Bioindication and Biomarker Responses of Earthworms: A Tool for Soil Pollution Assessment

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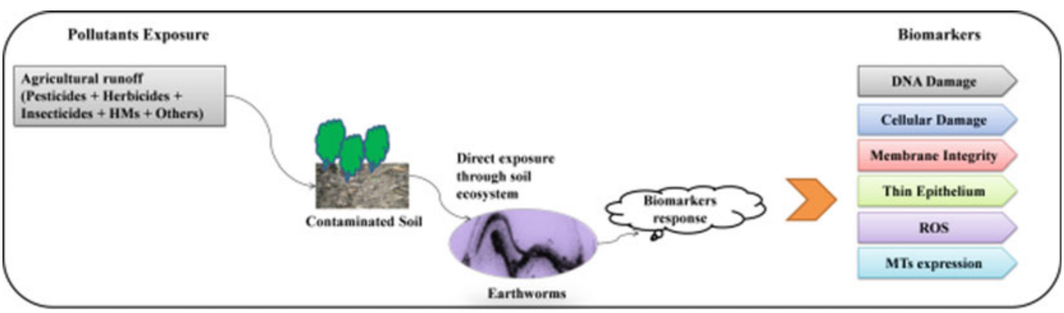
Abstract

Hazardous pollutants from anthropogenic activities are continually delivered into various natural spheres including the terrestrial biological system which is a highly influenced ecological sphere confronting the genuine contamination problem. Synthetics like pesticides, insecticides, herbicides, vinasse, polyaromatic hydrocarbons, heavy metals, agrochemicals, dioxins, and toxic sewage are among the potentially harmful pollutants that alter the physicochemical characteristics of the soil by chemical interactions with the soil environment and its dwelling biota, hence upsetting the typical functioning. Accordingly, these pollutants must be checked and monitored to revamp the health of the soil and henceforth utilization of earthworms gives an alternative yet stunning, novel, and biological monitoring tool to evaluate the hazardous impacts of the pollutants through its biomarkers response and bioindication abilities. Earthworms end up being profoundly viable in monitoring the soil pollutants. This chapter significantly reviews the importance of earthworms in pollutants biomonitoring in special reference to the soil ecosystem.

Graphical Abstract



Keywords

Bioindication · Biomarkers · Earthworms · Monitoring · Pollutants · Response · Soil ecosystem

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23.1 Introduction

The soil ecosystem is actively involved in the regulation of biogeochemical cycles, disposal of waste, retention of carbon, water filtration, and temperature regulation. All these services are maintained by the inherent communities of the soils intrinsic to life and other fundamental activities on the earth (Pérès et al. 2011). But, in the present-day scenario, the excessive use of pesticides, insecticides, and broad-spectrum chemicals causes negative environmental consequences at physiological and chemical levels in the soil ecosystem (Lionetto et al. 2019). Besides, urban waste, toxic sludge, atmospheric deposition, and industrialization enhance soil pollution (Calisi et al. 2014). The researchers in the most recent decade's centers around the physio-chemical characterization of soil health; however, there is a prerequisite for exceptionally capable and proficient tools to detect the real-time impact of the pollutants as conventional methodologies are not all that compelling (Bünemann et al. 2018; Yang et al. 2020a). Nonetheless, various studies indicate the utility of soil biota as the early warning indicators of pollutants through their biomarkers response and bioindication abilities (Burger 2006; Parmar et al. 2016). Cortet et al. (1999) in their critical review discuss the relevancy of nematodes, mites, isopods, mollusks, and earthworms as exceptionally valuable life forms for contamination bioindication (Cortet et al. 1999).

The growing concerns of soil health account for the developing interest in the improvement of new-age bioindicators and early warning techniques. The soil pollution assessment analysis is a complex process (Ashraf et al. 2014). Subsequently, the use of earthworms as a bioindicator model provides a unique, novel, eco-friendly, cost–benefit, and convenient approach for soil pollution assessment. Earthworms are engaged with the pedogenesis and customarily utilized as agents to indicate the soil fertility, land use impact, and organic matter breakdown (Calisi et al. 2011, 2014). They are straightforwardly confronting the toxic impacts of the soil pollutants through their permeable and highly sensitive skin for the pollutants. Additionally, they ingest the defiled soil particles and accordingly impact the pollutants availability (Wallwork 1983; Jager et al. 2003; Vijver et al. 2003). Because of their higher relevance in standard toxicity testing protocols, earthworms discover their utilization as soil contamination bioindicators. Their mechanism of response and biomarkers generation toward the stress produced by the toxic soil pollutants can provide more extensive information in accessing the level of soil health. Therefore, we, here in this chapter, have focused to explore the bioindication and biomarkers response of earthworms in pollution assessment of the soil ecosystem.

23.2 Biological System and Pollution Biomarkers

The pollution assessment and measuring their toxic impacts in different environmental spheres is a highly difficult process (Ashraf et al. 2014). For doing such complex estimations, nature furnished us with extraordinary sentinel living beings that can distinguish the continuous changes that occurred in the environment. The sentinel species can biosense the extent of pollution by making specific changes in the form of biomarkers response. These biomarkers are expressed as ``alterations' in the body of sentinel species. These changes can be best utilized to express the toxicological impacts of a particular pollutant. Therefore, it is used as an early warning indicator. When the biological sentinel species got exposed to a particular or variety of pollutants, adverse and toxic effects have been seen at the molecular and cellular levels. These changes are represented by the molecular and cellular biomarkers in the bioindicator species. These biomarkers viably give the necessary information on the bioavailability and the adverse effects of the contaminants on the environment as these biomarkers can assist us with understanding the biochemical processes of absorption, transportation, and biotransformation of pollutants in the sentinel organisms as well as the environment. Below are the various categories of biomarkers that are useful in measuring the level of pollution and toxicity of particular pollutant existed in the environment.

23.2.1 Exposure

These are the class-specific biomarkers as they are expressed in the body of sentinel species in response to a specific class of pollutants (Scott-Fordsmand and Weeks 2000). Genetic alterations, circulating antibodies, DNA and protein adducts, altered proteins, metallothioneins levels, altered cholinesterase activity, ethoxyresorufin-O-demethylase activity, and altered gene expression are some of the main exposure biomarkers that reflect their expression in response to pollutants exposure in the animal's body.

23.2.2 Histological

These are the biomarkers with a defined cellular origin (Kilty et al. 2007). Elevated troponin levels, altered alanine aminotransferase, transaminase activity, thinning of epithelium lining, altered lysosomal-cytoplasm ratio, and basophil-digestive cell ratio are some of the histological biomarkers that define the morphological damage in the organism's body when exposed to different pollutants (Reddy 2012).

23.2.3 Stress

As the name indicated, these biomarkers are expressed in the animal's body in response to the physiological stress instigated by the toxic impacts of the pollutants (Etteieb et al. 2019). The generation of heat shock proteins in response to tackling temperature variations, acute phase proteins, cortisol, cytokines, alpha-amylase, reactive oxygen species level, MDA levels in the serum and plasma, altered GHS, SOD, thioredoxin reductase, and glutathione peroxidase activities are some of the well-known stress biomarkers in the animal's body (Colacevich et al. 2011; Ali and Naaz 2013).

23.2.4 Genotoxicity

A few pollutants, for example, PAHs, naphthalene, and phenanthrene are notable for their genotoxic potential when exposed to living organisms. These agents cause DNA damage and consequently promote mutations in the organism's body (Hirano and Tamae 2010). These pollutants cause DNA alterations through the phenomenon of oxidative respiration and altered metabolic reactions. The damage to genetic material persists in the form of chromosomal abnormality, distorted sister chromatids, abnormal DNA-DNA crosslinks, and DNA–protein binding. To monitor the genotoxic damage on the exposure of toxic pollutants, there are several genotoxic biomarkers with the help of which we can assess the toxic potential of a particular toxin. For example, increased micronucleus formation, chromosomal aberrations, comet formation, and toxicogenomic signatures are some of the known biomarkers which serve as good genotoxic biomarkers for toxicity assessment (Vasseur and Bonnard 2014; Muangphra et al. 2015).

23.3 Effects of Soil Pollutants on Earthworms

Earthworms are perceived as suitable candidates for the biomonitoring purpose of soil pollutants (Hirano and Tamae 2011). Different investigations have been done to signify the role of earthworms as bioindicators of soil pollution (Haeba et al. 2013). Scientists examined the effect of natural and depleted uranium on the earthworms and noticed genetic and cytotoxic alterations in their body tissues (Giovanetti et al. 2010). It has been discovered that the earthworms have incredible bioaccumulation potential, thus, proved to be helpful for heavy metals biomonitoring (Usmani and Kumar 2015). The study by Natal-de-Luz et. al. observed the centrality of earthworms in the ecological risk assessment of mixed chemical compounds (Natal-da-Luz et al. 2011). Likewise, Qiu et al. utilize *Aporrectodea calignosa* (gray worm) for toxicity assessment of binary mixture of zinc and cadmium (Bart et al. 2018). One of the study reported the bioaccumulation of mixture form of nickel and chlorpyrifos in the body tissues of the earthworm, thus, describes the assimilation pathway of these pollutants in the *lumbricid* earthworms body (Lister et al. 2011). Different studies that evaluated the effects of a variety of soil pollutants on different earthworm species have been presented in Table 23.1.

23.4 Pollutant-Induced Biomarker Responses in Earthworm

As stated above, earthworms are highly sensitive toward pollutants exposure and proved helpful in their biomonitoring. When exposed to pollutants, the body of earthworms reacts to them by expressing cellular, behavioral, morphological, genetic, and biochemical biomarkers (Fig. 23.1). Different pollutants induce a different kind of biomarkers response. Different research reports signify the expression of a variety of biomarkers responses concerning a specific pollutant. These are discussed below.

23.4.1 Methiocarb

The insecticide methiocarb is a carbonic acid derived organic ester that is synthesized from the condensation of 3, 5-dimethyl-4 (methylsulfonyl) phenol with methyl carbamic acid (Fig. 23.2) Researchers investigated the biomarkers response of the *Lumbricus terrestris* toward the methiocarb exposure.

The study was performed under controlled experimental conditions at a temperature of 18 ± 1 °C with a 16:8 h photoperiod ratio of light and dark The exposure of the insecticide was given at different time intervals of 0, 7, and 14 days. The study involves the measurement of altered lysosomal permeability, MTs expression, and granulocyte morphogenetic analysis. With these analyses, other parameters like growth, reproduction, and survival capacity will also be taken into consideration. The results of the study concluded that the used model species of earthworms was very sensitive toward the methiocarb exposure and different biomarkers of effect such as enlarged granulocytes, and destabilized lysosomal membrane was observed to be the potential biomarkers that are helpful in biomonitoring of this specific insecticide (Calisi et al. 2011).

23.4.2 Imidacloprid

Imidacloprid influences the soil health and local soil life forms by enhancing the pollution levels in the terrestrial environment (Knoepp et al. 2012). The native earthworm species *Eisenia fetida* was exceptionally influenced when exposed to Imidacloprid. The risk evaluation of this particular insecticide was evaluated by researchers, and observed genotoxic effects on *Eisenia fetida*. The DNA damage and sperm deformity were observed to be the relevant genotoxic and physiological biomarkers expressed in this particular earthworm species in response to Imidacloprid exposure in the terrestrial ecosystem (Zang et al. 2000).

23.4.3 Pesticides

Certain studies conducted mutual toxicity testing of regularly utilized pesticides. Aldicarb, chlorfluazuron, cypermethrin, metalaxyl, and atrazine are some of the commonly used pesticides that are significantly important in causing soil pollution (Mosleh et al. 2003; Miglani and Bisht 2019). Experimental studies showed the environmental consequences of these pesticides and correlate the expression of the different biomarkers in the earthworm's body with the toxic impacts of these chemicals. For instance, researchers in a study observed the deleterious effects of these pesticides on the earthworm, *Aporrectodea calignosa*, and observed that the soluble protein in the earthworm's body was

Table 23.1 List of investigations on the adverse effects of common soil pollutants in different earthworm species after pollutants exposure

Earthworm Species	Soil Pollutants	Effects	References
Eisenia fetida	1,2,4-trichlorobenzene	• Alterations observed in the ultrastructure of skin and cuticle • Low mucus production and finally disappears	Wu et al. (2012)
	Cadmium and Lead	• Weight loss • Delayed sexual maturity	Urionabarrenetxea et al. (2020)
	Tetraethyl Lead and Lead Oxide	• Inflexible metameric segmentation • Rupturing of skin and cuticle • Coelomic fluid extrusion is observed	Rao et al. (2003)
	Benomyl	• Regeneration of posterior segment is influenced • Teratogenic effects • Groove anomalies • Development of two tails at the posterior end	Zoran et al. (1986), Drewes et al. (1987), Sorour and Larink (2001)
	Carbamates	• Development of tumors and swelling in the body	Yadav et al. (2017)
	Propoxur, Methidathion, Triazophos, Endosulfan, Carbofuran	• Swelling, bursting, and bleeding of the sores have been observed	Dureja et al. (1999), Dureja and Tanwar (2012)
	Integrated toxic effects of Cd, Cu, Pb, and Zn	• Higher mortality rate • Altered sexual activities	Spurgeon and Hopkin (1996)
	Pentachlorophenol	• Affect cocoon production • Infertile cocoons	Van Gestel et al. (1989), Landrum et al. (2006)
	PCBs	• Damaged genetic material • Influence the activity of CAT, POD, and SOD • Altered carbohydrate metabolism • Disrupted osmotic function	Åslund et al. (2011), Duan et al. (2017)
	2, 2′, 4, 4′-tetrabromodiphenyl ether (BDE-47) clothianidin,	• SOD gene transcripts upregulation • Suppressed catalase activity	Xu et al. (2015)
	Imidacloprid, thiacloprid, nitenpyram, and, acetamiprid,	• The altered activity of catalase enzyme • Lower fecundity rate	Wang et al. (2015)
Lampito mauritii	Phosphamidon	• Hyperactivity in the body	Bharathi and Rao (1986), Dureja and Tanwar (2012)
	Monocrotophos and Dichlorvos	• Inhibited and altered AChE activity • Damaged intestinal villi • Degenerated nucleus	Datta et al. (2016), Samal et al. (2019), Kavitha et al. (2020)

(continued)

Table 23.1 (continued)

Earthworm Species	Soil Pollutants	Effects	References
		• Weight loss • Blood sinuses congestion	
Lumbricus terrestris	Benomyl	•Hindered AChE activity • Impairment in locomotion •Mitosis inhibition	Byrde and Richmond (1976), Stringer and Wright (1976), Subaraja and Vanisree (2015)
Lumbricus rubellus and Lumbriculus variegatus	C60 Fullerene Nanoparticles	• Damaged musculature, epidermis, and cuticular part	Van der Ploeg et al. (2011,,
Aporrectodea rosea	Cadmium and Lead	• Inhibition of total antioxidant capacity	Sinkakarimi et al. (2020)
Pontoscolex corethrurus	benzo(a)pyrene	• Loss of weight • Low survival rate	Hernández-Castellanos et al. (2013)
Eisenia andrei	Oil contaminated soil	• Higher mortality rate is observed	Hentati et al. (2013)
Drawida willsi	Carbofuran and malathion	• Lowering acetylcholine esterase activity	Panda and Sahu (2004)
Allolobophora chlorotica	Carbendazim	• Disrupted functioning of giant nerve fibers • Altered burrowing behavior	Ellis et al. (2010)
Perionyx excavatus	Chlorpyrifos and carbofuran	• Highly toxic • Death of earthworms	De Silva and van Gestel (2009)
Enchytraeus crypticus	Nylon microplastics debris	• Significant reduction in the reproduction activity of earthworms	(Lahive et al. 2019)
Aporrectodea tuberculata	Copper and Zinc	• Decreased cytochrome CYP1A and GST activities	Lukkari et al. (2004)
Aporrectodea caliginosa	Pentachlorophenol, copper, and cadmium	• DNA and lysosomal damage are observed	Klobučar et al. (2011)
Aporrectodea rosea and Aporrectodea trapezoides	Cadmium and lead nitrate	• DNA damage • Lipid peroxidation • Decrease in total antioxidant capacity	Sinkakarimi et al. (2020)
Octolasion cyaneum	Glyphosate	• Glutathione S-transferase activity observed to be declined	Salvio et al. (2016)

decreased from the initial proteins levels. Besides this observation, the GPT, AcP, and GOT enzyme activities are at their low. These biochemical changes are considered as important biomarkers response of this snail species which were found to be suitable in determining the environmental toxicity (Mosleh et al. 2003).

23.4.4 Polystyrene Microplastics

Microplastics are one of the major and emerging soil pollutants that are known for their serious environmental consequences (Kumar et al. 2020). They are ubiquitous and non-biodegradable (Smith et al. 2018; Mammo

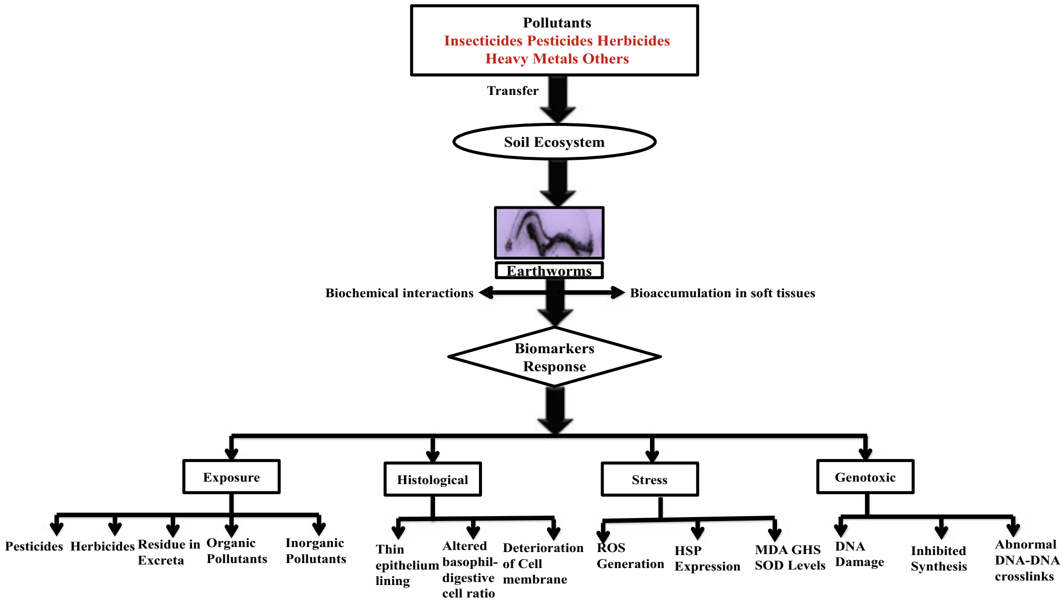


Fig. 23.1 Diagrammatic representation of the different biomarkers response of earthworms to common soil pollutants

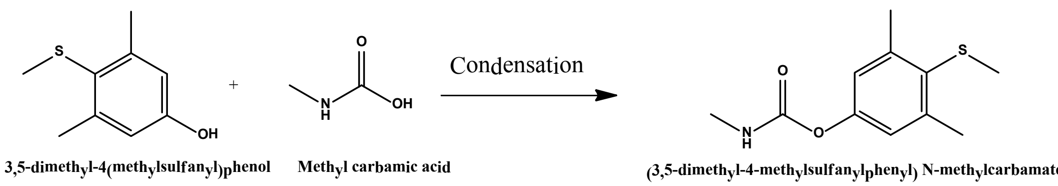


Fig. 23.2 Chemical synthesis of methiocarb. Adapted after: Calisi et al. 2011, Gupta 2011

et al. 2020). Studies on the toxic effects of these polystyrene-based microplastics are one of the enthusiastic areas of research. These polystyrene microplastics (PsMPs) bioaccumulates in the soft and delicate tissues of the soil creatures through the natural way of food chain interactions and causes adverse metabolic functioning (Wang et al. 2019). A recent study shows the toxic consequences of PsMPs on the earthworm species *Eisenia fetida*. Their exposure to *Eisenia fetida* initiates the expression of biomarkers response in the form of DNA damage and oxidative stress. Consequently, the study indicates the histopathological alterations in the intestinal wall of earthworms (Jiang et al. 2020).

23.4.5 Antibiotics

These are widely used biologically active molecules that interact with the soil ecosystem in their pure form (Manyi-Loh et al. 2018; Cycoń et al. 2019). They enter the terrestrial environment through medical waste dumping, domestic sludge, and human excretion (Larsson 2014; Kraemer et al. 2019). In a recent study, researchers explored the environmental effects on the soil ecosystem and the native earthworm species. The study involves the use of different exposure concentrations of ciprofloxacin to the earthworm *Eisenia fetida*, and it was observed that a concentration of 1–2 g/kg of ciprofloxacin exposure causes deformity in DNA while the other biomarkers such as antioxidant enzymatic activity, mRNA expression, HSP 70, MTs, etc. were upregulated (Yang et al. 2020b).

23.4.6 Thifluzamide

The extensive use of fungicides imposes serious environmental concerns (Mahmood et al. 2016). Apart from target organisms, their toxic nature also influences the non-target species present in the soil (Gill and Garg 2014). The fungicide Thifluzamide is one of the commonly used fungicides which are chemically characterized as amide (Yang et al. 2016; Yao et al. 2020). This fungicide disturbs the SDH metabolism in the organisms (Yang et al. 2017). A recent study evaluates the biomarkers response of *Eisenia fetida* concerning the stress induced by the Thifluzamide with different concentrations ranges from 0 to 10 mg/kg. It has been observed that this particular fungicide induces DNA damage, ROS generation, inhibited activities of GST, CAT, POS, and SOD enzymes in the body of *Eisenia fetida* (Yao et al. 2020).

23.4.7 Neonicotinoid Insecticides and Heavy Metals

The extensive use of neonicotinoid insecticides (For example, dinotefuran, thiamethoxam) and heavy metals (e.g., cadmium, zinc, copper) addition in the soil ecosystem causes serious environmental pollution across the globe (Goulson 2013). Recent studies analyzes the mutual toxic impacts of neonicotinoid insecticides and heavy metals on *Eisenia fetida.* This earthworm species is proved to be a very sensitive bioindicator species against the impact of the mutual pollutants. The development of ROS, cellular and DNA damage, deformed midgut cell lining, and disturbed MDA activity are some of the known biomarkers response of *Eisenia fetida* toward the mutual toxic impact of these neonicotinoid insecticides and heavy metals (Yan et al. 2020).

23.4.8 Sunfentrazone

Modern agricultural practices use some specific herbicides. The Sunfentrazone is one of the herbicides that have a wide range of applicability in modern agriculture (Gehrke et al. 2020). Studies reported the toxic effects of this herbicide on some of the aquatic organisms while amphibians also develop abnormalities toward its toxicity (Giesy et al. 2000; Graymore et al. 2001; Mann et al. 2009). Recently, researchers analyze its toxic potential by using *Eisenia fetida* as a model organism for its environmental biomonitoring. Different concentrations ranging from 0.2 to 5.0 mg/kg of the Sunfentrazone have been prepared and the earthworms are exposed to this herbicide in the soil under set laboratory protocols. During the study, the researchers observed the generation of reactive oxygen species in the earthworm's body which was one of the well-established biomarkers of this species toward soil pollutants exposure. Various other biomarkers such as GST, catalase, SOD, guaiacol peroxidase altered activities, and DNA damage are the prominent biomarkers that are highly useful for Sunfentrazone biomonitoring and its associated environmental impacts on soil health (Li et al. 2020). Table 23.2 represents the common, trade name, IUPAC nomenclature, molecular formula, and chemical structures of several soil pollutants.

23.5 Conclusion

It was observed that numerous earthworm species engaged with the biomonitoring and early warning of the soil pollutants. In recent years, the study of earthworm biomarkers proved their utility in contamination detection in terrestrial environments. The DNA damage, anomalous enzymatic functioning, heat shock proteins expression, MTs expression, and so forth are observed to be the prominent biomarkers that help in providing a scientific understanding of earthworm's biomarkers response toward soil pollutants exposure. This article proved to be beneficial for the development and promotion of

Table 23.2 List of common, trade, IUPAC nomenclature, molecular formula, and chemical structures of several soil pollutants (Adapted after: El-Gendy et al. 2020)

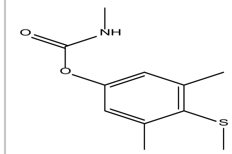
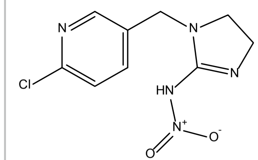
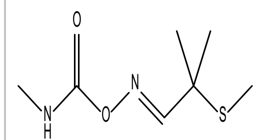
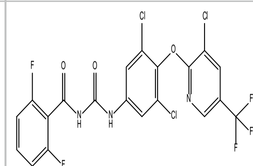
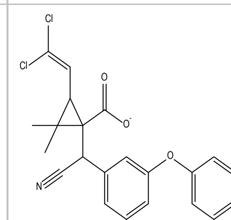
Soil Pollutants Class	Pollutants	Trade Name	Action	IUPAC Nomenclature	Molecular Formula	Chemical Structures
Carbamate Insecticide	Methiocarb	Metacil	Activity against insects, rodents, snails, and birds	4-(Dimethylamino)-3-methyl phenyl Nmethylcarbamate	$C_{11}H_{15}NO_2S$	
Systemic Insecticide	Imidacloprid	Premise 75, Confidor Provado Admire	Activity against cane beetles, locusts, stink bugs, termites, fleas, aphids, etc.	*N*-{1-[(6-Chloro-3-pyridyl)methyl]-4,5-dihydroimidazol-2-yl} nitramide	$C_9H_{10}ClN_5O_2$	
Carbamate insecticide	Aldicarb	Temik, ENT 27093, OMS 771, and UC 21149	Activity against Lygus, cane beetles, locusts, spider mites, stink bugs, termites, fleas, aphids, flea hoppers, etc.	2-Methyl-2-(methylthio) propanal *O*-(*N*-methyl carbamoyl)oxime	$C_7H_{14}N_2O_2S$	
Organochlorine, benzoylurea, Organofluorine insecticide	Chlorfluazuron	Atabron	Inhibit the synthesis of chitin in insects Helps in control of insects such as *Lepidoptera*	*N*-[[3,5-dichloro-4-[3-chloro-5-(trifluoromethyl) pyridin-2-yl] oxyphenyl]carbamoyl]-2,6-difluorobenzamide	$C_{20}H_9Cl_3F_5N_3O_3$	
Synthetic pyrethroid pesticide	Cypermethrin	Auzar 25 EC	Kills house termites, cockroaches, etc.	[Cyano-(3-phenoxyphenyl)methyl]3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate	$C_{22}H_{19}Cl_2NO_3$	

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Table 23.2 (continued)

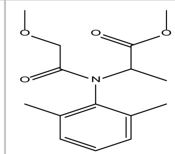
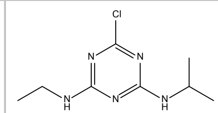
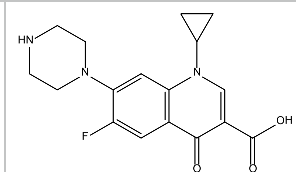
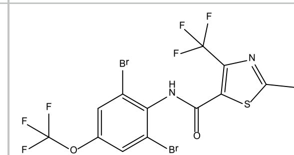
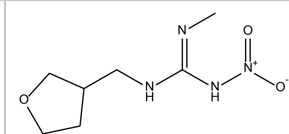
Soil Pollutants Class	Pollutants	Trade Name	Action	IUPAC Nomenclature	Molecular Formula	Chemical Structures
Systemic, phenylamide fungicide	Metalaxyl	Mefenoxam	Control on *Phytophthora infestans*	methyl 2-[(2,6-dimethylphenyl)(methoxyacetyl)amino] propanoate	$C_{15}H_{21}NO_4$	
Triazines	Atrazine	Solaro	Inhibit the normal functioning of photosynthesis in the broadleaf weeds by altering the mechanism of photosynthetic electron transport	1-Chloro-3-ethylamino-5-isopropylamino-2,4,6-triazine	$C_8H_{14}ClN_5$	
Antibiotic	ciprofloxacin	Cipro, Cipro XR, and ProQuin XR	Activity against bacterial infections	1-cyclopropyl-6-fluoro-4-oxo-7-piperazin-1-ylquinoline-3-carboxylic acid	$C_{17}H_{18}FN_3O_3$	
Aromatic fungicide	Thifluzamide	Pulsor	Control *Rhizoctonia*	*N*-[2,6-dibromo-4-(trifluoromethoxy) phenyl]-2-methyl-4-(trifluoromethyl)-1,3-thiazole-5-carboxamide	$C_{13}H_6Br_2F_6N_2O_2S$	
Neonicotinoid	*Dinotefuran*	Oshin 20 SG	Control insects like mole cricket, sawflies, lace bugs, thrips, etc.	2-methyl-1-nitro-3-[(tetrahydro-3-furanyl) methyl] guanidine	$C_7H_{14}N_4O_3$	

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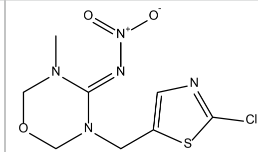
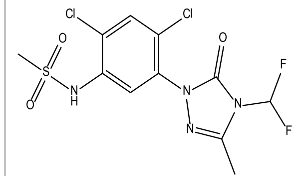
Table 23.2 (continued)

Soil Pollutants Class	Pollutants	Trade Name	Action	IUPAC Nomenclature	Molecular Formula	Chemical Structures
Neonicotinoid	Thiamethoxam	Evident 25% WG	Control leaf and soil-dwelling pests.	3-[(2-Chloro-1,3-thiazol-5-yl)methyl]-5-methyl-*N*-nitro-1,3,5-oxadiazinan-4-imine	$C_8H_{10}ClN_5O_3S$	
Herbicide	24 Sulfentrazone	Acetochlor	Control weeds, sedges, etc.	*N*-{2,4-Dichloro-5-[4-(difluoromethyl)-3-methyl-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl]phenyl} methanesulfonamide	$C_{11}H_{10}Cl_2F_2N_4O_3S$	

the earthworm-based biosensing approach for soil pollution assessment.

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