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A Comprehensive Review on Effects of Soil Pollutants on Pheretima spp. of Earthworm

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Abstract: Soil pollution due to addition of heavy metal has increased to a large extent that survival of human is under threat in such areas. These heavy metals are entering to the environment mainly as a result of anthropogenic activities and becoming hazardous to all living organism. Earthworms are important organisms regarded as macro invertebrates inhabiting the soil and contributing to soil fertility and enhancing organic matter availability to soil. But due to the presence of heavy metals in soil, populations of earthworms are influenced and decreased in areas having high concentrations of heavy metals. Earthworms are also important for ecotoxicological studies as they are indicator of soil pollution. Earthworms have ability to bioaccumulate Sewage water in their body tissues and there are several studies in this regard in which earthworms are used to remove heavy metals from soil known as vermicomposting. Although the toxicity of metal contaminated soils has been assessed with various bioassays, more information is needed about the biochemical responses, which may help to elucidate the mechanisms involved in metal toxicity. In this review, we describe recent studies concerning the relationship between earthworms and soil pollutants, and discuss the possibility of using the earthworm as a bio-monitoring organism for soil pollution.
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Review of Literature

About 800 genera and 8000 species of earthworms are recorded in the world which belongs to the order Oligochaeta (Edwards, 2004). Earthworms are nocturnal in their behavior as they dig soil that have well moisture and found in terrestrial environment therefore, they are called earthworms. The body of earthworms is covered with ring like structures. They are soft bodied and elongated like a thread (Gajalakshmi and Abbasi, 2004). Earthworms play an important role in enhancing the soil fertility through chemical and physical methods as they mix the organic matter into the soil and also degrade it into such forms that is easily available. Earthworms occupy various habitats that are well moisture. Earthworms mix the soil with other particles and stabilize these particles as they mix water in it so helps in controlling soil erosion (Edwards, 2004). Earthworms are one of those organisms that act as bio monitors as they bio accumulate pollutants in their bodies from environment in which they live (Zhang and Zheng, 2009).

The macro fauna of soil depends on earthworms for nutrient availability as earthworm accumulate the pollutants so indicator of pollution. The species that is mostly used for the assessment of eco toxic studies is *Eisenis fetida* as it has ability to build up large number of pollutants in their bodies (Brulle et al., 2006).

Earthworms also called “ecosystem engineers” this is due to because they play an important role in enhancement of soil management and soil richness (Guggenberger and Zech, 1996).

The world population is increasing continuously, it has been doubled for the last forty years (>7 billion in 2013) and it is estimated that if it will happen on continuous bases in the 21st century then it will destroy the soil fertility and nutrient contents which will lead to decline in economy. A great management is required for healthy ecosystem with good natural resources for survival of organisms. But unfortunately, human activities like industrial activities, agricultural practices and waste disposal play a vital role in soil pollution (Alexander, 2000).

From the anthropogenic sources a large number of heavy metals emit such as the mining and smelting industry, use of pesticides and mineral fertilizers and sewage sludge application (Michael *et al.*, 2007). In the present days, researchers have a main focused on heavy metal concentrations all over the world. Both positively and negatively heavy metals are affecting the living organisms (Ozturk *et al.*, 2011).

Metal pollution specially from industries is destroying the soil invertebrates’ habitats and effecting the soil ecosystem. Earthworms are acting as good bio indicators of soil contamination therefore, these pollutants severely affecting the earthworm population. The bio accumulative ability of earthworms enables them to be used as living organisms for the bio monitoring of soil pollution (Hirano and Tamae, 2011). Soil pollutants have a great impact on weight and biomass which cause the decline in individual numbers because mortality rate increases which surely decrease the population size (Davies et al., 2003).

Earthworms are major part of terrestrial invertebrates representing (80%) of biomass which are contributing in soil management by increasing the richness of soil. They are acting as good indicators and therefore, severely effecting by the soil pollutants. They provide basic information about the decline in soil quality by accumulating its pollutants (Bustos-Obregon and Goicochea, 2002). Earthworms act as bio indicators play an important role for conserving the natural resources of environments and in this way conserve the human ecosystem (Beeby, 2012).

Earthworms are acting as suitable bio indicators of soil toxicity because they consume a considerable amount of the organic matter and other material present in soil therefore, they play an important role in richness of topsoil. It has observed that earthworm skin is a major route of contaminants uptake (Sandoval et al., 2001). may disturb soil ecosystems by affecting

Among the soil creatures, earthworms are significant as ecosystem engineers. In moist and clayey soils, they form a chief portion of the animal biomass (Lavelle and Spain, 2001), and play important roles in biological material cessation and in soil structure upgrading (Lavelle et al., 1989; Boyle et al., 1997; Lavelle and Spain, 2001). Earthworms are also important in food webs, as they are regular target items for vertebrate hunters, such as badgers and the little owl (Goszczynski et al., 2000; Van den Brink et al., 2003). Terrestrial earthworms have progressed from aquatic organisms. They are soft-bodied organisms, and prerequisite moist situations to sustain their hydrostatic pressure and prevent scarcity (Laverack, 1963). After a heavy rain fall, there are many observations of earthworms expelled from their burrows (Darwin, 1881; Laverack, 1963; Sims and Gerard, 1999). The objective of this study was to determine earthworm population dynamics in a floodplain system, in relation to frequency and duration of flooding events.

Metallic contamination may interrupt land ecosystems by distressing the arrangement of soil inhabitants. Successful defense of communities loads knowledge of the ecophysiology of metals in invertebrates and their vulnerability to metallic intemperance (Dallinger and Rainbow, 1993; Hopkin, 1989).

The prospective threats to soil invertebrates from environmental contaminants have been evaluated in current years by the use of the 'earthworm acute toxicity test' (OECD, 1984; EEC, 1985). The earthworm *Eisenia fetida* used for 14-day LCs0 testing that has been vital for peril valuation and instruction of fresh and current chemicals (Becker *et al.*, 1992).

The end opinion of the 'earthworm acute toxicity test' is transience. However, death is doubtful to be whichever the most sensitive or ecologically applicable parameter for envisaging effects on populations. Reproductive/growth conflicts are more possibly to intercede population possessions (Moriarty, 1983). Because of its control on population dynamics, reproduction is likely to be of precise key in ecotoxicological estimation (Joosse & Verhoef, 1983; Kooijman & Metz, 1984; Denneman & Straalen, 1991).

Traditional environmental risk assessment (ERA) models for contaminants are usually established on facts about the vulnerability of species cast-off in standard toxicity tests. These standard tests relate individual endpoints such as mortality, growth and reproduction, rather than population or ecosystem level effects. Ecological connections and progressions are rarely taken into account and ERA methods frequently grieve from a nonexistence of ecological relevance (Forbes et al., 2001; Van Straalen, 2002). Calculation of effects at the ecosystem level is therefore still a rising field in ecotoxicology (e.g., Bogomolov et al., 1996; Knacker et al., 2004; Slijkerman et al., 2004; Winding et al., 2005).

Population density, in general, influences the impact of toxicants on life history traits, e.g., survival, reproduction and contaminant effects on key species might have great implications for ecosystem processes. For example, leaf litter decomposition in metal-polluted rivers was inhibited by reducing one functionally dominant type of invertebrate shredder species

(Carlisle and Clements, 2005) and effects of metals on earthworms and enchytraeids negatively impact soil microbial processes (Bogomolov et al., 1996; Salminen et al., 2002). Few experiments, however, have investigated the consequences of population density of such species on functional variables.

Earthworms play an important role in maintaining soil structure and promoting soil ecosystem processes and plant production (e.g., Lawton, 1994; Derouard et al., 1997; Binet et al., 1998). They can be easily used in laboratory experiments. The objective of our study was to investigate the effects of a pollutant (zinc) on soil ecosystem processes in the presence of different densities of the earthworm Lumbricus rubellus Hoffmeister 1843. L. rubellus is a key species in European soils in temperate regions and has colonized many similar habitats elsewhere in the world.

Heemsbergen et al. (2004) demonstrated its disproportional stimulating effect in soils compared to other soil invertebrates. However, mortality is unlikely to be either

Earthworms significantly impact many soils physicochemical and biological processes through their continuous burrowing and casting activities (Blouin et al., 2013). Changes in nutrient availability, soil respiration, microbial biomass and composition, and bacterial-to-fungal ratio are ecological endpoints commonly used to assess the impact of earthworm activity in soil. However, alterations that these annelids may cause in soil extracellular enzymes have been comparatively paid less attention (Kizilkaya et al., 2010).

Most of the studies agree that earthworm casts (or excreta) display higher levels of hydrolytic activity than the bulk soil (Tao et al., 2009), although this difference is highly dependent on earthworm diet (Flegel and Schrader, 2000) and enzyme type (Kizilkaya and Hepsen, 2004; Dempsey et al., 2013). Extracellular enzyme activities are critical in the decomposition of soil organic matter and nutrient cycling (Arnosti et al., 2014).

Accordingly, most research efforts have been addressed to understand the functional role of hydrolytic enzymes involved in the biogeochemical cycles of C (e.g., b-glucosidase, cellulase), N (e.g., urease, proteases), P (e.g., phosphomonoesterases, phosphodiesterases), and S (e.g., arylsulfatase). Over the past two decades, other extracellular enzyme activities, particularly oxidases, have gained widespread relevance to bio-remediate contaminated soils (Bollag, 1992; Gianfreda and Rao, 2008; Burns et al., 2013).

Lubricants in the form of motor oil or engine oil are consumed largely in automobiles to protect the internal combustion engines. Generally, motor oil consists of 90% petroleum fractions and 10% additives. Different brands of engine oils have different additives that belong to several groups such as antioxidants, detergents, dispersants, corrosion inhibitors and viscosity index improvers (Caines and Haycock, 2004). The internal combustion engine acts like an oxidator and the hydrocarbons in the engine oil are partially oxidized when contacted with oxygen at elevated temperatures for long periods. Metals like iron (Fe), copper (Cu), lead (Pb), nickel (Ni), etc. used in the manufacture of the engine are effective oxidation catalysts and increases the rate of oxidation of the engine oil. The oxidation process produces acidic bodies within the motor oil which are corrosive to typical Cu, Pb, and cadmium (Cd) engine bearings. Furthermore, oxidation products contribute to the formation of sludges within the motor oil and an overall breakdown of viscosity characteristics of the lubricant. One liter of used motor oil can pollute up to 3784 m2 of soil,

making it non-productive for farming or plant growth for up to 100 yrs. (Chin et al., 2012). Petroleum hydrocarbons, the main components of the used oils, spread horizontally on the groundwater surface and partition into water, soil pore, air space, and to the surfaces of soil particles (Plohl et al., 2002). The entry of used oil will alter some of the soil properties resulting in poor aeration, immobilization of nutrients, and lowering of pH which are largely responsible for change in fertility of soil (Shukry et al., 2013). These alterations may lead to mass mortality of animals living in the top most layers of soil, ultimately disrupting biological equilibrium of soil (Das and Chandran, 2011). Thus, soil becomes less productive when exposed to used motor oil (US EPA, 1996). Used oils are, therefore, considered as one of the most hazardous mainstream categories of environmental pollutants, posing a major threat to the environment and public health.

Recently, the effect of earthworms on plant growth has been reported (Scheu, 2003). Earthworms generally increase plant shoot biomass, while the response of root biomass is lesser clear; however, the shoot–root ratio was uniformly increased in the presence of earthworms. These organisms can improve the soil structure, increasing the microporosity, which in turn has effects on aeration, water dynamics, and the provision of paths for root exploration (Amador and Gorres, 2005; Wen et al., 2006).

They can alter the ecology of the soil, suppressing plant pathogens and/or promoting the growth of microflora and fauna that may be beneficial to crops (Clapperton et al., 2001). Moreover, they are capable of increasing the bioavailability of several soil nutrients such as C, N and P (Cheng and Wong, 2002; Chaoui et al., 2003). Similarly, earthworms' gut-associated processes may also increase metal availability; it has been reported that, following treatment with earthworms, the distribution of heavy metals in soil fractions was significantly changed (Devliegher and Verstraete, 1996; Cheng and Wong, 2002; Ma et al., 2002; Wen et al., 2006; Udovic and Lestan, 2007). Moreover, some earthworms can survive in heavy-metal-contaminated soils and can even accumulate metals, such as Cd, Cu, Zn and Pb, in their tissues (Morgan et al., 1989; Lanno et al., 2004; Yu et al., 2005).

Conventional agricultural practices can contaminate soil ecosystems with a large number of organic contaminants (OCs). Pesticides are introduced into soils through direct application as pest control chemicals and as seed coatings. Pesticides, pharmaceuticals (PhCs) and personal care products are introduced into soils by way of contaminated fertilizing materials, such as biosolids from municipal wastewater treatment plants (Mompelat et al., 2009; Tadeo et al., 2010) and farm animal wastes (Christian et al., 2003).

Agricultural soils are thus contaminated by OCs from a wide array of chemical classes present at very low concentrations (Sanchez- Brunete et al., 2004; Wu et al., 2010). The effects of OCs on soil biota have been documented. However, experiments are often carried out under conditions that are not representative of environmental conditions, i.e., single chemicals at high concentration (Yasmin and D'Souza, 2010). An increasing body of literature also highlights the importance of considering long-term exposure to low concentrations, as opposed to acute toxicity tests, when addressing the impact of OCs on soil biota (Eggen et al., 2004; Nash et al., 2004).

1,1,1-Trichloro-2,2′bis(p-chlorophenyl) ethane (DDT) was the first widely used synthetic pesticide and was liberally applied around the world for decades due to its high efficiency and low cost. Their inadvertent use and strong tendency to bioaccumulate has caused considerable harm to ecosystems, and its use was prohibited beginning worldwide in 1972 [1]. However, because of their extreme persistence, exposure to DDT remains widespread [2–4]. Inputs to ecosystems continue, both by illegal use and the continuing production and application in some countries such as India and in Africa as a very effective antimalarial. DDT even predominate over all organic chemicals among all matrices, especially in soils [5]; current levels in some biotic tissues can be comparable to the high levels found 30 years ago [6]. So, the hazard of DDT in the ecosystem is not negligible.

Assessing risk from DDT-contaminated soils is particularly challenging since a mixture of chemicals including pesticides, industrial products and heavy metals is generally present and the bioavailability varies with the soil characteristics [7]. The risk of DDT in contaminated soils could be assessed more quantitatively by identifying the responses of biomarkers to DDT in both a simplified simulated ecosystem and the complex soils themselves.

Earthworms play a key role in biogeochemical processes in soil ecosystems. Biomarkers for assessing the toxicity of chemicals on earthworms such as the survival, growth and reproduction are reliable, however, a great portion of these have been tested in simplified paper contact or artificial soils [8–13] and may lack ecological significance; so that it is essential to conduct tests with contaminated natural soils to allow the laboratory data to be interpreted and extrapolated to the contaminated field.

Organisms have defense mechanisms to protect the cell from the toxin or to defend it against adverse effects. Biochemical response capacities of defense system enzymes such as biotransformation system phase II enzyme glutathione-S-transferase (GST), the antioxidative enzymes such as catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD) provide results that are sensitive, informative, reproducible and can indicate potential toxicity in organisms such as fish, bivalves, aquatic worms, African common toad, mussels exposed to toxicant [14–17]. Some evidences indicate that the induction of the CAT activity is considered valid biomarkers of ambient oxidative pollutants in earthworm toxicology.

Sewage sludge can be defined as the solid or semi-solid residue left over after the treatment of wastewater. In literature it can be defined as by-product, yet it shall be treated as a waste in the process of wastewater treatment. Sewage sludge may be used as a source of energy (anaerobic digestion, thermal treatment), treated and used on land as a fertilizer and soil conditioner, or may even be used as a source to extract valuable compounds (phosphorous recovery). A significant number of wastewater treatment plants (WWTPs) compost dewatered sewage sludge under aerobic conditions with green wastes or other bulking agents or dry it in heat drying facilities up to 95% dry mass for use as fertilizer or fuel.

Hence the major question is what kind of contaminants can be found nowadays in sewage sludge? There are several papers focusing on new serious threats to human health and ecosystem occurring in sewage sludge e both chemicals (polyaromatic hydrocarbons (PAH), hydrocarbons; polychlorinated biphenyl (PCB), Perfluorinated Surfactants (PFCs), Personal Care Products (PCPs), Pharmaceuticals (PhCs), Benzotriazoles) and biologicals (Legionella, Yersinia, Escherichia coli O157:H7). However only some countries, e.g., Sweden initiated a program to systematically sample, analyze and bank sewage sludge (Olofsson et al., 2012).

In modern agriculture, cultivated fields are manipulated agroecosystems in which high yields are achieved through conventional management strategies based on the use of pesticides and fertilizers besides mechanical treatment. The repeated application of pesticides has led to chronic contaminations of cropped soils and thereby of their biota such as earthworms (Lumbricidae) (Lee,1985; Redondo et al., 1994; Luchini et al., 2000; Gevao et al., 2001; Al Mughrabi and Qrunfleh, 2002).

The persistence of several pesticides, such as the herbicide atrazine (Solomon et al., 1996; Giddings et al., 2005), raises environmental concern not only about residual contamination in cropped soils and their leaching potential to water bodies, but also about adverse effects on the soil biota. Earthworms often represent the largest soil biota biomass and are commonly entitled "ecosystem engineers". They contribute to pedogenesis and influence many key parameters of the soil, such as nutrient availability and turnover, soil porosity, and microbial activity (Jones et al., 1997; Binet et al., 1998; Monard et al., 2008; Bottinelli et al., 2010).

Earthworms' biodiversity however is reduced in intensively cultivated fields (Smith et al., 2008), with the use of pesticides evidenced as one of the responsible factors in laboratory toxicity studies (Springett and Gray, 1992; Yasmin and D'Souza, 2010). Consequently, a reduction in pesticide input could increase earthworm populations again (Pelosi et al., 2013a). Despite the hazards of these chemicals on earthworms, some species persist in intensively cultivated fields, in particular endogeic species such as Aporrectodea and Allolobophora spp (Jordan et al., 2004; Smith et al., 2008; Pelosi et al., 2013a).

This strongly suggests that long-term impacts of pesticide residues on some earthworm species are likely to induce adaptation processes. Adaptation processes have been described in metal exposed earthworms Dendrobaena octaedra, facilitated by a higher expression of metallothioneins (Fisker et al., 2013). Those adaptation processes may include physiological changes including increased detoxification activity and energy allocation, and behavioural changes, e.g., avoidance reactions, yet they are poorly understood. This study focuses on the two endogeic earthworms Aporrectodea caliginous and Allolobophora chlorotic, which are common species that persist in agricultural impacted soils, hence environmentally relevant for studies concerning adaptation mechanisms.

Earthworms' exposure to xenobiotics including pesticides can occur directly via uptake from soil interstitial water (porewater) through the outer skin and from ingestion of contaminated soil particles through gut epidermis (Belfroid et al., 1993, 1996). Exposure to pesticides then may induce biotransformation and detoxification mechanisms in earthworms, like in other organisms, to alter or scavenge them, aiding excretion (Rodríguez-Castellanos and Sanchez-Hernandez, 2007). Both, a constitutively higher expressed (due to selection over multiple generations) or a faster inducible detoxification mechanism, such as cytochrome P450 monooxygenases, esterase's or glutathione-S-transferases may facilitate coping with pesticides. These mechanisms have allowed multiple tolerance against pesticides not only in target, but also in non-target organisms (Field and Foster, 2002; Penilla et al., 2006; Huang and Han, 2007; Brausch and Smith, 2009).

The metabolism involved in increased detoxification is costly in terms of energy (Medina et al., 2007; Fisker et al., 2011). As a result, reductions in annelids' energy resources have been associated with detoxification of pollutants: in Dendrobaena octaedra, glycogen storage was depleted by internal regulation of metals like Al or Ni (Holmstrup et al., 2011). Lipids, carbohydrates and consequently growth and fecundity were markedly reduced in metal-resistant Nereis diversicolor (Pook et al., 2009; Holmstrup et al., 2011). Aim of this study was to assess the capacity of earthworms to adapt to and face multiresidue soil contamination, which is a key issue to predict ecosystem sustainability and resilience.

Perfluorooctane sulfonate (PFOS) has been widely used in consumer and industrial products, including food packaging, insecticides, protective coatings for fabrics and carpets, paper coatings, paints, cosmetics and fire-fighting foams (Moody et al.,2002; Noorlander et al., 2011). As a result, a pervasive presence of PFOS in food, drinking water and natural waters has been detected (Noorlander et al., 2011; Kannan et al., 2005; Lechner and Knapp, 2011; Skutlarek et al., 2006; Hansen et al., 2002; Taniyasu et al., 2004; Simcik and Dorweiler, 2005; So et al., 2007). PFOS is not readily biologically degradable and is often released in industrial wastewater directly into the aquatic environment or indirectly via canalization and sewage treatment plants. PFOS is emitted from wastewater treatment plants partially in the effluent and is partially adsorbed on sewage sludge. The use of sewage sludge as a fertilizer in agriculture can result in the contamination of soil with PFOS (Lechner and Knapp, 2011; Sinclair and Kannan, 2006).

Due to the persistent and bio accumulative nature of PFOS, its toxicities to aquatic organisms, animals and cells have attracted much attention. PFOS exposure in aquatic organisms and mammals has been shown to cause adverse effects in many systems, including hepatotoxicity, immunotoxicity, reproductive and developmental toxicity, neurotoxicity and increased tumorigenic potential of the liver, pancreas and breast (Harada et al., 2005; Cui et al., 2009).

Based on the 72-h oral LD50 of this compound on honeybees, PFOS was classified as "highly toxic" by the International Commission for Bee Botany. However, very few studies have focused on the potential harm of PFOS to terrestrial ecotoxicity (Zhao et al., 2011), despite the high levels of PFOS found in agricultural soils (Renner, 2009). The bioavailability and toxicity of contaminants varies among different soils and knowledge of this variance is essential to develop soil environmental quality guidelines (Yan et al., 2011). Therefore, to develop a comprehensive toxicity profile for PFOS, the toxicity of PFOS-contaminated soils should be assessed with various bioassays.

Organisms such as fish, snails and plants have been employed as bio monitors (Dorts et al., 2011; Li, 2009). A study showed that the survival after 21 d EC50 for PFOS was lowest for onions and highest for soybeans in a test of seven species of plants. Although this approach is useful and promising, it is somewhat limited because it is based on a specific combination of living organisms with certain substances (Hirano and Tamae, 2011).

Earthworms, the most prevalent animal species in soil, play an irreplaceable role in maintaining the ecological functions of soil. They have been used as important bio monitors to assess the ecological risks of toxic substances in the terrestrial environment (Fent, 2003; Xu et al., 2010).

The molecular, cellular and physiological levels of earthworms change significantly when they are under contamination stress. These reactions produce a specific biological signal, called a biomarker. The biomarkers can indicate the impact of pollutants on the individuals before the lethal effect becomes apparent and can also be used to monitor soil pollution and provide an eco-toxicological diagnosis as an early warning system (Spurgeon et al., 2003). However, few studies have focused on the potential toxicity of PFOS to earthworms in the soil (Xu et al., 2011). In this paper, Eisenia foetida (widely used in ecotoxicological tests of earthworms) was used as a model organism to study the effects of PFOS on the change in body-weight, antioxidant defense system and DNA damage.

Many genotoxic pollutants have been introduced into soils through anthropogenic pathways such as improper disposal of industrial wastes, wastewater irrigation (Chen et al., 2004), pesticide application and accidental leakage/spillage occurring during transport and storage of industrial materials. Particularly, mutagenic aromatic hydrocarbons and their derivatives (e.g., PAHs, nitroarenes, quinolines, thiophenes, etc.) from industrial and domestic combustion of fossil fuels and biomass contribute considerably to the pollution. These compounds can not only induce carcinogenesis, teratogenesis, and embryotoxicity in animals, but may also adversely affect ecosystem health (Mitchelmore and Chipman, 1998).

With increasing in industrial production and organic wastes release, more attention needs to be focused on the ecological risks of genotoxic organic pollutants in terms of their chronic long-term presence in the soil environment (White and Claxton, 2004). Assessment of the genotoxicity of compounds in terrestrial ecosystems presents a number of challenges, due to the diverse and complex nature of these environments. In vivo bioassays provide a more reliable assessment of toxicity because they are able to better preserve the natural soil conditions, allowing integration of a broad range of factors that contribute to the overall toxic effects (Alexander, 2000; Donnelly et al., 2004).

For contaminants whose principal effects are on the genetic functioning of organisms, quantification of the genetic damage in terms of the number of DNA strand breaks (DSBs) is a promising approach. The comet assay is capable of examining DSBs in individual eukaryotic cells after in vivo or in vitro exposure and is considered to be a sensitive biomarker for identification and quantification of genotoxicity (Faust et al., 2004). The consequences of exposure and metabolism of carcinogens can be assessed by DNA damage as revealed by the level of DNA bulky adducts or by the number of DSBs (Farmer et al., 2003; Husgafvel-Pursiainen, 2004).

The diversity of earthworms as well abundance has decreased as a result of human activities. Earthworms are sensitive to change in environment and therefore, their population in polluted soil is lower than in undisturbed areas. Many scientists have worked on it (Curry *et al.*, 2002).

Paoletti *et a.*, (1998) studied earth-worms population in 72 various ecosystems having vineyards and more other three types of orchards which included kiwi apple and peach. The effect of toxic metals was observed with other soil factors. The amount of copper was observed higher in vineyards. Both growth and abundance negatively associated with the copper accumulation. These factors decrease with the increases of copper. *Aporrectodea caliginous* was observed negatively correlated with the increasing amount of all metals. But *decrease chlorotic*. Was observed badly correlated with only by copper up take. Lumbricus rubellus and lumbricus were almost absent in tilled orchards but it seems that they were not much decrease by copper up take. The survival rate and growth both were badly affected by the copper up take.

Surgeon *et al*., (1999) studied the 56 days experiment by using the earthworm *Eisenia fetida.* Earthworm was treated with the toxic metals. For the experiment artificial soil prepared with the OCED recommended protocol. Morphological features were tested which include the biomass development body weight and death rates. No observed effect concentrations were also estimated mainly for copper and cadmium. The weights of earthworm showed negative behavior in all cases also in control conditions during the treatment, because the deficiency of proper food in the OECD which was used in soil medium.

According to Edwards (2002) that a great number of pollutants comes in the clean soil through human activities such as use of fertilizers industrial activities and their effluents which flow away without proper management. These are harming the nature severely. Ecologically these impacts are harming the survival of individual their biomass and development rate, also harming badly at the community level and also at ecosystem level, badly changing the food chains, food webs and natural cycles. There effects also observed at the landscape level. A large impairment occurred in terrestrial environment.

Lev. S. M., et al (2010) Earthworms have the potential to act as trophic links for pollutants that accumulate in urban soils. However, many pollutants may act as micronutrients at low concentrations and toxins at higher concentration. When pollutants are also micronutrients, bioaccumulations may initially increase trophic transfer as pollutant concentration increase, but at higher levels toxic effects may limit population size and the potential for trophic transfer. We found support for this model among earthworms exposed to a range of soil Zn levels. Worms showed increasing bioaccumulation of Zn with increasing Zn soil concentrations, but at higher Zn levels worm growth rates decreased.

Rongquan. Z., et al (2008) To provide basic toxicity data for formulating risk characterization benchmarks, the effects of lead on survival, locomotion, and sperm morphology were investigated in the Asian earthworm *Pheretima guillelmi*. The LC50 of *P. guillelmi* for 7 and 14 d were 4285_339 mg/kg and 3207_248 mg/kg, which shows *P. guillelmi* can tolerate a higher concentration of lead nitrate. The average weight of the surviving earthworms decreased at concentration of 2800 mg Pb/kg soil, and the locomotor ability of earthworms exposed to a range of soil Pb concentrations showed a general decrease with increasing Pb concentrations. We also presented data depicting the sperm morphology of earthworms, which shows potential as a sensitive biomarker for measuring the of heavy metal on reproduction.

Adlouni. C. E., et al (1994) Our interest in detecting genotoxic exposure in earthworms led us to isolate high quality DNA from the Eiseniafetida species. For that, we compared a modification of the conventional phenol-chloroform extraction procedure, usually referred to as the Maniatis procedure, to two commercially available kits reportedly eliminating multiple partitions in phenol and chloroform, namely the Qiagen and Nucleon protocols. From the 260 nm optical density values, the commercial kits extract hinted toward higher DNA recovery with those procedures. However, the 260/280 nm ratios indicated that the quality of the DNA isolated with the, modified Maniatis procedure was purer than that isolated with the commercial kits, the latter being most probably contaminated by proteins and/or RNA. The Maniatis procedure was slightly modified by the introduction of a potassium acetate step for protein precipitation and by shortening the proteinase K treatment from 12-18 h to only 2 h. The higher quality of the DNA isolated by phenol-chloroform extraction was confirmed by quantification with the fluorescent 3,5-diaminobenzoic acid assay. Preliminary results suggest that the modified Maniatis procedure herein described is not only applicable for DNA adducts studies using 32P-postlabelling techniques but is also suitable for DNA extraction from other earthworm species such as Lumbricus Terrestre's. (Mol Cell Beachem 142: 19-23, 1995).

Mostafaii. G. R., et al (2015) The use of earthworms to bioremediate soil results in decreasing the pollutant concentration through a bioaccumulation mechanism of the contaminants in the earthworm's body. The present work is an empirical study that was carried out on soils contaminated with chromium and cadmium. Organic matter in the amount of 5% and 9% of soil weight was added. Chromium and cadmium concentrations in soil and in the body of worms were measured at two time periods of 21 and 42 days. According to the results, increasing from 5% to 9% the organic material of the soil contaminated with chromium at the initial concentration of 0.06 mg/g, the removal efficiency decreased by 5%. In 0.1 mg/g concentration the bioremediation efficiency decreased by 20%, showing that the earthworms probably have more tendency to consume the organic material and low tendency for consuming the soil contaminated by metal. Results showed that, considering the increased mortality of worms in the soil at a concentration of 0.08 mg/g of chromium, using this method is not recommended. For cadmium we require more study, though we can say that the organic material had no influence on the bioremediation of the soil.

Morales. M. E., et al (2015) Maintenance of genomic integrity is critical for cellular homeostasis and survival. The active transposable elements (TEs) composed primarily of three mobile element lineages LINE-1, Alu, and SVA comprise approximately 30% of the mass of the human genome. For the past 2 decades, studies have shown that TEs significantly contribute to genetic instability and that TE-caused damages are associated with genetic diseases and cancer. Different environmental exposures, including several heavy metals, influence how TEs interact with its host genome increasing their negative impact. This minireview provides some basic knowledge on TEs, their contribution to disease, and an overview of the current knowledge on how heavy metals influence TE-mediated damage.

Zheng. K., et al (2013) The aim of this study was to evaluate the toxicological responses of earthworm (Eisenia fetida) induced by field-contaminated, metal-polluted soils. Biochemical responses and DNA damage of earthworm exposed to two multi-metal-contaminated soils in a steel industry park and a natural reference soil in Zijin Mountain for 2, 7, 14, and 28 days were studied. Results showed that three enzyme activities, including superoxide dismutase (SOD), acetylcholinesterase (AChE), and cellulase, in earthworm in metal-contaminated soils were significantly different from those of the reference soil. Cellulase and AChE were more sensitive than SOD to soil contamination. The Olive tail moment of the comet assay after 2-day exposure increased 56.5 and 552.0 % in two contaminated soils, respectively, compared to the reference soil. Our findings show that cellulase and DNA damage levels can be used as potential biomarkers for exposure of earthworm to metal-polluted soils.

Lin. D., et al (2014) Triclosan (TCS) is released into the terrestrial environment via the application of sewage sludge and reclaimed water to agricultural land. More attention has been paid to its effect on non-target soil organisms. In the present study, chronic toxic effects of TCS on earthworms at a wide range of concentrations were investigated. The reproduction, DNA damage, and expression levels of heat shock protein (Hsp70) gene of earthworms were studied as toxicity endpoints. The results showed that the reproduction of earthworms was significantly reduced (p\0.05) after exposure to the concentrations ranges from 50 to 300 mg kg-1, with a half-maximal effective concentration (EC50) of 142.11 mg kg-1. DNA damage, detected by the comet assay, was observed and there was a clear significant (R2 = 0.941) relationship between TCS concentrations and DNA damage, with the EC50 value of 8.85 mg kg-1. The expression levels of Hsp70 gene of earthworms were found to be up-regulated under the experimental conditions. The expression level of hsp70 gene increased, up to about 2.28 folds that in the control at 50 mg kg-1. The EC50 value based on the Hsp70 biomarker was 1.79 mg kg-1. Thus, among the three toxicity endpoints, the Hsp70 gene was more sensitive to TCS in soil.

Lahr. J., et al (2008) said that in traditional environmental risk assessment for soils, interactions between biota, contaminants and soil functioning are seldom taken into account. Also, single species toxicity tests are conducted with a fixed number of test animals. The objective of this study was to investigate effects of zinc (0–620mg Zn kg1 dry soil) on soil ecosystem processes at different densities of the earthworm Lumbricus rubellus. Experiments were conducted using 1-liter microcosms equipped with respirometers. The presence of L. rubellus stimulated relevant soil processes and parameters: litter fragmentation, leaf litter mass loss from the soil surface, soil organic matter (SOM) content and soil respiration. Zinc was not lethal to L. rubellus, but negatively impacted soil respiration at the highest concentrations. Litter mass loss from the soil surface was also decreased by zinc and there was a significant interaction with worm density. The results of the study demonstrate that the impact of zinc on soil processes depends on the presence and densities of key soil organisms such as earthworms that influence decomposition and SOM content. The outcome of this research can be used to make existing models for site-specific risk assessment more ecologically relevant, linking effects of contaminants on soil fauna populations with effects on ecosystem functioning.

Ramadass. K., et al (2015) The nontarget effects of fresh and used motor oil were studied in a soil test system involving such criteria as earthworm survival, response of soil dehydrogenase and urease, and nitrification. When earthworms were exposed to motor oil-contaminated soil for 4 weeks, the observed median lethal concentrations (LC50) were 40.33 and 3.88 g kg1 soil for fresh and used oil, respectively. Only fresh motor oil application increased earthworms' body weight even at the higher dose of 19 g kg1 soil. Gas chromatography/mass spectrometry revealed that used motor oil contained more of aromatic hydrocarbons and heavy metals than fresh oil. This disparity in the chemical composition might be the factor responsible for the significant toxicity of used motor oil towards earthworms. Activities of soil dehydrogenase and urease were significantly enhanced in presence of both the motor oils, while there was a significant inhibition in nitrification by the used motor oil even at a low concentration of 0.2 g kg 1 soil. This study clearly demonstrated that earthworm survival and nitrification could serve as suitable indices to assess motor oil pollution in soil.

Li. M., et al (2008) There are rising concerns about the hazardous effects of cadmium (Cd) and lead (Pb) on the environment in China. Biochemical and comet assays were conducted on the earthworm Eisenia fetida, a suitable bio-indicator organism for evaluating soil pollution after exposure to two heavy metals, Cd and Pb. Protein Content increased at low Cd concentrations ($p < 0.05$) and decreased at the highest Concentration of 10 mg kg¡1, compared to control ($p < 0.05$). Pb showed an inhibitory effect on protein content at low concentrations but demonstrated no significant effect at higher concentrations. There were no significant Differences between control and treated groups at the doses of 1 and 10 mg kg¡1 Cd while at a dose of 0.1 mg kg¡1 Cd the cellulose activity was significantly increased compared to control. Cellulase Activities of Pb-treated E. fetida increased in a dose dependent fashion. Results of the comet assay indicated Toxicant induced DNA damage. Cd exposure caused significant differences between control and treatment groups (ANOVA, $p < 0.05$, $p < 0.01$) and a positive dose-response profile. As for Pb treatment, there were no significant differences between the groups treated with 50 and 500 mg kg¡1 of Pb and the control. Results showed that DNA damage from Cd was more serious than that from Pb. And this indicated that the earthworm was more sensitive to the effects of Cd.

Mirmonsef. H., et al (2017) studied Contaminated soil is a problem throughout the industrialized world, and a significant proportion of these sites are polluted with heavy metals such as copper. Ecological risk assessment of contaminated sites requires ecotoxicological studies with spiked soils as well as in-situ ecological observations. Here, we report laboratory and field assessment of copper toxicity for earthworms at a Danish site (Hygum) exclusively contaminated with an increasing gradient in copper from background to highly toxic levels (> 1000 mg kg−1 dry soil). More specifically, we report effects on field populations, body contents of copper, hatching of earthworm cocoons and reproduction of the common species Aporrectodea tuberculate. Abundance of earthworms and cocoons decreased significantly from about 400–150 m−2 along the gradient as the soil copper concentration increased from ca. 50 to ca. 1000 mg kg−1. At lower concentrations, the population was dominated by endogeic species, whereas at high concentrations the population was dominated by epigenic species. At high copper contents the internal concentration of copper was in the range 100–160 mg kg−1 dry tissue. Despite the high internal copper contents, hatchability of field collected cocoons was not impaired in any species. The EC50 reproduction value of A. tuberculate was about 220 mg copper kg−1 dry soil in the first two exposure periods, but nearly doubled in the third period suggesting that an acclimation response had occurred. Also in the laboratory reproduction test, cocoon hatchability was not reduced, but rather slightly stimulated by copper. Based on these results we discuss the possibility that acute exposure in laboratory experiments is more detrimental than exposure in a field situation, perhaps because increased tolerance may be acquired through natural selection and genetic adaptation through increased use of defense mechanisms such as metallothioneins. Further, we discuss that the rather high tissue copper level of earthworms from the Hygum site may have smaller effects in these free ranging worms than it would have in acute-exposure laboratory tests because the copper is more efficiently sequestered and detoxified in the field situation where populations have been exposed for many generations.

Qiao. M., et al. (2007) In this study, DNA damage to earthworms (Eisenia fetida) after in vivo exposure to contaminated soils was measured by detecting DNA strand breakages (DSBs) and causality was analyzed through fractionation-based bioassays. A non-linear dose-response relationship existed between DNA damage and total soil PAHs levels. DNA damage, measured with the comet assay, and its repair process, were observed. To identify the chemical causality, an in vitro comet assay using coelomocytes was subsequently performed on the fractionated organic extracts from soils. The results showed that the PAHs in the soils were responsible for the exerting genotoxic effects on earthworms. When normalized to benzo(a)pyrene toxic equivalent (TEQBaP), the saturation dose in the dose-response curve was about 10 ng TEQBaP g_1 soil (dw).

Xu. D. et al (2012) The use of earthworms as a sublethal endpoint has significantly contributed to the ecological risk assessment of contaminated soils. Few studies have focused on the potential toxicity of PFOS to earthworms in the soil. In this work, artificial soils were tested, and contact filter paper studies were used. The results showed that earthworm growth was generally inhibited. The antioxidant activities of the enzyme's superoxide dismutase, peroxidase, catalase and glutathione peroxidase were initially activated and then inhibited. Reduced glutathione content was observed, and malondialdehyde content was elevated over the duration of the exposure. These results suggested that PFOS induced oxidative stress in earthworms. In addition, the values of olive tail moment, tail DNA% and tail length using SCGE showed similar frequency distributions and increased with increases in the PFOS concentration. These results suggest that all concentrations of PFOS cause DNA damage.

Rorat. A., et al (2013) Dendrobaena veneta is an earthworm species capable of consuming a wide range of organic wastes which may be used as a field indicator of municipal sewage sludge applied to land. The aim of the present 8-week laboratory experiment was to check viability, reproduction and the immune system of D. veneta maintained in soil without food additions (control 0s group) or in soil amended with 25% or 50% municipal sewage sludge (25s and 50s groups, respectively). Reproduction and immunity are important physiological functions whose detailed study can provide information on the effects of pollutants. After the 8-week exposure period, earthworm mortality (2 out 20 individuals) was recorded only in the 50s group. Reproduction was high in the 25s group (44 cocoons and 41 juveniles) whereas reproduction was almost completely inhibited both in the food-deprived control 0s group (1 cocoon, 3 juveniles) and in the 50s group containing a high amount of sludge (2 cocoons). Significantly increased numbers of non-invasively extruded coelomocytes were recorded 3 weeks after the start of the experiment in the 50s group, but they dropped to the food-deprived control level by the end of 8 weeks likely due to exhaustion of the immune system coping with sludge-derived microbes and/or toxins. In contrast, numbers of coelomocytes in the 25s group increased gradually reaching the maximum at the end of the experiments. In conclusion, high amounts of municipal sewage sludge are detrimental to worms, inhibiting reproduction and inflicting mortality. A moderate amount of municipal sewage sludge provides a good source of nutrients for D. veneta, supporting their growth and reproduction for at least 8 weeks. Immunological parameters might serve as useful indicators of earthworm exposure to sewage sludge.

Campos. J. R., et al (2014) Earthworms can accelerate the removal of contaminants from soil. Earthworms change the physical and chemical properties of soil by mixing it with organic material and through their burrowing they improve aeration and render contaminants available for microorganisms. The presence of earthworms in contaminated soil indicate that they can survive a wide range of different organic contaminants, such as pesticides, herbicides, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and crude oil, at least when concentrations of the contaminant are not too high. The improvement of the soil due to their activity and the microorganisms in their digestive track can contribute to the accelerated removal of contaminants from soil, but sometimes their casts adsorb the pollutant so that its dissipation is delayed. There are limits, however, on how earthworms can be used to remediate soil, which will be discussed in this review.

Wang. K., et al (2015) Imidacloprid is a well-known pesticide and it is timely to evaluate its toxicity to earthworms (Eisenia fetida). In the present study, the effect of imidacloprid on reproduction, growth, acetylcholinesterase (AChE) and DNA damage in earthworms was assessed using an artificial soil medium. The median lethal concentration (LC50) and the median number of hatched cocoons (EC50) of imidacloprid to earthworms was 3.05 and 0.92 mg/kg respectively, the lowest observed effect concentration of imidacloprid about hatchability, growth, AChE activity and DNA damage was 0.02, 0.5, 0.1 and 0.5 mg/kg, respectively.

Hiranob. T., et al (2009) studied Earthworms can be used as a bio-indicator of metal contamination in soil, Earlier reports claimed the bioaccumulation of heavy metals in earthworm tissues, while the metal-induced mutagenicity reared in contaminated soils for long duration. But we examined the metal-induced mutagenicity in earthworms reared in metal containing culture beddings. In this experiment we observed the generation of 8-oxoguanine (8-oxo-Gua) in earthworms exposed to cadmium and nickel in soil. 8-oxo-Gua is a major premutagenic form of oxidative DNA damage that induces GC-to-TA point mutations, leading to carcinogenesis.

Adlouni. C. E. et al (1994) Our interest in detecting genotoxic exposure in earthworms led us to isolate high quality DNA from the *Eiseniafetida* species. For that, we compared a modification of the conventional phenol-chloroform extraction procedure, usually referred to as the Maniatis procedure, to two commercially available kits reportedly eliminating multiple partitions in phenol and chloroform, namely the Qiagen and Nucleon protocols. From the 260 nm optical density values, the commercial kits extract hinted toward higher DNA recovery with those procedures. However, the 260/280 nm ratios indicated that the quality of the DNA isolated with the, modified Maniatis procedure was purer than that isolated with the commercial kits, the latter being most probably contaminated by proteins and/or RNA. The Maniatis procedure was slightly modified by the introduction of a potassium acetate step for protein precipitation and by shortening the proteinase K treatment from 12-18 h to only 2 h. The higher quality of the DNA isolated by phenol-chloroform extraction was confirmed by quantification with the fluorescent 3,5-diaminobenzoic acid assay.

Preliminary results suggest that the modified Maniatis procedure herein described is not only applicable for DNA adducts studies using 32P-postlabelling techniques but is also suitable for DNA extraction from other earthworm species such as *Lumbricus Terrestre's.*

Raja. I. A., et al (2017) The present study was designed to elucidate the effect of soil pollutants on diversity and abundance of earthworms in a winter vegetable crop (cauliflower). Collection was done through November to January and samples were anesthetized in alcohol, preserved in 10% formalin solution. The soil and water analysis were performed by atomic absorption spectrophotometry. The Shannon-Weiner Diversity Index (H′) of sampling site (Chack 204) was 1.803, evenness was 0.302 and dominance was 0.831. A significant difference of abundance was observed (t=12.62, p=0.0066 at $p<0.05$). The pH of sewage water was recorded as 5.833 and sewage water irrigated soil was 6.28, whereas pH of canal water was 7.66 and canal water irrigated soil was 7.88. A significant difference of physico-chemical characteristics of sewage water and canal water was observed as following pH, p=0.0043), DO (p=0.0112), TDS (p=0.043), TSS (p=0.015), turbidity (p=0.0028), Pb (t=11.4 p=0.0076), Zn (p=0.0192) except temperature and heavy metals including Co, Cd, Cr, Mg and Ca. Similarly, significant difference of physico-chemical characteristics of sewage water irrigated soil and canal water irrigated soil was recorded as for Pb (p=0.0014), Zn (p=0.0001), Cr (p=0.0366), Mg (p=0.001) and Ca (p=0.0003) except temperature, Co and Cd. Significantly positive correlation occurred between earthworms' abundance and temperature at site 1 (p=0.0246) and at sampling site 2 (p=0.0156). It is conceivable from the present data that earthworm population proves to be better and beneficial for non-polluted soil. Environmental factor, such as temperature is very important for earthworm management. The abundance of earthworms was low in cold months of winter. The harsh condition of winter seems to be limiting factor for abundance of earthworm. Acidic pH and high concentration of heavy metals badly affect the diversity and abundance of earthworms.

Jatwani. C., et al (2015) Heavy metals act as toxicants to soil and crops at elevated level. Earthworms help in bioremediation process they remove heavy metal from the soil and accumulate them in their body tissues especially yellow cells. Depending upon the concentrations of heavy metals the body of earthworm get affected. An experiment was conducted to check the effect of Hg and Co on Eiseniafetida. Nine concentrations of heavy metals were sprayed i.e., Hg @0.02,0.04,0.06 ppm, Co @0.02,0.04,0.06 ppm and Hg+Co @0.01 ,0.02,0.03 ppm for two months. Bio-molecular parameters were calculated at an interval of 15 days for 2 months and it was concluded that Hg at 0.006 ppm affect the biomolecular concentration of body of earthworm than Co and combination of both. The carbohydrates level has been decreased from 17.65% in 0.06 Hg, 17.05% in 0.06 Co and 17.32% in combination of 0.03Hg+0.03Co. At 0.06ppm of Hg lipid content decreased by 41.25%, 23.26% at 0.06ppm Co and 32.44% at 0.03Hg +0.03 Co whereas protein concentration was decreased by 42.47% at 0.06ppm Hg, 35.27% at 0.06ppm Co and 38.07% at 0.03Hg+0.03Co. So, it was concluded that Hg is more toxic to earthworm not only bio-molecular parameter it affects cocoon production, coelomocytes, body weight, length also.

Uwizeyimana. H., et al (2017) Earthworms are the key soil organisms, contribute to many positive ecological services that could be degraded by pesticides and other soil pollutants such as heavy metals. Chemicals usually occur as mixtures in the environmental systems which can lead synergistic effects. The assessment and characterization of soil pollutants that effects risks are very difficult due to the complexity of soil matrix, poor understanding about the fate and effects of chemical combinations like pesticide and metal mixtures in terrestrial systems, and scarcity of toxicological data on mixtures of pollutants. In this review we summarized the current studies on individual and joint effects of pesticides and metals on earthworms and indicate the mixture that cause the synergistic interactions. The review explores the methods and models used previously to evaluate the toxicity of chemical mixtures, and suggests the perspective approaches for a better knowledge of combine effects as well as research methods The summarized report indicates that pesticide and metal mixtures at all organization levels affect the earthworms negatively. Whereas, the combined pollution generated by mixtures of pesticides and metal ions could induce the DNA damage, disruption in enzyme activities, reduction in individual survival, production and growth rate, change in individual behavior such as feeding rate, and decrease in the total earthworm community biomass and density. Among the pesticides organophosphates were identified the most toxic pesticides causing the synergistic effects. The findings indicate the scarcity of toxicological data concerning the assessment of pesticide and metal mixtures at genome level; while the mechanisms causing synergism were still not sufficiently explored.

Song. Y., et al (2009) To evaluate atrazine (2-chloro-4-ethylamino-6-isopropylamino-1, 3, 5-triazine) ecotoxicology in soil, the effect of atrazine on the activity of antioxidative enzymes (superoxide dismutase, SOD; catalase, CAT; and guaiacol peroxidase, POD) and DNA damage induced by atrazine were investigated in earthworms. Atrazine was added to artificial soil at rates of 0, 2.5, 5 and 10 mg per kg of soil. Earthworm tissues exposed to each treatment were collected on the 7th, 14th, 21st, and 28th day of the treatment. Compared to the controls, the CAT activity was stimulated at 2.5 mg kg_1 treatment except on the 14th day, and inhibited at 5, 10 mg kg_1 atrazine except 5 mg kg_1 on the 28th day and 10 mg kg_1 on the 21st day; the overall SOD activity was inhibited, while the POD activities were stimulated by all atrazine concentrations in 28 days. The olive tail moments of single-cell gel electrophoresis of coelomocytes, as an indication of DNA damage, were increased after treatment with different doses of atrazine on the 7th, 14th, 21st, and 28th day, and significant differences were found compared to the controls. In conclusion, atrazine induces oxidative stress and DNA damage on earthworms, and the adverse effects may be the important mechanisms of its toxicity to earthworms.

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