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Review

The eco-toxic effects of pesticide and heavy metal mixtures towards earthworms in soil

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ABSTRACT

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.

1. Introduction

Pollutants are continuously accumulated in the form of complex mixtures in the natural systems through different anthropogenic sources including industries, mining, agricultural, and waste water treatment plant (Altenburger et al., 2004). The metals and pesticides are two kinds of chemicals/toxicants globally known, and are likely to occur in most of agricultural soil ecosystems (Wang et al., 2012a). The development of intensive agriculture in many countries involves the application of various pesticides which may lead to a heavy burden on the environment especially in agriculture soil ecosystems. A variety of pesticides used in the agriculture sector to improve the annual agriculture production, however their residues increase soil contamination which may be directly or indirectly stressful for soil organisms (Choung et al., 2013). A significant portion of these pesticides may be carried out by runoff into aquatic environments or pass slowly through soil lower soil layers and ground waters (Rial-Otero et al., 2004). Beside pesticides, heavy metal pollution in soil environments has become another major challenge across the world due to their increase in geologic and anthropogenic activities. Worldwide heavy metals production has been very scaring increase and openly spread in the environment since industrial revolution (Nriagu and Pacyna, 1988). The huge contamination of metals and pesticides in soils, water and air and their imminent transfer to higher organisms through the food chain continues to be an environmental issue which may involves diverse health risks for future generation.

Earlier the scientists have been relying only on individual compounds to assess the potential risks of environmental contaminants; however assessing joint effects of chemical mixtures by considering data obtained exclusively from single chemical toxicity, tends to over or underestimate the level of the joint toxicity. Assessments that take into account of combined actions of pollutants reflect better the existent impact of environmental exposures than the assessments that evaluate toxicity of single chemicals (Schnug et al., 2014). Joint toxic effects of

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chemical mixtures on organisms and ecosystems need to be seriously addressed, however but the evaluation of every possible chemical mixture is impossible practically. Thus the usage of computer-based models is considered to be a powerful tool that may accurately predict the joint toxicity of mixtures simulated from individual compound data. The concentration addition (CA) and independent action (IA) are two classical models that are frequently used to predict the joint toxicity of different chemical mixtures. Both these models used the non-interactions approach between the mixture and individual components, which means that there is no occurrence of interactions between compounds with the mixture. But each compound can individually contribute to the joint toxicity. The joint toxicity of toxic mixtures can be similar (additive), stronger (synergistic) or weaker (antagonistic) depending of on the sum of effects from individual exposures (Warne and Hawker, 1995). Most of the studies indicate that most of the metal mixtures frequently produce synergistic effects (Cedergreen, 2014). The applications of CA and IA models are limited to predict the actions of non-interactive mixtures. To challenge this limitation, the scientists have designed the second category of model that can be applied to mixtures to show synergism or antagonism interactions between the individual compounds of the mixture. The combination index (CI)-isobologram equation is the widely used model to indicate the type of interaction between components of a certain mixture (Chou, 2006). This model facilitates quantitative prediction of chemical interactions within the mixture.

However many studies have been reported on the toxicities of single chemicals, there is a great need to improve our knowledge about the harmful effects of exposures to chemical mixtures. The pollutants are generally the same in water and soil systems, but their fate and effects on living organisms may be varied in the two ecosystems. Soil is considered a complex terrestrial ecosystem containing a large variety of organisms, mineral particles and organic matter (Cachada et al., 2016). The binding of toxic compounds to some fractions of minerals and humus can reduce their mobility and bioavailability, and thus modulating their toxicity. Moreover, the availability and toxicity of soil pollutants are influenced by the weather and age of the soil as well. Consequently, the assessment of chemical toxicity toward terrestrial organisms is more difficult than that of aquatic organisms. It is also impossible to extrapolate toxicity in soil ecosystems from studies that use aquatic organisms. Specific approaches and models are always required to evaluate the toxic effects of soil pollutants toward the terrestrial biodiversity. Approaches conducting field studies or carrying out natural or artificial soil experiments are recommended in order to explain the availability of toxicant and their relative conditions of exposures (Spurgeon et al., 2003). The most challenging issue concerning exposures to soil contaminants is to measure the level on which the living organisms can be contaminated, and evaluate the toxic effects of those contaminants at individual, population and community levels. Therefore, any study that will evaluates the individual and joint toxicity of soil pollutants must be of great importance in the ecological risk assessment.

Earthworms are among the commonly known organisms that are found in the soil ecosystems where they play a crucial role in the improvement of soil quality (Spurgeon et al., 2003). The decrease of earthworms in the soil ecosystem may reduce nutrient cycling and their availability for plant uptake (Rizhiya et al., 2007). Earthworm as bioindicators of soil pollution have been considered as the key organism for ecological risk assessment (Song et al., 2009). Pollutants including pesticides and heavy metals accumulated by earthworms may be transferred to higher trophic-level organisms through food chain. Accumulation of contaminants in earthworms may not cause significant effects to the earthworm, but may have severe impact to other organisms and humans as well (Reinecke and Reinecke, 1996). The earthworms like *Eisenia fetida* is usually improving soil structure and their vulnerability towards soil contaminants, this great role of earthworms have made them to be considered as the best biological indicators for soil contamination. Moreover, earthworm species are better to be used in the ecotoxicological laboratory-based tests for short exposures (Fourie et al., 2007).

In recent years, with the broad application of chemical compounds in agricultural ecosystem, the study on the earthworm eco-toxicology has become increasingly widespread. The researchers around the world have built up a lot of earthworms-based eco-toxicological methods to test the presence of chemical materials (OECD, 1984). Different studies about eco-toxicological mechanisms of the earthworm toward pollutants, such as heavy metals, pesticides (Stenersen, 1979) and combined pollution of different heavy metals (Spurgeon et al., 2003), metals and pesticides mixtures (Xu et al., 2006), have been reported worldwide. However, studies on the interactions between toxic effects of pesticides and heavy metals are still very limited. Therefore, in this review we summarize the current studies on single and joint effects of pesticides and metals on earthworms at genome, cellular, individual population and community levels. We also overview the interactions between pesticides and metals in earthworms and make an overall judgment of the likelihood for synergistic effects, and highlight the current knowledge gaps. Finally, we explore the methods and models used to evaluate the toxicity level of chemical mixtures, and provide perspective approaches for a better understanding of joint effects as well as research methods.

2. Effects of heavy metals

Heavy metals released into the environment from various anthropogenic activities are toxic to soil organisms and affect the abundance, diversity, and distribution of the soil organisms. Earthworms inhabiting in the soils contaminated by heavy metals, due to their behavioral characteristic such as burrowing and feeding activities, are more susceptible to metal pollution than other groups of terrestrial invertebrates.

Exposure of earthworms to higher concentrations of heavy metals in soil ecosystems may affect cocoon production, growth and sexual development of worms, life behavior, viability and density (Andre et al., 2010). Heavy metals including zinc (Zn),lead (Pb), cadmium (Cd), mercury (Hg) and copper (Cu) are mostly bioaccumulate and results diverse effects in earthworms which may credits the earthworm as a suitable biological indicator to investigate the heavy metals pollution (Zhang et al., 2009). The most reported toxic effects of the heavy metals toward earthworm species include growth rate reduction, reproduction decline,DNA damage, pathological damage of nephridia, alterations in cytokinesis and antioxidant enzymes (Liang et al., 2011). Metals like Cd exposure may cause mortality in earthworms which ultimately lead reduction in the earthworm population size in contaminated soils. Genomic impairment in earthworm's cells due to metal is also used as early biomarker of metal effects at the individual level. Metal exposure induces very significant DNA damage in earthworms and cause alterations in the protein content especially metallothioneins and heat shock proteins (Maity et al., 2008). Cd a widely occurring metal pollutant, has been extensively studied globally and is well known to affect the physiological functions of earthworms, in terms of bioaccumulation, decrease in weight and oxidative DNA damage generation (Table 1). Multiple studies have reported metallothionein overespression in coelomocytes of *Eisenia fetida* after long-term exposure to metals particularly Cd contaminated soils (Brulle et al., 2011). However, not all heavy metals are very toxic toward earthworms. Nakashima et al. (2008) reported that among the heavy metal nickel (Ni) usually does not accumulate in the tissues of earthworm, even after long-time exposure, and did does cause any significant effects on earthworm's growth. In Table 1 we summarized the most recent reports on effects of different heavy metals including Cd, Zn, Cu, Hg, Pb and Ni towards different earthworm species; where the responses of earthworms to heavy metal exposures are the same for all earthworm species. In their study, Spurgeon and Hopkin (1996) evaluated the effects of heavy metals particularly