

Environmental Fate Studies of Fullerene C₆₀: Sorption to Soil, Desorption from Soil,
Biodegradation and Plant Uptake

by

Raghavendhran Avanasani Narasimhan, B.TECH

A Thesis

In

ENVIRONMENTAL TOXICOLOGY

Submitted to the Graduate Faculty
of Texas Tech University in
Partial Fulfillment of
the Requirements for
the Degree of

MASTER OF SCIENCE

Approved

Todd A. Anderson
(Chair)

Andrew Jackson

George P. Cobb

Fred Hartmeister
Dean of the Graduate School

August, 2010

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ACKNOWLEDGMENTS

I take this opportunity to thank each and everyone who has helped me in some way or the other for the successful completion of my Master's thesis research. Firstly, I would like to thank Dr. Todd Anderson, my thesis advisor, for all the professional help and the timely guidance that he provided. I thank him for all the time that he spent on mentoring me. I truly learnt a lot under his mentorship and I am always grateful to him. I would also like to thank my committee members including Dr. George Cobb and Dr. Andrew Jackson for their direction and advice. I thank them for raising challenging questions which made me work even further and come up with quality research.

I would also like to acknowledge the entire faculty at TIEHH who were involved in the purchase of the radiolabeled fullerene material. Without it, my research wouldn't have been possible. I also want to thank Dr. Jaclyn Cañas for helping with some of the experiments. I would also like to thank Dr. Philip Smith for giving me access to his lab for the experiments. I would like to thank my friends, the faculty and the support staff at TIEHH for their constant encouragement and mental support. Without their presence, my success wouldn't have been possible. Finally, I would like to thank my parents and my brother for their continued love and support.

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ABSTRACT

Buckminster fullerene (C_{60}) is the most representative among all fullerenes. It has been widely studied for its potential applications. Because its use in the medical, electronic, and materials industries is ever expanding, its release into the environment is inevitable. Studying the environmental fate of C_{60} fullerene is essential in order to better assess its potential for exposure to aquatic and terrestrial organisms. Our goal was to determine the basic environmental fate processes for C_{60} including sorption to soil, desorption from soil, biodegradation, and uptake into plants. Information on the fate of C_{60} in the environment and its potential transport to other trophic levels were critical data gaps that we addressed. Aqueous suspensions of ^{14}C -labeled C_{60} were prepared by two methods 1) extended mixing in water and 2) solvent exchange with THF. The aqueous suspensions of $^{14}C_{60}$ were characterized using a filtration experiment. It was observed that the aqueous suspension produced by extended mixing in water did not have aggregates in the nanometer range, whereas a majority (~60%) of aggregates in the aqueous suspension prepared by solvent exchange was in the nanometer range. The aqueous solution prepared by solvent exchange was used for all subsequent fate studies. Sorption and desorption parameters in three different soil types (sandy loam, silt loam, and a loam) were studied using the batch equilibrium method. Koc values for sandy loam (3684.8 mL/g) and silt loam (3719.65 mL/g) showed the expected consistency, while the Koc value for C_{60} in the loam was dramatically higher (10083.4 mL/g). Desorption from these soil types was determined as a percentage of the sorbed C_{60} . We found maximum desorption from sandy loam (85% at 24 h). The loam and silt loam showed around 35% C_{60} desorption at 24 h. Plant uptake experiments in sand and a hydroponic substrate

showed very low uptake of C_{60} ($< 10\%$). $^{14}C_{60}$ was distributed in the root $>$ tuber $>$ stem $>$ leaf. Biodegradation of C_{60} in the sandy loam and the silt loam was monitored for more than a year. No mineralization of C_{60} was observed. Our results on some basic environmental fate parameters for C_{60} have helped to fill data gaps related to the potential for fullerene nanomaterials to impact the environment. As is the case in nanomaterials research, there are some uncertainties in the results we obtained owing to the ability of C_{60} to aggregate and subsequently behave as a particulate.

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CHAPTER I

INTRODUCTION

1.1. Nanotechnology and Nanomaterials

Nanotechnology is defined as “the understanding and control of matter at dimensions of roughly 1-100 nm, where unique properties make novel applications possible” (NNI). The manufacture and use of nanoparticles has seen tremendous growth owing to the myriad of technological applications that have been proposed.

Nanoparticles in general can be grouped into natural and anthropogenic. Another basis of their classification is chemical composition. Based on this, nanoparticles may be carbon-containing or inorganic. Anthropogenic nanoparticles can either be produced intentionally for a specific property of interest or they can be formed as a by-product of combustion and other anthropogenic processes. Intentionally produced nanoparticles are termed engineered nanoparticles; examples include fullerenes, TiO₂, silver, etc. (Nowack and Bucheli, 2007).

Most of the interest in nanotechnology rests on engineered nanoparticles as they can be designed based on the intended application. Maynard et al. in 2006 predicted a production of 58,000 tons of nanomaterials between 2011 and 2020. With such huge increases in their production, release into the environment through spillage, waste releases from production facilities, and use of products such as electronics, paints, fabrics, personal care products, etc., is eminent. Most research on nanomaterials has focused on their further exploitation in different fields; less thought has been given to the potential environmental effects of their use (Ray et al., 2009). Although various toxicity

studies are being conducted, there are very few complementary environmental fate studies. The transport and fate of nanoparticles in the environment as a result of their intentional or unintentional release is uncharted territory in terms of nanoparticle research.

A model of the fate and transformation pathways of nanoparticles in the environment is illustrated in Figure 1. The figure also depicts how nanoparticles can undergo trophic transport once released into the environment from various sources. The source of the release could be a non-point source (use of commercial nanoparticle-based products) or a point source (industrial contamination or by-products of human activity). Once released into the environment, nanoparticles can be transported between different phases or transformed by the action of oxidants, microorganisms, light, etc. (Pérez et al., 2009). One other important fate parameter to be considered is the aggregation of individual nanoparticles to form clusters, hence losing some of the special properties owing to the structure of individual molecules.

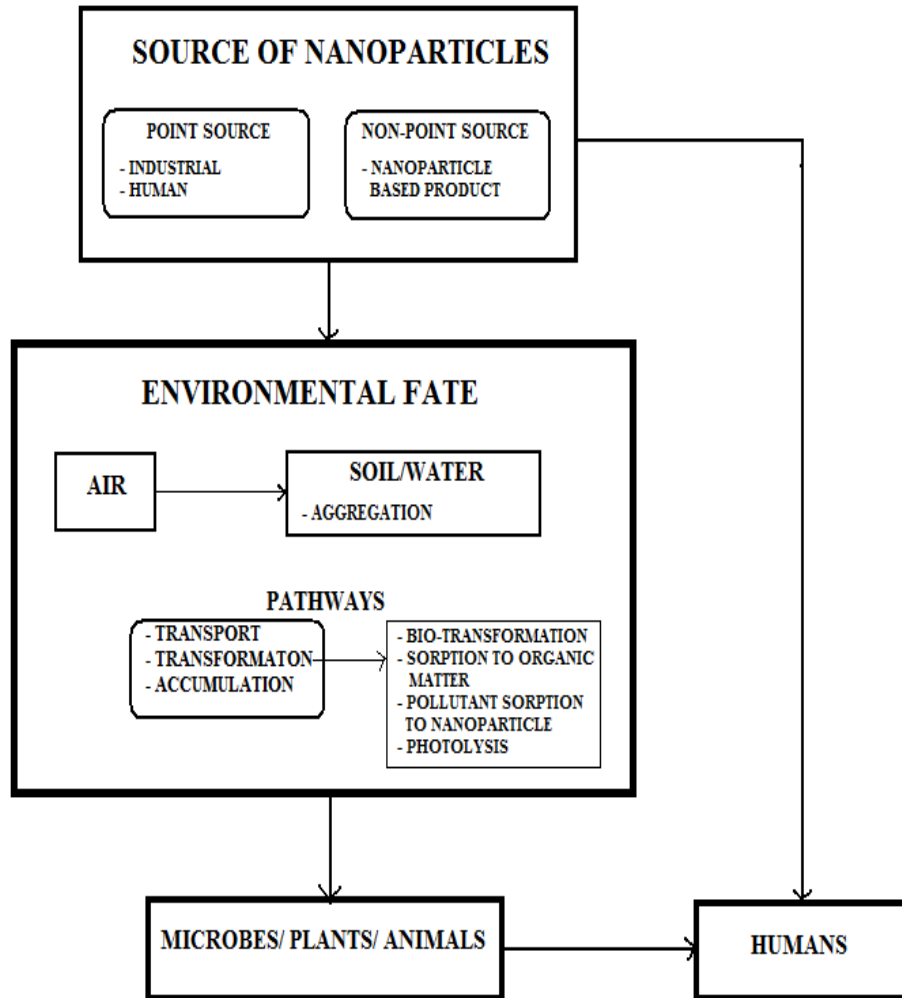


Figure 1: A model of fate pathways and transformations of nanoparticles in the environment (adapted from (Simonet and Valcárcel, 2009)).

Once in the environment, nanoparticles may be taken up by microorganisms, flora, and fauna. Trophic transfer of nanomaterials from soil and water to higher organisms and humans through the food web is a possibility which needs to be researched.

1.2. Fullerenes

Fullerenes were discovered by researchers in 1985 as another allotropic form of carbon. Fullerenes can be classified as natural as well as anthropogenic carbon-containing nanoparticles. Fullerenes were formed naturally millions of years ago and have been found in geological deposits from the cretaceous-tertiary boundary (Pérez et al., 2009), but it was only after their discovery in 1985 that scientists further studied their physical and chemical properties.

Fullerenes were initially discovered by scientists at Rice University and the University of Sussex in England who were studying the condensation of carbon vapors and discovered the formation of hollow truncated icosahedrons made of only carbon atoms. Following this, the formation of a number of these structures with varying numbers of carbon atoms was discovered. These molecules were named fullerenes because the structure of the C_{60} molecule resembled the geodesic dome that was built by Buckminster Fuller. Other species in the fullerene family have different geometric structures based on their mass (Kroto et al., 1985).

C_{60} is the most representative among all the fullerenes because of its abundance; it constitutes 75% of all the fullerenes produced by the Hufmann-Kratschmer arc-discharge method. It has 60 carbon atoms forming a soccer-ball like structure with 20 hexagons and

12 pentagons. Based on their structure, properties, and numerous possible derivatives, applications in the medical, electronic, chemical, and cosmetic industry have been proposed for fullerenes (Bosi et al., 2003).

Mass production and use of fullerenes will likely release them into the environment. Ju-Nam and Lead, in 2008, clearly discuss the lack of substantial data on fate and impact of nanoparticles in the environment. In order to perform valid risk assessments of nanoparticles, it is necessary to have quality data that describe the toxicity of nanoparticles and transport in the environment as well as among environmental and biological compartments.

Our goal was to study the environmental fate processes of C_{60} including sorption to soil, desorption from soil, biodegradation, and uptake into plants. Information on where C_{60} will end up in the environment and what are the chances for it to be transported to other trophic levels were critical data gaps that we addressed.

CHAPTER II

BACKGROUND

2.1. Structure of the C₆₀ Molecule

C₆₀ buckminsterfullerene has 60 carbon atoms which form a truncated icosahedral structure. This structure resembles a soccer ball with 20 hexagons and 12 pentagons made of carbon. All the electrons are paired and as a result all the valences satisfied. As a consequence, the inner and outer surfaces are covered with pi electrons which render the structure stability and aromaticity. Due to the stability of the structure and the presence of a hollow cavity in the center which can hold different kinds of atoms, a number of manipulations to the structure are possible (Kroto et al., 1985). The availability, high symmetry, and relatively cheap price make C₆₀ the most used and widely studied among all fullerenes (Billups, 2007).

2.2. Applications of C₆₀ and its Derivatives

C₆₀ derivatives have found applications in medicine, electronics, and materials industries. Adding functional groups such as -OH, -COOH, -NH₂, -NO₂, -CH₃ and various metals to the base C₆₀ structure modifies its solubility and surface charge, producing various unique compounds with semi conductive, superconductive, electrochemical, optical, and magnetic properties. Thus, C₆₀ can be used in the electronics, textile, and the other materials industries (Billups, 2007). Biological properties of fullerenes include antimicrobial activity (Lyon et al., 2006), antioxidant activity, neuroprotective activity, antiapoptotic activity, anti-HIV activity, enzyme inhibition, and osteoporotic therapy (Bosi et al., 2003).

2.3. Aqueous C₆₀ Suspensions

Ruoff et al., (1993) studied the solubility of C₆₀ in a number of solvents and concluded that C₆₀ has very low solubility in polar solvents like water ($< 10^{-9}$ mg/L). Andrievsky et al. (1995) found that C₆₀ can form stable aggregates in water called FWS (fullerene water suspensions) or nano-C₆₀ (nC₆₀). The aggregates have a net negative surface charge which stabilizes them in solution. Andrievsky and co-workers were able to produce C₆₀ suspensions in water by sonication. They took a solution of C₆₀ in toluene and added it to Milli-Q water. This was then sonicated until all the toluene was evaporated and the C₆₀ was in solution with water.

Deguchi et al. (2001) developed a method, later modified (Fortner et al., 2005), to introduce C₆₀ into an aqueous phase indirectly through a solvent exchange process using tetrahydrofuran (THF), in which C₆₀ aggregated with diameters around 5 to 500 nm. These aggregates were named nano-C₆₀. The method that they followed involved taking a solution of C₆₀ in THF, adding water, and mixing. The THF was then evaporated. These steps were repeated several times until all the C₆₀ was in water and all the THF had been removed. This procedure has been widely accepted and used. The aggregation of C₆₀ allows for concentrations up to 100 mg/L (Fortner et al., 2005), 11 orders of magnitude more than the estimated molecular solubility. Other methods used to produce nC₆₀ in water include extended mixing of C₆₀ in water (Cheng et al., 2005). This involves the stirring of C₆₀ in water for a long period of time (> 30 d).

Brant et al. (2006) compared the properties of C₆₀ aqueous suspensions prepared by various methods including extended mixing in water, solvent exchange using THF,

and the sonication procedure. They found that the aggregates produced by the sonication procedure were mostly smaller than 450 nm, whereas those produced by extended mixing and THF solvent exchange varied, with aggregate sizes ranging from a few hundred nm to a few thousand nm. They concluded that the size, shape, and chemical composition of fullerene aggregates varied among the different methods. Thus, it was difficult to generalize about the properties of aqueous fullerene suspensions. There has been considerable debate on whether using the solvent exchange method to get C₆₀ in water is the proper method to obtain aqueous suspensions since studies have shown the presence of trace levels of organic solvent in the aggregates even after evaporation.

2.4. Toxicity of C₆₀ Fullerene

A number of studies have been conducted to determine the toxicity of fullerenes in general and C₆₀ in particular. Duncan et al. (2007) clearly describe how a number of studies with aqueous suspensions of fullerenes (produced by a solvent exchange procedure with various organic solvents) have shown toxicity to fish, bacteria, and some human cell types. These results have been questioned by others who suggest that the organic solvent trapped in the fullerene aggregate structure is responsible for the toxicity observed. Oberdorster (2004) suggested that fullerene C₆₀ (prepared by the solvent exchange process with THF) induced lipid peroxidation in the brain of largemouth bass (*Micropterus salmoides*). Sayes et al. (2004) showed cytotoxicity of C₆₀ and its derivatives to two human cell lines: skin and liver. They also compared the toxicity of the C₆₀ molecule to its derivatives and found that C₆₀ was more toxic when compared to its derivatives. Fortner et al. (2005) reported the adverse effects of C₆₀ in water to both gram negative and gram positive bacteria. Sayes et al. (2005) described the toxicity of fullerene

nano-suspensions through lipid peroxidation caused by reactive oxygen species being produced. Usenko et al. (2007) found *in vivo* toxicity of C₆₀ to embryonic zebra fish.

Blickley and McClellan-Green in 2008 studied the movement of aqueous C₆₀ aggregates and their toxicity to a marine teleost. The study suggested no toxicity of aqueous C₆₀ to various life stages of the organism up to concentrations of 10 mg/mL. Andrievsky et al. (2005) conducted a review of all the toxicity studies done to date using nano-C₆₀ and concluded that most of the toxicity effects observed for nano-C₆₀ (prepared by solvent exchange method with THF) were due to the presence of the organic solvent within the C₆₀ aggregate structure. They claimed that pristine C₆₀ in water (a FWS; Fullerene Water Suspension), is non-toxic and in fact possesses some positive biological effects. Zhu et al. in 2006 studied the acute toxicity of C₆₀ fullerene aqueous suspensions prepared by solvent exchange (THF) and extended stirring in water to *Daphnia magna* and adult fathead minnow, *Pimephales promelas*. They found that for *Daphnia magna* the 48 h LC₅₀ of C₆₀ in water (prepared by solvent exchange with THF) was 0.8 ppm and that of C₆₀ in water (prepared by extended mixing) was > 35 ppm, indicating a higher toxicity for C₆₀ prepared by the solvent exchange method. For the adult male fathead minnows, exposure to 0.5 ppm of C₆₀ in water (solvent exchange with THF) caused death in 18 h, whereas the C₆₀ in water (extended mixing) did not cause death but produced increased lipid peroxidation.

Tao et al. in 2009 studied the sub-lethal reproductive responses and accumulation of nano-C₆₀ in *Daphnia magna*. They estimated sub-lethal concentrations of 0.4 mg/L for adult daphnids and 0.2 mg/L for neonate daphnids using a standard 48 h exposure test. They observed that at sub-lethal concentrations, C₆₀ caused increased mortality of

neonate daphnids during their development and the exposed mothers were not able to reproduce further. In addition, the accumulation of C₆₀ by daphnids depended on the lipid content of the individuals. Their results highlight the fact that acute C₆₀ toxicity is not the only concern; sub-lethal effects and chronic effects should also be considered. Lovern et al. in 2007 studied the behavioral effects of nano-C₆₀ to *Daphnia magna* and found that nano-C₆₀ caused higher heart rate, hopping frequency and appendage movement, all activities that might increase the risk to predation. Such an increase in activities also suggests decreased reproductive success, indirectly affecting the aquatic food web.

Ringwood et al. in 2009 studied the effects of C₆₀ fullerene aqueous suspension on Oyster, *Crassostrea virginica*. They used an aqueous C₆₀ solution prepared by solvent exchange with toluene. Results of their experiments showed C₆₀ toxicity to oyster embryonic development as well as lysosomal destabilization; lipid peroxidation was not observed when they exposed hepatopancreatic tissue to C₆₀. The observed effects on lysosomal function suggested that chronic effects of fullerene exposure are possible and need to be studied.

Valant et al. (2009) studied the *in vivo* toxicity of C₆₀ fullerenes to the terrestrial invertebrate, *Porcellio scaber*, and compared the effects with other engineered nanoparticles including zinc and titanium based nanoparticles. They found that C₆₀ (prepared by extended mixing in DI water) had the highest biological effect (stability of the hepatopancreatic cell membrane) among all the nanomaterials studied.

From all these studies and reviews, it is still unclear if the C_{60} molecule by itself is toxic or the combined action of the C_{60} molecule and the organic solvent trapped in the aqueous nano- C_{60} aggregates produces the toxicity.

2.5. Environmental Fate of C_{60} Fullerene

In contrast to the number of studies on C_{60} fullerene toxicity, there have been far fewer studies on the environmental fate of C_{60} and other nanomaterials (Pérez et al., 2009). Estimation of risk can be achieved only by integrating the exposure (availability) data and the toxicity data. In order to study the potential for exposure to C_{60} , environmental fate parameters such as octanol-water partition coefficient (K_{ow}) and organic carbon partition coefficient (K_{oc}) (among others) must be determined. An experimental octanol-water partition coefficient ($\log K_{ow}$) value of 6.67 for C_{60} has been determined (Jafvert and Kulkarni, 2008). This suggests very high lipophilicity and the subsequent potential to accumulate in organisms. Chen and Jafvert (2009) calculated a soil partition coefficient (from an ethanol-water solution) and then used it to predict the soil/water partition coefficient of molecular C_{60} . They used an ethanol-water mixture to obtain a solution of C_{60} in the molecular form and not an aggregate. They performed sorption experiments for C_{60} in ethanol-water mixtures (at varying mole fractions) using different soil types and calculated a partition coefficient between the soil and the ethanol-water mixture. The organic carbon partition coefficient (K_{oc}) was calculated at varying mole fractions of ethanol-water. This was extrapolated to calculate K_{oc} at an ethanol mole fraction of zero (where the mixture contains only water and no ethanol) which represented the K_{oc} in pure water. The $\log K_{oc}$ value predicted corresponded closely to their previously predicted $\log K_{ow}$ value for C_{60} . Although this method appears to

provide a good procedure for predicting the partition coefficient of molecular C₆₀, it probably does not represent the actual behavior of C₆₀ in the environment because of the ability of the C₆₀ molecule to form nano-aggregates in polar solutions. When aggregation is considered, the apparent soil partition coefficient for C₆₀ might vary largely from ideal behavior.

Studying the partitioning behavior of nano-C₆₀ adds another dimension to the environmental fate of C₆₀ that has not been studied thus far. Information on the partition coefficient of C₆₀ for both the molecular and aggregate forms will be of great value when assessing the overall exposure potential of these nanomaterials in aquatic and terrestrial environments and the processes that dictate their bioavailability to aquatic and terrestrial organisms.

2.6. The Use of Radiolabeled C₆₀ for Fate Studies

Radiolabeling chemicals and using them to monitor movement and distribution in various living systems has been widely used owing to the simplicity in detection and quantification. Assessing the environmental fate of chemicals in the laboratory using labeled tracers is an efficient technique. Scrivens et al. (1994) came up with the idea of using ¹⁴C₆₀ for experiments involving transport and uptake of C₆₀; they used ¹⁴C₆₀ in monitoring the uptake by human keratinocytes. ¹⁴C₆₀ can be detected and quantified using liquid scintillation spectroscopy. This technique can aid in locating the C₆₀ in samples and also reduces the laborious and time consuming methods often required for the analysis of non-labeled compounds. Recently, Nikolic et al. (2009) studied the biodistribution of ¹²⁵I-labeled nano-C₆₀ in rats and observed higher accumulation of C₆₀

in liver and spleen among other organs such as thyroid, stomach, lungs, and intestine. They concluded that the use of radiolabeled C_{60} in studying biodistribution as well as environmental fate is an efficient technique.

CHAPTER III

MATERIALS AND METHODS

3.1. Reagents and Chemicals

¹⁴C-labeled fullerene C₆₀ (37.06 μCi; 4.71 μCi/mg; 98.8% radiochemical purity; 96.9% chemical purity) was obtained via custom synthesis from Research Triangle Institute (Research Triangle Park, NC). Fullerene C₆₀ (99.5% pure) used for dilution was purchased from Sigma Aldrich (St. Louis, MO). A Beckmann LS 6500 liquid scintillation spectrometer was used to determine radioactivity in all samples. Scintiverse™ BD cocktail (Fisher Scientific) and disposable 5-mL polypropylene scintillation vials (Fisher) were also used. ACS grade THF (tetrahydrofuran) was obtained from EMD Chemicals (Gibbstown, NJ). 18 MΩ Milli-Q water was provided by a Nanopure™ Infinity water purification system. Miracle-Gro® all purpose plant food was used in the plant uptake experiments as a nutrient supplement.

A sandy loam (Terry County, Texas), a silt loam (Harlan County, Nebraska), a loam (Benton County, Oregon), and laboratory (Ottawa) sand (Fisher Scientific) were used as test “soils” in various experiments (described below). Physicochemical properties of these test materials were determined by Midwest Laboratories (Omaha, NE).

3.2. Methods

3.2.1. Liquid Scintillation Spectroscopy

A Beckmann LS 6500 liquid scintillation spectrometer was used to determine radioactivity in samples. Sample aliquots (in duplicate) were added to vials containing 5 mL of Scintiverse™ BD cocktail, vortexed to mix the contents, and counted for 5 minutes.

3.2.2. Preparation and Characterization of Aqueous $^{14}\text{C}_{60}$ Suspension

The ^{14}C -labeled C_{60} was provided in a 25 mL toluene solution with a total radioactivity of 37 μCi . The stock (12.5 mL, 18.53 μCi) was diluted with 20.9 g non-radiolabeled C_{60} to a final concentration of 1.48 $\mu\text{Ci}/\text{mL}$. Following this initial dilution, aqueous suspensions of fullerene C_{60} were prepared by two methods. First, an aqueous suspension of C_{60} was prepared using a solvent exchange procedure with THF described in Fortner et al., (2005). The $^{14}\text{C}_{60}$ stock solution described above (8.5 mL, 12.6 μCi) was mixed with 1L of THF and stirred. To 250 mL of this solution, an equal volume of water was added and stirred. This was followed by evaporation of THF using a rotary evaporator. The process was repeated until all the THF was evaporated and a final volume of 2 L of C_{60} in water was obtained. For the other method, extended mixing of the $^{14}\text{C}_{60}$ stock solution with water was used. The stock (12.5 mL) was N_2 evaporated to ≈ 0.5 mL and then added to 1 L of water and stirred for 30 days. After this, another 950 mL of water was added and stirred to obtain a final volume of 1950 mL.

In order to characterize both aqueous suspensions for the size of C_{60} aggregates, a filtration test was conducted. These two C_{60} aqueous suspensions were passed through

100, 200, 450, 1000 and 1500 nm filters and radioactivity in the filtrates were determined. We were not able to obtain the same filtration material in all pore sizes, so the filters consisted of different material including nylon, PTFE, PVDF, and glass fiber. Specifically, the 200 and 450 nm filters were nylon and PTFE; the 450 and 100 nm filters were PVDF; and the 1500 and 1000 nm filters were glass fiber.

The filtration experiment revealed that more than 90% of C_{60} aqueous suspension prepared using extended mixing in water had aggregate dimensions larger than 1500 nm, while the C_{60} aqueous suspensions produced by the solvent (THF) exchange process had aggregate sizes primarily (60%) smaller than 200, 450, 1000, and 1500 nm. Although the size of these aggregates did not appear to exactly match the literature values (Fortner et al., 2005), we proceeded with use of the aqueous suspension prepared by the solvent exchange method in all our experiments.

3.2.3. Sorption of C_{60} to Soil

Sorption of C_{60} to sandy loam, silt loam, loam, and laboratory sand was determined in batch equilibrium experiments. An experiment was conducted with sandy loam to determine the time point at which sorption equilibrium occurs. Sandy loam (0.1 g) was added to a 50 mL vial containing 17 mL 0.01 M $CaCl_2$ solution ($CaCl_2$ was used in order to provide ionic strength to the solution, thereby facilitating the settling of soil particles). To this, 3 mL of the C_{60} aqueous suspension was added and mixed. Controls had no soil and were used to monitor the “loss” of C_{60} to the vials by either sticking or settling. C_{60} in the supernatant was monitored at various time points including 24, 48, and 72 h. At each time point, readings were taken at 2 min, 1 h and 24 h hold times (after the

mixing was stopped). It was observed that, 24 h is long enough for sorption equilibrium to occur; therefore it was decided to use the 24 h time point to measure sorption in all soil types. Although the C_{60} in the supernatant varied with hold time (suggesting that some particles remained suspended), 2 min was considered an optimum hold time to measure sorption, as longer hold times brought natural and particle induced settling into consideration.

A total of 5 replicate test vials and 2 control vials (no soil or sand) were used in these experiments. Soil (0.1 g for the three soils and 1 g for sand experiment) was added to a 50 mL vial containing 17 mL 0.01 M $CaCl_2$ solution ($CaCl_2$ was used in order to provide ionic strength to the solution, thereby allowing soil particles to settle quickly). To this, 3 mL of the C_{60} aqueous suspension was added and mixed. After allowing the soil particles to settle for 2 min, the supernatant (0.5 mL) was collected and added to scintillation cocktail and counted. These were the 0 h readings. Subsequently, sorption equilibrium was allowed to occur by mixing the vials (150 rpm) for 24 h. At 24 h, 0.5 mL of the supernatant was collected and added to scintillation cocktail and counted. Using the 0 h and 24 h readings, we calculated the soil-water distribution coefficient (K_d) which is given by the ratio of concentration of C_{60} in soil to that in water.

$$K_d \text{ (mL/g)} = [(DPMs \text{ at } 0 \text{ h} - DPMs \text{ at } 24 \text{ h}) / \text{weight of soil}] / [(DPMs \text{ at } 24 \text{ h}) / \text{volume of water}]$$

The K_d value, along with the percent organic carbon content of each soil type was used to calculate the organic carbon distribution coefficient (K_{oc}).

$$K_{oc} = (K_d / \text{percent organic carbon}) * 100$$

The control vials (no soil) were used to determine how much C_{60} was either lost to the glass walls or aggregated and settled from the water column. This was expressed as the percent C_{60} lost to the vial,

$$\% C_{60} \text{ lost to the vial} = (\text{Total DPMs at 0 h} - \text{Total DPMs at 24 h}) / \text{Total DPMs at 0 h}$$

3.2.4. Desorption of C_{60} from Soil

Desorption of the sorbed C_{60} from the different soil types was determined using the same batch equilibrium method. A total of 5 replicate test vials and 2 control vials (no soil or sand) were used in these experiments. After the initial 24 h sorption period, water containing the un-sorbed C_{60} was removed and fresh water solution (0.01 M $CaCl_2$) was added to the vials. The vials were then mixed. After 2 min, 0.5 mL of the supernatant was removed from the vials, mixed with scintillation cocktail, and counted. This was the 0 h desorption count. Following this, 24 h, 48 h, and 72 h desorption counts were obtained in a similar fashion while the vials were mixed (150 rpm). Desorption values were expressed as the percentage of the sorbed C_{60} that desorbed over the respective time period.

3.2.5. Preliminary Plant Uptake Experiment

The uptake of C_{60} into radish (*Raphanus sativus*) was initially studied using three different substrates. The substrates included an inert rockwool substrate (Grodan®), a sand substrate, and a hydroponic solution. A preliminary uptake experiment conducted with the rockwool substrate suggested that the use of this material reduced the availability of C_{60} for uptake into plants. In order to increase the availability of C_{60} to plants, sand and hydroponic substrates were used in subsequent plant uptake studies.

3.2.6. Plant Uptake Study - Sand Substrate

Laboratory sand was used as the substrate to grow radish plants. Two separate plant uptake experiments with sand were conducted; one with 3 plants and the other with 6 plants. All plants were grown under controlled conditions of light and aeration in a Percival-Intellus environmental chamber. Radish seeds were germinated in a Petri dish and then sown in 250 mL beakers containing a known amount of sand. Seeds were watered regularly. When plants reached the 4 leaf stage, they were dosed with 5 mL of the C₆₀ solution (approximately 46,500 DPMs). After ≈2 weeks, plants are harvested and then separated into root, tuber, stem, and leaf. The individual plant part wet weights were determined, and these parts were allowed to dry. Dry plant parts were weighed and then extracted with THF. The extraction step included grinding plant parts using a mortar and pestle followed by shaking the ground parts in THF for 48 to 72 h. Plant extracts were then counted for radioactivity. Total DPMs in the plants were calculated based on the sum of DPMs in plant parts. Portions of the sand substrate (5 g) were extracted in triplicate with THF by shaking for 72 h. Radioactivity in the sand extracts was determined and used to calculate the total DPMs remaining in the sand. Radioactivity in plants and sand was compared to the initial C₆₀ radioactivity added in order to calculate a mass balance.

3.2.7. Plant Uptake Study - Hydroponic Substrate

Uptake of C₆₀ into plants under conditions of maximum bioavailability was studied using a hydroponic system. Plants (n = 7) were first grown in containers using potting soil. When radish plants reached the 4 leaf stage, they were transferred to beakers

containing water. Hydroponic plant systems were dosed with 5 mL of the C₆₀ solution (approximately 46,500 DPMs). After ≈2 weeks, plants are harvested and then separated into root, tuber, stem, and leaf. The individual plant part wet weights were determined, and these parts were allowed to dry. Dry plant parts were weighed and then extracted with THF. The extraction step included grinding the plant parts using a mortar and pestle followed by shaking the ground parts in THF for 48 to 72 h. Plant extracts were then counted for radioactivity. Total DPMs in the plants were calculated based on the sum of DPMs in plant parts. Radioactivity remaining in the hydroponic substrate was determined. Radioactivity in plants and the hydroponic solution was compared to the initial C₆₀ radioactivity added in order to calculate a mass balance.

3.2.8. Biodegradation

Biodegradation of C₆₀ was studied in the sandy loam and the silt loam soil types. 50 g of each soil was added to glass incubation bottles (4 replicates per soil type). Soils were moistened with a solution of 9 mL water and 6 mL of the C₆₀ aqueous suspension. Soils were mixed well and then a vial containing 10 mL of 0.1M KOH solution was placed in each bottle to trap CO₂. Incubation bottles were closed with a lid and incubated in the dark at room temperature. The KOH trap was replaced at approximately 30 day intervals. The KOH trap (0.5 mL) was removed, added to scintillation cocktail, and radioactivity was determined. Biodegradation studies were carried out for 754 days in silt loam and 328 days in sandy loam.

3.2.9. Analyses of Results

Results were analyzed using SigmaPlot (Version 10.0, Systat Software, Inc). Statistical analyses were performed using R (Version 2.8.1, The R Foundation for Statistical Computing).

CHAPTER IV

RESULTS

4.1. Characterization of Aqueous $^{14}\text{C}_{60}$ Suspensions: Estimation of Aggregate Size

The aqueous suspensions of $^{14}\text{C}_{60}$ prepared by (1) extended mixing in water and (2) solvent exchange process were characterized by filtering them through a range of filters: 100, 200, 450, 1000, 1500 nm. Results for the $^{14}\text{C}_{60}$ aqueous suspension prepared by extended mixing (Table 2) indicated loss of around 98% of the C_{60} in the 100, 200, and 450 nm filters made of nylon, polytetrafluoroethylene (PTFE), and polyvinylidene fluoride (PVDF) compared to unfiltered controls. The 1000 and 1500 nm filters (glass fiber, GF) retained 96% and 94% of the $^{14}\text{C}_{60}$, respectively.

Filtration tests on the aqueous suspension prepared by solvent exchange with THF (Table 3) showed $\approx 47\%$ loss of $^{14}\text{C}_{60}$ with 100, 200 (PTFE), and 450 (PTFE and PVDF) nm filters. The nylon 200 nm and 450 nm filters retained 94% and 75%, respectively, of the total $^{14}\text{C}_{60}$ compared to controls. With the 1000 nm and 1500 nm GF filters, only 39% of the $^{14}\text{C}_{60}$ was lost. These results suggest that the $^{14}\text{C}_{60}$ aqueous suspension prepared by solvent exchange contained aggregates that were in the nano range; hence we used this $^{14}\text{C}_{60}$ solution for all of our fate studies.

4.2. Sorption of C_{60} to Soil

From the initial sorption experiment with sandy loam to determine the time for equilibrium, it was found that the counts of C_{60} in the supernatant did not vary among the

individual time points of 24, 48 and 72 h. This suggests that 24 h was long enough for sorption equilibrium to occur. However, counts at time point + 2 min, time point + 1 h, time point + 24 h showed a consistent decrease in the amount of C_{60} in suspension as the hold time increased (Table 4). This suggests a role of the suspended soil particles in the settling of unsorbed C_{60} in suspension. In addition, the control vials showed that some of the C_{60} was lost to the vial either by sticking to glass or settling by aggregation. It was found that approximately $11.2\% \pm 1.2\%$ of the C_{60} was lost between two time points (24 h). Also, from the control vials, the loss of C_{60} to the water column was monitored over time and the results (Figure 2) revealed a steep increase in the loss of C_{60} between the 0 h and the 24 h + 2 m time point (0 to 40%) followed by a gradual increase with a maximum loss of around 75% at the 72 h + 24 h time point. Table 5 shows the K_{oc} values for each individual time point. We find that the K_{oc} values at specific hold times (at different time points) are similar, while those at specific time points (at different hold times) are different from each other. This supports the possibility of natural and soil particle assisted settling of C_{60} over time.

A series of batch equilibrium experiments were used to determine the soil-water partition coefficient of C_{60} in sandy loam, silt loam, loam, and laboratory sand (Table 6). The K_d value for C_{60} determined in the sandy loam soil was 47.9 ± 11.8 mL/g. For the silt loam soil, the K_d value for C_{60} was determined to be 92.9 ± 29 mL/g. For the loam soil, the K_d was 252.09 ± 51.08 mL/g, and for sand, the K_d for C_{60} was 5.71 ± 1.07 mL/g. The organic carbon partition coefficient (K_{oc}) for C_{60} , which is normalized using the percent organic carbon (%OC) content of the soil, was also calculated for each soil type. Given that the K_{oc} value is normalized for %OC, K_{oc} values should not vary with

soil type assuming OC is primarily responsible for sorption of the compound. Koc values for C₆₀ in sandy loam soil and silt loam soil were nearly equal (3684.8 ± 908.2 and 3719.65 ± 1036.9 mL/g, respectively). However, the Koc value for C₆₀ in the loam soil was 10083.4 ± 1827.4 mL/g. This suggests the role of factors other than percent organic carbon in the sorption process for C₆₀ in this soil type. The Koc value for sand using an assumed organic content of 0.1% yielded a Koc value of 5713.25 ± 1074.5 mL/g. From the control vials, the percentage C₆₀ lost from the water column by aggregate settling or sticking to glass was on average $14.6\% \pm 6$ of the total radioactivity added. The Koc value for C₆₀ suggests strong to very strong sorption (EPA, 2005) and slight mobility (Swann et al., 1983) in soil.

Statistical analyses were performed to compare the Koc values for the different soil types. The ANOVA results indicated a significant difference in the Koc value among the soil types ($p < 0.0001$). In order to compare the individual soil types, a Tukey's multiple comparison test was performed on the data. It was found that there was a significant difference in the Koc value for the loam and the sandy loam ($p < 0.0001$), the loam and the silt loam ($p < 0.0001$), and the loam and sand ($p < 0.0001$). The Koc values for C₆₀ in the sandy loam and the silt loam were not significantly different ($p = 0.999$). In addition, the Koc values for C₆₀ in the sandy loam and sand were not significantly different ($p = 0.129$). Finally, the Koc values for C₆₀ in the silt loam and sand were not significantly different ($p = 0.139$). Results of these statistical comparisons are consistent with OC acting as the primary driver for C₆₀ sorption to soil. The exception was the loam, in which something in addition to OC is participating in the sorption process for C₆₀, leading to a higher than expected Koc value.

4.3. Desorption of C₆₀ from Soil

Desorption of the sorbed C₆₀ was measured at 24-h intervals over 72 h using the same batch equilibrium technique. The desorption percentages were adjusted for natural settling of C₆₀ by using the percent C₆₀ lost in the control vials of the sorption experiments. It was observed that for the sandy loam, 74.4% of the sorbed ¹⁴C₆₀ desorbed immediately after the addition of fresh water and 85.4% desorption was observed after 24 h. For the silt loam soil, 36.4% desorption was observed at 0 h and subsequently, no further desorption was observed (Table 7).

For the loam, it was found that the desorption counts exceeded the sorption counts, suggesting that there were other factors which contributed to the increased settling of C₆₀. A soil control was prepared by shaking loam soil in water (at the same w/v ratio as the test) and removing the supernatant without the soil. Sorption tests were performed with the supernatant and ¹⁴C₆₀ counts were taken at 0 h, 24 h, and 24 h+15 m. The soil control showed 62% reduction of counts in the supernatant between the 24 h and 24 h+15 m. There was no loss of ¹⁴C₆₀ from the water column of the control sample. This result indicates that the presence of soil facilitates the loss of ¹⁴C₆₀ from the water column and not simply ¹⁴C₆₀ aggregation and settling. So, for the desorption experiment with loam soil, vials were kept undisturbed for 15 min prior to sampling. The percent desorption was calculated with total C₆₀ in soil (24 h+15 m count) as the basis, thereby, including the soil assisted settling process to the actual sorption process. It was observed that 42.4% of the sorbed ¹⁴C₆₀ desorbed after 24 h followed by a maximum of 52.4% desorption after 72 h.

ANOVA was used to determine if there was any difference in the mean percent desorption among the soil types at time = 0 h. The ANOVA result indicated a significant difference in the percent desorption among the soil types at time = 0 h ($p < 0.0001$). Data were further analyzed using a Tukey's multiple comparison test to identify differences among individual soil types. There was no significant difference in desorption between the silt loam and the loam soils ($p = 0.4959$). However, there was a significant difference between the sandy loam and the loam ($p = 0.0003$) and silt loam ($p < 0.0001$).

4.4. Plant Uptake of C_{60} from Sand and Hydroponic Substrates

Plant uptake studies with sand substrate were conducted in two sets of experiments. The overall plant results are summarized in Table 8. The first experiment included 3 plants. From the results of this experiment, we were able to account for 67% of the mass balance for $^{14}C_{60}$ (COV = 15%). Of the radioactivity accounted for, 11% \pm 6.3 of the $^{14}C_{60}$ was found in plants and 89% \pm 6.3 remained in the sand (Figure 3). Of the radioactivity found in plants, it was distributed among the different plant parts as follows: 44% in root, 23% in tuber, 22% in leaf, and 11% in stem (Table 9). The variation in uptake we observed among the plant replicates was higher than desired, so we repeated the experiment with six plants.

In the second experiment, a total of 6 plants were used to study uptake of $^{14}C_{60}$. We obtained a $^{14}C_{60}$ mass balance of 59% (COV = 14%). Of the total $^{14}C_{60}$ added, 4% of the $^{14}C_{60}$ was taken up by plants (Figure 5). The rest remained in the sand (SD = 0.8). Of the radioactivity contained in plants, 38% of the $^{14}C_{60}$ was in root, 21% in tuber, 22% in leaf, and 19% in stem (Table 9). From the plant uptake experiments using sand as the

substrate, we observed very little $^{14}\text{C}_{60}$ being taken up into plants from sand despite the potential for nearly maximum bioavailability.

In order to determine the uptake of $^{14}\text{C}_{60}$ into plants under maximum bioavailability, we set up a plant uptake experiment under hydroponic conditions. A total of 7 plants grown hydroponically were used to study $^{14}\text{C}_{60}$ uptake over a period of 2 weeks. A $^{14}\text{C}_{60}$ mass balance of 110% (COV = 12%) was observed. Seven percent of the $^{14}\text{C}_{60}$ was taken up by the plants (SD = 3) (Figure 7). Of the $^{14}\text{C}_{60}$ taken up by the plants, 48% was in the root, 24% in the tuber, 16% in the leaves, and 12% in the stem (Table 9).

A t-test was performed to determine if the higher bioavailability of $^{14}\text{C}_{60}$ caused an increase in the overall uptake of $^{14}\text{C}_{60}$ by plants. The uptake of $^{14}\text{C}_{60}$ by plants grown in the sand was compared to uptake from a hydroponic solution. Welsh's two sample T-test was performed with a confidence interval of 95%. The resulting p-value of 0.0539 indicates that there was no significant difference in the uptake of $^{14}\text{C}_{60}$ by plants grown in the two substrates.

4.5. Biodegradation of C_{60} in Soil

Mineralization ($^{14}\text{CO}_2$ production) of $^{14}\text{C}_{60}$ in silt loam and sandy loam soils has been monitored at regular intervals for 754 and 328 days, respectively. No radioactivity above background has been detected in KOH traps from either soil type. These results indicate that C_{60} appears to be resistant to mineralization in soil for at least 6 months and in one case for at least 1.5 years. At the end of the experiment, ^{14}C remaining in the soil samples was determined by extracting the soil with THF followed by scintillation counting of the extracts. A mass balance of total ^{14}C was then determined. For the sandy

loam, $53\% \pm 4\%$ of the total initial $^{14}\text{C}_{60}$ was recovered and for the silt loam, $51\% \pm 25\%$ was recovered.

Table 1: Physicochemical properties of soils used in studies on the environmental fate of $^{14}\text{C}_{60}$ fullerene nanomaterials.

Soil Type	% OC	% Sand	% Silt	% Clay	pH
Sandy loam	1.3	74	10	16	8.3
Silt loam	2.5	34	54	12	7
Loam	2.5	38	48	14	5.9
Sand	0.1*	100	0	0	7

*Assumption

Table 2: Percentage of $^{14}\text{C}_{60}$ in aqueous solution (prepared by extended mixing in water) lost to filters made of different materials and different pore sizes.

Filter Size (nm)	Filter Type	Percent $^{14}\text{C}_{60}$ lost
unfiltered		0.0
200	Nylon	98.7
450	Nylon	98.9
200	PTFE ¹	98.6
450	PTFE ¹	98.6
450	PVDF ²	96.8
100	PVDF ²	98.2
1000	GF ³	96.5
1500	GF ³	93.8

¹polytetrafluoroethylene

²polyvinylidene fluoride

³glass fiber

Table 3: Percentage of $^{14}\text{C}_{60}$ in aqueous solution (prepared by solvent exchange with THF) lost to filters made of different materials and different pore sizes.

Filter Size (nm)	Filter Type	Percent $^{14}\text{C}_{60}$ lost
unfiltered		0.0
200	Nylon	93.8
450	Nylon	75.0
200	PTFE ¹	47.3
450	PTFE ¹	46.8
450	PVDF ²	46.8
100	PVDF ²	46.6
1000	GF ³	39.3
1500	GF ³	38.5

¹polytetrafluoroethylene

²polyvinylidene fluoride

³glass fiber

Table 4: Sorption equilibrium determination- Percentage of free $^{14}\text{C}_{60}$ found in the supernatant at different time points and different hold times.

Time Point	Percent $^{14}\text{C}_{60}$ in the supernatant at 2 min	Percent $^{14}\text{C}_{60}$ in the supernatant at 1 h	Percent $^{14}\text{C}_{60}$ in the supernatant at 24 h
24 h	50.5	22.7	11.6
48 h	50.1	24.0	8.8
72 h	36.8	11.0	6.3

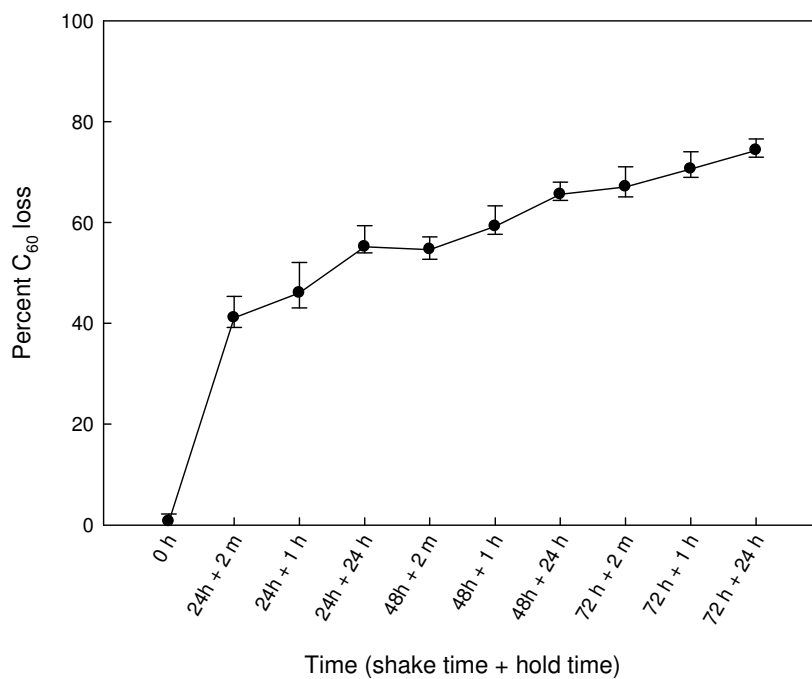


Figure 2: Percentage of $^{14}\text{C}_{60}$ lost to the control vial from time 0 h to 72 h + 24 h in the sorption equilibrium experiment.

Table 5: Sorption Equilibrium Experiment: Koc (mL/g) of $^{14}\text{C}_{60}$ at different time points and hold times.

Time Point	Koc of $^{14}\text{C}_{60}$ at 2 min (mL/g)	Koc of $^{14}\text{C}_{60}$ at 1 h(mL/g)	Koc of $^{14}\text{C}_{60}$ at 24 h(mL/g)
24 h	7233 \pm 2362	24138 \pm 5924	50153 \pm 6220
48 h	6123 \pm 1371	18434 \pm 3142	56384 \pm 8129
72 h	8755 \pm 2619	37800 \pm 6792	63010 \pm 9820

Table 6: K_d (mL/g) and K_{oc} (mL/g) values for $^{14}\text{C}_{60}$ fullerene nanomaterial sorption in three soil types and laboratory sand. Values are the mean \pm standard error of 5 replicates.

Soil Type	K_d (mL/g)	%OC	K_{oc} (mL/g)
Sandy loam	48 ± 12	1.3	3685 ± 908
Silt loam	93 ± 29	2.5	3720 ± 1037
Loam	252 ± 51	2.5	10083 ± 1827
Sand	5.7 ± 1	0.1*	5713 ± 1075

*Assumption

Table 7: Percentage of the sorbed $^{14}\text{C}_{60}$ that desorbed from various soil types over 0 to 72 h. Values are the mean \pm standard error, n = 5 replicates.

Time (h)	% $^{14}\text{C}_{60}$ Desorbed from Silt Loam Soil	% $^{14}\text{C}_{60}$ Desorbed from Silt Loam Soil	% $^{14}\text{C}_{60}$ Desorbed from Loam Soil
0	74.4 \pm 10	36.4 \pm 16	42.4 \pm 4
24	85.4 \pm 19	32.4 \pm 19	35.4 \pm 4
48	85.4 \pm 12	24.4 \pm 12	42.4 \pm 3
72	85.4 \pm 18	30.4 \pm 18	52.4 \pm 5

Table 8: Mean uptake of $^{14}\text{C}_{60}$ into plants from three plant uptake experiments: two sand substrate experiments and one hydroponic substrate experiment.

Plant Uptake Experiment	% $^{14}\text{C}_{60}$ Uptake into Plants
Sand Substrate 1	11.2 ± 6.3 (n=3)
Sand Substrate 2	4.3 ± 0.8 (n=6)
Hydroponic Substrate	7 ± 2.6 (n=7)

*n= number of replicates

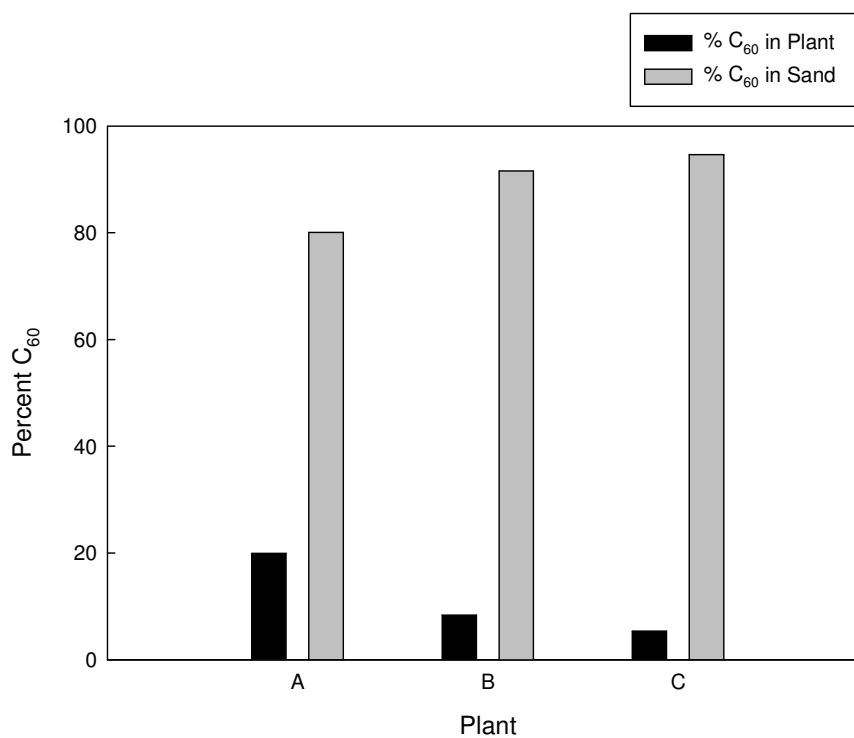


Figure 3: Results of Plant Uptake Experiment 1- The percentage of ¹⁴C₆₀ that was taken up into plants or remaining in the sand for the plant uptake experiment with sand as substrate.

Table 9: Results of the plant uptake experiments with sand as substrate and hydroponic substrate. The distribution of the $^{14}\text{C}_{60}$ taken up by the plants among the different plant parts is shown. Values are the mean \pm standard error.

Plant Part	Uptake Experiment 1 $^{14}\text{C}_{60}$ (% of Total Uptake)	Uptake Experiment 2 $^{14}\text{C}_{60}$ (% of Total Uptake)	Uptake Experiment 3 $^{14}\text{C}_{60}$ (% of Total Uptake)
Root	43.7 \pm 8	37.7 \pm 5	47.6 \pm 11
Stem	11.4 \pm 11	18.7 \pm 2	12.1 \pm 3
Tuber	23.3 \pm 5	21.3 \pm 2	23.8 \pm 10
Leaf	21.5 \pm 4	22.3 \pm 3	16.5 \pm 8

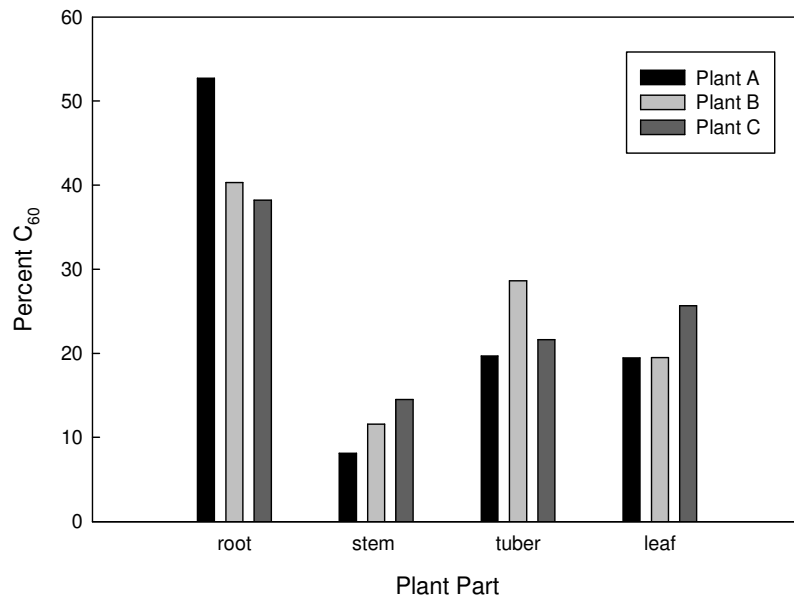


Figure 4: Plant uptake experiment with sand as substrate 1. Percent $^{14}\text{C}_{60}$ in each plant part for each plant tested.

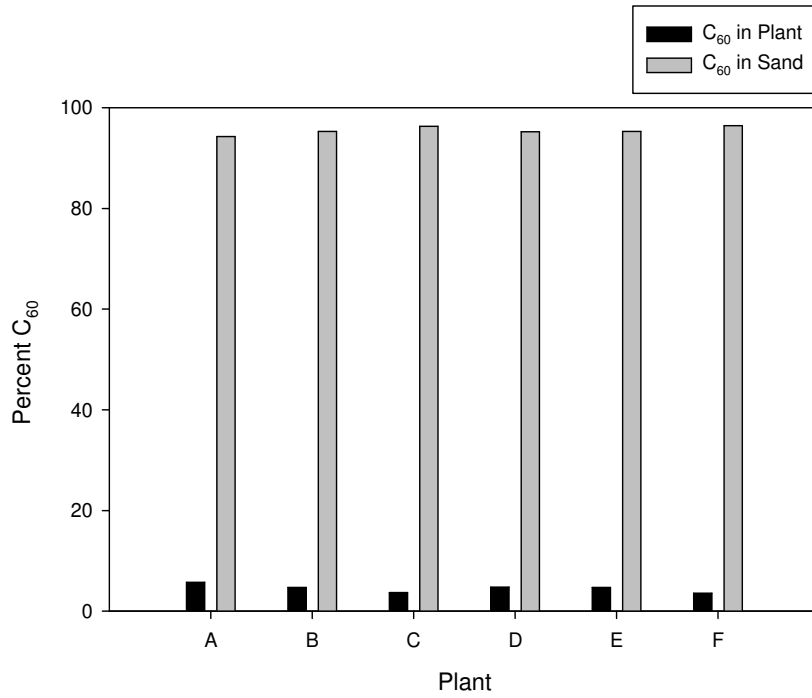


Figure 5: Results of the Plant Uptake Experiment 2- The percentage of ¹⁴C₆₀ that was taken up into plants or remained in the sand for the plant uptake experiment with sand as substrate.

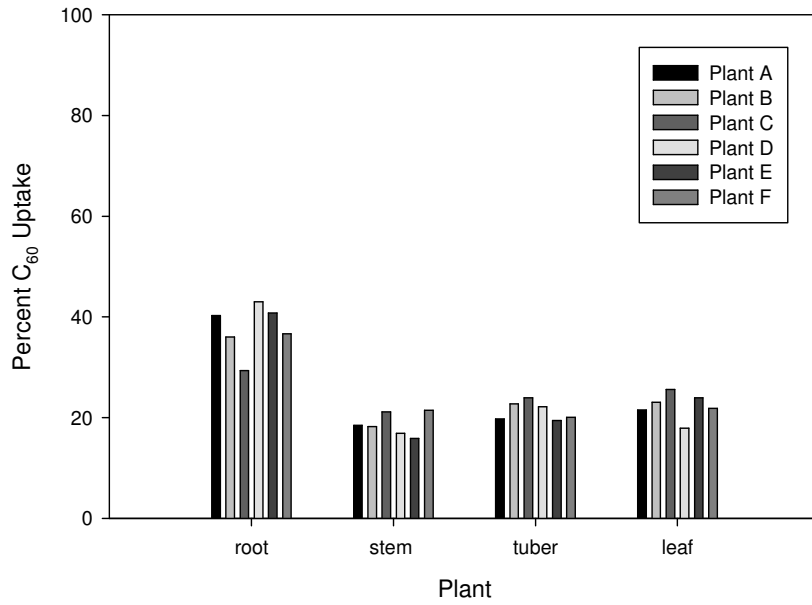


Figure 6: Plant uptake experiment with sand as substrate 2. Percent ¹⁴C₆₀ in each plant part for each plant tested.

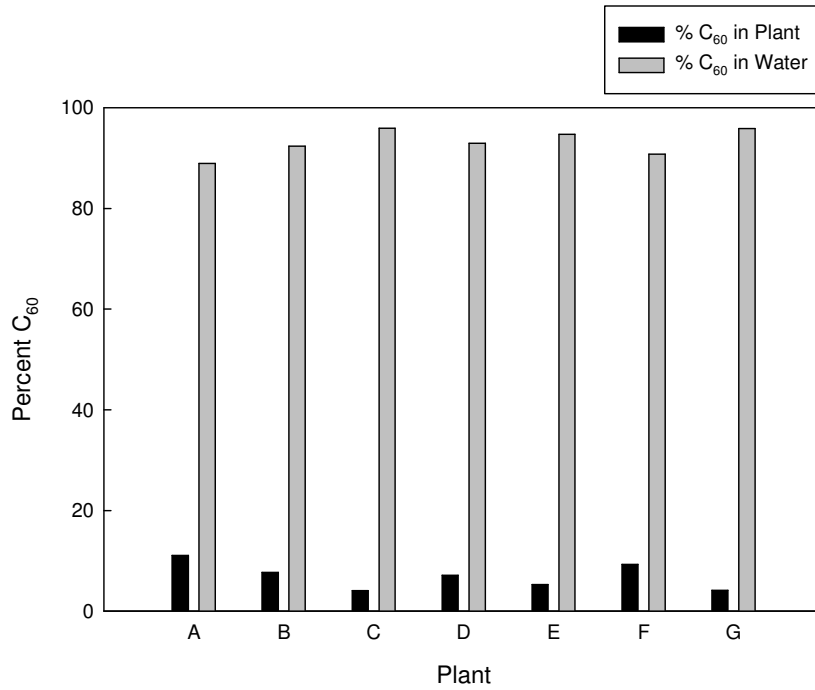


Figure 7: Results of the Plant Uptake Experiment 3- The percentage of ¹⁴C₆₀ that was taken up into plants or remaining in water for the plant uptake experiment in hydroponic solution.

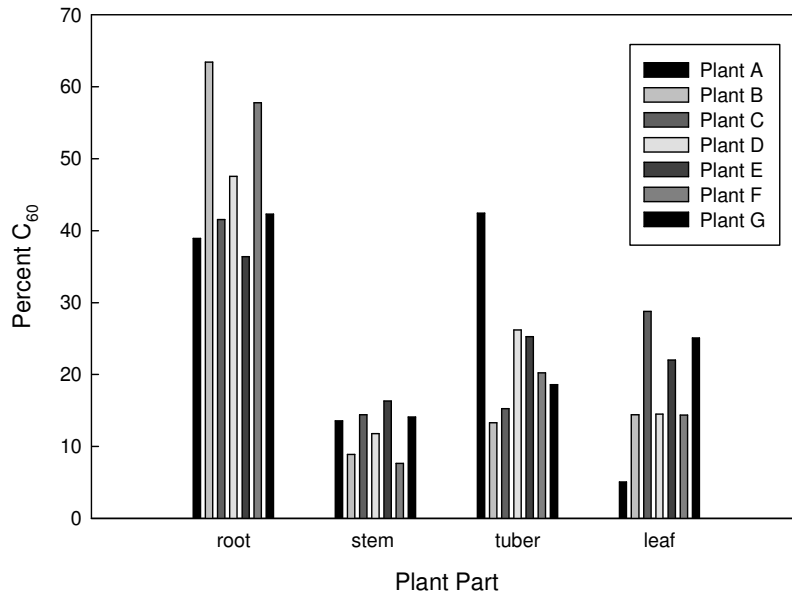


Figure 8: Plant uptake experiment with a hydroponic substrate. Percent $^{14}\text{C}_{60}$ in each plant part for each plant tested.

CHAPTER V

DISCUSSION

Since their discovery in 1985, fullerenes have been used in a number of fields owing to their size, physical, and chemical properties. As predicted, the use of fullerenes in industry has grown tremendously in the last decade and more utilization of these nanomaterials is expected in the future. Given their widespread use, it is likely that fullerene nanomaterials will eventually enter the environment. Therefore, it is prudent to determine the toxicity, behavior, and fate of fullerenes to estimate the risk that they may pose to humans as well as the environment.

Fullerenes in molecular form are highly lipophilic (Jafvert and Kulkarni, 2008). However, once in a polar environment, they can form aggregates that are nano in size. These “solutions” are actually water suspensions in which the fullerene aggregates have a net negative surface charge making them slightly polar (Andrievsky et al., 1995). Thus, fullerenes exhibit dual properties which make them very difficult to measure or quantify in natural environments. Isaacson and co-workers in 2009 also describe how the duality in properties of fullerenes makes it extremely difficult to use a single analytical method to study their fate and distribution in different natural environmental systems. The use of radiolabeled fullerenes to study fate in the environment under controlled laboratory conditions is not an exact representation of their behavior in natural systems; however, radioisotopes provide some significant analytical advantages. Ultimately, the use of radiolabeled fullerene nanomaterials provides a simple and efficient technique to begin to

gain some insight into fullerene behavior in different environmental systems (Isaacson et al., 2009).

We used a solvent exchange method (Fortner et al., 2005) and an extended mixing method to prepare aqueous suspensions of fullerenes. The solutions were characterized for aggregate size using a filtration experiment. The results from these experiments showed that the aggregates in the suspension prepared by extended mixing in water had sizes larger than the defined nanomaterial range. The suspension prepared using the solvent exchange method with THF had a majority of the aggregates in the nanometer range. The results also showed that the use of nylon filters increased the loss of C₆₀ aggregates to the filter compared to PTFE, PVDF, and GF filters suggesting that the filter material also influences apparent aggregate size. Variability of the results among different filters may be explained by the different properties of the filters including surface charge, variability of pore size, extent of clogging in different filter materials etc. Although only about 60% of the C₆₀ aggregates corresponded to the expected size of 5 to 500 nm (Fortner et al., 2005), taking the role of the filter material and the ambiguity of the actual form of fullerene aggregates in the natural environment (may be nanometer size or larger) into consideration, it was decided to proceed with using the aqueous ¹⁴C₆₀ solution prepared by solvent exchange for all the fate experiments. Other unpublished studies performed by Dr. George Cobb's research group (TIEHH, Texas Tech University) also showed variability in the size of fullerene aggregates in a solution of THF.

In the sorption equilibrium experiment, it was determined that the 24 h, 48 h and 72 h time points had similar counts of C₆₀ in the supernatant at individual hold times,

suggesting that 24 h was long enough for all the sorption to take place. Although the counts for the 72 h time point were slightly lower compared to the 24 and 48 h, this can be explained by the reduction in the volume of water over time (each vial had a total of 20 mL of water initially and 1 mL was removed from for each time point + hold time). By the time the 72 h time point readings were taken, 7 mL of the free C_{60} containing supernatant had been removed reducing the free C_{60} in solution. The readings at individual hold times suggested that some aggregates stick to suspended particles and settle over time in addition to the natural loss of C_{60} to the vials (control). Thus by choosing the 24 h + 2 min hold time, we are likely underestimating sorption (because we are counting C_{60} aggregates stuck to suspended soil particles as part of the C_{60} not yet sorbed), but at the same time, longer hold time readings would have included natural aggregate settling and the particle-assisted settling in the sorption estimate, leading to an over estimation of sorption (as seen in Table 5).

The control vials in the sorption experiment were used to determine the loss of $^{14}C_{60}$ to the vials by the processes of settling or sticking to the vial walls (causing an over-estimation of sorption). In spite of using a 0.01 M $CaCl_2$ solution to provide ionic strength to the solution and in turn encourage soil particle settling, the control vials showed an average of $14.6\% \pm 6.02$ loss of $^{14}C_{60}$ from the water column. The “loss” of $^{14}C_{60}$ from solution was likely caused by increased aggregation and settling from the change in ionic strength; it is also possible that $^{14}C_{60}$ was lost from solution by sorption to the walls of the glass containers. Considering the settling of C_{60} in control vials, the partition coefficient value for $^{14}C_{60}$ determined in sand can be considered negligible.

The organic carbon partition coefficient (Koc) values for $^{14}\text{C}_{60}$ determined experimentally corresponded to “strong/ very strong sorption” (EPA, 2005) and “slight mobility” (Swann et al., 1983). Koc, being a normalized value, was expected to be consistent for all soil types. The Koc value for $^{14}\text{C}_{60}$ in sandy loam, silt loam, and sand were numerically and statistically similar but the Koc value for $^{14}\text{C}_{60}$ in the loam was nearly three-fold higher. The organic carbon partition coefficient to sand was calculated with an assumed organic carbon content of 0.1%. Given the assumption, it would be reasonable to expect that the Koc for $^{14}\text{C}_{60}$ in sand might be different from the values in soil. However, that was not the case. Also, when we compare our log Koc estimates (3.6 - 4 mL/g) with a previously published log Koc estimate (7.1 mL/g) (Chen and Jafvert, 2009), we find that our estimate is around three orders of magnitude lower. It should be noted that Chen and Jafvert estimated the Koc value for molecular C_{60} by extrapolation of the partition coefficient between soil and ethanol/water mixture at varying mole fractions. Although this is a very good measure of Koc of molecular C_{60} , in natural polar environments C_{60} has a tendency to form nano-aggregates; the sorption of these nano-aggregates to organic matter might vary from that of the molecular form, as seen from our results.

Although the organic carbon content of the silt loam and the loam were the same, $^{14}\text{C}_{60}$ had a higher Koc value in the loam soil than in the silt loam. This suggests that soil compartments other than organic carbon participate in the sorption of fullerene nanomaterials. It is possible that since C_{60} nano-aggregates have a surface charge, other interactions besides simple partitioning may contribute to C_{60} 's interaction with soil (Isaacson et al., 2009). The potential role of soil mineral type and content (in addition to

organic content) should be considered when assessing the sorption of C₆₀ to different soil types. It is also possible that other factors like pH play a role in the apparent sorption of C₆₀ aggregates to soil. It has been shown that C₆₀ aggregates that are formed in the aqueous solution prepared by solvent exchange have a net negative surface charge (Isaacson et al., 2009). As a result, the sorption of these molecules to soils with acidic pH might be higher when compared to those with alkaline pH.

Considering the fact that we used the 24 h + 2 min time point to measure sorption and desorption, it can be said that we partially underestimated sorption and in turn, partially overestimated desorption (since we are using the sorption values to estimate percent desorption). C₆₀ sorbed to sandy loam desorbed very easily and quickly when compared to the silt loam and loam soil types. 85.4% of the C₆₀ sorbed to the sandy loam desorbed by 24 h. This indicates a weak association of C₆₀ aggregates, probably to the outer surfaces of the soil particle. Desorption of C₆₀ from the silt loam and loam was less and occurred at a slower rate compared to desorption of C₆₀ from the sandy loam. This indicates a strong association of the C₆₀ to the soil particles, possibly in the internal pores of the soil structure (Pignatello, 2000). Although C₆₀ adsorbs readily to soil, desorption does occur, even in soils with relatively high organic carbon content.

There are some uncertainties in the sorption and desorption results that we obtained, owing to the uncertainties brought in by the natural loss of C₆₀ from the water column (by sticking to glass, aggregation, and settling) and particle assisted settling over time. Nevertheless, it is a good estimate of what can be expected following an intentional or unintentional release of C₆₀ into the natural environment.

Plant uptake studies using both sand and hydroponic substrates indicated very low (< 10%) uptake of C₆₀ into radish plants, despite conditions of maximum bioavailability of C₆₀ to the plant; there was no significant difference between uptake of C₆₀ from sand substrate and hydroponic substrate. Despite the use of ¹⁴C₆₀ for the plant studies, the mass balance of C₆₀ in the two plant uptake experiments using sand substrate could not be closed. We hypothesized that some ¹⁴C₆₀ may have remained in the containers following extraction, despite efforts to efficiently remove it. Others have commented that extraction of C₆₀ using solvents is not a completely efficient process (Isaacson et al., 2009). For the plant uptake studies in the hydroponic substrate, the mass balance was closed. The efficiency of the methodology used to extract the plant parts could produce uncertainty in 2 important measurements that were part of the plant uptake study: (1) the determination of ¹⁴C₆₀ concentrations in plant compartments (the magnitude of plant uptake), and (2) the determination of the ¹⁴C₆₀ mass balance. Isaacson et al. suggested that the presence of a polar "cloak" around the hydrophobic fullerene molecules in aqueous solutions could cause low efficiency in the recovery/extraction of fullerenes from samples using organic solvents. This reduced extraction efficiency may lead to not all of the fullerenes taken up by the plant being quantified and as a result an underestimation of the uptake of C₆₀ by plants. There is a need for more understanding of the properties of fullerenes in polar environments and subsequently better extraction methods.

Topp and co-workers (1986) showed that chemicals with high log K_{ow} bind very tightly to soil and hence are typically not taken up by plants as their availability in soil is small. Results of the sorption test indicate a strong association of fullerenes with soil.

This is consistent with the minor uptake of C_{60} in plants that we observed. In addition, it was observed that a higher proportion of the $^{14}C_{60}$ taken up by plants remained in the root. This is also consistent with conclusions of Topp and co-workers in that higher log K_{ow} compounds tend to stick to the root leading to less of the chemical being translocated to the upper plant parts.

The biodegradation experiment results showed no production of $^{14}CO_2$ in either the silt loam or sandy loam. Although there is no evidence that C_{60} can be mineralized in soil, the possibility of partial breakdown of the C_{60} structure cannot be excluded. It is possible that the microbial flora in the soil could not break the C_{60} molecule completely, but was able to at least break some bonds in the structure. The photolytic breakdown of C_{60} cages (in aqueous suspensions) has been studied by Gelca et al., (2010). They observed the formation of open cage derivatives with carbonyl groups and hydrogen atoms (MS and FTIR spectra). This suggests possible degradation of C_{60} molecules in contact with air and water. Since only a KOH trap was used to capture production of $^{14}CO_2$, there was no way that any partially degraded C_{60} molecules could be captured and detected. Also, the detection method in these studies (liquid scintillation spectroscopy) can only detect the presence of ^{14}C , and makes no distinction as to chemical form of the ^{14}C .

CHAPTER VI

CONCLUSIONS

Our results describing basic environmental fate parameters for C₆₀ have helped to fill in some data gaps related to the potential for fullerene nanomaterials to impact the environment. We obtained information on the association of C₆₀ with soil, the magnitude and rate of desorption of C₆₀ from soil, uptake and distribution of C₆₀ in vegetation, and the potential for trophic transport of C₆₀ to higher organisms.

C₆₀ has a strong tendency to associate with soil types based on their texture and other properties. Desorption of C₆₀ from soil also varied based on the soil type and the strength of sorption. But, as is the case in nanomaterials research, there are some uncertainties in the results we obtained owing to the number of ambiguities that nanomaterials bring into the system (due to their unusual physio-chemical properties). Factors like aggregation, solubility in different solvents, inefficient extraction efficiency need to be studied in detail before more certain conclusions can be obtained.

With respect to the possibility of trophic transfer of fullerenes from soil to higher organisms through plants, it is likely only for organisms that consume below-ground plant parts; although some C₆₀ is translocated to above-ground vegetation. Invertebrates dwelling in soil may be exposed to C₆₀ and in turn cause exposure to small birds and mammals through secondary exposure. The high lipophilicity of C₆₀ fullerene also suggests the possibility of bio-magnification, but to date this has not been evaluated.

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