, pyrethroids cause excitation of the sodium and potassium channels of neurons and the delay of closing of the channels during the phase of depolarization and organophosphates inhibit the action of enzyme acetylcholinesterase (AChE) leading to accumulation of acetylcholine, excessive stimulation of the cholinergic receptors and disruption of neural activity (Stenersen 2004; Casida, 2009; Ribera et al., 2001; Gracia et al., 2011; Nasr & Badawy, 2015). Jeyanthi et al. (2016) reported that Carbaryl at higher concentration (50 kg/ha) decreases protein content and antioxidant enzymes glutathione-S-transferase (GST). The antibiotics, carbamates and organophosphates induced intermediate toxicity response to earthworms. Wang et al., (2012) reported that the neonicotinoids are the most toxic to *Eisenia Foetida* among the six chemical classes followed by pyrethroids, while IGRs exhibited the lowest toxicity. Organophosphates are not very toxic to earthworms. Considering the high efficacy of neonicotinoids against target organisms, environmental managers should carefully evaluate the use of them in integrated pest management (IPM) programs to avoid serious damage to earthworms.

**Impact of insecticides on earthworm growth and reproduction**

Various reproductive parameters such as maturation, cocoon production, viability, hatching and spersms production were studied with reference to the genotoxicity when exposed to different types of insecticides and other chemical classes (Espinoza-Navarro & Bustos, 2004; Govindarajan & Prabaharan, 2014). Pawar & Ahmad (2013) reported that the effect of Chlorpyriphos which is an organophosphate insecticide with the exposure period of 7, 14, 21, 28, and 35 days, the dose concentration of 0.1 and 0.2 showed less effect on growth with the exposure period of 7 and 14 days, but effected earthworms growth when exposed more than 14 days.

Booth & O’Halloran (2001) found significant reduction in growth of *A. Caliginosa* by exposure to two organophosphates, diazinon and chlorpyrifos, at 60 and 28 kg/ha dose. Rajshree et al. (2014) also found that Methyl parathion and phorate are very toxic to earthworms and showed progressive symptoms of toxicity such as coiling, curling and excessive mucous secretion with sluggish movements, swelling of the clitellum, degenerative changes in nervous system and loss of pigmentation which is elicited by organophosphorous insecticide. Malathion, the organophosphate, showed a significant reduction in body weight and negative impact on the male reproductive organs that alter the cell proliferation and affect the DNA structure of spermatogonia of earthworms (Espinoza-Navarro, 2004). Sperm count is also a sensitive marker (Mosleh et al., 2003; Venter & Reinecke 1985). Malathion could affect the sperm count, but in addition, its metabolites could affect the sperm quality (Espinoza-Navarro, 2004). Mosleh et al. (2003) assumed that the weight loss may indicate a reduced food intake, by which earthworms regulate intake of pesticides and leads to growth inhibition.

Mosleh et al. (2003) investigated that the toxicity of aldicarb, cypermethrin, profenofos, chlorfluazuron, atrazine, endosulfan and metalaxyl in the earthworms *Aporrectodea caliginosa* and *Lumbricus terrestris* causes a reduction in growth rate. Zhou et al. (2007) assessed and found that chlorpyrifos had an adverse effect on growth in earthworm exposed to 5 kg/ha chlorpyrifos after eight weeks. Some studies have shown that the growth of earthworms appeared to be more severely affected at juvenile stage than the adult stage. Chlorpyrifos exposure had a significant effect on reproduction in earthworm as it shows the effect on fecundity when exposed to 5 kg/ha after eight weeks (Zohu et al., 2006). According to Zohu et al., 2008 reproduction of earthworm appeared to be more severely affected by cypermethrin at juvenile stage than at adult stage. Application of 20 kg/ha cypermethrin caused significant toxic effects in the reproduction of worms. Apart from the above mentioned facts there are many more effects and responses that have been studied by various researchers (Table 6).

**Effect of insecticides on earthworm gut bacteria and cast production**

In soil, earthworms explicate soil property and regulate biochemistry of terrestrial soil. The cast of earthworms contribute significantly to cyclic processes carried out in soil ecosystem by supplying nutrient to the plant roots and maintain pedological characteristics of the soil. The earthworms are voracious feeders and the nutrient-rich organic matter along with the soil flows through earthworms gut. The gut of earthworms is a straight tube bioreactor and maintains stable temperature by the regulatory mechanism (Karthekeyan et al., 2004). The gut of earthworm is known as ideal habitat for many agriculturally important microbes (Wolter & Scheu, 1999) and mostly derives its energy and nutrient from gut-specific microbiota rather than from microbiota present in ingested soil (Sampredo et al., 2006). Shi et al. (2007) examined that earthworm exposed to deltamethrin for 14 days exposure showed dose-dependent toxic effect on growth and cellulose activity. A decrease in cast production was found in *L. Terrestris* when exposed to methomyl, carbaryl, and imidacloprid respectively for 7 days (Capoweiz et al., 2010).

**Conclusions**

The study highlights the use of pesticides in agriculture system results in many ecological problems. There is clear evidence that the population of earthworm and other non-target soil biota are influenced by pesticides and fertilizers use and the impact is wide-ranging and causing the unwanted shift in the community. Initially, pesticides were used for the benefit to human life by an increase in agricultural productivity and by controlling infectious diseases but their adverse effect on human health and environment were ignored. Multifarious and tremendous uses of pesticides are causing harm to the environment and its components. Some of the adverse effects emerged...
Table 6. Response of various insecticides on earthworm species at different concentrations

| Insecticide                  | Concentration of Insecticide/exposure | Species                  | Responses                                                                 | References                                      |
|------------------------------|----------------------------------------|---------------------------|---------------------------------------------------------------------------|------------------------------------------------|
| Aldrin, Endrin, DDE, parathion, and carbaryl | LD$_{50}$ value 45 µg/g      | Lumbricus terrestris     | With drawl responses and discoloration of the skin                        | Cathey, 1982                                    |
| Endrin                       | LD$_{50}$ value 45 µg/g              | Lumbricus terrestris     | With drawl responses and discoloration of the skin                        | Cathey, 1982                                    |
| DDE                          | LD$_{50}$ value 46 µg/g              | Lumbricus terrestris     | With drawl responses and discoloration of the skin                        | Cathey, 1982                                    |
| Parathion                    | LD$_{50}$ value 34 µg/g              | Lumbricus terrestris     | With drawl responses and discoloration of the skin                        | Cathey, 1982                                    |
| Carbaryl                     | LD$_{50}$ value 28 µg/g              | Lumbricus terrestris     | With drawl responses and discoloration of the skin                        | Cathey, 1982                                    |
| Chlorpyrifos                 | LC$_{50}$ value 0.063 mg/cm$^2$      | Eisenia fetida           | Inhibition of acetylcholinesterase activity, Behavioral and morphological abnormalities | Espinoza-Navarro & Bustos-Obregón, 2004         |
| Malathion                    | LD$_{50}$ value 880 mg/kg soil       | Eisenia fetida           | Decreased the spermatic viability in spermatheca, altering the cell proliferation and modifying the DNA Structure of spermatogonia. | Espinoza-Navarro & Bustos-Obregón, 2004         |
| Carbaryl                     |                                        | Metaphire posthuma       | Sperm head abnormalities                                                  | Gupta & Saxena, 2003                            |
| Dieldrin                     | LC$_{50}$ value 100 mg/kg            | Eisenia fetida (Juveniles) | Cilietum development retarded, Influencing reproduction. Growth was retarded even at the agricultural dose of 5kg/ha | Venter & Reinecke, 1985                         |
| Imidacloprid                 | LC$_{50}$ value 25.53 mg/kg          | Eisenia andrei           | Retarded development, reduced fertility, and teratogenic effects reveal qualitative and quantitative changes in earthworm population, mortality does not occur | Alves et al., 2013                              |
| Dimethoate                   | LC$_{50}$ Value 28 mg/kg d.w.        | Eisenia fetida           | Significantly reducing earthworm weight and showing an avoidance response at soil concentrations | Rico et al., 2016                               |
| Profenofos                   | LC$_{50}$ Value 4.56 and 3.55 µg/cm$^2$ | Eisenia fetida | Body ruptures, bloody lesions, and internal excessive formation of glandular cell mass and disintegration of circular and longitudinal muscles, which failed to regulate the internal coelomic pressure, leading to fragmentation in earthworms | Reddy & Rao, 2008                              |
| Dichlorvos                   | LC$_{50}$ Value 76 mg/kg d.w.        | Eisenia fetida           | The weight of earthworm decreases. Reproduction and avoidance behavior significantly affected. | Farrukh & Ali, 2011                            |
| Cypermethrin                 | LC$_{50}$ Value 0.008 mg/kg          | Perionyx excavatus       | Order of toxicity – cypermethrin> endosulfan> carbaryl> chlorpyrifos> aldicarb> monocrotophos | Gupta et al., 2010                              |
| Endosulfan                   | LC$_{50}$ Value 0.03 mg/kg           | Perionyx excavatus       | Order of toxicity – cypermethrin> endosulfan> carbaryl> chlorpyrifos> aldicarb> monocrotophos | Gupta et al., 2010                              |
| Carbaryl                     | LC$_{50}$ Value 6.07 mg/kg           | Perionyx excavatus       | Order of toxicity – cypermethrin> endosulfan> carbaryl> chlorpyrifos> aldicarb> monocrotophos | Gupta et al., 2010                              |
| Chlorpyrifos                 | LC$_{50}$ Value 7.3 mg/kg            | Perionyx excavatus       | Order of toxicity – cypermethrin> endosulfan> carbaryl> chlorpyrifos> aldicarb> monocrotophos | Gupta et al., 2010                              |
| Aldicarb                     | LC$_{50}$ Value 10.63 mg/kg          | Perionyx excavatus       | Order of toxicity – cypermethrin> endosulfan> carbaryl> chlorpyrifos> aldicarb> monocrotophos | Gupta et al., 2010                              |
| Monocrotophos                | LC$_{50}$ Value 13.04 mg/kg          | Perionyx excavatus       | Order of toxicity – cypermethrin> endosulfan> carbaryl> chlorpyrifos> aldicarb> monocrotophos | Gupta et al., 2010                              |
| Chlorpyrifos                 | LC$_{50}$ Value 0.5 mg/kg            | Eisenia foetida          | Effects on growth and weight of earthworms                                 | Pawar & Shahzad, 2013                           |
| Lambda-cyhalothrin, Cypermethrin, Didcot, Termitoc | LC$_{50}$ ranging from 0.000ml–0.002 ml | Lumbricus terrestris | Presents the highest number of mortality in all concentration            | Yuguda et al., 2015                             |
| Methyl Parathion and Phorate | Conc 0.05g/500 g of soil and Methyl parathion 0.12g/500 g | Edurilus eugeniae | Coiling, curling and excessive mucous secretion with sluggish movements, Swelling of the cilietum, Extrusion of coelomic fluids resulting in bloody lesions. Earthworms also showed degenerative changes in the anterior part of the nervous system. The disappearance of metameric segmentations and loss of pigmnetations. | Rajashree et al., 2014                           |
| Dimethoate                   | LC$_{50}$ Value 300 mg/kg            | Eisenia foetida          | The decrease in cocoon production and cocoon viability                     | Pal & Patidar, 2013                             |
| Carbofuran                   | LC$_{50}$ Value 23.5 and 9.3 mg/kg   | Eisenia Andrei and Pontoscolex corethrinus | After 7 days biomass reduction was observed only with E.andrei and after 14 days a biomass of both the species reduced significantly | Buch et al., 2013                               |
| Chlorpyrifos (pure)          | LC$_{50}$ Value 80 mg/kg soil       | Eisenia Fetida           | Adverse impact on growth and reproduction                                  | Zhou et al., 2007                               |
| Parathion                    | LC$_{50}$ Value 1478 mg/kg soil     | Eisenia Fetida           | Adverse effect on cocoon production, cocoon viability and hatching success rate. | Bustos-Obreg & Goicochea, 2002                  |

World of earthworms with pesticides and insecticides
Rashi Miglani and Satpal Singh Bisth

ISSN: 1337-6853 (print version) | 1337-9569 (electronic version)
in the form of an increase in resistant pest population, decline in beneficial soil microorganisms, predators, pollinators and earthworms. Earthworm which is one of the important soil fauna is extremely at the edge of the exposure to pesticides. Such sensitivity of earthworms to pesticides, especially to the major class of pesticides i.e. the insecticides, is well documented in the present review. The toxicity of the insecticides to earthworms varies with the category of chemicals affecting the earthworm life cycle parameters. The persistent nature of pesticides has impacted our ecosystem too that have entered into various food chains and into the higher trophic levels such as that of humans and other large mammals. In order to reduce the effect of pesticides there should be input of sufficient organic manures instead of chemical fertilizers with minimal disturbances in soil and can be adapted for optimum activity of earthworms in the soil for healthy and fertile soil. A little effort has been made to provide a comprehensive review of the toxicity level of insecticide to one of the non-target taxa i.e. earthworm. Therefore farmers must be educated regarding the beneficial role of earthworms because of its importance and to reduce or minimize the use of pesticide to provide the threshold to the environment and biodiversity.

Acknowledgements

The authors are grateful to the Department of Biotechnology (DBT), Ministry of Science and Technology New Delhi i.e. Vide Order No-BT/PR24972/NER/95/932/2017 for financial assistance.

REFERENCES

Akter W, Sengupta D, Chowdhury A. (2009). Impact of pesticides uses in agriculture: their benefits and hazards. Interdisc Toxicol 2(1): 1–12.
Alves PRL, Cardoso EJB, Martins AM, Sousa JP, Pasini A. (2013). Earthworm ecotoxicological assessments of pesticides used to treat seeds under tropical conditions. Chemosphere 90: 2674–2682.
Agropages. (2015). Markets and Markets Research Private Limited. http://report.agropages.com/Report-Detail-1875.htm.
Aveling C. (1981). The role of Anthocoris species (Hemiptera: Anthocoridae) in the integrated control of the damson-hop aphid (Phorodon humuli). Annals of Appl Biol 97(2): 143–153.
Baishya K. (2015). Impact of agrochemicals application on soil quality degradation—a review. ICSTM, pp. 776–786.

Table 6. Continued...

| Insecticide     | Concentration of Insecticide/exposure | Species             | Responses                                                      | References               |
|-----------------|--------------------------------------|---------------------|----------------------------------------------------------------|--------------------------|
| Imidacloprid    | LC50 Value 0.77 mg/kg dry soil        | Eisenia fetida      | Adult survival decreased significantly                         | Silva et al., 2017       |
| Thiacloprid     | LC50 Value 7.1 mg/kg dry soil         | Eisenia foetida     | Acting on sub-lethal endpoints leading to a reduction in the number of offspring. | Silva et al., 2017       |
| Cycloxaprid     | LC50 of 10.21 mg/kg dry soil          | Eisenia fettida     | It induced tissue damage to the epidermis, gut, and neurochord at sublethal doses and also induce oxidative stress | Suzhen et al., 2018      |

Bertrand M, Barot S, Boulin, Whalen J, de Oliveira T, Roger-Estrade J. (2015). Earthworm services for cropping systems. A Review. Agron Sustain Dev 35: 553–567.
Bhaduria T, Saxena KG. (2010). Role of Earthworms in Soil Fertility Maintenance through the Production of Biogenic Structures. Appl and Environ Soil Sci. pp.7.
Bhattacharya A, Sahu SK. (2013). Acute Toxicity of Dimethoate on soil health: A Study of its impact on Earthworm. In. J Bioci pp. 98–106.
Bindesbol AM, Bayley M, Damgaard C and Holmstrup M. (2009). Impacts of heavy metals Pahs and pesticides on freeze tolerance of the earthworm Dendrobaena octaedra. Environ. Toxicol. Chem 28: 2341–2347.
Booth LH, O’Halloran K. (2001). A comparison of biomarker responses in the earthworm Aporectodea caliginosa to the organophosphorous insecticides diazinon and chlorpyrifos. Environ Toxicol Chem 20: 2494–2502.
Bouché MB. (1977). Stratégies lombriciennes. In: Lohm, U., Persson, T.G. (Eds.), SoilOrganisms as Components of Ecosystems, Ecol Bull, Stockholm, pp. 122-132.
Bustos-Obregón E, Goicochea RI. (2002). Pesticide soil contamination mainly affects earthworm male reproductive parameters. Asian J of Andro 4(3): 195–200.
Buch AC, Brown GG Niva CC, Sautter KD, Sousa JP. (2013). Toxicity of three pesticides commonly used in Brazil to Pontoscoles corethraeus (Muller, 1857) and Eisenia andrei (bouche, 1972). App Soil Ecol 69: 32–38.
Capowiez Y, Rault M, Costagliola G, Mazziola C. (2005). Lethal and sublethal effects of imidacloprid on two earthworm species (Aporrectodea nocturna and Allolobophora chlorotica). Biol and Ferti of Soil 41(3): 133–143.
Capowiez Y, Bastardie F, Costagliola G. (2006). Sublethal effects of Imidacloprid on burrowing behavior of two earthworm species L: Modification of the 3D burrow systems in artificial cores and consequences on gas diffusion in soil. Soil Biol and Biochem 38(2): 285–293.
Capowiez Y, Dittbrenner N, Rault M, Triebkorn R, Hedde M, Mazziola C. (2010). Earthworm cast production as a new behavioural biomarker for toxicity testing. Environ. Pollut 158: 388–393.
Caselli F, Gastaldi L, Gambi N, Fabбри E. (2006). In vitro characterization of cholinesterases in the earthworm Eisenia andrei. Comp Biochem Physiol C: Toxicol Pharmacol 143: 416–421.
Cathey B. (1982). Comparative toxicities of five insecticides to the earthworm Lumbricus terrestris. Agric and Environ 7(1): 73–81.
Canesi L, Procházková Š. (2014). The invertebrate immune system as a model for investigating the environmental impact of nanoparticles. In: Boraschi D, Duschl A (eds), Nanoparticles and the immune system, safety and effects, Academic Press, Oxford, pp. 91–112.
CCOHS (2018). Canadian center for occupational health and safety. https://www.ccohs.ca/oshanswers/chemicals/id50.html.
Cortet J, Gillon D, Joffre R, Ourcival J.M, Poinsot-Balaguer N. (2002). Effects of pesticides on organic matter recycling and microarthropods in a maize field: use and discussion of the litter-bag methodology. Eur J Soil Biol 38: 261–265.
Daam MA, Leitão S, Cerejeira MJ, Paulo Sousa J. (2011). Comparing the sensitivity of soil invertebrates to pesticides with that of Eisenia fetida. Chemoresistant 85: 1040–1047.
Datta S, Singh J, Singh S, Singh J. (2016). Earthworms, pesticides and sustainable agriculture: a review. Environ Sci Pollut Res 23(9): 8227–43.
De Arnab, Bose R, Kumar A, Mojmard S. (2014). Targeted Delivery of Pesticides Using Biodegradable Polymeric Nanoparticles, Springer Briefs in Molecular Science, DOI: 10.1007/978-81-322-1689-6_2.
Devkota D, Dhakal SC, Dhakal D, Dhakal DD, Ojha RB. (2014). Economics of Production and Marketing of Vermicompost in Chitwan, Nepal. Int J of Agri and Soil Sci 2(7): 112–117.

De Silva PMCS, VanGestel CAM. (2009). Comparative sensitivity of Eisenia andrei and Perionyx excavatus in earthworm avoidance tests using two soil types in the tropics. Chemosphere 77: 1609–1613.

De silva PMCS. (2009). Pesticide effects on earthworms: A tropical perspective. PhD Thesis. Department of Ecological Science, VU University, Amsterdam, The Netherlands. pp.117.

DGCI&S. (2017). Directorate General of Commercial Intelligence and Statistics, Kolkata, WB, Ministry of Commerce. http://ppqs.gov.in/divisions/pesticides-monitoring-documentation.

Dureja P, Tanwar RS. (2012). “Pesticides: Evaluation of Environmental Pollution,” ed. by H. S. Rathore and L. M. L. Nollet, Chapt. 12, CRC Press, Boca Raton, Florida, U.S.A., pp. 337–359.

Dutta A, Dutta H. (2016). Some Insights into the effect of Pesticides on Earthworms. Int Res J Environ Sci 5(4): 61–66.

Edwards CA, Bohlen PJ. (1992). The effect of toxic chemicals on earthworms. Rev. Environ. Contam. Toxicol 125: 23–99.

Edwards CA, Bohlen PJ. (1996). Biology and Ecology of Earthworms 3rd edition. London : Chapman and Hall.

EPA. (2009). Registering Pesticides. Available online: http://www.epa.gov/pesticides/regulating/re-gistering/index.htm (accessed on 1 April 2011).

Espinoza-Navaroo O, Bustos-Obregon E. (2004). Sublethal doses of malathion after male reproductive parameters of Eisenia fetida. Ecotoxicol & Environ Safety 50(3): 180–188.

Espinoza-Navaroo O, Bustos-Obregon E. (2004). Effect of malathion on the male reproductive organ of earthworms, Eisenia fetida. Asian J Androl 7: 97–101.

Farrukh S, Ali AS. (2011). Effects of dichlorovos organophosphate on growth, reproduction, and avoidance behavior of earthworm Eisenia fetida. Iran J Toxicol 5: 495–501.

FICCI. Federation of Indian chambers of commerce and Industry (2014). Safe and judicious use of agrochemicals and applications of green chemistry. pp.1–32.

FICCI – Federation of Indian chambers of commerce and Industry. (2015). A Report on Indian Agrochemical Industry. pp. 64.

Fingerman M. (1984). Pollution our enemy. Proc. Symp. Physiol. Resp. Anim. Poll. pp.1–6.

Frampton GK, Jansch S, Scott-Fordsmand JJ, Rombke J, Van den Brink P.J. (2006). Effects of pesticides on soil invertebrates in laboratory studies: a review and analysis using species sensitivity distributions. Environ Toxicol Chem 25: 2480–2489.

Gambi N, Pasteris A, Fabbri E. (2007). Acetyl choline esterase activity in the earthworm Eisenia andrei at different conditions of carbaryl exposure. Comp Biochem Physiol C:Toxicol Pharmacol 145: 678–885.

Garcia M, Schefczyk A, Garcia A. (2008). Effects of three pesticides on the avoidance behavior of earthworms in laboratory tests performed under temperate and tropical conditions. Environ Pollut 153: 450–456.

Garcia M, Schefczyk A, Garcia A, Rombke J. (2011). The effects of the insecticide lambda-Cyhalothrin on the earthworm Eisenia fetida under experimental conditions of tropical and temperate regions. Environ Pollut 159: 398–400.

Gevo B, Jones KC, Semple KT, Craven A, Burael P. (2003). Non extractable pesticide residues in soil. Environ Sci Technol 1: 159–144.

Gill H.K and Garg H. (2014). Pesticides: Environmental Impacts and Management Strategies. Pesticides – Toxic Aspects, Marcelo L. Larramendy and So-nia Solonese, IntechOpen, DOI: 10.5772/57399.

Govindarajan B, Prabaharan V. (2014). The effect of pesticides on reproductive parameters of male reproductive organ of earthworms, Eisenia fetida. Bull Environ Con trol Toxicol 85: 83–86.

Gupta KD, Saxena PN. (2003). Carbaryl-induced behavioral and reproductive abnormalities in the earthworm Metaphire posthuma: a sensitive model, *Alternatives to Laboratory Animals* 31(6): 587–593.

Guruge KS, Tanabe S. (2001). Contamination by persistent organochlorines and butyltin compounds in the west coast of Sri Lanka. Mar Pollut Bull 42: 179–186.

Hassan SA. (1989). Testing methodology and the concept of the IOBC/WPRS working group. p. 1–18. In:”Pesticides and Non-target Invertebrates” (P.C. Jepson, ed.). Intercept, Wimborne, Dorset, UK, pp.240.

Hole DG, Perkins AJ, Wilson JD, Alexander IH, Grice PV, Evans AD. (2005). Does organic farming benefit biodiversity? *Biol Conservat* 122: 113–130.

Huang PM, Iskandar IK (1999) Soils and Groundwater Pollution and Remediation: Asia, Africa, and Oceania. CRC Press. ISBN 9781566704526.

Hussain S, Siddique T, Saleem M, Arshad M, Khalid A. (2009). Impact of pesticides on soil microbial diversity, enzymes, and biochemical reactions. Adv. in Agronomy 102: 159–200.

IRAC – Insecticides Resistance Action Committee (2016). Insecticide Mode of Action Classification Scheme. Version 8.1. www.irac-online.org. pp 1–26.

ISO. International Standards Organization (1993). Soil-quality-effects of Pollutants on Earthworms (*Eisenia fetida*). Part 1 Determination of Acute Toxicity using Artificial Soil Substrate. International Standardization Organization, Geneva, Switzerland, ISO11268–1.

Jansch S, Garcia M, Rombke J. (2005). Acute and chronic isopod testing using tropical Perionelloides pruinosus and three model pesticides. *Eu J Soil Biol* 41: 143–152.

Jager T, van der Wal L, Fleuren RH, Barendrecht A, Hermens JL. (2005). Bioaccumulation of organic chemicals in contaminated soils: evaluation of bio-assays with earthworms. *Environ. Sci. Technol* 39: 293–298.

Jeyanthi V, Paul JAJ, Selvi BK, Karmegam N. (2016). Comparative Study of Biochemical Responses in Three Species of Earthworms Exposed to Pesticide and Metal Contaminated Soil. Environ Process 3: 167–178.

Johnson M, Franke L, Lee R, Holloway S. (1999). Bioaccumulation of 2,4,6-trinitrotoluene and polychlorinated biphenyls through two routes of exposure in a terrestrial amphibian: is the dermal route significant? *Environ Toxicol and Chem* 18(5): 873–876.

Julka, JM. (2001). Earthworm diversity and its role in agroecosystem. VII National symposium on soil biology and ecology. Bangalore University of Agricultural Sciences, Bangalore, pp.13–17.

Karthikeyan S, Ramesh PT, Nagamani B, Kumar GM. (2004). Earthworms. Agro, India, 7: 34–35.

Kathireswari P. (2016). DNA Barcoding of Earthworms. In Science Communicators meet (103rd ISCA), Mysore.

Kaulsh BR, Bisho SPS, Kalia S. (1995). Population dynamics of the earthworm *Amythnas alexandri* (Megascolidae: Amelidida) in cultivated soils of the Kumaun Himalayas. *Appl Soil Ecol* 2(1): 125–30.

Lavelle P. (1983). “The structure of earthworm communities,” in Earthworm Ecology. J. E. Satchell, Ed., Chapman and Hall, London, UK, pp. 449–466.

Lee KE. (1985). Earthworms. Their Ecology and Relations to Soils and Land Use, First. Academic Press, Sydney, pp. 411.

Lim SL, Wu TY, Lim PN, Shank KPY (2015b). The use of vermicompost in organic farming: overview, effects on soil and economics. *J Sci Food Agric* 95(6):1143–1156.

Liang W, Beattie GCA, Meats A, Spooner-Hart R. (2007). Impact on soil-dwelling arthropods in citrus orchards of spraying horticultural mineral oil, carbaryl or methidathion. *Aust J Entomol* 46: 79–85.

Lim MP, Cardoso DNO, Soares A.M, Loureiro S. (2015). Carbaryl toxicity prediction to soil organisms under high and low-temperature regimes. *Ecotoxicol Environ Saf* 114: 263–272.

Lo CC. (2010). Effect of pesticides on soil microbial community. *J. Environ. Sci. Health, B: Pesticides* 45: 348–359.

Maurya P, Malik D. (2016). Accumulation and distribution of organochlorine and organophosphorus pesticide residues in water, sediments and fishes, Heteropneustis fossilis and Puntius ticto from Kali River, India. *J of Toxico and Environ Health Sci* 8(5): 30–40.

Mangala P, De Silva CS, Pathiratne A, Van Gestel CAM. (2009). Influence of Temperature and Soil Type on the Toxicity of Three Pesticides to Eisenia andrei. *Chemosphere* 76: 1410–1415.

Mahmod I, Imadi SR, Shazidi K, Gul A, Hakeem KR. (2016). Effect of Pesticides on environment. Springer International Publishing, Switzerland. DOI: 10.1007/978-3-319-27455-3_13.

Mathur SC. (1999). Future of Indian pesticides industry in next millennium. *Pesticide Information* 24(4): 9–23.
Mayanglambam T, Vig K, Singh DK. (2005). Quinolaphos persistence and leaching under field conditions and effects of residues on dehydrogenase and alkaline phosphomonoesterases activities in soil. Bull of Environ Contam and Toxicol 75: 1067–1076.

Milanović J, Milutinović T, Stojanović M. (2014). Effects of three pesticides on the earthworm Eisenia Fetida (Savigny 1826) under laboratory conditions: assessment of mortality, biomass, and growth inhibition. Eur J Soil Biol 62: 127–131.

Mosleh YY, Ismail SMM, Ahmed MT, Ahmed YM. (2003). Comparative toxicity and biochemical responses of certain pesticides to the mature earthworm Aporrectodea Caliginosa under laboratory conditions. Environ Toxicol, 8: 338–346.

Munoz-Leoz B, Ruiz-Romera E, Antiguedad I, Garbisiu C. (2011). Tebuconazole application decreases soil microbial biomass and activity. Soil Biol and Biochem, 43: 2176–2183.

Nasr HM, Badawy El. (2015). Biomarker Response and Biomass Toxicity of Earthworms Aporrectodea caliginosa Exposed to IGs Pesticides. J Environ Anal Toxic 5: 1–2.

Nasreen A, Ashfaq M, Mustafa G. (2000). Intrinsic Toxicity of some Insecticides to egg parasitoid Tricogramma chilonis (Hym Trichogrammatidae). Bull Inst Trop Agr Kyushu Univ 23: 41–44.

Nahmani J, Hodson ME, Black S. (2007). A review of studies performed to assess metal uptake by earthworms. Environ. Pollut 145: 402–424.

OECD. (1984). Guideline for Testing of Chemicals, No. 207, Earthworm Acute Toxicity, Paris, France.

OECD. (2004). Guideline for Testing of Chemicals, No. 222, Earthworm Reproduction Test (Eisenia fetida/Eisenia andrei), Paris, France.

OECD. (2015). Guidelines of testing of chemical No. 222 Earthworm Reproduction Test (Eisenia fetida/Eisenia andrei). Drafted 12.06.2015, Paris France.

Pal A, Patidar P. (2013). Effect of insecticide dimethoate on the reproduction of Eisenia fetida (earthworm). Adv pharmacol toxicol 143(4): 57–64.

Panda S, Sahu SK. (1999). Effects of malathion on the growth and reproduction of Dendroba Williis (Oligochaeta) under laboratory conditions. Soil Biol Biochem 31: 363–366.

Panda S, Sahu S. (2004). Acute toxicity assessment of three pesticides to the earthworm Dendroba willisii. J of Ecotoxicol and Environ Monitor 12(3): 215–223.

Pan Germany. (2012). Pesticide and health hazards. Facts and figures 116 (www.pan-germany.org/download/Vergift_EN-201112-web.pdf).

Pathangray AJ. (2014). Vermicompost: beneficial tool for sustainable farming. Asian J Multidisciplinary Stud 2(8): 254–257.

PAN Pesticide action network. (2010). Pesticide database. (last accessed on 56). PAN Pesticide action network. (2010). Pesticide database. (last accessed on 48).

Piccolo A, Conte P, Scheunert I, Paci M. (1998). Atrazine interactions with soil micrroorganisms. J Agric Environ Ethics (2007) Comparative effects of lindane and lirunon persistence in soil amended with vermicompost derived from spent grape marc and treated with urea. Appl Soil Ecol 44: 198–204.

Rosell G, Quero C, Coll J, Guerero A. (2008). Biorational insecticides in pest management. J of Pesticide Sci 33: 103–121.

Sampedro L, Jeannotte R, Whalen JK. (2006). Transgenic transfer of fatty acids from gut microbiota to the earthworm Lumbricus Terrestres L. July. Soil Biol and Biochem 38(8): 2188–2198.

Sánchez-Bayo F, Van den Brink Pj, Mann RM. (2011). Ecological Impacts of Toxic Chemicals. Bentham Science Publishers Ltd. Online, pp.281.

Sánchez-Bayo F, (2012) Insecticides Mode of Action in relation to their toxicity to Non-Target Organisms. J Environ Analytic Toxicol 54: 092.

Sarav SSS, Shokrzadeh M. (2011). Role of pesticides in human life in the modern age: a review. In: Stoytcheva M (ed) Pesticides in the modern world-risks and benefits. In Tech pp.4–11.

Saxena PN, Gupta SK, Murthy RC. (2014). Comparative toxicity of carbaryl, carbofuran, cypermethrin and fenvalerate in Metaphire posthumus and Eise- nia fetida—a possible mechanism. Ecotoxicol Environ Saf 100: 218–225.

Sabra F.S, Mekena E.S. (2015) Pesticides Toxicity in Fish with Particular Refer- ence to Insecticides. Asian J Agric Food Sci. Vol.3, pp.60–69.

Schaef er M. (2004). Assessing 4, 6-trinitrotoluene (TNT) contaminated soil using three different earthworm test methods. Ecotoxicol Environ Saf 57: 74–80.

Schreck E, Geret F, Gontier L, Treihou M. (2008). Neurotoxic effect and meta- bolic responses induced by a mixture of six pesticides on the earthworm Aporrectodea caliginosa nocturna. Chemosphere 71: 1832–1839.

Senapati BK, Biswal J, Sahu SK, Pan IC. (1991). Impact of malathion on Dra- widi willsi, a dominant earthworm in Indian rice fields. Pedobiology. 35: 117–128.

Sorou J, Larink O. (2001). Toxic effects of benomyl on the ultrastructure dur- ing spermatogenesis of the earthworm Eisenia Foetida. Ecotoxicol. Environ. Safa 50: 80–88.

Shi Y, Shi Y, Wang X, Lu Y, Yan S. (2007) Comparative effects of lindane and deltamethrin on mortality, growth, and cellulase activity in earthworms (Eisenia fetida). Pesticide Biochem and Physiol 89(1): 31–38.

Silva CL, Brennan N, Brouwer JM, Commandeur D, Verweij RA. (2017). Com- parative toxicity of imidacloprid and thiacloprid to different species of soil invertebrates. Ecotoxicology 26: 555–564.

Solaimalai A, Ramesh RT, Baskar M. (2004). Pesticides and environment. In: Environ contam and bioreclam, pp.197–204.

Stanley J, Chandrasekaran S, Preetha S, Kuttalams S, Jasmine RS. (2016). Selec- tive toxicity of diafenthiuron to non-target organisms: honey bees, coci- nellids, chelonus, earthworms, silkworms and fish. J Plant Protect Research 56(1): 1–5.

Stephenson G, Wren C, Middlerson I, C.J.Warner J. (1997). Exposure of the earthworm Lumbricus terrestris, to diazinon, and the relative risk to passer- ine birds. Soil Biol and Biochem 29(3/4): 717–720.

Surana T, Sharma J, Bhatt S. (2012). Sunidhi Research, Agrochemical research (A Technical Report). pp 1–106.

Suzhen Q, Donghui W, Lichen Z, Miaomiao T, Chengui W, Xiaofeng X, Lim- ing WU. (2018). Effects of a novel neonicotinoid insecticide cycloxaprid on earthworm, Eisenia fetida. Environ Sci and Poll Res 25: 1438–1447.

Thapa P, Zalawadia A, Pohkarar OV, Sattam SS. (2015). Classification of pesti- cides and its damaging effects: a review. BioLife 4(1): 13–24.

Ribera D, Narbonne JF, Arnaud C, Saint-Denis M. (2001). Biochemical re- sponses of the earthworm, Eisenia fetida, exposed to contaminated soil, effects of carbaryl. Soil Biol Biochem 33: 1123–1130.

Rico A, Sabater C, Castillo MA. (2016). lethal and sub-lethal effects of five pesticides used in rice farming on the earthworm Eisenia fetida. Ecotoxicol and Environ Saf 127: 222–229.

Ritz K, McNicol JW, Nunan N, Grayston S, Millard P, Atkinson D, Gollotte A, Habeshaw D, Boag B, Clegg CD, Griffiths BS, Wheatley RE, Glover LA, Mc- Caig AE, Prosser J. (2004). Spatial Environ Sci Pollut Resit structue in soil and microbiological properties in upland grassland. FEMS Micro- biol Ecol 49(2): 191–205.

Roberts B, Dorough W. (1984). Relative toxicities of chemicals to the earth- worm Eisenia Fetida. Environ Toxicol and Chem 3: 67–78.

Rodríguez-Campos J, Dendooven L, Alvarez-Bernal D, Contreras-Ramos SM. (2014). Potential of earthworms to accelerate removal of organic contami- nants from soil: A review. App Soil Ecol 79: 10–25.

Romero E, Fernández-Bayo J, Díaz JMC, Nogales R. (2010). Enzyme activities and diuron persistence in soil amended with vermicompost derived from spent grape marc and treated with urea. Appl Soil Ecol 44: 198–204.

Rosa G, Guero C, Coll J, Guerero A. (2008). Biorational insecticides in pest management. J of Pesticide Sci 33: 103–121.
Van Gestel C, Weeks J. (2004). Recommendations of the 3rd International Workshop on earthworm ecotoxicology, Aarhus, Denmark, August 2001. *Ecotoxicol and Environ Saf* **57**: 100–105.

Venter JM, Reinecke AJ. (1985). Dieldrin and growth and development of the Earthworm, *Eisenia fetida* (oligochaeta), *Bull of Environ Contam and Toxicol* **35**: 652–659.

Vickerman GP. (1988) Farm scale evaluation of the long-term effects of different pesticide regimes on the arthropod fauna of winter wheat. In: Greeves MP, Grieg-Smith PW, Smith BD (eds.) Field methods for the environmental study of the effects of pesticides. BCPC Monograph No. 40 British Crop Protection Council, Farnham, UK; pp.127–135.

Wang Y, Cang T, Zhao X, Yu R, Chen L, Wu C and Wang Q. (2012). Comparative acute toxicity of twenty-four insecticides to earthworm, *Eisenia fetida*. *Ecotoxicol Environ Saf* **79**: 122–128.

Wang Y, Wu S, Chen L, Wu C, Yu R, Wang Q and Zhao X. (2012a). Toxicity assessment of 45 pesticides to the epigeic earthworm *Eisenia fetida*. *Chemosphere* **88**: 484–491.

Ware GW. (1980). Effects of pesticides on nontarget organisms. *Residue Reviews* **76**: 173–201.

Ware GW and Whitacre DM. (2004). The Pesticide Book, 6th Ed. Meister Media Worldwide, Willoughby, Ohio. pp. 496.

Walter C and Scheu S. (1999). Changes in bacterial numbers and hyphal lengths during the gut passage through *Lumbricus terrestris* (Lumbricidae, Oligochaeta). *Pedobiologia* **43**: 891–900.

Yasmin S and D’Souza D. (2010). Effects of Pesticides on the Growth and Reproduction of Earthworm: A Review. *Appl and Environ Soil Sci* **2010**: Article ID 678360, 1–9.

Yuguda AU, Abubakar ZA, Jibo AU, AbdulHameed A and Nayaya AJ. (2015). Assessment of Toxicity of Some Agricultural Pesticides on Earthworm (*Lumbricus Terrestris*). *Am Eurasian J Sustain Agric* **9**: 49–59.

Zhang C, Liu X, Dong F, Xu J, Zheng Y, Li J. (2010). Soil microbial communities response to herbicide 2,4-dichlorophenoxyacetic acid butyl ester. *Eur J Soil Biol* **46**: 175–180.

Zhou S, Duan CP, Wang XH, Michelle WHG, Yu ZF, and Fu F. (2008). Assessing cypermethrin-contaminated soil with three different earthworm test methods. *J Environ Sci* **20**: 1381–1385.

Zhou Sp, Duan Cq, Fu Hui, Chen Yh, Wang Xh and Yu Zf. (2007). Toxicity assessment for chlorpyrifos-contaminated soil with three different earthworm test methods. *J Environ Sci* **19**: 854–858.

Zhou X, Zhang QR and Liang JD. (2006) Toxic effects of acetochlor and methamidophos on earthworm *Eisenia Fetida* in Phaiozem, northeast China. *J Environ Sci* **18**: 741–745.