

# Role of earthworms as potent bioindicator of soil pollution: A Review

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## ABSTRACT

The use of pesticides and insecticides has increased globally since the last few decades, which directly or indirectly influence soil structure and vegetation. Heavy metals like Nickel, Cadmium, Zinc and Lead released from industries, automobiles and other sources also acts as the major contributors of soil pollution. Crops cultivated in such soil are harmful for consumption and can result in severe health issues. So, it is necessary to have knowledge about the levels of toxicity present in the soil where farming has to be done. Earthworms are directly exposed to these toxicants. They show a wide range of changes in their behavioral, physiological and biochemical parameters in response to contaminated soil. This makes them a promising bioindicator for determining soil toxicity.

*Key words : Bioindicator, Earthworms, Heavy metals, Soil nematodes, Soil pollution*

## Introduction

Earthworms, often called 'Nature's Ploughman', are major inhabitants of the soil. They are present on almost all types of soil but they mostly prefer loose loamy soil which is rich in nitrogen and other organic matter. They are cosmopolitan in distribution but mostly found in the garden, grassland, field, dump yard and muddy banks of the water bodies. They are considered as a good representative of the soil fauna (Connor 1998). Earthworms are classified into three types on the basis of their niche: Epigeic-which dwell on the surface of the soil; Endogeic-which live on the topsoil layer and Anecic-which are subsoil dwellers and generally make deep burrows. They play a crucial role in agricultural practices by

modifying the structure and quality of the soil, promoting proper cycling of nutrients, maintaining the porosity, pH, aeration and water holding capacity of the soil (Kiyasudeen *et al.*, 2016; Boulin *et al.*, 2013). Hence, they are also referred to as 'ecosystem's engineers'. Since the last few decades there is a considerable increase in the use of insecticide, pesticide and chemical fertilizers in order to gain healthy and pest-free crop yield. Moreover, heavy metals like Cadmium (Cd), Nickel (Ni), Zinc (Zn) and Lead (Pb) released from industries and automobile emission also get accumulated in the nearby field and vegetation areas. These factors have negatively contributed to the increase in the soil toxicity by many folds which has endangered the life of floral, faunal and microscopic communities (Carolene *et al.*, 2001).

These polluted soils are not suitable for agriculture. These highly toxic chemicals enter into our food chain and finally reach the higher trophic level which creates immense problems of environmental quality as well as human health (Otitolaju *et al.*, 2009). These factors have led to an increase in awareness about sustainable agricultural practices (Eyhorn *et al.*, 2019; Reganold and Wachter, 2016). To maintain these practices, it is crucial to understand the negative effects of toxic chemicals on the soil and determine the level of toxicity of that soil where cultivation has to be done (Datta *et al.*, 2016).

Many chemical analyses are performed on the soil to determine its toxicity. But the best indicators are those organisms which are themselves exposed to these toxicants (Svendsen *et al.*, 2004).

One such bioindicator is earthworm. They absorb the soil moisture through their skin pores. So, their skin becomes an important route for the accumulation of toxic materials in their body. They also ingest the organic matter present in the soil and therefore their gut and alimentary surface are also exposed to contamination (Sanchez-Hernandez, 2006). They show various responses to soil contamination and therefore are used as tools for bioindicators of soil health (Carolene *et al.*, 2001). The proper study of their responses (behavioural, morphological and physiological etc.) against the soil contaminants would provide an insight about the conditions of its habitat soil. This would help to determine whether the soil is fit for cultivation or not.

### Types of Soil Pollutants

**PAH:** It stands for Polycyclic aromatic hydrocarbon. It contains only carbon and hydrogen atoms. The major source of accumulation of PAH comes from the coke processing industries and due to the extraction of shale oil (Pope *et al.*, 2000; Zhang *et al.*, 2006; Xiao *et al.*, 2014; Li *et al.*, 2020). They can cause cardiovascular diseases and several forms of cancer in humans.

**Industrial wastes :** Common industrial wastes which cause soil pollution include dioxins, polychlorinated biphenyls etc. Benzene and methylbenzene, which are a form of petroleum hydrocarbon, also act as major soil pollutants. They are carcinogenic in nature (Zhang *et al.*, 2020).

**Pesticides:** They include herbicides like triazines, carbamates etc. which are used to kill herbs and unwanted weeds (Qisse *et al.*, 2020), insecticides like

arsenic containing compounds, used for controlling insect pests and fungicides like mercury containing compounds used for killing fungi (Pérez-Mayán *et al.*, 2020; Sudoma *et al.*, 2021). They cause disorders of the central nervous system.

**Heavy metals:** They include copper, cadmium, zinc, arsenic etc. They are highly toxic to the environment and can cause severe health related issues.

**Microplastics:** Microplastics have a diameter of five millimetres (0.2 inch) or less. They are frequently discharged when water bottles, shopping bags, or other plastic objects break down. Plastic trash can enter terrestrial soils through a variety of routes, according to recent research. Hurley and Nizzetto distinguished three types of sources in this context: (I) fragmentation of existing plastic trash in the environment, (II) deposition and runoff from the surroundings, and (III) inputs from agricultural operations. Piehl *et al.* (2018) discovered 206 macroplastic pieces per hectare and 0.34 0.36 microplastic particles per kilogramme dry weight of soil in their study. Polyethylene is the most common polymer, followed by polystyrene and polypropylene in that order.

### Earthworms' Responses Against Soil Contaminants

#### Morphological changes

Earthworms which are exposed to heavy metals show symptoms like oozing out of coelomic fluid, swelling of posterior segments, curling, excessive secretion of mucus and clitellar bulging (Annapoorani, 2020).

#### Survivability

Abundance and survivability of earthworms can give crucial information about the soil and its fertility (Fründ *et al.*, 2010; Paoletti 1999; Pérès *et al.*, 2011). It has been noted that when concentration of copper exceeds 500 mg/gm and the organic matter remains less than 3.5% then the cocoon production and viability decreases (Avila *et al.*, 2009).

#### Behavioral Responses

Earthworms tend to move away from that part of the soil which is more toxic to that part of the soil which is comparatively less toxic. This behavioral response of earthworms could be studied by conducting an 'Avoidance Test'. It is evaluated on the

basis of abundance of earthworm on both the test and reference soil. If the ratio exceeds or is equal to 20%:80% in the test and reference soil respectively, then it indicates toxicity of the soil. This test is used for determining the toxicity of floodplain soil and can be performed on such fields where there is abundance of endergonic earthworm and where the main contaminant is copper (Fründ *et al.*, 2004).

Earthworms show changes in their burrowing activity, feeding and egestion behaviour if they are exposed to toxic soil. These behavioural responses could be studied by a test called 2D Terraria Test, also called Evan's boxes test. This test is performed in soil filled cuvettes (Evans, 1947). They allow us to visualise the activity of earthworms easily. So, they are used to study changes in burrowing patterns, feeding and egestion of earthworm (Evans, 1947; Schrader and Joschko, 1991; Schrader, 1993). Burrow length of *Aporrectodea nocturna* and *Aporrectodea icterica* decreases with increase in sublethal insecticide concentrations (Capwiz, 2003).

#### Cytological Response

Earthworm's lysosomes are sensitive to different organochlorines; 'lysosomal stability assessment test' is performed and the staining of cell lysosomes with the neutral red dye is seen and NRRT (Neutral red retention time) is calculated. It is used to determine copper (Cu) contamination (Maboeta *et al.*, 2003).

#### Genetic Response

Earthworms, namely *Lumbricus terrestris* and *Eisenia fetida* showed strand breakage and DNA adducts respectively on being exposed to dioxin and its derivative soil contaminated with organic pollutants and X rays (Saint-Denis *et al.*, 2000; Zhang *et al.*, 2000).

#### Enzymatic Responses

HSP (Heat Shock Protein) gets induced on earthworms on being exposed to contaminants. In *Lumbricus rubellus* HSP-60,70 and 90 get induced in those earthworms which live in metalliferous soils (Mario *et al.*, 1999). In *Lumbricus terrestris*, HSP-70 gets induced on being exposed to pentachlorophenol and heavy metals (Nadeau *et al.*, 2001). Transfer of earthworms from clean to metalliferous soil results in overexpression of HSP (Mario *et al.*, 1999). Activities of antioxidants like SOD (Superoxide Dismutase), CAT (Catalase) and GR (Glutathione reductase) can be used to evaluate the influence of contaminants on the antioxidants of earthworms. In

*Eisenia fetida*, exposure to Lead and Uranium can inhibit the activity of Catalase but they do not have any effect on SOD (Labrot, 1996). Levels of GST (Glutathione-S-Transferase) increases after being exposed to organic chlorides such as Aldrin, Endosulfan etc. (Hans *et al.*, 1993).

#### Role of Earthworms in Bioremediation of Toxic Soil

The earthworms not only act as bioindicator for soil pollution but its interaction with the soil microorganisms also aids in the bioremediation of the toxic soil. Bioremediation is defined as an approach to recover the land which is degraded or contaminated by pollutants (Marillo and Villaverde, 2017). The earthworms are known to effectively interact with the soil microorganisms like bacteria, fungi and actinomycetes (Brown and Doobie, 2004). This interaction is generally through their burrowing activity, casting and ingestion of soil along with the microorganisms. This behaviour aids in the improvement of soil structure and soil fertility (Edwards and Bohlen, 1996; Butt *et al.*, 1999; Haimi, 2000; Lowe and Butt, 2003; Butt *et al.*, 2004). The earthworms' movement through the soil enhances the distribution and transport of bacteria and also in the dispersal of soil inoculants (Daane *et al.*, 1997; Doobie *et al.*, 1994; Hampson and Coombes 1989; Hutchinson and Kannel 1956; Singer *et al.*, 1999; Stephens *et al.*, 1994; Thorpe *et al.*, 2003). They also secrete mucilaginous secretions which prime the soil and increase the nutrient availability (Wolters, 2000). Through these activities earthworm aids in bioremediation of the degraded soil and increases its fertility.

Nematodes are a major microorganism of the soil. Some of these nematodes are parasitic to the plant. They form gall like structures on the root nodes of the plant and retard their growth. Earthworms accidentally or selectively ingest these nematodes along with organic matter and thus contribute to their biocontrol (Hyvöen *et al.*, 1994).

#### Discussion

Earthworms belong to soil macrofauna. They perform various functions which aids in the improvement of the soil quality and fertility. They can increase the fertility of the soil by forming a layer of organic matter in the topsoil (Georgescu *et al.*, 2004). They incorporate and break down the organic residues in the soil by their burrowing activity. So, they

are considered as a very important soil organism.

In recent years, there is an increase in the use of pesticides and insecticides which has led to soil contamination. The 'German National Academy of Sciences' released a paper in 2018 called 'The silent spring' on the need for sustainable plant protection in which it was concluded that "the methods and strategies implemented at present in order to curb the adverse effects of insecticides and pesticides are not effective enough" (Schäfer *et al.*, 2018).

Earthworm, being a very sensitive bioindicator can help to determine the level of toxicity in the soil. They show different changes on being exposed to different types of contaminants. This can help in determining the type of the contaminant.

Earthworms also interact effectively with other soil microorganisms. They help in transport and distribution of bacteria from one part of the soil to other through their movement. They ingest the soil nematodes along with the soil and act as their biocontrol agents. Earthworms have also been shown to positively influence the rate of growth of certain plants by increasing the soil aeration and fertility. Achieving proper knowledge about earthworms, its habits and its response to soil pollution would provide better opportunities for sustainable agriculture.

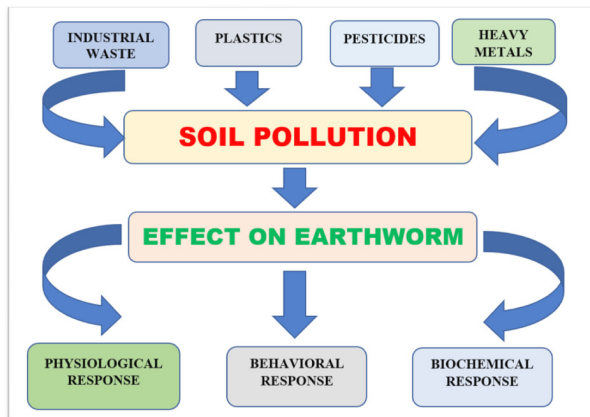


Fig. 1. Effects of soil pollution on earthworm

## Conclusion

Earthworms play vital role in detecting the levels of soil contamination. They can indicate the quality of the soil by their abundance and species composition at a particular site; behavioral changes on coming in contact with the toxic soil; biochemical, immunological and cytological stress and by the amount and level of accumulation of toxic chemicals from the

soil into their body. This makes them an effective model organism to study the quality of the soil.

Moreover, some species of earthworm are also said to act as a good bio-control of soil parasites. Parasitic nematodes, which form gall like structure in the root nodes of plants can cause a great damage to their proper growth and render huge loss of crops and other vegetation. Several chemical control methods are implemented which provide results but they are too costly to meet the financial ability of the farmers and they also cause soil toxicity.

Earthworms ingest nematodes along with the organic matter present in soil and act as an efficient bio-control agent for these nematodes. Using them would be a cost effective and eco-friendly method to mitigate the harmful effects of these plant parasites. Further, proper study of different species would also help to establish the best species for controlling them. Such earthworms could be introduced into the fields before irrigation which would help in their control efficiently and in a cost-effective way.

## References

- Annapoorani, C.A. 2020. Toxic effects of aluminum on reproduction and survival of *Eudriluseugeniae* (Kinberg) on the leaf litter. *Int. J. Curr. Microbiol. App. Sci.* 3 (5) : 493-500
- Avila, G.G., Gaete, H.H., Sauve, S.S. and Neaman, A.A. 2009. Organic matter reduces copper toxicity for the earthworm *Eisenia fetida* in soils from mining areas in central Chile. *Chilean J. Agric. Res.* 69 : 252-259.
- Blouin, M., Hodson, M.E., Delgado, E.A., Baker G., Broussard L., Butt K.R., Dai J., Dendooven L., Peres G, Tondoh J.E., Cluzeau, D. and Brun, J.J. 2013. A review on earthworm impact on soil function and ecosystem services. *Eur. J. Soil. Isci.* 64: 161-182. <https://doi.org/10.11111/egss.12025>.
- Brown, G.B., Doobie, B.M. and Edwards, C.A. 2004. In: *Earthworm Ecology*. Second ed. Boca Raton, FI: CRC Press. p. 213-239.
- Butt, K.R., Fredricson, J. and Lowe, C.N. 1999. Colonisation, survival and spread of earthworms on a partially restored landfill site. *Jena.* 43 : 1-7.
- Butt, K.R., Lowe, C.N., Fredricson, J. and Moffat A.J. 2004. The development of sustainable earthworm populations at Calvert landfill site, UK. *Land Degrad Dev.* 15: 27-36.
- Capoweize, Y., Rault, M., Mazzia, C. and Balzunees, L. 2003. Earthworm behaviour as biomarker: study case with imidacloprid. *Jena.* 47 : 542-547
- Caroline, J.L., Trevor, G.P., Andrew, A.M. and Krik, T.S. 2001. Survival and behaviour of the earthworms



- Lumbricus rubellus* and *Dendrodrilus rubidus* from arsenate contaminated and non-contaminated sites. *Soil Biol. Biochem.* 33 : 1239-1244.
- Connor, G.O.J. and Environ, Quail, 1998. *Doobie and Brown Earthworm Ecology* Eel.17: 215.
- Daane, L.L., Molina, J.A.E., Berry, E.C. and Sadoswsky M.J. 1997. Plasmid transfer between spatially separated donor and recipient bacteria in earthworm containing soil microcosms. *Appl. Environ. Microbiol.* 63: 679-686.
- Datta, S., Singh, J., Singh, S. and Singh J. 2016. Earthworms, pesticides and sustainable agriculture: a review. *Environ Sci Pollut. Res.* 23: 8227-8243. <https://doi.org/10.1007/s11356-016-6375-0>.
- Doobie, B.M., Ryder, M.H. and Davoren, C.W. 1994. Enhanced root nodulation of subterranean clover (*Trifolium subterraneum*) by Rhizobium *Leguminosarum biovar trifolii* in the presence of the earthworm *Aporrectodea trapezoides* (Lumbricidae). *Biol Fertil Soils.* 18: 169-174.
- Edwards, C.A. and Bohlen, C.J. 1996. *Biology and Ecology of Earthworm.* 3<sup>rd</sup> ed. Codon: Chapman and Hall.
- Evans, A.C. 1947. A method of studying the burrowing activity of earthworms. *Annu Mag Nat Hist.* 14 : 643-650.
- Eyhorn, F., Muller, A., Reganold, J.P., Frison E., Herren H.R., Luttkholt L, Mueller A., Sanders J., Scialabba N.E., Seufert, V. and Smith, P. 2019. Sustainability in global agriculture driven by organic farming. *Nat. Sustain.* 2: 253-255. <https://doi.org/10.1038/s41893-019-0266-6>.
- Fründ, H.C., Egbert, E. and Dumbeck, G. 2004. Spatial distribution of Earthworms (Lumbricidae) in recultivated soils of the Rhenish Lignite-mining area, Germany. *J Plant Nutr. Soil Sci.* 167 : 494-502.
- Fründ, H.C., Graefe, U. and Tischer, S. 2010. *Earthworms Bioindicators of Soil Quality. Biology of Earthworms.* Springer, Berlin. [https://doi.org/10.1007/978-3-642-14636-7\\_16](https://doi.org/10.1007/978-3-642-14636-7_16).
- Georgescu, B., Carmen Georgescu and Victoria Cosier, D. 2004. The role of stress-responsive genes in Ecotoxicology. *Bul. USAMV Cluj-Napoca. Seria Zootehnie și Biotehnoologii.* 60 : 39 6-397.
- Haimi, J. 2000. Decomposing animals and bioremediation of soils. *Environ Pollut.* 107 : 233-238.
- Hampson, M.C. and Coombes, J.W. 1989. Pathogenesis of *Synchytrium endobioticum*. VII. Earthworms as vectors of wart disease of potato. *Ant and Soil.* 116: 147-150.
- Hans, R.K., Khan, M.A., Farooq, M. and Beg, U. 1993. Glutathione-s-transferase activity in an earthworm (*Pheretima posthuma*) exposed to three insecticides. *Soil Biol and Biochem.* 25: 509-511.
- Hurley, R. R. and Nizzetto, L. 2018. Fate and occurrence of micro(nano)plastics in soils: Knowledge gaps and possible risks. *Curr. Opin. Environ. Sci. Heal.* 1: 6–11.
- Hutchinson, S.A. and Kamel, M. 1956. The effect of earthworm on dispersal of soil fungi. *Journal of the Soil Sci Soc Am J.* 7 : 213-218.
- Hyvönen, R., Andersson, S., Clarholm, M. and Persson, T 1994. Effects of lumbricidae and enchytraeids on nematodes in lined and unlined coniferous mor humus. *Bio Fertil Soils.* 17 : 201-205.
- Kiyasudeen, K., Ibrahim, M.H., Quail, S. and Ismail S.A. 2016. Vermicomposting: an earthworm mediated waste treatment technique. Prospects of organic waste management and the significance of earthworms. *AESE. Springer. Cham.* 167-199. <https://doi.org/10.1007/978-3-319-24708-3>.
- Labrot, F.D., Ribera, M., Saint-Denis, J. and Narbonne, F. 1996. *In vitro* and *in vivo* studies of potential biomarkers of lead and Uranium contamination: lipid peroxidation, acetylcholinesterase, catalase and glutathione peroxidase activities in three non-mammalian species. *Biomarkers.* 1 : 21-28.
- Li, Y., Hu, J., Liu, H. and Zhou, C. 2020. Electrochemically reversible foam enhanced flushing for PAHs- contaminated soil: Stability of surfactant foam, effects of soil factors and surfactant reversible recovery. *Chemosphere.* 260: 127645.
- Lowe, C.N. and Butt, K.R. 2003. Inoculation of earthworms into reclaimed soils: experiences from Britain. *Proceedings Sardinia 2003, Ninth International Waste Management and Landfill Symposium.*
- Maboeta, M.S., Reinecke, S.A. and Reinecke, A.J. 2003. The relationship between lysosomal biomarker and organismal responses in acute toxicity tests with *Eisenia fetida* (oligochaeta) exposed to the fungicide copper oxychloride. *Environ Res.* 96 : 95-101.
- Marino, F., Winters, C. and Morgan, A.J. 1999. Heat shock protein (HSP 60, HSP 70 and HSP 90) expression in earthworms exposed to metal in the field and laboratory. *Jena.* 43 : 615-624.
- Morillo, E. and Villaverde, J. 2017. Advanced technologies for the remediation of pesticide - contaminated soils. *Sci. Total Environ.* 586 : 576-597.
- Nadeau, D., Corneau, S., Plantae G., Morrow R.M. and Tanguay, 2001. Evaluation for HSP 70 as a biomarker of effect of pollutants on earthworms *Lumbricus terrestris*. *Cell Stress Chaperone.* 6 : 153-163.
- Otitoloju, A.A. and Ajikobi, A.O. and Eon Moran, R.I. 2009. *The Open Environmental Pollution and Toxicology Jarrot.* 1: 79-88.
- Paoletti, M.G. 1999. The role of earthworms for assessment of sustainability and as Bioindicators. *Agric. Ecosys. Environ.* 74: 137-155. [https://doi.org/10.1016/S0167-8809\(99\)00934-1](https://doi.org/10.1016/S0167-8809(99)00934-1).
- Pérès, G., Vandenbulcke, F., Guernion, M., Jesse, M., Beguiristian, T., Douay, F., Houot, S., Piron, P., Richard, A., Bispo, A., Grand, C., Galsomies, L. and Cluzeau, D. 2011. Earthworm indicators as tools for soil monitoring. Characterisation and risk assess-

- ment. An example from the National Bioindicator Programme (France). *Pedobiol. Int. J. Soil. Bio.* 54: S77-S87. <https://doi.org/10.1016/j.pedobi.2011.09.105>.
- Pérez-Mayán, L., Ramil, M., Cala, R. and Rodriguez, I. 2020. Multiresidue procedure to assess the occurrence and dissipation of fungicide and insecticides in vineyard soils from Northwest Spain. *Chemosphere.* 261: 127696.
- Piehl, S., Leibner, A., Löder, M.G.J., Dris, R., Bogner, C. and Laforsch, C. 2018. Identification and quantification of macro- and microplastics on an agricultural farmland. *Sci Rep.* 8: 17950. <https://doi.org/10.1038/s41598-018-36172-y>
- Pope, C.J., Peters, W.A. and Howard, J.B. 2000. Thermodynamic driving forces for PAH isomerization and growth during thermal treatment of polluted soils. *J. Hazard. Mater.* 79(1-2) : 189-208.
- Qisse, N., EL Alouani, M., E L Azzouzi, L., E.L. Faeil, I., Saifi, H, E.L. and Azzouzi, M. 2020. Adsorption of imazole herbicide onto Moroccan agricultural soils: Kinetic and isolation adsorption studies. *Ground. Sustain. Dev.* 11: 100468.
- Reganold, J. P. and Wachter, J.M. 2016. *Organic Agriculture in the Twenty-First Century.* Nature Publishing Group. 2: 1-8 <https://doi.org/10.1038/2015.221>.
- Saint-Denis, M.A., Pfohl-Leszkowicz, J.F. and Narbonne, D., Ribera, 2000. Dose response and kinetics of the formation of DNA adducts in the earthworm *Eisenia fetida* exposed to B(a)P-contaminated artificial soil. *Polycyclic Aromat Compd.* 18: 117-127.
- Sanchez-Hernandez, J.C. 2006. Earthworm biomarker in ecological risk assessment. *Rev Environ Contam Toxicol.* 188 : 85-126.
- Schrader, S. 1993. Semi-automatic image analysis of earthworm activity in 2D sections. *Geoderma.* 56 : 257-264.
- Schäffer, A., Filser J., Frische, T., Gessner M., Kock, W., Liess, M., Nuppenau, E.A., Rob-Nickoll, M., Schafer, R. and Scheringer, M. 2018. The silent spring-on the need for sustainable plant protection (Issue 16). German National Academy of Sciences, Leopoldina.
- Schrader, S. and Joschko, M. 1991. A method for studying the morphology of earthworm burrows and their function in respect to water movement. *Jena.* 35 : 185-190.
- Singer, A.C., Crowley, D.E. and Menge, J.A. 1999. Anecic earthworms as a means for delivery of biocontrol agent and antagonism towards *Phytophthora cinnamomi*. *Jena.* 43 : 771-775.
- Sudoma, M., Pestalova, N. and Bilkova, Z. 2021. Ageing effect on conazole fungicide bioaccumulation in arable soils. *Chemosphere.* 262: 127612.
- Stephens, P.M., Davoren, C.W., Ryder, M.H. and Doobie, B.M. 1994. Influence of the earthworm *Aporrectodea trapezoides* Lumbricidae on the colonisation of alfalfa (*Medicago sativa* L.) roots by *Rhizobium meliloti* L5-30R and the survival of *R. meliloti* L5-30R in soil. *Biofertil Soils.* 18 : 63-70.
- Svensden, C., Spurgeon, D.J., Hankard, P.K. and Weeks J.M. 2004. A review of lysosomal membrane stability measured by neutral red retention: is it a workable earthworm biomarker? *Ecotoxicol. Environ. Saf.* 57: 20-29.
- Thorpe, I.S., Prosser, J.I., Glover, L.A. and Killham K. 2003. The role of the earthworm *Lumbricus terrestris* in the transport of bacteria inocula through soil. *Biol Fertil Soils.* 23: 132-139.
- Wolters, V. 2000. Invertebrate control of soil organic matter stability. *Biol Fertil soils.* 31: 1-19.
- Xiao, R., Bai, J., Wang, J., Lu, Q., Zhao, Q., Cui, B. and Liu, X. 2014. polycyclic aromatic hydrocarbons (PAH) in wetland soils under different land uses in a coastal estuary: Toxic levels, sources and relationships with the soil organic matter and water stable aggregates. *Chemosphere.* 110: 8-16.
- Zhang, H.B., Luo, Y.M., Wong, M.H., Zhao, Q.G. and Zhang, G.L. 2006. Distribution and concentration of PAHs in Hongkong soils. *Environ. Pollut.* 141(1) : 107-114.
- Zhang, Y., Wang, X. and Ji, H. 2020. Stabilization process and potential of agro-industrial waste on Pb-contaminated soil around Pb-Zn mining. *Environ. Pollut.* 200: 114069.
- Zhang, Y., Zhan, Y. and Luo, 2000. Genotoxicity of two novel pesticides for the earthworm *Eisenia fetida*. *Environ. Pol.* 108 : 271-277.