EFFECTS, FATE AND UPTAKE OF NANOPESTICIDES IN THE TERRESTRIAL ENVIRONMENT

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Abstract

Over the past few decades a significant body of work has been done to understand the ecological and health risks of synthetic pyrethroid pesticides (SPs). Recently the use of nano-encapsulated SPs has been proposed. As nanoparticles can behave very differently from dissolved chemicals, it is possible that the effects, fate and uptake of these nano-formulated pesticides could be very different from the conventional SPs. This study therefore investigated the effects of nano-encapsulation on the fate and uptake of bifenthrin, a widely used third generation synthetic pyrethroid, in soil systems. Studies were performed, using Organisation for Economic Co-operation and Development (OECD) Guidelines and similar methods, on five soil types with different properties (total organic carbon and texture) to determine dissipation half-lives in soil, soil-water partition coefficients and uptake and depuration in the earthworm *Eisenia fetida* using analytical grade bifenthrin, a conventional bifenthrin formulation (Capture LFR) and two nano-formulations.

Persistence, sorption and uptake behavior of all the study materials varied across soil types. Generally, the persistence, sorption and uptake of bifenthrin in the conventional formulation were similar to the behavior of the non-formulated active ingredient. However, nanoencapsulation significantly affected the behavior of the bifenthrin. Results for the two nanoformulations were similar to each other but these showed enhanced persistence, decreased sorption and increased rates of uptake and depuration in the earthworms compared to the analytical grade material and the conventional formulation. We therefore anticipate that the distribution and impacts of the nanoformulation in natural soil systems will be different from currently used formulations. The observed differences in persistence and sorption behavior are possibly due to the polymer capsule 'protecting' the active ingredient from microbes and soil binding sites. Differences in uptake might be explained by differences in distribution of the bifenthrin within the organism (i.e., the nanoformulation is accummulating in the earthworm gut while bifenthrin in the conventional and non-formulated treatments is being internalised).

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Authors Declaration

The work presented in this thesis is original work and completed by myself as a PhD candidate at the Environment Department, University of York under the supervision of Professor Alistair B. A. Boxall and Professor Mark E. Hodson (January 2013 – December 2016).

The work presented in **Chapter 3** was presented at the 2015 Early Career Researchers' (ECR) conference which was held at the University of York on the 7th and 8th of April 2015.

The results presented in **Chapter 5** were presented at the SETAC Europe 26th Annual Meeting held in Nantes, France from the 22nd to 26th of May 2016.

This thesis has not been submitted for any other award at this or any other institution.

Chapter 1 Introduction

1.1 General introduction

In 1959, an American theoretical physicist Richard Feynman from the California Institute of Technology (CalTech) introduced the concept of nanotechnology in his famous talk entitled "There's Plenty of Room at the Bottom" describing a process that would allow a scientist to manipulate and control individual atoms and molecules (National Nanotechnology Initiative (NNI), accessed 2016). In 1974, over a decade later, Professor Norio Taniguchi from Tokyo University of Science coined the term nanotechnology. Taniguchi studied the developments in machining techniques over the period from 1940 until the early 1970s and predicted (correctly) that by the late 1980s techniques would have evolved to a degree that dimensional accuracy of better than 100 nanometers would be achievable. He then applied the term nanotechnology to this (Whatmore, 2006). With the development of the scanning tunnelling microscope (STM) in the early 1980s that could 'see' individual atoms, the modern nanotechnology era began. The term nanotechnology refers to the particular technological goal of precisely manipulating atoms and molecules for fabrication of macroscale products (Drexler, 1992; 1986). A more generalised description of nanotechnology was then accepted by referring nanotechnology as science, engineering and technology that are conducted at the nanoscale with materials with at least one dimension sized between 1 to 100 nanometers (USEPA, 2007). Nanotechnology as defined by size is naturally very broad. This includes the fields of science as diverse as surface science, semiconductor physics, microfabrication, organic chemistry and molecular biology (Saini *et al*., 2010).

Engineered nanoparticles (ENPs) are one of the results of the application of nanotechnology in controlling and manufacturing particles in the nanometer size range. They have the potential to be used in many areas such as energy generation and storage, agriculture and environmental remediation (Kookana *et al*., 2014). The agricultural field has received a great deal of attention in terms of the use of ENPs especially in the pesticide sector with the development of new plant protection products that are known as "nanopesticides". The term nanopesticide is used in order to explain the involvement of either very small particles of a pesticide active ingredient or other small engineered structures that are claimed to have useful pesticidal properties (Kookana *et al*., 2014). Nanopesticides could offer a wide range of benefits over conventional pesticides such as reducing the amount of active ingredient that needs to be used as well as increasing the efficacy and durability of a product (Pérez-de-Luque and Rubiales, 2009).

For a conventional pesticide, before it can be placed on the market for the use on farms and plantations, a tiered approach to environmental risk assessments (ERA) focusing on key drivers of impact is typically used (Kookana *et al*., 2014; Figure 1.1). Risk is determined using exposure and effects data (Kookana *et al*., 2014) while a tier is a step in the risk assessment approach. At lower tiers, conservative assumptions and simple studies are used to assess risk, if risk if found to be acceptable at these tiers, then the assessment can stop, if not the assessment will move to higher tiers which get increasingly more complex. For pesticide risk assessment, four tiers are typicall used with the first tier of the assessment involving a simple exposure model with defined scenarios. This includes a wide selection of organisms (i.e., aquatic and terrestrial). For aquatic organisms, tests can be done using microalgae (e.g., diatoms), invertebrates (e.g., *Daphnia magna*), fish and plants (e.g., monocotyledon). While for terrestrial organisms, earthworms (e.g., *Enchytraeus* sp.) and springtails (e.g., *Folsomia candida*) are commonly used.

The second tier involves a more complex exposure modelling including more complex laboratory effect studies such as modified exposure studies and/or toxicokinetics/toxicodynamics (TKTD) experiments. The development of species sensitivity distributions (SSD) using additional non-standards test species, preferably from taxa that is not included in the initial set of studies is also employed in the second tier. The assessment of processes such as biomagnification and indirect effects using semi-realistic exposure regimes and biological communities through the use of semi-field experiments such as microcosms and mesocosms are studied in the third tier. The fourth tier involves the determination of the conventional pesticide concentrations and effects from field application. The disadvantage of the field monitoring studies is sometime that the findings may not be able to provide a clear view of the effects of a single pesticide as the presence of multiple stressors in agro-ecosystems may interrupt the effects of any particular pesticide.

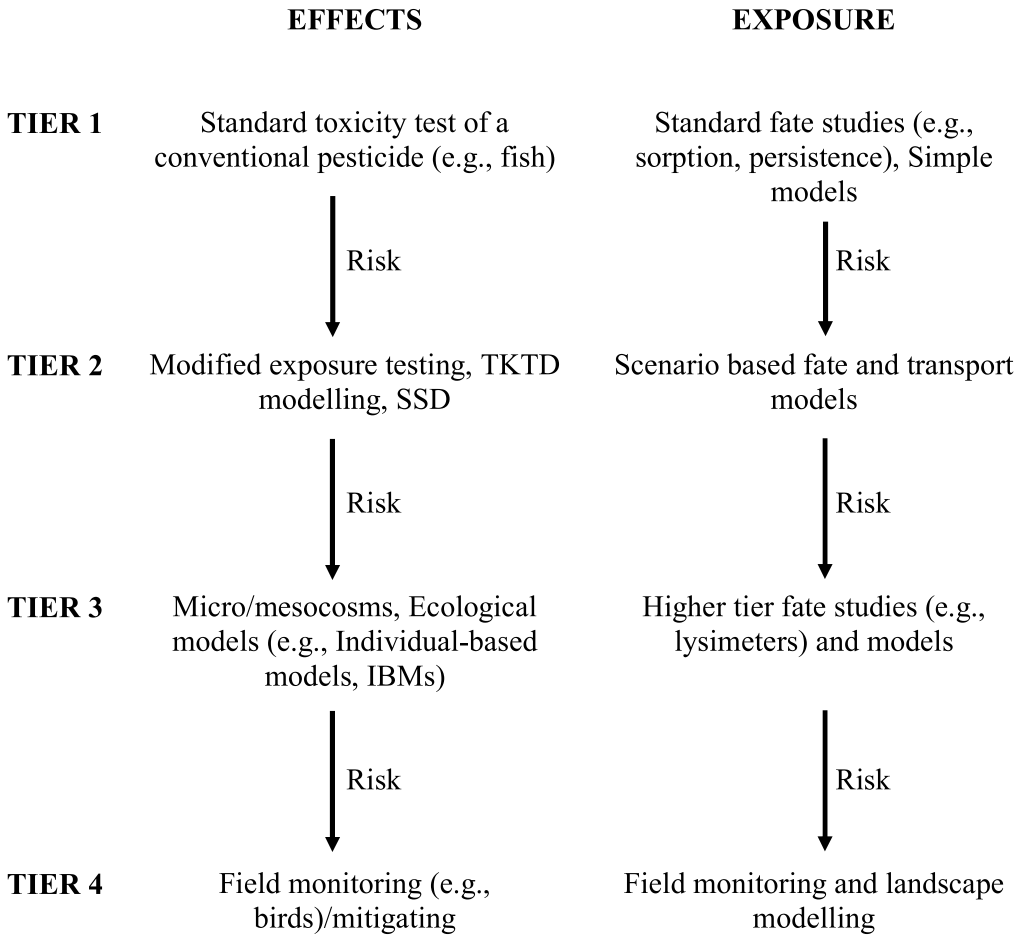


Figure 1.1 Flow of a tiered approach to environmental risk assessment (ERA) of a conventional pesticide

However, for nanopesticides, concerns have been raised about the potential risk of these new plant protection products. The fact that the fate and behavior of ENPs in the environment could be very different from the conventional products has led to concerns over the impacts of ENPs on the natural environment. The question of how the environmental risk of these products should be assessed for regulatory purpose also needs to be answered.

1.2 Aims and hypotheses of the study

The overall aim of this PhD study was therefore to better understand the impact and behavior of a nanopesticide in the terrestrial environment. The main objectives were to:

1. Review the current knowledge on the application of nanomaterials in the pesticides sector and on the fate and impact of nanomaterials, especially in the terrestrial environment (*Chapter* 2);
2. Perform a preliminary study to explore the uptake and effects of nanopesticides in the earthworm, *Eisenia fetida* in order to inform work in Objectives 3 – 5 (*Chapter* 3);
3. Explore how nanoencapsulation affects the fate (sorption and persistence) of pesticide active ingredients in different soil types (*Chapter* 4);
4. Explore how nanoencapsulation impacts the uptake and depuration kinetics and distribution of pesticide active ingredients in *E. fetida* and *Lumbricus terrestris* (*Chapter* 5); and
5. Investigate the effects of soil properties on the uptake kinetics of a nano-encapsulated pesticide active ingredient in *E. fetida* (*Chapter* 6).

The hypotheses for this study were:

1. The sorption of a nano-encapsulated pesticide active ingredient to soil particles is expected to be lower compared to the conventional formulation hence increasing the bioavailability of the active ingredient in soil pore water;
2. Nanoencapsulation of a pesticide active ingredient will enhance the persistence of the active ingredient in soils compared to conventional formulations;
3. The uptake of a nano-encapsulated active ingredient from soil into earthworms will be higher compared to the conventional formulation due to the material being more bioavailable in the soil pore water;
4. The distribution of a nano-encapsulated active ingredient in the earthworm body could be different compared to the conventional formulation due to the different routes (i.e., through passive diffusion and/or ingestion of soil particles) of uptake by earthworm; and

5. The fate (sorption and persistence), uptake and effects of a nano-encapsulated pesticide in the terrestrial environment will be affected by the properties, including pH, organic matter and texture, of the test soil.

1.3 Test compounds

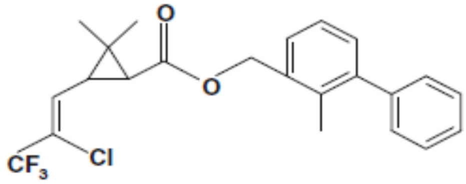
Recently, nano-encapsulated synthetic pyrethroids (SPs) have been developed by Vive Crop Protection Inc. based in Toronto, Canada. These nano-encapsulated formulations are prepared using the Allosperse^{TM} targeted delivery system. The preparation starts with the dissolution of negatively charged polymers in water. Since the polymers have the same charges, they repel each other leading the polymers to spread out in the solution. A precursor containing positively charged ions is added to the solution, hence neutralising the negative charge on the polymers causing them to collapse around the ion. At this stage, the polymers are cross-linked to create Allosperse^{TM} particles. The ions are filtered, leaving Allosperse^{TM} polymer particles with a hydrophobic (water-repelling) core and a water-soluble shell. These particles can then be loaded with a variety of active ingredients such as pesticides to make the apparent solubility of the active ingredients greater than the actual active ingredient on its own. This process has been adopted in order to prepare two different nano-encapsulated formulations of bifenthrin, a third generation synthetic pyrethroid (SP) (Table 1.1 – 1.2).

SPs chemicals are synthetic esters derived from the naturally-occurring pyrethrins and have been widely used as insecticides and acaricides (Briggs, 1992). Their use has increased in the recent years because they are selective in action. This is mainly due to the differences in uptake and distribution and the fact that they are easily degradable in the environment compared to other classes of pesticides (Casas *et al*., 2007; Stenersen, 2004). Commercially-available SPs includes bifenthrin, a widely used third generation synthetic pyrethroid. This group of SP is characterised by their greater photostability and insecticidal activity compared to earlier generation pyrethroids (Mokry and Hoagland, 1989). Pyrethroids utilise a number of different pathways to cause nervous system damage in invertebrates (Miller and Salgado, 1985). The interference with sodium channel gating in the nerve cell endings is one example (Narahashi, 2002; Miller and Salgado, 1985; Lund and Narahashi, 1981). They effectively paralyze organisms by severely limiting neuro-transmission by acting on the sodium channels to depolarise the pre-synaptic terminals (Salgado *et al*., 1983).

SPs have also been shown to inhibit ATPase enzyme production which is one reason why they are considered extremely toxic to fish and aquatic organisms (USEPA, 2003; Briggs, 1992; Clark and Matsumura, 1982). Ionic balance and osmoregulation should be maintained by the aquatic organisms in an extremely dilute environment, therefore active transport at cellular walls is needed to maintain critical cellular ion levels against a concentration gradient. Pyrethroids inhibit ATPase enzymes, resulting in the breakdown of the critical concentration gradient, leading to the death of the organisms. Pyrethroids have the most serious effects on fish and gill breathing aquatic insects because of the availability of the large surface area to de-ionise after ATPase inhibition (Siegfried, 1993).

The conventional formulation of bifenthrin used in the study was Capture LFR which is a patented formulation with Liquid Fertilizer Ready® technology is prepared to help provide an even application by keeping the active ingredient in suspension. Capture LFR helps to controls seed and seedling pests, such as wireworms, cutworms, grubs, armyworms, seedcorn maggots and common stalk borers.

Table 1.1 Properties of the analytical grade bifenthrin used in the study

Properties	**Bifenthrin PESTANAL®[a]**
Ingredients	Bifenthrin
Percentage, % (w/w)	98.9
Chemical formula[b]	$C_{23}H_{22}ClF_3O_2$
Molecular weight (g mol^{-1})[b]	422.88
Solubility in water at 20 °C (mg L^{-1})[b]	0.001
Log K_{ow} at pH 7, 20 °C[b]	6.6
Vapour pressure at 25 °C (mPa)[b]	0.0178
Henry's law constant at 25 °C (Pa m^3 mol^{-1})[b]	7.74×10^{-5}
K_d (mL g^{-1})[b]	992 – 5429
K_{oc} (mL g^{-1})[b]	130526 – 301611
Structure[c]	

[a] Bifenthrin PESTANAL® grade was purchased from Sigma-Aldrich (Dorset, UK)

[b] (University of Hertfordshire., 2016)

[c] (Sharma and Singh, 2012)

Table 1.2 Properties of the different bifenthrin formulations used in the study

Bifenthrin formulation	**Ingredients**	**Concentration in formulation (% w/w)**
Capture LFR[a]	Bifenthrin[c]	17.15
	Others	82.15
Nano A[b]	Water	40 – 70
	Bifenthrin	10 – 30
	Acrylates copolymer	3 – 7
	Sodium methyl oleoyl taurate	1 – 5
Nano B[b]	Water	40 – 70
	Bifenthrin	10 – 30
	Acrylates copolymer	3 – 7
	Sodium alkylnaphthalenesulfonate, formaldehyde condensate	1 – 5

[a] Capture LFR was obtained from FMC Corporation (Philadelphia, USA)

[b] Nano A and Nano B were obtained from Vive Crop Protection Inc. (Toronto, Canada)

[c] cis isomers 97% minimum, trans isomers 3% maximum

1.3.1 Fate and behavior of synthetic pyrethroids (SPs)

The release of SPs into terrestrial environment occurs in many ways, largely through spray drift from both agricultural and non-agricultural applications. Direct application and accidental spills to soil surfaces are also considered sources of SP release (Palmquist *et al.*, 2012). Agricultural synthetic pyrethroids (e.g., allethrin, metofluthrin, bifenthrin, cypermethrin, fenvalerate, phenothrin, tetramethrin) have a very low vapor pressure (Vp) of around 10^{-8} mm Hg at 25 °C (Laskowski, 2002). Therefore, distribution of the SPs into the air compartment is considered less important. In addition, the Henry's law constants (the parameter used to estimate volatilisation process of a chemical) of SPs are low indicating that they have a low tendency for volatilisation into air compartment (Lyman *et al.*, 1990).

Generally, SPs show a high affinity to soils and organic matter, therefore they are unlikely to undergo significant migration from areas of direct application. However, SPs may be found in air attached to soil particles or may be transported to in spray drift. Basically, the movement of SPs in soil compartment is controlled by diffusion, convection and dispersion processeses (Katagi, 2012). The use of a column leaching study is normally conducted to investigate the mobility of SPs in soil compartment following the OECD guideline using a soil-packed columns or intact undisturbed soil cores treated with radio-label SPs to investigate potential leaching to groundwater (OECD, 2004). A study done by Singh (2012) involving five different SPs (i.e., bifenthrin, λ-cyhalothrin, cypermethrin, fenvalerate and deltamethrin) showed that these SPs were found to reside mostly in the top section of the columns (i.e., 0 – 5 cm). Soil characteristics such as organic matter content was found to play an important role in affecting the mobility of SPs (Ismail and Kalithasan, 2004). The study was done using permethrin in Malaysian soils and showed that the insignificant movement of permethrin was related with the higher soil-organic carbon coefficients (K_{oc}) resulting in a lower mobility in the column study.

In terms of persistence, half-lives of SPs are highly variable ranging from 12 days (cyfluthrin) to 96 days (bifenthrin) (Oros and Werner, 2005). Degradation processes for SPs can occur in different ways such as microbial degradation (i.e., biodegradation) by microbes living in the soil and photolysis. Biodegradation of SPs has been observed to be fastest in natural soils compared to sterilised soils. This indicates that the breakdown of SPs in soils is influenced by the biological processes. For example, Chapman *et al*. (1981) found that after eight weeks following application, the concentrations of cypermethrin, fenpropanate and permethrin in soils were reduced to 20% of the original amount in both natural mineral and organic soils, while in sterilised soils, the concentrations in soils were more than 80% of the initial concentrations. Another example of SPs degradation is reported by Fecko (1999) showing the potential degradation of bifenthrin resulting in the formation of metabolites (Figure 1.2).

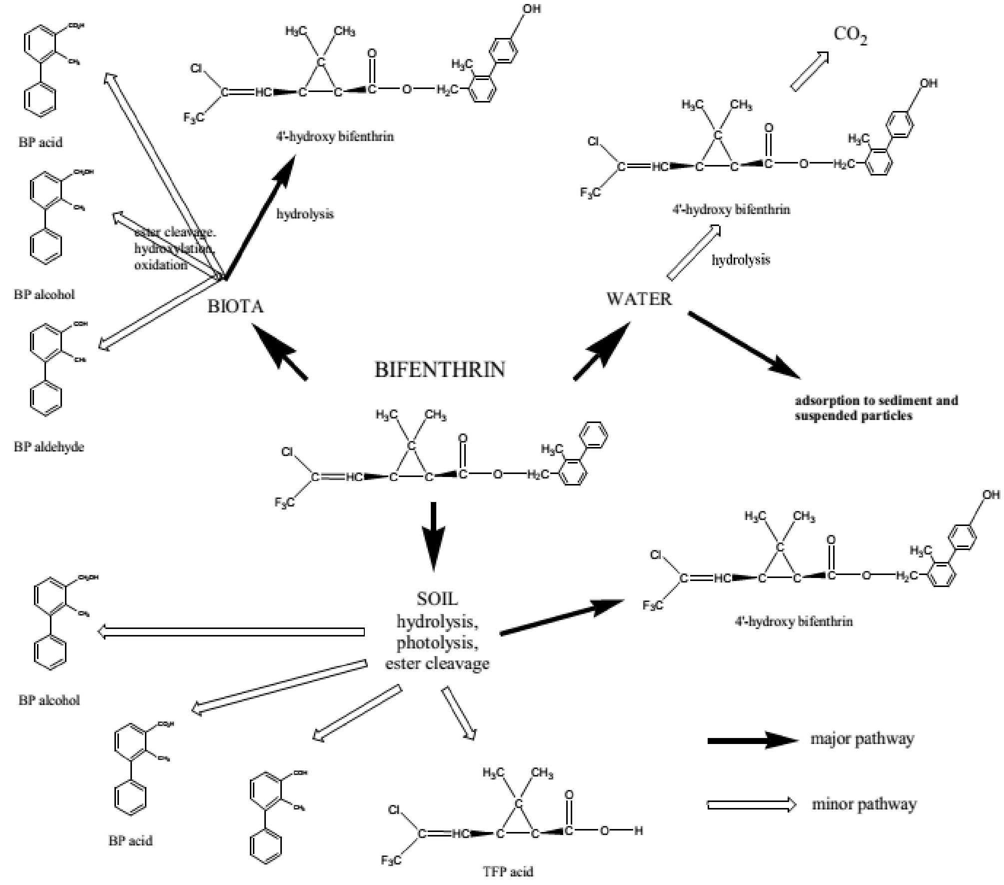


Figure 1.2 Potential degradation mechanisms of bifenthrin (Fecko, 1999)

A study was done by Tallur *et al*. (2008) investigating the biodegradation of cypermethrin using *Micrococcus* sp. (Figure 1.3). The organism was also found to utilise deltamethrin, fenvalerate and perimethrin as growth substrates. Cypermethrin was degraded by hydrolysis of the ester linkage to produce 3-phenoxybenzoate resulting in a loss of its insecticidal activity and further metabolised by diphenyl ether cleavage to produce protocatechuate and phenol. Both protocatechuate and phenol were then oxidised by the ortho-cleavage pathway. The authors concluded that *Micrococcus* sp. was a good candidate in detoxification resulting in a complete mineralisation of cypermethrin (Tallur *et al.*, 2008).

Photolysis is another degradation pathway for SPs in the soil and is influenced by soil characteristics (Palmquist *et al.*, 2012). Katagi (1991) found that the half-life of esfenvalerate was significantly increased under dark conditions in different soil types, with half-lives of 8 to 100 days being seen under continuous irradiation compared to 150 to 553 days in the dark conditions. Soil analysis was performed and revealed that esfenvalerate was largely present in complexes with humic acid indicating that photolytic degradation of SPs proceeds more slowly in highly organic soils.

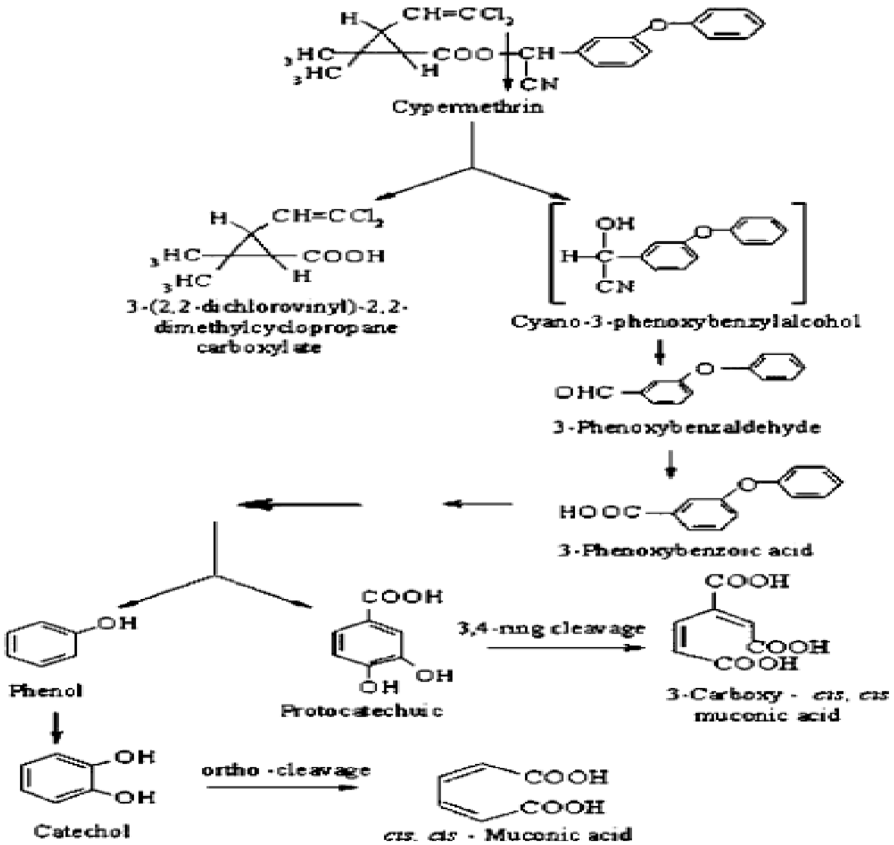


Figure 1.3 Proposed biodegradation pathway of cypermethrin by *Micrococcus* sp. strain CPN 1 (Tallur *et al.*, 2008)

1.4 Test species

The effects and uptake of analytical grade, conventionally-formulated and nano-encapsulated bifenthrin were assessed using two different earthworm species: *Eisenia fetida* and *Lumbricus terrestris* (Table 1.3). Earthworms are known to bio-magnify inorganic and organic soil contaminants, including pesticides, polycyclic aromatic hydrocarbons (PAHs), brominated flame retardants, and metals (Giovanetti *et al.*, 2010; Grumiaux *et al.*, 2010; Hinton and Veiga, 2008; Langdon *et al.*, 2005; Sellstrom *et al.*, 2005; Davies *et al.*, 2003; Matscheko *et al.*, 2002; Heikens *et al.*, 2001; Ma *et al.*, 1998, 1995; Janssen *et al.*, 1997; Van Gestel and Ma, 1988). The fact that earthworms can take up soil contaminants in number of ways (i.e., passive diffusion and dietary uptake), make them good candidates for being key indicator species with regards to understanding the toxicity of pollutants in soils.

Generally, *E. fetida* is an epigeic species which inhabit the top soil layers or on the soil surface residing in loose organic litter and debris (Edwards, 2004; 1996). On the other hand, *L. terrestris* is an anecic species which forms deep burrows in the soil and can go deep into the mineral soil layers. They feed primarily on decaying surface litter and therefore come to the soil surface more regularly.

1.4.1 *Eisenia fetida*

Eisenia fetida belongs to the Lumbricidae family of earthworms which is characterised by their segmented body. They are also known as redworm, brandling worm, panfish worm, trout worm, tiger worm, red wiggler worm and red californian earthworm. They are recommended as a good terrestrial animal for chemical testing by the Organisation for Economic Cooperation and Development (OECD) in Guidelines 207, 222, 317 (OECD, 2010, 2004, 1984). This is due to the fact that *E. fetida* is easily cultured in the laboratory, has a fast reproduction time and short generation time. *Eisenia fetida* is considered a robust species and found in a number of terrestrial environments in many regions, for example, European countries. In addition, they can tolerate a wide range of environmental conditions (i.e., soil pH (4.0 – 7.0), moisture (70 – 85%), and temperature (20 – 29 °C) (Gunadi *et al.*, 2003; Edwards, 1996). *Eisenia fetida* is mostly found in vermicomposting bins or on earthworm farms and can be considered a litter earthworm as it scavenges organic wastes. Because *E. fetida* is an epigeic species, it can also be found within the ground cover of leaves in the woods.

1.4.2 *Lumbricus terrestris*

Lumbricus terrestris also belongs to the Lumbricidae family of earthworms. *Lumbricus terrestris* is a larger species compared to *E. fetida*. They are known as the night crawler as they crawl to the surface of the soil during the night to feed. Compared to *E. fetida*, *L. terrestris* have a long generation time, require a stable burrow environment and are not able to tolerate a high culture density. Without a stable burrow, *L. terrestris* will face difficulties in both breeding and growing.

Table 1.3 Properties of *E. fetida* and *L. terrestris*

Parameter	***E. fetida***	***L. terrestris***
Family	Lumbricidae	Lumbricidae
Group	Epigeic	Anaecic
Length (mm)	60 – 120*	90 – 350*
Diameter (mm)	3 – 6*	6 – 10*
Number of segments (mm)	80 – 120*	140 – 155*
Location	Soil surface*	Deep burrows*
Soil pH tolerance	4.3 - 7.5*	6.2 - 10.0*

* (Sims and Gerard, 1985)

1.5 Description of thesis chapters

The thesis comprises seven chapters. A brief description of the contents of each Chapter is given below:

Chapter 2 reviews the current knowledge of the application of nanomaterials in the pesticide sector. The existing knowledge on the fate and impacts of nanomaterials in the terrestrial environment are also discussed and major knowledge gaps identified.

Chapter 3 describes experimental studies to understand the uptake and effects of bifentrin contained in nano and conventional formulations in the earthworm, *E. fetida*. This study was used to inform the design of the subsequent studies. In this study, earthworms were exposed to nano-encapsulated treatments (Nano A and Nano B) or Capture LFR. Soil pH, weight change, concentration in soil, concentration in soil pore water, production of cocoons and juveniles, and mortality of the *E. fetida* for the different treatments were measured every week during the experimental period and the degree of uptake of bifenthrin into *E. fetida* was determined at the experimental period.

Chapter 4 describes environmental fate studies (sorption and persistence) using nano and conventional formulations and analytical grade bifenthrin. This chapter explores the differences in the sorption coefficients (K_d) and half-life (DT_{50}) in soils treated with either analytical grade, conventionally-formulated or nano-encapsulated bifenthrin. The results are used to establish the release rates of bifenthrin from the nano-formulated bifenthrin (Nano A and Nano B) as well as the R_{50} values (time required for half the bifenthrin to be released from the nanocapsules).

Chapter 5 describes the uptake and depuration, and distribution of bifenthrin in the *E. fetida* body when exposed to either analytical grade bifenthrin or nano or conventional formulations of bifenthrin. This study was done in one type of soil chosen from the findings obtained in *Chapters* 3 and 4. The results from the uptake and depuration studies were fitted to a first order one-compartment model in order to calculate the uptake and depuration rates and bioconcentration factors (BCFs) for bifenthrin in the different treatments. For the determination of the distribution of the active ingredient bifenthrin in the earthworm body, two species of earthworms were used: *Eisenia fetida* and *Lumbricus terrestris*. This was done in order to explain the route of uptake of different bifenthrin treatments into earthworm by investigating the differences in distribution within the different species.

Chapter 6 explores the effects of soil properties on the uptake and depuration of the different bifenthrin treatments in *E. fetida*. This study was conducted following the same procedures as in *Chapter* 5 except no distribution studies were done. Results obtained were fitted with a first order one-compartment model to determine the uptake and depuration rates and BCF values of for bifenthrin for the different treatments.

Chapter 7 summarises the main findings as well as the conclusions of the study. The chapter describes broader implications of the use of nanopesticides in the terrestrial environment. Several recommendations for further studies concerning the use of nanopesticides in the terrestrial environment are also presented.

Chapter 2 Literature Review

2.1 Pesticides

Pesticides are the only group of chemicals that are purposely applied to the environment with the aim to suppress plant and animal pests and to protect agricultural and industrial products. According to the Food and Agriculture Organisation, FAO (2002) a pesticide is defined as any substance or mixture of substances intended for preventing, destroying or controlling any pest. This includes vectors of human or animal disease and unwanted species of plants or animals. Pesticides are applied to prevent any harm during or otherwise interfering with the production, processing, storage, transport or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs. Pesticides include both organic and inorganic moieties and may be classified into different groups based on their chemical composition. These include organochlorines, organophosphates, carbamates, formamidines, thiocyanates, organotins, dinitrophenols, synthetic pyrethroids and antibiotics (Bohmont, 1990).

A pesticide may be categorised on the basis of the organism (i.e., pest, pathogen, and parasite) curbed/controlled or killed by its application. For example, insecticides (e.g., organochlorines, organophosphates, and carbamates), act primarily by disrupting nervous system function, in particular, four nerve targets: acetylcholinesterase, voltage-gated chloride channel, the acetylcholine receptor and the γ-aminobutyric acid receptor (Kalia and Gosal, 2011). In contrast, herbicides often kill or injure plants by targeting plant-specific pathways. For example, by blocking photosynthesis, carotenoid synthesis or aromatic and branched chain amino acid synthesis in plants (DeLorenzo *et al*., 2001; Ecobichon, 1991). Herbicides affect mechanisms associated with vital processes such as photosynthesis, respiration, growth, cell and nucleus division or the synthesis of proteins, carotenoids or lipids (Ecobichon, 1991). Fungicides target various pathways that disrupt basic cellular functions, block fungal lipid biosynthesis, protein biosynthesis or essential enzymes (Casida, 2009).

As a result of their use patterns, it is inevitable that pesticides will be released into the natural environment. As they are bioactive molecules, these substances have the potential to impact non-target organisms. The degree of impact will depend on the use practices, environmental fate and intrinsic properties of the pesticide active ingredient which are discussed in the following sections.

2.1.1 Environmental fate of pesticides

The fate of pesticides in soil is controlled by the chemical, biological and physical dynamics of the soil (Sparks, 2003). These processes can be grouped into those that affect persistence, including chemical and microbial degradation, and those that affect mobility, involving sorption, plant and animal uptake, volatilisation, wind erosion, run-off and leaching (Andreu and Pico, 2004; Figure 2.1). Pesticides can be degraded by chemical and microbiological processes. Chemical degradation generally occurs in water or the atmosphere through reactions such as photolysis, hydrolysis, oxidation and reduction (Bavcon *et al*., 2003; Kodaka *et al*., 2003). Biological degradation takes place when soil organisms consume or break down pesticides (Sassman *et al*., 2004; Ghadiri *et al*., 2001). These microorganisms are mainly distributed in the top centimetres of the surface layer of the soil, where the organic matter acts as a food supply (Navarro *et al*., 2004). The extent of degradation ranges from formation of metabolites to decomposition to inorganic products (Kale *et al*., 2001).

Biodegradation involves a metabolic cooperation process which is known as the transfer of substrates and products within a well-coordinated microbial community (Abraham *et al*., 2002). Microorganisms such as fungi and bacteria are considered as the main pesticide transformers and degraders (Briceño *et al*., 2007). Generally, fungi biotransforms pesticides (e.g., SPs) in the soil by introducing minor changes on the molecular structure, rendering it nontoxic. While bacteria will further degrade the biotransformed pesticides (Diez, 2010). Both fungi and bacteria are known as the extracellular enzyme-producing microorganisms. These enzymes are involved in lignin degradation (e.g., lignin peroxidase, oxidases, manganese peroxidase).

Pesticide degradation is influenced by various parameters including pesticide and soil properties (Rosales-Conrado *et al*., 2002, LaPrade, 1992). The structure of pesticide such as the present of polar groups (e.g., -OH, -COOH and -NH_2) may induce microbial degradation. In contrast, the presence of halogen or alkyl substituents tends to make the pesticides more resistant to biodegradation (Shahgholi and Ahangar, 2014; Cork and Krueger, 1991). The physico-chemical properties of the pesticide such as sorption and volatilisation also play an important role in affecting the degradation of pesticide (Edwards, 1975). Other factor such as the soil properties including soil pH, clay and organic matter are also reported to affect the degradation of pesticides (Salvati *et al*., 2011; Regitano *et al*., 1997; Peter and Weber, 1985).

There is also increasing interest in the transformation products (TPs) of pesticides, because they can be present at higher levels in soil than the parent pesticide itself. Generally, pesticide TPs show lower toxicity to biota than the parent compounds (Nawab *et al*., 2003). However, in some cases, TPs are more toxic, so they represent a greater risk to the environment than the parent molecules (Sassman *et al*., 2004; Bavcon *et al*., 2003; Nawab *et al*., 2003; Pozo *et al*., 2001). Differences in the environmental behavior of many TPs compared to the parents mean that even though a TP is less toxic than its parent, it may still have the potential to produce an adverse impact on the environment (Sinclair *et al*., 2003; Patsias *et al*., 2002; Pozo *et al*., 2001). As a result, there is a need to consider TPs during the environmental risk assessment process.

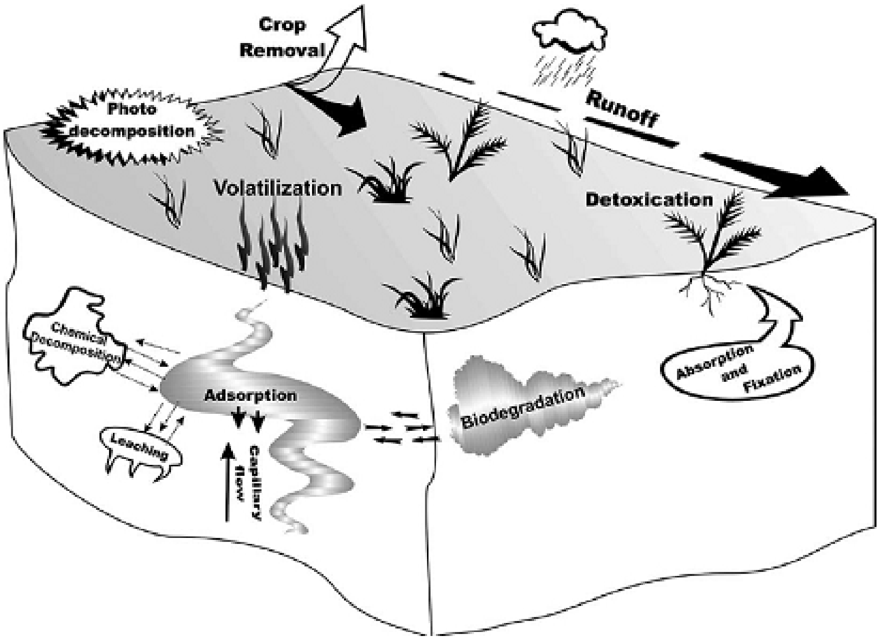


Figure 2.1 Fate of organic pollutants (e.g., pesticides) in soil compartment (Andreu and Pico, 2004)

2.1.2 Factors affecting the fate of pesticides

There are four major factors which affect the fate of pesticides: properties of the pesticide, properties of the environment, conditions of the site (e.g., humid or arid regions) and management practices (Rosales-Conrado *et al*., 2002; Perrin-Ganier *et al*., 2001; LaPrade, 1992). These will be discussed further in the next sub-sections.

2.1.2.1 Pesticide properties

Pesticide properties which affect movement to groundwater include solubility, adsorption behavior, volatility, and persistence. Chemicals which dissolve readily in water are said to be highly soluble. As water seeps downward through the soil, it carries with it water-soluble chemicals. This process is called leaching. Highly soluble pesticides, therefore, have a tendency to be leached from the soil to groundwater (Toth and Buhler, 2009; LaPrade, 1992). On the other hand, many pesticides do not leach because they are adsorbed or tightly held by soil particles. Adsorption depends not only on the chemical, but also on the soil type and amount of soil organic matter present. Highly volatile chemicals are easily lost to the atmosphere, similar to the evaporation of water (Sparks *et al*., 2003). If a pesticide is highly volatile and not very water soluble, it is likely to be lost to the atmosphere, and less will be available for leaching to groundwater (Kerle *et al*., 2007). Highly volatile compounds may become groundwater contaminants, however, if they are highly soluble in water.

2.1.2.2 Soil properties

The movement of pesticides is influenced by several soil properties including soil texture, soil permeability, soil pH, dissolved organic matter (DOM) and organic matter content (Salvati *et al*., 2011; Cáceres-Jensen *et al*., 2009; Li *et al*., 2005; Dolaptsoglou *et al*., 2007; Kerle *et al*., 2007; Tiryaki and Aysal, 1999; Regitano *et al*., 1997; Lee *et al*., 1990). Soil texture is determined by the relative proportions of sand, silt, and clay. Texture affects movement of water through soil and, therefore, affects the movement of dissolved chemicals, such as pesticides. The coarser the soil, the faster the movement of the percolating water, and the less opportunity for adsorption of dissolved chemicals. Soils with more clay and organic matter tend to hold water and dissolved chemicals longer (Kerle *et al*., 2007; Navarro *et al*., 2004;

Tiryaki and Aysal, 1999). These soils also have far more surface area on which pesticides can be adsorbed.

Soil permeability is a measure of how fast water can move downward through a particular soil. Water moves quickly through soils with high permeability (Patsias *et al*., 2002). They also lose dissolved chemicals with the percolating water. In highly permeable soils, therefore, the timing and methods of pesticide application need to be carefully designed to minimise leaching losses (LaPrade, 1992). Soil pH also plays an important role affecting the fate of ionisable pesticides. Soil pH governs the ionisation of many organic molecules. For example, Cáceres-Jensen *et al.* (2009) found that glyphosate, which can exist as an anion in soil, sorbed more strongly to the solids when soil pH decreased. Soil organic matter influences how much water a soil can hold and how well it will be able to adsorb pesticides (Tiryaki and Aysal, 1999; Kozak *et al*., 1983). Increasing the soil's organic content, through practices such as application of manure or ploughing under of cover crops, increases the soil's ability to hold both water and dissolved pesticides in the root zone where they will be available to plants and to eventual degradation (Salvati *et al*., 2011).

2.1.2.3 Site conditions

The conditions of the site where a pesticide is applied can also affect the movement of the pesticide. Such conditions include the depth to groundwater, geologic conditions, and climate (Gustafson, 2011; Fritz and Hoffmann, 2008; Nolan *et al*., 2008). The shallower the depth to groundwater, the less soil there will be to act as a filter. Also, there will be fewer opportunities for degradation or adsorption of pesticides in the soil. Therefore, extra precautions need to be taken to protect groundwater in areas where it is close to the ground surface. In humid regions, groundwater may be only a few metres below the surface of the soil (Racke *et al*., 1997). If rainfall is high and soils are permeable, water carrying dissolved pesticides may take only a few days to percolate downward to groundwater (LaPrade, 1992). In arid regions, groundwater may be several hundred metres below the soil surface, and leaching of pesticides to groundwater may be a much slower process (Shunthirasingham *et al*., 2010). In addition to depth to groundwater, it is important to look at the permeability of the geologic layers between the soil and groundwater.

Highly permeable materials, such as gravel deposits, allow water and dissolved pesticides to freely percolate downward to groundwater.

2.1.2.4 Management practices

The method used to apply the pesticide and the rates and timing of application are important factors affecting the fate of pesticides (Larsbo *et al*., 2009; Triantafyllidis *et al*., 2009; Locke *et al*., 2008; Kerle *et al*., 2007; Reichenberger *et al*., 2007; Gustafson, 1989). Injection or incorporation into the soil, as in the case of nematicides, makes the pesticide most readily available for leaching. Most of the pesticides that have been detected in groundwater are ones which are incorporated into the soil rather than being sprayed onto growing crops. The rate and timing of a pesticide's application are also critical in determining whether it will leach to groundwater (McBride, 1989). The larger the amount used and the closer the time of application to a time of heavy rainfall or irrigation, the more likely it is that some of the pesticide will leach to groundwater.

2.1.3 Environmental effects of pesticides

Due to their bioactive nature, pesticides can negatively impact non-target organisms in the environment. For example, negative impacts on soil microbial flora such as killing certain specific group(s) of microorganisms have been reported following exposure to pesticides (Araujo *et al*., 2003). A consequent decrease in microbe numbers can disturb specific processes in ecosystems performed by an individual or group, and disrupt the components relying upon it. A series of modifications is triggered in most microbial flora/fauna groups, which leads to changed prey-predator relationships that may cause changes in soil aggregation, soil chemistry, pH and structure as soil organic matter is depleted (Bossuyt *et al*., 2001). The degraded soil becomes more prone to erosion to surface water bodies following heavy rain and also responds less to ever high fertilizer input. Soil becomes barren over a period of few decades because only negligible organic matter is left to sustain microbial growth and development (Chowdhury *et al*., 2008). Pesticides which are more resistant to degradation by abiotic (i.e., physical, chemical, and other factors) and biotic (i.e., living organisms) processes are not being adsorbed to surface soils which then will leach into the lower strata of the soil, are taken up by plant roots, accumulate in the food chain and ultimately biomagnified in the food web.

Previous studies have confirmed the accumulation of pesticide residues in plant (Waliszewski *et al*., 2008; Babu *et al*., 2003) and animal tissues (Nakata *et al*., 2002; Hans and Farooq, 2000; Sofina *et al*., 1993). The biomagnification of pesticides in plant and animal tissues (particularly in lipid bodies) makes their use hazardous to health and may lead to several ailments. Over the decades, there has been a considerable increase in pesticide use and a simultaneous increase in the problem of biomagnification has been encountered in soil (Hans and Farooq, 2000), in plant and animal products such as cereals (Babu *et al*., 2003), fruits and vegetables (Waliszewski *et al*., 2008), and in milk and milk products (Kannan *et al*., 1997).

In addition, there is the emerging problem of the development of pesticide-resistant pests, which may resist even higher concentrations of pesticides. A field study on the movement of isomers of the organchlorine compounds Dichlorodiphenyltrichloroethane (DDT) and Hexachlorocyclohexane (HCH) was performed by Waliszewski *et al*. (2008). This study showed the diffusion of organochlorine pesticide from agricultural soils to growing carrot plants and the adsorption of organochlorine vapours by the leaves. Organochlorine pesticides accumulate within the carrot plant, especially in the root peel, which shows levels 3 – 7 times higher than those in the flesh of the root (Waliszewski *et al*., 2008). The principal source of these residues is considered to be their deposition in agricultural soils from where they are subsequently adsorbed by the roots, although they may also be adsorbed by the leaves of growing plants on volatilisation (Bidleman and Leone, 2004).

In conclusion, the environmental fate and effects of conventional pesticides are two of the main drivers determining the impact on the terrestrial environment. By altering formulations so that the fate and behavior of a pesticide are altered, the risks posed by the pesticide could be mitigated. One of the solutions is by incorporating the conventional pesticides with other components that can be used and applied to the crops for a better performance while keeping the risks to a minimum. One way to alter how a pesticide will behave is to use nanotechnology which employs engineered nanoparticles (ENPs) to develop formulation of pesticide with better performance compared to the conventional form. The application of nanotechnology for pesticide formulation is discussed in the following section.

2.2 Nanopesticides

2.2.1 Engineered nanoparticles (ENPs)

ENPs are synthesised products which are often developed with the aim of having better performance compared to the non-nano form of a substance due to their unique properties relating specifically to their dimensions. The term nano is defined as a scale of a billionth (10^{-9}), thus a nanometre (nm) is a billionth of a metre. ENPs can be referred to as particles that have at least one dimension ranging from 1 to 100 nm in size (Auffan *et al.*, 2009). ENPs are synthesised either via a structured organisation of atoms and molecules, or reduction of a macroscopic material to the nm scale. Due to the fact that particles show different or specific properties when they are at the nm scale, research into understanding the potential applications of ENPs has been widespread. The specific properties may include chemical, biological, structural, optical and mechanical effects. It is noted that particles in the nm scale have always existed. For example, Deoxyribonucleic acid (DNA) has a diameter of approximately 2.5 nm and viruses have diameters of 10 – 60 nm. Particles such as oil fumes, fumes from volcanic activity and certain atmospheric dusts are other examples of materials within the nm scale that already exist in the environment as well as the unintended products from human activity such as industrial blast furnace emissions and welding fumes (Teague, 2004).

Over the last few years, ENPs have begun to be used in numerous product types (Posner, 2009). For example: silver ENPs are now widely used in many commercial products such as textiles and medical devices; fullerenes are used in medical, cosmetic, electronic and optic applications (Bradford *et al.*, 2009; Isaacson *et al.*, 2009); cerium dioxide (CeO_2) ENPs are used in the automotive area as a catalyst (Hoecke *et al.*, 2009); zinc oxide (ZnO) ENPs are used in solar cell devices (i.e., photoelectrode) (Chen *et al.*, 2009b); and titanium dioxide (TiO_2) ENPs are used in sunscreens, paints, coatings and cosmetics (Fang *et al.*, 2009; Aarthi and Madras, 2008). The increasing use of ENPs will eventually lead to the spread of these ENPs into the terrestrial environment (Geranio *et al.*, 2009).

2.2.2 What is nanopesticide?

The idea of preparing novel plant protection products, known as nanopesticides, as an alternative to conventional pesticides is growing. The definition of a nanopesticide is highly debated. In general, a nanopesticide is defined as a pesticide nanoformulation that involves either very small particles of pesticide active ingredient or other small engineered structures (i.e., ENPs) with useful pesticidal properties (Kookana *et al*., 2014).

Overall, the development of nanopesticides is expected to increase the apparent solubility of poorly soluble pesticide active ingredients and/or result in the release of the pesticide active ingredient in a slow/targeted manner to the target area and/or protect the pesticide active ingredient from premature degradation. Increasing the solubility of poorly soluble pesticide active ingredient can be done using additives (i.e., surfactants) or changing the solid structure of the pesticide active ingredient (Horn and Rieger, 2001). Both of these approaches have been successfully used leading to an increase in the bioavailability of the pesticide active ingredient. This has motivated researchers to develop new nanoformulations for better delivery (e.g., nanoemulsions and nanodispersions).

Studies have been performed in order to investigate the impact of nanoemulsions on the environment which include a possible higher efficacy and, reduced hydrolysis and volatilisation of the pesticide active ingredient (Anjali *et al*., 2010; Song *et al*., 2009; Yang *et al*., 2009). However, nanoemulsions have been reported to have a relatively high kinetic stability and are often considered to be metastable (Gutierrez *et al*., 2008). Because the preparation of nanoemulsion requires high-energy input, work has been performed to develop a low-energy method using spontaneous emulsification and phase inversion temperature methods (Anton *et al*., 2008). To prepare nanoemulsions that are stable over time remains challenging.

Nanodispersion describes a method of incorporating the active ingredient with other components through the dispersion process of nanoparticles in liquid media. This method was proposed in order to produce a high surface area (relative to volume) of the particles which then can be expected to increase the dissolution and solubility of the pesticide active ingredient (Müller and Junghanns, 2006). A novel nanodispersion of lambda-cyhalothrin active ingredient formulation was successfully prepared by Cui *et al*. (2015) using the combination of melt-emulsification and high-speed shearing. With a lower size range (21.7 nm diameter particles), this nanodispersion formulation was reported to increase the dispersibility, stability and bioavailability of the active ingredient compared to the conventional formulation, thus improving the efficiency of the pesticide.

In the next section, several types of nanopesticides are discussed including the incorporation of pesticide active ingredient with clays, layered double hydroxides (LDHs), porous hollow silica, metals and metal oxides, solid lipids and polymers.

2.2.3 Types of nanopesticides

The potential applications of nanotechnology in the agricultural area including the incorporation of pesticides active ingredient with nanomaterials (i.e., encapsulated active ingredient by nanomaterials), stabilisation of biopesticides with nanomaterials, slow release and nanomaterial assisted delivery of genetic material for crop improvement (Ghormade *et al*., 2011) are summarised in Table 2.1. Among the inorganic nanomaterials, AgO, MgO, TiO_2 and ZnO are of interest due to their physically and optically stable properties (Makhluf *et al*., 2005; Stoimenov *et al*., 2002). Studies performed by Baruah and Duta (2009) and Makhluf *et al*. (2005) reported that the photocatalytic properties of TiO_2 and ZnO, and microbiocidal of AgO and MgO ENPs could be employed for pesticide degradation, detection and to control food spoilage. Other applications of ENPs include their use as nanosensors for the detection of plant pathogens and for pesticide, and soil remediation or conservation.

Table 2.1 Examples of the application of pesticide active ingredient incorporated with ENPs in the agricultural area

Application/ active ingredient	ENPs	Reference
Pesticide delivery		
2,4-dichlorophenoxyacetate (2,4-D)	Zn-Al layered double hydroxide (LDH)	Hussein *et al*., 2005
Avermectin	Porous hollow silica	Li *et al*., 2007
Atrazine	Magnetic carbon coated ENPs	Cifuentes *et al*., 2010
	Polyhydroxybutyrate-co-hydroxyvalerate	Grillo *et al*., 2010
Ethiprole or phenylpyrazole	Poly-caprolactone	Boehm *et al*., 2003
Gamma cyhalothrin	Solid lipid	Frederiksen *et al*., 2003
Tebucanazole/ chlorothalonil	Polyvinylpyridine and polyvinylpyridine-co-styrene	Liu *et al*., 2001
Validamycin	Porous hollow silica	Liu *et al*., 2006
Pesticide sensor		
Carbofuran/ triazophos	Gold	Guo *et al*., 2009
Dichlorodiphenyltrichloroethane (DDT)	Gold	Lisa *et al*., 2009
Dimethoate	Iron oxide	Gan *et al*., 2010
	Zirconium oxide	
Organophosphate	Zirconium oxide	Wang *et al*., 2009
Paraoxon	Silica	Ramanathan *et al*., 2009
	Carbon nanotubes	Joshi *et al*., 2005
Pyrethroid	Iron oxide	Kaushik *et al*., 2009
Pesticide degradation		
Lindane	Iron sulfide	Paknikar *et al*., 2005
Imidacloprid	Titanium oxide	Guan *et al*., 2008
Fertilizer delivery		
NPK controlled delivery	Nano-coating of sulfur	Wilson *et al*., 2008
	Chitosan	Corradini *et al*., 2010
Genetic material delivery		
DNA	Gold	Torney *et al*., 2007
	Gold	Vijayakumar *et al*., 2010
	Starch	Liu *et al*., 2008
Double stranded RNA	Chitosan	Zhang *et al*., 2010

2.2.3.1 Clays and layered double hydroxides (LDHs)

Inorganic components such as clays and LDHs are some of the best candidates for preparing formulations aimed at slow/targeted release of pesticides in the agricultural area (El-Nahlal *et al*., 1999). Clay nanoparticles have a structure of stacked platelets with one dimension in the nm scale while nano-clays show a high aspect ratio that can promote more interactions on the surfaces when exfoliated and dispersed well in the formulation matrices. Nano-clay formulations have been found to reduce the dispersion of soil pollutants and remove toxic compounds in the environment (El-Nahlal *et al*., 1999).

Investigations of the behavior of ethofumesate, a pre- and post-emergence herbicide for grasses and broad-leaved weed control based on the association of an agropolymer (wheat gluten) and montmorillonite (MMT) were done by Chevillard *et al*. (2012). The results showed a great affinity between the layered silicates and wheat gluten which indicated a key parameter to achieve a well-exfoliated nanocomposite structure. They also found that the entrapment of ethofumesate in the clay aggregates was affective in order to reduce the diffusion of ethofumesate through the composite. They concluded that the release mechanism was dominated by the ethofumesate/MMT and not by wheat gluten/MMT interactions. Due to the hydrophobic nature of ethofumesate, they suggested that the slow release was greatly influenced by the presence of the hydrophobic clays in the composite.

Investigations involving LDHs, which are anionic clays made of stacks of octahedral sheets in the nm scale, have been performed with agrochemical products such as pesticides, fertilizers and plant growth promoters (Bin Hussein *et al*., 2009; Choy *et al*., 2007; Lakraimi *et al*., 2000; Olanrewaju *et al*., 2000). The use of surfactants in preparing LDH-based pesticide formulation has also been studied (Qiu *et al*., 2009). They investigated the intercalation of a non-anionic avermectin which resulted in a formulation with a dimension approximately 400 – 600 nm. They also found that the release of avermectin was influenced by the pH, temperature and presence of electrolyte.

2.2.3.2 Porous hollow silica

A controlled release formulation using porous hollow silica ENPs as a carrier for avermectin was investigated by Li *et al*. (2007). They found that the release of avermectin was affected by pH, temperature and shell thickness. However, the encapsulation formulation showed a multistage pattern of release where the release was associated with different parts of the particles including in the internal core, pore channels and external part of the particles. In another study, Liu *et al*. (2006) investigated the controlled delivery of validamycin. The results obtained show a similar multistage pattern of release as in the previous study (Li *et al*., 2007) where they suggested this could be explained by the different adsorption locations of validamycin on the porous hollow silica ENPs. They also found that such release was also dependent on the dissolution medium conditions and enhanced by either increasing pH or temperature. They concluded that the release behavior of this formulation has made these porous hollow silica ENPs a promising carrier in agriculture, especially for controlled delivery of pesticides.

In another study, Cao *et al*. (2016) successfully synthesised mesoporous silica nanoparticles (MSNs) with particle diameters and pore sizes of approximately 110 nm and 3.7 nm, respectively via a liquid crystal templating mechanism. A water-soluble chitosan (CS) derivative (N-(2-hydroxyl)propyl-3-trimethyl ammonium CS chloride, HTCC) was successfully capped on the surface of pyraclostrobin-loaded MSNs. The electrostatic interactions and hydrogen bonding were found to be the major forces responsible for the formation of HTCC-capped MSNs. They found that the pyraclostrobin-loaded nanoparticles showed an initial burst which was followed by sustained release behavior. The formulation prepared was found to reduce the amount of technical grade pyraclostrobin to half of the required dose for the same effect on fungicidal activity against *Phomopsis asparagi* (a plant pathogen). They suggested that this will help to reduce the amount of pesticide needed to be applied and enhance the utilization efficiency. They concluded that HTCC-decorated MSNs demonstrated great potential as nanocarriers in agrochemical applications.

2.2.3.3 Metals and metal oxides

Nanometals, such as nano-Ag, have been proposed for use as pesticides due to their known antimicrobial activity and effects on the growth of plant pathogens (Chun *et al*., 2010). There are several potential advantages of nano-Ag over synthetic fungicides including the reduction in human toxicity, the development of resistance and pollution (Jo *et al*., 2009). However, before nano-Ag solutions can be officially used in agricultural area, the risks of nano-Ag should be assessed due to the toxicity of nano-Ag which may depend on the morphology and surface properties of nano-Ag (Jung *et al*., 2010). Investigations on nanometal oxides were successfully done via an incorporation of different proportions of nano-TiO_2 using polymer-based microcapsules as carriers (Guan *et al*., 2011). This nanoformulation was aimed at promoting the photocatalysis of the active ingredient after being released from the microcapsules to reduce the residues on plants and in the soil compartment (Guan *et al*., 2011). These microcapsules were then loaded with avermectin as the active ingredient. The results obtained show the nanoformulations were slightly more efficient than conventional formulations.

In another study, Xue *et al*. (2014) reported a study to enhance pesticide efficiency and eliminate residues. Results showed 0.25 g/L ZnO ENPs with 0.01 g/L thiram could inhibit the fungal growth through a synergistic interaction. They also found that the 0.01 g/L thiram was completely degraded by ZnO ENPs under simulated sunlight radiation within 6 h. They found that the adsorption of ZnO ENPs to fungi and the cellular internalisation of ZnO-thiram formulation played an important role in synergy. Data on oxidative stress showed ZnO-induced oxidative damage was influenced by thiram which resulted in a synergistic antifungal effect. They concluded that this formulation could control plant disease and reduce the risks to human health due to the subsequent faster degradation of the pesticide active ingredient.

2.2.3.4 Solid lipids and polymers

Organic ENPs such as lipids and polymers are useful for multiple purposes. Solid lipid ENPs comprise of aqueous dispersion of lipids such as triglycerides, steroids, long chain fatty acids and emulsifiers prepared by a high pressure homogenisation technique. They are reported to be useful in pesticide delivery and as biocidal essential oils (Lai *et al*., 2006; Frederiksen *et al*., 2003). Lai *et al*. (2006) prepared *Artemisia arborescens* L essential oil-loaded solid lipid ENPs and found that solid lipid nanoformulations were stable over a two-month period. They concluded that the loss of active ingredient could be reduced due to less evaporation compared to the conventional formulations.

Due to their stability and easily modifiable surfaces, polymer ENPs have received a great deal of attention for the purpose of nanopesticides production especially with insecticide active ingredients (Kah and Hofmann, 2014; Herrero-Vanrell *et al*., 2005; Vauthier *et al*., 2003; Table 2.2). According to the patent analysis done by Green and Beestman (2007), it is possible to incorporate the use of polymers in the pesticide formulations. Biodegradable polymers are mainly studied for preparing a controlled release formulation of active ingredients (e.g., pesticides and fertilizers) in order to allow a better delivery to the target area. The method of preparation for ENPs-based polymers is important in order to show different function such as release characteristics for the best delivery or encapsulation of the active ingredient.

Generally, polymer-based nanopesticides can be divided into two major types of formulations: nanospheres and nanocapsules (Anton *et al*., 2008). For nanospheres, the location or distribution of the pesticide active ingredient is not specified in contrary with nanocapsule formulations, where nanocapsules provide a core-shell structure that can be filled with the pesticide active ingredient dissolved in a polar or non-polar solvent. For this type of formulation, it is known to increase the stability of the spraying solution and spraying surface, uptake by target organisms, and to reduce phytotoxicity owing to a more homogeneous distribution during application. However, to design a nanocapsule in the nm size range is challenging. Yin *et al*. (2012) reported the preparation of nano-capsules of lansiumamide B (NCLB) by the microemulsion polymerisation method in order to improve the nematicidal efficacy of lansiumamide B (LB). The mean particle size was in the range of 38.50±0.64 nm. The release profile showed that the accumulated release of LB in NCLB reached up to 82% within 96 h. The nematicidal activity of NCLB was found to significantly increase compared to the non-formulated formulation. However, it was unclear whether the potential toxicity was influenced by the formulation ingredients (i.e., sodium dodecyl sulfate, N-amyl alcohol and chloroform). Based on this study, they concluded that NCLB, as a novel nematicides nanoformulation has the potential to perform more efficiently and provide longer effective maintenance against plant parasitic nematodes (Yin *et al*., 2012).

A study investigating the incorporation of polymers with a pesticide active ingredient was done recently to prepare a slow release microcapsule formulation of chlorpyrifos insecticide using poly(butyl acrylate-co-styrene) (poly(BA/St)) and poly(BA/St/ethylene glycol dimethacrylate (EGDMA)) by emulsion polymerisation (Wang *et al*., 2015). Based on this study, it was reported that the microcapsule particle size remained mostly constant (88.36 – 101.8 nm). However, the extent of sustainable release of the formulations was different with the different ratio of the surfactants used. It was reported, the extent of sustainable release was decreased with the increasing content of BA, St, or chlorpyrifos in the oil phase. In contrast, an adequate degree of cross-linking with EGMDA (0.5 – 2.5%) was found to increase the extent of sustainable release. However, at higher levels of cross-linking with EGDMA (5 – 10%), the extent of sustainable release was reduced. They concluded that the release of chlorpyrifos from specific microcapsules (monomer ratio 1:2 with 0.5% EGDMA or 5 g chlopyrifos) had a potential to be a diffusion-controlled process. This study indicates that microcapsule formulations tend to exhibit a controlled-release of an active ingredient that could give an advantage in the terrestrial environment in term of better delivery to the target organisms.

On the other hand, nanosphere formulations have been reported in the literatures that can improve the delivery of pesticide active ingredients to plants. Boehm *et al.* (2000) prepared nanosphere formulations with various amounts of poly(epsilon-caprolactone) using a nanoprecipitation method. This formulation was prepared to improve the delivery into plants as the primary objective. They found that the release of the pesticide active ingredient showed a similar pattern as in the classical suspension. The effects of increasing the amount of the surfactants were not to significantly influence the nanospheres formation, but the stability studies revealed that the surfactants were needed in order to avoid crystallization of the pesticide active ingredient for over a two-month period. Later, Boehm *et al.* (2003) prepared nanosphere formulations using an insecticide active ingredient and tested this on cotton plants infested by aphids. The results obtained showed that even with the average particle size of 135 nm and encapsulation rate of 3.5%, the speed of action and sustained release did not show any improvement compared to the classical suspension. However, the small particle size governed by the nanosphere formulations enhanced the penetration of the insecticide active ingredient through the plants. They concluded that an improved systemicity of the insecticide active ingredient could be achieved by nanosphere formulations.

In 2008, a group of researchers proposed a new technique in controlling the particle size of pesticide active ingredients to achieve a better controlled and efficient release of bifenthrin (Liu *et al*., 2008). They prepared a polymer-stabilized bifenthrin nanoparticle using a multi-inlet vortex mixer (MIVM) to provide rapid micromixing, high supersaturation and rapid nucleation and growth of bifenthrin nanoparticles known as Flash NanoPrecipitation. In this study, they also investigated several polymeric stabilisers. Results obtained showed the nanoparticle size increased from 100 to 200 nm with an increase in pesticide loading from 50 to 91%. The stability test revealed that the nanoparticle dispersions were followed for more than 12 days. They suggested that the steric stability affected by the corona structure of the hydrophilic block of the polymers helped to prevent the nanoparticle aggregation. They also found that the slow particle size growth could be attributed to the Ostwald ripening mechanism.

From these studies, it appears that polymer-based nanopesticides show the greatest potential due to their greater efficacy compared to the conventional formulations. By using polymers as the component in preparing nanopesticide, it would allow a wide range of objectives to be achieved such as: to increase the solubility of the active ingredient; provide a slow release formulation for better delivery; and protect active ingredients from premature degradation. This could be useful information for the development of more polymer-based nanopesticides in the future in order to overcome the drawbacks posed by the conventional formulations of pesticides.

Table 2.2 Examples of polymer-based nanopesticides

Insecticide	Polymer	Reference
Aldicarb	Lignin	Kok *et al*., 1999
Azadirachtin	Carboxymethyl chitosan-ricinoleic acid	Feng and Peng, 2012
Bifenthrin	Poly(acrylic acid)-b-poly(butyl acrylate)	Liu *et al*., 2008
	Polyvinyl alcohol	
	Polyvinylpyrrolidone	
Carbaryl	Carboxymethylcellulose	Isiklan, 2004
	Glyceryl ester of fatty acids	Quaglia *et al*., 2001
Carbofuran	Poly(methyl methacrylate)-poly(ethylene glycol)	Chin *et al*., 2011
	Polyvinylpyrrolidone	
	Polyethyleneglycol-dimethyl esters	Shakil *et al*., 2010
Chlorpyrifos	Polyvinylchloride	Liu *et al*., 2002
	Poly(butyl acrylate-co-styrene) with a cross-linker ethylene glycol dimethacrylate	Wang *et al*., 2015
	Calcium alginate-starch using CaCl2 as crosslinker	Roy *et al*., 2009
Beta-Cyfluthrin	Polyethylene glycol	Loha *et al*., 2012
Cypermethrin	Methyl methacrylate and methacrylic acid with and without 2-hydroxy ethyl methacrylate crosslinkage	Rudzinski *et al*., 2003
Deltamethrin	Polyethylene	Frandsen *et al*., 2010
Endosulfan	Starch-based polyethylene	Jana *et al*., 2001
Etofenprox	Chitosan	Hwang *et al*., 2011
Garlic essential oil	Polyethylene glycol	Yang *et al*., 2009
Imidacloprid	Lignin	Fernandez-Perez *et al*., 2011
	Lignin-polyethylene glycol-ethylcellulose	Flores-Cespedes *et al*., 2012
	Alginate-bentonite	Fernandez-Perez et al., 2011
	Poly (styrene–diacetone crylamide)	Qian *et al*., 2012
Itraconazole	Acrylic acid-Bu acrylate	Goldshtein *et al*., 2005

***Lippia sidoides* essential oil**	Chitosan-angico gum	Paula *et al*., 2010
***Moringa oleifera* extract**	Cashew gum	Paula *et al*., 2012
Neen seed oil	Alginate-glutaraldehyde	Kulkarni *et al*., 1999
Novaluron	Anionic surfactants (sodium linear alkyl benzene sulfonate, napthalene sulfonate condensate sodium salt and sodium dodecyl sulfate)	Elek *et al*., 2010
Pheromones	Polyamide	Hellmann *et al*., 2011
	Vinylethylene and vinylacetate	Wright, 1997
Piperonyl butoxide	Polyethylene	Frandsen *et al*., 2010
Rotenone	N-(octadecanol-1-glycidyl ether)-O-sulfate chitosan-octadecanol glycidyl ether	Lao *et al*., 2010
Tebucanazole	Amphiphilic copolymers of gelatin grafted with MMA	Salma *et al*., 2010
Thiamethoxam	Poly(ethylene glycols) and various aliphatic and aromatic diacids	Sarkar *et al*., 2012
Thiram	Poly(ethylene glycol)	Kaushik *et al*., 2013
Triclosan	Polyvinylpyrrolidone	Narayanan *et al*., 2008

2.3 Environmental fate and effects

2.3.1 ENPs

With the rapid development of the use ENPs, including nanopesticides, it has been predicted that a significant increase in the amount of various ENPs will be released into the environment. A significant fraction of ENPs is already estimated to enter the terrestrial environment through use as photocatalysts for water treatment or human activities (Fang *et al*., 2009; Joo *et al*., 2009). As the nanopesticide sector grows emissions of these materials to the soil environment is also inevitable. While, properties of nanomaterials are useful for product application e.g. as discussed earlier for nanopesticides, concerns have been raised over the potential risks of ENPs to the natural environment. It is also now recognised that many of the approaches and paradigms we use for environmental risk assessment of chemicals may not be appropriate for ENPs. In the next sub-sections, the fate (i.e., aggregation, stability, sorption and leaching) and effects of ENPs on terrestrial organisms (i.e., plants and animals) are discussed.

2.3.1.1 Aggregation

Soils are mainly composed of organic matter, minerals, air and water (Brady and Weil, 1999). Of all these components, air and water compose soil pore space where the mobilisation and stabilisation of dispersed ENPs takes place (Peralta-Videa *et al*., 2011). When ENPs are released to the terrestrial environment, the ENPs may interact with solid components such as organic matter and minerals. The fate of ENPs in soil compartment is reported to vary depending on the physico-chemical characteristics of the ENPs (Darlington *et al*, 2009). ENPs are reported to favor aggregation processes in saturated porous media due to the fact that ENPs are considered to behave like colloids, thus the fate and transport of ENPs could be changed (Praetorius *et al*., 2014; Solovitch *et al*., 2010).

The aggregation process of CeO_2 ENPs when exposed to a solution with a pH of 7.4 showed aggregates of the ENPs with a mean size of 400 nm. Another study was done by investigating the aggregation process of FeOOH ENPs (Gilbert *et al*., 2007). The ENPs formed stable suspended clusters under a range of aqueous solution conditions. Scattering analysis revealed that the suspended fractal nanoclusters were formed between pH 5 and 6.6 with a mean diameter size ranging from 25 to approximately 1000 nm. The most significant observation was that the nanoclusters retained a very high surface area and persisted in the suspension for at least 10 weeks. However, it was found that the aggregation process was irreversible as optically transparent suspensions were obtained when the ENPs settled at pH > 7 were changed to pH 4 without stirring. In addition, they found that there was no further dispersion from the aggregates formed after 1 month which indicated that ENPs could form stable nanoclusters in groundwater with implications for the transport of surface sorbed components (i.e., nutrients) and soil contaminants.

However, ENPs may show complex colloid and aggregation behavior in the soil compartment as aggregation is likely to be influenced by size, particle shape, and surface area, charge and coatings (Handy *et al.*, 2008a; Pettibone *et al.*, 2008). Soil pH, ionic strength and dissolved organic matter could also influence the aggregation of these ENPs in the soil compartment (French *et al.*, 2009; Jaisi and Elimelech, 2009; Baalousha *et al.*, 2008; Doshi *et al.*, 2008; Handy *et al.*, 2008a; Saleh *et al.*, 2008). Particles in the size range 4 – 5 nm of TiO_2 were found to readily form stable aggregates at pH 4.5 with an average diameter size ranging from 50 – 60 nm in a NaCl suspension when the ionic strength was adjusted to 0.0045 M (French *et al.*, 2009). When the ENPs were exposed to an increased ionic strength of 0.0165 M while keeping the pH constant, formation of micron-sized aggregates was observed after 15 min. At low ionic strength (0.0084 – 0.0099 M NaCl), micron-sized aggregates were formed in less than 5 min for all pH values tested (5.8 – 8.2). The study was also performed by changing NaCl suspension to $CaCl_2$ at pH 4.8 and ionic strength of 0.0128 M. They observed that aggregates were formed after 5 min which was faster compared to the observed aggregates formed for NaCl suspension even at the same pH and ionic strength. They concluded that pH and ionic strength greatly influenced the aggregation of ENPs in the soil compartment.

2.3.1.2 Stability

Stability is a function of the surface energy and ENPs are considered to be more stable when they have a low surface energy (Qafoku, 2010). This argument is in an agreement with anatase being a more stable phase of nanocrystalline TiO_2 compared to the higher surface energy posed by rutile particles (Naicker *et al.*, 2005). The surface energies of ENPs are determined by both their electrostatic energy charged surfaces and interfacial energy (Finnegan *et al*., 2007). These energies can be modified and manipulated through solution chemistry in order to gain control of the ENPs phase stability and their transformation kinetics (Finnegan *et al*., 2007). Investigation of the thermodynamic data on the surface energy of one of the most common metal oxides in all terrestrial environments, Fe oxides, has been studied (Navrotsky *et al*., 2008). They concluded that the size-driven crossovers in stability would help to explain the patterns of different Fe oxides occurring in the environment.

Previous studies have reported that solution pH is one of the most important variables controlling ENP stability (e.g., Finnegan *et al*., 2007). For example, small size rutile is more stable compared to anatase in very acidic conditions. In contrast, in very basic conditions, anatase is found to be more stable relative to rutile and brookite (Finnegan *et al*., 2007). The authors of this study concluded that the activity of potential determining ions (i.e., protons or hydroxyl groups) could control the TiO_2 ENPs phase stability in aqueous solutions depending on the pH values. Geranio *et al*. (2009) performed a study exploring Ag release from the nano-products. This study was conducted by investigating the effects of several parameters such as pH, Oxidising agents and surfactants. The results showed that dissolved Ag concentrations were about 10 times lower at pH 10 compared to pH 7. Oxidising agents were found to accelerate the dissolution of Ag.

2.3.1.3 Sorption

Soil colloids are of great environmental importance where they can carry many sorbed particles including soil pollutants and other materials, and can also facilitate the fate and transport of ENPs (Wilson *et al*., 2008). Dissolved organic matter (i.e., humic and fulvic acids) and ionic strength have been reported to enhance the colloidal stability of nanomaterials, hence affecting the transport of ENPs (Jaisi and Elimelech, 2009). This is in agreement with a study done by Solovitch *et al*. (2010), where they concluded that the chemistry of soil solution such as pH, ionic strength and natural organic matter strongly affect the interactions between ENPs and the soil media. This suggests that soil solution chemistry influences the balance between the free migration of particles and the deposition of ENPs. As a result of H^+ dissociation from carboxylic (-COOH) or phenolic (-OH) groups, the colloidal surfaces of organic matter (i.e., humus) are negatively charged. The sorption process that occurs between organic matter and the ENPs through electrostatic attraction and ligand exchange therefore reduces the aggregation process which then can alter their movement in the soil solution (Peralta-Videa *et al*., 2011). Sorption of humic acids onto the surface of the TiO_2, Al_2O_3 and ZnO ENPs was investigated by Yang *et al.* (2009). They found that as the result of the sorption process, the decreases in zeta potential meant that humic acid-coated nano-oxides could be more easily dispersed and suspended in solution because of their enhanced electrostatic repulsion.

2.3.1.4 Leaching

ENPs may also reach groundwater (Fang *et al*., 2009). Studies performed in soil columns have shown that the transport distances of TiO_2 in different soil types were in the range of 41.3 to 370 cm (Fang *et al*., 2009). This indicates there is possibility of ENPs to be transferred from the soil compartment to the groundwater. ENPs can be coated with both inorganic and organic compounds such as citrates, carbonates or surfactants in order to maintain the stability of the ENPs which suggests that surface modification will also influence the mobility and transport of ENPs. Mobility of single-walled carbon nanotubes (SWCNTs) under saturated flow conditions was investigated by Jaisi and Elimelech (2009). Results obtained suggest that SWCNTs will not exhibit substantial transport and infiltration in soils because of an effective retention by the soil properties (i.e., DOM).

2.3.1.5 Effects on organisms

The environmental effects of ENPs on terrestrial organism such as plants has also been investigated with most studies focussing on the toxicity of ENPs on seed germination and root elongation (Lopez-Moreno *et al*., 2010; Lin and Xing, 2008). Lin and Xing (2008) observed the uptake and phytotoxicity of ZnO ENPs via roots in different plants. Ryegrass was found to have reduced biomass production in the presence of the ZnO ENPs. This was explained by the observation that the root tips shrank, and root epidermal and cortical cells were found to collapse in the presence of ENPs. The study revealed that the phytotoxicity of the particular tested ENPs was not a direct result of their limited dissolution in the bulk nutrient solution or rhizosphere but that the ENPs were found to adhere to the root surface. The accumulation of ENPs into plants was successfully investigated by Kurepa *et al.*

(2010). They found that the modified TiO_2 was taken up by plants and distributed in different specific subcellular locations.

Investigations of the impact of ENPs on the nematode, *Caenorhabditis elegans* have also been reported (Roh *et al*., 2009; Wang *et al*., 2009). Studies show that *C. elegans* can accumulate Al_2O_3, Ag, TiO_2 and ZnO ENPs which then causes different degrees of toxic effects including a decrease in the reproduction and growth of the *C. elegans*. It was also reported that different forms of ENPs show different behaviour in terms of bioaccumulation of the ENPs. For example, Pipan *et al*. (2010) show that the accumulation of ZnO ENPs by terrestrial isopods was probably related to the presence of Zn^+ through dissolution and not from uptake of the ZnO ENPs. Studies on the accumulation of Ag, ZnO and $^{14}C_{60}$ ENPs in terrestrial invertebrates such as the earthworms, *E. fetida* and *Lumbricus rubellus* have also been reported (Diez-Ortiz *et al*., 2015; Gupta and Yadav, 2014; Ha *et al*., 2014; Li *et al*., 2010). Data obtained show that accumulation of ENPs in *L. rubellus* is influenced by both Ag^+ and Ag ENPs. Size of ENPs was also reported to influence the accumulation of ZnO ENPs.

These studies prove that the accumulation of ENPs into terrestrial organisms (i.e., plants and animals) is possible, thus the impact of ENPs should be assessed carefully in order to minimise the potential risks to the terrestrial environment. It is important to know how serious the impact of ENPs on the terrestrial environment is, therefore more studies should be carried out in order to understand the behavior of ENPs in the environment including studies on a wide range of soil properties (e.g., soil pH, texture, and organic matter) and on the behavior of different ENPs.

2.3.2 Nanopesticide fate and impacts

There are a limited numbers of studies available in the literature concerning the fate and effects of nanopesticides in soil. Most studies involving pesticide nanoformulations have focussed on the slow release mechanism to increase the efficacy of the nanoformulations (Liu *et al*., 2016; Boyandin and Volova, 2015; Jiang *et al*., 2015; Liu *et al*., 2015; Pereira *et al*., 2015; Pereira *et al*., 2014; Céspedes *et al*., 2013; Galán-Jiménez *et al*., 2013; Grillo *et al*., 2012; Hussein *et al*., 2012; Li *et al*., 2012). While data on the efficacy of nanopesticides are becoming available in the literature, studies into the nanoecotoxicology of nanopesticides are still being developed. As the fate and effects of nanoparticles show a difference compared to conventional formulation, the impact of nanopesticides could also be different in the environment. Therefore, it is necessary to develop an understanding of the ecotoxicology area involving nanopesticides. In the following sub-sections, the data on the fate and effects of nanopesticides that are available in the literature are discussed. Examples of studies into the fate and effects of nanopesticides are summarised in Table 2.3.

2.3.2.1 Sorption

A study investigating the sorption behavior of a nanoformulation of paraquat was performed by Silva *et al*. (2011). The sorption studies were performed using the free and associated paraquat with nanoparticles (NPs). They found that the association of paraquat with NPs changed the behavior of the active ingredient. This showed the nanoformulation reduced the sorption to soil particles, thus improving the herbicidal activity in soil. This is not in agreement with a study done by Trigo *et al*. (2010). They investigated the sorption behavior of organoclay-based formulations of atrazine in Buse loam soil. They found that the organoclay formulations showed a stronger sorption behavior compared to the standard formulation of atrazine. They concluded that as organoclay formulations were tightly adsorbed to soil particles, they were less likely to be transported in the soil compartment, thus reducing the potential to contaminate the groundwater.

In another study, Kah *et al*. (2014) investigated the sorption of a polymer-based nanoformulation of atrazine. Based on the study, the partition coefficients were determined using two different methods (batch equilibrium and centrifugation methods), in two different soils (sandy and loam soils) with different organic carbon content. Both of the methods showed that the sorption coefficients (K_ds) for a nanoformulation of atrazine were significantly higher compared to the analytical grade material. This suggests that nanoformulation of atrazine showed stronger sorption behavior which is similar to what was seen in the previous study done by Trigo *et al*. (2010). They also found that the K_ds were higher in a loam type of soil compared to the sand soil which suggests that sorption process was more favored in soils containing more organic carbon content. This trend is in agreement with a study done by the same authors on three different nano-encapsulated bifenthrin formulations: NFA and NFB contained polymer nanoparticles derived from a 75:25 copolymer consisting of poly(methacrylic acid-*ran*-butylmethacrylate); and NFC contains polymer nanoparticles derived from a 90:10 copolymer consisting of poly(methacrylic acid-*ran*-ethylacrylate) (Kah *et al*., 2016). They found that two of the nano-encapsulated bifenthrin showed higher K_ds in loam soil compared to the formulated (CF) and analytical grade bifenthrin (AI). The sorption increased in the order of NFC < AI < CF < NFA < NFB. However, studies in sandy soil showed a different trend where all the nano-encapsulated bifenthrin showed lower K_ds compared to the AI and CF treatments (NFC < NFB < NFA < CF < AI).

2.3.2.2 Leaching

A study investigating the impact on leaching of organoclay-based formulations of diuron was performed by Trigo *et al*. (2009). The formulations were applied to two soils, sampled from experimental olive fields located in Seville, Spain. Using column leaching tests they compared the behavior of the nanoformulations with a standard commercial formulation of diuron. They found that the organoclay formulations reduced the leaching potential in soils compared to the standard commercial formulation. They suggested that the use of organoclay-based formulations could then reduce the extensive transport losses associated with the application of diuron in the terrestrial environment.

In another study, Salma *et al*. (2010) prepared amphiphilic core/shell nanoparticles containing tebuconazole using copolymers of gelatin grafted with methyl methacrylate (MMA). The nanoformulation was applied into wood up to 85% efficiency. They found that the treated wood with the tebuconazole-containing nanoparticles formulation reduced the leaching of tebuconazole compared to the wood treated with tebuconazole solutions only. However, in their study, they also found that the nanoparticles were subject to aggregation of the ungrafted gelatin, thus reducing the delivery efficiency of the nanoparticles into wood.

2.3.2.3 Degradation

Investigations into the effect of incorporating LDHs with cinnamate, a chemical substance that could potentially be used as fungicide, on degradation of the compound has been done by Park *et al*. (2010). Cinnamate is a natural antibiotic that is rarely used because it is considered as a fast degrading pesticide. Results obtained show the incorporation of LDHs with cinnamate can prolong the retention of cinnamate in soil. This trend is similar to a study done by Kah *et al*. (2016) where nano-encapsulation of bifenthrin enhanced the degradation time of bifenthrin compared to the AI and CF treatments in loam and sandy soils. In contrast, a study done by Guan *et al*. (2010) found that the nanoformulations of imidacloprid were degraded faster in soil than a suspension concentrate (half-life in soil: 2.8 and 6.2 days, respectively). However, they found that the degradation rates in soyabean plants were the opposite way round (half-life in plants: 4.5 d and 1.9, respectively). A more recent study by Kah *et al*. (2014) investigated the degradation of a nanoformulation of atrazine. The results showed the degradation behavior of the nanoformulation and analytical grade atrazine was similar. They concluded that in terms of degradation, nanoformulation showed no effect compared to the analytical grade atrazine.

In another studies, Kumar *et al*. (2010) and Shakil *et al*. (2010) prepared poly(ethylene glycol) (PEG) based amphiphilic copolymers for formulation of azadirachtin and carbofuran, respectively. Half-lives (DT_{50}) of the azadirachtin nanoformulation in water were reported to range from approximately 3.05 to 42.80 d in different matrices. For the carbofuran nanoformulation, the DT_{50} in water ranged from 7.5 to 55.0 d compared to the conventional granule formulation where the DT_{50} was approximately 3.2 d. These results suggest that the application rate of these nanoformulations could be optimised by changing the polymer properties (i.e., molecular weight) in order to achieve an insect control formulation at the desired level and period.

2.3.2.4 Effects of surfactants on the fate of nanopesticides

In this section, it is also worth highlighting the potential effects of surfactants on the fate of nanopesticides. Surfactants are reported to be one of the important components that could potentially alter the fate and behavior of pesticide active ingredient (Katagi, 2008). The physico-chemical properties such as solubility, dissociation and volatilisation could be affected by the presence of surfactants. Thus, the fate and behavior of prepared nanoformulations with surfactants in the terrestrial environment could be changed.

The effect of surfactants was investigated by Liu *et al.* (2011). They found that the kinetic stability of bifenthrin in oil-in-water (O/W) nanoemulsion was affected by the presence of different surfactants. The two surfactants used in this study were di-potassium monododecyl phosphate [$C_{12}H_{25}OPO_3K_2$] (MAPK, molecular weight (MW) = 342, hydrophile-lipophile balance (N_{HLB}) = 13.5) and polyoxyethylene 3-lauryl ether [$C_{12}H_{25}O(CH_2CH_2O)_3H$] ($C_{12}E_3$, MW 318, N_{HLB} = 6.8). They found that the optimum nanoemulsion prepared was at an HLB value pf 10.82 and a mixing ratio of 6:4 (MAPK:$C_{12}E_3$) and 10 wt% surfactant mixture. For this mixture, the nanoemulsion showed high kinetic stability where the droplet size increased from 200.7 to 218.6 nm after 180 d at 25 °C. They suggested that the instability mechanism for the nanoemulsion could be attributed to Oswald ripening and the rate rose with the increase of the surfactant concentration (Liu *et al.*, 2011). In another study, the effect of surfactants on sorption behavior was investigated using soil columns (Hua *et al.*, 2009). Based on this study, the presence of anionic surfactants greatly increased the mobility of bentazone in a sandy loam soil. In contrast, the non-ionic surfactants decreased the mobility compared to the application of bentazone in sandy loam soil without surfactant.

2.3.2.5 Effects on organisms

Studies into the effects of a post-emergence herbicide comprising poly(epsilon-caprolactone) (PCL) nanocapsules containing atrazine were performed by Oliveira *et al.* (2015). The nanopesticides were applied to *Brassica juncea* species as the target plant. Generally, the atrazine-containing PCL nanocapsules showed very effective post-emergence herbicidal activity. After 72 h of exposure, they found that at exposure of 1 mg/mL of the nanopesticides, the net photosynthesis and PSII maximum quantum yield in the chlorophyll parameter were decreased. However, the leaf lipid peroxidation analysis was increased, leading to shoot growth inhibition and the development of severe symptoms (Oliveira *et al.*, 2015). When the plant was exposed to the nanocapsules without atrazine, no effects was observed. They concluded that the use of nano-encapsulated atrazine could be useful in the herbicide application at lower dosage, without any loss of efficiency, thus reducing the effects in the environment.

Bioactivity of nanoformulation of chlorfenapyr was investigated by Song *et al*. (2012) using dispersible silica nanoparticles as the carrier. The results obtained revealed that the LC_{50} of a nanoformulation of chlorfenapyr using bollworm larvae was approximately 2.25 times higher than the microformulation. They concluded that the use of silica nanoparticles as the carrier significantly enhanced the insecticidal activity. This indicates that incorporation of pesticide active ingredients with silica ENPs could improve the performance of the active ingredient. In another study, Son *et al*. (2015) investigated the toxicity of encapsulated λ-cyhalothrin using *Daphnia magna* as the test organism. When *D. magna* was exposed to a maximum condition of hydrodynamic diameter (HDD) or zeta potential in the overlying water, the results showed the worst-case exposure condition to *D. magna* was when the encapsulated λ-cyhalothrin was either stable or small in the overlying water (under the stable condition: the effective concentration, EC_{50} = 0.063 μg/L) (Son *et al*., 2015). The results suggest that water quality could modify the fate and toxicity of encapsulated λ-cyhalothrin in the aquatic environment, therefore the understanding in the interactions between water and the effects applied chemicals is critical for environmental risk assessment.

Table 2.3 Example of environmental fate and effects of nanopesticides

ENPs-based pesticides formulation	Summary	Reference
Clays and layered double hydroxides (LDHs)	Enhanced persistence (DT_{50} for formulation with LDHs was 17 d compared to cinnamic acid was only 6 d in aqueous solution)	Park *et al*., 2010
	No significant change in bioavailability in soil compared to free atrazine	Trigo *et al*., 2010
	Reduced leaching but no significant change in persistence for diuron organoclay formulation compared to conventional diuron	Trigo *et al*., 2009
	Slower release of ethofumesate incorporated with montmorillonite (MMT)	Chevillard *et al*., 2012
Porous hollow silica	Slower degradation caused by UV-shielding	Li *et al*., 2007
	Pyraclostrobin-loaded NPs showed slow release behaviour	Cao *et al*., 2016
	Insecticidal toxicity of nanoformulation of chlorfenapyr was twice as higher compared to microformulation	Song *et al*., 2012
Metals and metal oxides	Nano-TiO_2 using polymer-based microcapsules as carriers to reduce the residues by promoting photocatalysis	Guan *et al*., 2011
	No significant change in DT_{50} in plant and soil compared to conventional chlorfenapyr in concentrated suspension	Cao *et al*., 2005
Solid lipid and polymers	Loss via evaporation of *Artemisia arborescens* L essential oil was low compared to the emulsion formulation	Lai *et al*., 2006
	Solid lipid ENPs and polymeric nanocapsules using carbendazim and tebuconazole reduced toxicity compared to active ingredients	Campos *et al*., 2015
	Reduced leaching from treated wood compared to terbuconazole in aqueous solution	Salma *et al*., 2010
	Reduced sorption and enhanced persistence of bifenthrin	Kah *et al*., (2016)

2.4 Uptake of nanopesticides in the terrestrial environment

To the best of our knowledge, there are no previous studies investigating the uptake of nanopesticides in the terrestrial environment. The bioavailable fractions of the nanopesticide formulations may be taken up by soil dwelling organisms such as invertebrates (i.e., earthworms). Bioavailability of the nanopesticides is likely to be greatly affected by their fate in soil compartment such as sorption and persistence. From previous studies in this review, the polymer-based nanoformulations are aimed at better delivery and to increase the solubility of poorly soluble pesticides active ingredient. As a nanopesticide is more soluble, it will likely show a higher fraction in the soil pore water compared to being adsorbed to the soil particles. This could lead to a higher bioavailability compared to chemical compounds that are highly adsorbed to the soil particles (i.e., highly hydrophobic compounds). In addition, a more persistent chemical compound which remains in the soil for a long time could show the potential to accumulate to a higher concentration and thus have a greater chance to be taken up by an organism.

As has been discussed earlier, the environmental fate and behavior concerning nanopesticides are likely to be complex and will probably depend on the numerous soil properties (e.g., pH, ionic strength, and organic matter content) and processes that affect the behavior of the nanoparticles (e.g., growth, stability, aggregation, and aging). Therefore, the factors affecting the bioavailability and uptake of the nanopesticides in the terrestrial environment are likely to be multifaceted. Previous studies have demonstrated the possibility of organic chemical compounds being bioavailable and taken up by earthworms (Carter *et al*., 2014; Belfroid *et al*., 1993; Van Gestel and Ma, 1988). There are also studies involving the uptake of nanoparticles into earthworms (Diez-Ortiz *et al*., 2015; Gupta and Yadav, 2014; Ha *et al*., 2014; Li *et al*., 2010). However, no studies were found on the uptake of polymer-based nanopesticides into earthworms. Therefore, in this study the fate and uptake of nanopesticides will be investigated in different soil types in order to assess any potential risks of using nanopesticides in the terrestrial environment.

2.5 Conclusion

The previous work presented in this review confirms that nanomaterials exhibit differences in their fate and behavior compared to conventional forms of the substances. As the developments in the nanopesticide area are increasing, it is important to assess their possible impact on the terrestrial environment. To do these assessments requires an understanding of the fate and uptake of nanopesticides in the terrestrial organisms such as plants and animals. While increasing amounts of data are becoming available on the fate, uptake and effects of other ENPs including TiO_2, ZnO, Ag and CuO in terrestrial systems, information on nanopesticides is still limited. Therefore, it is important to develop an understanding on the fate, uptake and effects of nanopesticides in order to assess the potential risks of nanopesticides in the terrestrial environment. In the next sub-section, recommendations for future work are presented based on the previous work in this chapter.

2.5.1 Recommendations

Although some information on the fate and effects of nanopesticides are available in the literature and reviewed in this chapter, there are still many research gaps. These are discussed below:

1. Reviews of the previous works on nanopesticides show that most attention has been focused on insecticides. Studies are required using different types of pesticides as data on one type pesticide may not be informative of wider impacts of nanopesticides. Effects of surfactants are also reported to influence the fate and behavior of nanopesticides. Therefore, more studies involving the interactions of a wide range of surfactants with nanopesticides are also required.
2. Techniques for characterising nanopesticides are still limited. Methods for the characterisation of nanopesticides in terms of particle shape, size and surface properties could enhance our understanding of how these materials behave in the environment. Advanced techniques could be adopted for the characterisation of nanopesticides including nanoparticle tracking analysis (NTA), scattering and centrifugal methods.
3. Information on the fate, uptake and effects of nanopesticides on the terrestrial environment is still limited. The level of toxicity of nanopesticides could be

different compared to a conventional pesticide. Therefore, more studies should be carried out in order to investigate the uptake and effects of nanopesticides into soil organism such as earthworm.

4. The previous works indicate that soil properties can play an important role in affecting the fate and behavior of nanopesticides in the terrestrial environment although the range of soils used has been very limited. Studies should be carried out using a wider range of environmental conditions including soil pH, organic matter content and texture as well as the laboratory and field experiments to give a better understanding of the impact of nanopesticides in real agricultural land.

In the following parts of this thesis we aim to address some of these knowledge gaps. The experimental chapters begin with the toxicity studies using *E. fetida* as the test organism (*Chapter 3*), the thesis then moves onto fate (sorption and persistence) studies (*Chapter 4*), and then to studies into the uptake and distribution in *E. fetida* and *L. terrestris* (*Chapter 5*). Effects of soil properties on uptake into *E. fetida* are then described (*Chapter 6*) and finally a discussion sums up the findings of the different studies (*Chapter 7*).

Chapter 3 Uptake and Effects of Nanopesticides in the Earthworm, *E. fetida*

3.1 Introduction

The literature review presented in the previous chapter (*Chapter 2*) highlighted that one major knowledge gap for nanopesticides is around the effects in the terrestrial environment. The application of pesticides (e.g., insecticides) in agricultural fields is one of the factors that contributes to an increase in food production (Kranthi *et al*., 2002). Most pesticides are not only toxic to target organisms but also to non-target organisms due to the structural and physiological similarities between the pest and non-pest organisms (Santos *et al*., 2010; Walker *et al*., 2010). There is therefore concern over soil contamination caused by the extensive use of insecticides (Garcia *et al*., 2011; Santos *et al*., 2011; De Silva *et al*., 2010; De Silva and Van Gestel, 2009). The extensive use of pesticides can also result in resistance in target organisms leading to a loss in the efficacy of these chemical compounds as well as negative impacts on the natural environment (Wang *et al*., 2008).

A wide range of soil organisms has been proposed as good indicators for evaluating the effects of soil contamination each have advantages and drawbacks (Zhu *et al*., 2008). One of the proposed organisms is the earthworm. Earthworms have been used as a key index of ecotoxicology diagnosis although they are becoming extinct in many agriculture soils (Wang *et al*., 2012). They are common soil organisms in the soil compartment and play an important role in improving soil formation and fertility (Bartlett *et al*., 2010). The ability of earthworms to modify soil organic matter, mix litter with soil, facilitate the formation and stabilisation of soil aggregates, and improve soil porosity have made them a suitable bioindicator organism for evaluating the risk of contaminants in terrestrial systems and for deriving safety thresholds for contaminants in soils (Wang *et al*., 2012; Lourenco *et al*., 2011; Suthar *et al*., 2008; Landrum *et al*., 2006). Bioaccumulation of contaminants into earthworms can also cause harm to higher trophic levels that feed on the earthworms (Van Gestel *et al*., 2011; Hobbelen *et al*., 2006).

Many ecotoxicity studies have been done using earthworms and our knowledge of the factors and processes affecting the uptake and effects of contaminants in earthworms is well developed. Most work has been done on metals, polychlorinated biphenyls (PCBs) and conventional insecticides such as organochlorines, organophosphates and carbamates (Wang *et al*., 2012). The uptake and effects of some nanomaterials in/on terrestrial organisms has also been studied including ZnO NPs (García-Gómez *et al*., 2014; Hu *et al*., 2010; Tourinho *et al*., 2012), Ag NPs (Waalewijn-Kool *et al*., 2014; Tourinho *et al*., 2012; Schlich et al., 2013), CeO_2 NPs (Lahive *et al*., 2014), TiO_2 and Fe_2O_3 NPs (Tourinho *et al*., 2012). When combined, the data from the studies indicate that the introduction of metal NPs into the terrestrial environment results in bioaccumulation by soil invertebrate such as earthworms and can affect the survival and reproduction parameters including cocoon and juvenile production of earthworms.

Recently, the investigation of the effects of size (20, 35 and 50 nm) and surface coating (bovine serum albumin (BSA), chitosan (Chit) and polyvinylpyrrolidone (PVP)) on the bioaccumulation and toxicity of Ag NPs to the earthworm, *L. rubellus* have been investigated (Makama *et al*., 2016). After a 28 d exposure at 0 – 250 mg Ag/kg soil, total Ag tissue concentration in the *L. rubellus* was the highest when exposed to Ag NP/BSA especially at the lower concentration range. The survival, growth and cocoon production were affected by the exposure to all types of Ag NPs at the high test concentration with Ag NP/BSA being the most toxic. The effects of size on the uptake and toxicity were significantly affected by the Ag NP/PVP but not for Ag NP/BSA or the Ag NP/Chit. The authors concluded that the physico-chemical properties of Ag NPs as well as the influence of surface coating and effects of particle sizes (20 – 50 nm) under environmentally relevant conditions were important in affecting uptake and toxicity in *L. rubellus*. While data like this are now becoming available for metal and metal oxide NPs, no information is available for the effects of nanopesticides on earthworms.

It is possible that changes in the fate and toxicity of the active ingredient in a nano-formulated product compared to conventional materials could actually increase the environmental risks of the active ingredient. For example, in the case of nanopesticides, it is expected that phase partitioning, the relationship of mass concentration to particle concentration, uptake into biota and distribution within organisms are highly dependent on concentration (Kookana *et al*., 2014). Therefore, it is important to investigate the fate, uptake and effects of these new plant protection products in the terrestrial environment in order to assess the potential risks posed by these manmade products. The overall aim of this study therefore was to explore the fate, uptake and effects of nanopesticides in earthworms and to compare the behavior with that of an equivalent conventional bifenthrin formulation. The study was done using two different nano-encapsulated (Nano A and Nano B) products containing bifenthrin together with a conventional formulation (Capture LFR) and using the earthworm, *E. fetida*.

3.2 Materials

3.2.1 Chemicals

The different bifenthrin formulations (Capture LFR, Nano A and Nano B) used in this study were the same as those introduced in the previous *Chapter 1* (see section 1.3; Table 1.2). Acetonitrile (99.9 %) was purchased from Fisher Scientific (Loughborough, UK).

3.2.2 Test soils

Five different soil types were used in this study and were obtained from Landlook (Midlands, UK). These soils were chosen based on recommendations made by the Organisation for Economic Co-operation and Development (OECD) 106 Guideline in order to study the sorption behavior of chemicals under a variety of environmental conditions (OECD, 2000). Prior to use in experimental studies, the soils were air dried, sieved to ≤ 2 mm to ensure homogeneity within the soils matrix and stored at room temperature.

Soil pH was determined in water following the ISO 10390 (1994) protocol and water holding capacity (WHC) was determined following the ISO 11465 (1993) protocol. Soil moisture was determined by oven drying at 105 °C for 24 h and weight loss on ignition was determined by heating at 500 °C for 24 h, as a proxy to organic matter content. Total organic carbon (OC) was calculated using the *Van Bemmelen* constant based on the assumption that organic matter contains approximately 58% carbon; therefore, a factor of 1.724 can be used to convert organic matter to OC (Nelson and Sommers, 1982). Cation exchange capacity (CEC) was determined following the ISO 11260 & 14254 protocols using a dual view ICP-OES (Thermo iCAP 6500 duo). Soil particle sizes were determined using a Malvern Laser Granulometer (Hydro 2000MU, UK). Characteristics of the study soils are shown in Table 3.1.

Table 3.1 Test soils characteristics (±standard deviation (S.D), n = 4)

Parameter/soil	**1**	**2**	**3**	**4**	**5**
pH (water)	7.48±0.1	4.71±0.1	6.49±0.1	7.66±0.2	6.51±0.2
Water holding capacity (WHC), %	67.7±3.7	56.2±4.1	53.6±1.3	60.8±4.7	42.2±2.3
Soil moisture, %	9.4±0.7	6.2±0.8	8.1±0.7	9.1±1.6	2.5±1.8
Total organic carbon (OC), %	2.78±0.2	5.16±0.4	1.91±0.3	1.86±0.2	1.22±0.2
Cation exchange capacity (CEC), cmol$^+$/kg*	27.6	5.0	16.2	25.0	4.0
Clay, %	1.5	-	1.6	0.4	1.6
Silt, %	37.9	13.7	56.6	21.6	48.5
Sand, %	60.6	81.1	41.8	78.1	49.9
Texture§	Sandy loam	Loamy sand	Silt loam	Loamy sand	Sandy loam

* Analysis of CEC was done by Forestry Commission's Research Agency United Kingdom (Forest Research UK)

§ According to FAO and the US systems (FAO and US, 1962)

3.2.3 Test organism

Earthworms, *E. fetida* were obtained from Blades Biological Ltd. (Kent, UK). The earthworms were cultured in a medium of horse manure and peat (50:50) and kept moist with deionised water at room temperature (20±3 °C). The horse manure used was collected from horses that were not under medication to avoid any toxic effects on the earthworms. The earthworms were fed twice weekly with homogenised mashed potato powder which was added to the surface of the culture.

3.3 Experimental procedures

3.3.1 Toxicity test of bifenthrin treatments to *E. fetida*

The experiment was carried out as described in the OECD 222 guideline for assessing the effects of chemicals in soil on the reproductive output of the earthworm species (OECD, 2004) except 400 g of dry soil was used as opposed to 500 g of dry soil. The concentration of bifenthrin used for each of the bifenthrin treatments was 100 µg/g active ingredient. For each soil type, there were four replicate vessels per treatment with a further four control replicates containing no pesticide, making 80 glass containers in all. Groups of ten *E. fetida* were weighed (average weight of 8.21±0.48 g) and introduced to each vessel. The earthworms were kept at 20±2 °C under controlled light-dark cycles (preferably 16 h light and 8 h dark). Five grams of Smash® instant mashed potato powder was added as food to the surface of the soil in each glass container at the start of the experiment and then added once a week for the duration of the test (28 d).

At each time point (7, 14, 21 d) any food from previous additions that was still remaining on the soil surface was carefully removed, and surviving earthworms were removed, weighed and then returned to the glass containers to continue the exposure. Mortality was also recorded. Soil pH, moisture content, concentration of bifenthrin in soil and concentration in soil pore water were recorded in quadruplicates at each time point (0, 7, 14, 21 and 28 d) using a sub-sample of soil from the containers.

After 28 d, the surviving earthworms were removed and depurated for 48 h on moist filter papers. The moist filter papers were changed twice a day (in the morning and evening) to allow the earthworms to void their gut content (Dalby *et al*., 1996). Then, the earthworms were killed by freezing and then thawed prior to bifenthrin analysis. The cocoons produced were hand-sorted and returned to the glass containers containing the same test soils and incubated for another 28 d to determine the cocoon viability. After 56 d, the juveniles that had hatched were counted and recorded.

3.3.2 Sample preparation and analysis

Soil samples were extracted by liquid extraction. For all bifenthrin treatments, 5±0.5 g of soil was extracted into 15 ml acetonitrile and shaken on an orbital shaker (250 oscillations/min) at room temperature (20±2 °C) for 2 h. The samples were then allowed to settle and 2 mL aliquots of supernatant were taken for analysis. Soil pore water was extracted by placing 10±1 g of soil in a glass syringe with a layer of 3 cm of glass wool inserted into the bottom. Then, the syringe was inserted into a glass centrifuge tube and centrifuged for 20 min at 3000 rpm. The soil pore water was collected from the centrifuge tube for analysis.

Defrosted earthworms were extracted using 5 mL of acetonitrile and then the earthworm/acetonitrile mixture was homogenised for 5 minutes in a beaker using a

LabGen Series 7 homogeniser. The suspension was transferred from the beaker to a glass vial and the beaker was then rinsed with an additional 5 mL of acetonitrile which was combined with the suspension to give a total extract volume of 10 mL. The extracts were centrifuged for 20 min at 3000 rpm. The samples were then filtered and a 2 mL aliquot of the supernatant was taken for further analysis.

Soil and pore water and earthworm extracts were analysed using High-performance Liquid Chromatography (HPLC) on a Perkin Elmer Flexar coupled to a photodiode array detector and equipped with an automated injection system. The separation was performed with a SUPELCO 516 C-18-DB 5 µm, 4.6 x 150 mm column with a mobile phase flow rate of 1.5 mL/min. The mobile phase for the soil and pore water analyses comprised 15% water and 85% acetonitrile while for the earthworm extracts the mobile phase comprised 85% acetonitrile and 15% water each containing 0.1% formic acid. The detection wavelength was 250 nm and quantification was achieved using a calibration curve developed from known standards.

3.3.2.1 Analytical method validation

The analytical methods for HPLC developed were evaluated for different parameters such as linearity, relative standard deviation (RSD, %), limit of detection (LOD) and limit of quantification (LOQ). The linearity was qualified by the linear correlation coefficient, R^2 from the calibration curves using a series of concentrations for each bifenthrin treatments. Blank samples were tested to determine the specificity of the analytical method. For soil methods, the recoveries for each bifenthrin treatment were calculated using three different concentrations (2, 6 and 10 µg/g) and for earthworm methods, the concentrations applied were 1, 3 and 6 µg/g for each bifenthrin treatments. The LODs and LOQs were calculated for each bifenthrin treatment using 3 (for LOD) and 10 (for LOQ) times the signal to noise ratio.

3.3.3 Data analysis

3.3.3.1 Determination of sorption coefficient, K_d

Sorption coefficient, K_d values were calculated at each time point in each soil type for each bifenthrin treatment (Equation 1).

$$C_{pw} = \frac{\mathrm{Cs}}{\left(Kd\bullet\left(\frac{\%soil}{\%water}\right)\bullet bulk\ density\right)+1} \tag{1}$$

Where: C_{pw} and C_{soil} are the concentrations of the compound in pore water (μg/mL) and soil (μg/g), respectively; and %soil and %water are calculated based on the moisture content of the soil.

3.3.3.2 Determination of half-life, DT$_{50}$

The amount of time needed for the bifenthrin in each treatment to decay to half of its original concentration (DT$_{50}$) was determined using simple first-order degradation kinetics. Model parameters were calculated with Microsoft® Excel. The rate constant can then be used to derive the half-life $t_{1/2}$ using Equation 2.

$$t_{1/2} = \frac{\ln 2}{k} \tag{2}$$

3.3.3.3 Determination of soil based bioaccumulation factor, BSAF

BSAFs were calculated for earthworms (after 28 d) in each soil type for each bifenthrin treatment by dividing the concentration of bifenthrin in the earthworm by the concentration in the soil (Equation 3) (Rich *et al*., 2015).

$$BSAF = \frac{\mathrm{Cearthworm}}{\mathrm{Csoil}} \tag{3}$$

Where: $C_{earthworm}$ is concentration of bifenthrin in earthworm (μg/g); and C_{soil} is concentration of bifenthrin in soil (μg/g).

3.3.3.4 Statistical analysis

Statistical analysis was performed using SigmaPlot (v. 12.5). Data for K_ds, DT$_{50}$s, cocoon and juvenile production, and uptake by earthworms were first tested for normality using a Shapiro-Wilk test and then for equal variance. If these passed, then a one-way ANOVA was performed to assess the differences in the values. If the normality test failed, the analysis of variance was performed using Kruskal-Wallis analysis on ranks.

Between treatment differences in soil pH, weight change, mortality, concentration in soil and soil pore water from the toxicity study were first tested for normality using a Shapiro-Wilk test and then for equal variance. If these passed, a two-way repeated measure ANOVA was performed to assess the differences in the values among the groups. If these failed then a repeated measure ANOVA on ranks and ANOVA on ranks was performed for each treatment and each sampling date, respectively, to assess the differences in the values among the treatment and sampling date groups.

A linear regression analysis was performed to investigate the relationships between soil properties (soil pH, OC, CEC, clay, silt and sand) and the experimental observations (i.e., fate, uptake and effects) of the different bifenthrin treatments.

3.4 Results

3.4.1 Analytical method evaluation

In the blank samples, no peaks were observed at the same retention time of the analyte bifenthrin indicating that there were no false positive signals. The method validation studies for different bifenthrin treatments in water showed that average recoveries ranged from 95.3% (Capture LFR) to 106.8% (analytical grade bifenthrin) and the limit of detection (LOD) values ranged from 1.2 ng/mL (analytical grade bifenthrin) to 2.1 ng/mL (Nano B). For the soil extraction method, the recoveries ranged from 86.5% (Soil 4; analytical grade bifenthrin) to 108.6% (Soil 2; analytical grade bifenthrin) while the LODs ranged from 10.2 ng/g (Soil 5; Nano B) to 21.3 ng/g (Soil 4; Nano A). The recoveries for the earthworm, *E. fetida* extraction method ranged from 84.3% (Capture LFR) to 102.1% (analytical grade bifenthrin) while the LODs ranged from 58.2 ng/g (Nano A) to 194.8 ng/g (Capture LFR). Analytical method development information and a summary of the recoveries, LODs and LOQs can be found in Appendix 3.1.

3.4.2 Soil characteristics

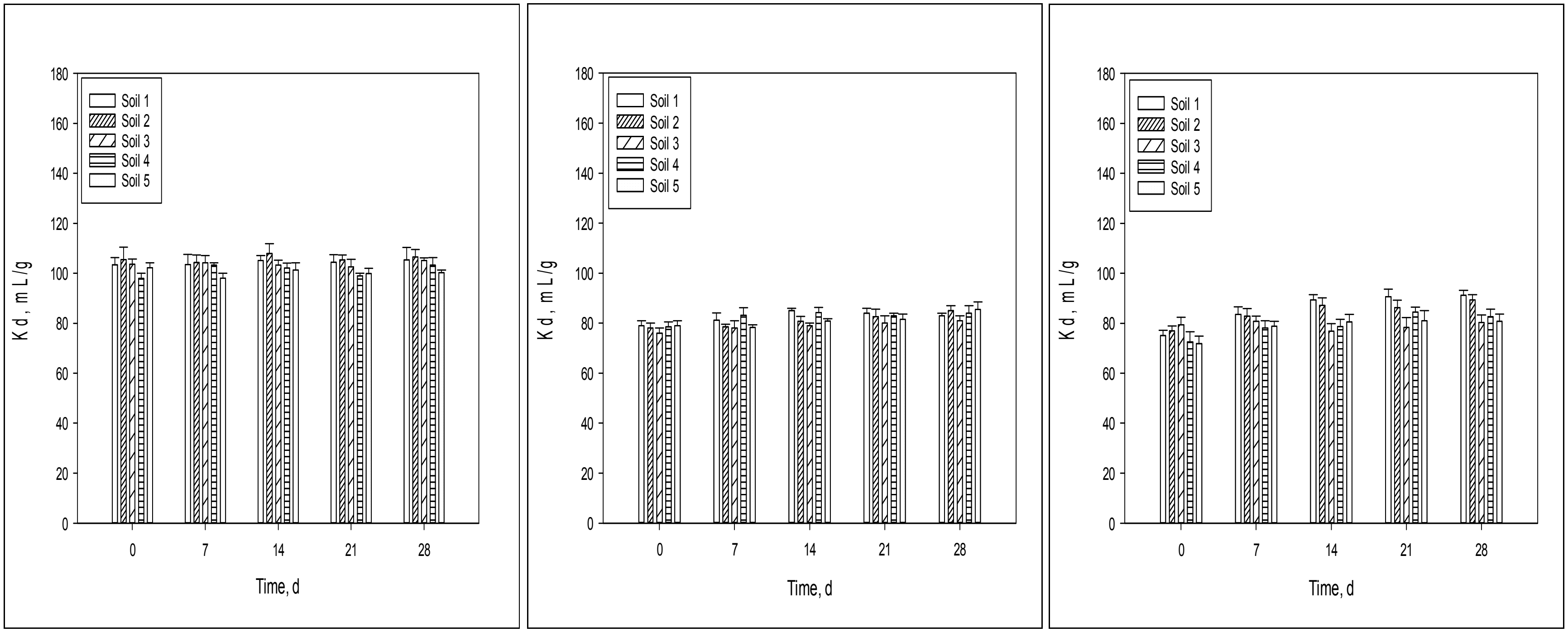
Generally, the bifenthrin treatments affected the soil pH over the 28 d experimental period (Table 3.2). With the exception of Soil 2, there was a significant difference in soil pH between the treatments for the 28 d experimental period ($F>26.61$; d.f.=12; $p<0.001$). Concentrations of bifenthrin in soil and soil pore water were found to decrease during the experimental period (Appendix 3.2 and 3.3, respectively) which could be attributed to the physico-chemical processes that occurred and uptake into the earthworms. Statistical analysis showed a significant difference in both the concentration of bifenthrin in soil and soil pore water in all tested soils over time ($F>18.09$; d.f.=12; $p<0.001$).

3.4.3 Fate of bifenthrin treatments

It was found that the K_ds varied across the soil and treatment types (Figure 3.1). This showed Capture LFR was more highly adsorbed to the soil particles compared to the two nanoformulation treatments across soil types ($F=10.99$; d.f.=4, $p<0.001$). No significant difference was observed for Nano A and Nano B treatments ($F<7.21$; d.f.=4, $p>0.125$). The DT_{50} values determined in this study also varied across soil types. The nanoformulation treatments (Nano A and Nano B) had significantly enhanced persistence compared to the Capture LFR in each soil type ($F>70.20$; d.f.=2; $p<0.001$, Table 3.3).

Table 3.2 Mean soil pH (±S.D, n = 4) for the five soil types at different time points and treatments during the study (for columns, values with same lower case letter have no significant difference, (p>0.05); for rows, values with same upper case letter have no significant difference, (p>0.05))

Soil/bifenthrin treatment	**Day**				
	0	**7**	**14**	**21**	**28**
Soil 1					
Control	7.49±0.07aA	7.48±0.06aA	7.45±0.13aA	7.51±0.07aA	7.49±0.04aA
Capture LFR	7.29±0.19bA	7.39±0.13bB	7.48±0.17aC	7.45±0.12bD	7.48±0.17aC
Nano A	7.39±0.04cA	7.52±0.03aB	7.53±0.03aB	7.54±0.05aB	7.48±0.17aC
Nano B	7.84±0.05dA	7.71±0.07cB	7.73±0.04bB	7.79±0.06bA	7.54±0.05aA
Soil 2					
Control	4.63±0.06aA	4.51±0.19aB	4.56±0.19aB	4.48±0.24aBC	4.46±0.24aBC
Capture LFR	4.59±0.17aA	4.57±0.17aA	4.62±0.24abA	4.69±0.17bAB	4.63±0.06bA
Nano A	4.62±0.03aA	4.67±0.06bB	4.66±0.05abC	4.51±0.06aD	4.54±0.09aE
Nano B	4.66±0.05aA	4.63±0.04abA	4.57±0.05aB	4.66±0.09bAC	4.66±0.05bAD
Soil 3					
Control	6.51±0.19aA	6.49±0.21aA	6.39±0.39aB	6.41±0.18aA	6.51±0.18aA
Capture LFR	6.58±0.08bA	6.54±0.22aB	6.55±0.26bB	6.47±0.15aC	6.44±0.13aD
Nano A	6.71±0.02cA	6.62±0.02bB	6.49±0.02cC	6.48±0.32aC	6.58±0.08bD
Nano B	6.71±0.02cA	6.68±0.12cB	6.63±0.06dC	6.65±0.09bBC	6.63±0.12cC
Soil 4					
Control	7.62±0.29aA	7.57±0.23aB	7.59±0.21aA	7.49±0.29aB	7.66±0.17aA
Capture LFR	7.74±0.26bA	7.63±0.24bB	7.66±0.36aC	7.78±0.22bA	7.62±0.31aB
Nano A	7.82±0.09bA	7.86±0.15cB	7.75±0.08bC	7.71±0.19bC	7.63±0.24aD
Nano B	7.79±0.08bA	7.69±0.06bB	7.77±0.11bC	7.72±0.05bD	7.66±0.17aE
Soil 5					
Control	6.59±0.34aA	6.71±0.29aB	6.69±0.25aB	6.52±0.19aC	6.55±0.27aD
Capture LFR	6.49±0.23bA	6.58±0.11bB	6.69±0.17abC	6.58±0.24aB	6.69±0.25bC
Nano A	6.78±0.07cA	6.74±0.06aB	6.75±0.06aB	6.69±0.04bC	6.71±0.08bD
Nano B	6.72±0.17dA	6.77±0.11aB	6.76±0.13acB	6.69±0.19bA	6.74±0.14bC



A) Capture LFR B) Nano A C) Nano B

Figure 3.1 Sorption coefficient, K_d (mL/g) (±S.D, n = 4): A) Capture LFR; B) Nano A; and C) Nano B

Table 3.3 Mean (±S.D, n = 4) sorption coefficients (K_ds), rates of degradation and half-life values (DT_{50}) values for the different bifenthrin treatments

Bifenthrin treatment/soil	**Persistence**	
	Rate, d^{-1}	**Half-life, DT_{50}, d**
Capture LFR		
1	-0.032±0.0003	21±2
2	-0.016±0.0002	43±2
3	-0.025±0.0001	28±1
4	-0.024±0.0002	28±2
5	-0.024±0.0002	29±2
Nano A		
1	-0.017±0.0004	42±1
2	-0.009±0.0003	82±3
3	-0.016±0.0001	43±3
4	-0.018±0.0001	39±2
5	-0.017±0.0002	41±2
Nano B		
1	-0.019±0.0003	37±3
2	-0.013±0.0001	52±2
3	-0.019±0.0002	37±1
4	-0.021±0.0002	33±1
5	-0.017±0.0001	40±2

3.4.4 Uptake of bifenthrin treatments into *E. fetida*

In all soil types, bifenthrin was not detected in the earthworm tissues from the control treatments (Figure 3.2). However, bifenthrin was detected in the earthworm tissues when exposed to different bifenthrin treatments. Within soil type, concentrations in earthworms exposed to Capture LFR were significantly higher than the concentrations in earthworms exposed to the two nanoformulation treatments ($F>5.89$; d.f.=2; $p<0.023$). However, there was no significant difference between uptake of bifenthrin into the earthworms across the two nanoformulation treatments.

Across the soil types, the uptake of bifenthrin into the earthworms in Soils 1, 3, 4 and 5 was similar for each bifenthrin treatment whereas uptake into Soil 2 was significantly lower ($F>5.89$; d.f.=2; $p<0.023$). Soil based bioaccumulation factors (BSAF) for each bifenthrin treatment in each soil were also determined (Figure 3.3). The data obtained showed that the BSAFs for Capture LFR were higher compared to the two nanoformulation treatments (Nano A and Nano B) in all soil types. There was a significant difference in the BSAFs among the bifenthrin treatments in each soil type (F>891.95; d.f.=2; p<0.001).

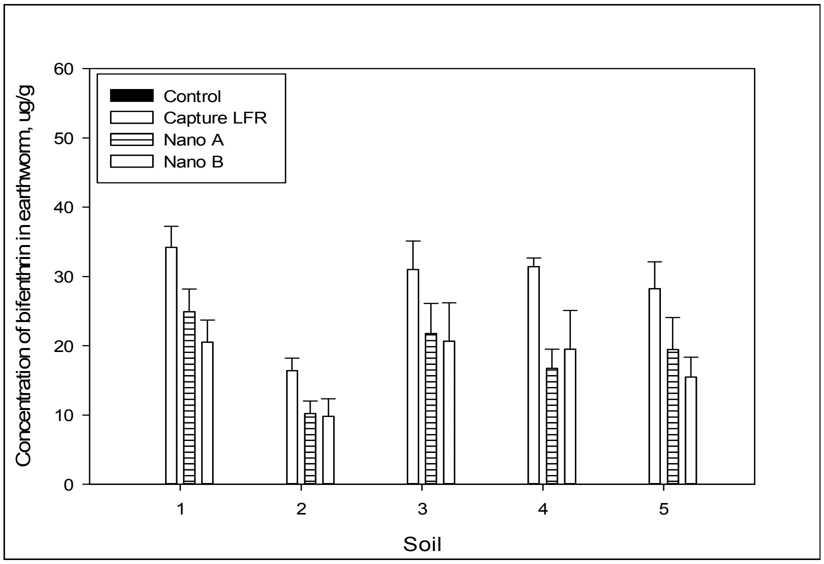


Figure 3.2 Mean uptake of bifenthrin (±S.D, n = 4) from different bifenthrin treatments into *E. fetida* (µg/g) after 28 d

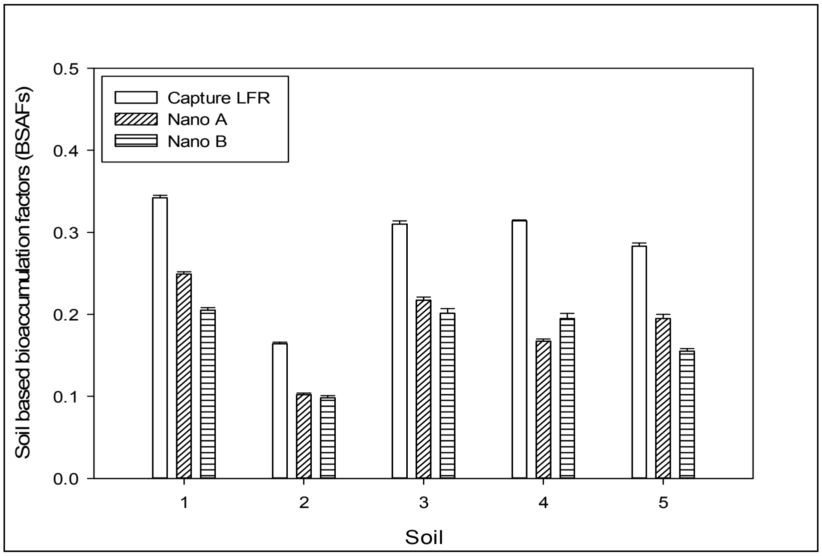


Figure 3.3 Mean BSAFs (±S.D, n = 4) from different bifenthrin treatments after 28 d

3.4.5 Effects of bifenthrin treatments on *E. fetida*

Generally, the weight of surviving earthworms in all treatments for each soil type changed significantly during the 28 d experimental period (F>2.21; d.f.=12; p<0.026; Table 3.4). With the exception of exposure in Soil 2 for all treatments, the weight of surviving earthworms increased during the 28 d experimental period. In Soil 2, when the earthworms were exposed to control and Capture LFR treatments, the weight of the earthworms was found to decrease ($F>16.00$; d.f.=4; $p<0.003$) whereas the exposure to Nano A and Nano B showed the weight of the earthworms remained the same ($F<2.18$; d.f.=4; $p>0.312$). This suggests that the earthworm growth was affected in some treatments during the 28 d experimental period.

Earthworm mortality was recorded during the 28 d experimental period (Figure 3.4). Kaplan-Meier Survival LogRank analysis showed that in each soil type, after 28 d, there was no significant difference in earthworm mortality when exposed to the different bifenthrin treatments ($F<8.82$; d.f.=3; $p>0.05$). No mortality was recorded for control treatments for all soil types. Low numbers of cocoons and juveniles were recorded in all bifenthrin treatments compared to controls after 28 d and 56 d across soil types (Figure 3.5(A) and (B), respectively). Statistical analysis showed a significant difference in the cocoon production between the treatments for Soil 2 and Soil 5 ($F>5.61$; d.f.=3; $p<0.012$). For juvenile production, only Soil 1 and Soil 5 showed a significant difference between the bifenthrin treatments compared to controls ($F>3.62$; d.f.=3; $p<0.045$). Across the soil types, Soil 2 showed the lowest degree in cocoon and juvenile production ($F>3.61$; d.f.=3; $p<0.045$).

Table 3.4 Mean weight of earthworms, g (±S.D, n = 4) for the five soil types at different time points and treatments during the study (for columns, values with same lower case letter have no significant difference, (p>0.05); for rows, values with same upper case letter have no significant difference, (p>0.05))

Soil/bifenthrin treatment	**Day**				
	0	**7**	**14**	**21**	**28**
Soil 1					
Control	0.79±0.03aA	0.84±0.04aB	0.88±0.05aC	0.91±0.04aC	0.95±0.05aD
Capture LFR	0.83±0.03aA	0.89±0.03aB	0.95±0.04aC	0.97±0.04aC	1.03±0.07aC
Nano A	0.83±0.03aA	0.85±0.03aA	0.86±0.04aA	0.88±0.05aAB	0.92±0.02aB
Nano B	0.82±0.03aA	0.84±0.04aAB	0.88±0.07aB	0.88±0.06aB	0.97±0.09aB
Soil 2					
Control	0.78±0.09aA	0.76±0.08aA	0.74±0.08aA	0.72±0.08aA	0.68±0.06aB
Capture LFR	0.84±0.07aA	0.82±0.06aAB	0.79±0.06aBC	0.81±0.09aCD	0.79±0.08aD
Nano A	0.83±0.03aA	0.81±0.05aA	0.80±0.13aA	0.82±0.08aA	0.83±0.11aA
Nano B	0.85±0.05aA	0.83±0.04aA	0.82±0.01aA	0.84±0.05aA	0.84±0.12aA
Soil 3					
Control	0.79±0.03aA	0.86±0.03aB	0.92±0.02abC	0.94±0.02aD	0.99±0.01aE
Capture LFR	0.83±0.03abA	0.86±0.04aB	0.89±0.04aC	0.91±0.04aD	0.98±0.08aE
Nano A	0.82±0.04abA	0.86±0.05aB	0.89±0.06aC	0.92±0.06aD	1.02±0.08aD
Nano B	0.89±0.02bA	0.94±0.01bB	0.98±0.03bC	0.98±0.03aC	1.01±0.03aC
Soil 4					
Control	0.75±0.05aA	0.86±0.04aB	0.94±0.04aC	0.98±0.04aD	1.02±0.03aE
Capture LFR	0.82±0.03abA	0.83±0.02aA	0.84±0.02bAB	0.86±0.04bB	0.89±0.04bC
Nano A	0.85±0.03bA	0.85±0.03aA	0.87±0.03bAB	0.89±0.08bB	0.97±0.06bC
Nano B	0.81±0.04abA	0.81±0.03aA	0.82±0.02bA	0.85±0.07bB	0.91±0.05bC
Soil 5					
Control	0.79±0.06aA	0.85±0.06aAB	0.93±0.08aBC	0.97±0.07aC	0.99±0.02aCD
Capture LFR	0.83±0.04aA	0.86±0.04aAB	0.91±0.05aB	0.93±0.05aB	0.94±0.08bB
Nano A	0.83±0.04aA	0.85±0.04aA	0.88±0.05aB	0.91±0.06aB	0.94±0.06abC
Nano B	0.83±0.03aA	0.84±0.03aA	0.84±0.02aA	0.87±0.06aB	0.97±0.08bC

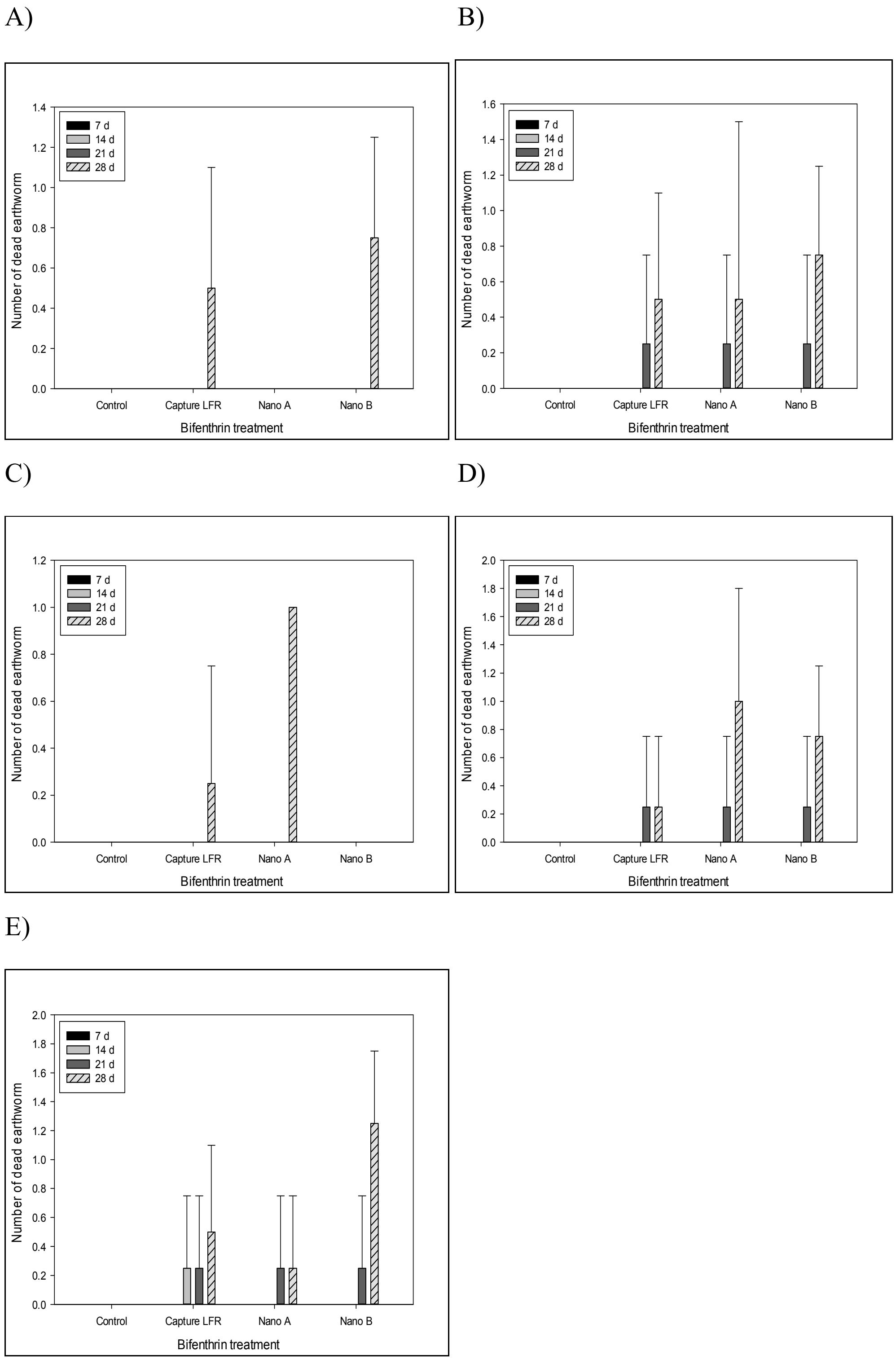
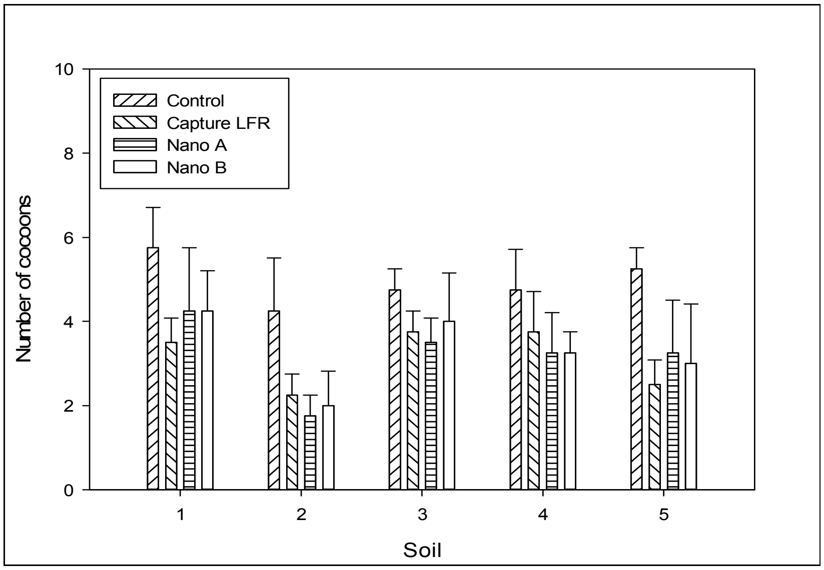


Figure 3.4 Mean mortality (±S.D, n =4) of the earthworms during 28 d; A) Soil 1, B) Soil 2, C) Soil 3, D) Soil 4, and E) Soil 5

A)



B)

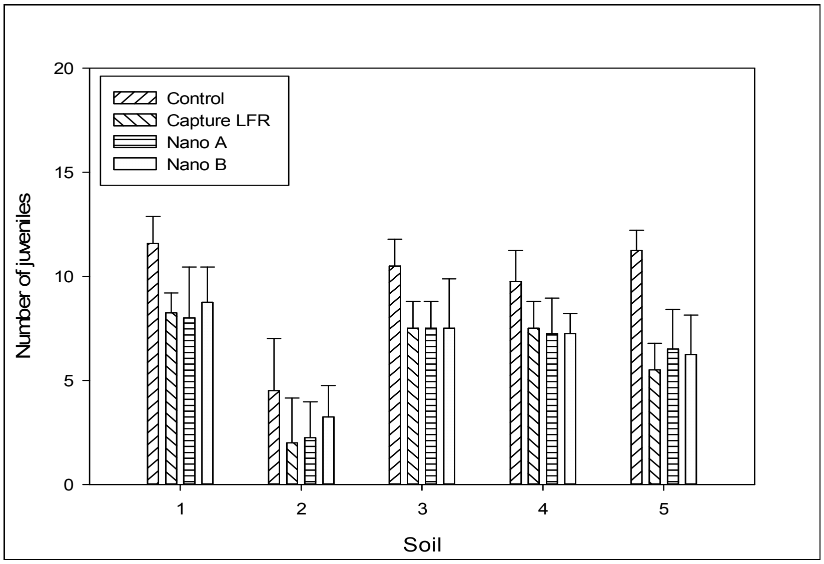


Figure 3.5 Mean cocoon (A) and juvenile (B) production (±S.D, n = 4) after 28 and 56 d, respectively

3.4.6 Relationships between soil properties and experimental observations

3.4.6.1 Fate and uptake of bifenthrin

Linear regression analysis between soil properties and the sorption coefficient, K_d values (average over 28 d) showed that only organic carbon was found to be related to the sorption behavior of Capture LFR ($R^2=0.839$, $p=0.039$) whereas no other relationships were observed between soil properties and K_ds for all the other bifenthrin treatments ($p>0.154$; Appendix 3.4). Soil pH and organic carbon content were found to be related with the DT_{50} values for all bifenthrin treatments and Nano A treatment, respectively ($R^2>0.823$, $p<0.034$). No relationships between the rest of soil properties and DT_{50} values were observed for any of the bifenthrin treatments ($R_2<0.698$; $p>0.078$; Appendix 3.5). A further analysis was performed to investigate the relationship of sorption with persistence in this study. Linear regression analysis showed that the sorption coefficient, K_d values (average 28 d) were not related to half-life, DT_{50} values ($p>0.172$; Appendix 3.6). This suggests that the persistence of different bifenthrin treatments was not influenced by the sorption process.

For the uptake of bifenthrin into earthworms, it was found that soil pH showed a significant relationship with uptake of bifenthrin from the Capture LFR and Nano B treatments ($R^2>0.813$; $p<0.036$) (Appendix 3.7). In addition, clay content was also found to show a relationship with uptake of bifenthrin from the Nano A treatment ($R^2=0.787$; $p=0.045$). In this study, the relation between the concentration of bifenthrin in soil for each bifenthrin treatments and BSAF values was also investigated. With the exception of Nano A treatment, there was a significant relationship found between the concentration of bifenthrin in soil (average of 28 d) and BSAF values ($R^2>0.807$; $p<0.038$).

3.4.6.2 Effects on earthworm mortality and juvenile production

No mortality was recorded in the control treatments, therefore linear regression was done only for the different bifenthrin treatments. The regression analysis showed soil properties had no effect on earthworm mortality ($p>0.089$) (Appendix 3.8). In terms of juvenile production, the regression analysis showed that the soil pH had clear relationships with the juvenile production when exposed to different bifenthrin treatments compared to controls ($R^2>0.829$; $p<0.032$) (Appendix 3.9). Soil organic carbon was also found to show a relationship with juvenile production in the Capture LFR treatment ($R^2=0.785$; $p=0.045$).

3.5 Discussion

3.5.1 Fate and uptake of bifenthrin treatments

In the present study, the fate, uptake and effects of nanoformulations of bifenthrin (Nano A and Nano B) were investigated together with traditionally formulated bifenthrin (Capture LFR) in order to assess the impact of pesticide nanoformulation on the terrestrial environment. Previous studies reported that the K_d and DT_{50} values of active ingredient bifenthrin were in the range of 882 – 6000 mL/g and 122 – 345 d, respectively across soil types (USEPA, 2010; FMC 50429-025, 1983; Froelich, 1983). Generally, the nano-encapsulation of bifenthrin affected the sorption and persistence behavior across soil types. In terms of sorption to soil particles, Capture LFR treatment showed higher K_ds compared to the two nanoformulation treatments across soil types ($p<0.001$; Figure 3.1) which suggested Capture LFR was highly adsorbed to the soil particles compared to the two nanoformulation treatments. As bifenthrin is a hydrophobic pesticide (log $K_{ow}=6$; Hansch *et al*., 1995), bifenthrin shows a great affinity towards organic carbon resulting in the significant in the K_ds.

For the Capture LFR treatment, the sorption of the bifenthrin was significantly lower compared to the reported K_d values for bifenthrin. This is probably due to the effect of the surfactants in the Capture LFR formulation that may alter the fate and behavior of pesticides active ingredient (Katagi, 2008). Lower K_ds in the nanoformulation treatments could be attributed to the nano-encapsulation of the bifenthrin protecting the active ingredient from soil binding sites, thus increasing the bioavailability in the pore water for uptake by earthworms. The findings in this study are in agreement with another study, where surfactants were found to affect the sorption of the chlorsulfuron and tribenuron methyl using both technical grade and commercial formulations in sand, loam and clay loam soils (Földényi *et al*., 2013). The extent of the sorption was higher for the technical grade compared to the commercial formulations.

The DT_{50} values obtained in this study were also found to be significantly lower across soil types (Table 3.3) compared to the reported DT_{50} values in the literature for bifenthrin (DT_{50}=122 – 345 d; FMC 50429-025, 1983). This could be explained by the abundance of soil microbes present in the soils tested which greatly influenced the degradation rates of the bifenthrin treatments. The nano-encapsulation was found to significantly enhance the persistence of bifenthrin in all soil types ($p<0.001$). In this study, the relationships between sorption and persistence of bifenthrin treatments showed that the persistence of different bifenthrin treatments was not influenced by the sorption process (see section 3.4.6.1). However, due to very little data being available in literature about the degradability of active ingredients loaded onto polymer-based nanocarriers, the explanation of these findings could be too simplistic. Therefore, an improved characterisation of the distribution and release of the active ingredient under a wide range of conditions should be carried out in order to understand completely about the degradation involving nanoformulations.

In this study, the uptake of the bifenthrin into earthworms, *E. fetida* was also determined (Figure 3.2). After the 28 d experimental period, it was seen that the uptake of bifenthrin from the two nanoformulation treatments (Nano A and Nano B) was low compared to the formulated bifenthrin (Capture LFR). As a result of the differences in the uptake, the BSAFs for the nanoformulation treatments were lower than the Capture LFR (Figure 3.3). A linear regression analysis between the concentration of bifenthrin in the soil (average of 28 d) and BSAFs for different bifenthrin treatments showed that there were relationships observed across the soil types in Capture LFR and Nano B treatments (see section 3.4.6.1). This could be explained by the sorption process that took place in the soil compartment, hence affecting the uptake into the earthworms. Based on this finding, it is likely that the uptake of bifenthrin treatments was not only driven by passive diffusion from soil pore water, but also through dietary uptake (i.e., ingestion of soil particles) (Jager *et al*., 2003; Belfroid *et al*., 1994a,b).

According to the study done by Chang *et al*. (2016), bifenthrin was found to accumulate in the earthworm, *E. fetida*. After 28 d of exposure at concentrations of 10 and 50 mg/kg soil, the calculated BSAF values were less than 0.3. They also found that the BSAFs showed an increasing trend with decreasing soil concentration for the low concentration (10 mg/kg soil) and exhibited peak-shaped curves. This could be attributed to the continuous uptake-elimination process in the earthworm or the adsorption and desorption of chemicals in soil. In contrast, for the exposure at high concentration (50 mg/kg soil), the trend of BSAFs was relatively stable. They suggested that this may be related to the quick elimination response for the earthworm when exposed to high concentration soil.

Investigation of the bioaccumulation of NPs in terrestrial organisms has also been reported (García-Gómez *et al*., 2014; Waalewijn-Kool *et al*., 2014; Hu *et al*., 2010). García-Gómez *et al*. (2014) investigated the toxicity and bioaccumulation of ZnO NPs, ZnO bulk and $ZnCl_2$ on *E. fetida*. They found that the effects of ZnO NPs and ZnO bulk on fecundity were similar and lower than the $ZnCl_2$. However, ZnO NPs were found to affect the fertility of the earthworms compared to ZnO bulk. Although the internal concentrations of Zn in earthworms from the ZnO NPs were greater compared to the ZnO bulk and $ZnCl_2$, the BSAF of all treatments were consistently <1. The observed BSAF values were in agreement with a study done by Hu *et al*. (2010) where the BSAF of ZnO NP was 0.26 when the *E. fetida* was exposed to the same concentration of 1,000 mg/kg soil dry weight. Another study involving toxicity and bioaccumulation of Ag NP and $AgNO_3$ was successfully performed on the soil arthropod *Folsomia candida* by Waalewijn-Kool *et al*. (2014). They found that the exposure to both forms of Ag caused a fast uptake of Ag. However, they also observed that the elimination rate of Ag was higher for Ag NP compared to $AgNO_3$. The calculated BSAFs for Ag NP and $AgNO_3$ were on average of 1.12 and 5.64, respectively. Based on our study, while no data available for nanopesticides, it is likely that our observations of the nanoformulation treatments are similar to the observations seen with metal, metal oxide NPs and bulk materials.

3.5.2 Effects of bifenthrin treatments on *E. fetida*

Throughout the 28 d experimental period, it was observed that the earthworm weight was increased in some cases when exposed to the bifenthrin treatments (Table 3.4). This indicated that the bifenthrin treatments were affecting the growth of the earthworms in some cases throughout the 28 d experimental period. The result in this study is in agreement with a study done by Song *et al*. (2015) where the exposure of lower concentration of deltamethrin increased the earthworm, *E. fetida* weight. In contrast, at high application rates (>30 mg/kg) the earthworm growth had slowed down.

When these findings are compared with the exposure involving NPs, most of the previous reported studies showed that the growth of adult earthworms were not affected by the metal NPs dispersed in soil (Kwak and An, 2015; Hooper *et al*., 2011). Unrine *et al*. (2010b) also found that the growth of *E. fetida* was not affected by the differences in the particle size of Cu NPs. In contrast, a study done by Lebedev *et al*. (2015) showed that the Zn NPs were found to affect the earthworm, *E. fetida* weight by decreasing the weight during the experimental period. They observed that after 14 d of exposure, the earthworm weight decreased was in the range of 4.2 to 7.4 % of the original earthworm weight.

The mortality of *E. fetida* was recorded throughout the 28 d experimental period (Figure 3.4). During the study, no mortality was recorded in the control treatments. However, mortality was observed when the earthworms were exposed to bifenthrin treatments. This suggests that the mortality could be attributed to the bifenthrin mode of action. In general, exposure to synthetic pyrethroids (SPs) could lead to incoordination, convulsions and paralysis of the exposed animals by disrupting the normal function of the peripheral nervous system resulting in mortality (Soderlund and Bloomquist, 1989; Miller and Salgado, 1985). Therefore, it is strongly suggested that the mortality was due to the active ingredient bifenthrin from all the treatments. When *E. fetida* was exposed to other type of SP (i.e., α-cypermethrin), a lower toxic effect was observed (Hartnik and Styrishave, 2008). This could be explained by the rapid metabolism shown by α-cypermethrin. The α-cypermethrin may have metabolised in the earthworm body where α-cypermethrin exerted its toxicity. The other possible metabolism is when α-cypermethrin reversibly bound to the receptor and redistributed between receptors and other tissues where this metabolism keeps the concentration in the earthworm tissue low, hence reduced the mortality of the earthworm (Hartnik and Styrishave, 2008).

However, work from previous studies investigating the impact of metal NPs was dissimilar to the findings in the present study. When the earthworms, *E. fetida* and *L. terrestris* were exposed to Ag NPs, no mortality was recorded (Barua *et al*., 2013; Schlich *et al*., 2013; Heckmann *et al*., 2011; Lapied *et al*., 2010). These studies were in agreement with a study done by Van der Ploeg *et al*. (2011) where they confirmed that earthworms, *L. rubellus* exposed to C_{60} showed no effects on the survival of adult earthworms. Our results suggest that the use of surfactants (e.g., polymers) together with the active ingredient bifenthrin for the bifenthrin treatments may have shown some effects on earthworm. According to previous study, Stanley and Joy (2014) investigated the impacts of polyoxyethylene alkyl amine (POEA) surfactant together with active ingredient glyphosate on earthworm, *Nsukkadrilus mbae*. They found that with increasing concentration and time of exposure, the percentage of mortality was increased. Overall, more work should be carried out involving a wide range of surfactants used (e.g., inorganic and organic materials) and different active ingredient in order to understand the impacts of surfactants on the terrestrial organism such as earthworm.

The reproductive parameters such as cocoon and juvenile production are two of the most common endpoints observed in order to assess the effect of soil contaminants in earthworms (Amorim *et al*., 2005; 1999). Generally, the introduction of bifenthrin treatments decreased the number of cocoon and juvenile produced compared to the control treatments after 28 d experimental period (Figure 3.5(A) and (B), respectively). This trend is the same as observed wih the exposure of deltamethrin to *E. fetida* where the exposure to SP decreased the cocoon production (Song *et al*., 2015). Across the soil types, it was found that the degree of cocoon and juvenile produced in Soil 2 were significantly lower than the other soil types ($F>5.61$; $d.f.=3$; $p<0.012$). This could be probably explained by the low pH value for Soil 2 ($pH=4.71\pm0.1$). Previous studies reported that soil pH played an important role in affecting the survival of adult earthworm and thus the production of juveniles (Amorim *et al*., 2005; 1999). Reported work in the literature confirms that the exposure of earthworms to NPs can affect the reproduction parameter. For example, ZnO NPs were found to affect the reproduction of *E. fetida* and *E. veneta* (Heggelund *et al*., 2014; Hooper *et al*., 2011). Coleman *et al*. (2010) was successfully performed both subchronic and avoidance tests for Al_2O_3 NPs. The reported results showed that the reproduction of *E. fetida* was adversely affected when the concentration of Al_2O_3 was exceeded 3000 mg/kg soil. However, some authors also reported that some negligible ecotoxicological effects of TiO_2 NPs occur in earthworm such as the reproduction (i.e., cocoon production and viability, and hatching rate) (Hund-Rinke *et al*., 2012; McShane *et al*., 2012) and the growth of juvenile (McShane *et al*., 2012).

It is noted that, these previous studies reported in the literature were performed using metal NPs as the test chemicals. To the best of our knowledge, no studies have been reported on the impact of pesticide nanoformulation on growth, survival, uptake and reproduction of adult earthworms. Therefore, more studies should be carried out under a wide range of conditions to assess the impact of pesticide nanoformulation on the terrestrial organisms as these ecotoxicological effects could also be influenced by the complex physico-chemical processes that could affect the distribution of the pesticide nanoformulation in the soil compartment.

3.5.3 Do soil properties affect the fate, uptake and effects of bifenthrin treatments on *E. fetida*?

In this study, as bifenthrin is a non-polar pesticide and shows a great affinity to organic carbon, it was expected that soil organic carbon content might play an important role in affecting the fate, uptake and effects of bifenthrin formulations in the terrestrial environment. However, based on the linear regression analysis, soil organic carbon content was found to be affecting the sorption behavior and juvenile production in Capture LFR treatment only.

Soil organic carbon was found to be related to the sorption of Capture LFR in the tested soils which could be explained by the interactions between surfactants in the Capture LFR formulation and the soil organic carbon. On the other hand, no relationships were observed between other soil properties on the sorption and persistence behavior. According to previous studies, a weak correlation between the sorption and degradation processes greatly influences the probabilistic analysis of leaching through soil (Kah *et al.*, 2007; Beulke and Brown, 2006; Wu *et al.*, 2011). In this study, the result obtained from linear regression analysis showed that there was no relationship between the sorption and persistence behavior.

In the uptake study, it was found that only soil pH played a role in affecting the uptake of bifenthrin into earthworms in Capture LFR and Nano B treatments while clay content only showed a relationship with the uptake in Nano A treatment. No relationships were found between other soil properties and the uptake. An analysis between the concentrations of bifenthrin in soil was performed together with the BSAFs obtained in this study for all bifenthrin treatments. It was observed that the soil concentration (average of 28 d) showed relationships with the Capture LFR and

Nano B treatments. This could be the reasonable explanation that in this study, the uptake of bifenthrin into earthworms could also greatly influenced by the ingestion of soil particles as well as the passive diffusion across the earthworm skin into their body (Jager *et al*., 2003).

Effects of soil properties on the survival of adult earthworms and reproduction parameters were also investigated. The regression analysis showed that the soil properties did not influence the mortality of the earthworms after 28 d experimental period in all bifenthrin treatments across the soil types. On the other hand, the juvenile production after 56 d experimental period in the bifenthrin treatments compared to controls was greatly influenced by the soil pH. Soil pH was reported to play an important role in affecting the production of juveniles (Amorim *et al*., 2005; 1999). In addition, soil organic carbon was also found to affect the juvenile production in the Capture LFR treatment whereas no other relationships were found between other soil properties and juvenile production. However, in order to understand the toxic effect of different pesticide formulations involving nanoformulations, more studies should be carried out such as using a wide range of soil pH, temperature, different condition such as tropical and temperate, and different nanoformulations of any interest active ingredient.

3.6 Conclusion

In this study, it is demonstrated that the effects of nanoformulations (Nano A and Nano B) compared with the formulated of bifenthrin (Capture LFR). The findings showed the formulated and nanoformulation treatments of bifenthrin had no significant effect on earthworm mortality whereas significant effects were observed for the cocoon and juvenile production compared to the controls. There was no difference in the toxicity of the different bifenthrin treatments, even though uptake of bifenthrin from the conventional treatment was significantly greater than the nanoformulation treatments. The results indicate that while nanopesticides are accumulated less than conventional pesticide, the toxicity (based on soil concentration) does not differ. The most significant result from this study was the uptake of bifenthrin from different bifenthrin treatments into earthworms after the 28 d experimental period. The results demonstrated the uptake by earthworms was significantly greater in the formulated treatment compared to the nanoformulation treatments of bifenthrin.

In order to understand completely the fate and uptake of nanoformulations, more work should be carried out investigating the fate of nanoformulation (i.e., sorption and persistence) and determination of the uptake kinetics as well as the distribution of the active ingredient in the earthworms. Therefore, in the next chapter, the fate (sorption and persistence) of nanoformulations (Nano A and Nano B) is explored.

Chapter 4 Sorption and Persistence of Nanopesticides in Soil-Water Systems

4.1 Introduction

The previous chapter (*Chapter 3*) explored the fate, uptake and effects of different bifenthrin treatments in the terrestrial environment using earthworms as the test organism. In terms of fate characteristics, the nanoformulation treatments showed a decrease in sorption and enhanced persistence compared to the non-nanoformulation treatments. The fate of pesticides is an important parameter that influences the distribution of pesticides in the terrestrial environment, thus affecting the uptake of pesticides into soil invertebrate such as earthworm (Kerle *et al*., 2007). In the terrestrial environment, pesticides are applied due to their ability to control pests that affect the agricultural crops as well as the pests in the home, yards and gardens. Once applied, pesticides will undergo fate processes that are controlled by their physical and biological properties of the environment and the chemical properties of the pesticides (Tang *et al*., 2012; Triantafyllidis *et al*., 2010; Sparks, 2003). The fate processes for pesticides are grouped into those that could affect mobility including sorption, plant and animal uptake, volatilisation, run-off and leaching, and those that could affect persistence including chemical and microbial degradation (Shipitalo *et al*., 2000; Baker and Mickelson, 1994; Beestman and Deming, 1974).

Sorption refers to the loss of a solute from aqueous solution (Sposito and Schindler, 1986). This includes adsorption, a general term describing the disappearance of solutes (i.e., pesticides) from solution with the presumption of adsorption on a solid phase. This process involves either physical or chemical interaction with the surface (e.g., solid) which then could determine the distribution of the pesticides in the terrestrial environment. As sorption describes the partitioning behavior between soil and water (Gawlik *et al*., 1997), it is a parameter that is measured experimentally in order to determine the availability of the active ingredient in the soil pore water for uptake by terrestrial organisms and the effectiveness of a pesticide (Kah and Brown, 2007; Walker, 2000). A standard batch equilibrium method (OECD 106, 2000) is normally used in order to determine the sorption coefficient (K_d) of conventional pesticides. The advantages of this method are that the soil and solution can be separated effectively and a large volume of solution is obtained for analysis.

However, some important experimental design aspects such as type of vessels, type of shaking (i.e., orbital or horizontal), soil solution ratios and temperature are not completely standardised. This could potentially result in different K_ds which are difficult to compare between different studies. In addition, the high soil solution ratios used in these studies (i.e., 1:2 to 1:100) are not typical of field soil moisture conditions and the results obtained may not adequately reflect the sorption processes in field-moist or unsaturated soil (Kah and Brown, 2007).

Alongside sorption, persistence is reported to be an important parameter for predicting the fate and transport of organic compounds in soils (Boesten and Van der Linden, 1991). Persistence can be explained by both chemical and microbiological degradation processes. Chemical degradation occurs through chemical reactions such as photolysis, hydrolysis, oxidation and reduction (Bavcon *et al*., 2003; Kodaka *et al*., 2003). On the other hand, microbiological degradation takes place when soil microorganisms consume or break down pesticides (Sassman *et al*., 2004; Ghadiri and Rose, 2001). Standard laboratory and field dissipation studies are normally performed in order to assess the rate of degradation expressed as a first-order half-life or DT_{50} (time required for 50% of the initial dose to be degraded). Soil column experiments are more analogous to field experiment but normally they will take a long time to complete and pose some drawbacks including a non-uniform flow within the column and the fact that it is difficult to differentiate between processes that occur in the column such as adsorption and degradation (Celorie *et al*., 1989).

There are many factors reported in the literature that influence both sorption and degradation processes mainly associated with the properties of the pesticide and properties of the soil (Rosales-Conrado *et al*., 2002; Perrin-Ganier *et al*., 2001; LaPrade, 1992). There are four main structural factors of the properties of pesticides that could affect the fate and transport of pesticide in the terrestrial environment. These include the nature of functional groups such as carboxyl, carbonyl alcoholic hydroxyl and amino groups. Generally, sorption is increased when chemicals have functional groups such as R_3N^+, $-CONH_2$, $-OH$, $-NHCOR$, $-NH_2$, $-OCOR$ and $–NHR$ (Khan, 1980). Another structural factor is the nature of substituting groups that could alter the behavior of the functional groups. Third, the position of substituting groups with respect to the functional groups that could enhance or hinder the intra-molecular bonding. This factor shows the potential to permit the coordination with any transition metal ions. The presence and magnitude of unsaturation in the molecule could also affect the lyophilic-lyophobic balance (Khan, 1980).

Other factor such as soil pH also plays an important role in determining the fate of pesticides in the terrestrial environment (Regitano *et al*., 1997; Shimizu *et al*., 1992; Piccolo and Celano, 1993; Fontaine *et al*., 1991; Lee *et al*., 1990). Soil pH can affect the ionisation of many organic molecules. For example, acidic pesticides are proton donors, which at high pH (one or more pH unit above the pKa of the acid) become anions due to dissociation (Piccolo and Celano, 1993). Soil clay content, organic matter and dissolved organic matter are also reported to affect the fate and transport of pesticides (Salvati *et al*., 2011; Peter and Weber, 1985). A study done by Nennemann *et al*. (2001) showed that metolachlor could be adsorbed by raw and purified bentonites, but they found that the amount adsorbed depended on the type of bentonite and pre-treatment reactions. They concluded that adsorption could be enhanced by modifying the bentonite or montmorillonite.

For the past years, the development in the engineered nanoparticle (ENPs) production has brought a great deal of attention on the production of nanopesticides or novel plant protection products. This is due to the potential of ENPs incorporated with pesticide active ingredient to create pesticides that have better performance such as improved the delivery compared to conventional pesticides. However, questions have also been raised on how to assess the possible impact (i.e., fate and transport) of these nanopesticides in the terrestrial environment. As nanoparticles can behave differently from dissolved chemicals, it is possible that the fate and transport of nanopesticides could be very different compared to conventional pesticides. Several studies have investigated the potential fate of ENPs (Ju-Nam and Lead, 2008; Klaine *et al*., 2008). One of the main important processes that could influence the fate and behavior of ENPs is the aggregation process. Aggregation of ENPs is likely to be influenced by size, particle shape, and surface area, charge and coatings (Handy *et al*., 2008; Pettibone *et al*., 2008). Soil properties including soil pH, ionic strength and dissolved organic matter could also influence the aggregation of these ENPs in the soil compartment (French *et al*., 2009). Four to five nm diameter TiO_2 ENPs were found to readily form stable aggregates at pH 4.5 with an average diameter size ranging from 50 – 60 nm in a NaCl suspension when the ionic strength was adjusted to 0.0045 M (French *et al*., 2009). When the ENPs were exposed to an increased ionic strength of 0.0165 M while keeping the pH constant, formation of micron-sized aggregates was observed after 15 min. At low ionic strength (0.0084 – 0.0099 M NaCl), micron-sized aggregates were formed in less than 5 min for all pH values tested (5.8 – 8.2). French *et al*. (2009) concluded that pH and ionic strength were greatly influencing the aggregation of ENPs in the soil compartment.

In the present study, the sorption and persistence were determined following a standard batch equilibrium method and a previous persistence study on bifenthrin as described in the OECD method 106 (2000) and Sharma and Singh (2012), respectively. The bifenthrin treatments tested including a conventional formulation bifenthrin (Capture LFR) and two nano-encapsulated formulations (Nano A and Nano B). Studies were also done on analytical grade bifenthrin. The use of OECD guideline in this study was aimed to estimate the sorption behavior of a chemical substance using a range of different concentrations on soils. The K_d values can then be used to predict partitioning behavior under a variety of environmental conditions (e.g., pH, organic carbon content, texture and temperature). In addition, analytical grade bifenthrin was used to provide a baseline for a comparison in terms of sorption behavior with the different bifenthrin treatments which was not performed in the previous chapter (*Chapter 3*).

On the other hand, persistence studies were aimed at providing an understanding of mechanisms using sterile and non-sterile treatments. The use of the analytical grade material for the persistence study would also allow us to understand the release rate of bifenthrin from the nano-encapsulated treatments (Nano A and Nano B) as bifenthrin is located within the nanocapsules and it was assumed that the increase in persistence observed in the previous *Chapter 3* was a result of the slow release of the bifenthrin from the capsules.

4.2 Materials

The chemicals and soils used in this study were the same as those used in *Chapter 3* with an additional analytical PESTANAL® grade bifenthrin (98.9%, w/w) purchased from Sigma-Aldrich (Dorset, UK). Detailed descriptions of the chemicals and soils can be found in section 3.2.1 and 3.2.2, respectively in *Chapter 3*.

4.3 Experimental procedures

4.3.1 Sorption studies

Sorption coefficients (K_d, mL/g) were determined using a batch equilibrium method based on OECD method 106 (2000). Five different concentrations were used in order to determine the K_d values for each bifenthrin treatment with four replicates per concentration. These concentrations were chosen based on the recommendation in the OECD guideline to choose an initial concentration that is at least two orders of magnitude higher than the detection limit of the analytical method. Previous studies showed that bifenthrin is highly adsorbed to the soil particles (Froelich, 1983), and it is also expected that the phase partitioning involving nanopesticides are highly concentration dependent (Kookana *et al*., 2014), hence higher concentrations were chosen in this study considering the amount of bifenthrin that may adsorb to the soil particles with time.

A preliminary experiment was done in order to identify the optimum soil/solution ratio and contact time for each of the bifenthrin treatment. For this experiment, the analytical grade bifenthrin or bifenthrin formulations were prepared in 0.01 M $CaCl_2$ solution and applied to 1±0.1 g of soil contained in glass centrifuge tubes or 1 L Duran bottles to give a concentration of 10 µg/mL. The $CaCl_2$ solution was chosen in order to improve centrifugation and minimise the cation exchange between the bifenthrin treatments and the soil components. For each bifenthrin treatment, experiments were done in quadruplicate for each soil/solution ratio (i.e., soil/solution = 1:5, 1:30, 1:50, 1:100, etc.) and time point (i.e., 2, 4, 6, 8 and 24 h). The glass tubes or Duran bottles were then covered with aluminium foil to avoid any photochemical reactions occurring and shaken on an orbital shaker (250 oscillations/min) at room temperature (20±2 °C) for each time point. After the shaking process was completed, the glass tubes were centrifuged for 15 min at 3000 g. In the case of samples prepared in Duran bottles, at the end of the shaking process, a total volume of 50 mL was transferred into glass centrifuge tubes and followed by the centrifugation at the same time and speed. After the centrifugation, the mixtures were allowed to settle and aliquots of supernatant were taken for analysis for bifenthrin. Controls without soil (only 0.01 M $CaCl_2$ solution) were used to determine dissipation in the test vessels and blanks (only soil) were used to confirm the absence of background.

In order to develop the sorption isotherms, a definitive experiment was performed based on the preliminary results. For this experiment, five different concentrations of 20, 40, 60, 80 and 100 µg/mL were applied to the soil/solution ratios selected (Table 4.1). The contact time chosen for this definitive experiment was 24 h as this was the time needed for the bifenthrin to achieve equilibrium between the soil and aqueous phases.

Table 4.1 Selected soil to solution ratios used for the sorption studies for different bifenthrin treatments

Soil	**Soil/solution ratio**			
	Analytical grade bifenthrin	**Capture LFR**	**Nano A**	**Nano B**
1	1:600	1:75	1:75	1:75
2	1:800	1:100	1:100	1:100
3	1:400	1:50	1:50	1:50
4	1:400	1:50	1:50	1:50
5	1:400	1:50	1:50	1:50

4.3.2 Persistence studies

Persistence was assessed following a method described by Sharma and Singh (2012) except that 5±0.5 g of dry soil was used as opposed to the 25 g dry soil; tests were done with four replicates rather than three; and the period of incubation was 112 d compared to 40 d in the previous study. This period was chosen as bifenthrin is highly adsorbed and the dissipation of bifenthrin could be hindered by the adsorption process (Froelich, 1983). Persistence of the bifenthrin formulations and analytical grade material was determined in both sterile and non-sterile soils. For the sterile treatments, soils were autoclaved using a Classic Prestige Medical autoclave at 121 °C for 30 min, for three times with an interval of 3 d. This was done to eliminate any active microorganisms in the test soils. Soils were added into glass vials under laminar flow conditions for sterile soils and under normal environmental condition for non-sterile soils. Sterile and non-sterile soils were spiked with the bifenthrin treatments at a level of 10 µg/g of dry soil. The mixtures of soil and bifenthrin treatments were then incubated in the dark at 20±2 °C for the whole period of the study. This temperature is recommended for all test substances which may reach the soil in temperate climates (OECD 307, 2002). The soil moisture content was maintained every day by the addition of 0.2 mL of sterile deionised water for the sterile soils and normal deionised water for non-sterile soils. At each sampling point (0, 7, 14, 28, 42, 56, 70, 84, 98 and 112 d), four replicates of sterile and non-sterile soils were taken along with four replicates of the controls for analysis for bifenthrin.

4.3.3 Sample preparation and analysis

Soil samples and samples of solution from the persistence and sorption studies were extracted and analysed using the analytical method described in section 3.3.2 in *Chapter 3*.

4.3.4 Data analysis

4.3.4.1 Sorption isotherms

The data obtained from sorption studies were fitted with linear, Freundlich and Langmuir isotherms. For the linear isotherms, the distribution coefficients (K_d) were determined from the slopes of the graphs plotted based on Equation 4.

$$\mathrm{Kd} = \frac{Qe}{Ce} = (\frac{ms}{maqueous}) \bullet (\frac{V0}{msoil}) \qquad (4)$$

Where: K_d is sorption coefficient (mL/g); Q_e is pesticide sorbed to the soil (µg/g); C_e is concentration of pesticide in water (µg/mL); m_s is mass of the test substance adsorbed on the soil at equilibrium (µg); $m_{aqueous}$ is mass of the test substance in the solution at equilibrium (µg); V_0 is initial volume of the aqueous phase in contact with the soil (mL); and m_{soil} is quantity of the soil phase, expressed in dry mass of soil (g) (OECD 106, 2000).

For the Freundlich isotherm, a linear form of the Freundlich equation (Equation 5) was used to derive the Freundlich parameters.

$$\mathrm{Log\ Qe} = \mathrm{Log\ Kf} + \left(\frac{1}{\mathrm{n}}\right) \mathrm{Log\ Ce} \qquad (5)$$

Where: Q_e is pesticide sorbed to the soil (µg/g); K_f is Freundlich isotherm constant (µg/g); C_e is concentration of pesticide in water (µg/mL); and n is adsorption intensity. From a plot of Log Q_e versus Log C_e, $1/n$ (a function of the strength of adsorption in the adsorption process) and the constant K_f (an approximate indicator of adsorption capacity) were determined (Voudrias *et al*., 2002).

Langmuir adsorption parameters were determined using a linear form of the Langmuir equation (Equation 6).

$$\frac{1}{\mathrm{Qe}} = \left(\frac{1}{KL} \bullet Q0\right) \bullet \frac{1}{Ce} + \frac{1}{Q0} \tag{6}$$

Where: Q_e is pesticide sorbed to the soil (µg/g); K_L is Langmuir isotherm constant (mL/µg); Q_o is maximum monolayer coverage capacity (µg/g); and C_e is concentration of pesticide in water (µg/mL).

A coefficient of determination (R^2) obtained from these isotherms was then used to determine the best-fitting isotherm (Desta, 2013; Dada *et al.*, 2012; Ho, 2004; Ho *et al.*, 2002).

4.3.4.2 Determination of half-life, DT_{50}

Half-life, DT_{50} for each bifenthrin treatment for each soil type was determined as described in section 3.3.3.2 in *Chapter 3*.

4.3.4.3 Determination of release rates and half-life, R_{50}

The release rates of bifenthrin from nanoformulation treatments (Nano A and Nano B) were determined in both sterile and non-sterile soils from the persistence study using a sequential first-order model (Figure 4.1).

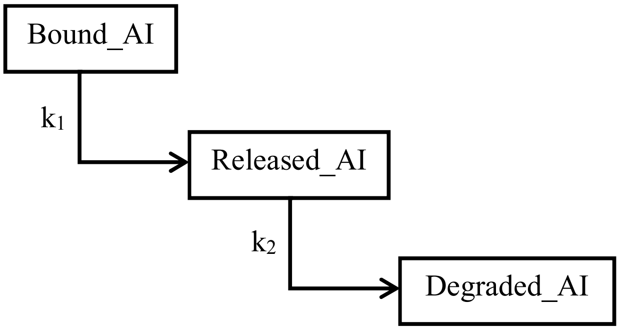


Figure 4.1 Schematic of the model used to estimate the release rate of bifenthrin from nanoformulation treatments, where k_1 and k_2 are the release and degradation rates, respectively

By using the fixed degradation rate, k_2 of the analytical grade bifenthrin, the parameter k_1 was estimated using ModelMaker (v. 4.0, Cherwell Scientific Ltd., Oxford, UK). Detailed descriptions on the model can be found in Appendix 4.1. The time required for half of the bifenthrin to be released from the nanocapsules (R_{50}) were calculated from the estimated rate constant, k_1 using Equation 7.

$$R_{50} = \frac{\ln 2}{k_1} \qquad (7)$$

4.3.4.4 Statistical analysis

Statistical analysis of the data was performed using SigmaPlot (v. 12.5). Data on the K_d, DT_{50} and R_{50} values were first tested for normality using a Shapiro-Wilk test and then for equal variance. If these passed, a one-way ANOVA was performed to assess the differences in the values among the bifenthrin treatments in each soil type and across soil types. If these tests failed, analysis of variance was performed using Kruskal-Wallis analysis on ranks (ANOVA on ranks).

A linear regression analysis was performed to investigate the relationships between soil properties (soil pH and the percentage of OC, CEC, clay, silt and sand) and the K_d and DT_{50} values of the different bifenthrin treatments.

4.4 Results

4.4.1 General trend of sorption isotherms of different bifenthrin treatments

The linear isotherm model showed the best R^2 values (Appendix 4.2), therefore, the results of the linear isotherm model were chosen in order to explore the differences in the sorption behavior of the different bifenthrin treatments in different soil types (Figure 4.2). Generally, the K_d values for the different bifenthrin treatments, based on batch experiments, increased in order Nano B < Nano A < Capture LFR < analytical grade bifenthrin and varied with the different soil types. The observed trend in the K_d values was in agreement with the previous findings in *Chapter 3* where the Capture LFR treatment showed higher K_d values compared to both Nano A and Nano B treatments. In this study, the range of the K_d values for analytical grade bifenthrin, Capture LFR, Nano A and Nano B treatments were 1800±41 – 7200±218, 190±10 – 470±61, 64±3 – 100±5 and 52±5 – 150±11 mL/g, respectively. In each soil type, there was a significant difference in the K_d values between the bifenthrin treatments ($H>13.10$; d.f.=12; $p<0.004$) and across soil types ($H=16.27$, d.f.=3, $p<0.001$). When K_d values were normalised to organic carbon content, K_{oc} values were found to range from 115600– 144700 mL/g for analytical grade bifenthrin, 9012– 15330 mL/g for Capture LFR, 1977– 5246 mL/g for Nano A and 2926– 4262 mL/g for Nano B (Table 4.2).

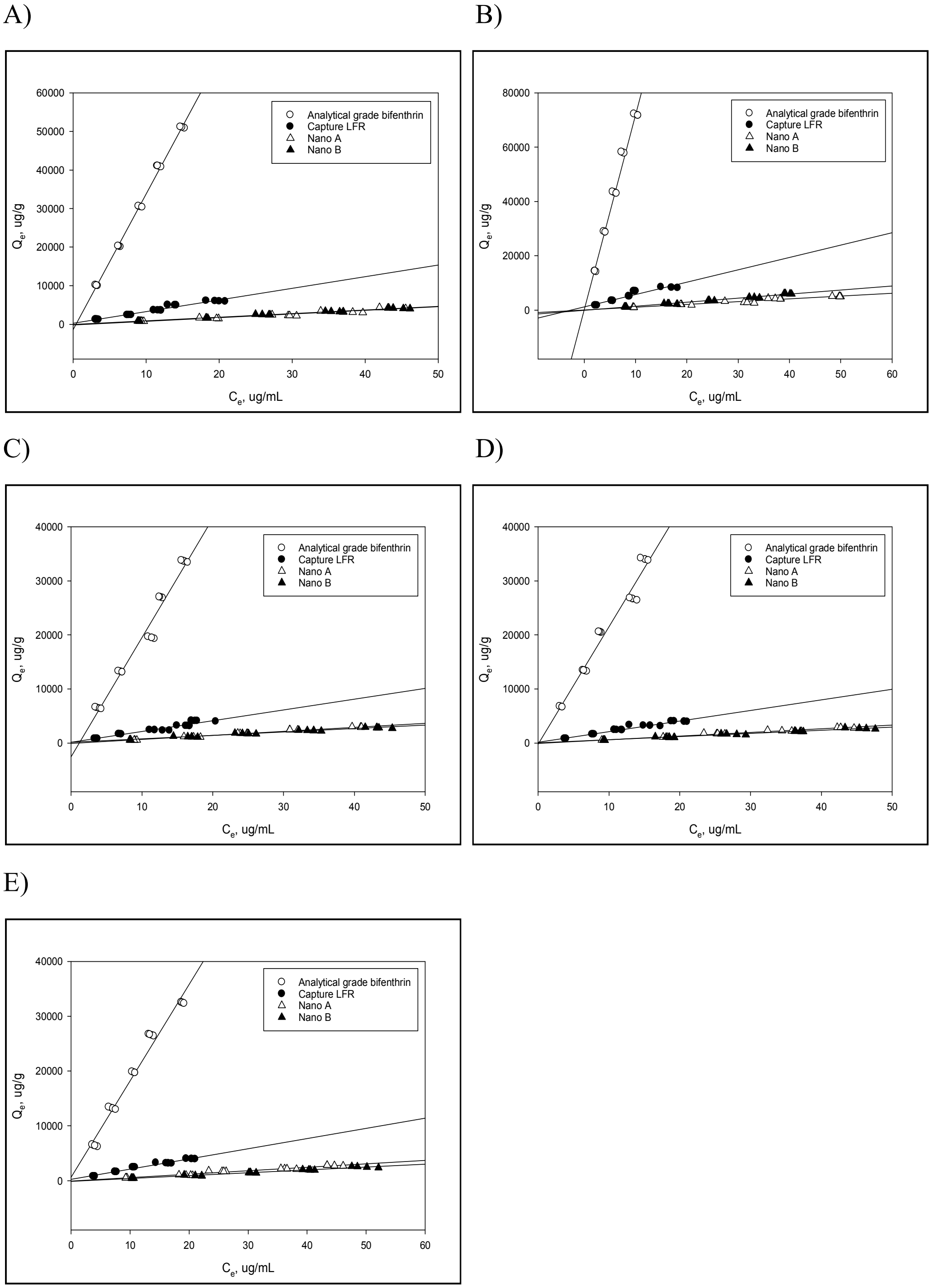


Figure 4.2 Linear sorption isotherms for bifenthrin in all bifenthrin treatments in A) Soil 1, B) Soil 2, C) Soil 3, D) Soil 4 and E) Soil 5 (n = 4)

Table 4.2 K_d and K_{oc} values (±S.D, n = 4) for different bifenthrin treatments in different soil types

Bifenthrin treatment	Soil	K_d (mL/g)	K_{oc} (mL/g)
Analytical grade bifenthrin	1	3500±85	126600±3058
	2	7200±218	130000±4225
	3	2200±51	115600±2670
	4	2200±31	116800±1667
	5	1800±41	144700±3361
Capture LFR	1	300±24	10900±863
	2	470±61	9012±1182
	3	200±13	10520±681
	4	200±11	10600±591
	5	190±10	15330±820
Nano A	1	96±6	3453±216
	2	100±5	1977±97
	3	76±8	3979±419
	4	68±5	3656±269
	5	64±3	5246±246
Nano B	1	94±7	3381±252
	2	150±11	2926±213
	3	64±4	3351±209
	4	59±5	3172±269
	5	52±5	4262±410

4.4.2 Persistence studies of different bifenthrin treatments

Concentrations of bifenthrin in both sterile and non-sterile soils at the start of the study were similar. Concentrations of bifenthrin in both sterile and non-sterile soils then decreased but the rate of decrease was faster in the non-sterile soils compared to the sterile ones. Dissipation in all treatments was modelled well using first order kinetics (Appendix 4.3). DT_{50} values for different bifenthrin treatments in different soil types in both sterile and non-sterile condition of soils were different with the degradation being significantly fastest in the non-sterile soils compared to sterile soils ($H>6.43$; d.f.=1; $p<0.014$; Table 4.3). This showed nanoformulation treatments were more persistent in the tested soils compared to the non-nanoformulation treatments. Across the soil types, Soil 2 showed the slowest degradation of bifenthrin while Soil 5 showed the fastest degradation for different bifenthrin treatments in both sterile and non-sterile soils ($H=24.33$; d.f.=9; $p=0.004$).

Table 4.3 Rate constant, k and DT$_{50}$ values (±S.D, n = 4) for different bifenthrin treatments

Soil	**Bifenthrin treatment**	**Rate constant, k**		**DT$_{50}$**	
		Sterile	**Non-sterile**	**Sterile**	**Non-sterile**
1	Analytical grade bifenthrin	-0.0022±0.0002	-0.0052±0.0003	320±9	140±2
	Capture LFR	-0.0024±0.0003	-0.0053±0.0004	290±10	130±1
	Nano A	-0.0012±0	-0.0025±0	580±0	280±0
	Nano B	-0.0013±0.0001	-0.0024±0.0001	560±26	290±6
2	Analytical grade bifenthrin	-0.0020±0.0002	-0.0050±0	350±9	140±0
	Capture LFR	-0.0023±0.0003	-0.0052±0.0002	300±7	140±3
	Nano A	-0.0011±0.0002	-0.0022±0.0004	650±32	320±9
	Nano B	-0.0012±0.0002	-0.0024±0.0002	600±30	300±7
3	Analytical grade bifenthrin	-0.0025±0.0005	-0.0053±0.0003	280±7	130±2
	Capture LFR	-0.0025±0	-0.0051±0.0001	280±0	140±1
	Nano A	-0.0015±0	-0.0026±0.0002	460±0	260±5
	Nano B	-0.0014±0	-0.0026±0.0002	500±0	270±6
4	Analytical grade bifenthrin	-0.0029±0.0003	-0.0058±0.0004	240±5	120±2
	Capture LFR	-0.0024±0.0005	-0.0048±0.0004	300±7	150±3
	Nano A	-0.0017±0.0003	-0.0028±0.0003	410±13	250±5
	Nano B	-0.0014±0.0002	-0.0025±0.0003	510±19	280±7
5	Analytical grade bifenthrin	-0.0027±0.0002	-0.0058±0.0001	260±8	120±2
	Capture LFR	-0.0024±0.0003	-0.0054±0.0001	290±6	130±1
	Nano A	-0.0018±0.0003	-0.0031±0.0001	390±11	230±4
	Nano B	-0.0015±0.0001	-0.0029±0	460±14	240±0

4.4.2.1 R_{50} values

Generally, the data showed the R_{50} values of bifenthrin were different between the sterile and non-sterile conditions (Table 4.4). In each soil for both sterile and non-sterile soils, there was no significant difference in the R_{50} values of Nano A and B ($F<2.67$; d.f.=1; $p>0.154$). Across the soil types, there was no significant difference among the Nano A and Nano B treatments in both sterile and non-sterile soils ($H=2.67$; d.f.=1; $p=0.333$).

Table 4.4 R_{50} values (±S.D, n = 4) for Nano A and Nano B treatments in sterile and non-sterile soils

Soil	**Bifenthrin treatment**	**R_{50} (d)**	
		Sterile	**Non-sterile**
1	Nano A	68±2	81±3
	Nano B	68±3	79±3
2	Nano A	68±1	83±4
	Nano B	67±3	79±5
3	Nano A	67±3	77±2
	Nano B	69±3	76±4
4	Nano A	67±3	75±4
	Nano B	69±4	78±3
5	Nano A	64±5	71±5
	Nano B	68±6	73±4

4.4.3 Relationships between soil properties with sorption and persistence

4.4.3.1 Soil properties with K_d values

A significant relationship was found between K_d values for the different bifenthrin treatments and soil organic carbon content of the different soils ($R^2>0.791$; $p<0.043$) (Appendix 4.4). No relationships were found between K_d and the other soil properties.

4.4.3.2 Soil properties with DT_{50} values

Results of the correlation analyses between soil properties and DT_{50} are provided in Appendix 4.5. There was a significant relationship between organic carbon content and degradation half-lives for Nano A in the non-sterile treatments ($R^2=0.949$; $p=0.005$). However, organic carbon content also showed relationships between the degradation of Nano A and Nano B in sterile condition of soil ($R^2>0.862$; $p<0.023$.

The clay, silt and sand content also showed a significant relationship with the degradation of Capture LFR in the sterile treatments ($p<0.036$).

4.5 Discussion

4.5.1 Effect of nanoformulation on sorption behavior

In general, the data obtained from sorption studies showed that the bifenthrin treatments behaved differently from each other in all soil types. This study showed that analytical grade bifenthrin was more strongly adsorbed to the soil particles compared to the nano and conventionally formulated bifenthrin treatments in all soil types (Table 4.2). The strong sorption capacity of analytical grade bifenthrin to the soil particles is probably explained by its very high hydrophobicity ($\log K_{ow} = 6$). For Capture LFR treatment, the sorption of the bifenthrin was significantly reduced compared to the analytical grade material. This is probably due to the effect of surfactants used in preparing the Capture LFR formulation. Surfactants are reported to be important components that could potentially alter the fate and behavior of pesticide active ingredients (Katagi, 2008). Physico-chemical properties such as solubility, dissociation and volatilisation could all be affected by the presence of surfactants in a formulation. For example, in a study done by Hua *et al*. (2009), the effect of surfactants on sorption behavior was investigated using soil columns. They found that the presence of anionic surfactants greatly increased the mobility of bentazone in a sandy loam soil. In contrast, non-ionic surfactants appeared to reduce pesticide mobility. In Capture LFR, propylene glycol, a non-ionic surfactant is used alongside the bifenthrin active ingredient to keep the active ingredient bifenthrin in the suspension. This could be the reason the sorption was reduced due to the competition between the surfactants with the organic carbon in the soil.

The effect of formulation on pesticide active ingredient was investigated by Khan (2016) using propyzamide in two different soil types. The effect of formulation on sorption behavior was significant in sandy loam soil ($p<0.001$) compared to sandy silt soil using a centrifugation method. The observed concentrations of the commercial formulation of propyzamide in the soil pore water were greater compared to the technical grade propyzamide indicating that less sorption of propyzamide from the formulated formulation. Just like our finding in this study involving the formulation of bifenthrin (Capture LFR) and analytical grade bifenthrin, the author suggested that the presence of co-formulants would favour keeping the active ingredient in the suspension (Khan, 2016; Oukali-Haouchine *et al*., 2013; Beigel and Barriuso, 2000). Although surfactants are reported to reduce sorption of the active ingredient to soil, Pose-Juan *et al.* (2010) reported that a formulated penconazole (water-oil emulsion formulation of penconazole, WOEP) enhanced the sorption compared to the technical grade using a classic batch equilibrium method. The found that about 70% of the active ingredient retained in the suspension was sorbed by soil. They suggested that the presence of the surfactants either enhanced the penetration of the penconazole onto the less polar sites of the soil organic matter or by the co-adsorption process within the soil-surfactant mixture (Khan, 2016).

K_d values for both of the nanoformulation treatments were significantly lower compared to the analytical grade bifenthrin and Capture LFR in all soil types. This is likely explained by the effect of nano-encapsulation. As bifenthrin is protected from soil binding sites by the nano-capsules, the nanoformulation treatments (Nano A and Nano B) showed lower K_ds compared to the non-nanoformulation treatments (analytical grade bifenthrin and Capture LFR). However, to consider that the active ingredient bifenthrin was loaded onto the nanocapsules to be completely protected from the soil binding sites might be too simplistic, therefore improved characterisation of the distribution and release of the active ingredient will be necessary (Kah *et al*., 2014).

The trend shown in this study is in agreement with a study done by Kah *et al*. (2016), where the sorption behavior was affected by the nano-encapsulation of bifenthrin. The sorption studies by Kah *et al*. (2016) were performed using three different nano-encapsulated bifenthrin formulations: NFA and NFB contained polymer nanoparticles derived from a 75:25 copolymer consisting of poly(methacrylic acid-*ran*-butylmethacrylate); and NFC contains polymer nanoparticles derived from a 90:10 copolymer consisting of poly(methacrylic acid-*ran*-ethylacrylate). Studies were also performed using a formulated bifenthrin (CF) and the corresponding active ingredient (AI). According to their study, the sorption was increased in the order of NFC < AI < CF < NFA < NFB in the loam soil, while the sorption in sand soil increased in the order of NFC < NFB < NFA < CF < AI. In their study, they found that although nanoformulation significantly affected the sorption behavior of the different formulations, not all discrepancies were significant. For example, the K_ds of NFA were not significantly different from NFB and CF in both soil types. This could be explained by the difference in soil organic carbon content in both soils where loam exhibited higher organic carbon content, thus favoring more sorption leading to higher K_d values.

In another study, the sorption behavior of nanoformulations of atrazine with analytical material has also been reported by Kah *et al*. (2014). Based on the study, the partition coefficients were determined using two different methods (batch equilibrium and centrifugation methods), in two different soils (sandy and loam soils) with different organic carbon content. Both of the methods showed that the K_ds for nanoformulations of atrazine, a herbicide for controlling weeds in numerous crops, were significantly higher compared to the analytical grade material, which is not in agreement with the current study with bifenthrin. A possible explanation given by Kah *et al*. (2014) was that large aggregates formed in the nano-formulated atrazine and that these settled during the centrifugation step. This observation could also be explained by the difference in physico-chemical properties of both compounds. Bifenthrin which is a more hydrophobic compound (log K_{ow} = 6) tends to be more adsorbed to soil particles compared to atrazine which is more hydrophilic (log K_{ow} = 2.7) (University of Hertfordshire, 2016). K_ds were found to be higher in a loam type of soil compared to the sand soil which suggests that sorption process was more favored in soils containing more organic carbon content. Comparing the results based on the two different methods, it was found that the centrifugation method produced results that could be considered as a more realistic soil-to-solution ratio whilst avoiding the disadvantages of column experiments and the method is thought to be particularly relevant to nanopesticides (Walker, 2000; Walker and Jurado-Exposito, 1998).

Concerns have been raised relating to the use of nanoformulations in the field of agriculture due to the different behavior of these nanoformulations compared with the conventional formulations. As nanoparticles can behave very differently from dissolved chemicals, it is possible that nanoformulations could have different behavior compared to the conventional formulations. The observed results in this study showed that the nanoformulation treatments (Nano A and Nano B) may be more mobile and bioavailable compared to the non-nanoformulation treatments (analytical grade bifenthrin and Capture LFR). This could lead to potential for increased risks of the nanoformulation in the terrestrial environment. Mobile compounds could travel deeper in soil depth leading to a possible risk to disturb groundwater system.

4.5.2 Effect of nanoformulation on persistence behavior

DT_{50} values for the non-nanoformulation treatments (analytical grade bifenthrin and Capture LFR) in both sterile and non-sterile condition of soils were almost the same as each other (Table 4.3). In contrast, the nanoformulation treatments (Nano A and Nano B) showed longer half-lives. Just like the sorption results, this is likely explained by the role of polymer capsules 'protecting' the active ingredient bifenthrin from being attacked and degraded by water (hydrolysis) and/or soil microorganisms (biodegradation). The degradation that was observed is probably explained by the slow release mechanism of the bifenthrin from the polymer capsules in Nano A and Nano B. The findings in this persistence study were in agreement with a study done by Kah *et al.* (2016). They found that nanoformulation of bifenthrin (NFA, NFB and NFC) prolonged the persistence of bifenthrin in both loam and sandy soils with faster degradation being observed in the loam compared to sand soil possibly due to the higher microbial activity in the loam which greatly influenced the degradation process. They observed that the DT_{50} for all bifenthrin treatments ranged from 125±13 d (loam soil; AI) to 228±16 d (sand soil; NFA). In our study, the DT_{50} in sterile treatments ranged from 240±5 d (Soil 4; analytical grade bifenthrin) to 650±32 d (Soil 2; NA) while in the non-sterile treatments ranged from 120±2 d (Soil 4; analytical grade bifenthrin) to 320±9 d (Soil 2; NA). Although no sterile treatments were performed in the degradation studies performed by Kah *et al*. (2016), our results and the results obtained by Kah *et al.* (2016) in the non-sterile treatments suggest that the enhancement in persistence by the nanoformulations could be attributed to the slow release of bifenthrin from the nanocarriers hence delaying the bioavailability of bifenthrin for degradation.

Based on an investigation deriving release rates involving slow release formulation by Ford *et al*. (2007), Kah *et al*. (2016) determined the release rates of bifenthrin for the formulated bifenthrin. They found that the was a significant effect of the type of formulation on the kinetics of release, however no consistent trend was observed among the formulations. The R_{50} increased in the order of NFB < NFA < CF < NFC in the loam soil while in the sand soil, the R_{50} increased in the order of NFC < NFB < CF < NFA. From the findings, they suggested that the effect of soil type may have played an important role in affecting the release rate of bifenthrin from different bifenthrin formulations.

In this study, we estimated the release rates of bifenthrin from the nanoformulation treatments (Nano A and Nano B) as described in the section 4.3.4.3. From the results, there was no difference in R_{50} values between Nano A and Nano B in both sterile and non-sterile soils ($p>0.154$; Table 4.4). Based on our findings, the R_{50} values for Nano A and Nano B ranged from 64±5 d (Soil 5; Nano A) to 69±4 d (Soil 3; Nano B) in sterile soils while in non-sterile soils, the R_{50} values ranged from 71±5 d (Soil 5; Nano A) to 83±4 d (Soil 2; Nano A). In a study performed by Kah *et al.* (2016), the R_{50} values for the nanoformulations of bifenthrin (NFA and NFB) ranged from 32±4 d (sand soil; NFB) to 58±9 d (loam soil; NFA) while the DT_{50} values ranged from 182±4 d (loam soil; NFB) to 442±26 d (sand soil; NFA). Our results suggest that the pattern for R_{50} values were the same as in previous study performed by Kah *et al.* (2016).

The observed R_{50} values in our study and previous study (Kah *et al.*, 2016) suggest that this could be explained by the role of the nanocapsules protecting the active ingredient bifenthrin from being release to the soil compartment. However, in order to explain the encapsulated active ingredients or active ingredients loaded onto polymer nanocarriers to be completely protected by the nanocarriers from degradation, either by hydrolysis or biodegradation could be too simplistic. The physico-chemical properties of the nanocapsules or nanocarrier materials may play a role in affecting the fate (e.g., degradation) of the active ingredient. For example, previous studies on the degradation of nanoformulation of atrazine have been reported by Kah *et al.* (2014) and Grillo *et al.* (2012). A poly(ε-caprolactone) was used as the nanocarrier of atrazine and found to be easily biodegraded in soil (Eubeler *et al.*, 2010) leading to no difference in degradation kinetics of nanoatrazine and the corresponding active ingredient atrazine. This could be explained by the promotion of degradation process of the polymer matrix towards the active ingredient atrazine by cometabolism; the simultaneous degradation of two compounds, in which the degradation of the second compound (the secondary substrate) depends on the presence of the first compound (the primary substrate). This was also supported by the fact that soil microbes affected the degradation process of atrazine by cometabolism (Mudhoo and Garg, 2011).

Therefore, the need of an improved characterisation of the distribution and the release of the active ingredients from the carrier materials under a wide range of conditions seems to be necessary before any assumption could be made involving polymer-based nanoformulation. Insufficient data concerning the degradability of the active ingredients loaded onto polymer-based nanocarriers made the explanation of the degradation of the Nano A and Nano B is difficult. In order to be more accurate explaining the degradation and release rates of active ingredient of polymer-based nanoformulations, studies concerning different type of polymer base or carrier materials (e.g., biodegradable polymers) and different active ingredients (e.g., hydrophilic active ingredient) should be conducted. This step could improve the understanding on degradation when dealing with nanoformulation.

4.5.3 Do soil properties affect the fate of nanoformulation in soil?

Previous studies concerning conventional organic chemical compounds such as pesticides revealed that soil properties such as clay content (Davies and Jabeen, 2002; Nennemann *et al*., 2001; Aguer *et al*., 2000; Laird and Fleming, 1999; Laird *et al*., 1992; Jaynes and Boyd, 1991; Peter and Weber 1985) and organic carbon content and dissolved organic matter (Williams *et al*., 2006; Albarrán *et al*., 2004; Ben-Hur *et al*., 2003; Cox *et al*., 2001; Chiou *et al*., 1983; Kozak *et al*., 1983; Means *et al*., 1982) affect the fate and behavior. Below the relationships between the soil properties and the K_d and DT_{50} values for the non-nano and nanoformulation treatments are discussed.

4.5.3.1 Effects on sorption behavior

It was found that organic carbon content of the soils showed a significant relationship to sorption behavior of the analytical grade bifenthrin and other bifenthrin treatments. This could be explained by the fact that bifenthrin is a non-polar organic chemical compound (hydrophobic) with a high log K_{ow} value 6, therefore it has the ability to dissolve easily in fats, oils and organic carbon (or organic matter) in the soil. This argument is in agreement with previous studies that showed organic carbon content was found to be a more relevant contributor to the sorption process between non-polar chemical compounds and soil particles compared to other components (Williams *et al*., 2006; Albarrán *et al*., 2004; Kozak *et al*., 1983; Lambert, 1968, Lambert, 1967; Lambert *et al*., 1965).

Nanoformulations of bifenthrin (Nano A and Nano B) were found to share the same trend. Just like the K_d values, the analytical grade bifenthrin displayed higher K_{oc} values (Table 4.2) compared to the rest of the bifenthrin treatments. For Capture LFR and both of the nanoformulation (Nano A and Nano B) treatments, the lower K_{oc} values could be related to the impacts of surfactants in the formulations. Khan and Schnitzer (1972) investigated the ability of extracted humic acid from soils in order to retain the hydrophobic organic compounds (HOC) and found this could be explained by the sorption mechanism on the external surfaces and in internal voids of molecular sieve-type soils (Delle Site, 2001). They suggested that this might be due to the trapping of hydrophobic molecules by internal voids which were formed between hydrogen bonding and Van der Waals attraction with humic polymers (Senesi, 1992; Hamaker and Thompson, 1972; Khan and Schnitzer, 1972).

Based on the findings using linear regression analysis, it is likely that the presence of organic carbon can affect the fate and transport of bifenthrin treatments in the terrestrial environment. However, a new regulatory approach concerning the nanopesticides should be considered as nanosize particles can behave very differently from any dissolved chemical compounds. This might or might not lead to the risks of reaching the groundwater by these new plant protection products.

4.5.3.2 Effects on persistence behavior

Previous studies confirmed that soil properties such as soil pH (Kah and Brown, 2006; Price *et al*., 2001; Walker *et al*., 2001; Singles *et al*., 1999; Ladlie *et al*., 1976), organic carbon (Kah *et al*., 2007; Henriksen *et al*., 2004; Pinna *et al*., 2004; Said-Pullicino *et al*., 2004) and clay (Kah *et al*., 2007) greatly influenced the degradation of pesticides. However, most of these studies were performed using acidic and basic pesticide formulations. In this study, bifenthrin, a neutral pesticide shows a different type of relationships with the soil properties as mentioned in section 4.4.3.2.

In this study, bifenthrin from all bifenthrin treatments was degraded fastest in the soil exhibiting the lowest sorption (Soil 5, OC%=1.22±0.2). This finding is not in agreement with a study done by Kah *et al*. (2014) where they found the active ingredient and nanoformulation of atrazine were degraded fastest in the soil exhibiting the greatest sorption. This could be explained by the organic carbon content having opposing effects on degradation, either via an increase in sorption or via an increase in microbial activity (Kah and Brown 2006). It is noted that, a weak correlation between the sorption and degradation processes greatly influences the probabilistic analysis of leaching through soil (Wu *et al*., 2011; Kah *et al*., 2007; Beulke and Brown, 2006). This could be explained by the sorption process reducing the chemical bioavailability in the soil leading to a positive relationship between K_d and DT_{50}. Thus, it is important to assess the possible relation between the two processes.

Linear regression analysis has been used previously in order to find out the relation of adsorption on degradation behavior (Kah *et al*., 2014; Wu *et al*., 2011). Therefore, in this study, linear regression analysis was performed in order to study the relation between sorption and degradation in non-sterile soils (Appendix 4.6). Based on the analysis, degradation of bifenthrin from analytical grade, Capture LFR and Nano B treatments did not appear to be affected by the sorption process in all soil types ($R^2<0.636$; $p>0.106$) while for Nano A treatment, a significant relationship was observed between sorption and degradation ($R^2=0.853$; $p=0.025$). It was also observed that the analytical grade bifenthrin and both of the nanoformulation treatments (Nano A and Nano B) showed a similar trend of increasing degradation time with sorption, however from the regression analysis this relationship was not significant, only significant for Nano A treatment (Appendix 4.6).

4.6 Conclusion

The present study showed a significant difference in the fate of bifenthrin in soils between analytical grade material, conventionally formulated (Capture LFR) material, and two different nanoformulations (Nano A and Nano B). Sorption studies based on batch equilibrium method revealed that K_d values, increased in order Nano B < Nano A < Capture LFR < analytical grade bifenthrin. The result suggested that analytical grade bifenthrin has a strong sorption capacity to the soil particles due to its being particularly hydrophobic (log K_{ow} = 6). While for nanoformulations of bifenthin, the polymer capsules seemed to protect the active ingredient from the soil binding sites, thus lowering the sorption to the soil particles. The lower sorption of the bifenthrin in the conventional formulation compared to the analytical grade might be explained by the presence of surfactants in the formulation. Persistence studies showed that the nano-formulated bifenthrin dissipated more slowly in both sterile and non-sterile conditions compared to the analytical grade and formulated bifenthrin. This behavior could be explained by the role of polymer capsule 'protecting' the active ingredient from microbes. The DT_{50} values in both sterile and non-sterile increased in the order analytical grade bifenthrin < Capture LFR < Nano A < Nano B. Bifenthrin is reported to be strongly adsorbed to soil so this could result in less availability of bifenthrin for microbial degradation. But a vast difference of half-life in sterile and non-sterile soil under study is a positive indication for potential microbial degradation of this persistent insecticide.

Overall, the findings suggest that nanoformulations could exhibit greater mobility and enhanced persistence in soil compared to analytical grade material and a conventional formulation. The use of nanoformulations could therefore increase the bioavailability and length of exposure of the active ingredient from nanoformulations in the environment. From the findings, it is also expected that the uptake of bifenthrin from the nanoformulations by organisms could be higher compared to the corresponding active ingredient, leading to a higher BCF values. However, further investigations should be carried out under a wide range of conditions to understand the distribution and uptake of nanoformulations in the terrestrial environment. Therefore, in the next chapter, the uptake of nanoformulations (Nano A and Nano B) is explored using earthworms as the test organism.

Chapter 5 Comparison of the Uptake, Distribution and Depuration of Conventional and Nano-encapsulated Bifenthrin in the Earthworm, *E. fetida* and *L. terrestris*

5.1 Introduction

The previous chapter (*Chapter 4*) explored the effects of nanoformulation on the fate of bifenthrin in different soil types. Based on the results, the nanoformulation treatments exhibited reduced sorption compared to the analytical grade material and the conventional formulation. Thus, it would be expected that the uptake into terrestrial organisms could be different compared to the non-nanoformulation treatments. It is possible that changes in the persistence, mobility and uptake of the active ingredient in a nano-formulated product compared to conventional materials could actually increase the environmental risks of the active ingredient.

One group of organisms that will be exposed to nanopesticides are terrestrial invertebrates such as earthworms. Earthworms are known to bio-magnify inorganic and organic soil contaminants, including pesticides, polycyclic aromatic hydrocarbons (PAHs), brominated flame retardants, and metals (Davies *et al*., 2013; Giovanetti *et al*., 2010; Grumiaux *et al*., 2010; Hinton and Veiga, 2008; Langdon *et al*., 2005; Sellstrom *et al*., 2005; Matscheko *et al*., 2002; Heikens *et al*., 2001; Ma *et al*., 1998; Van Gestel and Ma, 1998; Janssen *et al*., 1997). Earthworms take up contaminants in a number of ways: through living in the soil environment, by direct contact with the soil and soil pore water and therefore uptake via dermal contact from these two media is possible. The ingestion of soil particles may also lead to chemicals passing across the gut wall and into the tissue. Earthworms are considered as key organisms in the terrestrial environment; their presence is central to a healthy and sustainable soil environment. For example, earthworms help to establish and maintain the structure and fertility of the soil (Edwards, 2004; Killham, 1994). The physical motion of earthworm burrowing can bury plants deep in the soil which is crucial for the recycling of nutrients whilst the structure of the burrows is important in draining and aerating the soil (Edwards, 1996). Earthworms being at the base of a food chain hold an integral position. Uptake and accumulation of contaminants into earthworms not only poses a risk to the earthworm directly, but bioaccumulation and contaminant transfer through the food chain to top predators such as birds has the potential to result in secondary poisoning of mammals and birds (Spurgeon and Hopkin, 1996). As a result of their ecological importance, earthworms are a good candidate for being key indicator species with regard to toxic levels of pollutants in soils.

Despite the fact that they are a keystone species, only limited work has been done on the impacts of ENPs on earthworms. Carbon-based nanomaterials have been shown to negatively affect the reproduction of *Eisenia veneta*, *E. fetida* and *L. rubellus* which could result in a significant decrease in the population growth rate (Van der Ploeg *et al*., 2011; Li *et al*., 2010; Scott-Fordsmand *et al*., 2008). C_{60} fullerenes have been shown to bioaccumulate in *E. fetida* whereas carbon nanotubes did not accumulate in *E. fetida* (Li and Alvarez, 2011; Petersen *et al*., 2008). Ecotoxicological screening of metal-based nanomaterials indicated significant reproductive failure in *E. fetida* exposed to silver, zinc or copper nanoparticles (NPs) (Heckmann *et al*., 2011; Li *et al*., 2011). Different types (size and coatings) of silver NPs disturb earthworm's reproductive capacity at 500-1000 mg/kg soil in *E. fetida* (Shoults-Wilson *et al*., 2011b,c). Titanium dioxide (TiO_2) NPs are also found to affect earthworm reproductive activity by abolishing the circannual rhythm that depresses reproduction of *Eisenia andrei* (Schlich *et al*., 2012). Bioaccumulation of silver NPs in earthworms seems relatively low, however, more sensitive endpoints indicate that earthworm's physiological traits are affected at sub-100 mg/kg soil, for example, avoidance, oxidative stress responses and tissue apoptosis (Coutris *et al*., 2012; Tsyusko *et al*., 2012; Shoults-Wilson *et al*., 2011a; Lapied *et al*., 2010). However, despite the fact that it is inevitable that earthworms will be exposed to nanopesticides, no work has yet been done on this topic.

Investigations determining the distribution of non-pesticide NPs in the earthworm body have shown that following exposure, NPs are distributed across the earthworm body with high concentrations being observed in the earthworm gut (Gupta and Yadav, 2014; Gupta *et al*., 2014; Bystrzejewska-Piotrowska *et al*., 2012; Reddy *et al*., 2012; García-Alonso *et al*., 2011; Hu *et al*., 2010). For example, Yadav (2015) investigated the uptake and bioaccumulation of ZnO NPs in earthworm, *E. fetida*. After 24 h of exposure, the earthworms were sampled and analysed using Transmission Electron Microscopy (TEM). Based on the findings, they found that the ZnO NPs were highly accumulated in the earthworm gut. They suggested that the uptake of ZnO NPs into *E. fetida* was mainly through ingestion of soil particles. In another study, Khalil (2016) observed the same accumulation trend of Ag NPs in the earthworm, *Aporrectodea caliginosa* where the Ag NPs were highly accumulated in the earthworm gut. During the 28 d of experimental period, they found that Ag NPs were not lethal to earthworms with the exposure of 50 – 1000 mg/kg. However, they observed that cocoon production and hatchability, biomass and cellulase activity were significantly decreased when exposed to Ag NPs compared to control treatments.

Based on the previous studies, it can be concluded that the exposure and bioaccumulation of metal and metal oxide NPs by terrestrial organisms can cause some toxic effects, thus potentially affecting the balance in their population. It is noted that while studies like this are becoming available in the literature, no data are available for nanopesticides. Therefore, the aim of this study was to investigate the uptake, depuration and distribution of a pesticide active ingredient from conventional and nano-formulated products into earthworms with a view to understanding potential differences in behavior. The study focused on the synthetic pyrethroid bifenthrin and used nanopesticide products from Vive Crop Protection Inc. where bifenthrin is encapsulated in a polymer nanoparticle and which aims to better target the active ingredient.

5.2 Materials

The bifethrin formulations and analytical grade material were the same as used in *Chapters 3* and *4*. The uptake studies were done in Soil 5 whose characteristics are described in *Chapter 3*. *Eisenia fetida* were cultured according to the methods described in *Chapter 3*. In addition to *E. fetida*, studies were performed on *L. terrestris* for the distribution experiments. *Lumbricus terrestris* were obtained from Blades Biological Ltd. (Kent, UK) and cultured in a medium of moist soil (8 kg of Soil 5 in a plastic bucket). They were kept moist with deionised water under laboratory condition (20±2 °C for *E. fetida* while 13±2 °C for *L. terresris*). *Lumbricus terrestris* were fed twice weekly with birch leaves distributed on the surface of the moist soil. Detailed descriptions of the chemicals, soil and *E. fetida* culture can be found in the section 3.2.1, 3.2.2 and 3.2.3, respectively in *Chapter 3*.

5.3 Experimental procedures

5.3.1 Uptake and depuration studies

The uptake and depuration experiments were done following OECD Guideline 317 'Bioaccumulation in Terrestrial Oligochaetes' (OECD, 2010). Experiments were performed in glass jars at a concentration of 10 µg/g of active ingredient where each jar contained 50±1 g of test soil and kept in an incubator at 20±2 °C, using a 16:8 light/dark cycle. Before the earthworms were exposed to the analytical grade bifenthrin or the formulations, they were acclimated to the experimental conditions in the incubator for 48 h using non-treated soil. The different bifenthrin treatments were then mixed with the soil using deionised water as solvent carrier to achieve a moisture content between 60 – 70 % of the maximum water holding capacity (MWHC).

For each bifenthrin treatment, 45 glass jars of treated soil were prepared. At the start of the uptake phase, one mature adult *E. fetida* with a visible clitellum was added to each glass jar. Glass jars were then covered with garden fleece (to prevent earthworms from escaping while allowing sufficient air supply to be maintained) attached with an elastic band. The uptake phase of the experiment lasted for 21 d with triplicate samples being taken at 0 and 6 h and 1, 3, 7, 10, 14, 21 d. *Eisenia fetida* in the remaining glass jars were then transferred to clean soil for another 21 d for the depuration phase with samples being taken at 6 h and 1, 3, 7, 10, 14, 21 d after transfer. At each time point in both phases, the earthworm weight change and mortality were recorded. Soil moisture content of the soil in each glass jars was monitored throughout both phases, and adjusted, where necessary, by adding deionised water to ensure that it remained between 60 – 70 % of the MWHC. The pH of the soils was measured at the beginning and end of the uptake phase and at the end of the depuration phase. Earthworms were fed weekly with five grams Smash® instant mashed potato powder.

Once samples were collected, earthworms were removed, rinsed with deionised water, blotted dry, weighed and then placed for 48 h on moist filter papers to allow the earthworms to purge their gut contents (Dalby *et al.*, 1996). The moist filter papers were changed twice a day (in the morning and evening). The earthworms were then killed by freezing and were subsequently defrosted prior to analysis. Samples of soil were also taken for soil analysis and for extraction of soil pore water.

5.3.2 Distribution of bifenthrin in earthworms

The distribution of bifenthrin following exposure to the different treatments was assessed using both *E. fetida* and *L. terrestris*. Experiments were performed in glass jars at a concentration of 10 µg/g of active ingredient. Single *E. fetida*, were exposed to 50±1 g of soil treated with each bifenthrin treatment or soil only while single *L. terrestis* were exposed to 350±5 g of test soil containing each bifenthrin treatment or soil only. For each species-treatment combination, six jars were prepared and kept in an incubator at 20±2 °C for *E. fetida* while 13±2 °C for *L. terrestris*, using a 16:8 light/dark cycle. Jars were covered with fleece to prevent the worms escaping.

Earthworms were then exposed for 10 d after which three samples from each earthworm/treatment combination, including controls, were taken for analysis. Earthworms from the remaining were transferred into clean soil for a further 7 d to explore the depuration of the bifenthrin after which samples were taken for analysis. Soil moisture content of the soil in each glass jar was monitored throughout both phases, and adjusted, where necessary, by adding deionised water to ensure that it remained between 60 – 70 % of the MWHC. The pH of the soils was measured at the beginning and end of the uptake phase and at the end of the depuration phase. Earthworms were fed weekly with five grams Smash® instant mashed potato powder (for *E. fetida*) and birch leaves (for *L. terrestris*).

At each sampling time point, the earthworms were removed, rinsed with deionised water, blot dried then weighed and depurated for 48 h on moist filter papers to allow the earthworms to purge their gut contents (Dalby *et al*., 1996). The moist filter papers were changed twice a day (in the morning and evening). Then, the earthworms were killed by freezing and subsequently defrosted prior to analysis. Samples of soil were also taken for soil analysis and for extraction of soil pore water.

Prior to analysis, earthworms were placed on a dissecting tray with their dorsal side facing upwards. Using a pair of dissecting scissors, a shallow incision was made below the clitellum. Working from this cut the posterior section of the earthworm was then dissected to expose the earthworm gut. The chloragogue, gut and remaining tissues (for *L. terrestris*), and gut + chloragogue and remaining tissues (for *E. fetida*) of the earthworms were separated, washed and collected in a glass centrifuge tube. The collected organs were then centrifuged at 3000 rpm for 15 min and the water phase was collected and analysed for remaining bifenthrin.

5.3.3 Sample preparation and analysis

Soil samples, pore water, defrosted earthworms and dissected earthworm tissues were extracted and analysed following the analytical method in section 3.3.2 in *Chapter 3*. The analytical method for *L. terrestris* was validated in the same way as for *E. fetida* in section 3.3.2.1 in *Chapter 3* and recoveries ranged from 89.9% (Nano B) to 106.5% (Nano A) while the LODs ranged from 86.3 ng/g (Nano A) to 231.5 ng/g (analytical grade bifenthrin).

5.3.4 Data analysis

5.3.4.1 Determination of sorption coefficient, K_d

The sorption coefficient, K_d for each bifenthrin treatment for each soil type was determined as described in section 3.3.3.1 in *Chapter 3*.

5.3.4.2 Determination of half-life, DT_{50}

Half-life, DT_{50} for each bifenthrin treatment for each soil type was determined as described in section 3.3.3.2 in *Chapter 3*.

5.3.4.3 Earthworm kinetic modelling

Previous studies have shown the uptake of hydrophobic organic chemicals such as pesticides is mainly by passive diffusion through the body wall of the earthworm (Carter *et al*., 2014a; Jager *et al*., 1998; Belfroid *et al*., 1995; Van Gestel and Ma, 1988; Lord *et al*., 1980). Therefore, in this study, a first order one-compartment model was used to estimate the uptake and depuration rates for each bifenthrin treatment from soil pore water. The toxicokinetic model (Equation 8), was fitted to measured internal earthworm concentration data and kinetic using the methods outlined by Ashauer *et al*., (2010).

$$\frac{\mathrm{d}C\mathrm{organism}}{\mathrm{d}t} = k\mathrm{in} \bullet C\mathrm{water}\ (t) - k\mathrm{out} \bullet C\mathrm{organism}\ (t) \qquad (8)$$

Where: $C_{organism}$ is the internal concentration (µg g^{-1}); C_{water} the concentration in the pore water (µg mL^{-1}); and k_{in} and k_{out} are the uptake rate constant (mL g^{-1} h^{-1}) and the elimination rate constant (h^{-1}), respectively. The parameters k_{in} and k_{out} were estimated by least-squares minimisation (Levenberg-Marquardt algorithm) without weighting data. Bioconcentration factors (BCFs) were calculated by setting the concentration in pore water equal to 1 and running the simulation to beyond steady state. Parameter estimations and simulations were carried out using ModelMaker (v. 4.0, Cherwell Scientific Ltd., Oxford, UK).

5.3.4.4 Statistical analysis

Statistical analysis of the data was performed using SigmaPlot (v. 12.5). Data on the K_d, DT_{50} and BCF values were first tested for normality using a Shapiro-Wilk test and then for equal variance. If these passed, a one-way ANOVA was performed to assess the differences in the values among the groups. If this failed, the analysis of variance was performed using Kruskal-Wallis analysis on ranks. Data on the concentration of bifenthrin detected in the organs (chloragogue, gut and remaining tissues) for each earthworm species were first tested for normality using a Shapiro-Wilk test and then for equal variance. If these passed, a two-way ANOVA was performed to assess the difference among the bifenthrin treatments.

5.4 Results and discussion

At the end of the uptake and depuration experiment, no mortality was recorded in any of the treatments. The uptake and depuration studies passed the validity criteria (based on earthworm weight change and mortality) according to the principle outlined in the OECD 317 (2010). Generally, throughout the uptake phase, there was a decrease in concentration of bifenthrin in the soil and soil pore water which was associated with an increase in the concentration of bifenthrin in the earthworms (Figure 5.1). A mass balance was performed to describe the fate of the bifenthrin in the experiment using the measurements in soil, soil pore water and *E. fetida*. At the end of the uptake phase, > 60% of bifenthrin was extracted from the soil for each bifenthrin treatment with the Nano A treatment showing the highest percentage of 82% (Figure 5.2). For the dissipated bifenthrin from the soil, the percentages ranged from 16 – 32% for different bifenthrin treatments with Nano A showing the lowest percentage of 16%. This could possibly be explained by the transformation of bifenthrin into its metabolites through degradation in soil compartment. The contributions of soil pore water and uptake into earthworms were found to be low with the percentages ranged from 0.8 – 1.5% and 0.3 – 0.7%, respectively for different bifenthrin treatments.

Studies on the degradation of bifenthrin using cyclopropyl- and phenyl-^{14}C labelled bifenthrin showed that the formation of CO_2 up to 50% showing that bifenthrin is well mineralised in soil (Fecko, 1999) and the bound residues were remained below 25% after 120 d. Other metabolites determined were 4'-hydroxybifenthrin, 3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropane carboxylate (TFP acid), 2-methyl-3-phenylbenzyl alcohol (BP alcohol) and 2-methyl-3-phenylbenzoic acid (BP acid) with no single metabolite ever exceeded an amount of 10%. The maximum percentage was announced for 4'-hydroxybifenthrin with 8.2 % (Smith, 1991; Reynolds, 1986; Bixler, 1984).

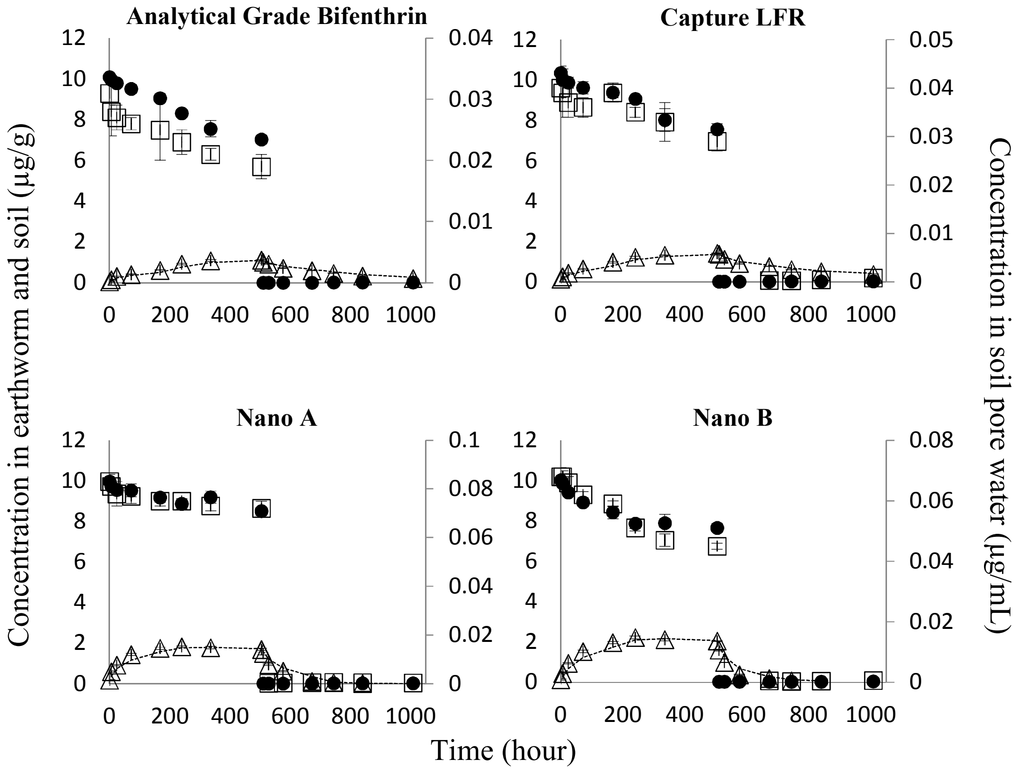


Figure 5.1 Uptake and depuration curves for *E. fetida* exposed to different bifenthrin treatments. Measured concentrations, µg/g (±S.D, n = 3) in *E. fetida*, soil and soil pore water are represented by open triangles, closed circles and open squares, respectively. The data lines represent the first-order model fit

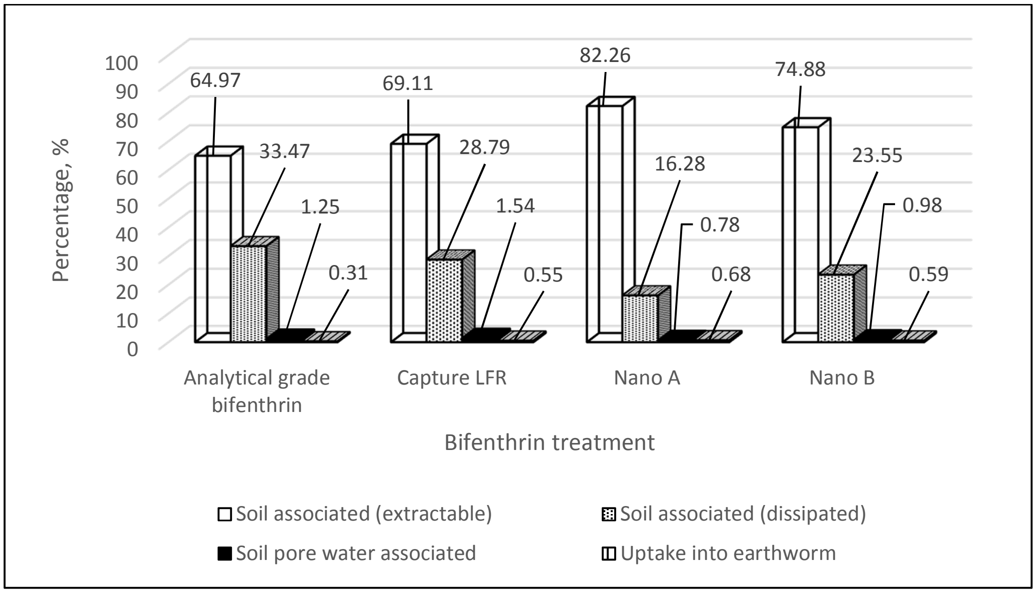


Figure 5.2 Percentage of bifenthrin associated with different compartments in the soil−earthworm systems at the end of the uptake phase of the experiment in comparison to applied bifenthrin at 0 h

Fate of bifenthrin treatments (i.e., sorption and persistence) may have played an important role in determining the uptake of bifenthrin into *E. fetida*. During the uptake phase, the concentration of bifenthrin in soil and soil pore water was also determined. Sorption coefficient (K_d) and half-life (DT_{50}) values were then calculated and tabulated in Table 5.1. The observed trend in the K_d and DT_{50} values was in agreement with the previous findings in *Chapter 3 – 4* where the non-nanoformulation treatments (analytical grade bifenthrin and/or Capture LFR) increased the sorption and reduced the persistence compared to the nanoformulation treatments (Nano A and Nano B).

Previous studies reported that K_ds for bifenthrin are in a range of 882 to 6000 mL/g in different soil types (USEPA, 2010; Froelich, 1983). From this study, the K_d values increased in the order of Nano A < Nano B < Capture LFR < analytical grade bifenthrin. There was a significant difference between the K_d values of the different bifenthrin treatments (H=13.058, d.f.=3, p=0.005). This could be well explained by the strong sorption capacity of bifenthrin to the soil particles due to its being particularly hydrophobic (log K_{ow}=6; Hansch *et al*., 1995) leading to a higher K_d for the non-nanoformulation treatments (analytical grade bifenthrin and Capture LFR) compared to the nanoformulation treatments (Nano A and Nano B). The result suggests that nano-encapsulation formulations affect the fate of bifenthrin in soil compartment; the polymer capsules acting as a “protector” to protect bifenthrin from being sorbed to the soil particles, leading to a lower values of K_d. This leads to a different pattern of uptake of bifenthrin into *E. fetida*. Since bifenthrin is highly adsorbed to soil particles, this means the bioavailability of bifenthrin from analytical grade bifenthrin and Capture LFR treatments is low compared to the nanoformulation treatments (Nano A and Nano B). Just like the sorption results, the nano-encapsulation does affect the persistence behavior of bifenthrin in soil. In this study, the DT_{50} values for nanoformulation treatments were found significantly higher than the non-nanoformulation treatments ($F=700.81$; d.f.=3; $p<0.001$; Table 5.1). Previous studies reported that the DT_{50} for bifenthrin was in the range of 122 – 345 d in different soil types (FMC 50429-025, 1983; Froelich, 1983). This could be explained by the active ingredient bifenthrin being ‘protected’ by the nanocapsules from being attacked and degraded by water (hydrolysis) and/or soil microorganisms (biodegradation).

E. fetida was found to accumulate bifenthrin from the different bifenthrin treatments. However, there was a difference in the accumulation pattern from different treatments. The analytical grade bifenthrin and Capture LFR treatments showed slower uptake rates into the earthworms than the two nanoformulation treatments (Nano A and Nano B). At the end of the uptake phase, 1.12±0.01 μg/g of bifenthrin was accumulated by *E. fetida* exposed to the analytical grade material. Exposure to Capture LFR, showed a similar pattern with 1.41±0.02 μg/g of bifenthrin found to be accumulated by *E. fetida* at the end of uptake phase. In contrast, *E. fetida* exposed to the nanoformulation treatments showed a higher uptake rate. *E. fetida* was found to accumulate 1.69±0.05 and 2.04±0.01 μg/g of bifenthrin concentration after exposure to Nano A and Nano B, respectively. Concentrations of bifenthrin at 240 h following exposure to the Nano A and Nano B treatments also appeared to be at the steady state ($F<1.13$; d.f.=1; $p>0.103$) while steady state did not appear to have been reached in the analytical grade bifenthrin and Capture LFR exposure. Elimination of bifenthrin from the earthworms exposed to the Nano A and Nano B treatments was faster compared to the analytical grade bifenthrin and Capture LFR treatments. At the end of the depuration phase, the bifenthrin accumulated from the nanoformulation treatments (Nano A and Nano B) was no longer detectable in the earthworm while for the analytical grade bifenthrin and Capture LFR treatments, bifenthrin was still detected with the analytical grade bifenthrin and Capture LFR accounting for 20% and 26%, respectively of the highest amount of bifenthrin taken up by *E. fetida* during the uptake phase. This means at the end of the depuration phase, *E. fetida* exposed to the analytical grade bifenthrin and Capture LFR were eliminating about 80% and 74%, respectively of bifenthrin out from their body.

In a study done by Diao *et al*. (2011), a rapid uptake of both (+) and (-)-enantiomers of α-cypermethrin into earthworm, *E. fetida* was observed during the 10 d exposure. Following the exposure duration (after 10 d), the concentration declined and reached a steady state (Diao *et al*., 2011). The BSAF values for both (+) and (-)-enantiomers were 0.43 and 0.54 mg/kg$_{\mathrm{wwt}}$, respectively. From their findings, they concluded that the uptake of α-cypermethrin into *E. fetida* was found to be influenced by the different enantiomer with the uptake of (-)-enantiomer was preferentially accumulated compared to the (+)-enantiomer by *E. fetida*. A study investigating the accumulation of the radiolabelled α-cypermethrin in earthworm, *E. fetida* was done by Hartnik and Styrishave (2008). The results showed that the uptake of α-cypermethrin increased with increasing concentrations in soil. During the whole exposure period, the ^{14}C activity in earthworms increased up to 28 d (for Steinskogen soil, Norway) or 50 d (for Askov soil, Denmark) without reaching a steady state. This observation is likely same as the pattern presented in this study involving the analytical grade bifenthrin and Capture LFR. Further exposure period showed that the ^{14}C activity did not change significantly with the increasing exposure time. They found that the accumulation of α-cypermethrin was significantly higher in Askov soil compared in Steinskogen soil with the BSAF value was almost a factor 3 higher in Askov soil compared in Steinskogen soil.

Previous studies have shown that soil pore water mainly influences the uptake of organic chemicals into the body of earthworm, therefore in this study the uptake and depuration kinetics from soil pore water were characterised (Belfroid *et al*., 1995). The first order one-compartment model fitted the uptake and depuration well (Figure 5.1). From the kinetic analysis, the rates of uptake and elimination were determined. It was found that the rates of uptake for Nano A and Nano B (±S.D, n = 3) were 0.5592±0.0696 and 0.6551±0.0779 mL g^{-1} h^{-1}, respectively which were higher than analytical grade bifenthrin and Capture LFR (0.2216±0.017 and 0.2049±0.0204 mL

g^{-1} h^{-1}, respectively). The rates of elimination for Nano A and Nano B were also higher compared to analytical grade bifenthrin and Capture LFR (0.0237±0.003 and 0.0173±0.0021 h^{-1}, and 0.0038±0.0004 and 0.0042±0.0005 h^{-1}, respectively). By using the optimised rates, the BCF values were determined by running the simulation beyond steady state. As a result of the differences in uptake and elimination rate constants, BCFs for the bifenthrin for the Nano A and Nano B treatments were lower than the analytical grade bifenthrin and Capture LFR treatments (Table 5.1). There was a significant difference in the BCFs of the different bifenthrin treatments (F=158.64, d.f.=3, $p<0.001$). These findings showed the rates of uptake and elimination were found to be affected by the nano-encapsulation formulations.

Table 5.1 K_d, DT_{50}, R_{50} values, uptake (K_{in}) and elimination (K_{out}) rates, and pore water based BCFs (±S.D, n = 3) for different bifenthrin treatments

Bifenthrin treatment	**K_d (mLg^{-1})**	**DT_{50} (d)**	**R_{50} (d)**	**K_{in} (mLg^{-1}h^{-1})**	**K_{out} (h^{-1})**	**$BCF_{pore\ water}$ (at steady state)**
Analytical grade bifenthrin	250±11	39±2	-	0.222±0.017	0.004±0.001	58±2
Capture LFR	180±7	41±3	-	0.205±0.020	0.004±0.001	48±2
Nano A	110±2	94±2	11±3	0.560±0.070	0.024±0.003	26±3
Nano B	130±3	54±5	9±2	0.655±0.078	0.017±0.002	38±1

When the uptake, distribution and depuration of bifenthrin into the two earthworm species was explored, the same pattern of concentration decline seen in the uptake phase as in the uptake and depuration studies was observed (Figure 5.3). From the distribution and depuration studies, it was found that *L. terrestris* accumulated more bifenthrin compared to *E. fetida* (Figure 5.4). In this study, the earthworm species tested were an anecic (*L. terrestris*) and epigeic (*E. fetida*) species. Both of these species showed a different behavior where *L. terrestris* resides in the soil column and burrows deeply and come to surface to feed unlike *E. fetida*, that is mostly found on the top soil and in the soil litter (Sims and Gerard, 1985a,b). Based on this study, the bifenthrin treatments were mixed with the soil to achieve an even distribution of the bifenthrin treatments, thus the differences in the ecology between these two species are probably not the major factor for the observed differences in the uptake between these species. It has been reported that for hydrophobic chemical compound (e.g., bifenthrin), the uptake via gut route became important (Jager *et al*., 2003; Belfroid *et al*., 1994a,b). Therefore, the dietary uptake (i.e., ingestion of soil particles) could be responsible for the differences in the uptake for a large species such as *L. terrestris* as well as the passive diffusion through the skin compared to *E. fetida*.

Previous work on the uptake of organic chemical compound into different earthworm species has been successfully performed (Carter *et al*., 2016). The finding was not in agreement with the presented work here where the bioaccumulation of a more hydrophobic chemical compound (i.e., orlistat with log K_{ow}=8.95) showed that the soil pore water based BCF value for *E. fetida* (63.03) was higher compared to *L. terrestris* (11.93). They found that *L. terrestris*, a larger species in comparison to *E. fetida* showed a minimal uptake through passive diffusion and the hydrophobic nature of orlistat restricting the uptake to primarily across the gut wall, hence reducing the bioaccumulation of orlistat in *L. terrestris*. They also suggested that the bioaccumulation of hydrophobic chemical compound such as orlistat was related to the lipid content of the organism. Based on their findings, orlistat was found to highly accumulate in *E. fetida* due to higher lipid content (5.1 % wet weight).

In *L. terrestris*, following the uptake phase, bifenthrin from the analytical grade and Capture LFR treatments was found to accumulate more in the chloragogue compared to the gut and remaining tissues which had similar bifenthrin concentrations (Figure

5.5). There was a significant difference in the uptake of bifenthrin between the chloragogue, gut and remaining tissues of each earthworm species and the bifenthrin treatments after the uptake phase (*E. fetida*, F=233.21, d.f.=3, p<0.001; *L. terrestris*, F=371.91, d.f.=6, p<0.001). The bifenthrin in the earthworms exposed to the nanoformulation treatments was distributed completely different with much higher concentrations being observed in the gut compared to the chloragogue and remaining tissues. At the end of the 7 d depuration phase, for the analytical grade and Capture LFR treatments, highest concentrations of bifenthrin were seen in the chloragogue while for the nanoformulation treatments highest concentrations were seen in the gut. There was no significant difference between the concentration of bifenthrin detected in the gut, chloragogue and remaining tissues of the *E. fetida* species and the bifenthrin treatments after the elimination phase (F=1.064, d.f.=3, p=0.392) while a significant difference was found between the gut, chloragogue and remaining tissues of the *L. terrestris* species and the bifenthrin treatments (F=90.97, d.f.=6, p<0.001).

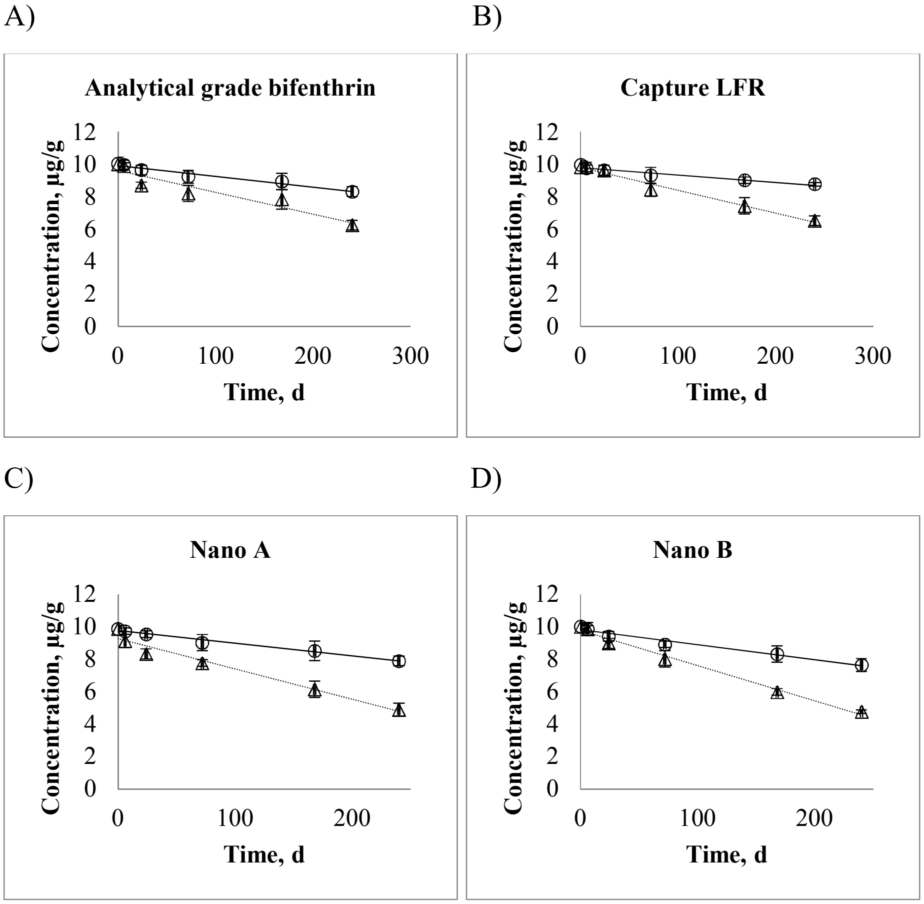


Figure 5.3 Concentration of bifenthrin, µg/g (±S.D, n = 3) for different bifenthrin treaments in soil when exposed to *E. fetida* (open circles) and *L. terrestris* (open triangles) during uptake phase (distribution studies). The data lines represent the first-order model fit

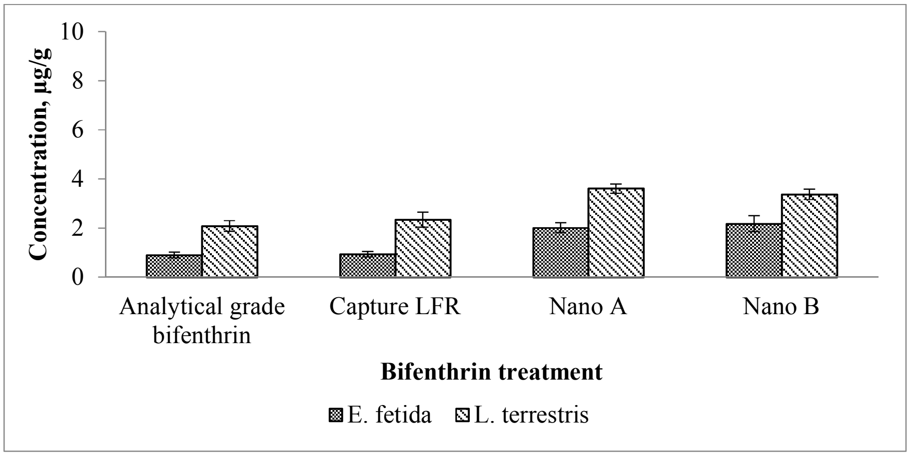


Figure 5.4 Normalised total concentrations of bifenthrin, µg/g (±S.D, n = 3) detected in earthworms species at the end of the uptake phase (distribution studies)

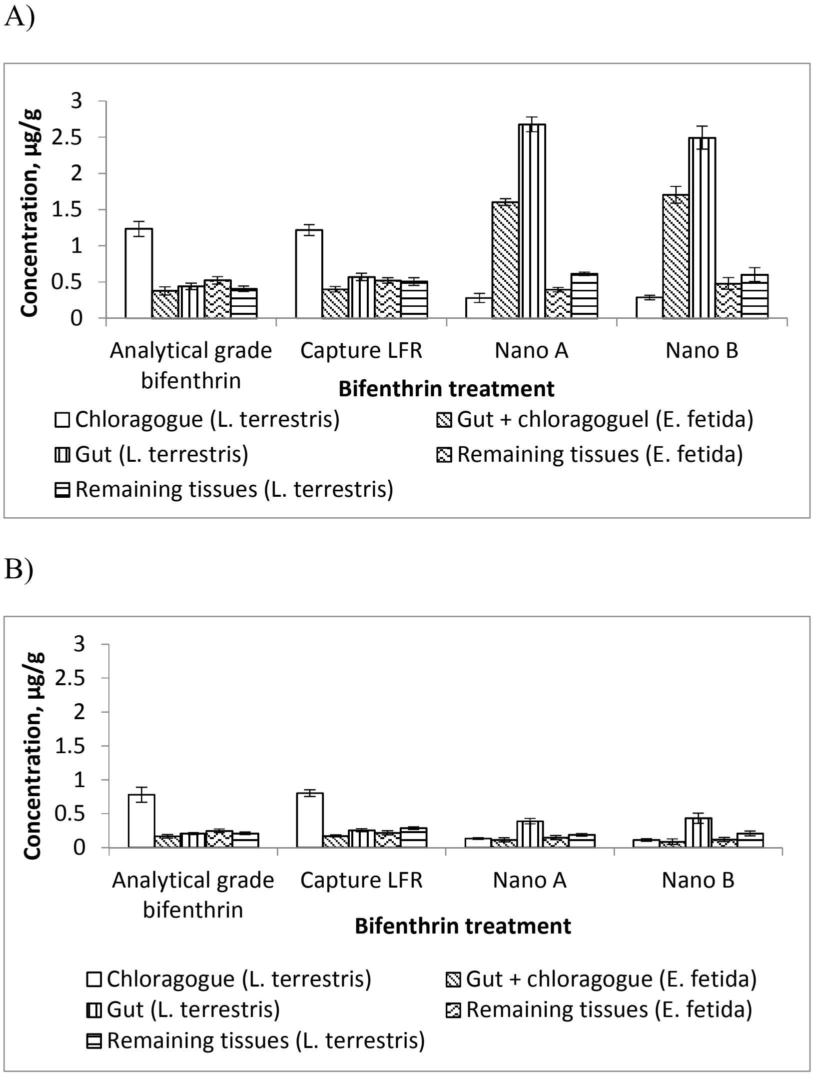


Figure 5.5 Concentration of bifenthrin, µg/g (±S.D, n = 3) measured in different organs of earthworms; A) after 10 d exposure to contaminated soil and B) after 7 d depuration in clean soil

Unfortunately, it was not practically possible to separate out the chloragogue of *E. fetida* from the gut so it was only possible to distinguish between bifenthrin in the remaining tissues and in the gut combined with the chloragogue. For the analytical grade and Capture LFR treatments, concentrations in the gut + chloragogue and in the remaining tissues were similar (Figure 5.5). Again, the distribution of bifenthrin from nanoformulation treatments was different with more bifenthrin accumulated in the gut combined with the chloragogue than the remaining tissues. Following the depuration phase, concentrations of bifenthrin in the gut + chloragogue and remaining tissues in all treatments were similar (Figure 5.5).

This study is in agreement with previous studies investigating the bioaccumulation of NPs into soil invertebrates such as earthworm which suggest that the accumulation of NPs was the greatest in the earthworm gut. Unrine *et al*. (2010) investigated the bioavailability and biodistribution of Au NPs within earthworm, *E. fetida*. Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) analysis showed the Au was distributed throughout the earthworm cross section. A significant result was found where the earthworm gut showed the greatest region with high intensity of Au NPs. They also found that the bioaccumulation of Au NPs into earthworm was greatly influenced by the size of the NPs. Another previous study concerning the bioaccumulation of metal NPs was done by Diez-Ortiz *et al*. (2015). Using an established approach of oral gluing, a toxicokinetics study was done in order to investigate the uptake routes of Ag NPs and Ag ion in earthworm, *L. rubellus*. The results indicated that a significant part of the Ag uptake in the earthworms is through oral/gut uptake for both Ag ions and NPs. Thus, sealing the mouth reduced Ag uptake by between 40% and 75% (Diez-Ortiz *et al*., 2015). This could be the evidence that uptake route of nanoformulations can be influenced by dietary uptake and not only via passive diffusion.

Based on this study, it is believed that the bifenthrin from analytical grade and Capture LFR treatments was internalised in the earthworm body while bifenthrin from the nanoformulation treatments was accumulating more in the earthworm guts as well as being internalised in the earthworm body. As the active ingredient bifenthrin will release from the nanocapsules over time, we believe that the earthworms may have taken up this ‘free’ bifenthrin, hence accumulating in the tissues. Therefore, the accumulated bifenthrin from the nanoformulation treatments in the tissues may cause the same effects as the non-nanoformulation treatments. Based on our findings in *Chapter 3*, we observed the toxicity on earthworm *E. fetida* did not differ among the bifenthrin treatments applied (i.e., nanoformulation treatments showed the same effects as Capture LFR treatment). Thus, this supports our explanation that the accumulation of the ‘free’ bifenthrin in the earthworm body may have shown the same effects as the non-nanoformulation treatments.

The observations could have important implications in terms of toxicity. In the nanoformulation treatments, it is noted that as the active ingredient bifenthrin is ‘protected’ by the nanocapsules, this means that the nanoformulation treatments could prolong the exposure as the bifenthrin remains in the nanocapsules, thus increasing the effects to the organism over time (i.e., bifenthrin will be released from the nanocapsules over time). For example, Choi and Park (2015) investigated the toxicological effect of citrate-coated silver NPs in *E. fetida*. The results showed that although no abnormal behavior was found at low Ag NPs concentrations, oxidative stress-related indicators were present. They concluded that Ag NPs may have regulated oxidative-stress related mechanisms in the earthworm. However, no studies were found on the bioaccumulation of polymer-based nanopesticides on the uptake into soil invertebrates. Therefore, more studies should be carried out involving different types of nanopesticide (e.g., different active ingredients with different properties and different nanocarriers) under a wide range of environmental conditions (e.g., pH, organic carbon content, moisture and temperature) in order to assess the possible impact of nanopesticides in the terrestrial environment.

5.5 Conclusion

The work presented here demonstrates that pesticide nanoformulations present in soils can be taken up by the earthworm, *E. fetida*. In terms of fate, the nanoformulation treatments (Nano A and Nano B) reduced the sorption and hence increased the bioavailability in the soil pore water for the uptake by the *E. fetida*. The uptake pattern of the nanoformulation treatments was different compared to the analytical grade bifenthrin and Capture LFR treatments. This showed Nano A and Nano B treatments were exhibiting higher uptake and elimination rates compared to the analytical grade bifenthrin and Capture LFR treatments. Based on the assumption that uptake into soil invertebrates such as earthworm occurs mainly through passive diffusion from the soil pore water, a simple one-compartment first order model was fitted to the uptake of different pesticide treatments into *E. fetida*. The differences in the rate constants resulting in the lower BCFs for both Nano A and Nano B treatments compared to the analytical grade bifenthrin and Capture LFR treatments. The distribution studies showed that the bifenthrin from both Nano A and Nano B treatments was accumulating more in the *E. fetida* and *L. terrestris* guts as well as being internalised while bifenthrin from analytical grade bifenthrin and Capture LFR treatments was internalised in both earthworm species.

Ecotoxicity of a conventional pesticide is normally related to the active ingredient mass concentration, thus risk is determined using exposure and effects data in terms of mass per volume or mass per mass of active ingredient (Kookana *et al*., 2014). In contrast, for nanopesticides, parameters such as particle size distribution (PSD), particle number concentration and, the ratio of free and nanoparticle-bound active ingredient may be important parameters in order to determine the bioavailability and toxicity. Taking into account that NPs could also aggregate within time, it is therefore important to characterise the nanopesticides at different stages throughout the fate and effects studies. In the future, it is recommended to perform more studies concerning nanopesticides under a wide range of environmental conditions (i.e., soil pH, texture, organic carbon content, moisture and temperature) and characterise the nanopesticides according to the parameters that are already discussed above. Therefore, in the next chapter the effects soil properties on the uptake and depuration of nanoformulations (Nano A and Nano B) are explored.

Chapter 6 Effects of Soil Properties on the Uptake and Depuration of Nanopesticides in Soil-Earthworm Systems

6.1 Introduction

The previous chapter (*Chapter* 5) explored the uptake and depuration in the earthworm *E. fetida* and uptake distribution and depuration in *L. terrestris* of different bifenthrin treatments. The study focused on one soil type and showed that uptake of bifenthrin from nanoformulations was lower than for a non-nanoformulation or analytical grade material. However, it is known that even the same chemical compound can behave very differently across soil types (Monteiro and Boxall, 2009) so the observations for one soil type may not reflect behavior more broadly in the environment.

Previous studies have shown that several soil properties can influence the uptake of soil-associated contaminants into earthworms, these include factors such as the clay (Chung and Alexander, 1998; White *et al*., 1997) and organic matter content (Chung and Alexander, 1998; Gobas and Zhang, 1994; Weber and Weed, 1968). Soil organic matter content influences the sorption potential of the soil and the level of biological activity (Fukushima and Tatsumi, 2001). Organic matter can bind with volatile organic chemicals, metals, nutrients, pesticides, and some pathogens (Gavrilescu, 2005). This will reduce the bioavailable fraction of the chemical compounds in the soil pore water for the uptake process. The very small pore sizes governed by clay particles and their tremendous surface area available for the sorption of cations mean that they are good for chemicals attenuation (i.e., pollutants). Some pesticides are also reported to be inactivated and degraded by sorption to clay colloids (Gavrilescu, 2005).

Other parameters such as soil texture (i.e., clay, silt and sand), soil pH and CEC have also been reported to influence the bioavailability of chemical compounds in the terrestrial environment (Franco *et al*., 2009; Gavrilescu, 2005; Guangyao *et al*., 2005; Hyun and Lee, 2005; Hyun and Lee, 2004; Di Vincenzo and Sparks, 2001; Erickson *et al*., 1994). For example, soil texture greatly influences the rate of water movement through the soil and the active surface area of the soil. Soils with fine texture show greater surface area and lower permeability, therefore providing longer contact time and a greater sorption area for pollutants attenuation. For ionisable chemical compounds, soil pH and CEC are particularly important parameters in affecting the fate and bioavailability of the chemical compounds in the soil compartment.

Studies have been performed in order to investigate the factors that could potentially affect the uptake of contaminants including both organic and inorganic chemicals into terrestrial organisms such as earthworm (Carter *et al*., 2016; Peijnenburg, 2001; Sample *et al*., 1997). For example, Janssen *et al*. (1997) investigated the chemical availability and BCF of heavy metals (Cd, Cu, Cr, Ni, Pb and Zn) to earthworms in different soil types. A multiple regression analysis was performed to investigate the relationships between the soil properties and the uptake into earthworm, *E. andrei*. Based on their findings, they suggested that the uptake of metals was either from the soil pore water or indirect through an uptake route closely related to soil pore water (Janssen *et al*., 1997).

As passive diffusion across the skin of the earthworms from the soil pore water has been reported to be the main uptake route for chemicals in the soil environment (Belfroid *et al*., 1995a,b; Belfroid *et al*., 1993a,b), differences in uptake across soils is believed to be explained by differences in the sorption of a substance across soils (Wu *et al*., 2011; Yu *et al*., 2006). For example, highly hydrophobic chemical compounds tend not to move within the soil and show a great affinity to the organic matter content in the soil, this can lead to the chemical compounds being more highly adsorbed to the soil particles hence decreasing the bioavailable fraction of the chemical compounds in the soil pore water. Yu *et al.* (2006) investigated the relationship between sorption and the uptake of different pesticides (butachlor, myclobutanil and chlorpyrifos) into earthworm, *Allolobophora caliginosa* using linear regression analysis. They found that for both butachlor and myclobutanil pesticides, the analyses showed the sorption coefficient (K_d) had a significant effect on the uptake into *A. caliginosa*. While for chlorpyrifos, no clear relationships were observed between the K_d and the BCF values. They suggested that this could be explained by the physico-chemical properties of chlorpyrifos with higher log K_{ow} made the pesticide to be highly adsorbed to the soil particles and the accumulation could be influenced by other factors such as soil properties (i.e., organic matter) and possible dietary uptake (ingestion of soil particles). This study is in agreement with a previous study performed by Davis (1971) with high log K_{ow} pesticides (dieldrin and dichlorodiphenyltrichloroethane, DDT) that showed the accumulation by earthworms, *L. terrestris* and *A. caliginosa* was influenced by soil properties. Regression analyses were performed between the soil properties (i.e., pH and organic matter) showed that organic matter had a significant effect on the uptake of DDT into *A. caliginosa*.

Studies on the uptake of nanomaterials (i.e., metal and metal oxide nanoparticles, NPs) in the terrestrial environment have been widely explored (García-Gómez *et al*., 2014; Lahive *et al*., 2014; Waalewijn-Kool *et al*., 2014; Tourinho *et al*., 2012; Hu *et al*., 2010). This includes the impact of nanomaterials on the earthworm such as the toxicity effects as described in the previous *Chapter 2* (see section 2.3.1.5). However, as NPs are behaving like colloids, the fate and uptake of these NPs could be very differently compared to the conventional chemicals. Processes such as aggregation and stability of NPs were found to influence the fate (i.e., sorption and bioavailability), thus affecting the accumulation of NPs in the terrestrial organisms (i.e., plants and animals). Aggregation of NPs is likely to be influenced by size, particle shape, surface area, charge and coatings (Handy *et al*., 2008a; Pettibone *et al*., 2008). Soil pH, ionic strength and dissolved organic matter could also influence the aggregation of these NPs in soil compartment (see reviews on the fate of NPs in section 2.3.1.1 in *Chapter 2*). With these type of studies are available in the literature, no information was obtained for the uptake of nanopesticides into earthworm.

Therefore, it is important to investigate the factors affecting the uptake of nanopesticides in the terrestrial environment. In this study, the uptake and depuration of different bifenthrin treatments in the earthworm, *E. fetida* were explored in different soil types to assess the relationships between the soil properties and uptake. This study was performed using two different bifenthrin nanoformulations (Nano A and Nano B) as well as the non-nanoformulated (Capture LFR) and corresponding active ingredient bifenthrin.

6.2 Materials

The chemicals, soils (Soil 1 – 4) and earthworm, *E. fetida* used in this study were the same as those used in *Chapter 3* with an additional analytical PESTANAL® grade bifenthrin (98.9%, w/w) purchased from Sigma-Aldrich (Dorset, UK). Detailed descriptions of the chemicals, soils and *E. fetida* culture can be found in section 3.2.1, 3.2.2 and 3.2.3, respectively in *Chapter 3*.

6.3 Experimental procedures

6.3.1 Uptake and depuration studies

The uptake and depuration experiments were done following OECD Guideline 317 'Bioaccumulation in Terrestrial Oligochaetes' (OECD, 2010) as described in the previous *Chapter 5*. Experiments were performed in glass jars at a concentration of 10 μg/g of active ingredient and exposed to the earthworm, *E. fetida* for 21 d and then depurated for another 21 d. Details on the uptake and depuration procedures can be found in section 5.3.1 in *Chapter 5*.

6.3.2 Sample preparation and analysis

Soil samples, pore water and defrosted earthworms were extracted and analysed following the analytical method in section 3.3.2 in *Chapter 3*.

6.3.3 Data analysis

6.3.3.1 Determination of sorption coefficient, K_d

Sorption coefficient, K_d for each bifenthrin treatment for each soil type was determined as described in section 3.3.3.1 in *Chapter 3*.

6.3.3.2 Determination of half-life, DT_{50}

Half-life, DT_{50} for each bifenthrin treatment for each soil type was determined as described in section 3.3.3.2 in *Chapter 3*.

6.3.3.3 Earthworm kinetic modelling

A first order one-compartment model was used to estimate the uptake and depuration rates for each bifenthrin treatment from soil pore water as described in the previous *Chapter 5*. Detailed descriptions on the earthworm kinetic modelling can be found in section 5.3.4.2 in *Chapter 5*.

6.3.3.4 Statistical analysis

Statistical analysis was performed using SigmaPlot (v. 12.5). Data obtained for the K_d, DT_{50} and BCF values were tested for normality using a Shapiro-Wilk test and then for equal variance. If these passed, a one-way ANOVA was performed to assess any significant difference between the different bifenthrin treatments in the different soil types. If these failed, a one-way ANOVA was performed on ranks (Kruskal-Wallis).

A linear regression analysis was performed to investigate the relationships between soil properties (soil pH and the percentage of OC, CEC, clay, silt and sand) on the BCF values of the different bifenthrin treatments.

6.4 Results and discussion

6.4.1 Mass balance

A mass balance was performed to account for the bifenthrin in the experiment using the measurements in soil, soil pore water and *E. fetida*. Based on the uptake and depuration data, the contribution of soil pore water and uptake by earthworms in the mass balance analysis were found to be low with the percentages ranging from 0.4 – 1.3% and 0.6 – 1.5%, respectively for different bifenthrin treatments. At the end of the uptake phase, approximately 50 – 80% of bifenthrin was extracted from all soil types for different bifenthrin treatments (Figure 6.1). For the dissipated bifenthrin from the soil, the percentages ranged from 14 – 37% for different bifenthrin treatments.

The observed dissipation could be explained by the transformation of bifenthrin into different metabolites during degradation process. The transformation of bifenthrin in soil compartment has been reported using cyclopropyl- and phenyl-^{14}C labelled bifenthrin (Smith, 1991; Reynolds, 1986; Bixler, 1984). This showed some mineralisation of bifenthrin into the formation of CO_2 up to 50% (Fecko, 1999). The bound residues were remained below 25% and the other metabolites were 4’-hydroxybifenthrin, 3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropane carboxylate (TFP acid), 2-methyl-3-phenylbenzyl alcohol (BP alcohol) and 2-methyl-3-phenylbenzoic acid (BP acid) with no single metabolite ever exceeded an amount of 10% after 120 d. The 4’-hydroxybifenthrin metabolite was found to give the maximum percentage of 8.2% (Smith, 1991; Reynolds, 1986; Bixler, 1984).

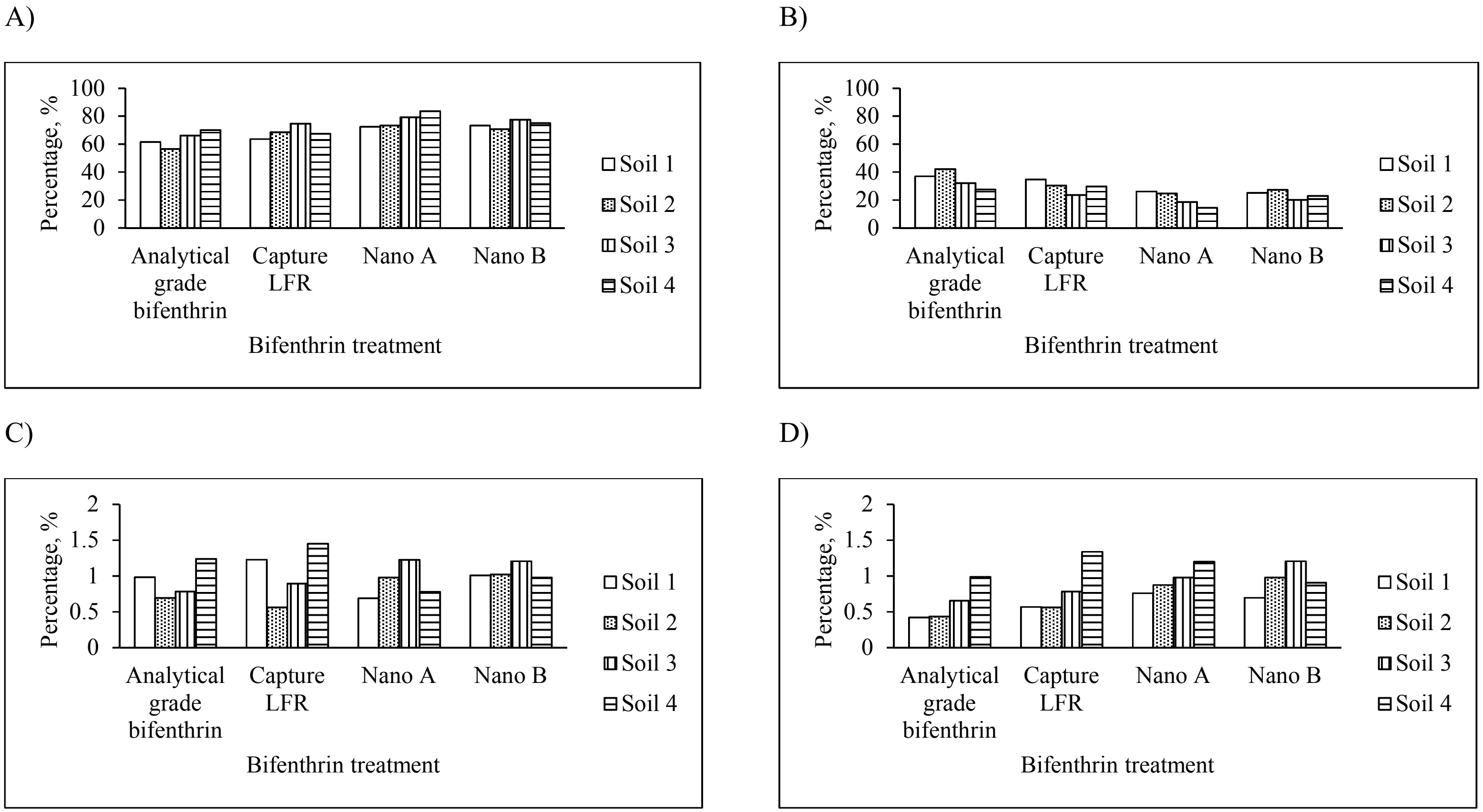


Figure 6.1 Percentage of bifenthrin associated with different compartments in the soil−earthworm systems at the end of the uptake phase of the experiment in comparison to applied bifenthrin at 0 h for A) Soil associated (extractable), B) Soil associated (dissipated), C) Soil pore water associated and D) Uptake into earthworm

6.4.2 Fate of bifenthrin treatments

Generally, nano-encapsulation does affect the sorption and persistence behavior of bifenthrin treatments to the soil particles. The observed trend in the K_d and DT_{50} values were in agreement with the previous findings in *Chapter 3 – 5* where the nanoformulation treatments (Nano A and Nano B) reduced the sorption and enhanced the persistence compared to the non-nanoformulation treatments (analytical grade bifenthrin and/or Capture LFR). In terms of sorption, this could be explained by the role of polymer capsules 'protecting' the active ingredient bifenthrin from soil binding sites, thus increasing the bioavailability in the pore water for uptake by earthworms. In this study, the K_ds for bifenthrin treatments increased in the order of Nano A (110 – 130 mL/g) < Nano B (140 – 160 mL/g) < Capture LFR (240 – 410 mL/g) < analytical grade bifenthrin (360 – 560 mL/g). This showed the non-nanoformulation treatments (analytical grade bifenthrin and/ or Capture LFR) were highly adsorbed to soil particles compared to the nanoformulation treatments (Nano A and Nano B) in each soil type ($F>461.35$; d.f.=3; $p<0.003$; Table 6.1). There was a significant difference in the K_d values across soil types ($H=13.432$, d.f.=3, $p=0.004$). Previous studies have reported that pyrethroid pesticides are easily adsorbed by soil particles (Perez-Fernandez *et al.*, 2010; Wong, 2006; Maund *et al.*, 2002) with the K_ds are in a range of 882 to 6000 mL/g in different soil types (USEPA, 2010; Froelich, 1983), thus affecting the uptake by earthworms.

Overall, the DT_{50} values of the different bifenthrin treatments were found to be lower across soil types (Table 6.1) compared to the reported DT_{50} values in the literature for bifenthrin (DT_{50}=122 – 345 d; FMC 50429-025, 1983; Froelich, 1983). This could be explained by the uptake into earthworm that reduced the available fraction of bifenthrin for degradation as well as the sorption that took place during the experimental period. In this study, the DT_{50} values of the nanoformulation treatments were significantly higher than the non-nanoformulation treatments in each soil type ($F>179.00$; d.f.=3; $p<0.001$; Table 6.1). Across soil types, there was a significant difference in the DT_{50} values between the bifenthrin treatments ($F=5.30$; d.f.=3; $p=0.015$). Just like the sorption results, this is likely explained by the role of polymer capsules 'protecting' the active ingredient bifenthrin from being attacked and degraded by water (hydrolysis) and/or soil microorganisms (biodegradation).

Table 6.1 K_d, DT_{50} and R_{50} values (±S.D, n = 3) for different bifenthrin treatments in different soil types

Soil	Bifenthrin treatment	K_d (mL g^{-1})	DT_{50} (d)	R_{50} (d)
1	Analytical grade bifenthrin	360±31	30±2	-
	Capture LFR	240±20	31±2	-
	Nano A	120±11	42±3	11±4
	Nano B	140±9	50±4	11±2
2	Analytical grade bifenthrin	560±27	26±2	-
	Capture LFR	410±18	42±2	-
	Nano A	130±4	51±3	12±1
	Nano B	140±8	48±2	11±3
3	Analytical grade bifenthrin	390±10	36±3	-
	Capture LFR	280±12	50±3	-
	Nano A	110±4	63±2	11±4
	Nano B	160±21	58±3	11±2
4	Analytical grade bifenthrin	410±43	42±2	-
	Capture LFR	270±12	41±2	-
	Nano A	130±5	77±4	11±3
	Nano B	150±3	49±4	10±1

6.4.3 Uptake and depuration studies

At the end of the uptake and depuration experiment, the earthworm weight change and mortality passed the validity criteria (based on earthworm weight change and mortality) according to the principle outlined in the OECD 317 (2010). Throughout the uptake phase, there was a decrease in concentration of bifenthrin in the soil and soil pore water which was associated with an increase in the concentration of bifenthrin in the earthworms (Figure 6.2 – 6.5).

Throughout the uptake phase, the earthworm, *E. fetida* was found to accumulate bifenthrin from the different bifenthrin treatments. The uptake and depuration patterns for the different bifenthrin treatments were similar for each soil type. However, there was a difference in the accumulation pattern between different treatments (non-nanoformulations and nanoformulations). The non-nanoformulation treatments (analytical grade bifenthrin and Capture LFR) showed a slower uptake rates into the earthworms than the two nanoformulation treatments (Nano A and Nano B). At the end of the uptake phase, for each bifenthrin treatment, *E. fetida* had accumulated approximately 0.7 – 1.06 μg/g of bifenthrin from exposure to the analytical grade material in all soil types. For the exposure of Capture LFR, a range of 0.79 – 1.96 µg/g of bifenthrin concentration was seen in the *E. fetida*. While for the Nano A and Nano B, the concentration of bifenthrin accumulated in the *E. fetida* ranged from 0.67 – 1.53 µg/g and 0.64 – 1.73 µg/g, respectively. However, the uptake of bifenthrin at 168 – 240 h of exposure from Nano A and Nano B treatments showed a steady state ($F<2.53$; d.f.=1; $p<0.251$) which was not observed for the exposure of the analytical grade bifenthrin and Capture LFR to *E. fetida* (Figure 6.2 – 6.5). Elimination of bifenthrin from the earthworms exposed to the nanoformulation treatments was faster compared to the non-nanoformulation treatments. At the end of the depuration phase, the bifenthrin accumulated from the nanoformulation treatments (Nano A and Nano B) was no longer detectable in the earthworm while for the analytical grade bifenthrin and Capture LFR treatments, bifenthrin was still detected.

Based on previous studies that showed soil pore water was mainly influenced the uptake of organic chemicals into the body of earthworm, therefore in this study the uptake and depuration kinetics were characterised (Belfroid *et al.*, 1995a,b). The first order one-compartment model fitted the uptake and depuration well (Figure 6.2 – 6.5). From the kinetics analysis, the rates of uptake and elimination were determined. In general, the rates constant for nanoformulation treatments (Nano A and Nano B) were higher than the non-nanoformulation treatments (analytical grade bifenthrin and Capture LFR). The rates of uptake increased in the order of analytical grade bifenthrin (0.162 – 0.249 $mLg^{-1}h^{-1}$) < Capture LFR (0.149 – 0.242 $mLg^{-1}h^{-1}$) < Nano A (0.34 – 1.027 $mLg^{-1}h^{-1}$) < Nano B (0.207 – 1.293 $mLg^{-1}h^{-1}$). Similarly, the rates of elimination followed the same pattern as the rates of uptake where increased in the order of analytical grade bifenthrin (0.002 – 0.005 h^{-1}) < Capture LFR (0.002 – 0.005 h^{-1}) < Nano A (0.029 – 0.044 h^{-1}) < Nano B (0.015 – 0.055 h^{-1}). These findings showed the rates of uptake and elimination was found to be affected by the nano-encapsulation. By using the optimised rates, the BCFs were determined by running the simulation beyond steady state. As a result of the differences in uptake and elimination rate constants, BCFs for the bifenthrin for the nanoformulation treatments were lower than the non-nanoformulation treatments and varied across soil types ($F>117.87$; d.f.=1; $p<0.003$; Table 6.2).

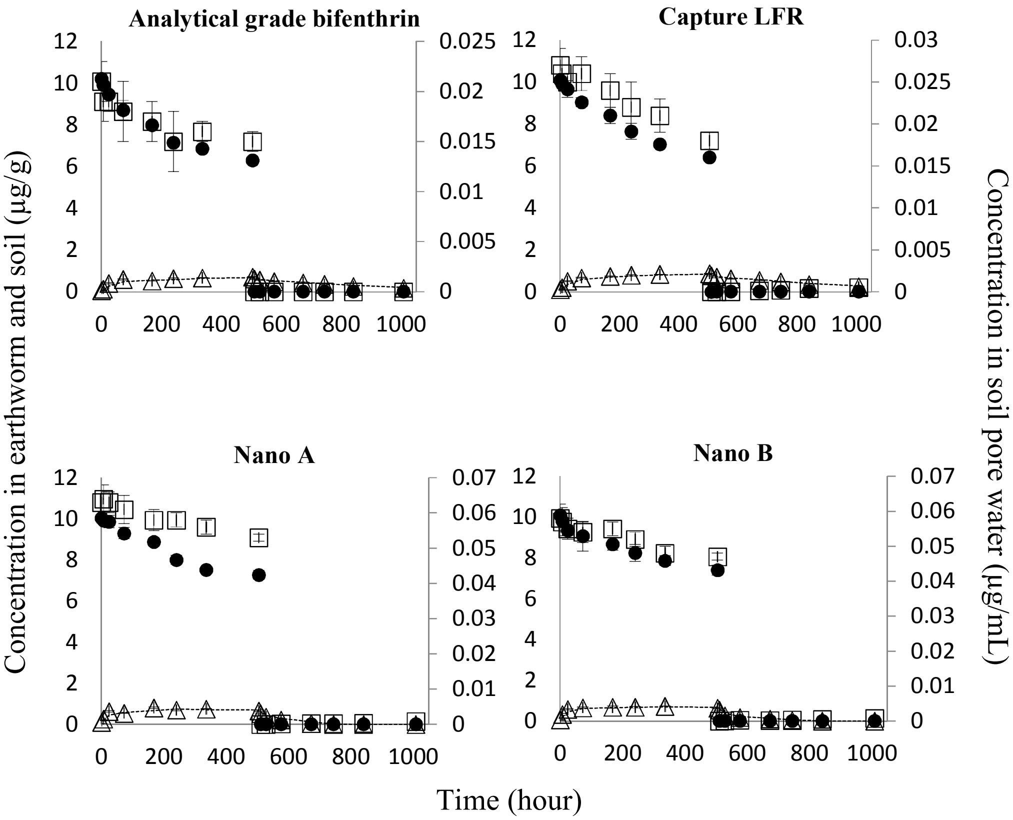


Figure 6.2 Uptake and depuration curves for *E. fetida* exposed to different bifenthrin treatments in Soil 1. Mean measured concentrations (±S.D, n = 3) in *E. fetida*, soil and soil pore water are represented by open triangles, closed circles and open squares, respectively. The data lines represent the first-order model fit

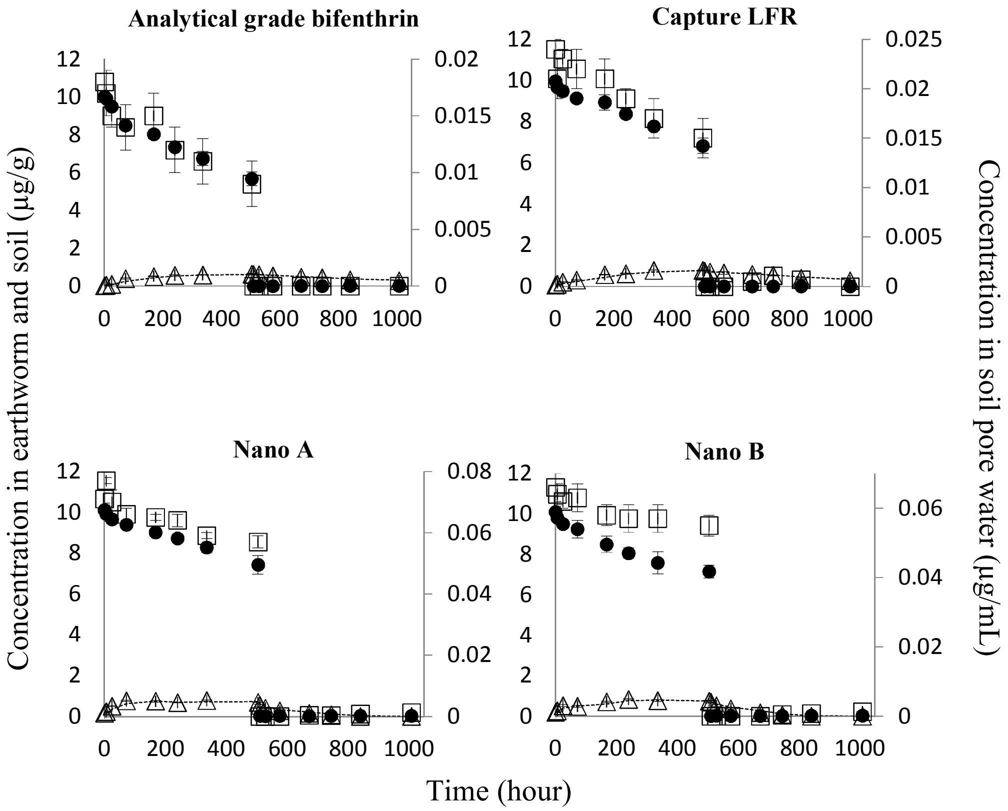


Figure 6.3 Uptake and depuration curves for *E. fetida* exposed to different bifenthrin treatments in Soil 2. Mean measured concentrations (±S.D, n = 3) in *E. fetida*, soil and soil pore water are represented by open triangles, closed circles and open squares, respectively. The data lines represent the first-order model fit

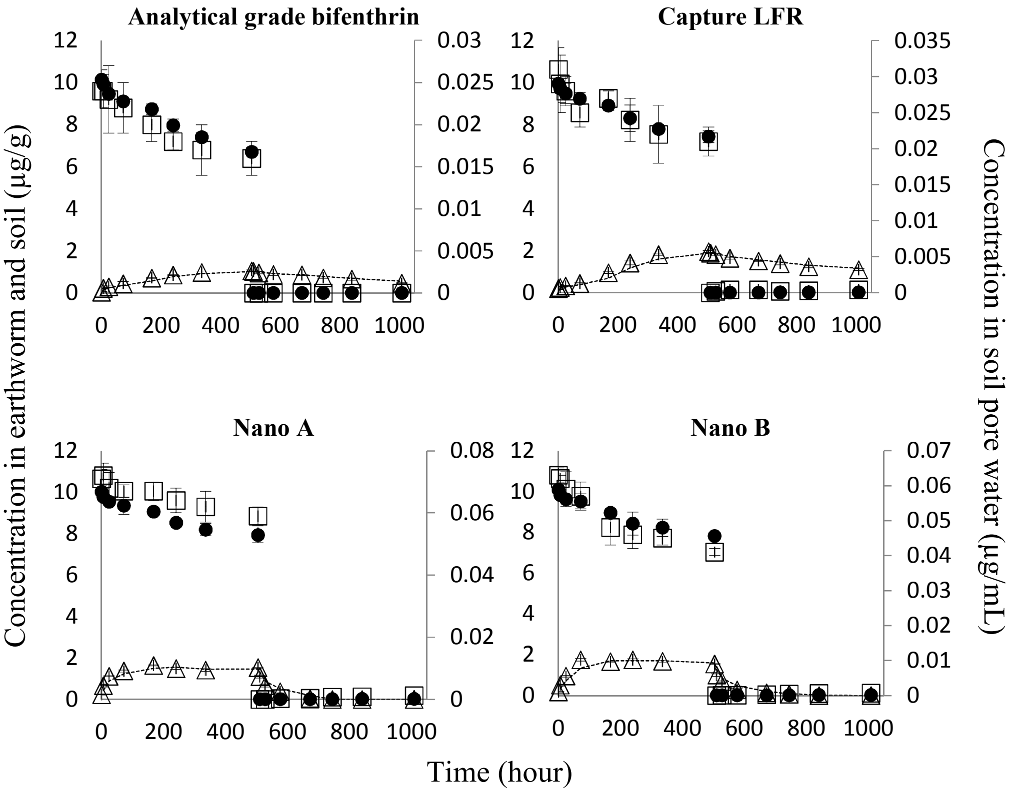


Figure 6.4 Uptake and depuration curves for *E. fetida* exposed to different bifenthrin treatments in Soil 3. Mean measured concentrations (±S.D, n = 3) in *E. fetida*, soil and soil pore water are represented by open triangles, closed circles and open squares, respectively. The data lines represent the first-order model fit

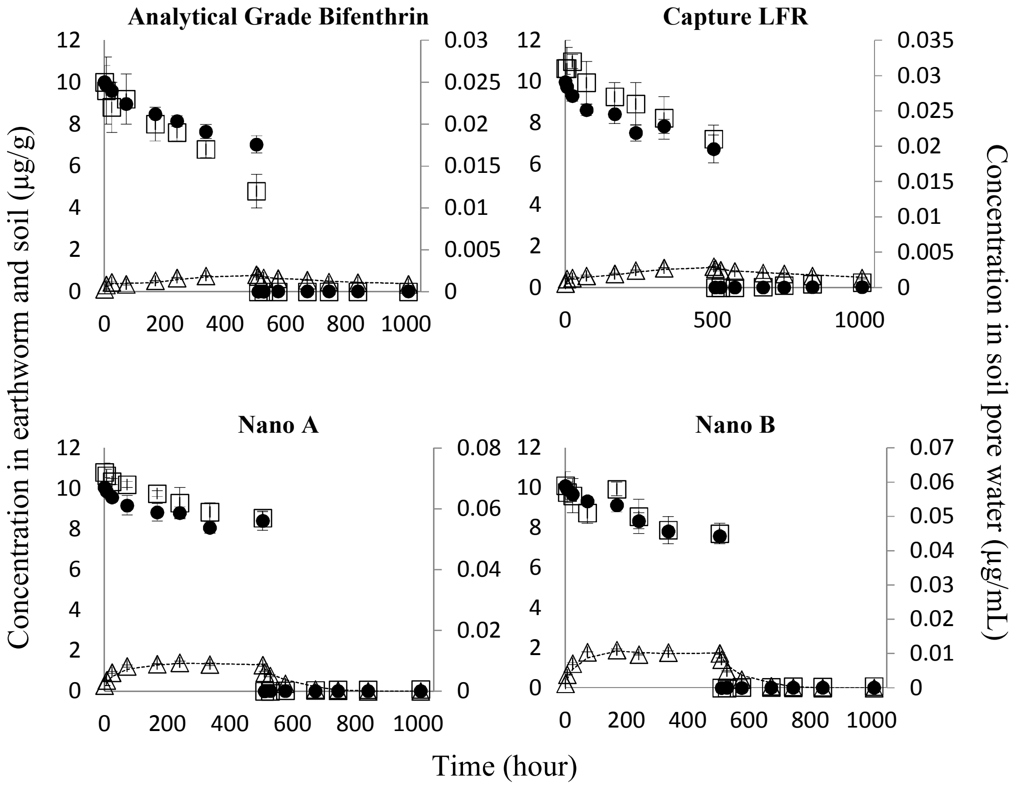


Figure 6.5 Uptake and depuration curves for *E. fetida* exposed to different bifenthrin treatments in Soil 4. Mean measured concentrations (±S.D, n = 3) in *E. fetida*, soil and soil pore water are represented by open triangles, closed circles and open squares, respectively. The data lines represent the first-order model fit

Table 6.2 Rates of uptake (K_{in}) and elimination (K_{out}), and pore water based BCFs (±S.D, n = 3) for different bifenthrin treatments

Soil	Bifenthrin treatment	K_{in} (mL g^{-1} h^{-1})	K_{out} (h^{-1})	$BCF_{pore\ water}$ (at steady state)
1	Analytical grade bifenthrin	0.249±0.049	0.005±0.001	52±4
	Capture LFR	0.220±0.041	0.005±0.001	59±3
	Nano A	0.507±0.114	0.040±0.009	15±4
	Nano B	0.736±0.132	0.055±0.010	17±4
2	Analytical grade bifenthrin	0.206±0.020	0.002±0.001	84±7
	Capture LFR	0.163±0.013	0.003±0.001	64±8
	Nano A	0.340±0.068	0.029±0.006	14±4
	Nano B	0.207±0.043	0.015±0.003	17±6
3	Analytical grade bifenthrin	0.197±0.018	0.002±0.001	96±9
	Capture LFR	0.242±0.015	0.002±0.001	151±3
	Nano A	1.027±0.171	0.044±0.007	27±6
	Nano B	1.014±0.126	0.030±0.004	34±4
4	Analytical grade bifenthrin	0.162±0.026	0.002±0.001	66±5
	Capture LFR	0.149±0.025	0.003±0.001	56±8
	Nano A	0.679±0.123	0.031±0.006	23±3
	Nano B	1.293±0.172	0.036±0.005	36±4

In another study involving different SPs, Ye *et al.* (2016) investigated the biaccumulation of fenvalerate (FV) and esfenvalerate (ESFV) using *E. fetida* as test organism. From the study, the found that the concentration of FV and ESFV in *E. fetida* tissues increased up to 14 d. The first sample point (2 d) showed the uptake of FV and ESFV was 9.9 and 17.9 µg/kg, respectively indicating that *E. fetida* were rapidly taken up both of the SPs. The samples taken at 14 d showed that the concentration in the *E. fetida* tissues for FV and ESFV were 36.8 and 32.6 µg/kg, respectively. After 14 d, the uptake of FV was mildly increased, just like the observation in this study involving analytical grade bifenthrin and Capture LFR, while for ESFV the uptake declined throughout the exposure duration (28 d). Further study revealed that the uptake of FV and ESFV was enantioselective and followed previous studies on α-cypermethrin (Diao *et al.*, 2011; Hartnik and Styrishave, 2008).

Previous studies involving the exposure of NPs showed that earthworms can also accumulate metal NPs (Ha *et al.*, 2014). The biota-sediment accumulation factor (BSAF) values of Zn and Ag in the earthworms were determined after *E. fetida* were exposed to the contaminated soils. After the 7 d of elimination phase, BSAF for ZnO

NPs (0.06) was 31 times lower than that for Zn ion (1.86), suggesting that ZnO NPs was less bioavailable than its ionic form from contaminated soil. In contrast, BSAFs for Ag NPs coated with polyvinyl pyrrolidone (PVP) (0.12) or with citrate (0.11) were comparable to those for Ag ion (0.17). This indicates that Ag from contaminated soil was bioavailable in a similar rate regardless of its chemical forms (Ha *et al*., 2014). A study investigating the effect of nanoparticles size was done by Gupta and Yadav (2014). The study demonstrated that the bioavailability of ZnO NPs was very high in the earthworm in all exposures of NPs particularly at exposure of 10 nm sized ZnO NPs. They also reported that the intact NPs can be taken up by earthworm from soil. Based on our study, while no data available elsewhere for nanopesticides, it is likely that our observations of the nanoformulation treatments are similar to the observations seen with metal and metal oxide NPs and bulk materials.

Bioaccumulation of $^{14}C_{60}$ by *E. fetida* was reported by Li *et al*. (2010) in the high organic carbon content. The BSAF increased initially to 0.190 after 7 d and then decreased to 0.093 over the 28 d experimentally period. They found that high organic carbon content accelerated the accumulation only in the initial stage but did not affect the accumulation after 28 d exposure. Another study concerning the bioaccumulation of metal NPs was done by Diez-Ortiz *et al*. (2015). Using an established approach of oral gluing, a toxicokinetics study was done in order to investigate the uptake routes of Ag NPs and Ag ion in earthworm, *L. rubellus*. The results indicated that a significant part of the Ag uptake in the earthworms is through oral/gut uptake for both Ag ions and NPs. Thus, sealing the mouth reduced Ag uptake by between 40% and 75% (Diez-Ortiz *et al*., 2015). This could be the evidence that uptake route of nanoformulations can be influenced by dietary uptake and not only via passive diffusion. In our study, the uptake of bifenthrin from the nanoformulation treatments is similar with the findings seen in metal NPs where bifenthrin was accumulating more in the earthworm gut.

6.4.4 Do soil properties affect the uptake into earthworm, *E. fetida*?

Bifenthrin is a non-polar chemical compound and shows a great affinity to organic carbon, therefore it was expected that organic carbon content would play an important role in affecting the fate and uptake of bifenthrin in the terrestrial environment. Based on the correlation analyses, there was no relationship found between the soil properties and the uptake of bifenthrin treatments into earthworms in all soil types ($F<5.432$; d.f.=1; $p>0.145$; Appendix 6.1), although the test soils selected exhibited different organic carbon contents ranging from 1.86 to 5.16%. Based on previous studies, clay has also been reported to influence the bioavailability of organic pollutants in soil compartment (Chung and Alexander, 1998; White *et al*., 1997; Gobas and Zhang, 1994; Weber and Weed, 1968). However, the clay content in all test soils selected were in a range of 0 – 1.5 % which were very low, hence this could be excluded as one of the factors that could influence the uptake into earthworms in this study. Since linear regression analysis showed no clear relationships between the soil properties and the BCFs, this could be explained by a complex interaction of a variety factors and physico-chemical processes (i.e., sorption and degradation) that took place in the soil compartment. Just like in the recommendations made in previous *Chapter 5*, the uptake route into organisms of hydrophobic chemical compounds can also be taken up through dietary uptake (Jager *et al*., 2003; Belfroid *et al*., 1994a,b). Therefore, an investigation between the concentration in soil and the uptake should also be considered to explore what are the parameters that influence the uptake process.

In order to understand the accumulation and the factors that might influence the process, the combination of both soil and soil pore water parameters should be considered. This could give a better explanation on the uptake process. Information from colloid science can be a useful guidance to provide information on the fate and behavior of nanomaterials (Lead and Wilkinson, 2006). The behavior of nanoparticles in the environment is expected not only to depend on both of the physical and chemical characteristics of the nanomaterial, but also the characteristics of the receiving environment (Saleh *et al*., 2008; Chen and Elimelech, 2006). It is generally known that small particles tend to aggregate or agglomerate with other dissolved, colloidal and particulate matter present in the environment. This could change the behavior of nanomaterials and made them different from the conventional materials. In order to assess the relationships between the soil properties and uptake of nanopesticides into terrestrial organisms such as earthworm, additional studies should be carried out under a wide range of soil properties (i.e., pH, texture and organic carbon content) and the different nanopesticides (i.e., active ingredients and nanocarrier materials).

6.5 Conclusion

In order to assess the potential risk of nanoformulations in the terrestrial environment, information on the effect of soil properties on the uptake process is important. In the current research, it was found that that there are no clear relationships between soil properties and the uptake of different pesticide formulations into earthworms, *E. fetida*. Although previous studies mentioned that soil properties showed to influence the fate of chemical compounds in the environment, but this is only true for conventional chemicals. As nanoparticles can behave very different from the conventional particles, the explanation on the fate and uptake involving nanoformulation chemicals is difficult. Organic matter content has shown a great influence in determining the uptake of chemical compounds into organisms due to sorption of chemicals to the soil particles hence decreasing the bioavailable fraction in the soil pore water (Carter *et al*., 2016). However, in this study this was not the case. Since distribution of nanoformulation between soil and soil pore water is different (Praetorius *et al*., 2014), hence the uptake of nanoformulation could not be explained mainly by the soil pore water parameter. A combination of both soil and soil pore water parameters should also be considered. For example, in our findings in *Chapter 3*, we found that the concentration of bifenthrin in soil had a clear relationship with the BSAF obtained except for the Nano A treatment ($p<0.038$), which indicates that the uptake of bifenthrin into *E. fetida* could also be influenced by the dietary uptake.

Another factor that could influence the uptake of nanoformulations into organisms is the exposure concentrations or doses applied. All forms of nanoformulations exist such as free nanoparticles and nanoparticles-bound active ingredient should be considered (Kookana *et al*., 2014). It is also important to realize that nanomaterials might concentrate due to their tendency to aggregate/agglomerate and potentially to adsorb to organic matter in soil compartment. These parameters are important in determining the active ingredient bioavailability (Kümmerer *et al*., 2010; French *et al*., 2009; Sharma, 2009).

In the next chapter, the summary of the findings from *Chapter 3 – 6* are discussed. This includes the overall conclusion from these studies, limitations specific to this study and recommendations for future work to explore the fate and behavior of nanopesticides in the terrestrial environment.

Chapter 7 Discussion

7.1 General discussion

Advances in science and technology could offer solutions to many problems in the field of agriculture. Numerous technologies (i.e., slow- or controlled-release fertilizers and pesticides, and nano-additives for animal feed) have been developed that have the potential to increase farm productivity and at the same time reducing the potential risks to the environment (Ying *et al.*, 2005; Frederiksen *et al.*, 2003). The development of a new plant production products based on nanotechnology (the so called ‘nanopesticides’) is one of these exciting technologies. The overall aim of developing a nanopesticide is to either: increase the solubility of the active ingredient to have more contact with the target area or organism; prevent the active ingredient from premature degradation; reduce the pesticide application rate on crops; or minimise the potential risks to the environment. As nanoparticles can behave very differently from dissolved chemicals, nanopesticides could show very different behavior in the soil environment than conventional pesticides.

Therefore, in the present study, the impact of nanoencapsulation on the fate, effects and uptake of the synthetic pyrethroid, bifenthrin, were explored. The study focused on the effects, fate (sorption and persistence) and uptake into earthworms. In order to characterise the effects of nanoformulation on the fate and uptake of pesticide nanoformulation in the environment, a series of laboratory studies were performed using conventionally formulated bifenthrin (Capture LFR), two different nano-encapsulated formulations (Nano A and Nano B) together with the corresponding analytical grade bifenthrin. The data obtained from the study revealed that the nanoformulation treatments (Nano A and Nano B) did behave differently in the soil environment compared to the non-nanoformulation treatments (analytical grade bifenthrin and Capture LFR) (Findings from the different experimental chapters are summarised in Table 7.1 – 7.5).

Table 7.1 Summary of the effects data to *E. fetida* from *Chapter 3* (±S.D, n = 4) of different treatments and soil types

Soil	**Treatment**	**Mortality (after 28 d)**	**Cocoon (after 28 d)**	**Juvenile (after 56 d)**
1	Control	0	5.75±0.96	11.50±1.29
	Capture LFR	0.50±0.60	3.50±0.58	8.25±0.96
	Nano A	0	4.25±1.50	8.00±2.45
	Nano B	0.75±0.50	4.25±0.96	8.75±1.71
2	Control	0	4.25±1.26	4.50±2.52
	Capture LFR	0.50±0.60	2.25±0.50	2.00±2.16
	Nano A	0.50±1.00	1.75±0.50	2.25±1.71
	Nano B	0.75±0.50	2.00±0.82	3.25±1.50
3	Control	0	4.75±0.50	10.50±1.29
	Capture LFR	0.25±0.50	3.75±0.50	7.50±1.29
	Nano A	1.00±0	3.50±0.58	7.50±1.29
	Nano B	0	4.00±1.15	7.50±2.38
4	Control	0	4.75±0.96	9.75±1.50
	Capture LFR	0.25±0.50	3.75±0.96	7.50±1.29
	Nano A	1.00±0.80	3.25±0.96	7.25±1.71
	Nano B	0.75±0.50	3.25±0.50	7.25±0.96
5	Control	0	5.25±0.50	11.25±0.96
	Capture LFR	0.50±0.60	2.50±0.58	5.50±1.29
	Nano A	0.25±0.50	3.25±1.26	6.50±1.91
	Nano B	1.25±0.50	3.00±1.41	6.25±1.89

Table 7.2 Summary of the sorption coefficient, K_d (mL g^{-1}) data from *Chapters 3* and *4* (±S.D, n = 4) and *Chapters 5* and *6* (±S.D, n = 3) of different bifenthrin treatments and soil types

Soil	Bifenthrin treatment	Chapter 3	Chapter 4	Chapters 5 and 6
1	Analytical grade bifenthrin	-	3500±85	360±31
	Capture LFR	100±3	300±24	240±20
	Nano A	82±4	96±6	120±11
	Nano B	86±4	94±7	140±9
2	Analytical grade bifenthrin	-	7200±218	560±27
	Capture LFR	110±6	470±61	410±18
	Nano A	81±3	100±5	130±4
	Nano B	85±4	150±11	140±8
3	Analytical grade bifenthrin	-	2200±51	390±10
	Capture LFR	100±7	200±13	280±12
	Nano A	79±4	76±8	110±4
	Nano B	79±6	64±4	160±21
4	Analytical grade bifenthrin	-	2200±31	410±43
	Capture LFR	100±4	200±11	270±12
	Nano A	83±5	68±5	130±5
	Nano B	79±7	59±5	150±3
5	Analytical grade bifenthrin	-	1800±41	250±11
	Capture LFR	100±9	190±10	180±7
	Nano A	81±4	64±3	110±2
	Nano B	79±5	52±5	130±3

Table 7.3 Summary of the half-life, DT_{50} (d) data from *Chapters 3* and *4* (±S.D, n = 4) and *Chapters 5* and *6* (±S.D, n = 3) of different bifenthrin treatments and soil types

Soil	**Bifenthrin treatment**	**Chapter 3**	**Chapter 4**		**Chapters 5 and 6**
			Sterile	**Non-sterile**	
1	Analytical grade bifenthrin	-	320±9	140±2	30±2
	Capture LFR	21±2	290±10	130±1	31±2
	Nano A	42±1	580±0	280±0	42±3
	Nano B	37±3	560±26	290±6	50±4
2	Analytical grade bifenthrin	-	350±9	140±0	26±2
	Capture LFR	43±2	300±7	140±3	42±2
	Nano A	82±3	650±32	320±9	51±3
	Nano B	52±2	600±30	300±7	48±2
3	Analytical grade bifenthrin	-	280±7	130±2	36±3
	Capture LFR	28±1	280±0	140±1	50±3
	Nano A	43±3	460±0	260±5	63±2
	Nano B	37±1	500±0	270±6	58±3
4	Analytical grade bifenthrin	-	240±5	120±2	42±2
	Capture LFR	28±2	300±7	150±3	41±2
	Nano A	39±2	410±13	250±5	77±4
	Nano B	33±1	510±19	280±7	49±4
5	Analytical grade bifenthrin	-	260±8	120±2	39±2
	Capture LFR	29±2	290±6	130±1	41±3
	Nano A	41±2	390±11	230±4	94±2
	Nano B	40±2	460±14	240±0	54±5

Table 7.4 Summary of the uptake kinetics data from *Chapters 5* and *6* (±S.D, n = 3) of different bifenthrin treatments and soil types

Soil	**Bifenthrin treatment**	**K_{in} (mL g^{-1} h^{-1})**	**K_{out} (h^{-1})**	**BCFs**
1	Analytical grade bifenthrin	0.249±0.049	0.005±0.001	52±4
	Capture LFR	0.220±0.041	0.005±0.001	59±3
	Nano A	0.507±0.114	0.040±0.009	15±4
	Nano B	0.736±0.132	0.055±0.010	17±4
2	Analytical grade bifenthrin	0.206±0.020	0.002±0.001	84±7
	Capture LFR	0.163±0.013	0.003±0.001	64±8
	Nano A	0.340±0.068	0.029±0.006	14±4
	Nano B	0.207±0.043	0.015±0.003	17±6
3	Analytical grade bifenthrin	0.197±0.018	0.002±0.001	96±9
	Capture LFR	0.242±0.015	0.002±0.001	150±3
	Nano A	1.027±0.171	0.044±0.007	27±6
	Nano B	1.014±0.126	0.030±0.004	34±4
4	Analytical grade bifenthrin	0.162±0.026	0.002±0.001	66±5
	Capture LFR	0.149±0.025	0.003±0.001	56±8
	Nano A	0.679±0.123	0.031±0.006	23±3
	Nano B	1.293±0.172	0.036±0.005	36±4
5	Analytical grade bifenthrin	0.222±0.017	0.004±0.001	58±2
	Capture LFR	0.205±0.020	0.004±0.001	48±2
	Nano A	0.560±0.070	0.024±0.003	26±3
	Nano B	0.655±0.078	0.017±0.002	38±1

Table 7.5 Summary of the R_{50} (d) data from *Chapter 4* (±S.D, n = 4) and *Chapters 5* and *6* (±S.D, n = 3) of different bifenthrin treatments and soil types

Soil	**Bifenthrin treatment**	**Chapter 4**		**Chapters 5 and 6**
		Sterile	**Non-sterile**	
1	Nano A	68±2	81±3	11±4
	Nano B	68±3	79±3	11±2
2	Nano A	68±1	83±4	12±1
	Nano B	67±3	79±5	11±3
3	Nano A	67±3	77±2	11±4
	Nano B	69±3	76±4	11±2
4	Nano A	67±3	75±4	11±3
	Nano B	69±4	78±3	10±1
5	Nano A	64±5	71±5	11±3
	Nano B	68±6	73±4	9±2

Over the past few years, the understanding around the environmental risks of nanomaterials has rapidly developed (see reviews by Kah and Hoffman, 2014; Kookana *et al*., 2014; Jahnel *et al*., 2013; Kah *et al*., 2013; Kuempel *et al*., 2012; Peralta-Videa *et al*., 2011; Savolainen *et al*., 2010). With the increased use of engineered nanoparticles (ENPs) in the agricultural field (i.e., nanopesticide), concerns have been raised over the potential risks posed by these ENPs on the natural environment. One of the main concerns is that regulatory approaches that have been used to assess the risks of conventional chemicals may not be appropriate for ENPs. Therefore, it is highly recommended to modify the existing regulatory approaches for risk assessment of ENPs (Handy *et al*., 2102). In the following sections we discuss the implications of the findings reported in *Chapters 3 – 6* for the environmental distribution and effects of pesticide active ingredients and for the environmental risk assessment process.

In the present study, toxicity of the non-nano and nanoformulations of bifenthrin was assessed by investigating the effects in soil on the mortality and reproductive output of the earthworm, *E. fetida* (*Chapter 3*). Generally, across the soil types, formulated (Capture LFR) and nanoformulations (Nano A and Nano B) of bifenthrin had a significant effect on the cocoon production compared to the control treatments. Throughout the experiment, it was observed that the concentration of bifenthrin from the bifenthrin treatments detected in soil and soil pore water was decreased over the experimental period. For all the bifenthrin treatments, the toxicity observed was not significantly different across the treatments even though the uptake of bifenthrin from the Capture LFR was significantly greater compared to the Nano A and Nano B. The results indicate that while nanopesticides are accumulated less than the formulated pesticide, the toxicity (based on soil concentration) does not differ and the toxicity of the different bifenthrin treatments is influenced by the bifenthrin's mode of action on terrestrial organism (Miller and Salgado, 1985).

Results obtained from the fate studies (*Chapter 4*) concluded that bifenthrin was highly adsorbed to soil particles. The K_ds of the analytical grade bifenthrin ranged from 1800 – 7200 mL/g across soil types. This indicates that bifenthrin tends not to move in the soil, which will minimise runoff into water sources, although bifenthrin bound to sediment could contaminate runoff. In contrast, nanoformulations of bifenthrin showed a significant lower K_d values with K_ds for both of the nanoformulation treatments ranging from 50 – 150 mL/g. The K_ds obtained in this study were found to share the same trend with the K_ds obtained from *Chapters 3*, *5* and *6* where the nanoformulation treatments reduced the K_ds. This suggests that the nanoformulation treatments are not so highly adsorbed to the soil particles, possibly leading to be more mobility within the soil compartment and that these materials may have the ability to reach and contaminate the groundwater.

In terms of sorption, the K_ds obtained from *Chapter 4* showed higher values compared to the K_ds obtained in *Chapters 3*, *5* and *6* which could be explained by the difference approach in determining the K_ds. For example, in *Chapter 4*, the K_ds were determined following a standard batch equilibrium method (OECD 106, 2000) while the K_ds determined in the *Chapters 3*, *5* and *6* followed a centrifugation method using a sub-sample of soil. It is noted that for the OECD method, some important experimental design aspects such as type of vessels, type of shaking (i.e., orbital or horizontal) and soil solution ratios are not completely standardised (Kah and Brown, 2007). This could potentially result in different K_ds which are difficult to compare between different studies. In addition, the high soil solution ratios used in *Chapter 4* (i.e., 1:50 to 1:800) are not typical of field soil moisture conditions and the results obtained may not adequately reflect the sorption processes in field-moist or unsaturated soil (Kah and Brown, 2007).

However, phase partitioning of nanopesticides may not be appropriate to explain the fate and movement of nanopesticides within the soil compartment. Several studies have been done to determine the partition coefficients for ENPs (Forouzangohar and Kookana, 2011; Petersen *et al*., 2010). In some cases, it was recognised that such measurements were not appropriate in determining the partitioning behavior of ENPs. The partitioning concept has been reported to not be appropriate for fullerenes (C_{60}) (Forouzangohar and Kookana, 2011) which was viewed as a borderline case involving organic molecules and nanoparticles. They found that the behavior of C_{60} did not follow the behavior that was expected for a dissolved hydrophobic molecule where the interactions with soil particles were dominated by the deposition and aggregation mechanisms instead of equilibrium partitioning mechanism. Partitioning between octanol and water to determine the partition coefficient (K_{ow}) was also reported to be not appropriate for ENPs. Hristovski *et al*. (2011) reported that ENPs did not dissolve and undergo partitioning between both of the octanol and water phases. However, another phase at the interface between octanol and water was observed.

This previous work suggests that partitioning behavior of ENPs is not relevant for determining the distribution of ENPs such as the K_ds in soil compartment based on the current regulatory protocol for testing chemicals, thus a new design or guideline of testing chemicals involving ENPs is needed. For example, approach such as the Derjaguin-Landau-Verwey-Overbeak (DLVO) theory describing the interactions between colloidal particles (i.e., nanoparticles) could be adopted for the measurement of fate of nanopesticides in the terrestrial environment. This approach combines both of the Van der Waals attraction and electrostatic repulsion forces in order to explain that the stabilisation of nanoparticles could be achieved in disperse systems. With further research, this approach could be adopted for nanopesticides in order to determine the partitioning behavior of nanopesticide, thus allowing such explanations on the fate and distribution of nanopesticides in the terrestrial environment.

In the current studies, persistence was determined in both sterile and non-sterile soils. The half-life (DT_{50}) for each bifenthrin treatment in each soil was determined. Data obtained showed that the nanoformulation treatments enhanced the persistence in both sterile and non-sterile soils compared to the non-nanoformulation treatments. In contrary to sorption behavior, the nanoformulation treatments were found to enhance the persistence and this trend was observed to be consistent across the experimental chapters (*Chapter 3*, *5* and *6*). It was also observed that the persistence for analytical grade bifenthrin and Capture LFR treatments showed the same trend. The difference in the DT_{50} values for both non-nano and nanoformulation treatments could be explained by the role of polymer capsules 'protecting' the active ingredient bifenthrin from being attacked by the soil microbes, thus the rate of degradation was decreased.

Although the DT_{50} values shared the same trend across the experimental chapters, it was observed that the DT_{50} values obtained in the *Chapters 3*, *5* and *6* were lower compared to the DT_{50} values in the *Chapter 4*. It is noted that in *Chapters 3*, *5* and *6*, the presence of earthworm may have play an important role in affecting the degradation rate of all bifenthrin treatments. Earthworm stimulates and increases microbial activity through creating favorable conditions for bacteria and improves soil aeration leading to a more contact of bacteria with chemical compounds in soil (Asgharnia *et al*., 2014). Thus, the degradation of chemical compounds could be improved with the presence of earthworms. For example, Monard *et al*. (2008) found that the degradation of atrazine was improved with the presence of earthworm, *L. terrestris*. In another study, Butenschoen *et al*. (2009) found that an endogeic earthworm, *Octolasion tyrtaeum* improved the microbial activity and mineralisation of phenolic compounds. This could be the explanation with our observations on the DT_{50} values which showed different values across the experimental *Chapters 3*, *4*, *5* and *6*.

In this study, the R_{50} values were also estimated using a model simulation (see section 4.3.4.3). The results for both of the nanoformulation treatments (Nano A and Nano B) were consistent across soil types. When the R_{50} values in *Chapter 4* (non-sterile treatments) were compared with the experimental *Chapters 5* and *6*, the R_{50} values in *Chapters 5* and *6* were lower compared to the R_{50} values in *Chapter 4* (non-sterile treatments). Just like the degradation, the release of bifenthrin from the nanocapsules could also be related to the presence of earthworms in the test soils. As the earthworms improve the ventilation and motivate the microbial action, thus the degradation may be increased (Asgharnia *et al*., 2014). The soil microbes may have increase the degradation rate of the surfactants (i.e., polymers in the nanocapsules) resulting in the less time required for the bifenthrin to be released from the nanocapsules. Previous studies involving the slow release of pesticide active ingredient strongly suggested that the combination of loaded active ingredient with the carriers (i.e., organic or inorganic components), release medium (i.e., soil or water media) and physico-chemical properties of the active ingredient (i.e., solubility) affected the release mechanism (Qing *et al*., 2013; Chen *et al*., 2011; Celis *et al*., 2005; Udy, 1977). Therefore, the need of an improved characterisation of the distribution and the release of the active ingredients from the carrier materials under a wide range of environmental conditions (i.e., soil pH, texture, organic carbon content, moisture and temperature) is necessary involving polymer-based nanoformulation. This step could improve the understanding on the degradation when dealing with nanoformulation.

The uptake and depuration of the nano and non-nanoformulation treatments were also investigated (*Chapter 5* and *6*). Data obtained in both of the chapters showed the rates of uptake and elimination of nanoformulation treatments were significantly higher compared to the non-nanoformulation treatments. In addition, at some point, the uptake by *E. fetida* showed a steady state which was not observed for the exposure of non-nanoformulation treatments. This pattern was similar in all soil types in both *Chapters 5* and *6*. Levels of bifenthrin in the *E. fetida* exposed to the nanoformulation treatments seemed to reach a steady state more quickly than the non-nanoformulation treatments. Determined BCFs showed an increase in the order of Nano A < Nano B < Capture LFR < analytical grade bifenthrin in all soil types. This suggests that *E. fetida* accumulated more bifenthrin from non-nano compared to the nanoformulation treatments. A more detailed study was done in order to determine the distribution of bifenthrin from different bifenthrin treatments in two species of earthworm, *E. fetida* and *L. terrestris* in sandy loam soil (Soil 5; *Chapter 5*). Unfortunately, it was not practically possible to separate out the internal organs of *E. fetida* from the gut so it was only possible to distinguish between bifenthrin in the remaining tissues and in the gut combined with the chloragogue. It was found that bifenthrin was internalised in both species when exposed to non-nanoformulation treatments. In contrast, bifenthrin from nanoformulation treatments was found to accumulate more in the earthworm gut. This suggests that the uptake of bifenthrin from the nanoformulation treatments by earthworm is not only dependent on passive diffusion from soil pore water but also possible dietary uptake (i.e., ingestion of soil particles).

Based on our findings, we believe that the bifenthrin from the nanoformulation treatments was internalised as well as being accumulated in the earthworm guts. This could be related to the uptake of ‘free’ bifenthrin which release from the nanocapsules over time by the earthworm. This observation could have important implications for in terms of toxicity. Effects on organisms feed on the exposed earthworm with nanopesticides (e.g., birds) could also be different as the active ingredient is ‘protected’ by the nanocapsules, this means that the nanopesticides could also prolong the exposure, thus increasing the risks to the organisms over time. In order to understand the potential risks of nanopesticides in the terrestrial environment, more studies should be carried out (i.e., using different active ingredients and nanocarriers) as the physico-chemical properties of these materials could also influence the fate and distribution of nanopesticides in the terrestrial environment. Development of models such as exposure models that could differentiate the 'free' active ingredient and bound-nanopesticides as well as the determination of the size distribution is therefore needed. These models (e.g., incorporated with toxicological and/ or fate processes including sorption and degradation over time) can then apply in order to understand the distribution and exposure of nanopesticide in the terrestrial environment.

7.2 Conclusion

As a conclusion, a series of laboratory studies into the effects, fate and uptake of nano-encapsulated formulations together with the analytical grade and formulated bifenthrin (Capture LFR) was successfully performed in different soil types. Relationships between soil properties such as pH, total organic carbon, cationic exchange capacity and textures, and experimental parameters were also investigated. Based on the presented work, it is noted that sorption process plays an important role in affecting the uptake of nanopesticides in earthworm. The fate data in each experimental chapter showed the same trend where nanoformulation treatments reduced the sorption and enhanced the persistence compared to the non-nanoformulation treatments. The work performed on the bioaccumulation of nanopesticides in earthworm, *E. fetida* showed the BCFs increased in the order of Nano A < Nano B < Capture LFR < analytical grade bifenthrin in all soil types which also showed the same pattern with the BSAFs obtained in *Chapter 3* where the BSAFs increased in the order of Nano B < Nano A < Capture LFR. This finding could be related to the uptake of bifenthrin into the earthworm species through ingestion of soil particles as well as uptake through passive diffusion from soil pore water.

A more detailed study was performed to investigate the distribution of bifenthrin from different bifenthrin treatments in two earthworm species: *E. fetida* and *L. terrestris* (*Chapter 5*). The distribution of bifenthrin in the earthworm body showed significant difference between the non-nano and nanoformulation treatments. This could be explained by difference route of uptake by the earthworms where the uptake of bifenthrin from the nanoformulation treatments was mainly from the dietary uptake (i.e., ingestion of soil particles) while the uptake of bifenthrin from the non-nanoformulation treatments was through the passive diffusion from soil pore water.

Based on this study, it is anticipated that the accumulation and internalisation of nanopesticides by the earthworm could show some implications in the food chain when an animal (i.e., birds and mammals) feed on those exposed earthworms. Thus, there is a possibility of birds and mammals are affected by the use of nanopesticides. Development of models to estimate the fate and uptake of nanopesticides could therefore be used to explore the effects of nanopesticide on the terrestrial organisms. For example, the toxicokinetics/toxicodynamics (TKTD) modelling approach could be adopted with a combination of relevant fate (i.e., sorption and degradation), physico-chemical properties (e.g., aggregation and surface charge of nanoparticles) and uptake data for determining the effects of nanopesticides.

It is believed that this approach could therefore be a first step to improve the current environmental risk assessment (ERA) approaches to estimate the effects of nanopesticides in the terrestrial organisms such as earthworm. As the fate, uptake and effects of nanopesticides could be related to the physico-chemical properties of the active ingredients and the nanocarriers, we therefore recommend that this model could also be applied to different pesticide active ingredients and nanocarriers.

7.3 Limitations specific to this study

Throughout this study there are a number of limitations related to the experimental procedures which may have an impact on the findings of this study and which should be studied further. Below, these limitations are discussed and recommendations provided on how these could be addressed in the future:

1. In these studies, only five different soil types were selected. Studies were performed at soil pH values ranging from 4.71 – 7.66, total organic carbon (1.22 – 5.16 %) and clay (0 – 1.5 %). Following the OECD recommendation on choosing different soil types, it would have been better if the test soil represented a wider range of e.g. soil pH values in order to evaluate the test compounds mobility and persistence (OECD 312, 2004). It is also recommended to choose soils that cover both temperate and tropical regions

if necessary to differentiate the fate and behavior of the test compounds when applying the substance in different regions.

2. In *Chapter 4*, the persistence studies were carried out involving the sterilisation of soils using an autoclave. It is known that using autoclave can influence the texture of the soils due to the soil expansion during the heating process. Thus, the alteration of the soil texture through heating and cooling process might affect the results of the experiments conducted. However, due to the limitation of instruments available, the autoclave approach was selected for the sterilisation process. Alternatively, soils could have been irradiated through the use of radioactive component such as Cobalt-60 (^{60}Co). Radiation using ^{60}Co is well-known to sterilise soils in order to remove soil microbes including bacteria, fungi and viruses while maintaining the texture of the sterilised soils.
3. In the presented work, the experiments were conducted using the same soils, to allow comparability of results, which were stored for < 2 years prior to the start of the experiments at laboratory condition (20±2 ºC), except for the toxicity (*Chapter 3*) and persistence studies (*Chapter 4*) where fresh soils were used. Due to the long time of soil storage, it is possible that soil properties may have changed over time. For example, a reduction in the content of organic matter in the soils might be expected. Ideally it would have been better to test fresh soils that have the same characteristics – something that is probably impossible given the scale of the studies performed.

7.4 Recommendations

The work performed in this study has produced novel information on the effects, fate and uptake of two different nano-encapsulated formulations together with the analytical grade and formulated bifenthrin (Capture LFR). Below we provide recommendations on future work that builds on the findings in the thesis:

1. Studies were performed using only two different bifenthrin nanoformulations. In order to have a better understanding on the impact of nanoformulations in the terrestrial environment, further experiments with other combinations of active ingredients with differing properties (i.e.,

hydrophilic) and nanocarriers (i.e., biodegradable polymer or clay) should be carried out to evaluate whether the observations seen here are applicable to nano-encapsulated pesticides more generally.

2. Better characterisation of nanoformulations of pesticides is also needed as this additional information could help to explain the fate and behavior of active ingredients in soils treated with nanoformulations. As nanoparticles tend to aggregate over time, characterisation at different stages in their life cycle throughout the fate studies may be very important. Approaches such as scattering methods (e.g., DLS) and particle tracking analysis (i.e., NTA) could be used to improve the understanding on the behavior of the nanoformulations. Another important approach that could also determine the fate and behavior of nanoformulations is the use filtration. Nanoparticles size effects are also should be considered in order to explain the effect of size on the fate and behavior of nanoformulations.
3. Toxicity studies were performed at one concentration which was 100x higher than normal bifenthrin field application rate (*Chapter 3*). It is recommended in the future to have a wider range of concentration in order to allow the estimation of toxicity parameters such as effect concentration for 50% effect (EC_{50}), median lethal concentration (LC_{50}), lowest observed effect concentration (LOEC) and no observed effect concentration (NOEC). The use of estimated Predicted Environmental Concentration (PEC) which shows the estimated concentration of an active chemical compound (i.e., pesticide active ingredients) in the environmental compartments such as soil, surface water and ground water, can then be compared with the values mentioned above (i.e., EC_{50} and NOEC) to derive a risk characterisation ratio. The ratio can then be used to compare with the regulatory trigger value in order to allow such assumptions on the fate and effects of nanopesticides in the terrestrial or aquatic environments.
4. Sorption studies showed nanoformulations were not as highly adsorbed to the soil particles compared to the non-nanoformulations. Therefore, the partitioning coefficient (i.e., K_d and K_{ow}) are not appropriate to determine to distribution of nanopesticides in the environment. Nanoparticles are thermodynamically unstable making them easily aggregated over time, thus K_d is not really appropriate to describe the partitioning between soil and

water. The use of DLVO theory describing the interactions between colloidal particles (i.e., nanoparticles) and their aggregation behavior could be adopted for the measurement of fate and exposure of nanopesticides in the terrestrial environment. As described in *Chapter 4*, we demonstrated a model to estimate the release of the active ingredient bifenthrin from the nanocapsules over time. With further research, a combination of these approach (i.e., DLVO and release of active ingredient) with the fate of nanoparticles (e.g., aggregation) could then be used to estimate the partitioning behavior and explaining the fate and distribution of nanopesticides in the terrestrial environment.

5. The uptake and depuration studies were performed using only two different earthworm species, *E. fetida* and *L. terrestris*. It is recommended to investigate the uptake and depuration on other organisms such as a typical small nematode (2.5 mm in size) species to observe any possible uptake of nanoformulations. Nematodes are present in the terrestrial environment and reported to accumulate organic chemicals such as pesticides (Winter *et al*., 2002). It would be interesting to investigate the impact of nanopesticides on this species. Following this, in the future it is best to study the uptake on aquatic organisms. Bifenthrin is considered toxic to aquatic organisms. Based on our study, it is likely that nanopesticides could show the same effects on the earthworm as observed for non-nanopesticides due to the ability of the nanopesticides to prolong the exposure duration of the earthworm. Thus, questions have been raised on how the fate and uptake by the aquatic organisms. Since nanoformulations are likely to be more mobile, there is possibility that they will reach the groundwater and eventually this will lead to reach the aquatic environment. Therefore, studies including aquatic organisms should also be considered.
6. Future studies on the implications of nanopesticides in the food chain (secondary poisoning) should also be considered. As bifenthrin from all bifenthrin treatments is likely to internalise in the earthworm body (*Chapter 5*), therefore there is a possibility of active ingredient bifenthrin to be transferred in different trophic levels (i.e., earthworms to birds). As nano-encapsulated bifenthrin can prolong the persistence of active ingredient, this will increase the exposure duration to organisms when exposed to

nanoformulations compared to the conventional pesticides. One way to investigate this is by adopting the TKTD approach as described earlier which includes the relevant fate data such as sorption. It is noted that the applied chemicals or pesticides in the terrestrial environment could show some uptake into plants. Therefore, it is also recommended that to include the uptake parameter into plants in the approach for estimating the distribution in the soil compartment. In addition, the accumulation of nanopesticides by plants may represent an important exposure of nanopesticides in the food chain thus present potential risk to humans which feed on them. An acceptable daily intake (ADI) value can be used to calculate the amount of chemical compound (e.g., nanopesticides) that can be consumed by a human without causing any risk to human health.

7. BCFs in uptake studies were derived from the uptake and elimination rate constants. This was done following to the previous studies that the bioaccumulation of chemicals in soil compartment was mainly via passive diffusion from soil pore water. However, distribution studies on *E. fetida* and *L. terrestris* revealed that there is a possibility of uptake by ingestion of food (dietary uptake). In future, it is best to include the soil based accumulation factor (BSAF) values to describe the uptake of nanoformulations by organisms. BSAF is a parameter describing bioaccumulation of sediment-associated organic compounds or metals into tissues of ecological receptors. This could help to improve the understanding from soil chemistry perspective of the interactions between the nanoformulations and soil compartment, and then the uptake into organisms.

Appendix

Appendix 3.1 Methods development for HPLC

Method: The analytical methods were performed based on a method developed at The Food and Environment Research Agency (FERA), York, United Kingdom for soil, soil pore water and earthworm tissues. The solvent used in this analytical method was acetonitrile.

For standard calibration, five different concentrations ranged from 2 – 10 µg/mL of each bifenthrin treatment were prepared in acetonitrile. A 2 mL of the aliquots of supernatant was taken for further analysis. For soil extraction method, 5±0.5 g of each soil type was spiked with 2, 4, 6, 8 or 10 µg/g of each bifenthrin treatment. After 1 d, the mixtures were shaken at room temperature (20±2 °C) on an orbital shaker for 2 h with a ratio of 1:3 (soil/acetonitrile). The samples were then allowed to settle and 2 mL of the aliquots of supernatant was taken for further analysis.

For earthworm extraction method, one adult earthworm, *E. fetida* (~ 1±0.5 g) and *L. terrestris* (~ 4±0.5 g) were injected with 1, 2, 3, 4, 5 or 6 µg/g of each bifenthrin treatment. After 4 h, *E. fetida* and *L. terrestris* were extracted using 5 mL and 20 mL of acetonitrile, respectively and then the mixture was homogenised for 5 minutes using a LabGen Series 7 homogeniser in a beaker. The suspension was transferred from the beaker to a glass vial and the beaker was then rinsed with an additional 5 mL of acetonitrile was combined with the suspension to give a total extract volume of 10 mL (*E. fetida*) and 25 mL (*L. terrestris*). The extracts were centrifuged for 20 min at 3000 rpm. The samples were then filtered and a 2 mL aliquot of the supernatant was taken for further analysis.

Table 3.1A Recoveries±RSD (n = 3), LODs and LOQs for the analytical methods

Concentration, µg/mL	**Bifenthrin treatment**							
	Analytical grade bifenthrin		**Capture LFR**		**Nano A**		**Nano B**	
	Recovery±RSD, %	**LOD (LOQ) (ng/mL)**	**Recovery±RSD, %**	**LOD (LOQ) (ng/mL)**	**Recovery±RSD, %**	**LOD (LOQ) (ng/mL)**	**Recovery±RSD, %**	**LOD (LOQ) (ng/mL)**
2	106.8 (2.1)	1.2 (3.7)	95.3 (1.2)	1.5 (4.7)	95.5 (0.5)	1.9 (5.9)	101.2 (5.3)	2.1 (6.5)
6	98.6 (0.7)		94.9 (0.6)		89.7 (0.2)		99.8 (4.3)	
10	89.7 (1.3)		98.8 (0.9)		93.2 (2.1)		96.4 (1.8)	

Table 3.1B Recoveries±RSD (n = 3), LODs and LOQs for each bifenthrin treatment in each soil

Soil	**Concentration, µg/g)**	**Bifenthrin treatment**							
		Analytical grade bifenthrin		**Capture LFR**		**Nano A**		**Nano B**	
		Recovery±RSD, %	**LOD (LOQ) (ng/g)**	**Recovery±RSD, %**	**LOD (LOQ) (ng/g)**	**Recovery±RSD, %**	**LOD (LOQ) (ng/g)**	**Recovery±RSD, %**	**LOD (LOQ) (ng/g)**
1	2	94.1 (2.1)	12.3 (38.1)	98.9 (3.2)	14.1 (43.7)	97.8 (0.7)	15.2 (47.1)	91.9 (0.3)	13.9 (43.1)
	6	101.1 (4.2)		96.5 (2.1)		93.0 (0.8)		102.2 (1.4)	
	10	98.7 (0.4)		102.9 (4.8)		98.2 (1.2)		93.9 (2.2)	
2	2	108.6 (4.3)	13.2 (40.9)	95.6 (1.2)	12.7 (39.4)	104.1 (5.2)	17.5 (54.3)	106.1 (4.9)	16.2 (50.2)
	6	102.3 (2.8)		97.6 (1.7)		98.7 (0.7)		102.2 (4.2)	
	10	98.5 (0.6)		89.4 (0.5)		103.6 (2.2)		97.6 (3.3)	
3	2	89.9 (2.0)	11.8 (36.6)	91.1 (3.8)	16.8 (52.1)	94.5 (1.9)	14.8 (45.9)	96.2 (2.7)	13.2 (40.9)
	6	92.4 (3.1)		103.2 (2.2)		97.3 (2.8)		92.2 (1.8)	
	10	89.4 (0.4)		99.3 (1.5)		99.0 (3.1)		94.6 (1.9)	
4	2	86.5 (4.3)	14.2 (44.0)	105.2 (3.2)	11.2 (34.7)	88.8 (2.6)	21.3 (66.0)	97.3 (0.7)	12.4 (38.4)
	6	90.1 (3.7)		102.7 (2.6)		92.3 (2.6)		96.4 (1.1)	
	10	89.5 (5.2)		97.8 (2.1)		95.4 (93.0)		99.2 (2.0)	
5	2	94.3 (1.2)	15.9 (49.3)	89.4 (3.2)	12.1 (37.5)	93.6 (2.1)	11.6 (36.0)	102.7 (5.4)	10.2 (31.6)
	6	98.2 (0.8)		87.5 (2.9)		92.1 (2.9)		98.4 (3.9)	
	10	102.4 (6.2)		91.3 (2.1)		96.7 (3.2)		96.5 (2.5)	

Table 3.1C Recoveries±RSD (n = 3), LODs and LOQs for each bifenthrin treatments in each earthworm species

Earthworm	**Concentration, µg/g)**	**Bifenthrin treatment**							
		Analytical grade bifenthrin		**Capture LFR**		**Nano A**		**Nano B**	
		Recovery±RSD, %	**LOD (LOQ) (ng/g)**	**Recovery±RSD, %**	**LOD (LOQ) (ng/g)**	**Recovery±RSD, %**	**LOD (LOQ) (ng/g)**	**Recovery±RSD, %**	**LOD (LOQ) (ng/g)**
E. fetida	1	102.1 (3.2)	152.1 (471.5)	84.3 (3.3)	194.8 (603.9)	92.3 (3.0)	58.2 (180.4)	88.9 (0.7)	76.6 (237.5)
	3	98.5 (2.9)		98.7 (2.8)		97.5 (2.9)		90.2 (0.9)	
	6	97.4 (2.2)		89.6 (1.5)		98.6 (2.1)		86.9 (1.7)	
L. terrestris	1	92.1 (0.6)	231.5 (717.7)	102.4 (1.9)	195.5 (606.1)	106.5 (5.5)	86.3 (267.5)	89.9 (1.6)	93.4 (289.5)
	3	102.4 (0.2)		97.4 (1.6)		99.2 (3.2)		92.6 (2.2)	
	6	98.2 (3.2)		99.3 (2.1)		102.2 (2.7)		96.3 (1.8)	

Appendix 3.2 Concentration of bifenthrin in soil

Table 3.2A Mean concentration of bifenthrin in soil, µg/g (±S.D, n = 4)

Soil/bifenthrin treatment	**Day**				
	0	**7**	**14**	**21**	**28**
Soil 1					
Capture LFR	101.9±5.69	96.10±5.57	75.86±2.93	73.24±1.76	37.59±4.68
Nano A	102.7±3.13	95.18±3.54	79.32±1.78	75.76±3.91	64.70±1.82
Nano B	101.2±0.28	94.06±1.72	85.07±2.91	70.36±3.33	60.53±2.30
Soil 2					
Capture LFR	101.9±4.87	90.97±3.04	75.37±3.48	71.19±3.00	65.82±1.51
Nano A	103.3±4.52	95.26±4.93	91.60±5.26	85.34±2.17	81.02±2.01
Nano B	105.2±4.71	99.45±1.15	92.44±3.09	79.76±1.85	73.57±2.63
Soil 3					
Capture LFR	100.1±5.26	97.93±5.84	74.95±9.24	67.35±4.57	50.29±1.97
Nano A	100.7±4.49	92.79±3.69	83.41±2.61	72.20±1.89	64.42±3.15
Nano B	101.6±0.58	95.67±1.76	88.47±2.41	77.66±3.99	58.36±2.04
Soil 4					
Capture LFR	100.9±4.97	91.21±2.70	74.44±3.82	62.40±2.83	51.86±1.35
Nano A	102.8±4.11	96.08±3.16	86.45±1.71	76.08±2.11	60.92±5.75
Nano B	101.2±0.79	93.98±1.81	83.19±1.70	70.70±2.56	55.76±3.65
Soil 5					
Capture LFR	101.9±4.95	96.56±4.57	78.28±3.18	63.46±2.08	54.74±4.86
Nano A	100.3±2.56	93.77±4.82	79.77±2.62	70.72±1.64	64.17±2.93
Nano B	101.5±0.81	95.73±2.73	85.64±3.33	75.42±2.22	62.60±2.42

Appendix 3.3 Concentration of bifenthrin in soil pore water

Table 3.3A Mean concentration of bifenthrin in soil pore water, μg/mL (±S.D, n = 4)

Soil/bifenthrin treatment	**Day**				
	0	**7**	**14**	**21**	**28**
Soil 1					
Capture LFR	0.93±0.08	0.81±0.08	0.74±0.08	0.57±0.05	0.46±0.05
Nano A	0.92±0.08	0.77±0.04	0.66±0.05	0.55±0.08	0.49±0.03
Nano B	1.08±0.08	0.93±0.03	0.69±0.06	0.56±0.06	0.45±0.06
Soil 2					
Capture LFR	0.79±0.07	0.69±0.08	0.57±0.04	0.42±0.07	0.28±0.03
Nano A	0.95±0.07	0.82±0.04	0.72±0.05	0.65±0.04	0.59±0.03
Nano B	1.11±0.09	0.97±0.08	0.87±0.06	0.79±0.06	0.72±0.04
Soil 3					
Capture LFR	1.13±0.08	0.99±0.05	0.77±0.05	0.65±0.04	0.53±0.06
Nano A	0.97±0.13	0.89±0.09	0.73±0.08	0.54±0.04	0.49±0.06
Nano B	1.13±0.09	0.97±0.08	0.82±0.04	0.69±0.03	0.62±0.03
Soil 4					
Capture LFR	1.03±0.08	0.89±0.08	0.74±0.06	0.57±0.04	0.39±0.04
Nano A	1.01±0.06	0.88±0.03	0.71±0.02	0.63±0.02	0.55±0.04
Nano B	1.12±0.12	0.93±0.06	0.79±0.08	0.65±0.05	0.57±0.04
Soil 5					
Capture LFR	0.92±0.08	0.80±0.08	0.69±0.04	0.59±0.03	0.44±0.04
Nano A	0.94±0.09	0.80±0.05	0.73±0.04	0.58±0.04	0.54±0.05
Nano B	1.01±0.06	0.90±0.06	0.74±0.03	0.64±0.05	0.59±0.06

Appendix 3.4 Relationships between soil properties and K_ds

Table 3.4A Summary of linear regression between soil properties and K_ds

Correlation parameter	**Bifenthrin treatment**	**p**	**R^2**
Soil pH	Capture LFR	0.317	0.323
	Nano A	0.438	0.210
	Nano B	0.720	0.049
OC, %	Capture LFR	0.039	0.839
	Nano A	0.937	0.002
	Nano B	0.154	0.546
CEC, cmol$^+$/kg	Capture LFR	0.983	<0.001
	Nano A	0.437	0.211
	Nano B	0.725	0.045
Clay, %	Capture LFR	0.597	0.104
	Nano A	0.590	0.107
	Nano B	0.691	0.060
Silt, %	Capture LFR	0.600	0.102
	Nano A	0.299	0.343
	Nano B	0.450	0.200
Sand, %	Capture LFR	0.679	0.065
	Nano A	0.247	0.407
	Nano B	0.491	0.170

Appendix 3.5 Relationships between soil properties and DT_{50}s

Table 3.5A Summary of linear regression between soil properties and DT_{50}s

Correlation parameter	**Bifenthrin treatment**	**p**	**R^2**
Soil pH	Capture LFR	0.031	0.830
	Nano A	0.034	0.823
	Nano B	0.012	0.910
OC, %	Capture LFR	0.167	0.523
	Nano A	0.018	0.880
	Nano B	0.078	0.698
CEC, $cmol^+/kg$	Capture LFR	0.169	0.521
	Nano A	0.339	0.300
	Nano B	0.165	0.527
Clay, %	Capture LFR	0.156	0.543
	Nano A	0.190	0.487
	Nano B	0.391	0.250
Silt, %	Capture LFR	0.310	0.331
	Nano A	0.254	0.398
	Nano B	0.447	0.203
Sand, %	Capture LFR	0.381	0.259
	Nano A	0.332	0.307
	Nano B	0.547	0.133

Appendix 3.6 Relationships between K_ds and DT_{50}s

Table 3.6A Summary of linear regression between K_ds and DT_{50}s

Bifenthrin treatment	**p**	**R^2**
Capture LFR	0.172	0.172
Nano A	0.835	0.016
Nano B	0.446	0.001

Appendix 3.7 Relationships between soil properties and BSAFs

Table 3.7A Summary of linear regression between soil properties and BSAFs

Correlation parameter	**Bifenthrin treatment**	**p**	**R^2**
Soil pH	Capture LFR	0.017	0.887
	Nano A	0.164	0.529
	Nano B	0.036	0.813
OC, %	Capture LFR	0.118	0.613
	Nano A	0.235	0.422
	Nano B	0.183	0.497
CEC, cmol$^+$/kg	Capture LFR	0.146	0.559
	Nano A	0.320	0.320
	Nano B	0.095	0.659
Clay, %	Capture LFR	0.198	0.475
	Nano A	0.045	0.787
	Nano B	0.277	0.369
Silt, %	Capture LFR	0.309	0.332
	Nano A	0.141	0.569
	Nano B	0.345	0.294
Sand, %	Capture LFR	0.388	0.253
	Nano A	0.176	0.510
	Nano B	0.423	0.222

Appendix 3.8 Relationships between soil properties and earthworm mortality

Table 3.8A Summary of linear regression between soil properties and earthworm mortality

Correlation parameter	Bifenthrin treatment	p	R^2
Soil pH	Capture LFR	0.512	0.155
	Nano A	0.961	0.001
	Nano B	0.982	0.0002
OC, %	Capture LFR	0.487	0.172
	Nano A	0.790	0.027
	Nano B	0.919	0.004
CEC, cmol$^+$/kg	Capture LFR	0.481	0.177
	Nano A	0.876	0.009
	Nano B	0.600	0.102
Clay, %	Capture LFR	0.970	0.001
	Nano A	0.628	0.088
	Nano B	0.865	0.011
Silt, %	Capture LFR	0.778	0.031
	Nano A	0.965	0.001
	Nano B	0.637	0.084
Sand, %	Capture LFR	0.841	0.016
	Nano A	0.934	0.003
	Nano B	0.089	0.089

Appendix 3.9 Relationships between soil properties and juvenile production

Table 3.9A Summary of linear regression between soil properties and juvenile production

Correlation parameter	**Bifenthrin treatment**	**p**	**R^2**
Soil pH	Control	0.081	0.691
	Capture LFR	0.022	0.863
	Nano A	0.028	0.842
	Nano B	0.032	0.829
OC, %	Control	0.045	0.785
	Capture LFR	0.169	0.520
	Nano A	0.084	0.683
	Nano B	0.199	0.474
CEC, $cmol^+/kg$	Control	0.421	0.224
	Capture LFR	0.095	0.660
	Nano A	0.195	0.480
	Nano B	0.110	0.629
Clay, %	Control	0.065	0.729
	Capture LFR	0.263	0.387
	Nano A	0.163	0.530
	Nano B	0.197	0.476
Silt, %	Control	0.147	0.557
	Capture LFR	0.356	0.283
	Nano A	0.244	0.411
	Nano B	0.325	0.315
Sand, %	Control	0.201	0.471
	Capture LFR	0.437	0.210
	Nano A	0.316	0.324
	Nano B	0.397	0.245

Appendix 4.1 Descriptions of the release model (example of k_1 estimation for Nano A treatment in non-sterile in Soil 1)

Main

- Bound_AI Unconditional
  dBound_AI/dt = – FB_R
  Initial Values = 10.01

- Released_AI Unconditional
  dReleased_AI/dt = +FB_R – FR_D
  Initial Values = 0.0

- Degraded_AI Unconditional
  dDegraded_AI/dt = +FR_D
  Initial Values = 0.0

- ↗ FB_R Unconditional
  Flow from Bound_AI to Released_AI
  FB_R = k_1 * Bound_AI

- ↗ FR_D Unconditional
  Flow from Released_AI to Degraded_AI
  FR_D = k_2 * Released_AI

Figure 4.1A Definition of each compartment and flow (unconditional model) to estimate release rate of bifenthrin from the nanoformulation treatments

Appendix 4.2 Isotherm parameters determined for sorption studies

Table 4.2A Linear, Freundlich and Langmuir isotherms (±S.D, n = 4)

Bifenthrin treatment	**Soil**	**Linear isotherm**			**Freundlich isotherm**				**Langmuir isotherm**		
		K_d, mL/g	**R^2**	**p**	**K_f, μg/g**	***n***	**R^2**	**p**	**K_L**	**R^2**	**p**
Analytical grade bifenthrin	1	3500±85	0.997	<0.0001	3000±231	0.94±0.03	0.996	<0.0001	-0.010±0.0002	0.921	<0.0001
	2	7200±218	0.993	0.0003	6600±670	0.96±0.04	0.987	<0.0001	-0.010±0.0001	0.892	<0.0001
	3	2200±51	0.982	0.0019	1400±301	0.88±0.06	0.969	0.0012	-0.015±0.0002	0.911	0.0001
	4	2200±31	0.986	0.0014	2000±230	0.97±0.04	0.972	0.0003	-0.006±0.0004	0.892	0.0002
	5	1800±41	0.983	0.0011	1600±348	0.95±0.07	0.977	0.0021	-0.015±0.0003	0.898	0.0004
Capture LFR	1	300±24	0.982	0.0015	430±52	1.12±0.06	0.967	0.0002	0.021±0.0002	0.912	0.0005
	2	470±61	0.968	0.0111	930±91	1.24±0.08	0.911	0.0004	0.042±0.0003	0.932	0.0005
	3	200±13	0.973	0.0041	270±12	1.10±0.02	0.944	<0.0001	0.012±0.0001	0.935	0.0002
	4	200±11	0.986	0.0016	220±16	1.02±0.04	0.966	0.0045	0.001±0.0005	0.961	<0.0001
	5	190±10	0.986	0.0014	230±17	1.05±0.04	0.977	0.0005	-0.001±0.0002	0.972	0.0001
Nano A	1	96±6	0.976	0.0046	73±7	0.95±0.04	0.953	0.0222	-0.001±0.0003	0.965	<0.0001
	2	100±5	0.971	0.0010	120±17	1.04±0.05	0.954	<0.0001	0.001±0.0002	0.942	0.0001
	3	76±8	0.989	<0.0001	47±7	0.89±0.04	0.988	0.0014	-0.006±0.0001	0.928	<0.0001
	4	68±5	0.988	0.0001	48±7	0.92±0.05	0.983	0.0015	-0.005±0.0005	0.891	<0.0001
	5	64±3	0.971	0.0003	48±3	0.94±0.02	0.965	0.0040	-0.003±0.0004	0.959	0.0007
Nano B	1	94±7	0.994	<0.0001	89±7	0.99±0.03	0.992	0.0016	-0.001±0.0003	0.974	<0.0001
	2	150±11	0.993	<0.0001	150±26	1.00±0.05	0.992	0.0018	0.001±0.0001	0.934	<0.0001
	3	64±4	0.983	0.0002	79±12	1.04±0.04	0.975	0.0017	0.001±0.0003	0.925	<0.0001
	4	59±5	0.994	<0.0001	57±7	0.99±0.05	0.993	0.0003	-0.002±0.0002	0.978	<0.0001
	5	52±5	0.992	<0.0001	39±8	0.94±0.06	0.991	0.0001	-0.002±0.0002	0.982	<0.0001

Appendix 4.3 First-order kinetic models for the dissipation of different bifenthrin treatments in different soils

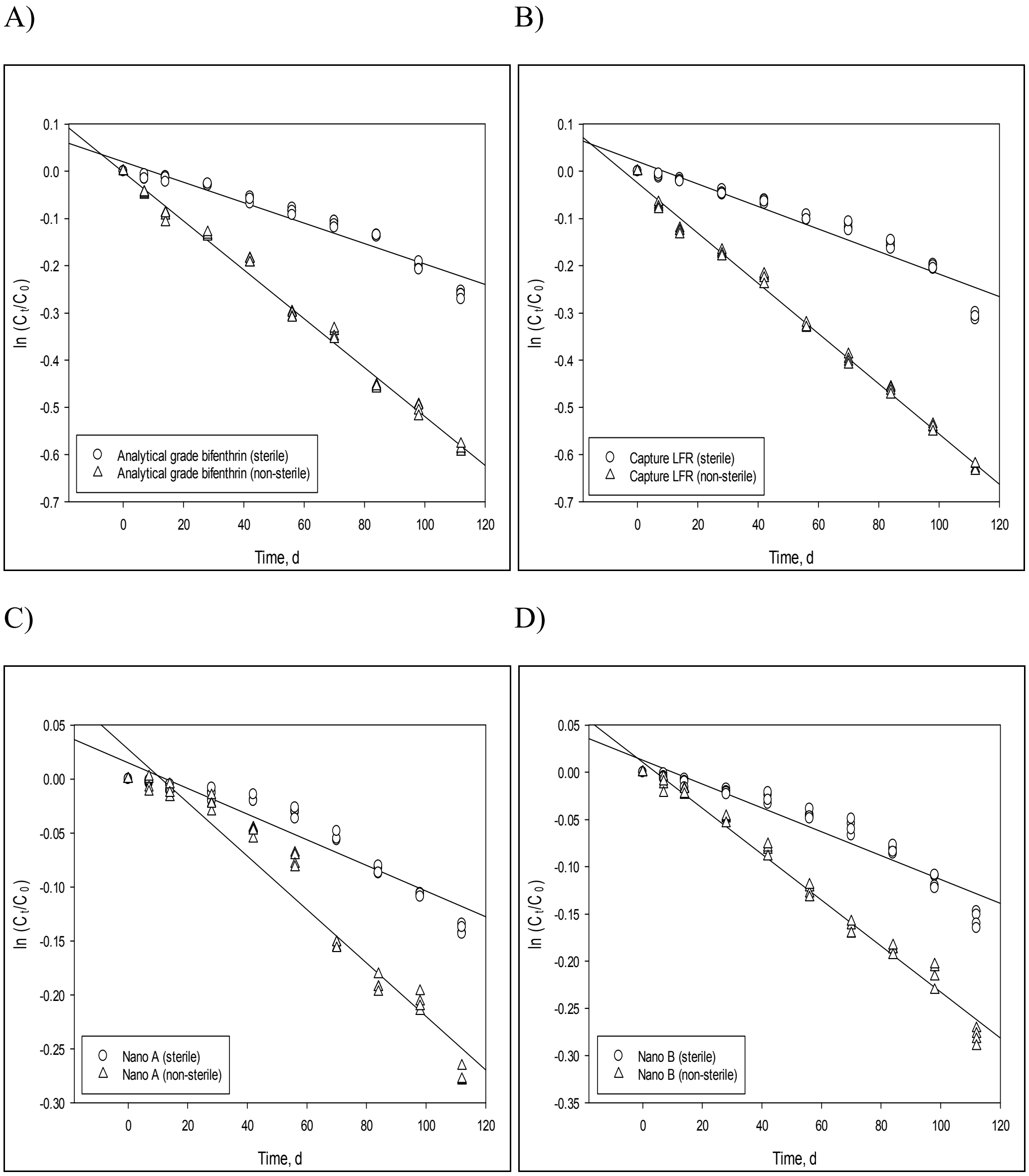


Figure 4.3A Curves ln (C_t/C_0) versus time (n = 4) for different bifenthrin treatment in sterile and non-sterile soils in Soil 1: A) analytical grade bifenthrin; B) Capture LFR; C) Nano A; and D) Nano B

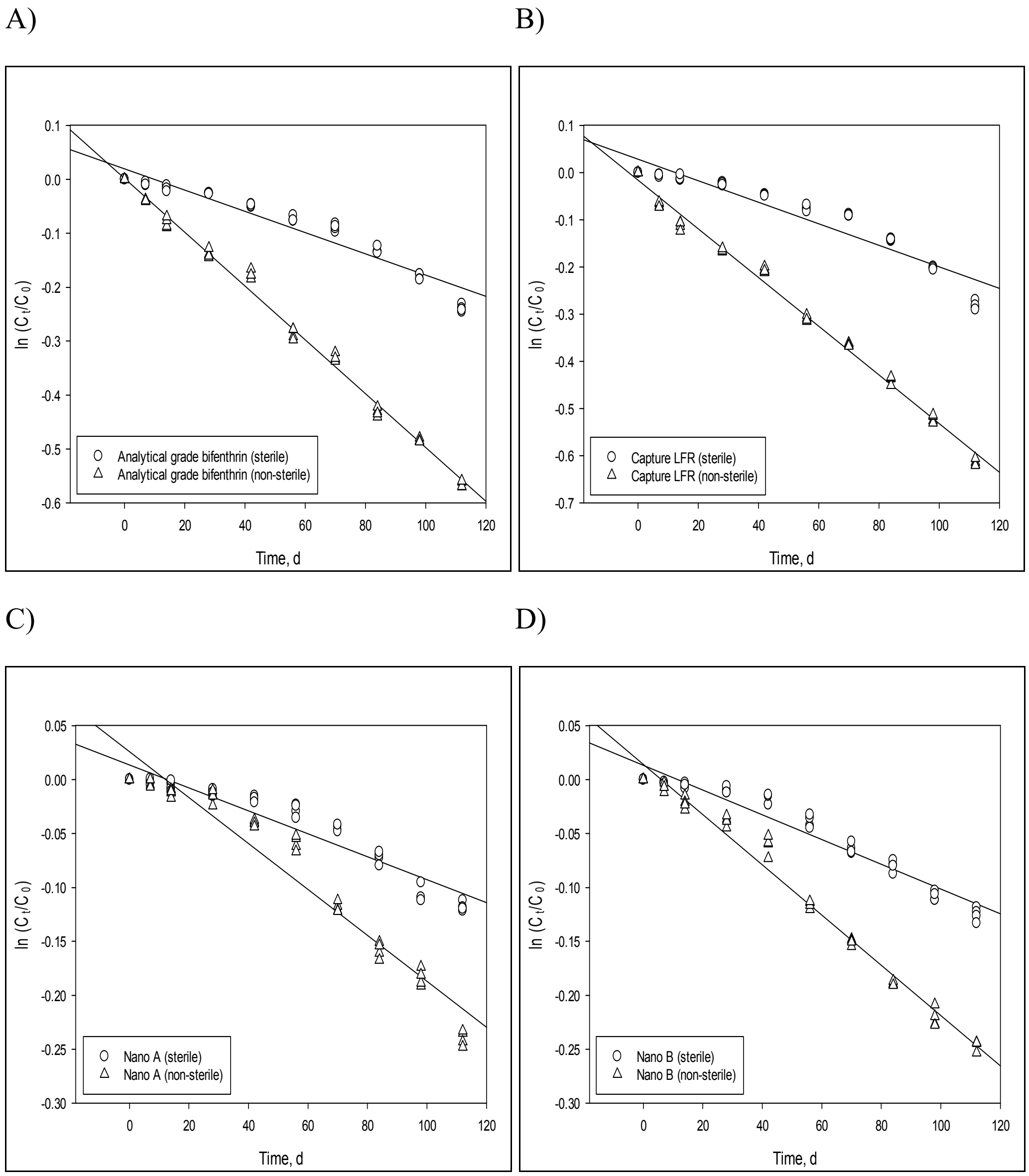


Figure 4.3B Curves ln (C_t/C_0) versus time (n = 4) for different bifenthrin treatment in sterile and non-sterile soils in Soil 2: A) analytical grade bifenthrin; B) Capture LFR; C) Nano A; and D) Nano B

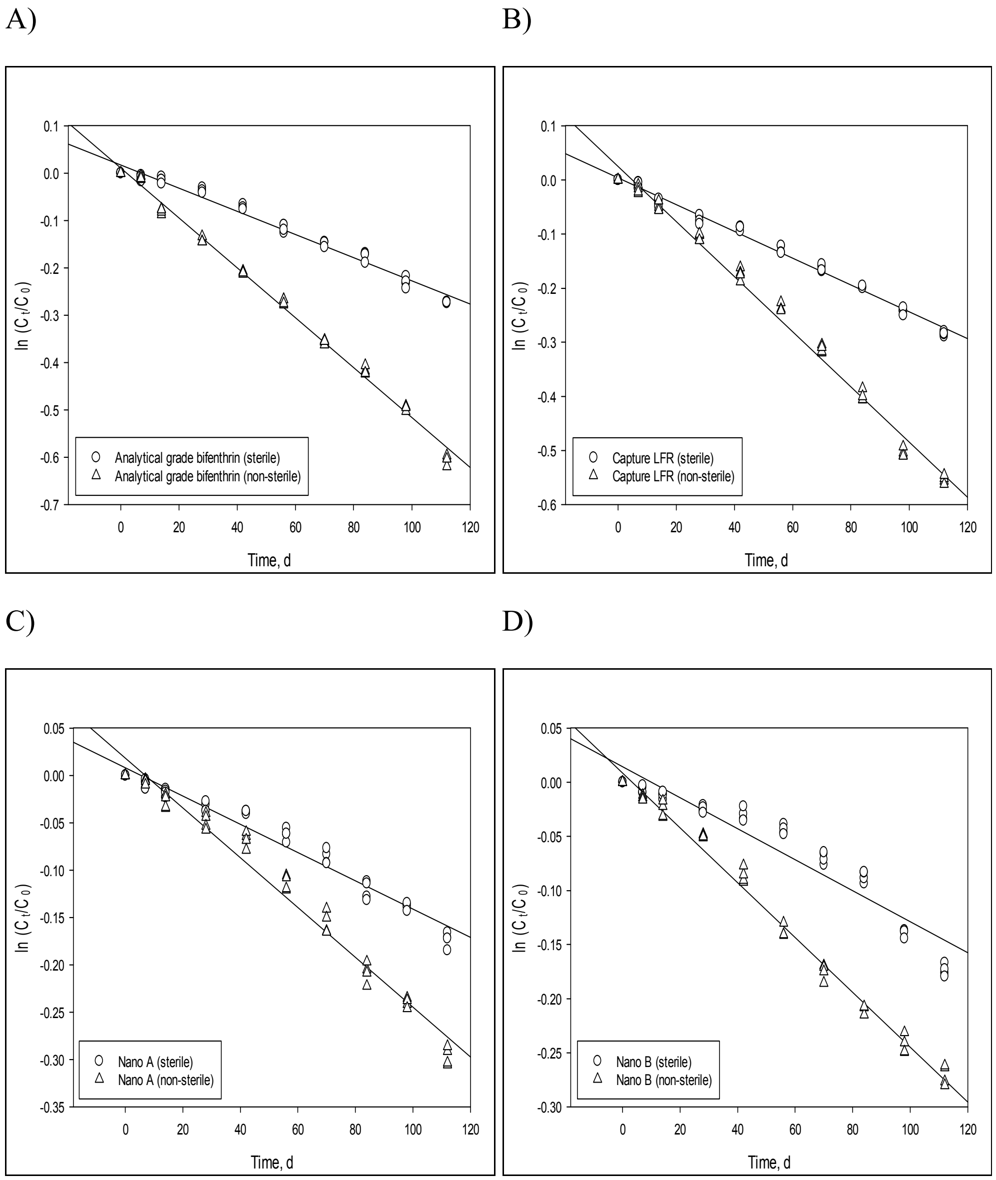


Figure 4.3C Curves ln (C_t/C_0) versus time (n = 4) for different bifenthrin treatment in sterile and non-sterile soils in Soil 3: A) analytical grade bifenthrin; B) Capture LFR; C) Nano A; and D) Nano B

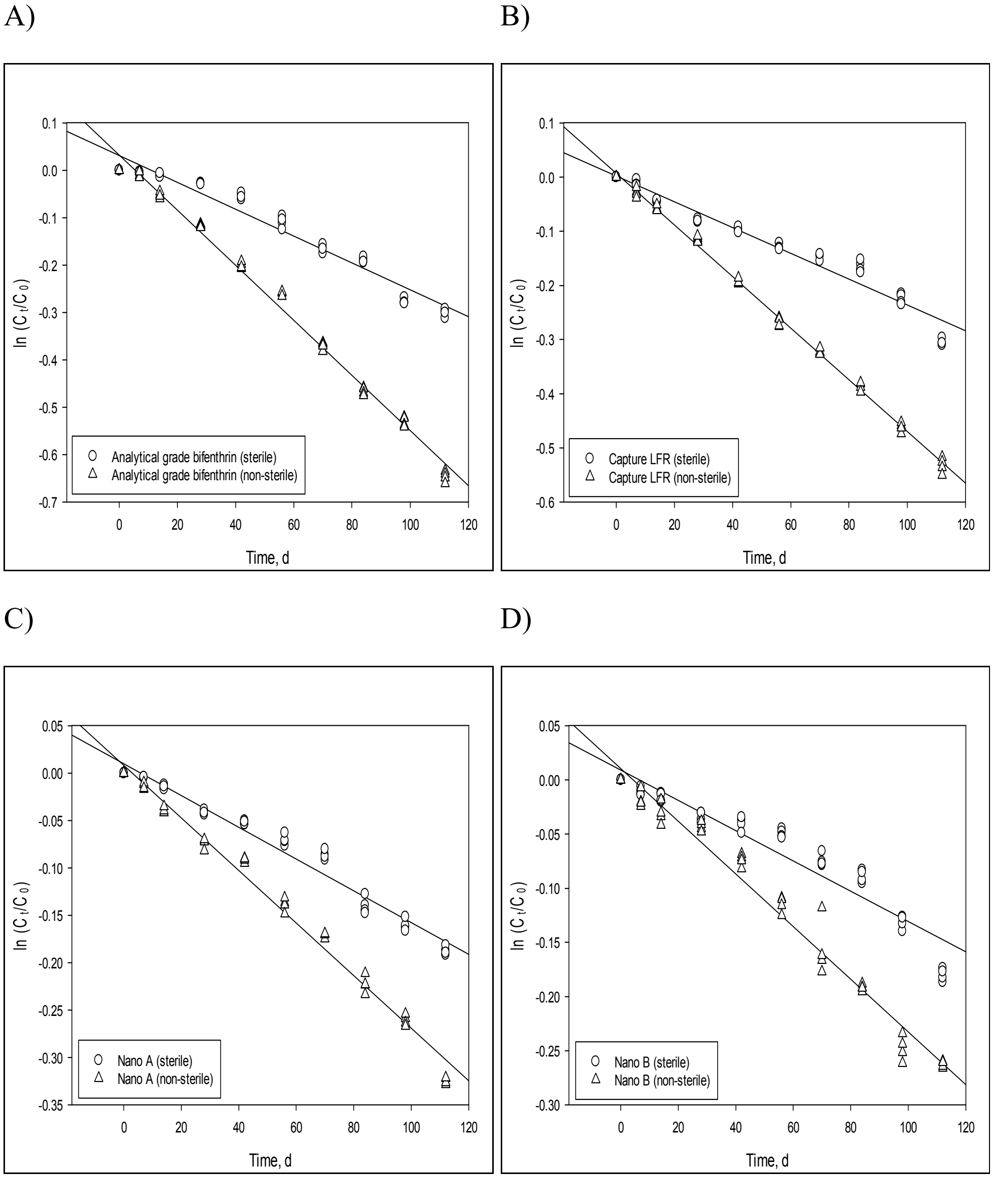


Figure 4.3D Curves ln (C_t/C_0) versus time (n = 4) for different bifenthrin treatment in sterile and non-sterile soils in Soil 4: A) analytical grade bifenthrin; B) Capture LFR; C) Nano A; and D) Nano B

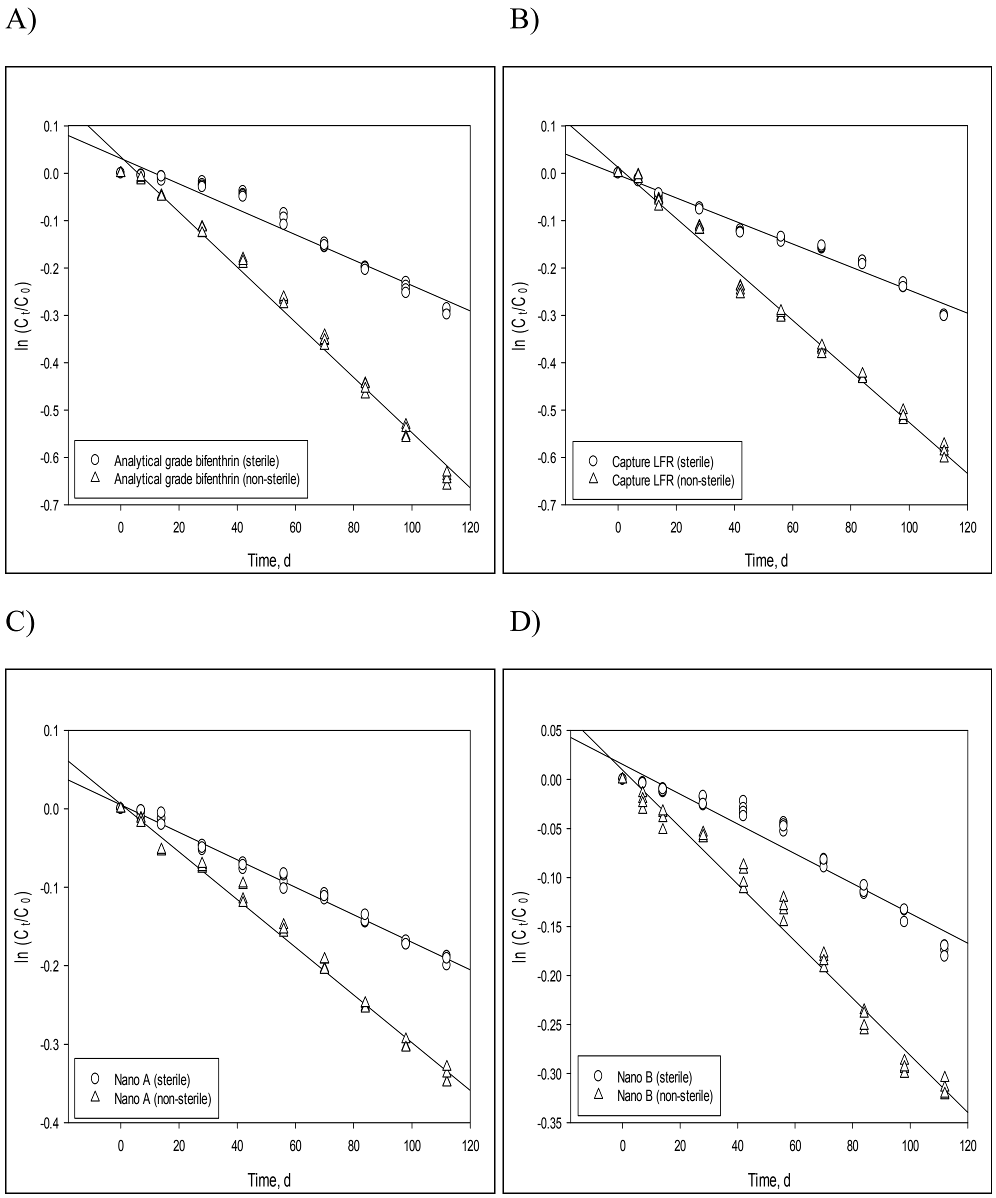


Figure 4.3E Curves ln (C_t/C_0) versus time (n = 4) for different bifenthrin treatment in sterile and non-sterile soils in Soil 5: A) analytical grade bifenthrin; B) Capture LFR; C) Nano A; and D) Nano B

Appendix 4.4 Relationships between soil properties and K_ds

Table 4.4A Summary of linear regression between soil properties and K_ds

Correlation parameter	**Bifenthrin treatment**	**p**	**R^2**
Soil pH	Analytical grade bifenthrin	0.125	0.598
	Capture LFR	0.170	0.518
	Nano A	0.415	0.224
	Nano B	0.162	0.533
OC, %	Analytical grade bifenthrin	<0.001	0.989
	Capture LFR	0.001	0.978
	Nano A	0.043	0.791
	Nano B	<0.001	0.988
CEC, cmol$^+$/kg	Analytical grade bifenthrin	0.590	0.107
	Capture LFR	0.653	0.076
	Nano A	0.948	0.002
	Nano B	0.672	0.068
Clay, %	Analytical grade bifenthrin	0.199	0.473
	Capture LFR	0.259	0.391
	Nano A	0.545	0.134
	Nano B	0.256	0.396
Silt, %	Analytical grade bifenthrin	0.192	0.485
	Capture LFR	0.216	0.448
	Nano A	0.428	0.218
	Nano B	0.226	0.434
Sand, %	Analytical grade bifenthrin	0.257	0.394
	Capture LFR	0.282	0.363
	Nano A	0.493	0.168
	Nano B	0.294	0.349

Appendix 4.5 Relationships between soil properties and DT_{50}s

Table 4.5A Summary of linear regression between soil properties and DT_{50}s

Correlation parameter	**Bifenthrin treatment**	**Sterile**		**Non-sterile**	
		p	**R^2**	**p**	**R^2**
Soil pH	Analytical grade bifenthrin	0.260	0.391	0.332	0.307
	Capture LFR	0.445	0.204	0.725	0.048
	Nano A	0.332	0.307	0.224	0.438
	Nano B	0.392	0.249	0.269	0.379
OC, %	Analytical grade bifenthrin	0.044	0.789	0.083	0.686
	Capture LFR	0.134	0.581	0.955	0.001
	Nano A	0.023	0.862	0.005	0.949
	Nano B	0.012	0.910	0.154	0.545
CEC, cmol$^+$/kg	Analytical grade bifenthrin	0.799	0.025	0.957	0.001
	Capture LFR	0.764	0.035	0.536	0.139
	Nano A	0.953	0.001	0.861	0.012
	Nano B	0.935	0.003	0.468	0.186
Clay, %	Analytical grade bifenthrin	0.632	0.086	0.724	0.048
	Capture LFR	0.036	0.815	0.329	0.311
	Nano A	0.472	0.184	0.274	0.373
	Nano B	0.250	0.403	0.241	0.414
Silt, %	Analytical grade bifenthrin	0.549	0.132	0.690	0.060
	Capture LFR	0.007	0.939	0.498	0.165
	Nano A	0.380	0.260	0.284	0.361
	Nano B	0.185	0.495	0.212	0.455
Sand, %	Analytical grade bifenthrin	0.635	0.084	0.775	0.032
	Capture LFR	0.014	0.897	0.443	0.205
	Nano A	0.448	0.202	0.349	0.290
	Nano B	0.229	0.431	0.222	0.441

Appendix 4.6 Relationships between K_ds and DT_{50}s

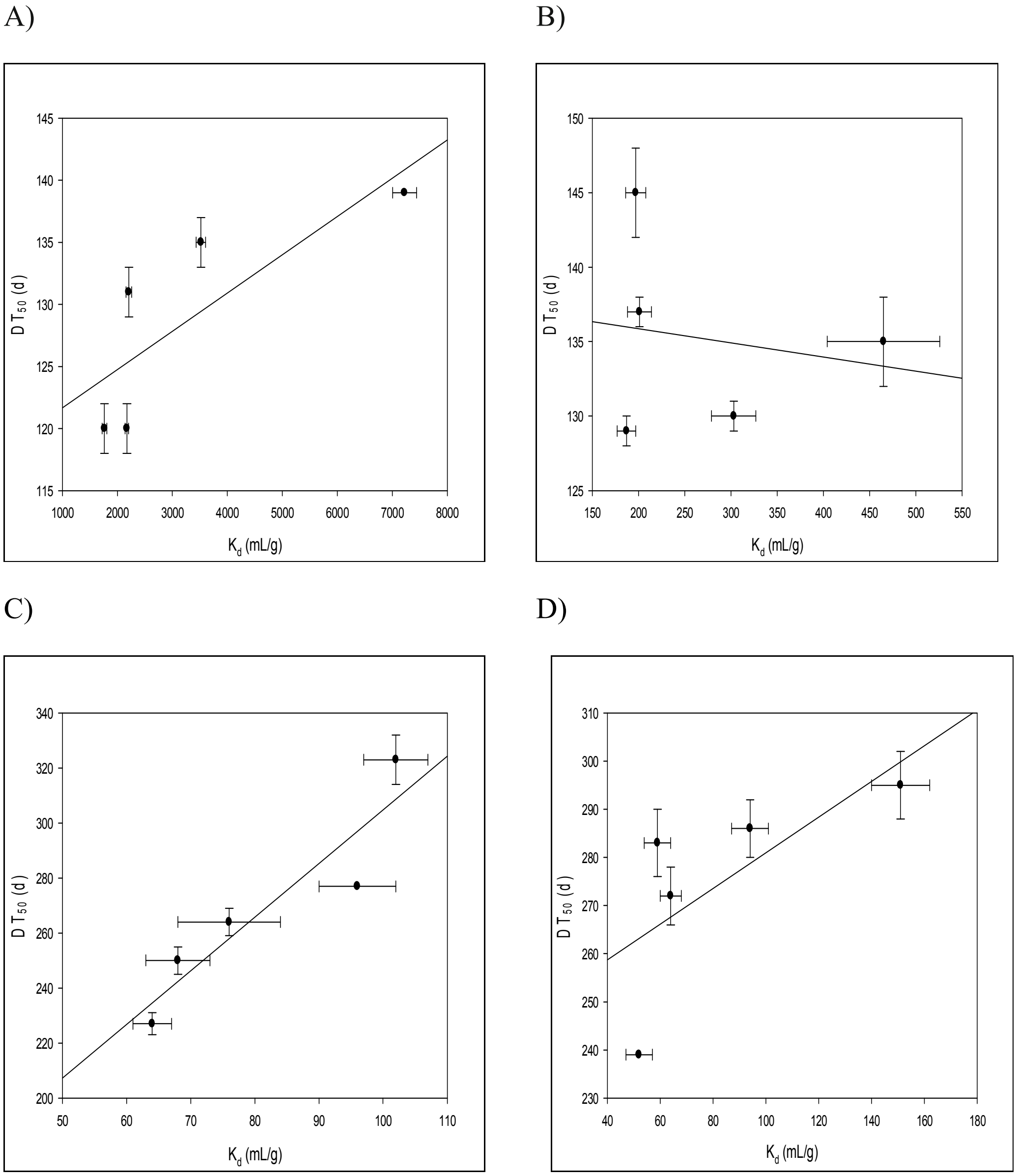
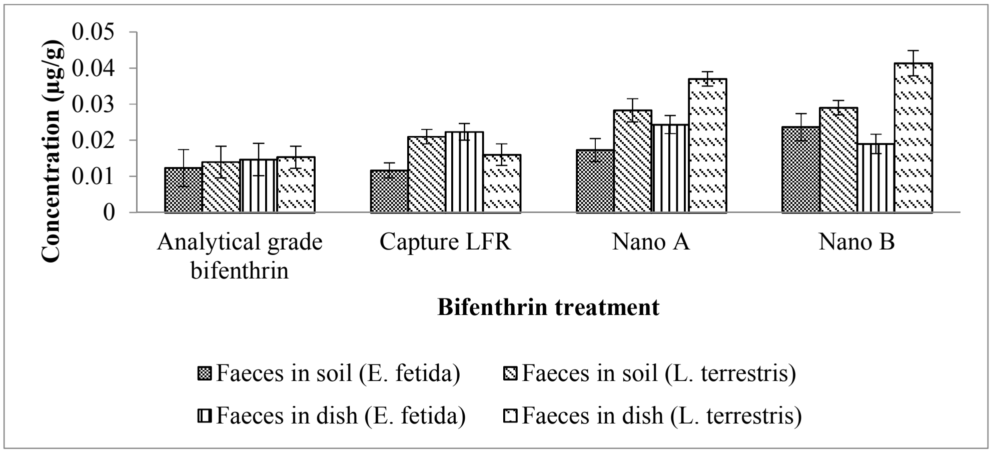


Figure 4.6A Linear regression between K_ds and DT_{50}s (±S.D, n = 4)

Appendix 5.1 Bifenthrin in earthworm faeces

A)



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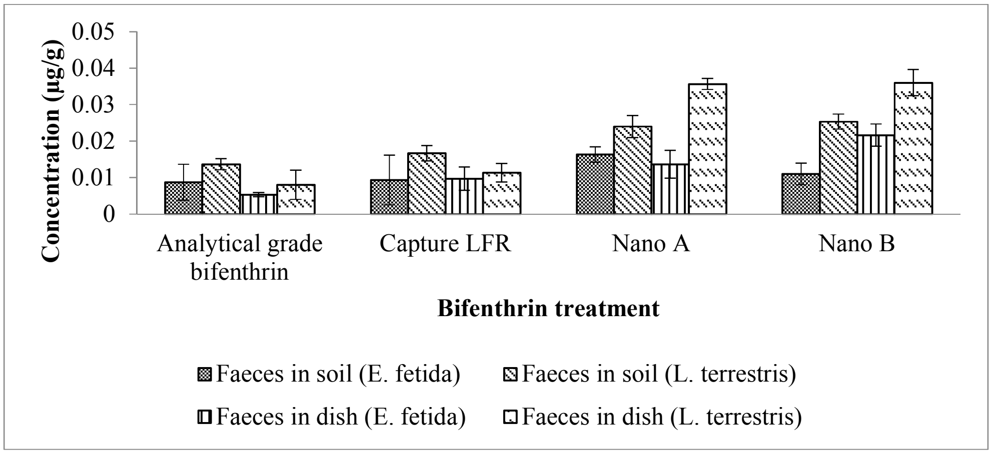

Figure 5.1A Mean concentration of bifenthrin, µg/g (±S.D, n = 3) detected in earthworm faeces (distribution studies): A) after uptake phase; and B) after depuration phase

Appendix 6.1 Relationships between soil properties and BCFs

Table 6.1A Summary of linear regression between soil properties and BCFs

Correlation parameter	**Bifenthrin treatment**	**p**	**R^2**
Soil pH	Analytical grade bifenthrin	0.376	0.389
	Capture LFR	0.885	0.013
	Nano A	0.634	0.134
	Nano B	0.555	0.198
CEC, $cmol^+/kg$	Analytical grade bifenthrin	0.264	0.542
	Capture LFR	0.790	0.044
	Nano A	0.798	0.041
	Nano B	0.730	0.073
OC, %	Analytical grade bifenthrin	0.870	0.017
	Capture LFR	0.627	0.139
	Nano A	0.213	0.619
	Nano B	0.223	0.603
Clay, %	Analytical grade bifenthrin	0.941	0.005
	Capture LFR	0.418	0.339
	Nano A	0.616	0.147
	Nano B	0.868	0.018
Silt, %	Analytical grade bifenthrin	0.719	0.079
	Capture LFR	0.172	0.686
	Nano A	0.381	0.383
	Nano B	0.656	0.118
Sand, %	Analytical grade bifenthrin	0.661	0.115
	Capture LFR	0.145	0.731
	Nano A	0.424	0.332

List of Abbreviations

BCF	Bioconcentration factor
BSAF	Soil based bioaccumulation factor
CEC	Cation exchange capacity ($cmol^+/kg$)
DOM	Dissolved organic matter
DT_{50}	Half-dissipation time
ENP	Engineered nanoparticle
ERA	Environment Risk Assessment
K_d	Soil sorption distribution coefficient (mL/g)
K_{oc}	Soil organic-carbon coefficient (mL/g)
LDH	layered double hydroxides
LOEC	Low observed effect concentration
Log K_{ow}	Octanol-water partition coefficient (measure of hydrophobicity)
R_{50}	Time required for the bifenthrin to be released from nanocapsules
SP	Synthetic pyrethroid
STM	Scanning tunnelling microscope
TOC	Total organic carbon (%)
TP	Transformation product

References

Aarthi, T., Madras, G., 2008. Photocatalytic reduction of metals in presence of combustion synthesised nano-TiO_2. Cataly. Comm. 9, 630 – 634.

Abdelmonem M. Khalil, A.M., 2016. Physiological and genotoxic responses of the earthworm *Aporrectodea caliginosa* exposed to sublethal concentrations of AgNP. Basic Appl. Zoology. 74, 8 – 15.

Aguer, J.P., Blachere, F., Boule, P., Garaudee, S., Guillard, C., 2000. Photolysis of dicamba (3,6-dichloro-2- methoxybenzoic acid) in aqueous solution and dispersed on solid supports. Photoenergy. 2.

Aitken, R.J., Chaudhry, M.Q., Boxall, A.B.A., Hull, M., 2006. Manufacture and use of nanomaterials: current status in the UK and global trends. Occup. Med. 56, 300 – 306.

Albarrán, A., Celis, R., Hermosín, M.C., López-Piñeiro, A., Cornejo, J., 2004. Behaviour of simazine in soil amended with the final residue of the olive-oil extraction process. Chemosphere. 54, 717 – 724.

Amorim, M.J.B., Rombke, J., Soares, A.M.V.M., 2005. Avoidance behaviour of *Enchytraeus albidus*: effects of benomyl, carbendazim, phenmedipham and different soil types. Chemosphere. 59 (4), 501 – 510.

Amorim, M.J.B., Sousa, J.P., Nogueira, A.J.A., Soares, A.M.V.M., 1999. Comparison of chronic toxicity of lindane (γ-HCH) to *Enchytraeus albidus* in two soil types: the influence of soil pH. Pedobiologia. 43 (6), 635 – 640.

Andreu, V., Pico, Y., 2004. Determination of pesticides and their degradation products in soil: critical review and comparison of methods. Trends in Analytical Chemistry. 23.

Anjali, C.H., Sharma, Y., Mukherjee, A., Chandrasekaran, N., 2012. Neem oil (*Azadirachta Indica*) nanoemulsion as potent larvicidal agent against *Culex Quinquefasciatus*. Pest Manage. Sci. 68(2), 158 – 163.

Anjali, C.H., Sudheer, K., Margulis-Goshen, S., Magdassi, K., Mukherjee, S., A., Chandrasekaran, N., 2010. Formulation of water-dispersible nanopermethrin for larvicidal applications. Ecotox. Environ. Safety. 73, 1932 – 1936.

Anton, N., Benoit, J.P., Saulnier, P., 2008. Design and production of nanoparticles formulated from nano-emulsion templates: A Review. Controll. Release. 128, 185 – 199.

Araújo, A.S.F., Monteiro, R.T.R., Abarkeli, R.B., 2003. Effect of glyphosate on the microbial activity of two Brazilian soils. Chemosphere. 52, 799 – 804.

Arias-Estevez, M., López-Periago, E., Martínez-Carballo, E., Simal-Gándara, J., Juan-C, M., García-Rió, L., 2008. The mobility and degradation of pesticides in soils and the pollution of groundwater resources. Agric, Ecosyst. Environ. 123, 247 – 260.

Asgharnia, H., Jafari, A.J., Kalantary, R.R., Nasseri, S., Mahvi, A., Yaghmaeian, K., Esrafili, A., Shahamat, Y.D., 2014. Influence of bioaugmentation on biodegradation of phenanthrene-contaminated soil by earthworm in lab scale. Environmental Health Science Engineering. 12, 150.

Ashauer, R., Caravatti, I., Hintermeister, A., Escher, B.I., 2010. Bioaccumulation kinetics of organic xenobiotic pollutants in the freshwater invertebrate *Gammarus Pulex* modelled with prediction intervals. Environ. Toxicol. Chem. 29 (7), 1625 – 1636.

Auffan, M., Rose, J., Bottero, J.Y., Lowry, G.V., Jolivet, J.P., Wiesner, M.R., 2009. Towards a definition of inorganic nanoparticles from an environmental, health and safety perspective. Nature Nanotechnol. 4, 634 – 664.

Baalousha, M., Manciulea, A., Cumberland, S., Kendall, K., Lead, J., 2008. Aggregation and surface properties of iron oxide nanoparticles: Influence of pH and natural organic matter. Environ. Toxicol. Chem. 27, 1875.

Babu, G.S., Farooq, M., Ray, R.S., Joshi, P.C., Viswanathan, P.N., Hans, R.K., 2003. DDT and HCH residues in basmati rice (*Oryza sativa*) cultivated in Dehradun (India). Water Air Soil Pollution. 144, 149 – 157.

Baker, J.L., Mickelson, S.K., 1994. Application technology and best management practices for minimizing herbicide runoff. Weed Technology. 8 (4), 862 – 869.

Banfield, J.F., Welch, S.A., Zhang, H.Z., Ebert, T.T., Penn, R.L., 2000. Aggregation-based crystal growth and microstructure development in natural iron oxyhydroxide biomineralization products. Science 289, 751 – 754.

Barriuso, E., Houot, S., Serra-Wittling, C., 1997. Influence of compost addition to soil on the behaviour of herbicides. Pestic. Sci. 49, 65 – 75.

Bartlett, M.D., Briones, M.J.I., Neilson, R., Schmidt, O., Spurgeon, D., Creamer, R.E., 2010. A critical review of current methods in earthworm ecology: from individuals to populations. Soil Bio. 46, 67 – 73.

Barua, S., Konwarh, R., Bhattacharya, S.S., Das, P., Devi, K.S., Maiti, T.K., Mandal, M., Karak, N., 2013. Non-hazardous anticancerous and antibacterial colloidal "green" silver nanoparticles. Colloids and Surfaces B: Biointerfaces. 105, 37 – 42.

Baruah, S., Dutta, J., 2009. Nanotechnology applications in pollution sensing and degradation in agriculture: A review. Environ. Chem. Lett. 7, 161 – 204.

Bavcon, M., Trebše, P., Zupančić-Kralj, L., 2003. Investigations of the determination and transformations of diazinon and malathion under environmental conditions using gas chromatography coupled with a flame ionisation detector. Chemosphere. 50 (5), 595 – 601.

Beestman, G.B., Deming, J.M., 1974. Dissipation of acetanilide herbicides from soils1. Agron. 66, 308 – 311.

Beigel, C., Barriuso, E., 2000. Influence of formulation on triticonazole solubilisation and sorption in a soil-water system. Pestic. Manag. Sci. 56, 271 – 276.

Belfroid, A., Meiling, J., Drenth, H., Hermens, J., Seinen, W., Van Gestel, K., 1995a. Dietary uptake of superlipophilic compounds by earthworms (*Eisenia Andrei*). Ecotox. and Environ. Safety. 31 (3), 185 – 191.

Belfroid, A., Meiling, J., Sijm, D., Hermens, J., Seinen, W., Van Gestel, K., 1994a. Uptake of lipophilic organic chemicals from food by earthworms (*Eisenia Andrei*). Arch. Environ. Contam. Toxicol. 27, 260 – 265.

Belfroid, A., Seinen, W., Van Den Berg, M., Hermens, J., Van Gestel, K., 1995b. Uptake, bioavailability and elimination of hydrophobic compounds in earthworms *(Eisenia Andrei)* in field contaminated soil. Environ. Toxicol. Chem. 14 (4), 605 – 612.

Belfroid, A., Seinen, W., Van Gestel, K., Hermens, J., 1993a. The acute toxicity of chlorobenzenes for earthworms (*Eisenia Andrei*) in different exposure systems. Chemosphere. 12, 2265 – 2277.

Belfroid, A., Sikkenk, M., Van Gestel, K., Se1nen, W., Hermens, J., 1994b. The toxicokinetic behaviour of chlorobenzenes in earthworms (*Eisenia Andrei*), Experiments in soil. Environ. Toxicol. Chem. 13, 93 – 99.

Belfroid, A., Van Wezel, A., Sw, Kenk, M., Van Gestel, K., Seinen, W., Hegmens, J., 1993b. The toxicokinetic behaviour of chlorobenzenes in earthworms (*Eisenia Andrei*): Experiments in water. Ecotox. Environ. Safety. 25, 154 – 165.

Belfroid, A.C., Scinen, W., Van Gestel, K.C.A.M., Hermens, J.L.M., Van Leeuwen, K.J., 1995. Modelling the accumulation of hydrophobic organic chemicals in earthworms – Application of the equilibrium partitioning theory. Environ. Sci. and Pollut. Res. 2 (1), 5 – 15.

Bending, G.D., Lincoln, S.D., Sorensen, S.R., Morgan, J.A.W., Aamand, J., Walker, A., 2003. In-field spatial variability in the degradation of the phenyl-urea herbicide isoproturon is the result of interactions between degradative *Sphingomonas spp*. and soil pH. Appl. Environ. Microb. 69, 827 – 834.

Ben-Hur, M., Letey, L., Farmer, W.J., Williams, C.F., Nelson, S.D., 2003. Soluble and solid organic matter effects on atrazine adsorption in cultivated soils. Soil Sci. Soc. 67, 1140 – 1146.

Bergeson, L.L., 2010. Nanosilver: USEPA's pesticide office considers how best to proceed? Environ. Qual. Manage. 19 (3), 79 – 85.

Beulke, S., Brown, C.D., 2006. Impact of correlation between pesticide parameters on estimates of environmental exposure. *Pest management science.* 62 (7), 603 – 609.

Bidleman, T.F., Leone, A.D., 2004. Soil–air exchange of organochlorine pesticides in the southern United States. Environ Pollut. 128, 49 – 57.

Bin Hussein, M.Z., Hashim, N., Yahaya, A.H., Zainal, Z., 2009. Controlled release formulation of agrochemical pesticide based on 4-(2,4-dichlorophenoxy)butyrate nanohybrid. Nanosci. Nanotechnol. 9, 2140 – 2147.

Bin Hussein, M.Z., Yahaya, A.H., Zainal, Z., Kian, L.H., 2005. Nanocomposite-based controlled release formulation of an herbicide, 2,4-dichlorophenoxyacetate incapsulated in zinc-aluminium-layered double hydroxide. Sci. Technol. Adv. Mater. 6, 956 – 962.

Bintein, S., Devillers, J., Karcher, W., 1993. Non-linear dependence of fish bioconcentration on n-octanol-water partition coefficient. SAR QSAR Environ. Res. 1, 29 – 39.

Bixler, T.A. 1984. Fate of alcohol (phenyl)-14C FMC 54800 in soil after 120 days. FMC Corporation, document No. P-0800

Boehm, A.L., Martinon, I., Zerrouk, R., Rump, E., Fessi, H., 2003. Nanoprecipitation technique for the encapsulation of agrochemical active ingredients. Microencapsulation. 20, 433 – 441.

Boehm, A.L.L., Zerrouk, R., Fessi, H., 2000. Poly epsilon-caprolactone nanoparticles containing a poorly soluble pesticide: formulation and stability study. Microencapsulation. 17, 195 – 205.

Boesten, J.J.T.I., Van der Linden, A.M.A., 1991. Modelling the influence of sorption and transformation on pesticide leaching and persistence. Environ. Qual. 20, 425 – 435.

Bohmont, B.L., 1990. The standard pesticide user's guide. Englewood Cliffs, NJ: Prentice Hall.

Bossuyt, H., Denef, K., Six, J., Frey, S.D., Merckx, R., Paustian, K., 2001. Influence of microbial populations and residue quality on aggregate stability. Appl Soil Ecol. 16 (3), 195 – 208.

Boyandin AN, Volova TG (2015) Application of polyhydroxyalkanoates for development of targeted forms of pecticides. Int J Gen Chem 1:10–14

Bradford, A., Handy, R.D., Readman, J.W., Atfield, A., Mühling, M., 2009. Impact of silver nanoparticle contamination on the genetic diversity of natural bacterial assemblages in estuarine sediments. Environ. Sci. Technol. 43, 4530 – 4536.

Brady, N.C., Weil, R.R., 1999. The Nature and Properties of Soils, twelfth ed., London.

Brar, S.K., Verma, M., Tyagi, R.D., Surampalli, R.Y., 2010. Engineered nanoparticles in wastewater and wastewater sludge – evidence and impacts. Waste Manag. 30, 504 – 520.

Burgos, M.G., Winters, C., Sturzenbaum, S.R., Randerson, P.F., Kille, P., Morgan, A.J., 2005. Cu and Cd effects on the earthworm *Lumbricus rubellus* in the laboratory: multivariate statistical analysis of relationships between exposure, biomarkers, and ecologically relevant parameters. Environmental 19. Science and Technology. 39, 1757 – 1763.

Butenschoen, O., Ji, R., Schaffer, A., Scheu, S., 2009. The fate of catechol in soil as affected by earthworms and clay. Soil Biol. Biochem. 41, 330 – 339.

Bystrzejewska-Piotrowska, G., Asztemborska, M., Giska, I., Mikoszewkski, A., 2012. Influence of earthworms on extractability of metals from soil contaminated with Al_2O_3, TiO_2, Zn, and ZnO Nanoparticles and microparticles of Al_2O_3. Pol. J. Environ. Stud. 21 (2), 313 – 319.

Cáceres-Jensen, L., Gan, J., Báez, M., Fuentes, R., Escudey, M., 2009. Adsorption of glyphosate on variable-charge, volcanic ash-derived soils. Environmental Quality. 38, 1449 – 1457.

Campos, E.V.R., De Oliveira, J.L., Da Silva, C.M.G., Pascoli, M., Pasquoto, T., Lima, R., Abhilash, P.C., Fraceto, L.F., 2015. Polymeric and solid lipid nanoparticles for sustained release of carbendazim and tebuconazole in agricultural applications. Scientific Reports. 5, 13809.

Cao, L., Zhang, H., Cao, C., Zhang, J., Li, F., Huang, Q., 2016. Quaternized chitosan-capped mesoporous silica nanoparticles as nanocarriers for controlled pesticide release. Nanomaterials. 6, 126.

Cao, Y.S., Chen, J.X., Wang, Y.L., Liang, J., Chen, L.H., Lu, Y.T., 2005. HPLC/UV analysis of chlorfenapyr residues in cabbage and soil to study the dynamics of different formulations. Science of the Total Environment. 350, 38 – 46.

Carter, L.J., Ashauer, R., Ryan, J.J., Boxall, A.B.A., 2014b. Minimised bioconcentration tests: A useful tool for assessing chemical uptake into terrestrial and aquatic invertebrates? Environ. Sci. Technol. 48 (22), 13497 – 13503.

Carter, L.J., Garman, C.D., Ryan, J., Dowle, A., Bergström, E., Thomas-Oates, J., Boxall, A.B.A., 2014a. Fate and uptake of pharmaceuticals in soil−earthworm systems. Environ. Sci. Technol. 48, 5955 – 5963.

Carter, L.J., Ryan, J.J., Boxall, A.B.A., 2016. Effects of soil properties on the uptake of pharmaceuticals into earthworms. Environmental Pollution. 213, 922 – 931.

Casida, J.E., 2009. Pest toxicology: The primary mechanisms of pesticide action. Chem. Res. Toxicol. 22 (4), 609 – 619.

Celis, R., Facenda, G., Hermosín, M.C., Cornejo, J., 2005. Assessing factors influencing the release of hexazinone from clay-based formulations. Environ. Anal. Chem. 85 (15), 1153 – 1164.

Celorie, J.A., Woods, S.L., Vinson, T.S., Istok, J.D., 1989. A comparison of sorption equilibrium distribution coefficients using batch and centrifugation methods. Environ. Qual. 18, 307 – 313.

Céspedes FF, Garcı´a SP, Sa´nchez MV, Pe´rez MF (2013) Bentonite and anthracite in alginate-based controlled release formulations to reduce leaching of chloridazon and metribuzin in a calcareous soil. Chemosphere 92:918–924

Chang, J., Wang, Y., Wang, H., Li, J., Xu, P., 2016. Bioaccumulation and enantioselectivity of type I and type II pyrethroid pesticides in earthworm. Chemosphere. 144, 1351 – 1357.

Chapman, R. A., Tu, C. M., Harris, C. R. and Cole, C., 1981. Persistence of five pyrethroid insecticides in sterile and natural, mineral and organic soil. Bulletin of Environmental Contamination and Toxicology. 26, 513 – 519.

Chen, B., Zhang, H.Z., Gilbert, B., Banfield, J.F., 2007. Mechanism of inhibition of nanoparticle growth and phase transformation by surface impurities. Phys. Rev. Lett. 98, 4.

Chen, J., Li, C., Song, J.L., Sun, X.W., Lei, W., Deng, W.Q. 2009. Bilayer ZnO nanostructure fabricated by chemical bath and its application in quantum dot sensitized solar cell. Appl. Surf. Sci. 255, 7508 – 7511.

Chen, J., Wang, W., Xu, Y., Zhang, X., 2011. Slow-release formulation of a new biological pesticide, pyoluteorin, with mesoporous silica. Agric. Food Chem. 59 (1), 307 – 311.

Chen, K.L., Elimelech, M., 2006. Aggregation and deposition kinetics of fullerene (C_{60}) nanoparticles. Langmuir. 22 (26), 10994 – 11001.

Chen, S., Luo, J., Hu, M., Geng, P., Zhang, Y., 2012. Microbial detoxification of bifenthrin by a novel yeast and its potential for contaminated soils treatment. PLoS ONE. 7 (2), 30862.

Chevillard, A., Angellier-Coussy, H., Guillard, V., Gontard, N., Gastaldi, E., 2012. Controlling pesticide release via structuring agropolymer and nanoclays based materials. Hazardous Materials. 205 – 206, 32 – 39.

Chin, C.P., Wu, H.S., Wang, S.S., 2011. New approach to pesticide delivery using nanosuspensions: Research and applications. Industrial & Engineering Chemistry Research. 50, 7637 – 7643.

Chiou, C.T., Porter, P.E., Schmedding, D.W., 1983. Partition equilibria of nonionic organic compounds between soil organic matter and water. Environ. Sci. Technol. 17(4), 227 – 231.

Choi, J.S., Park, J.W., 2015. Molecular characterisation and toxicological effects of citrate-coated silver nanoparticles in a terrestrial invertebrate, the earthworm (*Eisenia fetida*). Molecular and Cellular Toxicology. 11 (4), 423 – 431.

Choudhury, S.R., Pradhan, S., Goswami, A., 2012. Preparation and characterisation of acephate nano-encapsulated complex. Nanosci. Methods. 1, 9 – 15.

Chowdhury, A., Pradhan, S., Saha, M., Sanyal, N., 2008. Impact of pesticides on soil microbiological parameters and possible bioremediation strategies. Ind J Microbiol. 48, 114 – 127.

Choy, J.H., Choi, S.J., Oh, J.M., Park, T., 2007. Clay minerals and layered double hydroxides for novel biological applications. Appl. Clay Sci. 36, 122 – 132.

Chun, J.P., Choi, J.S., Ahn, Y.J., 2010. Utilization of fruit bags coated with nano-silver for controlling black stain on fruit skin of ‘niitaka’ pear (*Pyrus pyrifolia*). Horticulture Environment and Biotechnology. 51, 245 – 248.

Chung, N.H., Alexander, M., 1998. Differences in sequestration and bioavailability of organic compounds aged in dissimilar soils. Environ. Sci. Technol. 32, 855 – 860.

Cifuentes, Z., Custardoy, L., De La Fuente, J.M., Marquina, C., Ibarra, M.R., Rubiales, D., De Luque, A.P., 2010. Absorption and translocation to the aerial part of magnetic carbon-coated nanoparticles through the roots of different crop plants. Nanobiotechnol. 8 (26), 1 – 8.

Coleman, J.G., Johnson, D.R., Stanley, J.K., Bednar, A.J., Weiss, C.A. Jr., Boyd, R.E., Steevens, J.A., 2010. Assessing the fate and effects of nano aluminium oxide in the terrestrial earthworm, *Eisenia fetida*. Environ ToxicolChem. 29 (7), 1575 – 1580.

Connell, D.W., Hawker, D.W., 1988. Use of polynomial expressions to describe the bioconcentration of hydrophobic chemicals by fish. Ecotoxicol. Environ. Saf. 16, 242 – 257.

Connell, D.W., Markwell, R.D., 1990. Bioaccumulation in the soil to earthworm system. Chemosphere. 20, 91 – 100.

Cork, D.J., Krueger, J.P., 1991. Microbial transformation of herbicides and pesticides. Adv. Applied Microbiol. 36, 1 – 66.

Corradini, E., Moura, M.R., Mattoso, L.H.C., 2010. A preliminary study of the incorporation of NPK fertilizer into chitosan nanoparticles. Express Polymer Lett. 4, 509 – 515.

Coutris, C., Joner, E.J., Oughton, D.H., 2012. Aging and soil organic matter content affect the fate of silver nanoparticles in soil. Sci Total Environ. 420, 327 – 333.

Cox, L., Cecchi, A., Celis, R., Hermosfn, M., Koskinen, W., Cornejo, J., 2001. Effect of exogenous carbon on movement of simazine and 2,4-D in soils. Soil Sci. Soc. 65, 1688 – 1695.

Cui, B., Feng, L., Pan, Z., Yu, M., Zeng, Z., Sun, C., Zhao, X., Wang, Y., Cui, H., 2015. Evaluation of stability and biological activity of solid nanodispersion of lambda-cyhalothrin. PLoS ONE. 10 (8), e0135953.

Cui, B., Feng, L., Wang, C., Yang, D., Yu, M., Zeng, Z., Wang, Y., Sun, C., Zhao, X., Cui, H., 2016. Stability and biological activity evaluation of chlorantraniliprole solid nanodispersions prepared by high pressure homogenisation. PLoS ONE. 11 (8), 0160877.

Cukalevski, R., Lundqvist, M., Oslakovic, C., Dahlback, B., Linse, S., Cedervall, T., 2011. Structural changes in apolipoproteins bound to nanoparticles. Langmuir. 27 (23), 14360 – 14369.

Dada, A.O., Olalekan, A.P, Olatunya, A.M., Dada, O., 2012. Langmuir, Freundlich, Temkin and Dubinin–Radushkevich isotherms studies of equilibrium sorption of Zn^{2+} unto phosphoric acid modified rice husk. Applied Chemistry. 3 (1), 38 – 45.

Dalby, P.R., Baker, G.H., Smith, S.E., 1996. Filter paper method’’ to remove soil from earthworm intestines and to standardise the water content of earthworm tissue. Soil Biol. Biochem. 28, 685 – 687.

Darlington, T.K., Eeigh, A.M., Spencer, M.T., Nguyen, O.T., Oldenburg, S.J., 2009. Nanoparticle characteristics affecting environmental fate and transport through soil. Environ. Toxicol. Chem. 28, 1191 – 1199.

Davies, J.E.D., Jabeen, N., 2002. The adsorption of herbicides and pesticides on clay minerals and soils. Part 1. Isoproturon. Inclusion Phenomena and Macrocyclic Chemistry. 43 (3), 329 – 336.

Davies, N.A., Hodson, M.E., Black, S., 2003. The influence of time on lead toxicity and bioaccumulation determined by the OECD earthworm toxicity test. Environmental Pollution. 121, 55 – 61.

De Silva, P.M.C.S., Pathiratne, A., Van Gestel, C.A.M., 2010. Toxicity of chlorpyrifos, carbofuran, mancozeb and their formulations to the tropical earthworm *Perionyx excavatus*. Appl. Soil Ecol. 44, 56 – 60.

De Silva, P.M.C.S., Van Gestel, C.A.M., 2009. Comparative sensitivity of *Eisenia andrei* and *Perionyx excavatus* in earthworm avoidance tests using two soil types in the tropics. Chemosphere. 77, 1609 – 1613.

Dean-Ross, D., 1983. Methods for the assessment of the toxicity of environmental chemicals to earthworms. Regul Toxicol Pharmacol. 3 (1), 48 – 59.

Delle Site, A., 2001. Factors affecting sorption of organic compounds in natural sorbent/water systems and sorption coefficients for selected pollutants. A review. Phys. Chem. Ref. Data. 30, 187 – 439.

DeLorenzo, M.E., Scott, G.I., Ross, P.E., 2001. Toxicity of pesticides to aquatic microorganisms: A review. Environmental Toxicology and Chemistry. 20, 84 – 98.

Desta, M.B., 2013. Batch sorption experiments: Langmuir and Freundlich isotherm studies for the adsorption of textile metal ions onto teff straw (Eragrostis tef) agricultural waste. Thermodynamics. 2013, 6.

Di Vincenzo, J.P., Sparks, D.L., 2001. Sorption of the neutral and charged forms of pentachlorophenol in soil: Evidence for different mechanisms. Arch Environ Contam Toxicol. 40, 445 – 450.

Diao, J., Xu, P., Liu, D., Lu, Y., Zhou, Z., 2011. Enantiomer-specific toxicity and bioaccumulation of alpha-cypermethrin to earthworm *Eisenia fetida*. Hazardous Materials. 192 (3), 1072 – 1078.

Diez-Ortiz, M., Lahive, E., Kille, P., Powell, K., Morgan, A.J., Jurkschat, K., Van Gestel, C.A.M., Mosselmans, J.F.W., Svendsen, C., Spurgeon, D.J., 2015. Uptake routes and toxicokinetics of silver nanoparticles and silver ions in the earthworm *Lumbricus Rubellus*. Environmental Toxicology and Chemistry. 34 (10), 2263 – 2270.

Dolaptsoglou, C., Karpouzas, D.G., Menkissoglu-Spiroudi, U., Eleftherohorinos, I., Voudrias, E.A., 2007. Influence of different organic amendments on the degradation, metabolism, and adsorption of terbuthylazine. Environmental Quality. 36, 1793 – 1802.

Domingos, R.F., Tufenkji, N., Wilkinson, K.J. 2009a. Aggregation of titanium dioxide nanoparticles: Role of a fulvic acid. Environ. Sci. Technol. 43, 1282 – 1286.

Doshi, R., Braida, W., Christodoulatos, C., Wazne, M., Oconnor, G., 2008. Nanoaluminum: Transport through sand columns and environmental effects on plants and soil communities. Environ. Res. 106, 296 – 303.

Drexler, K.E., 1986. Engines of Creation: The Coming Era of Nanotechnology. Doubleday. ISBN 0-385-19973-2.

Drexler, K.E., 1992. Nanosystems: Molecular machinery, manufacturing, and computation. New York: John Wiley & Sons. ISBN 0-471-57547-X.

EC (2009) Regulation (EC) No. 1107/2009 of the European parliament and the council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. Off. J. Eur. Union 2009, 309, 1−50

Ecobichon, D.J., 1991. Toxic effects of pesticides. In: Amdur MO, Donl J, Klassen CD, editors. Casarett and Doull's toxicology. 4th edition. New York: Pergamon Press. P 2 – 18.

Edwards, C.A., 1975. Factors that affect the persistence of pesticides in plants and soils. Pure and Applied Chemistry. 42 (1), 39 – 56.

Edwards, C.A., 1996. Biology and Ecology of Earthworms. 3rd Ed, Chapman and Hall, London. Springer.

Edwards, C.A., 2004. Earthworm Ecology, 2nd ed. CRC Press, Boca Raton, FL, London, New York, Washington.

Edwards, C.A., Bohlen, P.J., 1996. Biology and Ecology of Earthworms. Springer.

Edwards, R., Millburn, P., 1985. The metabolism and toxicity of insecticides to fish: In Progress in Pesticide Biochemistry and Toxicology, Vol. 5sInsecticides, Hutson, D. H., Roberts, T. R., Eds.; Wiley: New York. P 249 – 274.

Elek, N., Hoffman, R., Raviv, U., Resh, R., Ishaaya, I., Magdassi, S., 2010. Novaluron nanoparticles: Formation and potential use in controlling agricultural insect pests. Colloids and Surfaces A-Physicochemical and Engineering Aspects. 372, 66 – 72.

El-Nahhal, Y., Nir, S., Margulies, L., Rubin, B., 1999. Reduction of photodegradation and volatilisation of herbicides in organoclay formulations. Appl. Clay Sci. 14, 105 – 119.

El-Temsah, Y.S., Joner, E.J., 2012. Ecotoxicological effects on earthworms of fresh and aged nano-sized zero-valent iron (nZVI) in soil. Chemosphere. 89, 76 – 82.

Erickson, R.J., Bills, T.D., Clark, D.J., Hansen, J., Knezovich, J.F.L., Mayer, McElroy, A.E., 1994. Synopsis of discussion session on physicochemical factors affecting toxicity. In: Bioavailability Physical, Chemical, and Biological Interactions, edited by J.L. Hamelink, P.F. Landrum, H.L. Bergman, and W. H. Benson. Lewis Publishers, Boca Raton. P 31 – 38.

Eubeler, J.P., Bernhard, M., Knepper, T.P., 2010. Environmental biodegradation of synthetic polymers II. Biodegradation of different polymer groups. TrAC-Trends Anal Chem. 29 (1), 84 – 100.

Fang, J., Shan, X.Q., Wen, B., Lin, J.M., Owens, G., 2009. Stability of titania nanoparticles in soil suspensions and transport in saturated homogeneous soil columns. Environ. Pollut. 157, 1101 – 1109.

FAO and US, 1962. Soil Texture Classification: Weed Science, 33, Suppl. 1 (1985) and Soil Sci. Soc. Amer. Proc., 26, 305.

Fecko, A., 1999. Environmental Fate of Bifenthrin. Environmental Monitoring and Pest Management Branch Department of Pesticide Regulation 830 K St. Sacramento, CA 95814.

Feng, B.H., Peng, L.F., 2012. Synthesis and characterisation of carboxymethyl chitosan carrying ricinoleic functions as an emulsifier for azadirachtin. Carbohydrate Polymers. 88, 576 – 582.

Fernandez-Perez, M., Garrido-Herrera, F.J., Gonzalez-Pradas, E., 2011. Alginate and ligninbased formulations to control pesticides leaching in a calcareous soil. Hazardous Materials. 190, 794 – 801.

FIFRA-SAP, 2009. Evaluation of the hazard and exposure associated with nanosilver and other nanometal pesticide products. U.S. Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP); http://www.epa.gov/scipoly/sap/meetings/2009/november/110309ameetingminutes.pdf

Finnegan, M.P., Zhang, H.Z., Banfield, J.F., 2007. Phase stability and transformation in titania nanoparticles in aqueous solutions dominated by surface energy. Phys. Chem. C. 111, 1962 – 1968.

Finnegan, M.P., Zhang, H.Z., Banfield, J.F., 2008. Anatase coarsening kinetics under hydrothermal conditions as a function of pH and temperature. Chem. Mater. 20, 3443 – 3449.

Flores-Cespedes, F., Figueredo-Flores, C.I., Daza-Fernandez, I., Vidal-Pena, F., Villafrancasanchez, M., Fernandez-Perez, M., 2012. Preparation and characterisation of imidacloprid lignin-polyethylene glycol matrices coated with ethylcellulose. Agricultural and Food Chemistry. 60, 1042 – 1051.

FMC Corporation., 1983. Application for experimental use permit, Product chemistry. DRR report no. 50429-024, Department of pesticide regulation, Sacaramento CA.

Földényi, R., Tóth, Z., Samu, G., Érsek, C., 2013. Study of sorption of two sulfonylurea type of herbicides and their additives on soils and soil components. Environ. Sci. Health, Part B: Pesticides, Food Contaminants, and Agricultural Wastes. 48, 758 – 766.

Fontaine, D.D., Lehmann, R.G., Miller, J.R., 1991. Soil adsorption of neutral and anionic forms of a sulfonamide herbicide, flumetsulam. Environmental Quality. 20, 759 – 762.

Food and Agriculture Organisation of the United Nations (FAO)., 2002. International Code of Conduct on the Distribution and Use of Pesticides. https://en.wikipedia.org/wiki/Pesticide. Accessed on September 2016.

Ford, S.C., Price, O.R., Terry, A.S., 2007. Transport of pesticides to water from slow release formulations: application of current pesticide fate models. In: Del Re, A.A.M., Capri, E., Fragoulis, G., Trevisan, M., (eds) Environmental fate and ecological effects of pesticides. La Goliarica Pavese, Pavia, IT

Forouzangohar, M, Kookana, R.S., 2011. Sorption of nano-C60 clusters in soil: hydrophilic or hydrophobic interactions? Environ. Monit. 13 (5), 1190 – 1194.

Franco, A., Fu, W.J., Trapp, S., 2009. Influence of soil pH on the sorption of ionizable chemicals: Modeling advances. Environ. Toxicol. Chem. 28, 458 – 464.

Frandsen, M.V., Pedersen, M.S., Zellweger, M., Gouin, S., Roorda, S.D., Phan, T.Q.C., 2010. Piperonyl butoxide and deltamethrin containing insecticidal polymer matrix comprising HDPE and LDPE. Patent Number WO 2010015256 A2 20100211.

Frederiksen, H.K., Kristensen, H.G., Pedersen, M., 2003. Solid lipid microparticle formulations of the pyrethroid gamma-cyhalothrin-incompatibility of the lipid and the pyrethroid and biological properties of the formulations. Control Release. 86, 243 – 252.

Frederiksen, H.K., Kristensen, H.G., Pedersen, M., 2003. Solid lipid microparticle formulations of the pyrethroid gamma-cyhalothrin-incompatibility of the lipid and the pyrethroid and biological properties of the formulations. Control. Release. 86 (2 – 3), 243 – 252.

French, R.A., Jacobson, A.R., Kim, B., Isley, S.L., Penn, R.N., Baveye, P.C., 2009. Influence of ionic strength, pH, and cation valence on aggregation kinetics of titanium dioxide nanoparticles. Environ. Sci. Technol. 43 (5), 1354 – 1359.

Fritz, B.K., Hoffmann, W.C., 2008. Atmospheric effects on fate of aerially applied agricultural sprays. Agric. Eng. Int. 10, PM08 008.

Froelich, L.W., 1983. Soil adsorption/ desorption characteristics of FMC 54800. FMC report P-0797; EPA MRID 141203.

Fukushima, M., Tatsumi, K., 2001. Functionalities of humic acid for the remedial processes of organic pollutants. Anal. Sci. 17, 821 – 823.

Gala´n-Jime´nez MdC, Mishael Y-G, Nir S, Morillo E, Undabeytia T (2013) Factors affecting the design of slow release formulations of herbicides based on clay-surfactant systems A methodological approach. PloS One 8(3):e59060

Gan, N., Yang, X., Xie, D., Wu, Y., Wen, W.A., 2010. Disposable organophosphorus pesticides enzyme biosensor based on magnetic composite nanoparticles modified screen printed carbon electrode. Sensors. 10, 625 – 638.

Garcia, M., Scheffczyk, A., Garcia, T., Rombke, J., 2011. The effects of the insecticide lambda-cyhalothrin on the earthworm *Eisenia fetida* under experimental conditions of tropical and temperate regions. Environ. Pollut. 159, 398 – 400.

García-Alonso, J., Khan, F.R., Misra, S.K., Turmaine, M., Smith, B.D., Rainbow, P.S., Luoma, S.N., Valsami-Jones, E., 2011. Cellular internalisation of silver nanoparticles in gut epithelia of the estuarine polychaete *Nereis diversicolor*. Environ. Sci. Technol. 45 (10), 4630 – 4636.

García-Gómez., C., babin, M., Obrador, A., Álvarez, J.M., Fernández, M.D., 2014. Toxicity of ZnO nanoparticles, ZnO bulk, and $ZnCl_2$ on earthworms in spkied natural soil and toxicological effects of leachates on aquatic organisms. Arch. Environ. Contam. Toxicol. 67, 465 – 473.

Gavrilescu, M., 2005. Fate of pesticides in the environment and its bioremediation. Eng. Life Sci. 5 (6).

Gawlik, B.M., Sotiriou, N., Feicht, E.A., Schulte-Hostede, S., Kettrup, A., 1997. Alternatives for the determination of the soil adsorption coeffcient Koc, of non-ionic organic compounds-a review. Chemosphere. 34 (12), 2525 – 2551.

Geranio, L., Heuberger, M., Nowack, B., 2009. The behaviour of silver nanotextiles during washing. Environ. Sci. Technol. 43, 8113 – 8118.

Gevao, B., Mordaunt, C., Semple, K.T., Piearce, T.G., Jones, K.C., 2001. Bioavailability of nonextractable (bound) pesticide residues to earthworms. Environ. Sci. Technol. 35, 501 – 507.

Ghadiri, H., Rose, C.W., 2001. Degradation of endosulfan in a clay soil from cotton farms of western Queensland. Environ. Manage. 62, 155 – 169.

Ghormade, V., Deshpande, M.V., Paknikar, K.M., 2011. Perspectives for nano-biotechnology enabled protection and nutrition of plants, biotechnology advances. 29 (6), 792 – 803.

Gilbert, B., Lu, G.P., Kim, C.S., 2007. Stable cluster formation in aqueous suspensions of iron oxyhydroxide nanoparticles. Colloid Interface Sci. 313, 152 – 159.

Giovanetti, A. Fesenko, S., Cozzella, M.L., Asencio, L.D., Sansone, U., 2010. Bioaccumulation and biological effects in the earthworm *Eisenia fetida* exposed to natural and depleted uranium. Environ. Radioact. 101 (6), 509 – 516.

Gobas, F.A.P.C., Zhang, X., 1994. Interactions of organic chemicals with particulate and dissolved organic matter in the aquatic environment. In: Bioavailability Physical, Chemical, Biological Interactions, edited by J.L. Hamelink, P.F. Landrum, H.L. Bergman, and W. H. Benson. Lewis Publishers, Boca Raton. P 83 – 91.

Gogos, A., Knauer, K., Bucheli, T., 2012. Nanomaterials in plant protection and fertilization: current state, foreseen applications, and research priorities. Agric. Food Chem. 60, 9781 – 9792.

Goldshtein, R., Jaffe, I., Tulbovich, B., 2005. Hydrophilic dispersions of nanoparticles of inclusion complexes of amorphous compounds. Patent Number US 20050249786 A1 20051110.

Green, J.M., Beestman, G.B., 2007. Recently patented and commercialized formulation and adjuvant technology. Crop Protection. 26, 320 – 327.

Grillo, R., AdE, S.P., Silva de Melo, N.F., Porto, R.M., Feitosa, L.O., Tonello, P.S., Dias Filho, N.L., Rosa, A.H., Lima, R., Fraceto, L.F., 2011. Controlled release system for ametryn using polymer microspheres: Preparation, characterisation and release kinetics in water. Hazard Mater. 186 (2 – 3), 1645 – 1651.

Grillo, R., Melo, N.F.S., De Lima, R., Lourenço, R.W., Rosa, A.H., Fraceto, L.F., 2010. Characterisation of atrazine-loaded biodegradable poly(hydroxybutyrate-co-hydroxyvalerate) microspheres. Polym. Environ. 18, 26 – 32.

Grillo, R., Pereira dos Santos, N.Z., Maruyama, C.R., Rosa, A.H., De Lima, R., Fraceto, L.F., 2012. Poly(epsilon-caprolactone) nanocapsules as carrier systems for herbicides: Physico-chemical characterisation and genotoxicity evaluation. Hazard Mater. 231, 1 – 9.

Grumiaux, F., Demuynck, S., Schikorski, D., Lemiere, S., Lepretre, A., 2010. Assessing the effects of FBC ash treatments of metal-contaminated soils using life history traits and metal bioaccumulation analysis of the earthworm *Eisenia andrei*. Chemosphere 79, 156 – 161.

Guan, H. A., Chi, D. F., Yu, J., and Li, H. (2010). Dynamics of residues from a novel nano-imidacloprid formulation in soyabean fields. Crop Protection, 29, 942–946.

Guan, H., Chi, D., Yu, J., Li, X., 2008. A novel photodegradable insecticide: Preparation, characterisation and properties evaluation of nano-imidacloprid pesticide. Biochem. Physiol. 92, 83 – 91.

Guan, H.N., Chi, D.F., Yu, J., Zhang, S.Y., 2011. Novel photodegradable insecticide W/TiO(2)/avermectin nanocomposites obtained by polyelectrolytes assembly. Colloids and Surfaces B: Biointerfaces, 83, 148 – 154.

Guangyao, S., Yaning, Y., Minsheng, H., Kai, Y., 2005. Influence of pH on pesticide sorption by soil containing wheat residue-derived char. Environ Pollut. 134, 457 – 463.

Gunadi, B., Edwards, C.A., Blount IV, C., 2003. The influence of different moisture levels on the growth, fecundity and survival of *Eisenia fetida* (Savigny) in cattle and pig manure solids. Soil Biol. 39 (1), 19 – 24.

Guo, Y.R., Liu, S.H., Gui, W.J., Zhu, G.N., 2009. Gold immunochromatographic assay for simultaneous detection of carbofuran and triazophos in water samples. Anal Biochem. 389, 32 – 39.

Gupta, S., Kushwah, T., Yadav, S., 2014. Earthworm coelomocytes as nanoscavenger of ZnO NPs. Nanoscale Research Letters. 9, 259.

Gupta, S., Yadav, S., 2014. Bioaccumulation of ZnO-NPs in earthworm *Eisenia fetida* (Savigny). Bioremed Biodeg. 5, 250.

Gustafson, D.I., 1989. Groundwater ubiquity score: A simple method for assessing pesticide leachability. Environmental Toxicology and Chemistry. 8 (4), 339 – 357.

Gustafson, D.I., 2011. Climate change: a crop protection challenge for the twenty-first century. Pest Management Science. 67 (6), 691 – 696.

Gutierrez, J.M., Gonzalez, C., Maestro, A., Sole, I., Pey, C.M., Nolla, J., 2008. Nano-emulsions: New applications and optimization of their preparation. Current Opinion in Colloid & Interface Science. 13, 245 – 251.

Guyodo, Y., Mostrom, A., Penn, R.L., and Banerjee, S.K., 2003. From nanodots to nanorods: Oriented aggregation and magnetic evolution of nanocrystalline goethite. Geophys. Res. Lett. 30, 4.

Ha, L.S., Sung-Dae, K., Yi, Y.S., Byeong-Gweon, L., 2014. Bioaccumulation of Zn and Ag nanoparticles in the earthworms (*Eisenia fetida*). EGU General Assembly Conference Abstracts. 16, 1823.

Hamaker, J.W., Thompson, J.M., 1972. In: Organic Chemicals in the Soil Environment, edited by C. M. Goring and J. W. Hamaker, Marcel Dekker, New York, Vol. 1, pp. 49–143.

Handy, R.D., Cornelis, G., Fernandes, T.F., Tsyusko, O., Decho, A., Sabo-Attwood, T., Metcalfe, C., Steevens, J., Klaine, S.J., Koelmans, A.A., Horne, N., 2012a. Ecotoxicity test methods for engineered nanomaterials: Practical experiences and recommendations from the bench. Environ. Toxicol. Chem. 31 (1), 15 – 31.

Handy, R.D., Owen, R., Valsami-Jones, E., 2008. The ecotoxicology of nanoparticles and nanomaterials: current status, knowledge gaps, challenges, and future needs. Ecotoxicology. 17, 315 – 325.

Handy, R.D., Van den Brink, N., Chappell, M., Muhling, M., Behra, R., Dusinska, M., Simpson, P., Ahtiainen, J., Jha, A.N., Seiter, J., Bednar, A., Kennedy, A., Fernandes, T.F., Riediker, M., 2012b. Practical considerations for conducting ecotoxicity test methods with manufactured nanomaterials: what have we learned so far? Ecotoxicology. 21 (4), 933 – 972.

Hans, R.K., Farooq, M., 2000. Dissipation and accumulation kinetics of lindane in soil and earthworms. Pollut Res. 19, 394 – 396.

Hansch, C., Leo, A., Hoekman, D., 1995. Exploring QSAR – Hydrophobic, electronic, and steric constants. Washington, DC: American Chemical Society. P 180.

Harper, S.S., 1994. Sorption-desorption and herbicide behaviour in soil. Rev. Weed Sci. 6, 207 – 225.

Hartnik, T., Styrishave, B., 2008. Impact of biotransformation and bioavailability on the toxicity of the insecticides α-cypermethrin and chlorfenvinphos in earthworm. Agric. Food Chem. 56 (22), 11057 – 11064.

Heckmann, L.H., Hovgaard, M.B., Sutherland, D.S., Autrup, H., Besenbacher, F., Scott-Fordsmand, J.J., 2011. Limit-test toxicity screening of selected inorganic nanoparticles to the earthworm *Eisenia fetida*. Ecotoxicol. 20, 226 – 233.

Heggelund, L.R., Diez-Ortiz, M., Lofts, S., Lahive, E., Jurkschat, K., Wojnarowicz, J., Cedergreen, N., Spurgeon, D., Svendsen, C., 2014. Soil pH effects on the comparative toxicity of dissolved zinc, non-nano and nano ZnO to the earthworm *Eisenia fetida*. Nanotoxicology. 8 (5), 559 -572.

Heikens, A., Peijnenburg, W., Hendriks, A.J., 2001. Bioaccumulation of heavy metals in terrestrial invertebrates. Environ. Pollut. 113, 385 – 393.

Hellmann, C., Greiner, A., Wendorff, J.H., 2011. Design of pheromone releasing nanofibers for plant protection. Polymers for Advanced Technologies. 22, 407 – 413.

Henriksen, T., Svensmark, B., Juhler, R.K., 2004. Degradation and sorption of metribuzin and primary metabolites in a sandy soil. Environ. Qual. 33, 619 – 628.

Hermens, J.L.M., De Bruijn, J.H.M., Brooke, D.N., 2013. Environ. Toxicol. Chem. 32, 732 – 733.

Herrero-Vanrell, R., Rincón, A.C., Alonso, M., Reboto, V., Molina-Martinez, I.T., Rodríguezcabello, J.C., 2005. Self-assembled particles of an elastin-like polymer as vehicles for controlled drug release. Control Rel. 102, 113 – 122.

Hinton, J.J., Veiga, M.M., 2008. The influence of organic acids on mercury bioavailability: Insight from an earthworm assessment protocol. Environ. Bioindic. 3, 47 – 67.

Ho, Y.S., 2004. Selection of optimum sorption isotherm. Carbon. 42, 2115 – 2116.

Ho, Y.S., Porter, J.F., McKay, G., 2002. Equilibrium isotherm studies for the sorption of divalent metal ions onto peat: copper, nickel and lead single component systems. Water Air Soil Pollut. 141 (1 – 4), 1 – 33.

Hobbelen, P.H.F., Koolhaas, J.E., Van Gestel, C.A.M., 2006. Bioaccumulation of heavy metals in the earthworms *Lumbricus rubellus* and *Aporrectodea caliginosa* in relation to total and available metal concentrations in field soils. Environ. Pollut. 144, 639 – 646.

Hoecke, K.V., Quik, J.T.K., Mankiewicz-Boczek, J., Schamphelaere, K.A.C.D., Elsaesser, A., Meeren, P.V.D., Barnes, C., McKerr, G., Howard, C.V., Meent, D.V.D., Rydzynski, K., Dawson, K.A., Salvati, A., Lesniak, A., Lynch, I., Silversmit, G., de Samber, B., Vincze, L., Janssen, C.R., 2009. Fate and effects of CeO_2 nanoparticles in aquatic ecotoxicity tests. Environ. Sci. Technol. 43, 4537 – 4546.

Hooper, H.L., Jurkschat, K., Morgan, A.J., Bailey, J., Lawlor, A.J., Spurgeon, D.J., Svendsen, C., 2011. Comparative chronic toxicity of nanoparticulate and ionic zinc to the earthworm *Eisenia veneta* in a soil matrix. Environ Intern. 37 (6), 1111 – 1117.

Hou, W.C., Westerhoff, P., Posner, J.D., 2013. Biological accumulation of engineered nanomaterials: A review of current knowledge. Environ. Sci.: Processes Impacts. 15, 103 – 122.

Hristovski, K.D., Westerhoff, P.K., Posner, J.D., 2011. Environ. Sci. Health, Part A: Toxic/Hazard. Subst. Environ. Eng. 46, 636 – 647.

Hsiao, I.L., Hsieh, Y.K., Wang, C.F., Chen, I.C., Huang, Y.J., 2015. Trojan-horse mechanism in the cellular uptake of silver nanoparticles verified by direct intra- and extracellular silver speciation analysis. Environ. Sci. Technol. 49 (6), 3813 – 3821.

Hu, C.W., Li, M., Cui, Y.B., Chen, J., Yang, L.Y., 2010. Toxicological effects of TiO_2 and ZnO nanoparticles in soil on earthworm Eisenia fetida. Soil Biol Biochem. 42, 586 – 591.

Hua, R.M., Spliid, N.H., Heinrichson, K., Laursen, B., 2009. Influence of surfactants on the leaching of bentazone in a sandy loam soil. Pest Management Science. 65, 857 – 861.

Hund-Rinke, K., Schlich, K., Klawonn, T., 2012. Influence of application techniques on the ecotoxicological effects of nanomaterials in soil. Environ Sci. Eur. 24, 30.

Hussein MZ, Nazarudin NFB, Sarijo SH, Yarmo MA (2012) Synthesis of a layered organic-inorganic nanohybrid of 4-chlorophenoxyacetate-zinc-layered hydroxide with sustained release properties. J Nanomater 2012:1–9

Hwang, I.C., Kim, T.H., Bang, S.H., Kim, K.S., Kwon, H.R., Seo, M.J., Youn, Y.N., Park, H.J., Yasunaga-Aoki, C., Yu, Y.M., 2011. Insecticidal effect of controlled release formulations of etofenprox based on nano-bio technique. Fac. Agric. Kyushu Univ. 56 (1), 33 – 40.

Hyun, S., Lee, L.S., 2004. Factors controlling sorption of prosulfuron by variable-charge soils and model sorbents. J Environ Qual. 33, 1354 – 1361.

Hyun, S., Lee, L.S., 2005. Quantifying the contribution of different sorption mechanisms for 2,4-dichlorophenoxyacetic acid sorption by several variable-charge soils. Environ Sci Technol. 39, 2522 – 2528.

Isaacson, C.W., Kleber, M., Field, J.A., 2009. Quantitative analysis of fullerene nanomaterials in environmental systems: A critical review. Environ. Sci. Technol. 43, 6463 – 6474.

Isiklan, N., 2004. Controlled release of insecticide carbaryl from crosslinked carboxymethylcellulose beads. Fresenius Environmental Bulletin. 13, 537 – 544.

Ismail, B. S., Kalithasan, K., 2004. Adsorption, desorption, and mobility of permethrin in Malaysian soils. Environ. Sci. Health. B39, 419 – 429.

ISO., 1993. Soil quality: Determination of dry matter and water content on a mass basis-Gravimetric method. ISO 11465.

ISO., 1994. Soil quality: Determination of pH. ISO 10390.

Jager, T., 1998. Mechanistic approach for estimating bioconcentration of organic chemicals in earthworms (Oligochaeta). Environ. Toxicol. Chem. 17, 2080 – 2090.

Jager, T., Fleuren, R., Hogendoorn, E.A., De Korte, G., 2003. Elucidating the routes of exposure for organic chemicals in the earthworm, *Eisenia Andrei* (Oligochaeta). Environ. Sci. Technol. 37 (15), 3399 – 3404.

Jahnel, J., Fleischer, T., Seitz, S.B., 2013. Risk assessment of nanomaterials and nanoproducts – Adaptation of traditional approaches. Physics: Conference Series. 429 (2013) 012063.

Jaisi, D., Elimelech, M., 2009. Single-walled carbon nanotubes exhibit limited transport in soil columns. Environ. Sci. Technol. 43, 9161 – 9166.

Jana, T., Roy, B.C., Maiti, S., 2001. Biodegradable film: 6. Modification of the film for control release of insecticides. European Polymer. 37, 861 – 864.

Janssen, R.P.T., Posthuma, L., Baerselman, R., DenHollander, H.A., vanVeen, R.P.M., Peijnenburg, J.G.M., 1997. Equilibrium partitioning of heavy metals in Dutch field soils. II. Prediction of metal accumulation in earthworms. Environ. Toxicol. Chem. 16, 2479 – 2488.

Jaynes, W.F., Boyd, S.A., 1991. Hydrophobicity of siloxane surfaces in smectities as revealed by aromatic hydrocarbon adsorption from water. Clays and Clay Minerals. 39 (4), 428 – 436.

Jerobin, J., Sureshkumar, R.S., Anjali, C.H., Mukherjee, A., Chandrasekaran, N., 2012. Biodegradable polymer based encapsulation of neem oil nanoemulsion for controlled release of Aza-A. Carbohydr Polym. 90 (4), 1750 – 1756.

Jiang L, Mo J, Kong Z, Qin Y, Dai L, Wang Y, Ma L (2015) Effects of organobentonites on imidacloprid release from alginate-based formulation. Appl Clay Sci 105–106:52–59

Jino Son, Louisa A. Hooven, Bryan Harper, Stacey L. Harper, Effect of pH and ionic strength on exposure and toxicity of encapsulated lambda–cyhalothrin to Daphnia magna, Science of The Total Environment, Volume 538, 15 December 2015, Pages 683-691

Jo, Y.K., Kim, B.H., Jung, G., 2009. Antifungal activity of silver ions and nanoparticles on phytopathogenic fungi. Plant Disease. 93, 1037 – 1043.

Joo, S.H., Al-Abed, S.R., Luxton, T., 2009. Influence of carboxymethyl cellulose for the transport of titanium dioxide nanoparticles in clean silica and mineral-coated sands. Environ. Sci. Technol. 43, 4954 – 4959.

Joshi, K.A., Tang, J., Haddon, R., Wang, J., Chen, W., Mulchandani, A., 2005. A disposable biosensor for organophosphorus nerve agents based on carbon nanotubes modified thick film strip electrode. Electroanalysis. 17, 54 – 58.

Ju-Nam, Y., Lead, J.R., 2008. Manufactured nanoparticles: An overview of their chemistry, interactions and potential environmental implications. Sci. Total Environ. 400, 396 – 414.

Jung, J.H., Kim, S.W., Min, J.S., Kim, Y.J., Lamsal, K., Kim, K.S., Lee, Y.S., 2010. The effect of nano-silver liquid against the white rot of the green onion caused by *Sclerotium cepivorum*. Mycobiology. 38, 39 – 45.

Kaegi, R., Ulrich, A., Sinnet, B., Vonbank, R., Wichser, A., Zuleeg, S., Simmler, H., Brunner, S., Vonmont, H., Burkhardt, M., and Boller, M., 2008. Synthetic TiO_2 nanoparticle emission from exterior facades into the aquatic environment. Environ. Pollut. 156, 233 – 239.

Kah, M., Beulke, S., Brown, C.D., 2007. Factors influencing degradation of pesticides in soil. Agric. Food Chem. 55, 4487 – 4492.

Kah, M., Beulke, S., Tiede, K., Hofmann, T., 2013. Nano-pesticides: State of knowledge, environmental fate and exposure modelling. Crit. Rev. Environ. Sci. Technol. 43 (16), 1823 – 1867.

Kah, M., Brown, C.D., 2006. Adsorption of ionisable pesticides in soils. Rev. Environ. Contam. Toxicol. 188, 149 – 218.

Kah, M., Brown, C.D., 2007. Changes in pesticide adsorption with time at high soil to solution ratios. Chemosphere. 68, 1335 – 1343.

Kah, M., Hofmann, T., 2014. Nanopesticides research: Current trends and future priorities. Environ. Int. 63, 224 – 235.

Kah, M., Weniger, A.K., Hofmann, T., 2016. Impacts of (nano)formulations on the fate of an insecticide in soil and consequences for environmental exposure assessment. Environ. Sci. Technol. 50 (20), 10960 – 10967.

Kah, M., Machinski, P., Koerner, P., Tiede, K., Grillo, R., Fraceto, L.F., Hofmann, T., 2014. Analysing the fate of nanopesticides in soil and the applicability of regulatory protocols using a polymer-based nanoformulation of atrazine. Environ Sci Pollut Res Int. 21 (20), 11699 – 11707.

Kahru, A., Dubourguier, H.C., Blinova, I., Ivask, A., Kasemets, K., 2008. Biotests and biosensors for ecotoxicology of metal oxide nanoparticles: A mini review. Sensors. 8, 5153 – 5170.

Kale, S.P., Murthy, N.B.K., Raghu, K., 2001. Degradation of C14- carbofuran in soil using a continuous flow system. Chemosphere. 44, 893 – 895.

Kalia, A., Gosal, S.K., 2011. Effect of pesticide application on soil microorganisms, Archives of Agronomy and Soil Science. 57 (6), 569 – 596.

Kang, M.A., Seo, M.J., Hwang, I.C., Jang, C., Park, H.J., Yu, Y.M., Youn, Y.N., 2012. Insecticidal activity and feeding behavior of the green peach aphid, *Myzus Persicae*, after treatment with nano types of pyrifluquinazon. Asia-Pac Entomol. 15 (4), 533 – 541.

Kannan, K., Tanabe, S., Giesy, J.P., Tatsukawa, R., 1997. Organochlorine pesticides and polychlorinated biphenyls in foodstuffs from Asian and oceanic countries. Rev Environ Contam Toxicol. 152, 1 – 55.

Katagi, T., 1991. Photodegradation of the pyrethroid insecticide esfenvalerate on soil, clay minerals, and humic acid surfaces. Agricultural and Food Chemistry. 39, 1351 – 1356.

Katagi, T., 2008. Surfactant effects on environmental behaviour of pesticides. Reviews of Environmental Contamination and Toxicology. 194, 71 – 177.

Katagi, T., 2012. Environmental behavior of synthetic pyrethroids. Top. Curr. Chem. 314, 167 – 202. OECD, 2004. OECD Guidelines for the Testing of Chemicals. Test No 312: "Leaching in Soil Columns". OECD Publishing.

Kaushik, A., Solanki, P.R., Ansarib, A.A., Malhotra, B.D., Ahmad, S., 2009. Iron oxide-chitosan hybrid nanobiocomposite based nucleic acid sensor for pyrethroid detection. Biochem Engineer. 46, 132 – 140.

Kaushik, P., Shakil, N.A., Kumar, J., Singh, M.K., Yadav, S.K., 2013. Development of controlled release formulations of thiram employing amphiphilic polymers and their bioefficacy evaluation in seed quality enhancement studies. Environ Sci Health B. 48 (8), 677 – 685.

Keller, A., Wang, H., Zhou, D., Lenihan, H.S., Cherr, G., Cardinale, B.J., Miller, R., Ji, Z., 2010. Stability and aggregation of metal oxide nanoparticles in natural aqueous matrices. Environ. Sci. Technol. 44, 1962 – 1967.

Kerle, E.A., Jenkins, J.J., Vogue, P.A., 2007. Understanding pesticide persistence and mobility for groundwater and surface water protection. Oregon State Univ Extension Service, EM8561-E.

Khan, M. A., 2016. Impacts of co-formulants on pesticide sorption and leaching through soil. PhD thesis, University of York.

Khan, S.U., 1980. Pesticide in the soil environment. Elsevier Scientific Publishing Company. P 29 – 56.

Khan, S.U., Schnitzer, M., 1972. The retention of hydrophobic organic compounds by humic acid. Geochim. Cosmochim Acta. 36, 745 – 754.

Khot, L.R., Sankaran, S., Maja, J.M., Ehsani, R., Schuster, E., 2012. Application of nanomaterials in agricultural production and crop protection: A review. Crop Prot. 35, 64 – 70.

Killham, K., 1994. Soil Ecology. Cambridge University Press, Cambridge.

Kim, B., Park, C.S., Murayama, M., Hochella, M.F., 2010. Discovery and characterisation of silver sulphide nanoparticles in final sewage sludge products. Environ. Sci. Technol. 44 (19), 7509 – 7514.

Klaine, S.J., Alvarez, P.J.J., Batley, G.E., Fernandes, T.F., Handy, R.D., Lyon, D.Y., Mahendra, S., McLaughlin, M.J., Lead, J.R., 2008. Nanomaterials in the environment: Behavior, fate, bioavailability, and effects. Environ. Toxicol. Chem. 27, 1825 – 1851.

Knowles, A., 2005. New developments in crop protection product formulation. Agrow Report.

Kodaka, R., Sugano, T., Katagi, T., Takimoto, Y., 2003. Clay-catalyzed nitration of a carbamate fungicide diethofencarb. Agric. Food Chem. 51 (26), 7730 – 7737.

Koelmans, A.A., Nowack, B., Wiesner, M.R., 2009. Comparison of manufactured and black carbon nanoparticle concentrations in aquatic sediments. Environ. Pollut. 157, 1110 – 1116.

Kok, F.N., Wilkins, R.M., Cain, R.B., Arica, M.Y., Alaeddinoglu, G., Hasirci, V., 1999. Controlled release of aldicarb from lignin loaded ionotropic hydrogel microspheres. Microencapsulation.16, 613 – 623.

Kookana, R.S., Boxall, A.B.A., Reeves, P.T., Ashauer, R., Beulke, S., Chaudhry, Q., Cornelis, G., Fernandes, T.F., Gan, J., Kah, M., Lynch, I., Ranville, J., Sinclair, C., Spurgeon, D., Tiede, K., Van Den Brink, P.J., 2014. Nanopesticides: Guiding principles for regulatory evaluation of environmental risks. Agricultural and Food Chemistry. 62 (19), 4227 – 4240.

Kozak, J., Weber, J.B., Sheets, T.J., 1983. Adsorption of prometryn and metolachlor by selected soil organic matter fractions. Soil Sci. 136, 94 – 101.

Kozak, J., Weber, J.B., Sheets, T.J., 1983. Adsorption of prometryn and metolachlor by selected soil organic matter fractions. Soil Science. 136, 94 – 101.

Kranthi, K.R., Jadhav, D.R., Kranthi, S., Wanjari, R.R., Ali, S.S., Russell, D.A., 2002. Insecticide resistance in five major insect pests of cotton in India. Crop Prot. 21, 449 – 460.

Kuempel, E.D., Geraci, C.L., Schulte, P.A., 2012. Risk assessment and risk management of nanomaterials in the workplace: Translating research to practice. Ann. Occup. Hyg. 56 (5), 491 – 505.

Kula, H., Larink, O., 1997. Development and standardization of test methods for the prediction of sublethal effects of chemicals on earthworms. Soil Biol Biochem. 29, 635 – 639.

Kulkarni, A.R., Soppimath, K.S., Aminabhavi, T.M., Dave, A.M., Mehta, M.H., 1999. Application of sodium alginate beads crosslinked with glutaraldehyde for controlled release of pesticide. Polymers News. 2, 285 – 286.

Kumar, J., Shakil, N.A., Singh, M.K., Pandey, A., Pandey, R.P., 2010. Development of controlled release formulations of azadirachtin: A employing poly(ethylene glycol) based amphiphilic copolymers. Environmental Science and Health Part B: Pesticides Food Contaminants and Agricultural Wastes. 45, 310 – 314.

Kümmerer, K., Menz, J., Schubert, T., Thielemans, W., 2010. Biodegradability of organic nanoparticles in the aqueous environment. Chemosphere. 82 (10), 1387 – 1392.

Kurepa, J., Paunesku, T., Vogt, S., Arora, H., Rabatic, B.M., Lu, J., Wanzer, M.B., Woloschak, G.E., Smalle, J.A., 2010. Uptake and distribution of ultrasmall anatase TiO_2 alizarin red S Nanoconjugates in *Arabidopsis Thaliana*. Nano Lett. 10, 2296 – 2302.

Kwak, J.II., An, Y.J., 2015. Ecotoxicological effects of nanomaterials on earthworms: A review. Human and Ecological risk Assessment: An International Journal. 21 (6), 1566 – 1575.

Laabs, V., Amelung, W., Pinto, A., Zech, W., 2002. Fate of pesticides in tropical soils of Brazil under field conditions. Environmental Quality. 31 (1), 256 – 268.

Ladlie, J.S., Meggitt, W.F., Penner, D., 1976. Effect of pH on microbial degradation, adsorption, and mobility of metribuzin. Weed Sci. 24, 477 – 481.

Lahive, E., jurkschat, K., Shaw, B.J., Handy, R.C., Spurgeon, D.J., Svendsen, C., 2014. Toxicity of cerium oxide nanoparticles to the earthworm *Eisenia fetida* subtle effects. Environmental Chemistry. 11 (3), 268 – 278.

Lai, F., Wissing, S.A., Müller, R.H., Fadda, A.M., 2006. *Artemisia Arborescens* L essential oil–loaded solid lipid nanoparticles for potential agricultural application: Preparation and characterisation. AAPS Pharm Scitech. 7, 1 – 9.

Laird, D.A., Barriuso, E., Dowdy, R.H., Koskinen, W.C., 1992. Adsorption of atrazine on smectites. Soil Sci. Soc. 56, 62 – 67.

Laird, D.A., Fleming, P.D., 1999. Mechanisms for adsorption of organic bases on hydrated smectite surfaces. Environ. Toxicol. Chem. 18, 1668 – 1672.

Lakraimi, M., Legrouri, A., Barroug, A., De Roy, A., Besse, J.P., 2000. Preparation of a new stable hybrid material by chloride-2,4-dichlorophenoxyacetate ion exchange into the zinc–aluminium–chloride layered double hydroxide. Mater Chem. 10, 1007 – 1111.

Lambert, S.M., 1967. Functional relation between sorption in soil and chemical structure. Agric. Food Chem. 15 (4), 572 – 576.

Lambert, S.M., 1968. Omega, a useful index of soil sorption equilibria. Agric. Food Chem. 16, 340 – 343.

Lambert, S.M., Porter, P.E., Schieferstein, H., 1965. Movement and sorption of chemicals applied to the soil. Weeds. 13, 185 – 190.

Landrum, M., Cañas, J.E., Coimbatore, G., Cobb, G.P., Jackson, W.A., Zhang, B.H., Anderson, T.A., 2006. Effects of perchlorate on earthworm (*Eisenia fetida*) survival and reproductive success. Sci. Total. Environ. 363, 237 – 244.

Langdon, C.J., Hodson, M.E., Arnold, R.E., Black, S., 2005. Survival, Pb-uptake and behaviour of three species of earthworm in Pb treated soils determined using an OECD-style toxicity test and a soil avoidance test. Environ. Pollut. 138, 368 - 375.

Lao, S.B., Zhang, Z.X., Xu, H.H., Jiang, G.B., 2010. Novel amphiphilic chitosan derivatives: Synthesis, characterisation and micellar solubilization of rotenone. Carbohydrate Polymers. 82, 1136 – 1142.

Lapied, E., Moudilou, E., Exbrayat, J.M., Oughton, D.H., Joner, E.J., 2010. Silver nanoparticle exposure causes apoptotic response in the earthworm *Lumbricus terrestris* (Oligochaeta). Nanomedicine. 5 (6), 975 – 984.

LaPrade, J.C., 1992. Extension Environmental Specialist, Alabama Cooperative Extension System, Community Resource Development, Auburn University. http://www.aces.edu/pubs/docs/A/ANR-0737/ANR-0737.pdf. Accessed on September 2016.

Larsbo, M., Stenstrom, J., Etana, A., Borjesson, E., Jarvis, N.J., 2009. Herbicide sorption, degradation, and leaching in three Swedish soils under long-term conventional and reduced tillage. Soil Tillage Res. 105, 200 – 208.

Laskowski, D. A., 2002. Physical and chemical properties of pyrethroids. Rev. Environ. Contam. Toxicol. 174, 49 – 170.

Lawrence, M.J., Rees G.D., 2000. Microemulsion-based media as novel drug delivery systems. Adv. Drug Delivery Rev. 45 (1), 89 – 121.

Lawrence, M.J., Warisnoicharoen, W., 2006. Recent advances in microemulsions as drug delivery vehicles. In V. P. Torchilin (Ed.), Nanoparticles as drug carriers. London, England: Imperial College Press.

Lead, J.R., Wilkinson, K.J., 2006. Aquatic colloids and nanoparticles: Current knowledge and future trends. Environ. Chem. 3, 159 – 171.

Lebedev, S., Yausheva, E., Galaktionova, L., Sizova, E., 2015. Impact of Zn nanoparticles on growth, survival and activity of antioxidant enzymes in *Eisenia fetida*. Modern Applied Science. 9 (10).

Lee, G., Tadros, T., 1982. Formation and stabilisation of emulsions produced by dilution of cationic and a non-ionic surfactants. Colloids Surf. 5, 105.

Lee, L.S., Rao, P.S.C., Nkedi-Kizza, P., Delfino, J.J., 1990. Influence of solvent and sorbent characteristics on distribution of pentachlorophenol in octanol-water and soil-water systems. Environ Sci Technol. 24, 654 – 661.

Li, D., Alvarez, P.J.J., 2011. Avoidance, weight loss, and cocoon production assessment for *Eisenia fetida* exposed to C_{60} in soil. Environmental Toxicology and Chemistry. 30, 2542 – 2545.

Li, D., Fortner, J.D., Johnson, D.R., Chen, C., Li, Q., Alvarez, P.J.J., 2010. Bioaccumulation of $^{14}C_{60}$ by the earthworm *Eisenia Fetida*. Environ. Sci. Technol. 44, 9170 – 9175.

Li, J., Yao, J., Li, Y., Shao, Y., 2012. Controlled release and retarded leaching of pesticides by encapsulating in carboxymethyl chitosan/bentonite composite gel. Environ. Sci. Health B. 47 (8), 795 – 803.

Li, K., Xing, B, Torello, W.A., 2005. Effects of organic fertilizer derived dissolved organic matter on pesticide sorption and leaching. Environmental Pollution. 134, 187 – 194.

Li, L.Z., Zhou, D.M., Willie, J.G.M., Peijnenburg, Van Gestel, C.A.M., Jin, S.Y., Wang, Y.J., Wang, P., 2011. Toxicity of zinc oxide nanoparticles in the earthworm, *Eisenia fetida* and subcellular fractionation of Zn. Environment International. 37 (6), 1098 – 1104.

Li, Z.Y., Zhang, Z.C., Zhou, Q.L., Gao, R.Y., Wang, Q.S., 2002. Fast and precise determination of phenthoate and its enantiomeric ratio in soil by the matrix solid-phase dispersion method and liquid chromatography. Chromatogr. A. 977, 17 – 25.

Li, Z.Z., Chen, J.F., Liu, F., Liu, A.Q., Wang, Q., Sun, H.Y., Wen, L.X., 2007. Study of UV-shielding properties of novel porous hollow silica nanoparticle carriers for avermectin. Pest Manag Sci. 63, 241 – 246.

Lin, D., Xing, B., 2008. Root uptake and phyotoxiciy of Zno nanoparticles. Environ. Sci. Technol. 42, 5580 – 5585.

Linde, C. D., 1994. Physico-Chemical Properties and Environmental Fate of Pesticides, Environmental Hazards Assessment Program, California, USA.

Lisa, M., Chouhan, R.S., Vinayaka, A.C., Manonmani, H.K., Thakur, M.S., 2009. Gold nanoparticles based dipstick immuno-assay for the rapid detection of dichlorodiphenyltrichloroethane: An organochlorine pesticide. Biosens Bioelectron. 25, 224 – 227.

Liu, B., Wang, Y., Yang, F., Wang, X., Shen, H., Cui, H., Wu, D., 2016. Construction of a controlled-release delivery system for pesticides using biodegradable PLA-based microcapsules. Colloids Surf. B Biointerfaces. 144, 38 – 45.

Liu, F., Wen, L.X., Li, Z.Z., Yu, W., Sun, H.Y., Chen, J.F., 2006. Porous hollow silica nanoparticles as controlled delivery system for water-soluble pesticide. Materials Research Bulletin. 41, 2268 – 2275.

Liu, J., Zhang, X., Zhang, Y., 2015. Preparation and release behavior of chlorpyrifos adsolubilized into layered zinc hydroxide nitrate intercalated with dodecylbenzenesulfonate. Appl. Mater. Interfaces. 7 (21), 11180 – 11188.

Liu, Y., Laks, P., Heiden, P., 2002. Controlled release of biocides in solid wood. II. Efficacy against *Trametes versicolor* and *Gloeophyllum trabeum* wood decay fungi. Applied Polymer Science. 86, 608 – 614.

Liu, Y., Tong, Z., Prud'homme, R.K., 2008. Stabilized polymeric nanoparticles for controlled and efficient release of bifenthrin. Pest Manag Sci. 64, 808 – 812.

Liu, Y., Wei, F., Wang, Y., Zhu, G., 2011. Studies on the formation of bifenthrin oil-in-water nano-emulsions prepared with mixed surfactants, Colloids and Surfaces A: Physicochemical and Engineering Aspects. 389 (1 – 3), 90 – 96.

Liu, Y., Yan, I., Heiden, P., Laks, P., 2001. Use of nanoparticles for controlled release of biocides in solid wood. Appl Polym Sci. 79, 458 – 465.

Locke, M.A., Zablotowicz, R.M., Reddy, K.N., Steinriede, R., 2008. Tillage management to mitigate herbicide loss in runoff under simulated rainfall conditions. Chemosphere. 70, 1422 – 1428.

Loha, K.M., Shakil, N.A., Kumar, J., Singh, M.K., Srivastava, C., 2012. Bio-efficacy evaluation of nanoformulations of β-cyfluthrin against *Callosobruchus Maculatus* (Coleoptera: Bruchidae). Environmental Science and Health Part B-Pesticides Food Contaminants and Agricultural Wastes. 47, 687 – 691.

Lopez-Moreno, M.L., De La Rosa, G., Hernandez-Viezcas, J.A., Castillo-Michel, H., Botez, C.E., Peralta-Videa, J.R., Gardea-Torresdey, J.L., 2010. Evidence of the differential biotransformation and genotoxicity of ZnO and CeO_2 nanoparticles on soybean (Glycine Max) plants. Environ. Sci. Technol. 44, 7315 – 7320.

Lord, K.A., Briggs, G.G., Neale, M.C., Manlove, R., 1980. Uptake of pesticides from water and soil by earthworms. Pest. Manag. Sci. 11, 401 – 408.

Lourenco, J.I., Pereira, R.O., Silva, A.C., Morgado, J.M., Carvalho, F.P., Oliveira, J.M., Malta, M.P., Paiva, A.A., Mendo, S.A., Gonc-alves, F.J., 2011.

Genotoxic endpoints in the earthworms sub-lethal assay to evaluate natural soils contaminated by metals and radionuclides. Hazardous Materials. 186, 788 – 795.

Luyts, K., Napierska, D., Nemery, B., Hoet, P.H.M., 2013. How physico-chemical characteristics of nanoparticles cause their toxicity: complex and unresolved interrelations. Environ. Sci.: Processes Impacts. 15, 23 – 38.

Lyman, W. J., Reehl, W. F., Rosenblatt, D. H., 1990. In: Handbook of chemical property estimation methods. Environmental behavior of organic compounds. American Chemical Society, Washington DC.

Ma, W.C., Immerzeel, J., Bodt, J., 1995. Earthworm and food interactions on bioaccumulation and disappearance in soil of polycyclic aromatic hydrocarbons: Studies on phenanthrene and fluoranthene. Ecotoxicol. Environ. Saf. 32, 226 – 232.

Ma, W.C., Van Kleunen, A., Immerzeel, J., de Maagd, P.G.J., 1998. Bioaccumulation of polycyclic aromatic hydrocarbons by earthworms: Assessment of equilibrium partitioning theory in in situ studies and water experiments. Environ. Toxicol. Chem. 17, 1730 – 1737.

Makama, S., Piella, J., Undas, A., Dimmers, W.J., Peters, R., Puntes, V.F., Van den Brink, N.W., 2016. Properties of silver nanoparticles influencing their uptake in and toxicity to the earthworm *Lumbricus rubellus* following exposure in soil. Environmental Pollution. 1 – 9.

Makhluf, S., Dror, R., Nitzan, Y., Abramovich, Y., Jelnek, R., Gedanken, A., 2005. Microwave-assisted synthesis of nanocrystalline MgO and its use as a bacteriocide. Adv Funct Mater. 15, 1708 – 1715.

Mano, D.M.S., Buff, K., Clausen, E., Langenbach, T., 1996. Bioaccumulation and enhanced persistente of the acaricide dicofol by *Azospirillum lipoferum*. Chemosphere. 33, 1609 – 1619.

Matscheko, N., Tysklind, M., de Wit, C., Bergek, S., Andersson, R., Sellstrom, U., 2002. Application of sewage sludge to arable land-soil concentrations of polybrominated diphenyl ethers and polychorinated dibenzo-p-dioxins, dibenzofurans, and biphenyls, and their accumulation in earthworms. Environ. Toxicol. Chem. 21, 2515 - 2525.

Maund, S.J., Hamer, M.J., Lane, M.C.G., Farrelly, E., Rapley, J.H., Goggin, U.M., Gentle, W.E., 2002. Partitioning, bioavailability, and toxicity of the pyrethroid insecticide cypermethrin in sediments. Environ. Toxicol. Chem. 21, 9 – 15.

McBride, D.K., 1989. Managing pesticides to prevent groundwater contamination. 10 SAF-2. North Dakota Cooperative Extension Service. North Dakota State University. Fargo, ND.

McKay, G., Otterburn, M.S., Sweeney, A.G., 1980. The removal of colour from effluent using various adsorbents. III. Silica: rate processes. Water Research. 14 (1), 15 – 20.

McShane, H., Sarrazin, M., Whalen, J.K., hendershot, W.H., Sunahara, G.I., 2012. Reproductive and behavioral responses of earthworms exposed to nano-sized titanium dioxide in soil. Environ Toxicol. Chem. 31 (1), 184 – 193.

Means, J.C., Wood, S.J., Hassett, J.J., Banwart, W.L., 1982. Sorption of amino- and carboxy-substituted polynuclear aromatic hydrocarbons by sediments and soils. Environ. Sci. Technol. 16, 93 – 98.

Miller, T.A. Salgado, V.L., 1985. The mode of action of pyrethroids on insects: In The Pyrethroid Insecticides. Leahy, J.P., Ed., Taylor & Francis, London. P 43 – 97.

Mogul, M.G., Akin, H., Hasirci, N., Trantolo, D.J., Gresser, J.D. Wise, D.L., 1996. Controlled release of biologically active agents for purposes of agricultural crop management. Resources, Conservation and Recycling. 16, 289 – 320.

Mokrey, L.E., Hoagland, K.D., 1989. Acute toxicities of five synthetic pyrethroid insecticides to *Daphnia magna* and *Ceriodaphnia dubia*. Environmental Toxicology and Chemistry. 9, 1045 – 1051.

Monard, C., Martin-Laurent, F., Vecchiato, C., Francez, A.J., Vandenkoornhuyse, P., Binet, F., 2008. Combined effect of bioaugmentation and bioturbation on atrazine degradation in soil. Soil Biol. Biochem. 40, 2253 – 2259.

Monkiedje, A., Spiteller, M., Bester, K., 2003. Degradation behavior and effects on microbial biomass of racemic mixture and enantiomeric forms of the fungicide metalaxyl in two soils. Environ. Sci. Technol. 37, 712.

Monteiro, S.C., Boxall, A.B.A., 2009. Factors affecting the degradation of pharmaceuticals in agricultural soils. Environ. Toxicol. Chem. 28, 2546 – 2554.

Moore, A., Waring, C.P., 2001. The effects of synthetic pyrethroid pesticide on some aspects of reproduction in Atlantic salmon (*Salmo salar* L.). Aquat Toxicol. 52, 1 – 12.

Moreau, J.W., Weber, P.K., Martin, M.C., Gilbert, B., Hutcheon, I.D., Banfield, J.F., 2007. Extracellular proteins limit the dispersal of biogenic nanoparticles. Science. 316, 1600 – 1603.

Mudhoo, A., Garg, V.K., 2011. Sorption, transport and transformation of atrazine in soils, minerals and composts: A review. Pedosphere. 21 (1), 11 – 25.

Mudunkotuwa, I.A., Pettibone, J.M., Grassian, V.H., 2012. Environmental implications of nanoparticle aging in the processing and fate of copper-based nanomaterials. Environ. Sci. Technol. 46 (13), 7001 – 7010.

Mueller, N.C., Nowack, B., 2008. Exposure modeling of engineered nanoparticles in the environment. Environ. Sci. Technol. 42, 4447 – 4453.

Müller, R.H., Junghanns, J.U.A.H., 2006. Drug nanocrystals/ nanosuspensions for the delivery of poorly soluble drugs. In V.P. Torchilin (Ed.), Nanoparticles as drug carriers (pp. 307–328). London, England: Imperial College Press.

Naicker, P.K., Cummings, P.T., Zhang, H.Z., Banfield, J.F. 2005. Characterisation of titanium dioxide nanoparticles using molecular dynamics simulations. Phys. Chem. B. 109, 15243 – 15249.

Nakata, H., Kawazoe, M., Arizono, K., Abe, S., Kitano, T., Shimada, H., Li, W., Ding, X., 2002. Organochlorine pesticides and polychlorinated biphenyl residues in foodstuffs and human tissues from China: status of contamination, historical trend, and human dietary exposure. Arch Environ Contam Toxicol. 43 (4), 473 – 480.

Narayanan, KS.., Jon, D., Patel, J., Winkowski, K., 2008. Aqueous composition for delivering bioactive hydrophobic nanoparticles. Patent Number WO 2008016837 A2 20080207.

National Nanotechnology Initiative (NNI), 2016. What is nanotechnology? http://www.nano.gov/nanotech-101/what/definition. Accessed October 2016.

Navarro, S., Vela, N., Garcia, C., Navarro, G.J., 2003. Persistence of simazine and terbuthylazine in a semiarid soil after organic amendment with urban sewage sludge. Agric and Food Chem. 51 (25), 7359 – 7365.

Navrotsky, A., Mazeina, L., Majzlan, J., 2008. Size-driven structural and thermodynamic complexity in iron oxides. Science. 319, 1635 – 1638.

Nawab, A., Aleem, A., Malik, A., 2003. Determination of organochlorine pesticides in agricultural soil with special reference to γ-HCH degradation by Pseudomonas strains. Biores. Technol. 88 (1), 41 – 46.

Nelson, D.W. and L.E. Sommers, 1982. Total carbon, organic carbon and organic matter: In: A.L. Page, R.H. Miller and D.R. Keeney) Methods of soil analysis. Part 2 Chemical and Microbiological Properties. P 539 – 579.

Nennemann, A., mishael, Y., Nir, S., Rubin, B., Polubesova, T., Bergaya, F., Damme, H.V., Lagaly, G., 2001. Clay-based formulation of metolachlor with reduced leaching. Applied Clay Science. 18, 265 – 275.

Nguyen, H.M., Hwang, I.C., Park, J.W., Park, H.J., 2012a. Enhanced payload and photo-protection for pesticides using nanostructured lipid carriers with corn oil as liquid lipid. Microencapsul. 29 (6), 596 – 604.

Nguyen, H.M., Hwang, I.C., Park, J.W., Park, H.J., 2012b. Photoprotection for deltamethrin using chitosan-coated beeswax solid lipid nanoparticles. Pest Manag Sci. 68 (7), 1062 – 1068.

Niemeyer, C.M., Doz, P., 2001. Nanoparticles, proteins, and nucleic acids: Biotechnology meets materials science. Angewandte Chemie Int Ed. 40, 4128 – 4158.

Nolan, B.T., Dubus, I.G., Surdyk, N., Fowler, H.J., Burton, A., Hollis, J.M., Reichenberger, S., Jarvis, N.J., 2008. Identification of key climatic factors regulating the transport of pesticides in leaching and to tile drains. Pest Management Science. 64 (9), 933 – 944.

Nowack, B., 2009. The behaviour and effects of nanoparticles in the environment. Environ. Pollut. 157, 1063 – 1064.

Nowack, B., Bucheli, T.D., 2007. Occurrence, behaviour and effects of nanoparticles in the environment. Environ. Pollut. 150, 5 – 22.

OECD, 1995. OECD Guidelines for the Testing of Chemicals, Section 1. Test No 107: “Partition Coefficient (n-octanol/water): Shake Flask Method”. OECD Publishing.

OECD, 2000. OECD Guideline for the Testing of Chemicals. Test No 106: “Adsorption−desorption using a batch equilibration method”. OECD Publishing.

OECD, 2002. OECD Guideline for the Testing of Chemicals. Test No 307: “Aerobic and Anaerobic Transformation in Soil”. OECD Publishing.

OECD, 2004. OCED Guidelines for the Testing Chemicals. Test No 222: “Earthworm Reproduction Test (*Eisenia fetida*/ *Eisenia andrei*)”. OECD Publishing.

OECD, 2004. OECD Guideline for the Testing of Chemicals. Test No. 312: “Aerobic and Anaerobic Transformation in Soil”. OECD Publishing.

OECD, 2004. OECD Guidelines for the Testing of Chemicals, Section 1. Test No 117: “Partition Coefficient (n-octanol/water), HPLC Method”. OECD Publishing.

OECD, 2006. OECD Guidelines for the Testing of Chemicals, Section 1. Test No 123: “Partition Coefficient (1-Octanol/Water): Slow-Stirring Method”. OECD Publishing.

OECD, 2010. OECD Guidelines for the Testing of Chemicals. Test No 317: “Bioaccumulation in Terrestrial Oligochaetes”. OECD Publishing.

Olanrewaju, J., Newalkar, B.L., Mamcino, C., Komarneni, S., 2000. Simplified synthesis of nitrate form of layered double hydroxide. Mater Lett. 45, 307 – 310.

Oliveira, H.C., Stolf-Moreira, R., Martinez, C.B.R., Grillo, R., de Jesus, M.B., Fraceto, L.F., 2015. Nanoencapsulation enhances the post-emergence herbicidal activity of atrazine against mustard plants. PLoS ONE. 10 (7), e0132971.

Oros, D. R. and Werner, I. 2005. Pyrethroid Insecticides: An Analysis of Use Patterns, Distributions, Potential Toxicity and Fate in the Sacramento-San Joaquin Delta and Central Valley. In White Paper for the Interagency Ecological Program, vol. SFEI Contribution 415. Oakland, CA: San Francisco Estuary Institute.

Oskam, G., 2006. Metal oxide nanoparticles: Synthesis, characterisation and application. Solgel Sci Technol. 37, 161 – 164.

Oudou, H.C., Alonso, R.M., Bruun, H.H.C., 2004. Voltammetric behaviour of the synthetic pyrethroid lamda cyhalothrin and its determination in soil and well water. Anal Chim Acta. 523, 69 – 74.

Oukali-Haouchine, O., Barriuso, E., Mayata, Y., Moussaoui, K. M., 2013. Factors affecting metribuzin retention in Algerian soils and assessment of the risks of contamination. Environ. Monit. Assess. 185, 4107 – 4115.

Özer, A., Pirinççi, H.B., 2006. The adsorption of Cd(II) ions on sulphuric acid-treated wheat bran. Hazardous Materials. 137 (2), 849 – 855.

Paknikar, K.M., Nagpal, V., Pethkar, A.V., Rajwade, J.M., 2005. Degradation of lindane from aqueous solutions using iron sulfide nanoparticles stabilized by biopolymers. Sci Tech Adv Mat. 6, 370 – 374.

Palmquist, K., Salatas, J., Fairbrother, A., 2012. Pyrethroid Insecticides: Use, Environmental Fate, and Ecotoxicology, Insecticides – Advances in Integrated Pest Management, Dr. Farzana Perveen (Ed.), ISBN: 978-953-307-780-2, InTech.

Pankaj, Shakil, N.A., Kumar, J., Singh, M.K., Singh, K., 2012. Bioefficacy evaluation of controlled release formulations based on amphiphilic nano-polymer of carbofuran against *Meloidogyne incognita* infecting tomato. Environ Sci Health B. 47 (6), 520 – 528.

Paraíba, L.C., Cerdeira, A.L., Da Silva, E.F., Martins, J.S., Da Costa Coutinho, H.L., 2003. Evaluation of soil temperature effect on herbicide leaching potential into groundwater in the Brazilian Cerrado. Chemosphere. 53 (9), 1087 – 1095.

Park, M., Lee, C.I., Seo, Y.J., Woo, S.R., Shin, D., Choi, J., 2010. Hybridization of the natural antibiotic, cinnamic acid, with layered double hydroxides (LDH) as green pesticide. Environmental Science and Pollution Research. 17, 203 – 209.

Park, S., Woodhall, J., Ma, G., Veinot, J.G.C., Cresser, M.S., Boxall, A.B.A., 2013. Regulatory ecotoxicity testing of engineered nanoparticles: Are the results relevant to the natural environment? Nanotoxicology. 8 (5), 583 – 592.

Patsias, J., Papadakis, E.N., Papadopoulou-Mourkidou, E., 2002. Analysis of phenoxyalkanoic acid herbicides and their phenolic conversion products in soil by microwave assisted solvent extraction and subsequent analysis of extracts by online SPE-liquid chromatography. Chromatogr. A. 959, 153 – 161.

Paula, H.C.B., Rodrigues, M.L.L., Ribeiro, W.L.C., Stadler, A.S., Paula, R.C.M., Abreu, F.O.M.S., 2012. Protective effect of cashew gum nanoparticles on natural larvicide from *Moringa Oleifera* seeds. Applied Polymer Science. 124, 1778 – 1784.

Paula, H.C.B., Sombra, F.M., Abreu, F.O.M.S., De Paula, R.C.M., 2010. *Lippia sidoides* essential oil encapsulation by angico gum/chitosan nanoparticles. The Brazilian Chemical Society. 21, 2359 – 2366.

Peijnenburg, W.J.G.M., 2001. Bioavailability of metals to soil invertebrates. In: Allen, H.E. (Ed.), Bioavailability of Metals in Terrestrial Ecosystems; Importance of Partitioning for Bioavailability to Invertebrates, Microbes and Plants. SETAC Press, Pensacola, FL, USA.

Penn, R.L., Erbs, J.J., Gulliver, D.M., 2006. Controlled growth of alpha-FeOOH nanorods by exploiting-oriented aggregation. Cryst. Growth. 293, 1 – 4.

Peralta-Videa, J.R., Zhao, L., Lopez-Moreno, M.L., De La Rosa, G., Hong, J., Gardea-Torresdey, J.L., 2011. Nanomaterials and the environment: A review for the biennium 2008–2010. Hazardous Materials. 186 (1), 1 – 15.

Pereira AES, Grillo R, Mello NFS, Rosa AH, Fraceto LF (2014) Application of poly(epsilon-caprolactone) nanoparticles containing atrazine herbicide as an alternative technique to control weeds and reduce damage to the environment. J Hazard Mater 268:207–215

Pereira EIc, Giroto AS, Bortoli A, Yamamoto CF, Marconcini JM, de Campos Bernardi AC, Ribeiro C (2015) Perspectives in nanocomposites for the slow and controlled release of agrochemicals: fertilizers and pesticides. In: Rai M (ed) Nanotechnology Food Agriculture Springer, p 241–265

Pérez-de-Luque, A., Rubiales, D., 2009. Nanotechnology for parasitic plant control. Pest Manage. Sci. 65, 540 – 545.

Perez-Fernandez, V., Angeles Garcia, M., Luisa Marina, M., 2010. Characteristics and enantiomeric analysis of chiral pyrethroids. Chromatogr. 1217, 968 – 989.

Perrin-Ganier, C., Schiavon, F., Morel, J., Schiavon, M., 2001. Effect of sludge-amendment or nutrient addition on the biodegradation of the herbicide isoproturon in soil. Chemosphere. 44, 887 – 892.

Peter, C.J., Weber, J.B., 1985. Adsorption, mobility and efficacy of alachlor and metolachlor as influenced by soil properties. Weed science. 33, 874 – 881.

Petersen, E.J., Huang, Q., Weber, W.J.Jr., 2010. Relevance of octanol-water distribution measurements to the potential ecological uptake of multi-walled carbon nanotubes. Environ. Toxicol. Chem. 29, 1106 – 1112.

Petersen, E.J., Huang, Q., Weber, W.J.Jr., 2008. Bioaccumulation of radio-labeled carbon nanotubes by *Eisenia foetida*. Environ Sci Technol. 42 (8), 3090 – 3095.

Pettibone, J. M., Cwiertny, D. M., Scherer, M., Grassian, V.H., 2008. Adsorption of organic acids on TiO_2 nanoparticles: Effects of pH, nanoparticle size, and nanoparticle aggregation. Langmuir. 24, 6659 – 6667.

Piccolo, A., Celano, G., 1993. Modification of infrared spectra of the herbicide glyphosate induced by pH variation. Environmental Science and Health Part B. 28, 447 – 457.

Pinna, M.V., Pusino, A., Gessa, C., 2004. Sorption and degradation of azimsulfuron on iron (III)-rich soil colloids. Agric. Food Chem. 52, 8081 – 8085.

Pipan, T.Z., Drobne, D., Jemec, A., Romih, T., Zidar, P., Bele, M., 2010. Zinc bioaccumulation in a terrestrial invertebrate fed a diet treated with particulate ZnO or $ZnCl_2$ solution. Toxicology. 269, 198 – 203.

Pose-Juan, E., Rial-Otero, R., López-Periago, E., (2010). Sorption of penconazole applied as commercial water–oil emulsion in soils devoted to vineyards. Hazard. Mater. 182, 136 – 143.

Posner, J.D., 2009. Engineered nanomaterials: Where they go, nobody knows. Nano Today. 4, 114 – 115.

Pozo, O., Pitarch, E., Sancho, J.V., Hernandez, F., 2001. Determination of the herbicide 4- chloro-2-methylphenoxyacetic acid and its main metabolite, 4-chloro-2- methylphenol in water and soil by liquid chromatography-electrospray tandem mass spectrometry. Chromatogr. A. 923 (1 – 2), 75 – 85.

Praetorius, A., Tufenkji, N., Goss, K.U., Scheringer, M., Von Der Kammer, F., Elimelech, M., 2014. The road to nowhere: Equilibrium partition coefficients for nanoparticles. Environ. Sci.: Nano. 1 (4), 317 – 323.

Price, O.R., Walker, A., Wood, M., Olivier, M.A., 2001. Using geostatics to evaluate spatial variation in pesticide/soil interactions. BCPC Symposium Proceedings No. 78: Pesticide BehaViour in Soils and Water; British Crop Protection Council: United Kingdom.

Puoci, F., Lemma, F., Spizzirri, U.G., Cirillo, G., Curcio, M., Picci, N., 2008. Polymer in agriculture: A Review. Am. J Agri Biol Sci. 3, 299 – 314.

Qafoku, N.P. 2010. Chapter Two - Terrestrial nanoparticles and their controls on soil-/ geo-processes and reactions, In: Donald L. Sparks, Editor(s), Advances in Agronomy, Academic Press. 107, 33 – 91.

Qian, K., Guo, Y., He, L., 2012. Controlled release of imidacloprid from poly(styrene-diacetone crylamide)-based nanoformulation. Nanoscience. 11, 6.

Qing, S., Yongli, S., Yuehong, Z., Ting, Z., Haoyan, S., 2013. Pesticide conjugated polyacrylate nanoparticles: Novel opportunities for improving the photostability of emamectin benzoate. Polym Adv Technol. 24(2), 137 – 143.

Qiu, D.P., Li, Y.H., Fu, X.Y., Jiang, Z., Zhao, X.Y., Wang, T., Hou, W.G., 2009. Controlled-release of avermectin from organically modified hydrotalcitelike compound nanohybrids. Chinese Journal of Chemistry. 27, 445 – 451.

Quaglia, F., Barbato, F., De Rosa, G., Granata, E., Miro, A., La Rotonda, M.I., 2001. Reduction of the environmental impact of pesticides: Waxy microspheres encapsulating the insecticide carbaryl. Agricultural and Food Chemistry. 49, 4808 – 4812.

Racke, K.D., Skidmore, M.W., Hamilton, D.J., Unsworth, J.B., Miyamoto, J., Cohen, S.Z., 1997. Pesticide fate in tropical soils. Pure and Appl. Chem. 69 (6), 1349 – 1371.

Radosevich, M., Traina, S.J., Tuovinen, O.H., 1996. Biodegradation of atrazine in surface soils and subsurface sediments collected from an agricultural research farm. Biodegradation. 7, 137 – 149.

Ramanathan, M., Luckarift, H.R., Sarsenova, A., Wild, J.R., Ramanculov, E.R., Olsen, E.V., Simonian, A.L., 2009. Lysozyme-mediated formation of protein–silica nano-composites for biosensing applications. Colloids Surf B Biointerfaces. 73, 58 – 64.

Reddy, L.H., Arias, J.L., Nicolas, J., Couvreur, P., 2012. Magnetic nanoparticles: Design and characterisation, toxicity and biocompatibility, pharmaceutical and biomedical applications. Chem. Rev. 112, 5818 – 5878.

Reed, B.E., Matsumoto, M.R., 1993. Modeling cadmium adsorption by activated carbon using the Langmuir and Freundlich isotherm expressions. Separation Science and Technology. 28 (13 – 14), 2179 – 2195

Regitano, J.B., Bischoff, M., Lee, L.S., Reichert, J.M., Turco, R.F., 1997. Retention of imazaquin in soil. Environ. Toxicol. Chem. 16, 397 – 404.

Reichenberger, S., Bach, M., Skitschak, A., Frede, H.G., 2007. Mitigation strategies to reduce pesticide inputs into ground- and surface water and their effectiveness: A review. Sci. Total Environ. 384, 1 – 35.

Reynolds, J.L., 1986. Characterisation of metabolites and bound residues obtained from soil treated with alcohol (phenyl ring) – 14C FMC 54800, FMC Corporation.

Rich, C.D., Blaine, A.C., Hundal, L., Higgins, C.P., 2015. Bioaccumulation of perfluoroalkyl acids by earthworms (Eisenia fetida) exposed to contaminated soils. Envrob. Sci. Technol. 49, 881 – 888.

Roh, J.Y., Sim, S.J., Yi, J., Park, K., Chung, K.H., Ryu, D.Y., Choi, J., 2009. Ecotoxicity of silver nanoparticles on the soil nematode *Caenorhabditis Elegans* using functional ecotoxicogenomics. Environ. Sci. Technol. 43, 3933 – 3940.

Rosales-Conrado, N., Leon-Gonzalez, M.E., Perez-Arribas, L.V., Polo-Diez, L.M., 2002. Determination of chlorophenoxy acid herbicide and their esters in soil by capillary high performance liquid chromatography with ultraviolet detection, using large volume injection and temperature gradient. Analitica Chimica Acta. 470, 147 – 154.

Roy, A., Bajpai, A.K., Bajpai, J., 2009. Dynamics of controlled release of chlorpyrifos from swelling and eroding biopolymers microspheres of calcium alginate and starch. Carbohydr. Polym. 76, 222 – 231.

Rudzinski, W.E., Chipuk, T., Dave, A.M., Kumbar, S.G., Aminabhavi, T.M., 2003. PH-sensitive acrylic-based copolymeric hydrogels for the controlled release of a pesticide and a micronutrient. Applied Polymer Science. 87, 394 – 403.

Römbke, J., Garcia, M.V., Scheffczyk, A., 2007. Effects of the fungicide benomyl on earthworms in laboratory tests under tropical and temperate conditions. Archives of Environmental Contamination and Toxicology. 53 (4), 590 – 598.

Saenz-de-Cabezon, I.F.J., Zalom, F.G., Moreno-Grijalba, F., Marco, V., Pérez-Moreno, I., Carvajal Montoya, L., 2008. Terrestrial bioaccumulation: experimental approaches on an arthropod prey-predatory mite system. ENDURE international conference on diversifying crop protection; 2008 Oct 12–15; La Grande-Motte, France

Said-Pullicino, D., Gigliotti, G., Vella, A.J., 2004. Environmental fate of triasulfuron in soils amended with municipal waste compost. Environ. Qual. 33, 1743 – 1752.

Saini, R., Saini, S., Sharma, Sugandha, 2010. "Nanotechnology: The Future Medicine". Cutaneous and Aesthetic Surgery. 3 (1), 32 – 33.

Sajjadi, H., Modaressi, A., Magri, P., Domańska, U., Sindt, M., Mieloszynski, J.L., Mutelet, F., Rogalski, M., 2013. Aggregation of nanoparticles in aqueous solutions of ionic liquids. Molecular Liquids. 186, 1 – 6.

Saleh, N., Kim, H.J., Phenrat, T., Matyjaszewski, K., Tilton, R.D., Lowry, G.V., 2008. Ionic strength and composition affect the mobility of surface-modified FeO nanoparticles in water-saturated sand columns. Environ. Sci. Technol. 42, 3349 – 3355.

Saleh, N.B., Pfefferle, L.D., Elimelech, M., 2008. Aggregation kinetics of multiwalled carbon nanotubes in aquatic systems: Measurements and environmental implications. Environmental Science and Technology. 42 (21), 7963 – 7969.

Salma, U., Chen, N., Richter, D.L., Filson, P.B., Dawson-Andoh, B., Matuana, L. Heiden, P., 2010. Amphiphilic core/shell nanoparticles to reduce biocide leaching from treated wood, 1 – Leaching and biological efficacy. Macromol. Mater. Eng. 295, 442 – 450.

Salvati, A., Aberg, C., Dos Santos, T., Varela, J., Pinto, P., Lynch, I., Dawson, K.A., 2011. Experimental and theoretical comparison of intracellular import of polymeric nanoparticles and small molecules: toward models of uptake kinetics. Nanomedicine. 7 (6), 818 – 826.

Sample, B.E., Beauchamp, J.J., Efroymson, R.A., Suter, G.W., Ashwood, T.L., 1997. Development and Validation of Bioaccumulation Models for Earthworms, ES/ER/TM-220, Oak Ridge Natl. Lab, Oak Ridge, Tenn

Santos, M.J.G., Ferreira, V., Soares, A.M.V.M., Loureiro, S., 2011. Evaluation of the combined effects of dimethoate and spirodiclofen on plants and earthworms in a designed microcosm experiment. Appl. Soil Ecol. 48, 294 – 300.

Santos, M.J.G., Soares, A.M.V.M., Loureiro, S., 2010. Joint effects of three plant protection products to the terrestrial isopod *Porcellionides pruinosus* and the collembolan *Folsomia candida*. Chemosphere. 80, 1021 – 1030.

Sarathy, V., Tratnyek, P.G., Nurmi, J.T., Baer, D.R., Amonette, J.E., Chun, C.L., Penn, R.L., Reardon, E.J., 2008. Aging of iron nanoparticles in aqueous solution: Effects on structure and reactivity. Phys. Chem. C. 112, 2286 – 2293.

Sarkar, D.J., Kumar, J., Shakil, N.A., Walia, S., 2012. Release kinetics of controlled release formulations of thiamethoxam employing nano-ranged amphiphilic PEG and diacid based block polymers in soil. Environ Sci Health A Tox Hazard Subst Environ Eng. 47 (11), 1701 – 1712.

Sassman, S.A., Lee, L.S., Bischoff, M., Turco, R.F., 2004. Assessing N,N'-dibutylurea (DBU) formation in soils after application of n-butylisocyanate and benlate fungicides. Agricultural and Food Chemistry. 52, 747.

Sasson, Y., Levy-Ruso, G., Toledano, O., Ishaaya, I., 2007. Nanosuspensions: Emerging novel agrochemical formulations. In: Ishaaya I, Nauen R, Horowitz AR, Editors. Insecticides Design Using Advanced Technologiesnetherlands: Springer-Verlag P. 1 – 32.

Satyanarayana, V.N.T., Kuchibhatla, A.S., Karakoti, D.R., Baer, S., Samudrala, M.H., Engelhard, J. E., Thevuthasan, A.S., Seal, S., 2012. Influence of aging and environment on nanoparticle chemistry: Implication to confinement effects in nanoceria. *Physical Chemistry C. 116* (26), 14108 – 14114.

Savolainen, K., Alenius, H., Norppa, H., Pylkkänen, L., Tuomi, T., Kasper, G., 2010. Risk assessment of engineered nanomaterials and nanotechnologies—A review. Toxicology. 269, 92 – 104.

Sayavedra-Soto, L.A., Gvakharia, B., Bottomley, P.J., Arp, D.J., Dolan, M.E., 2010. Nitrification and degradation of halogenated hydrocarbons – A tenuous balance for ammonia-Oxidising bacteria. Appl Microbiol Biotechnol. 86, 435 – 444.

Schlich, K., Klawonn, T., Terytze, K., Hund-Rinke, K., 2013. Effects of silver nanoparticles and silver nitrate in the earthworm reproduction test. Environ Toxicol Chem. 32 (1), 181 – 188.

Schlich, K., klawonn, T., Terytze, K., Hund-Rinke, K., 2013. Hazard assessment of a silver nanoparticle in soil applied via sewage sludge. Environ. Sci. Europe. 25, 17.

Schlich, K., Terytze, K., Hund-Rinke, K., 2012. Effect of TiO_2 nanoparticles in the earthworm reproduction test. Environmental Sciences Europe. 24, 5.

Scott-Fordsmand, J.J., Krogh, P.H., Schaefer, M., Johansen, A., 2008. The toxicity testing of double-walled nanotubes-contaminated food to *Eisenia veneta* earthworms. Ecotoxical. Environ. Saf., 71, 616 – 619.

Sellstrom, U., De Wit, C.A., Lundgren, N., Tysklind, M., 2005. Effect of sewage-sludge application on concentrations of higher-brominated diphenyl ethers in soils and earthworms. Environ. Sci. Technol. 39, 9064 – 9070.

Senesi, N., 1992. Binding mechanisms of pesticides to soil humic substances. Science of the Total Environment. 12, 63 – 76.

Shahgholi, H., Ahangar, A.G., 2014. Factors controlling degradation of pesticides in the soil environment: A review. Agric. Sci. Dev. 3 (8), 273 – 278.

Shakil, N.A., Singh, M.K., Pandey, A., Kumar, J., Parmar, V.S., Singh, M.K., Pandey, R.P., Watterson, A.C., 2010. Development of poly(ethylene glycol) based amphiphilic copolymers for controlled release delivery of carbofuran. Macromolecular Science, Part A: Pure and Applied Chemistry. 47, 241 – 247.

Sharma, D., Singh, S.B., 2012. Persistence of bifenthrin in sandy loam soil as affected by microbial community. Bull. Environ. Contam. Toxicol. 88 (6), 906 – 908.

Sharma, V.K., 2009. Aggregation and toxicity of titanium dioxide nanoparticles in aquatic environment − A review. Environ. Sci. Health, A. 44 (14), 1485 – 1495.

Shaw, L.J., Burns, R.G., 1998. Biodegradation of 2,4-D in a noncontaminated grassland soil profile. Environ. Qual. 27, 1464 – 1471.

Shimizu, Y., Yamazaki, S., Terashima, Y., 1992. Sorption of anionic pentachlorophenol (PCP) in aquatic environments: the effect of pH. Water Sci. Technol. 25, 41 – 48.

Shipitalo, M.J., Dick, W.A., Edwards, W.M., 2000. Conservation tillage macropore factors that affect water movement and the fate of chemicals. Soil Till. Res. 53, 167 – 183.

Shoults-Wilson, W.A., Reinsch, B.C., Tsyusko, O.V. Bertsch, P.M., Lowry, G.V., Unrine, J.M., 2011a. Effect of silver nanoparticle surface coating on bioaccumulation and reproductive toxicity in earthworms (*Eisenia fetida*). Nanotoxicology. 5, 432 – 444.

Shoults-Wilson, W.A., Reinsch, B.C., Tsyusko, O.V., Bertsch, P.M., Lowry, G.V., Unrine, J.M., 2011b. Role of particle size and soil type in toxicity of silver nanoparticles to earthworms. Soil Sci. Soc. 75, 365.

Shoults-Wilson, W.A., Zhurbich, O.I., McNear, D.H., Tsyusko, O.V., Bertsch, P.M., Unrine, J.M., 2011c. Evidence for avoidance of Ag nanoparticles by earthworms (*Eisenia fetida*). Ecotoxicology. 20 (2), 385 – 396.

Shunthirasingham, C., Mmereki, B.T., Masamba, W., Oyiliagu, C.E., Lei, Y.D., Wania, F., 2010. Fate of pesticides in the arid subtropics, Botswana, Southern Africa. Environ. Sci. Techno. 44 (21), 8082 – 8088.

Sijm, D.T.H.M., Seinen, W., Opperhuizen, A., 1992. Life-cycle biomagnification study in fish. Environ. Sci. Technol. 26, 2162 – 2174.

Silva MdS, Cocenza DS, Grillo R, de Melo NFS, Tonello PS, de Oliveira LC, et al. Paraquat-loaded alginate/chitosan nanoparticles: preparation, characterisation and soil sorption studies. J Hazard Mater 2011;190(1–3):366–74.

Sims, R.W., Gerard, B.M., 1985a. Family Lumbricidae, In: Kermack, D.M., Barnes, R.S.K. (Eds.), Earthworms, Synopses of the British Fauna (New Series). The Pitman Press, Bath, U.K, P. 80,106.

Sims, R.W., Gerard, B.M., 1985b. Earthworms: Keys and notes for the identification and study of the species. Brill Archive.

Sinclair, C.J., Boxall, A.B.A., 2003. Assessing the ecotoxicity of pesticide transformation products. Environ. Sci. Techno. 37, 4617 – 4625.

Singh, S., (2012). Downward movement of five synthetic pyrethroids in three different soils of Gujarat. Pesticide Research. 24 (1), 76 – 81.

Singles, S.K., Dean, G.M., Kirkpatrick, D.M., Mayo, B.C., Langford-Pollard, A.D., Barefoot, A.C., Bramble, F.Q., 1999. Fate and behavior of flupyrsulfuron-methyl in soil and aquatic systems. Pestic. Sci. 55, 288 – 300.

Sitea, A.D., 2001. Factors affecting sorption of organic compounds in natural sorbent/ water systems and sorption coefficients for selected pollutants. A Review. Phys. Chem. Ref. Data. 30 (1).

Smith, A.D., 1991. Metabolism studies: aerobic soil metabolism of bifenthrin (FMC 54800) in a silt loam soil. FMC Corporation, document No. P-1978.

Smith, T.M., Stratton, G.W., 1986. Effects of synthetic pyrethroid insecticides on nontarget organisms. Residue Review. 97, 93 – 120.

Soderlund, D.M., Bloomquist, J.R., 1989. Neurotoxic actions of pyrethroids insecticides. Annual Review of Entomology. 34, 77 – 96.

Sofina, L.I., Kolycheva, S.S., Zaria, N.I.U., 1993. Pesticide levels in food of pregnant women and newborn in rice growing areas of Kransnodar region. Vopr Pitan. 4, 53 – 54.

Soil Texture Classification (US and FAO systems): Weed Science, 33, Suppl. 1 (1985) and Soil Sci. Soc. Amer. Proc., 26, 305 (1962).

Solomon, K.R., Giddings, J.M., Maund, S.J., 2001. Probabilistic risk assessment of cotton pyrethroids: I. distributional analysis of laboratory aquatic toxicity data. Environmental Toxicology and Chemistry. 20 (3), 652 – 659.

Solovitch, N., Labille, J., Rose, J., Chaurand, P., Borschneck, D., Wiesner, M.R., Bottero, J., 2010. Concurrent aggregation and deposition of TiO_2 nanoparticles in a sandy porous media. Environ. Sci. Technol. 44, 4897 – 4902.

Song, M.R., Cui, S.M., Gao, F., Liu, Y.R., Fan, C.L., Lei, T.Q., Liu, D.C., 2012. Dispersible silica nanoparticles as carrier for enhanced bioactivity of chlorfenapyr. Pestic. Sci. 37 (3), 258 – 260.

Song, S.L., Liu, X.H., Jiang, J.H., Qian, Y.H., Zhang, N., Wu, Q.H., 2009. Stability of triazophos in self-nanoemulsifying pesticide delivery system. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 350, 57 – 62.

Song, Y., Kai, J., Song, X., Zhang, W., Li, L., 2015. Long-term toxic effects of deltamethrin and fenvalerante in soil. Hazardous Materials. 289, 158 – 164.

Sopena, F., Morillo, E., Villaverde, J., Maqueda, C., 2008. Reduction of pesticide pollution in agricultural soils by using controlled release formulations: Efficacy and persistence studies. Fresenius Environmental Bulletin. 17, 1705 – 1710.

Spagnoli, D., Banfield, J.F., Parker, S.C., 2008. Free energy change of aggregation of nanoparticles. Phys. Chem. C. 112, 14731 – 14736.

Sparks, R., 2003. *Environmental soil chemistry*. Amsterdam: Elsevier, Amsterdam, The Netherlands.

Sposito, G., Schindler, P.W., 1986. Reactions at the soil colloid-soil solution interface In: Landner, L. (ed.), Speciation of Metals in Water, Sediment and Soil Systems; Springer Verlag, Berlin, pp. 683–699.

Spurgeon, D. J., Hopkin, S.P., 1996. Risk assessment of the threat of secondary poisoning by metals to predators of earthworms in the vicinity of a primary smelting works. Sci. Total Environ. 187, 167 – 183.

Spurgeon, D.J., Lofts, S., Hankard, P.K., Toal, M., McLellan, D., Fishwick, S., Svendsen, C., 2006. Effect of pH on metal speciation and resulting metal uptake and toxicity for earthworms. Environ. Toxicol. Chem. 25, 788 – 796.

Stanley, O.N., Joy, O.A., 2014. Histopathological effects of glyphosate and its toxicity to the earthworm *Nsukkadrilus mbae*. British Biotechnol. 4 (2), 149 – 163.

Stenersen, J., 1984. Detoxification of xenobiotics by earthworms. Comp. Biochem. Physiol. 78c, 249 – 252.

Stoimenov, P.K., Klinger, R.L., Marchin, G.L., Klabunde, K.J., 2002. Metal oxide nanoparticles as bactericidal agents. Langmuir. 18, 6679 – 6686.

Stone, D., Harper, B.J., Lynch, I., Dawson, K., Harper, S.L., 2010. Exposure assessment: Recommendations for nanotechnology-based pesticides. Int. J. Occup. Environ. Health. 16 (4), 467 – 474.

Sun, C., Shu, K., Wang, W., Ye, Z., Liu, T., Gao, Y., Zheng, H., He, G., Yin, Y., 2014. Encapsulation and controlled release of hydrophilic pesticide in shell cross-linked nanocapsules containing aqueous core. Pharmaceutics. 463 (1), 108 – 114.

Suthar, S., Singh, S., Dhawan, S., 2008. Earthworms as bioindicator of metals (Zn, Fe, Mn, Cu, Pb and Cd) in soils: Is metal bioaccumulation affected by their ecological category? Ecol. Eng. 32, 99 – 107.

Tallur, P. N., Megadi, V. B., Ninnekar, H. Z., 2008. Biodegradation of cypermethrin by Micrococcus sp. strain CPN 1. Biodegradation. 19, 77 – 82.

Tang, X., Zhu, B., Katou, H., 2012. A review of rapid transport of pesticides from sloping farmland to surface waters: processes and mitigation strategies. Environ Sci 24, 351 – 361.

Taniguchi, N., 1974. "On the Basic Concept of 'Nano-Technology'," Proc. Intl. Conf. Prod. Eng. Tokyo, Part II, Japan Society of Precision Engineering.

Teague, E.C., 2004. Responsible development of nanotechnology national nanotechnology initiative meeting. National Nanotechnology Initiative. P 29.

Theng, B.K.G., Yuan, G., 2008. Nanoparticles in the soil environment. Elements. 4, 395 – 399.

Tilak, K.S., Veeraiah, K., Vardhan, K.S., 2003. Toxicity and residue studies of fenvalerate to the freshwater fish Channa punctatus. Bull Environ Contam Toxicol. 71, 1207 – 1212.

Tiryaki, O., Aysal, P., 1999. Bound pesticide residues in soil and plant and their determination. The Turkish Journal of Nuclear Sciences. 26 (2), 43 – 60.

Tomlin, C.D.S., ed. Bifenthrin (82657-04-3). In: The e-Pesticide Manual, 15th Edition, Version 5.0.1 (2010). Surrey UK, British Crop Protection Council.

Torney, F., Trewyn, B.G., Lin, V.S., Wang, K., 2007. Mesoporous silica nanoparticles deliver DNA and chemicals into plants. Nature Nanotechnol. 2, 295 – 300.

Toth, S.J., Buhler, W.G., 2009. Environmental effects of pesticides, Department of Entomology and Horticultural Science, North Carolina State University.

Tourinho, P.S., Van Gestel, C.A., Lofts, S., Svendsen, C., Soares, A.M., Loureiro, S., 2012. Metal-based nanoparticles in soil: fate, behavior, and effects on soil invertebrates. Environ. Toxicol. Chem. 31, 1679.

Triantafyllidis, V., Hela, D., Salachas, G., Dimopoulos, P., Albanis, T., 2009. Soil dissipation and runoff losses of the herbicide Pendimethalin in tobacco field. Water Air Soil Pollution. 201, 253 – 264.

Triantafyllidis, V., manos, S., Hela, D., Manos, G., Konstantinou, L., 2010. Persistence of trifluralin in soil of oilseed rape fields in Western Greece. Environmental Analytical Chemicatry. 90, 344 – 356.

Trigo, C., Celis, R., Hermosin, M.C., Cornejo, J., 2009. Organoclay-based formulations to reduce the environmental impact of the herbicide diuron in olive groves. Soil Science Society of America Journal. 73, 1652 – 1657.

Trigo, C., Koskinen, W.C., Celis, R., Sadowsky, M.J., Hermosin, M.C., Cornejo, J., 2010. Bioavailability of organoclay formulations of atrazine in soil. Agricultural and Food Chemistry. 58, 11857 – 11863.

Tsyusko, O.V., Hardas, S.S., Shoults-Wilson,W.A., Starnes, C.P., Joice, G., Butterfield, D.A., Unrine, J.M., 2012. Short-term molecular-level effects of silver nanoparticle exposure on the earthworm, *Eisenia fetida*. Environ. Pollut. 171, 249 – 255.

Udy, P.B., 1977. Factors influencing the release of insecticides from granular formulations and their subsequent diffusion into a range of New Zealand soils. Agric. Research. 20 (1), 79 – 85.

University of Hertfordshire., 2016. The pesticide properties data base developed by the Agriculture & Environment Research Unit, University of Hertfordshire, 2006 – 2016. http://sitem.herts.ac.uk/aeru/iupac/Reports/78.htm Accessed Sep 2016.

Unrine, J.M., Hunyadi, S.E., Tsyusko, O.V., Rao, W., Shoults-Wilson, W.A., Bertsch, P.M., 2010a. Evidence for bioavailability of Au nanoparticles from soil and biodistribution within earthworms (*Eisenia fetida*). Environ. Sci. Technol. 44, 8308 – 8313.

Unrine, J.M., Tsyusko, O.V., Hunyadi, S.E., Judy, J.D., Bertsch, P.M., 2010b. Effects of particle size on chemical speciation and bioavailability of copper to earthworms (*Eisenia fetida*) exposed to copper nanoparticles. Environ Qual. 39, 1942 – 1953.

US Environmental Protection Agency (USEPA)., 2007. Nanotechnology White Paper. Report EPA 100/B-07/001.Washington, DC, USA.

US Environmental Protection Agency (USEPA)., 2009. Targeted National Sewage Sludge Survey Statistical Analysis Report. EPA-822-R-08-018. Washington, DC: Office of Water.

US Environmental Protection Agency (USEPA)., 2010. Environmental fate and effects division. Environmental fate and ecological risk assessment problem formulation in support of registration review for bifenthrin.

Vaal, M., Van der Wal, J.T., Hoekstra, J., Hermens, J.L.M., 1997. Variation in the sensitivity of aquatic species in relation to the classification of environmental pollutants. Chemosphere. 35, 1311 – 1327.

Van der Ploeg, M.J.C., Baveco, J.M., Van der Hout, A., Bakker, R., Rietjens, I.M.C.M., Van den Brink, N.W., 2011. Effects of C_{60} nanoparticle exposure on earthworms (*Lumbricus rubellus*) and implications for population dynamics. Environ Poll. 159, 198 – 203.

Van der Ploeg, M.J.C., Baveco, J.M., Van der Hout, A., Bakker, R., Rietjens, I.M., Van den Brink, N.W., 2011. Effects of C60 nanoparticle exposure on earthworms (*Lumbricus rubellus*) and implications for population dynamics. Environ Pollut. 159 (1), 198 – 203.

Van Gestel, C.A.M., Ma, W., 1990. An approach to quantitative structure-activity relationships (QSARs) in earthworm toxicity studies. Chemosphere. 21, 1023 – 1033.

Van Gestel, C.A.M., Ma, W.C., 1988. Toxicity and bioaccumulation of chlorophenols in earthworms, in relation to bioavailability in soil. Ecotoxicol. Environ. Saf. 15, 289 – 297.

Van Gestel, C.A.M., Ortiz, M.D., Borgman, E., Verweij, R.A., 2011. The bioaccumulation of molybdenum in the earthworm *Eisenia andrei*: Influence of soil properties and ageing. Chemosphere 82, 1614 – 1619.

Van Gestel, C.A.M., Van Dis, W.A., 1988. The influence of soil characteristics on the toxicity of 4 chemicals to the earthworm *Eisenia fetida andrei* (Oligachaeta). Biology and Fertility of Soils. 6, 262 – 265.

Vauthier, C., Dubernet, C., Chauvierre, C., Brigger, I., Couvreur, P., 2003. Drug delivery to resistant tumors: The potential of poly(alkyl cyanoacrylate) nanoparticles. Control Rel. 93, 151 – 160.

Vijayakumar, P.S., Abhilash, O.U., Khan, B.M., Prasad, B.L.V., 2010. Nanogold-loaded sharp-edged carbon bullets as plant-gene carriers. Adv Funct Mater. 20, 2416 – 2423.

Viswanathan, P.N., and Krishnamurti, C.R., 1989. Effects of temperature and humidity on ecotoxicology of chemicals: In Ecotoxicology and Climate with Special Reference to Hot and Cold Climates, P. Bourdeau, J. A. Haines, W. Klein, and C. R. Krishanamurti, Eds. John Wiley & Sons, New York, NY, USA. P 139 – 154.

Voudrias, E., Fytianos, K., Bozani, E., 2002. Sorption – desorption isotherms of dyes from aqueous solutions and wastewaters with different sorbent materials. Global Nest: the Int. J. 4 (1), 75 – 83.

Waalewijn-Kool, P.L., Klein, K., Mallenco, R., Van Gestel, C.A.M., 2014. Bioaccumulation and toxicity of silver nanoparticles and silver nitrate to the soil arthropod *Folsomia candida*. Ecotoxicology. 23 (9), 1629 – 1637.

Waliszewski, S.M., Carvajal, O., Gomez-Arroyo, S., Amador-Munoz, O., Villalobos-Pietrini, R., Hayward-Jones, P.M., Valencia-Quintana ,R., 2008. DDT and HCH isomer levels in soils, carrot root and carrot leaf samples. Bull Environ Contam Toxicol. 81, 343 – 347.

Walker, A., 2000. A simple centrifugation technique for the extraction of soil solution to permit direct measurement of aqueous phase concentrations of pesticide. In: Cornejo, J., Jamet, P. (Eds.), Pesticide Soil Interactions-some current research methods. P 173 – 178.

Walker, A., Jurado-Exposito, M., 1998. Adsorption of isoproturon, diuron and metsulfuron-methyl in two soils at high soil:solution ratios. Weed Res. 38, 229 – 238.

Walker, A., Jurado-Exposito, M., Bending, G.D., Smith, V.J.R., 2001. Spatial variability in the degradation rate of isoproturon in soil. Environ. Pollut. 111, 407 – 415.

Walker, A.N., Golden, R., Horst, M.N., 2010. Morphologic effects of in vivo acute exposure to the pesticide methoprene on the hepatopancreas of a non-target organism, *Homarus americanus*. Ecotoxicol. Environ. Saf. 73, 1867 – 1874.

Wang, C.M., Baer, D.R., Amonette, J.E., Engelhard, M.H., Antony, J., Qiang, Y., 2009. Morphology and electronic structure of the oxide shell on the surface of iron nanoparticles. Am. Chem. Soc. 131, 8824 – 8832.

Wang, H., Wang, J., Choi, D., Tang, Z., Wu, H., Lin, Y., 2009. EQCM immunoassay for phosphorylated acetylcholinesterase as a biomarker for organophosphate exposures based on selective zirconia adsorption and enzyme-catalytic precipitation. Biosens Bioelectron. 24, 2377 – 2383.

Wang, H., Wick, R.L., Xing, B., 2009. Toxicity of nanoparticulate and bulk ZnO, Al_2O_3 and TiO_2 to the nematode *Caenorhabditis Elegans*. Environ. Pollut. 157, 1171 – 1177.

Wang, L.J., Li, X.F., Zhang, G.Y., Dong, J.F., Eastoe, J., 2007. Oil-in-water nanoemulsions for pesticide formulations. Colloid and Interface Science 314, 230 – 235.

Wang, T.C., Hoffman, M.E., 1991. Degradation of organophosphorus pesticides in coastal water. Association of Official Analytical Chemists. 74 (5),883 – 886.

Wang, Y., Cang, T., Zhao, X., Yu, R., Chen, L., Wu, C., Wang, Q., 2012. Comparative acute toxicity of twenty-four insecticides to earthworm, *Eisenia fetida*. Ecotoxicology and Environmental Safety. 79, 122 – 128.

Wang, Y., Gao, Z., Shen, F., Li, Y., Zhang, S., Ren, X., Hu, S., 2015. Physicochemical characteristics and slow release performances of chlorpyrifos encapsulated by poly(butyl acrylate-*co*-styrene) with the cross-linker ethylene glycol dimethacrylate. Agric. Food Chem. 63 (21), 5196 – 5204.

Wang, Y.H., Gao, C.F., Zhu, Y.C., Chen, J., Li, W.H., Zhuang, Y.L., Dai, D.J., Zhou, W.J., Ma, C.Y., Shen, J.L., 2008. Imidacloprid susceptibility survey and selection risk assessment in field populations of *Nilaparvata lugens* (Homoptera: Delphacidae). Econ. Entomol. 101, 515 – 522.

Ware, G.W., Whitacre, D.M., 2004. The Pesticide Book, 6th Ed. New York: Meister Publishing Company.

Weber, J.B., Weed, S.B., 1968. Adsorption and desorption of diquat paraquat and prometone by montmorillonitic and kaolinitic clay minerals. Soil Sci. Soc. Am. Proc. 32, 485.

Wenninger, J.A., Canterbery, R.C., McEwen, G.N.Jr., 2000. International cosmetic ingredient dictionary and handbook, 7th Ed, Vol 1–2. Washington, DC: CTFA.

Whatmore, R.W., 2006. "Nanotechnology – What is it? Should we be worried?". Occup. Med. 56 (5), 295 – 299.

White, J.C., Kelsey, J.W., Hatzinger, P.B., Alexander, M., 1997. Factors affecting sequestration and bioavailability of phenanthrene in soils. Environ. Toxicol. Chem. 16, 2040 – 2045.

Whitley, A.R., Levard, C., Oostveen, E., Bertsch, P.M., Matocha, C.J., Von der Kammer, F., Unrine, J.M., 2013. Behavior of Ag nanoparticles in soil: Effects of particle surface coating, aging and sewage sludge amendment. Environ. Poll. 182, 141 – 149.

Williams, R.G., Roussenov, V., Follows, M.J., 2006. Nutrient streams and their induction into the mixed layer. Global Biogeochem. Cycles. 20

Wilson, M.A., Tran, N.H., Milev, A.S., Kannangara, G.S.K., Volk, H., Lu, G.Q.M., 2008. Nanomaterials in soils. Geoderma. 146, 291 – 302.

Winter, M.D., McPherson, M.J., Atkinson, H.J., 2002. Neural uptake of pesticides disrupts chemosensory cells of nematodes. Parasitology. 125 (Pt 6), 561 – 565.

Wong, C.S., 2006. Environmental fate processes and biochemical transformations of chiral emerging organic pollutants. Anal. Bioanal. Chem. 386, 544 – 558.

Wright, J.E., 1997. Formulation for insect sex pheromone dispersion. Patent Number US 5670145 a 19970923.

Wu, X.M., Li, M., Long, Y.H., Liu, R.X., Yu, Y.L., Fang, H., Li, S.N., 2011. Effects of adsorption on degradation and bioavailability of metolachlor in soil. Soil Science and Plant Nutrition. 11 (3), 83 – 97.

Wu, X.M., Li, M., Long, Y.H., Liu, R.X., Yu, Y.L., Fang, H., Li, S.N., 2011. Effects of adsorption on degradation and bioavailability of metolachlor in soil. Soil Science and Plant Nutrition. 11 (3), 83 – 97.

Xu, J., Fan, Q.J., Yin, Z.Q., Li, X.T., Du, Y.H., Jia, R.Y., Wang, K.Y., Lv, C., Ye, G., Geng, Y., Su, G., Zhao, L., Hu, T.X., Shi, F., Zhang, L., Wu, CL., Tao, C., Zhang, Y.X., Shi, D.X., 2010. The preparation of neem oil microemulsion (*Azadirachta indica*) and the comparison of acaricidal time between neem oil microemulsion and other formulations in vitro. Veterinary Parasitology. 169 (3 – 4), 99 – 403.

Xu, L., Liu, Y., Bai, R., Chen, C., 2010. Applications and toxicological issues surrounding nanotechnology in the food industry. Pure Appl. Chem. 82, 349 – 372.

Xue, J., Luo, Z., Li, P., Ding, Y., Cui, Y., Wu, Q., 2014. A residue-free green synergistic antifungal nanotechnology for pesticidethiram by ZnO nanoparticles. Scientific Reports 4, Article number: 5408.

Yadav, S., 2015. Comparative study of impact of ZnO-NPs on *Caenorhabditis elegans* and *Eisenia foetida*. Micro. Appl. Sci. 4 (4), 1106 – 1113.

Yang, F.L., Li, X.G., Zhu, F., Lei, C.L., 2009. Structural characterisation of nanoparticles loaded with garlic essential oil and their insecticidal activity against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). Agric. Food Chem. 57 (21), 10156 – 10162.

Yang, F.L., Li, X.G., Zhu, F., Lei, C.L., 2009. Structural characterisation of nanoparticles loaded with garlic essential oil and their insecticidal activity against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). Agric. Food Chem. 57, 10156 – 10162.

Yang, K., Lin, D., Xing, B.S., 2009. Interactions of humic acid with nanosized inorganic oxides. Langmuir. 25, 3571 – 3576.

Yasmin, S., D'Souza, D., 2010. Effects of pesticides on the growth and reproduction of earthworm: A Review. Appl. Environ. Soil Sci. 9.

Yazgan, M.S., Wilkins, R.M., Sykas, C., Hoque, E., 2005. Comparison of two methods for estimation of soil sorption for imidacloprid and carbofuran. Chemosphere. 60, 1325 – 1331.

Ye, X., Xiong, K., Liu, J., 2016. Comparative toxicity and bioaccumulation of fenvalerate and esfenvalerate to earthworm *Eisenia fetida*. Hazardous Materials. 310, 82 – 88.

Yin, Y.H., Guo, Q.M., Han, Y, Wang, L.J., Wan, S.Q., 2012. Preparation, characterisation and nematicidal activity of lansiumamide B nano-capsules. Integr. Agric. 11 (7), 1151 – 1158.

Ying, S.H., Zi, X.R., Jian, F.L., Cai, H.H., Mei, X.S., 2005. In vitro adsorption of aflatoxin adsorbing nano-additive for aflatoxin B1, B2, G1, G2. Scientia Agricultura Sinica. 38 (5), 1069 – 1072.

Yun, L.Y., Xiao, M.W., Shao, N.L., Hua, F., Hai, Y.Z., Jing, Q.Y., 2006. An exploration of the relationship between adsorption and bioavailability of pesticides in soil to earthworm. Environ. Poll. 141 (3), 428 – 433.

Zacharia, J.T., 2011. Identity, Physical and Chemical Properties of Pesticides, Pesticides in the Modern World-Trends in Pesticides Analysis, Dr. Margarita Stoytcheva (Ed.), ISBN: 978-953-307-437-5, InTech, Available from:

http://www.intechopen.com/books/pesticides-in-the-modern-world-trends-in-pesticidesanalysis/identity-physical-and-chemical-properties-of-pesticides

Zeng, H., Li, X.F., Zhang, G.Y., Dong, J.F., 2008. Preparation and characterisation of beta-cypermethrin nanosuspensions by diluting O/W microemulsions. Disp. Sci. Technol. 29, 358 – 361.

Zhang, X., Zhang, J., Zhu, K.Y., 2010. Chitosan/ double-stranded RNA nanoparticle-mediated RNA interference to silence chitin synthase genes through larval feeding in the African malaria mosquito (*Anopheles gambiae*). Insect. Mol. Biol. 19, 683 – 693.

Zhu, J., Lu, Y.T., Chen, W.C., 2008. Single and joint toxic effects of cadmium and phenanthrene on enchytraeid *Fridericia bulbosa*. Soil Biol. 44, 260 – 265.

Zhu, X., Chang, Y., Chen, Y., 2009. Toxicity and bioaccumulation of TiO_2 nanoparticle aggregates in *Daphnia magna*. Chemosphere. 78, 209 – 215.