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Vermiculture in animal farming: A review on the biological and nonbiological risks related to earthworms in animal feed

Patrick Byambas^{1,2*}, Jean Luc Hornick¹, Didier Marlier³ and Frederic Francis⁴

Abstract: Earthworms are part of natural diet of some farm animals such as poultry. They are a protein source. Unfortunately, earthworms can accumulate some toxic substances occurring in the soil and animals can be contaminated through feed containing these earthworms.

The biological and nonbiological risks related to earthworms depend on the type of substrates through which they live. Earthworms are usually contaminated in polluted soil by various types of toxic and poisonous substance. The most common toxic compounds are heavy metals, pesticides and microbial toxins, which can lead to toxic or poisonous contamination of earthworms and then animals, such as poultry, fish and swine, through feeding.

This risk cannot be identified in earthworm body without its destruction. But according to toxic substance targeted, there are many ways of risks detection, in soil, earthworms and animals.

To protect farm animals, different technics are available. To limit heavy metal contamination, soil should be analyzed before collecting earthworms. Many treatments are available. By heating at 80°C for 2–4 h, microorganisms can be destroyed. Nevertheless, this technique needs to be adapted with certain types of bacteria whose enterotoxin withstands temperatures of 100°C for 30 min, or more. To limit

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Patrick Byambas has published peer-reviewed articles in the areas of porcine parasitology, poultry nutrition and earthworm. He has conducted experimental breeding of earthworm for poultry feeding. The information in the current paper are important for using earthworm in poultry feeding. The use of earthworm to feed poultry, under certain conditions, can be a hazard for farm animals. These information can help to prevent risk of contamination of poultry and humans.

PUBLIC INTEREST STATEMENT

Earthworms are source of feed for many omnivores. They are also known as a good source of protein, biological value of which is similar to fish profile. Despite its interesting nutritional properties, earthworms are able to accumulate some toxic compounds stemming from soil. The ground pollutants described as biological and nonbiological groups could be transmitted or accumulated in higher trophic levels, through food chain. All these chemical substances and biological organisms can be responsible for intoxications or intonations.

Although it is an interesting source of nutrient for future feeding concern for farming animals and human food, earthworms collected from uncontrolled soil may be a contamination risk for animals and if human eat these animals, it may become a public health concern.

all these risks, the use of earthworms reared from vermiculture can help to prevent contamination of poultry through feeding.

Subjects: Plant & Animal Ecology; Soil Sciences; Environment & Resources; Environmental Change & Pollution; Food Additives & Ingredients

Keywords: earthworms; intoxication; intoxication; omnivores; vermiculture; review

1. Introduction

Earthworms are burrowing animals that are grouped into 13 families. Although more than 5,000 species have been described, many are still unknown, mainly in the tropics (Brown et al., 2013). These invertebrates are found in soil in many parts of the world, both in temperate and tropical regions, where they represent a large fraction of living biomass, providing agroecosystem sustainability (Pelosi, Barot, Capowiez, Hedde, & Vandenbulcke, 2014). It is often used in vermicomposting process, especially in tropical and subtropical regions (Kumar Middha et al., 2012). Earthworms also are a part of livestock feed (Sonaiya & Swan, 2004) and can be used fresh or in milled form as additive source of nutrient (Chiripasi, Moreki, Nsoso, & Letso, 2013; Francis et al., 2003; Sogbesan, Ugwumba, & Madu, 2007). Because of their nutritional value (Sogbesan & Ugwumba, 2008), they have been used as additive feeding, for poultry in Vietnam (Francis et al., 2003), for fish in Malaysia (Abd Rahman Jabir, Razak, & Vikineswary, 2012), in Nigeria (Sogbesan & Madu, 2008), in the United Kingdom (Rawling et al., 2012) and for swine in Brazil (Vieira, Ferreira, and Donelle 2004).

Earthworms could be collected in soil or obtained from earthworm farming. According to Sogbesan et al. (2007), after harvesting, earthworms are dried and milled, then added to omnivore animals' diet. For example, it can be incorporated up to 25% in poultry feed to substitute fish meal without adverse effects on growth (Abd Rahman Jabir et al., 2012). They are also known as a good source of protein, the biological value of which is similar to fish profile (Agbédé, Nguekam, & Mpoame, 1994; Morin, 2004; Sogbesan et al., 2007).

Despite its interesting nutritional properties (Sogbesan & Ugwumba, 2008), earthworms are able to accumulate some toxic compounds stemming from soil (Suthar & Singh, 2008) and to which it is constantly exposed. Across skin and gastrointestinal tract, the main routes of entry of contaminants inside earthworms (Araneda, Undurraga, Lopez, Saez, & Barra, 2016), the ground pollutants described as biological and nonbiological groups, then could be transmitted or accumulated in higher trophic levels, through food chain (Gall, Boyd, & Rajakaruna, 2015). In the nonbiological group are found heavy metals and pesticides with particular emphasis on bioaccumulation of heavy metals (Dureja, Patra, Johnson, & Tomar, 1999; Heikens, Peijnenburg, & Hendriks, 2001; Rodriguez-Castellanos & Sanchez-Hernandez, 2007; Garg, Suthar, & Yadav, 2012; Pelosi et al. 2013; Soobhany, Mohee, & Garg, 2015; Araneda et al., 2016; K. Wang et al., 2018). In the biological group are bacteria and fungus on which relatively few researches have been carried out (Brito-Vega & Espinosa-Victoria, 2009; Schuch, Pelzek, Kan, & Fischetti, 2010; Stenfors Arnesen, Fagerlund, & Granum, 2008). All these chemical substances and biological organisms can be responsible for intoxications or intoxications (Araneda et al., 2016; Gall et al., 2015; Organji, Abulreesh, Elbanna, Osman, & Khider, 2015). According to Larousse agricole (2002), food intoxication refers to disorders that occur after intake of food containing bacteriotoxine or mycotoxine. Generally, microorganisms involved in cases of intoxication are bacteria or fungus. Food intoxication refers to disorders caused by absorption of food containing "heavy metals" or pesticides (Larousse, 2017).

Although it is an interesting source of nutriment for future feeding concern for farming animals (Sonaiya & Swan, 2004), earthworms collected from uncontrolled soil may be a contamination risk for animals (Spurgeon, Hopkin, & Jones, 1994) and may become a public health concern (Gall et al., 2015; Soobhany et al., 2015). Accurate methods are available to detect eubiotic or xenobiotic "heavy metals" (Delgado Arroyo, Miralles De Imperial Hornedo, Alonso Peralta, Rodriguez Almestre, & Martin Sanchez, 2014), pesticides (Masía, Suarez-Varela, Llopis-Gonzalez, & Picó, 2016) and

microorganisms (Organji et al., 2015; Wojewoda et al., 2013) in earthworms, but prevention remains a priority route to limit risks in food chain.

Knowing that there is a lack of information about the risk to use earthworm in animal feeding, the purpose of this work is to synthesize the current knowledge about foodborne diseases associated to this invertebrate. The transfer of biological and nonbiological elements from soils to earthworms and then to animals, ending with the risk of contamination in humans, will be examined in this review.

2. Earthworms risk assessment in animal feeding

The use of earthworms in animal feed is one of the pathways in search of alternative sources of protein for farming animal feeding (Sonaiya & Swan, 2004). Although earthworms are already incorporated as feed supplements for poultry, fish and swine, under certain conditions, they remain a nutritional source at risk for animals. Among the most important contamination sources that enter animal through earthworms, there are “heavy metals”, pesticides, bacteria and fungi. They can end up in humans through food chain as summarized in Figure 1. Some nematodes for whom earthworms are intermediate hosts are particular case in contamination of animals such as poultry.

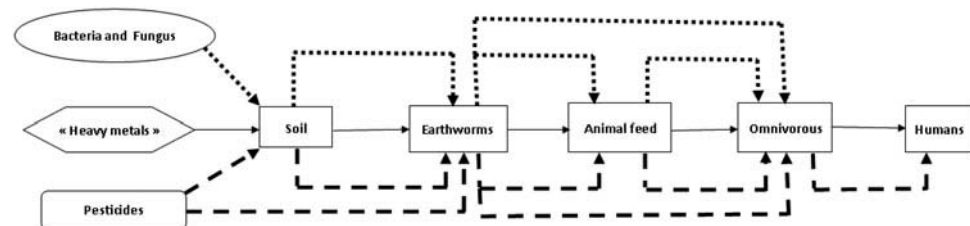
3. Risks

3.1. Nonbiological risks

“Heavy metals” refer to naturally occurring metals having atomic number (Z) greater than 20 and an elemental density greater than 5 g/cm^3 (Ali & Khan, 2018). Many studies have shown that earthworms are important bio-accumulators and bioindicators toward “heavy metals” in soil (Antunes, Castro, Nunes, Pereira, & Gonçalves, 2008; Mohee & Soobhany, 2014; Wang, Zhou, Cang, Li, & Zhu, 2009). Moreover, earthworm is able to concentrate metals that are more likely to transfer to other animals. The main metals identified in earthworm body are aluminum, beryllium, cadmium, chromium, copper, iron, manganese, nickel, lead, mercury, strontium, uranium and zinc (Antunes et al., 2008; Scott-Fordsmand, Krogh, Schaefer, & Johansen, 2008; Shkinev, Vitaly, & Dinu, 2012; Suthar & Singh, 2008; Wang et al., 2009). Depending on their biological function, two types of metals are considered: the essential metals (copper, nickel, zinc and iron) being beneficial only at very low concentration, and the toxic metals (lead, mercury and cadmium) being toxic even at very low concentration (Duffus, 2002; European Commission, 2003; Okoye, Ibeto, & Ihedioha, 2011). The most dangerous pollutants among “heavy metals” are mercury, lead, aluminum, cadmium and arsenic (World Health Organization, 2007). Annapoorani (2014) found that aluminum can have severe toxic effects on earthworms. It appears to impair the different stages of the earthworm reproductive cycle. At that time, earthworms can represent an important risk for animals. Metals are mainly located in the tissue of earthworms exposed to contaminant (Soobhany et al., 2015). According to the type of substrate on which earthworms grow, the concentration of metals in body differs. Delgado Arroyo et al. (2014) observed an increase in cadmium, zinc and mercury in earthworms exposed to sawdust poultry manure compared with those who were exposed to natural soil.

The fact that earthworms can act as a vector of toxic substances like most dangerous “heavy metal” listed above shows that the use of earthworms containing that kind of toxic is at risk for

Figure 1. Pathways of biological and nonbiological risk in food chain, adapted from Gall et al. (2015).



farming animals such as poultry (Spurgeon et al., 1994; Suthar & Singh, 2008). Accumulation of toxic metals in poultry, for example, can represent a public health risk for humans (Delgado Arroyo et al., 2014). Included in diet at excessively high concentrations, several toxic mineral elements may have adverse effects on animals and humans (Okoye et al., 2011). Earthworms are able to accumulate variable amount of “heavy metals” according to the type of substrate. After vermicomposting process, Soobhany et al. (2015) obtained concentration range from 6,900 to 6,901 mg/kg for cadmium, 2,051 to 2,068 mg/kg for copper and 621 mg/kg for zinc, in tissue of earthworms. From poultry manure, Delgado Arroyo et al. (2014) obtained different mean concentration at 1.2 mg/kg for cadmium, 15 mg/kg for copper and 140 mg/kg for zinc. More recently, Wang et al. (2018) reported range from 99.9 to 646 mg/kg for zinc and 8.60 to 157 mg/kg for copper and concluded that tissue metal concentration increases with increasing concentrations in soil what means that “heavy metals” in soil is positively correlated to earthworm tissue. Similar results have been obtained by Schlich, Klawonn, Terytze and Hund-Rinke (2013) about the correlation between Ag concentration in earthworm tissue and soil. According to the European Commission (2003), the maximum acceptable concentration of “heavy metals” in animal feed is 1, 100 and 500 mg/kg for cadmium, copper and zinc, respectively (Okoye et al., 2011). Cadmium, which is one of the most toxic metals, can easily contaminate omnivorous feed through earthworms. By contrast, zinc has biological benefit effects and can be used as an efficient tool for enhancing physiological status of some animals (Amen & Al-Daraji, 2011; European Commission, 2003). Another benefit of dietary zinc demonstrated by Yang et al. (2017) is that at 40 mg/kg, it improves carcass and muscle yields, increases fat content in thigh muscle and decreases accumulation of heavy toxic metals like lead and chromium in breast and thigh muscles.

The intensification of agricultural practices and, especially, the use of pesticides affect earthworms and contribute to loss of biodiversity (Hole et al., 2005). Pesticides threat animal species worldwide, and especially lombrics and animals that feed on them, such as poultry, fish and pigs (Givaudan, 2015). When pesticides occur in environment, earthworms usually accumulate preferentially the less toxic pesticides (Chang, Wang, Wang, Li, & Xu, 2016). Earthworms accumulate pesticides by ingestion or epidermal contact in crop (Pelosi, Joimel, & Makowski, 2013). All pesticides do not have the same impact on soil, earthworms and animals (Pelosi et al., 2014) and Chang et al. (2016) reported that organophosphorus pesticides are more toxic than pyrethroid pesticides.

Although the ways of contamination are different, earthworms may play a role in this process. Cox (1991) reported cases of disruption of thyroidal hormone activity and genetic damages in quails that eat earthworms exposed to pesticides such as malathion.

In Canada, cases of death occurred as a result of contamination of wild birds by insecticides such as carbofuran, diazinon and fensulfothion and earthworms were identified as vectors of toxics in the body of birds. The use of neonicotinoid insecticides such as imidacloprid affects insects and earthworms which are a part of poultry feed. This involves the decline of the population of 15 species of birds of 3.5% per year between 2003 and 2010 in the Netherlands, where the amount of pesticides used in agricultural practices has been multiplied by 10 in 10 years. Because of its harmfulness for the environment, imidacloprid has been forbidden in Europe since December 2013 (Hallmann, Foppen, Van Turnhout, De Kroon, & Jongejans, 2014).

Pesticides accumulated along the food chain, especially endocrine disruptors, pose a long-term risk to animals such as poultry (Givaudan, 2015). It is known that diet influences the composition of tissues and poultry eggs. Therefore, toxic substances in feed could enter in body of poultry or in eggs (Schuch et al., 2010) and therefore end up in humans.

3.2. Biological risks

Soil is an appropriate environment for the development of eukaryotes (algae, fungi, protozoa) and prokaryotes (bacteria, archaea) (Brito-Vega & Espinosa-Victoria, 2009). A part of the diet of the earthworms consists in microbial including fungi and bacteria that appear during the process of

Table 1. Abundance of microbial diversity in intestinal tract of earthworms and associated habitats, as reported in literature (Brito-Vega & Espinosa-Victoria, 2009)

Earthworms species	Bacteria	Actinomycetes	Habitats	References
<i>Eisenia fetida</i>	91		Industrial	Kim, Shin, Cha and Hur (2004)
<i>Lumbricus rubellus</i>	95		Crop soil	Furlong, Singleton, Coleman and Whitman (2002)
<i>Lumbricus rubellus</i>		76	Forest soil	Kritufek, Ravask and Pizl (1993)
<i>Octalasion montanum</i>		175	Forest soil	Kristufek et al. (1993)
<i>Eisenia luceus</i>		145	Forest soil	Gerrettson-Cornell and Gwalter (1985)

composting (Gómez-Brandón, Lores, & Domínguez, 2012). Unfortunately, some microorganisms mainly produce mycotoxins that induce food intoxication when animals feed contains contaminated earthworms. Bacteria are mainly located in the posterior section of intestine tract of earthworms because this portion should have adequate conditions for their development (Brito-Vega & Espinosa-Victoria, 2009). The microbial diversity in intestinal tract of earthworms is presented in Table 1.

Staphylococcus aureus and *Bacillus cereus* belong to a group of bacteria which are responsible for various food intoxication (Augustin & Carlier, 2004). Other bacteria from soil are also involved in food intoxication such as *Bacillus licheniformis*, *Bacillus mycoides*, *Bacillus thuringiensis* and *Brevibacillus laterosporus* (Organji et al., 2015). Bacteria produce toxins in the food, which are relatively large problems even after disappearance of the bacteria from which they arise. The dangerousness of *Bacillus* spp. and *Staphylococcus* spp. lies in the fact that they sporulate, conferring their ability to withstand environmental conditions (cooking, cleaning, drying, curing) and that increases their contamination capacity. *B. cereus* is present in the digestive tract of earthworms and it is responsible for several cases of animal food contamination (Schuch et al., 2010). *B. cereus* is widespread in nature and frequently isolated from soil, but it is also well adapted for growth in the intestinal tract of some invertebrates. From these habitats it can easily spread to poultry feed, where it may have an emetic effect (Stenfors Arnesen et al., 2008). Parkes and Shilton (2015) reported that bacteria of the genus *Clostridium* are also responsible for food intoxication. *Clostridium botulinum* is responsible for botulism, which is caused by a neurotoxin which affects poultry and many other animal species. And Souillard et al. (2014) stated that botulinum toxin has been identified in many invertebrates, including earthworms, operating in contaminated soils. This means that earthworms could be an important way of transmission of this toxin to other animal species such as poultry or fish for whom alimentary chain includes lombrics.

3.3. Nematodiasis

Apart from metals and chemicals, the earthworm can be vector of parasites that can lead to specific animal concerns, especially poultry. Earthworm is an intermediate host of some nematodes that do not produce toxins *sensu stricto* but whose amounts in animal, especially birds bred on rangelands, cause disease (Villate, 2001). Thus, the use of feed containing contaminated earthworms should be the starting point for parasite infestation in poultry. Various pathologies have been reported by Guerin, Balloy and Villate (2011). Syngamosis, which is caused by *Syngamus trachea* and *Syngamus merulae*, is ingested at their larval form, by earthworms. Histomoniasis is caused by the flagellated protozoan *Histomonas meleagridis* contaminating especially turkey earthworms consume encysted eggs by the way of Heterakis nematode intake. Capillariasis is caused by different types of nematodes pathogenic for chickens, turkeys and guinea fowl. They include *Capillaria annulata* and *Capillaria contorta* and contamination of poultry can be done indirectly via earthworms.

Parasitic infestation by feeds highlights the risk of using earthworms in feed manufacture.

4. Risks detection

Cases of intoxication and intoxication through animal diet are more common in wild birds than among poultry or fish. The proven poisoning is more common in areas where the use of toxic products for agriculture is abundant (Hallmann et al., 2014). Contamination of animals depends on the type of pathogen vector, agent or substance, incubation period, infestation rate, quantity and feed preparation process. Then the ways of risks detection, in soil, earthworms and animals are various according to toxic substance targeted (Hallmann et al., 2014).

4.1. Detection of “heavy metals”

Determinate of “heavy metal” in soil may be performed by the use of the Environmental Protection Agency (EPA) techniques (Delgado Arroyo et al., 2014; USEPA, 1994).

Various studies reported detection methods of “heavy metals” accumulated in the body of earthworms (Shkinev et al., 2012; Wang et al., 2009). The technique using the atomic spectrophotometer absorption is commonly used to determine the concentration of “heavy metals”. It consists of digesting earthworms according to the EPA Method 3051 (EPA, 1996) before analysis. Then the analysis of “heavy metals” is carried out by atomic spectrophotometer absorption (Domínguez-Crespo et al., 2012; EPA, 1996; Jeyanthi, Arockia John Paul, Karunai Selvi, Biruntha, & Karmegam, 2016). It is also possible to keep earthworms in a hot air oven to get them dried. For analysis, the dried residues are dissolved in 0.7 M HNO₃ and this dissolved solution is used to estimate the metal concentration in atomic absorption spectrophotometer (Welz & Sperling, 2007).

4.2. Detection of pesticides

According to the clearly demonstrated toxicity of pesticides, it is essential to get accurate and reliable methods of detecting their presence in ingredients used for animal feed manufacturing. The diversity of pesticides has led to the development of different detection methods. Sensitive and reliable techniques such as chromatographic methods like gas chromatography, high performance liquid chromatography along with mass spectrometry (MS) were developed. However, procedures are complex and take long time for sample treatments, they required highly trained technicians and they cannot perform on-field detection (Bhadekar, Pote, Tale, & Nirichan, 2011). Later, Yi et al. (2013) developed a novel label-free SiQDs-based sensor for the detection of pesticides. This method should be suitable for on-field pesticides detection. It's simple, rapid, highly sensitive and capable of anti-interference in pesticide detection. In the search of precision in pesticides detection, Masiá et al. (2016) reported that among the different methods, the QuEChERS approach (Quick, Easy, Cheap Effective Rugged and Safe) is a reference for determining residues in food. In order to have more precision, older methods have been improved. The new techniques use more sensitive devices like chromatographic techniques with various detection methods, electro analytical techniques, chemical and biosensors, spectroscopic techniques and flow injection analysis. Spectrophotometric detection methods were found suitable for detection of organo-pesticides such as malathion, phorate and dimethoate from food samples (Bhadekar et al., 2011).

4.3. Identification of microorganisms in earthworms

Laboratory methods are necessary to identify fungi and bacteria occurring in soil (Brito-Vega & Espinosa-Victoria, 2009) or in intestine tract of earthworms (Gómez-Brandón et al., 2012). The detection of *B. cereus* strains may occur in a laboratory at 37°C. The routine detection and identification of *B. cereus* involves the use of selective solid media such as mannitol-egg-yolk-polymyxin agar and polymyxin pyruvate-egg yolk-mannitol-bromothymol blue-agar that usually facilitates the detection of *B. cereus* lecithinase production and lack of mannitol fermentation (Organji et al., 2015). Polymerase Chain Reaction (PCR) is routinely used to identify colonies of *B. cereus* (Kotiranta, Lounatmaa, & Haapasalo, 2000; Organji et al., 2015). The isolation of more than 105 microorganisms/g of suspected food is, according to Murray, Baron, Jorgensen, Pfaller and Tenenbaum (2003), the limit leading to the recommendation to avoid that feed for animals.

The poisoning by *S. aureus* can be diagnosed by culture in the suspected food containing earthworms (Murray et al., 2003). The multiplex PCR using test like GeneXpert MRSA/SA helps to identify single pathogen such as *S. aureus* (Buchan et al., 2015). Others authors suggested to combine multiplex PCR and Matrix-Assisted Laser Desorption/Ionization Time Flight (MALDI-TF) MS directly on the bacteria collected in the culture to improve the efficiency of test (Wojewoda et al., 2013). Mainly used for the detection of foodborne pathogens, the use of biosensors was tested to identify *S. aureus* (Tokel et al., 2015). A biosensor is an analytical tool composed of two inseparable parts, the bio-receiver or probe of biological or bioinspired nature on the one hand, and the transducer on the other hand (Lazcka, Del Campo, & Muñoz, 2007).

The diagnosis of *C. botulinum* varies according to the mode of contamination. For food intoxication, diagnosis was based on the detection of the toxin in the serum or in the suspected feed (Acha & Szyfres, 2003). The bioassay in mice is the most reliable detection method in the presence of botulinum toxin but ELISA method can also be used to detect toxins (Souillard et al., 2014).

5. Means to preventing risks

Prevention methods differ depending on the nature and origin of the risky molecules such as toxins associated with microorganism, “heavy metals” or other chemicals. Whatever the risk origin, it is already necessary to avoid feeding animals with fresh earthworms collected from soils that may be contaminated, such as landfills, intensive agricultural land and industrial area.

Vermicomposting is actually the best way to limit contamination of earthworms by metals even if Soobhany et al. (2015) had shown that some vermicompost can contain “heavy metals” according to the origin of soil used. Some complementary cautions have also been applied for “heavy metals” and pesticides contents according to the origin of the soil used. Earthworm production based on adapted rearing techniques should be developed to reduce the risk of contamination for further animal production.

For microorganisms in general, heat treatment used in earthworm transformation processes into meal should be sufficient to destroy microorganisms in the food produced.

Nevertheless, some bacteria can develop resistance to conventional heat treatments. This is the case of *C. botulinum*, the staphylococcal enterotoxins and the emetic toxin of *B. cereus*. Stronger temperature and heating duration should be then adapted to manage earthworms as safety material to include in animal feed. Staphylococcal enterotoxin withstands temperatures of 100°C for 30 min; the emetic toxin of *B. cereus* resists 90 min at 126°C. The botulinum toxin is destroyed at 85°C for 5 min and after sporulation, it should keep on for at least 3 min at 121°C.

6. Conclusions

The search for sources of cheap and available protein in nature has encouraged the development of nonconventional farms for some omnivore’s diet, and this has led to the rise in earthworm production for animal feeding these last few years.

But the use of earthworms in some animals feeding, such as poultry, fish and pigs, has its limitations because of contamination risks. Earthworms can act as an intermediate host of many pathogen agents for those animals. High risk exists when earthworm is collected from uncontrolled soil which could contain toxics or toxins and distributed as fresh feed.

Technique of vermiculture and the process of transforming into earthworm powder, aimed to be incorporated in animal feed, are supposed to decrease the risk of contamination. The use of earthworm collected from controlled vermiculture is suitable for poultry feed, for example, and would not present a major risk of poisoning and intoxication to animals and humans.

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Competing Interests

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